Natural gradients in temperature and nitrogen: Iceland represents a unique environment to clarify long-term global change effects on carbon dynamics

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Clarification of contribution

I hereby declare that that the writing of the following thesis and the five accompanying papers (here referred to with their Roman Chapter numerals) is my work, done under supervision of Prof. Ivan A. Janssens, Prof. Bjarni D. Sigurdsson and Dr. Sara Vicca.

The contribution of Niki I. W. Leblans to the papers included in this thesis consists of:

Chapter II: Leblans collected a substantial part of the data presented in the paper. The paper was drafted by Sigurdsson, and was further revised by all co-authors. Sigurdsson was responsible for correspondence with the scientific journal.

Chapter III: Leblans collected the data presented in the paper. Leblans joined all data and performed the statistical analyses. All co-authors contributed to the to the interpretation of the data. Leblans drafted the paper, further revised by all co-authors.

Chapter IV: Leblans collected a substantial part of the data presented in the paper and performed the statistical analyses. All co-authors contributed to the to the interpretation of the data. Leblans drafted the paper, further revised by all co-authors. Leblans was responsible for correspondence with the scientific journal.

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Niki I. W. Leblans

Abstract

Leblans, N. I. W. 2016. Natural gradients in temperature and nitrogen: Iceland represents a unique environment to clarify long-term global change effects on carbon dynamics. Joint doctoral thesis at Antwerp University and Agricultural University of Iceland.

Global change is one of the greatest challenges of our generation. Surface temperatures are rising as a consequence of anthropogenic greenhouse gas (GHG) emissions, but projections remain highly uncertain as solid knowledge on potent feedback mechanisms from ecosystems to the climate system is limited. One such potentially powerful feedback mechanism is the warming-induced transfer of soil organic carbon (SOC) to the atmosphere, exacerbating the warming. This is especially true for high northern latitude ecosystems (≥ 60° N) where both the highest amount of SOC is stored and where warming is most pronounced. In this region, SOC storage is also strongly linked to nitrogen (N) cycling, as plant productivity, the primary source of SOC, is generally N limited in cold ecosystems. During the past decades, northern temperate and some boreal ecosystems have already been absorbing about 10% of the anthropogenic C emissions due to an N-driven increase in productivity. At high northern latitudes, the N inputs, which have remained relatively low up to now, are expected to increase substantially, potentially transforming northern ecosystems to important contributors of the N-induced C sink in the near future. However, the future evolution of both warming-induced SOC losses and the N-dependent C sink is highly uncertain due to the scarcity of empirical observations of long-term effects.

In this thesis, natural gradients were used to obtain empirical observations of long-term warming and N input effects on C dynamics in subarctic grasslands, which cover ca. 10% of the global terrestrial surface. The primary advantage of natural gradients is that equilibrium states can be observed. Further, their typical wide and continuous nature allows to study the full range of global change projections and to detect non-linearities in the response. Here, I investigated natural (geothermal) soil temperature gradients (+0 - +20 °C) at the ForHot research site (www.forhot.is) in southwest Iceland, where the presence of both short-term (formed in 2008) and long-term (≥ 50 years) gradients allowed to separate transient (short-term) from permanent (long-term) responses. Natural gradients in N inputs were investigated on islands of Vestmannaeyjar (off the south coast of Iceland) with differing soil age (and thus different N-accumulation time; 50 vs. 1,600 years) and amounts of seabird N inputs.

Firstly, I aimed to evaluate the potential of natural gradients of geothermal soil warming and N inputs as global change laboratories and secondly to assess and compare their short-term and long-term effects on the C dynamics of subarctic grasslands, with focus on plant phenology and SOC sequestration.

The geothermal soil temperature gradients proved to be a valuable complement to other warming techniques to improve our understanding of long-term warming effects on ecosystems, overcoming important difficulties that are typical for climate manipulation experiments, such as limited duration of the warming and a limited number of warming levels.

The length of the growing season (LOS), a phenological parameter of great importance in the C balance of northern ecosystems, extended linearly with increasing soil temperature by on average 15.6 ± 4.7 (SE) days $°C^{-1}$. This was primarily due to an advance in the start of the growing season, while the end of the growing season was largely unresponsive to warmer soil temperatures. This persistency of the warming-induced extension of LOS, which has recently been questioned, has important implications for the C-sink potential of subarctic grasslands under climate change.

SOC stocks showed a large and linear decline under soil warming (4.1 ± 0.5 SE % $°C^{-1}$), both after 5 and ≥ 50 years of warming. Central to the observed SOC loss was a loss of physical stabilization in soil aggregates. This was an important finding and such mechanism is presently not embedded in any Earth System Model, but its inclusion could possibly improve model projections. Further, the similar SOC loss after short- and long-term warming suggested that warming of subarctic grassland soils could cause a rapid positive feedback to climate warming.

In contrast to soil warming, increasing N inputs increased the short-term SOC storage rate of early successional and mature subarctic grasslands (from 0.018 to 0.29 and from 0.30 to 0.44 ton SOC ha^{-1} yr^{-1} respectively), as well as the long-term SOC storage rate of the mature grasslands (from 0.12 to 0.16 ton SOC ha^{-1} yr^{-1}). This suggested that the N-induced C sink of subarctic grasslands could be maintained for many centuries, which contradicts the current hypothesis that the N-deposition driven terrestrial CO_2 sink is likely to saturate in the near future. Further, the early successional ecosystems were proportionally more responsive to increased N inputs than mature ecosystems, and had a larger SOC storage potential.

I conclude that future climate warming can transform subarctic grassland soils into large C sources over a short time span. The N-induced increase in the C uptake of subarctic

grasslands could continue during the next centuries, but will probably be insufficient to offset the warming-induced C losses.

Keywords: Global change, subarctic grasslands, geothermal soil warming, organic nitrogen inputs, carbon sequestration, phenology

Samenvatting

Leblans, N. I. W. 2016. Natuurlijke temperatuur- en stikstofgradiënten: IJsland biedt unieke mogelijkheden om lange-termijn effecten van klimaatverandering op koolstofdynamieken te bestuderen. Dubbel doctoraat aan de Universiteit van Antwerpen en de Landbouw Universiteit van IJsland.

Klimaatverandering is een van de grootste uitdagingen van onze generatie. Oppervlakte-temperaturen stijgen als gevolg van antropogene boeikasgas uitstoot, maar voorspellingen zijn hoogst onzeker door de gelimiteerde kennis van krachtige terugkoppelingsmechanismen van ecosystemen naar het klimaatsysteem. Een voorbeeld van een potentieel belangrijk terugkoppelingsmechanisme is de transfer van organische bodemkoolstof (SOC) naar de atmosfeer als gevolg van stijgende temperaturen, wat de opwarming verder zou versterken. Dit is in het bijzonder het geval voor noordelijke ecosystemen (≥ 60° N), die niet alleen de grootste hoeveelheid SOC bevatten maar ook de sterkste opwarming ondergaan. In deze ecosystemen is SOC-opslag ook sterk gekoppeld aan de stikstof- (N-) cyclus, omdat plantproductie, wat de hoofdbron is van SOC, in koude ecosystemen over het algemeen gelimiteerd is door de beschikbaarheid van N. Noordelijke gematigde ecosystemen hebben de voorbije decaden al ongeveer 10% van de antropogene C emissies opgenomen door een N-gestimuleerde toename in productiviteit. Omdat verwacht wordt dat de N-input op hoge noordelijke breedtegraden - die tot nu toe relatief laag was - sterk zal toenemen, kunnen noordelijke ecosystemen in de nabije toekomst potentieel sterk bijdragen aan deze N-gestimuleerde C-sink. Desondanks is de toekomst van zowel de door opwarming geïnduceerde SOC verliezen als van de N-afhankelijke C opslag bijzonder onzeker als gevolg van een gebrek aan empirische observaties van lange-termijn effecten.

In deze thesis werden natuurlijke gradiënten gebruikt om empirische observaties te verwerven van lange-termijn effecten van opwarming en N-input op de C-dynamiek van subarctische graslanden, die ca. 10% van het globale terrestrische oppervlak omvatten. Het belangrijkste voordeel van natuurlijke gradiënten is de mogelijkheid om evenwichts-toestanden te bestuderen. Daarnaast zijn natuurlijke gradiënten typisch zeer breed en geleidelijk, waardoor het volledige bereik van de klimaatvoorspellingen onderzocht kan worden en niet-lineaire responsen waargenomen kunnen worden. Meer specifiek werden natuurlijke (geothermische) bodem-temperatuurgradiënten (van +0 tot +20 °C) onderzocht aan de ForHot onderzoek site (www.forhot.is) in zuidwest IJsland, waar de aanwezigheid van

korte- (ontstaan in 2008) en lange-termijn (≥ 50 jaar oud) gradiënten het mogelijk maakte om een onderscheid te maken tussen transiënte (korte-termijn) en permanente (lange-termijn) responsen. Natuurlijke N input gradiënten werden onderzocht op verschillende locaties op de Vestmannaeyjar (een eilandengroep voor de zuidkust van IJsland) met verschillende bodemouderdom (en dus verschillende N-accumulatietijd; 50 vs. 1,600 jaar) en met verschillende hoeveelheden zeevogel N-input.

Het doel van deze thesis was om eerst het potentieel te evalueren van natuurlijke gradiënten in geothermische bodemopwarming en in N-input als klimaatveranderings-laboratoria om daarna hun korte- en lange-termijn effecten op de C-dynamiek van subarctische graslanden te bestuderen en te vergelijken, met focus op plant fenologie en SOC-opslag.

Geothermische gradiënten in bodemtemperatuur bleken een waardevolle bijdrage te kunnen leveren, naast andere opwarmingstechnieken, om onze kennis van lange-termijn opwarming op ecosystemen te verbeteren, omdat ze toelaten verschillende moeilijkheden te omzeilen die typisch zijn voor klimaat manipulatie experimenten, zoals een gelimiteerde opwarmingsduur en aantal opwarmingsniveaus.

De lengte van het groeiseizoen, een fenologische parameter die van groot belang is in de C balans van noordelijke ecosystemen, liep lineair op met gemiddeld 15.6 ± 4.7 (SE) dagen per °C toename in bodemtemperatuur. Dit was hoofdzakelijk het gevolg van een vervroeging van de start van het groeiseizoen, terwijl het einde van het groeiseizoen grotendeels onafhankelijk was van bodemtemperatuur. Deze persistente verlenging van het groeiseizoen met toenemende temperatuur, die recent in vraag werd gesteld, heeft belangrijke implicaties voor de potentiële C opname van subarctische graslanden in een toekomstig, warmer klimaat.

Toenemende bodemtemperatuur veroorzaakte een sterke lineaire afname in SOC-opslag, met een gemiddeld van 4.1 ± 0.5 SE % per °C bodemtemperatuurstijging, zowel na 5 als na ≥ 50 jaar opwarming. Een belangrijk aspect in het SOC-verlies was de afname van fysische stabilisatie in bodem aggregaten. Dit was een belangrijke bevinding en dit mechanisme is momenteel niet opgenomen in klimaatveranderingsmodellen (Earth System Models). Zijn inclusie heeft potentieel om de modelvoorspellingen te verbeteren. Verder toonde het gelijkaardige verlies aan SOC na korte- en lange-termijn opwarming aan dat de opwarming van subarctische graslandbodems een snelle en positieve terugkoppeling naar klimaatopwarming zou kunnen veroorzaken.

In tegenstelling tot bodemopwarming zorgden toenemende N-inputs voor een stijging in de korte-termijn SOC-opslagsnelheid van subarctische graslanden, zowel in een vroeg successie stadium als in een matuur stadium (van 0.018 naar 0.29 en van 0.30 naar 0.44 ton SOC ha^{-1} yr^{-1} respectievelijk). Ook de lange-termijn SOC-opslagsnelheid in mature graslanden nam toe (van 0.12 naar 0.16 ton SOC ha^{-1} yr^{-1}). Dit suggereert dat de door N gestimuleerde C-sink van subarctische graslanden voor vele eeuwen zou kunnen aanhouden, in tegenstelling tot de huidige hypothese dat de door N-afhankelijke terrestrische C-sink zou satureren in de nabije toekomst.

Samenvattend kan klimaatopwarming subarctische graslandbodems over een korte tijdsspanne transformeren tot grote C bronnen. De door N gestimuleerde toename in C opname van subarctische graslanden kan aanhouden gedurende de volgende eeuwen, maar zal waarschijnlijk niet volstaan om de C verliezen als gevolg van opwarming te compenseren.

Trefwoorden: Klimaatverandering, subarctische graslanden, geothermische bodem-opwarming, organische stikstof input, koolstofopslag, fenologie

Útdráttur

Leblans, N. I. W. 2016. Náttúrulegir stiglar í jarðvegshita og köfnunarefnisákomu : Ísland býr yfir einstökum náttúrulegum aðstæðum til rannsókna á áhrifum hnattrænna umhverfisbreytinga á kolefnishringrás vistkerfa. Sameiginleg doktorsrigerð við Landbúnaðarháskóla Íslands og Antverpenháskóla.

Hnattrænar umhverfisbreytingar eru meðal stærstu áskorana sem núlifandi kynslóðir standa frammi fyrir. Hitastig jarðar stígur jafnt og þétt vegna losunar manna á gróðurhúsalofttegundum (GHG), en mismunandi loftslagslíkön spá mjög ólíkt fyrir um hraða hlýnunarinnar í framtíðinni vegna mikillar óvissu um ýmsa hitastigsháða ferla í náttúrunni sem geta bæði temprað eða magnað hlýnunina. Eitt slíkt ferli er hvað verður um jarðvegskolefni (SOC) eftir að loftslag hefur hlýnað umtalsvert. Ef hlýnunin leiðir til aukins niðurbrots og losunar til andrúmslofts þá gæti það leitt til jákvæðrar mögnunar á gróðurhúsaáhrifunum. Þetta er sérstakt áhyggjuefni á norðurslóðum (≥ 60° N), þar sem magn SOC er mest og mestri hlýnun er einnig spáð á heimsvísu. Kolefnisbinding í jarðvegi norðurslóða er hinsvegar mjög tengd hringrás köfnunarefnis (N), en nettó frumframleiðni (NPP) vistkerfa norðurslóða er almennt stýrt af framboði N sem losnar hægt vegna lágs jarðvegshita og stutts vaxtartíma. Það er talið að jarðvegur norðurslóða hafi bundið um 10% af heildarlosun manna á GHG síðustu áratugi, einkum vegna aukningar á NPP í kjölfar aukinnar ákomu N. Frekari aukningu á N-ákomu er spáð á norðurslóðum, en það óvíst er hvort það mun halda áfram að auka kolefnisbindingu vistkerfa þar til framtíðar og hvernig þau áhrif munu vega á móti áhrifum hlýnunar. Því skortir mjög frekari rannsóknir á langtímaáhrifum N-ákomu og hlýnunar á kolefnisforða norðlægra vistkerfa.

Í þessari ritgerð eru teknar saman rannsóknir sem unnar voru á graslendum á Íslandi á áhrifum náttúrulegra stigla (e. gradients) í N-ákomu og hitafari á kolefnishringrás þeirra, en graslendi þekja um 10% af flatarmáli jarðar. Meginkostur við að nýta náttúrulega stigla eru að áhrif umhverfisþáttana hafa verið til staðar um langan tíma og svörun vistkerfanna því oft orðin stöðugri en í stýrðum tilraunum sem standa yfirleitt stutt. Einnig gera stiglarnir það yfirleitt mögulegt að rannsaka áhrif umhverfisþáttanna við aðstæður sem spanna þær umhverfisbreytingar sem spáð er á komandi öld og þar með að kanna hvort líklegt er að svörunin vistkerfanna verði ólínuleg. Stiglarnir sem ég rannsakaði voru áhrif jarðhita (+0 til +20 °C) í graslendum á rannsóknasvæði ForHot (www.forhot.is) við Hveragerði, en þar eru til staðar bæði jarðhitastiglar frá Suðurlandsskjálftanum 2008 (5-6 ára) og eldri stiglar í Grændal

(>50 ára). Með því að bera svörun vistkerfa saman á milli þessara tveggja svæða má greina á milli skammtíma- og langtímaáhrifa hlýnunar. Náttúrulegir stiglar í N-ákomu voru rannsakaðir í Vestmannaeyjum, í Surtsey, Elliðaey og á Heimaey. En með því að bera saman svæði með og án sjófuglaáhrifa á Surtsey annarsvegar og sambærileg svæði á hinum eyjunum hinsvegar var hægt að greina á milli áhrifa aukinnar N-ákomu við tvö framvindustig graslenda (50 vs. 1,600 ár).

Fyrsta markmið mitt var að kanna hvort nota mætti jarðhitastigla og stigla í N-ákomu af völdum sjófugla sem „náttúrulegar rannsóknastofur“ á áhrifum hnattrænna umhverfisbreytinga (hlýnun jarðar og aukin N-ákoma af mannavöldum) og síðan að mæla og bera saman skammtíma- og langtímaáhrif slíkra umhverfisbreytinga á vaxtarferla og kolefnisforða norðlægra graslendisvistkerfa.

Niðurstöðurnar voru þær að nota mætti jarðvegshitastigla ForHot til rannsókna á áhrifum loftslagsbreytinga, og að þeir gæfu verðmætar upplýsingar sem aðrar stýrðar loftslagsrannsóknir gefa sjaldan; það er að lýsa bæði langtímaáhrifum hlýnunar (5 - >50 ár) og niðurstöðum á áhrifum af fleiri en einni eða í mesta lagi tveimur auknum hitastigum, eins og flestar stýrðar rannsóknir þurfa að takmarkast við vegna kostnaðar og tæknilegra takmarkana.

Einnig kom í ljós að lengd vaxtartíma jókst línulega með aukinni hlýnun, eða um 15,6 daga ± 4,7 (SE) á hverja gráðu sem hlýnaði að meðaltali í lofti og jarðvegi. Lengingin varð einkum vegna þess að grasvöxtur hófst fyrr að vori þar sem hlýrra var, en lok vaxtartímans að hausti breyttust tiltölulega lítið við hlýnunina. Nýlega hafa verið settar fram tilgátur um að norðlæg vistkerfi séu hætt að lengja vaxtartíma sinn vegna þeirrar hlýnunar sem þegar er orðin eða er að verða, en verkefni mitt styður ekki þær tilgátur. Lenging vaxtartíma af þeirri stærðargráðu sem fannst í þessu verkefni mun hafa mikil áhrif á framleiðni og kolefnisjöfnuð norðlægra graslenda í framtíðinni ef loftslag heldur áfram að hlýna.

Aukin jarðvegshlýnun leiddi einnig til mikils og línulegs taps á SOC (4.1 ± 0.5 SE % $°C^{-1}$), bæði eftir fimm ára og ≥ 50 ára hlýnun. Minnkun samkorna (e. aggregates) í jarðvegi útskýrði að hluta þetta mikla tap á SOC. Slíkar breytingar á jarðvegsbyggingu er ekki að finna í spálíkönum um loftslagsbreytingar (Earth System Models) í dag, en gætu mögulega verið mikilvægar og aukið spágetu slíkra líkana. Hversu hratt þessar breytingar gerðust í graslendum ForHot og það að graslendin sem höfðu verið upphituð lengur með jarðhita sýndu sama tap á SOC bendir til frekari loftslagsbreytingar geti valdið hraðri losun á SOC úr jarðvegi norðlægra graslenda sem gæti þýtt að þau gætu valið jákvæðri mögnun (e. positive feedback) á hraða loftslagsbreytinganna í framtíðinni.

Öfugt við áhrif hlýnunar þá leiddi aukin N-ákoma frá sjófuglum til aukins hraða kolefnisbindingar í efstu lögum jarðvegs, bæði við frumframvindu og síðframvindu graslenda (úr 0.018 í 0.29 tonn SOC ha^{-1} $ári^{-1}$ í Surtsey og úr 0.30 í 0.44 tonn SOC ha^{-1} $ári^{-1}$ á eldri eyjunum). Eftir 1600 ára óraskaða framvindu á eldri eyjunum leiddi aukna N-ákoman einnig til meiri heildaruppsöfnunar SOC í öllu jarðvegssniðinu (0,12-0,16 tonn SOC ha^{-1} $ári^{-1}$). Þetta bendir til þess að aukin N-ákoma geti hvatað kolefnisbindingu norðlægra graslenda í margar aldir og því séu áhyggjur óþarfar af því að það muni verða mettun á kolefnisbindigetu slíkra vistkerfa við áframhaldandi aukna N-ákomu á næstu áratugum. Hinsvegar er einnig ljóst að framvindustig skiptir einnig miklu máli hversu mikil slík kolefnisbinding getur orðið og bæði bindigetan og bindihraðinn er mestur í frumframvindu.

Ég dreg þá ályktun af þessum rannsóknum að hlýnandi loftslag muni hugsanlega valda hröðu og miklu tapi á kolefniforða jarðvegs á norðurslóðum, en að aukin N-ákoma muni auka frumframleiðni og bindingu kolefnis í jarðvegi. Hinsvegar sé ólíklegt að áhrif N-ákomunnar nái að vega á móti hlýnuninni.

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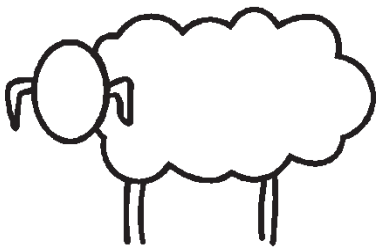
Bedankt! Thanks! Takk fyrir kaerlega!

September 2016

Niki Leblans



... and of course, a special word of thanks to the Grændalur sheep for keeping me company during the long days in the field.

Contents

List of Original Papers

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

Chapter II.

Sigurdsson B. D., Leblans, N. I. W., Gunnarsdótter, G. E., Dauwe, S. D., Sigurdsson, P., Gudmundsdóttir, E., Oddsdóttir, E. S. and Janssens, I. A., Geothermal ecosystems as natural climate change experiments: the ForHot research site in Iceland as a case study, *Submitted for publication in Icelandic Agricultural Sciences*

Chapter III.

Leblans, N. I. W., Sigurdsson, B. D., Vicca, S., and Janssens, I. A.: Persistent warming-induced extension of the growing season in subarctic grasslands, *In preparation.*

Chapter IV.

Leblans, N. I. W., Sigurdsson, B. D., Vicca, S., Soong, J., Weedon, J., Poeplau, C., Maljanen M., Gundersen, P., Marañón-Jiménez, S., Verbruggen, E., Wallander, H., Zhanfeng, L., Bååth, E. Holmstrup, M., Ilieva Makulec, K., Kätterer, T., Ostonen, I., Penuelas, J., Richter, A., Van Bodegom, P., Dauwe, S., Van de Velde, K ., Janssens, I. A.: Fast and persistent soil carbon reductions in naturally-warmed grasslands, *In preparation for resubmision to Nature.*

Chapter V.

Leblans, N. I. W., Sigurdsson, B.D., Roefs, P., Thuys, R., Magnússon, B. and Janssens, I.A. 2014. Effects of seabird nitrogen input on biomass and carbon accumulation after 50 years of primary succession on a young volcanic island, Surtsey. *Biogeosciences* 11, 1-14.

Chapter VI.

Leblans, N. I. W., Sigurdsson, B.D., Aerts, R., Vicca, S., Magnússon, B. and Janssens, I.A.: Icelandic grasslands as long-term C sinks under elevated organic N inputs, *Submitted for publication in Biogeochemistry.*

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Chapter I

Introduction

1 Introduction

1.1 Setting the scene - Northern high latitudes as carbon reservoirs

When thinking of northern high latitudes, the first images that come to people's mind are vast ice-covered landscapes inhabited by polar bears and seals. A region with a short bright summer and a long dark winter illuminated by flaring northern lights. Altogether, a harsh world for adventurers and explorers, rather remote from our daily lives. However, the recent growing concern about climate change and its consequences, has brought the 'far north' much closer to our attention (see § 1.2).

Northern high latitudes are generally defined as "the area confined by the 10°C July isotherm" (Vavrus et al., 2012), a climatic border that corresponds reasonably well with the latitudinal treeline (Beyens, 2016) and with the area north of 60°N. This area includes both the 'Arctic' and the 'subarctic' (Serreze et al., 2000) and while most people are more familiar with the Arctic, the subarctic covers the largest area (Fig. 1). Moreover, its position on the border-zone of the Arctic makes it the first region that will be impacted by global warming when cooling thresholds are passed (see further).

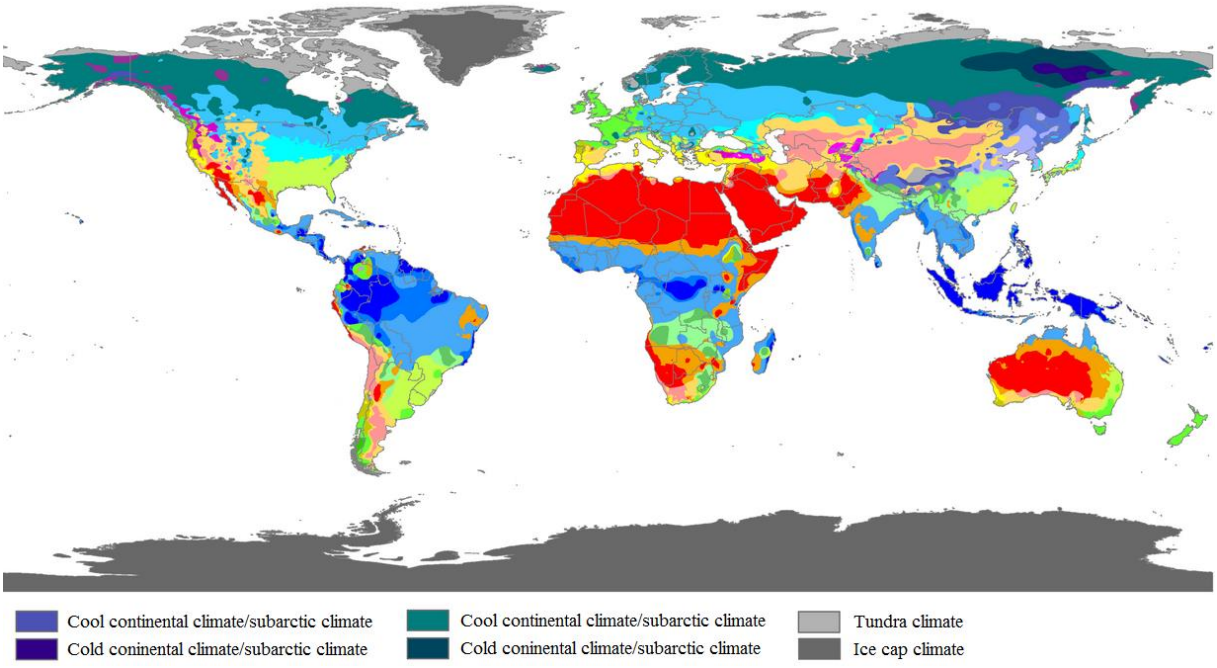


Figure 1: Distribution of the subarctic and arctic regions. Source: Peel et al. (2007)

High northern latitudes are not only characterised by low temperatures, but also (in general) by wet conditions, resulting from the low evapotranspiration in the region and the impenetrable permafrost layer (permanently frozen soil) that underlies large parts of the area (Cable et al., 2014). The typical cold and wet conditions strongly constrain decomposition of organic material, thereby slowing down biochemical cycles (McGuire et al., 2009). Over the course of many millennia, this has led to the build-up of thick organic soils. As a consequence, northern high latitude soils, which occupy only 5% of the terrestrial global soil surface (Ims et al., 2013), contain almost 30% of the global soil carbon (C) stocks (Hiederer and Köchy, 2011, Scharlemann et al., 2014) (Fig. 2). This equals the amount of C contained in the global atmosphere (IPCC, 2013). Nevertheless, the fate of this large northern C stock under global change (especially global warming and increased anthropogenic nitrogen (N) inputs) is highly uncertain (Kirschbaum, 1995, Friedlingstein et al., 2006, Todd-Brown et al., 2013) (see § 1.3), and changes can be expected soon. This is especially true for the sub-arctic region, due to its location at the border between two climate zones.

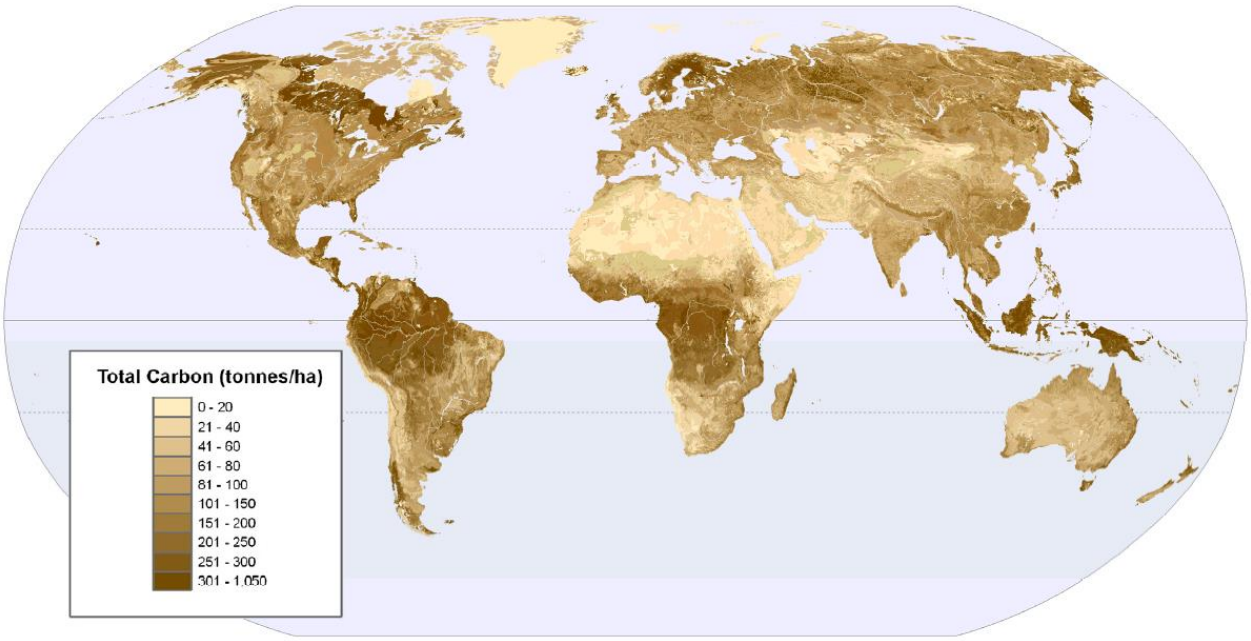


Figure 2: Global map of terrestrial C stocks. Source: Kapos et al. (2008)

1.2 Global change

Global change is one of the greatest challenges of our generation (Iyer et al., 2015). After decades of intense debate, initially about the reality of global change and later about the human influence on the global climate (Cook et al., 2013), little scientific dispute remains on either of these questions. One of the key aspects of global change is the increasing global air temperature as a consequence of increased greenhouse gas (GHG) emissions (IPCC, 2013). Global land surface temperatures have been rising on average 0.07°C per decade since the start of the industrial revolution (Bekryaev et al., 2010). A further increase in global land surface temperatures is expected, with estimates ranging from 1.2 ± 0.6 to 4.8 ± 0.9°C by the end of this century (IPCC, 2013).

1.2.1 Warming at high northern latitudes

Not all regions on earth are experiencing warming to the same extent. The northern hemisphere is currently warming faster than the southern hemisphere (IPCC, 2013). This is a consequence of its higher proportion of land masses, which warm more rapidly than ocean surfaces and the redistribution of heat towards the northern hemisphere by ocean circulation (Hutchinson et al., 2015).

Within the northern hemisphere, high latitude regions are subjected to the highest warming levels (Fig. 3). Already in 1896, Arrhenius predicted that a CO_2 driven climate warming would lead to disproportionally strong warming at higher latitudes (Arrhenius, 1896). This disproportional warming results from various positive feedback mechanisms to warming at high latitudes, a phenomenon called 'Polar amplification' (PA; Bekryaev et al., 2010). The proportional importance of the different mechanisms that drive the PA is not yet fully clarified, but it is agreed that changes in albedo and heat transport play a key role. Albedo decreases due to snow and ice melt, vegetation transitions, and altered cloud cover and black aerosol concentrations, whereas decreasing sea ice extent affects ocean-air heat fluxes as well as atmospheric and oceanic heat transports (Hwang et al., 2011, Serreze and Barry, 2011). As a consequence of PA, terrestrial high northern latitudes have been warming approximately twice as fast as the global average during the past century, with an average warming of 0.14 versus 0.07°C per decade (Bekryaev et al., 2010). During the past decades, the warming at northern high latitudes has even accelerated, reaching an average of 0.5°C per decade since 1978 (IPCC, 2013), and this strong warming trend is projected to continue during the coming century (IPCC, 2013).

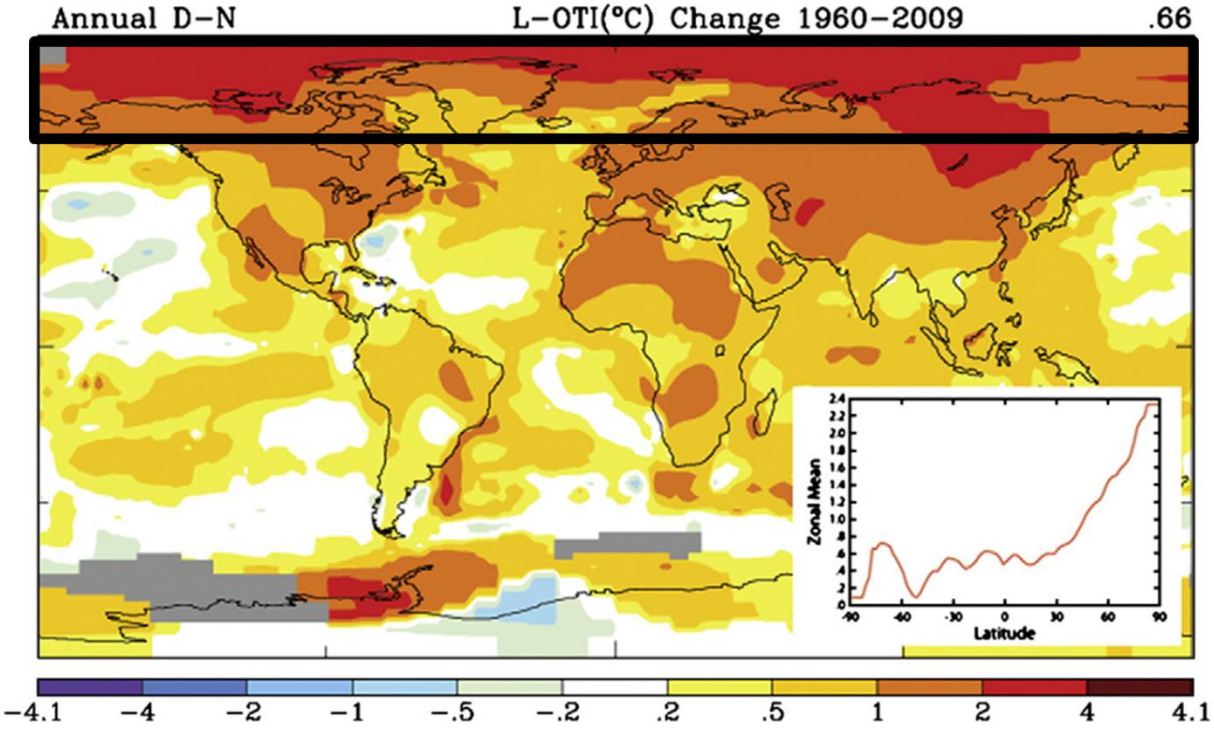


Figure 3: Global map of linear trends in annual mean surface air temperatures for the period 1960–2009, based on the National Aeronautics and Space Administration Goddard Institute for Space Sciences (NASA GISS) temperature analysis (http://data.giss.nasa.gov/gistemp). The inset shows linear trends over the 50-year analysis period averaged by latitude. Black marking shows the disproportional high warming at high northern latitudes due to the polar amplification. Source: Serreze and Barry (2011)

1.2.2 Increasing anthropogenic nitrogen deposition at northern latitudes

The start of the industrial revolution did not only initiate a drastic increase in anthropogenic CO_2 emissions causing the global climate to warm (see §1.2.1), it also amplified anthropogenic N deposition. Since the end of the nineteenth century, a three- to fivefold increase in anthropogenic N deposition has been recorded (Galloway et al., 2008). As with global warming, the intensity of anthropogenic N deposition is not equally spread over the globe. Its distribution is strongly dependent on point sources (mainly fertilizer production, agricultural intensification and fossil fuel combustion for industry and transport (Galloway et al., 2008, Gundale et al., 2014)) and wind and precipitation patterns (Penner et al., 1991). At northern high latitudes, N deposition rates are currently low (0.25 – 12 kg ha^{-1} yr^{-1}; Galloway et al., 2008, Gundale et al., 2011) (Fig. 4), yet in most regions substantially higher than the natural background N deposition (< 0.5 kg ha^{-1} yr^{-1}; Dentener et al., 2006). However, a further increase of the N input rates is expected, following to the disclosure of 'the high north' as a

consequence of global warming (see § 1.2.1), enabling more intensive agriculture, industry and transport (Hermans et al., 2010, Gudmundsdottir et al., 2011a, Lamarque et al., 2011).

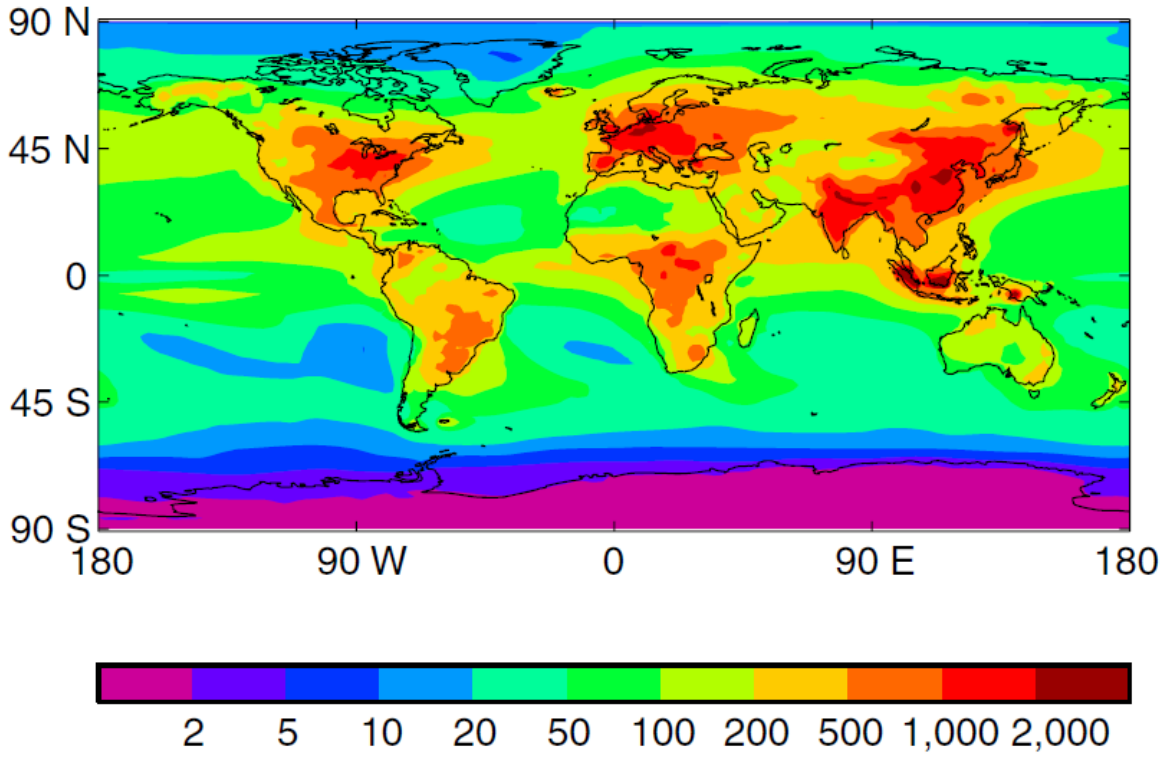


Figure 4: Global map of total N deposition between 2000 and 2010 (mg m^{-2} yr^{-1}). Source: (Penuelas et al., 2013).

1.3 Impact of global change on carbon storage at northern high latitudes

1.3.1 Warming effects on carbon storage

The consequences of warming at northern high latitudes are not restricted to the local scale, such as the loss of specific habitats, threatening complete ecosystems to extinction (Callaghan et al., 2004), but resonate around the globe. For one, the melt of large masses of land ice causes the global sea level to rise, putting numerous coastal cities and ecosystems at risk (Zambo, 2008). In addition, and maybe even more important, warming at high northern latitudes could switch on different feedback mechanism that can amplify global warming beyond the reach of human power (Bekryaev et al., 2010). An example of such feedback mechanisms is the large scale change in albedo caused by the melt of vast areas of snow and ice and by major changes in vegetation composition (see also §1.2.1).

One of the most potent feedback mechanisms of ecosystems at northern high latitudes to the global climate, is the transfer of CO_2 from soils to the atmosphere (Friedlingstein et al.,

2006, Todd-Brown et al., 2013). Warming is known to stimulate both CO_2 uptake (via plant growth) and the release of CO_2 (via decomposition) (Kirschbaum, 1995, Shaver et al., 2000, Melillo et al., 2002, Jones and Donnelly, 2004) and the relative importance of these two processes determines whether a warmed ecosystem becomes a CO_2 source or a CO_2 sink. As high northern latitude soils store a vast amount of C (see § 1.1), even small changes in the C balance of these soils can have far-reaching impacts on the global climate system (Jenkinson et al., 1991). Moreover, a recent meta-analysis of long-term warming studies showed that C rich soils, typically found in northern regions, are more susceptible to warming-induced C losses than C poor soils, releasing a larger proportion of their initial C stocks (Crowther et al., submitted).

This potentially powerful feedback is generally acknowledged, and many studies have been focussing on warming effects on the C dynamics of northern ecosystems (Dolman et al., 2010, Jahn et al., 2010). However, although many studies have been focussing on either C inputs (plant productivity) or C outputs (decomposition) in isolation, studies on the total ecosystem C balance are rare (Bradshaw and Warkentin, 2015). Most studies on productivity in northern ecosystems have found a significant increase in plant growth under warming, both in controlled experiments (e.g. Bret-Harte et al., 2002, Elmendorf et al., 2012, Campioli et al., 2013) and in time series (e.g. Kimball et al., 2007, Pouliot et al., 2009, Bhatt et al., 2010). The temperature sensitivity of decomposition depended on the turnover time of the soil organic C (SOC) and the approach that was used to study the response (incubation studies, field studies or cross-site studies) (Conant et al., 2011). In most cases, increasing temperatures stimulated decomposition rate and the temperature sensitivity of decomposition was highest in cold ecosystems (Davidson and Janssens, 2006b, Bradford et al., 2016, Crowther et al., Submitted).

The total C balance, however, depends on the relative temperature stimulation of productivity versus decomposition and can be studied by measuring SOC stocks, which integrate the net C inputs and outputs to the ecosystem if no perennial woody biomass is present. The few studies that investigated the temperature dependence of SOC stocks in northern ecosystems reported a decrease of SOC with increasing temperature of, on average, 2.3 % per °C, but with a standard error larger than the mean (2.4 %) (Saleska et al., 2002, Luo et al., 2009, Li et al., 2011, He et al., 2012, Sjögersten et al., 2012, Gill, 2014, McDaniel et al., 2014). This relatively large uncertainty can be attributed to the large variation among the studies in many aspects, of which the most important differences are the duration of the warming treatment (generally short-term), the warming technique, the range of the warming

levels (generally much lower than the current IPCC projections for northern ecosystems), the soil sampling depth (topsoil, i.e. active root layer, and subsoil, i.e. below the active root layer, SOC stocks can behave very different under warming; Tan et al., 2014) and the type of SOC analysis. Moreover, the measured SOC concentrations are only seldom converted to absolute SOC stocks, neglecting changes in soil structure and bulk density, which can strongly influence the actual change in SOC storage (Barcena et al., 2014). As a consequence, long-term C losses from the soil to the atmosphere remain a key uncertainty in climate projections (Kirschbaum, 1995, Friedlingstein et al., 2006, Todd-Brown et al., 2013).

The uncertainty about the fate of SOC in northern ecosystems is also obvious from a recent comparison study (the CMIP5 exercise; Sistla et al., 2013), which synthesized the projections of different earth system models (ESMs). Using the highest IPCC temperature increase scenario (RCP 8.5; IPCC, 2013), the variation in model projections for cold ecosystems was extreme. For the end of this century, the predicted ecosystem C-balance values ranged from -9 to +85 Pg C, with an average of 26 ± 32 Pg (Todd-Brown et al., 2013). This large uncertainty is a consequence of several inherent, logistical and financial constraints related to temperature manipulation experiments (see § 1.4), resulting in warming levels typically falling well below the projected temperature increase (especially in northern regions) and of the lack of empirical observations. Altogether, these restrictions have hampered the development of a good mechanistic understanding of the important processes that determine the ecosystem C balance in a warmer world, such as the temperature sensitivity of decomposition, belowground C allocation pathways and the priming mechanism (Todd-Brown et al., 2013).

1.3.2 Impact of increasing nitrogen deposition on carbon storage

As with warming, increasing N deposition at northern high latitudes has both local and global consequences. An example of a local impact is a loss of biodiversity (Gundale et al., 2011) following the dominance of more fast growing, competitive species when ecosystems are released from N limitation, which is typical for northern regions (Aerts and Chapin, 2000, Reich and Oleksyn, 2004). On global scale, the release of N limitation can have far reaching influences on the C cycle and thus global change.

It is known that increasing N inputs greatly stimulate plant productivity (C input) at northern high latitudes in the short-term (Sillen and Dieleman, 2012). On the other hand, decomposition (C output), can be stimulated as well as inhibited by increasing N inputs, depending on the natural background N deposition, the applied N input and the litter quality

(Aerts et al., 2003, Knorr et al., 2005, Hobbie, 2008, Zhang et al., 2008). Further, C partitioning into pools with different levels of recalcitrance can strongly influence the C balance (Adair et al., 2008). Fertilization experiments indicated that increasing N inputs can increase the C sink function of northern ecosystems (mainly by stimulating primary productivity) (Maaroufi et al., 2015).

It is believed that the N-induced increases in productivity have already played a major role in the global C cycle in recent years, explaining a significant part of the famous "missing C sink" (Esser et al., 2011). This "missing C sink" refers to the imbalance between anthropogenic C emissions and the atmospheric C increase during the past decades that remained unsolved for many years (Gifford, 1994). The atmospheric increase in CO_2 was over 50% lower than the actual anthropogenic emissions (4.0 vs. 8.9 Pg C y^{-1}; IPCC, 2013). Today it is known that this imbalance is caused by increasing C uptake by natural terrestrial and oceanic ecosystems, each taking up approximately 2.5 Pg C per year (IPCC, 2013). Northern temperate and boreal ecosystems have been responsible for 30% of the terrestrial C sink (White et al., 2000, Le Quere et al., 2009) and it is widely accepted that the N-induced increase in productivity has been an important driver of this terrestrial sink (Hudson et al., 1994, Lloyd, 1999, Schlesinger, 2009), together with land-use change driven processes (Nabuurs et al., 2013) and possibly positive feedbacks of enhanced atmospheric CO_2 concentration and longer growing seasons on the annual C uptake (White et al., 1999, Euskirchen et al., 2009, Zhao et al., 2015). Even if the increase in anthropogenic N deposition at high northern latitudes has been modest during the past decades (Galloway et al., 2008), limiting their contribution to this N-driven C sink, a substantial increase in anthropogenic N deposition is expected in this region in the near future (Lamarque et al., 2011; see also § 1.2.2). This implies that high latitude northern ecosystems could become significant N-driven C sinks during the next century.

However, despite the general recognition of the importance of the N-driven C sink, its long-term perspective remains highly uncertain. The uncertainty is partly caused by the lack of studies investigating the effect of increased N inputs on total ecosystem C storage, even if an extensive amount of high latitude research has been devoted to (short-term) N addition effects on C input rates (gross primary production (GPP) or net primary production (NPP)) and C output rates (litter decomposition and heterotrophic and autotrophic respiration) (Wookey et al., 2009, Bouskill et al., 2014). Further, and more importantly, long-term N-addition studies are rare because of multiple constraints (see § 1.4). The few studies that have applied long-term (> 5y) N-addition report contradicting conclusions, varying with

fertilization rate and litter quality (Hyvonen et al., 2007, Hopkins et al., 2009, Janssens et al., 2010, Nilsson et al., 2012, Gundersen et al., 2014), and are often confounded by superficial soil sampling (see e.g. Rumpel and Kogel-Knabner, 2011, Fornara et al., 2013, Olson and Al-Kaisi, 2015). Focusing on topsoil can lead to incorrect estimations of the actual long-term C storage in the soil, as topsoil and subsoil differ strongly in C dynamics and their responses to N addition (Appling et al., 2014, Batjes, 2014, Tan et al., 2014). Topsoil C dynamics are mainly driven by nutrient supply, while subsoil decomposition is limited by energy as the proportion of recalcitrant litter increases with soil depth (Fontaine et al., 2007, Wutzler and Reichstein, 2008).

1.4 Natural gradients as an alternative for manipulation experiments

Temperature and N manipulation experiments have provided valuable contributions to our understanding of the implications of global change (see §1.3). Nevertheless the interpretation and extrapolation of their findings is often restricted by various artefacts and inherent limitations (De Boeck et al., 2015). In this section, a brief overview of the most important limitations of manipulation experiments will be given, before exploring if natural gradients in temperature and N can offer solutions to some of these difficulties.

1.4.1 Limitations of manipulation experiments

The ideal setup for manipulation experiments (both in the laboratory and in the field) is often compromised by logistical and/or financial constraints. One consequence is the typical short duration of the manipulations (experiments with >5 years of continuous treatment are already considered as "long-term"; Smith et al., 2015), although exceptions do exist, both for warming experiments (Luo et al., 2011, Leppalammi-Kujansuu et al., 2014, DeAngelis et al., 2015) and for fertilization experiments (Anttonen et al., 2002, Hopkins et al., 2009, Nilsson et al., 2012, Gundersen et al., 2014). The short time span of most experiments compromises the potential to predict the long-term evolution of the observed response. Both under- and overestimations of the manipulation effect are possible, the former in the case of delayed ecosystem responses and the latter in the case of logarithmic responses and overshoot scenario's (De Boeck et al., 2015) (Fig. 5). Moreover, the short-term nature of manipulation experiments limits their power to predict slow structural adaptations to climate change, such as changes in vegetation composition and in soil structure (Beier et al., 2012).

Logistical and/or financial constraints also generally cause a trade-off between the action span of the study and its statistical power by restricting the number of treatment levels

and replicates that can be employed in manipulation experiments. Broadening the number of treatment levels should be prioritised if one aims to investigate the response curve in function of treatment intensity or to examine treatment interactions in detail (in the case of multi-treatment studies). This is currently one of the main focusses in climate change related ecosystem research, as previous studies were generally restricted to one or two treatment levels, limiting the interpretation of responses to linear relationships (Kreyling et al., 2014). However, a minimum number of replicates (which limits the effort and resources that can be allocated to expand the number of treatment levels) should be maintained to secure the statistical power of the study (De Boeck et al., 2015). One solution would be to replace the conventional 'block treatments approach' that is connected to ANOVA analyses and require a minimum number of replications per treatment level by a 'continuous treatment approach', which can be investigated with regression functions that are not restricted to minimum replication. So far, very few studies have applied this technique (Kreyling et al., 2014) as it requires highly accurate and frequent monitoring of the treatment factor under investigation.

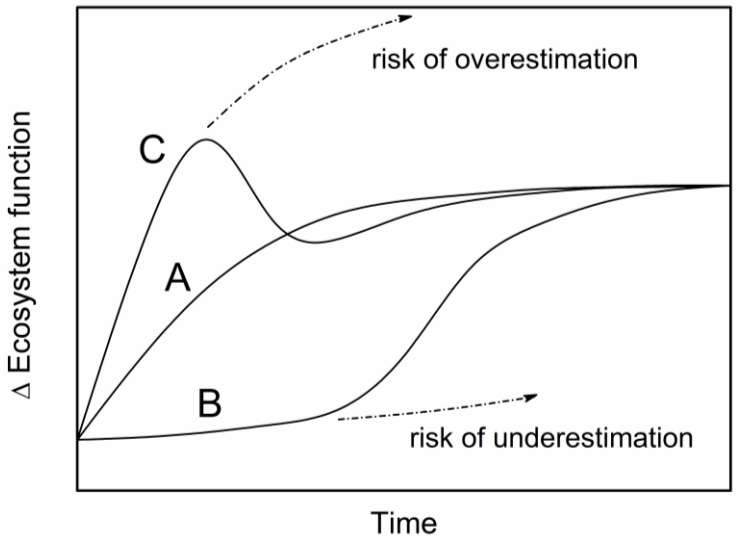


Figure 5: Examples of ecosystem response functions that lead to incorrect conclusions when short-term responses are extrapolated: (a) logarithmic response, (b) delayed response, and (c) overshoot scenario followed by stabilization. All responses ultimately align in this example. Source: De Boeck et al. (2015)

Another inherent trade-off in the design of manipulation studies is the incompatibility between treatment control and experimental realism (De Boeck et al., 2015). Treatment control, in combination with the exclusion of co-varying factors, is necessary to investigate the causality of responses and is optimally employed in laboratory conditions. However, there is evidence that laboratory studies, precisely due to their typically controlled conditions,

rarely replicate what happens in natural ecosystems where different influential factors are usually strongly linked and often interact (Crain et al., 2008). This is opposed to field experiments, where experimental realism and predictive power are higher, as environmental factors are allowed to co-vary and natural variability is taken into account. However, this limits their potential to uncover causal relations.

Finally, climate manipulation experiments are characterized by an initial step-change in the treatment factor, as opposed to the actual gradual change in climate. It is known that the initial phase of environmental changes often act as a disturbance (Wookey, 2008) and molecular research has revealed that responses to these changes can depend on their abrupt or gradual nature (Ambrosone et al., 2011).

1.4.2 Natural gradients in soil temperature and soil nitrogen as an alternative

Some of the abovementioned issues that are connected to climate manipulation experiments can be overcome by the use of natural gradients. Opposed to the typical short duration of manipulation experiments (see § 1.4.1), natural gradients are often long-persisting, which increases the reliability of long-term projections. Further, natural gradients are typically gradual, allowing a wide range of treatment levels. In addition, the gradients are often small scale, keeping background variables, such as climate and bedrock, constant and thus offering both reasonable treatment control and experimental realism. However, one should note that even over small distances environmental factors might co-vary (e.g. temperature and nutrient availability) and that additional controlled laboratory experiments might be necessary to identify causal relationships. Finally, natural gradients are low cost, saving the expenses of treatment application (O'Gorman et al., 2014, De Boeck et al., 2015). In this thesis, soil temperature gradients (see § 1.4.2.1) and soil N gradients (see § 1.4.2.2) were used as model examples.

1.4.2.1 Natural gradients in soil temperature

The most widely applied type of natural gradients in temperature used in global change research are ‘space for time’ substitutions. In space for time substitutions, latitudinal or altitudinal gradients in temperature are used as a proxy for climate warming (e.g. Dunne et al., 2004, De Frenne et al., 2013, Liu et al., 2014, Philben et al., 2016). The strength of these gradients is the long-term nature of the gradient, making it possible to observe equilibrium responses, their experimental realism and the absence of the initial step-increase in temperature that is typical for manipulation experiments (see § 1.4.1). On the other hand, these studies are inherently spread over large geographical areas, covering a wide range of

climates, soil conditions and other physical-chemical variables (Dunne et al., 2004). This could interfere with - and obscure direct temperature responses.

An alternative, that overcomes this spatial scale issue, is the use of geothermal gradients in soil temperature. Geothermal temperature gradients are characterized by large temperature variations over small distances and can thus be studied at a spatially small scale, keeping climate, bedrock, elevation and other confounding environmental factors reasonably constant. Geothermal areas, that exhibit soil warming levels relevant for climate change research can be found all over the globe in tectonically active areas and cover a wide range of climate regions and ecosystem types (O'Gorman et al., 2014; Fig. 5). Typical soil warming levels at 10 cm depth range from normal soil temperatures at greater distance from the heat source to >50°C where the geothermal channel is closest to the surface (O'Gorman et al., 2014). This broad range does not only allow to investigate the temperature response over the full range of current climate change predictions (opposed to manipulation experiments, where the applied warming levels are usually well below the predicted range for (sub)arctic regions (see § 1.3.1)), but also to assess the mechanisms behind the response by using the higher warming levels. Moreover, the study of more extreme warming levels can reveal thresholds in physiology, community dynamics and biogeochemistry, which is becoming increasingly important as extreme warming levels are expected to occur more frequently in the future (Kayler et al., 2015). Further, the gradual nature of the soil warming gradients in these geothermal systems makes it possible to investigate the shape of the temperature response in detail. Also the time-dependence of the temperature response can be evaluated in many cases, as a consequence of the dynamic nature of geothermal warming. Tectonic events can cause existing geothermal systems to shift towards previously unwarmed soils, resulting in short-term soil warming gradients which can be compared to nearby long-term geothermal warming gradients that have been constant over a longer period of time. This makes it possible to investigate the evolution of the response rate in time (Fig. 5). When studying short-term gradients, one has to bear in mind that the initial step change in temperature might influence the ecosystem responses (see also § 1.4.1).

Some caution is mandatory, however, when selecting geothermal sites to investigate climate change. In some systems, the geothermal water is not confined to the bedrock. When such water, which is often contaminated by magma degassing and high concentrations of dissolved minerals (Guo, 2012, Hernandez et al., 2012), enters the biosphere, it can affect biological, chemical and physical processes, thereby confounding the warming effects. Another restriction connected to the use of geothermal gradients is their typical volcanic soil

(Andosol), which has particular soil properties, such as strong bounds with soil organic matter, resulting in high C contents,and high water holding capacity (see § 1.5.2). Also, even if geothermal areas are well-spread over the globe, they do not cover all existing ecosystem types. Further, the small spatial scale of these gradients, which is an asset for limiting environmental variability, also limits the size of the "warm islands", potentially restricting biodiversity responses. Finally, and most importantly, one has to bear in mind that geothermal warming always causes asymmetrical warming, with high soil temperature elevations, compared to limited air warming. This limits the potential to investigate processes that are strongly dependent on air temperature. However, even if geothermal soil warming systems have some caveats of their own, they are a powerful tool to investigate the consequences of global warming (O'Gorman et al., 2014, De Boeck et al., 2015).

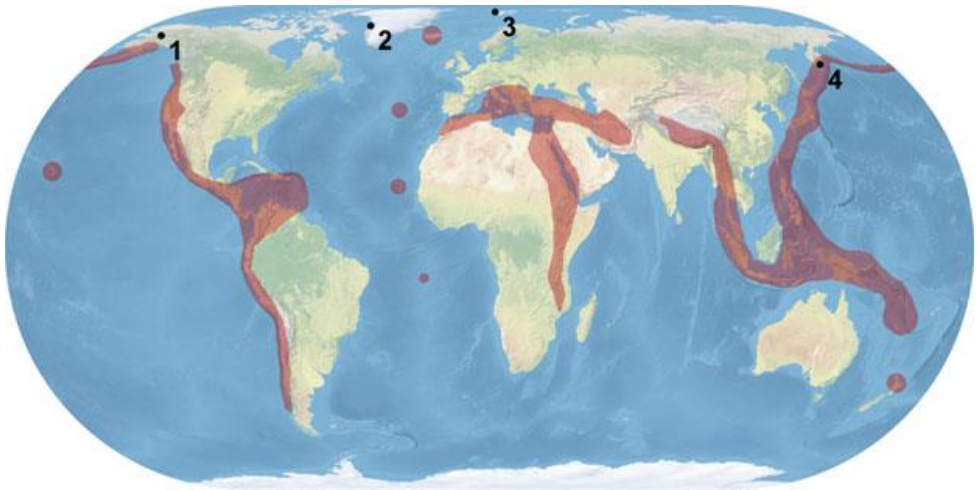


Figure 6: Map of active geothermal areas around the world (indicated in red; underlying data adapted from the US Geological Survey). Numbered black points show geothermal sites at high northern latitudes that were identified by Davis et al. (2013) as suitable locations for natural global change experiments. Source: O'Gorman et al. (2014)

1.4.2.2 Natural gradients in soil nitrogen

Natural gradients in soil N can occur under different conditions. A common type of soil N gradients can be found at sites with different soil age. Soil age has an important impact on both the total soil N stocks and the N availability for plants (Crocker and Major, 1955, White et al., 2004, Rhoades et al., 2008, Smithwick et al., 2009). Newly formed surfaces contain very little N (Vitousek and Howarth, 1991) and during the subsequent soil development, soils gradually accumulate N via atmospheric N deposition and biological N fixation (Vitousek and Howarth, 1991). Moreover, the parallel increase in the soil organic matter content improves the ion exchange capacity of the soil, leading both to improved N retention and increasing N

availability for plants (Deluca and Boisvenue, 2012). Such "slow soil formation (age) gradients" are, however, also confounded by many other factors, such as changes in biodiversity and ecosystem structure (Schleicher et al., 2011, Vilmundardottir et al., 2015b), soil water holding capacity (Deluca and Boisvenue, 2012, Kögel-Knabner et al., 2013), disturbance history (Kroël-Dulay et al., 2015) and often expand across different climatic conditions, similar to the natural elevation gradients mentioned earlier. This can compromise the detection of causal effects of the enhanced N-inputs alone.

Therefore, such gradients should preferably be formed on similar aged soils, but with contrasting N-input rates. One type of such natural gradients in N inputs can be found around dense seabird nesting colonies (Garcia et al., 2002, Zwolicki et al., 2013, Havik et al., 2014). Seabirds generally collect food in marine ecosystems and deposit the major part of their droppings (guano) close to their nesting sites (Polis and Hurd, 1996). As a consequence, natural (seabird-derived) gradients in N input take shape, with the highest inputs at the centre of dense nesting sites and a gradual decrease towards the rim (Ellis et al., 2006). Only few studies have attempted to quantify seabird-derived N inputs, but the existing reports show that seabird-derived N input rates can equal or even surpass anthropogenic N deposition rates in industrial areas (~60 kg ha^{-1} y^{-1}; Galloway et al., 2008) (Bancroft et al., 2005, Breuning-Madsen et al., 2010). Seabird guano does not only contain N, but also many other elements. However, the effect of these other elements on ecosystem C dynamics is not investigated in this thesis, as their influence is expected to be negligible compared to the effect of N, by far the most limiting nutrient for plant growth in northern ecosystems (Aerts and Chapin, 2000, Schlesinger, 2009).

As with geothermal warming studies, the persistent nature of both gradient types offers the possibility to evaluate the long-term outcome of increased soil N. Also, the broad and continuous range of soil N gives the opportunity to investigate the appropriate soil N scale in the scope of the study and to expose non-linearity in the responses.

1.5 Natural gradients in temperature and nitrogen inputs in Iceland

All studies that are presented in this thesis were conducted in Iceland, a volcanic island of 103.000 km^2 that is located on the Mid-Atlantic ridge, just south of the Arctic Circle (63°23′6" to 66°32′3" N; 13°29′6" to 24°32′1" W) (Fig. 7). Iceland has different characteristics that make it an ideal location to study long-term global change effects. First of all, Iceland offers an abundance of natural gradients in global change related factors

(O'Gorman et al., 2014). For this thesis, natural gradients in soil temperature (caused by geothermal activity, see § 1.5.2 and 1.5.4) and N inputs (caused by soil age and varying seabird influence, see § 1.5.5) were selected. In addition, thanks to the volcanic character of Iceland, a wide gradient of successional stages can be found, which is especially interesting as it is expected that global change will affect early and late successional stages differently (Kroël-Dulay et al., 2015) (see § 1.5.6).

Secondly, Iceland is located in the subarctic climatic region, which is expected to experience climate change faster and stronger than the world average (see § 1.2.1). Even if Iceland itself is an exception to this general rule, being projected to warm at approximately the global rate (IPCC, 2013), it offers a great opportunity to improve our knowledge on warming impacts on other subarctic and Arctic regions. The climate in Iceland ranges from subarctic in the lowlands to Arctic in the highlands (above 400 – 600 m.a.s.l.). In this thesis, I focus on the subarctic area, as this climate region is expected to experience the strongest shifts with gradual warming, due to its position on the border between Arctic and temperate climates (Gamache and Payette, 2005).

Thirdly, many Icelandic ecosystems are dominated by species with a circumpolar distribution (Kristinsson and Sigurdsson, 2010), improving the generalizability of the ecosystem responses to the natural climate gradients. In this thesis unmanaged subarctic grasslands are studied. The studied grasslands were dominated by species that are common in boreo-arctic and even temperate regions (see § 1.5.3). Despite the abovementioned assets of choosing Iceland for natural gradient studies, one has to note, however, that Iceland has a specific soil type (volcanic soils, see § 1.5.3), which might limit the extrapolation potential of the observations to other regions.

In the next sub-chapters, I will first briefly introduce the specific climatologic, pedogenic and vegetational background of the study area, bearing in mind that these factors play an important role for most processes in the carbon cycle (Jobbagy and Jackson, 2000, Ostle et al., 2009) (§ 1.5.1 to 1.5.3). Thereafter, I will further review the use of natural gradients in soil temperature, N input and successional stages in more detail, placing them in the context of Iceland (§ 1.5.4 to 1.5.6). For each type of gradient, a concise description of the research sites that were selected for this thesis will be given, but more information can be found in Chapter II - IV for the natural temperature gradients and in Chapter V – VI for the N input and successional gradients. Finally, an overview is given of the most closely related studies and their findings (§ 1.5.7).

Figure 7: Location of Iceland just south of the Arctic circle. Source: http://www.emapsworld.com

1.5.1 Iceland: Climatology

Iceland is located at the border of the Arctic area (Fig. 7), and its climate ranges from subarctic at the coastal lowlands to Arctic at the highlands (Fig. 8) (Vavrus et al., 2012). The weather in Iceland is very variable due to its position at the average location of the polar front, which brings frequently alternating mild and moist air masses from the southwest and cold and dry Arctic air masses from the northeast (Wanner et al., 2001). The oceanic influence moderates the temperature fluctuations, resulting in unusually small seasonal differences. The incoming oceanic air masses also lead to high precipitation, resulting in moist conditions. In the southwestern part of Iceland, where the research presented here was conducted, the monthly mean temperatures over the past ten years (2006 – 2015) of the coldest and the warmest month were -0.3°C (December) and 10.6°C (July) on the average (Icelandic.Meteorological.Office, 2016). The mean annual precipitation during the same period amounted to ~1400 mm and the mean wind speed was 5.5 m s^{-1} for the calmest month (July) and 7.8 m s^{-1} for the most windy month (February) (Icelandic.Meteorological.Office, 2016).

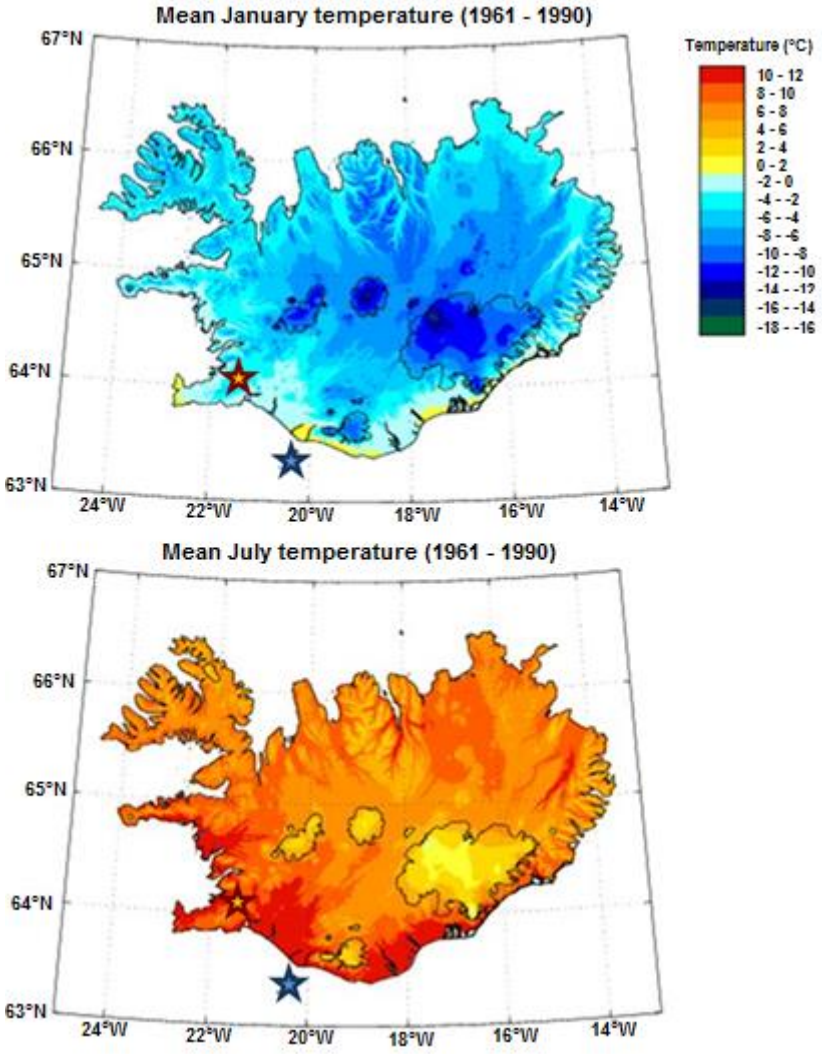


Figure 8: Mean temperature of the coldest month (January; upper panel) and the warmest month (July; lower panel) between 1961 and 1990 in Iceland. In the central highlands, the mean temperature of the warmest month never exceeds 10°C threshold which defines the Arctic climate (Beyens, 2016). The coastal lowlands do exceed this threshold and are classified as subarctic. The locations of the natural soil temperature gradient and N-input gradient that were studied in this thesis are indicated with a red and blue star respectively. Source: Icelandic Meteorological Office (www.vedur.is)

1.5.2 Iceland: Geology, volcanism and pedology

The oldest parts of Iceland were formed between 16 and 18 million years BP by a combination of volcanic activity connected to the divergence of the Eurasian and North American plates and local hot-spot activity (Arnalds, 2015). Iceland still hosts 30 active volcanic systems, featuring practically all volcano types known on Earth (Thordarson and Larsen, 2007) and the major part of these systems are located on a southwest – northeast oriented belt, where the plates diverge at a rate of 2 cm y^{-1} (Fig. 9). No less than 205 volcanic

eruptions have been recorded during historical times (since the settlement of Iceland in 874 AD) (Thordarson and Larsen, 2007).

The typical volcanic (basaltic) bedrock, together with the regular covering of the land surface by volcanic ash, has given rise to specific soil types with unique physical, chemical and mineralogical properties (Arnalds, 2015). Two large soil orders can be distinguished: Andosols and Histosols (Arnalds, 2015). During the weathering of basalt, allophanes and ferrihydrates are formed, two clay minerals with a large capacity to bind organic material. The binding of organic material to these clay minerals results in stable silt-sized aggregates (Arnalds, 2015). As a consequence, Andosols and Histic Andosols have the highest carbon content of all soil types, with the exception of Histosols (Óskarsson et al., 2004). Within these soil types, the highest carbon content (12 – 20%) can be found in Histic Andosols at undisturbed sites at large distances from ash sources. The high organic content of the soil results in a low bulk density, a high water holding capacity and a high cation exchange capacity. Further, bases released during the weathering of the basaltic bedrock give rise to a relatively neutral soil pH, ranging from 5.5 to 6.5 (Arnalds, 2015). The soils in the study area can be classified as Brown Andosols, a soil type typical for unsaturated environments and characterized by a carbon content of <12% and an allophane content of > 6% (Arnalds, 2015).

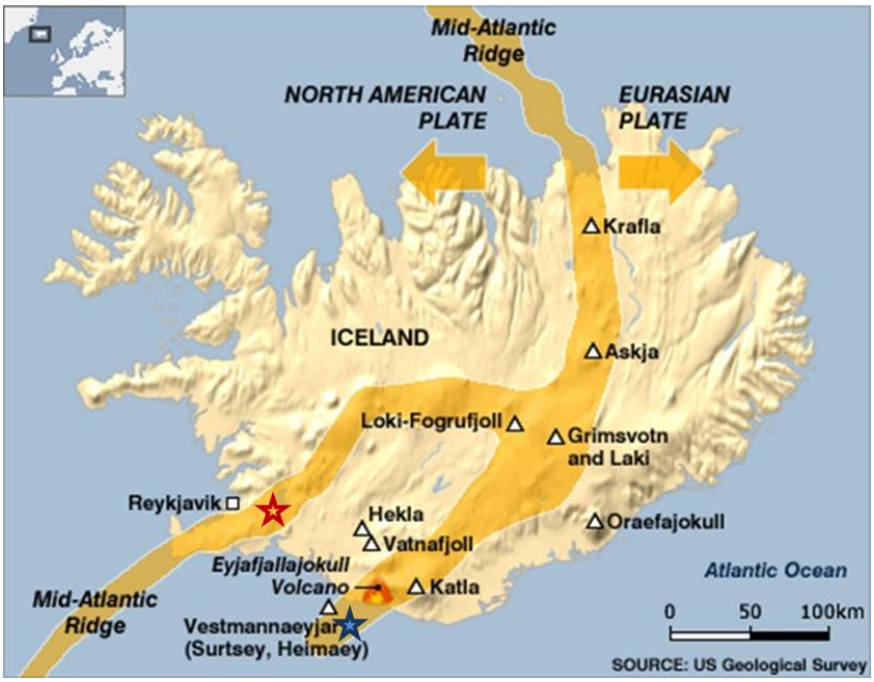


Figure 9: Icelands major volcanos and tectonic plate boundaries. The locations of the natural soil temperature gradient and N-input gradient that were studied in this thesis are indicated with a red and blue star respectively. Source: US Geological Survey

1.5.3 Vegetation of Iceland

The vegetation types and distribution in Iceland are mainly determined by climate, volcanism and historical grazing pressure (Ólafsdóttir et al., 2001). While the highlands in the centre, typically defined as the areas >400 m a.s.l. and covering 55% of Iceland's surface, are sparsely vegetated due to adverse climatic conditions and active volcanism, causing volcanic ash to cover and abrade the present vegetation (mostly tundra and moss heath), the lowlands along the coast are predominantly vegetated. The main vegetation types in the lowlands are forb meadows and grassland vegetation, wood- and shrublands, and wetland vegetation. Further, coastal- and permanent geothermal areas have their own specific vegetation types (Kristinsson and Sigurdsson, 2010).

In this thesis, forb meadows and grassland vegetation (hereafter "grasslands") were studied. Northern grasslands are an interesting system to study, as they cover an extensive area (ca. 10 % of the global terrestrial surface; Chapin et al., 2011) and have a large SOC storage potential (Aerts et al., 2003, Sui and Zhou, 2013), with >95 % of the total C stored as SOC (Grace, 2004). In Iceland, grasslands can be found in humid and relatively fertile areas and are generally relatively species rich (Kristinsson and Sigurdsson, 2010). Grasses and forbs occupy the largest share of the surface, sparsely supplemented by ferns and dwarf shrubs and commonly having a relatively large moss cover in the field layer. Common vascular plant species are viviparous sheep's-fescue (*Festuca vivipara*), sweet vernal-grass (*Anthoxanthum odoratum*), kentucky bluegrass (*Poa pratensis*), meadow buttercup (*Ranunculus acris*), mouse-ear chickweed (*Cerastium fontanum*), bentgrasses (*Agrostis capillaris* and *Agrostis stolonifera*) and alpine lady's-mantle (*Alchemilla alpina*) (Kristinsson and Sigurdsson, 2010). Most of these species have a circumpolar distribution in boreo-arctic and temperate regions, except for the last two, which are confined to Eurosiberia (Preston et al., 2002).

1.5.4 Natural soil temperature gradients - The ForHot research site

1.5.4.1 The ForHot research site as a model system

Iceland, as a volcanic island (see §1.5.2), offers extensive possibilities to study natural gradients in soil temperature as the geothermal hot-spots that are connected to the volcanic activity are often surrounded by a zone with warmed soils. For this thesis, the Hengill volcanic system was selected as a model system (Figs. 8 and 9). The Hengill volcanic system is located ~40 km east of Reykjavik on the junction of the Reykjanes Volcanic Zone, the West

Volcanic Zone and the South Iceland Seismic Zone (Hernandez et al., 2012). It is an active volcanic system with mount Hengill (803 m.a.s.l.) as central volcano.

In 2011 the ForHot research site was established in the southwestern part of the Hengill volcanic system. This part of the area is volcanologically extinct, albeit seismologically active. The area is highly permeable and hosts an unusual abundance of hot springs and fumaroles, to which the name of the nearby village 'Hveragerdi' or 'Hot spring settlement' refers. The ForHot research site (www.forhot.is; *Natural soil warming in natural grasslands and a Sitka spruce forest in Iceland*) is managed with an open research policy and since its establishment, the site has been visited by scientists from 18 different institutions. The overall aim of the ForHot research consortium is to unravel soil temperature effects on whole ecosystem functioning. More detailed information on the properties and the setup of the ForHot research site can be found in Chapter II - IV, but its main characteristics are briefly introduced in the next paragraphs.

There are different reasons why the geothermal area in which the ForHot research site is located is ideal to investigate the effect of increasing temperature on subarctic ecosystems (not all geothermal areas qualify for this purpose). For one, the fumaroles in the area emit primarily H_2O, with only trace amounts of CO_2, H_2S, H_2, N_2, CH_4 and He (Hernandez et al., 2012), which is important to exclude confounding contamination effects. Geothermal sites with high amounts of CO_2 emissions (without large emissions of the other gasses) could be of interest in climate change research too, as the combination of increasing soil temperature and elevated CO_2 concentrations would allow to study interaction effects between those two important climate change related factors. This is, however, not the case at the ForHot research site, where only temperature effects are studied. Another property of the study area, that is of great importance for the relevance of our study, is the fact that the soils are warmed by heat conduction from the underlying bedrock that is warmed from within by heated groundwater (Árnason et al., 1967, Gudmundsdottir et al., 2011a). This avoids the soil to be contaminated by mineral-rich geothermal groundwater. Further, the limited variability in possible confounding factors, such as soil moisture and pH along the soil temperature gradients (Maljanen et al., Submitted; see also Chapter II) made it an ideal area to study soil temperature effects alone. Finally, sufficient locations with the aimed soil temperature range (unwarmed to ~+20°C) were available on lands owned by the Agricultural University of Iceland.

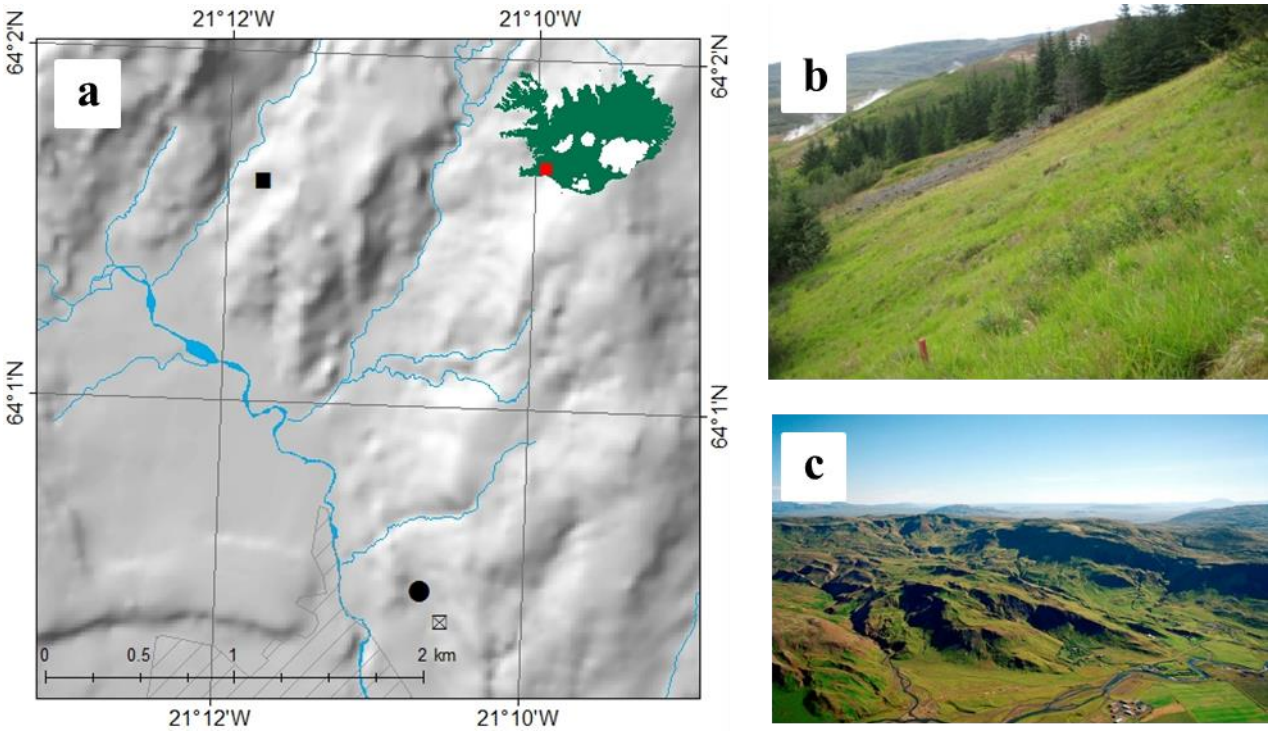


Figure 10: (a) Location of the short-term warmed natural subarctic grassland sites (crosshatched squares), the long-term warmed natural subarctic grassland sites (solid squares) and the short-term warmed forest site (solid circle). The hatched area represents the village of Hveragerdi. Source: Maljanen et al. (Submitted). (b) Picture of one of the two short-term warmed natural subarctic grassland sites. (c) Picture of the area of the long-term warmed subarctic grassland sites (Grændalur). The sites are located in the top-left valley on the picture.

Geothermal activity and the soil temperature gradients that are connected to it, are not always constant in space and time (see also § 1.4.2.1). The warming can be highly influenced by seismological and volcanic activity and by underground structures that facilitate or obstruct heat conductance. As we aimed to study effects of long-term soil warming, it was crucial to select sites that had been warmed for long periods of time. Therefore, we selected the sites in a valley called 'Grændalur' or 'Green valley' (Fig. 10), which refers to the lush and green vegetation around the geothermal hot-spots outside the normal growing season. This place name dates (at least) from 1708, to our knowledge the first time that it was mentioned in a historical document (Magnússon and Vídalín, 1918-1921). Further, we based our selection of locations that had not been subjected to major changes in soil temperature over the past 50 years, based on a survey of the geothermal activity in the valley in 1963-1965 (Kristján Sæmundsson, pers. comm.), regular soil temperature measurements in various

hotspot in the area since 2005 (Daebeler et al., 2014) and a detailed geothermal mapping of the valley in 2008 (Thorbjörnsson et al., 2009).

Not all geothermal hot-spots in the area have been stable over the past 50 years. After a major earthquake on the 29th of May 2008 (magnitude 6.3 on the Richter scale; Halldorsson and Sigbjornsson, 2009), different thermal systems close to its epicentre changed. At different locations, the earthquake formed new belowground geothermal channels within the bedrock, resulting in the warming of previous 'unwarmed' soils (Thorbjörnsson et al., 2009).

As was mentioned earlier, the ForHot research site consists of natural subarctic grasslands and a planted 50-years old Sitka spruce (*Picea sitchensis*) forest. The forest is located in the area that has been warmed since 2008 and will not be studied in this thesis. The natural subarctic grassland sites were selected both in the area that has been warmed since 2008 (hereafter short-term warmed grassland; consisting of two separated sites) and the area that had been warmed for at least 50 years (hereafter long-term warmed grassland; consisting of two separate sites) (Fig. 10). This made it possible to investigate and compare the effects of both long-term soil warming and short-term soil warming on natural subarctic grasslands.

1.5.4.2 The ForHot research site – Focus of this thesis and background

In this thesis, the ForHot research site was used for two purposes. Firstly, the site was used to investigate the suitability of geothermal warming gradients as climate change laboratories (Chapter II). Afterwards, the natural warming gradients were used to study effects on two ecosystem processes in detail (see also § 1.6). More specifically, warming effects on plant phenology (the length of the growing season as a proxy for C uptake potential; Chapter III) and on the net C balance (Chapter IV) of subarctic grasslands was investigated. Here, I will frame this research in the current knowledge on climate change in subarctic regions and in particular in Iceland.

Plant phenology, the timing of recurrent biological events and its biotic and abiotic causes (Lieth, 1974), is highly sensitive to climate change (Richardson et al., 2013). In turn, changes in the phenology of plant communities, especially in the length of the growing season, can induce strong feedbacks to the climate system. One of the most potent feedbacks of growing season changes to the climate system is an alteration of CO_2 fluxes between the atmosphere and the biosphere (Gu et al., 2003, Cleland et al., 2007, Ahlstrom et al., 2012, Richardson et al., 2013), as the length of the growing season (i.e. the period of photosynthetic activity) determines the C uptake potential of the ecosystem (Richardson et al., 2013). Therefore, a thorough understanding of climate change effects on the length of the growing

season is highly important. Yet, the current predictive capacity of phenological responses to climate change is still poor (Zhao et al., 2013, Fu et al., 2014a, Keenan and Richardson, 2015).

During the past decades, warming has in most regions caused an extension of the lengtht of the growing season (Linderholm, 2006), and the strongest response has been observed at high northern latitudes (Zhao et al., 2015). However, there are recent indications that this strong extension of the growing season is saturating (Zhao et al., 2015). The potential for further extension of the growing season of warming has profound consequences for the C uptake potential of northern latitudes, and is therefore studied in detail in Chapter III.

The importance of studying warming effects on the net C balance of high northern latitude ecosystems has already been indicated extensively (see § 1.3.1). Here, I present two studies that have made use of natural geothermal gradients to study subjects related to the C balance of northern ecosystems to set the stage for the research in this thesis. The first study (Gudmundsdottir et al., 2011a) was conducted close to the ForHot research site, about 5 km westward in the same volcanic system. Gudmundsdottir et al. (2011a) studied the effect of increasing temperature (from ambient temperature to + 15°C) on the biomass of primary producers (~ C input) in naturally warmed spring-fed streams. They found that the bryophyte biomass in the streams increased exponentially with temperature up to a warming level of ~ +10°C, above which it stabilized. The vascular biomass in the streams was very low and remained unchanged. This study suggests that the temperature response of C uptake is not linear with temperature, and that, when a certain threshold temperature is reached, the positive effect of increasing temperature stabilizes. In addition, this study showed that the temperature effect differed among species, causing the species community to shift along the temperature gradient. This could affect the C balance of the ecosystem, by changing the community averaged C allocation, litter properties and microclimate (Metcalfe et al., 2011).

The second study (Daebeler et al., 2014) was conducted in the same valley as the long-term warmed grassland of the ForHot research site, albeit in plant communities on more water-saturated histic Andosols. The study investigated the effect of increasing temperature on the coupling of the C and N cycle in these wetlands by studying the competition between nitrifiers and methane oxidizers. As the outcome of this competition determines whether the ecosystem becomes a sink or a source of methane and of gaseous N oxides (Stein and Klotz, 2011), this is an important aspect of the C balance of northern wetland ecosystems as well as their potential for emitting greenhouse gasses. Daebeler et al. (2014) concluded that the regulation of nitrification and methane oxidation was primarily driven by the availability of

mineral nitrogen. As the availability of mineral nitrogen is expected to increase with increasing temperatures (Homann et al., 2007), this regulatory mechanism could have far reaching consequences for the C and greenhouse gas balances under future warmer conditions.

1.5.5 Natural gradients in soil nitrogen – The Vestmannaeyjar archipelago

1.5.5.1 The Vestmannaeyar as a model system for natural gradients in soil nitrogen

Iceland hosts the two types of soil N gradients (gradients in soil age and gradients in seabird N inputs) that were discussed in § 1.4.2.2. Soil N gradients shaped by contrasting soil age (hereafter "early developmental" and "mature") can be widely found in Iceland, as the volcanic activity frequently shapes new surfaces or covers existing surfaces under thick ash layers. Iceland is also rich in seabird-derived N gradients, hosting many dense but well confined seabird colonies along its coast and on its countless coastal islands. In this thesis, both types of gradients were studied. Paired sites were selected: one pair of contrasting seabird influence on an early developmental soil and another on a mature soil. More detailed information on the properties and the setup of the soil N gradients can be found in Chapter V - VI, but their main characteristics are briefly introduced in the next paragraphs.

All the study sites were located on the Vestmannaeyjar (63°25' N; 20°17' W), an archipelago at the south coast of Iceland (Figs. 8, 9 and 11). The sites with the early developmental soils were located on Surtsey, the youngest island of the archipelago and of the whole of Iceland. Surtsey was formed during a submarine volcanic eruption that lasted from 1963 to 1967 and the island has been protected from human influences ever since (Baldurson and Ingadóttir, 2007). Scientific campaigns, that have taken place on a yearly basis since the emergence of the island, have yielded an extensive record on its biological and geological evolution (Baldurson and Ingadóttir, 2007). Two large areas could be distinguished on the island: One area had not been affected by seabird breeding, while the other area had been hosting a breeding colony of mainly lesser black-backed seagulls (*Larus fuscus*) since 1986. The sites with well-developed, mature soils were located on Heimaey and Ellidaey, at sites with a surfacing bedrock of ~5,900 years (Mattsson and Hoskuldsson, 2005), and an undisturbed soil profile down to an ash layer from ca. 395 AD (Larsen, 1984). The site on Heimaey is unsuitable for seabird breading due to unfavourable topographical conditions, and had in all likelihood always been free of major seabird influence. Ellidaey, on the other hand, hosted the second largest puffin colony (*Fratercula arctica*) of the archipelago, with 16,400

breeding pairs (Hansen et al., 2011), and its topographical shape and location has in all likelihood made it a preferable site for dense seabird colonies soon after its emergence.

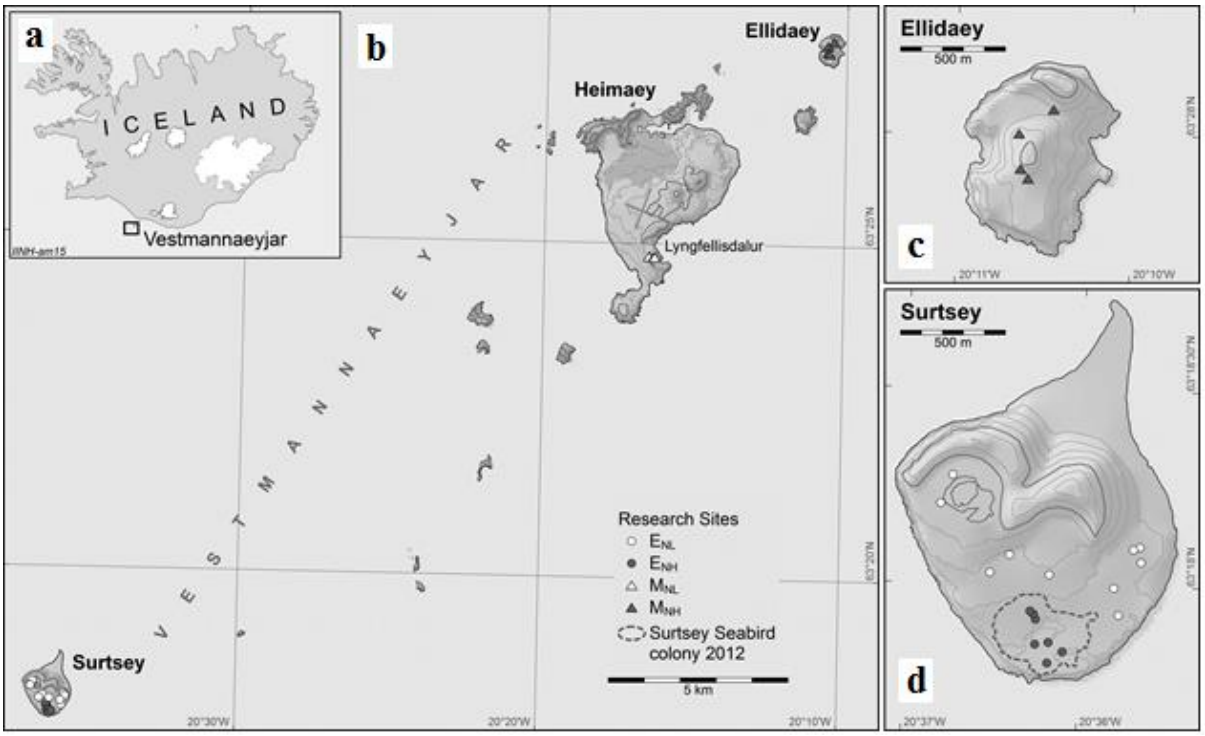


Figure 11: Topographical map of the studied soil N gradients on the Vestmannayjar. (a) and (b) Location of the Vestmannaeyjar including the three study islands, Surtsey, Heimaey and Ellidaey at the southwest coast of Iceland. (c) and (d) show the islands Ellidaey and Surstey in more detail. Dots show the research plots at early soil developmental stage under low (○) and high (●) N inputs. Triangles show the research plots at sites with mature soils under low (Δ) and high (▲) N inputs respectively. Map designed by Anette Th. Meier.

1.5.5.2 Nitrogen gradients on the Vestmannaeyar – Focus of this thesis and related research

In this thesis, the natural N gradients on the Vestmannaeyar were used to investigate the short-term and long-term effects of increased N inputs on biomass production and soil C storage (see § 1.6). The importance of improving our knowledge on the role of N in C storage in high northern ecosystems has been discussed in § 1.3.2. Here, I review different N addition studies that have been conducted in Iceland (thus with similar climate and pedology as our study sites) and that have investigated the effect of elevated N on different aspects of the C cycle.

The setup of the first study (Vilmundardottir et al., 2015a) was very similar to the study setup at the Vestmannaeyar, using both a chronosequence and a gradient in bird influence as N input gradients. The chronosequence, however, was shorter (up to 120 years)

and the bird influence was restricted to isolated hummocks rather than whole nesting colonies. One of the main aims of their study was to investigate the effect of (long-term) N inputs on SOC accumulation. They found very little effect along the 120 year long chronosequence, while the SOC stocks increased drastically with bird influence. The second study (Gudmundsson et al., 2004) was not a natural gradient study, but an exceptionally long (43 years) N fertilization study on permanent grasslands. Similar to my thesis study and the study of Vilmundardottir et al. (2015b), their study investigated the accumulation of SOC under long-term increased N inputs (75 and 120 kg N ha^{-1}, compared to 43 - 63 kg N ha^{-1} on the seabird sites on the Vestmannaeyar). They found that N fertilization increased SOC accumulation in the long-term, without indications for saturation, implying that positive effects of N inputs on C storage could last for at least half a century.

Also other Icelandic ecosystem types have been exposed to N fertilization to investigate its effects on biomas production: a freshwater stream ecysotem and a planted black cotonwood (*Populus trichocarpa*) forest. The study on freshwater stream ecosystems (Gudmundsdottir et al., 2011a; see also § 1.5.4.2) was conducted close to the ForHot research site. In their study, N was dripped to the streams and after one year of N fertilization, its effects on the biomass of primary producers was assessed. Already after this relatively short treatment time, an overall increase in biomass was found in the N fertilized streams, which was mainly caused by a strong positive response of bryophytes to N addition. The response, however, was highly class- and species-specific. This could also greatly influence the C balance of the ecosystem due to interspecific differences in C allocation and litter properties (Metcalfe et al., 2011). The study on the planted black cottonwood (Sigurdsson et al., 2001c) also studied the effect of relatively short-term (two to four years) N addition (from 40 to 120 kg N ha^{-1} yr^{-1}) on productivity. They too found a positive effect on whole plant biomass, as well as on the relative growth rates of stems and branches. Interestingly, the amount of branchwood was also increased per unit leaf area, locking the C in more slow-cycling compartments and thereby altering the C dynamics of the system.

To conclude, as expected for these strongly N-limited ecosystems, all the studies found a significant increase in biomass under N fertilization. The biomass response was already visible shortly after onset the fertilizer application (Sigurdsson et al., 2001c, Gudmundsdottir et al., 2011a) and also continued after long-term N addition (Gudmundsson et al., 2004, Vilmundardottir et al., 2015b). These long-term responses show that no N saturation had occurred yet and that N fertilization can stimulate productivity over long timescales (at least 43 years under relatively high N inputs; Gudmundsson et al., 2004).

1.5.6 Successional gradients– The Vestmannaeyjar

Iceland covers a broad range of successional stages as a consequence of its frequent volcanic activity (see §1.5.2) and historical soil erosion episodes (Arnalds et al., 2016). Volcanic eruptions do not only lead to the formation of new land surface but also regularly reset the development of ecosystems by covering them under lava or thick ash layers. The presence of such ash layers in (undisturbed) soil profiles makes it possible to estimate the timeframe during which the current soil and ecosystem has been developing. Such a high variety of successional stages is interesting in climate change research, as it makes it possible to investigate climate change effects on different developmental states in detail.

Few ecosystems are maintained over longer periods in their most mature state, as a consequence of former disturbances that have reset the ecosystem to an earlier stage (Prach and Walker, 2011). Nevertheless, successional stage is often overlooked in climate change studies (Kroël-Dulay et al., 2015). Moreover, the research gap on successional dynamics contributes for a substantial part to the uncertainties in global C dynamics and their responses to climate change (Chapin et al., 2011). In this thesis, I study the importance of successional stage on the response of soil C storage to increased N inputs. The relation between increased N inputs and soil C storage is expected to change during the course of soil maturation (Crocker and Major, 1955, Saynes et al., 2005, Seedre et al., 2011, Appling et al., 2014), as both total N stocks and plant available N increase during the soil maturation process (Crocker and Major, 1955, White et al., 2004, Rhoades et al., 2008, Smithwick et al., 2009). This is especially true for northern N-limited ecosystems (Aerts and Chapin, 2000, Reich and Oleksyn, 2004).

The early successional ecosystems were located on the young island Surtsey (see §1.5.5). The area outside the seabird colony showed only limited soil formation and was in an early successional stage between barrens and a subarctic grassland (Magnússon et al., 2014). Within the seabird-influenced area, however, an early developmental soil profile had developed that consisted of an O horizon and a premature A horizon (max. 10 cm deep) and the plant community had already reached a late successional subarctic grassland stage (Magnússon et al., 2014). The mature ecosystems were located on Heimaey and Ellidaey (see §1.5.5). Both islands had developed a mature soil profile, classified as ‘Brown Andosol’ (Arnalds, 2015). Further, also in terms of vegetation composition, both islands had reached a mature stage (Magnússon et al., 2014). The site on Heimaey, which was unsuitable for seabird breeding, was covered with a mature species-rich subarctic grassland community typical for low nutrient conditions (Magnússon et al., 2014), while the site on Ellidaey, which was

naturally ‘fertilized’ by seabird breeding, had developed into a mature species-poor subarctic grassland community (Magnússon et al., 2014). More detailed information on the properties and the setup of the successional gradients can be found in Chapter VI.

1.6 Research objectives and thesis structure

The main objective of my PhD study was to assess the long-term effects of warming and increased N inputs on the net C balance in high latitude unmanaged subarctic grasslands.

The specific aims were:

- To explore the possibilities of using geothermal ecosystems as natural climate change experiments (Chapter II).
- To evaluate the importance of soil warming for aboveground phenology of high latitude subarctic grasslands, an important factor in the carbon exchange potential of these ecosystems (Chapter III).
- To assess the impact of soil warming on net soil organic C (SOC) storage in high latitude subarctic grasslands (Chapter IV).
- To investigate how increased N inputs affect net SOC storage in early successional high latitude subarctic grasslands (Chaper V).
- To investigate how increased N inputs affect long-term SOC storage in high latitude subarctic grasslands as a function of successional stage (Chapter VI).

The papers written for this PhD thesis are separated into two main blocks. The first block (**Chapters II - IV**) covers the use of natural soil temperature gradients as climate change laboratories, with focus on soil temperature effects on C dynamics. In the second part (**Chapters V - VI**), natural gradients in N are used to study the importance of N in net SOC storage.

In **Chapter II**, the use of geothermal soil temperature gradients as laboratories to study long-term ecosystem responses to climate warming is introduced. The assets and possibilities of these systems are highlighted, together with their inherent limitations. In this evaluation, the ForHot research site in southwest Iceland is presented as a model system.

In **Chapter III**, the effect of warming on aboveground plant phenology is studied. Plant phenology has an important influence on the C exchange between plants and atmosphere, mainly trhough changes in the length of the growing season (i.e. C uptake potential). During the past decades, global change has induced major changes in plant

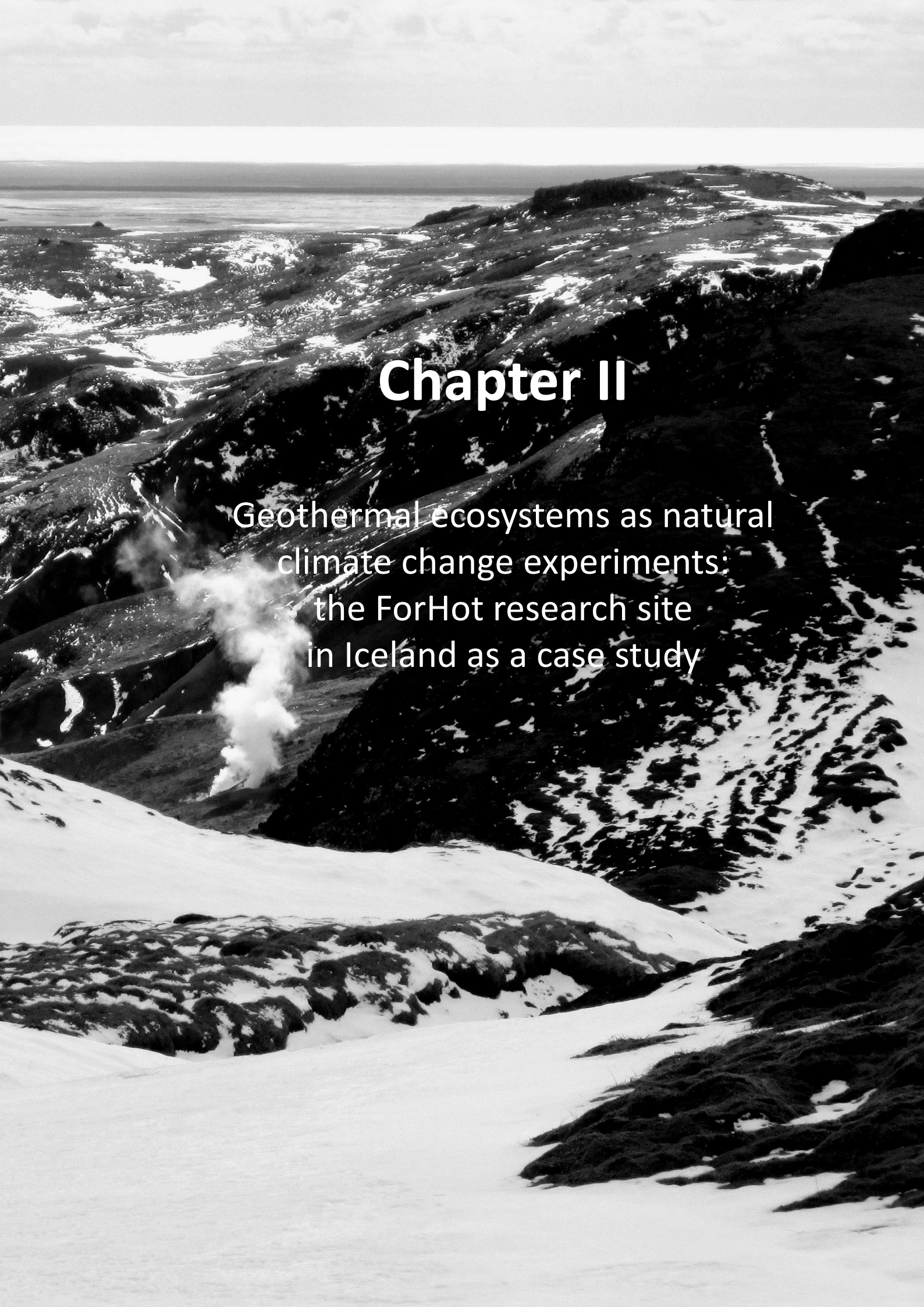
phenology all over the globe, with the largest shifts occuring at high northern latitudes. Nevertheless, the future of the warming-inuced extension of the growing season is highly uncertain. Here, the ForHot research site is used to study the responses of subarctic grassland phenology to warming.

In **Chapter IV**, warming-induced transfers of soil C to the atmosphere are addressed. This transfer is one of the largest uncertainties in climate change projections. Especially empirical evidence of long-term warming effects on SOC stocks is lacking. Using short-term and long-term geothermal soil temperature gradients allowed to clarify the time dependence of warming-induced changes in soil C. Further, the presence of wide temperature gradients made it possible to encompass the full gradient of IPCC warming scenarios and to evaluate the shape of the temperature response.

In **Chapter V**, the effects of increased N inputs on biomass production and net SOC storage in early successional subarctic grasslands are assessed. Early- and intermediate successional ecosystems are often neglected in studies on the net ecosystem C balance while it is known that N induced C uptake and storage can change drastically over the course of ecosystem succession.

Chapter VI focuses on long-term effects of increased N inputs on SOC storage. The anthropogenic N deposition at high northern latitudes is expected to increase substantially in the near future (see § 1.2.2) and increasing N inputs can stimulate net C uptake of northern latitude ecosystems, implying that these ecosystems could soon significantly contribute to the acknowledged N-induced terrestrial C sink (Esser et al., 2011; see § 1.3.2). However, the perspective of this C sink is highly uncertain as knowledge of long-term effects of N on the C cycle is limited due to the lack of long-term N input studies.

Finally, in **Chapter VII**, the key findings and implications of Chapters II - VI are combined in a general discussion and conclusion, and suggestions for future research are proposed.



Chapter II

Geothermal ecosystems as natural climate change experiments: the ForHot research site in Iceland as a case study

2 Geothermal ecosystems as natural climate change experiments: the ForHot research site in Iceland as a case study

Sigurdsson B. D., Leblans, N. I. W., Gunnarsdótter, G. E., Dauwe, S. D., Sigurdsson, P., Gudmundsdóttir, E., Oddsdóttir, E. S. and Janssens, I. A.

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2.1 Abstract

This article describes how natural geothermal soil temperature gradients in Iceland have been used to study terrestrial ecosystem responses to soil warming. The experimental approach was evaluated at three study sites in S-Iceland; at one grassland site that has been warm for at least 50 years (GO), and other comparable grassland (GN) and a Sitka spruce plantation (FN) sites that have both been warmed since an earthquake took place in S-Iceland in 2008. Within each ecosystem type, five ca. 50 m long replicated transects, with six permanent study plots each, were established across the soil warming gradients, spanning the unwarmed control conditions and gradually warmer soils. It was attempted to select the plots so the annual warming levels would be ca. +1, +3, +5, +10 and +20 °C within each transect. Results on continuous measurements of soil temperature (Ts) from 2013-2015 revealed that the soil warming was relatively constant and followed the seasonal Ts cycle of unwarmed control plots. Volumetric water content in the top 5 cm of soil was repeatedly surveyed during 2013-2016. The grassland soils were wetter than the Sitka spruce forest soils, but they also showed some significant warming-induced drying in the surface layer of the warmest plots, contrary to FN. Soil chemistry did not show any indications that geothermal water had reached the root-zone, but soil pH did increase somewhat with warming, which was probably linked to vegetation changes. As expected, potential decomposition rate of organic matter increased significantly with warming. It was concluded that the natural geothermal gradients at the ForHot sites in Iceland offered realistic conditions to study terrestrial ecosystem responses to warming with minimal artefacts.

2.2 Introduction

Impacts of changing climatic conditions on terrestrial ecosystems at high latitudes are a very active research field today (Hyvönen et al. 2007, Way & Oren 2010, Kayler et al. 2015). It has even been stated that climate change impacts are becoming the single most important environmental and policy concern of the 21st century (Rustad 2008).

Many different experimental approaches have been used to evaluate the effects of future warming on ecosystem structure and function, including, i) monitoring of natural variation in temperature and ecosystem variables, ii) climate gradient studies, iii) process-based modelling of plant- and ecosystem responses to warming, and iv) experimental indoor or outdoor warming of plants, soils or whole ecosystems (cf. Rustad 2008, De Boeck et al. 2014). Each method has its pros and cons; but there is a general consensus among ecosystem ecologists that experimental *in situ* warming of ecosystem components and/or whole ecosystems are the most powerful tools that allow for the elucidation of cause-and-effect relationships and provide for a mechanistic understanding of responses of ecosystems to global change (Rustad, 2008; Way & Oren 2010, De Boeck et al. 2014). Manipulation experiments tend, however, to be technically challenging and expensive to run, which most often limits their duration to only few years and include only one or few warming levels (De Boeck et al. 2014).

Rustad (2008) stated in her review that there is a great need to conduct longer-term warming studies in order to better understand changes that occur on multiannual timescales. Recently it has also been stressed that far too few *in situ* ecosystem warming experiments have been designed to impose warming gradients to identify possible response thresholds in ecosystem responses (De Boeck et al. 2014, Kayler et al. 2015). Such experiments could guide mechanistic models, with the ultimate aim of projecting when climate extremes will provoke non-linear responses or cause ecosystems to pass tipping points.

One possibility to meet both these requirements, i.e. studying long-term effects to warming and include large warming gradients, without requiring excessive funds and technological complexity, is to use ecosystems affected by natural geothermal activity. Such conditions could potentially offer a range of soil temperatures within a single small biogeographic area, which would better allow us to isolate soil temperature from other drivers compared to e.g. elevational gradient studies (O'Gorman et al. 2014).

Some earlier manipulation experiments have shown that N-limited northern ecosystems may respond to warming mainly through effects on soil processes (cf. Way &

Oren, 2010). E.g. in a recent large-scale warming experiment on mature Norway spruce (*Picea abies*) forest in N-Sweden it was discovered that only increasing the air temperature did not significantly change tree volume growth (Sigurdsson et al., 2013), while when only the soils were warmed at the same site, the forest productivity responded strongly (Strömgren & Linder 2002). This was explained with the strong N-limitation that exists in most high-latitude ecosystems, which can override the direct effects of air temperature on aboveground processes. Recently, there have been various large experiments with soil-only warming in different alpine and northern ecosystems to look at climate change effects (cf. Rustad 2001, Strömgren & Linder 2002, Patil et al. 2013, Streit et al. 2013, Schindlbacher et al. 2015) as well as a few experiments warming both soils and above ground parts separately (Bronson & Gower 2010, Krassovski et al. 2015).

Some research on both experimental and natural *in situ* warming has been done previously in Iceland, but never at such a large spatial scale or looking at as many terrestrial ecosystem levels as the ForHot project (e.g. Sigurdsson 2001b, Bergh et al. 2003, Elmarsdottir et al. 2003, Jónsdóttir et al. 2005, Dalebeler et al. 2014, 2015). The most comparable work in Iceland would be an ongoing project on naturally warmed stream ecosystems in the nearby Hengill area (e.g. Woodward et al. 2010, O'Gorman et al. 2014).

In this paper we address how suitable the geothermal systems at the ForHot sites in southern Iceland were for studying effects of warming on terrestrial ecosystem structure and function and how other potential cofounding drivers, such as a pollution by geothermal water or large changes in soil humidity, were affected by the geothermal warming.

2.3 Methods

2.3.1 Experimental setup

The study sites are located in south Iceland, close to the village of Hveragerði (64.008°N, 21.178°W; 83-168 m a.s.l.), at the grounds of the Agricultural University of Iceland campus at Reykir (Figure 1). Between 2003 and 2015, the closest synoptic station at Eyrabakki (9 km S of Hveragerdi) had a 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}, respectively (Icelandic Meteorological Office, 2016). The mean temperature of the warmest and coldest months, July and December, were 12.2 °C and -0.1 °C for the same period. The mean monthly precipitation during May-July was 75 mm $month^{-1}$, while it was on average 135 mm $month^{-1}$ for the remaining months at Eyrabakki (Icelandic Met Office, 2016). During the period of 1972-1999

both precipitation and air temperature were measured at the Reykir campus. During that period the MAT was found to be similar (0.1 °C warmer), but the MAP was on average 13% higher at Reykir (1616 mm $year^{-1}$) than at Eyrabakki (Icelandic Meteorological Office, 2016). The growing season normally starts in late May and ends in late August. Snow cover is not permanent during winters due to the mild oceanic climate, but the soil typically freezes for at least couple of months during mid-winter.

On the 29 May, 2008, a major earthquake (magnitude 6.3 on the Richter scale) occurred in S Iceland (Halldorsson & Sigbjörnsson 2009). The earthquake caused substantial damages to infrastructures and affected geothermal systems close to its epicenter. One such geothermal system at Reykir moved to a previously unwarmed area (Þorbjörnsson et al. 2009), where the new geothermal bedrock channels resulted in increasing temperature (Ts) in the soil above by radiative heating (O'Gorman et al. 2014).

The recently warmed area is covered by two different ecosystem types: a) a Sitka spruce forest (*Picea sitchensis*, provenances Seward and Homer from Alaska) that was planted in 1966-1967 (Böðvar Gudmundsson, pers. comm.), hereafter termed "FN" (Forest New) and b) unmanaged treeless grasslands dominated by *Agrostis capillaris* grass, some herbs and moss (Table 1), hereafter termed "GN" (Grassland New). The soil type at both sites is Brown Andosol (a volcanic soil type; Arnalds, 2015). It is silty loam in structure and has the typical characteristics of such soils in Iceland (Table 1; Arnalds 2015). The FN plantation was established mainly for shelter and has never been thinned. Therefore, it had a relatively high stand density, basal area and leaf area index (LAI) compared to typical managed spruce forests in Iceland or Scandinavia (Table 1; Snorrason & Einarsson 2002, Weslien et al. 2009).

The third study site "GO" (Grassland Old) is located 2.0-2.5 km NW of GN and FN on older geothermal Ts gradients, in Grændalur (Figure 1). It is covered by the same grassland type as GN and on the same soil type (Table 1). There, the earliest survey of geothermal hot-spots was made in 1963-1965 (45 years prior to the 2008 earthquake; Kristján Sæmundsson, pers. comm.). In autumn 2008, after the 2008 earthquake, the locations of the new and old geothermal hot-spots in the area were remapped (Þorbjörnsson et al. 2009). This survey was used to choose the GO, GN and FN sites for the ForHot study. Some hot-spots at GO have been monitored since 2005 by regular field measurements of Ts in another study in Grændalur (Daebeler et al. 2014, 2015). The geothermal activity has most likely been persistent in Grændalur (e: Green valley) for centuries, as according to local knowledge its name comes from the fact that the subarctic grasslands on the warmest hot-spots stay green during early and late parts of winter. The oldest historical document that mentions this place name was

written in 1708 (Magnússon & Vídalín, 1918-1921). Additional evidence for persistent geothermal warming at GO are the geothermal clay layers found at various depths in the subsoil profile (Leblans, unpublished). This indicates that over longer time periods, the warming may have fluctuated somewhat, as was observed at other nearby hot-spots following the 2008 earthquake (Daebeler et al. 2014).

In autumn 2012 and spring 2013, twenty-five permanent study plots were established in each of the three ecosystems (FN, GN and GO), around one main hot-spot in FN, but in two separate areas for GN and GO (Figure 1). The plots were placed along five ca. 50 m long transects placed perpendicular to the soil temperature gradients ranging from ambient soil temperature to ~ +10°C, placing five replicate plots at different warming levels (WLs) on each transect (~ +0, 1, 3, 5 and 10 °C warming; termed levels A (unwarmed control), B, C, D, and E, respectively). In GN and GO the plots were 2 × 2 m in size, but in FN they were 1 × 1 m and placed in between trees. In spring 2014 one additional WL was installed at each transect at ~ +20 °C (termed level F), but those plots were all 1 × 1 m in size due to the steeper soil warming gradients at the highest temperatures. This increased the number of permanent study plots to 30 per ecosystem, or 90 across all three.

2.3.2 Field measurements

Soil temperatures were measured hourly adjacent to each plot at 10 cm soil depth using HOBO TidbiT v2 Water Temperature Data Loggers (Onset Computer Corporation, USA). Air temperature was measured close to the surface (at 2 cm and 15 cm) at two plots at each warming level in each ecosystem and at 2 m height in one to two places in each system, using the same type of loggers and logging frequency, but protected from direct sunlight with radiation shelters. Vertical soil temperature profiles were measured in late June 2014 by 90 cm long temperature probe placed at different depths (Digi-Sense Type K Thermometer Probe, Oakton Instruments, IL, USA). Volumetric soil water content in the top 0-5 cm of soil was measured during campaigns in 2013 to 2016 by a handheld Theta Probe (Model ML3, Delta-T Devices Ltd., Cambridge, England), with a "mineral soil factory calibration curve" that has been found to give realistic results for Icelandic Andosols in S-Iceland (Berglind Orradottir, pers. comm.). Soil depth was measured using a 1 m long metallic rod pushed down until hitting a rock at 11 places along the S edge of each permanent plot, but recorded as 100 cm when deeper. A relative measure for exchangeable sulphur (S) was obtained using exchange membranes (PRS™ probes, Western Ag Innovations Inc., Saskatoon, SK, Canada). The membranes continuously absorb charged ionic species over the burial period, and the S

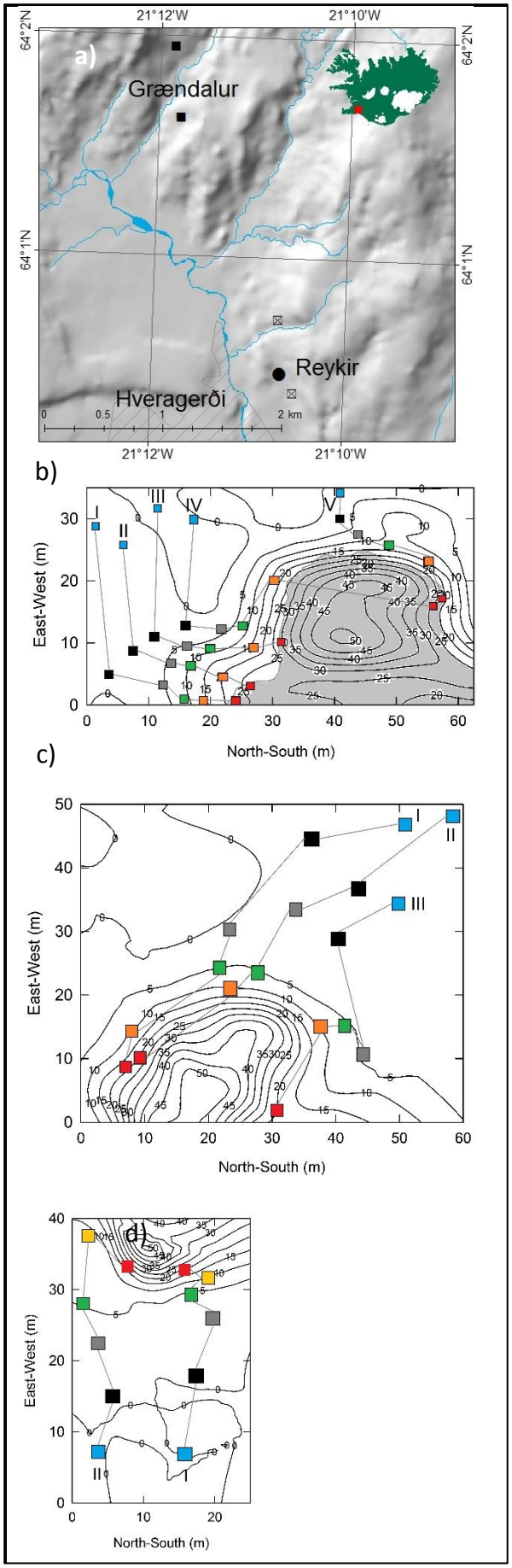


Figure 1. a) Location of the ForHot study sites in S Iceland. Filled circle is the recently warmed forest site (FN), crosshatched squares are the recently warmed grasslands (GN) and the filled squares are the near-by grasslands with long-term soil warming (GO) in Grændalur. The hatched area represents the village of Hveragerdi. b) Soil warming isotherms (°C) at five transects within the FN site in spring 2012 and at c) three transects of the GN and d) two transects of the GO sites in spring 2014. Warming levels are A (unwarmed control; blue), B (black), C (grey), D (green), E (orange) and F (red). For evaluation of warming levels see Table 2. In FN, the natural background soil temperature (Ts) varied varied from -2 to +2 °C because of stand density variation in the unwarmed forest stand and the actual geothermal warming was therefore ca. 2-4 °C lower than the isolines indicate. The shaded area for FN represents the area where Sitka spruce had died off in 2012.

Table 1. Main plant and soil structural characteristics of the control plots (A plots) in the recently warmed forest (FN) and grassland (GN) and the long-term warmed grassland (GO) in 2013. Data from Cilio (2014), Guðmundsdóttir et al. (2014), Michielsen (2014) and Leblans (2016).

	FN	GN	GO
Soil type	Brown Andosol	Brown Andosol	Brown Andosol
Soil texture	Silt loam	Silt loam	Silt loam
Clay:Silt:Sand ratio [a]	8:61:31%	6:53:41%	8:62:30%
Stoniness in top 10 cm	2.1%	1.6%	0.4%
Bulk density in top 10 cm	0.62 g cm^{-3}	0.70 g cm^{-3}	0.55 g cm^{-3}
Topsoil C/N ratio [b]	15.1	10.9	11.5
Three most dominant vascular plant species	*Picea sitchensis* Understory: *Equisetum arvense* – *Geranium sylvaticum*	*Agrostis cappillaris* – *Galium boreale* – *Anthoxantum odoratum*	*Agrostis capillaris* – *Galium boreale* – *Ranunculus acris*
Vascular plant cover [c]	7%	46%	79%
Moss cover	5%	88%	62%
LAI^{max} of veg. > 3 cm [d]	5.4	6.0	5.8
Dominant height	10.3 m	-	-
Diameter at Breast Height	12.6 cm	-	-
Basal Area	49 m^2 ha^{-1}	-	-
Stand density	4.461 trees ha^{-1}	-	-

[a] The standard methodology used here gives an underestimation for the true clay fraction in Andosol (Arnalds 2015); [b] 5-10 cm depth (only mineral soil); [c] Only including the ground vegetation in FN; [d] Determined by a LAI2200 instrument on 15-20 Sept. 2016.

availability is calculated as soil S flux over time. Four sets of membranes were inserted at 0-10 cm depth for 89 days (23 May to 20 August 2013) in each permanent plot. Afterwards, they were sent to Western Ag Innovations Inc. (Saskatoon, SK, Canada) for further analyses. Soil pH in H_2O and 1M KCl was determined for sieved (2 mm) soil samples, taken from 0-10 cm layer in all permanent plots in July 2014, after dissolving the samples in a 1:2.5 (per mass) solution, shaking for 20 min, and shaking shortly again after two hours before measuring pH with a Two Channel Benchtop pH/mV/ISE Meter (Hanna Instruments, Temse, Belgium). Finally, the potential decomposition rate of easily decomposable organic matter was determined following the TBI method (Keuskamp et al. 2013), by incubating four Lipton Green teabags at 5-7 cm depth in each permanent plot from late May to the middle of September (110 days) in 2014 and then drying them at 85 °C for 48 hours, weighing them and comparing their weight to stored control bags.

2.3.3 Statistical analysis

Individual permanent study plots in each ecosystem were used as the unit of replication in all ecosystems (n = 25 or 30, without and with the F treatment plots, respectively), except when standard deviations of all observations were calculated. Then all individual measurements

were included. One-Way ANOVA was used to test for differences between the three ecosystems, and when significant, it was followed by Fisher's Least Significant Difference (LSD) pairwise tests (SAS, version 9.4; SAS Institute Inc., Cary, NC, USA). Potential effect of soil warming on different parameters was evaluated with a linear regression analysis using mean annual Ts' measured for each plot.

2.4 Results

The mean annual soil temperature (MATs) of the unwarmed soil was 6.3 °C in the two grasslands, but 5.3 °C under the dense forest cover in 2014 (Figure 2; Table 2). The July and January Ts' were on average 12.4 °C and 1.0 °C in the unwarmed grasslands, but 10.0 °C and 0.9 °C at FN, respectively (Table 2). Frozen soil below 10 cm depth in winter, indicated with Ts being stable around 0 °C did occur. While this only lasted for limited time in the grasslands, FN showed longer periods with frozen soil during winter (Figure 2).

All the warming levels (WLs) were similar during the three years of study and followed the natural Ts seasonal cycle, with relatively stable offset and no periods with frozen soil for the WLs of C to F (Figure 2). We were not successful to place the permanent plots at the exactly same MATs in each ecosystem, but the average WL across the three ecosystems was +0.6, +1.9, +3.2, +7.7 and +16.8 °C for B, C, D, E and F, respectively (Table 2). Both natural unwarmed Ts and the WLs showed some fluctuations, but for A, B, C and D the hourly mean Ts was more or less always within ±3 °C of the WL annual average for each ecosystem, while the fluctuations became somewhat larger for E and F, where the hourly mean Ts was within ±3 °C for 79% and 59% of the time across the three ecosystems (Table 2). The absolute annual peak value in hourly maximum and minimum warming during 2015 for each WL is also shown in Table 2.

A survey measuring vertical Ts profiles at all WLs in all three ecosystems showed that the Ts measured at 10 cm depth was relatively constant in all WLs down to ca. 20-25 cm depth, which represents the most active root layer. Only in the warmest level (F; Figure 3), the Ts increased more promptly with depth and the Ts at 10 cm clearly underestimated the warming in the 15-30 cm layer. The spatial variability among the five replicated plots within each WL increased with Ts (Figure 3), indicating that it became increasingly difficult to place all plots on exactly similar Ts' as the geothermal warming gradients became steeper. Figure 3 illustrates the differences in soil depth between the three ecosystems, where GN had significantly shallower soils than both GO and FN, and GO had significantly the deepest soils

(Table 3). More importantly, however, there was no systematic difference in soil depth across the WLs within each of the three ecosystems (Table 3; regressions not significant).

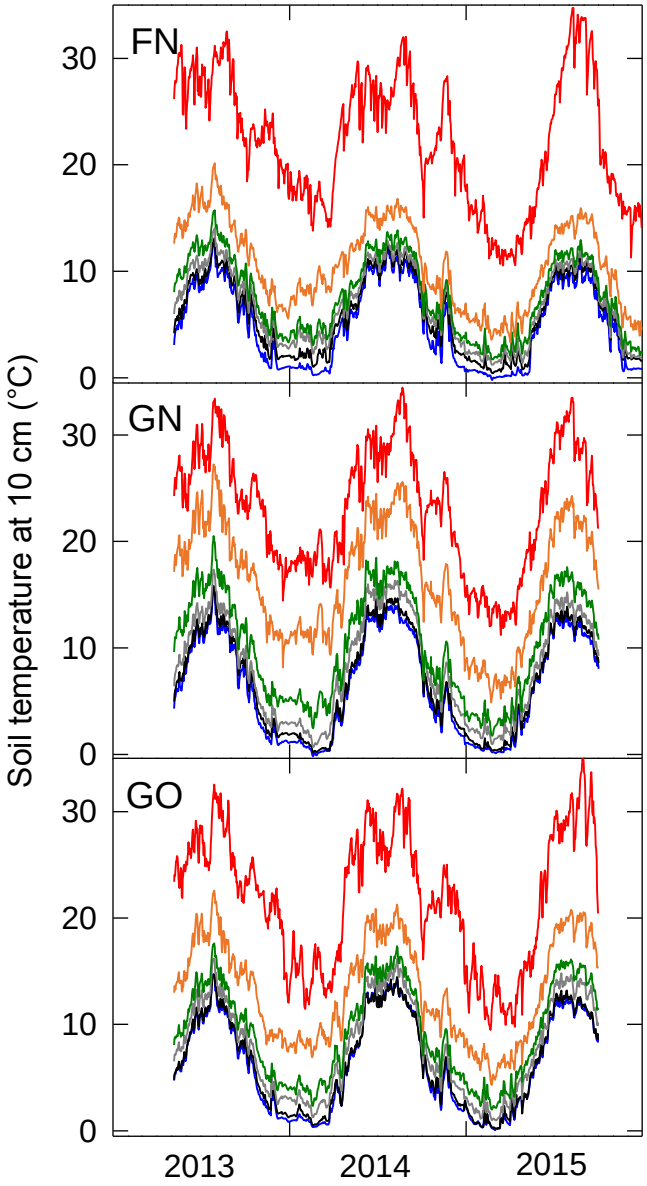


Figure 2. Changes in daily mean soil temperature (Ts) at 10 cm depth in the six warming levels (A-F) of recently warmed forest (FN; top), grassland (GN; middle) and long-term warmed grassland (GO; bottom). Warming levels are A (unwarmed control; dark blue), B (black), C (grey), D (green), E (orange) and F (red). For evaluation of warming levels see Table 2.

Table 2. Mean annual soil temperature at 10 cm depth (MATs, °C; ±Sd) and mean annual warming (MAW, °C) in 2014 and the maximum and minimum hourly warming (W_{min}, W_{max} ; °C) relative to average MATs on unwarmed (A) plots and different soil warming levels (B-F). Fraction of hourly MATs data from 2014 within 1, 3, 5 and 10 °C of the annual mean (Variation; ±1, ±3, ±5 and ±10 °C), July mean Ts in 2013, 2014, 2015 and January mean soil temperature (Ts) in 2014 and 2015 of the recently warmed forest (FN) and grassland (GN) and the long-term warmed grassland (GO). All averages and standard deviations are for n=5 plots per warming level (WL).

		2014		2014		Variation in 2014				July Ts			January Ts	
Sites	WL	MATs	MAW	W_{min}	W_{max}	±1 °C	±3 °C	±5 °C	±10 °C	2013	2014	2015	2014	2015
FN	A	5.3 ±0.2	**0.0**	-0.9	1.0	99%	100%	.	.	10.0	10.5	9.4	1.0	0.7
	B	6.2 ±0.5	**1.0**	-0.2	3.3	88%	100%	.	.	10.6	11.0	9.9	2.0	1.6
	C	7.2 ±0.5	**1.9**	0.2	4.3	73%	99%	100%	.	11.6	11.5	10.5	3.6	2.3
	D	8.0 ±0.2	**2.7**	0.7	5.4	73%	96%	100%	.	13.1	12.4	11.5	4.5	3.2
	E	11.1 ±0.7	**5.8**	2.7	10.3	50%	86%	98%	100%	17.2	15.1	14.0	7.9	5.4
	F	22.8 ±2.2	**17.5**	9.4	25.0	25%	49%	85%	99%	27.8	25.7	28.3	17.8	15.7
GN	A	6.3 ±0.3	**0.0**	-1.5	1.4	95%	100%	.	.	12.5	12.9	12.5	1.1	0.8
	B	6.8 ±0.3	**0.5**	-1.4	1.9	96%	100%	.	.	12.9	13.5	13.0	1.7	1.2
	C	8.3 ±1.4	**2.1**	-0.2	4.1	35%	93%	100%	.	14.3	15.0	14.2	2.8	2.3
	D	10.2 ±0.3	**3.9**	0.3	7.4	49%	97%	100%	.	16.9	16.4	16.5	5.1	3.6
	E	16.7 ±2.5	**10.5**	3.7	15.0	22%	68%	97%	100%	23.3	22.1	22.5	11.5	8.1
	F	23.6 ±1.3	**17.3**	8.8	24.2	30%	75%	93%	100%	29.4	28.3	30.5	18.2	14.6
GO	A	6.3 ±0.6	**0.0**	-1.8	1.9	82%	99%	100%	.	11.8	12.9	11.9	1.1	1.1
	B	6.5 ±0.6	**0.2**	-1.8	2.6	77%	100%	.	.	12.0	12.7	12.4	1.6	1.2
	C	7.8 ±0.5	**1.6**	-0.4	3.5	74%	100%	.	.	13.4	14.0	13.8	3.0	2.2
	D	9.1 ±0.3	**2.9**	0.2	5.2	71%	100%	.	.	14.7	15.0	15.4	4.2	3.6
	E	13.0 ±2.0	**6.8**	2.7	10.4	28%	82%	99%	100%	19.4	18.6	19.2	8.3	7.3
	F	21.9 ±3.3	**15.6**	6.5	25.7	19%	53%	70%	95%	27.7	26.3	28.7	15.6	14.2

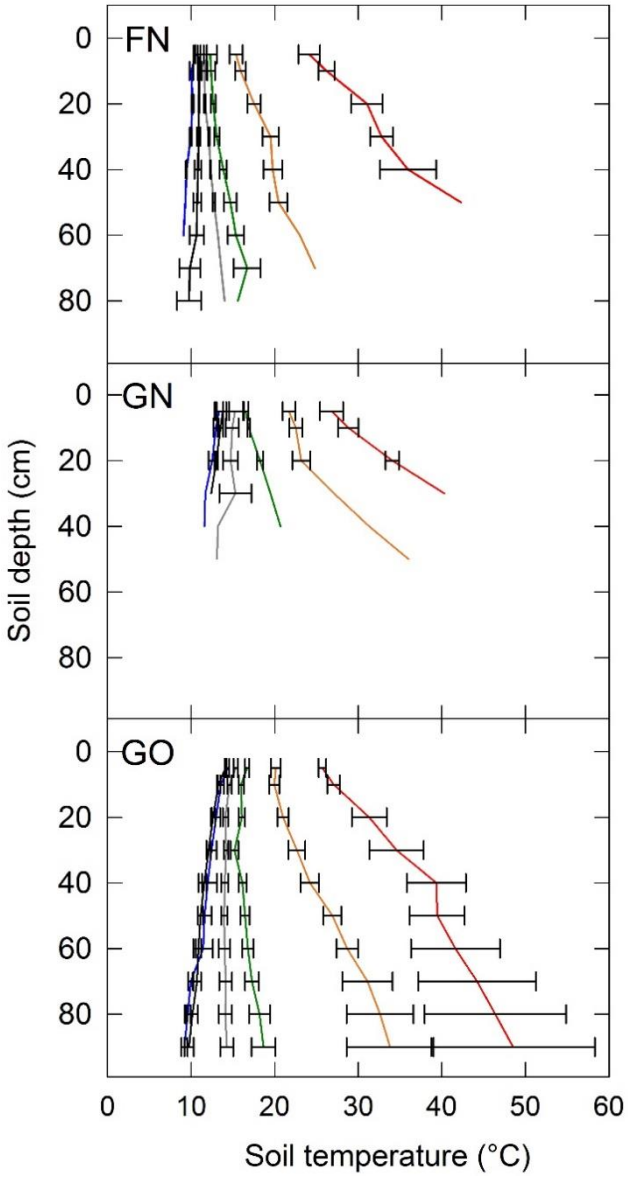


Figure 3. Vertical profiles (5, 10, 20, 30, … cm) of soil temperature in the six warming levels (A-F) of the recently warmed forest (FN; top) and grassland (GN; middle) and the long-term warmed grassland (GO; bottom) at 27 June 2014. Warming levels are A (unwarmed control; dark blue, thicker line), B (black), C (grey), D (green), E (orange) and F (red). SEs of Ts measurements from 2-5 plots are shown as lateral bars.

Measurements of air temperature (Ta) at 2 m height at different places in FN showed no effects of the soil warming (data not shown) and therefore Ta at 2 m was only measured at one location in GN and GO. At 2 and 15 cm height above the surface in GN and GO the regression relationships between average Ts (°C) and average Ta warming (ΔTa; °C) were significant (Figure 4; ΔTa_{15}: $r^2 = 0.32$; $P = 0.01$; ΔTa_{02}: $r^2 = 0.65$; $P < 0.001$):

$\Delta Ta_{15} = 0.03 \times Ts - 0.12,$ (1)

$\Delta Ta_{02} = 0.07 \times Ts - 0.53.$ (2)

The above relationships indicate that air warming was substantially less than the soil warming, and a 20 °C increase in Ts only elevated average Ta by 0.95 °C and 0.48 °C at 2 cm and 15 cm height above the surface, respectively (Figure 4).

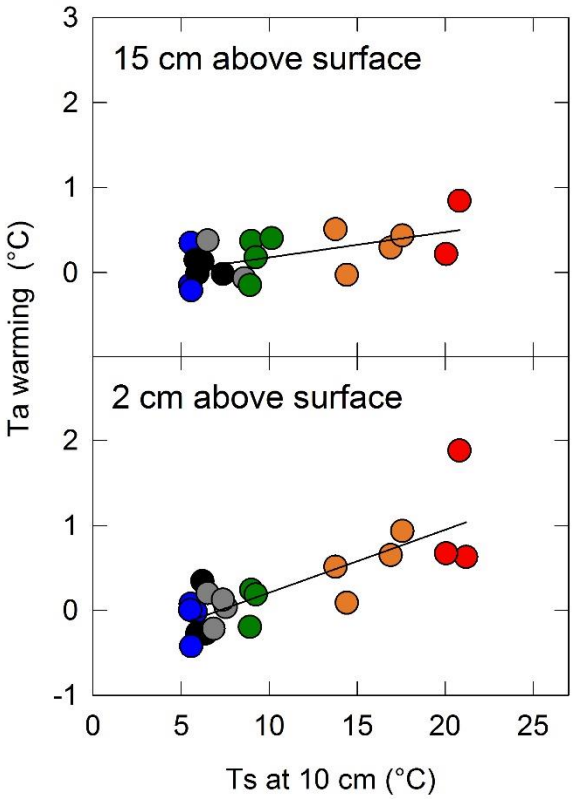


Figure 4. The relationship between mean soil temperature (Ts) at 10 cm depth and average air temperature (Ta) warming compared to Ta measured at 2 m height for the period 15 February to 30 June 2015 in the ForHot grasslands. Warming levels are A (unwarmed control; dark blue), B (black), C (grey), D (green), E (orange) and F (red). The lines represent significant regression relationships.

Measurements of pH_{H2O} of the top 10 cm of mineral soil did not show any indications that geothermal water had reached the root-zone in any of the ecosystems, i.e. it did not show any signs of acidification (Table 3). In the unwarmed soil, pH was significantly lower in the coniferous forest plantation than in the two grasslands, which did not differ (pH 5.2 in FN vs. ca. 5.9 in GN and GO). Soil pH did, however, increase somewhat with Ts and this change was significant in the recently warmed ecosystems (FN and GN), but not in the long-term warmed ecosystem (GO). $\Delta pH_{KCl-H2O}$, which was also significantly lower in FN than in the two

grasslands, increased significantly with WL in all three ecosystems (Table 3). A lack of geothermal contamination was further supported by the lack of significant regressions between Ts and exchangeable sulphur (S) in the soil in any of the ecosystems (Table 3). The overall level of exchangeable S was, however, much higher in GO, where higher number of geothermal vents are found within the same valley, than in the recently warmed systems.

All ecosystems had the highest volumetric water contents (WC) early in the spring and the lowest measured surface WCs in the middle of summer (Figure 5). Average surface WCs of the unwarmed control treatments (WC_{meanA}) during the period of 11 April to 1 September 2016 were 52.2%, 38.8% and 31.1% in GO, GN and FN, respectively (Figure 5), and similar values were obtained in 2013-2015 (Table 4). When the plot-level WCs for individual measurement days were compared across the soil warming gradients in each ecosystem, a significant linear drying effect with warming was observed for the surface soil for a part of the dates in both GN and GO, but only a few times in FN, which was the driest ecosystem (regression analysis; data not shown).

During 2013-2016, the surface WC did not change significantly (2014-2016) or was even significantly increased with increasing Ts (2013) in FN, when averaged over the whole growing season (ranging between +0.33 to +2.46%WC $°C^{-1}$; Table 4). The increase in WC at FN was especially pronounced at warming level E. In GN, however, the seasonal mean surface WC was always significantly reduced across the Ts gradients (-0.71 to -0.77%WC $°C^{-1}$), while in GO it shifted between no significant change to significantly reduced (-0.20 to -0.73%WC $°C^{-1}$; Figure 5; Table 4). It should, however, be noted that the observed surface WCs in the warmest treatments of GO were still substantially higher than the unwarmed control FN soil (e.g. 41.8% vs. 31.1% WC in 2016). The warmest GN plots were usually similar to the unwarmed FN soil, on average (30.0% vs. 31.1% WC, respectively, in 2016; Figure 5).

To indicate how ecosystem processes were affected by the soil warming, potential decomposition rate of organic matter is shown (Table 3). Green tea, used for these measurements, was decomposed faster in the two grasslands than in the forest ecosystem in unwarmed soil. The soil warming, significantly increased the decomposition rate in all ecosystems, but to a different degree. The slope of the temperature response was almost identical for the two recently warmed systems (FN and GN), but it was ca. 60% lower in the long-term warmed grassland. The net result was therefore that the observed mean decomposition potential across all WLs was significantly highest in GN, second in GO and significantly lowest in FN (Table 3).

Table 3. Mean soil depth, pH_{H2O}, $\Delta pH_{KCl-H2O}$, exchangeable sulphur, and potential decomposition rate for unwarmed (A) plots and different soil warming levels (B-F) in the recently warmed forest (FN), and grassland (GN) and the long-term warmed grassland (GO). Significant differences in ecosystem means are indicated by different letters (post-ANOVA LSD tests). The lower part of the table shows a regression analysis of the effect of soil temperature on the different variables. * = *P* < 0.001, ** *P* < 0.01, * = *P* < 0.05, (ns) = *P* < 0.10, ns = *P* > 0.10. Significant changes across Ts-levels are indicated in bold.**

	Soil depth (cm)			$pH_{H2O,\ 0\text{-}10\ cm}$			$\Delta pH_{KCl\text{-}H2O,\ 0\text{-}10\ cm}$			Exchangeable S [a]			Potential decomposition [b]		
Ecosystem	FN	GN	GO	FN	GN	GO	FN	GN	GO	FN	GN	GO	FN	GN	GO
A	76.3	54.1	98.2	5.3	5.6	5.7	-1.35	-1.49	-1.53	51.6	77.7	222.6	47%	53%	54%
B	72.4	38.6	91.5	5.1	5.8	5.7	-1.19	-1.63	-1.52	108.7	89.1	123.0	45%	58%	55%
C	68.1	42.7	75.0	5.1	5.8	5.5	-1.25	-1.62	-1.56	102.4	78.2	142.4	51%	58%	55%
D	68.6	28.7	90.5	5.0	5.9	5.7	-1.13	-1.56	-1.62	73.5	56.5	177.7	49%	56%	59%
E	69.3	27.4	82.9	5.2	6.3	6.1	-1.25	-1.95	-1.73	87.2	61.7	197.3	52%	69%	58%
F	-	-	-	5.7	6.3	6.1	-1.81	-2.00	-2.02	-	-	-	64%	69%	61%
Mean	70.9	38.3	87.6	5.25	5.95	5.82	-1.33	-1.71	-1.66	84.7	72.6	172.6	51%	61%	57%
LSD	a	b	c	a	b	b	a	b	b	A	a	b	a	b	c
Regression analysis (ANOVA)															
Intercept	79.8	60.1	97.6	4.905	5.524	5.532	-0.99	-1.39	-1.42	74.8	96.47	97.57	0.415	0.491	0.527
Slope	-1.2	-2.2	-1.2	**0.034**	**0.036**	0.026	**-0.03**	**-0.02**	**-0.02**	1.30	8.90	-1.16	**0.0098**	**0.0096**	**0.0039**
n	25	25	25	30	30	30	30	30	30	25	25	25	30	30	30
r^2	0.02	0.16	0.02	0.22	0.37	0.06	0.36	0.42	0.19	0.01	0.02	0.02	0.64	0.73	0.22
P	ns	(ns)	ns	*	***	ns	***	***	*	Ns	ns	ns	***	***	**

[a] Unit: Δg S 10 cm^{-2} 89 summer $days^{-1}$ [b] = Relative mass loss after 110 days of incubation (spring-autumn)

Table 4. Mean volumetric soil water content (%) in the top 5 cm in unwarmed soil (WC_{meanA}) and across all soil temperature (Ts) levels (WC_{mean}) and the corresponding standard deviation (WC_{Sd}) in 2013 to 2016 in the recently warmed forest (FN), and grassland (GN) and the long-term warmed grassland (GO). The lower part of the table shows a regression analysis of the effect of mean soil temperature on the mean seasonal plot-wise WC. *** = $P < 0.001$, ** $P < 0.01$, * = $P < 0.05$, (ns) = $P < 0.10$, ns = $P > 0.10$. Significant seasonal increases or reductions in WC with Ts are indicated in bold.

	2013			2014			2015			2016		
Ecosystem	FN	GN	GO	FN	GN	GO	FN	GN	GO	FN	GN	GO
Campaigns	4	4	4	12	8	8	4	6	6	11	10	10
WC_{meanA} (%)	31.2	38.3	36.4	33.3	49.4	53.5	28.6	46.4	54.0	31.1	38.4	52.3
WC_{mean} (%)	33.9	36.6	33.9	44.4	47.9	52.1	37.5	42.4	51.5	35.5	36.6	46.0
WC_{Sd} (%)	9.0	7.3	9.0	15.3	13.7	14.1	15.6	13.5	14.9	15.9	13.6	15.7
Regression analysis (ANOVA)												
Intercept (%)	15.25	43.8	37.89	39.4	56.7	60.0	30.6	51.6	58.4	31.8	44.9	52.0
Slope (% $°C^{-1}$)	**+2.46**	**-0.75**	-0.20	+0.50	**-0.74**	**-0.73**	+0.68	**-0.77**	**-0.65**	+0.33	**-0.71**	**-0.56**
n	25	25	25	30	30	30	30	30	30	30	30	30
r^2	0.61	0.40	0.02	0.10	0.44	0.22	0.12	0.57	0.24	0.03	0.37	0.15
P	***	***	ns	(ns)	***	**	(ns)	***	**	ns	***	*

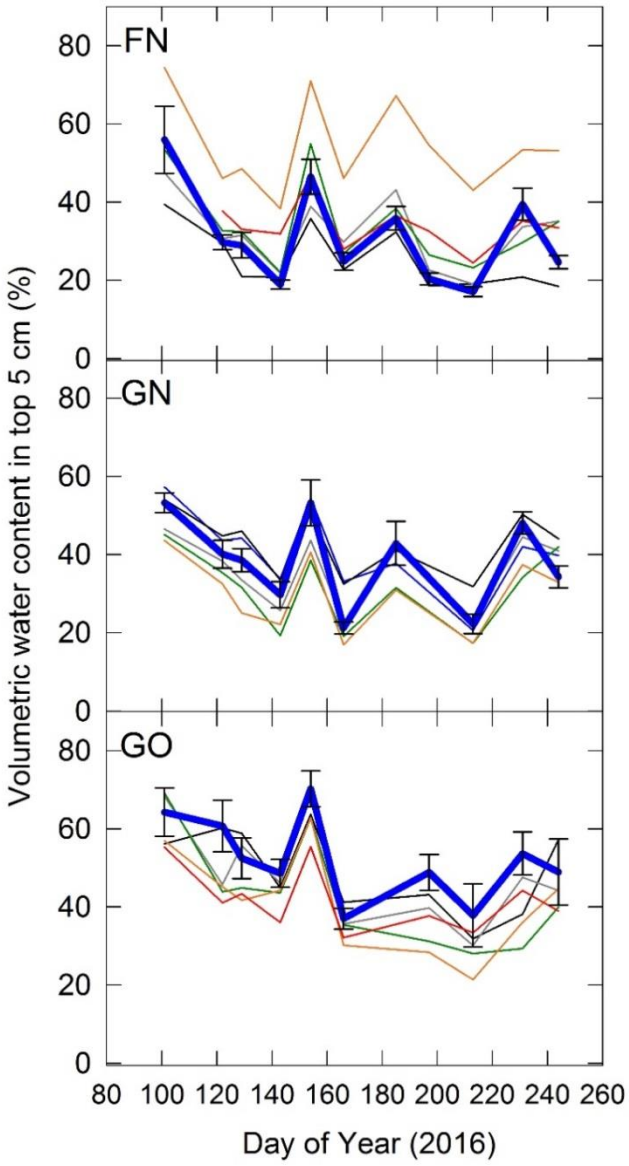


Figure 5. Changes in mean volumetric water content (WC) in the top 5 cm in the six warming levels (A-F) of the recently warmed forest (FN; top) and grassland (GN; middle) and the long-term warmed grassland (GO; bottom) from April to August 2016. Warming levels are A (unwarmed control; dark blue, thicker line, including SE of the mean), B (black), C (grey), D (green), E (orange) and F (red).

2.5 Discussion

2.5.1 Realism of the soil warming

The main concern when starting the ForHot project was if the geothermal water, rich in various dissolved minerals, would reach the root zone in the soils, thereby creating difficult conditions for many organisms. If this would be the case, observed changes in processes and ecosystem structure would not necessarily be driven by changes in temperature, but rather by other chemical drivers. Our results for pH and exchangeable S do not show any indications of this happening in any of the ForHot sites. This was further confirmed in a recent thesis on seasonal soil water chemistry below the root zone at the FN site that did not show any signals of geothermal contamination (Edlinger 2016). A second concern was that the natural seasonal patterns of Ts would flatten out at the warmed plots. This was not the case as can clearly be seen in Figure 2. Lastly we also expected the vertical Ts gradients in geothermally affected plots to become very different from the ones in unwarmed conditions. The difference was, however, marginal (except for the warmest plots), especially for the top 20-30 cm of soil, containing most of plant roots.

All the above findings support the theory that the geothermal gradients at ForHot offer comparable conditions to study effects of warming as e.g. manipulation studies where heating cables have been inserted into the soil to warm it up and simulate future climate change (Rustad 2001, Strömgren & Linder 2002, Bronson & Gower 2010, Patil et al. 2013, Streit et al. 2013, Krassovski et al. 2015, Schindlbacher et al. 2015).

2.5.2 Stability of the soil warming

It is not expensive nor difficult to maintain the soil warming in the ForHot sites, but since the warming is entirely passive there is no way to control it. During the study period of 2013-2015 the geothermal gradients remained relatively stable at all three sites. Geothermal systems, however, tend to be dynamic in nature (Carotenuto et al. 2016), as was also witnessed in the present study by the creation of the new geothermal gradients during the large 2008 earthquake (Þorbjörnsson et al. 2009). This complicates the estimation of the duration of unchanged warming in Grændalur (GO). Based on the survey in autumn 2008 by Þorbjörnsson et al. (2009), showing little changes in distribution of hotspots and geothermal vents in those areas (especially for transects 1-4 of GO), and Kristjánsson's first mapping of the geothermal hotspots in the same area during 1963-1965, it was assumed that they have existed for at least 50 years. However, even though the area of Grændalur has had those geothermal hot spots for centuries, they may have changed both spatially and thermally over

time. To further study the temporal and spatial history of the gothermal warming at GO, there is an ongoing activity using HPLC (High Performance Liquid Chromatography) analysis to study recalcitrant soil bacteria lipids in soil profiles from GO, which may be used to reconstruct historical soil temperature (De Jonge, pers. comm.). Such measurements have been successfully used as paleo-climate proxies of soil temperature elsewhere (De Jonge et al. 2014).

2.5.3 Warming induced drying

A general concern with all terrestrial manipulation experiments involving soil or air warming is that their warming treatments are almost inevitably increasing evapotranspiration and therefore potentially induce drought (Lu et al. 2012, De Boeck et al. 2015), which could confound the "warming responses". If such drying will induce some strong biological or biogeochemical responses, depends largely on the hydrological conditions of each site. The annual precipitation tends to be high at Reykir (between 1134 and 2023 mm in 1972-2000; Icelandic Met Office, 2016) and due to the relatively short summer and the high water storage potential of the sites' soil type (Arnalds 2015), it was not expected that drought would be a major driver at the ForHot sites.

The driest surface soils were found in the FN forest, where the dense forest stand likely both had higher transpiration and much higher evaporation from intercepted rainfall than the grasslands (cf. Koivusalo et al. 2006). However, no signs of water stress were observed when the stomatal conductance of the Sitka spruce trees was measured (André & Bondesson 2014). Further, no significant drying was observed at higher WLs in FN. On the contrary, there was a strong "wetting" there, especially at ca. +10 °C soil warming. This occurred exactly at the interface where warming induced tree mortality was taking place (O'Gorman et al. 2014), and where the stand had opened up, but where still little ground vegetation had colonized (Gudmundsdottir et al. 2014; Cilio 2014). At the F plots, all Sitka trees had died and the ground was covered with lush herbaceous vegetation (Gudmundsdottir et al. 2014). We interpret this as a relatively higher reduction in tree transpiration and intercepted rainfall in the warmest treatments in FN compared to the warming-induced drying.

The grasslands did, however, show a significant over-all drying of the surface layer with warming in most years, especially GN, which had thinner soils and therefore less water storage potential. This drying was not always significant and apparently became stronger during and just after the infrequent dry-spells in mid- to late summer, when the water content

of the unwarmed control plots was also reduced. Such drying of surface soil may, however, not necessarily cause physiological stress for plants, especially not those that have roots that extend below 5 cm. In fact, the soil water status of the "driest" treatments in the grasslands was still similar to the unwarmed control plots in FN. Further, when stomatal conductance (*gs*) was measured for *Ranunculus acris*, *Poa pratensis* and *Agrostis capillaris* in GO and GN, no significant (drought-induced) stomatal closure was observed across the WLs (Michielsen 2014). The more shallow-rooted *Poa* and *Agrostis* grasses had, however, on average significantly lower *gs* in GN than GO, which fits well with our measurements of their water status.

2.5.4 Soil warming vs. air warming

All soil warming techniques have only limited effects on air temperature (Lu et al. 2012, Patil et al. 2013) and this was also observed in our air temperature measurements. There was, however, a significant warming detected in the top 15 cm above the soil surface, but on average, the actual surface air warming was an order of magnitude smaller than the warming at 10 cm soil depth. This calls for some caution when findings of aboveground processes measured in soil-warming experiments are extrapolated in relation to future climate warming. An example is the effect of warming on tree canopy gas exchange which is measured at ca. 8 m height above the soil surface (André & Bondesson 2014). There the effects of the soil warming are interesting to better understand the potential effects soil temperature can have on such aboveground processes, but it is highly doubtful that the responses could be used to predict photosynthesis or transpiration of spruce in a future climate. This is a general issue with soil warming experiments, and ForHot is neither better nor worse in that respect than other recent or ongoing soil warming manipulation studies (Patil et al. 2013, Streit et al. 2013).

2.5.5 Effects on potential decomposition

At higher latitudes (>60 °N), soils contain the largest part of the ecosystem organic matter (Dixon et al. 1994). This is also the case for Andosols of Iceland (Arnalds 2015). Understanding the dynamics of organic matter (C) pools, including turnover of litter (Davidson & Janssens 2006a), roots (Leppälammi-Kujansuu et al. 2014) and biota in soil (Clemmensen et al. 2013) is therefore the key to make sound predictions of the future ecosystem C balance under changing climatic conditions. In this paper, which mainly focused on methodological issues, we only show warming effects on one process; the potential decomposition rate of easily decomposable organic material. As expected, the potential

decomposition rate was significantly enhanced with increasing Ts in all three ecosystems. Interestingly, however, the slope of the temperature response was much lower in GO, than in GN and FN. This might indicate some acclimation in the GO grasslands in terms of the rate of organic matter breakdown. A recent review of Lu et al. (2012) found indications that the temperature sensitivity of microbes declines at higher temperature levels in manipulation experiments or that the microbes themselves may acclimatize to high temperatures. Further studies where different aspects of the C-cycle of the ForHot grassland sites are being conducted (Leblans 2016; Maljanen et al. 2016; Poeplau et al. 2016).

2.5.6 Other observed effects of warming

The soil pH_{H2O} increased significantly with warming in both GN and FN, but not in GO. This might have been driven by a decrease in vascular plant root-litter inputs at the higher temperatures (Way & Oren 2010) and/or a gradual depletion of partly decomposed humic materials in the warmer soils of FN and GN after the warming was initiated in 2008. The reverse process, i.e. a gradual decrease of pH_{H2O} as vegetation cover or productivity increases is well known in Icelandic grassland and forest soils (Sigurðsson et al. 2005, Sigurdsson & Magnusson 2010, Vilmundardottir et al. 2015a).

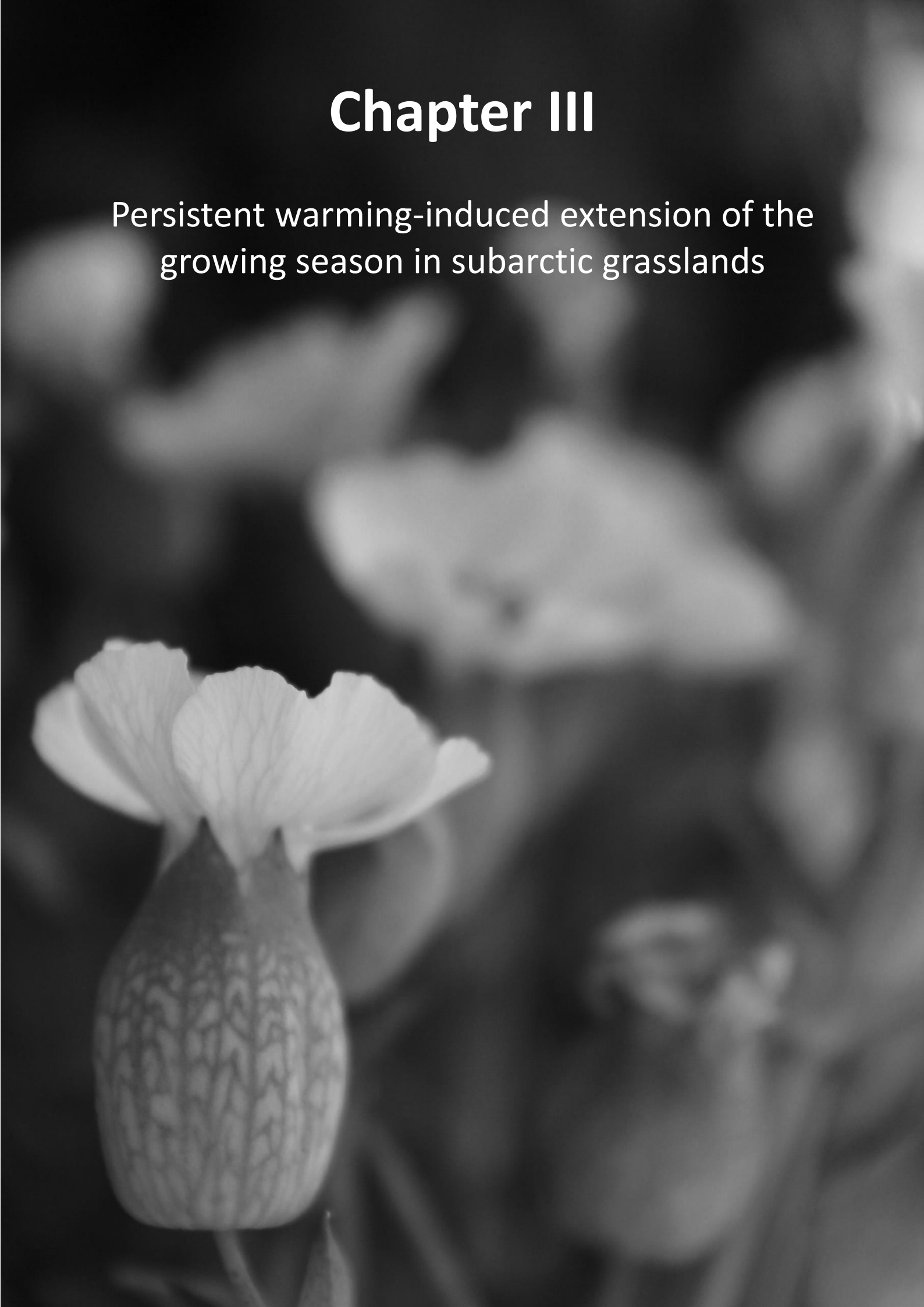
Another interesting finding was the gradually increased $\Delta pH_{KCl-H2O}$ with warming in all three ecosystems. This so-called "reserve acidity" is generally caused by additional Al^{3+} and H^{+} being released from exchange sites in the soil (Arnalds 2015). This increase may therefore indicate certain soil structural change due to warming, which increased the amount of such exchange sites being exposed. Indeed, a warming-induced change in soil structure and break-down of soil aggregates has recently been found to be an important driver for the observed alterations in soil organic matter at GN, apart from the increasing decomposition potential caused by higher temperatures (Poeplau et al. 2016). This is a very interesting finding and may change our understanding of how climate change is likely to affect soil organic matter.

2.6 Conclusions

We conclude that the natural geothermal gradients at the ForHot sites in Iceland offered realistic conditions to study terrestrial ecosystem responses to warming with minimal artefacts.

Chapter III

Persistent warming-induced extension of the growing season in subarctic grasslands



3 Persistent warming-induced extension of the growing season in subarctic grasslands

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In preparation

3.1 Abstract

Vegetation phenology, and in particular the length of the growing season (LOS), is highly sensitive to climate change and could in turn induce potent feedbacks to the climate system, e.g. by altering ecosystem albedo and C balance. During the past decades, the largest extensions of LOS have been observed at high northern latitudes. However, there are indications that this northern warming-induced extension of LOS is saturating. Subarctic grasslands cover a vast area and have a large C storage potential, but the evolution of their LOS under warming is highly uncertain. Here, we study the phenology of subarctic grasslands along short-term (5-7 years) and long-term (≥50 years) natural geothermal soil warming gradients, using ground-level NDVI measurements. The LOS extended with increasing temperature upon both short-term and long-term exposure to higher temperatures (on average with 15.5 days per °C), indicating that no saturation of the warming response had yet been reached. The fact that short-term and long-term warmed grasslands responded similarly to the warming indicated a strong physiological control of the subarctic grasslands' phenological response to warming, while genetic adaptations and community changes were likely of minor importance. We conclude that the warming-driven extension of the LOS of subarctic grasslands was not saturated yet, and that it can still extend if warming continues (unless genetic adaptations or species shifts do occur). This persistency of the warming-induced extension of LOS has important implications for the C-sink potential of subarctic grasslands under climate change.

3.2 Introduction

Vegetation phenology (the timing of recurrent biological events and its biotic and abiotic causes; Lieth, 1974) is highly sensitive to climate change (Henry and Molau, 1997, Tucker et al., 2001, Linderholm, 2006, Richardson et al., 2013), and shifts in vegetation phenology could in turn induce strong feedbacks to the climate system by altering fluxes of CO_2, water, energy and biogenic organic compounds, as well as by changing the albedo, surface roughness length and canopy conductance (Gu et al., 2003, Cleland et al., 2007, Ahlstrom et al., 2012, Richardson et al., 2013). The major driver of these potent phenological feedbacks to the climate system are changes in the length of the growing season (LOS), which follow from shifts in the start and/or the end of the growing season (SOS and EOS, respectively).

During the past decades, climate warming has generally extended LOS through the combined responses of SOS and EOS (Linderholm, 2006), and the largest extensions have occurred at high northern latitudes (Raynolds et al., 2015, Zhao et al., 2015, Gonsamo and Chen, 2016), where temperatures are rising fastest (IPCC, 2013). This warming-induced extension of LOS at high northern latitudes has been primarily driven by an advance in SOS, while EOS was generally less responsive to temperature (Cleland et al., 2007, Zhao et al., 2015). The advance of SOS under warming, however, has recently been shown to saturate for seedlings of broadleaf and evergreen trees (Morin et al., 2010) and for different mature broadleaf species across Europe (Fu et al., 2015). Also for northern latitude ecosystems, there are indications that the advance of SOS is slowing down, despite the continuous increase in temperature (Zhao et al., 2015). However, it remains highly uncertain how the timing of SOS will respond to further warming (Kimball et al., 2007, Zhao et al., 2013, Fu et al., 2014b, Keenan and Richardson, 2015).

Grassland ecosystems cover a large area of the global terrestrial surface (ca. 40 %, whereof 25 % at northern high latitudes; Chapin et al., 2011) and have a high C sink capacity (Soussana et al., 2007, Yoshitake et al., 2015). Grassland phenology is relatively understudied compared to forest phenology (Steinaker and Wilson, 2008, Li et al., 2016), but their C uptake, and thus their potential feedback strength to the climate system, is highly sensitive to changes in phenology, more than that of deciduous and needle leaf forests (Richardson et al., 2013). In this study, empirical observations of warming-induced changes in the LOS of unmanaged subarctic grasslands were made, using ground-level NDVI measurements at natural geothermal soil temperature gradients.

The study was conducted in southwest Iceland, at the ForHot research site (www.forhot.is), where natural geothermal soil temperature gradients of different age are studied. Some of the temperature gradients were 5 to 7 years old, formed after a major earthquake in 2008, which initiated geothermal warming in previously unwarmed areas (short-term warmed, hereafter "ST"). The other gradients had been present for at least 50 years, and most likely many centuries (long-term warmed, hereafter "LT") (www.forhot.is). In both areas, soil warming gradients from ambient to about +10°C were investigated. The contrast in warming time between ST and LT was especially interesting, as it allowed to separate between transient and permanent phenological responses to warming, which remains an uncertainty in phenology projections (Kimball et al., 2007).

Based on existing knowledge, we expected that the LOS of these subarctic grasslands would extend with increasing temperatures, and that this extension would be mainly driven by an advance in SOS. At high warming levels, however, the advance of SOS was expected to saturate. We hypothesized that the temperature responses of ST and LT would be similar, based on previous observations of fast phenological responses to warming in alpine grasslands (Frei et al., 2014) as well as in many other ecosystem types (e.g. Byers and Quinn, 1998, Williams et al., 2008, Morin et al., 2010, De Frenne et al., 2011). Alternatively, the response in LT could have been mitigated due to genetic adaptations and/or community changes, causing a smaller response in LT compared to ST.

3.3 Material and methods

3.3.1 Site description

The study sites were located in the Hengill geothermal area, 40 km east of Reykjavik, Iceland (64°00′01″ N, 21°11′09″ W; 100 – 225 m a.s.l.) and are part of the ForHot research site (www.forhot.is). The ambient mean annual air temperature at the sites amounted to 4.9 °C during the study period (2013 - 2015) and the mean temperature of the coldest and warmest month were -1.0 and 11.7 °C, respectively. The mean annual precipitation was 1431 mm (Icelandic Meteorological Office; www.vedur.is). More detailed climatological information for each measurement year (2013, 2014 and 2015) is shown in Fig. 1.

We studied two sets of sites, located within 2.5 km from each other, which had been subjected to geothermal soil warming for different periods of time. One set of sites had been warmed since the 29th of May 2008, when a major earthquake caused geothermal systems to shift to previously unwarmed areas (hereafter "short-term warmed grassland": "ST"), while

the other set of sites has been warmed for at least 50 years and probably for centuries (hereafter "long-term warmed grassland": "LT") (www.forhot.is; Sigurdsson et al., submitted). The soil warming originated from heat conduction from the underlying bedrock, which was warmed by geothermally heated groundwater (Sigurdsson et al., submitted). No signs of soil contamination by geothermal by-products were found. The degree of warming was relatively constant throughout the study period, and warming did not cause large changes in soil moisture or pH (Sigurdsson et al., submitted). The main vegetation type at both sites was unmanaged subarctic grassland, dominated by *Agrostris capillaris*, *Ranunculus acris* and *Equisetum pratense.* Further description of the study sites can be found in O'Gorman et al. (2014), Michielsen (2014), Gudmundsdóttir et al. (2014), Poeplau et al. (2016) and Sigurdsson et al. (submitted).

3.3.2. Study design

In autumn 2012, twenty-five 2 x 2 m plots were established at the subarctic grassland sites (ST and LT) along the soil temperature gradients, ranging from ambient soil temperature to ~ +10°C, placing 5 replicate plots at 5 temperature levels (approximately +0, 1, 3, 5 and 10 °C). Soil temperatures were measured hourly at 10 cm soil depth using HOBO TidbiT v2 Water Temperature Data Loggers (Onset Computer Corporation, USA). Air temperature was measured at 2 m height and at 2 cm above the soil surface, using the same type of loggers and logging frequency. All air temperature loggers were protected from direct sunlight, while allowing sufficient air circulation. The surface temperatures (at 2 cm) were measured at 10 out of 25 plots in both ST and LT, thus, to derive the plot specific surface temperature for all individual plots, a correlation was made between the difference in air temperature at 2 m and 2 cm height (for the available data) and the plot-specific soil temperature at 10 cm depth (Fig. 3). Prior to this, infrequent extreme temperature deviations at 2 cm (i.e. difference between 2 cm and 2 m measured air temperature of > +5 or -5°C), which were caused by e.g. direct insolation or radiation frosts on clear nights, were set to +5 or -5°C. This was done because such episodes could not be excluded from the data as continuous temperature data were needed for GDD calculations (see § 2.5). The frequency of these extreme temperature deviations showed no significant correlation with average soil temperature (SI, Fig. S.1).

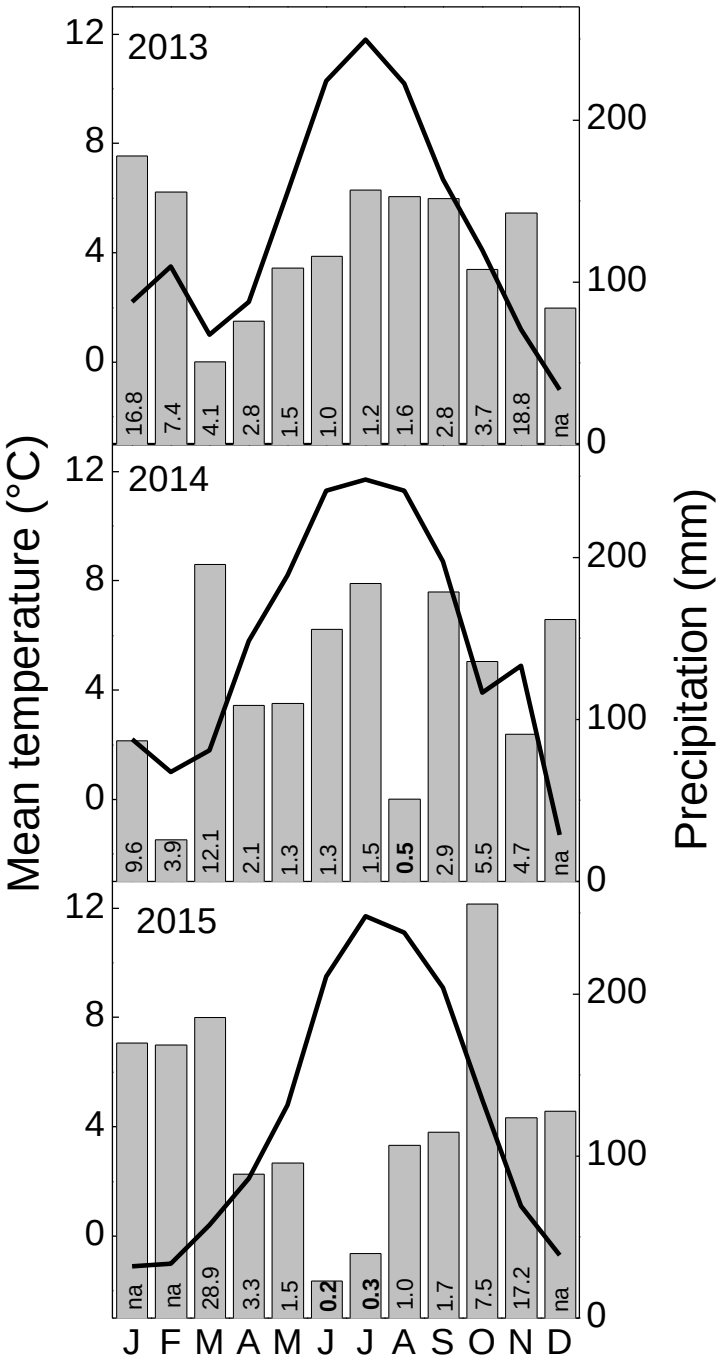


Figure 1: Monthly mean air temperature (black line), precipitation (grey bars) and water balance (precipitation / potential evapotranspiration (PET); numbers) at the ForHot research site for 2013, 2014 and 2015. Temperatures were measured at the ForHot research site. Precipitation was measured at the weather station of Eyrarbakki, 12 km south of the ForHot research site. (Source: www.vedur.is). Potential evapotranspiration was calculated following the approach of Thornthwaite (1948). The water balance values below one (in bold) indicate potential drought. Na: PET Equation not applicable because of below zero average temperatures.

3.3.3 NDVI-measurements

In 2013, 2014 and 2015, plot-specific NDVI measurements were made from April to November, using a hand-held SpectroSense 2+ four channel sensor (Skye instruments, UK). The measurements were carried out weekly, except in periods of continuous snow cover. Each measurement was conducted at a fixed location in each plot by placing the sensor pole in a pre-marked corner of the plot and tilting the pole in the direction of the opposite diagonal corner. A uniform tilt, corresponding to a measurement height of 2 m and a measurement surface of 0.62 m^2, was acquired by using a level bubble. The NDVI was calculated as in Tucker (1979), using the following Equation:

$$NDVI = \frac{\rho_{840} - \rho_{660}}{\rho_{840} + \rho_{660}} \quad \text{Eqn. 1}$$

Where ρ_{840} and ρ_{660} is the surface reflectance of the selected infrared wavelength (840 nm) and the visible red wavelength (660 nm), respectively.

3.3.4. Function fitting and determination of the start and end of the growing season

Each plot yielded three NDVI time series (Example: Fig. 2), one for each measurement year (2013, 2014 and 2015), which were scaled to a maximum value of one. From each time series, two phenological key dates were derived: (1) the start of growing season (SOS) and (2) the end of growing season (EOS). To retrieve these dates, two logistical functions were fitted to the NDVI time series, one for the greening and one for the senescence part of the data, according to the approach of Zhang et al. (2003) (Fig. 2). All functions yielded good fits over the whole soil temperature gradient ($R^2 \geq 0.88$, mean $R^2 = 0.96 \pm 0.01$ (SE)).

The SOS was detected by calculating the second derivative of the first part of the logistic greening function, thereby exposing the functions' highest change in curvature, following Zhang et al. (2003). For EOS, the timing of 10% senescence was used, as the real end of the growing season (the time point where the logistic senescence function levels of) could not be derived due to a lack of data in early winter. We selected the 10% senescence threshold because this point was reached in >90% of all data series and because the photosynthetic activity after this time point was expected to be negligible due to low light and temperature conditions. The EOS was calculated using the following equation:

$$EOS = \ln\left(\frac{a+d}{(a+d)*0.9}\right) - \left(\frac{b}{-c}\right) \quad \text{Eqn. 2}$$

Where EOS corresponds to the timing of 10% senescence and a, b, c and d are the parameters of the logistic senescence function (Zhang et al., 2003).

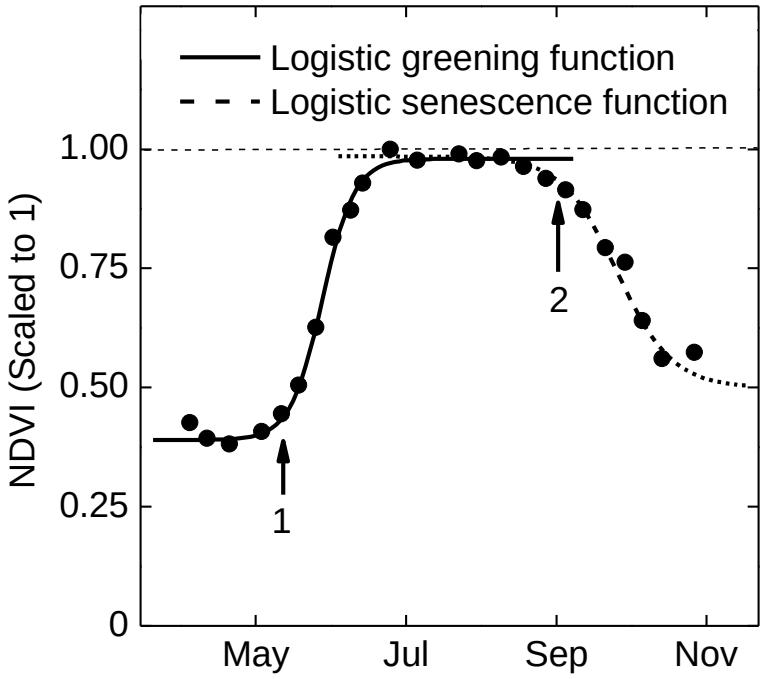


Figure 2: Example of NDVI measurement series (data points) with logistic function fits of the greening period (full line) and the senescence period (dashed line). Arrows indicate (1) the start of the growing season (SOS) and (2) the end of the growing season (EOS), corresponding to the timing of 10% senescence. Data show the 2014 NDVI time series of a plot with ambient soil temperature from the long-term warmed grassland.

3.3.5 Calculation of cumulative growing degree days at the start of the growing season

The cumulative growing degree days (GDDs) at the SOS were calculated using both soil temperatures ("soil GDDs", temperatures measured at 10 cm soil depth) and surface temperatures ("surface GDDs", temperatures measured at 2 cm above the soil surface). The cumulative GDDs were calculated according to (McMaster and Wilhelm, 1997), using the approach where the daily mean temperature is set equal to the base temperature when it is lower than the base temperature. The GDDs depend strongly on the chosen base temperature and on the starting date of GDD summation. We therefore first determined the optimal base temperature and starting date.

Hence, we compared the actual GDD of each unwarmed plot with its expected GDD (based on the average GDD of all other unwarmed plots) across all combinations of 16 base temperatures (ranging -5 to +10 °C, with an increment of 1 °C) and 9 starting dates (from the 1st of January to the 9th of May, which is the date of the first observed SOS, with an increment of 15 days). Then, the combination of base temperature and starting date that yielded the smallest difference between actual GDD and expected GDD across all unwarmed plots was

selected. The optimal base temperature based on this exercise was -1 and -3 °C for soil and surface GDD, respectively, and in both cases, the 15th of February was the optimal starting date (SI, Fig. S.2). These parameters were used to calculate the cumulative surface GDDs of all plots (both unwarmed and warmed). The cumulative soil GDD could only be calculated for 2014 and 2015, as no soil temperature data were available for the first months of 2013.

3.3.6. Data analyses

To test the influence of soil warming on surface temperatures, the relationship between soil warming and surface air warming (i.e. the temperature difference between the near-surface air temperature (at 2 cm above the soil surface) and the air temperature (at 2 m above the soil surface)) was tested with a linear regression model. The relationships between LOS, SOS or EOS and average soil or surface air temperature were tested with linear mixed models with temperature, warming time (ST and LT) and measurement year (2013, 2014 and 2015) as fixed factors. A logarithmic relationship for LOS, SOS and EOS vs. temperature was fitted when this yielded a better fit than the linear relationship (based on Akaike information criterion comparison with correction for finite samples (AICc comparison)). Further, a mixed linear model was used to test differences in cumulative soil GDD at the SOS between the different years, warming times and warming treatments (unwarmed and +1, +3, +5 and +10°C). Non-significant interactions were excluded from the model and a Tukey test was performed on the final model to test specific differences. Differences between cumulative surface GDDs at the SOS were tested in the same way. The relationship between SOS and EOS was tested with a linear mixed model with temperature, warming time and measurement year as fixed variables. The relationship between ΔSOS and ΔEOS was tested within measurement year with linear mixed models, with temperature and warming time as fixed variables. Requirements for normality and homoscedasticity were met in all cases. All tests were performed in R software (R-core-team, 2014) and null hypotheses were rejected at $p < 0.05$.

3.4 Results

3.4.1 Soil warming effects on surface air temperature

Soil warming at 10 cm soil depth caused a slight surface air warming (at 2 cm above the soil surface compared to ambient air temperature at 2 m height) of approximately 0.074 °C per °C soil warming (Fig. 3). At 15 cm height, the effects of soil warming were much lower (data not shown). This relationship between surface air warming and soil warming (based on

measurements at 20 out of the 50 plots) was used to calculate plot-specific surface temperatures for all plots where air temperature was not measured at 2 cm height. These estimates were then used to study surface temperature effects on phenology.

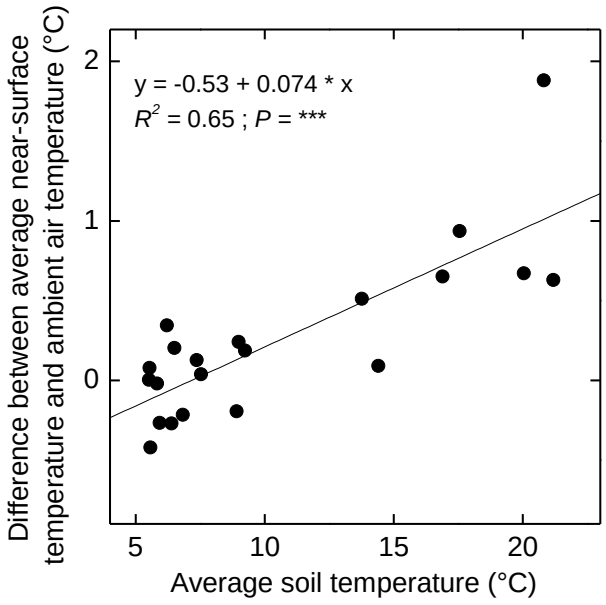


Figure 3: Temperature difference between average near-surface temperature (measured at 2 cm height) and average ambient air temperature (measured at 2 m height) vs. average soil temperature. All temperatures are averaged between the 15th of February and the 30th of June 2015. Significant source variables (p < 0.05) are indicated with an asterisk: *p is 0.05–0.01, **p is 0.01–0.001, ***p < 0.001.

3.4.2 Temperature effects on LOS, SOS and EOS of subarctic grasslands

During all three years in both ST and LT, the LOS of the unwarmed plots was approximately 120 days (four months), except in ST in 2013, where the growing season was about a month shorter (Fig. 4; upper panels). In all cases, warming extended LOS significantly, with an average prolongation of 2.1 ± 0.3 (SE) days per °C of soil warming (Fig. 4; upper row; Table 1), or 29 ± 5 (SE) days per °C of near-surface air warming (Table 1). There was no consistent difference in the response of ST and LT, since the three years showed a stronger (2013), weaker (2014) or no difference (2015) in warming response of ST compared to LT.

The SOS of unwarmed plots was initiated in late May in 2013 and 2014, and about a month later during the much cooler spring in 2015 (Fig. 4; middle panels). In all cases, warming significantly advanced SOS, following a logarithmic relationship. The response rate of ST and LT did not differ significantly in 2013 or 2014, with an advance rate of roughly 2.1 and 1.3 days per °C soil warming, respectively (corresponding to advance rates of 28 and 17

days per °C surface air warming; Table 1), while in 2015, the response rate of ST was slightly higher than that of LT (1.9 vs. 1.6 days per °C soil warming or 23 vs. 19 days per °C surface air warming, respectively; Fig. 4, Table 1).

The EOS of most unwarmed plots in 2013 and 2014 took place in late September (Fig. 4; lowest panels). The EOS of ST in 2013 was advanced with about a month compared to LT. In 2015, when SOS was late, EOS of the unwarmed plots was delayed with half a month compared to 2013 and 2014. In 2013 and 2014, there was no significant warming effect on EOS, while in 2015, soil warming slightly delayed EOS in a similar way for ST and LT (0.93 days per °C soil warming or 12.6 days per °C surface air warming).

3.4.3 Cumulative growing degree days at the start of the growing season

The cumulative soil GDDs at the SOS (calculated from soil temperatures at 10 cm depth) increased significantly with soil warming in all cases (Fig. 5, upper panels), except for the lowest warming treatment (+1°C; Table 2). The warmest plots (~ +10 °C soil warming) had e.g. received about three times the amount of soil GDD compared to unwarmed plots. Further, the cumulative soil GDDs at the SOS were significantly higher during the year with the coldest spring (2015). The cumulative surface GDDs at the SOS (measured at 2 cm height), on the contrary, decreased significantly with soil warming (Fig. 5, lower panels), except for the lowest warming treatment (+1°C, Table 2). Also here, the year with the coldest spring (2015) had a significantly higher surface GDD than the two warmer years (Table 2). Further, the surface GDDs at ST were slightly lower than at LT (Table 2).

3.4.4 Coupling between the end of the season and the start of season

The relationship between EOS and SOS was tested both for all data pooled and for the data excluding ST 2013, because this apparently was an outlier year (Fig. 6, main panel). Including all data, no influence of warming treatment on the relationship between EOS and SOS occurred, but warming time (ST and LT), and measurement year (2013, 2014 and 2015) did significantly influence the relationship (both $P < 0.001$). When excluding ST 2013, the outcome was very similar, but the interannual difference disappeared.

There was a significant interannual coupling between SOS and EOS (Fig. 6, main panel), both with and without ST 2013, with a slope of 0.66 and 0.73, respectively. Within years, however, the relationship between EOS and SOS disappeared (in 2013 and 2014) or was even reversed (in 2015) (Fig. 6, side panels), with a slope of -0.41. Warming time (ST or LT) did not influence the relationship.

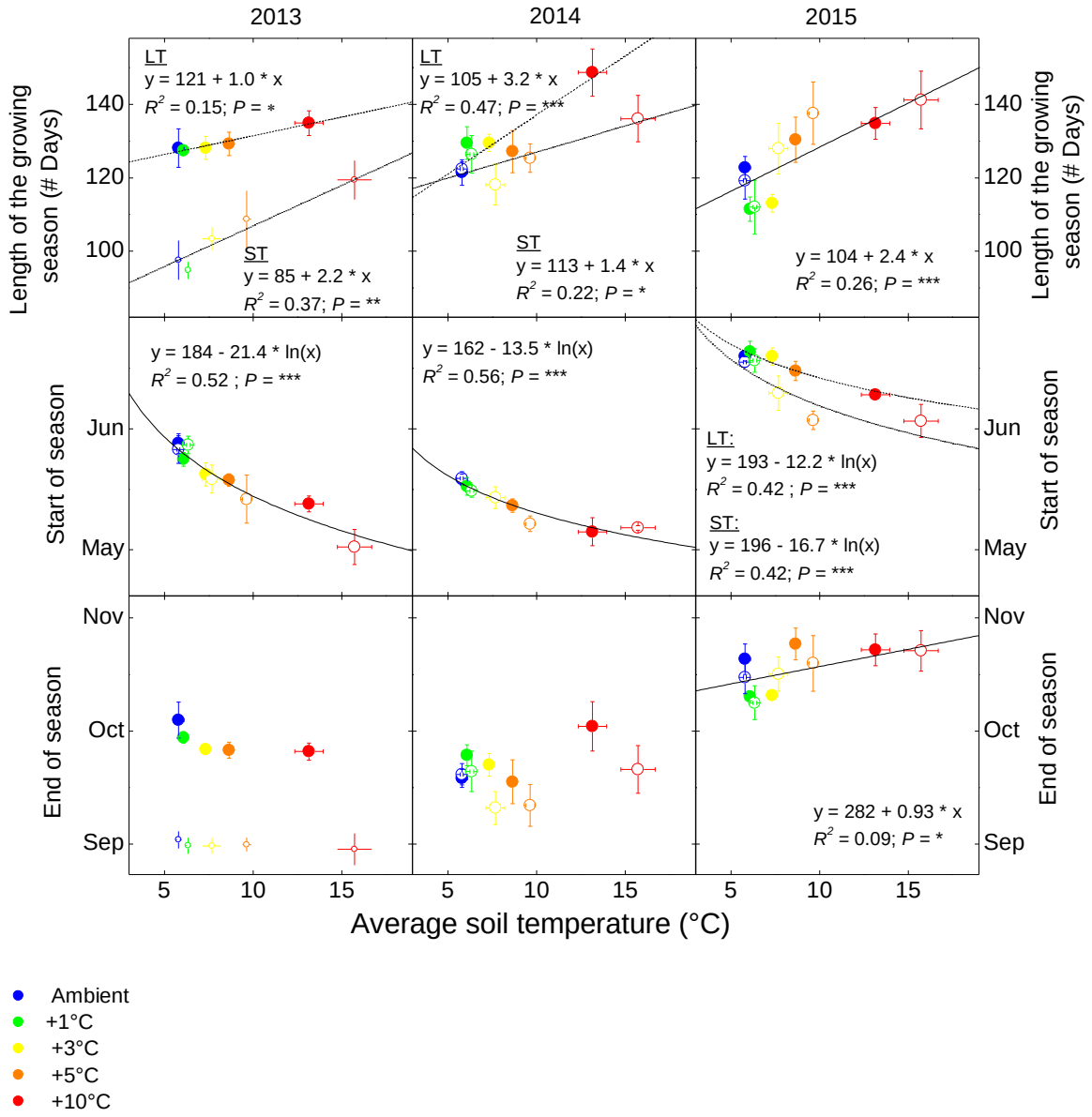


Figure 4: Length of the growing season (LOS; upper panels), start of season (SOS; middle panels) and end of season (EOS; lower panels) versus average soil temperature in 2013 (left column), 2014 (middle column) and 2015 (right column). The average soil temperature was calculated from May 2013 to May 2015. The short-term warmed grassland (ST) and the long-term warmed grassland (LT) are shown as open and filled circle respectively. The color gradient indicates the different soil warming treatments (ambient, +1°C, +3°C, +5°C and +10°C). For LOS and EOS for ST 2013, symbols are smaller to indicate that EOS was likely initiated by a local pest and not by a climatological cue, and should therefore be interpreted with caution. Error bars show SE's. Significant relationships are shown with a full line when no significant

interaction between ST and LT occurred. In case of interaction, the respective relationships are shown with a dotted line (ST) and a dashed line (LT). Equations for SOS and EOS are expressed in day of the year. Significant source variables (p < 0.05) are indicated with an asterisk: *p is 0.05–0.01, **p is 0.01–0.001, ***p < 0.001.

Table 1: Changes in the length of the growing season (LOS), the start of the growing season (SOS) and the end of the growing season (EOS) in days per °C soil warming (upper part) and near surface warming (lower part). Soil temperatures were measured at 10 cm soil depth and surface temperatures were measured 2 cm above the soil surface. The relationships were tested with mixed linear models with surface temperature, warming time (short-term "ST" and long-term "LT") and measurement year (2013, 2014 and 2015) as fixed variables. Significant source variables (p < 0.05) are indicated between brackets: *p is 0.05–0.01, **p is 0.01–0.001, ***p < 0.001. Errors indicate SE's.

	Change in days per °C surface air temperature increase			
	2013	2014	2015	Average
Soil warming				
LOS				
ST	+2.2 (**)	+1.4 (*)	+2.4 (***)	+2.1 ± 0.3
LT	+1.0 (*)	+3.2 (***)		
SOS				
ST	-2.1 (***)	-1.3 (***)	-1.3 (**)	-1.6 ± 0.2
LT			-1.3 (***)	
EOS				
ST	ns.	ns.	+0.93 (*)	-
LT	ns.	ns.		
Near surface warming				
LOS				
ST	+28 (**)	+20 (*)	+33 (***)	+29 ± 5
LT	+13 (○)	+48 (***)		
SOS				
ST	-28 (***)	-17 (***)	-19 (***)	-22 ± 2
LT			-23 (***)	
EOS				
ST	ns.	ns.	+12 (*)	-
LT	ns.	ns.		

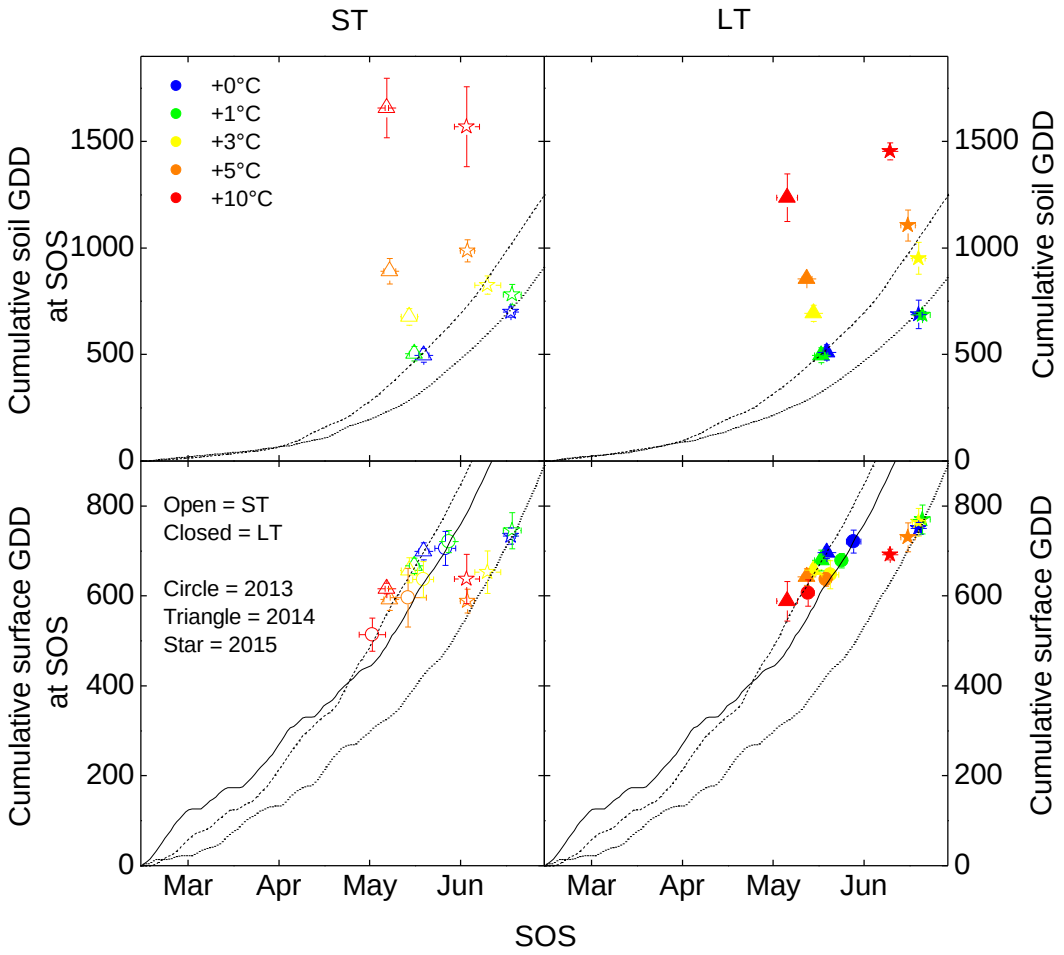


Figure 5: Cumulative soil growing degree days (GDDs) at the start of the growing season (SOS) versus SOS (upper panels) and cumulative surface GDDs at SOS versus SOS (lower panels) for the short-term warmed grassland (ST; left panels) and the long-term warmed grassland (LT; right panels) and for the three measurement years (2013: circles; 2014 triangles; 2015: stars). No cumulative soil GDDs could be calculated for 2013, due to a lack of soil temperature measurements in early 2013. Cumulative soil GDD corresponds to the GDD at 10 cm soil depth (base temperature: -1°C, starting date: 15th of February) and cumulative surface GDD corresponds to the GDD at 2 cm above the soil surface (base temperature: -3°C, starting date: 15th of February). Lines show the cumulative GDD for the unwarmed plots for 2013 (full line), 2014 (dashed line) and 2015 (dotted line). The color gradient indicates the different soil warming treatments (ambient, +1°C, +3°C, +5°C and +10°C). Error bars show SE's.

Table 2: Results of mixed linear models with cumulative soil or surface GDD as response variable, and year (2013, 2014 and 2015), warming time (short-term "ST" and long-term "LT") and warming treatment (unwarmed, +1, +3, +5 and +10°C soil warming) as fixed factors. No significant interactions occurred. Significant source variables (p < 0.05) are indicated with an asterisk: *p is 0.05–0.01, **p is 0.01–0.001, ***p < 0.001.

	Diff.	Lower	Upper	*Q-value*	*P-value*
Cumulative soil GDD					
Year					
2014 - 2015	173.1	105.8	240.5	1.41	***
Warming time					
ST - LT	-41.4	-108.8	26.0	1.41	0.23
Warming treatment					
+1°C - unwarmed	18.2	-131.1	167.5	0.05	1.00
+3°C – unwarmed	188.5	39.2	337.7	0.52	**
+5°C – unwarmed	361.2	211.9	510.4	1.00	***
+10°C – unwarmed	880.8	731.6	1030.1	2.44	***
Cumulative surface GDD					
Year					
2013 – 2014	0.3	-34.5	35.2	0.01	1.00
2013 – 2015	62.6	27.6	97.4	1.74	***
2014 – 2015	62.1	27.3	97.0	1.73	***
Warming time					
ST - LT	38.6	14.9	62.4	1.41	**
Warming treatment					
+1°C - unwarmed	-20.1	-72.6	32.4	0.39	0.83
+3°C – unwarmed	-62.2	-114.7	-9.7	1.21	*
+5°C – unwarmed	-91.6	-144.1	-39.1	1.79	***
+10°C – unwarmed	-125.6	-178.1	-73.1	2.45	***

3.5 Discussion

3.5.1 Phenological responses of subarctic grassland to warming

LOS extended consistently under warming, with limited interannual variation in the magnitude of the temperature response, confirming that temperature is an important driver of LOS in these subarctic grasslands. This is in line with earlier research on phenological drivers of northern ecosystems (Richardson et al., 2013). Averaged across all warming gradients, for the short-term and the long-term warmed grassland together, LOS extended with 15.5 days per °C, when the effects of soil and surface air temperature on LOS were averaged. This is an approximation, as the extension rate depended strongly on the place where the temperature was measured (in the soil or at the surface; see also § 4.3). Nevertheless, this average rate fits well in the range of what has previously been reported for middle and high latitude ecosystems of the northern hemisphere (on average 12.3 days per °C, ranging from 1.2 to 26.6 days per °C; Zhao et al., 2015).

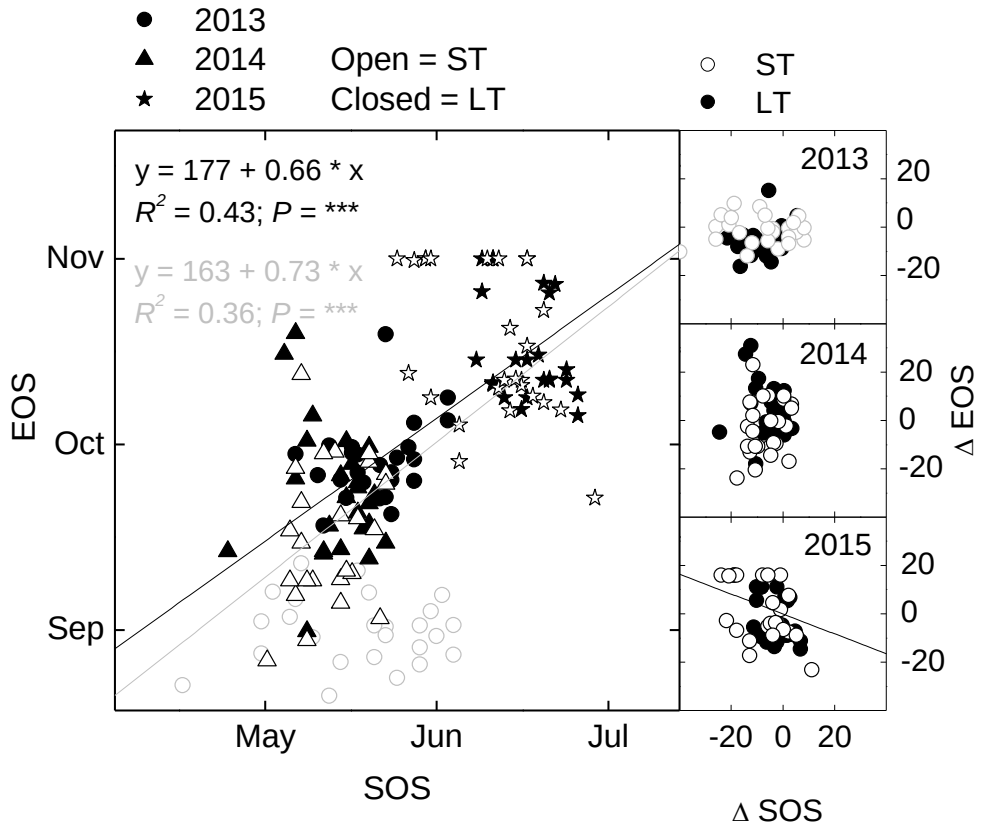


Figure 6: Main panel: Relationship between the end of the season (EOS) and the start of the season (SOS) across all measurement years for the short-term warmed grassland (ST) and the long-term warmed grassland (LT) together (Main panel). The relationship was tested both for all data together, and for the dataset without ST 2013 data (marked in grey), as the EOS in ST 2013 might be caused by a local pest and not by a climatological cue. Side panels: Relationship between changes in the end of the season (ΔEOS) and the start of the season (ΔSOS) per measurement year (2013, 2014 and 2015) for ST and LT. Changes are expressed in relation to the timing of SOS and EOS unwarmed plots from the same grassland and during the same year. Significant relationships are shown with a full line and the equation of the relationship between ΔEOS and ΔSOS in 2015 was y = -0.41 * x with R^2 = 0.12 and *P* = **. Significant source variables (p < 0.05) are indicated with an asterisk: *p is 0.05–0.01, **p is 0.01–0.001, ***p < 0.001.

The extension of LOS was mainly driven by an advance in SOS. EOS was only slightly delayed, as was expected, possibly to avoid a premature halt in the recovery of nutrients by early frost events (Estiarte and Penuelas, 2015). This agreed with what has been found for grasslands in cold climates specifically (Richardson et al., 2013) and for a wide range of northern ecosystems, using ground observations, temperature manipulation experiments and remote sensing techniques (Cleland et al., 2007, Zhao et al., 2015). Interestingly, the strong similarity between the response of ST and LT showed that the response occurred shortly after the onset of the warming and stabilized soon afterwards

(remaining similar for at least 50 years). This is an important finding with respect to the uncertainty of the time-dependence of phenological responses to warming (Kimball et al., 2007).

Importantly, although a saturation of the advance of SOS with warming has recently been found in some northern regions (Zhao et al., 2015), our observations indicate that these subarctic grassland had not yet reached the limit of their responsiveness. Contrary to our expectations, even the highest warming levels showed no clear saturation. We did observe a deceleration of the advance in SOS at the highest warming levels (as indicated by the non-linearity of the temperature response in Fig. 4, middle panel), but this might have been (partly) caused by an increasing decoupling of soil and air temperatures along the soil warming gradient. The smaller air warming compared to soil warming (Fig. 3), which is a typical artefact in soil warming experiments (Patil et al., 2013), might have played a role in constraining the advance in SOS, especially since no strong indications for other constraints were found (§ 4.2). The lack of a clear saturation of the phenological response agrees with an earlier study on alpine grasslands (which avoided this artefact by using a transplant approach), which found a linear advance of SOS over a range of 4°C (Frei et al., 2014). However, it opposes studies on certain broadleaf trees in Europe as well as in North America, which did find a saturation of the SOS response to warming (Morin et al., 2009, 2010). If the advance in SOS with warming of the subarctic would indeed persist, as was observed in this study, this would have far reaching consequences for the C storage potential of this region in a warmer climate, and should be taken into account in further model improvements of ecosystem:climate change feedbacks.

Finally, the delay of EOS with warming, albeit modest, showed that these subarctic grasslands have the potential to extend their growth in autumn, if thermal conditions allow, thereby strengthening the warming-induced extension of LOS. This contrasts to earlier studies, which found a mitigating effect of EOS on the extension of LOS, due to a coupling with SOS, which could be caused by a fixed leaf longevity or a depletion of soil water reserves by early spring greening (Fu et al., 2014a, Keenan and Richardson, 2015). In this study, this coupling appeared to mitigate interannual differences in LOS, but it did not restrict the warming-induced extension of LOS along the warming treatments within the years.

3.5.2 Drivers of the advance in SOS and potential environmental constraints

There are different mechanisms that may have underlain the advance in SOS, and the presence of ST and LT allowed us to speculate about their relative importance. The strong

similarity between ST and LT showed that the response happened in a short timeframe, agreeing with what is found in many common garden experiments and transplant studies (e.g. Byers and Quinn, 1998, Williams et al., 2008, Morin et al., 2010, De Frenne et al., 2011, Frei et al., 2014). This indicates a strong physiological control of the subarctic grasslands' phenological response to warming (i.e. the ability of a given genotype to produce variable phenotypes in different environments; Agrawal, 2001), which can act quickly (Jump and Penuelas, 2005), played a major role in the warming response.

While genetic adaptations and community changes do play a role in phenological responses in certain cases (Høye et al., 2007, Chen et al., 2014), the similarity between ST and LT indicated that it is unlikely that such slower acting mechanisms (Jump and Penuelas, 2005) were active in the warming response in this study. Unfortunately, no data are available to confirm the hypothesis that genetic adaptations were minimal at LT, but vegetation surveys did indeed find little change in community composition of both ST and LT up to warming levels of +5°C, and even at the highest warming level (+10°C), no changes in dominant plant species occurred (Gudmundsdóttir et al., 2014, Michielsen, 2014, Meynzer, In preparation).

Different environmental factors (next to temperature) have been proposed to play a role in the spring phenology of northern ecosystems (i.e. day length, precipitation, snow cover and lack of chilling; Richardson et al., 2013), mainly by delaying SOS or restricting warming-induced advances. These factors could thus potentially have caused the slight saturation of the advance of SOS and the interannual comparisons in this study made it possible to evaluate their importance. Day length constrains SOS in certain high latitude ecosystems as a frost avoidance mechanism (Høye et al., 2007), however, the considerable difference in the timing of the saturation between 2013 and 2014 (beginning of May) versus 2015 (beginning of June) indicated that day length was not the main driver of the saturation of the temperature response in these Icelandic subarctic grasslands. Precipitation can play a role in the SOS of northern and alpine grasslands, although its effect is not consistent, varying between non-existent (Piao et al., 2011), positive (Fu et al., 2014b), negative (Chen et al., 2014, Sha et al., 2016) and dependent on the specific situation (Zhang et al., 2015). In this study, no major variation in soil water status occurred along our temperature gradients (Sigurdsson et al., submitted), especially in early spring, making it unlikely that precipitation has been an important determinant of the saturating advance of SOS. There were, however, overall differences in spring precipitation that affected all treatments equally, as will be discussed in § 4.4.

Further, snow cover delays plant growth until the timing of spring melt (Richardson et al., 2013), but it did not limit the advance in SOS in this study, as no permanent snow cover

occurs at these Icelandic sites due to its relatively mild oceanic winter climate (Sigurdsson et al., submitted). Lastly, chilling requirements for SOS could be compromised by warming (Li et al., 2016). In grasslands, however, chilling requirements are generally believed to be of little importance, as grass leaf phenology is likely opportunistic (Li et al., 2016).

3.5.3 Location of the actual temperature response of SOS

We assume that the temperature response of SOS in these subarctic grasslands was primarily driven by meristem temperatures, in line with what has been found for maize grown in cold temperate climates (Stone et al., 1999). Our speculation is based on the responsiveness of SOS to temperatures at 10 cm soil depth and at 2 cm above the soil surface (where the warming was less extreme, Fig. 3). Firstly, while the advance in SOS with soil warming was in the lower range of previous reports for mid- and high northern latitudes (on average -1.7 days per °C vs. 0.2 to 16 days per °C; Zhao et al., 2015), the advance with surface air warming (on average -22 days per °C) was more extreme than any previous study that we are aware of. This low and high responsiveness, respectively, suggests that the responsive tissues were likely located in between.

Secondly, it is likely that the responsive tissues had a constant cumulative GDD requirement for SOS over the whole temperature gradient (in line with many earlier studies, e.g. Sigurdsson, 2001a, Fu et al., 2014b, Liu et al., 2014, Li et al., 2016), or potentially an increasing cumulative GDD requirement with warming (to avoid precocious SOS). If GDD does indeed remain constant or increased moderately with warming, our data suggests that the temperatures measured in the soil where too high and at the surface too low to calculate the true GDD requirements for the responsive tissues. When using soil temperatures, the cumulative GDD at the SOS increased too drastic to be caused by a saturation of the advance (a tripling over a gradient of 10°C), while when using the surface air temperature, the cumulative GDD at SOS decreased with warming.

Grassland meristems, which are the tissues where the greening starts (Pautler et al., 2013), are indeed located in the layer with intermediate warming (the topsoil and litter layer; Benson et al., 2004), and this was also true for these subarctic grasslands (personal observations). Unfortunately, we could not verify the hypothesis that meristem temperature was indeed the primary driver of the SOS response to warming, as meristem temperature was not measured. We advise to take this into account in further phenological studies, and a better knowledge of meristem physiology could greatly enhance model performances, as knowledge

on physiological mechanisms that drive warming-induced phenolgical responses remains one of the key restraints for further model improvements (Zhao et al., 2013).

3.5.4 Contribution of secondary effects to LOS

The interpretation of warming-induced changes in LOS can be confounded by overruling secondary effects. We were able to detect two such events by comparing data from different years. Firstly, we found that the temperature control on the SOS could be overruled by spring drought in these subarctic grasslands (across all treatments). This was revealed by the higher cumulative GDD requirements during the coldest year (2015) (both for soil and surface GDD), which was contra intuitive, as GDD requirements tend to be lower under colder conditions due to increasing energy use efficiency (Liang and Schwartz, 2014, Liu et al., 2014). Hence, it is likely that the unusually low precipitation during the late spring of 2015 (Fig. 1) delayed the greenup beyond the timing of 'GDD fulfillment', leading to a higher cumulative GDD at the time of SOS. This overruling effect of spring precipitation on the timing of SOS agreed with earlier studies on subarctic grasslands (Chen et al., 2014) and on grasslands in general (Sha et al., 2016).

Secondly, the early EOS in ST compared to LT in 2013, while climatic conditions were very similar for both sites, probably indicated the importance of non-climatic factors. We presume that the discrepancy was caused by a local pest in ST, such as a fungal infection or the mite *Penthaleus major*, that is known to affect Icelandic perennial grasslands in summer (Gudleifsson et al., 2002). In our study, the analyses were, however, robust against the exclusion of ST 2013. To conclude, the SOS in 2015 and the EOS in 2013 in ST may not be warming-driven, highlighting a potential caveat in studies on warming effects on phenology (and other ecosystem processes).

3.5.5 Opportunities of geothermal soil temperature gradients for phenological research

Despite some drawbacks of natural geothermal soil temperature gradients, such as the decoupling of soil- and air warming (§ 4.1 and 4.2), which, nevertheless, allowed us to locate the actual site of the phenological response (§ 4.3), these 'natural experiments' have proven highly valuable to investigate phenological responses to warming. Firstly, the presence of ST and LT, which was caused by the dynamic nature of geothermal systems (O'Gorman et al., 2014), made it possible to observe the temporal dynamics of the warming response, and offered clues on the underlying mechanisms. The similarity between ST and LT showed that the phenological response to warming occurred soon after the onset of the warming and was

maintained for at least 50 years and allowed us to speculate that the response was mainly driven by phenotypic plasticity and not by genetic adaptations or community changes.

Secondly, the gradual increase in soil temperature, typical for geothermal soil temperature gradients, made it possible to detect non-linear trends in the response. The warming-induced advance in SOS showed a slight indication for saturation at higher temperatures, while EOS was fairly unresponsive to soil warming, even at the highest warming levels. Observing long-term effects and non-linear changes is often not possible in climate manipulation experiments, where the warming time and the number of warming treatments is strongly constraint by logistical and financial limitations (De Boeck et al., 2015).

Finally, the typical small spatial scale of geothermal soil temperature gradients allowed to keep most environmental variables largely constant along the warming gradients (Sigurdsson et al., submitted). This allowed us to identify the contribution of secondary (non-warming) effects on LOS (see § 4.4). This limited complexity of environmental factors in our study offered an advantage compared to space for time studies, which often have to deal with a multitude of confounding factors (De Boeck et al., 2015).

3.6 Conclusions

The extension of LOS under warming, and the lack of a clear saturation of the advance of SOS, which was the main driver of LOS changes, showed that these subarctic grasslands were still far from a temperature-saturation in their phenological response. The similarity between ST and LT allowed us to speculate that phenotypic plasticity was the primary driver of the temperature response, and that the contribution of genetic adaptations and changes in community composition was minor. Also other environmental factors were found to be of little importance. Further, we hypothesize that meristem temperature was the major determinant of changes in SOS and we urge for a better understanding of meristem physiology to improve projections of feedbacks from ecosystem phenology to the climate system. In addition, phenology studies should be careful not to misinterpret confounding factors (such as drought spells or pest outbreaks) as phenological responses to warming.

This study indicated that the warming-induced extension of LOS of subarctic grasslands could still continue if warming persists (unless genetic adaptations or species shifts occur that would change that pattern). The lack of a clear saturation to warming in our study has important implications for the C uptake potential of these subarctic grasslands under

future climate change. This is a powerful ecosystem feedback to the climate system and should be studied in more detail.

Supplementary information

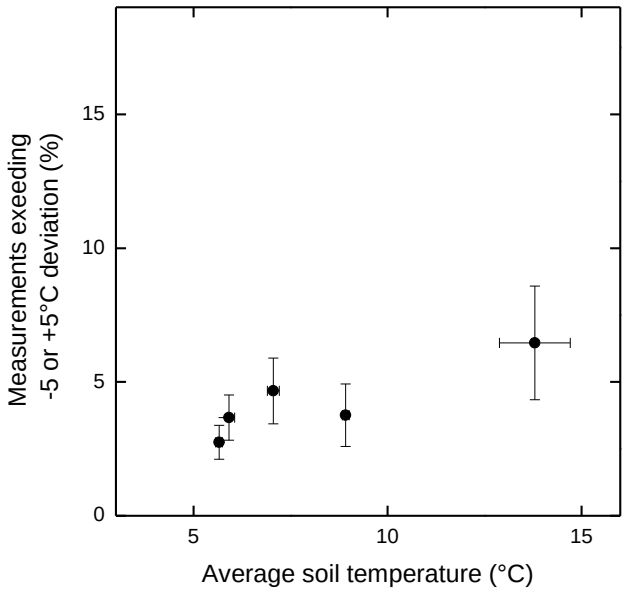


Figure S.1: Proportion of surface temperature measurements (at 2 cm above the soil surface) that exeeded a deviation of -5 or +5°C compared to the 2 m air temperature versus average soil temperature for the period of the the 15th of February to the 30th of June 2015.

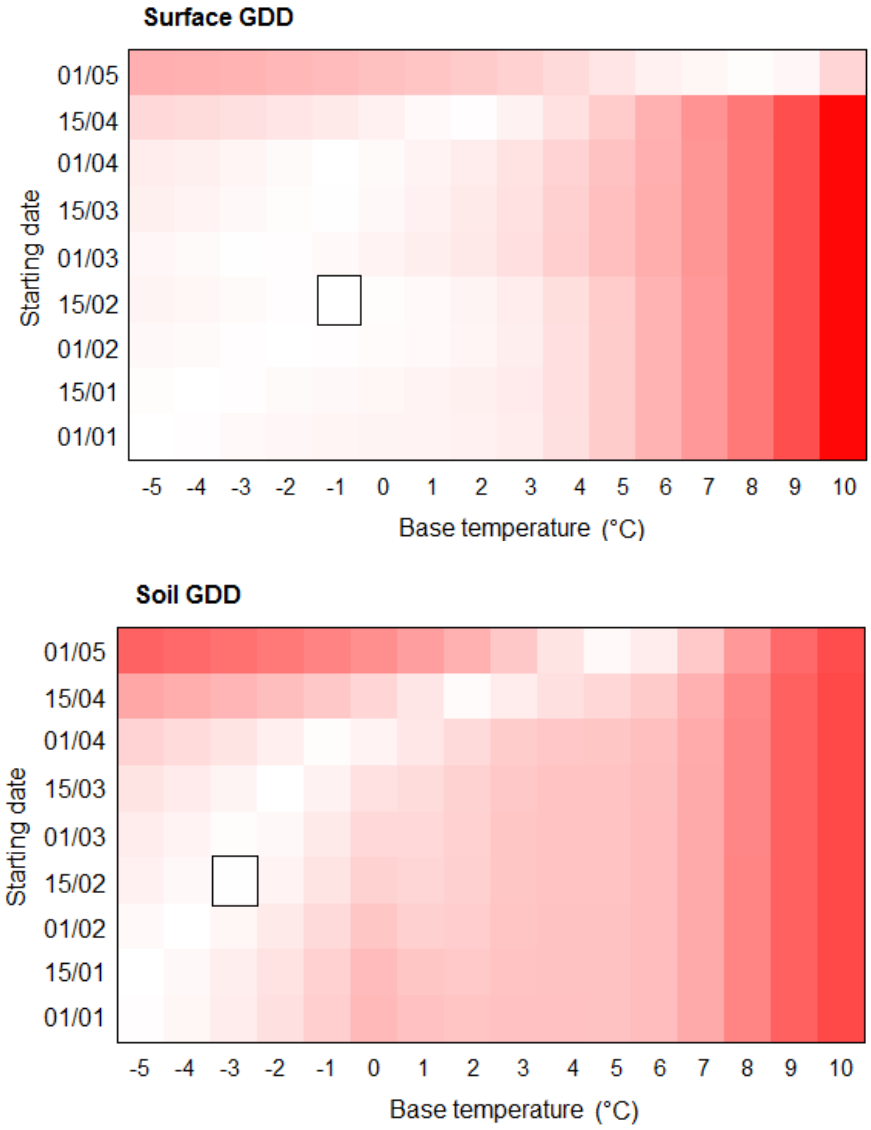
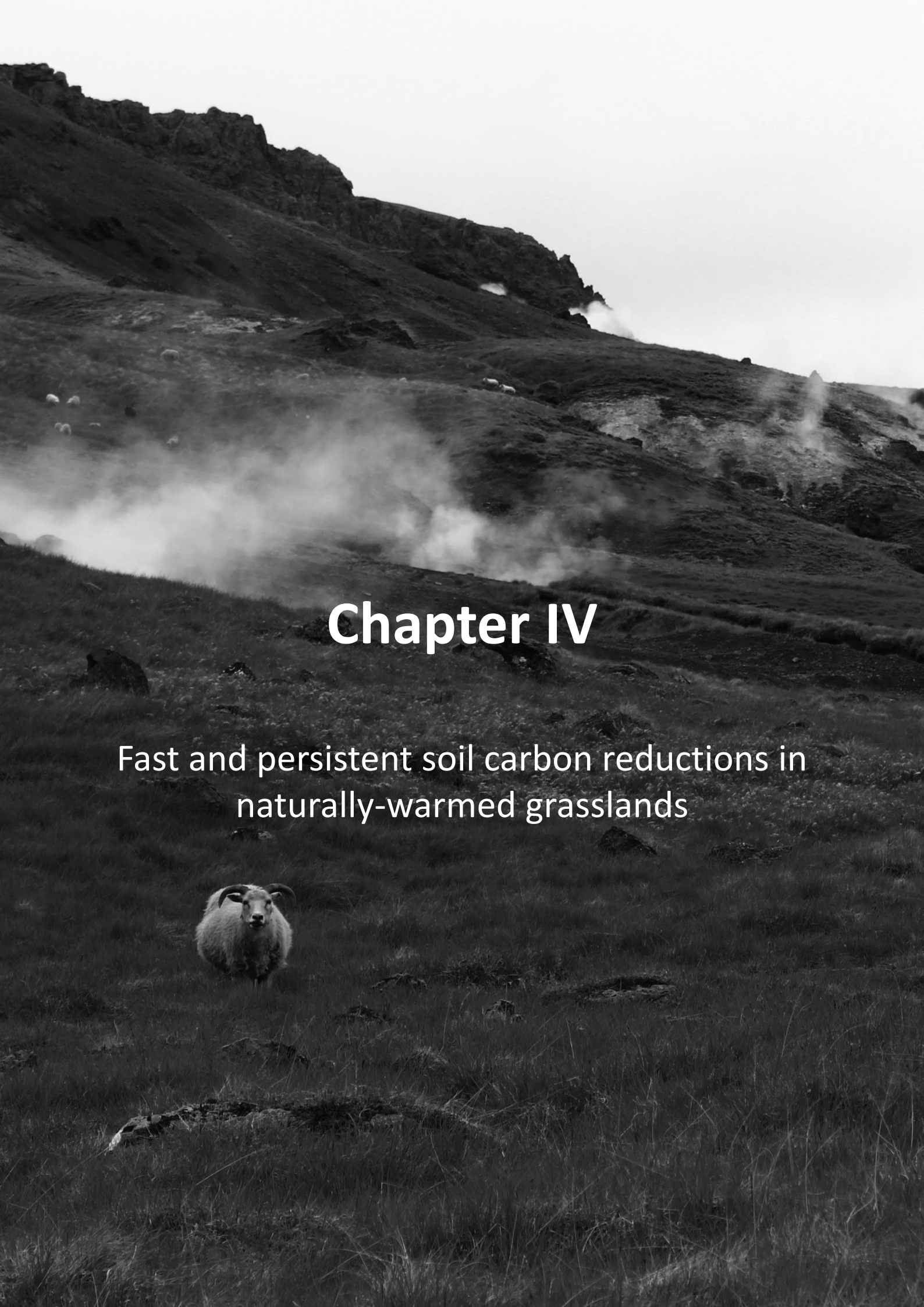


Figure S.2: Analysis of the optimal base temperature and starting date for calculations of surface GDD (upper panel) and soil GDD (lower panel). The colour scale shows the average discrepancy for all unwarmed plots between the actual cumulative GDDs and the predicted cumulative GDD, with lighter colours indicating lower discrepancies. The predicted cumulative GDD was based on the actual cumulative surface GDDs of all other unwarmed plots. All combination of 16 base temperatures (ranging -5 to +10 °C, with an increment of 1 °C) and 9 starting dates (from the 1st of January to the 9th of May) were tested.



Chapter IV

Fast and persistent soil carbon reductions in naturally-warmed grasslands

4 Fast and persistent soil carbon reductions in naturally-warmed grasslands

Leblans, N. I. W., Sigurdsson, B. D., Vicca, S., Soong, J. L., Weedon, J. T., Poeplau, C. , Van de Velde K., Verbruggen, E., Wallander, H., Liu, Z. F., Marañón-Jiménez, S., Gundersen, P., Maljanen M., Guenet, B., Dauwe, S., Kätterer, T., Oddsdóttir, E. S., Ostonen, I., Penuelas, J., Richter, A., Van Bodegom, P. M., Walker, T. N. and Janssens, I. A.

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4.1 Summary

Global warming could elicit transfers of carbon from soils to the atmosphere, which would exacerbate the warming. There is a scarcity of empirical evidence for long-term warming impacts on carbon transfers from soils to the atmosphere, contributing to high uncertainty in future climate projections. Here, we provide empirical evidence for large (4.1 % $°C^{-1}$) soil organic carbon (SOC) losses upon both short-term (5 years) and long-term (≥ 50 years) geothermal warming of northern grassland soils, over a soil temperature gradient that encompasses the current IPCC projections. Central to this observed SOC loss was a decline in the physical stabilization of SOC in soil aggregates. We therefore urge inclusion of soil physical structure in Earth System Models. Importantly, similar SOC losses after 5 and ≥ 50 years of soil warming indicate that SOC losses did not continue after five years of warming, suggesting a rapid positive feedback to climate warming by warming of northern soils.

4.2 Introduction

Soils store three times more carbon (C) than the atmosphere (Jobbagy and Jackson, 2000), with a disproportionally high contribution from northern high latitude ecosystems, where decomposition is strongly constrained by cold and wet conditions (McGuire et al., 2009). Global warming could induce major shifts in soil organic C (SOC) storage, because biomass production typically increases less with temperature than decomposition (Kirschbaum, 1995, Jones and Donnelly, 2004) , thereby constituting a powerful positive feedback to the climate system (Woodwell, 1983, Jenkinson et al., 1991). However, there is little empirical evidence to constrain modeled temperature-induced SOC losses, yielding high uncertainty in projections of both future SOC stocks and the magnitude of the feedback (Friedlingstein et al., 2006, Kirschbaum, 2006, Todd-Brown et al., 2013).

Many warming experiments have been conducted to study the impact of warming on ecosystem SOC stocks (Dieleman et al., 2012, Lu et al., 2013). Most *in situ* warming studies have been of short duration (Luo et al., 2011), although exceptions do exist (Luo et al., 2011, Leppalammi-Kujansuu et al., 2014, DeAngelis et al., 2015), and therefore lack structural adaptations to warming that are typically slow, such as changes in vegetation composition and in soil structure (Beier et al., 2012). Hence, uncertainty remains as to the long-term persistence of the responses observed in short-term studies. Furthermore, practical and financial constraints usually allow only a small number of warming levels, making it challenging to observe non-linear responses of SOC to warming. Finally, in most studies, the applied warming falls below the range of warming projected by the IPCC climate models (IPCC, 2013). This is especially true in northern regions, where climate warming is predicted to far exceed the target mean global warming threshold of 2°C.

Geothermally active areas offer long-lasting, continuous and large soil temperature gradients, and provide a solution to many of the practical difficulties associated with manipulative warming experiments (O'Gorman et al., 2014). The use of geothermally warmed sites as a proxy for climate change has some drawbacks (De Boeck et al., 2015), but nonetheless offers a unique and robust opportunity to study the direct response of SOC dynamics to temperature change. In this study, geothermally warmed subarctic grasslands were used as model ecosystems to study the magnitude, shape and mechanism of the temperature response of SOC storage in northern soils, taking advantage of natural gradients in soil warming ranging from normal soil temperature for Iceland to +20°C. Critical insights into the temporal dynamics of the warming response were provided by including thermal

gradients of contrasting age, one long-term warmed for ≥50 years (but probably for centuries (see Methods)) and one short-term warmed for five years (see Methods and Table 1).

4.3 Results and discussion

4.3.1 Large, fast and permanent decline in SOC

Across five replicated soil temperature gradients, we observed a linear loss of 1.28 ± 0.16 (± SE) t SOC ha^{-1} per degree of warming from the topsoil (upper 10 cm) over the entire soil temperature gradient in the short-term warmed grassland ($P < 0.001$; Fig. 1; Extended data Fig. 1 and 2). These losses, which were already statistically significant at 3°C warming, were equivalent to 4.1 ± 0.5 % $°C^{-1}$ of the SOC stock under ambient soil temperature, and are within reported ranges for short-term warming experiments in a recent meta-analysis (Lu et al., 2013). Strikingly, the SOC loss in the short-term warmed grassland was not different from the warming-induced SOC loss observed in the topsoil of five replicated temperature gradients in the adjacent valley with long-term warming (Fig. 1), indicating that SOC stock responses to warming occur within years and equilibrate quickly to a new steady state.

Warming-induced SOC losses from the subsoil (10-30 cm depth) of the long-term warmed grassland (the short-term warmed grassland soil was too shallow to study subsoil changes) were smaller ($P = 0.07$) than those in the topsoil: 0.81 ± 0.38 t SOC ha^{-1} $°C^{-1}$ ($P = 0.04$; equivalent to 2.6 ± 1.2 % of the SOC stock measured under ambient soil temperatures) (Fig. 1). This lower sensitivity compared to the topsoil could be due to a lower relative increase in decomposition, following: (i) increasing energy limitation of the microbial community with depth caused by a lower input of fresh root litter; and/or (ii) greater physical and chemical protection of SOC in the subsoil (Fontaine et al., 2007, Rumpel and Kogel-Knabner, 2011). This highlights the necessity of treating topsoil and subsoil C-dynamics separately (Rumpel and Kogel-Knabner, 2011).

4.3.2 Soil aggregate loss as a key mechanism

The decline in SOC in response to warming was associated with significant compaction in the topsoil (increasing bulk density; Fig. 2), and this compaction was consistent with loss of soil aggregation (Kozlowski, 1999). Reduced soil aggregation in response to warming is well known (Harris et al., 1966) and has been previously observed in grassland ecosystems (Wang et al., 2015). The associated reduction of the physical protection of SOC (Six et al., 2002) typically accelerates its mineralization (Rillig et al., 2015, Wang et al., 2015). However, the importance of this process in warming-induced SOC losses is not well studied (Conant et al.,

2011). In our study, the reduction of SOC occurred predominantly in the sand- and aggregate fractions of soil organic matter (Fig. 3). Given that sand particles are not capable of binding much organic C, reduced soil aggregation was primarily responsible for the observed SOC loss from this fraction, as well as from the total soil.

Several mechanisms potentially contribute to the decline in soil aggregation and subsequent SOC loss in response to warming; aggregate formation, stabilization and longevity can all be affected (Fig. 4). Aggregate formation depends on belowground C inputs (Six et al., 2004), and in our case both fungal and plant-derived C inputs decreased with temperature. We observed a strong linear decrease in fungal biomass (Extended data Fig. 3 and S.4), and fine root biomass declined by 5-6 % per °C (Extended data Fig. 5.b). It is unlikely that this decline in root biomass was compensated for by increased root inputs from accelerated root turnover, as the observed decline exceeds the Q_{10} of fine root turnover in grasslands by almost 200% (Gill and Jackson, 2000). Furthermore, aboveground biomass did not change significantly with temperature (Extended data Fig. 5.a). Aggregate stability and longevity generally decrease in response to warming (Harris et al., 1966) (Fig. 4).

In soils, aggregates can be stabilized by fine roots, filamentous saprotrophic fungi[21] and arbuscular mycorrhizal fungi[25]. Our study revealed strong declines in all three groups (Figs. S.3, S.4 and S.5.b). Combined with the reduced production of aggregates by the lower soil carbon inputs and the direct negative effect of temperature on aggregate longevity[18], this loss of aggregate-stabilizing agents is likely to explain why soil carbon was predominantly lost from the soil aggregate fraction.

The SOC stocks at higher soil temperatures were not only more available for decomposition due to the reduced physical protection in soil aggregates, they were also subjected to the typical direct temperature-driven stimulation of decomposition rates (Davidson and Janssens, 2006b). This was revealed by an *in situ* standardized teabag decomposition experiment (Extended data Fig. 6) and a laboratory soil incubation study (Marañón-Jiménez, unpublished results), which together showed that increasing temperatures accelerated the decomposition rate of both new incoming litter and of resident SOC. Accelerated decomposition, in combination with lower C inputs and lower stabilization potential at higher temperatures (Fig. 4), resulted in strongly reduced SOC stocks.

4.3.3 Projections and modelling implications

Earth system models (ESMs) participating in a recent comparison study (the CMIP5 exercise; Taylor et al., 2012) predicted on average an increase of SOC stocks in cold ecosystems by the

end of the 21st century, even for the highest temperature increase scenario (RCP 8.5 scenario), albeit with large variation in the magnitude and even the direction of the response (26±32 Pg for tundra; Todd-Brown et al., 2013). The large uncertainties in the model projections underline the need for empirical observations from cold biomes to better understand the response of SOC to temperature increase and thus better constrain the ESMs (Nishina et al., 2014). Our data, in contrast to most ESMs, suggest rapid and substantial SOC losses at warming levels that are expected by the end of this century. Following the projected temperature change for the subarctic (+2.2°C and +8.3°C warming for RCP 2.6 and 8.5, respectively), our results indicate that subarctic topsoil SOC stocks could decrease by 9.0 to 34.0%, assuming that our observations of temperature responses can be generalized.

There are several potential explanations for this difference between the ESMs and our observations. For one, we provide observations from only one region and one specific soil type, and it is possible that our results cannot be extrapolated to other soil and vegetation types. A remarkable contrast with most ESM predictions is the absence of temperature-stimulation of aboveground biomass production at our study site (Extdended data Fig. 5.a). This unexpected response might be caused by the observed lack of N retention following the warming-induced increased mineralization rate of soil organic matter: SON losses were exactly proportional to SOC losses, i.e. no change was observed in the soil C/N ratio (Extended data Fig. 7). This dramatic N loss from the system, both through gaseous emissions (Extended data Fig. 8a) and leaching (Extended data Fig. 8b), could be caused by the temperature-induced collapse of the fungal community (Extended data Fig. 3 and 4), which is typically the dominant microbial group in northern soils (Sistla et al., 2013), and whose decline has been linked to lower retention of labile nutrients (Gordon et al., 2008). In addition, the lack of aboveground productivity response to warming may also partly result from the absence of canopy warming in such soil warming gradients, although soil warming did result in higher aboveground productivity in two long-term warming experiments in northern forests (Bergh and Linder, 1999, Melillo et al., 2011).

However, the difference between our observations and the ESM projections could be of greater importance, as we detected a dominant role for a warming-induced SOC-destabilizing mechanism (aggregate breakdown) that is currently not simulated in the ESMs. The absence of this mechanism in ESMs (Todd-Brown et al., 2013) makes them unable to reproduce observations explained by modifications of soil structure. Including soil aggregation in ESMs (Todd-Brown et al., 2013) could bring model projections closer to our field observations. However, soil aggregation is a complex phenomenon occurring at very

fine spatiotemporal scales that are incompatible with the current ESM scales. Thus, before this phenomenon can be included in ESMs, empirical studies need to define overarching relationships between aggregate dynamics and relevant driving factors (e.g. temperature, precipitation and soil carbon inputs).

4.4 Conclusions

Long-term C losses from the soil to the atmosphere are a key uncertainty in climate projections (Friedlingstein et al., 2006, Kirschbaum, 2006, Todd-Brown et al., 2013). This study provides the first empirical evidence allowing comparison of short-term and long-term SOC responses to warming in subarctic grasslands, encompassing the full gradient of IPCC warming scenarios and revealing the dominant processes contributing to eventual future warming-induced SOC losses. Because the majority of the SOC loss in our study occurred in the stable aggregate fraction, we conclude that soil aggregates play a central role in the temperature response of SOC stocks, responding directly and indirectly to increasing temperature and thereby decreasing the physical stabilization of SOC in stable aggregates. The associated reduction of SOC-protection in soil aggregates, combined with the direct temperature-driven increase in decomposition rate, stimulated SOC loss and consequently reduced SOC stocks. Moreover, our two warming gradients of different age revealed that SOC losses were surprisingly rapid and that no further losses (or recovery) occurred upon longer-term exposure to higher warming. This implies that, at least in these subarctic grasslands on volcanic soils, SOC stocks equilibrate within a few years to warming temperatures, causing a rapid positive feedback to climate warming.

4.5 Tables

Table 1 | Environmental characteristics and site properties. Environmental characteristics and site properties of the short-term warmed grassland (left) and the long-term warmed grassland (right). Soil properties are given for the upper 10 cm of unwarmed soils (n = 5). Errors (±) refer to SEs.

	Short-term warmed grassland	Long-term warmed grassland
Coordinates	64°00′01″ N; 21°11′09″ W	
Altitude	100 - 225 m a.s.l.	
Mean annual air temperature (°C)[a]	5.7	
Mean annual soil temperature (°C)[b]	5.8	5.5
Mean annual precipitation (mm)	1431[c]	
Nitrogen deposition (kg N ha^{-1} yr^{-1})	1.3-1.4(Leblans et al., 2014)	
Vegetation type	Unmanaged grassland	
Soil type	Brown Andosol(Arnalds, 2015)	
Soil pH	5.6 ± 0.1	5.4 ± 0.1
SOC (%)	6.3 ± 0.4	7.5 ± 1.1
SON (%)	0.52 ± 0.03	0.61 ± 0.12
Clay (%)	6.2 ± 0.7	8.3 ± 0.5
Silt (%)	52.8 ± 2.4	63.0 ± 2.1
Sand (%)	41.0 ± 2.9	28.7 ± 2.5

SOC is soil organic carbon
SON is soil organic nitrogen
[a]Measured hourly at 2 m height between May 2013 and May 2015.
[b]Measured hourly at 10 cm soil depth between May 2013 and May 2015
[c] Icelandic meteorological office

4.6 Figures

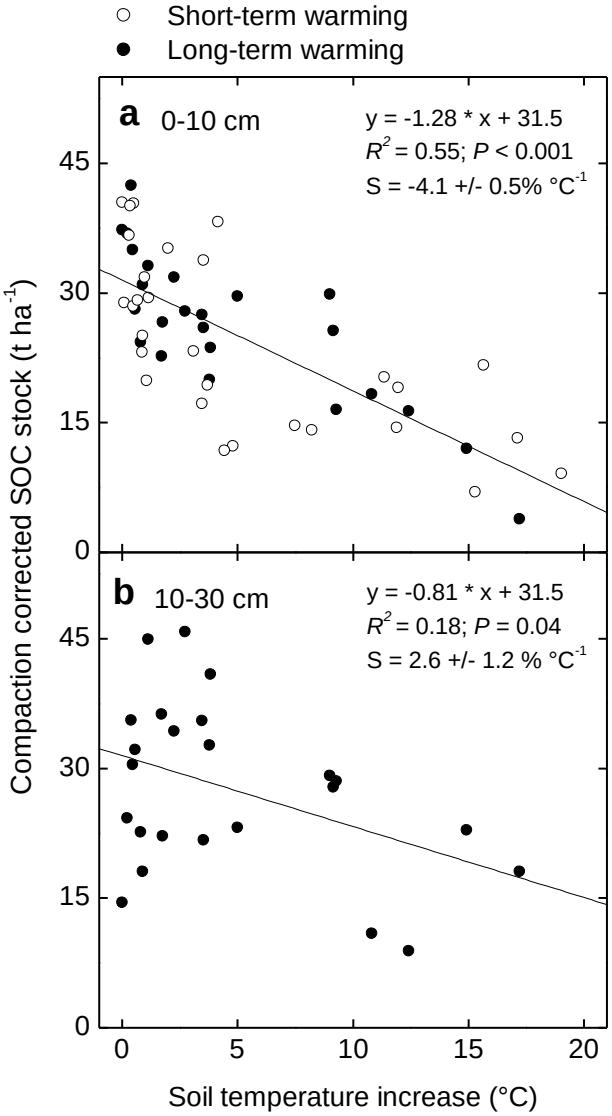


Figure 1 | Reduction of soil organic carbon (SOC) stock with increasing soil temperature, corrected for soil compaction, for (a) Topsoil (0-10 cm) and (b) subsoil (10-30 cm). Soil temperature increase is expressed relative to ambient soil temperature (both at 10cm depth), averaged from May 2013 to May 2015. For the subsoil (b), only the long-term warmed grassland is shown (the soil of the short-term warmed grassland was too shallow). The compaction correction is visualized in Extended data Fig. 2. The uncorrected SOC stocks yielded qualitatively similar conclusions (Fig. S1). Because the warming-duration × temperature interaction was not statistically significant (*P* = 0.50), the short- and long term warmed grasslands were combined in one statistical model (applies to (a) only). The sensitivity (S) was calculated as the linear % loss of SOC stock per °C as % of the ambient stock. Uncertainty levels (±) refer to SEs.

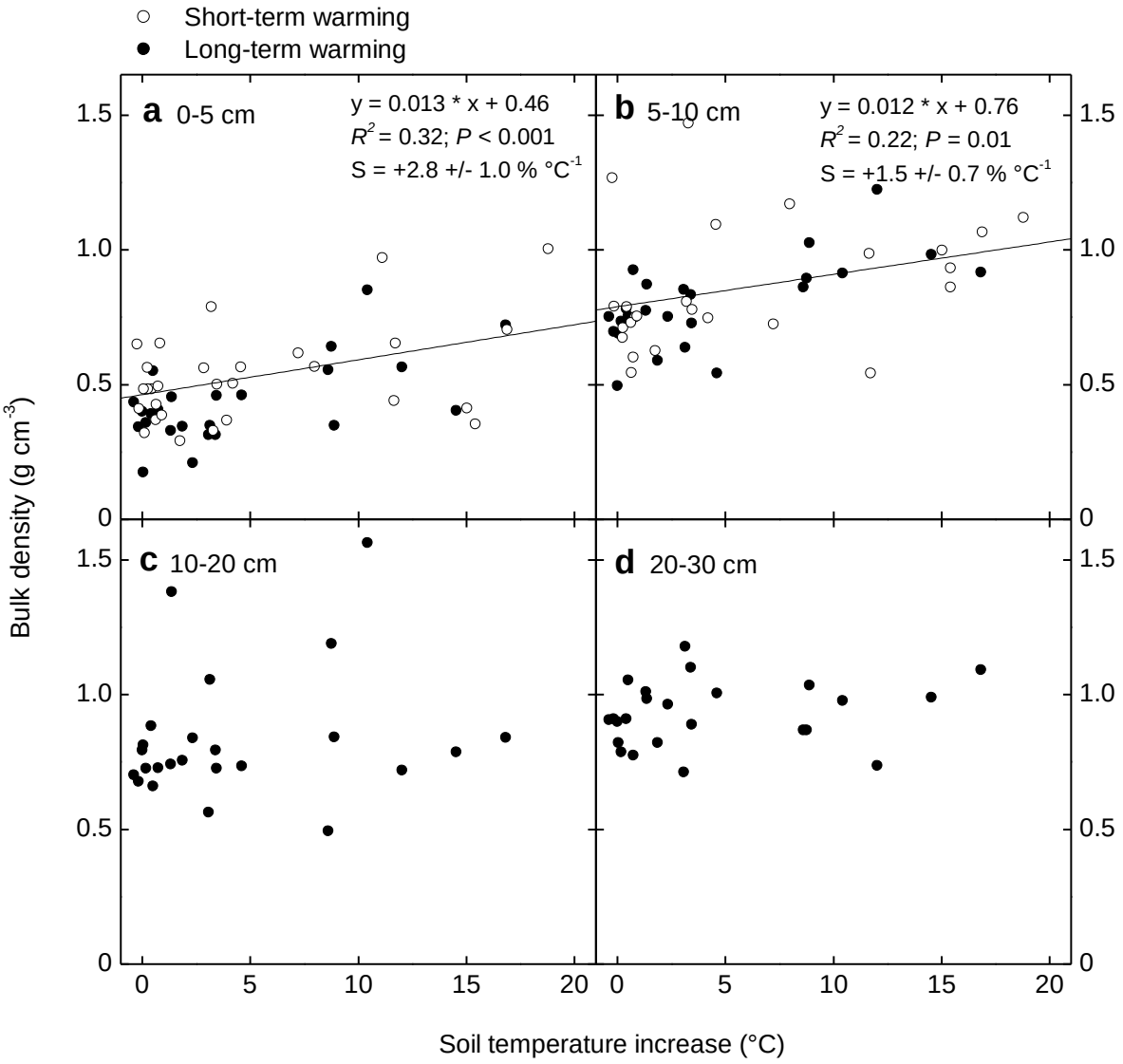


Figure 2 | Changes in bulk density with increasing soil temperature. Soil temperature increase is expressed relative to ambient soil temperature (at 10cm depth), averaged from May 2013 to May 2015. Bulk density is separated for (a) 0-5, (b) 5-10, (c) 10-20 and (d) 20-30 cm depth. Because the warming-duration × temperature interaction was not statistically significant, the short- and long term warmed grasslands were combined in one statistical model for (a) and (b) (*P* = 0.47 and 0.37, respectively). The sensitivity (S) was calculated as the linear % increase in bulk density per °C as % of the ambient bulk density. Uncertainty levels (±) refer to SEs. Relationships were considered statistically significant at *P* < 0.05.

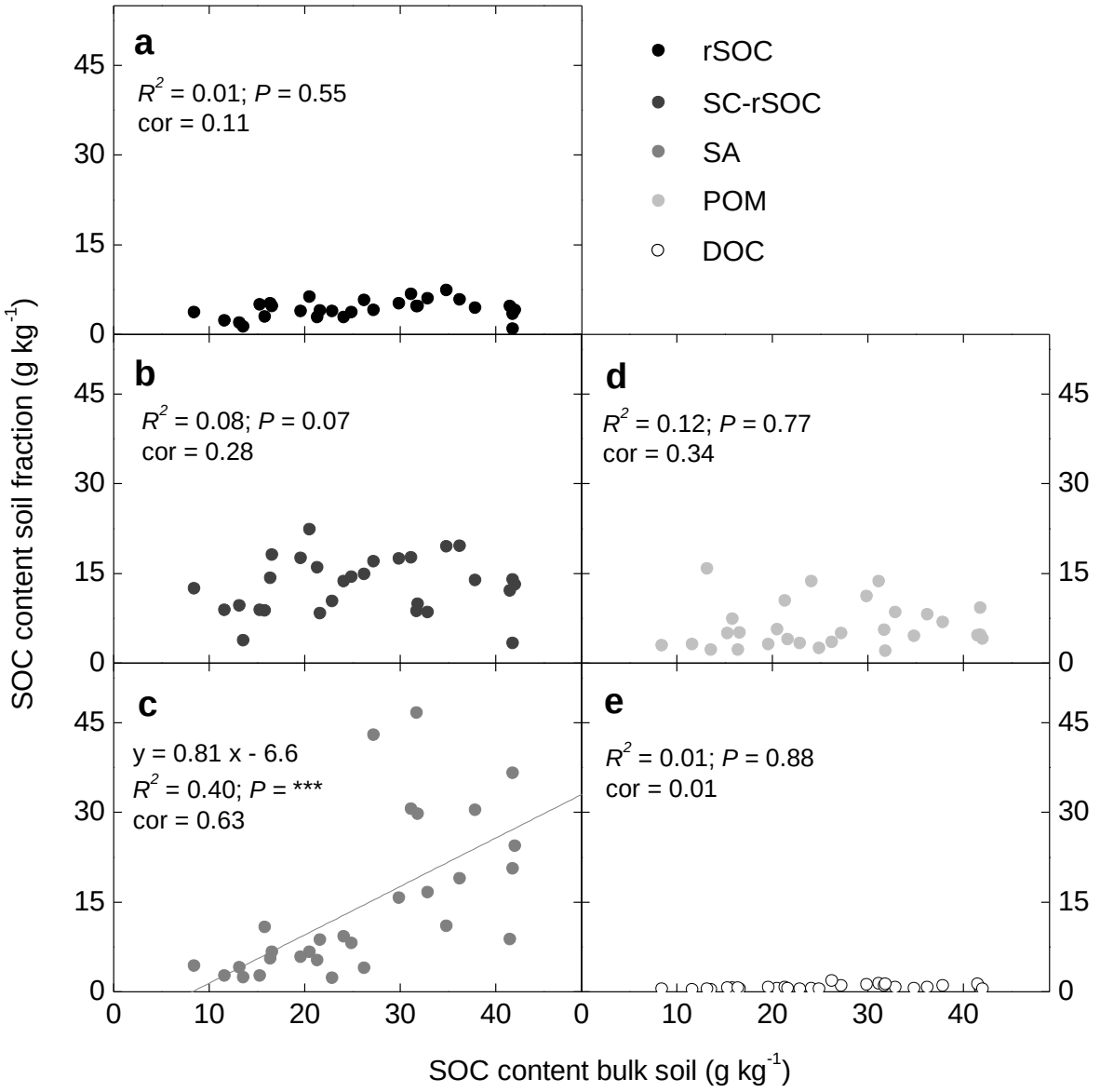


Figure 3 | Relationship between carbon in bulk soil and in different soil size fractions. Soil organic carbon (SOC) content in different soil fractions versus the SOC stock in the bulk soil in the different plots of the short-term warmed grassland. Warmed soils lost most SOC and are thus situated on the left side of each panel. (a) rSOC is NaOCl-resistant SOC. (b) SC-rSOC is the silt and clay fraction. (c) SA is the sand and stable aggregates fraction. (d) POM is particulate organic matter. (e) DOM is dissolved organic matter. Relationships were considered statistically significant at $P < 0.05$.

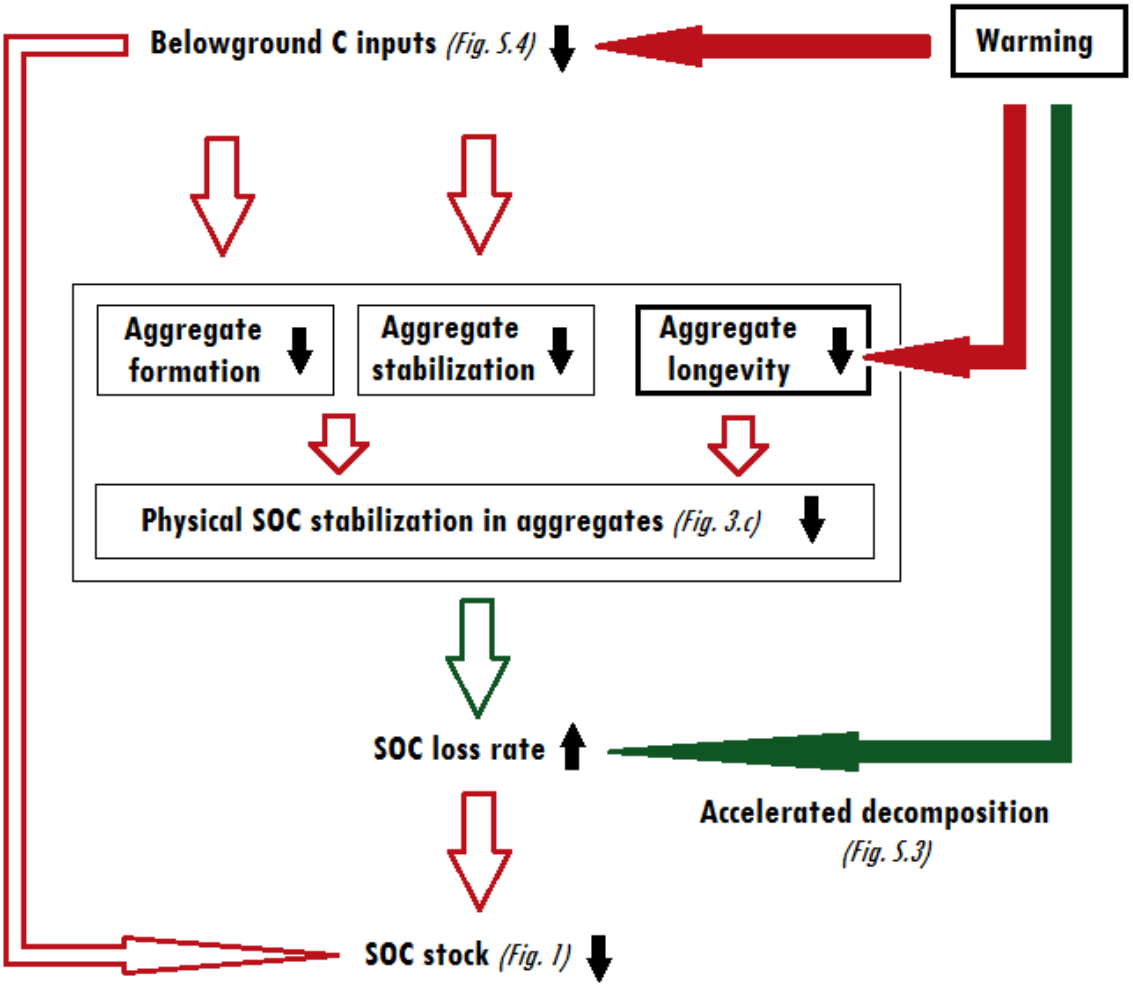
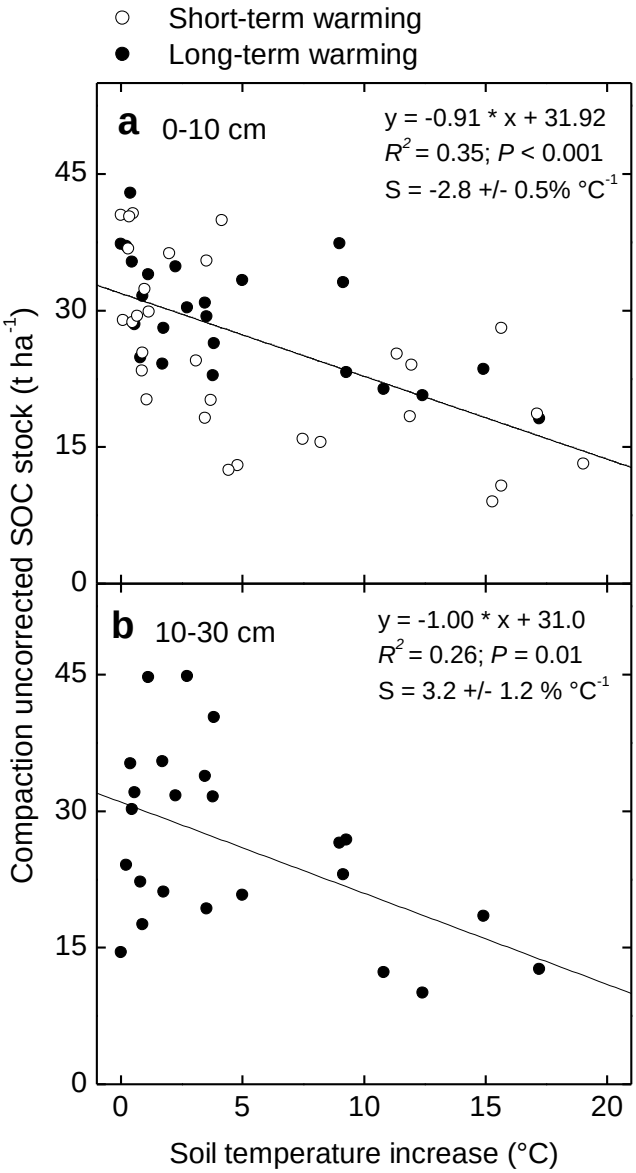
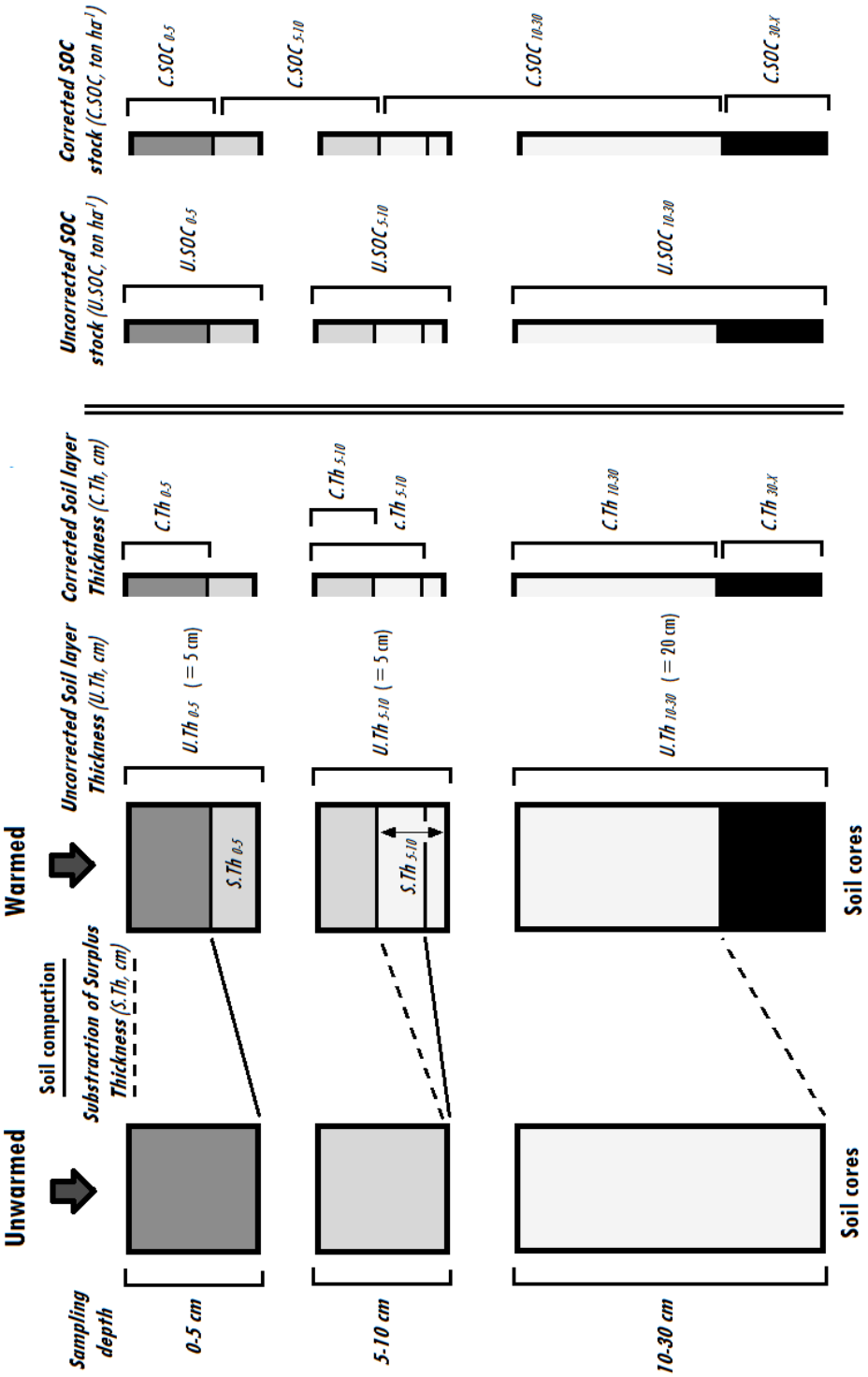


Figure 4 | Schematic representation of the soil organic C (SOC) loss mechanisms as identified in this study. Black arrows show positive (upward) and negative (downward) responses. Green and red arrows show positive and negative temperature effects respectively. Filled colored arrows show direct temperature effects and open colored arrows show indirect temperature effects. Figures referred to in the schematic can be found in the main text and in the supplementary material of the article.

4.7 Extended data



Extended data Figure 1 | Reduction of soil organic carbon (SOC) stock with increasing soil temperature, uncorrected for soil compaction, for (a) Topsoil (0-10 cm) and (b) subsoil (10-30 cm). Soil temperature increase is expressed relative to ambient soil temperature (both at 10cm depth), averaged from May 2013 to May 2015. For the subsoil (b), only the long-term warmed grassland is shown (the soil of the short-term warmed grassland was too shallow). The compaction correction is visualized in Extended data Fig. 2. Because the warming-duration × temperature interaction was not statistically significant (P = 0.56), the short- and long term warmed grasslands were combined in one statistical model (applies to (a) only). The sensitivity (S) was calculated as the linear loss of SOC stock per °C as % of the ambient stock. Uncertainty levels (±) refer to SEs.



Extended data Figure 2 | Corrections for soil compaction.

Due to significant soil compaction (increasing bulk density (BD)) with increasing soil temperature in the upper soil layers (Fig. 2), a certain soil depth in unwarmed soil corresponded to ever shallower soil depths at warmer soil temperatures. Therefore, the SOC and SON stocks were corrected for soil compaction.

Corrections 0-5 cm soil layer

First, the corrected soil layer thickness of the 0-5 cm layer ($C.Th_{0\text{-}5}$) was calculated for the warmed soils:

$$C.Th_{0-5} = U.Th_{0-5} * \frac{U.BD_{0-5}}{C.BD_{0-5}} \quad \text{(Eqn. S.1)}$$

Where $U.Th_{0\text{-}5}$ is the uncorrected soil layer thickness of the 0-5 cm layer (5 cm), $U.BD_{0\text{-}5}$ is the uncorrected BD of the 0-5 cm layer (which corresponds to the BD at ambient soil temperature) and $C.BD_{0\text{-}5}$ is the corrected BD for the 0-5 cm layer for a certain soil temperature increase. $C.BD_{0\text{-}5}$ was calculated using the linear relationship between BD and temperature (Fig. 2).

Then, the corrected SOC stocks of the 0-5 depth layer ($C.SOC_{0\text{-}5}$) were calculated:

$$C.SOC_{0-5} = U.SOC_{0-5} * \frac{C.Th_{0-5}}{U.Th_{0-5}} \quad \text{(Eqn. S.2)}$$

Where $U.SOC_{0\text{-}5}$ is the uncorrected SOC stock in the 0-5 cm depth layer, calculated with Eqn. 2 (see Materials and Methods) and the second term corresponds to the proportional thickness of the corrected layer compared to the uncorrected layer.

Corrections 5-10 cm soil layer

First, the thickness of the surplus soil layer from the 0-5 cm layer ($S.Th_{0\text{-}5}$) was calculated:

$$S.Th_{0-5} = (U.Th_{0-5} - C.Th_{0-5}) * \frac{U.BD_{0-5}}{C.BD_{0-5}} \quad \text{(Eqn. S.3)}$$

The second term is a correction factor for the soil compaction of the surplus soil layer.

Then, the corrected thickness of the 5-10 soil layer, not yet taking the surplus soil sampled from the 0-5 cm layer (= S.Th0-5) into account, ($c.T_{5\text{-}10}$) was calculated:

$$c.Th_{5-10} = U.Th_{5-10} * \frac{U.BD_{5-10}}{C.BD_{5-10}} \quad \text{(Eqn. S.4)}$$

Where $U.Th_{5\text{-}10}$ is the uncorrected soil layer thickness of the 5-10 cm layer (5 cm), $U.BD_{5\text{-}10}$ is the uncorrected BD of the 5-10 cm layer (which corresponds to the BD at ambient soil temperature) and $C.BD_{5\text{-}10}$ is the corrected BD for the 5-10 cm layer for a certain soil temperature increase. $C.BD_{5\text{-}10}$ was calculated using the linear relationship between BD and temperature (Fig. 2).

Subsequently, we took into account the thickness of the surplus soil sampled from the 0-5 cm layer to calculate the final corrected soil thickness of the 5-10 cm soil layer ($C.Th_{5\text{-}10}$). Hence, $C.T_{5\text{-}10}$ is the part of the 5-10 cm layer that remains after (i) correcting for soil compaction and (ii) subtracting the thickness of the surplus soil sampled at the 0-5 cm layer:

$$C.Th_{5-10} = c.T_{5-10} - S.Th_{0-5} \quad \text{(Eqn. S.5)}$$

Subsequently, the corrected SOC stock for the 5-10 cm layer ($C.SOC_{5\text{-}10}$) was calculated:

$$C.SOC_{5-10} = (U.SOC_{0-5} - C.SOC_{0-5}) + \left(U.SOC_{5-10} * \frac{C.Th_{5-10}}{U.Th_{5-10}}\right) \quad \text{(Eqn. S.6)}$$

Where $U.SOC_{5\text{-}10}$ is the uncorrected SOC stock in the 5-10 cm depth layer, calculated with Eqn. 2 (see Materials and Methods) and the last term corresponds to the proportional thickness of the corrected layer compared to the uncorrected layer.

Corrections 10-30 cm layer (only applicable to the long-term warmed grassland)

Corrections for the 10-30 cm soil layer are analogous to the 5-10 cm soil layer. First, the thickness of the surplus soil layer that from the 5-10 cm layer ($S.T_{5\text{-}10}$) was calculated:

$$S.Th_{5-10} = (U.Th_{5-10} - C.Th_{5-10}) * \frac{U.BD_{5-10}}{C.BD_{5-10}} \quad \text{(Eqn. S.7)}$$

The second term is a correction factor for the soil compaction of the surplus soil layer.

Then, the corrected thickness of the 10-30 soil layer ($C.T_{10\text{-}30}$) was calculated:

$$C.Th_{10-30} = U.Th_{10-30} - S.Th_{10-30} \quad \text{(Eqn. S.8)}$$

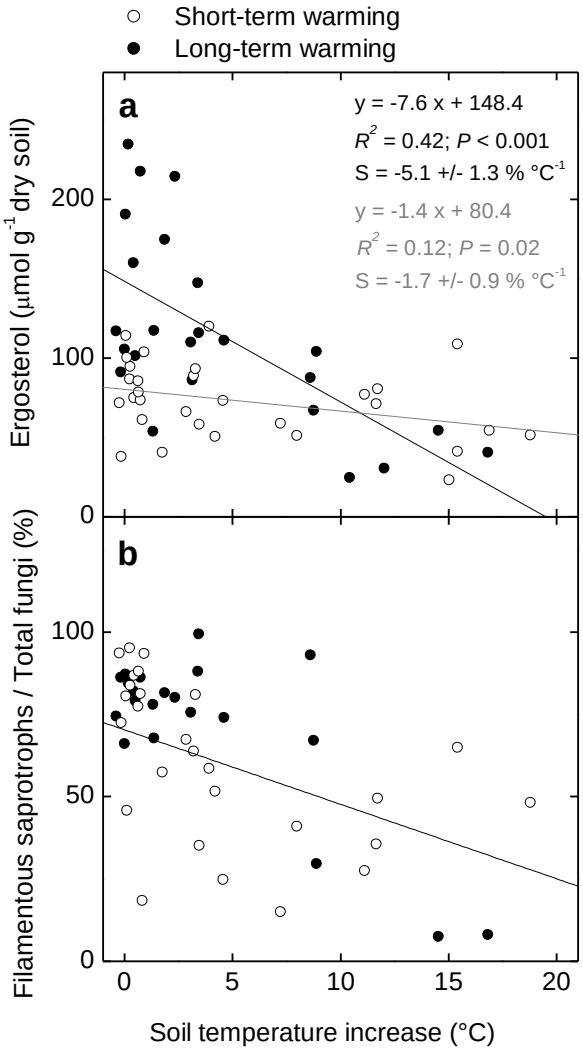
Where $U.Th_{10\text{-}30}$ is the uncorrected soil layer thickness of the 10-30 cm layer (20 cm).

Finally, the corrected SOC stock for the 10-30 cm layer ($C.SOC_{10\text{-}30}$) could be calculated:

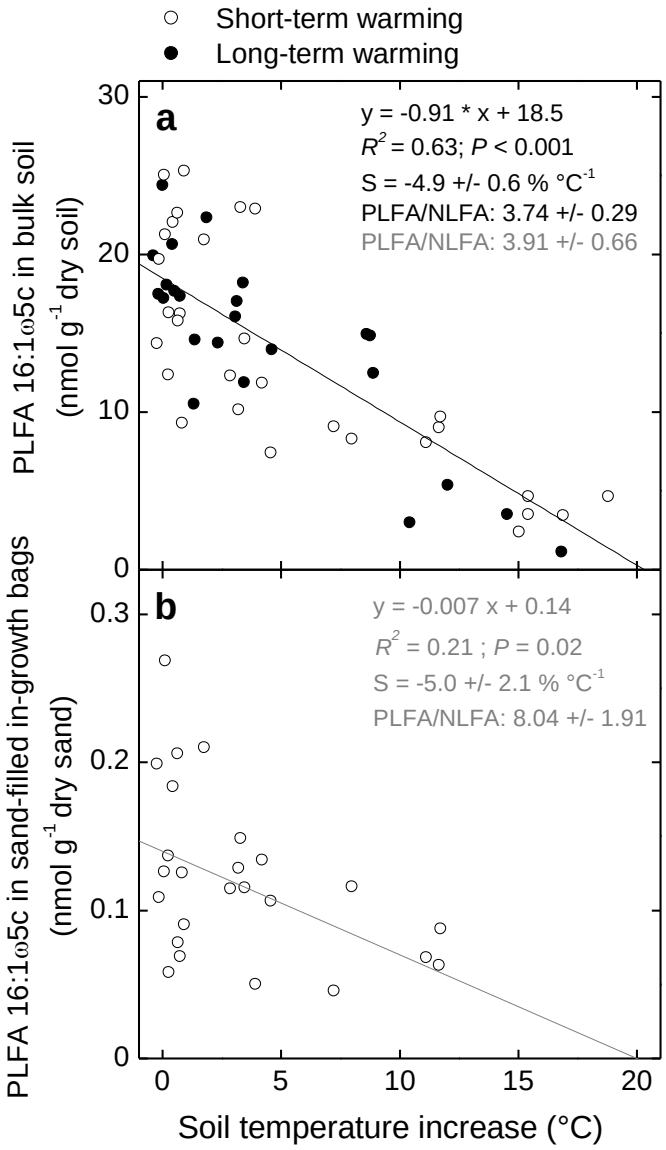
$$C.SOC_{10-30} = (U.SOC_{5-10} - C.SOC_{5-10}) + \left(U.SOC_{10-30} * \frac{C.Th_{10-30}}{U.T_{h10-30}}\right) \quad \text{(Eqn. S.9)}$$

Where $U.SOC_{10\text{-}30}$ is the uncorrected SOC stock in the 10-30 cm depth layer, calculated with Eqn. 2 (see Materials and methods) and the last term corresponds to the proportional thickness of the corrected layer compared to the uncorrected layer.

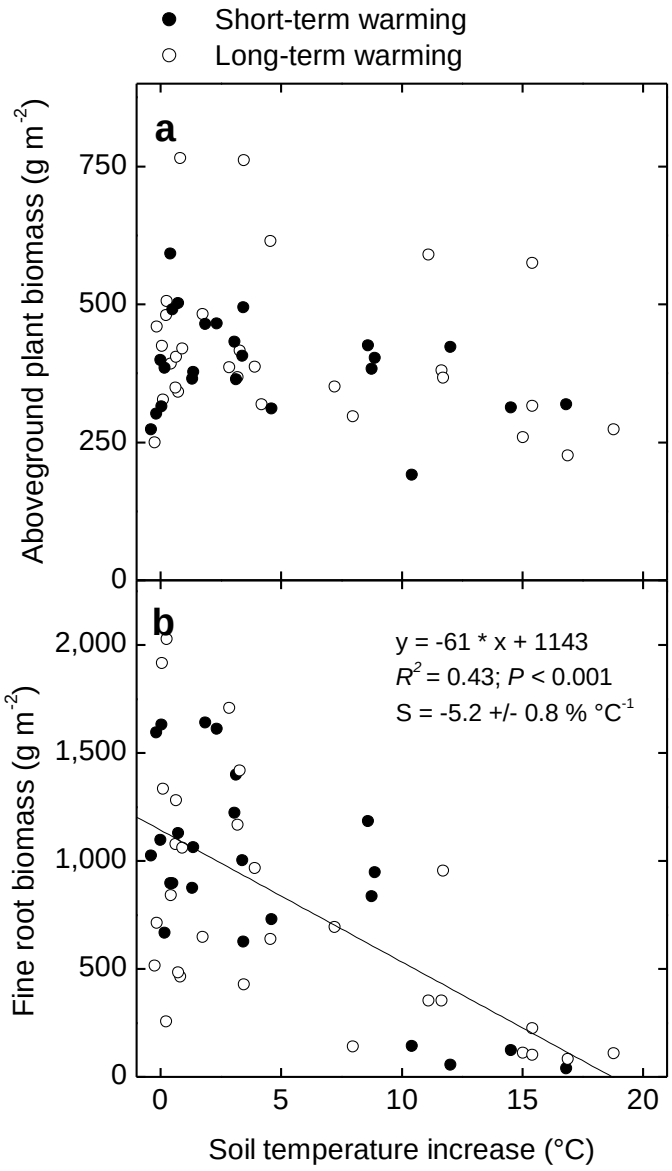
The SON stocks were calculated in the same way as the SOC stocks.



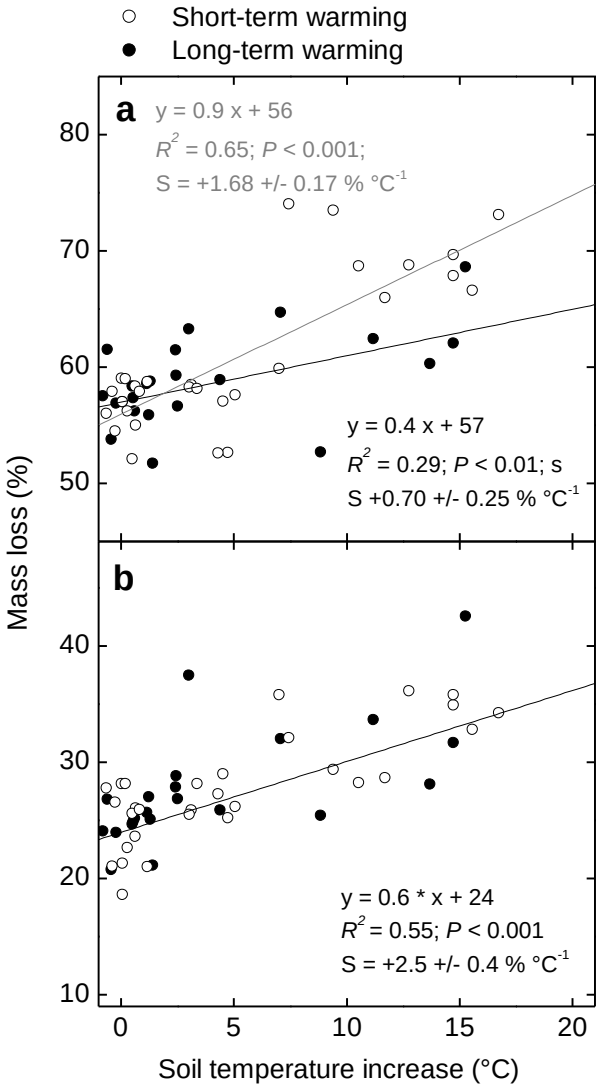
Extended data Figure 3 | Changes in fungal biomass with increasing soil temperature. Soil temperature increase is expressed relative to ambient soil temperature (at 10cm depth), averaged from May 2013 to May 2015. (a) Ergosterol is used as a proxy for ergosterol-producing fungi. Ergosterol producing fungi accounted for 94.8 ± 1.0 (SE) % of the total fungal community, based on amplicon read counts in the fungal community analysis, assuming that all fungi except Arbuscular mycorrhizal fungi (AMF) produced ergosterol (Olsson et al., 2003). (b) Proportion of filamentous saprothrophs in the total fungal community (based on amplicon read counts in the fungal community analysis). Because the warming-duration × temperature interaction was statistically significant in (a) ($P < 0.01$), separate equations are given for short-term warming (grey) and long-term warming (black). In the case of (b), the warming-duration × temperature interaction was not statistically significant ($P = 0.13$), so the short- and long term warmed grasslands were combined in one statistical model. The sensitivity (S) was calculated as the linear change per °C as % of the ambient value. Uncertainty levels (±) refer to SEs.



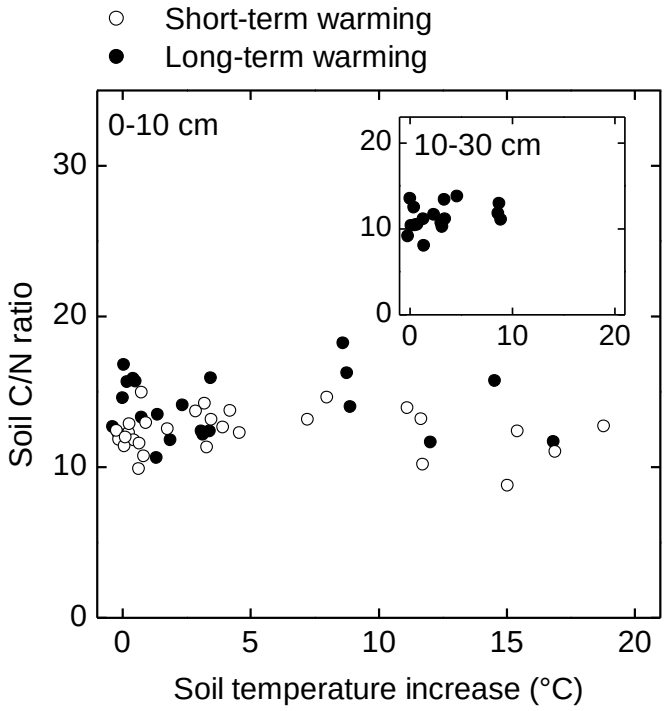
Extended data Figure 4 | Changes in the biomass of arbuscular myorrhizal fungi (AMF) with increasing soil temperature. Soil temperature increase is expressed relative to ambient soil temperature (at 10 cm depth), averaged from May 2013 to May 2015. Phospholipid fatty acids (PLFA) 16:1ω5c is used as proxy for (a) AMF in bulk soil and (b) AMF in sand-filled in-growth bags. While PLFA 16:1ω5c is produced by both AMF and bacteria, the ratios of PLFA 16:1ω5c and neutral lipid fatty acids (NLFA) 16:1ω5c (NLFA/PLFA) pointed to fungal dominance over bacteria, as this ratio varies between 1 and 200 in AMF and is below 1 in bacteria (Olsson et al., 2003). The in-growth bag study was only conducted in the short-term warmed study site. Because the warming-duration × temperature interaction was not statistically significant in (a) (P = 0.63), the short- and long term warmed grasslands were combined in one statistical model. The sensitivity (S) was calculated as the linear change per °C as % of the ambient value. Uncertainty levels (±) refer to SEs.



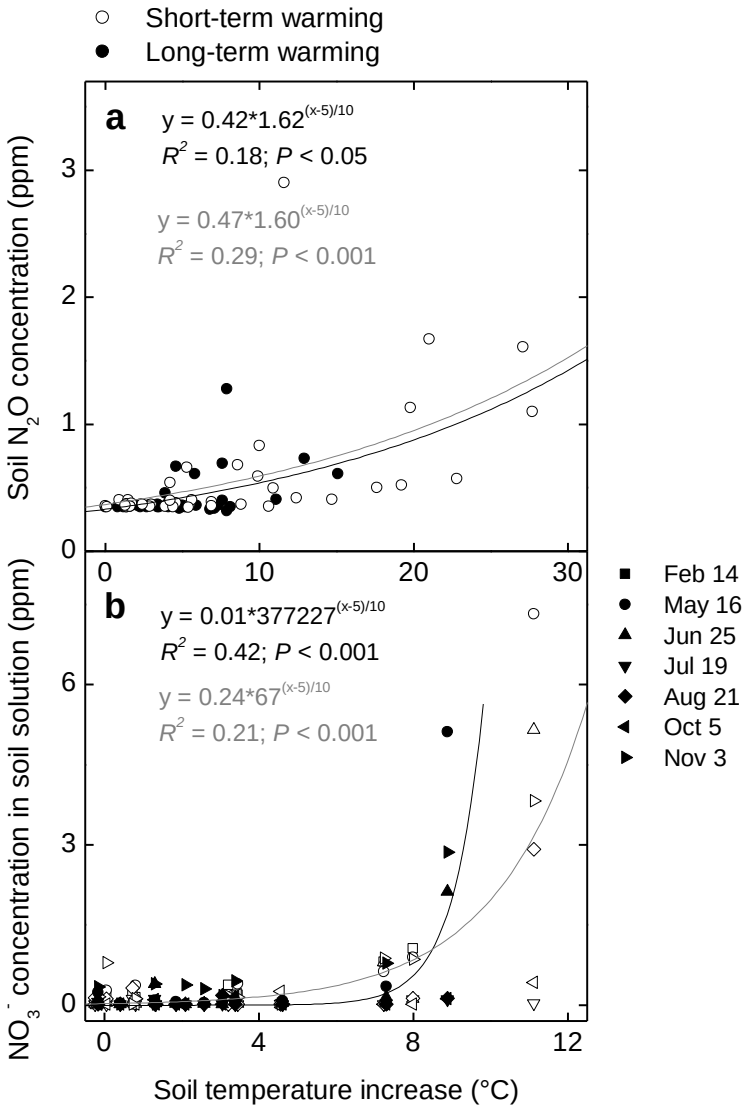
Extended data Figure 5 | Changes in above- and belowground plant biomass with increasing soil temperature. Soil temperature increase is expressed relative to ambient soil temperature (at 10 cm depth), averaged from May 2013 to May 2015. (a) Aboveground plant biomass, including vascular vegetation (monocotyls, dicotyls, ferns and equisetums), mosses and lichens. (b) Fine root biomass in the topsoil (upper 10 cm). The upper 10 cm of soil contained 80.7 ± 2.2 (SE) % of the total fine root biomass in the long-term warmed grassland. The short-term warmed grassland was too shallow to have deeper root growth. Because the warming-duration × temperature interaction was not statistically significant in (b) (P = 0.49), the short- and long term warmed grasslands were combined in one statistical model. The sensitivity (S) was calculated as the linear change per °C as % of the ambient value. Uncertainty levels (±) refer to SEs. Relationships were considered statistically significant at $P < 0.05$.



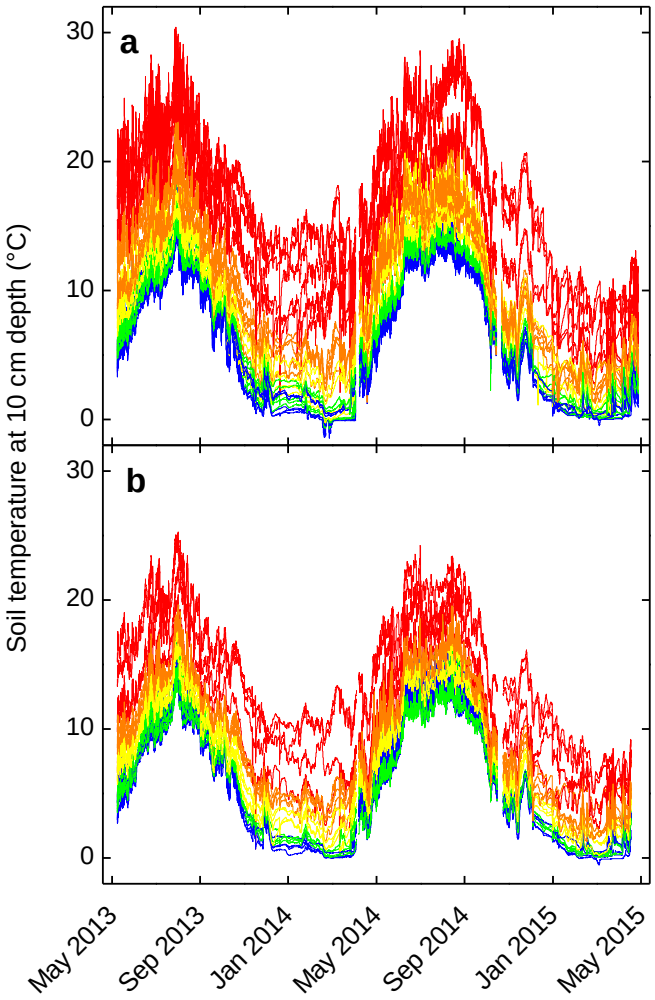
Extended data Figure 6 | Direct temperature stimulation of the decomposition rate of easily decomposable and recalcitrant litter. Soil temperature increase is expressed relative to ambient soil temperature (at 10cm depth), averaged over the litter burial time. Mass loss is used as a proxy for the decomposition rate of (a) easily decomposable litter (green tea, which contains a high proportion of easily degraded compounds such as sugars, starches and proteins) and of (b) recalcitrant litter (red tea, which contains a high proportion of recalcitrant materials such as cellulose, fats, waxes and tannins). Because the warming-duration × temperature interaction was statistically significant in (a) (*P* = 0.01), separate equations are given for short-term warming (grey) and long-term warming (black). In the case of (b), the warming-duration × temperature interaction was not statistically significant (*P* = 0.74), so the short- and long term warmed grasslands were combined in one statistical model. The sensitivity (S) was calculated as the linear change per °C as % of the ambient value. Uncertainty levels (±) refer to SEs.



Extended data Figure 7 | Proportional soil C and N losses under increasing soil temperature. Soil temperature increase is expressed relative to ambient soil temperature (at 10cm depth), averaged from May 2013 to May 2015. The main graph shows the soil C/N ratio in the topsoil (upper 10 cm). The inset graph shows the soil C/N ratio in the subsoil (10-30 cm depth) for the long-term warmed grassland. The soil of the short-term warmed grassland was too shallow to measure the soil C/N ratio below 10 cm soil depth.



Extended data Figure 8 | Increasing gaseous and liquid N concentrations with increasing soil temperature. Soil temperature increase is expressed relative to ambient soil temperature (at 10 cm depth), averaged from May 2013 to May 2015. (a) Soil N_2O concentrations and (b) nitrate (NO_3^-) concentrations in the soil solution at different collection dates. Equations show the Q_{10} relationships with soil temperature for short-term warming (grey) and long-term warming (black). Relationships were considered statistically significant at $P < 0.05$.



Extended data Figure 9 | Soil temperature elevations and seasonal dynamics. Hourly soil temperatures at 10 cm depth from May 2013 to May 2015 in every measurement plot in (a) the short-term warmed grassland and (b) the long-term warmed grassland. Colors show targeted soil temperature elevations: blue for unwarmed soils, green for +1°C, yellow for +3°C, orange for +5°C and red for +10°C.

4.8 Material and methods

4.8.1 Study sites

This study was conducted at the ForHot research site, located in the Hengill geothermal area, 40 km east of Reykjavik, Iceland (64°00′01″ N, 21°11′09″ W; 100 – 225 m a.s.l.). The mean annual temperature between 2004 and 2014 was 5.2 ± 0.1 (SE) °C, and mean annual daily minimum and maximum temperatures were 2.2 ± 0.1 (SE) and 8.6 ± 0.1 (SE) °C. The mean annual precipitation during the same period was 1431 ± 54 (SE) mm (Icelandic Meteorological Office). The main vegetation type is unmanaged grassland, dominated by *Agrostris capillaris*, *Ranunculus acris* and *Equisetum pratense* and the underlying soil is classified as Brown Andosol (Arnalds, 2015).

The site is divided into two areas that have been subjected to geothermal soil warming for different periods of time. One area (hereafter "short-term warmed") has been warmed since May 2008, when a large earthquake shifted geothermal systems to previously unwarmed soils. The second area (2.5 km North-east from first site; hereafter "long-term warmed") was already referred to as a warmed area in 1708 (Magnússon and Vídalín, 1708) and has thus likely been warmed for centuries. Warming at this area was officially registered in a census during the 1960s, and no change in the location of the hotspots has been recorded during the past 50 years (Personal communication of the person who conducted the census). The soil warming increment at both sites is relatively constant throughout the year and extreme deviations are rare (Extended data Fig. 9). Soil warming is caused by heat conduction from the underlying bedrock that is warmed from within by hot groundwater (Gudmundsdottir et al., 2011b). The geothermal water is confined within the bedrock and no signs of soil contamination by geothermal byproducts have been found. Soil moisture and pH (mean: 5.5 ± 0.1 (SE)) did not vary significantly along the warming gradients.

4.8.2 Experimental design

We established five replicate transects in each area (long-term warmed and short-term warmed) in 2012. Each transect consists of six soil temperature (Ts) levels including unwarmed soil (MAT: 5.7 ± 0.1 ºC) and approximately +1, +3, +5, +10 and +20 °C, yielding 60 plots in total. At each Ts level, a 2 x 2 m permanent measurement plot was established, accompanied by two 0.5 x 0.5 m subplots for destructive measurements. Plot-specific Ts were recorded hourly at 10 cm soil depth using HOBO TidbiT v2 Water Temperature Data Loggers (Onset Computer Corporation, USA). Because the intended soil warming intensities were not

always achieved, we did not work with soil warming classes, but adopted a regression approach (see statistics below).

4.8.3 Vegetation and soil sampling and analyses

We clipped all aboveground biomass from the subplots in July 2013 and 2015. Tissue was dried for 48 h at 40°C, weighed, and used to calculate dry weight per unit area (g m^{-2}) based on the sampling surface area. Data from 2013 and 2015 were averaged as no significant inter-annual difference was found (two-way ANOVA; soil temperature and year as fixed variables). In July 2013 and 2014, two 0-10 cm soil cores (corer ø = 5.12 cm) were taken within each destructive subplot. In the short-term warmed grassland, soils were too shallow for deeper cores, but additional 10-30 cm cores were taken in the long-term warmed grassland. Cores were analyzed for: (1) fine root biomass (< 2 mm; all roots belonged to this size class); (2) soil C and N concentrations; (3) pH (topsoil only); (4) grain size distribution (topsoil only); (5) soil bulk density (BD); and (6) SOC and SON stocks.

We obtained fine roots and soil particles > 2 mm ($s_{>2mm}$) (necessary to calculate BD) from the first core by washing the cores over two sieves with mesh sizes 2 mm and 0.5 mm. Roots and $s_{>2mm}$ were dried and weighed to gain fine root biomass (g m^{-2}) (as for aboveground vegetation) and the volume of $s_{>2mm}$ (g cm^{-3}) was measured by the water displacement method (Pang et al., 2011). The second soil core was first dried and weighed (as for aboveground vegetation), following which the core was sieved to obtain soil particles < 2 mm ($s_{<2mm}$) and split into three aliquots. One aliquot of 2 g was milled (Retsch MM301 Mixer Mill, Haan, Germany) and analyzed for C and N content (%) by dry combustion (Macro Elemental Analyser, model vario MAX CN, Hanau, Germany). A second aliquot of 10 g was analysed for pH. A third aliquot of 10 g was analyzed for grain size distribution (%) (Mastersizer 2000, Malvern, UK) after the removal of organic constituents following Kunze and Dixon (1982). Finally, BD (g cm^{-3}), SOC and SON stocks (t ha^{-1}) were calculated according to the approach described in Gundersen et al. (2014) (see details in SI, Eq. 1 and 2).

A soil depth correction of the SOC and SON stocks was necessary to compare stock changes across the Ts gradients as soil compaction increased with increasing Ts in the upper soil layers (increasing BD; Fig. 2), implying that soil depths in unwarmed soil corresponded to shallower soil depths at warmer Ts. For more information, see Extended data Fig. 2.

4.8.4 Soil carbon fractionation

We used 20 g sieved soil (<2mm; from an additional soil sampling) from all subplots of the short-term warmed grassland to perform an SOC fractionation following Zimmermann et al.

(2007). Briefly, a soil suspension, created using ultrasonic dispersion, was wet-sieved over a 63 µm sieve to separate the fine silt and clay (SC) fraction from a coarse fraction (>63 µm). The decanted water (200 ml) was filtered through a 0.45 µm membrane filter and analysed for DOC (On-Line TOC-Vcsh analyzer, Shimadzu, Japan). The coarse fraction (>63 µm) was further separated by density fractionation (sodium polytungstate solution with a density of 1.6 g cm^{-3}) into sand and stable aggregates (SA) and a particulate organic matter (POM) fraction. Finally, a chemically resistant fraction (rSOC) was isolated from the SC fraction by oxidation at room temperature (3 x 18 hours) with 6% NaOCl. We thus obtained five fractions: POM, DOC, SA, SC-rSOC and rSOC, all of which were analysed for C content (%) via dry combustion.

4.8.5 Teabag decomposition study

The temperature effect on decomposition rate (mass loss over time) was assessed following the standardized teabag decomposition protocol (http://www.decolab.org/tbi/). Teabags were buried for 110 days (June to September 2014). For each measurement plot, four replicates of both green (easily decomposable litter) and red tea (recalcitrant litter) were used. Data is presented as average mass loss (%) per plot over the total burial period.

4.8.6 Soil N_2O sampling

Soil N_2O concentrations (ppm) were measured in campaigns in June 2013 in the short-term and the long-term warmed grasslands. As the sampling points were partly located outside the measurement plots, Ts at 10 cm depth was recorded for each measurement. A stainless steel probe (ø 3 mm, length 30 cm) connected to a 60 ml syringe (Terumo) was used to sample (30 ml) soil gas at 10 cm depth. Samples were injected into 12 ml Labco pre-evacuated vials (Labco Excetainer®) within 12 h for gas analysis on a gas chromatograph (Agilent 6890N, Agilent Technologies, USA) equipped with an ECD detector (Gilson, USA).

4.8.7 NO_3^- sampling from soil solution

Soil water samples were collected from three transects and four Ts levels in the short-term and long-term warmed grasslands (unwarmed and ca. +3, +5 and +10°C warming) with teflon suction cup lysimeters (Prenart Super Quartz, Prenart Equipment Aps, Frederiksberg, Denmark). The lysimeters were installed one month prior to the first sampling to allow them to equilibrate with the soil solution and soil water was extracted and discarded three times before the first sampling. Samples were first taken in February 2015 and then monthly between May and November 2014. Nitrate concentrations (NO_3^-) (ppm) were determined by

ion chromatography (Shimadzu, Kyoto, Japan). Concentrations were assumed to represent concentrations in soil water leaching from the root zone (Hansen et al., 2007).

4.8.8 Fungal biomass and community analyses

Ergosterol content μmol g^{-1} dry soil) was used as a proxy to measure fungal biomass in soil samples according to the approach outlined in Bahr et al. (2013). In addition, sand-filled in-growth bags were used to determine the production of mycorrhizal mycelia following Wallander et al. (2001). Concentrations of PLFAs (phospholipid fatty acids) 16:1ω5 and NLFA (neutral lipid fatty acid) 16:1ω5 were measured following 10,11 and their ratio was used as a marker for arbuscular mycorrhizal biomass in the in-growth bags.

In May 2013, 2 g soil samples were collected from 5-10 cm soil depth and immediately freeze-dried. DNA was later extracted using the PowerSoil DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA, USA). We used 1 μl of DNA extract as a template for a 25 μl PCR containing 1 X Phusion High-Fidelity Mastermix (Thermo Scientific, Waltham, MA, USA) and 200 nM barcoded general fungal primers ITS1f and ITS2 (ref. Smith and Peay, 2104). PCR conditions were as follows: 98°C for 30 s, followed by 30 cycles of 98 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, with an additional final extension of 72 °C for 10 min. Duplicate successful amplification products were pooled and cleaned using Agencourt AMPure XP (Beckman Coulter, Brea, CA, USA), quantified using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA), pooled to equimolar concentrations, loaded onto a 1% agarose gel, excised to remove remaining primers, cleaned using the QIAquick Gel Extraction Kit (Qiagen, Venlo, the Netherlands) and again quantified. Samples were then sequenced on an Illumina MiSeq for 300 cycles in the forward direction and 12-cycles for indexing in presence of a 20% spike of PhiX.

DNA sequences were analysed using the UPARSE pipeline (Edgar, 2013) with the following steps/settings. Reads were first trimmed to 200 bp and filtered to allow a maximum estimated error of 1% (leaving 4,150,777 sequences). Reverse primers were removed and padded with N's, singletons were removed, and the remaining reads were clustered to 97% similarity. Chimeras were filtered *de novo* as well as through the UNITE database of ITS1 sequences (as implemented in UCHIME), removing 679 and 28 OTUs (operational taxonomic units), respectively. This left a total of 3,618 non-chimeric OTUs. Representative sequences for each OTU were aligned to all fungal representative species in the UNITE database (Abarenkov et al., 2010) using BLAST and assigned according to lowest E-value with a minimum alignment length of 75 bp. Representative sequences were then classified to

taxonomic levels depending on identity percentages: >90% for genus; >85% for family; >80% for order; and >75% for class (Tedersoo et al., 2014). OTUs were assigned to functional groups when assigned to genus level if they matched genera with known lifestyles (Tedersoo et al., 2014). In doing so we classified non-yeast saprobes as "filamentous saprotrophs". All original sequences were mapped against these OTUs with a similarity threshold of 97% for inclusion, and samples were rarefied to the minimum number of reads (5,274).

4.8.9 Data analyses

The Ts dependence of SOC stocks (corrected and uncorrected for soil compaction), BD, soil ergosterol content, proportion of filamentous fungi, PLFA 16:1ω5c in bulk soil and in-growth bags, aboveground biomass, fine root biomass, mass loss and soil C/N ratio were tested with linear mixed models using Ts and warming-duration (short-term vs. long-term) as explanatory variables and transect as a random variable. In case of a significant interaction effect between Ts and warming-duration, a linear mixed model was fitted for the short-term and the long-term warmed grassland separately, with Ts as explanatory variable and transect as a random variable. Associations between SOC stocks in the bulk soil and in the different soil size fractions were tested with a linear mixed model, using transect as a random variable. In all cases, criteria for normality and homoscedasticity were met. Based on AIC comparison, we selected a Q_{10} function to describe the relationships between Ts and soil N_2O and Ts and NO_3^- concentrations, where Q_{10} was calculated as in Vicca et al. (2009) (see details in SI, Eq. 3), but with a basal temperature of 5°C. All tests were performed using R software (R-core-team, 2014).

Chapter V

Effects of seabird nitrogen input on biomass and carbon accumulation after 50 years of primary succession on a young volcanic island, Surtsey

5 Effects of seabird nitrogen input on biomass and carbon accumulation after 50 years of primary succession on a young volcanic island, Surtsey

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Modified from Biogeosciences, 2015, 11, 6237–6250

5.1 Abstract

What happens during primary succession after the first colonizers have occupied a pristine surface largely depends on how they ameliorate living conditions for other species. For vascular plants the onset of soil development and associated increase in nutrient (mainly nitrogen, N) and water availability is especially important. Here, we report the relation between N accumulation and biomass- and ecosystem carbon (C) stocks in a 50-year-old volcanic island, Surtsey, in Iceland, where N stocks are still exceptionally low. However, a 27-year-old seagull colony on the island provided nutrient-enriched areas, which enabled us to assess the relationship between N stock and biomass- and ecosystem C stocks across a much larger range in N stock. Further, we compared areas on shallow and deep tephra sands as we expected that deep-rooted systems would be more efficient in retaining N. The sparsely vegetated area outside the colony had accumulated 0.7 kg N ha^{-1} y^{-1}, which was ca. 50-60% of the estimated N input rate from wet deposition. This approximates values for systems under low N input and bare dune habitats. The seagulls have added, on average, 47 kg N ha^{-1} y^{-1}, which induced a shift from belowground to aboveground in ecosystem N and C stocks and doubled the ecosystem 'N use efficiency', determined as the ratio of biomass and C storage per unit N input. Soil depth did not significantly affect total N stocks, which suggests a high N retention potential. Both total ecosystem biomass and C stocks were strongly correlated with N stock inside the colony, which indicated the important role of N during the first steps of primary succession. Inside the colony, the ecosystem biomass C stocks (17-27 ton C ha^{-1}) had reached normal values for grasslands, while the soil organic carbon stocks (SOC; 4-10 ton C ha^{-1}) were only a fraction of normal grassland values. Thus, it will take a long time until the SOC stock reaches equilibrium with the current primary production, during which conditions for new colonists may change.

5.2 Introduction

Primary succession, the chain of processes in which an ecosystem develops on an unvegetated substrate that lacks a developed soil (Vitousek et al., 1989, Walker and Del Moral, 2003), is a complex process that depends on the interplay of numerous factors, both biotic and abiotic (Raab et al., 2012). Most studies on primary succession of volcanic islands and inland volcanos have focused on community changes in flora and fauna (e.g. Walker et al., 2003, Magnússon et al., 2009, Marteinsdottir et al., 2010), but fewer have looked into changes in underlying environmental drivers for community change (Walker and Del Moral, 2003). Yet, such drivers often play a crucial role in the onset of ecosystem development (Kögel-Knabner et al., 2013) and are also often a function of long-term ecosystem processes, such as soil development (Long et al., 2013a).

The buildup of biomass leads to the accumulation of soil organic carbon (SOC), which is an indicator of soil development (Kögel-Knabner et al., 2013). SOC greatly improves the physical and chemical properties of soil, such as water holding capacity and ion exchange capacity, thereby enhancing nutrient retention and exchange (Deluca and Boisvenue, 2012). As the successful germination, establishment and growth rate of many species strongly depends on these soil properties, the presence of well-developed soils forms an ecological threshold for many species (Long et al., 2013b). Consequently, biomass and carbon (C) accumulation form an important step in the course of primary succession that enables less stress-tolerant and often more productive species to arrive (Grime, 1977). A positive reciprocal relationship can be expected between SOC (and its source, biomass production) and nutrient accumulation (Li and Han, 2008). On the one hand, SOC facilitates the retention of nutrients, while on the other hand improved nutrient supply has a positive effect on plant biomass in all terrestrial systems (Gruner et al., 2008), which leads to increased SOC accumulation. Therefore, we expected the amount of nutrient input to be an important determinant of biomass and C accumulation during primary succession. Once the system has reached a sufficient level of fertility for, often more productive, secondary colonizers to establish, they will enhance SOC production and a positive feedback loop between biomass production (SOC) and nutrient accumulation will initiate. Subsequently, the rate of primary succession has been shown to increase following such fertility change (Olff et al., 1993, Vitousek et al., 1993, Sigurdsson and Magnússon, 2010).

Of all nutrients, nitrogen (N) is most frequently the limiting nutrient for plant growth in high latitude terrestrial ecosystems due to the adverse effect of cold temperatures on

biogeochemical processes (Aerts and Chapin, 2000, Reich and Oleksyn, 2004, Storm and Suess, 2008), and this is even more pronounced on young substrates (Vitousek et al., 1993, Vitousek and Farrington, 1997, Elser et al., 2007). Therefore, N is expected to be strongly linked with SOC accumulation during primary succession of young volcanic islands at high latitudes.

Surtsey is a volcanic island which surfaced during a submarine eruption between 1963 and 1967, and has from the very beginning been strictly protected for research (Baldurson and Ingadóttir, 2007). This makes it an exceptionally suitable location to study the natural course of successional processes. As Surtsey is still a young volcanic island, its vegetation is expected to be N limited (Vitousek et al., 1993, Vitousek and Farrington, 1997, Elser et al., 2007). Nitrogen has indeed been identified as a strong limiting factor for plant growth and succession at Surtsey (Hendriksson, 1976, Magnússon and Magnússon, 2000), and the N:P ratio of five dominant plant species on Surtsey generally indicates a strong N limitation (N:P<14; Aerts and Chapin, 2000) (Thuys et al., 2014).

The importance of nutrients became very visible after the establishment of a seabird colony in 1986 (mainly lesser black backed gull, *Larus fuscus*) on the SW side of the island (Magnússon and Magnússon, 2000) (Fig. 1). Seabirds are known to transfer nutrients, primarily ammonium and nitrate, from sea to land (Polis and Hurd, 1996, Anderson and Polis, 1999, Ellis et al., 2006, Nie et al., 2014). The effect of enhanced nutrient inputs has been studied by comparing surfaces that were unaffected by seabirds with the seabird-colonized area (Magnússon and Magnússon, 2000, Magnússon et al., 2009, Sigurdsson and Magnússon, 2010). In the seabird colony, the rapid increase in soil N elicited subsequent increases in plant cover and species richness. These earlier studies did, however, not attempt to quantify the rate of N-accumulation or study the development of the whole ecosystem biomass, C or nutrient stocks.

Within the seabird colony at Surtsey, the earlier studies have revealed diverging successional trajectories of vascular plant communities between areas with deep tephra sand or with surfacing lava rocks (Magnússon and Magnússon, 2000). This has become even more pronounced during the past decade (Del Moral and Magnússon, 2014), showing faster development of species richness and plant cover in seabird colony plots on deep tephra sand.
The main goal of this study was to quantify the rate of N accumulation in different permanent study plots on Surtsey and investigate how this compared to changes in the whole ecosystem biomass and C stocks. Despite the fact that earlier studies have revealed drastic effects of the seabird-derived N input on the ecosystem structure and functions on Surtsey (Magnússon and

Magnússon, 2000, Kristinsson and Heidmarsson, 2009, Magnússon et al., 2009, Petersen, 2009, Sigurdsson and Magnússon, 2010, Del Moral and Magnússon, 2014, Thuys et al., 2014) no data exist on the annual N input coming from seabirds, except from a coarse estimate of (Magnússon et al., 2009), who combined excretion and food models for the lesser black-backed gull (*Larus fuscus*) and the herring gull (*Larus argentatus*) derived from Hahn et al. (2007), and nest counts on Surtsey. We therefore attempted to obtain a more precise approximation of the seabird derived N input by measuring total N stocks and correcting for the total colonization time. Our null hypothesis was that the annual net N accumulation rate inside the seabird colony amounts to ca. 30 ton N ha^{-1}, as was estimated by Magnússon et al. (2009).

Further, we tested four hypotheses in detail:

(i) Atmospheric N deposition is the main N source outside the seabird colony. As we do not expect that this sparsely vegetated area is very efficient in N retention, large amounts of N can be lost due to leaching during large rain events. Field observations in different ecosystems with low N input rates, as is the case outside the colony, revealed an average N retention of 75% (Thomas et al., 2013). Therefore, we hypothesized that total N stocks outside the seabird colony would approximate 75% of the total estimated accumulated atmospheric N deposition during the past 50 years.

(ii) Among similar vegetation types, those with deeper root systems were shown to be more effective in avoiding N leaching losses than their shallower rooting equivalents (Bowman et al., 1998). On Surtsey, most N that leaches through the upper substrate layer is removed from the system by seeping into cracks and fissures in the lava bedrock. Therefore, we hypothesized that plots on deep tephra sand show higher N retention compared to plots on shallow tephra sand.

(iii) Nutrient rich systems are known to invest relatively more in aboveground plant growth than nutrient poor systems (e.g. Warembourg and Estelrich, 2001, Wang et al., 2008, Gao et al., 2011, Zhou et al., 2014). Therefore we expected to find a shift from belowground to aboveground biomass, C and N stocks inside the seagull colony, compared to outside the colony.

(iv) An important factor in the process of soil development, the process in which bedrock material is transformed into a soil with defined horizons by a combination of climate, biota, topography, parent material and time, is the accumulation of SOC (Kögel-Knabner et al.,

2013). As SOC is a product of biomass degradation, we hypothesized that the increased productivity within the seagull colony enhances the rate of soil development.

5.3 Material & Methods

5.3.1 Site description

Samples were collected on Surtsey (63°18´11"N 20°36´17"W) in mid July 2012 and mid July 2013. The island surfaced during an eruption between 1963 and 1967 and was 1.41 km^2 in 2004. Surtsey is the youngest island of the Vestmannaeyjar archipelago, which is located on the insular shelf 32 km off the south coast of Iceland. The archipelago constitutes a separate volcanic system, which initiated activity around 100.000 years BP (Jakobsson, 1979). Climate conditions on the archipelago are humid sub-arctic. Winter and summer average temperatures between 1971 and 2000 were 1.5-2 °C and 10 °C, respectively (Icelandic Meteorological Office). Total annual precipitation during the same period was on average 1600 mm and fell mainly between October and March. The prevailing wind direction is East, and wind speed exceeds hurricane force (>32.7 m s^{-1}) on average 15 days per year (Icelandic Meteorological Office). The bedrock of the higher parts of Surtsey consists of basalt tuff, whereas in its lower parts it is made out of basaltic lava (Jakobsson, 1968). The island is still too young to have well developed soils. The lava is partly filled with tephra sand and silt, which originates from the eruption, from erosion of the bedrock material and by aeolian transport from the mainland. The vegetation on these sandy areas is dominated by *Honkenya peploides* and *Leymus arenaria* (Magnússon et al., 2014, Stefansdottir et al., 2014). In 1986, a seabird colony of lesser black backed gulls (*Larus fuscus*) was established in a confined area on the SW edge of the island (Petersen, 2009), and has been expanding in size ever since. The dominant plant species inside the seagull colony are *Poa pratensis, P. annua* and *Festuca richardsonii* (Magnússon et al., 2009).

5.3.2 Sample sites

Between 1990 and 1995, permanent 10x10 m plots were established on Surtsey to survey ecosystem changes in and outside the seabird colony (Fig. 1). Out of these plots, we selected 18 representative plots inside and outside the seabird colony, partly on deep (≥ 30 cm deep) and partly on shallow tephra sand (< 30 cm deep). Ten plots were situated outside the colony (of which six were sampled in 2012 and four in 2013) and eight plots were situated inside the colony (of which four were sampled in 2012 and four in 2013). In every case, half of the plots were located on shallow and the other half on deep tephra sand. We placed three subplots

(20x50 cm) for destructive sampling 0.5 m outside the southern edge of each permanent plot ($n_{outside\ colony} = 30$; $n_{inside\ colony} = 24$).

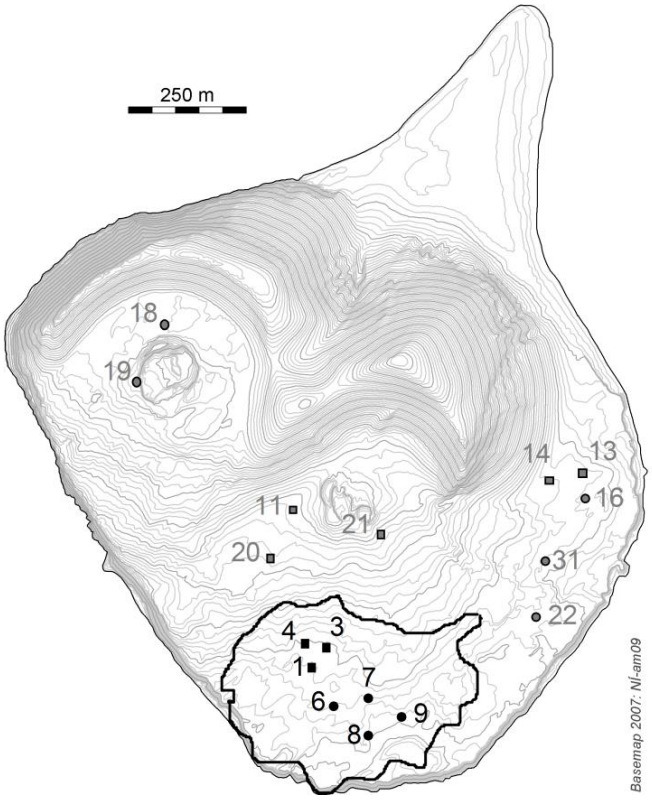


Figure 1. Location of the permanent study plots on Surtsey that were used in this study, shown on a topographical map from 2007. Contour intervals are 2m, the highest point on the island is 152m a.s.l.. The Eastern crater is located close to plots 18 and 19, the Western crater is located close to plot 21. North of the craters, a high rim of palagonite tuff was formed. The dense vegetation within the seabird colony in 2012 is marked with a black line (Approximation from aerial and sattelite images, by Anette Th. Meier; Magnússon et al., 2014). Grey = Outside seabird colony; Black = Inside colony; Squares = Deep tephra sand; Circles = Shallow tephra sand.

5.3.3 Vegetation & soil sampling

During the two expeditions to Surtsey, all vascular plants were cut in each subplot (20x50 cm) and subsequently separated into species in the field. Next, the litter layer was collected from the same plots, except in the more vegetated seabird colony, where it was collected from a 20x20 cm subplot within the harvest plot. Soil was then sampled from where the litter had been removed using a hand-driven soil corer (8.67 cm diameter). Consecutive soil samples of 5 cm depth were taken down to 30 cm depth or until the bedrock was reached. All samples were air dried awaiting further analyses.

5.3.4 Sample preparation and chemical analysis

Fine roots (all roots were of diameter < 2 mm) were picked manually from soil samples in the laboratory, for maximum 60 minutes per sample. In case where litter was found during the sorting process, it was added to the litter sample. The root-free soil was sieved through a 2 mm sieve in order to separate the coarse soil fraction (> 2 mm) from the sand, silt and clay particles (< 2 mm). All fractions (1) roots, (2) soil particles < 2 mm and (3) soil particles > 2

mm) were dried for 48 h at 40 °C. The coarse fraction was assumed to contain only a marginal amount of organic C or N. In few cases where the sample weight was too small for analysis (< 1 g dry weight), samples from the same layer were merged by neighboring subplots within the same plot. The basaltic tephra sand in Surtsey does not contain any carbonates (mineral C), and inorganic N was assumed to be negligible compared to the total SON (soil organic nitrogen) stock (Ponnameruma et al., 1967), and was therefore not measured.

After drying all biomass samples for 48 h at 40 °C, dry mass of (1) all aboveground vascular plants (separated in monocotyledons, eudicotyledons and ferns and horsetails), (2) moss, (3) litter and (4) fine roots (in 0-5, 5-10, 10-20 and 20-30 cm depth layers) was weighed. For each sample, 1 g dry mass (DM) was milled using a ball mill (Retsch MM301 Mixer Mill, Haan, Germany) and then total C and N concentrations were analysed by dry combustion on Macro Elementary Analyzer (Model Vario MAX CN, Hanau, Germany). C and N concentration was determined for (1) all aboveground vascular plant parts together, (2) litter, (3) moss, (4) roots and (5) soil particles (< 2 mm) per aforementioned soil layers. Soil samples were then dried at 105 °C for 48 hours and weighed again to get the correct DM.

5.3.5 C and N stocks and accumulation rates

Carbon- and N stocks in (1) aboveground vascular plant biomass, (2) litter and (3) moss were estimated by multiplying total DM with the respective concentrations and were expressed per unit area. Carbon- and N stocks in (1) roots and (2) soil particles (< 2 mm) per unit area were calculated per soil layer by multiplying total DM < 2mm of the soil layer with the respective concentrations.

For the plots outside the seabird colony, N accumulation rate was calculated by dividing the total N stock by the number of years since the start of accumulation (from 1963, the year of the surfacing of the island), assuming a constant N accumulation rate. For the plots inside the seabird colony, a separate estimate was made for the N accumulation rate of seabird derived N. Therefore the total N stock of plots inside the colony was first subtracted by the amount of total N stock outside the colony, assuming that this equals the non-bird N input over the whole island. Subsequently, the remaining N stock was divided by the years since seagulls started to breed within 1000 m^2 of each plot; information that was available from (Magnússon and Magnússon, 2000, Magnússon et al., 2009).

5.3.6 Data analyses

Differences in ecosystem N stocks, biomass and C stocks were tested with two-way ANOVA's in the R software (R Development Core Team, 2012), with seabird colony (yes – no) and tephra depth (deep-shallow) as fixed variables. Differences in N accumulation rate and in proportional distribution of SON and root N per soil depth layer were tested in the same way. The correlations between (1) biomass stocks, (2) ecosystem C stocks and (3) root/shoot ratios (R/S ratios) and ecosystem N stocks were tested separately for plots within and outside the seagull colony. Therefore, we used a correlation test, applying the "Spearman" method, whenever the assumptions of homoscedasticity (tested with residual plot) or linearity (tested with the runs test (Turlach, 2011, Trapletti et al., 2012, Zeileis et al., 2012)) were not met. Null hypotheses were rejected when $p < 0.05$.

5.4 Results

5.4.1 Ecosystem N stock and distribution

The total N stock outside and inside the seabird colony after 50 years of island development and ca. 26-27 years after the first lesser black backed gull colonization differed significantly, by a factor of approximately 30 (Fig. 2, Table 1). Soil depth had no significant effect on total accumulated N stock (in biomass and soil), neither outside nor inside the seabird colony. Also, the N stock of the living compartment (shoots, roots and moss together) and of shoots, roots and moss separately did not differ between deep and shallow tephra sand. However, inside the seagull colony, the N stock of the dead OM (organic material) compartment (litter and SON combined), and of the litter and SON separately, was significantly higher in deep tephra sand than in shallow tephra sand on lava (Fig. 2, Table 1).

Of the total N stock outside the colony, approximately 96% of the N was stored belowground in roots and SON, while within the colony this was reduced to about 78 and 88% in deep and shallow tephra sand, respectively (Fig. 2). The amount of N stored as SON was 24 (on deep tephra sand) and 11 (on shallow tephra sand) times higher inside than outside the colony (Fig. 2, Table 1). Despite this drastic effect on absolute SON stocks, the seagull colony did not change the relative distribution of SON trough the upper 30 cm of the soil profile. Irrespective of the bird influence, about 35% of the total SON in deep tephra sands was located in the upper 5 cm, and decreased gradually to about 5% at 25-30 cm depth (Fig. 3, Table 2).

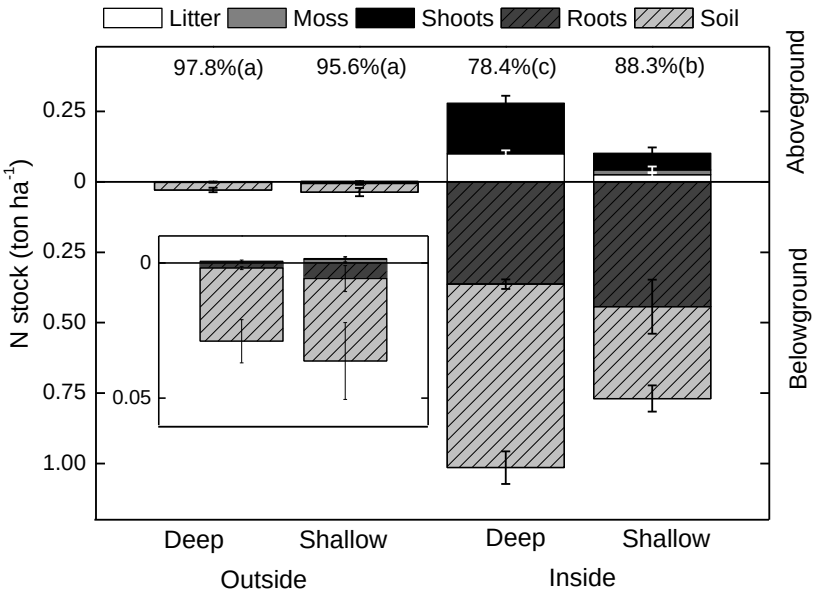


Figure 2. Mean (±SE) values of the nitrogen (N) stocks in litter, shoots, roots (to 30 cm depth) and soil organic nitrogen (SON) (to 30 cm depth) in four ecosystem types on Surtsey. The inserted graph gives the small N stock outside the colony in more detail. 'Shoots' include all aboveground living plant parts. Statistical results for are given in Table 1. Percentages above the graph indicate the % of total N that is located belowground. Letters show statistical differences.

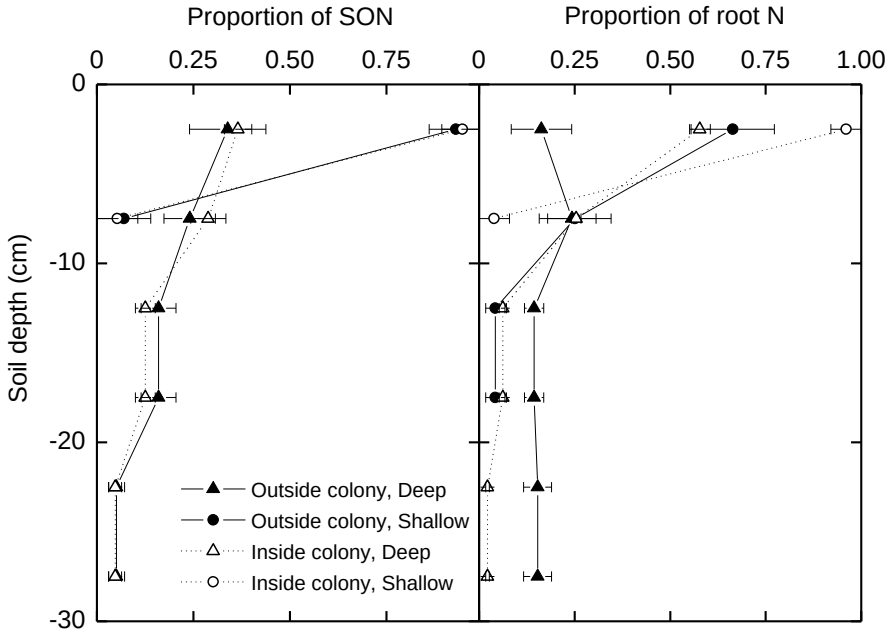


Figure 3. Depth distribution of soil organic nitrogen (SON) and root N in four ecosystem types on Surtsey. Mean (±SE) proportions at each depth layer were calculated relative to the total SON or root N content in the top 30 cm. Statistical results can be found in Table 2.

5.4.2 Nitrogen accumulation rate

The average rate of total N accumulation (in both living and dead compartments) over the 50 years of island succession was 0.7 kg ha^{-1} y^{-1} outside the seagull colony and the effect of tephra depth was not significant (Fig. 4, Table 1). In sharp contrast, the seabirds accelerated the N accumulation rate, with a factor of almost 50 - 100, to 36 - 58 kg ha^{-1} y^{-1} (Fig. 4). Within the seabird colony, there was no difference in N-accumulation rate between tephra depths (Table 2).

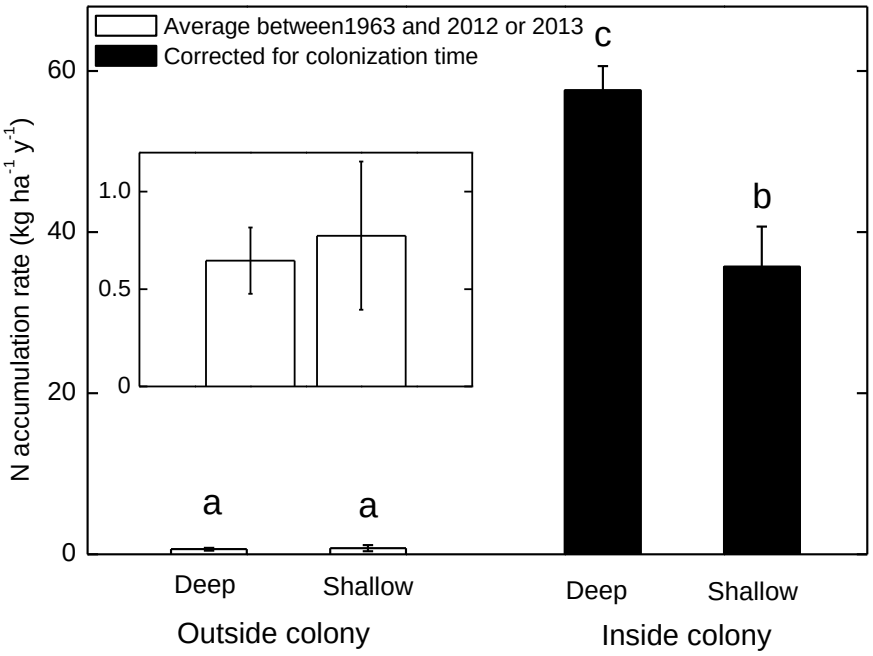


Figure 4. Mean (±SE) values of the nitrogen (N) accumulation rate in four ecosystem types on Surtsey. The inserted graph gives values outside the colony in more detail. White bars show the average N accumulation values between 1963 and the sampling year (2012 or 2013). Black bars show the N accumulation after the start of seagull colonization. Statistical results are shown in Table 1.

5.4.3 Ecosystem biomass and C stocks and SOC concentrations

The area inside the colony contained 50 times more biomass than the plots outside of the bird colony, irrespective from tephra depth. For C, the area inside the colony contained 29 times more C than the area not affected by the seabirds on deep tephra sand, and 16 times more C on shallow tephra sand (Fig. 5, Table 1).

The seabird colony increased all individual stocks of biomass and C, both belowground as aboveground (Fig. 5, Table 1). The living biomass stock was over 50 times larger (0.7 vs 36 ton ha^{-1} outside and inside the colony, respectively), and the living biomass

C stock was almost 70 times higher (0.19 vs 13 ton ha^{-1} outside and inside the colony, respectively) (Fig. 5). The proportion of C stored in living biomass compared to SOC also shifted drastically; from a 24:76 distribution outside the colony, to a 68:32 distribution inside the colony.

Tephra depth, however, had only an effect inside the colony. There, the plots on deep tephra sand stored more litter, litter C and SOC than those on shallow sand layers (Fig. 5, Table 1). The SOC stock in deep tephra sand (10 ton C ha^{-1}) was more than twice as large than on shallow tephra sand (4 ton C ha^{-1}) (Fig. 5).

On deep tephra sand, the seabird colony had a pronounced effect on the relative distribution of the roots (Fig. 3). Outside the colony, the roots were evenly distributed throughout the upper 10 cm of the soil. Inside the colony, however, the roots were concentrated in the top 5 cm (57% of the total root weight within 30 cm depth), and declined to a fraction of only 4% between 20 and 30 cm depth.

The SOC concentration in the upper 5 cm of the tephra sand outside the colony was 0.12 ± 0.02 % (error is SE; data not shown). Inside the colony, there was a significant depth effect on SOC concentration in the upper 5 cm of the tephra sand. On deep tephra sand, the SOC concentration was 0.9 ± 0.3 %, whereas it was as high as 4.6 ± 0.4 % on shallow tephra sand (errors are SE's; data not shown).

5.4.4 Impacts of ecosystem N stock on biomass and C stocks

There was a highly significant positive linear relationship within the seagull colony between total ecosystem biomass and C stock on the one hand and total ecosystem N stock on the other hand (Fig. 6), while almost half of the N and C was located in the soil (50 and 40% on deep and shallow soils respectively), and was therefore not directly related to the biomass. An increase of one g of N could support extra 32 g biomass and 20 g of ecosystem C. Outside the colony, neither biomass nor C showed a significant correlation with total N stock (here approximately 80% of N and C was located in the soil), but for one g N, a median of 17 and 12 g of biomass or C stock were found (Fig. 6). There was no significant correlation between R/S ratio and ecosystem N stock, outside, neither inside the colony (Fig. 7). The variation in the measured R/S ratio was large. The values ranged from 0.1 to 194 outside the colony and from 1.8 to 96 inside (Fig. 7). The median R/S ratios were 18 and 5, outside and inside the seabird colony, respectively.

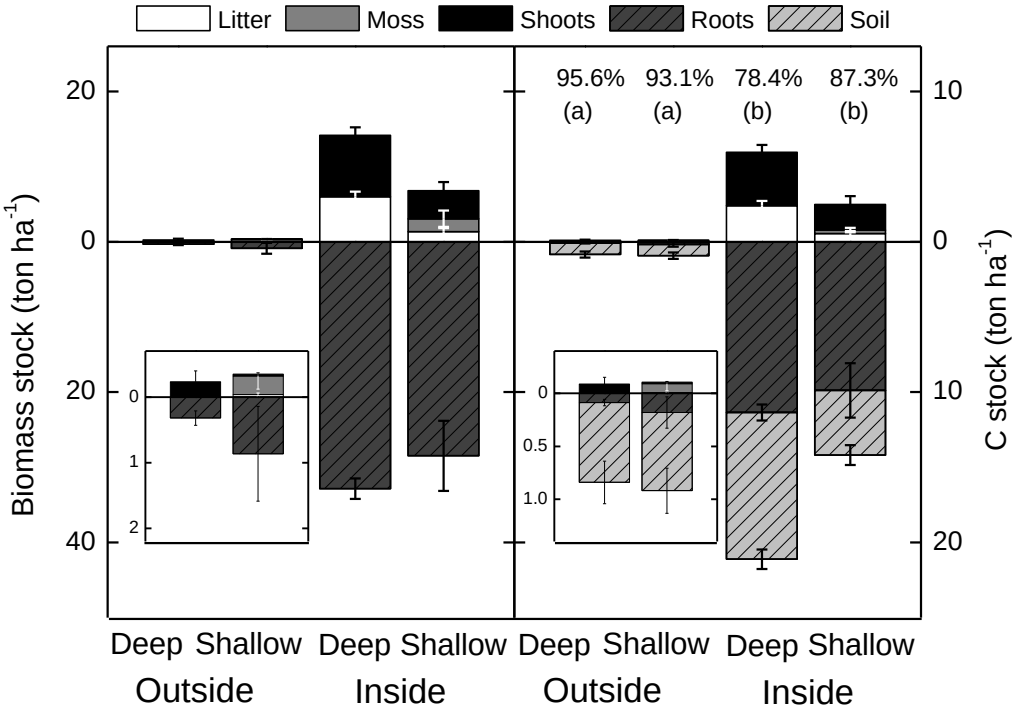


Figure 5. Mean (±SE) values of the biomass stock and carbon stock of litter, shoots, roots (to 30 cm depth) and soil organic carbon (SOC) (to 30 cm depth) in four ecosystem types on Surtsey. The inserted graphs give the values outside the seabird colony in more detail.. 'Shoots' include aboveground living vascular plant parts. Statistical results are shown in Table 1. Percentages above the graph indicate the % of total C that is located belowground. Letters show statistical differences.

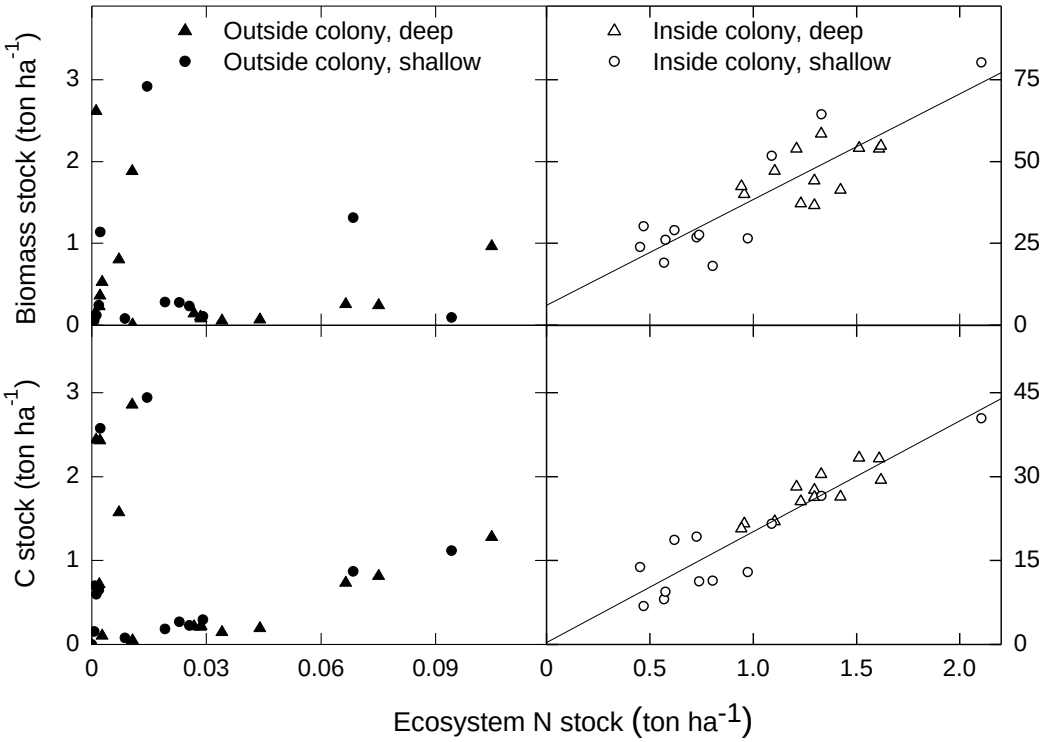


Figure 6. Relationships between ecosystem biomass stocks (upper graphs) and ecosystem carbon stocks (bottom graphs) and ecosystem N stocks. Left graphs show the relationship outside the seagull colony, right graphs show the relationship and inside the seagull colony. Note the difference in scale of the y-axis between the left and the right panel. Correlation function for biomass inside the colony: Biomass = 6 (± 4) + 32 (± 4) * ecosystem N stock (r = 0.86; t = 8.01; Df = 22; p < 0.001). Correlation function for C inside the colony: C = 0.4 (± 1.9) + 19.8 (± 1.7) * ecosystem N stock (r = 0.93; t = 11.75, Df = 22, p < 0.001). Errors = SE's.

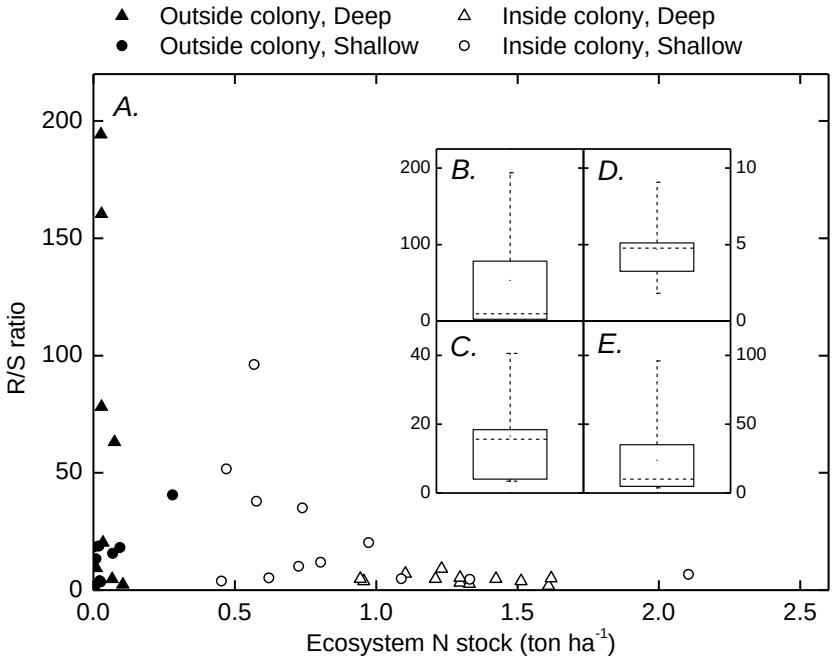


Figure 7. Relationship between R/S ratio (Root/Shoot ratio) and N stock (A). Central figure shows boxplots of the R/S ratio on deep tephra sand outside the colony (B), shallow tephra sand outside the colony (C), deep tephra sand inside the colony (D) and shallow tephra sand inside the colony (C). Boxes show 25, 50 and 75 percentile, whiskers show 5 and 95 percentile. Note the differences in scale of the y-axes. Samples lacking roots or shoots were excluded from the graphs.

Table 1. Results of two-way ANOVA's for N stock, biomass stock, C stock and N accumulation rate, using seabird colony and tephra depth as fixed variables. 'Shoots' include all aboveground living plant parts, 'Living' includes 'Shoots' and 'Roots' and 'Dead' includes 'Litter' and soil organic nitrogen (SON) or soil organic carbon (SOC). Significant source variables (p < 0.05) with respect to ecosystem parts are indicated with asterisks: ns = p >0.05, * p =0.05−0.01, ** p =0.01−0.001, *** p <0.001.

	N stock (ton ha^{-2})							
Source	Shoots	Roots	Moss	Living	Litter	SON	Dead	Total
Colony x depth								
Df Numerator	1	1	1	1	1	1	1	1
Df Denominator	15	15	15	15	15	15	15	15
F-value	5.82	0.24	1.62	0.03	13.63	7.20	9.56	3.29
p-value	*	ns	ns	ns	**	*	**	ns
Colony								
Df Numerator	-	1	1	1	-	-	-	1
Df Denominator	-	17	17	17	-	-	-	17
F-value	-	0.00	1.15	40.40	-	-	-	83.14
p-value	-	***	ns	***	-	-	-	***
Depth								
Df Numerator	-	1	1	1	-	-	-	1
Df Denominator	-	16	16	16	-	-	-	16
F-value	-	0.28	1.87	0.01	-	-	-	2.32
p-value	-	ns	ns	ns	-	-	-	ns
Colony effect on deep tephra sand								
Df Numerator	1	-	-	-	1	1	1	-
Df Denominator	8	-	-	-	8	8	8	-
F-value	0.00	-	-	-	11.97	0.00	0.00	-
p-value	***	-	-	-	*	***	***	-
Colony effect on shallow tephra sand								
Df Numerator	1	-	-	-	1	1	1	1
Df Denominator	7	-	-	-	7	7	7	7
F-value	3.21	-	-	-	5.02	0.00	0.00	3.21
p-value	ns	-	-	-	ns	***	***	ns
Depth effect outside colony								
Df Numerator	1	-	-	-	1	1	1	-
Df Denominator	8	-	-	-	8	8	8	-
F-value	0.53	-	-	-	1.83	0.02	0.02	-
p-value	ns	-	-	-	Ns	ns	ns	-
Depth effect inside colony								
Df Numerator	1	-	-	-	1	1	1	-
Df Denominator	7	-	-	-	7	7	7	-
F-value	5.16	-	-	-	11.97	6.57	8.68	-
p-value	ns	-	-	-	*	*	*	-

Table 1. Continued

	Biomass stock (ton ha^{-2})					
Source	Shoots	Roots	Moss	Living	Litter	Total
Colony x depth						
Df Numerator	1	1	1	1	1	1
Df Denominator	15	15	15	15	15	15
F-value	1.83	0.10	1.29	0.21	8.02	0.50
p-value	ns	ns	ns	ns	*	ns
Colony						
Df Numerator	1	1	1	1	-	1
Df Denominator	17	17	17	17	-	17
F-value	10.95	23.30	0.89	23.86	-	25.03
p-value	**	***	ns	***	-	***
Depth						
Df Numerator	1	1	1	1	-	1
Df Denominator	16	16	16	16	-	16
F-value	1.91	0.04	2.29	0.12	-	0.34
p-value	ns	ns	ns	ns	-	ns
Colony effect on deep tephra sand						
Df Numerator	-	-	-	-	1	-
Df Denominator	-	-	-	-	8	-
F-value	-	-	-	-	17.35	-
p-value	-	-	-	-	**	-
Colony effect on shallow tephra sand						
Df Numerator	-	-	-	-	1	-
Df Denominator	-	-	-	-	7	-
F-value	-	-	-	-	1.20	-
p-value	-	-	-	-	ns	-
Depth effect outside colony						
Df Numerator	-	-	-	-	1	-
Df Denominator	-	-	-	-	8	-
F-value	-	-	-	-	0.00	-
p-value	-	-	-	-	ns	-
Depth effect inside colony						
Df Numerator	-	-	-	-	1	-
Df Denominator	-	-	-	-	7	-
F-value	-	-	-	-	8.95	-
p-value	-	-	-	-	*	-

Table 1. Continued

	C stock (ton ha^{-2})								N accumulation rate
Source	Shoots	Roots	Moss	Living	Litter	SOC	Dead	Total	
Colony x depth									
Df Numerator	1	1	1	1	1	1	1	1	1
Df Denominator	15	15	15	15	15	15	15	15	15
F-value	2.78	0.24	0.63	0.03	13.63	25.42	37.69	3.29	5.92
p-value	ns	ns	ns	ns	**	***	***	ns	*
Colony									
Df Numerator	1	1	1	1	-	-	-	1	-
Df Denominator	17	17	17	17	-	-	-	17	-
F-value	22.06	32.70	0.40	40.40	-	-	-	83.14	-
p-value	***	***	ns	***	-	-	-	***	-
Depth									
Df Numerator	1	1	1	1	-	-	-	1	-
Df Denominator	16	16	16	16	-	-	-	16	-
F-value	2.66	0.28	2.73	0.01	-	-	-	2.32	-
p-value	ns	ns	0.12	ns	-	-	-	ns	-
Colony effect on deep tephra sand									
Df Numerator	-	-	-	-	1	1	1	-	1
Df Denominator	-	-	-	-	8	8	8	-	8
F-value	-	-	-	-	11.25	126.8	206.2	-	171.29
p-value	-	-	-	-	*	***	***	-	***
Colony effect on shallow tephra sand									
Df Numerator	-	-	-	-	1	1	1	-	1
Df Denominator	-	-	-	-	7	7	7	-	7
F-value	-	-	-	-	5.14	30.95	24.78	-	24.69
p-value	-	-	-	-	ns	***	**	-	**
Depth effect outside colony									
Df Numerator	-	-	-	-	1	1	1	-	1
Df Denominator	-	-	-	-	8	8	8	-	8
F-value	-	-	-	-	1.89	0.00	0.00	-	0.10
p-value	-	-	-	-	ns	ns	ns	-	ns
Depth effect inside colony									
Df Numerator	-	-	-	-	1	1	1	-	1
Df Denominator	-	-	-	-	7	7	7	-	7
F-value	-	-	-	-	11.25	28.48	40.17	-	5.15
p-value	-	-	-	-	*	**	***	ns	ns

Table 2. Results of two-way ANOVA's of the depth distribution of SON (soil organic nitrogen) and Root N (root nitrogen) per soil depth, using colony and tephra depth as fixed variables. Significant source variables (p < 0.05) with respect to soil depth are indicated with asterisks: ns = p >0.05, * p =0.05−0.01, ** p =0.01−0.001, *** p <0.001.

	SON				Root N			
	Soil depth (cm)							
Source	0-5	5-10	10-20	20-30	0-5	5-10	10-20	20-30
Colony x depth								
Df Numerator	1	1	n/a	n/a	1	1	1	n/a
Df Denominator	11	11	n/a	n/a	15	15	15	n/a
F-value	0.01	0.63	n/a	n/a	0.28	2.06	0.20	n/a
p-value	ns	ns	n/a	n/a	ns	ns	ns	n/a
Colony								
Df Numerator	1	1	1	1	1	1	1	1
Df Denominator	12	12	6	6	16	16	16	17
F-value	0.25	0.15	0.49	0.13	16.18	1.15	6.83	4.59
p-value	ns	ns	ns	ns	***	ns	*	*
Depth								
Df Numerator	1	1	n/a	n/a	1	1	1	n/a
Df Denominator	13	13	n/a	n/a	17	17	17	n/a
F-value	55.15	10.45	n/a	n/a	10.76	1.49	4.54	n/a
p-value	***	**	n/a	n/a	**	ns	*	n/a

n/a: Not applicable: No SON or Root N in shallow tephra sand

5.5 Discussion

5.5.1 Nitrogen accumulation outside the seabird colony

The mean N accumulation rate in soil and biomass outside the seabird colony during 50 years since Surtsey first emerged (0.7 kg ha^{-1} y^{-1}, Fig. 4), represents about 50-60% of the estimated annual atmospheric N deposition rate in Iceland corrected to 1600 mm annual precipitation (1.3 – 1.4 kg N ha^{-1} y^{-1}) (Gislason et al., 1996, Sigurdsson et al., 2005c). This estimate of 50-60% retention is somewhat low compared to the average N retention in systems under low N input (75%, Thomas et al., 2013) and even more so compared to the >80% N retention in bare dune habitats reported by ten Harkel et al. (1998). This could be explained by the initial lack of plants on the island, resulting in higher leakage during the first years. After establishment of the primary colonizers in the 1970s and 1980s, the total aboveground vegetation cover outside the colony has remained low and stable (3.0% ±0.3) since the start of detailed measurements in 1994 (Magnússon, unpublished data). Despite the very sparse aboveground vegetation cover, it became evident during sampling that the unvegetated tephra sand areas were fully colonized by roots. Another study, which took place on and around *Leymus* dunes in the same area outside the seagull colony, but not within our permanent study plots, found

similar dense root distribution (Stefansdottir et al., 2014). Therefore, we can assume that after vegetation establishment the N retention on Surtsey has increased and is now close to the 75-80% reported for ecosystems with low N inputs (Thomas et al. 2013).

We assume atmospheric deposition to be the main source of N outside the seabird colony. Additional mechanisms of N input could be: (1) N inputs from volcanic processes, (2) chemical weathering of N-containing bedrock, (3) N input from overflying birds, (4) symbiotic N_2 fixation (5) free living N_2 fixation and (6) N fixation by lichens and biological soil crusts (BSC). Mechanisms (1) and (2) can be largely rejected, since Ponnameruma et al. (1967) showed that the tephra sands of Surtsey did not contain any organic N and very little inorganic NH_4 and NO_3; the latter probably mostly originating from rainfall during the first 4 years since the eruption started. This study did report some inorganic N in recently deposited tephra, which could indicate volcanic N sources (cf. Huebert et al., 1999). However, the concentrations were lower in the older tephra layers, probably indicating leaching due to initial lack of biological activity to retain the NO_3. Further, we argue that N deposited by overflying birds (3) was of minor importance, because, as was stated above, total plant cover did not increase between 1994 and 2012 in any of the permanent plots outside the seabird colony (Magnússon, unpublished data). A steady increase of vegetation cover after 1986 (the establishment of the colony) would be expected if overflying birds added a substantial amount of N. Also N input mechanisms (4) and (5) were probably of lesser importance as well, since to date no plant species known to have N_2-fixing symbionts have colonized Surtsey (Magnússon et al., 2014), and because the low water retention of the tephra sand and the low soil temperatures would not support large populations of free-living N_2 fixing microbes (Zielke et al., 2005, Sorensen et al., 2006). The mean annual soil temperature and volumetric water content were 7.5 °C and only 8.4%, respectively, at 5 cm depth during 2010-2012 (unpublished data). Mechanism (6), N-fixation by lychens or biological soil crusts, must be occurring on Surtsey, because Kristinsson and Heidmarsson (2009) found known N_2-fixing species on the island. However, these species had a very limited distribution on the island, and their N input to the ecosystem is therefore assumed to be negligible for the island as a whole.

5.5.2 Nitrogen accumulation inside the seabird colony

The SON values from Surtsey ranged from 0.03 to 0.65 ton ha^{-1}, and span the whole range that Lin et al. (2000) modeled for Iceland (0.0 - 0.5 ton ha^{-1}). This shows well the large effect the seabirds have had on the N stock on Surtsey in only 27 years. The apparent lack of other major N inputs on Surtsey, which typically complicate the quantitative estimation of seabird-

driven N input rates, enabled us to calculate a reliable estimate of N input rates by seabirds from the measured N stocks and seabird colonization duration.

The seabirds increased the ecosystem N-stock 30-fold during 26-27 years, compared to the area outside the seagull colony, equivalent to an input rate of on average 47 kg ha^{-1} y^{-1}. This value was much higher than we originally hypothesized, but it corresponded almost exactly with the estimate of Bancroft et al. (2005) (50.9 kg ha^{-1} y^{-1}) for a Wedge-tailed Shearwaters (*Puffinus pacificus*) colony on Rottnest Island (West-Australia), who used guano traps to estimate seabird-derived N deposition. We are not aware of other studies that attempted to estimate seabird N input rates, but our own calculations, based on a study of Breuning-Madsen et al. (2010), who measured the N stocks in a 24-years old Cormorant colony in the Horsens Fjord (Denmark), resulted in even higher N input rates (an average of 292 kg ha^{-1} y^{-1}).

The SON concentration in the upper 10 cm of soil inside the bird colony at Surtsey (2.5 mg g^{-1} DM) was higher than what was found by Ligeza and Smal (2003) in perennial colonies of piscivorous birds in northern and eastern Poland (~0.6 mg g^{-1} DM), but was very similar to that reported by Anderson and Polis (1999) for seabird colonies on hyperarid, naturally nutrient-poor islands in the Gulf of California (~2 mg g^{-1} DM). It must be noted that both these studies investigated longer-lived bird colonies. Given the high organic-matter retention capacity of the andosols at Surtsey, it might be that SON concentrations increase even further, but this remains speculation.

5.5.3 Effect of tephra-layer depth on N retention

Even if SON and litter N accumulation were significantly higher at the deeper tephra sands within the seagull colony, we did not find a significant effect of tephra-layer depth on total N stock, when biomass-N was included. This suggests a high N retention potential in the upper centimeters of the tephra sand. This finding contradicts with our second hypothesis, that deeper substrates would positively affect N retention, as was shown by Selmants et al. (2014). Faster succession rates on the deeper tephra sands in Surtsey (Magnússon and Magnússon, 2000, Del Moral and Magnússon, 2014) can therefore not be explained solely by increased N accumulation rates.

5.5.4 Shifts from belowground to aboveground plant biomass

Despite the fact that the median R/S ratio outside the colony (18) was almost three times higher than the upper extreme for cool temperate deserts reported by (Mokany et al., 2006), it corresponded closely with the mean ratio of 19 reported by (Stefansdottir et al., 2014) for the

Leymus area outside the seabird colony. For the grassland inside the colony, the median R/S of 5 did not differ much from the median reported by Mokany et al. (2006) for cool temperate grasslands without bird colonies. Given the more severe nutrient limitation expected for cool temperate grasslands compared to grasslands in a seabird colony, this similar R/S was unexpected. We therefore assume that the low water retention capacity of the sands on Surtsey caused frequent droughts and that the positive impact of drought on R:S offset the negative effect of seabird-driven nutrient inputs (see discussion below).

Changes in nutrient availability can often be detected by looking at R/S ratios (Levang-Brilz and Biondini, 2003, Chu et al., 2006), as increased nutrient availability promotes investment in aboveground plant parts. Therefore, we hypothesized (hypothesis iii) a decrease in R/S ratio with increasing ecosystem N stock. Despite the fact that previous research has shown negative correlations between R/S ratio and nutrient availability in grassland ecosystems (Levang-Brilz and Biondini, 2003, Chu et al., 2006), no such relationship was found on Surtsey, neither inside, nor outside the colony. Outside the seabird colony, such a potential relationship might be concealed by the patchiness of the aboveground vegetation, the homogeneous root distribution (Stefansdottir et al., 2014) and the relatively small size of the measurement frames. The combination of these three factors probably explains why we measured several unrealistically high R/S ratio's, ranging up to 194. Inside the seabird colony, the lack of relationship between R/S and N stock is possibly due to other factors, such as limiting water availability, overruling the effect of N availability on the R/S ratio. Drought has been reported to be an important determinant of R/S ratios (Donkor et al., 2002, Gianoli et al., 2009, Dreesen et al., 2012) and water infiltration in Andosols is rapid compared to most other soils (Basile et al., 2003), especially when the carbon content is below 12% as is the case on Surtsey (Arnalds, 2008). Therefore, we attribute the absence of a clear nutrient influence on R/S to an overruling effect of drought in all occurring nutrient situations.

5.5.5 Shifts from belowground to aboveground N and C and 'N use efficiency'

The clear shift in both N and C from belowground to aboveground stocks inside the colony agrees with our hypothesis iii that the increase in nutrient availability inside the colony would stimulate the system to invest more in aboveground growth. This relationship between nutrient availability and growth investment confirms many earlier studies (e.g. Warembourg and Estelrich, 2001, Wang et al., 2008, Gao et al., 2011, Zhou et al., 2014). This shift from below- to aboveground was accompanied by a doubling of the N use efficiency; the grassland

inside the colony supported ~ 2 times more biomass and biomass-C per g N, compared to the almost unvegetated area outside the colony. This large increase in the 'N use efficiency' indicated a positive feedback between N stock and the storage of both biomass and C. This increase was caused by the drastic shift in C distribution: from a 76:24 towards a 32:68 proportion in SOC and biomass, respectively. The C/N ratio of SOC was smaller than that of biomass (10.4 ± 0.6 vs. 39.1 ± 2.3 (mean ± SE)), in line with the C/N ratios for undisturbed soils reported by Chapin et al. (2011) (~14) and for leaves reported by Sterner and Elser (2002) (~36). Hence, the overall C/N ratio of ecosystems with most C stored in SOC (such as the non-colonized area) is typically smaller than the overall C/N ratio of ecosystems with a high fraction of C stored in living biomass (such as in the seabird colony).

The reason why higher N-stocks can support more biomass and C per unit N remains to be better explored. Several studies on immature volcanic soils in New Zealand, Chile and Japan have found a positive relationship between total N stock and net N-mineralization rates (and consequently plant available N) (Parfitt et al., 2005, Hirzel et al., 2010, Yamasaki et al., 2011). In the last study, the increased N-mineralization following N-addition was proved to be correlated with the inhibition of microbial N-immobilization (and consequently decreased competition between plant and microbial N-uptake). This inhibition of N uptake by microbes was proposed to result from (1) nitrification-induced acidification, (2) increased soil osmotic potential to toxic values and (3) an inhibition of ligninolytic enzyme production. The significant acidification inside the seabird colony on Surtsey (Sigurdsson and Magnússon, 2010) supports this hypothesis, however, further study is needed to investigate the role of microbial N-immobilization in N-availability for plants on Surtsey.

5.5.6 Seabird effect on biomass and C stocks

Aboveground biomass outside the colony (0.13 ± 0.08 ton ha^{-1}) was very small and has almost not changed since the first measurements of aboveground biomass in the permanent plots in 1999 (0.1 ton ha^{-1}) (Magnússon et al., 2009). This invariable aboveground biomass probably also indicates relatively little changes in belowground biomass. As biomass degradation is an important source of SOC (Kögel-Knabner et al., 2013), the low biomass production rates outside the seagull colony explain largely the quasi constant SOC concentration and the lack of soil formation. Since 1986, the seabird influence caused an over 50-fold increase of total biomass, from 0.7 to 36 ton ha^{-1}, which corresponds to a shift from values typical for deserts (0-20 ton DM ha^{-1}) to values typical for grassland ecosystems (20 to 50 ton DM ha^{-1}) (Larcher, 2003). This large increase in only 25 years can largely be attributed to seabird N

input, as there was a strong positive relationship between total ecosystem biomass stock and N stock (as proxy for available N) inside the seabird colony.

The aboveground biomass has been increasing nearly linearly from 1.4 ton ha^{-1} in 1999 to 4.1 ton ha^{-1} in 2007 (Magnússon et al., 2009) to 5.9 ± 0.9 ton ha^{-1} (error = SE) in 2012-2013 . This steady increase indicates that aboveground limitations on growth, such as competition for light and space, were minor compared to the belowground limitations for water and nutrients. This could be expected in a natural grassland ecosystem with relatively low nutrient levels (Kiaer et al., 2013). This was also supported as well by the strong link between biomass and N stock.

The C stock in living biomass showed a clear shift as well, from a typical desert C stock outside the colony (0.19 ton ha^{-1}; desert ecosystems: 0-10 ton ha^{-1}; Larcher, 2003) to a stock typical for grassland ecosystems inside the colony (13 ton ha^{-1}; grassland ecosystems: 10-25 ton ha^{-1}; Larcher, 2003). However, despite the fact that biomass and living C-stock both have been developing from desert to grassland, the SOC accumulation was lagging behind, and remained far below the average for temperate grassland soils according to (Schlesinger, 1997) (see also 5.5.7).

5.5.7 Did the N accumulation accelerate soil development?

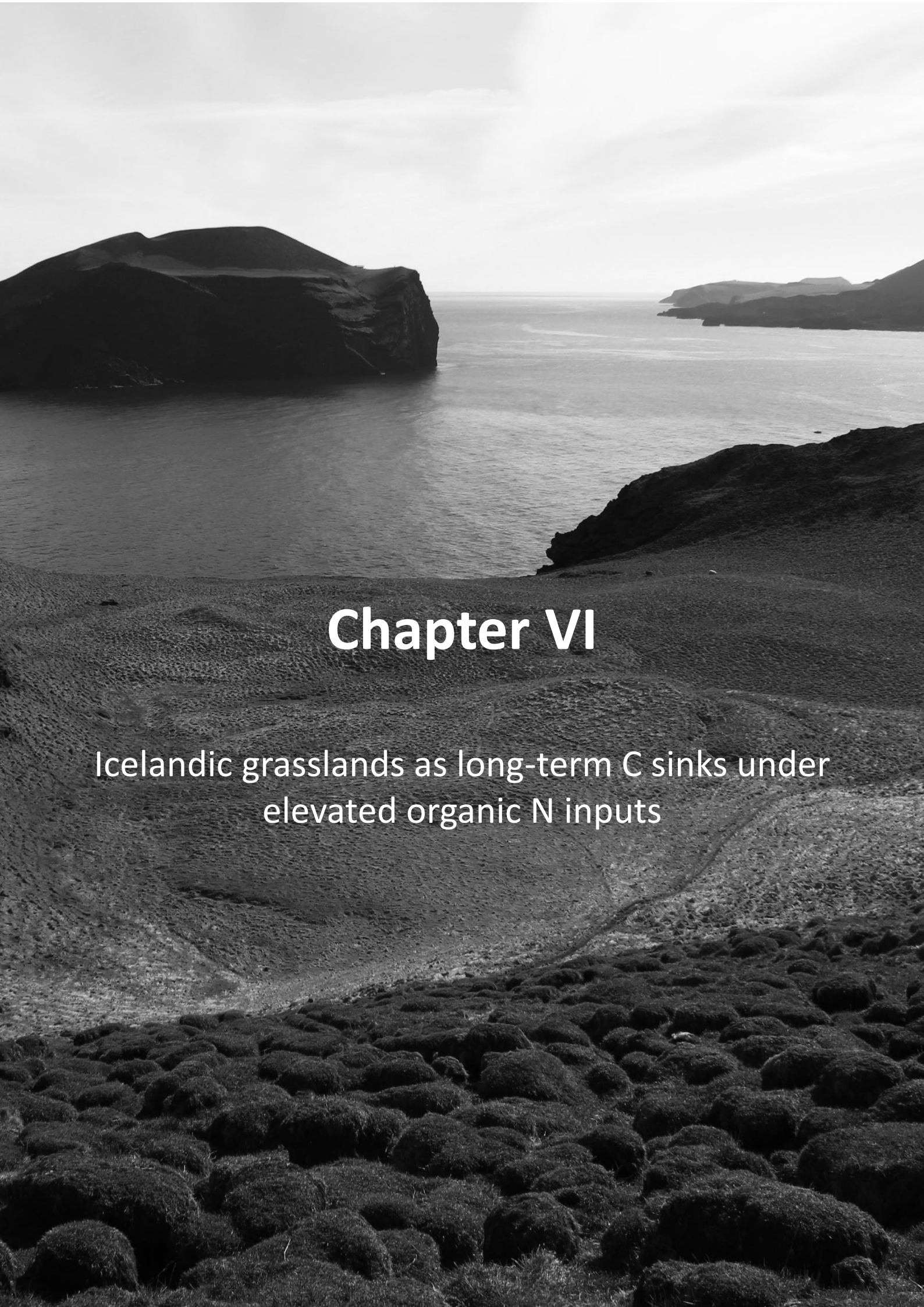
Outside the colony, the soil development has been proceeding very slowly, as the SOC concentration, which is a reliable indicator of soil development (Kögel-Knabner et al., 2013), had only increased slightly since 1986 in the upper 5 cm of the tephra sand (0.12 ± 0.02% SOC vs. 0.10 ± 0.02% SOC; errors are SE's) (Arnalds, unpublished data). When Surtsey had just emerged, the SOC concentration was 0.012 ± 0.009% (error is SE) (Ponnameruma et al., 1967).

Inside the colony, the SOC concentration has been increasing with a factor of 10 to 60 since the establishment of the colony, from 0.08% (taking the 1986 value as baseline SOC concentration as this was the first year of permanent seabird colonization) to 0.9 ± 0.3 % on deep tephra sand an 4.6 ± 0.4 % on shallow tephra sand (errors are SE's). This large increase agrees with (Stockmann et al., 2013) who hypothesized that priming effects, such as N addition, might stimulate SOC accumulation in grasslands with a high R/S. However, Stockmann et al. (2013) reported that grassland soils typically contain more than 1 - 3% SOC. Yang et al. (2014), for instance, measured a SOC concentration of 28 up to 58% in an arid grassland in North China. So despite the rapid buildup, the area inside the bird colony has not yet been lifted to a SOC concentrations that are typical for grassland ecosystems. Also the

current stock of SOC of about 4 (shallow) to 10 (deep) ton ha^{-1} is still marginal compared to the average SOC stock in temperate grassland soils of 192 ton ha^{-1} according to Schlesinger (1997). This indicates that the soil development at Surtsey is still in its first phase. Therefore, we expect that the SOC content will continue to accumulate for many centuries before it reaches an equilibrium with the aboveground productivity.

5.6 Conclusion

The calculated annual N accumulation rate outside the seabird colony amounted to ca. 50-60% of the estimated atmospheric N deposition during the past 50 years. This approximates values for systems under low N input and bare dune habitats. The seabird derived N input was higher than was expected based on earlier estimates for Surtsey. There was no difference in N retention between shallow en deep tephra sands, when total N stocks in biomass and soil were compared. Different succession rates on deep tephra sands within the seagull colony can therefore not be explained by increased N retention there. There was a clear shift from belowground to aboveground biomass, C and N inside the colony. Consequently, the R/S decreased from very high values outside the colony to more moderate values inside. Further, the 'N-use efficiency' or the amount C fixed per g N, was twice as high inside the colony. The accumulation of SOC, which is an indicator of soil development, was enhanced inside the seabird colony. However, despite the fact that the biomass and C stock of living material reached the typical range of grasslands, the SOC stock remained still small compared to SOC stocks in well-developed grasslands. Therefore we expect that the SOC stock will continue to increase for many centuries until it reaches an equilibrium with the litter production.



Chapter VI

Icelandic grasslands as long-term C sinks under elevated organic N inputs

6 Icelandic grasslands as long-term C sinks under elevated organic N inputs

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6.1 Abstract

About 10 % of the anthropogenic CO_2 emissions have been absorbed by northern temperate and boreal terrestrial ecosystems during the past decades. It has been hypothesized that part of this increasing carbon (C) sink is caused by the alleviation of nitrogen (N) limitation by increasing anthropogenic N inputs. However, little is known about this N-dependent C sink. Here, we studied the effect of chronic seabird-derived N inputs (47–67 kg N ha^{-1} yr^{-1}) on the net soil organic C (SOC) storage rate of unmanaged Icelandic grasslands on the volcanic Vestmannaeyjar archipelago by using a stock change approach in combination with soil dating. We studied both early developmental soils (50 years) and mature soils (1,600 years), and for the latter we separated between decadal (topsoil) and millennial (total soil profile) responses, where the SOC stocks in the topsoil accorded to 40–50 years of net SOC storage and those in the total soil to 1,600 years of net SOC storage. We found that enhanced N availability - either from accumulation over time, or seabird derived - increased the net SOC storage rate. Under low N inputs, the early developmental soils were weak decadal C sinks (0.018 ton SOC ha^{-1} yr^{-1}), but this increased quickly under elevated N inputs to 0.29 ton SOC ha^{-1} yr^{-1}, thereby equalling the decadal SOC storage rate of the unfertilized mature site. Furthermore, at the mature site, chronic N inputs not only stimulated the decadal SOC storage rate, but also the millennial SOC storage was consistently higher at the high N input site. Hence, our study suggests that Icelandic grasslands, if not disturbed, can remain C sinks for many centuries under current climatic conditions and that chronically elevated N inputs can induce a permanent strengthening of this sink.

6.2 Introduction

The global C cycle plays a prominent role in climate change and is greatly influenced by anthropogenic C emissions (IPCC, 2013). During the past 20 years, terrestrial ecosystems have been absorbing ca. 30 % of the total anthropogenic C emissions; a sink that has been increasing (Le Quere et al., 2009, IPCC, 2013). However, the future evolution of the terrestrial sink-source balance is highly uncertain, and depends on a multitude of factors, such as land use and nutrient dynamics (Poulter et al., 2011, IPCC, 2013, Fernandez-Martinez et al., 2014, Wieder et al., 2015).

Northern temperate and boreal terrestrial regions (>50° N) are important C sinks (Ciais et al., 1995, Poulter et al., 2011), accounting for about 30 % of the global net terrestrial C uptake (White et al., 2000). It has been hypothesized that this observed C sink is to a large extent caused by the alleviation of widespread N limitation in these ecosystems (Hudson et al., 1994, Lloyd, 1999, Schlesinger, 2009) due to the three to five fold increase in anthropogenic N deposition during the past century (Galloway et al., 2008, Gundale et al., 2014). Although the increase in anthropogenic N deposition at high northern latitudes has been modest during the past decades (Galloway et al., 2008), limiting their contribution to this N-driven C sink, a substantial increase in anthropogenic N deposition is expected in this region in the near future (Lamarque et al., 2011). This implies that high latitude northern ecosystems could become significant N-driven C sinks during the next century. The continuation of the northern C sink is highly debated, with estimates ranging from a current decline to a steady increase until at least the middle of this century (Lloyd, 1999, White et al., 2000, Cramer et al., 2001, Bachelet et al., 2003, Pepper et al., 2005, Friedlingstein et al., 2006, Canadell et al., 2007, Morales et al., 2007, Le Quere et al., 2009, Tao and Zhang, 2010, IPCC, 2013, Todd-Brown et al., 2013, Arora and Boer, 2014). This underlines the necessity for a better understanding of the N-induced stimulation of long-term C storage in northern ecosystems.

Uncertainties in the further development of this N-dependent northern C sink are related to several aspects. First of all, in spite of the large number of high latitude studies that investigate the short-term effect of N addition on the rates of aboveground C input fluxes (GPP) and ecosystem C output fluxes (litter decomposition and heterotrophic and autotrophic respiration) (Wookey et al., 2009, Bouskill et al., 2014), only a few studies have investigated the effect of long-term N fertilization on total ecosystem C stocks. Rare studies on this subject

report contradicting conclusions, which vary with fertilization rate and litter quality (Hyvonen et al., 2007, Hopkins et al., 2009, Nilsson et al., 2012, Gundersen et al., 2014).

Further, although it is crucial to understand the long-term effects of N on net ecosystem C storage, the few studies have investigated the effect of chronic N inputs (>5–8 y) on SOC and ecosystem C stocks (Hyvonen et al., 2007, Janssens et al., 2010, Leblans et al., 2014) are typically confounded by superficial soil sampling (see e.g. Hobbie et al., 2002, Rumpel and Kogel-Knabner, 2011, Fornara et al., 2013, Olson and Al-Kaisi, 2015). Soil organic carbon (SOC) dynamics and their responses to N addition are regulated by contrasting mechanisms in the topsoil and the subsoil (Mack et al., 2004, Zehetner, 2010, Appling et al., 2014, Batjes, 2014, Tan et al., 2014). Topsoil decomposition is mainly regulated by nutrient supply, while subsoil decomposition is limited by energy as the proportion of recalcitrant litter increases with depth (Fontaine et al., 2007, Wutzler and Reichstein, 2008). Therefore, focusing only on the upper soil layers can lead to incorrect estimations of the actual long-term SOC storage.

Finally, while most ecosystems are in an intermediate successional stage as a consequence of former disturbances (Kroël-Dulay et al., 2015), this is often neglected in studies on net ecosystem C storage. Ignoring ecosystem successional and soil developmental stage likely contributes to a substantial part of the uncertainties in global C dynamics (Chapin et al., 2011). For instance, the link between elevated N inputs and net ecosystem C storage might change during the course of soil maturation (Crocker and Major, 1955, Saynes et al., 2005, Seedre et al., 2011, Appling et al., 2014). This is especially true for N-limited ecosystems (Aerts and Chapin, 2000, Reich and Oleksyn, 2004), where total N stocks and plant available N increase during the soil maturation process (Crocker and Major, 1955, White et al., 2004, Rhoades et al., 2008, Smithwick et al., 2009).

Unmanaged northern grasslands have an extensive coverage (10 % of the global terrestrial surface; Chapin et al., 2011) and have a large SOC storage potential (Aerts et al., 2003, Sui and Zhou, 2013), whereof >95 % of the total C is stored as SOC (Grace, 2004). Moreover, it is likely that the response of these typically N limited systems (LeBauer and Treseder, 2008) to long-term N fertilization will be more pronounced than more southern grassland sites with higher background N deposition (Hopkins et al., 2009). However, the role of chronic N inputs in the net SOC storage in these ecosystems is yet unclear.

In this study we therefore investigated the effect of chronically elevated N inputs on the northern C sink by quantifying the total ecosystem C stocks and net SOC storage rates of unmanaged Icelandic grasslands. We studied two site pairs with contrasting natural N inputs

and soil developmental stage on the volcanic Vestmannaeyjar archipelago (south Iceland, Fig. 1). Variations in natural N inputs were caused by the topographical preferences of seabirds to form breeding colonies at specific locations. As N is by far the most limiting element in the ecosystems under investigation (Aerts and Chapin, 2000, Leblans et al., 2014), the influence of other seabird derived nutrient inputs was assumed to be negligible. Next to nutrients, seabird guano also contains organic C (~ 25% at our study sites; R. Aerts, personal communication), which is important to take into consideration in a study that investigates C storage. Nonetheless, also the influence of seabird-derived organic C inputs on total C storage was assumed negligible, as it was found to account for only 1.2 to 1.8% of the total organic C inputs (assuming a vegetation turnover rate of 1 year, a realistic value for alpine grasslands; Perez and Frangi, 2000). Further, the size of this contribution is expected not to change during the C stabilization process, as previous studies found no difference between the C storage efficiency of biomass residues and bird manure (Rahman, 2013, Hua et al., 2014, Rahman, 2014). We distinguished between decadal (topsoil; 40–50 years) and millennial (total soil profile; 1,600 years) responses of net SOC storage to chronic N inputs by soil layer dating. Finally, we focused on the importance of soil developmental stage, comparing early developmental (E) versus mature (M) soils under chronically low (E_{NL} and M_{NL}) and high (E_{NH} and M_{NH}) N input conditions.

We expected that soil N stocks and N availability would be smaller at sites with low natural N input rates than at sites with high natural N inputs, but we also expected that the early developmental stage with high N input rates (E_{NH}) would not have reached the total N stock and availability of the mature soils with low N inputs (M_{NL}). Hence, we expected the following sequence of soil N stocks and availability: $E_{NL} < E_{NH} < M_{NL} < M_{NH}$. Further we hypothesized that N availability would better explain soil C inputs (plant production) than soil N stocks in these N limited systems, as a large part of the N in andosols is generally inaccessible for plant roots (Gudmundsson et al., 2004).

We hypothesized that the decadal net SOC storage rate of mature Icelandic grasslands would be stimulated by chronically elevated N inputs. We expected that this stimulation would persist at the millennial timescale, but that the effect would be smaller, as a consequence of SOC saturation processes in the subsoil (Zehetner, 2010, Olson and Al-Kaisi, 2015). Early developmental Icelandic grasslands with low natural N inputs were expected to have lower decadal net SOC storage rates compared to mature grasslands because of their relatively higher N limitation. We did expect, for the same reason, that the relative positive effect of chronic N inputs would be more pronounced in the early developmental grassland

sites. These hypotheses result in the following order of net SOC storage at the decadal timescale E_{NL} <<< E_{NH} < M_{NL} < M_{NH} and at the millennial timescale M_{NL}< M_{NH}.

6.3 Material and methods

6.3.1 Study sites

This study was performed on three islands of the volcanic Vestmannaeyjar archipelago (63°25' N, 20°17' W; south Iceland; Fig. 1) in mid-July 2012 and 2013. The climate is cold temperate, with a mean annual temperature at Stórhöfði (meteorological station on the main island, Heimaey) of 5.1 °C between 1963 and 2012, and a min. and max. monthly average of 1.3 and 9.6 °C. Mean annual precipitation during the same period was 1600 mm (Icelandic Meteorological Office; www.vedur.is). The main vegetation type on the Vestmannaeyjar archipelago are lush grasslands, except in areas that are unsuitable for seabird colonization where heathlands, herb slopes or dry meadows can be found (Magnússon et al., 2014).

Two pairs of sites with low and high natural N inputs (negligible and major seabird influence respectively) were established on islands where soils were either at an early developmental stage (E) or on islands with mature soils (M) (Magnússon et al., 2014). The low N sites (E_{NL} and M_{NL}) received on average 1.3–1.4 kg N ha^{-1} yr^{-1} in the form of natural background N input by atmospheric deposition (Sigurdsson and Magnússon, 2010). No symbiotic N_2 fixing vascular plant species were found in any of the study plots (Magnússon et al., 2014). The high N input sites (E_{NH} and M_{NH}) received on average 47 kg N ha^{-1} yr^{-1} in the case of E_{NH} (Leblans et al., 2014) and 67 kg N ha^{-1} yr^{-1} in the case of M_{NH}, an estimation based on a bioenergetics model of Wilson et al. (2004) and Blackall et al. (2007) in combination with nesting densities from Hansen et al. (2011). All sites had similar bedrock characteristics (see further) and were located within 25 km distance from each other so that the influence of climate could be assumed to be negligible.

The E_{NL} and E_{NH} sites were located on the island Surtsey (Fig. 1), a 50-year-old volcanic island that was formed in an eruption between 1963 and 1967. Both E_{NL} and E_{NH} were located on the lower plain of basaltic lava flows that are partly filled with sand and silt (Jakobsson et al., 2007). While E_{NL} was virtually free of seabird influence, E_{NH} was located inside the confines of a well-defined permanent breeding colony of lesser black backed seagulls (*Larus fuscus*), great black-backed gulls *(Larus matitiumus*) and herring gulls (*Larus argentatus*) that was established in 1986 on the southwestern part of the island (Leblans et al., 2014, Magnússon et al., 2014). Limited soil formation had taken place at E_{NL}, while the E_{NH}

soil profile consisted of an O horizon, on top of a premature A horizon (max. 10 cm deep) and was classified as an Andosol (Arnalds, 2015). The pH at E_{NL} was significantly higher than at E_{NH} (7.6 vs. 6.6; Sigurdsson and Magnússon, 2010). The plant community at E_{NL} was in an early successional transitional state between barrens and grassland, while the plant community at E_{NH} had reached an early successional grassland stage (Magnússon et al., 2014).

The M_{NL} site was located on Heimaey, the largest island of the Vestmannaeyjar archipelago (13.4 km^2) (Fig. 1). It was established in Lyngfellisdalur, a valley on the southeastern part of the island. The valley is visually isolated from the sea, which makes it an unsuitable breeding location for seabirds. No seabird colonies were found within the valley and it is highly unlikely that they ever existed in the past because of the topographical conditions. The surfacing basaltic bedrock dates back to 5,900 AD (Mattsson and Hoskuldsson, 2005) and is covered by a mature soil classified as 'Brown Andosol' (Arnalds, 2008), which typically have a pH between 5.5 and 7.5 (Arnalds, 2015). The M_{NL} site hosts a species-rich grassland community, typical for low nutrient conditions (Magnússon et al., 2014). The M_{NH} site was located on the nearby island Ellidaey (0.46 km^2) (Fig. 1) which hosts the second largest puffin colony (*Fratercula arctica*) of the archipelago, with 16,400 breeding pairs (Hansen et al., 2011). Due to its topographical conditions it is highly likely that the island has served as breeding ground for seabirds from early times. The M_{NH} site has similarly aged bedrock and soil characteristics as the nearby M_{NL} site on Heimaey (Mattsson and Höskuldsson, 2003, Magnússon et al., 2014), but the nutrient-rich conditions have given rise to the development of a species-poor grassland community (Magnússon et al., 2014).

The soils of M_{NL} and M_{NH} contained two well-defined volcanic ash layers that could be used to date the profile; the lower one from a volcanic eruption in ca. 395 AD, which most probably originated from the mainland volcano Katla (Larsen, 1984), while the upper one originated from an eruption on Heimaey in 1973 (Morgan, 2000). Both ash layers varied in thickness between 0.5 and 5 cm. The 395 AD layer was located at 110 ± 5 (SE) cm soil depth at M_{NL} and at 160 ± 5 (SE) cm soil depth at M_{NH}, and coincided with the maximum depth of undisturbed soil, as below it an eroded gravel layer was found. The 1973 AD layer was located at 6.4 ± 0.4 (SE) and 11.4 ± 1.7 (SE) cm soil depth at M_{NL} and M_{NH}, respectively, and could be considered as the separation between topsoil and subsoil. At both sites, the topsoil contained over 70 % of the roots. At E_{NL} and E_{NH}, the vegetation and soil development was too recent to detect the 1973 AD ash layer, and, as no subsoil was developed yet, all soil was considered as topsoil.

6.3.2 Experimental setup

At E_{NL} and E_{NH}, all measurements were performed at ten and eight permanent 10x10 m survey plots, respectively. These permanent survey plots were established on Surtsey between 1990 and 1995 (Magnússon et al., 2014) and have been followed closely ever since, yielding an extensive amount of published data on important background variables such as vegetation development, seabird nesting history, soil parameters and gas exchange. To keep the permanent survey plots undisturbed for future research, destructive soil- and vegetation sampling (see § 2.4, 2.5 and 2.6) were always done outside the confines of the permanent survey plots. Therefore, three 0.2x0.5 m subplots were placed adjacent to each permanent survey plot. In both M_{NL} and M_{NH}, three 0.2x0.5 m subplots were placed adjacent to four 10x10 m research plots (n = 4) that were established in 2013 and will be used in the future for further research. The subplots at M_{NL} and M_{NH} were protected against possible human and livestock influence prior to the measurements (early May – late July) by covering them with 1x1 m enclosure cages. The grazing pressure of M_{NL} and M_{NH}, prior to the installation of the sheep-exclosures, was similar. Therefore, we assume that no major differences in trampling and consequential disruption of soil aggregation took place and that the grazing influence on C storage was minor. Further, additional C and N inputs from livestock were minimal, as no extra feeding was provided, and no signs of major C and N redistributions were observed, as grazing and manure dropping were not separated in space. No such protection against livestock was needed for E_{NL} and $E_{NH,}$ since neither tourists nor domestic animals are permitted on the Surtsey island (Baldurson and Ingadóttir, 2007). Nevertheless, also the early successional sites could be considered ‘grazed’, as graylag geese have colonized the island, feeding upon the grasslands there (Magnússon et al., 2014).

6.3.3 N availability

A relative measure for N availability was obtained using cation- and anion-exchange membranes (PRS™ probes, Western Ag Innovations Inc.; Saskatoon, SK, Canada). The membranes continuously absorb charged ionic species over the burial period, and the N availability is calculated as soil N flux over time. Four sets of membranes were inserted for one week in the topsoil (0–10 cm depth) of each main study plot in mid-July 2013. Afterwards, they were sent to Western Ag Innovations Inc. (Saskatoon, SK, Canada) for further analyses.

6.3.4 Plant analyses

All aboveground parts of vascular plants were harvested in each 0.2x0.5 m subplot, while litter and moss were collected in a 0.2x0.2 m section of the subplot. Subsequently, all vegetation samples were dried for 48 h at 40°C or until weight loss stopped, weighed, and milled using a ball mill (Retsch MM301 Mixer Mill, Haan, Germany) in preparation for further C and N analyses by dry combustion (Macro Elemental Analyser, model vario MAX CN, Hanau, Germany).

In each of the permanent survey plots, the height of three individuals of *Cerastium fontanum* was measured, as it was the only plant species that was common between all four treatments. Further, 2 g dry weight of mature healthy leaves of *C. fontanum* were collected for analyses of N by dry combustion (NC2100 C/N analyser; Carlo Erba Instruments, Italy) and of P by inductively coupled plasma procedure (sequential ICP-OES spectrometer; Jobin Yvon Ultima 2, France), respectively.

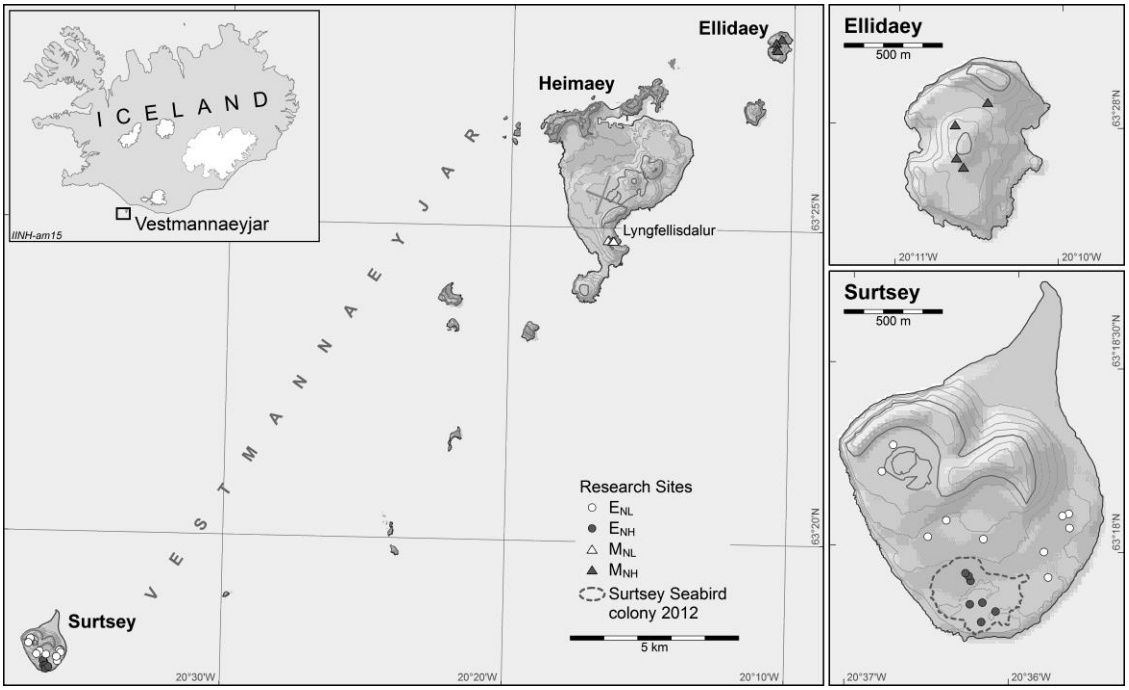


Figure 1: Topographical map of the research area. (A) Location of the Vestmannaeyjar including the three study islands, Surtsey, Heimaey and Ellidaey at the southwest coast of Iceland. (B) and (C) show the islands Ellidaey and Surstey in more detail. The dotted outline on panel C shows the contours of the seabird colony (that was established anno 1986) in 2012. Dots show the research plots at early soil developmental stage under low (○ = E_{NL}) and high (● = E_{NH}) seabird-derived N inputs. Triangles show the research plots at sites with mature soils under low (Δ = M_{NL}) and high (▲ = M_{NH}) seabird-derived N inputs respectively. Map designed by Anette Th. Meier.

6.3.5 Soil analyses

Underneath the vegetation sampling subplots (see Sect. 2.4), two parallel soil cores (8.67 cm diameter) were taken and split into 0–5, 5–10, 10–20 and 20–30 cm depth segments, where depth to the bedrock allowed this at E_{NL} and E_{NH}. At M_{NL} and M_{NH}, two additional 4.82 cm diameter soil cores were extracted down to the 395 AD ash layer from three out of the four main plots with a closed split corer and separated in segments of 30 cm. Each pair of soil cores was used to retrieve the dry weight and C and N concentrations of the fine roots and of the soil fraction < 2 mm, to calculate the stoniness of the soil (the soil fraction > 2 mm; the C and N content of soil particles > 2 mm was assumed to be negligible) and the soil bulk density (BD) and to analyse the grain size distribution of the soil. From the first of the two soil cores, the fine roots were washed out on a 0.5 mm sieve and subsequently treated identically to the aboveground vegetation. From the same core, the stoniness of the soil (fraction of soil particles > 2 mm) was derived using a sieve with a mesh size of 2 mm. The second soil core was dried for 48h at 40°C or until weight loss stopped, and the soil bulk density (dry weight of soil < 2 mm per sampling core volume) was calculated using the following equation:

$$BD\ s_{<2mm} = \frac{(DW\ s_{total} - DW\ s_{>2mm} - DW\ r)}{V\ s_{total}} \quad (1)$$

Where $BDs_{<2mm}$ is the bulk density of the portion of soil particles < 2 mm, DW is dry weight, s_{total} is total soil core, $s_{>2mm}$ is the portion of soil particles > 2 mm, and r are the fine roots and V is the volume.

Afterwards 2 g of soil < 2 mm was sieved from the second soil core and milled with a ball mill (Retsch MM301 Mixer Mill, Haan, Germany) as preparation for further C and N analyses.

Finally, a second aliquot of ~ 2 g of dried soil < 2 mm was sieved from the second soil core for soil texture analyses. Prior to the analyses, organic matter was removed from the samples by treatment with H_2O_2 and iron oxides were disrupted by HCl in accordance to (Pansu, 2003). Subsequently, all samples were analysed for grain size distribution using a particle size analyser (Mastersizer 2000, Malvern instruments, United Kingdom).

6.3.6 Calculation of C and N stocks and net SOC storage rates

The C and N stocks (in ton ha^{-1} and kg ha^{-1} respectively) of vegetation and roots were calculated by multiplying the respective C and N concentration with the dry weight of the sample and correcting for the respective sampling size and depth. The C and N stocks of the soil were calculated by multiplying the respective C and N concentration with the bulk

density of the soil (see equation 1) and transformed to the same units by correcting for the respective core size and depth.

The net SOC storage rate was calculated for both the topsoil (decadal net SOC storage rate) and the total soil profile (millennial net SOC storage rate). The topsoil was defined as the soil layer on top of the 1973 ash layer (accumulated over 40 years and containing > 70 % of the roots; see § 2.1) at the mature sites (M_{NL} and M_{NH}). At the early developmental sites (E_{NL} and E_{NH}), no subsoil had been formed yet. Therefore the total soil profile, which had a comparable age as the topsoil of the mature sites (45 years, accumulated since the eruption ended in 1967), was classified as topsoil. For the mature sites, the total soil profile reached until the 395 AD ash layer (see § 2.1).

The topsoil (decadal) net SOC storage rate (ton SOC ha^{-1} yr^{-1}) was calculated by dividing the topsoil SOC stocks by their respective accumulation time in year. This was 45 years for E_{NL}, 40 years for M_{NL} and M_{NH} (see earlier). For E_{NH}, where the soil had been accumulating under increase N inputs for ~26 years (since the initial seabird colonization of each plot (1986-2012; Magnússon et al., 2014)), the net SOC storage rate was calculated by firs correcting the SOC stock for the amount of SOC accumulated before the start of the colonization, which was derived from the stocks in the E_{NL} site (assuming a constant accumulation rate) and dividing the resulting SOC stock by the respective accumulation time. The SOC net storage rate over the total soil profile (the millennial net SOC storage rate) at M_{NL} and M_{NH} was calculated for consecutive cumulative soil ages, with 200 years intervals, down to the 395 AD ash layer. The age of the soil was calculated by assuming a constant soil accumulation rate between the two ash layers (0.67 and 0.97 mm yr^{-1} at M_{NL} and M_{NH}, respectively). The time resolution of 200 years was chosen because a detailed dating of soil profiles in S-Iceland (distant from active soil erosion areas (Gisladottir et al., 2010)), showed a reasonably stable average accumulation rate over this time scale. The reader should, however, be aware that this is an imprecise calculation .

6.3.7 Data analyses

The effects of the chronically elevated N inputs and soil developmental stage on N stocks, N availability, total aboveground biomass, N/P ratio and plant height of *C. fontanum*, ecosystem C stocks and net SOC storage rate in the topsoil, were tested with a two-way ANOVA, with N input (low/high) and soil developmental stage (early/mature) as fixed factors. In case of significant interaction, the pairwise differences were tested by post hoc LSD tests or Wilcoxon signed rank tests when the requirements of normality and homoscedasticity were

not met. The change in net SOC storage rate with increasing cumulative soil age was tested for M_{NL} and M_{NH} with a two-way ANOVA, with N input (low/high) and cumulative soil age as fixed variables. The correlation between net SOC and net SON storage rate was tested with a Pearson correlation test (conditions of normality and homoscedasticity were met). All tests were performed in R software (R-core-team, 2014) and null hypotheses were rejected at $p < 0.05$.

6.4 Results

6.4.1 N availability and N limitation

The PRS-derived N availability in the main rooting zone (0–10 cm) was significantly lower at the low N input sites (E_{NL} and M_{NL}) than at the high N input sites (E_{NH} and M_{NH}) (Fig. 2.A; Table 1). Accordingly, plot-scale biomass, the height of *Cerastium fontanum* and leaf N/P ratios were significantly higher for the high N input sites (Fig. 3; Table 2). Nonetheless, leaf stoichiometry at the high N input sites still indicated N limitation (Fig. 3.A).

Soil developmental stage had a marginally significant effect on topsoil N availability, with the M having slightly higher N availability than the E (Fig. 2.A; Table 1). Plant height of *C. fontanum* and total aboveground biomass were significantly higher in M than in E, but leaf stoichiometry did not reveal a significant influence of soil developmental stage (Fig. 3; Table 2).

6.4.2 N stocks

The effect of chronically elevated N inputs on total N stocks followed contrasting patterns when only topsoil was taken into account or when the total soil profile above the 395 AD ash layer was included. In the topsoil, elevated N inputs increased the total ecosystem N stocks significantly, from 34 to 1080 kg ha^{-1} for E_{NL} and E_{NH} and from 870 to 2200 kg ha^{-1} for M_{NL} and M_{NH}. This N input effect was significant for both plant biomass and soil stocks (Fig. 2.B; Table 1). When the total soil profile was considered (only applicable to M_{NL} and M_{NH}), chronically elevated N inputs significantly increased N stock by 38 %, compared to 121 % in the topsoil (Fig. 2.C; Table 1). This difference was, however, only significant when the N stocks were compared per cumulative soil age (similar to the analysis of the net C storage rate Sect. 2.6 and 2.7; data not shown), and not when the N stock in the bulk soil was considered as a whole (Table 1).

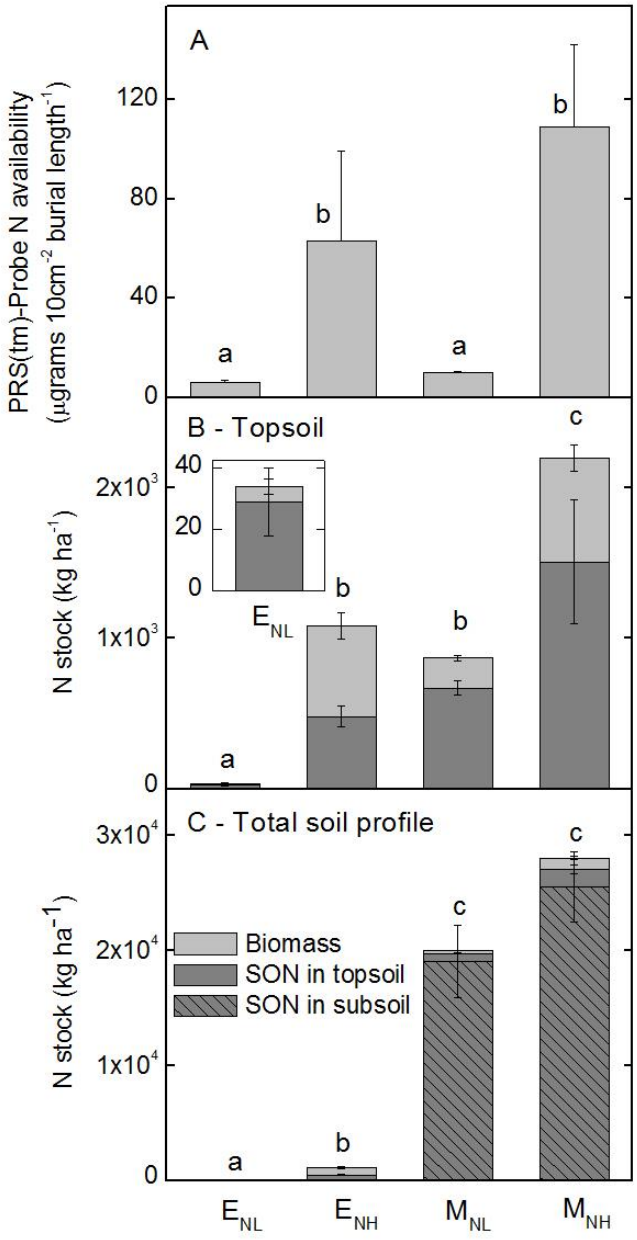


Figure 2: (A) PRS-probe derived N availability, measured by cation- and anion-exchange membranes that continuously absorb charged ionic species over the burial period, and expressed as soil N flux over time. (B and C) Nitrogen stocks in biomass (shoots + roots) and soil organic nitrogen (SON) in unmanaged Icelandic grasslands under low and high N inputs at an early soil developmental stage (E_{NL} and E_{NH}) and at sites with mature soils (M_{NL} and M_{NH}). The SON stocks are shown separately for the topsoil (B; since 1963 for E_{NL} and E_{NH}, above the 1973 ash layer for M_{NL} and M_{NH}) and the total soil profile including the subsoil (C; above the 395 AD ash layer). The inserted graph shows the N stock at E_{NL} in detail and is valid for both panels B and C, as E_{NL} had no subsoil. Letters show significant differences in total ecosystem N stocks. Error bars indicate SE's. Statistical details can be found in Table 1.

Table 1: Results of the two-way ANOVA's for N availability and the N stock in biomass, soil (soil organic nitrogen; SON) and the total ecosystem, using N input and soil developmental stage as fixed variables. The topsoil refers to the upper 30 cm or down to the bedrock for E_{NL} and E_{NH} (accumulated since 1963) and to the soil layer above the 1973 ash layer for M_{NL} and M_{NH}. The total soil profile is only applicable to M_{NL} and M_{NH} and refers to the soil profile above the 395 AD ash layer. Significant source variables ($p < 0.05$) are indicated with an asterisk: *p is 0.05–0.01, **p is 0.01–0.001, ***p < 0.001.

	N availability	N stock (kg ha^{-1})				
			Topsoil		Total soil profile[b]	
		Biomass	SON	Total[c]	SON	Total[d]
N input x soil developmental stage						
Df numerator	1	1	1	1	-	-
Df denominator	21	23	23	23	-	-
F-value	0.47	0.58	2.03	0.74	-	-
p-value	0.50	0.45	0.17	0.40	-	-
N input						
Df numerator	na	1	1	1	1	1
Df denominator	na	25	24	25	6	6
F-value	0[a]	68.1	19.60	25.37	2.80	3.54
p-value	***	***	***	***	0.15	0.11
Soil developmental stage						
Df numerator	na	1	1	1	-	-
Df denominator	na	24	24	24	-	-
F-value	101[a]	4.69	21.05	38.47	-	-
p-value	0.06	*	***	***	-	-

[a] Non parametrical Wilcoxon signed rank test: W-value
[b] Only applicable to mature soils (M_{NL} and M_{NH})
[c] Cumulation of biomass N and SON in topsoil
[d] Cumulation of biomass N and SON in total soil profile

6.4.3 Soil texture

Following the USDA-NRCS Soil texture classes (USDA, 2016), all soils were classified as sand, loamy sand or sandy loam, being very poor in clay (0.0 – 0.2 %), containing 5 – 34 % of silt and being rich to very rich in sand (64 – 94 %). The grain size distribution of M_{NL} and M_{NH} was very similar at all soil depths (Fig. 5), and any differences could be explained by different depths of the 1973 ash layer (shallower at M_{NL}, deeper at M_{NH}; see § 2.1), which has a course structure. The early successional sites had a similar soil texture to the mature sites in the upper soil layers (0-10 cm), but had a coarser texture in the lower layers (10-30 cm) (Fig. 5), where no soil development had occurred yet.

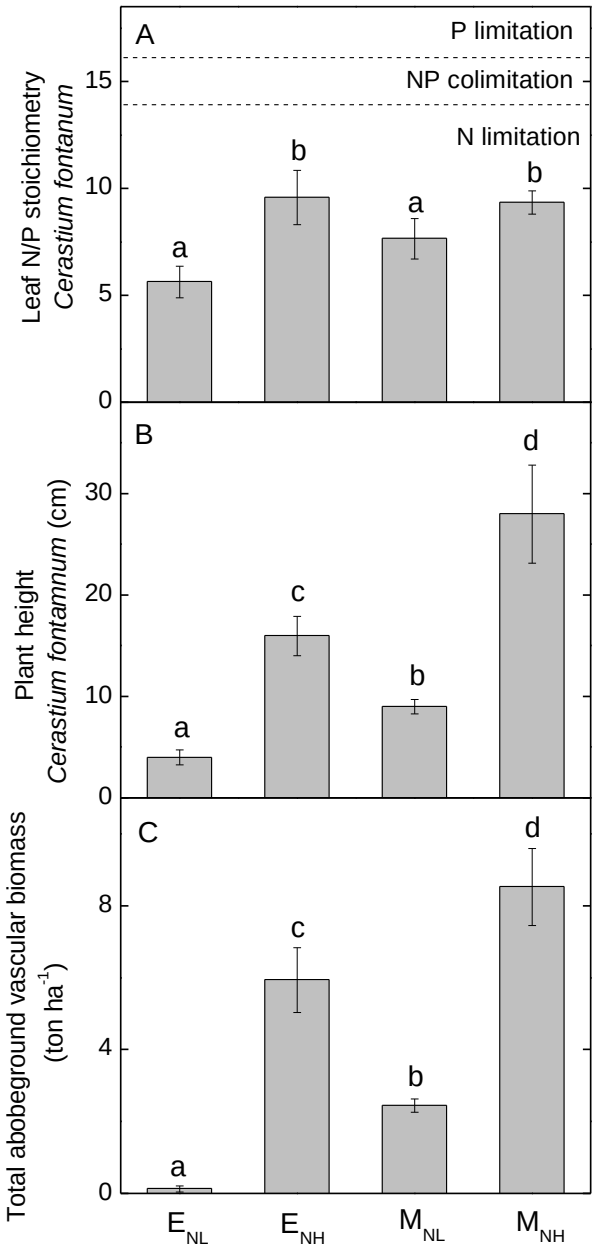


Figure 3: (A) Leaf N/P stoichiometry of *Cerastium fontanum* in mature healthy leaves. Dotted lines show the borders of N limitation (N/P < 14), NP co-limitation (14 < N/P < 16) and P limitation (N/P > 16) for higher plant communities (Aerts and Chapin, 2000). (B) Plant height of *C. fontanum*. (C) Total aboveground vascular biomass (monocots and dicots). Different bars shown unmanaged Icelandic grasslands under low and high N inputs at an early soil developmental stage (E_{NL} and E_{NH}) and at sites with mature soils (M_{NL} and M_{NH}). Error bars indicate SE's. Letters show significant differences. Further statistical details can be found in Table 2.

Table 2: Results of the two-way ANOVA's for leaf N/P stoichiometry of mature healthy leafs of *Cerastium fontanum*, plant height of *C. fontanum* and total aboveground vascular plant biomass using N input and soil developmental stage as fixed variables. Significant source variables (p < 0.05) with an asterisk: *p is 0.05–0.01, **p is 0.01–0.001, ***p < 0.001.

	Leaf N/P stoichiometry *C. fontanum*	Plant height *C. fontanum*	Total aboveground vascular biomass
N input x soil developmental stage			
Df numerator	1	1	1
Df denominator	12	25	74
F-value	1.12	2.29	0.04
p-value	0.31	0.14	0.85
N input			
Df numerator	1	1	1
Df denominator	14	27	76
F-value	7.16	33.29	75.23
p-value	*	***	***
Soil developmental stage			
Df numerator	1	1	1
Df denominator	13	26	75
F-value	0.15	14.41	12.23
p-value	0.70	***	***

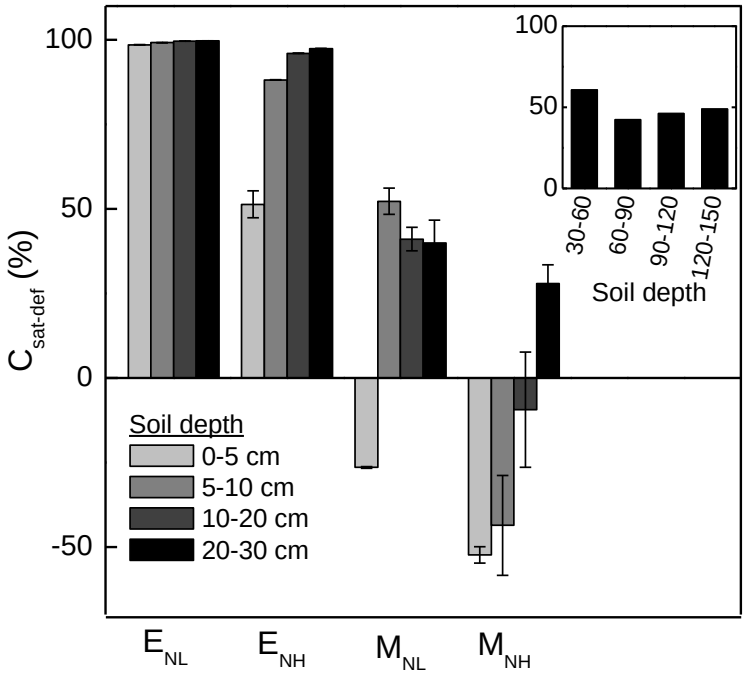


Figure 4: Carbon saturation deficit ($C_{sat-def}$) at the different study sites for different soil depth increments (0-5, 5-10, 10-20 and 20-30 cm). The inserted graph shows the Csat-def for the deeper soil (> 30 cm depth) of M_{NH}. The $C_{sat-def}$ of M_{NL} could not be calculated as no grain size data were available, which are necessary to calculate $C_{sat-def}$ (Wiesmeier et al., 2014). The $C_{sat-def}$ is the difference between the potential C saturation and the current C concentration and was calculated following Wiesmeier et al. (2014).

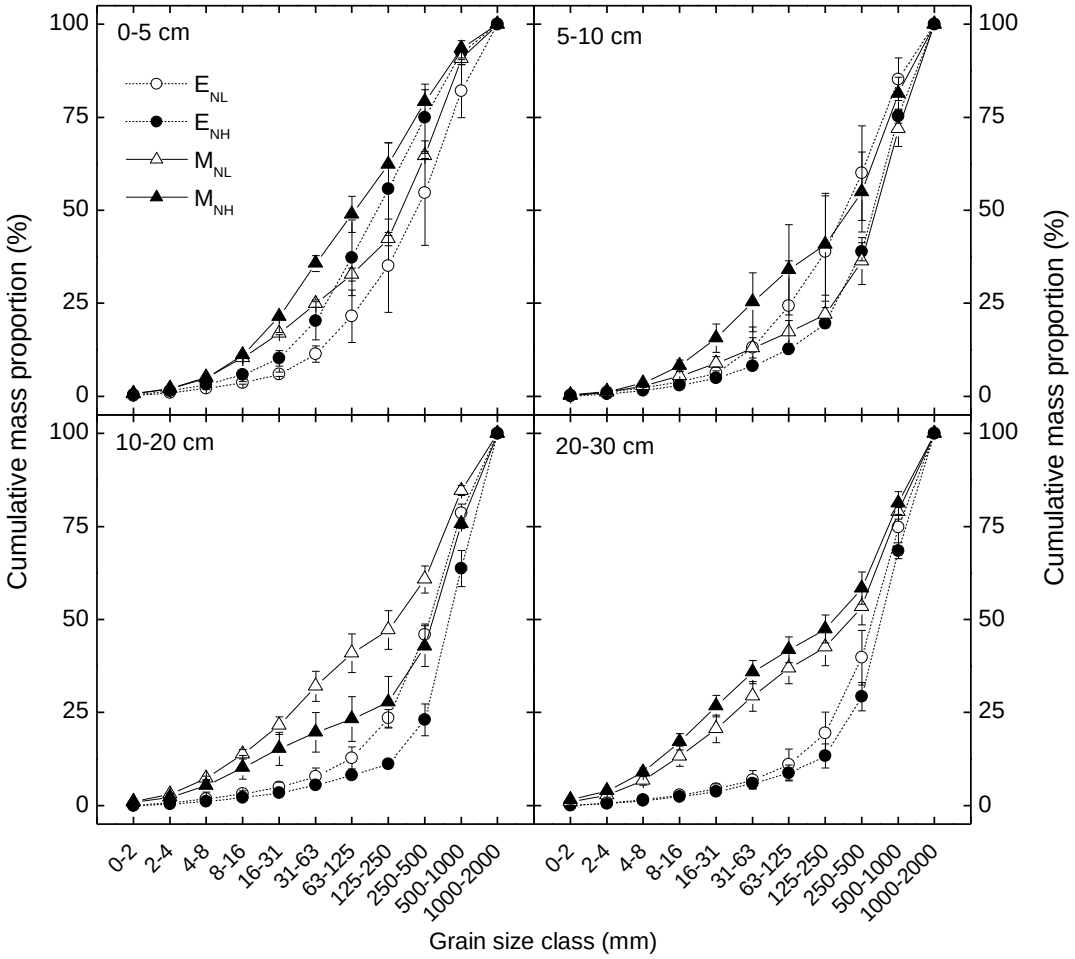


Figure 5: Cumulative mass proportion of clay, silt and sand (clay < 2 µm, silt 2-63 µm, sand 63-2000 µm) at different soil depths (0-5, 5-10, 10-20 and 20-30 cm) for unmanaged Icelandic grasslands under low and high N inputs at an early soil developmental stage (E_{NL} and E_{NH}) and at sites with mature soils (M_{NL} and M_{NH}).

6.4.4 Effect of chronically elevated N inputs on C stocks and storage in biomass and topsoil

At the sites with mature soils (M_{NL} and M_{NH}), chronically elevated N inputs led to a doubling of the ecosystem biomass plus topsoil C stock, from 17 to 35 ton C ha^{-1} (Fig. 6.A; Table 3). This significant effect of chronically elevated N input on the total C stocks was also visible in almost all individual C stocks: the SOC stock doubled from approximately 10 to about 20 ton C ha^{-1} and the C stock in aboveground vascular plant biomass ("shoots") increased almost 4 times, from 1.0 to 3.4 ton C ha^{-1} (Fig. 6.A; Table 3). The increase in root C stock in the topsoil of M_{NH} compared to M_{NL} was only marginally significant, but the total root C stock, including deeper roots, was significantly higher at M_{NH} (Fig. 6.A and 4.B; Table 3). The litter C stock remained stable at approximately 1 ton ha^{-1} and the moss C stock decreased from 1 to 0.2 ton ha^{-1} between M_{NL} and M_{NH} (Fig. 6.A, Table 3). The net SOC storage rate in the topsoil

of the sites with mature soils was 50 % higher under chronically increased N inputs (from 0.30 to 0.44 ton C ha^{-1} yr^{-1}; Fig. 7.B; Table 4).

For the sites in early soil developmental stage (E_{NL} and E_{NH}), the effects of chronically elevated N input were even more pronounced, with all C stocks in biomass and soil significantly higher in E_{NH} than in E_{NL} (Fig. 6.A, Table 3). Biomass C stocks increased from 0.2 to 15.0 ton ha^{-1}, total ecosystem C stock increased by from 1.0 to 22.1 ton ha^{-1} and SOC storage in topsoil increased from 0.02 to 0.28 ton C ha^{-1} yr^{-1}.

Regarding the effect of developmental stage, comparison of the early developmental and mature sites over the same timeframe (the last 40 to 50 years) revealed that the total C stock of E_{NL} was only a fraction of M_{NL} (1.0 vs. 30 ton ha^{-1}; Fig. 6.A; Table 3), and that its net SOC storage rate was a mere 9 % of the rate in M_{NL} (Fig. 7.B; Table 4). At the high N inputs sites, on the contrary, all biomass C stocks of E_{NH}, had reached the same level as M_{NH}. This was not the case for the SOC stocks, which, nevertheless, reached half of the M_{NH} stock (Fig. 6.A; Table 3). In terms of net SOC storage rate, E_{NH} stored half as much SOC as M_{NH} per unit time, and had reached the same rate as M_{NL} (Fig. 7.B; Table 4).

6.4.5 SOC stocks and storage in the total soil profile

The SOC stocks and storage rates in the total soil profile (since 395 AD) could only be studied at the sites with mature soils (M_{NL} and M_{NH}). At both sites, the C stocks and C storage rate decreased significantly with increasing soil depth (Fig. 7; Table 4). However, in ca. 1000 years old soil layers, the C storage reached an equilibrium of 0.12 and 0.16 ton C ha^{-1} yr^{-1} for M_{NL} and M_{NH} respectively. These results also indicate that the chronically elevated N inputs caused a significant increase in net SOC storage of M_{NH} compared to M_{NL}, and that this was maintained throughout the soil profile down to the 395 AD ash layer (Fig. 7.B; Table 4). The effect size of chronically elevated N inputs on net C storage rate decreased with cumulative soil age, as was shown by the significant interaction between N inputs and cumulative soil age (Table 4), until an equilibrium of ca. + 0.04 ton SOC ha^{-1} yr^{-1} (equivalent to +25 %) was reached. When the SOC stocks of M_{NL} and M_{NH} were compared as simple bulk values, integrating the entire soil profile, the differences between M_{NL} and M_{NH} were not statistically significant (Fig. 6.B; Table 3).

Finally, taking the whole measurement depth into account (30 cm for E_{NL} and E_{NH} and up to the 395 AD ash layer for M_{NL} and M_{NH}), the net storage rates of SOC and SON were strongly linked over all treatments, with an average C/N ratio of 12 (Fig. 8).

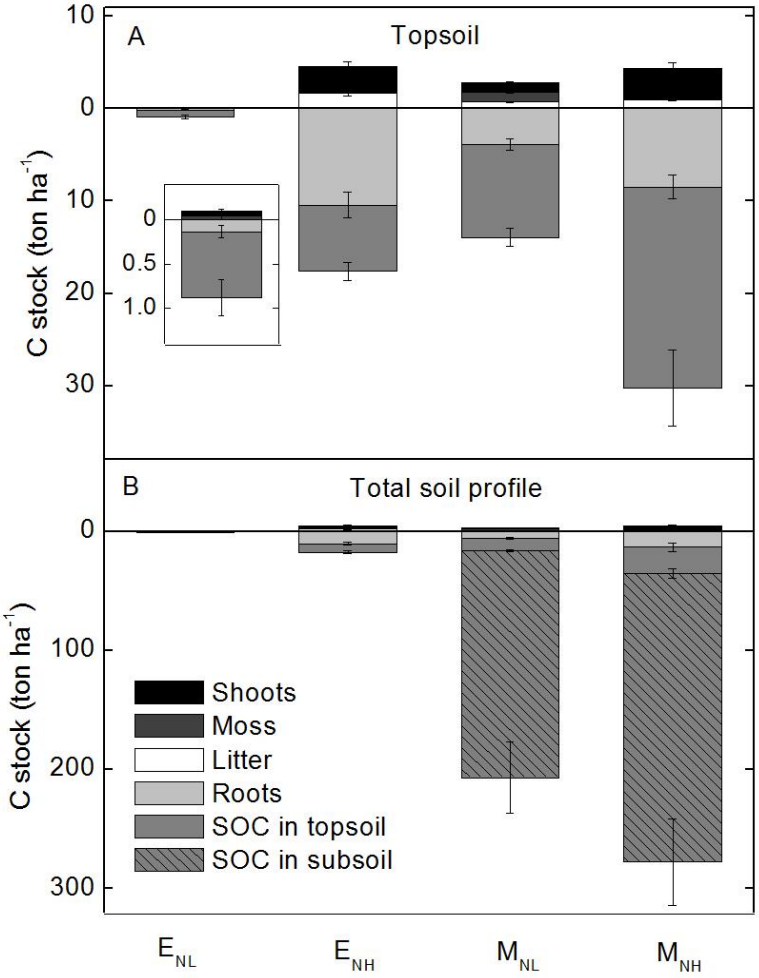


Figure 6: Carbon stocks in biomass (shoots, moss, litter and roots) and soil organic carbon (SOC) in unmanaged Icelandic grasslands under low and high N inputs at an early soil developmental stage (E_{NL} and E_{NH}) and at sites with mature soils (M_{NL} and M_{NH}). The SOC stocks are shown separately for the topsoil (A; since 1963 for E_{NL} and E_{NH}, above the 1973 ash layer for M_{NL} and M_{NH}) and for the total soil profile including the subsoil (B; above the 395 AD ash layer). The inserted graph shows the C stock at E_{NL} in detail and is valid for both panels as E_{NL} had no subsoil. Error bars indicate SE's. Statistical details can be found in Table 3.

Table 3: Results of the two-way ANOVA's for C stocks in different ecosystem parts, using N input and soil developmental stage as fixed variables. In case of significant interaction, no overall effects of N input or soil developmental stage could be derived and the pairwise differences were tested by post hoc LSD tests or Wilcoxon signed rank tests (lower part of the table). Topsoil C stocks corresponded to C stocks accumulated since 1963 for E_{NL} and E_{NH}, and to C stocks that were accumulated since the deposition of the 1973 ash layer for M_{NL} and M_{NH}. Total soil profile was only applicable to M_{NL} and M_{NH} (E_{NL} and E_{NH} have no subsoils yet), so that the influence of developmental stage could not be tested. It corresponded to the SOC stocks above the 395 AD ash layer. "Shoots" include all aboveground living vascular plant parts; SOC is Soil Organic Carbon. Significant source variables ($p < 0.05$) with respect to ecosystem parts are indicated with an asterisk: *p is 0.05–0.01, **p is 0.01–0.001, ***p < 0.001.

	C stock (ton ha^{-1})								
				Topsoil			Total soil profile[a]		
	Shoots	Moss	Litter	Roots	SOC	Total[b]	Roots	SOC	Total[c]
N input x soil developmental stage									
Df numerator	1	1	1	1	1	1	-	-	-
Df denominator	23	23	22	23	23	23	-	-	-
F-value	0.06	6.58	7.08	6.58	3.17	0.34	-	-	-
p-value	0.81	*	*	*	0.08	0.56	-	-	-
N input									
Df numerator	1	-	-	-	1	1	1	1	1
Df denominator	25	-	-	-	25	25	6	6	6
F-value	33.30	-	-	-	10.15	8.57	11.38	0.68	2.42
p-value	***	-	-	-	**	**	*	0.24	0.17
Soil developmental stage									
Df numerator	Na	-	-	-	1	1	-	-	-
Df denominator	Na	-	-	-	25	25	-	-	-
F-value	111[d]	-	-	-	27.26	34.56	-	-	-
p-value	0.07	-	-	-	***	***	-	-	-
Effect of N input at early soil developmental stage									
Df numerator	-	na	1	na	-	-	-	-	-
Df denominator	-	na	16	na	-	-	-	-	-
F-value	-	35[d]	24.24	0[d]	-	-	-	-	-
p-value	-	0.28	***	***	-	-	-	-	-
Effect of N input at mature soils									
Df numerator	-	1	1	na	-	-	-	-	-
Df denominator	-	6	6	na	-	-	-	-	-
F-value	-	62.69	1.98	1[d]	-	-	-	-	-
p-value	-	***	0.21	0.06	-	-	-	-	-
Effect of soil developmental stage at low N input									
Df numerator	-	na	1	na	-	-	-	-	-
Df denominator	-	na	12	na	-	-	-	-	-
F-value	-	40[d]	261.5	40[d]	-	-	-	-	-
p-value	-	**	***	**	-	-	-	-	-
Effect of soil developmental stage at high N input									
Df numerator	-	na	1	1	-	-	-	-	-
Df denominator	-	na	10	11	-	-	-	-	-
F-value	-	19[d]	1.58	1.07	-	-	-	-	-
p-value	-	0.93	0.23	0.32	-	-	-	-	-

[a] Only applicable to mature soils (M_{NL} and M_{NH})
[b] Cumulation of biomass C and SOC in the topsoil
[c] Cumulation of biomass C and SOC in the total soil profile
[d] Non parametrical Wilcoxon signed rank test: W-value

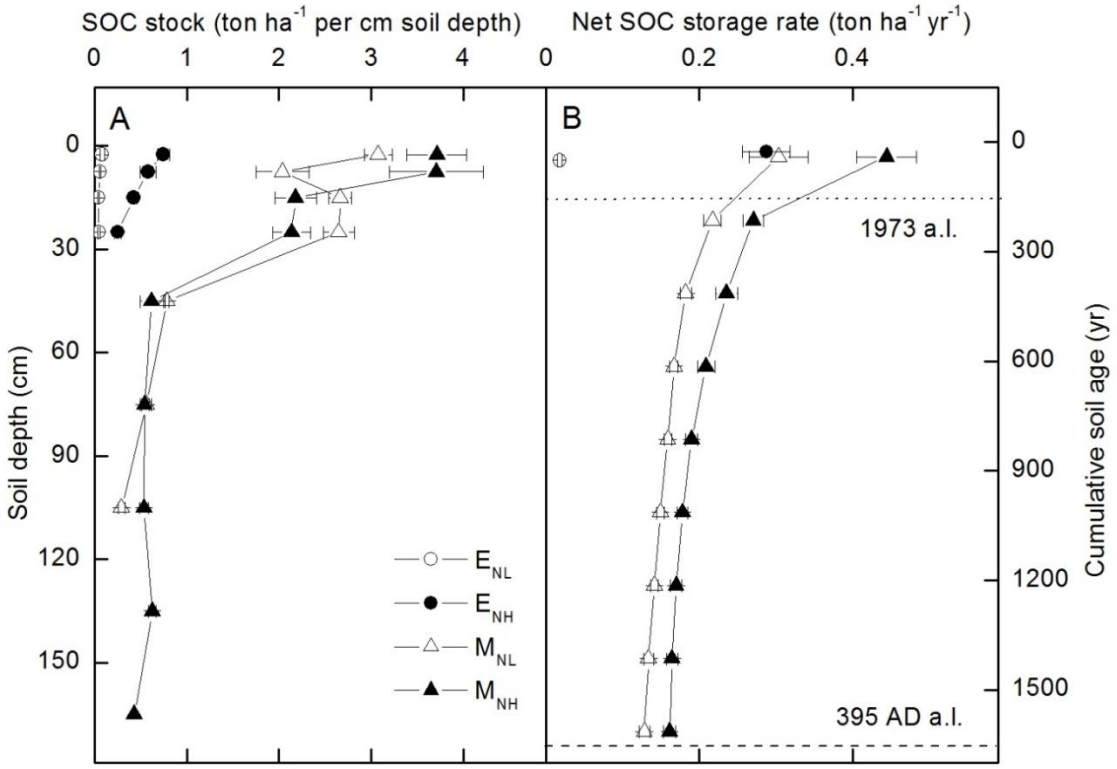


Figure 7: Soil organic carbon (SOC) stocks and the derived net SOC storage rates in unmanaged Icelandic grasslands under low and high N inputs at an early soil developmental stage (E_{NL} and E_{NH}) and at sites with mature soils (M_{NL} and M_{NH}). (A) Depth profile of the SOC stocks per soil layer of one cm, derived from layers of 0–5, 5–10, 10–20, 20–30, 60–90, 90–120, 120–150 and 150–180 cm soil depth. For E_{NL} and E_{NH}, the SOC stocks were measured down to the bedrock or down to 30 cm soil depth, for M_{NL} and M_{NH} up to the 395 AD ash layer. Note the difference in depth of the 395 AD ash layer between M_{NL} and M_{NH}. (B) Net SOC storage rate per cumulative soil age, where soil age was calculated assuming a linear soil accumulation between the 395 AD and 1973 ash layer. The dotted line indicates the 1973 ash layer, which marks the border between the topsoil and the subsoil. The dashed line indicates the 395 AD ash layer, which marks the lower limit of undisturbed soil. Note that E_{NL} and E_{NH} only contain topsoil, as they were still too young to have developed a deep organic soil. Error bars indicate SE's. Statistical details can be found in Tables 3 and 4.

Table 4: Results of the two-way ANOVA's for the net soil organic carbon (SOC) storage rate in the topsoil (decadal net SOC storage rate) and the total soil profile (millennial net SOC storage rage). For the topsoil (left columns), N input and soil developmental stage were used as fixed factors. Topsoil corresponded to the net SOC storage rate since 1963 (down to 30 cm soil depth or down to the bedrock) in the case of E_{NL} and E_{NH}, and to the net SOC storage rate in the soil above the 1973 ash layer for M_{NL} and M_{NH}. For the total soil profile (right columns; only applicable to M_{NL} and M_{NH}), N input and cumulative soil age were used as fixed factors. Total soil profile corresponded to the soil above the 395 AD ash layer. Significant source variables ($p < 0.05$) are indicated with an asterisk: ns. is $p > 0.05$, *p is 0.05–0.01, **p is 0.01–0.001, ***$p < 0.001$.

Net SOC storage rate in the topsoil (decadal storage rate) (ton ha^{-1} yr^{-1})		Net SOC storage rate in the total soil profile (millennial storage rate) (ton ha^{-1} yr^{-1})[a]	
N input x soil developmental		N input x cumulative soil age	
Df numerator	1	*Df numerator*	1
Df denominator	23	*Df denominator*	68
F-value	3.39	*F-value*	4.15
p-value	0.08	*p-value*	*
N input		N input	
Df numerator	1	*Df numerator*	1
Df denominator	24	*Df denominator*	68
F-value	47.61	*F-value*	17.00
p-value	***	*p-value*	***
Soil developmental stage		Cumulative soil age	
Df numerator	1	*Df numerator*	1
Df denominator	24	*Df denominator*	68
F-value	33.39	*F-value*	93.22
p-value	***	*p-value*	***

[a] Only applicable to mature soils (M_{NL} and M_{NL})

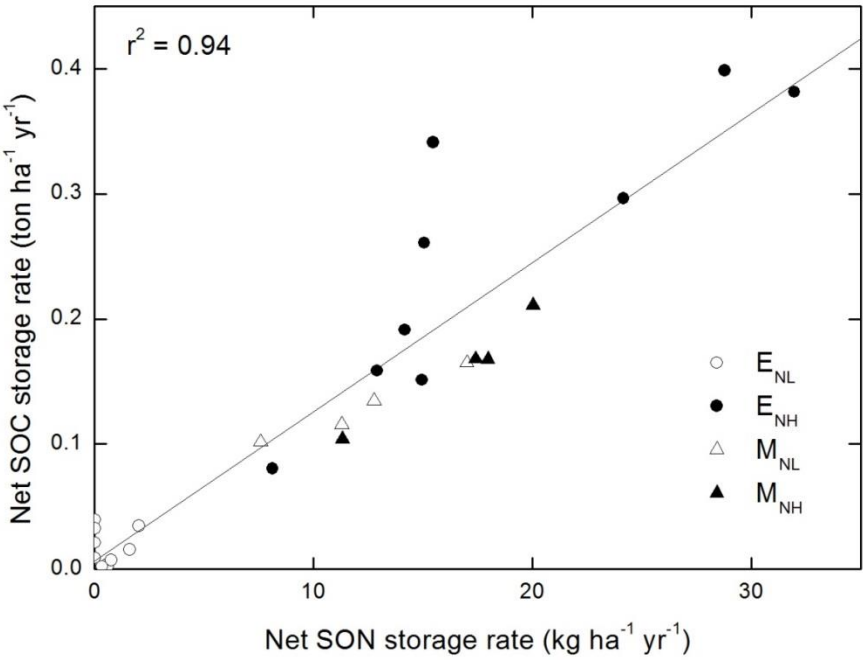


Figure 8: Linear relationship between net soil organic carbon (SOC) storage rate and net SON storage rate for unmanaged Icelandic grasslands under low and high N inputs at an early soil developmental stage (E_{NL} and E_{NH}) and at sites with mature soils (M_{NL} and M_{NH}). The net SOC and SON storage rates were calculated over the whole soil profile (down to the bedrock or down to 30 cm for E_{NL} and E_{NH} and down to the 395 AD ash layer for M_{NL} and M_{NH}). The linear relationship was highly significant ($p < 0.001$), with a slope of 0.12 (± 0.02).

6.5 Discussion

6.5.1 Similarities and dissimilarities between anthropogenic and seabird derived organic N

In this study, a comparison between seabird-affected and unaffected plots was used to test the effects of N enrichment on unmanaged Icelandic grasslands. Seabird-derived N inputs have as main drawback that they differ from the typical anthropogenic N deposition because N is in organic form (Schmidt et al., 2004) and because bird excrements also contain other nutrients (Zwolicki et al., 2013). In our case, the influence of other nutrients is assumed negligible because these ecosystems are strongly N limited (LeBauer and Treseder, 2008; see also § 4.2). Despite these potential drawbacks, comparing seabird-affected plots with control plots has different advantages. Firstly, the amount of N inputs is similar to the highest observed atmospheric N deposition (Lamarque et al., 2013). Secondly, and most importantly, the duration of the fertilization is exceptionally long and much longer than what is common for fertilizer addition experiments of which hardly any run long enough to infer long-term effects

of anthropogenic N deposition (but see e.g. Hyvonen et al., 2007, Hopkins et al., 2009, Nilsson et al., 2012, Gundersen et al., 2014).

6.5.2 N availability and N stocks in unmanaged Icelandic grasslands under organic N inputs

The N availability and the total SON stocks were greatly increased in the high N input sites compared to the low N input sites. Interestingly, even though this higher N availability and N stocks at the high N input sites clearly stimulated biomass production and C storage (total aboveground biomass, plant height of *C. fontanum* and ecosystem C stocks were significantly higher), the plant N/P ratios of *C. fontanum* indicated that plant growth remained N limited, in spite of the relatively high chronic N input rates in both E_{NH} and M_{NH} (~47 and ~67 kg N ha^{-1} yr^{-1}, respectively).

Contrary to our expectations that millennia of soil development would increase N availability and N stocks more than a few decades of allochtonous N inputs, the N status was clearly more closely related to the annual seabird-derived N input than to ecosystem maturation. However, even if the influence was smaller, there were strong indications that thousands of years of N retention and recycling had (partly) alleviated the N-limitation in M_{NL} and M_{NH} compared to E_{NL} and $E_{NH,}$ respectively, as both the total aboveground biomass and plant height of *C. fontanum* were significantly higher at the mature sites. However, the N availability was only slightly higher compared to the early developmental sites. The N stock, on the other hand, had indeed increased significantly in M_{NL} compared to E_{NL}. The observation that M_{NL} was still greatly N-limited (indicated by the low plant N/P stoichiometry of *C. fontanum*), supported our presumption that the largest part of the accumulated ecosystem N stock was locked up in undecomposed soil organic matter or in biomass. In the high N input sites, the short timeframe in which E_{NH} had received increased N inputs had been sufficient to bring the vegetation N stocks of E_{NH} and M_{NH} at the same level. The SON stocks, however, were still significantly higher in M_{NH}, which shows that the SON stock were not yet in equilibration with the N input rates in E_{NH}.

We expected that N availability would better explain differences in N limitation and biomass production than N stocks, because of the typical strong binding of N to soil particles in volcanic soils (Gudmundsson et al., 2004). Nonetheless, both had a comparable explanatory value.

6.5.3 Net SOC storage in mature Icelandic grasslands under chronically elevated N inputs

6.5.3.1 Decadal time scale

Our study was conducted on Andosols, which have specific characteristics, including high concentrations of Al, Fe and Si that can e.g. bind SOM in 'metal-humus' complexes (Arnalds, 2015). Nonetheless, the decadal net SOC storage rate in the mature grasslands (0.30 and 0.44 ton ha^{-1} yr^{-1} at M_{NL} and M_{NH} respectively) corresponded well with the average topsoil SOC storage in a broad range of soil types under previously SOC depleted perennial temperate grasslands (0.33 ton ha^{-1} y^{-1}; Post and Kwon, 2000).

As hypothesized, chronically elevated N inputs stimulated decadal SOC storage in mature soils, but to a lesser extent than in early developmental soils (an increase of 50 % from MNL to MNH, compared to an increase of 250 % from ENL to ENH; Leblans et al., 2014). The magnitude of the response is in line with an earlier long-term N addition study on managed grasslands in east Iceland, which reported a > 50 % increase in SOC concentration in the upper 10 cm of the soil after 43 years of fertilization by 120 kg N ha^{-1} yr^{-1} (Gudmundsson et al., 2004). The strong positive effect of chronically elevated N inputs on decadal net SOC storage (i.e. SOC storage in the soil layer that was accumulated after 1973), is in line with the theory that the recent northern C sink is at least partly caused by increasing N deposition (Hudson et al., 1994, Lloyd, 1999, Schlesinger, 2009).

The net response of decadal SOC storage to chronically elevated N inputs depends on the responses of the individual processes that influence carbon input to and release from the topsoil: (1) net primary productivity (NPP), (2) C partitioning (3) decomposition rate (Mack et al., 2004, Batjes, 2014) and (4) the C storage capacity of the soil. (1) Chronically elevated N inputs greatly stimulated NPP in the present study, which agreed with what is generally found in studies that investigate the effects of N inputs on productivity in northern grasslands (Sillen and Dieleman, 2012). (2) The root/shoot C partitioning was similar for M_{NL} and M_{NH} (average root/shoot ratios of ~10; data not shown). However, since the total amount of belowground C allocation is potentially also affected by changes in root turnover rates (Aerts et al., 1992, Milchunas et al., 2005), changes in exudation and mycorrhizal activity (Vicca et al., 2012) and changes in microbial C use efficiency (Wieder et al., 2013), the total belowground C inputs cannot be derived from the present data. (3) The response of decomposition to chronically elevated N inputs was not assessed in the present study, but previous studies have reported both positive and negative N input effects on the decomposition rate in the topsoil of northern grasslands. The direction of the response

depended on natural background N deposition, N input rate and litter quality (Aerts et al., 2003, Knorr et al., 2005, Hobbie, 2008, Zhang et al., 2008). In any case, the increased SOC stocks clearly indicate that the increase in topsoil C input rate surpassed potential increases in decomposition rate in our study. (4) The C storage capacity of the soil determines the maximum amount of C that can be stabilized in the soil, and is strongly dependent on soil texture, where particles ≤20 μm play the most important role in C stabilization (Hassink, 1997, Wiesmeier et al., 2014). The soil texture in the upper soil layers of M_{NL} and M_{NH} was very similar (Fig. 5) and their C storage potential was not yet reached, showing a C deficit of ~50%. Therefore, we assume that the C storage capacity of the soil has not had a significant influence on the C storage in M_{NL} and M_{NH}.

6.5.3.2 Millennial time scale

The total SOC stocks (down to the 395 AD ash layer; 220–280 ton C ha^{-1}) in the present study did not only correspond closely to a previous estimation for Brown Andosols in Iceland (227 ton ha-1; Óskarsson et al., 2004), but were also in line with non-volcanic temperate grassland soils, where estimates range from 197 (Schlesinger, 1997) to 236 ton ha^{-1} (Janzen, 2004). The observed millennial net SOC storage rates (0.12–0.16 ton C ha^{-1} yr^{-1}) corresponded well with those of deep SOC rich soils in northern regions (0.15–0.30 ton ha^{-1} yr^{-1}; Trumbore and Harden, 1997) and with the long-term SOC storage in temperate grassland ecosystems in China (0.11 ton ha^{-1} y^{-1}; He and Tang, 2008).

As hypothesized, the millennial net SOC storage rate was much lower than the decadal storage rate. This agrees with a recent review study of Matus et al. (2014) that showed a general decrease in net SOC storage rate with depth in Andosols and with the general observation that net SOC storage rates decline when soils approach their mature state (Post and Kwon, 2000). However, there was no evidence that the mature sites in this study had reached an SOC steady state, as the decline in net SOC storage rate with depth (or with increasing cumulative soil age) stabilized around 1000 years before present and did not decline to zero. Also the potential C storage of M_{NL} and M_{NH} in the deeper soil layers (Fig. 4) showed no indication of C saturation. Both mature sites showed a C deficit of ~50% in the deeper soil layers (> 10 cm depth) and even up to the 395 AD ash layer.

The stable C/N ratio of about 12 in the total soil profile of both M_{NL} and M_{NH} suggested that the total SOC stock could continue to increase with elevating N inputs even after millennia of soil maturation, providing that N can be retained. This was supported by our observation that millennial net SOC storage rate was still increased under chronically elevated N inputs, albeit

to a lesser extent than the decadal storage rate (25 % vs. 50 % increase, respectively). The modest increase in SOC storage rate under chronically elevated N inputs, however, was consistent throughout the soil profile and added up to a considerable strengthening of the C sink over a long time span while a thicker soil was developed.

6.5.4 Importance of soil developmental stage for net SOC storage in Icelandic grasslands

As expected, the decadal net SOC storage rate in mature soils under low natural N inputs was substantially higher compared to early developmental soils under low natural N inputs. The observed increase in SOC storage (~0.30 ton ha^{-1} yr^{-1}) was within the range of estimates for the transformation from early developmental to mature Andosols which range from 0.1 ton ha^{-1} yr^{-1} (Vilmundardottir et al., 2015b). This might be an underestimation as the older soils in this study had not reached their mature state yet, but mature soils in Iceland have been found to have a SOC storage rate of 0.6 ton ha^{-1} yr^{-1}, on average (Óskarsson et al., 2004). The maturation-driven increase in net SOC storage rate is not only an Andosol feature, but is also generally found in other soil orders (e. g. Lichter, 1998, Foote and Grogan, 2010, Kabala and Zapart, 2012, Kalinina et al., 2013) and is caused by centuries of N-accumulation, the stimulation of internal N cycling through biomass and the gradual increase in SOC stock (Vitousek and Reiners, 1975, Kirschbaum et al., 2003). Increasing SOC improves the N exchange capacity and the water holding capacity of the soil (Deluca and Boisvenue, 2012), thus stimulating plant growth and net SOC storage. In addition, the soil development in M_{NL} has shifted the soil texture to a more fine-grained structure compared to E_{NL} (Fig. 5), especially in the deeper soil layers (> 10 cm soil depth), causing M_{NL} to have a higher potential C saturation (Hassink, 1997, Wiesmeier et al., 2014). This more fine-grained structure allows M_{NL} to retain a larger proportion of the incoming C by binding organic matter with clay and silt particles.

We did not expect that only 28 years of elevated N inputs at the E_{NH} site would have created a positive effect on decadal net SOC storage rate similar to that reached at M_{NL} after thousands of years of slow N retention and recycling. In fact, the current annual SOC storage rate of E_{NH} most likely surpassed M_{NL}, as it was probably higher in recent years than its average rate calculated since the start of seabird colonization. Indeed, E_{NH} showed a 10-years delay in the establishment of full surface cover (and consequently net SOC storage) after the initiation of the allochthonous N inputs (Magnússon and Magnússon, 2000). Hence, E_{NH} was likely approaching the decadal net SOC storage rate of M_{NH}. This supposition is supported by the similar biomass C stocks at E_{NH} and M_{NH}, but it can be expected that the development and

stabilization of soil processes that regulate net SOC storage will need longer time to come into equilibration with the C input rates (Post and Kwon, 2000, Creamer et al., 2011).

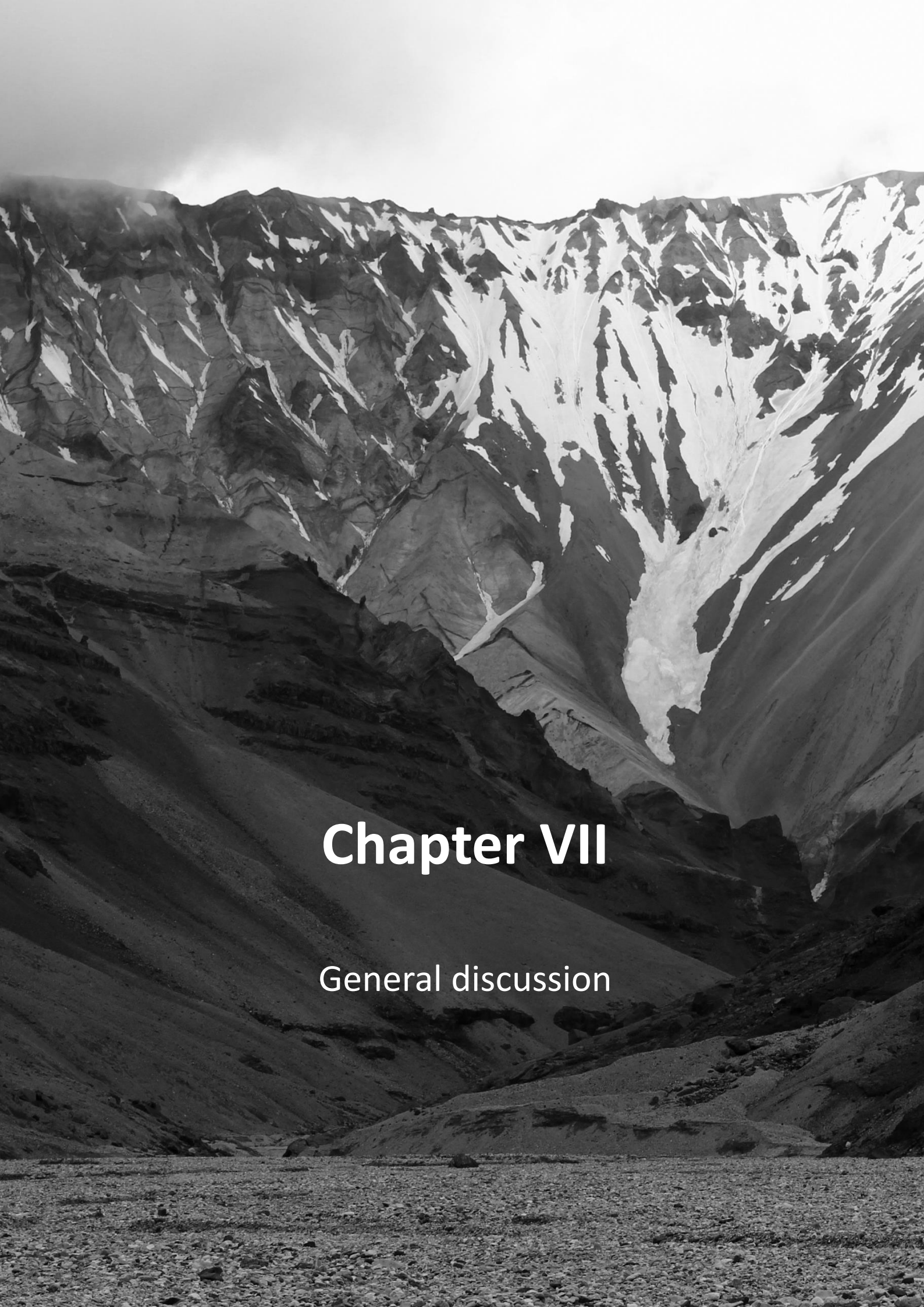
If we assume that, with time, the early developing soils will evolve into similar soils as the soils on the older islands, then the difference in total SOC stock provides an idea of the future 'SOC gap' (Kramer and Gleixner, 2008). This gap amounted to 220–260 ton SOC ha^{-1} in the present study and will be gradually filled during the process of soil maturation by the input of organic material at the soil surface and into the rooting zone. Chronically elevated N inputs induced only a slight (20 %) increase in this SOC gap, but did have a large influence on the time frame in which the gap could be filled (by enhancing the net SOC storage rate at E_{NH} by a factor 16). A key question in relation to this is when the apparent SOC gap will saturate. It has been shown that this depends on the status of the SOC stabilizing processes in the subsoil (Fontaine et al., 2007, Rumpel and Kogel-Knabner, 2011, Pausch and Kuzyakov, 2012, Wiesmeier et al., 2014) and on the frequency of soil disturbance processes (Baldocchi, 2008). Until recently, it was assumed that all mature soils were SOC-saturated (Wutzler and Reichstein, 2007). However, this supposition has been challenged by various observations of continuously increasing SOC stocks in old (> 1,000 years) undisturbed soils (e.g. Harden et al., 1992, Wardle et al., 1997) and model-based predictions that the equilibration process of SOC stocks could take millennia (Wang and Hsieh, 2002). Also our mature study sites (M_{NL} and M_{NH}), which considered the past 1,600 years (since a large-scale disturbance before 395 AD), did not seem to have reached SOC saturation yet (see Sect. 4.2.2.).

Considering the large difference in C dynamics that was observed between early developmental and mature sites, it is important to bear in mind that many ecosystems are in an intermediate developmental stage, following past disturbances. Therefore, we stress the importance of taking soil developmental stage into account when estimating net SOC storage rates.

6.6 Conclusion

In our study, the decadal net SOC storage rate of mature Icelandic grasslands was greatly stimulated by chronically elevated organic N inputs, which supported the theory that the high northern terrestrial ecosystems could become an important contributor to the northern terrestrial N-driven C sink in the near future, when N inputs are expected to increase in this region. The positive influence of chronic N inputs on the net SOC storage rate also persisted at a millennial timescale in the present study, albeit to a smaller extent. This indicates not only

that mature Icelandic grasslands, if not disturbed, could become C sinks if the current climate conditions prevail, but also that chronically elevated N inputs could induce a permanent strengthening of this sink.



Chapter VII

General discussion

7. General discussion

The main aim of this thesis was to assess the long-term effects of global change-related factors (warming and increasing N inputs) on net soil organic C (SOC) storage in subarctic grasslands, using natural gradients in soil temperature and N inputs as study systems.

In the first part, natural (geothermal) gradients in soil temperature were studied. A thorough evaluation of these gradients confirmed that these are a promising tool to improve our understanding of long-term warming effects on ecosystems, overcoming important difficulties that are typical for climate manipulation experiments such as limited duration of the warming and a limited number of warming treatments (see § 7.1.1). Warming increased length of the growing season (and thereby the C storage capacity) of these subarctic grasslands, and despite recent indications for a saturation of the phenological warming response at northern latitudes, no clear saturation of the response was found (see § 7.1.2). Notwithstanding the longer growing season at higher soil temperatures in these subarctic grasslands (suggesting an increase in C inputs), increasing soil temperatures drastically reduced SOC stocks. Similar SOC losses after short-term and long-term soil warming showed that these SOC losses occurred very fast (within 5 years). Decreasing physical protection of SOC in stable soil aggregates was identified as a key mechanism in the observed warming-induced SOC loss (see § 7.1.3), which was a novel finding. These observations indicate that subarctic grassland soils, when warmed, could induce a rapid positive feedback to climate change by releasing substantial amounts of soil-derived CO_2.

In the second part, natural gradients in N input rates were studied, using contrasting soil age (N accumulation time) and seabird N inputs as drivers. A longer N accumulation time (without seabird influences) did increase total N stocks and N availability, although to a lesser extent than seabird N inputs, which greatly increased the N status of the ecosystems. Nevertheless, all sites remained N limited (see § 7.2.1). Early successional sites were fast-forwarded by increased seabird-derived N inputs from a sparsely vegetated desert to a subarctic grassland ecosystem, with plant species composition and aboveground biomass similar to the mature grasslands with seabird influence (see § 7.2.2). However, even if they were drastically increased, their SOC stock remained much lower than typical stocks for mature subarctic grasslands, indicating that it would still need centuries to equilibrate with the biomass inputs. Mature subarctic grassland sites (with an undisturbed soil profile of 1,600 years), had also significantly increased SOC stocks under high N input conditions (see § 7.2.3), albeit to a smaller relative extent than was the case at the early successional sites.

Hence, increased N inputs could stimulate SOC storage over many centuries, although early successional subarctic grasslands have a larger potential for SOC storage.

In the previous sections, temperature increase and increasing N inputs were treated separately. In many cases, however, they will increase in parallel. A theoretical exercise, based on the results of this thesis, to obtain an estimate of their combined effect on SOC storage in subarctic grasslands, suggested that the negative effect of increasing temperature will overrule the positive effect of increasing N inputs on SOC stocks, leading to a substantial release of SOC from subarctic grassland soils in a warmer world (§ 7.3).

7.1 Natural geothermal gradients in soil temperature

7.1.1 Evaluation of natural geothermal gradients in soil temperature as global change laboratories

Natural (geothermal) gradients in soil temperature have been proposed as a valuable addition to climate manipulation experiments and space-for-time studies (O'Gorman et al., 2014, De Boeck et al., 2015). One of their benefits is the improved balance between experimental realism and treatment control. This is difficult to reach in manipulation experiments, as experimental realism is optimally reached under field conditions, while treatment control is best obtained in laboratory conditions (Chapter I, § 1.4.1). In natural geothermal gradients, however, the small spatial scale and the absence of climate manipulation (which often also influences factors other than temperature; De Boeck et al., 2015), suggest a reasonable treatment 'control' (i.e. little confounding factors), and at the same time a conservation of the experimental realism.

To test the validity of this assumption, two areas with geothermal soil warming in southwest Iceland were selected as model systems (the ForHot research site; www.forhot.is): one short-term warmed (since 2008) and one long-term warmed (≥ 50 years). Possible confounding factors were measured along the soil temperature gradients in order to verify that soil warming was the main differentiating factor (indicating reasonable treatment 'control') (Chapter II). Changes in soil pH and soil moisture were limited, with a slight increase in soil pH and a slight drying with warming. It is unlikely that the warming-induced drying has caused substantial drought effects at the warmer sites, since even the warmest plots remained more wet than a nearby unwarmed forest. Further, it was confirmed that geothermal water had no major influence on the upper soil layers, as different surveys with cation- and anion-exchange membranes (PRS™ probes, Western Ag Innovations Inc.; Saskatoon, SK, Canada)

gave no indication of soil S- or B-enrichment or K-depletion (typical characteristics of geothermal soil water in the Hellisheiði geothermal system; Gunnarsson, 2012).

Soil depth did not change consistently along the soil temperature gradients, although the short-term warmed site was shallower than the long-term warmed site, a difference that has to be taken into account when interpreting the consequences of different warming duration. Further, the small spatial scale of the study sites minimised variability in climate and bedrock, factors that often complicate interpretation of space-for-time studies. A factor that did change along the temperature gradient was soil bulk density (Chapter IV, Fig. 2). This change was, however, identified to be a consequence of temperature-induced disintegration of soil aggregates, leading to soil compaction (Kozlowski, 1999), and was thus considered as part of the treatment effect. To conclude, possible confounding factors were reasonably constant along the soil temperature gradients, confirming the assumption that natural geothermal soil temperature gradients do combine treatment 'control' and experimental realism to a large extent. In the context of experimental realism, it is important to remember, however, that geothermal gradients expose the ecosystem to soil warming only, restricting the study of processes that depend more on air temperature (see later).

Another interesting aspect of natural soil temperature gradients is the costless continuous (year-round) warming, which is in line with the current projections for climate change. The continuity of the warming was assessed by performing hourly soil temperature measurements along the soil temperature gradients from spring 2013 to the present (Chapter II). Also the stability of the warming levels was evaluated, as it is not only crucial that the warming is continuous in nature, but also that the warming levels remain reasonably stable over the seasons and between years, especially if one wants to investigate long-term warming effects. The soil temperature increase proved to be relatively continuous during the year. Also the warming levels were fairly constant over all seasons and over the whole experimental period (2013 – 2016), independent of seasonal fluctuations of the absolute 'unwarmed' soil temperature and extreme deviations were rare (Chapter II: Fig. 2; Table 2). Therefore, we can conclude that geothermal soil warming is sufficiently stable to be used as a costless soil warming treatment. One has to bear in mind, however, that major tectonic events can affect both the location and the intensity of the warming (Khodayar and Bjornsson, 2014).

One of the main assets of natural geothermal warming gradients, as opposed to most climate manipulation experiments, is their wide temperature range. The soil temperature at the ForHot research site ranged from normal soil temperatures for southwest Iceland to temperature increases of more than 50°C (Chapter II). In the first place, this allows for a large

number of warming levels, which are generally limited in manipulation experiments due to logistical and/or financial constraints (O'Gorman et al., 2014, De Boeck et al., 2015). Using more temperature levels makes it possible to elucidate the shape of the temperature response. An additional advantage from the wide temperature range is the possibility to study the full range of global change predictions and beyond. The lowest temperature levels provide data that are relevant to reach a better understanding of the ecosystem dynamics in a warmer world, while the higher temperature levels offer valuable information on the mechanisms behind the observed responses. In this thesis, for instance, the lower temperature levels provided information on soil C storage at realistic warming levels, while the warmest soils were a valuable addition to identify the loss of protection of soil C in stable aggregates as a key factor in the observed soil C loss (Chapter IV).

The final, but maybe most important advantage of natural soil temperature gradients, is the long-term nature of many of these gradients. This makes it possible to evaluate the long-term outcome of temperature responses. This is one of the main difficulties in manipulation experiments, as long-term manipulation experiments are rare and extrapolating from short-term experiments to long-term predictions is questionable due to the non-linear time dependency of many temperature responses (De Boeck et al., 2015; Introduction Fig. 5). Not all natural soil temperature gradients have been present for a long time. Major tectonic events may create new gradients at previously unwarmed sites, and the presence of both short-term and long-term soil temperature gradients in a small area makes it possible to evaluate the temperature dependency of the responses. An example from this thesis that shows the benefit of investigating both short-term and long-term soil temperature gradients are the findings on soil C stocks along both gradients. Soil C loss was very similar after short-term and long-term warming, indicating that the loss happened shortly after the onset of the warming and that the soil C stocks stabilized after the initial loss (Chapter IV). Other ecosystem properties, however, such as soil respiration (Walker et al., In preparation), microbial community (Weedon, In preparation), arthropod community (Holmstrup et al., In preparation), nematode community (Ilieva-Makulec et al., In preparation) and vegetation community (Michielsen, 2014a), showed a clear time dependency of the temperature response, while e.g. methane (CH_4) and nitrous oxide (N_2O) fluxes were also similar (Maljanen et al., Submitted).

One has to bear in mind, however, that the "generality" of the soil temperature gradients at the ForHot research site is restricted by some common drawbacks that are connected to the use of geothermal gradients in climate change research. For one, the site has a volcanic soil (Andosol) (Chapter I, § 1.5.2) with specific characteristics (Arnalds, 2015) that

could play a role in the temperature responses compared to warming of other soil types. Secondly, ecosystems on newly formed soil temperature gradients (short-term soil temperature gradients) are subjected to a stepwise change in temperature instead of a gradual change such as will be the case under climate change. Comparison between short-term and long-term warming effects, however, can help disentangling this issue. The close similarity of short- and long-term warming effects on the studied ecosystem properties (see § 7.1.2 and 7.1.3) together with the absence of temperature thresholds along the short-term soil temperature gradients (see § 7.1.2 and 7.1.3), supported the notion that no major artefacts of the step-change in temperature occurred. Finally, the decoupling of soil warming from air warming has to be taken into account, especially while studying plant processes that are driven more by air temperature. Many manipulation studies have used warming cables to warm the soil (Strömgren and Linder, 2002, Bronson et al., 2008, Patil et al., 2013), so this issue is not limited to geothermal gradient studies such as ForHot.

To summarize, the ForHot research site met to a large extent the anticipated benefits of the use of natural soil temperature gradients as global change laboratories. This confirmed that natural soil temperature gradients can provide a valuable complement to climate manipulation experiments, elevational gradients and space for time studies and are promising tools to improve our knowledge on ecosystem responses to long-term warming. This positive evaluation allowed us to investigate the effect of increasing soil temperature on e.g. aboveground plant phenology (see § 7.2) and SOC storage (§ 7.3) at the ForHot research site.

7.1.2 Long-term effects of soil warming on aboveground plant phenology in a subarctic grassland

Aboveground phenology, amongst others the start and the end of the growing season, is highly sensitive to climate change (Henry and Molau, 1997, Tucker et al., 2001, Linderholm, 2006, Richardson et al., 2013), and can in turn induce powerful feedbacks to the climate system (Gu et al., 2003, Cleland et al., 2007, Ahlstrom et al., 2012, Richardson et al., 2013) (Chapter III). Especially at high northern latitudes, large extensions in growing season length have occurred during the past decade due to the strong warming in this region (Raynolds et al., 2015, Zhao et al., 2015, Gonsamo and Chen, 2016). There have been indications that this northern warming-induced extension of the growing season is saturating (Zhao et al., 2015), potentially reducing their C uptake potential under warmer conditions. However, its future evolution is highly uncertain (Kimball et al., 2007, Zhao et al., 2013, Fu et al., 2014, Keenan and Richardson, 2015).

The length of the growing season of the subarctic grasslands under investigation extended consistently with warming (with on average 15.5 days per °C). This was mainly caused by an advance of the start of the growing season (Chapter III, Fig. 4). The larger temperature sensitivity of the start compared to the end of the growing season agrees with what has generally been found for northern ecosystems (Cleland et al., 2007, Zhao et al., 2015). The persistent extension of the growing season over the full warming gradient showed that these subarctic grasslands could still extend their growing season length if temperatures would continue to increase.

Interestingly, the response after short-term warming did not differ significantly from the response after long-term warming. Firstly, this indicated that the step-change in temperature, that had occurred at the short-term warmed site in 2008, had not induced major artefacts on vegetation physiology (see also § 7.1.1). Secondly, this suggested that quick acting responses, such as phenotypic plasticity (i.e. the ability of a given genotype to produce variable phenotypes in different environments; Agrawal, 2001), were more important in the phenological response to warming than slower acting mechanisms, such as genetic adaptations or changes in community composition (Jump and Penuelas, 2005). Unfortunately, no genetic data were available, but different plant inventory studies showed little changes in dominant species within the studied temperature range (Gudmundsdóttir et al., 2014, Michielsen, 2014b, Meynzer, In preparation).

Further, the vertical temperature gradient in these geothermal systems, which is also typical for other soil warming experiments (Patil et al., 2013), made it possible to speculate about actual location of the temperature response. The relatively low responsiveness of phenology towards soil temperatures (+2.1 days per °C), and the high responsiveness towards surface air temperatures (+29 days per °C) (Chapter III, Table 1) compared to earlier remote sensing studies at mid and high latitudes (1.2 to 24 days per °C; Zhao et al., 2015), suggested that the responsive tissues were located in between, in the upper soil layer and the litter layer. Indeed, this layer contained the grass meristems, which are the tissues where the growth starts in spring (Pautler et al., 2013). A better understanding of meristem physiology, and especially its response to warming, could therefore greatly improve the current projections of phenological shifts under climate change.

Finally, were detected two factors that can overrule warming effects on subarctic grassland phenology using interannual comparisons. This highlights a potential caveat of warming studies and cautions for misinterpretation of such phenological shifts as warming responses. Firstly, spring drought was found to delay the start of the growing season beyond

the point when the thermal requirements were met. This was clear from the consistently higher thermal requirement in the cold spring of 2015, compared to the two warmer springs (Chapter III, Fig. 5), which is contra intuitive, as thermal requirements are generally lower under colder conditions as a consequence of increased energy use efficiency (Liang and Schwartz, 2014, Liu et al., 2014), together with the exceptionally dry conditions in early summer 2015 (Chapter III, Fig. 1). Secondly, the end of the growing season set in exceptionally early in the short-term warmed grassland in 2013, while no major climatological differences occurred with the long-term warmed grassland (Chapter III, Fig. 4). We presume that the discrepancy was caused by a local pest in ST, such as a fungal infection or the mite *Penthaleus major*, that is known to affect Icelandic perennial grasslands in summer (Gudleifsson et al., 2002).

To conclude, this study indicated that the warming-induced extension of LOS of subarctic grasslands could still continue if warming persists (unless genetic adaptations or species shifts occur that would change that pattern). The lack of a clear saturation to warming in our study has important implications for the C uptake potential of these subarctic grasslands under future climate change. This is a powerful ecosystem feedback to the climate system and should be studied in more detail.

7.1.3 Long-term effects of soil warming on carbon storage in a subarctic grassland

Warming-induced carbon transfers from the soil to the atmosphere, mainly from northern ecosystems, are one of the largest uncertainties in global change projections (Kirschbaum, 1995, Friedlingstein et al., 2006, Todd-Brown et al., 2013) (see also Chapter I, § 1.3.1). This uncertainty is primarily caused by the lack of empirical observations of long-term warming effects, which is a consequence of practical and financial limitations that are connected to climate manipulation experiments that are typically performed to study this subject (see Chapter I, § 1.4.1, Chapter II and Chapter VII, § 7.1.1). In this thesis, the use of natural geothermal soil temperature gradients made it possible to provide the first empirical evidence for long-term (>50 years) SOC losses and to reveal the dominant processes contributing to eventual future warming-induced SOC losses (see also Chapter IV). Sub-arctic grasslands were used as model ecosystems to study the magnitude, the shape and the temporal dynamics of the temperature effects on SOC storage in northern soils.

I hypothesized that increasing soil temperature would substantially reduce the SOC stocks, as the temperature sensitivity of decomposition (SOC loss) is generally higher than that of primary production (SOC input) (Kirschbaum, 2000, Brady and Weil, 2008). I

expected that the accumulated SOC loss after short-term soil warming would be smaller than after long-term soil warming, because of the slow decomposition rates of the physically and chemically protected SOC in soils (Davidson and Janssens, 2006b). Moreover, I expected that the warming-induced SOC loss would be (partly) mitigated by an increase in C inputs due to (i) the alleviation of the strong N-limitation of the productivity of northern ecosystems (LeBauer and Treseder, 2008) as a consequence of increased decomposition and N mineralization (Schimel et al., 1994) and (ii) the higher temperatures that could accelerate plant growth and lengthen their growing season (Churkina et al., 2005).

As expected, I found a significant loss of SOC with increasing temperature in the topsoil (upper 10 cm) of the study sites. Surprisingly, the warming-induced SOC loss after 5 years of soil warming was very similar to the loss after ≥ 50 years of soil warming and amounted to 1.28 +/- 0.16 (SE) ton SOC ha^{-1} $°C^{-1}$ (equivalent to 4.1 +/- 0.5 (SE) % of the SOC stock measured under ambient soil temperatures) over a soil temperature gradient of 20°C (Chapter IV, Fig. 1.A). The SOC loss in the subsoil (10-30 cm depth) of the long-term warmed grassland (the soil of the short-term warmed grassland was too shallow to study subsoil changes), was less (P = 0.07): 0.81 +/- 0.38 (SE) ton SOC ha-1 $°C^{-1}$ (equivalent to 2.6 +/- 1.4 (SE) % of the SOC stock measured under ambient soil temperatures) (Chapter IV, Fig. 1.B). This smaller effect in the subsoil could be due to a lower relative increase of decomposition compared to the topsoil. This could be a consequence of (i) increasing energy limitation of the microbial community with depth caused by a lower input of fresh root litter and (ii) a greater physical and chemical protection of SOC in the subsoil (Fontaine et al., 2007, Rumpel and Kogel-Knabner, 2011). This smaller effect size in the subsoil could be due to a lower relative increase of decomposition compared to the topsoil. This could be a consequence of (i) increasing energy limitation of the microbial community with depth caused by a lower input of fresh root litter and (ii) a greater physical and chemical protection of SOC in the subsoil (Fontaine et al., 2007, Rumpel and Kogel-Knabner, 2011). This highlights the necessity of treating topsoil and subsoil SOC-dynamics separately (Rumpel and Kogel-Knabner, 2011), especially when designing climate change models to project SOC-climate feedback mechanisms. Taking only topsoil or subsoil into account or treating topsoil and subsoil as one SOC compartment could lead to large under- or overestimations of the feedback, depending on the approach of the study.

In this study, I found indications for three mechanisms that could explain the observed loss of SOC: (a) an accelerated decomposition, (b) a reduction of C-inputs and (c) a reduction of the physical SOC stabilization in aggregates:

(a) As hypothesized, a direct temperature-driven increase in decomposition rates (Davidson and Janssens, 2006b) likely contributed to the SOC loss. The occurrence of this typical temperature response of decomposition in the temperature gradients in this study was confirmed by both a standardized teabag decomposition experiment (Chapter IV, Extended data Fig. 6) and a soil incubation study (Marañón-Jiménez, in preparation), where warming accelerated the decomposition of both new incoming litter and SOC.

(b) In contrast to the expectation that C-inputs would increase due to direct temperature stimulation of productivity, alleviation of the N limitation of plant productivity and the lengthening of the growing season (see Chapter III), my results show a clear decrease in total C inputs (Net Primary Productivity; NPP) with temperature. While the aboveground biomass did not change significantly with temperature, the fine root biomass declined with 5-6% per °C (Chapter IV, Extended data Fig. 5.B). As this decline was twice as fast as the Q_{10} of fine root turnover in grasslands (Gill and Jackson, 2000), it is highly likely that the belowground C-inputs decreased as well. This unexpected lack of increasing C-inputs with increasing temperature could be caused by persistent N-limitation on productivity; for a yet unknown reason, N availability did not increase with temperature, thereby failing to stimulate NPP. Soil N stocks declined with temperature, and this loss was exactly proportional to the SOC loss (i.e. no change in soil C/N ratio; Chapter IV, Extended data Fig. 7).

The dramatic N loss could be caused for an important part by the collapse of the fungal community (Chapter IV, Extended data Fig. 3 and 4), which is typically the dominant microbial group in northern soils (Rillig et al., 2015), and whose decline has been linked to lower retention of labile nutrients (Kozlowski, 1999). There are indications that the N loss occurred via both increased gaseous N losses as via N leaching (Chapter IV, Extended data Fig. 8), which are the two most important pathways for soil N losses (Rillig et al., 2001). Another possible explanation for this large loss of soil N might be found in the different limiting factors for microbes versus plants. While plants are generally N-limited in northern ecosystems (N/P ratios in the study sites were also in the range of N limitation (Appendix, Fig. 1), indicating severe N limitation (Aerts and Chapin, 2000)), a soil incubation experiment of soils from the short-term warmed grassland revealed that the microbial community was clearly not N or P limited, but energy limited (Appendix, Fig. 2). It is possible that this strong energy limitation has stimulated the use of inorganic N as energy source, and the subsequent release of N from the system as N_2O, NO and HNO_2. The increase in soil N_2O concentrations (Chapter IV, Extended data Fig. 8) supports this hypothesis.

(c) A third important and not anticipated cause for the drastic temperature-driven loss of SOC at the ForHot research site was a temperature-induced decline in soil aggregation. Soil aggregates offer physical protection to decomposition (Six et al., 2002), and a decrease in soil aggregation can thus increase mineralization of SOC (Rillig et al., 2015). Five lines of evidence demonstrate that a temperature-induced decrease in soil aggregate formation and stability played an important role in the observed SOC loss. (i) In the upper soil layers (0-10 cm), soil compaction increased with increasing temperature (increase in bulk density; Chapter IV, Extended data Fig. 2). This reduction in soil pore space is consistent with loss of soil aggregation level (Kozlowski, 1999). (ii) An analysis of the distribution of SOC over the different soil size fractions showed that the reduction of SOC with temperature occurred predominantly in the sand and aggregate fraction (Chapter IV, Fig. 3). As the C content of sand is negligible, the main SOC loss can be attributed to the loss of C from stable aggregates. (iii) The fine root biomass, which plays an important role in both aggregate formation and stabilization (Rillig et al., 2015), decreased drastically with temperature in the main root zone (Chapter IV, Extended data Fig. 5.B). (iv) Also total fungal biomass decreased with temperature (Chapter IV, Extended data Fig. 3.A) and within this group, filamentous saprotrophs declined even more (Chapter IV, Extended data Fig. 3.B). As filamentous fungi are important contributors to aggregate stabilization due to their tensile strength and the long-lasting nature of their hyphae (Rillig et al., 2015), this could have contributed to aggregate breakdown. Also the abundance of arbuscular mycorrhiza (AMF), which are known to improve aggregate formation and stabilization through multiple mechanisms (Rillig et al., 2001), showed a steep decline with temperature, both in the topsoil community as in in-growth bags (Chapter IV, Extended data Fig. 4). (v) Finally, the comparable loss of SOC and soil organic N (SON) with temperature (Chapter IV, Extended data Fig. 7), suggest that abiotic loss mechanisms, such as loss of soil aggregation, played an important role in the temperature-driven loss of SOC. A schematic representation of the SOC loss mechanisms as identified in this study can be found in Chapter IV, Fig. 4.

The wide range of soil temperatures at the ForHot research site made it possible to measure SOC losses across the full range of RCP scenario's (which predict a temperature increase of 2.2 to 8.3°C for the subarctic by the end of the 21st century) and compare my observations to the data of the 'Coordinated Modelling Intercomparison Project Phase 5' (CMIP5) exercise (Taylor et al., 2012). The CMIP5 exercise involved 20 climate modelling groups from around the world and made a synthesis of a broad set of Earth System Models (ESMs). For the RCP 8.5 scenario (the highest temperature increase scenario), the ESM's

participating in the CMIP5 exercise predicted on average an increase of the SOC stocks in cold ecosystems, but with multi-model standard deviation higher than the multi model mean (26±32 Pg over the 21st century for tundra) (Todd-Brown et al., 2013). This large uncertainty underlines the necessity of empirical observations in cold biomes to better understand the response of SOC to temperature increase and thus better constrain the ESMs (Nishina et al., 2014). In contrast to most ESM projections, my data suggest rapid and substantial SOC losses at warming levels that are expected by the end of this century, even if the soil warming also induced a longer growing season by affecting plant phenology (Chapter III). For the subarctic (+2.2 to +8.3°C by the end of this century), my results indicate that topsoil SOC stocks could decrease with 9.0 to 34.0 %, providing that my temperature responses can be generalized.

There are several potential explanations for this difference between the ESMs and my observations. For one, I provide observations from only one region and one specific soil type, and it is well possible that my results should not be extrapolated to the whole subarctic region. On the other hand, this study detected several important warming-response mechanisms that are currently not embedded in ESMs. Firstly, in contrast to the ESM predictions N limitation was not alleviated by warming in this case (Chapter III). Nitrogen is known to be a much more important determinant of northern ecosystem productivity than warming (Hyvonen et al., 2007), but several ESMs still lack a nitrogen cycle. Secondly, and more importantly, this thesis clearly identified the impact of soil warming on soil physical structure and aggregation as an important contributing mechanism to the observed SOC stock decrease. This is an important finding, as the absence of this mechanism in ESMs (Todd-Brown et al., 2013), makes them unable to reproduce observations explained by modifications of soil aggregate dynamics.

The last two important take-home messages from this study are drawn from the very similar topsoil SOC loss after short-term (5 years) and long-term (≥ 50 years) soil warming. Firstly, it shows that the short-term warming effects, which had been caused by a step-wise temperature increase, were not confounded by major artefacts. Therefore, the gradually increasing effect along the temperature gradients is likely to be translated into similar response to gradually increasing warming as the real climate change progresses. Secondly, the similar response after short- and long-term warming indicates that the loss of SOC could occur very rapidly after the onset of warming. Therefore, it can be expected that the lag-time between global warming and SOC loss to the atmosphere will be short (few years maximum), causing a rapid positive feedback via the emissions of CO_2 from cold ecosystems.

7.2 Long-term effects increased N inputs on carbon storage in subarctic grasslands

7.2.1 The importance of studying long-term N input gradients

Terrestrial ecosystems and soils have been absorbing about 30% of the anthropogenic CO_2 emissions during the past decades (Le Quere et al., 2009, IPCC, 2013), thereby mitigating climate change. Northern terrestrial ecosystems (>50° N) have accounted for at least one third of this terrestrial C sink (White et al., 2000). It is generally accepted that the large northern C sink has been (partly) caused by an increase in the productivity of northern temperate terrestrial ecosystems as a consequence of the alleviation of the widespread N limitation (Hudson et al., 1994, Lloyd, 1999, Schlesinger, 2009) by increasing anthropogenic N deposition (Galloway et al., 2008, Gundale et al., 2014). Currently, the anthropogenic N deposition in Iceland and many other high latitude regions is still low (Galloway et al., 2008). It is, however, expected that a substantial part of these regions will experience an increase in N deposition in the near future, as a consequence of the disclosure of 'the high north' by global warming, enabling more intensive agriculture, industry and transport (Lamarque et al., 2011). Considering the typical strong N limitation in these northern ecosystems, this could cause a significant contribution to the terrestrial C sink. However, due to the lack of long-term N addition studies, the future of this important N-dependent C sink remains unclear (See Chapter I, § 1.3.2). Predictions range from a current saturation of the positive N effect to a steady increase of its effect up to at least the middle of this century (Lloyd, 1999, White et al., 2000, Cramer et al., 2001, Bachelet et al., 2003, Pepper et al., 2005, Friedlingstein et al., 2006, Canadell et al., 2007, Morales et al., 2007, Le Quere et al., 2009, Tao and Zhang, 2010, Todd-Brown et al., 2013, Arora and Boer, 2014).

In this thesis, I aimed to elucidate the long-term effect of increased N inputs on the net SOC storage in subarctic grasslands, using long-existing natural gradients in N inputs. Also, different successional stages (early successional and mature) were assessed, as the effect of increased N inputs is expected to change during the successional development (Crocker and Major, 1955, Saynes et al., 2005, Seedre et al., 2011, Appling et al., 2014), and as it is acknowledged that ignoring ecosystem successional and soil developmental stage contributes a substantial part to the uncertainties in global C dynamics (Chapin et al., 2011).

7.2.2 Natural gradients in N inputs – impact on N status of subarctic grasslands

As natural N input gradients, I selected one site pair with a short N accumulation time and one with a long N accumulation time. Each pair included one site with low and one with high seabird derived N inputs. The site pair with a short N accumulation time (the "young" sites) were located on the young island Surtsey that was formed ~50 years ago, during a submarine eruption from 1963 – 1967. The site pair with a long N accumulation time (the "old" sites; located on Heimaey and Ellidaey) had a bedrock of 5,900 years, but the maximum age of undisturbed soil (marked with an ash layer from 395 AD; Larsen, 1984) was approximately 1,600 years. Their soil profiles also contained an ash layer dating from the nearby 1973 Eldfell eruption, allowing to separate the last four decades of N accumulation from the millennial accumulation.

The N input rate at the sites with minor seabird influence (both young and old) was initially assumed to approximate the natural atmospheric N deposition in the area, amounting to 1.3 to 1.4 kg N ha^{-1} y^{-1} (the estimated annual atmospheric N deposition rate in Iceland corrected to 1600 mm annual precipitation; Gislason et al., 1996, Sigurdsson et al., 2005b). Investigation of the total ecosystem N stocks in the early successional dune vegetation stage on Surtsey, however, revealed an actual yearly N retention of 0.7 kg N ha^{-1} (ca. 50–60%), which approximates the N retention capacity of bare dune habitats under low N inputs (Thomas et al., 2013). The additional seabird-derived N inputs that were retained in plants and soil of the young Surtsey sites with major seabird influence was calculated using the total N stocks and detailed knowledge of the seabird colonization time on 1000 m^2 areas around each plot (ca. 20 – 26 years, depending on the location of the measurement plot; Magnússon, personal communication), and amounted on average to 47 kg N ha^{-1} y^{-1}. For the older site with major seabird influence, the additional seabird-derived N input was calculated from seabird nesting densities (Hansen et al., 2011) and a bioenergetics model that yielded an estimation of N input rates per breeding pair (Wilson et al., 2004, Blackall et al., 2007). This resulted in an estimation of on average 67 kg N ha^{-1} y^{-1}.

Before addressing the effect of seabird-derived N inputs on the N status of the sites (inducing total SON stocks and N availability), an important remark on the use of seabird manure as natural N input source should be made, especially in the context of SOC storage. Guano is N rich, but contains next to N also other nutrients, as well as organic C. At these study sites, the influence of other guano-derived nutrients on SOC storage was assumed to be negligible, as N was by far the most limiting element for plant productivity (Chapter VI, Fig. 3.A; Aerts and Chapin, 2000). Also the contribution of guano-derived organic C to the total

SOC storage was assumed to be insignificant. A conservative estimate of its contribution to the total organic C inputs amounted to only 1.2 to 1.8% (Chapter V) and its contribution to soil organic C was probably even lower, as C storage efficiency of bird manure tends to be lower than that of biomass residues (Rahman, 2013, Hua et al., 2014, Rahman, 2014). Therefore, N could be considered as the primary manure-derived factor influencing SOC storage.

It was expected that an increased N accumulation time (of ca. 1600 years) would have a larger effect on the ecosystem N status (increasing the total SON stocks, enhancing N availability and alleviating N limitation) than a few decades (20 - 26 years) of increased N inputs. However, the N status was clearly more closely related to the rate of annual seabird-derived N input than to ecosystem age. The total SON stocks and N availability were greatly increased in the high N input sites compared to the low N input sites in both age classes of islands. Interestingly, even though the N stocks and N availability at the high N input sites were strongly increased, the plant N/P ratios indicated that plant growth remained N limited (Chapter VI, Fig. 3.A), resulting in a continuous strong response to higher N-inputs with increasing biomass production and SOC storage (biomass stocks, plant height and ecosystem C stocks were significantly higher at the high N input sites (Chapter VI, Fig. 3.B, 3.C and 6)). Thousands of years of N retention and recycling did, however, slightly alleviate the N limitation in the older sites compared to the younger with similar seabird N inputs, as was shown by their significantly higher standing biomass and plant height (Chapter VI, Fig. 3.B and C). The N availability was only marginally higher in older sites compared to the younger sites, when estimated with ion exchange raisins (PRS probes; Chapter VI, Fig. 2.A). Finally, it is interesting to note that the vegetation N stocks reacted much more quickly to increased N inputs on the young sites on Surtsey than the SON stocks did. During the few decades (20 – 26 years) during which the young site had received increased N inputs, it had reached the same level of vegetation N stocks as the older high N input site where bird droppings increased N inputs probably for over a millennium. The SON stocks, however, were still significantly lower (over 35 times; Chapter VI, Fig. 2.B and C), which shows that the SON stock in the young site was not yet in equilibration with the N input rates and would still need centuries to fill this ‘SON gap’.

To conclude, N accumulation time and seabird N inputs had a strong influence on the N status of the ecosystems, with seabird N inputs having the largest effect. However, all sites remained N-limited, indicating that no N saturation had been reached yet, even after 1,600 years of N-accumulation and seabird-derived N inputs.

7.2.3 Effects of increased N inputs on net SOC storage in early successional subarctic grasslands

The response of N-limited ecosystems (such as the subarctic grasslands that are investigated in this thesis see § 7.2.1) to increased N inputs is expected to change during the course of succession, as total N stocks and N availability increase during the soil maturation process (Crocker and Major, 1955, White et al., 2004, Rhoades et al., 2008, Smithwick et al., 2009). To investigate this successional evolution, I selected subarctic grasslands with contrasting successional stages. The sites on the young island Surtsey were in an early successional stage, while the sites on the older islands (Heimaey and Ellidaey) were in their mature stage.

In early successional stages, the rate of SOC accumulation depends strongly on the rate in which plants can colonize the area, as plant material is the primary source of SOC (Kögel-Knabner et al., 2013). The colonization of vascular plants, however, also depends on soil development and the associated increase in nutrient (mainly N) and water availability (Gruner et al., 2008). At the early successional site on Surtsey with low N inputs, the biomass stocks were still very low (Chapter V, Fig. 5). The aboveground biomass (0.13 ± 0.08 ton ha^{-1}; errors = SE) was in the range of desert ecosystems (0–20 ton ha-1; Larcher, 2003) and had hardly changed since the first measurements of aboveground biomass were made in 1999 (0.1 ton ha^{-1}) (Magnússon et al., 2009). As expected, the plant growth was strongly constrained by N availability, as was shown by the low leaf N/P ratio of *C. fontanum* (Chapter VI, Fig. 3.A) and by the exceptionally high root/shoot (R/S) ratio (Chapter V, Fig. 7). Nutrient limitation can often be detected by looking at R/S ratios (Levang-Brilz and Biondini, 2003, Chu et al., 2006), as low nutrient availability promotes investment in belowground plant parts.

At the high N input site on Surtsey, only 26 years of increased N inputs had increased the biomass stock over 50 times (from 0.7 to 36 ton ha^{-1}) to a value that is typical for a mature grassland ecosystem (20 to 50 ton ha-1; Larcher, 2003). The increasing N inputs had played an important role in the biomass increase, as was shown by the strong positive relationship between biomass stock and N stock among the measurement plots and by the increase in leaf N/P ratio of *C. fontanum* (Chapter VI, Fig. 3.A) and the decrease of the ecosystem R/S ratio (Chapter V, Fig. 7). In addition, it was clear that the belowground limitations for nutrients and water were more important than aboveground limitations, such as competition for light and space, as the aboveground biomass had been increasing nearly linearly from 1.4 ton ha^{-1} in 1999 to 4.1 ton ha^{-1} in 2007 (Magnússon et al., 2009) to 5.9 ± 0.9 ton ha^{-1} (error = SE) in 2012-2013. This is typical for natural grassland ecosystems with relatively low nutrient levels (Kiaer et al., 2013).

The large increase in biomass under increased N inputs had strongly stimulated the accumulation of SOC. The SOC stock had increased from 0.7 ton ha^{-1} under low N inputs to 4-10 ton ha^{-1} under high N inputs (see Chapter V, Fig. 5 and Chapter VI, Fig. 6). However, even if the SOC stock had greatly increased, it was still only a fraction of the average SOC stock of mature temperate grassland soils (194 ton ha-1; Schlesinger, 1997). Consequently, I expect that the SOC stock will continue to accumulate on Surtsey for many centuries under both low and high N input scenario's) before it reaches an equilibrium with the aboveground productivity. However, the timeframe in which this 'SOC gap' will be filled will be very different for both scenarios, which becomes clear when the respective net SOC storage rates are calculated from the respective total SOC stocks and SOC accumulation times. The net SOC storage rate (and thus 'SOC gap filling rate') was more than 15 times higher at the high N input site (0.29 vs. 0.018 ton SOC ha^{-1} yr^{-1}; Chapter VI, Fig. 7.B). This stimulation of the SOC storage rate under increased N inputs and the topic of SOC equilibrium/'saturation' will be discussed further in § 7.4.2.

In conclusion, early successional sites respond strongly to increased N inputs in terms of biomass accumulation and SOC storage. In a very short time frame (26 years in this study), the biomass stock increased from values typical for desert ecosystems to a value in the range of mature grasslands. The SOC stocks, however, although strongly increased under increased N inputs, were much lower than those of mature grassland soils. This indicated that the SOC stock still had the potential to increase until an equilibrium state is reached with the biomass inputs. Increased future N deposition could therefore stimulate removals of CO_2 from the atmosphere to SOC stocks in early successional grassland ecosystems, partly counteracting the expected negative effects of higher future temperatures on SOC stocks (see also § 7.3).

7.2.4 Effects of increased N inputs on net SOC storage in mature subarctic grasslands

For mature grasslands, I used the presence of different dated ash layers to separate into 'decadal SOC storage' (SOC storage above the 1973 ash layer, which can be considered as 'short-term SOC storage') and 'millennial SOC storage' (SOC storage above the 395 AD ash layer, which can be considered as 'long-term SOC storage') (See Chapter VI, § 6.3.6). This separation allowed to compare decadal (short-term) and millennial (long-term) N input effects on SOC storage. In this section, I will first discuss N input effects on the decadal SOC storage, where after the millennial SOC storage will be discussed.

Despite their specific characteristics (including high concentrations of Al, Fe and Si that can e.g. bind SOM in 'metal-humus' complexes (Arnalds, 2015)), the Andosols in this

study showed a decadal SOC storage rate (0.30 - 0.44 ton ha^{-1} yr^{-1}) in the range of the average topsoil SOC storage in a broad range of soil types under previously SOC depleted perennial temperate grasslands (0.33 ton ha-1 y-1; Post and Kwon, 2000).Increased N inputs significantly increased the decadal net SOC storage rate, although less than at the early successional sites on Surtsey (50% vs. 250% increase in net SOC storage; see § 7.2.2). This accorded to my expectation that the early successional sites would be more N limited than the mature sites. The positive effect of N on net SOC storage corresponded well with an earlier long-term N addition study on managed grasslands in east Iceland, which reported a > 50 % increase in SOC concentration in the upper 10 cm of the soil after 43 years of fertilization (Gudmundsson et al., 2004).

The net response of SOC storage to increased N inputs is the product of the responses of the individual processes that influence SOC inputs and outputs from the topsoil: net primary productivity (NPP; SOC inputs), root/shoot C partitioning (SOC inputs and outputs) and decomposition rate (SOC outputs) (Mack et al., 2004, Batjes, 2014). The SOC inputs were greatly increased in the mature subarctic grasslands under high N inputs, as was indicated by the four times higher aboveground biomass at the high N input sites (Chapter VI, Fig. 3.C). This stimulation of NPP under elevated N inputs agreed with what is generally found in studies that investigate the effects of N inputs on productivity of subarctic grasslands (Sillen and Dieleman, 2012). Root/shoot C partitioning, of which changes could lead to net SOC outputs or inputs, depending on the recalcitrance of the tissues that are invested in, was similar under low and high N inputs (average root/shoot ratios of ~10; data not shown). However, since the total amount of belowground C allocation is potentially also affected by changes in root turnover rates (Aerts et al., 1992, Milchunas et al., 2005), changes in exudation and mycorrhizal activity (Vicca et al., 2012) and changes in microbial C use efficiency (Wieder et al., 2013), the total belowground C inputs could not be derived from the present data. Changes in SOC outputs (decomposition) under elevated N inputs were not assessed in the present study, but previous studies have reported both positive and negative effects of enhanced N input on the decomposition rate in the topsoil of subarctic grasslands (Aerts et al., 2003, Knorr et al., 2005, Hobbie, 2008, Zhang et al., 2008). The direction of the response seems to depend on e.g. natural background N deposition, N input rate and duration and litter quality. In any case, the clear increase in SOC stocks under increased N inputs in the Icelandic mature grasslands indicated that the N-induced increase in C inputs surpassed the potential increases in C outputs in my study.

The millennial net SOC storage had led to the buildup of SOC stocks (220–280 ton C ha^{-1}) that were in the range of a previous estimation for Brown Andosols in Iceland (227 ton ha-1; Óskarsson et al., 2004) and of non-volcanic temperate grassland soils (estimates range from 197 to 236 ton ha-1; Schlesinger, 1997, Janzen, 2004). Also the net annual SOC storage rate (0.12 – 0.16 ton C ha^{-1} yr^{-1}) corresponded well with previous studies; both with studies on deep SOC rich soils in northern regions (0.15–0.30 ton ha-1 yr-1; Trumbore and Harden, 1997) and with studies on long-term SOC storage in temperate grassland ecosystems in China (0.11 ton ha-1 y-1; He and Tang, 2008). Increased N inputs did increase the millennial net SOC storage rate, albeit to a smaller relative extent than it stimulated the decadal storage rate (25 % vs. 50 % increase, respectively). This "modest" increase in SOC storage rate under chronically elevated N inputs, however, was consistent throughout the soil profile and added up to a considerable strengthening of the SOC sink over a long time span while a thicker soil was developed (Chapter VI, Fig. 7.B).

To conclude, the SOC storage rate in mature subarctic grasslands increased under increasing N inputs, both in decadal as millennial timescale. The positive effect on decadal SOC storage supported the hypothesis that northern temperate grasslands have played an important role in mitigating anthropogenic CO_2 emissions during the past decades by increasing their SOC storage rate as a consequence of increased anthropogenic N deposition (Hudson et al., 1994, Lloyd, 1999, Schlesinger, 2009). Moreover, it suggests that subarctic grasslands can become an important contributor to the terrestrial C sink when N deposition at high northern latitudes increases, which is expected to happen in the near future (Hermans et al., 2010, Gudmundsdottir et al., 2011a, Lamarque et al., 2011). The positive effect of increased N inputs on the millennial SOC storage implies that the mitigating effect on anthropogenic CO_2 emissions could last for many centuries to come, but the duration of such enhanced terrestrial sink has been highly debated (Lloyd, 1999, White et al., 2000, Cramer et al., 2001, Bachelet et al., 2003, Pepper et al., 2005, Friedlingstein et al., 2006, Canadell et al., 2007, Morales et al., 2007, Le Quere et al., 2009, Tao and Zhang, 2010, Todd-Brown et al., 2013, Arora and Boer, 2014), as mentioned earlier. This last finding could be an important contribution to that discussion.

7.2.5 Importance of successional stage for net SOC storage in subarctic grasslands

In subarctic grassland ecosystems that did not receive a substantial amount of allochthonous N inputs, mature grasslands had a much higher SOC storage rate than early successional grasslands (Chapter VI, Fig. 7.B). This maturation-driven increase in net SOC storage rate is

generally found in many soil types and ecosystems (e. g. Lichter, 1998, Foote and Grogan, 2010, Kabala and Zapart, 2012, Kalinina et al., 2013) and is caused by an increasing soil development along the successional gradient. The soil development is associated with increasing SOC concentrations (Vitousek and Reiners, 1975, Kirschbaum et al., 2003), which improve the N exchange capacity and the water holding capacity of the soil (Deluca and Boisvenue, 2012), thereby stimulating plant growth and SOC storage. Increasing N inputs, however, changed this situation in a short timeframe. After only 26 years, increased N inputs had lifted the net SOC storage rate of the early successional grassland to the level of the mature grasslands (Chapter VI, Fig. 7.B).

Even if the net SOC storage rate of the early successional grassland under increased N inputs approached that of the mature high N input grasslands (Chapter VI), its total SOC stock was still much smaller (with 220–260 ton SOC ha^{-1}; Chapter VI, Fig. 6.B). This 'SOC gap' (Kramer and Gleixner, 2008) will be gradually filled during the process of soil maturation by the input of organic material at the soil surface and into the rooting zone. The timeframe in which this process will be completed depends on the net SOC storage rate of the early successional site. Increased N inputs will thus strongly shorten this timeframe. A key question in relation to this is when the apparent SOC gap will saturate. Until recently, it was assumed that all mature soils were SOC-saturated (Wutzler and Reichstein, 2007). However, this supposition has been challenged by various observations of continuously increasing SOC stocks in old (> 1,000 years) undisturbed soils (e.g. Harden et al., 1992, Wardle et al., 1997) and model-based predictions that the equilibration process of SOC stocks could take millennia (Wang and Hsieh, 2002). Also in this thesis study there were indications that the mature grasslands (on 1,600 years old undisturbed soils) had not yet reached SOC saturation. This claim is firstly supported by the finding that the decline in net SOC storage rate with depth stabilized around 1000 years before present and did not decline to zero (Chapter VI, Fig. 7.B). Secondly, the deeper soil layers showed a ~50 % SOC deficit, based on their potential SOC storage calculated according to Wiesmeier et al. (2014) (Chapter VI, Fig. 4). Finally, the stable C/N ratio of about 12 in the total soil profile, up to the deepest soil layers, suggested that the total SOC stock could continue to increase with elevating N inputs even after millennia of soil maturation, providing that N can be retained.

In summary, the early successional subarctic grasslands were smaller C sinks than the mature subarctic grasslands, but increased N inputs could reduce or even eliminate this difference over a short period of time. Moreover, the early successional subarctic grasslands (and early successional ecosystems in general; Kalinina et al., 2015) were characterized by a

large ‘SOC gap’ that will be filled during future soil development at Surtsey and thus provide a vast potential for future C storage. Mature ecosystems do not have this ‘SOC gap’, but my study indicated that mature subarctic grasslands were still net C sinks, even after 1,600 years of soil development, and that increased N inputs could induce a long-lasting strengthening of this sink. This is an important finding as it is still uncertain whether the current N-driven terrestrial sink for atmospheric CO_2 at northern latitudes could saturate in coming decades or not, as discussed earlier.

7.3 Combined increase in temperature and N inputs – an exercise

In this thesis, warming and increasing N inputs were studied in isolation. In reality, however, temperature and anthropogenic N inputs are expected to increase in parallel in many areas. It has long been acknowledged that the responses to single climate change related factors might interact (e.g. Long, 1991, Woodward, 1992), especially responses to N inputs and responses other factors (Hyvonen et al., 2007). Moreover, the findings in this thesis suggest that the effects of warming and increasing N inputs will act in a similar timeframe (shortly after the initiation of the change), indicating that their effects will influence each other. Temperature increases reduced the SOC stocks in less than 5 years, equilibrating to a similar level as soils that had been warmed for at least 50 years (Chapter IV). Increased N inputs, on the other hand, had drastically increased SOC stocks within 26 years in my study (Chapter V), and the response became apparent shortly after the initiation of the additional N-inputs (Magnússon and Magnússon, 2000). They showed that within 10 years of increased N inputs on Surtsey, a full vegetation cover was reached, which is closely related with SOC storage as vegetation residues are the main supply of soil organic matter (Kögel-Knabner et al., 2013).

An increasing number of studies acknowledges the importance of interactions between climate change related factors and has been investigating the combined effect of increasing temperature and N inputs. However, while a substantial number of short-term factorial warming and N addition studies have been conducted (e.g. Gunnarsson et al., 2004, Niboyet et al., 2011, de Sassi et al., 2012, Liu et al., 2013, Kaldy, 2014), not many have been investigating net SOC storage, and most of them have been conducted under laboratory conditions. Long-term factorial studies on SOC storage are even more rare (Luo et al., 2011). Further, both short-term and long-term manipulation studies are usually limited in the number of temperature x N additions to a single, or in the best case a few combinations (De Boeck et al., 2015).

Short-term studies have found both additive and antagonistic effects of warming and N addition on different variables connected to ecosystem C cycling (Dukes et al., 2005, Gudmundsdottir et al., 2011a, Tao and Song, 2013a, Graham et al., 2014) and there were indications that the combined effect depended on the level of warming and N addition (Tao et al., 2013b). Also in long-term studies, the type of interaction between warming and N addition is not consistent. While DeMarco et al. (2014) found an additive effect on productivity (C inputs), Olid et al. (2014) found a synergistic effect on both C inputs and C accumulation and Fröberg et al. (2013) and Zong et al. (2013) found antagonistic effects of warming and N addition on total ecosystem C stocks and ecosystem respiration, respectively. In the study of Campioli et al. (2012), the effect on plant growth (C inputs) could be additive, antagonistic or synergistic, depending on the species and on the growth parameter selected. To conclude, the combined effect of warming and N addition on C cycling is complex and depends on a broad variety of factors.

As an exercise, I here use the findings of this thesis to make an estimation of the cumulative effects of soil warming and increased soil N. For this theoretical exercise, I made two important assumptions: (1) warming and N addition effects are additive (no interactions), and (2) effects of whole system warming are similar to effects of soil warming only. I made a conservative estimate, based on an average temperature increase scenario and a maximum N input increase scenario. I acknowledge that this exercise is highly error-prone but it does give a rough idea of the relative importance of warming and N status on C storage in subarctic grasslands.

Based on the findings in Chapter IV, it is estimated that a temperature increase of 5°C (the average predicted temperature increase of the four RCP scenario's for (sub)arctic regions by the end of this century (IPCC, 2013)), would reduce topsoil SOC stocks by 20.5 ± 2.5 (SE) %. (Using the highest temperature increase scenario (RCP 8.5; + 8.3°C) the decrease in SOC would even amount to 34.0 ± 4.2 (SE) %.) The maximum estimate for N input increases in Iceland by the end of this century is ~10 kg N ha^{-1} y^{-1} (Lamarque et al., 2011). In Chapter VI, an increase in N inputs of 67 kg N ha^{-1} y^{-1} increased the topsoil SOC stocks with ~ 50%. Therefore, an increase of 10 kg N ha^{-1} y^{-1} would approximately lead to an increase in topsoil SOC stocks of 7.5 % (assuming that the relationship between N inputs and C stock is linear). Putting these estimates together, a combined temperature increase of 5 °C and increase in N inputs of 10 N kg ha^{-1} y^{-1}, would lead to a topsoil SOC loss of ~13 %. Hence, if the abovementioned assumptions hold, my data suggest that the negative temperature effect on

SOC stocks will offset the positive effects of increasing N inputs in these subarctic grasslands during the next century.

7.4 Conclusions

- Natural gradients in global change related factors are a promising complement to other approaches in global change research, offering the possibility to study equilibrium states (long-term global change impacts).
- Soil and surface temperatures were identified as an important determinant of aboveground grassland phenology. Soil warming increased the length of the growing season, mainly by advancing the start of the season. The longer growing, however, could not compensate for the warming induced SOC losses via increased decomposition (see below).
- Soil warming induced large and fast SOC losses from Andosols under subarctic grasslands. Equal SOC losses from short-term and long-term warmed soils suggested that warming of such subarctic soils could induce a rapid positive feedback to climate warming.
- Central to the observed SOC loss of the grassland soils was the loss of physical protection of SOC in soil aggregates. This mechanism is currently not embedded in Earth System Models, and its inclusion could reduce the model uncertainty.
- Increased seabird-derived N inputs stimulated plant productivity and SOC storage in early developmental Icelandic Andosols. After the initiation of enhanced N-inputs, plant productivity quickly reached the level of mature grassland ecosystems, while SOC stocks needed many centuries to build up. Therefore, early developmental soils have a large SOC storage potential, and their SOC storage rate can be strongly increased by increasing N inputs.
- In mature Icelandic grassland Andosols, increased N inputs also stimulated plant productivity and SOC storage. This indicates that the N induced C storage potential of the studied andosols had not been reached after millennia of N accumulation, and that the potential N-induced northern C sink (that could mitigate a substantial part of the anthropogenic C emissions in the near future) could continue for many centuries.

7.5 Future research

- In Chapter III, the effect of increased soil temperatures on the length of the aboveground growing season was studied with regular *in situ* NDVI measurements. The NDVI data could also be coupled more closely to changes in aboveground net primary productivity (ANPP) by using the integrated NDVI over the total growing season, especially in combination with repeated biomass harvests (data not used in this thesis). ANPP is a crucial factor in the understanding in ecosystem C storage, and its estimation is currently only based on standing biomass at the top of the growing season (see Chapter IV). Using the combined NDVI and biomass harvest data could greatly improve our estimation of the ANPP and would help to better answer the question how warming affected the ANPP. These analyses are planned during the coming months.

- At the ForHot research site, the observed temperature-induced SOC losses were strongly linked to the disintegration of soil aggregates (see Chapter IV). However, the global importance of this mechanism is unclear. Since the loss of soil aggregation and the associated loss of SOC was completed after only five years of soil warming, the universality of this mechanism may be verified by performing soil texture and soil C fractionation studies at a broad range of established temperature manipulation experiments worldwide.

- The stimulation of N mineralization by increased soil temperatures was expected to enhance plant-available N in the soil and thereby plant growth, as the grassland ecosystem was clearly N limited. It was further hypothesized that a major part of this additional available N would be immobilized by microbes and plants and thereby retained in the ecosystem. However, equal relative losses of soil N and SOC were observed and there was no significant increase in plant biomass with increasing temperature (see Chapter IV). We did find indications for both higher gaseous N_2O emissions and N leakage to the groundwater at higher soil temperatures, but the relative importance of these two processes remains unclear. Therefore, a better understanding of the grassland N cycle would greatly increase our understanding of the observed biomass and C-cycle responses to warming. Therefore, closing the ecosystem N cycle should be an important focus in future ForHot research, an effort that has already been initiated in the ongoing work of Steven Dauwe (N – temperature interactions), Sara Marañón-Jiménez (microbial N dynamics), Per Gundersen (aquatic

N losses), Marja Maljanen (HONO and N_2O emissions), Martin Maddison (N_2 emissions) and colleagues.

- A surprizing finding in this thesis was the drastic decrease in belowground biomass (roots) with increasing soil temperature without a change in aboveground biomass (Chapter IV). This imbalance could be (partly) explained by an allocation shift with enhanced N availability, but also possibly with a change in root turnover rate and phenology. A minirhizotron study could offer a better understanding of these processes. Therefore, minirhizotron tubes were installed at the ForHot research site in autumn 2014, but still await further study. A better understanding of how belowground NPP is affected by global change factors is critical, especially for high latitude ecosystems where such processes tend to govern aboveground processes in biogeochemical cycles.

- A key finding in this thesis was the long-term (ca. 1,600 years) stimulation of net SOC sequestration by increased N inputs. This observation contributes to the understanding of the future of the N-dependent terrestrial C sink and opposes the theory that this sink would saturate in the coming decades. Comparable studies should be performed in other ecosystem types to strengthen this understanding.

- In this thesis, the effects of long-term soil warming and increased N inputs were investigated in isolation (Chapters II-IV vs. Chapters V-VI respectively). In reality, however, temperature, atmospheric CO_2 concentration and anthropogenic N depositions are increasing in parallel. In the ecosystems under investigation, soil warming and increased N inputs exerted counter-acting impacts, with warming strongly decreasing soil C stocks and N inputs stimulating soil C storage. Their cumulative effect (which is not necesseraly additive; e.g. Chapin et al., 1995, Hyvonen et al., 2007) could be studied by combining the natural temperature gradients with a N addition experiment (preferably run in the long-term, >10 years). This project has been initiated in 2015 at ForHot in the PhD thesis work of Steven Dauwe.

- One further step would be to investigate the long-term effect of increasing CO_2 levels in combination with the currently studied long-term temperature and N effects. The increase of atmospheric CO_2 levels is a third global change factor that exerts a strong impact on the C dynamics of ecosystems (Hyvonen et al., 2007). Long-term CO_2 fertilization can be found in certain types of geothermal areas (Fridriksson et al.,

2006), and can therefore be combined with natural gradients in soil temperature that can be found at the same locations. However, sites with pure CO_2 fertilization (without contamination by other geothermally produced gasses) are rare (e.g. Weinlich et al., 1999, Werner et al., 2008, Hernandez et al., 2012). Moreover, CO_2 concentrations are often variable in time due to changes in geothermal activity and wind speed and –direction. Therefore, great care has to be taken in the site selection. Another option is to artificially increase the atmospheric CO_2 level (e.g. with free air CO_2 enrichment (FACE) or open top chambers (OTC's)). However, such systems should be maintained for a long period to reach the long-term equilibrium ecosystem response that could be studied without delay close to natural CO_2 sources.

- Other global change related factors that might be interesting to study in combination with long-term warming and/or nitrogen inputs are changes in precipitation, and, at high northern latitudes, changes in snow cover (IPCC, 2013).

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8 References

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Appendix

9. Appendix

9.1 Supplementary Figures

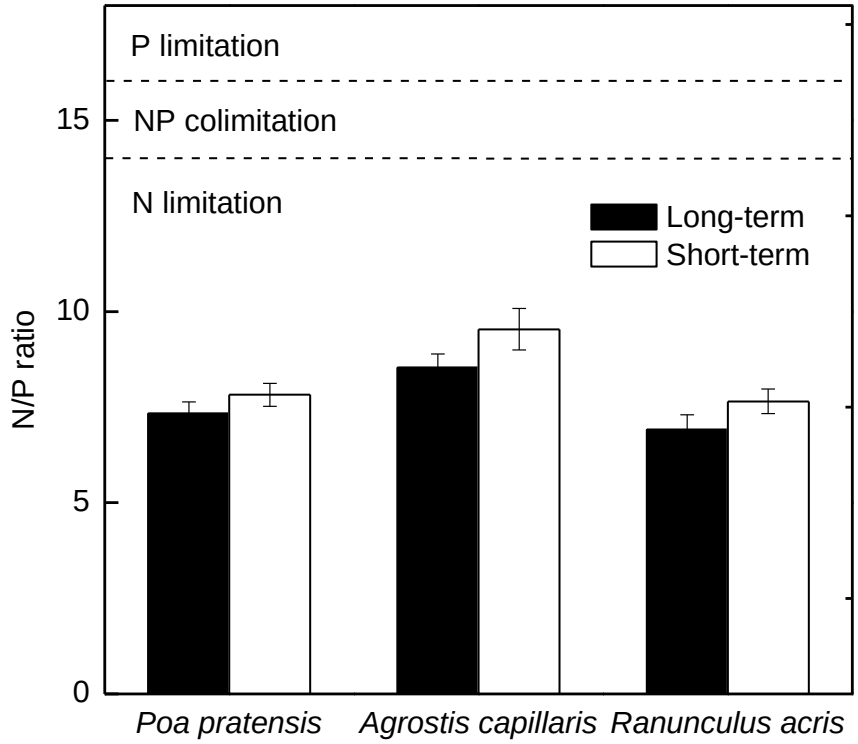

Figure 1: N/P ratios of mature healthy leaves of three dominant plant species (*P. pratensis*, *A. capillaris* and *R. acris*) in the long-term warmed grassland (black) and the short-term warmed grassland (white) at the ForHot research site. Dotted lines show the borders of N limitation (N/P < 14), NP co-limitation (14 < N/P < 16) and P limitation (N/P > 16) (Aerts and Chapin, 2000). N/P Error bars indicate SE's.

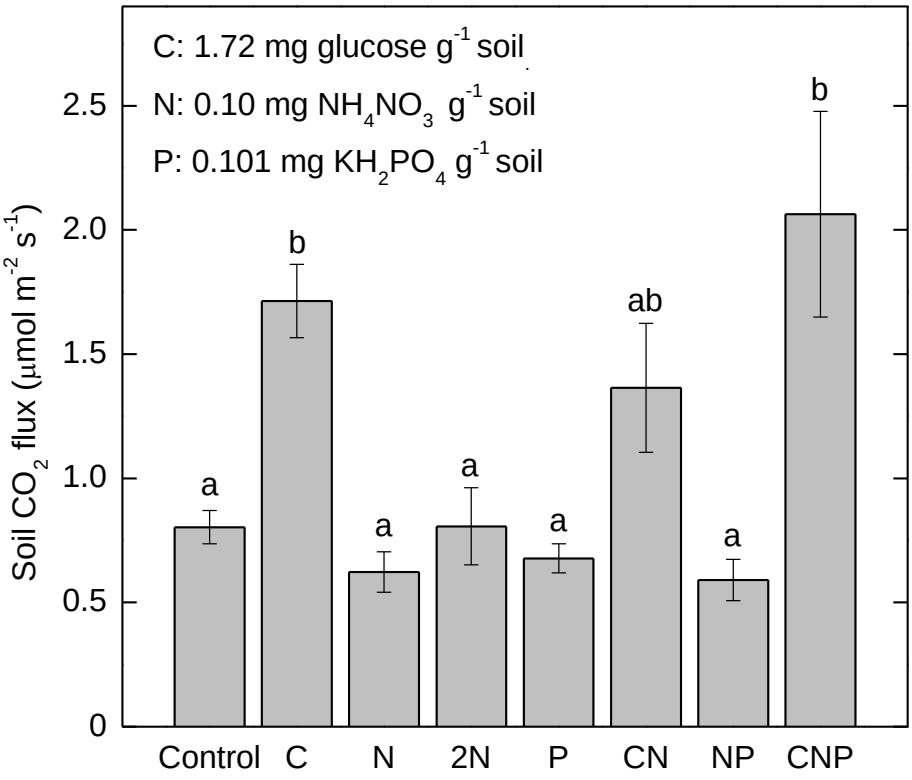

Figure 2: The effect of nitrogen (N), phosphorus (P) and carbon (C) addition on soil respiration of topsoils (0-10 cm) coming from unwarmed soils from short-term warmed grassland site at the ForHot research site, after 12 h of lab incubation at the natural mean annual soil temperature (5°C), keeping soil moisture to 60% of the field capacity. Letters show statistical differences. Differences were considered as statistically significant when $p < 0.05$. (Sara Marañón-Jiménez, Unpublished data)

9.2 Curriculum Vitae

Niki I. W. Leblans

Personal

Date of birth: June, 22 1988
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Education and research activities

2012 - present:	Joined PhD student at the University of Antwerp (Belgium) and the Agricultural University of Iceland - FWO funded. Project title: Natural gradients in temperature and nitrogen: Iceland represents a unique environment to clarify long-term global change effects on carbon dynamics.
2012	Umeå University (Sweden): M. course in Arctic Geo-ecology
2011	Umeå University (Sweden): M. course in Arctic Ecosystems
2009 – 2011	University of Antwerp (Belgium): M. Sc. in Biology (Ecology & Environment)
2006 – 2011	University of Antwerp (Belgium): B. Sc. in Biology

Publications

Accepted

Leblans, N. I. W., Sigurdsson, B.D., Roefs, P., Thuys, R., Magnússon, B. and Janssens, I.A.: Effects of seabird nitrogen input on biomass and carbon accumulation after 50 years of primary succession on a young volcanic island, Surtsey, Biogeosciences, 11, 1-14, doi: doi:10.5194/bg-11-6237-2014. Impact factor: 3.978

Poeplau, C., Kätterer, T., Leblans, N. I. W. and Sigurdsson, B. D.: Sensitivity of soil carbon fractions and their specific stabilisation mechanisms to extreme soil warming in a subarctic grassland, Global Change Biology, DOI: 10.1111/gcb.13491-2016. Impact factor: 8.444

Sigurdsson, B. D., Leblans, N. I. W., Oddsdóttir, E. S., Marjanen, M. and Janssens, I. A.: Effects of geothermal soil warming on soil carbon and nutrient processes in a Sitka spruce plantation. Working Papers of the Finnish Forest Research Institute, 316, 11-13. ISBN 978-951-40-2513-6, 2014.

Campioli, M., Leblans, N. I. W. and Michelsen, A.: Stem Secondary Growth of Tundra Shrubs: Impact of Environmental Factors and Relationships with Apical Growth, Arcitc Antarctic and Alpine Research, 44: 16-25, doi:10.1657/1938-4246-44.1.16-2012, Impact factor: 1.515

Campioli, M., Leblans, N. I. W. and Michelsen, A.: Twenty-Two Years of Warming, Fertilisation and Shading of Subarctic Heath Shrubs Promote Secondary Growth and Plasticity but Not Primary Growth, Plos One, doi:10.1371/journal.pone.0034842-2012. Impact factor: 3.730

Campioli, M., Schmidt, N. M., Albert, K. R., Leblans, N. I. W., Ro-Poulsen, H. and Michelsen, A.: Does warming affect growth rate and biomass production of shrubs in the High Arctic? Plant Ecology, 214: 1049-1058, doi: 10.1007/s11258-013-0230-2013. Impact factor: 1.463

Submitted

Leblans, N. I. W., Sigurdsson, B.D., Aerts, R., Vicca, S., Magnússon, B. and Janssens, I.A.: Northern grasslands as long-term C sinks under rising N inputs, submitted for publication in Biogeochemistry

Sigurdsson B. D., Leblans, N. I. W., Gunnarsdótter, G. E., Dauwe, S. D., Sigurdsson, P., Gudmundsdóttir, E., Oddsdóttir, E. S. and Janssens, I. A., Geothermal ecosystems as natural climate change experiments: the ForHot research site in Iceland as a case study, Submitted for publication in Icelandic Agricultural Sciences

Maljanen, M., Yli-Moijala, H., Biasi, C., Leblans, N. I. W., De Boeck, H. J., Bjarnadóttir, B. & Sigurdsson, B. D.: The emissions of N2O and CH4 from natural soil temperature gradients in a volcanic area in southwest Iceland. submitted for publication in Soil Biology & Biochemistry.

In preparation

Leblans, N. I. W., Sigurdsson, B. D., Vicca, S., Soong, J., Weedon, J., Poeplau, C., Maljanen M., Gundersen, P., Marañón-Jiménez, S., Verbruggen, E., Wallander, H., Zhanfeng, L., Bååth, E. Holmstrup, M., Ilieva Makulec, K., Kätterer, T., Ostonen, I., Penuelas, J., Richter, A., Van Bodegom, P., Dauwe, S., Van de Velde, K ., Janssens, I. A.: Large, fast and persistent reductions of soil carbon stocks in naturally-warmed grasslands, in preparation for resubmission to Nature

Leblans, N. I. W., Sigurdsson, B. D., Vicca, S., and Janssens, I. A.: Persistent warming-induced extension of the growing season in subarctic, in preparation

Holmstrup, M., Ehlers, B., Slotsbo, S., Sigurdsson, B. D., Leblans, N. I. W., Ilieva-Makulec, K. and Berg, M.: Shift in community composition of Collembola in a warmer world is linked to functional traits, in preparation

Walker, T., Kaiser, C., Leblans, N. I. W., Sigurdsson, B. D., Janssens, I. A. & Richter, A.: Microbial community adaptation halts sustained soil carbon loss under climate warming, in preparation

Ilieva-Makulec, K., Holmstrup, M., Ehlers, B., Sigurdsson, B. D., Leblans, N. I. W.: Relating nematode taxonomic and functional diversity to natural soil warming gradient: a case study in Iceland, in preparation

Conferences

Leblans, N. I. W., Sigurdsson, B. D. and Janssens, I. A.: Natural gradients in temperature, CO_2 and soil age in Iceland: A study of long-term global change effects on nutrient dynamics, vegetation and microbial communities. ClimMani Workshop, Furka pass, Switzerland, September 2012, Oral presentation

Leblans, N. I. W., Sigurdsson, B. D. and Janssens, I. A.: ForHot: Effects of natural temperature gradients on soil organic carbon and nutrient stocks and dynamics in grassland and forest ecosystems. Soil Carbon conference, Reykjavik, Iceland, May 2013

Leblans, N. I. W., Sigurdsson, B. D. and Janssens, I. A.: Effects of seabird nitrogen input on biomass and carbon accumulation during 50 years of primary succession on a young volcanic island, Surtsey. Surtsey 50th Anniversary Conference, Reykjavik, Iceland, August 2013, Oral presentation

Leblans, N. I. W., Sigurdsson, B. D. and Janssens, I. A.: ForHot: Effects of natural temperature gradients on soil organic carbon and nutrient stocks and dynamics in grassland and forest ecosystems. Increase/Interact symposium, Copenhagen, Denmark, October 2013

Leblans, N. I. W., Sigurdsson, B. D. and Janssens, I. A.: Effects of seabird nitrogen input on biomass and carbon accumulation during 50 years of primary succession on a young volcanic island, Surtsey. Icelandic Biology conference, Reykjavik, Iceland, November 2013, Oral presentation

Leblans, N. I. W., Sigurdsson, B. D. and Janssens, I. A.: Natural gradients in soil temperature and soil age: Iceland represents a unique environment to clarify long term global change effects on nutrient dynamics and vegetation. ForHot project meeting on Joint ecosystem assessment of warming impacts, Leiden, The Netherlands, February 2014, Oral presentation

Leblans, N. I. W., Sigurdsson, B. D. and Janssens, I. A.: Effects of short-term and long-term soil warming on ecosystem phenology of a sub-arctic grassland: an NDVI-based approach. European Geosciences Union, Vienna, Austria, April 2014

Leblans, N. I. W., Sigurdsson, B. D. and Janssens, I. A.: Forhot: Natural soil warming in natural grasslands and a Sitka spruce forest in Iceland. Imbalance-P kick-off meeting, Barcelona, Spain, January 2015, Oral presentation

Leblans, N. I. W., Dauwe, S., Sigurdsson, B. D. and Janssens, I. A.: Impact of warming and N enrichment on carbon stocks and ecosystem structure and function in Icelandic grasslands. Imbalance-P kick-off meeting, Barcelona, Spain, January 2015, Oral presentation

Leblans, N. I. W., Sigurdsson, B. D. and Janssens, I. A: Impact of natural soil warming on carbon stocks and ecosystem structure and function in Icelandic grasslands – The ForHot research project. Icelandic Ecology conference, Stykkisholmur, Iceland, March 2015, Oral presentation

Leblans, N. I. W., Sigurdsson, B. D. and Janssens, I. A.: ForHot: Short- and long-term natural soil warming in natural grasslands in Iceland. Biology Research day at the University of Antwerp, Antwerp, Belgium, September 2015

Leblans, N. I. W., Sigurdsson, B. D., Vicca, S. and Janssens, I. A.: Northern grassland as long-term carbon sinks under rising nitrogen inputs. Second annual ClimMani meeting on nutrients in terrestrial ecosystems and their role in climate change feedbacks, Poznan, Poland, September 2015, Oral presentation

Leblans, N. I. W., Sigurdsson, B. D. and Janssens, I. A.: ForHot: Short- and long-term natural soil warming in natural grasslands in Iceland. Biology research day at the institute of Natural Sciences, Reykjavik, Iceland, November 2015, November

Leblans, N. I. W., Sigurdsson, B. D., Vicca, S. and Janssens, I. A.: Natural gradients in soil temperature: Iceland represents a unique environment to clarify long-term global change effects on soil C storage. Soil Ecology and Planetary Boundaries PhD workshop, Lunteren, The Netherlands, January 2016

Leblans, N. I. W., ., Sigurdsson, B. D., Vicca, S. and Janssens, I. A.: Using natural gradients in soil temperature to clarify long term global change effects on vegetation, carbon and nutrient dynamics. Forhot Meeting, Antwerp, Belgium, March 2016, Oral presentation

Poeplau, C., Sigurdsson, B. D., Leblans N. I. W., Kätterer, T.: Quantitative and qualitative responses of soil organic carbon to six years of extreme soil warming in a subarctic grassland, Iceland. Presenation at the EGU conference, Vienna, Austria, April 2016

Maljanen, M., Sigurdsson, B. D., Leblans, N. I. W., De Boeck, H., Bjarnadóttir, B., Yli-Moijala, H., Biasi, C.: The emissions of N2O and CH4 from natural soil temperature gradients on a volcanic area in Southwestern Iceland. Presentation at the EGU conference, Vienna, Austria, April 2016

Walker, T., Leblans, N. I. W., Sigurdsson, B. D. and Richter, A.: Long-term versus short-term warming effects on microbial processes. Presentation at the EGU conference, Vienna, Austria, April 2016

Leblans, N. I. W., ., Sigurdsson, B. D., Vicca, S. and Janssens, I. A.: Natural gradients in soil temperature: Iceland represents a unique environment to clarify long-term global change effects on net C storage. Annual MC meeting and Natural gradient workshop, Novi Sad, Servia, October 2015

Supervised Master dissertations

Michielsen, L.: Plant communities and global change: adaptation by changes in present species composition or adaptation in plant traits, a case study in Iceland. Master thesis at the University of Antwerp, 2013-2014

Van De Velde, K.: Effects of short-term and long-term natural soil warming gradients on plant productivity, carbon and nitrogen stocks of a sub-arctic grassland, a case study in Iceland. Master thesis at the University of Antwerp, 2013-2014

Thuys, R.: Evolution of plant traits along an island chronosequence at the South coast of Iceland. Master thesis at the University of Antwerp, 2013-2014

Roefs, P.: Distribution of carbon and nitrogen in plants and soil along an island chronosequence at the South coast of Iceland. Master thesis at the University of Antwerp, 2013 - present

Van Loock, S.: Short-term and long-term changes in plant biomass in response to natural soil warming and nitrogen availability in a sub-arctic grassland. Master thesis at the University of Antwerp, 2015-present

Meynzer, W.: Short-term and long-term changes vascular plant community composition and biodiversity in response to natural soil warming and nitrogen availability in a sub-arctic grassland. Master thesis at the University of Antwerp, 2015-present

Awards

2011 *Actua price* (University of Antwerp)