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Mineralization responses at near-zero temperatures in three alpine soils

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Abstract Cold-season processes are known to contribute substantially to annual carbon (C) and nitrogen (N) budgets in continental high elevation and high-latitude soils, but their role in more temperate alpine ecosystems has seldom been characterized. We used a 4-month lab incubation to describe temperature (−2, 0, 5°C) and moisture [50, 90% water-holding capacity (WHC)] effects on soil C and N dynamics in two wet and one dry meadow soil from the Sierra Nevada, California. The soils varied in their capacity to process N at and below 0°C. Only the dry meadow soil mineralized N at −2°C, but the wet meadow soils switched from net N consumption at −2°C to net N mineralization at temperatures ≥0°C. When the latter soils were incubated at −2°C at either moisture level (50 or 90% WHC), net NO_3^- production decreased even as NH_4^+ continued to accumulate. The same pattern occurred in saturated (90% WHC) soils at warmer temperatures (≥0°C), suggesting that dissimilatory processes could control N cycling in these soils when they are frozen.

Keywords Cold-season processes · Nitrogen · Nitrate consumption · Ammonification · Soil moisture · Sierra Nevada

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Introduction

In many high latitude and high-elevation ecosystems, the strong seasonal variation in soil temperature and moisture regulates patterns of belowground respiration (Grogan and Chapin 1999) and net N mineralization (Giblin et al. 1991; Hobbie and Chapin 1996; Brooks et al. 1996; Schimel et al. 2004). For years, it was assumed that biological activity was suspended when soils were frozen, but more recent work indicates that microbes can remain physiologically active as long as free water

remains in the soil (Coxson and Parkinson 1987; Mikan et al. 2002), and thus can metabolize C at temperatures well below 0°C (Mikan et al. 2002). An increasing number of studies have demonstrated that microbial activity persists at ecologically significant levels throughout the winter, accounting for a large (20–70%) proportion of annual C and N turnover where winters are long (Sommerfeld et al. 1993; Brooks et al. 1997; Monson et al. 2006). In soils that approach 0°C during the winter months but rarely freeze, the dynamics of overwinter C and N mineralization are largely undescribed. Most studies to date have focused on soil freeze-thaw dynamics (e.g., Skogland et al. 1988; Schimel and Clein 1996; Lipson and Monson 1998), in situ estimates of N mineralization (e.g., Giblin et al. 1991; Brooks et al. 1996), or temperature effects on respiration (e.g., Mikan et al. 2002; Schimel and Mikan 2005) in soils that reach temperatures well below 0°C during the winter months (e.g., a minimum of −14°C in Colorado alpine soils; Brooks et al. 1996; to −20°C in Arctic tundra soils; Sturm et al. 2005). However, many soils experience less extreme cold season conditions than the Arctic and alpine, and are instead characterized by near-zero winter temperatures (EPA 2004).

We evaluated the effects of soil temperature and moisture on C and N mineralization in two alpine and one subalpine meadow soil from the southern Sierra Nevada, California. Approximately 80–90% of annual precipitation falls as snow at these sites (Sickman et al. 2001), and soils stay at or near 0°C during the winter months, insulated by the snowpack until late spring-early summer. The microbial processes that occur in cold soils under the snowpack set the conditions for the biogeochemical processes that occur at snowmelt, the hydrological event in alpine ecosystems that controls catchment-scale NO_3^- export (Foster et al. 1989). Using temperature and moisture regimes that approximate winter-spring conditions in the field, we examined the potential of the Sierra soils for supporting microbial respiration and N mineralization, and identified controls on N availability and release. We were specifically interested in the interaction between temperature and moisture as soil temperatures fell below 0°C. Finally, we used our experimental findings to speculate on the processes that may control N export in this ecosystem.

Methods

Soil characteristics

Soils were collected from two high elevation catchments located on the western slope of the southern Sierra Nevada, in Sequoia-Kings Canyon National Park: a subalpine site in the Emerald Lake watershed (36°35′49″N, 118°40′29″W, elev. 2,800 m), and an alpine site in the Topaz Lake watershed (36°37′30″N, 118°38′11″W, elev. 3,218 m). The sites are characterized by a springtime precipitation maximum and deep snowpacks (mean annual precipitation 1,510 mm at the subalpine site; 954 mm at the alpine site) that constrain the length of the growing season (Sickman et al. 2001). While all soils remain above 0°C once a snowpack develops, alpine soils are ~0.2–0.5°C colder than subalpine soils during the winter months (November–June; Fig. 1).

The subalpine soils were collected from a wet meadow dominated by Sierra willow (*Salix orestera* Schneid.) and bluejoint reedgrass [*Calamagrostis canadensis* (Michx.) Beauv.]. The alpine soils were from a dry meadow dominated by low-growing graminoids and shrubs, including arctic willow (*Salix arctica* Pallas) and dwarf bilberry (*Vaccinium caespitosum* Michx.), and from a wet meadow dominated by sedges (*Carex* spp.). Meadow soils were chosen for this study because they are believed to be areas of active C and N cycling in these bedrock-dominated watersheds, and because most surface runoff in our study sites flows through extensive meadow areas before reaching streams and lakes. All soils are classified as Lithic Cryumbrepts underlain by slightly weathered granodiorite (Huntington and Akeson 1987). Bulk density ranges from 1.0 to 1.2, and pH ranges from 4.7 to 5.0 (Huntington and Akeson 1987).

Experimental design and analyses

We used a factorial design of soil moisture × temperature to examine potential respiration and N mineralization rates in each soil type. Surface soils (0–10 cm) were collected in October 2003 from five representative areas within each site. The area sampled at each site ranged from 1.5 to 2.5 ha. Soils were returned to the lab where they were sieved to 4 mm and hand sorted to remove rock fragments, detritus, and plant material, including rhizomes and

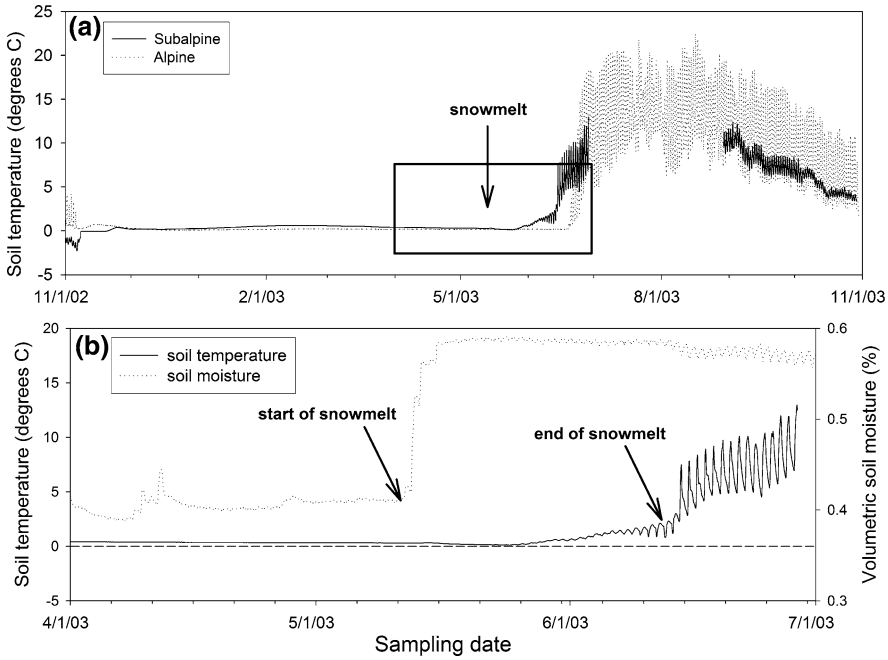

Fig. 1 (**a**) Seasonal variation in field soil temperature (5 cm) at subalpine and alpine meadow sites, November 2002–November 2003. (**b**) Variation in soil temperature and moisture at the time of snowmelt in the subalpine meadow site (Emerald Lake watershed). *Arrows* mark the approximate start and end of the snowmelt period

roots. A 4 mm sieve was used because coarse fragments comprise 25–60% of soil by mass (Hutchison and Akeson 1987).

Soils were composited by site, homogenized by hand and stored at 5°C until the start of the incubation. We bulked soils from each site to reduce sample variance when estimating the mean response to temperature and moisture treatments. We were interested in how soil processes vary with environmental conditions, and our approach assumes that the samples used to generate the composite were representative of the site. However, we cannot rule out the possibility that a different subset of soils from each site could have produced a different outcome. Because we lack an estimate of variance by site (i.e., no field replication), our statistical inferences apply only to the average soil from a site, rather than to the sites themselves, and are relevant only for the temperature × moisture treatment combinations.

Experimental treatments consisted of 10–20 g dry weight equivalent of soil in loosely capped 50 mL centrifuge tubes incubated at two levels of soil moisture [50, 90% water-holding capacity (WHC)] and three temperatures (−2, 0, 5°C), using a circulating glycol bath as described in Mikan et al. (2002). We defined 100% WHC as the gravimetric water content of soil that was first saturated and then allowed to drain over 6 h. We used temperature and moisture treatments that bracket the range of conditions experienced by soils between fall freeze-thaw and spring snowmelt (October–June), with soils remaining at or slightly above 0°C for 16–24 weeks each year (Fig. 1a; J. Sickman, unpublished data). The 5°C treatment is representative of soil temperatures at the end of snowmelt and during the early summer transition when diurnal variation in soil temperature begins to increase markedly (Fig. 1b). The −2°C treatment represents the low end of the temperature range for soils in the field and was of interest because respiration responses in arctic soils have indicated a shift in substrate use near 0°C (Michaelson and Ping 2003; Schimel and Mikan 2005). The soil moisture treatments consisted of a 'moist' treatment at 50% WHC (equivalent to ∼40% volumetric water content), which was assumed to be within the optimal range for microbial activity (Robertson et al. 1999), and a 'wet' treatment at 90% WHC (equivalent to ∼60% volumetric water content), which is representative of early snowmelt conditions, e.g., in late May-early June (Fig. 1b).

Within 3 days of the start of the experiment, we analyzed a subset of soils from each site (n = 6) for organic matter content (%SOM) by loss on ignition and for %C and %N using a Carlo-Erba CHN

analyzer. Microbial C and N were determined on a second subset (n = 3) using the chloroform fumigation-extraction technique (Vance et al. 1987) with a 5-day fumigation period and were analyzed for total C and N using a persulphate digestion (Doyle et al. 2004). We did not correct for extraction efficiency, and thus our values represent the actively cycling, chloroform-labile pool of C and N rather than total microbial biomass. We harvested a third subset of soils (n = 3) from each soil × treatment combination 3 days after the start of the incubations when respiration rates had stabilized—this was considered the initial value for the mineralization responses. Soils were extracted with 0.5 M K_2SO_4 (5:1 extraction) and filtered through pre-rinsed Whatman no. 1 filters for determination of NH_4^+ (diffusion method; Lachat Method 31-107-06-5-A) and NO_3^- (Griess–Ilovsay reaction; Lachat Method 12-107-k04-1-B).

Soil respiration was measured on all soils weekly for the first 4 weeks, and every 2 weeks thereafter over the course of the 16-week incubation. At each sampling interval, soils were capped with gas-tight lids containing rubber septa and the increase in headspace CO_2 was measured over 72 h, using an infrared gas analyzer with an in-line injection system (Li-Cor LI-6252). Headspace volume was ~35–40 mL, or 70–80% of container volume. Q_{10} values for respiration, the factor by which a 10°C increase in temperature will increase metabolism, were calculated for cumulative CO_2 release over the 16-week incubation. Cumulative CO_2 release was calculated for each soil × treatment combination by applying measured respiration rates across each sampling interval, and then summing across all sampling intervals (n = 4). A standard exponential rate equation was used over a defined temperature interval to calculate the respiration coefficient, Q_{10},

$$B = \frac{\ln\left(\frac{R_2}{R_1}\right)}{(T_2 - T_1)} \cdots \text{and} \cdots Q_{10} = e^{(10\times B)},$$

where, R_1 and R_2 are respiration rates at temperatures T_1 and T_2, respectively. Following each set of respiration measurements, soils were vented to prevent headspace CO_2 concentrations from exceeding 2%. Soils were kept loosely capped between measurements.

After 16 weeks, all soils were brought up to 5°C for 48 h for processing. One subset of soils was harvested for determination of final microbial biomass and inorganic N pools for each soil × treatment combination in each of the three soils (n = 4), as above. A second set was leached for 12 h at 5°C with HPLC-grade water for determination of soluble C and N (n = 4). Samples were filtered through ashed GF/F filters (0.7 μm), and analyzed for NH_4^+, NO_3^-, and total dissolved N (TDN) (Valderrama 1981), as above. Dissolved organic N (DON) was calculated as TDN minus NH_4^+ and NO_3^-. Dissolved organic C (DOC) was determined on a Shimadzu TOC-V (Shimadzu, Columbia, MD, USA) at the University of California, Riverside. Nitrification potential, using the shaken-slurry method over 4 h intervals (Belser and Mays 1980), and denitrification enzyme activity (DEA) over 6 h intervals (Smith and Tiedje 1979) were assayed at 5°C on a third set of soils from each soil × treatment combination (n = 3). Nitrification potentials determined by this method measure NO_3^- accumulation in a dilute ammonium phosphate solution over a fixed time interval and have been used to estimate maximum nitrification rates at a given temperature, as well as nitrifier population size (Hart et al. 1994).

We used a Monte Carlo-type approach to calculate a range of values for respiration Q_{10}s over each temperature interval for each soil and moisture combination. We had four replicate respiration measurements at each temperature and calculated a Q_{10} for each possible combination of replicates (16 total pairs). This provided both an estimate of the average Q_{10} and an estimate of the variability to compare Q_{10}s across temperature intervals. Treatment (temperature, moisture) effects on soil C and N dynamics were analyzed within each soil type using a general linear model in SAS (PROC GLM; SAS Institute, Carey NC, USA). Variables were log-transformed as necessary to meet assumptions of normality and homoscedasticity. A Tukey's Studentized Range (HSD) test was used to examine a posteriori differences among treatment means in each soil at a Bonferroni-adjusted significance level of α = 0.001. All figures show untransformed means.

Results

Microbial C and N

The soils had similar SOM, C, and N contents at the start of the experiment (Table 1). Microbial C did not change significantly over the course of the incubation (Fig. 2a), but microbial N decreased in subalpine wet meadow soils ($F = 55.2$, $df = 1$, $P < 0.001$; Fig. 2b). Temperature and moisture had no consistent effect on microbial pools at 16 weeks, although microbial C increased in the alpine dry meadow soils at 90% WHC (Fig. 2a; Table 2), and microbial N decreased in the alpine wet meadow soils over the course of the experiment (Fig. 2b; Table 2).

C and N mineralization

Carbon mineralization (cumulative CO_2 release over 16 weeks) was lowest at −2°C and increased with temperature in all soils (Fig. 3; Table 2). When soils were maintained at −2°C and 90% WHC, respiration rates were very low. When compared to rates at 0°C, the resulting Q_{10}-values exceeded 1,000 (Table 3). These values are too large to represent only the direct effects of temperature and suggest that indirect effects (e.g., diffusion limitation) could also be operating (Mikan et al. 2002).

In contrast to respiration rates, DOC concentrations were greatest at −2°C where they accounted for up to 60% of total C release (respiration + DOC losses) across all soil types (Fig. 3, Table 4). DOC losses were also greatest at 90% WHC in the subalpine wet and alpine dry meadow soils (Table 4), although significant interaction terms for all soils indicate that temperature and moisture effects on DOC were not independent.

Microbial processing of N at −2°C was dominated by net NH_4^+ production (ammonification) in all soil types and by net NO_3^- consumption in wet meadow soils (Fig. 4a–c). The interaction of these processes determined whether soils showed overall net N mineralization or immobilization (Fig. 4a) and thus the amount of soluble N recovered from frozen soils (Table 4). As a result of high net NO_3^- consumption in wet meadow soils, little, if any, N mineralization occurred at −2°C even though soils were producing NH_4^+. In comparison, low net NO_3^- consumption in dry meadow soils, paired with NH_4^+ production, resulted in measurable net N mineralization at −2°C.

In wet meadow soils at 0°C and 50% WHC, net NO_3^- production equaled or exceeded NH_4^+ production (Fig. 4c). At 5°C, wet meadow soils in the 50% WHC treatment showed maximum net nitrification and N mineralization rates (Fig. 4a, c). Wet meadow soils at 90% WHC showed net NO_3^- consumption at both 0 and 5°C. Dry meadow soils showed little change in net NH_4^+ or NO_3^- production at either 0 or 5°C.

Consistent with NH_4^+ production in frozen soils, soluble N concentrations at −2°C and 90% WHC were dominated by NH_4^+ and DON, with contributions from NO_3^- comprising only 3–4% of TDN (Table 4). However, at warmer temperatures, and even at −2°C and 50% WHC, water-extractable NO_3^- comprised ~50–90% of TDN in wet meadow soils. In dry meadow soils at 5°C, NO_3^- contributions to TDN peaked at ~20–24%, but inorganic N concentrations were very low.

Nitrification and denitrification potentials

Nitrification potentials decreased with temperature in all soils, but were not affected by moisture (Fig. 5a; Table 2). However, little net nitrification

Table 1 Organic matter content and C : N ratios of bulk soils by mass (mean ± 1 SE)

Soil properties	Wet meadow Subalpine	Wet meadow Alpine	Dry meadow Alpine
SOM (%)	34.3 (4.0)	29.5 (2.5)	26.9 (4.3)
C (%)	15.1 (1.9)	13.8 (2.2)	12.4 (2.3)
N (%)	0.9 (0.1)	1.0 (0.1)	0.8 (0.1)
C:N	16.5 (0.4)	14.4 (0.4)	16.0 (0.5)

All variables were measured at the beginning of the incubation

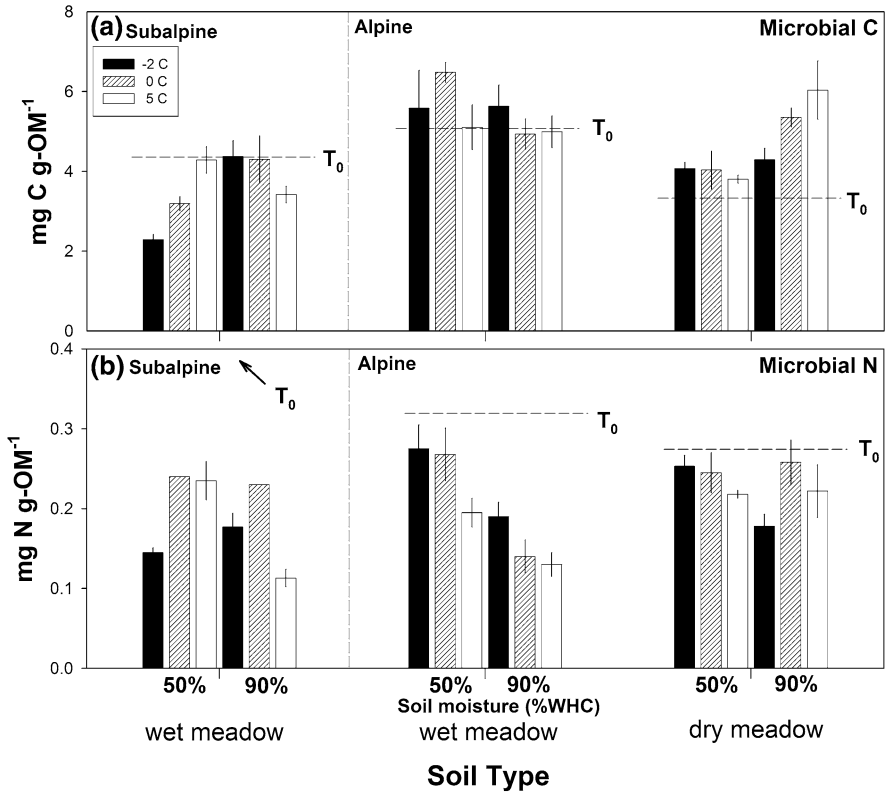

Fig. 2 (**a**) Microbial C and (**b**) N pools per g soil organic matter (*OM*) at 16 weeks (mean ± 1 SE). Soil moisture (50%, 90% WHC) treatments are indicated on the *X*-axis. *Horizontal dashed* lines indicate mean pool sizes at T_0; subalpine wet meadow microbial N pools were ~0.5 mg N g-OM^{-1} at T_0

occurred at −2°C, suggesting that any NO_3^- produced was rapidly immobilized or consumed. DEA, an index of the biomass of denitrifying enzymes in the soil, increased with temperature in wet meadow soils at 90% WHC (Fig. 5b; Table 2). Denitrification potentials were comparable across soils maintained at 50% WHC regardless of temperature.

Discussion

Microbial activity was maintained at −2°C in all soils. Respiration declined in the frozen soils, but substantial N processing continued to occur. We found two general patterns of behavior that were notable: (1) in all soils maintained at 50% WHC, net N mineralization responses differed above and below 0°C; and (2) in wet meadow soils, frozen (−2°C) and saturated (90% WHC) soils induced similar N mineralization responses (NH_4^+ production and NO_3^- consumption), suggesting that similar processes occurred under each set of conditions.

N mineralization responses above and below 0°C

Soils maintained at 50% WHC showed divergent net N mineralization responses (net N mineralization or N consumption) above and below 0°C. The wet meadow soils mineralized N only at temperatures ≥0°C, whereas dry meadow soils showed the reverse pattern, mineralizing N only at −2°C. Estimates of net N mineralization for the Sierra soils cannot be used to infer site-level differences (see *Methods*), but do suggest fundamental differences in the way that these soils process N. Similar disparities have been observed in arctic and continental alpine tundra soils; i.e., arctic wet tundra soils show substantial N mineralization at temperatures above 0°C (Giblin et al. 1991; Clein and Schimel 1995; Weintraub and Schimel 2003), whereas arctic dry tundra soils show the majority of annual net N production when frozen (Giblin et al. 1991; Brooks et al. 1996; Schimel et al. 2004).

The Sierra dry meadow soils mineralized N only at −2°C, suggesting that (a) soils could be N-limited at temperatures above 0°C, and (b) available N could

Table 2 Effect of temperature (Temp) and moisture (WHC) treatments on soil C and N dynamics measured at 16 weeks

	Subalpine wet meadow		Alpine wet meadow		Alpine dry meadow	
	Source	*F*	Source	*F*	Source	*F*
Net N mineralization	Temp	7.8**	Temp	3.4	Temp	5,116.7***
	WHC	201.6***	WHC	56.4***	WHC	1,935.8***
	$T \times W$	88.9***	$T \times W$	13.6***	$T \times W$	1,951.0***
Net nitrification	Temp	111.3***	Temp	20.5***	Temp	1.1
	WHC	320.6***	WHC	62.7***	WHC	0.1
	$T \times W$	92.5***	$T \times W$	15.2***	$T \times W$	0.0
Net ammonification	Temp	358.2***	Temp	354.4***	Temp	2,496.2***
	WHC	13.6**	WHC	0.1	WHC	1,106.4***
	$T \times W$	8.2**	$T \times W$	0.4	$T \times W$	1,123.8***
Microbial C	Temp	1.2	Temp	0.8	Temp	1.8
	WHC	7.0*	WHC	1.4	WHC	15.1**
	$T \times W$	8.5**	$T \times W$	1.2	$T \times W$	3.3
Microbial N	Temp	4.2*	Temp	4.1*	Temp	1.8
	WHC	3.4	WHC	22.1***	WHC	1.1
	$T \times W$	12.8**	$T \times W$	0.9	$T \times W$	2.6
Nitrification potential	Temp	15.6***	Temp	22.3***	Temp	5.3*
	WHC	0.0	WHC	0.4	WHC	0.6
	$T \times W$	3.3	$T \times W$	2.3	$T \times W$	0.4
Denitrification potential	Temp	23.5***	Temp	22.3***	Temp	0.2
	WHC	1.5	WHC	0.4	WHC	11.1*
	$T \times W$	28.6***	$T \times W$	2.3	$T \times W$	0.3
Cumulative CO_2 release	Temp	124.7***	Temp	61.9***	Temp	78.1***
	WHC	0.1	WHC	5.4*	WHC	8.7**
	$T \times W$	7.8**	$T \times W$	9.0**	$T \times W$	3.0

The interaction term ($T \times W$) is shown below main effects

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

increase as soils freeze. Ratios of C respired to N mineralized in the dry meadow soils are consistent with this hypothesis. At 0 and 5°C, high ratios of mineralized C : N (~820–2,100) suggested a high-microbial demand for N (cf. Nadelhoffer et al. 1991). At −2°C, the C : N ratios were lower (~10–100 in 50% WHC soils), suggesting that microbes could be accessing a greater proportion of N-rich materials. A shift by microbes to N-rich materials at subzero temperatures has been observed in arctic soils, where the increased use of the labile microbial biomass and products pool appears to result from microbial cold acclimation strategies (Schimel and Mikan 2005; Schimel et al. 2007). We hypothesize that an analogous change could be occurring in our frozen alpine dry meadow soils. If so, the shift in overwinter substrate-use patterns could result in important ecosystem-level nutrient dynamics at snowmelt.

In some alpine soils, the nearly complete turnover of the microbial community following spring snowmelt drives seasonal N dynamics (Lipson et al. 1999). The resultant summer and winter microbial communities differ in their temperature tolerances (Lipson et al. 2000) and appear to utilize C sources of differing quality (Lipson et al. 2002; Schadt et al. 2003). Continuous field incubations at the subalpine wet and alpine dry meadow sites have indicated a crash in microbial pools during the fall-winter

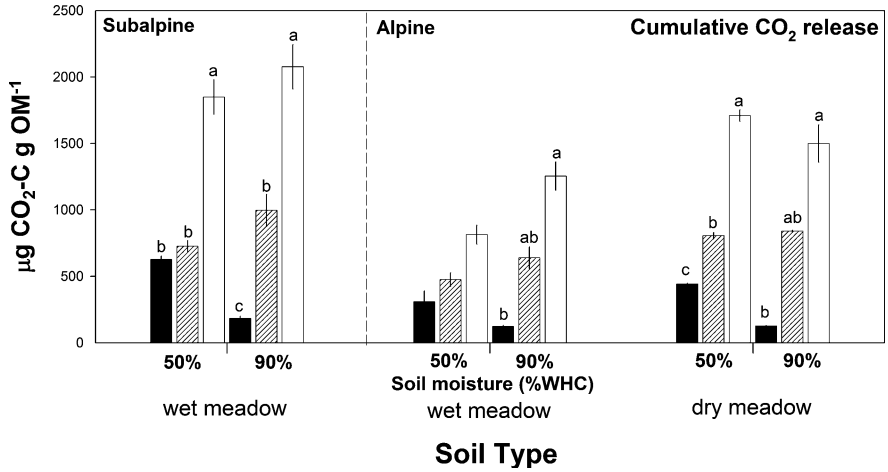

Fig. 3 Cumulative C mineralized over the 16 weeks incubation (mean ± 1 SE). Soil respiration was measured over a 24-h period every 7 days for the first 4 weeks, and every 14 days thereafter. Lower case letters denote temperature effects within moisture treatments ($\alpha = 0.001$)

Table 3 Q_{10}-values for cumulative C mineralized (µg C/g soil) at 50 and 90% WHC in subalpine and alpine meadow soils at 16 weeks (mean ± 1 SE)

Soil type	Q_{10}-values			
	−2 to 0°C		0 to 5°C	
	Water content (% WHC)		Water content (% WHC)	
	50%	90%	50%	90%
Subalpine wet meadow	2.5 (0.4)	>1,000	6.8 (0.6)	5.0 (0.6)
Alpine wet meadow	10.2 (5.6)	>1,000	3.2 (0.4)	4.5 (0.6)
Alpine dry meadow	20.8 (1.6)	>1,000	4.5 (0.2)	2.6 (0.3)

Q_{10}-values were calculated from the actual response over each temperature interval and normalized to the equivalent exponential response for a 10°C interval

transition and, at the subalpine site, a decline of ~40% in microbial N during the spring transition, when snowmelt begins (A. Miller, unpublished data 2002–2004). These field observations suggest that a turnover of the microbial pool in the Sierra soils could be induced by freeze-thaw events in the fall (Fig. 1a), and/or by the rapid increase in soil water at the start of snowmelt (Fig. 1b). In the dry meadow soils, for example, the mean increase in mineralized N between 5 and −2°C (~50 µg N g-OM^{-1} at 90% WHC) was only slightly greater than the reduction in the microbial N pool (~45 µg N g-OM^{-1}), suggesting the potential for increased utilization of microbial N as soils froze.

N mineralization responses in wet and frozen soils

In the Sierra wet meadow soils, saturated (e.g., 90% WHC) and frozen (−2°C) soils supported similar levels of NO_3^- consumption, suggesting the development of anaerobic conditions in each set of treatments. Microbes can assimilate NO_3^- when they are N-limited, but NO_3^- assimilation does not occur when excess NH_4^+ is present (Rice and Tiedje 1989). Since NH_4^+ accumulated at −2°C and 90% WHC while NO_3^- was being consumed, we suggest that the NO_3^- consumption could not have been due to assimilation, but instead must have been dissimilatory. Because the two possible dissimilatory pathways [denitrification and dissimilatory NO_3^- reduction to ammonium (DNRA)] are both anaerobic (Atlas and Bartha 1987), we hypothesize that the soils became anaerobic when either flooded or frozen. Flooding is known to induce anaerobiosis, but ice also limits the diffusion of O_2 in soils, particularly at higher moisture contents (Öquist et al. 2004). Even at lower moisture contents, however, freezing has been shown to produce anaerobic conditions in boreal and

Table 4 Variation in soluble C and N pools at 16 weeks (mean ± 1 SE)

	Subalpine-wet		Alpine-wet		Alpine-dry	
	Water content (% WHC)		Water content (% WHC)		Water content (% WHC)	
	50%	90%	50%	90%	50%	90%
NO_3^- (μg N g-OM^{-1})						
−2°C	44.2 (11.4)^{c}	1.8 (0.4)	27.5 (6.1)^{b}	1.5 (0.1)	1.5 (0.0)	1.5 (0.0)
0°C	148.2 (17.2)^{b}	3.3 (0.5)	93.8 (13.9)^{a,b}	3.2 (0.3)	1.6 (0.1)	1.6 (0.1)
5°C	241.9 (2.1)^{a}	2.5 (0.7)	139.6 (7.8)^{a}	4.2 (0.6)	3.1 (0.8)	1.5 (0.0)
	Temp***; WHC***; $T \times W$***		Temp***; WHC***; $T \times W$***			
DIN (μg N g-OM^{-1})						
−2°C	69.4 (14.6)^{b}	29.3 (1.1)^{a}	45.4 (6.7)^{b}	17.5 (0.9)^{a}	4.0 (0.4)	25.3 (0.7)^{a}
0°C	152.9 (18.1)^{a,b}	4.2 (0.3)^{b}	95.4 (14.2)^{a,b}	3.5 (0.3)^{b}	2.1 (0.1)	2.0 (0.1)^{b}
5°C	256.6 (3.3)^{a}	3.7 (0.7)^{b}	142.8 (8.1)^{a}	4.6 (0.7)^{b}	4.9 (2.1)	1.8 (0.0)^{b}
	Temp***; WHC***; $T \times W$***		Temp***; WHC***; $T \times W$***		Temp***; WHC***; $T \times W$***	
DON (μg N g-OM^{-1})						
−2°C	18.2 (1.6)^{b}	28.1 (1.2)^{a}	20.5 (1.4)	15.1 (0.7)^{a}	16.4 (1.3)^{a}	29.0 (0.0)^{a}
0°C	21.0 (3.8)^{b}	7.1 (0.9)^{b}	12.1 (1.9)	6.6 (0.5)^{b}	6.9 (0.4)^{b}	6.9 (0.1)^{b}
5°C	61.9 (2.2)^{a}	10.3 (1.1)^{b}	14.6 (2.3)	6.0 (1.1)^{b}	8.0 (0.6)^{b}	5.7 (0.1)^{b}
	Temp***; WHC***; $T \times W$***		Temp***; WHC***		Temp***; WHC***; $T \times W$***	
DOC (μg C g-OM^{-1})						
−2°C	145.8 (10.1)^{a}	281.4 (27.0)^{a}	138.8 (14.8)^{a}	116.8 (4.7)	170.9 (10.2)^{a}	255.4 (19.5)^{a}
0°C	68.7 (4.9)^{b}	88.0 (13.2)^{b}	62.8 (2.0)^{b}	76.5 (5.5)	88.9 (1.4)^{b}	109.1 (11.1)^{b}
5°C	89.6 (4.3)^{b}	132.8 (14.5)^{a,b}	60.3 (2.6)^{b}	79.7 (6.8)	103.5 (8.5)^{b}	113.2 (4.1)^{b}
	Temp***; WHC***; $T \times W$**		Temp***; $T \times W$*		Temp***; WHC***; $T \times W$**	

Different superscript letters denote differing temperature effects within moisture treatments ($\alpha = 0.001$). Main effects and temperature × moisture ($T \times M$) interactions are shown below each set of means

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

arctic soils (Clein and Schimel 1995; Öquist et al. 2004).

In our study, all frozen soils supported substantial NH_4^+ production in spite of significant decreases in respiration. Furthermore, wet meadow soils at −2°C and 90% WHC showed increased net NO_3^- consumption and decreased NO_3^- production relative to soils incubated at temperatures ≥0°C and 50% WHC. As a result, net NO_3^- production decreased even though NH_4^+ continued to accumulate at −2°C. Our findings are consistent with other studies of cold and/or wet soils in which the decrease in NO_3^- and increase in NH_4^+ and N_2O have been attributed to anaerobiosis, as indicated by increased N_2O (Dorland and Beauchamp 1991; Chantigny et al. 2002) and decreased O_2 (Chantigny et al. 2002).

The observed increase in NH_4^+ and decrease in NO_3^- in the Sierra soils could have resulted from ammonification coupled with denitrification, DNRA, or both. If ammonification and denitrification were occurring, NH_4^+ would be expected to accumulate in frozen soils due to N mineralization (Giblin et al. 1991; Schimel et al. 2004), while NO_3^- would be consumed by dentrifiers. If DNRA was occurring, then the increase in NH_4^+ would result directly from the reduction of NO_3^-. Denitrification has been widely documented in cold, organic soils where N_2O production can occur at temperatures below 0°C (Dorland and Beauchamp 1991; Öquist et al. 2004; Koponen et al. 2004). Activity often peaks when soils thaw, presumably due to increased availability of soluble substrate (Nyborg et al. 1997; Koponen et al. 2004). As soils

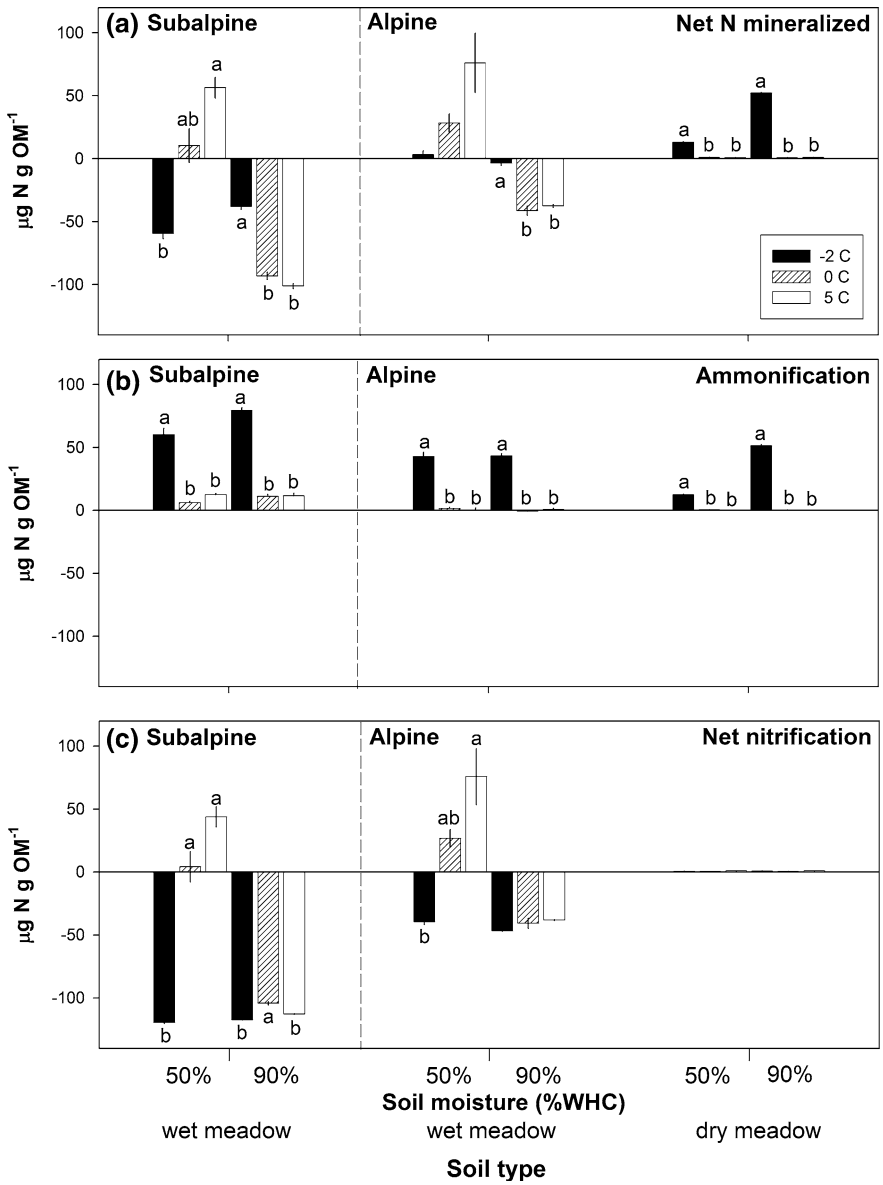

Fig. 4 (**a**) Net N mineralization, (**b**) net ammonification, and (**c**) net nitrification over the 16 week incubation (mean ± 1 SE). Values represent mineralized pools. Temperature effects within moisture treatment are as in Fig. 3

freeze, solutes become concentrated in the free water surrounding soil particles (Stähli and Sadler 1997). Encapsulated by ice, these unfrozen, solute-rich microsites become anaerobic, promoting denitrification. As soils thaw, high-soil moisture (e.g., ≥60% WHC; Öquist et al. 2004) should continue to support denitrifier activity, while low soil moisture should promote a return to aerobic conditions suitable for nitrifier activity. DNRA, on the other hand, has been observed only at temperatures well above 0°C (e.g., Silver et al. 2001; Ma and Aelion 2005), and it is unclear whether it can occur at lower temperatures (W. Silver, personal communication). If DNRA was occurring, we would expect the increase in NH_4^+ to be approximately equivalent to the decrease in NO_3^-, but in the subalpine wet meadow soil we saw NO_3^- disappear at roughly twice the rate that NH_4^+ appeared.

In the Sierra wet meadow soils, NO_3^- consumption could have been due to denitrification at temperatures ≥0°C (90% WHC), but at −2°C and 90% WHC the processes controlling NO_3^- consumption were less clear. Denitrifier activity measured as

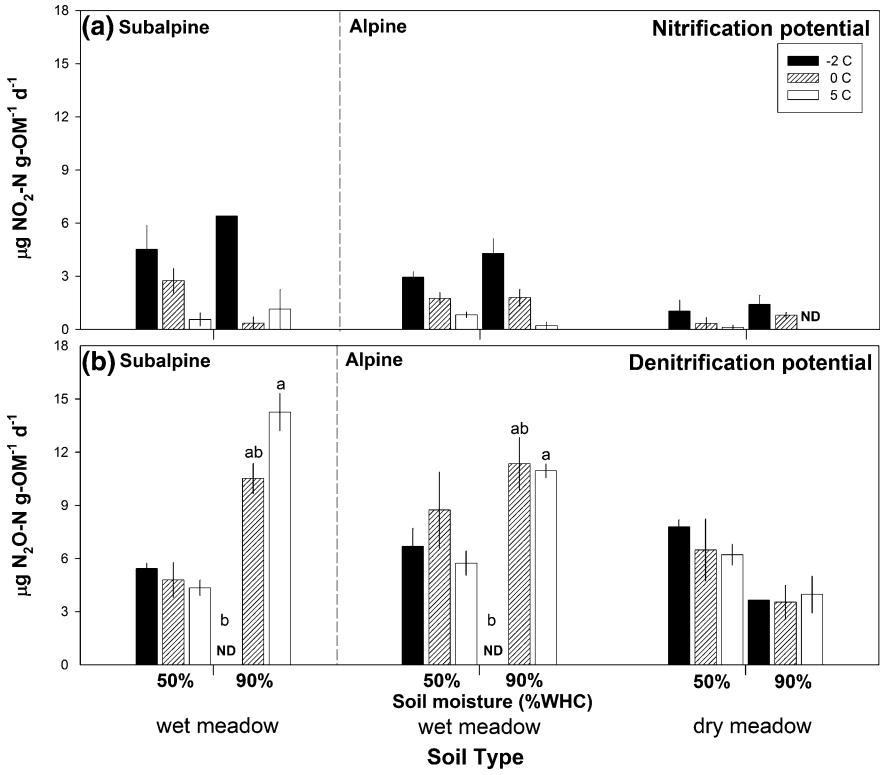

Fig. 5 (**a**) Nitrification and (**b**) denitrification potentials (denitrification enzyme activity) per g soil organic matter at 16 weeks (mean ± 1 SE). ND denotes values that were below detection limits. Temperature effects within moisture treatment are as in Fig. 3

DEA was below detection limits in the −2°C and 90% WHC soils, yet NO_3^- consumption was comparable to that at −2°C and 50% WHC, where DEA ranged from 5.4 to 6.7 μg N_2O g-OM^{-1} day^{-1}. It is possible that denitrification occurred in the −2°C and 90% WHC soils even though measured enzyme activities were low, but NO_3^- losses via DNRA are also possible.

Relationship of experimental results to catchment-scale N cycling

In many seasonally snow-covered catchments, snowmelt- and rainfall-driven export of NO_3^- can comprise 50–90% of annual catchment yield, the majority of which is derived from soil N (Campbell et al. 2002; Sickman et al. 2003; Piatek et al. 2004). In the Emerald Lake watershed, the majority of snowmelt NO_3^- is released to streams several weeks after the release of base cations, silica, and sulfate (Leydecker et al. 1999), suggesting that the flushed N is not transported through soils during the early stages of snowmelt but is released during the transition from low to high runoff (Sickman et al. 2001). During this time, soils are thought to shift from an anaerobic to aerobic condition as the soils are first saturated and then flushed by oxygenated waters (Sickman et al. 2001). This flushing would be expected to inhibit dissimilatory processes, stimulate nitrification, and leach any NO_3^- produced.

Current climate change scenarios for the northern California Coast Range and the Sierra Nevada predict increased cold season precipitation, increased rainfall at high elevations, and earlier snowmelt dates (Stewart et al. 2004; Kim 2005), all of which could influence N export during snowmelt. Once insulated by snow, the Sierra soils currently remain at temperatures just above 0°C (e.g., 0.1–0.6°C) for the duration of the winter. Increased snow depth and warmer winter temperatures forecast by climate models could support greater rates of gross C and N processing in these soils in the future, but the specific nature of N cycling processes would be expected to be sensitive to the characteristics of the soil microclimate.

If the Sierra soils were to remain at temperatures just above 0°C over the course of the winter, we would expect soil moisture content to be a critical

driver of mineralization and the fate of soil N. In particular, the timing of the transition from anaerobic to aerobic processes (cf. Sickman et al. 2001) could control the magnitude of the NO_3^- pulse during snowmelt by determining the length of time that soils either produced or consumed N. It is notable that model simulations applied to the Emerald Lake watershed have previously overestimated NO_3^- export from these soils, perhaps due to an overestimation of nitrification and an underestimation of dissimilatory processes in the early stages of melt (Meixner and Bales 2002).

If the Sierra soils were to drop to temperatures below 0°C, possibly as a result of later snowpack formation in the autumn or discontinuous snowpack conditions in the early winter, we would expect that freezing-induced anaerobiosis could occur regardless of soil moisture. Thus, the occurrence of dissimilatory processes in the −2°C soils at even 50% water holding capacity could determine whether soils at near-zero temperatures show net production or consumption of N, and could play an important role in regulating future winter processes in this and other alpine ecosystems.

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