

Nitrogen availability increases in a tundra ecosystem during five years of experimental permafrost thaw

VERITY G. SALMON¹, PATRICK SOUCY¹, MARGUERITE MAURITZ², GERARDO CELIS², SUSAN M. NATALI³, MICHELLE C. MACK^{1,2} and EDWARD A. G. SCHUUR^{1,2}

¹Biology Department, University of Florida, Gainesville, FL 32611, USA, ²Center for Ecosystem Science and Society, Northern Arizona University, Flagstaff, AZ 86011, USA, ³Woods Hole Research Center, Falmouth, MA 02540, USA

Abstract

Perennially frozen soil in high latitude ecosystems (permafrost) currently stores 1330–1580 Pg of carbon (C). As these ecosystems warm, the thaw and decomposition of permafrost is expected to release large amounts of C to the atmosphere. Fortunately, losses from the permafrost C pool will be partially offset by increased plant productivity. The degree to which plants are able to sequester C, however, will be determined by changing nitrogen (N) availability in these thawing soil profiles. N availability currently limits plant productivity in tundra ecosystems but plant access to N is expected improve as decomposition increases in speed and extends to deeper soil horizons. To evaluate the relationship between permafrost thaw and N availability, we monitored N cycling during 5 years of experimentally induced permafrost thaw at the Carbon in Permafrost Experimental Heating Research (CiPEHR) project. Inorganic N availability increased significantly in response to deeper thaw and greater soil moisture induced by Soil warming. This treatment also prompted a 23% increase in aboveground biomass and a 49% increase in foliar N pools. The sedge *Eriophorum vaginatum* responded most strongly to warming: this species explained 91% of the change in aboveground biomass during the 5 year period. Air warming had little impact when applied alone, but when applied in combination with Soil warming, growing season soil inorganic N availability was significantly reduced. These results demonstrate that there is a strong positive relationship between the depth of permafrost thaw and N availability in tundra ecosystems but that this relationship can be diminished by interactions between increased thaw, warmer air temperatures, and higher levels of soil moisture. Within 5 years of permafrost thaw, plants actively incorporate newly available N into biomass but C storage in live vascular plant biomass is unlikely to be greater than losses from deep soil C pools.

Keywords: carbon balance, decomposition, *Eriophorum vaginatum*, linear mixed effect model, moist acidic tussock tundra, natural abundance $\delta^{15}\text{N}$, plant productivity

Received 31 July 2015; revised version received 19 November 2015 and accepted 1 December 2015

Introduction

Climate change is expected to dramatically alter carbon (C) cycling in high latitude ecosystems by inducing thaw of permafrost soils that remain frozen year-round. Globally, permafrost currently stores an estimated 1330–1580 Pg C in the form of frozen soil organic matter (Hugelius *et al.*, 2014; Schuur *et al.*, 2015). The thaw and potential decomposition of this large C pool is globally important because Earth's atmosphere currently contains 829 Pg C and is increasing by 4 Pg C per year due to fossil fuel combustion and land use change (IPCC AR5, 2013). Global warming is expected to thaw 246–436 Pg of permafrost C by 2100 (Harden *et al.*, 2012) and an estimated 120–195 Pg of this is vulnerable to decomposition upon thaw (Schuur *et al.*, 2015). High latitude ecosystems are already responding to global warming: permafrost temperatures in the discontinuous

permafrost zone have increased since the 1980s by 0.5–1.5 °C (Osterkamp & Romanovsky, 1999), and shrub encroachment into graminoid tundra has been observed over the last 60 years (Sturm *et al.*, 2001a). The release of permafrost C into the atmosphere in the form of carbon dioxide (CO₂) and methane (CH₄) is expected to contribute to further warming as the atmospheric concentrations of these greenhouse gases increase (Koven *et al.*, 2011). Current research in the field of permafrost C cycling is focused on estimating the magnitude of future permafrost C losses and determining whether released C will be in the form of CO₂ or CH₄ (Koven *et al.*, 2015; Schuur *et al.*, 2015; Treat *et al.*, 2015). To fully understand how permafrost C losses will feed-back to future climate warming, however, we must understand the degree to which increased plant productivity under warmed conditions will offset these losses (Sistla *et al.*, 2013; Natali *et al.*, 2014).

Tundra plants root in a seasonally thawed active layer of soil that lies above permafrost. The growth of

Correspondence: Verity G. Salmon, tel. +1 925 878 9681, fax +1 352 392 3704, e-mail: vsalmon@ufl.edu

these plants is limited by temperature but also by nitrogen (N) availability (Shaver *et al.*, 1992; Chapin *et al.*, 1995; Van Wijk *et al.*, 2003). Nitrogen fixation and deposition rates are low in tundra ecosystems so plant access to N is heavily dependent on soil microbial turnover of N from plant litter during decomposition (Chapin, 1983; Cleveland *et al.*, 1999). Low N availability limits plant productivity and causes plant litter inputs to be low in quantity, slow to decompose, and generally N poor (Hobbie, 1992). As a result, tundra ecosystems have tightly coupled N and C cycles and competition between soil microbes and plants for available N is intense (Schimel & Chapin, 1996). Increases in the depth of permafrost thaw have been associated with higher levels of N availability and plant productivity (Schoor *et al.*, 2007). Organic matter in permafrost is generally older, more highly decomposed, and contains higher levels of dissolved N than active layer soils (Hicks Pries *et al.*, 2012; Keuper *et al.*, 2012). Dissolved N from thawed permafrost is biologically available for plant uptake (Keuper *et al.*, 2012) but runoff from thawing landscapes is known to transport N into streams and away from plant roots (Bowden *et al.*, 2008; Reyes & Loughheed, 2015). The process of permafrost thaw is expected to increase N availability on a landscape scale, but the degree to which this N is spatially or temporally available for incorporation into plant biomass has not been quantified.

The dynamic relationship between nutrient availability and temperature has been a central focus of manipulative field experiments in high latitude ecosystems since the early 1980s (Van Wijk *et al.*, 2003). Experiments in Alaska, Sweden, and Canada all found that N fertilization has a stronger and more immediate impact on plant productivity than increased growing season air temperatures (Shaver *et al.*, 1986; Chapin *et al.*, 1995; Press *et al.*, 1998; Graglia *et al.*, 2001; Van Wijk *et al.*, 2003). When warming and fertilization treatments were applied together, several of these experiments saw a positive interaction between treatments (Van Wijk *et al.*, 2003). This implies that plants require ample nutrients to fully take advantage of warmed conditions. At Toolik Lake Long Term Ecological Research (LTER) site in Alaska, long-term fertilization and warming of moist acidic tussock tundra both shifted the sedge dominated tundra community to one in which deciduous shrubs play a major role. In fertilized plots, this transition took place after 9 years but in greenhouse warmed plots it was only observed after 14 years (Chapin *et al.*, 1995; Sistla *et al.*, 2013). After 20 years of fertilizer application, Mack *et al.* (2004) found ecosystem C storage to be significantly lower than controls and most of this change was attributed to faster decomposition of soil organic matter. In contrast,

20 years of warming at this site increased C storage in plant biomass and active layer mineral soils (Sistla *et al.*, 2013). Greenhouse warming, though useful for understanding the role temperature plays on plant growth and soil C storage, did not simulate the year round air or soil temperatures that these ecosystems are expected to experience under future climate regimes. Greenhouse warming experiments therefore cannot fully inform our understanding of decomposition and permafrost thaw dynamics.

An important observation from the warming component of the Toolik Lake LTER experiment was that increased abundance of shrub species caused snow accumulation during winter. As a result of snow insulation, soil temperatures that were previously unaffected by greenhouse warming increased (Sturm *et al.*, 2001b; Deslippe *et al.*, 2012; Sistla & Schimel, 2013). Feedbacks between snow depth, shrub growth, and soil temperatures were investigated by a series of field experiments in which snow fences were used to build up snow banks to passively warm soils (Walsh *et al.*, 1997; Wahren *et al.*, 2005; DeMarco *et al.*, 2011; Leffler & Welker, 2013). Results from these experiments indicated that plant available N increased in warmed soils, but the snow banks also increased hydrological inputs, delayed spring melt out, and altered plant phenology (Wipf & Rixen, 2010). Experimental consequences such as these are important when investigating feedbacks between snow depth and plant community composition but they do present an obstacle to using these experimentally warmed systems as an analogue for how plants and soils will respond to a warmer climate.

Greenhouse warming experiments elucidated the important role the aboveground environment plays in determining the plant community and long-term soil nutrient availability. Snow fence experiments, on the other hand, demonstrated that changes to the belowground environment have important implications for nutrient availability and abiotic feedback between the plant canopy structure, snow cover, soil temperature, and soil N availability. These two types of field manipulations therefore highlighted two important mechanisms that together will determine the future carbon balance of high latitude ecosystems. To better understand interactions between these two mechanisms, the Carbon in Permafrost Experimental Heating Research (CiPEHR) project began manipulating above and belowground temperatures in the winter of 2008 (Natali *et al.*, 2011). Snow fences passively warmed tundra soils in winter and excess snow was removed in the spring to avoid the experimental artifacts associated with previous snow fence experiments. Warmer winter soil temperatures translated to deeper thaw during the growing season as well as mean annual soil tempera-

tures that were 0.85–1.00 °C warmer. In addition to soil warming, an air warming treatment was implemented using open top chambers (OTCs) that warmed the canopy air by 0.42 °C during the growing season. The two treatments were applied alone and in combination so that the relative impact of air vs. soil warming could be teased apart. The application of both warming treatments together mimics the conditions these ecosystems will experience in the near future: Alaskan mean annual temperatures are projected to increase by 0.4–2.8 °C by 2035 (IPCC AR5, 2013). Previously published research from the first 3 years of warming at CiPEHR has focused on plant productivity, phenology, net ecosystem exchange, and sources of ecosystem respiration (Natali *et al.*, 2011, 2012; Hicks Pries *et al.*, 2015). The impact of 5 years of warming on soil temperature and moisture, wintertime ecosystem respiration and moss community dynamics has also been previously published (Deane-Coe *et al.*, 2015; Natali *et al.*, 2015). The study presented here documents changes to vascular plant biomass, foliar chemistry, foliar N pools, and soil inorganic N availability throughout 5 years of manipulative warming. We seek to answer the following questions:

1. How does the plant available N pool change as permafrost begins to thaw?
2. Do warmer air temperatures during the growing season alter the way plants and soils respond to permafrost thaw?
3. Which plant species benefit the most from increased N availability during permafrost thaw?

Based on results from other field experiments, we predicted that soil warming would induce permafrost thaw while Air warming would only impact above-ground temperatures. We hypothesized that plant biomass pools (g biomass m⁻² of ground) and foliar N pools (g N m⁻² of ground) would both respond positively to Soil warming treatment because of increased N availability associated with accelerated decomposition in the warmed active layer as well as the release of dissolved N from thawing permafrost. Air warming was expected to have little impact on plants when applied alone because N availability would still be limiting plant growth in these plots. We hypothesized that plant biomass would be highest in Air & Soil warmed plots due to the release of plants from N limitation and warmer growing conditions. Vegetation at a natural thaw gradient (Schuur *et al.*, 2007) led us to predict that the initial stages of permafrost thaw would see an increase in biomass and foliar N pools for graminoid species but eventually deciduous shrubs would dominate the response to permafrost thaw.

Materials and methods

Study site

The CiPEHR project is located in Interior Alaska near Denali National Park and Eight Mile Lake (EML, 63°52'59"N, 149°13'32"W). This site has mean annual temperature of –1.45 °C (±0.25, 1977–2013 average) and is at the southernmost extent of the discontinuous permafrost zone. Soils are gelisols with a 45–65 cm thick organic horizon overlaying a cryoturbated mixture of glacial till and loess. The active layer thickness is from 50 to 60 cm in undisturbed areas. Intensive monitoring of soil temperature, soil moisture, and CO₂ effluxes at a natural permafrost gradient near EML has been ongoing since 2004 (Vogel *et al.*, 2009). The vegetation at this site is moist acidic tussock tundra dominated by the sedge *Eriophorum vaginatum*. Other vascular species present include *Andromeda polifolia*, *Betula nana*, *Carex bigelowii*, *Empetrum nigrum*, *Oxycoccus microcarpus*, *Rhododendron subarcticum*, *Rubus chamaemorus*, *Vaccinium uliginosum*, and *V. vitis-idaea* (Schuur *et al.*, 2007).

Experimental design

Manipulative warming at the CiPEHR project started in the winter of 2008–2009 and comprised two warming treatments. The soil warming treatment (“Soil”) was applied using snow fences that are 1.5 m tall × 8 m long. In winter, the snow banks that developed on the leeward side of the fences insulated soil from cold winter air. Every spring, snow on the Soil-warmed side of the fence was removed so that the depth of the snowpack matched the surrounding landscape. The air warming treatment (“Air”) was applied during the growing seasons using OTCs that passively warmed canopy air. In addition to being applied alone, the two warming treatments were applied in combination with one another (“Air & Soil”). “Control” plots were not warmed and served as procedural controls. Plots measure 60 cm × 60 cm and were placed in a split plot design across six replicate snow fences organized in three blocks. Soil warming was applied at the fence level ($n = 6$) while Air warming was applied at the plot level ($n = 12$). Previously published research from the CiPEHR project contains more details regarding the experimental design (Natali *et al.*, 2011, 2012) although it should be noted that treatment names have changed. Formerly, Soil warming was “winter warming,” Air warming was “summer warming,” and Air & Soil warming was “annual warming.”

Environmental monitoring

Soil temperature and soil volumetric water content (“soil moisture”) were monitored continuously from October 2008 through October 2013. Soil temperature was measured at 5, 10, 20, and 40 cm using constantan-copper thermocouples. Air temperature was measured during the growing season (June through September) at a height of 10 cm using a thermistor. Growing season measurements of soil moisture were made using depth integrated probes (Campbell Scientific CS616,

Logan, UT, USA) installed from 0 to 20 cm in the soil profile. Data from all sensors were logged half-hourly using Campbell Scientific CR1000. Maximum thaw depth at all plots was measured annually in mid-September using a 3 mm diameter metal rod.

Aboveground vascular plant biomass

Aboveground biomass of vascular plants (herein "biomass") was measured at the peak of the 2009–2013 growing seasons using a nondestructive point intercept method (Schuur *et al.*, 2007). A 60 cm × 60 cm aluminum frame with a 7 cm string grid was suspended over each plot. At each of the 49 string intersections a rod was vertically lowered into the vegetation and the species at each point of contact was recorded. Previously published species-specific allometries from this site (Schuur *et al.*, 2007) were updated for this study with data from additional destructive harvest plots. Updated allometric relationships were then used to convert the average number of contact points per species to dry weight of biomass (g m^{-2}). Data describing updated allometric relationships is archived and available for download (<http://dx.doi.org/10.6073/pasta/4446e37f339ffdac6912030d83e2f61f>). Changes in total biomass and *E. vaginatum* biomass (Δ Total Biomass, Δ *E. vaginatum* Biomass) during 5 years of experimental manipulation were calculated by subtracting 2013 biomass from 2009 biomass.

Foliar chemistry

In 2008–2013 leaves of *B. nana*, *C. bigelowii*, *E. vaginatum*, *R. chamaemorus*, *R. subarcticum*, and *V. uliginosum* were collected at the peak of the growing season for elemental and isotopic analysis. These six species comprise approximately 80% of vascular plant biomass at this site. In 2013, foliar samples from four additional, nondominant species (*A. polifolia*, *E. nigrum*, *V. vitis-idaea*, *O. microcarpus*) were also collected. For all collections, the peak of the growing season was considered mid-July and care was taken to select current year's growth only. Samples from replicate treatment plots at each fence were combined at the species level to minimize damage to the plants ($n = 6$). Following collection, leaves were dried, ground, and analyzed for foliar %C, %N, C : N, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$. Analyses were performed using a Costech elemental analyzer (Valencia, CA, USA) and a Thermo Finnigan MAT Delta Plus XL continuous flow isotope ratio mass spectrometer (ThermoScientific Inc., Waltham, MA, USA).

Foliar N pools

To investigate whether any observed changes in biomass were synchronized with changes in N acquisition, annual foliar N pools were calculated from leaf biomass and foliar %N. Leaf biomass was calculated as a proportion of total plant biomass based on species-specific ratios established in destructive harvests. We do not consider the ratios of leaf biomass: total biomass to have changed through the course of this experiment because (i) the aboveground biomass of the dominant species *E. vaginatum* is made up almost entirely of leaf tissue and (ii)

previous snow fence experiments have seen no change in this ratio for any of the shrub species found at CiPEHR (Addis, 2014).

The foliar N pool for each species (g N m^{-2}) was calculated by multiplying the species-specific leaf biomass (g m^{-2}) at each plot by foliar %N measured for the corresponding species, treatment, fence, and year. In 2008–2012 foliar samples from nondominant species were not collected, so in these years the foliar N was calculated using the average species-specific foliar %N from the 2013 Controls. Total foliar N was calculated as the sum of the foliar N pools of all species present in a plot.

Soil inorganic N availability

The availability of ammonium and nitrate in surface soils was measured using cation and anion sorbing resins (Giblin *et al.*, 1994). Polyester mesh bags measuring 7 cm × 7 cm were filled with 3 g of anion (AG 1-X8, Bio-Rad, Hercules, CA, USA) or cation (AG 50W-X8, Bio-Rad) binding resin beads. Prior to installation in the field, resin bags were acid washed and soaked in 2 M NaCl solution to clean and charge the binding surface of the resins. In both 2012 and 2013, a pair of anion and cation resin bags were deployed in each plot at 10 cm depth for the length of the growing season (June–September) and the winter season (September–June). Following collection, resin bags were extracted with a 2 M NaCl/0.1 M HCl solution. Extracts were filtered through GF/A filters and ammonium and nitrate concentrations were determined using an Astoria Pacific Autoanalyzer (Clackamas, OR, USA). Resin bags made in 2012 were acid washed, recharged, and reused in 2013. During the 2013 growing season contamination during extractions reduced sample size by one-third for all treatments but for all other sampling periods $n = 12$ per treatment.

Statistical analysis

Linear mixed effect models were used to analyze the response of plants and soils to CiPEHR warming treatments. All analyses were completed in R using the LME4 package (Bates *et al.*, 2014; R Development Core Team, 2014). Soil warming, Air warming, and the interaction between Air warming and Soil warming were included as discrete fixed effects. Fence, Soil warming nested in fence, and plot nested in Soil warming were included as random effects so that the split plot design and repeated measures were taken into account. Year was treated as a continuous fixed effect or a random effect, depending on the question the model was designed to address. When the model needed to test for a change in a response variable through time, year was included as a fixed effect in the model and interactions with treatment effects were tested. When testing for a significant overall response to 5 years of warming treatments, however, year was included in the model as a random effect. For all models, the significance of fixed effects was assessed by calculating 95% confidence intervals for model coefficients using bootstrapping techniques (1000 iterations). Effects were considered

statistically significant if the 95% confidence interval of the coefficients did not include zero (Pinheiro & Bates, 2000). Unwarmed control plots served as the reference in all models and therefore were represented in model intercept values. The percent change in the response variable due to a warming treatment was calculated as the fixed effect coefficient for that warming treatment divided by the model intercept. Percent change was calculated to facilitate comparison between the relative response of biomass, foliar N pools, and soil inorganic N availability to 5 years of manipulative warming.

To test whether the Soil warming had progressively greater impact on thaw depth through the course of the experiment, the thaw depth model included year as a continuous fixed effect. Year was centered and rescaled to have a mean of zero and range of (−0.5, 0.5). Centering and rescaling the year covariate minimized the correlation between the model's slope and intercept terms and ensured that coefficients for fixed effects summarized change over the entire 5 year period. Interactions between year and treatments were tested for inclusion using forward step-wise regression.

Annual soil temperature, growing season soil moisture, growing season air temperature, biomass, and total foliar N pools were individually tested to see if 5 years of warming had a significant impact on the average values of these response variables. The models used to assess the impact of warming on these response variables did not examine whether the responses to warming changed through time: instead they tested the total impact of 5 years of warming and included year as a random effect. Fixed effect coefficients from these models summarize the average change in the response variable relative to unwarmed plots across the entire 5 year observation period.

Soil inorganic N availability during the fourth and fifth years of the experiment were grouped by season before being analyzed in linear mixed effect models that included year as a random effect. The fixed effect coefficients summarize how warmed plots differ from unwarmed plots in a given season during the final 2 years of warming.

Eriophorum vaginatum foliar $\delta^{15}\text{N}$ varied greatly through the 5 years of warming but this variance was not well-explained by experimental treatments. Due to the fact that this species was strongly correlated with increases in total biomass (see Results), environmental drivers of the observed variance in foliar $\delta^{15}\text{N}$ were explored using forward stepwise regression of a linear mixed effect model. Variables in the initial model included categorical warming treatments, average annual soil temperatures, average maximum thaw depth, and average growing season soil moisture. A five point improvement of the Akaike Information Criterion (AIC) was used to justify inclusion of fixed effects and interaction terms were tested throughout the model selection process (Zuur *et al.*, 2009).

Results

Environmental monitoring

Soil warming significantly increased the maximum depth of thaw and the impact of this treatment

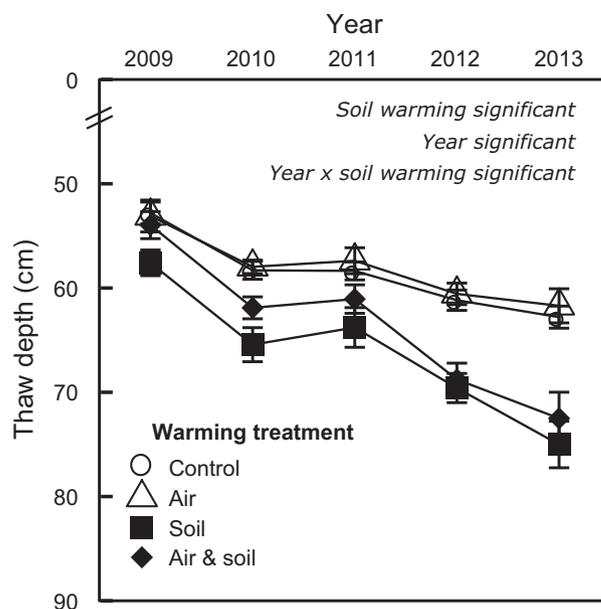


Fig. 1 Impact of warming treatments on thaw depth through 5 years of experimental manipulation. The interface between soil and moss surface is defined as 0 cm. Soil warming increased thaw depth significantly through the 5 years of manipulation and this effect increased with time.

increased as the experiment progressed (Fig. 1). All plots experienced thaw through time (8.54 cm, 95% CI from 6.88 to 10.22 cm) but Soil warmed plots did thaw significantly more than Controls (7.60 cm, 95% CI from 3.64 to 11.47 cm). The effect of Soil warming increased significantly through the course of the 5 year experiment (8.06 cm, 95% CI from 5.64 to 10.45 cm). Air warming did not significantly impact thaw depth alone though there was a tendency for thaw to be shallower in Air & Soil warmed plots (−2.10 cm, 95% CI from −5.37 to 1.28 cm).

Models fitted to 5 year soil temperature datasets showed that the Soil warming treatment significantly increased the annual average soil temperature of the top 40 cm of the active layer by 0.85–1.00 °C (Tables 1 and S1). When growing season and winter soil temperatures were analyzed separately, it became clear that shallow soils were only impacted by Soil warming during the winter. At 5 cm depth, wintertime average soil temperatures were 1.69 °C higher in Soil warmed plots (95% CI from 1.15 °C to 2.22 °C) while at 10 cm depth they were 1.31 °C higher (95% CI from 1.04 °C to 1.59 °C). During the growing season neither 5 nor 10 cm soil temperatures were affected by soil warming. Deeper in the active layer, however, both growing season and winter soil temperatures showed significant effects of Soil warming. At 20 cm depth, growing season temperatures in Soil warmed plots were 0.60 °C

Table 1 Soil temperature and soil moisture averaged by treatment for 5 years of warming

Year	Treatment	Soil temperature, 5 cm (°C)		Soil temperature, 10 cm (°C)		Soil temperature, 20 cm (°C)		Soil temperature, 40 cm (°C)		Soil moisture (%)	
		<i>n</i>	Annual average	<i>n</i>	Annual average	<i>n</i>	Annual average	<i>n</i>	Annual average	<i>n</i>	Growing season average
2009	Control	12	0.27 ± 0.23	12	0.20 ± 0.12	6	-0.49 ± 0.13	6	-0.82 ± 0.17	6	38.06 ± 0.84
	Air warming	12	0.33 ± 0.12	12	-0.01 ± 0.11	6	-0.68 ± 0.12	6	-0.96 ± 0.10	6	39.93 ± 1.40
	Soil warming	12	1.57 ± 0.13*	12	0.96 ± 0.12*	6	0.26 ± 0.11*	6	-0.20 ± 0.06*	6	41.52 ± 2.19*
	Air & Soil warming	12	1.33 ± 0.12	12	0.76 ± 0.12	6	0.18 ± 0.08	6	-0.31 ± 0.05	6	41.27 ± 1.57
2010	Control	12	0.15 ± 0.11	12	-0.32 ± 0.10	6	-1.11 ± 0.13	6	-1.32 ± 0.13	6	42.15 ± 1.09
	Air warming	12	0.11 ± 0.13	12	-0.27 ± 0.08	6	-0.96 ± 0.07	6	-1.40 ± 0.06	6	44.26 ± 1.98
	Soil warming	12	1 ± 0.08*	12	0.20 ± 0.29*	6	-0.22 ± 0.13*	6	-0.64 ± 0.11*	6	46.30 ± 1.36*
	Air & Soil warming	12	0.69 ± 0.11	12	0.23 ± 0.06	6	-0.40 ± 0.12	6	-0.65 ± 0.08	6	48.47 ± 1.63
2011	Control	12	-0.25 ± 0.12	12	-0.66 ± 0.08	6	-1.21 ± 0.12	6	-1.30 ± 0.14	6	40.37 ± 1.24
	Air warming	12	-0.39 ± 0.10	12	-0.64 ± 0.09	6	-1.14 ± 0.11	6	-1.37 ± 0.08	6	41.27 ± 1.63
	Soil warming	12	1.39 ± 0.13*	12	0.87 ± 0.14*	6	0.42 ± 0.05*	6	-0.02 ± 0.05*	6	44.01 ± 1.48*
	Air & Soil warming	12	1.49 ± 0.07	12	0.94 ± 0.06	6	0.31 ± 0.11	6	-0.06 ± 0.07	6	45.99 ± 1.59
2012	Control	12	1.15 ± 0.15	12	0.69 ± 0.11	6	-0.07 ± 0.12	6	-0.52 ± 0.15	6	42.57 ± 2.42
	Air warming	12	1.04 ± 0.13	12	0.69 ± 0.13	6	0.06 ± 0.14	6	-0.45 ± 0.10	6	42.91 ± 1.86
	Soil warming	12	2.03 ± 0.07*	12	1.60 ± 0.06*	6	1.07 ± 0.06*	6	0.54 ± 0.10*	6	47.42 ± 2.54*
	Air & Soil warming	12	1.89 ± 0.11	12	1.52 ± 0.08	6	1.09 ± 0.12	6	0.52 ± 0.11	6	51.88 ± 1.95
2013	Control	12	2.02 ± 0.10	12	1.39 ± 0.09	6	0.45 ± 0.09	6	-0.14 ± 0.09	6	37.85 ± 1.41
	Air warming	12	1.93 ± 0.13	12	1.34 ± 0.06	6	0.49 ± 0.14	6	-0.16 ± 0.11	6	38.54 ± 1.46
	Soil warming	12	2.34 ± 0.05*	12	1.76 ± 0.08*	6	1.01 ± 0.09*	6	0.46 ± 0.13*	6	42.72 ± 3.00*
	Air & Soil warming	12	2.23 ± 0.11	12	1.60 ± 0.06	6	1.06 ± 0.17	6	0.41 ± 0.12	6	46.14 ± 2.17

Annual treatment means are reported ±SE. Bold type and asterisk (*) designate statistical significance based on whether or not the 95% confidence interval of linear mixed effect model coefficient for a given treatment included zero. Note that models tested for an overall treatment effect of 5 years of warming but did not quantify changes within the 5 year period.

higher (95% CI from 0.06 °C to 1.14 °C) while in the winter they were 1.21 °C higher (95% CI from 0.92 °C to 1.50 °C). Growing season Soil temperatures at 40 cm depth increased with Soil warming by 0.68 °C (95% CI from 0.25 °C to 1.13 °C) and in the winter they increased by 0.89 °C with Soil warming (95% CI from 0.61 °C to 1.17 °C). In addition to experiencing increased soil temperatures, Soil warmed plots also had significantly higher growing season soil moisture (4.20%, 95% CI from 0.04% to 8.49%) (Tables 1 and S1). Increased soil moisture in these plots is likely a function of subsidence since springtime snow removal ensures water inputs are similar across treatments.

Air warming significantly increased growing season average daily air temperatures by 0.42 °C (95% CI from 0.05 °C to 0.78 °C) (Tables 2 and S1). The Air warming treatment did not significantly impact annual soil temperatures or growing season soil moisture (Table 1). During the growing season, however, Air warming tended to cool soils at 40 cm (-0.16 °C, 95% CI from -0.45 °C to 0.13 °C). This finding was consistent with the trend toward shallower thaw in these plots.

Aboveground vascular plant biomass

Total biomass increased significantly during 5 years of Soil warming but did not respond to Air warming (Fig. 2). Soil warming was shown to increase total biomass by 114.51 g m⁻² relative to non-Soil warmed plots (Table 4, 95% CI from 7.34 to 222.94 g m⁻²). This effect translates to a 23% increase in biomass with 5 years of Soil warming. The coefficient for the interaction between Air warming and Soil warming was negatively skewed, though this response was not significant (-67.30 g m⁻², 95% CI from -222.71 to 89.35 g m⁻²). The negative skew of this interaction coefficient means that Air & Soil warmed plots tended to have lower biomass than their Soil warmed counterparts.

Across all treatments, changes in *E. vaginatum* biomass between 2009 and 2013 ($\Delta E. vaginatum$) explained 91% of the changes in total biomass (Δ Total Biomass, Fig. 3). The linear relationship (dashed line) between $\Delta E. vaginatum$ biomass and Δ Total Biomass had a slope of 1.06 (95% CI from 0.96 to 1.15) and an $R^2 = 0.91$. No individual plant species had a significant change in bio-

Table 2 Average growing season air temperature during 5 years of warming

Year	Treatment	n	Air temperature (°C)	
			Growing season average	
2009	Control	12	12.7 ± 0.4	
	Air warming	12	13.0 ± 0.4*	
	Soil warming	12	12.7 ± 0.4	
	Air & Soil warming	12	12.9 ± 0.4	
2010	Control	12	11.9 ± 0.2	
	Air warming	12	12.2 ± 0.2*	
	Soil warming	12	11.6 ± 0.1	
	Air & Soil warming	12	12.3 ± 0.1	
2011	Control	12	10.9 ± 0.3	
	Air warming	12	11.5 ± 0.3*	
	Soil warming	12	11.1 ± 0.3	
	Air & Soil warming	12	11.5 ± 0.3	
2012	Control	12	10.6 ± 0.1	
	Air warming	12	10.8 ± 0.1*	
	Soil warming	12	10.4 ± 0.2	
	Air & Soil warming	12	11 ± 0.1	
2013	Control	12	12.2 ± 0.2	
	Air warming	12	12.9 ± 0.4*	
	Soil warming	12	12.7 ± 0.4	
	Air & Soil warming	12	13.1 ± 0.4	

Treatment means are reported ±SE. Bold type and asterisk (*) designate statistical significance based on whether or not the 95% confidence interval of linear mixed effect model coefficient for a given treatment included zero. Note that models tested for an overall treatment effect of 5 years of warming but did not quantify changes within the 5 year period.

mass in response to warming despite the significant positive impact soil warming had on total biomass. When the biomass dataset was filtered to exclude plots containing 10 g m^{-2} of *E. vaginatum* (removing 10 plots out of a total 48), there was a significant effect of Soil warming on *E. vaginatum* biomass (118.18 g m^{-2} , 95% CI from 1.95 to 236.09 g m^{-2}).

Foliar chemistry

Even though *E. vaginatum* contributed heavily to changes in total biomass during 5 years of warming, this species did not show any significant effect of warming treatments on foliar %N, %C, C : N, $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ (Table 3). Variance in *E. vaginatum* foliar $\delta^{15}\text{N}$ increased drastically throughout the course of the experiment: in 2009, values ranged from 0.43‰ to 2.55‰ across all treatments while in 2013 they ranged from -2.68‰ to 2.82‰. Stepwise linear regression revealed that annual maximum thaw depth was the

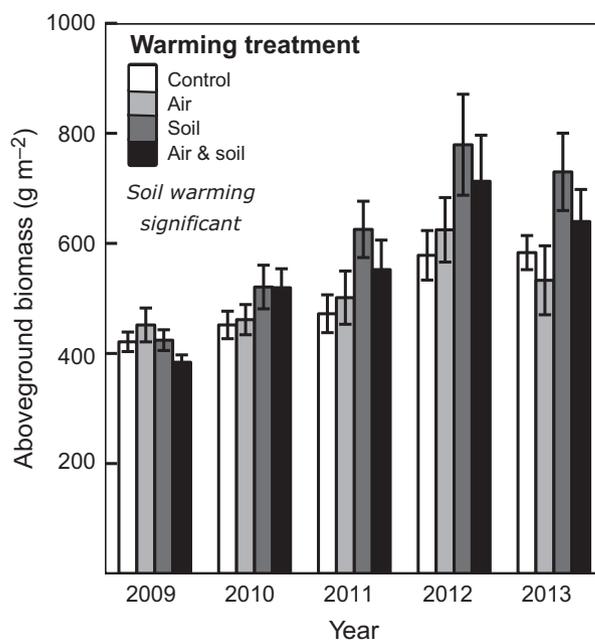


Fig. 2 Aboveground vascular plant biomass during 5 years of experimental warming. Five years of Soil warming significantly increased plant biomass by 23% or 114.51 g m^{-2} (95% CI from 7.34 to 222.94 g m^{-2}). Error bars represent SE based on annual treatment means.

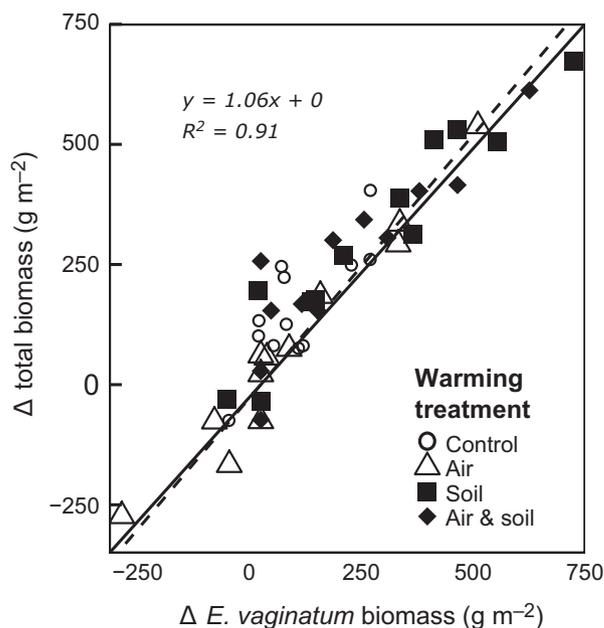


Fig. 3 Changes in aboveground vascular plant biomass during 5 years of experimental manipulation were correlated to changes in the dominant sedge, *Eriophorum vaginatum* during the same time period. The solid line represents the 1 : 1 line while the dashed line shows a linear model fit to the data (slope is not significantly different from 1 based on 95% CI from 0.96 to 1.15).

Table 3 *Eriophorum vaginatum* foliar chemistry through 5 years of experimental warming

Year	Treatment	<i>n</i>	<i>Eriophorum</i> foliar %C	<i>Eriophorum</i> foliar %N	<i>Eriophorum</i> foliar C : N	<i>Eriophorum</i> foliar $\delta^{13}\text{C}$	<i>Eriophorum</i> foliar $\delta^{15}\text{N}$
2009	Control	6	45.74 ± 0.38	1.73 ± 0.14	27.33 ± 2.27	-25.54 ± 0.41	1.31 ± 0.19
	Air warming	6	45.65 ± 0.25	1.67 ± 0.11	27.97 ± 1.85	-25.35 ± 0.21	1.15 ± 0.18
	Soil warming	6	45.52 ± 0.36	1.85 ± 0.04	24.67 ± 0.37	-25.93 ± 0.16	1.68 ± 0.23
	Air & Soil warming	6	45.27 ± 0.43	1.84 ± 0.12	25.13 ± 1.47	-25.40 ± 0.47	1.38 ± 0.24
2010	Control	6	45.41 ± 0.11	1.87 ± 0.13	24.91 ± 1.85	-26.28 ± 0.13	1.05 ± 0.19
	Air warming	5	44.86 ± 0.19	1.75 ± 0.07	25.78 ± 0.87	-25.81 ± 0.27	0.89 ± 0.35
	Soil warming	6	45.42 ± 0.18	1.93 ± 0.06	23.65 ± 0.66	-26.13 ± 0.11	1.13 ± 0.29
	Air & Soil warming	4	45.40 ± 0.41	2.01 ± 0.18	23.08 ± 1.97	-25.97 ± 0.48	2.00 ± 0.16
2011	Control	6	45.30 ± 0.36	1.93 ± 0.09	23.69 ± 1.04	-25.87 ± 0.18	1.19 ± 0.05
	Air warming	6	45.86 ± 0.13	1.99 ± 0.06	23.19 ± 0.62	-26.24 ± 0.21	1.28 ± 0.18
	Soil warming	6	45.91 ± 0.22	1.93 ± 0.09	24.09 ± 1.04	-26.01 ± 0.31	1.43 ± 0.24
	Air & Soil warming	6	44.83 ± 0.50	1.85 ± 0.08	24.44 ± 0.86	-26.05 ± 0.36	1.87 ± 0.24
2012	Control	6	45.96 ± 0.12	1.72 ± 0.09	27.11 ± 1.33	-26.01 ± 0.21	0.13 ± 0.27
	Air warming	6	45.97 ± 0.13	1.59 ± 0.18	32.21 ± 5.92	-25.87 ± 0.27	0.55 ± 0.20
	Soil warming	6	45.81 ± 0.11	1.86 ± 0.06	24.74 ± 0.79	-26.14 ± 0.18	0.52 ± 0.32
	Air & Soil warming	6	45.60 ± 0.27	1.84 ± 0.10	25.21 ± 1.40	-26.23 ± 0.34	0.98 ± 0.20
2013	Control	6	46.07 ± 0.32	2.06 ± 0.06	22.43 ± 0.61	-25.42 ± 0.52	1.66 ± 0.33
	Air warming	6	43.20 ± 1.70	1.97 ± 0.07	22.01 ± 0.90	-25.57 ± 0.37	1.58 ± 0.29
	Soil warming	6	44.72 ± 0.74	2.04 ± 0.08	22.14 ± 0.87	-25.83 ± 0.20	0.01 ± 0.75
	Air & Soil warming	6	46.15 ± 0.29	2.17 ± 0.10	21.51 ± 1.00	-26.22 ± 0.69	0.31 ± 0.63

Annual treatment means are reported \pm SE. No treatment coefficients were found to be significant because the 95% confidence interval of linear mixed effect model coefficients all included zero. Note that models tested for an overall treatment effect of 5 years of warming but did not quantify changes within the 5 year period.

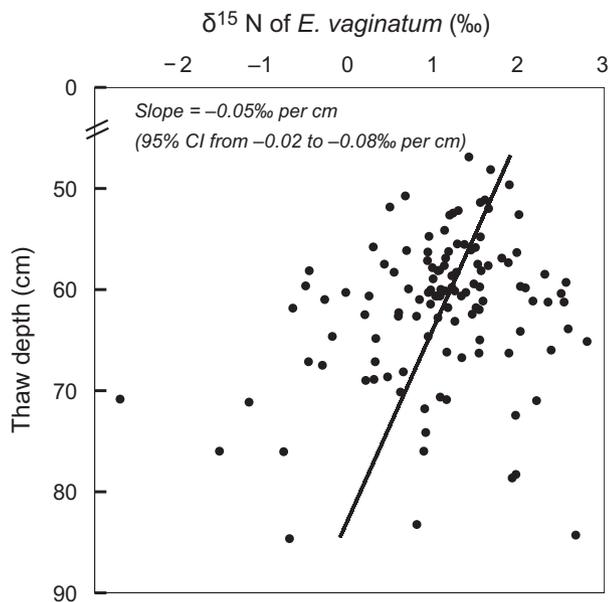


Fig. 4 Thaw depth vs. foliar $\delta^{15}\text{N}$ signature of *Eriophorum vaginatum* during 5 years of experimental warming. Line represents slope and intercept of linear mixed effect model fit with random effects for year, fence, Soil warming (nested in fence), and Air warming (nested in Soil warming). The interface between soil and moss surface is defined as 0 cm.

only important factor for predicting *E. vaginatum* foliar $\delta^{15}\text{N}$ (Fig. 4). Each centimeter increase in thaw depth significantly decreased *E. vaginatum* foliar $\delta^{15}\text{N}$ by 0.05‰ (95% CI from -0.02‰ per cm to -0.08‰ per cm), indicating that *E. vaginatum*'s N source became isotopically lighter as thaw progressed.

Several vascular plant species exhibited significant changes in their foliar chemistry in response to warming treatments despite the fact that their biomass did not change (Table S2). The deciduous shrub *Betula* had a significant increase in foliar %N with Soil warming (0.16 increase in foliar %N, 95% CI from 0.03 to 0.29) but the interaction between Air warming and Soil warming decreased foliar %N by 0.34 (95% CI from -0.53 to -0.15). Similarly, the C : N of *B. nana* foliar samples decreased significantly in response to Soil warming (-1.93, 95% CI from -3.58 to -0.28) and increased when warming treatments were combined (4.58, 95% CI from 2.21 to 7.01). The interaction between Air warming and Soil warming significantly decreased the $\delta^{15}\text{N}$ signature of *B. nana* leaves by 1.26‰ (95% CI from -1.97‰ to -0.52‰). The $\delta^{13}\text{C}$ of *B. nana* leaves decreased significantly with Soil warming by -0.49‰ (95% CI from -0.96‰ to -0.02‰). The dynamic response of *B. nana*'s foliar chemistry to warming indi-

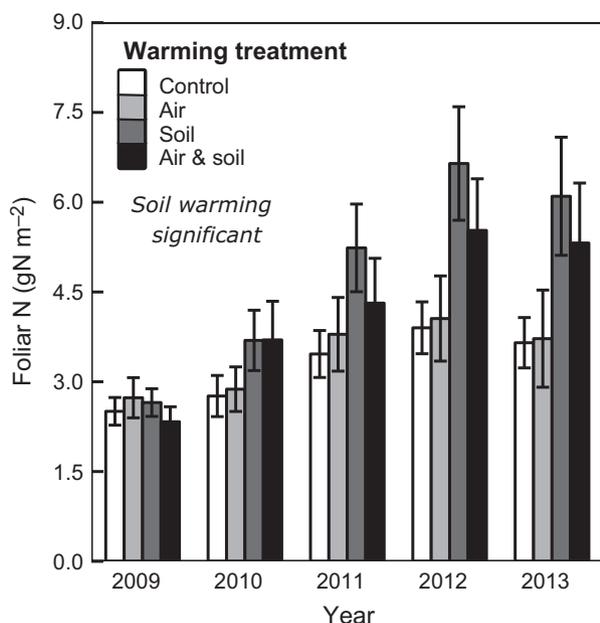


Fig. 5 Foliar N pools during 5 years of warming. Five years of Soil warming significantly increased foliar N by 49% or 1.61 g N m^{-2} (95% CI from 0.15 to 3.09 g N m^{-2}). Error bars represent SE based on annual treatment means.

states that C and N cycling within this species was altered by 5 years of warming despite the fact the *B. nana* biomass did not change.

Foliar N pools

The foliar N pool of the vascular plant community increased by 1.61 g N m^{-2} in response to 5 years of soil warming (Fig. 5, 95% CI from 0.15 to 3.09 g N m^{-2}). This effect was significant and translates to a 49% increase in foliar N pools. The interaction between Air warming and Soil warming did not impact the foliar N

pool significantly, but the value for this coefficient tended to be negative (-0.81 g N m^{-2} , 95% CI from -2.85 to 1.25 g N m^{-2}).

Soil inorganic N availability

Growing season soil inorganic N availability increased dramatically in response to Soil warming (Fig. 6a). Inorganic N on resin bags deployed from June to September increased by $4.78 \mu\text{g N g}^{-1}$ dry resin with Soil warming (Table 4, 95% CI from $0.99 \mu\text{g N g}^{-1}$ dry resin to $8.72 \mu\text{g N g}^{-1}$ dry resin). This effect represents a 136% increase in growing season soil inorganic N availability with permafrost thaw. The interaction between Air warming and Soil warming treatments, however, was significant and negative. This negative model coefficient means that the inorganic N availability decreased when the two warming treatments were applied together ($-5.21 \mu\text{g N g}^{-1}$ dry resin, 95% CI from $-1.24 \mu\text{g N g}^{-1}$ dry resin to $-9.26 \mu\text{g N g}^{-1}$ dry resin). This reduction was large enough to negate all increases associated with Soil warming. When applied alone, Air warming did not have a significant effect on growing season inorganic N availability.

During the winter, Soil warming also increased soil inorganic N availability (Fig. 6b). Total inorganic N bound to resins deployed from September to June increased by $5.77 \mu\text{g N g}^{-1}$ dry resin (Table 4), 95% CI from $0.81 \mu\text{g N g}^{-1}$ dry resin to $10.67 \mu\text{g N g}^{-1}$ dry resin) This effect translates to an 86% increase in inorganic N availability. Air warming applied alone or in conjunction with Soil warming did not significantly impact inorganic N availability during the winter. Inorganic N availability was higher during the winter than during the growing season and within both seasons 2013 values were lower than those observed in 2012.

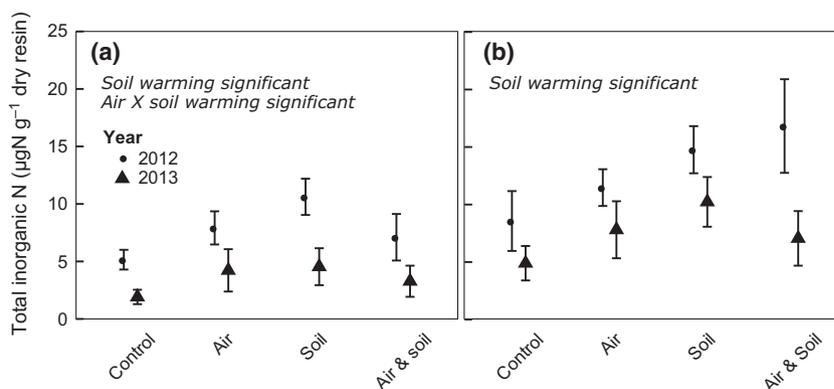


Fig. 6 Inorganic N availability measured with anion and cation binding resins deployed in surface soils during the growing season (a) and winter (b). During both seasons, Soil warming significantly increased inorganic N availability. Growing season inorganic N availability was significantly decreased by the interaction between Air & Soil warming treatments.

Table 4 Linear mixed effect model coefficients and 95% confidence intervals for biomass, foliar N, and inorganic N availability during the growing season and winter

	Coefficient	Lower CI (2.5%)	Upper CI (97.5%)	Significance
Biomass model, 2009–2013				
Intercept	501.36	383.40	616.87	Yes
Air	13.10	−97.76	122.08	No
Soil	114.51	7.34	222.94	Yes
Air × Soil	−67.30	−222.71	89.35	No
Foliar N model, 2009–2013				
Intercept	3.26	1.86	4.60	Yes
Air	0.18	−1.23	1.58	No
Soil	1.61	0.15	3.09	Yes
Air × Soil	−0.81	−2.85	1.25	No
Growing season inorganic N availability model, 2012–2013				
Intercept	3.51	−1.27	8.19	No
Air	2.51	−0.40	5.42	No
Soil	4.78	0.99	8.72	Yes
Air × Soil	−5.21	−9.26	−1.24	Yes
Winter inorganic N availability model, 2012–2013				
Intercept	6.68	0.43	12.82	Yes
Air	2.98	−1.98	8.05	No
Soil	5.77	0.81	10.67	Yes
Air × Soil	−3.67	−10.52	3.01	No

Bold type designates statistical significance based on whether or not the 95% confidence interval of linear mixed effect model coefficient included zero. Note that models tested for an overall treatment effect of 5 years of warming but did not quantify changes within the 5 year period.

Discussion

Environmental changes associated with experimental warming

The treatments applied at the CiPEHR experiment effectively simulated a future warmer climate: air temperature was elevated, on average, by 0.42 °C during the growing season while annual soil temperatures increased 0.82–1.00 °C relative to unwarmed controls. Alaskan mean annual temperatures are projected to increase by 0.4–2.8 °C by 2035 (IPCC AR5, 2013). Given these predictions, the CiPEHR experiment should be considered a conservative simulation of Alaskan projected climate in 2035. The environmental changes induced by snow fences at CiPEHR were not limited to changes in the mean annual temperatures, however; several seasonal patterns were altered by this manipulative treatments. Winter snow accumulation insulated soils and significantly elevated the average temperature of the entire soil profile during winter and continued to impact soil temperatures at 20 and 40 cm depth during

the growing season. Increased soil temperatures translated to significantly deeper seasonal thaw that extended further into the soil profile each growing season. This suggests that even after 5 years, permafrost thaw in this ecosystem has not equilibrated to the new energy balance induced by our manipulative treatments.

The Soil warming treatment induced dramatic permafrost thaw at CiPEHR. While all plots exhibited some degree of progressive thaw through the 5 year study, that depth in the CiPEHR Control plots was shallower than an adjacent area with minimal degradation (63.7 cm vs. 58.6 cm, $P < 0.001$) and the change in thaw in the Control plots during this period was within the range of interannual variation observed at these sites. The Soil warming treatment did impose substantial increases in thaw and permafrost degradation above and beyond changes occurring locally, increasing the maximum depth of thaw depth by to 20% through course of this study.

In addition to altering soil temperatures and the depth of thaw, Soil warming also significantly increased growing season soil moisture. Wetter soils in areas experiencing permafrost thaw were likely due to ground subsidence since spring time snow removal ensures water inputs are similar across all CiPEHR treatments. As permafrost thaws, ice lenses melt and the soil subsides into the space previously occupied by ice. After subsidence, the distance between the soil surface and the water table is reduced due to compression of the active layer (Jorgenson & Osterkamp, 2005). Soil subsidence is reflected in increased soil bulk density values at 55–65 cm in the Soil warming treatment (E. Pegoraro, unpublished data). Increased soil moisture and subsidence were unexpected consequences of warming and permafrost thaw at CiPEHR but these physical changes to the soil environment have important implications for decomposition (Hicks Pries *et al.*, 2013) and plant access to soil nutrients.

The OTCs deployed during the growing season at CiPEHR effectively increased air temperatures but had little impact on soil moisture or temperature of surface soils. The 0.42 °C increase in the average growing season air temperature observed at CiPEHR was less dramatic than the 1.2–1.8 °C average increase observed in the ITEX experiments (Marion *et al.*, 1997), possibly due to the smaller size of the CiPEHR OTCs. Air & Soil warmed plots tended to have more shallow thaw than Soil warmed plots (−2.10 cm, 95% CI from −5.37 to 1.28 cm), but this nonsignificant trend was present in pretreatment thaw measured at the end of the 2008 growing season (−4.37 cm, 95% CI from −9.50 to 0.47 cm). We therefore conclude that the impact of Air warming on the belowground environment is minimal

and the tendency for shallow in Air & Soil warmed plots reflects landscape heterogeneity.

Plant available N increases with permafrost thaw

By the fourth and fifth years of Soil warming, soil inorganic N availability had increased relative to Controls by 136% during the growing season and by 86% during the winter (Fig. 6). Since soil temperature and soil moisture both increased with Soil warming at CiPEHR, the relative importance of these factors in controlling soil inorganic N availability is difficult to tease apart. Across all treatments, resins deployed in winter accumulated almost twice as much inorganic N than those deployed during the growing season. Such a large seasonal disparity could be attributed to a longer deployment period for winter resins (8.5 months vs. 3.5 months), lack of competition from plant roots for available N during the winter, and the decomposition of fall litter inputs and turnover of fine roots (Hobbie & Chapin, 1996; Kielland *et al.*, 2006). In 2012, inorganic N availability was higher than in 2013 during both the growing season and the winter. However, foliar N pools (Fig. 5) in 2012 and 2013 do not indicate that there was any change in plant access to N and all CiPEHR treatments exhibited the same decline in soil inorganic N availability from 2012 to 2013. Together, these observations suggest that observed decline in soil inorganic N availability might not have been a response experimental warming but rather a methodological artifact, potentially due to decreased binding efficiency in reused resins. We therefore conclude that soil inorganic N increased dramatically as CiPEHR soils became warmer, wetter, and more deeply thawed.

The coordinated increase in soil inorganic N availability and foliar N pools with Soil warming indicates that permafrost thaw allowed plants to access previously unavailable N sources. Moreover, the increase in the foliar N pool was large enough that it cannot be explained by translocation of existing plant N: instead, it is best interpreted as an increase in plant uptake of soil N. The foliar N pool of vascular plant species in CiPEHR Control plots in 2009 was 2.51 g N m^{-2} , and work by Sullivan *et al.* (2007) has shown that moist acidic tussock tundra contains approximately 250 g m^{-2} fine root biomass that is, on average, 1.27% N by weight. Based on these values, the total N in leaves (measured at 2.51 g N m^{-2}) and fine roots (estimated at 3 g N m^{-2}) is likely to have been around 6 g N m^{-2} before warming treatments were applied at CiPEHR. In the fourth and fifth year of warming, however, the foliar N pool alone averaged over 6 g N m^{-2} in Soil warmed plots. Reduced plant allocation to belowground structures is likely with increased N

availability, but such a complete translocation is improbable over a 5 year time period. The fact that Soil warmed plants have utilized new sources of N, however, has important implications for plant productivity in these N limited ecosystems.

As expected, plant biomass increased in response to increased N availability and the altered environmental conditions associated with Soil warming treatments. The magnitude of the response over the 5 year period, however, was not as great as that observed in soil inorganic N availability. This can be partially explained by the fact that soil inorganic N availability was measured only during the last 2 years of warming when treatment effects had accumulated. When plant responses from 2012 and 2013 were analyzed without the preceding 3 years of data, the Soil warming effect on these response variables was stronger: biomass increased 30% rather than 23% and foliar N increased 69% rather than 49%. These responses, however, were still smaller in magnitude compared to the response of growing season soil inorganic N to Soil warming (136%). The disparity between plant and soil responses to warming at CiPEHR is similar to the pattern observed across ecosystems by Rustad *et al.* (2001); their global meta-analysis of manipulative warming studies showed that 2–9 years of warming elevated soil N mineralization rates by 46% and plant productivity by 19%. The differential response of plants vs. soils to CiPEHR soil warming is therefore typical and could be attributed to DIN bound to resin bags in surface soils failing to reflect plant available N throughout the soil profile. Other possible reasons for this disparity include increased turnover of biomass and foliar N pools with warming, limitation by another nutrient or environmental condition, or a delay in the response of plants to increased soil N availability.

Air & Soil warming interact to reduce soil inorganic N availability

Our hypothesis that Air warming alone would have little impact on plant growth was supported: neither plant biomass nor foliar N pools significantly increased in response to this treatment. The lack of response could be because the $0.42 \text{ }^{\circ}\text{C}$ increase in growing season air temperature was too weak to trigger increased plant growth. Alternatively, plant productivity in Air warmed plots could have been limited by other environmental variables, namely soil temperature, soil moisture, or soil inorganic N availability. Disentangling the role of these environmental factors is complicated by the fact that all three increased with the Soil warming treatment and none of them were significantly impacted by Air warming. When Air & Soil warming

were combined, however, soil inorganic N during the growing season was significantly reduced compared to the Soil warming treatment alone. This unexpected decrease in soil inorganic N availability was paired with a tendency for reduced biomass and foliar N pools in Air & Soil warmed treatment compared to Soil warming alone. It is unlikely that the trend in biomass or foliar N pools was caused by moisture or temperature limitation on plant growth since Air warming did not impact either one of these variables. Additionally, none of the plant species present had any increases in foliar $\delta^{13}\text{C}$ in response to Air warming. If plant species were experiencing water stress, foliar $\delta^{13}\text{C}$ signatures would have increased due to prolonged periods of stomatal closure (Farquhar & Richards, 1984). As a result, we propose that diminished plant response was driven largely by significantly lower soil inorganic N availability in Air & Soil warmed plots.

Thaw in Air & Soil warmed plots was significantly deeper than Controls but two treatments appeared to have similar soil inorganic N availability during the growing season. The depth of permafrost thaw therefore does not directly predict soil inorganic N availability at CiPEHR. Air & Soil warmed plots had significantly higher soil moisture, air temperature, and aboveground biomass than Control plots, so we propose that higher rates of evapotranspiration in Air & Soil warmed plots may have increased movement of soil solution and potentially altered soil microbial dynamics. Overall, we found a strong positive relationship between the depth of permafrost thaw and plant available N in the tundra but the low soil inorganic N availability observed when warming treatments were combined could have important implications for predicting how tundra plant and soil dynamics will respond to future climate.

Plant species responses to experimental warming

We found support for our hypothesis that graminoid species would be the first to respond to permafrost thaw: a linear regression between Δ *E. vaginatum* biomass and Δ total vascular plant biomass showed *E. vaginatum* explained 91% of the changes in total biomass from 2009 to 2013. *E. vaginatum* is a dominant species in the plant community, but there was spatial heterogeneity of this species at the plot scale. Across all plots, Soil warming did not significantly increase *E. vaginatum* biomass. However, when plots with $<10 \text{ g m}^{-2}$ *E. vaginatum* ($\sim 2\%$ of total vascular biomass) were excluded from the model the response to Soil warming was significant (118.18 g m^{-2} , 95% CI from 1.95 to 236.09 g m^{-2}) and similar in scale the response observed for changes in total biomass across the entire

dataset (114.51 g m^{-2}). Changes in the biomass of plots with low *E. vaginatum* biomass may have been difficult to detect given the inherently low spatial resolution of the point intercept method.

The role of *E. vaginatum* as the primary responder to permafrost thaw was first observed at CiPEHR after 2 years of Soil warming (Natali *et al.*, 2012) and was consistent with the plant community structure changes observed at a natural gradient of permafrost degradation at EML. Schuur *et al.* (2007) saw that graminoid biomass increased from sites where permafrost was minimally thawed (45.9 cm) to sites where permafrost was moderately thawed (20 years of thaw, 49.7 cm). By the time permafrost was extensively thawed (>30 years of thaw, 65.3 cm), deciduous shrubs dominated and graminoid biomass was reduced. Though *E. vaginatum* foliar chemistry was not significantly impacted by the warming treatments, the high variance of foliar $\delta^{15}\text{N}$ values for *E. vaginatum* was best explained by an increase in thaw depth (Fig. 4). The $\delta^{15}\text{N}$ values of bulk soil organic matter increase with depth at this site (Hicks Pries *et al.*, 2012), suggesting there is not a direct connection between bulk soil N exposed by thawing permafrost and foliar $\delta^{15}\text{N}$ values. The relationship between depth and the $\delta^{15}\text{N}$ values of dissolved N is unknown and is likely to differ from bulk organic matter values. In the boreal forest, dissolved organic N was found to have a negative $\delta^{15}\text{N}$ value so increased thaw could have increased *E. vaginatum*'s uptake of this N source (Mayor *et al.*, 2012). Regardless of the exact mechanism behind the correlation between thaw depth and foliar $\delta^{15}\text{N}$, this result supports our conclusion that increased thaw at CiPEHR has altered N cycling and increased plant access to N.

Changes in the foliar chemistry of the deciduous shrub *B. nana* with warming are of particular interest given this species key role in response of long-term warming experiment at Toolik Lake, Alaska (Chapin *et al.*, 1995; Shaver *et al.*, 2001). At CiPEHR, there was no significant response of *B. nana* biomass to any of the warming treatments (Table S2). However, Soil warming did prompt *B. nana* to have a significant decrease in foliar $\delta^{13}\text{C}$, a significant increase in foliar %N, and a significant decrease in foliar C : N. Together these changes suggest that as permafrost thaws, *B. nana* has access to more N and either more access to water or higher C assimilation rates (Farquhar *et al.*, 1989). When Air warming and Soil warming were combined, *B. nana* leaves exhibited decreased $\delta^{15}\text{N}$, decreased %N, and increased C : N. These findings indicate that *B. nana* is experiencing exacerbated N limitation in Air & Soil warmed plots compared to Soil warmed plots. The lower foliar $\delta^{15}\text{N}$ signature in Air & Soil warmed plots could due to *B. nana* relying more heavily on N trans-

ferred via ectomycorrhizal symbionts since this exchange discriminates against the heavier isotope (Hobbie & Colpaert, 2003; Hobbie & Hobbie, 2006). Increased reliance on mycorrhizal symbionts was observed in response to long-term warming by greenhouses at Toolik Lake (Deslippe *et al.*, 2012) and would be logical at CiPEHR based on the low soil inorganic N availability observed in Air & Soil warmed plots. As warming continues across high latitude ecosystems, we expect mycorrhizae to play an increasingly important role in determining plant access to N.

Plant biomass changes in response to soil carbon loss

Increased plant biomass in response to permafrost thaw at CiPEHR translates to an increase in the plant C pool of 157 g C m^{-2} (95% CI from 10 to 306 g C m^{-2}) over the 5 year manipulation if we assume that (i) aboveground plant biomass was 45% C and (ii) belowground plant allocation of C was twice as high aboveground (Shaver & Chapin, 1991). This estimate does not take into account changes that may have occurred in allocation, rhizome production, or turnover of plant tissues. Projections from an isotope partitioning model of ecosystem respiration at CiPEHR suggests that $35\text{--}55 \text{ g C m}^{-2}$ would be lost from old, deep soils every growing season (Hicks Pries *et al.*, 2015). On this basis, 5 years of thaw could induce $175\text{--}275 \text{ g C m}^{-2}$ from old, deep soils and is therefore unlikely to be fully offset by observed 157 g C m^{-2} increase in plant C. Increased C storage in litter pools at CiPEHR would provide additional compensation for C losses but we do not have the data to sufficiently characterize litter production, pool size or turnover at CiPEHR. Additionally, the observed high level of N availability in warmed soils would likely increase turnover of active layer soils (Mack *et al.*, 2004), adding to potential C losses from young, shallow soils. The difference between increases in the plant C pool vs. losses from the deep soil C pool therefore does not represent the net change in the ecosystem carbon balance during permafrost thaw. The growing net ecosystem exchange (NEE) of CO_2 balance at CiPEHR has monitored during the growing season by an autochamber system (Natali *et al.*, 2011, 2014) and the contribution of winter respiration has been modeled based on several datasets of winter respiration at the EML site (Belshe *et al.*, 2012; Natali *et al.*, 2014; Webb *et al.*, 2016). By combining growing season NEE data from autochambers and models of wintertime respiration, Natali *et al.* (2014) determined that CiPEHR Soil warmed plots acted as a C sink in the first 2 years of warming but acted as a C source by year three. Natali *et al.*'s conclusion this

warmed landscape has transitioned to a C source provides import context for our conclusion that increases in the plant C pool are unlikely to be greater than losses from the deep soil C pool during the first 5 years of permafrost thaw.

Conclusions

The research presented here documented changes in plant productivity and N cycling associated with 5 years of Air & Soil warming in a tundra ecosystem. Experimental Soil warming at this site induced permafrost thaw and increased soil moisture due to subsidence of the active layer. Plant access to N increased dramatically within 5 years of permafrost thaw, as evidenced by significant increases in soil inorganic N availability during the fourth and fifth years of warming. Increased N availability and permafrost thaw were associated with large increases in aboveground biomass (23%) and foliar N (49%) over the same 5 year period. The 114 g C m^{-2} increased in the plant biomass induced by Soil warming was not enough to compensate for estimated losses of C from deep soils in these plots (Hicks Pries *et al.*, 2015). Surprisingly, Air warming interacted negatively with Soil warming to significantly reduce soil inorganic N availability during the growing season despite the fact that Air warming treatments did not significantly impact the belowground environment. This unexpected finding suggests that N cycling in tundra ecosystems is impacted not only by soil temperatures but also the disparity between Air & Soil temperatures during the growing season. The dominant sedge *E. vaginatum* made up most of the changes in total biomass in response to Soil warming, suggesting that any shift from graminoid to deciduous shrub dominance would take more than 5 years to develop. Collectively, we found that 5 years of permafrost thaw increased plant access to N but the concurrent increases in the plant C pool were unlikely to be greater than losses from deep soil C.

Acknowledgements

The authors would like to thank Chris Wilson, Christina Schädel, Elaine Pegoraro, Jennie Demarco and three anonymous reviewers for valuable help with data analysis and manuscript preparation.

References

- Addis CE (2014) Effects of increased snow on growth and allocation patterns of arctic plants. MS thesis. University of Alaska, Fairbanks, 39 pp.
- Bates D, Maechler M, Bolker B, Walker S (2014) lme4: linear mixed-effects models using Eigen and S4.
- Belshe EF, Schuur EA, Bolker BM, Bracho R (2012) Incorporating spatial heterogeneity created by permafrost thaw into a landscape carbon estimate. *Journal of Geophysical Research: Biogeosciences*, **117**, G01026.

- Bowden WB, Gooseff MN, Balsler A, Green A, Peterson BJ, Bradford J (2008) Sediment and nutrient delivery from thermokarst features in the foothills of the North Slope, Alaska: potential impacts on headwater stream ecosystems. *Journal of Geophysical Research: Biogeosciences*, **113**, 1–12.
- Chapin FS (1983) Direct and indirect effects of temperature on arctic plants. *Polar Biology*, **2**, 47–52.
- Chapin FS, Shaver GR, Giblin AE, Nadelhoffer KJ, Laundre A (1995) Responses of arctic tundra to experimental and observed changes in climate. *Ecology*, **76**, 694–711.
- Cleveland CC, Townsend AR, Schimel DS *et al.* (1999) Global patterns of terrestrial biological nitrogen (N_2) fixation in natural ecosystems. *Global Biogeochemical Cycles*, **13**, 623–645.
- Deane-Coe KK, Mauritz M, Celis G, Salmon V, Crummer KG, Natali SM, Schuur EA (2015) Experimental warming alters productivity and isotopic signatures of tundra mosses. *Ecosystems*, **18**, 1070–1082.
- DeMarco J, Mack MC, Bret-Harte MS (2011) The effects of snow, soil microenvironment, and soil organic matter quality on N availability in three Alaskan arctic plant communities. *Ecosystems*, **14**, 804–817.
- Deslippe JR, Hartmann M, Simard SW, Mohn WW (2012) Long-term warming alters the composition of Arctic soil microbial communities. *FEMS Microbiology Ecology*, **82**, 303–315.
- Farquhar G, Richards R (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Australian Journal of Plant Physiology*, **11**, 539.
- Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, **40**, 503–537.
- Giblin AE, Laundre JA, Nadelhoffer KJ, Shaver GR (1994) Measuring nutrient availability in arctic soils using ion exchange resins: a field test. *Soil Science Society of America Journal*, **58**, 1154.
- Graglia E, Jonasson S, Michelsen A *et al.* (2001) Effects of environmental perturbations on abundance of subarctic plants after three, seven and ten years of treatments. *Ecography*, **24**, 5–12.
- Harden JW, Koven CD, Ping CL *et al.* (2012) Field information links permafrost carbon to physical vulnerabilities of thawing. *Geophysical Research Letters*, **39**, L15704.
- Hicks Pries CE, Schuur EAG, Crummer KG (2012) Holocene carbon stocks and carbon accumulation rates altered in soils undergoing permafrost thaw. *Ecosystems*, **15**, 162–173.
- Hicks Pries CE, Schuur EA, Vogel JG, Natali SM (2013) Moisture drives surface decomposition in thawing tundra. *Journal of Geophysical Research: Biogeosciences*, **118**, 1133–1143.
- Hicks Pries CE, Schuur EAG, Natali SM, Crummer KG (2015) Old soil carbon losses increase with ecosystem respiration in experimentally thawed tundra. *Nature Climate Change*. doi: 10.1038/nclimate2830.
- Hobbie SE (1992) Effects of plant species on nutrient cycling. *Trends in Ecology & Evolution*, **7**, 336–339.
- Hobbie SE, Chapin FS (1996) Winter regulation of tundra litter carbon and nitrogen dynamics. *Biogeochemistry*, **35**, 327–338.
- Hobbie EA, Colpaert JV (2003) Nitrogen availability and colonization by mycorrhizal fungi correlate with nitrogen isotope patterns in plants. *New Phytologist*, **157**, 115–126.
- Hobbie JE, Hobbie EA (2006) ^{15}N in symbiotic fungi and plants estimates nitrogen and carbon flux rates in arctic tundra. *Ecology*, **87**, 816–822.
- Hugelius G, Strauss J, Zubrzycki S *et al.* (2014) Improved estimates show large circum-polar stocks of permafrost carbon while quantifying substantial uncertainty ranges and identifying remaining data gaps. *Biogeochemical Discussions*, **11**, 4771–4822.
- IPCC AR5 (2013) Chapters 6 & 14. In: *Climate Change 2013: The Physical Science Basis* (eds Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley P), pp. 467–553; 1219–1256. Cambridge University Press, Cambridge, UK and New York, NY.
- Jorgenson MT, Osterkamp TE (2005) Response of boreal ecosystems to varying modes of permafrost degradation. *Canadian Journal of Forest Research*, **35**, 2100–2111.
- Keuper F, Bodegom PM, Dorrepaal E, Weedon JT, Hal J, Logtestijn RSP, Aerts R (2012) A frozen feast: thawing permafrost increases plant-available nitrogen in subarctic peatlands. *Global Change Biology*, **18**, 1998–2007.
- Kielland K, Olson K, Ruess RW, Boone RD (2006) Contribution of winter processes to soil nitrogen flux in taiga forest ecosystems. *Biogeochemistry*, **81**, 349–360.
- Koven CD, Ringeval B, Friedlingstein P *et al.* (2011) Permafrost carbon-climate feedbacks accelerate global warming. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 14769–14774.
- Koven CD, Lawrence DM, Riley WJ (2015) Permafrost carbon-climate feedback is sensitive to deep soil carbon decomposability but not deep soil nitrogen dynamics. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, 3752–3757.
- Leffler AJ, Welker JM (2013) Long-term increases in snow pack elevate leaf N and photosynthesis in *Salix arctica*: responses to a snow fence experiment in the High Arctic of NW Greenland. *Environmental Research Letters*, **8**, 025023.
- Mack MC, Schuur EAG, Bret-Harte MS, Shaver GR, Chapin FS (2004) Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. *Nature*, **431**, 440–443.
- Marion G, Henry G, Freckman D *et al.* (1997) Open-top designs for manipulating field temperature in high-latitude ecosystems. *Global Change Biology*, **3**, 20–32.
- Mayor JR, Schuur EAG, Mack MC, Hollingsworth TN, Bååth E (2012) Nitrogen isotope patterns in Alaskan black spruce reflect organic nitrogen sources and the activity of ectomycorrhizal fungi. *Ecosystems*, **15**, 819–831.
- Natali SM, Schuur EA, Trucco C, Hicks Pries CE, Crummer KG, Baron Lopez AF (2011) Effects of experimental warming of air, soil and permafrost on carbon balance in Alaskan tundra. *Global Change Biology*, **17**, 1394–1407.
- Natali SM, Schuur EA, Rubin RL (2012) Increased plant productivity in Alaskan tundra as a result of experimental warming of soil and permafrost. *Journal of Ecology*, **100**, 488–498.
- Natali SM, Schuur EA, Webb EE, Pries CE, Crummer KG (2014) Permafrost degradation stimulates carbon loss from experimentally warmed tundra. *Ecology*, **95**, 602–608.
- Natali SM, Schuur EAG, Mauritz M *et al.* (2015) Permafrost thaw and soil moisture driving CO_2 and CH_4 release from upland tundra. *Journal of Geophysical Research: Biogeosciences*, **120**, 525–537.
- Osterkamp TE, Romanovsky VE (1999) Evidence for warming and thawing of discontinuous permafrost in Alaska. *Permafrost and Periglacial Processes*, **10**, 17–37.
- Pinheiro JC, Bates DM (2000) Mixed-effects models in S and S-PLUS. In: *Statistics and Computing* (eds Chambers J, Eddy W, Hardle W, Sheather S, Tierney L), p. 537. Springer, New York, NY.
- Press M, Potter J, Burke MJW, Callaghan TV, Lee J (1998) Responses of a subarctic dwarf shrub heath community to simulated environmental change. *Journal of Ecology*, **86**, 315–327.
- R Development Core Team (2014) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Reyes FR, Loughheed VL (2015) Rapid nutrient release from permafrost in Arctic aquatic ecosystems. *Arctic, Antarctic, and Alpine Research*, **47**, 35–48.
- Rustad L, Campbell J, Marion G *et al.* (2001) A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia*, **126**, 543–562.
- Schimel JP, Chapin FS (1996) Tundra plant uptake of amino acid and NH_4^+ nitrogen *in situ*: plants complete well for amino acid N. *Ecology*, **77**, 2142–2147.
- Schuur EA, Crummer KG, Vogel JG, Mack MC (2007) Plant species composition and productivity following permafrost thaw and thermokarst in Alaskan tundra. *Ecosystems*, **10**, 280–292.
- Schuur EA, McGuire AD, Schädel C *et al.* (2015) Climate change and the permafrost carbon feedback. *Nature*, **520**, 171–179.
- Shaver GR, Chapin FS III (1991) Production: biomass relationships and element cycling in contrasting arctic vegetation types. *Ecological Monographs*, **61**, 1–31.
- Shaver GR, Chapin FS III, Gartner BL (1986) Factors limiting seasonal growth and peak biomass accumulation in *Eriophorum vaginatum* in Alaskan tussock tundra. *Journal of Ecology*, **74**, 257–278.
- Shaver GR, Billings W, Chapin FS III, Giblin AE, Nadelhoffer KJ, Oechel WC, Rastetter EB (1992) Global change and carbon balance of arctic ecosystems. *BioScience*, **42**, 433–441.
- Shaver GR, Syndergaard Bret-Harte M, Jones MH, Johnstone J, Gough L, Laundre J, Stuart Chapin F (2001) Species composition interacts with fertilizer to control long-term change in tundra productivity. *Ecology*, **82**, 3163–3181.
- Sistla SA, Schimel JP (2013) Seasonal patterns of microbial extracellular enzyme activities in an arctic tundra soil: identifying direct and indirect effects of long-term summer warming. *Soil Biology and Biochemistry*, **66**, 119–129.
- Sistla SA, Moore JC, Simpson RT, Gough L, Shaver GR, Schimel JP (2013) Long-term warming restructures Arctic tundra without changing net soil carbon storage. *Nature*, **497**, 615–618.
- Sturm M, Racine C, Tape K (2001a) Climate change: increasing shrub abundance in the Arctic. *Nature*, **411**, 546–547.
- Sturm M, McFadden JP, Liston GE *et al.* (2001b) Snow–shrub interactions in arctic tundra: a hypothesis with climatic implications. *Journal of Climate*, **14**, 336–344.
- Sullivan PF, Sommerkorn M, Rueth HM, Nadelhoffer KJ, Shaver GR, Welker JM (2007) Climate and species affect fine root production with long-term fertilization in acidic tussock tundra near Toolik Lake, Alaska. *Oecologia*, **153**, 643–652.

- Treat C, Natali SM, Ernakovich J *et al.* (2015) A pan-Arctic synthesis of CH₄ and CO₂ production from anoxic soil incubations. *Global Change Biology*, **21**, 2787–2803.
- Van Wijk MT, Clemmensen KE, Shaver GR *et al.* (2003) Long-term ecosystem level experiments at Toolik Lake, Alaska, and at Abisko, Northern Sweden: generalizations and differences in ecosystem and plant type responses to global change. *Global Change Biology*, **131**, 105–123.
- Vogel J, Schuur EA, Trucco C, Lee H (2009) Response of CO₂ exchange in a tussock tundra ecosystem to permafrost thaw and thermokarst development. *Journal of Geophysical Research: Biogeosciences*, **114**, G04018.
- Wahren CHA, Walker MD, Bret-Harte MS (2005) Vegetation responses in Alaskan arctic tundra after 8 years of a summer warming and winter snow manipulation experiment. *Global Change Biology*, **11**, 537–552.
- Walsh N, McCabe T, Welker J, Parsons A (1997) Experimental manipulations of snow-depth: effects on nutrient content of caribou forage. *Global Change Biology*, **3**, 158–164.
- Webb EE, Schuur EAG, Natali SM *et al.* (2016) Increased wintertime CO₂ loss as a result of sustained tundra warming. *Journal of Geophysical Research: Biogeosciences*. doi: 10.1002/2014JG002795.
- Wipf S, Rixen C (2010) A review of snow manipulation experiments in Arctic and alpine tundra ecosystems. *Polar Research*, **29**, 95–109.
- Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York, NY.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Linear Mixed effect model coefficients and 95% confidence intervals for annual average soil temperature, soil volumetric water content, air temperature and thaw depth.

Table S2. Linear Mixed effect model coefficients and 95% confidence intervals for foliar %C, %N, C : N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of dominant species.