

Net ecosystem CO₂ exchange and plant biomass responses to warming and N addition in a grass-dominated system during two years of net CO₂ efflux

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Abstract

Background and Aims Climate warming and increased atmospheric nitrogen (N) deposition both have the potential to increase plant productivity over the next century, yet they can also increase decomposition and respiration. Our aim was to examine the extent to which warming and N addition can, on balance, alter net ecosystem CO₂ exchange (NEE) in a grass-dominated system.

Methods We measured NEE responses to warming and N addition over two growing seasons in a temperate old field using steady-state flow-through chambers, which allowed for the integrated measurement of respiration and photoassimilation effects on net CO₂ flux over diel periods. We also assessed the relationship between NEE and plant biomass responses to the warming and N treatments.

Results In both years, our study system was a net source of carbon (C) during the snow-free season. N addition did not significantly affect diel NEE or dark respiration in either year, despite a doubling in aboveground plant biomass in response to N addition in the second year, and a corresponding increase in peak daily net CO₂ photoassimilation in N addition plots. The warming treatment also had no significant effect on NEE, although the flow-through chambers

required warming to be temporarily halted during NEE measurements.

Conclusions Overall, our results both highlight the potential divergence of plant and soil responses to N addition and demonstrate the capacity for a grass-dominated system to function as a net source of C in consecutive years.

Keywords Atmospheric N deposition · Biomass · Canopy · Climate warming · CO₂ flux

Introduction

Although terrestrial ecosystems have the potential to contribute to a positive feedback between increasing atmospheric CO₂ concentrations and temperature over the next century, the magnitude of their contribution to this feedback remains uncertain (Heimann and Reichstein 2008). This uncertainty exists, in part, because net plant productivity and subsequent inputs to soil organic matter (the main terrestrial carbon (C) sink) and heterotrophic respiration (the main non-anthropogenic terrestrial C source) are both expected to increase across many ecosystems with increasing temperature (Rustad et al. 2001). In addition, the indirect effects of warming on net primary production and respiration, mediated through changes in soil moisture, nutrient availability, growing season length, and plant species composition, may be more important than the direct effects (Shaver et al. 2000).

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Plant growth in many terrestrial ecosystems is nitrogen (N) limited (LeBauer and Treseder 2008), and indeed, at a global scale, ecosystem C accumulation is predicted to be constrained by N availability (Hungate et al. 2003; Luo et al. 2004). Therefore, climate warming effects must be considered in the context of increases in atmospheric N deposition, particularly in temperate systems, where some of the largest increases in N deposition are expected over the next century (Galloway et al. 2004). With respect to plant litter quality, increased N content often increases decomposition rates (Vitousek et al. 1997), yet there is also evidence that N addition can directly suppress microbial activity on plant litter (Carreiro et al. 2000). Possibly as a result of these opposing effects, N enrichment has had no statistically significant effect on litter decomposition when averaged across studies (Knorr et al. 2005). While increased N deposition could promote increased C storage in woody biomass in forested systems (Pregitzer et al. 2008; Vicca et al. 2012), there are doubts that the additional C sequestration resulting from N inputs will keep pace with increased CO₂ emissions at a global scale (Schlesinger and Andrews 2000; Reay et al. 2008).

There is the potential for ecosystems to respond synergistically to the combination of warming and N addition (Press et al. 1998). Multifactorial experiments have addressed the combined effects of these factors on C sequestration in the field, but there has been a tendency for aboveground plant responses and soil responses to be considered separately in these experiments, both conceptually and analytically, despite evidence that above and below ground processes are closely linked and can interact (Heimann and Reichstein 2008). Most notably, measures of soil respiration in field experiments are often taken from closed chambers placed on soil collars that are positioned to exclude plant shoots, and these collars can also affect CO₂ fluxes by cutting through roots (Heinemeyer et al. 2011). In some cases plant shoots have been clipped, and the absence or reduction of plant biomass could affect soil microbial biomass and community composition, either directly (e.g. by reducing rhizodeposition) or indirectly (e.g. by altering soil moisture and temperature). In experiments where closed chambers can be fitted over low-statured vegetation, the contribution of plant photoassimilation to net ecosystem CO₂ exchange (NEE) can only be assessed over short time intervals. In contrast, the eddy covariance method can provide continuous diel measures of NEE integrated over both plant and soil, but

these measures are integrated over a relatively large area (Subke and Tenhunen 2004), and thus cannot be used to assess flux responses in plots at the scale of a few square meters in field experiments. A useful compromise between the shortcomings of closed chamber methods and eddy covariance methods can be reached through the use of steady-state flow-through canopy chambers, which provide a means of measuring NEE at the plot level in global change experiments (Alterio et al. 2006).

We used steady-state flow-through chambers over two growing seasons in a temperate old field to test the hypothesis that NEE would vary in response to warming and N addition. We also examined the relationship between NEE and aboveground plant biomass responses to the experimental treatments. We predicted that the old field would be a net C sink on an annual basis, and that daily influx would be the highest in mid-summer, leading up to the peak in aboveground plant biomass. We also predicted increased C sequestration in response to N addition, and that this increase would coincide with increased plant biomass. Although we anticipated a positive correlation between soil temperature and nighttime CO₂ efflux, we nonetheless predicted that warming would indirectly increase C influx on a diel basis, based on the assumption that warming would increase plant biomass.

Materials and methods

Site description

We conducted our research as part of a long-term warming and N addition experiment located within the Agriculture and Agri-Food Canada Southern Crop Protection and Food Research Centre in London, Ontario, Canada (43° 04' N, 81° 20' W, elevation 264 m) between March 2009 and October 2010. The field site was previously used as an agricultural field but had not been ploughed, fertilized or mowed in the past 25 years. The mean annual air temperature for the site was 7.5 °C, with a mean daily low of -6.3 °C in January and a mean daily high of 20.5 °C in July, and mean annual precipitation was 595 mm (Canadian Climate Normals 1971–2000, Environment Canada, National Climate Data and Information Archive). The soil was characterized as imperfectly drained silt loam glacial till, with an

average pH of 7.5 and soil organic content (loss-on-ignition at 500 °C for 12 h) of 7.1 % (Bell et al. 2010). The perennial grasses *Poa pratensis* L. and *Bromus inermis* Leyss. dominated the site, with the forbs *Cirsium arvense* L. and *Lotus corniculatus* L. also common in patches within some of the plots.

Warming and N addition treatments

In 2006, we initiated a combination of warming and N addition treatments in a randomized factorial block design (see Turner and Henry (2009) for a description of the full experiment; only a subset of the blocks was used in the current study, resulting in $n=6$ for each treatment combination). Each circular plot was 113 cm in diameter, with a 10 cm buffer zone around the perimeter, which also received the experimental treatments. Plots were separated by a minimum of 80 cm within blocks, and blocks were spaced a minimum of 10 m apart. Each year, we added N at a rate of $6 \text{ g m}^{-2} \text{ y}^{-1}$, with 2 g m^{-2} aqueous ammonium nitrate added in late March and 4 g m^{-2} added in late May using slow-release pellets (Florikan ESA, Sarasota, FL, USA). The N addition treatment was designed to reflect approximate atmospheric deposition rates in this region by the year 2050 (Galloway et al. 2004). We administered warming year-round using 150 W ceramic infrared heaters (Zoo-Med Laboratories, San Luis Obispo, CA, USA) suspended 50 cm above the plot centers. We measured soil temperature hourly in each of the plots using 107-BAM-L temperature probes at 2 cm depth, and we measured soil moisture using CS616-L time-domain reflectometry probes located from 0–15 cm depth (both probes from Campbell Scientific Canada Corp., Edmonton, AB, Canada). Warming increased soil temperatures at 2 cm depth by an average of $0.7 \text{ }^\circ\text{C}$ over the plant growing season (April through the end of October) over the two years. A given heater was temporarily turned off and moved an hour in advance of a NEE measurement to accommodate the canopy chamber, and the warming effect at 2 cm soil depth (relative to unheated plots) dissipated in less than 90 min. Thus, any potential effects of the warming treatment on our NEE measurements would have been indirect (e.g. resulting from effects of warming on soil moisture, soil nutrients, plant and microbial biomass and community composition, etc...), and not a direct result of the heating. Heating was resumed upon removal of the chamber the following day. The effect of warming on volumetric

water content was not significant in 2009, and in 2010 there was a significant decrease in mean volumetric water content in warmed plots of $0.018 \text{ m}^3 \text{ m}^{-3}$.

CO₂ flux measurements

We measured net ecosystem CO₂ flux using four CPY-3 open canopy chambers (PP Systems, Amesbury, MA, USA). Each chamber consisted of a 0.5 m diameter \times 1 m high polyethylene terephthalate (PET) cylinder with an aluminum base ring. Two fans circulated the air within the chamber, and each chamber contained an air temperature sensor and a PAR sensor. Air exchange rates were adjustable between 20 and 80 L m^{-1} , and condensation of water on the walls of the chambers was uncommon. An infrared gas analyzer measured the concentration of CO₂ at 10 min intervals, and at each interval it separately analysed the reference sample and the analysis sample for 30 s each (Fig. 1). For each sampling day, we placed a chamber in each plot within a single block (one chamber per treatment combination). We began measuring CO₂ flux at approximately 0930 h, then relocated the chambers to another block at approximately 0830 h the next day. We continued moving the

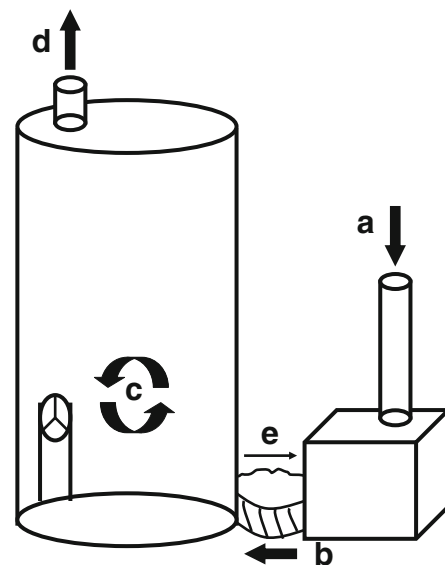


Fig. 1 Diagram of air flow through the control interface module (CIM) and flux chamber. *a*) Air was drawn into the chimney of the CIM, then *b*) pumped into the chamber at a rate of between 20 and 80 L m^{-1} , where it *c*) was circulated by fans and *d*) expelled passively through a vent at the top of the chamber. Every 10 min, ambient air entering the CIM was sampled for 30 s, then *e*) air from the chamber was drawn back into the CIM at a rate of 0.35 L m^{-1} and sampled for 30 s

chambers to new blocks for a total of six consecutive days, and re-sampled these same blocks over 6 day periods approximately every second week of the snow-free seasons in 2009 and 2010, resulting in 90 and 66 days of CO₂ flux sampling, respectively (three non-consecutive weeks of sampling scheduled for 2010 were missed as a result of technical problems). Chamber positions were randomized among treatments on each sampling day, and there was no evidence of a chamber effect on measures of CO₂ flux.

Plant biomass measurements

We estimated green aboveground biomass monthly from May to September from permanent 113 cm² sampling rings within each plot using the combination of measured shoot abundance data and shoot height-mass allometry data (see Hutchison and Henry (2010) for the detailed method). Biomass estimates were restricted to the grasses *B. inermis* and *P. pratensis*, which combined accounted for >95 % of the aboveground biomass at our site. We also sampled total root biomass in early October 2009 from single 2 cm diameter by 15 cm deep cores collected from each plot. Based on data from Hutchison and Henry (2010), <5 % of the root biomass in our plots occurs below 15 cm depth.

Statistical analyses

For each chamber on each sampling day, we summed across the data collected at 10 min intervals to calculate NEE on a diel basis. For each year, we also estimated total NEE over the snow-free season (April through October) using multiple linear regression (details below). For analyzing treatment effects on NEE we used randomized block repeated measures ANOVAs (one for each year) that included warming, N addition and their interaction term as between-subjects factors, and date and all interactions between date and warming and N addition as within-subjects factors (subject (i.e. plot ID) was included as a random factor nested in warming and N addition). We ran the analyses using the restricted maximum likelihood (REML) method of the Fit Model platform in JMP 4.0 (SAS Institute). Individual days within a given sampling week were used as replicates for calculating mean treatment responses for that sampling interval ($n=6$), such that both the spatial location of plots and the day of sampling within the week were

combined in the statistical block effect. We square-root transformed the data prior to analysis to satisfy the assumptions of normality and homogeneity of variances. Changes in NEE during the day result from a combination of changes in PAR and changes in temperature. In order to examine the correlation between soil temperature and NEE in the absence of the confounding effects of PAR, we also calculated mean rates of nighttime NEE (NEE_{dark}) for each sampling date by excluding measurements taken during daylight hours. We then examined the relationship between NEE_{dark} and nightly mean soil temperature for each year using Pearson correlations. We also calculated the most negative hourly NEE (or lowest hourly NEE, in the absence of net influx) for each day a plot was measured (NEE_{daily min}), in order to assess net CO₂ flux under periods corresponding with peak daily photosynthetic activity. Treatment effects on both NEE_{dark} and NEE_{daily min} were examined as described above for measurements of diel NEE. As a convention, we used negative NEE values to indicate net influx of CO₂.

We also used repeated measures analyses (see above) to assess treatment effects on aboveground biomass. We conducted separate analyses for total aboveground biomass, *P. pratensis* biomass and *B. inermis* biomass. We used a randomized block two-way ANOVA to analyze the effects of warming and N addition on root biomass. For all biomass data, we square-root transformed the data to satisfy the assumptions of normality and homogeneity of variances.

Modeling of NEE

In order to obtain estimates of total NEE over the snow-free season, we used multiple linear regressions to model NEE hourly based on soil temperature, soil moisture and solar radiation. Separate regressions using air temperature instead of soil temperature were also run, but were weaker at explaining variation in CO₂ flux (see Results). Solar radiation data were obtained from a weather station at the study site, and were used instead of PAR because data for the latter were only available for days when the chambers were active. Although the ratio of solar radiation to PAR varies among clear and overcast skies, and with atmospheric aerosol content, changes in this ratio are typically less than 10 % (Jacovides et al. 2003). During our CO₂ flux measurements, the regression between solar radiation (kW m⁻²) and PAR (μmoles m⁻² s⁻¹) was PAR=2.04×(solar

radiation)+11.90 ($r^2=0.959$). We developed separate models for each year, and since the effects of N addition and the (indirect) effects of warming on NEE were not significant (see Results), hourly NEE was averaged over all chambers. Separate models for each season (spring, summer and fall) were also examined in order to account for seasonal variability in the relationship between NEE and temperature, but these models did not produce NEE estimates that were substantially different than the model that incorporated all seasons simultaneously. NEE was transformed using logarithmic or root transformations to ensure linearity (Table 2). The relationship between NEE (transformed) and solar radiation was only linear during daylight hours (solar radiation $>0.0055 \text{ kW m}^{-2}$). Therefore, separate regressions for dark and daylight hours were used to model NEE, with solar radiation removed from the dark model. All interactions between factors were initially included in the regression models, with the non-significant interactions with $P>0.4$ subsequently removed. We used the final regression equations to model NEE over the snow free season for each plot based on the plot specific soil temperature and moisture data. For each year, we then analyzed the effects of the warming and N treatments on soil temperature, soil moisture and modeled total NEE using two-way ANOVA.

Results

Net CO₂ flux measurements

In 2009, the growing season (April to October) mean temperature (14.4 °C) and total precipitation (620 mm) were close to normal (14.4 °C and 583 mm, respectively; Canadian Climate Normals 1971–2000, Environment Canada, National Climate Data and Information Archive), whereas in 2010, the mean growing season temperature (16.3 °C) was 1.9 °C warmer than normal, and the total precipitation (669 mm) was 86 mm greater than normal, but with extended periods of drought in mid and late summer. Chamber air temperatures were 0.3 °C warmer on average than ambient air temperatures, and this difference did not exceed 2 °C in full sun. The presence of the chambers had no significant effect on soil temperature in the dark, but a slight cooling effect on soil temperature occurred in chambers during the day (mean of 0.4 °C \pm 0.2 °C at noon), with the greatest cooling on sunny days. In both 2009 and 2010 our study system was

a net source of C (Fig. 2a). In 2009, measurements of mean diel NEE (Fig. 2a) started near zero in early spring, then rose with increases in soil temperature (Fig. 3b) and plant biomass (Fig. 4) until late June, at which point they remained relatively stable over the rest of the summer, then fell sharply in mid-September. In 2010, measurements of mean diel NEE increased in spring more rapidly than in 2009, but declined earlier in the summer (Fig. 2a) during an extended drought period (Fig. 3c).

In both years, there were no significant effects of the N addition and warming treatments or their interaction on measurements of mean diel NEE, nor were there any significant interactions between the treatments and sampling date (Table 1). Although daily variation in weather increased variability among replicates within each sampling week, examination of treatment effects within individual weeks, where daily variation in weather was accounted for by a blocking factor, also failed to reveal significant treatment effects. Likewise, measurements of NEE_{dark} were not significantly correlated with the warming or N addition treatments (Table 1, Fig. 2b). However, the latter measurements were conducted with the heaters turned off, and across all sampling dates, mean nightly soil temperatures (square-root transformed) were positively correlated with NEE_{dark} (2009: $r^2=0.54$, $P<0.001$; 2010: $r^2=0.22$, $P<0.001$). In addition, there was a significant effect of N addition on NEE_{daily min} ($P=0.049$), which was consistent with increased daily peak photoassimilation rates in these plots (Table 1, Fig. 2c). On balance, this increased photoassimilation did not significantly alter the effects of N addition on measurements of mean diel NEE (Fig. 2a), because of the dominant influence of high nighttime CO₂ efflux (NEE_{dark}; Fig. 2b), which was insensitive to N addition.

Modeling of Net CO₂ flux

In ambient plots, total C efflux modeled over the snow-free season was estimated to be 127 \pm 14 (s.e.) g C m⁻² in 2009 and 101 \pm 6 (s.e.) g C m⁻² in 2010 (derived from the CO₂ efflux data in Table 3). The direct effects of warming were modeled by applying soil temperature and moisture data from warmed plots to the regression models in Table 2, and comparing these model results with those obtained using data from ambient plots. Modeled warming was estimated to increase NEE significantly by 19 % over the 2009

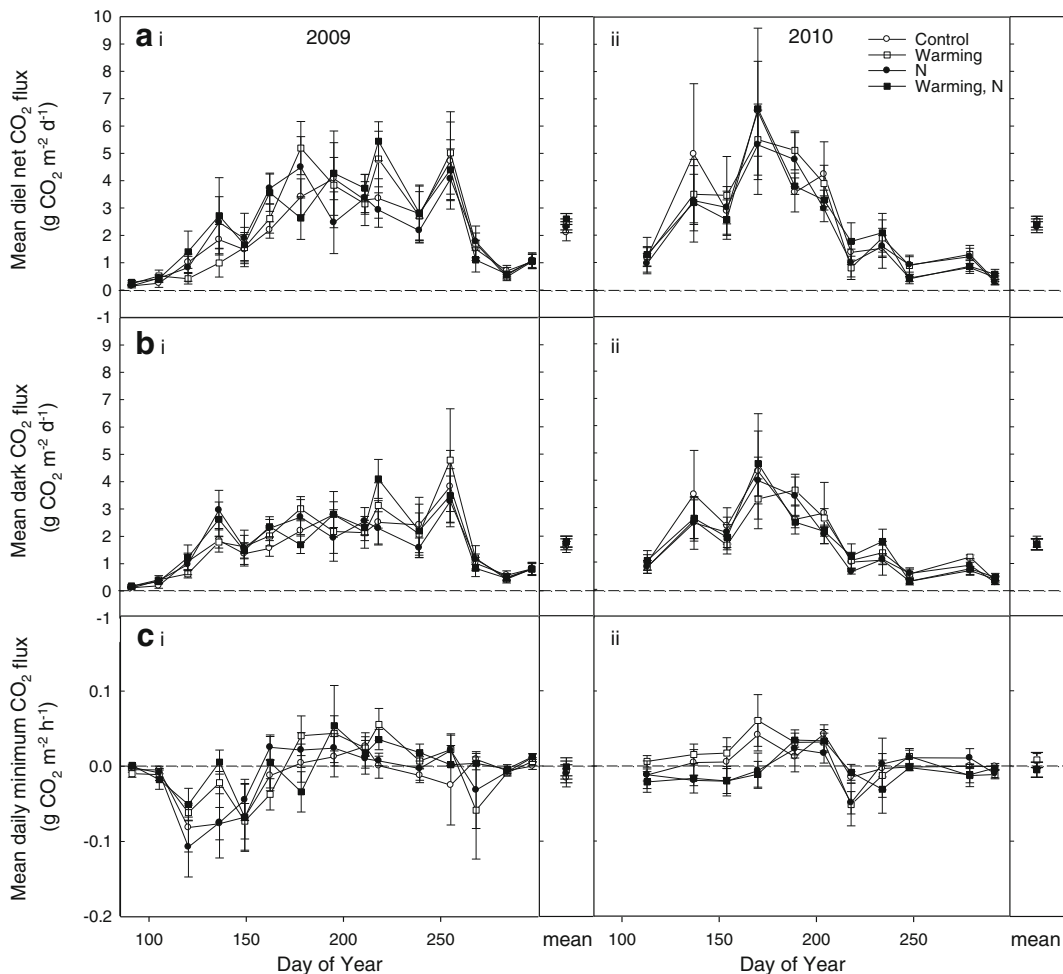


Fig. 2 **a** Mean diel net ecosystem CO₂ exchange (NEE), **b** mean dark net ecosystem CO₂ exchange (NEE_{dark}) and **c** mean daily minimum CO₂ flux (NEE_{daily min}) for each sampling period over the i) 2009 and ii) 2010 growing seasons (each point represents the mean of 6 different plots over 6 days – positive values indicate net CO₂ efflux; mean values for the

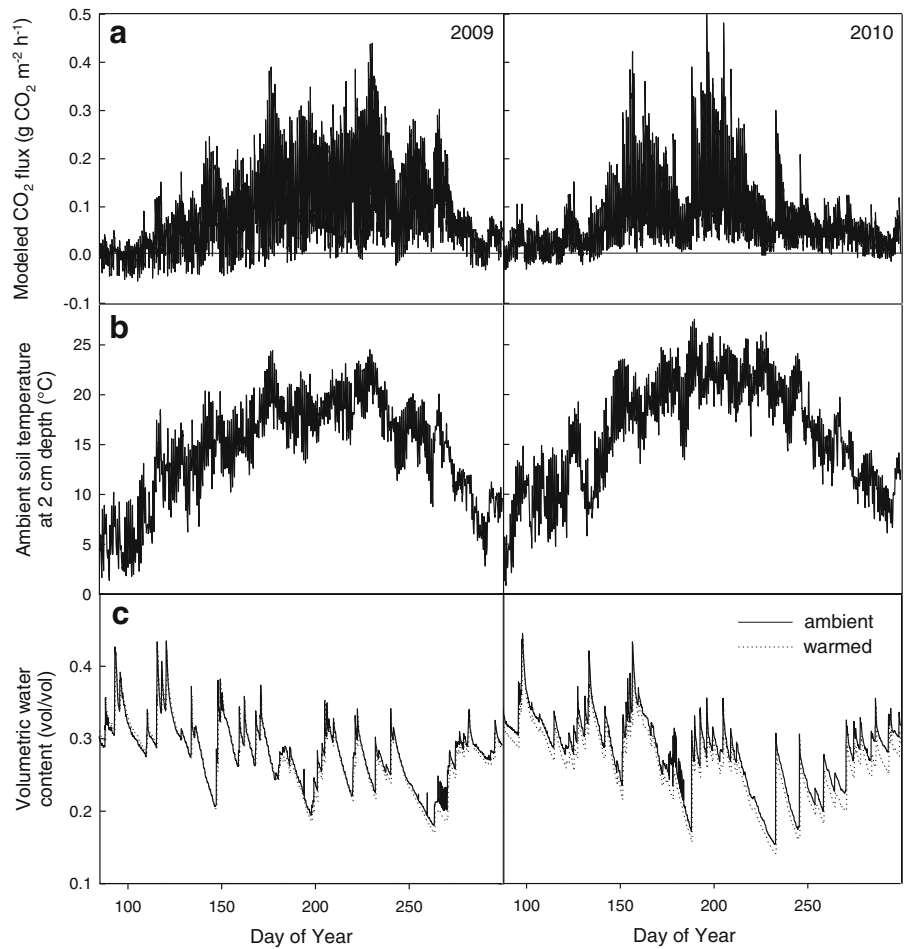
overall plot means are displayed in a separate box after each time series). Data are presented for ambient temperature plots (*circles*) and warmed plots (*squares*), both with added N (closed symbols) and without added N (open symbols). Raw means are displayed and error bars denote standard error. Associated significance tests are provided in Table 1

snow-free season ($P < 0.001$), as a result of a 0.9 °C increase in soil temperature ($P < 0.001$; Table 3). In 2010, a milder mean temperature increase of 0.6 °C ($P = 0.003$) combined with a significant decrease in volumetric water content ($P < 0.027$) resulted in a trend of decreased modeled NEE in warmed plots, but this result was only marginally significant ($P = 0.091$; Table 3). Based on the r^2 values, the percentages of the CO₂ flux data explained using air temperature in the models were less than the percentages explained using soil temperature in all cases (2009_{dark}: 46 % vs. 58 %; 2009_{light}: 52 % vs. 53 %; 2010_{dark}: 11 % vs. 44 %; 2010_{light}: 20 % vs. 30 %).

Plant biomass

In 2009, there were no significant effects of N addition or warming on aboveground biomass (Table 1). Averaged across all plots, aboveground biomass peaked at $619 \pm 29 \text{ g m}^{-2}$ (*P. pratensis*: $303 \pm 13 \text{ g m}^{-2}$; *B. inermis*: $316 \pm 34 \text{ g m}^{-2}$) during late June (\pm denotes standard error). In 2010, peak aboveground biomass approximately doubled in response to N addition ($P_N = 0.027$), but there was no significant effect of warming ($P_W = 0.68$) (Table 1; Fig. 4). The aboveground biomass response to N addition resulted primarily from increases in *P. pratensis* biomass, whereas *B. inermis* biomass did not significantly increase with N

Fig. 3 **a** Estimates of hourly NEE obtained from multiple regression models based on variation in soil temperature, soil moisture and solar radiation (Table 2). **b** soil temperature at 2 cm depth, **c** volumetric water content from 0–15 cm depth, in ambient plots. In **c**, the warming treatment is denoted by a dotted line. Refer to Table 3 for other treatment effects on modeled NEE, soil temperature and soil moisture



addition (*P. pratensis*: $P_N=0.002$; *B. inermis*: $P_N=0.29$). Root biomass was significantly higher in N addition plots than in unfertilized plots in 2009 ($605 \pm 44 \text{ g m}^{-2}$ versus

$518 \pm 34 \text{ g m}^{-2}$, respectively: $P_N=0.008$), but there were no other significant treatment effects on root biomass ($P_W=0.36$, $P_{W \times N}=0.37$).

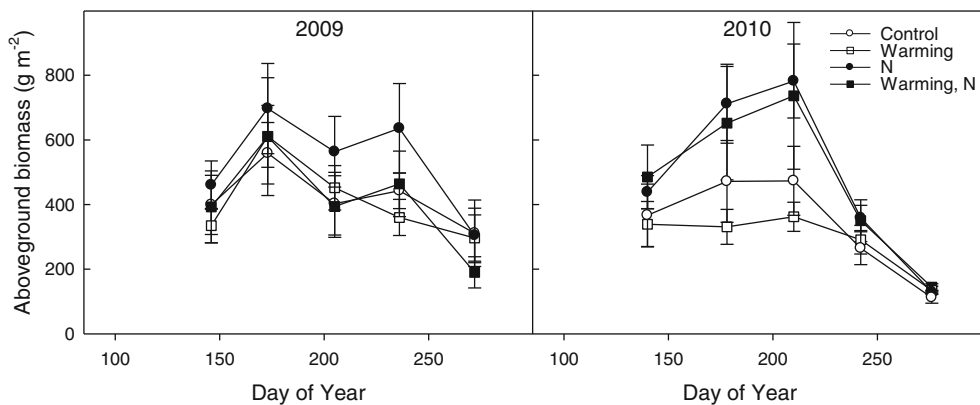


Fig. 4 Aboveground biomass over the 2009 and 2010 growing seasons. Data are presented for ambient temperature plots (*circles*) and warmed plots (*squares*), both with added N (closed

symbols) and without added N (open symbols) ($N=6$). Raw means are displayed and error bars denote standard error. Associated significance tests are provided in Table 1

Table 1 Summary of ANOVA P-values for repeated measures analyses of warming and N addition effects on net ecosystem CO₂ exchange (NEE), nighttime-only NEE (NEE_{dark}), daily minimum NEE (NEE_{daily min}) and aboveground biomass

Effect	2009				2010			
	NEE	NEE _{dark}	NEE _{daily min}	Biomass	NEE	NEE _{dark}	NEE _{daily min}	Biomass
Between-subjects								
W	0.413	0.512	0.153	0.294	0.259	0.291	0.924	0.677
N	0.769	0.713	0.485	0.379	0.897	0.763	0.049*	0.027*
W×N	0.958	0.928	0.941	0.379	0.946	0.962	0.882	0.904
Within-subjects								
D	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
W×D	0.814	0.973	0.301	0.8	0.989	0.993	0.949	0.201
N×D	0.499	0.798	0.961	0.289	0.95	0.93	0.153	0.199
W×N×D	0.957	0.623	0.645	0.793	0.567	0.607	0.532	0.927

W Warming, N Nitrogen, D Date

Asterisks denote significance (* P <0.05; ** P <0.01; *** P <0.001)

Discussion

Annual net ecosystem exchange

Contrary to our prediction that the study system would be a net C sink, based on our models we estimated net effluxes equal to 127 g C m⁻² and 101 g C m⁻² in 2009

and 2010, respectively, in ambient plots over the snow free season. Even leading up to the point of peak above-ground plant biomass in mid-summer, the contribution of photoassimilation to NEE was minor relative to the increased CO₂ efflux under warmer temperatures, and the plots only served as marginal net C sinks during the peak daylight hours, if at all. Our NEE estimates did not

Table 2 Multiple linear regression models of hourly CO₂ flux (transformed) based on soil temperature (T; °C), soil moisture (M; vol vol⁻¹) and solar radiation (S; kW m⁻²)

2009				2010			
<i>Dark model (solar radiation <0.0055 kW m⁻²)</i>				<i>Dark model (solar radiation <0.0055 kW m⁻²)</i>			
Transformed DV=CO ₂ flux ^{0.3} ; $r^2=0.58$				Transformed DV=CO ₂ flux ^{0.15} ; $r^2=0.44$			
Term	Estimate	S.E.	P	Term	Estimate	S.E.	P
Intercept	0.39	(0.03)	<0.001	Intercept	0.27	(0.03)	<0.001
T	0.024	(0.001)	<0.001	T	0.016	(0.001)	<0.001
M	-0.57	(0.09)	<0.001	M	0.74	(0.09)	<0.001
				(T-15.3)×(M-0.282)	0.075	(0.026)	0.004
<i>Light model (solar radiation >0.0055 kW m⁻²)</i>				<i>Light model (solar radiation >0.0055 kW m⁻²)</i>			
Transformed DV=log ₁₀ (CO ₂ flux+0.2); $r^2=0.53$				Transformed DV=log ₁₀ (CO ₂ flux+0.8); $r^2=0.30$			
Term	Estimate	S.E.	P	Term	Estimate	S.E.	P
Intercept	-0.55	(0.02)	<0.001	Intercept	-1.37	(0.06)	<0.001
T	0.011	(0.001)	<0.001	T	0.019	(0.001)	<0.001
M	-0.53	(0.06)	<0.001	M	0.75	(0.15)	<0.001
S	-0.24	(0.01)	<0.001	S	-0.29	(0.02)	<0.001
(T-14.7)×(M-0.283)	-0.048	(0.012)	<0.001	(T-18.0)×(M-0.284)	0.14	(0.05)	0.003
(T-14.7)×(S-0.358)	-0.0067	(0.0018)	<0.001	(M-0.284)×(S-0.368)	-1.1	(0.5)	0.042

Transformed DV Transformed dependent variable

Table 3 Treatment effects on plot means of mean hourly soil temperature, mean hourly soil volumetric water content and total modelled NEE, from 1 Apr. to 30 Nov. Standard error denoted in parentheses ($n=6$)

	2009			2010		
	Soil temperature (°C)	Volumetric water content	Modelled NEE (g CO ₂ m ⁻²)	Soil temperature (°C)	Volumetric water content	Modelled NEE (g CO ₂ m ⁻²)
Ambient	11.8 (0.1)	0.289 (0.008)	465 (12)	13.3 (0.1)	0.293 (0.010)	371 (6)
Warming	12.8 (0.2)	0.278 (0.007)	575 (20)	13.8 (0.2)	0.270 (0.011)	345 (13)
N	11.8 (0.1)	0.280 (0.007)	492 (13)	13.2 (0.1)	0.288 (0.007)	351 (8)
Warming and N	12.6 (0.2)	0.280 (0.004)	561 (17)	13.8 (0.2)	0.270 (0.008)	343 (11)
Factorial ANOVA	$P_W < 0.001$	$P_W = 0.42$	$P_W < 0.001$	$P_W = 0.003$	$P_W = 0.027$	$P_W = 0.091$
	$P_N = 0.64$	$P_N = 0.58$	$P_N = 0.69$	$P_N = 0.63$	$P_N = 0.83$	$P_N = 0.26$
	$P_{W \times N} = 0.79$	$P_{W \times N} = 0.38$	$P_{W \times N} = 0.22$	$P_{W \times N} = 0.84$	$P_{W \times N} = 0.76$	$P_{W \times N} = 0.34$

W Warming, N Nitrogen

account for CO₂ fluxes from December through the end of March, and given the lack of plant activity over winter, these months would have added to our total annual efflux estimates. Although extrapolation of our data from the late fall and early spring suggests that net fluxes over these months might be low on average relative to the summer, potential respiratory bursts at spring thaw can increase the relative contribution of winter CO₂ efflux to the annual total (Skogland et al. 1988). Winter CO₂ efflux can contribute to as much as half of the total annual CO₂ flux (Monson et al. 2006), but this occurs in systems that feature longer winters compared to those experienced at our site.

While the net C efflux we observed was within the range of +400 g C m⁻² y⁻¹ (ecosystem efflux) to -800 g C m⁻² y⁻¹ (ecosystem influx) reported as annual totals for other grass-dominated systems (see table in Novick et al. 2004), net annual efflux was unexpected for our site given its agricultural legacy, with tillage typically depleting soil C (Morell et al. 2010). Nevertheless, high interannual variability in NEE, in some cases including years of net C efflux, is also characteristic of grassland systems (Novick et al. 2004; in this study, 11 out of 30 years reported for different grasslands exhibited net C efflux), and our two year time series therefore should not be interpreted as evidence of sustained long-term C efflux from our system. In particular, our system features aboveground biomass production of over 800 g m⁻² y⁻¹ (Hutchison and Henry 2010), which equates to approximately 320 g C m⁻² y⁻¹ (for comparison, approximately 3,000 g C m⁻² is in the top 10 cm of soil at our site; M. Turner, unpublished data). The surface litter decomposes (or at least fragments) rapidly,

and by fall, only 100 g m⁻² on average of leaf litter from the previous year's growing season remains on the soil surface (Mensink 2009). Furthermore, relative to species in other grass-dominated systems, the species at our site have a shallow rooting depth (see Canadell et al. 1996), which facilitates decomposition by increasing the proportion of labile organic C that remains near the soil surface (Raich and Potter 1995). Taken as a whole, these data suggest that a large proportion of the annual CO₂ efflux in our system may be derived from plant litter, in addition to soil organic matter, causing years following high litter production to be characterized by net CO₂ efflux. This hypothesis is supported by our observation that the year prior to our study featured very high aboveground biomass production, of approximately 1,200 g m⁻² averaged over all treatments (Hutchison and Henry 2010). Although the second year of our study was warmer and wetter than the climate normal for the region, the first year of our study featured normal temperature and precipitation, so our net annual C efflux results cannot be explained on the basis of unusual weather during the period of observation.

Although annual C effluxes from grasslands have been reported previously for sites with high carbonate soils, and the soils at our site are situated on a limestone base, large contributions of carbonate dissolution to total CO₂ efflux may be restricted to arid and semi-arid systems (Emmerich 2003). Furthermore, the isotopic signature of the soil C at our site ($\delta^{13}\text{C} = -25.2\text{‰}$; M. Turner, unpublished data) suggests that the bulk of the soil C was derived from C₃ plant residue, rather than from carbonates. Severe drought can also promote CO₂ efflux (Meyers 2001), but we observed the opposite

trend in response to a moderate drought in our system (further discussion below). Likewise, while lateral transport of dissolved C in leachate or runoff can provide C inputs to some systems (Ciais et al. 2006), our study site was relatively flat.

An additional possible explanation for our measurements of net C efflux over both summers is that there were artifacts associated with the presence of the chambers (e.g. increased temperature and humidity, pressure differentials or reduced PAR) that increased respiration or decreased photoassimilation. An advantage of open flow-through chambers is that near ambient air temperatures and humidity can be maintained (Lund et al. 1999). In our experiment, chamber air temperatures were 0.3 °C warmer on average than ambient air temperatures, and this difference did not exceed 2 °C in full sun. In addition, there was a slight cooling effect on soil temperatures within the chambers under full sun. The soil cooling effect may have resulted from increased convective heat losses associated with the air flow through the chambers, or possibly from shading caused by the PET film or the non-transparent parts of the chamber infrastructure (e.g. the frame and fans). The latter shading would have reduced photoassimilation, although PAR transmission into the chambers is >85 % (PP systems, manufacturer's specifications). While negative pressure in chambers can produce artificially high rates of CO₂ flux from soil, flow-through chambers like those used in our study create positive pressure (Davidson et al. 2002), which would potentially cause an underestimate of soil CO₂ flux (and thus would not explain our observation of net CO₂ efflux over both summers). Conversely, the use of fans in chambers to ensure adequate mixing of the air can potentially dislodge CO₂ within the litter layer and cause overestimates of net efflux, but this problem primarily affects instantaneous measures of soil CO₂ flux (Butnor et al. 2005). Pressure-induced effects on CO₂ flux are minimized though the use of relatively large volume chambers, and by venting that is resistant to backpressure from wind, both of which were features of our chambers, but variation in pressure effects among different types of chambers can nevertheless make comparison among published rates of CO₂ fluxes difficult (Butnor et al. 2005).

In both growing seasons, NEE and NEE_{dark} were correlated positively with soil temperature, which is consistent with the general dependence of soil organic matter decomposition on temperature in laboratory incubations (Kirschbaum 1995). However, in the field, the

temperature sensitivity of soil C turnover can be overestimated or underestimated when variation in labile C pools is in phase with that of temperature (Gu et al. 2004). In mid-August 2010, a moderate drought resulted in a sharp fall in measured NEE, despite the continuation of warm summer temperatures. Such a dependence of respiration on soil temperature, but only when soil moisture is adequate, is common in other grass-dominated systems (Flanagan and Johnson 2005). Nevertheless, our measurements of NEE did not decline in response to decreased soil moisture at all times during the growing season, even when such a response was predicted by our NEE model. In particular, while the regression model in 2010 was highly sensitive to soil moisture (Table 2), and a decrease in soil moisture on approximately day 180 (Fig. 3c) resulted in a sharp decline in modeled NEE (Fig. 3a), there was no comparable decrease in measured NEE at that time (Fig. 2a). It was only later in the summer that the measured and modeled NEE responses to decreased soil moisture were in agreement, at a time when changes in soil moisture were confounded with plant senescence.

Given the strong influence of ecosystem respiration on NEE in our study, further insights could be gained by considering the typical responses of individual respiration components. Root respiration often accounts for at least half of soil respiration (e.g. Kuzyakov and Larionova 2005), although the studies upon which this estimate is based only measured heterotrophic respiration from the rhizosphere, primarily in managed systems; thus they would not include respiration associated with the decomposition of a thick surface litter layer, as was present in our system. In grasslands, root respiration has exhibited high sensitivity to soil moisture (Gomez-Casanovas et al. 2012), and to nutrient availability (Wang et al. 2010), but less sensitivity to soil temperature. Likewise, the mycorrhizal and rhizosphere components of heterotrophic respiration have been tied closely to photosynthetic activity, with relatively low responsiveness to temperature (Moyano et al. 2007). In contrast, total heterotrophic soil respiration is often strongly influenced by both soil temperature and soil moisture (Arevalo et al. 2010; Gomez-Casanovas et al. 2012).

N and warming effects on NEE

As we predicted, N addition increased aboveground biomass in 2010, yet surprisingly, there was no corresponding effect of N on NEE, as observed in previous

studies (e.g. Volk et al. 2011). This result did not reflect a simple balancing of increased photoassimilation and increased respiration in response to N addition, because N addition had no effect on NEE_{dark} . However, the significant effect of N addition on $NEE_{\text{daily min}}$ confirmed there was an increase in the peak rates of photoassimilation that corresponded with increased aboveground biomass in these plots. The combination of these results indicates that CO_2 efflux overwhelmed any effect of photoassimilation on NEE in our system, which is consistent with our earlier observation that net influx was only occasionally observed at midday during periods of peak aboveground biomass. Nevertheless, it remains surprising that there were also no indirect effects of increased plant biomass on NEE caused by microbial responses to changes in root biomass or increased shading of the ground. Divergent effects of experimental treatments on vegetation versus ecosystem respiration have been observed elsewhere, as in the case of snow depth manipulations in arctic tundra (Rogers et al. 2011).

Our results also imply that the direct effects of N on soil microbial activity were minimal, which is consistent with the results of Bell et al. (2010), who detected no significant shifts in microbial extracellular enzymatic activity or microbial biomass C in response to N addition in our experiment. While belowground biomass increased significantly in response to N addition in 2009, increases in aboveground biomass that year were not statistically significant. Nevertheless, as described above, the aboveground biomass response to N addition was very strong the previous year (Hutchison and Henry 2010), and the resulting increase in litter layer thickness in N addition plots in 2009 may have suppressed shoot growth, although a potential role of interannual variation in phosphorus limitation cannot be ruled out. Similar oscillating patterns of annual growth responses to N addition have been observed in other multi-year global change field experiments (e.g. Dukes et al. 2005).

Contrary to the results obtained in our experiment in 2008 (Hutchison and Henry 2010), there was no significant effect of warming on aboveground biomass in either 2009 or 2010. In 2008, earlier spring snowmelt in the warmed plots appeared to be the most important factor driving increased plant productivity, but in 2009 and 2010, snowmelt was mostly synchronous between the warmed and ambient temperature plots. Therefore, while phenological changes in response to warming can potentially alter NEE by lengthening the plant growing

season (Shaver et al. 2000), in both years of our study there was no enhanced NEE response to the indirect effects of warming (e.g. possible changes in plant or microbial biomass or community composition) in early spring or late fall. This result is consistent with those of previous years, when there were no effects of warming on soil extracellular enzyme activities, fungal to bacterial biomass ratios or microbial biomass N in our experiment (Bell et al. 2010). Although warming did not indirectly affect NEE in our study, we estimated from our multiple regression model that net CO_2 efflux would have been 19 % higher in the warmed plots than in the ambient plots in 2009 if the heating had been maintained during the NEE measurements. In contrast, a lack of a significant difference in modeled NEE between warmed and ambient plots in 2010 occurred because the effects of reduced soil moisture counteracted warming-induced increases in soil respiration.

Conclusions

Our study is among the first to report CO_2 flux data obtained using steady-state flow-through canopy chambers within the context of a warming and N addition experiment. The lack of a relationship between biomass and total NEE responses to N addition was surprising, and indicates a divergence of plant and soil responses to global change factors in our system. However, we observed this lack of a relationship between biomass and total NEE responses in years of net CO_2 efflux, which may not be representative of the coupling between biomass and total NEE in years of net CO_2 influx. Although our study is not the first to reveal a grass-dominated system potentially being a source of C over the snow-free season, our result challenges the assumption that the establishment of an old field following the abandonment of agriculture consistently results in net annual C sequestration. It also reinforces the observation by Novick et al. (2004) that there are large uncertainties regarding the year to year function of grass-dominated systems as CO_2 sources or sinks. Nevertheless, the two years over which we documented net CO_2 efflux can be considered atypical in that CO_2 influx is expected for the majority of years in grasslands. In addition, given the potential influences of chamber design on net CO_2 flux measurements, caution must be applied in comparing fluxes among studies.

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