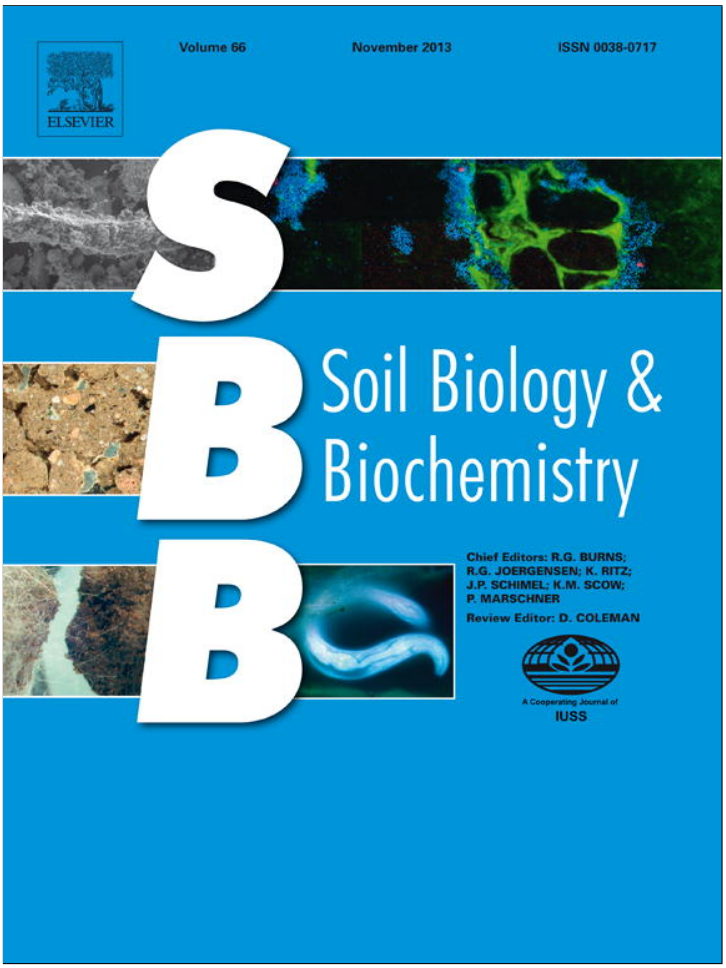

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Seasonal patterns of microbial extracellular enzyme activities in an arctic tundra soil: Identifying direct and indirect effects of long-term summer warming

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ABSTRACT

Arctic systems, which store ~50% of global soil carbon, are undergoing rapid climatic warming that may drive significant carbon release to the atmosphere. To better understand how warming impacts arctic decomposition, we characterized the effects of a twenty-two year long tundra greenhouse warming experiment on decomposer-produced extracellular enzymes, nutrients, and microbial biomass across a year. This experiment, which is the longest running tundra ecosystem warming study in existence, was previously shown to have altered the plant and soil communities. The greenhouse treatment has also changed the seasonal soil temperature regime by indirectly increasing winter soil temperature, an effect that was likely facilitated through an increase in snow-trapping shrub biomass. Irrespective of the warming treatment, we observed that peak nutrient pools, microbial biomass, and hydrolytic enzyme activities all occurred from the late winter through thaw. This pattern was decoupled from peak oxidative enzyme activities, which occurred during the summer. The greenhouse treatment amplified the natural seasonal cycle of extracellular enzyme activities, suggesting that tundra decomposer communities maintain a temporal niche space which is critical to understanding how arctic biogeochemical cycling will respond to warming. A spatial separation was also observed; extracellular enzyme activities in the deeper soil horizons were more sensitive to warming than at the surface. Direct greenhouse warming did not strongly stimulate decomposition: only oxidative enzyme activities in the surface horizon increased during the summer. Unexpectedly, the strongest treatment effect observed was a stimulation of hydrolytic enzyme activities at depth in the mineral horizon from the late winter through thaw (which also affected extracellular enzyme stoichiometry, increasing C:N and C:P acquisition activities), before the greenhouse treatment was directly active. This effect declined during senescence and was reversed in early winter, suggesting that negative biotic-abiotic feedbacks may curtail increased decomposer activity in warming arctic systems.

1. Introduction

Arctic and boreal soils are among the largest stores of soil organic carbon (SOC) globally, representing nearly 50% of the world's soil C stocks (Tarnocai et al., 2009). SOC accumulated because cold soil temperatures limit decomposer activity, which constrains nitrogen (N) release, thereby further limiting both plant and microbial growth (Chapin et al., 1995; Mack et al., 2004; Sistla et al., 2012). The arctic is rapidly warming, however, and temperatures will continue to increase over the next century (Moritz et al., 2002). By stimulating decomposers, warming increases plant growth and woody dominance in tundra systems (Walker et al., 2006; Elmendorf et al., 2012; Natali et al., 2012). These coupled changes can facilitate longer-term affects on seasonal soil temperature patterns and soil community structure (Blok et al., 2011; Deslippe et al., 2012; Sistla et al., 2013).

A major uncertainty in projecting the net effect of warming on tundra soil C stocks is identifying whether changes in the plant community will dampen or accelerate the direct positive effects of warming on decomposers (Elmendorf et al., 2012). Winter soil temperatures are projected to increase disproportionately in tundra systems (Sturm et al., 2005; Schuur et al., 2008), when the decomposer community is likely the most sensitive to warming. At

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a finer resolution, microbially-synthesized extracellular enzymes directly catalyze the break down of polymeric soil compounds (Burns, 1982; Schimel and Weintraub, 2003). In Arctic systems, extracellular enzyme activity (EEA) is strongly temperature limited (Wallenstein et al., 2009). Therefore, EEA should increase as Arctic soils warm. Notably, tundra EEA remains relatively low during the summer, even when soil temperatures rise above freezing (Wallenstein et al., 2009). This phenomenon suggests that when warmer temperatures would otherwise accelerate decomposition, nutrient availability may limit EEA by regulating microbial growth (Weintraub and Schimel, 2005; Sistla et al., 2012) or the activity of specific members of the decomposer community.

Because suites of extracellular enzymes are associated with different soil and decomposer characteristics, seasonal patterns of EEA—and EEA stoichiometry, which correlates with resource acquisition demand (Sinsabaugh et al., 2008)—may change under sustained warming. Oxidative enzymes are associated with nutrient mining of recalcitrant N sources and tend to break down compounds with irregular molecular bonds (Talbot et al., 2012). Their activity is negatively correlated with inorganic N-availability and positively correlated with fungal dominance, in particular the presence of ectomycorrhizal (ECM) fungi (Cusack et al., 2010; Sinsabaugh, 2010; Talbot et al., 2012). Hydrolytic enzymes tend to degrade SOM constituents with regularly arranged, hydrolyzable bonds (e.g. cellulose, protein), and their activity tends to be positively associated with high N-availability, and dominance by saprotrophic fungi and bacteria (Waldrop and Zak, 2006; Sinsabaugh, 2010; Talbot et al., 2012). EEA therefore represents an integrated response to both soil temperature changes and longer-term feedbacks between plant-derived soil inputs, the decomposer community, and the soil environment.

Coupled biotic-abiotic feedbacks to warming take many years to develop in tundra systems (Lamb et al., 2011); therefore, long-running warming studies represent a particularly useful tool to identify the effects of these feedbacks on arctic decomposer activity and biogeochemical cycling. We characterized EEA and related biogeochemical characteristics in the 22nd year of an ecosystem tundra warming experiment. This summer-warming greenhouse (GH) experiment was initiated in 1989 at the Toolik Field Station, AK, and is the longest tundra warming study in existence. Twenty years of warming increased plant biomass and shrub dominance (Sistla et al., 2013). The GH treatment also increased ECM fungal biomass and the ratio of fungi to bacteria abundance in the organic horizons (Clemmensen et al., 2006; Deslippe et al., 2011, 2012). While warming tends to increase soil C- and N-mineralization (Hobbie and Chapin, 1998; Rustad et al., 2001), the GH treatment increased summertime C-mineralization only in the mineral horizon; it decreased surface soil active microbial biomass, and did not detectably alter N pools in any horizon (Sistla et al., 2013).

These counterintuitive effects may reflect feedbacks between the warming-driven changes in plant community structure and its effects on seasonal soil temperature patterns. Over time, the GH treatment has altered the soil temperature regime and increased maximum thaw depth (Deslippe et al., 2011; Sistla et al., 2013). While the direct summer-warming effects of the GH treatment have been dampened (an effect that is likely to be caused by increased shading and greater litter insulation of the soil surface), soils are now warmed in the winter into the mineral horizon (Blok et al., 2011; Deslippe et al., 2011; Sistla et al., 2013). This winter warming effect is likely driven by increased shrub biomass in the GH plots facilitating the formation of soil-insulating snow drifts, which is hypothesized to stimulate over-winter nutrient mineralization, thereby stimulating further shrub expansion (Schimel et al., 2004; Sturm et al., 2005).

Disentangling the effects of warming on tundra soil biogeochemical cycling is thus complicated by the altered biotic and abiotic conditions that develop as shrub expansion occurs. Further, the majority of over-winter soil C mineralization occurs when the soils are not deeply frozen—in the early winter and at thaw— periods that are also experiencing disproportionately strong climate warming (Sturm et al., 2005). However, our understanding of how sustained warming affects seasonal arctic soil biogeochemical patterns remains sparse. We identified how twenty-two years of GH warming affected the seasonal patterns of a suite of hydrolytic and oxidative EEA, microbial biomass, and nutrient pools. Specifically, we asked whether long-term summer warming: **1)** Indirectly stimulates decomposer activity during the winter; **2)** Differently affects seasonal patterns of hydrolytic and oxidative EEA, soil nutrients, and microbial biomass across depth; **3)** Drives positive or negative feedbacks to warming-acceleration of decomposition at an annual timescale?

2. Methods

2.1. Site description

The experimental GH study was established in 1989 on a moist acidic tundra (MAT) site near Toolik Lake, Alaska (68o38'N, 149o34'W). The experiment is maintained by the Arctic Long Term Ecological Research site. The tussock-forming sedge *Eriophorum vaginatum* forms regular vegetation patterns, with the dominant deciduous shrub *Betula nana* growing between the tussocks (Chapin and Shaver, 1989). There is approximately 0.9×10^6 km^2 of tussock tundra vegetation worldwide, which is found across the northern Alaska, Canada, and eastern Siberia (Oechel et al., 1993). MAT soil is classified as coarse-loamy, mixed, acidic, gelic Typic Aquiturbels, with a 30–50 cm thick organic horizon underlain by silty mineral soil (Romanovsky et al., 2007; National Cooperative Soil Survey Program, USDA).

2.2. Greenhouse treatment

The GH treatment consists of four replicate blocks that include spatially paired control and GH plots. Passive summer warming is created by erecting clear polyethylene-sheets over permanent wooden frames ($2.5 \times 5 \times 1.5$ m) when the ground is snow-free (~June 1st). The GH treatment does not detectably affect soil moisture (Deslippe et al., 2011; Sistla et al., 2013), and uneven microtopography allows air circulation beneath the greenhouse bases (Clemmensen et al., 2006). The GH reduces photosynthetically active radiation and direct precipitation inputs, but does not negatively influence plant growth (Deslippe et al., 2011).

Air temperature in the GH is elevated 2.1 °C on average over the summer, and its influence on soil temperature extends into the mineral horizon (Deslippe et al., 2011; Sistla et al., 2013). Soil temperature was measured at ~10, 20, and 40 cm using a Campbell CR21x data logger which recorded two profiles of soil temperature per treatment in one block with copper/constantan thermocouple wires. Soil temperature data over the 22 year experimental period is patchy; however, a changing soil warming regime is detectable over the treatment period. The GH treatment initially warmed the soil into the mineral horizon during the summer (when the GH treatment was active). The summer soil warming effect has declined over the course of the experiment, while a winter warming effect occurs into the mineral horizon (Deslippe et al., 2011; Sistla et al., 2013).

2.3. Soil sampling and processing

We measured an array of soil biogeochemical properties over a year period corresponding to arctic seasons (Table 1; April 28 [late winter], May 19 [thaw], July 3 [summer], September 10 [senescence], and November 10 [early winter] (Olsson et al., 2003; Sturm et al., 2005)). We harvested soils in ~5 cm^2 blocks. The soil sampling and analyses were separated into three horizons. Visible loose green surface litter was removed, and the soil was separated into three depth increments, including litter (recognizable dead plant material), 0–5 cm organic, and >5 cm depth organic to the mineral soil, following the protocol of Mack et al. (2004) and Sistla et al. (2013). The organic soil was harvested with a serrated knife, and the mineral soil (ranging from 5 to 10 cm below the organic–mineral interface) was harvested using a hand corer when the soil was thawed (July and September sampling dates). When the soils were frozen and snow covered (April, May, November), snow pits were dug to the soil surface (SI Plate 1) and the soil was sampled with a hammer and mason's chisel. The deepest snow was observed in late winter (snow pits dug for sampling to the surface of the soil were 100 ± 14 cm for the GH and 80 ± 11 cm for the control).

Soil samples were maintained at either at 4 °C (July, September) or frozen (April, May, November) and shipped to our laboratory (University of California, Santa Barbara), where they were maintained at their respective conditions for less than 48 h prior to processing (in a 4 °C room). Soils were hand homogenized by horizon at the block level, and live coarse roots and rocks were removed. The soils were then sub-sampled for potential EEA, extractable nutrients, microbial biomass, and gravimetric water content. Soil temperature data for the GH experiment was acquired from the Toolik LTER database (Shaver and Laundre, 2010).

2.4. Extracellular enzyme assays

We measured the potential activity of six hydrolytic and two oxidative soil extracellular enzymes (Table 2). During the enzyme assays, soils were stored in coolers with ice packs, and all assays were incubated at 1 °C, to maintain slurry temperatures within ambient soil conditions. Incubation times ranged from 1.5 h to ~18 h for the hydrolytic enzyme (fluorescence) assays, and up to 24 h for the oxidative enzyme (colorimetric) assays. Hydrolytic enzyme assays were conducted by following a 96-well microplate flurometric technique (Saiya-Cork et al., 2002; Wallenstein et al., 2009; Cusack, 2013). In brief, 10 g (wet weight) of surface organic soil, 3 g of deep organic soil, and 3 g of mineral soil were homogenized for one minute with 100 ml 50 mM sodium acetate buffer (pH 5.0) in a Waring blender for 1 min. The slurries were then placed on a stir plate and 100 μl subsamples were added to the microplates. One hundred μl of 200 μM fluorescing substrate was added to each assay.

Table 1
Soil sampling periods.

Seasonal period	Description (adapted from Sturm et al., 2005)
Late winter (April)	Active soil layer frozen, soil and air temperature in phase
Thaw (May)	Minimum air temperature above freezing, soil temperature rapidly rising, snowpack melting
Summer (July)	Active soil layer thawing, greatest plant biomass
Senescence/Early snow (September)	Maximum soil temperature and depth of active layer
Early winter (November)	Active layer freezes (from surface down and permafrost up)

Seasons that the soils were sampled in; late winter, thaw, and early winter are considered periods that are particularly susceptible to warming (Sturm et al., 2005).

Table 2
Hydrolytic and oxidative extracellular enzymes assayed.

Extracellular enzyme	Type	Targets
α-Glucosidase (AG)	C-targeting hydrolytic	Soluble saccharides
Cellobiohydrolase (CBH)	C-targeting hydrolytic	Cellulose (for disaccharides)
β-Glucosidase (BG)	C-targeting hydrolytic	Cellulose (for glucose)
β-Xylosidase (BX)	C-targeting hydrolytic	Hemicellulose (for sugar monomes)
N-acetylglucosaminidase (NAG)	N-targeting hydrolytic (also liberates C)	Chitin
Acid posphatase (AP)	P-targeting hydrolytic	Phosphorous
Phenol oxidase	Recalitrant N and C	Lignin and other complex compounds
Peroxidase	Recaclitrant N and C	Lignin and other complex compounds

A description of the extracellular enzymes assayed and their substrate targets, with abbreviations for extracellular enzyme names given in parentheses.

Eight replicates of each enzyme substrate were tested with each soil. Eight control quench replicates were made for each soil (soil slurry plus 4-methylum-bellifferone [MUB]). Background fluorescence of soils, substrates, and standard curves of MUB were also measured. Reactions were stopped with 30 μL NaOH, to bring the pH to 8.0, and the microplates were scanned on a Perkin–Elmer Victor2 plate reader (excitation filter at 365 nm and emission filter at 450 nm).

Oxidative enzymes were measured using a colorimetric assay. Eight replicates of 600 μl of soil slurry were pipetted into 2 mL deep-well plates, and 400 μL of 25 mM L-3,4-dihydroxy-phenylalanine (DOPA) substrate were added. Thirty μL of 0.3% H_2O_2 were added to initiate the peroxidase activity. Absorbance was read on the Perkin–Elmer Victor2 plate reader at 450 nm. Background absorbance of DOPA was measured, and an extinction coefficient was calculated using a standard curve of DOPA degraded with mushroom tyrosinase. In the early winter (November) sampling, both phenol oxidase and peroxidase extracellular activity were below detection threshold across the soil samples. Peroxidase activity was also below detection limit during thaw (May).

2.5. Extractable C, N and microbial biomass

Chloroform-extractable (CFE) microbial biomass was characterized by horizon as previously described (Fierer and Schimel, 2003; Cusack, 2013). The CFE and K_2SO_4-only extracts were analyzed for extractable organic C (EOC) and extractable N using a total organic C/total N (TOC/TN) analyzer (Shimadzu Corporation, Series V Model CSN analyzer). Extractable microbial biomass C and N was calculated as the difference in total dissolved C and N between the chloroform-exposed subsample and the corresponding K_2SO_4-only extracted subsample. Total microbial biomass C or N was then calculated by dividing the measured extractable biomass values by correction factors of 0.45 for C (Beck et al., 1997) and 0.54 for N (Brookes et al., 1985). Microbial biomass in the control soil mineral horizon was below detection limits at senescence (September) and was not included in the analyses. Extractable organic nitrogen (EON) was estimated as K_2SO_4-only extracted TN minus K_2SO_4–NH_4^+ and K_2SO_4–NO_3^-. Extracts were analyzed for NH_4^+ and NO_3^- by flow injection analysis (LACHAT Instruments, Mequon, WI, USA). NO_3^- concentrations were often below detection limits and were not included in the analysis; low NO_3^- levels are common in these soils (Schimel et al., 2004).

2.6. Statistical analyses

Ratios (resource acquisition activities) of C-acquiring to P-acquiring EEA (ln(AG) : ln(AP), ln(BG) : ln(AP), ln(BX): ln(AP),

ln(CBH) : ln(AP)), C-acquiring to N-acquiring EEA (ln(AG) : ln(NAG), ln(BG) : ln(NAG), ln (BX) : ln(NAG), ln(CBH) : ln(NAG)) and N-acquiring to P-acquiring EEA (ln (NAG) : ln(AP)) were calculated for each horizon, treatment, and season for each block. These ratios provide an index of enzymatic resources directed towards acquisition of organic P and organic N relative to C (Sinsabaugh et al., 2008). The effect of season, GH treatment, and their interaction on potential EEA, resource acquisition activities, extractable nutrients, and microbial biomass across the soil profile was tested using a mixed model ANOVA, with horizon and block specified as random effects and treatment, season, treatment*season, and enzyme type or ratio (when relevant) specified as fixed effects. Non-significant interactions were removed from the analysis. A Tukey HSD post hoc test was used to test for significant differences between seasons following the ANOVA.

The effect of the GH treatment on the dependent variables (including soil temperature) in each soil horizon and season was tested separately tested using a blocked ANOVA. Due to random data loss and shifting in temperature probe depth with free–thaw cycles, temperature analysis used the monthly average of the 2 temperature probes (for GH and control in one block of the experiment, respectively). A GH effect size on potential EEA for each enzyme (separated by season and horizon) was calculated as the natural log of the response ratio of GH: control for each set of paired plots that make a block. Logarithmic ratios standardize the treatment effect symmetrically around zero, whereas the non-transformed ratio is asymmetric (i.e. the non-transformed response ratio must be greater than 0, but if response $A <$ response B, the ratio will always be constrained $0 \leq A: B < 1$). Response ratios for individual enzymes and average hydrolytic and oxidative EEA were tested for significant difference from zero (Cusack et al., 2010). Data were tested for normality (Shapiro–Wilks), and log transformed when necessary to meet assumptions of normality prior to analysis. An α of 0.1 was chosen to balance the risk of making Type I versus Type II errors, given the low replicate number ($n = 4$). The JMP 7.0 package (SAS Institute Inc., Core, NC, USA) was used for all statistical tests.

3. Results

3.1. Seasonal pattern of average potential extracellular enzyme activity

On average across the soil profile and treatment, potential hydrolytic and oxidative EEA significantly varied across seasons ($P < 0.001$ in both cases; Table 3). Enzyme type was a significant factor for both analyses ($P < 0.0001$; data not shown). Average potential hydrolytic EEA was greatest in the late winter (April; Tukey post hoc comparison) and declined through the thaw (May) into the summer (July). Average hydrolytic EEA did not differ significantly from summer through senescence (September) and early winter (November). In contrast to hydrolytic EEA, potential oxidative EEA was lowest during the late winter and increased through the summer (July), before declining during plant senescence to undetectable levels in early winter (November). Potential EEA for individual enzymes, treatments, and horizons are presented in SI Table 1.

Table 3
Average extracellular enzyme activity by season across horizons and treatment.

Seasonal period	Hydrolytic enzyme activity	Oxidative enzyme activity
Late winter (April)	3608 ± 609 (A)	7319 ± 1360 (c)
Thaw (May)	1491 ± 229 (B)	62,258 ± 18,631 (b)
Summer (July)	365 ± 53 (C)	142,638 ± 16,494 (a)
Senescence/Early snow (September)	904 ± 172 (C)	119,577 ± 20,680 (a,b)
Early winter (November)	1273 ± 622 (C)	No detectable activity

Average extracellular enzyme activity (nmol g^{-1} soil h^{-1}) ± one standard error pooled across the soil profile and treatment. Seasons that significantly differ from each other are indicated by letter for hydrolytic (capital) and oxidative (lower case) potential enzyme activity (repeated measures ANOVA $P < 0.001$ in both cases, followed by Tukey HSD).

3.2. Greenhouse effects on potential enzyme activity

The GH treatment increased average potential hydrolytic ($P = 0.04$) and oxidative ($P = 0.03$) EEA across the soil profile over the year. Across the soil profile, there was also a significant treatment by date interaction ($P < 0.0001$) for average potential hydrolytic EEA, which was significantly greater in the GH treatment than control in the thaw period ($P < 0.0001$), but significantly lower than control in the early winter ($P = 0.004$). A GH effect size for each enzyme was calculated as the natural log of the response ratio of GH: control for each paired plot by enzyme, season, and horizon (Figs.1–2). The GH treatment significantly affected average hydrolytic EEA in the mineral horizon across all seasons except during senescence ($P < 0.02$ in all cases), in the deep organic horizon activity during thaw and early winter ($P < 0.001$, $P = 0.02$, respectively), and in the surface organic activity in late winter ($P = 0.02$; Fig. 1a). Average hydrolytic EEA in the surface organic tended to be reduced relative to ambient conditions during the late winter, in contrast to the mineral horizon. There was a distinct seasonal pattern to the GH treatment effects on the deeper horizon's hydrolytic EEA: a positive effect in the deep organic and mineral horizon during late winter and thaw that declined through the summer and was reversed in the early winter (Fig. 1a). Oxidative enzyme responses to the GH treatment were less consistent; in the mineral and deep organic horizons, they showed a pattern similar to hydrolytic EEA, but the effects were not significant. The only significant oxidative EEA response to the GH treatment was in the surface organic horizon during the summer (Fig. 1b).

There was no overall effect of the treatment on the ratios of C-acquiring to P-acquiring EEA, C-acquiring to N-acquiring EEA, or N-acquiring to P-acquiring EEA pooled across seasons, horizons, and specific enzyme types. However, season significantly affected C-acquiring to P-acquiring EEA ($P = 0.004$) and N-acquiring to P-acquiring EEA ($P = 0.0001$). Average C-acquiring to P-acquiring EEA (pooled across treatments and horizons) peaked in late winter (0.66 ± 0.02, but did not significantly differ from thaw or summer), and was significantly greater than early winter (0.52 ± 0.04) and senescence (the annual minimum; 0.49 ± 0.05). Average N-acquiring to P-acquiring EEA also peaked in late winter (0.8 ± 01, but did not significantly differ from any other season from thaw through senescence), and was significantly lower in the early winter than the rest of the year (0.62 ± 0.03).

When separated by enzyme, season, and horizon (Fig. 2), the only significant effect of the GH treatment on potential oxidative EEA was increased peroxidase activity in the surface organic during the summer (Fig. 2g). In contrast, the GH treatment affected potential EEA of specific hydrolytic enzymes across seasons and horizons. The GH treatment reduced BX EEA in the late winter surface organic horizon relative to the control (Fig. 2a). This treatment effect corresponded to decreased surface organic horizon C:N and C:P acquisition activities, as reflected by a reduction in ln(BX) : ln(NAG); $P = 0.05$ and ln(BX) : ln(AP); $P = 0.07$. NAG EEA was significantly reduced relative to control conditions in the early

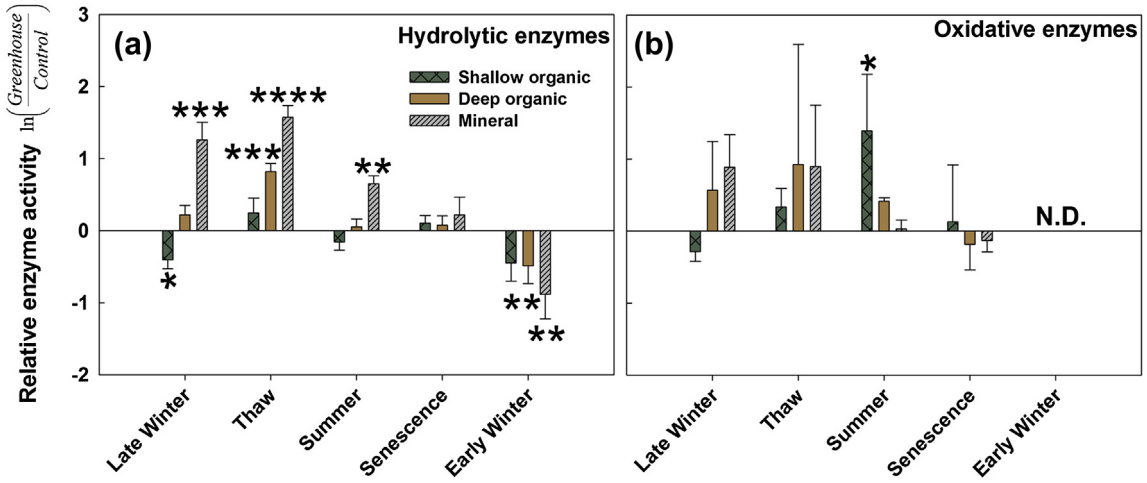

Fig. 1. The greenhouse effect on average potential hydrolytic **(a)** and oxidative **(b)** extracellular enzyme activity for each season and horizon. Data represents means ± one standard deviation. A * denotes that ln (*green house*/*contol*) enzyme activity differs from 0 (an enzyme activity ratio of 1:1) using a one-sample *t*-test at $P \leq 0.1$, with ** = $P < 0.05$, *** = $P < 0.01$, and **** = $P < 0.001$. No data denoted by N.D.

winter (Fig. 2m), although this did not significantly affect C:N or C:P acquisition activities.

The GH effects on deep organic and mineral horizon hydrolytic EEA paralleled each other, but were stronger in the mineral soil. Although no significant effects of the GH on individual EEA were detected in the deep organic horizon during the late winter, the treatment decreased both C:N and C:P acquisition activities. Relative to control, reductions in GH ln(CBH): ln(NAG) ($P = 0.08$) and ln(CBH) : ln(AP) ($P = 0.01$) (due to a decline in CBH EEA and increase in NAG and AP EEA), and a reduction in ln(BG): ln(AP) ($P = 0.05$) (due to an increase in AP relative to BG) were observed in the GH deep organic horizon during the late winter. During thaw, AG EEA in the deep organic horizon was significantly elevated in the GH treatment (Fig. 2e), which increased GH C:P acquisition activity (ln(AG): ln(AP), $P = 0.04$) and C:N acquisition activity (ln(AG): ln(NAG), $P = 0.08$) relative to control. The GH treatment also increased deep organic horizon N:P acquisition activity (ln(NAG): ln(AP), $P = 0.09$), due to an increase in NAG EEA relative to AP EEA during thaw. No significant effects of the GH on individual EEA were detected in the deep organic horizon during July; however, the treatment decreased C:N acquisition activity (ln(BG): ln(NAG), $P = 0.08$), due to a relative reduction in BG EEA and increase in NAG EEA. Stimulation of hydrolytic EEA declined as the year progressed, with deep organic CBH activity reduced in the early winter (Fig. 2n); however, there was no significant treatment effect on the C:N or C:P acquisition activities.

Similar to the overlying organic soil, although no significant treatment effect was detected on individual EEA in the mineral horizon during the late winter, the treatment did decrease C:P acquisition activity (ln(BG): ln(AP), $P = 0.06$) due to a greater increase in AP relative to BG. During thaw, AG, BG, and CBH activities were elevated in the GH mineral horizon relative to the control (Fig. 2f). These effects corresponded to increases in GH C:N acquisition activities (ln(AG): ln(NAG), $P = 0.05$ and ln(CBH): ln(NAG), $P = 0.09$) and increases in GH C:P acquisition activities (ln(CBH): ln(AP); $P = 0.03$; ln(AG): ln(AP); $P = 0.03$; and ln(BX): ln(AP), $P = 0.1$). An increase in GH mineral horizon N:P acquisition activity (ln(NAG): ln(AP)), due to a greater increase in NAG relative to AP was also observed during thaw ($P = 0.04$). A slight stimulation in GH mineral horizon C:P acquisition activity (ln(BX): ln(AP), ($P = 0.1$)) occurred in July, although no other treatment effects on hydrolytic EEA were detected during the peak growing season. From senescence through early winter, the GH stimulation of mineral horizon hydrolytic EEA declined, with a significant reduction on CBH EEA (Fig. 2l, o), corresponding to a decline in GH C:P acquisition activity (ln(CBH): ln(AP)) relative to control during senescence ($P = 0.04$).

3.3. The effects of season and treatment on extractable nutrients and microbial biomass

There was no overall GH treatment effect on extractable nutrients or microbial biomass across the soil profile; however, season had a significant effect on these pools across the soil profile ($P < 0.0001$ in all cases). Across horizons, EOC, EON, NH_4^+, and chloroform-extractable MB and MBN pools were the highest in the late winter and thaw periods, before declining from summer through early winter, where a tendency for the EOC and NH_4^+ pools to recover to late winter/thaw sizes was observed (Table 4). When separated by season and horizon, GH surface organic horizon EOC was more than 4-fold greater than control during senescence (Fig. 3a; $P = 0.1$). MBC and MBN in the GH deep organic horizon during the summer were 2.2- and 2.6-fold greater than control, respectively (Fig. 3k; $P = 0.08$ in both cases) and MBC was 2.9-fold greater during senescence ($P = 0.03$). In the early winter, the GH treatment significantly reduced surface organic MBN by 76% ($P = 0.1$).

3.4. GH treatment effects on soil temperature and water content

In the 22nd year of the GH treatment, the strongest warming effect occurred from January through March, when the greenhouses insulating plastic sheets were not up (SI Fig. 1). This winter soil warming effect presumably resulted from shrubs trapping thicker snow that insulated the soil (Sturm et al., 2005). During the winter, the GH soil averaged 3.2 °C, 3.3 °C, and 3.0 °C warmer than control at 10 cm, 20 cm, and 40 cm depth ($P = 0.03$ in all cases). This winter warming effect declined from later winter through thaw (April though May), when the top 10 cm of the GH soil averaged ~1 °C warmer than ambient conditions ($P = 0.1$), but no other significant effects were detected. When the GH treatment was active (June through August) the top 10 cm of the GH soil averaged ~1.7 °C warmer than ambient conditions ($P = 0.03$), but the GH did not significantly affect the deeper soils. The GH treatment did not significantly affect soil temperature at any depth from September

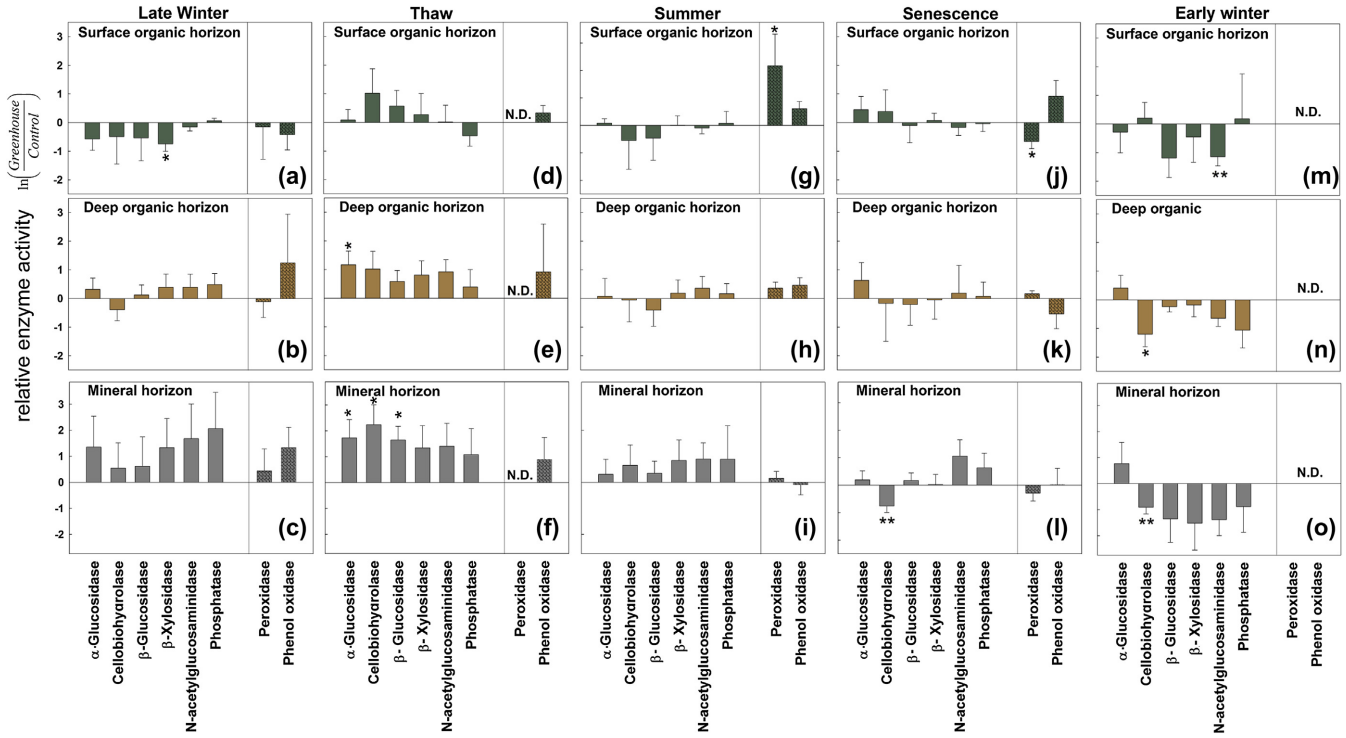

Fig. 2. The greenhouse effect on potential hydrolytic and oxidative extracellular enzyme activity for each enzyme, season, and horizon. Data represents means ± one standard deviation. A * denotes that ln (*green house*/*contol*) enzyme activity differs from 0 (a greenhouse:control enzyme activity ratio of 1:1) using a one-sample *t*-test at $P \leq 0.1$, with ** = $P < 0.05$. No data denoted by N.D.

Table 4
Average extractable nutrients and microbial biomass.

Seasonal period	Extractable organic C (mg g^{-1} soil)	Extractable organic N (mg g^{-1} soil)	NH_4^+ (mg g^{-1} soil)	Microbial biomass C (mg g^{-1} soil)	Microbial biomass N (mg g^{-1} soil)
Late winter (April)	2.8 ± 0.4 (A)	0.1 ± 0.02 (A)	0.009 ± 0.0009 (A)	9.1 ± 1.4 (A)	0.6 ± 0.08 (A)
Thaw (May)	3.5 ± 0.6 (A)	0.2 ± 0.03 (A)	0.01 ± 0.003 (A)	8.5 ± 1.5 (A)	0.7 ± 0.1 (A)
Summer (July)	0.6 ± 0.08 (B,C)	0.07 ± 0.01 (B)	0.0006 ± 0.0001 (B)	1.6 ± 0.3 (B)	0.2 ± 005 (B)
Senescence/Early snow (September)	0.7 ± 0.2 (C)	0.04 ± 0.008 (C)	1e-4 ± 5e-5 (C)	4.8 ± 1.4 (B)	0.2 ± 0.05 (B)
Early winter (November)	1.4 ± 0.4 (B)	0.04 ± 0.0009 (C)	0.006 ± 0.002 (A)	2.1 ± 0.7 (B)	0.1 ± 0.05 (B)

Average extractable nutrients and microbial biomass (mg g^{-1} soil) ± one standard error across the soil profile and treatment (microbial biomass in the mineral horizon during September was not included in the analysis). Seasons that significantly differ from each other are indicated by letter (repeated measures ANOVA $P < 0.001$ in all cases, followed by Tukey HSD).

through November. Water content did not significantly differ between greenhouse and control in any horizon across dates sampled.

4. Discussion

Winter warming more strongly accelerates the net loss of CO_2 from arctic tundra soil to the atmosphere than summer warming (Natali et al., 2011), suggesting that it is critical to understand how decomposer activity shifts among the seasons and whether decomposers respond differently to warming in different seasons. We found that arctic soil processes change qualitatively between growing and non-growing season and that twenty-two years of GH warming had different effects on oxidative and hydrolytic EEA across seasons and horizons. Treatment effects increased deeper in the profile, with minimal effects in surface organic soil. In the deeper layers, GH warming amplified the natural seasonal cycle in hydrolytic EEA: high in the late winter and at thaw but decreasing through the growing season. Warming increased activities early in the year, but decreased them late in the growing season and in the early winter. This seasonal pattern of hydrolytic EEA paralleled those of extractable nutrient pools and microbial biomass. Oxidative enzymes showed an inverse seasonal cycle, being low early in the year but increasing through the growing season; greenhouse warming, however, had little effect on these enzymes.

4.1. Seasonal patterns on enzyme activity and nutrient availability

Averaged across soil horizons and treatment, peak hydrolytic enzyme activity, microbial biomass and nutrient availability were temporally decoupled from summertime peak plant productivity. A similar seasonal pattern for extractable nutrients and hydrolytic EEA has been noted in both arctic and alpine sites (Lipson et al., 2000; Weintraub and Schimel, 2005; Wallenstein et al., 2009); as well as for hydrolytic (although not oxidative) EEA in a temperate grassland, where peak activity correlated with spring thaw (Bell et al., 2010). The cause of the growing season decline in extractable nutrients and hydrolytic EEA is uncertain, but may reflect a crash in soil nutrients due to increased nutrient uptake by plant roots and/or increased microbial uptake (Weintraub and Schimel, 2005) coupled with a decline in labile C substrate availability as soil temperatures rapidly climb above freezing (Lipson et al., 2000; Averill and Finzi, 2011).

As the tundra soils begin to freeze in the early winter, the proportion of microbial respiration derived from recycled microbial biomass products increases (Mikan et al., 2002), which may contribute to the buildup of nutrients over the winter observed in our study. The subsequent increase in soil nutrients and hydrolytic activity in the late winter and thaw periods may be caused by this recycling; additionally, water film expansion during thaw can cause the release of frozen substrate, thereby increasing the availability of relatively high quality, labile organic matter (Sturm et al., 2005). Supporting this hypothesis, average C:P and N:P acquisition activities across the soil profile peaked in late winter and was lowest during senescence and early winter (respectively), suggesting that decomposer C- and N-demand are tightly coupled in a seasonally specific manner, with maximum decomposer C-demand occurring in late winter (Buckeridge and Grogan, 2008). The increase in P-demand relative to C-demand observed in the transition from growing season to winter likely reflects declining available P following the beginning of the growing season (Chapin et al., 1978).

The seasonal pattern of oxidative EEA contrasted with the seasonal changes in hydrolytic EEA, microbial biomass, and extractable nutrients. Across horizons and treatment, oxidative enzyme activity was at the lowest detectable level during the late winter, peaked in the summer (correlated with the maximum plant growth), marginally declined at senescence, and was not detectable in the late winter. The oxidative enzyme activity pattern may reflect that lower labile nutrient levels tend to promote oxidative enzyme expression by saprotrophic fungi (Sinsabaugh, 2010). Further, ECM fungi produce oxidative enzymes; their activity is phenologically tied to the timing of plant growth and would therefore be expected to increase through the growing season (Wookey et al., 2009; Talbot et al., 2012). The temporal separation between peak hydrolytic and oxidative EEA and the warming-driven amplification of this pattern also suggests that tundra decomposer communities maintain a niche space which may be critical to understanding biogeochemical cycling (and its response to global change phenomena) both in arctic biomes and in other highly seasonally-defined ecosystems.

4.2. Greenhouse warming effects on decomposer activity and extractable nutrients

Tussock tundra soil EEA was sensitive to warming, an effect which has been demonstrated in other systems, including lower latitude Mediterranean shrubland and peatland soils (Fenner et al., 2005; Sardans et al., 2008). The GH treatment increased plant biomass and shrub dominance, indicating that warming had increased plant-available nutrients (Deslippe et al., 2011; Sistla et al., 2013). In contrast, after 22 years of summer GH warming, the only significant treatment effects during the summer were an increase in peroxidase activity in the surface organic soil (correlated with warmer surface soil temperature) and greater microbial biomass C and N in the deep organic horizon. The dominant treatment effects on decomposer activity occurred in the deep organic and mineral horizons during late winter and thaw (before the GH treatment was active) and in the early winter period (after the GH treatment was removed). These effects reflect a spatial (in terms of depth) and temporal decoupling between the direct application of the GH treatment and its biogeochemical consequences. Therefore, the warmer winter soil temperatures which have developed over the two decades that the GH treatment has been active (Sistla et al., 2013) more strongly influence decomposer activity than the direct summertime warming effect.

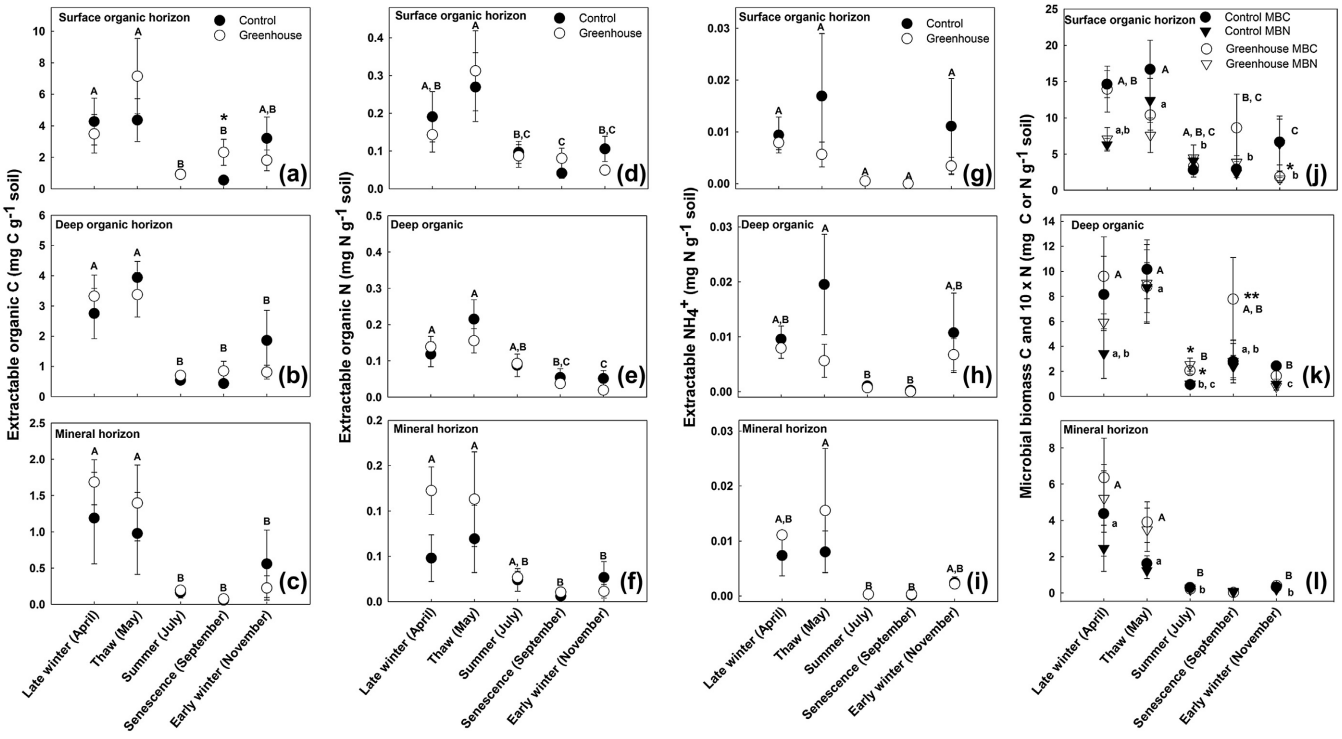

Fig. 3. Extractable organic C (**a**–**c**), extractable organic N (**d**–**f**), NH_4^+ (**g-i**), and microbial biomass (**j**–**l**) for the greenhouse and control soils, for each season and horizon (surface organic [**a, d, g, j**]; deep organic [**b, e, f, k**]; mineral [**c, f, i, l**]). Data represents means ± one standard deviation. Treatment effects are denoted by * = $P \leq 0.1$ and ** = $P < 0.05$. Letters indicate results of Tukey's HSD post hoc test of significant difference between seasons (for microbial biomass, upper case letters mark microbial biomass C, lower case represent microbial biomass N); bars that share the same letter are not significantly different ($\alpha \leq 0.1$). Control microbial biomass in the mineral horizon during senescence was below detectable levels, and was not included in the analysis.

The average hydrolytic EEA in the GH mineral soil was elevated relative to control from thaw through summer. This complements previous findings of greater C-mineralization rates, active microbial biomass, and an expansion of the soil food web in the GH mineral soil (Sistla et al., 2013). Greater average hydrolytic EEA in the mineral horizon were not sustained; by early winter (November), EEA was lower than the control. A similar (although less robust) seasonal pattern was observed in the GH deep organic horizon. The early winter reduction in hydrolytic EEA paralleled a decline in the difference between GH soil and control soil temperatures; however, monthly average GH mineral horizon temperature was never lower than control. Surface organic soil hydrolytic enzyme activities also tended to be lower than ambient conditions in the early winter, when the GH soil temperature was comparable to ambient conditions, suggesting that temperature alone did not drive the observed treatment effects on EEA.

Intriguingly, a similar seasonal pattern of EEA response to warming was shown in a Mediterranean shrubland, where 6 years of nighttime warming increased hydrolytic EEA only during the winter and spring, when soil moisture was the highest (Sardans et al., 2008). Although we did not detect treatment effects on soil moisture, the seasonal differences in the influence of the GH experiment may be explained by treatment-driven changes in coupled abiotic-biotic conditions, including greater late winter through thaw soil temperatures and increased (likely higher quality, lower C:N) litter inputs to the soil (Sistla et al., 2013). Water soluble organic substrates in mineral soils underlying shrub-dominated tundra are higher quality than tussock tundra substrates (Michaelson, 2003), which may contribute to the enhanced decomposer activity that is observed in deeper shrub soils (Sturm et al., 2005).

Greater shrub biomass in the GH treatment (Deslippe et al., 2012; Sistla et al., 2013) has likely increased the quality of leaf litter derived substrates (Weintraub and Schimel, 2003). If this material is transported downwards through water microfilms as the late winter GH soil temperatures rapidly exceed control conditions, this may explain why the GH deeper organic and mineral horizon hydrolytic EEA was stimulated during later winter and thaw, while the surface organic activity was reduced in the late winter. Supporting this hypothesis, the GH treatment decreased C:N and C:P acquisition activities in the organic horizons, and decreased C:P acquisition activity in the mineral horizon in the late winter. This effect was caused by increases in N and P acquisition activities relative to C-acquisition activity in the deep organic and mineral horizons and a decline in C-acquisition activity at the surface.

The seasonal reversal in nutrient acquisition stoichiometry in the GH soil may reflect that from the summer through the late winter, the GH microbial community was experiencing increasing substrate depletion relative to control conditions, which can induce decomposers to down-regulate extracellular enzyme production (Sinsabaugh and Moorhead, 1994). By the early winter, GH soil extractable C and N were consistently (although not statistically significantly) lower than control across all horizons. During this season, the GH surface organic horizon also had reduced MBN coupled to a decline in NAG EEA, while CBH EEA declined in the deep organic and mineral horizons. If warming-driven increases in substrate demand following thaw could not be satisfied, this may have driven the decline in the GH treatment stimulation of hydrolytic EEA observed from senescence through early winter in the deeper soil horizons.

In contrast to hydrolytic EEA, the strongest effects of the GH treatment on oxidative EEA occurred in the surface organic horizon. During the summer (the period of maximum plant growth), peroxidase activity was significantly increased relative to control. This effect may reflect increased oxidative enzyme substrate availability coupled to greater ECM biomass in the GH organic soils, both of which is promoted by greater *B. nana* shrub dominance in the GH experiment (Deslippe et al., 2011, 2012). Peroxidase activity declined in the GH surface organic relative to control when the plants senesced in September, which was also correlated with marginally greater EOC availability. Because increased labile C and N availability can down-regulate oxidative enzyme synthesis (Szklarz et al., 1989; Sinsabaugh, 2010), it is possible that greater labile C production due to higher summertime EEA (and/or increased labile C inputs from greater plant litter) down-regulated oxidative enzyme production during senescence.

5. Conclusion

Microbial activity in tussock tundra organic soil is nutrient-limited during the summer (Sistla et al., 2012); our study suggests that long-term warming may not alleviate this limitation, thereby limiting the effect of summer warming on decomposer activity. Further, the stimulation and subsequent depression of EEA relative to control conditions that was observed with both suites of enzymes suggests that at an annual scale, warming-driven, seasonally-linked changes in extracellular enzyme substrate and product availability may create stabilizing feedbacks to increased decomposer activity. These feedbacks, which would have been overlooked in more traditional (growing season, surface soil) sampling regimes, may constrain warming-driven soil C loss in arctic tundra systems. Supporting this hypothesis, the GH experiment slightly *increased* mineral horizon C stock (although summertime mineral horizon C-mineralization rate was also elevated) after 20 years of warming, but did not alter overall soil C storage (Sistla et al., 2013). Similarly, experimental warming (which drove soil drying) depressed decomposer abundance and activity in a boreal forest site (Allison and Treseder, 2008), suggesting that a negative effect of warming on decomposition may extend to other permafrost systems.

Understanding the effects of global change perturbations on decomposer activity is increasingly recognized as a critical factor in projecting soil C cycling responses to a changing environment (Allison et al., 2010). Research characterizing the influence of global change manipulations on EEA and related biogeochemical traits often focus on single time periods, seasons, and soil horizons (Waldrop et al., 2004; Allison and Treseder, 2008; Cusack et al., 2010; Averill and Finzi, 2011). In contrast, this study revealed complex patterns across season and soil depth in soil nutrient cycling, highlighting the potential sensitivity of deeper tundra soils to the coupled abiotic-biotic changes that are driven by sustained warming. As such, our results demonstrate the importance of further understanding the mechanisms by which warming may promote both positive and negative feedbacks to decomposer activity on an annual timescale.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2013.07.003.

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