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Long-Term Wheat-Soybean Rotation and the Effect of Straw Retention on the Soil Nutrition Content and Bacterial Community

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Abstract: Straw retention and wheat-soybean rotation play critical role in maintaining soil quality. However, the correlation between bacterial diversity and community structure, and soil nutrients is unknown, and a systematic understanding of their responses to straw retention is lacking. In the field experiment, the straw retention treatments included no straw (NS), half straw (HS), and total straw (TS) retention during long-term wheat-soybean rotation. The mean contents of soil total nitrogen (TN), nitrate-N (NO_3^--N), and microbial biomass nitrogen (MBN) increased by 15.06%, 21.10%, and 38.23%, respectively, with straw retention relative to NS, while that of ammonium-N (NH_4^+-N) reduced by 3.68%. The concentration of carbon components increased as straw retention increased. The levels of soil dissolved organic carbon (DOC), microbial biomass carbon (MBC), and soil organic carbon (SOC) increased by 4.34%, 7.63%, and 9.34%, respectively, with straw retention relative to NS. Soil bacterial *alpha* diversity was reduced with straw retention. Soil pH and nutrient content were identified as the main factors affecting the soil microbial diversity and structure at the phylum level. Accordingly, straw retention and soybean-wheat rotation enable sustainable agriculture in the dryland of northern China.

Keywords: straw retention; wheat-soybean rotation; carbon and nitrogen components; bacterial community; sustainable agriculture

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1. Introduction

Straw retention and soybean-wheat rotation are widespread agricultural planting models that have been practiced for thousands of years in northwest China. These methods significantly increase rainfed crop productivity in dry climates, and are the essence of traditional agriculture in China [1]. The annual crop straw output in China is approximately 700–800 million tons, thereby serving as a vital agricultural resource. The return of straw into agricultural soil is one of the most important management practices in China [2]. Crop straw can be used as an organic fertilizer, significantly increasing the average soil organic carbon (SOC) content by 13.97% [3]. Accordingly, this fertilizer has a remarkable potential for replacing chemical fertilizers [4]. Straw mulching can increase soil water storage (26.5 mm), grain yield (28.7%), and water-use efficiency (26.6%), which are considered beneficial in Guanzhong, China [5]. Approximately 4 billion metric tons of crop residues are produced annually worldwide. The retention of such large amount of residues on agricultural land can be beneficial for soil carbon sequestration and SOC promotion globally [3,6]. Straw retention is the main driver for improved crop production (4.0–28.0%, median: 8.2%); as a result, straw retention plays an essential role in achieving sustainable land use, and the concomitant achievement of relatively high grain yields [7,8]. However, some researchers have found that crop yield decreased with straw retention. In fact, straw return did not improve crop yield in wheat/maize mono-cropping systems and decreased the crop yield by 2.42% in wheat-maize double cropping systems of Chinese upland soils [2]. Significant increases of 0.5–8.7% and 1.4–27.7% in soil CO_2 emission were also observed during all growth stages of summer soybean and winter wheat in the total straw regime, compared with that observed with no straw retention [9]. Straw retention can enhance carbon bioavailability and immobilize microbial nitrogen (N) in soil [10]. However, the response to the change in the content of soil nitrogen and carbon components upon addition of wheat and soybean straw is uncertain.

The efficient utilization of biological nitrogen by soybean, a legume crop, is an agrotechnical practice that improves the soil environment [11]. Soybean plant not only increases nitrogen (N) content, but also carbon (C) content in soils. Soil microbial community structures and their diversity were found to be altered by the long-term use of soybean [12]. Generally, soybean straw with high nitrogen content and low C/N ratio is easily decomposed in soil, and can release more mineral nitrogen and improve soil microbial biomass [13]. Soybean is an economically important green manure crop because of its high biological nitrogen fixation capacity [14]. Furthermore, the application of N fertilization, which is widely used in intercropping and rotation systems, can be reduced. Long-term implementation of crop rotation and straw input improves the soil environment and quality [15]. By performing crop rotation with soybean, more significant net nitrogen mineralization and increases in residual soil nitrogen content were achieved [16]. Lower soil CO_2 emissions and higher water use efficiency in soybean fields than in maize are particularly beneficial in the dryland cropping systems of northern China [17]. Thus, the effects of straw retention on soil carbon and nitrogen dynamics during long-term wheat-soybean rotation should be comprehensively elucidated.

Soil microorganisms play an essential role in the soil ecological and biogeochemical processes, including carbon and nitrogen cycling for plant nutrient uptake [18]. The complexity of the microbial network increases significantly with straw return [19]. Soybean straw application can improve soil fertility and alter soil bacterial community structure [20]. However, the importance of the microbiome for plant performance in agricultural systems is often ignored. Fertilization eliminates the dependency of plants on plant-beneficial microbial processes [21], such as soil nitrogen-cycling microbes and biological nitrogen fixation by legumes crops [22]. Most areas in China are associated with agricultural production situations such as excessive chemical fertilizer application, insufficient investment in organic fertilizer, and degradation of soil quality [23]. The dissolved organic carbon (DOC), SOC, and microbial biomass carbon and nitrogen (MBC and MBN) content of the soil are the primary drivers of microbial community composition [24]. The total nitrogen (TN), nitrate-N (NO_3^--N), and ammonium-N (NH_4^+-N) contents of the soil are dynamic quantitative values that are essential for the improvement of crop yield and comprehensively reflect soil microbial biomass during nitrogen mineralization and nitrogen retention in the microecological environment [25]. Soil organic carbon and available nitrogen (NO_3^--N and NH_4^+-N) in the soil have a significant impact on the abundance of the bacterial and fungal communities [26]. In summary, soil bacterial diversity and community composition are crucial for soil health and plant growth [27]. Soil bacterial communities under straw retention and long-term winter wheat- summer soybean rotation, and their dynamics in response to soil carbon and nitrogen changes are poorly understood.

Soil physical and chemical properties change owing to differences in straw retention under long-term wheat-soybean rotation, and may influence the variation in soil bacteria diversity and community structure [28]. Based on this, we opted to understand how soil quality can be retained while maintaining productivity during winter wheat-summer soybean rotation. The effects of straw retention or rotation on SOC and TN pools in winter wheat and soybean management have been determined [28]. However, in the long-term rotation process of legumes and Gramineae, there is a lack of relevant studies on the seasonal dynamic changes in soil nitrogen components and their response to straw return. In this experiment, the contents of soil nitrogen and carbon components were measured throughout different periods during winter wheat-summer soybean growth over two years. The aims of this study were to (1) identify the impact of straw retention on soil NO_3^--N, NH_4^+-N, MBN, TN, SOC, DOC, and MBC contents; (2) determine the effect of straw retention on soil microbial diversity; and (3) establish a relationship between bacterial community structure and soil nitrogen and carbon components during straw retention under wheat-soybean rotation.

2. Materials and Methods

2.1. Study Site

The study was performed at the agricultural experimental field at Northwest A&F University (34°12′ N and 108°7′ E), Shaanxi Province, north-west China. The station is located 520 m above sea level. The historical mean annual rainfall is 630 mm, and mean monthly temperature is 23.4 °C (Figure 1). The texture of the soil (0–20 cm) at the experimental site is silt clay loam, and is classified as Lou soil (anthrosol) [29]. The properties of the soil at the 0–20 cm depth at the start of the experiment in October of 2016 were as follows: bulk density, 1.40 g·cm^{-3}; saturated soil water content, 43.5%; and field capacity, 24.1%. The soil nutrient concentrations were as follows: 0.87 g·kg^{-1} TN, 8.99 g·kg^{-1} organic matter, 15.63 mg·kg^{-1} alkali hydrolysable nitrogen, 22.37 mg·kg^{-1} available phosphorus, and 165.33 mg·kg^{-1} available potassium.

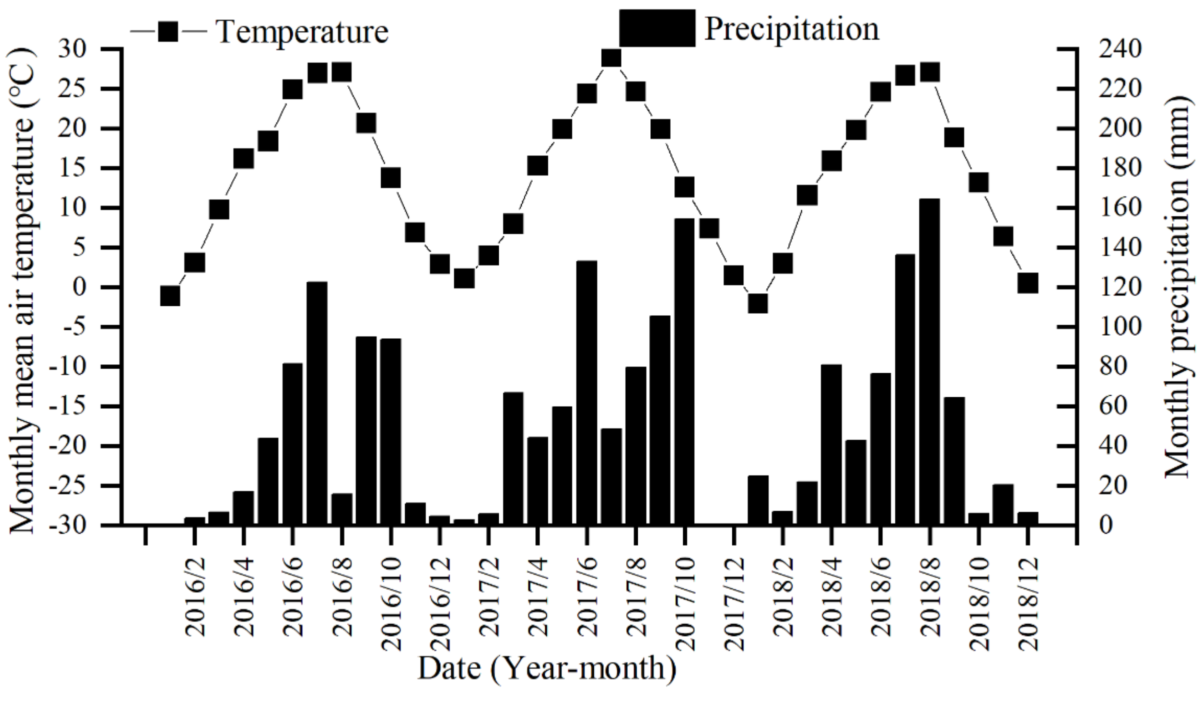

Figure 1. Variation of monthly air temperature and monthly precipitation in the research area during the crop growing seasons from January 2016 to June 2018.

2.2. Experimental Design and Management

This study was carried out in 2008 as a field experiment on straw retention and winter wheat–summer soybean rotation. This study is also part of a long-term experiment conducted between April 2016 and June 2018. Rotation modes have been widely adopted in local agricultural production, and rotation-crop systems included winter wheat (*Triticum aestivum* L.) as an autumn sown crop, and soybean (*Glycine max* (Linn.) Merr.) as a summer sown crop. The research plots were subjected to three straw retention treatments: no straw (NS), half straw (HS), and total straw (TS) retention throughout the field experiment during long-term wheat-soybean rotation. For NS, all straws from winter wheat and summer soybeans were removed. For HS, half the amount of the TS coverage was removed, and similar to the TS treatment, the straw was chopped to provide even coverage. For TS, all straws from winter wheat and summer soybean were retained. The amount of straw retained for the different treatments is outlined in Table 1. In the research area, winter wheat was sown in October and harvested at the beginning of June of the following year. During the winter wheat harvest, the wheat straw was chopped into pieces of 3–5 cm (length) using a straw chopping machine, mulched on the soil surface, and maintained for the next summer soybean crop. Summer soybean was sown in the middle of June and harvested in September. Soybean straw was chopped into pieces of 3–5 cm (length), and returned to the soil by rotary tilling (depth of 0–10 cm) prior to winter wheat sowing. Fertilizer was applied before winter wheat sowing, and urea and diammonium phosphate were used according to local practices. Fertilizing practices and amounts were the same for every plot, and consisted of 118 kg $P_2O_5{\cdot}ha^{-1}$ and 135 kg $N{\cdot}ha^{-1}$ as base fertilizer.

Table 1. Experiment design of different straw retention treatments.

Crop Type	TC, TN Content of Straw (%)		Amount of Straw Retention ($kg{\cdot}ha^{-1}$)		
	TC	**TN**	**NS**	**HS**	**TS**
Winter wheat	44.4	0.66	0	1900	3800
Summer soybean	45.35	1.05	0	3200	6400

For the field experiment, major local varieties of winter-wheat (Xinong 889) and summer soybean (Dongdou 339) were selected. The row spacing was 20 cm for winter-wheat, and the plant density was 15 cm × 60 cm for summer soybean. The experiment comprised three replicates and each plot was 12 m in length and 5.1 m wide. The crop cultivation and farming system, and the management measures for all three treatments were in accordance with the local conventional cultivation practices. Irrigation was not performed beyond rainfall throughout the experimental period.

2.3. Measurement of Nitrogen and Carbon Indices of Soil

In this study, soil samples from soil depths of 0–20 and 20–40 cm were collected during the growth of winter wheat and summer soybean. Samples were collected randomly from three different locations in each plot, and soils from the same depth were mixed to form a composite sample [30]. These samples were divided into two parts: one sample was used for the analysis of soil nutrients, and the other fresh soil sample was used to investigate the abundance, diversity, and community composition of bacteria. A portion of each soil sample was immediately shipped from the field to the laboratory in an icebox and immediately stored at −80 °C for DNA extraction. The remaining portion of the soil sample was dried at room temperature and stored for physicochemical analysis [31]. All measurements were performed in triplicate.

The SOC content was determined according to the $K_2Cr_2O_7$–H_2SO_4 digestion method. Total nitrogen NO_3^--N, NH_4^+-N, MBC and MBN were determined as described in a previous study [32].

2.4. Soil DNA Extraction and High-Throughput Sequencing

Soil DNA was extracted from each soil sample using a FastDNA spin kits for soil (MP Biomedical, Carlsbad, CA, USA) according to the manufacturer's directions. For each sample, the total genomic DNA was extracted from 1.5 g (0.5 g × 3) fresh soil and then mixed. A NanoDrop spectrophotometer (ND-One, NanoDrop Technologies, Wilmington, DE, USA) was used to determine the quantity and quality of DNA. Thereafter, 2% agarose gel electrophoresis was performed to confirm the integrity of the DNA extracts. The DNA extracted from the soil was stored at −80 °C. The details of test are described by XU [31].

Soil bacterial diversity and relative abundance were analyzed using the Illumina Hiseq high-throughput sequencing technology to determine the bacterial sequences. Polymerase chain reaction (PCR) amplification of the bacterial 16S rRNA gene V3–V4 region was performed using the forward primer, 338F (5′-ACTCCTACGGGAGGCAGCA-3′), and reverse primer, 806R (5′-GGACTACHVGGGTWTCTAAT-3′) [33]. Sequencing, bioinformatics, and statistical analysis were performed at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China).

2.5. Statistical Analyses

All data were subjected to analysis of variance (ANOVA) using IBM SPSS Statistics 21 software (SPSS Institute Inc., 2008 IBM, Armonk, NY, USA). The stratification ratio is the ratio of soil nutrient content in the surface layer to that in the deeper layer under the same soil conditions. To assess the relative factor of soil nitrogen fractionations (such as NO_3^--N, NH_4^+-N, MBN, TN, the ratio of NO_3^--N to TN, the ratio of NH_4^+-N to TN, the ratio of MBN to TN, and the ratio of NO_3^--N to NH_4^+-N) and carbon fractionations (such as MBC, DOC, SOC, the ratio of MBC to SOC, the ratio of SOC to TN, the ratio of MBC to MBN) on bacterial diversity and community structure, correlations were determined using redundancy analysis.

3. Results

3.1. Soil Nitrogen Content

Plants can directly take up soil inorganic nitrogen (NO_3^--N and NH_4^+-N). The concentration of soil inorganic nitrogen varied markedly with soil depth and seasons. Soil NO_3^--N, NH_4^+-N, MBN, and TN contents increased significantly owing to straw retention under wheat-soybean rotation (Figure 2 and Figure S1). Soil NO_3^- -N and NH_4^+-N contents displayed a regular dynamic change, with gradual depletion during winter wheat growth in spring (from March to May) and an increase in the crest value from October to December, after the end of the summer soybean period (i.e., when the fertilizer was added before winter wheat sowing) (Figure S1). The NO_3^--N content ranged from 1.64 to 22.76 mg/kg and was significantly affected by different straw return levels (Figure S1). The mean contents of NO_3^--N were 9.30, 11.18, and 11.54 mg/kg for NS, HS, and TS at the 0–20 cm soil depth, respectively (Figure 2a). The content of MBN increased during spring, and was low in the winter. Consequently, the content variation trend of soil MBN was opposite to that of NO_3^--N. Soil MBN content ranged from 2.34 to 47.14 mg/kg under the different straw return treatments at different stages (Figure S1). The mean MBN content was 24.34, 30.42, and 32.75 mg/kg with NS, HS, and TS, respectively, at the 0–20 cm soil depth (Figure 2c). There were no significant differences in the mean soil NO_3^--N content during the study period among the three straw retention treatments. However, there were significant differences in the soil NO_3^--N content among the three treatments at the different test times. The content of NH_4^+-N, MBN, and TN displayed the same trend (Figures 2 and S1).

Straw retention has a marked effect on the quantity and formation of mineral nitrogen in the soil. Soil NO_3^--N, MBN, and TN content increased after straw retention; however, no significant differences were found among the three straw retention strategies (Figure 2). Compared with that for NS, the average increase in NO_3^--N, MBN and TN contents in the 0–20 cm soil layer were 16.86, 24.46, and 25.02% with HS, and 34.56, 7.33, and 14.52% with TS treatments, respectively. The average increase in NO_3^--N, MBN and TN contents in 20–40 cm soil layer were 14.55, 28.54, and 46.34% with HS, 46.98, 13.76, and 24.70%, respectively, with TS compared with NS. The mean soil TN, NO_3^--N, and MBN contents with straw return treatment were 15.06, 21.10, and 38.23% higher than those with NS, respectively.

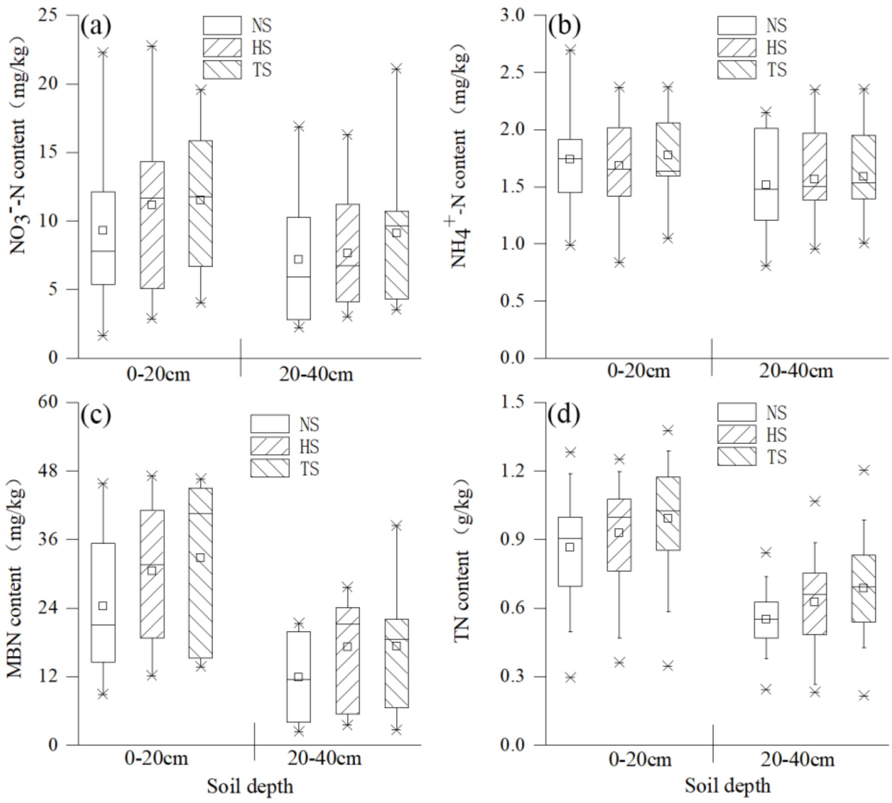

Figure 2. Effect of different straw applications on mean content of soil nitrogen components. (**a**), NO_3^--N content, NO_3^--N, nitrate-N; (**b**), NH_4^+-N content, NH_4^+-N, ammonium-N; (**c**), MBN content, MBN, microbial biomass nitrogen; (**d**), TN content, TN, Soil total nitrogen.

The NH_4^+-N content was reduced by the straw retention treatments. Soil NH_4^+-N content ranged from 0.81 to 2.70 mg/kg under the different straw returning treatments, and the variation coefficient of soil NH_4^+-N content was less than that of NO_3^--N (Figure S1). The mean content of NH_4^+-N was 1.74, 1.69, and 1.77 mg/kg with NS, HS, and TS at 0–20 cm soil depth, respectively (Figure 2b). Compared with that in NS, the content of NH_4^+-N was decreased in the HS (7.92 and 4.49%) and TS treatments (2.18 and 0.06%) at the 0–20 and 20–40 cm soil depths, respectively. The NH_4^+-N content of the straw return treatment was reduced by 3.68% compared with that of the NS treatment.

The distribution of TN and MBN in the soil layer was usually the highest in the surface soil and gradually decreased as the depth increased. In this experiment, the stratification ratios of MBN were 2.94, 2.27 and 2.68, whereas those of TN were 1.60, 1.51, and 1.47 with NS, HS, and TS, respectively. The stratification ratios of TN decreased as the return amount increased (Figure 2d). The mean ratios of soil NO_3^--N to TN were 0.98, 1.09, and 1.08%, while those of NH_4^+-N to TN were 0.25, 0.23, and 0.22% with NS, HS, and TS. The ratios of NO_3^--N to NH_4^+-N were 5.23, 6.11, and 6.46, and those of soil MBN to TN were 2.28%, 2.71%, and 2.75% with NS, HS, and TS, respectively. The mean soil nitrogen bioavailability increased as straw retention increased.

3.2. Different forms of Soil Carbon Content

The increase in crop yield after straw return is closely related to the improvement in SOC, soil structure, and nutrients [2]. The contents of SOC, MBC, and DOC, which are important indices reflecting soil quality and soil fertility, were highest with TS and were significantly promoted by straw return treatments. The coefficient of variation of the carbon components with TS was larger than that with NS. Soil MBC content ranged from 49.06 to 522.07 mg/kg at the 0–40 cm soil depth under different straw returning treatments, varying considerably in different periods (Figure S2a). The mean contents of MBC were

285.5, 315.3, and 320.7 mg/kg with NS, HS, and TS, respectively, at the 0–20 cm soil depth (Figure 3a). Soil MBC was lower in September to December and higher in March to May of spring during wheat growth, but decreased after wheat harvest. The variation in MBC content was more extensive in the surface soil than in the deeper soil under the different straw retention treatments. Soil MBC content increased by 10.45 and 14.13% with HS and TS, respectively, compared with that of NS at the 0–20 cm soil depth and increased by 2.96 and 2.98% with HS and TS, respectively, at the 20–40 cm soil depth. The average ratio of soil MBC to SOC (soil microbial entropy) was 2.72, 2.79, and 2.58% for NS, HS, and TS, respectively.

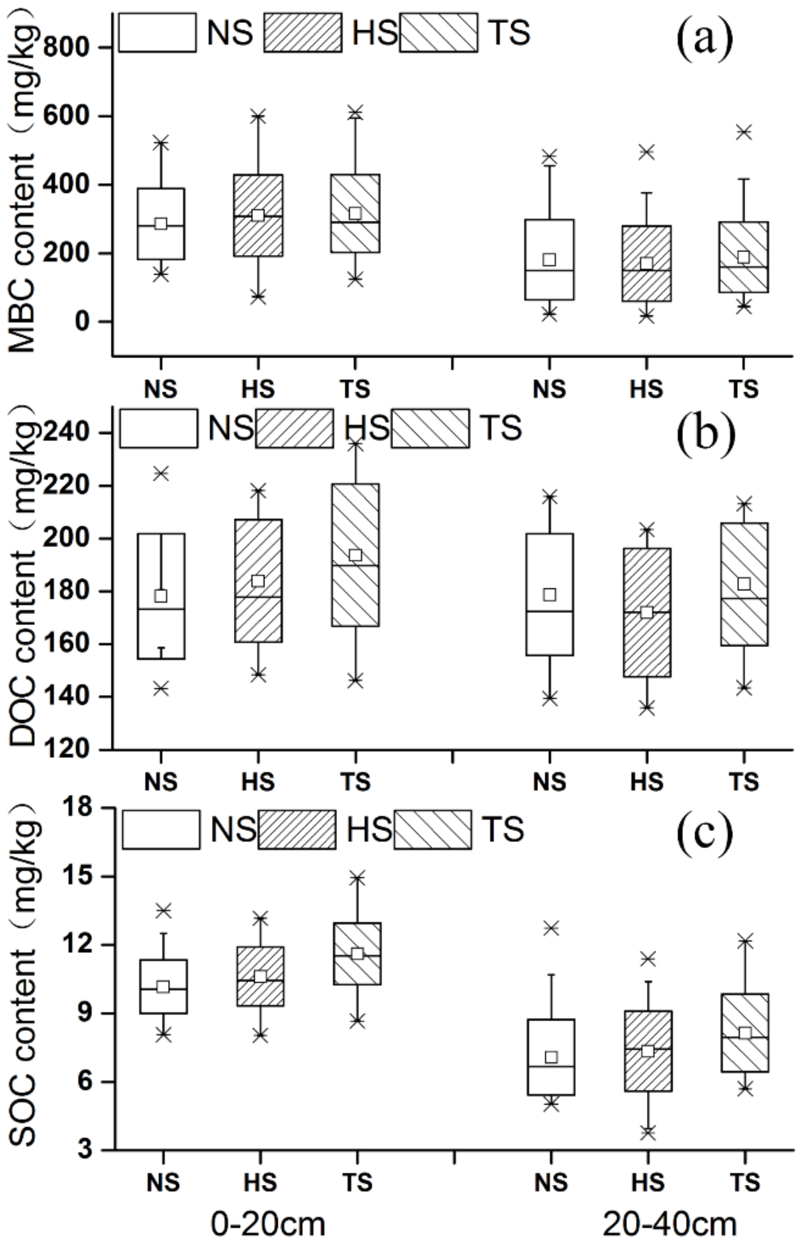

Figure 3. Effect of different straw application levels on mean MBC, DOC, SOC content. (**a**), MBC content. MBC, microbial biomass carbon; (**b**), DOC content, DOC, dissolved organic carbon; (**c**), SOC content. SOC soil organic carbon.

Soil DOC content ranged from 135.82 to 236.07 mg/ kg (Figure S2b), increasing by 3.28 and 8.76% with HS and TS at the 0–20 cm soil depth and 0.73 and 4.60% with HS and TS at the 20–40 cm soil depth, compared with that of NS, respectively (Figure 3b). The mean DOC content were 178.1, 184.0, and 193.7 mg/ kg for NS, HS, and TS, respectively, at the 0–20 cm soil depth (Figure 3b). The ratio of DOC to SOC were 2.11, 2.06, and 1.96% for NS, HS, and TS, respectively. The content of SOC ranged from 5.06 to 12.71 g/kg at the 0–40 cm soil depth under different straw returning treatments (Figure S2c). The mean SOC content were10.2, 10.6, and 11.6 g/kg for NS, HS, and TS, respectively, at 0–20 cm soil depth (Figure 3c). The SOC content increased by 4.41 and 14.15% at the 0–20 cm soil depth and 3.78 and 15.00% at the 20–40 cm soil depth for HS and TS, respectively, compared with that for NS.

The concentrations of soil MBC, DOC, and SOC gradually increased as the amount of straw return increased, with increases of 7.63, 4.34, and 9.34% in soil MBC, DOC, and SOC content under the straw return treatment, respectively, compared with those in the non-returning treatment. The variation of soil DOC and SOC content during the different periods was smaller than that of MBC. The ratio of SOC to TN increased as the straw application level increased, with average values of 11.10, 12.25, and 13.02 for NS, HS, and TS, respectively. The average ratio of MBC to MBN decreased as straw retention levels increased, with values of 13.49, 10.04, and 9.79 in the NS, HS, and TS treatments, respectively.

3.3. Diversity of Soil Bacteria

The *alpha* diversity of soil bacterial communities is affected by straw retention with winter wheat and soybean. The *alpha* diversity scores indicated a decrease in bacterial abundance and diversity under straw retention. The diversity indices valuesof *Chao1* and *Observed species* represent richness. The *chao1* index values ranged from 2069.6 to 3682.6, with averages of 2906.5, 2910.6, and 2626.8 for NS, HS, and TS, respectively. (Figure 4a). The *Observed species* index values ranged from 1876 to 3141, with averages of 2549.1, 2546.0, and 2273.9 for NS, HS, and TS, respectively (Figure 4b). The diversity indices, *Simpson* and *Shannon*, showed variations. The *Simpson* index values ranged from 0.9973 to 0.9991, with averages of 0.9987, 0.9985, and 0.9986 for the NS, HS, and TS treatments, respectively (Figure 4c). The *Shannon* index values ranged from 9.95 to 10.82, with averages of 10.41, 10.34, and 10.23 for NS, HS, and TS, respectively (Figure 4d). *Faith's* phylogenetic diversity(*'PD'*) indicates diversity based on evolution and had values ranging from 161.6 to 245, with averages of 201.3, 201.9, and 188.7 for NS, HS, and TS, respectively (Figure 4e). *Pielou's evenness* index represents the uniformity and values ranged from 0.905 to 0.932, with averages of 0.9215, 0.9160, and 0.9195 for NS, HS, and TS, respectively (Figure 4e). The *Good's coverage* index values ranged from 0.9717 to 0.9897, with averages of 0.9811, 0.9798, and 0.9836 for NS, HS, and TS, respectively (Figure 4g). Straw retention decreased the soil microbial taxonomic diversity.

3.4. Structure of Soil Bacteria

HiSeq sequencing revealed that at the phylum level, the predominant phyla were *Proteobacteria*, *Actinobacteria*, and *Acidobacteria*, which accounted for more than 66% of the relative abundance. The proportions of bacteria were as follows: *Proteobacteria* (28.14, 27.40, and 28.16%) > *Acidobacteria* (22.90, 24.86, and 24.82%) > *Gemmatimonadetes* (14.35, 13.52, and 14.20%) > *Actinobacteria* (11.06, 10.94, and 9.91%) > *Chloroflexi* (7.17, 6.94, and 6.59%) > *Bacteroidetes* (4.08, 4.08, and 4.00%); the percentages in parentheses indicate the mean relative abundance of soil bacteria for NS, HS, and TS, respectively (Figure 5a). The relative abundance of *Acidobacteria* differed significantly between the three treatments. The proportions of bacteria at the class level were as follows: *Subgroup 6* (13.63, 15.43, and 15.25%) > *Gammaproteobacteria* (13.19, 12.40, and 13.19%) > *Gemmatimonadetes* (11.74, 11.06, and 11.57%) for NS, HS, and TS, respectively (Figure 5b). The relative abundance of soil bacteria of *Subgroup6* and *Deltaproteobacteria* significantly differed ($p < 0.05$) at the class level among the three treatments.

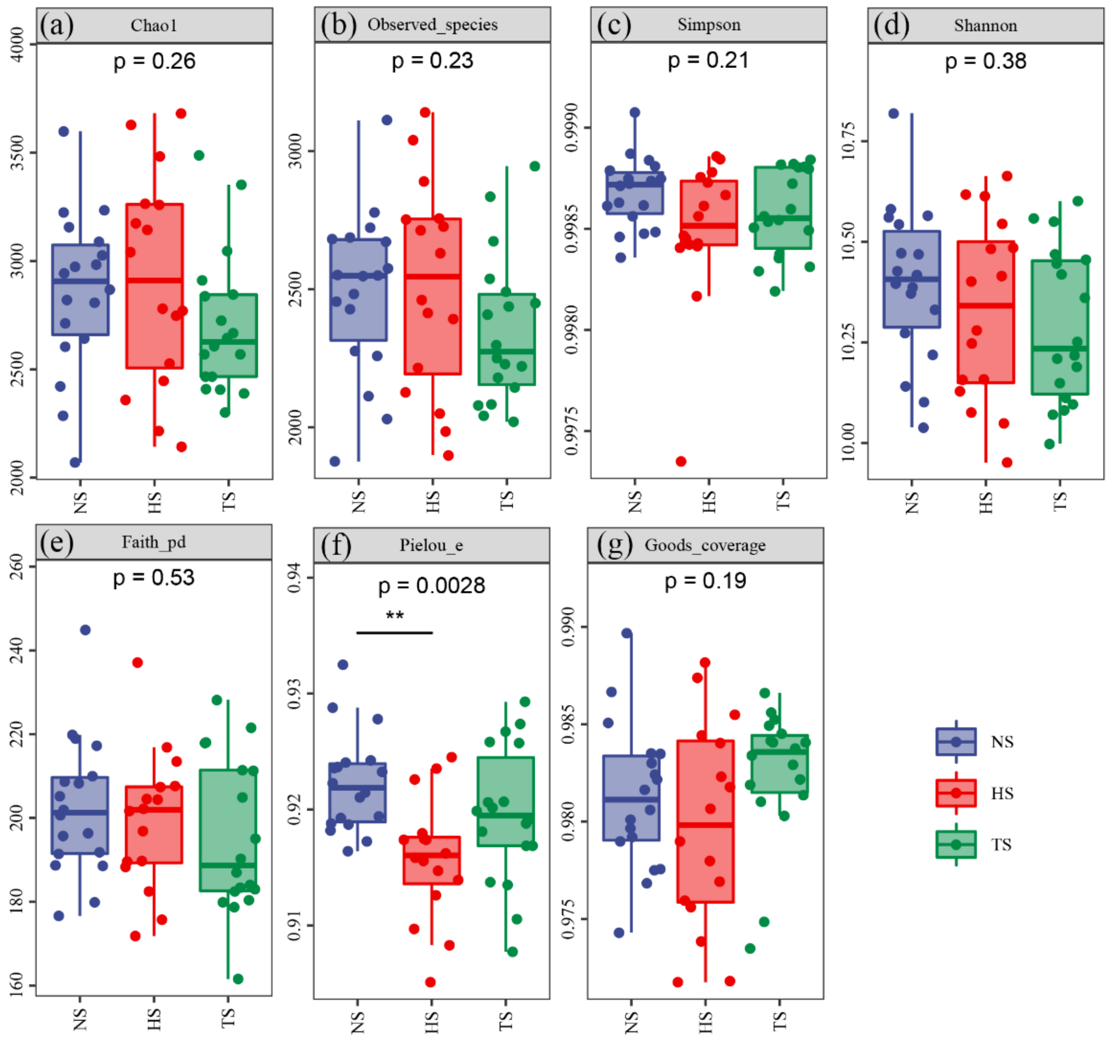

Figure 4. Effect of different fertilizer and straw applications on bacterial diversity based on *chao1*, *Shannon*, and *Simpson* indices. (**a**), *Chao1*; (**b**), *Observed species*; (**c**), *Simpson*; (**d**), *Shannon*; (**e**), *Faith's PD*; (**f**), Pielou e; (**g**), Good's coverage. Significant differences are denoted by ** $p < 0.01$).

The relative abundance of soil bacteria at the order level (*Subgroup 6* and *Betaproteobacteriales*) was significantly different ($p < 0.05$) among the three treatments. The proportions of bacteria were as follows the sequence: *Subgroup 6* (13.58, 15.37, and 15.21%) > *Gemmatimonadales* (11.74, 11.06, and 11.57%) > *Betaproteobacteriales* (8.49, 7.56, and 8.25%) for NS, HS, and TS, respectively (Figure 5c). The relative abundance of soil bacteria, *Nitrosomonadaceae* and *Subgroup 6*, differed significantly ($p < 0.05$) between the three treatments at the family level. The relative abundance of soil bacteria at the family level was as follows: *Subgroup 6* (13.58, 15.37, and 15.21%) > *Gemmatimonadaceae* (11.74, 11.06, and 11.57%) > *Nitrosomonadaceae* (4.61, 3.84, and 4.81%) for NS, HS, and TS, respectively (Figure 5d).

The relative abundance of soil bacteria at the genus level (*Subgroup 6* and *MND1)* was differed significantly ($p < 0.05$) between the three treatments (Figure 5e). The proportion of bacteria were as follows: *Subgroup 6* (13.58, 15.37, and 15.21%) > *MND1* (3.64, 3.04, and 3.84%) > *Nitrosomonadaceae* (3.39, 3.25, and 3.34%) for the NS, HS, and TS treatments, respectively. The relative abundance of soil bacteria at the species level were as follows: *Gemmatimonadetes bacterium* (0.80, 0.78, and 0.89%) > *Subgroup 6 Acidobacteria bacterium* (0.33, 0.40, and 0.40%) > *Lysobacter dokdonensis* (0.11, 0.12, and 0.10%) for NS, HS, and TS, respectively (Figure 5f).

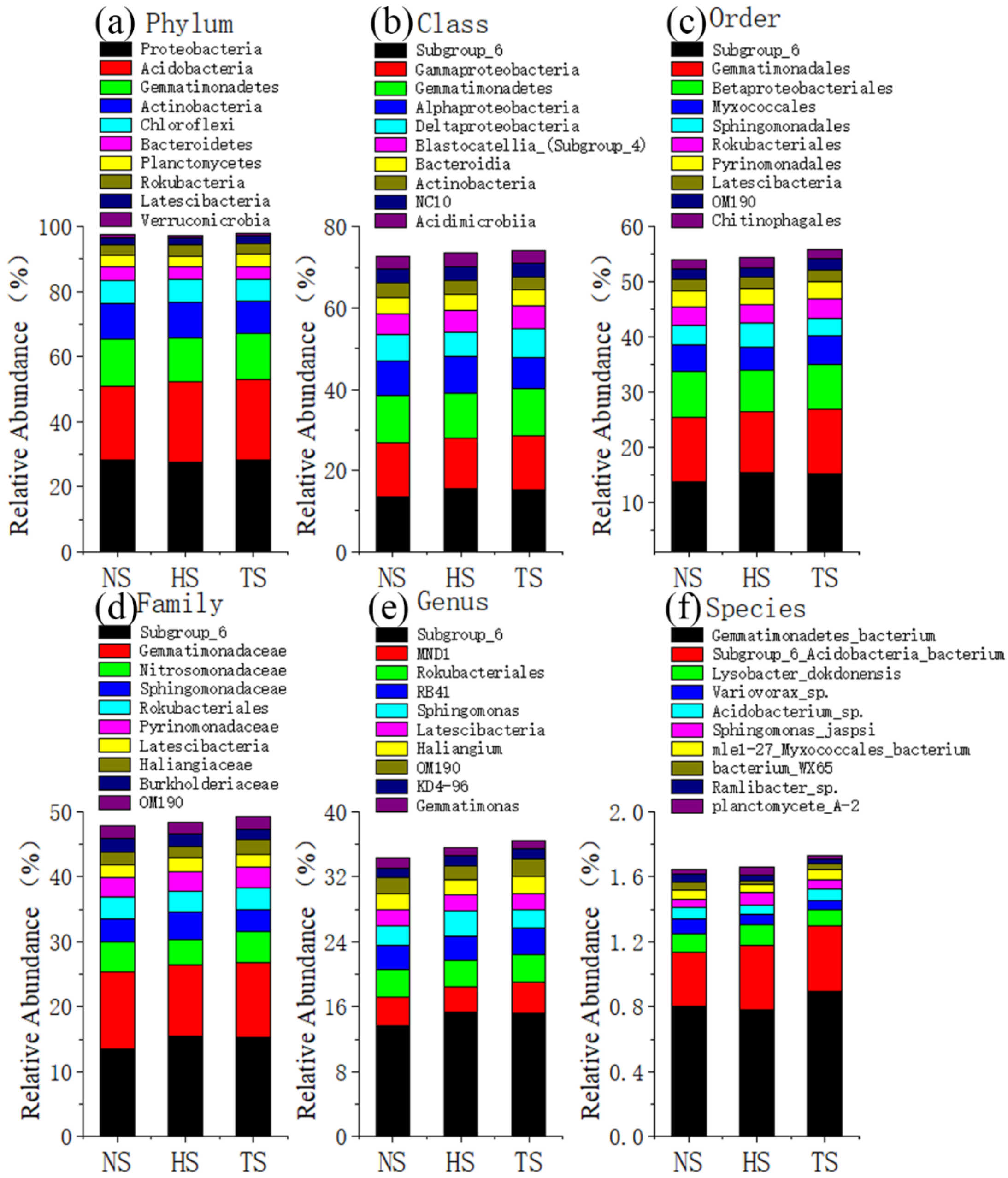

Figure 5. Effect of straw retention on the relative abundance of soil bacteria. (**a**), the relative abundance of soil bacteria at the Phylum level; (**b**), the relative abundance of soil bacteria at the Class level; (**c**), the relative abundance of soil bacteria at the Order level; (**d**), the relative abundance of soil bacteria at the Family level; (**e**), the relative abundance of soil bacteria at the Genus level; (**f**), the relative abundance of soil bacteria at the Species level.

3.5. Correlation between Bacterial Community Structure

The microbial community structure is associated with soil environmental factors, including MBN, SOC, MBC, DOC/SOC ratio, C/N ratio, MBC/MBN ratio, pH, and moisture (Figure 6). The OTU of bacteria had a significant positive correlation with MBC/MBN ratio, with a correlation coefficient of 0.326. The *Simpson* index for soil bacteria had a significant negative correlation with SOC content, and a significant positive correlation with the ratio of DOC to SOC, with correlation coefficients of −2.71 and 0.269, respectively. The *Chao1*, *ACE*, and *Shannon* indices were positively correlated with the ratio of MBC/MBN, with correlation coefficients of 0.272, 0.293, and 0.325, respectively.

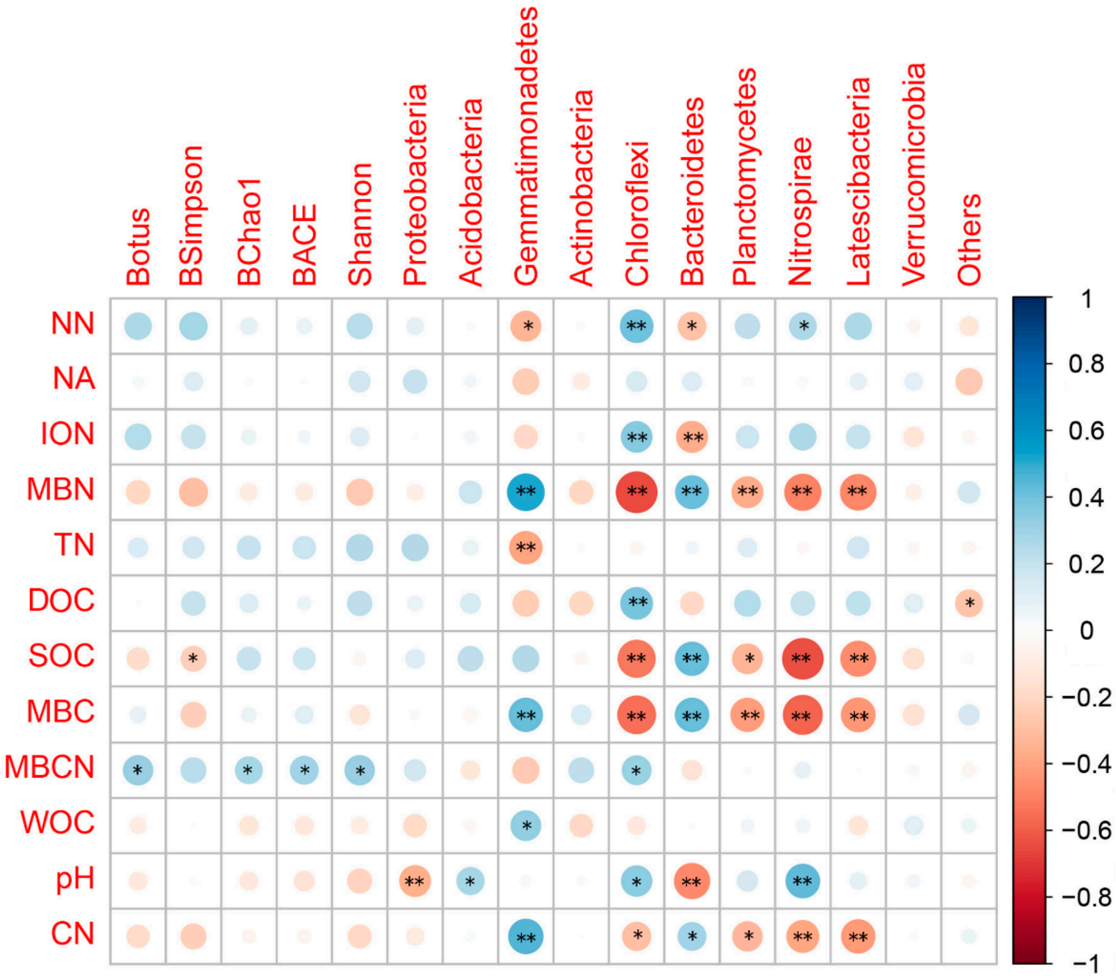

Figure 6. Correlation between soil nitrogen and carbon components and bacterial community structure. Capital letters on the ordinate represent soil nutrients. NN, nitrogen-N; NA, ammonium-N; AN, soil inorganic nitrate; ION, rate of nitrogen to ammonium; TN, total nitrogen; MBN, microbial biomass nitrogen; DOC, dissolved organic carbon; SOC, soil organic carbon; DSC, rate of DOC to SOC; IC, soil inorganic carbon; MBC, microbial biomass carbon; MBCN, rate of MBC to MBN; WOC, soil moisture; pH, soil pH; CN, Rate of SOC to TN. The abscissa letters indicate the *Alpha* diversity communities and relative abundance of soil bacteria at the phylum level. B OTU, the operational taxonomic unit of soil bacteria; B *Simpson*, *Simpson* index of soil bacteria; B *Chao1*, *Chao1* index of soil bacteria; B ACE, ACE estimator of soil bacteria; B *Shannon*, *Shannon* index of soil bacteria. And other was bacterial community structures at the phylum level. Blue indicate the positive effects; and red indicate the negative effects. "**", correlation is signification at the $p < 0.01$ level, "*" correlation is signification at the $p < 0.05$ level.

Soil pH is one of the critical factors affecting the composition of bacterial communities. In this experiment soil pH decreased as straw usage increased (Figure S3). *Proteobacteria* was the most abundant bacteria in the soil at the phylum level, and had a significant negative correlation with pH value, and a little correlation with NH_4^+-N and TN content in the soil. A significant positive correlation was found between the relative abundance of *Acidobacteria* and soil pH, with a correlation coefficient of 0.269. A significant positive correlation was found between the relative abundance of *Gemmatimonadetes* in soil bacteria and MBN, MBC, and the soil C/N ratio, with correlation coefficients of 0.516, 0.422, and 0.451, respectively. The relative abundance of *Chloroflexi* was positively correlated with the NH_4^+-N and DOC content, and rate of NO_3^--N to NH_4^+-N, and negatively correlated with soil MBN, MBC, and SOC. The influence of *Chloroflexi* was opposite to that of *Bacteroidetes*.

4. Discussion

4.1. Effect of Straw Retention on Soil Nitrogen

Soil nitrogen is the primary nutrient limiting plant growth in the northwest of China. The transformation of N_2 into other forms that can be assimilated by plants is mediated by biological nitrogen fixation in soybean [34]. Crops require soluble inorganic nitrogen (NO_3^--N and NH_4^+-N) for growth [35]. Previous studies have revealed that microbes may assimilated carbon from the straw and release straw-derived nitrogen via rapid mineralization and nitrification [36]. Straw retention has also been reported to significantly increase soil TN content [37]. In fact, soil available nitrogen was higher under straw retention than under residue removal [38]. This study revealed that straw retention has different effects on the content of soil nitrogen, with mean soil TN, NO_3^--N, and MBN contents 15.06, 21.10, and 38.23% higher in the straw return treatments than in the NS treatment at the 0–40 cm soil depth, respectively. The NH_4^+-N content was reduced by 3.68% owing to the straw return treatment compared with that of the NS treatment at a depth of 0–40 cm soil. This may be due to NH_4^+-N being the main nitrogen consumed by soil microorganisms; the number and activity of microorganisms increased after straw return, which led to competition between the microflora and plants. Liu [39] reported that the NH_4^+-N content was significantly higher in the HS returning treatment than in the TS return = treatment. Nelissen [40] also revealed that NH_4^+-N was consumed at a higher rate (333–508%) with biochar addition to cropland compared to that in the treatment with no biochar addition.

Soil nitrogen is crucial for plant growth; however, the level of inorganic nitrogen in soil is unknown. Without the addition of artificial fertilizer, plants can continuously produce nitrogen from the organic nitrogen of straw residue via mineralization processes [41]. Winter wheat grain yield increases as the soil NO_3^--N content increases [11]. In this experiment, the inorganic nitrogen content in the soil was low in spring, and increased in winter. The sharp decrease in the inorganic nitrogen content in spring might be due to absorption by wheat, leaching and losses via volatilization, higher temperature stimulating microbial activity, and transformation to organically bound nitrogen or immobilization by soil microbes, which generally enable the maintenance of low levels of inorganic nitrogen. Ball [42] reported that organic nitrogen becomes available for crop growth in spring as temperature increases. Furthermore, soil microbial production was stimulated and the concentration of MBN rapidly increases with the onset of the warmer weather in spring. The main reason for the high level of inorganic nitrogen in the 9–12 months might be the addition of fertilizer at winter wheat sowing in October, nitrogen fixation by nodule bacteria in soybean crops in autumn, mineralization of soybean straw, excretion by summer soybean living roots into the rhizosphere, and low nitrogen absorption by wheat at the seedling stage in winter. Collectively, these factors may have resulted in abnormally high concentrations of inorganic nitrogen in the soil for 9–12 months. The TN in the soil increased after long-term straw retention [43]. this indicates that the respective mean content of NO_3^--N was 9.30, 11.18, 11.54 mg/kg and that of NH_4^+-N was 1.74, 1.69, 1.77 mg/kg for NS, HS, and TS, respectively, at a soil depth of 0–20 cm. Further, the average ratios of NO_3^--N to NH_4^+-N were 5.23, 6.11 and 6.46 with NS, HS, and TS, respectively. This may be due to the ability of straw retention to effectively reduce soil bulk density, improve soil aeration, facilitate the nitrification process of nitrogen, and promote the conversion of NO_3^--N to NH_4^+-N, thereby decreasing NH_4^+-N content. The content of NO_3^--N was also reported to be higher than that of NH_4^+-N in croplands, whereas the opposite was found in forest soil [44]. Most previous studies indicate that inorganic nitrogen content is lower in cropland than in forest soil, while the NH_4^+-N content (29.84 mg/kg) is greater than the NO_3^--N content (12.67 mg/kg) in forest soil [45].

Mineralization or maintenance of soil nitrogen by soil microbes is crucial for the sustainability of available soil nitrogen [46]. Straw combined with the chemical fertilizer nitrogen, phosphorus, and potassium led to significantly higher MBN and MBN/TN at soil depths of 10–40 cm [43]. In this study, soil microbial biomass and activity increased as straw retention increased. The mean content of MBN was 24.34, 30.42, and 32.75 mg/kg; and the rate of MBN to TN was 2.76, 3.24, and 3.19% for NS, HS, and TS at a soil depth of 0–20 cm, respectively. However, the MBN concentration in farmland was lower than that in the nearby forest soil at depths of 0–20 cm [47].

4.2. Straw Retention Stimulates Soil Carbon and Stoichiometry

Soil organic carbon plays a central role and is a master indicator of soil function [48]. Straw retention plays an increasingly important role in improving soil quality, reducing agricultural inputs, and improving environmental sustainability [49]. The increase in crop yield after straw return is closely related to the improvement of soil organic carbon, soil structure, and nutrients [2]. DOC was identified as the most important influential factor, which explained the 42.2% variation in soil microbial community [50]. Soil MBC, DOC, and SOC increased by 7.63%, 4.34%, and 9.34%, respectively, with straw returning compared with non-returning treatment during long-term wheat-soybean rotation. However, a meta-analysis revealed that the increase in MBC was higher than that in SOC with straw application [51]. According to other researchers, straw return to the field significantly increases the SOC content by an average of 13.97% [3]. Similarly, a previous meta-analysis reported that crop straw retention significantly increased in SOC pool (12.3–36.8%) [52] and led to an SOC sequestration rate of 11.3% in China's croplands [53], a value higher than that obtained in this study. This difference may be due to the different durations of straw return and the SOC content in the soil. Wang [53] revealed a decreases in the SOC sequestration rate with increasing in experimental durations.

The availability and amount of carbon source application and nitrogen fertilizer may affect the SOC/TN ratio of the soil [43]. The SOC/TN ratios of straw input was 67.3 and 43.2 for winter wheat and summer soybean, respectively. The amounts of nitrogen input were 41.10 and 82.14 kg N·hm^{-2}, while those of carbon were 2282.5 and 4564.9 kg·C·hm^{-2} from straw retention in the HS and TS treatments each year (Table 1), respectively. The artificial fertilizer input for each plot was 135 kg·N·hm^{-2}. According to the calculation, the SOC/TN ratio of the input was 12.96, and 21.02 for HS and TS, respectively. A previous study revealed that approximately 11.5% of maize straw carbon is sequestered in the soil [37]. The average ratio of SOC to TN was 11.10, 12.25, and 13.02 for NS, HS, and TS at soil depths of 0–40 cm, respectively, and was lower than that in forest soil at high elevations in the Central Austrian Alps [54]. Zhang [55] reported that the mean soil C/N ratio was significantly higher with straw incorporation than that without. Straw retention might lead to a higher C/N ratio than that found in soil, and its application to fields can affect carbon and nitrogen cycling in the soil [56]. Straw retention increases not only carbon sources, but also soil microbial activity. However, mineralized nitrogen is reabsorbed by the microorganisms during growth. Soil surface CO_2 flux was higher with low instead of high N fertilization rates during wheat-soybean rotation [57]. Thus, the amount of fertilizer nitrogen needed to overcome nitrogen deficiency during straw application must be determined.

The mean content of MBC was 285.5, 315.3, and 320.7 mg/kg and the ratio of soil MBC to SOC was 2.72, 2.79, and 2.58% for NS, HS, and TS at soil depths of 0–20 cm, respectively. Furthermore, the MBC content was always aligned with the concentration of SOC. The content of MBC in the farmland soil at 0–20 cm was lower than that at the same depth in the forest soil [47]. This result may explain why straws with highly available carbon sources can provide energy for microorganisms and increase carbon and nitrogen immobilization [43]. The ratio of soil MBC to SOC was higher than that in upland soil reported by Wei [58], and a higher than that in forest soil of high elevation in the Central Austrian Alps [54]. The stoichiometry of the MBC/MBN ratios can affect soil carbon storage, and higher ratio of MBC to SOC may be detrimental to soil carbon sequestration [47], leading to high soil nitrogen bioavailability if the ratio of MBC/MBN is low [54]. The mean ratio of MBC to MBN were 13.49, 10.04, and 9.79 for NS, HS, and TS, respectively. Based on the observed decrease in the MBC/MBN ratio with the increase in straw return, the soil nutrient bioavailability was improved with increases in straw return. The highest ratio of MBC/MBN with NS reflects greater N losses through nitrate leaching and denitrification [58]. Soil nutrition vertical distribution and composition in the soil profile are known to be affected by straw retention and tillage systems [59]. The stratification ratio indicates the relationship between the nutrition content in the first soil layer and the nutrition content of deeper layers, and can be used as an indicator to evaluate soil quality [60]. Based on this experiment, the stratification ratios (0–20cm/20–40 cm) were as follows: MBN, 2.94, 2.27, and 2.68; MBC, 2.21, 2.07, and 1.94; TN, 1.60, 1.51, and 1.47; DOC, 1.02, 1.05, and 1.06; SOC, 1.48, 1.52, and 1.47 for NS, HS, and TS, respectively. Depending on the surface soil temperature and ventilation, the stratification ratios of MBN and MBC were higher than those of TN, SOC, and DOC. A previous study revealed that straw retention significantly increased the stratification ratio of SOC and TN [55,61], which differs from the results of this study. This discrepancy can be explained by the different levels of straw and artificial fertilizer application, duration of straw retention and wheat-soybean rotation. Furthermore, the abundance of nitrogen and SOC from surface soil leaching in soil at a deeper depth in farmland may also explain this difference.

4.3. Effect on Soil Bacterial Diversity and Structure

Soil microbiota diversity, which is essential for maintaining the health of terrestrial systems [62], contributes to plant growth and productivity and is involved in the improved uptake of nutrients, regulation of plant metabolism, and activation of plant responses to biotic and abiotic stresses [63]. Thus bacterial diversity is a key component of soil health [64]. Most previous studies have reported that straw retention has the most significant effect on soil microbial diversity by changing the SOC and TN contents [65,66]. Further research has revealed that soil water content, DOC, and NH_4^+-N are important factors in the determination of bacterial community diversity and composition [67]. However, inconsistent results have been observed in this study. Our finding also indicates that straw retention changed the soil nutrients and water availability, which were the main factors for decreased soil diversity. The bacterial diversity based on the *Chao1* (−1.67%), *Faith's PD* (−2.49%), *Good's coverage* (−0.01%), *Shannon* index (−0.82%), *Simpson* (−0.01%), *Pielou's evenness* (−0.50%), and *Observed species* (−2.30%) indices under straw treatments were compared to the NS treatment. A previous study revealed that straw addition decreased the *Shannon* (−11.83%) and *Chao1* (−11.57%) indices [68]. This may be due to the direct return of straw into the field without decomposition, which increases the porosity of the surface soil. The *Simpson* index for soil bacteria had a significant negative correlation with SOC content, and a significant positive correlation with the ratio of DOC to SOC, with correlation coefficients of −2.71, and 0.269, respectively. The *Chao1*, *ACE*, and *Shannon* indices were positively correlated with the ratio of MBC/MBN, with correlation coefficients of 0.272, 0.293, and 0.325, respectively.

Microorganisms are the main decomposers of straw in the soil and play an important role in the decomposition and transformation process of straw [69]. Soil microbial communities are fundamental for the maintenance of key soil processes associated with litter decomposition, nutrient cycling, and plant productivity [70]. The mean relative abundance of soil bacteria at the phylum level increased by 8.47, 4.33, and 1.43% (i.e., *Acidobacteria*, *Planctomycetes*, and *Latescibacteria*, *respectively*) with straw retention compared to that with NS. However, the levels of *Proteobacteria* (−1.28%), *Gemmatimonadetes* (−3.44%), *Actinobacteria* (−5.76%), *Chloroflexi* (−5.59%), *Bacteroidetes* (−1.11%), *Rokubacteria* (−2.63%), and *Verrucomicrobia* (−14.28%) reduced with straw retention compared to NS, respectively (Figure 6). Straw addition favors the growth of *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes*, and inhibits the growth of *Acidobacteria* and *Nitrospirae* [68].

Changes in vegetation composition, quantity, and quality of straw retention alter SOC, TN, and alkali nitrogen, and have a more substantial positive impact on soil bacterial communities [54,71]. A significant positive correlation was found between the relative abundance of *Gemmatimonadetes* in the soil and MBN, MBC, and soil C/N ratio, with correlation coefficients of 0.516, 0.422, and 0.451, respectively. The relative abundance of *Chloroflexi* was positively correlated with the NH_4^+-N content, rate of NO_3^--N to NH_4^+-N, and DOC, and negatively correlated with soil MBN, MBC and SOC. The influence of *Chloroflexi* was opposite to that of *Bacteroidetes*. Soil pH, organic carbon quality, and quantity are important factors that can influence the composition of soil bacterial communities [72]. Changes in the organic matter decomposition of crop straw retention might lead to a higher concentration of H^+, which decreases soil pH, leading to soil acidification [56,68]. Furthermore, in this study, soil pH at the 20–40 cm depth was found to decrease with increased straw usage (Figure S3), consistent with the results of previous research, but soil pH increased with HS at the 0–20 cm soil depth, compared to that with NS. *Proteobacteria* were the most abundant bacteria at the phylum level in the soil. The abundance of *Proteobacteria* had a significant negative correlation with the pH of the soil, and a little correlation with NH_4^+-N and TN form content in the soil. A significant positive correlation was found between the relative abundance of *Acidobacteria* and soil pH, with a correlation coefficient of 0.269.

5. Conclusions

Based on long-term wheat-soybean rotation and the straw retention cropping patterns, soybean has a remarkable capacity to fix N_2 from the atmosphere and transform ammonia to enable its utility by plants. The soil N used by wheat during its growth period can be replenished by the application of a small amount of fertilizer and N_2 fixation by soybean. Straw retention increased SOC, TN, MBC, and MBN contents, with significant differences found among different straw return strategies. The contents of TN (10.51 and 19.61%), NO_3^--N (15.71 and 26.50%), MBN (35.68 and 40.77%), SOC (4.10 and 14.58%), DOC (2.01 and 6.68%), MBC (6.71 and 7.67%) were found to increase with HS and TS compared to those with NS; however, the NH_4^+-N content was reduced by 3.68% with the straw return strategies compared to that with NS. Soil pH, and carbon and nitrogen contents were the dominant factors affecting the diversity and composition of bacteria. Soil bacterial *alpha* diversity reduced in response to the straw retention. Using the different straw retention strategies, *Proteobacteria*, *Acidobacteria*, and *Gemmatimonadetes* were identified as the dominant bacterial communities at the phylum level. Long-term straw retention with winter wheat-summer soybean rotation affects N and C cycling by changing microbial processes. These processes may reduce the amount of nitrogen fertilizer, and induce markedly higher nitrogen efficiency. Thus, when a large amount of straw is returned to the field, nitrogen fertilizer should be added to overcome the nitrogen depletion induced by straw decomposition and maintain nitrogen balance in the soil. These results could serve as a valuable reference for the national soybean development plan, the expansion of soybean cultivated area and production, and reduction in soybean import by China.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy12092126/s1, Figure S1. Effect on soil nitrogen fractions under different straw level application. (a), NO_3^--N content of the soil 0–20 cm; NO_3^--N, nitrate-N; (b), NO_3^--N content of the soil 20–40 cm; NO_3^--N, nitrate-N; (c), NH_4^+-N content of the soil 0–20cm, NH_4^+-N, ammonium-N; (d), NH_4^+-N content of the soil 20–40 cm, NH_4^+-N, ammonium-N; (e), MBN content of the soil 0–20cm, MBN, microbial biomass nitrogen; (f), MBN content of the soil 20–40 cm, MBN, microbial biomass nitrogen; (g), TN content of the soil 0–20 cm; TN, Soil total nitrogen; (h), TN content of the soil 20–40 cm; TN, Soil total nitrogen. Bars show means ± s. e. m. Different lowercase letters indicate significant difference amount of fertilization treatments under same time at $p < 0.05$ level, the same as below. Figure S2. Soil organic carbon fractions content affected by different straw level application. (a), MBC content of the soil 0–20 cm; MBC, microbial biomass carbon; (b), MBC content of the soil 20–40 cm; MBC, microbial biomass carbon; (c), DOC content of the soil 0–20 cm, DOC, dissolved organic carbon; (d), DOC content of the soil 20-40cm, DOC, dissolved organic carbon; (e), SOC content of the soil 0–20cm, SOC, soil organic carbon; (f), SOC content of the soil 20–40 cm, SOC, soil organic carbon. Figure S3. Effect on soil pH and soil water content under different straw application levels. (a), soil water content; (b) soil pH.

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