https://doi.org/10.56946/jspae.v3i1.404
ISSN: 2957-9082
Journal of Soil, Plant and Environment

ORIGINAL RESEARCH

# Synergistic Effects of Rice Straw Return and Nitrogen Fertilizer on Rhizosphere Bacterial Communities and Soil Fertility

Nasita Rahman Borny[1,*], Golam Mohammod Mostakim[2], Asif Raihan[3,*], Md. Shoaibur Rahman[4]

[1]Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh.

[2]Graduate Training Institute, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh.

[3]Institute of Climate Change, National University Malaysia, Bangi 43600, Selangor, Malaysia.

[4]Department of Agroforestry & Environment, Hajee Mohammad Danesh Science & Technology University (HSTU), Dinajpur 5200, Bangladesh.

*Corresponding authors' email: nasitaborny14@gmail.com; asifraihan666@gmail.com

Received: 27 April 2024
Revised: 01 June 2024
Accepted: 02 June 2024

**ABSTRACT:** Returning straw to the field combined with nitrogen (N) fertilizer application is an effective way to enhance soil fertility. While previous studies have focused on soil's physical and chemical properties, the impact of straw returning on the microbial community has been less explored. In this study, we used four treatments including control (CK), nitrogen 150 kg ha$^{-1}$ (N), straw return 10 tonnes ha$^{-1}$ (SR), and combined SR and N (SRN= straw return 5 tonnes ha$^{-1}$ + nitrogen 75 kg ha$^{-1}$) to understand the effects of N fertilizer application and straw returning on bacterial community structure. Using high-throughput sequencing, we analyzed the bacterial community under different treatments and identified the main factors influencing soil bacterial communities. Results showed that soil properties such as pH, soil organic carbon (SOC), and available phosphorous (AP) were significantly higher in SR+N treatments. While AP, available nitrogen (AN), available potassium (AK), and total nitrogen (TN) were higher in sole N applied treatments. The results of high-throughput sequencing analyses demonstrated that the main bacteria at the phylum level were *Actinobacteria* (31-34%), *Proteobacteria* (25-30%), *Acidobacteria* (15-21%), and *Chloroflexi* (13-16%) across the treatments. Furthermore, the SR+N treatment exhibited the highest relative abundances of *Dependentiae, Proteobacteria, Chloroflexi*, and *Bacteroidetes* compared to all other treatments. Our results indicated that the combined application of straw return and N fertilizer enhanced soil fertility and increased the abundance of beneficial soil bacteria. Additionally, SOC emerged as the primary factor influencing variations in soil bacterial communities. However, several beneficial bacteria were less abundant in the combined treatment and more prevalent in the sole SR or sole N treatments. Thus, further research is necessary to develop new straw return strategies that optimize agricultural yields while minimizing ecological impacts.

**KEYWORDS:** Nitrogen, soil properties, soil bacteria, straw return, rice



## 1. Introduction

Chemical fertilizers are widely used to quickly and effectively supplement essential nutrients for crop growth, significantly boosting the levels of nitrogen (N), phosphorus (P), and potassium (K) in the soil (Ali et al., 2020; Ali et al., 2019). These elements are critical for increasing agricultural yields. However, excessive use of chemical fertilizers can lead to environmental pollution and soil degradation, including salinization and acidification, which damage soil structure and cause soil crusting (Ahmad et al., 2022). To mitigate these adverse effects, a combined application of chemical fertilizers and organic matter has

been recommended. Crop straw is the most common organic material in agricultural production and combining it with chemical fertilizers can enhance the organic matter content of black soil, improving its physical properties (Luo et al., 2020; Zhu et al., 2022; Han et al., 2018). Studies have shown that straw returning can increase soil porosity, reduce bulk density, improve soil permeability, enhance water and fertilizer retention, boost crop yields, and reduce pollution from straw burning (Gao et al., 2024; Yin et al., 2018; Wu et al., 2023; Sarkar et al., 2020; Zheng et al., 2015; Sing et al., 2022).

Soil microorganisms, the most abundant life forms in soil, are essential for maintaining ecological functions such as biogeochemical cycles (Garaycochea et al., 2024), litter decomposition (Bonilla et al., 2012), and plant growth (Khan et al., 2024; Ahmad et al., 2023; Ahmad et al., 2022). Their sensitivity to environmental changes makes them valuable indicators of ecological shifts (Yu et al., 2016; Le et al., 2024). The decomposition and transformation of straw in the soil are driven by these microorganisms, which benefit from the carbon sources provided by straw, thereby increasing their abundance and diversity (Su et al., 2020). Research has shown that straw returning can alter the bacterial community structure and enhance the abundance of bacteria involved in organic matter degradation (Hao et al., 2019). Compared to the sole application of chemical fertilizers, the combination of straw returning, and chemical fertilizers significantly improves soil fertility, increases soil enzyme activity and bacterial abundance, and modifies the bacterial community structure (Chen et al., 2017).

While previous studies have documented the impact of chemical fertilizers and straw returning on soil microbial communities, most relied on traditional methods that do not provide detailed insights into microbial community dynamics (Su et al., 2020). Additionally, few studies have examined the effects of reducing nitrogen fertilizer in conjunction with long-term straw returning on soil microbial communities in farmlands (Shi, et al., 2014). This study aims to fill this gap by evaluating the effects of continuous rice straw return and reduced nitrogen fertilizer application on the bacterial communities in farmland soils. Using high-throughput sequencing technology, we characterized the impact of combining nitrogen fertilizer application with straw returning on soil microbial community structure and diversity and explored their relationship with soil chemical properties.

This study aimed to investigate the effects of different treatments on soil properties and bacterial communities in paddy soil and their relationship to soil properties. Specifically, the objectives were to analyze the community structure of soil bacteria and evaluate the alpha and beta diversity of these bacterial communities. The treatments included control (CK), nitrogen application (N), straw return (SR), and a combination of straw return and nitrogen (SRN). All treatments utilized rice straw to replace the removed straw in the field. Through this research, we sought to understand how these agricultural practices influence soil microbial ecology.

## 2. Materials and methods

### 2.1 Experimental design and treatment details

A randomized complete block experiment was conducted at the Microbiology and Biocontrol Lab, Bangladesh Agricultural University, Mymensingh, Bangladesh, with four treatments, each replicated three times. The details of the experimental treatments are as follows: control (CK), nitrogen 150 kg $ha^{-1}$ (N), straw return 10 tonnes $ha^{-1}$ (SR), and combined SR and N (SRN= straw return 5 tonnes $ha^{-1}$ + nitrogen 75 kg $ha^{-1}$). All of the removed straw was replaced in the field, and the straw that was brought back was rice straw. The amount of total nitrogen (TN), total phosphorus (TP), and total potassium (TK) in 8–10 cm of crushed rice straw was 0.089, 0.0276, and 2.0 g $kg^{-1}$, respectively. Urea was used for N fertilizer N content of 46%. Other fertilizers such as potassium sulfate ($K_2O$ content 50%) and double superphosphate ($P_2O_5$ content 46%) were applied to all treatments with the rate of 120 kg $ha^{-1}$ and 80 kg $ha^{-1}$ respectively. N fertilizer was divided into three different doses, 50% before transplanting, 30% during tillering, and 20% at the heading stage. All standard agronomic practices, including irrigation and herbicide and insecticide applications, were the same for all plots.

### 2.2 Soil sampling

In each plot, three randomly designated 5 $m^2$ areas were marked out before the rice was harvested in the second year of 2021. Five soil samples (0–15 cm topsoils) were collected from each area using a soil auger, and the samples were then combined into one composite sample. The composite soil sample was subsequently divided into two parts: one part was crushed, air-dried, and passed through a 2 mm sieve for the determination of soil chemical properties; the second part was stored at −80 °C for sequencing.

Soil pH was determined using a pH meter with a soil-to-water ratio of 1:2.5 w/v. Soil organic carbon (SOC) was analyzed following the method outlined by Yeomans and Bremner, (1988). Total nitrogen (TN) was measured using an elemental analyzer (Elementar, Langenselbold, Germany). Available nitrogen (AN) was sequentially digested in $H_2SO_4$-$HCLO_4$, 0.05 M $NaHCO_3$, and 2.0 M KCL. Available phosphorus (AP) was quantified using a colorimetric method after extraction with 0.5 M $NaHCO_3$ (Zhu et al., 2021). Total phosphorus (TP), total potassium (TK), available nitrogen (AN), and available potassium (AK) were measured using continuous flow analysis (SAN++, Skalar Analytical, Breda, The Netherlands).

### 2.3. DNA extraction and 16S rRNA sequencing

DNA was extracted using the FastDNA™ Spin Kit for Soil (MP Biomedicals, US) according to the manufacturer's instructions. DNA concentration was measured with a NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA), and the quality of PCR products was verified by 2% agarose gel electrophoresis. The V3–V4 region of the 16S rRNA gene was amplified using the primer pairs 515F (GTGCCAGCMGCCGCGG) and 907R (CCGTCAATTCMTTTRAGTTT). PCR and sequencing were carried out by the Microbiology and Biocontrol Lab, Bangladesh Agricultural University, Mymensingh, Bangladesh.

### 2.4. Processing of Illumina Sequencing Data

The paired reads were merged using FLASH (version 1.2.3) software to combine the sequences before assembling a gene segment (Magoc and Salzberg, 2011). Chimeric sequences were identified and removed using a de novo method with USEARCH (version 8.1.1861) (Edgar, 2010). After removing the chimeras, high-quality bacterial sequences were collected for subsequent analysis.

Effective bacterial sequences were subsampled separately for each sample for further statistical analysis. Following subsampling, the data were processed using a modified SOP pipeline based on USEARCH and the software package QIIME (Quantitative Insights Into Microbial Ecology v1.8.0) (Tian et al., 2015). Briefly, the selected sequences were clustered into operational taxonomic units (OTUs) using a two-stage clustering algorithm with USEARCH (version 8.1.1861) at 97% sequence identity (Edgar, 2010). Representative sequences in each OTU were aligned to the SILVA reference alignment (Yilmaz et al., 2014). Taxonomy was assigned to each representative sequence using the RDP classifier with a minimum confidence threshold of 85%.

An OTU-based analysis method was used to evaluate bacterial diversity (alpha diversity) in each sample. To estimate the diversity index and species richness for each sample, OTU richness, and Chao1 and Shannon indices were calculated using QIIME software (v1.8.0) at a sequencing depth of 3%. Statistical analysis was performed using ANOVA to determine significant differences in diversity indices and species richness among the plant rhizosphere soil samples. Rarefaction and rank abundance curves were calculated at a 97% similarity level of the OTUs.

Beta diversity analysis was conducted to determine the similarity of community structure among all samples. At the OTU level, beta diversity was calculated using weighted UniFrac distances and visualized through principal coordinate analysis (PCoA). The weighted UniFrac distance matrices were clustered and evaluated using QIIME software (v1.8.0) to show phylogenetic relationships among various communities and their abundance in the respective samples.

### 2.5. Statistical Analysis

Statistics 8.1 analytical software was used to perform analysis of variance (ANOVA) among the treatments for each variable. Alpha diversity of bacteria, including Shannon and Chao1 indices, was calculated using QIIME software (v1.8.0). Rarefaction curves for species richness were plotted against the number of sequences, and the analysis of dominant phyla was conducted using Microbiome Analyst (Dhariwal et al., 2017). Correlation analysis among soil properties and soil microbial abundance was performed using R (3.2) software.

## 3. Results

### 3.1 Soil Properties

Soil properties were significantly affected by nitrogen (N), straw return (SR), and combined SR and N (SR+N) treatments (Table 1). Soil pH showed the highest value of 6.4 in the combined treatment (SR+N), followed by N (5.9), compared to sole SR and CK treatments. Soil organic carbon (SOC) increased by 37% in the SR+N treatment

compared to CK, while total nitrogen (TN) increased by 22% in the individual N treatment compared to the SR treatment. Similarly, available nitrogen (AN), available phosphorus (AP), and available potassium (AK) increased in the N treatment by 21%, 13%, and 30%, respectively, compared to the control. In contrast, total phosphorus (TP) and total potassium (TK) were highest in the SR treatment, with values of 1.58 $g \cdot kg^{-1}$ and 0.96 $g \cdot kg^{-1}$, respectively, compared to all other treatments. The lowest values of TP, TN, and TK were recorded in the CK treatment. Overall, these results showed that soil properties are greatly influenced by the type of treatment applied, highlighting the importance of combined nitrogen and straw return for enhancing soil health.

### 3.2 Alpha and Beta Diversity of Soil Bacteria

The soil bacterial alpha diversity indices, namely Chao1 and Shannon indices, demonstrated notable variations across the different treatments (Figure 1 A and B). Statistically significant differences were observed among the four treatments ($p < 0.05$). The SR treatment yielded the highest Shannon index of 4.9, with CK and N treatments following, and the SR+N treatment showing the lowest value (Figure 1A). Similarly, the Chao1 index reached its peak at 585.4 in the SR treatment, followed by the CK and N treatments.

Table 1. Changes in soil properties under the treatments of straw return, nitrogen application, and their combined treatments.

| Treatments | CK | SR | N | SR+N |
|---|---|---|---|---|
| pH | 5.8± 0.13 c | 5.4± 0.32 c | 5.9± 0.14b | 6.4± 0.24 a |
| SOC ($g\ kg^{-1}$) | 1.89± 0.05 c | 1.76± 0.13 c | 2.1± 0.14 b | 2.6± 0.08 a |
| TN ($g\ kg^{-1}$) | 1.77± 0.22 c | 1.58± 0.09 c | 1.94± 0.24 a | 1.82± 0.12 b |
| TP ($g\ kg^{-1}$) | 0.76 ± 0.02 c | 0.96 ± 0.00 a | 0.84 ± 0.01b | 0.73 ± 0.03c |
| TK ($g\ kg^{-1}$) | 15.43 ± 0.41c | 21.03 ± 0.55 a | 19.51 ± 0.13b | 19.67 ± 0.81b |
| AN ($mg\ kg^{-1}$) | 187.46 ± 4.05b | 180.57 ± 3.06b | 209.09 ± 2.33a | 148 ± 7.71c |
| AP ($mg\ kg^{-1}$) | 45.52 ± 0.65b | 46.61 ± 3.53b | 51.78 ± 0.34 a | 51.91 ± 1.10 a |
| AK ($mg\ kg^{-1}$) | 167.15 ± 6.14c | 162.46 ± 5.43 c | 218.66 ± 4.76 a | 186.59 ± 5.94b |

Note: SOC-Soil organic carbon, TN-total nitrogen, TP-total phosphorus, TK-total potassium, AN-available nitrogen, AP-available phosphorus. AK-available potassium. N- nitrogen, SR-straw return. Different lowercase letters indicate significant differences according to one-way ANOVA coupled with the LSD test ($p < 0.05$).

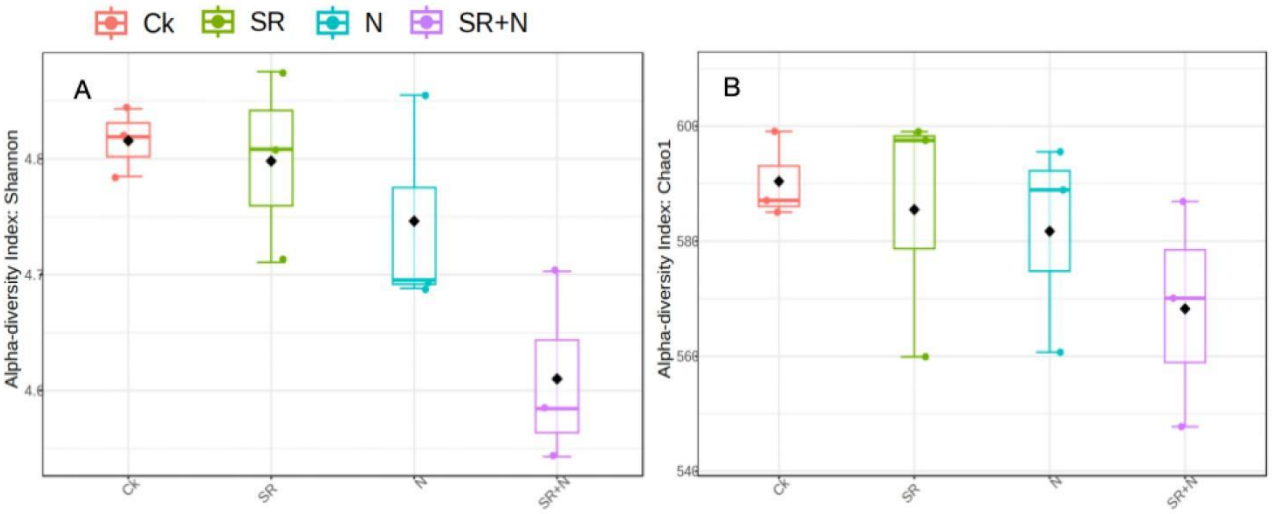


Figure 1. Changes in the abundance and diversity of OTUs from soil samples under straw return and nitrogen fertilizers.

The soil bacterial alpha diversity indices, namely Chao1 and Shannon indices, demonstrated notable variations across the different treatments (Figure 1 A and B). Statistically significant differences were observed among the four treatments ($p < 0.05$). The SR treatment yielded the highest Shannon index of 4.9, with CK and N treatments following, and the SR+N treatment showing the lowest value (Figure 1A). Similarly, the Chao1 index reached its peak at 585.4 in the SR treatment, followed by the CK and N treatments.

Figure 2 shows the principal coordinate analysis of the four studied treatments using the bray-curtis distance index. The analysis explained 70% of the total variation among the replicated samples of each treatment, with pcoA1 explaining 51.5 % and pcoA 2 explaining 18.5% variation. The analysis showed greater differences among the treatments with Sr+N treatment being the most dissimilar in terms of bacterial diversity. The CK and SR treatments were somehow similar as indicated by the distribution of replicated samples on the quadrants. However, the changes observed between these treatments were statistically non-significant.

Figure 3 shows the non-metric dimensional scaling (NMDS) analysis of the four studied treatments using the bray-curtis distance index.

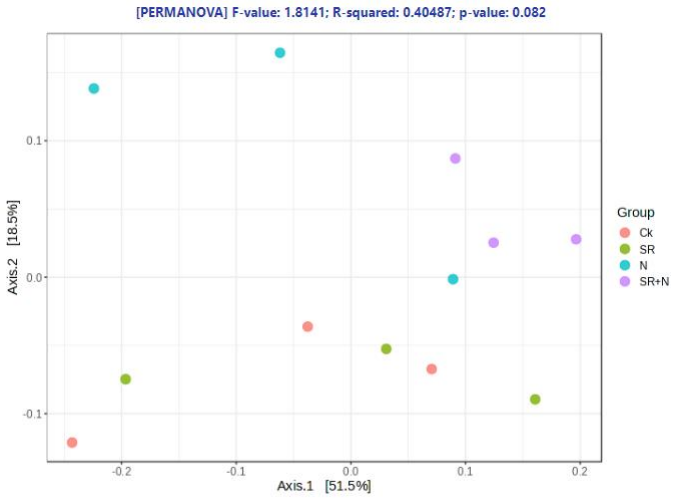


Figure 2. Principal coordinate analysis of the four studied treatments using the bray-curtis distance index.

The analysis showed greater differences among the treatments with Sr+N treatment

being the most dissimilar in terms of bacterial diversity. The CK and SR treatments were somehow similar as indicated by the distribution of replicated samples on the quadrants. The changes observed in between these treatments were statistically non-significant. However, the stress value of 0.035 shows that the analysis is a good fit for the interpretation.

### 3.3 Community Structure of Soil Bacteria

To calculate rarefaction curves, bacterial richness, and diversity, OTUs with 97% genetic similarity were discovered. The rarefaction curves showed that the sequencing effort was enough to characterize the majority of the variety in soil samples (Figure 4).

Figure 5 represents the relative abundance of soil bacterial phyla influenced by SR, N, and their combined treatments. Across the treatments, the relative abundance of the top phyla in descending order was *Actinobacteria* (31-34%), *Proteobacteria* (25-30%), *Acidobacteria* (15-21%), and *Chloroflexi* (13-16%).

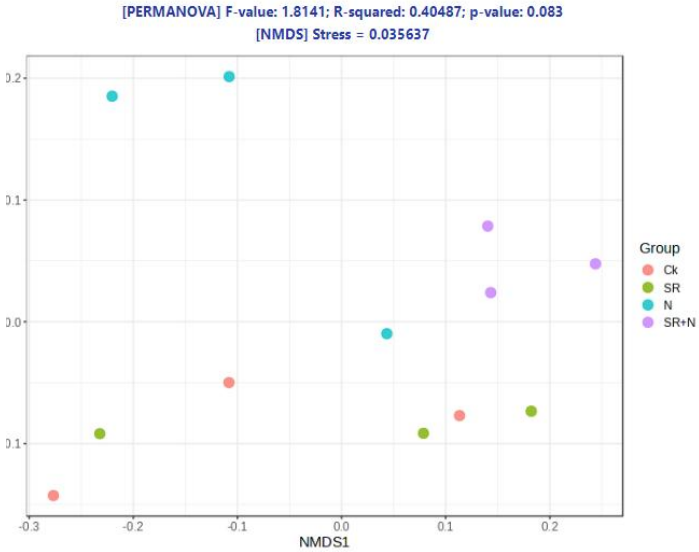


Figure 3. Non-metric dimensional scaling (NMDS) analysis of the four studied treatments using the bray-curtis distance index.

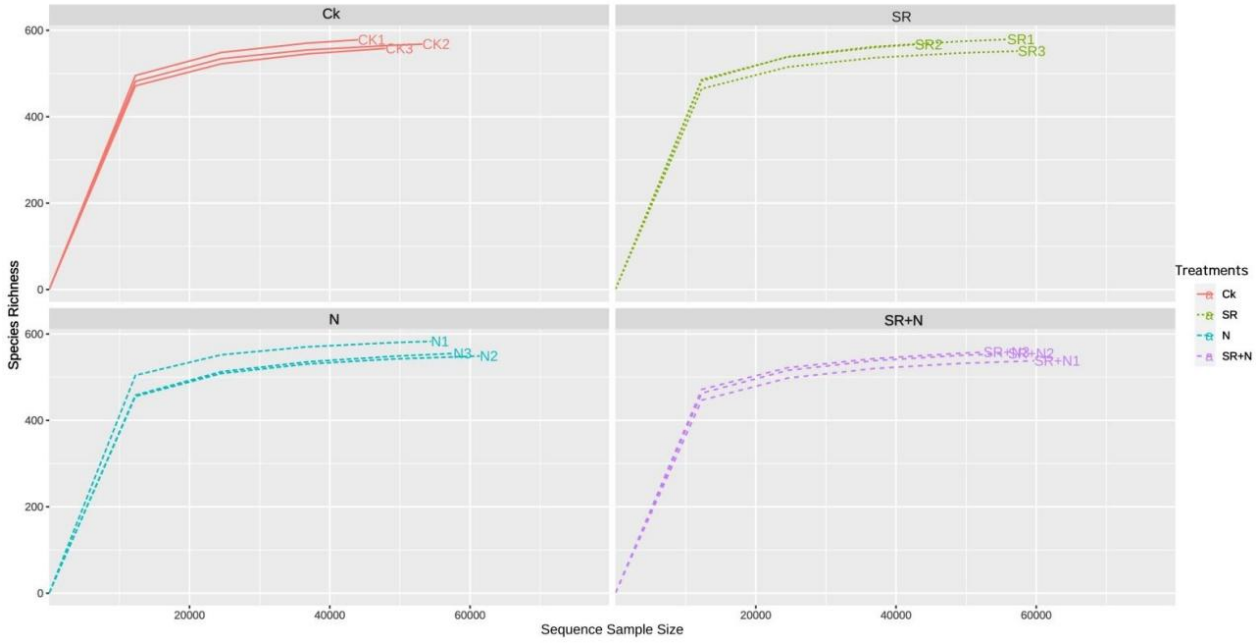


Figure 4. Rarefaction curves of 16S rRNA sequencing depth and number of species numbers in soil depth (0–20 cm). Control (CK), nitrogen 150 kg ha−1 (N), straw return 10 tonnes ha−1 (SR), and combined SR and N (SRN).

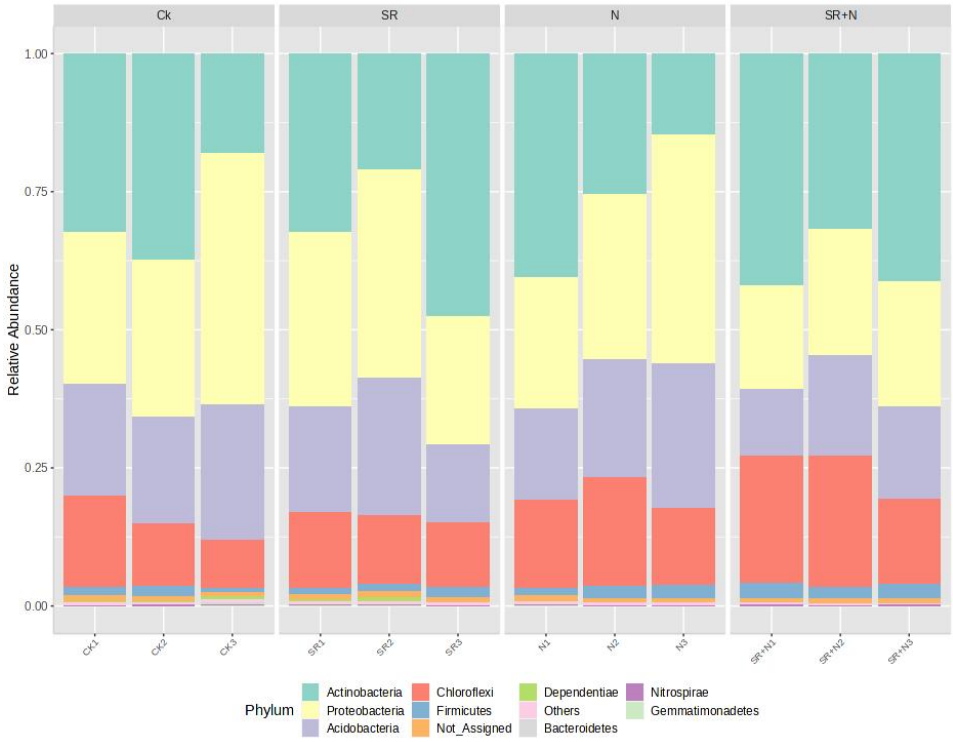


Figure 5. Based on the 16S rRNA gene the relative abundance of soil bacterial community composition at phylum level. Each strip represents the mean of three replicates.

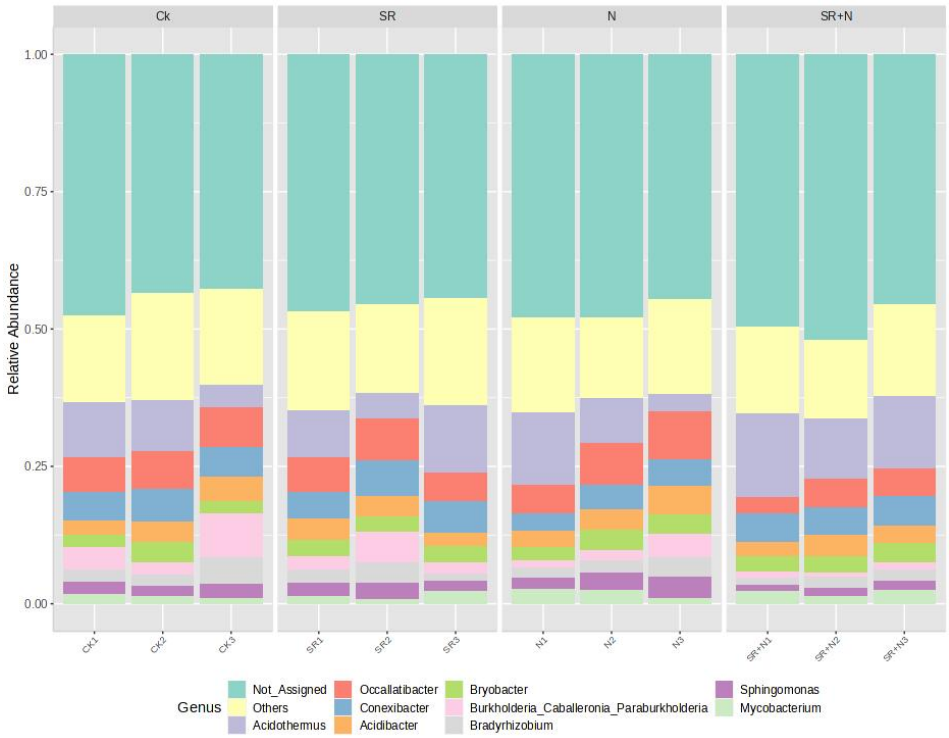


Figure 6. Based on the 16S rRNA gene the relative abundance of soil bacterial community composition at genus level. Each strip represents the mean of three replicates.

The abundance of other bacterial phyla, including *Firmicutes*, Not_Assigned, *Bacteroidetes*, *Dependentiae*, *Gemmatimonadetes, Nitrospirae*, and Others, ranged from 0.1% to 1% At the genus level, the top 10 bacteria were *Dependentiae, Nitrospirae, Proteobacteria, Gemmatimonadetes, Chloroflexi, Actinobacteria, Acidobacteria, Firmicutes,* Not_Assigned, *Bacteroidetes*, and Others (Figure 6). Among these genera, *Dependentiae, Nitrospirae, and Proteobacteria* were the most abundant across all treatments, with relative abundances of 40-48%, 15-20%, and 7-12%, respectively.

The SR+N (Straw return + nitrogen) treatment exhibited the highest relative abundances of *Dependentiae, Proteobacteria, Chloroflexi,* and *Bacteroidetes,* with values of 49.09%, 49.09%, 3.08%, and 2.18%, respectively. In contrast, *Nitrospirae* showed the highest relative abundance of 17% in the sole SR treatment. *Gemmatimonadetes* and *Actinobacteria* were most abundant in the N treatment, with values of 7.01% and 4.20%, respectively.

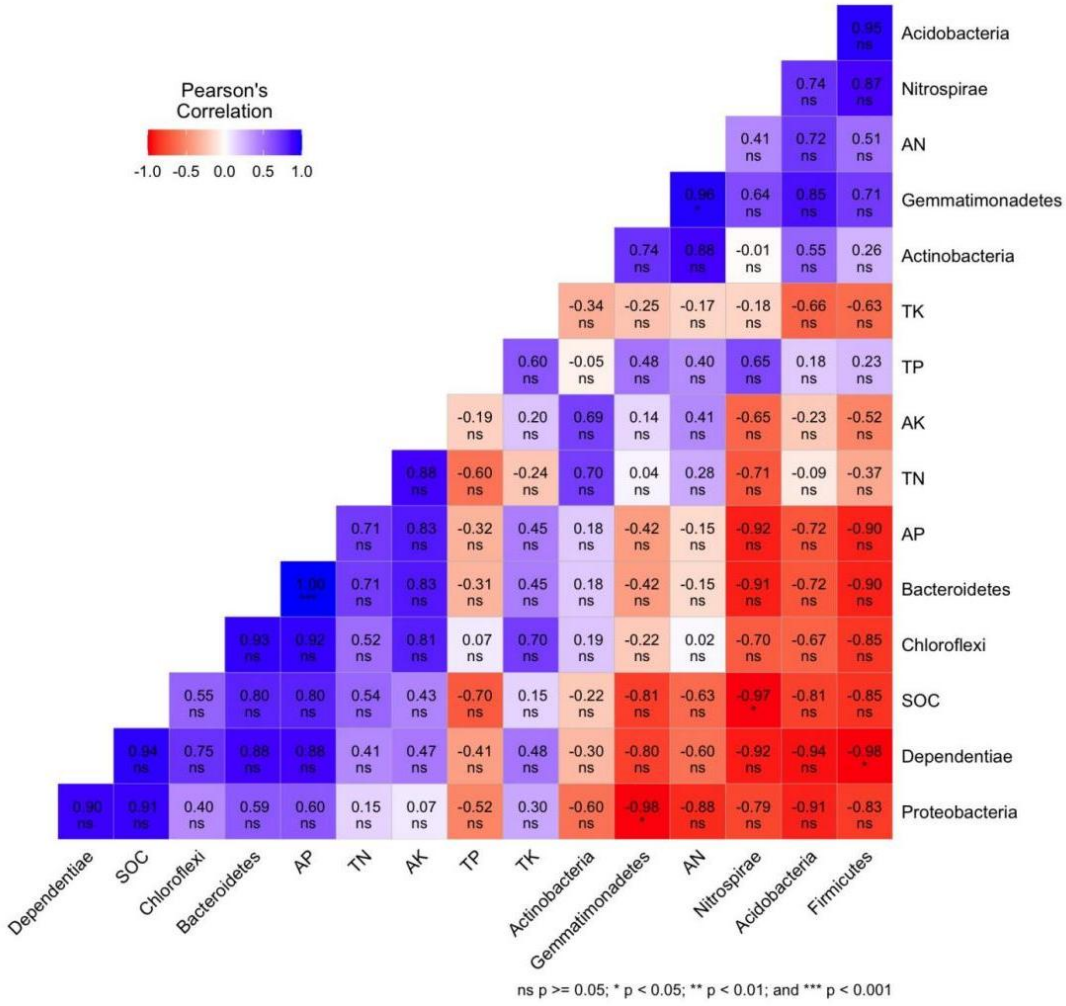


Figure 7. Correlation analysis among soil microbial abundance and soil properties.

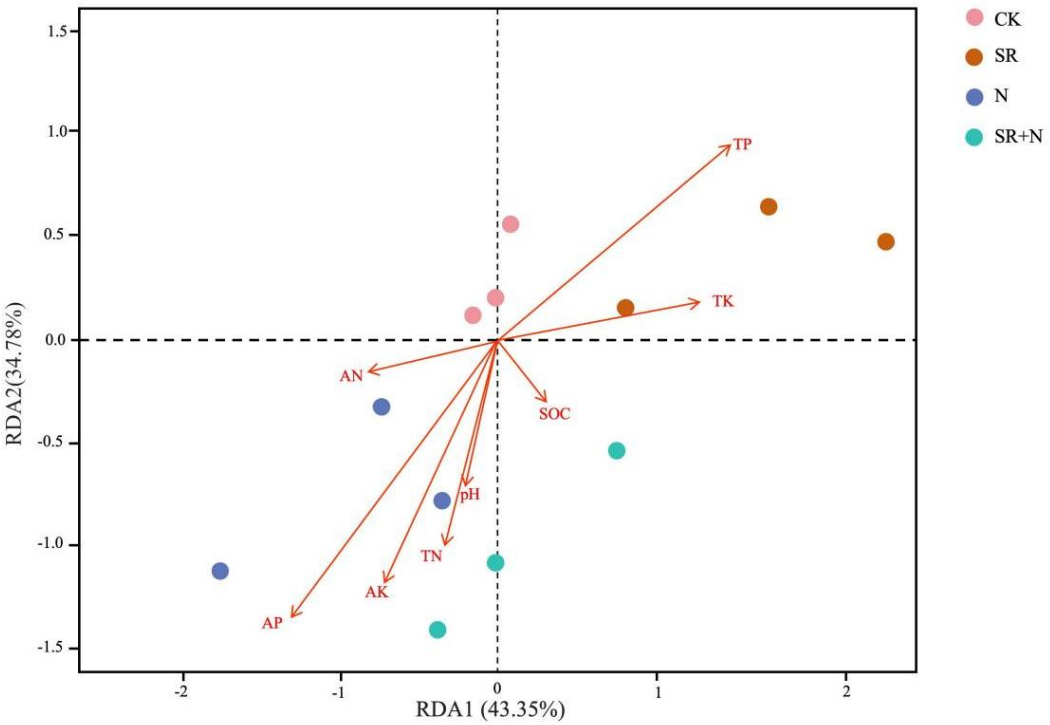


Figure 8. Correlation analysis among soil microbial abundance and soil properties.

### 3.4 Correlation of soil properties and soil-abundant bacteria

Figure 7 shows the Pearson correlation heatmap among the most abundant bacteria and soil properties. Soil properties, including SOC (R = 0.91), AP (R = 0.60), and TN (R = 0.15), were strongly positively correlated with *Proteobacteria*. However, soil TP (R = −0.52) and AN (R = −0.88) were strongly negatively correlated with *Proteobacteria*. Furthermore, the abundance of soil bacteria, including *Dependentiae* (R = 0.947), *Chloroflexi* (R = 0.55), and *Bacteroidetes* (R = 0.80), was strongly positively correlated with the SOC of paddy rice, whereas the abundance of *Actinobacteria Gemmatimonadetes,* and *Firmicutes* showed a negative relationship with SOC

RDA was employed to assess the strength of the relationship between soil pH, SOC, TN, TP, TK, AN, AP, and AK concentration and soil bacterial diversity. Figure 8 indicates the correlation between bacterial communities (at the phyla level) and soil characteristics under various treatments. Soil pH, AN, AP, AK, and AP all occurred in the same quadrant, indicating that nitrogen fertilizer and straw return had a major impact on soil characteristics. Where AP and SOC occurred in the same quadrant as DR, which indicated that SR alone with the maximum amount of application can improve SOC and AP. The four treatments were administered in four distinct quadrants, demonstrating that the fertilization treatments had a significant impact on the composition of soil microorganisms. Straw return combined with N fertilizer has a substantial association with soil characteristics.

## 4. Discussion

Soil properties play a vital role in soil bacterial diversity, activity, and function, influencing nutrient cycling, organic matter decomposition, and overall soil health. In the current study soil pH, SOC, and AP were increased with the combined treatment of SR+N. The observed increase in soil pH with the combined SR+N treatment can be attributed to several mechanisms. Firstly, straw return can increase the microbial decomposition of organic matter, which produces organic acids that buffer soil pH (Zhao et al., 2016). Secondly, nitrogen fertilizers often contain alkaline substances such as lime (calcium carbonate), which neutralize soil acidity and increase pH (Nasedjanov et al., 2012). This combined effect can create a more favorable environment for microbial activity and nutrient availability, thus enhancing soil health and fertility (Pan et al., 2021). Additionally, the combined SR+N treatment enhances soil organic carbon (SOC) by promoting microbial activity and organic matter incorporation and increases available phosphorus (AP) due to the improved mineralization of organic phosphorus compounds (Wang et al., 2022; Guo et al., 2024; Yuan et al., 2021).

In the current study, soil total nitrogen (TN), available phosphorus (AP), and available potassium (AK) were recorded higher in treatments where nitrogen (N) was applied alone compared to treatments with straw return (SR), control (CK), and the combination of SR and CK. The higher levels of TN, AP, and AK in the sole N treatments can be attributed to the direct addition of nutrients in readily available forms that are immediately accessible to plants and soil microorganisms. In contrast, the SR and combined treatments may result in a slower release of nutrients as organic matter decomposes over time, leading to lower immediate availability of these key nutrients (Palm et al., 1997; Guan et al., 2020; Siedt et al., 2021). The findings underscore the importance of considering both the immediate and long-term effects of different fertilization practices on soil nutrient dynamics and overall soil health (Guan et al., 2020; Siedt et al., 2021). In contrast, TP and TK were higher in the sole treatment SR, which indicated that the incorporation of organic material from straw can enhance the availability of these nutrients by promoting mineralization and reducing nutrient losses through leaching (Wang et al., 2021).

Soil microorganisms play a vital role in soil nutrient availability (Ahmad et al., 2023; Ahmad et al., 2022). In the present study, the SR+N treatment (Straw return + nitrogen) exhibited the highest relative abundances of Dependentiae, Proteobacteria, Chloroflexi, and Bacteroidetes genera. This increase in bacterial abundance can be attributed to the combined effects of organic matter from straw and nitrogen fertilization, which enhance soil nutrient availability and create a more favorable environment for microbial growth (Huang et al., 2021; Mohammadi et al., 2011; Chen et al., 2021). The incorporation of straw provides a carbon source that supports the growth of heterotrophic bacteria like Proteobacteria and Bacteroidetes, while nitrogen fertilization improves the overall nutrient status of the soil, benefiting a broad range of microbial taxa (Zhang et al., 2019; Xiaoping et al., 2019;

Zhu et al., 2022). These bacteria play crucial roles in nutrient cycling, organic matter decomposition, and promoting soil health, which can enhance plant growth and productivity (Ali et al., 2022; Khan et al., 2022a; Khan et al., 2022b: Khan et al., 2021; Song et al., 2022).

In contrast, *Nitrospirae* genera showed the highest relative abundance of 17% in the sole SR treatment. *Gemmatimonadetes* and *Actinobacteria* genera were most abundant in the N treatment, with values of 7.01% and 4.20%, respectively. The increased abundance of *Nitrospirae* in the SR treatment can be attributed to the enhanced availability of organic substrates from straw decomposition, which supports the growth of nitrifying bacteria involved in nitrogen cycling (Daims et al., 2015; Luo et al., 2017; Wang et al., 2021; Guan et al., 2023). The high levels of *Gemmatimonadetes* and *Actinobacteria* in the N treatment are likely due to the improved soil nutrient status from nitrogen fertilization, which favors these bacteria known for their roles in nutrient turnover and organic matter decomposition (Ren et al., 2020; Janssen, 2006; Xu et al., 2020; Gu et al., 2021; Zhang et al., 2014). These microbial groups contribute significantly to soil health by promoting nutrient cycling, enhancing soil structure, and supporting plant growth through the decomposition of organic materials and the release of nutrients.

The combination of straw return and nitrogen fertilizer can induce physicochemical changes in the soil, resulting in alterations in the composition of the bacterial community (Jiao et al., 2023). In the present study, we observed that straw return amendments significantly influenced soil properties, as shown in Table 1. Furthermore, it has been reported that soil quality traits are positively correlated with the structure and composition of the bacterial community (Wu et al., 2020). Figure 7 illustrates the relationship between the bacterial community at the genus level and soil traits, including TN, SOC, TP, TK, AN, AP, and AK, for different treatments. Our findings indicate that dominant bacteria at the genus level, such as *Proteobacteria*, *Chloroflexi*, and *Acidobacteria*, are positively correlated with SOC and AP. This suggests that bacterial growth is strongly influenced by N fertilizer, and regulating the type and proportion of straw return is an effective strategy for enhancing bacterial growth. In conclusion, the application of straw return amendments in conjunction with nitrogen fertilizer may create a more favorable environment for bacterial growth, thereby improving the bacterial community structure and soil fertility.

## 5. Conclusions

The results demonstrated that the combination of straw return and nitrogen fertilizer significantly improved soil pH, SOC, and AP. In contrast, sole straw return enhanced TP and TK, while sole nitrogen application increased AP, AN, and TN. The bacterial Chao1 and Shannon indices were highest in treatments with only straw return, followed by those with only nitrogen application. The ten most abundant genera across all treatments were *Dependentiae, Nitrospirae, Proteobacteria, Gemmatimonadetes, Chloroflexi, Actinobacteria, Acidobacteria,.Firmicutes*, Not_Assigned,.and *Bacteroid*

*etes*. Moreover, variations in soil bacterial communities were closely linked to changes in soil SOC, TN, AP, and AK, indicating that the effects of combined straw return and nitrogen application on bacterial communiti es were driven by changes in soil chemical properties. These findings provide valuable insights and a foundational understanding of improving paddy soil through the combined use of straw return and nitrogen fertilizer, highlighting its promising application potential.

## Author Contribution:

**Nasita Rahman Borny:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, **Asif Raihan:** review & editing, Visualization, Formal analysis, Data curation. **Golam Mohammod Mostakim:** Conceptualization, Methodology, Formal analysis, Resources, review & editing, Supervision, Funding acquisition. **Md. Shoaibur Rahman:** Conceptualization, Methodology, Project administration, Resources, Formal analysis, Resources, Writing–review & editing, Supervision, Funding acquisition. Funding acquisition. All authors read and approved the final manuscript.

**Acknowledgments:** Not applicable (N/A)

**Conflicts of Interest:** The authors declare no conflict of interest.

**Availability of Data and Materials:** Data will be available on a formal request from the corresponding authors.

**Data Funding:** Not Applicable (N/A)

## REFERENCES

Ahmad, R., Gao, J., Gao, Z., Khan, A., Ali, I., & Fahad, S. Influence of biochar on soil nutrients and associated Rhizobacterial communities of mountainous apple trees in northern loess plateau China. Microorganisms. (2022). 10(10), 2078. https://doi.org/10.3390/microorganisms10102078

Ahmad, R., Gao, J., Li, W., Zhang, Y., Gao, Z., Khan, A., Fahad, S. Response of soil nutrients, enzyme activities, and fungal communities to biochar availability in the rhizosphere of mountainous apple trees. Plant and Soil. (2023). 489(1), 277-293. https://doi.org/10.1007/s11104-023-06016-4

Ali, I., Khan, A. A., Imran, Inamullah, Khan, A., Asim, M., Iqbal, B. Humic acid and nitrogen levels optimizing productivity of green gram (Vigna radiate L.). Russian agricultural sciences. (2019). 45, 43-47. https://doi.org/10.3103/S1068367419010051

Ali, I., Yuan, P., Ullah, S., Iqbal, A., Zhao, Q., Liang, H., Jiang, L. Biochar amendment and nitrogen fertilizer contribute to the changes in soil properties and microbial communities in a paddy field. Frontiers in microbiology. (2022). 13, 834751. https://doi.org/10.3389/fmicb.2022.834751

Bonilla, N., Gutiérrez-Barranquero, J. A., de Vicente, A., Cazorla, F. M. Enhancing soil quality and plant health through suppressive organic amendments. Diversity. (2012). 4(4), 475-491. https://doi.org/10.3390/d4040475

Chen, Q., Liu, Z., Zhou, J., Xu, X., & Zhu, Y. Long-term straw mulching with nitrogen fertilization increases nutrient and microbial determinants of soil quality in a maize-wheat rotation on China's Loess Plateau. Science of The Total Environment. (2021). 775, 145930. https://doi.org/10.1016/j.scitotenv.2021.145930

Chen, Y., Xin, L., Liu, J., Yuan, M., Liu, S., Jiang, W., Chen, J. Changes in bacterial community of soil induced by long-term straw returning. Scientia Agricola. (2017). 74, 349-356.https://doi.org/10.1590/1678-992x-2016-0025

Daims, H., Lebedeva, E. V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., ... & Wagner, M. Complete nitrification by Nitrospira bacteria. Nature. (2015). 528(7583), 504-509. https://doi.org/10.1038/nature16461

Edgar, R. C. Search and clustering orders of magnitude faster than BLAST. Bioinformatics, (2010). 26(19), 2460-2461. https://doi.org/10.1093/bioinformatics/btq461

Gao, Y., Shao, Y., Wang, J., Hu, B., Feng, H., Qu, Z., Liu, Y. Effects of straw returning combined with blended controlled-release urea fertilizer on crop yields, greenhouse gas emissions, and net ecosystem economic benefits: A nine-year field trial. Journal of Environmental Management. (2024). 356, 120633. https://doi.org/10.1016/j.jenvman.2024.120633

Garaycochea, S., Altier, N. A., Leoni, C., Neal, A. L., Romero, H. Abundance and phylogenetic distribution of eight key enzymes of the phosphorus biogeochemical cycle in grassland soils. Environmental Microbiology Reports. (2023). 15(5), 352-369. https://doi.org/10.1111/1758-2229.13159

Gu, Y., Wang, J., Cai, W., Li, G., Mei, Y., & Yang, S. Different amounts of nitrogen fertilizer applications alter the bacterial diversity and community structure in the rhizosphere soil of sugarcane. Frontiers in Microbiology. (2021). 12, 721441. https://doi.org/10.3389/fmicb.2021.721441

Guan, X. K., Wei, L., Turner, N. C., Ma, S. C., Yang, M. D., & Wang, T. C. Improved straw management practices promote in situ straw decomposition and nutrient release, and increase crop production. Journal of Cleaner Production. (2020). 250, 119514. https://doi.org/10.1016/j.jclepro.2019.119514

Guan, Y., Wu, M., Che, S., Yuan, S., Yang, X., Li, S., Wu, Z. Effects of continuous straw returning on soil functional microorganisms and microbial communities. Journal of Microbiology. (2023). 61(1), 49-62. https://doi.org/10.1007/s12275-022-00004-6

Guo, Z., Ye, W., Wang, H., He, W., Tian, Y., Hu, G., ... & Zhuge, Y. Straw and phosphorus applications promote maize (Zea mays L.) growth in saline soil through changing soil carbon and phosphorus fractions. Frontiers in Plant Science. (2024). 15, 1336300. https://doi.org/10.3389/fpls.2024.1336300

Han, X., Xu, C., Dungait, J. A., Bol, R., Wang, X., Wu, W., Meng, F. Straw incorporation increases crop yield and soil organic carbon sequestration but varies under different natural conditions and farming practices in China: a system analysis. Biogeosciences. (2018). 15(7), 1933-1946. https://doi.org/10.5194/bg-15-1933-2018

Hao, M., Hu, H., Liu, Z., Dong, Q., Sun, K., Feng, Y., Ning, T. Shifts in microbial community and carbon sequestration in farmland soil under long-term conservation tillage and straw returning. Applied Soil Ecology. (2019). 136, 43-54. https://doi.org/10.1016/j.apsoil.2018.12.016

Huang, T., Yang, N., Lu, C., Qin, X., & Siddique, K. H. Soil organic carbon, total nitrogen, available nutrients, and yield under different straw returning methods. Soil and Tillage Research. (2021). 214, 105171. https://doi.org/10.1016/j.still.2021.105171

Janssen, P. H. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Applied and environmental microbiology. (2006). 72(3), 1719-1728.

https://doi.org/10.1128/AEM.72.3.1719-1728.2006

Jiao, F., Zhang, D., Chen, Y., Wu, J., & Zhang, J. Effects of Long-Term Straw Returning and Nitrogen Fertilizer Reduction on Soil Microbial Diversity in Black Soil in Northeast China. Agronomy. (2023). 13(8), 2036. https://doi.org/10.3390/agronomy13082036

Khan, A., Gillani, S. W., Jiang, H., Wei, Y., Li, M., Yu, Z., Adnan, M., Zhang, M. Combine application of N and biofertilizers improved plant morphology and soil environment via regulating rhizosphere bacterial community under sugarcane monocropping. Industrial Crops and Products. (2024). 210, 118074. https://doi.org/10.1016/j.indcrop.2024.118074

Khan, A., Jiang, H., Bu, J., Adnan, M., Gillani, S. W., & Zhang, M. An insight to rhizosphere bacterial community composition and structure of consecutive winter-initiated sugarcane ratoon crop in Southern China. BMC Plant Biology. (2022a). 22(1), 74. https://doi.org/10.1186/s12870-022-03463-6

Khan, A., Jiang, H., Bu, J., Adnan, M., Gillani, S. W., Hussain, M. A., & Zhang, M. Untangling the Rhizosphere bacterial community composition and response of soil physiochemical properties to different nitrogen applications in sugarcane field. Frontiers in Microbiology. (2022b). 13, 856078. https://doi.org/10.3389/fmicb.2022.856078

Khan, A., Wang, Z., Chen, Z., Bu, J., Adnan, M., & Zhang, M. Investigation of soil nutrients and associated rhizobacterial communities in different sugarcane genotypes in relation to sugar content. Chemical and Biological Technologies in Agriculture. (2021). 8, 1-13. https://doi.org/10.1186/s40538-021-00244-5

Le Geay, M., Lauga, B., Walcker, R., Jassey, V. E. A meta-analysis of peatland microbial diversity and function responses to climate change. Soil Biology and Biochemistry. (2024). 189, 109287. https://doi.org/10.1016/j.soilbio.2023.109287

Luo, X., Han, S., Lai, S., Huang, Q., & Chen, W. Long-term straw returning affects Nitrospira-like nitrite oxidizing bacterial community in a rapeseed-rice rotation soil. Journal of Basic Microbiology. (2017). 57(4), 309-315. https://doi.org/10.1002/jobm.201600400

Luo, Y., Iqbal, A., He, L., Zhao, Q., Wei, S., Ali, I., Jiang, L. Long-term no-tillage and straw retention management enhances soil bacterial community diversity and soil properties in southern China. Agronomy. (2020). 10(9), 1233. https://doi.org/10.3390/agronomy10091233

Magoc, T., and S. L. Salzberg. "FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. (2011). 27, 2957e2963. https://doi.org/10.1093/bioinformatics/btr507

Mohammadi, K., Heidari, G., Khalesro, S., & Sohrabi, Y. Soil management, microorganisms and organic matter interactions: A review. African Journal of Biotechnology. (2011). 10(86), 19840. https://doi.org/10.5897/AJBX11.006

Nasedjanov, M. The effects of lime on pH values of soil at different pH levels. United Nations University Land Restoration Training Programme, [final project] http://www. unulrt. is/static/fellows. (2012).

Palm, C. A., Myers, R. J., & Nandwa, S. M. Combined use of organic and inorganic nutrient sources for soil fertility maintenance and replenishment. Replenishing soil fertility in Africa. (1997). 51, 193-217. https://doi.org/10.2136/sssaspecpub51.c8

Pan, Y. Q., Tung, S. A., Yang, L., Wang, Y., & Zhou, X. B. Effect of straw return and nitrogen application rate on the photosynthetic characteristics and yield of double-season maize. Journal of Soil Science and Plant Nutrition. (2021). 1-14. https://doi.org/10.1007/s42729-021-00676-w

Ren, N., Wang, Y., Ye, Y., Zhao, Y., Huang, Y., Fu, W., & Chu, X. Effects of continuous nitrogen fertilizer application on the diversity and composition of rhizosphere soil bacteria. Frontiers in microbiology. (2020). 11, 1948. https://doi.org/10.3389/fmicb.2020.01948

Sarkar, S., Skalicky, M., Hossain, A., Brestic, M., Saha, S., Garai, S., Brahmachari, K. Management of crop residues for improving input use efficiency and agricultural sustainability. Sustainability. (2020). 12(23), 9808. https://doi.org/10.3390/su12239808

Shi, J., Xu, F., Wang, Z., Stiverson, J. A., Yu, Z., & Li, Y. Effects of microbial and non-microbial factors of liquid anaerobic digestion effluent as inoculum on solid-state anaerobic digestion of corn stover. Bioresource Technology. (2014). 157, 188-196. https://doi.org/10.1016/j.biortech.2014.01.089

Siedt, M., Schäffer, A., Smith, K. E., Nabel, M., Roß-Nickoll, M., & Van Dongen, J. T. Comparing straw, compost, and biochar regarding their suitability as agricultural soil amendments to affect soil structure, nutrient leaching, microbial communities, and the fate of pesticides. Science of the Total Environment. (2021). 751, 141607. https://doi.org/10.1016/j.scitotenv.2020.141607

Singh, V. K., Malhi, G. S., Kaur, M., Singh, G., Jatav, H. S. Use of organic soil amendments for improving soil ecosystem health and crop productivity. Ecosystem Services. Nova Publisher. (2022). ISBN: 978-1-68507-614-6

Song, Y., Zhao, Q., Guo, X., Ali, I., Li, F., Lin, S., & Liu, D. Effects of biochar and organic-inorganic fertilizer on pomelo orchard soil properties, enzymes activities, and microbial community structure. Frontiers in Microbiology. (2022). 13, 980241. https://doi.org/10.3389/fmicb.2022.980241

Su, Y., Lv, J. L., Yu, M., Ma, Z. H., Xi, H., Kou, C. L., Shen, A. L. Long-term decomposed straw return positively affects the soil microbial community. Journal of Applied Microbiology. (2020). 128(1), 138-150. https://doi.org/10.1111/jam.14435

Su, Y., Lv, J. L., Yu, M., Ma, Z. H., Xi, H., Kou, C. L., Shen, A. L. Long-term decomposed straw return positively affects the soil microbial community. Journal of Applied Microbiology. (2020). 128(1), 138-150. https://doi.org/10.1111/jam.14435

Tian, B. Y., Cao, Y., & Zhang, K. Q. Metagenomic insights into communities, functions of endophytes and their associates with infection by root-knot nematode, Meloidogyne incognita, in tomato roots. Scientific reports. (2015). 5(1), 17087. https://doi.org/10.1038/srep17087

Wang, K., Ren, T., Yan, J., Zhu, D., Liao, S., Zhang, Y., Lu, J. Straw returning mediates soil microbial biomass carbon and phosphorus turnover to enhance soil phosphorus availability in a rice-oilseed rape rotation with different soil phosphorus levels. Agriculture, Ecosystems & Environment. (2022). 335, 107991. https://doi.org/10.1016/j.agee.2022.107991

Wang, L., Wang, C., Feng, F., Wu, Z., & Yan, H. Effect of straw application time on soil properties and microbial community in the Northeast China Plain. Journal of Soils and Sediments. (2021). 21(9), 3137-3149. https://doi.org/10.1007/s11368-021-03009-7

Wu, D., Feng, Z., Gu, W., Wang, Y., Liu, Z., Wang, W., Chen, W. Could continuous rice cropping increase soil fertility and rice productivity by rice straw carbonized utilization in cold areas? -A 6-year field-located trial. Environmental Science and Pollution Research. (2023). 30(51), 110674-110686. https://doi.org/10.1007/s11356-023-30097-4

Xiaoping, Wang, L., Hou, Y., Wang, H., Tsang, Y.F., & Wu, J. Responses of Soil Microbial Community Structure and Activity to Incorporation of Straws and Straw Biochars and Their Effects on Soil Respiration and Soil Organic Carbon Turnover. Pedosphere. (2019). 29(4), 492-503. https://doi.org/10.1016/S1002-0160(19)60813-1

Xu, A., Li, L., Coulter, J. A., Xie, J., Gopalakrishnan, S., Zhang, R., ... & Khan, S. Long-term nitrogen fertilization impacts on soil bacteria, grain yield and nitrogen use efficiency of wheat in semiarid loess plateau, China. Agronomy. (2020). 10(8), 1175. https://doi.org/10.3390/agronomy10081175

Yeomans, J. C., & Bremner, J. M. A rapid and precise method for routine determination of organic carbon in soil. Communications in soil science and plant analysis. (1988). 19(13), 1467-1476. https://doi.org/10.1080/00103628809368027

Yilmaz, P., Parfrey, L. W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., & Glöckner, F. O. The SILVA and "all-species living tree project (LTP)" taxonomic frameworks. Nucleic acids research. (2014). 42(D1), D643-D648. https://doi.org/10.1093/nar/gkt1209

Yin, H., Zhao, W., Li, T., Cheng, X., Liu, Q. Balancing straw returning and chemical fertilizers in China: Role of straw nutrient resources. Renewable and Sustainable Energy Reviews. (2018). 81, 2695-2702. https://doi.org/10.1016/j.rser.2017.06.076

Yu, Z., Liu, J., Liu, J., Jin, J., Liu, X., Wang, G. Responses of ammonia-oxidizing bacterial communities to land-use and seasonal changes in Mollisols of Northeast China. European journal of soil biology. (2016). 74, 121-127. https://doi.org/10.1016/j.ejsobi.2016.03.012

Yuan, G., Huan, W., Song, H., Lu, D., Chen, X., Wang, H., & Zhou, J. Effects of straw incorporation and potassium fertilizer on crop yields, soil organic carbon, and active carbon in the rice-wheat system. Soil and Tillage Research. (2021). 209, 104958. https://doi.org/10.1016/j.still.2021.104958

Zhang, C., Tayyab, M., Abubakar, A. Y., Yang, Z., Pang, Z., Islam, W., Zhang, H. Bacteria with different assemblages in the soil profile drive the diverse nutrient cycles in the sugarcane straw retention ecosystem. Diversity. (2019). 11(10), 194. https://doi.org/10.3390/d11100194

Zhang, X., Wei, H., Chen, Q., & Han, X. The counteractive effects of nitrogen addition and watering on soil bacterial communities in a steppe ecosystem. Soil Biology and Biochemistry. (2014). 72, 26-34. https://doi.org/10.1016/j.soilbio.2014.01.034

Zhao, S., Li, K., Zhou, W., Qiu, S., Huang, S., & He, P. Changes in soil microbial community, enzyme activities and organic matter fractions under long-term straw return in north-central China. Agriculture, Ecosystems & Environment. (2016). 216, 82-88. https://doi.org/10.1016/j.agee.2015.09.028

Zheng, L., Wu, W., Wei, Y., Hu, K. Effects of straw return and regional factors on spatio-temporal variability of soil organic matter in a high-yielding area of northern China. Soil and Tillage Research. (2015). 145, 78-86. https://doi.org/10.1016/j.still.2014.08.003

Zhu, F., Lin, X., Guan, S., & Dou, S. Deep incorporation of corn straw benefits soil

organic carbon and microbial community composition in a black soil of Northeast China. Soil Use and Management. (2022). 38(2), 1266-1279. https://doi.org/10.1111/sum.12793

Zhu, F., Lin, X., Guan, S., & Dou, S. Deep incorporation of corn straw benefits soil organic carbon and microbial community composition in a black soil of Northeast China. Soil Use and Management. (2022). 38(2), 1266-1279. https://doi.org/10.1111/sum.12793

Zhu, X., Zhang, L., Zuo, Y., Liu, J., Yu, J., Yuan, F., Xu, X. Wetland reclamation homogenizes microbial properties along soil profiles. Geoderma. (2021). 395, 115075. https://doi.org/10.1016/j.geoderma.2021.115075

**How to cite this article:**

Borny, N.R., Mostakim, GM., Raihan, A., Rahman, S. Synergistic Effects of Straw Return and Nitrogen Fertilizer on Soil Bacterial Communities and Fertility. Journal of Soil, Plant and Environment. (2024). 3(1), 41–58. https://doi.org/10.56946/jspae.v3i1.404