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Published in:
Environmental Science & Technology

DOI:
10.1021/acs.est.9b03089

Print publication: 05/11/2019

Document Version
Peer reviewed version

Citation for pulished 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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Journal:	*Environmental Science & Technology*
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

Manuscript submitted to *Environmental Science & Technology*

Type of contribution: Research Article

Oxygen regulates nitrous oxide production directly in agricultural soils

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Abstract

Oxygen (O_2) plays a critical and yet poorly understood role in regulating nitrous oxide (N_2O) production in well-structured agricultural soils. We investigated the effects of *in situ* O_2 dynamics on N_2O production in a typical intensively managed Chinese cropping system under a range of environmental conditions (temperature, moisture, ammonium, nitrate, dissolved organic carbon etc.). Climate and management (fertilization, irrigation, precipitation and temperature), and their interactions significantly affected soil O_2 and N_2O concentrations ($P<0.05$). Soil O_2 concentration was the most significant factor correlating with soil N_2O concentration (r= -0.71) when compared with temperature, water-filled pore space and ammonium concentration (r= 0.30, 0.25 and 0.26, respectively). Soil N_2O concentration increased exponentially with decreasing soil O_2 concentrations. The exponential model of N treatments and fertilization with irrigation/precipitation events predicted 74-90% and 58% of the variance in soil N_2O concentrations, respectively. Our results highlight that soil O_2 status is the proximal, direct and the most decisive environmental trigger for N_2O production outweighing the effects of other factors, and could be a key variable integrating the aggregated effects of various complex interacting variables. This study offers new opportunities for developing more sensitive approaches to predicting and through appropriate management interventions mitigating N_2O emissions from agricultural soils.

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

Introduction

Agricultural emissions of the greenhouse gas nitrous oxide (N_2O) have become a global concern given its role as the second largest non-carbon dioxide (CO_2) climate forcing agent following methane (CH_4) and the most significant ozone-depleting gas emitted to the stratosphere.[1-2] Agricultural soils are responsible for around 60% of global anthropogenic N_2O emissions.[3-5] Although it is known that microbial nitrification and denitrification are main processes of N_2O production in soils,[6] the key biological mechanisms of N_2O production, and the interaction between regulating environmental variables, remain difficult to predict.

Of these, soil oxygen (O_2) is the key proximal factor simultaneously controlling nitrification and denitrification by influencing these processes at the cellular-level, and further determining the partitioning of the end products between dinitrogen (N_2) and N_2O.[7-8] Other major factors particularly soil moisture, nitrogen (N) and oxidizable carbon (C), together with soil texture and aggregate structure play a role primarily through their influence on the availability of O_2.[7] Thus, soil texture and aggregate structure together determine soil physical factors such as the total porosity, air-filled porosity, water retention, and tortuosity and interconnectivity of the pore system that determine O_2 diffusion rates into the soil, and the O_2 availability varying across the aggregate radius. This is recognized as a major driving force in the fate of N transformations and N_2O production in aggregates.[9-11] Despite the central role of O_2 in determining the processes and rates of N_2O production, there is little quantitative evaluation of the effects of O_2 on N_2O formation in soils particularly in field conditions, and how these relations are affected by the complex interactions between soil, climate and management factors. As a result, there is a missed opportunity to use O_2 as a powerful predictor of N_2O production, and improve understanding of underlying processes.[12]

For a given site, O_2 dynamics would be mainly regulated by changing climate factors within the year (temperature and precipitation), and agronomic management (cropping systems, fertilization, irrigation etc.). A limited number of studies have measured O_2 dynamics in contrasting wetland ecosystems, especially paddy soils, humid forest soils, urine-amended pastures and riparian wetlands.[8,13-17] Unlike aquatic systems experiencing nearly constant anoxia throughout the year, many agricultural soils have been shown to have both spatially and temporally fluctuating redox status and experience intermittent low redox potentials associated with precipitation or irrigation events.[13,18] However, few studies have considered the changes in O_2 concentration that occur in agricultural soils which are typically associated with well-aerated conditions,[19] and thus impede our understanding of how O_2 responses to climate and management regulate soil trace gas emissions.

Knowledge regarding O_2-regulated N_2O production is derived mostly from pure culture and soil microcosm studies.[20-22] Nitrification was found to be the main source of N_2O at O_2 concentrations greater than 0.35%.[21-22] The amount of N_2O-N generated as per unit of N nitrified is highly sensitive to O_2 concentrations and can increase nearly tenfold from 0.16% to 1.48% when O_2 concentration is reduced from 20.4% to 0.8%, indicating that N_2O produced by nitrification could be a significant source process at reduced O_2 concentrations mainly via nitrifier denitrification, especially in ammonium (NH_4^+)-N fertilized soils.[12,22] As soil O_2 concentrations decrease, the denitrification rates also increase, however, the macropore-O_2 content must fall below 0.5% to result in a large increase in denitrification rate.[23] Given high spatio-temporal heterogeneity of O_2 dynamics in the *in situ* upland agricultural soils in this study, the role of O_2 in regulating N_2O production remains challenging to explain.[12]

In well-structured soils under frequent drying-wetting cycles, coupled with the spatio-temporal changes of climate and management factors in the *in situ* upland cropping systems, we hypothesized that: (1) soil O_2 concentration regulates N_2O production directly following certain quantitative correlations; (2) the strength of the correlations depends on the combination of fertilization, irrigation and precipitation events. The objectives of this study were therefore: (1) to quantify the effects of soil O_2 and other soil environment variables on N_2O production in the *in situ* upland agricultural soils and (2) to establish robust empirical models between soil O_2 and N_2O concentrations under the coupling spatio-temporal changes of the climate and management factors.

Materials and Methods

Experiment site and design

Our study site was located at the China Agricultural University Research Station in Shangzhuang (39°48’N, 116°28’E) near Beijing, in the North China plain. This site is representative of upland agricultural soils in this region.[24] The altitude of this site is 40

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

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

(1) Zero N (N_0), no fertilizer N input as a control;

(2) Optimum N (N_{opt}), chemical N fertilizer applied at optimum rates calculated by the mineral N (Nmin) test method based on the synchronization of crop N demand and soil N supply;

(3) Conventional N (N_{con}), chemical N fertilizer applied at rates of 260 and 300 kg N

ha^{-1} for maize and wheat, respectively, according to local conventional farming practice;[26]

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

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

Soil gas (O_2, N_2O, CO_2, CH_4) sampling and measurements

In each plot, we established a subplot for gas sampling covering an area of 9 m^2 (3m*3m) including two 1 m width guard rows as borders alongside the footpath to avoid disturbance of the crop and soil (Figure S2). In every subplot, two soil-air equilibration samplers were installed vertically in the soil to a depth of 5-20 cm. The two gas samplers were positioned randomly in the subplot in wheat and in the N fertilizer band in maize (Figure S2), respectively. The soil-air equilibration sampler was modified from Wang et al[30] and consisted of a polyvinylchloride (PVC) tube with a 2.5 cm inner diameter, a PVC dust cap, a rubber plug and a microbore polytetrafluoroethylene (PTFE) tube (inner diameter 0.25 cm) fitted with a three-way stopcock to connect with the sampling syringe at the soil surface (Figure S3). The PVC tube was perforated, which ensured air diffusion and exchange between the sampler and the surrounding soil. We drilled a 3.0 cm diameter hole by soil auger prior to the installation of the sampler and backfilled the soil after inserting it in the hole. The soil-air equilibration samplers were dug out prior to each crop harvest and inserted back after the sowing of the next crop. To avoid connection of atmospheric air to soil air, three-way stopcocks of the samplers were closed on non-sampling days ensuring the representatives of soil gases inside the samplers.

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

The concentration of O_2 was measured directly by a portable O_2 content analyzer (G100 Range, Geotechnical Instruments Ltd., UK) linking to the samplers immediately after gas sampling. N_2O, CO_2 and CH_4 concentrations were analyzed within 24 h after sampling by a gas chromatograph (GC) (Agilent 6820, USA), see details in SI (S2.3) and previously published papers.[25-26,28,31] Detailed measurements of soil temperature, water-filled pore space (WFPS), mineral N (NH_4^+, NO_2^-, NO_3^-), dissolved organic C and N (DOC and DON) and pH, crop aboveground biomass, N uptake, grain yield and source of climate data are reported in SI (S2.4-S2.6).

Data analyses

Differences in average soil O_2, N_2O and CO_2 concentrations between different management factors and grain yield between different treatments were analyzed by a one-way ANOVA procedure for least significant differences (LSD) at $P < 0.05$ (Figures 2 and S22, Tables S7-S10). Effects of agronomic event, N rate, straw management and their interactions on soil O_2, N_2O and CO_2 concentrations were analyzed by multi-way ANOVA for LSD at $P < 0.05$ (Table S6). Pearson analysis was performed to evaluate the correlation between soil N_2O concentration and soil environmental parameters (Table S11). Stepwise multiple linear regression was used to determine the variation in soil N_2O concentration that could be explained by soil O_2 concentration, moisture and temperature, with a criteria of $P<0.05$ to accept variables (Table S12). The above statistical analyses were undertaken using IBM SPSS Statistics 21 (SPSS Inc., Chicago, IL, USA). Regression models of soil N_2O and CO_2 concentration responding to soil O_2 concentration, WFPS and temperature were fitted by SigmaPlot 14.0 (Systat Software Inc., Erkrath, Germany) (Figure 3, Figures S16-S17). Selection of the best function and a related boundary line analysis are described in SI (S2.7).

Results

Climatic parameters

Annual mean air temperature and precipitation in the experimental year (2016-2017) were 14.1 ℃ and 679 mm, respectively (Figure S4 a), leading overall to warmer and wetter conditions compared to the corresponding long-term (1981-2015) averages of 13.0 ℃ and 540 mm at the study site (Figure S23 a). The summer period (June-September) in 2016-2017 had consistently higher air temperature (25.8 ℃) and far higher precipitation (553 mm) in relation to the average of 24.5 ℃ and 427 mm between 1981and 2015 (Figure S23 a). An extreme rainfall event of 253 mm occurring on 20 July accounted for 37% of the annual precipitation in 2016-2017 (Figure S4 a). Although the total annual precipitation in 2016-2017 was not exceptional, the extreme rainfall event was the highest daily precipitation since 1981 and was nearly 2-8 times higher than other recorded maximum daily precipitation events between 1981 and 2015 (Figure S23 c-d). Total precipitation exceeding 300 mm in July is highly unusual and has only happened with a frequency of 6% over the past 35 years (Figure S23 b). The winter period (December-February) was distinctly colder and drier than the summer with a mean air temperature of 0.3 ℃ and precipitation of 5.6 mm in 2016-2017, which were similar to the corresponding long-term (1981-2015) averages of -1.3 ℃ and 9.2 mm, respectively (Figure S23 a). Annual evaporation in 2016-2017 was 1336 mm, including 533 mm occurring in summer (June-September) nearly equivalent to precipitation while evaporation during winter (December-February) was 190 mm and far greater than the precipitation.

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

Concentrations of soil O_2 and N_2O

Clear patterns of soil O_2 depletion and concurrent N_2O production occurred in N treatments following fertilization with subsequent irrigation or precipitation (named as

Fer.+Irr./Pre. event in this study) in the field (Figure 1 a-b, d-e). In particular, the extreme rainfall following fertilization on 20 July 2016 resulted in the lowest O_2 concentration of 6% and highest N_2O concentration up to 140 μL L^{-1} in N_{con} under waterlogging conditions, even though less N fertilizer was applied at this event compared to others, which normally led to an O_2 concentration of 15% to 18% and a N_2O concentration of between 5 μL L^{-1} and 35 μL L^{-1} in N treatments under aerated soil conditions. The fertilization occurring separately (Fer. event) reduced the O_2 concentration to 18.3-18.5% and led to N_2O peaks of 5.2-5.9 μL L^{-1} in N_{con}, N_{con}+S and N_{bal}+M+S when the WFPS ranged between 40-45% on 6 August 2016. Irrigation or precipitation (Irr./Pre. event) slightly decreased soil O_2 concentrations to 19% but did not stimulate N_2O production except when the irrigation on 21 June 2016 was followed by continuous rainfall which reduced O_2 concentrations to 17.2% and brought about a small N_2O pulse of 8.1 μL L^{-1} in N_{con}+S. Only when fertilization was coupled with irrigation or precipitation, were there intense episodes of soil O_2 depletion resulting in increased N_2O production.

Based on the results of a multi-way ANOVA analysis, we found that agronomic event (*E*), N rate (*N*) and their interactions (*E*N*) were the significant management factors regulating soil O_2 and N_2O concentrations in the plough layer ($P<0.05$) (Table S6). On average across all treatments, Fer.+Irr./Pre. event reduced the soil O_2 concentration to 16.8% which was significantly lower than the concentration of 19.5% in both Fer. and Irr./Pre. events and 20.4% at other time ($P<0.05$) (Table S7, Figure 2 a). The corresponding average soil N_2O concentration following Fer.+Irr./Pre. event was 10.5

μL L^{-1} which was up to 4-15 times higher than that in Fer. (2.4 μL L^{-1}) and Irr./Pre. (0.9 μL L^{-1}) events and other time (0.7 μL L^{-1}) (Figure 2 b). Intriguingly, soil O_2 and N_2O levels matched well with N rates under the Fer.+Irr./Pre. event. Mean soil O_2 concentration declined from 17.9% to 17.2% and 16.0% as N rates increased from zero to optimum and conventional, respectively, and the relative soil N_2O concentration in these three N levels were 1.3, 7.5 and 22.8 μL L^{-1} (Table S10, Figure 2 d-e). However, when Fer. or Irr./Pre. events occurred, soil O_2 concentration ranged from 19.4%-19.7% showing no significant difference between N rates, and N_2O concentration remained low (4 μL L^{-1}) even with conventional N input (Tables S8-9). This was probably because there was an ample O_2 supply from the atmosphere under the soil dry conditions of the Fer. event. In the Irr./Pre event without N additions, a weak O_2 depletion indicated that the air-filled porosity physically replaced by water could recover instantly.

Therefore, a small depletion in the soil O_2 concentration could still be a strong environmental trigger for disproportionate N_2O production, especially when there were sufficient available substrates and moisture caused by the Fer.+Irr./Pre. event. Our results indicate that soil O_2 depletion is the proximal and direct driver underlying the effects of climate and management factors on N_2O production. See S3.3 in SI for concentrations of soil CO_2 and CH_4.

Correlations between soil O_2 and N_2O concentrations

Soil O_2 concentration, temperature, WFPS, and NH_4^+ concentration were significant environmental factors controlling N_2O production in the studied upland soil under field conditions ($P<0.01$) (Table S11). However, soil O_2 concentration was the strongest factor correlating with N_2O concentrations (r=-0.71) when compared to temperature, WFPS and NH_4^+ content (r=0.30, 0.25 and 0.26, respectively). The strength of the correlations between soil O_2 and N_2O concentrations was evidently affected by agronomic event, N rate, and crop season, but not by straw and manure applications. Regarding agronomic event, soil O_2 and N_2O concentrations were the most closely correlated under Fer.+Irr./Pre. event (r=-0.68) when compared to Irr./Pre., Fer. and other time (r=-0.55, -0.41 and -0.31, respectively). The correlations under conventional and optimum N rates were similar (r=-0.89 and -0.86, respectively) but were much larger than the strength in zero N rate (r=-0.56). The correlation between soil O_2 and N_2O concentrations in maize (r=-0.68) was greater than that in wheat (r=-0.45). The widely different correlation coefficients indicated that soil N_2O production would be exclusively dependent on soil O_2 concentration when soil temperature, moisture and NH_4^+ substrate were not limiting in the Fer.+Irr./Pre. event, N treatments and maize growth season.

Using stepwise multiple regression analysis between soil environmental parameters and N_2O concentrations, regression models derived from all the measurement data within the study year showed that soil O_2 concentration was the most significant variable ($P<0.01$) rather than temperature and WFPS (Table S12). Soil O_2 concentration alone could explain 49-84% of the variance in soil N_2O concentration, and the explanation of variance was only marginally improved by adding soil temperature and

WFPS. The regression model of conventional N treatment which simultaneously included soil O_2 concentration, temperature and WFPS as variables provided a prediction of soil N_2O concentration which was very close to the *in situ* observed values (Figure S8).

Given the strong correlations between soil O_2 and N_2O concentrations, we further explored the response of soil N_2O concentration to soil O_2 concentration throughout the experimental period. Generally, soil N_2O concentration increased as soil O_2 concentration decreased and this response was best fitted by an exponential model (Table S13, Figure S9). The N_2O production rate per unit O_2 of depletion (slope of the curve) was relatively low (less than 6 μL L^{-1} N_2O per unit O_2 depleted) at O_2 levels higher than 12%, but below this point the rate increased steeply to 23 μL L^{-1} N_2O per unit O_2 depleted as O_2 concentration was reduced to 6% (Figure 3 a). Thus, we infer that an O_2 concentration of 12% in bulk soil air might be a critical transition point between the dominance of aerobic versus anaerobic processes in structured field soils. The exponential model for zero, optimum and conventional N rates explained 31%, 74% and 90% of the variance in soil N_2O concentration, respectively (Figure 3 b). As soil O_2 concentration decreased from 21% to 10%, soil N_2O concentration increased from zero to 2, 20 and 60 μL L^{-1} in zero-, optimum- and conventional-N rate, respectively. Similarly, the exponential model performed better in Fer.+Irr./Pre. (R^2=0.58) than in Irr./Pre. (R^2=0.34), Fer. (R^2=0.17) and other time (R^2=0.10) (Figure 3 c). Soil N_2O concentration rose by up to 40 μL L^{-1} when soil O_2 concentration was reduced from 21% to 10% in Fer.+Irr./Pre., but there was no significant increase in Fer., Irr./Pre. and other time when the soil O_2 concentration was above 16%. This indicated that the exponential increase in soil N_2O concentration responding to soil O_2 depletion was more robust under high inputs of N and water, which provided implications for new approaches to simulation and mitigation of N_2O in agricultural soils.

Discussion

Understanding O_2 dynamics in upland agricultural soils

Field measurements of soil O_2 and N_2O concentrations demonstrated a highly dynamic temporal and spatial pattern which was driven by changes in climate and management. Precipitation and irrigation alter soil O_2 concentration mainly through physical replacement of soil air by water which significantly slows down O_2 diffusion in the water phases.[32-34] In saturated layers, the O_2 diffusion rate would be reduced to 1/10000 of that in air, which could not replace the microbial consumption of O_2, leading to the reduced redox potential at the centre of aggregates stimulating denitrification in these microsites.[11] Field observations in upland forest soils have demonstrated this concept,[13] and modelling has shown that the anaerobic fraction of soils can account for 10% of soil volume at 65% WFPS but increase sharply once the WFPS exceeds 80%.[35]

Fertilization coupled with irrigation or precipitation simultaneously promotes microbial O_2 consumption and physical inhibition of O_2 diffusion.[14] Soil waterlogging driven by extreme rainfall can cause severe O_2 depletion in soil even without N addition as shown by the lowest O_2 concentration of 7% in the control treatment in our study. In these circumstances O_2 can remain low until gas exchange recovers with soil drainage.[13,15] Although a completely anoxic bulk soil environment did not develop in our study and there were few observations with extremely low O_2 concentration (Figure 3), the oxic, hypoxic and completely anoxic microsites might nevertheless co-exist in the soil.[19] Once the concentration of O_2 fell below the intermediate value of 12% N_2O production increased sharply following fertilization coupled with irrigation or precipitation (Figure 3 a). Therefore, we speculate that the generated N_2O resulted from a combination of nitrification, denitrification and coupled nitrification denitrification that occurred simultaneously in the soil matrix.[36-37]

Studies in repacked soils have shown that anaerobic microsites appear surrounding added fertilizer N, organic matter, plant residue and rhizosphere, or within soil aggregates in well-structured soils.[37-41] Fragments of plant residue can also be anoxic by absorbing water from adjacent soil[41] (See S4.1 in SI for formation mechanisms of anaerobic microsites in soils). These anaerobic microsites may facilitate significant N_2O production by inducing denitrification and coupled nitrification denitrification, which has been taken into account in some modelling approaches.[9-11] In spite of the observations from laboratory studies, soil microsite development and measurements of O_2 concentration have rarely been reported in the field. The conceptual scheme to visualize O_2 diffusion, transformations of C and N substrates in well-structured soils under different moisture conditions are described in SI.

Ammonia oxidation, the first step of nitrification, actively consumes soil O_2, which has been shown to increase linearly as urea input increases in a robotized incubation experiment using similar soil, implying it could be another important reason for O_2

depletion in the soil matrix.[42] Urea or ammonium-based fertilization actively consumed O_2 in soil especially at high N rates by ammonia oxidation.[42] Our results showed that O_2 consumption proceeded on a similar time-scale and trend between N rates, but was smaller than that reported by Huang et al[42], probably because the O_2 supply in the field could be replenished from the atmosphere. This process of replenishment was also reported by Zhu et al[37] from the calculated O_2 consumption by nitrification that far outweighed depletion of O_2 in soil. In a pasture field, soil O_2 concentration at 10 cm depth showed diurnal variation and reached a minimum of 13% after urine application together with irrigation but recovered to the pre-application level only after 24 h.[16] Similarly, as a consequence of biochemical reactions and supply by diffusion, soil O_2 concentration may vary significantly on a diurnal basis, as shown by our results.[14-15]

Role of O_2 in regulating N_2O production in situ

Oxygen plays a critical and yet poorly understood role in regulating N_2O production in well-structured upland agricultural soils. From the perspective of nitrogen cycling, O_2 concentration in the soil pore space is a key controlling factor of the nitrification process (oxidation of ammonium to nitrate) by nitrifying organisms. Insufficient O_2 will lead to the incomplete oxidation of ammonium to nitric oxide and nitrite instead of nitrate. This may increase the risk of N_2O loss through nitrifier denitrification and coupled nitrification denitrification in soil.

Several previous pure cultures and soil microcosm studies have identified the role of O_2 in regulating N_2O production under laboratory conditions,[12,20-22,42] see S4.2 in SI for detailed mechanisms. In a clay loam soil amended with urea, N_2O production increased by a factor of 19 as O_2 concentration decreased from 21% to 3%.[12] Previous studies based on field measurements consistently point to a significant correlation between O_2 concentration and N_2O production in soils of various textures and environments,[15,17,19] although the data is still highly limited and inadequate to establish a robust empirical response of N_2O to O_2.

Our results established the inverse relationship between O_2 concentration and N_2O production in upland agricultural soils. The nonlinearity of the O_2-N_2O relationship suggested that N_2O was generated from a complex combination of source processes. Nitrification involving nitrifier nitrification, nitrifier denitrification and coupled nitrification denitrification, are the main sources of N_2O in NH_4^+ or urea based fertilizer amended soil especially under limited O_2 conditions.[12,20-21,42-43] Nitrifier denitrification can account for the majority (up to 60-70%) of total N_2O production and far exceed that from nitrification and coupled nitrification denitrification in soils that have received urea or NH_4^+-N fertilizers.[12,42] However, the absolute amount of N_2O produced by nitrifier denitrification increased 50 to 80-fold as the O_2 concentration was reduced from 21% to 0.5%.[12,20] Khalil et al[22] established a regression (R^2=0.94, n=25) of O_2 consumption rates versus nitrification rates under five O_2 concentrations between 0.8% and 20.4% with a slope of 2.02±0.12 mol O_2 consumed per mol N nitrified. This was almost equivalent to the theoretical value for O_2 consumption by nitrification (2 mol O_2 per mol N), implying that the amount of O_2 consumed as per unit of N that was nitrified was relatively constant over a wide range of O_2 concentration. They also found that the production of N_2O by nitrification (i.e. the amount of N_2O-N evoked per unit N nitrified) increased rapidly by a factor of 9 when O_2 concentration fell from 20.4% to 0.8%. These findings suggested that the yield of N_2O per unit O_2 consumed by nitrification increased many times as the O_2 concentration was reduced. This implies that nitrification plays a dominant role in N_2O production and that the ratio of N_2O emitted in nitrification increases with O_2 depletion.

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

The exponential response of N_2O production to soil O_2 depletion was more significant under high rates of N with irrigation or precipitation. It could be speculated that ammonia oxidation with abundant NH_4^+ rapidly consumed soil O_2 and accumulated NO_2^- (the substrate for nitrifier denitrification), and irrigation or precipitation contributed directly to O_2 depletion, leading to anoxic conditions and promoting nitrifier denitrification, coupled nitrification denitrification or heterotrophic denitrification.

The characteristics of the climate, soil and cropping system in this study are widely distributing across the world's farmlands, such as the well-known corn belt in the US Midwest.[48-49] Such cropping systems are subject to intensive management involving high inputs of N fertilizers, and the results of this study therefore help understand the underlying mechanism linking such management to N_2O production.[50] Maize is a particularly important crop in this context and our results therefore have a direct relevance to N_2O production in such cropping systems at the global scale .[51] Thus, the established relationships between O_2 and N_2O concentrations should represent and could be used in modelling global agricultural soils, particularly alkaline soils, with a clay loam texture and a low organic carbon content.

Understanding the role of O_2 in regulating N_2O production is central to improving efficiency of C, N and water management.[52] We propose that avoiding severe O_2 depletion is the key to reducing N_2O formation in agricultural soils. Adopting optimum rates of fertilization and irrigation which meet the crop demand, and applying improved water management using drip or sprinkle irrigation rather than flooding could be options maintaining soil aeration.[53-54] Extreme rainfall caused the largest O_2 depletion and N_2O production even with low N rates, which highlight the linkage between climate and management factors on N_2O production.[50] This enhancement of intense episodes of O_2 depletion facilitating increased N_2O production will feed back to extreme weather events under future global change.

Comparison between WFPS and O_2 as a predictor for N_2O production

Soil moisture has been widely adopted as a proxy of O_2 availability, and our results also showed that soil O_2 concentration decreased quadratically with increases in WFPS (Figure S16 a). However, the changes in WFPS explained only 19% of variance in soil O_2 concentration, which indicated that WFPS could not be an effective predictor for soil O_2 concentration in the field. This is because soil O_2 changes not only depend on soil moisture but also on soil structure and biological respiration. The calculation of WFPS does not take into account the distribution of macropores and micropores, the effects of pore connectivity and tortuosity on gas diffusion, and thus could not reliably predict microsite O_2 concentration.[12,55] Soil WFPS also poorly predicted the soil N_2O and CO_2 concentrations by weak Gaussian functions (R^2=0.05-0.11) in our study (Figure S16 b, S17 b). Measurements in a wetland soil suggested that O_2 was the dominant predictor for N_2O production.[8] Hall et al[56] suggested a need to decouple soil moisture from O_2 availability for predicting production of trace gases, and to reevaluate the representations of moisture in N_2O models, because water addition generated high spatial and temporal variation in soil moisture without significant effect on soil O_2 concentration, and the redox-sensitive GHGs (CO_2, CH_4, N_2O) displayed a weak, non-deterministic relationship with moisture in the forest soil. The predictions of other soil environmental parameters for N_2O production are discussed in SI (S4.4).

The optimal soil water content (calculated from the regression or boundary line equations) for production of N_2O and CO_2, and consumption of O_2 in our study was consistently around 60% WFPS. This intermediate water content was surprisingly same as that deemed to be optimal for aerobic processes, e.g. nitrification, O_2 uptake and CO_2 production by microbial respiration, and also the threshold inducing anaerobic denitrification in the previously established classic conceptual model of the relation between soil water content and microbial activity.[56] In that model, the optimal value of 60% WFPS represented the intersection of increasing availability of C and N and decreasing availability of O_2. Conceptually, a soil moisture of around 60% WFPS offers favorable conditions for aerobic processes (e.g. nitrification) when the diffusion of both substrates and gases (O_2) are not restricted.[55,57] The optimum conditions for N_2O emissions via denitrification are considered to exist within 70-90% WFPS.[12,57-58] The consistency between our observations and the conceptual optimal soil WFPS model explains the tight link between soil N_2O (or CO_2) and O_2 concentration induced by the complex combination of source processes in soil. See S4.3 in SI for correlations between soil O_2 and CO_2 concentrations.

WFPS is calculated using total porosity and defined as the proportion of the total pore space filled with water, and hence the actual fraction of the entire soil volume filled with water or air may differ across soils with different total porosities whilst having the same WFPS.[59] Therefore, WFPS cannot be considered as a single measure to describe the effects of soil water on all processes and should not be applied across soils with varying bulk density, texture and structure.[56] WFPS must be combined with other structural parameters to adequately predict diffusion in soils. These include descriptions of soil structure, tortuosity and connectivity, especially when up-scaling models to regional or continental scales.[59] By contrast, O_2 is a more universally predictive measure given that it is the direct factor regulating the various processes generating N_2O no matter where the site or what the climate is. Our results provide future opportunities for the utilization of soil O_2 concentration to predict N_2O emission more efficiently when dealing with the complicated and interacting factors of climate, soil, agricultural managements, growth of plant and microorganisms under real field conditions. See S4.5 in SI for implications of considering O_2 effects into modeling for better N_2O prediction.

Acknowledgements

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

Supporting Information Available

The supporting information includes additional introduction, materials and methods, results and discussion, supplementary figures and tables (Figures S1-S23, Tables S1-S14), and the conceptual scheme of O_2 diffusion, transformations of C and N in well-structured soils under different moisture conditions. This information is available free of charge via the Internet at http://pubs.acs.org.

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Figure Captions

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

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

errors are shown in Table S7 and S10.

Figure 3. Response of soil nitrous oxide (N_2O) concentration to soil oxygen (O_2) concentration at 7-20 cm depth based on all the measurement data (a), data of different N rates (b) or agronomic events (c) during the period from April 2016 to April 2017. Zero, Optimum and Conventional in (b) are same as that in Figure 2. Fer., Irr./Pre., Fer.+Irr./Pre. and Others in (c) represent the same as that in Figure 2. Detailed response equations and the 95% confidence interval (CI) for (b) and (c) are shown in Figure S12 and S13, respectively. Significance level: $^{**}P<0.01$.

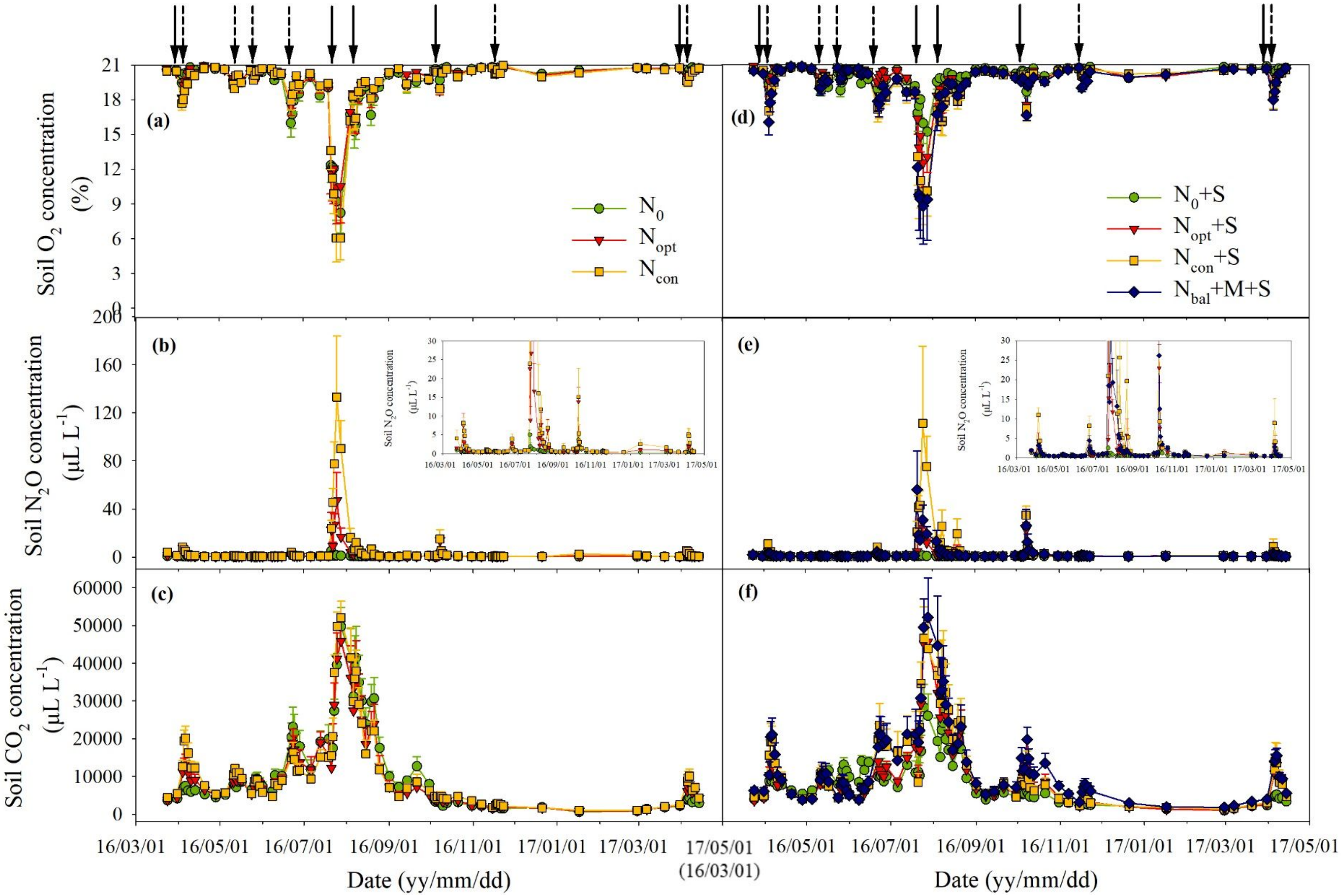
Soil O2 concentration (%)
Soil N2O concentration (μL L-1)
Soil CO2 concentration (μL L-1)
(a)
(b)
(c)
(d)
(e)
(f)
N0
Nopt
Ncon
N0+S
Nopt+S
Ncon+S
Nbal+M+S
21
18
15
12
9
6
3
0
200
160
120
80
40
0
60000
50000
40000
30000
20000
10000
0
30
25
20
15
10
5
0
16/03/01
16/05/01
16/07/01
16/09/01
16/11/01
17/01/01
17/03/01
17/05/01
(16/03/01)
Date (yy/mm/dd)

Figure 1

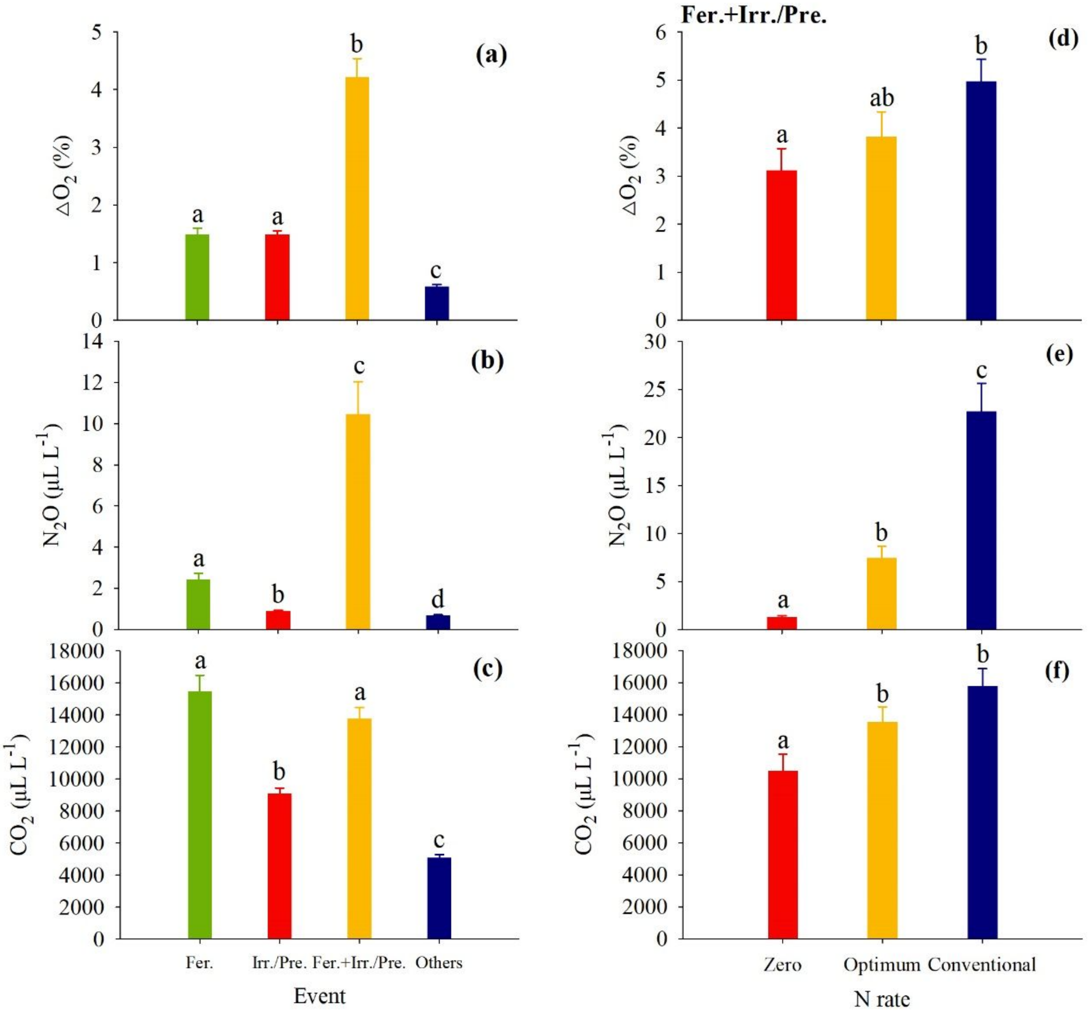
Fer.+Irr./Pre.
(a)
(b)
(c)
(d)
(e)
(f)
ΔO_2 (%)
N_2O (μL L^{-1})
CO_2 (μL L^{-1})
a
a
b
c
a
b
c
d
a
b
a
c
a
ab
b
a
b
c
a
b
b
Fer.
Irr./Pre.
Fer.+Irr./Pre.
Others
Event
Zero
Optimum
Conventional
N rate

Figure 2

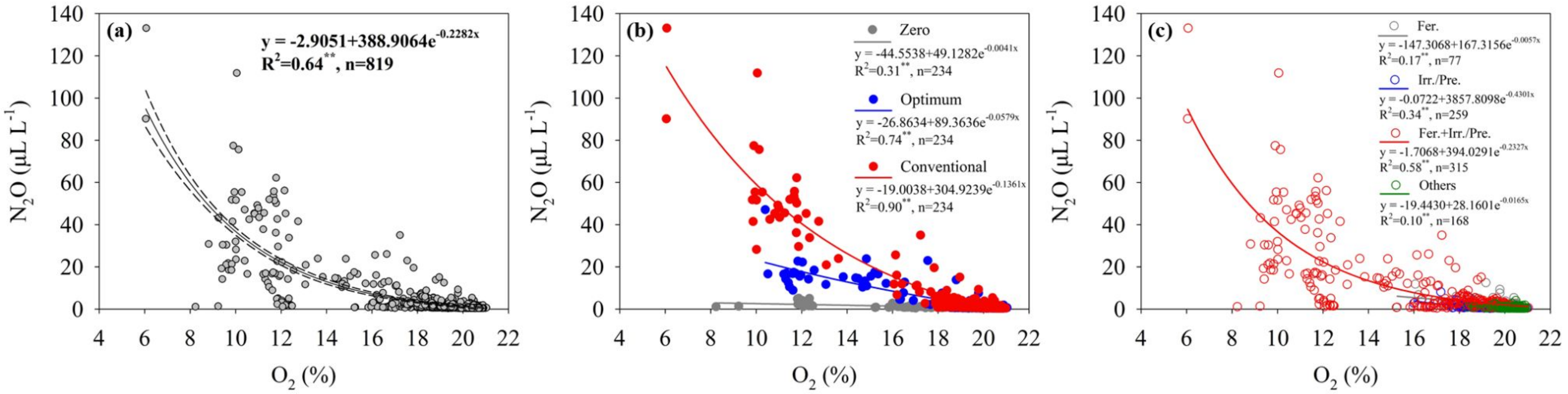
(a)
y = -2.9051+388.9064e^-0.2282x
R²=0.64**, n=819
(b)
Zero
y = -44.5538+49.1282e^-0.0041x
R²=0.31**, n=234
Optimum
y = -26.8634+89.3636e^-0.0579x
R²=0.74**, n=234
Conventional
y = -19.0038+304.9239e^-0.1361x
R²=0.90**, n=234
(c)
Fer.
y = -147.3068+167.3156e^-0.0057x
R²=0.17**, n=77
Irr./Pre.
y = -0.0722+3857.8098e^-0.4301x
R²=0.34**, n=259
Fer.+Irr./Pre.
y = -1.7068+394.0291e^-0.2327x
R²=0.58**, n=315
Others
y = -19.4430+28.1601e^-0.0165x
R²=0.10**, n=168
N2O (μL L-1)
0
20
40
60
80
100
120
140
O2 (%)
4
6
8
10
12
14
16
18
20
22

Figure 3

Abstract Art