



HAL
open science

Global Change Could Amplify Fire Effects on Soil Greenhouse Gas Emissions

Audrey Niboyet, Jamie R. Brown, Paul Dijkstra, Joseph C. Blankinship, Paul W. Leadley, Xavier Le Roux, Laure Barthes, Romain L. Barnard, Christopher B. Field, Bruce A. Hungate

► **To cite this version:**

Audrey Niboyet, Jamie R. Brown, Paul Dijkstra, Joseph C. Blankinship, Paul W. Leadley, et al.. Global Change Could Amplify Fire Effects on Soil Greenhouse Gas Emissions. PLoS ONE, 2011, 6 (6), pp.e20105. 10.1371/journal.pone.0020105 . halsde-00723483

HAL Id: halsde-00723483

<https://hal.science/halsde-00723483v1>

Submitted on 29 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Global Change Could Amplify Fire Effects on Soil Greenhouse Gas Emissions

Audrey Niboyet^{1*}^{‡a}, Jamie R. Brown², Paul Dijkstra², Joseph C. Blankinship², Paul W. Leadley¹, Xavier Le Roux^{3‡b}, Laure Barthes¹, Romain L. Barnard⁴, Christopher B. Field⁵, Bruce A. Hungate²

1 Laboratoire Ecologie, Systématique et Evolution, UMR 8079 Université Paris-Sud 11/CNRS/AgroParisTech, Université Paris-Sud 11, Orsay, France, **2** Department of Biological Sciences and Merriam-Powell Center for Environmental Research, Northern Arizona University, Flagstaff, Arizona, United States of America, **3** Laboratoire d'Ecologie Microbienne, UMR 5557 CNRS, USC 1193 INRA, Université Lyon 1, Villeurbanne, France, **4** Institute of Plant Sciences, ETH Zürich, Zürich, Switzerland, **5** Department of Global Ecology, Carnegie Institution for Science, Stanford, California, United States of America

Abstract

Background: Little is known about the combined impacts of global environmental changes and ecological disturbances on ecosystem functioning, even though such combined impacts might play critical roles in shaping ecosystem processes that can in turn feed back to climate change, such as soil emissions of greenhouse gases.

Methodology/Principal Findings: We took advantage of an accidental, low-severity wildfire that burned part of a long-term global change experiment to investigate the interactive effects of a fire disturbance and increases in CO₂ concentration, precipitation and nitrogen supply on soil nitrous oxide (N₂O) emissions in a grassland ecosystem. We examined the responses of soil N₂O emissions, as well as the responses of the two main microbial processes contributing to soil N₂O production – nitrification and denitrification – and of their main drivers. We show that the fire disturbance greatly increased soil N₂O emissions over a three-year period, and that elevated CO₂ and enhanced nitrogen supply amplified fire effects on soil N₂O emissions: emissions increased by a factor of two with fire alone and by a factor of six under the combined influence of fire, elevated CO₂ and nitrogen. We also provide evidence that this response was caused by increased microbial denitrification, resulting from increased soil moisture and soil carbon and nitrogen availability in the burned and fertilized plots.

Conclusions/Significance: Our results indicate that the combined effects of fire and global environmental changes can exceed their effects in isolation, thereby creating unexpected feedbacks to soil greenhouse gas emissions. These findings highlight the need to further explore the impacts of ecological disturbances on ecosystem functioning in the context of global change if we wish to be able to model future soil greenhouse gas emissions with greater confidence.

Citation: Niboyet A, Brown JR, Dijkstra P, Blankinship JC, Leadley PW, et al. (2011) Global Change Could Amplify Fire Effects on Soil Greenhouse Gas Emissions. PLoS ONE 6(6): e20105. doi:10.1371/journal.pone.0020105

Editor: Justin Wright, Duke University, United States of America

Received: September 16, 2010; **Accepted:** April 25, 2011; **Published:** June 8, 2011

Copyright: © 2011 Niboyet et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: The Jasper Ridge Global Change Experiment was supported by the U.S. National Science Foundation (<http://www.nsf.gov>), the U.S. Department of Energy (<http://www.energy.gov>), the Carnegie Institution for Science (<http://carnegiescience.edu>) and the Jasper Ridge Biological Preserve at Stanford University (<http://jrpb.stanford.edu>). This work was supported by a CNRS - Etats Unis grant and by funding from Université Paris-Sud 11 (<http://www.u-psud.fr>), Université Lyon 1 (<http://www.univ-lyon1.fr>), CNRS (<http://www.cnrs.fr>), INRA (<http://www.inra.fr>) and the U.S. National Science Foundation (DEB-0092642, DEB-0445324). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: audrey.niboyet@grignon.inra.fr

^{‡a} Current address: Laboratoire Biogéochimie et Ecologie des Milieux Continentaux, UMR 7618 Université Pierre et Marie Curie/CNRS/AgroParisTech, AgroParisTech, Thiverval Grignon, France

^{‡b} Current address: French Foundation for Biodiversity Research, Paris, France

Introduction

Human-caused global environmental changes, such as rising atmospheric CO₂ concentration, climate change [1] and enhanced nitrogen (N) deposition [2–3] have increasingly recognized impacts on the functioning of terrestrial ecosystems [4]. However, the combined effects of these environmental changes on ecosystem processes are poorly understood [5–6], and understanding these combined effects remains critical for predicting ecosystem response to concurrent changes in the environment [7]. Furthermore, the effects of these environmental changes have not yet been examined in combination with natural disturbances such as fires, although such combined effects might play important roles in

shaping ecosystem processes [8–9]. Here, we examine the response of soil emissions of nitrous oxide (N₂O) – a potent and long-lived greenhouse gas [10] – to the interactive effects of simulated global environmental changes and fire in a Mediterranean grassland ecosystem.

Global environmental changes and fire can both enhance the release of N₂O from soils through their effects on the two main microbial processes contributing to soil N₂O production [11–12] – nitrification [13–14] and denitrification [15]. Rising atmospheric CO₂ concentration has been found to stimulate soil N₂O production [16], an effect mainly attributed to increased denitrification-associated N₂O efflux [17–19]. This effect could result from enhanced root-derived soil carbon (C) providing

energy for heterotrophic denitrification [17–18] and increased soil moisture reducing soil oxygen concentration and enhancing anaerobic denitrification [19]. Enhanced atmospheric N deposition and N fertilization have also been reported to increase soil N_2O production [20], an effect associated with increased soil ammonium and nitrate contents leading to increased in both nitrification- and denitrification-associated N_2O efflux [21]. Finally, fire can also stimulate the release of N_2O from soils [20]. This effect has been attributed to increased nitrification-associated N_2O efflux resulting from increased levels of ammonium in fire-impacted soils [22], and has been shown to persist up to several months after fire [23–25]. Thus, there is evidence that elevated CO_2 , enhanced N supply and fire can increase soil N_2O production, but whether their effects will be additive, synergistic (amplifying each other) or antagonistic (counteracting each other) has not been studied.

We investigated the interactive effects of simulated global environmental changes and fire on soil N_2O emissions as part of the Jasper Ridge Global Change Experiment (CA, USA). This field experiment, initiated in 1998, was initially designed to assess the interactive effects of four global environmental changes - elevated CO_2 , warming, increased precipitation, and enhanced N supply - at levels projected for the second half of the 21st century in an annual grassland ecosystem [6,26–27]. However, almost five years after the start of the experiment, an accidental, rapid and low-intensity fire burned two of the eight replicates [8–9]. This provided a unique opportunity to investigate the interactive effects between fire and global environmental changes. Warming, the treatment that had the weakest effects on nitrification and denitrification prior to the fire [28], was discontinued in the burned plots in order to increase the number of replicates for each treatment combining fire and other global environmental changes. Thus, the new field experiment consisted of a complete factorial design with four factors at two levels – burn (unburned vs. burned),

CO_2 (ambient vs. $680 \mu\text{mol mol}^{-1}$), precipitation (ambient vs. +50% above ambient) and N supply (ambient vs. +7 g $N\text{-Ca}(\text{NO}_3)_2 \text{ m}^{-2} \text{ yr}^{-1}$) – and a total of 16 treatment combinations.

Here, we report the response of soil N_2O emissions to the combined effects of fire and elevated levels of CO_2 , precipitation, and N supply during the three years following the fire disturbance at the Jasper Ridge Global Change Experiment. We also report the responses of related N cycling processes, including nitrification and denitrification, and of their main drivers to the treatments. Our objectives were (i) to assess the effects of the fire disturbance on soil N_2O emissions and investigate the interactive effects of fire and global environmental changes on soil N_2O emissions, (ii) to identify the mechanisms controlling the response of soil N_2O emissions to the treatments, and (iii) to assess the duration of the response of soil N_2O emissions to the fire disturbance.

Results and Discussion

Soil N_2O emissions responses to fire and global environmental changes

Responses of soil N_2O emissions to the interactive effects of fire and elevated levels of CO_2 , precipitation, and N supply were investigated during the three years following the fire (i.e. 9, 15, 19, 21 and 33 months after fire). Prior to analysis, we verified that, prior to the fire, soil and plant characteristics were indistinguishable between the plots that later burned and those that did not (Table S1). We also verified that, for each measurement date, soil N_2O emissions were indistinguishable between the previously warmed burned plots and the previously not-warmed burned plots (Table S2). This ensured that any significant effects of the burn “treatment” on soil N_2O emissions could be attributed to the fire disturbance.

Fire did not significantly alter soil N_2O emission rates at the end of the first year following the disturbance (Fig. 1). In contrast,

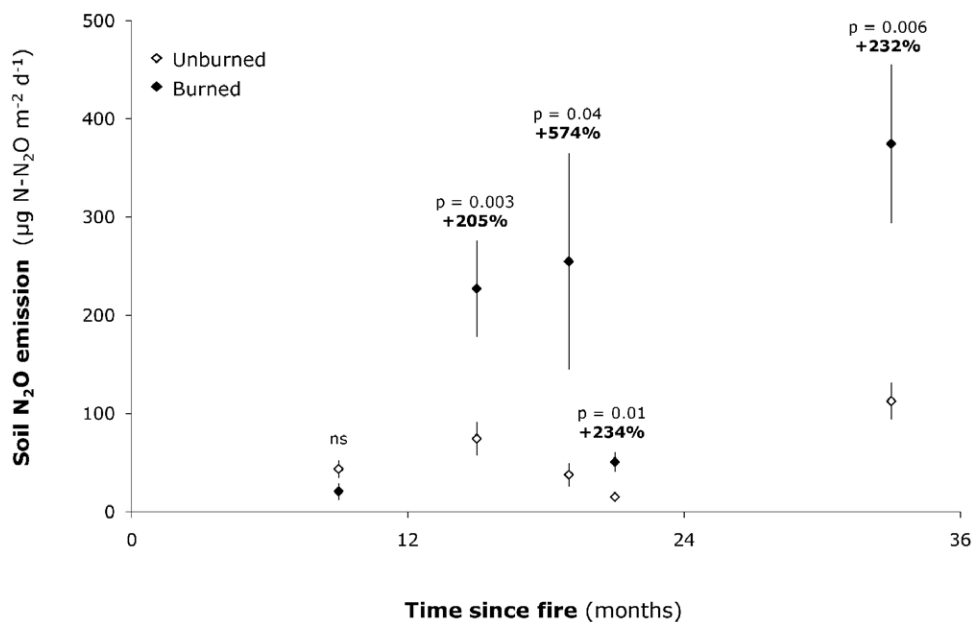


Figure 1. Soil N_2O emission rates in the unburned and burned plots in relation to time since fire. For each measurement date (i.e. 9, 15, 19, 21 and 33 months after fire), soil N_2O emission rates are grouped by burn treatments (unburned: open symbols, burned: closed symbols) and averaged across the CO_2 , precipitation and N treatments. Error bars denote standard error ($n = 48$ for unburned plots; $n = 32$ for burned plots). Results from mixed model analysis testing for a fire effect (ns: not significant) as well as relative fire effect sizes (calculated as: % effect = $100 \times [\text{burned} - \text{unburned}] / [\text{unburned}]$) are indicated. doi:10.1371/journal.pone.0020105.g001

during the second and third years after fire, we observed large increases in soil N_2O emission rates in the burned compared to unburned plots (+205% 15 months after fire, $P=0.003$; +574% 19 months after fire, $P=0.04$; +234% 21 months after fire, $P=0.01$; +232% 33 months after fire, $P=0.006$; Fig. 1). As a result, averaged across all measurement dates, soil N_2O production rates were substantially higher in the burned compared to unburned plots ($56 \pm 30 \mu\text{g N-N}_2\text{O m}^{-2} \text{d}^{-1}$ in the unburned plots vs. $185 \pm 145 \mu\text{g N-N}_2\text{O m}^{-2} \text{d}^{-1}$ in the burned plots, +227%, $P=0.001$; Tables S3 and S4). In line with other grassland studies [29], soil N_2O emission rates showed high temporal variation at our site: the lowest soil N_2O emission rates occurred 9 months and 21 months after fire (Fig. 1) when soil moisture was low (12% and 18%, respectively), while the highest emission rates occurred 33 months after fire (Fig. 1) when soil moisture was high (25%). Soil N_2O emission rates also showed particularly high variation in the burned plots (Fig. 1). We therefore checked whether the response of soil N_2O emissions to fire was driven by a response of specific burned plots. We found that, for each date, some of the burned plots showed very high soil N_2O emission rates compared to the others, which explains the very large variance, but the burned plots showing these very high soil N_2O emission rates differed depending on measurement date (not shown), indicating that the response of soil N_2O emissions to fire was not driven by a specific response of some of the burned plots.

Soil N_2O emission rates exhibited a strongly non-additive response to fire and other global environmental changes (Fig. 2). Increases in soil N_2O emission rates were larger in the burned plots when also exposed to enhanced N supply ($P=0.01$ for the Burn \times N interaction; Table S3; Fig. 2). Furthermore, the largest increases in soil N_2O emission rates occurred in the treatment combining fire, elevated CO_2 and enhanced N supply ($P=0.0007$ for the Burn \times CO_2 \times N \times Time interaction; Table S3; Fig. 2): soil N_2O emission rates were little affected by elevated CO_2 (-30%) or enhanced N supply (-25%) in isolation, were approximately

doubled in the burned plots (+124%), and increased by 516% in the burned plots exposed to elevated CO_2 and enhanced N supply (Fig. 2). This interactive effect was most pronounced 19 months after fire: at this time, CO_2 , N, and burn treatments tended to decrease soil N_2O emission rates when considered in isolation (-59%, -75% and -89%, respectively), but the combination of CO_2 , N, and burn treatments increased soil N_2O emission rates by 958% ($P=0.04$ for the Burn \times CO_2 \times N interaction).

Microbial processes mediating the responses of soil N_2O emissions to fire and global environmental changes

To tease apart the microbial processes for the increases in soil N_2O emission rates in the burned plots, especially when exposed to elevated levels of N and CO_2 , we investigated the responses of related N cycling processes to the treatments – including the responses of N mineralization, nitrification and denitrification – and the responses of the main drivers of these microbial processes. These drivers included soil ammonium and nitrate concentrations, soil environmental variables – soil moisture, soil temperature, soil pH – and soil CO_2 emission rates, as an indicator of soil heterotrophic activity (Fig. 3, Tables S4, S5, S6, S7, S8, S9).

Three lines of evidence indicated that increases in soil N_2O emissions in the burned plots during the second and third years after fire were caused by higher denitrification rates. First, potential denitrification rates were higher in the burned compared to unburned plots (+37% on average, $P=0.03$; Tables S4, S6 and S7; Fig. 3), whereas gross rates of N mineralization and gross and potential rates of nitrification either exhibited slightly negative responses or were non-responsive to fire (Tables S4, S8 and S9; Fig. 3). In particular, and in contrast to previous studies reporting increases in soil N_2O emission rates after fire [22–23,25], soil ammonium concentrations and nitrification rates were not increased in the burned compared to unburned plots (Tables S4, S8 and S9 and Fig. 3). Second, and consistent with this, soil N_2O emission rates were significantly and positively correlated with

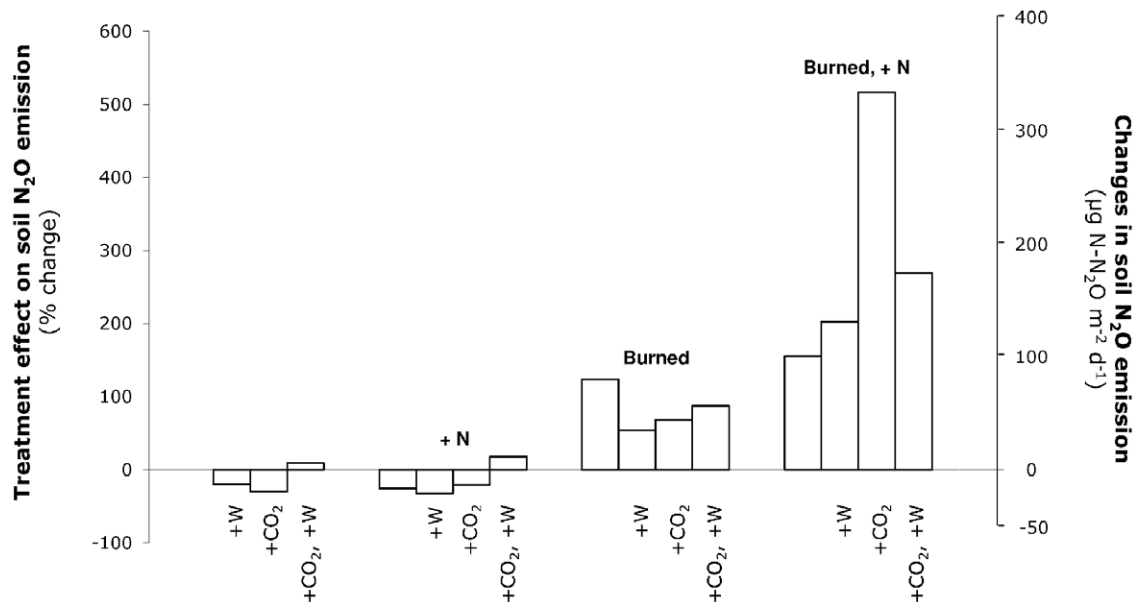


Figure 2. Treatment effects on soil N_2O emission rates. Treatments are increased precipitation (+W), elevated CO_2 (+ CO_2), enhanced N supply (+N), burn (Burned), and all their combinations. Treatment effects were calculated across the five measurement dates (i.e. 9, 15, 19, 21 and 33 months after fire) as: % effect = $100 \times [\text{treatment} - \text{control}] / [\text{control}]$. In the control treatment, all factors are ambient. Changes in soil N_2O emission rates in each treatment combination relative to the control treatment are also indicated. In the control treatment, soil N_2O emission rates were $65 \pm 103 \mu\text{g N-N}_2\text{O m}^{-2} \text{d}^{-1}$ (values indicate mean \pm pooled standard error; $n=6 \times 5$ sampling dates). doi:10.1371/journal.pone.0020105.g002

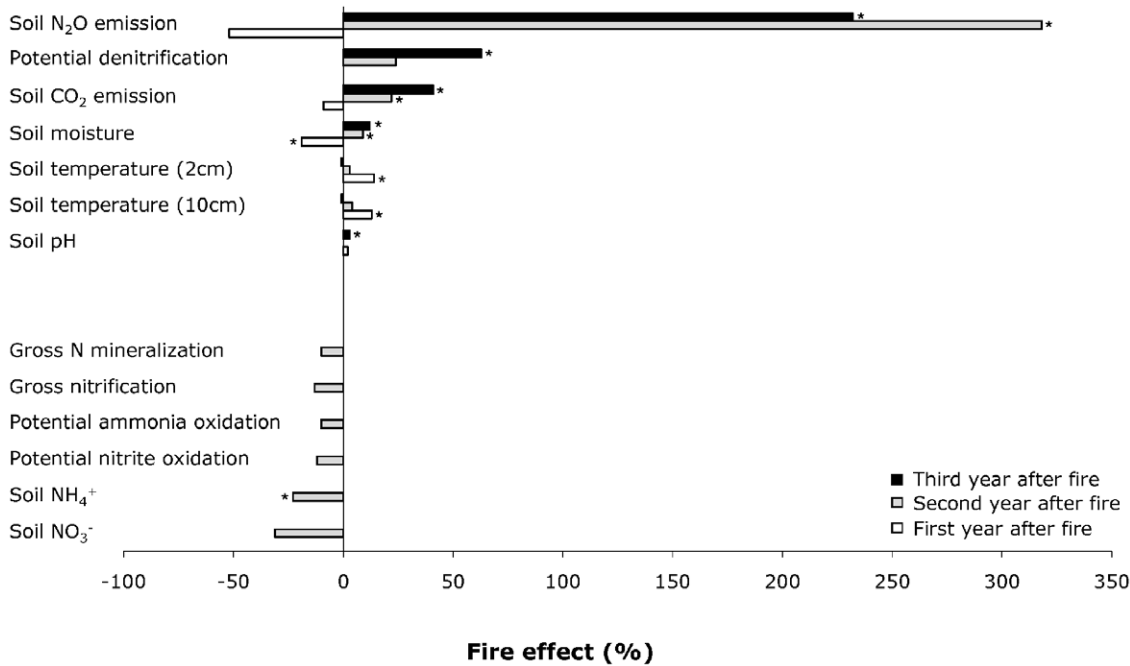


Figure 3. Effects of fire on soil N₂O emissions, soil characteristics and soil nitrogen cycling. For each variable, bars are arranged chronologically from bottom to top: white bars correspond to data collected during first year after fire (i.e. 9 months after fire), grey bars to data collected for several dates during second year after fire (i.e. 15, 19 and 21 months after fire), and black bars to data collected during third year after fire (i.e. 33 months after fire). The top section shows the response to fire of soil N₂O emissions, potential denitrification and soil characteristics measured over the three-year period following the fire (with the exception of year one for potential denitrification and of year two for soil pH). The bottom section shows the response to fire of soil N cycling variables measured for several dates in year two after fire. Fire effect sizes were calculated as: % effect = 100 × [burned – unburned] / [unburned]. Asterisks next to bars indicate significant effects of fire ($P < 0.05$). doi:10.1371/journal.pone.0020105.g003

potential denitrification rates ($P < 0.0001$), and with well known drivers of denitrification [15]: soil moisture ($P < 0.0001$) - an indicator of availability of anaerobic niches, and soil CO₂ production rates ($P < 0.0001$) - an indicator of labile C availability. Third, potential denitrification also exhibited a non-additive response to fire and enhanced N supply ($P < 0.0001$ for the Burn × N interaction; Tables S6 and S7; Fig. 4). Thus, burn or elevated N alone had only small effects on potential denitrification (+2% and +15%, respectively), but the combination of burn and elevated N almost doubled potential denitrification (+94%). This interactive effect of fire and enhanced N supply on potential denitrification likely explained why increases in soil N₂O emission rates were largest in the burned plots also exposed to elevated N (Fig. 4).

Possible mechanisms underlying the responses of soil N₂O emissions and denitrification to fire and global environmental changes

Our results suggest that the interactive responses of soil N₂O emission and denitrification rates to fire and enhanced N supply (Fig. 4) resulted from the simultaneous relaxation of multiple constraints on denitrifying microorganisms in the burned and fertilized plots. In particular, denitrifying microorganisms may have benefited from decreased soil oxygen concentration and increased soil C and N availability in these plots.

Indeed, we found that the burn treatment significantly increased soil moisture during the second and third years after fire (by 9% year two after fire, $P = 0.02$, Table S6; by 12% year three after fire, $P = 0.004$, Table S7; Fig. 3). Resulting decreases in soil oxygen concentration likely stimulated anaerobic denitrification, as suggested

by the significant positive relationship between soil moisture and potential denitrification rates ($P < 0.0001$, $R^2 = 0.35$), and as has been reported in other grassland studies [28,30]. Increases in soil moisture in the burned plots may have resulted from post-fire decreases in evapotranspiration or increases in infiltration. Decreases in evapotranspiration in the burned plots during the second and third years after fire are however unlikely at our site. Indeed, fire increased plant production when combined with enhanced N supply and suppressed the negative effect of elevated CO₂ on plant production at the end of the first year after the disturbance [8], but the responses of plant production to fire were short-lived and were no longer detected two or three years after fire [31–32]. Increases in soil moisture could thus reflect increases in infiltration in the burned plots resulting from changes in soil structure, e.g. from increases in soil aggregation and porosity as has been observed at some post-fire sites [33]. Post-fire increases in soil moisture may have enabled greater denitrification and N₂O emission rates. However, other mechanisms must have been in play. Indeed, though elevated CO₂ also significantly increased soil moisture (by 14% year three after fire, $P = 0.009$; Table S7), we observed no significant increases in denitrification or soil N₂O emissions in the elevated CO₂ plots (Tables S3, S6 and S7).

In addition to its effect on soil moisture, the burn treatment may have relaxed C limitation of denitrification [15]. This idea is in agreement with the increases in soil CO₂ emission rates in the burned plots during the second and third years after fire (+22% year two after fire, $P = 0.004$, Table S6; +41% year three after fire, $P = 0.009$, Table S7; Fig. 3), suggesting higher soil labile C availability in these plots. This hypothesis is further supported by previous work at our site reporting decreases in the activity of extracellular enzymes involved in C acquisition in the burned plots

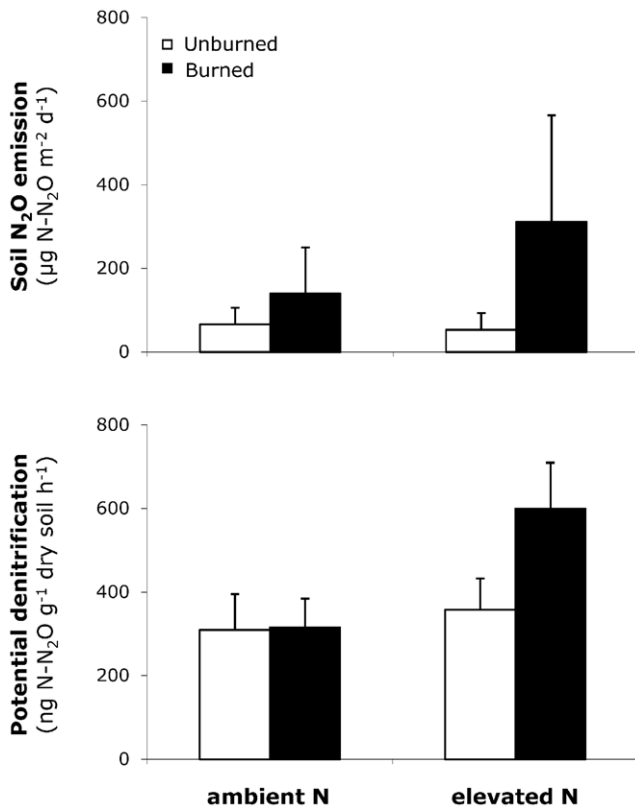


Figure 4. Effects of fire on soil N₂O emission and potential denitrification rates under ambient and elevated nitrogen supply. The top section shows the effects of burn and N on soil N₂O emission rates, and the bottom section shows the effects of burn and N on potential denitrification rates. The data of soil N₂O emission and potential denitrification rates are grouped by burn treatment (unburned: open bars, burned: closed bars) and by N treatment (ambient vs. elevated). Each bar is the average of data collected 15, 19, 21, and 33 months after fire. Error bars denote pooled standard error (for each N treatment, n=24×4 sampling dates for unburned plots; n=16×4 sampling dates for burned plots). doi:10.1371/journal.pone.0020105.g004

during the second year after fire [9], though the response was not detected three years after fire [9]. Increased C availability in the burned plots, possibly reflecting increased C inputs to the soil from accelerated soil organic matter decomposition or stimulated primary productivity following burning [8], might have reduced microbial C demand and thereby activity of C acquisition enzymes [9]. Furthermore, the burn treatment may also have increased the availability of other nutrients (e.g. phosphorus (P)). Indeed, at the end of the first year after fire, decreased tissue N:P ratio of grasses in the burned plots suggested increased P availability in these plots [8,34].

Increases in soil N₂O emission rates in response to fire were substantially larger when fire was combined with enhanced N supply and elevated CO₂ (Fig. 2). In the burned plots exposed to elevated N, denitrifying microorganisms likely benefited from higher substrate N availability, as evidenced by large significant increases in soil nitrate concentrations (+771% on average, $P < 0.0001$; Table S8) and in gross and potential nitrification rates (+72% on average, $P = 0.003$; Table S8 and +50% on average, $P < 0.0001$, Table S9) in response to added N. Increases in denitrification and soil N₂O emissions in the burned and fertilized plots (Fig. 4) thus probably resulted from increased soil moisture combined with greater C and N availability in these plots. The

additional increase in soil N₂O emission rates with elevated CO₂ in the burned and fertilized plots (Fig. 2) could have resulted from further increases in soil moisture (Table S7) due to decreased plant transpiration [35–36] or from increases in soil temperature in the burned plots exposed to elevated CO₂ ($P = 0.04$ for the Burn×CO₂ interaction; Tables S6 and S7). Indeed, elevated CO₂ reduced soil temperature in the unburned plots but increased soil temperature in the burned plots (+0.3°C at 2 cm depth; +0.5°C at 10 cm depth) which may have further stimulated denitrifying microorganisms, thus leading to even greater soil N₂O emissions. In our experiment, it is unlikely that the increase in soil N₂O emissions with elevated CO₂ in the burned fertilized plots resulted solely from an increase in soil labile C. Indeed, and in contrast with other studies [37], elevated CO₂ did not induce significant increases in root biomass production at our site [27], and even suppressed root allocation when combined with other treatments for some years [6]. Furthermore, elevated CO₂ neither substantially increased soil CO₂ emission rates (Tables S5, S6 and S7) nor substantially decreased extracellular enzyme activities involved in C acquisition [9].

Responses of soil N₂O emissions to fire over time

Soil N₂O emissions responses to fire changed over time: soil N₂O emissions were not significantly altered in the burned plots 9 months after fire, but were significantly increased in the burned plots during the second and third years after fire (Fig. 1).

The lack of increase in soil N₂O emissions in the burned plots 9 months after fire is probably due to the decrease in soil moisture and absence of increased C availability in the burned plots at this time. Indeed, at the end of the first year after fire, fire significantly decreased soil moisture (by 19%, $P = 0.01$; Table S5; Fig. 3), presumably due to the removal of surface litter and to the subsequent increase in soil temperature [8], and did not significantly alter soil CO₂ emission rates (Table S5) or extracellular enzyme activities [9]. Soil N₂O emissions were then consistently higher in the burned compared to unburned plots and remained elevated by 232% in the burned plots almost 3 years after the fire (Fig. 1). Similarly, potential denitrification rates remained elevated 33 months after fire (+63%, $P = 0.003$; Table S7; Fig. 3), consistent with the microbial process invoked for the increase in N₂O emissions in the burned plots. This long-lasting response of soil N₂O emissions to the fire disturbance deserves attention. First, this response is more persistent than previously reported for chaparral or forest ecosystems, in which increased soil N₂O emissions after fire lasted only several months and were associated with transient increases in soil inorganic N concentrations [22–25]. Second, this long-lasting response raises the question of whether increases in N₂O production in the burned plots persisted beyond year three after fire. Soil moisture and soil CO₂ emission rates remained significantly elevated in the burned plots 33 months after fire (+12%, $P = 0.004$ and +41%, $P = 0.009$; Table S7; Fig. 3). If the mechanisms proposed as responsible for increases in N₂O production in the burned plots (i.e. greater soil moisture due to changes in soil properties and greater soil C or P availability) have persisted beyond year three after fire, or if other long-lasting mechanisms have occurred (e.g. post-fire shifts in the soil microbial communities, as observed by [38]), then increases in denitrification-associated N₂O efflux may have lasted even longer. This is especially true for the burned fertilized plots, where N inputs could have sustained increased soil N₂O emissions.

Conclusions and implications

Our study provides evidence that global environmental changes could strongly amplify fire effects on soil N₂O production. We

found that over a three-year period following a fire in a California grassland, soil N₂O emission rates were increased by a factor of two in the burned plots (+124%) and by a factor of six in the burned plots exposed to elevated CO₂ and enhanced N supply (+516%). We also provide evidence that the underlying mechanism for this response was increased microbial denitrification, resulting from increased soil moisture and soil C and N availability in the burned and fertilized plots. The responses of soil N₂O emission rates to fire were large and persisted for at least three years after the fire. Thus, our results clearly stress the need to further explore the interactions between fire disturbances and global environmental changes. First, they indicate that limiting global change studies to undisturbed ecosystems could underestimate the impacts global environmental changes may have on soil greenhouse gas production. Second, they suggest that the interactive effects of fire and global environmental changes could play a significant role in controlling the greenhouse gas balance of grassland ecosystems and promote them as significant sources of N₂O.

Materials and Methods

The study site and experimental design

This study took place at the Jasper Ridge Global Change Experiment (JRGCE) in the Jasper Ridge Biological Preserve, which is located in the eastern foothills of the Santa Cruz Mountains in northern California (37°24'N, 122°14'W). The site experiences a Mediterranean-type climate with a cool, wet growing season from November to March, and a hot, dry summer from June to October. The dominant species are annual grasses (especially *Avena barbata* and *Bromus hordeaceus*) and annual forbs (especially *Geranium dissectum* and *Erodium botrys*) [26]. The soil is a fine, mixed, Typic Haploxeralf developed from Franciscan complex alluvium sandstone [9].

The JRGCE was initiated in November 1998 and provides a complete factorial design of four treatments at two levels (ambient vs. elevated): atmospheric CO₂ concentration (ambient vs. 680 μmol mol⁻¹), temperature (ambient vs. soil surface warming of 0.8–1.0°C), precipitation (ambient vs. +50% above ambient), and N supply (ambient vs. +7 g N m⁻² yr⁻¹) [6,26–27]. These treatments were selected to mimic conditions predicted to occur at the end of the 21st century for central California, and to allow a more comprehensive understanding of mechanisms driving the grassland responses to future changes in the environment [6,26–27]. CO₂ is elevated with a free air CO₂ enrichment (FACE) system delivering pure CO₂ at the plant height. Temperature is increased using overhead infrared heaters. Precipitation was enhanced at first with drip irrigation (1998–2000) and then with overhead sprinklers (2001–2006). N is applied twice per year as Ca(NO₃)₂, with an initial application of 2 g N m⁻² in solution early in the growing season (each November) and an additional application of 5 g N m⁻² as slow-release fertilizer (Nutricote 12-0-0, Agrivert, Riverside, CA, USA) later in the growing season (each January) [27]. Treatments are organized in a randomized block split-plot design, with CO₂ and temperature treatments applied at the plot level (in 2 m-diameter circular plots) and precipitation and N treatments applied at the subplot level (in 0.78 m²-quadrants – each plot being divided in four quadrants with 0.5 m solid belowground barriers and mesh aboveground partitions).

Each of the 16 possible treatment combinations was initially replicated eight times. However, in July 2003, an accidental, rapid and low-intensity fire ashed all aboveground litter in two of the eight blocks of the experiment [8–9]. Warming was discontinued in the burned plots immediately after the fire, thus leading to a new complete factorial design with four “treatments” at two levels:

burn (unburned vs. burned), CO₂, precipitation and N supply (ambient vs. elevated). Combinations of the CO₂, precipitation and N treatments were replicated six times in the unburned plots and four times in the burned plots [8–9].

Soil N₂O and CO₂ emission rates

Soil N₂O and CO₂ emission rates were measured during the three years following the fire disturbance: at the end of each of the three growing seasons (i.e. at the time of peak biomass of plants: 9, 21 and 33 months after fire), and for multiple dates during the second growing season after fire (at early germination: 15 months after fire; at mid vegetative stage: 19 months after fire). Measurements performed 15 months after fire were conducted at four dates following a simulated 20-mm rainfall event marking the end of the dry season (an average of data collected was used for analysis); measurements performed 19 months after fire were conducted at two dates (an average of data collected was used for analysis).

Measurements were made using a static chamber approach [39], with chambers (1.8 L/V) constructed from 10.2 cm-diameter PVC pipe closed with a PVC cap. At each measurement date, chambers were placed in each quadrant into a permanent respiration ring. Three subsequent 15 mL-headspace air samples were then taken at 15-minute intervals through a septum installed at the top of each chamber using a nylon syringe. Samples were then analyzed for N₂O and CO₂ concentrations on a gas chromatograph system (Agilent 6890 GC System, Palo Alto, CA) with Haysep Q 60/80 and Porapak Q 60/80 packed columns, equipped with an electron capture detector to determine N₂O concentrations and a flame-ionization detector with a methanizer to determine CO₂ concentrations. Field N₂O and CO₂ emission rates were calculated using linear regression analysis of concentrations over time.

Soil sampling

Soil cores (5 cm diameter×5 cm deep) were sampled in each quadrant at each date where soil N₂O emission rates were measured, i.e. 9, 15, 19, 21, and 33 months after fire. Soil samples collected 15 months after fire were collected prior to the simulated rainfall event. In addition, soil cores (2.2 cm diameter×15 cm deep) were collected 3 months before fire (i.e. at the end of the last growing season preceding the wildfire) and were used as control samples of the pre-burn conditions [28]. At each sampling date, large roots and rocks were removed by hand, and soil sample heterogeneity was reduced by thorough mixing. Soil samples were used for measurements of soil environmental variables (soil moisture, soil pH and soil NH₄⁺ and NO₃⁻ concentrations), as well as for measurements of gross or potential rates of N mineralization, nitrification and denitrification.

Soil environmental variables

Gravimetric soil moisture was determined at each soil sampling date by comparing the mass of a 5-g soil sample before and after drying at 105°C. Soil temperature data were obtained at hourly intervals from thermocouples buried at 2 cm and 10 cm below the soil surface in each quadrant, and averaged over each soil N₂O efflux measurement date. Soil pH was measured in 1:1 mixture of soil and distilled water on soil samples collected during year one and year three after fire (i.e. 9 and 33 months after fire). Soil NH₄⁺ and NO₃⁻ concentrations were measured on soil samples collected during year two after fire (i.e. 15, 19 and 21 months after fire). At each date, NH₄⁺ and NO₃⁻ were extracted in 25 mL of 0.25 M K₂SO₄ from 10 g soil samples, which were vigorously shaken for 30 min. Extracts were filtered and analyzed colorimetrically for

NH_4^+ and NO_3^- concentrations using an autoanalyzer (Lachat Quickchem FIA+8000).

Gross rates of N mineralization and nitrification

Gross N mineralization and nitrification rates were determined using ^{15}N pool dilution [40] on soil samples collected during year two after fire (i.e. 19 and 21 months after fire for gross N mineralization; 15, 19 and 21 months after fire for gross nitrification). At each date, two 50-g soil samples from each quadrant were placed in separate plastic bags, and 3 mL of either ^{15}N - $(\text{NH}_4)_2\text{SO}_4$ or ^{15}N - $\text{Ca}(\text{NO}_3)_2$ were added (99 atom % ^{15}N) and thoroughly mixed to each, producing target concentrations of $1 \mu\text{g } ^{15}\text{N g}^{-1}$ dry soil. One 10-g soil subsample was immediately taken and extracted with 25 mL 0.25 M K_2SO_4 for determination of the initial NH_4^+ and NO_3^- concentrations. After a 24 h incubation period in the field, a second 10-g subsample was taken and equally extracted. Extracts were filtered and analyzed colorimetrically for NH_4^+ and NO_3^- concentrations using an autoanalyzer (Lachat Quickchem FIA+8000). A diffusion procedure onto acidified filter disks was used to separate NH_4^+ and NO_3^- in soil extracts [41] and filter disks were then analyzed for ^{15}N - NH_4^+ and ^{15}N - NO_3^- contents by isotope ratio mass spectrometry at the Colorado Plateau Stable Isotope Laboratory (<http://www.mpcer.nau.edu/isotopelab/>). Gross N mineralization rates were calculated based on NH_4^+ and ^{15}N - NH_4^+ concentrations at time 0 and time 24, and gross nitrification rates based on NO_3^- and ^{15}N - NO_3^- concentrations at time 0 and time 24, according to ^{15}N pool dilution equations [40].

Potential rates of nitrification and denitrification

Potential nitrification and denitrification rates were measured on soil samples collected 3 months prior to the fire (data prior to the fire are from [28]) and on soil samples collected during year two and year three after fire (i.e. 19 and 21 months after fire for potential nitrification; 15, 19, 21 and 33 months after fire for potential denitrification). Measurements of potential rates of nitrification and denitrification are proxies of measurements of the concentrations of the nitrifying and denitrifying enzymes in soils [40,42]. These concentrations of enzymes (i) are determined by the *in situ* environmental constraints to which nitrifying and denitrifying microorganisms were exposed in the field prior to soil sampling; and (ii) are measured in laboratory incubations during which substrates are made non-limiting and environmental conditions are made optimal for the reaction considered, over time periods where *de novo* synthesis of enzymes does not occur [42–44]. Measurements of potential rates of nitrification and denitrification thus reflect the direction and magnitude of the environmental constraints in the field on the nitrifying and denitrifying microorganisms. Potential rates of nitrification and denitrification are thought to be more constant over time than *in situ* rates of nitrification and denitrification [44–45], and have been widely used to provide information on the impacts of environmental changes on the size of the nitrifying and denitrifying microbial communities.

Potential rates of nitrification were measured on soil samples collected prior to the fire according to [28]. Both potential rates of ammonia oxidation and nitrite oxidation – the two distinct steps of nitrification – were measured on soil samples collected year two after fire. Potential ammonia oxidation rates were measured as NO_2^- production rates from soil samples amended with NH_4^+ and NaClO_3 , an inhibitor of the microbial oxidation of NO_2^- into NO_3^- [46]: 5 g equivalent dry soil were supplied with 50 mL of a solution of 0.18 mM $(\text{NH}_4)_2\text{SO}_4$, 0.8 mM K_2HPO_4 , 0.1 mM KH_2PO_4 and 0.01 M NaClO_3 , and were incubated at 28°C for

9 h with agitation at 150 rpm. NO_2^- concentration was measured after 0 h, 3 h, 6 h and 9 h on a spectrophotometer (Uvikon 800, Leeds, UK) at 520 nm using the Griess reagent. Rates of NO_2^- production were constant during ammonia oxidation assays (data not shown). Potential nitrite oxidation rates were measured as NO_2^- consumption rates from soil samples amended with NO_2^- [47]: 5 g equivalent dry soil were supplied with 50 mL of a solution of 0.36 mM NaNO_2 and were incubated at 28°C for 30 h with agitation at 150 rpm. NO_2^- concentration was measured after 0 h, 9 h, 24 h and 30 h as described above. Rates of NO_2^- consumption were constant during nitrite oxidation assays (data not shown).

Potential denitrification rates were measured on soil samples collected prior to the fire [28] and on soil samples collected year two and year three after fire as N_2O production rates from soil samples amended with NO_3^- and labile C, and in which N_2O reductase was inhibited with acetylene [48]: 5 g equivalent dry soil were placed in 150 mL plasma flasks sealed with rubber stoppers and amended with 1 mg C-glucose g^{-1} dry soil, 1 mg C-glutamic acid g^{-1} dry soil and 0.1 mg N- $\text{NO}_3^- \text{g}^{-1}$ dry soil. The atmosphere of the flask was replaced by a He: C_2H_2 mixture (90:10) to ensure anaerobic conditions and inhibition of N_2O reductase. Flasks were incubated at 27°C for 8 h. N_2O concentration was measured every two hours on a gas chromatograph equipped with an electron capture detector (Agilent Micro GC, P200). Rates of N_2O production were constant during denitrification assays (data not shown).

Plant and litter biomass

End-season plant and litter biomass were measured at the end of the growing season preceding the fire (i.e. 3 months before fire) and at the end of the three growing seasons following the fire (i.e. 9, 21 and 33 months after fire). At each measurement date, all aboveground plant matter was collected in a 141 cm^2 area and separated into live and litter material, and root biomass was determined by separating live roots out of 15 cm-depth soil cores taken in the area of the aboveground biomass harvest [8,27]. Aboveground biomass, senesced aboveground tissue and belowground biomass were oven-dried at 70°C before weighing. Total plant biomass was estimated as the sum of above- and belowground biomass, and litter biomass as the biomass of senesced aboveground tissue. Data on biomass prior to the fire are from [27]; data 9 months after the fire are from [8]; preliminary data 21 and 33 months after the fire are available on request from Nona Chiariello (nonajrbp@stanford.edu).

Statistical analysis

All statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC, USA). We analyzed our data with PROC MIXED using a repeated four-way split-plot analysis of variance in order to assess the overall effects of the burn and other global environmental changes treatments on soil N_2O emissions over the three year-period following the fire, as well as the temporal variability of these treatment effects. The burn and CO_2 treatments were included as whole-plot effects, while the precipitation and N treatments were included as split-plot effects. We also analyzed our data for each individual measurement date using a full factorial split-plot mixed model in order to assess the treatment effects at each date. As the responses of soil N_2O emissions to the burn treatment differed depending on year since fire, we analyzed the effects of the treatments on the other variables for each individual year after fire. Data collected year one or year three after fire were analyzed using a full factorial split-plot mixed model. Data collected year two after fire were analyzed

using a repeated four-way split-plot analysis of variance (all measurement dates were included, except for soil moisture for which data collected 15 months after fire were excluded from the analysis as measurements of soil N₂O emissions were performed following a wet-up experiment, while measurements of soil moisture were conducted on soil samples collected prior to the wet-up experiment). In addition, we analyzed the data collected 3 months before fire using the same full factorial split-plot model to verify that prior to the fire, microbial and plant characteristics were indistinguishable between the plots that later burned and those that did not (Table S1). We also analyzed the response of the soil N₂O emissions to the “previously warmed” treatment in the burned plots by carrying out analyses of variance with PROC MIXED to verify that soil N₂O emissions were indistinguishable between the previously warmed burned plots and the previously un-warmed burned plots (Table S2). Data were square-root or log transformed prior to analysis to correct non-homogeneity of variance (a square-root transformation was used for soil N₂O and CO₂ emission rates and potential nitrification; a log transformation was used for other variables). Effects with $P < 0.05$ are referred to as significant.

Finally, we performed correlation analyses between soil N₂O emission rates, rates of microbial processes contributing to N₂O production in soils (i.e. gross or potential rates of nitrification and denitrification), and main drivers of these microbial processes (i.e. soil environmental variables, gross N mineralization and soil CO₂ emission rates) to provide insights into the mechanisms controlling the response of the soil N₂O emissions to the treatments. Given the large number of correlations performed, we applied Bonferroni corrections by dividing α ($\alpha = 0.05$) by the number n of correlations ($n = 11$) and by checking significance at $P < \alpha/n$ (i.e. $P < 0.0045$).

Supporting Information

Table S1 Treatment effects on potential nitrification and denitrification, and litter and plant biomass prior to the fire ($n = 80$). Treatments are elevated CO₂ (CO₂), increased precipitation (W) and N supply (N). The treatment “B” (plots that burned in July 2003) was included in the analysis to verify that the variables measured in April 2003 were indistinguishable between the plots that later burned and those that did not. Significant responses are indicated in bold ($\alpha = 0.05$). As shown in the table, prior to the fire, no measured variable was significantly different between the plots that later burned and those that remained untouched by the fire (“B”: $P > 0.05$ in all cases). Data of potential N rates prior to the fire are from [28]; data of plant biomass prior to the fire are from [27].
(DOC)

Table S2 Effect of the “previously warmed” treatment on soil N₂O emission rates in the burned plots ($n = 32$ for each measurement date). As shown in the table, for each measurement date, soil N₂O emission rates were not significantly different between the previously warmed burned plots and the previously un-warmed burned plots ($P > 0.05$ in all cases).
(DOC)

Table S3 Treatment effects on soil N₂O emission rates over the three years following the fire ($n = 80 \times 5$ sampling dates – 9, 15, 19, 21 and 33 months after fire). Treatments are burn (B), elevated CO₂ (CO₂), increased precipitation (W), and N supply (N). Significant responses are indicated in bold ($\alpha = 0.05$). The overall effect of the burn treatment was calculated as: % effect = $100 \times [\text{burned} - \text{unburned}] / \text{unburned}$ ($n = 32 \times 5$ in the burned plots, $n = 48 \times 5$ in the unburned plots). The overall effects of the CO₂, precipitation, and N treatments were calculated as: % effect = $100 \times [\text{elevated} - \text{ambient}] / \text{ambient}$ ($n = 40 \times 5$ in the elevated and ambient plots).
(DOC)

the unburned plots). The overall effects of the CO₂, precipitation, and N treatments were calculated as: % effect = $100 \times [\text{elevated} - \text{ambient}] / \text{ambient}$ ($n = 40 \times 5$ in the elevated and ambient plots).
(DOC)

Table S4 Mean values of soil N₂O and CO₂ emission rates and soil N cycling variables in the unburned and burned plots. For each variable, data were grouped by burn treatment (unburned vs. burned) and averaged across the CO₂, precipitation and nitrogen treatments. Values indicate mean (averaged across all available measurement dates) \pm pooled standard error. The number of data in the unburned and burned plots multiplied by the number of measurement dates for each variable is indicated in parentheses. Refer to Tables S3 and Tables S5 to S9 for the results from mixed model analysis testing for a fire effect on the variables presented in Table S4.
(DOC)

Table S5 Treatment effects on soil CO₂ emission rates, soil moisture and soil temperature (at 2 cm depth) year one after fire ($n = 80$). Treatments are burn (B), elevated CO₂ (CO₂), increased precipitation (W), and N supply (N). Significant responses are indicated in bold ($\alpha = 0.05$). The overall effect of the burn treatment was calculated as: % effect = $100 \times [\text{burned} - \text{unburned}] / \text{unburned}$ ($n = 32$ in the burned plots, $n = 48$ in the unburned plots). The overall effects of the CO₂, precipitation, and N treatments were calculated as: % effect = $100 \times [\text{elevated} - \text{ambient}] / \text{ambient}$ ($n = 40$ in the elevated and ambient plots).
(DOC)

Table S6 Treatment effects on potential denitrification, soil CO₂ emission rates, soil moisture and soil temperature (at 2 cm depth) year two after fire ($n = 80 \times 3$ sampling dates – 15, 19 and 21 months after fire, except for soil moisture where $n = 80 \times 2$ sampling dates – 19 and 21 months after fire). Treatments are burn (B), elevated CO₂ (CO₂), increased precipitation (W), and N supply (N). Significant responses are indicated in bold ($\alpha = 0.05$). The overall effect of the burn treatment was calculated as: % effect = $100 \times [\text{burned} - \text{unburned}] / \text{unburned}$ ($n = 32 \times 3$ in the burned plots, $n = 48 \times 3$ in the unburned plots). The overall effects of the CO₂, precipitation, and N treatments were calculated as: % effect = $100 \times [\text{elevated} - \text{ambient}] / \text{ambient}$ ($n = 40 \times 3$ in the elevated and ambient plots).
(DOC)

Table S7 Treatment effects on potential denitrification, soil CO₂ emission rates, soil moisture and soil temperature (at 2 cm depth) year three after fire ($n = 80$). Treatments are burn (B), elevated CO₂ (CO₂), increased precipitation (W), and N supply (N). Significant responses are indicated in bold ($\alpha = 0.05$). The overall effect of the burn treatment was calculated as: % effect = $100 \times [\text{burned} - \text{unburned}] / \text{unburned}$ ($n = 32$ in the burned plots, $n = 48$ in the unburned plots). The overall effects of the CO₂, precipitation, and N treatments were calculated as: % effect = $100 \times [\text{elevated} - \text{ambient}] / \text{ambient}$ ($n = 40$ in the elevated and ambient plots).
(DOC)

Table S8 Treatment effects on gross N mineralization, gross nitrification and soil NH₄⁺ and NO₃⁻ concentrations year two after fire ($n = 80 \times 3$ sampling dates – 15, 19 and 21 months after fire, except for N mineralization where $n = 80 \times 2$ sampling dates – 19 and 21 months after fire). Treatments are burn (B), elevated CO₂ (CO₂), increased precipitation (W), and N supply (N). Significant responses are indicated in bold ($\alpha = 0.05$). The overall effect of the burn treatment was calculated as: % effect = $100 \times [\text{burned} - \text{unburned}] / \text{unburned}$ ($n = 32 \times 3$ in the burned plots, $n = 48 \times 3$ in the unburned plots). The overall effects of the CO₂, precipitation, and N treatments were calculated as: % effect = $100 \times [\text{elevated} - \text{ambient}] / \text{ambient}$ ($n = 40 \times 3$ in the elevated and ambient plots).
(DOC)

precipitation, and N treatments were calculated as: % effect = $100 \times [\text{elevated} - \text{ambient}] / \text{ambient}$ ($n = 40 \times 3$ in the elevated and ambient plots).

(DOC)

Table S9 Treatment effects on potential ammonia and nitrite oxidation year two after fire ($n = 80 \times 2$ sampling dates – 19 and 21 months after fire). Treatments are burn (B), elevated CO₂ (CO₂), increased precipitation (W), and N supply (N). Significant responses are indicated in bold ($\alpha = 0.05$). The overall effect of the burn treatment was calculated as: % effect = $100 \times [\text{burned} - \text{unburned}] / \text{unburned}$ ($n = 32 \times 2$ in the burned plots, $n = 48 \times 2$ in the unburned plots). The overall effects of the CO₂, precipitation, and N treatments were calculated as: % effect = $100 \times [\text{elevated} - \text{ambient}] / \text{ambient}$ ($n = 40 \times 2$ in the elevated and ambient plots).

(DOC)

References

- IPCC (2007) Climate change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the IPCC; Solomon S, Qin D, Manning M, Chen Z, Marquis M, et al., eds. Cambridge, United Kingdom and New York, New York, USA: Cambridge University Press. 1009 p.
- Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, et al. (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry* 70: 153–226.
- Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, et al. (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320: 889–892.
- IPCC (2007) Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change; Parry ML, Canziani OF, Palutikof JP, van der Linden PJ, Hanson CE, eds. Cambridge, United Kingdom and New York, New York, USA: Cambridge University Press. 976 p.
- Reich PB, Hobbie SE, Lee T, Ellsworth DS, West JB, et al. (2006) Nitrogen limitation constrains sustainability of ecosystem response to CO₂. *Nature* 440: 922–925.
- Shaw MR, Zavaleta ES, Chiariello NR, Cleland EE, Mooney HA, et al. (2002) Grassland responses to global environmental changes suppressed by elevated CO₂. *Science* 298: 1987–1990.
- Norby RJ, Rustad LE, Dukes JS, Ojima DS, Parton WJ, et al. (2007) Ecosystem responses to warming and interacting global change factors. In: Canadell J, Pataki D, Pitelka L, eds. *Terrestrial Ecosystems in a Changing World*. Berlin, Germany: Springer. pp 23–36.
- Henry HAL, Chiariello NR, Vitousek PM, Mooney HA, Field CB (2006) Interactive effects of fire, elevated Carbon dioxide, Nitrogen deposition, and precipitation on a California annual grassland. *Ecosystems* 9: 1066–1075.
- Gutknecht JLM, Henry HAL, Balsler TC (2010) Inter-annual variation in soil extra-cellular enzyme activity in response to simulated global change and fire disturbance. *Pedobiologia* 53: 283–293.
- Forster P, Ramaswamy V, Artaxo P, Bernsten T, Betts R, et al. (2007) Changes in Atmospheric Constituents and in Radiative Forcing. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, et al., eds. *Climate Change 2007: The Physical Science Basis Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, United Kingdom and New York, USA: Cambridge University Press. pp 129–234.
- Smith KA (2010) *Nitrous Oxide and Climate Change*. London, UK: Earthscan. 256 p.
- Kool DM (2010) On the origin of nitrous oxide and its oxygen [thesis]. Wageningen, Netherlands: Wageningen University. 199 p.
- Shaw LZ, Nicol GW, Smith Z, Fear J, Prosser JI, et al. (2006) *Nitrosospora* spp. can produce nitrous oxide via a nitrifier denitrification pathway. *Environmental Microbiology* 8: 214–222.
- Wrage N, Velthof GL, van Beusichem ML, Oenema O (2001) Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biology and Biochemistry* 33: 1723–1732.
- Tiedje JM (1988) Ecology of denitrification and dissimilatory nitrate reduction to ammonium. In: Zehnder AJB, ed. *Environmental Microbiology of Anaerobes*. New York, New York, USA: John Wiley and Sons. pp 179–244.
- Kamman C (2001) Impact of rising atmospheric CO₂ concentrations on the fluxes of the greenhouse gases N₂O and CH₄ in a grassland ecosystems [thesis]. Giessen, Germany: University of Giessen. 228 p.
- Baggs EM, Richter M, Cadisch G, Hartwig UA (2003) Denitrification in grass swards is increased under elevated atmospheric CO₂. *Soil Biology and Biochemistry* 35: 729–732.
- Ineson P, Coward PA, Hartwig UA (1998) Soil gas fluxes of N₂O, CH₄ and CO₂ beneath *Lolium perenne* under elevated CO₂: the swiss free air carbon dioxide enrichment experiment. *Plant and Soil* 198: 89–95.
- Arnone JA, Bohlen PJ (1998) Stimulated N₂O flux from intact grassland monoliths after two growing seasons under elevated atmospheric CO₂. *Oecologia* 116: 331–335.
- Skiba U, Smith KA (2000) The control of nitrous oxide emissions from agricultural and natural soils. *Chemosphere - Global Change Science* 2: 379–386.
- Barnard R, Leadley PW, Hungate BA (2005) Global Change, nitrification, and denitrification: a review. *Global Biogeochemical Cycles* 19: 1–13.
- Levine JS, Wesley R, III C, Sebacher DI (1988) The effects of fire on biogenic soil emissions of nitric oxide and nitrous oxide. *Global Biogeochemical Cycles* 2: 445–449.
- Anderson IC, Levine JS, Porter JH, Riggan PJ (1988) Enhanced biogenic emissions of nitric oxide and nitrous oxide following surface biomass burning. *Journal of Geophysical Research* 93: 3893–3898.
- Weitz AM, Veldkamp E, Keller M, Neff J, Crill PM (1998) Nitrous oxide, nitric oxide, and methane fluxes from soils following clearing and burning of tropical secondary forest. *Journal of Geophysical Research* 103: 28047–28058.
- Melillo JM, Steudler PA, Feigl BJ, Neill C, Garcia D, et al. (2001) Nitrous oxide emissions from forests and pastures of various ages in the Brazilian Amazon. *Journal of Geophysical Research* 106: 34179–34188.
- Zavaleta ES, Shaw MR, Chiariello NR, Thomas BD, Cleland EE, et al. (2003) Grassland responses to three years of elevated temperature, CO₂, precipitation, and N deposition. *Ecological Monographs* 73: 585–604.
- Dukes JS, Chiariello NR, Cleland EE, Moore LA, Shaw MR, et al. (2005) Responses of grassland production to single and multiple global environmental changes. *PLoS Biology* 3: 1829–1837.
- Barnard R, Le Roux X, Hungate BA, Cleland EE, Blankinship JC, et al. (2006) Several components of global change alter nitrifying and denitrifying activities in an annual grassland. *Functional Ecology* 20: 557–564.
- Cantarel AAM, Bloor JMG, Deltroy N, Soussana JF (2011) Effects of Climate Change Drivers on Nitrous Oxide Fluxes in an Upland Temperate Grassland. *Ecosystems* 14: 223–233.
- Attard E, Recous S, Chabbi A, De Berranger C, Guillaumeud N, et al. (2011) Soil environmental conditions rather than denitrifier abundance and diversity drive potential denitrification after changes in land uses. *Global Change Biology* 17: 1975–1989.
- Lunch CK (2009) Primary productivity in an annual grassland ecosystem: Responses to global change and local environmental variation [thesis]. Stanford/California, USA: Stanford University. 99 p.
- Niboyet A (2008) Impact des changements globaux sur le cycle de l'azote [thesis]. Orsay, France: Université Paris-Sud 11. 251 p.
- Wohlgemuth PM, Hubbert K, Arbaugh MJ (2006) Fire and Physical Environments Interactions: Soil, Water, and Air. In: Sugihara NG, van Wagtenonk JW, Shaffer KE, Fites-Kaufman JA, Thode AE, eds. *Fire in California's Ecosystems*. University of California Press. pp 75–93.
- Dukes JS, Shaw MR (2007) Responses to changing atmosphere and climate. In: Stromberg M, Corbin J, D'Antonio C, eds. *Ecology and Management of California Grasslands*. Berkeley/California, USA: University of California Press. pp 218–229.
- Field CB, Jackson RB, Mooney HA (1995) Stomatal responses to increased CO₂: implications from the plant to the global scale. *Plant, Cell and Environment* 18: 1214–1225.
- Zavaleta ES, Thomas BD, Chiariello NR, Asner GP, Shaw MR, et al. (2003) Plants reverse warming effect on ecosystem water balance. *Proceedings of the National Academy of Sciences of the United States of America* 100: 9892–9893.

Acknowledgments

The authors would like to thank Jeffrey S. Dukes, Hugh A. Henry, and Benjamin Z. Houlton for constructive comments on this manuscript, Stéphane Robin for help with statistical analysis, Annick Ambroise, Sandrine Fontaine and Nadine Guillaumeud for help with laboratory measurements, and Christian Andreassi, Nona Chiariello, Noel Gurwick, Jessica Gutknecht, Yuka Otsuki Estrada and Alison Rountree for help at the JRGCE.

Author Contributions

Conceived and designed the experiments: CBF. Performed the experiments: AN JRB PD JCB PWL XLR LB RLB CBF BAH. Analyzed the data: AN. Wrote the paper: AN BAH.

37. de Graaff M, van Groenigen K, Six J, Hungate BA, van Kessel C (2006) Interactions between plant growth and soil nutrient cycling under elevated CO₂: a meta-analysis. *Global Change Biology* 12: 2077–2091.
38. Yeager CM, Northup DE, Grow CC, Barns SM, Kuske CR (2005) Changes in nitrogen-fixing and ammonia oxidizing bacterial communities in soil of a mixed conifer forest after wildfire. *Applied and Environmental Microbiology* 71: 2713–2722.
39. Hutchinson GL, Mosier AR (1981) Improved soil cover method for field measurement of nitrous oxide fluxes. *Soil Science Society of America Journal* 45: 311–316.
40. Hart SC, Stark JM, Davidson EA, Firestone MK (1994) Nitrogen mineralization, immobilization, and nitrification. In: Weaver R, ed. *Methods of Soil Analysis Part 2 - Microbiological and Biochemical Properties*. Madison Wisconsin, USA: Soil Science Society of America. pp 985–1018.
41. Stark JM, Hart SC (1996) Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. *Soil Science Society of America Journal* 60: 1846–1855.
42. Tiedje JM (1982) Denitrification. In: Page AL, Miller RH, Keeney DR, eds. *Methods of Soil Analysis, Part 2*. Madison Wisconsin, USA: American Society of Agronomy. pp 1011–1026.
43. Pinay G, Barbera P, Carreras-Palou A, Fromin N, Sonié L, et al. (2007) Impact of atmospheric CO₂ and plant life forms on soil microbial activities. *Soil Biology and Biochemistry* 39: 33–42.
44. Niboyet A, Barthes L, Hungate BA, Le Roux X, Bloor JMG, et al. (2010) Responses of soil nitrogen cycling to the interactive effects of elevated CO₂ and inorganic N supply. *Plant and Soil* 327: 35–47.
45. McGill BM, Sutton-Grier AE, Wright JP (2010) Plant trait diversity buffers variability in denitrification potential over changes in season and soil conditions. *PLoS One* 5: e11618.
46. Belser LW, Mays EL (1980) Specific inhibition of nitrite oxidation by chlorate and its use in assessing nitrification in soils and sediments. *Applied and Environmental Microbiology* 39: 505–510.
47. Wertz S, Degrange V, Prosser JI, Poly F, Commeaux C, et al. (2007) Decline of soil microbial diversity does not influence the resistance and resilience of key soil microbial functional groups following a model disturbance. *Environmental Microbiology* 9: 2211–2219.
48. Smith MS, Tiedje JM (1979) Phases of denitrification following oxygen depletion in soil. *Soil Biology and Biochemistry* 11: 261–267.