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Effects of interactive global changes on methane uptake in an annual grassland

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[1]   The future size of the terrestrial methane (CH_4) sink of upland soils remains uncertain, along with potential feedbacks to global warming. Much of the uncertainty lies in our lack of knowledge about potential interactive effects of multiple simultaneous global environmental changes. Field CH_4 fluxes and laboratory soil CH_4 consumption were measured five times during 3 consecutive years in a California annual grassland exposed to 8 years of the full factorial combination of ambient and elevated levels of precipitation, temperature, atmospheric CO_2 concentration, and N deposition. Across all sampling dates and treatments, increased precipitation caused a 61% reduction in field CH_4 uptake. However, this reduction depended quantitatively on other global change factors. Higher precipitation reduced CH_4 uptake when temperature or N deposition (but not both) increased, and under elevated CO_2 but only late in the growing season. Warming alone also decreased CH_4 uptake early in the growing season, which was partly explained by a decrease in laboratory soil CH_4 consumption. Atmospheric CH_4 models likely need to incorporate nonadditive interactions, seasonal interactions, and interactions between methanotrophy and methanogenesis. Despite the complexity of interactions we observed in this multifactor experiment, the outcome agrees with results from single-factor experiments: an increased terrestrial CH_4 sink appears less likely than a reduced one.

1. Introduction

[2]   Methanotrophic bacteria in upland soils remove about 22 teragrams (Tg = 10^{12} g) of the greenhouse gas methane (CH_4) from the atmosphere per year [*Dutaur and Verchot*, 2007]. The annual global CH_4 budget is currently balanced within 1 Tg [*Denman et al.*, 2007], so future reductions in terrestrial CH_4 uptake may feedback to reinforce global warming. Although many studies have investigated the response of soil CH_4 uptake to individual components of global environmental change, including altered precipitation regime, warming, rising atmospheric CO_2 concentration, and increased atmospheric nitrogen deposition, few studies have investigated responses to expected combinations of multiple components of global change. Do simultaneous global changes elicit quantitatively important interactive responses in soil CH_4 uptake that could be incorporated into atmospheric models?

[3]   Most single-factor manipulations of precipitation, temperature, atmospheric CO_2, and N deposition find either a reduction in terrestrial methane consumption or no change

(Table 1). Reductions in CH_4 consumption associated with increased precipitation and elevated CO_2 are often attributed to increased soil moisture [*Castro et al.*, 1994a; *Billings et al.*, 2000; *Borken et al.*, 2000; *Phillips et al.*, 2001; *Davidson et al.*, 2004]. Higher soil moisture content increases resistance to atmospheric CH_4 transport (i.e., decreases rates of diffusion) and can induce substrate (i.e., CH_4) limitation for methanotrophic organisms [*Koschorreck and Conrad*, 1993; *Striegl*, 1993; *Bowden et al.*, 1998]. Reductions caused by increased N deposition are attributed to ammonium [*Adamsen and King*, 1993; *King and Schnell*, 1994] and nitrite inhibition of methane monooxygenase [*King and Schnell*, 1994; *Wang and Ineson*, 2003], the mechanisms of which are not completely understood, and are more likely to occur with higher rates of N deposition [*Bradford et al.*, 2001]. Effects of warming on field CH_4 flux are less studied. Both multiyear warming experiments find no effect [*Torn and Harte*, 1996; *Rustad and Fernandez*, 1998]. Methanogenesis generally increases with increasing temperature and is more responsive to temperature than methanotrophy [*Conrad*, 1996; *Le Mer and Roger*, 2001]. Collectively, results from single-factor global change experiments predict either no change in the future strength of the terrestrial CH_4 sink or a weaker sink that may act as a self-reinforcing feedback to global warming. The exception to this global pattern will occur in ecosystems if diffusion increases as a result of lower precipitation [*Billings et al.*, 2000; *Borken et al.*, 2000, 2006;

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Table 1. A Review of Effects of Individual and Interactive Global Change Treatments on Soil Methane Consumption[a]

Global Change Treatment	Reference	Ecosystem Type	Duration of Treatment (years)	Effect on CH$_4$ Consumption (%)
Precip.	*Davidson et al.* [2004]	tropical forest	4.2	−79
Precip.	*Billings et al.* [2000]	boreal forest (dry)	7.3	−54
Precip.	*Borken et al.* [2000]	coniferous forest	2.0	−46
Precip.	*Borken et al.* [2006]	deciduous forest	1.0	−6
Precip.	*Billings et al.* [2000]	boreal forest (wet)	7.3	0
Temp.	*Torn and Harte* [1996]	alpine meadow	3.0	0
Temp.	*Rustad and Fernandez* [1998]	coniferous forest	3.0	0
Temp.	*Peterjohn et al.* [1994]	deciduous forest	0.5	+14
Temp.	*Hart* [2006]	coniferous forest	1.1	+38
CO$_2$	*Ineson et al.* [1998]	grassland	2.0	−67
CO$_2$	*Phillips et al.* [2001]	coniferous forest	3.0	−47
CO$_2$	*Mosier et al.* [2002]	grassland	3.5	0
CO$_2$	*Kanerva et al.* [2007]	grassland	2.0	0
CO$_2$	*Mosier et al.* [2003]	grassland	5.0	+10
N	*Castro et al.* [1994b]	coniferous forest	4.0	−80
N	*Castro et al.* [1995]	coniferous forest	6.0	−62
N	*Sitaula et al.* [1995]	coniferous forest	1.0	−38
N	*Castro et al.* [1995]	deciduous forest	6.0	−36
N	*Chan et al.* [2005]	deciduous forest	8.0	−35
N	*Mosier et al.* [1991]	grassland	14.0	−34
N	*Steudler et al.* [1989]	deciduous forest	0.5	−33
N	*Willison et al.* [1995]	grassland	138.0	0
N	*Börjesson and Nohrstedt* [1998]	coniferous forest	27.0	0
N	*Börjesson and Nohrstedt* [1998]	coniferous forest	23.0	0
N	*Steinkamp et al.* [2001]	coniferous forest	3.0	0
N	*Bradford et al.* [2001]	deciduous forest	1.0	0
CO$_2$ × N	*Ambus and Robertson* [1999]	deciduous forest (ambient N)	2.0	−67 (CO$_2$ effect)
CO$_2$ × N	*Ambus and Robertson* [1999]	deciduous forest (increased N)	2.0	0 (CO$_2$ effect)

[a]Precip., increased precipitation; Temp., increased temperature; CO$_2$, increased atmospheric carbon dioxide concentration; N, increased atmospheric nitrogen deposition; % effect = 100% * (treatment − control)/control; "0" indicates no significant effect. For precipitation treatments, effect sizes were standardized so that the "treatment" reflected higher precipitation and the "control" reflected lower precipitation.

Davidson et al., 2004] or warming-induced soil drying [*Peterjohn et al.*, 1994; *Hart*, 2006].

[4] It is unknown whether effects of multiple global change factors on terrestrial CH$_4$ uptake are additive, cancel each other out, or enhance the individual effects. We are aware of only one other study that has tested effects of interactive global changes on CH$_4$ fluxes. *Ambus and Robertson* [1999] included elevated CO$_2$ and N deposition in a full factorial design for 2 years and found that elevated CO$_2$ caused a reduction in CH$_4$ uptake under ambient nitrogen, but not under increased nitrogen. These results, and interactions found with ecosystem carbon and water cycling [*Luo et al.*, 2008], suggest that predicting the response of CH$_4$ uptake to potential interactive effects of simultaneous global changes is not straightforward.

[5] Here we report on the effects of 6–8 years of four global change factors on methane flux in a California annual grassland. Field CH$_4$ fluxes were measured five times in 3 consecutive years after exposure to every combination of ambient and elevated levels of precipitation, temperature, atmospheric CO$_2$ concentration, and N deposition. Our sampling strategy was designed to test for main effects and their interactions, but specifically not meant to construct quantitative annual budgets. Thus, we focused on sampling all treatment combinations exhaustively, rather than sampling individual treatments at frequencies required to construct annual budgets. Soil CH$_4$ consumption was measured at optimal moisture in laboratory, at the same times we measured CH$_4$ fluxes in the field. Lab incubations were compared with field fluxes to separate the direct effects of

treatment-induced changes in soil microclimate from the indirect effects of changes in methanotrophic activity.

2. Methods

2.1. Site Description

[6] The Jasper Ridge Global Change Experiment (JRGCE) is located at Stanford University's Jasper Ridge Biological Preserve in central California (37°24′N, 122°13′W, elevation 150 m). The experiment was established in a moderately fertile annual grassland (loam soil texture derived from sandstone), dominated by annual grasses (*Avena barbata* and *Bromus hordeaceus*) and forbs (*Geranium dissectum* and *Erodium botrys*), and experiences a Mediterranean-type climate with a mean annual temperature of 14°C and 80% of mean annual precipitation (652 mm) falling between November and March. Field and laboratory CH$_4$ fluxes were measured on five sampling dates: late in the growing season in spring 2004, 2005, and 2006; early in the growing season in fall 2004; and the middle of the growing season in winter 2005.

[7] JRGCE treatments began in November 1998 with all combinations of ambient and elevated levels of four simulated global changes in full factorial design [*Shaw et al.*, 2002; *Zavaleta et al.*, 2003]. Briefly, 32 experimental plots (2 m diameter) were arranged in a split-plot design, by dividing each plot into four 0.78 m^2 quadrants. Treatments at the plot level included ambient and elevated atmospheric CO$_2$ concentration using FACE emitter rings (ambient and ambient + 380 μmol mol^{-1}) and temperature using infrared lamps (ambient and ambient + 80 W m^{-2}, resulting in

approximately 1.0°C soil surface warming) [*Rillig et al.*, 2002]. Treatments randomized within plots included ambient and elevated rainfall using a spray/drip system (ambient and ambient + 50% per event + 3 week elongation of growing season) and simulated atmospheric nitrate (NO$_3^-$) deposition using slow-release fertilizer (ambient and ambient + 7 g N-Ca(NO$_3$)$_2$ m^{-2} yr^{-1}). There were eight replicates of all 16 combinations of CO$_2$, temperature, precipitation, and N treatments, but two replicates were accidentally burned in 2003 [*Henry et al.*, 2006] and excluded from this analysis ($n = 6$ for this analysis).

2.2. Field CH$_4$ Flux

[8] We measured field CH$_4$ flux in all 96 experimental quadrants between 9:00 A.M. and 4:00 P.M. on 30 April 2004, 13 October 2004, 25 February 2005, 21 April 2005, and 3 May 2006 using the static chamber approach [*Hutchinson and Mosier*, 1981]. Plots were sampled by block to avoid confounding time (i.e., afternoon warming) with treatment. Chambers were constructed from a 10.2 cm-diameter PVC pipe closed at one end with a PVC cap, with a total headspace volume of 1.8 L. The bottom 3 cm of each chamber was filed to allow the chamber to slide smoothly into a permanent PVC ring in each quadrant, and dense closed-cell foam rings created an airtight seal between the chamber and the ring. With the chamber in place, headspace air (15 ml) was sampled through a rubber septum installed at the top of each chamber using a 20 ml nylon syringe. We collected three additional headspace gas samples at 15 min intervals to determine net rates of CH$_4$ uptake or emission. Field CH$_4$ fluxes were expressed as mg CH$_4$-C m^{-2} d^{-1}.

2.3. Laboratory CH$_4$ Flux

[9] Soil cores (0–5 cm deep) were collected and homogenized within 24 h after measurement of field CH$_4$ flux, and large roots and rocks (>2 mm diameter) were removed by hand. Gravimetric water content (g H$_2$O g^{-1} soil) was determined beforehand by oven-drying soil subsamples at 105°C. After overnight storage in sealed plastic bags at room temperature, soils (15 g) were placed in 250 ml screw-top glass serum bottles. Soils were first air-dried for 48 h to water contents below 35% water-holding capacity (WHC). About 2 h before the incubation started (within 72 h of soil collection), deionized water was added with a spray bottle to standardize samples at 35% WHC (about 21% gravimetric water content for this grassland), an assumed optimum [*Gulledge and Schimel*, 1998]. Soil WHC was determined as the mass of water absorbed after draining saturated, sieved soil for 2 h over Grade 2 filter paper (15 g air-dried soil, $n = 8$, WHC = 0.61 ± 0.06 g H$_2$O g^{-1} soil).

[10] After standardizing soil moisture, incubation bottles were sealed with screw caps lined with airtight Teflon®-silicone septa. To reduce substrate limitation, CH$_4$ concentrations inside the bottles were increased to about 10× ambient concentrations (18 ppmv) by adding 1 ml of CH$_4$ in air at a concentration of 4800 ppmv. A CH$_4$ concentration of 18 ppmv is high enough to reduce substrate limitation to high-affinity CH$_4$-oxidizing bacteria, which have half-saturation constants (K$_m$) between 10 and 80 ppm in temperate meadow and forest soils [*Bender and Conrad*, 1993; *Czepiel et al.*, 1995; *Benstead and King*, 1997; *Gulledge et al.*, 2004], but low enough to have little effect on low-

affinity CH$_4$-oxidizing bacteria [*Bender and Conrad*, 1992]. Optimal moisture and elevated CH$_4$ concentrations enhanced rates of CH$_4$ oxidation relative to CH$_4$ production, thereby increasing the possibility of observing a treatment-induced change in methanotrophic activity. As an assay of methanotrophic enzyme activity and population size, we expected a positive correlation between laboratory CH$_4$ consumption and field CH$_4$ uptake.

[11] The laboratory incubations ran for 48 h in the dark in a Revco BOD-50A incubator at 25°C. Methane fluxes were calculated from three 15 ml headspace samples taken 30–60 min, 24 h, and 48 h after sealing bottles and adding CH$_4$. Soil CH$_4$ flux was expressed per g soil dry weight. Soil dry weight was corrected for the presence of stones and roots by sieving soil through a 2 mm mesh sieve at the end of the incubations.

2.4. Gas Sample Storage, Analysis, and Flux Calculations

[12] Gas samples were immediately injected into sealed preevacuated 12 ml glass vials with capped 20 mm butyl rubber stoppers that are known to be airtight for at least 10 weeks (data not shown), and were overpressurized (+3 ml) so that any leaks would be evident when vials were analyzed. Gas samples were analyzed in Flagstaff, AZ within 4 weeks on a gas chromatograph system (Agilent 6890 GC System, Palo Alto, CA, USA) using Haysep Q 60/80 and Porapack Q 60/80 packed columns equipped with a flame-ionization detector with methanizer for determining CH$_4$ concentrations. Field CH$_4$ fluxes were calculated using linear regression analysis of concentrations over time (mg CH$_4$-C m^{-2} d^{-1}). The linear model was the best fit for changes in CH$_4$ concentrations over the 45 min sampling period, with an average R^2 value of 0.75. Laboratory CH$_4$ fluxes were calculated from differences in concentrations over 48 h, and converted to ng CH$_4$-C g^{-1} soil h^{-1}, after correction for pressure differences between Flagstaff (elevation 2106 m) and Palo Alto (150 m). Net CH$_4$ emission was not observed in the laboratory, but occurred often in the field. Control bottles with distilled water (i.e., no soil) showed no significant changes in CH$_4$ concentrations during the incubation period due to leakage or water absorption (data not shown). Soil respiration (μg CO$_2$-C g^{-1} soil h^{-1}) was measured at standardized moisture and temperature in the same incubations used to measure laboratory CH$_4$ consumption on the same gas chromatograph system.

2.5. Statistical Analysis

[13] Field CH$_4$ fluxes, laboratory CH$_4$ fluxes, laboratory CO$_2$ production rates, and gravimetric soil water contents (0–5 cm) from the five sampling dates were analyzed with the MIXED procedure in SAS 9.1 (SAS Institute, Cary, NC) using a repeated-measures split-plot analysis of variance (ANOVA) that included: (1) CO$_2$ and temperature as between-plot factors; (2) precipitation and nitrogen as within-plot factors; (3) interaction terms for all treatment combinations; and (4) repeated-measures time analysis. Means were calculated as least squares means, and the denominator degrees of freedom of subplot effects were determined using the Kenward-Roger technique. Nonequal variances in laboratory CH$_4$ fluxes were corrected by square-root transformation. Relative treatment effect sizes were calculated as: %

Table 2. Linear Regression Analysis of Field CH$_4$ Flux[a]

Predictor Variable	All Dates	April 2004	October 2004	February 2005	April 2005	May 2006
Laboratory CH$_4$ Consumption (0–5 cm)						
F ratio	0.53	0.04	0.68	1.02	0.05	0.43
P value	0.46	0.85	0.41	0.25	0.83	0.52
R^2 value	<0.01	<0.01	0.01	0.02	<0.01	0.01
Soil Water Content (0–5 cm)						
F ratio	0.25	0.37	1.06	0.41	0.53	0.85
P value	0.62	0.55	0.31	0.52	0.47	0.36
R^2 value	<0.01	<0.01	0.01	<0.01	0.01	0.01
Soil Water Content (15–30 cm)						
F ratio	4.42	0.13	1.13	0.76	2.53	2.85
P value	**0.03**	0.72	0.29	0.39	0.12	0.09
R^2 value	0.01 (+)	<0.01	0.01	0.01	0.03	0.03
Soil Water Content (30–45 cm)						
F ratio	3.73	0.17	0.01	1.28	1.66	1.84
P value	**0.05**	0.68	0.93	0.29	0.20	0.17
R^2 value	0.01 (+)	<0.01	<0.01	0.01	0.02	0.02

[a]With laboratory soil CH$_4$ consumption and gravimetric (0–5 cm deep) and volumetric soil water content (15–30 cm and 30–45 cm deep using time domain reflectometry) across all sampling dates and within individual sampling dates. Bold indicates significant at α level of 0.05. Parentheses indicate direction of slope. The denominator degrees of freedom equal 448, 89, 84, 88, 92, and 87 for all dates, April 2004, October 2004, February 2005, April 2005, and May 2006, respectively.

effect = 100% * (elevated mean − ambient mean)/ambient mean.

3. Results

3.1. Relationships Between Field CH$_4$ Flux, Laboratory CH$_4$ Flux, and Soil Water Content

[14] There was no significant correlation between field and laboratory CH$_4$ flux on any sampling date (Table 2). Increased precipitation, temperature, and N deposition did not affect soil water content (0–5 cm) on our sampling dates, but elevated CO$_2$ significantly increased soil water content later in the growing season in April 2004 and May 2006 (data not shown). There was no correlation between soil water content and field CH$_4$ flux across or within sampling dates. Across sampling dates, there was a significant positive correlation between field CH$_4$ flux and water content in deeper soils (15–45 cm), with lower net CH$_4$ uptake associated with higher soil water content at depth.

3.2. Temporal Differences in CH$_4$ Flux

[15] Field and laboratory methane fluxes were strongly dependent on sampling date (Table 3). Across all treatments, the mean field flux exhibited the highest rates of CH$_4$ uptake in April 2004 (−0.45 ± 0.14 mg CH$_4$-C m^{-2} d^{-1}), significantly lower rates in February 2005 (−0.13 ± 0.08), and rates that were not significantly different from zero in October 2004, April 2005, and May 2006 (−0.15 ± 0.20, −0.06 ± 0.09, and +0.03 ± 0.13, respectively). A greater percentage of individual experimental quadrants showed net CH$_4$ emission (i.e., linear rates of increase of CH$_4$ concentration) on later sampling dates, which was consistent with the overall increase in ambient precipitation during the 3 years of sampling (Figure 1). In the laboratory, there was more CH$_4$ consumption in April 2004, April 2005, and May 2006 (−0.65 ± 0.02, −0.51 ± 0.02, and −0.65 ± 0.02 ng CH$_4$-C g^{-1} soil h^{-1}, respectively) than in October 2004 and February

2005 (−0.20 ± 0.03 and −0.24 ± 0.04, respectively). Laboratory incubations always showed net CH$_4$ uptake.

3.3. Response of Field CH$_4$ Flux to Global Change Treatments

[16] Increased precipitation was the only single-factor treatment that consistently reduced field CH$_4$ uptake across sampling dates (Table 2 and Figure 2). Across sampling dates and treatments, increased precipitation caused a 61% reduction in uptake from −0.22 ± 0.09 to −0.08 ± 0.08 mg CH$_4$-C m^{-2} d^{-1}. Precipitation exhibited significant interactions with temperature and N deposition (Figure 3); and with elevated CO$_2$ (Figure 4). The reduction in CH$_4$ uptake under increased precipitation did not depend on sampling date and was more pronounced in the presence of either increased N deposition or warming (effect sizes = −93% and −98%, respectively), and less pronounced in the absence or presence of both N deposition and warming (−24% and −30%, respectively). The interaction between precipitation and CO$_2$ was dependent on sampling date. Early and in the middle of the growing season (October and February), increased precipitation caused a reduction in CH$_4$ uptake under ambient CO$_2$ (from net uptake to zero uptake) and had no effect under elevated CO$_2$. At the end of the growing season (April and May), increased precipitation caused a reduction in CH$_4$ uptake under elevated CO$_2$ (from net uptake to zero uptake) and had no effect under ambient CO$_2$. The effect of the warming treatment also depended on sampling date. Warming reduced CH$_4$ uptake earlier in the growing season (from net uptake to zero uptake), but had no effect later in the growing season (Figure 5).

3.4. Response of Laboratory CH$_4$ Consumption to Global Change Treatments

[17] Variation in soil CH$_4$ consumption in the laboratory was primarily driven by sampling date. There was not a significant effect of increased precipitation, CO$_2$ concentration, or N deposition on laboratory CH$_4$ consumption.

Table 3. Repeated-Measures Split-Plot ANOVA Results[a]

Treatment	Field CH₄ Consumption		Laboratory CH₄ Consumption	
	Percent Effect	P Value (F Statistic)	Percent Effect	P Value (F Statistic)
Main-plot effects				
T	−42	0.21 (1.73)	+13	0.14 (2.42)
C	−14	0.76 (0.10)	−4	0.73 (0.12)
T × C		0.52 (0.42)		0.69 (0.17)
Subplot effects				
R	**−61**	**0.03 (4.88)**	+8	0.35 (1.30)
N	−15	0.76 (0.09)	+6	0.37 (0.80)
T × R		0.76 (0.09)		0.45 (0.58)
T × N		0.20 (1.65)		0.98 (<0.01)
C × R		0.47 (0.53)		0.78 (0.08)
C × N		0.63 (0.24)		0.63 (0.23)
R × N		0.89 (0.02)		0.13 (2.28)
T × C × R		0.10 (2.66)		0.25 (1.32)
T × C × N		0.33 (0.96)		0.68 (0.17)
T × R × N		**0.05 (3.24)**		0.51 (0.43)
C × R × N		0.59 (0.29)		0.52 (0.41)
T × C × R × N		0.43 (0.61)		0.56 (0.34)
Time effects		**<0.0001 (7.82)**		**<0.0001 (175.10)**
T × date		**0.04 (2.52)**		**0.01 (3.28)**
C × date		0.64 (0.64)		0.59 (0.71)
R × date		0.99 (0.06)		0.20 (1.51)
N × date		0.60 (0.68)		0.73 (0.50)
T × C × date		0.96 (0.17)		0.99 (0.06)
T × R × date		0.55 (0.76)		0.82 (0.38)
T × N × date		0.63 (0.65)		0.93 (0.22)
C × R × date		**0.02 (3.11)**		0.77 (0.45)
C × N × date		0.91 (0.25)		0.79 (0.43)
R × N × date		0.60 (0.68)		0.32 (1.17)
T × C × R × date		0.87 (0.31)		0.09 (2.40)
T × C × N × date		0.97 (0.12)		0.63 (0.65)
T × R × N × date		0.15 (1.64)		0.77 (0.46)
C × R × N × date		0.60 (0.69)		0.96 (0.15)
T × C × R × N × date		0.34 (1.12)		0.54 (0.77)

[a]Here $\alpha = 0.05$, indicated in bold. Single-factor effect sizes (% effect = 100% * (treatment − control)/control) for field and laboratory CH₄ flux in the Jasper Ridge Global Change Experiment on 30 April 2004, 13 October 2004, 25 February 2005, 21 April 2005, and 3 May 2006. T, increased temperature; C, elevated atmospheric CO_2; R, increased rainfall; N, increased nitrate deposition. Effect sizes are in terms of CH₄ consumption. A negative effect size indicates a reduction in net CH₄ consumption and a more positive CH₄ flux, whereas a positive effect size indicates the opposite. The numerator degrees of freedom equal 1 for main-plot and subplot effects and 4 for time effects. The denominator degrees of freedom equal 15 for main-plot effects. The denominator degrees of freedom for subplot and time effects equal 361 and 369 for field and laboratory CH₄ fluxes, respectively.
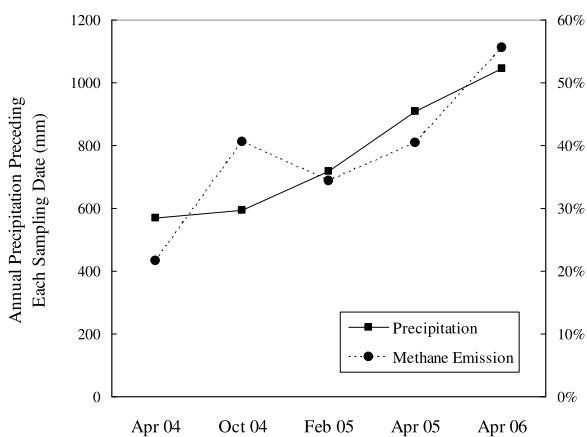

Figure 1. Total ambient precipitation received at the Jasper Ridge Global Change Experiment during the 365 days preceding each sampling date and the percentage of the 96 experimental quadrants in which net CH₄ emission was observed on each sampling date.
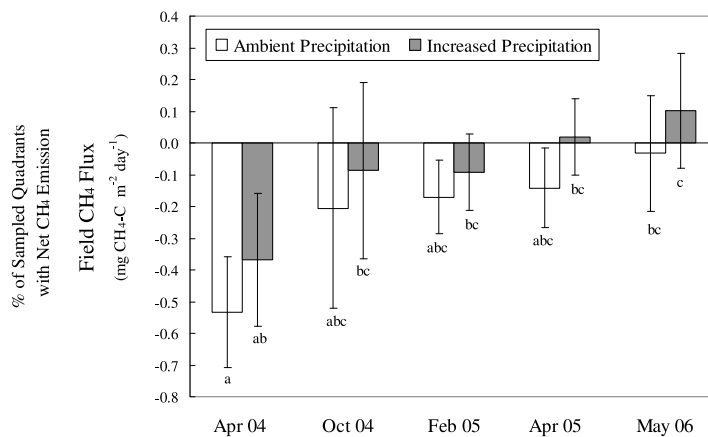

Figure 2. Reduced rates of field CH₄ consumption associated with increased precipitation (mean ±95% confidence interval; letters indicate significant differences in Tukey's HSD test; negative fluxes indicate net CH₄ consumption; positive fluxes indicate net CH₄ emission).
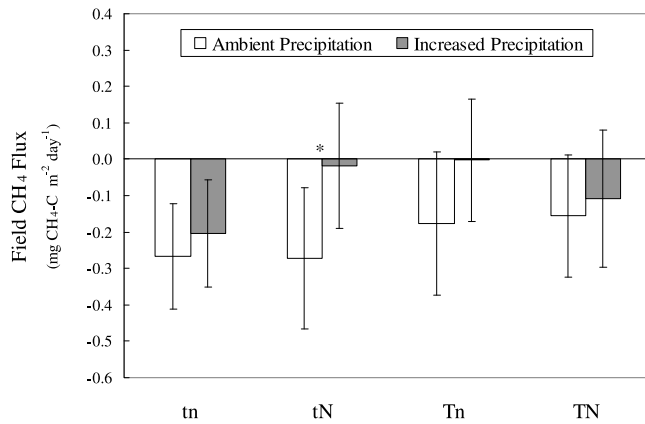
Figure 3. Response of the field CH_4 flux (mean ±95% confidence interval) to a significant three-way interaction between the precipitation, temperature, and N treatments that was independent of sampling date (asterisk indicates significant precipitation effect in a post-hoc one-way ANOVA at an alpha level of 0.05; t, ambient temperature; T, increased temperature; n, ambient nitrogen deposition; N, increased nitrogen deposition).

However, there was a significant effect of increased temperature that depended on sampling date. Warming caused a 32% reduction in CH_4 consumption earlier in the growing season (from -0.24 ± 0.04 to -0.16 ± 0.03 ng CH_4-C g^{-1} soil h^{-1}), but had no effect later in the growing season (Figure 6). Soil respiration was measured simultaneously in the same soil incubations and was about three times higher on sampling dates earlier in the growing season (data not shown). There was a significant positive correlation between laboratory CH_4 and CO_2 fluxes ($P < 0.0001$, $R^2 = 0.37$),
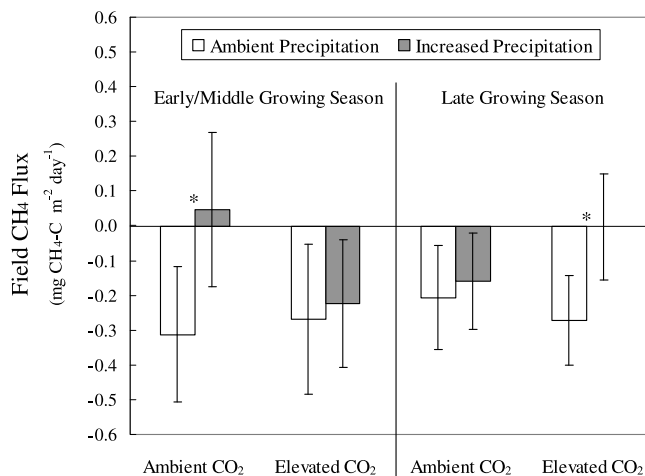

Figure 4. Response of the field CH_4 flux (mean ±95% confidence interval) to a significant two-way interaction between precipitation and CO_2 concentration that was dependent on sampling date (asterisk indicates significant precipitation effect in a post-hoc one-way ANOVA at an alpha level of 0.05; early and middle growing season is October 2004 and February 2005; late growing season is April 2004, April 2005, and May 2006).
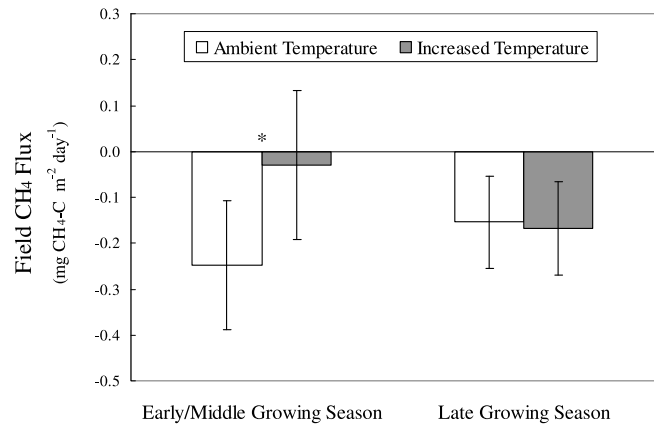

Figure 5. Season-dependent response of the field CH_4 flux (mean ±95% confidence interval) to warming (asterisk indicates significant precipitation effect in a post-hoc one-way ANOVA at an alpha level of 0.05; early and middle growing season is October 2004 and February 2005; late growing season is April 2004, April 2005, and May 2006).

with high CO_2 production associated with low CH_4 consumption.

4. Discussion

[18] Precipitation was the global change factor that had the greatest influence on methane flux. This effect was modified quantitatively by interactions with other global change factors. The overall 61% reduction in field CH_4 uptake under increased precipitation was similar in direction and magnitude to forest ecosystems [*Billings et al.*, 2000; *Borken et al.*, 2000; *Davidson et al.*, 2004]. This response, however, disappeared and reappeared in our grassland depending on temperature, N deposition, and atmospheric CO_2 concentration.

[19] The effect of precipitation on CH_4 flux was modulated by temperature and N deposition. Under ambient
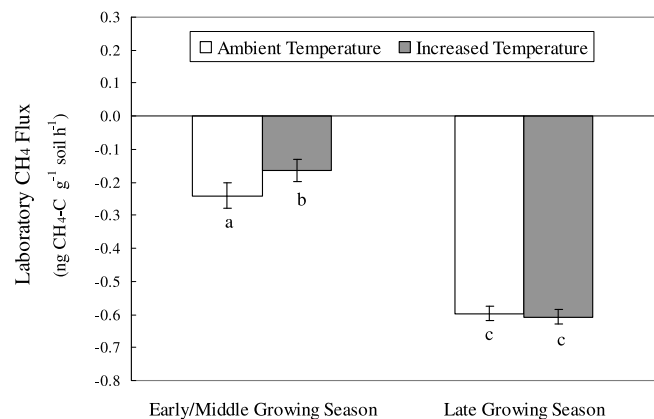

Figure 6. Season-dependent response of the laboratory soil CH_4 flux to warming (mean ±95% confidence interval; letters indicate significant differences in Tukey's HSD test; negative fluxes indicate net CH_4 consumption; early and middle growing season is October 2004 and February 2005; late growing season is April 2004, April 2005, and May 2006).

temperature and N deposition, the increased precipitation caused only a small reduction in CH$_4$ uptake. Small or no effects of precipitation have been found in other studies [*Billings et al.*, 2000; *Borken et al.*, 2006] and, along with the poor correlation between field CH$_4$ flux and surface soil water content (0–5 cm deep), suggest that CH$_4$ uptake in this grassland is not strongly affected by a direct effect of water addition on the diffusion of atmospheric CH$_4$ [*Benstead and King*, 1997]. The increased precipitation treatment did not affect the water content of surface soils on our particular sampling dates probably because measurements were conducted during relatively dry periods (i.e., no sampling in the rain) when enough time had passed (at least 24 h) for the added precipitation to infiltrate deeper than 5 cm or be taken up by plants. The soil CH$_4$ concentration seems ample for methanotrophic activity at any soil moisture. This hypothesis was tested in a 5 day laboratory incubation with soil collected from outside the experimental plots (data not shown, no effect of soil moisture was detected between a tested range of 10–80% of soil water-holding capacity). As further support, elevated atmospheric CO$_2$ consistently increased soil water content but did not affect field CH$_4$ flux individually.

[20] Although precipitation effects were absent under ambient temperature and N deposition, increased precipitation caused a substantial reduction in CH$_4$ uptake under either increased temperature or increased N deposition. This response was only observed in global change scenarios that included warming or increased N deposition individually, but not in combination. This is the first evidence of an interactive response in soil CH$_4$ flux involving three global change factors.

[21] The effect of precipitation on CH$_4$ flux was also affected by CO$_2$ concentration and depended on time of the year or growth stage of the vegetation. Unlike the previous interaction with temperature and N, this response was dependent on time of sampling. Earlier in the growing season, increased precipitation caused a reduction in CH$_4$ uptake under ambient CO$_2$. Later in the growing, increased precipitation caused a reduction in CH$_4$ uptake under elevated CO$_2$. *Ambus and Robertson* [1999] observed that elevated CO$_2$ caused a reduction in CH$_4$ uptake under ambient N deposition early in the growing season but not late. Our interactive response occurred both early and late in the growing season, but the nature of the interaction changed. A higher sampling frequency would be required to confirm this seasonal response and determine when exactly the nature of the interaction changes.

[22] Where increased precipitation caused a reduction in field methane uptake, the reduction was not caused by an indirect effect of moisture on methanotrophic activity in surface soil. The increased precipitation did not affect CH$_4$ consumption in the laboratory under standardized moisture. Neither was there a correlation between the rate of CH$_4$ flux observed in the field and CH$_4$ consumption in the lab incubations. The lack of correlation between field CH$_4$ flux and lab CH$_4$ consumption suggests that changes in the abundance and activity of methanotrophic organisms in surface soils were relatively unimportant in controlling net CH$_4$ uptake in this grassland. It is also likely that soil structure and CH$_4$ diffusional paths in the laboratory were altered from the undisturbed soils, thereby dampening

treatment effects observed in the field. This result supports the notion that laboratory CH$_4$ data should be extrapolated to field conditions with utmost care [*Bender and Conrad*, 1993; *Ambus and Robertson*, 1999]. The mean laboratory CH$_4$ consumption was about three times higher on sampling dates later in the growing season (perhaps due to higher soil respiration and lower oxygen availability earlier in the growing season), but this change in potential activity was not reflected in the field. There is evidence in this same grassland that methanotrophic bacteria may adapt to the wetter precipitation regime by a shift in community structure. *Horz et al.* [2005] found that the wetter regime decreased the relative abundance of Type II methanotrophic bacteria and increased the relative abundance of a novel clade of Type I methanotrophic bacteria, which tend to dominate methanotrophic communities under high and low CH$_4$ concentrations, respectively [*Amaral et al.*, 1995]. It is possible that this shift in community structure explains why methanotrophy was so resistant to a suite of global change scenarios.

[23] Because there is no evidence that precipitation effects were related to changes in methanotrophic activity or the diffusion of atmospheric CH$_4$ in surface soil, enhanced methanogenesis becomes a likely explanation. Gross CH$_4$ production is common in soils that typically exhibit net CH$_4$ uptake [*von Fischer and Hedin*, 2002], and our findings support results from other studies that an ecosystem can switch from net CH$_4$ uptake to net emission from one season to another [*Itoh et al.*, 2009] and under an altered precipitation regime [*Billings et al.*, 2000; *Davidson et al.*, 2004]. Methanogenic organisms can produce CH$_4$ within anoxic microsites of otherwise oxic soils [*von Fischer and Hedin*, 2002]. In fact, some atmospheric CH$_4$-oxidizing bacteria may depend on this endogenous CH$_4$ production for their growth and maintenance [*West and Schmidt*, 2002]. It is likely that the net CH$_4$ flux of an ecosystem is partly controlled by gross CH$_4$ production occurring in scattered anoxic soil microsites, deeper soil layers, and localized hot spots of death and decomposition [*Kammann et al.*, 2009]. *Henry et al.* [2005] found that decomposition at Jasper Ridge is primarily driven by changes in water availability. Taking this into consideration, along with the positive correlation between CH$_4$ emission and the water content of deeper soils, we hypothesize that the reduction in CH$_4$ uptake associated with increased precipitation was caused by an increase in endogenous CH$_4$ production.

[24] In light of the importance of methanogenesis in this grassland, we offer possible mechanisms for the interactive responses in CH$_4$ flux observed in the field. In the three-way interaction (precipitation × temperature × N deposition), the increased precipitation only caused a reduction in CH$_4$ uptake in combination with warming or increased nitrate deposition because these two treatments increase soil ammonium (NH$_4^+$) concentrations in this grassland [*Barnard et al.*, 2006], which may reduce N limitation on methanogenesis [*Kimura et al.*, 1992; *Lindau*, 1994]. In previous measurements at Jasper Ridge, warming and N deposition interacted to affect the soil NH$_4^+$ concentration: the warming treatment increased soil NH$_4^+$ under ambient N deposition, but decreased soil NH$_4^+$ under elevated N deposition, for unknown reasons [*Barnard et al.*, 2006]. The net CH$_4$ flux, therefore, may track N and water limitations on methano-

genesis deeper in the soil profile. However, when the increased precipitation was added to both increased temperature and increased N deposition, an even larger stimulation of methanogenesis may have reduced substrate limitation on low-affinity methanotrophy, thereby increasing gross CH$_4$ production and resulting in a smaller effect of precipitation on net CH$_4$ consumption. Elevated CO$_2$ has been shown to control the balance between CH$_4$ production and CH$_4$ consumption in a forest ecosystem [*McLain and Ahmann*, 2008], whereas we found that precipitation controlled the balance in a grassland ecosystem.

[25] In the two-way interaction (precipitation × CO$_2$ concentration), why may have endogenous CH$_4$ production in ambient CO$_2$ plots responded so strongly to an early season increase in precipitation? One possible explanation is that elevated CO$_2$ increases soil aggregate water stability in this grassland [*Rillig et al.*, 1999], and this resistance to crumbling may mean a resistance to anoxia under a wetter precipitation regime. Therefore, we would expect more gross CH$_4$ production under ambient CO$_2$. Later in the growing season, when elevated CO$_2$ increases soil moisture most [*Zavaleta et al.*, 2003], water accumulates in deeper soils and the combination of reduced plant evapotranspiration and a wetter precipitation regime pushes endogenous CH$_4$ production over an anoxic threshold. This nonadditive effect of precipitation and elevated CO$_2$ could be an important interactive response to global change concealed in single-factor experiments [e.g., *McLain and Ahmann*, 2008], especially in ecosystems with deep, clayey soil.

[26] The warming treatment by itself also caused a reduction in CH$_4$ uptake earlier in the growing season, but had no effect later in the growing season. It is possible that the long, hot summer was responsible for lower methanotrophic activity earlier in the growing season. We found that the warming treatment lowered activity even more, possibly explaining the reduction in field CH$_4$ uptake earlier in the growing season. Warming has been shown to decrease the relative abundance of Type II methanotrophic bacteria in this grassland [*Horz et al.*, 2005]. The lack of a correlation between field and lab fluxes does not support this hypothesis, but it also does not rule out the possibility that changes in methanotrophic population size or per capita activity indirectly affected the net CH$_4$ consumption (e.g., by not keeping pace with endogenous CH$_4$ production). The methanotrophic community appears to be more resistant to warming later in the growing season when overall activity is higher.

5. Conclusion

[27] The effect of increases in precipitation, temperature, CO$_2$ concentration, and N deposition on CH$_4$ flux in the field and laboratory were studied. Although precipitation generally reduced the CH$_4$ uptake in the field, this general response was significantly modulated by interaction with temperature, N deposition, and CO$_2$ concentration. Global change treatments either reduced CH$_4$ uptake or had no effect. Results from this four-way full factorial experiment suggest that different global change factors will not interact to enhance terrestrial CH$_4$ uptake. This study also supports previous findings that global environmental changes can impact the net CH$_4$ flux differently during different times of

the year, suspend terrestrial CH$_4$ uptake (i.e., switch CH$_4$ flux from net uptake to zero uptake), and induce changes in methanogenic activity.

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