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Temporal Variations Rather than Long-Term Warming Control Extracellular Enzyme Activities and Microbial Community Structures in the High Arctic Soil

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Abstract
In Arctic soils, warming accelerates decomposition of organic matter and increases emission of greenhouse gases (GHGs), contributing to a positive feedback to climate change. Although microorganisms play a key role in the processes between decomposition of organic matter and GHGs emission, the effects of warming on temporal responses of microbial activity are still elusive. In this study, treatments of warming and precipitation were conducted from 2012 to 2018 in Cambridge Bay, Canada. Soils of organic and mineral layers were collected monthly from June to September in 2018 and analyzed for extracellular enzyme activities and bacterial community structures. The activity of hydrolases was the highest in June and decreased thereafter over summer in both organic and mineral layers. Bacterial community structures changed gradually over summer, and the responses were distinct depending on soil layers and environmental factors; water content and soil temperature affected the shift of bacterial community structures in both layers, whereas bacterial abundance, dissolved organic carbon, and inorganic nitrogen did so in the organic layer only. The activity of hydrolases and bacterial community structures did not differ significantly among treatments but among months. Our results demonstrate that temporal variations may control extracellular enzyme activities and microbial community structure rather than the small effect of warming over a long period in high Arctic soil. Although the effects of the treatments on microbial activity were minor, our study provides insight that microbial activity may increase due to an increase in carbon availability, if the growing season is prolonged in the Arctic.

Keywords Arctic · Tundra · Extracellular enzyme activity · Bacterial community structure · Warming · Temporal variations

Introduction

Arctic soils store a large amount of organic matter, which is estimated to be in the range of 1330–1580 Pg [1, 2]. Slow decomposition of organic matter due to low temperatures leads the accumulation and stabilization of soil organic carbon in Arctic region [3]. Global warming in the Arctic region was the greatest over the past 30 years [4], and the IPCC 5th assessment report anticipates that temperatures will rise further, at least by more than 4 °C over the next 100 years based on the RCP 8.5 scenario, and precipitation in the Arctic has increased over the past century [5]. Stimulated activity of decomposers due to temperature and precipitation rise in the Arctic may enhance the emissions of greenhouse gases (GHGs; CO_2, CH_4, N_2O), which is expected to generate a 'biological positive feedback' to climate change [6–8]. This feedback is emerging as an important research topic in the Arctic because carbon (C) dynamics and plant-microbial-soil interactions under climate change still have large uncertainties due to the lack of mechanistic understanding [9–11]. Since microorganisms play a critical role in C dynamics, their community composition, activity, and mechanisms of decomposition should be investigated to better understand the intensity of the biological feedback under climate change.

Microorganisms produce extracellular enzymes to degrade polymeric organic compounds to acquire C, nitrogen (N), and other resources [12, 13]. As maintenance costs of enzyme production with limited resources are higher than

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those of microbial growth, the production of extracellular enzymes may be regulated by C and N availability [13]. As microorganisms sensitively react to low resource availability and low temperatures in Arctic ecosystems [14, 15], warming can increase microbial activity and decomposition rate due to increase in C and N availability [16, 17]. However, several studies have supported the idea that temperature rise will essentially decrease enzyme activities because temperature rise will affect soil moisture, which in turn may limit microbial activity and degradation of C. For example, warming can induce water-logging conditions [18, 19] or drought stress [20]; both can lower enzyme activities. In addition, increased precipitation can stimulate microbial activity by increase in inorganic N mineralization in the high Arctic [21]. Hence, the major uncertainty in the effect of warming and high precipitation on C stocks and GHG emissions in the Arctic soils is whether microorganisms accelerate or reduce production of enzymes and their activity. Although N availability [22], temperature [23], and the quality of C [24] have been proposed as key controlling variables for enzyme activities in Arctic soils, much less information is available for Arctic ecosystems than for other ecosystems, for example temperate forests or aquatic ecosystems [25–29].

Microbial community structures, which are of great importance for decomposition of organic matter and GHGs emissions, can be influenced by physico-chemical changes in the soil environment. Changes in temperature and soil moisture can cause changes in not only water availability which determines transport of C and nutrients but also microbial diversity in Arctic soils [30–32]. For example, increase in temperature and soil moisture can accelerate CH_4 production by increasing methanogen diversity in Arctic soils [33, 34]. These changes can influence considerable shifts in microbial community structures, as the microorganisms are sensitive to temperature and soil moisture and availability of water, C, and nutrients [35–38], which can in turn affect decomposition processes in soils. In addition, abundance of bacteria is generally higher than those of archaea or fungi in permafrost subsoils [39]. Thus, bacterial community structures are important in regulating decomposition processes in Arctic soils, and response of bacterial community structure to warming and high precipitation should be identified. However, the effects of warming and high precipitation on shifts in microbial community structure in Arctic soils are still elusive due to the complex interaction of the microbial community with environmental factors, which are of great importance for a correct understanding of C decomposition in the Arctic regions.

Arctic tundra soils are generally considered to be N-limited ecosystems [40]. The concentrations of inorganic N in soils are mainly determined by N mineralization and uptake by both plants and microbes [40, 41]. N limitation may hinder the activities of extracellular enzymes in Arctic tundra soils [23, 42]. However, rapid warming in Arctic regions may stimulate decomposition of soil organic matter and consequent mineralization of nutrients including N, resulting in the removal of N limitation [43]. In contrast, warming may limit microbial activity and reduce N availability due to higher nutrient uptake by vegetation [40, 44, 45]. It is not clear yet which would exert stronger influence on enzyme activities and microbial community structure.

In this study, we investigated the responses of extracellular enzyme activities and bacterial community to changes in temperature and moisture contents in tundra soils, and identified factors that regulate those characteristics. We artificially warmed and wetted tundra soils by using passive chambers and sprinkling water during every summer over 6 years. Effects of warming in Arctic ecosystems are typically assessed by passive warming system such as OTC. While this approach has a merit, typical warming temperature of 1–2 °C may be too small to observe any significant effects of warming. Another approach is to monitor the changes in ecosystem processes over a season where temperature variations are more drastic. In this case, however, other factors such as water and nutrient availability co-vary; thereby, direct effects of warming may not be accurately determined. In the present study, we attempted to employ both approaches to see how microbial community structure and activities are affected by temperature changes in Arctic soil. We monitored extracellular enzyme activities and chemical properties of soils 4 times during summer in 2018. The key questions we asked in this study were (1) how enzyme activities and bacterial community structures change in tundra soils that have been exposed to warming and higher precipitation; (2) how temporal variation of enzyme activities and bacterial community structures change during Arctic summer; and (3) what factors control enzyme activities and bacterial community structures during Arctic summer.

Materials and Methods

Study Site and Experimental Design

The study site was in the high Arctic dry tundra, which is a continuous permafrost zone, and was located in Cambridge Bay, Nunavut, Canada (69°07′48″N, 105°03′36″W). The daily mean temperature exceeds 3.3 °C from June to September in 2018, and the mean annual precipitation was 140 mm between 1971 and 2000. The vegetation is mainly composed of prostrate-dwarf shrubs, with the dominant species of *Dryas integrifolia* and *Carex* spp. We designed 4 treatments to change the temperature and moisture content of soil: control (C), precipitation increase (P), warming (W), and warming together with precipitation increase (WP). Two liters of

distilled water was sprinkled weekly on each plot that is a square with 2 m to increase precipitation, and we realized 0.5 mm of precipitation increase per week. We referred to a climate change model that predicts the precipitation will increase by 15.6 mm (0.52 mm per week) during growing season in 2040–2069 compared to that in 1971–2000 in Cambridge Bay [46]. Passive hexagonal chambers with a diameter of 2 m were set up to increase soil temperature by blocking wind [47]. The treatments consisted of 5 replicate blocks and were applied during every summer from 2012 to 2018. The manipulation experiment was conducted from early July to early October in 2012–2013 and from mid-June to mid-September in 2014–2018. In each sampling, soils from three points in the plot were collected and pooled to minimize the spatial variation. Soil samples were collected monthly from June to September 2018 (28 June, 14 July, 18 August, and 2 September), which covers the period from thawing to the end of summer. Soils were separated into organic layer, D1 (mostly 0–5 cm), and mineral layer, D2 (mostly within a range of 5–10 cm). Upon collection, soil samples were shipped to the laboratory on ice and stored at 4 °C until analyses. For DNA extraction, subsamples were stored at − 80 °C. Soil temperature was measured at 5-cm soil depth.

Soil Properties

Air temperature and soil temperature for each treatment plot were measured hourly using data logger (HOBO H21-002 Micro Station Data Logger, Onset, USA) at 20 cm height and at 5 cm depth, respectively. Gravimetric water content and organic matter content in soil were measured by drying at 105 °C for 24 h and loss-on-ignition after heating at 600 °C for 24 h, respectively. Dissolved organic carbon (DOC) in soil was extracted with distilled water, and the extract was filtered through a 0.45 µm pore size filter. Carbon content in the extract was measured using a total organic carbon analyzer (TOC-V_{CHP}; Shimadzu, Kyoto, Japan), and DOC content was expressed as mg C per dry weight of soil. Specific ultraviolet absorbance (SUVA) of the DOC extract was measured by a spectrophotometer at 254 nm (A_{254}) and 365 nm (A_{365}). The ratio of A_{254} to DOC concentration ($SUVA_{254}$) was calculated to estimate the humic fraction of DOC and was expressed as m^{-1} mg^{-1} L. The ratio of A_{254} to A_{365} (A_{254}/A_{365}), which is in inverse proportion to the molecular weight of the DOC, was also calculated. Inorganic N (NH_4^+, NO_3^-) was extracted with 2 M KCl and filtered through Whatman #42 paper. The filtrate was analyzed with an auto-analyzer (Quaatro; Seal Analytical, Inc., Norderstedt, Germany).

Measurement of Extracellular Enzyme Activities

The potential activities of four hydrolases and phenol oxidase were measured and the enzyme assays following a fluorometric technique [48, 49]. The hydrolases were β-glucosidase (BG) and cellobiase (CB), which are associated with C cycling, and β-N-acetyl-glucosaminidase (NAG) and Leu-aminopeptidase (LA), which are associated with N cycling. Soil samples (4 g) were mixed with 20 mL of 50 mM sodium acetate buffer (pH 5.0). After vortexing and centrifugation, the supernatant was used as an enzyme extract. Each extract (1 mL) was mixed with 5 mL of methylumbelliferyl (MUF), which is a substrate for hydrolases, and 1 mL of L-3,4-dihydroxyphenylalanine (L-DOPA), which is a substrate for phenol oxidase, and the mixture was incubated at 20 °C. After 15 min of incubation, the product of phenol oxidase was measured using absorbance at 460 nm (FLUO-star OPTIMA, BMG Labtech., Ortenberg, Germany). After 60 min, the products of hydrolases were measured using a fluorescent assay (excitation: 355 nm, emission: 460 nm; FLUO-star OPTIMA). Activity was calculated as nmol g^{-1} soil min^{-1} for hydrolases, and nmol diqc g^{-1} soil min^{-1} where diqc (3-dihydroindole-5,6-quinone-2-carboxylate) is the product of L-DOPA for phenol oxidase.

Soil Respiration Measurement

R_{eco} (ecosystem respiration; total CO_2 emission rate) was measured at each plot in four blocks, 16 plots in total, using opaque chambers. Chambers had the volume of 12 L, covering 850 cm^2 of soil surface. They were connected to LI-840 (LI-COR, Lincoln, NE, USA), which measures CO_2 concentration of the chamber headspace every 1 s. Concentration measurements were restricted to 2 min to avoid saturation effects [50].

Microbial Abundance

DNA was extracted from soil samples (0.25 g) by using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA quality and quantify were assessed by using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA). Then, real-time quantitative polymerase chain reaction (RT-qPCR) using CFX96 (Bio-Rad Laboratories, Hercules, CA, USA) was conducted targeting bacterial 16S rRNA. The forward and reverse primers were 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 927R (5′-CCG TCA ATT CCT TTR AGT TT-3′), respectively. Standards for RT-qPCR were tenfold dilution series of plasmids carrying the bacterial 16S rRNA region.

16S rRNA Gene Amplification and Sequencing Analysis

To determine soil bacterial community composition and diversity in each soil sample, an amplicon survey of a portion of the 16S rRNA gene was performed. The workflow for 16S rRNA library preparation was as follows: 1st stage PCR, clean-up of 1st PCR product, 2nd stage PCR, gel extraction of the 2nd PCR product, and pooling of all samples. The amplicon targeting V3–V4 region of bacterial 16S rRNA was amplified with primer set 341F/805R. The forward primer (5′-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3′) and reverse primer (5′-GTC TCG TGG GCT CGG AGA TGT GTA TA A GAG ACA GGA CTA CHV GGG TAT CTA ATC C-3′) contained Illumina adapters, which has high sequence coverage for bacteria and produces an appropriately sized amplicon (460 bp) for Illumina sequencing [51]. First PCR reactions were carried out in a total volume of 25 µl containing 2.5 µl of 10 × PCR buffer, 2.5 µl of dNTP (2 mM), 0.5 µl of Taq polymerase, 3 µl of $MgCl_2$, 0.5 µl of BSA, 5 µl of each primer (1 µM), 2.5 µl of template DNA, and 3.5 µl of ddH_2O. Extracted DNA from each sample was used as a template for the 1st PCR amplification, which was performed as follows: initial denaturation at 95 °C for 3 min followed by 10 cycles of 95 °C for 30 s, 67 °C for 30 s, and 72 °C for 1 min, followed by a 1 °C decrease in the annealing temperature at every cycle, and then 20 cycles 95 °C for 30 s, 57 °C for 30 s, 72 °C for 1 min, followed by a final extension at 72 °C for 5 min and subsequent cooling to 4 °C. All the 1st PCR products were cleaned up by using a GenElute PCR Clean-Up Kit (Sigma, St. Louis, MO, USA) and used as templates for the 2nd PCR amplification. Second PCR reactions were performed with primer set which contained sample specific barcodes and were carried out in a total volume of 50 µl containing 5 µl of 10 × PCR buffer, 5 µl of dNTP (2 mM), 1 µl of Taq polymerase, 6 µl of $MgCl_2$, 1 µl of BSA, 5 µl of each primer (1 µM), 5 µl of template DNA, and 17 µl of ddH_2O. The 2nd PCR was performed as follows: initial denaturation at 95 °C for 3 min followed by 8 cycles of 95 °C for 30 s, 57 °C for 30 s, 72 °C for 1 min, followed by a final extension at 72 °C for 5 min and subsequent cooling to 4 °C. Primer and primer dimers were separated out by electrophoresis on a 1.3% agarose gel, and 2nd PCR products were recovered using a QIA quick Gel Extraction Kit (Qiagen). PCR amplicons from all samples were then pooled at equimolar concentrations. Sequencing was conducted on an Illumina MiSeq sequencer at Macrogen, Inc., Seoul, Korea.

After sequencing, each sample was classified using MiSeq raw data, and FASTQ files were generated. Adaptor sequences were removed using fastp [52], and error correction was performed for overlapping pairs. Paired-end data of each sample were assembled using FLASH (1.2.11) [53]. Assembled sequences shorter than 400 bp or longer than 500 bp were removed. Sequences with errors such as low-quality, ambiguous, and chimeric sequences were removed using CD-HIT-OUT software [54], and operational taxonomic units (OTUs) were generated by clustering sequences with over 97% similarity. Representative sequences from each OTU were classified using BLASTN v2.2.25 [55], and taxonomic assignment was based on similarity to sequences from known organisms. When the query coverage and identity of the matched area were under 85%, best hit was not used for taxonomic assignment.

Statistical Analysis

Factorial ANOVA test was conducted with depths and blocks to assess whether enzyme activities, DOC, SUVA, water content, inorganic N, and microbial abundances were affected by time, treatments and time × treatment interactions. A Tukey HSD post hoc test was performed using SPSS 25 software (IBM Corporation, New York, NY, USA) at the 0.05 level when the differences were significant. Spearman correlation and multiple regression analysis were performed using the *corrplot* and *stats* package, respectively in R v.3.6.0 (R Core Team, 2019) to identify the relationships between enzyme activities and soil physico-chemical properties at each soil depth. Canonical correspondence analysis (CCA) and Adonis statistic for permutational multivariate analysis were performed by using the *vegan* package in R.

Results

Soil Properties

The average soil temperature was highest in July (9.01 °C), followed by August (5.59 °C), September (0.80 °C), and June (0.76 °C). The average soil temperature in the P, W, and WP plots was 0.45 °C, 0.43 °C, and 0.65 °C higher than that of the control plot, respectively. Although there were no significant differences in average soil temperature among treatments, the cumulative soil temperature of all treatments was higher than the control, with that of WP being the highest.

The soil properties (DOC, $SUVA_{254}$, A_{254}/A_{365}, inorganic N, and water content) were not significantly different among treatments, but significant differences among months were found (Table 1). While DOC content in D1 decreased over the summer, it peaked in July and then declined in late summer in D2 (Table 1). The quality of DOC was expressed using $SUVA_{254}$ and the A_{254}/A_{365} ratio. $SUVA_{254}$ differed by depth and month rather than by treatments. Overall, $SUVA_{254}$ was lower in D2 than in D1, and was significantly lower in July than in the other months at both depths

Table 1 Average soil properties in different soil layers

Month	DOC (mg C $g^{-1}_{dry\ soil}$)		$SUVA_{254}$ (m^{-1} mg^{-1} L)		A_{254}/A_{365}		Inorganic N (μg N g^{-1} soil)		Water content (%)	
	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2
June	0.252 ± 0.075^{a}	0.045 ± 0.018^{b}	4.216 ± 0.464^{a}	3.178 ± 0.709^{a}	5.463 ± 0.394^{bc}	5.836 ± 0.445^{a}	8.428 ± 2.781^{c}	1.607 ± 0.479	62.733 ± 5.524^{a}	20.881 ± 6.815^{a}
July	0.287 ± 0.114^{a}	0.104 ± 0.023^{a}	3.596 ± 0.855^{b}	1.584 ± 0.256^{c}	5.300 ± 0.529^{c}	4.497 ± 0.689^{b}	10.942 ± 2.201^{bc}	1.670 ± 0.792	56.072 ± 9.319^{b}	11.450 ± 3.064^{b}
August	0.185 ± 0.037^{b}	0.033 ± 0.009^{c}	3.955 ± 0.497^{ab}	2.797 ± 0.667^{ab}	5.731 ± 0.474^{ab}	6.037 ± 0.764^{a}	12.568 ± 3.485^{b}	1.824 ± 0.984	54.225 ± 5.618^{b}	11.610 ± 3.288^{b}
September	0.128 ± 0.035^{c}	0.034 ± 0.006^{bc}	4.002 ± 0.203^{a}	2.412 ± 0.595^{b}	5.800 ± 0.197^{a}	6.729 ± 2.403^{a}	15.680 ± 3.541^{a}	1.349 ± 0.663	54.743 ± 6.333^{b}	11.858 ± 2.969^{b}

Values are mean ± standard deviation derived from 20 replicate measurements. Superscript letters indicate significantly different ($P < 0.05$) groups between months. Statistical significance was determined using ANOVA and Tukey-HSD tests. *D1*, organic layer; *D2*, mineral layer

($P < 0.001$). The A_{254}/A_{365} ratio was also significantly lower in July than in the other months at both depths ($P < 0.001$). The concentration of inorganic N gradually increased in D1 ($P < 0.001$), whereas no significant differences were found in D2 (Table 1). Water content decreased over time at both depths ($P < 0.001$).

Extracellular Enzyme Activities

The enzyme activities were not significantly different among treatments, but significant differences among months were found except for phenol oxidase in D1 (Fig. 1). Activity of hydrolases declined significantly during growing season at both depths ($P < 0.001$), and these patterns were similar among treatments. Activity of hydrolases was the greatest at both depths in June, when soil started to thaw. Unlike in D1, activity of hydrolases in D2 was the lowest in August and then slightly increased except for the activity of NAG (Fig. 1). On the contrary, phenol oxidase activity was low in June and July in D2 and increased significantly toward the late summer ($P < 0.001$). However, no statistically significant differences were found in D1. Neither hydrolase nor phenol oxidase activity showed significant differences among warming and watering treatments.

Relationship Between Enzyme Activity and Soil Properties

Multiple linear regression analysis revealed that water content, soil temperature, SUVA, inorganic N, and bacterial abundance had significant correlations with the activity of BG in D1 ($R^2 = 0.722$, $P < 0.001$) (Table 2). Significant correlations were found between the activity of NAG and water content, inorganic N, and bacterial abundance in D1 ($R^2 = 0.611$, $P < 0.001$). In D2, the activities of both BG and NAG showed significant correlations with water content, soil temperature, inorganic N, and activity of phenol oxidase ($R^2 = 0.757$, $P < 0.001$; $R^2 = 0.781$, $P < 0.001$, respectively). Water content and concentration of inorganic N were common significant variables across the depth of the soil and activities of BG and NAG. Water content, DOC, bacterial abundance, and soil temperature had positive correlations with activity of hydrolases at both depths, while inorganic N had a weakly negative correlation with activity of hydrolases in D1 (Fig. S1).

Soil Respiration and Abundance of Bacteria

R_{eco} from the soil sampling date was measured during the growing season and was comparable across the months and treatments (Fig. 2). The R_{eco} was the highest in July and tended to decline thereafter and was significantly different among months ($P < 0.001$) and treatments ($P < 0.05$).

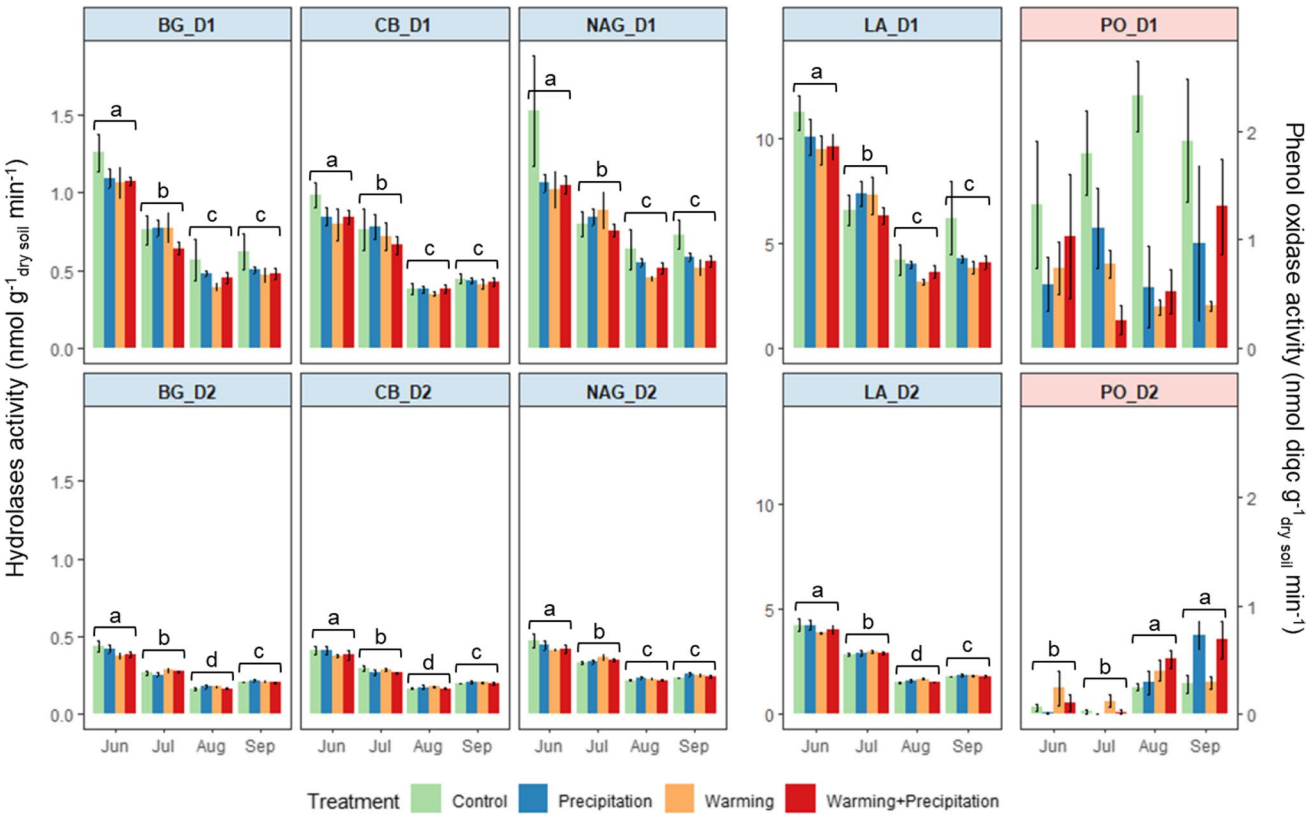

Fig. 1 Extracellular enzyme activities (BG, β-glucosidase; CB, cellobiase; NAG, β-N-acetyl-glucosaminidase; LA, leucine aminopeptidase; PO, phenol oxidase) in different treatments at organic layer (D1) and mineral layer (D2). Error bars are standard error of the mean ($n=5$), and letters denote statistically significant differences among months ($P<0.001$)

The bacterial abundance of each plot was measured over the growing season (Fig. 3). Overall, the abundance showed a significant difference along the month ($P<0.001$). The abundance tended to be similar at both depths, being more abundant at the beginning of the growing season than the end. Bacterial abundance in D1 declined until August and slightly increased in September, while it showed an abrupt decline from June to July and stable abundances afterward.

Bacterial Community Structure and Its Relationship with Soil Properties

We obtained 1,781,399 bacterial sequences from 160 samples, and the number of sequences sampled ranged between 885 and 30,931. All sequences were clustered into 5878 OTUs at the 97% similarity cutoff, and their number per sample ranged between 321 and 1658. Bacterial community structure varied significantly with depth and was also affected by month during the Arctic summer, but did not differ across the treatments at both depths (Fig. 4 and Fig. S2). While the relative abundances of *Acidobacteria* and *Bacteroidetes* increased over time, those of *Actinobacteria*, *Planctomycetes*, and *Verrucomicrobia* gradually decreased in D1 and that of *Proteobacteria* in D1 peaked in July (Fig. 4a). In contrast, the relative abundance of *Proteobacteria* in D2 was highest in August, while those of *Acidobacteria* decreased over time in D2 (Fig. 4b). Unlike in D1, the abundance of *Actinobacteria* was not significantly different among the months in D2.

CCA of 160 soil samples was performed to separate the microbial community structure along months and depths, and to identify factors that best explain the effects of soil properties on microbial community structure (Fig. 5). The first two axes explained 32.8% and 20.1% of the variations in D1 and D2, respectively ($P<0.05$ for both depths), and temporal variation during the growing season shifted the community structure at both depths (Adonis, $P=0.001$ for both depths). Water content and soil temperature were significant factors in determining microbial community composition in both D1 ($P<0.05$; $P=0.002$) and D2 ($P=0.001$; $P=0.012$). Furthermore, inorganic N ($P=0.001$), DOC ($P<0.05$), and bacterial abundance ($P=0.014$) were also significant factors in D1 only (Fig. 5a).

Table 2 Multiple regression analysis between soil properties and activity of BG and NAG

Dependent variable (units)	Depth	Variable	B (±SE)	*P*
β-glucosidase (BG) (nmol $g^{-1}_{dry\ soil}$ min^{-1})	D1	Water content	0.581 ± 0.074	**< 0.001**
		T	0.163 ± 0.076	**< 0.05**
		SUVA	0.170 ± 0.065	**0.010**
		Inorganic nitrogen	− 0.292 ± 0.071	**< 0.001**
		Bacterial abundance	0.170 ± 0.077	**< 0.05**
	$R^2 = 0.722$, Adj.$R^2 = 0.703$, $F = 38.38$, ***P* < 0.001**			
β-N-acetyl-glucosaminidase (NAG) (nmol $g^{-1}_{dry\ soil}$ min^{-1})	D1	Water content	0.428 ± 0.094	**< 0.001**
		DOC	0.127 ± 0.090	0.163
		SUVA	0.149 ± 0.076	0.053
		Inorganic nitrogen	− 0.241 ± 0.079	**0.003**
		Bacterial abundance	0.278 ± 0.092	**0.003**
		Phenol oxidase	0.116 ± 0.075	0.127
	$R^2 = 0.611$, Adj.$R^2 = 0.579$, $F = 19.08$, ***P* < 0.001**			
β-glucosidase (BG) (nmol $g^{-1}_{dry\ soil}$ min^{-1})	D2	Water content	0.764 ± 0.062	**< 0.001**
		T	0.265 ± 0.068	**< 0.001**
		Inorganic nitrogen	− 0.169 ± 0.061	**< 0.01**
		Phenol oxidase	− 0.134 ± 0.067	**< 0.05**
	$R^2 = 0.757$, Adj.$R^2 = 0.744$, $F = 58.54$, ***P* < 0.001**			
β-N-acetyl-glucosaminidase (NAG) (nmol $g^{-1}_{dry\ soil}$ min^{-1})	D2	Water content	0.676 ± 0.059	**< 0.001**
		T	0.375 ± 0.064	**< 0.001**
		Inorganic nitrogen	− 0.139 ± 0.058	**< 0.05**
		Phenol oxidase	− 0.168 ± 0.064	**0.010**
	$R^2 = 0.781$, Adj.$R^2 = 0.769$, $F = 66.91$, ***P* < 0.001**			

Values of variables were scaled before analysis and represented in bold are the significant values ($P < 0.05$). *B*, unstandardized coefficients; *SE*, standard error of estimate; *D1*, organic layer; *D2*, mineral layer; *T*, soil temperature

Discussion

Extracellular Enzyme Activities and Controlling Factors

Activity of hydrolases reportedly decreases during the growing season, which has been attributed to a decline in the availability of N and C [23, 42]. Our results are in accordance with these reports in that the overall activity of hydrolases tended to decline over summer in Arctic soil. This trend is consistent with decreases in C availability, water content, and bacterial abundance over time. In our study, activity of hydrolases was closely related to C availability. Microorganisms increase their allocation of resources to extracellular enzyme production to acquire C and N in extreme environments such as low temperatures

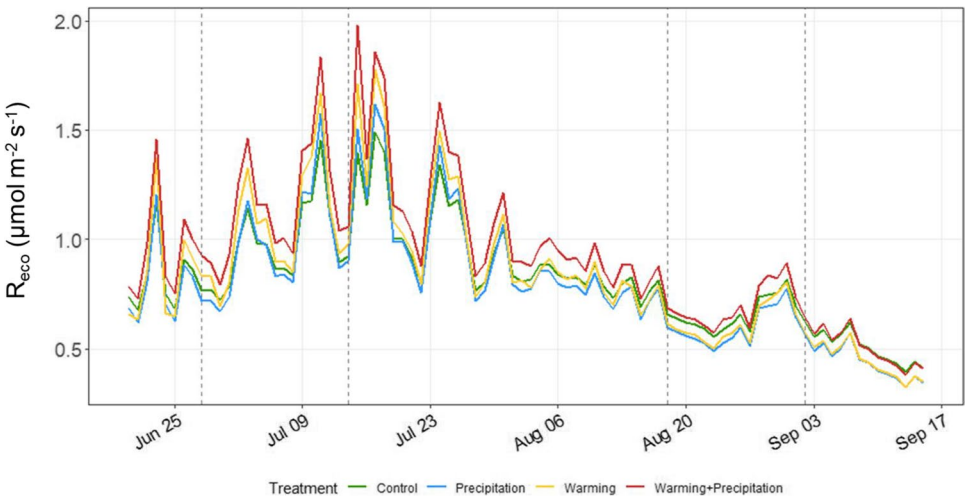

Fig. 2 Ecosystem respiration (R_{eco}) in different treatments ($P < 0.05$). Data are means ($n = 4$) and dashed line denote soil sampling date

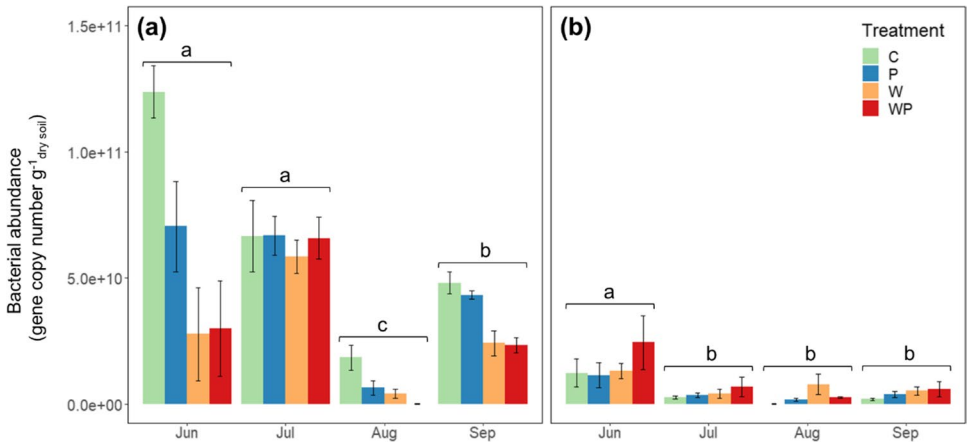

Fig. 3 Abundance of bacterial genes recorded on different soil treatments at **a** organic layer (D1) and **b** mineral layer (D2). Data are means with standard error bars ($n=5$). Small letters denote statistically significant differences among months ($P<0.001$)

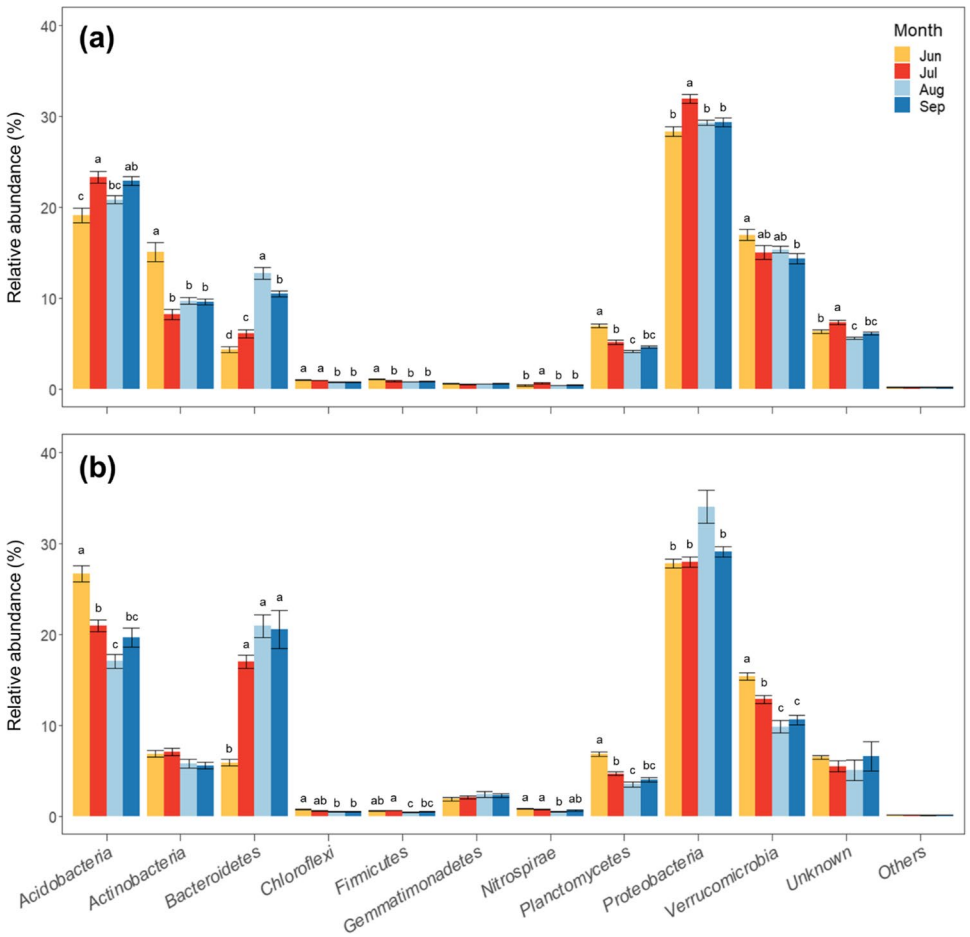

Fig. 4 Mean relative abundance of bacterial phyla for each month at **a** organic layer (D1) and **b** mineral layer (D2). Underlying data are based on 16S rRNA gene-encoding fragments recovered from metagenomic datasets. Values represent the abundance of each bacterial phylum as a proportion of the total bacterial community. Only the top 10 of phyla with a mean relative abundance are displayed

[56]. However, when C availability is low, they reduce the relative resource allocation to enzyme production to sustain their physiology [13, 57]. Therefore, enzyme pools in tundra soils in late summer could be smaller than early in the growing season due to decreased allocation to enzyme production. This implies that the physiology of microorganisms is closely associated with C availability.

We found that water content had a high positive correlation with the activity of hydrolases at both depths. Water availability is a well-known factor to control enzyme activity as it determines transport of substrates and water is required for hydrolysis [30, 32]. Thawing of frozen tundra soils, which begins in mid-June at our study site, may cause the release of frozen substrates, thereby increasing the availability of C and N and the activity of hydrolases.

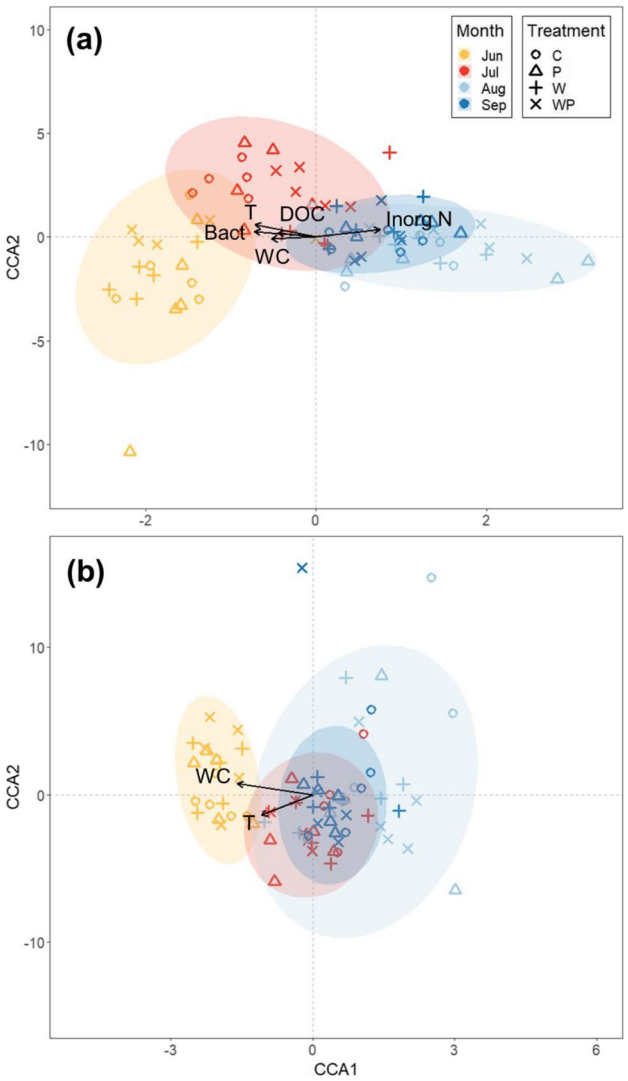

Fig. 5 Canonical correspondence analysis (CCA) biplot of community phylogenetic composition with environmental variables (T; soil temperature, DOC; dissolved organic carbon, WC; water content, Bact; bacterial abundance, Inorg N; inorganic nitrogen) at **a** organic layer (D1) and **b** mineral layer (D2). The arrows represent the relationship (direction and length) of the soil properties with the samples. The length of the arrow indicates the magnitude of the change in the corresponding variable, which increases along the arrow

Soil thawing causes accumulation of melt water; however, soil moisture may decrease over the growing season through evapotranspiration as well as drainage to a deeper soil layer, making surface soil drier [58, 59]. Hence, water availability at the end of growing season is lower than at the beginning, and hydrolases, which need water molecules for reactions they catalyze, may lose their activity during the growing season [47]. On the other hand, the activity of phenol oxidase gradually increased in D2 during the growing season. These results may also be explained by the temporal variation in water availability. Water drawdown in previously water-saturated anaerobic soil can increase oxygen availability, which stimulates oxidative enzymes [60]. We can suggest that water availability is a key driver of enzyme activities in Arctic soils during the growing season.

We also found that bacterial abundance was closely related the activity of hydrolases in D1. This could be expected because enzymes are synthesized and released by microorganisms [61, 62]. Our results suggest that the enzyme synthesis decreased over time due to a decrease in bacterial abundance. Indeed, R_{eco} decreased from July to August, suggesting that this decrease related to decline in bacterial abundance and their activity—decomposing organic matter to CO_2—towards the end of growing season.

The Increase in Inorganic N During the Summer

For many years, researchers have suggested that microbial activity may be limited by an extremely low concentration of N in Arctic tundra ecosystems [13, 22, 23, 42]. However, contrary to these studies, our results show that the concentration of inorganic N increased gradually during summer, even though N is generally known as a limiting resource in the Arctic. Several mechanisms could be responsible for this increase. First, uptake of inorganic N by microorganisms may dwindle over time during the growing season. This assumption is based on our finding that inorganic N accumulates over time, likely due to low microbial activity and bacterial abundance by a depletion of labile C. Thus, even if N-limitation to soil microbes is alleviated, the activity of hydrolases may decrease due to low concentration of C at the end of the growing season [20]. Second, soil organic N from dying and senescing plants may be mineralized during the growing season. Keuper et al. [63] demonstrated that thawing permafrost soil can release a biologically relevant amount of inorganic N during the growing season through continued microbial mineralization of organically bound N. Third, NH_4^+ could accumulate because of plant preference for NO_3^-. Our results show that the content of NH_4^+, which constituted a larger portion of inorganic N than did NO_3^-, greatly increased in D1 over time (Fig. S3). Previous studies found a high acquisition of NO_3^- in comparison to other N forms by tundra plants [64, 65]. The higher mobility of NO_3^- in soil may have contributed to high plant access to NO_3^-; for example, the diffusion rate of NO_3^- is five times those of NH_4^+ and amino acids [66]. Hence, our results demonstrated a declining trend in activity of hydrolases during summer despite N sufficiency, suggesting that enzyme activity is more influenced by C availability and water content than by N availability.

Microbial Community and Environmental Factors

Bacterial community structures changed gradually over summer, and the responses were distinct depending on soil layers and the environmental factors; water content and soil temperature affected the shift of bacterial community structures in both layers, whereas bacterial abundance, DOC, and inorganic nitrogen did so in the organic layer only. Microbial communities in the soil surface layer can be pre-adapted for rapid metabolism of labile C substrates and available nutrients [67]. As the organic layer contains more labile C and available nutrients than the mineral layer, microorganisms can take in more labile C and N by decomposing organic materials in the organic layer. In contrast, most available C and N may be already consumed in the mineral layer [68]. Microbes inhabiting deeper soil experience lower availability of C and nutrients than surface-living microbes [67]. These vertical distributions of available C and N can affect bacterial community and might explain higher bacterial abundance in D1 than in D2. This difference can explain why DOC, bacterial abundance, and inorganic N influenced the temporal shift of bacterial community structure more in D1 than D2. In addition, water content was mainly responsible for the shift of bacterial community structure at both depths, even if the temporal variation of water content during the growing season was small. Bacterial communities could be highly sensitive to changes in variability of water content and temperature in soil [69]. Seasonal change in water content and temperature alters microenvironment and microbial activity through impacts on oxygen concentration and nutrient availability [35, 70–72]. These microtopographic conditions at both depths can influence microbial community structure and function as well as enzyme activity [73, 74]. The shift in microbial community and physiology, in turn, can lead to changes of GHGs emissions [75, 76]. Given our results, the variability of DOC, bacterial abundance, and inorganic N are likely to be important factors controlling the composition of microbial communities in organic layer, where changes in available C and N can be larger in the organic layer than in the mineral layer. The microbial community structure in both organic and mineral layers may be responsive to the variability of environmental factors such as rapid changes in water content and temperature in Arctic soils.

Researchers have suggested that the bacterial community varied greatly across seasons in tundra soils [77, 78], which is consistent with our data on the changes in bacterial community structure during the growing season. *Acidobacteria* in D1 were more abundant at the end of the growing season than at the beginning. This can be explained by the oligotrophic characteristic of *Acidobacteria*, which can proliferate in an environment that offers very low levels of C and nutrients, decomposing recalcitrant C and storing the nutrients [79]. However, *Acidobacteria* in D2 were dominant when soil started to thaw. *Acidobacteria* can also proliferate in anaerobic conditions when soils are wet and enriched with organic C [77, 80]. In the mineral layer, the highest water content in June allows anaerobic microsites suitable for *Acidobacteria*. The increase in the relative abundance of *Bacteroidetes* over time at both depths may be related to soil temperature and labile C availability. As *Bacteroidetes* include many cold-tolerant degraders of complex substrates [80], they can be more active during the end of the growing season with more recalcitrant organic C than at the beginning. Hence, the *Bacteroidetes* may be involved in the activity of phenol oxidase, which contributes to the degradation of recalcitrant C compounds such as lignin. For example, Gittel et al. [81] demonstrated that *Bacteroidetes* has a positive correlation with phenol oxidase activity. Similarly, we found an increase in both the activity of phenol oxidase and the abundance of *Bacteroidetes* over time. The relative abundance of *Proteobacteria* in D1 peaked in July, concurrent with C availability. *Proteobacteria* are commonly associated with substrates rich in organic C [79, 82] and are some of the initial metabolizers of labile C inputs [83]. As such, rich organic C allowed *Proteobacteria* to thrive in July. Our results suggest that each bacterial phylum show different responses along with soil depth and characteristics, as they have different cell metabolism and survival strategies, which are essential adaptive response under certain environmental conditions.

Temporal Variations in Interactions Between Environmental Factors and Microbial Activity

We hypothesized that enzyme activities and microbial community structures would be more reactive to warming and high precipitation than to control during the growing season in Arctic soils. However, neither the activities of enzymes nor microbial community structure were affected by the treatments that lasted over 6 years. Instead, we found strong temporal variations of enzyme activity and microbial community structure from early summer to the beginning of fall. Similarly, previous studies have demonstrated no straightforward relationship between temperature and potential enzyme activity [60, 84–87], suggesting that temperature alone may not drive the observed treatment effects on microbial activity and community structures. Several interpretations of our findings are possible. The microbial activities are insensitive to small increases in soil temperature over the long period [85, 86], and are more responsive to seasonal variations than to the small effect of warming. Weedon et al. [87] demonstrated that effect of warming on soil enzymes and microbial community is less than that of seasonal differences, which are most likely to be mediated by the seasonality of substrate supply and microbial nutrient demand. In our study,

compared to the treatment of warming and precipitation, the temporal variations may have more effects on microbial activity and community due to larger variation of air and soil temperature than the treatment. However, the small effects of warming and precipitation might be induced by complex ecosystem responses between plant, microorganisms, and soil properties. For example, Jeanbille et al. [88] found a relationship between warming response ratio and C:N ratio, highlighting the legacy of vegetation on microbial response to warming although effect of warming was not detected. The other possibility is that increased evapotranspiration by warming can result in decrease in soil temperature by shading the ground and uptaking water from vegetation which have larger canopy and biomass by warming [89, 90]. These ecosystem responses may offset the effects of treatments by cooling the soil surface, which may result in small effects of each treatment on microbial activities and community. As ecosystem responses are complex, even if its effects are small, it is necessary to understand the effects of warming and precipitation on microbial activity and community for further understanding the mechanisms.

Conclusion

In this study, warming and precipitation treatments were performed during every growing season over 6 years in Arctic tundra soils. We hypothesized that enzyme activities and microbial community structures would be more reactive to warming and high precipitation than to control during the growing season in Arctic soils. We investigated the responses of extracellular enzyme activities and bacterial community to changes in temperature and moisture contents in tundra soils during summer in 2018 and identified factors that regulate those characteristics. Our results clearly show a significant decrease in activity of hydrolases and shift of the bacterial community structure during the growing season, and these changes were significantly affected by environmental factors such as water content, soil temperature, bacterial abundance, DOC, and inorganic nitrogen. In line with several previous reports, warming and precipitation treatments did not significantly affect enzyme activity and bacterial community structure, despite higher soil respiration of the treatment plots than the control plots, suggesting that the effect of warming on soil enzymes and microbial community is less than seasonal differences. Our results demonstrate the importance of understanding the mechanisms by which temporal variations may control extracellular enzyme activities and microbial community structure rather than the small effect of warming in Arctic tundra soils. However, given the rapid environmental changes such as rising temperatures, permafrost is currently thawing in the Arctic [91–93], which will strongly affect soil ecosystems. Therefore, future efforts should consider temporal interactions between microbial activity and environmental factors to improve predictions of the response of tundra ecosystems to climate change and the uncertainty of C pools.

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Data availability Not applicable.

Code Availability Not applicable.

Declarations

Ethics Approval Not applicable.

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Conflict of interest The authors declare no competing interests.

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