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A Study of Arctic Microbial Community Structure Response to Increased Temperature and Precipitation by Phospholipid Fatty Acid Analysis

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ABSTRACT

Climate change is more rapid in the Arctic than elsewhere in the world, and increased precipitation and warming are expected cause changes in biogeochemical processes due to altered microbial communities and activities. It is crucial to investigate microbial responses to climate change to understand changes in carbon and nitrogen dynamics. We investigated the effects of increased temperature and precipitation on microbial biomass and community structure in dry tundra using two depths of soil samples (organic and mineral layers) under four treatments (control, warming, increased precipitation, and warming with increased precipitation) during the growing season (June–September) in Cambridge Bay, Canada (69°N, 105°W). A phospholipid fatty acid (PLFA) analysis method was applied to detect active microorganisms and distinguish major functional groups (e.g., fungi and bacteria) with different roles in organic matter decomposition. The soil layers featured different biomass and community structure; ratios of fungal/bacterial and gram-positive/-negative bacteria were higher in the mineral layer, possibly connected to low substrate quality. Increased temperature and precipitation had no effect in either layer, possibly due to the relatively short treatment period (seven years) or the ecosystem type. Mostly, sampling times did not affect PLFAs in the organic layer, but June mineral soil samples showed higher contents of total PLFAs and PLFA biomarkers for bacteria and fungi than those in other months. Despite the lack of response found in this investigation, long-term monitoring of these communities should be maintained because of the slow response times of vegetation and other parameters in high-Arctic ecosystems.

Keywords: Climate change, High arctic, Microbial biomass, Phospholipid fatty acid analysis, Soil depth, Temporal changes

Introduction

At present, the warming trend in the Arctic is four times faster than the global average (IPCC, 2019). Frozen ground stores enormous amounts of carbon, which is vulnerable to enhanced microbial decomposition under warming conditions, and the released carbon dioxide and methane produced by microbial activities can contribute to atmospheric temperature increase (Schuur *et al.*, 2008; 2009). Climate change also includes changes to regional

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precipitation regimes. It is generally expected that precipitation will increase with atmospheric warming because more evapotranspiration induced by increased temperature leads to more water vapor in the air and poleward moisture transport (Bintanja, 2018; McCrystall *et al.,* 2021). Many studies have been conducted to investigate the effects of increased temperatures in the Arctic, but few have focused on the impacts of altered precipitation. Studies dealing with the combined effects of both these aspects are even more scarce, despite the significant interaction effects (Hu *et al.,* 2020; Liu *et al.,* 2022). Increased air temperature could enhance the amount of precipitation, but increased precipitation could in turn decease the soil temperature. Thus, it is critical to consider the important climate parameters of temperature and precipitation simultaneously.

Microorganisms are a very diverse organism group, and an understanding of microbial community structure is the first step in connecting ecosystem functions and microorganismal roles (Graham *et al.,* 2016). Microbial community structure has been characterized using several methods, such as culture-dependent techniques, traditional molecular fingerprinting methods, 16S rRNA gene-based sequencing techniques, and phospholipid fatty acid (PLFA) analysis (Spring *et al*., 2000; Vanwonterghem *et al.,* 2014). Traditional molecular fingerprinting methods, such as temperature gradient electrophoresis and terminal restriction fragment length polymorphisms, are time-consuming and do not provide exact information at the species level (Hugerth & Andersson, 2017). 16S-based sequencing techniques can provide details about species composition; however, it is not easy to connect the identification of species with their ecosystem functions (Vanwonterghem *et al.,* 2014). Even the relic DNA can be detected with these approaches (Carini *et al*., 2017; Lewe *et al*., 2021). In contrast, PLFA analysis can provide information on viable cell presence because it detects phospholipid fatty acids that are degraded immediately after cell death (Watzinger, 2015). This technique allows the extraction of information on microbial biomass and the main functional groups (Frostegård *et al.,* 1991; Joergensen, 2022; Joergensen & Emmerling, 2006). PLFA analysis cannot necessarily distinguish between microbial species but can separate important microbial functional groups (Joergensen, 2022; Joergensen & Wichern, 2008). However, most PLFA indicators are not specific to certain groups of microbes, and caution is therefore required when interpreting the analysis results (Joergensen, 2022).

Rapid climate change can lead to changes in plants, animals, and microorganisms in permafrost ecosystems (Bardgett *et al.,* 2008). The monitoring of microorganisms under climate change is of importance because of their functional connections to biogeochemical processes in ecosystems (Bardgett *et al.,* 2008; Van der Heijden *et al.,* 2008). Arctic microorganisms are generally adapted to cold environments and thus likely to be affected by increases in temperature (Patoine *et al.,* 2022). A large number of studies have been conducted on the response of microbial community structure to climate change. Deslippe *et al.* (2012) reported an altered composition of fungal and bacterial communities under long-term warming near Toolik Lake, Alaska. Rinnan *et al.* (2007) found that 15 years of warming decreased the relative abundance of fungal groups in the subarctic heath tundra. However, there have also been several studies that reported no response of the microbial community to warming. Yun *et al*. (2022) showed that the microbial community structure of high Arctic Canada did not vary under warming or precipitation treatments. Jung *et al.* (2020) found no differences in bacterial community structure investigated by 16S rRNA gene amplicon sequencing under warming in Northeast Greenland. No consistent conclusions have thus been drawn regarding the effects of warming on the microbial community structure in the Arctic. To help clarify this question, we therefore tested the combined effects of increased temperature and precipitation on the microbial community structure and biomass in dry tundra in the high Arctic using a PLFA approach.

Materials and Methods

Study site and climate manipulation experiment set-up

Our study was conducted in dry tundra at Cambridge Bay, Nunavut, Canada (69°07′48″N, 105°03′36″W), located on the southeast coast of Victoria Island. Between 2012 and 2018, annual mean temperature at this location ranged from −14.1 to −11.7°C, and annual total precipitation ranged from 121.6 to 196.4 mm. There were no particular directional changes in temperature or precipitation during the manipulation periods. The dominant vegetation was *Dryas integrifolia* and *Carex* spp., and the soil type at the study site was Turbic Cryosol (McLennan *et al.,* 2015). To alter the temperature and available water in the soil during the growing season (mid-June to the end of September), we designed a full factorial experiment with four treatments: control (C), increased precipitation (P), warming (W), and warming with increased precipitation (WP). The climate manipulation experiments began in 2012. Each treatment had five replicates, and the complete manipulation experiment thus consisted of 20 plots. A hexagonal open-top chamber with a diameter of 2 m was set up to increase the temperature for the warming treatments (W and WP). The C and P treatments took place in square plots of 4 m^2 (2×2 m). To simulate increased precipitation, two liters of distilled water was sprayed weekly into the plots with increased precipitation treatments (P and WP). Soil temperature in W and WP treatments was about 0.5°C higher than in the non-

warming treatments (Yun *et al.,* 2022). Soil samples were collected monthly from June to September 2018 (28th June, 14th July, 18th August, and 2nd September). At each sampling date, we sampled soil at two depths, an organic layer (mostly 0-5 cm) and a mineral layer within 10 cm depth, from three points in the plot and pooled samples to minimize spatial variation. Approximately 50 g of soil was sub-sampled for PLFA analysis after collection. All samples were shipped in a frozen state and stored at −20°C in the laboratory of South Korea until analysis. The subsamples for the PLFA analysis were freeze-dried before extraction.

PLFA analysis

Phospholipid fatty acids (PLFAs) were extracted using a modified version of the method described by Bligh and Dyer (1959) and Quideau *et al.* (2016). Half a milliliter of C19:0 fatty acid (0.1 mg mL^{-1}) was added to soil samples (0.5 g dry wt. in organic soil and 3 g dry wt. in mineral soil to acquire an adequate amount of fatty acids for analysis) as an internal standard before extraction. The Bligh and Dyer (1959) extractant (2 mL citrate buffer, 2.5 mL chloroform, and 5 mL methanol) was added to the soil sample and placed in a shaker for 2 hours after vortexing for 30 seconds. After centrifuging the sample at 226×*g* for 15 minutes, the supernatant was transferred to a 50-mL glass tube using a Pasteur pipette. This extraction step was then repeated. Five milliliters of chloroform and 5 mL of citrate buffer were added to a 50-mL glass tube containing the supernatant and placed overnight in a refrigerator in the dark after vortexing for 30 seconds. The chloroform phase was collected and evaporated under compressed nitrogen gas at room temperature. Samples were re-dissolved by adding chloroform (0.5 mL) and transferred to preconditioned solid-phase extraction columns (silica, 500 mg, 6 mL; Supelco, Bellefonte). Neutral lipids and glycolipids were discarded by sequentially passing 5 mL of chloroform and 5 mL of acetone through the SPE column, and the PLFA fractions were eluted by adding 5 mL of methanol. The fractions were evaporated under compressed nitrogen gas at room temperature, then chloroform (0.5 mL), methanol (0.5 mL), and methanolic KOH (1 mL) were added for methylation. The samples were placed in a water bath at 37°C for 30 minutes. Two milliliters of hexane and 0.2 mL of 1.0 M acetic acid was added to each sample and swirled for mixing, then 2 mL of HPLC-grade water was added. After centrifugation at 226×*g* for 2 minutes, the hexane phase (upper layer) was transferred to a 10 mL glass vial. Subsequently, 2 mL of hexane was added to the water phase (lower layer), and the previous process was repeated. The hexane phase was collected in 10 mL glass vials and evaporated under compressed nitrogen gas at room temperature. Extracted PLFA methyl esters were re-dissolved in 150 μL of hexane and transferred into a 2 mL gas chromatography vial, then stored at −80°C until analysis.

PLFA methyl ester was separated through a gas chromatography setup (GC) (7890B; Agilent, Santa Clara) equipped with a HP-ULTRA 2 capillary column (25 m×200 μm internal diameter ×0.33 μm film thickness) and a flame ionization detector (FID). Each peak detected by GC-FID was identified using the Sherlock Microbial Identification System (MIDI Inc.). Concentration of each PLFA was calculated by comparing peak areas of internal standard (C19:0 fatty acid) and expressed as nM g^{-1} of dry soil weight. The sum of all PLFAs was used as a proxy for microbial biomass (Fierer *et al.,* 2003). Monoenoic PLFAs and cyclopropane PLFAs, such as 14:1ω5c, 16:1ω9c, cy17:0, and cy19:0, were chosen to represent gram-negative bacteria (Quideau *et al.,* 2016). Branched saturated PLFAs such as i14:0, i15:0, i17:0, and a15:0 were used to represent gram-positive bacteria (Quideau *et al*., 2016). PLFA 18:1ω9c and 18:2ω6,9c were chosen as fungal biomarkers of fungi (Quideau *et al*., 2016). All PLFA biomarkers (Quideau *et al*., 2016; Spring *et al*., 2000; Zelles, 1997) used in this study are listed in Table 1. The concentrations of each PLFA biomarker are shown in Supplementary Tables 1 and 2.

Table 1. PLFA markers for soil microbial communities

Group	Biomarker	Reference
Gram positive	i13:0, a13:0, i14:0, i15:0, a15:0, i16:0, i17:0, a17:0	Zelles (1997)
Gram negative	12:0 2OH, 12:0 3OH 14:1ω5c, 16:1ω11c, 16:1ω9c, 16:1ω7c, 18:1ω7c, 18:1ω5c, 19:1ω11c, 20:1ω9c cy17:0, cy19:0	Zelles (1997); Spring *et al.* (2000)
Actinomycetes	10Me17:0, 10Me18:0, 10Me19:0	Quideau *et al.* (2016)
Sulphate-reducing bacteria	17:1ω8c	Spring *et al.* (2000)
Fungi	16:1ω5c, 18:1ω9c, 18:2ω6,9c, 18:3ω6,9,12c	Quideau *et al.* (2016)
Protozoa	20:4ω6,9,12,15c	Quideau *et al.* (2016)

PLFA, phospholipid fatty acid.

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Table 2. Results of three-way ANOVA with factors of season and changes in temperature and precipitation for the total PLFA concentration, sum of fatty acids concentrations indicating different microbial groups, and the ratios between microbial groups

Organic layer (nM g^{-1} soil)	Month				Treatment			
	June	July	August	September	C	P	W	WP
Organic layer								
Total PLFA	1,878.6 (410.7)	1,783.5 (594.9)	1,758.8 (395.3)	1,922.5 (499.5)	1,857.2 (460.1)	1,940.9 (490.9)	1,826.2 (527.2)	1,719 (436.0)
Gram+	191.9 (38.9)	184.8 (63.4)	177.4 (40.2)	199.2 (46.6)	191.6 (48.5)	198.2 (51.9)	186.1 (49.4)	177.3 (43.2)
Gram-	791.0 (169.3)	766.7 (242.6)	749.4 (159.1)	780.6 (206.2)	779.3 (192.7)	810.3 (206.9)	756.1 (211.0)	741.9 (170.7)
Actinomycetes	25.1 (5.8)	23.2 (9.7)	22.5 (6.0)	24.8 (6.1)	24.9 (8.2)	24.2 (6.1)	24.3 (8.4)	22.2 (5.2)
Fungi	383.3 (123.8)	343.8 (125.5)	355.5 (87.8)	400.1 (130.0)	371.0 (96.4)	399.9 (101.1)	389.3 (156.2)	322.6 (100.8)
Protozoa	5.8 (7.0)	5.5 (7.3)	7.4 (10.8)	11.7 (6.4)	8.2 (10.2)	7.6 (9.0)	6.8 (7.3)	7.8 (6.8)
SRB	19.4 (5.5)	18.4 (7.8)	18.0 (4.8)	20.4 (5.2)	19.0 (5.7)	20.2 (6.2)	18.8 (5.8)	18.2 (6.2)
F/B	0.38 (0.08)	0.35 (0.06)	0.38 (0.05)	0.4 (0.04)	0.38 (0.05)^{AB}	0.39 (0.05)^{A}	0.4 (0.08)^{A}	0.34 (0.04)^{B}
G+/G-	0.24 (0.01)^{b}	0.24 (0.02)^{b}	0.24 (0.02)^{b}	0.26 (0.01)^{a}	0.25 (0.02)	0.24 (0.02)	0.25 (0.02)	0.24 (0.02)
Mineral layer								
Total PLFA	233.7 (85.0)^{a}	141.3 (79.5)	137.9 (88.5)	127.8 (59.4)	144.8 (83.6)	158.8 (83.6)	173.9 (97.2)	163.3 (93.4)
Gram+	25.2 (9.5)^{a}	16.3 (9.1)	15.5 (9.5)	15.0 (6.5)	16.4 (9.4)	18.4 (9.3)	18.6 (10.0)	18.6 (10.1)
Gram-	81.1 (36.6)^{a}	51.6 (35.7)	48.9 (36.5)	46.3 (25.4)	51.4 (33.3)	57.0 (35.2)	60.5 (37.6)	58.9 (40.1)
Actinomycetes	2.5 (1.4)	1.6 (1.6)	1.4 (1.5)	1.7 (0.8)	1.6 (1.2)	1.9 (1.2)	1.8 (1.7)	1.8 (1.5)
Fungi	64.3 (23.1)^{a}	35.5 (12.6)	38.5 (20.2)	29.4 (11.5)	38.3 (19.6)	39.6 (20.1)	48.8 (26.4)	41.2 (20.6)
Protozoa	0.3 (0.5)	0.2 (0.5)	0.2 (0.6)	0.2 (0.4)	0.1 (0.3)	0.2 (0.3)	0.5 (0.8)	0.1 (0.4)
SRB	2.2 (1.1)^{a}	1.4 (1.0)^{ab}	1.5 (1.0)^{ab}	1.5 (0.7)^{ab}	1.5 (0.9)	1.7 (1.0)	1.8 (1.1)	1.7 (1.0)
F/B	0.65 (0.26)	0.63 (0.31)	0.7 (0.29)	0.51 (0.12)	0.62 (0.2)	0.61 (0.34)	0.67 (0.27)	0.58 (0.2)
G+/G-	0.32 (0.04)	0.33 (0.04)	0.34 (0.04)	0.34 (0.05)	0.33 (0.05)	0.34 (0.04)	0.33 (0.05)	0.34 (0.04)

Numbers in each column are averages, and numbers in parentheses are standard deviations. Different capital and small letters denote significant post-hoc differences among treatments and seasons, respectively. Values not marked in this way were not significantly different among seasons or treatments. PLFA, phospholipid fatty acid; C, control; P, increased precipitation; W, warming; WP, warming and increased precipitation; SRB, sulfur-reducing bacteria; F/B, fungal/bacterial ratio; G+/G-, gram-positive bacteria/gram-negative bacteria ratio.

Statistical analyses

All measured variables (dependent variables: total PLFA concentration, sum of fatty acid concentrations indicating different microbial groups, and ratios between microbial groups) differed considerably between the two soil sampling depths (organic and mineral soil layers). The differences between treatments and seasons (explanatory variables: season, temperature change, and precipitation change) were therefore analyzed separately for each layer in a three-way ANOVA. Additionally, there was no interaction effect of seasons with treatments, and therefore the interaction terms with seasons were ignored in the three-way ANOVA. When there was a significant effect ($P<0.05$) in the ANOVA, a post-hoc analysis (Tukey's HSD) was conducted. A principal component analysis (PCA) was conducted to describe differences in total PLFA concentrations by treatment effects, season changes, and depth effects. In this analysis, the substantial variation in PLFAs between the two sampling layers was compensated for by dividing PLFA concentrations by the soil organic matter content. ANOVA and PCA were performed using JMP (version 16.2.0; SAS institute Inc.) and R Statistical Software (version 4.0.2; R Foundation for Statistical Computing).

Results

Total PLFA content in the organic layer was eight times higher than in the mineral layer (Table 2). While the abundance of bacterial groups was 8-10 fold higher in the organic layer, the abundance of fungal groups was only six times higher and thus below the PLFA ratio. In contrast, the abundance of the protozoan group was approximately 20 times higher in the organic layer. Microbial abundance in both soil layers followed the order gram-negative bacteria >fungi >gram-positive bacteria.

Except for the fungal/bacterial (F/B) ratio in the organic layer, there were no statistically significant differences among treatments (Table 2). The interaction effect was significant for the F/B ratio; the F/B ratio in the P and W treatments was significantly higher than that in the WP treatment. Thus, the combination of warming and increased precipitation led to a higher F/B ratio than either factor on its own.

The total PLFAs were highest in September at 1,922.5 nM g^{-1} soil in the organic layer, but there were no significant differences among months (Table 2). In contrast, the total PLFAs in the mineral layer in June were significantly higher than those at any other sampling time. The major microbial groups (gram-negative bacteria, gram-positive bacteria, and fungi) followed the same trend as the total PLFAs in the mineral layer. However, the F/B and G+/G- ratios did not vary with sampling time.

PCA was performed using 67 of the identified PLFAs from all samples in both layers. The PC1 and PC2 axes accounted for 36.46% and 10.33% of total variance, respectively. The organic and mineral layers are divided along the PC2 axis in the score plot (Fig. 1A). The samples from the organic layer were mostly positioned on the upper side of PC2, whereas those from the mineral layer were

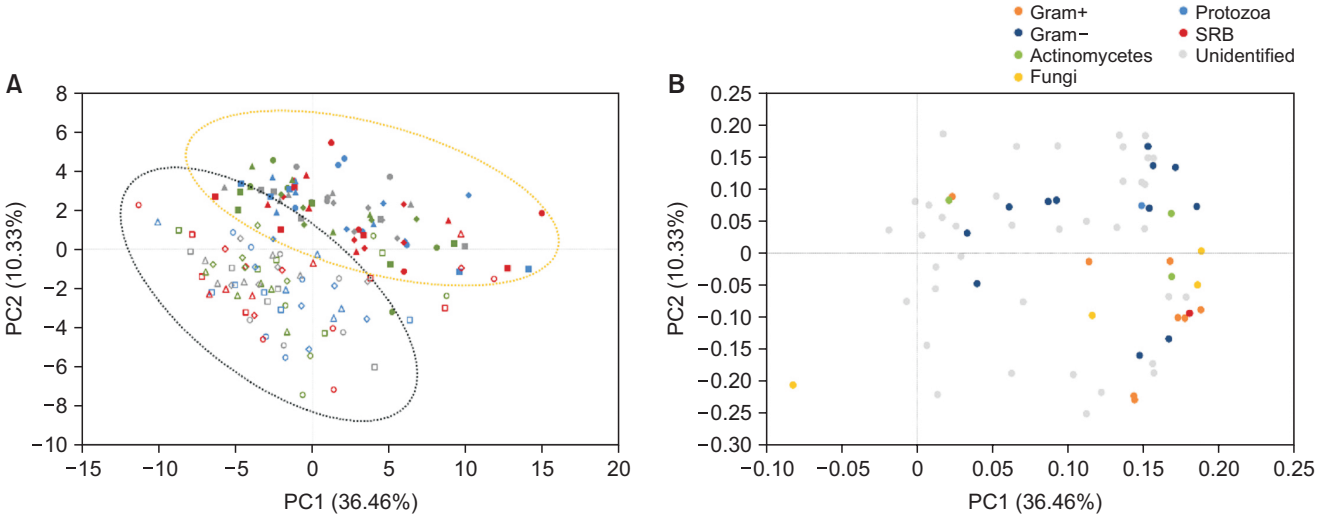

Fig. 1. Results of principal component analysis using the converted values based on the soil organic matter content of all detected PLFAs in both soil layers of all sampling periods. Each symbol in the score plot (A) represents a soil sample. Filled: organic layer (grouped by yellow ellipse), empty: mineral layer (grouped by black ellipse). Different colors denote the treatments: gray for control, blue for increased precipitation, red for warming, and green for both warming and increased precipitation. Symbols in the loading plot (B) represent detected phospholipid fatty acids from all samples. Different colors denote PLFAs assigned to different microbial groups. PLFA, phospholipid fatty acid; SRB, sulfur-reducing bacteria.

located on the lower side of PC2. No effects of treatment or seasonal changes were evident in the PCA results. The loading plot illustrates all PLFAs (Fig. 1B) responsible for the arrangement of the sample score plot. The biomarker PLFAs of gram-negative bacteria were located on the upper side of the loading plot, while those of gram-positive bacteria are plotted on the lower side.

Discussion

In this study, we examined the effects of increased temperature and precipitation on microbial biomass and community structure in the soil organic and mineral layers of dry tundra in the high Arctic using the PLFA method. Overall, the microbial biomass and community composition in both soil layers were not affected by climate manipulation during the growing seasons. However, microbial biomass and composition varied with the soil layer: a higher PLFA value, lower F/B ratio, and differences in the relative abundance of each microbial group were observed in the organic layer compared to the mineral layer.

Treatment effects

There were no significant effects of increased temperature or precipitation on the microbial biomass or community structure in either soil layer during the growing season (Table 2). In line with our results, Yun *et al.* (2022) did not find any significant effects of treatments on the bacterial community structure in the same soil samples analyzed by 16S rRNA gene sequencing. We therefore conclude that microbial community structure and biomass, as measured by fatty acid analysis and DNA sequencing, were not influenced by seven years of warming or increased precipitation in the dry tundra of Cambridge Bay, Canada. No particular responses to increased precipitation in this study were consistent with the lack of irrigation effects on the microbial community in High Arctic patterned ground soil (Newsham *et al.,* 2022). This is in agreement with several reports showing an absence of responses of microbial biomass or community structure to warming in the Arctic. In both *Cassiope* and *Salix* vegetation, Jung *et al.* (2020) found no effect on bacterial community composition after in Northeast Greenland after 8-9 years of warming. Zhang *et al.* (2020) observed warming effects on soil microbial communities in alpine *Kobresia* meadows, but not in alpine steppe meadows. A case of long-term (15 years) warming effects on microbial biomass and community has been reported, but there were no apparent effects after 5 years of warming at the same study site (Jonasson *et al.,* 1999; Rinnan *et al.,* 2007). This indicates that the duration of the experimental period and the specific ecosystem type could influence responses to treatments. The response of vegetation to the treatments and their subsequent effects on the quantity and quality of soil inputs may constitute the main pathways influencing the microbial community. Streit *et al.* (2014) suggested that the negligible warming effects on microbial biomass and community composition measured by PLFA in their experiments were the result of warming with insignificant impacts on the responses of plants and litter-derived carbon in alpine soils (Dawes *et al.,* 2011; Hagedorn *et al.,* 2013).

Seasonal changes

There were no significant seasonal changes in microbial biomass or microbial community structure in the organic layer (Table 2). However, in the mineral layer, the total PLFA content and the content of PLFA markers for gram-positive and gram-negative bacteria, fungi, and sulfur-reducing bacteria were significantly higher in June than in the other months (Table 2). This seasonal increase in PLFA abundance might be explained by the change from frozen to thawed soil at this time. A study on seasonal changes in microbial community structure by Buckeridge *et al.* (2013) showed a strong shift in the microbial community during the transition period from frozen to thawed soil in mesic tundra. In particular, higher levels of PLFA fungal biomarkers and F/B ratios were present in winter (Buckeridge *et al*., 2013). A higher content of fungal biomarkers in June thus accords with previous findings of studies of higher tundra fungal biomass in winter (Buckeridge *et al.,* 2013; Schadt *et al.,* 2003). These authors also reported relatively low variability in the microbial community from spring to fall despite dynamic changes in vegetation productivity. Wallenstein *et al.* (2007) also showed lack of variation in fungal and bacterial communities in Alaskan tussock tundra in late fall and early spring, using a DNA sequencing approach. Our results showed no significant variation in the microbial community in the organic layer during the sampling periods; most samples in the mineral layer similarly were consistent with previous results and supported the absence of strong changes in the microbial community in the soil surface during a single growing season. This facet of tundra microbial community ecology requires further detailed study to generate firm conclusions on whether there are any other specific microbial groups or species that are affected by plant growth, competition, soil environment, or similar factors.

Depth effects

Microbial biomass in the mineral layer was significantly lower than that in the organic layer for all treatments and months. The microbial communities in these two layers were distinctly separate from the calibrated PLFA values based on the organic matter content (Fig. 1). Substrate availability and soil microclimates vary significantly with soil depth, affecting the vertical distribution of microbes (Kim *et al.,* 2014; Ren *et al.,* 2022; Tripathi *et al.,* 2019).

Overall, the F/B ratio was higher in the mineral layer than in the organic layer (Table 2). Substrate quality affects the relative dominance of microbial groups, and recalcitrant substrates generally provide benefits to fungal groups over bacterial groups (Wardle *et al.,* 2004). Lower substrate quality would therefore have led to the dominance of fungi over bacteria in the mineral layer compared with the organic layer. In addition, the ratio of gram-positive to gram-negative bacteria was higher in the mineral layer than in the organic layer, which is consistent with previous studies (Fierer *et al.,* 2003; Li *et al.,* 2017). Gram-positive bacteria are better able to utilize recalcitrant substrates than are gram-negative bacteria, and are thus better able to adapt to unfavorable environments. In contrast, gram-negative bacteria are generally dominant in variants of upper soil layers, such as plant rhizospheres and locations with high amounts of organic substrates, owing to their preference for plant-derived organic matter (Fierer *et al.,* 2003). The higher ratio of gram-positive to gram-negative bacteria in the mineral layer than in the organic layer in our study is therefore in agreement with previous studies (Blume *et al.,* 2002; Fierer *et al.,* 2003).

At both depths, the microbial abundance showed an order of gram-negative bacteria >fungi >gram-positive bacteria. The dominance of gram-negative bacteria might be associated with the alkaline soil (pH 7.4-7.8) resulting from the parent materials in this study site (Jeong *et al.,* 2022). Soil pH is one of the most influential factors in determining microbial communities and also substantially drives the results of PLFA analyses (Pietri & Brookes, 2009; Rousk *et al.,* 2010). Grayston *et al.* (2004) showed that gram-negative bacteria were strongly connected to the higher pH in soil; in contrast, gram-positive bacteria are known to survive in acidic environments due to specific physiological mechanisms (Cotter & Hill, 2003). Additionally, the aerated conditions in the upper layer may favor gram-negative over gram-positive bacteria due to the latter group's association with wetlands and deeper soils, whereas gram-negative bacteria are found in well-aerated conditions (Bossio *et al.,* 2006; Li *et al.,* 2017).

Limitations and further research

In this study, we did not detect any changes in the microbial community of high Arctic dry tundra in response to temperature and precipitation manipulations. Because some studies have reported microbial responses to similar interventions after a long period of time, coupled with a lack of responses in the short term (Jonasson *et al.,* 1999; Rinnan *et al.,* 2007), it remains necessary to monitor the microbial community structure in the longer term and further measure vegetation responses and microbial activities to warming and increased precipitation. Such a longer observation will better reflect the eventual microbial response to climate change in the Arctic region.

It should also be noted that storage methods can affect phospholipid analysis results. Immediate extraction from field-moist soil or lyophilization has been suggested as the best method for PLFA analysis (Lee *et al.,* 2007; Veum *et al.,* 2019). We did our best to continuously keep soil samples in a frozen state until analysis to preserve PLFA contents. However, no deep freezer was available at the experimental site to store samples at −80°C or to conduct freeze drying on site, and it was not possible to constantly maintain sample temperature at −20°C during shipping. Sample storage duration in the −20°C freezer also varied for different sampling times depending on how customs clearance for prohibited imports progressed. It is thus possible that fatty acid contents in samples underwent alteration during transport. This might not lead to any significant differences among treatments, but still represents an unavoidable limitation in dealing with sampling and soil imports from the Arctic. During analysis, we assumed that these issues connected with sample transportation were equally applicable to all samples under the different treatments in each sampling period. The Canadian High Arctic Research Station is now fully functional, and soil pretreatment issues at Cambridge Bay can be resolved in situ in the future. A well-equipped research infrastructure is important for accurately assessing processes occurring in the Arctic field. Soil storage in RNAlater which is a stabilizing solution has been suggested as an alternative storage method for PLFA available to researchers working in Arctic and alpine regions (Schnecker *et al.,* 2012).

Conflict of Interest

The authors declare that they have no competing interests.

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