

Climate change reduces the net sink of CH₄ and N₂O in a semiarid grassland

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Abstract

Atmospheric concentrations of methane (CH₄) and nitrous oxide (N₂O) have increased over the last 150 years because of human activity. Soils are important sources and sinks of both potent greenhouse gases where their production and consumption are largely regulated by biological processes. Climate change could alter these processes thereby affecting both rate and direction of their exchange with the atmosphere. We examined how a rise in atmospheric CO₂ and temperature affected CH₄ and N₂O fluxes in a well-drained upland soil (volumetric water content ranging between 6% and 23%) in a semiarid grassland during five growing seasons. We hypothesized that responses of CH₄ and N₂O fluxes to elevated CO₂ and warming would be driven primarily by treatment effects on soil moisture. Previously we showed that elevated CO₂ increased and warming decreased soil moisture in this grassland. We therefore expected that elevated CO₂ and warming would have opposing effects on CH₄ and N₂O fluxes. Methane was taken up throughout the growing season in all 5 years. A bell-shaped relationship was observed with soil moisture with highest CH₄ uptake at intermediate soil moisture. Both N₂O emission and uptake occurred at our site with some years showing cumulative N₂O emission and other years showing cumulative N₂O uptake. Nitrous oxide exchange switched from net uptake to net emission with increasing soil moisture. In contrast to our hypothesis, both elevated CO₂ and warming reduced the sink of CH₄ and N₂O expressed in CO₂ equivalents (across 5 years by 7% and 11% for elevated CO₂ and warming respectively) suggesting that soil moisture changes were not solely responsible for this reduction. We conclude that in a future climate this semiarid grassland may become a smaller sink for atmospheric CH₄ and N₂O expressed in CO₂-equivalents.

Keywords: climate change, global warming potential, multifactor experiment, PHACE, positive feedback, water availability

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Introduction

Methane (CH₄) and nitrous oxide (N₂O) are powerful greenhouse gases that are 25 and 298 times more potent than carbon dioxide (CO₂) over a 100 year lifespan (Forster *et al.*, 2007). Terrestrial ecosystems are large sources and sinks of CH₄ and N₂O. Climate change may alter the source and sink strength of CH₄ and N₂O in terrestrial ecosystems with potentially important feedbacks to the global climate system (King, 1997; Xu-Ri *et al.*, 2012). Based on a meta-analysis including field, greenhouse and growth chamber studies, it was estimated that a rise in atmospheric CO₂ concentration projected for the mid- to end of this century would increase CH₄ and N₂O emission from terrestrial ecosystems equivalent to 1.1 Pg CO₂ per yr globally, thereby offsetting 16.6% or more of the concurrent increase in terrestrial C storage (van Groenigen *et al.*, 2011). Similarly, using a process-based model, it was estimated

that combined CH₄ and N₂O emission from terrestrial ecosystems in North America alone would increase by 0.7–1.3 Pg CO₂ eq. per yr by the end of this century in response to future climate change scenarios that were derived from three different global climate models (elevated CO₂ effects not included, Tian *et al.*, 2012).

Results from manipulative field experiments indicate that elevated CO₂ and warming effects on CH₄ and N₂O exchange with the atmosphere vary widely among different systems (Dijkstra *et al.*, 2012). Elevated CO₂ reduced CH₄ uptake in a temperate forest (Phillips *et al.*, 2001a; Dubbs & Whalen, 2010) and in a temperate grassland (Ineson *et al.*, 1998), but had no effect in other temperate and semiarid grasslands (Mosier *et al.*, 2002; Blankinship *et al.*, 2010). Reduced CH₄ uptake was associated with increased soil moisture impeding the supply of atmospheric CH₄ for oxidation by methanotrophs in the soil, and increasing CH₄ production by methanogens (Ineson *et al.*, 1998; Phillips *et al.*, 2001a). Elevated CO₂ increased N₂O emission in grasslands and agroecosystems that were fertilized with N (Ineson *et al.*, 1998; Baggs *et al.*, 2003; Kammann *et al.*, 2008;

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Lam *et al.*, 2011), but generally had no effect in non-fertilized systems (Phillips *et al.*, 2001b; Billings *et al.*, 2002; Mosier *et al.*, 2002; Larsen *et al.*, 2011; Niboyet *et al.*, 2011). Greater labile C inputs under elevated CO₂ could stimulate N₂O production by denitrifiers, at least when inorganic N is also abundant (Dijkstra & Morgan, 2012; Dijkstra *et al.*, 2012). However, in systems where N availability is low, elevated CO₂ could reduce N availability thereby constraining N₂O production (Hungate *et al.*, 1997; Mosier *et al.*, 2002). Warming increased CH₄ uptake in a temperate forest and several subarctic systems (Peterjohn *et al.*, 1994; Sjögersten & Wookey, 2002), most likely because the soil drying effect of warming enhanced diffusivity thereby stimulating the oxidation of atmospheric CH₄ in the soil. Warming increased N₂O emission in urban lawns and a heath land (Bijoor *et al.*, 2008; Larsen *et al.*, 2011), decreased N₂O emission in a wheat field and an alpine meadow (Hantschel *et al.*, 1995; Hu *et al.*, 2010), and had no effect on N₂O exchange in an annual grassland and temperate forests (Peterjohn *et al.*, 1994; McHale *et al.*, 1998; Niboyet *et al.*, 2011). Inconsistent warming effects could arise because warming affects multiple processes that may cause opposing effects on N₂O exchange. For instance, an increase in soil temperature could directly enhance the activity of nitrifiers and denitrifiers thereby stimulating N₂O emission, but a decrease in soil moisture could reduce microbial activity (McHale *et al.*, 1998; Bijoor *et al.*, 2008).

In semiarid grasslands, which comprise roughly 11% of the global land surface (Bailey, 1979), one of the most important drivers for biological activity is soil moisture, including the production and uptake of CH₄ and N₂O (Mosier *et al.*, 2008). Because soil moisture is low in most of the year, production and uptake of CH₄ and N₂O in semiarid grasslands can be very different from more mesic environments. For instance, in mesic environments net CH₄ uptake is often negatively related to soil moisture because soil moisture reduces CH₄ diffusivity into the soil for oxidation by methanotrophs, or increases CH₄ emission by methanogens (Del Grosso *et al.*, 2000; Phillips *et al.*, 2001a). In semiarid environments however, soils are often so dry that soil moisture effects on methanotroph activity can become more important than effects on CH₄ diffusivity or methanogen activity (von Fischer *et al.*, 2009). Therefore, under dry conditions net CH₄ uptake can increase with increased soil moisture (Mosier *et al.*, 2008; Dijkstra *et al.*, 2011). In mesic and wet environments N₂O emissions can be high because intermediate to high levels of soil moisture are conducive to nitrification and denitrification, both of which contribute to N₂O emissions (Klemetsson *et al.*, 1988; Davidson, 1992). Net N₂O emission in mesic environments is sometimes

restrained, however, because of N₂O consumption by denitrifiers (Holtgrieve *et al.*, 2006; Chapuis-lardy *et al.*, 2007). Consumption of N₂O has also been observed under dry conditions resulting in a net N₂O sink (Donoso *et al.*, 1993; Goldberg & Gebauer, 2009; Grover *et al.*, 2012; Stewart *et al.*, 2012). While the underlying mechanisms are still unclear, a possible explanation is that drier soil conditions enhance N₂O diffusion from the atmosphere into the soil where under limited inorganic N supply from the soil, atmospheric N₂O is reduced to N₂ by denitrifiers (Chapuis-lardy *et al.*, 2007).

Because biological processes strongly depend on soil moisture in semiarid grasslands we postulate that climate change effects on CH₄ and N₂O in these systems are largely mediated by changes in soil moisture. Previously we have shown that elevated CO₂ increased soil moisture in a semiarid grassland in Wyoming, USA, because of reduced stomatal conductance, while warming decrease soil moisture because of desiccation effects (Morgan *et al.*, 2011). During the first two growing seasons of the experiment CH₄ uptake was strongly controlled by soil moisture, and elevated CO₂ and warming effects could largely be explained by treatment effects on soil moisture (Dijkstra *et al.*, 2011). The drier soil conditions with warming reduced CH₄ uptake over the whole growing season. However, the increase in soil moisture under elevated CO₂ enhanced CH₄ uptake during dry periods of the growing season, but reduced CH₄ uptake during wet periods. When elevated CO₂ effects on CH₄ uptake were considered for the whole growing season, elevated CO₂ had no net effect. Interactive CO₂ × warming effects were also not observed during the first 2 years. Here, we present single and combined effects of elevated CO₂ and warming on CH₄ and N₂O fluxes during five growing seasons. None of the five growing seasons were exceptionally dry or wet (Table 1). We hypothesized that an increase in soil moisture under elevated CO₂ would have no effect on CH₄ uptake, but that a decrease in soil moisture with warming would decrease CH₄ uptake, similar to what we found during the first 2 years. We further hypothesized that elevated CO₂ would have no effect on N₂O emission because of a tighter N cycle under elevated CO₂ (Dijkstra *et al.*, 2010). On the other hand, we

Table 1 Total precipitation and mean temperature during the growing season for each year and averaged across all 5 years

	2007	2008	2009	2010	2011	Average
Precip. (mm)	288	349	353	242	363	319
Mean temp (°C)	17.4	15.9	12.5	13.3	13.9	14.6

hypothesized that drier soil conditions with warming would reduce N₂O emission, and with limited N available, potentially turning this system into an N₂O sink. Because the decrease in net N₂O emission would offset the decrease in net CH₄ uptake with warming, combined CH₄ and N₂O fluxes in this grassland expressed in CO₂ equivalents would be insensitive to climate change.

Materials and methods

Site description and experimental design

We conducted our study in the Prairie Heating And CO₂ Enrichment (PHACE) experiment in a semiarid grassland in Wyoming, USA (41° 11' N, 104° 54' W). Vegetation at the site is a northern mixed prairie dominated by the C₄ grass *Bouteloua gracilis* (H.B.K) Lag and the C₃ grasses *Pascopyrum smithii* (Rydb.) A. Love and *Hesperostipa comata* Trin and Rupr. Other species include the sedge *Carex eleocharis* L. Bailey, the forb *Sphaeralcea coccinea* (Nutt.) Rydb., and the subshrub *Artemisia frigida* Willd. The soil is a fine-loamy, mixed, mesic Aridic Argiustoll with a pH of 7.0 and a total organic C and N content of 1.7% and 0.16%, respectively, in the top 15 cm. Mean air temperature in January is -2.5 °C and 17.5 °C in July and the mean annual precipitation is 384 mm (132-year mean). Methane and N₂O fluxes were measured during the growing season (April–October) from 2007 to 2011 (see below). Total precipitation during the growing season varied between 242 mm in 2010 and 363 mm in 2011, while mean air temperature varied between 12.5 °C in 2009 and 17.4 °C in 2007 (Table 1).

In 2005 twenty 3.4 m diameter plots were established in a 2.5 ha fenced-off area that had been moderately grazed until 2005. A plastic flange was dug into the ground 60-cm deep around each plot preventing lateral water flow. We used Free Air CO₂ Enrichment technology (Miglietta *et al.*, 2001) to increase the atmospheric CO₂ concentration to 600 ppmv (±40 ppmv). The CO₂ was injected into the plot from a plastic pipe, perforated with 300-µm laser-drilled holes, surrounding the plot just above the plant canopy. The CO₂ treatment started in April 2006 and only occurred when plants were photosynthetically active (during light hours and during the growing season from April through October). We used ceramic infrared heaters (1000 W; Mor Electric Heating Assoc., Inc., Comstock Park, MI, USA) controlled by a proportional-integral-derivative feed-back loop (Kimball *et al.*, 2008) to increase the canopy temperature by 1.5 °C above ambient during the day and by 3 °C during the night. Heaters were attached to a metal triangular frame 1.5 m above the ground surface (six heaters per plot). The warming treatment started in April 2007 and ran continuously throughout the year. The CO₂ and warming treatments were implemented in a full factorial design with five replicates of each of the treatment combinations (ct: ambient CO₂ and ambient temperature, cT: ambient CO₂ and elevated temperature, Ct: elevated CO₂ and ambient temperature, and CT: elevated CO₂ and elevated temperature). Detailed information about the site and CO₂ and

warming treatments can be found elsewhere (Dijkstra *et al.*, 2010; Morgan *et al.*, 2011). In June 2008 we established 0.4 m⁻² subplots without plants in each of the 20 plots. These subplots were separated from the main plot by a metal sheet buried 30 cm into the ground. The vegetation was killed by lightly spraying with the broad spectrum systemic herbicide glyphosate. The N that we added with the glyphosate (<0.3 g N m⁻² compared to approximately 0.5 g N m⁻² as NH₄⁺ and NO₃⁻ in the top 15 cm of the soil, Dijkstra *et al.*, 2010) may have had some temporary impacts on soil N availability but most likely no or minor impacts in the long-term. New seedlings after spraying were regularly removed by hand.

CH₄ and N₂O flux measurements

We measured CH₄ and N₂O fluxes approximately every other week during five consecutive growing seasons (from April through October), starting in 2007 until 2011 (between 12 and 16 measurements per season) using static chambers (Hutchinson & Mosier, 1981). We inserted chamber anchors (diameter 20 cm, height 10 cm) 8 cm into the soil, two in each plot (one in an area with vegetation intact (with plants), and one in the area where we killed the vegetation (without plants)). Anchors were installed at least 1 month before the first measurements were taken. Measurements were taken during midmorning. After placement of the chambers on the anchors, gas samples were taken from the headspace after 0, 15, 30 and 45 min. Gas samples were analysed for CH₄ and N₂O concentration on a gas chromatograph (GC) equipped with a flame ionization detector for CH₄ and an electron capture detector for N₂O (Varian 3800; Varian, Inc., Palo Alto, CA, USA) usually within 2 days after sampling. The precision of the GC was about 1 ppb for both gases. The CH₄ and N₂O fluxes were calculated as the slope of linear regressions from the measured gas concentrations with time. The *r*² value of the regressions was sometimes below 0.4, particularly when fluxes were low. Because we did not want to create a bias against low fluxes, we did not discard fluxes with low *r*² values, unless CO₂ concentrations measured at the same time as CH₄ and N₂O did not show a clear increasing trend with time. We calculated cumulative fluxes of CH₄ (in mg C m⁻²) and N₂O (in mg N m⁻²) produced/consumed over the growing season by multiplying the average flux measured at two consecutive dates by the time interval, and by summing up the cumulative fluxes calculated for each time interval of the growing season. Because we did not start with the CH₄ and N₂O flux measurements in the subplots without plants until June in 2008, we only calculated cumulative fluxes of CH₄ and N₂O in the subplots without plants for 2009, 2010 and 2011. We calculated the cumulative Global Warming Potential from CH₄ and N₂O (in g CO₂ eq. per m²) by adding the cumulative GWP (Global Warming Potential) from CH₄ (cumulative flux of CH₄ in g CH₄ per m² multiplied by 25) and the cumulative GWP from N₂O (cumulative flux of N₂O in g N₂O per m² multiplied by 298).

Soil moisture, temperature and plot greenness

Soil moisture at 10-cm soil depth was measured in each plot (with plants only) with EnviroSMART probes (Sentek Sensor

Technologies, Stepney, Australia). Water filled pore space (WFPS) in the top 15 cm of the soil was calculated based on soil moisture measured at 10-cm soil depth and bulk densities measured at 0–5 and 5–15 cm soil depth in 2005. Soil temperature at 10-cm soil depth was measured in each plot (with plants only) with thermocouples. Soil moisture and temperature data were logged every hour throughout the year (CR10X data loggers; Campbell Scientific, Logan, UT, USA). We calculated seasonal average WFPS and soil temperature for each year by averaging the WFPS and soil temperature values recorded at the time of flux measurements.

We measured plot greenness as a degree of photosynthetically active plant biomass inside the static chambers at the time of CH₄ and N₂O flux measurements. In each plot (with plants only) digital photographs were taken of the surface area inside the anchors with a camera attached to a tripod from 50 cm above the ground. Photographs were taken directly after each time we sampled for CH₄ and N₂O. Photographs were then analysed for the total green area as a percentage of the total surface area inside the anchor (where the area that was not green was either bare soil or senesced plant material) using the software program SamplePoint (Booth *et al.*, 2006). Plot greenness varied between 0 (in early April and late October) and 62% (July–August) during the season (Fig. S2). Plot greenness measured in mid-July of 2007–2011 was positively related to aboveground green biomass harvested in mid-July of each year ($n = 100$, $P < 0.0001$, $r = 0.65$), suggesting that plot greenness provided a reasonable measure of photosynthetically active plant biomass.

Statistical analyses

We used a repeated measures analysis of variance (repeated measures ANOVA) to test for main effects of CO₂ (ambient vs. elevated), temperature (no warming vs. warming, both between-subjects factors), year (2007–2011, within-subjects factor), and their interactive effects on cumulative CH₄, N₂O and GWP from CH₄ and N₂O. We ran the repeated measures analysis separately for measurements with and without plants. We used *post-hoc* tests (Tukey's HSD) to test for differences in cumulative CH₄, N₂O and GWP from CH₄ and N₂O among the different CO₂ and warming treatment combinations. Repeated measures ANOVA was further used for WFPS, soil temperature and plot greenness measured in the subplots with plants only. We used linear and nonlinear regressions to relate CH₄ and N₂O fluxes to WFPS, soil temperature and plot greenness using data that were aggregated by date (i.e., averaged across the CO₂ and warming treatments) and by date and treatment (i.e., average of the five replicates of each treatment on each date). We further tested if relationships differed among the CO₂ and warming treatments using analysis of covariance (ANCOVA) with the CO₂ and warming treatment as main effects and either WFPS, soil temperature and plot greenness as the covariate. Because CH₄ fluxes showed bell-shaped relationships with WFPS and soil temperature, we included a quadratic term of the covariate in the ANCOVAs (Dijkstra *et al.*, 2011). Significant interactions between main effects and the covariate indicate that relationships between

CH₄ or N₂O fluxes with WFPS, soil temperature, or plot greenness were altered by the CO₂ and/or warming treatment. When necessary, data were log-transformed to reduce heteroscedasticity and improve assumptions of normality. All statistical analyses were performed with JMP (version 4.0.4; SAS Institute, Cary, NC, USA).

Results

Soils were a net sink for CH₄ throughout all five growing seasons (as indicated by negative fluxes). Variable CH₄ fluxes were observed within each season although CH₄ uptake tended to be highest mid to late summer and smallest at the start and end of the growing season (Fig. S1). In the presence of plants, cumulative CH₄ uptake during the growing season was smallest in 2007 (90 mg C m⁻² averaged across the CO₂ and warming treatments) and greatest in 2011 (130 mg C m⁻², Fig. 1). Elevated CO₂ increased cumulative CH₄ uptake in 2007 by 15%, but decreased it in all other years up to 12% in 2011 causing a significant CO₂ × year interactive effect ($P = 0.02$). Warming significantly reduced cumulative CH₄ uptake across all years ($P < 0.0001$) with an average decrease of 15%. Warming also significantly reduced cumulative CH₄ uptake without plants ($P = 0.01$), while elevated CO₂ had no effect on cumulative CH₄ uptake without plants. Cumulative CH₄ uptake was higher without than with plants.

Nitrous oxide fluxes in the presence of plants were small throughout the growing season and were often negative, particularly during mid to late summer (Fig. S1), suggesting soil uptake of N₂O at those times. Cumulative fluxes of N₂O were also sometimes negative (N₂O uptake), particularly during the last 2 years (Fig. 2a). Across years there was no significant elevated CO₂ effect on cumulative N₂O, but during the last 2 years N₂O uptake decreased under elevated CO₂ causing a significant CO₂ × year interactive effect ($P = 0.002$). There was no warming effect on cumulative fluxes of N₂O. Cumulative fluxes of N₂O were much larger without than with plants, particularly in 2009 and 2011 (Fig. 2b). Elevated CO₂ significantly reduced the cumulative flux of N₂O production without plants ($P = 0.006$).

Seasonal variability in WFPS, soil temperature and plot greenness was large (Fig. S2); where WFPS ranged between 11% and 41%, soil temperature between 2 and 29 °C, and plot greenness between 0% and 62% during the growing season. Growing season averages of WFPS were significantly higher under elevated CO₂ (absolute increase of 3.8% across all years, $P < 0.0001$) and significantly lower with warming (absolute decrease of 2.1% across all years, $P < 0.0001$, Table 2). Soil temperature was significantly higher with warming (increase of

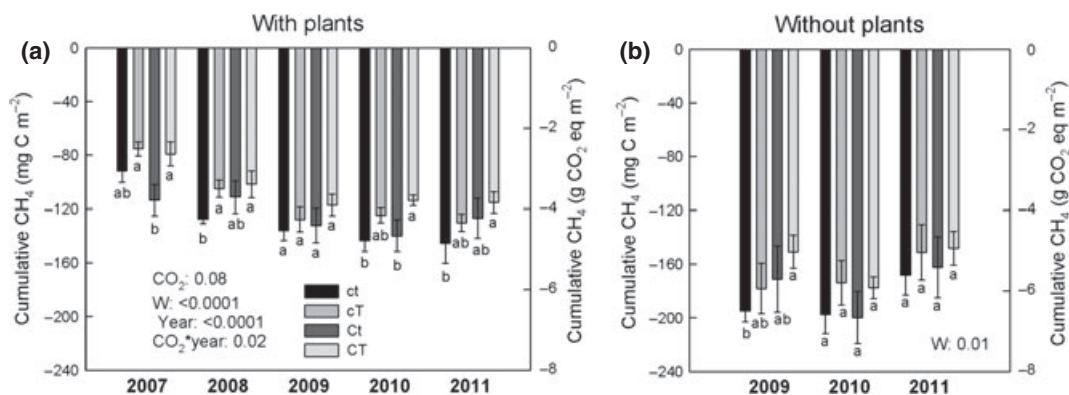


Fig. 1 Cumulative CH_4 in mg C m^{-2} (left y -axis) and in $\text{g CO}_2 \text{ eq. per m}^2$ (right y -axis) in plots with (a) and without plants (b) in response to elevated CO_2 and warming. Negative values indicate sinks. Treatments: ct: ambient CO_2 and ambient temperature; cT: ambient CO_2 and elevated temperature; Ct: elevated CO_2 and ambient temperature; CT: elevated CO_2 and elevated temperature. W: warming treatment. Error bars indicate ± 1 SE. ANOVA P -values are reported when $P < 0.05$ (in bold) or $P < 0.1$ (in italics). Different letters above bars indicate significant differences among the elevated CO_2 and warming treatments for each year separately ($P < 0.05$, Tukey's HSD test).

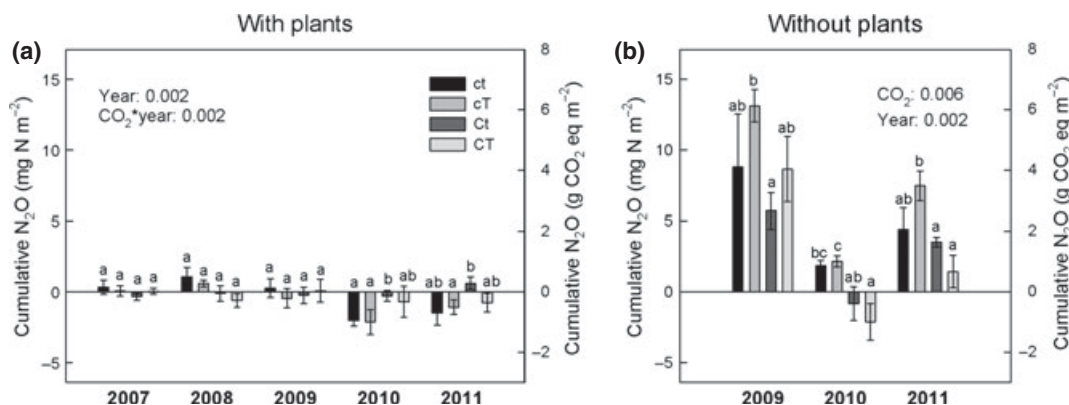


Fig. 2 Cumulative N_2O in mg N m^{-2} (left y -axis) and in $\text{g CO}_2 \text{ eq. per m}^2$ (right y -axis) in plots with (a) and without plants (b) in response to elevated CO_2 and warming. Negative values indicate sinks. Treatments: ct: ambient CO_2 and ambient temperature; cT: ambient CO_2 and elevated temperature; Ct: elevated CO_2 and ambient temperature; CT: elevated CO_2 and elevated temperature. W: warming treatment. Error bars indicate ± 1 SE. ANOVA P -values are reported when $P < 0.05$ (in bold) or $P < 0.1$ (in italics). Different letters above bars indicate significant differences among the elevated CO_2 and warming treatments for each year separately ($P < 0.05$, Tukey's HSD test).

1.9 °C, $P < 0.0001$), but was not affected by elevated CO_2 . Elevated CO_2 and warming also had no significant effect on plot greenness.

The temporal variation in CH_4 and N_2O fluxes in subplots with plants could to a large degree be explained by the temporal variation in WFPS (Fig. 3a and b). We observed a significant bell-shaped relationship for CH_4 with the largest CH_4 uptake at 24% WFPS and smaller uptake at higher and lower WFPS. We further observed significant interactions between WFPS and the CO_2 and warming treatments (Table S1), indicating that the relationships differed among the CO_2 and warming treatments. Individual regressions for each treatment showed that elevated CO_2 increased the

optimum WFPS (on average by 3.6%), while warming showed reduced CH_4 uptake at optimum WFPS (on average by $3.1 \mu\text{g C m}^{-2} \text{ hr}^{-1}$, Fig. S3, Table S1). Similar results were obtained during the first 2 years of the experiment (Dijkstra *et al.*, 2011). On the other hand the temporal N_2O fluxes showed a significant positive linear relationship with WFPS (Fig. 3b), while the slope of this relationship was lower under elevated CO_2 (Fig. S3, Table S1). Despite the large range in soil temperatures at the time of our measurements, soil temperature had no effect on CH_4 when data were aggregated across the CO_2 and warming treatment (Fig. 3c). However, CH_4 fluxes showed bell-shaped relationships with soil temperature, similar to the relationships with

Table 2 Water filled pore space (WFPS), soil temperature and plot greenness averaged by the elevated CO₂ and warming treatments measured during trace gas flux measurements

Year	WFPS (%)				Soil temp (°C)				Plot greenness (%)			
	ct	cT	Ct	CT	ct	cT	Ct	CT	ct	cT	Ct	CT
2007	21.8 ± 1.5	20.1 ± 0.7	26.1 ± 1.9	24.6 ± 2.0	16.3 ± 0.6	18.5 ± 0.6	16.3 ± 0.5	18.2 ± 0.6	20.2 ± 2.0	21.7 ± 1.7	21.7 ± 0.8	21.9 ± 1.1
2008	24.5 ± 1.2	21.6 ± 0.5	27.6 ± 1.2	25.4 ± 1.3	15.5 ± 1.0	17.6 ± 0.5	15.7 ± 0.6	16.9 ± 0.6	23.4 ± 1.6	23.7 ± 1.1	21.3 ± 0.8	22.8 ± 0.7
2009	24.3 ± 1.2	22.5 ± 0.8	29.1 ± 1.4	26.6 ± 1.2	14.5 ± 0.9	17.3 ± 1.0	15.0 ± 1.0	16.0 ± 0.7	21.8 ± 1.4	22.5 ± 1.6	18.6 ± 0.6	20.3 ± 1.2
2010	21.3 ± 0.9	20.2 ± 0.4	24.7 ± 0.9	23.2 ± 0.9	14.3 ± 0.5	16.6 ± 0.5	14.6 ± 0.5	16.0 ± 0.5	15.7 ± 0.8	18.3 ± 1.5	16.7 ± 1.7	16.0 ± 1.1
2011	24.3 ± 1.1	22.2 ± 0.3	28.3 ± 0.7	25.1 ± 1.0	13.4 ± 0.4	15.5 ± 0.5	13.3 ± 0.5	15.2 ± 0.1	23.4 ± 0.8	27.1 ± 1.4	25.7 ± 2.9	22.0 ± 0.6
ANOVA <i>P</i> -values												
CO ₂	<0.0001				0.24				0.16			
W	<0.0001				<0.0001				0.32			
CO ₂ × W	0.74				0.06				0.21			
Year	0.35				<0.0001				0.92			
CO ₂ × year	0.37				0.87				0.51			
W × year	0.75				0.99				0.77			

Ct, ambient CO₂ and ambient temperature; cT, ambient CO₂ and elevated temperature; CT, elevated CO₂ and ambient temperature; CT, elevated CO₂ and elevated temperature; W, warming treatment.

P-values are in bold when *P* < 0.05 and in italics when *P* < 0.1.

WFPS, when regressions were performed for each CO₂ and warming treatment (Fig. S3, Table S1). Interestingly, N₂O fluxes were significantly negatively related to soil temperature (Fig. 3d), while this relationship was not affected by the CO₂ and warming treatments (Fig. S3, Table S1). However, it should be noted that WFPS and soil temperature were also significantly negatively correlated (*P* < 0.0001, *r* = 0.51). Plot greenness had no effect on N₂O fluxes, but was significantly correlated with CH₄ fluxes (Fig. 3e and f) without differences among the CO₂ and warming treatments (Fig. S3, Table S1). Plot greenness was not correlated with WFPS (*P* > 0.1) but was positively related to soil temperature (*P* < 0.0001, *r* = 0.57).

This semiarid grassland was at all times a net sink regarding the combined cumulative GWP from CH₄ and N₂O (Fig. 4). Both elevated CO₂ and warming significantly reduced this sink (*P* = 0.04 for elevated CO₂ and *P* = 0.002 for warming). The reduced net sink of CH₄ and N₂O under elevated CO₂ increased with time (significant CO₂ × year interactive effect, *P* = 0.0003). Across years, elevated CO₂ and warming reduced the net sink for CH₄ and N₂O by 7.2% and 11%, respectively, with the largest reduction of 17% when elevated CO₂ and warming were combined.

Discussion

This grassland was a sink for CH₄ at all times during the growing season, while it was sometimes a source and sometimes a sink for N₂O (i.e., net N₂O uptake). When CH₄ and N₂O were combined in terms of CO₂ equivalents, this grassland was always a sink. Contrary to our hypothesis, both elevated CO₂ and warming reduced the net sink of CH₄ and N₂O in this grassland, and the largest net sink reductions occurred when elevated CO₂ and warming were combined (Fig. 4). Combined effects of elevated CO₂ and warming resulted in a significant decrease in the net sink of CH₄ and N₂O by 28% and 24% in 2010 and 2011 respectively. Our results suggest that this semiarid grassland causes a positive feedback to global warming by reducing the net sink of CH₄ and N₂O.

CH₄

Both elevated CO₂ and warming reduced CH₄ uptake in this semiarid grassland in most of the years; CH₄ uptake in plots exposed to elevated CO₂ and warming together was significantly lower than in the control plots in three of the 5 years (2008, 2010, and 2011, Fig. 1). The reduced CH₄ uptake under elevated CO₂ is in contrast to observations in a similar semiarid grassland where CH₄ uptake was not affected by elevated

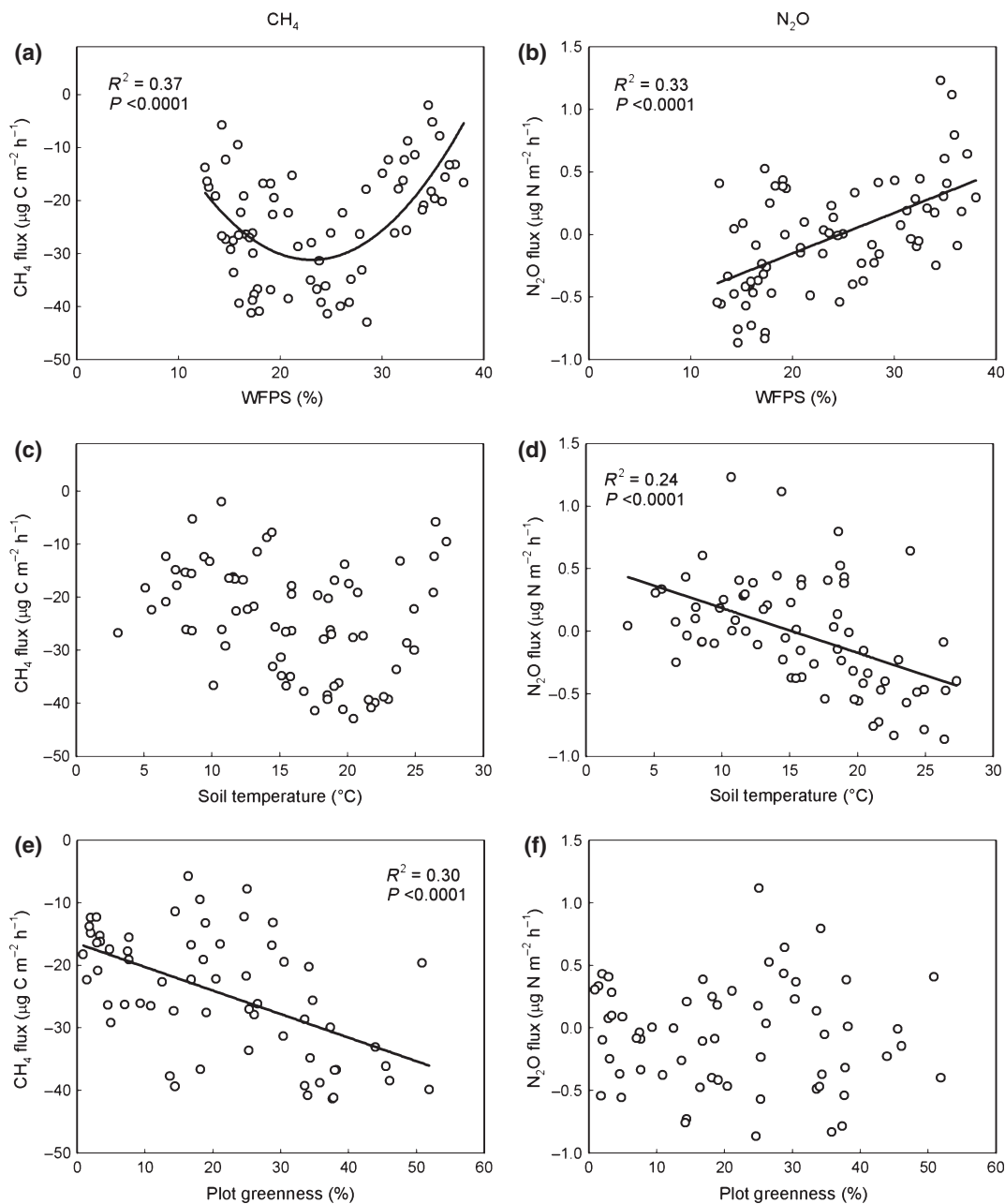


Fig. 3 Fluxes of CH₄ (a, c, e) and N₂O (b, d, f) as a function of water filled pore space (WFPS, a, b), soil temperature (c, d) and plot greenness (e, f). Each data point is the average CH₄ or N₂O flux, and average WFPS, soil temperature, or plot greenness measured across all treatments at a specific date between 2007 and 2011. Regression lines are only shown when significant ($P < 0.05$).

CO₂ (Mosier *et al.*, 2002), but supports results from studies in mesic environments (Ineson *et al.*, 1998; Phillips *et al.*, 2001a; Dubbs & Whalen, 2010). On the other hand, the reduced CH₄ uptake with warming in this semiarid grassland differed from studies in wetter environments where no or increased CH₄ uptake was found in response to warming (Peterjohn *et al.*, 1994; Sjögersten & Wookey, 2002; Blankinship *et al.*, 2010).

Treatment effects on CH₄ uptake may have been mediated by their effect on soil moisture. The CH₄ flux

showed a bell-shaped relationship with WFPS with an optimum CH₄ uptake rate at 24% (Fig. 3a). Soil moisture consistently increased under elevated CO₂ and consistently decreased with warming (Table 2), which could have caused opposing effects on CH₄ uptake depending on what side of the curve the change in soil moisture occurred (Dijkstra *et al.*, 2011). A soil moisture increase under elevated CO₂ could increase CH₄ uptake (through stimulating methanotroph activity) when soils are relatively dry, or decrease CH₄ uptake (through

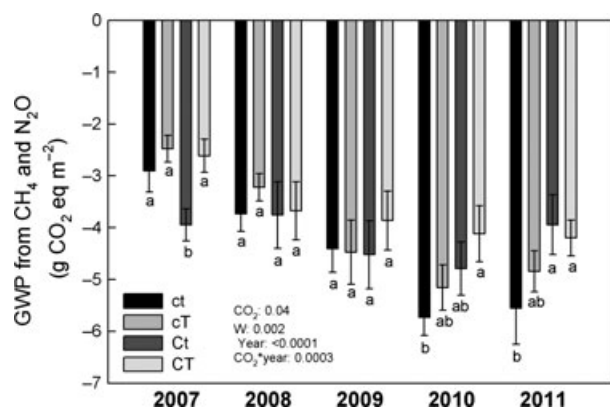


Fig. 4 Cumulative Global Warming Potential (GWP) in g CO₂ eq. per m² in plots with plants in response to elevated CO₂ and warming. Treatments: ct: ambient CO₂ and ambient temperature; cT: ambient CO₂ and elevated temperature; Ct: elevated CO₂ and ambient temperature; CT: elevated CO₂ and elevated temperature. W: warming treatment. Error bars indicate ± 1 SE. ANOVA *P*-values are reported when *P* < 0.05 (in bold) or *P* < 0.1 (in italics). Different letters above bars indicate significant differences among the elevated CO₂ and warming treatments for each year separately (*P* < 0.05, Tukey's HSD test).

decreasing CH₄ diffusivity into the soil) when soils are relatively wet, while a soil moisture decrease with warming should have the opposite effect. In contrast, we observed reductions in CH₄ uptake with elevated CO₂ and warming that are hard to explain by the treatment effects on soil moisture alone. For instance, when elevated CO₂ and warming were combined, average WFPS was only slightly higher than in the control plots (Table 2) because of opposing effects of the two treatments on soil moisture (Morgan *et al.*, 2011). Yet, plots exposed to elevated CO₂ and warming (CT plots) often showed the largest reduction in CH₄ uptake. The relationship between CH₄ uptake and WFPS differed somewhat among the treatments (Fig. S3, Table S1), further suggesting that other drivers than soil moisture alone must have contributed to the reduction in CH₄ uptake under elevated CO₂ and warming.

Another driver that could have influenced treatment effects on CH₄ uptake is soil temperature. An increase in soil temperature could enhance methanotroph activity, but could also enhance methanogen activity that tends to be more responsive to temperature (Topp & Pattey, 1997). Both processes usually occur at the same time (Yavitt *et al.*, 1995; von Fischer & Hedin, 2007), and stimulation of both processes by soil temperature may have cancelled out each other's effect. We observed no relationship between CH₄ uptake and soil temperature when the data were aggregated across all treatments (Fig. 3c), but found similar bell-shaped relationships as observed between CH₄ uptake and WFPS (Fig. S3). Because of the strong correlation between

WFPS and soil temperature, the relationships between CH₄ uptake and soil temperature for each treatment may have been driven partially by soil moisture. Nevertheless, as with WFPS, the reduced CH₄ uptake with elevated CO₂ and warming combined are difficult to explain by treatment effects on soil temperature alone (note that elevated CO₂ had no effect on soil temperature, Table 2).

Treatment effects on N cycling may also have affected CH₄ uptake. Ammonium (NH₄⁺) can suppress CH₄ oxidation in the soil because some of the CH₄ oxidizing microbes switch to oxidizing NH₄⁺ with increased availability of NH₄⁺ (Stuedler *et al.*, 1989; Hanson & Hanson, 1996). Elevated CO₂ significantly increased NH₄⁺ concentrations in our experiment (Carrillo *et al.*, 2012), which could have contributed to reduced CH₄ uptake that we observed in some years under elevated CO₂. It has also been suggested that soil nitrate (NO₃⁻) could stimulate CH₄ uptake at low CH₄ concentrations due to shifts in the CH₄ oxidizing bacteria community (Jang *et al.*, 2011). We observed a significant reduction in soil NO₃⁻ under elevated CO₂ (Carrillo *et al.*, 2012), suggesting that this too may have contributed to the reduced CH₄ uptake under elevated CO₂, particularly with a progressively tighter N cycle under elevated CO₂ (Dijkstra *et al.*, 2010). Soil NO₃⁻ was much higher in the subplots without plants (Carrillo *et al.*, 2012), suggesting that soil NO₃⁻ may also have played a role in the higher CH₄ uptake without plants (Fig. 1).

N₂O

The N₂O fluxes in this grassland were small (cumulative N₂O never exceeded 2 mg N m⁻²), while both production and uptake of N₂O occurred (Fig. 2a). Because of the small fluxes, elevated CO₂ and warming had no effect on N₂O across all years. However, elevated CO₂ significantly reduced the N₂O sink in the last 2 years. This is in contrast to studies conducted in a similar semiarid grassland where no elevated CO₂ effects on N₂O fluxes were observed (Mosier *et al.*, 2002). In temperate grasslands that received N fertilization elevated CO₂ increased N₂O emission (Ineson *et al.*, 1998; Baggs *et al.*, 2003; Kammann *et al.*, 2008). In these grasslands N fertilization may have alleviated N constraints, while at the same time increased supply of labile C under elevated CO₂ may have reduced C constraints on denitrification thereby causing the increase in N₂O emission (Dijkstra & Morgan, 2012; Dijkstra *et al.*, 2012). On the other hand, in systems without N fertilization and where N availability is limiting denitrification, an increase in labile C under elevated CO₂ may not increase N₂O emission. At our site, N₂O emission

appeared to be limited by N availability rather than by C availability. The absence of plant N uptake in subplots without plants significantly increased N availability (Carrillo *et al.*, 2012), which may have caused the significant increase in N₂O emission in these plots (Fig. 3). We found no relationship between plot greenness and N₂O fluxes (Fig. 3f). Assuming that plot greenness correlates with photosynthetically active plant biomass, and therefore with plant input of labile C (Leake *et al.*, 2006), the lack of a relationship between plot greenness and N₂O exchange suggests that seasonal variation in plant input of labile C had no effect on N₂O fluxes (although plot greenness may have related poorly to labile C input into the soil at the time of measurement). Furthermore, we often observed uptake of N₂O in the plots with plants, particularly during the last 2 years, suggesting low levels of available N in these plots. The process of N₂O uptake under dry conditions is still little understood (Chapuis-lardy *et al.*, 2007), but dry soil conditions may have enhanced diffusion of atmospheric N₂O into the soil where in the absence of NO₃⁻, N₂O was used as the electron acceptor for denitrification (Stewart *et al.*, 2012). The positive relationship between WFPS and N₂O flux (Fig. 3b) suggests that elevated CO₂-induced increases in soil moisture may have reduced the diffusion of N₂O into the soil and therefore reduced N₂O uptake in the last 2 years.

Nitrous oxide fluxes were negatively related to soil temperature (Fig. 3d). Often N₂O emissions increase with increased temperature (Dobbie & Smith, 2001; Mosier *et al.*, 2008). Most likely, N₂O fluxes in our study were driven more by soil moisture than by temperature, as WFPS was significantly negatively correlated with soil temperature ($P < 0.0001$, $r = 0.51$). Warming had also no effect on the N₂O flux. Possibly, the direct stimulatory effect of increased soil temperature with warming on the N₂O flux was offset by the indirect inhibitory effect of reduced soil moisture with warming (McHale *et al.*, 1998; Bijoor *et al.*, 2008).

Cumulative GWP

Both elevated CO₂ and warming significantly increased the cumulative GWP from CH₄ and N₂O combined. The largest increase in the cumulative GWP occurred when elevated CO₂ and warming were combined, resulting in a significant increase in 2010 and 2011 of 1.6 and 1.4 g CO₂ eq. per m², respectively, compared with the control plots (Fig. 4). Methane was the largest contributor to the increase in cumulative GWP in response to combined effects of elevated CO₂ and warming; 61% and 74% of the total increase in cumulative GWP in 2010 and 2011, respectively, was caused by reductions in CH₄ uptake. We hypothesized that

elevated CO₂ and warming would have opposing effects on the cumulative GWP from CH₄ and N₂O because of their opposing effects on soil moisture (Morgan *et al.*, 2011). Because CH₄ was the dominant contributor to the cumulative GWP, and because average soil moisture contents were on average near optimum CH₄ uptake rates in control plots, both elevated CO₂-induced increases and warming-induced decreases in soil moisture increased the cumulative GWP. Other drivers, such as soil N availability may also have played a role in causing synergistic rather than antagonistic effects of elevated CO₂ and warming on the net sink of CH₄ and N₂O in this system.

Our results show that, when expressed in CO₂-equivalents, both elevated CO₂ and warming reduced the net sink of CH₄ and N₂O in this semiarid grassland. Elevated CO₂ and warming effects on this sink may be different in exceptionally dry or wet seasons (i.e., seasons with less than 242 mm observed in 2010 or more than 363 mm observed in 2011), or in other semiarid grasslands with different rainfall regimes. Nevertheless, when we extrapolate our results of the last two seasons (which were the driest and wettest season during the 5-year period that we measured) to the global land surface of semiarid grasslands (11% of the global land surface or 16 383 400 km², Bailey, 1979), then between 22 and 26 Tg CO₂ eq. per yr less will be taken up as CH₄ and N₂O in response to elevated CO₂ and temperature at levels that are predicted for the mid- to end of this century. Combined effects of elevated CO₂ and warming also caused some of the largest losses in soil C at our site (E. Pendall, J. L. Heisler-White, D. G. Williams, F. A. Dijkstra, Y. Carrillo, J. A. Morgan, D. R. LeCain, in review). These results together with our results suggest that semiarid grasslands cause an important positive feedback to climate change.

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Supporting Information

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

Figure S1. Seasonal fluxes of CH₄ (a) and N₂O (b) during the growing season in 2007–2011 in response to elevated CO₂ and warming. Negative values indicate fluxes from the atmosphere to the soil. Treatments: ct: ambient CO₂ and ambient temperature; cT: ambient CO₂ and elevated temperature; Ct: elevated CO₂ and ambient temperature; CT: elevated CO₂ and elevated temperature. Error bars indicate Tukey's HSD.

Figure S2. Seasonal water filled pore space (WFPS) (a), soil temperature (b), and plot greenness (c) during the growing season in 2007–2011 in response to elevated CO₂ and warming. Treatments: ct: ambient CO₂ and ambient temperature; cT: ambient CO₂ and elevated temperature; Ct: elevated CO₂ and ambient temperature; CT: elevated CO₂ and elevated temperature. Error bars indicate Tukey's HSD.

Figure S3. Fluxes of CH₄ (a, c, e) and N₂O (b, d, f) as a function of water filled pore space (WFPS, a, b), soil temperature (c, d) and plot greenness (e, f). Each data point is the average CH₄ or N₂O flux, and average WFPS, soil temperature, or plot greenness measured across all five replicates for each treatment at a specific date between 2007 and 2011. Treatments: ct: ambient CO₂ and ambient temperature; cT: ambient CO₂ and elevated temperature; Ct: elevated CO₂ and ambient temperature; CT: elevated CO₂ and elevated temperature. Regression lines are only shown when significant ($P < 0.05$, Table S1).

Table S1. Results from ANCOVAs with water filled pore space (WFPS), soil temperature (Temp), and plot greenness (Greenness) as covariates, and as independent variables in regression analyses to predict CH₄ and N₂O fluxes for each treatment (ct: ambient CO₂ and ambient temperature; cT: ambient CO₂ and elevated temperature; Ct: elevated CO₂ and ambient temperature; CT: elevated CO₂ and elevated temperature).