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Repeated freeze–thaw cycles increase extractable, but not total, carbon and nitrogen in a Maine coniferous soil

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ABSTRACT

Northeastern North America has been experiencing warmer winters with reduced snow accumulation, with more frequent winter freeze–thaw cycles. We conducted a laboratory experiment to investigate how increased frequency of freeze–thaw cycles (FTC) would alter soil C and N availability. Organic (O) and mineral (B) horizon soils were collected from a coniferous forest in Maine, processed to exclude roots, and then frozen in the laboratory (−10 °C) with one (FTC-1), two (FTC-2), or six (FTC-6) thaw periods (+5 °C). Soils were analyzed for extractable ammonium (NH_4-N), water extractable organic carbon (WEOC), carbon dioxide flux (respiration), and total C and N. Extractable NH_4-N increased following FTC (all levels), for both horizons. While WEOC concentrations did not change for FTC vs. control, the WEOC in O horizons had a lower $SUVA_{254}$ in FTC soils compared to control, indicating a stronger microbial influence (i.e., microbial cell lysis) in these soils after FTC. Respiration in O horizon soils decreased post-incubation and did not differ between FTC and Control soils. In the B horizon, however, FTC soils showed greater respiration than Control soils, suggesting that the newly available nutrients may have stimulated microbial activity. In contrast to these results, total C and N remained unaltered by FTC, presumably because the FTC disturbances represented mostly a translocation of C and N from one pool into another, and losses due to respiration were too small to significantly influence the large TC and TN pools. The effect of FTC on NH_4-N did not change with FTC frequency, suggesting that a single FTC is sufficient to alter both C and N availability and/or quality, and that additional FTC may not have a significant further effect. This study provides fresh insights on how organic and mineral horizon soils might respond to increased freeze–thaw frequency in winter.

1. Introduction

Soil freeze–thaw cycles can disrupt winter biogeochemical processes in temperate and boreal forest systems (Matzner and Borken, 2008; Song et al., 2017). A well-developed snowpack insulates winter soil against freezing air temperatures, but winters in northeastern North America are becoming warmer with less snow accumulation (Contosta et al., 2019), increasing the occurrence of soil frost (Henry, 2008) and altering the timing of nutrient availability (Patel et al., 2020). Soil freezing and thawing have generally been found to increase soil C and N availability due to microbial stress and cell lysis, root mortality, and/or soil aggregate disruption (DeLuca et al., 1992; Herrmann and Witter, 2002). However, it is unclear how repeated freeze–thaw cycles will influence these responses – results from prior studies are inconsistent, potentially due to vegetation influences (Koponen and Bååth, 2016) or the intensity and frequency of freezing and thawing events (Matzner and Borken, 2008; Song et al., 2017). Most snow manipulation and soil freezing studies in northeastern North America have been conducted in hardwood stands (e.g. Campbell et al., 2014b) and coniferous stands have been relatively under-studied (e.g., Campbell et al., 2014a; Reinmann

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et al., 2012). Given the substantial coniferous cover in the region, it is essential to understand how these soils might respond to changes in winter snowpack dynamics.

Here we report findings from a laboratory experiment examining the effects of freeze–thaw in coniferous soils. We hypothesized that (H1) freeze–thaw cycles (FTC) would increase soil extractable C and N concentrations, (H2) respiration would increase as a result of enhanced substrate/nutrient availability, and (H3) these responses would be amplified by repeated freeze–thaw cycles.

We collected surface organic (O) and mineral (B) horizon soils from the University of Maine's Dwight B. DeMerritt Forest (44°56′ N, 68°40′ W). Soils were acidic well-drained, coarse-loamy, isotic frigid Typic Haplorthods, with O horizons 1–5 cm thick. Additional site information can be found in Appendix A1 and in Patel et al. (2018); Tatariw et al. (2017). We sampled soils from the O horizon and the B horizon to a depth of 10 cm. The soils were sieved through 6 mm screens (O horizon) or 2 mm screens (B horizon) and homogenized to form one bulk sample for each horizon. Coarse and fine roots were excluded from the samples while processing, by sieving or removed by hand. Each homogenized soil was split into twenty experimental units (five replicates in each treatment level, see below) and incubated in glass Mason jars in the dark for seven weeks. The jars were left uncovered to allow for gas exchange and to prevent anoxic conditions during the incubation.

Soils were incubated at three treatment levels: (a) frozen, with one freeze–thaw event (FTC-1); (b) frozen with two freeze–thaw events (FTC-2); (c) frozen, with six freeze–thaw events (FTC-6). Additionally, Control soils were kept continuously thawed at 5 °C. For the freezing periods of the incubation, FTC soils were kept at −10 °C in a commercial freezer. For the thawing periods of the incubation, soils were kept at 5 °C in a commercial refrigerator. These temperature values were chosen using data from a snow manipulation field study at the site (Tatariw et al., 2017). A thaw cycle consisted of a thaw for 24 h, after which soils were returned to the freezer. At the end of the incubation, all soils were brought to 5 °C for 24 h prior to extraction and analysis.

Soils were subsampled both pre- and post-incubation for chemical analyses: (a) extractable inorganic N (ammonium, NH_4^+-N and nitrate, NO_3^--N), extracted using 2 M KCl and determined colorimetrically; (b) water-extractable organic C (WEOC), extracted using deionized milliQ water determined by combustion catalytic oxidation; (c) specific ultraviolet absorbance (SUVA), determined using absorbance of WEOC extracts at 254 nm. SUVA has been correlated with aromaticity and is considered an indicator of WEOC quality, with higher values indicative of increased microbial utilization of organic C (Kalbitz et al., 2003); (d) total C and total N, determined by combustion on dry soils. (e) Soils were also analyzed for CO_2 flux by sealing the jars and collecting headspace samples over a 1-hour period. This was done both pre-incubation and post-incubation (6 h after the incubation ended). We chose 6 h because a pilot study indicated that CO_2 peaked around this time. CO_2 concentrations were measured on a LI-COR LI-700 gas analyzer. Detailed methods are provided in Appendix A1.

2. FTC vs. control

Consistent with hypothesis H1, FTC soils showed greater NH_4-N concentrations compared to control soils, for O and B horizons (Table 1). While root mortality in response to frost can increase labile C and N soil pools (Campbell et al., 2014b), we removed roots during sieving. Thus, this increase was likely largely due to microbial cell lysis (Campbell et al., 2014a; Vestgarden and Austnes, 2009) or soil aggregate disruption (Herrmann and Witter, 2002).

WEOC was significantly greater in post-incubation than pre-incubation soils (Table 1), likely because of FTC-induced microbial stress and cell lysis, or desiccation-induced destabilization of mineral-bound C during the incubation (Bailey et al., 2019). Interestingly, post-incubation WEOC differed between FTC and control only in the B horizon soils, with FTC about half that of the control. $SUVA_{254}$ also showed contrasting trends for O and B horizon soils. In the O horizon soils, $SUVA_{254}$ was lower in FTC than in control soils (as well as pre-incubation soils), indicating that the FTC organic carbon was more labile, possibly with a stronger microbial influence, consistent with other field (Haaland et al., 2008) and laboratory (Campbell et al., 2014a; Vestgarden and Austnes, 2009) studies. In the B horizon soils, however, $SUVA_{254}$ did not differ between FTC and control, suggesting that despite increases in WEOC concentrations, the *quality* of the WEOC did not differ between the two treatments. Our contrasting results suggest that mechanisms driving C availability and/or destabilization may be different for organic vs. mineral horizon soils. Freeze-thaw cycles may have caused microbial cell lysis or SOM breakdown in the organic, but not (or, to a lesser extent) in the mineral horizon soils. These differences between organic and mineral horizons were likely an effect of both the lower microbial biomass and lower SOM content typical of the B horizon (Fierer et al., 2003; Song et al., 2017).

The respiration results further highlight the differences between O and B horizon soils. Post-incubation, both FTC and control O horizon soils had significantly lower CO_2 flux compared to pre-incubation, although the CO_2 flux did not differ significantly between FTC and control soils (Table 1). The relatively large numerical differences between FTC and control, although not statistically significant, highlight the spatial variability seen in these soils, even in a highly controlled laboratory setting. The reduced respiration in the control soils was likely because of continuous consumption of more labile C during the seven-week incubation without fresh C inputs. In contrast, since the FTC soils were frozen for most of the incubation, we expect less C loss via mineralization (respiration). We suggest instead that the FTC may have compromised the microbial capacity for C-mineralization, compared to the pre-incubation conditions. Additionally, evaporative losses reduced the moisture content post-incubation, and although these changes were relatively minor (182% w/w pre-incubation vs. 148% post-incubation), we expect the non-uniform drying of the soils (especially at the surface) may have contributed to some of the respiration losses during the incubation. This desiccation effect would be greater in the control soils than in FTC, because the unfrozen samples lost more water than the

Table 1
Means (±standard error) of variables measured for pre-incubation and post-incubation soils. Different letters indicate significant differences among levels, at $\alpha = 0.05$. The FTC data are averaged across all three FTC levels. Soil NO_3-N concentrations were below the detection limit (0.1 mg kg^{-1}) for all soils and are therefore not reported here. Total C and total N data are not available for pre-incubation samples.

	pre-incubation	post-incubation	
		control	FTC
O horizon			
NH_4-N, mg kg^{-1}	43.2 ± 0.84 c	74.6 ± 0.86 b	158 ± 3.5 a
WEOC, mg kg^{-1}	213 ± 10.5 b	355 ± 7.8 a	322 ± 7.9 a
$SUVA_{254}$, L mg^{-1} m^{-1}	4.73 ± 0.09 a	4.94 ± 0.05 a	3.82 ± 0.07 b
CO_2 flux, mg C g^{-1} hr^{-1}	16.9 ± 1.97 a	1.78 ± 0.18 b	2.70 ± 0.47 b
total C, %	–	38.9 ± 0.35 a	39.3 ± 0.29 a
total N, %	–	1.22 ± 0.02 a	1.23 ± 0.01 a
moisture, % w/w	182	148	161
B horizon			
NH_4-N, mg kg^{-1}	2.05 ± 0.04 b	2.14 ± 0.02 b	7.39 ± 0.2 a
WEOC, mg kg^{-1}	4.12 ± 0.3 c	20.9 ± 4.09 a	10.3 ± 0.47 b
$SUVA_{254}$, L mg^{-1} m^{-1}	2.79 ± 0.23 a	1.13 ± 0.38 b	1.62 ± 0.06 b
CO_2 flux, mg C g^{-1} hr^{-1}	1.93 ± 0.12 a	0.98 ± 0.2 b	2.28 ± 0.11 a
total C, %	–	3.33 ± 0.05 a	3.29 ± 0.03 a
total N, %	–	0.14 ± 0 a	0.14 ± 0 a
moisture, % w/w	37.5	11.0	22.5

Table 2
Means (±standard error) of variables measured for post-incubation FTC soils. For all variables, values were not significantly different among treatment levels ($p > 0.05$). WEOC statistics for O horizon are driven by the low values in FTC-2. However, because FTC-2 had low WEOC content to begin with (Appendix A2), the WEOC accumulation over the incubation was not significantly different.

	FTC-1	FTC-2	FTC-6	F-statistic	p-value
O horizon					
NH_4-N, mg kg^{-1}	147 ± 5.37	161 ± 7.09	165 ± 2.19	3.57	0.081
WEOC, mg kg^{-1}	313 ± 13.81	309 ± 1.99	345 ± 15.9	5.08	0.042
$SUVA_{254}$, L mg^{-1} m^{-1}	3.93 ± 0.10	3.63 ± 0.06	3.90 ± 0.14	0.22	0.646
CO_2 flux, mg C g^{-1} hr^{-1}	3.92 ± 1.21	2.55 ± 0.23	1.63 ± 0.38	3.93	0.069
total C, %	39.6 ± 0.48	39.3 ± 0.61	38.9 ± 0.42	1.03	0.331
total N, %	1.24 ± 0.02	1.24 ± 0.02	1.20 ± 0.01	3.07	0.105
B horizon					
NH_4-N, mg kg^{-1}	6.79 ± 0.15	7.13 ± 0.24	8.25 ± 0.25	26.98	<0.001
WEOC, mg kg^{-1}	10.33 ± 0.89	10.99 ± 1.01	9.59 ± 0.53	0.88	0.365
$SUVA_{254}$, L mg^{-1} m^{-1}	1.60 ± 0.06	1.55 ± 0.08	1.71 ± 0.15	1.09	0.315
CO_2 flux, mg C g^{-1} hr^{-1}	2.14 ± 0.29	2.28 ± 0.08	2.42 ± 0.15	1.08	0.318
total C, %	3.35 ± 0.07	3.25 ± 0.06	3.27 ± 0.04	0.33	0.577
total N, %	0.14 ± 0	0.14 ± 0	0.14 ± 0	0.23	0.642

frozen FTC samples, as evidenced by the gravimetric moisture values in Table 1.

In the B horizon soils, respiration remained unchanged for FTC soils, although control soils showed lower respiration than FTC soils, perhaps because the more favorable and labile WEOC was consumed during the incubation. Evaporative losses also reduced the moisture content post-incubation, and this likely also drove some of the respiration responses we report here.

The trends reported here are consistent with other mesocosm freeze–thaw studies conducted in the region. Reinmann et al. (2012) reported increased soil extractable NH_4-N in both hardwood (10x increase) and softwood soils (6x increase), and decreased DOC leaching only in softwood stands. Campbell et al. (2014a) reported decreased $SUVA_{254}$ values in both hardwood and softwood stands following severe frost, comparable to the results we report here. In contrast to these studies, however, we did not observe changes in NO_3-N concentrations, because of the low nitrification rates in these soils (Patel et al., 2018).

3. Extractable vs. total C and N

Despite shifts in NH_4-N and WEOC following the FTC, there was no difference in total C or total N between control and FTC (Table 1). We attribute this to (a) FTC disturbance appears to translocate C and N from one pool to another (e.g., from microbial biomass to extractable pool, or from aggregate-protected to available pool), which would not impact total C or N estimates; and (b) likely changes in total C due to losses such as respiration would be unlikely to be detectable with such large total C pools.

4. Effect of multiple FTC

We expected that the magnitude of soil response would increase with FTC frequency (H3). Contrary to hypothesis H3, NH_4-N did not change significantly among the three FTC levels (Table 2). Although the WEOC analysis appeared to be influenced by FTC frequency ($p = 0.042$, Table 2), this was driven by the low WEOC concentrations in FTC-2 soils. These soils also had low WEOC concentrations pre-incubation (Appendix A2, A3), and thus there was, in fact, no significant influence of FTC levels on WEOC concentrations. This is consistent with the meta-analysis by Song et al. (2017), but is in contrast with previous studies that have reported greater C and N availability with more frequent FTCs (Freppaz et al., 2007; Wipf et al., 2015). However, the soil response is likely driven by other confounding environmental, pedological, and climatic factors (Kreyling et al., 2020), as well as the intensity and duration of the FTCs (Patel et al., 2018).

The lack of differences among the FTC levels (Table 2) suggests that the first FTC may cause the most disturbance, with minimal effect of subsequent cycles. Thus, if the increased NH_4-N and WEOC was caused by microbial cell lysis, it suggests that the first FTC caused the greatest damage to the microbial community, and the surviving microbial population was resistant to further damage in subsequent cycles, similar to results by Freppaz et al. (2007). While the precise mechanism of C and N availability is beyond the scope of the current study, our findings suggest that more frequent mid-winter thaws followed by refreezing may not strongly impact nutrient availability in these coniferous Maine soils.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data and processing scripts are available at https://github.com/kaizadp/DBDF_freeze_thaw (DOI: 10.5281/zenodo.5111906) and archived at the *Environmental Data Initiative* (edi.898.1, DOI: 10.6073/pasta/496a14364a69146ecb5c0f1774048e49).

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Appendix A1. Detailed methods

Site description

This study was conducted at the University of Maine's Dwight B. DeMerritt Forest (44°56′N, 68°40′W) in Old Town, Maine. Average annual air temperature (2005–2014) at the site is 6.4 °C and annual precipitation is 1184 mm; average winter [December–January–February (DJF)] air temperature and precipitation are −6.8 °C and 272 mm, respectively (Station GHCND: USW00094644; Menne et al. 2012a, 2012b). Vegetation at the site is dominated by *Pinus strobus* (eastern white pine), *Tsuga canadensis* (eastern hemlock), and *Picea rubens* (red spruce). Soils are acidic (pH-$CaCl_2$ = 3.2), well drained, coarse-loamy, isotic frigid Typic Haplorthods (Bangor series), with O horizons 1–5 cm thick.

Gravimetric moisture

Gravimetric moisture was determined by drying subsamples at 65/105 °C for 24 hours and calculating the amount of water lost.

$$\textit{gravimetric moisture}, \% = [(FM - OD)/OD]^{*}100$$

where FM = field moist weight, OD = oven-dry weight

Soil inorganic N

Soil inorganic N (ammonium, NH_4^+-N and nitrate, NO_3^--N) was extracted using 2M KCl (soil:extractant ratio 1:10), shaken for 30 min, and filtered through Whatman® 42 filter paper. Inorganic N concentration was determined colorimetrically on an Alpkem A/E Ion Analyzer (OI Analytics) at the Maine Agricultural and Forest Experiment Station (MAFES) Analytical Laboratory.

Water extractable organic C (WEOC)

Organic C was extracted using deionized water (soil:extractant ratio 1:10), and hand-shaken for one minute before centrifuging and filtration through Nuclepore™ 0.4 μm polycarbonate membranes (procedure modified from (Hunt and Ohno, 2007). WEOC concentration was measured on a Shimadzu TOC-L total organic carbon analyzer. WEOC absorbance was measured on a Shimadzu UV-1800 spectrophotometer, and specific ultraviolet absorbance (SUVA) was calculated from absorbance at 254 nm (Weishaar et al., 2003).

Total C and total N

Air-dried soils were ground to 2 mm using a Wiley Mill, and TC and TN were determined as percentage values by dry combustion (Sollins et al., 1999) on a LECO TruMac CN analyzer (MAFES Analytical Laboratory).

Soil respiration

Carbon dioxide (CO_2) flux was determined by the static chamber method of (Collier et al., 2014). The Mason jars were closed with lids fitted with rubber septa, and 15 mL gas samples were collected from the headspace every 20 minutes for one hour. The gas samples were stored in evacuated sealed vials (Exetainer®; Labco Limited, UK), and refrigerated until analysis (maximum storage time of 48 hours). The gas samples were analyzed for CO_2 concentrations on a LI-COR LI-7000 gas analyzer, and converted from volumetric to mass using the Ideal Gas Law:

$$PV = nRT$$

where P = pressure (1 atm), V = headspace volume, n = number of moles, R = Ideal Gas Constant (0.0821 atm L mol^{-1} K^{-1}), T = temperature (278 K).

We evaluated concentration-vs-time data for linearity, and used the slope to calculate flux, normalized to soil weight:

$$F = SV/W$$

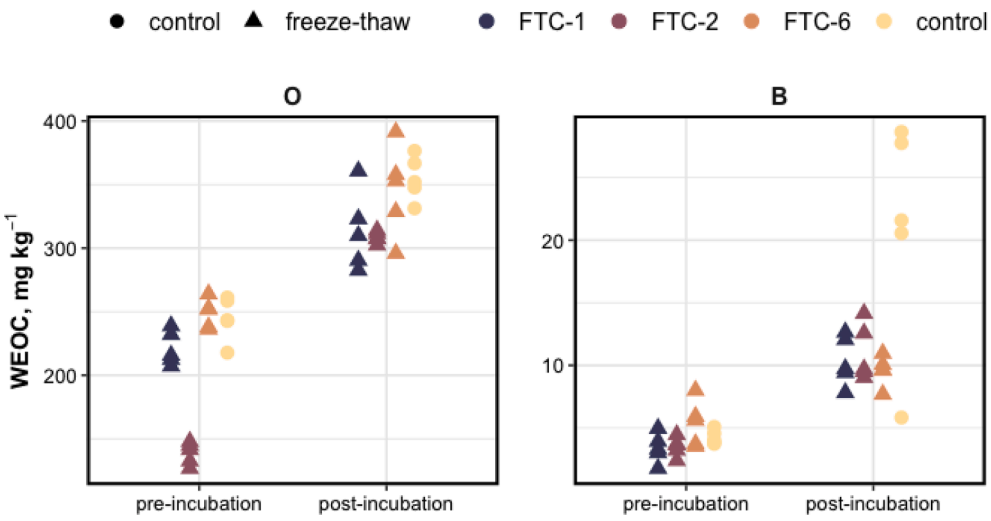
where F = flux (mol g^{-1} hr^{-1}), V = headspace volume, and W = air-dry equivalent soil weight.

Data analysis

We used one-way analysis of variance (ANOVA) to test for significant differences among treatment levels. To test overall effects of freeze-thaw cycles, the three FTC levels were combined and compared against the control. Statistical significance was determined at $\alpha = 0.05$. Data analysis was performed in R version 4.0.2 (2020-06-22) (R Core Team, 2020), using the *dplyr v1.0.4* package (Wickham et al., 2020) for data processing and *ggplot2 v3.3.3* (Wickham, 2016) and *PNWColors v0.1.0* (Lawlor, 2020) for data visualization.

Appendix A2. Water extractable organic carbon (WEOC)

Water-extractable organic carbon data for pre- and post-incubation, in O and B horizon soils. FTC-2 soils had lowest WEOC values pre-incubation. Note the different y-axis scales for the two horizons.



Appendix A3. Pre-incubation data

Pre-incubation data, separated by treatment. Values are reported as mean ± standard error.

	control	FTC-1	FTC-2	FTC-6
O horizon				
NH_4-N, mg kg^{-1}	43.6 ± 1.63	41.5 ± 0.87	41.1 ± 1.22	47.7 ± 1.24
WEOC, mg kg^{-1}	245 ± 7.72	221 ± 6.05	139 ± 3.94	248 ± 5.13
$SUVA_{254}$, L mg^{-1} m^{-1}	4.91 ± 0.02	4.33 ± 0.33	4.83 ± 0.04	4.85 ± 0.04
CO_2 flux, mg C g^{-1} hr^{-1}	17.5 ± 3.74	18.2 ± 4.62	15.0 ± 4.24	16.9 ± 4.39
B horizon				
NH_4-N, mg kg^{-1}	2.14 ± 0.07	1.98 ± 0.09	1.92 ± 0.07	2.18 ± 0.05
WEOC, mg kg^{-1}	4.25 ± 0.25	3.39 ± 0.53	3.49 ± 0.34	5.36 ± 0.82
$SUVA_{254}$, L mg^{-1} m^{-1}	2.23 ± 0.12	3.66 ± 0.61	3.05 ± 0.36	2.21 ± 0.32
CO_2 flux, mg C g^{-1} hr^{-1}	1.54 ± 0.23	2.01 ± 0.2	1.97 ± 0.19	2.2 ± 0.31

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