



## Article

# Short-Term Straw Return Combined with Nitrogen Fertilizer Alters the Soil Nitrogen Supply in Rice–Rapeseed Planting Systems

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**Abstract:** This study aimed to assess the influences of short-term straw return combined with nitrogen (N) fertilizer on crop yield, soil properties, the bacterial community, and soil nitrogen cycling gene abundance in a rice–rapeseed planting system. A two-year field experiment was conducted in a paddy field from 2019 to 2021. There were four treatments in the experiment: –N–S, no N applied with no straw return; –N+S, no N applied with straw return; +N–S, N applied with no straw return; and +N+S, N applied with straw return. The results showed that short-term straw return combined with N fertilizer could increase crop yield and N use efficiency. N fertilizer application had a positive effect on *Gemmatimonadota* and *Desulfobacterota* abundance. Straw returning had a positive effect on *Desulfobacterota* and *Proteobacteria* abundance. N fertilization significantly increased the abundance of *amoA*-AOA, *amoA*-AOB, and *nxB* in agricultural soils. Straw return and N fertilization were not conducive to denitrification. We conclude that short-term straw return combined with N fertilizer in rice-growing areas not only increase crop yield and improve crop N uptake but also increase SOM, total N, and  $\text{NH}_4^+$  and improve the soil microbial activity and N use efficiency.

**Keywords:** straw return; soil nitrogen cycling; rice–rapeseed planting system; bacterial community composition; nitrogen cycling gene abundance



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## 1. Introduction

Crop residue is a considerable renewable resource with abundant organic carbon and mineral nutrients [1,2]. As the largest agricultural producer, China produces more than 800 million tons of crop straw each year. Crop straw is rich in organic components and has been widely used in fields to promote carbon sequestration in the soil [3,4]. The Yangtze River Basin is the largest rice production area in China, with a sown area of approximately 20 million  $\text{hm}^2$  and a total output of 138 million tons, contributing 70% of the total rice. In addition, the Yangtze River basin is also the main rapeseed-producing area in China, with an area of more than 6 million  $\text{hm}^2$  planted with rapeseed, accounting for approximately 91% of the national rapeseed area [5,6].

Generally, a large amount of crop residue is burned directly in the field to rapidly dispose of the straw for the next planting. However, straw burning has many adverse environmental and ecological impacts. Straw burning releases large amounts of pollutants, such as  $\text{PM}_{2.5}$ ,  $\text{SO}_2$ ,  $\text{CO}$ ,  $\text{NH}_3$ , VOC, and  $\text{NO}_x$ , and has a significant impact on global warming [7]. In recent years, people's consciousness of environmental protection has gradually strengthened, laws and regulations prohibiting the burning of straw have been gradually improved, and straw is being increasingly used in agricultural production

activities [8]. Previous studies have documented the positive and significant impact of straw use on bacterial community structure and fungal community structure under short-term or long-term straw return [9,10].

Nitrogen transformations in the soil are complicated because nitrogen can occur in different forms, soluble and gaseous forms, or as organic and inorganic compounds [11]. Arable soils receive considerable amount of N from anthropogenic activities, which largely contributed to a stable increase in global grain and cash crop production in the past few years [12,13]. Previous studies have shown that N fertilization increases mineral N contents and particulate organic matter and decreases soil pH and microbial biomass [14–16]. Changes in soil properties such as soil texture, available nutrient content, organic matter, and aggregate stability can be induced by N fertilizer application to improve crop yields [17,18]. According to a systematic analysis of microbial communities affected by global change disturbances, 84% of the 38 studies showed a significant sensitivity of the soil microbial community composition to chemical fertilization [19]. The abundances of bacteria in the ureolytic and chitinolytic communities significantly changed in response to N fertilization. Therefore, N addition enhances soil ecosystem functionality by potentially impacting the composition, aggregation, stability, diversity, and activity [20–23] of microbial communities, which further contributes to soil carbon (C) availability, N mobilization, and cation exchange capacity [24–26]. In addition, the application of N fertilizer will directly affect the soil N cycling functional micro-organisms, including N fixing, nitrification, and denitrification. N fertilizer changed the living environment of the dominant microbial communities, thereby altering DNA retesting and gene copy number [27].

Additionally, studies have shown that straw return can effectively promote C and N cycling in soil, improve soil enzyme activity, increase the complexity of microbial networks, and improve soil ecology [27–29]. Nitrification and denitrification are the two main microbial pathways of  $\text{N}_2\text{O}$  emission from soil.  $\text{N}_2\text{O}$  is a by-product of nitrification and an intermediate product of denitrification [30,31]. The addition of straw provides a good environment for the survival of micro-organisms, stimulates the growth of micro-organisms, and affects nitrification and denitrification processes, thus affecting the concentrations of total N and  $\text{NO}_3^-$ -N in the soil, as well as  $\text{N}_2\text{O}$  emissions [32].

However, the effects of straw return on crop yield, soil nutrients, and the bacterial community in short-term trials are unclear, especially in rice–rapeseed planting systems, where there are still gaps in research on the sequential return of rice straw and rapeseed straw [33,34]. Therefore, the aim of this study was to investigate the dynamics of yield, nitrogen uptake, soil properties, the bacterial community, and N cycling functional genes in rice–rapeseed planting systems after different field management and N fertilizer application treatments, which could provide scientific data on the potential short-term impacts of these treatments for comparison with short-term field experiments.

## 2. Materials and Methods

### 2.1. Site Description

The field experiment was performed in Wuxue City ( $29^\circ 54' 17''$  N,  $115^\circ 30' 19''$  E), Hubei Province, China. This region has a subtropical monsoon climate with an annual mean temperature of  $16.8^\circ\text{C}$ , an annual mean precipitation of 1409 mm, and an annual mean sunshine duration of 1900 h. The soil type is classified as Albic Luvisol according to the FAO classification. The basic soil properties at a depth of 0–20 cm prior to planting were as follows:  $30.8\text{ g kg}^{-1}$  soil organic matter, pH 5.5,  $177.0\text{ mg kg}^{-1}$  alkali-hydrolyzable N,  $24.9\text{ mg kg}^{-1}$  available P, and  $104.7\text{ mg kg}^{-1}$  available K. The area has good irrigation and drainage conditions.

### 2.2. Experimental Design

The planting system was summer rice and winter rapeseed. The experiment was conducted from May 2019 to May 2021. There were four treatments in the field trial, each replicated across four plots 4 m in width and 5 m in length. These treatments included

no nitrogen application without and with straw return (−N−S and −N+S) and nitrogen application without and with straw return (+N−S and +N+S). The application rates of fertilizer and returned straw are shown in Table 1. Rapeseed straw (41.2% C, 0.81% N) was returned in rice season, and rice straw (36.4% C, 0.93% N) was returned in rapeseed season. The returned straw was incorporated into the soils at a depth of 0–20 cm. For rice, straw returned and planted was in June, harvested was in October. Then, 50% of the N fertilizer was applied as basal fertilizer, 30% was applied during the tillering stage, and the remaining 20% was applied during the booting stage. For rapeseed, straw was returned and planted in November, and harvest was in May. For the N fertilizer, 50% was applied as basal fertilizer, 20% was applied during the overwintering stage, and an additional 30% was applied during the initiation of the stem elongation stage. The P and K fertilizers were applied as basal fertilizers at 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>−1</sup> and 90 kg K<sub>2</sub>O ha<sup>−1</sup> before rice planting and at rates of 90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>−1</sup> and 120 kg K<sub>2</sub>O ha<sup>−1</sup> before rapeseed planting. The N, P, and K fertilizer form was urea (46% N), superphosphate (12% P<sub>2</sub>O<sub>5</sub>), and potassium chloride (60% K<sub>2</sub>O).

**Table 1.** The rate of N fertilizer and returned straw (kg hm<sup>−2</sup>).

| Treatment | Rice Season |                | Rapeseed Season |            |
|-----------|-------------|----------------|-----------------|------------|
|           | N           | Rapeseed Straw | N               | Rice Straw |
| −N−S      | 0           | 0              | 0               | 0          |
| −N+S      | 0           | 6000           | 0               | 7500       |
| +N−S      | 180         | 0              | 180             | 0          |
| +N+S      | 180         | 6000           | 180             | 7500       |

### 2.3. Grain Yield and N Uptake

During the harvest period, grain yield was measured from a 10 m<sup>2</sup> area in each plot via manual harvesting and machine threshing. Aboveground samples were collected from 8 plants per plot and divided into straw and grain components. All aboveground samples were oven dried at 60 °C to a constant weight and then weighed and analyzed for N concentration by using a H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub> digestion and flowing injection analyzer (AA3, Bran and Luebbe, Norderstedt, Germany). Total N uptake in plants and N use efficiency (NUE) were calculated using equations as follow [35]:

$$\text{N uptake} = N_{\text{Straw}} \times \text{Straw} + N_{\text{Grain}} \times \text{Grain}$$

$$\text{NUE} = (N_{\text{uptake in N add}} - N_{\text{uptake in control}}) / \text{N application rate}$$

where and N<sub>Straw</sub> and N<sub>Grain</sub> represent N concentration in straw and grain, respectively. Straw and Grain represent the biomass. N<sub>uptake in N add</sub> and N<sub>uptake in control</sub> represent N uptake of the added N fertilizer and the unfertilized control treatment, respectively.

### 2.4. Soil Sampling and Measurement

Soil sampling was carried out at rice and rapeseed harvest, and samples were collected from a depth of 0–20 cm. Five points were randomly sampled from each plot and thoroughly mixed to form a composite sample. The samples were divided into 2 parts: one part was stored at 4 °C for analysis of physicochemical properties, and the other part was stored at −80 °C for DNA extraction.

The soil pH was measured using a glass electrode with a soil-to-water ratio of 1:2.5. The soil organic matter (SOM) and total nitrogen (TN) contents were determined according to the methods outlined by Hu et al. [36]. Soil nitrate nitrogen (NO<sub>3</sub><sup>−</sup>) and ammonium nitrogen (NH<sub>4</sub><sup>+</sup>) were extracted with 2 M KCl solution and measured with a flow injection analyzer (AA3, Germany).

### 2.5. qPCR and High-Throughput Sequencing

Soil DNA was extracted from a 0.25 g soil sample by using the E.Z.N.A.<sup>®</sup> soil DNA Kit (Omega Biotek, Norcross, GA, USA). The concentration and purity of the extracted DNA were independently checked using an ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at 260 nm. qPCR of the nitrification functional gene (*amoA*-AOB and *amoA*-AOA), denitrification functional gene (*nxB*, *nirS*, and *nosZ*) and nitrogen-fixing functional gene (*nifH*) was performed using a LightCycler 480 (CFX96, Bio-Rad, Hercules, CA, USA). The primers used for each gene were determined with reference to Wang et al. [27]. The 15 µL PCR mixture contained 7.5 µL of SYBR Green Mix, 0.7 µL of each primer, and 2 µL of the DNA template. The bacterial 16S rRNA gene was paired-end sequenced (PE300) on an Illumina MiSeq platform according to standard protocols with the primer sets 338F and 806R. These operations were performed at Majorbio Bio Pharm Technology Co., Ltd. (Shanghai, China).

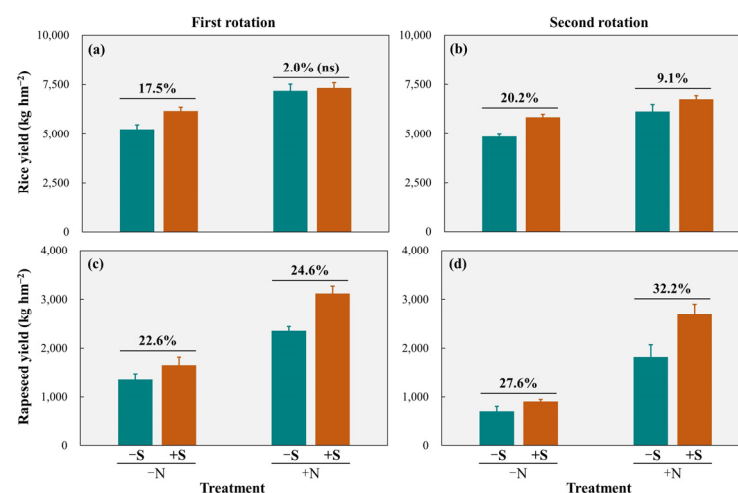
### 2.6. Data Analysis

Microsoft Excel (version 2013) was used for data processing. Origin (version 2021) was used for data visualization. Significant differences in the data were analyzed via ANOVA, and Duncan's multiple comparison test was used to compare the means for each variable via SPSS Statistics (version 20.0).

## 3. Results

### 3.1. Grain Yield

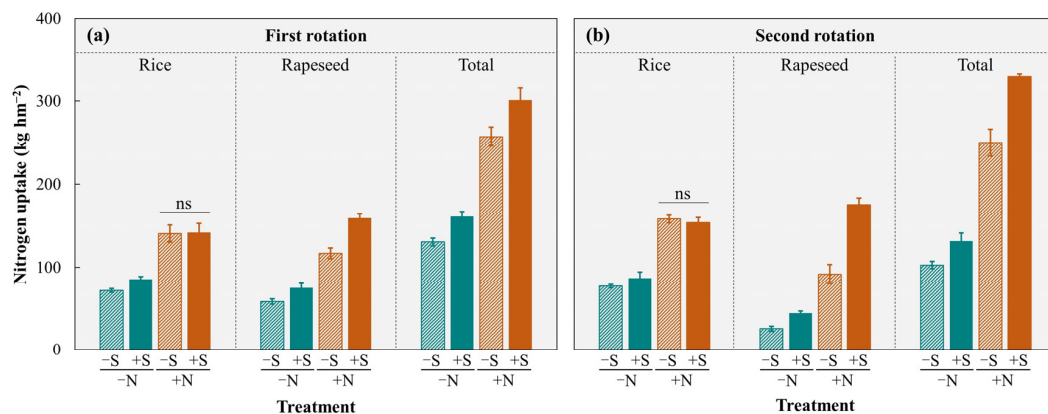
Straw return significantly altered the grain yield in the rice–rapeseed planting system under the different N application conditions (Figure 1). As expected, grain yields were improved by N fertilization, and the yield increase in rapeseed was greater than that in rice in all rotations. In the first rotation, the rice yield was the lowest in the −N−S treatment and increased by 17.5% in the −N+S treatment. However, there was no difference in rice yield between +N−S and +N+S. The yield increases in rapeseed were 22.6% and 24.6% without and with N fertilization, respectively. In the second rotation, the yield increases in both the rice and rapeseed plants caused by straw return were greater than those in the first rotation. The increase caused by straw return was greater in the −N treatment than in the +N treatment during the rice season, while the opposite was observed in the rapeseed season.



**Figure 1.** Effect of N fertilizer and straw return on rice yield (a,b) and rapeseed yield (c,d). The values on the lines represent the yield increase resulting from straw return. The “ns” on the lines represents that the yield difference was not significant between treatments.

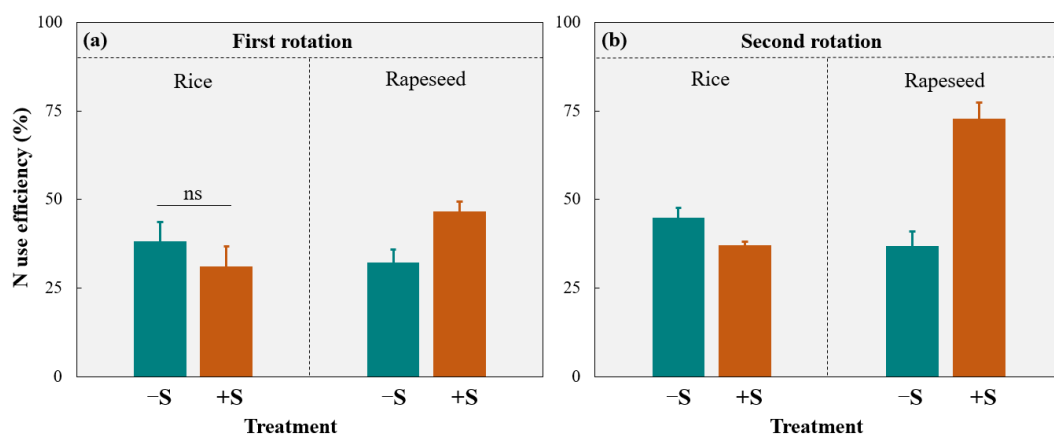
### 3.2. N Uptake and Use Efficiency

The effects of N fertilizer and straw return on the N uptake of rice and rapeseed were not consistent (Figure 2). Like those of grain yield, the N uptake of rice and rapeseed significantly increased in response to N application. Straw return improved N uptake in most cases, but not in the two rice seasons under N application. For the total annual N uptake, the lowest N uptake was observed in the  $-N-S$  treatment, and the highest was in the  $+N+S$  treatment. Straw return increased the total annual N uptake by 23.4% and 17.0% without and with N application, respectively, in the first rotation and improved the total annual N uptake to 27.9% and 31.6%, respectively, in the second rotation. For different crops, the response of nitrogen uptake in rice season to straw returning was effective without nitrogen fertilizer application. However, in the rapeseed season, the application of N fertilizer improved the increase in N uptake caused by straw return.



**Figure 2.** Effects of straw return and N fertilizer on the N uptake of rice and rapeseed in the first (a) and second (b) rotation. The “ns” on the lines represents that the yield difference was not significant between treatments. The unmarked yield data represent significant differences ( $p < 0.05$ ) between the  $-S$  and  $+S$  treatments under the same N application conditions.

As shown in Figure 3, the response of N use efficiency to straw return differed between the two rotations. In the first season, straw return did not markedly change the N use efficiency of rice but did significantly increase that of rapeseed. By the second season, the N use efficiency of rice had decreased from 44.9% in the  $-S$  treatment to 37.0% in the  $+S$  treatment, and the N use efficiency of rapeseed had nearly doubled in response to straw return.



**Figure 3.** Effect of straw return on N use efficiency in the first (a) and second (b) rotation. The “ns” on the lines represents that the difference was not significant. The unmarked data represent significant differences ( $p < 0.05$ ) between the  $-S$  and  $+S$  treatments.

### 3.3. Soil Properties

Two years of straw return had different effects on the soil properties (Table 2). The lowest SOM content was observed in the  $-N-S$  treatment, at 28.4 and 27.5 g kg<sup>-1</sup> in the rice and rapeseed harvest periods, respectively. Straw return obviously increased the SOM content, and the increase was greatest in the plots without N fertilization during the rapeseed season. The TN content did not differ during the rice season but increased in response to straw return during the rapeseed season, mainly because of the change in the NO<sub>3</sub><sup>-</sup> content. There was no difference in the pH value between the treatments.

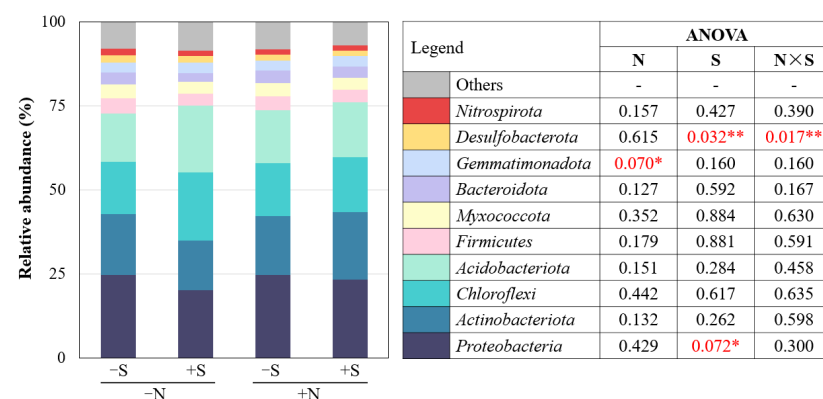
**Table 2.** Soil properties under different treatments in the second season for rice and rapeseed.

| Year-Stage                | Treatment | SOM<br>(g kg <sup>-1</sup> ) | TN<br>(g kg <sup>-1</sup> ) | NH <sub>4</sub> <sup>+</sup><br>(mg kg <sup>-1</sup> ) | NO <sub>3</sub> <sup>-</sup><br>(mg kg <sup>-1</sup> ) | pH     |
|---------------------------|-----------|------------------------------|-----------------------------|--------------------------------------------------------|--------------------------------------------------------|--------|
| 2020-<br>Rice harvest     | $-N-S$    | 28.4 c                       | 1.64 a                      | 3.70 b                                                 | 1.08 a                                                 | 6.33 a |
|                           | $-N+S$    | 29.7 bc                      | 1.63 a                      | 3.70 b                                                 | 1.06 a                                                 | 6.24 a |
|                           | $+N-S$    | 30.8 b                       | 1.66 a                      | 4.90 a                                                 | 1.16 a                                                 | 6.18 a |
|                           | $+N+S$    | 32.3 a                       | 1.66 a                      | 4.84 a                                                 | 1.15 a                                                 | 6.21 a |
| 2021-<br>Rapeseed harvest | $-N-S$    | 27.5 c                       | 1.51 c                      | 2.84 a                                                 | 1.27 b                                                 | 6.27 a |
|                           | $-N+S$    | 32.5 a                       | 1.91 a                      | 2.98 a                                                 | 1.44 b                                                 | 6.23 a |
|                           | $+N-S$    | 30.7 b                       | 1.68 b                      | 2.90 a                                                 | 1.28 b                                                 | 6.19 a |
|                           | $+N+S$    | 32.8 a                       | 1.82 a                      | 3.25 a                                                 | 1.87 a                                                 | 6.16 a |

Note: Different letters indicate statistical significance at the  $p = 0.05$  level for the same year in the same column. SOM, soil organic matter; TN, total nitrogen; NH<sub>4</sub><sup>+</sup>, ammonia nitrogen; NO<sub>3</sub><sup>-</sup>, nitrate nitrogen.

### 3.4. Bacterial Community Composition

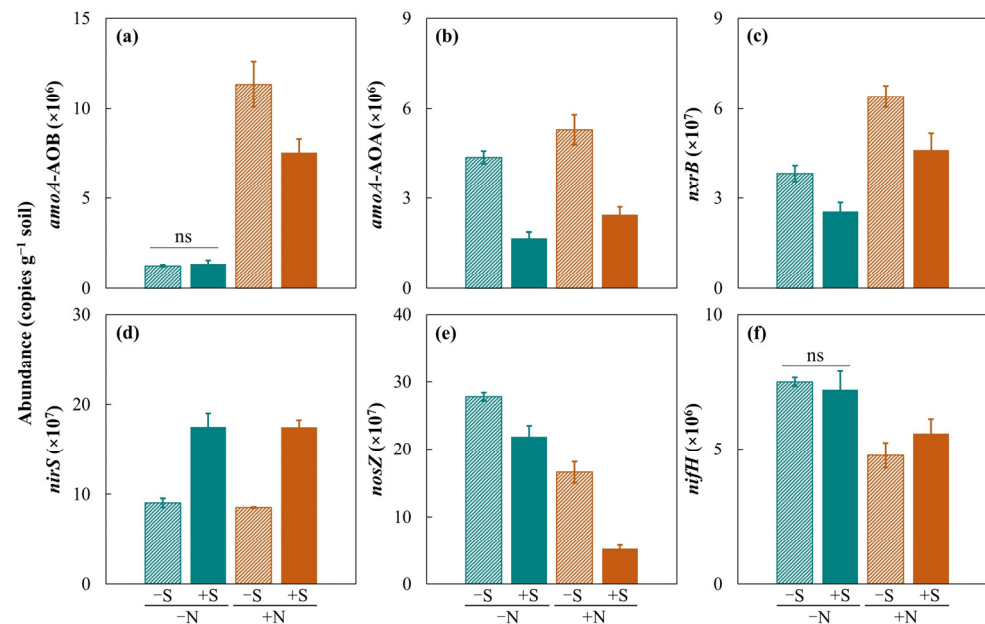
The relative abundances of different bacterial phyla are shown in Figure 4. The dominant soil bacterial communities were *Proteobacteria*, *Actinobacteriota*, *Chloroflexi*, *Acidobacteriota*, *Firmicutes*, *Myxococcota*, *Bacteroidota*, *Gemmatimonadota*, *Desulfobacterota*, and *Nitrospirota* in all the treatments, accounting for 91.43–92.96% of the total operational taxonomic units (OTUs). The relative abundances of the top four phyla (*Proteobacteria*, *Actinobacteria*, *Chloroflexi*, and *Acidobacteria*) were 72.65%, 73.79%, 75.02%, and 76.00%, respectively, in the  $-S-N$ ,  $+S-N$ ,  $-S+N$ , and  $+S+N$  treatments. The relative abundance levels of the bacterial phyla varied substantially among the different N and S treatments. Under the  $+S-N$  treatment, the relative abundance of *Proteobacteria* was significantly lower than that under the other three treatments, and the relative abundance of *Chloroflexi* and *Acidobacteriota* was significantly greater than that under the other treatments. Analysis of variance (ANOVA) revealed that  $+N$  was significantly and positively correlated with *Gemmatimonadota* ( $p < 0.1$ ), and  $+S$  was significantly and positively correlated with *Desulfobacterota* ( $p < 0.05$ ) and *Proteobacteria* ( $p < 0.1$ ).  $+S+N$  was significantly and positively correlated with *Desulfobacterota* ( $p < 0.05$ ).



**Figure 4.** Relative abundance levels of dominant bacterial groups (phylum level) under different nitrogen fertilizer and straw return treatments. \* represents a significant difference at  $p < 0.1$ , \*\* represents a significant difference at  $p < 0.05$ .

### 3.5. Soil Nitrogen Cycling Genes

The application of N fertilizer significantly increased the abundances of *amoA*-AOB, *amoA*-AOA, and *nirB*, especially at  $-S$ , which reached  $11.4 \times 10^6$ ,  $5.3 \times 10^6$ , and  $6.4 \times 10^7$  copies  $g^{-1}$  soil, respectively; these values were the highest among those of the four treatments (Figure 5). However, the abundances of *amoA*-AOA and *nirB* were the lowest in the  $-N+S$  treatment ( $1.7 \times 10^6$  and  $2.6 \times 10^7$  copies  $g^{-1}$  soil, respectively), indicating that the application of N fertilizer had a positive effect on the abundances of *amoA*-AOB, *amoA*-AOA, and *nirB*, and the abundances were the highest when they combined with no return to the field.



**Figure 5.** Variations in the copy numbers of nitrification (*amoA*-AOB (a), *amoA*-AOA (b), and *nirB* (c)), denitrification (*nirS* (d) and *nosZ* (e)), and nitrogen fixation (*nifH* (f)) genes after two years. The “ns” on the lines represents that the difference was not significant. The unmarked data represent significant differences ( $p < 0.05$ ) between the  $-S$  and  $+S$  treatments.

The *nirS* abundance reached a maximum value of  $17.5 \times 10^7$  copies  $g^{-1}$  soil in the  $+S$  treatment, and N fertilizer application had no obvious effect on *nirS* abundance. The abundance of *nosZ* at  $+N+S$  was the lowest, while that at  $-N-S$  was the highest ( $5.2 \times 10^7$  and  $27.8 \times 10^7$  copies  $g^{-1}$  soil, respectively). The abundance of *nifH* in the  $-N$  treatment was significantly greater than that in the  $+N$  treatment, while the abundance of *nifH* in the  $+N-S$  treatment was the lowest, while that in the  $-N-S$  treatment was the highest ( $4.8 \times 10^7$  and  $7.5 \times 10^7$  copies  $g^{-1}$  soil, respectively).

## 4. Discussion

### 4.1. Effects of Straw Return on Crop Yield and N Uptake

Previous studies have shown that straw return can effectively increase crop yield and nutrient uptake [37,38], which is consistent with the results of our study. Our study showed that straw return had a yield-increasing effect on both crops, with rapeseed showing a significantly greater yield increase than rice, especially under  $+N$  conditions. By investigating the reasons for the crop yield increase, it was found that straw return significantly increased the number of grains per spike and the number of seeds per pod of rapeseed. In addition, studies have shown that the uptake efficiency of N fertilizer also affects crop yield, especially during the rapeseed season [39]. In this study, N uptake during the rapeseed season was significantly greater in the  $+N+S$  treatment than in the other treatments, which is consistent with the yield characteristics of the rapeseed season. The rice season is characterized by high temperature, internal flooding, and active microbial activity, and the

concentrations of phenols and organic acids produced by straw decomposition are high, which has a certain toxic effect on the root system, thus limiting the yield increase in rice. In addition, the straw decomposition rate in the rapeseed growing season is relatively slow, the nutrient release cycle is long, and the toxic effect caused by straw decay is relatively weak, which is conducive to increasing the yield of rapeseed [40].

#### 4.2. Effects of Straw Return on Soil Properties

In this study, the soil pH has no significant changes after short-term straw return and N fertilization. Shan et al. [41] indicated that there was a negative correlation between soil pH and the amount of nitrogen applied; the more N was applied, the lower the soil pH was. This confirms the finding that N-containing fertilizers tend to acidify soils, mainly because most fertilizers first provide N in the form of  $\text{NH}_4^+$ , releasing  $\text{H}^+$  ions upon oxidation [42]. In addition, straw return significantly improved soil chemical properties, as indicated by the SOM, total N,  $\text{NH}_4^+$ , and  $\text{NO}_3^-$  content, and among these treatments, the +S treatment performed significantly better than the −S treatment. These results confirmed that straw return may be a potential option in agricultural practices for converting nitrate N to solid N, minimizing  $\text{N}_2\text{O}$  emissions, limiting  $\text{NO}_3^-$  and  $\text{NH}_4^+$  leaching, and better conserving nitrogen in the soil [43]. The application of N fertilizers or NP mineral fertilizers had different degrees of positive effects on the N content of the soil (total N,  $\text{NH}_4^+$ , and  $\text{NO}_3^-$ ), which may be due to the accumulation of N as a hydrolyzable unknown N fraction due to N fertilizer or NP mineral fertilizers [44].

#### 4.3. Effects of Straw Return on the Bacterial Community Composition

Interactions between plants, soils, and bacteria play a key role in nutrient cycling, and in the study of rice–rapeseed planting system, the process of soil nitrogen absorption and utilization is closely related to the bacterial community. Numerous previous studies have revealed that the SOC content and the availability of soil with inorganic N and pH are associated with a shift in the soil bacterial community structure [45,46]. In this study, the predominant bacterial phyla in the four treatment groups were *Proteobacteria* and *Actinobacteria*, with averages of 23.25% and 17.60%, respectively, which are consistent with the findings of Bay et al. [47]. Although straw return and N application resulted in differences in soil nutrient content, the effects of the two treatments on the main bacterial categories were not significant. *Proteobacteria*, *Nitrospirae*, *Firmicutes*, *Bacteroidota*, and *Actinobacteria* are considered copiotrophic groups, while *Acidobacteria* and *Chloroflexi* are typical oligotrophic bacteria [48]. Nitrogen is a key limiting factor for soil micro-organisms, and the application of N can change microbial biomass, activity, and species composition [28]. Dai et al. [49] reported that the relative abundances of *Actinobacteria* and *Proteobacteria* were significantly and positively correlated with N, which is consistent with our findings that the relative abundances of *Actinobacteria* and *Proteobacteria* were greater under +N conditions than under −N conditions in our study. However, the relative abundance of *Acidobacteria* was found to decrease under +N conditions in this study, especially under +N−S conditions, where the relative abundance of *Acidobacteria* was 19.8%, which was significantly greater than that under −N−S conditions (14.2%). These findings may be closely related to the soil pH. Previous studies in several ecosystems have also revealed that soil pH strongly affects changes in bacterial community structure and diversity [50]. The total N,  $\text{NH}_4^+$ , and  $\text{NO}_3^-$  contents in the soil increased slightly after N fertilizer application (+N) but became more pronounced after straw return (+S) increased, indicating that straw return better retained N in the soil, which may be due to the decomposition of straw to provide a source of N to the soil and simultaneously mitigated the toxic effects of nitrogen fertilizer application on microbial populations.

#### 4.4. Effects of Straw Return on Soil Nitrogen Cycling Genes

Previous studies have reported the effect of fertilization regimes on canonical ammonia oxidizers. The addition of N fertilizers increased the abundance of AOB, which was

consistent with this study. The abundance of AOB is inherently sensitive to environmental changes and is often treated as a pertinent biological indicator [51,52]. The cell size of AOB is larger than that of AOA, and there are different ammonia oxidation pathways between them. These factors may affect the ammonium availability in soil [53]. Taken together, our findings imply that AOB constitute a more important target group for N management to reduce N losses and improve N use efficiency. The abundance of *nxrB* increased in response to organic N fertilizers, which was consistent with the results of the present study [27]. The application of organic N fertilizers not only supports *Nitrospira* growth through the increased availability of nitrite, which drives *Chemolithotrophy*, but may also stimulate their growth through mixotrophic or heterotrophic metabolism [54].

Functional genetic analysis of the denitrification pathway reveals complex interactions between the soil environment and the denitrifying community. The *nirS* gene is the only functional gene significantly associated with the reduction in pH and macro- and micronutrients in soil [55], and soil moisture and temperature, total N concentration, soil  $\text{NO}_3^-$  concentration, soil organic matter (SOM), dissolved organic carbon (DOC), and pH all affect *nirS* abundance [56]. Henry et al. [57] reported that the application of N fertilizer under crop rotation conditions did not affect the abundance of the *nosZ* gene, an oxidative nitrite reductase that regulates the last step of the denitrification process. Studies involving denitrification genes in soil indicate that soil pH, moisture, C, and  $\text{NH}_4^+$  correlate with *nosZ* genes. Denitrification genes and rates are most abundant as the soil pH and moisture increase [56]. If the water, pH, and organic carbon conditions are not conducive to denitrification, the *nosZ* abundance will decrease. Previous studies have shown that there is no significant relationship between *nifH* abundance and N fertilizer application. The *nifH* abundance data likely include the abundance of symbiotic N-fixers, which live in plant-controlled environments and are therefore less likely to respond to environmental change. Straw return and N fertilizer can increase the soil C/N ratio, cause nitrogen limitation, and stimulate *nifH* gene expression [58].

## 5. Conclusions

In the present study, we found that short-term straw return in combination with N fertilizer significantly increased crop yield in a rice–oilseed rape rotation. Straw return and N fertilizer application improved N use efficiency, especially during the rapeseed season, when the N uptake rate was most significantly increased. After two years of experiments with different treatments, soil physicochemical properties such as SOM, total N,  $\text{NH}_4^+$ , and  $\text{NO}_3^-$  changed differently. Moreover, straw return and N fertilizer application can significantly impact the soil bacterial community, especially *Desulfobacterota*. N fertilization significantly increased the abundance of *amoA*-AOA, *amoA*-AOB, and *nxrB* in the soil. However, straw returning and nitrogen application are not conducive to denitrification, which will reduce the abundance of *nosZ*.

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