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Impacts of an anomalously warm year on soil nitrogen availability in experimentally manipulated intact tallgrass prairie ecosystems

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Abstract

Global climate change can potentially increase the frequency of climate anomalies. Anomalously warm years may cause an increase in soil nitrogen (N) availability by stimulating N mineralization. To date, most studies addressing the effects of ecosystem warming have been conducted in relatively cold ecosystems and few studies have addressed impacts of interannual as opposed to continuous, multiyear warming. In this study, 12 intact soil monoliths were excavated from a tallgrass prairie site near Purcell, Oklahoma, USA and divided among four large flux chambers (EcoCELLs). During the first year, all four EcoCELLs were subjected to Oklahoma air temperatures and precipitation. During the second year, air temperature in two EcoCELLs was increased by 4 °C throughout the year resulting in an increase in soil temperature of 2.3 °C at 7.5 cm depth. During the third and fourth years, temperatures in the warmed EcoCELL returned back to 'normal' conditions. During the warming year, vegetation N content was not significantly affected by the warming treatment suggesting no change in N availability. Other N availability indicators (soil solution chemistry, leaching, and N adsorption by ion exchange resins) did not show any effect of warming. Soil solution, leaching, and ion exchange resins showed a large pulse of NH_4^+ at the start of the study most likely due to disturbance caused by monolith excavation and transport but these effects were short-lived and had disappeared before the treatment started. The lack of a clear warming effect may be explained by a reduction in soil moisture in the warming treatments compared with the controls offsetting a potential stimulation of N mineralization in response to increased temperatures. As a result, effects of an anomalously warm year on N availability in warmer ecosystems may be small compared with colder ecosystems but are likely to depend on soil moisture status.

Keywords: ammonium, interannual climate variability, mesocosms, NH_4, nitrate, NO_3, nutrient uptake, tallgrass prairie

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Introduction

During the last several decades, atmospheric CO_2 concentrations have increased rapidly in response to fossil fuel combustion and changes in land use (Keeling *et al*., 1996). One of the potential consequences of this global climate change is an increase in the frequency of unusually warm, wet, or dry years (Easterling *et al*., 2000; IPCC, 2007). These climate anomalies are very likely to affect ecosystem carbon (C) and nitrogen (N) cycling on a variety of timescales. For instance, extreme years can affect aboveground net primary productivity (ANPP) in grassland ecosystems up to several years after the anomalous year (Wiegand *et al*., 2004). However, the mechanisms that cause these aboveground responses to

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climate anomalies are not well understood. Based on modeling analyses, Braswell *et al*. (1997) hypothesized that on a global scale, ecosystem NPP responses to climate anomalies could be caused by changes in soil N availability. They speculated that N availability would increase during a warm year due to increased N mineralization. This extra N could then be taken up in the subsequent normal year resulting in an increase in NPP following the warm year.

The hypothesis by Braswell *et al*. (1997) appears to be supported by a large number of studies. A meta-analysis of data compiled from a range of soil/ecosystem warming and gradient studies showed that indeed warming leads to a significant overall increase in N mineralization (Rustad *et al*., 2001). Most studies included in this analysis were, however, conducted in temperate, high-latitude environments. To date, only few studies have applied warming treatments in warmer ecosystems (e.g. Beier *et al*., 2004; Penuelas *et al*., 2007) and only two out of the 32 studies included in the meta-analysis conducted by Rustad *et al*. (2001) had an annual average temperature exceeding 10 °C. It is unclear if an increase in temperature in warm ecosystems has a similar effect on soil N availability as in colder ecosystems. Although relative effects of temperature on soil organic matter decomposition tends to decrease with increasing temperature (e.g. Kirschbaum, 1995), effects of temperature increases on N mineralization can show different patterns. For instance, Wang *et al*. (2006) found no effect of an increase in temperature on net N mineralization between −10 and 5 °C while N mineralization steadily increased at higher temperatures up to 35 °C agreeing with results found by Nicolardot *et al*. (1994) and Stark & Firestone (1996). These studies suggest that effects of temperature on N mineralization may be larger in warmer ecosystems. However, Rustad *et al*. (2001) hypothesized that in warmer environments, the indirect effects of temperature on soil moisture may be more important compared with colder ecosystems.

Most studies to date have employed multiyear warming treatments to assess potential long-term effects of increases in global average annual temperatures. Melillo *et al*. (2002) observed a strong increase in net N mineralization during the first year of a long-term soil warming study in a forested ecosystem, but this stimulation decreased over time. In contrast, Verburg & Van Breemen (2000) did not find an increase in net N mineralization until the second year of warming in a boreal forest ecosystem. Consequently, the effects of a 1-year temperature anomaly could have a much more pronounced impact on N availability in the ecosystem studied by Melillo *et al*. (2002) as opposed to the system studied by Verburg & Van Breemen (2000) even though both studies show increases in net N mineralization after prolonged warming.

In this paper, we present results from a mesocosm experiment designed to study the effects of an anomalously warm year on native Oklahoma tallgrass prairie ecosystems. We used tallgrass prairie because the grassland biome accounts for >20% of all terrestrial land surface and 10% of the C pools (mostly in soils) on a global scale (e.g. Schimel, 1995; Schlesinger, 1997). Our study differs from previous studies because average annual temperature at the site is higher (>15 °C) than many previous study sites. In addition, to date no studies have experimentally assessed ecosystem effects of interannual warming patterns on N availability under rigorously controlled environmental conditions.

In our study, large, intact, soil-vegetation monoliths from a native tallgrass prairie ecosystem were subjected to an anomalously warm year in a fully replicated design using the unique mesocosm-scale Ecologically Controlled Enclosed Lysimeter Laboratories (EcoCELL) at the Desert Research Institute (DRI; Griffin *et al*., 1996; Verburg *et al*., 2004, 2005). We present 4 years of data (one pretreatment 'normal' year, one 'warm' year, and two posttreatment 'normal' years). We quantified the effects of an anomalously warm year on N availability by measuring net plant uptake, soil solution chemistry, leaching, and N adsorption by ion exchange resins.

Materials and methods

Experimental system and design

We selected a native tallgrass prairie site at the Kessler Farm Field Laboratory of the University of Oklahoma near Purcell, Oklahoma, to extract twelve 12 000 kg soil monoliths (2.44 m × 1.22 m × 1.8 m) for transport back to DRI in Reno, Nevada, USA. The average annual temperature is 16 °C with a monthly mean temperature of 3.1 °C in January and 28.0 °C in July. Mean annual precipitation is 967.2 mm with most of the precipitation falling between March and September. Vegetation at the site is dominated by *Panicum virgatum* L., *Schizachyrium scoparium* (Michx.) Nash, *Andropogon gerardii* Vitman, *Sorghastrum nutans* (L.) Nash, *Ambrosia psilostachya* DC., *Xanthocephalum texanum* (DC.) Shinners, *Bromus japonicus* Thunb. ex Murr., and *Eragrostis* spp. The site has not been grazed for 20 years. Soils at the site belong to the Nash–Lucien complex, a mixture of loamy, mixed, thermic shallow Typic Haplustolls and coarse-silty, mixed, thermic Udic Haplustolls (sand: 32%; silt: 60%; clay: 8%) and are considered to be moderately fertile. Soil organic C contents in the 0–15 cm is 0.85 ± 0.12% ($n = 12$) while N content is 0.063 ± 0.01% ($n = 12$) with both C and N concentration decreasing exponentially

with depth. Total C stocks down to 90 cm depth equal $6.87 \pm 0.67\,kg\,C\,m^{-2}$ and total N stocks equal $0.53 \pm 0.05\,kg\,N\,m^{-2}$ ($n = 12$). Both C and N stocks were similar between monoliths assigned to the warmed vs. control treatments. In general, these native tallgrass prairie plant communities are considered to be N limited (Blair *et al*., 1998).

Most vegetative species were dormant at the time of the excavation (September and October 2001), and aboveground vegetation was clipped to a height of 10 cm above the soil surface before excavation. A detailed description of the excavation process and installation in the EcoCELLs is given by Verburg *et al*. (2005). The EcoCELL facility consists of four open-flow, mass-balance systems using the principles employed in leaf-level, gas-exchange experiments but at a much larger scale. Total volume of each chamber is $184\,m^3$ of which $40\,m^3$ is occupied by three monoliths plus 45 cm thick Styrofoam® (The Dow Chemical Company, Midland, MI, USA) insulation that extends from the top edge of each container to the floor of the EcoCELL. Each monolith was placed on four load cells (truck scales; one on each bottom corner of the monolith containers), each with a capacity of 5000 kg and a combined precision of ± 1 kg. Environmental controls include temperature, CO_2 concentration, and relative humidity. The chambers receive natural light, which is attenuated by 22%. A detailed description of the EcoCELL facility is given by Griffin *et al*. (1996).

During the experiment, temperatures inside the EcoCELLs were maintained based on 8-year (1993–2000), 5 min averages from a MESONET station (Brock *et al*., 1995) <1.6 km from the excavation site. Ambient air temperatures were adjusted weekly while maintaining diurnal patterns. Using heating and cooling underneath the monoliths, soil temperatures in the deepest horizons (1.45 m depth) were kept as close as possible to the 1993–2000 mean annual ambient air temperature (16 °C) measured at the excavation site to simulate realistic soil temperature profiles (Verburg *et al*., 2005). We installed 45 cm thick polystyrene insulation around all sides of the monolith containers to minimize lateral heat transfer. During the first year of the study, ambient air temperatures in all four EcoCELLs were kept the same. On 11 February 2003, we increased air temperature in two EcoCELLs by 4 °C and maintained this differential throughout the year to simulate an anomalously warm year. On 11 February 2004, temperatures returned back to the prewarming control normal values and remained at these temperatures for another 2 years. The 4 °C temperature increase was based on long-term records (1873–1999) of mean annual air temperatures measured at various locations in central Oklahoma. Anomalously warm years were evident in this record and had a mean annual temperature increase of 1.1–3.8 °C. In addition, annual temperature and precipitation were unrelated ($P > 0.80$, $r^2 < 0.15$), justifying a simple increase in temperature without altering the precipitation regime. Precipitation was applied using an overhead rain simulator with a fixed intensity. We based frequency and amounts on measurements from the MESONET site (same 8-year period used for temperature) with precipitation at each watering applied at the mean monthly total divided by the mean frequency for that month.

Soil water content and temperature

Soil water content was measured hourly in the center of each monolith using segmented time domain reflectometry probes (E.S.I. Environmental Sensors Inc., Sidney, Canada) at depth intervals of 0–15, 15–30, 30–60, 60–90, 90–120, and 120–150 cm. We used thermocouples to measure soil temperature every 5 min in the center of each monolith at depths of 7.5, 22.5, 45, 75, 105, and 145 cm.

Plant N content and soil N availability

We estimated soil N availability by quantifying (1) total N uptake by the vegetation, (2) N concentrations in soil solution and drainage water, and (3) N adsorption using two types of resin-based techniques. We used these specific methods as they minimized destructive sampling and thus soil disturbance.

We estimated N content of ANPP (ANPP-N) by clipping shoots to a depth of 10 cm above the soil surface in early August of each year and multiplying biomass with N concentrations. At the same time, biomass between 0 and 10 cm canopy height was sampled by clipping two $30\,cm \times 30\,cm$ areas in each monolith to the soil surface. During spring of the pretreatment year, *Vicia sativa* L. emerged in several monoliths. Because this species is considered invasive at the field site, we removed it from the monoliths. The N contained in the *Vicia* was not included in the ANPP-N numbers because *Vicia* is an N fixing species and $\delta^{15}N$ numbers indicated that most N was derived from the atmosphere rather than the soil. Any *Vicia* occurring during subsequent years was removed from the monoliths. The contribution of *Vicia* to total ANPP during any of the years was <15%.

We estimated N content of belowground net primary productivity (BNPP-N) using in-growth cores – two 4 cm diameter cores per monolith over 0–90 cm depth. To estimate BNPP-N during the growing season (February–August), we collected cores in August; to quantify BNPP during the cold 'dormant' seasons (September–late January), we collected cores in early February corresponding to start and end dates of warming. After sampling, we

back-filled existing holes with sieved soil from the excavation site. Total NPP-N was calculated as the sum of ANPP-N and BNPP-N. Biomass N concentrations of shoots and roots were measured at the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University.

Soil solution was sampled approximately monthly using ceramic cup tension lysimeters (Soil Moisture Inc., Santa Barbara, CA, USA). Lysimeters were placed in two diametrically opposite quadrants of each monolith at 10 and 40 cm. Samples were collected during a 1-week period by applying a vacuum to all sampling bottles. In order to quantify N losses from the ecosystems, we continuously collected leachate in buckets connected to the draining pans. Samples were analyzed on a weekly basis and more frequently during periods with high leaching. Soil solution and leachate samples were filtered using an Osmonics 0.45 μm nylon filter (General Electric Company, Trevose, PA, USA) and analyzed for NH_4^+ and NO_3^- at the Soil, Water and Forage Analytical Laboratory (SWFAL) at Oklahoma State University using a Lachat 8000 flow-injection analyzer with a Cetac xyz autosampler (CETAC Technologies, Omaha, NE, USA). Fluxes of NH_4^+ and NO_3^- were calculated by multiplying concentrations by the amount of leachate collected.

We installed mixed-bed cation/anion exchange resin capsules (Unibest PST-1; Yang & Skogley, 1992; Dobermann *et al.*, 1994) at 10 and 40 cm depth in the same quadrants used for the soil solution lysimeters. Resins were held in place using a polyvinyl chloride (PVC) tube with a removable rod inside the tube. The capsule was fixed at the end of each rod. We also installed resin membrane stakes in the same quadrant. These resin stakes were Plant Root Simulator (PRS™, Western Ag Innovations, Saskatoon, Canada) probes consisting of anion or cation exchange membranes imbedded in plastic stakes. Resin capsules and membranes were installed for a period of 1 month four times per year. We did not start the PRS™ probes until the beginning of the treatment year. At the end of the incubation period, the resin capsules and PRS probes were removed and extracted for NH_4^+ and NO_3^-. After washing off the soil adhering to the resin capsule surface, the capsules were extracted with three sequential 20 mL solutions of 2 M KCl on a platform shaker for 20 min each. The combined extractant (60 mL) was filtered through a Fisher P4 qualitative filter (Fisher Scientific, Pittsburg, PA, USA) and analyzed for NH_4^+ and NO_3^- on a Lachat 8000 flow-injection analyzer with CETAC xyz autosampler at the SWFAL. Values were expressed in $\mu mol\,N\,cm^{-2}$ of resin area. After washing off soil from the PRS probes, they were sent to Western Ag Innovations Inc. for extraction. At Western Ag, the probes were extracted with 17.5 mL of 0.5 M HCl for 1 h in a zip lock bag, and the extractant was analyzed for NH_4^+ and NO_3^- using a Technicon Autoanalyzer (Alpkem Technicon Autoanalyser Instruments, Saskatoon, Canada). The values were reported in units of $\mu mol\,N\,10\,cm^{-2}$ of membrane surface, and these were converted to $\mu mol\,N\,cm^{-2}$. We did not measure exchangeable N pools because systematic measurement of this pool would have required too much destructive sampling and caused too much soil to be removed, thereby potentially altering the ecosystems. We expected that both the resin capsules and PRS probes would have acted as sorption sites and would thus have been somewhat representative of the soil matrix.

Data analysis

To assess effects of the warming treatments during and after the treatment year, we analyzed our data using repeated-measures analysis of variance (ANOVA) with treatment as the main factor. We interpreted significant interactions between treatment and time (the repeat factor) as caused by the warming treatments. Significant treatment effects for individual measurements dates were tested using *t*-test. We analyzed data using EcoCELL means ($n = 2$) for all statistical analyses. We used DATADESK version 6.0 for all statistical analyses.

Results

Environmental parameters

During the first year (pretreatment), weekly average air temperature was similar in all four EcoCELLs. On 11 February 2003, air temperature was increased in two EcoCELLs causing an average increase in soil temperature of 2.3 °C at 7.5 cm depth (Fig. 1). After the treatment year, soil and air temperature in the previously warmed EcoCELLs closely tracked the control EcoCELLs again. Soil temperatures at 1.45 m depth remained close to the set point of 16 °C, with diurnal and seasonal oscillations in soil temperature increasing at shallower depths (Fig. 1). Occasional deviations from the set point temperature at 1.45 m were caused by malfunctions of the chilling unit regulating subsoil temperatures as a result of either power outages or ice formation inside the cooling coils.

Across all depths, soil water content was similar in all four EcoCELLs during the pretreatment year. During the treatment year, soil water content increased in the control EcoCELLs while soil moisture in the warmed EcoCELLs did not change compared with the pretreatment year. The increase in soil moisture in the control EcoCELLs was caused by a significant reduction in evapotranspiration (ET) from 917 ± 20 mm in the pretreatment year to 832 ± 19 mm in the treatment year ($P = 0.046$). In the warmed EcoCELLs, ET tended to decrease from 949 ± 15 mm in the pretreatment year to 897 ± 1 in the treatment year ($P = 0.09$). The relative reduction in soil

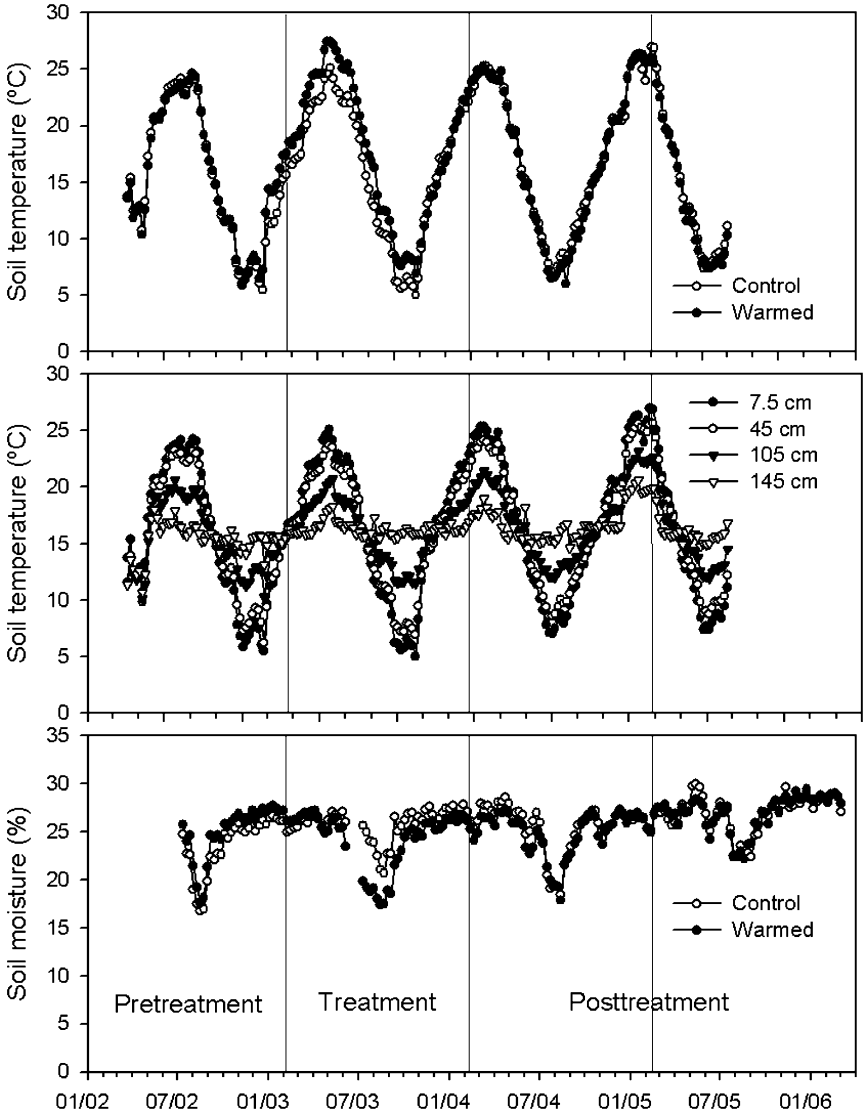

Fig. 1 Soil environmental conditions for 4 years in control and warmed EcoCELLs. Beginning 11 February 2003, air temperature in two out of four EcoCELLs was increased by 4 °C throughout the year. The first panel represents soil temperature at 7.5 cm depth. The second panel shows soil temperature with depth in the control EcoCELLs. The third panel shows soil moisture at 7.5 cm depth.

water content in the warmed EcoCELLs was most pronounced in the topsoil (Fig. 1). Soil moisture in the 0–15 cm soil layer in the first posttreatment year initially was slightly lower in the previously warmed than in the control EcoCELLs but became similar during spring and remained the same throughout the rest of the posttreatment.

Soil N availability

The warming treatment did not significantly affect plant N contents (Table 1). Before the warming treatment, ANPP-N was similar in all EcoCELLs (Fig. 2). During the treatment year, ANPP-N tended to be lower in the warmed EcoCELLs ($P = 0.057$) while in both postwarming years, ANPP-N was similar in all EcoCELLs. BNPP-N was similar throughout the study but tended to be lower in the second posttreatment year ($P = 0.092$) in the previously warmed EcoCELLs. Total NPP-N (ANPP-N + BNPP-N) did not show significant treatment effects but tended to be lower during treatment year in the warmed EcoCELLs ($P = 0.097$).

We did not observe any effects of the warming treatment on soil solution NH_4^+ and NO_3^- concentrations at any depth. Soil solution NH_4^+ concentrations were highest during spring of the pretreatment year but lowered to detection limit values throughout the remainder of the study (Fig. 3). There was a significant time × treatment

Table 1 *P*-values for repeated-measures analysis of variance for various parameters using each EcoCELL as a replicate ($n = 2$)

	Time	Treatment	Time × Treatment
ANPP-N	0.310	0.371	0.260
BNPP-N	0.749	0.236	0.540
NPP-N	0.822	0.248	0.393
Soil solution NH_4^+*	**<0.001**	0.655	**0.024**
Soil solution NO_3^-*	**<0.001**	0.934	1.000
Drainage NH_4^+†	**<0.001**	0.872	0.858
Drainage NO_3^-†	0.143	0.116	0.779
Drainage H_2O†	0.347	0.919	0.899
Resin capsule NH_4^+ (10 cm)	**<0.001**	**0.014**	0.885
Resin capsule NH_4^+ (40 cm)	**<0.001**	0.051	**0.002**
Resin capsule NO_3^- (10 cm)	**0.003**	0.438	0.395
Resin capsule NO_3^- (40 cm)	**<0.001**	0.627	0.215
PRS NH_4^+	**<0.001**	0.284	0.072
PRS NO_3^-	**<0.001**	0.583	0.060

Significant effects ($P < 0.05$) are presented in bold.
*Data for 10 and 40 cm depth combined.
†Statistics were done on cumulative annual leaching.

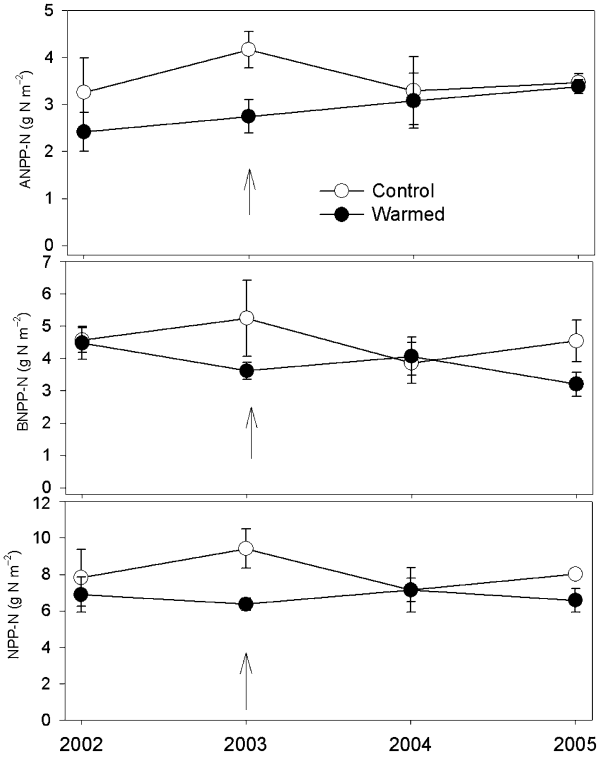

Fig. 2 Nitrogen content in aboveground, belowground, and total biomass. Error bars represent standard errors of the mean ($n = 2$). The arrow indicates the warming year. Significant differences ($P < 0.05$) between control and warmed EcoCELLs are indicated by an asterisk.

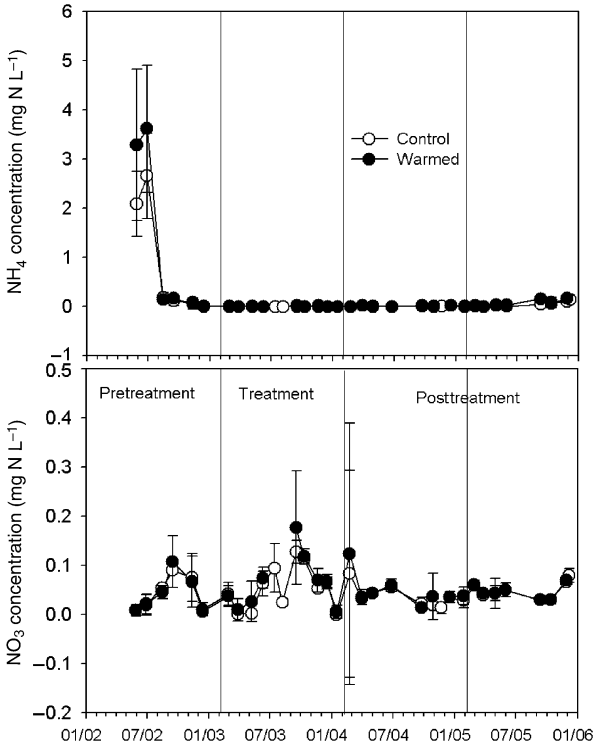

Fig. 3 Soil solution NH_4^+ and NO_3^- concentrations in soil solution during the 4 years of the study. Temperatures were increased in the warmed EcoCELLs during the second year. During the following 2 years, temperatures were similar in all EcoCELLs. Concentrations are averaged across 10 and 40 cm depths. Error bars represent standard errors of the mean ($n = 2$).

interaction for NH_4^+; however, that was most likely caused by the higher NH_4^+ concentration during the pretreatment year in the EcoCELLs that were assigned to the warming treatment. Soil solution NO_3^- did not show an initial peak, but concentrations were higher than NH_4^+ for most of the study (Fig. 3). Soil solution NO_3^- concentrations showed seasonal patterns with concentrations generally higher during summer than winter.

Dissolved inorganic N concentrations in drainage showed large temporal and spatial (between monoliths/EcoCELLs) variability, but we did not find significant effects of the warming treatment on annual N leaching fluxes during the 4 years (Table 1). During spring of the pretreatment year, NH_4^+ leaching was very high, but decreased during the first few months and remained low for the remainder of the study (Fig. 4). Following the initial NH_4^+ pulse, NO_3^- concentrations were higher than NH_4^+ concentrations. Drainage water fluxes were similar for all treatments (Fig. 4), so leaching patterns were not confounded by differences in total water flux. Total N losses through DIN leaching were very small compared with NPP, and the highest annual N loss was 26 mg N m^{-2} yr^{-1} for EcoCELL 1

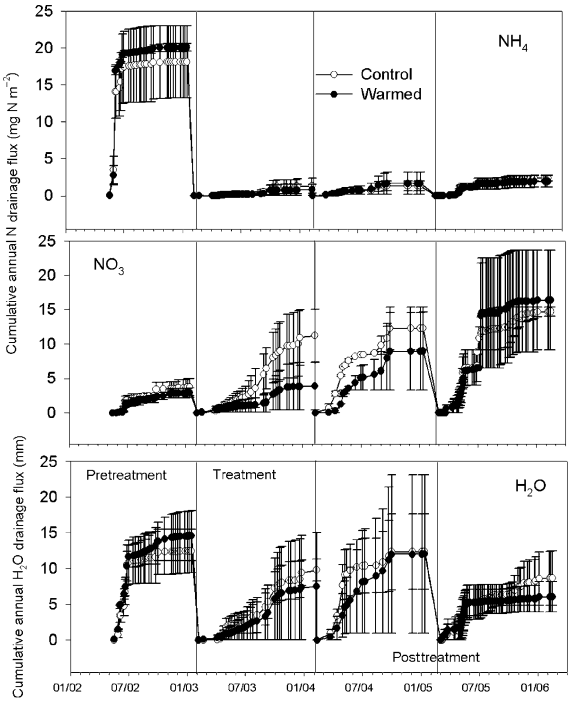

Fig. 4 Nitrogen concentrations and water flux in drainage. The numbers represent cumulative flux for each separate year. At the start of each year, total drainage flux is reset to zero. Error bars represent standard errors of the mean ($n = 2$).

during the second posttreatment year. Cumulative drainage NH_4^+ losses for the entire study were $23.2 \pm 6.9\,mg\,N\,m^{-2}\,yr^{-1}$ for the control EcoCELLs and $24.5 \pm 2.0\,mg\,N\,m^{-2}\,yr^{-1}$ for the warmed EcoCELLs. Drainage NO_3^- losses were $41.9 \pm 2.6\,mg\,N\,m^{-2}\,yr^{-1}$ for the control EcoCELLs and $32.1 \pm 2.5\,mg\,N\,m^{-2}\,yr^{-1}$ for the warmed EcoCELLs and NO_3^- leaching tended to be lower in the warmed EcoCELLs ($P = 0.056$).

Repeated-measures ANOVA analysis of the resin capsule data at 10 cm showed no significant time × treatment interactions indicating no effects of warming at that depth (Table 1). There was an overall treatment effect indicating preexisting differences in NH_4^+ availability between control and warmed EcoCELLs that were not caused by the warming treatments. At 40 cm, the ANOVA showed a significant time × treatment interaction. At both depths, NH_4^+ showed a clear peak during early spring of the pretreatment year; but this peak was significantly higher in the warmed EcoCELLs ($P = 0.02$; Fig. 5) at 40 cm. A second NH_4^+ peak appeared during September of the treatment year in all EcoCELLs at 10 and 40 cm, but these peaks were similar for warmed and control EcoCELLs. NO_3^- concentrations showed no significant treatment effects. At 10 cm, average NO_3^- concentrations appeared to be higher in the control than in the previously warmed EcoCELLs during summer and fall of the second posttreatment year; but the differences were not significant (Fig. 6). We did not observe significant effects of warming treatments on annual inorganic NO_3^- concentrations at any depth. NH_4^+ concentrations at 40 cm were lower in the two posttreatment years, however, compared with the pretreatment and treatment year at both depths.

Repeated-measures ANOVA suggested the presence of a treatment effect on NH_4^+ and NO_3^- for the PRS probes with treatment × time interactions being close to significant (Table 1; NH_4^+: $P = 0.072$; NO_3^-: $P = 0.060$). These patterns were, however, not consistent throughout the study. NH_4^+ concentrations significantly increased in fall of the treatment year in the warmed EcoCELLs ($P = 0.048$) followed by a decrease compared with the control EcoCELLs (Fig. 7). Average NO_3^- concentrations showed a similar pattern but the increase in NO_3^- during fall of the treatment year was not significant ($P = 0.061$). During the posttreatment years, inorganic N concentrations were similar in all EcoCELLs. We found no significant warming effects on annual N availability during or after the warming treatments as calculated by summing inorganic N concentrations of the individual incubation periods for each year.

Discussion

The overall goal of our study was to investigate effects of an anomalously warm year on soil N availability. Plant N content remained constant throughout the study, suggesting no change in soil N availability. Field studies carried out in tallgrass prairie ecosystems close to the location of the monolith excavation showed increases in total aboveground N content in response to 4 years of warming (An *et al*., 2005) but effects varied by year. In this same experiment, Wan *et al*. (2005) found that N mineralization increased in the first warming year but decreased in the second year. These studies suggest that total aboveground N content may not necessarily reflect soil N availability. Potentially, warming could affect vegetation N resorption efficiency resulting in changes in N use efficiency (NUE; amount of biomass produced per unit N in vegetation; Norby *et al*., 2000). In our study, NUE was similar in control and warmed EcoCELLs throughout the study. This is consistent with An *et al*. (2005) who did not find a change in NUE until the third year of warming in tallgrass prairie ecosystems. Several studies have shown that total aboveground N in tallgrass prairie depends on other factors besides soil N availability (e.g. Blair, 1997; Turner *et al*., 1997). As a result, attempts to relate NPP-N to soil N availability in multiyear warming studies can be confounded by interannual variability in ambient

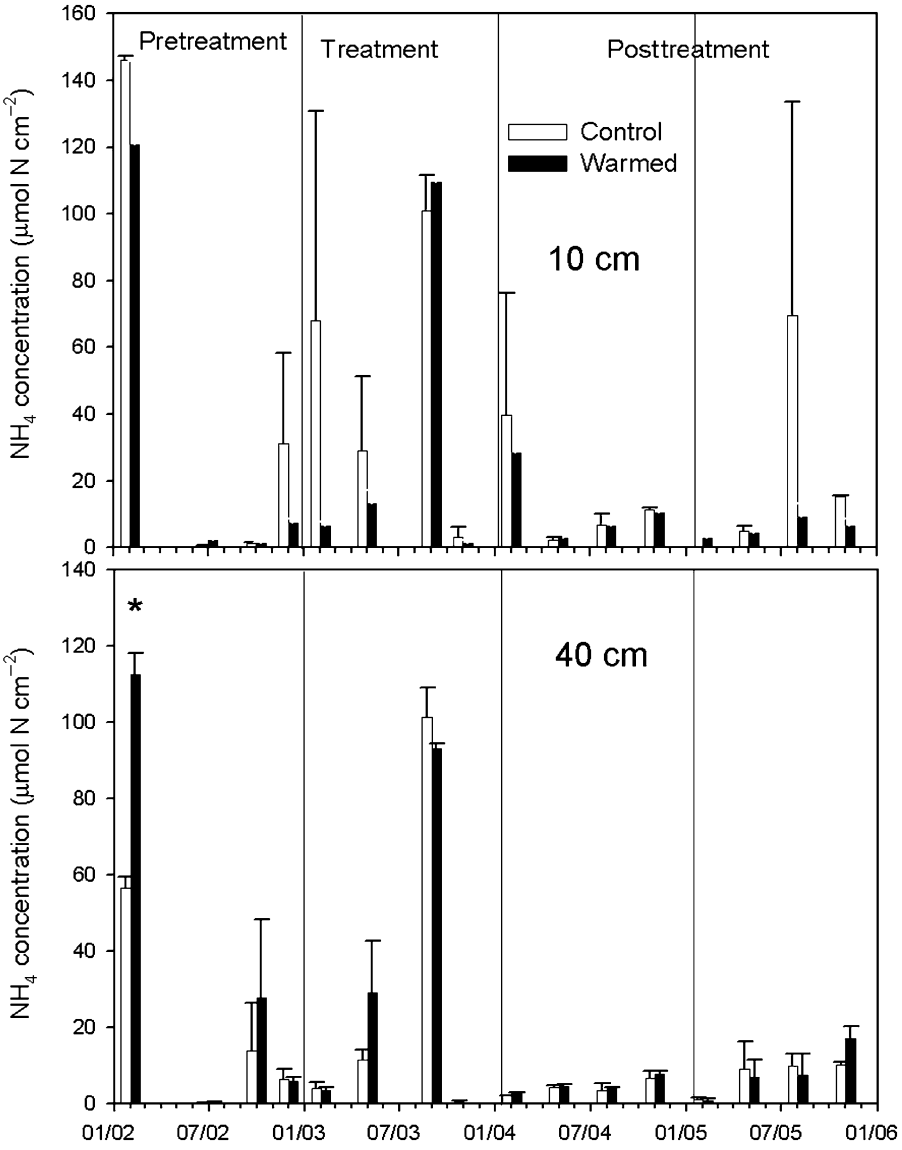

Fig. 5 Ammonium concentrations in resin capsules at 10 and 40 cm depth. The units reflect N concentrations per unit resin area. Error bars represent standard errors of the mean ($n = 2$). Significant differences ($P < 0.05$) between control and warmed EcoCELLs are indicated by an asterisk. Significant differences ($P < 0.05$) between control and warned EcoCELLs are indicated by an asterisk.

climate conditions. In our study, we only varied air temperature in 1 year while keeping ambient climate conditions constant throughout the study to eliminate interannual variability in environmental conditions. In addition, most studies do not include BNPP-N. In our study, BNPP-N constituted >50% of total NPP-N (Fig. 2). Despite the uncertainties in relating NPP-N to soil N availability, Turner *et al*. (1997) found that ANPP-N in tallgrass prairie ecosystems increased with fertilizer application, suggesting that if N availability would drastically change, vegetation N content would most likely reflect these changes. In addition, the low soil solution N concentrations and leaching fluxes support the notion by Blair *et al*. (1998) that these ecosystem are N limited, so we would have expected an increase in N uptake with increased soil N availability. Therefore, we conclude that the absence of any clear changes in NPP-N indicates that soil N availability did not significantly change.

The lack of response of NPP-N to warming is consistent with most other indicators of soil N availability used in this study. Soil solution DIN concentrations and DIN in leachate were very low throughout the study and were not affected by warming (Figs 3 and 4). Soil solution represents a small but potentially dynamic nutrient pool. Soil solution DIN concentrations fluctuated

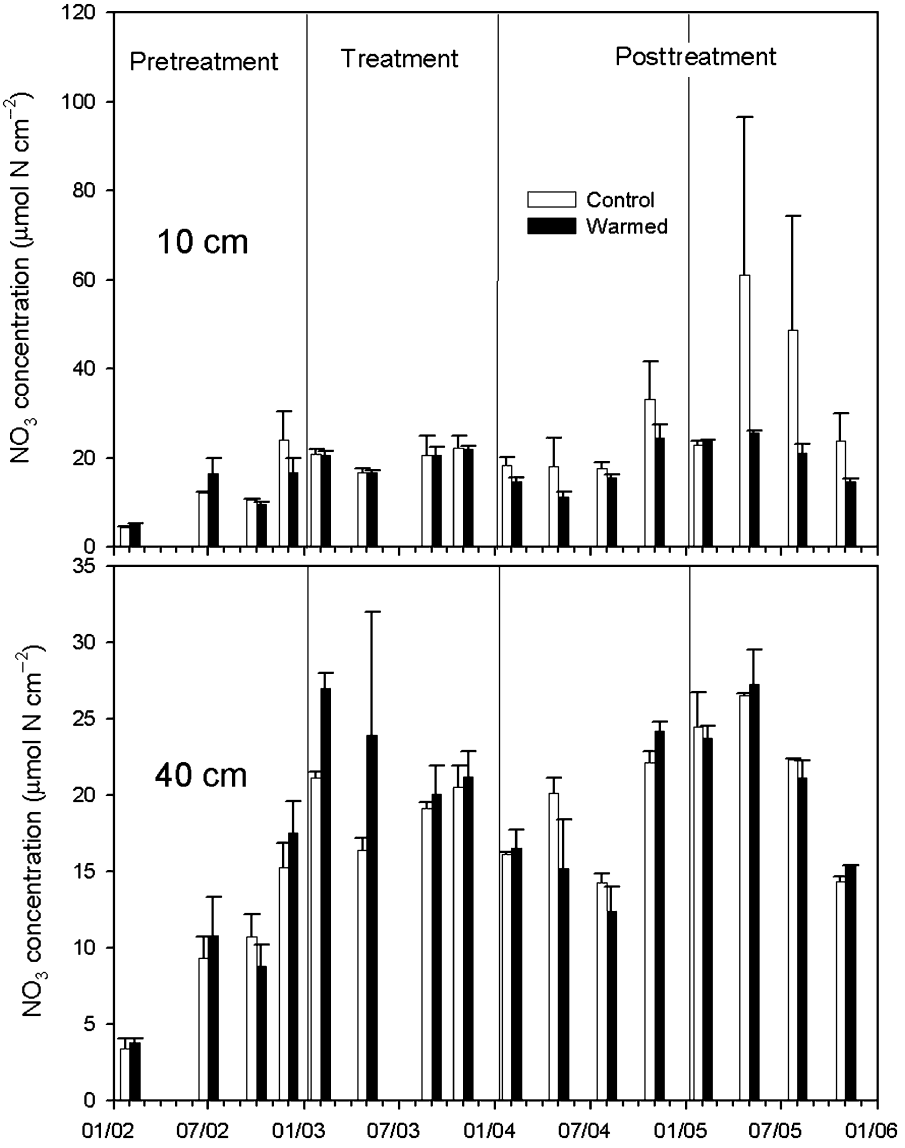

Fig. 6 Nitrate concentrations in resin capsules at 10 and 40 cm depth. The units reflect N concentrations per unit resin area. Error bars represent standard errors of the mean ($n = 2$).

throughout the study with average concentrations peaking at 0.18 mg L^{-1}. If one assumes total soil saturation at a pore space of 40%, the amount of water present in each monolith would equal approximately 673 L m^{-2}. This would result in a total amount of 0.12 g N m^{-2} in soil solution equivalent to approximately 1% of NPP-N. Despite this small pool size, several studies have shown changes in soil solution chemistry in response to experimental warming. Schmidt *et al*. (2004) and Verburg (2005) showed increased soil solution NO_3^- concentrations in response to experimental warming of N-limited northern European shrubland and forest ecosystems. Consistent with the soil solution responses, we did not see any effects of warming on annual N leaching losses. Over the entire experimental period, warming caused a reduction in NO_3^- leaching which could indicate a decrease in nitrification or increased denitrification rates but this did not become apparent until all years were combined. Overall, annual N leaching losses were very small compared with plant uptake (<0.3%). As with soil solution, leaching fluxes can be a sensitive indicator of changes in N status as they spatially integrate ecosystem responses. For instance, Lükewille & Wright (1997) showed increased NO_3^- leaching in a small forested catchment in southern Norway in response to soil warming even though soil solution concentrations and N mineralization measurements did not show clear warming effects.

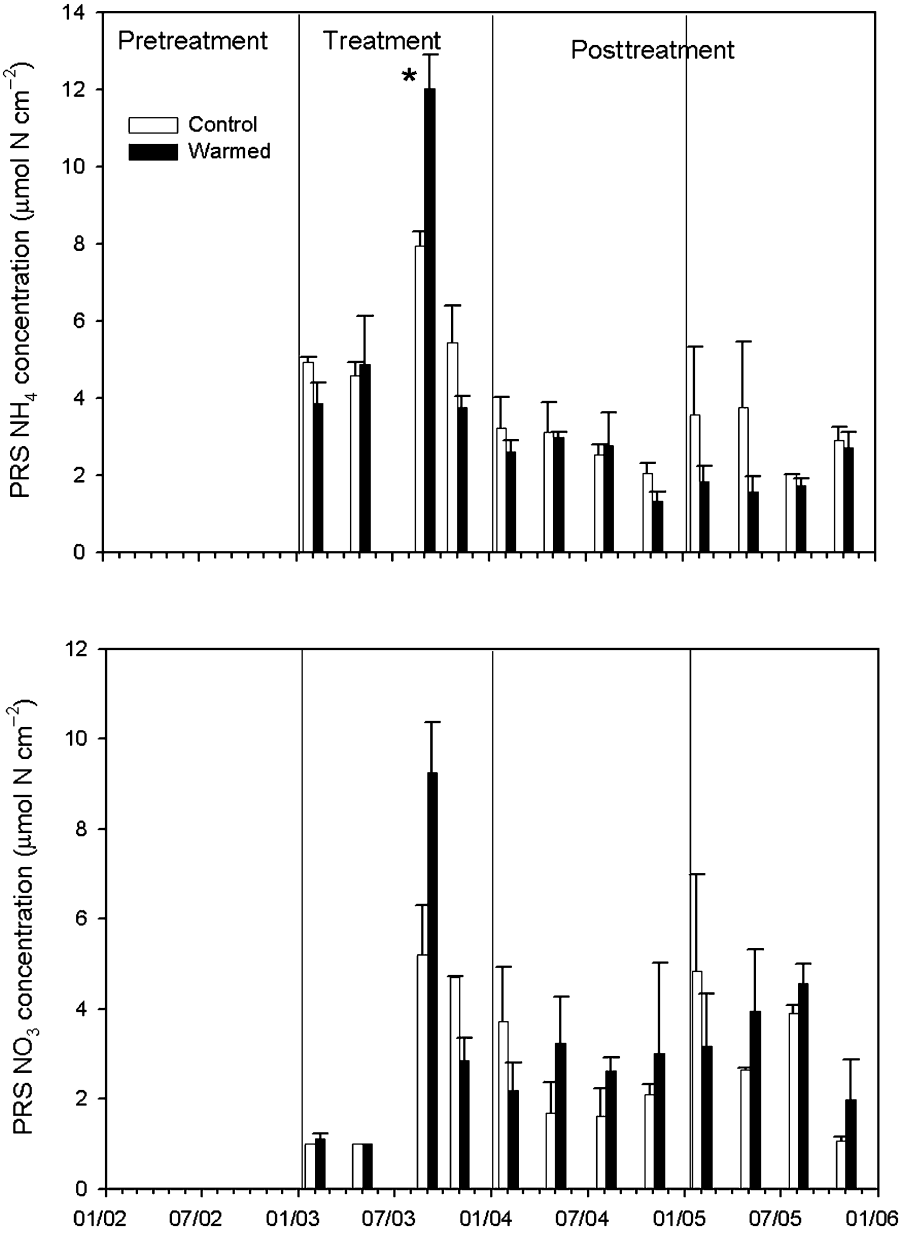

Fig. 7 Ammonium and nitrate concentrations in Plant Root Simulator (PRS) probes. The units reflect N concentrations per unit resin area. Error bars represent standard errors of the mean ($n = 2$). Significant differences ($P < 0.05$) between control and warned EcoCELLs are indicated by an asterisk.

The resin capsules also did not show clear changes in NH_4^+ or NO_3^- availability in response to the warm year. Both resin capsule and PRS data showed a large increase in NH_4^+ adsorption during fall of the warm year. During this time, only the PRS probes showed a higher inorganic N adsorption in the warmed than in the control EcoCELLs. Johnson *et al*. (2007) showed that N adsorbed by PRS probes was inversely related to plant uptake, indicating that in his study PRS probes measured N left over in the soil after plant uptake was satisfied. However, in our study, plant N was similar in control and warmed ecosystems during the warming year while PRS-N increased, suggesting a small increase in available N in the topsoil during this period. Combining the PRS probe data for the four incubation periods during the warming year showed that annual N availability was similar in control and warmed ecosystems consistent with the NPP-N data.

Both resin capsules and PRS probes showed that NH_4^+ availability decreased following the warming year compared with pretreatment and treatment years. The decrease in NH_4^+ availability over time was not

due to increased nitrification because the decrease in NH_4^+ availability was not balanced by an increase in NO_3^- availability. One potential explanation for the decrease in NH_4^+ availability over time may be that aboveground litter inputs and subsequent N mineralization decreased. As a means to simulate grazing, we removed all aboveground plant material during harvests in the second, third, and fourth years of the study and did not return this material back to the ecosystems. This continuous removal most likely contributed to depletion of the NH_4^+ pool. Still, this potential reduction of available N as measured in our study did not appear to impact N uptake by the vegetation as plant N content remained constant for the duration of the study. Under field conditions, grazing is likely to remove less biomass leaving more dead material to be added to the aboveground litter pool every year. In addition, some nutrients will be returned to the community via urine and feces deposition.

Potentially, changes in N mineralization rates could have resulted in changes in the amount of soil inorganic N. Verburg (2005) showed that warming-induced increases in net N mineralization resulted in a small but significant increase in inorganic N pools in two forested boreal catchments. In this study, we did not measure soil inorganic N pools due to the destructive nature of the sampling. The soils in our study had a low organic N content ($N < 0.10\%$) in the topsoil, but the total soil N pool was approximately $530\,g\,m^{-2}$. Given this large background and relatively small changes in N availability ($<0.5\%$ of the soil organic N pool), it is unlikely that these changes could have been detected given spatial variability in soil N pools and analytical uncertainty.

Soil solution, leaching, and resin data showed very high NH_4^+ availability at the start of the study which may have been a result of transportation of the monoliths from Oklahoma to Reno. The disturbance caused by the excavation and transportation could have caused an increase in N mineralization. The monoliths were excavated in fall 2001 and installed during winter into the EcoCELLs. During this period, plant growth and thus N uptake were small causing accumulation of NH_4^+ in the soil. The resin capsule data showed that the initial pulse was higher for the EcoCELLs that were going to be warmed. It is unclear why this differential response occurred. However, the initial pulse quickly dissipated when precipitation started and the ecosystems stabilized during the summer of the pretreatment year.

Our results do not agree with many field-warming studies conducted in colder ecosystems (e.g. Rustad *et al*., 2001), suggesting that warming effects may not be as pronounced in warmer ecosystems compared with ecosystems in colder climatic settings. Still, laboratory studies show consistent increases in N mineralization with increasing temperatures even at higher temperature ranges (e.g. Nicolardot *et al*., 1994; Wang *et al*., 2006). Potentially, the lower soil moisture in the warmed compared with the control ecosystems offset the increase in temperature as hypothesized by Rustad *et al*. (2001). Indeed, in a laboratory incubation using tallgrass prairie soils obtained from the study site, Johnson *et al*. (2005) showed that net N mineralization was positively correlated with both temperature (range 16–25 °C) and soil moisture (range 15–25%), showing that effects of temperature increases can be offset by decreases in soil moisture. Wang *et al*. (2006) observed strong, site-specific interactions between effects of temperature and moisture on N mineralization with relative temperature effects decreasing at lower soil moisture levels. Separating the temperature effects from moisture effects is difficult in our study because the two variables are highly correlated (Verburg *et al*., 2005). It is unlikely that the lack of response in our study was due to acclimation of N mineralization to higher temperatures. Melillo *et al*. (2002) showed that effects of warming on N mineralization decreased over a 5-year period perhaps due to depletion of labile soil N pools. In our study, we only warmed the ecosystems for 1 year followed by 2 ambient years limiting the potential for acclimation. Furthermore, Wan *et al*. (2005) found a large increase in net N mineralization in the first year of warming tallgrass prairie ecosystems.

The results from our study show that the impacts of interannual temperature variability on N availability in tallgrass prairie ecosystems may be small compared with colder (and potentially wetter) ecosystems. In colder ecosystems, temperature-induced changes in N availability can have important impacts on ecosystem C sequestration (e.g. Melillo *et al*., 2002). The importance of these N feedbacks on C sequestration may be much smaller in warmer, more mesic grassland ecosystems. Indeed, Sherry *et al*. (2008) showed that immediate and lagged responses of ANPP to an anomalously warm year in tallgrass prairie ecosystems could be explained by changes in soil moisture alone while nutrient feedbacks did not appear to be important. In addition, measurements of net ecosystem CO_2 exchange and NPP in the EcoCELL study showed that immediate and lagged responses to the anomalously warm year were caused by direct effects of changes in soil moisture on plant physiological processes (Arnone *et al*., 2008). Our results suggest, however, that effects of temperature anomalies on N availability and thus ecosystem C sequestration may be more important in more humid systems where impact of warming on soil moisture may be smaller.

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