

## PRIMARY RESEARCH ARTICLE

# Terrestrial N<sub>2</sub>O emissions and related functional genes under climate change: A global meta-analysis

Linfeng Li<sup>1,2</sup>  | Zhenzhen Zheng<sup>1</sup> | Weijin Wang<sup>2,3,4</sup> | Joel A. Biederman<sup>5</sup> |  
 Xingliang Xu<sup>6</sup>  | Qinwei Ran<sup>1</sup> | Ruyan Qian<sup>1</sup> | Cong Xu<sup>1</sup> | Biao Zhang<sup>1</sup> |  
 Fang Wang<sup>1,2</sup> | Shutong Zhou<sup>1</sup> | Lizhen Cui<sup>1</sup> | Rongxiao Che<sup>7</sup> | Yanbin Hao<sup>1,8</sup>  |  
 Xiaoyong Cui<sup>1,8</sup> | Zhihong Xu<sup>2</sup> | Yanfen Wang<sup>1,8</sup>

<sup>1</sup>College of Life Sciences, University of Chinese Academy of Sciences, Beijing, China

<sup>2</sup>Environmental Futures Research Institute, School of Environment and Science, Griffith University, Brisbane, Qld, Australia

<sup>3</sup>Department of Environment and Science, Brisbane, Qld, Australia

<sup>4</sup>School of Agriculture and Food Sciences, University of Queensland, Brisbane, Qld, Australia

<sup>5</sup>Southwest Watershed Research Center, Agricultural Research Service, Tucson, AZ, USA

<sup>6</sup>Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences (CAS), Beijing, China

<sup>7</sup>Institute of International Rivers and Eco-security, Yunnan University, Kunming, China

<sup>8</sup>CAS Center for Excellence in Tibetan Plateau Earth Sciences, Chinese Academy of Sciences (CAS), Beijing, China

## Correspondence

Yanbin Hao and Yanfen Wang, College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China. Emails: ybhao@ucas.ac.cn; yfwang@ucas.ac.cn

## Funding information

CAS Strategic Priority Research Programme (A), Grant/Award Numbers: XDA19030202 and XDA20050103; International Cooperation and Exchange of National Natural Science Foundation of China, Grant/Award Numbers: 31761123001 and 31761143018

## Abstract

Nitrous oxide (N<sub>2</sub>O) emissions from soil contribute to global warming and are in turn substantially affected by climate change. However, climate change impacts on N<sub>2</sub>O production across terrestrial ecosystems remain poorly understood. Here, we synthesized 46 published studies of N<sub>2</sub>O fluxes and relevant soil functional genes (SFGs, that is, *archaeal amoA*, *bacterial amoA*, *nosZ*, *narG*, *nirK* and *nirS*) to assess their responses to increased temperature, increased or decreased precipitation amounts, and prolonged drought (no change in total precipitation but increase in precipitation intervals) in terrestrial ecosystem (i.e. grasslands, forests, shrublands, tundra and croplands). Across the data set, temperature increased N<sub>2</sub>O emissions by 33%. However, the effects were highly variable across biomes, with strongest temperature responses in shrublands, variable responses in forests and negative responses in tundra. The warming methods employed also influenced the effects of temperature on N<sub>2</sub>O emissions (most effectively induced by open-top chambers). Whole-day or whole-year warming treatment significantly enhanced N<sub>2</sub>O emissions, but daytime, nighttime or short-season warming did not have significant effects. Regardless of biome, treatment method and season, increased precipitation promoted N<sub>2</sub>O emission by an average of 55%, while decreased precipitation suppressed N<sub>2</sub>O emission by 31%, predominantly driven by changes in soil moisture. The effect size of precipitation changes on *nirS* and *nosZ* showed a U-shape relationship with soil moisture; further insight into biotic mechanisms underlying N<sub>2</sub>O emission response to climate change remain limited by data availability, underlying a need for studies that report SFG. Our findings indicate that climate change substantially affects N<sub>2</sub>O emission and highlights the urgent need to incorporate this strong feedback into most climate models for convincing projection of future climate change.

## KEYWORDS

drought, nitrous oxide, precipitation, soil moisture, soil N cycle, warming

## 1 | INTRODUCTION

Rising atmospheric concentrations of greenhouse gases drives changes in both temperature and the hydrological cycle, including altered amounts and timing of precipitation. Although CO<sub>2</sub> is the most abundant greenhouse gas in the atmosphere, the global warming potential of N<sub>2</sub>O is 265 times greater than that of CO<sub>2</sub> (Stocker, 2014). In terrestrial ecosystems, temperature and precipitation changes alter soil microclimate (Liu et al., 2016), nutrient availability (Bai et al., 2013) and microbial ecology (Zhang et al., 2013), thereby impacting N<sub>2</sub>O (Dalal & Allen, 2008). Therefore, our ability to predict future climate depends in part on a comprehensive understanding of feedbacks between terrestrial N<sub>2</sub>O emissions and ongoing climate change.

Taken individually, past studies of N<sub>2</sub>O flux response to climate change show a wide variety of responses such as the positive (Cui et al., 2018), neutral (Li et al., 2016) and negative effects (Hu et al., 2010) on N<sub>2</sub>O emission under experimental warming. Possible reasons for such variation include differences in biome type, climate manipulation characteristics, and experimental methods, all of which may impact the microbial communities mediating N<sub>2</sub>O emission. Across biomes, variation in vegetation composition (i.e. shrubs, graminoids and bryophytes) often moderates climate change effects on greenhouse gas fluxes (i.e. CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O; Kuiper, Mooij, Bragazza, & Robroek, 2014; Ward et al., 2013), due to different plant and microbial traits (de Vries et al., 2012; Teuling et al., 2010). Climate manipulation characteristics affecting N<sub>2</sub>O emissions may include intensity, duration and timing (seasonal and/or diurnal) of an impact. These characteristics determine how the impact translates into soil microclimate (e.g. changes in soil moisture under different drought intensity by Li et al., 2018) and non-linear microbial responses to resultant stress (McHale, Mitchell, & Bowles, 1998). At the seasonal scale, warming outside the growing season (i.e. cold season) of a peatland tended to exert greater effects on N<sub>2</sub>O emissions than in the growing season (Ward et al., 2013). At the diurnal scale, warming applied at different times of the day may have different impacts on nitrogen pools and dynamics (Bai et al., 2013). When biome type and climate impacts are consistent, differences in N<sub>2</sub>O emissions may be related to nutrient availability (Dijkstra, Morgan, Follett, & Lecaen, 2013) and/or microbial community composition (Bijoor, Czimczik, Pataki, & Billings, 2008) with treatment duration. Finally, a wide variety of methods and equipment are employed in manipulative climate change experiments, potentially impacting soil microclimate, microbiology and, ultimately, N<sub>2</sub>O responses. For experimental warming, air and soil temperature can be manipulated with methods including open-top chambers (OTC), heating cables, infrared radiators, glasshouses and curtains. Each methodology has its specific characteristics and limitations (Aronson & McNulty, 2009), creating different ecological impacts on nitrogen cycling (Bai et al., 2013). Precipitation changes are most commonly imposed using fully or partly covered rainout shelters (Hoover, Wilcox, & Young, 2018), resulting in discrepant ecological effects (Hoover & Rogers, 2016).

Ultimately, the physical and biological characteristics of each experiment impact the microorganisms involved in soil N<sub>2</sub>O production. These microbes may now be quantified using recent innovations in molecular biology, providing insight into biotic mechanisms mediating the climate–N<sub>2</sub>O feedback (Kuypers, Marchant, & Kartal, 2018). The functional genes that encode N redox enzymes are widely used in environmental studies as gene markers for nitrifiers and denitrifiers (Figure S1). For example, Archaeal *amoA* and bacterial *amoA* genes are used as genetic markers for ammonia-oxidizing archaea and ammonia-oxidizing bacteria respectively (Francis, Roberts, Beman, Santoro, & Oakley, 2005). *NarG* is used as a gene marker for the detection of microorganisms that reduce nitrate to nitrite (Kandeler, Deiglmayr, Tschirko, Bru, & Philippot, 2006). *NirS* and *nirK* that encode cd1-NIR and Cu-NIR are used as gene markers for denitrifiers that reduce nitrite to NO (Henry et al., 2004; Kandeler et al., 2006). However, to date, there have been no reviews on how climate change affected the soil functional genes (SFGs) involved in nitrification and denitrification, limiting our understanding of the biotic drivers' impacts on responses of N<sub>2</sub>O flux to climate change.

Recently, several meta-analyses have examined N<sub>2</sub>O fluxes under climate changes, but these were limited in scope by a lack of data, a narrow focus on one type of climate change (e.g. warming by Bai et al., 2013 or drought by Homyak, Allison, Huxman, Goulden, & Treseder, 2017), or limitation to a specific biome type (e.g. Mediterranean climate cropping systems by Cayuela et al., 2017). Besides, few previous meta-analyses assessed how different treatment durations and intensities affected N<sub>2</sub>O flux responses. Given the diversity of biomes, climate manipulations and methods across individual site-based studies, there is a need for synthesis across the rapidly growing body of site-level N<sub>2</sub>O flux studies at a global scale.

To address these knowledge gaps in how climate change alters N<sub>2</sub>O flux from terrestrial ecosystems, we compiled 210 observational data points from 46 published articles (Table S2) to quantitatively synthesize N<sub>2</sub>O fluxes and the relevant SFGs under climate changes, including both warming and changes in precipitation regime. The objectives of this study were to (a) identify global-scale patterns of N<sub>2</sub>O emissions and the relevant SFG responses to various types of climate changes; (b) relate the variability in the responses among studies to different biomes, treatment methods, treatment time, treatment season as well as treatment duration and intensity; and (c) examine underlying biotic mechanisms for the alteration in N<sub>2</sub>O emissions in response to climate changes.

## 2 | MATERIALS AND METHODS

### 2.1 | Data selection

We collected publications that reported on responses of N<sub>2</sub>O or/and SFGs (i.e. archaeal *amoA*, bacterial *amoA*, *nosZ*, *narG*, *nirK* and *nirS*) to experimental precipitation and/or temperature

manipulations in the field until November 11, 2018 by searching Web of Science using a range of search terms and wildcards (Table S1). To better represent responses of N<sub>2</sub>O and SFGs under natural conditions, we did not include greenhouse mesocosm or laboratory experiments. Water control experiments using sewage and wastewater, or irrigation management studies in cropland (e.g. drainage and flood irrigation) were not included. Similarly, studies on altering water table levels in wetlands or fens, changes in snow cover or drying-rewetting cycles were also excluded. We limited our analyses to results from plots that solely manipulated precipitation or temperature; results from plots with changing precipitation or temperature combined with other resource manipulations were excluded. N<sub>2</sub>O fluxes show seasonal variations. To reduce bias caused by limited sampling dates, we only included studies that measured N<sub>2</sub>O fluxes for least 3 months. If a study reported results from experiments conducted in different locations or having multiple distinct treatments, these components were treated as individual case studies. Because effect size (natural log of the response ratio, Equation 1) could not be calculated for those observations with zero or negative values under either control or treatments, data from studies that contained zero or negative values were not included (21 out of 231 observational data points). In total, our meta-analysis included 46 published papers, including 72 precipitation or temperature manipulation case studies (Table S2).

The manipulative climate change studies in the field were grouped into four types of treatment: (a) increased precipitation, (b) decreased precipitation, (c) increased temperature and (d) prolonged drought (increased the intervals of precipitation but did not alter the precipitation quantity). For each selected study, we collected latitude, longitude, mean annual temperature (MAT) and precipitation (MAP), and one of five main biome types: forest, shrubland, grassland (including grassland, lawn, meadow, prairie, steppe and savannah), cropland (including wheat, rice, maize and apple) and tundra. The geographical locations of the studies involving N<sub>2</sub>O fluxes under climate changes spanned from 2°53'N to 56°29'N (Figure 1), with the MAT ranging from -5.2 to 26.0°C, and the MAP from 108 to 2,000 mm (Table S2). Studies reporting SFGs were conducted at nine geographically and climatically diverse sites including Inner Mongolia, Hebei and Guangdong in China, Iberian Peninsula in Spain, northern Minnesota in United States, Austria and Switzerland (Figure 1).

We also collected information on treatment time (whole-day, daytime or nighttime warming for increased temperature), treatment season (whole-year, growing season, winter and several-day for increased temperature; whole-year and growing season for increased precipitation), treatment duration and treatment method (heating cables, infrared radiators, curtains and OTCs for increased temperature; exclusion [excludes all ambient rainfall] or reduction [excludes a fixed proportion of the ambient rainfall] for decreased precipitation; Table S2). Control and treatment means ( $\bar{X}$ ), standard deviations ( $s$ ), and sample sizes ( $n$ ) of N<sub>2</sub>O, SFGs and corresponding soil water content and temperature were directly extracted or

recalculated from digitized figures of each study using Digitizer (Free Software Foundation, Inc.).

## 2.2 | Statistical analyses

Natural log of the response ratio ( $\ln R$ ), defined as the 'effect size', was used to assess the responses of N<sub>2</sub>O fluxes (average or cumulative fluxes over a specific measurement period), SFGs and other variables to climate change treatments, which were calculated as:

$$\ln R = \ln \left( \frac{\bar{X}_t}{\bar{X}_c} \right), \quad (1)$$

with a variation ( $v$ ) of:

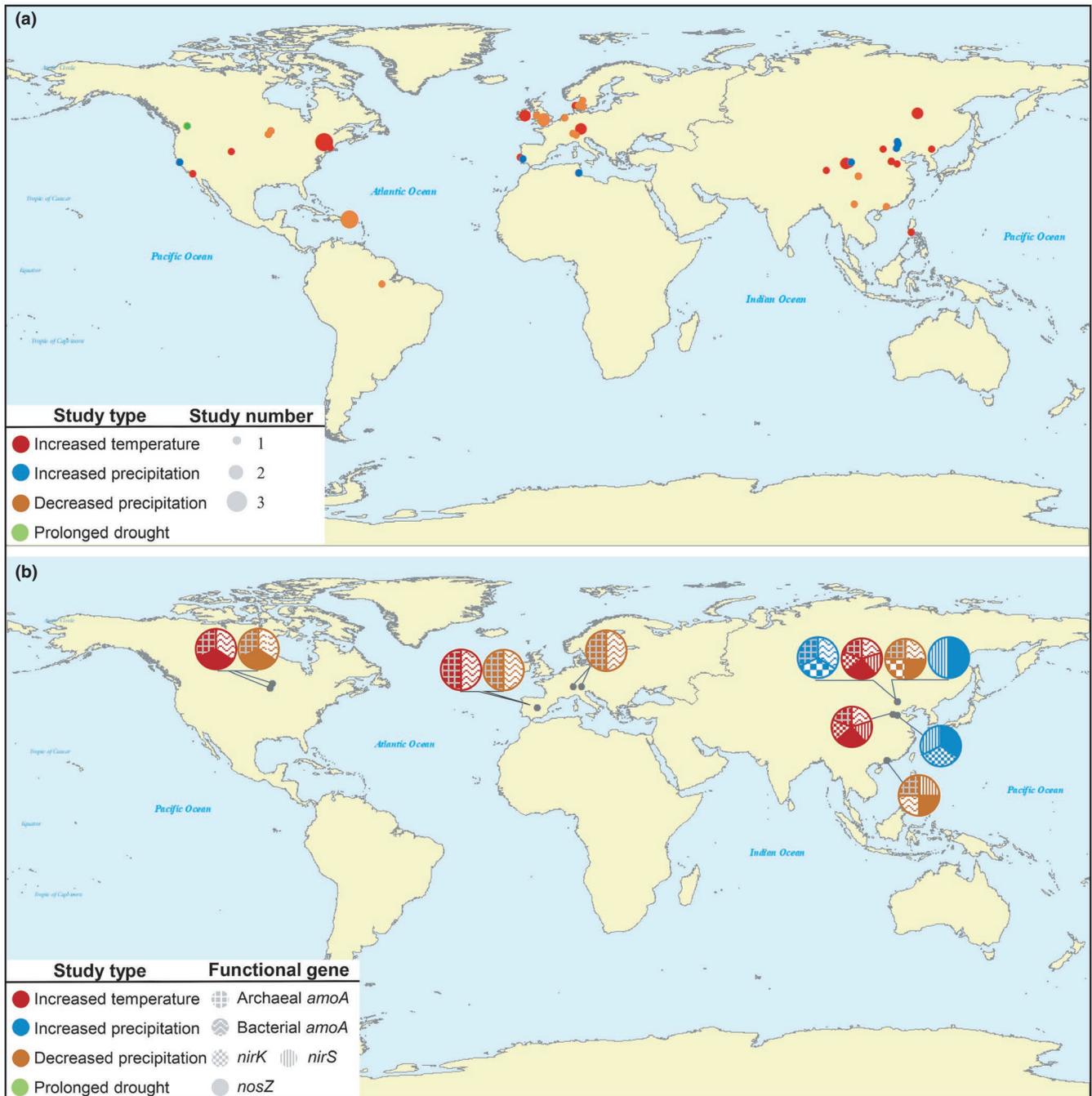
$$v = \frac{s_t^2}{n_t \bar{X}_t^2} + \frac{s_c^2}{n_c \bar{X}_c^2}, \quad (2)$$

where  $\bar{X}_t$  and  $\bar{X}_c$  are the means for the treatment and control groups,  $s_t$  and  $s_c$  are the standard deviations for all observations in the treatment and control groups respectively;  $n_t$  and  $n_c$  are the sample sizes for the treatment and control groups respectively.

We chose to use  $\ln R$  because it is not biased by the variations among replicates within a treatment (including control) and the absolute magnitude of N<sub>2</sub>O fluxes among experiments and generally follows a normal distribution (Gruner et al., 2008; Vidal & Murphy, 2018). The mean effect size ( $\overline{\ln R}$ ) of all observations for a treatment was calculated from  $\ln R$  using a categorical random effect model. The effect sizes in different phases within the same study were treated separately (e.g. one effect size per year in a multiyear study), because the mean effect sizes based on randomly selected individual effect sizes from these multiple-phase studies were highly similar to those based on the entire effect size data set (Table S3). This approach was similar to past meta-analysis studies (Butler, Elser, Lewis, Mackey, & Chen, 2018; Dijkstra & Adams, 2015).

Collectively, 98 effect sizes for N<sub>2</sub>O were derived from 51 N<sub>2</sub>O flux studies, including 68 effect sizes from 29 increased temperature experiments, 10 effect sizes from 7 increased precipitation experiments, and 18 effect sizes from 14 decreased precipitation experiments (Figure 2a-c). There was only one study with two effect sizes on N<sub>2</sub>O fluxes under prolonged drought, and no study tested SFG response to prolonged drought (Figure 2d).

Confidence interval (CI) of the effect size was generated using bootstrapping (9,999 iterations). Significance of effect size was assessed with the 95% CI. If the 95% CI of the effect size of a treatment on a variable overlapped "0," the treatment effect was considered nonsignificant; otherwise, the treatment effect was significant. Categorical random effect model was used to assess whether N<sub>2</sub>O fluxes and SFGs showed different responses to climate changes among different biomes, treatment times, treatment seasons and treatment methods. A significant between-group heterogeneity test ( $Q_b$ ) indicated a significant difference in the treatment effect between different groups (e.g. exclusion vs. reduction for decreased



**FIGURE 1** Global distributions of experiments reporting N<sub>2</sub>O (a) and soil function genes (b) included in this meta-analysis. Marker colours indicate four types of climate change. The size of the symbol in (a) represents the number of experiments per location. Symbol shading in (b) represents different soil functional genes at each location

precipitation). All statistical analyses described above were performed in MetaWin 2.1.

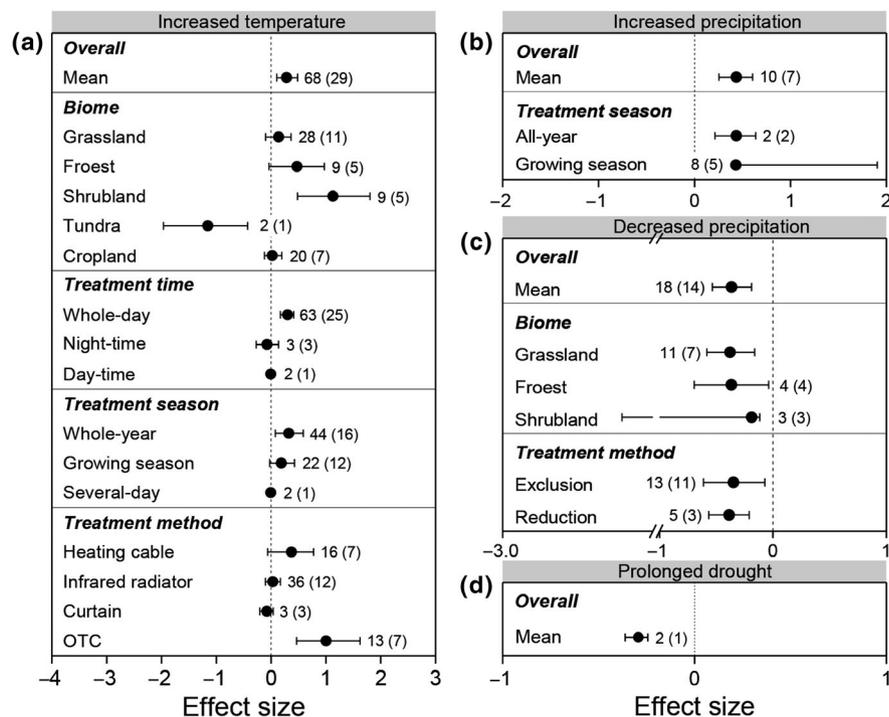
To facilitate explanation, the mean effect size ( $\overline{\ln R}$ ) was transformed back to the percentage change resulted from the treatment using the following formula:

$$\left(e^{\overline{\ln R}} - 1\right) \times 100\%. \quad (3)$$

In addition to the meta-analysis procedure, relationships between effect size on N<sub>2</sub>O or SFGS and changes in soil condition

and experimental treatment were also examined. According to the principle of maximum adjusted  $R^2$ , linear correlations were employed to examine the relationships between the effect size for N<sub>2</sub>O and changes in soil temperature, treatment duration in increased temperature studies and the effect size for soil moisture in decreased and increased precipitation studies. Similarly, binomial correlations were used to test the relationships between the effect size of SFGS and the effect size of soil moisture across decreased and increased precipitation studies.

**FIGURE 2** The mean effect sizes of experimental climate change on  $N_2O$  fluxes from soil. The variables are categorized into different groups based on the biomes, treatment times, seasons and methods respectively. Error bars represent 95% confidence intervals. The dashed line was drawn at mean effect size = 0. The effect was considered significant if the 95% CI of the effect size did not cover zero. The sample size for each variable is shown next to the point and the number of study is in the parentheses. OTC: open-top chamber



### 3 | RESULTS

#### 3.1 | $N_2O$ flux

On average, increased temperature and increased precipitation significantly increased  $N_2O$  emissions by 33% and 55% (95% CI: 11%–62% and 29%–83%), respectively, while decreased precipitation and prolonged drought suppressed  $N_2O$  emissions by 31% and 25% (95% CI: –41% to –17% and –30% to –22%) respectively (Figure 2).

Different biomes showed clear differences in  $N_2O$  response to increased temperature ( $p < .01$ ; Table 1). Increased temperature stimulated  $N_2O$  emission by 208% in shrublands (95% CI: 62%–507%), suppressed  $N_2O$  emission in tundras by 68% (95% CI: –85% to –35%), had various effects in forests (95% CI: –4% to 164%). Meanwhile, increased temperature did not significantly alter  $N_2O$  emissions in the biome groupings of grasslands (95% CI: –10% to 44%) or croplands (95% CI: –12% to 22%; Figure 2a). Additionally,  $N_2O$  responses to increased temperature depended significantly on treatment methods ( $p < .01$ ; Table 1). The use of OTCs consistently increased  $N_2O$  emissions (mean effect size: 172%; 95% CI: 59%–407%) while heating cables had no significant effect (mean effect size: 45%; 95% CI: –6% to 117%). In contrast, infrared radiators and curtains did not significantly increase  $N_2O$  emissions (Figure 2a). Although  $N_2O$  emission in response to increased temperature did not significantly change with treatment time and season ( $p = .53$  and  $.67$  respectively; Table 1), the treatments over whole-day (mean effect size: 35%; 95% CI: 11%–67%) or whole-year (mean effect size: 38%; 95% CI: 8%–80%) consistently had significant positive effects (Figure 2a).

The effect size of increased temperature on  $N_2O$  emissions increased significantly with increasing soil temperature in shrubland

( $R^2 = .43$ ,  $p = .03$ ) while there were no significant relationships between  $N_2O$  emissions and temperature in other biomes (Figure 3a). Besides, the effect size of increased temperature on  $N_2O$  emissions increased with marginal significance with treatment duration in studies with the OTC method ( $R^2 = .17$ ,  $p = .07$ ), decreased marginally with the duration in studies with the heating cable method ( $R^2 = .14$ ,  $p = .08$ ), but did not change significantly in studies with the infrared radiator method (Figure 3b). Additionally, the effect of increased temperature on  $N_2O$  emissions had a weakly positive relationship with latitude (Figure S2b).

The effects of increased precipitation on  $N_2O$  emission were consistently positive regardless of treatment seasons ( $p = .93$ ; Table 1; Figure 2b). Similarly, decreased precipitation reduced  $N_2O$  emission irrespective of biomes and treatment methods ( $p = .90$  and  $.85$  respectively; Table 1; Figure 2c). The effect size on  $N_2O$  emissions had a significant positive relationship with the effect size of soil moisture across increased and decreased precipitation studies ( $R^2 = .25$ ,  $p < .01$ ; Figure 3c). Additionally, the effect of decreased temperature on  $N_2O$  emissions had a significantly positive relationship with MAP, while there is no significant relationship between the effect of increased precipitation on  $N_2O$  emissions and MAP (Figure S2c).

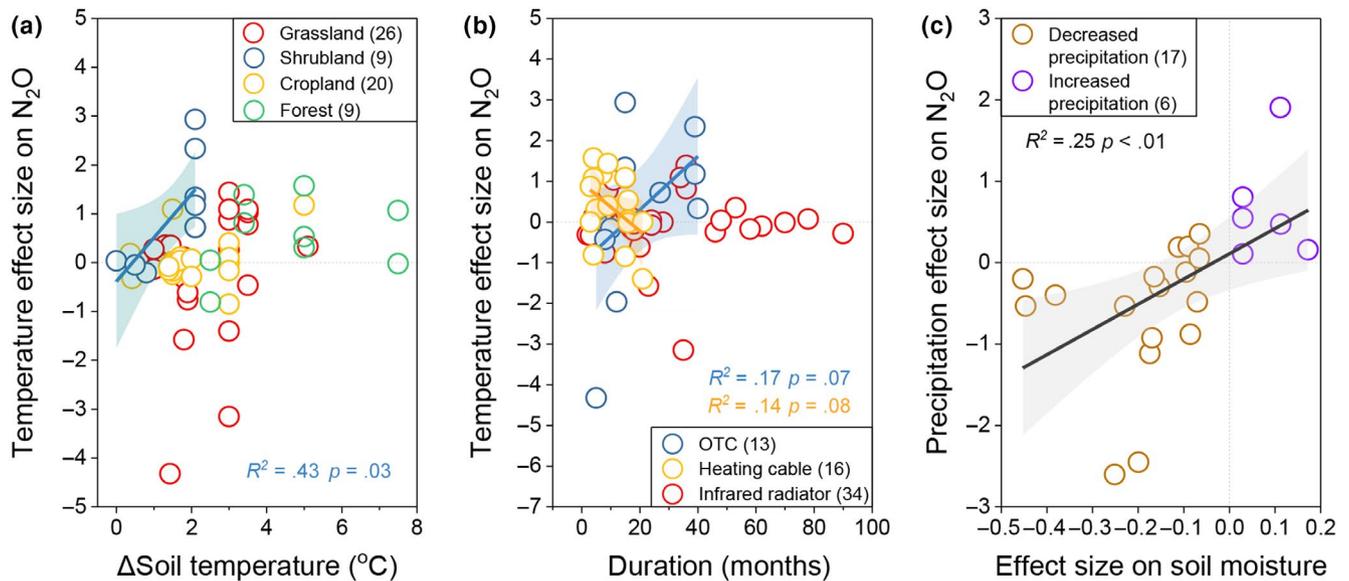
#### 3.2 | Soil functional genes

Increased temperature did not significantly affect abundance of archaeal *amoA*, bacterial *amoA* and *nosZ*, but significantly decreased the abundances of *nirK* and *nirS* by 26% (95% CI: –38% to –6%) and 31% (95% CI: –49% to –12%) respectively (Figure 4). There were no significant differences in increased temperature effects on SFGs between biome, treatment season and method groups (Figure S3; Table 1). Decreased precipitation had few effects on abundances of archaeal *amoA*, bacterial

**TABLE 1** The between-group heterogeneity ( $Q_b$ ) of climate change effects on various response variables

Manipulative type	Response variables	Categorical variables	$df_b$	$Q_b$	$p$ -Value
Increased temperature	N <sub>2</sub> O flux	Biomes	4	27.45	<b>&lt;.01</b>
		Treatment times	2	0.65	.53
		Treatment seasons	2	0.50	.67
		Treatment methods	3	21.61	<b>&lt;.01</b>
	Archaeal <i>amoA</i>	Biomes	2	0.32	.81
		Treatment seasons	1	0.39	.51
		Treatment methods	1	0.12	.72
	Bacterial <i>amoA</i>	Biomes	2	1.47	.54
		Treatment seasons	1	0.04	.84
		Treatment methods	1	<0.01	.97
<i>nosZ</i>	Biomes	2	0.96	.66	
	Treatment seasons	1	0.01	.96	
Increased precipitation	N <sub>2</sub> O flux	Treatment seasons	1	<0.01	.93
	<i>nirK</i>	Biomes	1	0.13	.62
	<i>nirS</i>	Biomes	1	4.95	.07
	<i>nosZ</i>	Biomes	1	0.42	.53
Decreased precipitation	N <sub>2</sub> O flux	Biomes	2	0.29	.89
		Treatment methods	1	0.05	.85
	Archaeal <i>amoA</i>	Biomes	1	0.73	.46
		Treatment methods	1	1.00	.42
	Bacterial <i>amoA</i>	Biomes	1	1.83	.23
		Treatment methods	1	0.97	.36
	<i>nosZ</i>	Treatment methods	1	0.47	.70

$p$ -values in bold are statistically significant to  $\alpha = .05$ .

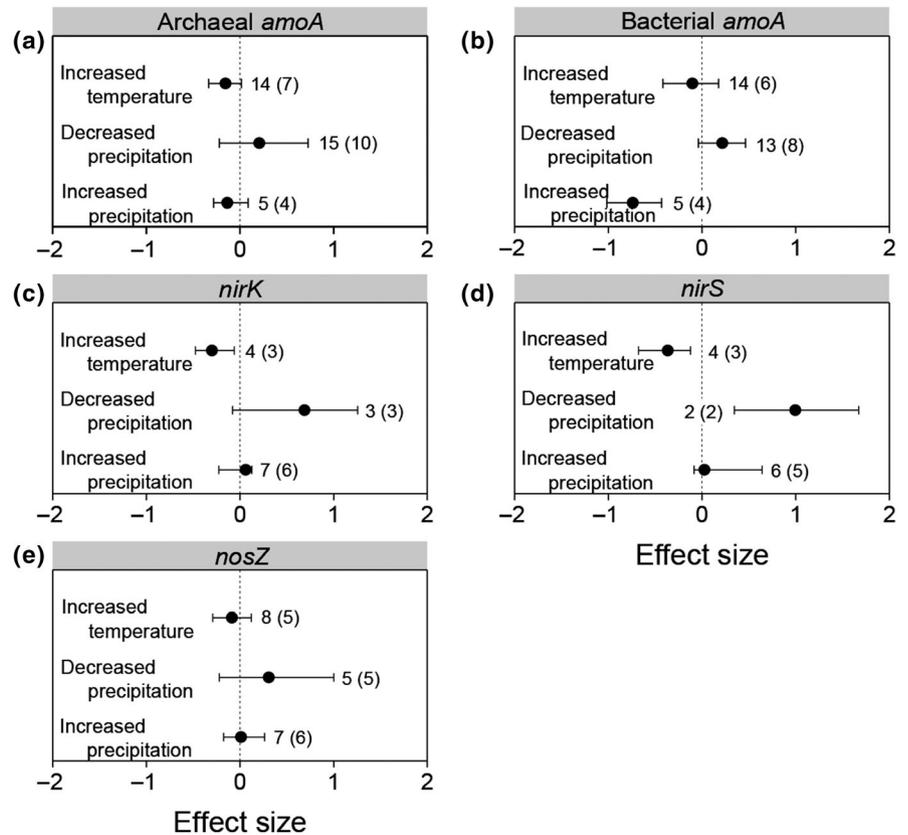


**FIGURE 3** Relationships between effect sizes on N<sub>2</sub>O emission and intensity and duration of climate change treatment. Only significant ( $p \leq .05$ ) or marginally significant ( $.05 < p < .10$ ) relationships were shown and corresponding shaded areas represent 95% confidence intervals. The sample size for each variable is shown in parenthesis. OTC: open-top chamber

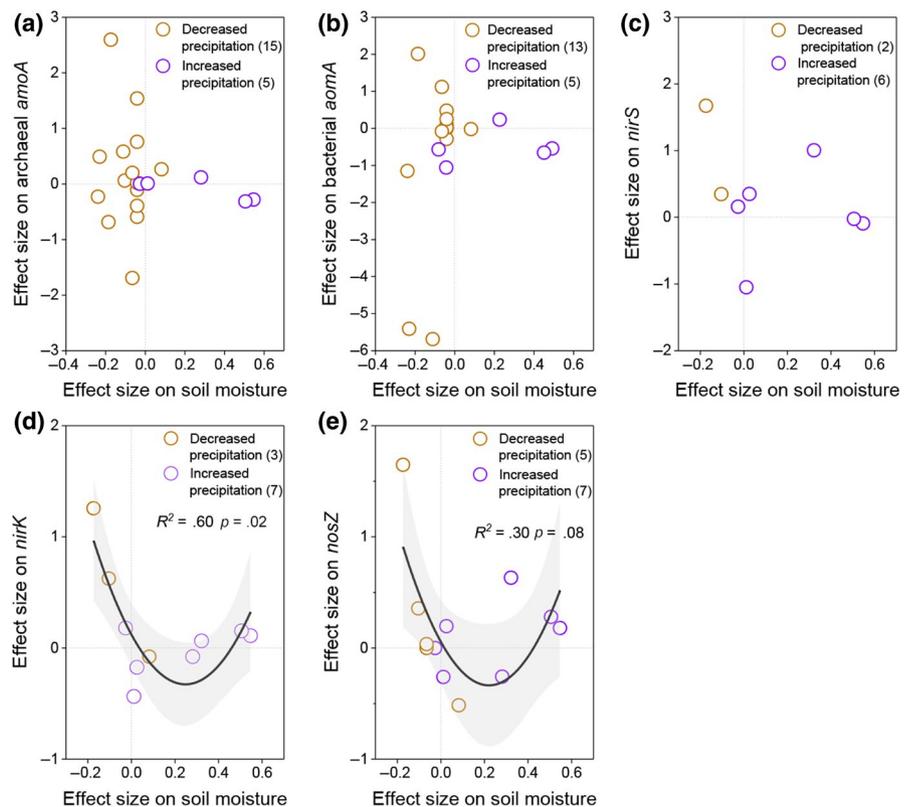
*amoA*, *nirK* and *nosZ* (Figure 4a–c,e; Figure S4) but showed positive effects on abundances of *nirS* based on two studies (mean effect size: 170%; 95% CI: 41%–432%; Figure 4d). Increased precipitation had little

effect on abundances of archaeal *amoA*, *nirK*, *nirS* and *nosZ* (Figure 4a,c–e; Figure S5) while showing negative effects on abundances of bacterial *amoA* (mean effect size: –52%; 95% CI: –64% to –35%; Figure 4b).

**FIGURE 4** The mean effect sizes of climate change on soil functional genes. Error bars represent 95% confidence intervals. The dashed line was drawn at mean effect size = 0. The effect was considered significant if the 95% CI of the effect size did not cover zero. The sample size for each variable is shown next to the point and the number of studies is shown in parenthesis



**FIGURE 5** Relationships between effect sizes of changed precipitation on soil functional genes and soil moisture. Only significant or marginally significant relationships (binomial relationships) were shown and the shaded areas represent 95% confidence intervals. The sample size for each variable is shown in parentheses



For increased temperature studies, there were no significant relationships between the effect size on SFGs (archaeal *amoA*, bacterial *amoA* and *nosZ*; *nirK* and *nirS* were not tested due to

limited sample size) and changes in soil temperature and treatment duration (Figure S6a–f). Across increased and decreased precipitation studies, *nirK* and *nosZ* showed significant and marginally

significant upward-convex relationships with soil moisture, and were lowest when the effect size on soil moisture was about 0.2 ( $R^2 = .60, p = .02$  and  $R^2 = .30, p = .08$  respectively; Figure 5d,e). In contrast, the relationships of the effect size on archaeal *amoA*, bacterial *amoA* and *nirS* to the effect size on soil moisture were not significant (Figure 5a–c).

## 4 | DISCUSSION

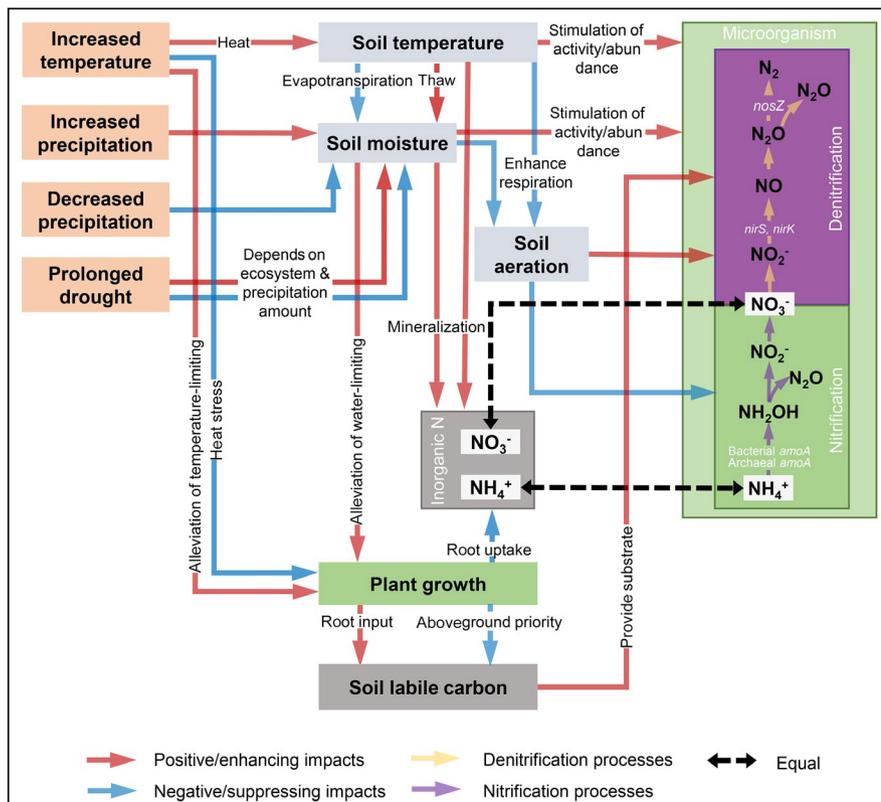
To our knowledge, this meta-analysis is one of the pioneering studies to provide a synthetic evaluation of N<sub>2</sub>O emissions from terrestrial ecosystems, especially relevant SFGs, under multiple types of climate change including increased temperature, increased precipitation, decreased precipitation and prolonged drought. Compared with previous meta-analysis studies, this study encompasses a more extensive database (e.g. 68 vs. 26 for N<sub>2</sub>O under increased temperature; Bai et al., 2013). In particular, this study fills a critical knowledge gap on how increased precipitation affects N<sub>2</sub>O emissions. Unfortunately, to date, prolonged drought effects on N<sub>2</sub>O and relevant SFGs still remain largely unclear due to insufficient studies (one study for N<sub>2</sub>O emission and no study for SFGs; Table S2).

### 4.1 | N<sub>2</sub>O emission stimulated by increased temperature

On average, our analysis indicated that experimentally increased temperature significantly increased N<sub>2</sub>O emission by 33% (Figure 2a).

This was much higher than the nonsignificant increase of 14% found in an earlier meta-analysis based on 26 measurements (Bai et al., 2013), confirming that warming could increase N<sub>2</sub>O emissions, consistent with expectations based on temperature-driven microbial kinetics.

There are several possible mechanisms for the positive effect of increased temperature on N<sub>2</sub>O emissions (Figure 6). First, elevated soil temperature can speed N mineralization and thereby inorganic N availability (Bai et al., 2013; Bijoor et al., 2008; Chen et al., 2017; Cui et al., 2018; Tu & Li, 2017), providing substrate for nitrification and denitrification. Second, increased temperature may benefit plant growth including root growth and turnover (Wu, Dijkstra, Koch, Penuelas, & Hungate, 2011). Labile carbon via root exudation and litter decomposition would facilitate N<sub>2</sub>O emissions (Liu et al., 2018) as denitrification is generally stimulated by high availability of labile carbon as a source of energy (Kuypers et al., 2018). Third, increased temperature can enhance root respiration, which consumes oxygen in soils and thus promotes N<sub>2</sub>O production through anaerobic denitrification (Saggar et al., 2013). Fourth, the activity and abundance of denitrifiers and nitrifiers could be directly stimulated by higher temperature to produce more N<sub>2</sub>O (Shi, Chen, Chen, Wu, & Wu, 2012). For example, warming boosted N<sub>2</sub>O emission as a result of increased bacterial *amoA* abundance in a boreal-temperate forest (Martins et al., 2017). Cantarel et al. (2012) suggested that increased temperature had positive effects on N<sub>2</sub>O emission due to not only a greater production of N<sub>2</sub>O but also less reduction of N<sub>2</sub>O in an upland grassland ecosystem, as demonstrated by the increased abundances of bacterial *amoA*, *nirK* as well as *nosZ* genes.



**FIGURE 6** Processes and mechanisms influencing impacts of climate change on N<sub>2</sub>O flux. Positive/enhancing impacts and negative/suppressing impacts between two variables are indicated by red and blue lines, respectively, on which relative mechanisms are listed. For example, increased temperature treatment would heat soil temperature which may increase or decrease soil aeration by enhancing respiration or by increasing soil moisture via snow thawing, thereby causing positive or negative effects on denitrification respectively. Nitrification and denitrification processes are indicated by purple and yellow lines, respectively, on which relative soil functional genes are listed. Black dashed lines indicate equal variables

In some permafrost regions, in addition to direct positive effects of increased soil temperature, soil thawing under increased temperature also further promoted N mineralization, plant growth and thus organic matter, and the activities of denitrifiers and nitrifiers, resulting in extra positive effects on N<sub>2</sub>O emission (Chen et al., 2017; Cui et al., 2018).

With the assembled data sets presently available, we were not able to pinpoint the soil microbial mechanisms underlying positive temperature effects on N<sub>2</sub>O emission at a global scale (Figure 4). The potential reasons for such a disconnection between temperature effects on N<sub>2</sub>O emission and on SFGs include data limitations and possible underlying mechanistic explanations. An important limitation of this meta-analysis is that N<sub>2</sub>O flux and SFGs were measured independently, presenting a possible barrier to finding their relation. To date, there remains a lack of sufficient field experiments measuring both N<sub>2</sub>O emissions and SFGs (e.g. there were only three studies with four observations investigating warming effects on *nirK* and *nirS*). Mechanistically, if soils are dried by increased temperature, we would expect decreased abundances of denitrifiers and nitrifiers, especially denitrifiers which are inhibited by aerobic conditions in drier soils (Waghmode et al., 2018). Additionally, enhanced plant growth with increased temperature may increase inorganic nitrogen uptake by the plants, thereby reducing soil nitrogen availability for N<sub>2</sub>O production through nitrification and denitrification (Carter et al., 2012; Dijkstra et al., 2012, 2013; Pereira et al., 2013; Zhu et al., 2015). In some cases, plants prioritize aboveground growth in the face of increased temperature while reducing belowground carbon supply (Dieleman et al., 2012), which may limit denitrifier proliferation and thus N<sub>2</sub>O emissions. These mechanisms could also underlie observed negative or null effects of increased temperature on N<sub>2</sub>O emission in some studies (Dijkstra et al., 2013; Hu et al., 2010; Liu et al., 2016). Overall, alteration of soil temperature and moisture under experimental warming may have interactive or cascading effects on plant growth and/or soil nitrogen and carbon contents, complicating relationships between N<sub>2</sub>O responses and SFGs (Figure 6). Further observational data are required.

#### 4.2 | High variation of N<sub>2</sub>O response to increased temperature across biomes and warming methods

It should be noted that the effect sizes of increased temperature on N<sub>2</sub>O emissions and the relationships with changes in soil temperature and treatment duration varied largely with different biomes and warming methods (Table 1; Figures 2a and 3a,b), consistent with a previous review suggesting that increased temperature had mixed effects on N<sub>2</sub>O emission (Dijkstra et al., 2012). Shrublands showed the strongest response to experimental increased temperature among all the biomes and the positive responses to rise in soil temperature were mainly recorded in a 3-year study conducted in a permafrost region, Northeast China (Cui et al., 2018). However, for extensively investigated grassland and forest ecosystems, we did not find any significant relationships between the effect size of increased temperature on N<sub>2</sub>O emission and changes in soil

temperature or warming duration. Therefore, the general patterns of increased temperature effect on N<sub>2</sub>O emissions in relation to warming intensity or duration remain largely unknown.

The OTC method appeared to be the most effective increased temperature method, followed by heating cables, in terms of promotion of N<sub>2</sub>O fluxes (Figure 2a). Interestingly, the effect size of increased temperature on N<sub>2</sub>O induced by OTCs and heating cables showed positive and negative relationships with treatment duration respectively (Figure 3b). This may be because the passive OTC method generally had less impacts on soil moisture when the temperature was gradually but continuously increased (Bai et al., 2013), and thus the direct positive temperature effect dominated among the driving factors to N<sub>2</sub>O emissions. In contrast, directly and actively heating soil with heating cables for a long time could substantially reduce soil moisture (Bai et al., 2013), thereby offsetting the positive effect of increased temperature on N<sub>2</sub>O emissions. Another potential reason for the high sensitivity of N<sub>2</sub>O emission to the OTC warming method is that this method was mainly used in cold ecosystems (Table S2), such as a permafrost region (MAT is -3.9°C; Cui et al., 2018), Tibetan Plateau (MAT is -5.2°C; Chen et al., 2017) and an alpine tundra (MAT is -1.6°C; Zhou et al., 2016). The OTCs were the most often used method to increase temperature in remote or high-latitude regions where electrical supply can be problematic (Aronson & McNulty, 2009). Although experimentally increased temperature could effectively ease the temperature limitation to microbial activities in these areas, infrared radiation, which is the best method for simulating natural warming for ecosystems (Aronson & McNulty, 2009), appeared to have less influence on N<sub>2</sub>O emissions. It might be attributed to the fact that this method was widely used in various environmental conditions (e.g. a large range of ambient temperature and precipitation, Table S2) and induced various effects on soil temperature as well as soil moisture and subsequent nitrogen cycling as discussed above. As a result, the temperature effect on N<sub>2</sub>O emission could have been offset or overshadowed by moisture effect in some cases (Liu et al., 2016; Tu & Li, 2017). Besides, only whole-day and whole-year warming treatment significantly enhanced N<sub>2</sub>O emission among all treatment times and seasons, respectively, suggesting that continued warming had the largest impacts on N<sub>2</sub>O emissions. Therefore, the high variability in N<sub>2</sub>O emission responses to increased temperature may bring great challenges to predict N<sub>2</sub>O fluxes under global warming and resultant future climate change at large spatial or temporal scales based on the field manipulative experiments.

#### 4.3 | N<sub>2</sub>O emission under changed precipitation regimes

Overall, our estimate of N<sub>2</sub>O emission suppression (-31%) by decreased precipitation (Figure 2b) is generally comparable to the recent synthesis of drought-induced decline in N<sub>2</sub>O emission (-53%) with a smaller data volume (15) and a different computing method for effect sizes (Hedge's *d*; Homyak et al., 2017). However, the corresponding 95% CI of estimates in this meta-analysis was

much smaller than that of Homyak et al. (2017;  $-0.53$  to  $-0.19$  vs.  $-1.38$  to  $-0.15$ ), providing a more well-constrained estimate of how precipitation regulates  $N_2O$  production globally. In contrast to the effect of decreased precipitation,  $N_2O$  emission was significantly promoted by increased precipitation (Figure 2c), which is in agreement with most previous observations (Brown et al., 2011; Du, Guo, Cao, & Li, 2016; Liu et al., 2015; Zhang, Hou, Guo, Li, & Xu, 2017). Together with the consistent response of  $N_2O$  emission to increased and decreased precipitation regardless of biomes, treatment methods and seasons (Figure 2b,c), as well as the strong positive relationship between the effect sizes of  $N_2O$  versus soil moisture (Figure 3c), we highlight that  $N_2O$  emissions from terrestrial ecosystems were strongly regulated by soil water availability.

Generally,  $N_2O$  emissions were more affected by decreased precipitation-induced negative effects including suppression of micro-organism activities and abundances (Auyeung, Martiny, & Dukes, 2015; Shi et al., 2012), decline in nitrogen mineralization (Larsen et al., 2011) and unfavourable aerobic environment for denitrifiers (Davidson, Nepstad, Ishida, & Brando, 2008), in comparison to the accompanying positive effects such as increase in C and N substrate import (Davidson, Ishida, & Nepstad, 2004) and favourable aerobic environment for nitrifiers (Carter et al., 2012). In contrast, the increased precipitation-induced positive effects tended to dominate over the negative effects on  $N_2O$  emissions (Brown et al., 2011; Liu et al., 2015; Zhang et al., 2017). Therefore, soil moisture could be considered as an important regulatory and thus predictive factor for  $N_2O$  emissions under climate change although current projections about precipitation variations in response to climate change remain uncertain (Lazenby, Todd, Chadwick, & Wang, 2018).

Interestingly, we found that both effect sizes of *nirK* and *nosZ* showed U-shaped relationships with the effect size of soil moisture (Figure 5d,e), in contrast to the perception that *nirK* and *nosZ* genes may be inhibited by lower soil moisture and corresponding higher soil oxygen concentration under drought (Delgado-Baquerizo et al., 2014; Homyak et al., 2017). The increases in the abundance of these two functional genes were observed when 67% of rainfall was excluded during a dry season in a 35 year old mixed species forest study (Chen et al., 2017). More studies are required to verify the effects of decreased precipitation on denitrifiers and nitrifiers as well as  $N_2O$  emissions.

The only prolonged drought study in this meta-analysis suggested that prolonged drought suppressed  $N_2O$  emissions by 25% (Fentabil et al., 2016). As with increased or decreased precipitation, we assumed that prolonged drought effects on  $N_2O$  emissions should also be strongly controlled by soil moisture (Figure 6). In other words,  $N_2O$  emission could be increased by prolonged drought-induced higher volume rainfall events necessary to accomplish the same seasonal precipitation with longer dry intervals or decreased by prolonged drought-induced dry soil conditions during drought. But soil moisture response to prolonged drought, both magnitude and direction, may depend on ecosystem types due to habitat-specific environmental conditions (e.g. moist or dry) and annual precipitation

amount (Knapp et al., 2008; Thomey et al., 2011). For example, larger but fewer rain events led to greater average soil moisture in a semi-arid shortgrass steppe in north eastern Colorado (Heisler-White, Blair, Kelly, Harmoney, & Knapp, 2009) while resulting in a lower temporally averaged soil water content in a semiarid steppe in Inner Mongolia (Liu et al., 2017).

## 5 | LIMITATIONS AND PERSPECTIVES

Globally, we found overall significant positive effects of experimentally increased temperature on  $N_2O$  emission, but there are still uncertainties across individual studies due to multiple regulation mechanisms. We were unable to quantify the direct and indirect effects among soil temperature, soil moisture, soil nitrate and ammonium concentrations, plant biomass (above and below portion) and, especially, soil function genes on  $N_2O$  fluxes (e.g. using structural equation model method), as few studies measured many of these variables simultaneously.  $N_2O$  emission was the primary targeted parameter in these studies, while the corresponding SFGs were rarely measured. Such limited data of SFGs currently available from regions did not allow us to draw general conclusions about how nitrifiers and denitrifiers respond to climate changes at a global scale. However, considering soil biotic communities play direct and fundamental roles in driving soil  $N_2O$  response to climate change, the SFG responses to simulated climate change are urgently needed to improve our understanding of biological mechanisms for  $N_2O$  emissions from various terrestrial ecosystems.

Our analysis did not consider the interaction between different climate changes. Increased temperature and decreased precipitation are often coupled in nature mainly through positive drought-temperature feedbacks (Ciais et al., 2005; De Boeck, Dreesen, Janssens, & Nijs, 2010; Seneviratne, Luthi, Litschi, & Schar, 2006), while both temperature and precipitation were predicted to increase in the future in some regions, such as East Africa (Stocker, 2014). Effects of changes in temperature and precipitation work together and may trigger unexpected outcomes. Besides, atmospheric nitrogen deposition is a primary global change and nitrogen enrichment has been shown to alter  $N_2O$  response to climate changes (Brown et al., 2011; Chen et al., 2017; Zhao et al., 2017). Evaluating interactive effects of multiple global change factors on ecosystem processes is more important for understanding ecological responses than looking into a single factor alone (Luo et al., 2008; Zhu, Chiariello, Tobeck, Fukami, & Field, 2016). Thus, it is essential and vital to adequately assess how  $N_2O$  fluxes would respond to interactions of these factors.

In reviewing field experiments in this meta-analysis, it became apparent that almost all studies included in this meta-analysis examined the impacts of long-term chronic climate change. For increased temperature studies, 16 of 29 studies were warming throughout the whole year and 12 studies were warming over the growing season or winter only, with only 1 heat wave study (extreme warming for

1 week; Li et al., 2016; Figure 2a). For increased precipitation studies, evenly increased precipitation throughout a year were tested in all studies apart from one with randomly increased precipitation during a 4 month treatment period. There is almost no study examining short-term but large increases in temperature and precipitation to simulate heat waves and heavy rainfalls. Although past studies have largely improved our understanding of how N<sub>2</sub>O flux responds to changes in precipitation and increases in temperature, much work is required to further our understanding of the climate change effects on N<sub>2</sub>O fluxes, as the ecological effects of climate extremes and those of long-term chronic climate change were markedly different (Jentsch, Kreyling, & Beierkuhnlein, 2007; Reyer et al., 2013). A typical example is the application of a constant heat sum on northern red oak seedlings with different amplitudes and duration, +3°C for 4 weeks, +6°C for 2 weeks and +12°C for 1 week, representing warming, moderate heat wave and severe heat wave respectively (Bauweraerts et al., 2013). Interestingly, these three treatments increased, did not change and decreased biomass accumulation, respectively, highlighting opposite ecosystem responses to long-term warming and short-term heat waves. Therefore, we suggest that future manipulative field experiments should investigate extreme climate event treatments.

## 6 | CONCLUSIONS

Understanding the responses of N<sub>2</sub>O emissions from soil under climate change is important for predicting future climate and nitrogen cycling in terrestrial ecosystems. Our synthesis based on an extensive new database showed that increased temperature significantly stimulates N<sub>2</sub>O emissions at a global scale, despite varying degrees of impact across different biomes, treatment time and warming methods, representing a positive global warming feedback. Additionally, as increased and decreased precipitation, respectively, promoted and suppressed N<sub>2</sub>O emission regardless of biomes, treatment methods and seasons, as well as the strong positive relationship between the effect sizes of N<sub>2</sub>O versus soil moisture, precipitation amount and corresponding soil moisture could be considered as a strong regulatory and thus predictive factor for N<sub>2</sub>O emissions under a future uncertain precipitation regime. To accurately project future climate, climate models should incorporate N<sub>2</sub>O feedback and field manipulative studies need to focus on biotic mechanisms (i.e. SFGs) underlying N<sub>2</sub>O emission in response to climate change.

## ACKNOWLEDGEMENTS

This project was funded by the CAS Strategic Priority Research Programme (A; Grant Nos. XDA20050103, XDA19030202) and the International Cooperation and Exchange of National Natural Science Foundation of China (Grant Nos. 31761123001, 31761143018). The authors thank members of the Inner Mongolia Grassland Ecosystem Research Station for their help.

## ORCID

Linfeng Li  <https://orcid.org/0000-0001-5831-8837>

Xingliang Xu  <https://orcid.org/0000-0003-2869-4932>

Yanbin Hao  <https://orcid.org/0000-0001-6821-0395>

## REFERENCES

- Aronson, E. L., & McNulty, S. G. (2009). Appropriate experimental ecosystem warming methods by ecosystem, objective, and practicality. *Agricultural and Forest Meteorology*, *149*(11), 1791–1799. <https://doi.org/10.1016/j.agrformet.2009.06.007>
- Auyeung, D. N., Martiny, J. B., & Dukes, J. S. (2015). Nitrification kinetics and ammonia-oxidizing community respond to warming and altered precipitation. *Ecosphere*, *6*(5), 1–17. <https://doi.org/10.1890/ES14-00481.1>
- Bai, E., Li, S., Xu, W., Li, W., Dai, W., & Jiang, P. (2013). A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics. *New Phytologist*, *199*(2), 431–440. <https://doi.org/10.1111/nph.12252>
- Bauweraerts, I., Wertin, T. M., Ameye, M., McGuire, M. A., Teskey, R. O., & Steppe, K. (2013). The effect of heat waves, elevated [CO<sub>2</sub>] and low soil water availability on northern red oak (*Quercus rubra* L.) seedlings. *Global Change Biology*, *19*(2), 517–528. <https://doi.org/10.1111/gcb.12044>
- Bijoor, N. S., Czimczik, C. I., Pataki, D. E., & Billings, S. A. (2008). Effects of temperature and fertilization on nitrogen cycling and community composition of an urban lawn. *Global Change Biology*, *14*(9), 2119–2131. <https://doi.org/10.1111/j.1365-2486.2008.01617.x>
- Brown, J. R., Blankinship, J. C., Niboyet, A., van Groenigen, K. J., Dijkstra, P., Le Roux, X., ... Hungate, B. A. (2011). Effects of multiple global change treatments on soil N<sub>2</sub>O fluxes. *Biogeochemistry*, *109*(1–3), 85–100. <https://doi.org/10.1007/s10533-011-9655-2>
- Butler, O. M., Elser, J. J., Lewis, T., Mackey, B., & Chen, C. (2018). The phosphorus-rich signature of fire in the soil-plant system: A global meta-analysis. *Ecology Letters*, *21*(3), 335–344. <https://doi.org/10.1111/ele.12896>
- Cantarel, A. A. M., Bloor, J. M. G., Pommier, T., Guillaumaud, N., Moirot, C., Soussana, J. F., & Poly, F. (2012). Four years of experimental climate change modifies the microbial drivers of N<sub>2</sub>O fluxes in an upland grassland ecosystem. *Global Change Biology*, *18*(8), 2520–2531. <https://doi.org/10.1111/j.1365-2486.2012.02692.x>
- Carter, M. S., Larsen, K. S., Emmett, B., Estiarte, M., Field, C., Leith, I. D., ... Beier, C. (2012). Synthesizing greenhouse gas fluxes across nine European peatlands and shrublands – Responses to climatic and environmental changes. *Biogeosciences*, *9*(10), 3739–3755. <https://doi.org/10.5194/bg-9-3739-2012>
- Cayuela, M. L., Aguilera, E., Sanz-Cobena, A., Adams, D. C., Abalos, D., Barton, L., ... Lassaletta, L. (2017). Direct nitrous oxide emissions in Mediterranean climate cropping systems: Emission factors based on a meta-analysis of available measurement data. *Agriculture, Ecosystems & Environment*, *238*, 25–35. <https://doi.org/10.1016/j.agee.2016.10.006>
- Chen, X., Wang, G., Zhang, T., Mao, T., Wei, D., Hu, Z., & Song, C. (2017). Effects of warming and nitrogen fertilization on GHG flux in the permafrost region of an alpine meadow. *Atmospheric Environment*, *157*, 111–124. <https://doi.org/10.1016/j.atmosenv.2017.03.024>
- Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogee, J., Allard, V., ... Valentini, R. (2005). Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature*, *437*(7058), 529–533. <https://doi.org/10.1038/nature03972>
- Cui, Q., Song, C., Wang, X., Shi, F., Yu, X., & Tan, W. (2018). Effects of warming on N<sub>2</sub>O fluxes in a boreal peatland of Permafrost region,

- Northeast China. *Science of Total Environment*, 616–617, 427–434. <https://doi.org/10.1016/j.scitotenv.2017.10.246>
- Dalal, R. C., & Allen, D. E. (2008). Greenhouse gas fluxes from natural ecosystems. *Australian Journal of Botany*, 56(5), 369–407. <https://doi.org/10.1071/bt07128>
- Davidson, E. A., Ishida, F. Y., & Nepstad, D. C. (2004). Effects of an experimental drought on soil emissions of carbon dioxide, methane, nitrous oxide, and nitric oxide in a moist tropical forest. *Global Change Biology*, 10(5), 718–730. <https://doi.org/10.1111/j.1529-8817.2003.00762.x>
- Davidson, E. A., Nepstad, D. C., Ishida, F. Y., & Brando, P. M. (2008). Effects of an experimental drought and recovery on soil emissions of carbon dioxide, methane, nitrous oxide, and nitric oxide in a moist tropical forest. *Global Change Biology*, <https://doi.org/10.1111/j.1365-2486.2008.01694.x>
- De Boeck, H. J., Dreesen, F. E., Janssens, I. A., & Nijs, I. (2010). Climatic characteristics of heat waves and their simulation in plant experiments. *Global Change Biology*, 16(7), 1992–2000. <https://doi.org/10.1111/j.1365-2486.2009.02049.x>
- de Vries, F. T., Liiri, M. E., Bjørnlund, L., Bowker, M. A., Christensen, S., Setälä, H. M., & Bardgett, R. D. (2012). Land use alters the resistance and resilience of soil food webs to drought. *Nature Climate Change*, 2(4), 276–280. <https://doi.org/10.1038/nclimate1368>
- Delgado-Baquerizo, M., Maestre, F. T., Escolar, C., Gallardo, A., Ochoa, V., Gozalo, B., & Prado-Comesaña, A. (2014). Direct and indirect impacts of climate change on microbial and biocrust communities alter the resistance of the N cycle in a semiarid grassland. *Journal of Ecology*, 102(6), 1592–1605. <https://doi.org/10.1111/1365-2745.12303>
- Dieleman, W. I., Vicca, S., Dijkstra, F. A., Hagedorn, F., Hovenden, M. J., Larsen, K. S., ... Dukes, J. S. (2012). Simple additive effects are rare: A quantitative review of plant biomass and soil process responses to combined manipulations of CO<sub>2</sub> and temperature. *Global Change Biology*, 18(9), 2681–2693. <https://doi.org/10.1111/j.1365-2486.2012.02745.x>
- Dijkstra, F., & Adams, M. (2015). Fire eases imbalances of nitrogen and phosphorus in woody plants. *Ecosystems*, 18(5), 769–779. <https://doi.org/10.1007/s10021-015-9861-1>
- Dijkstra, F. A., Morgan, J. A., Follett, R. F., & Lecain, D. R. (2013). Climate change reduces the net sink of CH<sub>4</sub> and N<sub>2</sub>O in a semiarid grassland. *Global Change Biology*, 19(6), 1816–1826. <https://doi.org/10.1111/gcb.12182>
- Dijkstra, F. A., Prior, S. A., Runion, G. B., Torbert, H. A., Tian, H., Lu, C., & Venterea, R. T. (2012). Effects of elevated carbon dioxide and increased temperature on methane and nitrous oxide fluxes: Evidence from field experiments. *Frontiers in Ecology and the Environment*, 10(10), 520–527. <https://doi.org/10.1890/120059>
- Du, Y., Guo, X., Cao, G., & Li, Y. (2016). Increased nitrous oxide emissions resulting from nitrogen addition and increased precipitation in an alpine meadow ecosystem. *Polish Journal of Environmental Studies*, 25(1), 447–451. <https://doi.org/10.15244/pjoes/60860>
- Fentabil, M. M., Nichol, C. F., Jones, M. D., Neilsen, G. H., Neilsen, D., & Hannam, K. D. (2016). Effect of drip irrigation frequency, nitrogen rate and mulching on nitrous oxide emissions in a semi-arid climate: An assessment across two years in an apple orchard. *Agriculture, Ecosystems & Environment*, 235, 242–252. <https://doi.org/10.1016/j.agee.2016.09.033>
- Francis, C. A., Roberts, K. J., Beman, J. M., Santoro, A. E., & Oakley, B. B. (2005). Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proceedings of the National Academy of Sciences of the United States of America*, 102(41), 14683–14688. <https://doi.org/10.1073/pnas.0506625102>
- Gruner, D. S., Smith, J. E., Seabloom, E. W., Sandin, S. A., Ngai, J. T., Hillebrand, H., ... Bracken, M. E. (2008). A cross-system synthesis of consumer and nutrient resource control on producer biomass. *Ecology Letters*, 11(7), 740–755. <https://doi.org/10.1111/j.1461-0248.2008.01192.x>
- Heisler-White, J. L., Blair, J. M., Kelly, E. F., Harmoney, K., & Knapp, A. K. (2009). Contingent productivity responses to more extreme rainfall regimes across a grassland biome. *Global Change Biology*, 15(12), 2894–2904. <https://doi.org/10.1111/j.1365-2486.2009.01961.x>
- Henry, S., Baudoin, E., López-Gutiérrez, J. C., Martin-Laurent, F., Brauman, A., & Philippot, L. (2004). Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR. *Journal of Microbiological Methods*, 59(3), 327–335. <https://doi.org/10.1016/j.mimet.2004.07.002>
- Homyak, P. M., Allison, S. D., Huxman, T. E., Goulden, M. L., & Treseder, K. K. (2017). Effects of drought manipulation on soil nitrogen cycling: A meta-analysis. *Journal of Geophysical Research: Biogeosciences*, 122(12), 3260–3272. <https://doi.org/10.1002/2017jg004146>
- Hoover, D. L., & Rogers, B. M. (2016). Not all droughts are created equal: The impacts of interannual drought pattern and magnitude on grassland carbon cycling. *Global Change Biology*, 22(5), 1809–1820. <https://doi.org/10.1111/gcb.13161>
- Hoover, D. L., Wilcox, K. R., & Young, K. E. (2018). Experimental droughts with rainout shelters: A methodological review. *Ecosphere*, 9(1). <https://doi.org/10.1002/ecs2.2088>
- Hu, Y., Chang, X., Lin, X., Wang, Y., Wang, S., Duan, J., ... Zhao, X. (2010). Effects of warming and grazing on N<sub>2</sub>O fluxes in an alpine meadow ecosystem on the Tibetan plateau. *Soil Biology and Biochemistry*, 42(6), 944–952. <https://doi.org/10.1016/j.soilbio.2010.02.011>
- Jentsch, A., Kreyling, J., & Beierkuhnlein, C. (2007). A new generation of climate-change experiments: Events, not trends. *Frontiers in Ecology and the Environment*, 5(7), 365–374. [https://doi.org/10.1890/1540-9295\(2007\)5\[365:ANGOCO\]2.0.CO;2](https://doi.org/10.1890/1540-9295(2007)5[365:ANGOCO]2.0.CO;2)
- Kandeler, E., Deiglmayr, K., Tschirko, D., Bru, D., & Philippot, L. (2006). Abundance of narG, nirS, nirK, and nosZ genes of denitrifying bacteria during primary successions of a glacier foreland. *Applied and Environmental Microbiology*, 72(9), 5957–5962. <https://doi.org/10.1128/AEM.00439-06>
- Knapp, A. K., Beier, C., Briske, D. D., Classen, A. T., Luo, Y., Reichstein, M., ... Weng, E. (2008). Consequences of more extreme precipitation regimes for terrestrial ecosystems. *BioScience*, 58(9), 811–821. <https://doi.org/10.1641/b580908>
- Kuiper, J. J., Mooij, W. M., Bragazza, L., & Robroek, B. J. M. (2014). Plant functional types define magnitude of drought response in peatland CO<sub>2</sub> exchange. *Ecology*, 95(1), 123–131. <https://doi.org/10.1890/13-0270.1>
- Kuypers, M. M. M., Marchant, H. K., & Kartal, B. (2018). The microbial nitrogen-cycling network. *Nature Review Microbiology*, 16(5), 263–276. <https://doi.org/10.1038/nrmicro.2018.9>
- Larsen, K. S., Andresen, L. C., Beier, C., Jonasson, S., Albert, K. R., Ambus, P. E. R., ... Stevnbak, K. (2011). Reduced N cycling in response to elevated CO<sub>2</sub>, warming, and drought in a Danish heathland: Synthesizing results of the CLIMAITE project after two years of treatments. *Global Change Biology*, 17(5), 1884–1899. <https://doi.org/10.1111/j.1365-2486.2010.02351.x>
- Lazenby, M. J., Todd, M. C., Chadwick, R., & Wang, Y. (2018). Future precipitation projections over central and southern Africa and the adjacent Indian Ocean: What causes the changes and the uncertainty? *Journal of Climate*, 31(12), 4807–4826. <https://doi.org/10.1175/JCLI-D-17-0311.1>
- Li, J., Jin, Y., Liu, Y., Zhang, Y., Grace, J., Song, Q., ... Fei, X. (2018). Effects of precipitation exclusion on N<sub>2</sub>O emissions in a savanna ecosystem in SW China. *Atmospheric Environment*, 187, 1–8. <https://doi.org/10.1016/j.atmosenv.2018.05.035>
- Li, L., Fan, W., Kang, X., Wang, Y., Cui, X., Xu, C., ... Hao, Y. (2016). Responses of greenhouse gas fluxes to climate extremes in a semi-arid grassland. *Atmospheric Environment*, 142, 32–42. <https://doi.org/10.1016/j.atmosenv.2016.07.039>
- Liu, L., Hu, C., Yang, P., Ju, Z., Olesen, J. E., & Tang, J. (2016). Experimental warming-driven soil drying reduced N<sub>2</sub>O emissions from fertilized

- crop rotations of winter wheat-soybean/fallow, 2009–2014. *Agriculture, Ecosystems & Environment*, 219, 71–82. <https://doi.org/10.1016/j.agee.2015.12.013>
- Liu, S., Ji, C., Wang, C., Chen, J., Jin, Y., Zou, Z., ... Zou, J. (2018). Climatic role of terrestrial ecosystem under elevated CO<sub>2</sub>: A bottom-up greenhouse gases budget. *Ecology Letters*, 21(7), 1108–1118. <https://doi.org/10.1111/ele.13078>
- Liu, W. J., Li, L. F., Biederman, J. A., Hao, Y. B., Zhang, H., Kang, X. M., ... Xu, C. Y. (2017). Repackaging precipitation into fewer, larger storms reduces ecosystem exchanges of CO<sub>2</sub> and H<sub>2</sub>O in a semiarid steppe. *Agricultural and Forest Meteorology*, 247, 356–364. <https://doi.org/10.1016/j.agrformet.2017.08.029>
- Liu, X., Dong, Y., Qi, Y., Peng, Q., He, Y., Sun, L., ... Liu, X. (2015). Response of N<sub>2</sub>O emission to water and nitrogen addition in temperate typical steppe soil in Inner Mongolia, China. *Soil and Tillage Research*, 151, 9–17. <https://doi.org/10.1016/j.still.2015.01.008>
- Luo, Y., Gerten, D., Le Maire, G., Parton, W. J., Weng, E., Zhou, X., ... Rustad, L. (2008). Modeled interactive effects of precipitation, temperature, and [CO<sub>2</sub>] on ecosystem carbon and water dynamics in different climatic zones. *Global Change Biology*, 14(9), 1986–1999. <https://doi.org/10.1111/j.1365-2486.2008.01629.x>
- Martins, C. S. C., Nazaries, L., Delgado-Baquerizo, M., Macdonald, C. A., Anderson, I. C., Hobbie, S. E., ... Field, K. (2017). Identifying environmental drivers of greenhouse gas emissions under warming and reduced rainfall in boreal-temperate forests. *Functional Ecology*, 31(12), 2356–2368. <https://doi.org/10.1111/1365-2435.12928>
- McHale, P. J., Mitchell, M. J., & Bowles, F. P. (1998). Soil warming in a northern hardwood forest: Trace gas fluxes and leaf litter decomposition. *Canadian Journal of Forest Research*, 28(9), 1365–1372. <https://doi.org/10.1139/x98-118>
- Pereira, J., Figueiredo, N., Goufo, P., Carneiro, J., Morais, R., Carranca, C., ... Trindade, H. (2013). Effects of elevated temperature and atmospheric carbon dioxide concentration on the emissions of methane and nitrous oxide from Portuguese flooded rice fields. *Atmospheric Environment*, 80, 464–471. <https://doi.org/10.1016/j.atmosenv.2013.08.045>
- Reyer, C. P., Leuzinger, S., Rammig, A., Wolf, A., Bartholomeus, R. P., Bonfante, A., ... Pereira, M. (2013). A plant's perspective of extremes: Terrestrial plant responses to changing climatic variability. *Global Change Biology*, 19(1), 75–89. <https://doi.org/10.1111/gcb.12023>
- Saggar, S., Jha, N., Deslippe, J., Bolan, N., Luo, J., Giltrap, D., ... Tillman, R. (2013). Denitrification and N<sub>2</sub>O: N<sub>2</sub> production in temperate grasslands: Processes, measurements, modelling and mitigating negative impacts. *Science of the Total Environment*, 465, 173–195. <https://doi.org/10.1016/j.scitotenv.2012.11.050>
- Seneviratne, S. I., Luthi, D., Litschi, M., & Schar, C. (2006). Land-atmosphere coupling and climate change in Europe. *Nature*, 443(7108), 205–209. <https://doi.org/10.1038/nature05095>
- Shi, F., Chen, H., Chen, H., Wu, Y., & Wu, N. (2012). The combined effects of warming and drying suppress CO<sub>2</sub> and N<sub>2</sub>O emission rates in an alpine meadow of the eastern Tibetan Plateau. *Ecological Research*, 27(4), 725–733. <https://doi.org/10.1007/s11284-012-0950-8>
- Stocker, T. (2014). *Climate change 2013: The physical science basis: Working Group I contribution to the Fifth assessment report of the Intergovernmental Panel on Climate Change*. Cambridge, UK: Cambridge University Press.
- Teuling, A. J., Seneviratne, S. I., Stöckli, R., Reichstein, M., Moors, E., Ciais, P., ... Wohlfahrt, G. (2010). Contrasting response of European forest and grassland energy exchange to heatwaves. *Nature Geoscience*, 3(10), 722–727. <https://doi.org/10.1038/ngeo950>
- Thomey, M. L., Collins, S. L., Vargas, R., Johnson, J. E., Brown, R. F., Natvig, D. O., & Friggens, M. T. (2011). Effect of precipitation variability on net primary production and soil respiration in a Chihuahuan Desert grassland. *Global Change Biology*, 17(4), 1505–1515. <https://doi.org/10.1111/j.1365-2486.2010.02363.x>
- Tu, C., & Li, F. (2017). Responses of greenhouse gas fluxes to experimental warming in wheat season under conventional tillage and no-tillage fields. *Journal of Environmental Science (China)*, 54, 314–327. <https://doi.org/10.1016/j.jes.2016.09.016>
- Vidal, M. C., & Murphy, S. M. (2018). Bottom-up vs. top-down effects on terrestrial insect herbivores: A meta-analysis. *Ecology Letters*, 21(1), 138–150. <https://doi.org/10.1111/ele.12874>
- Waghmode, T. R., Chen, S., Li, J., Sun, R., Liu, B., & Hu, C. (2018). Response of nitrifier and denitrifier abundance and microbial community structure to experimental warming in an agricultural ecosystem. *Frontiers in Microbiology*, 9, 474. <https://doi.org/10.3389/fmicb.2018.00474>
- Ward, S. E., Ostle, N. J., Oakley, S., Quirk, H., Henrys, P. A., & Bardgett, R. D. (2013). Warming effects on greenhouse gas fluxes in peatlands are modulated by vegetation composition. *Ecology Letters*, 16(10), 1285–1293. <https://doi.org/10.1111/ele.12167>
- Wu, Z., Dijkstra, P., Koch, G. W., Penuelas, J., & Hungate, B. A. (2011). Responses of terrestrial ecosystems to temperature and precipitation change: A meta-analysis of experimental manipulation. *Global Change Biology*, 17(2), 927–942. <https://doi.org/10.1111/j.1365-2486.2010.02302.x>
- Zhang, L., Hou, L., Guo, D., Li, L., & Xu, X. (2017). Interactive impacts of nitrogen input and water amendment on growing season fluxes of CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O in a semiarid grassland, Northern China. *Science of the Total Environment*, 578, 523–534. <https://doi.org/10.1016/j.scitotenv.2016.10.219>
- Zhang, X., Liu, W., Schloter, M., Zhang, G., Chen, Q., Huang, J., ... Han, X. (2013). Response of the abundance of key soil microbial nitrogen-cycling genes to multi-factorial global changes. *PLoS ONE*, 8(10), e76500. <https://doi.org/10.1371/journal.pone.0076500>
- Zhao, Z., Dong, S., Jiang, X., Liu, S., Ji, H., Li, Y., ... Sha, W. (2017). Effects of warming and nitrogen deposition on CH<sub>4</sub>, CO<sub>2</sub> and N<sub>2</sub>O emissions in alpine grassland ecosystems of the Qinghai-Tibetan Plateau. *Science of Total Environment*, 592, 565–572. <https://doi.org/10.1016/j.scitotenv.2017.03.082>
- Zhou, Y., Hagedorn, F., Zhou, C., Jiang, X., Wang, X., & Li, M. H. (2016). Experimental warming of a mountain tundra increases soil CO<sub>2</sub> effluxes and enhances CH<sub>4</sub> and N<sub>2</sub>O uptake at Changbai Mountain, China. *Scientific Reports*, 6, 21108. <https://doi.org/10.1038/srep21108>
- Zhu, K., Chiariello, N. R., Tobeck, T., Fukami, T., & Field, C. B. (2016). Nonlinear, interacting responses to climate limit grassland production under global change. *Proceeding of National Academy Sciences of the United States of America*, 113(38), 10589–10594. <https://doi.org/10.1073/pnas.1606734113>
- Zhu, X., Luo, C., Wang, S., Zhang, Z., Cui, S., Bao, X., ... Zhou, Y. (2015). Effects of warming, grazing/cutting and nitrogen fertilization on greenhouse gas fluxes during growing seasons in an alpine meadow on the Tibetan Plateau. *Agricultural and Forest Meteorology*, 214–215, 506–514. <https://doi.org/10.1016/j.agrformet.2015.09.008>

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Li L, Zheng Z, Wang W, et al.

Terrestrial N<sub>2</sub>O emissions and related functional genes under climate change: A global meta-analysis. *Glob Change Biol*. 2019;00:1–13. <https://doi.org/10.1111/gcb.14847>