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Effects of multiple global change treatments on soil N_2O fluxes

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Abstract Global environmental changes are expected to alter ecosystem carbon and nitrogen cycling, but the interactive effects of multiple simultaneous environmental changes are poorly understood. Effects of these changes on the production of nitrous oxide (N_2O), an important greenhouse gas, could accelerate climate change. We assessed the responses of soil N_2O fluxes to elevated CO_2, heat, altered precipitation, and enhanced nitrogen deposition, as well as their interactions, in an annual grassland at the Jasper Ridge Global Change Experiment (CA, USA). Measurements were conducted after 6, 7 and 8 years of treatments. Elevated precipitation increased N_2O efflux, especially in combination with added nitrogen and heat. Path analysis supported the idea that increased denitrification due to increased soil water content and higher labile carbon availability best explained increased N_2O efflux, with a smaller, indirect contribution from nitrification. In our data and across the literature, single-factor responses tended to overestimate interactive responses, except when global change was combined with disturbance by fire, in which case interactive effects were large. Thus, for chronic global environmental changes, higher order interactions dampened responses of N_2O efflux to multiple global environmental changes, but interactions were strongly positive when global change was combined with disturbance. Testing whether these responses are general should be a high priority for future research.

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Introduction

Nitrous oxide (N_2O) is an important greenhouse gas (Cicerone 1987), and soils are major sources of N_2O emissions to the atmosphere. N_2O emissions from soil are sensitive to global environmental change (reviewed in Barnard et al. 2005; van Groenigen et al. 2011), yet how N_2O emissions respond to multiple, simultaneous global changes is not well understood. The overarching goal of the work described here was to examine responses of N_2O emissions from soils to multiple global change drivers.

Nitrous oxide is produced in soils as a byproduct of nitrification and denitrification. Ammonia-oxidizers, the microorganisms that carry out the first step of nitrification (Hayatsu et al. 2008), can produce N_2O as a byproduct of the oxidation of ammonia to nitrite (NO_2^-), and during nitrifier-denitrification, where the nitrite produced is then reduced to N_2O as in denitrification (Wrage and Velthof 2001; Shaw et al. 2006). Denitrification produces NO, N_2O or N_2 as end products (Tiedje 1988). In well-aerated soils, N_2O efflux is mostly linked to nitrification, while water saturated soils mainly produce N_2O through denitrification (Wrage et al. 2004). Soil N_2O efflux rates are highly variable and depend on O_2 concentration in soil microsites, availability of inorganic N and labile carbon (C), and soil temperature, all of which can affect nitrification and denitrification (Weier et al. 1993). The physiological differences between nitrifiers and denitrifiers, as well as their different resource requirements, strongly suggest that they will differ in response to environmental change. Thus, accurately projecting future N_2O emissions from soils will require understanding how nitrification and denitrification contribute to soil-atmosphere N_2O flux in a changing environment. A second goal of the work presented here was to examine the microbial drivers of N_2O emissions from soil.

Many studies have been conducted to determine the effects of single global change treatments on soil N_2O efflux (reviewed in Barnard et al. 2005). Nitrogen addition stimulates both nitrification and denitrification (Barnard et al. 2005), enhancing N_2O emissions from soils in both natural and managed ecosystems (Bouwman et al. 2002; Stehfest and Bouwman 2006; van Groenigen et al. 2010). Elevated CO_2 has been shown to increase and decrease nitrification and denitrification enzyme activities (Barnard et al. 2005; Niboyet et al. 2010, 2011a), decrease and increase available N (Reich et al. 2006), increase C input to soil (de Graaff et al. 2006), and increase soil water content (Arnone and Bohlen 1998), all of which can affect N_2O efflux. Although there is considerable variation, on average, elevated CO_2 increases N_2O efflux from soils (van Groenigen et al. 2011). Warming has been shown to increase nitrification, denitrification, and soil N_2O emissions (Larsen et al. 2011). Altered precipitation can also influence N cycling (Jamieson et al. 1999), including soil N_2O efflux (Dijkstra et al. 2010).

Determining how soil N_2O efflux responds to multiple global environmental changes in combination is essential, because such changes are occurring simultaneously. Soil N_2O efflux results from nitrification and denitrification, processes requiring different resources and exhibiting distinct sensitivities to environmental conditions, such that responses of soil N_2O efflux to combined global environmental changes may not be simple functions of their effects measured in isolation. Individually, increased soil moisture or enhanced C input below ground may have minor effects on soil redox status, but together could promote anaerobic conditions, restricting nitrification, favoring denitrification, resulting in non-additive effects on N_2O efflux to increased precipitation and elevated CO_2 (Mummey et al. 1994). Amplifying interactions can also occur because of shifting resource limitations: if NO_3^- is scarce, denitrification may be insensitive to increased C availability, until the NO_3^- limitation is alleviated. In this case, the potential effect of elevated CO_2 on denitrification may only be apparent when combined with N addition, and the combined effect would not be predicted by the isolated, single-factor manipulations. Alternatively, limited capacities of biological enzyme systems could result in muted, or dampening, interactions (where combined effects are smaller than expected): N_2O production could be limited by the pool of available enzymes, such that combined resource additions have effects that are smaller than expected from the sum of single-resource additions.

Here, we investigated responses of soil N_2O efflux to four simulated global environmental changes, singly and in full factorial combination: increased CO_2, increased N, increased precipitation, and increased temperature in an annual grassland. We also examined which microbial drivers were most likely responsible for the observed responses. We tested the null hypothesis that the combined effects of global environmental changes are predictable from their effects measured in isolation. Tests of interactive effects in ecology are mostly conducted using analysis of variance (ANOVA), but N_2O emissions are highly variable in space and time, making it difficult to detect statistically significant interaction terms in ANOVA, unless they are quite large. Yet, small changes in N_2O emissions could have important implications for feedbacks from soils to climate and atmospheric change. In particular, ecologically significant interactive effects could be dismissed if not statistically significant according to ANOVA (Type II Error). Therefore, we focused not just on the statistical significance of interactive effects in ANOVA, but also on the magnitude of combined effects of environmental changes, and how these deviated from the expectation from single-factor responses. We used this same approach to synthesize past studies examining the effects of multi-factor global environmental change on soil N_2O efflux. Previous efforts to synthesize interactive effects of these global environmental changes on soil N_2O efflux were limited by insufficient data (Barnard et al. 2005). Since then, a number of studies have assessed combined global environmental changes on soil N_2O efflux. We synthesized these past studies to test whether responses of soil N_2O efflux to combined global environmental changes exhibit general responses, or if interactions render patterns idiosyncratic.

Materials and methods

Study site and experimental design

We conducted this study at Stanford University's Jasper Ridge Biological Preserve in central California (37°24′N, 122°13′W, elevation 150 m). Mean annual temperature is 14°C, and total annual precipitation averages 605 mm, most falling between November and March. Summers are dry. The soil is a fine, mixed, thermic Typic Haploxeralf, with a loam texture, pH of 6.5–7.0, and water-holding capacity of 21% (Gutknecht et al. 2010; Blankinship et al. 2010). Non-native C3 annual grasses (*Avena barbata*, *Avena fatua*, *Bromus hordeaceus*, and *Lolium multiflorum*) are the dominant vegetation, with non-native (*Geranium dissectum*, *Erodium botrys*, and *Crepis vesicaria*) and native annual forbs (*Hemizonia congesta* ssp. *luzulifolia* and *Epilobium brachycarpum*) also present (Zavaleta et al. 2003a).

Treatments consisting of two levels (ambient and elevated) of four global change factors began in November 1998 (Dukes et al. 2005). Briefly, 32 circular plots (2 m diameter) were arranged in split-plot design, by dividing each plot into four 0.78-m^2 quadrants. Treatments included *atmospheric CO_2 concentration* using free-air CO_2 enrichment (FACE) rings (ambient and 680 μmol mol^{-1}), *temperature* using infrared lamps (ambient and ambient + 80 W m^{-2}, resulting in a 0.8–1.0°C soil surface heating), *precipitation* using a spray/drip system (ambient and ambient + 50% per rain event + 3-week elongation of growing season), and *nitrogen addition* applied as nitrate in liquid form at the beginning of the growing season, and then using slow-release fertilizer (ambient and ambient + 7 g N m^{-2} $year^{-1}$ as $Ca(NO_3)_2$). Treatments were organized as a split-plot design, with the CO_2 and temperature treatments applied at the plot level, and the precipitation and nitrate treatments at the quadrant level. There were 6 replicates of all 16 combinations of CO_2, temperature, precipitation, and NO_3^- addition treatments (96 quadrants). The original design contained 8 replicates of all 16 possible treatment combinations. However a fire burned two replicate blocks in July of 2003 (Henry et al. 2006; Gutknecht et al. 2010). The impact of the fire treatment on soil N_2O emission rates and related processes is discussed elsewhere (Niboyet et al. 2011a), and is not included in this analysis.

Field N_2O fluxes

We measured soil N_2O fluxes in situ between 12:00 p.m. and 4:00 p.m. on 30 April 2004, 21 April 2005, and 3 May 2006 using the static chamber approach (Hutchinson and Mosier 1981). Each year, we sampled the full four-way factorial experimental design. Though our measurements capture only brief

periods in time for a highly dynamic microbial process, they were coordinated with intensive field campaigns, providing ancillary data for exploring mechanisms underlying responses to treatments, and occurred near the period of maximum plant biomass (April–May), a time when plant-mediated effects of global environmental change might be most apparent (e.g., Kammann et al. 2008). Thus, our sampling focused primarily on testing for interactive effects of global environmental changes, rather than constructing annual budgets of N_2O exchange.

Chambers (1.8 l) were constructed from 10.2 cm-diameter PVC pipe closed with a PVC cap. The bottom 3 cm of each chamber was tapered to allow the chamber to slide smoothly into PVC rings of similar diameter permanently located in each quadrant. Aboveground vegetation within these rings was regularly removed. Closed cell foam rings were used to create a seal between the chamber and the ring. Once the chamber was in place, headspace air (15 ml) was sampled through a rubber septum (fixed to the top of each chamber) using a 20-ml nylon syringe equipped with a nylon stopcock and a 23-gauge needle. Three subsequent headspace samples were taken at 15-min intervals for the determination of N_2O flux rates.

Headspace gas samples were immediately injected into pre-evacuated 12-ml glass vials capped with 20-mm butyl rubber stoppers. These vials are airtight for at least 10 weeks (Blankinship unpublished), and were over-pressurized (+3 ml) so that any leaks would be evident when vials were analyzed. Samples were analyzed within 4 weeks on a gas chromatograph system (Agilent 6890 GC System, Palo Alto, CA, USA) with Haysep Q 60/80 and Porapack Q 60/80 packed columns and equipped with an electron capture device to determine N_2O concentrations. Field fluxes were calculated using linear regression of concentrations over time. Values that were clearly outliers (±2SD) were removed. The mean r^2 value was 0.71. The flux rates were calculated as $\mu g\ N_2O\text{-}N\ m^{-2}\ day^{-1}$.

Drivers of N_2O efflux

During the field campaigns, ancillary measurements were conducted for potential drivers of soil N_2O emission on soil samples collected in each quadrant (0–5 cm depth, see Blankinship et al. 2010). At each date, we determined CO_2 production rate in the laboratory as described in Blankinship et al. (2010). These incubations were conducted at constant soil water content, so we used the rate of CO_2 production in the laboratory as a proxy for C availability belowground, a key driver of denitrification. We also measured gravimetric soil water content at each date (Blankinship et al. 2010). Potential denitrification and ammonia oxidation rates were measured in April 2005 and 2006 (Niboyet et al. 2011a).

Statistical analyses

Analysis of variance

Analysis of variance was carried out using SAS 9.2 (SAS Institute, Cary, NC, USA). We used a four-way split-plot analysis of variance with repeated measures analysis to assess the treatment effects on soil N_2O field emission rates over the 3 years of measurements, and the temporal variability of these treatment effects. We therefore constructed a model with PROC MIXED, that included CO_2 and temperature treatments as between-plot factors, precipitation and nitrate treatments as within-plots factors, and all the interactive terms between the four treatments. Soil N_2O flux data were square-root transformed prior to analysis to correct non-equal variances; effects with $P < 0.05$ are referred to as significant, and effects with $0.05 \leq P < 0.1$ as marginally significant.

Correlations and path analysis

Using correlation analysis conducted with PROC CORR in SAS 9.2, we determined which variables were significantly related to soil N_2O emission rates. These variables were potential rates of ammonia oxidation and denitrification—the two microbial processes involved in soil N_2O production—soil moisture, and laboratory soil CO_2 efflux as a proxy of soil C availability. We then used path analysis to assess potential causal relationships between the variables that were significantly correlated to soil N_2O emission rates. In particular, we hypothesized that ammonia oxidation, soil moisture, and soil CO_2 production were related to field N_2O emission rates, at least in part, through changes in denitrification. We therefore tested a path diagram presented Fig. 2 using PROC CALIS in SAS 9.2, and determined the coefficients of each path as the standardized coefficients calculated using the analysis of correlation matrices.

Observed versus expected interactive effects: Jasper Ridge Global Change Experiment

This study focused on testing interactive effects of global changes on soil N_2O emissions. ANOVA offers one basis for inference about interactive effects, in particular for determining whether interactive effects occur or not. In order to complement the ANOVA approach, we also analyzed how observed interactive effects differed from expected interactive effects (based on combinations of single-factor effect sizes), focusing on the direction and magnitude of the deviation (observed–expected). This analysis evaluates the direction and magnitude of interactions, whether significant or not. Analysis of variance tests for interactions that deviate from an additive expectation: in other words, ANOVA finds no interaction if the combined effect is indistinguishable from the sum of effects measured in isolation. Arguably, the null model for many ecological systems are non-additive (e.g., Wootton 1994), so we also considered a multiplicative null model, in which the null expectation is that effects in combination are the product of effects measured in isolation.

For the additive model, the effect size of a single factor (X_T) was determined as:

$$X_T = T - C, \quad (1)$$

where T is the mean N_2O flux for the treatment, and C is the mean N_2O flux for the control. For the multiplicative model, the effect size of a single factor (Y) was determined as the log of the response ratio:

$$Y_T = \ln(T/C). \quad (2)$$

Combined effects of multiple factors on N_2O efflux were calculated similarly. For example, the additive combined effect of Heat and Nitrogen was calculated as,

$$X_{HN} = HN - C, \quad (3)$$

where X_{HN} is the observed effect of the combined Heat + Nitrogen treatment, and HN is the mean N_2O flux for the combined Heat + Nitrogen treatment. Similarly, the observed combined effect of Heat + Nitrogen for the multiplicative model (Y_{HN}) was calculated as:

$$Y_{HN} = \ln(HN/C). \quad (4)$$

Expected combined effects were then estimated as the sum of the observed single-factor effect sizes for the additive model. Because we log-transformed the response ratios for the multiplicative model, the sum of log response ratios reflects a multiplicative expectation. For example, the expected Heat × Nitrogen combined effect, $E(Y_{HN})$ for the multiplicative model, was calculated as

$$E(Y_{HN}) = Y_H + Y_N. \quad (5)$$

For the additive model, the expected combined effect was calculated as:

$$E\ (X_{HN}) = X_H + X_N. \quad (6)$$

We then calculated the interaction term (I) as the difference between observed and expected combined effect sizes, either $I = X_{HN} - E(X_{HN})$ for the additive model, or $I = Y_{HN} - E(Y_{HN})$ for the multiplicative model. We used bootstrapping to estimate mean interaction terms and 95% confidence limits.

Observed versus expected interactive effects: meta-analysis

We used a similar approach to conduct a meta-analysis of the magnitude of interactions in past studies of interactive responses of N_2O efflux to global environmental change. Studies examining N_2O efflux in response to two or more experimental manipulations—in full factorial design—were gathered from the literature, using Google Scholar (Google Inc., Mountain View, CA, USA) to search for articles published before January 2011. A total of 15 studies were identified (see Table 3). Means and sample sizes were extracted for all treatment combinations, and estimates of standard deviation were recorded when available. We estimated expected combined effects, observed combined effects, and the deviation from the expectation—the interaction—as described above (Eqs. 2, 4, 5). For this literature review, we focused on multiplicative models, because the absolute values of the effect of global environmental changes on N_2O fluxes varied considerably as functions of study duration and study system. For the meta-analysis, we tested whether the observed combined effects differed significantly from the expected combined effect, in short testing whether the interaction term was significantly different from zero. We used the same interaction term for the multiplicative model described above, where $I = Y_{HN} - E(Y_{HN})$, as our effect size metric in the meta-analysis, and we weighted all

observations equally. We further tested whether particular global environmental changes were more likely to elicit significant interactions, using a categorical meta-analytic model and all factors present in the dataset with at least two observations (CO_2, Heat, Nitrogen, Water, and Fire). We used MetaWin 2.1 to conduct the meta-analyses.

Results

Field N_2O efflux

Nitrous oxide fluxes in the field ranged from −150 to 565 μg N_2O-N m^{-2} day^{-1}. Elevated precipitation increased N_2O efflux by +78% on average (Fig. 1; Table 1). The effect was most pronounced in April 2006 (W × time interaction, Table 1), the sampling campaign with the highest overall soil water content and the highest overall rates of N_2O emission (Table 2). For all three sampling dates, the high precipitation treatment had higher soil water content than the control (Table 2). The increase in soil N_2O efflux with elevated precipitation was largest in combination with heat and N addition (Precip × N, and Precip × Heat × N, Table 1; Fig. 1). Elevated CO_2 did not significantly affect N_2O emissions, either alone or in combination with other treatments (Table 1).

Drivers of N_2O efflux

Path analysis identified potential causal relationships between variables significantly correlated with soil N_2O emissions (Fig. 2). Path analysis supported denitrification as the major driver of soil N_2O production, with nitrification playing a secondary role (Fig. 2; Table 3). In the path analysis, potential ammonia oxidation was significantly related to potential denitrification and marginally significantly related to N_2O emissions. Path analysis revealed that potential ammonia oxidation influenced N_2O emissions mainly indirectly as a process providing substrate (NO_3^-) for denitrification. The path coefficient for the total effect of potential denitrification on soil N_2O emissions was higher than that of potential nitrification on soil N_2O emissions, suggesting that denitrification was the more important driver. Path analysis further identified both C availability and soil water content as drivers of denitrification and of N_2O emissions (Fig. 2; Table 3). The path coefficients indicated that changes in soil water content and labile soil C were the major drivers of altered soil N_2O emissions.

Combined effects and interactions—Jasper Ridge Global Change Experiment

Across all treatment combinations, observed combined effects were positively correlated with expected

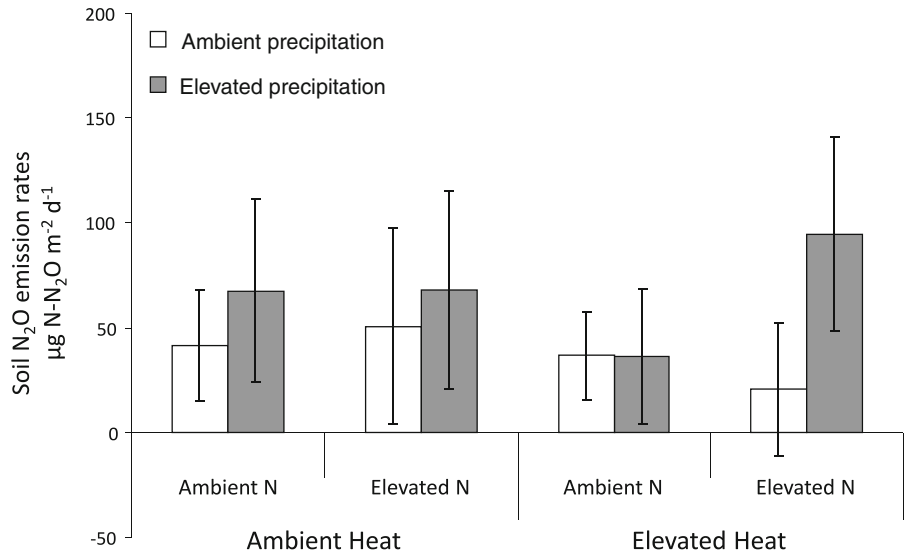

Fig. 1 Mean soil N_2O efflux across the precipitation, nitrogen, and heat treatments (±pooled standard errors of the mean) and all measurement dates, illustrating the significant N × Precipitation × Heat interaction

Table 1 Results from repeated-measures split-plot analysis of variance (three measurement dates) for in situ N_2O fluxes in response to multiple simulated global environmental changes

Treatment	Soil N_2O emission rates	
	Percent effect	*P* value
Treatment effects		
CO_2	+6	0.82
Heat	−17	0.40
CO_2 × Heat		0.51
Precip	**+78**	**0.002**
N	+29	0.24
CO_2 × Precip		0.50
CO_2 × N		0.47
Heat × Precip		0.42
Heat × N		0.45
Precip × N		**0.02**
CO_2 × Precip × N		0.44
Heat × Precip × N		**0.03**
CO_2 × Heat × Precip		0.64
CO_2 × Heat × N		0.77
CO_2 × Heat × Precip × N		0.18
Time effects		
Time		**0.0005**
Time × CO_2		0.17
Time × Heat		0.89
Time × CO_2 × Heat		0.91
Time × Precip		**0.001**
Time × N		0.20
Time × CO_2 × Precip		0.53
Time × CO_2 × N		0.43
Time × Heat × Precip		0.27
Time × Heat × N		0.22
Time × Precip × N		0.18
Time × CO_2 × Precip × N		0.78
Time × Heat × Precip × N		0.32
Time × CO_2 × Heat × Precip		0.26
Time × CO_2 × Heat × N		0.20
Time × CO_2 × Heat × Precip × N		0.93

Percent effect is the percent change in N_2O emissions (treatment − control)/control × 100%, shown for the main effects ($n = 48 \times 3$ sampling dates in the ambient and elevated treatments)

Bold values indicate statistically significant responses ($P < 0.05$)

combined effects in the additive and multiplicative models (Fig. 3a, c). Although the observed and expected combined effects were correlated, the observed effects consistently fell below the 1:1 line for both the additive and multiplicative models; the average interaction term was negative, with 90% confidence intervals that did not overlap zero (Fig. 3b, d). In sum, in our experiment, the effects of single-factor global changes on N_2O efflux were recapitulated, though muted, in multi-factorial combinations.

Combined effects and interactions—meta-analysis

Across studies conducted to date examining the effects of multiple, simultaneous global environmental changes on soil N_2O efflux (Table 4), observed combined effects ranged from −1.1 to 1.8, a more than two-fold greater range than found for expected combined effects, which ranged from −0.1 to 1.2 (test for unequal variances, $P = 0.014$). Overall, expected combined effects were poor predictors of observed combined effects ($r = 0.025$), because in some cases interactions were strongly positive (1.6 for CO_2 × N × Burn), and in others strongly negative (−1.6 for N × Heat). Interactions fell into two groups—positive interactions involving fire disturbance, and negative to near-zero interactions for other combinations of global change effects not involving fire (Fig. 4). For observations not including fire disturbance, the mean interaction term was negative with 95% confidence interval below zero (Fig. 4b), indicating that expected combined effects were larger than those actually observed. By contrast, when global changes were combined with fire disturbance, the expected combined effects underestimated observed effects on N_2O fluxes.

Discussion

Soil N_2O emissions and microbial drivers

Fluxes of N_2O we observed (−150 to 565 μg N m^{-2} day^{-1}) were well within the range of fluxes measured in other grassland ecosystems (Mosier et al. 2002; Huang et al. 2003), and of past measurements in California annual grasslands (Hungate et al. 1997; Davidson 1992). N_2O fluxes in grasslands are lower than in forests (Garcia-Montiel et al. 2002) and agricultural ecosystems (Li et al. 1996; Matson et al. 1998; del Grosso et al. 2005; Attard et al. 2010, 2011), but nevertheless contribute a significant amount (11%)

Table 2 Nitrous oxide flux rates (μg N_2O-N m^{-2} day^{-1}) and soil water contents (g H_2O g^{-1} × 100%), comparing values across low and high precipitation treatments over time

	April 2004	April 2005	April 2006
Nitrous oxide flux			
Low precipitation	33.11 ± 7.41	10.87 ± 4.26	69.43 ± 14.58
High precipitation	42.05 ± 9.61	14.17 ± 5.70	149.91 ± 19.90
Soil water content			
Low precipitation	11.94 ± 0.50	16.58 ± 0.46	22.33 ± 0.58
High precipitation	13.72 ± 0.56	18.31 ± 0.47	24.89 ± 0.64

Values are means ± standard errors ($n = 48$)

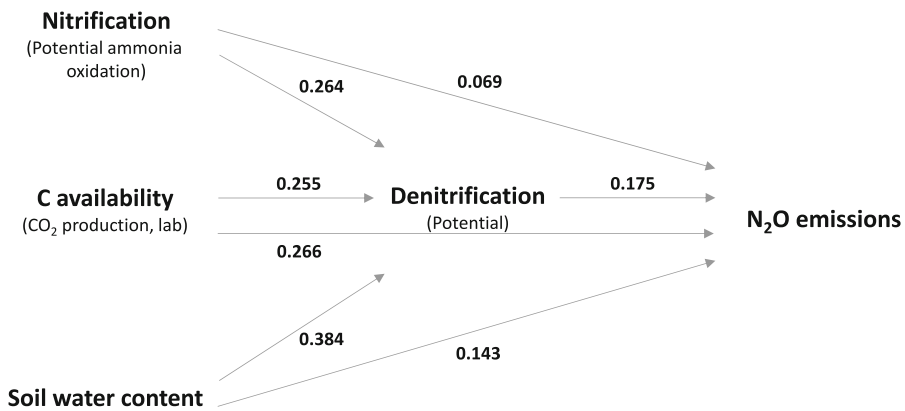

Fig. 2 Path diagram of the effects of nitrification (measured as potential ammonia oxidation rates), soil C availability (measured as soil laboratory-incubated CO_2 efflux), soil moisture (measured as gravimetric soil water content), and denitrification (measured as potential denitrification rates) on soil field N_2O emission rates. The path coefficients (values indicated next to the *arrows*) correspond to the standardized coefficients calculated based on the analysis of correlation matrices, and indicate by how many standard deviations the effect variable would change if the causal variable was changed by one standard deviation. The effect of nitrification, soil C availability, and soil moisture on soil N_2O emission rates are split between direct and indirect effects through changes in denitrification rates. The strength of the indirect effects can be calculated by the product of the coefficients along the path. The overall effect of a variable is the sum of its direct and its indirect effect. The significance of the direct, indirect, and total effects of the different variables presented in the path diagram on field soil N_2O emission rates are presented in Table 2

Table 3 Summary of path analysis (see the associated path diagram Fig. 2)

Response variable	Drivers	Total effect		Direct effect		Indirect effect	
		r	*P*	*r*	*P*	*r*	*P*
Denitrification	Ammonia oxidation	0.264	<.0001	0.264	<.0001	–	–
	Soil CO_2 production	0.255	0.0006	0.255	0.0006	–	–
	Soil water content	0.384	<.0001	0.384	<.0001	–	–
N_2O emissions	Denitrification	0.175	0.04	0.175	0.04	–	–
	Ammonia oxidation	0.115	0.08	0.069	0.31	0.046	0.06
	Soil CO_2 production	0.310	0.0003	0.266	0.003	0.045	0.08
	Soil water content	0.21	0.02	0.143	0.13	0.067	0.06

The path coefficients (*r*) correspond to the standardized coefficients calculated based on the analysis of correlation matrices. The significance of the total, direct or indirect effects of the exogenous variables on the responses variables is indicated

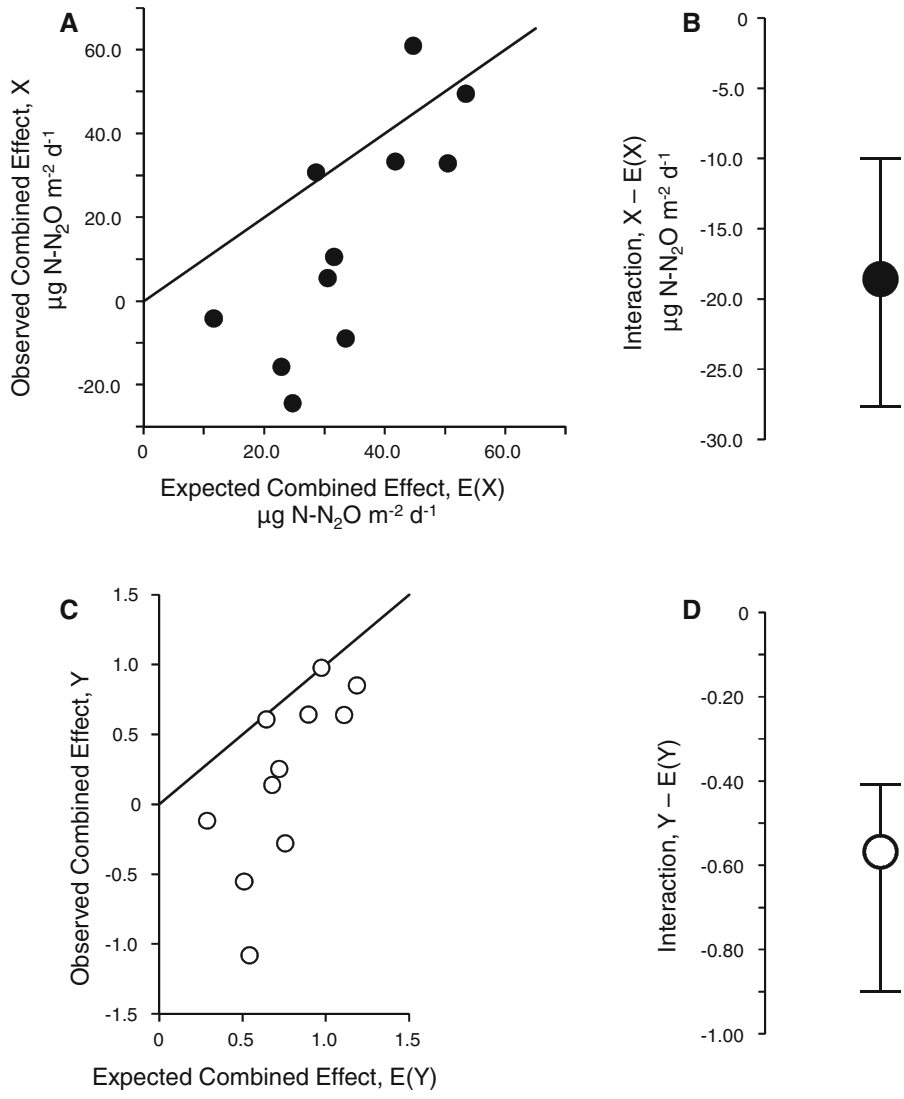

Fig. 3 Expected and observed combined effects and interaction terms for the additive (**a**, **b**) and multiplicative (**c**, **d**) models. **a** and **c** show observed combined effects as functions of expected combined effects, calculated from observed single-factor responses. *Solid lines* show 1:1, the expected relationships for no interactive effects. The departure from this 1:1 line is the interaction, shown in (**b**) and (**d**) (mean and 90% confidence intervals). Note that terms for the multiplicative model are unitless, because they are functions of response ratios

to terrestrial N_2O emissions from North America (Tian et al. 2010), and are an important component of the aggregated, global terrestrial N_2O source (Mosier et al. 1996; Xu et al. 2008).

The temporal variability we observed is typical of soil N_2O emissions, which can be quite dynamic (Kaiser et al. 1998; Marhan et al. 2011). Changes in N_2O efflux over time are caused in part by changes in conditions and substrate availability (Laville et al. 2011) and associated with changes in microbial communities (Chèneby et al. 2009). Past work has shown sensitivity of nitrifying and denitrifying communities to environmental change (Wallenstein et al. 2006; Braker et al. 2010; Attard et al. 2010; Szukics et al. 2010), including in the system examined here (Horz et al. 2004; Avrahami and Bohannan 2007).

Our results show not only that fluxes of N_2O are dynamic, but also that their responses to global change vary over time, consistent with past findings that effects of global change on microbial biomass and activity vary over time (Billings et al. 2002; Deiglmayr et al. 2004; Docherty et al. this issue). Our analysis provides correlative support for the notion that changes in conditions and resource availability (in this case, soil moisture and nitrate) at least partially explain the observed variation over time and between treatments, though the fairly low path coefficients suggest that other factors such as changes in microbial

Table 4 Summary of experiments examining interactive effects of global environmental change on soil N_2O emissions, by type of interaction examined, number of observations for each interaction type, and data source

Interaction	Number of observations	Data source
2-way		
CO_2 × Heat	3	1, 13
CO_2 × N	11	1–2, 4–11
CO_2 × Burn	1	15
CO_2 × Water	11	1, 3, 13, 14, 16
CO_2 × Ozone	1	12
N × Heat	1	1
N × Water	1	1
N × Burn	1	15
Water × Heat	1	1
Water × Burn	1	15
3-way		
CO_2 × Water × Heat	2	1, 13
CO_2 × Water × Burn	1	15
CO_2 × N × Burn	1	15
CO_2 × N × Heat	1	1
CO_2 × N × Water	1	1
N × Water × Heat	1	1
N × Water × Burn	1	15
4-way		
CO_2 × N × Water × Heat	1	1
CO_2 × N × Water × Burn	1	15

1. This study; Larsen et al. (2011); 2. Ambus and Robertson (1999); 3. Dijkstra et al. (2010); 4. Martin-Olmedo et al. (2002); 5. Baggs et al. (2003); 6. Baggs and Blum (2004); 7. Hagedorn et al. (2000); 8. Hungate et al. (1997); 9. Kettunen et al. (2005); 10. Kettunen et al. (2007a); 11. Kettunen et al. (2007b); 12. Kanerva et al. (2007); 2, 13. Larsen et al. (2011); 14. Liikanen et al. (2003); 15. Niboyet et al. (2011a); 16. Welzmiller et al. (2008)

community structure (Avrahami and Bohannan 2009) are also at play. Such dynamic microbial responses to global change compound the already difficult challenge of predicting microbial roles in modulating future ecosystem processes and feedbacks to climate (Todd-Brown et al. this issue). Nitrification and denitrification both contribute to N_2O emissions, with the latter increasing at soil water contents exceeding field capacity (Davidson 1992; Davidson et al. 2000). The stronger role of denitrification than nitrification in the path analysis of N_2O fluxes (Fig. 2) is consistent with the relatively high soil water contents measured during the 2005 and 2006 measurement campaigns, approximately 85% of soil water holding capacity for 2005, and 114% WHC for 2006 (Blankinship et al. 2010). Past work indicating that nitrification was the major source of N_2O production in these grasslands (Hungate et al. 1997; Avrahami and Bohannan 2007, 2009) was conducted at lower soil water contents. Furthermore, path analysis suggests that these sources of N_2O may be tightly coupled, with indirect effects of nitrification on total N_2O emissions, through production of NO_3^- that may be immediately reduced to N_2O during denitrification. Our measurements were not sufficient for estimating annual N_2O fluxes and their responses to treatments, but rather focused on dynamic microbial drivers of this key ecosystem process at a critical time of year, maximum aboveground plant biomass, the time when plant-mediated effects of global environmental change on soil processes may be most pronounced. A major challenge in this field is connecting these short-term and dynamic responses with temporally integrative measurements of N_2O emissions, a connection necessary to describe how particular microbial processes and microbial groups (e.g., nitrifiers vs. denitrifiers) influence emergent processes like N_2O emissions on longer time scales (Attard et al. 2011; Treseder et al. this issue; Todd-Brown et al. this issue).

Responses to precipitation and interactions with heat and nitrogen

Our finding that elevated precipitation increased soil production of nitrous oxide (N_2O) is consistent with the known sensitivity of N_2O production to water availability (Li et al. 1992; Mummey et al. 1994; Hungate et al. 1997; Davidson et al. 2004; Attard et al. 2011). The greater response in 2006, the measurement with the highest overall soil moisture and highest rates of N_2O emission, is consistent with denitrification being the major driver of soil N_2O production. Indeed, elevated precipitation also increased potential denitrification rates (Niboyet et al. 2011a), consistent with denitrification being an anaerobic process, thus more active when soil water content is high (Davidson 1992; Conrad 1996).

Co-limitation by water and N availability has also been shown to influence N_2O efflux (Mummey et al. 1994), and precipitation and temperature together explain continental-scale patterns of N_2O emissions

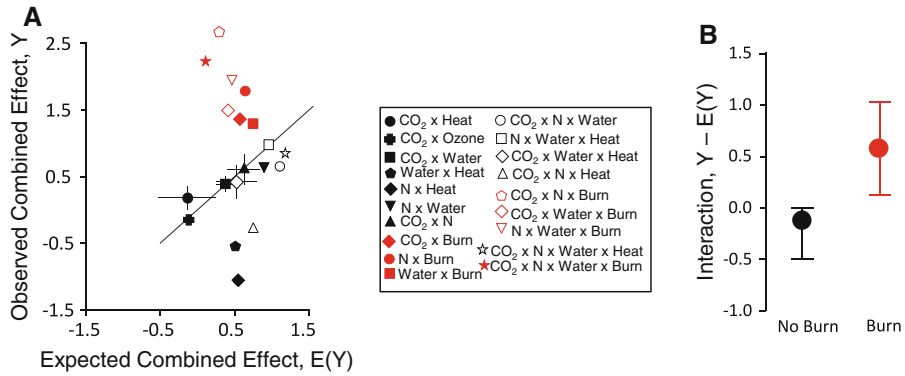

Fig. 4 **a** Observed combined effect as a function of the expected combined effect for the literature synthesis. *Symbols* indicate interactions tested, with global change factors shown in the legend. Two-way interactions are shown in *filled symbols*, three-way in *open symbols*, and four-way as *stars*. *Symbols in red* indicate interactions involving fire disturbance (burn). *Symbols with error bars* (standard errors) show interactions tested in multiple studies (CO_2 × Water and CO_2 × N, $n = 11$; CO_2 × Heat, $n = 3$; CO_2 × Water × Heat, $n = 2$; Table 3). *Solid line* shows the 1:1 relationship (no interaction). **b** Magnitude of the observed interaction terms for interactions involving burn (*red*) and those involving only chronic global change perturbations (*black*). *Symbols* are means and *bars* show 95% confidence limits determined using MetaWin

(Tian et al. 2010). The mechanism of the interactions found is straightforward: as denitrification is anaerobic, high soil moisture promotes denitrification when sufficient NO_3^- is available (significant Precip × N interaction, Table 1; Conrad 1996; Wrage et al. 2004). Furthermore, the combination of adequate water, nitrate, and higher temperature promotes N_2O efflux from intensive agricultural ecosystems (significant Precip × N × Heat interaction, Table 1; Dobbie et al. 1999). In laboratory incubations using this grassland soil, temperature, water content, and ammonia fertilizer additions interactively influenced soil N_2O emissions, in part by altering the community structure of nitrifying bacteria (Avrahami and Bohannan 2009). Thus, the 3-way interaction we observed makes biological sense, with the strongest stimulatory effect of precipitation on N_2O emissions occurring in combination with added N and heat (Fig. 1). Possibly, with denitrifiers being the major source of N_2O during these measurements, wetter soils with high precipitation were necessary before denitrifiers could respond to increased NO_3^- availability in the high nitrogen treatment (Wrage et al. 2004), and denitrification was more responsive to temperature than nitrification (Tian et al. 2010).

Responses to N, heat, and CO_2

The absence of a statistically significant main effect of N addition on N_2O efflux is surprising for four reasons: N was added in the form of nitrate, the substrate for denitrification; N significantly increased potential denitrification at our site (Niboyet et al. 2011a); path analysis clearly indicated that denitrification was an important source of N_2O production (Fig. 2); and N addition often increases soil N_2O efflux (Mosier 1994; Barnard et al. 2005; Bouwman et al. 2002; Stehfest and Bouwman 2006). N addition can significantly stimulate N_2O efflux from these grasslands (Hungate et al. 1997), though at N addition rates, 20 g N m^{-2} $year^{-1}$, approximately 3-times greater than those used here, 7 g N m^{-2} $year^{-1}$. Thus, the absence of a significant response to N addition alone may be explained by the lower amount of N added. However, N addition did increase N_2O production when combined with elevated precipitation and elevated temperature at our site (Fig. 1).

The temperature treatment caused a small increase in soil temperature (<1.0°C), which could explain why we found no significant effect of heat on N_2O efflux. Others have found that heat increases nitrification and denitrification (Tscherko et al. 2001; Malchair et al. 2010; Larsen et al. 2011), and N_2O emissions (Smith et al. 1998). However, past measurements in this grassland have found that nitrification and denitrification are insensitive to heat (Barnard et al. 2006; Niboyet et al. 2011a), consistent with our findings.

Across terrestrial ecosystems, elevated CO_2 increases N_2O emissions from soil, on average (van Groenigen et al. 2011), although responses are quite

variable (Barnard et al. 2005), so the lack of significant effect of elevated CO_2 on emissions here is not surprising. Furthermore, past work at this site examining effects of elevated CO_2 on N_2O emissions have shown no effect (Hungate et al. 1997), or only in combination with fire disturbance (Niboyet et al. 2011b).

As a whole, global change factors influenced N_2O efflux mainly through changes in soil moisture and C availability, whereas no significant relationship was observed with soil N concentration and the effect of nitrification on N_2O was indirect (likely by affecting denitrification). These results are consistent with path analysis results reported by Attard et al. (2011) indicating that soil organic carbon and water-filled pore space were the main drivers of potential denitrification, and ultimately in situ N_2O emission rate in temperate agro-ecosystems.

Interactive responses of soil N_2O emissions to global environmental change

Only two of 11 possible interactive effects of global environmental changes were statistically significant in our analysis, consistent with past experiments and syntheses that suggest few higher-order interactive responses to global environmental change (Zavaleta et al. 2003b; Luo et al. 2008; Larsen et al. 2011; Niboyet et al. 2011a). Using ANOVA as the sole basis for inference supports the interpretation that global environmental changes largely have additive effects on soil N_2O emissions. The two significant interactions we observed (precipitation $\times$ N and heat $\times$ precipitation $\times$ N) were both amplifying, where the observed combined effect was larger than the additive or multiplicative expectation. Yet, comparing the magnitude of effect sizes of all possible interactive effects suggests that treatment combinations tend to cause smaller changes in N_2O efflux than would be expected from single-factor manipulations (Fig. 3). Based on our review of the published literature, this response appears to be general for interactions involving combinations of chronic global change factors (heat, CO_2, N, precipitation), but not when combined with fire disturbance (Fig. 4).

Dampening interactive effects are consistent with the idea that processes producing N_2O exhibit saturating responses to multiple resource additions (C, NO_3^-, NH_4^+), and to alleviation of environmental constraints (temperature, water, redox status). Such responses could involve saturation of the enzyme pool with multiple resource additions, if sudden changes in resource additions were too rapid for microbial growth responses. This is unlikely to explain responses to chronic global environmental changes applied over multiple years. Alternatively, resources or conditions which are not altered by the combined experimental manipulations, or which become less favorable to N_2O production in combined manipulations, could impose limits on N_2O emissions not expressed when treatments occur singly. Finally, changes in the composition of N_2O producing communities could, in theory, yield novel responses surfaces for N_2O production in response to global change, causing dampening or amplifying interactions. The finding that fire disturbance causes amplifying interactions suggests that disturbance involves fundamentally different changes in conditions or resources regulating N_2O production when compared to chronic global environmental changes. Possibly, fire disturbance alleviates a broader array of resource constraints, improves soil conditions, or alters the community in ways that make N_2O production even more responsive to chronic global changes. The finding that fire disturbance shifts the nature of the interaction from dampening to amplifying is based on multiple interactions between fire and global environmental change reported in one study (Niboyet et al. 2011a), and thus should be extrapolated with caution. Nevertheless, the potential for strong amplifying interactions when global environmental change is combined with ecological disturbance underscores the need for more experimental work on this topic.

Understanding how simultaneous global changes affect microbial production and consumption of greenhouse gases is crucial to informing global change models (Treseder et al. this issue; Todd-Brown, this issue). Thus, we emphasize the need for long-term experiments and datasets examining responses of microbial processes to multiple, and potentially interacting, global changes (Docherty et al. this issue 1965).

Conclusions

We found that elevated precipitation increased soil emissions of N_2O, especially in combination with

added nitrogen and heat. Denitrification was the dominant microbial source of N_2O, and responded to increased soil water content and higher labile carbon availability. Nitrification indirectly influenced N_2O emissions, likely by providing substrate to denitrifiers. In our data, and in the literature we surveyed, statistically significant interactive effects of global environmental changes were infrequent. Yet, our analysis of effect sizes suggests that combined treatments alter N_2O emissions in unexpected ways: responses to combined chronic global changes may be smaller than expected based on responses to individual global change factors, whereas responses to interactions between global change and disturbance may be larger than expected. These findings suggest that N_2O emissions in a changing environment are unlikely to be simple functions of effects observed in single-factor manipulative experiments. In particular, our analysis suggests that experiments focusing on the interactions between multiple global environmental changes and ecological disturbance are needed in order to elucidate whether the trends we observed are general.

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