

 Open access • Journal Article • DOI:10.1111/GCB.14847

## Terrestrial N<sub>2</sub>O emissions and related functional genes under climate change: A global meta-analysis — [Source link](#)

[Linfeng Li](#), [Linfeng Li](#), [Zhenzhen Zheng](#), [Weijin Wang](#) ...+16 more authors

**Institutions:** [Griffith University](#), [Chinese Academy of Sciences](#), [University of Queensland](#), [Agricultural Research Service](#) ...+1 more institutions

**Published on:** 01 Feb 2020 - [Global Change Biology](#) (Glob Chang Biol)

**Topics:** [Global warming](#), [Climate change](#), [Climate model](#), [Terrestrial ecosystem](#) and [Tundra](#)

Related papers:

- [Effects of warming, grazing/cutting and nitrogen fertilization on greenhouse gas fluxes during growing seasons in an alpine meadow on the Tibetan Plateau](#)
- [Soil GHG fluxes are altered by N deposition: New data indicate lower N stimulation of the N<sub>2</sub>O flux and greater stimulation of the calculated C pools](#)
- [Effects of multiple global change treatments on soil N<sub>2</sub>O fluxes](#)
- [A review of nitrogen enrichment effects on three biogenic GHGs: the CO<sub>2</sub> sink may be largely offset by stimulated N<sub>2</sub>O and CH<sub>4</sub> emission.](#)
- [Nitrous oxide emissions from soils: how well do we understand the processes and their controls?](#)

Share this paper:    

View more about this paper here: <https://typeset.io/papers/terrestrial-n2o-emissions-and-related-functional-genes-under-14f5t2h5hb>

1

2 MR. LINFENG LI (Orcid ID : 0000-0001-5831-8837)

3 DR. XINGLIANG XU (Orcid ID : 0000-0003-2869-4932)

4 DR. YANBIN HAO (Orcid ID : 0000-0001-6821-0395)

5

6

7 Article type : Primary Research Articles

8

9

10 **Terrestrial N<sub>2</sub>O emissions and related functional genes under climate change: a global**  
11 **meta-analysis**12 **Running Title:** climate change effects on N<sub>2</sub>O13 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>,  
14 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  
15 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> and Yanfen Wang<sup>1,8,\*</sup>

16

17 <sup>1</sup>College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049,  
18 China;19 <sup>2</sup>Environmental Futures Research Institute, School of Environment and Science, Griffith  
20 University, Brisbane 4111, Australia;21 <sup>3</sup>Department of Environment and Science, Dutton Park, Brisbane, Queensland 4102,  
22 Australia;23 <sup>4</sup>School of Agriculture and Food Sciences, University of Queensland, Brisbane, Queensland  
24 4072, Australia;25 <sup>5</sup>Southwest Watershed Research Center, Agricultural Research Service, Tucson, AZ, 85719,  
26 USA;

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/gcb.14847](https://doi.org/10.1111/gcb.14847)

This article is protected by copyright. All rights reserved

27 <sup>6</sup>Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic  
28 Sciences and Natural Resources Research, Chinese Academy of Sciences (CAS), Beijing  
29 100101, China.

30 <sup>7</sup>Institute of International Rivers and Eco-security, Yunnan University, Kunming, Yunnan,  
31 650091, China.

32 <sup>8</sup>CAS Center for Excellence in Tibetan Plateau Earth Sciences, Chinese Academy of Sciences  
33 (CAS), Beijing 100101, China.

34 # These authors contributed equally to this work.

35 \*Author for correspondence tel +86 136 6124 1601; email [ybhao@ucas.ac.cn](mailto:ybhao@ucas.ac.cn);  
36 [yfwang@ucas.ac.cn](mailto:yfwang@ucas.ac.cn)

Author Manuscript

37 **ABSTRACT**

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

60

61 **KEYWORDS:** drought, nitrous oxide, precipitation, soil N cycle, soil moisture, warming

## 62 INTRODUCTION

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

72 Taken individually, past studies of N<sub>2</sub>O flux response to climate change show a wide  
73 variety of responses such as the positive (Cui et al., 2018), neutral (Li et al., 2016) and  
74 negative effects (Hu et al., 2010) on N<sub>2</sub>O emission under experimental warming. Possible  
75 reasons for such variation include differences in biome type, climate manipulation  
76 characteristics, and experimental methods, all of which may impact the microbial  
77 communities mediating N<sub>2</sub>O emission. Across biomes, variation in vegetation composition  
78 (i.e. shrubs, graminoids and bryophytes) often moderates climate change effects on  
79 greenhouse gas fluxes (i.e. CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O, Kuiper, Mooij, Bragazza, & Robroek, 2014;  
80 Ward et al., 2013), due to different plant and microbial traits (de Vries et al., 2012; Teuling et  
81 al., 2010). Climate manipulation characteristics affecting N<sub>2</sub>O emissions may include  
82 intensity, duration, and timing (seasonal and/or diurnal) of an impact. These characteristics  
83 determine how the impact translates into soil microclimate (e.g. changes in soil moisture  
84 under different drought intensity by Li et al., 2018) and nonlinear microbial responses to  
85 resultant stress (McHale, Mitchell, & Bowles, 1998). At the seasonal scale, warming outside  
86 the growing season (i.e. cold season) of a peatland tended to exert greater effects on N<sub>2</sub>O  
87 emissions than in the growing season (Ward et al., 2013). At the diurnal scale, warming  
88 applied at different times of day may have different impacts on nitrogen pools and dynamics  
89 (Bai et al., 2013). When biome type and climate impacts are consistent, differences in N<sub>2</sub>O  
90 emissions may be related to nutrient availability (Dijkstra, Morgan, Follett, & Lecain, 2013)  
91 and/or microbial community composition (Bijoor, Czimeczik, Pataki, & Billings, 2008) with  
92 treatment duration. Finally, a wide variety of methods and equipment are employed in  
93 manipulative climate change experiments, potentially impacting soil microclimate,  
94 microbiology and, ultimately, N<sub>2</sub>O responses. For experimental warming, air and soil  
95 temperature can be manipulated with methods including open-top chambers (OTC), heating

96 cables, infrared radiators, glasshouses and curtains. Each methodology has its specific  
97 characteristics and limitations (Aronson & McNulty, 2009), creating different ecological  
98 impacts on nitrogen cycling (Bai et al., 2013). Precipitation changes are most commonly  
99 imposed using fully or partly covered rainout shelters (Hoover, Wilcox, & Young, 2018),  
100 resulting in discrepant ecological effects (Hoover & Rogers, 2016).

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

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

125 To address these knowledge gaps in how climate change alters N<sub>2</sub>O flux from terrestrial  
126 ecosystems, we compiled 210 observational data points from 46 published articles  
127 (Supporting information, Table S2) to quantitatively synthesise N<sub>2</sub>O fluxes and the relevant  
128 SFGs under climate changes, including both warming and changes in precipitation regime.  
129 The objectives of this study were to (i) identify global-scale patterns of N<sub>2</sub>O emissions and

130 the relevant SFG responses to various types of climate changes; (ii) relate the variability in  
131 the responses among studies to different biomes, treatment methods, treatment time,  
132 treatment season as well as treatment duration and intensity; and (iii) examine underlying  
133 biotic mechanisms for the alteration in N<sub>2</sub>O emissions in response to climate changes.

Author Manuscript

## 134 MATERIALS AND METHODS

### 135 Data selection

136 We collected publications that reported on responses of N<sub>2</sub>O or/and soil functional genes (i.e.  
137 archaeal *amoA*, bacterial *amoA*, *nosZ*, *narG*, *nirK* and *nirS*) to experimental precipitation  
138 and/or temperature manipulations in the field until November 11<sup>th</sup>, 2018 by searching Web of  
139 Science using a range of search terms and wildcards (Table S1). To better represent responses  
140 of N<sub>2</sub>O and soil functional genes under natural conditions, we did not include greenhouse  
141 mesocosm or laboratory experiments. Water control experiments using sewage and  
142 wastewater, or irrigation management studies in cropland (e.g. drainage and flood irrigation)  
143 were not included. Similarly, studies on altering water table levels in wetlands or fens,  
144 changes in snow cover or drying-rewetting cycles were also excluded. We limited our  
145 analyses to results from plots that solely manipulated precipitation or temperature; results  
146 from plots with changing precipitation or temperature combined with other resource  
147 manipulations were excluded. N<sub>2</sub>O fluxes show seasonal variations. To reduce bias caused by  
148 limited sampling dates, we only included studies that measured N<sub>2</sub>O fluxes for least three  
149 months. If a study reported results from experiments conducted in different locations or  
150 having multiple distinct treatments, these components were treated as individual case studies.  
151 Because effect size (natural log of the response ratio, Eq. 1) could not be calculated for those  
152 observations with zero or negative values under either control or treatments, data from studies  
153 that contained zero or negative values were not included (21 out of 231 observational data  
154 points). In total, our meta-analysis included 46 published papers, including 72 precipitation or  
155 temperature manipulation case studies (Supporting Information, Table S2).

156 The manipulative climate change studies in the field were grouped into four types of  
157 treatment: (1) increased precipitation; (2) decreased precipitation; (3) increased temperature;  
158 and (4) prolonged drought (increased the intervals of precipitation but did not alter the  
159 precipitation quantity). For each selected study, we collected latitude, longitude, mean annual  
160 temperature (MAT) and precipitation (MAP), and one if five main biome types: forest,  
161 shrubland, grassland (including grassland, lawn, meadow, prairie, steppe, and savannah),  
162 cropland (including wheat, rice, maize, and apple), and tundra. The geographical locations of  
163 the studies involving N<sub>2</sub>O fluxes under climate changes spanned from 2°53' N to 56°29' N  
164 (Figure 1), with the MAT ranging from -5.2 to 26.0 °C, and the MAP from 108 to 2000 mm  
165 (Supporting Information, Table S2). Studies reporting SFGs were conducted at 9  
166 geographically and climatically diverse sites including Inner Mongolia, Hebei, and



167 Guangdong in China, Iberian Peninsula in Spain, northern Minnesota in USA, Austria and  
168 Switzerland (Figure 1).

169 We also collected information on treatment time (whole-day, day-time or night-time  
170 warming for increased temperature), treatment season (whole-year, growing season, winter  
171 and several-day for increased temperature; whole-year and growing season for increased  
172 precipitation), treatment duration, and treatment method (heating cables, infrared radiators,  
173 curtains and open-top chambers (OTC) for increased temperature; exclusion (excludes all  
174 ambient rainfall) or reduction (excludes a fixed proportion of the ambient rainfall) for  
175 decreased precipitation) (Supporting Information, Table S2). Control and treatment means  
176 ( $\bar{X}$ ), standard deviations ( $s$ ), and sample sizes ( $n$ ) of N<sub>2</sub>O, SFGs and corresponding soil water  
177 content and temperature were directly extracted or recalculated from digitized figures of each  
178 study using Digitizer (Free Software Foundation, Inc., Boston, MI, USA).

179

## 180 **Statistical analyses**

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

$$184 \quad \ln R = \ln \left( \frac{\bar{X}_t}{\bar{X}_c} \right) \quad (1)$$

185 with a variation ( $v$ ) of :

$$186 \quad v = \frac{s_t^2}{n_t \bar{X}_t^2} + \frac{s_c^2}{n_c \bar{X}_c^2} \quad (2)$$

187 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  
188 deviations for all observations in the treatment and control groups, respectively;  $n_t$  and  $n_c$   
189 are the sample sizes for the treatment and control groups, respectively.

190 We chose to use  $\ln R$  because it is not biased by the variations among replicates within a  
191 treatment (including control) and the absolute magnitude of N<sub>2</sub>O fluxes among experiments  
192 and generally follows a normal distribution (Gruner et al., 2008; Vidal & Murphy, 2018). The  
193 mean effect size ( $\overline{\ln R}$ ) of all observations for a treatment was calculated from  $\ln R$  using a  
194 categorical random effect model. The effect sizes in different phases within the same study  
195 were treated separately (e.g., one effect size per year in a multi-year study), because the mean  
196 effect sizes based on randomly selected individual effect sizes from these multiple-phase  
197 studies were highly similar to those based on the entire effect size dataset (Table S3). This  
198 approach was similar to past meta-analysis studies (Butler et al., 2018; Dijkstra & Adams,  
199 2015).

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

205 Confidence interval (CI) of the effect size was generated using bootstrapping (9999  
206 iterations). Significance of effect size was assessed with the 95% confidential interval (CI).  
207 If the 95% CI of the effect size of a treatment on a variable overlapped “0”, the treatment  
208 effect was considered non-significant; otherwise, the treatment effect was significant.  
209 Categorical random effect model was used to assess whether N<sub>2</sub>O fluxes and SFGs showed  
210 different responses to climate changes among different biomes, treatment times, treatment  
211 seasons and treatment methods. A significant between-group heterogeneity test (Q<sub>b</sub>)  
212 indicated a significant difference in the treatment effect between different groups (e.g.,  
213 exclusion vs. reduction for decreased precipitation). All statistical analyses described above  
214 were performed in MetaWin 2.1.

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

$$217 \quad (e^{\overline{\ln R}} - 1) \times 100\% \quad (3)$$

218 In addition to the meta-analysis procedure, relationships between effect size on N<sub>2</sub>O or SFGS  
219 and changes in soil condition and experimental treatment were also examined. According to  
220 the principle of maximum adjusted  $R^2$ , linear correlations were employed to examine the  
221 relationships between the effect size for N<sub>2</sub>O and changes in soil temperature, treatment  
222 duration in increased temperature studies and the effect size for soil moisture in decreased and  
223 increased precipitation studies. Similarly, binomial correlations were used to test the  
224 relationships between the effect size of SFGs and the effect size of soil moisture across  
225 decreased and increased precipitation studies.

226 **RESULTS**

227 **N<sub>2</sub>O flux**

228 On average, increased temperature and increased precipitation significantly increased N<sub>2</sub>O  
229 emissions by 33% and 55% (95% CI: 11 - 62% and 29 - 83%), respectively, while decreased  
230 precipitation and prolonged drought suppressed N<sub>2</sub>O emissions by 31% and 25% (95% CI: -41  
231 to -17% and -30 to -22%), respectively (Figure 2).

232 Different biomes showed clear differences in N<sub>2</sub>O response to increased temperature ( $P <$   
233 0.01, Table 1). Increased temperature stimulated N<sub>2</sub>O emission by 208% in shrublands (95%  
234 CI: 62 - 507%), suppressed N<sub>2</sub>O emission in tundras by 68% (95% CI: -85 to -35%), had  
235 various effects in forests (95% CI: -4 to 164%). Meanwhile, increased temperature did not  
236 significantly alter N<sub>2</sub>O emissions in the biome groupings of grasslands (95% CI: -10 to 44%)  
237 or croplands (95% CI: -12 to 22%) (Figure 2a). Additionally, N<sub>2</sub>O responses to increased  
238 temperature depended significantly on treatment methods ( $P <$  0.01, Table 1). The use of  
239 open-top chambers (OTCs) consistently increased N<sub>2</sub>O emissions (mean effect size: 172%;  
240 95% CI: 59 - 407%) while heating cables had no significant effect (mean effect size: 45%; 95%  
241 CI: -6 to 117%). In contrast, infrared radiators and curtains did not significantly increase N<sub>2</sub>O  
242 emissions (Figure 2a). Although N<sub>2</sub>O emission in response to increased temperature did not  
243 significantly change with treatment time and season ( $P =$  0.53 and 0.67, respectively, Table  
244 1), the treatments over whole-day (mean effect size: 35%; 95% CI: 11 - 67%) or whole-year  
245 (mean effect size: 38%; 95% CI: 8 - 80%) consistently had significant positive effects (Figure  
246 2a).

247 Increasing soil temperature in shrubland ( $R^2 =$  0.43,  $P =$  0.03) while there were no  
248 significant relationships between N<sub>2</sub>O emissions and temperature in other biomes (Figure 3a).  
249 Besides, the effect size of increased temperature on N<sub>2</sub>O emissions increased with marginal  
250 significance with treatment duration in studies with the OTC method ( $R^2 =$  0.17,  $P =$  0.07),  
251 decreased marginally with the duration in studies with the heating cable method ( $R^2 =$  0.14,  $P$   
252  $=$  0.08), but did not change significantly in studies with the infrared radiator method (Figure  
253 3b). Additionally, the effect of increased temperature on N<sub>2</sub>O emissions had a weakly  
254 positive relationship with latitude (Supporting Information, Figure S2b).

255 The effects of increased precipitation on N<sub>2</sub>O emission were consistently positive  
256 regardless of treatment seasons ( $P =$  0.93, Table 1 and Figure 2b). Similarly, decreased  
257 precipitation reduced N<sub>2</sub>O emission irrespective of biomes and treatment methods ( $P =$  0.90  
258 and 0.85, respectively, Table 1 and Figure 2c). The effect size on N<sub>2</sub>O emissions had a  
259 significant positive relationship with the effect size of soil moisture across increased and

260 decreased precipitation studies ( $R^2 = 0.25$ ,  $P < 0.01$ ; Figure 3c). Additionally, the effect of  
261 decreased temperature on N<sub>2</sub>O emissions had a significantly positive relationship with MAP,  
262 while there is no significant relationship between the effect of increased precipitation on N<sub>2</sub>O  
263 emissions and MAP (Supporting Information, Figure S2c).

264

### 265 **Soil functional genes**

266 Increased temperature did not significantly affect abundance of archaeal *amoA*, bacterial  
267 *amoA* and *nosZ*, but significantly decreased abundances of *nirK* and *nirS* by 26% (95% CI: -  
268 38 to -6%) and 31% (95% CI: -49 to -12%), respectively (Figure 4). There were no  
269 significant differences in increased temperature effects on SFGs between biome, treatment  
270 season and method groups (Supporting Information, Figure S3 and Table 1). Decreased  
271 precipitation had few effects on abundances of archaeal *amoA*, bacterial *amoA*, *nirK* and *nosZ*  
272 (Figures 4a, b, c, e and S4) but showed positive effects on abundances of *nirS* based on two  
273 studies (mean effect size: 170%; 95% CI: 41 - 432%, Figure 4d). Increased precipitation had  
274 little effect on abundances of archaeal *amoA*, *nirK*, *nirS* and *nosZ* (Figures 4a, c, d, e and S5)  
275 while showing negative effects on abundances of bacterial *amoA* (mean effect size: -52%; 95%  
276 CI: -64 to -35%, Figure 4b).

277 For increased temperature studies, there were no significant relationships between the  
278 effect size on SFGs (archaeal *amoA*, bacterial *amoA* and *nosZ*; *nirK* and *nirS* were not tested  
279 due to limited sample size) and changes in soil temperature and treatment duration  
280 (Supporting Information, Figures S6a-f). Across increased and decreased precipitation studies,  
281 *nirK* and *nosZ* showed significant and marginally significant upward-convex relationships  
282 with soil moisture, and were lowest when the effect size on soil moisture was about 0.2 ( $R^2 =$   
283  $0.60$ ,  $P = 0.02$  and  $R^2 = 0.30$ ,  $P = 0.08$ , respectively; Figures 5d and e). In contrast, the  
284 relationships of the effect size on archaeal *amoA*, bacterial *amoA* and *nirS* to the effect size  
285 on soil moisture were not significant (Figures 5a-c).

286 **DISCUSSION**

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

296

297 **N<sub>2</sub>O emission stimulated by increased temperature**

298 On average, our analysis indicated that experimentally increased temperature significantly  
299 increased N<sub>2</sub>O emission by 33% (Figure 2a). This was much higher than the non-significant  
300 increase of 14% found in an earlier meta-analysis based on 26 measurements (Bai et al.,  
301 2013), confirming that warming could increase N<sub>2</sub>O emissions, consistent with expectations  
302 based on temperature-driven microbial kinetics.

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

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

324 With the assembled datasets presently available, we were not able to pinpoint the soil  
325 microbial mechanisms underlying positive temperature effects on N<sub>2</sub>O emission at a global  
326 scale (Figure 4). The potential reasons for such disconnection between temperature effects on  
327 N<sub>2</sub>O emission and on SFGs include data limitations and possible underlying mechanistic  
328 explanations. An important limitation of this meta-analysis is that N<sub>2</sub>O flux and SFGs were  
329 measured independently, presenting a possible barrier to finding their relation. To date, there  
330 remains a lack of sufficient field experiments measuring both N<sub>2</sub>O emissions and SFGs (e.g.  
331 there were only three studies with four observations investigating warming effects on *nirK*  
332 and *nirS*). Mechanistically, if soils are dried by increased temperature, we would expect  
333 decreased abundances of denitrifiers and nitrifiers, especially denitrifiers which are inhibited  
334 by aerobic conditions in drier soils (Waghmode et al., 2018). Additionally, enhanced plant  
335 growth with increased temperature may increase inorganic nitrogen uptake by the plants,  
336 thereby reducing soil nitrogen availability for N<sub>2</sub>O production through nitrification and  
337 denitrification (Carter et al., 2012; Dijkstra et al., 2013; Dijkstra et al., 2012; Pereira et al.,  
338 2013; Zhu et al., 2015). In some cases, plants prioritize aboveground growth in the face of  
339 increased temperature while reducing belowground carbon supply (Dieleman et al., 2012),  
340 which may limit denitrifier proliferation and thus N<sub>2</sub>O emissions. These mechanisms could  
341 also underlie observed negative or null effects of increased temperature on N<sub>2</sub>O emission in  
342 some studies (Dijkstra et al., 2013; Hu et al., 2010; Liu et al., 2016). Overall, alteration of soil  
343 temperature and moisture under experimental warming may have interactive or cascading  
344 effects on plant growth and/or soil nitrogen and carbon contents, complicating relationships  
345 between N<sub>2</sub>O responses and SFGs (Figure 6). Further observational data are required.

346

### 347 **High variation of N<sub>2</sub>O response to increased temperature across biomes and warming** 348 **methods**

349 It should be noted that the effect sizes of increased temperature on N<sub>2</sub>O emissions and the  
350 relationships with changes in soil temperature and treatment duration varied largely with  
351 different biomes and warming methods (Table 1, Figures 2a, 3a and b), consistent with a  
352 previous review suggesting that increased temperature had mixed effects on N<sub>2</sub>O emission  
353 (Dijkstra et al., 2012). Shrublands showed the strongest response to experimental increased

354 temperature among all the biomes and the positive responses to rise in soil temperature were  
355 mainly recorded in a three-year study conducted in a permafrost region, Northeast China (Cui  
356 et al., 2018). However, for extensively investigated grassland and forest ecosystems, we did  
357 not find any significant relationships between the effect size of increased temperature on N<sub>2</sub>O  
358 emission and changes in soil temperature or warming duration. Therefore the general patterns  
359 of increased temperature effect on N<sub>2</sub>O emissions in relation to warming intensity or duration  
360 remain largely unknown.

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

388 may bring great challenges to predict N<sub>2</sub>O fluxes under global warming and resultant future  
389 climate change at large spatial or temporal scales based on the field manipulative experiments.

390

### 391 **N<sub>2</sub>O emission under changed precipitation regimes**

392 Overall, our estimate of N<sub>2</sub>O emission suppression (-31%) by decreased precipitation (Figure  
393 2b) is generally comparable to the recent synthesis of drought-induced decline in N<sub>2</sub>O  
394 emission (-53%) with a smaller data volume (15) and a different computing method for effect  
395 sizes (Hedge's *d*) (Homyak et al., 2017). However, the corresponding 95% confidence  
396 interval of estimates in this meta-analysis was much smaller than that of Homyak et al. (2017)  
397 (-0.53 to -0.19 vs. -1.38 to -0.15), providing a more well-constrained estimate of how  
398 precipitation regulates N<sub>2</sub>O production globally. In contrast to the effect of decreased  
399 precipitation, N<sub>2</sub>O emission was significantly promoted by increased precipitation (Figure 2c),  
400 which is in agreement with most previous observations (Brown et al., 2011; Du, Guo, Cao, &  
401 Li, 2016; Liu et al., 2015; Zhang, Hou, Guo, Li, & Xu, 2017). Together with the consistent  
402 response of N<sub>2</sub>O emission to increased and decreased precipitation regardless of biomes,  
403 treatment methods and seasons (Figures 2b and c), as well as the strong positive relationship  
404 between the effect sizes of N<sub>2</sub>O vs. soil moisture (Figure 3c), we highlight that N<sub>2</sub>O  
405 emissions from terrestrial ecosystems were strongly regulated by soil water availability.

406 Generally, N<sub>2</sub>O emissions were more affected by decreased precipitation-induced negative  
407 effects including suppression of microorganism activities and abundances (Auyeung, Martiny,  
408 & Dukes, 2015; Shi et al., 2012), decline in nitrogen mineralization (Larsen et al., 2011) and  
409 unfavourable aerobic environment for denitrifiers (Davidson, Nepstad, Ishida, & Brando,  
410 2008), in comparison to the accompanying positive effects such as increase in C and N  
411 substrate import (Davidson, Ishida, & Nepstad, 2004) and favourable aerobic environment for  
412 nitrifiers (Carter et al., 2012). In contrast, the increased precipitation-induced positive effects  
413 tended to dominate over the negative effects on N<sub>2</sub>O emissions (Brown et al., 2011; Liu et al.,  
414 2015; Zhang et al., 2017). Therefore, soil moisture could be considered as an important  
415 regulatory and thus predictive factor for N<sub>2</sub>O emissions under climate change although  
416 current projections about precipitation variations in response to climate change remain  
417 uncertain (Lazenby, Todd, Chadwick, & Wang, 2018).

418 Interestingly, we found both effect sizes of *nirK* and *nosZ* showed U-shaped relationships  
419 with the effect size of soil moisture (Figures 5d and e), in contrast to the perception that *nirK*  
420 and *nosZ* genes may be inhibited by lower soil moisture and corresponding higher soil  
421 oxygen concentration under drought (Delgado-Baquerizo et al., 2014; Homyak et al., 2017).



422 The increases in the abundance of these two functional genes were observed when 67% of  
423 rainfall was excluded during a dry season in a 35-year old mixed species forest study (Chen  
424 et al., 2017). More studies are required to verify the effects of decreased precipitation on  
425 denitrifiers and nitrifiers as well as N<sub>2</sub>O emissions.

426 The only prolonged drought study in this meta-analysis suggested that prolonged drought  
427 suppressed N<sub>2</sub>O emissions by 25% (Fentabil et al., 2016). As with increased or decreased  
428 precipitation, we assumed that prolonged drought effects on N<sub>2</sub>O emissions should also be  
429 strongly controlled by soil moisture (Figure 6). In other words, N<sub>2</sub>O emission could be  
430 increased by prolonged drought-induced higher-volume rainfall events necessary to  
431 accomplish the same seasonal precipitation with longer dry intervals or decreased by  
432 prolonged drought-induced dry soil conditions during drought. But soil moisture response to  
433 prolonged drought, both magnitude and direction, may depend on ecosystem types due to  
434 habitat-specific environmental conditions (e.g. moist or dry) and annual precipitation amount  
435 (Knapp et al., 2008; Thomey et al., 2011). For example, larger but fewer rain events led to  
436 greater average soil moisture in a semiarid shortgrass steppe in north eastern Colorado  
437 (Heisler-White, Blair, Kelly, Harmony, & Knapp, 2009) while resulting in a lower  
438 temporally averaged soil water content in a semiarid steppe in Inner Mongolia (Liu et al.,  
439 2017).

440

#### 441 **LIMITATIONS AND PERSPECTIVES**

442 Globally, we found overall significant positive effects of experimental increased temperature  
443 on N<sub>2</sub>O emission, but there are still uncertainties across individual studies due to multiple  
444 regulation mechanisms. We were unable to quantify the direct and indirect effects among soil  
445 temperature, soil moisture, soil nitrate and ammonium concentrations, plant biomass (above  
446 and below portion), and especially soil function genes on N<sub>2</sub>O fluxes (for example, using  
447 structural equation model method), as few studies measured many of these variables  
448 simultaneously. N<sub>2</sub>O emission was the primary targeted parameter in these studies, while the  
449 corresponding SFGs were rarely measured. Such limited data of SFGs currently available  
450 from several specific regions did not allow us to draw general conclusions about how  
451 nitrifiers and denitrifiers respond to climate changes at a global scale. However, considering  
452 soil biotic communities play direct and fundamental roles in driving soil N<sub>2</sub>O response to  
453 climate change, the SFGs responses to simulated climate change are urgently needed to  
454 improve our understanding of biological mechanisms for N<sub>2</sub>O emissions from various  
455 terrestrial ecosystems.

456 Our analysis did not consider the interaction between different climate changes. Increased  
457 temperature and decreased precipitation are often coupled in nature mainly through positive  
458 drought-temperature feedbacks (Ciais et al., 2005; De Boeck, Dreesen, Janssens, & Nijs,  
459 2010; Seneviratne, Luthi, Litschi, & Schar, 2006), while both temperature and precipitation  
460 were predicted to increase in the future in some regions, such as East Africa (Stocker, 2014).  
461 Effects of changes in temperature and precipitation work together and may trigger  
462 unexpected outcomes. Besides, atmospheric nitrogen deposition is a primary global change  
463 and nitrogen enrichment has been shown to alter N<sub>2</sub>O response to climate changes (Brown et  
464 al., 2011; Chen et al., 2017; Zhao et al., 2017). Evaluating interactive effects of multiple  
465 global change factors on ecosystem processes is more important for understanding ecological  
466 responses than looking into a single factor alone (Luo et al., 2008; Zhu, Chiariello, Tobeck,  
467 Fukami, & Field, 2016). Thus it is essential and vital to adequately assess how N<sub>2</sub>O fluxes  
468 would respond to interactions of these factors.

469 In reviewing field experiments in this meta-analysis, it became apparent that almost all  
470 studies included in this meta-analysis examined the impacts of long-term chronic climate  
471 change. For increased temperature studies, 16 out of 29 studies were warming throughout the  
472 whole year and 12 studies were warming over the growing season or winter only, with only  
473 one heat wave study (extreme warming for one week, (Li et al., 2016)) (Figure 2a). For  
474 increased precipitation studies, evenly increased precipitation throughout a year were tested  
475 in all studies apart from one with randomly increased precipitation during a four-month  
476 treatment period. There is almost no study examining short-term but large increases in  
477 temperature and precipitation to simulate heat waves and heavy rainfalls. Although past  
478 studies have largely improved our understanding of how N<sub>2</sub>O flux responds to changes in  
479 precipitation and increases in temperature, much work is required to further our  
480 understanding of the climate change effects on N<sub>2</sub>O fluxes, as the ecological effects of  
481 climate extremes and those of long-term chronic climate change were markedly different  
482 (Jentsch, Kreyling, & Beierkuhnlein, 2007; Reyer et al., 2013). A typical example is the  
483 application of a constant heat sum on northern red oak seedlings with different amplitudes  
484 and duration, + 3 °C for four weeks, + 6 °C for two weeks and + 12 °C for one week,  
485 representing warming, moderate heat wave and severe heat wave, respectively (Bauweraerts  
486 et al., 2013). Interestingly, these three treatments increased, did not change and decreased  
487 biomass accumulation, respectively, highlighting opposite ecosystem responses to long-term  
488 warming and short-term heat waves. Therefore, we suggest that future manipulative field  
489 experiments should investigate extreme climate event treatments.

490

491 **CONCLUSIONS**

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

Author Manuscript

505 **ACKNOWLEDGEMENTS**

506 This project was funded by the CAS Strategic Priority Research Programme (A) (Grant No.  
507 XDA20050103, XDA19030202) and the International Cooperation and Exchange of National  
508 Natural Science Foundation of China (Grant No. 31761123001, 31761143018). Great thanks  
509 for the help of the Inner Mongolia Grassland Ecosystem Research Station.

Author Manuscript

510 **REFERENCES**

- 511 Aronson, E. L., & McNulty, S. G. (2009). Appropriate experimental ecosystem warming  
512 methods by ecosystem, objective, and practicality. *Agricultural and Forest*  
513 *Meteorology*, 149(11), 1791-1799. doi:10.1016/j.agrformet.2009.06.007
- 514 Auyeung, D. N., Martiny, J. B., & Dukes, J. S. (2015). Nitrification kinetics and  
515 ammonia-oxidizing community respond to warming and altered precipitation.  
516 *Ecosphere*, 6(5), 1-17. doi:10.1890/ES14-00481.1
- 517 Bai, E., Li, S., Xu, W., Li, W., Dai, W., & Jiang, P. (2013). A meta-analysis of experimental  
518 warming effects on terrestrial nitrogen pools and dynamics. *New Phytologist*, 199(2),  
519 431-440. doi:10.1111/nph.12252
- 520 Bauweraerts, I., Wertin, T. M., Ameye, M., McGuire, M. A., Teskey, R. O., & Steppe, K.  
521 (2013). The effect of heat waves, elevated [CO<sub>2</sub>] and low soil water availability on  
522 northern red oak (*Quercus rubra* L.) seedlings. *Global Change Biology*, 19(2), 517-  
523 528. doi:10.1111/gcb.12044
- 524 Bijoor, N. S., Czimczik, C. I., Pataki, D. E., & Billings, S. A. (2008). Effects of temperature  
525 and fertilization on nitrogen cycling and community composition of an urban lawn.  
526 *Global Change Biology*, 14(9), 2119-2131. doi:10.1111/j.1365-2486.2008.01617.x
- 527 Brown, J. R., Blankinship, J. C., Niboyet, A., van Groenigen, K. J., Dijkstra, P., Le Roux,  
528 X., . . . Hungate, B. A. (2011). Effects of multiple global change treatments on soil  
529 N<sub>2</sub>O fluxes. *Biogeochemistry*, 109(1-3), 85-100. doi:10.1007/s10533-011-9655-2
- 530 Butler, O. M., Elser, J. J., Lewis, T., Mackey, B., & Chen, C. (2018). The phosphorus-rich  
531 signature of fire in the soil-plant system: a global meta-analysis. *Ecology Letters*,  
532 21(3), 335-344. doi:10.1111/ele.12896
- 533 Cantarel, A. A. M., Bloor, J. M. G., Pommier, T., Guillaumaud, N., Moirrot, C., Soussana, J.  
534 F., & Poly, F. (2012). Four years of experimental climate change modifies the  
535 microbial drivers of N<sub>2</sub>O fluxes in an upland grassland ecosystem. *Global Change*  
536 *Biology*, 18(8), 2520-2531. doi:10.1111/j.1365-2486.2012.02692.x
- 537 Carter, M. S., Larsen, K. S., Emmett, B., Estiarte, M., Field, C., Leith, I. D., . . . Beier, C.  
538 (2012). Synthesizing greenhouse gas fluxes across nine European peatlands and  
539 shrublands – responses to climatic and environmental changes. *Biogeosciences*, 9(10),  
540 3739-3755. doi:10.5194/bg-9-3739-2012
- 541 Cayuela, M. L., Aguilera, E., Sanz-Cobena, A., Adams, D. C., Abalos, D., Barton, L., . . .  
542 Lassaletta, L. (2017). Direct nitrous oxide emissions in Mediterranean climate

543 cropping systems: Emission factors based on a meta-analysis of available  
544 measurement data. *Agriculture, Ecosystems & Environment*, 238, 25-35.  
545 doi:10.1016/j.agee.2016.10.006

546 Chen, J., Xiao, G., Kuzyakov, Y., Jenerette, G. D., Ma, Y., Liu, W., . . . Shen, W. (2017). Soil  
547 nitrogen transformation responses to seasonal precipitation changes are regulated by  
548 changes in functional microbial abundance in a subtropical forest. *Biogeosciences*,  
549 14(9), 2513-2525. doi:10.5194/bg-14-2513-2017

550 Chen, X., Wang, G., Zhang, T., Mao, T., Wei, D., Hu, Z., & Song, C. (2017). Effects of  
551 warming and nitrogen fertilization on GHG flux in the permafrost region of an alpine  
552 meadow. *Atmospheric Environment*, 157, 111-124.  
553 doi:10.1016/j.atmosenv.2017.03.024

554 Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogee, J., Allard, V., . . . Valentini, R. (2005).  
555 Europe-wide reduction in primary productivity caused by the heat and drought in  
556 2003. *Nature*, 437(7058), 529-533. doi:10.1038/nature03972

557 Cui, Q., Song, C., Wang, X., Shi, F., Yu, X., & Tan, W. (2018). Effects of warming on N<sub>2</sub>O  
558 fluxes in a boreal peatland of Permafrost region, Northeast China. *Science of Total  
559 Environment*, 616-617, 427-434. doi:10.1016/j.scitotenv.2017.10.246

560 Dalal, R. C., & Allen, D. E. (2008). Greenhouse gas fluxes from natural ecosystems.  
561 *Australian Journal of Botany*, 56(5), 369-407. doi:10.1071/bt07128

562 Davidson, E. A., Ishida, F. Y., & Nepstad, D. C. (2004). Effects of an experimental drought  
563 on soil emissions of carbon dioxide, methane, nitrous oxide, and nitric oxide in a  
564 moist tropical forest. *Global Change Biology*, 10(5), 718-730. doi:10.1111/j.1529-  
565 8817.2003.00762.x

566 Davidson, E. A., Nepstad, D. C., Ishida, F. Y., & Brando, P. M. (2008). Effects of an  
567 experimental drought and recovery on soil emissions of carbon dioxide, methane,  
568 nitrous oxide, and nitric oxide in a moist tropical forest. *Global Change Biology*.  
569 doi:10.1111/j.1365-2486.2008.01694.x

570 De Boeck, H. J., Dreesen, F. E., Janssens, I. A., & Nijs, I. (2010). Climatic characteristics of  
571 heat waves and their simulation in plant experiments. *Global Change Biology*, 16(7),  
572 1992-2000. doi:10.1111/j.1365-2486.2009.02049.x

573 Delgado-Baquerizo, M., Maestre, F. T., Escolar, C., Gallardo, A., Ochoa, V., Gozalo, B., &  
574 Prado-Comesaña, A. (2014). Direct and indirect impacts of climate change on  
575 microbial and biocrust communities alter the resistance of the N cycle in a semiarid  
576 grassland. *Journal of Ecology*, 102(6), 1592-1605. doi: 10.1111/1365-2745.12303

577 de Vries, F. T., Liiri, M. E., Bjørnlund, L., Bowker, M. A., Christensen, S., Setälä, H. M., &  
578 Bardgett, R. D. (2012). Land use alters the resistance and resilience of soil food webs  
579 to drought. *Nature Climate Change*, 2(4), 276-280. doi:10.1038/nclimate1368

580 Dieleman, W. I., Vicca, S., Dijkstra, F. A., Hagedorn, F., Hovenden, M. J., Larsen, K. S., . . .  
581 Dukes, J. S. (2012). Simple additive effects are rare: a quantitative review of plant  
582 biomass and soil process responses to combined manipulations of CO<sub>2</sub> and  
583 temperature. *Global Change Biology*, 18(9), 2681-2693. doi:10.1111/j.1365-  
584 2486.2012.02745.x

585 Dijkstra, F., & Adams, M. (2015). Fire eases imbalances of nitrogen and phosphorus in  
586 woody plants. *Ecosystems*, 18(5), 769-779. doi:10.1007/s10021-015-9861-1

587 Dijkstra, F. A., Morgan, J. A., Follett, R. F., & Lecain, D. R. (2013). Climate change reduces  
588 the net sink of CH<sub>4</sub> and N<sub>2</sub>O in a semiarid grassland. *Global Change Biology*, 19(6),  
589 1816-1826. doi:10.1111/Gcb.12182

590 Dijkstra, F. A., Prior, S. A., Runion, G. B., Torbert, H. A., Tian, H., Lu, C., & Venterea, R. T.  
591 (2012). Effects of elevated carbon dioxide and increased temperature on methane and  
592 nitrous oxide fluxes: evidence from field experiments. *Frontiers in Ecology and the*  
593 *Environment*, 10(10), 520-527. doi:10.1890/120059

594 Du, Y., Guo, X., Cao, G., & Li, Y. (2016). Increased Nitrous Oxide Emissions Resulting  
595 from Nitrogen addition and increased precipitation in an alpine meadow ecosystem.  
596 *Polish Journal of Environmental Studies*, 25(1), 447-451. doi:10.15244/pjoes/60860

597 Fentabil, M. M., Nichol, C. F., Jones, M. D., Neilsen, G. H., Neilsen, D., & Hannam, K. D.  
598 (2016). Effect of drip irrigation frequency, nitrogen rate and mulching on nitrous  
599 oxide emissions in a semi-arid climate: An assessment across two years in an apple  
600 orchard. *Agriculture, Ecosystems & Environment*, 235, 242-252.  
601 doi:10.1016/j.agee.2016.09.033

602 Francis, C. A., Roberts, K. J., Beman, J. M., Santoro, A. E., & Oakley, B. B. (2005). Ubiquity  
603 and diversity of ammonia-oxidizing archaea in water columns and sediments of the  
604 ocean. *Proceedings of the National Academy of Sciences of the United States of*  
605 *America*, 102(41), 14683-14688. doi:10.1073/pnas.0506625102

606 Gruner, D. S., Smith, J. E., Seabloom, E. W., Sandin, S. A., Ngai, J. T., Hillebrand, H., . . .  
607 Bracken, M. E. (2008). A cross-system synthesis of consumer and nutrient resource  
608 control on producer biomass. *Ecology Letters*, 11(7), 740-755. doi:10.1111/j.1461-  
609 0248.2008.01192.x

- 610 Heisler-White, J. L., Blair, J. M., Kelly, E. F., Harmoney, K., & Knapp, A. K. (2009).  
611 Contingent productivity responses to more extreme rainfall regimes across a grassland  
612 biome. *Global Change Biology*, 15(12), 2894-2904. doi:10.1111/j.1365-  
613 2486.2009.01961.x
- 614 Henry, S., Baudoin, E., López-Gutiérrez, J. C., Martin-Laurent, F., Brauman, A., & Philippot,  
615 L. (2004). Quantification of denitrifying bacteria in soils by nirK gene targeted real-  
616 time PCR. *Journal of Microbiological Methods*, 59(3), 327-335.  
617 doi:10.1016/j.mimet.2004.07.002
- 618 Homyak, P. M., Allison, S. D., Huxman, T. E., Goulden, M. L., & Treseder, K. K. (2017).  
619 Effects of drought manipulation on soil nitrogen cycling: A Meta-Analysis. *Journal of*  
620 *Geophysical Research: Biogeosciences*, 122(12), 3260-3272.  
621 doi:10.1002/2017jg004146
- 622 Hoover, D. L., & Rogers, B. M. (2016). Not all droughts are created equal: the impacts of  
623 interannual drought pattern and magnitude on grassland carbon cycling. *Global*  
624 *Change Biology*, 22(5), 1809-1820. doi:10.1111/gcb.13161
- 625 Hoover, D. L., Wilcox, K. R., & Young, K. E. (2018). Experimental droughts with rainout  
626 shelters: a methodological review. *Ecosphere*, 9(1). doi:02088. 10.1002/ecs2.2088
- 627 Hu, Y., Chang, X., Lin, X., Wang, Y., Wang, S., Duan, J., . . . Zhao, X. (2010). Effects of  
628 warming and grazing on N<sub>2</sub>O fluxes in an alpine meadow ecosystem on the Tibetan  
629 plateau. *Soil Biology and Biochemistry*, 42(6), 944-952.  
630 doi:10.1016/j.soilbio.2010.02.011
- 631 Jentsch, A., Kreyling, J., & Beierkuhnlein, C. (2007). A new generation of climate-change  
632 experiments: events, not trends. *Frontiers in Ecology and the Environment*, 5(7), 365-  
633 374. doi:10.1890/1540-9295(2007)5[365:ANGOCE]2.0.CO;2
- 634 Kandeler, E., Deiglmayr, K., Tschirko, D., Bru, D., & Philippot, L. (2006). Abundance of  
635 *narG*, *nirS*, *nirK*, and *nosZ* genes of denitrifying bacteria during primary successions  
636 of a glacier foreland. *Applied and Environmental Microbiology*, 72(9), 5957-5962.  
637 doi:10.1128/AEM.00439-06
- 638 Knapp, A. K., Beier, C., Briske, D. D., Classen, A. T., Luo, Y., Reichstein, M., . . . Weng, E.  
639 (2008). Consequences of more extreme precipitation regimes for terrestrial  
640 ecosystems. *Bioscience*, 58(9), 811-821. doi:10.1641/b580908
- 641 Kuiper, J. J., Mooij, W. M., Bragazza, L., & Robroek, B. J. M. (2014). Plant functional types  
642 define magnitude of drought response in peatland CO<sub>2</sub> exchange. *Ecology*, 95(1), 123-  
643 131. doi:10.1890/13-0270.1



- 644 Kuypers, M. M. M., Marchant, H. K., & Kartal, B. (2018). The microbial nitrogen-cycling  
645 network. *Nature Review Microbiology*, *16*(5), 263-276. doi:10.1038/nrmicro.2018.9
- 646 Larsen, K. S., Andresen, L. C., Beier, C., Jonasson, S., Albert, K. R., Ambus, P. E. R., . . .  
647 Stevnbak, K. (2011). Reduced N cycling in response to elevated CO<sub>2</sub>, warming, and  
648 drought in a Danish heathland: Synthesizing results of the CLIMAITE project after  
649 two years of treatments. *Global Change Biology*, *17*(5), 1884-1899.  
650 doi:10.1111/j.1365-2486.2010.02351.x
- 651 Lazenby, M. J., Todd, M. C., Chadwick, R., & Wang, Y. (2018). Future precipitation  
652 projections over central and southern Africa and the adjacent Indian Ocean: What  
653 causes the changes and the uncertainty? *Journal of Climate*, *31*(12), 4807-4826.  
654 doi:10.1175/JCLI-D-17-0311.1
- 655 Li, J., Jin, Y., Liu, Y., Zhang, Y., Grace, J., Song, Q., . . . Fei, X. (2018). Effects of  
656 precipitation exclusion on N<sub>2</sub>O emissions in a savanna ecosystem in SW China.  
657 *Atmospheric Environment*, *187*, 1-8. doi:10.1016/j.atmosenv.2018.05.035
- 658 Li, L., Fan, W., Kang, X., Wang, Y., Cui, X., Xu, C., . . . Hao, Y. (2016). Responses of  
659 greenhouse gas fluxes to climate extremes in a semiarid grassland. *Atmospheric*  
660 *Environment*, *142*, 32-42. doi:10.1016/j.atmosenv.2016.07.039
- 661 Liu, L., Hu, C., Yang, P., Ju, Z., Olesen, J. E., & Tang, J. (2016). Experimental warming-  
662 driven soil drying reduced N<sub>2</sub>O emissions from fertilized crop rotations of winter  
663 wheat–soybean/fallow, 2009–2014. *Agriculture, Ecosystems & Environment*, *219*, 71-  
664 82. doi:10.1016/j.agee.2015.12.013
- 665 Liu, L., Wang, X., Lajeunesse, M. J., Miao, G., Piao, S., Wan, S., . . . Deng, M. (2016). A  
666 cross-biome synthesis of soil respiration and its determinants under simulated  
667 precipitation changes. *Global Change Biology*, *22*(4), 1394-1405.  
668 doi:10.1111/gcb.13156
- 669 Liu, S., Ji, C., Wang, C., Chen, J., Jin, Y., Zou, Z., . . . Zou, J. (2018). Climatic role of  
670 terrestrial ecosystem under elevated CO<sub>2</sub> : a bottom-up greenhouse gases budget.  
671 *Ecology Letters*, *21*(7), 1108-1118. doi:10.1111/ele.13078
- 672 Liu, W. J., Li, L. F., Biederman, J. A., Hao, Y. B., Zhang, H., Kang, X. M., . . . Xu, C. Y.  
673 (2017). Repackaging precipitation into fewer, larger storms reduces ecosystem  
674 exchanges of CO<sub>2</sub> and H<sub>2</sub>O in a semiarid steppe. *Agricultural and Forest Meteorology*,  
675 *247*, 356-364. doi:10.1016/j.agrformet.2017.08.029
- 676 Liu, X., Dong, Y., Qi, Y., Peng, Q., He, Y., Sun, L., . . . Liu, X. (2015). Response of N<sub>2</sub>O  
677 emission to water and nitrogen addition in temperate typical steppe soil in Inner

678 Mongolia, China. *Soil and Tillage Research*, 151, 9-17.  
679 doi:10.1016/j.still.2015.01.008

680 Luo, Y., Gerten, D., Le Maire, G., Parton, W. J., Weng, E., Zhou, X., . . . Rustad, L. (2008).  
681 Modeled interactive effects of precipitation, temperature, and [CO<sub>2</sub>] on ecosystem  
682 carbon and water dynamics in different climatic zones. *Global Change Biology*, 14(9),  
683 1986-1999. doi:10.1111/j.1365-2486.2008.01629.x

684 McHale, P. J., Mitchell, M. J., & Bowles, F. P. (1998). Soil warming in a northern hardwood  
685 forest: trace gas fluxes and leaf litter decomposition. *Canadian Journal of Forest  
686 Research*, 28(9), 1365-1372.

687 Martins, C. S. C., Nazaries, L., Delgado-Baquerizo, M., Macdonald, C. A., Anderson, I. C.,  
688 Hobbie, S. E., . . . Field, K. (2017). Identifying environmental drivers of greenhouse  
689 gas emissions under warming and reduced rainfall in boreal-temperate forests.  
690 *Functional Ecology*, 31(12), 2356-2368. doi:10.1111/1365-2435.12928

691 Pereira, J., Figueiredo, N., Goufo, P., Carneiro, J., Morais, R., Carranca, C., . . . Trindade, H.  
692 (2013). Effects of elevated temperature and atmospheric carbon dioxide concentration  
693 on the emissions of methane and nitrous oxide from Portuguese flooded rice fields.  
694 *Atmospheric Environment*, 80, 464-471. doi:10.1016/j.atmosenv.2013.08.045

695 Reyer, C. P., Leuzinger, S., Rammig, A., Wolf, A., Bartholomeus, R. P., Bonfante, A., . . .  
696 Pereira, M. (2013). A plant's perspective of extremes: terrestrial plant responses to  
697 changing climatic variability. *Global Change Biology*, 19(1), 75-89.  
698 doi:10.1111/gcb.12023

699 Saggarr, S., Jha, N., Deslippe, J., Bolan, N., Luo, J., Giltrap, D., . . . Tillman, R. (2013).  
700 Denitrification and N<sub>2</sub>O: N<sub>2</sub> production in temperate grasslands: processes,  
701 measurements, modelling and mitigating negative impacts. *Science of the Total  
702 Environment*, 465, 173-195. doi:10.1016/j.scitotenv.2012.11.050

703 Seneviratne, S. I., Luthi, D., Litschi, M., & Schar, C. (2006). Land-atmosphere coupling and  
704 climate change in Europe. *Nature*, 443(7108), 205-209. doi:10.1038/nature05095

705 Shi, F., Chen, H., Chen, H., Wu, Y., & Wu, N. (2012). The combined effects of warming and  
706 drying suppress CO<sub>2</sub> and N<sub>2</sub>O emission rates in an alpine meadow of the eastern  
707 Tibetan Plateau. *Ecological Research*, 27(4), 725-733. doi:10.1007/s11284-012-0950-  
708 8

709 Stocker, T. (2014). *Climate change 2013: the physical science basis: Working Group I  
710 contribution to the Fifth assessment report of the Intergovernmental Panel on Climate  
711 Change*: Cambridge University Press , Cambridge, UK and New York, NY.

- 712 Teuling, A. J., Seneviratne, S. I., Stöckli, R., Reichstein, M., Moors, E., Ciais, P., . . .  
713 Wohlfahrt, G. (2010). Contrasting response of European forest and grassland energy  
714 exchange to heatwaves. *Nature Geoscience*, *3*(10), 722-727. doi:10.1038/ngeo950
- 715 Thomey, M. L., Collins, S. L., Vargas, R., Johnson, J. E., Brown, R. F., Natvig, D. O., &  
716 Friggens, M. T. (2011). Effect of precipitation variability on net primary production  
717 and soil respiration in a Chihuahuan Desert grassland. *Global Change Biology*, *17*(4),  
718 1505-1515. doi:10.1111/j.1365-2486.2010.02363.x
- 719 Tu, C., & Li, F. (2017). Responses of greenhouse gas fluxes to experimental warming in  
720 wheat season under conventional tillage and no-tillage fields. *Journal of*  
721 *Environmental Science (China)*, *54*, 314-327. doi:10.1016/j.jes.2016.09.016
- 722 Vidal, M. C., & Murphy, S. M. (2018). Bottom-up vs. top-down effects on terrestrial insect  
723 herbivores: a meta-analysis. *Ecology Letters*, *21*(1), 138-150. doi:10.1111/ele.12874
- 724 Waghmode, T. R., Chen, S., Li, J., Sun, R., Liu, B., & Hu, C. (2018). Response of nitrifier  
725 and denitrifier abundance and microbial community structure to experimental  
726 warming in an agricultural ecosystem. *Frontiers in Microbiology*, *9*, 474.  
727 doi:10.3389/fmicb.2018.00474
- 728 Ward, S. E., Ostle, N. J., Oakley, S., Quirk, H., Henrys, P. A., & Bardgett, R. D. (2013).  
729 Warming effects on greenhouse gas fluxes in peatlands are modulated by vegetation  
730 composition. *Ecology Letters*, *16*(10), 1285-1293. doi:10.1111/ele.12167
- 731 Wu, Z., Dijkstra, P., Koch, G. W., Penuelas, J., & Hungate, B. A. (2011). Responses of  
732 terrestrial ecosystems to temperature and precipitation change: a meta-analysis of  
733 experimental manipulation. *Global Change Biology*, *17*(2), 927-942.  
734 doi:10.1111/j.1365-2486.2010.02302.x
- 735 Zhang, L., Hou, L., Guo, D., Li, L., & Xu, X. (2017). Interactive impacts of nitrogen input  
736 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  
737 grassland, Northern China. *Science of the Total Environment*, *578*, 523-534.  
738 doi:10.1016/j.scitotenv.2016.10.219
- 739 Zhang, X., Liu, W., Schloter, M., Zhang, G., Chen, Q., Huang, J., . . . Han, X. (2013).  
740 Response of the abundance of key soil microbial nitrogen-cycling genes to multi-  
741 factorial global changes. *PLoS One*, *8*(10), e76500. doi:10.1371/journal.pone.0076500
- 742 Zhao, Z., Dong, S., Jiang, X., Liu, S., Ji, H., Li, Y., . . . Sha, W. (2017). Effects of warming  
743 and nitrogen deposition on CH<sub>4</sub>, CO<sub>2</sub> and N<sub>2</sub>O emissions in alpine grassland  
744 ecosystems of the Qinghai-Tibetan Plateau. *Science of Total Environment*, *592*, 565-  
745 572. doi:10.1016/j.scitotenv.2017.03.082

- 746 Zhou, Y., Hagedorn, F., Zhou, C., Jiang, X., Wang, X., & Li, M. H. (2016). Experimental  
747 warming of a mountain tundra increases soil CO<sub>2</sub> effluxes and enhances CH<sub>4</sub> and N<sub>2</sub>O  
748 uptake at Changbai Mountain, China. *Scientific Reports*, 6, 21108.  
749 doi:10.1038/srep21108
- 750 Zhu, K., Chiariello, N. R., Tobeck, T., Fukami, T., & Field, C. B. (2016). Nonlinear,  
751 interacting responses to climate limit grassland production under global change.  
752 *Proceeding of National Academy Sciences of the United States of America*, 113(38),  
753 10589-10594. doi:10.1073/pnas.1606734113
- 754 Zhu, X., Luo, C., Wang, S., Zhang, Z., Cui, S., Bao, X., . . . Zhou, Y. (2015). Effects of  
755 warming, grazing/cutting and nitrogen fertilization on greenhouse gas fluxes during  
756 growing seasons in an alpine meadow on the Tibetan Plateau. *Agricultural and Forest  
757 Meteorology*, 214-215, 506-514. doi:10.1016/j.agrformet.2015.09.008

758 **Table 1** The between-group heterogeneity ( $Q_b$ ) of climate change effects on various response  
 759 variables.

Manipulative type	Response variables	Categorical variables	DF <sub>b</sub>	Q <sub>b</sub>	P-value
Increased temperature	N <sub>2</sub> O flux	Biomes	4	27.45	< <b>0.01</b>
		Treatment times	2	0.65	0.53
		Treatment seasons	2	0.50	0.67
		Treatment methods	3	21.61	< <b>0.01</b>
	Archaeal <i>amoA</i>	Biomes	2	0.32	0.81
		Treatment seasons	1	0.39	0.51
		Treatment methods	1	0.12	0.72
	Bacterial <i>amoA</i>	Biomes	2	1.47	0.54
		Treatment seasons	1	0.04	0.84
		Treatment methods	1	< 0.01	0.97
	<i>nosZ</i>	Biomes	2	0.96	0.66
		Treatment seasons	1	0.01	0.96
Increased precipitation	N <sub>2</sub> O flux	Treatment seasons	1	< 0.01	0.93
	<i>nirK</i>	Biomes	1	0.13	0.62
	<i>nirS</i>	Biomes	1	4.95	0.07
	<i>nosZ</i>	Biomes	1	0.42	0.53
Decreased precipitation	N <sub>2</sub> O flux	Biomes	2	0.29	0.89
		Treatment methods	1	0.05	0.85
	Archaeal <i>amoA</i>	Biomes	1	0.73	0.46
		Treatment methods	1	1.00	0.42
	Bacterial <i>amoA</i>	Biomes	1	1.83	0.23
		Treatment methods	1	0.97	0.36
<i>nosZ</i>	Treatment methods	1	0.47	0.70	

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

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

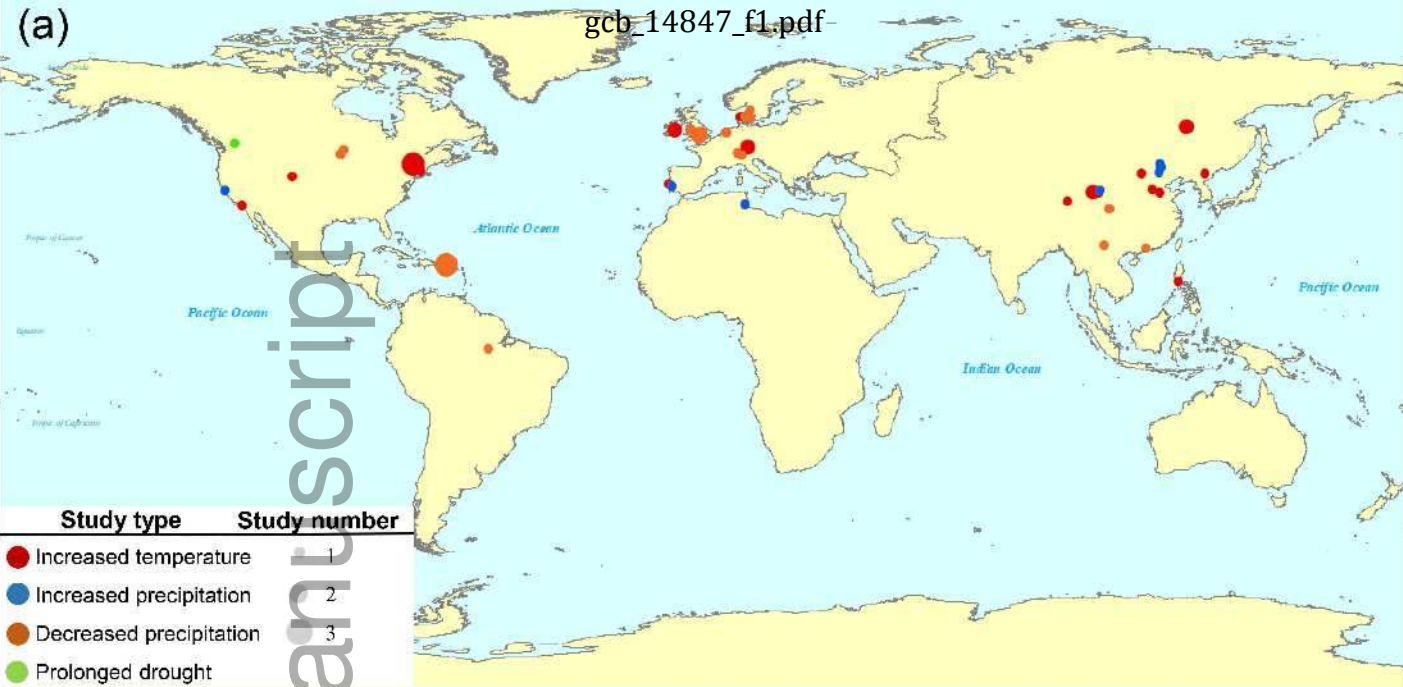
771 **Figure 3** Relationships between effect sizes on N<sub>2</sub>O emission and intensity and duration of  
772 climate change treatment. Only significant ( $P \leq 0.05$ ) or marginally significant ( $0.05 < P <$   
773  $0.10$ ) relationships were shown and corresponding shaded areas represent 95% confidence  
774 intervals. The sample size for each variable is shown in parenthesis. OTC: open-top chamber.

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

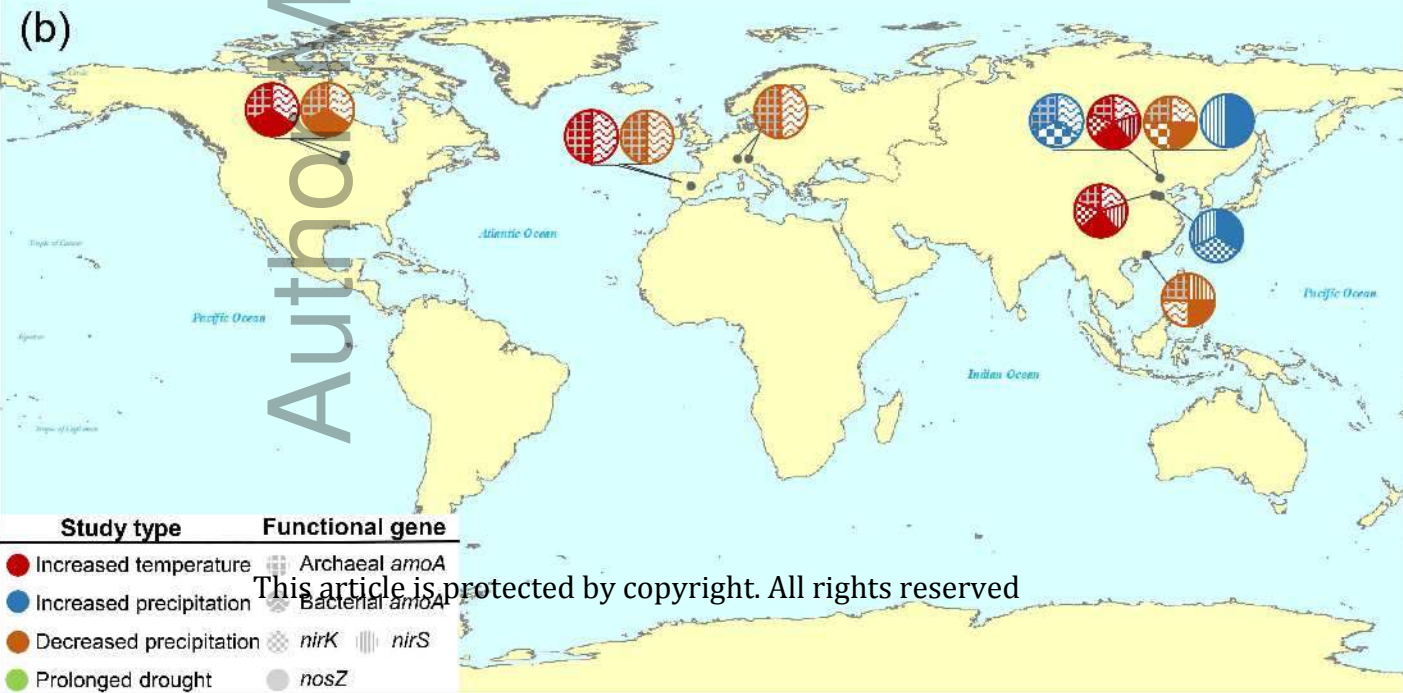
780 **Figure 5** Relationships between effect sizes of changed precipitation on soil functional genes  
781 and soil moisture. Only significant or marginally significant relationships (binomial  
782 relationships) were shown and the shaded areas represent 95% confidence intervals. The  
783 sample size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

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

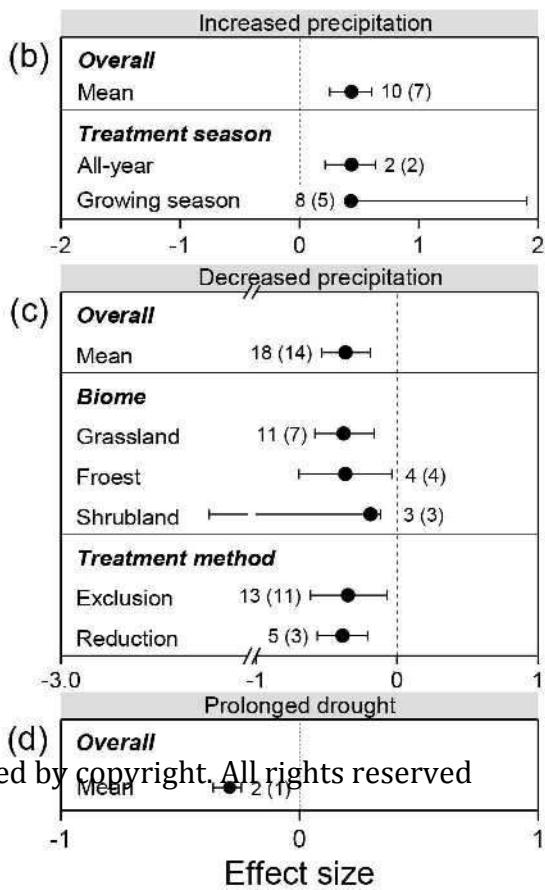
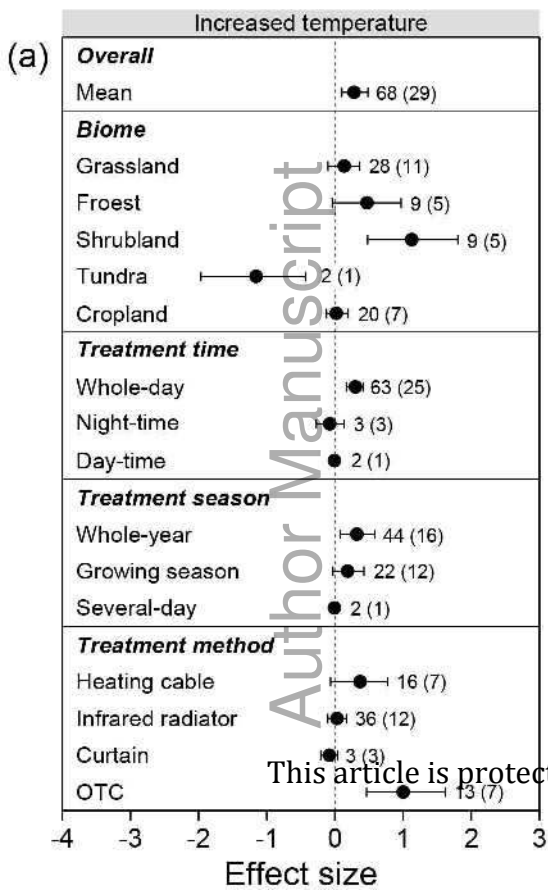
(a)



(b)

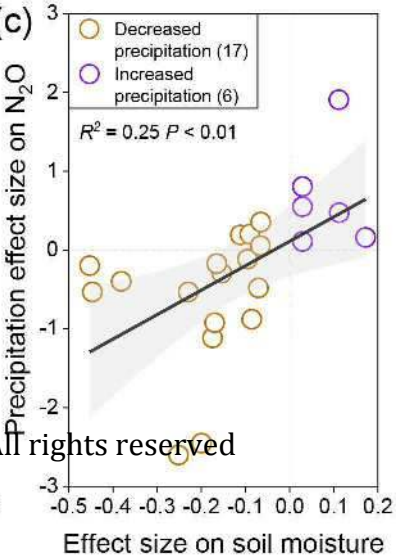
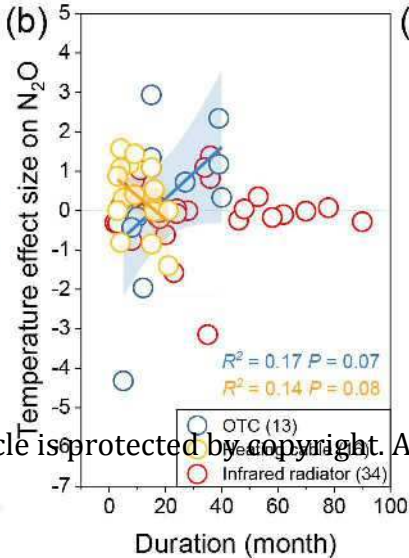
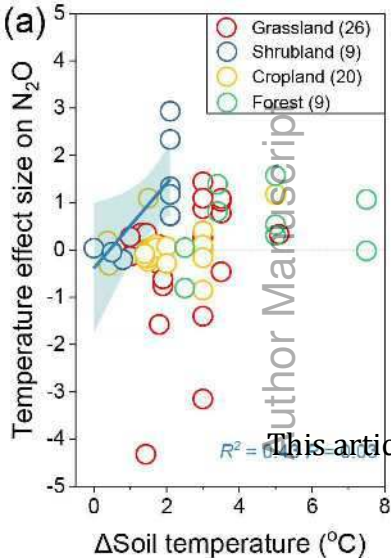


This article is protected by copyright. All rights reserved

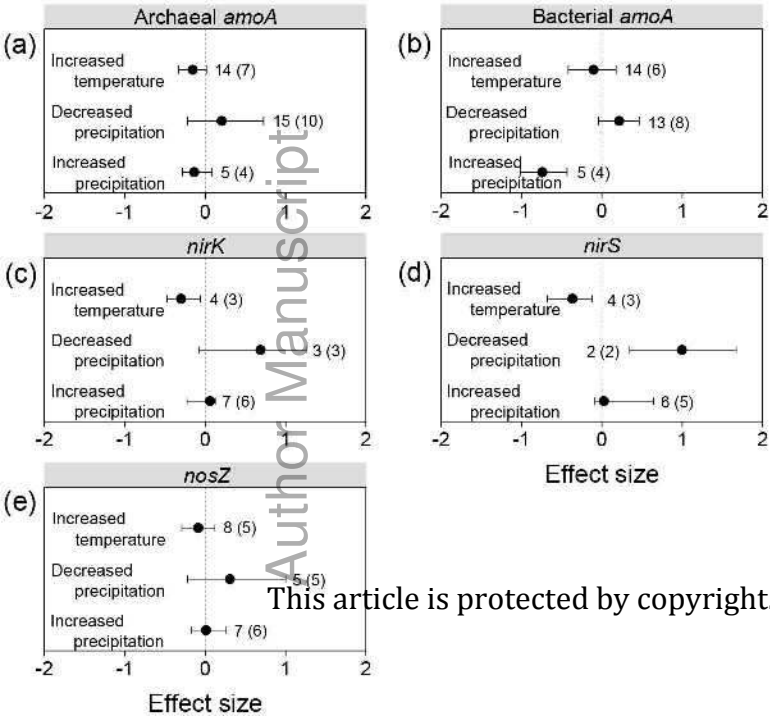


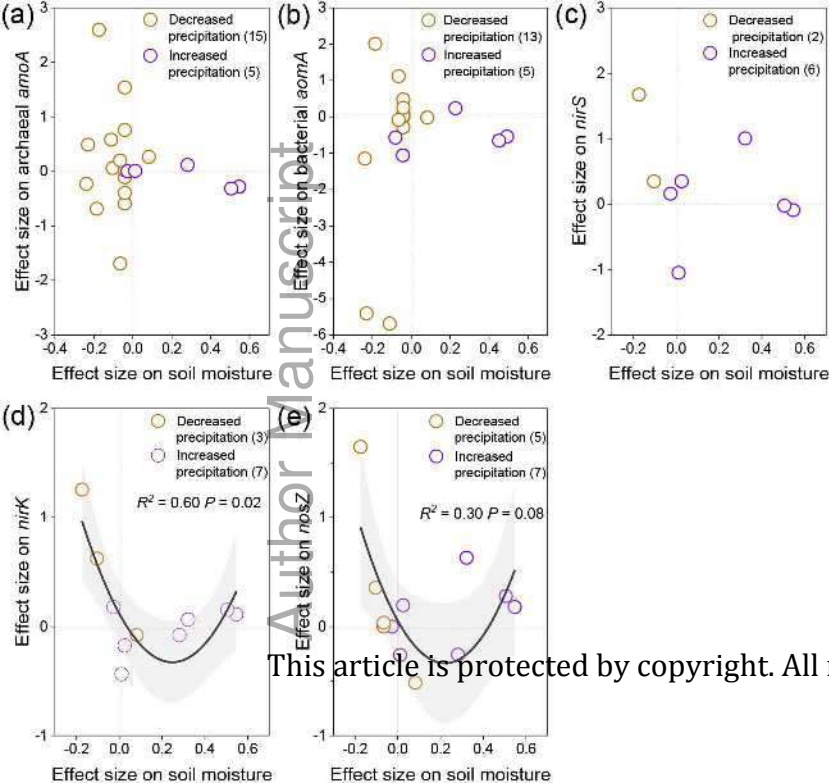
This article is protected by copyright. All rights reserved



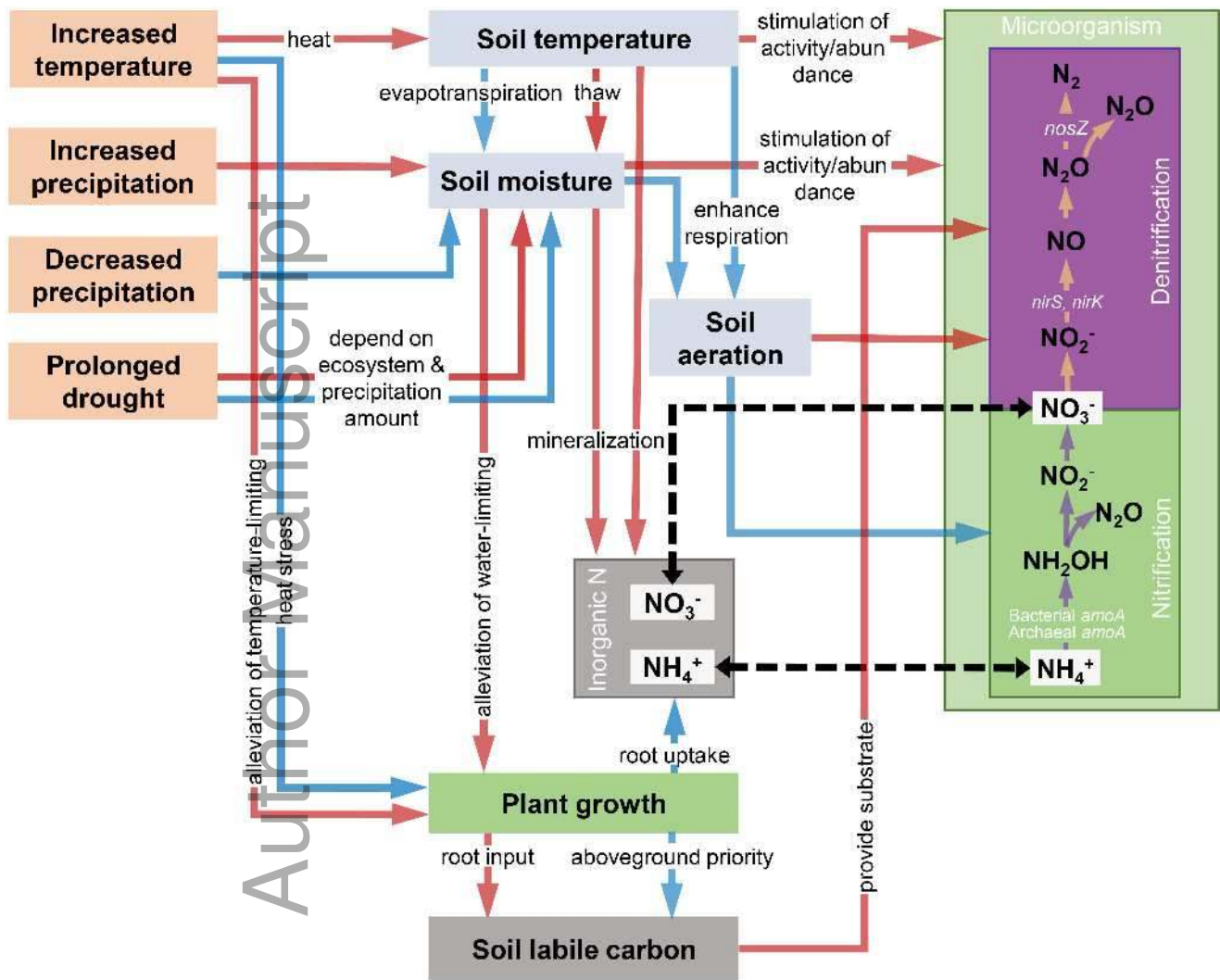


This article is protected by copyright. All rights reserved





Author Manuscript



This article is protected by copyright. All rights reserved

- ➔ Positive/enhancing impacts
- ➔ Negative/suppressing impacts
- ➔ Denitrification processes
- ➔ Nitrification processes
- ↔ Equal