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Authors
BIJOOR, NEETA S
CZIMCZIK, CLAUDIA I
PATAKI, DIANE E
et al.

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Effects of temperature and fertilization on nitrogen cycling and community composition of an urban lawn

NEETA S. BIJOOR*, CLAUDIA I. CZIMCZIK*, DIANE E. PATAKI*† and SHARON A. BILLINGS‡

*Department of Earth System Science, Croul Hall, University of California, Irvine, CA 92697-3100, USA, †Department of Ecology and Evolutionary Biology, Steinhaus Hall, University of California, Irvine, CA 92697-2525, USA, ‡Department of Ecology & Evolutionary Biology and Kansas Biological Survey, Higuchi Hall, University of Kansas, Lawrence, KS 66047, USA

Abstract

We examined the influence of temperature and management practices on the nitrogen (N) cycling of turfgrass, the largest irrigated crop in the United States. We measured nitrous oxide (N₂O) fluxes, and plant and soil N content and isotopic composition with a manipulative experiment of temperature and fertilizer application. Infrared lamps were used to increase surface temperature by 3.5 ± 1.3 °C on average and control and heated plots were split into high and low fertilizer treatments. The N₂O fluxes increased following fertilizer application and were also directly related to soil moisture. There was a positive effect of warming on N₂O fluxes. Soils in the heated plots were enriched in nitrogen isotope ratio (δ¹⁵N) relative to control plots, consistent with greater gaseous losses of N. For all treatments, C₄ plant C/N ratio was negatively correlated with plant δ¹⁵N, suggesting that low leaf N was associated with the use of isotopically depleted N sources such as mineralized organic matter. A significant and unexpected result was a large, rapid increase in the proportion of C₄ plants in the heated plots relative to control plots, as measured by the carbon isotope ratio (δ¹³C) of total harvested aboveground biomass. Our results suggest that an increase in temperature caused by climate change as well as the urban heat island effect may result in increases in N₂O emissions from fertilized urban lawns. In addition, warming may exacerbate weed invasions, which may require more intensive management, e.g. herbicide application, to manage species composition.

Keywords: C₃ and C₄, C₄ weeds, crabgrass, fescue, nitrous oxide, turfgrass, warming

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Introduction

Turfgrass ecosystems are expanding rapidly in conjunction with urbanization, which is expected to increase 79% in the United States in the next 25 years (Alig et al., 2004). As the largest irrigated crop in the United States, turfgrass currently covers 1.9% of the national surface area (Milesi et al., 2005). Turfgrass land cover can sequester carbon (C) (Qian & Follett, 2002), but can also cause significant emissions of nitrous oxide (N₂O) (Kaye et al., 2005), an important greenhouse gas that has a global warming potential 296 times greater than that of carbon dioxide (CO₂) (IPCC, 2001), and that has been increasing in the troposphere at a rate of about 0.2% yr⁻¹ (Weiss, 1981; Khalil et al., 2002). It is, therefore, important to understand nitrogen (N) cycling and N₂O emissions from turfgrass, and their potential responses to climate change.

It is difficult to predict how warming caused by climate change and the urban heat island effect (Arnfield, 2003) may influence N cycling of turfgrass. It is also difficult to predict how plant N concentrations will change in response to warming; field experiments have shown that foliar C/N changes can be dynamic or species-specific (Read & Morgan, 1996; Klein et al., 2007). Experimental warming manipulations in various ecosystems have shown that warming may significantly...
alter soil N budgets through increased N mineralization
(Shaver et al., 2000; Rustad et al., 2001). However, the
effects of elevated temperature on nitrification, denitrifi-
cation, and subsequent N$_2$O emissions are still unclear
due to the small number of field studies (Barnard et al.,
2005). The impact of warming on N$_2$O fluxes is compi-
lated by two possible effects – reduced soil moisture,
generally a negative effect, and increased soil tempera-
ture, a positive effect (Brumme, 1995; Skiba et al., 1998;
Flechard et al., 2007). Studies of fertilized soils are
particularly important, as agricultural crops are known
to be large sources of N$_2$O at global, national, and
statewide scales (Mosier et al., 1998; Bouwman et al.,
2002; Franco, 2002). There have been a surprisingly few
direct measurements of N$_2$O emissions from turfgrass
(Maggiotto et al., 2000; Kaye et al., 2004; Bremer, 2006),
such that the contributions of managed lawns to local
and regional greenhouse gas budgets are largely un-
constrained.

We initiated an experimental warming and fertiliza-
tion manipulation in a fescue-dominated lawn to un-
derstand how temperature, fertilizer, and their
combination influence N cycling. In addition to measur-
ing soil N$_2$O fluxes, we examined foliar and soil N
content and nitrogen isotope ratios ($\delta^{15}$N) in each treat-
ment in order to determine the effects on plant N
availability. $\delta^{15}$N is a useful indicator of plant N sources
and ecosystem N losses, as N derived from mineralized
organic matter may undergo microbial fractionation
(Shearer et al., 1974; Mariotti et al., 1980; Nadelhoffer
& Fry, 1988), and is likely to be lighter than fertilizer-
derived N, which is often isotopically enriched due to
Our study system was a commonly occurring mixture of
C3 fescue and C4 crabgrass; therefore, we measured
the carbon isotope ratio ($\delta^{13}$C) of each species separately
and in total harvested aboveground biomass to quantify
the relative abundance of each species in each treat-
ment. The $\delta^{13}$C of C4 biomass varies from −11‰ to
−15‰, while the $\delta^{13}$C of C3 biomass varies from −20‰
to −35‰ (Dawson et al., 2002). The isotopic composition
of each functional type is isotopically distinct; thus,
measurements of $\delta^{13}$C may be used to determine the
proportion of C3 vs. C4 plants in each treatment.

As increased temperature is expected to increase the
metabolic activity of microbes, we hypothesized that
increased temperature as well as fertilization would
increase N$_2$O fluxes in this ecosystem, with a corre-
sponding enrichment of $\delta^{15}$N in the soil. We also
hypothesized that plant C/N would decrease in re-
sponse to fertilizer addition and then remain relatively
constant in response to warming due to the large
amounts of fertilizer N applied to this system. While
in general, plants utilizing the C4 photosynthetic path-
way have a competitive advantage over C3 plants in
warmer environments based on the relationship be-
tween the quantum yield of photosynthesis and tem-
perature (Ehleringer & Bjorkman, 1977), we did not
expect the proportion of C3 and C4 biomass to change
greatly in our 15 month measurement period. Changes
in the proportion of C3 and C4 plants may be very
important for turfgrass management as C4 plants are
often weeds in C3 lawns, requiring the application of
herbicides to turfgrass in addition to fertilizer. Hence,
the impacts of warming and fertilization on N$_2$O fluxes,
ecosystem N cycling, and species composition are
highly relevant for turfgrass ecosystem management
as well as global change.

Materials and methods

Study site

This study was conducted at the University of Califor-
ia, Irvine, Arboretum (33.7°N 117.7°W, 30 m a.s.L) on a
turfgrass lawn dominated by tall fescue [Schedonorus
phoenix (Scop.) Holub], a cool season, C3 species, and
crabgrass (Digitaria Haller), a warm season, C4 species.
The site has a Mediterranean climate, with a mean
annual air temperature of 18.6°C and 352 mm of pre-
cipitation, primarily falling between November and
April. Tall fescue is a widespread turf species in the
United States adapted to cool and humid climates
(USDA-NRCS, 2007). Crabgrass is an annual weed that
commonly invades domesticated lawn grasses. Bermu-
da grass (Cynodon dactylon L.), a warm season, C4
species, was initially present at the site at low abun-
dance. The experimental site was fenced to exclude
grazing by wildlife, primarily rabbits. The soil type
was alkaline alo clay (USDA-NRCS, 2007). Before the
experiment, this site was managed as turfgrass for over
16 years (L. Lyons, personal communication, 2008).

Experimental design

Six plots of 1.5 m × 2 m were established on June 10,
2005. Pairs of plots were blocked by slope position at
three levels on a slight slope. For each pair, plots were
randomly assigned to control or heated treatments. Each
plot was split into two subplots that were randomly
assigned a low or high fertilizer treatment. In March
2006, these subplots were separated with 10 cm deep
plastic trenching materials inserted into the soil. Mea-
surements were concentrated in the central 2500 cm$^2$
of the subplots, while the surrounding area served as a
buffer zone. This design resulted in a 2 × 2 factorial
experiment of fertilizer and temperature with three
replicates (total of 12 plots). In this paper, the treatments
will be referred to as control (C), high temperature × low fertilizer (T), control temperature × high fertilizer (CN), and high temperature × high fertilizer (TN). The plots were watered with domestic water approximately three times a week during summer (May–September) and twice a week during the winter. Starting in July 2006, all plots received exactly equal amounts of water based on recommendations by Hartin et al. (2001). The heated subplots were subjected to an average increase in mean daily surface temperature of 3.5 ± 1.3 °C (average ± SD) by elevating ceramic infrared heaters (250 W, Exo Terra, Rolf C. Hagen Inc., Montreal, Canada) 1 m over the ground. Plots were heated from July 14, 2005 to December 1, 2006. The applied fertilizer was a common commercial formula of 29:3:4 NPK that contained 28% CON2H4, 1% NH4, 3% P2O5, 4% K2O, and 1% Fe (Vigoro Ultra Turf, Spectrum Group, St Louis, MO, USA). The δ15N of fertilizer was −0.64 ± 0.09%o. Low fertilizer plots received 76.4 kg N ha−1 yr−1 in two applications in 2006 (62.3 kg N ha−1 on April 2 and 14.0 kg N ha−1 on July 20) and high fertilizer plots received 118.5 kg N ha−1 yr−1 in four applications in 2006 (62.3 kg N ha−1 on April 2, 14.0 kg N ha−1 on May 21, 28.1 kg N ha−1 on July 20, and 14.0 kg N ha−1 on August 31). The treatments were based on the recommended fertilizer application rates for these varieties, which vary approximately from 50 to more than 200 kg N ha−1 yr−1 divided into two to six applications during the period from March to November (Reynolds & Flint, 2004). These are also plausible fertilization scenarios for householders based on Osmond & Hardy (2004), who found that five North Carolina communities apply 24–151 kg N ha−1 to turfgrass and had on average between 1.5 and 3 applications per year.

Measurements

Soil surface temperature (Tso) and volumetric soil water content (θ) were measured at the center of each plot. Surface temperature was measured at the mineral soil surface with copper–constantan thermocouples and θ was measured at 5 cm soil depth with water content reflectometers (CS616, Campbell Scientific Inc., Logan, UT, USA). Air temperature and relative humidity were continuously recorded at 1 m above ground in one location at the site starting March 22, 2006 (CS215 shielded by 41303-5a, Campbell Scientific). Before this, meteorological variables were obtained from the California Irrigation Management Information System (www.cimis.ca.gov, Irvine station #75). All environmental data were recorded every 30 min (AM25 and AM16/32 multiplexer, CR10x logger, Campbell Scientific).

The rate of N2O efflux from the soil surface was measured using a static, polyvinyl chloride (PVC) chamber lid (height 15 cm, inner diameter 26 cm) containing a septum port. The chamber was placed over the soil surface and the lower rim was surrounded by water-filled plastic tubes to prevent the diffusion of ambient air into the chamber. Gas samples were taken using a syringe at four timed intervals over a 15 or 21 min period, and were injected into airtight, pre-evacuated 12 mL vials. The samples were shipped to the University of Kansas where they were analyzed for N2O on a Varian CP3800 gas-chromatograph fitted with a 63Ni electron capture detector, operated at high temperature (300–400 °C) using N2 as the carrier gas. Rates of N2O–N loss were calculated as the rate of N2O accumulation over time in the chamber. Temperatures inside the N2O chambers were assumed to be equivalent with ambient air temperatures measured at the site.

Aboveground biomass production was clipped approximately every month to a height of 4 cm at the same time that the area outside the plots was mowed to the same height (clippings removed). The biomass was oven-dried at 70 °C for at least 48 h and weighed. Subsets of well-mixed bulk biomass, C3 only, and C4 only biomass material were removed from each harvest for C, N, and stable isotope analysis. Specific leaf area (SLA) was estimated on August 10, September 11, and October 12, 2006 by determining leaf area on a subset of harvested fresh leaves (IMAGEJ software, US National Institute of Health, http://rsb.info.nih.gov/ij/) and dividing leaf area by dry weight. Another subset of samples was ground to a fine powder and analyzed for % C, % N, δ13C, and δ15N with an elemental analyzer (Carlo Erba NA 1500 NC, Milan, Italy) coupled to an isotope ratio mass spectrometer (ThermoFinnigan Delta Plus, San Jose, CA, USA) at the University of California, Irvine, stable isotope facility. Isotope ratios were referenced to the PDB standard for C and the atmospheric standard for N. The precisions of these measurements were 0.14, 0.06, 0.18, and 1.00 (SD) for δ15N, δ13C, % N, and % C, respectively.

Soil samples were collected on July 17, 2005, June 6 and December 1, 2006 at 0–5 and 5–10 cm depths. In 2005, pretreatment samples were collected from the buffer zone of each plot before the plots were split into high and low fertilizer treatments (total of six samples). In June 2006, one sample was taken from the buffer zone of each subplot (total of 12 samples). Samples were collected from the plot buffer zone to minimize disturbance; measurements of the spatial variability of surface temperature in the plots indicated that the soil in the buffer zone was subjected to a similar increase in temperature as the main measurement area. Soil samples from 2005 were oven-dried at 60 °C, and samples from 2006 were freeze-dried. The different processing methods were related to other measurements not reported here and should not affect the determination.
of total soil C, N, and isotopic composition. After the removal of roots and litter, subsamples were ground to a fine powder and acid-treated for the removal of inorganic C. Two grams of each subsample were treated with 0.5 M HCl and shaken overnight, centrifuged to remove the remaining acid, washed with 20 mL of water, centrifuged and decanted, and freeze-dried. The acid-treated soils were analyzed for C and N content and isotope ratios. The precisions of these measurements were 0.20, 0.06, 0.23, and 1.64 (SD) for $^{15}$N, $^{13}$C, % N, and % C, respectively.

**Statistical analysis**

Data were analyzed using SAS software v. 9.1 (SAS Institute, Cary, NC, USA). Split-plot, repeated measures ANOVAs were used to determine treatment effects, as well as analyses of covariance (ANCOVAs), when volumetric soil water content co-varied with the dependent variable. Paired t-tests were used to analyze pre- vs. post-treatment differences, and ANOVAs were used to analyze the differences among treatments for individual sampling periods. For all analyses, P-values < 0.05 were considered significant.

**Results**

**$N_2O$ fluxes**

In all treatments, $N_2O$ fluxes were highly variable throughout the year (Fig. 1a). Heated plots had higher fluxes than control plots following the first and second fertilization events on April 3, April 7, and May 22, 2006 ($P = 0.0183$, $P = 0.0360$, and $P < 0.0001$). High N plots had significantly higher fluxes than low N plots on May 22 and July 27, 2006 ($P = 0.0148$ and 0.0327). The highest fluxes were 2.0 mg N m$^{-2}$ day$^{-1}$, and were measured in the heated (T and TN) subplots following fertilization in April 2006. The temporal pattern of $N_2O$ fluxes appeared to follow the seasonal pattern in daily average $\theta$ in the growing season (Fig. 1b). Before June, when average daily $\theta$ was 39.4 ± 0.42%, $N_2O$ fluxes increased immediately following fertilization events, and then decreased (Fig. 1a). Fluxes remained low despite two additional fertilizer applications after June, coincident with a decrease in soil moisture. Although the lawn was watered intensively during summer, soil moisture declined due to unusually high temperatures and high evaporative demand (Czimczik et al., submitted for publication). $N_2O$ fluxes were positively correlated with soil moisture in all four treatments (Fig. 2). An analysis of covariance (ANCOVA) with soil moisture as the covariate showed that there was no difference in the slope of this relationship among C, CN, and T treatments ($P > 0.05$); however, TN had a greater slope than the other treatments. $N_2O$ fluxes measured from T were higher than C and CN ($P = 0.0391$ and 0.0046, respectively). $N_2O$ fluxes measured from C and CN were not significantly different ($P > 0.05$). For all treatments, there was a decline in $N_2O$ fluxes over time ($P < 0.0001$), indicating that fluxes decreased with the decline in soil moisture in summer, probably caused by an increase in soil temperature during this period (Czimczik et al., submitted for publication). There were interactions of time with temperature on $N_2O$ fluxes before and after the initiation of high fertilizer treatments ($P = 0.0003$ and 0.0002), showing that $N_2O$ fluxes from warmed plots initially increased, but then decreased when soil moisture was low. The integrated flux over the measurement period was 75.2 mg N m$^{-2}$ for C and 107.7 mg N m$^{-2}$ for T. After the initiation of high fertilizer treatments, the integrated flux was 16.8, 11.4, 32.6, and 16.3 mg N m$^{-2}$ from C, CN, T, and TN, respectively.

**Plant isotopic and chemical composition**

In C$_4$ plants, leaf C/N increased with time before the fertilization of high N treatments (CN and TN), but then did not change afterwards (Fig. 3a and Table 1; repeated measures ANOVA, $P = 0.0002$ and $P > 0.05$, respectively). The application of fertilizer in the high N treatments decreased leaf C/N of C$_4$ plants (Table 1 and Fig. 3a; $P = 0.0028$). On October 12, 2006, there was a marginally significant decrease in C/N of C$_4$ plants in the high N plots ($P = 0.0613$). There was no effect of temperature on C/N in C$_4$ plants (Table 1; $P > 0.05$), although on October 12, 2006, heated plots had lower C/N ($P = 0.0498$). In C$_3$ plants, leaf C/N increased with time before the fertilization of high N plots, and then decreased with time afterwards (Fig. 3b and Table 1; repeated measures ANOVA, $P < 0.0001$ and $P = 0.0021$, respectively). At the end of the experiment, C$_3$ plants in heated (T and TN) plots had lower C/N than in the control (C and CN) plots (Fig. 3b; $P = 0.0410$). After the establishment of CN and TN plots, C/N of C$_3$ plants were lower than those of C$_4$ plants (repeated measures ANOVA, $P < 0.0001$). There was a negative correlation between C/N and $\delta^{15}$N of aboveground biomass for C$_4$ grasses in each treatment (Fig. 4). This relationship was particularly strong in the C treatment, where leaf $\delta^{15}$N was also the most isotopically depleted, with a value as low as –2.9%. An analysis of covariance (ANCOVA) with C/N as the covariate showed that there was no difference in the slope among treatments ($P > 0.05$). Heated plots were more enriched in $\delta^{15}$N ($P < 0.0001$) with no effect of fertilization ($P > 0.05$). For C$_3$ plants, C/N and $\delta^{15}$N were correlated only in the
In the C treatment, there was a significant relationship between $d^{13}$Co and plant biomass and soil moisture (Fig. 5a; $P < 0.0021$), and a marginally significant relationship for the CN treatment (Fig. 5b; $P = 0.0507$).

All treatments showed increases in $C_4$ aboveground biomass with time as estimated with $d^{13}$Co (repeated measures ANOVA; $P < 0.0001$). Fertilization and its interactions were nonsignificant for $d^{13}$C ($P > 0.05$), such that fertilization treatments were combined to evaluate the effect of warming (Fig. 6a). Before the warming treatment, the $d^{13}$C of bulk harvests from both control and treatment plots were similar ($-25.6 \pm 0.7\%$ and $-24.5 \pm 1.2\%$, respectively), indicating similar proportions of $C_3$ vs. $C_4$ species. In a period of approximately 7 months, the aboveground biomass in heated plots became isotopically enriched by 4.9% on average (Fig. 6a), indicating a greater proportion of $C_4$.
Fig. 2  The relationship between N₂O flux and volumetric soil moisture in each treatment. Treatments are abbreviated as in Fig. 1. Symbols represent different blocks. \( P < 0.0001 \) for C, T, and TN; \( P = 0.003 \) for CN; \( R^2 \) were 0.32, 0.33, 0.30, and 0.66 for C, CN, T, and TN treatments, respectively.

Fig. 3  C/N ratio of (a) C₄ aboveground biomass and (b) C₃ aboveground biomass. Dotted lines indicate fertilization events. Asterisks (*) show significant treatment differences due to temperature, and circles (○) show treatment differences due to fertilization. Treatments are abbreviated as in Fig. 1. Error bars show the standard error.
plant material. Repeated measures ANOVAs showed no effect of warming on δ13C before January 31, 2006 (P > 0.05), but an effect of warming on δ13C following January 31, 2006 (P = 0.0275). The isotope ratios of each species measured at each harvest were utilized as end-members to calculate the proportion of C3 vs. C4 biomass, and confirmed that changes in δ13C of the bulk harvest were dominated by changes in community composition, rather than changes in δ13C of C3 plants (Fig. 6b). The C4 biomass was 30 ± 16% greater in the heated plots by October 2006 (Fig. 6b).

Soil chemical and isotopic composition

There was no change in N and δ13C of soil in response to fertilization, temperature, or time (Fig. 7a and c, P > 0.05). However, after the warming treatment was applied, soil δ15N in heated plots were enriched relative to control plots at the 0–5 cm depth (Fig. 7b, P = 0.016 in June 2006) and at the 5–10 cm depth (Fig. 7b, P = 0.031 in June 2006 and P = 0.007 in December 2006).

Plant canopy properties

Specific leaf area averaged 181.9 ± 11.1 cm² g⁻¹ (mean ± SE) in C3 plants and 217.8 ± 5.6 (mean ± SE) in C4 plants. There were no significant effects of temperature, fertilizer, time, or their interactions on SLA for C3 or C4 plants (repeated measures ANOVA, P > 0.05). Leaf area index (LAI) changed significantly over time for C3 and C4 plants (P = 0.0367 and 0.0006 respectively), but was not affected by fertilization (P > 0.05). C3 plants in heated plots had lower LAI than control plots on two sampling dates (August 10 and September 11, P = 0.0088 and 0.0422, respectively). The LAI of C4 plants was unaffected by heating. Average total plot LAI in 2006 ranged from 0.60 ± 0.08 m² m⁻² (mean ± SE) in August–September to 0.80 ± 0.11 m² m⁻² (mean ± SE) in October.

Discussion

Measured N₂O fluxes were within the reported range for turfgrass (Maggiotto et al., 2000; Kaye et al., 2004; Bremer, 2006). As hypothesized, fertilizer application caused an increase in N₂O fluxes, although the effects were significant only on two sampling dates. Maggiotto et al. (2000) and Bremer (2006) also reported increased N₂O emissions from fertilized turfgrass plots relative to control plots. The positive effect of warming on N₂O fluxes was consistent with our hypothesis. We are not aware of prior warming manipulations in turfgrass, and field warming studies in other ecosystems have shown conflicting effects on N₂O fluxes. Kamp et al. (1998),

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<th>Table 1</th>
<th>F- and P-values for treatment effects on C₃ and C₄ foliar C/N for time periods before and after fertilization of high N plots (CN and TN)</th>
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Fig. 4  The relationship between $\delta^{15}$N and C/N ratio of C$_4$ aboveground biomass in each treatment. Treatments are abbreviated as in Fig. 1. Symbols represent different blocks. $P < 0.0001$, 0.0446, 0.0334, 0.0013 for C, CN, T, TN, respectively; $R^2$ were 0.66, 0.32, 0.18, 0.56 for C, CN, T, and TN treatments, respectively.

Fig. 5  Average volumetric soil water content before harvest vs. $\delta^{13}$C of C$_3$ aboveground biomass in (a) control (C) and (b) high N (CN) treatments. Symbols distinguish different blocks.
who applied a 3 °C warming to wheat and fallow fields, did not find differences in cumulative emissions of \( \text{N}_2\text{O} \), but did find that heated fallow plot emissions were three times higher than control plots during summer. Conversely, Hantschel et al. (1995) found lower \( \text{N}_2\text{O} \) fluxes in 3 °C heated wheat fields during winter in Germany. Peterjohn et al. (1994) did not find an effect of a 5 °C warming on \( \text{N}_2\text{O} \) fluxes in a deciduous hardwood forest. McHale et al. (1998) did not find a strong response of heating on \( \text{N}_2\text{O} \) fluxes in plots heated to 2.5, 5.0, or 7.5 °C above ambient in a northern hardwood forest. In a companion study, we found increases in ecosystem respiration in heated plots relative to control plots in winter when aboveground biomass production was low (Czimczik et al., submitted for publication). It is possible that heating increased heterotrophic respira-

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**Fig. 6** (a) The \( \delta^{13}\text{C} \) of bulk turfgrass aboveground biomass. (b) The percentage of total aboveground biomass comprised of C\(_4\) plants as calculated from the isotopic composition of C\(_3\) biomass, C\(_4\) biomass, and the bulk harvest. The dashed line indicates the beginning of the warming treatment, and dotted lines indicate fertilization events. High and low N treatments are combined as they were not statistically different. Asterisks (*) show significant treatment differences due to temperature. Error bars show the standard error.

**Fig. 7** (a) N (kg m\(^{-2}\)), (b) \( \delta^{15}\text{N} \), and (c) \( \delta^{13}\text{C} \) of soil measured at two depths at the beginning of the warming treatment (July 2005), following fertilization (June 2006), and at the end of the experiment (December 2006). Treatments are abbreviated as in Fig. 1. Asterisks (*) show significant treatment differences due to temperature. Error bars show the standard error.
tion and N-mineralization. Higher rates of N-mineralization in heated plots may have increased available ammonium pools, causing the positive response of N$_2$O fluxes to heating. In summer, N$_2$O fluxes declined, perhaps because low soil moisture suppressed the hydrolysis of applied urea fertilizer to ammonium, the substrate for N$_2$O production via nitrification. The activity of urease, the enzyme responsible for urea hydrolysis, peaks near field capacity and declines with decreasing soil moisture (Vlek & Carter, 1983; Sahrawat, 1984). In addition, urea hydrolysis strongly depends on the incorporation of urea into soil through diffusion of dissolved ammonia (Sadeghi et al., 1989). Thus, it is possible that fertilizer was unavailable for microbial processes during the period of low soil moisture in summer, resulting in low N$_2$O fluxes (Fig. 1). A reduction in soil denitrification, which requires anoxic conditions associated with soil moisture, may also explain the decline of N$_2$O fluxes when soil moisture was low.

Leaf C/N of C$_3$ plants decreased in the period following fertilization of high N plots, supporting our hypothesis (Fig. 3b). However, C/N of C$_4$ plants did not change over time in response to fertilization, contrary to our hypothesis (Fig. 3a), indicating plant N-limitation despite the additional application of fertilizer. The negative correlation between leaf C/N of C$_4$ grasses and leaf $\delta^{15}$N (Fig. 4) suggests the greater use of isotopically light forms of N when N was limiting. The labile soil organic matter that is fractionated by microbial processes during the period of low soil moisture in summer, resulting in low N$_2$O fluxes (Fig. 1). A reduction in soil denitrification, which requires anoxic conditions associated with soil moisture, may also explain the decline of N$_2$O fluxes when soil moisture was low.

Leaf C/N of C$_3$ plants decreased in the period following fertilization of high N plots, supporting our hypothesis (Fig. 3b). However, C/N of C$_4$ plants did not change over time in response to fertilization, contrary to our hypothesis (Fig. 3a), indicating plant N-limitation despite the additional application of fertilizer. The negative correlation between leaf C/N of C$_4$ grasses and leaf $\delta^{15}$N (Fig. 4) suggests the greater use of isotopically light forms of N when N was limiting. The labile soil organic matter that is fractionated by mineralization (Shearer et al., 1974; Mariotti et al., 1980; Nadelhoffer & Fry, 1988) is likely to be isotopically lighter than fertilizer N, which can undergo isotopic enrichment due to rapid gaseous loss through volatilization (Högberg, 1990, 1997; Robinson, 2001). As the soils in this study were alkaline (pH ranging from 7 to 9), ammonia volatilization may have been an important pathway for gaseous N loss (Kirchmann & Witter, 1989). Thus, the correlation between C/N of C$_4$ grasses and $\delta^{15}$N in all treatments suggests greater use of fertilizer-derived ammonium during wetter conditions earlier in the year vs. uptake of N mineralized from organic matter later in the year when applied fertilizer was not biologically available. Additional measurements of inorganic N forms and their isotopic composition would be required to validate this interpretation, as many processes may cause fractionation of plant and soil N (Evans, 2001; Robinson, 2001). Plants in the high N treatments showed greater isotopic enrichment and weaker correlations between $\delta^{15}$N and C/N than the C treatment, possibly because of increased fertilizer uptake. The T treatment also showed a weaker trend and greater isotopic enrichment than the C treatment, possibly because of greater N$_2$O and other gaseous losses such as NH$_3$, NO, and N$_2$ (Fig. 1). This is supported by the soil data, which shows enrichment of $\delta^{15}$N in heated plots (Fig. 7b).

The $\delta^{13}$C of leaves is determined by $c_i/c_a$, the ratio of CO$_2$ inside and outside of the leaf, which is in turn determined by the balance between photosynthesis and stomatal conductance (Farquhar et al., 1989). Previous studies have reported more positive $\delta^{13}$C values in response to reduced soil moisture in C$_3$ grasses, including tall fescue turfgrass (Johnson & Bassett, 1991; Ebdon et al., 1998; Johnson & Li, 1999). In the C and CN treatments, we found a direct correlation between $\delta^{13}$C and soil moisture in the C$_3$ plants (Fig. 5), which suggests stomatal closure in response to declining soil moisture. In the T and TN treatments, there was no correlation with variations in soil moisture. However, the $\delta^{13}$C of C$_3$ plants was significantly more enriched in the two high temperature treatments than the C and CN treatments (ANOVA, $P = 0.0385$), suggesting that plants in the T and TN treatments were generally more water-stressed, consistent with the lower soil moisture (Fig. 1b).

One of the most significant results of this study was unexpected: the temperature treatments facilitated a rapid (~7 months) shift in C$_4$ weed biomass relative to C$_3$ plant biomass, as shown by the time series of $\delta^{13}$C of total biomass (Fig. 6a). C$_4$ biomass was $30 \pm 16\%$ greater in the heated plots at the end of the experiment in October 2006 (Fig. 6b). Immediately following the first spring fertilizer application, the $\delta^{13}$C of total biomass in the control and high temperature plots had become more similar and were not significantly different, possibly because fertilization enabled the C$_3$ plants to compete more successfully with the C$_4$ species, which have higher N-use efficiency (Sage & Pearcy, 1987). A greater ability to take up isotopically heavy N early in the growing season may also have contributed to the competitive advantage of C$_4$ species in all plots, and particularly in heated plots, and may explain why C$_4$ C/N did not change over time. Because C$_3$ and C$_4$ plants can have different patterns of N uptake and allocation, changes in C$_3$/C$_4$ community composition could ultimately influence other aspects of N cycling such as N$_2$O fluxes, as both microbes and plants share the same soil N pool.

Plants utilizing the C$_4$ photosynthetic pathway have a competitive advantage over C$_3$ plants in warmer environments (Ehleringer & Bjorkman, 1977). Because most weeds are C$_4$ plants (Holm, 1977), there is great concern about the possibility of more widespread weed invasions as a result of global climate change (Patterson, 1995; Dukes & Mooney, 1999; Sage & Kubien, 2003; Schmitz, 2006). However, field experiments often fail to confirm simple predictions based on physiological
principles. For example, C₄ plants were predicted to be relatively unresponsive to elevated atmospheric CO₂, but this has been contradicted by experimental evidence (Dukes & Mooney, 1999). As most field warming experiments have been conducted in high latitude ecosystems where C₄ plants are largely absent, there have been few of these studies on C₃ vs. C₄ plants (White et al., 2000, 2001; Wan et al., 2005; Luo, 2007), particularly in agricultural and highly managed ecosystems, e.g. White et al., 2000, 2001; Ziska, 2000, 2003; Derner et al., 2003; Fuhrer, 2003. Weed expansion under higher temperatures is particularly relevant because herbicide is commonly applied to fescue lawns to control the invasion of C₄ weeds such as crabgrass, often at higher application rates than in other types of agriculture (Templeton et al., 1998).

Conclusions
This study showed reduced N₂O fluxes and plant N limitation with declining soil moisture and increases in N₂O fluxes with warming. Negative correlations between C/N of C₄ plants and plant δ¹⁵N suggest that N limitation was associated with the increased use of isotopically depleted N sources, such as mineralized organic matter. The increases in N₂O fluxes with warming suggest that soil N₂O fluxes could serve as a positive feedback to global warming in turfgrass. The strong influence of soil moisture on N₂O fluxes suggests that best management practices for turfgrass should optimize the tradeoff between soil moisture enhancement of urea fertilizer hydrolysis and gaseous N emissions. That is, soil moisture should be regulated so that plant use of fertilizer is maximized while gaseous N loss is minimized (e.g. Matson et al., 1998).

These results also provide in situ evidence for rapid, warming-induced C₄ weed expansion in turfgrass and suggest that other managed systems, such as agricultural crops, may experience rapid weed invasions or changes in community composition in response to warming. Because turfgrass is often associated with urban and suburban land cover, the urban heat island effect as well as climate change may exacerbate weed invasions, which would require more intensive management, e.g. herbicide application, to manage species composition.

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