Climate Change Impacts Upon Plants and Soils Along Environmental Gradients

Insights from Swedish subarctic tundra and boreal forests

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Cover: View of part of the elevational gradient on Mount Suorooaivi where experiments presented in papers I and II were conducted.
   (photo: T. Logan)

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Abstract

Climate change is altering ecosystems worldwide. Despite advances in our understanding of the effects of increasing temperature, little is known about how increased temperatures will impact upon plants and soils across environmental gradients. This thesis investigated soil legacies and plant defense along a subarctic tundra elevational gradient as well as soil microbial and nematode community and litter decomposition responses to interactions between plant functional group removal and warming along a post-fire successional chronosequence in boreal forest. In subarctic tundra, soil legacies were strongly temperature-driven, but these effects varied between vegetation types, with plants grown in meadow vegetation showing a strong unidirectional decline in growth when grown in soils from increasing elevation and plants grown in heath soils showing no response to elevation. Positive legacy effects were observed in soils from the lowest (i.e., warmest) elevation and these effects were primarily the result of soil abiotic, as opposed to biotic, effects. Subarctic plant defense was reduced by nitrogen (N) fertilization, while phosphorous (P) fertilization had few effects. Nitrogen fertilization reduced plant defense most at the lowest elevation. Along a boreal forest successional gradient, warming and plant functional group removal favored bacteria in the youngest forests. In contrast, the nematode community was impaired by plant functional group removal, but was not responsive to warming or successional stage. Litter decomposition was strongly influenced by understory plant functional groups. Mosses increased litter mass loss and reduced P loss, while shrubs decreased litter mass loss and immobilized more litter P; warming and successional stage were less significant drivers. Taken together, these results demonstrate that above- and belowground responses to temperature can vary considerably in subarctic tundra and boreal forest ecosystems. Therefore, the work presented in this thesis highlights the importance of considering environmental context (i.e., changes associated with increasing elevation/succession) when making predictions about how global climate change will affect plant and soil-mediated ecosystem processes.

Keywords: boreal forest, climate change, decomposition, elevational gradient, plant defense, plant functional group removal, plant-soil interactions, post-fire succession, soil community, soil legacy, subarctic tundra.

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Dedication

To the journey and to everyone I have encountered along the way.

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I De Long, J. R., Kardol, P., Sundqvist, M. K., Veen, G. F. (Ciska), and Wardle, D. A. (2015) Plant growth response to direct and indirect temperature effects varies by vegetation type and elevation in a subarctic tundra. *Oikos* 124, 772-783. DOI: 10.1111/oik.01764

II De Long, J. R., Sundqvist, M. K., Gundale, M. J., Giesler, R. and Wardle, D. A. (2015) Effects of elevation and nitrogen and phosphorus fertilization on place defence compounds in subarctic tundra heath vegetation. *Functional Ecology* (in press) DOI: 10.1111/1365-2435.12493

III De Long, J. R., Dorrepaal, E., Kardol, P., Nilsson, M. –C., Teuber, L. M., and Wardle, D. A. (2016) Contrasting responses of soil microbial and nematode communities to warming and plant functional group removal along a post-fire boreal forest successional gradient. *Ecosystems* (in press) DOI: 10.1007/s10021-015-9935-0.

IV De Long, J. R., Dorrepaal, E., Kardol, P., Nilsson, M. –C., Teuber, L. M., and Wardle, D. A. Plant functional groups and litter species identity are stronger drives of litter decomposition than warming along a boreal forest post-fire successional gradient. *Submitted manuscript*.

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Abbreviations

AMF	arbuscular mycorrhizal fungi
C	carbon
cm	centimeter
CO_2	carbon dioxide
CWA	community weighted average
g	gram
h	hours
km	kilometers
m	meter
mg	milligram
mL	milliliter
N	nitrogen
NH_4-N	ammonium-nitrogen
NO_3-N	nitrate-nitrogen
OTC	open top chamber
P	phosphorus
PCA	principal component analysis
PCC	protein complexation capacity
PLFA	phospholipid fatty acid
PO_4-P	phosphate phosphorus
RDA	redundancy analysis
SIR	substrate induced respiration
μm	micrometer

1 Introduction

1.1 Climate change

The Earth's climate is currently undergoing rapid change. Over the last century, surface temperatures have risen by approximately 1 °C and a further increase of 1-3 °C is expected by the end of the current century (IPCC 2013). Further, changes to temperature are likely to alter plant and soil community composition and functioning. Although a growing number of studies have explored the ramifications of increased temperature on above- and belowground communities and ecosystem processes (Bachelet *et al.*, 2001; Bardgett *et al.*, 2008; Garcia-Palacios *et al.*, 2015), there is a lack of knowledge on how temperature may interact with other environmental factors to impact upon plants and soils (Ostle *et al.*, 2009; Kardol *et al.*, 2012). Exploring multiple global change drivers is integral to understanding how our world is going to function as climate change advances.

1.2 Environmental gradients

Studying changes to above- and belowground processes that occur over long time scales is a challenge in terrestrial ecology. Experiments that monitor how ecosystems change over decadal timescales are uncommon (but see (Chapin *et al.*, 1994; Whittaker *et al.*, 1999; Silvertown *et al.*, 2002), while those that measure changes over centennial or millennial scales are non-existent. To circumvent this issue, ecologists often utilize chronosequence experiments or other natural environmental gradients. Properly designed chronosequence experiments that control for confounding variables (e.g., precipitation, parent soil, contrasting successional trajectories) are capable of answering ecological questions based upon "space for time substitution" over time scales that are impossible through other approaches (Fukami & Wardle, 2005; Walker *et al.*,

2010). For example, long-term changes to the plant and soil community associated with secondary succession generated by disturbances such as fire control a number of vital ecosystems processes. Studies along fire-driven forested chronosequences have shown that ecosystem processes such as community assembly, carbon (C) storage and decomposition can change predictably with increasing time since the most recent fire event (Wardle *et al.*, 2003a; Hart & Chen, 2006; Bartels *et al.*, 2016). Additionally, incorporating a climatic manipulation component to chronosequence experiments can enhance our capacity to predict how increasing temperatures generated by advancing climate change will impact upon ecosystems at different stages of chronological development.

Further, the use of elevational gradients has gained recent popularity for determining how factors such as nutrient availability, plant and soil community composition, and species interactions change with climate (Richardson *et al.*, 2005; Sanders *et al.*, 2007; Sundqvist *et al.*, 2011a; Bahram *et al.*, 2012). Similar to the chronosequence approach, the use of elevational gradients for assessing ecosystem responses to temperature change can have an advantage over manipulative experiments because they are better able to assess community and ecosystem processes over longer timeframes and larger spatial scales (Wolkovich *et al.*, 2012; Sundqvist *et al.*, 2013). However, it is essential that other environmental factors that change with elevation (i.e., precipitation, slope/aspect, parent soil, incoming solar radiation) that can drive ecosystem processes are controlled for when selecting elevational gradients. Both successional and elevational gradients have the potential to answer important questions concerning the effects of climate change on above- and belowground ecosystem components.

1.3   Soil legacy effects

Temperature is one of the strongest determinants of plant performance at both local and global scales and advancing global climate change is expected to increase global surface temperatures (IPCC 2013). However, plant performance is also driven by both soil abiotic and biotic factors, and these factors are constantly changing in tandem with the plant community (Aerts & Chapin, 2000; Bardgett & Wardle, 2010). Plants are capable of altering soil abiotic conditions such as mineral nutrient availability (Bezemer *et al.*, 2006), hydrological properties (Bardgett & Wardle, 2010) and soil structure (Angers & Caron, 1998). Additionally, biotic factors such as the densities and composition of soil pathogens (van der Putten *et al.*, 1993), mycorrhizal fungi (Klironomos, 2002), and the decomposer biota that mineralizes nutrients

required for plant growth (Ayres *et al.*, 2009) are also influenced by the plants growing in a particular locale. Soil legacy effects (i.e., the manner in which plants previously growing in a given locale alter soil abiotic and/or biotic conditions) that plant species exert on soil abiotic and biotic properties have consequences for subsequent plant growth and community development. For example, plants may alter successional trajectories by altering nutrient availability (Nilsson & Wardle, 2005; Manning *et al.*, 2008), the soil microbial community (Kardol *et al.*, 2007) or through species-specific priority effects (van de Voorde *et al.*, 2011).

Moreover, interactions between plants and soils also depend upon temporal scale (Kardol *et al.*, 2013) and climatic factors (Richardson *et al.*, 2005; Sanders *et al.*, 2007; Bahram *et al.*, 2012). For example, harsh climatic conditions often impair nutrient mineralization, which lead to plant communities that are adapted to nutrient limitation (Sundqvist *et al.*, 2011b) and soil communities that are adapted to decompose the recalcitrant litter that they produce (Northup *et al.*, 1998). Therefore, disentangling the role that climate plays in the creation of soil legacy effects and the role these effects have in controlling plant performance is vital for predicting how plants will respond to warmer global temperatures.

1.4 Plant defense

Plants have evolved myriad chemical and structural mechanisms to protect themselves from damage, which can be altered by environmental conditions such as climate. With advancing global climate change and resultant higher air surface temperatures, some research predicts plant defenses will increase (Peñuelas & Llusià, 2003), but the effect of increased temperature on plant chemical defense has been seldom explored and is not fully understood (Bidart-Bouzat & Imeh-Nathaniel, 2008).

Polyphenols are a broad class of secondary C-based compounds found in nearly all higher plants (Lattanzio *et al.*, 2006), which afford protection against UV radiation (Close & McArthur, 2002) and microbial pathogens (Scalbert, 1991). Polyphenols help deter herbivory in live foliage (Coley *et al.*, 1985), while after senescence certain classes of polyphenols (i.e., tannins) can regulate nutrient cycling through the formation of protein-stabile tannin compounds (Fierer *et al.*, 2001; Joanisse *et al.*, 2009).

Various theories have been proposed to explain how abiotic and biotic conditions work in tandem to drive the differences found in plant defenses between species and across ecosystems. For example, the 'Carbon Nutrient Balance' hypothesis (Bryant *et al.*, 1983) asserts that when nitrogen (N) is

limited, C is fixed in surplus, which causes increases in C-based defense compounds. Further, the 'Resource Availability' hypothesis (Coley *et al.*, 1985) proposes that slower-growing plants in nutrient-limited environments invest more in defenses to minimize tissue loss due to herbivory, while the 'Protein Competition' hypothesis (Jones & Hartley, 1999) takes a more physiological approach by suggesting trade-offs in the metabolic pathways of plants for the production of photosynthetic vs. defense compounds.
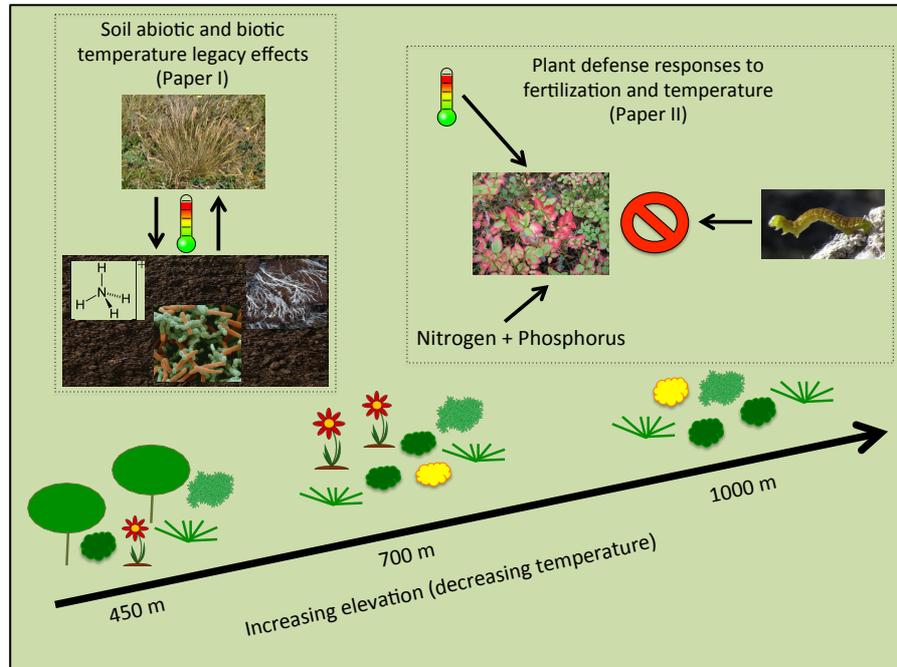

Figure 1. Papers I and II of this thesis utilized a subarctic elevational gradient to examine temperature driven abiotic and biotic soil legacy effects on plant performance and the effects of fertilization and temperature on plant chemical defense.

Despite myriad studies on the topic, there is considerable debate surrounding the predictive power of these theories (Hamilton *et al.*, 2001; Koricheva, 2002; Endara & Coley, 2011; Johnson, 2011). In line with most theories, higher N availability is typically associated with lower polyphenol production (Bryant *et al.*, 1983; Koricheva *et al.*, 1998; Kraus *et al.*, 2004), but the effect of N availability on polyphenols has been found to vary, particularly for subarctic and alpine plant species (Shevtsova *et al.*, 2005; Nybakken *et al.*, 2008; Sundqvist *et al.*, 2012). Further, changes in phosphorus (P) availability have been shown to have little effect on polyphenols (Koricheva *et al.*, 1998),

while others have found polyphenol concentrations in plants to increase (Feller, 1995), or decrease (Zhang *et al.*, 2012) with increasing P availability. Additionally, decreasing temperature and nutrient availability and increasing UV radiation (i.e., increasing physiological stress) associated with increasing elevation have been shown to have positive (Bernal *et al.*, 2013), negative (Wallis *et al.*, 2011) and neutral (Rasmann *et al.*, 2014) effects on plant chemical defenses. However, little is known concerning how the interactive effects of nutrient availability and temperature affect plant defense compounds in either live or senesced leaves in a natural ecosystem.

1.5   Soil communities and climate change

Advancing climate change has already generated an increase in surface temperatures worldwide, which is altering ecosystem functioning on a global scale (IPCC 2013). Different functional groups within the soil community may respond differently to interactions between climatic change and other environmental factors (Shaw *et al.*, 2002; Mikkelsen *et al.*, 2008). Responses to such interactions may depend upon factors such as successional stage. Soil microbes and nematodes are two important components of the soil community that play an essential role in driving nutrient cycling and C storage (Bardgett & Chan, 1999; Bardgett *et al.*, 2008), are indicative of ecosystem functioning, and are sensitive to changes in both climate and plant community composition (Wardle & Zackrisson, 2005; Kardol *et al.*, 2010).

Implications of the direct effects of climate change (i.e., increased temperature) for long-term ecosystem functioning will also depend upon indirect effects on the soil community mediated through plant functional groups (Cleland *et al.*, 2007; Kardol *et al.*, 2010; Langley & Megonigal, 2010) and these may also vary with successional stage. For example, in the boreal forest mosses and shrubs make up the majority of the understory plant community and both contribute significantly to controlling ecosystem productivity (Wardle *et al.*, 2003a; Nilsson & Wardle, 2005) and soil microbial communities (Bradley *et al.*, 2000; Lindo & Gonzalez, 2010). The resultant feedbacks of the effects of warming, successional stage and plant functional groups on the soil community (Bardgett *et al.*, 2008; van der Putten *et al.*, 2010) and the aboveground plant community (van der Putten *et al.*, 2013) have the potential to impact upon ecosystem functioning. Therefore, the consideration of both the direct effects of warming and its indirect effects mediated via plant functional groups along environmental gradients on different components of the soil community is vital for predicting how soil

organisms and the ecosystem processes that they control will respond to global warming.

1.6   Litter decomposition and climate change

Another important ecosystem process that may be affected by climate change in a context-dependent manner is litter decomposition, which serves as one of the primary controls of terrestrial C storage (Swift *et al.*, 1979; Kirschbaum, 1995) and nutrient release (Kalbitz *et al.*, 2000). As the climate warms (IPCC 2013), increasing temperatures are expected to accelerate decomposition rates, creating a positive feedback with climatic warming (i.e., through warming increasing decomposition, in turn releasing more CO_2, which leads to more warming) (Kirschbaum, 2000; Schuur *et al.*, 2008). However, there is a dearth of knowledge concerning how factors such as litter quality or vegetation-generated microclimates may influence the effects of increased temperature on decomposition (Davidson & Janssens, 2006; Schmidt *et al.*, 2011). Additionally, decomposition and nutrient release are affected by successional changes in the soil and vegetation (Wardle *et al.*, 2003a; Jonsson & Wardle, 2008), which emphasizes the need to consider successional context when predicting changes to decomposition processes.

Exploring decomposition rates of litters that differ in quality fosters understanding of ecosystem-level changes in C storage (Aerts, 2006). Some studies have shown that litter species identity is a stronger control over decomposition processes than is climate (Hobbie, 1996; Cornwell *et al.*, 2008), but whether this holds across ecosystems and for different plant functional groups remains to be tested. Further, different plant functional groups respond differently to changing climate (Chapin *et al.*, 1995; Hollister *et al.*, 2005) and the microclimate generated by different groups can impact upon decomposition (Straková *et al.*, 2011). Additionally, vegetation changes associated with succession can also alter decomposition processes. For example, the litter of the early successional shrub *Vaccinium myrtillus* is high in N and decomposes quickly, while *Empetrum hermaphroditum* and feather mosses (both more dominant in late succession) produce recalcitrant, slow decomposing litter (DeLuca *et al.*, 2002; Wardle *et al.*, 2003a), leading to higher C storage in old-aged boreal forests (Wardle *et al.*, 2003a). Therefore, investigating how the direct effects of warming and its indirect effects driven by changes in plant functional groups alter with succession is essential to predicting how decomposition and nutrient dynamics will be affected as climate change advances.
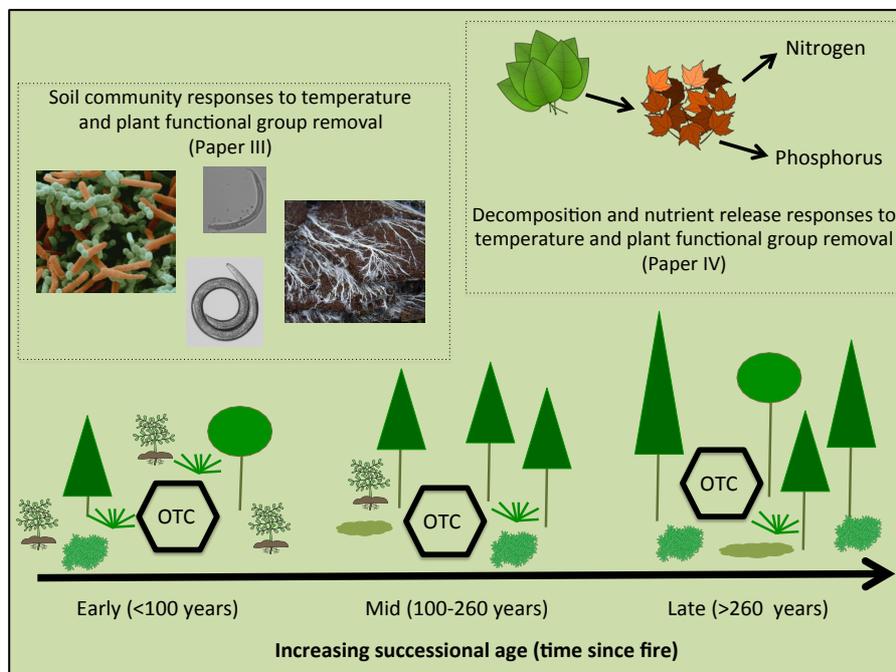
Figure 2. Papers III and IV of this thesis utilized an open top chamber (OTC) warming and plant functional group removal experimental set up along a post-fire successional gradient to investigate soil community and litter decomposition responses.

1.7 Objectives

The overall aim of this thesis is to better understand how plant and soil communities respond to changing temperatures across environmental gradients. Research included in papers I and II was conducted along an established subarctic elevational gradient near Abisko, Sweden (Figure 1). papers III and IV focused on a plant removal and warming experiment established along a post-fire successional chronosequence near Arvidsjaur, Sweden (Figure 2). Paper I investigated how the direct and indirect effects of temperature change with elevation and vegetation type to impact upon plant growth responses. Specifically, plants were grown in controlled growth chamber experiments in soils from two vegetation types from along the subarctic elevational gradient to tease apart the abiotic and biotic factors driving growth response. Paper II explored how plant defense traits responded to fertilization and changing temperature associated with increasing elevation. An established N and P fertilization experiment from along the elevational gradient was used to determine the contributions of temperature, fertilization and their interactions to plant defense. For papers III and IV, a fully factorial experiment in which plots were subjected to warming, moss removal and shrub removal was carried out in ten sites ranging from 48 to 368 years since the last

stand-replacing fire to investigate how interactions between these factors drive ecological processes. Specifically, paper III focused on how soil microbial and nematode communities responded to these treatments, while paper IV focused on how these treatments drive vascular plant litter decomposition and nutrient release.

The central questions of each of the papers in this thesis are as follows:

I.   Are plant growth responses along an elevational gradient driven by direct and/or indirect temperature effects, vegetation type or abiotic and biotic properties of the soil?
II.   How are plant chemical defenses affected by interactions between fertilization and the environmental changes associated with increasing elevation?
III.   How does the soil community respond to interactions between warming, moss removal, shrub removal and successional stage?
IV.   How does decomposition and nutrient release of vascular plant litter respond to interactions between warming, moss removal, shrub removal and successional stage?

This thesis addresses this set of questions to further our understanding of how climate change will interact with plants and soil along environmental gradients to drive ecosystem processes and functioning both above- and belowground. Considering the interactions of increasing temperatures with the abiotic and biotic environment is crucial for predicting how plants and soils and the ecosystem processes that they drive will respond to advancing climate change.

2 Material and Methods

2.1 Study system: Subarctic tundra elevational gradient

Research for papers I and II was conducted along a well-established elevational gradient (Sundqvist *et al.*, 2011a; Sundqvist *et al.*, 2011b; Milbau *et al.*, 2013) on the northeast-facing slope of Mount Suorooaivi, located approximately 20 km south of Abisko, Sweden (68°21'N, 18°49'E) (Figure 3). The growing season is approximately three months and the climate is subarctic with a mean annual temperature of approximately -2.2 °C in the region (Björk *et al.*, 2007). Mean growing season air temperature declines by around 3 °C from 450 to 1000 m along this elevational gradient (Sundqvist *et al.*, 2011a), making this gradient ideal for comparing climate and soil drivers of plant performance across a temperature range that is on par with expected future increases over the coming century (IPCC 2013). Mean annual precipitation measured at the Abisko Scientific Research Station was 310 mm between 1913-2000, with peak precipitation occurring in July (51 mm) and the lowest in April (12 mm) (Kohler *et al.*, 2006). Along this elevational gradient, precipitation ranges from 230-290 mm from June to October and shows no change with elevation (Sundqvist *et al.*, 2014), in line with other comparable elevational gradients in the region (Karlsson *et al.*, 2005). The tree line is found at 500-600 m and is composed of *Betula pubescens* ssp. *czerepanovii*. Parent soil material consists of salic igneous rocks and quartic and phyllitic hard schists.

A mosaic of two markedly contrasting ground-layer vegetation types are co-dominant at all elevations of this gradient. These vegetation types include heath, which is dominated by ericaceous dwarf-shrubs with sparse grass coverage, and meadow, which is dominated by graminoid and herbaceous species (Figure 4). The heath vegetation typically has lower pH, lower mineral N and higher P availability than does the meadow vegetation (Björk *et al.*, 2007; Sundqvist *et al.*, 2011a; Sundqvist *et al.*, 2011b). Studies along this

gradient have found contrasting responses to increasing elevation (i.e. decreasing temperature) between the two vegetation types. Specifically in the heath vegetation, with increasing elevation there are linear decreases in plant-available N and P, increasing fungal to bacterial ratios and low plant species turnover (Sundqvist *et al.*, 2011a). The meadow vegetation is associated with plant available P that declines with increasing elevation (Vincent *et al.*, 2014), idiosyncratic N availability, a peak in fungal to bacterial ratios at mid elevations, and higher plant species turnover with increasing elevation (Sundqvist *et al.*, 2011a; Sundqvist *et al.*, 2011b). Given the pan-arctic distribution of these two vegetation types (Walker *et al.*, 2005), it is vital to investigate how changes in climate (i.e., temperature) will impact upon both heath and meadow vegetation ecological processes.

In both experiments along this elevational gradient, the mean distance between plots within vegetation types and within elevations was between *c*. 10-48 m. Considering the high level of heterogeneity over short distances observed in this study system (Björk *et al.*, 2007), it is expected this distance is sufficient to ensure independence between plots (Sundqvist *et al.*, 2012). Further, all plots used in these experiments had the same slope and aspect.

Figure 3. Map showing the location of the research sites used in papers I and II. Sites at varying elevations along the slope of Mount Suorooaivi are marked on the topographic map inset.

2.1.1 Direct and indirect impacts of temperature on plant growth

Five plots of 1 m × 1 m were established at each of three elevations, i.e., 450, 700, and 900 m, for each of the heath and meadow vegetation types. Two fully factorial concurrent growth chamber experiments were set up using soils collected adjacent to each plot. In Experiment 1, field soils were processed by removing large stones and roots and then thoroughly homogenized within each vegetation type per elevation (Pizano *et al.*, 2011). Subsamples of soil were put into plastic pots (total volume of 490 mL), each containing a drainage layer at the bottom of 150 mL sterilized sand and the remaining volume filled with the collected field soil; there were 30 pots for each elevation × vegetation type combination. Two common grass species (*Deschampsia flexuosa*, which is more common in heath vegetation, and *Festuca ovina*, which is more common in meadow vegetation) that occur across the entire elevational gradient were selected as phytometers. Seeds were obtained from natural sources (Pratensis AB, Sweden; *Deschampsia flexuosa*: Småland, Sweden; *Festuca ovina*: Umeå, Sweden), surface-sterilized in a 1% sodium hypochlorite solution for 1 minute, rinsed, and germinated in autoclaved sand. Next, for each of the two species, five seedlings were transplanted into half of the pots for each elevation × vegetation type combination. For the 15 pots for each elevation × vegetation type × species combination, five replicate pots were placed in each of three growth chambers, whose conditions were set to mimic temperature conditions as observed at 450, 700, and 900 m along the elevational gradient during the 2012 growing season. Photosynthetically active radiation (PAR) and hours of daylight were set in each growth chamber to represent conditions measured from mid June to mid September 2012 in the vicinity of the elevational gradient. Pots were rotated systematically between chambers (i.e. entire temperature treatments moved between chambers) on a weekly basis to control for any artifacts resulting from differences between growth chambers that were independent of treatment. After 12 weeks the three largest individual seedlings in each pot were harvested. Upon harvest and before drying, a subsample of root tissue was taken from plants in all pots containing soils from the most extreme elevations (i.e., 450 and 900 m) that were grown in chambers that mimicked the 450 and 900 m temperature conditions, to measure the abundance of arbuscular mycorrhizal fungi (AMF) colonization. Roots were carefully washed and separated from the shoots. Samples were oven dried and weighed.

Experiment 2 sought to separate the contribution of abiotic and biotic components of soil effects from contrasting elevations and vegetation types. For Experiment 2, an equal subsample of soil from all plots within each vegetation type from all elevations was taken and combined into two bulk

soils, one representing heath and the other representing meadow. The bulk soils were then sent out for γ-irradiation, which is a method shown to effectively sterilize soils while exerting minimal impacts on other soil properties (Berns *et al.*, 2008). By comparing sterilized soils to reinoculated soils, the effects of abiotic vs. biotic soil factors on plant growth can be effectively separated (Kardol *et al.*, 2007). For each of the vegetation types, we prepared four inoculation treatments (i.e. inoculation with unsterilized soil from each of the three elevations or with sterilized soil) in order to explore the effects of soil biota and nutrients on plant growth. A volume of 10% unsterilized soil from each elevation or sterilized soil was mixed with the bulked sterilized soil. Plant performance in the pots inoculated with unsterilized soils relative to the corresponding sterilized soils allows for an assessment of the effect of soil biota (Kardol *et al.*, 2007; Gundale *et al.*, 2014b). Prepared soils were then placed into plastic pots as per Experiment 1, yielding a total of 30 pots per vegetation type × soil inoculum treatment, except for the bulked sterilized soils with sterilized inoculum for which there were 60 pots per vegetation type. Half of the pots of each soil treatment were planted with one of the two grass species, as per Experiment 1. Five replicates of all vegetation × inoculum × species combinations (or ten for the sterilized soil treatments) were placed in each of the three climate chambers set to mimic conditions observed along the gradient at 450, 700 and 900 m sites, as per Experiment 1. This resulted in a full-factorial experiment with four factors: 1) three growth chamber temperatures simulating the temperature characteristics of the three elevations; 2) soil from the two vegetation types, heath and meadow; 3) four soil inoculum treatments, including soil inoculum from each of the three elevations (i.e., 450, 700 and 900 m) and bulked sterilized soil without inoculum; and 4) two grass species (see above), with five replicates (or ten for sterilized uninoculated soil treatments) of each treatment combination. This yielded a total of 300 pots with each pot serving as a separate experimental unit. Maintenance, rotations, chamber settings, duration of experiment, harvesting and AMF measurements were as described in Experiment 1.

2.1.2 Effects of elevation and fertilization on plant chemical defense

For paper II we utilized plots from a fertilization experiment (along the same elevational gradient as described in paper I) first described by Sundqvist *et al.* (2014). During July 2008 a total of 48 plots measuring 1 × 1 m were established in heath vegetation at each of the three elevations along the gradient, i.e., 500, 800, and 1000 m. At each elevation 16 replicate plots were established, and divided into four blocks of four plots; the four plots in each

block were randomly assigned one of four fertilization treatments: control, N addition, P addition and N+P addition. Fertilizer was applied to the treatment plots annually in the amount of 10 g N m^{-2} yr^{-1} as NH_4NO_3 and 5 g P m^{-2} yr^{-1} as superphosphate $(Ca(H_2PO_4)_2 \cdot H_2O)$. The amounts of N and P fertilization used in this experiment are consistent with previous studies that have sought to study N and P limitation in arctic ecosystems (Jonasson, 1992; Chapin *et al.*, 1995; Mack *et al.*, 2004; Rinnan *et al.*, 2007).

To determine the effects of elevation and fertilization on leaf chemical and structural traits, approximately 3 g dry weight per species of both fresh and senesced fully expanded undamaged leaves of all major vascular plant species were collected from all plots during 2012. Leaves were sampled from each individual of each species in each plot, which is considered sufficient to capture total intraspecific variability (incorporating variability both among and within individuals) at the plot scale (Albert *et al.*, 2011; Lepš *et al.*, 2011; Violle *et al.*, 2012). The species selected represent over 80% of the vascular plant species cover at each elevation (Sundqvist *et al.* 2014) as determined by point quadrat analysis (Goodall, 1952). It must be noted that species growth form can confound the results of point quadrat analysis (Bråthen & Hagberg, 2004), which may have impacted upon the results obtained. This resulted in a total of eight species collected from the gradient: *Betula nana* (800, 1000 m), *Calamagrostis lapponica* (800, 1000 m), *Cassiope tetragona* (1000 m)*, Deschampsia flexuosa* (500 m), *Empetrum hermaphroditum* (500, 800, 1000 m), *Vaccinium myrtillus* (500 m)*, V. uliginosum* (800, 1000 m) and *V. vitis-idaea* (500, 800, 1000 m).

Subsamples of both fresh and senesced leaves from each species from each plot were dried and sorted in the lab prior to being ground. A subsample of each dried leaf sample was analyzed for foliar C, N, and P and for lignin using acid digestion. An additional subsample of leaf litter was extracted in methanol. Two sub aliquots of this subsample were each analyzed for total phenol and condensed tannin concentrations using the Prussian Blue technique with a catechin standard (Stern *et al.*, 1996) and the acid-butanol method with procyanidin standard B2 (Porter *et al.*, 1986), respectively. Another subsample of leaf litter was extracted in de-ionized water. This subsample was used to determine protein complexation capacity (PCC) to measure the amount of protein precipitated by each leaf extract (Gundale *et al.*, 2010). All of the above extractions were filtered through 0.2 µm filter paper under vacuum filtration, stored at -18 ºC until further analysis and removed from the freezer 24 h prior to analysis to eliminate temperature-induced interference with reaction kinetics.

Figure 4. An example of typical meadow vegetation from the 700 m elevation used in paper I. Photo: Paul Kardol

2.2 Study system: Boreal forest post-fire successional gradient

The ten field sites used in papers III and IV were located near Arvidsjaur in northern Sweden (65°35'-66°07'N, 17°15'-19°26'E) along a fire-driven boreal forest chronosequence (Figure 5). Time since last fire varies from 48 to 368 years along this chronosequence. In these experiments, the sites are divided into early, mid and late successional stages, with time since last fire of < 100 years (three sites), 100-260 years (four sites), and > 260 years (three sites), respectively (Jackson *et al.*, 2013). The overstory becomes increasingly dominated by *Picea abies* with an understory of *Empetrum hermaphroditum* as time since fire increases, while moss cover thickness increases (DeLuca *et al.*, 2002). Further, there is a decrease in nutrient availability (DeLuca *et al.*, 2002) and litter decomposition rates (Jackson *et al.*, 2013) as the chronosequence proceeds. The climate is cold temperate humid with cool summers and a mean annual temperature of -2 °C (July 12 °C and January -14 °C, monthly means) and mean annual precipitation is about 600 mm (Jackson *et al.*, 2013). Soils are classified as Typic or Entic Haplocryods (DeLuca *et al.*, 2002).

Both papers III and IV utilized an experiment that involved plant functional group removals and warming treatments using open top chambers (OTCs) along this chronosequence. Functional group removal experiments are useful for understanding how different plant groups affect above- and belowground

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ecosystem processes (Díaz *et al.*, 2003; McLaren & Turkington, 2010). The use of OTCs has been extensive in alpine and arctic environments to experimentally simulate climate warming during the growing season, and in these systems generally raises average air and soil temperatures over that period by approximately 1-2 °C (Marion *et al.*, 1997; Hudson *et al.*, 2011).

At each site, four hexagonal plots were established in June 2010 in relatively open patches of forest to avoid shading by dense continuous canopy to the south of the plot and thus interference with the OTC warming treatment. Selected plots had a homogeneous vascular understory representative for that site. Each plot was randomly assigned to one of four full-factorial combinations of warming (ambient or warming temperature) and shrub removal (shrubs present or removed). Each plot was then divided into two, and a moss removal treatment (mosses present or removed) was randomly assigned to one of the two subplots. Across all sites, this yielded a total of 80 subplots: ten sites (classified by successional stage as early, mid and late) × two temperature treatments (ambient or warming) × two moss treatments (present or removed) × two shrub treatments (present or removed).

Shrub and moss removal was performed by hand, with care taken to remove as much living biomass of the target functional group as possible with minimal disturbance to other plants and litter within each subplot, as per Wardle and Zackrisson (2005). Plots were allowed to recover from the initial removal disturbance for three growing seasons before measurements were taken, which is considered sufficient time to avoid any confounding effects of disturbance (Wardle & Zackrisson, 2005; Wardle *et al.*, 2013). Functional group removal was maintained as necessary on a yearly basis at the beginning of the growing season. For the plots subjected to OTC warming treatments, we used transparent, Perspex OTCs. One OTC was placed over each plot receiving the warming treatment (Figure 6) each year at the beginning of the growing season (early June) and removed at the end of the growing season (early October).

For paper III, plant community composition and air and soil temperatures were recorded in all subplots. For paper IV, air temperatures were recorded in moss present plots only. Plant community assessment included all vascular and non-vascular plants, as assessed by point quadrat analysis (Goodall, 1952) in each subplot. This was performed by recording the total number of times each species was intercepted from a total of 100 downward projecting points in each plot (Wardle *et al.*, 2003a). The total number of intercepts of each species in each plot was used as the measure for species abundance. Again, it must be noted that species growth form can confound the results of point quadrat analysis (Bråthen & Hagberg, 2004), which may have impacted upon the results obtained. Standing litter (which includes litter hanging in the vegetation

and resting on the soil surface) and bare humus in the plots were also recorded whenever intercepted. Air temperature data were gathered five cm above the soil surface (papers III and IV) and soil temperature data five cm below the soil surface (paper III) (or five cm below the surface of the living moss, when present in paper III). Plots receiving the OTC treatment in this experiment had early season mean air temperatures that were approximately 0.4 °C and 0.3 °C higher relative to the plots without OTCs in the 2013 and 2014 growing seasons, respectively. Although this temperature increase is somewhat lower than in OTC experiments in arctic and alpine systems (Marion *et al.*, 1997; Hudson & Henry, 2010), it is on par with similar warming studies that have shown significant ecosystem responses to comparable temperature changes (Rustad *et al.*, 2001).

Figure 5. Map showing the location of the research sites used in papers III and IV along a boreal forest successional gradient. Location of specific research sites is indicated on the detailed inset map.

2.2.1 Soil community response to multiple environmental drivers

In order to quantify the effects of successional stage, warming and plant functional group removal on the soil microbial and nematode communities, soil samples were collected from two locations in each subplot in 2013, using a 1.7 cm diameter corer to sample 10-15 cores to a maximum depth of 6 cm. The

soils were bulked per subplot, and kept at 4 °C until analysis. Prior to passing each bulked sample through a sieve with 4 mm mesh size to remove stones and plant matter, a subsample of 250 mL was taken for nematode extraction.

A subsample of soil from each subplot was analyzed for phospholipid fatty acid (PLFA) markers (Bligh & Dyer, 1959; White *et al.*, 1979). Different subsets of the microbial community are characterized by different PLFA markers, making these markers useful for answering questions about shifts in the total microbial community in response to environmental or treatment factors (Frostegård *et al.*, 1993; Ramsey *et al.*, 2006; Frostegård *et al.*, 2011). After freeze-drying and grinding, PLFAs were extracted from each subsample according to Frostegård *et al.* (1991). Different PLFAs represent different groups of soil microorganisms, such as bacteria (both gram positive and gram negative), fungi and actinomycetes (Frostegård & Bååth, 1996; Zelles, 1999). The ratio of fungal to bacterial PLFA markers was calculated from these data.

Nematodes were extracted from an unsieved, homogenized 250 mL soil subsample from each subplot by using a modified sugar floatation method (Jenkins, 1964); these nematodes were then fixed using a 4% formaldehyde solution. A minimum of 150 nematodes per subplot were identified to family (or super-family in the case of Dorylaimoidea) level and placed into one of four feeding groups (Yeates *et al.*, 1993), i.e. bacterial feeders, fungal feeders, plant feeders, and omnivore-carnivores. The ratio of fungal to bacterial feeding nematodes was calculated.

2.2.2 Vascular plant litter decomposition responses to multiple environmental drivers

For paper IV, freshly senesced leaf litter was collected from around Umeå, Sweden (63°49'N, 20°26'E) early September 2013 from *Vaccinium myrtillus*, *Pinus sylvestris*, and *Empetrum hermaphroditum*, which represent species with fast, medium and slow rates of decomposition, respectively (Wardle *et al.*, 2003b). Litter was cleaned, sorted and allowed to dry at room temperature (25 ºC) for five days. For each species a subsample of litter was retained for analysis of %N and %P (representing concentrations of nutrients prior to decomposition) by Kjeldahl analysis so that the amount of N and P lost or gained during decomposition could be calculated. Nylon bags (7 × 11 cm; 100 μm mesh) were filled with 1 g of dried litter of each species and closed with a metal tie. Two duplicates of each litter type were each placed on the surface of the moss layer, at the interface between the moss layer and the humus layer in each of the moss present subplots and on the surface of the bare humus in each of the moss removal subplots in October 2013. Placing litterbags in these three positions allows for simultaneous comparisons of how moss presence versus absence affects litter decomposition by impacting upon humus layer microbial

activity (i.e., litter bags placed at the moss/humus interface and litterbags placed on bare humus) and how freshly fallen litter is affected by moss presence versus absence (i.e., litter bags placed on the moss surface and litterbags placed on bare humus (Jackson *et al.*, 2013)). Between all sites, this yielded a total of 720 litterbags: ten sites × two temperature treatments (ambient or warming) × two shrub treatments (present or removed) × three bag positions (above moss surface, moss/humus interface, bare humus) × three litter types × two duplicates of each litter type.

All the litterbags were retrieved from the field in October 2014 after one year of decomposition and taken back to the lab where they were dried at 60 ºC for a minimum of 48 h. Each duplicate litterbag was weighed individually to determine mass loss and the values of the two duplicates were averaged to provide a single data point prior to analysis. Duplicates from each of the litter bag position treatments (i.e., above moss surface, moss/humus interface, bare humus) were bulked, ground, and analyzed for %N and %P, by Kjeldahl analysis. The N to P ratio of each litter sample was calculated. Mass loss and N and P concentration data were also used to determine the total amount of nutrients lost from the litter during decomposition (Wardle *et al.*, 2003b).

To analyze soil nutrient supply over the course of a year, a resin capsule containing approximately 1 g of mixed bed ionic resins was placed in the humus layer of each subplot approximately 5 cm deep (Gundale *et al.*, 2014a) in October 2013. These were then collected from the field in October 2014, and stored at 4 °C until ions were extracted with three extractions of 10 mL 1 M KCl and pooled into one sample. Extracts were frozen at -18 ºC until analyzed for NH_4-N, NO_3-N and PO_4-P.

Substrate-induced respiration (SIR) was used to quantify the relative active soil microbial biomass (Anderson & Domsch, 1978; Wardle, 1993). Three soil cores to a depth of 6 cm were collected from each subplot October 2014, using a 1.7 cm diameter corer. The three cores were bulked and kept at 4 °C until analysis. From this sample, a 1 g (dry weight equivalent) subsample of soil was placed into a 120 mL glass vial, amended to 100% moisture (dry weight basis), amended with 50 mg glucose, sealed and incubated at 22 °C. Measurements of CO_2 were taken at 1 h and 3 h by injecting a 5 mL subsample of headspace gas into a gas analyzer, which was used as a measure of SIR.

2.3 Soil property measurements

A number of soil properties were characterized in papers I, II and III to aid interpretation of the results. Soils were collected from each plot/subplot with a soil corer, bulked and kept at 4 °C overnight before being passed through a 4

mm mesh sieve to remove plant matter and stones. Soil pH was determined on a subsample of fresh soil (2.5 g dry weight) after shaking for 12 h in 40 mL deionized water (papers I, II, III). Gravimetric moisture content was determined after drying (105 °C, 24 h) and soil organic matter (SOM) content was determined after combustion in a muffle furnace (550 °C, 4 h) (papers I, II, III). A subsample of fresh soil (5 g dry weight equivalent) was extracted with 1 M KCl after shaking for 2 h; extracts were frozen until analyzed for NO_3-N, NH_4-N, and PO_4-P (papers II, III). A subsample of soil was dried (60 °C, 72 h), ground with a ball mill and analyzed for total C and N by dry combustion (papers I, II, III) and for P with nitric-perchloric acid digestion analyzed by inductively coupled plasma (Spark 1996) (papers I, II, III). In paper I, a subsample of soil from each plot was analyzed for phospholipid fatty acid (PLFA) markers (Bligh & Dyer, 1959; White *et al.*, 1979). Please see above for a full description of the PLFA analysis, outlined in section 2.2.1.

2.4 Statistical analyses

In paper I, two-way ANOVA was used to test for the effects of vegetation type and elevation on soil properties. For both experiments in paper 1, 4-way ANOVA was used to test for the effects of temperature, soil origin (or soil inoculation in Experiment 2), vegetation type and species, and all possible interactions (all as fixed factors, and with block as a random factor) on root biomass, shoot biomass, total biomass, root to shoot ratio and mycorrhizal colonization.

In paper II, three-way ANOVA was used to test for the effects of elevation, N addition, P addition and all possible interactions on soil abiotic properties, and on the measured leaf trait values of all species that occurred at two or more elevations. Elevation, N addition and P addition were considered fixed factors, with block as a random factor. To further examine the link between plant secondary metabolites and foliar nutrient ratios, Pearson's correlation coefficients were calculated.

Further, to explore changes in the fresh and senesced leaf trait values measured at the whole community level across the elevational gradient and in response to N and P addition in paper II, community weighted averages (CWA) were calculated as described by Garnier *et al.* (2007). The use of CWAs is based upon the idea that the contribution of each species to ecological processes at the whole community level is related to the proportion of total community biomass that it represents (Grime, 1998; Garnier *et al.*, 2004). For each leaf trait in each plot, CWAs were calculated as:

$$Weighted\ plot\text{-}average = \sum_{i=1}^{n} (p_i \times trait_i)$$

where p_i is the relative abundance of species i in a plot (as a proportion of the total abundance), and $trait_i$ is the measured leaf trait of species i. A three-way ANOVA was then used on the CWA values for total phenols, condensed tannins, PCC, lignin and foliar nutrient ratios. Further, the method developed by Lepš *et al.* (2011) was used to determine the relative contributions of intra- and interspecific trait variation on the response of weighted average trait values to elevation and nutrient treatments. Partitioning trait variance into intra- and interspecific components is important for determining their relative contributions to ecological processes (Albert *et al.*, 2011; Lepš *et al.*, 2011; Violle *et al.*, 2012).

In paper III, all soil abiotic, microbial and nematode data were analyzed using four-way mixed model ANOVA. Successional stage (early, mid, late), warming (ambient or warming temperature), moss removal (removed or present) and shrub removal (removed or present) were considered fixed factors. Site (i.e., the ten sites used in the experiment) and plot (i.e., each hexagonal plot which was divided into two separate subplots with moss removed and moss present, respectively) were considered random factors to account for variation between experimental sites and the subplot factor, respectively. When plot was not a significant random factor, it was dropped from the model. Temperature data was analyzed in the same way as for the other variables except that a repeated measures term was also included because daily temperature data was used (and the same model was also used for temperature data in paper IV).

In order to examine the effects of the experimental treatments on the plant community and therefore assist in the interpretation of the soil community data, Principal Component Analyses (PCA) were performed. These PCAs were also included in paper IV to help interpret the decomposition data. One PCA was run which included only mosses and non-shrub vascular plants (i.e., excluding all subplots receiving the moss removal treatment and excluding all shrubs). Another PCA was run which included only vascular plants (i.e., excluding all plots receiving the shrub removal treatment and excluding all moss species). Data were analyzed in separate ordinations to avoid confounding effects of the removal of plants from driving the ordination scores (Wardle *et al.*, 2013), and so that the effects of the warming and successional stage treatments could be more clearly depicted.

To determine how the experimental treatment effects as mediated through the plant community and soil abiotic properties were driving the microbial

community, and how the experimental treatment effects as mediated through the plant community, abiotic soil properties and microbial community were driving the nematode community, we used partial Redundancy Analysis (RDA). A partial RDA with site as a random factor was performed in both cases because the microbial and nematode communities were compositional response variables with 1 and 1.6 SD units, respectively, indicating a linear response (Šmilauer & Lepš, 2014). Only plant species with > 20% cover in one or more subplots (as well as standing litter and bare humus, which are known to impact upon soil microbial communities (Sayer, 2006)) were considered as predictor variables to avoid large influences of rare species on the ordination and to eliminate excessive zeros from the data matrix before analysis (Borcard *et al.*, 1992; Sundqvist *et al.*, 2011a); the species included accounted for at least 80% of the vegetation cover in each subplot. Stand productivity was also incorporated as a potential explanatory variable. All explanatory variables were then subjected to Monte Carlo permutation tests with 999 permutations. Plots were freely permuted and subplots were not permuted. Forward selection of significant explanatory variables was carried out, with each explanatory variable that explained a significant amount of variance ($p \leq 0.05$) retained in forward selection as per Blanchet *et al.* (2008).

In paper IV, litter data were analyzed using five-way mixed model ANOVA. Stand age (early, mid, late), warming (ambient or warming), shrub removal (removed or present), litterbag position (above moss surface, moss/humus interface, bare humus) and litter species (*E. hermaphroditum, P. sylvestris* and *V. myrtillus*) were considered fixed factors. Site (i.e., the ten sites used in the experiment) and plot (i.e., each hexagonal plot which was divided into two separate subplots with moss removed [litterbags only placed upon bare humus] and moss present [litterbags placed both upon the moss surface and at the moss/humus interface]) were considered random factors to account for variation between experimental sites and the plots, respectively. Resin capsule and SIR data were analyzed as above except there was no species factor and the bag position factor was replaced with a moss removal (present or removed) factor. When effects of site and/or plot were not significant, they were dropped from the model.

For all ANOVAs, whenever significant effects were found, differences among means were further explored using post hoc tests. For all analyses in all papers, data were transformed whenever necessary to meet the assumptions for parametric testing. All statistical analyses were performed in SPSS (PASW statistics 21.0, IBM Corporation, Armonk, New York, USA) or SAS (Hewlett-Packard 9.4 TS level 1M0, Cary, North Carolina, USA). Multivariate analyses

in papers III and IV were performed in CANOCO version 5.02 (ter Braak and Šmilauer 2012).

Figure 6. Open top chambers placed over plots receiving different plant functional removal treatments at one of the late successional stage boreal forest sites (Vaksliden; last fire was in the year 1711). Photo: Babs Stuiver

3   Results and Discussion

The chapters contained within this thesis aim to explore how climate impacts upon plants and soils along elevational and successional gradients. Along these gradients, climate often interacted with other factors such as vegetation type, fertilization and plant functional group removal to drive plant growth, plant defense, soil community composition and vascular plant litter decomposition. Below the major findings of each paper are presented and briefly discussed.

3.1   Direct and indirect impacts of temperature on plant growth

Plants grown in soils from the elevational gradient in the growth chamber experiment responded to both the direct effect of temperature and its indirect effect via soil legacies; these direct and indirect effects were generally decoupled (paper I). Vegetation type (i.e., different soil conditions) was a major determinant of plant responses to both the direct and indirect effects of temperature. Importantly, plants grown in soils from meadow vegetation generally showed a strong unidirectional decline in biomass with increasing elevation of soil origin regardless of temperature treatment, but plants grown in heath soils showed no such response to the soil legacy effects of temperature. The non-responsiveness of the plants grown in heath soils from contrasting elevations indicates a "buffering effect" of these soils against temperature variation, indicating that the plants growing in them may be more resistant in their response to temperature change (Hudson & Henry, 2010). However, this result must be interpreted cautiously given that the species selected as phytometers in this experiment were grasses and may therefore have been more responsive when grown in meadow soils, which were likely more favorable due to factors such as better matched arbuscular mycorrhizal fungal communities.

Further, the influence of soil biota (as tested with the sterilization and inoculum addition treatments) was independent of elevation, with a positive effect of inoculum on plant growth across all elevations regardless of soil origin for meadow soils but not for heath soils. However, the highest mycorrhizal colonization was often observed in plants grown in meadow soils collected from 900 m elevation and grown at 900 m temperatures. This indicates a more important role of mycorrhizae with increasing elevation resulting from the associated declines in nutrient availability and temperature (Pérez & Frangi, 2000). Taken together, this means that responses of plant growth to soil legacy effects of temperature across this elevational gradient were driven primarily by soil abiotic, and not biotic, factors. This indicates that, at least in the short term, abiotic soil conditions were stronger drivers of plant performance than biotic soil conditions. These findings highlight the importance of considering the direct, indirect, and interactive effects of climate, vegetation and soils to better understand and predict how subarctic ecosystems may respond to climate change (Kardol *et al.*, 2012).

3.2 Effects of elevation and fertilization on plant chemical defense

At the community level, N addition reduced condensed tannin concentrations and PCC in both fresh and senesced leaves and total phenol concentrations in senesced leaves, while P addition had few effects on plant secondary metabolites in subarctic heath vegetation (paper II). Further, at lower (i.e., warmer) elevations, N addition frequently decreased these plant defenses more, meaning that a warmer climate with increased nutrient availability will likely reduce plant defense concentrations. Both intraspecific and interspecific variation emerged as strong drivers of the response of plant defense to elevation, while intraspecific variation and its covariation with interspecific variation were the main components of defense response to N addition. These findings demonstrate the need to consider both sources of variation in explaining plant defense response to changes in environmental conditions. Our findings suggest that as temperatures warm and N availability increases due to global climate change, secondary metabolites in subarctic heath vegetation will decline.

Further, plant defense traits measured in both fresh and senesced leaves responded markedly similarly to the elevation and fertilization treatments, despite evidence that total polyphenol concentrations often decrease, while tannin concentrations often increase, considerably with senescence (Gallet & Lebreton, 1995; Hättenschwiler & Vitousek, 2000). Therefore, responses to

extrinsic factors of leaf chemistry in fresh leaves (which regulate herbivory) are capable of predicting responses of senesced leaf chemistry (which regulate decomposition and nutrient fluxes). This indicates that these two contrasting ecological processes seem to be tightly coupled in subarctic heath vegetation.

Subarctic heath vegetation has high levels of polyphenols (Gundale *et al.*, 2010) that act as defense against herbivory and impair plant litter decomposition rates. Responses of plant defenses in subarctic heath to increasing temperatures and N availability, particularly in low elevation communities that currently experience high invertebrate herbivory pressure (Hagen *et al.*, 2007), will likely lead to further increased herbivory and altered decomposition rates and nutrient fluxes (Bardgett & Wardle, 2003), which may result in feedbacks to the plant community (Facelli & Pickett, 1991). Further, increased N mineralization rates that are expected to result from climate warming in the arctic (Aerts, 2006), may down-regulate concentrations of tannins and PCC for some species which could change interactions between litter chemistry, decomposition, plant N availability and plant growth (Dorrepaal *et al.*, 2007). These results emphasize the need to consider interactions between temperature and nutrients in regulating the role that polyphenols play in fresh leaves (i.e., defense) and in senesced leaves (i.e., nutrient cycling) of subarctic heath plant communities, and the importance of addressing these interactions when predicting how future climate change will alter ecosystem functioning.

3.3   Soil community responses to multiple environmental drivers

Warming from the OTC treatment altered soil microbial communities, but the effect of warming was mediated by mosses and shrubs (i.e., plant functional group removal versus presence), and often varied with successional stage (paper III). These interactions between warming and plant functional removal often favored bacterial-based (versus fungal-based) microbial communities in early successional stages, which may help promote decomposition rates of labile organic substrates and more rapid nutrient cycling (Bardgett & Wardle, 2010). These stronger interactive effects in early successional boreal forests were likely due to both higher nutrient availability in early successional stages and the mediating effects of understory plant functional groups. However, the nematode community was primarily positively affected by the presence of mosses and shrubs, with these effects generally being independent of warming or successional stage. This consistent responsiveness of nematodes to the understory vegetation is likely due to the effects of this vegetation on organic inputs to the soil (Arpin *et al.*, 1995; Sayer, 2006) and soil moisture (Kardol *et*

al., 2010). Microbes in boreal forest soils appear to be more sensitive than nematodes to interactions between warming, plant functional group removal and successional stage, probably due in part to their limited mobility within the soil.

These results highlight that different types of soil microorganisms may respond in contrasting manners to interactions between warming and plant functional groups, with likely consequences for ecosystem functioning that can vary with successional stage. Therefore, changes to understory vegetation caused by disturbances that impact on forest successional stage will interact with warming and alter the magnitude of its effects. The examination of other guilds within the soil and litter community not studied here (i.e., mites, collembola, tardigrades, enchytraeids) and creating a food web that links their responses to the factors studied in this work would offer a more complete picture of how the soil community may change as surface temperatures continue to warm.

On the longer term, as the climate warms, temperature and its indirect effects via changes to the plant community will play an important role in reshaping the microbial community, with potentially important consequences for nutrient and C cycling (Bardgett *et al.*, 2008; Kardol *et al.*, 2010; Ward *et al.*, 2013). Alterations to these processes may in turn feed back to plant community composition (van der Putten *et al.*, 2013) and ultimately ecosystem C exchange (Bardgett *et al.*, 2008; Bardgett *et al.*, 2013; Ward *et al.*, 2013). Our results underscore that considering the context-dependent responses of different components of the soil community to interactions between warming and different plant functional groups (which are important drivers of ecosystem processes and function (Díaz *et al.*, 2004)) along successional gradients is crucial to the development of accurate predictions of effects of global warming in boreal ecosystems.

3.4   Vascular plant litter decomposition responses to multiple environmental drivers

Litter decomposition and nutrient loss were primarily driven by plant functional group removal and not by interactions with warming or successional stage (paper IV). The moss layer increased litter mass loss and reduced litter P loss, while shrub presence typically reduced litter mass loss, but caused greater immobilization of P for one of the three litter species tested. Litter N loss was unaffected by plant functional group removal. Warming interacted with stand age and the litter species decomposed, but these effects were uncommon and generally weak. Further, plant functional group removals and litter species

identity had different effects on the release of N versus P from the decomposing litter. Previous studies have shown litter species identity to be a stronger driver of decomposition than are the direct effects of climate (Hobbie, 1996; Aerts, 2006; Cornwell *et al.*, 2008), which is supported by our findings. Given that shrubs and mosses are dominant understory functional groups throughout the boreal zone, the effects observed here may also be relevant for boreal forests over large areas of the Northern Hemisphere.

The effects of moss and shrub removal on decomposition and nutrient release were dependent upon the litter species considered, thereby highlighting the species-specific decomposition responses to understory plant functional groups. As climate change advances, moss cover is expected to decrease (Rixen & Mulder, 2009; Sorensen *et al.*, 2012) and shrub cover is expected to increase (Myers-Smith *et al.*, 2011). Taken together with the results presented here, this suggests that as climate change advances the resultant lower moss cover will hinder decomposition rates but increase nutrient cycling rates, while the predicted increase in shrub cover will likely reduce decomposition rates and nutrient cycling. Further, these results emphasize the importance of considering how climate-induced shifts to plant community composition over the long-term (Chapin *et al.*, 1995) can have indirect effects that may differ for two interlinked ecosystems processes, namely litter decomposition and nutrient loss. As the climate continues to warm, temperature and its indirect effects via changes to the vegetation and subsequent changes to the microclimate created by the vegetation will play an important role in driving decomposition, with likely impacts on C storage and nutrient cycling (Straková *et al.*, 2011; Langley & Hungate, 2014). Our findings indicate that, at least in the short term, the decomposition of vascular plant litter is more strongly driven by plant functional groups and litter species identity than by stand age and warming.

3.5 Conclusions

The results presented in this thesis contribute to our understanding of climate impacts upon plant and soil driven ecosystem processes along environmental gradients. Although the works presented here advance our knowledge in this field, there are still many unanswered questions. Below are issues that merit further exploration that have been spawned by each of the papers in this thesis and that consider different spatial and temporal scales:

I.   If the growth chamber experiment from paper I were to be repeated using different species (i.e., forbs or dwarf shrubs instead of grasses), would the soil legacy effects of temperature have been the

same? Would executing a similar experiment in a natural community (versus in the growth chamber) yield similar results and conclusions? It is likely that plant species with different growth strategies and mycorrhizal associations will react differently to the legacy effects of temperature and that contrasting plant communities would show alternative responses to those observed in paper I. It would also be very interesting to consider a greater time scale to investigate if and when the legacy effects of temperature cease to be of importance for plant performance (i.e., when current environmental conditions become more important).

II.   If the experiment from paper II were to be repeated in a different community (i.e., meadow vegetation), would similar responses in leaf phenolic concentrations to fertilization and elevation occur? Considering that subarctic meadow vegetation has much lower polyphenol concentrations than heath vegetation, it is likely the effects of fertilization and elevation would be less pronounced in meadow vegetation. Does the duration of the fertilization interact with temperature to affect plant defense? Further, sampling along an elevational gradient with even more pronounced temperature differences (i.e., a longer gradient with greater temperature extremes) would likely yield stronger and more significant effects of elevation and fertilization on plant defense. Therefore, exploring fertilization effects over greater spatial and temporal scales across different soil types would allow us to make more accurate predictions about how increased nutrient availability and/or cycling rates will alter ecosystem functioning as climate change advances.

III.   Do the interactive effects of warming and plant functional group removal along a successional gradient on the microbial community become stronger over greater temporal scales than that considered in this study? Would high throughput sequencing of the soils examined in paper III allow for clearer resolution of how the microbial community was affected by the treatments? Importantly, delving deeper into determining the specific identity and function of different components of the nematode and microbial community would allow for a more accurate picture of the ecosystem processes these organisms drive. If the experiment in paper III were allowed to continue over a longer time scale, would the nematode community eventually be affected by interactions between warming, plant functional group removal and successional stage? With increasing experimental duration, it is likely an alternate

steady state would be reached, thereby reducing the effects of the treatments (i.e., the nematode community may never be affected by the warming treatment).

IV.   If the duration of the warming and plant functional group removal treatments were increased, would interactive effects on decomposition and nutrient release have been realized in paper IV? Would repeating this experiment utilizing litter from all dominant species from the boreal system realize different results, such as more multi-way interactions? The lack of warming effects on decomposition in paper IV were probably the result of a minimal warming effect from the OTCs. If temperature increases were higher and the experiment was allowed to run longer, greater effects would likely be observed. However, species identity would still likely trump environmental conditions in most cases, which would further underscore the necessity of considering community composition when discussing decomposition responses to climate.

Designing and implementing experiments that explore how increasing temperatures interact with both abiotic and biotic components is critical for building comprehensive predictions about what the functioning of terrestrial ecosystems will look like under the new climate regime.

Taken collectively, the results of this thesis provide us with a number of important conclusions about how climate (i.e., temperature) can interact with other environmental factors to impact upon ecological processes (Figure 7). Firstly, each of the studies in this thesis showed that the responses of plants, soils and the processes they control to changing temperature often varied according to environmental context. Importantly, these studies showed that there is no blanket response to changes in temperature regime. Instead, they reveal that the specific environmental context needs to be taken into consideration when making predictions of how advancing global climate change might alter ecosystem functioning. However, despite this seeming hindrance, the chapters of this thesis actually advance our predictive powers because they give us a platform upon which we can build more accurate, context-dependent climate change projections. The type of data that this thesis has generated could potentially make a useful contribution to informing models that predict the effects of increasing temperature on plants and soils.

Nonetheless, caution must be exercised because these experiments were conducted in only two different types of biomes: subarctic tundra and the boreal forest. Even across these two high-latitude systems, the responses of ecosystem processes to changes in temperature were not consistently negative,

positive or even idiosyncratic. Further, it also remains to be seen if the effects observed here are indeed representative of subarctic tundra and the boreal forest on the circumpolar scale. Additionally, expanding upon the work done here by comparing ecosystem responses to these drivers across different types of biomes elsewhere (e.g., temperate-deciduous, tropical, savannah) would aid in understanding the extent to which the main findings of this thesis are broadly applicable; that is, to what extent can generalization be made at the global scale. Addressing this would further enhance our understanding of the complex interactions between different components of each respective ecosystem (Kardol *et al.*, 2012).

Further, it must be noted that certain dominant species may have been particularly strong drivers of the responses observed in all chapters of this thesis. Plant species that are common to both the subarctic and boreal systems may have been especially influential. For example, *E. hermaphroditum* is known to produce high amounts of batatasin-III (Odén *et al.*, 1992), an allelopathic chemical known to impact upon soil microbial activity (Wardle *et al.*, 1998), inhibit seedling germination (Zackrisson & Nilsson, 1992) and negatively affect species richness (Pellissier *et al.*, 2010). However, it has been found that the detrimental effects of *E. hermaphroditum* on ecosystem processes can be reversed by disturbances such as wildfire or nutrient input (Bråthen *et al.*, 2010). Considering the potential of this dominant dwarf shrub species to act as a type of ecosystem engineer (Bråthen & Ravolainen, 2015) highlights the importance of considering the role dominant species may play in modifying the effects of temperature in other biomes.

In addition, our understanding of the role of the drivers considered in this thesis other than temperature (i.e., soil legacies, nutrient addition, plant functional groups, successional stage) in controlling ecosystems processes is far from comprehensive. No doubt myriad other factors can also be identified as important interactive partners with temperature. However, as interest in multiple factor experiments continues to grow, we must exercise restraint. Just because we have the resources to create gigantic experiments that consider a plethora of drivers and the statistical power to analyse the massive data sets that these experiments generate does not mean that the experiment itself will necessarily yield ecologically relevant results. For instance, it has been shown that as the number of measured ecosystem drivers increases the magnitude of observed response tends to decrease (Leuzinger *et al.*, 2011). The best starting place to build multi-factorial experiments is to identify factors that have been found to have a strong main effect on ecosystem processes and then determine whether or not interactions between such factors do indeed interactively drive the processes in question. We must temper feasibility with wisdom and

carefully select the most relevant factors that will likely yield the best explanation for the ecological patterns that we are attempting to understand (Templer & Reinmann, 2011).
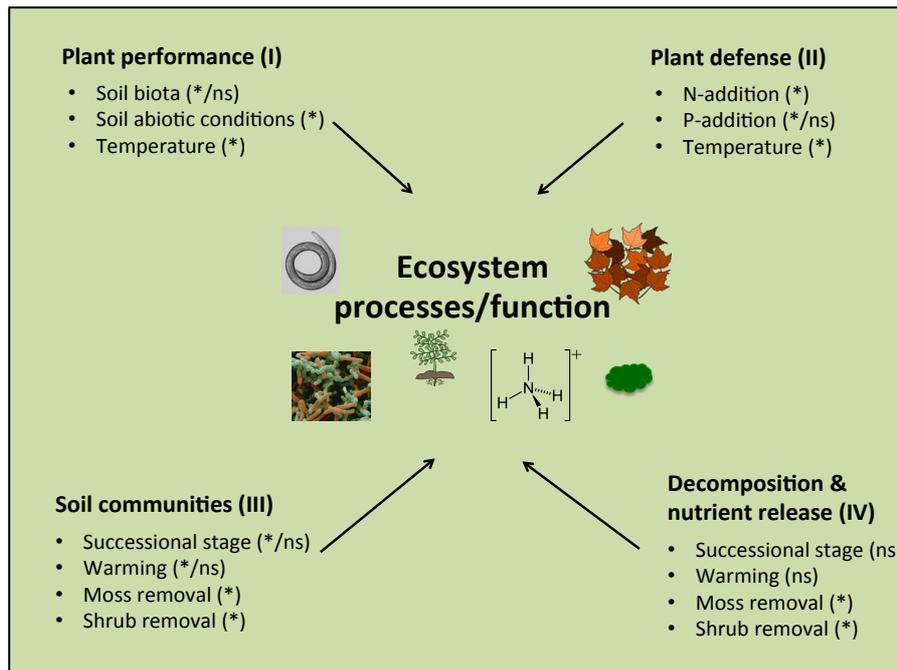

Plant performance (I)
- Soil biota (*/ns)
- Soil abiotic conditions (*)
- Temperature (*)

Plant defense (II)
- N-addition (*)
- P-addition (*/ns)
- Temperature (*)

Ecosystem processes/function

Soil communities (III)
- Successional stage (*/ns)
- Warming (*/ns)
- Moss removal (*)
- Shrub removal (*)

Decomposition & nutrient release (IV)
- Successional stage (ns)
- Warming (ns)
- Moss removal (*)
- Shrub removal (*)

Figure 7. The importance of different factors considered in this thesis in driving ecosystem processes and function. (*)= significant influence and (ns)= non-significant influence. Roman numerals in parentheses indicate thesis chapters.

Finally, there is increasing interest in experiments that take into account how multiple factors drive ecosystem processes and their response to climate change (Tylianakis *et al.*, 2008; Ostle *et al.*, 2009; Templer & Reinmann, 2011). The chapters contained in this thesis expand upon this topic by showing that plant performance and defense (papers I and II), soil communities (paper III) and litter decomposition and subsequent nutrient release (paper IV) were often affected by changes in temperature, but that these effects were in turn dependent upon other ecosystem components. However, this is far from the end of the story. The next phase is to delve deeper and seek a greater understanding of how these different components that are controlled by temperature drive ecosystem responses. Compiling and analyzing data from across ecosystems from different groups of organisms could build upon the results of this thesis and further our understanding of how ecosystem function will be altered as climate change advances.

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