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Regulation of the product stoichiometry of denitrification in intensively managed soils

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Abstract

Crop residue amendment in conjunction with synthetic nitrogen (N) fertilization is a common agricultural practice that increases soil fertility and crop yield. However, such a practice may also change soil denitrification process. Here, we conducted an incubation experiment with a robotized continuous flow N_2 free incubation system [using helium (He) and oxygen (O_2)] and measured fluxes of N_2O and N_2 at a high resolution in an intensively managed soil to examine the interaction effect of straw amendment and N fertilization on soil denitrification. Four treatments were set consisting of (a) a nonamended treatment (Control); (b) a Straw treatment (2 g straw kg^{-1} dry soil); (c) a KNO_3 treatment (KNO_3, 15 mM KNO_3); and (d) a Straw + KNO_3 treatment (2 g straw kg^{-1} dry soil and 15 mM KNO_3). During the oxic phase (80% He plus 20% O_2) of the experiment (initial 2 days), flux rates of N_2O were 0.54 and 0.38 kg N/ha day^{-1} in the Control and KNO_3 treatments, respectively. Meanwhile, straw amendment triggered N_2O fluxes immediately after the onset of treatments, which was more evident in the Straw + KNO_3 treatment. During the anoxic phase (100% He), both N_2O and N_2 emissions increased in all treatments, with the effect being more pronounced in the Straw and Straw + KNO_3 treatments. In line with the observed differences in gas fluxes, the abundances of *nirK*, *nirS*, and *nosZ* genes increased clearly in these treatments. Overall, the mean $N_2O/(N_2O + N_2)$ ratio (0.69 ± 0.03) in the Control treatment was significantly lower compared to the KNO_3 treatment (18.8%) and higher than the straw-amended soils (31.9% and 17.4% compared to the Straw and Straw + KNO_3 treatments, respectively). Taken together, our results suggest straw amendment significantly altered N_2O and N_2 fluxes by decreasing the $N_2O/(N_2O + N_2)$ ratio; however, the effects of straw amendment depended on soil nitrate content.

KEYWORDS

crop residue, denitrification, dinitrogen (N_2), nitrous oxide (N_2O), sustainable management

1 | INTRODUCTION

Nitrous oxide (N_2O), a powerful anthropogenic greenhouse gas and ozone-destructing substance, has received great attention because of its substantial concentration increase in the atmosphere since preindustrial times (IPCC, 2007; Ravishankara et al., 2009). Agricultural soils are one of the major sources of N_2O emissions, due to the associated intensive management practices including fertilization, irrigation, and residue incorporation (IPCC, 2007; Mosier et al., 1998; Tilman et al., 2002). The production of N_2O in soil is intimately related with nitrification and denitrification, where denitrification is the dominant biological process responsible for the increase in atmospheric N_2O (Baggs, 2008; Senbayram et al., 2009). For natural terrestrial ecosystems, denitrification plays a benign role in removing the accumulative nitrate in soil by the sequential reduction of NO_3^-, to NO_2^-, NO, N_2O, and finally to elemental N_2 (Galloway et al., 2004; Petersen et al., 2013; Seitzinger et al., 2006). But for agricultural soils, denitrification may also cause many economic and environmental problems via the enormous loss of nitrogen (N) fertilizer in various forms (Ju et al., 2009).

Straw incorporation is a widespread agricultural practice to improve the soil fertility; for instance, the straw application rate in the Taihu Lake Region in China increased from 0.1 Mg/ha per year in 1955 to 9 Mg/ha per year in 2004 (Wang et al., 2008). Proper management and utilization of straw play a pivotal role in maintaining and improving soil organic matter content and achieving high crop production (Bijay et al., 2008). Amending agricultural soils with straw provides labile carbon (C) and N, as well as several other nutrients, which stimulate microbial growth and activity (Tu et al., 2006). Therefore, the application of straw may trigger denitrification and the related nitrogenous trace gas emissions (Meijide et al., 2007; Senbayram et al., 2012). However, contradictory results have been reported showing positive or negative effects of straw incorporation on N_2O emissions (Baggs et al., 2000; Köbke et al., 2018; Pan et al., 2017; Xiao et al., 2018; Zou et al., 2005).Understanding to what extent straw incorporation affects denitrification and its product ratio may help us to estimate the environmental benefits of straw management and develop specific management practices to mitigate the associated greenhouse gas emissions.

Soil labile C content affects denitrification by supplying easily decomposable C to denitrifying micro-organisms and lowering the oxygen level at microsites where decomposition occurs (Lan et al., 2015). It is well accepted that high nitrate concentrations in soil inhibit N_2O reduction to N_2 (Blackmer & Bremner, 1978; Firestone et al., 1979; Qin et al., 2017; Senbayram et al., 2012; Wu, et al., 2018). Furthermore, Senbayram et al. (2019) found that high NO_3^- concentrations in soil (>44.9 mg N/kg soil) can almost completely inhibit N_2O reduction to N_2 (>90%), regardless of the soil pH. The labile C and NO_3^- contents are considered as "proximal factors" interactively affecting both the rate and the product stoichiometry of denitrification (Hu et al., 2016). Besides, nitrate reduction processes are closely related to soil microbial communities (Jones et al., 2014; Ma et al., 2017; Shan et al., 2016). Many studies have tried to explore the relationship between denitrifier community and N_2O emissions (Giles et al., 2012; Ruser & Schulz, 2015); however, few studies have examined the denitrifier community and their relation to N_2 emissions.

To meet the high agricultural production needs, vegetable cultivation in China, especially in the Yangtze River Delta Region (YRDR), often involves treatments that use excessive amounts of N fertilizer (Wang et al., 2008; Xiong et al., 2006; Zhu et al., 2011). Large inputs of N fertilizers in the YRDR have caused a series of eco-environment problems, which, however, do not cause an excess N load in soil because of the high rate of reactive N loss during heavy rain or irrigation period (Zhou et al., 2016). Although many studies have reported significant N loss by leaching, runoff, and gaseous N (NH_3, NO, and N_2O) in the YRDR (Min et al., 2011; Shi et al., 2009; Wang et al., 2011), only a few studies have been able to make a reliable mass balance calculation therein. One key reason for this might be that the total denitrification rate and gaseous N_2 loss in soil are very difficult to measure due to the high concentration of atmospheric N_2 and a lack of sensitive techniques. To quantify soil denitrification, a number of different approaches have been applied to indirectly determine the fluxes of soil-born N_2 including the acetylene inhibition technique (AIT) (Miller et al., 2008; Yuan et al., 2019) and ^{15}N isotope labeling method (Lewicka-Szczebak et al., 2017). However, both the aforementioned methods suffer from a series of disadvantages. The AIT method significantly underestimates denitrification rate as C_2H_2 inhibits nitrification process (Groffman et al., 2006), and NO is catalytically decomposed in presence of acetylene and oxygen (Bollmann & Conrad, 1997; Nadeem et al., 2013). With the ^{15}N isotope labeling method, the added ^{15}N-NO_3^- homogeneous mixing with the soil NO_3^- pool is difficult (Matheson et al., 2002), and nonhomogeneity of labeling can lead to underestimation of N_2 fluxes (Well et al., 2019). Recently, a number of air-tight soil incubation systems have been established to determine N_2 fluxes directly, based on replacing the headspace of the vessel's atmosphere with helium (He) (Cárdenas et al., 2003; Groffman et al., 2006; Köster et al., 2013; Molstad et al., 2007; Scholefield et al., 1997; Senbayram et al., 2018). Compared with the AIT and ^{15}N isotope labeling methods, the advantage of these He-flow systems is directly measuring soil-borne N_2 emissions with high resolution.

In this study, the effects of straw and N fertilizer (NO_3^--based) amendment on soil denitrification and the associated $N_2O/(N_2O + N_2)$ ratio in an intensively managed soil

of the YRDR were investigated. Specifically, incubation experiments were performed using a robotized continuous flow (RoFlow) system which enabled us to directly measure soil N_2O, N_2, and CO_2 fluxes at high temporal resolution. Furthermore, the abundances of denitrification-related functional genes (i.e., genes encoding *narG* of nitrate reductase, *nirK* of cytochrome cd1 nitrite reductase, *nirS* of copper-containing nitrite reductase, and *nosZ* of nitrous oxide reductase) were also studied aiming to understand the effects of straw and nitrate amendment at the molecular level.

2 | MATERIALS AND METHODS

2.1 | Site description and soil sampling

The soil was sampled from an intensively managed (plastic shed) vegetable field on 20 March 2018 nearby Changshu Agroecological Experimental Station of the Chinese Academy of Sciences, Jiangsu Province, China (31°33'16''N, 120°43'17"E). This site is located in a typical region of the subtropical monsoon, with an average rainfall of 990 mm in which about 60%–70% occurs between June and September. The annual average temperature is about 16.1°C. The plastic shed vegetable field was converted from a wheat–rice field 12 years ago and has received around 860 kg N/ha and 26.4 t/ha organic matter (straw and chicken manure) per year. The experimental soil was a typical Wushan Soil (Hydragric Anthrosols, based on the FAO World Reference Base, 2013) developed from lacustrine sediments of Taihu Lake. The soil contained 2.20 g N/kg as total N and 18.5 g C kg^{-1} as total C, with a pH (0.01M $CaCl_2$) value of 5.13. The original NH_4^+-N, NO_3^--N, and soil organic carbon (SOC) concentrations were 6.4, 93.4 mg N/kg, and 17.4 g C kg^{-1} dry soil, respectively. The study soil consisted of 27.2% sand (20–2,000 μm), 38.5% silt (2–20 μm), and 34.2% clay (<2 μm). Besides, the soil moisture in the field condition was extremely high due to the frequent irrigation, creating a wet and fully saturated situation favoring denitrification. The upper 2 cm of the soil and roots were removed, and soil was collected from the first 20 cm below the removed layer. The samples were air-dried to around 20% water filled pore space (WFPS), then sieved through a 2 mm mesh and stored at 4°C shortly before the soil incubation experiment.

2.2 | Automated soil incubation experiment

The incubation experiment was carried out in a RoFlow incubation system with 16 resinic vessels (Figure 1; Senbayram et al., 2018). The vessels were cylindrical, made from acrylic glass, with an inner diameter of 14 cm and a height of 15 cm and equipped with a polyamide filter membrane (EcoTech; pore size: 0.45 μm) at the bottom. Prior to incubation, the soil was pre-incubated for 5 days at around 45% WFPS to stabilize the microbial activity and eliminate the effect of drying soil. The orthogonal experimental design consisted of four treatments ($n = 3$): (a) a nonamended treatment (Control); (b) a Straw treatment; (c) a KNO_3 treatment; and (d) a Straw + KNO_3 treatment. Oven-dried rice straw [ground through a 2 mm mesh sieve (0.7% total N, 45.4% total C)] was mixed using a vertical mixer into the pre-incubated soils in the Straw and Straw + KNO_3 treatments at a rate of 2 g straw kg^{-1} dry soil. After mixing, 1 kg soil was packed into each vessel (with a density of 1.25 g/cm^3 dry weight). The incubation vessels were then sealed, and N fertilizer was applied in the form of KNO_3. The main aim of the study was to examine the denitrification rate and

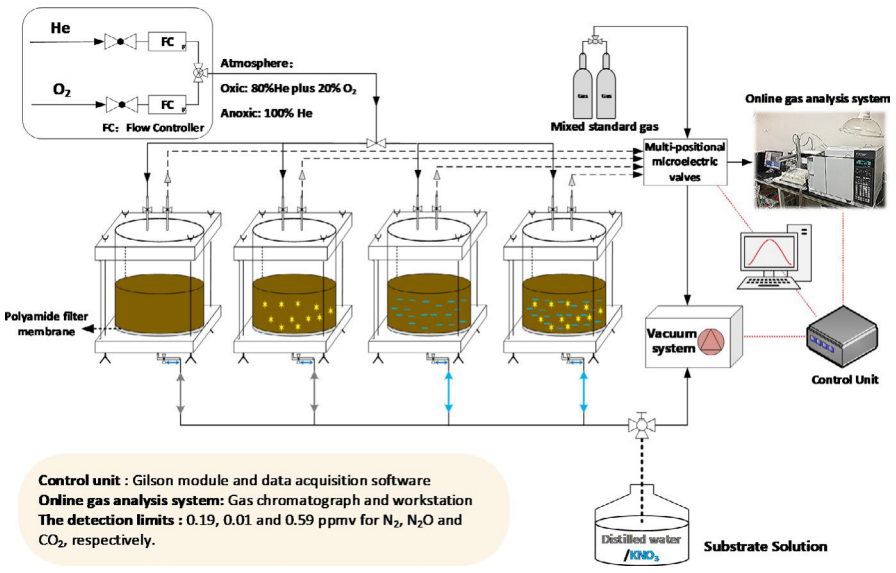

FIGURE 1 Schematic diagram of the robotized continuous flow (RoFlow) incubation system used in our experiment. The RoFlow system consists of 16 airproof cylindrical acrylic glass vessels, which is controlled by a Gilson module-based microcontroller unit equipped with two multi-positional micro-electric valves. By adjusting the position of micro-electric valves, the control unit gives the signals to the GC (online analysis) and computer (data acquisition)

its product stoichiometry in this intensively managed soil where nitrate was the predominant inorganic N form, and thus, KNO_3 was used as the N source to avoid the significant contribution of other N_2O emitting processes during the experiment. The amounts of straw and KNO_3 applied in these treatments can represent the average level of SOC and nitrate content in the soil after fertilization during each vegetable planting season. For that, soils were flooded with 15 mM KNO_3 solution (in the KNO_3 and Straw + KNO_3 treatments) or distilled water (in the Control and Straw treatments) and drained to ~80% WFPS (determined according to the field condition) by applying a vacuum from the bottom of the filter membrane. A flooding and draining procedure was used to achieve a homogenous nitrate concentration and keep a well-drained condition for all soils. The initial and final soil mineral N contents were shown in Table 1. The headspace of each vessel was continuously flushed with mixed air (80% He mixed with 20% O_2, ~25 ml air min^{-1}) during the oxic phase (0–2 days) and flushed with only He during the anoxic phase (3–26 days). The room temperature was set to 21°C throughout the whole incubation experiment. The main purpose of setting incubation conditions to aerobic headspace for 2 days was to measure N_2O and N_2 emission in steady-state conditions (typical wet conditions in vegetable fields after irrigation). Furthermore, we set up a relatively longer anoxic phase (24 days) to study complete denitrification potential and denitrification product stoichiometry of such intensively managed soil.

2.3 | Soil chemical analysis

At the end of incubation, fresh soil samples were extracted with 2M KCl solution under a soil/solution ratio of 1:5. The KCl extracts were then filtered through Whatman 602 filter paper and stored at −20°C until analysis. The concentrations of NH_4^+ and NO_3^- were determined with a continuous-flow analyzer (Smartchem 200S/N1104238, WESTCO, France). The soil pH was measured in 0.01 M $CaCl_2$ solution, at a soil/solution ratio of 1:5, using a pH meter (Sartorius).

2.4 | Gas kinetics measurements

The headspace concentrations of N_2O, N_2, and CO_2 were analyzed online by directing the outlet flow of each incubation vessel sequentially to a gas chromatograph via two multipositional valves (16 port micro actuator VICI valve, USA) controlled by Trilution software (Gilson, Inc.) and an interface module (508 Interface Module, Gilson, Inc.) (Figure 1). The gas chromatograph (Agilent 7890B) was equipped with an electron capture detector (for measuring concentrations of N_2O) and thermal conductivity detector (for measuring CO_2 and N_2 concentrations). The detection limits for N_2, N_2O, and CO_2 of the gas chromatograph were 0.19, 0.01, and 0.59 ppmv, respectively. Besides, to quantify the leakage of the system, an incubation experiment with empty vessels was conducted before the formal experiment, and one empty vessel was always measured during each experiment to check the N_2 background noise. The average leakage rate of the RoFlow system was about 0.07 ppmv N_2 hr^{-1}, and the N_2 background concentration in the incubation experiment was around 15 ppmv. The gas emissions from the outlet of each vessel were measured approximately every 3.6 hr, and the outlet flux rate for each incubation pot was measured manually every day with a portable gas flux meter (GFM Pro Gas Flowmeter, Thermo Fisher Scientific). Standard gases (N_2O, N_2, and CO_2) were measured at the end of each gas-measurement run for calibration.

2.5 | Quantification of denitrification functional genes copies

At the end of incubation (time point: 26 days), total DNA was extracted from the incubated soils and used for analyzing the abundances of denitrification-related functional genes. Briefly, soil DNA was extracted from 0.5 g freeze-dried soil subsamples with a Fast DNA SPIN kit for soil (MP biomedical), according to the manufacturer's instructions. The concentration of DNA was examined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). For quantification of the four functional gene (*narG*, *nirK*, *nirS*, and *nosZ*) copy numbers, a

TABLE 1 Soil NO_3^- and NH_4^+ concentrations at the end of the experiment in the nonamended treatment (Control), Straw, KNO_3 and Straw + KNO_3 treatments. Data are expressed as mean ± standard error ($n = 3$). Means denoted by a different letter in the same column differ significantly according to Tukey's HSD post hoc test at $\alpha = 0.05$

	Initial		Final	
Parameter	NH_4^+ mg N/kg dry soil	NO_3^- mg N/kg dry soil	NH_4^+ mg N/kg dry soil	NO_3^- mg N/kg dry soil
Control	6.4 ± 0.30[a]	93.4 ± 4.56[b]	17.6 ± 1.35[a]	3.9 ± 2.33[b]
Straw	6.4 ± 0.30[a]	93.4 ± 4.56[b]	20.7 ± 0.10[a]	0.9 ± 0.12[b]
KNO_3	6.4 ± 0.30[a]	159.8 ± 2.58[a]	11.9 ± 6.53[a]	44.5 ± 3.74[a]
Straw + KNO_3	6.4 ± 0.30[a]	159.8 ± 2.58[a]	12.3 ± 1.46[a]	0.2 ± 0.12[b]

CFX96™ Real-Time System (Bio-Rad) was used for quantitative PCR (*q*PCR). The primers and cycling conditions for *q*PCR are described in Chen et al. (2015). Standard curves were obtained with serial plasmid dilutions of a known amount of plasmid DNA containing a fragment of the *narG*, *nirK*, *nirK*, or *nosZ* gene (Chen et al., 2015) and then were applied in triplicate to each *q*PCR. The 25 μL reaction system contained 12.5 μL SYBR Premix Ex Taq (Takara, Japan), 0.2 mM of each primer and distilled water, and 5 μL DNA templates (1–10 ng). Each sample was analyzed in triplicate. For all functional genes, amplification efficiencies were 92%–99%, and the R^2 value for the standard curves was .995–.999. Appropriate negative controls containing no template DNA gave null values. To check the specificities of the amplification products, melting curve analysis was performed at the end of each real-time PCR run.

2.6 | Data processing and statistical analyses

The emission rates of N_2O, N_2, and CO_2 were calculated using the following equation:

$$F = \frac{C \times \text{flow rate} \times 60 \times 24}{A \times 10^6} \times \frac{M \times 273}{M_V \times (273 + T)}$$

where F is the N_2O, N_2, or CO_2 emission in units of g N or C ha^{-1} day^{-1}; C is the N_2O, N_2, or CO_2 gas concentration (for N_2 only, C was recalculated by the background noise and leakage rate of the system) (ppmv); "flow rate" is the flush gas flow rate (ml/min); 60 and 24 are the time conversions, which are minutes to hours, and hours to days, respectively; A is the surface area of the soil core (ha); 10^6 is the volume conversion, ppm to ml/ml; M is the weight of pure N or C molar mass of N_2, N_2O, or CO_2 (28, 28, and 12 g/mol, respectively); M_V is the molar volume of the gas at 273 K and 101.325 KPa (L/mol); and T is the average temperature in the incubation vessels (°C).

The cumulative gas emissions were calculated by linear interpolation based on the daily measured fluxes. Gas emission rates are expressed as the arithmetic mean of the three replicates. The differences in cumulative N_2O, N_2, and CO_2 emissions, the $N_2O/(N_2O + N_2)$ ratio, soil mineral N content, and the abundances of functional genes, were examined using a one-way analysis of variance (ANOVA), and the mean comparison was evaluated by Tukey's HSD post hoc test in the software IBM SPSS Statistics 21.0 (significance level was set at $p < .05$).

3 | RESULTS

3.1 | Soil mineral N

Final soil NH_4^+ concentrations varied from 11.9 ± 6.53 to 20.7 ± 0.10 mg/kg, which were slightly higher than the initial concentrations and with no significant difference among different treatments (Table 1). In the straw-amended treatments, final soil NO_3^- concentrations were below 1 mg NO_3^--N/kg dry soil, whereas in the Control and KNO_3 treatments the concentrations were 3.9 and 44.5 mg NO_3^--N/kg dry soil, respectively. At the end of the incubation, the order of soil NO_3^- concentrations followed the trend: KNO_3 > Control > Straw = Straw + KNO_3 (Table 1). Overall, the depletion of soil NO_3^- was 99.8%, 99.1%, 72.2%, and 95.8% in the Straw + KNO_3, Straw, KNO_3, and Control treatments, respectively.

3.2 | Gas emission kinetics

During the oxic phase, flux rates of N_2O increased slightly over time in the Control and KNO_3 treatments, whereas straw amendment caused a drastic increase in N_2O emissions in the Straw and Straw + KNO_3 treatments (Figure 2). The maximum N_2O peak was 4.07 kg N_2O-N/ha day^{-1} in the Straw + KNO_3 treatment, being 29% higher than that in the Straw treatment (Figure 2a–d). Switching conditions from oxic to anoxic increased N_2O fluxes sharply in all treatments. In the Control and KNO_3 treatments, N_2O fluxes continued to increase until day 12 and decreased gradually to the background levels toward the end of the experiment. However, in the straw-amended treatments, the highest N_2O peak was observed at day 3 and day 5 in the Straw and Straw + KNO_3 treatments, respectively, which then decreased to below the detection limit (> 4.35 g N_2O-N/ha day^{-1}) at day 8 and 18. Cumulative emissions of N_2O during the oxic phase were significantly ($p < .05$) higher in straw-amended soils than in the Control and KNO_3 treatments (Table 2). During the anoxic phase, however, cumulative N_2O emissions were the lowest in the Straw treatment and the highest in the Straw + KNO_3 treatment. Overall, total cumulative N_2O emissions during the 26-day incubation were 29.9 ± 3.09, 16.8 ± 1.17, 35.0 ± 1.67, and 55.8 ± 2.01 kg N_2O-N/ha in the Control, Straw, KNO_3, and Straw + KNO_3 treatments, respectively (Figure 4a). Compared with the Control treatment, total cumulative N_2O emissions decreased by 43.8% in the Straw treatment, while the cumulative N_2O emissions increased by 59.4% in the Straw + KNO_3 as compared to those in the KNO_3 treatment.

The fluxes of N_2 during the oxic phase were low in all treatments, with a slight increase over time (Figure 2a–d). Emissions of N_2 increased significantly ($p < .05$) when conditions were switched to anoxic in all treatments, with the effect being more pronounced in the Straw treatment. The main N_2 peak occurred at day 4 and day 18 in the Straw and Straw + KNO_3 treatments, respectively. The maximum N_2 peak was 9.29 kg N_2-N/ha day^{-1} in the Straw + KNO_3 treatment, being 4.5 times higher than that in the Straw treatment

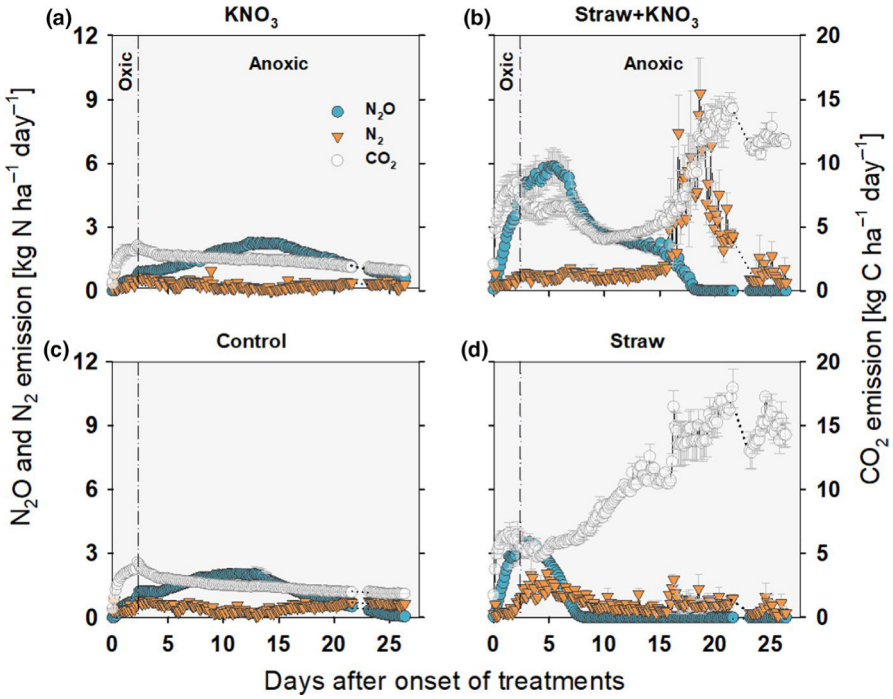

FIGURE 2 Daily emissions of N_2O, N_2, and CO_2 during the incubation period (26 days) in the nonamended treatment (Control, c), Straw (d), KNO_3 (a), and Straw + KNO_3 (b) treatments. Error bars show the standard error of each treatment ($n = 3$)

TABLE 2 Cumulative emissions of N_2O, N_2, CO_2, and the ratio of $N_2O/(N_2O + N_2)$ in oxic Phase I (0–2 days), and anoxic Phase II (3–26 days) in the nonamended treatment (Control), Straw, KNO_3, and Straw + KNO_3 treatments. Data are expressed as mean ± standard error ($n = 3$). Means denoted by a different letter in the same column differ significantly according to Tukey's HSD post hoc test at $\alpha = 0.05$

Treatment	N_2O kg N/ha	N_2 kg N/ha	$N_2O + N_2$ kg N/ha	$N_2O/(N_2O + N_2)$	CO_2 kg C ha^{-1}
	Oxic (0–2 days)				
Control	1.2 ± 0.08^{b}	0.9 ± 0.06^{a}	2.1 ± 0.11^{b}	0.55 ± 0.02^{b}	7.0 ± 0.25^{b}
Straw	4.4 ± 0.60^{a}	0.7 ± 0.07^{a}	5.1 ± 0.60^{a}	0.86 ± 0.02^{a}	13.0 ± 1.45^{ab}
KNO_3	0.8 ± 0.07^{b}	0.7 ± 0.13^{a}	1.5 ± 0.20^{b}	0.52 ± 0.03^{b}	6.1 ± 1.07^{b}
Straw + KNO_3	5.1 ± 0.47^{a}	0.9 ± 0.10^{a}	6.0 ± 0.58^{a}	0.86 ± 0.01^{a}	15.9 ± 3.14^{a}
	Anoxic (3–26 days)				
Control	28.2 ± 3.01^{b}	12.5 ± 0.72^{bc}	41.2 ± 3.02^{b}	0.69 ± 0.03^{b}	56.8 ± 2.54^{c}
Straw	12.1 ± 0.62^{c}	18.4 ± 1.87^{b}	30.8 ± 2.49^{b}	0.40 ± 0.01^{d}	283.7 ± 23.58^{a}
KNO_3	34.2 ± 1.68^{b}	7.0 ± 1.05^{c}	41.1 ± 1.58^{b}	0.83 ± 0.03^{a}	55.2 ± 1.14^{c}
Straw + KNO_3	50.7 ± 1.55^{a}	40.7 ± 1.58^{a}	91.4 ± 2.42^{a}	0.56 ± 0.01^{c}	182.5 ± 3.49^{b}

(Figure 2). In the KNO_3 treatment, N_2 emissions remained constantly low throughout the experiment, whereas in the Control treatment, they increased gradually over time until the end. Cumulative emissions of N_2 during the oxic phase were below 1 kg N_2-N/ha in all treatments, with no significant difference among treatments (Table 2). However, during the anoxic phase, cumulative N_2 emissions in the Straw + KNO_3 treatment were around two and five times higher than those in the Straw and KNO_3 treatments, respectively. Overall, cumulative N_2 fluxes in the 26-day incubation were 13.5 ± 0.79, 19.2 ± 1.89, 7.7 ± 1.19, and 41.5 ± 1.53 kg N_2-N/ha in the Control, Straw, KNO_3, and Straw + KNO_3 treatments, respectively (Figure 4a). In comparison with the Control treatment, cumulative N_2 fluxes increased by 42.2% and 207.4% in the Straw and Straw + KNO_3 treatments, respectively, but decreased by 43.0% in the KNO_3 treatment (Figure 4a).

The daily $N_2O/(N_2O + N_2)$ ratio during the oxic phase of the experiment was higher than 0.5 in all treatments, being the lowest in the KNO_3 and the highest in the Straw + KNO_3 treatment. During the anoxic period, the ratio decreased rapidly in straw-amended soils, with the effect being more significant in the Straw treatment than that in the Straw + KNO_3 treatment. However, in the Control and KNO_3 treatments, this ratio increased over time until day 13 and decreased gradually, almost to zero and to 0.7, respectively (Figure 3). Overall, the total ratios of $N_2O/(N_2O + N_2)$ during the 26-day incubation were 0.69 ± 0.03, 0.47 ± 0.01, 0.82 ± 0.03, and 0.57 ± 0.01 in the Control,

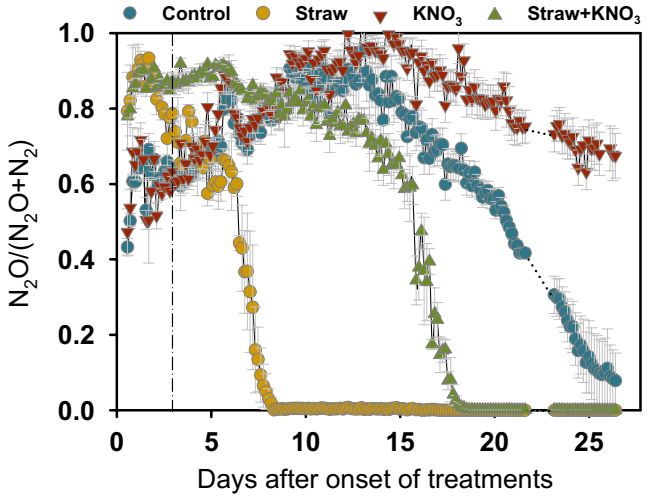

FIGURE 3 Dynamics of the ratio of $N_2O/(N_2O + N_2)$ during the incubation period (26 days) in the nonamended treatment (Control), Straw, KNO_3, and Straw + KNO_3 treatments. Error bars show the standard error of each treatment ($n = 3$)

Straw, KNO_3, and Straw + KNO_3 treatments, respectively (Figure 4a). The total ratios of $N_2O/(N_2O + N_2)$ decreased by 31.9% in the Straw treatment as compared to those in the Control treatment, while these ratios decreased by 30.5% in the Straw + KNO_3 treatment relative to those in the KNO_3 treatment.

During the oxic phase, CO_2 fluxes increased significantly over time in all treatments, with maximum rates in the straw-amended soils. In the Straw and Straw + KNO_3 treatments, CO_2 daily fluxes continued to increase in straw-amended soils, also during the anoxic period, whereas CO_2 fluxes decreased gradually in the Control and KNO_3 treatments. Total cumulative gaseous carbon emissions during the whole experiment period were 63.4 ± 2.35, 296.7 ± 24.88, 61.4 ± 0.84, and 198.4 ± 3.81 kg CO_2-C ha^{-1} in the Control, Straw, KNO_3, and Straw + KNO_3 treatments, respectively (Figure 4b).

3.3 | Denitrification-related gene abundance

There was a clear increase in the abundances of *nirK*, *nirS*, and *nosZ* genes in the Straw and Straw + KNO_3 treatments as compared to those in the Control and KNO_3 treatments. Especially, *nosZ* gene abundances in the Straw and Straw + KNO_3 treatments were around two- and threefold higher ($p < .05$) than those in the Control treatment, respectively (Figure 5). Likewise, in comparison with the Control treatment, the *nirK* gene abundance was 71.5% and 90.0% higher, and the *nirS* gene abundance increased by 68.5% and 43.0% in the Straw and Straw + KNO_3 treatments, respectively. However, compared to the Control treatment, the *nirK* and *nirS* gene abundances in the KNO_3 treatment were

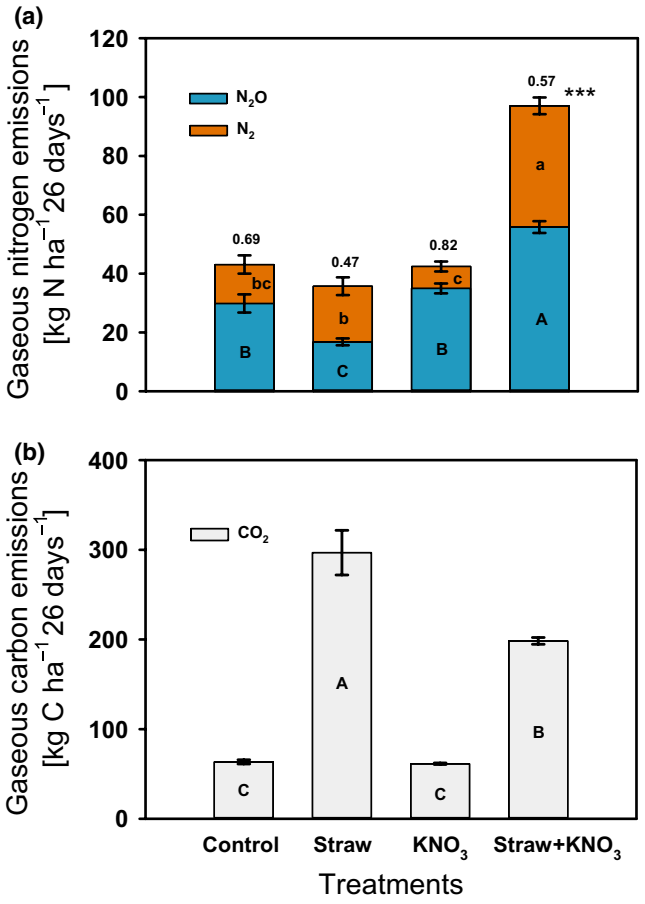

FIGURE 4 Soil cumulative N_2O and N_2 (a) and CO_2 (B) flux during incubation (26 days) in the nonamended treatment (Control), Straw, KNO_3, and Straw + KNO_3 treatments. The figures above the bar (A) showed the ratio of $N_2O/(N_2O + N_2)$ during the whole incubation period (0–26 days). Error bars show the standard error of each treatment ($n = 3$). Means denoted by a different letter (the labels "A, B" and "a, b" inside the bars (A) indicate significant differences within cumulative N_2O and N_2 emissions) differ significantly according to Tukey's HSD post hoc test at $\alpha = 0.05$ (***$p < .001$)

reduced by 25.9% and 13.3%, respectively. Gene abundances of *narG* increased significantly in the Straw treatment, but decreased in the nitrate-addition treatments (KNO_3 and Straw + KNO_3 treatments).

4 | DISCUSSION

4.1 | Soil respiration

Straw amendment stimulated microbial activity and significantly ($p < .05$) increased CO_2 emissions, regardless of nitrate supply (Table 2; Figure 2). Assuming that the native soil organic matter mineralization rate was unaffected by the straw amendment (i.e., no priming effect), cumulative CO_2 emissions can be used to estimate the mineralized fraction of the added rice straw C during the incubation period. The

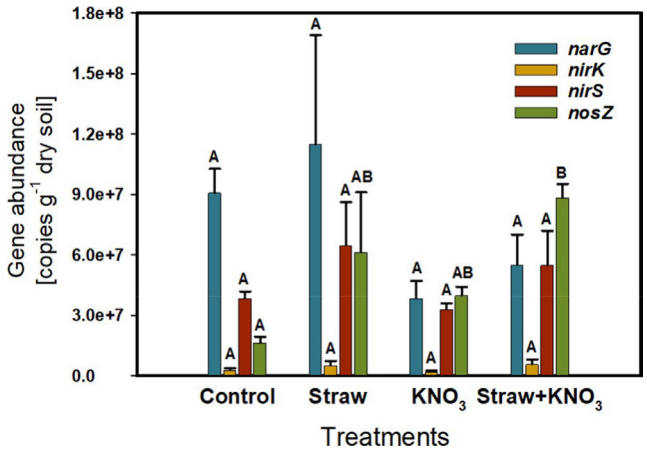

FIGURE 5 Denitrification-related functional gene (*narG*, *nirK*, *nirS,* and *nosZ*) abundances in the nonamended treatment (Control), Straw, KNO_3, and Straw + KNO_3 treatments at the end of incubation. Error bars show the standard error of each treatment ($n = 3$). Means denoted by a different letter differ significantly according to Tukey's HSD post hoc test at $\alpha = 0.05$

calculated share of amended rice straw C throughout the experiment was 40% and 23% in the Straw and Straw + KNO_3 treatments, respectively. In moist soils, electron donors (e.g., labile C) are considered to be the limiting factor for denitrification, directly controlling the potential denitrification rate (Bouwman et al., 2013; Senbayram et al., 2009). This is because denitrifying micro-organisms use labile C as an electron donor for all of the reduction steps from NO_3^- to N_2 (Butterbach-Bahl et al., 2013; Morley & Baggs, 2010). Therefore, we typically expect that the labile C released from straw decomposition triggers denitrification rate under moist situations.

4.2 | Interaction effect of straw and nitrate amendments on the denitrification rate

In our study, the difference of soil nitrate depletion and cumulative nitrogenous gas emissions between straw-amended and nonstraw-amended treatments (Tables 1 and 2) clearly showed that additional labile C supply in the straw-amended treatments caused an increase in denitrification rate. Interestingly, the availability of organic matter amendment was still the limiting factor for denitrification in the intensively managed (i.e., intensive input of organic fertilizer) soils even though organic matter was therein. This is likely due to the fact that labile C pools of organic matter can be rapidly mineralized when conditions facilitate (moist conditions and higher soil temperature, as in this intensively managed production system), and the organic matter becomes more recalcitrant over time, limiting the size of the electron donor pool (Bi et al., 2013; Hu et al., 2016; Sánchez-García et al., 2016; Wright & Snyder, 2009).

At the end of the incubation, N budgets in the different treatments were calculated showing that the $N_2O + N_2$ emissions accounted for 72.1%, 49.9%, 43.6%, and 93.8% of the total nitrate-N loss in the Control, Straw, KNO_3, and Straw + KNO_3 treatments throughout the experiment, respectively. This demonstrated that other nitrate-reducing processes [i.e., dissimilatory nitrate reduction to ammonium (DNRA) and anaerobic ammonium oxidation (anammox)] and N immobilization may occur and contribute to the mineral N loss in our study. Besides, final soil ammonium tended to increase compared to the original concentrations, which may be attributed to the occurrence of DNRA process under relatively high organic C/nitrate ratio (nitrate was gradually depleted by denitrifiers) and anaerobic conditions (Friedl et al., 2018; Kraft et al., 2014), as well as the mineralization of organic N.

4.3 | Effect of straw and nitrate amendments on N_2O and N_2 emissions

Straw amendment caused a drastic increase in N_2O emissions during the oxic phase of the experiment, whereas the application of nitrate alone showed almost no effect (Figure 2). This is particularly because the initial soil NO_3^- content was not a limiting factor for N_2O production, even in the Control and Straw treatments (with an initial NO_3^- content of 94.4 ± 4.56 mg NO_3^--N/kg dry soil), due to the high residual NO_3^-. Generally, N application rates in plastic shed vegetable soils are significantly greater than in all other land-use systems. Therefore, the present study suggests that inputs of organic matter in intensively managed soils with high residual NO_3^- content (as in this specific managed soil, or NO_3^--based N-fertilized soils) may trigger N_2O fluxes produced by nitrification or denitrification, which is in good agreement with a number of other studies (Hu et al., 2016; Köbke et al., 2018; Pfab et al., 2011; Zhang et al., 2019).

When the experimental condition switched to anaerobic, fluxes of N_2O increased in all treatments, with the effect being more evident in the Straw + KNO_3 treatment (Figure 2). With the increase of incubation time, however, N_2O emissions decreased gradually to almost zero in all treatments (except the KNO_3 treatment), with the decrease being more significant in the Straw treatment. Reduced N_2O flux rates coupled with significant increases in N_2 emission were observed in all treatments, while the highest N_2 fluxes being toward the end of the experiment were observed specifically in straw-amended soils. This phenomenon probably occurred because N_2O reduction began to exceed N_2O production after soil NO_3^- content fell below a certain threshold level (Hu et al., 2016; Senbayram et al., 2018). It should be noted that anammox process may also contribute to N_2 emissions; however, its potential contribution should be minor especially in

the Straw and Straw + KNO_3 treatments because anammox activities and the ratio of anammox to total N_2 production were negligible relative to denitrification under high C condition (Jin et al., 2012; Shan et al., 2018). Due to the more rapid decrease in soil NO_3^- content in straw-amended soils (owing to high denitrification loss), the relationship between denitrification and NO_3^- level at the denitrifying microsites shifted from zero-order (independent of NO_3^-) to first-order kinetics (dependent on NO_3^-). It is generally accepted that NO_3^- is preferentially used by denitrifiers over N_2O as an electron acceptor (Morley & Baggs, 2010; Senbayram et al., 2012). In straw-amended soils, however, the dominant form of emitted N shifted significantly from N_2O to N_2 (increasing N_2O reduction rates), due to the more rapid decrease in NO_3^- content compared to the nonstraw-amended treatments. Thus, we concluded that organic matter amendment can mitigate N_2O emissions in soils with low NO_3^- content (due to higher N_2O reduction), or trigger N_2O fluxes when coupled with high soil NO_3^- content under the moist situation. Earlier studies reported that straw amendment has the potential to mitigate N_2O emissions (Yao et al., 2017; Zou et al., 2005), while others showed that straw returning can increase N_2O losses (Pinheiro et al., 2019; Wu et al., 2018). In this context, we believe that our study contributes to the understanding of why contradictory observations on the impact of straw addition on N_2O fluxes have been reported. In addition, the lowest N_2O but high N_2 emissions in straw-amended treatments were observed during the whole incubation period, especially in soils with lower NO_3^- level (Figure 2). This result suggested that straw applied alone would lead to more N_2 emissions, and straw applied together with N fertilizer would result in more N_2O emissions in the intensively managed soil. To mitigate the higher N_2O emissions trigged by combined amendments of straw and N fertilizer, straw amendment should not be simultaneously applied with NO_3^- containing N fertilizer in intensively managed soils.

4.4 | Effect of straw and nitrate amendments on the $N_2O/(N_2O + N_2)$ ratio

It has frequently been suggested that labile C addition to soil may mitigate the N_2O emissions owing to its plausible effects on the N_2O reduction step of denitrification [i.e., lowering the $N_2O/(N_2O + N_2)$ ratio] (Frimpong & Baggs, 2010; Mathieu et al., 2006; Millar & Baggs, 2004). Our results clearly showed that even with very high inputs of organic matter, N_2O reduction is unaffected (during the oxic and initial phase of the anoxic phase) when soil NO_3^- content is high. Instead, N_2O production was trigged under such condition causing large N_2O peaks. On the other hand, during the later phase of the experiment, when NO_3^- started to co-limit denitrification, straw amendment triggered N_2O reduction, as evidenced by the delayed N_2 peak (~10 days) in the Straw + KNO_3 treatment compared to the Straw treatment. The size of both the N_2O and N_2 peaks increased in straw-amended soils in the presence of NO_3^-. This may be attributed to the fact that the size of the NO_3^- pool also continued to increase during the anoxic phase in the Straw + KNO_3 treatment (when NO_3^- was not limiting), initially producing mainly N_2O, and later mainly N_2. However, when NO_3^- started limiting denitrification, the denitrifying community shifted from a dominance of N_2O production to one of N_2O reduction, causing a rapid increase in N_2 loss. This change might have been due to a shift from a dominance of fungal denitrification to one of bacterial denitrification, which has been reported as a combined effect of straw amendment and NO_3^- limitation (Senbayram et al., 2018). In addition, this change could be also promoted by the anoxic condition because most fungi are obligate aerobes. Unlike bacterial denitrification, denitrifying fungi lack the N_2O reductase enzyme and fungal denitrification relies solely on the soil content of NO_3^- and NO_2^- as electron acceptors (Baggs, 2011). Previously, Senbayram et al. (2018) clearly showed that when the soil NO_3^- content started limiting the denitrification rate, there was a clear shift from fungal to bacterial denitrification, resulting in a lower $N_2O/(N_2O + N_2)$ ratio. In this study, a similar shift trend from fungal to bacterial denitrification could be expected although we did not distinguish the processes contributing to the N_2O production.

4.5 | Denitrifier community abundance and nitrate reduction

At the end of the experiment, the *narG*, *nirK*, *nirS,* and *nosZ* gene copy numbers in the straw-amended treatments were clearly higher than those in the Control or KNO_3 treatments, indicating that straw application probably increased the activity of certain denitrifiers such as N_2O reducing micro-organisms. It had been shown that soil C content is the key factor controlling soil denitrifier growth and denitrification rate (Luo et al., 1999). We also found that the increases in denitrifying-related gene abundance were in-line with higher cumulative CO_2 emissions and denitrification rates when the soil was amended with straw (Figures 4 and 5). Pan et al. (2017) found that straw amendment could significantly increase *nosZ* gene abundance, which was related to more N_2 production in agricultural soils. However, no significant correlations were found between denitrifier abundance and N_2O/N_2 emissions in this study, which were consistent with results of previous studies (Henderson et al., 2010; Miller et al., 2008). This may be due to the taxonomic diversity of denitrifiers since the gaseous N emissions were from the entire denitrifying community, rather than just the denitrifiers quantified

in this study. Besides, this may also be attributable to the limitation of gene abundance at the DNA level to represent denitrifier activities, because gene presence does not necessarily reflect activity (Lycus et al., 2017; Wallenstein et al., 2006). However, to some extent, the increase in gene abundance might be a reflection of the development of the denitrifier community, as well as the abundance of groups differing in their loaded enzymes. The highest copy numbers of all denitrification-related genes were found in the Straw and Straw + KNO_3 treatments (Figure 5), suggesting the growth of denitrifying microbes were triggered by conditions strongly favoring denitrification. In particular, *nosZ* abundance was augmented by straw amendment, possibly indicating that when NO_3^- becomes limiting in soils, N_2O reductases were favored in relation to N_2O producers, since their substrate (N_2O) would still be abundant, while NO_3^- would become increasingly limited. These phenomena were in agreement with increased N_2 production, as shown by Senbayram et al. (2018) where bacterial denitrification (with N_2O reductase participation) became dominant in soils when NO_3^- was below a certain threshold. It should be noted that recent studies have confirmed that *nosZ* gene could be identified into two clades, that is, clade I and clade II *nosZ* (Jones et al., 2013; Sanford et al., 2012), the primer set used in this study may be not adequate for comprehensive quantifying the *nosZ* abundance and will certainly miss some potentially important microbes carried with N_2O reductase if presented in the soil. Overall, the gene copy numbers used to examine the influence of straw and nitrate on soil denitrifiers were limited due to the soil heterogeneity and lack of high-frequency sampling.

5 | CONCLUSION

Our results demonstrated that the effect of straw amendment on N_2O emission was nitrate level-dependent. Straw amendment may drastically increase soil-born N_2O emissions in NO_3^--rich soil, while it may also induce effective N_2O reduction to N_2 when soil NO_3^- contents decrease under a certain threshold level. Based on our findings, straw amendment should be avoided to apply with N fertilizer simultaneously in most intensively managed soils with high nitrate residue, and otherwise, it may induce higher N_2O emissions by triggering the growth of denitrifiers.

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SUPPORTING INFORMATION

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

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