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Microbial abundances and carbon use under ambient temperature or experimental warming in a southern boreal peatland

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Abstract Organic peat soils occupy relatively little of the global land surface area but store vast amounts of soil carbon in northern latitudes where climate is warming at a rapid pace. Warming may result in strong positive feedbacks of carbon loss and global climate change driven by microbial processes if warming alters the balance between primary productivity and decomposition. To elucidate effects of warming on the microbial communities mediating peat carbon dynamics, we explored the abundance of broad microbial groups and their source of carbon (i.e. old carbon versus more recently fixed photosynthate) using microbial lipid analysis (δ^{13}C PLFA) of peat samples under ambient temperatures and before/after initiation of experimental peat warming (+ 2.25, + 4.5, + 6.75, and + 9 °C). This analysis occurred over a profile to 2 m depth in an undrained, ombrotrophic peat bog in northern Minnesota. We found that the total microbial biomass and individual indicator lipid abundances were stratified by depth and strongly correlated to temperature under ambient conditions. However, under experimental warming, statistically significant effects of temperature on the microbial community were sporadic and inconsistent. For example, 3 months after experimental warming the relative abundance of Gram-negative bacterial indicators across depth combined and > 50 cm depth and Gram-positive bacterial indicators at 20–50 cm depth showed significant positive relationships to temperature. At that same timepoint, however, the relative abundance of Actinobacterial indicators across depth showed a significant negative relationship to temperature. After 10 months of experimental warming, the relative abundance of fungal biomarkers was positively related to temperature in all depths combined, and the absolute abundance of anaerobic bacteria declined with increasing temperature in the 20–50 cm depth interval. The lack of observed response in the broader microbial community may suggest that at least initially, microbial community structure with peat depth in these peatlands is driven more by bulk density and soil water content than temperature. Alternatively, the lack of broad microbial community response may simply represent a lag period, with more change to come in the future. The long-term

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trajectory of microbial response to warming in this ecosystem then could either be direct, after this initial lag time, or indirect through other physical or biogeochemical changes in the peat profile. These initial results provide an important baseline against which to measure long-term microbial community and carbon-cycling responses to warming and elevated CO_2.

Keywords $\delta^{13}C$ PLFA · Boreal peatland · Microbial communities · Climate change · Carbon

Introduction

Arctic and boreal ecosystems play a major role in responses or feedbacks to climate change. Northern peatlands cover just 3% of the earth's land area but store approximately 30% of earth's total stored soil carbon (Gorham 1991; Yu 2012). Northern latitudes are also among the most quickly warming regions on earth, warming at up to twice the rate of other terrestrial land areas (Hartmann et al. 2013; Johnson and Stefan 2006), and northern boreal ecosystems, including carbon-rich peatlands, are projected to continue warming even under low emissions scenarios (Hayhoe et al. 2018; Marvel et al. 2023). Concurrent climate-change-associated changes in temperature, hydrology, vegetation, and microbial communities may alter decomposition and carbon cycling processes, shifting peatlands from carbon sinks to sources. This subsequent release of carbon dioxide, methane, and dissolved organic matter may create a positive feedback to climate change, meaning that warming that increased the release of greenhouse gases then leads to further warming (Allison and Treseder 2011; Ciais et al. 2013). However, there is a need to better understand responses of microbial communities and their carbon usage to warming, as microbial communities drive carbon cycling and are a central factor needed in the assessment of future peatland carbon cycling processes (i.e. Kluber et al. 2020).

Peatland microbial responses to warming occur within the context of how northern peatland physical and chemical properties respond to climate change. Belowground responses depend largely on water table dynamics, which are in turn affected by changing precipitation patterns, including snow versus rain, and associated changes in plant communities, as well as warming and consequent changes in plant evapotranspiration rates (Allison and Treseder 2011; Laiho 2006; Peltoniemi et al. 2009). For example, peatland warming paired with increased precipitation could lead to greater anaerobic decomposition of subsurface peat and increased carbon dioxide and methane emission. On the other hand, peatland warming with unchanged precipitation may result in longer periods of lowered water tables and drier conditions due to the higher plant respiration and evapotranspiration rates. Greater rates of decomposition favored by warm aerobic conditions may be offset by reduced nutrient availability or in extreme cases, microbial drought stress. Lower water table conditions could also lead to more ephemeral pulsed nutrient cycling or decomposition events when precipitation does occur (Allison and Treseder 2011).

Soil microbes may respond directly to warming through adaptations at the individual level, such as using internal energy stores or shifting enzyme production or through mutations, or at the community level with species shift or turnover. Warming may also affect microbial communities indirectly through changes in soil moisture status, vegetation, or availability of carbon pools (Bradford 2013; Manlick et al. 2024). Soil microbial communities have shown varying responses to warming and other environmental conditions expected under future climate change scenarios. For example, warming has led to shifts in soil microbial community composition in varying ecosystems (Cregger et al. 2014; Peltoniemi et al. 2009). Despite varying observed microbial community responses, climate change will likely alter fungal and bacterial communities in peatlands in some respect, with subsequent consequences for decomposition and carbon substrates, and further alteration of forest plant species composition (Andersen et al. 2013; Fernandez et al. 2017; Fernandez and Kennedy 2016).

Warming-associated changes in microbial community structure may then be associated with changes in carbon mineralization rates, carbon usage patterns, and carbon use efficiency (CUE), with implications for peatland stability (Hopple et al. 2020). Warming in northern latitudes can increase soil respiration (Lin et al. 1999; Niinistö et al. 2004), but these results can be varied or inconsistent, suggesting that site-specific conditions are key to modulating the response (Juszczak et al. 2013; Preston et al. 2012). Warming can also

influence decomposition of organic matter and the availability of different soil carbon pools and shift the microbial use of carbon toward either older or newer carbon sources (Andrews et al. 2000; Dorrepaal et al. 2009; Ofiti et al. 2022). Use of older versus newer carbon pools is important for peatland carbon fate, because much of the carbon in peatlands is found primarily in the relatively old peat, which is more stable and recalcitrant and represents thousands of years of carbon accumulation (Hobbie et al. 2017; Tfaily et al. 2014). Shifting of microbial carbon use towards older carbon could release massive amounts of carbon and signal decreasing stability of peatland carbon stores. However, the fate of peatland carbon stores is also affected by microbial biomass turnover and reallocation of carbon towards growth, which may be temperature-dependent (Asemaninejad et al. 2017; Ofiti et al. 2022).

Peatland microbial responses to climate change also occur within the strong vertical stratification of these ecosystems. Northern boreal peatlands form because decomposition of the mostly acidic dominant plant species is restricted by environmental conditions such as low mean temperature, saturation, and soil anoxia (Dorrepaal et al. 2005). As they form, peatlands become strongly vertically stratified, and along a typical peat depth profile, the peat becomes older and more decomposed with depth, resulting in higher bulk densities and greater enrichment in $\delta^{13}C$ (Boelter and Verry 1977; Esmeijer-Liu et al. 2012; Iversen et al. 2014). Peatlands also show vertical stratification of microbial functional groups. For example, ectomycorrhizal fungi (EMF) and ericoid mycorrhizal fungi have prolific growth in the surface peat and play a strong role in plant nutrient acquisition (Andersen et al. 2013; Smith and Read 2008; Thormann 2006). Bacteria also play an important role in soil decomposition and can predominate in the deeper anaerobic layers of the peat profile (Mandic-Mulec et al. 2014). Deeper in the peat profile, anaerobic methane and sulfur cycling bacterial communities dominate carbon cycling processes (Lin et al. 2014b). Because of this change in community structure and function with depth, measuring the responses of multiple microbial groups is important for understanding future peatland carbon dynamics.

In this study, we used $\delta^{13}C$ PLFA analysis to investigate the total microbial biomass, the abundance of key microbial groups, and microbial carbon use across peatland depth profiles in response to both ambient fluctuation and manipulation of peat temperatures with the aim of providing information about how deep peat warming may influence organism and ecosystem stability as well as changes in biogeochemical cycling of carbon. We obtained $\delta^{13}C$ PLFA profiles as part of the Spruce and Peatland Responses Under Changing Environments (SPRUCE; https://mnspruce.ornl.gov/) experiment in a southern boreal bog in northern Minnesota immediately before and at 3 months (when soil warming targets were achieved) and 10 months after the initiation of direct peat warming.

Our objectives in this study were to (1) Determine how microbial PLFA indicators and the $\delta^{13}C$ signatures of those indicators respond to natural temperature variation or to experimental warming treatments, (2) determine whether $\delta^{13}C$ PLFA profiles relationships with temperature are different at different peat depths (3) understand the relationships between $\delta^{13}C$ PLFA profiles and physical soil characteristics. We predicted that microbial community size (total lipid abundance), structure (lipid indicator absolute and relative abundance), and carbon use ($\delta^{13}C$ PLFA profiles) would be related to temperature under both ambient conditions and experimental warming treatments. We predicted that we would see even stronger relationships, corresponding to responsiveness or sensitivity, to temperature under experimental warming.

Materials and methods

SPRUCE site characteristics and experimental design

This research was performed in collaboration with the SPRUCE project, an ecosystem warming experiment that is sponsored by the Department of Energy. The experiment is cooperatively operated by the Oak Ridge National Laboratory and the USDA Forest Service. The SPRUCE site is located in the S1 bog in the USDA Forest Service Marcell Experimental Forest near Grand Rapids, Minnesota, USA (Sebestyen et al. 2011). The ombrotrophic bog formed after the Wisconsin glaciation and drains naturally to a stream and the surrounding groundwater aquifer in a 50-m deep outwash sand (similar to the nearby S2 bog; Verry and Janssens 2011). The S1 bog vegetation is dominated by black spruce (*Picea mariana*), tamarack

(*Larix laricina*) *Sphagnum* mosses (Norby et al. 2019; Walker et al. 2017), and ericaceous shrubs. The site is located along the southern boundary of boreal peatlands. Air temperatures on the Experimental Forest range from − 45 to 38 °C, with an average temperature of 18.9 °C in July and − 15.1 °C in January (Sebestyen et al. 2011). Average precipitation is 79 cm with more occurring in the summer months than in winter, and snowfall is about one third of the precipitation (Sebestyen et al. 2021).

The peat is a Greenwood series soil, which is a Typic Haplohemist (http://www.websoilsurvey.nrcs.usda.gov). In this type of peatland, physical properties are vertically stratified relative to the local surface of hollows in the hummock/hollow microtopography (Verry 1984). Specific physical properties of the SPRUCE site other than soil moisture content were characterized before the coring for this PLFA analysis (see references below). Peat depth is highly variable across the site, with an average depth of 2.5 m (Parsekian et al. 2012). The bulk density of the surface peat (0–20 cm) was characterized in 2012, and varies from 0.01 to 0.05 g cm^{-3}, reaching a maximum between approximately 20–100 cm where values range from 0.03 to 0.27 g cm^{-3} and decreasing slightly below 100 cm before stabilizing, with values between 0.12-0.43 g cm^{-3}. Soil pH steadily increases with depth, varying between 3.5 and 5.2 (Iversen et al. 2014). The water table fluctuates within the top 30 cm of the peatland during most years (Sebestyen et al. 2011), and this zone corresponds to fluctuating redox conditions, high rates of decomposition, and active biogeochemical cycling (Lin et al. 2014a, 2014b; Tfaily et al. 2014). The bulk peat $\delta^{13}C$ ranges from − 25 to − 30, and increases with depth, indicating that lower depths in the peat profile contain older stored carbon and surface depths contain newer, recently photosynthesized carbon (Hobbie et al. 2017). Peat bulk density, pH, and $\delta^{13}C$ are summarized in Table 1.

The warming treatments during the Deep Peat Heating (DPH) phase of the SPRUCE study (Jul. 2014 to Jun 2015) ranged from ambient to + 9 °C relative to an unheated reference plot (+ 0, + 2.25, + 4.5, + 6.75, + 9 °C with two plots each ambient or warmed level, 10 total plots; Hanson et al. 2017; Wilson et al. 2016). This study used a regression-based design, which favors additional treatment levels over replicates to characterize the response of various ecological variables to temperature. A regression-based approach generally provides more power than an ANOVA-based approach and produces quantitative response data that can be integrated into ecological models (Cottingham et al. 2005). The regression-based design of SPRUCE has been used to examine temperature responses of *Sphagnum* and other vegetation (Malhotra et al. 2020; McPartland et al. 2019, 2020; Norby et al. 2019; Smith et al. 2018), carbon balance (Wilson et al. 2016), methane and CO_2 emissions (Gill et al. 2017; Hanson et al. 2016), and decomposition (Fernandez et al. 2019). Deep Peat Heating was achieved using an array of vertical, low-wattage-heating elements (100 W; pipe thread core heaters, Indeeco, St Louis, MO) installed throughout each plot. Target warming levels were achieved at 2–3 m depth, with heat radiating upward through the profile. The experiment was established with 2 chambers per warming level: one to maintain ambient CO_2 levels and one to create CO_2 levels elevated approximately 500 ppm above ambient levels. However, elevated CO_2 treatments had not yet been initiated at the time of this study, resulting in n = 2 replicates at each warming level in the regression design.

Table 1 Pre-experimental bulk peat chemical and physical characteristics with an n of 16 pre-treatment core locations

Depth (cm)	Bulk density (g cm^{-3})		Bulk peat pH		Bulk peat $\delta^{13}C$ (‰)	
	Mean	SE	Mean	SE	Mean	SE
0–20	0.03	0.0025	3.73	0.0325	− 28.38	0.18
20–50	0.14	0.02	3.85	0.03	− 26.73	0.1
50–100	0.19	0.0075	3.99	0.025	− 26.3	0.0975
100–150	0.15	0.0025	4.23	0.0375	− 25.91	0.0525
150–200	0.17	0.0125	4.53	0.05	− 26.45	0.235
> 200	0.21	0.02	4.82	0.05	− 26.51	0.2575

Data summarized from data set published by Iversen et al. (2014)

Peat sampling

All SPRUCE plots were sampled on three days during June 2014, September 2014, and June 2015. Deep peat warming began just after initial sampling in June 2014 and stabilized within 0.5 °C of the target temperature by the second sampling date in September 2014 (Wilson et al. 2016). The final sampling date for this analysis was June 2015, 10 months after stabilization of DPH treatments. At each sampling date two peat cores were sampled from hollow locations (hummocks were avoided) from all 10 experimental plots to a depth of 250 cm (200 cm for June 2015). The first two depth increments (0-10 cm and 10–20 cm) were sampled using a rotary hole saw, and below 20 cm, a Russian corer (Jowsey 1966; model # 040903C, Eijkelkamp Soil & Water, Giesbeek, The Netherlands) was used (Iversen et al. 2014). Samples were bulked and homogenized in the field in increments of 10 cm until a depth of 100 cm and then in 25 cm increments from 100 cm to 250 cm. The peat was frozen on dry ice immediately upon sampling and was then stored frozen at – 80 °C until freeze drying and grinding.

Samples were then combined into the following depth increments for this analysis (with equal sample from contributing depth increments added to the combination): 0–20 cm, 20–50 cm, 50–100 cm, 100–150 cm, 150–200 cm, and 200–250 cm. These depth increments were based on previous research at this site indicating 30–50 cm as a transitional depth for peat physical properties (Verry et al. 2011), the microbial community, biogeochemical processes (Lin et al. 2014a, 2014b), and $\delta^{13}C$ isotopic signature (Hobbie et al. 2017).

Peat physical characteristics

Bulk density, gravimetric water content, and soil temperature data are all included to aid in understanding general patterns of microbial distribution with peat characteristics. For each variable, published datasets are available as part of the SPRUCE core experiment (Iversen et al. 2014; Kluber et al. 2016). Bulk density was measured in 2012 at all plots and depth increments by measuring the volume of each depth increment sample and weighing before and after drying at 70 °C for 48 hours (Iversen et al. 2014). Gravimetric soil moisture was collected from the same cores analyzed for PLFA. To determine gravimetric water content, each peat sample at each sample date was weighed, dried at 60 °C for 48 h, and weighed again dry to determine the amount of water present (Kluber et al. 2016). Water content was averaged between depths if needed for a particular core segment and is reported here as % moisture (g water g dry peat^{-1} × 100).

Soil temperature data was logged at depths of 0, 5, 10, 20, 30, 40, 50, 100, and 200 cm every 30 min using multipoint thermistor probes (W.H. Cooke & Co. Inc, Hanover, PA; Hanson et al. 2017). For this analysis, we used published temperature data (Kluber et al. 2016) from one of three probes using the probe that was closest to where peat was sampled within the experimental plot and with depth. Because the peat sampling depths do not correspond to temperature probe depths, linear interpolation was used to estimate temperature at each peat sampling interval. We then averaged temperature across the entire peat sampling interval for the 48-h periods before and the day of sampling. Where multiple core segments were pooled for PLFA, the average temperature estimate of contributing segments was used. Because logged soil temperatures only extend to 200 cm, the temperature for the 150–200 cm depth interval was used for the 200–250 cm interval for the purposes of statistical analysis.

$\delta^{13}C$ PLFA extractions and data correction

The amount of dry peat used for the PLFA extraction from each depth increment varied according to prior method testing and optimization (Blake 2017). Target weights were as follows: 2-g dry weight for the 0–20 cm depth increment, 6–8-g dry weight for increments 20–50 cm, 50–100 cm, and 100–150 cm, and 10-g dry weight for increments 150–200 cm and 200–250 cm.

The PLFA (Phospholipid Fatty Acid Analysis) method was modified from the Bligh and Dyer (1959) method both for $\delta^{13}C$PLFA analysis (Herman et al. 2011) and for extractions from peat (Blake 2017). An initial extraction was performed three times from each peat sample using a 0.9:1:2 of citrate buffer:chloroform ($CHCl_3$):methanol (MeOH). After a final additional centrifugation step to remove remnant peat particles, citrate buffer and $CHCl_3$ were added to the extract to bring the final ratio of

buffer:$CHCl_3$:MeOH to 0.9:1:1 to allow for phase separation overnight. The $CHCl_3$ layer was then evaporated and the phospholipid fraction was collected using silica column chromatography. Phospholipids were then methylated using an alkaline methylation procedure (Herman et al. 2012) and analyzed using an Agilent (Santa Clara, California) 7790A GC coupled with an Elementar (Langenselbold, Germany) IsoPrime100 IRMS system. 1,2-dinonadecanoyl-sn-glycero-3-phosphocholine (19:0 PC; Avanti Polar Lipids, Alabaster, Alabama, cat # 850367P) was used as a surrogate standard and tridecanoic acid methyl ester (C 13:0; Sigma-Aldrich, St. Louis, Missouri, cat # T062) was used as an internal standard used for converting peak areas to nmol fatty acid g^{-1} dry peat.

Signature fatty acids (biomarkers) that indicate the microbial groups included here are: Gram positive bacteria (G+ bacteria; 15:0 iso and 15:0 anteiso; Wilkinson et al. 2002), Gram negative bacteria (G− bacteria; 18:1 ω9t and 16:1 ω7c; Wilkinson et al. 2002), fungi (Fungi; 18:2 ω6,9c and 18:1 ω9c, Balser et al. 2005), Actinobacteria (Actinobacteria; 16:0 10 me and 18:0 10me; Kroppenstedt 1985), and Gram negative anaerobic bacteria (Anaerobic bacteria; 19:0 cyclo; Zelles et al. 1999). A preliminary exploration of this dataset demonstrated that this set of indicators represented community structure well (Blake 2017). When more than one lipid was used as an indicator for a given group, they were summed to determine abundance and relative abundance. Microbial biomass was determined by summing lipids equal to or less than 20:0 carbons in length, not including palmitic acid (16:0) as it is found in high levels in the *Sphagnum* dominating our study site (Koskimies and Simola 1980).

The $\delta^{13}C$ signature of fatty acids can be used to track microbial carbon use because the isotopic signature will reflect that of the carbon being taken up into microbial cells (Ofiti et al. 2022). At the SPRUCE site, the $\delta^{13}C$ is less enriched in the less decomposed surface peat than lower in the profile where peat is older and more decomposed (Hobbie et al. 2017; McFarlane et al. 2018; Table 1). In addition, recent photosynthate in the plant rhizosphere is likely even less enriched in $\delta^{13}C$ than surface peat (Hobbie et al. 2017). Raw $\delta^{13}C$ data for each lipid were first normalized using USGS40 international reference material (L-glutamic acid; $\delta^{13}C = -26.39 \pm 0.04‰$) and reported relative to Vienna Pee Dee Belemnite (VPDB). These data were then corrected for the additional carbon molecule added during the methylation of fatty acids to fatty acid methyl esters (FAME), by using the following formula: $\delta^{13}C_{PLFA} = [(C_{PLFA} + 1) \times \delta^{13}C_{FAME} - \delta^{13}C_{MeOH}]/C_{PLFA}$, where C_{PLFA} is the number of carbons in each individual lipid before methylation, $\delta^{13}C_{FAME}$ is the corrected $\delta^{13}C$ value of each measured individual lipid, and $\delta^{13}C_{MeOH}$ is the corrected $\delta^{13}C$ of the methanol used for the methylation steps determined using elemental combustion analysis (Butler et al. 2003). The methanol used during methylation had a measured $\delta^{13}C$ signature of -55.84‰. $\delta^{13}C$ of total biomass and indicator groups was calculated using a weighted average of the individual lipids that make up each group. Averages were weighted based on lipid concentrations and number of carbons using Eq. (1), where $\#C_{PLFA}$ is the number of carbon atoms in the PLFA, $concentration_{PLFA}$ is the concentration in the PLFA, and $\delta^{13}C_{PLFA}$ is the isotopic signature of the PLFA.

$$Weighted\ average \delta^{13}C_{microbial\ group} = \frac{\sum \#C_{PLFA} \times concentration_{PLFA} \times \delta^{13}C_{PLFA}}{\sum \#C_{PLFA} \times concentration_{PLFA}} \quad (1)$$

Statistics

Data were initially explored to check for outliers for subsequent statistics in RStudio version 1.2.5042 (RStudio Team 2020) using R software version 4.0.2 (R Core Team 2020). Outlier removal was performed separately on the total biomass abundance and $\delta^{13}C$ values, which were further subdivided by sampling date. For each subset of the data, the upper and lower bounds were set as the mean ± 3 standard deviations. Samples with outliers for total biomass abundance (n = 2 of 161) and $\delta^{13}C$ (n = 1 of 145) were removed from all absolute/relative abundance and $\delta^{13}C$ analyses, respectively.

In order to determine how lipid profiles and lipid $\delta^{13}C$ signatures were influenced by either natural temperature fluctuations or induced temperature treatments, the data were subdivided with separate statistics by sampling date: June 2014 (pretreatment, just before warming was initiated), September 2014 (just after DPH soil warming targets were achieved), and June 2015 (1 year after onset of peat warming, and

approximately 10 months after warming targets were achieved). Separate regression models were used for each grouping to determine significant linear relationships between microbial lipid data and other variables, including peat temperature across ambient or treated plots. These linear regressions were calculated for the dependent variables of total microbial abundance, total mean $\delta^{13}C$, bioindicator abundances, bioindicator relative abundances, and bioindicator $\delta^{13}C$ values using JMP software (SAS Institute Inc. 1989). Figures were also made with JMP software (SAS Institute Inc. 1989). Independent variables in the main model included depth and measured soil temperature. We also ran separate regression models to examine effects of peat soil moisture and bulk density, which covaried with depth (Tfaily et al. 2014), on microbial data that were pooled across sample timepoints (Table S1). To explore how microbial communities at different peat depths varied in sensitivity to changes in temperature, we tested for effects of measured soil temperature separately for three depth categories: 0–20 cm, 20–50 cm, and depths lower than 50 cm. These three depth categories created a balance between depth resolution and large enough datasets for robust regression analysis. For all statistical analyses, plot was included as a random factor to account for possible covariance with sampling location, and a significance level of 0.05 was used.

Results

Peat physical properties

Temperature, moisture content, and bulk density varied strongly with depth and somewhat over time (see data publications Hanson et al. 2015; Kluber et al. 2016). Average temperatures ranged from 12 °C at the surface to 3 °C at 200-cm depth in June 2014, from 13 °C at the surface to 6 °C at 200-cm depth in September 2014, and from 7 °C at the surface to 2 °C at 200-cm depth in June 2015. Gravimetric soil moisture contents ranged from 1330% at the surface to 555% at 200-cm depth in June 2014, from 1613% at the surface to 661% at 200-cm depth in September 2014, and from 1417% at the surface to 747% at 200-cm depth in June 2015. Bulk density, only measured in 2012, varied from 0.03 to 0.43 g cm^{-3} with lowest values generally at the surface and highest values appearing between approximately 20–100 cm.

Microbial relationships with depth and peat properties

All microbial indicators varied significantly with peat depth at least at one of the samplings in terms of abundance, relative abundance, or $\delta^{13}C$ (see data publication Gutknecht et al. 2024). Absolute abundance of total lipids and all indicators, except anaerobic bacteria in June 2015, decreased with depth across all three datasets, indicating decreased microbial biomass with depth (Fig. 1, Tables 2, 3, 4). The relative abundances of indicator lipids, which indicate shifts in community composition, also changed with depth (Fig. 2, Tables 2, 3, 4). Fungal relative abundance decreased with depth in all datasets ($P \leq 0.0011$), while actinobacterial relative abundance increased with depth in September 2014 ($P < 0.0001$) and June 2015 ($P = 0.0095$), and anaerobic bacteria relative abundance increased with depth in September 2014 ($P = 0.0267$). The isotopic signatures of lipids also showed significant relationships with depth (Tables 2, 3, 4). Total biomass, fungi, Gram-negative bacteria, and actinobacteria $\delta^{13}C$ were positively related to depth in June 2014, indicating a general ^{13}C enrichment of lipids with increasing depth. Total biomass, fungi, and actinobacteria lipid $\delta^{13}C$ were positively related to depth (total biomass $P \leq 0.0001$, fungi $P = 0.0002$, actinobacteria $P < 0.0001$), and anaerobic bacteria lipid $\delta^{13}C$ was negatively related to depth in September 2014 ($P = 0.0025$). Only total biomass and fungal indicators showed a significant relationship between $\delta^{13}C$ and depth in June 2015 (total biomass $P = 0.0228$, fungi $P = 0.0336$).

Microbial indicators also showed significant relationships to peat bulk density and soil moisture content, which covaried strongly with depth. Soil total microbial biomass and all indicator abundances significantly decreased with increasing peat bulk density ($P \leq 0.0099$) and total microbial biomass and abundance of all microbial indicators except for anaerobic bacteria indicators increased with increasing soil moisture content ($P \leq 0.0197$; Table S1). The total biomass, actinobacterial, and fungal lipid $\delta^{13}C$ signatures were positively related to bulk density ($P \leq 0.0013$). The $\delta^{13}C$ signature of total biomass and

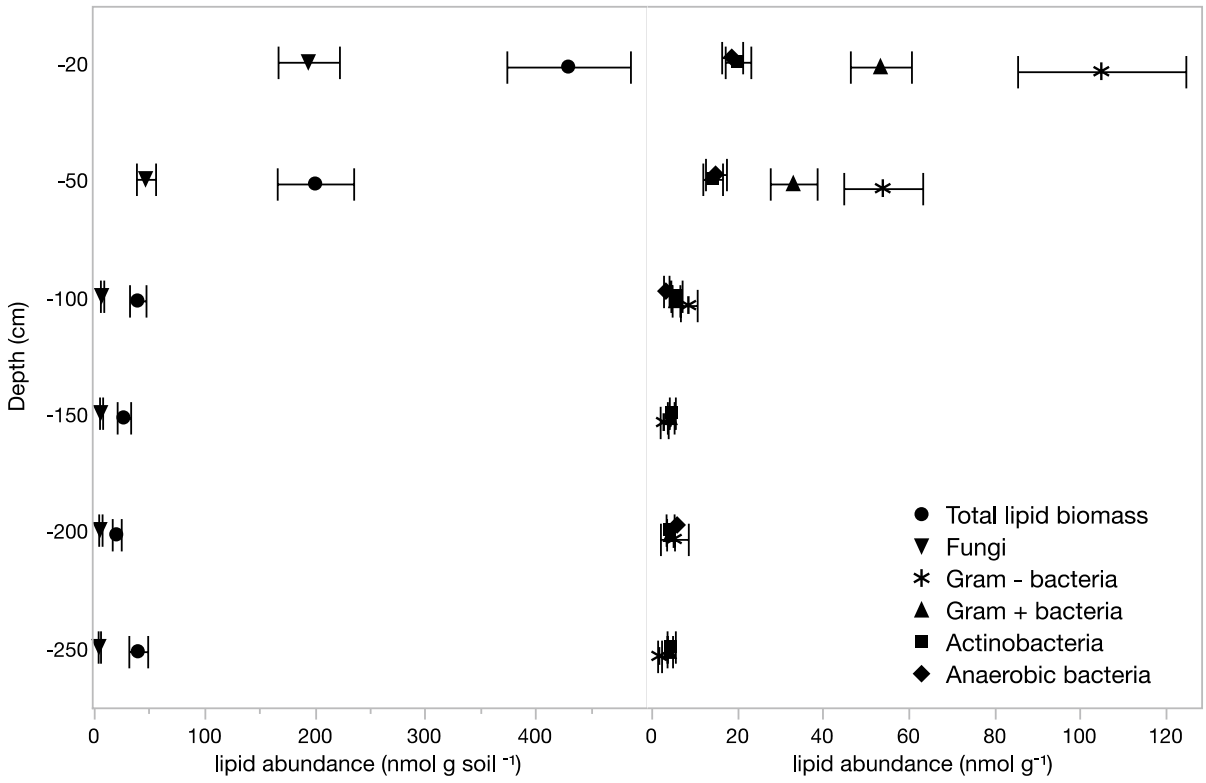

Fig. 1 Microbial lipid abundances (nmol g soil^{-1}) by depth (cm), June 2014 before warming began. The lefthand panel includes total (circles) biomass and lipid indicator biomass for fungi (inverted triangles) and the righthand panel includes Gram negative bacteria (Gram− bacteria; asterisks), Gram positive bacteria (Gram+ bacteria, triangles), Actinobacteria (squares) and anaerobic bacteria (diamonds). Data were averaged across treatment plots for this visualization, each symbol representing the mean and error bars representing one standard error from the mean

fungal lipid indicators were negatively related to percent soil moisture ($P \leq 0.0393$; Table S1, Fig. S1).

Microbial relationships to temperature

Microbial positive, negative, or nonexistent linear relationships with temperature were determined using linear regression analysis (see section "Statistics"), were analyzed at 3 separate dates: pretreatment data (June 2014 samples) were used to determine relationships between microbial lipid data and ambient temperatures; September 2014 data were used to examine acute responses immediately following equilibration of warming treatments; and June 2015 data were used to examine responses 10 months after equilibration of warming treatments.

In June 2014, total lipid biomass and the abundances of all indicator lipids except anaerobic bacteria were significantly positively related to peat temperature when all depths were combined ($P < 0.0001$; Table 2; Fig. 3). Anaerobic bacteria showed a significant negative relationship with temperature in the 0-20 cm interval ($P = 0.0159$). There were few relationships between the total biomass or indicator absolute abundances and temperature following peat warming (Tables 3, 4). Absolute lipid indicator abundances did not show any significant relationships to temperature in September 2014, and after 10 months of warming, in June 2015, only the absolute abundance of anaerobic bacterial indicators was negatively related to temperature in the 20-50 cm depth ($P = 0.0445$).

In June 2014, the relative abundances of indicators for all groups except anaerobic bacteria were also significantly related to temperature in ambient plots, with the fungi, Gram-negative, and Gram-positive bacteria demonstrating positive relationships (fungi $P < 0.0001$, Gram-negative $P = 0.0001$, Gram-positive

Table 2 Relationships between time, depth, and temperature on lipid indicators pretreatment, before the onset of warming treatments (June 2014)

Pretreatment lipid abundance																		
	Total biomass			Fungi			Gram− bacteria			Gram+ bacteria			Actino-bacteria			Anaerobic bacteria		
	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2
Temperature	**< 0.0001**	+	0.4752	**< 0.0001**	+	0.4103	**0.0001**	+	0.3724	**< 0.0001**	+	0.5127	**< 0.0001**	+	0.4138	0.2177	+	0.0788
Depth	**< 0.0001**	−	0.7515	**0.0002**	−	0.7330	**0.0073**	−	0.6991	**< 0.0001**	−	0.7587	**< 0.0001**	−	0.7071	**0.0279**	−	0.8585
Temperature: 0–20 cm	0.3455	−	0.3452	0.1774	−	0.2802	0.3301	−	0.1576	0.0944	−	0.3964	0.1025	−	0.3820	**0.0159**	−	0.7195
Temperature: 20–50 cm	0.703	−	0.0192	0.6758	−	0.0230	0.5459	−	0.0474	0.9172	−	0.0014	0.9575	+	0.0004	0.3284	−	0.1193
Temperature: > 50 cm	0.9949	−	0.0000	0.4456	+	0.0280	0.8853	+	0.0014	0.7427	+	0.0042	0.9201	+	0.0003	0.7654	+	0.0551
Pretreatment lipid relative abundance																		
	Fungi			Gram− bacteria			Gram+ bacteria			Actino-bacteria			Anaerobic bacteria					
	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2			
Temperature	**< 0.0001**	+	0.6373	**0.0001**	+	0.3662	**0.0005**	+	0.2426	**0.0274**	−	0.094	0.1163	−	0.1163			
Depth	**< 0.0001**	−	0.8959	**0.0003**	−	0.7947	**0.0149**	−	0.5348	**0.0025**	+	0.545	**< 0.0001**	+	0.9681			
Temperature: 0–20 cm	0.8646	−	0.0053	0.5059	+	0.0770	0.8773	+	0.0043	0.7854	−	0.013	0.8596	−	0.0069			
Temperature: 20–50 cm	0.5788	−	0.0402	0.3064	−	0.1299	0.0816	+	0.3314	**0.049**	+	0.402	0.2314	−	0.1733			
Temperature: > 50 cm	0.3788	+	0.0371	0.4121	+	0.0453	0.06	+	0.1294	0.0738	+	0.096	0.077	−	0.8520			
Pretreatment lipid $\delta^{13}C$																		
	Total biomass			Fungi			Gram− bacteria			Gram+ bacteria			Actino-bacteria			Anaerobic bacteria		
	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2
Temperature	**< 0.0001**	−	0.343	0.5924	−	0.0127	0.8933	−	0.0008	0.0768	−	0.0921	**0.0003**	−	0.3427	0.0525	+	0.2151
Depth	**< 0.0001**	+	0.856	0.0866	+	0.7689	0.7	+	0.4902	0.0411	+	0.6199	**< 0.0001**	+	0.9021	0.0812	−	0.8554
Temperature: 0-20 cm	0.1114	+	0.367	**0.0496**	+	0.5007	0.4551	+	0.0960	0.941	+	0.0010	0.2695	+	0.2356	0.3316	+	0.2335
Temperature: 20-50 cm	0.9098	+	0.316	0.3832	+	0.0962	0.0835	+	0.3281	0.6749	−	0.0231	0.5859	+	0.0445	0.7172	+	0.0199
Temperature: >50 cm	0.1442	+	0.050	0.9311	−	0.0017	0.7335	+	0.0323	0.0347	+	0.2644	0.4426	−	0.0398	0.2537	−	0.8494

P values in bold indicate significance at $P < 0.05$

Temperature responses were based on separate linear regressions, depth responses were categorical given incremental depth categories. ± indicates the direction of the response, with N/A representing inconclusive or insignificant trends

Table 3 Relationships between lipid indicators and temperature 3 months after onset of warming treatments at equilibrium (September 2014)

Equilibrium lipid abundance

	Total Biomass			Fungi			Gram− bacteria			Gram+ bacteria			Actino-			Anaerobic bacteria		
	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2
Temperature	0.2124	+	0.0322	0.3153	+	0.0288	0.5851	+	0.0094	0.0701	+	0.0760	0.3163	+	0.0304	0.8013	−	0.0027
Depth	**< 0.0001**	−	0.7660	**< 0.0001**	−	0.8053	0.0783	−	0.5970	**< 0.0001**	−	0.7703	**0.0244**	−	0.6469	**0.0033**	−	0.6933
Temperature: 0–20 cm	0.631	+	0.0302	0.5364	+	0.0496	0.8984	+	0.0022	0.6026	+	0.0354	0.8841	+	0.0028	0.5789	+	0.0401
Temperature: 20–50 cm	0.5275	−	0.0517	0.3718	−	0.1006	0.5137	−	0.0551	0.7361	−	0.0150	0.7169	−	0.0173	0.6343	−	0.0297
Temperature: > 50 cm	0.4965	−	0.0167	0.7754	+	0.0056	0.1915	+	0.1377	0.4404	+	0.0273	0.6803	−	0.0135	0.9259	+	0.0024

Equilibrium lipid relative abundance

	Fungi			Gram− bacteria			Gram+ bacteria			Actino-bacteria			Anaerobic bacteria		
	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2
Temperature	0.0875	+	0.0812	**0.0148**	+	0.1717	0.9664	+	0.0000	**0.005**	−	0.2148	0.5554	+	0.0147
Depth	**< 0.0001**	−	0.8692	0.0796	−	0.5959	0.0665	+	0.4817	**< 0.0001**	+	0.9012	**0.0051**	+	0.7792
Temperature: 0–20 cm	0.7832	+	0.0100	0.6785	−	0.0226	0.9428	+	0.0007	0.67	−	0.0239	0.7842	+	0.0099
Temperature: 20–50 cm	0.0717	−	0.3498	0.8371	+	0.0056	**0.0064**	+	0.6256	0.052	+	0.3939	0.2467	+	0.1633
Temperature: > 50 cm	0.064	+	0.2105	0.0356	+	0.3183	0.5162	+	0.0194	0.107	-	0.1874	0.5701	−	0.0871

Equilibrium lipid $\delta^{13}C$

	Total Biomass			Fungi			Gram− bacteria			Gram+ bacteria			Actino-bacteria			Anaerobic bacteria		
	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2
Temperature	**0.0296**	−	0.1156	0.659	+	0.0073	0.9733	+	0.0000	0.365	−	0.0257	**0.0029**	−	0.3135	0.1084	+	0.1368
Depth	0.2105	+	0.4342	**0.0022**	+	0.8362	**0.0241**	+	0.7105	0.4226	+	0.4453	**< 0.0001**	+	0.9414	**0.0013**	−	0.9352
Temperature: 0-20 cm	0.5315	+	0.0507	0.9935	+	0.0000	0.2551	+	0.1582	0.1668	+	0.2243	0.2315	+	0.1733	0.2794	+	0.1641
Temperature: 20-50 cm	0.112	+	0.2849	0.1564	+	0.2342	0.1566	+	0.2340	**0.0247**	+	0.4875	0.2634	+	0.1744	0.1842	+	0.2089
Temperature: >50 cm	0.1568	+	0.1026	0.3416	+	0.1294	0.2926	+	0.2683	0.8355	−	0.0037	0.8188	+	0.0115	NA	NA	NA

P values in bold indicate significance at $P < 0.05$

Temperature responses were based on separate linear regressions, depth responses were categorical given incremental depth categories. ± indicates the direction of the response, with N/A representing inconclusive or insignificant trends

Table 4 Relationships between lipid indicators and temperature 10 months after onset of warming treatments (June 2015).

June 2015 lipid abundance

	Total biomass			Fungi			Gram− bacteria			Gram+ bacteria			Actino-bacteria			Anaerobic bacteria		
	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2
Temperature	0.134	+	0.0539	0.2214	+	0.0616	0.4344	+	0.0268	0.8192	+	0.0022	0.6287	−	0.0113	0.2245	−	0.0855
Depth	**< 0.0001**	−	0.7009	**0.0043**	−	0.7856	0.205	−	0.5768	**0.0064**	−	0.7703	**0.0479**	−	0.7800	0.2612	−	0.7209
Temperature: 0-20 cm	0.8989	+	0.0021	0.9476	+	0.0006	0.5715	+	0.0417	0.5061	−	0.0571	0.6136	−	0.0384	0.3328	+	0.1868
Temperature: 20-50 cm	0.0735	−	0.3463	0.0721	−	0.3491	0.0876	−	0.3212	0.1204	−	0.2741	0.2147	−	0.1850	**0.0445**	−	0.4147
Temperature: > 50 cm	0.4831	+	0.0237	0.8155	+	0.0153	0.3057	−	0.3360	0.4309	+	0.1607	0.8497	−	0.0226	NA	NA	NA

June 2015 lipid relative abundance

	Fungi			Gram− bacteria			Gram+ bacteria			Actino-bacteria			Anaerobic bacteria		
	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2
Temperature	**0.0215**	+	0.2013	0.9629	+	0.0001	0.2401	−	0.0570	0.5195	−	0.0200	0.0967	−	0.1538
Depth	**0.0036**	−	0.7918	0.9657	−	0.1919	0.3043	+	0.5402	0.159	+	0.6950	**0.0002**	+	0.9742
Temperature: 0-20 cm	0.5208	+	0.0534	0.3516	+	0.1089	0.3626	−	0.1043	0.5683	−	0.0487	0.0828	−	0.4835
Temperature: 20-50 cm	0.4117	+	0.0857	0.3632	−	0.1041	0.1203	+	0.2743	0.0897	+	0.3177	0.1636	−	0.2272
Temperature: >50 cm	0.489	+	0.1265	0.5118	−	0.1551	0.4016	-	0.1802	0.994	−	0.0000	NA	NA	NA

June 2015 Lipid $\delta^{13}C$

	Total biomass			Fungi			Gram− bacteria			Gram+ bacteria			Actino-bacteria			Anaerobic bacteria		
	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2	P	±	R^2
Temperature	0.2617	−	0.0392	0.1625	−	0.1054	0.8929	+	0.0011	0.6515	−	0.0104	0.9691	−	0.0001	0.8676	−	0.0026
Depth	0.1633	+	0.5117	0.8013	+	0.4441	0.8006	−	0.4235	0.2845	+	0.6140	0.9987	+	0.1304	0.9935	+	0.1980
Temperature: 0-20 cm	0.1573	−	0.2333	0.1209	−	0.2735	0.1147	−	0.3167	0.1928	−	0.2018	0.1962	−	0.3077	0.0973	−	0.5376
Temperature: 20-50 cm	0.6638	+	0.0248	0.6575	+	0.0297	0.399	+	0.1033	0.6565	+	0.0260	0.3533	+	0.2158	0.3035	+	0.2082
Temperature: > 50 cm	0.5339	−	0.0331	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

P values in bold indicate significance at $P < 0.05$

Temperature responses were based on separate linear regressions, depth responses were categorical given incremental depth categories. ± indicates the direction of the response, with N/A representing inconclusive or insignificant trends

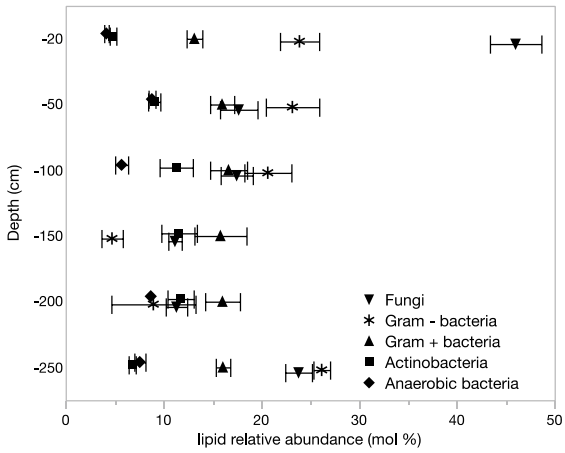

Fig. 2 Microbial lipid relative abundances by depth, June 2014 before warming began. The lipid indicator relative abundance (mol%) for fungi (inverted triangle Gram negative bacteria (Gram− bacteria; asterisks), Gram positive bacteria (Gram+ bacteria, triangles), Actinobacteria (squares) and anaerobic bacteria (diamonds). Data were averaged across treatment plots for this visualization, each symbol representing the mean and error bars representing one standard error from the mean

P = 0.0005) and the actinobacteria indicators demonstrating a negative relationship (actinobacteria P = 0.0274; Table 1) when all depths were combined. Only actinobacteria indicators were significantly related to temperature when separated by depth category, showing a positive relationship with temperature in the 20-50 cm interval (P = 0.0490). The relative abundances of Gram-negative, Gram-positive, and actinobacteria did show some significant relationships with temperature in September 2014 (Table 3). The relative abundance of the lipid indicators for Gram negative bacteria in all depths combined, Gram-negative bacteria in the >50 cm interval, and Gram-positive bacteria in the 20-50 cm interval were related positively to temperature (Gram-negative all depths P = 0.0148, Gram-negative >50 cm depth P = 0.0356, Gram-positive 20-50 cm depth P = 0.0064), while the actinobacterial indicator was related negatively to temperature when all depths were combined (P = 0.0050). In June 2015, the relative abundance of fungi was significantly positively related with temperature, but only the overall temperature relationship was significant (P = 0.0215), with no relationships within the three depth categories (Table 4).

In June 2014, the total biomass and actinobacteria $\delta^{13}C$ isotopic signatures had negative (P < 0.0001) relationship with ambient temperature when all depths were combined (Table 2). Gram-positive indicator $\delta^{13}C$ was positively related to temperature in the >50-cm depth interval (P = 0.0347, Table 2). In September 2014 there was a negative relationship between $\delta^{13}C$ and temperature for actinobacteria (P = 0.0195) in all depths combined (Table 3). Gram-positive $\delta^{13}C$ was positively correlated to temperature in the 20–50-cm depth interval (P = 0.0247; Table 3). $\delta^{13}C$ did not show a significant relationship to temperature in any lipid indicators in June 2015 (Table 4).

Discussion

Our objective was to determine the extent to which microbial community abundance and ^{13}C signatures are related to ambient or manipulated temperatures in a southern boreal peatland. We hypothesized that the microbial community, defined by the microbial lipid profile, would be strongly linked with both natural temperature fluctuations and experimental temperature manipulations. In contrast, we found that lipid indicators were more strongly associated with the temperature under ambient conditions, and that peat depth in general was a strong factor in shaping the microbial community and carbon use with only 1 year of experimental soil warming.

Microbial communities structured with depth

Peat depth was the strongest factor governing microbial absolute and relative abundances of microbial groups, and lipid $\delta^{13}C$ profiles (Tables 2, 3, 4, Figs. 1, 2). This finding aligns with previous studies showing the influence of peat depth on the structuring of microbial communities at the Marcell Experimental Forest (Lin et al. 2014a, b; Wilson et al. 2016) and at other boreal peatlands (Jaatinen et al. 2007; Sundh et al. 1997). The strong role of depth in shaping microbial communities makes sense in the context of peatland structuring, with vertical variations in redox conditions, peat chemical composition, bulk density, and other physical and chemical characteristics (Boelter and Verry 1977; Tfaily et al. 2014; Verry and Janssens 2011), which create distinct microbial

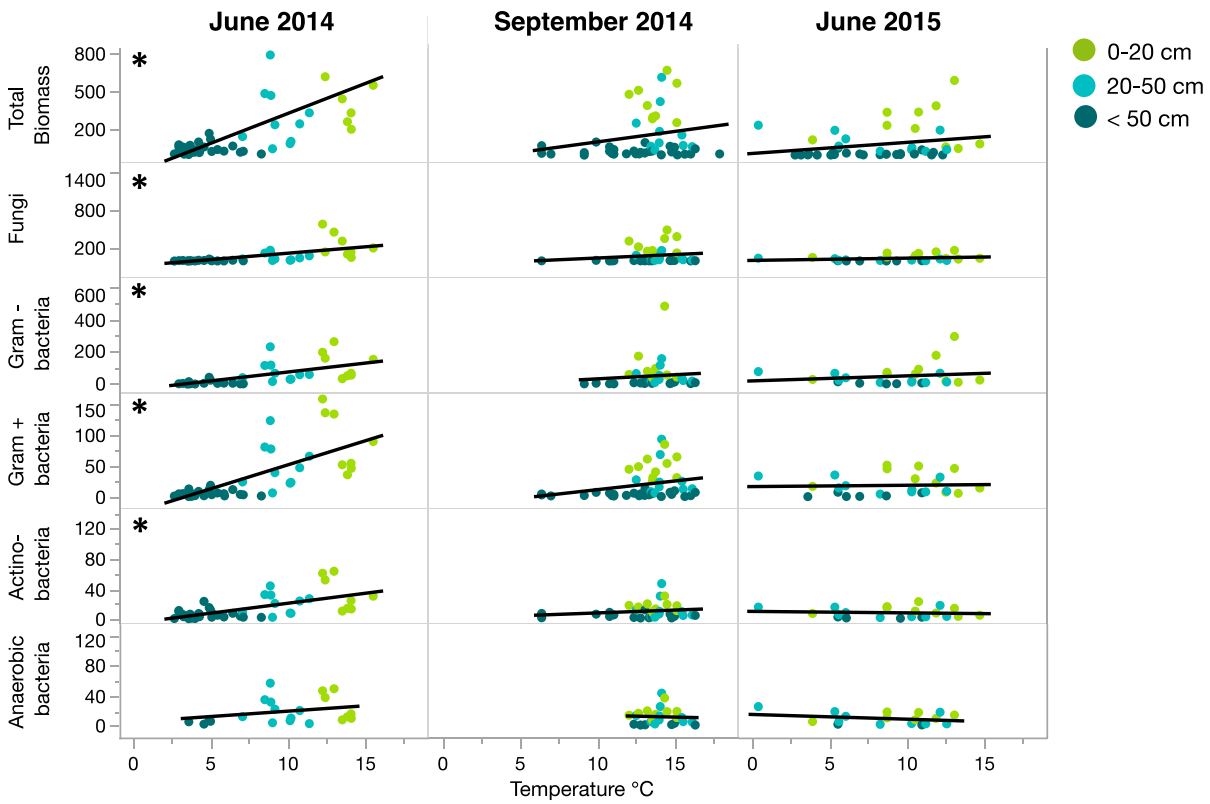

Fig. 3 Microbial lipid abundance (nmol g soil^{-1}) relationships with temperature. Asterisks indicate those indicators, within dates, that had significant relationships with temperature at P < 0.05. Temperature data used for this analysis were collected and processed as described in methods section "Peat physical characteristics", measured directly from peat and averaged across the 48-h period before during sampling. Black lines in each panel indicate the linear regression trend of each relationship

niches along the peat profile (Artz 2013), as microbial communities are strongly affected by environmental conditions, such as pH, temperature, organic matter composition, water, oxygen, and nutrient availability (Allison and Treseder 2011; Andersen et al. 2013; Davidson and Janssens 2006; Whitaker et al. 2014). For example, peat near the water table is a hot spot of microbial growth and activity because of the dynamically fluctuating oxidation states of available nutrients and energy sources, while the deeper, constantly saturated and more highly decomposed peat layers are characterized by a relatively static and inactive microbial community (Lin et al. 2014b), and previous studies have found functional gene profiles and microbial community composition corresponding to redox gradients and changes in carbon and nutrient chemistry along the peat profile at the SPRUCE site (Lin et al. 2014a, b; Wilson et al. 2016).

The isotopic signatures of the membrane lipids that we observed are likely reflective of vertical structuring of the peat caused by vegetative history. Because of changes in isotopic composition of CO_2 since approximately 1850 and stable isotope fractionation during the decomposition process, peat $\delta^{13}C$ is influenced by depth, with older, more decomposed peat generally more enriched in $\delta^{13}C$ (Esmeijer-Liu et al. 2012). However, $\delta^{13}C$ values are also affected by the dominant vegetation, climate, and atmospheric composition of carbon isotopes at the time of vegetation growth, confounding the depth trends that occur with decomposition (Alewell et al. 2011; Ehleringer et al. 2000; Hobbie et al. 2017). We observed total lipid $\delta^{13}C$ increasing from an average of − 32.2‰ at

the 0–20-cm sampling depth to a peak of − 22.3‰ at 150–200-cm before decreasing to − 24.1‰ at 200–250-cm depth, which is consistent with the general trend in previously reported bulk peat $\delta^{13}C$ attributed to vegetative history at the site (Hobbie et al. 2017); the depletion of atmospheric $\delta^{13}C$ due to anthropogenic fossil fuel combustion since approximately 1850 has resulted in recent photosynthate and plant material having depleted ^{13}C signatures (Ehleringer et al. 2000; Hobbie et al. 2017). Studies have shown that microbial biomass $\delta^{13}C$ is related to the isotopic signature of microbial carbon sources (Abraham et al. 1998; Abraham and Hesse 2003; Boschker and Middelburg 2002). Therefore, we expect the isotopic signature of microbial lipids to reflect those in the bulk peat where microbes are primarily consuming peat as opposed to dissolved organic carbon (DOC). Previous studies have shown that DOC at this site is primarily derived from recent photosynthate, even at depth (Tfaily et al. 2014). Accordingly, microbial DOC consumption would be expected to decouple peat and microbial $\delta^{13}C$. The generally lower $\delta^{13}C$ values for bioindicators compared to bulk peat are likely attributable to fractionation during metabolism and biosynthesis, which also differs between microbial groups and bioindicators (Abraham et al. 1998; Boschker and Middelburg 2002; Šantrůčková et al. 2000; Watzinger 2015). Usage of different carbon pools within a given group may also contribute to deviations from overall peat $\delta^{13}C$ and between microbial groups. For example, fungi and filamentous actinobacteria might be better able to break down older isotopically enriched carbon through extracellular enzyme production, which could be reflected in those indicators having higher $\delta^{13}C$ values.

Although fungal communities are expected to decrease in abundance quickly with peat depth and the disappearance of oxygen (Jaatinen et al. 2007), we observed fungal bioindicators at depth. Though anaerobic conditions at depth are unfavorable to many fungi, yeasts have been previously identified at the SPRUCE site, increasing in proportion of the fungal community with depth (Lin et al. 2014b). Numerous yeast taxa have been previously identified in other peatland soils as well (Thormann et al. 2007). Alternatively, we may have extracted lipids from microbial necromass that was sustained under the anaerobic nutrient poor conditions of the bog.

Microbial relationships with ambient temperature

We also explored how peatland microbial communities corresponded to ambient temperature prior to initiation of deep peat heating to provide context for interpreting experimental warming. Total microbial biomass and the absolute abundance of most lipid indicators were associated with temperature, showing a positive relationship with temperature when all depths were analyzed together. The relative abundance of fungal and Gram-negative and Gram-positive bacterial indicator lipids were also positively related to temperature. Temperature changes are likely to cause shifts in microbial communities, as the physiology of microbes (e.g. enzymes and membrane lipids) differ in their temperature optima, and temperature affects the availability and composition of available substrates (reviewed in Bradford 2013). Therefore, it is not surprising that warmer temperatures would be more favorable and allow for greater growth, especially when still within the range typically experienced by microbes (i.e. ambient conditions). The observed negative correlation between actinobacterial indicators and depth is likely a compositional effect driven by increases in relative abundance of fungal and other bacterial groups.

The observed correlation between lipid abundances and temperature could be part of the strong correlation between lipid abundances and peat depth, where depth, temperature, bulk density, and soil moisture all covaried (Table S1). Although the observed decrease in total microbial $\delta^{13}C$ signature with increasing ambient temperature may be related to shifts in microbial carbon usage, the most likely explanation of this pattern is also the correlation with depth, since microbes living at a given depth will reflect the $\delta^{13}C$ signature at that depth.

Microbial responses to experimental warming

Although microbial communities had strong relationships with temperature under ambient conditions, these relationships became insignificant when peat was experimentally warmed at depth. Analysis of microbial communities under direct peat warming at our site with 16S rRNA sequencing and quantitative PCR of 16S and 18S rRNA genes yielded a similar unresponsiveness to experimental warming, which was attributed to temperature-independent controls on

peat decomposition, such as chemical recalcitrance (thermodynamic limitation) (Wilson et al. 2016). The lack of response to experimental warming suggests that factors other than temperature that are associated with the depth profile, such as bulk density, moisture, and chemical composition, are stronger drivers of the microbial communities in this system than temperature and that the perceived relationship with temperature is actually just a relationship with depth. The onset of warming decoupled temperature and these other factors associated with peat depth, which were highly correlated under ambient conditions.

The lack of observed response to experimental warming may also reflect a lag period between the onset of warming treatments and microbial responses. The sample dates reflected 2 and 10 months after treatment initiation, respectively, which is a short period of time relative to the millennial timescale of peatland development (Verry and Janssens 2011). It could be that microbial growth takes a longer time to respond to warming than the 10 months of this sampling period. A similar lack of short-term responsiveness to warming treatments has been observed in other boreal warming experiments (Rinnan et al. 2007; Wang et al. 2022). This idea of a period before which microbial abundance or carbon use responds to experiment warming is supported by presence of these types of microbial responses in 2018, 2 years later (Ofiti et al. 2022). There is also evidence that ombrotrophic bogs, like the S1 bog, might generally be less responsive to climate change (Wu and Roulet 2014). Continuing from the earlier discussion of the many factors associated with the peat depth profile that drive microbial communities, the interactive effects of warming on peatland water table dynamics might be a more important driver than warming per se (Macrae et al. 2013). Under a warmer climate, peatland water tables could be lower, for longer durations of time during the growing season, meaning that the peat profile is aerobic or has lower soil moisture at a lower depth than pre-warming. A warmer climate may also lead to changes in plant evapotranspiration or water use, or changes in plant community composition, that indirectly alter peat soil moisture or chemistry (McPartland et al. 2019). In this study, direct peat warming did not occur for long enough to have observable effects on the water table, soil moisture or plant community through direct belowground responses to warming. In addition, only peat temperatures were warmed so that plant responses could not be observed. These are all reasons that in the future, there could be stronger microbial responses to peatland warming.

While the broader microbial community was generally unresponsive to experimental warming, fungal relative abundance was positively related to temperature at 10 months when all depths were pooled (Table 4). We hypothesize that the observed changes in the peat fungal relative abundance results from indirect temperature effects mediated by plants. Peat fungal communities are shaped as much by plant community structure as by physical attributes of the peat depth profile, potentially increasing the response of fungal growth and carbon use patterns relative to bacterial communities. The implications of fungal community shifts on carbon cycling and climate change depend on the types of fungi represented by the fungal lipid indicators, which can represent either saprotrophic or mycorrhizal fungi (MF). These two types of fungi are not distinguished by our PLFA indicators, but they have distinct ecological roles, roles in biogeochemical cycling, and responses to environmental drivers, and potentially different responses to changes in climate (Phillips et al. 2013; Zhou et al. 2022). Depending on the types of fungi present different mechanisms may be causing fungal community shifts.

Two types of mycorrhizal fungi are common in peatlands: ericoid mycorrhizal fungi (ErM) and ectomycorrhizal fungi (EMF). Ericoid mycorrhizal fungi are not as well studied but appear capable of forming mycorrhizal associations and degrading a wide range of organic compounds (Thormann 2006). Although evidence suggests that EMF may mine carbon for protein synthesis from organic nitrogen, they rely heavily on plant photosynthate for the carbon required for growth (Hobbie et al. 2014). On the other hand, saprotrophic fungi rely more heavily on decomposing older organic matter.

Previous research has found positive impacts of warming on EMF attributed to increased aboveground plant growth or increased allocation of carbon belowground (Clemmensen et al. 2006; Fujimura et al. 2008). However, ectomycorrhizal fungi have been found to be impacted both positively and negatively by warming (Compant et al. 2013). While we did not observe a significant relationship between

fungal $\delta^{13}C$ and temperature during the course of this study, fungal $\delta^{13}C$ can help us infer shifts in fungal community composition (EMF vs. saprotroph) and carbon usage, which could affect peatland stability.

Conclusions

In this study, we used $\delta^{13}C$-PLFA to track changes in carbon use and microbial community structure in peatlands under ambient environmental conditions with the aim of expanding on previous research characterizing peat isotopic composition and microbial community structure (Hobbie et al. 2017; Lin et al. 2014b; Wilson et al. 2016) and identifying susceptibility to alteration and feedbacks under future climate change scenarios. While the entire microbial community was closely related to the range of observed ambient temperatures, few indicators showed significant relationships with temperature following warming treatments. Because PLFA responds rapidly to microbial community changes, the lack of observed changes in response to the implementation of experimental warming suggests that the microbial community is not sensitive to the range of temperature changes introduced by warming treatments over the 10-month timescale of this research. However, while we focused on the microbial community structure, we did not look into changes in the activity of the microbial community, which would have implications for carbon and nutrient cycling and may be more sensitive to change. In conclusion, the abundances of broad microbial groups may not be sensitive to initial (less than 1 year) changes in temperature but instead might respond indirectly to temperature change through other physical changes in peat such as bulk density or soil moisture.

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Author contributions JG conceptualized and supervised this research project as well as contributing to the writing and data analysis. CB carried out the sample collection and lab analysis and contributed to data analysis and writing. SS contributed to the review and writing of the manuscript, provided background environmental data, and contributed study site and subject matter expertise. MF contributed to the data analysis, data curation, and writing.

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Declarations

Competing interests The authors declare that they have no conflict of interest.

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