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# Effects of Straw Amendment in Combination with Synthetic N Fertilizer Addition on $N_2O$, $N_2$, and Their Stoichiometric Ratios in Three Different Agro-Ecosystems

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**Abstract:** Nitrogen (N) fertilizer and crop residue amendments are important agricultural practices that could increase soil health, fertility, and crop yield. Such practices may also change soil denitrification processes where contradictory observations have been reported on soil $N_2O$ emissions with fewer studies on $N_2$ emissions due to its large atmospheric background concentrations limiting its soil-borne measurement. This study aims to investigate $N_2O$ production and reduction of $N_2$ emissions under a conducive denitrifying environment (like anaerobic microsites, 80% WFPS, available N and C) after rice straw amendment and $KNO_3$ application to three different soil types (fluvo-aquic, black, and paddy soils). In this regard, three treatments for three different soil types were set consisting of (a) a non-amended treatment (control), (b) a $KNO_3$ treatment ($KNO_3$, 20 mM $KNO_3$), and (c) a straw plus $KNO_3$ treatment (2.5 g rice straw $kg^{-1}$ dry soil and 20 mM $KNO_3$), which were incubated under 80% WFPS. Moreover, direct $N_2O$ and $N_2$ fluxes were measured over 17 days in the current incubation experiment with a robotized incubation system using a helium atmosphere. Results showed that rice straw amendment combined with N fertilizer increased both $N_2O$ and $N_2$ fluxes compared with control or $KNO_3$ treatments in all three soil types. Overall, compared with the black and paddy soils, the $N_2O$ and $N_2$ fluxes were higher in the fluvo-aquic soil, with a maximum of 234.2 ± 6.3 and 590.1 ± 27.3 g N $ha^{-1}$ from F_SK treatment, respectively, during the incubation period. The general trends in three soil types of both $N_2O$ and $N_2$ emissions were control < $KNO_3$ < rice straw plus $KNO_3$ treatments. Straw amendment in combination with $KNO_3$ can stimulate a high denitrification rate (less $N_2O$ and higher $N_2$), whereas their effect on stoichiometric ratios of $N_2O/(N_2O + N_2)$ highly depends on soil nitrate concentration, oxygen level, soil moisture content, and labile C. The current study underscores that the rice straw amendment in combination with N fertilizer can trigger denitrification with less increment on soil $N_2O$ but higher $N_2$ emissions under conditions favoring denitrification.

**Keywords:** N fertilizer; straw amendment; denitrification; $N_2O$ and $N_2$ emissions; agricultural soil



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## 1. Introduction

Nitrogen (N) is an important agricultural macronutrient for plant growth and a yield-limiting nutrient in agricultural production [1], and its remarkable contributions to food production worldwide to enhance food security are highly recognized. Since the discovery of the Haber–Bosch process in the early 20th century, whereby atmospheric dinitrogen ($N_2$) is artificially fixed for the production of synthetic N fertilizer, the consumption of global synthetic N increased mainly over the last five decades (1961–2020) from 11.5 to 113.3 Tg

(teragram = $10^{12}$ g) N $year^{-1}$ [2]. This artificial N fixation has converted a substantial amount of unreactive dinitrogen ($N_2$) to reactive N forms, allowing farmers to change infertile croplands to fertile croplands [3]. However, a high and uncontrolled supply of N into agricultural soil with reduced N use efficiency (NUE) pollutes the environment through air pollution, eutrophication in water bodies, and depletion of the ozone layer in the stratosphere causing global warming [4,5].

Agricultural soils are one of the major anthropogenic sources of $N_2O$ emissions to the atmosphere, mainly due to the related intensive management practices including fertilization, irrigation, and residue incorporation [6–8]. Fertilized agricultural soils are estimated to emit 4.1 Tg $N_2O$ annually [9], which makes it a significant anthropogenic source of $N_2O$ emissions. The growing evidence has shown that soil $N_2O$ production mainly results from nitrification and denitrification processes where denitrification is considered the main biological process [5,10–12]. Denitrification is a reduction process of nitrate ($NO_3^-$) and nitrite ($NO_2-$ through nitric oxide (NO) and $N_2O$ to $N_2$ [13–15], enabling the removal of accumulated reactive N in the biosphere [16]. Previous literature studies reported high uncertainties concerning the quantification of how much reactive N is converted back into $N_2$ via denitrification due to the lack of accurate or unbiased $N_2$ measurement techniques against high atmospheric $N_2$ background [17,18]. Since, N is one of the macronutrient limiting factors for crop growth [19], increasing rates of N fertilizer applications will also increase $N_2O$ and $N_2$ losses from agricultural soil [20]. Moreover, complete denitrification is a main environmentally useful pathway for converting reactive N into stable molecules of $N_2$ emissions. However, $N_2O$ measured simultaneously with $N_2$ after the addition of N fertilizer and rice straw amendment has not been well documented in agricultural soils. Therefore, the present study will shed more light on denitrification losses ($N_2O$ and $N_2$ emissions) when synthetic N fertilizer is concomitantly applied with rice straw in agricultural soils.

Straw return is generally considered an effective practice to improve soil health, soil quality, and crop yield [8,21]. In China, it has been highly encouraged to return crop straws due to the prohibition of burning them [21,22]. Therefore, crop straw return should be highly considered as an important agricultural practice to better utilize resources and protect the environment. Previous literature reported that agricultural soils amended with straw receive labile carbon (C) and N together with other micronutrients where microbial growth and activity are also stimulated due to high SOC enhancement [8,23]. This microbial growth and activity stimulation due to straw return are also beneficial for enhancing the complete denitrification process. In the meta-analysis, Liu et al. [24] reported that straw return has been widely recommended as an environmentally friendly practice to manage carbon sequestration in agricultural ecosystems. Chen et al. [25], in their research work about soil fertility and crop performance in winter wheat–summer maize crop rotation, reported that returning only wheat straw and removing maize straw would maintain improved soil nutrient contents. Straw amendment can stimulate denitrification by providing organic C as the energy source for microbial respiration, which also enhances anaerobic conditions, hence denitrification. However, contradictory observations concerning denitrification have been reported, showing positive or negative effects of straw amendment in combination with N fertilizer on $N_2O$ emissions [21,26–29], while the final denitrification product has not been included in the studies. For example, Zhou et al. [29] reported a 150% increase in $N_2O$ emissions in 2010 and a 35% decrease in 2011 in the maize season from the same field. Yao et al. [30] reported that straw return can stimulate $N_2O$ emissions by providing bioavailable C and N to soil denitrifiers, while Zhou et al. [31] indicated that straw return may inhibit $N_2O$ emissions by increasing the $N_2/N_2O$ ratio during denitrification and may also enhance biological N fixation. Bai et al. [21], after a two-year study of straw amendment combined with different N fertilizers, concluded that straw return is an environmentally friendly, high-crop-yielding, high-economic-benefit, and low-$N_2O$-emission production technology for arid and semiarid areas. Previous studies have reported that $N_2O$ emissions from soils can be lowered under conditions favoring $N_2O$ reduction to

$N_2$ [8,32,33]; however, it is still not clear to what extent straw amendment combined with N fertilizer would affect both production and reduction rate of $N_2O$. Interactive effects of straw amendment combined with N fertilizer under a controlled conducive denitrifying environment will help us better understand the environmental benefits of straw management and develop specific management practices to mitigate $N_2O$ emission.

Our current study will help us understand the influence of straw amendment combined with N fertilizer addition to $N_2O$ and $N_2$ emissions. In general, the incorporation of crop residue may affect soil temperature and moisture, soil N content, DOC content, and microbial activity, thus regulating soil $N_2O$ and $N_2$ emissions in a complex manner [8,34,35]. The helium direct measurement method is advantageous since it does not change soil properties by adding extra substrates such as $^{15}N$-labeled substrates or $C_2H_2$ in the $^{15}N$ isotope labeling method and $C_2H_2$ inhibition technique, respectively [36]. Predominantly, straws from rice, corn, and wheat have been reported to account for 90% of the total straw production in China [37], which is why—and also due to its high C/N ratio—rice straw was selected to be used in the current study. The main objectives of the current study are as follows: (a) to assess the influence of straw amendment in combination with synthetic N fertilizer addition on $N_2O$ and $N_2$ emissions; (b) to explore the main bacterial community composition stimulated by the rice straw amendment combined with synthetic N fertilizer addition, hence affecting $N_2O$ production and its reduction to $N_2$.

## 2. Materials and Methods

### *2.1. Experimental Sites*

In the current study, the three soil types sampled from different fields were known as two upland soils and one paddy soil. Soil samples collected were as follows: upland soil samples collected from long-term summer maize–winter wheat crop rotation (fluvo-aquic soil), upland black soil samples from summer maize monocropping (black soil), and paddy soil samples from rice monocropping (paddy soil).

Fluvo-aquic soil samples were collected from the Luanchenghe agro-ecosystem experimental station in Hebei Province, China (37°53′ N, 114°41′ E, 50 m). The soil category in this region is known as a silt loam Haplic Cambisol [38] with a temperate semiarid typical monsoon climate. The yearly average precipitation and temperature are 540 mm and 12.7 °C, respectively. This soil type received synthetic annual N fertilizer of 200 kg $ha^{-1}$.

Black soil samples were collected from Gongzhuling, Jilin Province, China (43°510′ N, 124°820′ E, 300 m). The soil category in this region is known as a Haplic Chernozem with a mollic horizon [38,39] with a cool temperate, subhumid continental monsoon climate. The yearly average precipitation is around 614 mm, where 75% occurs mainly in the summer season (June–September). The average yearly temperature is 6.9 °C, with monthly average temperatures ranging from −13.5 °C in January to 23.7 °C in July. The black soil in northeastern China is one of the dominant soils and it represents an important maize cultivation area in China, significantly contributing to the national maize production. This soil type also received synthetic annual N fertilizer of 200 kg $ha^{-1}$.

Paddy soil samples were collected from Hubei Province, China (31°10′ N, 114°58′ E). This rice riparian of the southeast region belongs to the middle and lower reaches of the Yangtze River with a long history of rice cultivation. This study area is characterized by a typical subtropical monsoon climate with a mean annual temperature of 16.8 °C, and a mean annual rainfall of 1258 mm, of which 60–70% occurs during the summer. This soil type also received synthetic annual N fertilizer of 200 kg $ha^{-1}$. The paddy soil in this region is classified as Lixisols [38,39].

### *2.2. Measurement of Soil Parameters*

The topsoil samples were collected from 0–20 cm from the three different soil types mentioned above with respect to three replicates for each soil type in 2022. Soil samples for each soil type were divided into three parts: one part was stored at −20 °C for total microbial population analysis; the second part was stored at 4 °C for the determination of

soil physicochemical properties, including soil ammonium ($NH_4^+$-N), nitrate ($NO_3^-$-N), dissolved organic carbon (DOC), and soil pH; and the third part was air-dried at room temperature to determine soil total nitrogen (TN) and total carbon (TC) concentrations.

$NH_4^+$ and $NO_3^-$ were extracted by shaking for 1 h a mixture of 10 g of fresh soil with 50 mL of 1 mol $L^{-1}$ KCl solution. Then, the soil extracts were filtered using Whatman 42 filter paper, and $NH_4^+$ and $NO_3^-$ concentrations were measured using a Smartchem 140 automatic analyzer and dual wavelength ultraviolet spectrophotometer, respectively [5,40]. Soil-dissolved organic carbon (DOC) was extracted by mixing 10 g of fresh soil cores with 50 mL of deionized water. Soil extracts were centrifugated for 10 min at 8000 rpm and the mixture was finally filtered and determined by Liqui TOCII analyzer (Elementar, Hanau, Germany) [41]. Soil TC and TN were measured after the soil was air-dried and ground by an elemental analyzer (Vario MAX; Elementar, Hanau, Germany). The soil water content (%) was gravimetrically measured by drying the soil at 105 ± 0.05 °C for 24 h and, then, by soil water-filled pore space (WFPS, %) calculations based on [41]. After the onset treatments of the incubation period, soil moisture was adjusted for all soil samples of all three soil types because soil samples were air-dried prior to the incubation period. Table 1 summarizes the soil properties of three soil types used in the current study.

**Table 1.** Soil physicochemical properties. Data are expressed as mean ± standard error (n = 3). Lowercase letters in the same column denote a significant difference between tested average values at $p < 0.05$.

| Parameter | DOC (mg C $kg^{-1}$) | $NO_3^-$ (mg N $kg^{-1}$) | $NH_4^+$ (mg N $kg^{-1}$) | pH | TC (g C $kg^{-1}$) | TN (g N $kg^{-1}$) | C/N | Bulk Density (g $cm^{-3}$) |
|---|---|---|---|---|---|---|---|---|
| Fluvo-aquic soil | 50.1 ± 2.4 $^a$ | 36.0 ± 0.8 $^a$ | 1.9 ± 0.6 $^a$ | 8.0 ± 0.1 $^a$ | 21.9 ± 0.8 $^a$ | 1.2 ± 0.1 $^a$ | 17.8 ± 1.5 $^a$ | 1.3 ± 0.1 $^a$ |
| Black soil | 48.3 ± 1.0 $^b$ | 31.9 ± 1.2 $^b$ | 1.2 ± 0.4 $^b$ | 6.6 ± 0.1 $^b$ | 16.8 ± 1.2 $^b$ | 1.1 ± 0.1 $^b$ | 15.0 ± 0.2 $^b$ | 1.2 ± 0.1 $^b$ |
| Paddy soil | 41.7 ± 1.1 $^c$ | 28.0 ± 1.1 $^c$ | 0.8 ± 0.3 $^c$ | 6.1 ± 0.1 $^c$ | 10.8 ± 0.5 $^c$ | 0.9 ± 0.1 $^c$ | 12.1 ± 1.9 $^c$ | 1.1 ± 0.1 $^c$ |

### *2.3. Incubation Experiment for Soil Gas Measurements*

This study consisted of three different treatments with three different soil types. Incubation procedures began after soil samples were air-dried and sieved through a 2 mm mesh to remove plant residue and other impurities in the laboratory. For each soil type, there were controls and treatments ($KNO_3$ only and $KNO_3$ with straw) with three replicates. Prior to incubation, each soil type was preincubated for two days at around 45% WFPS to stabilize the microbial activity and eliminate the effect of drying soil. The experimental design for fluvo-aquic soil (F), black soil (B), and paddy soil (P) samples consisted of three treatments (n = 3) each: (a) a non-amended treatment (control); (b) $KNO_3$ treatment; and (c) a straw plus $KNO_3$ treatment. In total, we had 27 replicates for gas measurements only. In detail, all treatments were designed as follows: for fluvo-aquic (F): F_CK, F_K, and F_SK; for black soil: B_CK, B_K, and B_SK and for paddy soil (P): P_CK, P_K, and P_SK).

Oven-dried rice straw was ground through a 2 mm mesh sieve with 0.7% of total N and 45% of total C. During the incubation time, the straw was mixed into the preincubated soils in the straw and straw plus $KNO_3$ treatments at a rate of 2.5 g straw per kg of dry soil. Then, the soil was packed into 30 g in each 120 mL serum flask. In the current study, N fertilizer was applied in the form of $KNO_3$. Therefore, $KNO_3$ was used as the N source to avoid other significant contributions of $N_2O$ emitting processes during the experiment. The soils were then flooded with 20 mM $KNO_3$ solution in the $KNO_3$ and straw plus $KNO_3$ treatments ($KNO_3$ and $KNO_3$ plus straw treatments: equivalent to 112 mg of $NO_3^-$-N per kg soil addition) or distilled water in the control and drained to approximately 80% WFPS by weighing the soil to keep the weight constant. The room temperature was set to 21 °C throughout the whole incubation period.

The serum flasks were then closed with butyl rubber and aluminum caps. Furthermore, these serum flasks were flushed with pure helium (99.99%) four times to make the headspace environment free from $N_2$ and oxygen ($O_2$ < 450 ppm). In each treatment, 3 replicated serum flasks were used for measuring the concentration of accumulated $N_2O$

and $N_2$ gases 3 times every day during the incubation period of 17 days. The cumulative concentrations of $N_2O$ and $N_2$ in the serum flasks were measured every 24 h using a robotized sampling and analysis system, which consisted of an autosampler, a peristaltic pump, and a gas chromatograph (GC, Agilent 7890A; Santa Clara, CA, USA). The $N_2O$ concentrations were analyzed using an electron capture detector while $N_2$ concentrations were also analyzed using a thermal conductivity detector. Therefore, direct $N_2O$ and $N_2$ fluxes were measured over 17 days in the current incubation experiment with a robotized incubation system using a helium atmosphere. The system details and the gas concentration computations were described previously [42]. Cumulative $N_2O$ and $N_2$ emissions were calculated by linear interpolation between measured fluxes.

### *2.4. DNA Extraction and 16s rRNA Sequencing*

For determining the denitrifying bacterial community, original soil samples were collected and stored at 20 °C before air-drying other portions of soil samples while incubated soil samples were collected at the end of the incubation experiment. DNA extraction for soil samples was performed using FastDNA Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) per the manufacturer's instructions. Soil total DNA was extracted from 0.5 g of soil using FastDNA Spin Kit for Soil (MP Biomedicals, USA) per the manufacturer's instructions with little modifications. The gDNA was checked for quantity and quality using a Nanodrop one spectrophotometer (NanoDrop, Thermo Fisher Scientific, Madison, WI, USA) and gel agarose electrophoresis, respectively. The DNA samples were then sent for 16s rRNA gene sequence at Personalbio Laboratories, Shanghai, China.

For 16s rRNA sequencing, the V3-V4 region of the bacterial 16S rRNA gene was amplified using primer sets 1369F, (5′-CGGTGAATACGTT CYCGG-3′) and 1492R, (5′-GGWTACCTTGTTACGACTT-3′) to investigate the bacterial community diversity and structure using high throughput sequencing technology. The main aim of the study was to analyze the denitrification rate and its product stoichiometry in this intensively managed soil type where nitrate was the major inorganic N form.

### *2.5. Statistical Analysis*

To compare cumulative $N_2O$ and $N_2$ emissions among treatments for each soil type and the three soil types, we performed a one-way ANOVA with Turkey's test. The difference was considered significant when $p < 0.05$. All figures and statistical tests were conducted using Origin Pro8 software.

## 3. Results

### *3.1. Soil Mineral N Variables from the Three Soil Types*

In straw plus N fertilized ($KNO_3$) treatments for all three soil types, final $NO_3^-$ concentrations were below 2 mg $NO_3^-$-N per kg, while in control and $KNO_3$ treatments for all three soil types, the concentrations were below 5 and 16 mg $NO_3^-$-N per kg, respectively (Table 2). At the end of the incubation period, the order of soil $NO_3^-$ concentrations was observed following the trend below: $KNO_3$ > Control > Straw plus $KNO_3$. Overall, in the three soil types, the soil $NO_3^-$ concentrations were more depleted in straw plus $KNO_3$, at more than 99%. Final soil $NH_4^+$ concentrations were low in all treatments for all three soil types with values ranging between 1 to 3 mg N per kg (Table 2) and they were slightly higher than initial concentrations.

**Table 2.** Soil $NO_3^-$ and $NH_4^+$ concentrations at the end of the experiment in the non-amended treatments (CK), $KNO_3$, and straw plus $KNO_3$ treatments. Data are expressed as mean ± standard error (n = 3).

| Parameter | | Final (mg N $kg^{-1}$) | |
|---|---|---|---|
| | | $NH_4^+$ | $NO_3^-$ |
| Fluvo-aquic soil | F_CK | 2.4 ± 0.1 | 4.2 ± 0.6 |
| | F_K | 2.2 ± 0.2 | 14.8 ± 0.1 |
| | F_SK | 2.9 ± 0.2 | 1.3 ± 0.1 |

**Table 2.** *Cont.*

| Parameter | | Final (mg N kg$^{-1}$) | |
|---|---|---|---|
| | | $NH_4^+$ | $NO_3^-$ |
| Black soil | B_CK | 2.3 ± 0.2 | 3.5 ± 0.6 |
| | B_K | 2.1 ± 0.1 | 15.9 ± 0.3 |
| | B_SK | 2.6 ± 0.3 | 1.4 ± 0.1 |
| Paddy soil | P_CK | 1.3 ± 0.2 | 2.7 ± 0.5 |
| | P_K | 1.1 ± 0.1 | 13.2 ± 0.1 |
| | P_SK | 1.7 ± 0.2 | 1.3 ± 0.1 |

### *3.2. Temporal Emissions of $N_2O$ and $N_2$ Emissions from Three Different Soil Types*

The $N_2O$ emissions increased in all treatments of the three soil types (fluvo-aquic, black, and paddy soils) shortly after $KNO_3$ was applied to each soil core, especially in the fluvo-aquic soil treatments (Figure 1). Comparing all fertilized $KNO_3$ plus straw amendment treatments from the three different soil types to unfertilized treatments, without straw amendment, the fertilized $KNO_3$ with straw amendment greatly enhanced $N_2O$ emissions (Figure 1). Generally, $N_2O$ emissions were the highest from SK treatments and lowest from CK treatments in all three soil types. Compared with the paddy soil and black soils, the $N_2O$ flux was higher in the fluvo-aquic soil, with a maximum of 27.3 ± 3 g N ha$^{-1}$ day$^{-1}$ from F_SK treatment. The average values of $N_2O$ emissions were 4.3 ± 0.4, 9.7 ± 1.2, and 13.8 ± 1.2 g N ha$^{-1}$ d$^{-1}$ from F_CK, F_K, and F_SK of fluvo-aquic soil treatments, respectively. The peak of $N_2O$ emissions of fluvo-aquic soil treatments was observed on the third day of the experiment. The average values of $N_2O$ emissions were 4.0 ± 0.4, 8.5 ± 1.1, and 12.1 ± 1.5 g N ha$^{-1}$ d$^{-1}$ from B_CK, B_K, and B_SK of black soil treatments, respectively. The peak of $N_2O$ emissions from black soil was also observed on the third day of the experiment. The average values of $N_2O$ emissions were 3.3 ± 0.4, 6.8 ± 0.8, and 10.6 ± 1.3 g N ha$^{-1}$ d$^{-1}$ from P_CK, P_K, and P_SK of paddy soil treatments, respectively. The peak of $N_2O$ emissions from paddy soil was also observed on the fourth day of the experiment.

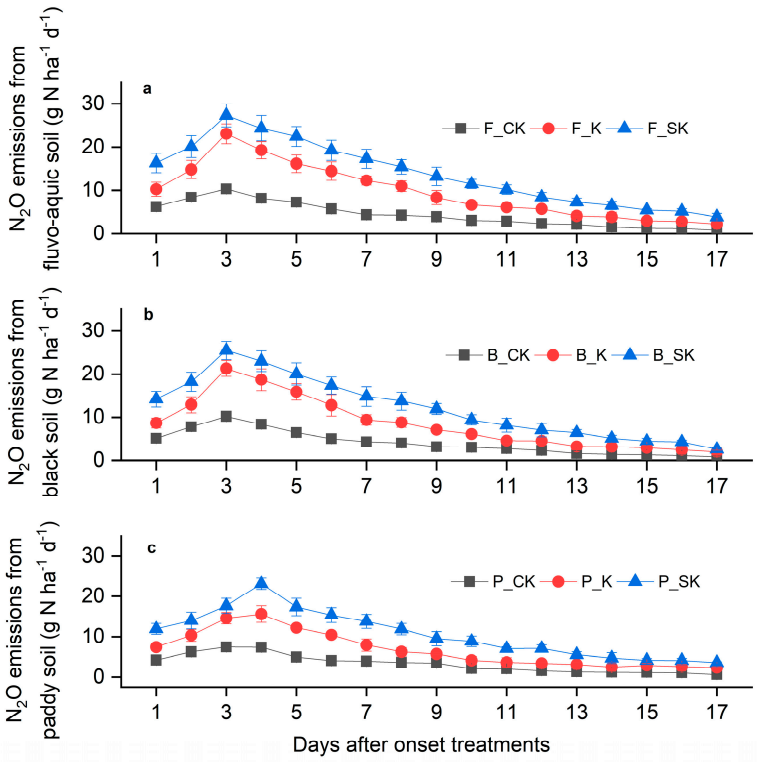


**Figure 1.** Daily $N_2O$ emissions during the incubation period in the non-amended treatment (CK), $KNO_3$, and straw plus $KNO_3$ treatments collected from (**a**) fluvo-aquic soil, (**b**) black soil, and (**c**) paddy soil. Error bars show the standard error of each treatment (n = 3).

$N_2$ emission peaks in all three soil types increased when the soil denitrifying environment was conducive a few days after $KNO_3$ was applied with straw addition (Figure 2) and then gradually decreased till the end of the incubation period. Our results showed that $N_2$ emissions observed from $KNO_3$-fertilized plus straw amendment treatments of the three soil types were higher than from other treatments. This is obvious due to the fact that organic C from straw enhanced the electron donor for $N_2O$ reduction where C availability also increased microbial respiration in soils along with a decreased $O_2$, thus creating anaerobic microsites for denitrifying microorganisms. Overall, compared with the black and paddy soils, the $N_2$ flux was higher in the fluvo-aquic soil, with a maximum of $57.0 \pm 8.0$ g N $ha^{-1}$ $day^{-1}$ from F_SK treatment. The average values of $N_2$ emissions were $14.3 \pm 2.5$, $18.3 \pm 2.3$, and $34.7 \pm 4.6$ g N $ha^{-1}$ $d^{-1}$ from F_CK, F_K, and F_SK of fluvo-aquic soil treatments, respectively. The peak of $N_2$ emissions of fluvo-aquic soil treatments was observed on the fifth day of the incubation period. The average values of $N_2$ emissions were $12.5 \pm 2.3$, $16.6 \pm 2.3$, and $30.0 \pm 4.5$ g N $ha^{-1}$ $d^{-1}$ from B_CK, B_K, and B_SK of black soil treatments, respectively. The peak of $N_2$ emissions from black soil was observed on the seventh day of the incubation period. The average values of $N_2$ emissions were $9.4 \pm 2.0$, $15.0 \pm 2.0$, and $25.0 \pm 3.4$ g N $ha^{-1}$ $d^{-1}$ from P_CK, P_K, and P_SK of paddy soil treatments, respectively. The peak of $N_2$ emissions from paddy soil was observed on the sixth day of the incubation period.

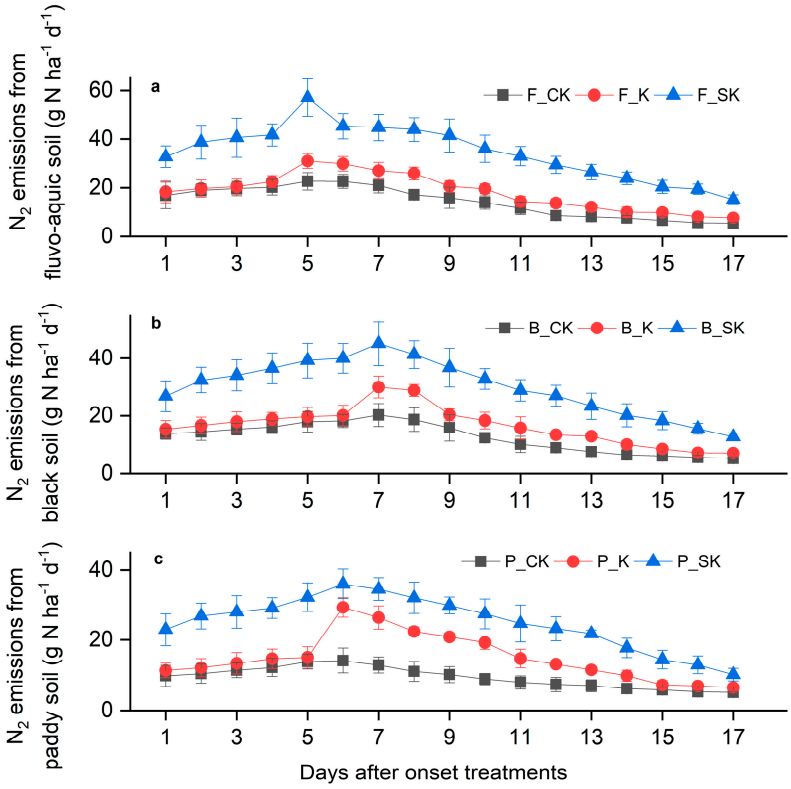


**Figure 2.** Daily $N_2$ emissions during the incubation period in the non-amended treatment (CK), $KNO_3$, and straw plus $KNO_3$ treatments collected from (**a**) fluvo-aquic soil, (**b**) black soil, and (**c**) paddy soil. Error bars show the standard error of each treatment (n = 3).

### *3.3. Cumulative $N_2O$ and $N_2$ Emissions from Three Different Soil Types*

The cumulative emissions of both $N_2O$ and $N_2$ from the three different soil types (fluvo-aquic, black, and paddy soils) were investigated during the incubation period of 17 days in our current study (Figures 3 and 4). Overall, compared with the black and paddy soils, the $N_2O$ and $N_2$ fluxes were higher in the fluvo-aquic soil, with a maximum of $234.2 \pm 6.3$ and $590.1 \pm 27.3$ g N $ha^{-1}$ from F_SK treatment, respectively, during the incubation period. The higher emissions observed from fluvo-aquic soil type were boosted

by the availability of a conducive denitrifying environment like higher soil pH and C/N ratio where soil moisture and required nitrate and temperature were also in acceptable ranges. The cumulative emissions of $N_2O$ were significantly different among all treatments and average values obtained were 73.6 ± 2.2, 164.2 ± 3.2, and 234.2 ± 6.3 g N $ha^{-1}$ from F_CK, F_K, and F_SK of fluvo-aquic soil treatments, respectively, during the incubation period. Annual average cumulative $N_2$ emissions were 242.7 ± 15.3, 311.0 ± 6.6, and 590.1 ± 27.3 g N $ha^{-1}$ from F_CK, F_K, and F_SK of fluvo-aquic soil treatments, respectively, during the incubation period. Second, the annual cumulative emissions of $N_2O$ were significantly different among all treatments and average values obtained were 68.3 ± 1.2, 144.0 ± 3.6, and 205.7 ± 13.2 g N $ha^{-1}$ from B_CK, B_K, and B_SK of black soil treatments, respectively, during the incubation period. Annual average cumulative $N_2$ emissions were 213.1 ± 7.6, 281.6 ± 15.0, and 511.4 ± 12.5 g N $ha^{-1}$ from B_CK, B_K, and B_SK of black soil treatments, respectively, during the incubation period. Third, the annual cumulative emissions of $N_2O$ were significantly different among all treatments and average values obtained were 56.7 ± 1.0, 115.6 ± 8.4, and 180.1 ± 6.4 g N $ha^{-1}$ from P_CK, P_K, and P_SK of paddy soil treatments, respectively, during the incubation period. Annual average cumulative $N_2$ emissions were 159.4 ± 2.1, 254.6 ± 4.7, and 424.6 ± 2.1 g N $ha^{-1}$ $d^{-1}$ from P_CK, P_K, and P_SK of paddy soil treatments, respectively, during the incubation period.

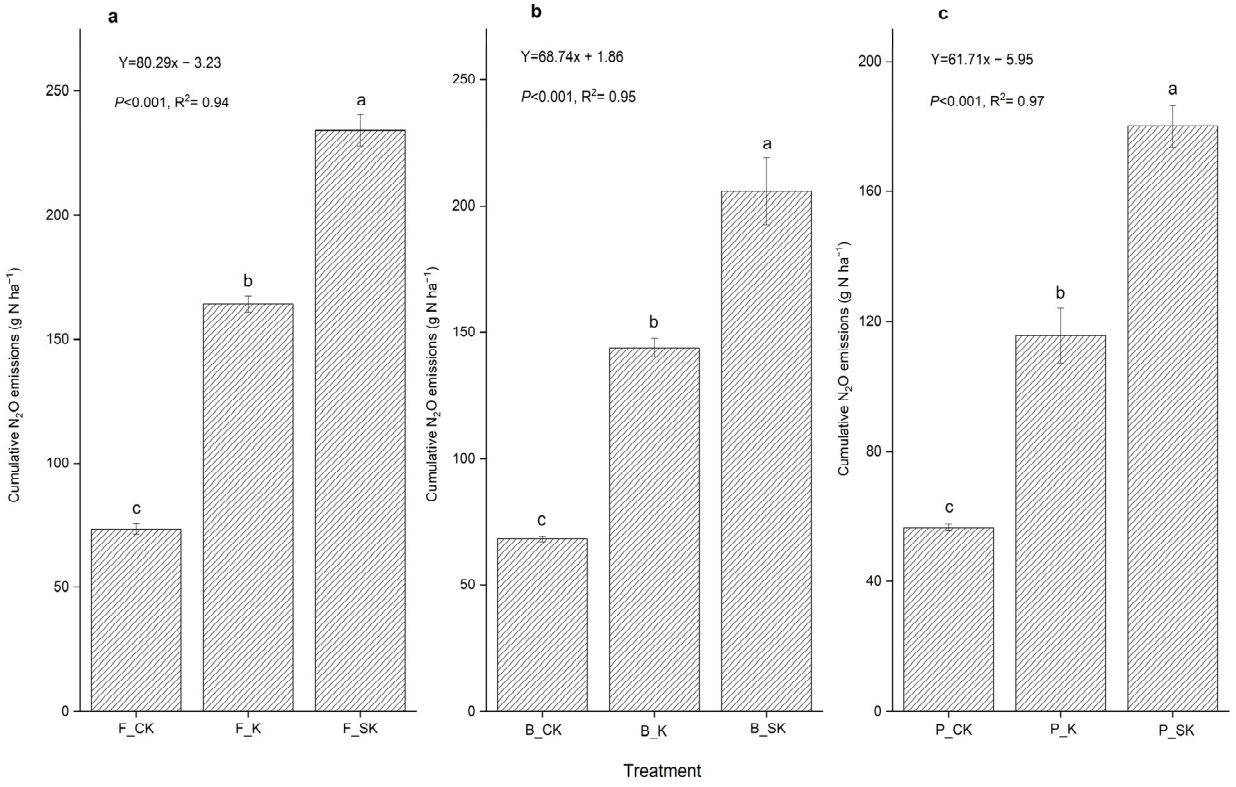


**Figure 3.** Cumulative $N_2O$ emissions during the incubation period in non-amended treatment (CK), $KNO_3$, and straw plus $KNO_3$ treatments collected from (**a**) fluvo-aquic soil, (**b**) black soil, and (**c**) paddy soil. Error bars show the standard error of each treatment (n = 3). Data are expressed as mean ± standard error (n = 3). Lowercase letters denote a significant difference between average values at $p = 0.05$.

The total cumulative $N_2O$ plus $N_2$ emissions were higher in fluvo-aquic soils and the lowest in paddy soil while more emissions were evident in $KNO_3$ plus straw treatments in all three soil types (Table 3). The total annual cumulative $N_2O$ and $N_2$ emissions in each soil type were significantly different between controls and the amended treatments with $KNO_3$ and $KNO_3$ plus straw treatments throughout the incubation period (Table 3).

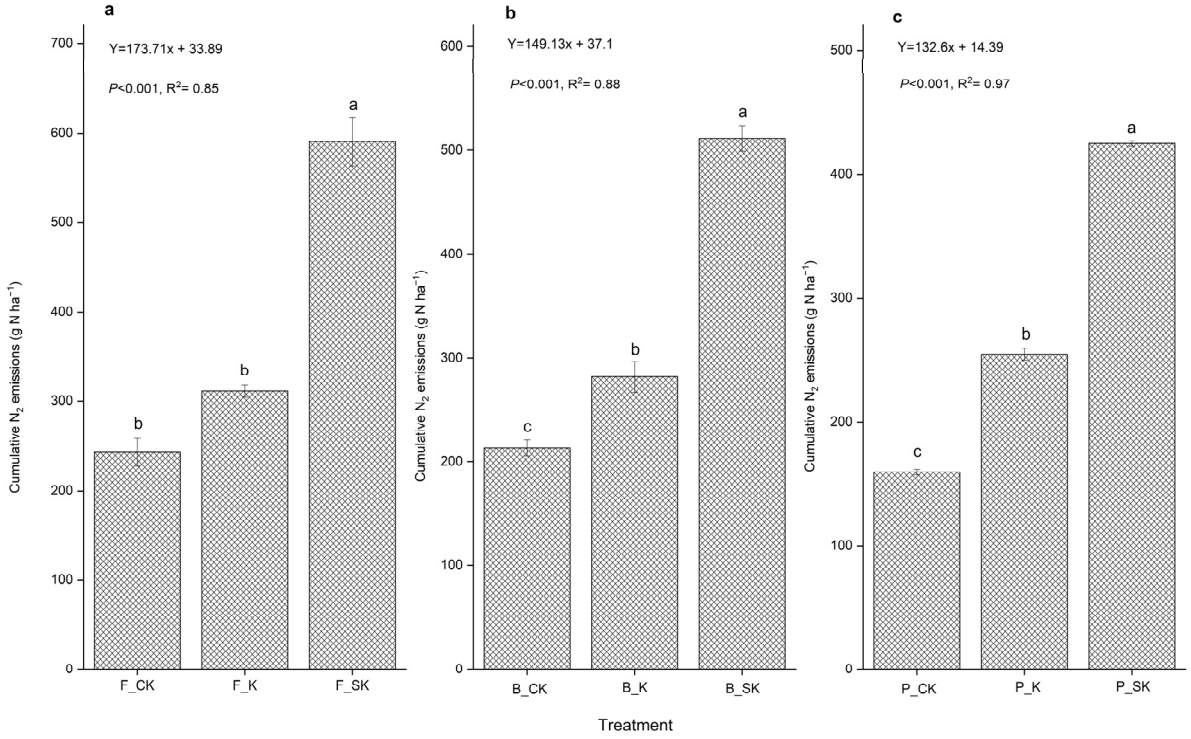


**Figure 4.** Cumulative $N_2$ emissions during the incubation period in non-amended treatment (CK), $KNO_3$, and straw plus $KNO_3$ treatments collected from (**a**) fluvo-aquic soil, (**b**) black soil, and (**c**) paddy soil. Error bars show the standard error of each treatment (n = 3). Data are expressed as mean ± standard error (n = 3). Lowercase letters denote a significant difference between average values at $p = 0.05$.

**Table 3.** Comparison of cumulative $N_2O$ and $N_2$ emissions and their stoichiometric ratios for the three soil types considering the column of the similar treatment (control, $KNO_3$, or straw plus $KNO_3$). Data are expressed as mean ± standard error (n = 3). Lowercase letters in the same column denote a significant difference between tested average values at $p = 0.05$.

| Parameter | $N_2O$ (g N ha$^{-1}$) | | | $N_2$ (g N ha$^{-1}$) | | | $N_2O + N_2$ (g N ha$^{-1}$) | | | $N_2O/(N_2O + N_2)$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control (CK) | $KNO_3$ (K) | Straw + $KN_3O$ (SK) | Control (CK) | $KNO_3$ (K) | Straw + $KN_3O$ (SK) | Control (CK) | $KNO_3$ (K) | Straw + $KN_3O$ (SK) | Control (CK) | $KNO_3$ (K) | Straw + $KN_3O$ (SK) |
| Fluvo-aquic soil | 73.6 ± 2.2 $^{a}$ | 164.2 ± 3.2 $^{a}$ | 234.2 ± 6.3 $^{a}$ | 242.7 ± 15.3 $^{a}$ | 311.0 ± 6.6 $^{a}$ | 590.1 ± 27.3 $^{a}$ | 316.4 ± 17.4 $^{a}$ | 475.2 ± 9.3 $^{a}$ | 824.4 ± 24.9 $^{a}$ | 0.23 ± 0.01 $^{c}$ | 0.35 ± 0.0 $^{a}$ | 0.28 ± 0.01 $^{a}$ |
| Black soil | 68.3 ± 1.2 $^{b}$ | 144.0 ± 3.6 $^{b}$ | 205.7 ± 13.2 $^{b}$ | 213.1 ± 7.6 $^{b}$ | 281.6 ± 15.0 $^{b}$ | 511.4 ± 12.5 $^{b}$ | 281.4 ± 8.3 $^{b}$ | 425.6 ± 18.2 $^{b}$ | 717.1 ± 14.0 $^{b}$ | 0.24 ± 0.01 $^{bc}$ | 0.34 ± 0.01 $^{a}$ | 0.29 ± 0.02 $^{a}$ |
| Paddy soil | 56.7 ± 1.0 $^{c}$ | 115.6 ± 8.4 $^{c}$ | 180.1 ± 6.4 $^{c}$ | 159.4 ± 2.1 $^{c}$ | 254.6 ± 4.7 $^{c}$ | 424.6 ± 2.1 $^{c}$ | 216.1 ± 1.3 $^{c}$ | 370.3 ± 8.1 $^{c}$ | 604.7 ± 7.8 $^{c}$ | 0.26 ± 0.01 $^{ab}$ | 0.31 ± 0.02 $^{a}$ | 0.30 ± 0.01 $^{a}$ |

### *3.4. Stoichiometric Ratios of $N_2O/(N_2O + N_2)$ from the Three Different Soil Types*

Overall, compared with all soil types in the current study, the $N_2O/(N_2O + N_2)$ product ratio was higher in the black soil with a maximum of 0.55, while in paddy soil, there was a minimum of 0.11. The daily emission ratios of $N_2O/(N_2O + N_2)$ were the highest in F_K and lowest in F_CK for fluvo-aquic soil type in the current study (Figure 5), while the average values were 0.22, 0.32, and 0.27 from F_CK, F_K, and F_SK treatments, respectively, with minimum and maximum ratios of 0.13 and 0.53.

The daily emission ratios of $N_2O/(N_2O + N_2)$ were the highest in B_K and lowest in B_CK for the black soil type in the current study (Figure 5), while the average values were 0.23, 0.31, and 0.27 from B_CK, B_K, and B_SK treatments, respectively, with minimum and maximum ratios of 0.14 and 0.55.

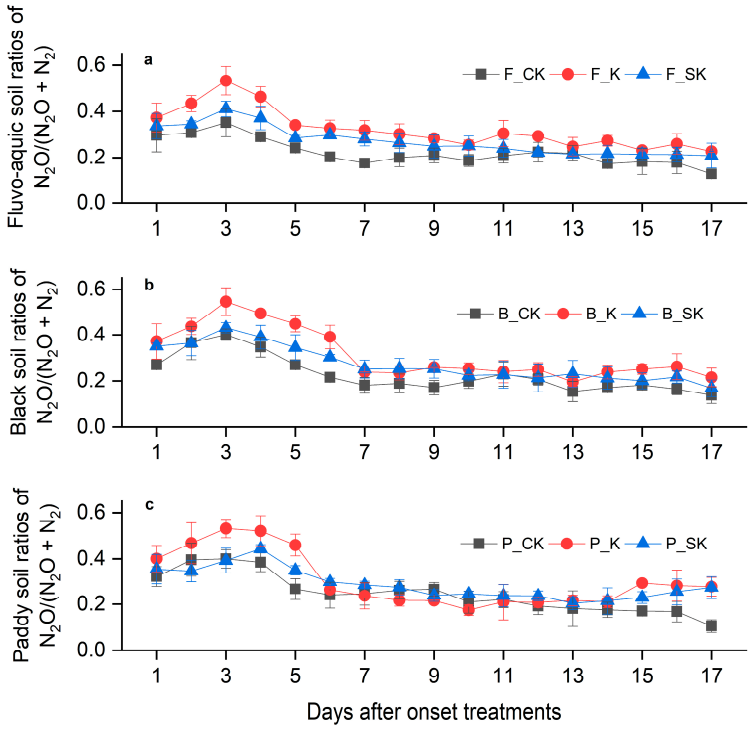


**Figure 5.** $N_2O/(N_2O + N_2)$ emission ratios during the incubation period in the non-amended treatment (CK), $KNO_3$, and straw plus $KNO_3$ treatments collected from (**a**) fluvo-aquic soil, (**b**) black soil, and (**c**) paddy soil. Error bars show the standard error of each treatment (n = 3).

The daily emission ratios of $N_2O/(N_2O + N_2)$ were the highest in P_K and lowest in P_CK for the black soil type in the current study (Figure 5), while the average values were 0.25, 0.31, and 0.29 from P_CK, P_K, and P_SK treatments, respectively, with minimum and maximum ratios of 0.11 and 0.53. In fact, after onset treatments, the product stoichiometric ratios increased in all treatments, with the effect being more pronounced in K treatments for all three soil types; then, the emission ratios gradually decreased till the end of the incubation period. Higher stoichiometric ratios of $N_2O/(N_2O + N_2)$ were observed in $KNO_3$ treatments and the lowest in controls for all three soil types. The differences in the stoichiometric ratios of $N_2O/(N_2O + N_2)$ between the soil types were small and insignificant (Table 3).

## 4. Discussion

### *4.1. Effect of Straw and Nitrate Amendments on $N_2O$ and $N_2$ Emissions and Their Stoichiometric Ratios*

It has been reported that straw application together with nitrate can increase the rate of denitrification [28] by supplying extra substrates known as electron donors in the form of an energy source [43,44]. In our experiment, with the increase in incubation time, straw amendment combined with $KNO_3$ caused a slight increase in $N_2O$ emissions (Figure 1) where the peaks occurred on the third incubation day and then gradually decreased to the minimum till the end of incubation in all treatments for all three soil types. At the same time, $N_2$ emissions (Figure 2) drastically increased with the increment being more significant in the straw plus $KNO_3$ treatments for all three soil types; after the peaks, the emissions slowly decreased till the end of incubation. This phenomenon probably occurred because $N_2O$ reduction began to exceed $N_2O$ production after the soil had met certain conducive denitrification conditions [5] and $NO_3^-$ content fell below a certain threshold level [45]. In the current study, we observed a decreasing trend between initial and final soil $NO_3^-$ (Table 2) where $NO_3^-$ depletion was attributed to the production and reduction of $N_2O$ emissions causing high $N_2$ emissions as a final denitrification product.

This is a valid phenomenon because in our study, the soil moisture was set at 80% WFPS in anaerobic conditions, resulting in more $N_2$ emissions. Our findings are in agreement with the previous studies that reported that the addition of crop residues could decrease and/or slightly increase $N_2O$ emissions and drastically increase $N_2$ emissions by lowering the stoichiometric ratio of $N_2O/(N_2O + N_2)$ and stimulating microbial immobilization in soil [8,46]. Our results contradicted denitrification observations that were previously reported by saying that high nitrate concentrations and high organic C inputs could still inhibit $N_2O$ reduction [44]. Perhaps because in moist soils, electron donors like labile C are known to be limiting factors for denitrification, thereby controlling the denitrification rate directly [27]. Another fact is that microorganisms responsible for denitrification use labile C as an electron donor for all reduction steps, especially from $NO_3^-$ to $N_2$ [18,47].

Due to the fact that lower $N_2O$ emissions coupled with significantly higher $N_2$ emissions were observed in all treatments for all three soil types. The highest $N_2$ emissions were more pronounced in the straw plus $KNO_3$ treatments for all three soil types where a more rapid decrease in soil $NO_3^-$ concentrations was caused by high denitrification loss. It was previously reported that $N_2O$ was not utilized by denitrifiers as a terminal electron acceptor [45,48]. However, the current results showed that straw plus $KNO_3$ treatments emitted gaseous N, which sightly increased $N_2O$ with a drastic increase in $N_2$ emissions due to more increment of $N_2O$ reduction rates with high nitrate. This was explained by a more rapid decrease in $NO_3^-$ content compared with the $KNO_3$ treatments alone for all three soil types (Table 2). Therefore, the results show that organic matter amendment can mitigate $N_2O$ emissions in soils with high $NO_3^-$ content due to higher $N_2O$ reduction to $N_2$ in the presence of conducive denitrifying conditions. The previous studies about straw return to soils reported controversial straw effects on mitigation of $N_2O$ emissions, mainly based on the nature of the soil—either paddy or upland—and, also, $NO_3^-$ content in the soil. For example, some studies showed that straw return can mitigate $N_2O$ emissions [30,49], while others showed that straw return can significantly increase $N_2O$ emissions [50,51]. Wei et al. [8] reported an increase in $N_2O$ and $N_2$ emissions from straw incorporation combined with synthetic N fertilizer.

Moreover, lower $N_2O$ (Figure 1) but higher $N_2$ (Figure 2) emissions in all treatments were observed during the whole incubation period for all three soil types (fluvo-aquic, black, and paddy soils), especially in straw plus $KNO_3$ treatments in the presence of conducive denitrification conditions (high soil moisture, high labile C, favorable temperature, etc). Our results also showed that straw plus $KNO_3$ treatments resulted in more $N_2O$ and $N_2$ emissions compared with controls, mainly because they favored more bacterial growth so that they could facilitate denitrification to occur in all three soil types. To sustainably obtain desired soil fertility, which facilitates crop production, the combination of straw plus $KNO_3$ in the same field should be promoted instead of burning straw regardless of the initial amount of $NO_3^-$ in the soil. However, the previous study reported that when the initial $NO_3^-$ is very high in the intensively managed soils, it was previously recommended to not simultaneously apply $NO_3^-$ with straw in order to avoid high $N_2O$ emissions [8]. This observation was due to the very high N fertilizer that was applied to that particular vegetable soil. Appropriate N use efficiency should be maintained where supplying the amount of N fertilizer that is needed by the crops at the right time should be encouraged just to avoid overloading $NO_3^-$ into farmlands. This will help to mitigate very high $N_2O$ emissions that may be emitted into the atmosphere.

### *4.2. Effects of Soil Moisture on $N_2O$, $N_2$ and Their Stoichiometric Ratios of $N_2O/(N_2O + N_2)$*

Gaseous N losses such as $N_2O$ and $N_2$ emitted from managed soil are due to the reduction in N available in the soil, potentially representing a substantial loss of applied N fertilizers [5,52]. In the current study, $NO_3^-$ concentrations were more depleted at a rate of more than 99% in treatments that combined both $KNO_3$ and straw for all three soil types (Table 2). One of the main factors for this high reduction with more $N_2$ emissions was a high soil moisture content of around 80% WFPS. In our current study, when compared

with the black and paddy soils, the cumulative $N_2O$ and $N_2$ fluxes were higher in the fluvo-aquic soil, with a maximum of 234.2 ± 6.3 and 590.1 ± 27.3 g N $ha^{-1}$ from F_SK treatment, respectively, during the incubation period. Additionally, average cumulative $N_2O$ and $N_2$ fluxes also showed the same trend (Table 3). Our findings were consistent with the previous literature where higher reductions of $N_2O$ to $N_2$ emissions were reported due to high soil moisture content compared with low soil moisture content [53,54]. Soil moisture is known to control microbial activities and is an important soil variable for soil gas emissions. Soils with less WFPS emit $N_2O$ emissions by nitrification with a maximum of 20% WFPS [55,56], while conducive microbial denitrification to produce and reduce $N_2O$ to $N_2$ emissions requires more than 60% WFPS [18,53,55]. When WFPS is greater than 60%, the soil pores are filled with water and this phenomenon limits the amount of available $O_2$ in those soil pores, leading to soil anaerobic conditions that are conducive to the production of $N_2O$ and even reduction of $N_2$ emissions. Under such conditions, the soil $NO_3^-$ is reduced by facultative anaerobic bacteria to $NO_2^-$, $NO_2^-$ to $N_2O$, and then $N_2O$ to $N_2$ emissions (Figures 6 and 7) [18,56,57]. However, the optimum WFPS for nitrification and denitrification processes to occur varies with different soil types [33,58]. Using German fine-loamy soil, Ruser et al. [59] analyzed how soil moisture levels between 40% and 98% WFPS affected $N_2O$ emissions, and the results showed that denitrification ($N_2O$ and $N_2$ emissions) increased when soil moisture was more than 60% WFPS. Nitrification was the main process producing $N_2O$ at 35–60% WFPS, while almost all $N_2O$ emitted through denitrification at 70% WFPS [55]. The study was conducted about the effects of different soil moisture levels on $N_2O$ and $N_2$ emissions in Australian soil [53]. Their results showed that when the soil was at 80% and 100% WFPS, $N_2$ emissions were more than $N_2O$ emissions by a factor of 8 and 17, respectively. It should be noted that optimum WFPS for $N_2O$ production and reduction of $N_2$ emissions to occur may vary with climatic zones.

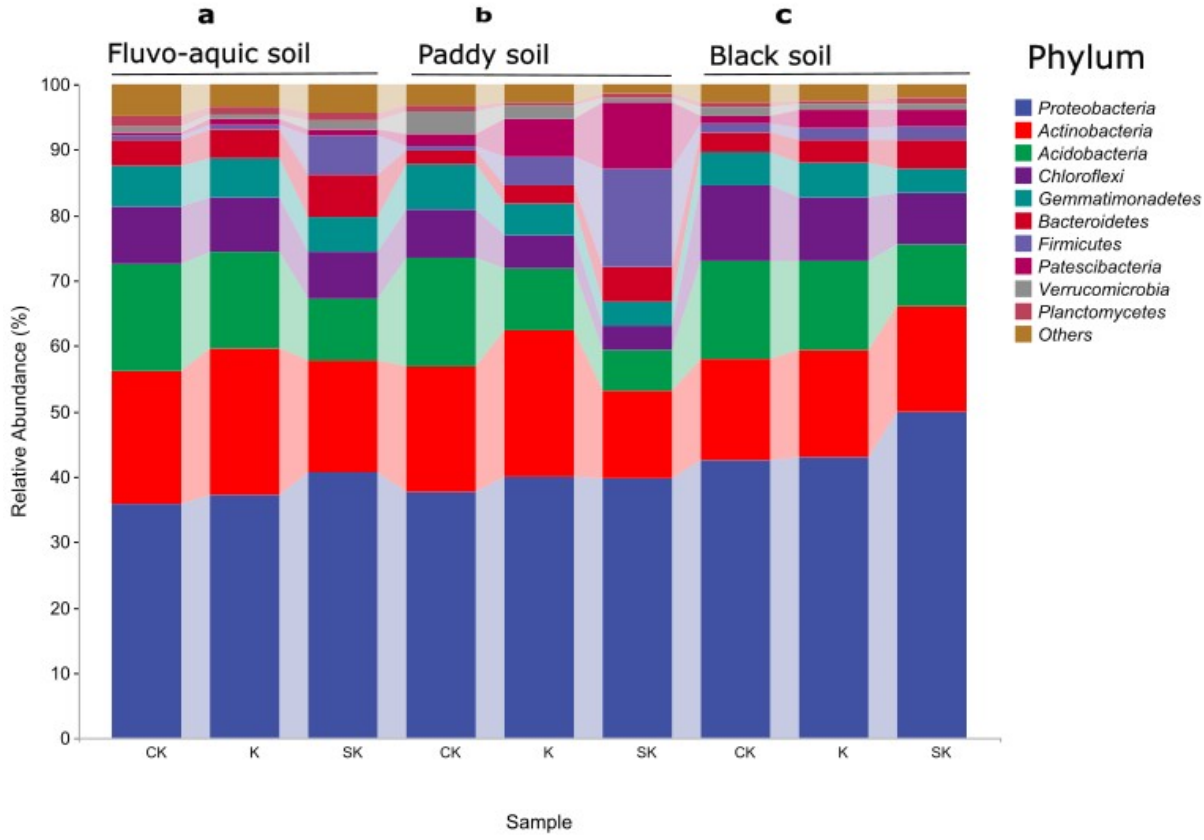


**Figure 6.** Bacterial community composition at phylum level at the end of incubation period in the non-amended treatment (CK), $KNO_3$, and straw plus $KNO_3$ treatments collected from (**a**) fluvo-aquic soil, (**b**) paddy soil, and (**c**) black soil.

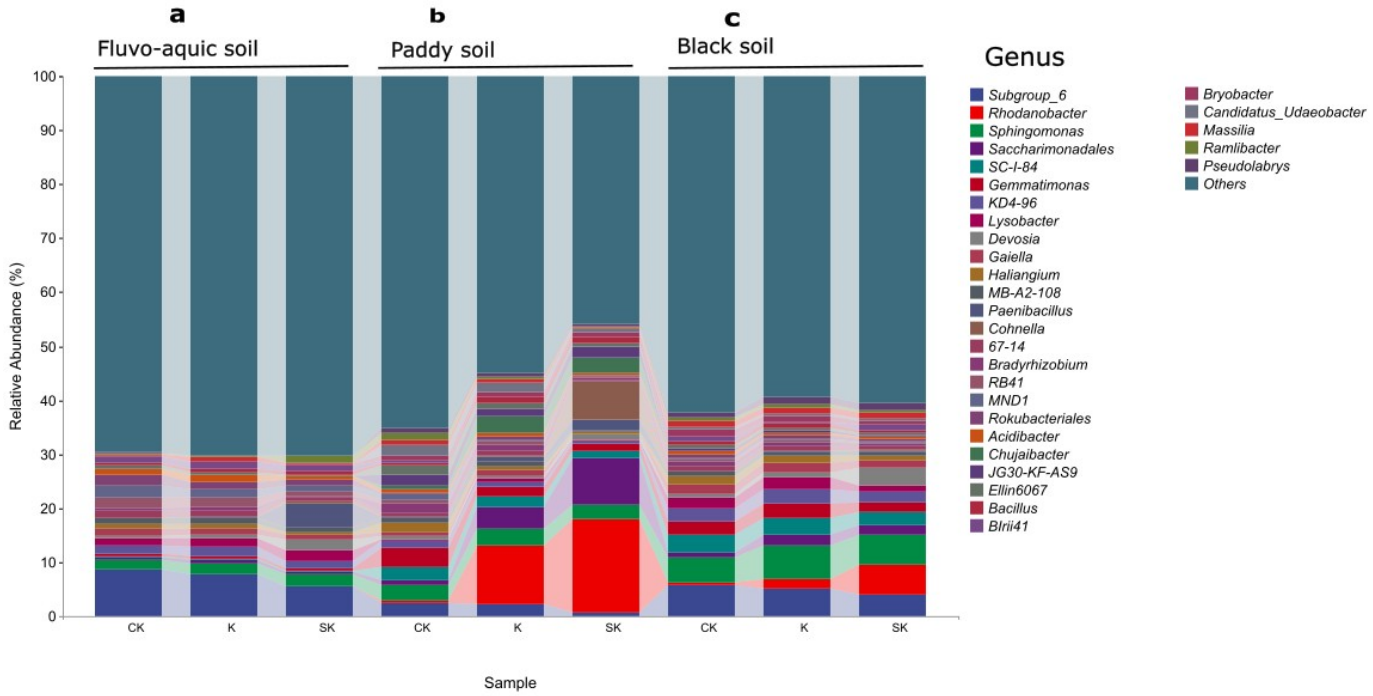


**Figure 7.** Bacterial community composition at genus level at the end of incubation period in the non-amended treatment (CK), $KNO_3$, and straw plus $KNO_3$ treatments collected from (**a**) fluvo-aquic soil, (**b**) paddy soil, and (**c**) black soil.

In the current study, stoichiometric ratios of $N_2O/(N_2O + N_2)$ were also influenced by the trends of $N_2O$ and $N_2$ emissions, with observed low values ranging from 0.11 to 0.55 for all three soil types. This observation is consistent with the previous literature that reported both $N_2O$ and $N_2$ emissions after straw and $KNO_3$ amendment [8].

### *4.3. Effect of Straw and Nitrate Amendments on Bacterial Community Composition*

Overall, cumulative $N_2O$ and $N_2$ emissions were higher in the fluvo-aquic soil compared with the black and paddy soils during the incubation period (Table 3). The higher emissions observed from the fluvo-aquic soil type were boosted by the availability of a conducive denitrifying environment like higher soil moisture, soil pH and C/N ratio, and required nitrate and temperature in acceptable ranges. This is obviously due to the fact that organic C from straw enhanced the electron donor for $N_2O$ reduction where C availability also increased microbial respiration in soils along with a decreased $O_2$, thus creating anaerobic microsites for denitrifying microorganisms in all three soil types. In our current results, bacterial community composition at the phylum level was dominated by Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, and Gemmatimonadetes (Figure 6). At the genus level, bacterial community composition was dominated by Subgroup_6, Sphingomonas, and Gemmatimonas (Figure 7). Microbial populations of any particular soil type are responsible for nitrification and denitrification processes [8,60,61]. The denitrification process is mainly carried out by microorganisms like phototrophs, organotrophs, and lithotrophs, which are responsible for delivering energy from light, organic carbon, and inorganic N, respectively [61]. In our results, more microbial populations observed were for denitrification mostly—such as Subgroup_6, Sphingomonas, Saccharimonadales, Paenibacillus, Bacillus, and Pseudolabrys. On the contrary, Rhodanobacter and Saccharimonadales were present more in paddy soil. However, autotrophic bacteria like *Nitrosomonas* and *Nitrobacter* that mainly carry out nitrification [62] were not observed in our studied soil types. Other soil environmental factors also play a key role in microbial activity whereby there are some suitable ranges for denitrifiers to operate. The influence of soil environmental factors—for example, soil moisture, soil pH, temperature, C/N ratio,

and dissolved $O_2$—were reported [5,54], but the suitable ranges for better denitrifying microorganisms to operate smoothly may vary from soil to soil.

Further evidence to support our current $N_2O$ and $N_2$ emissions was previously reported: e.g., Pan et al. [27] reported that straw amendment could significantly increase nosZ gene abundance, which was related to more $N_2$ production in agricultural soils. It was previously reported in studies that there was an inhibitory effect of $N_2O$ reduction to $N_2$ due to high $NO_3^-$ concentrations (over 40–50 mg $NO_3^-$-N $kg^{-1}$ dry soil), arguing that $N_2O$ was not utilized by denitrifiers as a terminal electron acceptor [32,45]. However, our current results clearly show that $N_2O$ was utilized by denitrifiers as a terminal electron acceptor, hence resulting in high $N_2$ emissions and low $N_2O/(N_2O + N_2)$ ratios in the current study. This may be because of high soil moisture (80% WFPS), available carbon due to straw amendment, and even more abundance and increased activity of recently identified clade II nosZ genes of denitrifiers for $N_2O$ reductase enzymes. These clade II nosZ genes of denitrifiers were reported to potentially consume $N_2O$ in soils and emit more $N_2$ [63,64]. In further research, straw quantity and quality, types of synthetic N fertilizers, and their rates may be compared when evaluating the impacts of crop straw incorporation on denitrification ($N_2O$ and $N_2$ emissions).

## 5. Conclusions

Rice straw amendment combined with N fertilizer increased both $N_2O$ and $N_2$ fluxes compared with control or $KNO_3$ treatments in all three soil types (fluvo-aquic, black, and paddy soils). The overall trends in the three soil types for both $N_2O$ and $N_2$ emissions were as follows: control < $KNO_3$ < rice straw plus $KNO_3$ treatments. Therefore, this indicates that straw amendment in combination with $KNO_3$ can stimulate a high denitrification rate (lower $N_2O$ and higher $N_2$), whereas their effect on stoichiometric ratios of $N_2O/(N_2O + N_2)$ highly depends on soil nitrate concentration, oxygen level, soil moisture content, and labile C. The current study underscores that rice straw amendment in combination with N fertilizer can generally trigger denitrification with less increment on soil $N_2O$ but higher $N_2$ emissions under conditions favoring denitrification regardless of the soil type. Therefore, we recommend incorporating crop straws and combining them with chemical N fertilizer in order to enhance agricultural economic benefits.

**Author Contributions:** Conceptualization, F.B. and C.H.; methodology, F.B.; software, F.B.; validation, C.H.; formal analysis, F.B.; investigation, F.B., A.T. and W.D.; resources, W.D. and X.L.; writing—original draft preparation, F.B.; writing—review and editing, F.B., W.D., A.T., M.R., X.L., Y.Z. and C.H.; visualization, F.B., A.T. and M.R.; supervision, C.H.; project administration, W.D., X.L., Y.Z. and C.H.; funding acquisition, C.H. and X.L. All authors have read and agreed to the published version of the manuscript.

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