DIRECT AND INDIRECT EFFECTS OF CLIMATIC CHANGES ON VEGETATION PRODUCTIVITY AND SPECIES COMPOSITION OF PERMAFROST PEATLANDS

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VRIJE UNIVERSITEIT

Direct and indirect effects of climatic changes on vegetation productivity and species composition of permafrost peatlands

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. L.M. Bouter,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de Faculteit der Aard- en Levenswetenschappen
op dinsdag 6 november 2012 om 15.45 uur
in het auditorium van de universiteit,
De Boelelaan 1105

door

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PhD-thesis VU University Amsterdam

Thesis 2012-06 of the Department of Ecological Science, VU University Amsterdam, The Netherlands
ISBN: 978-90-9027-153-8

This research was partially funded by the Darwin Center for Biogeosciences and the VU University Amsterdam

Layout and cover	Matti Kummu & Jonathan Ramsay
Printed by	Printservice Ede

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Chapter I

General introduction

1. Northern tundra ecosystems and climate change

Large anthropogenic emissions of greenhouse gases such as carbon dioxide (CO_2) and methane (CH_4) are currently causing global climate change by changing the radiative forcing of the planet (IPCC, 2007). There is extensive variation between geographic regions in the predicted magnitude and direction of climatic changes (Christensen *et al.*, 2007) and the strongest impacts are expected at higher latitudes (ACIA, 2004). Such changes in climate, including raising temperatures and changing precipitation patterns, are likely to affect primary productivity and decomposition rates (Fig. 1). If the balance changes among the processes that take up and release carbon, feedbacks through enhanced or decreased carbon emissions may occur. Northern tundra ecosystems are important in climate-induced feedbacks since they contain a large part of our global terrestrial soil carbon pool (Tarnocai *et al.*, 2009). These large carbon pools exist because of the low decomposition rates in tundra ecosystems compared to the amount of carbon sequestered in net primary production (ACIA, 2004, Aerts, 2006). Therefore, despite their low primary productivity, tundra ecosystems have been acting as a carbon sink (net uptake of carbon). If climatic change does affect the balance between primary productivity and decomposition rates, this has the potential to change the tundra from a carbon sink into a carbon source (net release of carbon). If such a change in carbon emission or sequestration is large enough, this would affect the global carbon cycle and thus feedback to climate. Hence, one of the key components in understanding and predicting the carbon cycle in northern tundra ecosystems is their plant productivity.

Within the range of northern tundra ecosystems (Walker *et al.*, 2005), northern peatlands are

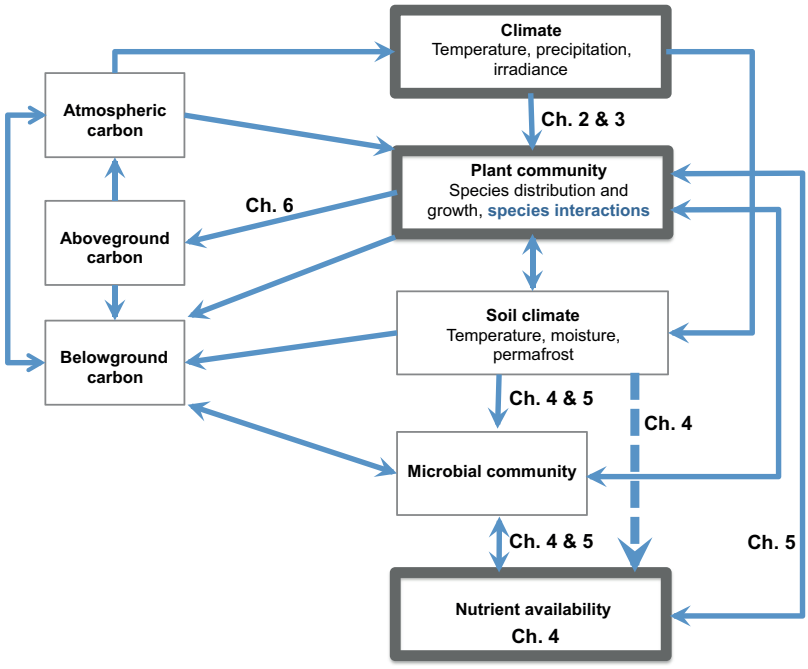

Figure 1. Schematic representation of the interactions between climate and permafrost peatland plant communities. The dark boxes in particular are discussed in this thesis and the Chapter numbers indicate the position of each chapter within the framework. The dashed arrow represents a (new) permafrost-thaw specific pathway which is presented in Chapter 4 of this thesis.

of particular interest because they contain around one third of the earth's terrestrial carbon (Gorham, 1991, Limpens *et al.*, 2008), more than half the current atmospheric stock of carbon dioxide (Rydin & Jeglum, 2006).

Permafrost, soil that is at or below the freezing point of water (0 °C) for two or more consecutive years (Brown *et al.*, 1998), is a common feature in high latitude regions and affects 24 % of all soil area in the northern hemisphere (16% of the global soil area). Of all permafrost area, approximately 19% is classified as peatland (adding up to approximately 3.6 x 10^6 km^2) (Tarnocai *et al.*, 2009). Particularly large stocks of organic C (around 280 billion ton C) and N are receding in these organic frozen soils (Batjes, 1996, Frey *et al.*, 2007, Kuhry *et al.*, 2010, Schuur *et al.*, 2008, Tarnocai *et al.*, 2009, Uhlirova *et al.*, 2007), which can be released when permafrost thaws as a result of climatic changes.

2. Factors determining plant productivity in permafrost peatlands

Plant productivity in northern tundra ecosystems in general is strongly constrained by the adverse physical environment. The main limiting factors include a short growing season, low temperature and low nutrient availability (Aerts *et al.*, 2006a, Chapin *et al.*, 1995, Elmendorf *et al.*, 2012, Shaver *et al.*, 2001), but water shortage is also frequently listed as a growth-limiting factor for tundra vegetation (Bliss *et al.*, 1994, Hodkinson *et al.*, 1999, Kade *et al.*, 2005, Ostendorf & Reynolds, 1998, Press *et al.*, 1998b, Qian *et al.*, 2010) (Fig. 1). The predominant limiting nutrient of terrestrial arctic ecosystems is nitrogen, of which the low availability in the soil severely limits plant growth at high-latitudes and in high-latitude peatlands in particular (Aerts *et al.*, 1992, Berendse & Jonasson, 1992, Chapin *et al.*, 1995, Hobbie *et al.*, 2002, Van Wijk *et al.*, 2004). Although tundra soils contain large stocks of nitrogen (Batjes, 1996, Limpens *et al.*, 2006), these stocks are largely tied up in unavailable, complex organic forms (Rydin & Jeglum, 2006), immobilized in microbial biomass (Jonasson *et al.*, 1996) or frozen in permafrost soils, and are therefore mostly unavailable to plants.

In permafrost soils, biotic processes are largely restricted to the shallow surface layer of the soil which is unfrozen in summer only (the active layer). Permafrost affects soil moisture, either because the water is in frozen state and thus unavailable to plants, or because the underlying frozen soil layer causes water-logging in the seasonally thawed active layer (Callaghan *et al.*, 2004). Additionally, permafrost affects microbial activity, plant rooting depth and plant-availability of nutrients in deeper soil layers (Camill *et al.*, 2001, Christensen *et al.*, 2004, Luoto *et al.*, 2004, Rydin & Jeglum, 2006, Turetsky *et al.*, 2000).

Lastly, in peatlands in particular, vegetation is typically dominated by *Sphagnum* peatmosses, which are competent ecosystem engineers and modify temperature, pH, nitrogen and moisture conditions of their surroundings. In general, these peatmoss interferences further restrict vascular plant productivity (Limpens *et al.*, 2008, Rydin & Jeglum, 2006).

3. Climatic changes affecting plant growth in permafrost peatlands

3a. Climatic changes affecting northern tundra ecosystems in general

In a changing climate, one or more of the above-mentioned limiting factors for plant productivity (and decomposition) are likely to change. The observed and predicted climatic changes in northern tundra ecosystems include rising air- and soil (permafrost) temperatures and changing precipitation regimes (ACIA, 2004, Akerman & Johansson, 2008, IPCC, 2007) can affect plant growth both directly and indirectly (Fig. 1). Higher air temperatures can directly affect plant growth in northern tundra ecosystems in general and can result in higher productivity (Elmendorf *et al.*, 2012, Myers-Smith *et al.*, 2012). If growth of tundra vegetation is water-limited, an increase in precipitation will also positively affect vegetation productivity (Hodkinson *et al.*, 1999, Qian *et al.*, 2010). An important indirect effect of rising temperatures on tundra vegetation involves changes in nutrient availability (Hungate *et al.*, 2003, Rustad *et al.*, 2001). Such increases are observed and predicted to occur through warmer soil temperatures, because mineralization rates of organic nitrogen can increase and concomitantly plant nutrient availability will become higher (Weedon *et al.*, 2012). Moreover, some observations suggest that increases in nutrient availability may occur through thawing of permafrost soil. These thawing soils may release nitrogen into tundra ecosystems, given that they contain large frozen organic N stocks (Mack *et al.*, 2010, Schuur *et al.*, 2007), although the fate and availability of this nitrogen source when unfrozen is not fully understood. Overall, these direct and indirect climatic changes may affect growth of different tundra species to different degrees, although the relative importance of each effect is currently not known. Finally, if growth differences occur, this can alter species interactions and thus potentially alter species composition, which in turn can cause important changes in C-sequestration through differences in intrinsic productivity and litter input of the species involved (Chapin *et al.*, 1996, Dorrepaal *et al.*, 2005).

3b. Carbon sink and source function of permafrost peatlands in particular

In the remainder of this thesis, the focus is on permafrost peatlands, because of their vast stores of carbon and as they are considered to be particularly sensitive to ongoing climatic changes (Schuur *et al.*, 2008, Tarnocai *et al.*, 2009). Extensive research in recent years has shown that carbon dioxide emissions from permafrost peatlands may increase due to higher soil decomposition rates of both shallow and deep organic matter in response to warming (Dorrepaal *et al.*, 2009, Kuhry *et al.*, 2010). Also, methane emissions can increase in response to thawing permafrost and changing hydrology (Christensen *et al.*, 2004, Olefeldt *et al.*, 2012). However, much less research effort has been spent on potential changes in carbon uptake (sink function) by permafrost peatlands than on their potential carbon emission changes (Olefeldt *et al.*, 2012). A climate change mediated increase in carbon uptake can, however, be expected in permafrost peatlands via positive vegetation responses to warming and increased precipitation (direct effects), as well as in response to increased nutrient availability (indirect effect) (Fig. 1).

There are several reasons why vegetation responses in permafrost peatlands to climatic changes may be significantly different from vegetation responses in other northern ecosystems, making direct extrapolation of findings found elsewhere difficult. Firstly, these peatlands are characterized by the presence of *Sphagnum* peatmosses which, because of their dominant ground cover, act as a 'living soil'. This 'living soil' is likely to respond to climatic changes, for example by increased vertical growth in response to warming, and thus potentially modifies the

impact of these changes on the vegetation community (Dorrepaal *et al.*, 2006). Hence, because responses to climatic change can be expected of both the vascular community as well as of the dominant peatmoss (Dorrepaal *et al.*, 2004), the net community responses, and ultimately carbon uptake, will depend both on interactions within the vascular community, as well as on interactions between the vascular community and the dominant peatmoss (Van Breemen, 1995). Secondly, the structure and function of permafrost peatlands is to a large extent determined by the permanently frozen soil layer. Thawing of this frozen soil will increase the thickness of the layer in which biotic processes can take place, and may alter soil structure, hydrology, and possibly nutrient availability. The latter is of particular interest because growth of the vegetation in these peatlands is strongly nitrogen-limited (Aerts *et al.*, 1992, Berendse & Jonasson, 1992). In addition, thawing could release the above-mentioned frozen organic N stocks, which will have consequences for growth of the N-limited peatland vegetation (Schuur *et al.*, 2007) if these stocks turn out to contain plant available N. Overall, permafrost peatlands are thus expected to respond strongly to climatic changes, and the effect of permafrost thawing is one of the biggest uncertainties for determining the magnitude and direction of the effect of climatic changes on their carbon sink function (Rydin & Jeglum, 2006, Turetsky, 2004, Wania *et al.*, 2009).

Yet, although increased temperature (Elmendorf *et al.*, 2012) and increased summer precipitation (Bengtsson *et al.*, 2011, Blok *et al.*, 2011, Dormann & Woodin, 2002) studies have been performed on many northern tundra ecosystems, none were so far performed on permafrost peatlands. Similarly, although many nutrient studies have been performed on northern tundra ecosystems in general (Aerts *et al.*, 1992, Chapin *et al.*, 1995, Hobbie *et al.*, 2002, Van Wijk *et al.*, 2004), little is known about the potential change in the amount of plant-available N due to thawing permafrost in northern permafrost peatlands.

4. General aim and specific research questions

The aim of this thesis is therefore to evaluate northern permafrost peatland vegetation response to climate change induced increases in temperature, precipitation and N-availability (through permafrost thawing) both at the community and the plant level (for both vascular and bryophyte plants). I aim to answer how these climate factors affect vegetation productivity in northern permafrost peatlands through species-specific growth and changes in species composition.

Specifically, answers are sought to the following research questions:

i. How do spring- and summer warming, and increased snow cover affect species-specific growth responses and species composition in northern permafrost peatlands?

ii. How does increased summer precipitation affect species-specific growth responses and species composition in northern permafrost peatlands?

iii. Can permafrost thawing affect species-specific growth responses and species composition in northern permafrost peatlands through a release of plant-available N?

5. Outline of the thesis

In Chapter 2, I show how permafrost peatland vegetation responds to manipulated changes in temperature and snow depth and duration over a period of eight years. Both vascular as well as bryophyte responses were studied and we propose a mechanism of how bryophyte responses to warming may modify the response of the vascular plant community. In Chapter 3, vegetation responses to increased summer precipitation are presented, and the responses in two distinct permafrost affected tundra ecosystems (shrub-dominated tundra and *Sphagnum*-dominated peatland) are compared. In Chapter 4, we present our findings on the amount of plant-available nitrogen that can be released from thawing permafrost peatlands. In Chapter 5 the actual in situ potential of permafrost peatland plants to utilize additional nutrients as released at the thaw front is discussed. Finally, in Chapter 6, the main findings of this thesis and their implications for the carbon balance are discussed.

Most experimental work in this thesis was performed on permafrost peatlands in the Abisko area, northern Sweden, with the exception of part of the work presented in Chapter 3 which was partly performed in the Kytalyk Reserve in north-eastern Siberia. The study sites are described in more detail in the individual chapters.

I

Chapter II

A Race for Space? How *Sphagnum fuscum* stabilizes vegetation composition during long-term climate manipulations

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Global Change Biology 17 (2011) 2162–2171

Abstract

Strong climate warming is predicted at higher latitudes this century, with potentially major consequences for productivity and carbon sequestration. Although northern peatlands contain one-third of the world's soil organic carbon, little is known about the long-term responses to experimental climate change of vascular plant communities in these *Sphagnum*-dominated ecosystems. We aimed to see how long-term experimental climate manipulations, relevant to different predicted future climate scenarios, affect total vascular plant abundance and species composition when the community is dominated by mosses. During 8 years, we investigated how the vascular plant community of a *Sphagnum fuscum*-dominated subarctic peat bog responded to six experimental climate regimes, including factorial combinations of summer as well as spring warming and a thicker snow cover. Vascular plant species composition in our peat bog was more stable than is typically observed in (sub)arctic experiments: neither changes in total vascular plant abundance, nor in individual species abundances, Shannon's diversity or evenness were found in response to the climate manipulations. For three key species (*Empetrum hermaphroditum*, *Betula nana* and *S. fuscum*) we also measured whether the treatments had a sustained effect on plant length growth responses and how these responses interacted. Contrasting with the stability at the community level, both key shrubs and the peatmoss showed sustained positive growth responses at the plant level to the climate treatments. However, a higher percentage of moss-encroached *E. hermaphroditum* shoots and a lack of change in *B. nana* net shrub height indicated encroachment by *S. fuscum*, resulting in long-term stability of the vascular community composition: in a warmer world, vascular species of subarctic peat bogs appear to just keep pace with growing *Sphagnum* in their race for space. Our findings contribute to general ecological theory by demonstrating that community resistance to environmental changes does not necessarily mean inertia in vegetation response.

Introduction

Growth and survival of plants in high-latitude ecosystems are limited by harsh environmental conditions, such as low nutrient availability, low temperatures and low irradiance during a large part of the year. Increases in both summer and spring temperatures and in winter snowfall have been recorded in these areas over the past decades and are predicted to continue in this century (IPCC, 2007). It is currently widely recognized that these climatic changes will have immediate and persistent effects on composition and structure of Arctic vascular plant communities (Wullschleger *et al.*, 2010). This notion is primarily based on the results of meta-analyses showing rapid and consistent responses to experimental climate warming in general across plant communities. Indeed, Walker *et al.* (2006) showed that across many sites, experimental warming of tundra ecosystems not only increased height and cover of deciduous and evergreen shrubs and graminoids, but also decreased species diversity and evenness. However, it is important to realize that these meta-analyses did not address climatic changes other than summer warming, nor have they included all predominant tundra ecosystem types, with strong underrepresentation of *Sphagnum*-dominated peatlands.

Few studies so far accounted for the impact of climatic changes both inside and outside the growing season, even though increased snow in winter and increased spring temperatures are expected to strongly affect plant performance in the Arctic. Indeed phenological responses to

short-term spring warming have been reported (Aerts *et al.*, 2006). Changes in the winter climate and increases in snow cover in particular have been linked with observed changes in Arctic shrub growth, possibly because overwintering shoots benefit from increased protection of a thicker snow layer and a concomitantly higher nutrient availability due to higher winter soil temperatures (Sturm *et al.*2001b; Sturm *et al.*, 2005; Tape *et al.*, 2006). Most experiments on impacts of a thicker snow cover so far have, however, made use of snow fences, which often cause an extreme and highly unrealistic increase in snow thickness (but see Johansson (2009)).

In addition to the paucity of data on the impact of realistic climatic changes outside the growing season, a distinction between peatland versus non-peatland vegetation responses is largely missing. Peatlands are important in the light of climate change because of their relatively large carbon storage capacity (one-third of the world's terrestrial carbon) (Gorham, 1991). In northern peatlands, vascular plant life is more controlled by the often dominant presence of peat mosses (*Sphagnum spp.*) than in other northern ecosystem types. By efficiently accumulating nutrients from atmospheric deposition and by slowing down soil organic matter decay and nutrient mineralization, *Sphagnum* mosses strongly reduce the availability of nutrients to vascular plants (Aerts *et al.*, 1992; Rydin *et al.*, 2006). In addition, the progressively increasing height of the *Sphagnum* forces vascular plants to keep pace in order not to become buried (Van Breemen, 1995; Rydin *et al.*, 2006; Dorrepaal *et al.*, 2006). Thus, both the abiotic as well as the biotic environment places extreme demands on vascular plant growth in high-latitude peatlands.

In non-*Sphagnum*-dominated ecosystems, monitoring studies have shown an increase in shrub abundance over the last decades (Tape *et al.*, 2006, but see Olofsson *et al.*, 2009). Moreover, changes in vegetation composition in response to experimental summer warming (Walker *et al.*, 2006) and winter snow addition (Wipf *et al.*, 2010) were largely dominated by changes in cover of the dominant shrubs. This strong impact of climate change is often attributed to a positive nutrient feedback favouring shrubs (Wookey *et al.*, 2009). A general negative correlation between vascular and non-vascular species biomass has been observed in studies which addressed responses of both plant types to warming (Press *et al.*, 1998; Cornelissen *et al.*, 2001; van Wijk *et al.*, 2004; Walker *et al.*, 2006). However, these studies were all performed in non-*Sphagnum*-dominated ecosystems. Moreover, this negative correlation between the responses of vascular and non-vascular species was largely dominated by a decrease in lichen biomass. Hence, extending these findings to *Sphagnum*-dominated peat bogs is not self-evident.

There are several reasons to expect that a dominant presence of the peat moss *Sphagnum* in an ecosystem will counteract the abovementioned effects of climate on plant community composition. In addition to its influences on nutrient dynamics and vascular plant growth, a positive growth response to climatic changes of *Sphagnum fuscum* itself might negatively impact shrub growth (Dorrepaal *et al.*, 2006), especially in the long term. Hence, in contrast with responses in other Arctic ecosystems (Walker *et al.*, 2006; Wipf *et al.*, 2010), climatic changes might not result in increased height or closure of the vascular canopy above thick *Sphagnum* carpets, despite positive effects of increased temperatures on shrub growth. Yet, no long-term data on responses of vegetation composition to climate manipulations in northern *Sphagnum*-dominated peatlands have been published thus far.

Here, we present, to our knowledge, the first long-term experimental climate change study to take into account both realistic and independent climate changes inside and outside the growing season as well as peatland-specific vascular plant community responses. Our aims were: (1) to

quantify, over the course of 8 years, how the vascular plant community of a subarctic peat bog changed in species composition, diversity and evenness in response to six different experimental climate scenarios (summer warming, increased winter snow, increased winter snow plus spring warming and combinations thereof); and (2) to clarify how interactions between dominant growth forms at the plant level may affect community responses and community resistance or resilience to induced climate change, by measuring growth responses of the two dominant shrubs (*Empetrum hermaphroditum* and *Betula nana*) and the dominant peatmoss (*Sphagnum fuscum*).

Methods

Study site

The study was performed on a slightly sloping sub-arctic bog on the southern shore of Lake Torneträsk, near the Abisko Scientific Research Station in northern Sweden (68°21'N, 18°49'E, alt. 340 m). The recent decade (1999–2008) showed a mean annual rainfall of 352 mm and mean monthly January and July temperatures of -9.7 °C and 12.3 °C, respectively, (meteorological data Abisko Scientific Research Station, Freschet *et al.*, 2010). The length of the growing season is 130 days (Karlsson *et al.*, 1996), and the site is permafrost underlain with an active layer thickness of approx. 55 cm. The dominant component of the bog vegetation is the peat moss *Sphagnum fuscum* (Schimp.) H. Klinggr. The vascular plant community is low-statured and open (maximum shrub height 15 cm, average cover 25%) and mainly consists of the evergreen dwarf shrubs *Empetrum hermaphroditum* Hagerup (41%) and *Andromeda polifolia* L. (5%), the deciduous dwarf shrubs *Betula nana* L. (22%) and *Vaccinium uliginosum* L. (6%), the grass *Calamagrostis lapponica* (Wahlenb.) Hartm. (5%) and the forb *Rubus chamaemorus* L. (10%).

Experimental design of the climate change experiment

Six climatic regimes were established in June 2000, relevant to different possible future climate scenarios in the Arctic (Sælthun, 2003; ACIA, 2004; Kohler *et al.*, 2008) and allowing to study separate and interactive effects of climate change in different seasons (Dorrepaal *et al.*, 2004). Our experimental manipulations led to full-factorial combinations of two summer treatments (ambient, warming) and three treatments during winter and spring (ambient, snow addition, snow addition plus spring warming)(n =5). For brevity, the latter are referred to as winter treatments (see Table 1). Warming treatments were imposed by passive warming using a modified, larger version of the transparent, hexagonal ITEX Open Top Chambers (OTCs; 50 cm high, 2.2-2.5 m bottom diameter; cf. Marion *et al.* 1997). For the spring treatment, OTCs were placed on the plots in late April and removed approx. 1 June. Summer warming was imposed by OTCs from around 1 June until approx. 1 October, and the winter treatment consisted of OTCs on the plots from approx. 1 October until the end of April (for treatment codes see Table 1). Average daily mean air temperature in the OTCs increased by 0.3-1.0 °C in spring (late-April-June) and by 0.2-0.9 °C in summer (June-October). Soil temperature responses generally tracked those of air temperatures, with an increase in spring soil temperature of about 1.0 °C, and a summer increase of 0.6-0.9 °C. During winter (October-April) the OTCs passively accumulated snow, thereby increasing the average temperatures by 0.5-2.8 °C and 0.5-2.2 °C at +5 and -5 cm respectively, and doubling the ambient (max. approx. 15 cm) snow-cover thickness. Average snow melt date was not affected by the treatments. Full details of the experimental procedures and their effects on summer and winter microclimate were presented by Dorrepaal *et al.* (2004, 2009).

Table 1. Experimental design and treatment codes used for the climate manipulations.

	Summer treatment	
Winter treatment	Ambient	Warming
Ambient	AA	WA
Snow addition	AS	WS
Snow addition plus spring warming	AS+	WS+

Vegetation abundance measurements

In the first two weeks of August 2000, 2002, 2006 and 2008, i.e. 0, 2, 6 and 8 years after the start of the experiment, vegetation cover was measured by means of the point-intercept method (Jonasson, 1988). We used a 60*60 cm metal frame with adjustable legs and a metal mobile double-strip with 11 holes in a row. By moving the double-strip 5 cm horizontally each time, a grid with 121 holes was created. The position of the frame was marked permanently and chosen at a minimum of 65 cm from the plot edge to prevent edge effects like reduced precipitation or clonal connections beyond the plots. A metal pin 5 mm in diameter was lowered through each hole in the strip and each contact of the pin with green living vegetation was recorded by species until the pin reached the moss substrate. Depending on the species, a hit related to a single bigger leaf (of e.g. *R. chamaemorus, C. lapponica, V. uliginosum, B. nana*) or to a single stem or branch with several smaller leaves (e.g. *E. hermaphroditum, A. polifolia, V. microcarpum*). Earlier studies have shown that point-intercept measurements are a good estimator of plant biomass (Jonasson, 1988; Hobbie *et al.*, 1999; Aerts, 2009).

Shrub length growth, shrub height above Sphagnum and Sphagnum vertical growth measurements

To assess the effects of changes in summer and winter climate on shrub growth and on interactions between shrubs and their living *S. fuscum* substrate, we performed additional measurements on *E. hermaphroditum, B. nana* and *S. fuscum*. Because the shrubs are long-lived perennials, the ramets of which are connected below the moss layer through overgrown stems, we defined a shoot as a stem that emerged above the moss layer (Chapin *et al.*, 1985).

Annual stem-length increments of ten randomly selected *E. hermaphroditum* shoots per plot were measured for years 7 and 8. Current-year growth was identified using colour differences of the stem, bud scars and changes in leaf length between the growth segments of the different years. Annual stem-length increment data for years 1 and 2 were taken from Dorrepaal *et al.* (2006). In addition, from a random subsample of 10 shoots per plot the percentage of shoots of which fewer than two year-segments or less than one year-segment was visible above the moss-layer was counted in year 8 (as an estimate of encroachment by *S. fuscum*).

For *B. nana*, the length of the current-year growth of the main axis of 10 randomly selected shoots per plot was measured in year 8. Current-year growth was identified using colour differences of the stem and scars of the terminal bud. Additionally, current-year stem-length growth data for years 1 and 2 were taken from Dorrepaal *et al.* (2006). Unlike *E. hermaphroditum* shoots, *B. nana* shoots in our plots do not grow perpendicular to the moss substrate. Therefore, *B. nana* shrub height after 9 years was measured as the vertically projected distance from the top of the shoot to the *S. fuscum* surface (five shoots per plot). *S. fuscum* length growth was measured

in all plots in year 9 using a modification (Dorrepaal *et al.*, 2004) of the cranked wire technique (Clymo, 1970), with five wires per plot. In addition, *S. fuscum* length-growth data for year 1 and 2 for the same plots were taken from Dorrepaal *et al.* (2004).

Statistical analysis

Prior to statistical analysis, plot means were calculated for all parameters. All plot mean data were tested for normality and homogeneity of variances by visual estimation of residual and probability plots. For the vegetation cover data ln-transformation improved the homogeneity of variances considerably. Transformation did not improve the distribution of *E. hermaphroditum* length increment data for years 7 and 8. As ANOVA is robust to considerable heterogeneity of variances as long as sample sizes are nearly equal (Zar, 1999), we included these data untransformed. All other data approximated normal distributions and homogeneous variance. All analyses were performed with SPSS 15.0 for Windows.

The effects of the summer and winter climate manipulations on vegetation cover (number of hits) over years 0-8 were analysed using repeated measures (RM-)ANCOVAs. 'Year' was the within-subject factor, 'initial cover' the covariate (to account for differences in initial cover), and 'summer treatment' and 'winter treatment' were the between-subject factors. 'Winter treatment' was ambient, snow addition or snow plus spring warming, the effects of which were separated by a Tukey's HSD post hoc test on data corrected for the covariate to allow post hoc testing. This analysis was performed for the total cover as well as for individual cover of each of the six most abundant species.

Likewise, the effects of the summer and winter climate manipulations on *E. hermaphroditum* annual stem-length increments over years 1, 2, 7 and 8 were analysed with RM-ANOVAs, with year as the within-subject factor, and summer treatment and winter treatment as between-subject factors. This same RM-ANOVA was also applied to *S. fuscum* vertical growth over years 1, 2 and 9. The data for *B. nana* growth in year 8 and *B. nana* shrub height in year 9 were analysed using an ANOVA with summer treatment and winter treatment as fixed factors. Treatment effects on the percentage of *E. hermaphroditum* shoots that had fewer than two year-segments or less than one year-segment visible above the moss in year 8 were analysed with the χ^2-test.

Results

Community parameters

Aboveground vascular plant abundance

There were no significant main treatment effects of the climate manipulations on total vascular plant abundance in the course of 8 years. The interaction between the effects of the summer and winter treatments (Fig. 1; Table 2) was most likely caused by the contrasting effects of the summer warming and winter ambient treatments vs. the snow addition plus spring and summer warming treatments. Correspondingly, no change in abundance in response to any climate manipulation was observed at the species level, as no changes were detected in the abundances of the main representatives of the growth forms in our system: evergreen shrubs (*E. hermaphroditum*, *A. polifolia*), deciduous shrubs (*B. nana*, *V. uliginosum*), graminoids (*C. lapponica*) and forbs (*R. chamaemorus*) in the course of 8 years (Table 2). Apparent trends ($P < 0.1$) of winter

and winter x summer treatments affecting the abundance of *C. lapponica* and *R. chamaemorus* respectively seem to be statistical artefacts caused by the low number of hits for these individual species, which made them particularly sensitive to variance in initial cover. Indeed, these apparent trends disappeared when cover was analysed as differences compared to initial cover in a RM-ANOVA. However, the apparent trend towards interaction between year and summer treatment for *E. hermaphroditum* was consistent in both analyses. *E. hermaphroditum* abundance increased under ambient conditions in the last 2 years of the experiment, but remained unchanged in any of the other year-treatment combinations.

Diversity & Evenness

The unaltered total vascular plant abundance and individual species abundances were reflected in the diversity indices, as none of the treatments affected the Shannon's diversity index (Table 2). Similarly, Shannon's evenness index remained unchanged in response to the treatments, although the higher order interaction between year, summer treatments and winter treatments indicated a minor influence caused by the combination of treatments in some years (Table 2).

Gross growth parameters

Apical growth of *E. hermaphroditum* showed a positive trend in response to the winter treatments, in particular to spring warming (Tukey's HSD, AS+/WS+ vs AS/WS, P =0.053) (Fig. 2; Table 2) and this effect became more pronounced in the later years of the experiment. Summer warming

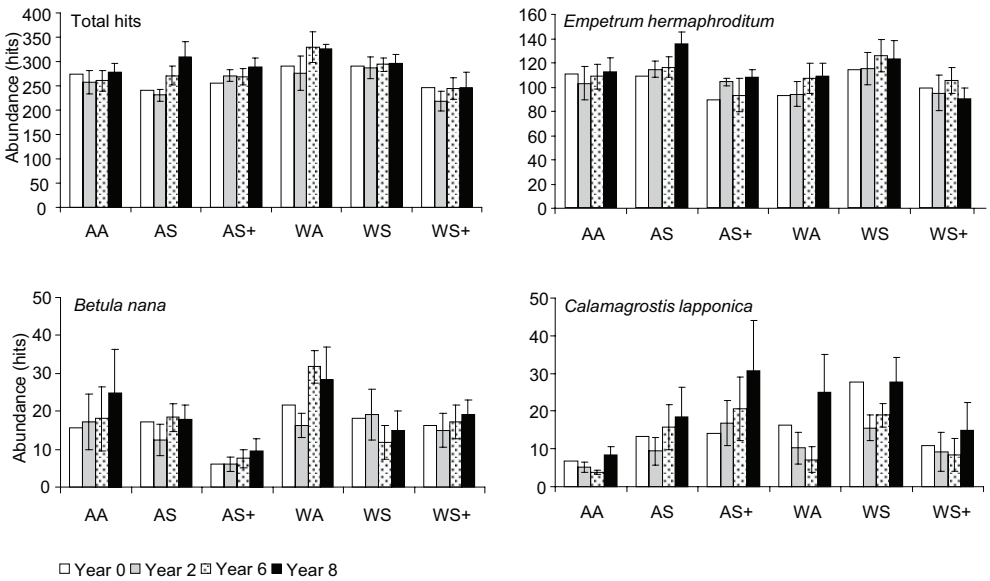

Figure 1. Mean (+/- 1SE) abundances (expressed as mean number of hits) of all vascular plants and three important individual species after 2, 6 and 8 years of simulated climate change compared to initial abundances (data from year 0 are without SE since these values were used as a covariate)(n =5). There were no significant main effects of summer or winter treatments or year of sampling (Table 2). For treatment codes see Table 1.

Table 2. *F*- statistics for repeated-measures ANCOVAs for the effects of summer (ambient, warming: SUM) and winter treatments (ambient, snow addition, snow addition plus spring warming: WIN) and initial values (INI) on the community parameters: abundance (number of point-intercept hits), Shannon's diversity and Shannon's evenness; and on the gross growth parameters: annual stem length increments of *Empetrum hermaphroditum* and *Betula nana*, and vertical growth of *Sphagnum fuscum*. The summer and winter treatments (SUM & WIN) were taken as between-subject factors, year as within-subject factor and initial densities (INI) as a covariate

	Source								
Variable and species	**SUM**	**WIN**	**INI**	**SUM x WIN**	**Year**	**Year x SUM**	**Year x WIN**	**Year x SUM x WIN**	**Year x INI**
Community parameters									
Years (0), 2, 6 & 8									
Abundance [a]									
Total number of hits	1·00	0·581	27·3 ***	3·608 *	1·28	2·13	0·406	0·771	1·19
Empetrum hermaphroditum	0·259	0·924	9·53 **	0·544	0·041	2.88 +	0·334	0·855	0·029
Betula nana	0·254	0·262	129·8 ***	0·10	2·66	0·011	0·511	3·10 *	0·663
Calamagrostis lapponica	1·24	3·21 +	62·0 ***	0·150	1·52	0·638	1·01	0·101	0·173
Rubus chamaemorus	1·28	0·686	32·5 ***	2·90 +	1·12	2·49	1·35	0·732	1·11
Vaccinium uliginosum	0·003	0·509	92·9 ***	1·01	4·01 *	1·16	0·479	0·141	3·79 *
Andromeda polifolia	1·16	0·248	36·6 ***	1·16	0·933	1·61	1·69	1·18	2·00
Diversity & Evenness [b]									
Shannon's Diversity (H)	0·410	1·44	33·6 ***	0·759	1·94	0·356	0·362	1·08	
Shannon's Evenness (J)	0·314	1·21	18·7 ***	2·50	1·34	0·040	0·679	2·59 *	
Gross growth parameters									
Apical growth [a, c]									
Empetrum hermaphroditum									
Years 1, 2, 7 & 8	2·61	3·13 +	-	0·071	16·5 ***	0·959	1·33	0·480	-
Years 1 & 2	2·50	0·550	-	0·060	44·7 ***	0·530	0·892	0·639	-
Years 7 & 8	1·57	4·24 *	-	0·110	11·6 ***	2·00	0·310	1·02	-
Betula nana									
Years 1, 2 & 8	12·1 **	0·559	-	0.019	4·82 *	0·211	0·199	0·864	-
Years 1 & 2	7·77 **	0·850	-	0·139	1·26	0·137	0·235	1·28	-
Year 8 [d]	6·04 *	0·021	-	0·510	-	-	-	-	-
Vertical growth [b, c]									
Sphagnum fuscum									
Years 1, 2, 9	6·42 *	0·235	-	1·69	24·8 ***	1·59	1·21	2·78 *	-
Years 1 & 2	7·11 *	0·132	-	2·00	41·4 ***	0·037	2·53	1·19	-
Year 9 [d]	2·03	0·391	-	2·41	-	-	-	-	-

Notes indicate: [a]: data ln-transformed; [b]: data untransformed; [c]: RM ANOVA; [d]: Two-way ANOVA. Asterisks indicate significance level of treatment effects: +: $P < 0.1$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

did not affect *E. hermaphroditum* stem growth. In contrast to the positive effect of spring warming on *E. hermaphroditum*, the winter treatments did not affect *B. nana* stem length growth. However, summer warming had a sustained positive effect on *B. nana* stem growth (Fig. 2; Table 2). *S. fuscum* vertical growth was higher in response to summer warming, but was not affected by the winter treatments (Fig. 3; Table 2), except for a higher order interaction effect with time.

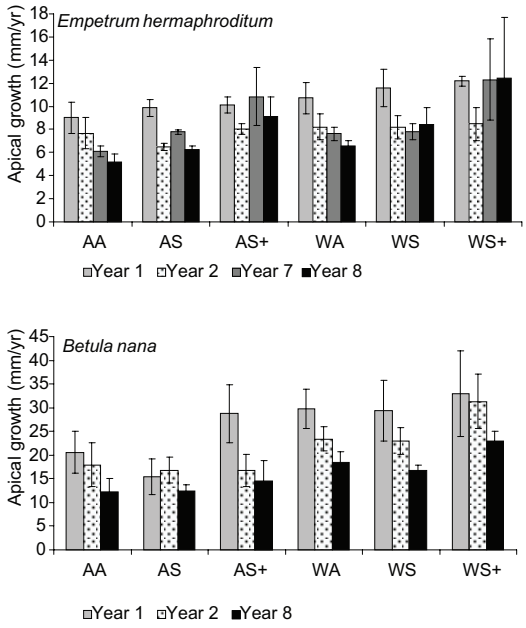

Figure 2. Apical annual growth (mean +/- 1SE) of *Empetrum hermaphroditum* and *Betula nana* in response to simulated environmental change after 1, 2, 7 and 8 years of simulated climate change (n =5). Tukey's post-hoc test at $P < 0.05$ on RM-ANOVA with between subjects-factors: summer (A, W) and winter (A, S, S+) indicated that AS+/WS+ vs. AS/WS differed significantly for *E. hermaphroditum*. *B. nana* growth was significantly affected by summer warming (Table 2). For treatment codes see Table 1.

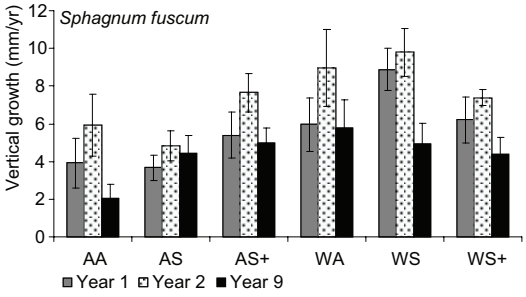

Figure 3. *Sphagnum fuscum* vertical annual growth (mean +/- 1SE) after 1, 2 and 9 years of simulated climate change (n =5). Only summer warming affected growth significantly (RM-ANOVA with between subjects-factors: summer (A, W) and winter (A, S, S+) followed by a Tukey's post-hoc test at $P < 0.05$). For treatment codes see Table 1.

Net growth parameters (year 8)

In year 8 of the treatment, more *E. hermaphroditum* branches were partly overgrown (i.e. a higher percentage of branches had less than one or two year segments above the moss) by *S. fuscum* in the manipulation treatments than in the ambient plots (χ^2-test-test, all six treatments, P <0.01). In particular, the summer warming treatment increased the percentage of partly overgrown branches (χ^2-test-test, AA/AS/AS+ vs WA/WS/WS+, P <0.001) (Fig. 4; Table 2).

Despite its increased apical growth rate in response to summer warming, net shrub height of *B. nana* (vertical distance from the shoot tip to the S.fuscum moss layer) remained the same under all climate manipulations (Fig. 4), as did the average stem inclination towards the moss-surface (results not shown).

Discussion

Vascular plant community composition in our peat bog was more resistant to climate manipulations than is typically observed in other (sub)arctic ecosystems: vascular plant abundance, individual species abundances, Shannon's diversity or evenness did not change across any growth form in response to 8 years of either winter snow addition or experimental spring or summer warming. This resistance occurred despite the fact that growth rates of *B. nana* and *E. hermaphroditum* responded strongly and significantly and sustained this response over 8 years of treatments. This paradox of no effects of the climate manipulations at the community level versus significant individual growth responses may be explained by the role of *S. fuscum* in modifying effects of climate change at the community level.

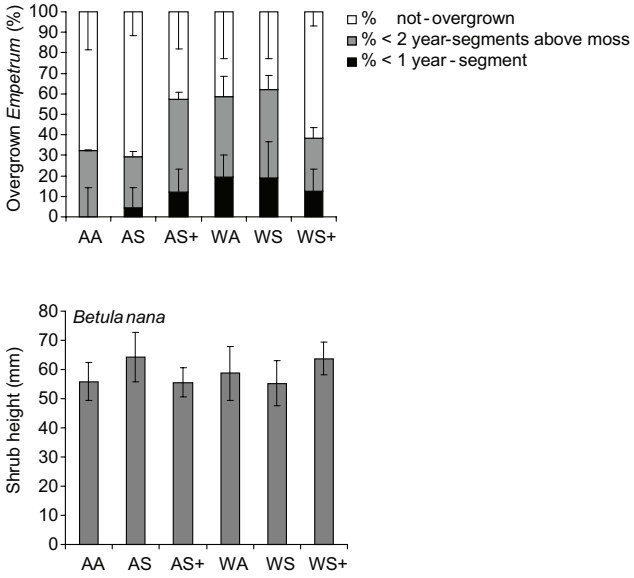

Figure 4. Measures of encroachment by *S. fuscum* of two key species after 8 years of simulated environmental change (n =5). a) *E. hermaphroditum*: percentage of branches with less than one or than two year-segments visible above the moss-surface. Summer warming increased this percentage (χ^2-test-test, AA/AS/AS+ vs. WA/WS/WS+, P < 0.001) and b) *B. nana* net shrub height above the moss-surface remained unaltered in response to all treatments. Error bars indicate SEM. For treatment codes see Table 1.

Climate change effects on the vascular plant community

Winter snow addition did not change our vascular plant community, which contrasts with the generally found pattern of increasing deciduous shrub abundance and decreased species richness in response to thicker snow cover (Sturm *et al.*, 2005; Tape *et al.*, 2006; Wipf *et al.*, 2010). A hypothesized mechanism behind observed increases in shrub abundance is a positive feedback loop leading from more shrubs to thicker snow cover to higher subnivian temperatures, with associated increases in decomposition and nutrient mineralization (Sturm *et al.*, 2001a). This mechanism seems a plausible explanation for systems with relatively large shrubs (e.g. *B. nana* up to approx. 0.3 m in Alaska, Happy Valley, and in Siberia, Kytalyk Reserve (F. Keuper, data unpublished), as compared to the shrubs in our system (*B. nana* up to approx. 0.15 m). Moreover, the vascular plant community on *S. fuscum* dominated peatlands is generally low and open due to competition between the vascular community and the peatmoss (e.g. 25% vascular plant cover in the current study). Accordingly, the experimental increase in snow thickness was much lower in our experiment than in most experimental snow increase studies so far (Wahren *et al.*, 2005; Borner *et al.*, 2008; but see Johansson *et al.*, 2009). The unresponsiveness of the vascular community and in particular of the dwarf shrubs to our moderate snow treatment may thus be explained by a combination of low initial shoot density and a rather moderate increase in insulation, insufficient for initiating a positive feedback loop as hypothesized by Sturm (2001a).

Furthermore, eight years of experimental warming in spring and/or summer did not change the vegetation community composition either. This also contrasts with previous findings of increased shrub abundance in response to (experimental) climate warming (Tape *et al.* 2006; Walker *et al.* 2006; but see van Wijk *et al.* 2004; Olofsson *et al.* 2009), and with the commonly observed decrease in subarctic species diversity and evenness (Press *et al.*, 1998; Walker *et al.*, 2006) as a result of such changes. The most likely explanation for these discrepancies is that our study is the first study looking closely at long-term effects of experimental warming on the vascular plant community of a *Sphagnum*-dominated peatland.

Effects of summer and winter treatments on plant growth rates

Even in the absence of changes at the community level, we did expect to find positive effects of the treatments at the plant level. Indeed, apical growth of *E. hermaphroditum* increased in response to the spring warming treatment in year 7 and 8 of the experiment, while it did not increase in response to summer warming. Empetrum is somewhat drought sensitive (Tybirk *et al.*, 2000) and our summer warming treatment slightly affected summer soil moisture (mean summer soil moisture by volume 26% in the summer warmed plots vs. 34% ambient; $P < 0.05$; Dorrepaal *et al.* 2009). The direct effect of warming on *E. hermaphroditum* apical growth might thus have been compensated by a negative growth response to drying of the peat. In contrast to Empetrum, length growth of *B. nana* showed a sustained increase to summer warming at the plant level, which is in line with previous reports (Bret-Harte *et al.*, 2001; Dorrepaal *et al.*, 2006). Unlike summer warming, snow addition did not affect the length growth of *B. nana*, *E. hermaphroditum* or *S. fuscum*, which again may be explained by the modest increase in snow cover in our winter treatment. This indicates, in addition to the absence of snow cover effects on the vascular plant community, that in this experiment summer warming seems to be a more likely driver behind possible future changes than increases in snow cover.

The role of Sphagnum fuscum in stabilizing vegetation composition during long-term climate manipulations

The cumulative difference in vertical growth of *S. fuscum* in warmed versus ambient plots was approximately 18 mm over 8 years (Fig. 3). The average difference in annual apical growth rates of *E. hermaphroditum* and *B. nana* between the warmed and the ambient treatments would add up to a cumulative difference over 8 years of respectively 12 mm and 64 mm (Fig. 2). These differences are remarkable, as the ambient average height of the shoot tip above the moss is roughly 20 mm for *E. hermaphroditum* and 60 mm for *B. nana* (with an average branch length of 90 mm, but an inclination of approx. 45°). Within-plot, between-year standard errors of the mean number of point intercept hits for both *E. hermaphroditum* and *B. nana* were low (respectively 2.3% and 8.3%) because the point-intercept frame was always placed in exactly the same position in the plot, indicating a high sensitivity to detect temporal vegetation dynamics. Moreover, these point-intercept standard errors are much smaller than the 60% increase in shoot length, suggesting that differences would most likely be detected (even though the number of point-intercept hits does not translate 1:1 to shoot lengths) if these increased growths had not been masked by a persistent increase in *S. fuscum* growth under warmed conditions.

Direct evidence for a 'race for space' is provided by the altered distribution of *E. hermaphroditum* year-segments above and below the moss, suggesting enhanced encroachment of *E. hermaphroditum* by *S. fuscum* in the summer warmed plots (Fig. 4), in correspondence to the higher growth of *S. fuscum* upon summer warming. Also for *B. nana*, the advancement of *S. fuscum* on the shrubs as a result of summer warming masked the increased apical growth of the shrub (Fig. 2) as a consequence of which no changes in height (i.e. vertical distance of the shoot tips to the moss) were detected (Fig. 4). Hence, the long-term absence of changes in net shrub height in response to the summer warming treatment exemplifies the role of the elevating moss surface: *S. fuscum*-mediated effects are long-term and strong enough to prevent vascular plant growth responses from becoming apparent at the community level.

In other, non-*Sphagnum*-dominated ecosystems, strong responses of *B. nana* abundance to either increased winter snow or summer warming have been ascribed to its high biomass production at a relatively low nitrogen investment, by which it may outcompete other species when subject to increased nitrogen availability (Shaver *et al.*, 2001; Sturm *et al.*, 2005). Reasons for the absence of this response to climatic changes in our *S. fuscum*-dominated system may be twofold: firstly, inorganic nitrogen pool sizes do not differ among our treatments, whereas the microbial-nitrogen pool is significantly higher in the warming (WA) and snow addition (WS+) treatment (J.T. Weedon, unpublished results). Hence, nutrient availability may not increase in response to climatic warming or snow addition because *Sphagnum* itself is a strong competitor for (and immobilizer of) nutrients, or because of a strong microbial sink for nutrients in organic (sub) arctic soils (cf. Jonasson *et al.* 1996; Schmidt *et al.* 1999). Secondly, and crucial to our findings, while greater biomass production by *B. nana* in non-*Sphagnum*-dominated systems often gives it competitive superiority through out-shading neighbouring plants, in a *Sphagnum*-dominated system the shrubs appear to only just keep pace with *Sphagnum* in their race for space.

Although some other studies also found discrepancies between responses at the plant-level and at the community level, these seemed idiosyncratic and have so far lacked a clear mechanistic explanation (Wahren *et al.*, 2005; Hudson *et al.*, 2010). In our system, we have provided mechanistic evidence that the overall unaltered vascular species composition may be a consequence of the

dominant positive growth responses of *S. fuscum* (the 'living soil') to experimental warming. This key finding adds another important new contributor to the list of mechanisms by which mosses, as key ecosystem engineers (sensu Lawton 1994), tightly regulate soil hydrology, biogeochemical cycling and climate (Van Breemen, 1995; Beringer *et al.*, 2001; Gornall *et al.*, 2007; Cornelissen *et al.*, 2007; Gavazov *et al.*, 2010).

Northern Sphagnum peatlands resistant to climate change?

Our key finding, that the vascular plant community composition of a *S. fuscum*-dominated peatland was resistant to the imposed climatic changes, does not imply that these northern peatlands are dormant players in the global carbon cycle under a changing climate. First, the observed growth responses of both the predominant vascular plants and *S. fuscum* itself indicate that the aboveground C pool might have increased in response to warming. Secondly, the unaltered aboveground vascular community composition does apparently not reflect responses of belowground processes, which were shown to be significant and of great relevance in the light of climate change (e.g. total ecosystem respiration rates accelerated by up to 60 % in response to 1 °C warming in our experiment) (Dorrepaal *et al.*, 2009). Finally, the resistance of the vegetation composition in direct response to different climate change scenarios imposed in this study does not mean that climate change does not threaten *S. fuscum*-dominated peatlands. Disturbances invoked by climate change, such as thawing permafrost and subsequently altered hydrology, may severely threaten these systems, as has already been observed in various peatlands in northern Sweden during this decade (Christensen *et al.*, 2004; Johansson *et al.*, 2006; Akerman *et al.*, 2008; Limpens *et al.*, 2008). Moreover, if the structure of the faster growing moss changes (Dorrepaal *et al.*, 2004), this may also affect small-scale local hydrology in the longer term. The local hydrology in this type of ecosystem is a strong determinant of moss survival (Granath *et al.*, 2010), moss species interactions (Sonesson *et al.*, 2002) and of vascular plant composition (Lang *et al.*, 2009). Climate change induced changes in hydrology may thus have a more pronounced effect on the vascular plant community than was observed under the experimentally altered climatic conditions in the current study. However, paleo records show that ombrotrophic bogs in general have been persistent in their presence ever since the Holocene (e.g. Kuhry, 1998; Sannel *et al.*, 2008). Furthermore, we know that our bog has undergone changes in mean annual temperature of 2.5°C over the last 100 years without major shifts in vegetation (Sonesson, 1974; Callaghan *et al.*, 2010). Our study might very well be a step forwards in explaining the resistance of subarctic *S. fuscum*-dominated peat bogs and their vascular plant community to the prevailing climatic changes. In a still broader context, by revealing the mechanisms of peatland species interactions in response to climate change, our findings also contribute to fundamental ecological theory, revealing important new insights into the mechanisms of ecosystem resistance and resilience to global changes. Specifically, we show that resistance to climate change, here in terms of stable community composition, does not necessarily mean vegetation inertia (cf. Grime *et al.*, 2000, Hudson *et al.*, 2010), but that community stability can also be achieved in a dynamic ecosystem, in our case with strong growth responses to warming.

Acknowledgements

We would like to thank M. Berg, M. de Beus, S. Bokhorst, E. Cruz Reno, D. Edo Matas, E. Krab, C. Kanters, M. Makkonen, G. Venhuizen and several students and field assistants for help with the vegetation measurements. We would like to thank the staff of the Abisko Scientific Research Station, especially M. Kardefeldt, L. Ericsson, A. Kristoffersson and A. Eriksson, for snow measurements and other technical assistance. The County Administrative Board at Luleå gave permission to perform the field experiments in the Abisko National Park. We thank J. Akkerman, S.I. Lang, W.G. Keuper and two anonymous reviewers for helpful discussions and/or comments on the manuscript. Funding for this research was provided to R. Aerts by the VUA-USF (grant 98.24), the Darwin Centre for Biogeosciences (grant 142.161.042), the Dutch Polar Program (ALW-NPP grant 851.30.023) and the EU-ATANS (grant FP6 506004); and to F. Keuper by the European Science Foundation (ESF) through the CLIMMANI Research Networking Programme (www.esf.org/climmani) and by the Abisko Scientific Research Station (Scholarship 2008).

II

Chapter III

Tundra in the Rain: Differential vegetation responses to three years of experimentally doubled summer-precipitation in Siberian shrub and Swedish bog tundra

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Ambio 41 (2012), 269-280

Abstract

Precipitation amounts and patterns at high latitude sites have been predicted to change as a result of global climatic changes. We addressed vegetation responses to three years of experimentally increased summer precipitation in two previously unaddressed tundra types: *Betula nana*-dominated shrub tundra (northeast Siberia) and a dry *Sphagnum fuscum*-dominated bog (northern Sweden). Positive responses to approximately doubled ambient precipitation (an increase of 200 mm yr^{-1}) were observed at the Siberian site, for *B. nana* (30% larger length increments), *Salix pulchra* (leaf size and length increments) and *Arctagrostis latifolia* (leaf size and specific leaf area), but none were observed at the Swedish site. Total biomass production did not increase at either of the study sites. This work corroborates studies in other tundra vegetation types and shows that despite regional differences at the plant-level, total tundra plant productivity is, at least at the short or medium-term, largely irresponsive to experimentally increased summer precipitation.

Introduction

Northern tundra ecosystems are important in the current context of a changing climate since they contain a large part of our global terrestrial soil carbon pool (Tarnocai *et al.*, 2009). Despite their low primary productivity, tundra ecosystems act as a carbon sink due to their low decomposition rates. Climatic changes, which are currently increasing decomposition rates of old carbon, threaten to change the tundra from a carbon sink into a source (Dorrepaal *et al.*, 2009). In contrast, enhanced vegetation productivity in response to climatic changes may increase net storage of carbon in these systems in the near-future (Qian *et al.*, 2010). Hence, climatic changes alleviating growth-limiting factors of tundra vegetation have a potential to feed back to the global carbon cycle.

Plant productivity in northern tundra ecosystems is strongly constrained by the adverse physical environment. In addition to low temperature and low nutrient availability (Aerts *et al.*, 2006a, Chapin *et al.*, 1995, Elmendorf *et al.*, 2012, Shaver *et al.*, 2001), water shortage is frequently listed as one of the growth-limiting factors for tundra vegetation (e.g. Bliss *et al.*, 1994, Hodkinson *et al.*, 1999, Kade *et al.*, 2005, Ostendorf & Reynolds, 1998, Press *et al.*, 1998b, Qian *et al.*, 2010). However, the notion that growth of tundra vegetation is water-limited is largely based on observational evidence: the best developed arctic plant communities are often found at sites where snow-melt water or seasonal streambeds keep the soil moist (Bliss *et al.*, 1994, Bliss *et al.*, 1984, Kade *et al.*, 2005). Additionally, dendrochronological research suggests summer precipitation as one of the main climate drivers for shrub (*Betula nana*) growth in northeast-Siberian tundra (Blok *et al.*, 2011). This is supported by modelling approaches, which reveal enhanced precipitation as the major driving factor for increasing growing season leaf area index trends in northeast-Siberian tundra as well as in northern Scandinavia from 1980 to 2000 (Piao *et al.*, 2006).

In contrast with these correlative or modelling studies that postulate water as a limiting factor for arctic plant growth, direct measurements of plant water potential suggest that water is not a growth-limiting factor, at least for Alaskan arctic tundra vegetation (Oberbauer & Miller, 1982). In addition, very few positive plant responses to experimentally increased summer precipitation

in arctic ecosystems have been documented (for an overview of performed experiments see Table 1). However, this experimental evidence is based on a limited number of experiments (seven) only, which were restricted to an even more limited number of geographic areas (Table 1). Moreover, only three of these increased summer precipitation experiments at higher latitudes were performed in tundra sites, as the other studies were executed in subarctic forest ecosystems (Table 1). As such, the tundra types covered in these three studies do not reflect the full diversity of tundra ecosystems (Walker *et al.*, 2005).

Climate change predictions include increasing precipitation at high latitudes for the coming decades (Bengtsson *et al.*, 2011, IPCC, 2007), while long-term trends of both increased precipitation amounts and intensity have already been observed (Callaghan *et al.*, 2010, IPCC, 2007). If low water availability indeed poses a limitation to plant productivity, this may critically affect carbon uptake of northern ecosystems. Thus, there is clearly a need for additional studies on tundra plant water limitation in previously unaddressed tundra types in order to corroborate the conclusions of previous experiments (Table 1). Two tundra types which have not been previously addressed are northeast-Siberian shrub tundra ('dry heath', van Huissteden *et al.*, 2005) and Swedish permafrost-underlain ombrotrophic bog tundra ('dry elevated palsa', Rydén, 1976). Together, these dry tundra types represent a large part of the tundra biome (approximately 30%) (Gorham, 1991, Walker *et al.*, 2005). Moreover, both shrub-tundra and bog tundra are important actors in the global carbon cycle: shrub tundra because of its long-term C-storage in woody biomass, and bog tundra because of their efficient C-storage capacity in slowly decomposing peat. Their responsiveness to increased moisture availability in a changing climate therefore needs to be known.

In this study, we assessed how increased summer precipitation, through modulating water availability, will affect vegetation productivity and species-specific growth responses in dry heath and dry elevated palsa types. Hence, we performed increased summer precipitation experiments in both habitats for three consecutive growing seasons and approximately doubled the ambient summer precipitation. If water indeed inhibits growth of tundra vegetation, we hypothesize vegetation productivity to increase in response to additional summer precipitation. Given the high spatial heterogeneity that is commonly observed in biomass sampling studies (Whittaker & Marks, 1975) (and accordingly low statistical power), total annual vascular production might not represent the most sensitive vegetation variable. We therefore also determined more sensitive species-specific stem growth responses of dominant species. Traits allow sensitive detection of vegetation responses to environmental drivers (e.g. Elmendorf *et al.*, 2012, Olefeldt *et al.*, 2012). Commonly measured traits that are known to be particularly sensitive to drought stress include leaf size and specific leaf area (SLA) of dominant individual species (Cornwell & Ackerly, 2009). Therefore, we hypothesized that leaf size would increase and SLA would decrease if moisture is indeed inhibiting growth in dry elevated palse and dry heath. In addition, because stem growth of sub-arctic shrub species has been shown to respond strongly to a release of inhibiting factors (Bret-Harte *et al.* 2001), we additionally determined more sensitive species-specific stem growth responses of dominant species.

Table 1. Summary of the results of summer water addition experiments on vascular arctic tundra vegetation, arranged by geographical region and vegetation type. Background shading colour indicates similarity between geographical regions. Natural summer precipitation (NP) values are given under 'treatment', unless unavailable (NA), while no data were available for influx from glaciers or thawing permafrost. Effect is indicated as positive (+), negative (-) or no effect (0), with the measured variable between parentheses. To the best of our knowledge, this list of experiments is complete. All studies listed have been reviewed previously by Dormann and Woodin (2002).

	Geographical region	Vegetation type	Treatment	Effect	Studies
1	Swedish Lapland (subarctic)	Birch forest, *Vaccinium vitis-idaea*	4.5 mm each forthnight (≈ 18 mm season^{-1}); NP = 120 mm	"0" (*V. uliginosum*); "+" (*V. vitis-idaea* leaf-weight/shoot-weight; number of leaves per shoot)	1
2	Swedish Lapland (subarctic)	dwarf shrub heath, open birch forest	4.5 mm week-1 (≈ 60 mm season^{-1}); NP = 120 mm	"0" (overall mean biomass; flowering frequency; shoot production)	2-6
3	Swedish Lapland (subarctic)	dwarf shrub heath, open birch forest	9 mm week-1 (≈ 100 mm season^{-1}); NP = 120 mm	"0" (overall stem growth and branching; cover percentage; leaf thickness *V. myrtillus* and *V. vitis-idaea*) "+"(flowering and berry production *V. myrtillus;* stem growth *E. hermaphroditum* in one out of four measured years)	7
4	Finnish Lapland (subarctic)	Birch forest, dwarf shrub heath, *V. vitis-idaea, Empetrum nigrum*	weekly watering (≈ 130 mm season^{-1}); NP = 240 mm	"+" (shoot production *V. vitis-idaea*); "-" (shoot production *Empetrum nigrum*)	8
5	Svalbard (high Arctic)	Polar *Dryas octopetala* semi desert community	45 mm season^{-1}, divided over 6 times, 60% increase compared to NP	"0"(cover; green biomass; all photosynthetic parameters; all reproductive parameters; seedling establishment); "+" (specific leaf area *Dryas octopetala*)	9-12
6	Canadian Arctic (high arctic)	1. wet sedge meadow; 2. mesic *Cassiope* heath; 3. dry-mesic *Dryas* heath	33 mm each ten days (≈ 100 mm season^{-1}); NP≈10 mm	"0" (green and total standing crop of any species, on all sites)	13
7	Alaska (high arctic)	Montane *Dryas octopetala* tundra, fellfield	5 mm 1-2x week^{-1} (≈ 30-50 mm season^{-1}); NP = NA	"0" (mean leaf number per shoot; shoot growth rates; aboveground production)	14

Methods

Study sites

Siberia

The first site (Fig. 1a) is a low-Arctic tundra site within the Kytalyk nature reserve in NE-Siberia, Russia (70°49`N, 147°28`E, elevation 48 m.a.s.l.), previously described by Van Huissteden *et al.* (2005). Mean annual precipitation is 205 mm, most of which falls during the summer months. July is the wettest month with an average precipitation of 32 mm (Tank *et al.*, 2002). Regional climate data (Chokurdakh weather station (WMO station 21946), http://climexp.knmi.nl/, 1948–2006) show mean annual air temperatures of -13.9 °C and average July temperatures of 10.5 °C. In 2007, the growing season was longer than usual, with snow melt occurring three weeks earlier than the other measured years and average temperatures were higher than normal. The following years were more typical for this region, with 2009 being the coldest of the studied three-year period (Parmentier *et al.*). The same holds true for wetness, where 2007 was the wettest, 2008 in between and 2009 the driest (pers. obs. F-JWP). The experiment was set up on an elevated dry palsa, with a maximum active layer thickness of around 25 cm (measured at the end of the growing season, in each plot at three replicate points, by probing a 0.5 cm diameter graduated stainless steel rod into the soil to refusal). The subsoil is silt clay overlain by 10–15 cm of highly organic soil, carpeted with an approximately 4–5 cm thick moss layer (consisting of 35-45% *Aulacomnium turgidum* (Wahlenb.) Schwägr.). The vascular plant community consists of the deciduous shrubs *Betula nana* subsp *exilis* (93% of total aboveground biomass) and *Salix pulchra* (4%), the graminoid *Arctagrostis latifolia* (2%), and the forbs *Saxifraga spp.* (<1%) and *Pyrola rotundifolia* (<1%) and has been classified as dry heath (van Huissteden *et al.*, 2005).

Sweden

The second study site (Fig. 1b) is at the Stordalen nature reserve in northern Sweden (68°21'N, 19°03'E, 351 m.a.s.l.) which has been described in full detail by Sonesson (1980). Annual precipitation in the area is around 300 mm yr^{-1} (1913–2006: Johansson *et al.* (2009)). Summer precipitation at the site in the years of the experiment equalled 287 mm in 2007, 228 mm in 2008,

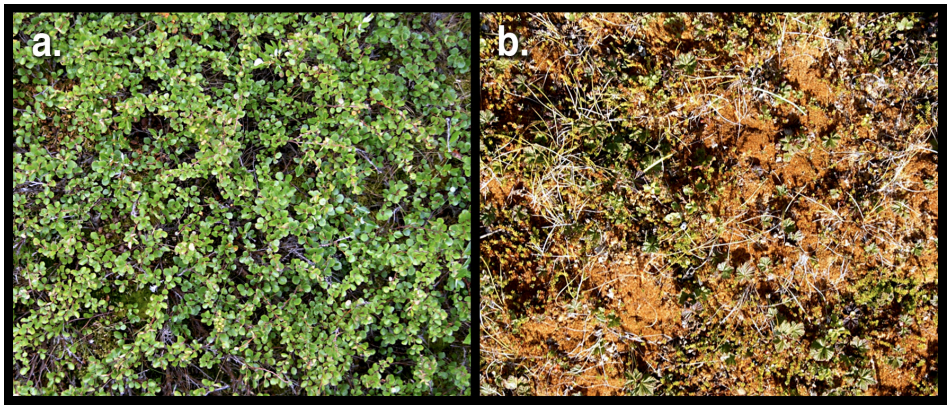

Figure 1. Tundra types addressed in this study, showing dense cover at the *Betula nana* dominated heath (northeast Siberia) site (a); and a low and open but *Sphagnum fuscum* dominated vegetation at the ombrotrophic palsa site (northern Sweden) (b). (Photos: F. Keuper and JL Ramsay)

and 190 mm in 2009; with a mean of 235 mm ± 49 SD (Olefeldt & Roulet, 2012). The mean summer temperature at the site is 7 °C, the mean winter temperature is -6 °C. The experiment was set up on an elevated, dry (Rydén, 1976, Sonesson, 1980), ombrotrophic *Sphagnum fuscum* (Schimp.)-dominated palsa, with an active layer thickness of around 50 cm. The vascular plant community consists of the evergreen dwarf shrubs *Empetrum hermaphroditum* L. (56% of total aboveground biomass) and *Andromeda polifolia* L. (9%), the deciduous dwarf shrubs *Betula nana* L.subsp. *nana* (6%) and *Vaccinium uliginosum* (7%), the forb *Rubus chamaemorus* L. (15%), and the graminoid *Eriophorum vaginatum* L. (8%) and has been classified as dry-growing elevated palsa vegetation by Madsen and Widell (1974).

Treatment and experimental design

At both sites, 16 visually similar plots of 1 m^2 were laid out at the beginning of the growing season in 2007. Following a completely randomized design, the plots were assigned to one of the precipitation treatments (ambient or increased precipitation, n =8). The plots with increased precipitation were evenly watered sixteen times during each growing season with 12.5 litres of water per plot per time. This resulted in a total of 200 mm extra precipitation per growing season; i.e., an approximate doubling of the ambient summer precipitation. Both experiments lasted three growing seasons.

At the Siberian site, water for additional precipitation was taken from nearby clear-water pools and the Berelekh (Yelon) River, a tributary of the Indigirka River. Total inorganic nitrogen was 0.4 ± 0.2 mg l^{-1} (data based on water samples used for the treatment, taken in 2007 and analysed using a SA-40 auto-analyser, Skalar, Breda, The Netherlands).

At the Swedish site, water for additional precipitation was taken from the stream adjacent to the experimental site and had a conductivity of 50.0 ± 11.5 µS cm^{-1}, pH 6.7 ± 0.2, dissolved organic carbon content of (DOC) 10.6 ± 2.6 mg l^{-1}, and total nitrogen of (TN) 0.3 ± 0.2 mg l^{-1} (data based on measurements from two separate spots in the stream from the end of April until the end of September, 2008) (Olefeldt & Roulet, 2012). Given these low nutrient concentrations in neither site, nutrient addition does not seem to have been a confounding factor in our set-up.

Vegetation measurements

Total annual productivity

We chose to present annual vascular production instead of total aboveground biomass, as the latter is bound to be largely unresponsive to perturbations due to dilution of the current season's growth by material produced in previous years (cf. Parsons *et al.*, 1994). After three growing seasons, all aboveground biomass, defined as all plant material emerging above the moss layer, was harvested. The total annual vascular production was subsequently calculated as the sum of: (1) the aboveground green biomass of all graminoids and forbs (*A. latifolia*, *Saxifraga spp.*, *P. rotundifolia* for the Siberian site and *E. vaginatum* and *R. chamaemorus* for the Swedish site); (2) the leaf biomass and length increment biomass of all deciduous shrubs (*B. nana* subsp. *exilis* and *S. pulchra* for the Siberian site and *B. nana* subsp. *nana* and *V. uliginosum* for the Swedish site); and (3) all new growth of the evergreen species, which were only present at the Swedish site. Total biomass of the evergreen's new growth was calculated by multiplying the total aboveground biomass with the ratio of current-year's growth biomass to total shoot biomass. This ratio was determined on samples of 20 randomly picked shoots for *E. hermaphroditum*, whereas the ratio

for *A. polifolia* was estimated to be 1:3 (pers. comm. R. Aerts; data from a site 200 m from the present study site).

Because the most productive species at the Swedish site is the peat moss *Sphagnum fuscum*, productivity of this bryophyte was measured separately. Hereto, length growth of *S. fuscum* was measured in all plots, using a modification (Dorrepaal *et al.*, 2004) of the cranked wire technique (Clymo, 1970), with four wires per plot. Although length growth was measured in the last two years of the experiments, bulk density of the moss was determined (destructively, on the top 1 cm of the moss carpet), in the last year of the experiment (on two samples per plot, dry weight per volume). Therefore, annual production was calculated only for the last year of the experiment (by multiplying bulk density with length growth). However, to check for temporal consistency, length growth was analysed with repeated measures (RM)-ANOVA (within-subject factor year and between-subject factor treatment) for the last two years of the experiment.

Species-specific growth responses

In addition to the integrative measure of total annual production, several more sensitive key variables of species-specific plant performance were measured (leaf size, specific leaf area, length increments, biomass per length increment).

Two traits known to be particularly responsive to drought, leaf size and specific leaf area (SLA), were measured in the last year of the experiment on randomly picked leaves (number of leaves per plot) of the most abundant broad-leaved species (*B. nana* subsp. *exilis* (twenty), *S. pulchra* (five) and *A. latifolia* (five) at the Siberian site, and *B. nana* subsp. *nana* (ten), *V. uliginosum* (ten) and *A. polifolia* (ten) at the Swedish site). Leaf size and SLA were measured and calculated according to the standard protocol described in Cornelissen *et al.* (2003).

Current-year apical stem length increments of the dominant shrub species were determined as measures of growth responses. At the Siberian site, all stem length increments of five randomly selected individual shrubs of *B. nana* subsp. *exilis* per plot were measured of the last two years' growth. The length of all shoots (both short and long shoots) of these individuals was determined in order to obtain a reliable measurement. Apical stem length increments of all *S. pulchra* plants per plot (min. three, max. nine) were measured during the last year of the experiment. Current-year length increments were identified using colour differences of the stem and scars of the terminal bud. At the Swedish site, length increments of ten randomly selected *E. hermaphroditum* shoots per plot were measured during the last two years of the experiment. Current-year growth was identified using colour differences of the stem, bud scars and changes in leaf length between the growth segments of the different years (Keuper *et al.*, 2011). *B. nana* subsp. *nana* length increments at the Swedish site were measured as the distance from a marked point on the stem until the tip of the shoot, both at the beginning (early June) and at the end (early September) of the growing season. Only the first two years were measured because the destructive end harvest in the last year of the experiment interfered with these measurements.

Statistics

All data were tested for normality and homogeneity of residual variances by visual inspection of residual and probability plots. Log-transformation improved the homogeneity of residual variances for the total vascular production data and for the *B. nana* subsp. *nana* length increment data. For all other variables residual variance was approximately normal and homogeneous.

Total vascular production in the last year of the experiment at both sites was analysed simultaneously with a two-factor ANOVA (with treatment and site as independent factors) in order to reduce the chance of Type I error.

Leaf size and SLA were analysed with separate MANOVAs for the two sites, with treatment as independent factor and the leaf traits (leaf size and SLA) as dependent variables with the species identities acting as multivariates.

Differences in length increments and biomass per new stem length of the dominant shrubs were analysed using separate repeated measures (RM)-ANOVAs for species and site when data for two years were available (*B. nana*, *E. hermaphroditum*), with year as a within-subject factor and treatment as a between-subject factor. A one-way ANOVA was used when only one year of measurements was available (stem length increments of *S. pulchra*, number of *B. nana* leaves on the new stem, weight per unit new stem length, and production of the dominant peat moss *S. fuscum* for the Swedish site).

Before statistical analysis, values for each variable were averaged per plot, and all analyses were performed with SPSS 15.0 for Windows. Given the high intrinsic variability of many of the vegetation response measures, the statistical power is generally low and therefore we also discuss results with $P < 0.1$.

Results

Total aboveground biomass production

Total annual aboveground biomass production of the vascular plant communities at the Siberian and the Swedish site was not affected by precipitation addition ($F = 2.0$; $P > 0.1$). Although vascular production was higher at the Siberian site (Fig. 2; $F = 85.7$; $P < 0.001$), there was no interaction ($F = 0.35$; $P > 0.1$) between site and treatment (Fig. 2).

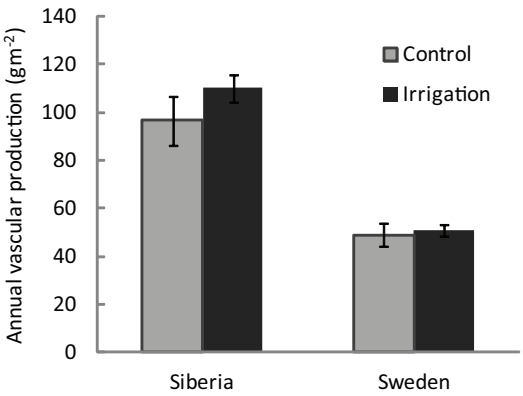

Figure 2. Mean (+/- 1 SE) annual vascular production after three years of water-addition compared to control plots ($n = 8$). There were no significant effects of the treatment (two-way ANOVA with independent factors site and treatment ($P > 0.1$).

Species-specific responses to the precipitation treatment

Leaf traits

At the Siberian study site, leaf size of *B. nana* subsp. *exilis* was not affected by the treatment ($F = 0.068$; $P > 0.1$), but leaf size of both *S. pulchra* ($F = 5.4$; $P < 0.05$) and *A. latifolia* ($F = 11.1$; $P < 0.01$) were 25% and 113% larger in the plots with additional precipitation, respectively (Fig. 3a, Table 2). In contrast to leaf size, specific leaf area (SLA in $cm^2\ g^{-1}$) of *B. nana* subsp. *exilis* was only slightly larger in the irrigated plots ($F = 3.75$; $P < 0.1$). SLA of *A. latifolia* was even approximately 25% larger in the irrigated plots ($F = 12.9$; $P < 0.001$). In contrast, SLA of *S. pulchra* was not affected by treatment ($F = 3.04$; $P > 0.1$) (Fig. 3c). At the Swedish site, neither leaf size nor SLA of any of the examined species (*B. nana* subsp. *nana*, *V. uliginosum*, *A. polifolia*) were affected by treatment ($P > 0.05$ for all species) (Fig. 3b and 3d).

Stem traits

At the Siberian study site, length increments of the dominant shrub *B. nana* subsp. *exilis* were about 30% larger in the irrigated plots both in the penultimate and in the last year of the experiment ($F = 17.1$; $P < 0.01$). There was a marginally significant difference in growth among the years ($F = 3.6$; $P < 0.1$), while there was no interaction between treatment and year ($P >$

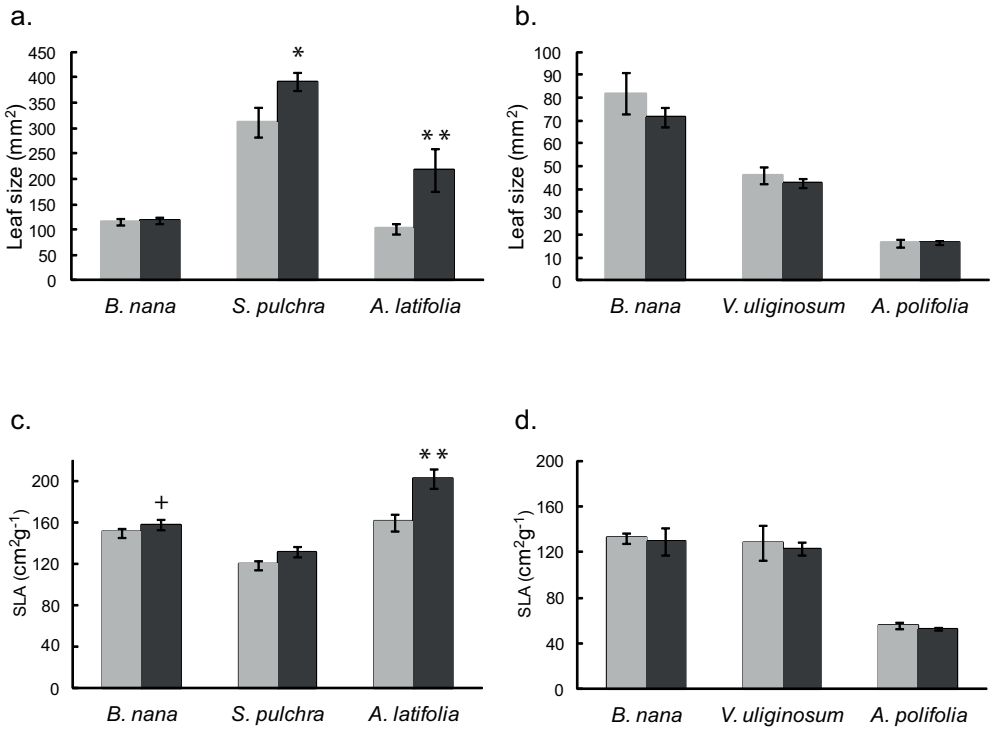

Figure 3. Mean (+/- 1 SE) leaf size and SLA at the Siberian (a, c) and the Swedish (b, d) study site in the third year of the experiment in water addition (black bars) compared to control (grey bars) plots ($n = 8$). Significant differences between water addition and controls per species are indicated with + ($P < 0.1$); * ($P < 0.05$); and ** ($P < 0.01$) (see main text for details on statistical analysis).

Table 2. Summary of species-specific responses to summer water addition in the two sites addressed in this study (see main text for details). Background shading colour indicates similarity between geographical regions including those presented in Table 1. Response is indicated as positive (+), negative (-) or no effect (0), with the species and measured variable between parentheses. See main text for effect sizes and *F*-values.

Geographical region	Vegetation type	Species-specific responses
Northeast Siberia (arctic)	*Betula nana*-dominated dwarf shrub heath	"**+**" *B. nana* SLA and length increments; *S. pulchra* leaf size and length increment; *A. latifolia* leaf size and SLA); "**0**" (*B. nana* leaf size and biomass per length growth; *S. pulchra* SLA and number of leaves per cm stem; biomass per length growth); "**-**" (number of *B. nana* leaves per stem)
Swedish Lapland (subarctic)	*Sphagnum fuscum*-dominated ombrotrophic peatland	"**0**" (*B. nana, V. uliginosum, A. polifolia* leaf size and SLA; *E. hermaphroditum, B. nana, S. fuscum* length increments; *E. hermaphroditum, S. fuscum* biomass per length growth)

0.1) (Fig. 4a). These elongated *B. nana* subsp. *exilis* stems produced about one leaf per cm stem length less (-25%, last experimental year data only) ($F = 13.7$; $P < 0.01$). The treatment did not affect biomass per unit length of the new stem growth ($F = 2.4$; $P > 0.1$). Length increment of the subdominant shrub *S. pulchra* at the Siberian site was also 32 % larger in the last year of the experiment (18.1 cm ± 3.9 SD for the irrigated plots versus 13.7 cm ± 3.4 SD for the control plots, $F = 4.8$; $P < 0.05$), but the number of leaves per new stem length remained unchanged (3 per cm stem, $F = 2.4$; $P > 0.1$) and biomass of the new stem growth was not affected either ($F = 0.73$; $P > 0.1$).

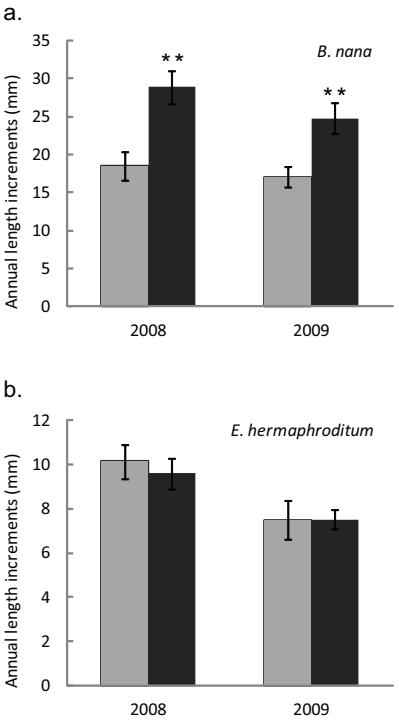

Figure 4. Mean (+/- 1 SE) apical annual growth of the dominant dwarf shrubs in both study sites in response to summer water addition (black bars) or control treatment (grey bars) in the last two years of the experiment: (a) *Betula nana* subsp. *exilis* at the Siberian site and (b) *Empetrum hermaphroditum* at the Swedish site ($n = 8$). Significant differences between water addition and controls per species are indicated with + ($P < 0.1$); * ($P < 0.05$); and ** ($P < 0.01$) (see main text for details on statistical analysis).

At the Swedish study site, the length increments of the dominant shrub *E. hermaphroditum* were not affected by additional precipitation ($F = 0.059$; $P > 0.1$) and there was no effect of year, nor an interaction between year and treatment ($P > 0.1$) (Fig. 4b). Biomass per unit length of the new growth was neither affected by treatment (1.49 ± 0.24 SD mg mm^{-1} yr^{-1} for control and 1.36 ± 0.26 SD mg mm^{-1} yr^{-1} for plots with additional precipitation; $F = 0.89$; $P > 0.1$) and no effect of year nor an interaction between year and treatment was observed ($P > 0.1$). Similarly with the other Swedish results but in contrast to the Siberian results for this species, *B. nana* subsp. *nana* length increments were not affected by additional precipitation at the Swedish site (5.9 ± 5.1 SD mm^{-1} yr^{-1} for control and 3.6 ± 2.7 SD mm^{-1} yr^{-1} for plots with additional precipitation; $F = 0.93$; $P > 0.1$), nor was there an effect of year ($F = 0.66$; $P > 0.1$) or an interaction between year and treatment ($F = 0.77$; $P > 0.1$). Length growth of the dominant bryophyte *Sphagnum fuscum* was not different from the control in the plots with additional precipitation during the last two years of the experiment (4.10 ± 2.2 SD mm yr^{-1} in the control plots and 4.73 ± 1.9 SD mm yr^{-1} in the treatment plots: $F = 0.57$; $P > 0.1$). *Sphagnum* length growth was significantly different in the two measured years ($F = 20.8$; $P = 0.00$), but there was no significant interaction between treatment and year ($F = 0.11$; $P > 0.1$). Biomass production of the peat moss was not affected by the precipitation treatment (288 ± 170 SD g m^{-2} in the control plots and 313 ± 141 SD g m^{-2} in the plots with additional precipitation: $F = 0.10$; $P > 0.1$).

Discussion

Water limited plant-growth in tundra? No response in total annual productivity

Three years of experimentally increased summer precipitation did not affect total aboveground vascular biomass production at either of the studied tundra sites. Total annual production is the integrative measure of all species-specific growth responses. Hence, this finding shows that overall, primary production of Siberian *B. nana* heath and Swedish ombrotrophic bog tundra vegetation is at the short to medium-term not limited by water availability, even though some specific-species growth responses to the treatment were observed. Our results extend the established pattern of negligible tundra plant productivity responses to experimentally increased summer precipitation (reviewed by Dormann and Woodin (2002)) with two previously unaddressed tundra types.

Although the investigated systems generally receive little natural precipitation (between 150 and 300 mm on average (Remer, 2009)), there are several potential explanations for their resistance in plant productivity to changes in summer precipitation. Firstly, plant productivity may be more limited by other factors such as nutrient availability and/or temperature to such extent that positive responses to alleviation of water limitation are hampered or masked. This explanation is supported by the study of Parsons *et al.* (1995) (Table 1), who only found a significant effect of watering on graminoid biomass if watering was applied in combinations with either increased temperature or fertilizer. Secondly, although summer precipitation in tundra may be low, total evapotranspiration is also low, ensuring that in general tundra soils remain relatively wet throughout the summer (polar deserts excluded). Moreover, soils are driest in late summer (Rydén, 1976), while most growth takes place in early summer (Karlsson, 1996, Sonesson, 1980). At the beginning of the growing season, moisture may still be available from snowmelt, while drainage is limited due to the shallowness of the active layer (McGraw, 1985). Lastly, total productivity responses to water addition might be hard to detect due to spatial heterogeneity in initial biomass and a relatively short duration of the experiment (i.e. three years).

Species-specific responses: signs of drought stress-release and growth responses

While the overall productivity was unaffected, species-specific growth responses to increased summer precipitation differed remarkably between species and sites (Table 2). The increased leaf size in response to irrigation observed for the deciduous shrub *Salix pulchra* and the grass *Arctagrostis latifolia* at the Siberian study site suggests that growth of these species is water-limited, in line with our hypotheses. Specific leaf area of *S. pulchra* remained constant, but for *A. latifolia* the increased leaf size co-occurred with thinner leaves, again in line with our hypotheses. Leaves of the dominant shrub *B. nana* subsp. *exilis* (93% of aboveground biomass) also became slightly thinner ($P < 0.1$) (Fig. 3b) while its leaf size remained unchanged in response to irrigation (Fig. 3a). While nutrient deficiency and drought stress are known to have very similar effects on vegetative plant characteristics (Small, 1973), the very low nutrient concentrations in our irrigation water decrease possible interferences by increased nutrient availability as confounding factor. Together, the species-specific vegetation responses to the water addition suggest that growth of the vegetation is water-limited at the Siberian dry heath. Alleviation of this water-limitation allowed length increments of *B. nana* subsp. *exilis* to increase with around 30%, while length increments of *S. pulchra* increased as well (Fig. 4a). The fact that these growth responses were not sufficient to increase the overall productivity at the Siberian site might be explained by the relatively small contribution of *B. nana* subsp. *exilis* length increments (4%) to total annual productivity, compared to the contribution of total *B. nana* subsp. *exilis* leaf biomass (84%, results not shown), which did not differ among treatments (results not shown). However, note should be taken that we did not take growth (thickening of the stems) into account. Such secondary growth can amount up to an annual production of approximately 15% of total standing biomass (Bret-Harte *et al.*, 2002) and has previously been reported responsive to natural summer precipitation patterns (Blok *et al.*, 2011). Given the relatively large contribution of the 'old' woody biomass to the total standing biomass at the Siberian site (84%, results not shown) secondary growth might have contributed considerably to annual total productivity in the plots with additional precipitation.

In contrast, at the Swedish site, the absence of any responses in leaf size and specific leaf area and the absence of increased length increments of all measured species, including *Sphagnum fuscum*, suggests that in this habitat, drought stress was not a condition that limited the plants during the period of leaf and stem development (Fig. 3b, 3d and 4b). *Empetrum* (56% of aboveground biomass at the Swedish site) is assumed to be tolerant to variations in soil water content (Bell & Tallis, 1973) and has previously been found unresponsive to experimentally increased summer precipitation (Shevtsova *et al.*, 1997)(Table 1). Although *S. fuscum* is sensitive to water availability and has previously been shown to respond to small increases in summer precipitation (up to 20 mm yr^{-1}) (Sonesson *et al.*, 2002), larger increases, such as provided in this study, may also negatively affect productivity through increased leaching of nutrients (Sonesson *et al.*, 2002). This would explain the absence of net effects of increased summer precipitation.

The difference in *B. nana* responsiveness among the sites may be due to the occurrence of different 'ecotypes' of *B. nana* at the two sites: different responses to experimental manipulation in different geographical areas (Alaska, Northern Sweden) have previously been observed for this species (Van Wijk *et al.*, 2004). Previous observations of plant responses to water drainage patterns in Alaskan tundra (Matthes-Sears *et al.*, 1988) showed that both *B. nana* as well as *S. pulchra* had higher aboveground biomass in water-tracks. Growth of *B. nana* at the Siberian site might also have been more drought-stressed ambiently, and might have contributed to drying of

the soil because it comprised 93% of the total biomass and thus had a relatively large transpiring leaf area. Finally, at the Siberian site, *B. nana* subsp. *exilis* supposedly had superficial roots only due to the shallow active layer thickness (max. 25 cm compared to 50 cm at the Swedish site) and could thus only exploit a much smaller reservoir of soil water.

Long-term implications for species composition

Despite species-specific responses to the treatment at the Siberian study site, no changes in species composition indices were found on either site (results not shown). Hence, the current study shows that even doubling of yearly summer precipitation does not induce a shift in species composition during the first three years. For the Swedish site, impacts on vegetation composition through species interactions are not expected due to the low total species cover in the plots and the complete absence of responses. However, at the Siberian site, the increased leaf size of *S. pulchra* and *A. latifolia* suggests that these species are drought stressed and may, if the tundra gets wetter, on the long-term benefit from wetter conditions. Enhanced responses in tundra ecosystems in terms of species composition and total productivity to additional precipitation seem even more likely in a future climate when changes in temperature and nutrient availability are predicted to co-occur with increased summer precipitation (Parsons *et al.*, 1994) and when permafrost degradation drastically affects local hydrology, the first signs of which are already apparent (Wright *et al.*, 2005).

Conclusions

The plant level responses for the Swedish site indicate that increased summer precipitation alone will not have major effects on ombrotrophic peatland vegetation productivity. For the Siberian *B. nana* dominated dry heath site on the other hand, the fact that several individual plant measurements show a positive response to the precipitation treatment, suggests that in the long-term biomass production may increase in response to increased summer precipitation. However, over the course of this three-year experiment, primary production did not increase in response to the treatment at either of the study sites. Hence, despite regional differences at the plant-level, our study shows that total tundra plant productivity shows at the short-term no response to experimentally increased summer precipitation, corroborating previous studies on tundra plant productivity responses to summer precipitation. This contrasts with the results of nutrient addition experiments and suggests that water will in most tundra ecosystems not be the primary growth-limiting factor but a co-limiting growth factor. This work extends the available knowledge with two previously unaddressed tundra types.

Acknowledgements

We are grateful to the staff of the BioGeoChemical Cycles of Permafrost Ecosystems Lab in Yakutsk for logistic support and to the staff of the Kytalyk State Resource Reservation for their permission and hospitality to conduct research in the Kytalyk reserve. Special thanks to Sergei Karsanaev, Roman Sofronov, Elena Ivanova, Lena Paryadina, Stanislav Ksenofontov and Trofim Maximov. We also thank the staff of the Abisko Research station for technical assistance and Milo Keuper for practical support. The helpful comments of two anonymous reviewers greatly improved the manuscript. Financial support was offered to FK by the Darwin Centre for Biogeosciences (grant 142.161.042) and ANS Scholarship 2008, and to RA by the Dutch Polar Program (ALW-NPP grant 851.30.023) and the EU-ATANS (grant FP6 506004).

Chapter IV

A Frozen Feast:
Thawing permafrost increases plant-available nitrogen in subarctic peatlands

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Global Change Biology 18 (2012) 1998-2007

Abstract

Many of the world's northern peatlands are underlain by rapidly thawing permafrost. Because plant production in these peatlands is often nitrogen (N)-limited, a release of N stored in permafrost may stimulate net primary production or change species composition if it is plant-available. In this study, we aimed to quantify plant-available N in thawing permafrost soils of subarctic peatlands. We compared plant-available N-pools and -fluxes in near-surface permafrost (0-10 cm below the thawfront) to those taken from a current rooting zone layer (5-15 cm depth) across five representative peatlands in subarctic Sweden. A range of complementary methods was used: extractions of inorganic and organic N, inorganic and organic N-release measurements at 0.5 and 11 °C (over 120 days, relevant to different thaw-development scenarios) and a bioassay with *Poa alpina* test plants. All extraction methods, across all peatlands, consistently showed up to seven times more plant-available N in near-surface permafrost soil compared to the current rooting zone layer. These results were supported by the bioassay experiment, with an eight-fold larger plant N-uptake from permafrost soil than from other N-sources such as current rooting zone soil or fresh litter substrates. Moreover, net mineralisation rates were much higher in permafrost soils compared to soils from the current rooting zone layer (273 mg N m^{-2} and 1348 mg N m^{-2} per growing season for near-surface permafrost at 0.5 °C and 11 °C respectively, compared to -30 mg N m^{-2} for current rooting zone soil at 11 °C). Hence, our results demonstrate that near-surface permafrost soil of subarctic peatlands can release a biologically relevant amount of plant available nitrogen, both directly upon thawing as well as over the course of a growing season through continued microbial mineralisation of organically bound N. Given the nitrogen-limited nature of northern peatlands, this release may have impacts on both plant productivity and species composition.

Introduction

Many of the world's northern peatlands, which contain one-third of the global soil organic carbon pool (Gorham 1991), are underlain by permafrost (Tarnocai *et al.* 2009). Recently, it was shown that thawing of permafrost soils occurs over large geographical areas due to climatic warming (ACIA 2004, IPCC 2007). Due to large stocks of carbon and nutrients in these frozen soils this has a potential to feed back to global biogeochemical cycles (Tarnocai *et al.* 2009, Kuhry *et al.* 2010).

Low soil nutrient availability limits plant growth at high-latitudes and in peatlands in particular (Aerts *et al.* 1992, Berendse & Jonasson 1992, Chapin *et al.* 1995, Hobbie *et al.* 2002, Van Wijk *et al.* 2004). Although peatland soils contain large stocks of nitrogen (Limpens *et al.* 2006), these stocks are largely tied up in unavailable, organic N forms (Rydin & Jeglum 2006), immobilized in microbial biomass (Jonasson *et al.* 1996) or in permafrost soils, and are therefore mostly not available to plants.

Thawing of permafrost soils, however, might cause a release of plant-available N into N-limited high-latitude peatlands. Although little is known about plant-available N concentrations in permafrost soils, increased uptake of nitrogen by plants in Alaskan thermokarst areas (Schuur *et al.* 2007), and increased organic nitrogen concentrations in Arctic rivers as a result of permafrost

degradation (Frey & McClelland 2009) suggest that plant-available nitrogen release from thawing permafrost is occurring in some areas. Also, N measurements in Yedoma soils of Northeast Siberia suggest a biologically relevant N release upon thaw (Mack *et al.* 2010). Despite this circumstantial evidence, little is known about the actual quantity and particularly about the quality of N stored in permafrost soils underlying peatlands.

Two historic processes may account for the high amount of potentially plant-available N in permafrost soil and cause two different mechanisms and time-spans through which plant-available N may be released from thawing near-surface permafrost soil. Firstly, the amount of dissolved, but frozen, plant-available N in near-surface permafrost (just below the thaw-front) may be high due to leaching of dissolved N from the active layer into the perennially frozen ground at the end of summer over the course of decennia (Mackay 1983, Allard & Rousseau 1999, Kokelj & Burn 2003) and because of a spatio-temporal separation of microbial N mineralisation and plant N-uptake (cf. Hobbie & Chapin 1996). This N will be readily available to plants immediately upon thaw and will most likely be used up rapidly by plants and/or microorganisms. A second release of plant-available N may come from organically bound yet potentially mineralisable N, accumulated as a result of historical conditions unfavourable to decomposition (cf. Weintraub & Schimel 2003). Mineralisation of this N may be activated in defrosted soils, creating a longer-term, sustained source of plant-available N.

The aim of this paper is therefore to provide insight both into the amount of readily plant-available N in thawing permafrost of peatlands in subarctic Sweden and into the longer-term release of plant-available N from permafrost soil through N-mineralisation. We hypothesized that near-surface permafrost soil of subarctic peatlands can release a biologically relevant amount of (1) readily plant-available N directly upon thawing (i.e. detectable with traditional extraction methods and as measurable plant-uptake) and of (2) plant-available N through mineralisation after thawing over the course of a growing season. Because this is the first study providing data on plant-available N release from subarctic peatland permafrost soils, we sampled a range of representative peatlands to estimate spatial variability. Given that methods for measuring plant-available N are debated (e.g. Van Duren & Pegtel 2000), we used two complementary methods to obtain a robust measure of directly biologically available N and test our hypothesis (1): extractions of inorganic and organic N and a bioassay with *Poa alpina* test-plants to measure net plant N-uptake (Quested *et al.* 2003, Dorrepaal *et al.* 2007). To assess the relative significance of this N-release from thawing permafrost soil, we compared it with the release from two other important N sources: current rooting zone soil and leaf litter.

To test our hypothesis (2), we performed a mineralisation incubation experiment at two temperatures. Because thawing of permafrost peatlands can progress as a mere gradual thickening of the active layer (ACIA 2004) or as thermokarst development and subsequent peatland collapse (Beilman & Robinson 2003, ACIA 2004, Luoto *et al.* 2004), the temperature aspect of two thaw-development scenarios was mimicked in order to compare its potential effects on the mineralisation of N from thawing of permafrost soil. Although multiple factors limiting N-mineralisation such as hydrology, biochemistry, oxygen availability and soil temperature (Robinson 2002, ACIA 2004, Schuur *et al.* 2008) may be affected differentially by such diverging thaw scenarios, we limit our research to the impact of temperature, an important determinant of microbial nitrogen mineralisation rates (Robinson 2002, Jones *et al.* 2009, Wallenstein *et al.* 2009). The incubation temperatures were 0.5 °C (deepening active layer scenario) and 11 °C (degrading palsa scenario) for near-surface permafrost and 11 °C for current rooting zone soil,

based on measurements of temperatures just above the thaw front during core extraction (0.5 °C) and on average ambient field summer rooting-zone soil temperatures (11 °C). Furthermore, because microbial immobilization of inorganic N can be a strong N-sink in organic soils, we also measured microbial biomass N.

Methods

Sampling area

To characterize spatial variability in N availability, soil samples were taken from five ombrotrophic permafrost peatlands (Table 1) in the Torneträsk region in the northernmost part of Sweden, which lies within the zone of sporadic permafrost (Brown *et al.* 1998). Mean annual precipitation in this area was around 350 mm in the most recent decade and mean annual air temperature around -0.6 °C (meteorological data 1999-2008, Abisko Scientific Research Station), but there is evidence that since 2000 the long-term trend of mean annual temperature has significantly exceeded the 0 °C threshold (Callaghan *et al.* 2010). The temperature of the permafrost in the study area is only a few degrees below zero (Akerman & Johansson 2008, Johansson *et al.* 2011), and mean summer soil temperature in the rooting zone (at 5-15 cm) of these peatlands is around 11 °C (unpublished data Dept. of Systems Ecology, VU University Amsterdam). In all five peatlands the vascular vegetation was low and open and dominated by the peatmoss *Sphagnum fuscum* (Schimp.) H. Klingg. The exact sampling locations were chosen for the presence of a living *S. fuscum* carpet and the vascular plant species *Empetrum hermaphroditum* Hagerup, *Andromeda polifolia* (evergreen dwarfshrubs), *Rubus chamaemorus* (forb), *Betula nana* and *Vaccinium uliginosum* (deciduous dwarf shrubs).

Soil sampling

Soil samples were taken in the second week of September 2008, within one week of the time of maximum seasonal thaw depth. A rectangular (5x6 cm) hardened stainless steel hand corer with a length of 1 m was driven through the active layer and forced into the frozen soil. Three to seven cores were extracted for each of the five peatlands (Table 1). Near-surface permafrost samples were cut 0-10 cm below the thawfront. For comparison, an equal volume of current rooting zone soil was taken from each soil core at 5-15 cm below the soil surface, which is where the bulk of the plant roots are concentrated (Rydin and Jeglum, 2006). Therefore, this is the most appropriate part of the active layer for plant N-uptake with which to compare the N availability for plants from near-permafrost. Therefore, this is the most relevant part of the active layer for plant N-uptake and thus to compare the N availability for plants from near-permafrost with.

Table 1. The peatland names, mean active layer thickness (ALT) and number of cores taken per peatland. The depth of the peat is similar (about 90 cm) at three of the five peatlands (Storflaket, Kursflaket and Abisko Naturreservat) (Akerman & Johansson 2008) and is at least > 70 cm at Stordalen and Torneträsk.

Peatland name	*Coordinates*		*Mean ALT*	*n° of cores taken*
A. Stordalen	68°21.428'N	19°03.181'E	50 cm	7
B. Torneträsk	68°13.423'N	19°44.621'E	56 cm	4
C. Storflaket	68°20.836'N	18°58.398'E	54 cm	5
D. Abisko	68°21.533'N	18°48.594'E	50 cm	5
E. Kursflaket	68°21.011'N	18°52.324'E	52 cm	3

The position of the thawfront was determined separately at three replicate points around each sampling location by probing a 0.5 cm diameter graduated stainless steel rod into the soil to refusal, because active layer thickness (ALT) as determined by probing was generally larger than when visually estimated from the soil cores. Mean ALT per peatland is presented in Table 1. The soil samples were transported unthawed to the laboratory and stored at -18 °C. Subsamples of each sample were analysed for moisture content, bulk density (dry mass per volume; Table SI 1, Supporting Information) and elemental N (by dry combustion of ground samples with a Flash EA1112 elemental analyser, Thermo Scientific, Rodana, Italy) for which the subsamples were oven dried at 60 °C for 48 hrs. Prior to all analyses, roots (>0.5 mm) were removed from the replicate soil samples and each replicate was homogenized. This was done in a cool room (11 °C) in order to minimize moisture loss and microbial mineralisation.

Nitrogen pool measurements

Nitrogen availability to plants is generally measured and expressed as extractable inorganic nitrogen (NO_3- and NH_4+). However, low molecular mass forms of organic nitrogen (e.g. amino acids) can also be considered plant-available, especially in (sub)arctic systems where ectomycorrhizal organic N uptake is abundant (McKane *et al.* 2002, Nasholm *et al.* 2009). Therefore, a range of complementary measures was used to compare the release of plant-available N from near-surface permafrost soils of subarctic peatlands to that from current rooting zone soil.

Nitrogen extractions

Both plant-available extractable inorganic as well as organic nitrogen content were determined by means of chemical extractions. Extractable inorganic N was determined after shaking 5 gram fresh soil for two hours in 25.0 ml 1 M KCl. Extractable organic N was calculated by subtraction of inorganic N from total extractable N. For these calculations inorganic N was also measured in a 0.5 M K_2SO_4-extract (5 gram fresh soil per 25 ml), and total extractable N of the same sample was determined after potassium persulfate digestion (Cabrera & Beare 1993). Ammonium and nitrate (inorganic N) concentrations in all extracts were measured using a SA-40 auto-analyser (Skalar, Breda, The Netherlands). Values were multiplied with their bulk density (Table SI 1) and expressed as mg N per m^2 for the layer investigated (10 cm soil depth).

Bioassay

In addition to the extractions and in order to assess the plant availability (i.e. uptake) of N in the presence of other nutrients as well as phytotoxic substances (e.g. polyphenols), we measured plant-available N by means of a bioassay (Clements & Goldsmith 1924).

We used the perennial grass *Poa alpina* (Mossberg *et al.* 1992) as a test species (henceforth 'phytometer') (Quested *et al.* 2003, Dorrepaal *et al.* 2007). The phytometers were picked directly from viviparous *Poa alpina* parent plants, which were collected in Abisko, northern Sweden (68°21'N, 18°49'E) in August 2008. A positive control experiment with increasing levels of N-addition confirmed the strong nutrient-limitation of *Poa alpina* phytometer growth (Fig. SI 1, Supporting Information). Upon planting, all phytometers received approximately 10 ml of a liquid mixture of peat and fine roots collected at the *Poa alpina*-parent community to provide an inoculum of natural soil organisms.

The phytometers (one per pot, *n* =13 per treatment) were grown for a period of 74 days and plant N-uptake from permafrost soil, current rooting zone soil, and a representative mix of fresh peatland litter were compared. The latter two treatments were added to test whether permafrost soil is a biologically relevant source of nitrogen. Therefore, phytometers were grown on a mixture of sand with either fresh, thawed soil (either permafrost or current rooting zone) or with a leaf litter substrate, equivalent to 3 g dry weight of each substrate per pot (0.6 dm^3). The litter treatment consisted of a mixture of the two common subarctic peatland species *Rubus chamaemorus* and *Betula nana* (resp. 2.4 g and 0.6 g). An additional control treatment consisted of sand only. The soil and litter samples for the bioassay were collected from one representative peatland (Peatland A, Fig. 1; Table SI 1, Supporting Information) in September and processed as described above.

The bioassay experiment was performed in a greenhouse at 14 °C and a random block design of pot positions was applied and updated bi-weekly. The phytometers were watered two times per week with de-ionized water. Upon harvest, the plants and roots (Fig. SI 2, Supporting Information) were cleaned, oven dried (60 °C, 48 h) and weighed. The total amount of elemental N per phytometer was determined in ground material by dry combustion with a Flash EA1112 elemental analyser (Thermo Scientific, Rodana, Italy), after which plant N-uptake was calculated by multiplying the plant N concentration with the total dry weight of the phytometer and expressed as N-uptake per gram substrate (permafrost or current rooting zone soil or litter) (μgN/gDW).

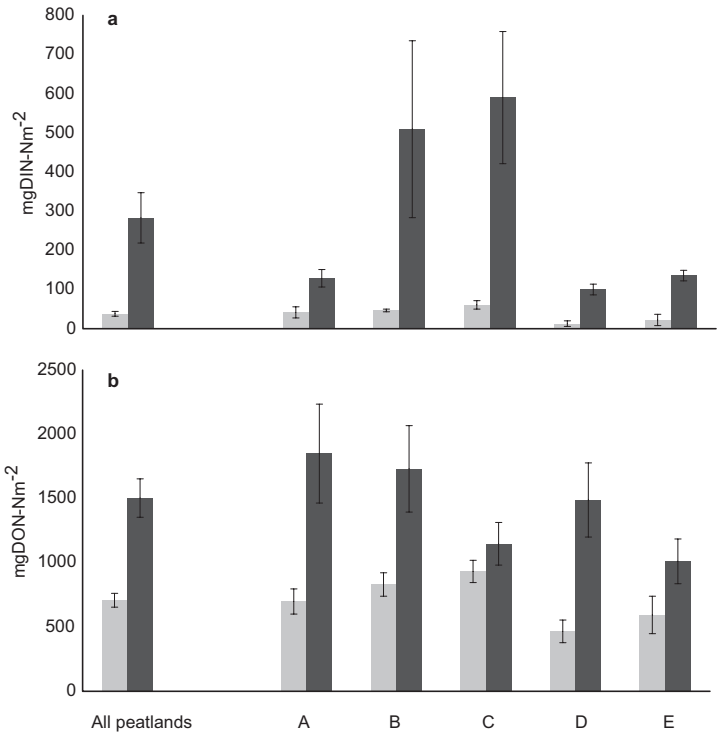

Figure 1. Extractable inorganic N (a) and extractable organic N (b) in current rooting zone soil (grey bars) and in near-surface permafrost (black bars) soil of five subarctic peatlands (Table 1) at the end of the growing season. Data are means ± SE, expressed per cm soil depth, with 3-7 replicates per peatland.

Mineralisation incubations: simulation of two permafrost thaw scenarios

To assess the longer-term plant-available N release from near-surface permafrost soil, two temperature scenarios relevant to different thaw-development in permafrost soils were mimicked by mineralisation incubations. In the first scenario we recreated a situation in which the defrosted near-surface permafrost soil temperature increases only to little above 0 ºC due to the continuing insulation provided by an overlying intact peat layer: the 'Deepening active layer' scenario (I). The second scenario sought to simulate the temperature in a situation where the peatland is subject to severe degradation and collapse and formerly frozen soil is exposed to current rooting zone soil temperatures: the 'Degrading palsa' scenario (II). Scenario I incubation temperatures were 0.5 °C for near-surface permafrost and 11 °C for current rooting zone soil, based on average ambient field summer rooting-zone soil temperatures and measurements of temperature at the thaw front during core extraction (equalling 0.35 °C +/- 0.2 °C). Under Scenario II, soil from both depths was incubated at average ambient field summer rooting-zone soil temperature (11 °C). The samples were incubated for 120 days for both scenarios, similar to the duration of the growing season in this area (Karlsson & Callaghan 1996).

At both temperatures, 15 gram fresh soil per sample was incubated in 50 ml pots and the positions of the bottles within the dark incubation chambers were randomized. Each pot was covered by parafilm with three small openings to minimize moisture loss but allow for gas exchange. Moisture loss was measured weekly by weighing the pots and when necessary returned to the initial weight with de-ionised water. Measurement of inorganic and organic N was performed as described above, at the beginning and the end of the experiment. In addition, the microbial biomass N pool was determined, for which 5 g subsample was chloroform-fumigated for 24 hours at ~20 °C in a darkened desiccation jar, and then extracted after two hours shaking in 25.0 ml 0.5 M K_2SO_4. Similarly, a second non-fumigated sample was extracted for determination of total extractable non-microbial N. Both samples were then oxidized by potassium persulfate digestion for determination of total extractable N (Cabrera & Beare 1993). Microbial biomass pool sizes were calculated as the differences between fumigated and non-fumigated extracts, using correction factor KE*N* = 0.40, representative for organic soils, to account for microbial tissue N that is not released by exposure to chloroform (Jonasson *et al.* 1996).

Calculation of mineralisation, immobilization and microbial biomass N dynamics

Net mineralisation and immobilization as well as net change in extractable organic N and microbial biomass N was calculated as the differences between the values obtained at the start and the end of the experiment.

Statistical analysis

All data were tested for normality and homogeneity of residual variances by visual inspection of residual and probability plots. Log-transformation improved the homogeneity of residual variances for $N\text{-}NO_3$, $N\text{-}NH_4$, inorganic N (NO_3 + NH_4), organic N and total N values and of the bioassay data. For all other variables residual variance was approximately normal and homogeneous.

The effects of the sampling depth (current rooting zone or near-surface permafrost) and sampling site (peatland) on all soil variables were analysed using repeated measures (RM-) ANOVAs, with

'depth' within a core as the within-subject factor and 'peatland' as the between-subject factor. The effects of the five different peatlands were separated by a Tukey's HSD post-hoc test. In a similar RM-design with 'peatland' as the between-subject factor, mineralisation rates were analysed with rates of current rooting zone soil at 11 °C vs. rates of near-surface permafrost soil at 0.5 °C as within-subject factor for Scenario I. For Scenario II, rates of current rooting zone soil at 11 °C vs. rates of near-surface permafrost soil incubated at 11 °C were applied as within-subject factor. The bioassay data were analysed with a one-way ANOVA between the four levels of substrate treatment and Tukey's HSD post-hoc test to identify differences in treatment means. All analyses were performed with SPSS 15.0 for Windows.

Results

Higher values of plant-available nitrogen were found in near-surface permafrost soils compared to soil taken from the current rooting zone (5-15 cm) independent of the measurement method used.

The amount of readily plant-available inorganic N was seven times larger in permafrost soil than in an equal volume of current rooting zone soil (Fig 1a). The inorganic N fraction constituted around 0.09% of the total N pool for both current rooting zone soil and the permafrost soil samples, due to a larger total N pool in near-surface permafrost soil (Table 2). In both soil layers only a small fraction of inorganic N was NO_3-N; the main part was NH_4-N (79% in current rooting zone soil and 89% in permafrost soil) (Table 2). The amount of readily plant-available organic N was about two times larger in the permafrost soil than in the current rooting zone soil layer (Fig. 1b). Permafrost soil therefore had a three times higher ratio of plant-available inorganic to organic N than current rooting zone soil. There were significant differences in the amount of plant-available inorganic N across the five different peatlands, but the overall pattern of higher N values for permafrost soils was consistent and highly significant across all peatlands (Table 2).

Measurements of net phytometer N-uptake from permafrost soil, current rooting zone soil and fresh leaf litter yielded a strikingly similar pattern in plant-available N. Mean plant N content was respectively 189, 113, 147 and 1072 μgN for plants grown on the control, the current rooting zone soil, the peatland litter mix and the near-surface permafrost treatments. When expressed as mean uptake per gram dry weight of added substrate, we found over eight-fold larger net N-uptake by phytometers grown on sand mixed with near-surface permafrost soil than by phytometers grown on sand mixed with either current rooting zone soil or fresh leaf litter ($P < 0.001$)(Fig. 2).

In response to Scenario I ('Deepening active layer'), significantly larger net mineralisation rates were observed in permafrost samples than in rooting zone samples (Fig. 3a). Also, net consumption rates of extractable organic N were six times higher in the current rooting zone samples than in permafrost samples (Fig 3b). Surprisingly, although initial microbial biomass values were higher in current rooting zone samples than in permafrost samples (Fig. 4), there was no significant effect of depth on changes in microbial N under Scenario I. This indicates similar net microbial N immobilisation rates (around 0.8 g N m^{-2} per growing season) at dissimilar incubation temperatures in near-surface permafrost and current rooting zone soil (incubated at 0.5 °C and 11 °C respectively, Fig. 4).

Table 2. Averages, standard errors (SE) and F- statistics for repeated-measures ANOVAs for the effects of depth (current rooting zone, permafrost) and sampling location (peatland) on the availability of different nitrogen species. Values are expressed per 10 cm soil depth. The sampling location (five peatlands) was taken as between-subject factor and depth (current rooting zone (AL), permafrost (PF)) as within-subject factor. All N values were log-transformed before analysis, and $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

	Mean		SE		F-values		
Variable	**AL**	**PF**	**AL**	**PF**	**Depth**	**Peatland**	**Depth X Peatland**
Initial N pools ($mgNm^{-2}$)							
$N\text{-}NO_3$	8	31	2	6	15.37***	3.61*	1.39
$N\text{-}NH_4$	30	252	5	66	55.86***	5.69**	2.73
DIN ($N\text{-}NO_3$ + $N\text{-}NH_4$)	38	283	6	64	55.41***	6.13**	0.54
DON	708	1504	54	150	21.94***	1.97	1.27
Nmic	2318	974	235	229	14.12***	2.13	1.82
Mineralisation/immobilization/change rates ($mgNm^{-2}$ per growing season)							
Scenario I: AL 11°C vs. PF0.5°C							
DIN	-30	273	6	122	6.20*	1.33	1.39
DON	-426	-69	39	115	10.11**	1.37	1.05
Nmic	835	802	116	235	0.00	1.61	1.45
Scenario II: AL 11°C vs. PF 11°C							
DIN	-30	1348	6	351	8.91**	3.78*	4.23*
DON	-426	90	39	111	19.29***	0.77	1.62
Nmic	835	1492	116	284	4.78*	1.05	0.54

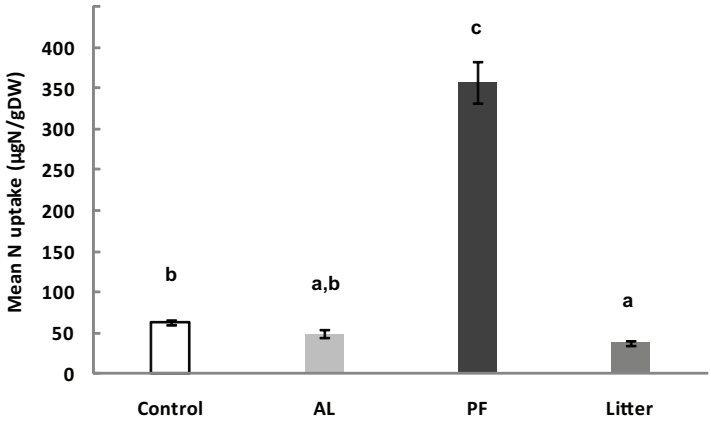

Figure 2. Phytometer plant-available N expressed as N-uptake per gram substrate (DW): near-surface permafrost soil (PF), current rooting zone soil (AL) or litter compared with control plant N-uptake without added substrate. Data are means ± SE, $n = 13$ per treatment, $P < 0.05$.

Under Scenario II, where samples from both soil layers had been exposed to ambient rooting zone temperatures ('Degrading palsa'), we found even larger differences in changes in N availability between the near-surface permafrost and current rooting zone samples. Firstly, a five-fold larger net release of extractable inorganic N was observed from permafrost samples

incubated at 11 °C (Scenario II) compared to permafrost samples incubated at 0.5 °C (under Scenario I), whereas only a net immobilization of inorganic N was observed in the current rooting zone soil samples incubated at 11 °C (Fig. 3a). In contrast, in the current rooting zone, similar rates of net immobilization occurred at 0.5 °C (results not shown). Secondly, extractable organic N change in the permafrost samples was 90 mgN m^{-2} as opposed to -426 mgN m^{-2} in an equal volume of current rooting zone soil per simulated growing season under Scenario II (Fig. 3b). In contrast to Scenario I, the net immobilisation rate of plant available N into microbial biomass was significantly larger in permafrost samples incubated at 11 °C compared to that in the current rooting zone soil samples incubated at 11 °C (Scenario II) (Fig. 4, Table 2). This indicates that total nitrogen fluxes in the permafrost samples at scenario II had even been higher. The overall significant effect of sampling depth on inorganic and organic pool change rates was consistent across all peatlands under both Scenario I and II (Table 2).

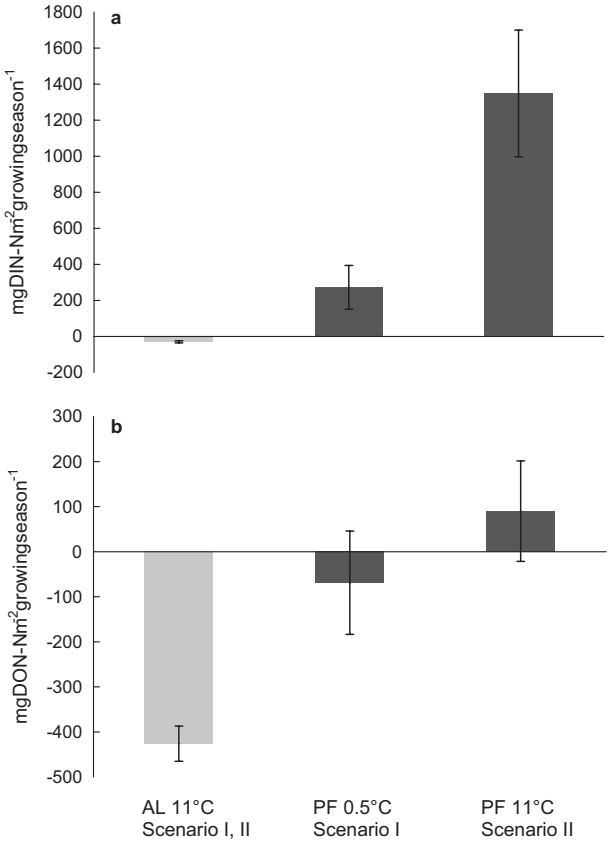

Figure 3. Net rate of change in extractable inorganic and organic N in current rooting zone soil (grey bars) vs. near-surface permafrost soil (black bars) under both a 'deepening active layer' scenario (I) and a 'degrading palsa' scenario (II) over one growing season. Data are means of five peatlands ± SE.

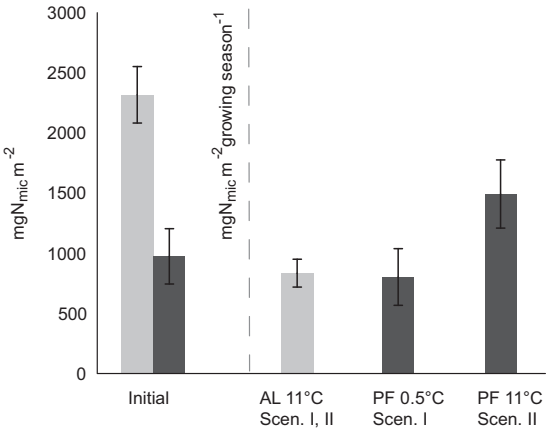

Figure 4. Pool sizes at the start of the incubation (initial) and rate of change in microbial biomass N (N_{mic}) in current rooting zone soil (grey bars) vs. near-surface permafrost soil (black bars) under both a 'deepening active layer' scenario (I) and a 'degrading palsa' scenario (II) over one growing season. Data are means of five peatlands ± SE.

Discussion

This study shows consistently higher concentrations of plant-available N in near-surface permafrost soils than in current rooting zone soils of subarctic peatlands. Moreover, we show that plant-available N released from near-surface permafrost can originate both from a limited but readily plant-available N pool (historically accumulated in frozen layers) as well as from N mineralisation of freshly thawed soil over the course of a growing season.

To our knowledge, this is the first study providing data on plant-available N in near-surface permafrost of subarctic peatlands. We do realize that the data may not be extrapolated directly to the field situation. Instead, our data serve as a first estimate on the relevance of plant-available N from permafrost soils. Below we will discuss our findings and their potential implications for the nutrient dynamics of sub-arctic peatlands underlain by thawing permafrost.

Fast-food: readily available plant-available N pools in near-surface permafrost soil

We found plant-available N concentrations in near-surface permafrost soils that were up to seven times higher than in current rooting zone samples (Fig. 1a, b). However, the samples were taken at the time of maximum thaw, i.e. at the end of the growing season. Higher plant-available N pool sizes might be expected in the rooting zone of tundra soils earlier in the season, i.e. directly after spring thaw but before plant uptake (Hobbie & Chapin 1996, Larsen *et al.* 2007). This would imply that the difference between the two soil layers may be smaller earlier in the growing season. Indeed, comparative measurements in the current rooting zone in mid-June and mid-September showed three-fold higher early season inorganic N values in the current rooting zone, while organic N values remained constant (results not shown). However, even taking these early-season N values into consideration, the plant-available N pool sizes are still up to 100% larger in near-surface permafrost soil than in soil taken from the current rooting zone layer (5-15 cm).

In line with the extraction results, plant N-uptake as measured by means of the bioassay was significantly stimulated by the near-surface permafrost soil substrate. In contrast, current rooting zone soil and fresh litter had very little or, in the case of plant litter, even negative effects on phytometer growth and N-uptake (Fig. 2), although these are considered the two primary plant-available N-sources in subarctic mires (Rosswall & Granhall 1980). Apparently, these N-sources contain less readily plant-available N, may have a slower turnover due to more structural carbon-substrates (i.e. the litter samples), or may have had phytotoxic (related to a high release of phenolic compounds) or immobilising effects that negatively affected plant N-uptake (Robinson 2002, Dorrepaal *et al.* 2007). Such negative effects did not occur for the phytometers which were grown on the near-surface permafrost substrate. However, it should be realized that in the longer term net litter N mineralisation will occur (Cadish & Giller 1997). Nevertheless, this comparison of net phytometer N-uptake from near-surface permafrost soil with two important other nutrient sources in subarctic peatland ecosystems corroborates our findings from the chemical extractions that thawed near-surface permafrost soil might provide an important 'new' plant-available N-source.

Our data thus show a consistent pattern of a significantly larger amount of readily plant-available N in near-surface permafrost than in current rooting zone soil, independent of measurement method or peatland. This N will become available immediately upon thawing, but will most likely be used up shortly after thawing.

Slow-food: longer-term release of plant-available N from permafrost soil

The relatively high net N-mineralisation rates in near-surface permafrost soil (Fig. 3a) over the course of a simulated growing season show that plant-available N in near-surface permafrost soil not only consists of historically accumulated, dissolved readily plant-available N, but also contains a considerable amount of organically-bound N. Upon thawing, this organically-bound N can be mineralised at significantly higher rates in near-surface permafrost soil than in current rooting zone soil, even under our most conservative temperature scenario (Scenario I).

Furthermore, the difference between mineralisation rates under Scenario I and II showed that the mineralisation of N in near-surface permafrost is highly temperature sensitive, with a five times increase over 0.5 ºC to 11 ºC, which is high, but not unrealistic (Davidson & Janssens 2006). Even though care should be taken when comparing in vitro with in situ conditions, and noting that the two scenarios presented here included temperature effects only, the different responses to the two imposed scenarios demonstrate that the mode of thaw-development is an important determinant of permafrost-thaw effects on nutrient cycling.

The observed high mineralisation rates in near-surface permafrost soil point to the presence of a potentially active microbial community. Complementary enzyme analyses on the same samples corroborate this finding: all permafrost samples showed high potential aminopeptidase activities and three out of four analysed potential activities were significantly larger in permafrost samples than in current rooting zone samples (Table SI 2, Supporting Information). Additionally, a strikingly similar increase in microbial biomass N was observed in soil from both depths under Scenario I in spite of the 10 ºC lower incubation temperature of permafrost soil ('Deepening active layer', Fig. 4)). Under Scenario II ('Degrading palsa'), the absolute increase in microbial biomass N in permafrost soil was even almost twice as large as in current rooting zone soil despite the higher net N mineralisation rates (Fig. 4). The highly active microbial community in the near-

surface permafrost may partially explain the high temperature sensitivity of N mineralisation rates of these soils, since the temperature response is higher than would be expected based on enzyme responses to temperature alone (Weedon *et al.* 2012). It is well-established that microbial activity can occur at sub-zero temperatures (Panikov *et al.* 2006, Wallenstein *et al.* 2009), and our results are the first to suggest that a microbial community at the thawfront can indeed actively alter the nutrient status of subarctic peatlands.

The frozen feast released

Based on an observed one cm thaw of permafrost per year (Akerman & Johansson 2008) and the data presented in this study, we can provide a preliminary estimate of the amount of plant-available N release per year in the near-future from thawing permafrost in subarctic peatlands. Following a one-off release of 0.03 g N m^{-2}, a slow but continuous release of 0.03 g N m^{-2} yr^{-1} (dissolved inorganic) can be expected from thawing permafrost under Scenario I, and of 0.13 g N m^{-2} yr^{-1} under Scenario II (Table 2). Moreover, these values would be cumulative in the near-future because due to ever increasing active layer thickness, every year more soil is seasonally thawed and releasing plant-available N. Hence, with progressing permafrost-thaw, an increasing yearly contribution, from 0.03 - 0.13 g N m^{-2} yr^{-1} in the first year to 0.3 – 1.3 g N m^{-2} yr^{-1} in the tenth year could be expected (assuming constant decomposition and mineralisation rates, growing season length and soil temperatures). Even if the mineralisation rates of a thawed layer would decrease over time (which may be expected given the apparent labile nature of the organic compounds mineralised in the near-permafrost soil), these values are considerable compared with the 0.8 g N m^{-2} yr^{-1} net N mineralisation in northern peatland ecosystems (Rosswall & Granhall 1980). Net annual N mineralisation in northern peatland ecosystems could increase by up to 46% as observed in climate warming experiments (in the range of 0.3 - 6.0 ºC; Rustad *et al.* 2001). Furthermore, atmospheric deposition of N in northern Scandinavia is around 0.1-0.2 g N m^{-2} yr^{-1} (Dentener *et al.* 2006). This shows that although ambient net N mineralisation will most likely still be the main source of plant-available N under both Scenario I and under Scenario II, the potential plant-available N release through permafrost thawing is at least in the same order of magnitude as other climate-change related increases in N input in northern peatlands.

Whether nutrients released at the thawfront will really become available to plants will ultimately depend on the ability of the local vegetation to reach this N source. Obviously, this is most critical under Scenario I, where the peatland structure stays intact. Although little information about the activity of plant roots at the permafrost thaw front is currently available, the roots of some graminoids are known to grow at the surface of the frozen soil as this surface recedes down the soil profile towards the permafrost during summer (Bliss 1956, Callaghan *et al.* 1991). Also, roots of *Rubus chamaemorus* were observed at the permafrost thaw front during sampling for this study (pers. obs. F. Keuper). Hence, indeed some plant species may be capable of foraging the thawfront for nitrogen.

These observations suggest that a sustained release of nutrients at the thaw front might thus be expected to selectively benefit specific, deep-rooting species and thereby alter species composition. Although many fertilization experiments have been carried out in subarctic tundra indicating a likely change in the vegetation community (Parsons *et al.* 1995, Michelsen *et al.* 1996, Press *et al.* 1998, Robinson *et al.* 1998, Shaver *et al.* 2001, Haugwitz & Michelsen 2011, Lamb *et al.* 2011), the vegetation responses to a release of nutrients in deeper soil layers such as presented

here cannot be inferred in a straightforward manner from studies where fertilizer was applied at the soil surface.

In addition to the local implications of our results for the vegetation of northern peatlands, N release by thawing permafrost might have larger-scale impacts, as northern peatlands are an important carbon sink. Carbon release due to decomposition of plant litter and soil organic matter is exceeded by carbon assimilation in primary production in these ecosystems, but both processes are restricted by the availability of nitrogen (Chapin *et al.* 1995, Robinson 2002, Mack *et al.* 2004, Reich *et al.* 2006) due to low external nitrogen inputs. A permafrost-thawing induced increase in plant-available N might therefore stimulate biomass production (Rustad *et al.* 2001), and thus C-uptake. On the other hand, litter quality might increase (lower C:N ratio) (Aerts 1997), potentially stimulating decomposition (Bragazza *et al.* 2006). Over the longer term, an internal release of N from thawing permafrost is likely to speed up internal N and C cycling in northern peatlands, and potentially change their function as carbon sink, although the magnitude and direction is hard to predict without further understanding of plant and microbial responses to an increased deep N input.

Altogether, our results suggest that this 'new' climate-change induced N input into subarctic peatlands is indeed potentially available to plants and moreover similar in magnitude to other climate change induced increased N inputs such as increased mineralisation in surface soil layers (current rooting zone) and atmospheric deposition. These results from permafrost underlain peatlands are thus important when predicting the response of these nutrient-limited northern ecosystems to climate warming.

Acknowledgements

The authors would like to thank S. Marozava for her irreplaceable help during sampling and S. Sjögersten for her generous offer to (ab)use her peat corer, which made this research possible. Many thanks go to M. Johansson for helpful discussions and to the staff of the Abisko Naturvetenskapliga Station for technical assistance. Financial support was offered to FK by the Darwin Centre for Biogeosciences (grant 142.161.042) and ANS Scholarship 2008, and to RA by the Dutch Polar Program (ALW-NPP grant 851.30.023) and the EU-ATANS (grant FP6 506004).

Supporting Information

Table SI 1. Mean bulk density values (dry soil mass per volume) per peatland for both current rooting zone soil (active layer, AL) and near-surface permafrost soil (PF).

Peatland	Depth	Bulk density *(mgDW/cm^3)*	
		Mean	*SE*
A. **Stordalen**	AL	57.4	4.2
	PF	134.8	18.5
B. **Torneträsk**	AL	57.6	9.3
	PF	205.8	5.1
C. **Storflaket**	AL	84.4	11.3
	PF	209.7	43.9
D. **Abisko**	AL	36.8	2.4
	PF	221.8	28.7
E. **Kursflaket**	AL	38.2	2.2
	PF	275.3	121.4
All peatlands	AL	274.4	4.5
	PF	1047.3	19.4

The values in the main text of this article are expressed as mg N per m^2 per 10 cm depth, which reflects the amount of plant-available nitrogen that may be released yearly in the near-future (e.g. decade, based on an annual 1 cm thaw rate (Akerman & Johansson 2008)). This is equivalent to 0.1 mg N m^{-3} and can be recalculated into other units for comparison with literature with the bulk density data provided in Table SI 1.

Total N values were calculated by multiplying the %N by weight with the bulk density. Note: total N represents all N, including non-extractable highly recalcitrant N-forms, whereas DON, DIN and N_{mic} (Table 2 in the main document) are all extractable N forms only. Hence, total dissolvable N is lower than total N.

Mean total N values per 10 cm soil depth were 41 g m^{-2} (± 6 SE) for current rooting zone soil and 297 g m^{-2} (± 20 SE) for near-surface permafrost soil. Total N was analysed with repeated-measures ANOVAs for the effects of depth (within-subject factor: current rooting zone, permafrost) and sampling location (between-subject factor, five peatlands): factor depth F = 259.24, $P < 0.001$; peatland $F = 6.64$, $P < 0.01$ and a significant interaction between the two main factors depth and peatland $F = 3.97$, $P < 0.05$.

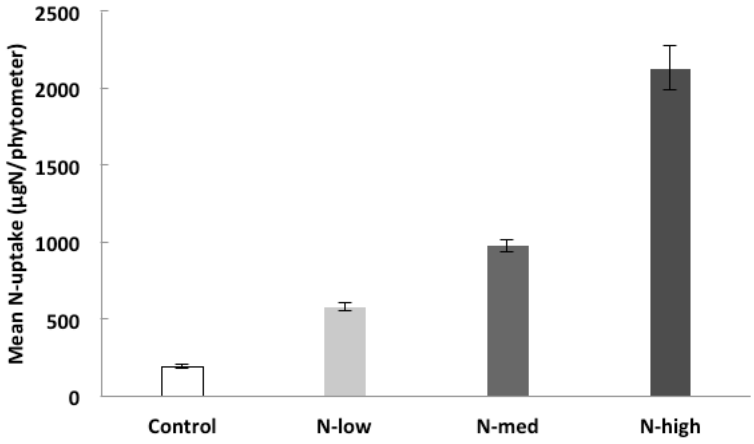

Figure SI 1. As a positive control of *Poa alpina* phytometer N-limitation, three N additions of 0.75 g N m^{-2} (N-low), 1.5 g N m^{-2} (N-med) and 3.0 g N m^{-2} (N-high) were applied. Fertilizer was given to the soil surface (79 cm^2) as NH_4NO_3 solution divided over 6 weekly 10 ml gifts. Controls, soil and litter treatments received 10 ml de-ionized water simultaneously to prevent moisture-related artefacts. Data are means ± SE, $n = 13$, all treatments differed significantly from each other ($P < 0.001$).

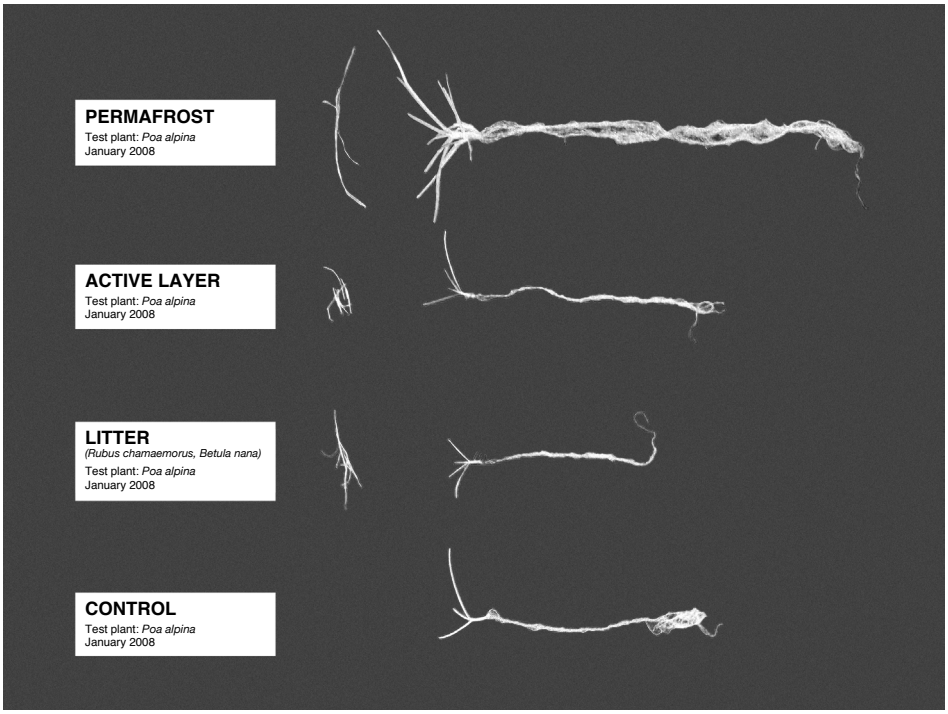

Figure SI 2. Phytometers grown on sand mixed with near-surface permafrost soil, current rooting zone (active layer) soil or litter, immediately after their harvest, showing remarkable differences in size. For an elaborate description of the historical use, pros and cons of the bioassay technique see Axmanova *et al.* (2011, *Plant and Soil*, 342, 183-194).

Table SI 2. Averages and *F*- statistics for repeated-measures ANOVAs for the effects of depth and sampling location on potential enzyme activities for four aminopeptidases in peat soils (see text for abbreviations) incubated at 4 °C (nmol/gDW/hr). Sampling location (peatland A-E) was taken as between-subject factor and depth (active layer, permafrost) as within-subject factor. Alanine data were log-transformed before analysis.

Substrate		Mean	SE	Depth	Peatland	Depth X Peatland
Alanine	*AL*	7.85	1.29	3.74+	0.79	3.02*
	PF	15.47	3.91			
Lys.ala	*AL*	3.35	0.25	21.02***	2.44	5.39**
	PF	6.58	0.59			
Leucine	*AL*	6.03	0.55	12.78**	2.51+	3.18*
	PF	11.16	1.18			
AAP	*AL*	318.08	40.50	36.53***	1.28	1.27
	PF	63.82	10.20			

Methods fluorometric enzyme assays

Enzyme assays were conducted according to the protocol of Steinweg and McMahon (http://enzymes.nrel.colostate.edu/) using substrates labelled with 7-amino-4-methylcoumarin (MUC). Specific substrates were L-leucine-7-amido-4-MUC (Leucine), L-alanine-7-amido-4-MUC (Alanine), L-lysine-alanine-7-amido-4-MUC (Lys-Ala), and L-alanine-alanine-phenylalanine-7-amido-4-MUC (AAP) (all substrates supplied by Sigma-Aldrich).

Soil slurries were prepared by blending 4 g (fresh weight) homogenized peat in 90 mL of 50 mM sodium acetate buffer (pH 5) for 60 seconds. 800 μL of slurry were incubated with 200 μL of 200 μM substrate in 96-deepwell plates at at 4 °C for 20 hours. Simultaneously 800 μL slurry was incubated in identical conditions with 200 μL volumes of a dilution series of MUC standard (0, 2.5, 5, 10, 25, 50, 100 μM). In this way standard curves could be constructed for each sample, thus controlling for between-sample variance in fluorescence quenching dynamics. After incubation, plates were centrifuged at 1500 rpm for 3 minutes, the supernatant transferred to fluorescence plates and pH adjusted with 5 μL 0.5M NaOH. Fluorescence was then immediately measured using a Spectramax Gemini XS microplate fluorometer (Molecular Devices, Sunnyvale, USA) with excitation wavelength 365 nm and emission wavelength 450 nm. Product accumulation was calculated by comparison with each sample's standard curve. When standard curves were non-linear separate curves were fitted to the separate linear portions and the appropriate regression equation used for calculating sample reaction rates. All measurements were converted to nanomols per gram dry weight per hour.

IV

Chapter V

Foraging the Thaw Front: Increased nutrient uptake at the permafrost surface enhances biomass production of deep-rooting subarctic peatland species

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Manuscript

Abstract

1. Plant production in subarctic peatlands is nitrogen (N)-limited. Climate warming increases N mineralization in superficial peat layers and recent results additionally show that permafrost thawing in these peatlands may substantially increase plant-available N at the thaw front. This might stimulate net primary production and affect species composition. However, the ability of individual peatland plant species to take up N from the permafrost thaw front has never been studied before.

2. We aimed to identify the potential impact of increased N-availability due to thawing permafrost on subarctic peatland plant productivity and species composition. We compared this impact with the effect of increased nutrient availability in shallower layers (e.g. through enhanced N-mineralization due to climatic warming). Therefore, we supplied ^{15}N-labeled nitrogen at the thaw front and performed a 3-year full-factorial belowground fertilization experiment with deep-fertilization at the thaw front at 45 cm depth and shallow-fertilization at 10 cm depth.

3. We found that only particular species (e.g. *Rubus chamaemorus*) are present with active roots at the thaw front. Further, if supplied with nitrogen at the thaw front, these species had higher aboveground biomass and N-content, whereas this was not the case for shallower-rooting species (e.g. *Empetrum hermaphroditum* and *Andromeda polifolia*). Moreover, the effects of increased nutrient availability at the thaw front on total aboveground biomass production were similar in magnitude to the effects of increased nutrient availability in shallower layers. Additionally, nutrient limitation of plant growth in subarctic peatlands appeared to be sufficiently strong for the effects of increased deep and shallow nutrient-availability on biomass production to be additive.

4. Synthesis Altogether, our results show that plant-available N released from thawing permafrost can be considered a true 'new' N source for deep-rooting sub-arctic plant species, which will increase their biomass production. As this is not the case for shallow-rooting species, the release of plant-available N from thawing permafrost has the potential to alter species composition on the long-term by benefitting specific deep-rooting species only.

Introduction

Many of the world's northern peatlands are underlain by permafrost (perennially frozen ground) which is thawing rapidly as a result of climate change (ACIA, 2004, Tarnocai *et al.*, 2009). Recently, we have shown thawing permafrost may release considerable amounts of plant-available nitrogen (N) into subarctic peatlands (up to 0.3 g N m^{-2} yr^{-1} in the coming decade) (Keuper *et al.*, 2012). This 'new' global-change induced N-source at the permafrost thaw front is additional to the predicted and observed increase in N-availability in shallower soil layers due to atmospheric deposition or increased N-mineralisation rates by higher air temperatures (Rustad *et al.*, 2001, Weedon *et al.*, 2012). Plant production in these northern peatlands is often N-limited (Aerts *et al.*, 1992, Berendse & Jonasson, 1992) and a climate-change induced increase in N availability in shallower soil layers stimulates primary production and alters species composition (Chapin *et al.*, 1995, Van Wijk *et al.*, 2004). A release of stored permafrost N due to thawing may likely have similar effects, if plants are able to access this new permafrost-thaw induced N-source.

So far, little is known about the ability of peatland plants to take up nutrients released at the thaw front, although some studies suggest that specific peatland graminoids and forbs extend their roots to deeper soil layers (up to 60 cm soil depth). This applies in particular to the forb *Rubus chamaemorus*, as observed by Rapp and Steenberg (1977) in (non-permafrost) *Sphagnum fuscum*-dominated peatlands in northern Norway, and the sedge *Eriophorum vaginatum* (Wein, 1973), as observed in peatlands of the British Isles. Moreover, roots of *Eriophorum angustifolium*, *E. vaginatum* and *Arctagrostis latifolia* are able to grow at the frozen soil surface while this recedes down the soil profile during the northern summers (Bliss, 1956, Callaghan *et al.*, 1991) and can resume their growth after having been frozen for several days (Billings *et al.*, 1976). Further, although the moment of maximum thaw depth coincides with the end of the growing season, most perennial sub-arctic species can store the N in belowground structures such as rhizomes and thick roots (Chapin *et al.*, 1990) and use the stored N in the next growing season. Additionally, observations of increased canopy N in Alaskan thermokarst areas suggest that increased plant N-uptake from thawing permafrost is indeed possible in some areas (Schuur *et al.*, 2007). However, so far it has not been tested whether or not species can effectively utilize nutrients released at the permafrost thaw front at the time of maximum thaw depth (end of the growing season) and how much they rely on this uptake for their total nutrient budget relative to their nutrient-uptake from shallower soil layers.

The deep belowground release of plant-available N from thawing permafrost makes this N-source fundamentally different from other climate-change related increases in N-input, which generally affect nutrient availability in the surface layer of the soil only (eg. increased mineralisation as a result of higher temperatures or increased atmospheric N deposition) (Rustad *et al.*, 2001, Weedon *et al.*, 2012). Nutrient utilisation in shallow layers (0-15 cm depth) is largely determined by species-specific plant traits related to their competitive ability for nutrient uptake, such as nutrient absorption capacity, specific root length and root allocation (Lambers *et al.*, 1998). Competition for the uptake of nutrients released from thawing permafrost would take the importance of these last two traits to a different level: in areas with thawing permafrost, community responses will first and foremost depend on whether and which specific species can reach this 'new' deep N source. Whether species composition in permafrost peatlands is affected by resource partitioning within the rooting zone (Berendse, 1982, Casper *et al.*, 2003, McKane *et al.*, 2002) is largely unknown. However, if such vertical resource partitioning occurs in permafrost peatlands, a release of nutrients at the thaw front will most likely benefit deeper rooting species only.

There may be interactive effects of climate-change induced increases in nutrient supply between shallow and deeper soil layers of northern peatlands, i.e. nutrient-uptake from deeper soil layers may be dampened when the vegetation's need for nutrients is already satisfied by increased nutrient supply in shallower, more easily accessible layers. However, given the strong nutrient-limitation of plant production in northern peatlands, the effects on plant production of increased nutrient supply in shallow- and deeper soil layers are most likely additive.

In this paper we aim to identify the potential impact of increased N-availability at the permafrost thaw front on subarctic peatland vegetation. We hypothesized that: a) only particular peatland species have roots present and active at the thaw front of subarctic peatlands; and that b) if presented with increased nutrient availability at the permafrost thaw front, only these deep-rooting species will show increased aboveground biomass and N-content. Moreover, we hypothesized that c) the effects on aboveground biomass production of subarctic peatland

vegetation of increased nutrient availability at the thaw front can be similar in magnitude to the effects of an equal increase in nutrient availability in shallower layers and that d) nutrient limitation of plant-growth in subarctic peatlands is strong enough for the effects of increased deep- and shallow-nutrient availability to be additive.

Methods

General approach

All experiments were performed at two *Sphagnum fuscum* dominated ombrotrophic peatlands in the Stordalen nature reserve in northernmost Sweden (68°21.428'N, 19°03.181'E). We first determined the vertical plant root distribution in the active layer down to the thaw front, which is at a depth of 45-50 cm in these peatlands. Subsequently, we analysed qualitative differences among vascular subarctic peatland species in their capability to take up N from the thaw front between the time of maximum thaw depth (coinciding with the end of the growing season) and the beginning of the next growing season, by injecting ^{15}N-ammonium at the thaw front. In addition, we performed a below-ground fertilization experiment to test whether increased plant-available nutrient supply at the thaw front can affect net aboveground primary production, plant N-content, and species composition, and to get insight in the relative importance of increased nutrient supply at the thaw front compared to increased nutrient supply in the current (main) rooting zone (5-15 cm) and potential interactions. The below-ground fertilization experiment included full-factorial combinations of fertilization in the active layer (at 10 cm depth) and fertilization at the thaw front (at 45 cm depth). After three growing seasons, we measured treatment effects on aboveground biomass and N-content of the dominant species.

Root presence down to the thaw front

Vertical root biomass distribution was determined in five randomly taken cores (measuring 6 x 5 cm, to a depth of 45 cm) which were extracted at the time of maximum thaw depth (second half of September) from the first peatland, where *Rubus chamaemorus*, *Empetrum hermaphroditum* and *Andromeda polifolia* were the key species. Upon extraction, each core was divided into four layers of each 10 cm at subsequent depths (5-15 cm; 15-25 cm; 25-35 cm and 35-45 cm, the latter representing the thaw front). All living roots (> 0.1 mm) were removed from the *Sphagnum* peat and sorted per species by means of visual estimation of differences in colour, diameter and structure. The roots were sorted in the lab, oven dried (48 hrs, 60 °C), weighed and root biomass was expressed as root dry weight per cm^3 soil volume.

^{15}N-uptake at the thaw front

To test for differences among subarctic peatland species in their capability to take up nitrogen from the thaw front between the time of maximum thaw depth and the beginning of the next growing season, a ^{15}N-pulse-labeling at the thaw front was performed. Six peatland plots were selected based on mutual presence of the vascular species *E. hermaphroditum*, *R. chamaemorus*, *Vaccinium uliginosum*, *A. polifolia*, and if possible *Eriophorum vaginatum* and *Betula nana*; and an active layer thickness of approximately 45 cm. At the time of maximum soil thaw (September 15th, 2008), 28 mg ^{15}N-ammonium chloride (98 atom% ^{15}N, Isotec, Miamisburg, OHIO, USA) dissolved in 60 ml deionized water, was injected at the thaw front in triangular grids, using three metal tubes (10 cm apart, 20 ml per tube) with small openings at the bottom of the tube. The

^{15}N-ammonium solution was inserted by connecting a syringe to the airtight tubes and forcing the liquid to exit the tube at the bottom under pressure (See picture, Supplementary material Fig. SI 1). Six control plots were selected and all plots were at least one meter apart from each other. At the start of the following growing season (June 12, 2009), leaf material of all vascular species present within the triangle was collected. The leaves were stored in separate paper bags, oven dried (at 60 °C) and ground. Additionally, on September 20, 2009, one year after the pulse labeling and at the time of maximum thaw, soil cores were extracted from the centre of every ^{15}N-plot and of four control plots to check for potential undesired vertical redistribution of the labeled nitrogen. Subsamples were taken at three depths per soil core (15, 30 and 45 cm depth) and roots were removed. Soil samples were then oven dried (48 hrs, 70 °C) and ground. Before further analysis of the plant material, all soil samples were analysed for the abundance of ^{15}N to check if ^{15}N-enrichment had not spread to layers other than the deepest layer (either through vertical redistribution via plant root litter or exudates, capillary rise or methodological contamination), using a cut-off value of 3.75 mg^{15}N gN^{-1}. Because we had no means to distinguish between the natural redistribution processes or methodological contamination, only plant samples from plots without any vertical redistribution of the labeled nitrogen were used in subsequent analyses, resulting in n = 5 for most species. Unfortunately, for both *E. vaginatum* and *B. nana* only two replicates remained. Atom percentages of ^{15}N of all plant and soil samples were determined with an elemental analyser (NC2500, ThermoQuest Italia, Rodano, Italy) coupled online to a stable isotope ratio mass spectrometer (DeltaPlus, ThermoQuest Finnigan, Bremen, Germany).

Three-year full-factorial belowground fertilization experiment

To quantitatively assess the relative importance of deep N availability vs. shallow N availability, a full-factorial belowground fertilization experiment was performed with factors 'thaw front (deep)-fertilization' (+/-) and 'current rooting zone (shallow)-fertilization' (+/-). This thus resulted in four treatments: 'shallow-fertilized' (S), 'deep-fertilized' (D), 'shallow- plus deep-fertilized' (SD) and a control treatment (C). Plots of 60 x 60 cm were randomly chosen within an ombrotrophic peatland dominated by *E. hermaphroditum* and *R. chamaemorus* (78% and 12% of total biomass, respectively), and each experimental treatment was replicated eight times. Maximum active layer thickness was measured at the time of maximum thaw depth (second half of September) in each plot by probing a steel rod into the soil until refusal, and was around 45 cm. In early June 2008, 11.8 g slow release fertilizer grains (NPK 17:3:11, with the 17% N consisting of 8.9% NH_4-N and 8.1% NO_3-N), equivalent to 8 gN m^{-2} (to be released slowly during the three years after insertion) were added in the shallow-fertilization treatment (10 cm depth). The grains were inserted through an aluminium tube (diameter 10 mm) in grids of nine points (3 x 3 design with insertion points 20 cm apart, see Supporting Information, Fig. SI 2). Control plots were equally grid-wise perforated, but did not receive fertilizer. As the deeper soil layers are hard-frozen at the beginning of the growing season, an equal amount of slow release fertilizer was inserted in the same grid of the deep-fertilization treatments (D and SD) at a depth of 45 cm at the time of maximum thaw depth prior to the shallow addition (September 2007). In this way, we synchronized the potential availability of shallow and deep N into the same growing season.

To verify treatment effects on nutrient availability, plant root simulator (PRS™) soil probes (Western Ag Innovations Inc., Saskatoon, Canada), commercially manufactured ion exchange resins, were inserted in four plots per treatment and at two depths (10-15 cm and 40-45 cm)

two years after the start of the experiment. The shallow- and the deep- inserted probes showed higher N-values at the depths where the fertilizer grains had been inserted but not at other depths (Supporting Information, Fig. SI 3). There were no interaction effects, showing that our treatments indeed had the intended effect on soil nutrient availability.

Effects of belowground fertilization on vegetation

Three growing seasons after insertion of the fertilizer, at the peak of the growing season 2010 (July 26th), total aboveground vascular biomass was harvested from two 20 x 20 cm subplots per plot (Supporting Information, Fig. SI 2) which were combined for further analysis. The samples were divided into leaves of *R. chamaemorus*, *E. hermaphroditum* (together 90% of total aboveground biomass) and the remaining species (*A. polifolia*, *Vaccinium microcarpum*, *V. uliginosum*). These species, which were all shallow-rooting, were grouped because of generally low abundance and because none of these remaining species were present in all plots. Leaves were oven dried (48 hours at 60 °C), weighed, ground and nitrogen concentrations were determined by dry combustion with a Flash EA1112 elemental analyser (Thermo Scientific, Rodana, Italy).

Additionally, to test whether roots at the thaw front are actively 'foraging' when presented with increased nutrient supply, changes in *R. chamaemorus* root biomass at the thaw front were determined by comparing root biomass in five randomly selected control (C) plots with eight deep-fertilization (D) plots at the time of maximum thaw depth in the last year of the experiment. In every plot one core (of 6 x 5 cm and a depth of 10 cm) was extracted at 35-45 cm (due to practical constraints not all treatments and depths were sampled). Roots (> 0.1 mm) were sorted in the lab, oven dried (48 hrs, 60 °C), weighed and root biomass was expressed as root dry weight per cm^3 (fresh) soil volume.

Statistical analysis

All data were tested for normality and homogeneity of residual variances by visual estimation of residual plots and normal probability plots. Log-transformation improved the homogeneity of residual variances for the ^{15}N labeling experiment data, as well as for the aboveground biomass, the N-content and the root biomass data of the full-factorial belowground fertilization experiment.

Due to the high number of zero-values, the ambient root biomass distribution data did not render enough power for credible significance-testing. We therefore chose to present the vertical distribution of mean root biomass and presence-absence data only graphically. ^{15}N-contents of plant leaves in the ^{15}N-labeling experiment were analysed with a repeated-measures (RM)-ANOVA with within-subject factor 'species' (*A. polifolia*, *V. uliginosum*, *E. hermaphroditum* and *R. chamaemorus*) and as between-subject factor '^{15}N-treatment'. The differences among species were subsequently analysed by paired t-tests. Aboveground biomass and N-content of the two dominant species (*R. chamaemorus*, *E. hermaphroditum*) in the full-factorial belowground fertilization experiment were analysed with a RM-ANOVA with 'species' (*R. chamaemorus*, *E. hermaphroditum*) as the within-subject factor, and 'shallow-fertilization' and 'deep-fertilization' as the between-subject factors. We performed the analyses on aboveground biomass and N-content data in the full-factorial belowground fertilization experiment on the two key species only (*R. chamaemorus* and *E. hermaphroditum*, together 90% of total biomass) because of many near-zero values in the 'rest-group'. Differences in root biomass at the thaw front in control plots vs. deep-fertilization plots were analysed with a one-way ANOVA with 'deep-fertilization' as an independent factor.

All analyses were performed with SPSS 15.0 for Windows.

Results

Roots at the thaw front

On the five randomly chosen locations where *Rubus chamaemorus*, *Empetrum hermaphroditum* and *Andromeda polifolia* were mutually present, we found strong interspecific differences in vertical root distribution patterns. At the thaw front, only roots of *R. chamaemorus* were present (Fig. 1). Root biomass of *R. chamaemorus* was relatively evenly distributed over the soil profile, with in the deepest layer an average root biomass of 0.8 ± 0.1 (SE) gDW cm^{-3} (n = 5). Roots of the other species were largely confined to the upper soil layer (up to 15 cm depth) and were scarcely (at 15-25 cm) or not at all found below 25 cm depth (Fig. 1). Thus the bulk of root biomass of *A. polifolia* or *E. hermaphroditum* occurred at 5-15 cm soil depth, with a steep vertical decrease in root biomass (e.g. for Empetrum 1.5 gDW cm^{-3} at 5-15 cm and only 0.15 gDW cm^{-3} at 15-25 cm) (Fig. 1).

N-uptake at the thaw front

R. chamaemorus, *E. hermaphroditum*, *A. polifolia* and *Vaccinium uliginosum* differed in their mean ^{15}N content as a result of uptake from the thaw front between the time of maximum thaw depth and the beginning of the following growing season ('species' F = 15.92; P < 0.005; 'species'x'treatment' F = 15.84; P < 0.005 and the between-subject factor 'treatment' F = 16.59; P < 0.005). The significant interaction indicates ^{15}N uptake by some species in the treatment only. Paired t-tests and Fig. 2 show that the significant differences among species were due to the significantly higher ^{15}N content in *R. chamaemorus* compared to each of the other species. Visual

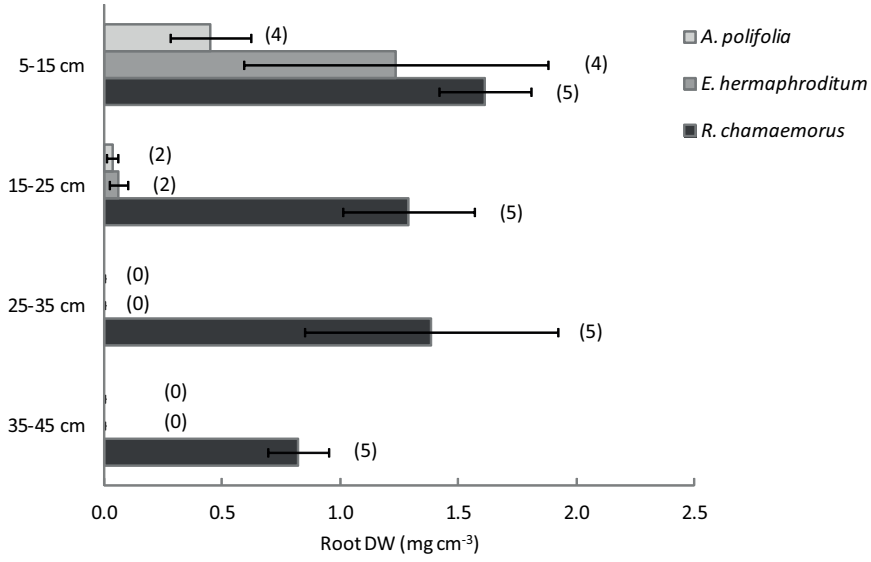

Figure 1. Mean (± SE) root dry weight for three subarctic peatland species at increasing soil depths, down to the thaw front, at the time of maximum thaw depth. Five cores were sampled; the number of cores in which roots were present is indicated in parentheses.

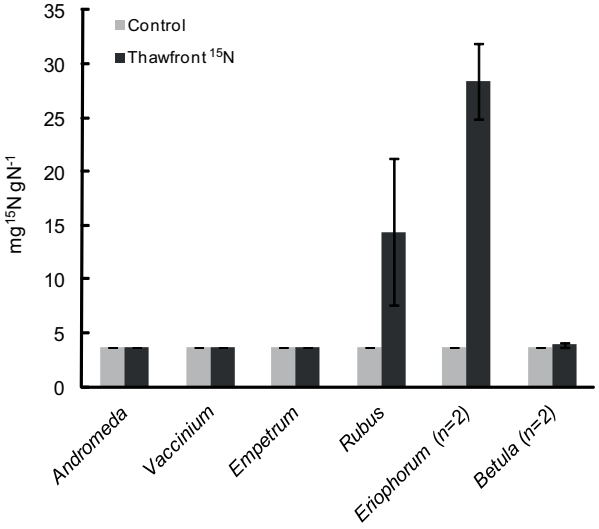

Figure 2. ^{15}N-content of early spring-leaves after insertion of ^{15}N-label at the thaw front at the time of maximum thaw depth in the previous year (end of the growing season). Data are means ± SE and $n = 5$ for all species except *Eriophorum vaginatum* and *Betula nana* ($n = 2$), which were excluded from the statistical analysis.

interpretation suggest that leaves of *Eriophorum vaginatum* were most likely also ^{15}N-enriched in response to insertion of labeled ^{15}N ammonium at the thaw-front. None of the shallow-rooting species had taken up the labeled nitrogen (Fig. 2). This could not be tested statistically due to the lack of power as a result of the low number of remaining replicates ($n = 2$; see Methods). As a control, an analysis in which all plots and all species were included (not accounting for potential contamination) showed the same pattern ('species' $F = 25.70$; $P < 0.005$; 'species' x 'treatment' $F = 25.75$; $P < 0.005$; 'treatment' $F = 98.38$; $P < 0.005$) but with both the deep rooting species *R. chamaemorus* and *E. vaginatum* significantly different from the other species.

Table 1. Results of a RM-ANOVA for the effects of between-subject factors shallow-fertilization and deep-fertilization and within-subject factor species (*R. chamaemorus* vs. *E. hermaphroditum*) on aboveground biomass and N-content of two subarctic peatland key-species (*Rubus chamaemorus* and Empetrum hermaphroditum). Data were log-transformed, * depicts $P \leq 0.05$; ** $P \leq 0.01$ and *** $P \leq 0.001$.

	Aboveground biomass (g m^{-2})	N-content (gN m^{-2})
Source	*F-value*	*F-value*
Species (*Rubus*, *Empetrum*)	352.3 ***	10.7 **
Shallow-fertilization	13.1 ***	4.9 *
Deep-fertilization	15.5 ***	20.6 ***
Species X shallow-fertilization	2.6	0.5
Species X deep-fertilization	17.5 ***	13.4 **
Shallow- X deep-fertilization	1.8	2.2
Species X shallow- X deep-fertilization	0.8	0.1

Full-factorial belowground fertilization experiment: aboveground biomass and N-content

Deep-fertilization caused significantly different responses of the two dominant species in both mean biomass and N-content (significant 'deep-fertilization x species' interaction, Table 1). Only the deep-rooting *R. chamaemorus* showed higher biomass and N content in response to fertilization at the thaw front, whereas this was not the case for the shallow-rooting *E. hermaphroditum* (Fig. 3). In contrast, shallow fertilization had the same positive effect on biomass and N-content of both species (i.e. no 'shallow-fertilization' x 'species' interaction, Table 1). Similar results were obtained when including the biomass data of the 'other species' as a third level in the within-subject factor (within-subject factor 'species' $F = 107.8$, $P < 0.001$; 'shallow-fertilization x species' $F = 1.3$, P = ns; 'deep-fertilization x species' $F = 4.0$, $P < 0.05$). Moreover, for all analyses, there was no interaction between the treatment effects: thus, the effects of the shallow- and deep-fertilization on mean aboveground biomass and N-content were additive (Fig. 3, Table 1).

Root biomass at the thaw front in response to increased nutrient supply

Despite an up to 100% increase of root biomass of *R. chamaemorus* at the thaw front in the deep-fertilized plots compared to the control plots (1.6 ± SD 0.8 mg cm^{-3} in the deep-fertilized plots vs. 0.8 ± SD 0.3 mg cm^{-3} in the control plots) this difference was only marginally significant ('deep-fertilization' $F = 4.6$; $P = 0.056$). This lack of significance despite the large difference was most likely due to the large spatial variability that is characteristic for root distribution patterns.

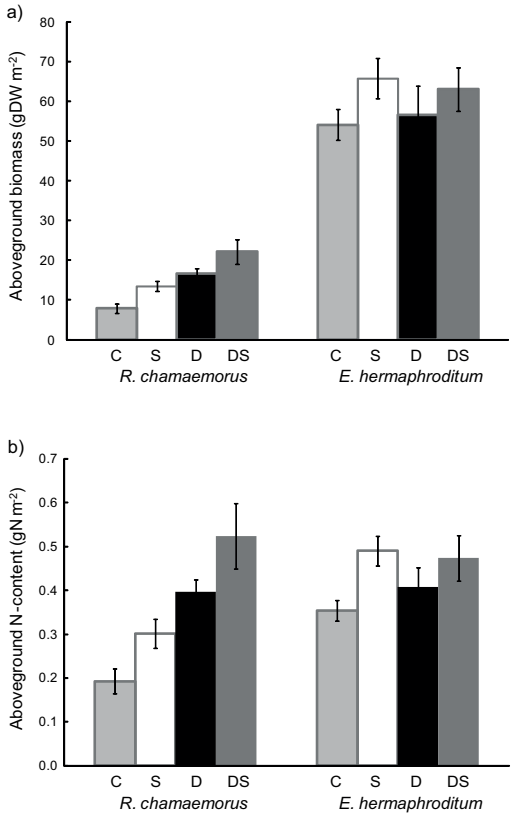

Figure 3. Mean (± SE) aboveground biomass (a) and canopy N-content (b) of the two key species *Rubus chamaemorus* and *Empetrum hermaphroditum* in control (C), shallow-fertilized (S), deep-fertilized (D) and shallow- plus deep-fertilized (SD) plots of the full-factorial belowground fertilization experiment (n =8, See Table 1 for RM-ANOVA statistics).

Discussion

Thawing permafrost is often mentioned as one of the main climate change induced threats to northern peatlands (ACIA, 2004). Recently, we showed that permanently frozen soils of subarctic permafrost peatlands contain considerable amounts of plant-available N (Keuper *et al.*, 2012), which may be released upon thawing. Changes in nutrient availability due to climate change are likely to have considerable influence on the vegetation composition and biomass of subarctic peatlands as these ecosystems are usually strongly N-limited (Dormann & Woodin, 2002, Limpens *et al.*, 2011). Here, we show for the first time that vascular peatland plants are able to utilize N at the permafrost thaw front to increase their biomass and N content. Moreover, our results show that increased nitrogen availability at the thaw front may induce a shift in species composition in the longer term because of a differential ability of peatland species with different rooting depths to take advantage of this new nitrogen source.

Roots at the thaw front: present and actively taking up nutrients

We found large differences in rooting depth among species and only one of our species, *Rubus chamaemorus*, had roots at the thaw front (hypothesis a; Fig. 1). These findings are consistent with earlier observations of deep-rooting *R. chamaemorus* (Rapp & Steenberg, 1977) and demonstrate that indeed only particular species have roots at the thaw front. Moreover, our labeling experiment showed that nutrients can be taken up by *R. chamaemorus* at the end of the growing season (Fig. 2). Given the asynchronicity of the plant growing season and the period in which the deeper layers are unfrozen, this species is most likely capable of storing nutrients that are acquired late in the growing season in rhizomes (Chapin *et al.*, 1990, Taylor, 1971), ready for quick use in early spring of the next growing season. Although our data do not allow for strong conclusions about other potentially deep-rooting species (but see Fig. 2), it is likely that other deep-rooting rhizomatous species like *E. vaginatum* or *Calamagrostis lapponica* (Callaghan *et al.*, 1991, Chapin *et al.*, 1990, Venhuizen, 2009) are also capable of utilizing nutrients released from thawing permafrost.

Impacts of differential uptake of nutrients from the thaw front on community composition

As hypothesized, both deep- and shallow-rooting species were responsive to shallow fertilization. Many studies have been performed on the effects of increased nutrient supply in shallow soil layers, usually with fertilizer applied to the soil surface (Haugwitz & Michelsen, 2011, Parsons *et al.*, 1995, Press *et al.*, 1998b, Shaver *et al.*, 2001). Such experimentally increased nutrient supply tends to result in an increased abundance of fast-growing, competitive species (mostly graminoids, deciduous shrubs and herbaceous species) at the expense of slow growers (mostly evergreen shrubs and mosses and lichens) (e.g. Aerts *et al.*, 2006b). Our results suggest that fertilizer applied deep-belowground affects a different combination of species than shallow fertilization, due to differences in vertical root distribution (Fig 1, 2), indicating that in this system, spatial niche differentiation seems to be more important than in other systems (de Kroon *et al.*, 2012). The additional N-uptake at the thaw front clearly provided the deep-rooting *R. chamaemorus* with a competitive advantage as it significantly increased its aboveground biomass and N-content in the belowground fertilization experiment after three years of treatment (Fig. 3; Table 1). Moreover, the impacts of shallow- and deep-fertilization on aboveground biomass of this deep-rooting species were remarkably similar in magnitude, given that the moment of maximum thaw depth coincides with the end of the growing season (hypothesis c, Fig. 3). The

importance of these observations is illustrated by the results of fertilizer experiments performed on bogs with a similarly low nutrient status in northern Norway, where *R. chamaemorus* responses to fertilizer applied to the surface of the soil were suppressed by strong responses to the same treatment of competitive deciduous dwarf shrubs in the bog (Taylor, 1971). The discrepancy between our results and the observations of Taylor (1971) demonstrate that competitive species interactions controlled by resource partitioning (niche differentiation between different soil layers) (Berendse, 1982, Casper *et al.*, 2003, McKane *et al.*, 2002) may be affected differentially by increased nutrient supply in shallower layers than by increased nutrient supply at the permafrost thaw front. Hence, increased nutrient supply at the thaw front, in combination with strong spatial niche differentiation caused by differential vertical rooting patterns, may lead in the long term to a species composition shift in favour of deep-rooting species in these nutrient-limited ecosystems.

Additive effects of increased shallow- and thaw-front-nutrient supply

There was no interaction between effects of the shallow-fertilization and the deep-fertilization treatments on aboveground biomass; the effects were additive (hypothesis d; Fig 3a,b; Table 1). Hence, shallow fertilization did not dampen uptake from the thaw front, which indicates that nutrient limitation in these peatlands is strong enough to avoid nutrient saturation, at least until other nutrients such as phosphorus become limiting (Aerts *et al.*, 2001). The amount of nutrients applied in this experiment was relatively large (an initial supply of 8 gN m^{-2}) compared to the amount that might be released from thawing permafrost of subarctic peatlands (up to 0.3 g N m^{-2} yr^{-1} in the coming decade) (Keuper *et al.*, 2012) or to the estimated increase in nutrient supply in shallower layers (e.g. climate-warming in the range of 0.3 - 6.0 ºC would induce an estimated 46% increase of the 0.8 g N m^{-2} yr^{-1} net N mineralisation in northern peatland ecosystems) (Rosswall & Granhall, 1980). Hence, it seems likely that the effects of 'natural' sources of increased N-supply in northern peatlands will also be additive.

Potential longer-term effects

So far, observational records on species composition shifts upon thawing permafrost exist only for two systems, with well documented thaw-histories (our study area: Malmer *et al.*, 2005, Alaska: Schuur *et al.*, 2007). These records show mixed results: in Alaska, a shift from graminoid-dominated tundra in the least disturbed site to shrub-dominated tundra in the oldest most subsided site was observed (Schuur *et al.*, 2007), while in a subarctic permafrost peatland site in our study area, dwarf-shrub-dominated hummock sites receded and gave way to wet sites dominated by graminoids (Malmer *et al.*, 2005). This illustrates that long-term vegetation responses to thawing permafrost are most likely not only due to changes in nutrient availability, but also to multiple related factors such as co-occurring changes in hydrology or soil temperature, which were not included in our experimental design. Therefore, we do not address these related factors in depth, but instead confine this discussion to potential long-term responses to permafrost-thaw-induced changes in nutrient availability.

Long-term plant-responses to permafrost-thaw-induced increases in nutrient availability can be affected by several factors. Firstly, vertical transport of nitrogen via root and leaf litter of deep rooting species from the thaw front to shallower layers may occur. Thus, permafrost-N can, in the longer term, become a new N-source also for shallower-rooting species, who might utilize this N after it has been redistributed through the soil layers. Secondly, microbial biomass is a

strong sink for nutrients in arctic soils (Jonasson *et al.*, 1996) and the competitive strength of the microbial community could increase in response to increased N-supply. However, within the scope of this experiment the large increases in vascular plant biomass in response to fertilization at the thaw front were not matched by similar increases in mean microbial biomass N. Instead, microbial biomass was not affected by the deep fertilization treatment and only slightly by the shallow fertilization treatment (Supporting Information, Fig. SI 4). This suggests that the microbial community benefits less than the vegetation from the additional deep N-source. This may increase the discrepancy between vegetation responses to climate-change-induced shallow- versus deep increases in N-supply in the longer-term. Thirdly, longer-term plant responses may differ from the results of this study due to changes in vertical root biomass distribution in response to increased deep nutrient supply (Hodge, 2004). In this experiment, root biomass at the thaw front tended to increase (up to 100%), but only for the deep-rooting species. This is similar to results obtained for crops where deep root biomass increased in response to localized belowground fertilization (Drew, 1975), and in line with plant 'foraging' as described by Callaghan *et al.* (1991) and McNickle *et al.* (2009). The observed altered root biomass distribution in response to a relatively short-term (three year) experiment suggests that, over the longer-term, the effects on plant productivity of increased nutrient supply at the thaw front may aggravate due to increased root biomass and thus uptake capacity at the thaw front. Overall, although plant responses to thawing permafrost in subarctic peatlands are affected by multiple factors, the experimental results presented in this study suggest that, in the longer term, a release of nutrients from thawing permafrost could accelerate N-cycling in these nutrient-deprived ecosystems.

In conclusion, we have shown for the first time that nitrogen released from thawing permafrost can be taken up by deep-rooting plant species and can result in increased biomass production of such species. Hence, we show that plant-available N from thawing permafrost can be considered a real plant-available 'new' N input. Moreover, our results show that the effect of a deep, belowground release of nutrients on subarctic peatland vegetation is fundamentally different from the effect of a shallow increase in nutrient availability. In the longer term, this climate change-induced new N source may lead to an accelerated N cycle and changes in species composition of subarctic peatlands.

Acknowledgements

The authors would like to thank M. Johansson and M. Thompson for inspiring discussions and G. Blume-Werry for helpful comments on the manuscript. Many thanks go to the staff of the Abisko Naturvetenskapliga Station for technical assistance and support. Further, this research would not have been possible without the luminous assistance of R. v. Bogaert, B. Hidding, and J. Mulder. Financial support was offered to FK by the Darwin Centre for Biogeosciences (grant 142.161.042) and ANS Scholarship 2008, and to RA by the Dutch Polar Program (ALW-NPP grant 851.30.023) and the EU-ATANS (grant FP6 506004).

Supporting Information

Figure SI 1. Injection of ^{15}N-ammonium solution through a metal tube into the thaw-front.

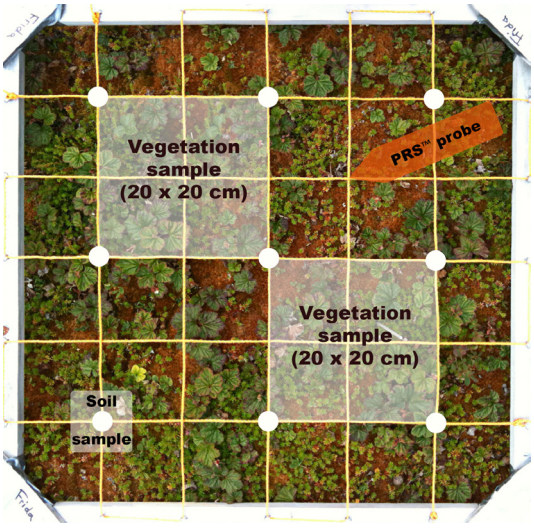

Figure SI 2. A plot of the thaw-front-fertilization experiment: the white dots represent the 9-points slow-release fertilizer insertion grid; the two large rectangles depict the biomass harvest areas; and the smaller rectangle is the root biomass and microbial biomass N soil-sampling site.

Thaw-front fertilization experiment: effects on N-supply

To verify treatment effects, plant root simulator (PRS™) soil probes (Western Ag Innovations Inc., Saskatoon, Canada), also known as ion exchange resins were inserted in four plots per treatment and at two depths: 5-15 cm and 35-45 cm deep, two years after the start of the experiment. The PRS probes were inserted at the time of maximum thaw depth (2nd half of September) and removed exactly one year later. After extraction from the soil, the probes were carefully cleaned with deionized water and analysed to obtain inorganic N exchange rates during the incubation period.

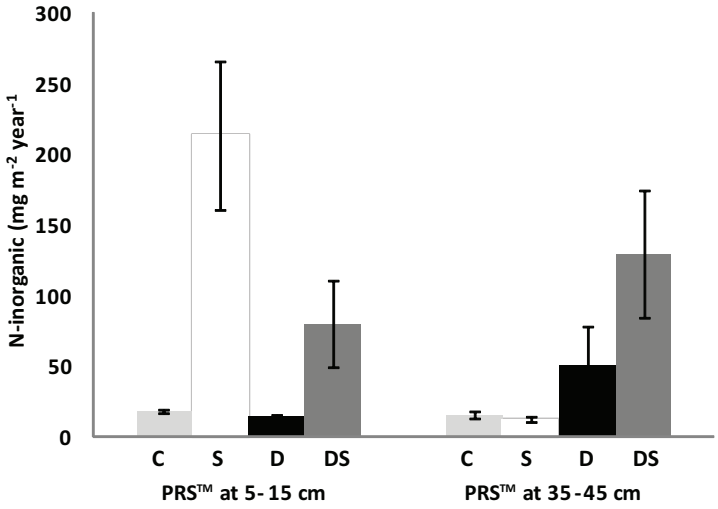

Figure SI 3. Mean PRSTM-probe N-exchange rate (± SE) data after a year-round burial in the thaw-front fertilization experiment at two depths (surface = 5-15 cm, deep = 35-45 cm; *n* = 4). Treatments are: control (C); shallow-fertilized (at 10 cm depth; S); deep-fertilized (at 45 cm deep; D); deep- and shallow-fertilized (DS).

Statistics (effects on N-supply)

Log-transformed N-exchange rates for the shallow and deep inserted probes, respectively, were analysed with two separate two-way ANOVAs to test for the effects of the four different treatments (control, shallow, deep, shallow-and-deep) which were separated by a Tukey's HSD post-hoc test. For the shallow-inserted probes, treatment effects were significant (*F*-value 16.92 and $P < 0.001$) and S>DS>C>D. For the deep-inserted probes treatment effects were significant as well (*F*-value 4.65 and $P < 0.05$) and DS>D>C>S, showing that our treatment had indeed the desired effect on soil nutrient availability (Fig. SI 3).

Microbial biomass N in the full-factorial below-ground fertilization experiment

Additionally to differential below-ground plant-species interactions at the thaw front and in shallower soil layers, below-ground plant-microbe interactions might also differ among these soil layers. For example, if less microbial immobilization of nutrients would occur at the thaw front due to a less active microbial community, nutrients released there would be relatively more plant-available than in shallower soil layers (Keuper *et al.*, 2012).

Because the microbial community can be a strong competitor for nutrients in subarctic ecosystems, we also determined microbial biomass N (N_{mic}) in soil samples taken both in shallow soil layers (5- 15 cm depth) as well as at the thaw front (35-45 cm depth) in the full-factorial belowground fertilization experiment in order to gain insight in potential changes in competitive strength of the microbial community in response to increased belowground nutrient supply.

We hypothesized that low initial microbial biomass would lead to no significant increase of the N immobilized by the microbial community at the thaw front, whereas we did expect an increase in N_{mic} in the shallower soil layers (at 5-15 cm depth) in response to shallow below ground fertilization.

Methods N_{mic} determination

After the final vegetation biomass harvest and at the time of maximum thaw-depth, soil samples for N_{mic} determination were taken in the full-factorial belowground fertilization experiment. One core per plot was extracted and two samples per core (5-15 cm and 35-45 cm) were transported to the lab in a cooling box with icepacks and subsequently frozen at -18 °C until further analysis. Subsamples of 3 x 3 x 3 cm were taken in the field from each sample for determination of fresh weight per soil volume (bulk density). For determination of the N_{mic} pool, two subsamples of each 5 gram fresh soil were taken, one of which was chloroform-fumigated for 24 hours at ~20 °C in a darkened desiccation jar. Both subsamples were then extracted after two hours shaking in 25.0 ml 0.5 M K_2SO_4, and oxidized by potassium persulfate digestion for determination of total extractable N (Cabrera & Beare, 1993). N_{mic} pool sizes were calculated as the differences between fumigated and non-fumigated extracts, using correction factor KEN = 0.40, representative for organic soils, to account for microbial tissue N that is not released by exposure to chloroform (Jonasson *et al.*, 1996). Values were expressed as μg N_{mic} cm^{-3} fresh soil.

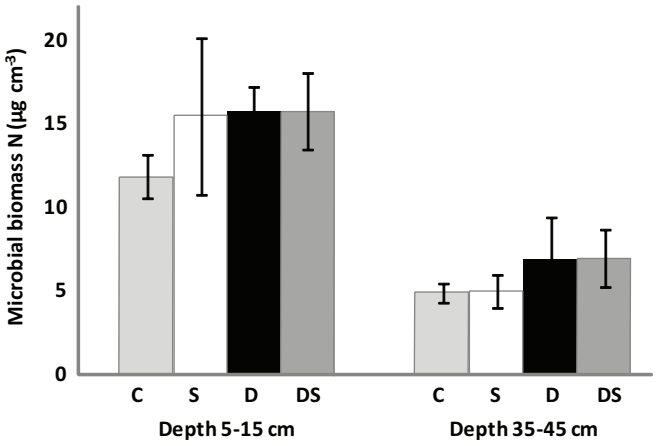

Figure SI 4. Mean (± SE) microbial biomass N pool sizes at two depths in the thaw-front fertilization experiment at the time of maximum thaw. Treatments are: control (C); shallow-fertilized (S); deep-fertilized (D) and shallow- plus deep-fertilized (SD). Only shallow-fertilization had a small positive effect ($P < 0.1$) on microbial biomass N (RM-ANOVA with 'sampling depth' as the within-subject factor and the two treatments 'shallow-fertilization' and 'deep-fertilization' as the between-subject factors, $n = 8$).

Statistical analysis N_{mic} determination in the full-factorial below-ground fertilization experiment

Microbial biomass N (N_{mic}) data were log(x+1) transformed to improve the homogeneity of residual variances. The N_{mic} data from the belowground fertilization experiment were analysed with an (RM)-ANOVA with 'sampling depth' (shallow/deep) as the within-subject factor and 'shallow-fertilization' and 'deep-fertilization' as the between-subject factors.

Results N_{mic} in the full-factorial belowground fertilization experiment

The mean microbial biomass N (N_{mic}) in the shallow rooting zone was more than twice as large as mean N_{mic} at the thaw front ('sampling depth' $F = 28.0$; $P < 0.001$). There were no significant interactions between the sampling depth and the treatments ('sampling depth x shallow-fertilization' $F = 0.04$; $P > 0.1$; 'sampling depth x deep-fertilization' $F = 0.16$; $P > 0.1$) and no significant effect of the deep fertilization treatment on microbial biomass N ($F = 0.13$; $P > 0.1$) but there was a positive trend of shallow fertilization on N_{mic} ($F = 3.9$; $P < 0.1$) (Fig. SI 4).

Chapter VI

General discussion

The aim of this thesis was to evaluate the responses of permafrost peatland vegetation at the community and the plant level (for both vascular and bryophyte plants) to several important aspects of climate change. Vegetation responses to direct effects of climate change (warming, increased precipitation, Chapter 2 and 3) as well as to indirect effects (increased nutrient availability as a result of permafrost thawing, Chapter 5) were investigated. In Chapter 4, the potential amount of plant-available N that can be released from thawing permafrost peatlands was presented, which is essential information for estimating the potential impact of permafrost thawing on tundra vegetation.

In this general discussion, the relative impacts of the investigated direct (warming, precipitation) and indirect effects (increased nutrient availability) of climate change on permafrost peatland vegetation will be analysed and compared. By means of conversion of the observed vegetation responses to units of carbon uptake, an answer is provided to the question: what is the relative contribution of the studied climate factors to changes in C-uptake by peatland vegetation (through changes in species specific growth and vegetation biomass) in northern permafrost peatlands? Moreover, an attempt is made to relate the findings presented in this thesis to the grand overarching climate change question: "what do these vegetation responses mean for the carbon sink-function of northern permafrost peatlands?"

1. Estimation of climate impacts on C-uptake

1a. Warming

In Chapter 2, we showed that our permafrost peatland vegetation community was more stable in response to manipulations of spring and summer temperature than is typically observed in other (sub-)arctic experiments (Elmendorf *et al.*, 2012). Even after eight years of climate manipulations we observed no changes in total vascular plant abundance, nor in individual species abundances, Shannon's diversity or evenness. These findings suggest that changes in carbon-sequestration through changes in species composition (differing in carbon turnover times) are unlikely for this system, which makes this system particularly different from other tundra ecosystems where often increases in shrub abundance are observed. Such changes may lead to increased C-storage in wood (Myers-Smith *et al.*, 2012). In our system, the proposed mechanism through which the stability was achieved is 'a race for space': increased growth of both vascular plants as well as the peatmoss. Hence, total biomass increased although this was not reflected in vascular plant community composition. Thus, despite community stability in response to the experimentally altered climate, carbon sequestration most likely did increase, as all separately measured individual species showed increased productivity at the individual plant level. Vascular plant biomass production increased by up to approximately 130 %, based on the average increase in aboveground *E. hermaphroditum* and *B. nana* productivity as weighted by their contribution to total aboveground vascular biomass. This would lead to an annual increase in C-uptake of 20 g C m^{-2}, which, when extrapolated to all permafrost peatland area, adds up to 70 Mt C annually (Box 1 and 2). Moreover, bryophyte (*Sphagnum fuscum*) productivity responses to higher summer temperatures increased with approximately 200% (based on vertical growth measurements, Chapter 2), leading to an impressive annual increase in C-uptake of 150 g C m^{-2} (based on an observed ambient productivity of 75 g C m^{-2} which is lower than but comparable to *S. fuscum* productivity values of 112 g C m^{-2} observed by Street (2012). When extrapolated to all permafrost peatland area, this adds up to 535 Mt C annually[1] (Box 1 and 2).

Box 1. Carbon-uptake by permafrost peatland vegetation

Assumptions

Permafrost peatland facts

1. Annual vascular production: 50 g m^{-2} (Ch. 3, this thesis)

2. Annual bryophyte production: 150 g m^{-2} (Ch. 3; Moore 1989; Street 2012)

3. Carbon percentage vegetation: 50 % (F. Keuper, unpublished data)

Maximum impact of studied climate factors on productivity

4. Warming: vascular plants 80 % increase (Ch. 2, this thesis)

5. Warming: bryophytes 200 % increase (Ch. 2, this thesis)

6. Below-ground fertilization: vascular 20 % increase (Ch. 5, this thesis)

Calculations of carbon-uptake by permafrost peatland vegetation

Maximum effect of warming (Ch. 1)

I. [Annual vascular C uptake] x [80 % (4)] = **20 g C m^{-2} yr^{-1}**

II. [Annual bryophyte C uptake] x [200 % (5)] = **150 g C m^{-2} yr^{-1}**

Maximum effect of permafrost thaw (indirect, via increased thaw-front N, Ch. 5)

III. [Total annual vascular C production] x [20 % (6)] = **5 g C m^{-2} yr^{-1}**

1b. Increased precipitation

In Chapter 3, we showed that the vegetation of the studied permafrost peatland was irresponsive (both in terms of vascular and bryophyte biomass and of species composition) to doubling of the ambient summer precipitation. Precipitation in the sampling area (Abisko) in the years of sampling (2008 and 2009) was lower than the long-term average, with 2009 being the 11th driest year since 1913 (Callaghan *et al.*, 2010, Olefeldt *et al.*, 2012). During dry years, an experimental increase in summer precipitation would have had a maximum effect on growth of the vegetation, yet no effect was observed. Moreover, our results for permafrost peatlands are in line with the established pattern of negligible tundra plant productivity responses to experimentally increased summer precipitation (Dormann & Woodin, 2002). Thus, in terms of carbon currency, no effects of increased precipitation are expected on vegetation performance of permafrost peatlands.

[1] Although we did not measure bulk density of the *Sphagnum* carpet, this could be negatively affected by the warming treatment (Dorrepaal, 2004). However, even when taking into account a potential decrease in bulk density of 20% (Dorrepaal, 2004), warming would still lead to an extra uptake of approximately 430 Mt C annually.

1c. Permafrost thaw induced increase in N-availability

In Chapter 4, we showed that thawing permafrost of subarctic peatlands can release plant-available N (up to 1.3 g N m^{-2} in the near-future). In contrast with the irresponsiveness of the permafrost peatland vegetation to experimentally increased precipitation, a rather large increase in total biomass production (up to 20%) of the vascular vegetation was observed in response to deep (40 cm) below-ground fertilization (Chapter 5). Note should be taken that this is a maximum value, as the amount of fertilizer added experimentally was higher than what can be expected to be released from thawing permafrost[2] (Chapter 4). However, although vegetation responses to a release of nutrients in deeper soil layers such as presented in this thesis cannot be inferred in a straightforward manner from studies where fertilizer was applied at the soil surface,

Box 2. Carbon-comparisons for sink and source strength of permafrost peatlands

Assumptions about C-sink strength in a changing climate

Total permafrost peatland area

1. 3.5 x 10^{12} m^2 (Tarnocai 2009)

Maximum effect of warming (Ch. 1)

2. Total area x effect on vascular C uptake [Box 1] = **70 Mt C yr^{-1}**

3. Total area x effect on bryophyte C uptake [Box 1] = **535 Mt C yr^{-1}**

Maximum effect of permafrost thaw (indirect, via increased thaw-front N, Ch. 5)

4. Total area x effect on vascular C uptake [Box 1] = **18 Mt C yr^{-1}**

Assumptions about C-source strength in a changing climate

1. Maximum C-source strength in response to warming:

100 Mt C yr^{-1} (Dorrepaal 2009)

2. Permafrost thaw induced C-source strength:

1000-2000 Mt C yr^{-1} (Schuur 2011)

Comparisons C-uptake by vegetation with C-source strength

1. In response to **warming**, C-uptake by vegetation (70 + 535 Mt C yr^{-1}) is **6 times larger** than C-release by the deeper soil layers.

2. The **N-availability related effect of permafrost thawing** on C-uptake by vegetation (18 Mt C yr^{-1}) is approximately **50-100 times smaller** than C-release by permafrost thawing.

the observed positive response in vegetation productivity (up to 20% increase) to increased nutrient availability is in line with many fertilization experiments that were carried out in subarctic tundra (Haugwitz & Michelsen, 2011, Lamb *et al.*, 2011, Parsons *et al.*, 1994, Shaver *et al.*, 2001). In carbon currency, 20% higher permafrost peatland vegetation productivity would mean an increased C-uptake of 5 g C m^{-2} yr^{-1} (18 Mt C yr^{-1} for all permafrost peatlands) (Box 1 & 2).

2. Permafrost peatlands and climate change: comparison of sink and source strengths

2a. Direct effects

The values for increased C-uptake (carbon sink function) through increased vegetation productivity in response to spring- and summer warming (yearly 70 Mt C for vascular plants and 535 Mt C for bryophytes), easily offset the predicted increase in carbon (CO_2) emissions based on measurements in the same experiment, for which the maximum given value is an annual increase of 100 Mt C per year (Dorrepaal *et al.*, 2009)(Box 2). No direct effect of warming on methane (CH_4) emission is expected in permafrost peatlands without a groundwater table and with relatively mild redox conditions[3] (Dorrepaal *et al.*, 2009, Roulet *et al.*, 1992). In wetter (permafrost) peatlands, temperature is one of the primary factors influencing CH_4 emission (Moore *et al.*, 2011) and can have a direct positive impact of up to 40% (based on Q_{10} ranging between 2 and 3) (Svensson & Rosswall, 1984, Williams & Crawford, 1984), although large variation exists in observed Q_{10} for the direct effect of warming on methane emission (Segers, 1998, van Hulzen *et al.*, 1999). Even so, estimations based on four warming scenarios (part of the IPCC fifth assessment report) show that most of the released carbon will be in the form of CO_2, with only about 2.7% in the form of CH_4 (Schuur *et al.*, 2011). In line with this, Lee *et al.* (2012) conclude that the direct potential effect on climate from permafrost C release is greater under aerobic (dry) conditions than under anaerobic (wet) conditions, even when taking the higher greenhouse warming potential[4] of CH_4 into account. Hence, the net direct effect of warming on C-uptake and release suggests that climatic warming will increase the carbon sink-strength of permafrost peatlands.

[2] An exact value can not be provided here because the fertilizer was added in the form of 'slow release grains', of which the exact release rate is not known. The inserted amount was equivalent to 8 g N m^{-2}.

[3] The permafrost peatlands studied in this thesis are ombrotrophic peatlands, which, by definition, do not have a groundwater table. Unfortunately, the available global area coverage data which are generally used for upscaling results of permafrost peatlands (Walker 2004; Tarnocai 2009; Gorham 1991) do not distinguish between ombrotrophic and non-ombrotrophic peatlands, nor is the dominant type of peatmoss specified. This is a major uncertainty in the calculations presented in this chapter. Fortunately, for the response to warming specific C-source data were available from the same experimental site (Dorrepaal 2009). However, for the C-source strength in response to permafrost thawing, I chose to use an educated guess provided by the permafrost community in Schuur (2004), which is not ombrotrophic peatland specific. I could have used 19% of this educated guess number, to represent the percentage of permafrost area that is covered by peatlands (Tarnocai 2009), but as most greenhouse gas emissions are expected to come from soils with high organic carbon contents, this correction would most likely give an underestimation. Hence, under the assumption that the main part of the C-source strength of permafrost will come from permafrost peatlands, I used the number provided by Schuur (2011).

[4] CH4 has a greater global warming potential (GWP CH4 = 25) than CO2 (GWP = 1) over a 100-year time period (IPCC, 2007).

2b. Indirect effects

The value for C-uptake by vegetation as a result of increased N-availability in response to permafrost thawing (18 Mt C per year, Box 2) is approximately 50 to 100 times smaller than the estimate for the increase in total C-emissions as a result of permafrost thaw (faster release of CO_2, CH_4 and DOC) based on an educated guess from the permafrost community (Schuur *et al.*, 2011). This estimation of carbon release from permafrost degradation was 30 billion to 63 billion ton of carbon by 2040 (equal to approximately 1000-2000 Mt C yr^{-1}) (Box 2). Hence, apart from being one order of magnitude smaller than the direct effect of warming, the effects of increased N-availability through permafrost thawing on the sink strength of permafrost peatlands would, according to these simple calculations, most likely not counteract a shift from sink to source function of permafrost peatlands as a result of permafrost thawing.

2c. Factors potentially affecting the calculations

The carbon calculations presented here are fairly crude. Firstly, vegetation changes were not observed within the scope of the performed experiments, and thus not taken into account in the calculations in Box 1 (purely based on the work performed in this thesis). However, in response to warming for example, vegetation changes may occur on the longer term in permafrost peatlands with a more dense vascular cover, where the here observed bryophyte-driven stability in the vascular community (Chapter 2) does not take place (Elmendorf *et al.*, 2012). Unfortunately, detailed information about vegetation density and bryophyte cover is not yet available for all area covered by permafrost peatlands (Walker *et al.*, 2005). For this reason, it remains challenging to upscale the experimental findings or estimate the longer-term implications for C-uptake of either vegetation changes or the observed bryophyte-vascular community interaction (Chapter 2) for permafrost peatlands. Another potential driver of longer-term vegetation shifts on the longer term which was beyond the scope of this thesis, is degrading palsa structure due to disappearance of the physical support provided by permafrost, and associated changes in hydrology. Accompanying shifts to a graminoid-dominated vascular community could lead to mildly increased C-sequestration by the vegetation of up to 4 g C m^{-2} yr^{-1} (7.3 %) (Malmer *et al.*, 2005). However, such changes in hydrology are also associated with faster CO_2 and CH_4 release and increased outflow of dissolved organic C from permafrost peatlands (Christensen *et al.*, 2004, von Deimling *et al.*, 2012). Moreover, such changes in hydrology could increase outflow of plant available N from permafrost peatlands (Frey & McClelland, 2009, McClelland *et al.*, 2007) and thus negatively affect our estimations of potential increased C-uptake by vegetation as a result of increased N-availability through thawing of permafrost soil.

A second simplification in our calculations is the omission of potential changes in belowground biomass. Since approximately 80% of vascular plant biomass in northern tundra ecosystems resides below-ground (Chapin & Ruess, 2001), not taking into account potential changes in this carbon pool is a major oversight (van Noordwijk *et al.*, 1998). This is most likely especially true for permafrost affected ecosystems, where plant root growth is restricted by the depth of the active layer (Billings *et al.*, 1976) that will increase as a result of permafrost thawing (Akerman & Johansson, 2008). If aboveground-belowground allometry remains stable (or decreases), increased belowground biomass (Chapter 5) and concomitant C-uptake can be expected. Hence,

not taking potential changes in belowground biomass into account may lead to a conservative estimate of C-uptake by the vegetation in response to climatic changes by permafrost peatlands.

Overall, the discussed interactions between different elements of the permafrost peatland system (plant-plant; plant-microbe; plant-permafrost soil) can be affected by climatic changes in ways that were not addressed in this thesis. Further, unforeseen feedback mechanisms may occur on the longer term and either increase or decrease C-sequestration. Yet, the calculations presented in this chapter are, to our knowledge, the first calculations specifically about the vegetation productivity component of the carbon balance of permafrost peatlands. Moreover, the straightforward comparison between C-uptake in response to specific experimental manipulations relevant to current climate change scenarios is, to our knowledge, unique. Hence, the insight these calculations provide in the sink-strength of these climate sensitive ecosystems is, albeit crude, highly relevant in order to direct future research.

My recommendations for future research include a stronger focus on belowground processes, both plant-root as well as microbe related. Moreover, ongoing research in long-term experiments is essential for detecting longer-term changes in vegetation community and microbial activity as a result of climatic changes. And lastly, since according to the calculations provided here (Box 1 & 2) the peatmoss *Sphagnum fuscum* can play a crucial role in the carbon-sink function of permafrost peatlands, more research should be performed on total land area coverage and on factors affecting the productivity of this 'living soil'.

3. Conclusions

In summary, the work presented here suggests that the direct effect of warming, and the indirect effect of thawing of permafrost soil via a release of plant available nutrients, will have a greater impact on carbon-uptake (sink-function) of permafrost peatland vegetation than an increase in summer precipitation. The sum of the here discussed direct effects (623 Mt C yr^{-1}) and indirect effects (18 Mt C yr^{-1}) of climate change on the C-sink value of permafrost peatlands is large enough to offset approximately half the predicted release of carbon from thawing permafrost (1000-2000 Mt C yr^{-1}, Schuur 2011). Hence, in depth understanding of the underlying processes of changes in carbon uptake as a result of climate change, as provided in this thesis[5], is of major importance to estimate future impacts of climatic changes on the terrestrial carbon balance.

[5] Note: according to the latest IPCC report (2007), the anthropogenic C-source strength is approximately 13.4 x 10^3 Mt C yr^{-1}. This is at minimum one order of magnitude larger than the projected climate driven release of greenhouse gases from permafrost soils and at least two orders of magnitude larger than the in this chapter presented maximum value for increased C-uptake by peatland vegetation in response to climatic changes. Hence, "despite the massive amount of carbon stored in permafrost soils, emissions from these soils are unlikely to overshadow those from the burning of fossil fuels, which will continue to be the main source of climate forcing" (Schuur, 2011). Increased C-uptake by permafrost peatland vegetation will not easily offset this.

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Summary

Strong climate warming and altered precipitation regimes are predicted to occur at higher latitudes this century, with potentially major consequences for vegetation productivity and carbon sequestration. Although northern permafrost peatlands contain one-third of the world's soil organic carbon, little is known about responses of their vascular plant communities to climatic changes. Both direct effects, of warming and altered precipitation, as well as indirect effects, through permafrost thawing and increased nutrient-availability, are expected. In this thesis I aimed to investigate how short- to long-term experimental climate manipulations, relevant to different predicted future climate scenarios, affect vascular plant productivity and species composition in permafrost peatlands. Specifically, answers were sought to the following research questions: (1) How do spring- and summer warming, and increased snow cover affect species-specific growth responses and species composition in northern permafrost peatlands; (2) How does increased summer precipitation affect species-specific growth responses and species composition in northern permafrost peatlands; and (3) Can permafrost thawing affect species-specific growth responses and species composition in northern permafrost peatlands through a release of plant-available N? To answer these questions three experiments were performed, investigating vegetation responses to: (a) spring- and summer warming and a thicker snow cover (Chapter 2); (b) increased summer precipitation (Chapter 3); and (c) increased nutrient availability at the permafrost thaw front (Chapter 5). Moreover, the amount of plant-available nitrogen (N) that can be released from thawing permafrost into these peatlands in the near-future was quantified (Chapter 4). All experiments were performed on *Sphagnum fuscum*-dominated permafrost peatlands the Abisko area, northern Sweden. The increased summer precipitation experiment (Chapter 3) was complemented by a sister-experiment in the Kytalyk Reserve in north-eastern Siberia.

I found that in response to 8 years of experimental spring- and summer warming, and (moderately) thicker snow cover, vascular plant species composition of *Sphagnum fuscum*-dominated permafrost peatlands was more resistant than is typically observed in (sub)arctic experiments: neither changes in total vascular plant abundance, nor in individual species abundances, Shannon's diversity or evenness were found in response to the climate manipulations. For three key species (*Empetrum hermaphroditum*, *Betula nana* and *S. fuscum*) it was also determined whether the treatments had a sustained effect on plant length growth responses and how these responses interacted. Contrasting with the stability at the community level, both key shrubs and the peatmoss showed sustained positive growth responses to the climate treatments. However, a higher percentage of moss-encroached *E. hermaphroditum* shoots and a lack of change in *B. nana* net shrub height indicated encroachment by *S. fuscum*, resulting in long-term stability of the vascular community composition. These findings show that in a warmer world, vascular species of subarctic peat

bogs appear to just keep pace with growing *Sphagnum* in their 'race for space'. They contribute to general ecological theory by demonstrating that community resistance to environmental changes does not necessarily mean inertia in vegetation response (Chapter 2).

Secondly, vegetation responses to three years of experimentally increased summer precipitation in two tundra types were investigated, in (a) *B. nana*-dominated shrub tundra (northeast Siberia) and (b) a dry *S. fuscum*-dominated bog (northern Sweden). These tundra types were not previously addressed in increased precipitation studies. Positive responses to approximately doubled ambient precipitation (an increase of 200 mm yr^{-1}) were observed at the Siberian site, for *B. nana* (30% larger length increments), *Salix pulchra* (leaf size and length increments) and *Arctagrostis latifolia* (leaf size and specific leaf area), but none were observed at the Swedish site. Total biomass production did not increase at either of the sites. This work corroborates studies in other tundra vegetation types and shows that despite regional differences at the plant-level, total tundra plant productivity is, at least at the short or medium-term, largely irresponsive to experimentally increased summer precipitation (Chapter 3).

Thirdly, the amount of plant-available nitrogen that can be released by near-surface permafrost soil of permafrost peatlands was quantified (Chapter 4), and how such a release can affect species-specific growth responses and species composition in northern permafrost peatlands was investigated (Chapter 5). Plant-available N-pools and -fluxes in near-surface permafrost soil samples (taken 0-10 cm below the thaw front) were compared to those taken from a current rooting zone layer (5-15 cm depth) across five representative permafrost peatlands in subarctic Sweden. A range of complementary methods was used: extractions of inorganic and organic N, inorganic and organic N-release measurements at 0.5 and 11 °C (over 120 days, relevant to different thaw-development scenarios) and a bioassay with *Poa alpina* test-plants. All extraction methods, across all peatlands, consistently showed up to seven times more plant-available N in near-surface permafrost soil compared to the current rooting zone layer. These results were supported by the bioassay experiment, with an eight-fold larger plant N-uptake from permafrost soil than from other N-sources such as current rooting zone soil or fresh litter substrates. Moreover, net mineralisation rates were much higher in permafrost soils compared to soils from the current rooting zone layer (273 mg N m^{-2} and 1348 mg N m^{-2} per growing season for near-surface permafrost at 0.5 °C and 11 °C respectively, compared to -30 mg N m^{-2} for current rooting zone soil at 11 °C). Hence, these results demonstrate that near-surface permafrost soil of subarctic peatlands can release a biologically relevant amount of plant available nitrogen, both directly upon thawing as well as over the course of a growing season through continued microbial mineralisation of organically bound N (Chapter 4). However, being plant-available does not necessarily imply that peatland plant species will be able to take up this N at the thaw front. Therefore, I identified the potential impact of increased N-availability due to thawing permafrost on subarctic peatland plant production and species composition. This impact was compared to the effect of increased nutrient availability in shallower layers (e.g. through enhanced N-mineralization due to climatic warming). To achieve this, ^{15}N-labeling of the thaw front was applied and a full-factorial belowground fertilization experiment (deep-fertilization at the thaw front at 45 cm depth and shallow-fertilization at 10 cm depth) was performed. I found that only particular species (e.g. *Rubus chamaemorus*) have active roots at the thaw front. Further, once supplied with nitrogen at the thaw front, these species had higher aboveground biomass and N-content, whereas this was not the case for shallower-rooting species (e.g. *E. hermaphroditum* and *Andromeda polifolia*). Moreover, the effects of increased nutrient availability

at the thaw front on total aboveground biomass production were similar in magnitude to the effects of increased nutrient availability in shallower layers. Additionally, nutrient limitation of plant growth in subarctic peatlands appeared to be strong enough for the effects of increased deep and shallow nutrient availability on biomass production to be additive. Altogether, these results show that plant-available N released from thawing permafrost can be considered a true 'new' N source for deep-rooting sub-arctic plant species, which will increase their biomass production. As this is not the case for shallow-rooting species, the release of plant-available N from thawing permafrost has the potential to alter species composition on the long-term by benefitting specific deep-rooting species only (Chapter 5).

This thesis was concluded by a comparison of response sizes to the different investigated direct and indirect effects of climate change on permafrost peatlands. The comparison was based on potential responses, expressed using carbon-uptake as a unifying currency. The largest response in carbon-uptake by permafrost peatland vegetation is to be expected from warming (approximately 170 g C m^{-2} yr^{-1}), mainly through increased productivity of the peatmoss *Sphagnum fuscum* (approximately 150 g C m^{-2} yr^{-1}), followed by the vascular vegetation responses to increased nutrient availability through thawing permafrost (5 g C m^{-2} yr^{-1}). No effects of increased precipitation are expected on vegetation performance of permafrost peatlands. Based on an estimated permafrost peatland area of 3.5 x 10^{12} m^2 (Tarnocai 2009), the potential increase in C-sink strength as a result of increased vegetation productivity calculated on the findings presented in this thesis would be at approximately 600 Mt C yr^{-1} in response to warming, and 18 Mt C yr^{-1} in response to increased nutrient availability as a result of permafrost thawing. Although these increases in C-sink strength are about halve the total global expected C-source strength as a result of permafrost thawing (1000-2000 Mt C yr^{-1}, Schuur *et al.* 2011), they are about 6 times higher than the projected increased C-source strength of permafrost peatlands in response to warming, for which the maximum given value is an annual increase of 100 Mt C yr^{-1} (Dorrepaal *et al.*, 2009). Therefore, further in depth understanding of the underlying processes of changes in carbon uptake as a result of climate change, as provided in this thesis, is of major importance for estimating future impacts of climatic changes on the terrestrial carbon balance.

Synopsis

Voor noordelijke streken worden nog binnen deze eeuw sterke klimaatsveranderingen voorspeld, zoals hogere temperaturen en veranderende neerslagpatronen. Deze veranderingen kunnen grote gevolgen hebben voor vegetatieproductiviteit en koolstofopslag. Een derde van de organische koolstof die mondiaal opgeslagen is in bodems bevindt zich in noordelijke permafrostbodems, met name in veengebieden. Er is nog weinig bekend over de reactie van plantengemeenschappen in deze veengebieden op klimaatsveranderingen. Men verwacht directe effecten van klimaatsverandering op deze plantengemeenschappen, bijvoorbeeld de effecten van opwarming en veranderende neerslagpatronen, maar ook indirecte effecten door het ontdooien van permafrostbodems en door toenemende nutriëntenbeschikbaarheid.

Voor dit proefschrift getiteld 'Directe en indirecte effecten van klimaatsverandering op vegetatieproductiviteit en soortensamenstelling van permafrost veengebieden' heb ik onderzocht hoe experimentele manipulaties van het klimaat, zowel op de lange als op de korte termijn en relevant voor verschillende klimaatscenarios de productiviteit en samenstelling van plantengemeenschappen in permafrost veengebieden beïnvloeden. In het bijzonder heb ik getracht de volgende onderzoeksvragen te beantwoorden: (1) Hoe beïnvloeden voorjaars- en zomeropwarming en een dikkere sneeuwlaag de soortspecifieke groeiresponsen en soortensamenstelling in noordelijke permafrost gebieden; (2) Hoe beïnvloedt meer zomerneerslag (regen) de soortspecifieke groeiresponsen en soortensamenstelling in noordelijke permafrost veengebieden en (3) kan het ontdooien van permafrost een effect hebben op soortspecifieke groeiresponsen en soortensamenstelling in noordelijke permafrost veengebieden via het vrijkomen van plantbeschikbaar stikstof (N)?

Om deze vragen te beantwoorden zijn er drie experimenten opgezet, om vegetatie responsen op de volgende factoren te onderzoeken: (a) voorjaars- en zomeropwarming en een dikkere sneeuwlaag (Hoofdstuk 2); (b) verhoogde zomerneerslag (Hoofdstuk 3) en (c) verhoogde nutriëntenbeschikbaarheid aan het permafrost dooifront (Hoofdstuk 5). Daarnaast heb ik gemeten hoeveel plantbeschikbaar N er door het ontdooien van de permafrost in noordelijke veengebieden vrij kan komen in de nabije toekomst (Hoofdstuk 4). Alle experimenten werden uitgevoerd in door *Sphagnum fuscum* (bruin veenmos) gedomineerde veengebieden nabij Abisko in Zweeds Lapland. Het verhoogde neerslag experiment werd daarnaast gecomplementeerd met een zusterexperiment in het Kytalyk Reservaat in noordoost Siberië.

Uit het eerste experiment is gebleken dat de soortensamenstelling van noordelijke *Sphagnum fuscum* veengebieden met permafrost beter bestand is tegen experimentele voorjaars- en zomeropwarming en een dikkere sneeuwlaag dan over het algemeen geobserveerd wordt in (sub)arctische klimaatveranderingsexperimenten: er waren geen veranderingen in abundantie

van alle vaatplanten samen, noch in de individuele abundanties van de specifieke soorten, Shannon's Diversity en Evenness in reactie op de klimaatmanipulaties. Voor drie sleutelsoorten (*Empetrum hermaphroditum*, *Betula nana* en *S. fuscum*) werd bovendien bepaald of de behandelingen effect hadden op de lengtegroeiresponsen en of deze responsen elkaar beïnvloedden. In tegenstelling tot de stabiliteit op het plantengemeenschapsniveau bleek de lengtegroei zowel van de twee struiksoorten als van het veenmos positief te reageren op de behandelingen. Echter, uit een hoger percentage door mos overwoekerde *E. hermaphroditum* (kraaiheide) takjes en het ontbreken van een toename in netto struikhoogte van *B. nana* (dwergberk), bleek overgroeiing door *S. fuscum*. Deze overgroeiing verklaart de stabiliteit van de vaatplantsamenstelling op de lange termijn. Onze bevindingen laten zien dat in een opwarmende wereld de vaatplantsoorten in subarctische veengebieden gelijk op gaan met het oprukkende veenmos in hun 'Race for Space'. De bevindingen dragen bij aan de algemene ecologische theorie door te laten zien dat weerstand van een plantengemeenschap tegen milieuveranderingen niet perse via inertie in vegetatieresponsen plaatsvindt.

In het tweede experiment werden de vegetatieresponsen op drie jaar experimenteel verhoogde zomerneerslag in twee typen toendra onderzocht, te weten (a) in *B. nana* gedomineerde struiktoendra (noordoost Siberie) en (b) in een droog *S. fuscum* gedomineerd veengebied (noord Zweden). Deze typen toendra werden niet eerder onderzocht in het kader van de invloed van een toename in neerslag. Op de Siberische onderzoekslocatie werden positieve plantresponsen op een experimentele verdubbeling (een toename van 200 mm per jaar) van de natuurlijke neerslag waargenomen, voor zowel *B. nana* (30 % meer lengtegroei) als voor Salix pulchra (toegenomen bladgrootte en lengtegroei) en Arctagrostis latifolia (grotere bladgrootte en 'Specific Leaf Area'). Op de Zweedse onderzoekslocatie was geen meetbare respons op de bewatering waarneembaar. De totale biomassaproductie bleef op beide locaties onveranderd. Dit werk ondersteunt studies in andere toendravegetatietypen en toont aan dat ongeacht regionale verschillen op het plantniveau, de totale productiviteit van toendraplanten, in ieder geval op de korte tot middellange termijn, grotendeels ongevoelig blijkt voor experimenteel verhoogde zomerneerslag (Hoofdstuk 3).

Verder werd de hoeveelheid plantbeschikbare stikstof die vrij kan komen uit permafrostbodem van noordzweedse veengebieden gemeten (Hoofdstuk 4). Hiertoe werden plantbeschikbare stikstof 'pools' en 'fluxes' in oppervlakkige permafrostbodemmonsters (genomen op 0-10 cm onder het dooifront) vergeleken met bodemmonsters uit de huidige bewortelde bodemlaag (op 5-15 cm bodemdiepte). Deze vergelijking werd gedaan in vijf representatieve permafrost veengebieden in subarctisch Zweden. Een scala aan complementaire technieken werd toegepast: extracties van inorganische en organische stikstof, meting van het vrijkomen van inorganische en organische stikstof zowel bij 0.5 °C als bij 11 °C (relevant voor verschillende dooi-scenarios) gedurende 120 dagen, en een bioassay gebruikmakend van Poa alpina testplanten. Alle extractietechnieken gaven, in alle bemonsterde veengebieden, een gelijksoortig beeld weer: tot zeven maal hogere plantbeschikbare stikstofwaarden in de permafrostbodem dan in de huidige bewortelde bodemlaag. Dit beeld werd bevestigd door de resultaten van het bioassay experiment, waar acht maal grotere stikstofopname door planten werd waargenomen op permafrostbodem vergeleken met opname van andere stikstofbronnen zoals bodem van de huidige bewortelde laag of vers bladstrooisel. Bovendien waren de netto stikstofmineralisaties opmerkelijk hoger in permafrostbodem vergeleken met de bodem uit de bewortelde laag (273 mg N m^{-2} en 1348 mg N m^{-2} per groeiseizoen voor permafrostbodem bij respectievelijk 0.5 °C en 11 °C, vergeleken met -30 mg N m^{-2} voor bodem uit de bewortelde laag bij 11 °C). Deze resultaten laten

zien dat er een biologisch relevante hoeveelheid plantbeschikbare stikstof kan vrijkomen uit permafrostbodems van noordelijke veengebieden, zowel direct na het ontdooien als gedurende de daaropvolgende groeiseizoenen door een voortdurende microbiele mineralisatie van organisch gebonden N (Hoofstuk 4).

In het derde experiment werd bestudeerd hoe het vrijkomen van stikstof uit ontdooiende permafrost de soortspecifieke groeiresponsen en plantensamenstelling in permafrost veengebieden kan beinvloeden (Hoofdstuk 5). Plantbeschikbare stikstof betekent binnen de context van een door permafrost aangedaan ecosysteem niet perse dat de plantensoorten deze stikstof ook daadwerkelijk kunnen opnemen, aan het permafrost dooifront. Daarom heb ik ook specifiek naar de mogelijke invloed van een toename in stikstofbeschikbaarheid aan het dooifront op subarctische veenplantproductie en soortensamenstelling gekeken. Deze mogelijke invloed heb ik vergeleken met het effect van een toename in stikstofbeschikbaarheid in ondiepe bodemlagen (zoals bijvoorbeeld kan ontstaan als gevolg van sterkere stikstofmineralisatie als gevolg van een warmer klimaat). Hiertoe heb ik ^{15}N-labeling van het dooifront toegepast en een full-factorial ondergronds bemestingsexperiment uitgevoerd (met diepe bemesting aan het dooifront op 45 cm diepte, en ondiepe bemesting op 10 cm bodemdiepte). Hieruit bleek dat slechts bepaalde soorten (bv. Rubus chamaemorus) actieve wortels aan het permafrost dooifront hebben. Verder bleek dat wanneer deze soorten stikstof aan het dooifront toegediend kregen, dit een positief effect had op de bovengrondse biomassa productie en op het bladstikstofgehalte. Dit was niet het geval voor de ondieper wortelende soorten (bv. *E. hermaphroditum* en Andromeda polifolia). Ook bleek dat de effecten op de totale biomassa van toegenomen nutrientenbeschikbaarheid aan het dooifront van gelijke grootte waren als de effecten van toegenomen nutrientenbeschikbaarheid in ondiepere lagen. Bovendien bleek de nutrientlimitatie van plantengroei in subarctische veengebieden dermate sterk te zijn dat de effecten van diepe en ondiepe bemesting op biomassa additief waren. Bij elkaar tonen deze resultaten aan dat de plantbeschikbare stikstof vrijkomend uit ontdooiende permafrost daadwerkelijk beschouwd kan worden als een 'nieuwe' stikstofbron voor diepwortelende subarctische plantensoorten, en hun biomassaproductie kan vergroten. Aangezien dit niet geldt voor ondiep-wortelende soorten kan het vrijkomen van stikstof uit ontdooiende permafrost op de lange termijn mogelijk de soortensamenstelling veranderen door slechts diepwortelende soorten te bevoordelen.

Ik sluit dit proefschrift af met een vergelijking van de respons-groottes in reactie op de verschillende onderzochte directe en indirecte effecten van klimaatsverandering op permafrost veengebieden. Deze vergelijking is gebaseerd op potentiele responsen, uitgedrukt in koolstof eenheden. De grootste respons in koolstofopname door vegetatie in permafrost veengebieden kan verwacht worden als gevolg van opwarming (ongeveer 170 g C m^{-2} per jaar), voornamelijk door verhoogde productiviteit van het veenmos *S. fuscum* (ongeveer 150 g C m^{-2} per jaar), gevolgd door vaatplantresponsen op toegenomen nutrientenbeschikbaarheid door ontdooiende permafrost (5 g C m^{-2} per jaar). Verhoogde neerslag heeft waarschijnlijk geen effect op de opname van koolstof door vegetatie in permafrost veengebieden. Gebaseerd op een geschatte mondiale oppervlakte van permafrost veengebieden van 3.5 x 10^{12} m^2 (Tarnocai 2009) zou de potentiele toename in koolstof-'sink' sterkte als gevolg van grotere vegetatieproductiviteit berekend op basis van de in dit proefschrift beschreven bevindingen ongeveer 600 Mt C per jaar zijn als gevolg van opwarming, en 18 Mt C per jaar als gevolg van grotere nutrientbeschikbaarheid door ontdooiende permafrost. Al zijn deze toenames in koolstof-'sink' sterkte slechts half zo groot als de totale verwachte koolstof- 'source' sterkte als gevolg van het ontdooien van permafrost

(1000-2000 Mt C per jaar, Schuur et al 2011), ze zijn zes keer hoger dan de voorspelde toename in koolstof-'source' sterkte van permafrost veengebieden in reactie op opwarming (100 Mt C per jaar, Dorrepaal 2009). Een grondiger begrip van de onderliggende processen van veranderingen in koolstofopname als gevolg van klimaatverandering, zoals dit proefschrift biedt, is dus van groot belang bij het inschatten van toekomstige effecten van klimaatsverandering op de terrestrische koolstofbalans.


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Caminante, no hay camino,
se hace camino al andar.
Al andar se hace camino,
y al volver la vista atrás
se ve la senda que nunca
se ha de volver a pisar.
Caminante, no hay camino,
sino estelas en la mar.

Antonio Machado, 1912

Abcnowledgements

A-F

A is voor Rien **A**erts, die dit onderzoek initieerde, chocolade op Stordalen verstopte, en het sferisch perspectief in mijn leven bracht. Rien, dankjewel.

B is voor Peter van **B**odegom, wiens open en bruut eerlijke aanpak van wetenschap en management ik bewonder. Peter, ontzettend bedankt dat je me wilde begeleiden bij het voltooien van dit project. Ik heb het ervaren als de wind in de rug, en er veel van geleerd op precies die fronten waar ik daar behoefte aan had.

C is for my dear co-authors Terry **C**allaghan and Hans **C**ornelissen. Thank you both for bringing twinkles in this work and in my eyes!

D en **E** zijn voor **E**llen **D**orrepaal, van wie niet alleen de initialen het dichtst bij de mijne staan. Ellen, al is het een dooddoener: het is onmogelijk te verwoorden wat jij voor me betekent. *Festina lente*. De precisie, het plezier en de rust waarmee jij je werk doet contrasteren met de snelheid waarop je je doelen bereikt. Ik weet nog steeds niet hoe je het doet, maar het fascineert en inspireert me. Ik voel me gelukkig dat ik de komende jaren met je zal samenwerken. Tot gauw in Abisko!

F is for **f**riends and **f**amily. Please swap the page and enjoy the good memories :) Thank you all!

G-Z

I had in mind to express verbal thanks only to my formal supervisors (A-E). Yet, this work would not have been possible without the professional help of many others. I would like to express special thanks to: My Mate Marika Makkonen, Matti Kummu, Richard van Logtestijn, Jurgen van Hal, James Weedon, Margareta Johansson, the Staff of the Abisko Vetenskapliga Station, Janny Akkerman, Wessel Keuper, Frans-Jan Parmentier, Daan Blok, Rob Broekman, Karin Uyldert, Michel Groen, Jemima Mensah, Gemma Venhuizen, Sviatlana Marozava and **J**onathan **R**amsay, and to my paranymphs Diana Edo Matas and Eric Massey. Thank you en superbedankt!


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