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Warming and drought reduce temperature sensitivity of nitrogen transformations

DOLAPORN S. NOVEM AUYEUNG*, VIDYA SUSEELA*† and JEFFREY S. DUKES*‡§

*Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907, USA, †School of Agricultural, Forest and Environmental Sciences, Clemson University, Clemson, SC 29634, USA, ‡Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, USA, §Department of Biology, University of Massachusetts, Boston, MA 02125, USA

Abstract

Shifts in nitrogen (N) mineralization and nitrification rates due to global changes can influence nutrient availability, which can affect terrestrial productivity and climate change feedbacks. While many single-factor studies have examined the effects of environmental changes on N mineralization and nitrification, few have examined these effects in a multifactor context or recorded how these effects vary seasonally. In an old-field ecosystem in Massachusetts, USA, we investigated the combined effects of four levels of warming (up to 4 °C) and three levels of precipitation (drought, ambient, and wet) on net N mineralization, net nitrification, and potential nitrification. We also examined the treatment effects on the temperature sensitivity of net N mineralization and net nitrification and on the ratio of C mineralization to net N mineralization. During winter, freeze–thaw events, snow depth, and soil freezing depth explained little of the variation in net nitrification and N mineralization rates among treatments. During two years of treatments, warming and altered precipitation rarely influenced the rates of N cycling, and there was no evidence of a seasonal pattern in the responses. In contrast, warming and drought dramatically decreased the apparent Q_{10} of net N mineralization and net nitrification, and the warming-induced decrease in apparent Q_{10} was more pronounced in ambient and wet treatments than the drought treatment. The ratio of C mineralization to net N mineralization varied over time and was sensitive to the interactive effects of warming and altered precipitation. Although many studies have found that warming tends to accelerate N cycling, our results suggest that warming can have little to no effect on N cycling in some ecosystems. Thus, ecosystem models that assume that warming will consistently increase N mineralization rates and inputs of plant-available N may overestimate the increase in terrestrial productivity and the magnitude of an important negative feedback to climate change.

Keywords: apparent Q_{10}, Boston-Area Climate Experiment, climate change, multifactor experiment, nitrogen cycling, old-field community

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Introduction

Terrestrial nitrogen (N) cycling rates depend strongly on soil temperature and moisture and could be affected by climate change (IPCC, 2007). Processes such as N mineralization and nitrification play important roles in determining the availability of soil inorganic N. In N-limited ecosystems, the amount of plant-available N may constrain increases in carbon (C) sequestration by terrestrial plants in response to rising levels of atmospheric carbon dioxide (CO_2). Thus, changes in N cycling and availability can affect the magnitude of an important negative feedback in response to elevated CO_2 concentrations (Luo *et al.*, 2004; Reich *et al.*, 2006; Norby *et al.*, 2010). Because changes in N availability can affect C cycling feedbacks to climate change, it is important to understand the responses of N cycling to warming and altered precipitation.

Although many studies have examined how N mineralization and nitrification rates respond to warming or altered precipitation alone, few have examined their responses to different combinations of these factors. Many experimental studies have found that N mineralization increased with warming (Rustad *et al.*, 2001) while warming has increased (Grundmann *et al.*, 1995; Verburg *et al.*, 1999; Larsen *et al.*, 2011) or had no effect on nitrification rates (Shaw & Harte, 2001; Niboyet *et al.*, 2011). Similarly, drought or reduced soil moisture has generally decreased N mineralization rates (Emmett *et al.*, 2004; Larsen *et al.*, 2011) while precipitation effects on nitrification have been highly variable (Grundmann *et al.*, 1995; Breuer *et al.*, 2002; Larsen *et al.*, 2011). Results from the few field studies that have examined the interactive effects of warming and altered precipitation on N mineralization and nitrification have

Correspondence: Dolaporn S. Novem Auyeung, Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907, USA, tel. +1 765 409 0853,
fax +1 765 494 9461 e-mail: novema@gmail.com

been inconsistent. One study found that drought diminished the positive effect of warming on gross N mineralization (Larsen *et al.*, 2011); other studies found no interactive effects of warming and altered precipitation on N mineralization or nitrification rates (Niboyet *et al.*, 2011; Shiqiang Wan, personal communication). Thus, continued research in multifactor settings may provide insight into the conditions that determine whether N mineralization and nitrification rates will respond to the interactive effects of warming and altered precipitation.

Although many researchers have described the temperature sensitivity (Q_{10}) of C mineralization under different environmental conditions, few have examined changes in the Q_{10} of net N mineralization or nitrification due to warming or changes in precipitation. Previous work has found reductions in the Q_{10} of C mineralization when soils were incubated at higher temperatures (Kirschbaum, 1995; Bekku *et al.*, 2003; Koch *et al.*, 2007; Craine *et al.*, 2012) and in drought conditions (Davidson & Janssens, 2006; Suseela & Dukes, 2012), suggesting that soil respiration is less responsive to warming at higher temperatures and in drier conditions. Similarly, a handful of studies have found lower Q_{10} values for net N mineralization when soils were incubated at higher temperatures (Kirschbaum, 1995; Dalias *et al.*, 2002; Koch *et al.*, 2007). However, soil moisture effects on the Q_{10} of net N mineralization or net nitrification have been highly variable (e.g., Sierra, 1997; Wang *et al.*, 2006), and few studies have compared the Q_{10} of C mineralization to the Q_{10} of net N mineralization (e.g., Kirschbaum, 1995; Koch *et al.*, 2007). To better understand some of the mechanisms underlying the responses of N cycling to climate change, we propose examining how the Q_{10} values of N transformations respond to different warming and precipitation treatments and how these Q_{10} values compare to the Q_{10} values of C mineralization.

In some ecosystem models (e.g., Thornton & Rosenbloom, 2005; Thornton *et al.*, 2007), N cycling is driven by the breakdown of different C pools. By examining how warming and altered precipitation affect the ratio of C mineralization to net N mineralization in soil organic matter, we can gain insight into how environmental conditions affect the efficiency of microbial respiration for N mineralization. This information could be used to refine ecosystem models so that they better predict future shifts in N cycling and availability and their consequences for C cycle feedbacks.

Experimental studies of soil N cycling in temperate ecosystems are often conducted during the growing season (e.g., Shaw & Harte, 2001; Ma *et al.*, 2011; Niboyet *et al.*, 2011), but recent studies have suggested that warming and changes in precipitation during the winter can affect N cycling and the annual N budget. Variations in winter temperature and soil moisture can directly influence net N mineralization, net nitrification, and potential nitrification rates (Miller *et al.*, 2007). Changes in temperature and precipitation can also affect environmental parameters thought to influence N transformations, such as snow depth (Schimel *et al.*, 2004; Borner *et al.*, 2008) and freeze–thaw events (DeLuca *et al.*, 1992; Henry, 2008). During winter, because there is little plant N uptake, increases in N mineralization and nitrification could lead to greater N losses through leaching or gaseous loss (Turner & Henry, 2010). Therefore, it is important to understand N responses to global changes during the winter as well as the growing season.

We tested three main hypotheses. First, we hypothesized that warming would increase N cycling rates throughout the year and that rates would be more sensitive to warming during cold sampling periods due to changes in snow depth, soil frost depth, and the frequency of freeze–thaw cycles during winter. Second, we hypothesized that increased precipitation would increase N cycling rates throughout the year and that warming would increase N cycling rates to a greater extent in ambient and wet treatments. We expected N cycling to proceed faster and be more temperature sensitive in wetter soils because microbial processes would rarely be limited by soil moisture or the diffusion of substrates. Third, we expected the temperature sensitivity of net N mineralization and net nitrification to be lower in the warmed treatments and in the drought treatment. To test these hypotheses, we examined the effects of 12 combinations of warming and altered precipitation on net N mineralization, net nitrification, and potential nitrification rates in an old-field ecosystem, a grassland community that was once used for agricultural production. Responses were monitored throughout the year to characterize seasonal differences and to examine whether the treatments affected the temperature sensitivity of N transformations.

Materials and methods

Site description & experimental design

This research was conducted at the Boston-Area Climate Experiment (BACE) in an old-field ecosystem in Waltham, Massachusetts (42°23′ N, 71°13′ W). Prior to the start of this experiment and since the 1960s, the site was periodically mowed. Mean annual precipitation at the site is 1194 mm yr^{-1}, and mean annual temperature is 9.3 °C based on data from 1960 to 2008 (NOAA National Climatic Data Center Cooperative Station ID 190535). Soils were classified as mesic Typic Dystrudepts, and the top 30 cm were loam soils that consist of 45% sand, 46% silt, and 9% clay with a pH of 5.9 ± 0.1. The bulk density of the top 10 cm of soil was

0.98 g cm^{-3}. The species composition at the site consisted of a mix of native and introduced annual, biennial, and perennial grasses and forbs, and seedlings of four tree species (*Acer rubrum*, *Betula lenta*, *Pinus strobus*, *Quercus rubra*) were planted in designated 0.5 m-by-0.5 m subplots (Hoeppner & Dukes, 2012).

The BACE used a full-factorial, split-plot design to provide three levels of precipitation and four levels of warming in three replicate blocks (36 experimental plots). Each block contained three precipitation zones: drought (50% below ambient year-round), ambient, and wet (50% above ambient during the growing season). Four warming treatments were nested within each zone: high (target of +4.0 °C), medium (+~2.7 °C), low (+~1.0°C), and unwarmed. In the drought treatment zones, clear, corrugated polycarbonate slats covered 50% of the roof area of the rainout shelter. In nonfreezing months, water intercepted by the roofing slats during rain events was fed into storage tanks and immediately pumped onto the wet treatment through sprinklers. From mid-November to early May, sprinklers were turned off to avoid damage from frozen pipes, which meant that the wet treatment was not in effect from mid-November to early May. Roofing slats in the drought treatment section reduced incoming photosynthetically active radiation by ~5%, so deer fencing was placed over the ambient and wet treatment zones to approximate this reduction. Because the roof ranged from ~3.0 to 4.3 m above the plots and the sides of the structure remained open, the rainout shelters did not have a measureable effect on relative humidity or the evenness of rainfall distribution in the drought treatment zones. Warming treatments were achieved using infrared heaters with different wattages (200, 600, and 1000 W) placed 1 m above the ground, facing downward at a 45º angle, at all four corners of the 2 m-by-2 m plots to promote uniform warming (see Kimball *et al*., 2012). Infrared radiometers (IRR-PN; Apogee Instruments, Logan, UT, USA) measured the canopy temperature in the center of the unwarmed and high warming plots, and these measurements were fed into a feedback control program (Labview, National Instruments, Austin, TX, USA) that controlled heaters for all three warmed plots within each zone on a single circuit. Drought treatments began in January 2007, wet treatments began in June 2008, and warming treatments began in July 2008.

Field measurements of volumetric soil moisture and soil temperature were taken regularly to ensure that the warming and precipitation treatments effectively changed soil temperature and moisture, respectively. Pairs of time-domain reflectometry (TDR) waveguides were permanently installed vertically in the southeast corner of each plot to provide integrated measures of volumetric soil moisture in the top 10 cm (starting in April 2009) and top 30 cm (starting in April 2008). Measurements were taken weekly during the growing season and biweekly during other parts of the year using a portable TDR-100 (Campbell Scientific, Logan, UT, USA). Starting in October 2008, soil temperature was monitored using custom-made linear temperature sensors placed at 2 cm and 10 cm below the soil surface in the northeast corner of each plot. Measurements were recorded every 30 min throughout the year. The number of freeze–thaw events was calculated based on the frequency with which average daily soil temperatures at either 2 cm or 10 cm changed from below to above 0 °C during the 2008–2010 winter sampling periods (October to January and January to April). Starting in 2009, snow depth was measured twice weekly and immediately after snow events or snow melt during months when there was snow (December to March). Average snow depth was calculated from snow depth data only during sampling days when there was snow on any of the plots within the 2009–2010 winter sampling periods. Starting in 2009, soil frost tubes filled with methylene blue dye solution, as described in Hardy *et al.* (2001), were installed into vertical, 45–50 cm deep holes in each plot to collect weekly measurements of soil freezing depths during colder months (December to March). We estimated the cumulative soil freezing depth, an index of the intensity of soil freezing, during the 2009–2010 winter sampling periods. Cumulative soil freezing depth was calculated as the sum of the weekly measurements of soil freezing depths within each of the sampling periods.

Net N mineralization and net nitrification rates

Soil samples were collected in October 2008; August and October 2009; and January, April, June, August, and October 2010. Samples that were collected in April, June, and August were incubated *in situ* for 2 months. Samples that were collected in October and January were incubated *in situ* for 3 months because N cycling rates were expected to be lower during colder months. For simplicity, we will refer to the sampling periods as early winter (October to January), late winter (January to April), spring (April to June), summer (June to August), and fall (August to October). One soil core (5 cm diameter × 10 cm depth), or the initial sample, was collected from each experimental plot at the beginning of each sampling period and immediately extracted for NH_4^+ or NO_3^- analyses. A second soil core (2.54 cm diameter × 10 cm depth), or the incubated sample, was placed intact into a PVC tube in each plot. Each incubated sample was capped with one ion-exchange resin bag on top and two ion-exchange resin bags on the bottom, following the methods of DiStefano & Gholz (1986) and Turner & Henry (2010). The top- and bottommost ion-exchange resin bags intercepted incoming NH_4^+ or NO_3^- to prevent contamination of the incubated sample. The ion-exchange resin bag that was directly below the incubating soil core trapped NH_4^+ or NO_3^- that leached through the incubated sample to prevent the underestimation of net N mineralization or nitrification rates.

In the lab, soil samples were homogenized, and soils (20 g) and resin bags (3 g of resin) were extracted with 100 ml of 2M KCl. Both extracts were analyzed for NH_4^+ or NO_3^- on a Lachat QuikChem 8500 Flow Injection Analysis System (Loveland, CO, USA). We estimated the net N mineralization rate by taking the sum of extracted NH_4^+ and NO_3^- from the incubated sample and bottom resin bag and subtracting the sum of extracted NH_4^+ and NO_3^- from the initial sample. Similarly, net nitrification rates were estimated based on the difference in extracted NO_3^-. Rates are reported in $\mu g\ N\ g^{-1}\ day^{-1}$. The annual net N mineralization and annual net nitrification rates were calculated by summing the net N mineralization rate and net nitrification rate, respectively, for all the incubation dates from October 2009 to October 2010 (Subler *et al.*, 1998; Zhang *et al.*, 2008). Annual net N mineralization and annual net nitrification rates are reported in μg N g^{-1} yr^{-1}.

Potential nitrification rates

Subsamples of sieved soils collected for net N mineralization and net nitrification measurements were used to estimate potential nitrification rates in January, April, June, August, and October 2010. While net nitrification rates provided an estimate of *in situ* nitrification rates over a 2- or 3-month period, potential nitrification rates provided an estimate of the maximum potential nitrification over a 24-h period. Potential nitrification rates were determined using the shaken soil-slurry method described in Hart *et al.* (1994) with slight modifications. The filtered solution was analyzed for NO_3^- as above. The potential nitrification rate was calculated as the difference in NO_3^--N concentration per gram of dry soil between the 24 h sample and the initial sample in μg N g^{-1} day^{-1}.

Ratio of C mineralization to net N mineralization

We estimated the ratio of C mineralization rates to N mineralization rates during each sampling period from August 2009 to October 2010. Estimates of C mineralization were based on field measurements of microbial respiration from plant-exclusion collars that excluded plants, roots, and fresh litter input, as described in Suseela *et al.* (2012). Briefly, a 25 cm diameter PVC collar was installed to a depth of 30 cm in each plot to exclude roots starting in November 2007. Using a LI-COR 6400-09 soil CO_2 flux chamber attached to a 6400 portable photosynthesis system, respiration measurements were collected monthly from a smaller PVC collar (10 cm diameter × 5 cm height) that was installed within the larger root-exclusion collar. Soil temperature at 5 cm depth was measured simultaneously using a thermocouple probe. Because net N mineralization rates were measured every 2 or 3 months, we compared the ratio of cumulative C mineralization to the net N mineralization rate within each 2- or 3-month period. C mineralization rates were converted from μmol CO_2 m^{-2} s^{-1} to μg C g^{-1} per 2- or 3-month period, assuming our measurements captured soil CO_2 efflux down to a depth of 30 cm. In some plots, net N mineralization rates were negative, which indicated that N immobilization exceeded gross N mineralization. Because we were mainly interested in amount of C respired per net N mineralized, we excluded plots with negative net N mineralization rates (n = 6) from the analysis.

Statistical analyses

The main and interactive effects of warming and altered precipitation on soil temperature, soil moisture, net N mineralization, net nitrification, and potential nitrification across all sampling periods were analyzed in a repeated measures, split-plot ANOVA using PROC MIXED in SAS 9.2 (SAS Institute, Inc., Cary, NC, USA). The main and interactive effects of warming and precipitation on the frequency of freeze–thaw events, average snow depth, and cumulative soil freezing depth during winter sampling periods (from 2008 to 2010 for the frequency of freeze–thaw events, from 2009 to 2010 for all other variables) were analyzed in a repeated measures, split-plot ANOVA using PROC MIXED. We tested these effects in a mixed model using the restricted maximum likelihood (REML) method, and df were calculated using the Kenward–Rogers method. Warming and precipitation treatments were fixed effects, and blocks were random effects. Tukey's HSD post hoc test was used to determine if there were significant differences (α = 0.05) between individual treatments. Using a mixed model REML analysis, we also examined the treatment effects on net N mineralization, net nitrification, and potential nitrification within each sampling period after finding a significant interactive effect between the sampling period and the treatments, and we examined the treatment effects on annual net N mineralization and annual net nitrification. If needed, data were normalized using the log or square root transformation before analysis.

To determine how well soil temperature, soil moisture, freeze–thaw events, snow depth, or cumulative soil freezing depth could predict net N mineralization or net nitrification rates, separate regression models were fitted to each of the predictors. For soil temperature, regression models were fitted using the average of the soil temperatures at 2 and 10 cm within each plot, during each sampling period. For the regression models, a simple linear function:

$$R = a + bx \tag{1}$$

and exponential function:

$$R = a\mathrm{e}^{bx} \tag{2}$$

were used, where R is the net N mineralization or net nitrification rate, a is the net N mineralization or net nitrification rate when x is zero, b is the sensitivity of net N mineralization or net nitrification to x, and x is the soil temperature, soil moisture, the frequency of freeze–thaw events, average snow depth, or cumulative soil freezing depth. These functions were chosen based on previous studies of the dependence of N mineralization or nitrification on soil temperature or moisture (Sierra, 1997; Leirós *et al.*, 1999; Breuer *et al.*, 2002; Dessureault-Rompré *et al.*, 2010). Previous studies have used logistic (Dessureault-Rompré *et al.*, 2010) and s-shaped (De Neve *et al.*, 1996) functions to describe the dependence of N mineralization on soil temperature and logistic (De Neve & Hofman, 2002) and Gaussian (De Neve & Hofman, 2002; Sleutel *et al.*, 2008) functions to describe the dependence of N mineralization on soil moisture. However, because we lacked data in the upper soil moisture and temperature ranges, where rates were expected to plateau (according to the logistic function) or reach an optimum and decline (according to the s-shaped or Gaussian functions), these functions were not a good fit for our data (data not shown). For all the regression models, the coefficient of determination (r^2) was used to assess the goodness-of-fit.

To determine the soil temperature response of net N mineralization and net nitrification in each of the 12 treatments, we fitted Eqn (2) to data within each treatment across all sampling periods from 2008 to 2010. We used Eqn (2) based on our observations that an exponential model best described the temperature dependence of net N mineralization and net nitrification for the temperature ranges we observed at our site. For each treatment, we calculated the temperature sensitivity (Q_{10}) or the change in net N mineralization or net nitrification rates with every 10 °C increase in temperature. Because our samples were collected over time in the field, where changes in substrate and moisture availability and microbial activity could also influence N transformation rates, the Q_{10} values we obtained are apparent Q_{10} values, rather than intrinsic $Q_{10.}$ The apparent Q_{10} of each treatment was calculated using the following equation and using b, or the temperature sensitivity parameter, from Eqn (2):

$$Q_{10} = e^{10b} \tag{3}$$

We then fitted Eqn (2) to data within each plot across all sampling periods, calculated the Q_{10} using Eqn (3), and used a mixed model REML analysis to determine whether the Q_{10} differed among treatments. To compare the Q_{10} values of net N mineralization and net nitrification with the Q_{10} values of C mineralization within each treatment, we also fitted Eqn (2) to data from only the sampling periods from August 2009 to October 2010 (as there were no C mineralization data available during early winter 2008–2009).

Results

Microclimate

Precipitation altered volumetric soil moisture in the top 10 cm and top 30 cm during all sampling periods from 2009 to 2010 and from 2008 to 2010, respectively ($P < 0.05$, Table S1; Fig. 1). From 2008 to 2010, the warming treatments increased the average soil temperature at a depth of 2 cm and 10 cm ($P < 0.0001$, Table S1; Fig. S1). On average, across all sampling periods, warming increased the soil temperature at 2 cm by 1.3, 2.1, and 3.2 °C in the low, medium, and high treatments, respectively, and the soil temperature at 10 cm by 0.41, 1.7, and 2.9 °C in the low, medium, and high treatments, respectively. Volumetric soil moisture and soil temperature varied with sampling period ($P < 0.0001$, Table S1). In general, volumetric soil moisture was higher during colder sampling periods (October to April). In addition, the warming treatment decreased the volumetric soil water content in the top 10 cm and top 30 cm by 21% and 7.2%, respectively, and the gravimetric soil water content by 21% across all sampling periods ($P < 0.05$, Table S1). The average soil temperature at 2 cm below the soil surface was also 1.2 and 1.4 °C higher in the drought plots than in the ambient and wet plots, respectively ($P < 0.05$, Table S1). During colder sampling periods, warming decreased the frequency of freeze–thaw events by 42%

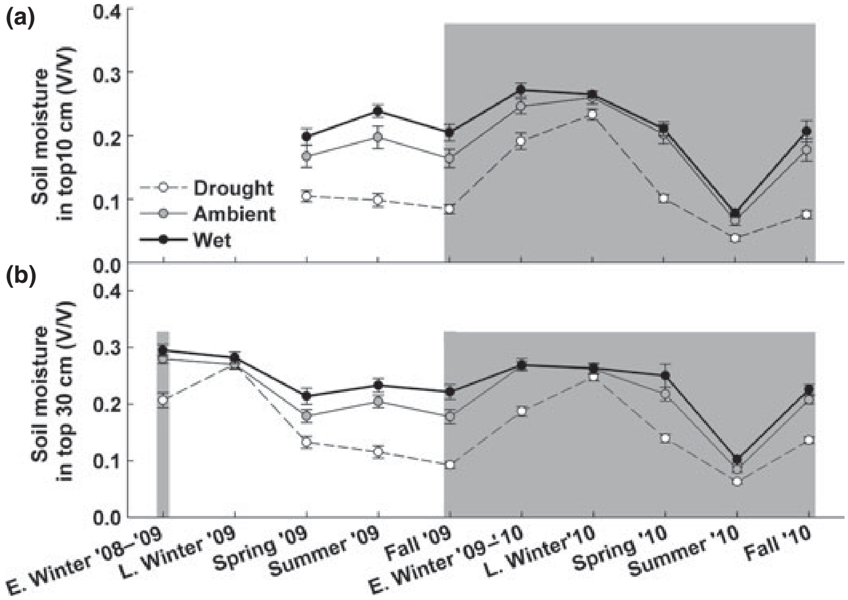

Fig. 1 Volumetric soil moisture in (a) the top 10 cm and (b) top 30 cm from October 2008 to October 2010 with sampling periods highlighted in gray. 'E. Winter' represents the early winter sampling period. 'L. Winter' represents the late winter sampling period. See 'Materials and Methods' for more details on the sampling periods. Values represent the means of the weekly or biweekly soil moisture within each sampling period (n = 12 for each precipitation treatment during each sampling period) ±SE.

($P = 0.0055$, Fig. S2), average snow depth by 76% ($P < 0.0001$, Fig. S2), and cumulative freezing depth by 86% ($P < 0.0001$, Fig. S2) in the high warming treatment relative to the unwarmed treatment. Drought also decreased the average snow depth by 22% ($P = 0.0011$, Fig. S3) and cumulative soil freezing depth by 38% ($P = 0.0208$, Fig. S3) relative to the ambient precipitation treatment.

Net N mineralization rates

Net N mineralization rates ranged from −0.014 to 2.8 μg N g^{-1} day^{-1} across all sampling periods and varied with sampling period; on average, rates were highest during summer 2010 and lowest during late winter 2010 (Table 1; Fig. 2a). Warming did not affect N mineralization, and the effect of altered precipitation

Table 1 A summary of the P-values and numerator and denominator degrees of freedom, respectively (in parentheses), from a mixed model split-plot ANOVA with repeated measurements testing for the treatment effects on net N mineralization, net nitrification, potential nitrification, and the ratio of C mineralization to net N mineralization ('$C_{min} : N_{min}$') across all sampling periods. Significant effects are in bold ($P < 0.05$) and marginally significant effects are in italics ($0.05 \leq P < 0.1$)

Transformation	Net N mineralization $\ln(x + 0.05)$	Net nitrification $\ln(x + 0.05)$	Potential nitrification $\sqrt{x}$	$C_{min} : N_{min}$ $\ln(x)$
Precipitation (P)	0.7487 (2, 22.1)	0.1478 (2, 3.95)	0.4942 (2, 6)	0.6453 (2, 3.92)
Warming (W)	0.2321 (3, 22.1)	*0.0736* (3, 18.1)	0.2762 (3, 18)	0.1608 (3, 18.3)
P × W	0.3153 (6, 22.1)	0.6301 (6, 18.1)	0.9638 (6, 18)	**0.0039** (6, 18.3)
Sampling period (T)	**<0.0001** (6, 143)	**<0.0001** (6, 143)	**<0.0001** (4, 96)	**<0.0001** (5, 116)
P × T	**0.0139** (12, 143)	**<0.0001** (12, 143)	**0.0003** (8, 96)	**0.0349** (10, 116)
W × T	0.1126 (18, 142)	*0.0631* (18, 143)	0.3219 (12, 96)	0.2577 (15, 116)
P × W × T	0.3539 (36, 142)	0.7907 (36, 143)	0.2860 (25, 96)	0.3225 (30, 116)

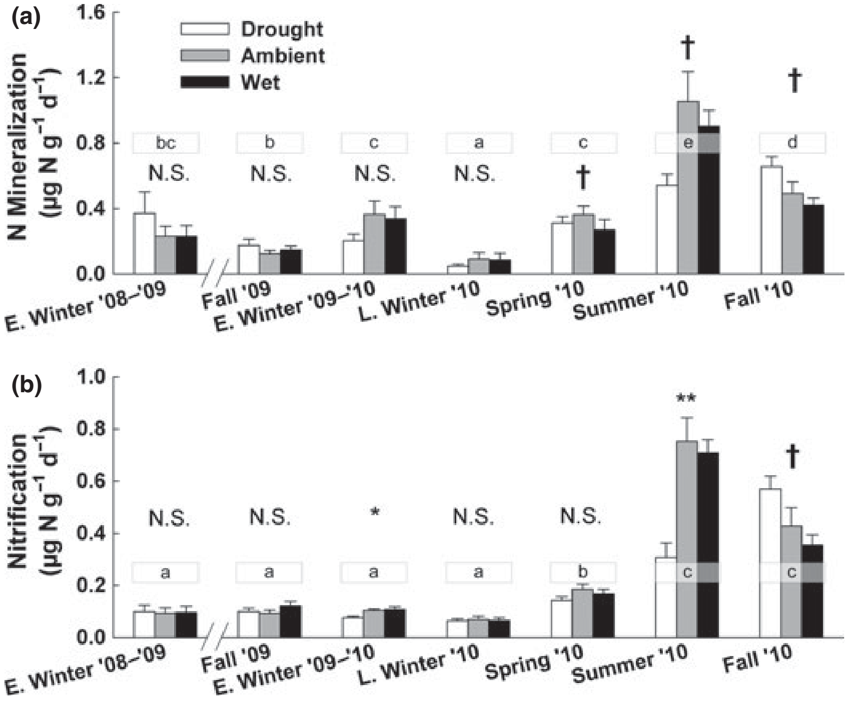

Fig. 2 Net N mineralization (a) and net nitrification rates (b) in μg N g^{-1} day^{-1} of each precipitation treatment for all sampling periods. 'E. Winter' represents the early winter sampling period. 'L. Winter' represents the late winter sampling period. See 'Materials and Methods' for more details on the sampling periods. Values represent means ($n = 12$ for each precipitation treatment at each sampling period, except for the dry treatment during early winter 2008–2009 and the wet treatment during spring 2010 where $n = 11$) ±SE. P-values of the precipitation effect at each sampling period from the mixed model analysis are also noted: N.S. is not significant, †$P < 0.1$, *$P < 0.05$, and **$P < 0.01$. Letters a through d indicate a significant difference (Tukey's HSD, $\alpha = 0.05$) between the average net N mineralization or nitrification rate across all treatments among the sampling dates.

depended on the sampling period (precipitation × sampling period; Table 1; Fig. 2a). Warming also did not affect annual net N mineralization rates, and precipitation effects were only marginally significant (Table S2).

When data from each sampling period were analyzed separately, warming and altered precipitation only had marginally significant effects on net N mineralization during some sampling periods (Table S2). During summer 2010, there was an interactive effect of precipitation and warming (Table S2; Fig. S4), but none of the treatments were significantly different based on post hoc pairwise comparisons, and the effect of warming on net N mineralization did not appear to be more pronounced in the wet treatment than in other precipitation treatments.

Of all the regression models fitted, the exponential model of the dependence of net N mineralization on soil temperature had the best fit ($r^2 = 0.27$). Based on both the linear model using Eqn (1) and exponential model using Eqn (2), soil temperature, freeze–thaw events, and average snow depth were positively correlated with net N mineralization while soil moisture and cumulative soil freezing depth were negatively correlated with N mineralization (Figs 3 and 4).

Net nitrification rates

Net nitrification rates ranged from −0.021 to 1.4 μg N g^{-1} day^{-1} across all sampling periods and varied with sampling period; on average, rates were highest during summer 2010 and lowest during late winter in 2010 (Table 1; Fig. 2b). The effects of precipitation on net nitrification depended on the sampling period (precipitation × sampling period; Table 1; Fig. 2b), and the warming by sampling period interaction was marginally significant. Neither treatment affected annual net nitrification rates (Table S2).

Net nitrification rates responded to precipitation treatments only during some sampling periods (Table S2, Fig. 2b). Drought decreased net nitrification rates during early winter of 2009–2010 (27% and 30% lower

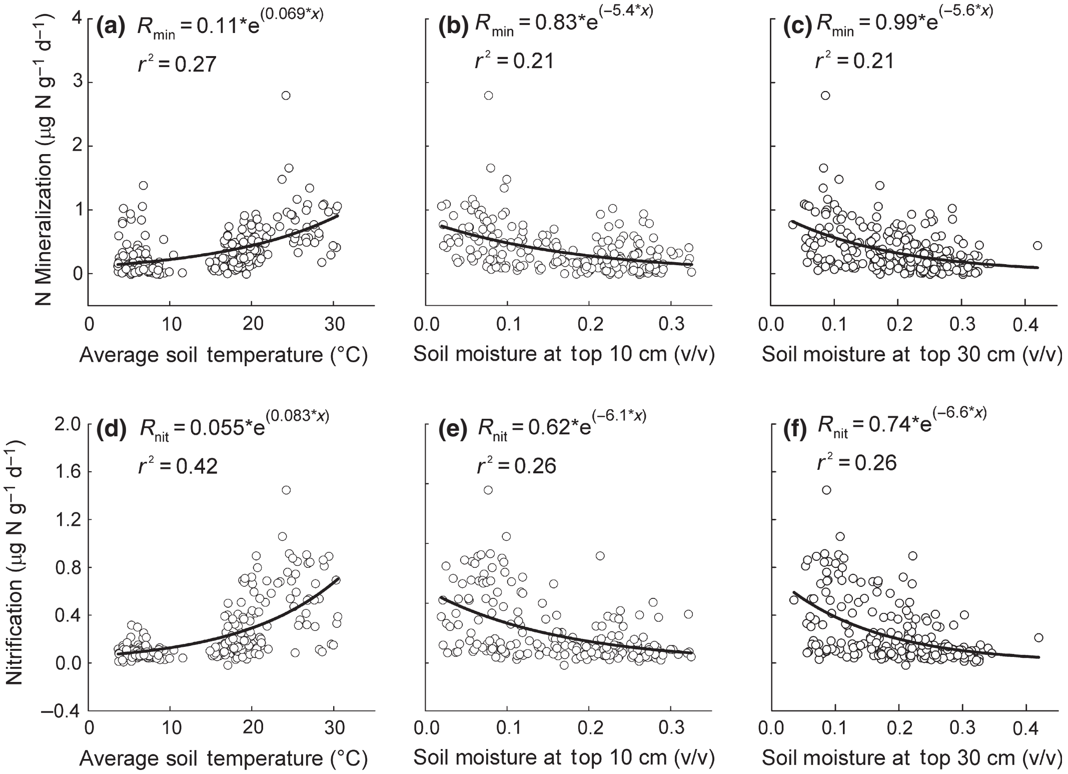

Fig. 3 The dependence of net N mineralization (R_{min}, μg N g^{-1} day^{-1}) year-round on (a) average of soil temperatures at 2 cm and 10 cm, (b) soil moisture at the top 10 cm, and (c) soil moisture at the top 30 cm and the dependence of net nitrification (R_{nit}, μg N g^{-1} day^{-1}) year-round on (d) average of soil temperatures at 2 cm and 10 cm, (e) soil moisture at the top 10 cm, and (f) soil moisture at the top 30 cm. Graphs only show the regression model with the highest coefficient of determination or r^2. For all the regression models shown, $P < 0.05$. Points represent individual plot measurements from all sampling periods from 2008 to 2010 (for average soil temperature and soil moisture at the top 30 cm, $n = 250$) and from all sampling periods from August 2009 onwards (for soil moisture at the top 10 cm, $n = 215$).

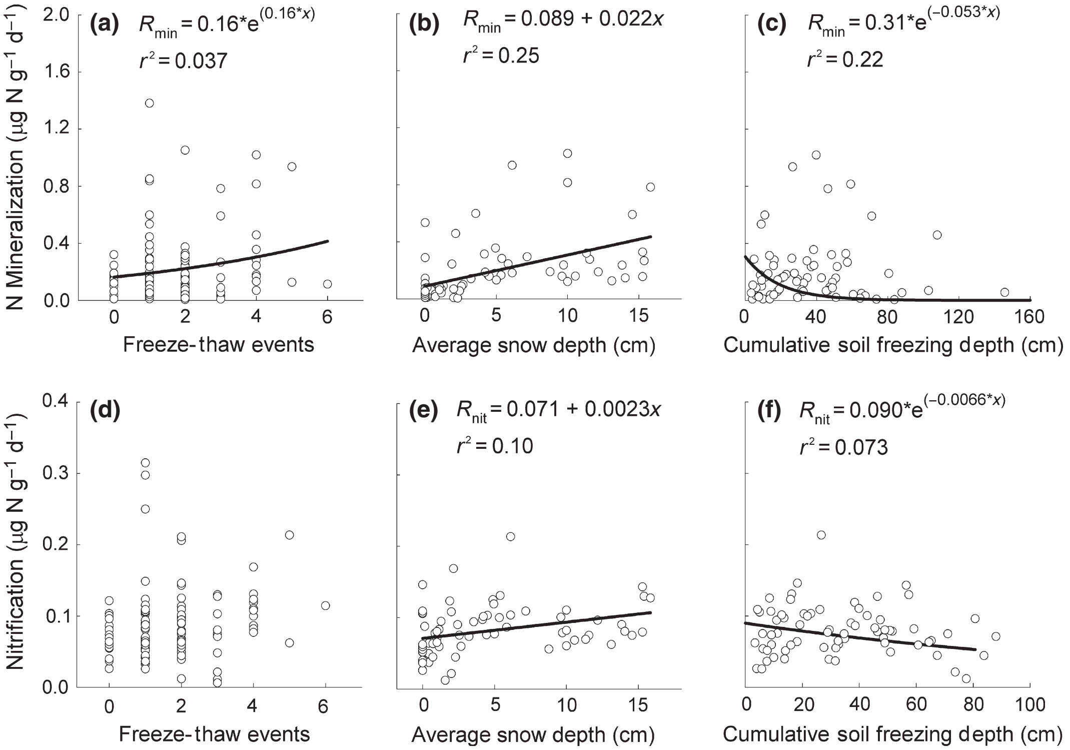

Fig. 4 The dependence of net N mineralization (R_{min}, μg N g^{-1} day^{-1}) during the colder sampling periods on the (a) frequency of freeze–thaw events, (b) average snow depth, and (c) cumulative soil freezing depth and the dependence of net nitrification (R_{nit}, μg N g^{-1} day^{-1}) in colder sampling periods on (d) frequency of freeze–thaw events, (e) average snow depth, and (f) cumulative soil freezing depth Graphs only show the regression model with the highest coefficient of determination or r^2. Only regression models with a significant *P*-value ($P < 0.05$) are shown. For (d), none of the regression models had a significant *P*-value ($P < 0.05$) so no regression model is shown. Points represent individual plot measurements from early winter 2008–2009, early winter 2009–2010, and late winter 2010 for the frequency of freeze–thaw events ($n = 108$) and from early winter 2009–2010 and late winter 2010 for average snow depth and cumulative soil freezing depth ($n = 72$).

in drought plots relative to ambient and wet plots, respectively, $P = 0.0489$) and summer 2010 (59% and 57% lower in drought plots relative to ambient and wet plots, respectively, $P = 0.0021$). During those sampling periods, the drought treatments significantly decreased net nitrification rates relative to ambient plots ($P < 0.05$, Tukey's HSD) while there was no significant difference between the ambient and wet treatments ($P > 0.05$, Tukey's HSD). Net nitrification rates responded to warming only during fall 2010 ($P = 0.0423$, Table S2). During that sampling period, net nitrification rates were 16%, 58%, and 68% higher in the low, medium, and high plots relative to unwarmed plots.

Of all the regression models for net nitrification, the exponential relationship with soil temperature fit best ($r^2 = 0.42$). Based on both the linear model using Eqn (1) and exponential model using Eqn (2), soil temperature and average snow depth were positively correlated with net nitrification; soil moisture, and cumulative soil freezing depth were negatively correlated with net nitrification (Figs 3 and 4).

Potential nitrification rates

Potential nitrification rates varied by sampling period (Table 1; Fig. 5), as did the potential nitrification response to precipitation (precipitation × sampling period; Table 1; Fig. 5). Potential nitrification rates ranged from 0.22 to 5.0 μg N g^{-1} day^{-1}; on average, rates were highest in August 2010 and lowest in April 2010. When data from each sampling period were analyzed separately, precipitation increased potential nitrification rates in June 2010 ($P = 0.0408$; Table S3; Fig. 5). During that time, potential nitrification rates were 27% lower in drought plots relative to both ambient and wet plots.

Soil temperature response of net N mineralization and net nitrification

Soil temperature was exponentially correlated with N transformations in most of the treatments (Table 2), but the shape of this response differed among the

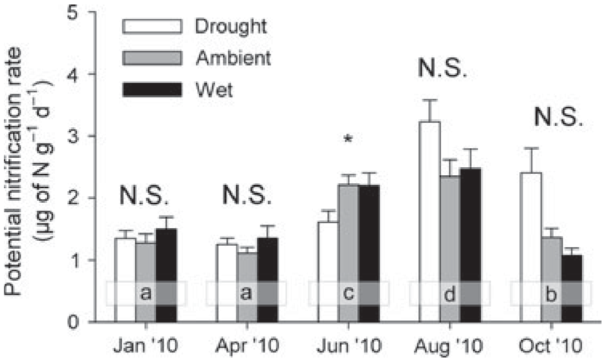

Fig. 5 Potential nitrification rates in μg N g^{-1} day^{-1} of each precipitation treatment for all sampling periods during 2010. Values represent means (n = 12 for each precipitation treatment at each sampling period) ±SE. P-values of mixed model analysis of each individual date are also noted: N.S. is not significant, $^*P < 0.05$. Letters a through d indicate a significant difference (Tukey's HSD, $\alpha = 0.05$) between the average potential nitrification rates across all treatments among the sampling dates.

treatments (Fig. 6). Warming and drought both suppressed the Q_{10} values for net N mineralization [$Q_{10\,(\mathrm{Min})}$] and net nitrification [$Q_{10(\mathrm{Nit})}$] (Table 3), and warming also suppressed these Q_{10} values to a greater extent in the ambient and wet plots than in the drought plots (precipitation × warming; Table 3; Figs 6 and 7). Values of $Q_{10(\mathrm{Min})}$ and $Q_{10(\mathrm{Nit})}$ tended to be greatest in the control plots and smallest in drought and medium or high warming plots. Based on the Q_{10} values calculated using data from August 2009 to October 2010, $Q_{10(\mathrm{Min})}$ and $Q_{10(\mathrm{Nit})}$ were higher than the Q_{10} of C mineralization in all treatments except the ambient and low warming and drought and medium warming treatments (Table S4).

Ratio of C mineralization to net N mineralization

The ratio of C mineralization to net N mineralization ranged from 1.1 to 720 and varied by sampling period (Table 1; Fig. 8). The average ratio was the highest during late winter 2010 (145 ± 30) and lowest during summer 2010 (4.7 ± 0.5). Across all sampling periods, warming appeared to decrease the ratio of C mineralization to net N mineralization in the wet plots to a greater extent than in the ambient or dry plots (precipitation × warming; Table 1; Fig. 9) although there were no significant post hoc differences between treatments. The effects of altered precipitation also differed among sampling periods (precipitation × sampling period; Table 1; Fig. 8). When we analyzed each sampling period individually, we found that drought decreased the ratio of C mineralization to net N mineralization relative to the ambient and wet plots only during fall 2010 ($P = 0.0047$, −35% and −31% in drought plots relative to ambient and wet plots, respectively).

Discussion

Consistent with our expectations, the temperature sensitivity of net N mineralization and net nitrification declined due to warming and drought; however, this led to the lack of a strong warming or precipitation effect on N cycling rates, which was contrary to our expectations and may have consequences for climate change feedbacks if the phenomenon is widespread. We found little evidence of interactive effects of warming and altered precipitation on N cycling rates, and changes in snow depth, soil frost depth, and the frequency of freeze–thaw explained little of the variation in N cycling rates during the winter. Warming also decreased the ratio of C mineralization to N mineralization to a greater extent in the wet treatment than in the ambient or dry treatments, and the ratio differed among sampling periods. Although our technique has limited resolution, these results suggest that the amount of C respired for a given amount of N mineralized may change depending on the temperature and moisture conditions and may vary over time within a site.

Table 2 Exponential model of (a) net N mineralization and (b) net nitrification as a function of soil temperature within each treatment across all sampling periods from 2008 to 2010 and apparent Q_{10} values

	Drought	Ambient	Wet
(a) Net N mineralization			
Unwarmed	$R_{\mathrm{min}} = 0.99\mathrm{e}^{0.071\mathrm{T}}$, $r^2 = 0.62$, $Q_{10} = 2.0$	$R_{\mathrm{min}} = 0.016\mathrm{e}^{0.19\mathrm{T}}$, $r^2 = 0.56$, $Q_{10} = 6.7$	$R_{\mathrm{min}} = 0.025\mathrm{e}^{0.15\mathrm{T}}$, $r^2 = 0.55$, $Q_{10} = 4.4$
Low	$R_{\mathrm{min}} = 0.11\mathrm{e}^{0.063\mathrm{T}}$, $r^2 = 0.39$, $Q_{10} = 1.9$	$R_{\mathrm{min}} = 0.15\mathrm{e}^{0.054\mathrm{T}}$, $r^2 = 0.14$, $Q_{10} = 1.7$	$R_{\mathrm{min}} = 0.021\mathrm{e}^{0.14\mathrm{T}}$, $r^2 = 0.68$, $Q_{10} = 4.0$
Medium	$R_{\mathrm{min}} = 0.23\mathrm{e}^{0.020\mathrm{T}}$, $r^2 = 0.025$, $Q_{10} = 1.2$	$R_{\mathrm{min}} = 0.051\mathrm{e}^{0.097\mathrm{T}}$, $r^2 = 0.61$, $Q_{10} = 2.6$	$R_{\mathrm{min}} = 0.074\mathrm{e}^{0.099\mathrm{T}}$, $r^2 = 0.35$, $Q_{10} = 2.7$
High	$R_{\mathrm{min}} = 0.18\mathrm{e}^{0.040\mathrm{T}}$, $r^2 = 0.14$, $Q_{10} = 1.5$	$R_{\mathrm{min}} = 0.077\mathrm{e}^{0.087\mathrm{T}}$, $r^2 = 0.55$, $Q_{10} = 2.4$	$R_{\mathrm{min}} = 0.13\mathrm{e}^{0.060\mathrm{T}}$, $r^2 = 0.37$, $Q_{10} = 1.8$
(b) Net nitrification			
Unwarmed	$R_{\mathrm{nit}} = 0.054\mathrm{e}^{0.074\mathrm{T}}$, $r^2 = 0.41$, $Q_{10} = 2.1$	$R_{\mathrm{nit}} = 0.0066\mathrm{e}^{0.21\mathrm{T}}$, $r^2 = 0.73$, $Q_{10} = 7.9$	$R_{\mathrm{nit}} = 0.013\mathrm{e}^{0.16\mathrm{T}}$, $r^2 = 0.87$, $Q_{10} = 5.1$
Low	$R_{\mathrm{nit}} = 0.052\mathrm{e}^{0.084\mathrm{T}}$, $r^2 = 0.44$, $Q_{10} = 2.3$	$R_{\mathrm{nit}} = 0.025\mathrm{e}^{0.12\mathrm{T}}$, $r^2 = 0.68$, $Q_{10} = 3.4$	$R_{\mathrm{nit}} = 0.011\mathrm{e}^{0.16\mathrm{T}}$, $r^2 = 0.82$, $Q_{10} = 5.0$
Medium	$R_{\mathrm{nit}} = 0.11\mathrm{e}^{0.029\mathrm{T}}$, $r^2 = 0.061$, $Q_{10} = 1.3$	$R_{\mathrm{nit}} = 0.031\mathrm{e}^{0.11\mathrm{T}}$, $r^2 = 0.56$, $Q_{10} = 2.9$	$R_{\mathrm{nit}} = 0.023\mathrm{e}^{0.13\mathrm{T}}$, $r^2 = 0.77$, $Q_{10} = 3.8$
High	$R_{\mathrm{nit}} = 0.083\mathrm{e}^{0.050\mathrm{T}}$, $r^2 = 0.23$, $Q_{10} = 1.6$	$R_{\mathrm{nit}} = 0.041\mathrm{e}^{0.10\mathrm{T}}$, $r^2 = 0.69$, $Q_{10} = 2.7$	$R_{\mathrm{nit}} = 0.047\mathrm{e}^{0.092\mathrm{T}}$, $r^2 = 0.61$, $Q_{10} = 2.5$

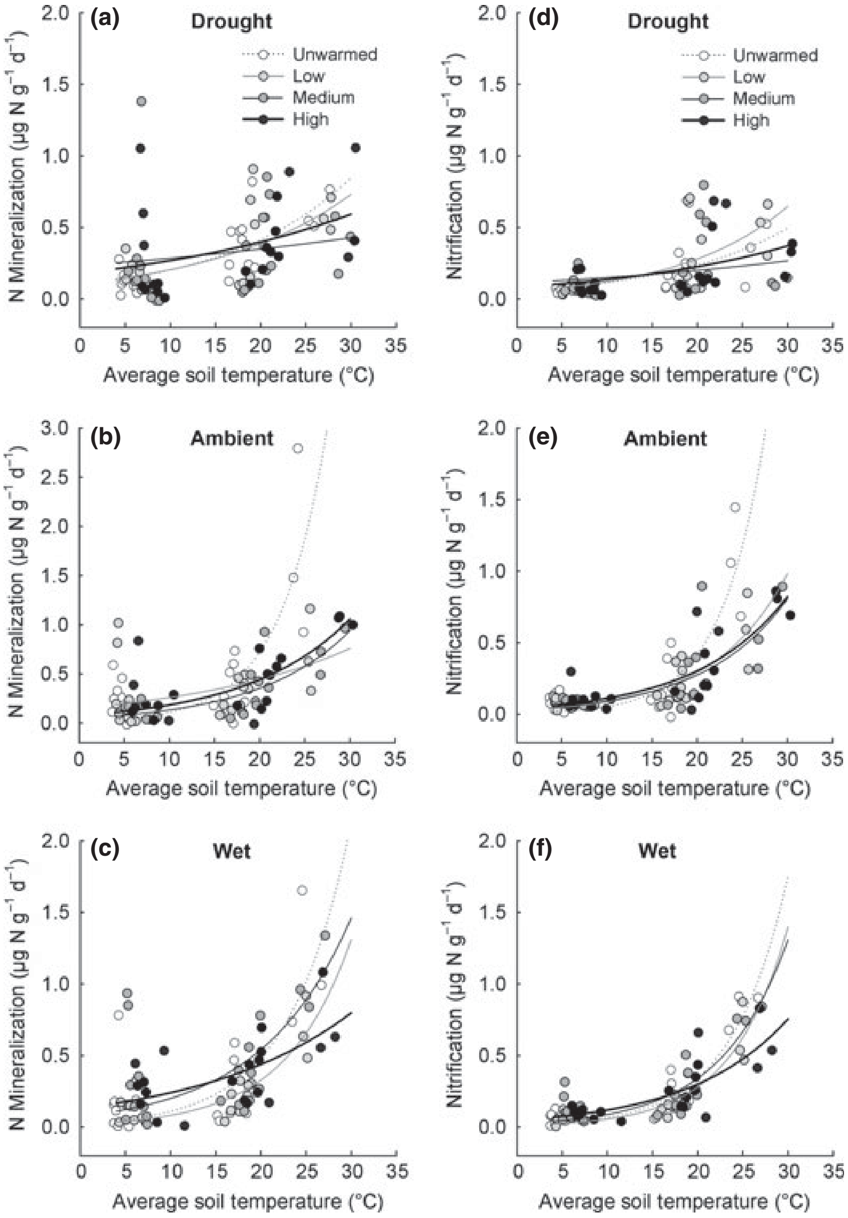

Fig. 6 The exponential relationship between soil temperature and net N mineralization in the drought (a), ambient (b), and wet (c) treatments and between soil temperature and net nitrification in the drought (d), ambient (e), and wet (f) treatments during October 2008–January 2009 and August 2009–October 2010. Points represent individual plots (n = 21 for each warming treatment within the precipitation treatments, except n = 20 for the drought and low warming and wet and unwarmed treatments). Details are in Table 2.

Responses of N cycling rates

In general, seasonal changes in temperature explained much of the variation in average N cycling rates, and rates increased with temperature (Fig. 3). Warming decreased the frequency of freeze–thaw events, snow depth, and soil freezing depth (Fig. S2); however, these changes only explained a small portion of the observed changes in net N mineralization and net nitrification. Other studies have reported similar results; the N cycling responses to freeze–thaw events are highly variable and appear to play a small role in changes in N dynamics compared to other indirect effects of warming such as changes in plant composition (Joseph & Henry, 2008; Matzner & Borken, 2008; Hentschel *et al.*, 2009).

The warming treatments rarely influenced N cycling rates at our site. This lack of a consistent positive effect of warming on net N mineralization rates contrasts with results from some experiments (Rustad *et al.*,

Table 3 A summary of the P-values and denominator degrees of freedom ('DDF') from a mixed model split-plot ANOVA testing for the main and interactive effects of precipitation ('P') and warming ('W') on the Q_{10} of net N mineralization [$Q_{10(\mathrm{Min})}$] and net nitrification [$Q_{10(\mathrm{Nit})}$] from Eqn (3). The numerator degrees of freedom are: 2 (precipitation), 3 (warming), and 6 (precipitation × warming). Significant effects are in bold ($P < 0.05$). For significant effects, a positive response of $Q_{10(\mathrm{Min})}$ or $Q_{10(\mathrm{Nit})}$ to increased precipitation or warming was indicated with an upward pointing arrow (↑) and a negative response was indicated with a downward pointing arrow (↓)

		P		W		P × W	
Value	Transformation	DDF	P-value	DDF	P-value	DDF	P-value
$Q_{10(\mathrm{Min})}$	None	24	**0.0007** ↑	24	**<0.0001** ↓	24	**0.0080**
$Q_{10(\mathrm{Nit})}$	ln(x)	24	**<0.0001** ↑	24	**0.0005** ↓	24	**0.0221**

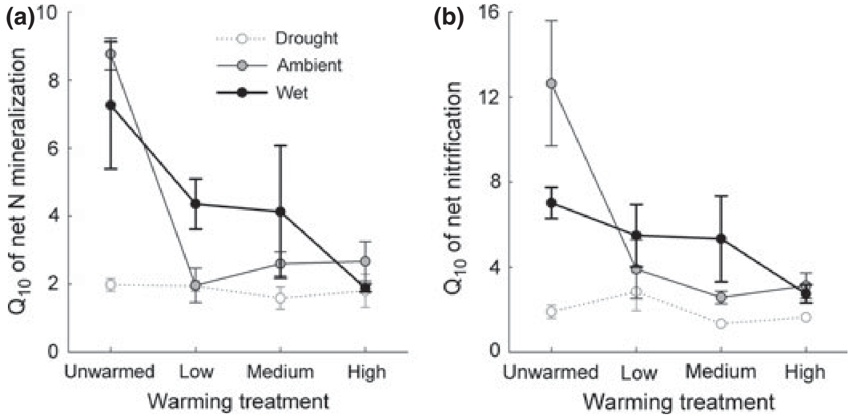

Fig. 7 The interactive effects of warming and altered precipitation on the apparent Q_{10} of (a) net N mineralization and (b) net nitrification across all sampling periods from 2008 to 2010. Values represent the average of the apparent $Q_{10(\mathrm{Min})}$ and $Q_{10(\mathrm{Nit})}$ of each replicate plot ($n = 3$ for all treatments) ±SE.

2001; Schmidt *et al.*, 2002; Butler *et al.*, 2011; Ma *et al.*, 2011), but several other studies have also found no warming effects on N mineralization (Emmett *et al.*, 2004; Keller *et al.*, 2004; Zhang *et al.*, 2005; Niboyet *et al.*, 2011). Even within the same site, warming can have potentially contrasting effects. For example, a study conducted in a subalpine meadow found that net N mineralization responded to warming in xeric microsites, but not in mesic microsites (Shaw & Harte, 2001). Like N mineralization, nitrification responses to warming were also highly variable, although fewer studies have reported these responses in the field. Similar to our results, Ma *et al.* (2011) found that net nitrification increased in response to warming in a temperate grassland, but others have found no response (Shaw & Harte, 2001; Emmett *et al.*, 2004). Examining potential nitrification, Malchair *et al.* (2010) found no response to warming in experimental grassland topsoils, similar to our findings, but they found that warming increased potential nitrification in soils 6–10 cm below the surface, and Larsen *et al.* (2011) found a positive effect on soils in the top 10 cm. Currently, there is little information on why warming has strongly influenced N mineralization and nitrification rates at some sites and not others; below, we propose that the responses of the temperature sensitivity of N transformations play a role in explaining this difference.

At the BACE, net N mineralization and net nitrification rates were negatively correlated with soil moisture when all measurement dates were considered together (Fig. 3), in contrast with previous studies of soil moisture effects on N cycling (Leirós *et al.*, 1999; De Neve & Hofman, 2002). The analysis provided this counterintuitive result because it included data from all seasons, and soils were wettest in the winter (Fig. 1) when biological activity was at its lowest (e.g., Suseela *et al.*, 2012). Similar to the warming treatments, the precipitation treatments rarely influenced N cycling rates and did not have consistently strong effects in either the warmer or colder months.

Responses of the temperature sensitivity of N transformations

To our knowledge, this is the first study to examine the responses of the temperature sensitivity of net N

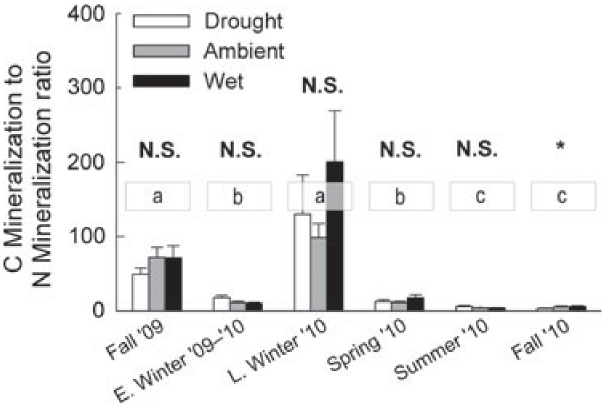

Fig. 8 Ratio of N mineralization to cumulative C mineralization of each precipitation treatment for all sampling periods from August 2009 to October 2010. 'E. Winter' represents the early winter sampling period. 'L. Winter' represents the late winter sampling period. See 'Materials and Methods' for more details on the sampling periods. Values represent means (n = 12 for each precipitation treatment at each sampling period, except n = 11 during fall 2009, summer 2010, fall 2010 and n = 9 during late winter 2010) ±SE. P-values of mixed model analysis of each individual date are also noted: N.S. is not significant, $^*P < 0.05$. Letters a through c indicate a significant difference (Tukey's HSD, $\alpha = 0.05$) between the ratio of N mineralization to cumulative C mineralization across all treatments among the sampling dates.

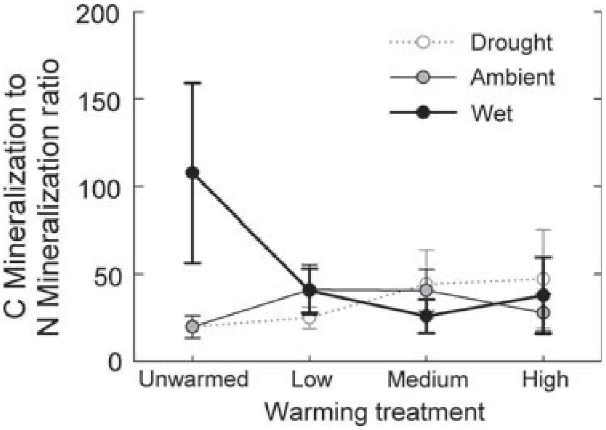

Fig. 9 The interactive effects of warming and altered precipitation on the ratio of N mineralization to cumulative C mineralization across all sampling periods from August 2009 to October 2010. Values represent means (n = 18, except n = 17 for the drought and low warming, drought and medium warming, ambient and high warming, wet and unwarmed treatments and n = 16 for the ambient precipitation and unwarmed treatment) ±SE.

mineralization and net nitrification rates to the main and interactive effects of warming and altered precipitation. Given that we determined the temperature sensitivity over a span of 2 years, we acknowledge that many other factors such as changes in substrate quality and the plant and microbial community may have contributed to the changes in net N mineralization and net nitrification rates in addition to temperature. Nonetheless, soil temperature explained a considerable amount of variation in net N mineralization and net nitrification rates in most of the treatments. Also, because very little is known regarding the effects of warming and altered precipitation on the temperature sensitivity of net N mineralization and net nitrification, we argue that this study takes an important first step to determining how the responsiveness of net N mineralization and net nitrification to warming may change under different background temperatures and precipitation regimes.

Across most treatments, the apparent $Q_{10(\mathrm{Min})}$ and apparent $Q_{10(\mathrm{Nit})}$ values were greater than the apparent Q_{10} values of C mineralization at our site, which suggests that net N mineralization and nitrification were more sensitive to temperature than C mineralization. Consistent with previous studies (Kirschbaum, 1995; Dalias *et al*., 2002; Koch *et al*., 2007), the $Q_{10(\mathrm{Min})}$ and $Q_{10(\mathrm{Nit})}$ values decreased at higher temperatures; a similar pattern to that of heterotrophic respiration. The reductions in the $Q_{10(\mathrm{Min})}$ and $Q_{10(\mathrm{Nit})}$ values under warming may explain why warming did not accelerate net N mineralization or net nitrification.

Because the apparent $Q_{10(\mathrm{Min})}$ and apparent $Q_{10(\mathrm{Nit})}$ values were greater than four for many of the treatments, it is highly likely that other factors, in addition to temperature, influenced net N mineralization and net nitrification rates. At our site, the warming treatments decreased the soil moisture content. Because moisture stress can limit the diffusion of substrates and constrain microbial activity (Manzoni *et al*., 2011), moisture stress likely contributed to the suppression of these temperature sensitivities in the warmed plots. Warming can also influence rates of soil organic matter decomposition, and changes in soil organic matter content have been shown to correlate with the Q_{10} of N mineralization (Koch *et al*., 2007), so the temperature sensitivity of N mineralization may have responded in part to warming-induced changes in substrate availability. In addition, the metabolic activities of microbial communities can acclimate to warming (Bradford *et al*., 2008; Allison *et al*., 2010), so shifts in microbial physiology or composition in the warmed plots may have led to a decrease in temperature sensitivity. This suggests that sites with higher substrate or microbial turnover rates or sites with faster growing, short-lived plant communities may be more likely to acclimate to warming and consequently appear to have no response to warming treatments. For example, sites that found no warming effects on N mineralization had faster growing herbaceous species (this study; Zhang *et al.*, 2005; Niboyet *et al.*, 2011) or were mesic (this study; mesic site from Shaw & Harte, 2001), so they may have experienced faster turnover rates or more rapid shifts in the plant and/or microbial community, which led to a decrease in the temperature sensitivity of N mineralization due to warming. Sites that found a strong warming effect on N mineralization were at the higher latitudes (Rustad *et al.*, 2001; Schmidt *et al.*, 2002), had slower growing hardwoods (Butler *et al.*, 2011), or experienced drier conditions (xeric site from Shaw & Harte, 2001; Ma *et al.*, 2011), so they may have had slower turnover rates and a less dramatic change in the temperature sensitivity of N mineralization due to warming.

Similar to the apparent Q_{10} of heterotrophic respiration at our site (Suseela *et al.*, 2012), soil moisture appears to strongly influence the temperature sensitivity of N cycling rates. In drought plots, the temperature sensitivity of net N mineralization and net nitrification was lower than that in ambient or wet plots. Thus, similar to the warmed plots, moisture stress likely depressed the temperature sensitivity of net N mineralization and net nitrification in the drought plots. In addition, there was evidence of nonadditive effects of warming and altered precipitation on the temperature sensitivity of N cycling rates. The warming-induced decrease in temperature sensitivity was much more pronounced in the wet and ambient plots than the drought plots; temperature sensitivity in the drought plots was already suppressed by water stress, ensuring that any decrease in the temperature sensitivity due to warming would be slight compared with the decreases seen in the ambient or wet plots.

Response of the ratio of C mineralization to N mineralization

At our site, the ratio of C mineralization to net N mineralization was sensitive to changes in temperature and precipitation and varied among sampling periods. Based on the effect sizes, however, the temporal differences in the ratio of C mineralization to net N mineralization were far greater than the treatment effects. The ratio of C mineralization to net N mineralization peaked during winter 2010 and was 30 times greater than when it was at its minimum during summer 2010. In contrast, drought decreased the ratio of C mineralization to net N mineralization by at most 35% relative to the ambient and wet plots. Thus, the sampling period appears to play a more dominant role in determining the amount of C respired per amount of N mineralized than the warming or precipitation treatments. There were also interannual differences in the ratio of C mineralization to N mineralization; the ratio decreased noticeably in fall 2010 compared to fall 2009, and the ratio appeared to generally decrease as the experiment progressed. Because C mineralization and N mineralization are dependent on the availability of different substrates and the activities of different microbial communities, it is possible that shifts in substrate availability or microbial physiology or composition over the duration of the experiment led to changes in the efficiency of C mineralization per net N mineralized. For example, a decline in the availability of labile C relative to labile N may have led to the decrease in the ratio of C mineralization to net N mineralization. Our C mineralization measurements were taken from plant-exclusion collars that excluded fresh litter inputs, and in general, labile C has been found to decrease over time in warming experiments (Xu *et al.*, 2012). Our net N mineralization measurements, on the other hand, were based on incubations of new soil cores collected every 2–3 months, ensuring that there was a fresh supply of substrates. In addition, microbial communities responsible for C mineralization differ from microbial communities responsible for N mineralization and immobilization, and these communities may have responded differently to warming and altered precipitation.

Implications for predicting N cycling responses in future climates

Based on previous studies, N cycling rates are generally expected to increase in a future, warmer world. Indeed, many ecosystem models assume that warming will accelerate N mineralization (e.g., TEM, Raich *et al.*, 1991; Century, Parton *et al.*, 1993; Biome-BGC, Running & Hunt, 1993), potentially increasing simulated productivity. However, our results, along with results from other studies (Shaw & Harte, 2001; Schmidt *et al.*, 2002; Niboyet *et al.*, 2011), suggest that the level of warming projected for later this century can have small or insignificant effects on N cycling in some ecosystems due in part to decreases in the temperature sensitivity of net N mineralization and nitrification as the temperature rises. Meta-analyses have found that warming generally increases primary productivity (Rustad *et al.*, 2001; Lin *et al.*, 2010; Wu *et al.*, 2011), but the magnitude of this increase may depend on a number of limiting factors, including plant N availability at N-limited sites (Rustad *et al.*, 2001). Thus, ecosystem models that assume warming will increase N cycling rates and inputs of plant available N may overestimate the magnitude of the negative feedback to climate change in some ecosystems.

Although some ecosystem models assume that rates of N mineralization and decomposition respond similarly to changes in temperature and moisture (e.g., LM3V, Gerber *et al.*, 2010) or that the ratio of C mineralization to N mineralization remains constant within a given site (e.g., CENTURY, Parton *et al.*, 1993; Biome-BGC, Thornton & Rosenbloom, 2005; CLM, Thornton *et al.*, 2007), our results suggest that the responses of N transformations to warming and altered precipitation may not mirror those of C mineralization. Extrapolating the responses of N cycling rates to climate change from the modeled responses of C mineralization rates provides a simple way to implement N transformation in models, but results from this study suggest that this method may fail to capture important differences in microbial process responses. Until researchers explore these relationships in other ecosystems, and with more precise techniques, we will have limited confidence that these model formulations accurately represent N availability in future climates.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Soil temperature at (a) 2 cm below the soil surface and (b) 10 cm below the soil surface from October 2008 to 2010 with sampling periods highlighted in gray. Values represent the average of the daily soil temperature within each sampling period ($n = 9$ for each warming treatment during each sampling period) ±SE. Open circles and dotted lines represent unwarmed plots, light grey circles and dashed lines represent low warmed plots, dark grey circles and thin solid lines represent medium warmed plots, and black circles and thick solid lines represent high warmed plots.

Figure S2. The (a) total number of freeze-thaw cycles in each warming treatment at both 2 cm and 10 cm below the soil surface across all winter sampling periods (October 2008–January 2009, October 2009–January 2010, January–April 2010). Values represent means ($n = 27$ for each warming treatment) ±SE. The (b) average snow depth, and (c) cumulative soil freezing depth in each warming treatment across winter sampling periods in 2009–2010 (October 2009–January 2010, January–April 2010). Values represent means ($n = 18$ for each warming treatment) ±SE. Letters a through d represent a significant difference (Tukey's HSD, $\alpha = 0.05$) between the warming treatments.

Figure S3. The (a) average snow depth and (b) cumulative soil freezing depth in each precipitation treatment across winter sampling periods in 2009–2010 (October 2009–January 2010, January–April 2010). Values represent means ($n = 24$ for each warming treatment) ±SE. Letters a and b represent a significant difference (Tukey's HSD, $\alpha = 0.05$) between the precipitation treatments.

Figure S4. Interactive effects of precipitation and warming on N mineralization or N nitrification during April to June 2010. Values represent means ($n = 3$ for each treatment, except for the unwarmed, wet treatment in April to June 2010 where $n = 2$) ±SE. Open circles represent dry plots, gray circles represent ambient plots, and black circles represent wet plots.

Table S1. A summary of the P-values and numerator and denominator degrees of freedom, respectively (in parentheses), from a repeated measures mixed model ANOVA testing for the treatment effects on the gravimetric and volumetric soil moisture and soil temperature across all sampling periods. Significant effects are in bold ($P < 0.05$). The gravimetric and volumetric soil moisture (in %) and soil temperature (in °C) within each of the warming treatments and each of the precipitation treatments across all sampling dates is also summarized. Values are means ±SE ($n = 9$ for the warming treatments, $n = 12$ for the precipitation treatments).

Table S2. A summary of the P-values and denominator degrees of freedom ('DDF') from a mixed model split-plot ANOVA testing for the main and interactive effects of precipitation ('P') and warming ('W') on net N mineralization ('Min.') and net nitrification ('Nit.') within each sampling period from 2008–2010, the ratio of C mineralization to net N mineralization ('$C_{min} : N_{min}$') within each sampling period from 2008 to 2010, and the annual rates of net N mineralization and net nitrification from October 2009 to 2010. The numerator degrees of freedom are: 2 (precipitation), 3 (warming), and 6 (precipitation × warming). Significant effects are in bold ($P < 0.05$) and marginally significant effects are in italics ($0.05 \leq P < 0.1$). For significant effects, a positive response of net N mineralization or net nitrification to increased precipitation or warming was indicated with an upward pointing arrow (↑) and a negative response was indicated with a downward pointing arrow (↓).

Table S3. A summary of the P-values and denominator degrees of freedom ('DDF') from a mixed model split-plot ANOVA testing for the main and interactive effects of precipitation ('P') and warming ('W') on potential nitrification rates within each sampling period. The numerator degrees of freedom are: 2 (precipitation), 3 (warming), and 6 (precipitation × warming). Significant effects are in bold ($P < 0.05$) and marginally significant effects are in italics ($0.05 \leq P < 0.1$). For significant or marginally significant effects, a positive response of potential nitrification to increased precipitation or warming was indicated with an upward pointing arrow (↑).

Table S4. Exponential model of (a) net N mineralization, (b) net nitrification, (c) C mineralization as a function of soil temperature within each treatment across all sampling periods from August 2009 to October 2010 and apparent Q_{10} values.