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Comments

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RESEARCH ARTICLE

Soil microbial legacies influence freeze–thaw responses of soil

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Abstract

1. Warmer winters with less snowfall are increasing the frequency of soil freeze–thaw cycles across temperate regions. Soil microbial responses to freeze–thaw cycles vary and some of this variation may be explained by microbial conditioning to prior winter conditions, yet such linkages remain largely unexplored. We investigated how differences in temperature history influenced microbial community composition and activity in response to freeze–thaw cycles.
2. We collected soil microbial communities that developed under colder (high elevation) and warmer (low elevation) temperature regimes in spruce-fir forests, then added each of these soil microbial communities to a sterile bulk-soil in a laboratory microcosm experiment. The inoculated high-elevation cold and low-elevation warm microcosms were subjected to diurnal freeze–thaw cycles or constant above-freezing temperature for 9 days. Then, all microcosms were subjected to a 7-day above-freezing recovery period.
3. Overall, we found that the high-elevation cold community had, relative to the low-elevation warm community, a smaller reduction in microbial respiration (CO_2 flux) during freeze–thaw cycles. Further, the high-elevation cold community, on average, experienced lower freeze–thaw-induced bacterial mortality than the warm community and may have partly acclimated to freeze–thaw cycles via increased lipid membrane fluidity. Respiration of both microbial communities quickly recovered following the end of the freeze–thaw treatment period and there were no changes in soil extractable carbon or nitrogen.
4. Our results provide evidence that past soil temperature conditions may influence the responses of soil microbial communities to freeze–thaw cycles. The microbial community that developed under a colder temperature regime was more tolerant of freeze–thaw cycles than the community that developed under a warmer temperature regime, although both communities displayed some level of resilience. Taken together, our data suggest that microbial communities conditioned to less extreme winter soil temperatures may be most vulnerable to rapid changes in freeze–thaw regimes as winters warm, but they also may be able to quickly recover if mortality is low.

KEYWORDS
freeze–thaw cycles, legacies, microbial biomass, microbial communities, respiration, soil nitrogen, temperate forest soils, winter climate change

1 | INTRODUCTION

As the climate warms, winters in temperate ecosystems are experiencing reductions in snow cover depth and duration (Burakowski et al., 2022; Demaria et al., 2016; IPCC, 2021; Kreyling & Henry, 2011). Because snow insulates soils, snow cover regulates patterns of soil freeze–thaw cycles and plays a key role in moderating soil nutrient cycling (Blankinship & Hart, 2012; Campbell et al., 2014; Groffman et al., 2001). Thus, warming will increase the frequency and intensity of soil freeze–thaw cycles in many temperate ecosystems. While the stress of changing winters on a diversity of organisms (Williams et al., 2015), from trees (Deschênes et al., 2019; Rustad et al., 2020) to hibernating mammals (Fietz et al., 2020; Inouye et al., 2000), has been well characterized, there has been less focus on belowground dynamics and how important microbial processes are changing (Semenova et al., 2016). The expansion and contraction of water during freeze–thaw cycles disrupts soil aggregates and structure (Oztas & Fayetorbay, 2003; Xiao et al., 2019), fractionates plant litter and damages roots (Kreyling et al., 2012), and impacts microbes (Sorensen et al., 2018; Yanai et al., 2004), with cascading effects on ecosystem nitrogen (N) and carbon (C) cycling (Nielsen et al., 2001; Song et al., 2017; Urakawa et al., 2014). Microbial community composition and activity are highly sensitive to temperature and moisture changes (Ren et al., 2020; Wallenstein & Hall, 2012), and thus microbial communities conditioned to local soil temperature regimes may be disrupted by changing winter temperatures and more frequent freeze–thaw cycles. Yet, how legacies of past soil temperature conditions influence the responses of microbial communities to freeze–thaw cycles remain unclear.

Microbial responses to experimentally imposed freeze–thaw cycles are varied. Some studies find minimal or no detectable effects (Groffman et al., 2001; Koponen et al., 2006; Männistö et al., 2009; Meisner et al., 2021), while others find that freeze–thaw cycles significantly affect the activity (Sorensen et al., 2018; Yang et al., 2019), community structure (Feng et al., 2007; Ji et al., 2022; Ren et al., 2020; Yergeau & Kowalchuk, 2008), and death of soil microbes (Sorensen et al., 2018; Yanai et al., 2004; Yang et al., 2019). Ruptured microbial cells release nutrients and organic C, which become available to the surviving community when the soil thaws (Herrmann & Witter, 2002; Schimel & Clein, 1996; Skogland et al., 1988; Yergeau & Kowalchuk, 2008). Physical disruption during these freeze–thaw cycles releases labile substrates and nutrients from soil aggregates (Feng et al., 2007). Together, microbial cell death and physical disruption of existing soil C can lead to a respiratory pulse by surviving microbes who can quickly use the newly available substrates (Feng et al., 2007; Herrmann & Witter, 2002; Schimel & Clein, 1996; Skogland et al., 1988; Yergeau & Kowalchuk, 2008).

Local environmental conditions influence plant, animal and microbial tolerance to extreme or changing temperatures (Boyles et al., 2011; Ruelland et al., 2009; Sendall et al., 2015; Stres et al., 2010; Yergeau & Kowalchuk, 2008), which could explain why some communities respond strongly to freeze–thaw cycles while others are more resistant. Like many organisms, microbes physiologically respond to cold and subzero temperatures by regulating intracellular solutes. Soil microbes can produce cryoprotectants or antifreeze proteins (Drotz et al., 2010; Lorv et al., 2014; Walker et al., 2006), create cold-adapted enzymes (Margesin et al., 2009; Margesin & Miteva, 2011; Siddiqui & Cavicchioli, 2006) and adjust their lipid membrane composition to maintain membrane fluidity (Drotz et al., 2010; Margesin & Miteva, 2011; Shivaji & Prakash, 2010). The ability to physiologically respond to freezing allows some microbes to survive and quickly recover function after a freeze–thaw stress (Nielsen et al., 2001). Differential response to freezing events by individual microbes in a community can, over a season or after multiple-events across time, shift a community to favour more cold-tolerant community members (Monson et al., 2006; Schimel et al., 2007; Walker et al., 2006). For instance, communities may become more fungal dominant as fungi are often more tolerant to freezing than bacteria (Aanderud et al., 2013; Perez-Mon et al., 2020). As a result of variable cold-tolerance development, microbial survival and activity during and following soil freeze–thaw cycles may be greater for microbial communities with a history of lower soil temperatures or frequent freeze–thaw cycles compared to those conditioned to a higher or narrower soil temperature range. Communities with colder soil histories may therefore be better conditioned to tolerate increases in the frequency and intensity of soil freeze–thaw cycles as winter climate changes, with perhaps less perturbation of biogeochemical cycles and ecosystem functions.

To date, research on whether climate-driven microbial legacies influence freeze–thaw responses is compelling but limited. Further, most work in this area has focused on soils in polar and high alpine regions, despite the fact that temperate and lower elevation ecosystems may be more responsive to freeze–thaw cycles (Gao et al., 2018). Prior thermal conditioning of a soil community can influence microbial responses (i.e. community structure, survival, activity) to freeze–thaw events, but there is a lot of observed variation in microbial response (Jurburg et al., 2017; Perez-Mon et al., 2020; Stres et al., 2010; Yergeau & Kowalchuk, 2008) and, more generally, the effects of freeze–thaw cycles on soil microbes remain poorly understood (Ji et al., 2022). This variation likely results from different experimental approaches, variable seasonal timing of soil collection, and environmental factors like soil type (Henry, 2007). Exposing different soil microbial communities to standardized freeze–thaw regimes while controlling for soil characteristics may overcome these

limitations and allow us to conclude whether microbial legacies influence soil responses to freeze–thaw cycles.

Here, we determined how soil microbial legacies in temperate forests influence responses of microbial activity, community composition, and soil extractable N and C to freeze–thaw cycles. Using soil microbial communities adapted to a low- and a high-elevation spruce-fir forest in the northeastern USA, we established a freeze–thaw microcosm experiment (Figure 1). We selected spruce-fir forest sites that had similar soil properties and plant communities and used a sterile bulk-soil in each microcosm. We inoculated each microcosm with a soil microbial community from either the high (colder) or low (warmer) elevation site (hereafter "high-spruce" and "low-spruce"). After inoculation, we subjected microcosms to either diurnal freeze–thaw cycles or a constant, above-freezing temperature for 9 days. Following the experimental manipulation, we allowed all microcosms, regardless of treatment, to undergo a 7-day above-freezing recovery period. We measured microbial respiration over every 24-h period and, at the end of the freeze–thaw and recovery periods, we measured total soil inorganic nitrogen (TIN), extractable organic nitrogen, and extractable organic carbon. We measured microbial biomass and community composition at the end of the freeze–thaw period. We hypothesized that a microbial community from the colder high-spruce site would be more tolerant of freeze–thaw cycles and therefore experience lower mortality and impairment of activity compared to a community from the warmer low-spruce site.

2 | MATERIALS AND METHODS

2.1 | Sampling sites

We obtained soil for the freeze–thaw microcosm experiment from a low- and a high-elevation temperate forest (8.6 km apart, Figure 1a) in Vermont, USA. These two forests, which have

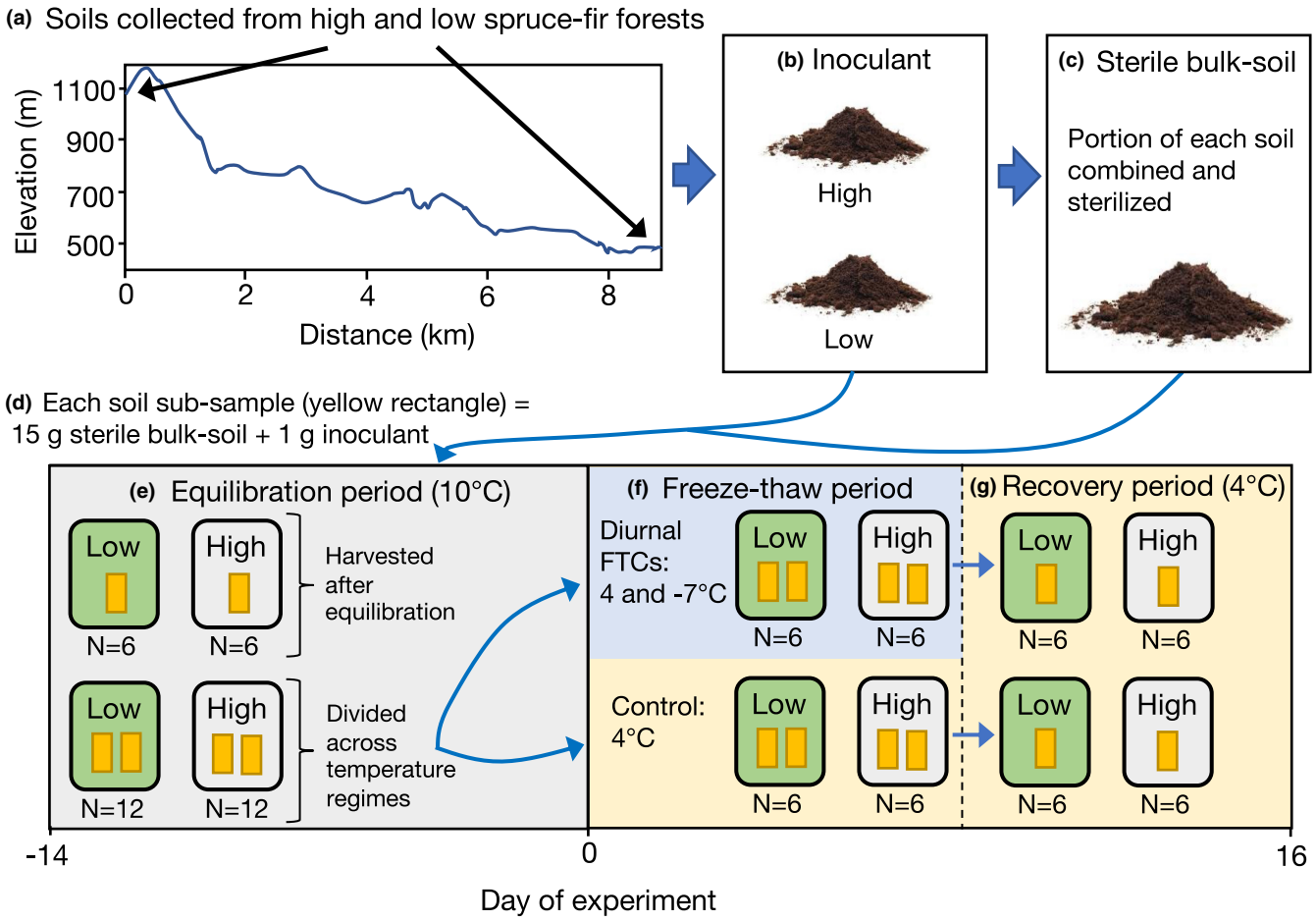

FIGURE 1 Schematic overview of the experiment. Soils from high- and low-elevation spruce-fir forests (a) were used as inoculant (b) and a portion of each soil was combined and autoclaved to create a common bulk-soil (c). A small amount of inoculant was added to the same sterile bulk-soil in each microcosm sub-sample (d). 12 microcosms were harvested after the two-week equilibration period (e) to measure initial conditions, and the remaining 24 microcosms were subjected to either 9 diurnal freeze–thaw cycles or a constant above-freezing temperature (f). After the 9 freeze–thaw cycles, one soil sub-sample per microcosm was harvested to measure soil properties, microbial biomass, and community composition. The microcosms then underwent a 7-day recovery period (g), after which all remaining soil sub-samples were harvested for soil properties. Microbial respiration was measured over each 24-h period during the freeze–thaw and recovery periods.

experienced different winter climate conditions, are dominated by red spruce *Picea rubens* and balsam fir *Abies balsamea*. The high-elevation site is on a west-facing slope near the peak of Camel's Hump in the Green Mountains in Huntington, Vermont (1074 m a.s.l., 44°19′19″N, 72°53′27″W), and the low-elevation site is located on a Vermont Land Trust site in Duxbury, Vermont (498 m a.s.l., 44°15′40″N, 72°49′26″W). Permission for fieldwork was granted by the Vermont Land Trust and the State of Vermont Agency of Natural Resources (licence 22,556). The Camel's Hump site has Spodosols (Lyman-Marlow complex) with a sandy loam texture (Soil Survey Staff, 2022); soil pH was 4.33 (measured via Mettler Toledo S220 SevenCompact benchtop pH/ion meter in supernatant with soil: deionized water ratio of 1:3; Mettler Toledo, LLC) and mineral soil (0–10 cm) total C was 20.2% and total N was 1.2% (measured by dry combustion; UNICUBE elemental analyser). Soils at the low-elevation site are also Spodosols (Colonel fine sandy loam) with a sandy loam texture (Soil Survey Staff, 2022); soil pH was 4.64 and total C was 6.4% and total N was 0.4% (measured as above).

The high-spruce site experienced lower soil and air temperatures for a longer portion of the year compared to the low-spruce site (Figure S1). Mean daily minimum and maximum air temperatures averaged 0.4 ± 0.1°C and 11.1 ± 0.1°C, respectively, at the low-spruce site, and −0.9 ± 0.1°C and 8.2 ± 0.1°C at the high-spruce site from 2000–2019 (Daymet data; Thornton et al., 2016). We measured local subcanopy air and soil temperatures at the site of soil collection with shielded Thermochron iButton temperature sensors and dataloggers (DS1922L, Maxim Integrated Products, Inc.). Sensors were hung from trees 1.5 m above the ground surface and buried 5 cm beneath the soil surface (5–6 sensors per site). Air and soil temperatures were logged in 1-h intervals from November 2020 through June 2021. Mean daily minimum air temperatures were −4.5 ± 0.6°C at the high-spruce site and −1.5 ± 0.6°C at the low-spruce site (Figure S1b). Mean daily minimum soil temperatures were 2.5 ± 0.2°C at the high-spruce site and 4.6 ± 0.3°C at the low-spruce site (Figure S1e). Soil temperatures never went below 0°C during the year of soil collection, although soil freezing can occur in this region. Thirty-seven percent of hourly soil temperature measurements at the high-spruce site were below 1°C, compared to <1% at the low-spruce site (Figure S1d).

2.2 | Soil sampling

To obtain soil microbial communities, we extracted mineral soil cores (0–10 cm) starting beneath the organic layer on Nov. 5–6, 2020. We collected six soil cores per site using a soil corer (AMS mini soil probe; 2 cm diameter; AMS, Inc.) and combined them in a plastic bag (with soils from each site kept separate). Soil from each site was immediately transported to the lab, homogenized by sieving (mesh size 2 mm), and stored at −20°C until use as an inoculant. When it is not possible to use fresh soil, other freeze–thaw incubation studies have stored soil in frozen conditions (e.g. Herrmann & Witter, 2002; Yergeau & Kowalchuk, 2008), and studies investigating effects of pre-treatment storage conditions on soil and microbial properties recommend storage at −20°C to prevent gradual depletion of substrates while limiting storage effects on the microbial communities (Lee et al., 2007; Stenberg et al., 1998). We acknowledge that this may have influenced freeze-sensitive microbes, but both microbial communities were collected during the cold season, were exposed to the same storage conditions, and were allowed an equilibration period to recover from storage.

To create a single sterile bulk-soil for use in all microcosms, we collected additional mineral soil from each site (0–10 cm) using a small shovel and transported it to the lab on ice. We separately homogenized the soil for each site by sieving (mesh size 2 mm). We mixed soil from both sites (1:1 by volume) by hand and dried the combined soil to 60% field capacity. We then autoclaved the combined soil twice at 123°C for 30 min (separated by 24 h). This sterile, mixed field soil (Figure 1c) was used in all microcosms. Total soil C and N concentrations were 13.9% and 0.9%, respectively (measured as above).

2.3 | Microcosm experiment and microbial respiration measurements

We established a microcosm experiment to determine whether soil microbial legacies in temperate forests influence freeze–thaw responses (Figure 1). First, we mixed sterile bulk-soil with either high-spruce or low-spruce inoculum (15/1 mixture, Figure 1d). Next, we added 16 g of either high-spruce or low-spruce inoculated soils into uncapped 60 ml Nalgene polyethylene bottles, creating 30 high-spruce and 30 low-spruce inoculated soil sub-samples. Two high-spruce or low-spruce soil sub-samples were placed into 24 1-L glass canning jars fitted with aluminium lids (i.e. microcosms). This method resulted in a factorial design with 2 microbial communities/sites (high-spruce and low-spruce) × 2 treatments (control or freeze–thaw cycles) × 6 replicates for a total of 24 microcosms (each containing 2 sub-samples) and a sample size of 6 microcosms for each treatment combination (Figure 1f). To measure initial microbial biomass, community composition and soil properties (i.e. at the end of the equilibration period but before the freeze–thaw period began), we established an additional 12 microcosms, 6 containing a high-spruce inoculated sub-sample and 6 containing a low-spruce inoculated sub-sample (Figure 1e).

Microcosms were incubated at constant 10°C (mean minimum growing season temperature at low-spruce site from 2000–2019; Daymet data; Thornton et al., 2016) for 2 weeks prior to the freeze–thaw period to allow microbial communities to establish and equilibrate. At the end of the equilibration period, we harvested the 12 microcosms containing one soil sub-sample each (Figure 1e) to measure initial gravimetric soil moisture, microbial biomass and community composition, TIN and extractable organic C and N. We subjected the remaining 24 microcosms to one of two temperature treatments using dark, thermostat regulated incubators: (1) diurnal

freeze–thaw cycles that alternated every 12 hours between 4°C and −7°C (mean maximum and minimum cold season air temperatures at the low-spruce site from 2000–2019; Daymet data; Thornton et al., 2016); or (2) constant 4°C (Figure 1f). This freeze–thaw period lasted for 9 days, followed by a 7-day recovery period at 4°C (Figure 1g) to assess lagged effects of freeze–thaw cycles on microbial respiration. At the end of the freeze–thaw period and again at the end of the experiment, one soil sub-sample from each of the 24 microcosms was harvested to measure gravimetric soil moisture, TIN and extractable organic C and N. We also measured microbial biomass and community composition in the soil sub-samples collected after the freeze–thaw period.

During the freeze–thaw and recovery periods, we measured microbial respiration over each full 24-h period using an infrared photoacoustic spectroscopy gas analyser (PAS; Model 1412i, Innova Air Tech Instruments) (Adair et al., 2019). We inserted two stainless infusion needles in jar lids through separate septa, with one needle connected to the PAS inlet port and the other to the outlet port. To calculate the change in CO_2 concentration over each 24-h period (i.e. respiration rate), we sampled the headspace at time = 0 h (initial measurement) and time = 24 h (final measurement) each day. Respiration measurements were collected in duplicate and averaged. Immediately after each day's final measurement (time = 24 h), each jar was uncapped and flushed for 1 min with ambient air by fanning before being recapped to take an initial measurement for the next 24-h period. This process was repeated daily. Septa were changed halfway through the experiment during the flushing time. Average temperature of the room during measurements was 17°C, and microcosms were exposed to this temperature for ≈5 min total each day including final + initial measurements and flushing.

The difference between each day's initial and final CO_2 concentrations over time (typically ≈24 h) was used to calculate microbial respiration rates (CO_2 flux):

$$F = \frac{\Delta C}{\Delta t} \times m \times \frac{V}{D} \times MW \times \rho \times \alpha,$$

where F is CO_2 flux (mg CO_2-C kg dry soil^{-1} h^{-1}), $\Delta C / \Delta t$ is the difference in CO_2 concentration (μmol mol^{-1}) divided by the difference in time (h) between the initial and final measurements for the day, m is a molar fraction (1/10^6 mol μmol^{-1}), V is headspace volume (0.000968 m^3), D is dry soil mass (kg), MW is the molecular weight of CO_2 (44,010 mg mol^{-1}), ρ is gas density at 20°C and 0.101 MPa (1/0.02404 mol m^{-3}), and α is the mass ratio of C to CO_2 in a CO_2 molecule (12.01/44.01). Gas density was calculated at 20°C because that is the temperature at which the PAS measures gas concentration.

2.4 | Nutrient extraction and chemical analyses

Soil subsamples were extracted after the equilibration, freeze–thaw, and recovery periods by shaking 5 g wet soil with 50 ml 2 M KCl for 1 h followed by gravity filtration using pre-leached Whatman No. 1 filter paper. Extracts were stored at −20°C until nutrient analysis. We determined gravimetric soil moisture by drying a 5 g wet soil subsample at 60°C for 48 h (adapted from Topp & Ferré, 2002).

We measured the extracts for concentrations of total organic C and total (organic + inorganic) N via combustion using a total organic carbon analyser (TOC-L, Shimadzu). Since those analyses were performed on filtered soil extracts, they yielded concentrations of extractable organic C and total (organic + inorganic) extractable N. We performed colorimetric analyses for soil NH_4^+-N and NO_3^--N on a microplate reader (BioTek Synergy HTX, BioTek Instruments, Inc.). NO_3^--N was measured after conversion to nitrite and/or nitric oxide using vanadium (III) (adapted from Doane & Horwáth, 2003; Hood-Nowotny et al., 2010). NH_4^+-N was measured using the Berthelot colour reaction (adapted from Forster, 1995). NO_3^--N levels were very low (3 μg NO_3^--N g dry soil^{-1} vs. 118 μg NH_4^+-N g dry soil^{-1} on average) and unaffected by treatments. Thus, we combined NH_4^+-N and NO_3^--N to calculate TIN, which we used in figures and analyses. We calculated the amounts of extractable organic N by subtracting TIN from total extractable N. We calculated all soil extractable C and N values on a per gram dry soil basis.

2.5 | PLFA

We measured microbial biomass and community composition after the equilibration and freeze–thaw periods using phospholipid fatty acid (PLFA) analysis. We extracted microbial lipids from 1.5 g of sieved, root-free, fresh weight soil that had been stored at −20°C until analysis began. We used a modified Bligh and Dyer (1959) extraction of chloroform with a phosphate buffer for the initial collection (Guckert et al., 1985; White et al., 1979). We fractionated these lipid extracts on silicic acid columns into neutral, glyco-, and polar lipids. Only polar lipids were collected and then methylated with 0.2 M methanolic KOH to form fatty acid methyl esters (FAMEs). We identified and quantified FAMEs based on retention time data with known standards for each marker of interest using a Varian 3800 GC-FID.

We chose i15:0, a15:0, i16:0, i17:0, a17:0, 16:1ω7c, 18:1ω7 and cy19:0 PLFAs to indicate bacterial biomass (Frostegård & Bååth, 1996; Ruess & Chamberlain, 2010; Vestal & White, 1989; Zelles, 1997). Of those markers, we assigned the first five to Gram-positive bacteria and the last three to Gram-negative bacteria (O'leary & Wilkinson, 1988; Ruess & Chamberlain, 2010; Vestal & White, 1989; Wilkinson, 1988). We chose 18:2ω6,9 and 18:1ω9 PLFAs to indicate fungal biomass (Bååth, 2003; Ruess & Chamberlain, 2010; Vestal & White, 1989). We calculated fungal:bacterial ratios (F:B) from the sum of the PLFAs characteristic of their groupings. We chose 10Me16:0 to indicate actinomycetes (Vestal & White, 1989) and did not include this PLFA in any other taxonomic microbial groupings or ratios. We also calculated ratios of Gram-positive:Gram-negative (Gram+:Gram−) and saturated:monounsaturated fatty acids (Sat:Mono) as potential stress indicators (Willers et al., 2015). We calculated fatty acids on a per gram dry soil basis.

2.6 | Statistical analyses

For daily microbial respiration rates, we separately analysed data from the freeze–thaw period and from the recovery period. We used linear mixed effects models for full factorial ANOVA repeated measures, including main effects of day (as a continuous variable; within-subjects), temperature regime (hereafter "temperature"; between-subjects), microbial community/site (hereafter "site"; between-subjects), and all interactions of the above. Microcosm identity and microcosm identity × day were each nested within temperature and site as random effects to account for nonindependent measurements from the same microcosm over time. For all other responses (microbial biomass, TIN, extractable organic N and C), we conducted separate full factorial ANOVAs for the post-freeze–thaw and, when applicable, post-recovery data, including main effects of temperature and site and a temperature × site interaction. Initial differences in microbial biomass, TIN, and extractable organic N and C between the 6 high- and 6 low-spruce microcosms harvested after equilibration were analysed with T-tests. Data were checked for assumptions of normality and homogeneity of variances. We conducted all analyses with statistical analysis software (JMP Pro 15.0.0, 2019, SAS Institute Inc.).

3 | RESULTS

3.1 | Microbial respiration

Freeze–thaw cycles reduced daily microbial respiration rates in all microcosms, with a 53% reduction for low-spruce compared to a 37% reduction for high-spruce during the freeze–thaw period (temperature × site: $p = 0.01$, Table 1, Figure 2). The magnitude of the response modestly varied for both high- and low-spruce microcosms by day but was always negative (temperature × day: $p = 0.004$, Table 1, Figure 2). These declines, while different in magnitude between the two microbial communities, reduced respiration to similar absolute rates. Specifically, while respiration was 23% higher on average for low-spruce controls compared to high-spruce controls, freeze–thaw cycles reduced respiration rates of low-spruce to 0.83 ± 0.05 mg CO_2-C kg soil^{-1} h^{-1} and high-spruce to 0.89 ± 0.03 mg CO_2-C kg soil^{-1} h^{-1} on average (Figure 2). During the recovery period, we observed a pulse in respiration for high-spruce during the first three recovery days (i.e. the freeze–thaw group's respiration surpassed the control group's respiration), followed by a return to baseline levels and a modest reduction on the final day (temperature × site × day: $p = 0.03$, Table 1, Figure S2). Respiration rates of low-spruce recovered to baseline levels on the first recovery day and did not exhibit a pulse (Table 1, Figure S2). The declines in absolute respiration rates over time were likely due to gradual depletion of substrates (Figure 2).

3.2 | Soil extractable nitrogen and carbon

The freeze–thaw treatment did not significantly influence TIN, extractable organic N, or extractable organic C after either the freeze–thaw or recovery period (Table S1, Figure S3), although there was a trend of greater extractable organic N and C after freeze–thaw cycles for low-spruce microcosms (Figure S3c–f). TIN, but not extractable organic N or C, was greater in high-spruce relative to low-spruce microcosms before and throughout the experiment (Tables S1 and S2, Figure S3).

3.3 | Microbial biomass and community composition

Community composition for low- and high-spruce was similar prior to the freeze–thaw period (Table S2). The only significant difference was 17% lower actinomycetes biomass for low-spruce relative to high-spruce ($p = 0.03$, Table S2).

We were unable to detect an effect of freeze–thaw cycles on total (fungal + bacterial) biomass (temperature: $p = 0.11$, Table 2, Tables S3 and S4), although freeze–thaw cycles reduced biomass by 14% on average for low-spruce (Table S4). When bacterial and fungal biomass were analysed separately, we detected a negative effect of freeze–thaw cycles on bacteria, which was most evident in the low-spruce microcosms (temperature: $p = 0.08$, Table 2,

	Freeze–thaw period		Recovery period	
Effect	*F*	*p* > *F*	*F*	*p* > *F*
Temperature	135.15	**<0.0001**	0.89	0.36
Site	3.61	0.07	16.79	**<0.001**
Day	543.61	**<0.0001**	19.64	**<0.001**
Temperature × Site	7.65	**0.01**	2.28	0.15
Temperature × Day	10.75	**0.004**	0.28	0.60
Site × Day	1.88	0.19	3.05	0.10
Temperature × Site × Day	0.00	0.99	5.67	**0.03**

Note: $p < 0.05$ bolded to draw attention to the most important terms in the model. *df* = 1 for all factors and interactions.

TABLE 1 Linear mixed effects model probabilities ($p > F$) for microbial respiration rates during the freeze–thaw and recovery periods.

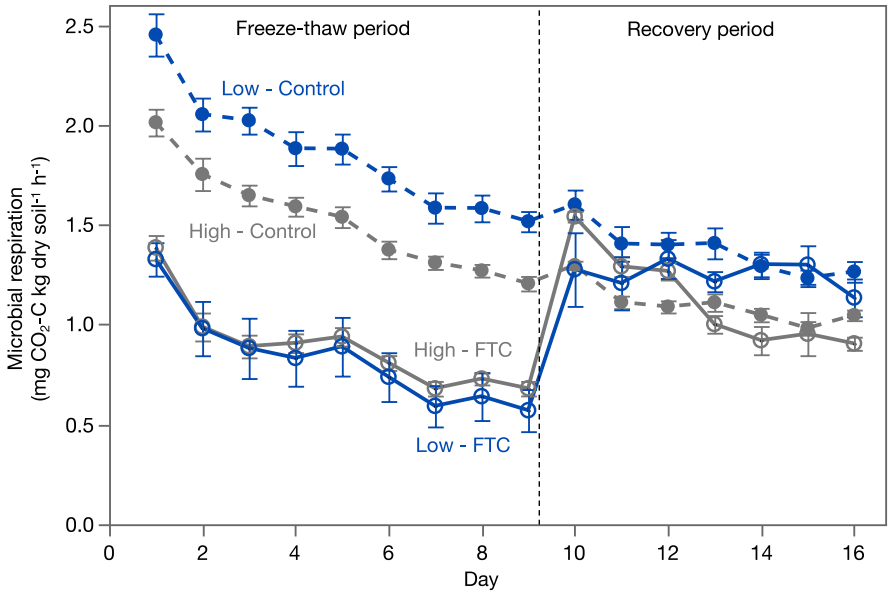

FIGURE 2 Daily microbial respiration rates for high-spruce (grey) or low-spruce (blue) microcosms exposed to constant 4°C (closed circles, dashed lines) or to diurnal freeze–thaw cycles (FTC, open circles, solid lines) for the first 9 days of the experiment (freeze–thaw period). During the next 7 days, all microcosms were exposed to constant 4°C (recovery period). Points show means ±1 SE. $N = 6$ microcosms per point. Results of statistical analyses are reported in Table 1.

TABLE 2 Full factorial ANOVA model probabilities ($p > F$) for lipid biomass of total fungi, total bacteria, fungal: bacterial (F:B) ratio, total fungi+bacteria, actinomycetes, Gram-positive bacteria, Gram-negative bacteria, Gram-positive:Gram:negative ratio and saturated:monounsaturated fatty acids (Sat:Mono). *F*-ratios are shown in Table S3.

	Fungal	Bacterial	F:B	Total (F + B)	Actino	Gram +	Gram −	Gram +: Gram −	Sat:Mono
Effect	***p* > *F***	***p* > *F***	***p* > *F***	***p* > *F***	***p* > *F***	***p* > *F***	***p* > *F***	***p* > *F***	***p* > *F***
Temperature	0.47	0.08	0.35	0.11	0.31	**0.02**	0.22	**0.03**	**0.02**
Site	0.18	**0.049**	0.94	0.06	0.44	0.39	**0.04**	0.83	0.56
Temperature × Site	0.20	0.21	0.28	0.19	0.91	0.80	0.16	0.56	0.10

Note: $p < 0.05$ bolded to draw attention to the most important terms in the model. $df = 1$ for all factors and interactions.

Tables S2 and S4, Figure 3a). Freeze–thaw cycles reduced Gram-positive bacteria in both high- and low-spruce microcosms by 24% on average (temperature: $p = 0.02$, Table 2, Tables S3 and S4, Figure 3b), which was accompanied by an average 25% decrease in Gram+:Gram- for high-spruce and 16% decrease for low-spruce microcosms relative to controls (temperature: $p = 0.03$, Table 2, Table S3 and S4, Figure 3c). Freeze–thaw cycles also reduced Sat:Mono by 13% for high-spruce and 3% for low-spruce microcosms on average relative to controls (treatment: $p = 0.02$, Table 2, Tables S3 and S4, Figure 3d).

4 | DISCUSSION

Winters are becoming more variable with less snowpack and more frequent freeze–thaw cycles in temperate ecosystems, potentially impacting the functional composition of soil microbial communities that regulate nutrient availability. However, soil microbial communities with histories of cold winter soil conditions may be cold-conditioned and respond less to soil freeze–thaw cycles than communities that have developed under less variable and warmer soil conditions. We explored how microbial communities that developed under different temperature regimes responded to freeze–thaw cycles in a microcosm experiment. We hypothesized that a microbial community from the colder high-spruce site would be more tolerant of freeze–thaw cycles and therefore experience lower freeze–thaw-induced mortality and impairment of activity compared to a community from the warmer low-spruce site.

We found that freeze–thaw cycles reduced microbial respiration relatively consistently across days and, as hypothesized, those reductions were smaller for the high-spruce microcosms, which experienced an average 37% decrease, compared to the low-spruce microcosms, which experienced a 53% decrease. Respiration rates of the low-spruce control were higher than those of the high-spruce control at 4°C despite similar initial community composition, possibly due to different temperature sensitivities between communities of specific taxa (Wang et al., 2021). It is interesting that while freeze–thaw cycles reduced low-spruce respiration more than high-spruce respiration as hypothesized, the respiration rates reached were similar between microbial communities. Perhaps freeze–thaw cycles acted as an equalizer, reducing rates of the two communities to a suppressed activity state or limiting activity to a subset of microbes that were cold-tolerant and present in both communities, ideas that should be tested in future studies.

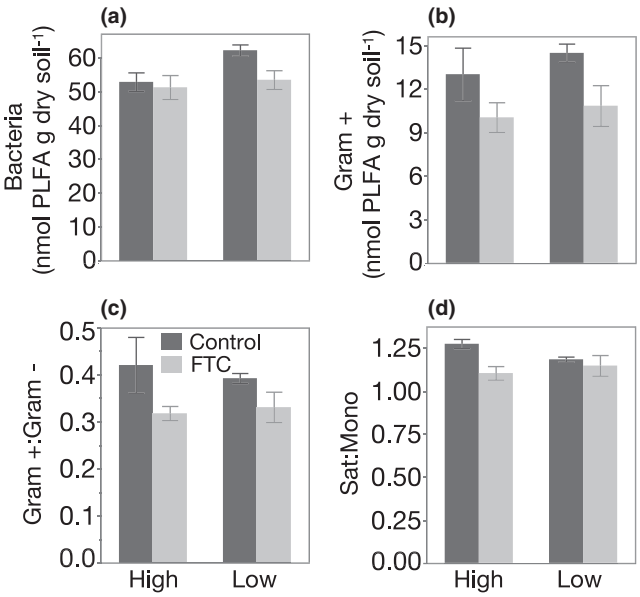

FIGURE 3 PLFA contents of high-spruce or low-spruce microcosms after exposure to constant 4°C (control, dark grey) or freeze–thaw cycles (FTC, light grey) for 9 days. Lipid biomass is shown for total bacteria (a), Gram-positive bacteria (b), the Gram-positive:Gram-negative ratio (Gram +:Gram −, c), and saturated:monounsaturated fatty acids (Sat:Mono, d). Bars show means ±1 standard error. $N = 6$ microcosms per bar. Values for the other PLFA biomarkers are shown in Table S2 and results of statistical analyses are reported in Table 2 and Table S3.

The high-spruce community, in addition to a smaller impairment of respiration during freeze–thaw cycles, exhibited a modest respiratory pulse during the first 3 days of recovery. As we do not have evidence for an increase in soil resources to fuel the observed pulse (discussed below), we hypothesize that the high-spruce community contains taxa that are able to temporarily take advantage of a sustained increase in temperature. Respiration rates of the low-spruce community recovered on the first day of the recovery period and did not exhibit a pulse. The additional amount of C lost via the respiratory pulse of the high-spruce community is small relative to the magnitude of soil C stocks, however such losses could scale meaningfully over decades as periods of freeze–thaw cycles become more frequent.

The rapid recovery of both communities indicates that impaired activity under freeze–thaw cycles was readily reversible and largely driven by physiological responses rather than mortality. Indeed, we did not detect overall changes in total (fungal + bacterial) microbial biomass in response to freeze–thaw cycles, in agreement with freeze–thaw experiments using peat, agricultural, and alpine dry meadow soils (Koponen et al., 2006; Lipson et al., 2000). Despite no effect of freeze–thaw cycles on total microbial biomass, we did detect modest changes in bacterial, but not fungal, biomass, as in other temperate forest studies (Sang et al., 2021). Specifically, freeze–thaw cycles reduced bacterial biomass in the less freeze–thaw tolerant low-spruce microcosms and reduced Gram-positive biomass in all microcosms. The resulting decrease in the Gram-positive to Gram-negative ratio is consistent with the greater freeze–thaw tolerance of Gram-negative bacteria (Margesin et al., 2009).

Modest microbial mortality in our study may help explain why we did not observe strong freeze–thaw-induced pulses of soil extractable nutrients or respiration, which have been associated with microbial mortality after freeze–thaw cycles in other studies (Herrmann & Witter, 2002; Schimel & Clein, 1996; Skogland et al., 1988; Yergeau & Kowalchuk, 2008). Although we did not detect significant effects of freeze–thaw cycles on soil extractable C or N, in contrast to the increases observed in other studies (Freppaz et al., 2007; Gao et al., 2018, 2021; Sanders-DeMott et al., 2018; Sang et al., 2021; Watanabe et al., 2019), there was a trend of modestly greater extractable organic C and N after freeze–thaw cycles relative to controls in the low-spruce microcosms. This may have resulted from ruptured bacterial cells (Gao et al., 2018), but it did not contribute to a respiratory pulse. Other studies show that freeze–thaw cycles release more nutrients in soils from locations like our low-spruce site with warmer, more stable winter soil temperature histories relative to sites with colder soils (Kreyling et al., 2020; Wipf et al., 2015). However, previous observed effects of freeze–thaw cycles may have been related to differing soil characteristics among sites and thus differing soil structure disruption, whereas our study used a common bulk-soil. Additionally, although our microbial communities developed under different temperature regimes, the soil extractable C and N trends in our study may have been limited because both microbial communities still experienced cold winters and were thus likely conditioned to low temperatures, but to different extents.

For instance, greater physiological acclimation of the high-spruce community to freeze–thaw cycles may have contributed to its higher resiliency compared to the low-spruce community. The reduced ratio of saturated to monounsaturated fatty acids in response to freeze–thaw cycles was most notable in the high-spruce microcosms and may indicate adjustments of lipid membrane composition to increase fluidity during freezing conditions. This shift may also have resulted from a change in microbial community composition that favoured species with a greater degree of unsaturation in their lipid membranes (Drotz et al., 2010). Either change could indicate greater resiliency of the high-spruce community to freeze–thaw cycles. Although the low-spruce community appeared to be less resilient to freeze–thaw cycles, we emphasize that it was still robust in that respiration rapidly recovered, effects on microbial biomass were modest, and there were no significant changes in soil extractable C or N.

Overall, our findings improve our understanding of how soil microbial legacies influence freeze–thaw responses. The greater vulnerability of the low-spruce community to freeze–thaw cycles may be related to a history of warmer winter soil temperatures relative to the more cold-tolerant high-spruce community. It is possible that conditioning to soil moisture histories in the field also affected microbial responses to freeze–thaw cycles, although we controlled for soil moisture during the experiment. Whether less cold-tolerant microbial communities, like that from our low-spruce site, will eventually adjust to more frequent freeze–thaw conditions via physiological

and/or community structural changes to better maintain activity, and the timescales over which such adjustments would happen, is an important area for future research. Despite changes in microbial respiration and community composition in response to freeze–thaw cycles, some variables (e.g. fungal biomass, soil extractable C and N) were unresponsive and respiration rapidly recovered, indicating that microbial communities may concurrently exhibit vulnerabilities and resilience to freeze–thaw cycles. Taken together, our results suggest that soil microbial communities conditioned to warmer winter soil temperatures may be most vulnerable to rapid changes in freeze–thaw regimes as winters warm and snow cover disappears, but that they may be able to quickly recover if mortality is low. Determining how climate histories will affect microbial responses to winter climate change across systems and whether those effects scale to influence nutrient availability, water quality, and ecosystem and regional C balances remains a critical unmet challenge for future investigations.

AUTHOR CONTRIBUTIONS

All authors conceived the ideas and designed the methodology. Melissa A. Pastore, Karin Rand, Marie E. English and Melissa A. Knorr collected the data. Melissa A. Pastore analysed the data. Melissa A. Pastore led the writing of the manuscript, and Aimée T. Classen, E. Carol Adair, Serita D. Frey, Karin Rand and Melissa A. Knorr contributed to the drafts. All authors gave final approval for publication.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data are available from the Environmental Data Initiative (EDI): https://doi.org/10.6073/pasta/f7a45325935bb69c36c29601e953a9e1 (Pastore et al., 2023).

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REFERENCES

Aanderud, Z. T., Jones, S. E., Schoolmaster, D. R., Jr., Fierer, N., & Lennon, J. T. (2013). Sensitivity of soil respiration and microbial communities to altered snowfall. *Soil Biology and Biochemistry*, *57*, 217–227.

Adair, E. C., Barbieri, L., Schiavone, K., & Darby, H. M. (2019). Manure application decisions impact nitrous oxide and carbon dioxide emissions during non-growing season thaws. *Soil Science Society of America Journal*, *83*, 163–172.

Bååth, E. (2003). The use of neutral lipid fatty acids to indicate the physiological conditions of soil fungi. *Microbial Ecology*, *45*, 373–383.

Blankinship, J. C., & Hart, S. C. (2012). Consequences of manipulated snow cover on soil gaseous emission and N retention in the growing season: A meta-analysis. *Ecosphere*, *3*, 1–20.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*, 911–917.

Boyles, J. G., Seebacher, F., Smit, B., & McKechnie, A. E. (2011). Adaptive thermoregulation in endotherms may alter responses to climate change. *Integrative and Comparative Biology*, *51*, 676–690.

Burakowski, E. A., Contosta, A. R., Grogan, D., Nelson, S. J., Garlick, S., & Casson, N. (2022). Future of winter in northeastern North America: Climate indicators portray warming and snow loss that will impact ecosystems and communities. *Northeastern Naturalist*, *28*, 180–207.

Campbell, J. L., Socci, A. M., & Templer, P. H. (2014). Increased nitrogen leaching following soil freezing is due to decreased root uptake in a northern hardwood forest. *Global Change Biology*, *20*, 2663–2673.

Demaria, E. M., Roundy, J. K., Wi, S., & Palmer, R. N. (2016). The effects of climate change on seasonal snowpack and the hydrology of the northeastern and Upper Midwest United States. *Journal of Climate*, *29*, 6527–6541.

Deschênes, É., Brice, M.-H., & Brisson, J. (2019). Long-term impact of a major ice storm on tree mortality in an old-growth forest. *Forest Ecology and Management*, *448*, 386–394.

Doane, T. A., & Horwáth, W. R. (2003). Spectrophotometric determination of nitrate with a single reagent. *Analytical Letters*, *36*, 2713–2722.

Drotz, S. H., Sparrman, T., Nilsson, M. B., Schleucher, J., & Öquist, M. G. (2010). Both catabolic and anabolic heterotrophic microbial activity proceed in frozen soils. *Proceedings of the National Academy of Sciences of the United States of America*, *107*, 21046–21051.

Feng, X., Nielsen, L. L., & Simpson, M. J. (2007). Responses of soil organic matter and microorganisms to freeze–thaw cycles. *Soil Biology and Biochemistry*, *39*, 2027–2037.

Fietz, J., Langer, F., & Schlund, W. (2020). They like it cold, but only in winter: Climate-mediated effects on a hibernator. *Functional Ecology*, *34*, 2098–2109.

Forster, J. C. (1995). Soil nitrogen. In K. Alef & P. Nannipieri (Eds.), *Methods in applied soil microbiology and biochemistry* (pp. 79–87). Academic Press.

Freppaz, M., Williams, B. L., Edwards, A. C., Scalenghe, R., & Zanini, E. (2007). Labile nitrogen, carbon, and phosphorus pools and nitrogen mineralization and immobilization rates at low temperatures in seasonally snow-covered soils. *Biology and Fertility of Soils*, *43*, 519–529.

Frostegård, A., & Bååth, E. (1996). The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils*, *22*, 59–65.

Gao, D., Bai, E., Yang, Y., Zong, S., & Hagedorn, F. (2021). A global meta-analysis on freeze-thaw effects on soil carbon and phosphorus cycling. *Soil Biology and Biochemistry*, *159*, 108283.

Gao, D., Zhang, L., Liu, J., Peng, B., Fan, Z., Dai, W., Jiang, P., & Bai, E. (2018). Responses of terrestrial nitrogen pools and dynamics to different patterns of freeze-thaw cycle: A meta-analysis. *Global Change Biology*, *24*, 2377–2389.

Groffman, P. M., Driscoll, C. T., Fahey, T. J., Hardy, J. P., Fitzhugh, R. D., & Tierney, G. L. (2001). Effects of mild winter freezing on soil nitrogen and carbon dynamics in a northern hardwood forest. *Biogeochemistry*, *56*, 191–213.

Guckert, J. B., Antworth, C. P., Nichols, P. D., & White, D. C. (1985). Phospholipid, ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. *FEMS Microbiology Ecology*, *1*, 147–158.

Henry, H. A. (2007). Soil freeze–thaw cycle experiments: Trends, methodological weaknesses and suggested improvements. *Soil Biology and Biochemistry*, *39*, 977–986.

Herrmann, A., & Witter, E. (2002). Sources of C and N contributing to the flush in mineralization upon freeze–thaw cycles in soils. *Soil Biology and Biochemistry*, *34*, 1495–1505.

Hood-Nowotny, R., Umana, N. H.-N., Inselbacher, E., Oswald-Lachouani, P., & Wanek, W. (2010). Alternative methods for measuring inorganic, organic, and total dissolved nitrogen in soil. *Soil Science Society of America Journal*, *74*, 1018–1027.

Inouye, D. W., Barr, B., Armitage, K. B., & Inouye, B. D. (2000). Climate change is affecting altitudinal migrants and hibernating species. *Proceedings of the National Academy of Sciences of the United States of America*, *97*, 1630–1633.

IPCC. (2021). Summary for policymakers. In V. Masson-Delmotte, P. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J. B. R. Matthews, T. K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, & B. Zhou (Eds.), *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 3–32). Cambridge University Press. https://doi.org/10.1017/9781009157896.001

Ji, X., Liu, M., Yang, J., & Feng, F. (2022). Meta-analysis of the impact of freeze–thaw cycles on soil microbial diversity and C and N dynamics. *Soil Biology and Biochemistry*, *168*, 108608.

Jurburg, S. D., Nunes, I., Brejnrod, A., Jacquiod, S., Priemé, A., Sørensen, S. J., Van Elsas, J. D., & Salles, J. F. (2017). Legacy effects on the recovery of soil bacterial communities from extreme temperature perturbation. *Frontiers in Microbiology*, *8*, 1832.

Koponen, H. T., Jaakkola, T., Keinänen-Toivola, M. M., Kaipainen, S., Tuomainen, J., Servomaa, K., & Martikainen, P. J. (2006). Microbial communities, biomass, and activities in soils as affected by freeze thaw cycles. *Soil Biology and Biochemistry*, *38*, 1861–1871.

Kreyling, J., & Henry, H. A. (2011). Vanishing winters in Germany: Soil frost dynamics and snow cover trends, and ecological implications. *Climate Research*, *46*, 269–276.

Kreyling, J., Peršoh, D., Werner, S., Benzenberg, M., & Wöllecke, J. (2012). Short-term impacts of soil freeze-thaw cycles on roots and root-associated fungi of Holcus lanatus and Calluna vulgaris. *Plant and Soil*, *353*, 19–31.

Kreyling, J., Schumann, R., & Weigel, R. (2020). Soils from cold and snowy temperate deciduous forests release more nitrogen and phosphorus after soil freeze–thaw cycles than soils from warmer, snow-poor conditions. *Biogeosciences*, *17*, 4103–4117.

Lee, Y. B., Lorenz, N., Dick, L. K., & Dick, R. P. (2007). Cold storage and pretreatment incubation effects on soil microbial properties. *Soil Science Society of America Journal*, *71*, 1299–1305.

Lipson, D. A., Schmidt, S. K., & Monson, R. K. (2000). Carbon availability and temperature control the post-snowmelt decline in alpine soil microbial biomass. *Soil Biology and Biochemistry*, *32*, 441–448.

Lorv, J. S., Rose, D. R., & Glick, B. R. (2014). Bacterial ice crystal controlling proteins. *Scientifica*, *2014*, 1–20.

Männistö, M. K., Tiirola, M., & Häggblom, M. M. (2009). Effect of freeze-thaw cycles on bacterial communities of Arctic tundra soil. *Microbial Ecology*, *58*, 621–631.

Margesin, R., Jud, M., Tscherko, D., & Schinner, F. (2009). Microbial communities and activities in alpine and subalpine soils. *FEMS Microbiology Ecology*, *67*, 208–218.

Margesin, R., & Miteva, V. (2011). Diversity and ecology of psychrophilic microorganisms. *Research in Microbiology*, *162*, 346–361.

Meisner, A., Snoek, B. L., Nesme, J., Dent, E., Jacquiod, S., Classen, A. T., & Priemé, A. (2021). Soil microbial legacies differ following drying-rewetting and freezing-thawing cycles. *The ISME Journal*, *15*, 1207–1221.

Monson, R. K., Lipson, D. L., Burns, S. P., Turnipseed, A. A., Delany, A. C., Williams, M. W., & Schmidt, S. K. (2006). Winter forest soil respiration controlled by climate and microbial community composition. *Nature*, *439*, 711–714.

Nielsen, C. B., Groffman, P. M., Hamburg, S. P., Driscoll, C. T., Fahey, T. J., & Hardy, J. P. (2001). Freezing effects on carbon and nitrogen cycling in northern hardwood forest soils. *Soil Science Society of America Journal*, *65*, 1723–1730.

O'Leary, W. M., & Wilkinson, S. G. (1988). Gram-positive bacteria. In C. Ratledge, & S. G. Wilkinson (Eds.), *Microbial Lipids* (Vol. 1, pp. 117–201). Academic Press.

Oztas, T., & Fayetorbay, F. (2003). Effect of freezing and thawing processes on soil aggregate stability. *Catena*, *52*, 1–8.

Pastore, M. A., Classen, A. T., English, M. E., Frey, S. D., Knorr, M. A., Rand, K., & Adair, E. C. (2023). Freeze-thaw microcosm experiment data 2021 ver 1. *Environmental Data Initiative*. https://doi.org/10.6073/pasta/f7a45325935bb69c36c29601e953a9e1

Perez-Mon, C., Frey, B., & Frossard, A. (2020). Functional and structural responses of arctic and alpine soil prokaryotic and fungal communities under freeze-thaw cycles of different frequencies. *Frontiers in Microbiology*, *11*, 982.

Ren, Y., Zhang, L., Yang, K., Li, Z., Yin, R., Tan, B., Wang, L., Liu, Y., Li, H., & You, C. (2020). Short-term effects of snow cover manipulation on soil bacterial diversity and community composition. *Science of the Total Environment*, *741*, 140454.

Ruelland, E., Vaultier, M.-N., Zachowski, A., & Hurry, V. (2009). Cold signalling and cold acclimation in plants. *Advances in Botanical Research*, *49*, 35–150.

Ruess, L., & Chamberlain, P. M. (2010). The fat that matters: Soil food web analysis using fatty acids and their carbon stable isotope signature. *Soil Biology and Biochemistry*, *42*, 1898–1910.

Rustad, L. E., Campbell, J. L., Driscoll, C. T., Fahey, T. J., Groffman, P. M., Schaberg, P. G., Hawley, G. J., Halm, I., Bowles, F., & Leuenberger, W. (2020). Experimental approach and initial forest response to a simulated ice storm experiment in a northern hardwood forest. *PLoS ONE*, *15*, e0239619.

Sanders-DeMott, R., Sorensen, P. O., Reinmann, A. B., & Templer, P. H. (2018). Growing season warming and winter freeze–thaw cycles reduce root nitrogen uptake capacity and increase soil solution nitrogen in a northern forest ecosystem. *Biogeochemistry*, *137*, 337–349.

Sang, C., Xia, Z., Sun, L., Sun, H., Jiang, P., Wang, C., & Bai, E. (2021). Responses of soil microbial communities to freeze–thaw cycles in a Chinese temperate forest. *Ecological Processes*, *10*, 1–18.

Schimel, J., Balser, T. C., & Wallenstein, M. (2007). Microbial stress-response physiology and its implications for ecosystem function. *Ecology*, *88*, 1386–1394.

Schimel, J. P., & Clein, J. S. (1996). Microbial response to freeze-thaw cycles in tundra and taiga soils. *Soil Biology and Biochemistry*, *28*, 1061–1066.

Semenova, T. A., Morgado, L. N., Welker, J. M., Walker, M. D., Smets, E., & Geml, J. (2016). Compositional and functional shifts in arctic fungal communities in response to experimentally increased snow depth. *Soil Biology and Biochemistry*, *100*, 201–209.

Sendall, K. M., Reich, P. B., Zhao, C., Jihua, H., Wei, X., Stefanski, A., Rice, K., Rich, R. L., & Montgomery, R. A. (2015). Acclimation of photosynthetic temperature optima of temperate and boreal tree species in response to experimental forest warming. *Global Change Biology*, *21*, 1342–1357.

Shivaji, S., & Prakash, J. S. (2010). How do bacteria sense and respond to low temperature? *Archives of Microbiology*, *192*, 85–95.

Siddiqui, K. S., & Cavicchioli, R. (2006). Cold-adapted enzymes. *Annual Reviews of Biochemistry*, *75*, 403–433.

Skogland, T., Lomeland, S., & Goksøyr, J. (1988). Respiratory burst after freezing and thawing of soil: Experiments with soil bacteria. *Soil Biology and Biochemistry*, *20*, 851–856.

Soil Survey Staff. (2022). Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at the following link: http://websoilsurvey.sc.egov.usda.gov/

Song, Y., Zou, Y., Wang, G., & Yu, X. (2017). Altered soil carbon and nitrogen cycles due to the freeze-thaw effect: A meta-analysis. *Soil Biology and Biochemistry*, *109*, 35–49.

Sorensen, P. O., Finzi, A. C., Giasson, M.-A., Reinmann, A. B., Sanders-DeMott, R., & Templer, P. H. (2018). Winter soil freeze-thaw cycles lead to reductions in soil microbial biomass and activity not compensated for by soil warming. *Soil Biology and Biochemistry*, *116*, 39–47.

Stenberg, B., Johansson, M., Pell, M., Sjödahl-Svensson, K., Stenström, J., & Torstensson, L. (1998). Microbial biomass and activities in soil as affected by frozen and cold storage. *Soil Biology and Biochemistry*, *30*, 393–402.

Stres, B., Philippot, L., Faganeli, J., & Tiedje, J. M. (2010). Frequent freeze–thaw cycles yield diminished yet resistant and responsive microbial communities in two temperate soils: A laboratory experiment. *FEMS Microbiology Ecology*, *74*, 323–335.

Thornton, P. E., Thornton, M. M., Mayer, B. W., Wei, Y., Devarakonda, R., Vose, R. S., & Cook, R. B. (2016). *Daymet: Daily surface weather data on a 1-km grid for North America; version 3*. ORNL DAAC. http://dx.doi.org/10.3334/ORNLDAAC/1328

Topp, G., & Ferré, P. (2002). 3.1 water content. *Methods of Soil Analysis: Part 4 Physical Methods*, *5*, 417–545.

Urakawa, R., Shibata, H., Kuroiwa, M., Inagaki, Y., Tateno, R., Hishi, T., Fukuzawa, K., Hirai, K., Toda, H., & Oyanagi, N. (2014). Effects of freeze–thaw cycles resulting from winter climate change on soil nitrogen cycling in ten temperate forest ecosystems throughout the Japanese archipelago. *Soil Biology and Biochemistry*, *74*, 82–94.

Vestal, J. R., & White, D. C. (1989). Lipid analysis in microbial ecology. *Bioscience*, *39*, 535–541.

Walker, V. K., Palmer, G. R., & Voordouw, G. (2006). Freeze-thaw tolerance and clues to the winter survival of a soil community. *Applied and Environmental Microbiology*, *72*, 1784–1792.

Wallenstein, M. D., & Hall, E. K. (2012). A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. *Biogeochemistry*, *109*, 35–47.

Wang, C., Morrissey, E. M., Mau, R. L., Hayer, M., Piñeiro, J., Mack, M. C., Marks, J. C., Bell, S. L., Miller, S. N., & Schwartz, E. (2021). The temperature sensitivity of soil: Microbial biodiversity, growth, and carbon mineralization. *The ISME Journal*, *15*, 2738–2747.

Watanabe, T., Tateno, R., Imada, S., Fukuzawa, K., Isobe, K., Urakawa, R., Oda, T., Hosokawa, N., Sasai, T., & Inagaki, Y. (2019). The effect of a freeze–thaw cycle on dissolved nitrogen dynamics and its relation to dissolved organic matter and soil microbial biomass in the soil of a northern hardwood forest. *Biogeochemistry*, *142*, 319–338.

White, D., Davis, W., Nickels, J., King, J., & Bobbie, R. (1979). Determination of the sedimentary microbial biomass by extractible lipid phosphate. *Oecologia*, *40*, 51–62.

Wilkinson, S. (1988). Gram-negative bacteria. In *Microbial lipids* (pp. 299–488). Academic Press.

Willers, C., Jansen van Rensburg, P., & Claassens, S. (2015). Phospholipid fatty acid profiling of microbial communities–a review of interpretations and recent applications. *Journal of Applied Microbiology*, *119*, 1207–1218.

Williams, C. M., Henry, H. A., & Sinclair, B. J. (2015). Cold truths: How winter drives responses of terrestrial organisms to climate change. *Biological Reviews*, *90*, 214–235.

Wipf, S., Sommerkorn, M., Stutter, M. I., Wubs, E. J., & Van Der Wal, R. (2015). Snow cover, freeze-thaw, and the retention of nutrients in an oceanic mountain ecosystem. *Ecosphere*, *6*, 1–16.

Xiao, L., Zhang, Y., Li, P., Xu, G., Shi, P., & Zhang, Y. (2019). Effects of freeze-thaw cycles on aggregate-associated organic carbon and glomalin-related soil protein in natural-succession grassland and Chinese pine forest on the Loess Plateau. *Geoderma*, *334*, 1–8.

Yanai, Y., Toyota, K., & Okazaki, M. (2004). Effects of successive soil freeze-thaw cycles on soil microbial biomass and organic matter decomposition potential of soils. *Soil Science and Plant Nutrition*, *50*, 821–829.

Yang, K., Peng, C., Peñuelas, J., Kardol, P., Li, Z., Zhang, L., Ni, X., Yue, K., Tan, B., & Yin, R. (2019). Immediate and carry-over effects of increased soil frost on soil respiration and microbial activity in a spruce forest. *Soil Biology and Biochemistry*, *135*, 51–59.

Yergeau, E., & Kowalchuk, G. A. (2008). Responses of Antarctic soil microbial communities and associated functions to temperature and freeze–thaw cycle frequency. *Environmental Microbiology*, *10*, 2223–2235.

Zelles, L. (1997). Phospholipid fatty acid profiles in selected members of soil microbial communities. *Chemosphere*, *35*, 275–294.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Full factorial ANOVA model probabilities (P>F) for total soil inorganic N, extractable organic N, and extractable organic C after freeze-thaw and recovery periods.

Table S2. PLFA, total soil inorganic N, extractable organic nitrogen, and extractable organic carbon from 12 microcosms harvested after equilibration. For PLFA, values show mean lipid biomass ± 1 standard error (nmol g dry soil^{-1}) for total fungi, total bacteria, fungal:bacterial (F:B) ratio, total fungi + bacteria, actinomycetes, Gram-positive bacteria, Gram-negative bacteria, Gram-positive:Gram:negative ratio, and saturated fatty acids:monounsaturated fatty acids (Sat:Mono). For total inorganic nitrogen and extractable organic nitrogen, values show means ± 1 standard error (μg N g dry soil^{-1}). For extractable organic carbon, values show means ± 1 standard error (μg C g dry soil^{-1}).

Table S3. F-ratios corresponding to Table 2 from full factorial ANOVA model probabilities (P>F) for lipid biomass of total fungi, total bacteria, fungal:bacterial (F:B) ratio, total fungi + bacteria, actinomycetes, Gram-positive bacteria, Gram-negative bacteria, Gram-positive:Gram:negative ratio, and saturated fatty acids:monounsaturated fatty acids (Sat:Mono).

Table S4. Mean lipid biomass ± 1 standard error (nmol g dry soil^{-1}) for total fungi, total bacteria, fungal:bacterial (F:B) ratio, total fungi + bacteria, actinomycetes, Gram-positive bacteria, Gram-negative bacteria, Gram-positive:Gram:negative ratio, and saturated fatty acids:monounsaturated fatty acids (Sat:Mono) for high-spruce or low-spruce microcosms after exposure to constant 4°C (control) or freeze-thaw cycles (FTC) for 9 days. Results of statistical analyses are shown in Tables 2 and S3.

Figure S1. Mean, minimum, and maximum daily air (a-c) and soil (d-f) temperatures recorded at the low (blue) and high (grey) elevation sites from November 2020 through June 2021. Dashes indicate a break in the x-axis from day 179-310 during the warm-season when data were not recorded. Data from each pair of air or soil sensors were averaged together per hour; one soil sensor at the high-elevation site failed and is not included.

Figure S2. Relative recovery of respiration on each day of the recovery period (constant 4°C) for high-spruce (grey) or low-spruce (blue) microcosms. Relative recovery for high-spruce and low-spruce was calculated as the mean respiration rate of the freeze-thaw treatment

(FTC) divided by that of the control group. The dashed line at 1.0 indicates where mean respiration of the freeze-thaw treatment equals that of the control (i.e., full recovery). Error bars show uncertainty in the ratios calculated via error propagation of standard deviation. Points for each day are slightly offset to improve clarity of the error bars.

Figure S3. Total soil inorganic nitrogen (a, b), extractable organic nitrogen (c, d), and extractable organic carbon (e, f) contents for high-spruce or low-spruce microcosms exposed to constant 4°C (dark grey) or freeze-thaw cycles (FTC, light grey) after the freeze-thaw period (a, c, e) and after the recovery period (b, d, f). Bars show means ±1 SE. N=6 microcosms per bar. Note that the range of the Y-axis differs among panels. Results of statistical analyses are reported in Table 2.

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