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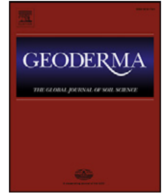

Warming and increased precipitation enhance phenol oxidase activity in soil while warming induces drought stress in vegetation of an Arctic ecosystem

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A B S T R A C T

Global climate change models predict that surface temperature and precipitation will increase in the Polar Regions. Arctic tundra soils contain a large amount of carbon, which may be vulnerable to decomposition under potential climate change. However, mechanistic understanding of the decomposition process and the consequent changes remains lacking. In the present study, we conducted a manipulation experiment at an arctic soil system in Cambridge Bay, Canada, where temperature and precipitation were increased artificially by installing open top chambers and adding distilled water during growing seasons. After one and half year of environmental manipulation, we investigated extracellular enzyme activities, which are related to decomposition, and analyzed stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) in soils and plants, which are related to water and nitrogen availability. Hydrolase (β-D-glucosidase, cellobiase, N-acetyl-glucosidase and aminopeptidase) activity did not differ significantly under different treatments. However, phenol-oxidase showed higher activity under warming combined with increased precipitation than under other treatments. Stable isotope ratio ($\delta^{13}C$) in plants revealed that drought stress in vegetation was induced under warming. We concluded that in the long term, climate change may amplify the feedback of soil to climate change in arctic tundra soil.

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1. Introduction

Arctic soil is an important ecosystem that contains a huge amount of carbon stock because of the low rate of decomposition in cold temperatures. The magnitude of this carbon stock is quite uncertain but is currently estimated at 1672 Pg of organic carbon (Tarnocai et al., 2009). This organic carbon stored in the Tundra is thought to be vulnerable to climate change, and the emission of even a small fraction of this carbon stock may significantly increase the atmospheric greenhouse gas (GHG) concentration. Moreover, global climate change models predict the highest increase in temperature and slight increase in precipitation in the Arctic region (IPCC, 2007). These phenomena can lead to substantial changes in carbon balance and climate change feedback in the system.

Microorganisms produce enzymes that are directly involved in organic matter decomposition. Extracellular enzymes produced by microbial organisms decompose polymerized or macromolecular substrates into small molecules to absorb substrates into the cell. Phenol oxidase is particularly important because it is responsible for recalcitrant carbon mineralization, which is often the rate-limiting step in organic matter mineralization (Freeman et al., 2001, 2004). Because recalcitrant phenolic compounds can inhibit the activity of hydrolases, phenol oxidase activation can augment overall extracellular enzyme activity. Previous studies have shown that warming and precipitation change may lead to destabilization of old organic carbon, but little is known about the effect on enzyme process (Davidson and Janssens, 2006; Kim et al., 2012; Kwon et al., 2013).

Factors influencing enzyme activity include temperature, moisture, redox potential, pH, salinity, substrate availability, biomass, adsorption by clay minerals, and humus (Freeman et al., 1998; Gianfreda et al., 1996; Kang et al., 2009; Vo and Kang, 2012). Some of the factors are related to enzyme producer propagation and some are related to the reaction rate of discharged enzymes (Burns, 1982). In Arctic or boreal/alpine regions, climate changing factors including temperature increase, CO_2 elevation, and nitrogen enrichment have been considered as factors influencing enzyme activity. Moorhead and Linkins (1997) reported that elevated CO_2 levels alter enzymic characteristics of root surface, suggesting that arctic plants respond to elevated CO_2 by increasing nutrient acquisition activity. An investigation of seasonal variation in enzyme activity found that it showed low sensitivity to temperature in summer and suggested that N-limitation is a reason for this (Wallenstein et al., 2009). Koch et al. (2007) suggested that temperature sensitivity of enzyme activity is higher in winter. Sistla and Schimel (2013) reported

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that warming treatment amplified the seasonal cycle of extracellular enzyme activity. Wang et al. (2010) found that nitrogen fertilization did not influence enzyme activity.

The indirect effects of warming constitute another important issue. In addition to the direct effects of higher temperature, warming may cause drought stress in microorganisms and plants via accelerated evapotranspiration. Allison and Treseder (2008) raised soil temperature, which resulted in soil moisture decline. In this study, warming and drying suppressed microbial activity including enzyme activity, and bacterial and fungal abundance. This suppression can be assessed by using stable isotope signatures of plant materials. Heavy carbon isotope (^{13}C) is depleted to greater extents in plant product when the stomata are wide open owing to sufficient moisture supply during photosynthesis. Therefore, natural abundance of ^{13}C is a good indicator of moisture availability or drought stress in plants (Stewart et al., 1995; Warren et al., 2001).

In spite of the importance of having a mechanistic understanding of the decomposition process and the resulting changes in arctic soil under global climate change, our understanding remains lacking. The aim of this study is to reveal the effects of warming and precipitation change on 1) microbial enzyme activity in soils and 2) stable isotopic signature of plant in an arctic ecosystem by employing open-top chamber methods.

2. Material and methods

2.1. Study site

Our study site is in Cambridge Bay, Nunavut, Canada. Cambridge Bay is included in the Arctic Circle, and the study site is located at 69°07′48″ N and 105°03′36″W. The minimum daily mean temperature observed in January is −33 °C, and the daily mean temperature rises above zero from June to August. The average precipitation between 1971 and 2000 was 140 mm per year. The vegetation consists of mosses, lichens, herbs and shrubs of height less than 10 cm. *Dryas integrifolia* and *Carex* spp. dominate the vegetation.

2.2. Climate change manipulation

A climate change model (Bell et al., 2003) predicts that mean air temperature will rise by 2.8 °C and precipitation will increase by 15.6 mm (0.52 mm per week) during growing season (April to October) in 2040–2069 compared to that in 1971–2000 in Cambridge Bay. Considering this prediction, we conducted a manipulation experiment to investigate the impact of climate change on SOM (soil organic matter) decomposition that is relevant to GHG emission. A radiator or heating wire can raise the temperature by as much as predicted, but they may disturb or dry the soil system; therefore, we used an open-top chamber, which allows for air circulation, to increase air temperature. Moreover, we realized the precipitation increase of 0.5 mm per week.

We considered four types of treatments for climate change manipulation: no treatment (control), increased precipitation, warming, and warming with increased precipitation. In the warming plot, a hexagonal, lucent open-top chamber made of polycarbonate having a diameter of 2 m was installed, and in the increased precipitation plot, 2 L of distilled water was added to soil in a square plot (2 × 2 m) every week. In the warming with increased precipitation plot, the soil in the installed open-top chamber was watered. The increased precipitation plot received additional precipitation of 4 mm per year compared with control plot which received average 147 mm of total precipitation in 2012–2013. We set five blocks (plot groups) with relatively uniform active layer depths in each block and conducted four kinds of treatments in each of the five blocks. The manipulation experiment was conducted from mid-July to early October in 2012 and from late June to early October in 2013. Monitored air temperature in the warming plot increased by 1–2 °C, but no change in soil moisture content was detected in the increased precipitation plot because soil moisture content varied largely with locations. Given that 2 mm and 4 mm of weekly water addition were undetectable in a previous study (Sullivan et al., 2008), and that the precipitation in July and August ranged 14–56 mm per month, 0.5 mm per week of water addition might be too low to detect.

2.3. Soil and plant sampling

2.3.1. Soil

Soil from two depth layers (D1: 0–5 cm, D2: 5–10 cm) were collected from three points in a plot and combined to represent a sample for each plot. The first sampling was conducted shortly before the start of manipulation in July 2012. The second sampling was conducted in the middle of the second year's manipulation period (August 2013).

2.3.2. Plant leaves

Warming and precipitation addition can change plant biomass and coverage as observed by Wahren et al. (2005). We did not measure the biomass per se, but surveyed surface coverage of plant in July

Table 1
Soil properties at depths of 0–5 cm (D1) and 5–10 cm (D2) before manipulation (2012) and after manipulation (2013).

Water content (%)	2012		2013	
	D1[a]	D2[b]	D1[a]	D2[b]
No treatment	54.6(6.1)	42.1(7.9)	55.0(3.8)	34.2(8.7)
Precipitation	60.8(1.9)	49.4(5.5)	54.1(3.9)	40.9(7.7)
Warming	60.0(6.0)	45.8(5.9)	51.8(6.7)	41.4(5.0)
W + P	60.7(4.0)	51.2(8.0)	52.7(5.5)	39.6(6.8)
Organic matter (%)	2012		2013	
	D1[a]	D2[b]	D1	D2
No treatment	51.9(9.0)	34.4(8.9)	54.4(6.0)	32.3(13.6)
Precipitation	58.8(5.5)	43.9(6.6)	52.8(7.9)	49.2(22.3)
Warming	60.4(9.2)	38.5(5.6)	52.5(11.4)	35.9(8.2)
W + P	58.5(7.7)	49.0(7.6)	53.1(8.3)	33.1(6.1)
DOC (mg g^{-1} soil)	2012		2013	
	D1[a]	D2[b]	D1[a]	D2[b]
No treatment	0.18(0.03)	0.10(0.02)	0.20(0.03)	0.11(0.03)
Precipitation	0.23(0.01)	0.13(0.02)	0.22(0.03)	0.14(0.03)
Warming	0.22(0.03)	0.14(0.02)	0.22(0.05)	0.12(0.02)
W + P	0.24(0.02)	0.13(0.03)	0.20(0.03)	0.11(0.02)
SUVA254 (m^{-1} mg^{-1} L)	2012		2013	
	D1	D2	D1[a]	D2[b]
No treatment	5.0(0.2)	4.0(0.2)	3.1(0.2)	2.3(0.3)
Precipitation	4.7(0.3)	4.4(0.4)	3.0(0.3)	2.3(0.1)
Warming	4.7(0.3)	4.2(0.3)	3.0(0.1)	2.8(0.2)
W + P	4.6(0.1)	5.2(0.4)	3.1(0.2)	2.6(0.2)
A254/A365	2012		2013	
	D1[a]	D2[b]	D1[a]	D2[b]
No treatment	6.6(0.1)	11.4(0.9)	5.7(0.2)	6.5(0.1)
Precipitation	7.1(0.2)	11.3(2.0)	5.3(0.4)	6.5(0.1)
Warming	7.0(0.4)	10.3(1.0)	5.8(0.2)	6.1(0.2)
W + P	7.7(0.7)	9.8(1.0)	5.8(0.2)	6.5(0.1)

Letters (a and b) denote the significant difference between depths ($P < 0.05$).
( ): standard error.

2012 and August, 2013. No significant differences were found among treatments (data not shown).

We selected *Pedicularis spp.* growing in each plot in order to investigate the effects of climate change on vegetation. *Pedicularis spp.* is an annual or biennial herb, and it is easy to distinguish young leaves that might have grown during the manipulation period. A few young leaves of *Pedicularis spp.* were collected and pooled as one sample for each plot in the middle of the second year's manipulation period (August 2013).

2.4. Soil properties

Soil water content (WC) was measured by oven-drying at 105 °C for 24 h, and organic matter (OM) content was determined by loss-on-ignition at 600 °C for 24 h. WC and OM content were expressed as percentages of soil dry weight. Extractable dissolved organic carbon (DOC) content was measured by extracting soil with distilled water, filtering through a 0.45-μm filter, and then measuring organic carbon concentration with a total organic carbon (TOC) analyzer (TOC-V, Shimadzu). DOC content was expressed as the amount of C (mg) per soil dry weight. Specific ultraviolet absorbance (SUVA) of the DOC extract was measured using a spectrophotometer at 254 nm (A254) and 365 nm (A365). The ratio of A254 to DOC concentration (SUVA 254), which is associated with the humic fraction of DOC, and the ratio of A254 to A365 (A254/A365), which is inversely proportional to the molecular weight of the DOC compound, were calculated (Ågren et al., 2008; USEPA, 2005). SUVA 254 was expressed as m^{-1} mg^{-1} L, and A254/A365 was expressed as a dimensionless ratio.

2.5. Extracellular enzyme activity

To investigate extracellular enzyme activity, we analyzed the specific substrate consumption rates of four hydrolases and one oxidase (phenol oxidase). Target hydrolases were β-D-glucosidase and cellobiase, which are associated with carbon cycling, and N-acetyl-glucosidase (β-N-acetyl-glucosaminidase) and aminopeptidase, which are associated with nitrogen cycling. Soil enzymes were extracted with acetate butter (50 mM, pH 5.0), and the extract was mixed with methylumbelliferyl (MUF)-linked substrate solution for hydrolases and l-3,4-dihydroxyphenylalanin (l-DOPA) solution for phenol oxidase (DeLaune et al., 2013; Kwon et al., 2013; Paul, 2007). The amount of MUF substrate consumed was measured using a fluorescent assay (excitation, 355 nm; emission, 460 nm; FLUO-star OPTIMA, BMG LABTECH) after incubation at 25 °C. Hydrolase activity was expressed as nmol g^{-1} soil min^{-1}. The amount of L-DOPA consumed was measured with a colorimetric assay (absorption at 460 nm; FLUO-star OPTIMA, BMG LABTECH) after incubation at 25 °C. Phenol oxidase activity was expressed as nmol diqc g^{-1} soil min^{-1}. Because the soil samples were collected in different periods of 2012 and 2013, to exclude the seasonal effect, we estimated relative enzyme activity. The ratio of activity under each treatment and depth to activity in the controlled plots were calculated (100% at D1 in no treatment plot).

2.6. Stable isotope ratio

2.6.1. $\delta^{13}C$ and $\delta^{15}N$ values of soil and plant leaves

Soil samples from depths of 0–5 cm (D1) were dried at 105 °C for 24 h, and plant leaves were dried at 60 °C for 72 h. The dried samples were ground using a ball mill (Mixer Mill MM 400, Retsch) before measurement. $\delta^{13}C$ (‰, PDB) and $\delta^{15}N$ (‰, air) values of soil and plant leaves were analyzed with an elemental analyzer (vario MICRO cube, Elemental) and isotope ratio mass spectrometer (Isoprime100, Isoprime) system (EA-IRMS). The δ values of reference gases linked to the EA-IRMS system were calibrated according to international standards prescribed by International Atomic Energy Agency, namely, IAEA-600, IAEA-CH-3, and IAEA-N-1. We analyzed each sample thrice and used the average values for further analyses.

2.6.2. ^{15}N enrichment factor

In a chemical reaction, the heavy stable isotope is depleted in the product rather than in the substrate. The degree of depletion is different for each reaction and depends on many factors such as substrate concentration, reaction rate, and type of catalysis or enzyme. The degree of depletion is defined as the stable isotope enrichment factor between substrate and product (Fry, 2007). We calculated ^{15}N enrichment factor (ε) between soil (D1) and plant for each plot by subtracting the $\delta^{15}N$ value of soil from the $\delta^{15}N$ value of plant leaves (Emmett et al., 1998; Nilsson et al., 2006).

$$\varepsilon = \delta^{15}N_{plant} - \delta^{15}N_{soil}$$

2.7. Statistics

A two-way ANOVA test was conducted to compare soil properties and enzyme activity at two depths across the four treatments, and a one-way ANOVA test was conducted to compare the stable isotope date across the four treatments (SPSS 18.0). In addition, we performed Tukey and LSD post-hoc analysis at the 0.05 level when the differences among treatments were significant. Normality (Kolmogorov–Smirnov statistics) and variance homogeneity were evaluated before the ANOVA analysis.

3. Results

3.1. Soil properties

WC, OM content, DOC content, humic fraction, and molecular weight of DOC were mostly higher at D1 than at D2, although there was some

Table 2
Results of two-way ANOVA of soil properties at two depths and under four treatments.

Water content	2012		2013	
	F value	*P* value	*F* value	*P* value
Treatment	0.641	0.594	0.076	0.973
Depth	7.899	0.008	10.626	0.003
Treatment * Depth	0.057	0.982	0.262	0.852
Organic matter	**2012**		**2013**	
	F value	*P* value	*F* value	*P* value
Treatment	0.697	0.561	0.206	0.892
Depth	8.692	0.006	3.584	0.067
Treatment * depth	0.232	0.874	0.256	0.856
DOC	**2012**		**2013**	
	F value	*P* value	*F* value	*P* value
Treatment	1.634	0.201	0.375	0.772
Depth	31.965	<0.001	18.375	<0.001
Treatment * depth	0.332	0.802	0.024	0.995
SUVA254	**2012**		**2013**	
	F value	*P* value	*F* value	*P* value
Treatment	0.872	0.466	0.569	0.639
Depth	1.818	0.187	14.414	0.001
Treatment * depth	2.605	0.069	0.667	0.579
A254/A365	**2012**		**2013**	
	F value	*P* value	*F* value	*P* value
Treatment	0.128	0.943	0.364	0.780
Depth	27.552	<0.001	22.826	<0.001
Treatment * depth	0.708	0.554	1.401	0.260

variation in the significance (Tables 1 and 2). Higher SUVA254 means higher humic fraction of DOC, and lower A254/A365 means higher molecular weight. This trend was observed both in 2012 and 2013, i.e., before and after manipulation, respectively. Soil properties were uniform in the four treatment plots initially (2012), and they did not show any difference after one and half year of manipulation (2013).

3.2. Extracellular enzyme activities

Hydrolase (β-D-glucosidase, cellobiase, N-acetyl-glucosidase, and aminopeptidase) activity in soil was higher at D1 than at D2 (Figs. 1 and 2; $P < 0.05$). This trend was more striking in 2013, but there were no significant differences among treatments (Table 3).

In contrast, phenol oxidase activity showed significant differences among treatments after manipulation. Phenol oxidase activity was not different across all depths and treatments in the initial state (2012); however, it was higher in the warming with increased precipitation plot than in the non-warming plots after one and half year of manipulation (Fig. 3).

3.3. Stable isotope ratios

The carbon isotope signature ($\delta^{13}C$) of *Pedicularis* leaves was analyzed only in 2013 after manipulation. In a one-way ANOVA test, differences between the groups were not significant at the 0.05 level ($F = 3.043$, $P = 0.059$), but the LSD post-hoc test showed some trends. The $\delta^{13}C$ of young leaves was higher (less negative) in the warming plots than in the increased precipitation plot without warming (Fig. 4).

The plant–soil ^{15}N enrichment factor (ε) did not differ significantly among treatments ($F = 1.257$, $P = 0.322$). The enrichment factor of the warming with increased precipitation plot was slightly higher than that of the other plots (Fig. 5).

4. Discussion

We investigated extracellular enzyme activity and soil condition at two depths in an arctic ecosystem under warming and/or precipitation increase. As can be inferred from the soil properties (Table 1), soil corresponding to D1 is considered as the organic layer and soil corresponding to D2 is considered as the mineral or less organic layer. While soil samples from D1 were observed to be dark brown to black in color and have the properties of the organic layer, soil samples from D2 were generally observed to be brown and have the texture of sandy soil. WC, OM content, and DOC content of D1 soil were generally higher than those of D2 soil. Moreover, the former moist and organic layer is considered as a hotspot of microbial activity. The hydrolase activity in our sites differed significantly between D1 and D2 (Figs. 1 and 2). This trend was also observed in other studies involving arctic tundra soil (Svalbard

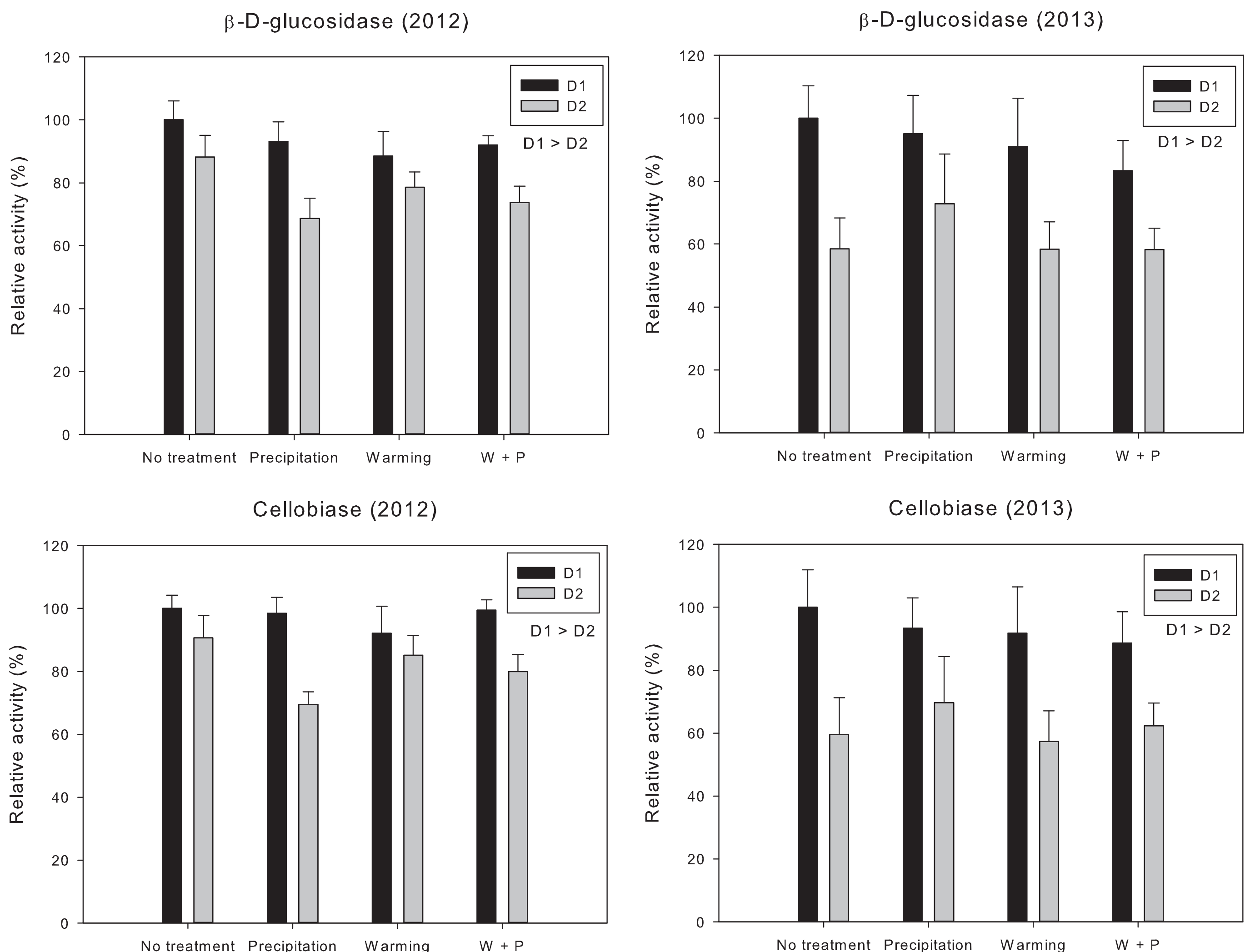

Fig. 1. Relative activity of hydrolases (β-D-glucosidase and cellobiase) at depths of 0–5 cm (D1) and 5–10 cm (D2) before (2012) and after (2013) manipulation. Treatments are no treatment, (increased) precipitation, warming, and W + P (warming with increased precipitation). D1 showed higher enzyme activity than D2 ($n = 5$, $P < 0.05$).

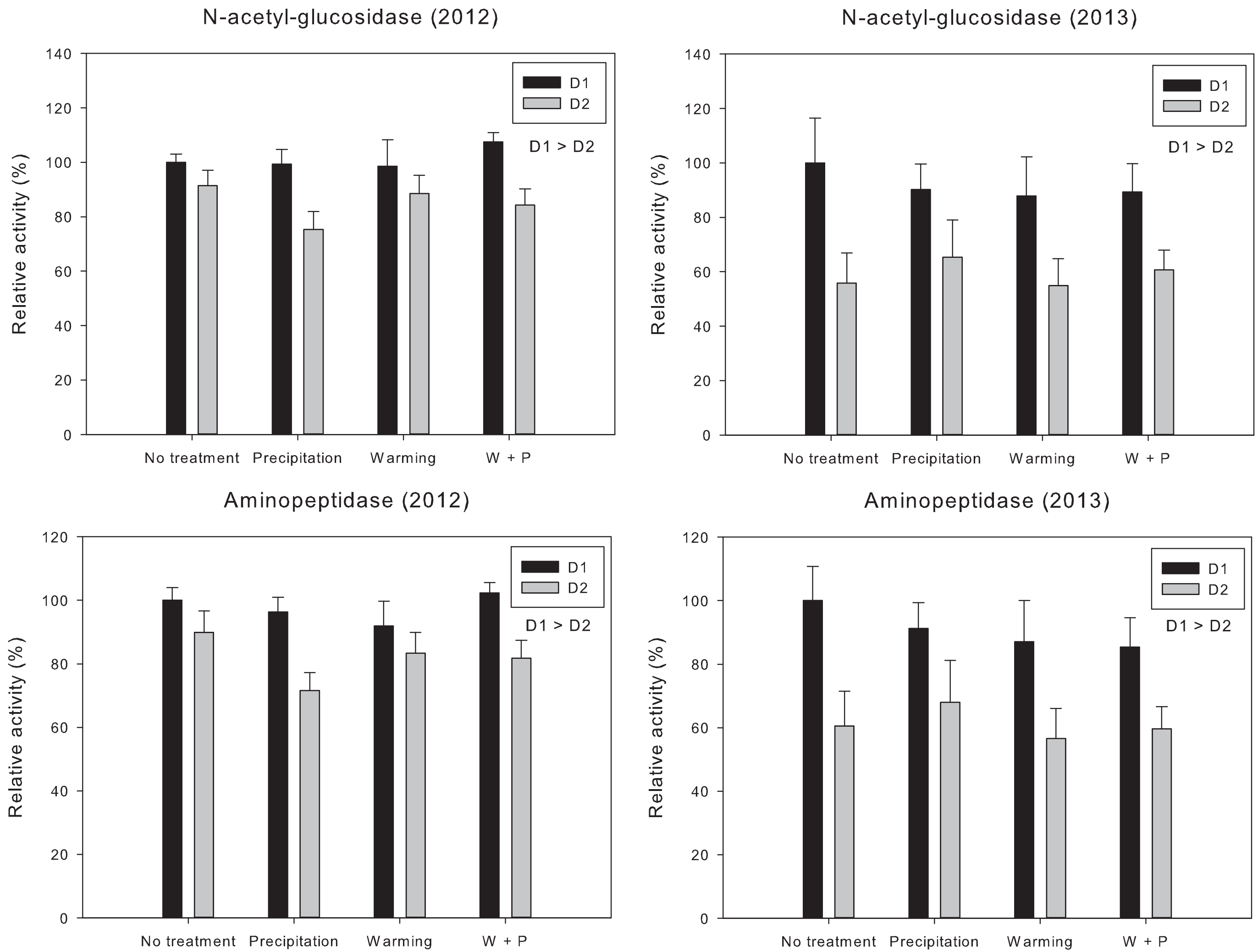

Fig. 2. Relative activity of hydrolases (N-acetyl-glucosidase and aminopeptidase) at depths of 0–5 cm (D1) and 5–10 cm (D2) before (2012) and after (2013) manipulation. Treatments are no treatment, (increased) precipitation, warming, and W + P (warming with increased precipitation). D1 showed higher enzyme activity than D2 ($n = 5$, $P < 0.05$).

archipelago, Norway) and alpine meadows (Lee et al., 2013; Wang et al., 2010). In the aforementioned studies, enzyme activity was generally higher in the upper soil layers than in the lower soil layers. Lee et al. (2013) reported that bacterial and archaeal communities, too, differed between the organic and mineral layers.

Although enzyme activity is influenced by multiple factors, we investigated changes in temperature and precipitation in an arctic ecosystem in this study. After one and half year of manipulation, only phenol oxidase activity differed among treatments (Fig. 3). The activity tended to increase in the warming plots, particularly at D1. It is reported that oxidative enzyme activity in the surface horizon increased during summer due to greenhouse warming treatment in arctic tundra soil near Toolik Lake in Alaska (Sistla and Schimel, 2013). Temperature increase may support the production of phenol oxidase and may increase the reaction rate of phenol oxidase.

Meanwhile, low water availability might affect organisms under warming treatments, likely owing to increased evaporation. The $\delta^{13}C$ value of *Pedicularis* leaves was higher (less negative) in the warming plots than in the increased precipitation plot (Fig. 4). We analyzed young leaves considered to have grown during the manipulation period. Natural abundance of ^{13}C is enriched when water availability of plants is low. Because soil water content changes over the long-term and varies greatly with location (Sharp et al., 2013; Sullivan et al., 2008), ^{13}C ratio of leaves could be a good indicator of water availability at the time of photosynthesis (Stewart et al., 1995; Warren et al., 2001). We could conclude that water availability of plants decreased relatively in the warming plots and soil enzymes were under drought stress, although soil water content did not change significantly. There was a similar report in the high arctic (Pituffik Peninsula in Greenland); leaf $\delta^{13}C$ data showed slight but significant enrichment with warming in three species, but measured soil water content was governed by regional differences (Sharp et al., 2013).

Hydrolases, which need water molecules for reaction, might essentially be inhibited under drought; on the other hand, oxidase is activated or facultative under drought in optimum levels, resulting in aerobic conditions (Kang et al., 1997; Kwon et al., 2013; Williams et al., 2000). In this study, warming treatment enhanced phenol oxidase activity but not hydrolase activity. In general, however, enzyme activity can be enhanced by increasing the temperature. We suggest that the indirect effect of warming led to drought stress offset temperature sensitivity of hydrolases in this arctic soil.

Because phenol oxidase is related to recalcitrant carbon mineralization, which is responsible for the activity of other enzymes (enzyme latch theory), phenol oxidase could be a key factor of organic matter decomposition (Freeman et al., 2001, 2004). If phenol oxidase activity is enhanced in the long term, a secondary effect on hydrolases and GHG emissions could appear.

While surface temperature in the warming plot appeared to be 1–2 °C higher than that in the control plot and overall surface temperature was below 15 °C, we compared potential enzyme activity at 25 °C.

Table 3
Results of two-way ANOVA of enzyme activity at two depths and under four treatments.

β-D-Glucosidase	2012		2013	
	F value	*P* value	*F* value	*P* value
Treatment	1.973	0.138	0.488	0.693
Depth	14.554	0.001	14.011	0.001
Treatment * Depth	0.612	0.612	0.284	0.837
Cellobiase	2012		2013	
	F value	*P* value	*F* value	*P* value
Treatment	1.333	0.281	0.173	0.914
Depth	16.091	0.000	14.934	0.001
Treatment * depth	1.562	0.218	0.227	0.877
N-acetyl-glucosidase	2012		2013	
	F value	*P* value	*F* value	*P* value
Treatment	0.841	0.481	0.132	0.940
Depth	14.402	0.001	15.023	0.000
Treatment * depth	0.908	0.448	0.247	0.863
Amino peptidase	2012		2013	
	F value	*P* value	*F* value	*P* value
Treatment	1.433	0.251	0.371	0.775
Depth	15.642	0.000	16.310	0.000
Treatment * depth	0.942	0.432	0.235	0.871
Phenol oxidase	2012		2013	
	F value	*P* value	*F* value	*P* value
Treatment	1.478	0.239	6.701	0.001
Depth	2.879	0.099	1.059	0.311
Treatment * depth	1.590	0.211	0.459	0.713

Although potential enzyme activity is not equal to in situ activity, both are closely related. Potential activity indicates overall enzyme concentration and enzyme production (Wallenstein and Weintraub, 2008). An empirical model of in situ activity of an arctic soil system based on potential activity, temperature sensitivity, and daily soil temperature has been developed (Wallenstein et al., 2009). According to this model, not only higher potential activity but also higher temperature itself further enhances the in situ activity. Therefore, higher potential activity under warming in our study suggests higher possibility of SOM mineralization under warming.

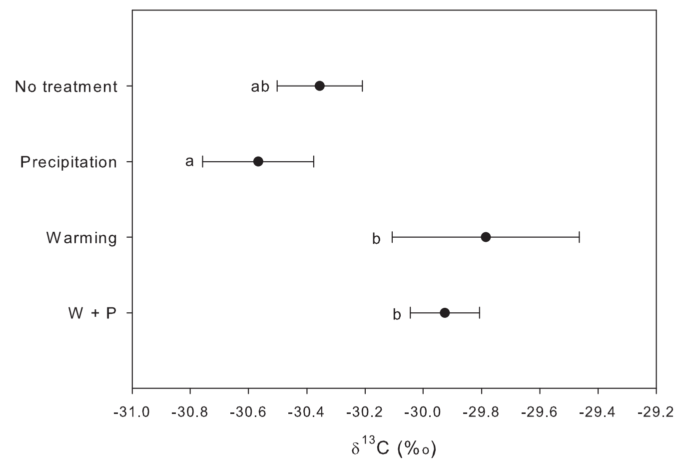

Fig. 4. Carbon isotope signature ($\delta^{13}C$) of *Pedicularis* leaves under four treatments after manipulation (2013). Treatments are no treatment, (increased) precipitation, warming, and W + P (warming with increased precipitation). Based on LSD comparison, letters (a and b) denote the difference among treatments (LSD, n = 5, $P < 0.05$; one-way ANOVA, $F = 3.043$, $P = 0.059$).

In many ecosystems, plant–soil ^{15}N enrichment factor was correlated with nitrogen deposition gradient or nitrogen mineralization rate (Emmett et al., 1998; Nilsson et al., 2006). We examined the plant–soil ^{15}N enrichment factor as an indicator of nitrogen availability. The ^{15}N enrichment factor did not differ significantly among treatments after one and half year of manipulation (Fig. 5). However, nitrogen availability could be enhanced by mineralization under climate change, and mineralized nitrogen could enhance microbial activity. In contrast, nitrogen scarcity can limit enzyme activity in arctic soil in spite of a temperature increase (Wallenstein et al., 2009). Therefore, changes in nitrogen availability and enzyme activity need to be observed over the long term.

5. Conclusions

We conducted a climate change experiment by simulating warming and/or precipitation increase in an arctic ecosystem to investigate the change in extracellular enzyme activity, which is responsible for organic matter decomposition. Phenol oxidase showed higher activity under warming, whereas hydrolases did not. This can possibly be attributed to the drought effect due to warming. Arctic tundra soils containing

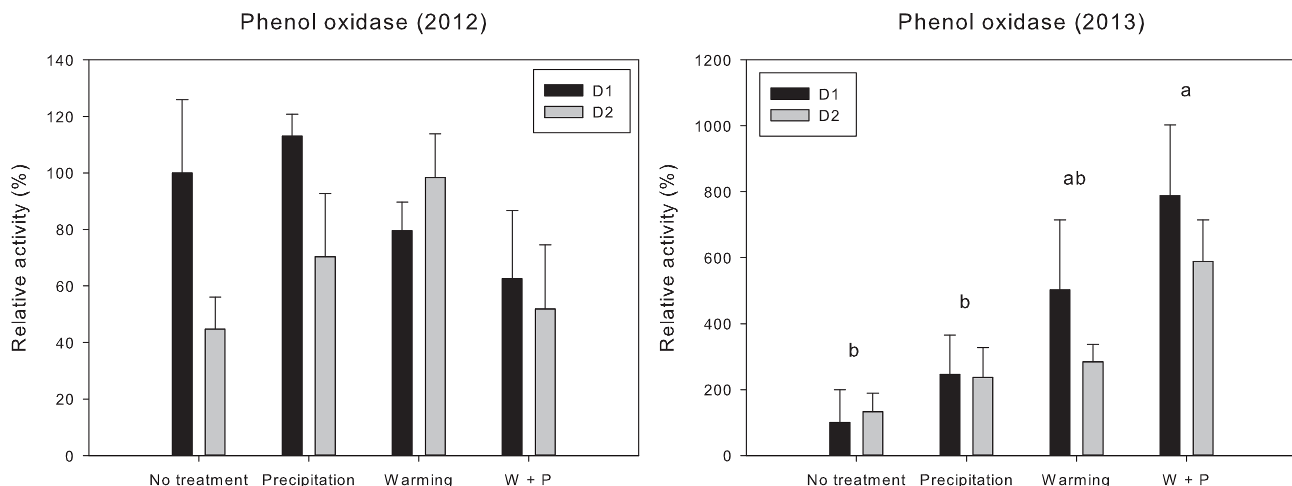

Fig. 3. Relative activity of phenol oxidase at depths of 0–5 cm (D1) and 5–10 cm (D2) before (2012) and after (2013) manipulation. Treatments are no treatment, (increased) precipitation, warming, and W + P (warming with increased precipitation). Based on Tukey comparison, letters (a and b) denote the difference among treatments (n = 5, $P < 0.05$).

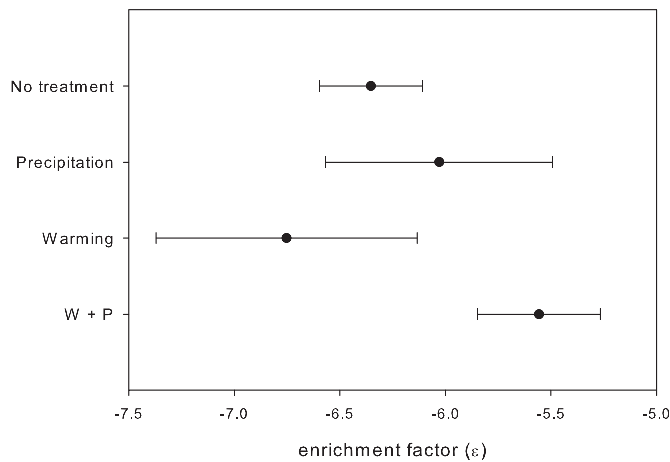

Fig. 5. ^{15}N enrichment factor (ε) between plant (*Pedicularis*) and soil (D1) under four treatments after manipulation (2013). Treatments are no treatment, (increased) precipitation, warming, and W + P (warming with increased precipitation). No significant differences were found (one-way ANOVA, n = 5, $F = 1.257$, $P = 0.322$).

large amounts of carbon are vulnerable to decomposition under potential climate change, and enhanced phenol oxidase activity could accelerate climate change. Because the results presented herein were obtained for a manipulation period of one and half years, long-term observation is necessary.

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References

Ågren, A., Buffam, I., Berggren, M., Bishop, K., Jansson, M., Laudon, H., 2008. Dissolved organic carbon characteristics in boreal streams in a forest-wetland gradient during the transition between winter and summer. J. Geophys. Res. Biogeosci. 113 (G3), G03031.
Allison, S.D., Treseder, K.K., 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. Glob. Change Biol. 14 (12), 2898–2909.
Bell, A., Collins, N., Ells, C., de Romily, G., Rossiter, A., Young, R., 2003. Evaluation of the ClimAdapt Guide to Incorporating Climate Change Into the Environmental Impact Assessment Process. Canadian Environmental Assessment Agency.
Burns, R.G., 1982. Enzyme Activity in Soil: Location and a Possible Role in Microbial Ecology. Soil Biol. Biochem. 14 (5), 423–427.
Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440 (7081), 165–173.
DeLaune, R.D., Reddy, K.R., Richardson, C.J., Megonigal, J.P., 2013. Methods in Biogeochemistry of Wetlands. Soil Science Society of America, Madison.
Emmett, B.A., Kjønaas, O.J., Gundersen, P., Koopmans, C., Tietema, A., Sleep, D., 1998. Natural abundance of 15N in forests across a nitrogen deposition gradient. For. Ecol. Manag. 101 (1–3), 9–18.
Freeman, C., Nevison, G.B., Hughes, S., Reynolds, B., Hudson, J., 1998. Enzymic involvement in the biogeochemical responses of a Welsh peatland to a rainfall enhancement manipulation. Biol. Fertil. Soils 27 (2), 173–178.
Freeman, C., Ostle, N., Kang, H., 2001. An enzymic 'latch' on a global carbon store. Nature 409 (6817), 149.
Freeman, C., Ostle, N.J., Fenner, N., Kang, H., 2004. A regulatory role for phenol oxidase during decomposition in peatlands. Soil Biol. Biochem. 36 (10), 1663–1667.
Fry, B., 2007. Stable Isotope Ecology. Springer, New York.
Gianfreda, L., Bollag, J., Stotzky, G., 1996. Influence of natural and anthropogenic factors on enzyme activity in soil. Soil Biochem. 9, 123–193.
IPCC, 2007. Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Intergovernmental Panel on Climate Change, Geneva, Switzerland.
Kang, H.-J., Freeman, C., Emmett, B.A., 1997. Effects of long term nitrogen additions on soil enzyme activities in a Sitka spruce forest. Int. J. Ecol. Environ. Sci. 23, 75–80.
Kang, H., Kang, S., Lee, D., 2009. Variations of soil enzyme activities in a temperate forest soil. Ecol. Res. 24 (5), 1137–1143.
Kim, S.-Y., Freeman, C., Fenner, N., Kang, H., 2012. Functional and structural responses of bacterial and methanogen communities to 3-year warming incubation in different depths of peat mire. Appl. Soil Ecol. 57, 23–30.
Koch, O., Tscherko, D., Kandeler, E., 2007. Temperature sensitivity of microbial respiration, nitrogen mineralization, and potential soil enzyme activities in organic alpine soils. Glob. Biogeochem. Cycles 21 (4), GB4017.
Kwon, M.J., Haraguchi, A., Kang, H., 2013. Long-term water regime differentiates changes in decomposition and microbial properties in tropical peat soils exposed to the short-term drought. Soil Biol. Biochem. 60, 33–44.
Lee, S.-H., Jang, I., Chae, N., Choi, T., Kang, H., 2013. Organic layer serves as a hotspot of microbial activity and abundance in Arctic tundra soils. Microb. Ecol. 65 (2), 405–414.
Moorhead, D., Linkins, A.E., 1997. Elevated CO_2 alters belowground exoenzyme activities in tussock tundra. Plant Soil 189 (2), 321–329.
Nilsson, L.O., Wallander, H., Baath, E., Falkengren-Grerup, U., 2006. Soil N chemistry in oak forests along a nitrogen deposition gradient. Biogeochemistry 80 (1), 43–55.
Paul, E.A., 2007. Soil Microbiology, Ecology and Biochemistry. 3rd ed. Elsevier Science, Burlington.
Sharp, E.D., Sullivan, P.F., Steltzer, H., Csank, A.Z., Welker, J.M., 2013. Complex carbon cycle responses to multi-level warming and supplemental summer rain in the high Arctic. Glob. Change Biol. 19 (6), 1780–1792.
Sistla, S.A., Schimel, J.P., 2013. Seasonal patterns of microbial extracellular enzyme activities in an arctic tundra soil: identifying direct and indirect effects of long-term summer warming. Soil Biol. Biochem. 66, 119–129.
Stewart, G., Turnbull, M., Schmidt, S., Erskine, P., 1995. 13C natural abundance in plant communities along a rainfall gradient: a biological integrator of water availability. Funct. Plant Biol. 22 (1), 51–55.
Sullivan, P.F., Welker, J.M., Steltzer, H., Sletten, R.S., Hagedorn, B., Arens, S.J.T., Horwath, J.L., 2008. Energy and water additions give rise to simple responses in plant canopy and soil microclimates of a high arctic ecosystem. J. Geophys. Res. Biogeosci. 113 (G3), G03S08.
Tarnocai, C., Canadell, J.G., Schuur, E.A.G., Kuhry, P., Mazhitova, G., Zimov, S., 2009. Soil organic carbon pools in the northern circumpolar permafrost region. Glob. Biogeochem. Cycles 23 (2), GB2023.
USEPA, 2005. Determination of Total Organic Carbon and Specific UV Absorbance at 254 nm in Source Water and Drinking Water. U.S. Environmental Protection Agency.
Vo, N.X.Q., Kang, H., 2012. Regulation of soil enzyme activities in constructed wetlands under a short-term drying period. Chem. Ecol. 29 (2), 146–165.
Wahren, C.H.A., Walker, M.D., Bret-Harte, M.S., 2005. Vegetation responses in Alaskan arctic tundra after 8 years of a summer warming and winter snow manipulation experiment. Glob. Change Biol. 11 (4), 537–552.
Wallenstein, M.D., Weintraub, M.N., 2008. Emerging tools for measuring and modeling the in situ activity of soil extracellular enzymes. Soil Biol. Biochem. 40 (9), 2098–2106.
Wallenstein, M.D., McMahon, S.K., Schimel, J.P., 2009. Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. Glob. Change Biol. 15 (7), 1631–1639.
Wang, C., Long, R., Wang, Q., Liu, W., Jing, Z., Zhang, L., 2010. Fertilization and litter effects on the functional group biomass, species diversity of plants, microbial biomass, and enzyme activity of two alpine meadow communities. Plant Soil 331 (1–2), 377–389.
Warren, C., McGrath, J., Adams, M., 2001. Water availability and carbon isotope discrimination in conifers. Oecologia 127 (4), 476–486.
Williams, C., Shingara, E., Yavitt, J., 2000. Phenol oxidase activity in peatlands in New York State: response to summer drought and peat type. Wetlands 20 (2), 416–421.