Abiotic and biotic effects of long-term straw retention on reactive nitrogen runoff losses in a rice–wheat cropping system in the Yangtze Delta region

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ABSTRACT

Straw retention has been widely recommended as a nutrient-conserving measure that effectively increases soil fertility and soil nitrogen (N) availability. However, despite its efficacy in reducing reactive N (Nr) runoff losses, there is currently little information available regarding the influence of distinct straw return modes with respect to potential Nr runoff loss. In this study, based on a 6-year period of straw incorporation in a field under rice–wheat rotation with a yield of two crops a year, we sought to evaluate the overall responses of Nr runoff losses and N changes in the surface water to soil biotic and abiotic factors. Four different straw return modes (CK: no straw, W: wheat straw, R: rice straw, and S: both wheat and rice straw) were examined. The results revealed that compared with the CK treatment, the three modes of crop residue incorporation significantly reduced N runoff loss by 4.21 %–11.59 % ($P < 0.05$), of which the S mode of straw returning was found to be the most effective. The reductive effect of straw return was observed during the early period of rice growth (the basal and tiller fertilization stages, $P < 0.05$). Significant differences in both the soil abiotic and biotic factors were observed not only between the tillering and ripening stages of rice growth ($P < 0.01$), but also among the four straw return modes ($P < 0.05$). Structural equation modelling revealed that soil inorganic nitrogen (SIN, $r = 0.53$, $P = 0.007$) had the most direct positive effect on Nr runoff loss, but the effect was statistically compromised by the overall negative effects of soil organic carbon (SOC, $r = -0.33$, $P = 0.034$), pH ($r = -0.15$, $P > 0.05$), and bacterial community composition ($r = -0.10$, $P = 0.041$), and to a less extent by α-diversity ($r = -0.04$, $P > 0.05$). During the tillering stage of rice growth and in response to the S mode of straw return in particular, there was a significant enrichment of *Proteobacteria* ($P < 0.01$), thereby indicating the potentially prominent role of microbial N immobilization in reducing N losses. The links among N contents in paddy standing water, surface runoff, and soil biotic and abiotic factors established in this study will inform straw return strategies to reduce the levels of N-based pollution.

1. Introduction

From the perspective of both environmental and human health, N has become "too much of a good thing" owing to its dispersion from agricultural ecosystems and further conversion into pollutants, thereby contaminating the surrounding land and water bodies and impairing human health and well-being (Drinkwater and Snapp, 2007; Sutton et al., 2011). In monetary terms, Nr pollution is estimated to cause damage amounting to 0.3 %–3.0 % of the global gross domestic product (GDP) (Sutton et al., 2013). With respect to rice cultivation, the excessive release of Nr tends to be associated with lower nitrogen-use efficiency (NUE), particularly in China (approximately 31 %) (Galloway et al., 2008; Zhang et al., 2012), and notably in the Yangtze Delta region owing to the intensive nature of cultivation in this area (Zhao et al., 2012b), which has led to substantial Nr losses through surface runoff and leaching pathways (Zhao et al., 2012a; Xue et al., 2014; Zhang et al., 2016a).

The control and reduction of Nr runoff losses from their sources are the most effective preventive measures of N pollution (Zhang et al., 2016b). For instance, Nr runoff losses have been found to be reduced by 15 %–82 % via optimizing agronomic management practices (Zhang et al., 2016b), among which crop residue retention has been

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demonstrated to be significantly effective in reducing Nr runoff losses (Sainju et al., 2007; Tan et al., 2011; Liu et al., 2017). However, previous analyses of agricultural N losses have mostly been conducted in the absence of sufficient field monitoring activity, thereby resulting inaccuracy and large differences of Nr loss among studies (Xue et al., 2014; Xia et al., 2016; Wang et al., 2018a). In addition, it has been observed that Nr losses can also differ markedly due to site-specific peculiarities related to precipitation, topography, soil properties, and management (Basso and Ritchie, 2005; Fang et al., 2006; Liang et al., 2011). However, even when straw return is undertaken with an equivalent input of fertilizer, substantial differences in Nr loss have been observed in the same region (Liang et al., 2011; Xue et al., 2014; Xia et al., 2016; Wang et al., 2018a), and accordingly it is necessary to determine the optimal modes of straw return. It has been established that returning crop straw with a high C/N ratio ($>$30) can stimulate the microbial immobilization of soil ammonium (NH_4^+) and nitrate (NO_3^-) (Aulakh et al., 2001; Xia et al., 2016; Cheng et al., 2017), but whether soil microbiomes play an important role in regulating N concentration in the surface water of paddy field and closely relate to Nr runoff loss still remains poorly understood. It is also worth noting that soil bacterial communities can differ markedly according to the stage of rice growth (Edwards et al., 2015; Wang et al., 2016); therefore, it is reasonable to speculate that straw return could reduce Nr runoff loss during the specific period of rice growth. Based on the present knowledge about the soil bacterial communities in response to straw return, we further hypothesize that, with the amendment of straw, the enhanced soil C pool and stimulated bacterial phyla would directly or indirectly contribute in decreasing Nr runoff loss, and their effects might be more significant with the mode of consecutive seasonal straw return for the rice-wheat double cropping system.

In this study, following the 6-year program of straw incorporation, a field experiment was conducted to investigate the effects of different straw retention modes in the rice–wheat rotation system, with the following specific aims: (1) to quantify the Nr runoff loss loads and evaluate their potential characteristics during the entire rice production period; (2) to characterize soil physicochemical factors and bacterial community structure at different stages of rice growth; and (3) to investigate the direct and indirect relationships between Nr runoff losses and key soil abiotic and biotic factors. The findings from this study will provide new insights into the efficacy of straw return measures with respect to Nr runoff loss and the underlying mechanism.

2. Materials and methods

2.1. Description of the site and long-term experiment

The long-term straw retention experiment was conducted at Changshu Agro-Ecological Station (31°32′93″N, 120°41′88″E), a member station of the Chinese Ecosystem Research Network (CERN), Chinese Academy of Sciences, in the Yangtze Delta region. The site is a typical rice-wheat double cropping area of China and characterized by a subtropical monsoon, with a mean annual air temperature (MAT) of 23.7 °C and mean annual precipitation (MAP) of 1097.1 mm (2008–2018) of which approximately 70 % occurs during the summer rice season. The experimental paddy soil is classified as Anthrosol (FAO, 2006) derived from lacustrine sediments.

The long-term experiment was initiated in 2012 and set up as a randomized block design. Four experimental treatments (Table 1) with three replicate plots (size: 43.7 m^2) characterized by an equivalent input of NPK fertilizer were examined: (1) CK, without straw return; (2) W, wheat straw return only (in the rice-growing season); (3) R, rice straw return only (in the wheat-growing season); and (4) S, both wheat and rice straw return (in rice- and wheat-growing seasons of the rotation, respectively). In 2018, the tested summer rice (*Oryza sativa* L) was transplanted on June 18th and harvested on November 3rd. The synthetic N fertilizer (in the form of urea) was split into: 40 % as basal

Table 1

Field experimental treatments and managements during the rice growth season.

Treatment	CK	W	R	S
Chemical fertilizer application rate(N:P:K, kg ha^{-1})	240:15:60	240:15:60	240:15:60	240:15:60
Split N application ratio	4:2:4	4:2:4	4:2:4	4:2:4
Split K application ratio	5:0:5	5:0:5	5:0:5	5:0:5
Wheat/rice straw input rate (kg dry matter ha^{-1})	0/0	5500/0	0/10,000	5500/ 10,000

fertilizer, 20 % as tiller fertilizer, and 40 % as panicle fertilizer. Phosphorus (in the form of calcium superphosphate) was applied as basal fertilizer. Potassium (in the form of potassium chloride) was split into: 50 % as basal and 50 % as panicle fertilizer. Rice and wheat residues were chopped into pieces (5~10 cm in length) after each harvesting and fully rotary incorporated into the corresponding plots before the next crop transplantation.

To monitor the Nr runoff losses, identical cubic concrete tanks (1.5 $\times$ 1.2 $\times$ 1.2 m^3) were built beside the field for each plot through a piping system (Fig. S1). As dictated by local customs, flooding conditions were maintained after the rice plant sprouted leaves and the standing water layer was kept 3~5 cm depth, except for duration of later tiller stage and last two weeks before harvest according to local customs. The upper water-level holes were set up 7 cm above the ground surface for collecting runoff during the rice season.

2.2. Water and soil sampling

At the beginning the two weeks after each fertilization (Jun 12th, July 4th, and Aug 4th in 2018), for every two days, surface water was collected about 100 mL from five random locations in each plot, after which the sampling frequency was changed to once a week, once every two weeks, and finally once a month during the rice season from June to September in 2018. As for the runoff water, the runoff was carefully mixed in each tank and then sampled into a 100 mL polyethylene bottle after each runoff event during the whole experimental period, before which the depth of water in each tank was recorded. To prepare for the next runoff collection, the remaining water was discharged and cleaned. Half of per sample was filtered through a 0.45 μm membrane and the two portions were stored at 4 °C for chemical analysis and assayed within 24 h after sampling.

In 2018, soil samples were collected from depths of 0–15 cm at the rice tillering stage on July 10th and after rice ripening on September 27th (a week before rice harvest). For each plot, five cores (5 cm in diameter) were sampled randomly, mixed separately, and then homogenized by being gently passed through a 2 mm sieve to remove large roots and gravels. A portion of each sample was stored at 4 °C for additional air drying and subsequent chemical analysis and the other part was stored at -80 °C until molecular analysis was performed.

2.3. Chemical properties analysis

The filtered water sample was assayed for nitrate nitrogen (NO_3^--N) by dual-wavelength ultraviolet spectrometric method and ammonium nitrogen (NH_4^+-N) by indophenol blue method (Committee of Analytical Method of Water and Wastewater, 2002). The unfiltered part was measured for total nitrogen (TN) through alkaline potassium persulfate oxidation-ultraviolet spectrometric method (Rayment and Higginson, 1992).

Soil moisture was determined by oven drying at 105 °C for 12 h. Soil inorganic nitrogen (SIN, include NH_4^+, NO_3^- and NO_2^-) were extracted with 2 M KCl followed measurement by continuous-flow analyzer (San++, Skalar, The Netherlands). Soil organic matter (SOM) was analyzed by the $K_2Cr_2O_7$ oxidation method (Bao, 2000). Soil total nitrogen (SN) was determined by semi-micro Kjeldahl digestion method

(Lu, 2000). Soil available phosphorus (AP) was extracted using sodium bicarbonate and then measured by the molybdenum-blue method. Soil available potassium (AK) was measured using flame atomic absorption spectrophotometry. Soil pH and electrical conductivity (EC) was determined with a soil to water ratio of 1:2.5 and 1:5, respectively.

2.4. Molecular methods bioinformatics analysis

For each replicate of soil sample, genomic DNA was extracted from 0.5 fresh soil using a Soil DNA Kit (MP Biomedicals, Santa Ana, CA, United States) according to the manufacturer's protocols. The DNA concentration was monitored by Qubit 3.0 Fluorometer. The bacterial V3-V4 hypervariable region of the 16S rRNA gene was amplified by PCR using the primers "CCTACGGRRBGCASCAGKVRVGAAT" and "GGAC-TACNVGGGTWTCTAATCC." PCR reactions of 16S rDNA were performed in triplicate 25-μL mixture containing 2.5 μL of TransStart Buffer, 2 μL of dNTPs, 1 μL of each primer, and 20 ng of template DNA. Meanwhile, indexed adapters were added to the ends of the 16S rDNA amplicons at the same time to generate indexed libraries ready for downstream NGS sequencing on Illumina Miseq. High-throughput sequencing of total bacterial 16S rRNA genes was performed on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) using PE250 paired-end according to manufacturer's instructions.

The bacterial 16S rRNA gene data were processed using the Quantitative Insights Into Microbial Ecology (QIIME) 1.9.1 pipeline (Caporaso et al., 2010). Briefly, the sequences were binned into OTUs using a 97 % identity threshold, and the most abundant sequence from each OTU was selected as a representative sequence for that OUT. Taxonomy was assigned to bacterial OTUs against a subset of the Greengene 13.8 database. Usearch was used to remove chimera and aligned OTU representative sequences (Edgar, 2013). A subset of 47,176 sequences per sample was selected for downstream analyses. The raw sequences are available through the NCBI Sequence Read Archive (Accession No. PRJNA592598).

2.5. Statistical calculation and analysis

The volume (V) of runoff and loss flux (Q) of various forms of was calculated by the follows:

$$V_i\,(L) = D_i \times S \times 1000 \tag{1}$$

$$Q_i\,(kg\,ha^{-1}) = \sum_{i=1}^{n} C_i \times \frac{V_i}{43.7} \times 10^{-2} \tag{2}$$

where D_i (m) is the depth of water recorded per collection tank recorded after each runoff-producing rainfall event. S (m^2) equals to 1.8, which is area of the bottom of tank. C_i (mg L^{-1}) is the concentration of TN, NO$_3$-N and NH$_4^+$-N and i is the frequency number of runoff events occurred during whole rice growth season.

One way analysis of variance (ANOVA, *Duncan* multiple comparisons) was performed using SPSS 18.0 to separately compare the differences of N runoff losses, soil properties, α-diversity indexes and bacterial relative abundance at phylum level in response to four treatments and two rice growing stages. The ACE richness index and Shannon diversity index were generated using Mothur (Schloss et al. 2009). Analysis of the differential OTU abundance was performed using Wilcoxon rank sum tests with relative abundance from top ten phyla, and corresponding P values were corrected for multiple tests using a FDR set at 0.05 using R package edgeR (Robinson et al., 2010). Comparisons of the genera from top five bacteria phyla among the different groups (straw return modes and growth stages) were separately performed using STAMP (Parks et al., 2014). The bacterial community assemblage was also visualized by principal coordinate analysis (PCoA) based on the matrix weighted unifrac distance and also estimated by the permutation analysis of variance (PERMANOVA) test, which was conducted in R 3.2.1 (http:

//www.rproject.org/) with the VEGAN package (Dixon, 2003). Structural equation modelling (SEM) were conducted to evaluate the direct and indirect relationships of environmental factors, bacterial alpha and beta diversity on the Nr loss, prior to which a Pearson correlation analysis (SPSS 18.0) between TN loss and soil properties (two-tailed test) was performed to simplify the model. Here, the overall goodness of model fit was validated by the chi-square test (the model has a good fit when χ2 /df < 3 and $P > 0.05$), the (adjusted) goodness of fit index (GFI and AGFI; the model has a good fit when GFI > 0.9 and AGFI > 0.9) and the root mean square error of approximation (RMSEA; the model has a good fit when RMSEA < 0.05) (Schermelleh-Engel et al., 2003). In addition, standard total effects of observed variables were calculated (Delgado-Baquerizo et al., 2016). The endogenous variables of SEM were ln-transformed to improve normality and all data satisfied normal distribution and homogeneity of variance before the ANOVA analysis.

3. Results

3.1. Nr runoff losses and the N dynamic characteristics in the surface water

It was found that Nr loss via surface runoff was clearly influenced by straw return (Fig. 1 B, D, and F), with the observed seasonal TN cumulative loads under rice cultivation showing the following pattern: S ≈ W < R < CK ($P < 0.05$), of which an average between 76.2 % and 87.0 % was discharged in the NH$_4^+$-N form. Relative to the CK treatment, the W, R, and S treatments resulted in TN load reductions of 9.83 %, 4.21 %, and 11.59 %, respectively ($P < 0.05$).

In order to identify the processes contributing to the observed reductions in N loss, the Nr concentrations were closely monitored in paddy surface water throughout the rice-growing season (Fig. 1A, C, and E). Consistent with the N composition of runoff, NH$_4^+$-N comprised the largest proportion of Nr in the surface water. With the exception of the basal stage, the Nr content peaked after each fertilizer application, although urea was applied at the 4:2:4 ratio for the three stages, and rapidly decreased over time. The results revealed the following pattern of TN concentration in the surface water averaged over the entire rice-growing season: CK ≥ R ≥ S ≈ W (Table S1, $P < 0.05$). This pattern was found to be similar to the TN levels at the basal and tiller stages, whereas it was opposite to those at the panicle stage. This accordingly illustrated that a decrease in Nr loss occurred during the early stage after incorporating straw, which exceeded the enhanced effect observed at the panicle stage.

3.2. Soil physicochemical properties

Soil samples were collected at the tillering and ripening stages to further examine the variations in N characteristics, and accordingly found that soil properties showed significant differences between these two rice growth stages, and also among the modes of residue return (Table 2). Generally, the EC, SOC, SN, AP, AK, and particularly SIN content decreased, whereas pH increased with the progression of rice development from the tillering to the ripening stage. Compared with the CK treatment, over a 6-year period of straw incorporation, either single- (W and R) or double- (S) season returns significantly increased the SOC, SN, AP, and AK content at both stages ($P < 0.05$), which generally showed the following pattern: S ≥ R ≈ W > CK. However, the SIN content showed distinct changes. For instance, relative to the CK treatment, the SIN content under the S treatment was lower at the tillering stage but higher at the ripening stage ($P < 0.05$). As a consequence of residue retention, both pH and EC reduced at the tillering stage, but in the ripening stage, pH reduced while EC was not significantly different among the four straw return modes.
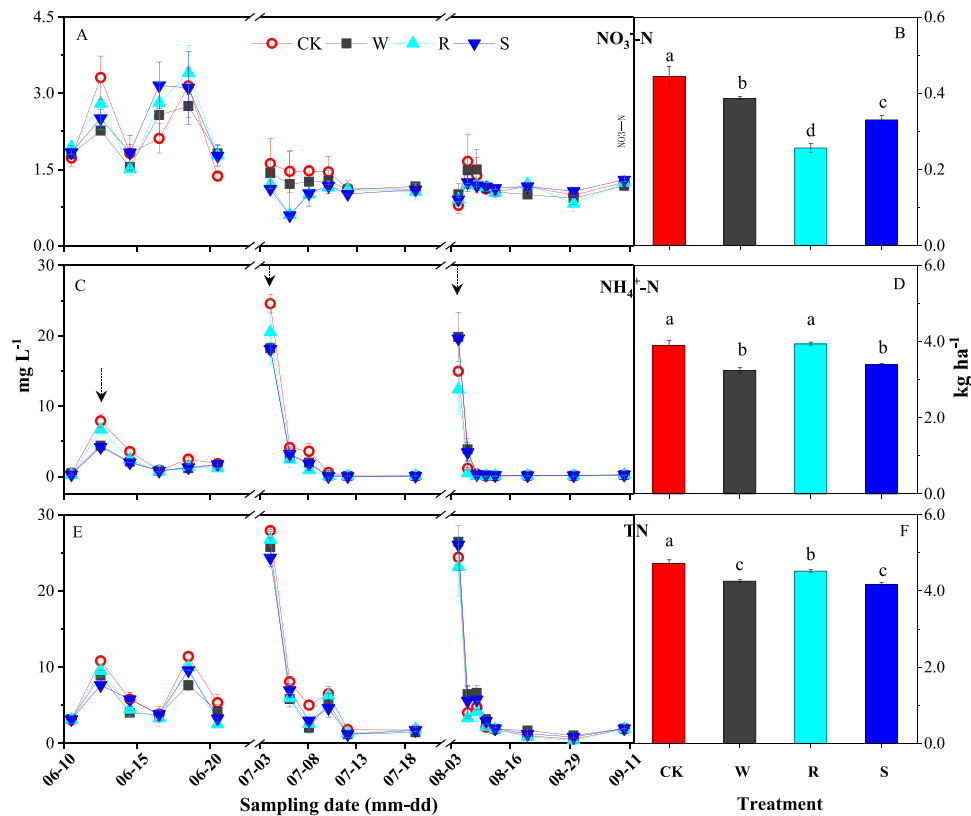
Fig. 1. Surface water changes and cumulative runoff losses of nitrate nitrogen (NO$_3^-$-N, A, B), ammonium nitrogen (NH$_4^+$-N, C, D), and total nitrogen (TN, E, F) during the entire rice-growing period. The arrows indicate fertilizer applications. Error bars represent standard errors of the mean values. Different letters above the bars indicate statistically significant differences at $P < 0.05$.

3.3. Bacterial α-diversity and community composition

To assess the effect of the different straw return modes on bacterial diversity at different stages of rice growth, the ACE and Shannon index values were determined (Fig. S2). However, although the differences were not significant, the values of both indices were lower from the tillering to the ripening stage. Unexpectedly, the Shannon index showed almost no appreciable changes in response to the treatments under the different modes of straw return, whereas ACE values for soil under residue treatments R and S were found to be higher than those determined for the CK treatment ($P < 0.05$).

The analysis of bacterial community composition at the phylum level provided further evidence of differences in response to different treatments at both the tillering and ripening stages (Fig. 2). These communities were predominated by bacteria of the phyla *Proteobacteria* (34.6 %–39.7 %), *Acidobacteria* (20.2 %–23.7 %), and *Bacteroidetes* (8.88 %–12.7 %), whereas the proportion of the remaining seven assessed phyla ranged between 1% and 10 %. Among the five most predominant phyla, it was observed that compared with the ripening stage, *Proteobacteria*, *Nitrospirae*, and *Chloroflexi* were enriched in the tillering stage, whereas the populations of *Acidobacteria* and *Bacteroidetes* were depleted (Fig. 3). Furthermore, the STAMP results revealed that during the tillering stage, seven (including *Thioalkalispira*, *Haliangium*, *Sideroxydans*, and *Devosia*) among the twelve genera showed a significant difference belonged to *Proteobacteria* (Fig. 4). All the straw return treatments (W, R, and S), particularly the double-season straw return (S), enhanced the *Proteobacteria* and *Nitrospira* abundances but reduced the *Latescibacteria* abundance during the tillering stage (Figs. 2 and 3, $P < 0.05$). Similar differences in response were also detected at the generic level (Fig. S3), with genera belonging to *Proteobacteria* showing the most significant responses to the W, R, and S treatments, and those in the *Acidobacteria* phylum being the most affected by the CK treatment.

The PCoA analysis clearly revealed distinctions in microbial distributions at both tillering and ripening stages of rice growth in response to the different straw return modes (Fig. 5). Responses at the tillering and ripening stages were separately clustered in the lower and upper portions of the PCoA plot, respectively. The PC1 axis indicted treatment-specific distribution at both stages of rice growth. Intriguingly, the PC1 axis data indicated that responses to the CK treatment differed from those to all the three straw return treatments, with distances generally following the order S > R > W. The PERMANOVA analysis confirmed the significant differences between the two growth stages and among the four treatments (Table 3). More importantly, the tillering stage was found to be a more important period with respect to bacterial community differentiation in response to the different treatments.

3.4. Relationships between Nr runoff loss and soil abiotic and biotic factors

The Pearson correlation analysis (Table S2) revealed the various interrelationships between different soil environmental factors and N runoff loss, whereas the SEM analysis (Fig. 6, Table 4) further indicated the direct and indirect effects of key soil biotic and abiotic variables on Nr loss. Datasets from the tillering and ripening stages of rice growth under each of the four treatments were fitted with the final model (Fig. 6), which explained 94 % of the N runoff losses. Generally, the straw return modes ($r_d = 0.08$, r_d: direct regression weight coefficient) and rice growth stages ($r_d = 0.11$) were found to directly affect runoff losses. SOC directly ($r_d = -0.33$) and indirectly ($r_i = -0.05$, r_i: indirect regression weight coefficient) affected the Nr loss by mediating α-diversity ($r = 0.98$); SIN ($r_d = 0.53$) showed the maximum positive coefficient directly affecting the N runoff loss and contributed to reductions in N loss by mediating bacterial community composition ($r = -0.76$), as did soil pH ($r_d = -0.15$, $r = -0.47$). However, only direct and weaker

Table 2
Soil properties for four field treatments after 6 years of fertilization.

Treatment	pH		EC (µm cm⁻¹)		SOC (g kg⁻¹)		SN (g kg⁻¹)		SIN (mg kg⁻¹)		AP (mg kg⁻¹)		AK (mg kg⁻¹)	
	Tillering	Ripening	Tillering	Ripening	Tillering	Ripening	Tillering	Ripening	Tillering	Ripening	Tillering	Ripening	Tillering	Ripening
CK	7.02a	7.11a	134a	98a	22.8c	21.1c	2.07c	1.96c	17.5a	9.1c	15.9b	13.5b	134b	133b
W	6.93ab	7.07ab	108c	93a	24.9b	23.0b	2.30b	2.10b	16.1ab	10.7ab	18.4ab	14.9b	140b	142ab
R	6.73bc	6.90bc	121b	100a	26.8a	24.6a	2.32b	2.30a	16.4ab	10.0bc	19.3ab	14.4b	157b	132b
S	6.51c	6.77c	114bc	94a	27.3a	25.3a	2.57a	2.31a	15.0b	11.3a	21.2a	17.4a	188a	148a

EC, soil electric conductivity; SOC, soil organic carbon; SN, soil total nitrogen; SIN, soil inorganic nitrogen; AP, available P; AK, available K. Different letters in the same column indicate statistically significant differences ($P < 0.05$) according to the Duncan test.

effects were detected with respect to bacterial community composition (r_d = -0.10) and α-diversity (r_d = -0.04).

4. Discussion

4.1. Response of Nr runoff losses risks to straw retention

Prominent differences in N losses through the hydrologic pathways are driven by regional differences in climatic conditions (Pandey et al., 2018), whereas appropriate fertilization measures have been found to effectively reduce N runoff loss (Wang et al., 2015a; Xia et al., 2016; Li et al., 2018; Zhang et al., 2018b). In the experimental rice–wheat cropping system investigated in the present study, we found that all straw return treatments significantly reduced N loss loads, although our results indicated that single-season straw incorporation (W and R) was less effective in this regard than double-season incorporation (S). Previous studies have speculated that this reductive effect could be attributed to the stimulation of bacterially mediated N immobilization (Moreno-Cornejo et al., 2014; Xia et al., 2017; Zhao et al., 2018a), which is consistent with findings that increases in the soil N immobilization rate and soil microbial biomass nitrogen (MBN) promote a decrease in N runoff and leaching (Wang et al., 2015b; Xia et al., 2018). In the present study, we noted that Nr loss was the lowest across all treatments at the basal fertilization stage, when urea was applied at a 4:2:4 (basal: tillering: panicle fertilization) ratio. We further discovered that the reduction in N loss indeed occurred during the earlier stages following the incorporation of crop residues, which exceeded the increasing effect at the panicle fertilization stage. These observed reductions in N concentrations can be explained in terms of the absorptive properties of straw and microbial immobilization of inorganic N (Kahlon et al., 2013), the latter of which has been shown to be strongly stimulated by residues with a high C:N ratio early after straw application. Furthermore, the immobilization of inorganic N temporarily exceeded the amount of Nr released from straw decomposition (Trinsoutrot et al., 2000; Nicolardot et al., 2001; Zhao et al., 2018b). Despite these findings, evidence for more direct and comprehensive causal factors is needed.

4.2. Changes in soil properties and bacterial communities

Physical and chemical gradients in agricultural soils are known to be dependent on crop growth and development over time (Krause et al., 2010). In the present study, we observed decreasing trends for all the assessed soil chemical factors (SOC, SN, SIN, AP, and AK) from the rice tillering to ripening stage. This pattern was anticipated, given the periodic supply of exogenous fertilizer in conjunction with a high microbial activity, which contributed to the effective tillering and prolific root growth in rice, is associated with a high accumulation and consumption of critical nutrients during the tillering stage (Sheehy et al., 1998; Ye et al., 2013). Expectedly and consistent with the findings of most previous studies, we found that 6 years of straw return, either as a single- or double-season application, promoted an increase in soil SOC, SN, AP, and AK (Smith et al., 2007; Powlson et al., 2008; Blanco-Canqui and Lal, 2009). More importantly, we found that crop residues, particularly those with double-season application, significantly reduced the SIN content during the tillering stage, whereas it promoted an increase during the ripening stage; the latter effect has also been found in a global meta-analysis (Wang et al., 2018b). Crop growth and N uptake could directly benefit from a higher content of organic matters continually supplied by seasonal straw input of the S mode (Wang et al., 2018b; Xia et al., 2018). These findings accordingly indicate that there may be a higher NUE during the tillering period or under the S mode, and thus a concomitant reduction in Nr losses (Staver and Brinsfield, 1998).

Fertilization regimes can have notable effects on the structure of soil bacterial communities, as previously demonstrated by long-term field experiments (Zhou et al., 2015; Zhang et al., 2018a). Generally, in the present study, there was little evidence of substantial changes in
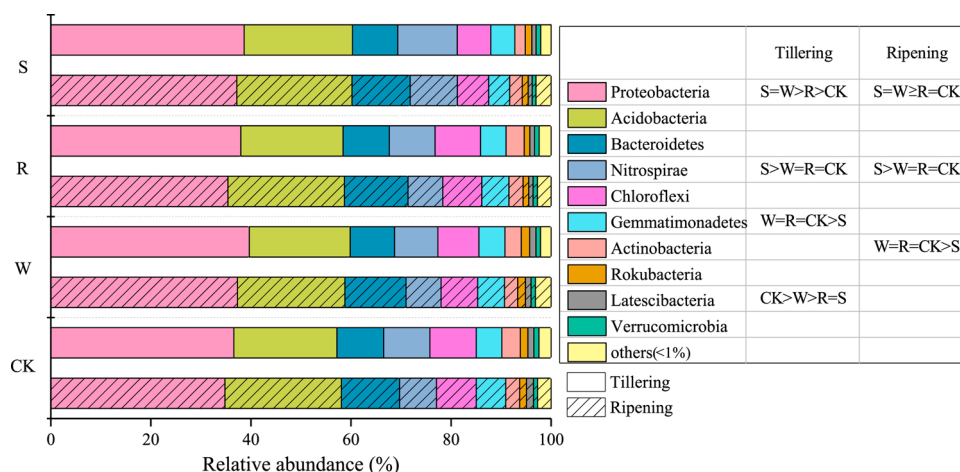
Fig. 2. Relative abundances of bacterial phyla under straw return during the two rice growth stages. The differences among treatments at the same stage were analyzed using Duncan test ($P < 0.05$).
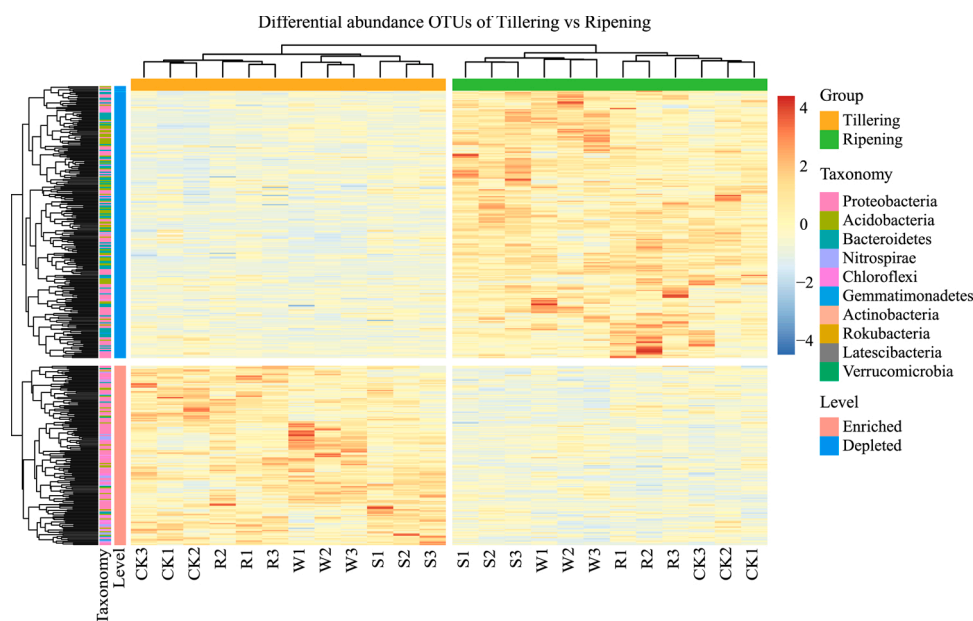

Fig. 3. Hierarchical clustering and heat map showing the percentage abundances (log2 scale) of the top 10 phyla (rows) during the two rice growth stages (columns). Phyla that were significantly more abundant (enriched) or less abundant (depleted) in samples with respect to stages are denoted in different blocks. The phyla are color coded as in Fig. 2.

bacterial α-diversity indices among different treatments at the tillering and ripening stages of rice growth, which is consistent with the findings of Wang et al. (2019a). This can probably be attributed to the fact that there was little change in soil pH, which is the primary factor affecting microbial richness and diversity (Guo et al., 2010; Siciliano et al., 2014; Zeng et al., 2016), and is influenced by the interactions among fertilizer release, uptake of nutrients by rice, and diverse root secretions (Ramanathan and Krishnamoorthy, 1973; Huang et al., 2014; Riah-Anglet et al., 2015; Li et al., 2019). Nevertheless, we observed significant differences in the bacterial community in response to different straw return modes and at different stages of rice growth, which could be affected by variations in soil hydrothermal conditions (Karhu et al., 2014). In addition, the PCoA and PERMANOVA analyses indicated that differences in bacterial community compositions associated with distinct straw return modes were the most apparent during the tillering stage, which again is consistent with the findings of Wang et al. (2019b). Others also established that the soil bacterial community is relatively

stable during the latter stages of rice growth (Noll et al., 2005; Wang et al., 2019a). In the present study, we found that, when compared with the CK treatment in which only N fertilizer was applied to soil, all straw-return treatments significantly contributed to an increase in the relative abundances of *Proteobacteria* and *Nitrospirae* during the early (tillering) stage. We suspect that this may be indirect evidence indicating a lower SIN content and reduced Nr loss potential during the tillering period, given that the phylum *Proteobacteria* includes numerous N-fixing bacteria, which play vital roles in enhancing N retention (Wang et al., 2015c; Yang et al., 2015). In this regard, the species of *Nitrospirae* are known to be sensitive to soil N profiles (Fierer et al., 2012), which determine N metabolism and the nitrification process that are of importance in the biogeochemical N cycling (Daebeler et al., 2014; Kumar et al., 2018). A positive relationship between the *Nitrospirae* community and available nutrients, including SIN, AP, and AK, has been previously reported in a long-term straw-return experiment (Luo et al., 2017).
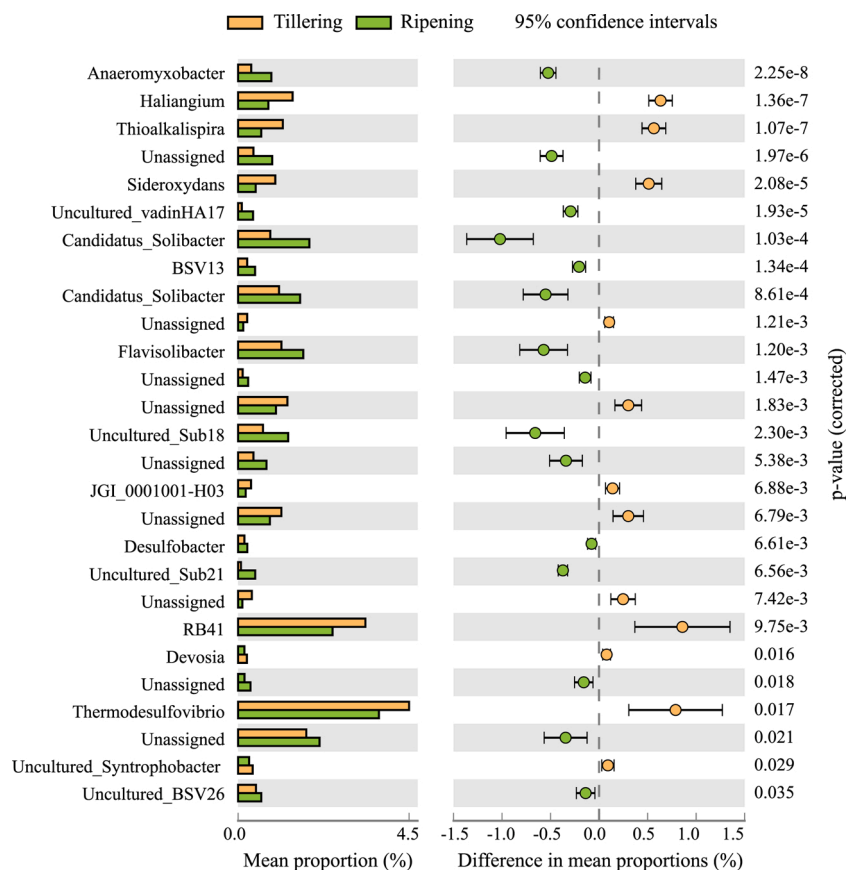
Fig. 4. Significant genera of top five phyla between the tillering and ripening stages as determined by Welch's *t*-test at the 95 % confidence interval ($P < 0.05$, n=3).
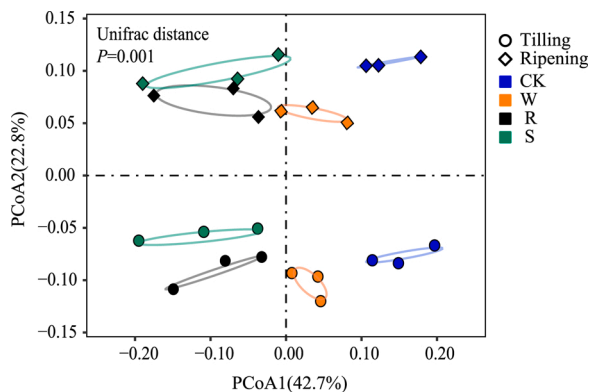

Fig. 5. Principal coordinate analysis (PCoA) plot of the bacterial community.

Table 3
Dissimilarity test of bacterial communities using Permutational multivariate (PERMANOVA) analysis based on Bray-Curtis distance.

PERMANOVA test	MeanSqs	R^2	P value
Tillering VS Ripening	0.211	0.190	0.005
Four treatments (Tillering)	0.054	0.439	0.006
Four treatments (Ripening)	0.046	0.452	0.030
Four treatments (the whole stage)	0.086	0.309	0.012

4.3. Relationship between soil biotic and abiotic factors and N runoff losses

Although Nr runoff losses showed a significantly higher reduction

during the rice tillering stage, relationship coefficients with Nr loss were found to be higher for straw return treatments, as indicated by our SEM analysis. With respect to the interactive effect of straw return modes and growth stages, we anticipated that the SIN content would be the most significantly positive factor relevant to Nr runoff loss, which was reduced by straw application during the tillering stage. In contrast, both SOC and pH were observed to have a significant negative effect on Nr loss. In this regard, it has previously been reported that an enhancement in soil C sequestration may contribute to a decrease in Nr loss (Xia et al., 2018) by facilitating a better synchronization between crop demand and soil nutrient supply under conditions of straw return (Shan et al., 2008; Blanco-Canqui and Lal, 2009). Abiotic factors should also be taken into account, given that N undergoes a cycle of transformation in response to the addition of crop residues to soil (Chen et al., 2014; Zhang et al., 2016a). The interaction among N mineralization, immobilization, and nitrification in soil regulates the amounts of plant-available N and thus influences the loss of Nr from soils (Wang et al., 2015b), and in this respect, we found that bacterial community composition had weaker but significant relationships with Nr runoff loss. The microbial N immobilization promoted by straw return contributes to a reduction in N loss loads via it effects on hydrological pathways (Cheng et al., 2017; Xia et al., 2017), which is consistent with our observation of a significant increase in the abundance of *Proteobacteria* in response to the double-season treatment, particularly during the tillering stage.

5. Conclusions

Our results obtained from a field experiment following the 6-year program of straw retention yielded solid data related to soil abiotic and biotic factors for us to elucidate putative mechanisms underlying the reduction in Nr runoff loss associated with straw retention. We found that all three modes of straw return evaluated in the present study were
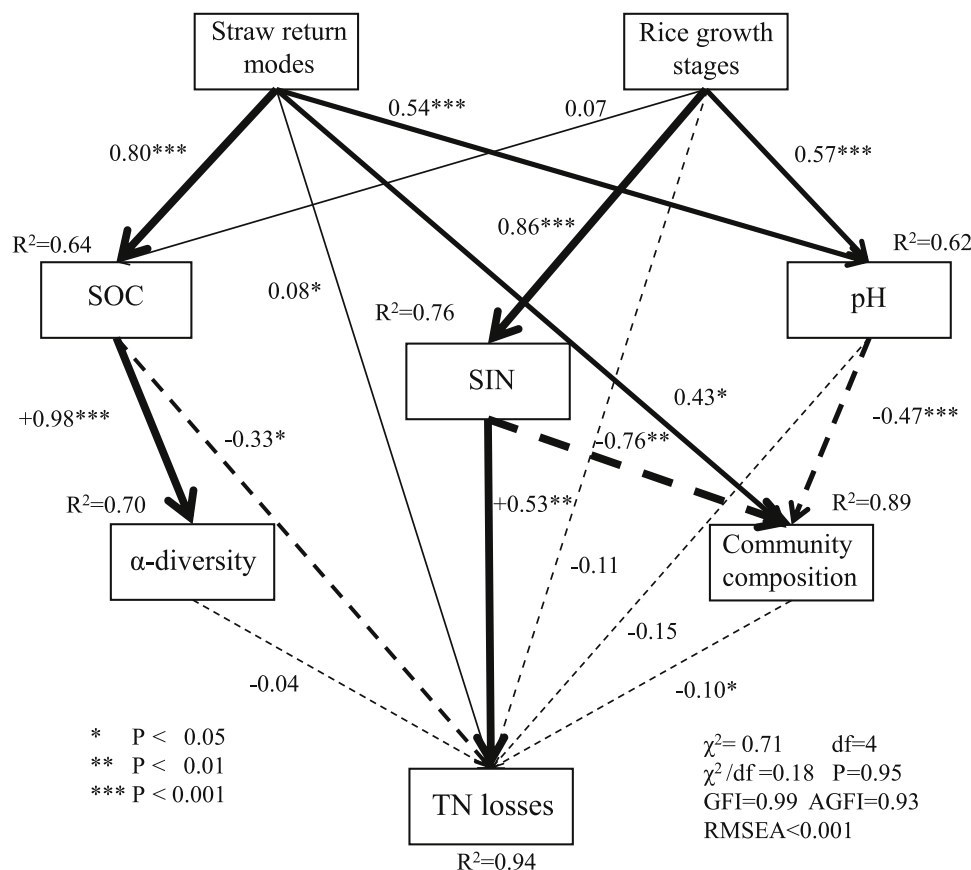
Fig. 6. Direct and indirect effects of soil parameters on reactive nitrogen losses. Single-headed arrows represent causal relationships. Solid and dotted arrows indicate positive and negative relationships, respectively (except arrows from "straw return modes" and "rice growth stages", which are set by categorical variables). Numbers adjacent to arrows are standardized path coefficients. The path widths are scaled proportionally to the path coefficient.

Table 4
Standardized regression weights of direct, indirect, and total effects of the key abiotic and biotic environmental factors on reactive nitrogen (Nr) runoff losses.

	Rice growth stages	Straw return modes	SIN	SOC	pH	Community composition	α-diversity
Direct effects	−0.11	0.08	0.53	−0.33	−0.15	−0.10	−0.04
Indirect effects	−0.60	−0.39	0.05	−0.05	0.05	0.00	0.00
Total effects	−0.71	−0.32	0.58	−0.39	−0.11	−0.10	−0.04

effective in reducing Nr discharge loads in the rice growing season, particularly with the double-season straw return mode and during the rice tillering stage in the rice-wheat cropping system in the Yangtze Delta region. Furthermore, we found that both soil physicochemical and bacterial properties play important roles in influencing N runoff loss. Our assessment of the interaction between straw return modes and rice growth stages indicated that the SOC content and pH had direct negative effects on Nr runoff loss, whereas the SIN content showed the most direct positive effect. A significant increase in the abundance of *Proteobacteria*, particularly at the tillering stage in response to double-season straw return, indicated the prominent effect of microbial N immobilization in reducing N loss potential under straw return. These findings support our research hypothesis and highlight the significance of investigations into the relationships between Nr runoff loss and soil factors under different straw retention modes. However, given the complexities of N cycling and the diverse N fates, further studies focusing on N transformation processes and the associated specific microbial communities, based on functional gene analyses, will be necessary to gain a more comprehensive understanding of the mechanisms underlying the beneficial effect of straw amendment with respect to N losses from soil.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agee.2020.107162.

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