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Adding Depth to Our Understanding of Nitrogen Dynamics in Permafrost Soils

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Key Points:

- Soil microbes are nitrogen (N) limited in surface soils, but deeper layers are N rich; permafrost thaw will alter this stratification
- Warming will release inorganic N (1) by thawing N-rich permafrost and (2) by accelerating N mineralization within active layer soils
- These two sources of inorganic N are similar in magnitude during the first 5 years of thaw and together exceed plant N demand

Supporting Information:

- Supporting Information S1

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Abstract Losses of C from decomposing permafrost may be offset by increased productivity of tundra plants, but nitrogen availability partially limits plant growth in tundra ecosystems. In this soil incubation experiment carbon (C) and nitrogen (N) cycling dynamics were examined from the soil surface down through upper permafrost. We found that losses of CO_2 were negatively correlated to net N mineralization because C-rich surface soils mineralized little N, while deep soils had low rates of C respiration but high rates of net N mineralization. Permafrost soils released a large flush of inorganic N when initially thawed. Depth-specific rates of N mineralization from the incubation were combined with thaw depths and soil temperatures from a nearby manipulative warming experiment to simulate the potential magnitude, timing, and depth of inorganic N release during the process of permafrost thaw. Our calculations show that inorganic N released from newly thawed permafrost may be similar in magnitude to the increase in N mineralized by warmed soils in the middle of the profile. The total release of inorganic N from the soil profile during the simulated thaw process was twice the size of the observed increase in the foliar N pool observed at the manipulative experiment. Our findings suggest that increases in N availability are likely to outpace the N demand of tundra plants during the first 5 years of permafrost thaw and may increase C losses from surface soils as well as induce denitrification and leaching of N from these ecosystems.

Plain Language Summary Arctic plants are rooted in an active layer of soil that thaws during the summer months and is often nutrient-poor because of slow decomposition in these cold ecosystems. Beneath the active layer, there is a layer of soil that remains frozen year-round (permafrost). In this experiment, we collected soil cores that spanned the entire active layer and upper permafrost and incubated these soils in the lab so we could monitor their decomposition. We focus on nitrogen cycling because this is a key nutrient for the growth of arctic plants and soil microbes. We found nitrogen availability was low in shallow surface soils but high deep in the active layer and permafrost. Our results show that arctic warming will impact nitrogen release from two locations in the soil profile: at the bottom of the soil profile when nitrogen-rich permafrost soil thaws for the first time and with the active layer when decomposition is accelerated by warmer temperatures. Our calculations suggest that these two sources of nitrogen are similar in size during the first five years of permafrost thaw, exceed plant demand for nitrogen, and are likely to contribute to losses of nitrogen from warming arctic ecosystems.

1. Introduction

Anthropogenic forcing of global climate disproportionally warms high-latitude ecosystems and the thaw of permafrost is expected to have a dramatic impact on the global carbon (C) cycle in coming decades (Intergovernmental Panel on Climate Change AR5, 2014). Temperatures in high-latitude ecosystems are currently increasing by 0.6 °C per decade, a rate twice that observed at other latitudes (Cohen et al., 2014). Rising temperatures in these ecosystems are of global concern due to the 1,330–1,580 Pg C currently stored in perennially frozen ground or permafrost (Hugelius et al., 2014; Schuur et al., 2015). An estimated 120–195 Pg C will be gradually released to the atmosphere by 2100 due to the thaw and subsequent decomposition of permafrost C (Schuur et al., 2015). The magnitude of this C flux is comparable to current estimates for C emissions associated with global land use change, yet permafrost C dynamics are largely absent from large-scale climate models. Changing nitrogen (N) availability is a key source of uncertainty when modeling the future C balance of tundra ecosystems because N availability influences plant productivity as well as decomposition of soil organic matter (SOM) by the soil microbial community (Mack et al., 2004; Nowinski et al., 2008; Shaver et al., 1992).

Nitrogen is an essential nutrient for both plants and soil microbes but is in limited supply in the tundra and boreal communities overlying the permafrost zone (Hobbie et al., 2002). A shallow active layer of soil thaws during the short growing season and refreezes in winter, providing the rooting zone where plant root and microbes compete for N (Iversen et al., 2015; Nordin et al., 2004; Schimel & Chapin, 1996). There is little deposition of anthropogenic N at high latitudes, and biological N fixation rates are low in the tundra (Cleveland et al., 1999; Galloway et al., 2004). As a result, tundra plants and soil microbes rely on N released during decomposition of active layer SOM. Low temperatures, however, slow decomposition and restrict the release of biologically available N (Shaver et al., 1992). As a result, access to N constrains both plant productivity and microbial decomposition in tundra ecosystems (Chapin & Shaver, 1996; Mack et al., 2004; Nowinski et al., 2008; Van Wijk et al., 2003).

Deep C cycling in tundra ecosystems is sensitive to climate change (Hicks Pries et al., 2012; Sistla & Schimel, 2013), but our understanding of N cycling in deep soils is limited. Past work in tundra systems has focused on the organic-rich rooting zone in the upper 10-20 cm of the soil profile (DeMarco et al., 2011; Schaeffer et al., 2013; Schimel & Chapin, 1996; Schimel & Mikan, 2005; Weintraub & Schimel, 2003). Permafrost soils, however, contain high levels of biologically available N in deeper, perennially frozen layers (Finger et al., 2016; Keuper et al., 2012; Wild et al., 2013), and thawing landscapes are associated with increased transport of N from soils to streams (Abbott et al., 2015; Bowden et al., 2008; Harms & Jones, 2012). There is evidence that plants access more N during permafrost thaw (Keuper et al., 2017; Lafreniere et al., 2017; Salmon et al., 2016; Schuur et al., 2007), but the location, timing, and magnitude of increased N availability in warming tundra profiles are not well documented, and plant uptake from deep N pools is not included in current Earth system models (Koven et al., 2015).

The relationship between C losses and N release during the decomposition of SOM varies for different portions of the soil profile. In arctic tundra, soil age tends to increase with depth (Hicks Pries et al., 2012; Schirrmeister et al., 2011). Deep soils are often slower to decompose than shallow soils (Bracho et al., 2016; Schadel et al., 2014), which could be because they contain SOM that has experienced a high degree of decomposition during past periods of thaw (Berg & Staaf, 1980), because labile SOM is adsorped to charged surfaces or occluded in micropores of mineral soils (Mar n-Spiotta et al., 2014; Schmidt et al., 2011) or because of low microbial activity (Waldrop et al., 2010). There is evidence from aquatic systems that organic material transported from thawing permafrost landscapes is highly decomposable (Grewer et al., 2015; Mar n-Spiotta et al., 2014; Pautler et al., 2010) so physical protection and microbial abundance may limit decomposition of otherwise labile SOM deep within the soil profiles. During decomposition, N in SOM is either immobilized and incorporated into microbial biomass or mineralized and released into the soil solution. Net mineralization of N only occurs if microbial N demand is exceeded by the N released in SOM decomposition (Davidson et al., 1992; Vitousek & Matson, 1984; Waksman, 1932). Substrate quality can also influence the relationship between soil C:N and decomposition. In fresh SOM and leaf litter, low C:N materials are associated with high C losses due to the N available to support a large and active microbial community (Enr quez et al., 1993). In a metaanalysis of soils from permafrost landscapes, however, Schadel et al. (2014) found low C:N ratios to be associated with low rates of C turnover. Permafrost soils span a wider range of the decomposition stages than leaf litter: The relationship between slow decomposition and low C:N in permafrost soils can therefore be attributed to loss of quickly cycling C substrates during past thaw events. Low C:N permafrost soils therefore contain ample N but only slowly cycling C substrates. Soils below the current rooting zone in tundra ecosystems vary in their thaw and decomposition histories, C quality, and C:N so investigating the relationship between C losses and N availability by depth is crucial to understanding how these soil profiles may be altered by a warming climate.

The current limitation low N availability places on tundra plant productivity as well as microbial decomposition indicates that increases in N availability have the potential to impact C sequestration in these ecosystems. The relative location, timing, and magnitude of N released during from permafrost and during decomposition of warmed active layer soils will likely determine whether these fluxes of N are utilized by plants, or microbes, or are vulnerable to vertical or lateral transport. To better understand the relationship between soil depth, C losses, N availability, and warming in a permafrost soil profile, we collected soil samples spanning the entire active layer and upper permafrost at the Eight Mile Lake (EML) site in Interior Alaska. We then performed a long-term soil incubation study aimed at understanding the following three questions:

QI). How does the mineralization of C and N change throughout a permafrost soil profile?
Q2). What soil characteristics are associated with high rates of net N mineralization during a long-term soil incubation?
Q3). How does the flux of N released during the initial thaw of permafrost compare to potential N released by accelerated decomposition in a warmer active layer soils?

We focused on fluxes of inorganic N through the course of this incubation since this portion of the dissolved N pool represents N in excess of microbial demands. Evidence from ^{15}N label studies in the field have shown that plant uptake of the organic N pool is low when incubations are short enough to preclude microbial processing (Nordin et al., 2004) and is similar to plant uptake of inorganic N when incubations are extended for more than 1 day (Schimel & Chapin, 1996). Soil from 10-cm depth increments throughout the soil profile was incubated at 15 ℃, and C and N mineralization were measured throughout the laboratory experiment. We hypothesized that surface soils would have high C mineralization and low rates of N mineralization, while permafrost soils would have low C mineralization and high N mineralization (QI). We expected N mineralization to increase with and depth and hypothesized that depth would be the most important predictor for N mineralization per unit C lost due to decreasing C:N and carbon decomposability down the soil profile (Q2). Field observations of thaw depth and soil temperature at the Carbon in Permafrost Experimental Heating Research (CiPEHR) project were used to estimate the impact of 5 years of soil warming on potential N mineralization of active layer soils and inorganic N released during the initial thaw of permafrost. We anticipated the flux of inorganic N from the initial thaw of permafrost soils to be greater than the release of N from warmed active layer soils (Q3). Comparing these two fluxes of inorganic N and linking them to observed increases in the foliar N pool at CiPEHR allows us to consider the fate of N released during the process of permafrost thaw and place deep soil N dynamics into a valuable context for understanding the response of tundra ecosystems to warming.

2. Materials and Methods

2.1. Site Description

The research presented here was conducted at the EML study site located in interior Alaska at the southernmost extent of the discontinuous permafrost zone (63° 52'59"N, 149° 13'32"W). This site has mean annual temperature of -1.45 ℃ (±-0.25, 1977-2013 average), and soils are Gelisols with a 35- to 65-cm-thick organic horizon overlaying a cryoturbated mixture of glacial till and loess (Schuur et al., 2009). The active layer thickness ranges from 50 to 60 cm in undisturbed areas. Intensive monitoring of soil temperature, soil moisture, and CO_2 effiuxes along a natural permafrost gradient at EML has been ongoing since 2004 (Belshe et al., 2012; Lee et al., 2010; Schuur et al., 2009; Trucco et al., 2012; Vogel et al., 2009). The vegetation at EML is characterized as moist acidic tussock tundra and is dominated by the tussock forming sedge *Eriophorum vaginatum.* Other vascular species present include *Andromeda po/ifo/ia, Betula nana, Carex bigelowii, Empetrum nigrum, Oxycoccus microcarpus, Rhododendron subardicum, Rubus chamaemorus, Vaccinium uliginosum,* and *Vacdnium vitis-idaea* (Schuur et al., 2007). The nonvascular community is dominated by mosses *Sphagnum fuscum, Dicranum* spp., and *Pleurozium schreberi* (Deane-Coe et al., 2015; Schuur et al., 2007).

2.2. Soil Core Samples

In June of 2013, seven soil cores were collected from randomly selected locations within a 1-ha area of undisturbed tussock tundra at the EML site. The thawed upper 20 cm of the soil profile was sampled with a knife, while deeper frozen soil was sampled using a gasoline engine powered auger. Drilling proceeded to a depth of 85 cm, so cores span the active layer (-0-55 cm) as well as upper permafrost (55-85 cm). After collection all soils were placed in a freezer and shipped frozen to the University of Florida. In the lab, soil cores were split into 10-cm depth intervals (9 depth intervals per core, 7 cores, 63 soil samples in total). Subsamples were taken for determination of bulk soil %(, o/oN, bulk density, and moisture content. Bulk soil o/oC and o/oN were measured on a Costech elemental analyzer (Valencia, CA, USA), and organic soils were distinguished from mineral soils using a cutoff of 20% bulk soil o/oC.

2.3. Incubation Conditions and CO_2 Flux Measurements

Soil from each depth interval of each core was placed into eight replicate vials to allow destructive subsampling over the course of the incubation. Each vial contained approximately 10 g of soil that was rolled in a punctured sheath of aluminumfoil and perched on top of glass beads lining the bottom of the vial. Soils were not homogenized prior to incubation to preserve soilstructure.Throughout the course of the 241-day incubation, distilled water was added to the vials every 1-2 weeks to maintain field capacity moisture conditions. Water was free to drain through aluminum foil sheath and glass beads ensured the soil was not sitting in excess water and additions of water were small (1-2 ml) so N was not leached from the soils. The eight replicate vials for each soil sample were placed into one 1 L Mason jar (*n* = 63 jars). Jars were placed in an Automated Soil Incubation System (ASIS; Bracho et al., 2016). ASIS maintained the temperature inside the jars at 15 °C and measured fluxes of CO_2 from each jar. Details of ASIS flux measurements and system design are similar those described in Bracho et al. (2016). Fifteen degrees C was chosen as incubation temperature because it was the low temperature used in a separate experiment that characterized the Q_{10} of C respiration on a different set of soilcores collected at the CiPEHR experiment (Bracho et al., 2016). CO_2 fluxes were monitored continuously in ASIS for the first 30 days of the incubation. Through day 74 measurements were made every 1-2 weeks. During the remaining incubation period, measurements were made on days 146, 201, and 239. Between measurements, jars were stored in a dark growth chamber at 15 °C with lids that allowed free gas exchange. Measured flux rates for the entire incubation period were linearly interpolated to get daily flux rates, and total CO_2-C losses were determined by summing daily fluxes over the entire incubation period. The incubation was conducted for 241 days to ensure both C and N cycling dynamics had stabilized across all depths (supporting information Figures SI and S2).

2.4. Nutrient Extractions

At six timepoints during the incubation experiment, replicate vials of soil from each depth interval of each core were destructively harvested to determine extractable dissolved organic C (DOC) and dissolved inorganic N (DIN). Extractions for DOC and DIN were performed on incubation days 16, 34, 55, 82, 152, and 241. The soil extractions performed on day 16 were taken to represent the pool of initial extractable DIN following the soil's full transition from a frozen to a thawed state. Deep soils were frozen in the field when collected in June, but at this point in the spring surface soils had already thawed. All soils were frozen for transport to the laboratory. We therefore considered this 16-day window a thaw equilibration period. Initial extractable DIN values may reflect some degree of postthaw microbial processing, but the equilibration period allows comparison across depths while minimizing the confounding effect of time since thaw. Soils were extracted with 2M KCI using a 5:1 ratio for fresh soil weight (g) to volume (ml), and extracts were vacuum filtered through preleached Whatman GF/A filters (1.6-μm pore size). Ammonium and nitrate concentrations were determined on an Astoria Pacific Autoanalyzer (Clackamas, Oregon, USA). Extracts were analyzed for DOC using a nonpurgeable organic C method on a Shimadzu TOC-L analyzer (Kyoto, Japan). DIN and DOC frozen in ice-rich permafrost were included in the initial extractable DIN and DOC pools. Though organic N is an important component of N cycling within tundra soils (Schimel & Bennett, 2004), we focused on inorganic N in this study because net N mineralization indicates that N availability exceeds of microbial demand. N limitation of microbial communities in tundra soils has been documented (Nordin et al., 2004; Schimel & Chapin, 1996; Weintraub & Schimel, 2005) so it is ecologically significant to determine where in the soil profile N demand is met and net N mineralization dominates.

Daily rates of N mineralization were calculated as change in DIN concentration between two consecutive harvests divided by the number of days between the harvests. Net N mineralization was calculated as sum of all available daily rates of N mineralization. Similar calculations were performed to determine daily and net rates of DOC production. For both N mineralization and DOC production, positive values represent net microbial release, while negative values represent net immobilization.

2.5. Microbial Biomass

At the end of the incubation, microbial biomass was measured using a chloroform slurry method as described in Fierer and Schimel (2003). For each soil, four replicate 5-g samples of homogenized soil were extracted with 25-ml 2M KCl. Two of the replicates were amended with 0.25-ml chloroform to lyse microbial cell membranes, and soil extract was performed as described above. Extracts were sparged for 30 min to volatilize any chloroform present. Carbon in soil microbial biomass was calculated as the difference in DOC between extracts performed with and without chloroform.

2.6. Estimating the Impact of Permafrost Thaw on Fluxes of DIN

Warmer temperatures in high-latitude ecosystems are expected to impact fluxes of DIN by both increasing temperature within the active layer and by increasing the depth of the active layer. We utilized soil temperature and thaw depth data from the CiPEHR project to estimate the impact changes in soil temperature, and active layer thickness would have on fluxes of DIN. A soil warming treatment at CiPEHR uses snow fences to passively keep soil warm in winter (Natali et al., 2011). In the spring, the insulating layer of snow that accumulated is shoveled off the plots to avoid delaying snowmelt or adding water to soil-warmed plots. Insulation by the snow pack increases mean annual soil temperatures by 0.85-1.00 °C (2009-2013, Salmon et al., 2016). Mean annual temperatures for Alaska are expected to increase by 0.4-2.8 °C by 2035 (Intergovernmental Panel on Climate Change AR5, 2014) so the CiPEHR soil warming treatment can be considered a conservative analogy for the temperatures and thaw the EML region will experience in the next 20 years.

To relate field conditions at CiPEHR to the C and N fluxes measured in this incubation study, we utilized daily soil temperature and weekly thaw depth measurements from control and soil-warmed plots during the 2009-2013 growing seasons. Weekly thaw depth measurements were linearly interpolated to determine daily thaw depths. The length of the thaw period for each 10-cm depth interval was then calculated as the number of days remaining in the growing season after thaw of the deepest point of a given depth interval. The end of the growing season was defined as 1 October based on historic analysis of soil and air temperatures at the EML site (Osterkamp et al., 2009). To determine the temperature of 10-cm depth interval when thawed, daily average soil temperatures at 5, 10, 20, and 40 cm were extrapolated to the full depth of soil core samples using linear interpolation between each soil temperature measurement. The temperature of stable permafrost at this site (1 m, -0.2 °C, G. Celis, personal communication, 2017) was used to anchor the temperature-depth relationship and extend the soil temperature profile from 40 down to 85 cm. The average growing season soil temperatures measured at CiPEHR and the depth and temperature of stable permafrost at the EML site together exhibit a classic trumpet curve of permafrost soil temperature with depth (Andersland & Ladanyi, 1994; Romanovsky et al., 2010). Linear interpolation of soil temperature between these points was therefore justified. The resulting time series of soil temperature for each 10-cm depth interval was then filtered to only include days when thaw measurements indicate the depth interval was thawed. The average temperature during thaw (T_{thaw}) was then calculated under both soil-warmed and control conditions.

The length of the thaw period and the temperature upon thaw were used to estimate the amount of N a given depth interval of soil would mineralize during a simulated growing season (herein *Seasonal N mineralization).* This term was calculated by applying a site-specific Q_{10} to the average daily N mineralization rate so that the new rate reflected the average temperature that depth interval experienced upon thaw (Table S1). We used a Q_{10} of 2.6 based on the average temperature sensitivity of CO_2 fluxes from soils collected at the EML site (Bracho et al., 2016), which is consistent with the Q_{10} values reported for N cycling enzymes in another tundra site (Wallenstein et al. (2009). A metaanalysis by Kirschbaum (1995) found N mineralization in laboratory incubations Q_{10} values tended to be between 1.5 and 4 with no discernable difference temperature sensitivity of N cycling processes compared to C mineralization. Temperature corrected daily net N mineralization rates were multiplied by the number of days the depth interval was thawed:

$$\text{Seasonal N mineralization} = \left[\frac{\text{net N mineralization}}{241\ \text{days}}\right] \times 2.6^{\left(5^\circ C - T_{thaw}\right)/10} \times \text{days thawed}$$

We assume soil that remained frozen year-round had a Seasonal N mineralization of 0. Since the field conditions for each depth interval (days thawed and soil temperature upon thaw) were averaged prior to use in calculation of Seasonal N mineralization, the variability in Seasonal N mineralization came solely from variation in net N mineralization across the seven incubated soil cores. Seasonal N mineralization of the entire active layer was calculated as the sum of Seasonal N mineralized for all thawed depth intervals during a

Table 1
Initial Properties of Incubated Soils From the Eight Mile Lake Site

Depth (cm)	Proportion samples organic (>20% C)	Zone	Bulk soil (%C)	Bulk soil (%N)	Bulk soil (C:N)	Bulk density (g/cm3)	Gravimetric soil moisture (%)
0-5	7/7	active layer	42.64 ± 0.33	1.06 ± 0.07	41.13 ± 2.77	0.08 ± 0.01	75.28 ± 4.44
5-15	7/7	active layer	41.44 ± 0.56	0.99 ± 0.08	43.91 ± 3.61	0.08 ± 0.01	83.18 ± 1.56
15-25	7/7	active layer	39.54 ± 0.93	1.29 ± 0.13	32.77 ± 3.64	0.12 ± 0.02	81.84 ± 1.71
25-35	5/7	active layer	30.47 ± 4.60	1.42 ± 0.23	21.92 ± 0.59	0.36 ± 0.08	66.17 ± 5.81
35-45	1/7	active layer	11.87 ± 2.57	0.50 ± 0.11	24.36 ± 0.80	0.90 ± 0.09	34.40 ± 3.57
45-55	0/7	active layer	12.32 ± 1.74	0.47 ± 0.06	26.23 ± 0.80	0.73 ± 0.09	41.54 ± 3.17
55-65	1/7	permafrost	17.46 ± 2.88	0.73 ± 0.14	24.49 ± 0.96	0.38 ± 0.03	61.79 ± 2.73
65-75	3/7	permafrost	17.62 ± 3.15	0.72 ± 0.15	25.37 ± 0.90	0.29 ± 0.02	68.25 ± 1.26
75-85	4/7	permafrost	20.59 ± 3.22	0.79 ± 0.14	26.48 ± 1.13	0.41 ± 0.07	60.58 ± 4.71
Impact of increasing depth:			%C decreases	%N decreases	C:N decreases	BD increases	% decreases
significance of horizon (organic versus mineral):			organic higher	organic higher	organic higher	organic lower	organic higher
significance of zone (permafrost versus active layer):			permafrost lower	n.s	permafrost lower	n.s	n.s

Note. Values are plus or minus standard error based on seven replicate soil cores. The proportion of soils that were organic was the number of replicate samples from the seven cores for which the bulk soil had greater than 20% C. The impact of depth, soil horizon (organic versus mineral), and soil zone (permafrost versus active layer) was tested using separate linear mixed effect models. Depth was treated as a continuous variable, while horizon and zone were categorical variables. Model results are summarized in the last three rows of the table with statistically significant effects described in bold. n.s denotes non significant effects.

year. It should be noted that Seasonal N mineralization must be characterized as a potential rate: We did not account for temperature dependence of N mineralization versus immobilization, we do not consider changes to soil moisture that could accompany thaw of a permafrost soil profile, and the incubation experiment clearly lacks the influence of plant root may have on microbial decomposition.

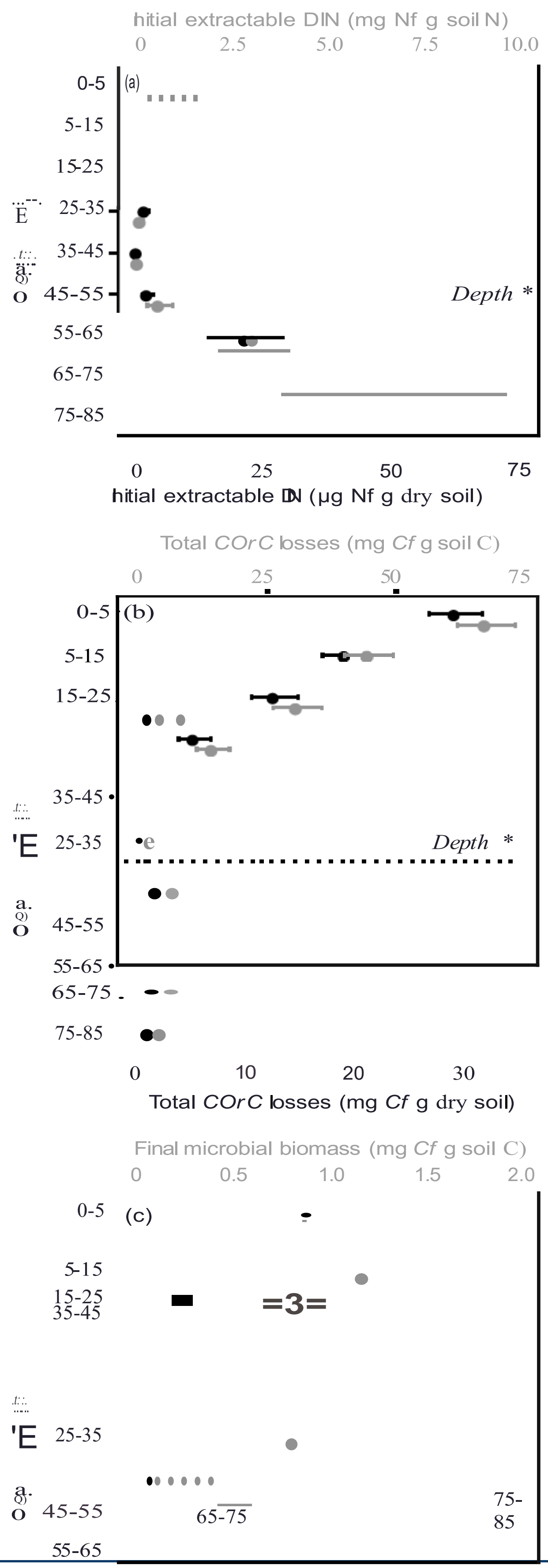
2.7. Statistical Analysis

Linear mixed effect models were used to analyze the effect of depth, time, and simulated growing season conditions on response variables. All analyses were completed in R using the lme4 package (Bates et al., 2014; R Development Core Team, 2014). Soil core was included in all models as a random effect, and data were natural log-transformed to meet assumptions of normality. The significance of the fixed effects was determined by bootstrapping parameter estimates (1,000 iterations) to establish 95% confidence intervals (CI). If the CI of a fixed effect parameter did not intersect 0, the fixed effect was considered significant (Pinheiro & Bates, 2000).

Differences in soil properties between soil horizons (organic versus mineral) and soil zones (active layer versus permafrost) were determined using linear mixed effect models that included either soil horizon or soil zone as discrete fixed effects. The impact of depth on soil properties was determined using linear mixed effect models that included depth as a continuous fixed effect.

To determine the soil properties that best predict rates of net N mineralization, a linear mixed effect model was constructed that included depth, bulk soil %N, bulk soil C:N, bulk density, total CO_2-C losses, and net DOC production as continuous fixed effects. Backward stepwise regression proceeded with an improvement in the Akaike information criterion of four as criteria for inclusion of fixed effect in final model (Zuur et al., 2009). Interactions between fixed effects were tested throughout the model selection process.

Seasonal N mineralization (g N $\cdot m^{-2} \cdot year^{-1}$) throughout the soil profile during 5 years of simulated soil-warmed and control conditions was analyzed using a linear mixed effect model that included year as a continuous fixed effect, soil warming as a discrete fixed effect, and depth as a discrete fixed effect. Depth was a discrete effect in this model due to its nonlinear relationship with Seasonal N mineralization as well as our desire to test for a significant impact of soil warming on specific depth intervals. Repeated measures were accounted for by including soil sample as a random effect nested within core. Seasonal N mineralization summed across the entire active layer was analyzed using a linear mixed effect model that included year and soil warming as fixed effects so that the impact of the treatment through time could be understood.



3. Data

3.1. Soil Properties Varied Greatly by Depth, Horizon, and Zone

Soil properties across the seven cores exhibited a high degree of variability and were generally influenced by depth, whether the soil was classified as organic or mineral and whether the soil was part of the active layer or permafrost zone. Due to cryoturbation at this site, organic soils were found in both active layer and permafrost soils (Table 1). To avoid confounding the effects of depth, horizon (organic versus mineral) and zone (active layer versus permafrost), we opted to independently test for differences across depths, soil horizons, and soil zones. All soils deeper than 55 cm were considered permafrost based on the active layer depth at the EML site at the outset of the field experiment. Across all the cores, soils from the top 25 cm of the soil profile were organic. From 35 to 55 cm the proportion of samples that were organic decreased and the bottom of the active layer (45-55 cm) was mineral soil in all cores. Below 65-cm depth, however, roughly half of the samples were organic but had significantly higher bulk densities than organic soils found in the active layer.

The bulk soil o/oC decreased significantly across depth intervals and permafrost soil had significantly lower o/oC than the active layer (18.56 ± 1.72 o/oC versus 29.71 ± 2.22 o/oC). Bulk soil o/oN was significantly lower deeper in the soil profile, and organic soils had higher bulk soil o/oN than mineral soils (1.20 ± 0.06 o/oN versus 0.49 ± 0.03 o/oN). Soil C:N decreased significantly with depth, and organic soils and active layer soils had higher C:N than their mineral and permafrost counterparts. Bulk density increased significantly with depth, but the bulk density of permafrost soils did not differ significantly from active layer soils, potentially due to ice volume present in permafrost soils. Initial gravimetric soil moisture decreased with depth and was significantly higher in organic soils than in mineral soils (75.54 ± 1.66% versus 48.84 ± 2.81, Table 1).

3.2. C0$_2$ Losses, Nutrient Extracts, and Microbial Biomass

Cumulative losses of COi-C during the incubation period decreased significantly with depth on both a per g dry soil basis *(mass basis)* as well as on a per gram soil C basis (C *basis,* Figure 1 and Tables 2 and 3). One gram of deep soil therefore respired less C than a gram of shallow soil, and the deep soils also lost a smaller proportion of their total C than shallow soils. C0$_2$-C losses from permafrost soils represented 10% of losses from active layer soils when compared on a mass basis and approximately 20% when compared on C basis. Deep soils and permafrost therefore had lower rates of COi-C release as well as lower rates of C turnover compared to shallow soils and soils in the active layer.

Depth *

0 0.2 0.4 0.6 0.8

Final microbial biomass (mg *Cf* g dry soil)

Figure 1. Depth effects on carbon and nitrogen cycling in incubated soils. Depth had a significant impact on (a) initial extractable DIN, (b) total CO_2-C losses, and (c) microbial biomass at the end of the incubation. Initial extractable DIN increased significantly with depth while total CO_2-C losses and final microbial biomass decreased significantly with depth. These depth patterns were statistically significant on a per gram dry soil basis (black points, bottom axis) as well as when analyzed in proportion to the soil C or soil N present in the bulk soil (gray points, top axis). Dotted lines indicate the active layer depth.

Table 2
Elemental Fluxes During Laboratory Incubation for 241 Days at 15°C

Depth (cm)	Proportion samples organic (>20% C)	Zone	Net N mineralization (mg N/g soil N)	Net DOC production (µg C/g soil C)	Total C02-2 losses (mg C/g soil C)
0-5	717	active layer	0.75 ± 1.20	-251.37 ± 296.48	68.44 ± 5.67
5-15	717	active layer	0.20 ± 0.14	-314.27 ± 147.75	4-0.47 ± 4.38
15-25	717	active layer	2.76 ± 2.10	-280.10 ± 55.29	31.63 ± 4.83
25-35	5/7	active layer	4.05 ± 1.35	-269.58 ± 82.54	15.19 ± 32.1
35-45	1/7	active layer	7.86 ± 3.42	46.38 ± 64.05	6.32 ± 1.47
45-55	0/7	active layer	10.06 ± 2.62	-25.24 ± 19.95	5.21 ± 0.59
55-65	1/7	permafrost	6.71 ± 3.26	-70.75 ± 68.45	5.75 ± 0.65
65-75	3/7	permafrost	3.40 ± 2.18	-355.58 ± 117.57	5.94 ± 1.06
75-85	4/7	permafrost	3.79 ± 1.68	-302.47 ± 63.01	4.49 ± 0.51
Impact of increasing depth:			n.s	n.s	COr2 losses decrease
significance of horizon (mineral versus organic):			n.s	organic lower	organic higher
significance of zone (active layer versus permafrost):			n.s	n.s	permafrost lower

Note. Values are plus or minus standard error based on the seven replicate cores. The impact of depth, soil horizon (organic versus mineral), and soil zone (permafrost versus active layer) was tested using separate linear mixed effect models. Depth was treated as a continuous variable, while horizon and zone were categorical variables. Model results are summarized in the last three rows of the table with statistically significant effects described in bold. n.s denotes non-significant effects.

Initial extractable DIN increased significantly with depth and reflects the potential pool of DIN available for plant uptake, microbial utilization, or physical transport following the first seasonal thaw of active layer soil or the initial thaw of permafrost soil (Figure 1). The pattern of increasing initial extractable DIN down through the soil profile was similar on a mass basis and on a per g soil N basis *(N basis).* Initial extractable DIN from permafrost soils was almost 10 times higher than initial extractable DIN from active layer soils on a mass basis and was over 12 times higher when compared on an N basis. In contrast, initial extractable DIN from mineral and organic soils did not differ significantly. Though initial extractable DIN exhibited a strong relationship with depth, net N mineralization did not (Table 2). Surface soils down to 15 cm had very low rates of net N mineralization, and several samples at these depths exhibited net immobilization of N, meaning microbial uptake of soil mineral N exceeded microbial production rates. Net N mineralization rates peaked at 45-55 cm, corresponding with the bottom of the active layer. Active layer soils and permafrost soils, on average, had similar rates of net N mineralization. Net N mineralization in organic soils and mineral soils was also similar. Nitrate levels in soil extracts were consistently low (-0.3-µg N/g dry soil or approximately 1% of the extractable DIN pool) so nitrification and subsequent N losses were assumed to be negligible. The contrasting

Table 3
Unear Mixed Effect Model Parameters for Incubation Data

Model	Model term	Coefficient	Lower Cl (2.5%)	Upper Cl (97.5%)
Thaw released DIN (In transformed, µg N/g soil N)	Intercept	-2.43	-3.27	-1.56
	Depth (cm)	3.93E-2	2.26E-2	5.53E-2
Net N mineralization (In transformed +3, mg N/g soil N)	Intercept	1.29	0.81	1.76
	Depth (cm)	7.63E-3	-1.27E-3	1.66E-2
Net N mineralization (In transformed +3, mg N/g soil N)	Intercept	2.06	1.79	2.32
	$C0_2$ lost (mg C/g soil Q	-1.74E-2	-2.63E-2	-8.40E-3
Total C02-C losses (In transformed, mg C/g soil Q	Intercept	4.02	3.72	4.32
	Depth (cm)	-3.51E-2	-4.11E-2	-2.92E-2
Net DOC production (µg C/g soil C)	Intercept	6.39	6.07	6.71
	Depth (cm)	3.43E-3	-2.86E-3	9.SOE-3
Final Microbial Biomass (In transformed, mg C/g soil Q	Intercept	0.41	-1.56E-2	8.64E-1
	Depth (cm)	-2.BSE-2	-3.77E-2	-2.00E-2

Note. Depth was tested as a model parameter for all response variables and significant effects are bold. Backward stepwise regression based on Akaike information criterion (see section 2) revealed net N mineralization was best predicted by cumulative $C0_2$-C losses and the final model fit is presented here.

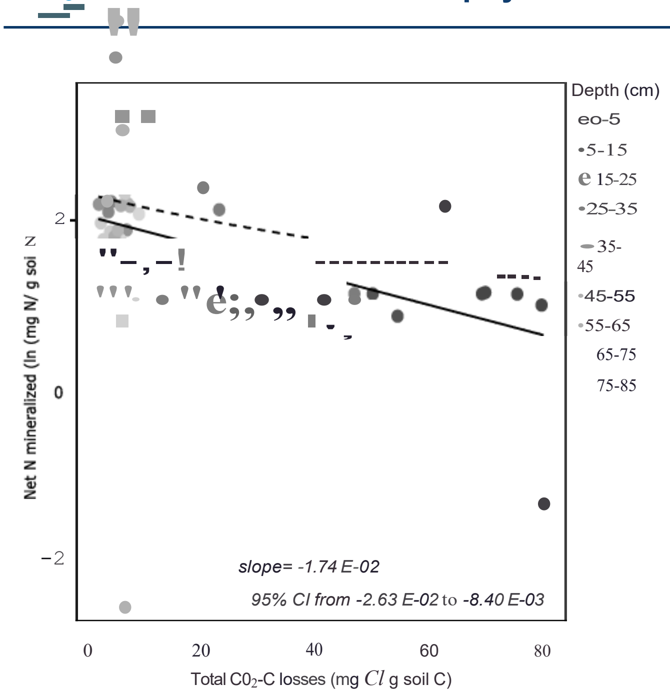


Figure 2. Rate of net N mineralization in relation to total C02-C losses. Net N mineralization is shown on a natural log scale. The solid line represents linear mixed effect model prediction, and dashed lines represent the model's 95% CI.

patterns of initial extractable DIN and net N mineralization within the soil profile suggests DIN production and DIN storage do not occur at the same depths and movement of DIN takes place within the soil profile. Measurements of net DOC production were negative in most soils, indicating microbial immobilization was greater than production of DOC during this incubation. Net DOC production on a C basis did not differ with depth or soil zone but was significantly lower in organic soils than mineral soils (-256.95 ± 82.36-µg C/g soil C versus -109.53 ± 46.25-µg C/g soil C, Tables 2 and 3). Like the pattern observed for net N mineralization, net DOC production peaked in the lower portion of the active layer (35-45 cm). The soil microbial community from 35 to 55 cm therefore had a low capacity for immobilization of both C and N despite having similar biomass to the microbial community below 55 cm at the end of the incubation (Figure 1).

Microbial biomass measured at the end of the incubation period decreased significantly with depth on both a mass and C basis, much like the pattern observed in total COrC losses (Figure 1 and Tables 2 and 3). Permafrost soils had significantly lower microbial biomass than active layer soils, and organic soils had significantly higher microbial biomass than mineral soils, and these findings were consistent on both a mass and C basis. One gram of soil C located in the permafrost, mineral horizon, or deep in the soil profile therefore supports a smaller amount of microbial biomass than its counterpart in the active layer, organic horizon, or upper portion of the soil profile.

3.3. Drivers of Net N Mineralization

Model selection showed that the only covariate important for predicting net N mineralization was cumulative COi-C losses (Figure 2 and Table 3). Depth, bulk soil C:N, soil o/oN, bulk density, and net DOC production did not significantly improve the model for net N mineralization. Cumulative COi-C losses exhibited a negative relationship with proportion of soil N mineralized (Figure 2 and Table 3, slope -1.74E-02, 95% CI from -2.63E-2 to -8.40E-3). This model was fit to natural log-transformed data, so a negative slope of this magnitude means that doubling the proportional loss of COrC from the mean observed value reduced the net N mineralization flux by 66%. Halving the COi-C losses from the mean observed value increased net N mineralization by 43%. The negative slope observed was largely driven by high *COi-C* losses and net N immobilization in soils above 25 cm (Figure 2), but it is important to note that COi-C losses outperformed depth as an accurate predictor for net N mineralization in the model selection process.

3.4. Changes in Seasonal N Mineralization During Permafrost Thaw

Field data from the CiPEHR warming experiment showed that the length of the thaw period and average temperature a soil experienced when thawed varied by depth, year, and treatment (Table SI). Five years of soil warming significantly increased the maximum depth of thaw from around 55 to 75 cm though all plots did experience some degree of progressive thaw (Salmon et al., 2016). In addition to increasing the maximum depth of thaw, the soil warming treatment increased the number of days soils was thawed and the average temperature soils maintained during the thaw period (Table SI). Detailed analysis of soil warming's impact on the soil environment can be found in Salmon et al. (2016) and (Mauritz et al., 2017).

Our calculation of Seasonal N mineralization showed that soils in the middle of the soil profile had the highest potential for producing DIN due to the high rates of net N mineralization these soils exhibited in the lab and the extended period of thaw they experienced in the field. Seasonal N mineralization was significantly elevated at 35-45 cm compared to other points in the soil profile (Figure 3 and Table 4). Soil warming significantly increased Seasonal N mineralization from 25 to 65 cm, as evidenced by the significant positive interaction terms between soil warming and these depth intervals (Figure 3 and Table 4). The significant positive interaction between the 35- and 65<m depth intervals and year shows this portion of the soil profile also had higher Seasonal N mineralization through time (Figure 3 and Table 4). The model also showed a

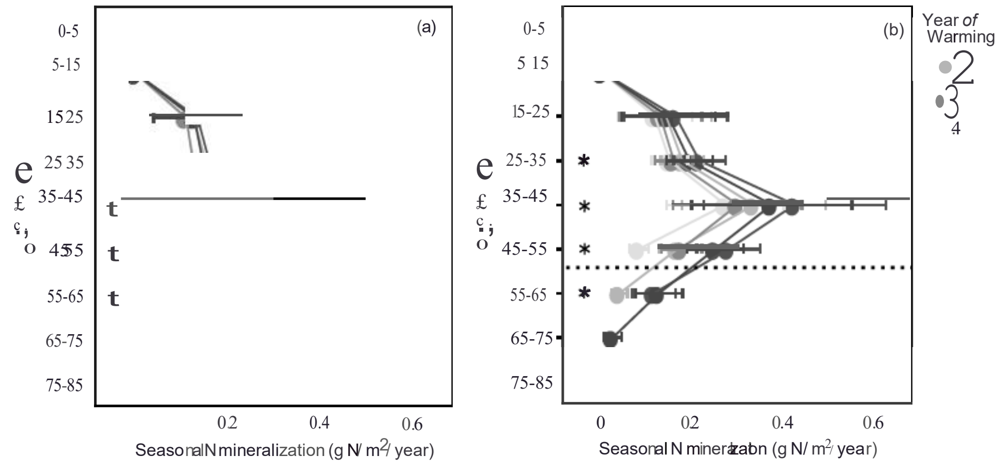


Figure 3. Simulated Seasonal N mineralization over 5 years of (a) control conditions and (b) soil-warmed conditions. The dashed line represents the interface between the active layer and permafrost at the beginning of the experiment. Depths marked with (t) in (a) had a significant interaction with year, while depths marked with (*) in (b) had a significant interaction with soil warming, indicating significantly higher N mineralization with warming at these depths. Year, soil warming, and the interaction between year and soil warming were also significant model parameters.

significant positive interaction between year and soil warming, indicating that the impact of the soil warming treatment increased through time (8.68E-3, 95% CI from 4.05E-3 to 1.32E-2). Deep, permafrost soils had very high rates of net N mineralization when incubated at 15 °C (Table 2), but in the field they thawed only for a short, cold period (<2 °C) so Seasonal N mineralization from these soils was very low (Tables 2 and S1). Similarly, soils at the surface were thawed for long, warm periods in the field but had low rates of net N mineralization in the lab and therefore low rates of Seasonal N mineralization. Soil warming's positive impact on Seasonal N mineralization of soils from 25- to 65-cm depths indicates that permafrost thaw will stimulate N availability in soils that are currently part of the rooting zone in addition to releasing N from permafrost soils (Iversen et al., 2015; Keuper et al., 2012).

Both year and the interaction between year and soil warming were found to have a significant positive impact on the total Seasonal N mineralization of the active layer (Figure 4 and Table 4). The positive year parameter (5.51E-2, 95% CI from 2.56E-2 to 8.57E-2) indicates that under both soil-warmed and control conditions, active layer Seasonal N mineralization increased with time. Dividing the year coefficient by the model intercept reveals that the trend through time in active layer Seasonal N mineralization represents an annual increase of 12%. The positive interaction between soil warming and year (7.81E-2, 95% CI from 3.69E-2 to 1.18E-1) demonstrates that the increase through time was significantly greater under soil-warmed conditions and represents an additional 17% increase in Seasonal N mineralization.

4. Discussion of Results

The results of this long-term soil incubation study allow us to understand how C and N cycling differ throughout a permafrost soil profile, the soil characteristics associated with high rates of N mineralization, and how the flux of N released during the initial thaw of permafrost may compare to the potential release of N from warming active layer soils. We found that the cumulative losses of C in the form of CO_2 decreased dramatically with soil depth, and a similar pattern was seen in microbial biomass C. In contrast, net N mineralization during the incubation peaked in the middle of the soil profile, near the bottom of the current active layer at this site. Net N mineralization across the entire soil profile was best predicted by CO_2-C losses, and the relationship between these two variables was negative. Soils with a quickly cycling C pool therefore immobilize a higher proportion of their N pool than soils with slowly cycling C. The initial release of N during the thaw of shallow permafrost soils was greater than the release observed from active layers soils. When field and lab data were combined to simulate 5 years of Seasonal N mineralization, the results showed N mineralization potential peaks in the middle of the soil profile due to the combined effect of high rates of net N mineralization in the lab and extended periods of thaw in the field.

Table 4
Unear Mixed Effect Madel Parameters for Seasonal N Mineralization Calculated for 5 Years of CiPEHR Manipulative Warming

Model	Model term	Coefficient	Lower Cl (2.5%)	Upper Cl (97.5%)
Seasonal N mineralization (g N/m^2/year)	Intercept	2.12E-2	-9.46E-2	1.34E-1
	15-cm depth	-5.67E-3	-1.68E-1	1.61E-1
	25-cm depth	948E-2	-6.48E-2	2.55E-1
	35-cm depth	1.1OE-1	-5.25E-2	2.74E-1
	45-cm depth	1.77E-1	761E-3	3.46E-1
	55-cm depth	-1.60E-2	-1.81E-1	1.43E-1
	65-cm depth	-5.96E-2	-2.20E-1	1.11E-1
	75-cm depth	-1.62E-2	-1.77E-1	145E-1
	85-cm depth	-8.15E-3	-1.73E-1	1.57E-1
	Soil warming	-2.60E-2	-492E-2	-2.38E-3
	Soil warming x 15-cm depth	193E-4	-2.77E-2	2.80E-2
	Soil warming x 25-cm depth	190E-2	-7.91E-3	4.74E-2
	Soil warming x 35-cm depth	3.66E-2	9.31E-3	6.43E-2
	Soil warming x 45-cm depth	9.46E-2	6.67E-2	122E-1
	Soil warming x 55-cm depth	876E-2	5.98E-2	1.16E-1
	Soil warming x 65-cm depth	5.39E-2	2.64E-2	821E-2
	Soil warming x 75-cm depth	5.32E-3	-2.18E-2	331E-2
	Soil warming x 85-cm depth	-7.30E-5	-2.71E-2	2.77E-2
	Year	-429E-3	-1.21E-2	3.44E-3
	Year x 15-cm depth	3.37E-5	-9.76E-3	1.00E-2
	Year x 25-cm depth	6.35E-3	-345E-3	166E-2
	Year x 35-cm depth	8.33E-3	-1.64E-3	187E-2
	Year x 45-cm depth	2.07E-2	1.07E-2	3.12E-2
	Year x 55-cm depth	3.75E-2	2.73E-2	4.78E-2
	Year x 65-cm depth	182E-2	8.16E-3	2.83E-2
	Year x 75-cm depth	2.65E-3	-7.39E-3	1.28E-2
	Year x 85-cm depth	-5.03E-5	-9.99E-3	1.04E-2
	Year x soil warming	868E-3	405E-3	132E-2
Active layer Seasonal N mineralization (g N/m^2/year)	Intercept	4.67E-1	2.57E-1	6.86E-1
	Soil warming	6.37E-2	-7.01E-2	1.98E-1
	Year	5.51E-2	2.56E-2	857E-2
	Year x soil warming	781E-2	3.69E-2	1.1BE-1

Note. Significant fixed effects are in bold. The intercept in Season N mineralization model corresponds to Seasonal N mineralization in soils at 5-cm depth under control conditions.

4.1. Thaw of Permafrost Will Release a Large Amount of Frozen DIN

The upper 10 cm of permafrost (55-65 cm) released approximately 7 times more DIN upon thaw than active layer soils in the 5- to 15-cm rooting zone (Figure la). When compared on an area basis, the difference between permafrost and active layer soils is further accentuated: Initial extractable DIN from upper permafrost soil was 35 times higher than initial extractable DIN in rooting zone. Keuper et al. (2012) observed similar values for KCl extractable DIN from shallow permafrost soils in Sweden, though the observed difference between rooting zone and permafrost soils was greater in our study. The increase in initial extractable DIN with depth suggests that thaw of even a thin layer of permafrost can have important implications for the N available to the plant and microbial community in the active layer. The pool of extractable DIN in the current active layer (0-55 cm) was 034 ± 0.14 g N/m^2. If permafrost in the SS- to 65-cm depth interval thaws, the active layer pool of extractable DIN would more than double to 1.06 ± 0.34 g N/m^2. Should thaw progress through permafrost from 65- to 75-cm depths, the active layer pool would further increase to 2.01 ± 0.61 g N/m^2. Thaw down to 85 cm would increase the extractable DIN pool to 4.05 ± 1.04 g N/m^2: an increase that over 10 times higher than the current pool. Release of this large pool of frozen DIN from permafrost could increase plant productivity and the turnover of active layer SOM, but local topography and hydrology are likely to dictate losses from this pool via lateral flow and or denitrification. Elevated DIN levels in aquatic systems have been linked to permafrost thaw (Bowden et al., 2008; Harms et al., 2014; Louiseize et al., 2014) so thawing soil profiles are unlikely to retain the entire pool of DIN previously stored in frozen permafrost.

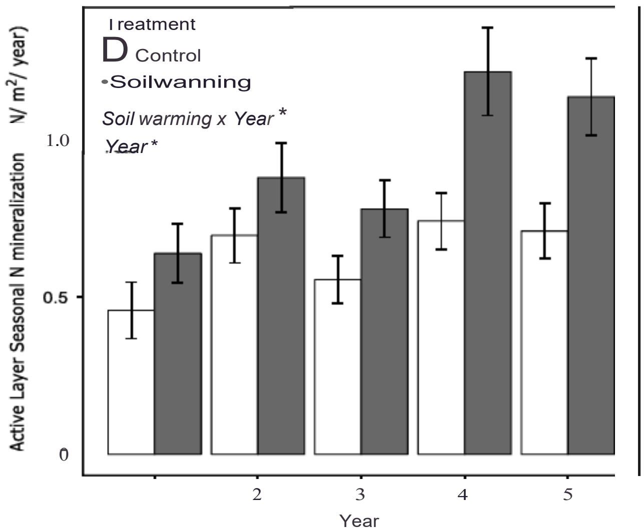


Figure 4. Simulated active layer Seasonal N mineralization through 5 years of control and soil-warmed conditions. Seasonal N mineralization was modeled using field conditions at the Carbon in Permafrost Experimental Heating Research warming experiment and combined with incubation rates of net N mineralization. Year had a significant positive effect, and there was a significant positive interaction between year and soil.

4.2. Soil Microbes Are C Limited in Deep Soils and N Limited in Surface Soils

The opposing patterns of decreasing CO_2-C losses and increasing inorganic N availability down the soil profile support our initial hypothesis (Ql) and suggests that microbial activity is constrained by N availability in surface soils and by C decomposability in deep soils. Our 241-day incubation saw losses of around SO mg C!g soil C at 0- to 1S-cm depth, which represents S.4% of the initial soil C present in these soils. Other studies that have performed similar incubations of shallow tundra soils have observed somewhat higher cumulative C losses (240-mg C/g soil C in Lavoie et al., 2011, and 1SO-mg C!g soil C in Bracho et al., 2016) but similar proportional losses (6% of the initial soil C pool present in Schadel et al., 2014). Permafrost soils in this study lost approximately *05%* of the initial soil C pool.

Surface soils exhibited distinct signs of N limitation: their initial extractable pool of DIN was small and the upper 2S cm of the soil profile mineralized only a small proportion of soil N. These low pools of extractable DIN and net N mineralization in surface soils were not, however, due to slow decomposition in these soils. Shallow soils supported a large microbial community that mineralized a substantial portion of the soil C pool present (Figure 1c and Table 2). Soils below 2S cm in contrast had a large pool of initial extractable DIN despite being associated with slow decomposition and C turnover. The small microbial biomass pool and high rate of N mineralization in deep soils suggest that the deep microbial community was limited by access to decomposable soil C, a finding that is supported by the decrease in extractable DOC with depth and slow turnover of deep soil C.

We initially hypothesized that depth would be the most important predictor of net N mineralization (Q2) but CO_2-C losses captured more of the variation in net N mineralization than the other predictors considered. We attribute this to CO_2-C losses being a more consistent indicator a soil's decomposition stage than depth. Cryoturbation within the active layer and root inputs of fresh SOM could introduce variability substrate quality and C:N across the cores collected at this site. Overall, however, deep soil at the EML site is markedly older than soil found at the surface. Hicks Pries et al. (2012) found soil from 0 to S cm to be less than SO years old, while soil at 80 cm was 8,000-10,000 radiocarbon years old. COi-C losses in our cores decreased significantly with depth, and so the relationship between COi-C losses and N mineralization can be attributed in part to depth. Our findings support the conclusions of Wild et al. (201S) who documented a strong decrease in N limitation with depth within Siberian soil profiles that was greater than the variation in N cycling parameters across surface soils that spanned a latitudinal gradient from boreal forest to tundra.

The depth-<lependent patterns in C and N mineralization in this experiment suggest that C and N cycling in surface soils is currently decoupled from deep soil cycling. Permafrost thaw therefore has the potential to connect C and N cycling throughout the soil profile. DOC from surface soils could trickle down into newly thawed deep soils where it would accelerate the decomposition of old soil C. Transport of N from frozen deep soils to the surface could similarly be achieved by vertical flow, a fluctuating water table, or plant uptake and deposition of N-rich litter. Mack et al. (2004) found that long-term fertilization of moist acidic tussock tundra reduced soil C stocks and a majority of this change could be attributed to accelerated decomposition (Sullivan et al., 2007). The strong depth-<lependent trends in N and C cycling observed in this study suggest that transport of C and N within the soil profiles will be a key factor when predicting the C storage potential of these soils under a warmer climate.

4.3. Seasonal N Mineralization During Progressive Permafrost Thaw

Seasonal N mineralization represents a speculative value for plant available inorganic N, but calculating this potential flux during progressive permafrost thaw using site-specific environmental data and temperature correction provides an important context for the soil pools and fluxes measured during our incubation.

Scaling up incubation data from high-latitude regions is an established practice due to the scarcity of field measurements data, the dramatic warming these regions are experiencing, and the simplistic representation of permafrost dynamics in large-scale ecosystem models (Schadel et al., 2014; Treat et al., 2015). Progressive permafrost thaw had the largest impact on the Seasonal N mineralization of soils from 35- to 65-cm depths. Soils in the upper 25 cm of the soil profile did not exhibit a response to soil warming nor any change through time, likely because the soil warming treatment had little effect on growing season surface soil temperatures (Salmon et al., 2016). Low net N mineralization or net N immobilization was common in these surface soils (Table 2) so our calculation of Seasonal N mineralization in surface soils relies heavily on our assumption that N mineralization and N immobilization have similar temperature dependencies. Some soils below 65 cm thawed for the first time under soil warming, but the short thaw period and cold temperatures at these depths meant that Seasonal N mineralization was extremely low. Our assertion that progressive permafrost thaw will primarily impact N mineralization from soils at 35- to 65-cm depths does not consider C or N inputs associated with leaf litter deposition on the soil surface or root litter deposition in the rooting zone. If leaf or root litter pool sizes or turnover times are impacted by the process of permafrost thaw, the transition we observed between N limitation in surface soils to and C limitation in deep soils could be altered dramatically. Inputs of root C to deep soils and inputs of litter N to surface soils might cause this depth-dependent gradient to weaken.

Soil warming significantly increased active layer Seasonal N mineralization through 5 years of simulated soil warming (Figure 4 and Table 4). Understanding a soil's capacity for N mineralization and how this characteristic varies with depth is valuable given the N limited nature of these tundra ecosystems and the lack of studies that focus on deep soil N cycling dynamics. When active layer Seasonal N mineralized by control soils were summed together for the entire 5-year period, active layer soils had the potential to mineralize 3.16 g N/m^2 (±-0.43 g N/m^2). During the same 5-year period, soil-warmed soils had the potential to mineralize 4.65 g N/m^2 (±-0.56 g N/m^2). Soil warming therefore increased the potential pool of mineralized N within the active layer by 1.49 g N/m^2 in 5 years. The majority of this newly mineralized N would be located at 35-65 cm, well within the reach of plant roots growing in soil-warmed plots (Iversen et al., 2015). Nitrogen mineralized in the active layer during 5 years of CiPEHR soil warming, however, is not the only source contributing to increases in the plant available N pool in these plots. Soil warming also increased the depth of thaw at CiPEHR from 55 to 75 cm and released previously frozen DIN. We estimate that this would increase the amount of DIN released during annual thaw by 1.67 g N/m^2. By combining increases in N mineralized by the active layer with increases in initial extractable DIN, we estimate that 5 years of permafrost thaw would increase DIN by approximately 3 g N/m^2. Tundra plant species are known to take up and use organic forms of N (Chapin et al., 1993) so this could be considered a conservative estimate of the increase in the total plant available N pool.

Foliar N pools at CiPEHR increased by 1.61 g N/m^2 during 5 years of soil warming (95% CI from 0.15 g N/m^2 to 3.09 g N/m^2; Salmon et al., 2016). This observed increase in foliar N was similar in scale to the increase in Seasonal N mineralization we calculated for the same period (1.49 g N/m^2) as well as the increase in initial extractable DIN from thawing permafrost soils (1.67 g N/m^2). The increase in foliar N at CiPEHR can therefore not be interpreted as direct evidence of plant uptake of N directly from thawing permafrost since this increase could have been sustained entirely by accelerated N mineralization in warmed active layer soils from 35 to 65 cm. Comparing these pools suggests that increases in plant available N during permafrost thaw are likely outpace N uptake by the plant community, potentially resulting in a loss of N from these terrestrial ecosystems.

4.4. Locating the Release of DIN in Space and Time

During the first 5 years of permafrost thaw, we found that the initial pulse of DIN released during the initial thaw of permafrost is likely to be similar in size to the DIN released through accelerated decomposition of warmed active layer soils. This finding stands in contrast to our initial hypothesis that thaw released DIN would be the greater of the two fluxes (Q3). Though we found these two sources of DIN to be similar in magnitude, their seasonal timing and location differ. The 1.67-g N/m^2 pulse of DIN released during the thaw of initial the top 20 cm of permafrost is likely to diminish as these soils are integrated into the annually thawing active layer. DIN in permafrost may therefore be a transient N pool that is available primarily in the fall season. Permafrost thaw at 75 cm within the CiPEHR experiment took place in late August, long after aboveground plant structures had started to senesce (data not shown). Phenological studies in the Arctic, however, suggest belowground productivity extends well beyond that of aboveground structures (Blume-Werry et al., 2016; Radville et al., 2016). Uptake of N at the thaw front in late has been directly observed for deeply rooted species (Keuper et al., 2017) so DIN released directly from permafrost cannot be considered temporally inaccessible to plants. Deeply rooted plant species and species with spongy, aerenchymous roots capable of penetrating the saturated soils often present at the thaw front, however, may have a competitive advantage when it comes to accessing this pool. In contrast to the deep DIN released from thawing permafrost, the accelerated decomposition and subsequent increase in Seasonal N mineralization (1.49-g N/m^2 DIN) is located from 35 to 65 cm and would be primarily accessible during June, July, and August. The time of year Seasonal N mineralization occurs, and its location in the soil profile may make this newly mineralized N accessible to a larger proportion of the tundra plant community.

The dramatic increases in plant available N associated with permafrost thaw have important implications. Plant N uptake and productivity at CiPEHR did increase in the presence of this additional N and increase in plant biomass offset some, but not all, losses from the permafrost C pool during this time period (calculations in Mauritz et al., 2017; Salmon et al., 2016). The estimated 3-g N/m^2 increase in the DIN pool associated with the progressive thaw is unlikely to be taken up entirely by the plant community. Denitrification and leaching into aquatic systems could remove some of the newly released DIN from the soil profile, and a portion of this N could make its way to surface soils either via surface flow or plant uptake and subsequent deposition of N-rich litter on the soil surface (Abbott et al., 2015). The relationship between depth, net N mineralization, and CO_2-C losses observed in this study suggests that the microbial community in surface soils is highly N limited. If the newly available DIN from permafrost thaw and warming-induced mineralization was transported to the soil surface, decomposition of the surface soil C pool could be further accelerated.

5. Conclusions

Our results suggest that the DIN pool increased by around 3 g N/m^2 within 5 years of permafrost thaw. This increase could be equally attributed to the pulse of DIN released following the initial thaw of permafrost soils and the increase in N mineralization from warmer active layer soils. The estimated 3-g N/m^2 increase in the DIN pool was larger than the 1.61-g N/m^2 increase in foliar N pool observed during the first 5 years of experimental warming at this site. We expect that decomposition of surface soils will be accelerated as DIN released from thawing permafrost is taken up by plants and deposited at the surface as plant litter, since the microbial community in shallow soils is currently N limited. Carbon and N cycling within these tundra soil profiles will be profoundly impacted by the process of permafrost thaw, and by understanding interactions between these two cycles, we will be better able to predict the future C balance of these high-latitude ecosystems.

Acknowledgments
The data used are listed in the references, tables, and supporting information and are available through the LTER data catalogue (CiPEHR soil temperature 001:10.6073/pasta/4ecdacb7e011bc5d0b5b8813ec324ab, CiPEHR thaw depth 001: 10.6073/pasta/d0fb84d0ca48864b482 ddaecc4ffbf76, soil incubation soil properties and final microbial biomass DOI: 10.6073/pasta/8c8a28a8d8ed4f443cb7Sed00aabd647, soil incubation CO_2 fluxes DOI: 10.6073/pasta/42154f55124b9774632094ff6242f7d3, soil incubation DIN and DOC extracts DOI: 10.6073/pasta/eef7d20be08bfc20f1 ea0540e2a1431). Supporting information can be found in supporting information Table Sl. The authors state no conflict of interest and would like to thank Patrick Soucy, Grace (rummer, Julia Reiskind, Jack Hutchings, April Melvin, and Jason Downing for supporting lab analyses, fieldwork, and data archival. This research was supported financially by Denali National Park Research Fellowship (V.G Salmon); US Department of Energy, Office of Biological and Environmental Research, Terrestrial Ecosystem Science (TES) Program, Award DE-SC0006982 (E. A.G. Schuur); National Parks Inventory and Monitoring Program (E.A.G. Schwr); National Science Foundation Bonanza Creek LTER Program, Award 1026415 (E. A.G. Schuur); National Science Foundation Office of Polar Programs, Award 1203777 (E. A.G. Schuur). Completion of this manuscript by V.G. Salmon was also supported by the Office of Biological and Environmental Research in the U.S. Department of Energy's Office of Science through Oak Ridge National Laboratory. This manuscript has been authored by UT-Battelle, LLC under contract DE-AC05-000R22725 with the U.S. Department of Energy. The US. Government retains and the publisher, by accepting the article for publication, acknowledges that the U.S. Government retains a nonexclusive, paid-up, irrevocable, worldwide license to publish or reproduce the published form of this manuscript, or allow others to do so, for U.S. Government purposes. The Department of Energy will provide public access to these results of federally sponsored research in accordance with the DOE Public Access Plan (http://energy.gov/download s/doe-public-access-plan).

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