Differential responses of soil nitrogen-oxide emissions to organic substitution for synthetic fertilizer and biochar amendment in a subtropical tea plantation

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Abstract
Tropical and subtropical acidic soils have been well documented as hotspots of global soil nitrogen (N) oxide (i.e., nitrous oxide (N2O) and nitric oxide (NO) emissions). While the effectiveness of possible mitigation options has been extensively examined in croplands, little is known about their effectiveness in reducing N-oxide emissions from acidic soils of rapidly expanding tea plantations in China. Here, we conducted a 2-year field experiment to investigate how organic substitution for synthetic fertilizer and biochar amendment affect soil N-oxide emissions from a subtropical tea plantation. Across the 2-year measurement period, full organic substitution for synthetic fertilizer significantly increased N2O emissions by an average of 17% while had a lower NO emission compared to synthetic fertilizer alone. Our global meta-analysis further revealed that full or partial organic fertilizer substitution resulted in a 29% (95% confidence interval: 5%–60%) increase of N2O emissions from acidic soils. In contrast, irrespective of fertilizer type, biochar amendment significantly reduced N2O emissions by 14% in the first but not second experimental year, suggesting a transient effect. The trade-off effect of full organic substitution on N2O and NO emissions may be attributed to the favorable conditions for N2O production due to the stimulated activity of nitrifiers and denitrifiers. The suppression of N2O emission following biochar amendment was probably due to promoted further reduction of N2O to dinitrogen. The fertilizer-induced emission factor (EF) of N2O (2.1%) in the tea plantation was greater than the current IPCC default value, but the EF of NO (0.8%) was comparable to the global estimate. Taken together, while biochar amendment could have mitigation potential, cautions are needed when applying organic substitution for synthetic fertilizer as mitigation options for acidic soils as hotspots of N-oxide emissions.

KEYWORDS
acids soil, climate-smart practice, hotspots, mitigation option, sustainable agriculture, trace gas
1 INTRODUCTION

Nitrous oxide (N$_2$O) and nitric oxide (NO) are two trace gases of great concern, directly or indirectly involved in global warming and adversely impacts human health and ecosystem functioning (Erisman et al., 2011; Stocker et al., 2013). Agricultural soils are key anthropogenic sources of N$_2$O and NO, as nitrogen (N) fertilizer applied in farming production is vital to the increase of substrate for nitrification and denitrification processes (Davidson, 2009; Firestone & Davidson, 1989; Galloway et al., 2008). Over the recent decade (2007–2016), anthropogenic sources contributed to, on average, 43% of the total N$_2$O emission (7.3 Tg N year$^{-1}$), of which directly and indirectly emissions from N inputs in agriculture accounted for around 52% (Tian et al., 2020). Moreover, NO emissions from N fertilizer application in agriculture were estimated to be 3.7 Tg N year$^{-1}$, accounting for approximately 10% of global total (Stocker et al., 2013). Therefore, to achieve the 1.5°C target by 2100, it will be required that greenhouse gas (GHG) emissions from all related sectors, including the agricultural sector, will be strongly reduced, thereby putting soil N-oxide emissions from fertilized soils in focus (Rogl et al., 2018).

Tea plantations are hotspots of soil N-oxide emissions. Tea (Camellia sinensis L.) is mainly cultivated in well-drained and acidic soils and grown in subtropical and tropical climates. Tea-planted soils generally receiving high N fertilization (even at the annual rate of up to 1200 kg N year$^{-1}$) are more vulnerable to acidification and turn into a significant source of potent N-oxide emissions (Liu et al., 2017; Wang et al., 2020). For example, the global mean value of fertilizer N-induced direct emission factor (EF) of N$_2$O from global tea plantations is 2.31%, being two times higher than the IPCC default value of 1% (Hergoualc’h et al., 2019; Wang et al., 2020). The EF of NO emission was about 70-fold greater in tea plantations than in an adjacent cereal cropping system (Yao et al., 2018). China is the largest tea-growing country, and its area under tea plantation accounts for 70% of the world total (FAOSTAT, 2020). Previous studies have shown that tea plantations in subtropical climates are hotspots of soil N-oxide emissions in the agricultural sector (Wang et al., 2020; Yao et al., 2018). Given such significant and unneglectable emissions, there is an urgent need to develop effective mitigation options for N-oxide emissions from rapidly expanding tea plantations.

Organic substitution for synthetic fertilizer is a sustainable measure for improving soil fertility and alleviating environmental deterioration (Leiber et al., 2017; Mäder et al., 2002). Through mediating the availability of soil inorganic N and bioavailable organic C as substrates for microbial N-oxide production and consumption, organic fertilizer application may have inconsistent effects on soil N-oxide emissions in individual studies (He et al., 2019; Yao et al., 2018; Zhou et al., 2017). Previous studies have reported that manure application effects on N$_2$O and NO emissions are related to climate, agricultural practices, manure characteristics, and specific initial soil properties (Liu et al., 2017; Zhou et al., 2017). Although several individual studies have investigated the effect of synthetic fertilizer replaced by organic fertilizer on N-oxide emissions in subtropical tea plantations, a general conclusion has not been made due to the high variation of manuring effects across different experimental sites. For example, in a tea plantation in southeast China, He et al. (2019) reported that livestock manure could suppress soil N$_2$O emissions due to relatively low decomposability, while soybean cake manure can remarkably increase its emissions due primarily to its low C/N ratios and high decomposability. Similarly, other studies observed the stimulation effect of oilcake manure application on soil N$_2$O emissions (Deng et al., 2017; Yao et al., 2018). Therefore, detailed knowledge about how substituting synthetic fertilizers with animal manures affects soil N-oxide emissions is critical to developing an effective mitigation option.

Over the past decade, the use of biochar in cropland management has attracted great attention due to its high potential to sequester carbon and regulate non-CO$_2$ emissions (Cayuela et al., 2015; Wang et al., 2016). For example, it is estimated that a suitable distribution of biochar across global croplands may contribute to a decrease of 3%–14% of reactive N losses from global croplands (Liu et al., 2019). However, the biochar effect on soil N$_2$O emissions is highly variable, depending on the type and application rate of biochar, soil characteristics, and environmental conditions (Borchard et al., 2019; Cayuela et al., 2014, 2015). The decreased N$_2$O emissions following biochar addition were mainly attributed to increased soil pH, improved soil aeration, enhanced N immobilization, or a toxic effect induced by biochar organic compounds on nitriﬁers and denitriﬁers (Brassard et al., 2016). Conversely, increases in N$_2$O emissions may be ascribed to biochar-induced increases in soil water content, which favors denitriﬁcation or biochar embodied-N release (Mukherjee & Lal, 2014). Besides, few field studies examined the effects of biochar addition on soil NO emissions and showed inconsistent results. For example, some studies showed a significant reduction of soil NO emissions with biochar amendment (Ji et al., 2020; Zhang et al., 2019), whereas others reported no difference (He et al., 2019; Xiang et al., 2015). Consequently, there is a need to explore the overall effect and highly complex mechanisms of biochar amendment on N-oxide emissions in subtropical tea plantations toward better use of biochar.

In this study, we present the field measurement results of two consecutive years in which fluxes of N$_2$O and NO, environmental factors, and functional genes involved in soil N-cycling were measured simultaneously in a subtropical tea plantation in southeast China. We hypothesized that (i) compared to synthetic fertilizer, full organic substitution for
synthetic fertilizer would induce an increase in \( \text{N}_2\text{O} \) but a decrease in \( \text{NO} \) emissions and that (ii) biochar application would reduce \( \text{N}_2\text{O} \) emissions but have no detectable effect on \( \text{NO} \) emissions. The main objectives of this study were (i) to quantify annual \( \text{N}_2\text{O} \) and \( \text{NO} \) emissions as well as their direct EFs of applied N for different fertilizer managements; (ii) to improve understanding of the underlying mechanisms and factors controlling their emissions under various fertilization management practices; and (iii) to assess the effects of biochar amendment on their emissions, thus identifying the most promising management regime for mitigating \( \text{N}_2\text{O} \) in subtropical tea plantations.

2 | MATERIALS AND METHODS

2.1 | Experimental site and design

The field experiment was conducted over two annual cycles from August 2018 to August 2020 in a typical subtropical tea plantation (c. 18 years old), which was located in the Nonglin tea planting farm in Zhenjiang City, China (106 m a.s.l., 31°97′N, 119°14′E). The region has a subtropical monsoon climate with a mean annual air temperature and precipitation of 16.2°C and 1192 mm, respectively (2003–2019; National Meteorological Information Center, http://data.cma.cn/en). The clay loam soil at the study site is classified as Planosols (FAO, 1981), with 36.1 ± 2.0% sand, 28.0 ± 4.0% silt, and 35.9 ± 2.1% clay. Mean soil bulk density is 1.21 g cm\(^{-3}\). The soil of this site had a strongly acidic pH of 4.5, total organic C of 2.14 ± 0.03%, and total N of 0.32 ± 0.01%.

The field experiment was established in July 2018. Five treatments consist of conventional compound fertilizer (N: P\(_2\text{O}_5\): K\(_2\text{O} = 16:16:16\); CF), organic fertilizer (cattle manure; OF), CF plus biochar at a rate of 20 t ha\(^{-1}\) (CF+Bc), OF plus biochar at a rate of 20 t ha\(^{-1}\) (OF+Bc), and control without fertilization (Control). The treatments were arranged in a randomized complete block design with three replications (plot size: 18 m\(^2\)). The width of the canopy of tea plants was 0.8 m, and the distance between the canopies was 0.3 m. The N application rate was 450 kg N ha\(^{-1}\) year\(^{-1}\) in all fertilized plots. One-third of the total N fertilizer was applied as basal fertilizer on October 12 in both years, and the remaining as top-dressing was applied on February 28, 2019. Note that the top-dressing in 2020 was not applied because the fieldwork was not allowed during the COVID-19 pandemic period. According to local practices, fertilizers and biochar were evenly applied as band application in the soil between rows with a width of 0.2 m and then incorporated to a depth of 0.1 m below the soil surface. The biochar was only applied on October 12, 2018, over the soil between rows together with basal fertilizers. The organic fertilizer with a pH of 7.85, total N of 2.76%, and had a C/N ratio of 7.6. The biochar was produced from wheat straw and slow-pyrolyzed at 400°C in a mobile bench-scale pyrolyzer (SSSBP-5000A). The biochar had a pH of 9.61, an ash content of 14.6%, total C of 71.3%, total N of 0.63%, H:C\(_{\text{org}}\) of 0.04, and surface area of 13.95 m\(^2\) g\(^{-1}\) (Bian et al., 2016). Spring trimming was conducted at the end of May every year, and most of the trimmed leaves and branches were left on the soil surface. The harvests of tea leaves were in early April.

2.2 | Measurements of \( \text{N}_2\text{O} \) and \( \text{NO} \) fluxes

Fluxes of \( \text{N}_2\text{O} \) and \( \text{NO} \) were measured from the soil between rows and under the canopy within each treatment using the static chamber-gas chromatography (GC) method (De Klein & Harvey, 2012). Rectangular (0.8 m × 0.6 m) and circular (diameter 0.3 m) polyvinyl chloride (PVC) resin collars were inserted into the soil between rows and under the canopies in each plot to a depth of 0.15 m, respectively. The top edge of the collars had a groove for filling with water to seal the rim of the chamber. To minimize the possibility of influence on gas fluxes from the temporary installation of chamber bases, they were inserted into the soil one month before the start of flux measurements and maintained in place throughout the experimental period. The gas sampling chamber with a bottom area of 0.6 m × 0.8 m and a height of 0.8 m to cover one tea plant on the hill. The chamber size for the plots between rows was designed as 0.3 m in diameter and 0.8 m in height. PVC resin opaque chambers were covered with insulating foam to minimize air temperature changes inside the chamber during sampling. The top of each chamber was fitted with a three-way stopcock and equipped with a circulating fan to ensure complete gas mixing. Five gas samples were collected per chamber with a pre-evacuated 1.5-L gas sampling bag (Delin Gas Packing Co., LTD) at a 5-min interval after chamber enclosed the PVC resin collar and stored for laboratory analysis within 24 hr. Gases were sampled from local time 8:00 through 10:00 LST weekly and three times a week during the fertilization period.

Nitrous oxide concentrations were determined with a gas chromatograph (Agilent 7890A) fitted with an electron capture detector (ECD) to analyze \( \text{N}_2\text{O} \) at 330°C. A standard \( \text{N}_2\text{O} \) gas with a concentration of 350 ppbv \( \text{N}_2\text{O} \) (National Center for Standard Matters) was used to calibrate \( \text{N}_2\text{O} \) concentration. Concentrations of NO were analyzed with a model 42i chemiluminescence NO-NO\(_x\)-NO\(_x\) analyzer (Thermo Environmental Instruments Inc.) as described previously (Yao et al., 2015). The NO\(_x\) analyzer instrument was calibrated once every 2 or 3 months using the same manufacture’s calibration system and the standard gas from the National Center of Standard Matters. \( \text{N}_2\text{O} \) and NO fluxes were calculated by a nonlinear fitting approach as described previously (Kroon et al., 2008). The cumulative emissions (kg N ha\(^{-1}\)) of N-oxide from the soil between rows (\( E_{\text{rows}} \)) and under the canopy (\( E_{\text{canopy}} \)) of tea plants were
approximated by applying the trapezoid rule on time intervals between measured flux rates, assuming constant flux rates per day. The total emissions of N-oxide for the entire field \( (E) \) were calculated by accounting for their area ratio of the whole plot:

\[
E = 0.27 \times E_{\text{rows}} + 0.73 \times E_{\text{canopy}},
\]

(1)

Using the IPCC’s default Tier 1 approach (Hergoualc’h et al., 2019), the EFs (%) of \( \text{N}_2\text{O} \) and NO emissions were calculated using the following equation:

\[
\text{EF} = \frac{\text{N-oxide emissions from the fertilized treatment} - \text{N-oxide emissions from the control}}{\text{N applied}} \times 100
\]

(2)

### 2.3 Soil sampling and physicochemical analysis

Parallel to gas sampling, soil temperature and moisture were measured at a depth of 10 cm. Soil temperature was measured using a handheld thermometer, while soil volumetric water content (v/v, %) was determined using a portable frequency domain reflectometer (MPM-160) and expressed as water-filled pore space (WFPS) calculated by dividing volumetric water content by total porosity (Linn & Doran, 1984), where total soil porosity was calculated as (1- (bulk density/particle density)) using a soil particle density of 2.65 g cm\(^{-3}\).

Surface soil samples (0–20 cm) were collected at 2-week intervals from each plot with stainless steel corers. Fresh soils for each plot were pooled and passed through a 2-mm sieve to remove visible gravel and plant residue. One portion was stored at 4°C to await further analysis, and the other portion was stored at −80°C until DNA extraction. Soil water content was determined gravimetrically by drying soil in an oven (24 h, 105°C). Soil pH was determined with a soil-to-water ratio of 1:2.5. Exchangeable ammonium (\( \text{NH}_4^+ \)) and nitrate (\( \text{NO}_3^- \)) of fresh soils were extracted using 1 M KCl (1:5 w/v) and dissolved organic carbon (DOC) was extracted using ultrapure water. The concentrations of \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) in the extracts were determined via the colorimetric salicylate procedure of Mulvaney (1996) and the vanadate method of Miranda et al. (2001), respectively, using a UV-VIS spectrophotometer (U-2900, Hitachi). Total organic carbon concentration was determined using a TOC analyzer (TOC-L, Shimadzu). Soil total C and N were measured with a EuroEA 3000 elemental analyzer (EuroVector).

### 2.4 Soil DNA extraction and quantitative PCR assay

The DNA of soil samples collected from the between-rows position was extracted to analyze the abundances of nitrifying and denitrifying functional genes. Soil DNA samples were extracted using the DNeasy PowerSoil Kit (Qiagen Inc.) according to the manufacturer’s protocol. The concentration and quality of extracted DNA were measured with a Nanodrop ND-100 spectrophotometer (Thermo Scientific). The abundances of genes encoding the key enzymes involved in ammonia oxidation (\( \text{AOA-amoA} \) and \( \text{AOB-amoA} \)) and denitrification (\( \text{nirK, nirS, and nosZ} \)) were quantified by quantitative PCR using the StepOnePlus™ real-time PCR system with 96-well plates (Applied Biosystems). The thermal conditions and gene-specific primers for amplification of the genes are described in Table S1. The specific amplification of PCR products was checked using a melting curve analysis.

### 2.5 Meta-analysis and statistical analysis

To test the generality of our finding, we conducted a comprehensive literature search of published papers that reported \( \text{N}_2\text{O} \) emissions following organic fertilizer application using the ISI Web of Science (Thomson Reuters). “Nitrous oxide” OR “\( \text{N}_2\text{O} \)” AND “organic fertilizer” AND “organic substitution” AND “soil” were used as the searching keywords. We extracted original experimental results from the identified studies in acidic soils (pH ≤ 6.5), providing that soil \( \text{N}_2\text{O} \) fluxes were continuously measured over at least one cropping season. The following information was extracted: \( \text{N}_2\text{O} \) emissions, experimental duration, dominated vegetation, soil characteristics, N application rates in the paired organic and synthetic fertilizer treatments, and manure properties. The final dataset comprised 238 field measurements sourced from 31 studies (Table S2).

We performed a meta-analysis and meta-regression to assess the response of soil \( \text{N}_2\text{O} \) emissions to organic substitution for synthetic fertilizer in global acidic soils. The effect of organic substitution treatments was quantified using the natural log of response ratio (RR), which effect sizes are calculated as:

\[
\text{RR} = \ln \left( \frac{X_i}{X_c} \right),
\]

(3)

where \( X_i \) and \( X_c \) represent the mean of total \( \text{N}_2\text{O} \) emissions from organic substitution and synthetic fertilizer treatments, respectively. Similar to the previous studies (van Groenigen et al., 2011; Wang et al., 2018), we used the number of replications for weighting:

\[
w = \frac{N_c \times N_i}{N_c + N_i},
\]

(4)

where \( N_c \) and \( N_i \) are the numbers of replications in the organic substitution and synthetic fertilizer treatments, respectively. For ease of interpretation, RR and its corresponding
95% confidence intervals (CIs) were transformed to a percent change between organic substitution and synthetic fertilizer treatments as \((e^{RR} - 1) \times 100\%\). The effect of organic substitution on soil \(N_2O\) emissions was considered significant if the CIs did not cover zero. Means of categorical variables were considered significantly different from each other if their 95% CIs did not overlap.

All data were tested for normal distribution (using Shapiro–Wilk’s test) and equality of variance (using Levene’s test), and parameters with non-normal distributions or unequal variances were either logarithmically or square-root transformed when necessary. For analysis of time-series data (i.e., repeated measurements of soil environmental and biotic factors and N-oxide fluxes), we used linear mixed-effects models (LME, package LME4; Bates et al., 2015) to test for the fixed effects of different treatments with the spatial replication (experimental plots) and time (sampling days) included as random effects. If the Akaike’s information criterion (AIC) showed an improvement in the LME models, we included a first-order temporal autoregressive function to account for the decreasing correlation of the measurements with increasing time and/or a variance function (varIdent) to account for heteroscedasticity in the fixed-factor variances (Crawley, 2013). Multiple comparisons were made using the Tukey HSD test. Missing values were excluded from analyses, and effects were accepted as statistically significant if \(p \leq 0.05\). The non-parametric Kruskal–Wallis test was used to examine the differences in microbial functional genes between treatments within each experimental year. All analyses were performed in R version 4.0.3 (R Core Team, 2020).

3 | RESULTS

3.1 | Climate and soil conditions

During the 2018–2019 and 2019–2020 experimental periods, the annual air temperature was comparable with a mean air temperature of 16.9 and 17.4°C, respectively (Figure S1). The distribution pattern of precipitation was similar, whereas total rainfall was slightly different between the two experimental periods (1006 vs. 1226 mm). Note that we mainly focused on the data from the soil between rows since none of the fertilizer type and biochar amendment affected soil properties or N-oxide emissions from the soil under the canopy (Figures S2 and S3; Table S2). Soil temperature across all treatments showed no significant difference \((p > 0.05\); Table S2) and followed a clear seasonal pattern, with the highest value observed in July and the lowest in February (Figure 1a). Soil WFPS ranged from 36.2% to 98.5% during the whole experimental period \((p > 0.05\), but there were no detectable differences between treatments (Figure 1b).

Soil pH under synthetic fertilizer plots was generally lower than the soil under organic fertilizer plots \((p < 0.05\), Figure 1c). Soil \(NH_4^+\) concentrations across all treatments varied strongly in response to N addition and increased rapidly following basal fertilization in autumn, and remained high until top-dressing fertilization in spring (Figure 1d).

Soil \(NH_4^+\) concentrations were more abundant under CF plots (12.6–205.4 mg N kg\(^{-1}\)) than under OF plots (2.4–174.0 mg N kg\(^{-1}\)), whereas biochar amendment tended to have less \(NH_4^+\) concentrations as compared to the corresponding fertilization treatment \((p < 0.001\). Compared with the synthetic fertilizer plots, organic fertilizer application significantly increased soil \(NO_3^-\) concentrations \((p < 0.01\); Figure 1e). Biochar amendment had greater soil \(NO_3^-\) concentrations regardless of fertilizer type. The highest content of DOC was found in the CF treatment, while there was no consistent pattern among other treatments (Figure 1f).

3.2 | Soil N-oxide emissions

During the entire experimental period, \(N_2O\) fluxes from the soil between rows showed a similar pattern but with remarkable differences across all treatments, which were highly and temporally variable and followed a sporadic and pulse-like pattern \((p < 0.05\); Figure 2). Substantial \(N_2O\) emissions among the treatments were observed in spring and summer, with the significant losses in June relating to favorable soil conditions (i.e., high soil moisture, temperature, and mineral N concentrations). Several \(N_2O\) peaks were observed in response to changes in environmental conditions (e.g., rainfall events) and management activities such as fertilization and pruning. For example, the highest \(N_2O\) flux peak was observed in the OF treatment with a large amount of rainfall in the previous week during the respective experimental period, amounting to 5750 and 2074 µg N m\(^{-2}\) h\(^{-1}\) in June 2019 and 2020, respectively (Figure 2a; Figure S1). However, the highest \(N_2O\) flux peak was observed in the CF treatment accompanied by rainfall events in summer with 1548 µg N m\(^{-2}\) h\(^{-1}\) in 2019 and 804 µg N m\(^{-2}\) h\(^{-1}\) in 2020 (Figure 2b; Figure S1).

Because of different rates of N application between the two experimental years due to the COVID-19 pandemic, this study focused on differences in N-oxide emissions between the treatments within each year rather than their interannual variations (Table 1). Annual N-oxide emissions from the whole plots strikingly different between treatments within each experimental year \((p < 0.05\). Regardless of biochar amendment, organic fertilization increased soil \(N_2O\) emissions by an average of 17% across both years. On average, biochar amendment resulted in a 14% reduction of \(N_2O\) emissions in 2018–2019, but this effect disappeared in 2019–2020. In contrast, organic fertilization contributed to the decreased NO emissions by an average of 18% compared to the synthetic fertilizer treatments in 2018–2019, irrespective of biochar addition. Biochar amendment combined with
FIGURE 1 Temporal variability of soil temperature (a) and water-filled pore space (WFPS; b), pH (c), NH$_4^+$ (d), NO$_3^-$ (e), and dissolved organic carbon (DOC; f) contents from the soil between rows in a subtropical tea plantation. Values represent mean ± SEM (n = 3). Control, unfertilized plots; CF, plots with chemical fertilizer; OF, plots with composted cattle manure; CF+Bc, CF plots with wheat straw-derived biochar amendment; OF+Bc, OF plots with wheat straw-derived biochar amendment. The shading area indicates the lack of sampling campaign during the lockdown period (January 15 to April 25, 2020) due to the COVID-19 pandemic.

3.3 | Abundances of bacterial and N-cycling functional genes

Across the 2-year measurement period, different fertilization practices significantly influenced the variations of abundances of bacterial and functional genes associated with N-cycling ($p < 0.01$; Figure S4; Table S2). The abundances of the bacterial gene were marginally greater (2018–2019, $p = 0.085$; Figure 3) or significantly greater (2019–2020, $p < 0.001$; Figure S5) in the organic fertilizer treatments than in the synthetic fertilizer treatments. The numbers of ammonia oxidizers (AOA-amoA and AOB-amoA), nitrite reducers ($nirK$ and $nirS$) were generally greater in the organic fertilizer plots than in the ones with synthetic fertilizer, but there was no difference in the numbers of N$_2$O-reducers. Relative to synthetic fertilizer, organic fertilizer application significantly increased the AOB/bacteria ratio but had no effects on nosZ/bacteria and ($nirK+nirS$)/nosZ ratios. Compared to the unamended plots, biochar amendment appeared to have lower numbers of bacteria, ammonia oxidizers, and nitrite reducers, albeit nonsignificant. Regardless of fertilizer type, biochar amendment enhanced the numbers of N$_2$O-reducers, which led to an increased nosZ/bacteria ratio but a decreased ($nirK+nirS$)/nosZ ratio.

3.4 | Effect of organic substitution on N$_2$O emissions from global acidic soils

Based on 128 paired measurements of 31 field studies, our meta-analysis showed that organic substitution for synthetic fertilizer significantly increased N$_2$O emissions by an average of 29.3% (95% CIs: 4.9%–59.5%; Figure 4a). Although synthetic fertilizer instead of organic fertilizer insignificantly lowered NO emissions by an average of 12% compared to the unamended treatment. Across the 2-year measurement period, soil N-oxide emissions were dominated by N$_2$O at all treatments (Figure S3), and the ratio of annual NO/N$_2$O emissions was 0.42 in 2018–2019 and 0.31 in 2019–2020. The EFs of N$_2$O, NO, and N$_2$O+NO emissions from the studied tea plantation without biochar amendment were 2.12%, 0.80%, and 2.92%, respectively (Table 1).
there were no significant differences between either crop types or manure types, the positive impact of organic substitution on N$_2$O emissions tended to have less uncertainty in the cereal cropping system (16.8%, 95% CIs: –13.1% to 56.9%) or with cattle manure application (27.8%, 95% CIs: –8.2% to 78.0%). Notably, when compared to the partial substitution, full organic substitution for synthetic showed a significant and positive effect on soil N$_2$O emissions (30%, 95% CIs: 1.4%–66.7%). Changes in the organic substitution effect on soil N$_2$O emissions were negatively correlated with soil clay content ($r$ = –0.56, $p < 0.001$) and soil organic C ($r$ = –0.43, $p < 0.001$). When only studies with full organic substitution were considered, there was a negative relationship between the response ratio of N$_2$O emission and the C/N ratio of organic fertilizer ($r$ = –0.27, $p = 0.03$; Figure 4d).

**4 | DISCUSSION**

**4.1 | Response of soil N-oxide emissions to fully organic fertilizer substitution**

Over a 2-year experimental observation, we found that organic fertilizer substitution significantly increased soil N$_2$O emissions in the subtropical tea plantation. Our meta-analysis supported this finding that organic substitution for synthetic fertilizer had an overall significant and positive impact on N$_2$O emissions from global acidic soils (Figure 4a). In accord with our findings, a previous study has also found such effects in acidic soils rather than neutral and alkaline soils (Zhou et al., 2017). Moreover, our meta-analysis demonstrated that full organic substitution for synthetic fertilizer tends to have a remarkable effect on N$_2$O emissions from acidic soils. We made the following comparison between studies in tea plantations to explore whether this finding is universal. In contrast to our conclusion, full cattle manure substitution for synthetic fertilizer can result in a 57% reduction of soil N$_2$O emissions of a tea plantation in the same region (He et al., 2019). This discrepancy may be ascribed to the difference in the C/N ratio of cattle manure (13 vs. 7.5; Figure 4d), leading to the relatively lower decomposability in their study compared to ours. Another possible reason is that the fertilization-induced increments of soil denitrification and N$_2$O emissions tend to be greater in medium-textured as in our study than in coarse-textured soils (Wang et al., 2018). In the subtropical region of central China, however, soil N$_2$O emissions increased by 1.5 times following full oilcake substitution for synthetic fertilizer (Yao et al., 2018), which is likely
### TABLE 1  Emissions of soil N-oxide and their emission factors (EF) from a subtropical tea plantation during the 2018–2020 experimental period

| Treatment       | 2018–2019 | 2019–2020 |
|-----------------|-----------|-----------|
|                 | $E_{\text{rows}}$ | $E_{\text{canopy}}$ | Whole plot | $E_{\text{rows}}$ | $E_{\text{canopy}}$ | Whole plot | $E_{\text{rows}}$ | $E_{\text{canopy}}$ | Whole plot | N$_2$O | NO | N$_2$O+NO |
| Control         | 11.83 ± 0.32$^d$ | 5.86 ± 0.30$^b$ | 7.49 ± 0.27$^d$ | 3.19 ± 0.33$^c$ | 3.22 ± 0.05$^c$ | 3.22 ± 0.12$^c$ | 9.72 ± 0.13$^d$ | 3.41 ± 0.14$^b$ | 5.13 ± 0.09$^e$ | 1.97 ± 0.22$^b$ | 1.24 ± 0.06$^b$ | 1.44 ± 0.13$^b$ |
| CF              | 36.40 ± 1.93$^b$ | 10.14 ± 0.83$^a$ | 17.39 ± 1.13$^b$ | 14.65 ± 1.31$^a$ | 5.98 ± 0.43$^a$ | 8.34 ± 0.65$^a$ | 12.27 ± 0.84$^c$ | 4.94 ± 0.35$^a$ | 6.94 ± 0.43$^b$ | 3.63 ± 0.24$^a$ | 2.19 ± 0.17$^a$ | 2.59 ± 0.15$^a$ |
| OF              | 46.21 ± 1.31$^a$ | 10.82 ± 0.37$^a$ | 20.48 ± 0.36$^a$ | 10.50 ± 0.70$^b$ | 4.92 ± 0.22$^b$ | 6.43 ± 0.15$^b$ | 19.60 ± 0.84$^c$ | 4.26 ± 0.26$^c$ | 8.45 ± 0.38$^a$ | 3.77 ± 0.28$^a$ | 1.78 ± 0.36$^a$ | 2.32 ± 0.30$^a$ |
| CF+Bc           | 29.88 ± 0.94$^c$ | 9.50 ± 0.36$^a$ | 15.06 ± 0.16$^c$ | 13.14 ± 0.36$^a$ | 5.15 ± 0.40$^b$ | 7.33 ± 0.20$^b$ | 12.91 ± 0.46$^c$ | 4.82 ± 0.22$^a$ | 7.03 ± 0.17$^b$ | 3.24 ± 0.31$^a$ | 1.93 ± 0.27$^a$ | 2.29 ± 0.25$^a$ |
| OF+Bc           | 38.15 ± 0.99$^b$ | 9.82 ± 0.06$^d$ | 17.54 ± 0.31$^b$ | 10.20 ± 0.26$^d$ | 5.03 ± 0.21$^b$ | 6.44 ± 0.09$^b$ | 38.15 ± 0.99$^b$ | 9.82 ± 0.06$^d$ | 17.54 ± 0.31$^b$ | 10.20 ± 0.26$^d$ | 5.03 ± 0.21$^b$ | 6.44 ± 0.09$^b$ |

Note: $E_{\text{rows}}$ and $E_{\text{canopy}}$ represent N-oxide emissions from the soil between rows and under the canopy, respectively. Values represent mean ± SEM ($n$ = 3). Control, unfertilized plots; CF, plots with chemical fertilizer; OF, plots with composted cattle manure; CF+Bc, CF plots with wheat straw-derived biochar amendment; OF+Bc, OF plots with wheat straw-derived biochar amendment. Values with different lowercase letters for each column during each experimental period indicate significant differences between treatments at $p < 0.05$. 


due to the more decomposability of oilcake with a C/N ratio of 6.1 (Huang et al., 2004).

Changes in quantity and quality of inorganic N substrate and environmental conditions from organic fertilizer applications may regulate soil N$_2$O production and emission (Butterbach-Bahl et al., 2013). There are several reasons why organic substitution for synthetic fertilizer aggravated N$_2$O emissions from acidic soils. First, the increase in C and N substrates’ availability by organic fertilizer application could enhance N$_2$O production of nitrifiers and denitrifiers (Firestone & Davidson, 1989; Van Groenigen et al., 2005). As expected, we found greater soil mineral N content and higher abundances of functional genes involved in autotrophic nitrification and encoding nitrite reductase during denitrification (Figures 3 and 5). Second, organic fertilization provides more labile organic C compounds as energy for microbial activity, thereby stimulating N$_2$O production derived from heterotrophic nitrification (Figure 5). This explanation could be supported by higher soil NO$_3^-$ concentration under manure plots because of enhanced direct oxidation of organic N to NO$_3^-$ via soil heterotrophic nitrification. Support for this explanation comes from a $^{15}$N tracing model study where the heterotrophic nitrification process plays an essential role in soil mineral N supply and correlates significantly with N$_2$O production in acidic tea soils (Zhu et al., 2014). Third, the liming effect of organic fertilizer application could be another key regulator of N$_2$O emissions from acidic soils. This was supported by the fact that increased soil pH in organic fertilization plots may have contributed to promoting nitrifiers and heterotrophic denitrifiers (Figures 1 and 3). It should be noted that the unchanged abundance of gene encoding N$_2$O reductase might be due to the unfavorable pH condition (<5; Bakken et al., 2012). Fourth, the significant and negative relationship between the C/N ratio of organic fertilizer and the response of soil N$_2$O emissions highlights the importance of its quality in controlling N$_2$O emission (Figure 4d). The lower C/N ratio of organic fertilizer means more decomposability, thereby tending to stimulate N$_2$O emissions.
Conversely, there was an apparent decrease in soil NO emissions following organic fertilizer compared to synthetic fertilizer (Table 1). Our finding agrees with previous studies where full organic substitution for synthetic fertilizer can result in up to 60% decrease in soil NO emissions in subtropical tea plantations (He et al., 2019; Yao et al., 2018). A recent global meta-analysis also reported that organic fertilizer alone or in combination with inorganic fertilizer has a less stimulative effect on soil NO emission than synthetic fertilizer (Liu et al., 2017). On the one hand, the increase in the availability of C and N substrates by organic fertilizer application could enhance microbial activity and oxygen consumption, hence creating an anoxic condition in the soil due to oxygen depletion and facilitate denitrification, thereby increasing NO further reduction to N₂O as discussed above. On the other hand, the reduced soil pH following synthetic fertilizer application probably enhanced the contribution of chemodenitrification to soil NO emissions (Venterea et al., 2005). Additionally,
we speculated that soil receiving organic fertilizers reduced NO emissions due to increased NO consumption via aerobic co-oxidation actions in heterotrophic bacteria (Dunfield & Knowles, 1998).

4.2 Effect of biochar amendment on soil N-oxide emissions

Biochar amendment significantly reduced soil N₂O emissions by approximately 13% during the first experimental year, independent of the type of fertilizer (Table 1). This is consistent with the finding of a meta-analysis (Cayuela et al., 2015) that biochar addition under field conditions can result in 12%–44% reductions in soil N₂O emissions. Biochar used in this study was also in accord with their conclusions that herbaceous material-derived biochar applied at a rate of between 1% and 2% is sufficient to reduce N₂O emissions significantly. Nevertheless, our result was at the lower end of their range, which may be explained by the lowest emission reduction effect often observed in strongly acidic soils following biochar addition (Borchard et al., 2019; Cayuela et al., 2014). However, in the second experimental year, we found that biochar amendment did not affect soil N₂O emissions. This finding was in good agreement with previous studies that biochar-induced reductions of N₂O emissions were transient with a tendency to be negligible within 1 year (Borchard et al., 2019; Spokas, 2013). Since very few studies have attempted to investigate the persistence of the N₂O emissions suppressing effect (Hagemann et al., 2017), our understanding of whether single biochar application would be sufficient to reduce soil N₂O emissions in the long run remains elusive (Verhoeven et al., 2017).

Our results suggest that the stimulated N₂O further reduction to N₂, as indicated by the decreased ratio of \((\text{nirK}+\text{nirS})/\text{nosZ}\), may be mainly responsible for reduced N₂O emissions after biochar application (Figure 5). This result is consistent with Cayuela et al. (2013), who found that biochar enhances the last step of denitrification (i.e., a lower N₂O/N₂ ratio) using the \(^{15}\text{N}\) gas-flux method, acting as an “electron shuttle” that facilitates the transfer of electrons to denitrifying communities (Kappler et al., 2014). Previous studies measuring denitrification gene expression have also suggested that the lower N₂O emissions from biochar amended soil are caused by stimulation of microbial N₂O reduction by both classical and novel N₂O-reducing microorganisms (Harter et al., 2014, 2016). Our wheat-straw biochar with an H:Corg ratio of 0.04 further supports the argument that biochar with low molar H:Corg ratios and pyrolyzed at 400–700°C tend to show the highest capacity to accept and donate electrons, thereby having greater mitigation capacity (Cayuela et al., 2015; Klüpfel et al., 2014). Collectively, our results and those findings are in accord with the explanations that whether biochar amendment can mitigate soil N₂O emissions is associated with the dominant processes responsible for N₂O production. That is, biochar amendment may contribute to N₂O mitigation in the soil where denitrification is the dominant producing pathway but not the soil where nitrification is dominated (Sánchez-García et al., 2014). Since the molar NO/N₂O emission ratios were generally lower than unity (Figure S3), we inferred that denitrification is the dominant process involved in the observed soil N-oxide emissions (Davidson et al., 2000; Skiba et al., 1993). This speculation is well supported by previous studies where they demonstrated that denitrification plays a dominant role in N₂O emissions from acidic tea plantations (Ji et al., 2020; Yamamoto et al., 2014; Zou et al., 2014). It is worthy to note that despite the absence of biochar amendment effect on soil NO emissions, current lesser understanding of how biochar addition would affect NO emissions from various soils underlines more research is needed.

4.3 Hotspots of soil N-oxide emissions from subtropical tea plantations

As expected, annual soil N-oxide emissions from the present tea plantation (Table 1) were remarkably greater than those from the cereal cropping system (Wang et al., 2011) and comparable to those from intensive vegetable cropping systems in this region (Geng et al., 2021; Wang et al., 2015). On average, annual soil N₂O emissions (17.1 kg N ha⁻¹) and background emissions (2.8 kg N ha⁻¹) of our tea plantation fell within the corresponding range of 13.1–21.3 and 1.4–5.2 kg N ha⁻¹ from global tea plantations (Wang et al., 2020). Although the observed EFs of soil N₂O were similar to the global mean EF of 2.31% (95% CI: 1.91%–2.71%), it was distinctly lower than the IPCC default value of 1.0% (95% CI: 0.1%–1.8%; Hergoualc’h et al., 2019). Similarly, we found that annual NO emissions were greater than the mean value of 4.06 kg N ha⁻¹ across global terrestrial ecosystems (Liu et al., 2017). Owing to the corresponding large background emissions, the EFs of NO emission were comparable with the global mean value of 1.16%. Therefore, the EF of N₂O+NO emissions from our tea plantation falls within the upper end of global estimates (1.81%–3.35%; Liu et al., 2017).

In China, tea was planted on 2310.8 Kha in 2018, equivalent to c. 1.6% of the total cropland area (NBSC, 2019). Based on the latest national questionnaire survey, the recommended N application rate for tea plantations across China is 450 kg N year⁻¹ (Ni et al., 2019). Using the averaged EFs and background emissions in this study (Table 1), we estimated total N₂O and NO emissions from national tea plantations are approximately 36.7 and 13.7 Gg N year⁻¹, respectively, following the IPCC’s default Tier 1 approach. These estimates...
account for 6%–17% of the national total direct N₂O emissions (range: 211–620 Gg N year⁻¹; Zhou et al., 2014; Zou et al., 2010) and 2–6% of total NO emissions from national terrestrial ecosystems (range: 243–657 Gg N year⁻¹; Wang et al., 2005; Yan et al., 2003). Consequently, our results suggest that tea plantations in China are major contributors to soil N-oxide emissions in the agricultural sector, highlighting the necessity for priority emission reductions.

4.4 Mitigation options for soil N-oxide emissions from subtropical tea plantations

This study had two key implications for climate-smart tea plantations in a subtropical climate. First, full organic substitution for synthetic fertilizer can trigger substantial N₂O emissions from subtropical tea plantations, suggesting that caution is needed in substituting organic fertilizer as an optimal fertilization measure on a large scale. Our field study and global meta-analysis suggested that full organic substitution, especially organic fertilizers with a lower C/N ratio (c. 15), may not be suitable as alternative fertilization methods. Considering the strongly increased NO emissions following synthetic N fertilization, partial organic substitution combined with chemical nitrification inhibitors could be an option to simultaneously reduce N₂O and NO emissions (Figure 4a; Akiyama et al., 2010). Second, wheat straw-derived biochar can contribute to N₂O mitigation from our acidic tea plantation, but such an effect is transient. Although the findings of our study and previous meta-analysis of field studies suggest the presence of biochar-induced reduction in N₂O emissions, there is little knowledge about the long-term impact of single biochar application on soil N₂O emissions (Borchard et al., 2019; Verhoeven et al., 2017). This knowledge gap may have contributed to taking no account of the impact of biochar addition on soil N₂O emissions when evaluating its role as negative emission techniques in curbing climate change (Miller-Robbie et al., 2015; Smith, 2016). Nevertheless, there is evidence that frequent biochar applications can maintain their suppression effect on N₂O emissions from acidic soils (Ji et al., 2020). Notably, further research is needed to assess the feasibility of the practical and/or economic aspects regarding frequent biochar applications.

In summary, our 2-year field experiment indicated that full organic substitution for synthetic N fertilizer increased N₂O but lowered NO emissions as compared to equal inorganic N fertilization. The global meta-analysis further supported this finding and suggested that the decomposability of organic fertilizers is crucial in determining whether full organic substitution for synthetic fertilizer would stimulate N₂O emissions from acidic soils. The increased N₂O emissions were mainly attributed to the stimulated activity of nitrifiers and denitrifiers following organic fertilizer application. Irrespective of fertilizer type, biochar amendment had no significant effects on NO emissions but reduced soil N₂O emissions, while such suppression effect disappeared in the second experimental year. Taken together, acidic soils of tea plantations in a subtropical climate are hotspots of soil N-oxide emissions and that adopting the replacement of synthetic fertilizer by manure or biochar amendment as a potential way to reduce chemical N input and combat climate change should be taken with caution.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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