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*Published in:*  
Environmental Science & Technology

*DOI:*  
[10.1021/acs.est.9b03089](https://doi.org/10.1021/acs.est.9b03089)

Print publication: 05/11/2019

*Document Version*  
Peer reviewed version

[Link to publication](#)

*Citation for published version (APA):*  
Song, X., Ju, X., Topp, CFE., & Rees, RM. (2019). Oxygen regulates nitrous oxide production directly in agricultural soils. *Environmental Science & Technology*, 53(21), 12539-12547.  
<https://doi.org/10.1021/acs.est.9b03089>

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### **Oxygen regulates nitrous oxide production directly in agricultural soils**

Journal:	<i>Environmental Science &amp; Technology</i>
Manuscript ID	es-2019-03089t.R1
Manuscript Type:	Article
Date Submitted by the Author:	08-Sep-2019
Complete List of Authors:	Song, Xiaotong; China Agricultural University, College of Resources and Environmental Sciences Ju, Xiaotang; China Agricultural University, College of Resources and Environmental Sciences Topp, Cairistiona ; Scotland's Rural College Rees, Robert; Scotland's Rural College

SCHOLARONE™  
Manuscripts

1 Manuscript submitted to *Environmental Science & Technology*

2 Type of contribution: Research Article

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4 **Oxygen regulates nitrous oxide production directly in agricultural soils**

5

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**16 Abstract**

17 Oxygen (O<sub>2</sub>) plays a critical and yet poorly understood role in regulating nitrous oxide  
18 (N<sub>2</sub>O) production in well-structured agricultural soils. We investigated the effects of *in*  
19 *situ* O<sub>2</sub> dynamics on N<sub>2</sub>O production in a typical intensively managed Chinese cropping  
20 system under a range of environmental conditions (temperature, moisture, ammonium,  
21 nitrate, dissolved organic carbon etc.). Climate and management (fertilization,  
22 irrigation, precipitation and temperature), and their interactions significantly affected  
23 soil O<sub>2</sub> and N<sub>2</sub>O concentrations ( $P < 0.05$ ). Soil O<sub>2</sub> concentration was the most  
24 significant factor correlating with soil N<sub>2</sub>O concentration ( $r = -0.71$ ) when compared  
25 with temperature, water-filled pore space and ammonium concentration ( $r = 0.30, 0.25$   
26 and  $0.26$ , respectively). Soil N<sub>2</sub>O concentration increased exponentially with  
27 decreasing soil O<sub>2</sub> concentrations. The exponential model of N treatments and  
28 fertilization with irrigation/precipitation events predicted 74-90% and 58% of the  
29 variance in soil N<sub>2</sub>O concentrations, respectively. Our results highlight that soil O<sub>2</sub>  
30 status is the proximal, direct and the most decisive environmental trigger for N<sub>2</sub>O  
31 production outweighing the effects of other factors, and could be a key variable  
32 integrating the aggregated effects of various complex interacting variables. This study  
33 offers new opportunities for developing more sensitive approaches to predicting and  
34 through appropriate management interventions mitigating N<sub>2</sub>O emissions from  
35 agricultural soils.

36 **Keywords:** soil oxygen, nitrous oxide, nitrogen fertilization, extreme rainfall, irrigation,  
37 *in situ* upland soil

## 38 **Introduction**

39 Agricultural emissions of the greenhouse gas nitrous oxide ( $\text{N}_2\text{O}$ ) have become a global  
40 concern given its role as the second largest non-carbon dioxide ( $\text{CO}_2$ ) climate forcing  
41 agent following methane ( $\text{CH}_4$ ) and the most significant ozone-depleting gas emitted to  
42 the stratosphere.<sup>1-2</sup> Agricultural soils are responsible for around 60% of global  
43 anthropogenic  $\text{N}_2\text{O}$  emissions.<sup>3-5</sup> Although it is known that microbial nitrification and  
44 denitrification are main processes of  $\text{N}_2\text{O}$  production in soils,<sup>6</sup> the key biological  
45 mechanisms of  $\text{N}_2\text{O}$  production, and the interaction between regulating environmental  
46 variables, remain difficult to predict.

47 Of these, soil oxygen ( $\text{O}_2$ ) is the key proximal factor simultaneously controlling  
48 nitrification and denitrification by influencing these processes at the cellular-level, and  
49 further determining the partitioning of the end products between dinitrogen ( $\text{N}_2$ ) and  
50  $\text{N}_2\text{O}$ .<sup>7-8</sup> Other major factors particularly soil moisture, nitrogen (N) and oxidizable  
51 carbon (C), together with soil texture and aggregate structure play a role primarily  
52 through their influence on the availability of  $\text{O}_2$ .<sup>7</sup> Thus, soil texture and aggregate  
53 structure together determine soil physical factors such as the total porosity, air-filled  
54 porosity, water retention, and tortuosity and interconnectivity of the pore system that  
55 determine  $\text{O}_2$  diffusion rates into the soil, and the  $\text{O}_2$  availability varying across the  
56 aggregate radius. This is recognized as a major driving force in the fate of N  
57 transformations and  $\text{N}_2\text{O}$  production in aggregates.<sup>9-11</sup> Despite the central role of  $\text{O}_2$  in  
58 determining the processes and rates of  $\text{N}_2\text{O}$  production, there is little quantitative  
59 evaluation of the effects of  $\text{O}_2$  on  $\text{N}_2\text{O}$  formation in soils particularly in field conditions,

60 and how these relations are affected by the complex interactions between soil, climate  
61 and management factors. As a result, there is a missed opportunity to use O<sub>2</sub> as a  
62 powerful predictor of N<sub>2</sub>O production, and improve understanding of underlying  
63 processes.<sup>12</sup>

64 For a given site, O<sub>2</sub> dynamics would be mainly regulated by changing climate factors  
65 within the year (temperature and precipitation), and agronomic management (cropping  
66 systems, fertilization, irrigation etc.). A limited number of studies have measured O<sub>2</sub>  
67 dynamics in contrasting wetland ecosystems, especially paddy soils, humid forest soils,  
68 urine-amended pastures and riparian wetlands.<sup>8,13-17</sup> Unlike aquatic systems  
69 experiencing nearly constant anoxia throughout the year, many agricultural soils have  
70 been shown to have both spatially and temporally fluctuating redox status and  
71 experience intermittent low redox potentials associated with precipitation or irrigation  
72 events.<sup>13,18</sup> However, few studies have considered the changes in O<sub>2</sub> concentration that  
73 occur in agricultural soils which are typically associated with well-aerated conditions,<sup>19</sup>  
74 and thus impede our understanding of how O<sub>2</sub> responses to climate and management  
75 regulate soil trace gas emissions.

76 Knowledge regarding O<sub>2</sub>-regulated N<sub>2</sub>O production is derived mostly from pure  
77 culture and soil microcosm studies.<sup>20-22</sup> Nitrification was found to be the main source  
78 of N<sub>2</sub>O at O<sub>2</sub> concentrations greater than 0.35%.<sup>21-22</sup> The amount of N<sub>2</sub>O-N generated  
79 as per unit of N nitrified is highly sensitive to O<sub>2</sub> concentrations and can increase nearly  
80 tenfold from 0.16% to 1.48% when O<sub>2</sub> concentration is reduced from 20.4% to 0.8%,  
81 indicating that N<sub>2</sub>O produced by nitrification could be a significant source process at

82 reduced O<sub>2</sub> concentrations mainly via nitrifier denitrification, especially in ammonium  
83 (NH<sub>4</sub><sup>+</sup>)-N fertilized soils.<sup>12,22</sup> As soil O<sub>2</sub> concentrations decrease, the denitrification  
84 rates also increase, however, the macropore-O<sub>2</sub> content must fall below 0.5% to result  
85 in a large increase in denitrification rate.<sup>23</sup> Given high spatio-temporal heterogeneity of  
86 O<sub>2</sub> dynamics in the *in situ* upland agricultural soils in this study, the role of O<sub>2</sub> in  
87 regulating N<sub>2</sub>O production remains challenging to explain.<sup>12</sup>

88 In well-structured soils under frequent drying-wetting cycles, coupled with the  
89 spatio-temporal changes of climate and management factors in the *in situ* upland  
90 cropping systems, we hypothesized that: (1) soil O<sub>2</sub> concentration regulates N<sub>2</sub>O  
91 production directly following certain quantitative correlations; (2) the strength of the  
92 correlations depends on the combination of fertilization, irrigation and precipitation  
93 events. The objectives of this study were therefore: (1) to quantify the effects of soil O<sub>2</sub>  
94 and other soil environment variables on N<sub>2</sub>O production in the *in situ* upland  
95 agricultural soils and (2) to establish robust empirical models between soil O<sub>2</sub> and N<sub>2</sub>O  
96 concentrations under the coupling spatio-temporal changes of the climate and  
97 management factors.

98

## 99 **Materials and Methods**

### 100 *Experiment site and design*

101 Our study site was located at the China Agricultural University Research Station in  
102 Shangzhuang (39°48'N, 116°28'E) near Beijing, in the North China plain. This site is  
103 representative of upland agricultural soils in this region.<sup>24</sup> The altitude of this site is 40

104 m. Long-term (1981-2015) mean annual precipitation and air temperature was 540 mm  
105 and 13.0 °C, respectively. Soil properties in the top 0-20 cm layer are: bulk density 1.31  
106 g cm<sup>-3</sup>, clay loam texture with 28% clay, 32% silt and 40% sand (USDA standard),  
107 organic C content 7.9-13.7 g kg<sup>-1</sup>, total N 0.8-1.2 g kg<sup>-1</sup>, C/N ratio 9.5-11.0, and pH 7.5  
108 (1:2.5, soil/water). Soil total porosity is 51%, and air-filled porosity ranges from 12%  
109 to 42% along with the varying volumetric water content (9-39%) during the observation  
110 year, in which the hydraulic conductivity of the soil would be lower than 20 cm d<sup>-1</sup> (See  
111 S2.4 in Supporting Information (SI) for calculations of these physical parameters). The  
112 studied winter wheat-summer maize rotation is the main cropping system in this  
113 region,<sup>25</sup> in which wheat is sown at the beginning of October and harvested at the  
114 beginning of June in the following year, and then maize is immediately sown and  
115 harvested at the end of September (See S1.1 in SI for introduction of general soil-  
116 climatic conditions in the North China plain).

117 This study was based on a long-term field experiment established in October 2006,  
118 which was designed with four N rates (zero, optimum, conventional N and calculated  
119 N balance with manure) combining with two straw managements (straw removal and  
120 straw return). The four N rates were as follows:

- 121 (1) Zero N ( $N_0$ ), no fertilizer N input as a control;
- 122 (2) Optimum N ( $N_{opt}$ ), chemical N fertilizer applied at optimum rates calculated by the  
123 mineral N ( $N_{min}$ ) test method based on the synchronization of crop N demand and  
124 soil N supply;
- 125 (3) Conventional N ( $N_{con}$ ), chemical N fertilizer applied at rates of 260 and 300 kg N



126  $\text{ha}^{-1}$  for maize and wheat, respectively, according to local conventional farming  
127 practice;<sup>26</sup>

128 (4) Calculated N balance with manure ( $N_{\text{bal}+\text{M}}$ ), composted cattle manure applied with  
129 supplementary chemical N fertilizer based on N-balanced calculations, i.e. the rate  
130 of chemical N fertilizer equals to crop N uptake and soil residual mineral N minus  
131 available manure-N and soil initial mineral N.

132 We selected seven treatments from the long-term field experiment including the zero,  
133 optimum and conventional N levels with straw removal ( $N_0$ ,  $N_{\text{opt}}$ ,  $N_{\text{con}}$ ) and straw return  
134 ( $N_0+\text{S}$ ,  $N_{\text{opt}}+\text{S}$ ,  $N_{\text{con}}+\text{S}$ ), and the N balanced treatment with manure and straw return  
135 ( $N_{\text{bal}+\text{M}+\text{S}}$ ) (see Table S1). Each treatment was replicated three times in a randomized  
136 block arrangement with an area of  $64 \text{ m}^2$  ( $8 \text{ m} \times 8 \text{ m}$ ) per plot. Urea was used as the N  
137 source because it was the main N fertilizer used by farmers in this region. We carried  
138 out this study throughout a whole year from the middle stage of the 2015-2016 wheat  
139 (April 2016) to the middle stage of the 2016-2017 wheat (April 2017). Detailed rates  
140 of each N fertilization and irrigation, soil chemical properties of each treatment, and  
141 management activities are described in SI (S2.1-S2.2, Tables S2-S3 and Figure S1) and  
142 previously published papers.<sup>25-29</sup>

143

#### 144 *Soil gas ( $\text{O}_2$ , $\text{N}_2\text{O}$ , $\text{CO}_2$ , $\text{CH}_4$ ) sampling and measurements*

145 In each plot, we established a subplot for gas sampling covering an area of  $9 \text{ m}^2$  ( $3 \text{ m} \times 3 \text{ m}$ )  
146 including two 1 m width guard rows as borders alongside the footpath to avoid  
147 disturbance of the crop and soil (Figure S2). In every subplot, two soil-air equilibration

148 samplers were installed vertically in the soil to a depth of 5-20 cm. The two gas samplers  
149 were positioned randomly in the subplot in wheat and in the N fertilizer band in maize  
150 (Figure S2), respectively. The soil-air equilibration sampler was modified from Wang  
151 et al<sup>30</sup> and consisted of a polyvinylchloride (PVC) tube with a 2.5 cm inner diameter, a  
152 PVC dust cap, a rubber plug and a microbore polytetrafluoroethylene (PTFE) tube  
153 (inner diameter 0.25 cm) fitted with a three-way stopcock to connect with the sampling  
154 syringe at the soil surface (Figure S3). The PVC tube was perforated, which ensured air  
155 diffusion and exchange between the sampler and the surrounding soil. We drilled a 3.0  
156 cm diameter hole by soil auger prior to the installation of the sampler and backfilled the  
157 soil after inserting it in the hole. The soil-air equilibration samplers were dug out prior  
158 to each crop harvest and inserted back after the sowing of the next crop. To avoid  
159 connection of atmospheric air to soil air, three-way stopcocks of the samplers were  
160 closed on non-sampling days ensuring the representatives of soil gases inside the  
161 samplers.

162 On each sampling day, we collected 20 ml gas samples between 9:00 am and 11:00  
163 am using 50 ml plastic syringes connecting to the samplers through the three-way  
164 stopcocks. Before the gas samples were collected, we sampled 20 ml of soil air inside  
165 the sampler using the syringe and injected it out to flush the syringe, then carefully took  
166 another 20 ml soil air sample and injected back to the sampler and repeated this  
167 procedure three times to evenly mix air inside the sampler. Gas samplings were  
168 undertaken on days 1, 2, 3, 5, 7, 10 after fertilization, days 1, 2, 3, 5, 7 after irrigation,  
169 and days 1, 3, 7 after precipitation (>20 mm). For the remaining periods, gas was

170 sampled weekly, except during the winter period (December-February) when the gas  
171 was sampled monthly.

172 The concentration of O<sub>2</sub> was measured directly by a portable O<sub>2</sub> content analyzer  
173 (G100 Range, Geotechnical Instruments Ltd., UK) linking to the samplers immediately  
174 after gas sampling. N<sub>2</sub>O, CO<sub>2</sub> and CH<sub>4</sub> concentrations were analyzed within 24 h after  
175 sampling by a gas chromatograph (GC) (Agilent 6820, USA), see details in SI (S2.3)  
176 and previously published papers.<sup>25-26,28,31</sup> Detailed measurements of soil temperature,  
177 water-filled pore space (WFPS), mineral N (NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>), dissolved organic C  
178 and N (DOC and DON) and pH, crop aboveground biomass, N uptake, grain yield and  
179 source of climate data are reported in SI (S2.4-S2.6).

180

#### 181 *Data analyses*

182 Differences in average soil O<sub>2</sub>, N<sub>2</sub>O and CO<sub>2</sub> concentrations between different  
183 management factors and grain yield between different treatments were analyzed by a  
184 one-way ANOVA procedure for least significant differences (LSD) at  $P < 0.05$  (Figures  
185 2 and S22, Tables S7-S10). Effects of agronomic event, N rate, straw management and  
186 their interactions on soil O<sub>2</sub>, N<sub>2</sub>O and CO<sub>2</sub> concentrations were analyzed by multi-way  
187 ANOVA for LSD at  $P < 0.05$  (Table S6). Pearson analysis was performed to evaluate  
188 the correlation between soil N<sub>2</sub>O concentration and soil environmental parameters  
189 (Table S11). Stepwise multiple linear regression was used to determine the variation in  
190 soil N<sub>2</sub>O concentration that could be explained by soil O<sub>2</sub> concentration, moisture and  
191 temperature, with a criteria of  $P < 0.05$  to accept variables (Table S12). The above

192 statistical analyses were undertaken using IBM SPSS Statistics 21 (SPSS Inc., Chicago,  
193 IL, USA). Regression models of soil N<sub>2</sub>O and CO<sub>2</sub> concentration responding to soil O<sub>2</sub>  
194 concentration, WFPS and temperature were fitted by SigmaPlot 14.0 (Systat Software  
195 Inc., Erkrath, Germany) (Figure 3, Figures S16-S17). Selection of the best function and  
196 a related boundary line analysis are described in SI (S2.7).

197

## 198 **Results**

### 199 *Climatic parameters*

200 Annual mean air temperature and precipitation in the experimental year (2016-2017)  
201 were 14.1 °C and 679 mm, respectively (Figure S4 a), leading overall to warmer and  
202 wetter conditions compared to the corresponding long-term (1981-2015) averages of  
203 13.0 °C and 540 mm at the study site (Figure S23 a). The summer period (June-  
204 September) in 2016-2017 had consistently higher air temperature (25.8 °C) and far  
205 higher precipitation (553 mm) in relation to the average of 24.5 °C and 427 mm between  
206 1981 and 2015 (Figure S23 a). An extreme rainfall event of 253 mm occurring on 20  
207 July accounted for 37% of the annual precipitation in 2016-2017 (Figure S4 a).  
208 Although the total annual precipitation in 2016-2017 was not exceptional, the extreme  
209 rainfall event was the highest daily precipitation since 1981 and was nearly 2-8 times  
210 higher than other recorded maximum daily precipitation events between 1981 and 2015  
211 (Figure S23 c-d). Total precipitation exceeding 300 mm in July is highly unusual and  
212 has only happened with a frequency of 6% over the past 35 years (Figure S23 b). The  
213 winter period (December-February) was distinctly colder and drier than the summer

214 with a mean air temperature of 0.3 °C and precipitation of 5.6 mm in 2016-2017, which  
215 were similar to the corresponding long-term (1981-2015) averages of -1.3 °C and 9.2  
216 mm, respectively (Figure S23 a). Annual evaporation in 2016-2017 was 1336 mm,  
217 including 533 mm occurring in summer (June-September) nearly equivalent to  
218 precipitation while evaporation during winter (December-February) was 190 mm and  
219 far greater than the precipitation.

220 Soil temperature at 10 cm depth tended to reflect air temperature, except in spring  
221 (April-June) when it was slightly lower than air temperature (Figure S4 a). Soil WFPS  
222 was between 60% and 75% following irrigation in April and November, and the  
223 extreme rainfall in July (Figure S4 b). Irrigation in May and June, and frequent light  
224 rainfall in the summer period resulted in a lower WFPS within the range of 40-60%  
225 owing to high rates of evaporation and transpiration. Drier soil moisture conditions in  
226 which WFPS dropped down to values between 20-30% occurred episodically from late  
227 April to middle September when there was no irrigation or precipitation. The relatively  
228 high temporal heterogeneity of climatic factors in the study year provided advantaged  
229 good platform for testing our hypotheses. Dynamics of soil matrix (mineral N, pH,  
230 organic C and N), crop aboveground biomass, N uptake and grain yield are described  
231 in SI (S3.1-S3.2).

232

### 233 *Concentrations of soil O<sub>2</sub> and N<sub>2</sub>O*

234 Clear patterns of soil O<sub>2</sub> depletion and concurrent N<sub>2</sub>O production occurred in N  
235 treatments following fertilization with subsequent irrigation or precipitation (named as

236 Fer.+Irr./Pre. event in this study) in the field (Figure 1 a-b, d-e). In particular, the  
237 extreme rainfall following fertilization on 20 July 2016 resulted in the lowest O<sub>2</sub>  
238 concentration of 6% and highest N<sub>2</sub>O concentration up to 140 μL L<sup>-1</sup> in N<sub>con</sub> under  
239 waterlogging conditions, even though less N fertilizer was applied at this event  
240 compared to others, which normally led to an O<sub>2</sub> concentration of 15% to 18% and a  
241 N<sub>2</sub>O concentration of between 5 μL L<sup>-1</sup> and 35 μL L<sup>-1</sup> in N treatments under aerated  
242 soil conditions. The fertilization occurring separately (Fer. event) reduced the O<sub>2</sub>  
243 concentration to 18.3-18.5% and led to N<sub>2</sub>O peaks of 5.2-5.9 μL L<sup>-1</sup> in N<sub>con</sub>, N<sub>con</sub>+S and  
244 N<sub>bal</sub>+M+S when the WFPS ranged between 40-45% on 6 August 2016. Irrigation or  
245 precipitation (Irr./Pre. event) slightly decreased soil O<sub>2</sub> concentrations to 19% but did  
246 not stimulate N<sub>2</sub>O production except when the irrigation on 21 June 2016 was followed  
247 by continuous rainfall which reduced O<sub>2</sub> concentrations to 17.2% and brought about a  
248 small N<sub>2</sub>O pulse of 8.1 μL L<sup>-1</sup> in N<sub>con</sub>+S. Only when fertilization was coupled with  
249 irrigation or precipitation, were there intense episodes of soil O<sub>2</sub> depletion resulting in  
250 increased N<sub>2</sub>O production.

251 Based on the results of a multi-way ANOVA analysis, we found that agronomic event  
252 (*E*), N rate (*N*) and their interactions (*E*\**N*) were the significant management factors  
253 regulating soil O<sub>2</sub> and N<sub>2</sub>O concentrations in the plough layer (*P*<0.05) (Table S6). On  
254 average across all treatments, Fer.+Irr./Pre. event reduced the soil O<sub>2</sub> concentration to  
255 16.8% which was significantly lower than the concentration of 19.5% in both Fer. and  
256 Irr./Pre. events and 20.4% at other time (*P*<0.05) (Table S7, Figure 2 a). The  
257 corresponding average soil N<sub>2</sub>O concentration following Fer.+Irr./Pre. event was 10.5

258  $\mu\text{L L}^{-1}$  which was up to 4-15 times higher than that in Fer. ( $2.4 \mu\text{L L}^{-1}$ ) and Irr./Pre. ( $0.9$   
259  $\mu\text{L L}^{-1}$ ) events and other time ( $0.7 \mu\text{L L}^{-1}$ ) (Figure 2 b). Intriguingly, soil  $\text{O}_2$  and  $\text{N}_2\text{O}$   
260 levels matched well with N rates under the Fer.+Irr./Pre. event. Mean soil  $\text{O}_2$   
261 concentration declined from 17.9% to 17.2% and 16.0% as N rates increased from zero  
262 to optimum and conventional, respectively, and the relative soil  $\text{N}_2\text{O}$  concentration in  
263 these three N levels were 1.3, 7.5 and  $22.8 \mu\text{L L}^{-1}$  (Table S10, Figure 2 d-e). However,  
264 when Fer. or Irr./Pre. events occurred, soil  $\text{O}_2$  concentration ranged from 19.4%-19.7%  
265 showing no significant difference between N rates, and  $\text{N}_2\text{O}$  concentration remained  
266 low ( $4 \mu\text{L L}^{-1}$ ) even with conventional N input (Tables S8-9). This was probably  
267 because there was an ample  $\text{O}_2$  supply from the atmosphere under the soil dry  
268 conditions of the Fer. event. In the Irr./Pre event without N additions, a weak  $\text{O}_2$   
269 depletion indicated that the air-filled porosity physically replaced by water could  
270 recover instantly.

271 Therefore, a small depletion in the soil  $\text{O}_2$  concentration could still be a strong  
272 environmental trigger for disproportionate  $\text{N}_2\text{O}$  production, especially when there were  
273 sufficient available substrates and moisture caused by the Fer.+Irr./Pre. event. Our  
274 results indicate that soil  $\text{O}_2$  depletion is the proximal and direct driver underlying the  
275 effects of climate and management factors on  $\text{N}_2\text{O}$  production. See S3.3 in SI for  
276 concentrations of soil  $\text{CO}_2$  and  $\text{CH}_4$ .

277

#### 278 *Correlations between soil $\text{O}_2$ and $\text{N}_2\text{O}$ concentrations*

279 Soil  $\text{O}_2$  concentration, temperature, WFPS, and  $\text{NH}_4^+$  concentration were significant

280 environmental factors controlling N<sub>2</sub>O production in the studied upland soil under field  
281 conditions ( $P<0.01$ ) (Table S11). However, soil O<sub>2</sub> concentration was the strongest  
282 factor correlating with N<sub>2</sub>O concentrations ( $r=-0.71$ ) when compared to temperature,  
283 WFPS and NH<sub>4</sub><sup>+</sup> content ( $r=0.30$ ,  $0.25$  and  $0.26$ , respectively). The strength of the  
284 correlations between soil O<sub>2</sub> and N<sub>2</sub>O concentrations was evidently affected by  
285 agronomic event, N rate, and crop season, but not by straw and manure applications.  
286 Regarding agronomic event, soil O<sub>2</sub> and N<sub>2</sub>O concentrations were the most closely  
287 correlated under Fer.+Irr./Pre. event ( $r=-0.68$ ) when compared to Irr./Pre., Fer. and  
288 other time ( $r=-0.55$ ,  $-0.41$  and  $-0.31$ , respectively). The correlations under conventional  
289 and optimum N rates were similar ( $r=-0.89$  and  $-0.86$ , respectively) but were much  
290 larger than the strength in zero N rate ( $r=-0.56$ ). The correlation between soil O<sub>2</sub> and  
291 N<sub>2</sub>O concentrations in maize ( $r=-0.68$ ) was greater than that in wheat ( $r=-0.45$ ). The  
292 widely different correlation coefficients indicated that soil N<sub>2</sub>O production would be  
293 exclusively dependent on soil O<sub>2</sub> concentration when soil temperature, moisture and  
294 NH<sub>4</sub><sup>+</sup> substrate were not limiting in the Fer.+Irr./Pre. event, N treatments and maize  
295 growth season.

296 Using stepwise multiple regression analysis between soil environmental parameters  
297 and N<sub>2</sub>O concentrations, regression models derived from all the measurement data  
298 within the study year showed that soil O<sub>2</sub> concentration was the most significant  
299 variable ( $P<0.01$ ) rather than temperature and WFPS (Table S12). Soil O<sub>2</sub> concentration  
300 alone could explain 49-84% of the variance in soil N<sub>2</sub>O concentration, and the  
301 explanation of variance was only marginally improved by adding soil temperature and



302 WFPS. The regression model of conventional N treatment which simultaneously  
303 included soil O<sub>2</sub> concentration, temperature and WFPS as variables provided a  
304 prediction of soil N<sub>2</sub>O concentration which was very close to the *in situ* observed values  
305 (Figure S8).

306 Given the strong correlations between soil O<sub>2</sub> and N<sub>2</sub>O concentrations, we further  
307 explored the response of soil N<sub>2</sub>O concentration to soil O<sub>2</sub> concentration throughout the  
308 experimental period. Generally, soil N<sub>2</sub>O concentration increased as soil O<sub>2</sub>  
309 concentration decreased and this response was best fitted by an exponential model  
310 (Table S13, Figure S9). The N<sub>2</sub>O production rate per unit O<sub>2</sub> of depletion (slope of the  
311 curve) was relatively low (less than 6 μL L<sup>-1</sup> N<sub>2</sub>O per unit O<sub>2</sub> depleted) at O<sub>2</sub> levels  
312 higher than 12%, but below this point the rate increased steeply to 23 μL L<sup>-1</sup> N<sub>2</sub>O per  
313 unit O<sub>2</sub> depleted as O<sub>2</sub> concentration was reduced to 6% (Figure 3 a). Thus, we infer  
314 that an O<sub>2</sub> concentration of 12% in bulk soil air might be a critical transition point  
315 between the dominance of aerobic versus anaerobic processes in structured field soils.  
316 The exponential model for zero, optimum and conventional N rates explained 31%, 74%  
317 and 90% of the variance in soil N<sub>2</sub>O concentration, respectively (Figure 3 b). As soil  
318 O<sub>2</sub> concentration decreased from 21% to 10%, soil N<sub>2</sub>O concentration increased from  
319 zero to 2, 20 and 60 μL L<sup>-1</sup> in zero-, optimum- and conventional-N rate, respectively.  
320 Similarly, the exponential model performed better in Fer.+Irr./Pre. (R<sup>2</sup>=0.58) than in  
321 Irr./Pre. (R<sup>2</sup>=0.34), Fer. (R<sup>2</sup>=0.17) and other time (R<sup>2</sup>=0.10) (Figure 3 c). Soil N<sub>2</sub>O  
322 concentration rose by up to 40 μL L<sup>-1</sup> when soil O<sub>2</sub> concentration was reduced from  
323 21% to 10% in Fer.+Irr./Pre., but there was no significant increase in Fer., Irr./Pre. and

324 other time when the soil O<sub>2</sub> concentration was above 16%. This indicated that the  
325 exponential increase in soil N<sub>2</sub>O concentration responding to soil O<sub>2</sub> depletion was  
326 more robust under high inputs of N and water, which provided implications for new  
327 approaches to simulation and mitigation of N<sub>2</sub>O in agricultural soils.

328

## 329 **Discussion**

### 330 *Understanding O<sub>2</sub> dynamics in upland agricultural soils*

331 Field measurements of soil O<sub>2</sub> and N<sub>2</sub>O concentrations demonstrated a highly dynamic  
332 temporal and spatial pattern which was driven by changes in climate and management.  
333 Precipitation and irrigation alter soil O<sub>2</sub> concentration mainly through physical  
334 replacement of soil air by water which significantly slows down O<sub>2</sub> diffusion in the  
335 water phases.<sup>32-34</sup> In saturated layers, the O<sub>2</sub> diffusion rate would be reduced to 1/10000  
336 of that in air, which could not replace the microbial consumption of O<sub>2</sub>, leading to the  
337 reduced redox potential at the centre of aggregates stimulating denitrification in these  
338 microsites.<sup>11</sup> Field observations in upland forest soils have demonstrated this concept,<sup>13</sup>  
339 and modelling has shown that the anaerobic fraction of soils can account for 10% of  
340 soil volume at 65% WFPS but increase sharply once the WFPS exceeds 80%.<sup>35</sup>

341 Fertilization coupled with irrigation or precipitation simultaneously promotes  
342 microbial O<sub>2</sub> consumption and physical inhibition of O<sub>2</sub> diffusion.<sup>14</sup> Soil waterlogging  
343 driven by extreme rainfall can cause severe O<sub>2</sub> depletion in soil even without N addition  
344 as shown by the lowest O<sub>2</sub> concentration of 7% in the control treatment in our study. In  
345 these circumstances O<sub>2</sub> can remain low until gas exchange recovers with soil

346 drainage.<sup>13,15</sup> Although a completely anoxic bulk soil environment did not develop in  
347 our study and there were few observations with extremely low O<sub>2</sub> concentration (Figure  
348 3), the oxic, hypoxic and completely anoxic microsites might nevertheless co-exist in  
349 the soil.<sup>19</sup> Once the concentration of O<sub>2</sub> fell below the intermediate value of 12% N<sub>2</sub>O  
350 production increased sharply following fertilization coupled with irrigation or  
351 precipitation (Figure 3 a). Therefore, we speculate that the generated N<sub>2</sub>O resulted from  
352 a combination of nitrification, denitrification and coupled nitrification denitrification  
353 that occurred simultaneously in the soil matrix.<sup>36-37</sup>

354 Studies in repacked soils have shown that anaerobic microsites appear surrounding  
355 added fertilizer N, organic matter, plant residue and rhizosphere, or within soil  
356 aggregates in well-structured soils.<sup>37-41</sup> Fragments of plant residue can also be anoxic  
357 by absorbing water from adjacent soil<sup>41</sup> (See S4.1 in SI for formation mechanisms of  
358 anaerobic microsites in soils). These anaerobic microsites may facilitate significant  
359 N<sub>2</sub>O production by inducing denitrification and coupled nitrification denitrification,  
360 which has been taken into account in some modelling approaches.<sup>9-11</sup> In spite of the  
361 observations from laboratory studies, soil microsite development and measurements of  
362 O<sub>2</sub> concentration have rarely been reported in the field. The conceptual scheme to  
363 visualize O<sub>2</sub> diffusion, transformations of C and N substrates in well-structured soils  
364 under different moisture conditions are described in SI.

365 Ammonia oxidation, the first step of nitrification, actively consumes soil O<sub>2</sub>, which  
366 has been shown to increase linearly as urea input increases in a robotized incubation  
367 experiment using similar soil, implying it could be another important reason for O<sub>2</sub>

368 depletion in the soil matrix.<sup>42</sup> Urea or ammonium-based fertilization actively consumed  
369 O<sub>2</sub> in soil especially at high N rates by ammonia oxidation.<sup>42</sup> Our results showed that  
370 O<sub>2</sub> consumption proceeded on a similar time-scale and trend between N rates, but was  
371 smaller than that reported by Huang et al<sup>42</sup>, probably because the O<sub>2</sub> supply in the field  
372 could be replenished from the atmosphere. This process of replenishment was also  
373 reported by Zhu et al<sup>37</sup> from the calculated O<sub>2</sub> consumption by nitrification that far  
374 outweighed depletion of O<sub>2</sub> in soil. In a pasture field, soil O<sub>2</sub> concentration at 10 cm  
375 depth showed diurnal variation and reached a minimum of 13% after urine application  
376 together with irrigation but recovered to the pre-application level only after 24 h.<sup>16</sup>  
377 Similarly, as a consequence of biochemical reactions and supply by diffusion, soil O<sub>2</sub>  
378 concentration may vary significantly on a diurnal basis, as shown by our results.<sup>14-15</sup>

379

#### 380 *Role of O<sub>2</sub> in regulating N<sub>2</sub>O production in situ*

381 Oxygen plays a critical and yet poorly understood role in regulating N<sub>2</sub>O production in  
382 well-structured upland agricultural soils. From the perspective of nitrogen cycling, O<sub>2</sub>  
383 concentration in the soil pore space is a key controlling factor of the nitrification process  
384 (oxidation of ammonium to nitrate) by nitrifying organisms. Insufficient O<sub>2</sub> will lead to  
385 the incomplete oxidation of ammonium to nitric oxide and nitrite instead of nitrate. This  
386 may increase the risk of N<sub>2</sub>O loss through nitrifier denitrification and coupled  
387 nitrification denitrification in soil.

388 Several previous pure cultures and soil microcosm studies have identified the role of  
389 O<sub>2</sub> in regulating N<sub>2</sub>O production under laboratory conditions,<sup>12,20-22,42</sup> see S4.2 in SI for

390 detailed mechanisms. In a clay loam soil amended with urea, N<sub>2</sub>O production increased  
391 by a factor of 19 as O<sub>2</sub> concentration decreased from 21% to 3%.<sup>12</sup> Previous studies  
392 based on field measurements consistently point to a significant correlation between O<sub>2</sub>  
393 concentration and N<sub>2</sub>O production in soils of various textures and environments,<sup>15,17,19</sup>  
394 although the data is still highly limited and inadequate to establish a robust empirical  
395 response of N<sub>2</sub>O to O<sub>2</sub>.

396 Our results established the inverse relationship between O<sub>2</sub> concentration and N<sub>2</sub>O  
397 production in upland agricultural soils. The nonlinearity of the O<sub>2</sub>-N<sub>2</sub>O relationship  
398 suggested that N<sub>2</sub>O was generated from a complex combination of source processes.  
399 Nitrification involving nitrifier nitrification, nitrifier denitrification and coupled  
400 nitrification denitrification, are the main sources of N<sub>2</sub>O in NH<sub>4</sub><sup>+</sup> or urea based fertilizer  
401 amended soil especially under limited O<sub>2</sub> conditions.<sup>12,20-21,42-43</sup> Nitrifier denitrification  
402 can account for the majority (up to 60-70%) of total N<sub>2</sub>O production and far exceed that  
403 from nitrification and coupled nitrification denitrification in soils that have received  
404 urea or NH<sub>4</sub><sup>+</sup>-N fertilizers.<sup>12,42</sup> However, the absolute amount of N<sub>2</sub>O produced by  
405 nitrifier denitrification increased 50 to 80-fold as the O<sub>2</sub> concentration was reduced  
406 from 21% to 0.5%.<sup>12,20</sup> Khalil et al<sup>22</sup> established a regression ( $R^2=0.94$ ,  $n=25$ ) of O<sub>2</sub>  
407 consumption rates versus nitrification rates under five O<sub>2</sub> concentrations between 0.8%  
408 and 20.4% with a slope of  $2.02\pm 0.12$  mol O<sub>2</sub> consumed per mol N nitrified. This was  
409 almost equivalent to the theoretical value for O<sub>2</sub> consumption by nitrification (2 mol O<sub>2</sub>  
410 per mol N), implying that the amount of O<sub>2</sub> consumed as per unit of N that was nitrified  
411 was relatively constant over a wide range of O<sub>2</sub> concentration. They also found that the

412 production of  $N_2O$  by nitrification (i.e. the amount of  $N_2O$ -N evoked per unit N nitrified)  
413 increased rapidly by a factor of 9 when  $O_2$  concentration fell from 20.4% to 0.8%. These  
414 findings suggested that the yield of  $N_2O$  per unit  $O_2$  consumed by nitrification increased  
415 many times as the  $O_2$  concentration was reduced. This implies that nitrification plays a  
416 dominant role in  $N_2O$  production and that the ratio of  $N_2O$  emitted in nitrification  
417 increases with  $O_2$  depletion.

418 Although heterotrophic denitrification occurs mainly in totally anoxic environments,  
419 this pathway might also make a contribution to the exponential  $N_2O$  increase,  
420 considering that pure heterotrophic denitrification under anoxic conditions produces 3-  
421 9 times more  $N_2O$  than other processes under low  $O_2$  conditions.<sup>12,22</sup> There have been  
422 studies showing that  $N_2O$  emissions can increase exponentially as anoxic conditions  
423 develop around the applied manure in soils, probably by denitrification.<sup>37,44</sup> In field  
424 environments, heterotrophic denitrification might proceed in anaerobic microsites or  
425 soil aggregates as discussed previously, especially when extreme rainfall or irrigation  
426 events result in soil waterlogging.<sup>41</sup> In addition, short term expression of denitrifying  
427 enzymes under anoxic conditions induced by transient flooding could lead to so-called  
428 aerobic denitrification with  $N_2O$  as a main end-product during the recovery of soil  $O_2$   
429 concentration.<sup>36</sup> Nitrate is a more favorable electron acceptor for denitrifiers than  $N_2O$ ,  
430 so  $N_2O$  generated from heterotrophic denitrification would not normally be reduced  
431 further to  $N_2$  in soils containing ample  $NO_3^-$ . Nitrate accumulated in our studied soils,  
432 which might have increased emissions of  $N_2O$  from heterotrophic denitrification.<sup>12,45-47</sup>  
433 The gradual increase in  $N_2O$  concentration per unit of  $O_2$  reduced also suggests a

434 progressively increasing contribution of heterotrophic denitrification to N<sub>2</sub>O generation  
435 in our study.

436 The exponential response of N<sub>2</sub>O production to soil O<sub>2</sub> depletion was more  
437 significant under high rates of N with irrigation or precipitation. It could be speculated  
438 that ammonia oxidation with abundant NH<sub>4</sub><sup>+</sup> rapidly consumed soil O<sub>2</sub> and accumulated  
439 NO<sub>2</sub><sup>-</sup> (the substrate for nitrifier denitrification), and irrigation or precipitation  
440 contributed directly to O<sub>2</sub> depletion, leading to anoxic conditions and promoting  
441 nitrifier denitrification, coupled nitrification denitrification or heterotrophic  
442 denitrification.

443 The characteristics of the climate, soil and cropping system in this study are widely  
444 distributing across the world's farmlands, such as the well-known corn belt in the US  
445 Midwest.<sup>48-49</sup> Such cropping systems are subject to intensive management involving  
446 high inputs of N fertilizers, and the results of this study therefore help understand the  
447 underlying mechanism linking such management to N<sub>2</sub>O production.<sup>50</sup> Maize is a  
448 particularly important crop in this context and our results therefore have a direct  
449 relevance to N<sub>2</sub>O production in such cropping systems at the global scale.<sup>51</sup> Thus, the  
450 established relationships between O<sub>2</sub> and N<sub>2</sub>O concentrations should represent and  
451 could be used in modelling global agricultural soils, particularly alkaline soils, with a  
452 clay loam texture and a low organic carbon content.

453 Understanding the role of O<sub>2</sub> in regulating N<sub>2</sub>O production is central to improving  
454 efficiency of C, N and water management.<sup>52</sup> We propose that avoiding severe O<sub>2</sub>  
455 depletion is the key to reducing N<sub>2</sub>O formation in agricultural soils. Adopting optimum

456 rates of fertilization and irrigation which meet the crop demand, and applying improved  
457 water management using drip or sprinkle irrigation rather than flooding could be  
458 options maintaining soil aeration.<sup>53-54</sup> Extreme rainfall caused the largest O<sub>2</sub> depletion  
459 and N<sub>2</sub>O production even with low N rates, which highlight the linkage between climate  
460 and management factors on N<sub>2</sub>O production.<sup>50</sup> This enhancement of intense episodes  
461 of O<sub>2</sub> depletion facilitating increased N<sub>2</sub>O production will feed back to extreme weather  
462 events under future global change.

463

#### 464 *Comparison between WFPS and O<sub>2</sub> as a predictor for N<sub>2</sub>O production*

465 Soil moisture has been widely adopted as a proxy of O<sub>2</sub> availability, and our results also  
466 showed that soil O<sub>2</sub> concentration decreased quadratically with increases in WFPS  
467 (Figure S16 a). However, the changes in WFPS explained only 19% of variance in soil  
468 O<sub>2</sub> concentration, which indicated that WFPS could not be an effective predictor for  
469 soil O<sub>2</sub> concentration in the field. This is because soil O<sub>2</sub> changes not only depend on  
470 soil moisture but also on soil structure and biological respiration. The calculation of  
471 WFPS does not take into account the distribution of macropores and micropores, the  
472 effects of pore connectivity and tortuosity on gas diffusion, and thus could not reliably  
473 predict microsite O<sub>2</sub> concentration.<sup>12,55</sup> Soil WFPS also poorly predicted the soil N<sub>2</sub>O  
474 and CO<sub>2</sub> concentrations by weak Gaussian functions ( $R^2=0.05-0.11$ ) in our study  
475 (Figure S16 b, S17 b). Measurements in a wetland soil suggested that O<sub>2</sub> was the  
476 dominant predictor for N<sub>2</sub>O production.<sup>8</sup> Hall et al<sup>56</sup> suggested a need to decouple soil  
477 moisture from O<sub>2</sub> availability for predicting production of trace gases, and to re-



478 evaluate the representations of moisture in N<sub>2</sub>O models, because water addition  
479 generated high spatial and temporal variation in soil moisture without significant effect  
480 on soil O<sub>2</sub> concentration, and the redox-sensitive GHGs (CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O) displayed a  
481 weak, non-deterministic relationship with moisture in the forest soil. The predictions of  
482 other soil environmental parameters for N<sub>2</sub>O production are discussed in SI (S4.4).

483 The optimal soil water content (calculated from the regression or boundary line  
484 equations) for production of N<sub>2</sub>O and CO<sub>2</sub>, and consumption of O<sub>2</sub> in our study was  
485 consistently around 60% WFPS. This intermediate water content was surprisingly same  
486 as that deemed to be optimal for aerobic processes, e.g. nitrification, O<sub>2</sub> uptake and CO<sub>2</sub>  
487 production by microbial respiration, and also the threshold inducing anaerobic  
488 denitrification in the previously established classic conceptual model of the relation  
489 between soil water content and microbial activity.<sup>56</sup> In that model, the optimal value of  
490 60% WFPS represented the intersection of increasing availability of C and N and  
491 decreasing availability of O<sub>2</sub>. Conceptually, a soil moisture of around 60% WFPS offers  
492 favorable conditions for aerobic processes (e.g. nitrification) when the diffusion of both  
493 substrates and gases (O<sub>2</sub>) are not restricted.<sup>55,57</sup> The optimum conditions for N<sub>2</sub>O  
494 emissions via denitrification are considered to exist within 70-90% WFPS.<sup>12,57-58</sup> The  
495 consistency between our observations and the conceptual optimal soil WFPS model  
496 explains the tight link between soil N<sub>2</sub>O (or CO<sub>2</sub>) and O<sub>2</sub> concentration induced by the  
497 complex combination of source processes in soil. See S4.3 in SI for correlations  
498 between soil O<sub>2</sub> and CO<sub>2</sub> concentrations.

499 WFPS is calculated using total porosity and defined as the proportion of the total

500 pore space filled with water, and hence the actual fraction of the entire soil volume  
501 filled with water or air may differ across soils with different total porosities whilst  
502 having the same WFPS.<sup>59</sup> Therefore, WFPS cannot be considered as a single measure  
503 to describe the effects of soil water on all processes and should not be applied across  
504 soils with varying bulk density, texture and structure.<sup>56</sup> WFPS must be combined with  
505 other structural parameters to adequately predict diffusion in soils. These include  
506 descriptions of soil structure, tortuosity and connectivity, especially when up-scaling  
507 models to regional or continental scales.<sup>59</sup> By contrast, O<sub>2</sub> is a more universally  
508 predictive measure given that it is the direct factor regulating the various processes  
509 generating N<sub>2</sub>O no matter where the site or what the climate is. Our results provide  
510 future opportunities for the utilization of soil O<sub>2</sub> concentration to predict N<sub>2</sub>O emission  
511 more efficiently when dealing with the complicated and interacting factors of climate,  
512 soil, agricultural managements, growth of plant and microorganisms under real field  
513 conditions. See S4.5 in SI for implications of considering O<sub>2</sub> effects into modeling for  
514 better N<sub>2</sub>O prediction.

515

## 516 **Acknowledgements**

517 This study was financially supported by the National Natural Science Foundation of  
518 China (41830751), Newton Fund N-Circle project (Grant Ref. BB/N013484/1), Sino-  
519 British Joint Research Innovation PhD Exchange Program funded by the China  
520 Scholarship Council (201603780112). We sincerely thank Leyi Wang, Dongrun Li,  
521 Hepu Liu, Zhiyong Suo for their assistance in the sampling and measurement work.

522

523 **Supporting Information Available**

524 The supporting information includes additional introduction, materials and methods,  
525 results and discussion, supplementary figures and tables (Figures S1-S23, Tables S1-  
526 S14), and the conceptual scheme of O<sub>2</sub> diffusion, transformations of C and N in well-  
527 structured soils under different moisture conditions. This information is available free  
528 of charge via the Internet at <http://pubs.acs.org>.

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**728 Figure Captions**

729 Figure 1. Dynamics of soil oxygen ( $O_2$ ) (a, d), nitrous oxide ( $N_2O$ ) (b, e) and carbon  
730 dioxide ( $CO_2$ ) (c, f) concentrations at 7-20 cm depth during the period from April 2016  
731 to April 2017.  $N_0$ ,  $N_{opt}$ ,  $N_{con}$  and  $N_0+S$ ,  $N_{opt}+S$ ,  $N_{con}+S$  represent the zero, optimum and  
732 conventional N treatments with and without straw removal, respectively.  $N_{bal}+M+S$   
733 represents the N balanced treatment with manure and straw return. Solid and dashed  
734 arrows represent fertilization and irrigation events, respectively. Vertical bars in (a)-(f)  
735 indicate standard errors (n=6).

736

737 Figure 2. Average reduction in soil oxygen ( $O_2$ ) concentration compared with the  
738 calibrated background  $O_2$  concentration in soil air (20.9%), average soil nitrous oxide  
739 ( $N_2O$ ) and carbon dioxide ( $CO_2$ ) concentrations at 7-20 cm depth under different  
740 agronomic events (a-c) or in different N rates under the Fer.+Irr./Pre. event during the  
741 period from April 2016 to April 2017. Fer., Irr./Pre., Fer.+Irr./Pre. and Others represent  
742 the data covering all treatments measured under fertilization, irrigation or precipitation,  
743 fertilization with irrigation or precipitation and other time, respectively. Fer. or  
744 Fer.+Irr./Pre. include measurement data in 10 days following the fertilization. Irr./Pre.  
745 includes data in 7 days following the irrigation or precipitation. Zero, Optimum and  
746 Conventional refer to the zero ( $N_0$ ,  $N_0+S$ ), optimum ( $N_{opt}$ ,  $N_{opt}+S$ ) and conventional  
747 ( $N_{con}$ ,  $N_{con}+S$ ) N treatments, respectively. Vertical bars in (a)-(f) indicate standard  
748 errors (n=42 in a-c, n=12 in d-f). Different letters above each bar indicate significant  
749 difference between events or N rates at  $P<0.05$ . Values of the columns and standard

750 errors are shown in Table S7 and S10.

751

752 Figure 3. Response of soil nitrous oxide ( $\text{N}_2\text{O}$ ) concentration to soil oxygen ( $\text{O}_2$ )  
753 concentration at 7-20 cm depth based on all the measurement data (a), data of different  
754 N rates (b) or agronomic events (c) during the period from April 2016 to April 2017.  
755 Zero, Optimum and Conventional in (b) are same as that in Figure 2. Fer., Irr./Pre.,  
756 Fer.+Irr./Pre. and Others in (c) represent the same as that in Figure 2. Detailed response  
757 equations and the 95% confidence interval (CI) for (b) and (c) are shown in Figure S12  
758 and S13, respectively. Significance level:  $**P < 0.01$ .

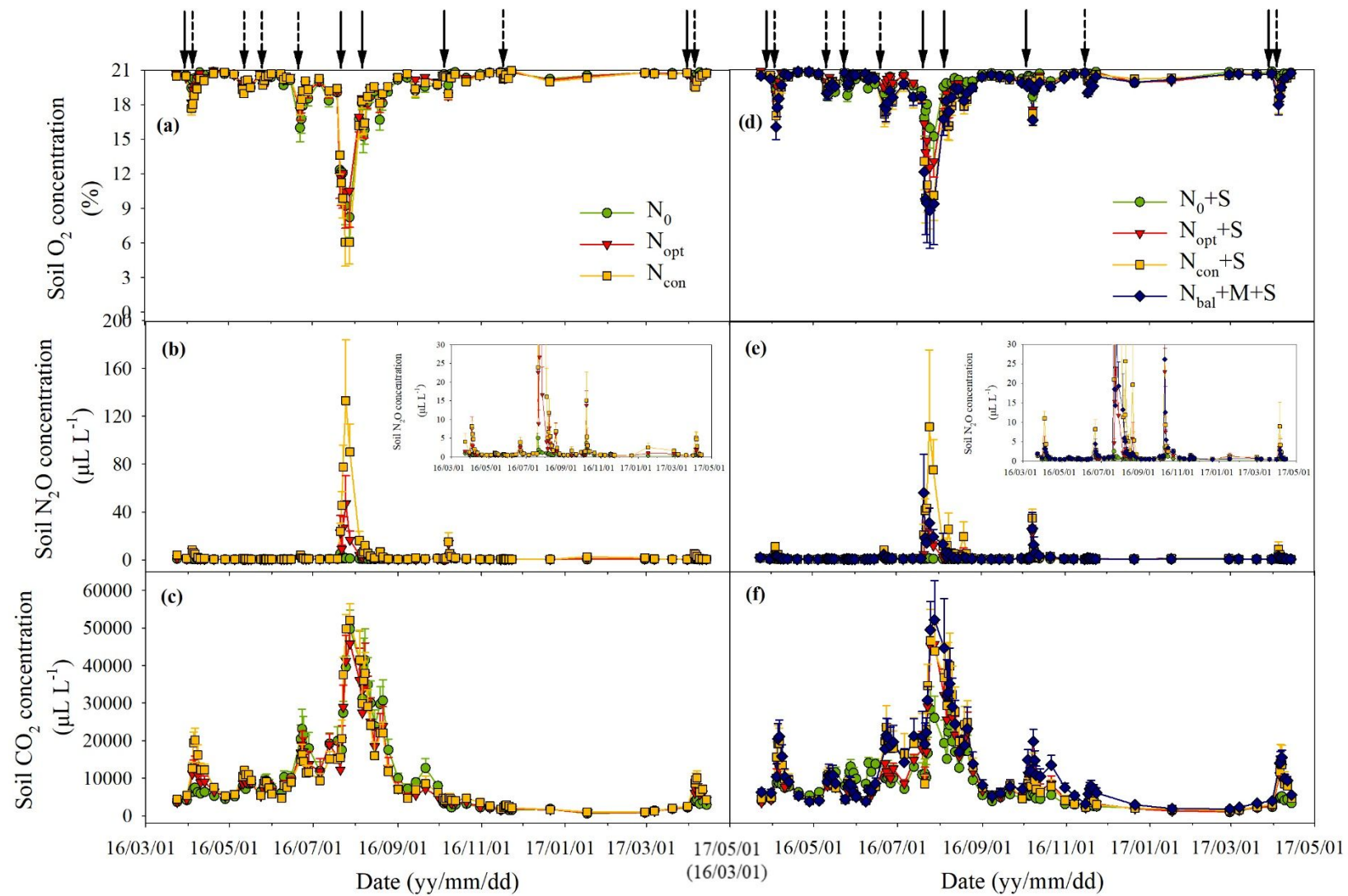


Figure 1



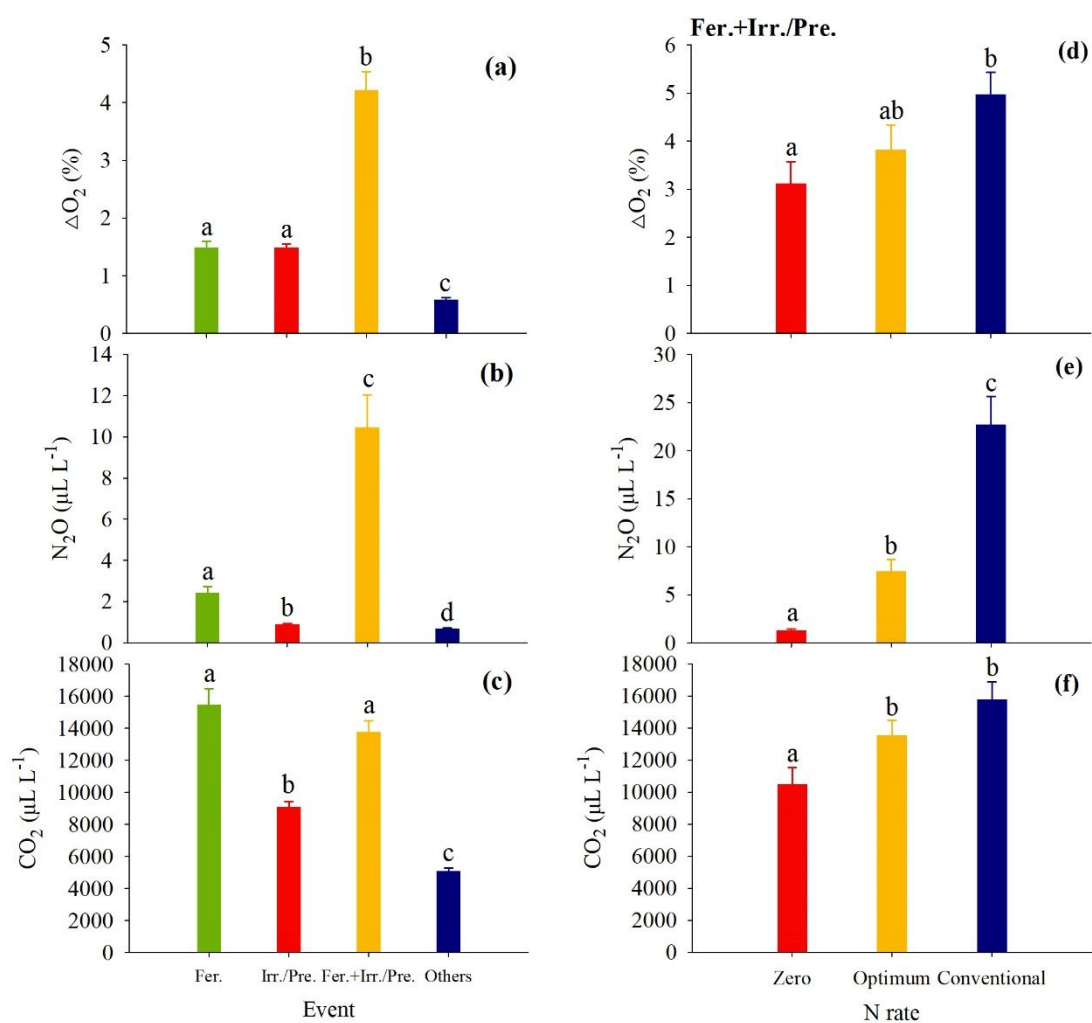


Figure 2

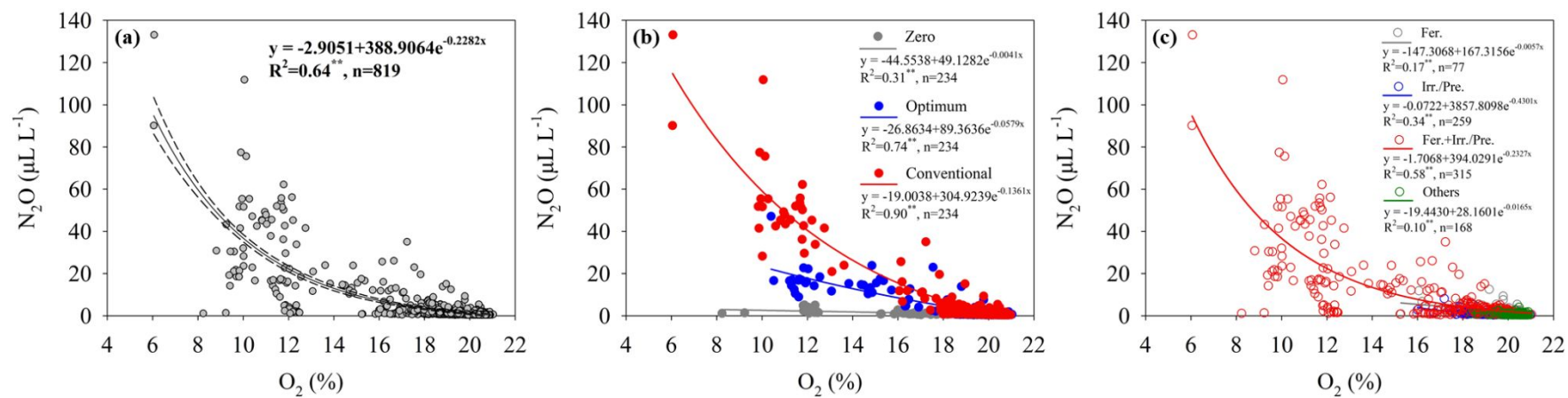


Figure 3

### Abstract Art

