UC Irvine

UC Irvine Previously Published Works

Title

Greenhouse gas fluxes under drought and nitrogen addition in a Southern California grassland

Permalink

<https://escholarship.org/uc/item/34q9687z>

Authors

Aronson, Emma L
Michael L. Goulden
Steven D. Allison

Publication Date

2019-04-01

DOI

10.1016/j.soilbio.2018.12.010

Peer reviewed

Highlights

Multi-year CA grassland drought experiment decreased N₂O & CO₂ emission, CH₄ uptake

Increased N deposition in the same experiment increased N₂O emission

Global warming potential of reduced CO₂ emission outweighed N₂O & CH₄ effects

Overall net GHG emissions dropped 17% with less rainfall and more N deposition

1 **Title Page**

2 Greenhouse gas fluxes under drought and nitrogen addition in a Southern California grassland

3

4 Emma L. Aronson^{1,2*} Michael L. Goulden³, and Steven D. Allison^{2,3}

5 ^{1*} Department of Microbiology and Plant Pathology, University of California, Riverside

6 3411 Boyce Hall

7 Riverside, CA 92521

8 (p) (951) 827-4201

9 (f) (951) 827-4294

10 emma.aronson@ucr.edu

11 ² Department of Ecology and Evolutionary Biology, University of California, Irvine

12 ³ Department of Earth System Science, University of California, Irvine

13 **Keywords**

14 Greenhouse gases; CH₄; N₂O; CO₂; drought; nitrogen deposition.

15

Abstract

Climate and regional air quality models predict that Southern California will experience longer and more severe droughts, and possibly wetter, more intense storms and changing nitrogen (N) deposition. We investigated how the three major soil greenhouse gas (GHG) fluxes respond to 4-6 years of exposure to a full-factorial experiment of reduced and augmented precipitation crossed with increased N in a semi-arid grassland in Irvine, CA, USA. The mean emission fluxes across all treatments were $249.8 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$, $-16.41 \text{ } \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$, and $2.24 \text{ } \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$. Added N plots released 3.5 times more N_2O than ambient N plots, and N treatment and soil moisture interacted, such that volumetric soil moisture in added N plots correlated positively with N_2O release. Soil moisture, which was higher in the added water plots, correlated positively with respiration. CH_4 consumption increased with soil moisture in the drought treatment, an opposite trend to that observed in most other studies.

Our data suggest that CH_4 consumption, N_2O production, and soil respiration will decline if Southern California grasslands experience more frequent and extreme droughts. However, when drought is followed by high rainfall, the additional moisture will likely increase CH_4 consumption and N_2O release in periodic pulses. Overall, climatic shifts in this ecosystem may lead to a decrease in overall soil GHG emissions to the atmosphere. However, increased N deposition to Southern California will likely lead to increased N_2O release and a shift in the dominant N loss pathway toward gaseous release of N. If N deposition continues to increase along with severity and duration of drought, our data predict a decrease in global warming potential (GWP) of 17.2% from this ecosystem.

1 Introduction

Increasing atmospheric greenhouse gas (GHG) concentrations have been projected to lead to large climatic and environmental changes (IPCC, 2013). Likewise, climate and environmental changes can influence variation in soil consumption and release of key GHGs (Liu and Greaver, 2009, Blankenship et al., 2010, van Groenigen et al., 2011). Carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) are currently increasing in the atmosphere at rapid rates due to human activities (IPCC, 2013). An improved understanding of the natural and anthropogenic cycles of GHGs is needed for better projections of future climate forcing and change.

More frequent and intense drought in the southwest US has been projected with climate change, as a result of possible shifts in precipitation patterns and increases in potential evapotranspiration (Seager and Vecchi, 2010). Increasing anthropogenic nitrogen (N) deposition, especially near growing urban or industrial centers, is a further environmental change in the southwest US (Fenn et al., 1998). N deposition is a particular concern in Southern California, as it can drive shifts in vegetation composition that correlate with increased fire frequency (Fenn et al., 2003a). Both drought and N deposition induce significant changes in soil biogeochemical processes and trace gas fluxes (Aronson and Helliher, 2010, Aronson and Allison, 2012, Allison et al., 2013), but these responses are poorly understood.

Carbon dioxide is a particularly abundant GHG, and soil respiration is the primary means by which CO₂ is released by terrestrial ecosystems to the atmosphere (Schlesinger and Andrews, 2000). Net soil CO₂ release is a function of soil organic matter stocks, root respiration, rate of decomposition, and composition of the microbial community. Recent studies suggest that microbes can adjust their respiration and substrate requirements to C and N availability

(Eberwein et al., 2015; Schimel and Weintraub, 2003; Manzoni et al., 2008). In aridland Mediterranean soils, the Birch effect, where large CO₂ pulses occur after rainfall following extended drought, accounts for a large percentage of CO₂ released (Birch, 1958; Jarvis et al., 2007).

Methane is responsible for over 20% of total greenhouse gas radiative forcing (IPCC, 2013). Environmental conditions that influence soil CH₄ emissions and consumption include the moisture and oxygen content of the soil, as well as the concentration of available N (Aronson et al., 2013b). Inorganic N inhibits CH₄ oxidation under most conditions, but can stimulate CH₄ oxidation under low N.

Many of the environmental factors that govern CH₄ cycling also affect the release rate of N₂O, another important GHG (Aronson and Allison, 2012). In general, production of N₂O and nitrogen gas (N₂) through denitrification is stimulated by anaerobic conditions and the presence of the precursor compounds, ammonia (NH₃), nitrite (NO₂⁻), nitrate (NO₃⁻), and nitric oxide (NO). Conversely, production of N₂O and N₂ through the process of nitrification is stimulated by aerobic conditions and the presence of ammonia (NH₃) for ammonia oxidation and nitrite (NO₂⁻) for nitrite oxidation.

Southern California is currently experiencing marked environmental change driven by climate trends, NO_x emission, and N deposition (Fenn et al., 2003a). Total N deposition is 25 to 40 kg N ha⁻¹ y⁻¹ in some regions, and has increased rapidly in recent decades (Fenn et al., 2010). Global Circulation and Regional Climate Models (GCMs and RCMs) almost uniformly project significant warming in California (IPCC, 2013), which is likely to increase frequency and severity of drought. Projections of future precipitation are less certain, with GCMs lacking agreement on future trends for the region (IPCC, 2013). Hence, studies are needed to determine

the interacting effects of increased N deposition and both increased and decreased precipitation.

We investigated how the three most important soil GHG fluxes respond to a fully-factorial experimental manipulation in a pulse-driven Mediterranean-climate grassland. We carried out our observations 4-6 years after the sustained manipulations began. Previous studies have often focused on the effects of recently initiated treatments, and our experiment allowed us to consider the longer-term effects of precipitation and nutrient shifts on soil-mediated gas exchange with the atmosphere.

We hypothesized that soil respiration would be reduced in drought plots due to decreased plant carbon inputs (Fuchslueger et al., 2014), limited availability of soluble C substrate (Sihi et al., 2018), and microbial dormancy (Salazar et al., 2017), while increased precipitation and N would increase CO₂ release. We further hypothesized that net CH₄ consumption should decrease with greater soil moisture due to decreased methanotroph access to oxygen and increasing methanogenic production of CH₄ (Aronson et al., 2013b). We hypothesized that N addition would lead to high soil N content, especially as ammonium, which would inhibit CH₄ consumption due to the ability of methanotrophs to oxidize both ammonium and CH₄ (Aronson and Helliker, 2010). Finally, we hypothesized that the release of N₂O due to nitrification and denitrification would be lower in the drought treatment, as dry soil decreases most microbial processes, particularly those like denitrification that require anaerobic conditions. Conversely, we hypothesized that release of N₂O would be stimulated by increased precipitation and inorganic N addition (Aronson and Allison, 2012) as a direct result of greater inorganic N entering the system, facilitated by additional water (Figure 1).

2 Materials and methods

2.1 Study System

We measured the response of a coastal grassland to N and water manipulations at the Loma Ridge Global Change Experiment in the Irvine Ranch National Landmark of the Santa Ana foothills in Southern California (33° 44' N, 117° 42' W, 365 m elevation). The soil is a deep, moderately well-drained Myford sandy loam with a pH of 6.8 (German et al., 2012). The plant community is primarily exotic annual grasses and forbs (Allison et al., 2013). The average annual rainfall at this site was 247 mm between 2006 and 2014, and the average observed temperature from 2009-2012 was 16.4°C.

2.2 Field Manipulations

This experiment manipulates grassland N and precipitation using a factorial design, with added precipitation, ambient precipitation, and drought, crossed with ambient and added N, to generate 6 treatments. The six combinations are replicated in eight blocks, resulting in 48 plots, each with dimensions of 6.1 m by 8.5 m. Nitrogen was added at 60 kg N ha⁻¹ y⁻¹, with 20 kg N ha⁻¹ delivered as 100-day release CaNO₃ before the growing season and 40 kg N ha⁻¹ soluble CaNO₃ during the growing season. This level of N addition is comparable to the higher levels of N deposition in the region (Fenn et al., 2003b) and is similar to that used in other experimental studies focused on the impacts of increased N deposition. For example, Aronson et al. (2012) applied 67 kg N ha⁻¹ y⁻¹, while Christensen et al. (1999) applied 50 and 100 kg N ha⁻¹ y⁻¹. The experiment uses rainout shelters with clear polyethylene retractable roofs to exclude ~50% of the annual precipitation from the low precipitation plots. The drought treatment, which has been applied since 2007, is achieved by shelter closure during a subset of winter storms. The water draining off the shelters is collected with metal gutters and PVC pipe and stored in large

polyethylene tanks. This water is subsequently applied to the increased precipitation plots. In the 2011-2012 water year, drought plot rainfall was reduced by 105 mm, from the ambient 250 mm to 145 mm, and added precipitation plot rainfall was increased by 78 mm, to 329 mm. In the 2012-2013 water year, drought plot rainfall was reduced by 56 mm, from the ambient 166 mm to 110 mm, and added precipitation plot rainfall was increased by 80 mm, to 246 mm.

2.3 Trace gas fluxes

GHG gas fluxes were measured on 14 dates from 28 October 2011 through 20 March 2013. In September, 2011, one month prior to initial trace gas flux measurements, non-reflective, opaque PVC soil collars were implanted ~3cm into the soil for static vented chamber CO₂, CH₄ and N₂O flux measurements. The soil collars, 25.4cm inner diameter and 15 cm high, were left implanted for the duration of the study period. Fluxes were measured using ~15 cm high PVC soil chambers with sampling ports and pressure compensation apertures, which were sealed onto the collars using closed-cell foam (Verchot et al., 2000). The enclosed headspace of the collars with chambers was ~13-14L, which is typical for forest and grassland CH₄ flux measurements (Steudler et al., 1989). The heights of collars above the soil were measured 4 times over the study period and volume calculations changed accordingly.

Gas samples were taken over 20 minutes at 10-minute intervals. The fluxes were measured at each of the 48 chamber bases on most sampling events, with a subset of sampling events only occurring in half of the plots. Samples were taken using a gas-tight luer-lock syringe, which was flushed twice with internal chamber air for mixing before filling to 30 ml. The sample of chamber gas was then transferred to 20 ml gas-tight crimp-sealed vials for N₂O measurement

and 5ml gas-tight crimp-sealed vials in duplicate for CH₄ and CO₂ quantification. The vial septa were further sealed with non-reactive vacuum grease.

Gas samples were brought back to UC Irvine and run within 48h of collection on a gas chromatograph (GC) for CH₄ and CO₂ and a separate GC for N₂O concentrations. We analyzed CH₄ and CO₂ concentrations with a GC2014 (Shimadzu Corporation, Kyoto, Japan) equipped with a 2 ml sample loop and a thermal conductivity detector for measuring CO₂ in series with a flame ionization detector to measure CH₄. We analyzed N₂O concentrations using a separate GC2014 with an electron capture detector, operated with an AOC-5000 Autoinjector (Shimadzu). We calculated flux rates for each plot and gas as the linear regression slope of the 3 concentration measurements over time.

2.4 Additional Measurements

In situ soil moisture and temperature measurements were made concurrently with the chamber incubations using a GS3 probe with a handheld ProCheck datalogger (Decagon Devices, Inc.). A nearby weather station recorded air temperature, pressure, relative humidity, and rainfall at 30 min intervals. Nitrate/nitrite and ammonia were measured on soil collected from all 48 subplots on March 20, 2013, by KCl extraction and vacuum filtration of field fresh soil, paired with gravimetric water content using a drying oven. The KCl extracts were analyzed for nitrate/nitrite and ammonia using a microplate reader (BioTek Synergy 4; Allison et al., 2008). Soil total N and C content were measured on dried soils collected on September 13, 2012 and March 20, 2013 by combustion analysis (CE Elantech Flash EA1112 elemental analyzer).

2.5 Statistical Analysis

Statistical analyses were performed in R (R development core team, 2017). We conducted the analysis as a split-plot, randomized block using a mixed ANCOVA model with a full factorial design. The N and precipitation treatments were fixed effects, soil moisture was a covariate, and plot was nested within block as the random effect. We report conditional r^2 values to account for the inclusion of the random variables in the ANCOVAs. We disregarded CO₂ flux data if the initial concentration was greater than 650 ppm, which we considered spurious. This removed 63 CO₂ flux measurements in all. We rank-transformed the CO₂ and N₂O data before running the ANCOVA model because the residuals were not normally distributed. We also performed one-way analyses of variance (ANOVAs) on each gas measured on each date of sampling, describing a selection of these in text, and on all ancillary variables, with those results shown in Table 1.

We compared the N treatment responses to other studies by converting the study average trace gas flux data into the study index T_i for CH₄ fluxes following Aronson and Helliker (2010) and for N₂O following Aronson and Allison (2012). We did not convert CO₂ flux data, as there is no published comparison meta-dataset for this study index on CO₂. The study index T_i is calculated in equation 1, where C_i and N_i indicate paired, average measurements of trace gas fluxes (either N₂O or CH₄) from control (ambient N) (C_i) and N amendment (added N) (N_i) plots. The study index is assigned a sign based on the direction of the difference in gas flux due to N amendment: a negative T_i indicates a decrease in release (or increase in consumption) due to N amendment, whereas a positive T_i indicates an increase in release (or decrease in consumption). The range of T_i is from -1 to 1, with an index of zero indicating no difference in flux between the control and treatment plots.

$$T_i = \pm \frac{|N_i - C_i|}{|C_i| + |N_i|} \quad (1)$$

198

199 Finally, we calculated the Global Warming Potential (GWP), or CO₂ equivalent for
200 atmospheric heating for a given time horizon, of all treatment combinations and various future
201 scenarios. GWP can be used to evaluate how the treatments and other factors impact the overall
202 GHG release from the ecosystem. We used the 20-year GWP, which is 86 for CH₄ (i.e. 86 times
203 that of CO₂), and 268 for N₂O (IPCC, 2013; Knoblauch et al., 2018). We used these 20-year
204 potentials rather than the often cited 100-year potentials to emphasize the short-term feedbacks
205 between GHGs and radiative forcing, as the 20-year potential for methane is more than double
206 the 100-year. We calculated these GWPs for average flux values across all sample dates by
207 treatment combination. We also attempted to disentangle the impacts of soil moisture, a
208 continuous factor, on GHG fluxes, to determine the GWP impact of dry vs. moist soils. We
209 compared calculated flux values based on the regression of flux by volumetric water content for
210 moistures at the high and low end of observed values. The different VWC values represent
211 different assumptions or scenarios based on our observations, and we acknowledge that there
212 may be substantial variance around these chosen values. At the low end we chose 2% (0.02 m³
213 m⁻³) VWC, which was a consistent lowest value across all treatments. At the high end, we chose
214 23% for most treatments, and 17% for the drought treatment, which did not have any
215 observations above this level.

216

217 **3 Results**

218 *3.1 Soil conditions and nutrients*

Soil moisture and temperature in the top 5 cm differed between the water treatments across all measurement dates, according to one-way ANOVA and Tukey's HSD test ($p < 0.0001$; Table 1). Soil moisture was significantly lower in the drought treatments than in the ambient plots (26.1% reduction) and added water plots (36.8% reduction), with no significant difference between the ambient and added water plots. Temperature was significantly higher in the drought vs. added water plots (3.27°C). Total N and C concentrations were significantly higher in the drought treatment than in the added water treatment at the end of the measurement period (27.5% and 25.9% increase, respectively; Table 1).

3.2 GHG fluxes

The mean CO_2 flux across all treatments was $250 \pm 11.4 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ (mean $\pm$ standard error of the mean; Figure 2). The mean CH_4 flux was $-16.4 \pm 6.71 \text{ } \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$, where negative numbers indicate uptake by the soil, while the mean N_2O flux was $2.24 \pm 0.52 \text{ } \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$. Added N plots released significantly more N_2O than ambient N plots across all flux measurements ($p < 0.0245$). The N_2O emissions from N added plots was 3.5 times that of the ambient N plots.

Soil moisture was the only significant variable ($p < 0.001$) in the CO_2 flux ANCOVA model ($r^2 = 0.18$). The precipitation interaction with soil moisture was significant ($p < 0.01$; Figure 3), and soil moisture itself was marginally significant ($p < 0.08$; $r^2 = 0.046$) in the ANCOVA for CH_4 flux. The precipitation treatment determined the response to soil moisture in a separate one-way ANOVA, with the drought treatment showing a significant increase in CH_4 consumption with greater soil moisture (Figure 3). The study index (Aronson and Helliker 2010) for average CH_4 flux was $T_i = -0.50$, indicating that N addition stimulated CH_4 uptake.

In the ANCOVA for N₂O flux, the N treatment ($p < 0.001$), soil moisture ($p < 0.006$), and the interaction of N treatment with soil moisture ($p < 0.005$) accounted for significant model variance ($r^2 = 0.07$ for all factors; Figure 4). The study index for the average N₂O flux response was $T_i = 0.53$, showing that N addition stimulated release of N₂O.

We used one-way ANOVAs to further investigate the seasonal and short-term impacts of treatments on GHG fluxes (Figure 5), and here describe five selected dates that sample a range of conditions. On 19 January 2012, after one month without rainfall and immediately following the addition of 40 kg ha⁻¹ soluble N and irrigation, there were no significant treatment effects. However, one week later and after a 12.2 mm storm, ambient N plots released significantly more CO₂ ($p < 0.036$) and less N₂O ($p < 0.033$) than added N plots. On 23 March 2012, nine days after irrigation and five after 2.84 cm rainfall with closed roofs, added precipitation plots released significantly more CO₂ ($p < 0.014$), and ambient N plots consumed significantly more CH₄ ($p < 0.034$). On 15 June 2012, two months after rainfall and five months after N addition, added N plots released significantly more CO₂ ($p < 0.025$), and consumed significantly more CH₄ ($p < 0.048$) than ambient N plots. Finally, on 14 December 2012, at the start of a 2.11 cm rainfall event with roofs closed, two weeks after irrigation and addition of 20 kg N ha⁻¹ as CaNO₃, added precipitation plots released significantly more CO₂ than drought plots ($p < 0.045$). Also, added N plots released significantly more CO₂ than ambient N plots ($p < 0.006$), while ambient precipitation plots consumed significantly more CH₄ than drought plots, which consumed more than added precipitation plots ($p < 0.019$).

The impacts of the precipitation manipulation on soil moisture were variable over time. Soil moisture, and the interactions between soil moisture and other factors, were more significant factors in determining flux responses than the precipitation treatment. We used GWP

conversions to get a more wholistic understanding of the magnitude of the impacts of our
 observed responses. When all GHGs were converted to CO₂ equivalents for comparison between
 gases, the ambient CO₂ flux was $2.50 \times 10^5 \mu\text{g CO}_2 \text{ eq m}^{-2} \text{ h}^{-1}$, the CH₄ flux was while CH₄ was -
 $1.41 \times 10^3 \mu\text{g CO}_2 \text{ eq m}^{-2} \text{ h}^{-1}$, and the N₂O flux was $268 \mu\text{g CO}_2 \text{ eq m}^{-2} \text{ h}^{-1}$, for a total of $2.487 \times$
 $10^5 \mu\text{g CO}_2 \text{ eq m}^{-2} \text{ h}^{-1}$. Under elevated N conditions, the increased N₂O emissions bring the GWP
 up to 2.493×10^5 equivalent $\mu\text{g CO}_2 \text{ eq m}^{-2} \text{ h}^{-1}$, a very small increase. We calculated GWPs
 based on assumed VWC values from regressions of selected treatment combinations to better
 demonstrate a potential future scenario in this ecosystem. A likely future scenario for Southern
 California includes elevated N deposition and extended periods of drought bringing more
 frequent extreme low soil moisture levels. Based on significant effects in the ANCOVAs, this
 scenario corresponds most closely to the drought plots at low soil moisture for CH₄, at 9.43×10^2
 equivalent $\mu\text{g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$, the added N plots at low soil moisture for N₂O, at 2.06×10^2
 equivalent $\mu\text{g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$, and the across-treatment low soil moisture conditions for CO₂, at 2.09
 $\times 10^5 \mu\text{g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ (Table 2). Relative to the current ambient plot observations reported here
 (Table 1), the likely future scenario corresponds to a 17.2% decrease in GWP. This decline is
 driven by a 17.4% decrease in CO₂ emissions slightly offset by a 2-fold increase in CH₄
 emissions.

4 Discussion

4.1 Magnitude of GHG fluxes and comparison to other studies

The GHG flux rates we measured were generally similar to or lower than previously
 reported for Mediterranean-climate, arid and semiarid grasslands. We found an average CO₂
 release of $250 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$, which is on par with emissions from other semiarid grasslands

(for example, Wang et al. (2016) measured an average of $311 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$). Our fluxes were lower than those observed in a more productive grassland during the growing season ($534\text{--}1050 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$) but far greater than those observed in the dormant season ($76.4 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$) (Frank et al., 2002).

The average CH_4 uptake (i.e. negative flux) we found was $-16.4 \text{ } \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$. Our measured consumption rates were just over one third of the average fluxes of -41.1 for grasslands and $-39.8 \text{ } \mu\text{g CH}_4 \text{ m}^{-2} \text{ h}^{-1}$ for deserts, reported by Aronson et al. (2013). Our average N_2O release was $2.28 \text{ } \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$, which was less than one third of the average of $7.53 \text{ } \mu\text{g N}_2\text{O m}^{-2} \text{ h}^{-1}$ based on a meta-analysis across grasslands (Kim et al., 2013). Our N_2O release rates under N addition were roughly half that observed in other grasslands.

Our experimental manipulation was based on a percentage of the ambient precipitation received during the study. The site received below-average ambient precipitation during the study (Kogan and Guo, 2015), which was further reduced in the drought plots, leading to extremely low water inputs relative to the historic conditions. The ambient plots received less water during the study than the historic average, and the added precipitation plots received precipitation inputs that approached the historic average. The relatively low rates of CH_4 uptake and N_2O release in our study may reflect the overall dry conditions. All of the treatments may have experienced some level of water limitation during the study, which led to similar N_2O emissions across treatments.

4.2 Direct and interacting effects of N addition and precipitation treatments on GHG fluxes

The precipitation treatments themselves (both reduced and added water) did not consistently influence GHG fluxes. However, soil moisture and the interaction between soil

moisture and water treatment were both significant variables in explaining the soil trace gas fluxes. Soil moisture controls the access of soil microorganisms and extracellular enzymes to substrates and products of enzyme-mediated reactions.

The CH₄ relationship was opposite what we expected: greater soil moisture correlated with greater CH₄ consumption under drought treatment, suggesting that CH₄ consumption at the site was limited by soil moisture. Soil moisture is known to limit CH₄ production due to the requirement for anaerobic microsites, but the potential for moisture limitation of methanotrophy has not been widely recognized (Aronson et al., 2013b). Extremely dry conditions with drought likely inhibited both CH₄ release and consumption (fluxes averaged near zero on the dates with the lowest moisture). The CH₄ uptake increase in response to greater soil moisture was weaker in the ambient plots, and nonexistent in the added water plots. The pattern of inhibition of methanotrophy with low soil moisture varied by precipitation treatment likely because ambient and added water plots received sufficient water for microbial activity, including CH₄ consumption and production. This increased uptake response to higher soil moisture has been observed in other desert and dryland systems (Angel and Conrad, 2009; Schnell and King, 1996; Torn and Harte, 1996).

We hypothesized that inorganic N addition would stimulate release of N₂O as a direct result of increased substrate availability for N₂O production. Consistent with this hypothesis, we found a study index of $T_i = 0.53$ for the N₂O flux response to N addition, which represents a somewhat stronger response than the average grassland response of $T_i = 0.41$ found by Aronson and Allison (2012). Nitrogen addition stimulates N₂O release across a range of ecosystems (Aronson and Allison, 2012), through increased nitrification, denitrification or both.

Methanotrophs may oxidize ammonia rather than CH_4 under ammonia abundance and we hypothesized that the added N plots would show reduced CH_4 consumption (Hanson and Hanson, 1996). However, we did not see a consistent increase in N content in the soil associated with chronic N additions in this study. On one date that was chosen for closer analysis, two months after N addition but within days of a rain event, we did see the expected trend of added N plots consuming less CH_4 , but this trend was not apparent on other sample dates. We found a marked spatial and temporal CH_4 flux variability (Table 2), suggesting N limitation of both methanotrophy and methanogeny (Leigh, 2000; Bodelier et al., 2000; Aronson et al., 2013a).

The impact of added N on soil inorganic N was minimal, despite the impact on N_2O release from the soil. This pattern may be unique compared to other grassland systems, where N amendments often lead to greater N_2O release while also increasing ammonium and nitrate concentrations (Neff et al., 1994; Crenshaw et al., 2008). However, many studies assume that adding nitrate and other fertilizers will increase soil available N without directly measuring soil N content after amendments have started (e.g. van Cleemput et al., 1994). In our system, available N may have been taken up by plants or lost quickly through leaching. Further, while total N would change slowly, the inorganic N indicators, NO_2/NO_3 and NH_4 , have the potential to vary rapidly, so our sampling dates may have missed any large build-ups. Elevated N_2O production may have accounted for some additional losses under N-fertilization, though these losses were three orders of magnitude lower than the N addition rate. The flux observations were not always during or soon after the N or water additions, and several were made after rain events. We used a sporadic sampling approach that may have missed some of the N_2O release pulses.

The negligible impact of N addition on available and total N content could also help explain why N treatment had no significant impact on CO_2 and CH_4 fluxes. We had hypothesized

that added N would increase soil respiration due to alleviation of microbial N limitation. Overall, this hypothesis was not supported, which may be because the microbial community was not N limited in this site. We did observe more complex interactions on specific sampling dates following recent N or water addition. Within 1 day of soluble N addition, and during irrigation in added precipitation plots, there was no significant N effect, but one week later after a storm, the ambient N plots showed greater respiration. Although we cannot directly explain the greater respiration from ambient N plots, the lack of observed increases in soil respiration after N and water input is consistent with rapid N loss from the system via leaching and N₂O release. During the dry season, and another two weeks after slow-release N was added, we observed greater respiration in added N plots. These findings suggest that the slow release N may continue to impact the soil microbial community.

Finally, we hypothesized that the release of N₂O due to nitrification and denitrification would be lower in the drought treatment due to reduced microbial activity related to N turnover. Conversely, we hypothesized that release of N₂O would be stimulated by increased precipitation, as a consequence of greater N mineralization with added water. Neither of these predictions was observed across all dates. Our findings contrast with a meta-analysis of drought treatment effects on soil nitrogen by Homyak et al. (2017) that found lower N₂O release and a build-up of ammonium in soils under drought treatment.

4.3 Short- vs. long-term effects of N additions and precipitation treatments on GHG fluxes

Our experimental design allowed us to compare the short-term responses to individual events (e.g., the effect of wetter soil following a recent rain or irrigation) vs. the long-term responses to treatments (e.g., the effect of wetter average soil in the water addition plots). When

all time points are taken together, the statistical analysis does not show significant impacts of most long-term treatments on GHG fluxes, with the exception that N addition increased N₂O release. However, GHG fluxes analyzed at individual time points demonstrated significant responses to recent water inputs.

One of our main hypotheses was that soil respiration would increase with long-term added precipitation due to increased plant carbon inputs. Overall, we found that greater soil moisture correlated with greater CO₂ release. However, the precipitation treatment did not significantly impact soil respiration across all dates. On two study dates we selected for closer investigation, which were during or immediately after water input, we did observe the expected treatment impact on CO₂ release. These results suggest that the effects of drought on carbon balance in this dryland system may be apparent only after rain events. The immediate effect of recent rainfall resulted in greater changes in respiration than the long-term effects of cumulative treatment across time, showing that important role of individual rainfall events in this pulse-type system.

The impacts of the precipitation manipulation on soil moisture were variable over time, and may have been short-lived (Table 2). Accordingly, soil moisture, and its interactions, were more significant factors in determining flux responses than the long-term treatment. We found that for low moisture values, CO₂ emissions were half those at high soil moisture. For CH₄, fluxes were slightly positive (emission) in the drought treatment plots under low soil moisture, whereas high moisture corresponded to a high rate of CH₄ consumption. In the added water plots, the CH₄ flux trend was reversed, with higher soil moisture corresponding to lower consumption rates and lower soil moisture corresponding to greater consumption rates. For N₂O,

we found that greater soil moisture corresponded to a much higher emission rate in added N plots, but in ambient N plots the difference was an order of magnitude smaller.

4.4 Implications for future GHG fluxes

Climate models project that California will continue to experience more frequent and severe droughts, with wetter and longer lasting storms. As a result, declining soil moisture over time may decrease CH₄ consumption. However, when drought is followed by high rainfall, the additional moisture will likely increase CH₄ consumption in periodic pulses. Overall, climatic shifts in this ecosystem may lead to temporal changes in the source-sink dynamics of CH₄.

Regional air quality models predict continued shifts in N deposition in Southern California, which could lead to increased N₂O release. However, in our study, soil moisture correlated positively with N₂O release, such that severe and extended droughts may reduce N₂O emissions. Therefore, as N deposition increases along with drought, these effects might cancel each other out, leading to no significant changes in N₂O fluxes from this ecosystem.

The treatment that most clearly impacted the Global Warming Potential (GWP) was N addition, as N₂O emissions were increased 3.5-fold by N addition. In our constructed equivalent GWP for a likely future scenario for Southern California, which includes elevated N deposition and extended periods of drought, we found a 17.2% decrease in GWP compared to the current ambient conditions. This decline is driven by a 17.4% decrease in CO₂ emissions slightly offset by a 2-fold increase in CH₄ emissions. While drought and added precipitation treatments did not impact CO₂ fluxes directly, our data indicate that drought-associated reductions in soil moisture could lead to future reduced soil respiration and decreased CH₄ consumption. Taken together, our results show that increased drought may reduce the overall GWP of GHG emissions from

semi-arid grasslands. We also found that N addition to this CA grassland led to increased N₂O release, which did not substantially change the GWP. In a potential future scenario of increased frequency and duration of drought along with increased N deposition, our data predict a significant reduction in the GWP, a negative feedback to climate change.

5 Acknowledgements

We thank NOAA for a Climate and Global Change Fellowship to EA. This work was funded by the UCI Center for Environmental Biology and the Office of Science, US Department of Energy, BER (DE-SC0016410). We thank Lab Manager Lucy Lu, undergraduate assistants Hira Akbar and Stephen Kim, and volunteer field assistant Jon Botthoff, for their field and lab contributions.

References

- Allison, S.D., Czimczik, C.I., Treseder, K.K., 2008. Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. *Global Change Biology* 14, 1156–1168.
- Allison, S.D., Lu, Y., Weihe, C., Goulden, M.L., Martiny, A.C., Treseder, K.K., Martiny, J.B., 2013. Microbial abundance and composition influence litter decomposition response to environmental change. *Ecology* 94, 714–725.
- Angel, R., Conrad, R., 2009. In situ measurement of methane fluxes and analysis of transcribed particulate methane monooxygenase in desert soils. *Environmental Microbiology* 11, 2598–2610.
- Aronson E.L., Dubinsky, E. Helliker, B.R., 2013a. Effects of nitrogen addition on soil microbial diversity and methane cycling capacity depend on drainage conditions in a pine forest soil. *Soil Biology and Biochemistry* 62, 119–128.
- Aronson, E.L., Allison, S.D., 2012. Meta-analysis of environmental impacts on nitrous oxide release in response to N amendment. *Frontiers in Terrestrial Microbiology* DOI: 10.3389/fmicb.2012.00272.
- Aronson, E.L., Allison, S.D., Helliker, B.R., 2013b. Environmental impacts on the diversity of methane-cycling microbes and their resultant function. *Frontiers in Terrestrial Microbiology* DOI: 10.3389/fmicb.2013.00225.
- Aronson, E.L., Helliker, B.R., 2010. Methane flux in non-wetland soils in response to nitrogen addition: a meta-analysis. *Ecology* 91, 3242–3251.
- Aronson, E.L., Vann, D.R., Helliker, B.R., 2012. Methane flux response to nitrogen amendment in an upland pine forest soil and riparian zone. *Journal of Geophysical Research – Biogeosciences* 117, G03012.

459 Birch, H.F. 1958. The effect of soil drying on humus decomposition and nitrogen availability.
 460 Plant and Soil 10, 9-32.

461 Blankinship, J.C., Brown, J.R. Dijkstra, P., Hungate, B.A., 2010. Effects of interactive global
 462 changes on methane uptake in an annual grassland. Journal of Geophysical Research 115,
 463 G02008.

464 Bodelier, P.L.E., Hahn, A.P., Arth, I.R., Frenzel, P., 2000. Effects of ammonium-based
 465 fertilisation on microbial processes involved in methane emission from soils planted with
 466 rice. Biogeochemistry 51, 225-257.

467 Christensen, T.R., Michelsen, A., Jonasson, S., 1999. Exchange of CH₄ and N₂O in a subarctic
 468 heath soil: effects of inorganic N and P and amino acid addition. Soil Biology &
 469 Biochemistry 31, 637-641.

470 Crenshaw, C.L., Lauber, C., Sinsabaugh R.L., Staveland, L.K., 2008. Fungal control of nitrous
 471 oxide production in semiarid grassland. Biogeochemistry 87, 17-27.

472 Eberwein, J.R., Oikawa, P.Y., Allsman, L.A., Jenerette, G.D., 2015. Carbon availability regulates soil
 473 respiration response to nitrogen and temperature. Soil Biology & Biochemistry 88, 158-164.

474 Fenn, M.E., Baron, J.S., Allen, E.B., 2003a. Ecological effects of nitrogen deposition in the
 475 western United States. Bioscience 53, 404-420.

476 Fenn, M.E., Haeuber, R., Tonnesen, G.S., Baron, J.S., Grossman-Clarke, S., Hope, D., Jaffe,
 477 D.A., Copeland, S., Geiser, L., Rueth, H.M., Sickman, J.O., 2003b. Nitrogen emissions,
 478 deposition, and monitoring in the western United States. Bioscience 53, 391-403.

479 Fenn, M.E., Poth, M.A., Aber, J.D., Bormann, B.T., Johnson, D.W., Lemly, A.D., McNulty,
 480 S.G., Ryan, D.F., Stottlem, R., 1998. Nitrogen excess in North American ecosystems:

481 Predisposing factors, ecosystem responses, and management strategies. *Ecological*
 482 *Applications* 8, 706–733.

483 Frank, A.B., Liebig, M.A., Hanson, J.D., 2002. Soil carbon dioxide fluxes in northern semiarid
 484 grasslands. *Soil Biology & Biochemistry* 34, 1235–1241.

485 Fuchslueger, L., Bahn, M., Fritz, K., Hasibeder, R., Richter, A., 2014. Experimental drought
 486 reduces the transfer of recently fixed plant carbon to soil microbes and alters the bacterial
 487 community composition in a mountain meadow. *New Phytologist* 201, 916–927.

488 German, D.P., Marcelo, K.R.B., Stone, M.M., Allison, S.D., 2012. The Michaelis-Menten
 489 kinetics of soil extracellular enzymes in response to temperature: a cross-latitudinal study.
 490 *Global Change Biology* 18, 1468-1479.

491 Hanson, R.S., Hanson, T.E., 1996. Methanotrophic bacteria. *Microbiological Reviews* 60, 439–
 492 471.

493 Homyak, P.M., Allison, S.D., Huxman, T.E., Goulden, M.L., Treseder, K.K., 2017. Effects of
 494 Drought Manipulation on Soil Nitrogen Cycling: A Meta-Analysis. *Journal of Geophysical*
 495 *Research – Biogeosciences* 122, 3260–3272.

496 IPCC, 2013. *Climate Change 2013: The Physical Science Basis. Contribution of Working Group*
 497 *I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (eds
 498 Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M. &
 499 Miller, H.L.). Cambridge University Press, Cambridge and New York, NY, pp. 996.

500 Jarvis, P., Rey, A., Petsikos, C., Wingate, L., Rayment, M., Pereira, J., Banza, J., David, J.,
 501 Miglietta, F., Borghetti, M., Manca, G., Valentini, R., 2007. Drying and wetting of
 502 Mediterranean soils stimulates decomposition and carbon dioxide emission: the "Birch
 503 effect." *Tree Physiology* 27, 929-940.

504 Kim, D.-G., Giltrap, D., Hernandez-Ramirez, G., 2013. Background nitrous oxide emissions in
 505 agricultural and natural lands: a meta-analysis. *Plant Soil* 373, 17–30.

506 Knoblauch, C., Beer, C., Liebner, S., Grigoriev, M.N., Pfeiffer, E.-M., 2018. Methane production
 507 as key to the greenhouse gas budget of thawing permafrost. *Nature Climate Change* 8, 309–
 508 312.

509 Kogan, F., Guo, W., 2015. 2006–2015 mega-drought in the western USA and its monitoring
 510 from space data. *Geomatics, Natural Hazards and Risk* 6, 651-668.

511 Leigh, J.A., 2000. Nitrogen fixation in methanogens: the archaeal perspective. *Curr Issues Mol*
 512 *Biol* 2, 125-131.

513 Liu, L.L., Greaver, T.L., 2009. A review of nitrogen enrichment effects on three biogenic GHGs:
 514 the CO₂ sink may be largely offset by stimulated N₂O and CH₄ emission. *Ecology Letters*
 515 12, 1103-1117.

516 Manzoni, S., Jackson, R.B., Trofymow, J.A., Porporato, A., 2008. The global stoichiometry of
 517 litter nitrogen mineralization. *Science* 321, 684-686.

518 Neff, J.C. Bowman, W.D., Holland, E.A., Fisk, M.C., Schmidt, S.K., 1994. Fluxes of Nitrous
 519 Oxide and Methane from Nitrogen-Amended Soils in a Colorado Alpine Ecosystem.
 520 *Biogeochemistry* 27, 23-33.

521 R Development Core Team. 2017. R: A language and environment for statistical computing. R
 522 Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL [http://www.R-](http://www.R-project.org)
 523 [project.org](http://www.R-project.org).

524 Salazar, A., Sulman, B.N. and Dukes, J.S., 2018. Microbial dormancy promotes microbial biomass and
 525 respiration across pulses of drying-wetting stress. *Soil Biology and Biochemistry* 116, 237-244.

526 Schimel, J., Weintraub, M.N., 2003. The implications of exoenzyme activity on microbial carbon

527 and nitrogen limitation in soil: a theoretical model. *Soil Biology and Biochemistry* 35, 549-
528 563.

529 Schlesinger, W. H., Andrews, J.A., 2000. Soil respiration and the global carbon cycle. *Biogeochemistry*
530 48, 7-20.

531 Schnell, S., King, G. 1996. Responses of methanotrophic activity in soils and cultures to water stress.
532 *Applied Environmental Microbiology* 62, 3203–3209.

533 Seager, R., Vecchi, G.A., 2010. Greenhouse warming and the 21st century hydroclimate of southwestern
534 North America. *Proceedings of the National Academy of Sciences USA*, 107, 21277–21282.

535 Sihi, D., Davidson, E.A., Chen, M., Savage, K.E., Richardson, A.D., Keenan, T.F. and Hollinger, D.Y.,
536 2018. Merging a mechanistic enzymatic model of soil heterotrophic respiration into an ecosystem
537 model in two AmeriFlux sites of northeastern USA.

538 Steudler, P.A., Bowden, R.D., Melillo, J.M., Aber, J.D., 1989. Influence of nitrogen-fertilization on
539 methane uptake in temperate forest soils. *Nature* 341, 314-316.

540 Torn, M.S., Harte, J. 1996. Methane consumption by montane soils: implications for positive and
541 negative feedback with climatic change. *Biogeochemistry* 32, 53–67.

542 van Cleemput, O., Vermoesen, A., de Groot, C-J., Ryckeghem, K., 1994. Nitrous oxide emission
543 out of grassland. *Environmental Monitoring and Assessment* 31, 145-152.

544 van Groenigen, K.J., Osenberg, C.W., Hungate, B.A., 2011. Increased soil emissions of potent
545 greenhouse gases under increased atmospheric CO₂. *Nature* 475, 214-218.

546 Verchot, L.V., Davidson, E.A., Cattanio, J.H., Ackerman, I.L., 2000. Land-use change and
547 biogeochemical controls of methane fluxes in soils of eastern Amazonia, *Ecosystems* 3, 41-56.

548 Wang Z., Ji, L., Hou, X., Schellenberg, M.P., 2016. Soil Respiration in Semiarid Temperate
549 Grasslands under Various Land Management. *PLoS ONE* 11, e0147987.

Figure Legends

Figure 1: Conceptual diagram of hypotheses about water and elevated N impacts on greenhouse gas fluxes measured in this study. Arrows indicate flux direction, and size indicates magnitude, such that larger arrows show greater rates of flux into or out of the soil.

Figure 2: Regression of CO₂ flux by volumetric soil moisture across all dates.

Figure 3: Regression of CH₄ flux (negative is soil consumption) on volumetric soil moisture in the a) added precipitation, b) ambient precipitation and c) drought treatment plots.

Figure 4: Regression of N₂O flux (positive is soil release) on volumetric soil moisture in the a) ambient nitrogen and b) added nitrogen treatment plots. Ambient nitrogen flux measurements are shown as gray circles, while added nitrogen treatment flux measurements are shown as black circles.

Figure 5: Treatment impacts on CH₄, N₂O, and CO₂ fluxes over time, showing the averages for all replicates for each treatment measured for each date. Significance data are from one-way ANOVAs of the N and precipitation treatments. Drought plots are shown in red circles, added water plots are shown in blue triangles, and ambient water plots are shown in black squares; N added plots are filled shapes, while ambient N plots are open; significant factors are shown as * for precipitation treatment, ^ for nitrogen treatment, and # for their interaction; double symbols indicate significance at $p < 0.05$, while single symbols indicate marginal significance at $p < 0.1$.

1 Table 1: Environmental and greenhouse gas flux measurements, with means, standard deviations,
2 date ranges and significance values (for ancillary data only) for one-way ANOVAs, as well as
3 GWP for each treatment combination. Treatment combinations are: H-X = drought, ambient N;
4 H-N+ = drought, added N; XX = ambient water, ambient N; XN+ = ambient water, added N;
5 H+X = added water, ambient N; H+N+ = added water, added N. Significantly different treatment
6 combinations for by precipitation treatment are indicated with lower case letters, whereas
7 differences by nitrogen treatment are shown in upper case letters (Tukey post-hoc tests).

Measurement Type (Units)	Date or Range	Mean +/- Standard Deviation of the Mean					
		H-X	H-N+	XX	XN+	H+X	H+N+
Temperature (°C) ^{^^}	10/2011- 03/2013	26.9±8.65 a	26.5±8.20 a	25.0±7.76 ab	24.9±7.78 ab	23.2±7.26 b	23.6±7.90 b
Volumetric Soil Moisture (%) ^{^^}	10/2011- 03/2013	5.64x10 ⁻² ±0.34 a	6.10x10 ⁻² ±0.04 a	7.62x10 ⁻² ±0.06 b	8.66x10 ⁻² ±0.06 b	9.78x10 ⁻² ±0.06 b	9.45x10 ⁻² ±0.06 b
Ammonium (µg g ⁻¹ dry soil)	03/2013	18.0±11.1	31.0±26.9	16.0±5.9	9.91±4.30	15.1±9.88	28.6±34.0
Nitrate/Nitrite (µg g ⁻¹ dry soil)	03/2013	9.97±12.5	23.7±24.3	18.1±27.1	2.68±2.82	12.8±23.3	18.7±35.9
N Conc. (%)	09/2012	4.15±1.14	3.86±1.56	3.39±0.59	4.09±1.07	3.68±0.54	4.30±1.56
C Conc. (%)	09/2012	46.8±14.7	41.2±20.0	38.1±10.5	45.7±14.6	41.4±7.06	50.5±22.3
N Conc. (%) ^{^^}	03/2013	4.58±1.11 a	4.46±0.73 b	3.59±0.62 ab	4.19±0.85 ab	3.43±0.43 b	3.66±0.65 b
C Conc. (%) ^{^^}	03/2013	50.2±12.4 a	46.4±8.02 a	39.2±7.96 ab	45.6±12.4 ab	37.1±4.55 b	39.7±7.62 b
C/N	09/2012	11.2±0.83	10.5±1.19	11.1±1.46	11.1±0.63	11.2±0.49	11.5±0.87
C/N*	03/2013	11.0±0.31 A	10.4±0.30 B	10.9±0.52 A	10.8±0.71 B	10.8±0.40 A	10.8±0.39 B
CO ₂ (mg m ⁻² h ⁻¹)	10/2011- 03/2013	212±193	233±253	253±278	287±277	263±240	251±227
CH ₄ (µg m ⁻² h ⁻¹)	10/2011- 03/2013	-11.6 ±151	-26.7 ±167	4.03±143	-24.7 ±162	-16.8 ±162	-22.5 ±150
N ₂ O (µg m ⁻² h ⁻¹)	10/2011- 03/2013	1.40±12.1	4.05±13.2	1.44±11.4	3.14±12.6	0.36±12.7	3.06±10.8
GWP (equivalent ug CO ₂ m ⁻² h ⁻¹)	10/2011- 03/2013	2.11x10 ⁵	2.32x10 ⁵	2.54x10 ⁵	2.86x10 ⁵	2.62x10 ⁵	2.50x10 ⁵

8 **N sig P<0.05; *N sig P<0.1; ^^Precipitation sig P<0.05; ^Precipitation sig P<0.1

Table 2

1 Table 2: Gas fluxes and corresponding greenhouse warming potentials (GWP) under different
2 volumetric water content (VWC %). For each treatment indicated, observed VWC, flux and
3 GWP are given at the lower and higher end of VWC content observed for that treatment.

Flux Data	CH ₄ drought		CH ₄ added water		N ₂ O ambient N		N ₂ O added N		CO ₂ all plots	
VWC assumption	2%	17%	2%	23%	2%	23%	2%	23%	2%	23%
Corresponding Flux (ug m ⁻² h ⁻¹)	11.0	-129	-35.8	-4.45	0.80	1.27	0.77	11.4	2.09 x10 ⁵	4.10 x10 ⁵
GWP (equivalent ug CO ₂ m ⁻² h ⁻¹)	9.43x10 ²	-1.11 x10 ⁴	-3.08 x10 ³	-3.83 x10 ²	2.15 x10 ²	3.40 x10 ²	2.06 x10 ²	3.04 x10 ³	2.09 x10 ⁵	4.10 x10 ⁵

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5