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Climate change reduces the net sink of CH_4 and N_2O in a semiarid grassland

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Abstract

Atmospheric concentrations of methane (CH_4) and nitrous oxide (N_2O) 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 CO_2 and temperature affected CH_4 and N_2O 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 CH_4 and N_2O fluxes to elevated CO_2 and warming would be driven primarily by treatment effects on soil moisture. Previously we showed that elevated CO_2 increased and warming decreased soil moisture in this grassland. We therefore expected that elevated CO_2 and warming would have opposing effects on CH_4 and N_2O fluxes. Methane was taken up throughout the growing season in all 5 years. A bell-shaped relationship was observed with soil moisture with highest CH_4 uptake at intermediate soil moisture. Both N_2O emission and uptake occurred at our site with some years showing cumulative N_2O emission and other years showing cumulative N_2O uptake. Nitrous oxide exchange switched from net uptake to net emission with increasing soil moisture. In contrast to our hypothesis, both elevated CO_2 and warming reduced the sink of CH_4 and N_2O expressed in CO_2 equivalents (across 5 years by 7% and 11% for elevated CO_2 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 CH_4 and N_2O expressed in CO_2-equivalents.

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

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Introduction

Methane (CH_4) and nitrous oxide (N_2O) are powerful greenhouse gases that are 25 and 298 times more potent than carbon dioxide (CO_2) over a 100 year lifespan (Forster *et al*., 2007). Terrestrial ecosystems are large sources and sinks of CH_4 and N_2O. Climate change may alter the source and sink strength of CH_4 and N_2O 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 CO_2 concentration projected for the mid- to end of this century would increase CH_4 and N_2O emission from terrestrial ecosystems equivalent to 1.1 Pg CO_2 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 CH_4 and N_2O emission from terrestrial ecosystems in North America alone would increase by 0.7–1.3 Pg CO_2 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 CO_2 effects not included, Tian *et al*., 2012).

Results from manipulative field experiments indicate that elevated CO_2 and warming effects on CH_4 and N_2O exchange with the atmosphere vary widely among different systems (Dijkstra *et al*., 2012). Elevated CO_2 reduced CH_4 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 CH_4 uptake was associated with increased soil moisture impeding the supply of atmospheric CH_4 for oxidation by methanotrophs in the soil, and increasing CH_4 production by methanogens (Ineson *et al*., 1998; Phillips *et al*., 2001a). Elevated CO_2 increased N_2O 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 CO_2 could stimulate N_2O 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 CO_2 could reduce N availability thereby constraining N_2O production (Hungate *et al*., 1997; Mosier *et al*., 2002). Warming increased CH_4 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 CH_4 in the soil. Warming increased N_2O emission in urban lawns and a heath land (Bijoor *et al*., 2008; Larsen *et al*., 2011), decreased N_2O emission in a wheat field and an alpine meadow (Hantschel *et al*., 1995; Hu *et al*., 2010), and had no effect on N_2O 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 N_2O exchange. For instance, an increase in soil temperature could directly enhance the activity of nitrifiers and denitrifiers thereby stimulating N_2O 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 CH_4 and N_2O (Mosier *et al*., 2008). Because soil moisture is low in most of the year, production and uptake of CH_4 and N_2O in semiarid grasslands can be very different from more mesic environments. For instance, in mesic environments net CH_4 uptake is often negatively related to soil moisture because soil moisture reduces CH_4 diffusivity into the soil for oxidation by methanotrophs, or increases CH_4 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 CH_4 diffusivity or methanogen activity (von Fischer *et al*., 2009). Therefore, under dry conditions net CH_4 uptake can increase with increased soil moisture (Mosier *et al*., 2008; Dijkstra *et al*., 2011). In mesic and wet environments N_2O emissions can be high because intermediate to high levels of soil moisture are conducive to nitrification and denitrification, both of which contribute to N_2O emissions (Klemedtsson *et al*., 1988; Davidson, 1992). Net N_2O emission in mesic environments is sometimes restrained, however, because of N_2O consumption by denitrifiers (Holtgrieve *et al*., 2006; Chapuis-lardy *et al*., 2007). Consumption of N_2O has also been observed under dry conditions resulting in a net N_2O 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 N_2O diffusion from the atmosphere into the soil where under limited inorganic N supply from the soil, atmospheric N_2O is reduced to N_2 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 CH_4 and N_2O in these systems are largely mediated by changes in soil moisture. Previously we have shown that elevated CO_2 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 CH_4 uptake was strongly controlled by soil moisture, and elevated CO_2 and warming effects could largely be explained by treatment effects on soil moisture (Dijkstra *et al*., 2011). The drier soil conditions with warming reduced CH_4 uptake over the whole growing season. However, the increase in soil moisture under elevated CO_2 enhanced CH_4 uptake during dry periods of the growing season, but reduced CH_4 uptake during wet periods. When elevated CO_2 effects on CH_4 uptake were considered for the whole growing season, elevated CO_2 had no net effect. Interactive CO_2 × warming effects were also not observed during the first 2 years. Here, we present single and combined effects of elevated CO_2 and warming on CH_4 and N_2O 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 CO_2 would have no effect on CH_4 uptake, but that a decrease in soil moisture with warming would decrease CH_4 uptake, similar to what we found during the first 2 years. We further hypothesized that elevated CO_2 would have no effect on N_2O emission because of a tighter N cycle under elevated CO_2 (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 N_2O emission, and with limited N available, potentially turning this system into an N_2O sink. Because the decrease in net N_2O emission would offset the decrease in net CH_4 uptake with warming, combined CH_4 and N_2O fluxes in this grassland expressed in CO_2 equivalents would be insensitive to climate change.

Materials and methods

Site description and experimental design

We conducted our study in the Prairie Heating And CO_2 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 C_4 grass *Bouteloua gracilis* (H.B.K) Lag and the C_3 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 N_2O 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 CO_2 Enrichment technology (Miglietta *et al.*, 2001) to increase the atmospheric CO_2 concentration to 600 ppmv ($\pm$40 ppmv). The CO_2 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 CO_2 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 CO_2 and warming treatments were implemented in a full factorial design with five replicates of each of the treatment combinations (ct: ambient CO_2 and ambient temperature, cT: ambient CO_2 and elevated temperature, Ct: elevated CO_2 and ambient temperature, and CT: elevated CO_2 and elevated temperature). Detailed information about the site and CO_2 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 NH_4^+ and NO_3^- 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.

CH_4 and N_2O flux measurements

We measured CH_4 and N_2O 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 CH_4 and N_2O concentration on a gas chromatograph (GC) equipped with a flame ionization detector for CH_4 and an electron capture detector for N_2O (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 CH_4 and N_2O 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 CO_2 concentrations measured at the same time as CH_4 and N_2O did not show a clear increasing trend with time. We calculated cumulative fluxes of CH_4 (in mg C m^{-2}) and N_2O (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 CH_4 and N_2O flux measurements in the subplots without plants until June in 2008, we only calculated cumulative fluxes of CH_4 and N_2O in the subplots without plants for 2009, 2010 and 2011. We calculated the cumulative Global Warming Potential from CH_4 and N_2O (in g CO_2 eq. per m^2) by adding the cumulative GWP (Global Warming Potential) from CH_4 (cumulative flux of CH_4 in g CH_4 per m^2 multiplied by 25) and the cumulative GWP from N_2O (cumulative flux of N_2O in g N_2O per m^2 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 CH_4 and N_2O 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 CH_4 and N_2O. 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 CO_2 (ambient vs. elevated), temperature (no warming vs. warming, both between-subjects factors), year (2007–2011, within-subjects factor), and their interactive effects on cumulative CH_4, N_2O and GWP from CH_4 and N_2O. 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 CH_4, N_2O and GWP from CH_4 and N_2O among the different CO_2 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 CH_4 and N_2O fluxes to WFPS, soil temperature and plot greenness using data that were aggregated by date (i.e., averaged across the CO_2 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 CO_2 and warming treatments using analysis of covariance (ANCOVA) with the CO_2 and warming treatment as main effects and either WFPS, soil temperature and plot greenness as the covariate. Because CH_4 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 CH_4 or N_2O fluxes with WFPS, soil temperature, or plot greenness were altered by the CO_2 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 CH_4 throughout all five growing seasons (as indicated by negative fluxes). Variable CH_4 fluxes were observed within each season although CH_4 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 CH_4 uptake during the growing season was smallest in 2007 (90 mg C m^{-2} averaged across the CO_2 and warming treatments) and greatest in 2011 (130 mg C m^{-2}, Fig. 1). Elevated CO_2 increased cumulative CH_4 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 CH_4 uptake across all years ($P < 0.0001$) with an average decrease of 15%. Warming also significantly reduced cumulative CH_4 uptake without plants ($P = 0.01$), while elevated CO_2 had no effect on cumulative CH_4 uptake without plants. Cumulative CH_4 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 N_2O at those times. Cumulative fluxes of N_2O were also sometimes negative (N_2O uptake), particularly during the last 2 years (Fig. 2a). Across years there was no significant elevated CO_2 effect on cumulative N_2O, but during the last 2 years N_2O uptake decreased under elevated CO_2 causing a significant CO_2 × year interactive effect ($P = 0.002$). There was no warming effect on cumulative fluxes of N_2O. Cumulative fluxes of N_2O were much larger without than with plants, particularly in 2009 and 2011 (Fig. 2b). Elevated CO_2 significantly reduced the cumulative flux of N_2O 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 CO_2 (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

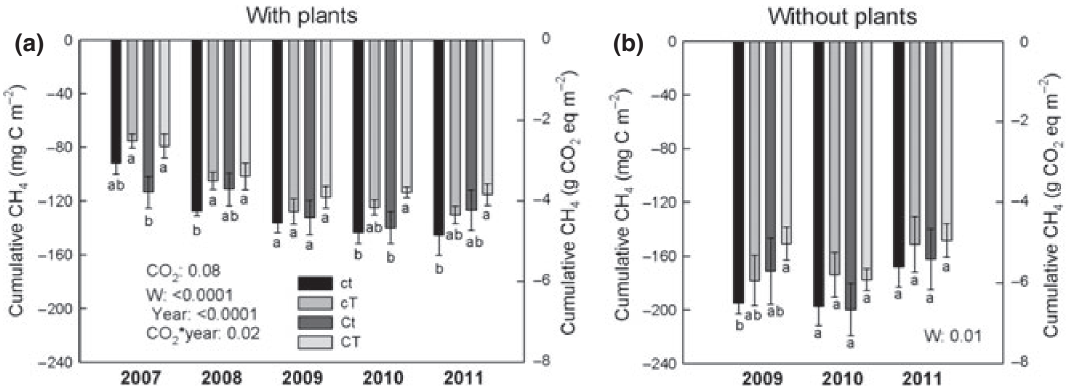

Fig. 1 Cumulative CH_4 in mg C m^{-2} (left *y*-axis) and in g CO_2 eq. per m^2 (right *y*-axis) in plots with (a) and without plants (b) in response to elevated CO_2 and warming. Negative values indicate sinks. Treatments: ct: ambient CO_2 and ambient temperature; cT: ambient CO_2 and elevated temperature; 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).

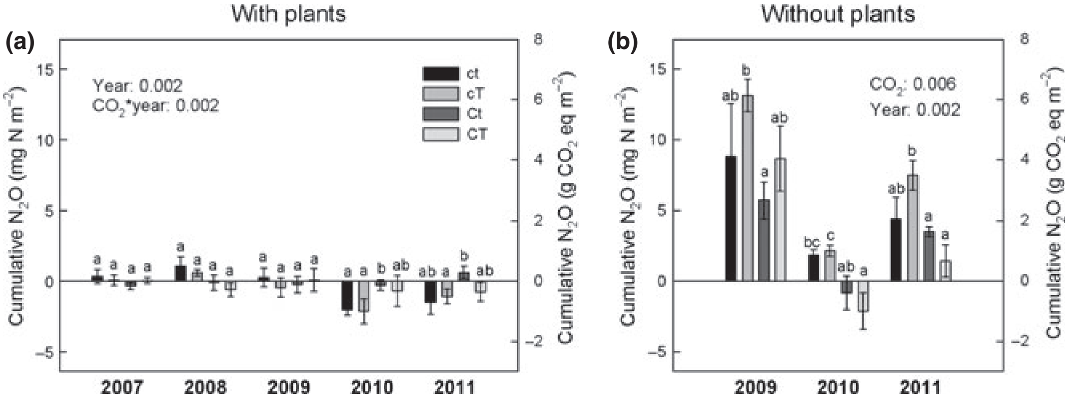

Fig. 2 Cumulative N_2O in mg N m^{-2} (left *y*-axis) and in g CO_2 eq. per m^2 (right *y*-axis) in plots with (a) and without plants (b) in response to elevated CO_2 and warming. Negative values indicate sinks. Treatments: ct: ambient CO_2 and ambient temperature; cT: ambient CO_2 and elevated temperature; 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 μg C m^{-2} 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 CO_2 and warming treatments measured during trace gas flux measurements

	WFPS (%)				Soil temp (°C)				Plot greenness (%)			
Year	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 *P*-values												
CO2	<**0.0001**				0.24				0.16			
W	<**0.0001**				<**0.0001**				0.32			
CO2 × W	0.74				*0.06*				0.21			
Year	0.35				<**0.0001**				0.92			
CO2 × year	0.37				0.87				0.51			
W × year	0.75				0.99				0.77			

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.

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

WFPS, when regressions were performed for each CO_2 and warming treatment (Fig. S3, Table S1). Interestingly, N_2O fluxes were significantly negatively related to soil temperature (Fig. 3d), while this relationship was not affected by the CO_2 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 N_2O fluxes, but was significantly correlated with CH_4 fluxes (Fig. 3e and f) without differences among the CO_2 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 CH_4 and N_2O (Fig. 4). Both elevated CO_2 and warming significantly reduced this sink ($P = 0.04$ for elevated CO_2 and $P = 0.002$ for warming). The reduced net sink of CH_4 and N_2O under elevated CO_2 increased with time (significant CO_2 × year interactive effect, $P = 0.0003$). Across years, elevated CO_2 and warming reduced the net sink for CH_4 and N_2O by 7.2% and 11%, respectively, with the largest reduction of 17% when elevated CO_2 and warming were combined.

Discussion

This grassland was a sink for CH_4 at all times during the growing season, while it was sometimes a source and sometimes a sink for N_2O (i.e., net N_2O uptake). When CH_4 and N_2O were combined in terms of CO_2 equivalents, this grassland was always a sink. Contrary to our hypothesis, both elevated CO_2 and warming reduced the net sink of CH_4 and N_2O in this grassland, and the largest net sink reductions occurred when elevated CO_2 and warming were combined (Fig. 4). Combined effects of elevated CO_2 and warming resulted in a significant decrease in the net sink of CH_4 and N_2O 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 CH_4 and N_2O.

CH_4

Both elevated CO_2 and warming reduced CH_4 uptake in this semiarid grassland in most of the years; CH_4 uptake in plots exposed to elevated CO_2 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 CH_4 uptake under elevated CO_2 is in contrast to observations in a similar semiarid grassland where CH_4 uptake was not affected by elevated

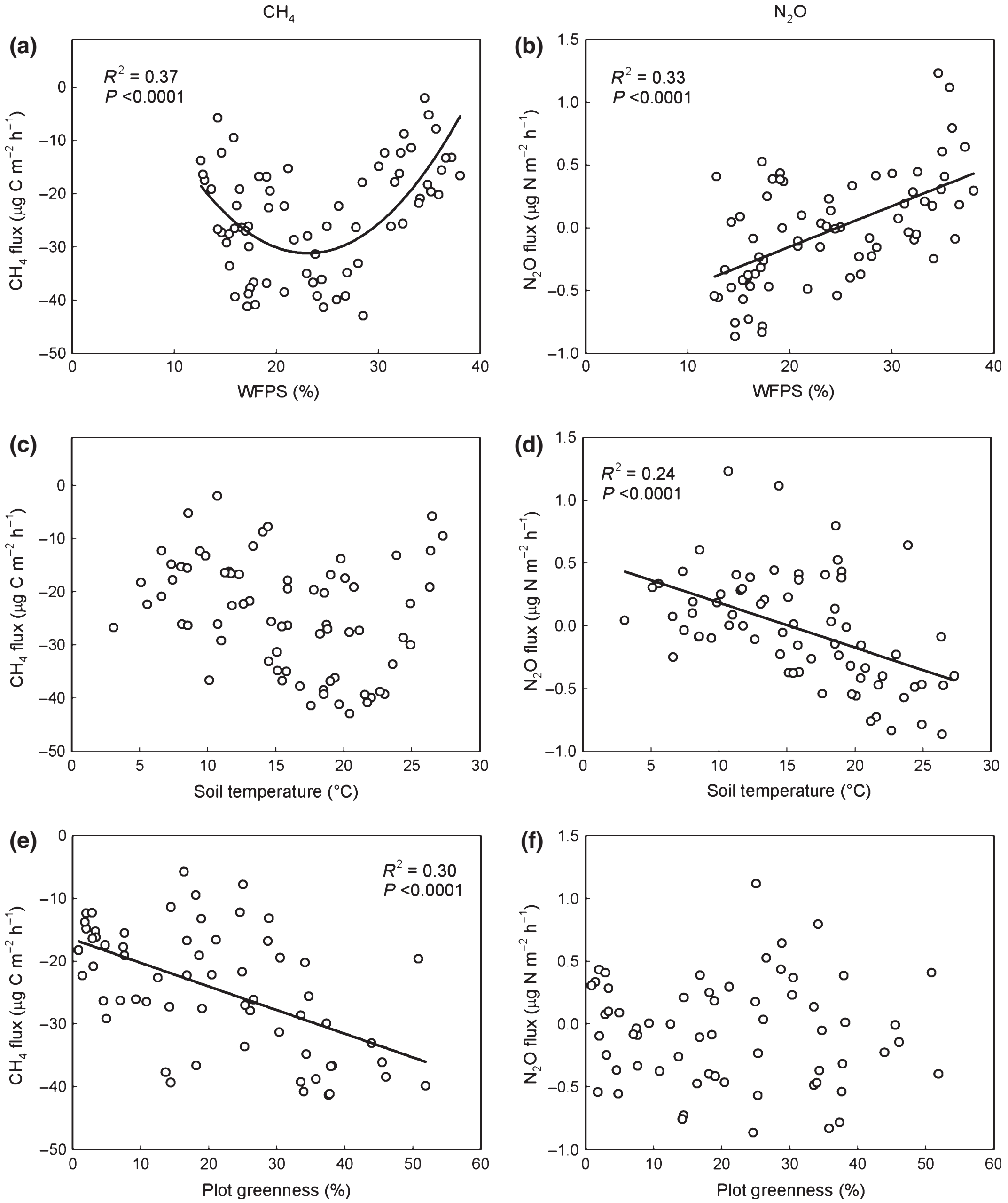

Fig. 3 Fluxes of CH_4 (a, c, e) and N_2O (b, d, f) as a function of water filled pore space (WFPS, a, b), soil temperature (c, d) and plot greenness (e, f). Each data point is the average CH_4 or N_2O flux, and average WFPS, soil temperature, or plot greenness measured across all treatments at a specific date between 2007 and 2011. Regression lines are only shown when significant ($P < 0.05$).

CO_2 (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 CH_4 uptake with warming in this semiarid grassland differed from studies in wetter environments where no or increased CH_4 uptake was found in response to warming (Peterjohn *et al.*, 1994; Sjögersten & Wookey, 2002; Blankinship *et al.*, 2010).

Treatment effects on CH_4 uptake may have been mediated by their effect on soil moisture. The CH_4 flux showed a bell-shaped relationship with WFPS with an optimum CH_4 uptake rate at 24% (Fig. 3a). Soil moisture consistently increased under elevated CO_2 and consistently decreased with warming (Table 2), which could have caused opposing effects on CH_4 uptake depending on what side of the curve the change in soil moisture occurred (Dijkstra *et al.*, 2011). A soil moisture increase under elevated CO_2 could increase CH_4 uptake (through stimulating methanotroph activity) when soils are relatively dry, or decrease CH_4 uptake (through

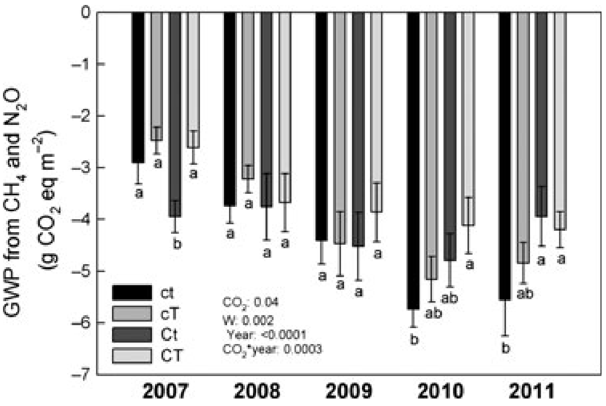

Fig. 4 Cumulative Global Warming Potential (GWP) in g CO_2 eq. per m^2 in plots with plants in response to elevated CO_2 and warming. Treatments: ct: ambient CO_2 and ambient temperature; cT: ambient CO_2 and elevated temperature; Ct: elevated CO_2 and ambient temperature; CT: elevated CO_2 and elevated 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).

decreasing CH_4 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 CH_4 uptake with elevated CO_2 and warming that are hard to explain by the treatment effects on soil moisture alone. For instance, when elevated CO_2 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 CO_2 and warming (CT plots) often showed the largest reduction in CH_4 uptake. The relationship between CH_4 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 CH_4 uptake under elevated CO_2 and warming.

Another driver that could have influenced treatment effects on CH_4 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 CH_4 uptake and soil temperature when the data were aggregated across all treatments (Fig. 3c), but found similar bell-shaped relationships as observed between CH_4 uptake and WFPS (Fig. S3). Because of the strong correlation between WFPS and soil temperature, the relationships between CH_4 uptake and soil temperature for each treatment may have been driven partially by soil moisture. Nevertheless, as with WFPS, the reduced CH_4 uptake with elevated CO_2 and warming combined are difficult to explain by treatment effects on soil temperature alone (note that elevated CO_2 had no effect on soil temperature, Table 2).

Treatment effects on N cycling may also have affected CH_4 uptake. Ammonium (NH_4^+) can suppress CH_4 oxidation in the soil because some of the CH_4 oxidizing microbes switch to oxidizing NH_4^+ with increased availability of NH_4^+ (Steudler *et al.*, 1989; Hanson & Hanson, 1996). Elevated CO_2 significantly increased NH_4^+ concentrations in our experiment (Carrillo *et al.*, 2012), which could have contributed to reduced CH_4 uptake that we observed in some years under elevated CO_2. It has also been suggested that soil nitrate (NO_3^-) could stimulate CH_4 uptake at low CH_4 concentrations due to shifts in the CH_4 oxidizing bacteria community (Jang *et al.*, 2011). We observed a significant reduction in soil NO_3^- under elevated CO_2 (Carrillo *et al.*, 2012), suggesting that this too may have contributed to the reduced CH_4 uptake under elevated CO_2, particularly with a progressively tighter N cycle under elevated CO_2 (Dijkstra *et al.*, 2010). Soil NO_3^- was much higher in the subplots without plants (Carrillo *et al.*, 2012), suggesting that soil NO_3^- may also have played a role in the higher CH_4 uptake without plants (Fig. 1).

N_2O

The N_2O fluxes in this grassland were small (cumulative N_2O never exceeded 2 mg N m^{-2}), while both production and uptake of N_2O occurred (Fig. 2a). Because of the small fluxes, elevated CO_2 and warming had no effect on N_2O across all years. However, elevated CO_2 significantly reduced the N_2O sink in the last 2 years. This is in contrast to studies conducted in a similar semiarid grassland where no elevated CO_2 effects on N_2O fluxes were observed (Mosier *et al.*, 2002). In temperate grasslands that received N fertilization elevated CO_2 increased N_2O 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 CO_2 may have reduced C constraints on denitrification thereby causing the increase in N_2O 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 CO_2 may not increase N_2O emission. At our site, N_2O 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 N_2O emission in these plots (Fig. 3). We found no relationship between plot greenness and N_2O 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 N_2O exchange suggests that seasonal variation in plant input of labile C had no effect on N_2O 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 N_2O in the plots with plants, particularly during the last 2 years, suggesting low levels of available N in these plots. The process of N_2O uptake under dry conditions is still little understood (Chapuis-lardy *et al.*, 2007), but dry soil conditions may have enhanced diffusion of atmospheric N_2O into the soil where in the absence of NO_3^-, N_2O was used as the electron acceptor for denitrification (Stewart *et al.*, 2012). The positive relationship between WFPS and N_2O flux (Fig. 3b) suggests that elevated CO_2–induced increases in soil moisture may have reduced the diffusion of N_2O into the soil and therefore reduced N_2O uptake in the last 2 years.

Nitrous oxide fluxes were negatively related to soil temperature (Fig. 3d). Often N_2O emissions increase with increased temperature (Dobbie & Smith, 2001; Mosier *et al.*, 2008). Most likely, N_2O 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 N_2O flux. Possibly, the direct stimulatory effect of increased soil temperature with warming on the N_2O 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 CO_2 and warming significantly increased the cumulative GWP from CH_4 and N_2O combined. The largest increase in the cumulative GWP occurred when elevated CO_2 and warming were combined, resulting in a significant increase in 2010 and 2011 of 1.6 and 1.4 g CO_2 eq. per m^2, 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 CO_2 and warming; 61% and 74% of the total increase in cumulative GWP in 2010 and 2011, respectively, was caused by reductions in CH_4 uptake. We hypothesized that elevated CO_2 and warming would have opposing effects on the cumulative GWP from CH_4 and N_2O because of their opposing effects on soil moisture (Morgan *et al.*, 2011). Because CH_4 was the dominant contributor to the cumulative GWP, and because average soil moisture contents were on average near optimum CH_4 uptake rates in control plots, both elevated CO_2–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 CO_2 and warming on the net sink of CH_4 and N_2O in this system.

Our results show that, when expressed in CO_2–equivalents, both elevated CO_2 and warming reduced the net sink of CH_4 and N_2O in this semiarid grassland. Elevated CO_2 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 km^2, Bailey, 1979), then between 22 and 26 Tg CO_2 eq. per yr less will be taken up as CH_4 and N_2O in response to elevated CO_2 and temperature at levels that are predicted for the mid- to end of this century. Combined effects of elevated CO_2 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 CH_4 (a) and N_2O (b) during the growing season in 2007–2011 in response to elevated CO_2 and warming. Negative values indicate fluxes from the atmosphere to the soil. 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. 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 CO_2 and warming. 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. Error bars indicate Tukey's HSD.

Figure S3. Fluxes of CH_4 (a, c, e) and N_2O (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 CH_4 or N_2O 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 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. 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 CH_4 and N_2O fluxes for each treatment (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).