Straw return affects the soil carbon and nitrogen pools and microbial structural and functional diversity

Haoying Wang[1] and Ting-liang Li[1]

[1]Affiliation not available

July 16, 2024

Abstract

Straw return into the field reduces the reliance on chemical fertilisers, promoting sustainable straw utilisation, and mitigating soil nodulation associated with excessive chemical fertiliser application. In this study, we investigated the effects of straw return on soil carbon and nitrogen fractions and the structural and functional diversity of soil microbial communities. We set up four treatments: S0 (0% straw returned to the field), S1/2 (50% straw returned), S1 (100% straw returned to the field), and S2 (200% straw returned to the field). The results revealed that most carbon and nitrogen fractions increased with increased amounts of straw returned into the field (soil organic carbon and total nitrogen: 6.4–39.8 % and 2.9–15.1 %, dissolved organic carbon and nitrogen: 1.4–37.9 % and 20.2–33.1 %, microbial biomass carbon and nitrogen: 26.3–129.3 % and 4.7–62.6 %, and light organic carbon and nitrogen: 52.4–155.9 % and 72.6–166.7 %). High-throughput sequencing and Biolog-ECO revealed that straw return significantly altered the relative abundance of bacterial and fungal communities and enhanced soil carbon metabolism. Redundancy and correlation analyses and partial least squares path modelling revealed that Proteobacteria ($P = 0.002$), Acidobacteriota ($P = 0.004$), Mortierellomycota ($P < 0.05$), and amino acid carbon sources ($P < 0.01$) were correlated with the changes in soil carbon and nitrogen fractions. Returning straw to the field significantly boosted the soil carbon and nitrogen fractions and affected soil carbon pools, particularly the active ones, by markedly influencing soil bacterial activity and consequently altering the soil carbon pool content. Our results provide a scientific and theoretical basis for enhancing nutrient content in dryland wheat calcareous brown soil, establishing a healthy soil ecological environment, and improving quality and efficiency in wheat production.

Straw return affects the soil carbon and nitrogen pools and microbial structural and functional diversity

Abstract

Straw return into the field reduces the reliance on chemical fertilisers, promoting sustainable straw utilisation, and mitigating soil nodulation associated with excessive chemical fertiliser application. In this study, we investigated the effects of straw return on soil carbon and nitrogen fractions and the structural and functional diversity of soil microbial communities. We set up four treatments: S0 (0% straw returned to the field), S1/2 (50% straw returned), S1 (100% straw returned to the field), and S2 (200% straw returned to the field). The results revealed that most carbon and nitrogen fractions increased with increased amounts of straw returned into the field (soil organic carbon and total nitrogen: 6.4–39.8 % and 2.9–15.1 %, dissolved organic carbon and nitrogen: 1.4–37.9 % and 20.2–33.1 %, microbial biomass carbon and nitrogen: 26.3–129.3 % and 4.7–62.6 %, and light organic carbon and nitrogen: 52.4–155.9 % and 72.6–166.7 %). High-throughput sequencing and Biolog-ECO revealed that straw return significantly altered the relative abundance of bacterial and fungal communities and enhanced soil carbon metabolism. Redundancy and correlation analyses and partial least squares path modelling revealed that Proteobacteria ($P = 0.002$), Acidobacteriota ($P = 0.004$), Mortierellomycota ($P < 0.05$), and amino acid carbon sources ($P < 0.01$) were correlated with the changes in soil carbon and nitrogen fractions. Returning straw to the field significantly boosted the soil carbon and

nitrogen fractions and affected soil carbon pools, particularly the active ones, by markedly influencing soil bacterial activity and consequently altering the soil carbon pool content. Our results provide a scientific and theoretical basis for enhancing nutrient content in dryland wheat calcareous brown soil, establishing a healthy soil ecological environment, and improving quality and efficiency in wheat production.

Keywords: Straw return; Soil carbon and nitrogen; Structural diversity of soil microorganisms; Soil microbial carbon metabolism; Functional diversity of soil microorganisms

1. Introduction

Straw return to the field is a sustainable alternative to chemical fertilisers for soil enrichment and improving crop growth because of its renewability and carbon sequestration benefits (Li et al., 2022a). Excessive use of chemical fertilisers leads to nitrate leaching and residual nutrients in the soil (Patle et al., 2019). Additionally, straw burning generates substantial amounts of fine particulate matter, affecting human health and safety (Huang et al., 2021). Returning straw to the field instead of using partial fertilisers can reduce the cost of agricultural production and air pollution caused by straw burning, improving soil quality. Chen et al. (2022) reported that straw application enhances soil carbon, nitrogen, and phosphorus cycling, improving soil physicochemical and biological properties. Straw application significantly increases the levels of stable and active carbon and nitrogen pools in the soil, increasing wheat yield (Hu et al., 2021; Cui et al., 2022). Furthermore, straw improves the metabolic function and diversity of microbial communities (Liu et al., 2020; Jin et al., 2020). However, the effect of straw application on soil nutrients and microorganisms depend on the amount of straw incorporated. Using a meta-analysis, Wang et al. (2021a) found that soil organic carbon considerably increased with increases in the quantity of straw incorporated. Moreover, Zhao et al. (2016) reported that incorporating a low amount of straw had marginal effects on soil microorganisms; however, incorporating a high amount increased total phosphorus fatty acids, resulting in changes in the microbial community structure, suggesting that incorporating optimal amounts of straw in the field can increase soil nutrients and alter the soil microbial diversity.

Soil carbon and nitrogen influence terrestrial ecosystems and global biogeochemical cycles (Han et al., 2020; Lewis et al., 2021). Studies on soil carbon and nitrogen contents have received attention from researchers owing to the rapid shifts in global climate and ecology. The cycling and accumulation of soil carbon and nitrogen are crucial for assessing soil nutrient content (Xiao et al., 2023). Soil carbon and nitrogen fractions are classified into stable and active pools. Changes in these indicators reflect soil quality and health, particularly in the context of active carbon and nitrogen pools. Active carbon and nitrogen pools are formed owing to the accumulation of carbon and nitrogen in the soil because organic matter is involved in biological and chemical processes. Although they constitute a relatively small proportion of soil organic carbon and nitrogen, active carbon and nitrogen pools are key indicators of soil quality (Ren et al., 2023a; Wang et al., 2022a; Benbi et al., 2012). Specifically, soil active carbon is a marker of the soil carbon cycle, carbon sequestration potential, and soil response to climate change (Wu et al., 2020a; Benbi et al., 2012). Furthermore, soil active nitrogen content is used to determine nitrogen transformation, nitrification, reduction, and fixation processes in the soil (Koković et al., 2021). Soil carbon and nitrogen is derived primarily from underground root exudation and decomposition of aboveground plant residues (Zhu et al., 2021). Soil microorganisms play a crucial role in element cycling by decomposing plant residues through metabolic activities, converting plant organic matter into soil organic matter. Microorganisms increase oil-stable carbon and nitrogen pools by decomposing organic matter in the rhizosphere soil (Adamczyk et al., 2019). The abundance and activity of microbial species influence the changes in the carbon and nitrogen fractions, regulating the stability and efficacy of soil carbon and nitrogen cycles (Wang et al., 2023a; Coonan et al., 2020). Straw application alters the soil microbial community by providing cellulose and lignin (Yan et al., 2019). Guan et al. (2023) reported that straw application stimulated the growth of soil microorganisms, affecting the structure and function of the microbial community. Wu et al. (2020b) found that straw application combined with the application of inorganic fertilisers considerably altered soil bacterial communities, hypothesised to be correlated with soil carbon and nitrogen contents, suggesting that straw application stimulates soil microorganisms. However, whether the straw application changes and optimises the soil nutrient fraction is unclear.

Therefore, the present study carried out a 5-year straw application experiment to determine its effect on the contents of soil carbon and nitrogen pool components, soil bacterial and fungal community diversity, and the capacity of soil microorganisms to metabolise carbon. Furthermore, the study aimed to determine the effects of incorporating different amounts of straw on soil carbon and nitrogen fractions and microbial structure and function. The study analyses the effects of soil microorganisms on carbon and nitrogen fractions under straw application. The results of this study provide a theoretical basis for the improvement of soil nutrient content and the soil ecological environment in dryland wheat production areas.

2. Materials and methods

2.1. *Study area*

The experiment was conducted from 2018 to 2023 in Hongtong County, Linfen City, Shanxi Province, China (36°15'34" N, 111°40'31" E). The study site is in a typical Loess Plateau dryland farming area and has a temperate continental monsoon climate with an average annual temperature of 12.6 and mean annual precipitation of 500 mm concentrated from June–September. The study area has calcareous brown soils with a physical clayiness of approximately 40%. The results of the physicochemical properties of the soil at the study site are presented in Table S1.

2.2. *Experimental design and sample collection*

The field experiment was laid in a randomised complete block design with four treatments: S0 (0% straw returned to the field), S1/2 (50% straw returned to the field), S1 (100% straw returned to the field), and S2 (200% straw returned to the field). The experiment had four blocks. Each plot measured 126 m^2 (30 m x4.2 m [length x width]). The experiment was set up in 2018. Wheat was harvested, and straw was returned to the field in June each year for the 5-year study period. Wheat was sown in mid-October, and fertiliser was spread in late October each year for the 5-year study period. We crushed the wheat straw into 2 cm pieces and incorporated it into the field by tilling using a tiller at 0–20 cm soil layer depth. The amount of straw returned to the field was calculated considering the straw yield of the previous harvest; therefore, the amount of straw returned to the field differed in each treatment in each recurrent year for the 5-year study period. Wheat was sown in furrows on a ridge and film. After fertiliser application and land preparation, the ridge was covered with a film, and the seeds were sown on the side of the film in the furrow. The wheat variety used was "Jinmai 47", planted at 150 kg/hm^2sowing rate without irrigation during the experiment.

The fertiliser application rate for each treatment was determined before the sowing of winter wheat by assessing the nitrate nitrogen in the 0–2 m soil layer and the available phosphorous and potassium in the 0–40 cm soil layer. The calculated fertiliser application rate was adjusted by subtracting the amount of nutrients contributed by the straw application obtain the final fertiliser application rate. Briefly, the nutrient content of the straw was measured after harvesting the wheat in each season. The nutrient release rates for straw nitrogen, phosphorus, and potassium were 50 %, 65 %, and 90 %, respectively, and the amount of nutrients provided by straw application was determined. Soil samples were collected in June 2023 from the 0–20 cm soil layer. The amount of fertilisers applied in each year of the 5-year experimental period are presented in Table S2. The soil at the experimental site had sufficient potassium. Therefore, only nitrogen and phosphorus fertilisers were applied. Urea (containing 46 % N) was applied as the nitrogen source, and calcium superphosphate (containing 16 % P_2O_5) was applied as the phosphorus source. Same fertilisers were applied throughout the experimental period. After harvesting the winter wheat in June 2023, four soil samples were randomly collected from each plot from a depth of 0—20 cm. Furthermore, soil samples were collected for subsequent microbiological analyses as described above. Samples for DNA high-throughput sequencing and Biolog-ECO analysis were stored in a refrigerator at -80 , and the remaining samples were stored in a refrigerator at -4 .

2.3. *Sample determination and methods*

(1) Analysis of soil carbon and nitrogen indicators

Soil organic carbon content was determined using the potassium dichromate external heating method (Bao, 2000). Soil total nitrogen (TN) content was determined using the Kjeldahl method. Soil microbial biomass carbon (MBC) and nitrogen (MBN) contents were determined using the K_2SO_4-chloroform fumigation method (Wu et al., 2006) and a total organic carbon (TOC) metre (Thermo Fisher Scientific, Waltham, MA, USA). Soil dissolved organic carbon (DOC) and nitrogen (DON) were extracted using 1 mol/L KCl and filtered through a 0.45-μm membrane filter (Thermo Fisher Scientific). DOC and DON were determined using a TOC metre (Thermo Fisher Scientific). For the determination of soil light fraction organic carbon (LFOC), heavy fraction organic carbon (HFOC), light fraction organic nitrogen (LFON), and heavy fraction organic nitrogen (HFON), recombinant soil samples were obtained by shaking and centrifuging 1.8 g/cm^3 of zinc bromide solution with the soil samples in a centrifuge tube thrice, shaking and centrifuging the soil samples with 95% ethanol thrice to wash out the excess zinc bromide, and finally shaking and centrifuging the soil samples with distilled water twice to wash out the excess ethanol. Subsequently, HFOC, HFON, and LFOC and LFON were determined by the potassium dichromate heating, Kjeldahl, and the difference subtraction methods.

(2) DNA extraction and sequencing

DNA from the soil samples was extracted using the E.Z.N.A.® soil DNA kit (Omega Bio-Tek, Norcross, GA, USA). DNA concentration and purity were determined using a NanoDrop2000 spectrophotometer (Thermo Fisher Scientific). Polymerase chain reaction (PCR) of the V3–V4 variable region of the 16 S rRNA gene was performed using the primers 338F (5'-ACTCCTACGGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The V4 variable region of the 16S rRNA gene was amplified using PCR. The PCR products were recovered on a 2% agarose gel (Agarose. Konya, Turkey) and purified using a DNA Gel Recovery and Purification Kit (PCR Clean-Up Kit, Omega Bio-tek, Shengzhen, China). The recovered products were quantified using Qubit 4.0 (Thermo Fisher Scientific) at the Shanghai Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China.

(3) Determination of soil microbial metabolism indicators

Soil microbial carbon metabolism capacity was assessed using a Biolog-ECO microplate (Thermo Fisher Scientific). Briefly, 10.00 g of the soil sample was added into a conical flask containing 90 mL of 0.85% sodium chloride solution and shaken for 1 min followed by a 1-min ice bath, and the procedure was repeated thrice. Next, the mixture was allowed to settle, and a 1000-fold dilution of the soil suspension was prepared, and 150 μL of the soil suspension was extracted and inoculated onto the Biolog-ECO microplate (Thermo Fisher Scientific). Finally, the microplate was placed in an incubator for continuous cultivation at 30 for 144 h.

Optical density was measured at 0, 6, 12, 24, 48, 72, 96, 120, and 144 h at the wavelength of 595 nm using an ELX 808 microplate reader system (Thermo Fisher Scientific). Subsequently, **Average well color development (AWCD)** was calculated, and the Shannon (H), Simpson (D), and Pielou indices (E) were calculated to characterise the functional diversity of the soil microbial community using the following formulas:

$$\mathbf{A\Omega^{\sim}\Delta = \Sigma\ (C_i\text{-}R)\ /\ 31\ (1)}$$

where C_i represents the absorbance of each well and R is the absorbance of the control well;

$$H = \text{-}\ (P_i \times \ln P_i)\ \mathbf{(2)}$$

where P_i = (C_i-R) / (C_i-R), representing the ratio of the difference between the absorbance value of the ith hole and the absorbance value of the control hole and the sum of the differences in the absorbance values of all holes;

D = **1-** P_i^2 **(3)**

E = H / ln S **(4)**

where S is the number of carbon sources utilised ,

with (C_i-R) > **0.25 pores per pore.**

2.4. *Statistical analysis*

Data processing was performed using Microsoft Excel 2023 (Microsoft Corporation, Redmond, WA, USA), and different treatments were subjected to analysis of variance and Principal Component Analysis (PCA) using SPSS 27.0 (IBM, Armonk, NY, USA). Graphical representations, including bar charts, box plots, line graphs, and PCA plots, were generated using Origin 2022 (OriginLab Corporation, Northampton, MA, USA). Alpha diversity of the microbial communities was assessed based on the operational taxonomic unit levels by calculating the Abundance-based coverage estimator (ACE) and Shannon indices. Beta diversity of the microbial communities was evaluated using Principal Coordinates Analysis (PCoA) using the "vegan" package in R (RStudio, Boston, MA, USA). Bidirectional cluster heatmaps were created using the "Complex Heatmap" package in R (RStudio). Pearson correlation analysis and visualisation of correlations were performed using the "corrplot" package in R (RStudio). The "plspm" package in R (RStudio) was utilised to construct the Partial Least Squares Path Models (PLS-PM) to predict the pathways of explanatory variables. Redundancy analysis (RDA) was performed using Canoco version 5 (Canoco, Boston, MA, USA).

3. Results

3.1. *Soil carbon and nitrogen fractions*

Table 1 presents the effects of different straw return rates on soil carbon fractions. S2 increased SOC content ($P < 0.05$) by 39.8% and 31.4% compared with those of S0 and S1/2, respectively. Soil DOC content was significantly ($P < 0.05$) higher (25.3%–37.9%) in S2 than those in the other treatments. Soil MBC content increased by 26.3% (S1/2, $P < 0.05$), 16.7% (S1, $P < 0.05$), and 55.3% (S2, $P < 0.05$) with increased straw return amount. The soil MBC in S2 was 129.3% higher than that in S0. S2 significantly increased the LFOC content by 155.9% and 67.9% and significantly decreased the HFOC content by 26.7% and 24.1% compared with those of S0 and S1/2, respectively) ($P < 0.05$).

All soil nitrogen fractions increased with increased straw return (Table 2). S2 significantly increased the soil TN content by 15.1% ($P < 0.05$) compared with that of S0. The DON content in S1 and S2 was significantly higher than that in S0 by 29.3% and 33.1% higher, respectively ($P < 0.05$). The S2 treatment resulted in an average increase of 55.4% in the MBN content compared with those of the S0, S1, and S1/2 treatments. ($P < 0.05$). MBN sensitivity in S2 was considerably higher than that in the other treatments. LFON increased by 72.6%, 20.3%, and 28.5% in S2, S1/2, and S1, respectively ($P < 0.05$), compared with that in S0. The HFON content increased with increasing amount of straw returned, with an average increase of 3.7%, but no significant difference was observed between the treatments ($P < 0.05$).

3.2. *Alpha and beta diversity of soil microbial communities*

The richness and diversity of soil microbial communities were assessed using the ACE and Shannon indices of soil microbial alpha diversity, respectively (Fig. 1). The results revealed that increasing the amount of straw returned affects the alpha diversity of soil fungal microbial communities (Fig. 1A). PCoA revealed that different straw return rates affected the soil bacterial and fungal community composition. No significant differences in beta diversity were detected between bacterial ($p = 0.365$, R-squared = 0.2796) and fungal communities ($p = 0.063$, R-squared = 0.3296) (Fig. S1).

3.3. *Structural diversity of soil microbial communities*

Figure 2 reveals that 10 dominant bacterial phyla and genera were detected in the different treatments. Proteobacteria accounted for 21.6%–28.8% of the total sequences at the bacterial phylum level, with maximum relative abundance values. The Proteobacteria relative abundance was significantly increased by 12.6% ($P < 0.05$) in S2 compared with that in S0. Acidobacteriota relative abundance was significantly reduced by 40.3% ($P < 0.05$) in S2 compared with that in S0. Additionally, the Bacteroidota relative abundance significantly differed between S1/2 and S2, and increasing the amount of straw returned significantly increased Bacteroidota relative abundance by 27.0% ($P < 0.05$). No significant difference between the treatments was observed at the bacterial genus level ($P > 0.05$). (Fig. 2b).

At the fungal phylum level, S2 significantly increased the Mortierellomycota relative abundance by 54.8% and 56.5% ($P < 0.05$), compared with those of S0 and S1/2, respectively (Fig. 2c). Additionally, the relative abundance of unclassified_k__Fungi differed significantly between S1/2 and S1 ($P < 0.05$). Ascomycota, Basidiomycota, Mortierellomycota, and unclassified_k__Fungi were highly enriched in all treatments, accounting for 98.1% to 99.2% of the total fungal relative abundance. S2 significantly reduced the relative abundance of*Chaetomium* by 48.9% ($P < 0.05$) compared with that of S1/2 (Fig. 2d). Furthermore, compared with those of S0 and S1/2, S1 and S2 significantly increased the relative abundance of*unclassified_o_Polyporales* ($P < 0.05$).

3.4. Functional diversity of soil microorganisms

Changes in soil microbial activity and functional metabolic indices under different straw return amounts after 192 h of incubation are presented in Fig. S2. The AWCD values of all treatments increased gradually with the extension of incubation time in the following sequence: S2 > S1 > S1/2 > S0, indicating that soil microbial metabolic activity increased with an increased amount of straw returned. The AWCD of each treatment increased rapidly at 24–72 h and stabilised after 120 h of incubation. Concurrently, the AWCD in S2 was significantly increased by 94.6% and 70.8% ($P < 0.05$) compared with those in S0 and S1/2 (Fig. S3a). The Shannon and Simpson indices demonstrated a progressive increase with increasing levels of straw returned, and significant differences were noted among the treatments ($P < 0.05$). No significant difference ($P > 0.05$) in the Pielou index was observed among the treatments (Fig. S3d). Principal component and 2-way cluster heatmap analyses were performed based on the AWCD values of 31 carbon sources at 120 h (Figs 3 and S4). Both analyses revealed similar carbon source utilisation patterns across the treatments, with S1/2 distinguishing between S0 and S1 and S2 distinguishing between all treatments. Based on the PCA, the correlation strength between the three principal components and each type of carbon source was further analysed (Table S3). The carbon sources with an absolute loading value >0.6 were the main carbon sources for soil microbial metabolism. The results revealed that the types of carbon sources contributing to the heterogeneity of soil microbial carbon metabolism were primarily carbohydrates, amino acids, and carboxylic acids, consistent with the cluster heatmap results.

3.5. Correlation between soil carbon and nitrogen fractions and soil microorganisms

Straw application affected the soil carbon and nitrogen fractions and soil microorganisms. It indirectly affected the soil carbon and reservoirs by affecting soil microorganisms. Bacteria and fungi with high relative abundances at the phylum and genus levels were selected for RDA (Fig. 4) and Pearson correlation heatmap analyses (Fig. 5), respectively. The correlation between the two was explained based on these analyses. The RDA results revealed that among the soil bacteria, Proteobacteria played a major role ($P = 0.002$) in explaining the changes in soil carbon fractions, whereas Acidobacteriota was the main factor ($P = 0.004$) causing changes in the soil nitrogen fractions. Among the soil fungi, Mortierellomycota explained 30.1 % and 29.3 % ($P < 0.05$) of the changes in soil carbon and nitrogen fractions, respectively. Compared to other carbon sources, carbon sources of amino acids had the highest explanatory power for the changes in soil carbon and nitrogen fractions (54.3 % and 48.5 %, respectively, $P < 0.01$).

Results of the correlation heatmap analysis were consistent with those of the RDA (Fig. 5). A significant ($P < 0.05$) or highly significant ($P < 0.001$) positive correlation was observed between Proteobacteria and

the soil carbon and nitrogen fractions and a significant ($P < 0.05$) or highly significant ($P < 0.001$) negative correlation was observed between Acidobacteriota and the soil carbon and nitrogen fractions. There was a strong correlation between the two bacteria and the DOC, MBC, and LFON variables ($P < 0.001$). Significant positive ($P < 0.05$) and negative ($P < 0.05$) correlations in the soil fungi and soil carbon and nitrogen fractions were observed between Mortierellomycota and Ascomycota, respectively, with a significant positive correlation ($P < 0.01$) between Mortierellomycota and DON. Carbon sources with strong correlations with the soil carbon and nitrogen pools were concentrated in amino acids, carbohydrates, carboxylic acids, and polymers (particularly amino acids). For example, amino acid carbon sources such as glycyl-L-glutamic acid and L-asparagine exhibited significant ($P < 0.05$ and $P < 0.01$) or highly significant positive correlations ($P < 0.001$) with DOC, MBC, and LFON. The PLS-PM results revealed that straw return was the main factor affecting the soil carbon and nitrogen fractions (Fig. 6). Moreover, straw return significantly ($P < 0.05$) affected the soil carbon fraction by influencing soil bacterial diversity.

4 Discussion

4.1. *Effects of straw return on soil carbon and nitrogen fractions*

Straw return increases soil carbon and nitrogen pools and improves the soil environment (Yu et al., 2020; Ren et al., 2023b). In the present study, straw return to the field increased the stable carbon and nitrogen pools (SOC, TN, and HFON) and active carbon and nitrogen pools (DOC, MBC, DON, MBN, LFOC, and LFON) in the 0–20 cm soil layer (Tables 1 and 2). Compared with those in the control, SOC and TN increased by 6.4%–39.8% and 2.9%–15.1 %, respectively, with increases in the amount of straw returned to the field. This was because straw applied to the soil as exogenous organic matter increases the soil SOC and TN storage; thus, crop residues are retained in the soil as derived carbon and nitrogen pools (Zhao et al., 2019), consistent with the findings by Dai et al. (2021).

The increase in soil active carbon and nitrogen pools as a result of straw return is a consequence of the straw degradation process releasing large amounts of nutrients and soluble substances and being microbially mediated and stimulating microbial growth (Zhang et al., 2020). Zhao et al. (2020a) demonstrated that straw is more readily available to microorganisms for utilisation and decomposition than biochar, increasing the unstable stable soil organic carbon pools (DOC, MBC, and labile carbon). Straw application induces the microbial turnover of dead material in the soil, increasing DON and MBN levels (Liu et al., 2021; Hassan, 2013), which is consistent with the results obtained in the present study.

Light (LFOC and LFON) and heavy (HFOC and HFON) fraction organic carbons and nitrogen are determined considering the carbon and nitrogen fractions after physical density fractionation, which have different compositions and turnover mechanisms (Crow et al., 2007). The results of the present study revealed that the LFOC content increased with an increase in the amount of straw returned, whereas the HFOC content decreased, which is inconsistent with the results of Hao (2023). This may be because the light fraction is mainly derived from undecomposed plant residues, and straw inputs increase the LFOC and LFON contents along with the increase in crop residues in the soil (Wang et al., 2021b; Yan et al., 2020). Although soil HFOC, as a stable carbon pool component, is usually adsorbed to various soil compounds or minerals, straw inputs stimulated HFOC mineralisation; however, its mineralisation rate was much lower than its formation rate; thus, HFOC decreased with increases in the amount of straw returned in the present study (Chen et al., 2019). Hu et al. (2018) revealed that the carbon dynamics of difficult-to-degrade organic carbon are correlated with the nature of organic matter. Organic matter with a higher content of lignin and polyphenols is more stable in carbon turnover. HFOC and HFON contents are correlated with the carbon and nitrogen turnover processes and they exhibited different variations depending on the microbial load, soil depth, and climatic conditions, which might explain why soil-heavy carbon and nitrogen exhibited different responses to straw return in the present study (Li et al., 2022b).

4.2. Effects of straw return on microorganism diversity

Straw application alters the biotic and abiotic environmental conditions of the soil and affects its local

ecology, which in turn affects the structural diversity of soil bacterial and fungal communities (Philippot et al., 2023). In the present study, soil fungal populations were significantly affected by straw return, as revealed by the results of the ACE and Shannon indices, whereas the alpha diversity of soil bacterial communities did not respond significantly to different straw return rates. Bacteria usually have shorter life cycles than those of fungi and decompose unstable nutrient pools, leading to the dominance of bacteria in the early stage of straw decomposition, whereas fungi have more stable population dynamics with straw return in the later stages of decomposition; thus, the alpha diversity of fungi was more considerably affected by straw return than that of bacteria (Yang et al., 2019; Wang et al., 2021c).

The returned straw is a substrate for microbial growth and improves environmental conditions, improving microbial activity (Yang et al., 2019; Shan et al., 2021). In the present study, the bacteria that were most responsive to the straw return were Proteobacteria, Acidobacteriota, and Bacteroidota (Fig. 2a, b). Kim et al. (2021) exhibited that the Proteobacteria and Acidobacteriota levels in the soil were correlated with soil pH, terminal electron acceptors, and toxic metals, with Proteobacteria enriched at high soil organic matter content and Acidobacteriota having contrasting results. The increase in straw return quantities provided organic material and improved the environmental conditions of the soil. Consequently, both factors exhibited significant differences across different straw return rates in the present study. Bacteroidota are carbohydrate degraders in the soil, breaking down complex compounds and playing a role in the breakdown of hemicellulose in the later stages of straw decomposition. The increase in straw polymers, along with the increase in microbial accessibility, increased Bacteroidota abundance in the late stage of straw decomposition (Huang et al., 2023). Fungal communities responded differently to low (S1/2) and high (S1 and S2) amounts of straw return. Ascomycota, Basidiomycota, and Mortierellomycota were predominant in the fungal community at the phylum level, and Mortierellomycota and *Chaetomium* exhibited significant variations between the treatments (Figs 2c and d). However, the relative abundance of each of these fungal groups varied in S1/2, inconsistent with the results in the other treatments. Ascomycota are saprophytic fungi that decompose plant and animal debris and organic matter. *Chaetomium* have an inhibitory effect on phytopathogenic fungi, and Mortierellomycota, which are saprophytic fungi, enhance phosphorus and iron in the soil. These fungi are eutrophic fungi (Abuduaini et al., 2021; Ozimek et al., 2020). Contrastingly, Basidiomycota, are important for lignin and substrate decomposition, are ectomycorrhizal fungi that consume more resources than they produce, and are oligotrophic (Li et al., 2021). In the present study, the Basidiomycota relative abundance increased (decrease) in S0–S1/2 and decreased (increase) in S1–S2, suggesting that low and high straw inputs may have different stimulatory effects on fungal communities. The high abundance of oligotrophic bacteria in the soil at the early stage of plant residue decomposition and that of eutrophic bacteria at the later stage of decomposition, coupled with the longer life cycle of fungi than that of bacteria, may have increased oligotrophic fungi with increasing amounts of straw returned to the field (Wang et al., 2022b), consistent with findings from this study. Additionally, the input of organic materials changes the structure of soil microorganisms and activates many soil-borne microorganisms, thereby increasing microbial diversity (Semenov et al., 2021). Microorganisms with high relative abundances did not show significant differences, probably due to their differences in periods of action in the decomposition process and competitiveness in utilising straw polymers (Huang et al., 2023).

Our results showed that straw application enhanced the ability of microorganisms to utilise carbon sources (AWCD) and enhanced their functional diversity (Shannon, Simpson, and Pielou indices) (Figs S2 and S3). This was because plant residues and root exudates under straw return provide substrates for microbial carbon metabolism, affecting the habitat of microorganisms and enhancing the carbon metabolism process (Zhang et al., 2022; Zhu et al., 2022). As a lignocellulosic biosaccharification feedstock, straw is composed of cellulose (28–45%), hemicellulose (12–32%), and lignin (5–24%) (Srivastava et al., 2023). Wheat straw has many carbohydrates and is rich in protein, minerals, silica, and ash (Tufail et al., 2021). Straw application increases the amounts of carbohydrates, amino acids, and carboxylic acids in the soil, considering composition and products of decomposition; thus, enhancing the biomass attachment sites for microorganisms on these substances. Therefore, the observed differences in microbial carbon metabolism between the different amounts of straw returned to the soil were correlated with increased carbohydrates, amino acids, and car-

boxylic acid. The results of PCA and cluster analyses, indicating the utilisation of carbon sources in the different treatments, exhibited that S1/2 was distinguished from S0 and S1, whereas S2 was distinguished from the rest of the treatments (Figs 3 and S4). This may be because of the low homogeneity of the microbial community in S1/2 and the difference in the types of microorganisms that decompose straw between the low and high amounts of straw returned to the field (Zhang et al., 2017). Compared to bacterial communities, fungal communities are more efficient at decomposing cellulose, hemicellulose, and lignin (Zhang et al., 2017). The fungal community analyses indicate that S1/2 is distinct from S0, S1, and S2, suggesting a correlation between the soil fungal community structure and the capacity for carbon metabolism.

4.3. Soil microbes influence changes in soil carbon and nitrogen fractions

Soil microorganisms alter the soil carbon and nitrogen stocks through decomposition and formation of soil organic matter (Fan et al., 2021). The results of the present study exhibited that straw return to the field significantly affected the soil carbon pool by affecting the abundance of bacteria such as Proteobacteria, Acidobacteriota, and Bacteroidota and consequently affecting the soil carbon pool content (Fig. 6). Wu et al. (2021) reported that straw application significantly increased the soil unstable carbon and nitrogen fractions and that bacterial communities responded differently to carbon and nitrogen mineralisation, with *Brevundidanas* (a member of the subzonal family of *Proteobacteria*) stimulating carbon mineralisation, suggesting that changes in the abundance of Proteobacteria affected the soil active carbon pool content, consistent with our results that revealed that Proteobacteria was strongly correlated with soil active carbon pools (DOC and MBC) (Fig. 5). Acidic bacteria are some of the most important microbial populations that dominate during soil denitrification, and they decrease soil NO and N_2O, thereby affecting soil N fractions (Pessi et al., 2022).Wang et al. (2023b) suggested that elevated levels of soil nitrogen supply increase the relative abundance of Proteobacteria and Bacteroidota, which are the key bacteria influencing soil nitrogen cycling and carbon effectiveness. In the present study, we observed positive correlations (to varying degrees) between the Proteobacteria and Bacteroidota and the soil carbon and nitrogen fractions. Mortierellomycota exhibited a high degree of interpretability considering changes in soil carbon and nitrogen pool content (Fig. 4). Mortierellomycota are mostly present in the soil as living organic material and are efficient in decomposing carbon sources such as chitin polymers and cellulose (Wolińska et al., 2022; Ozimek et al., 2020), indicating their importance in the soil nutrient pools. Zhao et al. (2020b) reported that compared to fungi, bacteria play a more important role in carbon and nitrogen cycling, where genes involved in carbon degradation are from bacteria in eutrophic phyla. In the present study, as oligotrophic fungi grew, bacteria had a stronger effect on the carbon pool in the PLS-PM results compared with that of fungi ($P < 0.05$) (Fig. 6); however, a greater functional potential may occur at a later stage of decomposition because of the strong degradation of fungi and their longer growth cycle. Additionally, the effects of microorganisms differ under different environmental conditions because of the differences in the mechanisms of carbon and nitrogen cycles. Fuchslueger et al. (2019) revealed that the microorganisms involved in the carbon cycle are more sensitive to temperature changes. Contrastingly, those involved in the nitrogen cycle are more affected by moisture. Microorganisms involved in the carbon and nitrogen cycles are affected by the fungal: bacteria ratio, soil environment and properties, and other factors (Ma et al., 2020).

In the present study, among the different types of carbon sources utilised by soil microorganisms, amino acid-based carbon sources explained the most variation in soil carbon and nitrogen fractions (Fig. 4). Amino acids circulate very rapidly in the soil cycle, and the strong dynamics of carbon and nitrogen in the soil are correlated with the high turnover rate of amino acids, which can be rapidly mineralised and assimilated by microorganisms compared with other types of substances; thus, amino acids are important nutrient drivers in the soil and had a strong correlation with the soil active carbon and nitrogen pools in our study (Jones et al., 2009) (Fig. 5). Ning et al. (2022) found that optimal supplementation of amino acid-based carbon sources favoured straw decomposition in the soil. For example, glycyl-L-glutamic acid played an important role in straw decomposition, especially in the pre-decomposition stage, which could provide favourable environmental conditions for soil microbial communities to proliferate and increase their populations and balance the soil carbon and nitrogen ratio. L-asparagine facilitates nutrient uptake by microorganisms (Han et al., 2022), and it is effective in the late stage of straw decomposition. Therefore,

we can speculate that these amino acid carbon sources can increase the active carbon and nitrogen pools of the soil by promoting nutrient uptake during different periods of straw decomposition. In the present study, we observed different degrees of significant positive correlations between these carbon sources and active carbon and nitrogen fractions (Fig. 5). The above analyses revealed that straw return significantly increased the soil carbon and nitrogen pools and stimulated the effect and driving mechanism of soil microorganisms. Therefore, the influence of straw input on soil nutrients should not be ignored.

5. Conclusion

The results of the 5-year continuous straw return experiment revealed that the straw return significantly increased the content of soil carbon and nitrogen pools, particularly active carbon pools, by enhancing the structural diversity of bacterial communities. Straw return also enhanced the ability of microorganisms to utilise carbohydrates, amino acids, and carboxylic acid carbon sources, with amino acid carbon sources more influential in explaining the changes in soil carbon and nitrogen pool contents. Additionally, we observed differences in the fungal community structure and microbial metabolic activity between low (S1/2) and high (S1, S2) amounts of straw returned to the field, indicating that the application of an optimal amount of straw to the field can regulate the soil microbiological environment, affecting the microbial diversity and consequently the content of soil carbon and nitrogen pools, improving the soil quality through biological regulation. Our study provides a theoretical basis for the sustainable development of high-efficiency and high-quality wheat production in drylands.

Acknowledgements:

This work was supported by the National Key Research and Development Program of China (2021YFD1900700) and the National Natural Science Foundation of China (42377356).

References

Abuduaini, X., Nuer, H., Zhao, Q.Q., et al., 2021. Effects of Bacillus subtilis J-15 secondary metabolites against Verticillium dahliae on diversity of soil fungi. Microbiol 48, 1997-2007(in Chinese).

Adamczyk, B., Sietiö, O.M., Straková, P., et al., 2019. Plant roots increase both decomposition and stable organic matter formation in boreal forest soil. Nature Communications 10(1), 3982.

Bao, Shidan., 2013. Soil Agricultural Chemistry Analysis. (Third Version) Beijing: China Agriculture Press (in Chinese).

Benbi, D.K., Brar, K., Toor, A.S., et al., 2012. Soil carbon pools under poplar-based agroforestry, rice-wheat, and maize-wheat cropping systems in semi-arid India. Nutrient Cycling in Agroecosystems 92, 107-118.

Chen, L., Sun, S., Yao, B., et al., 2022. Effects of straw return and straw biochar on soil properties and crop growth: A review. Frontiers in Plant Science 13, 986763.

Chen, X., Wu, J., Opoku-Kwanowaa, Y., 2019. Effects of organic wastes on soil organic carbon and surface charge properties in primary saline-alkali soil. Sustainability 11(24), 7088.

Coonan, E.C., Kirkby, C.A., Kirkegaard, J.A., et al., 2020. Microorganisms and nutrient stoichiometry as mediators of soil organic matter dynamics. Nutrient Cycling in Agroecosystems 117(3), 273-298.

Crow, S.E., Swanston, C.W., Lajtha, K., et al., 2007. Density fractionation of forest soils: methodological questions and interpretation of incubation results and turnover time in an ecosystem context. Biogeochemistry 85, 69-90.

Cui, H., Luo, Y., Chen, J., et al., 2022. Straw return strategies to improve soil properties and crop productivity in a winter wheat-summer maize cropping system. European Journal of Agronomy 133, 126436.

Dai, W., Wang, J., Fang, K., et al., 2021. Wheat straw incorporation affecting soil carbon and nitrogen fractions in Chinese paddy soil. Agriculture 11(8), 803.

Fan, X., Gao, D., Zhao, C., et al., 2021. Improved model simulation of soil carbon cycling by representing the microbially derived organic carbon pool. The ISME Journal 15(8), 2248-2263.

Fuchslueger, L., Wild, B., Mooshammer, M., et al., 2019. Microbial carbon and nitrogen cycling responses to drought and temperature in differently managed mountain grasslands. Soil Biology and Biochemistry 135, 144-153.

Gu, X., Fang, X., Xiang, W., et al., 2019. Vegetation restoration stimulates soil carbon sequestration and stabilization in a subtropical area of southern China. Catena 181, 104098.

Guan, Y., Wu, M., Che, S., et al., 2023. Effects of Continuous Straw Returning on Soil Functional Microorganisms and Microbial Communities. Journal of Microbiology 61(1), 49-62.

Han, G., Tang, Y., Liu, M., et al., 2020. Carbon-nitrogen isotope coupling of soil organic matter in a karst region under land use change, Southwest China. Agriculture, Ecosystems & Environment 301, 107027.

Han, M., Wang, S., Wu, L., et al., 2022. Effects of Exogenous L-Asparagine on Poplar Biomass Partitioning and Root Morphology. International Journal of Molecular Sciences 23(21), 13126.

Hao, X., Han, X., Wang, C., et al., 2023. Temporal dynamics of density separated soil organic carbon pools as revealed by $\delta^{13}C$ changes under 17 years of straw return. Agriculture, Ecosystems & Environment 356, 108656.

Hassan W., 2013. C and N mineralization and dissolved organic matter potentials of two contrasting plant residues: effects of residue type, moisture, and temperature, Acta Agriculturae Scandinavica, Section B. Soil & Plant Science 63:7, 642-652.

Hu, J., Wu, J., Qu, X., 2018. Decomposition characteristics of organic materials and their effects on labile and recalcitrant organic carbon fractions in a semi-arid soil under plastic mulch and drip irrigation. Journal of Arid Land 10, 115-128.

Hu, Y., Sun, B., Wu, S., et al., 2021. Soil carbon and nitrogen of wheat–maize rotation system under continuous straw and plastic mulch. Nutrient Cycling in Agroecosystems 119, 181-193.

Huang, J., Gao, K., Yang, L., et al., 2023. Successional action of Bacteroidota and Firmicutes in decomposing straw polymers in a paddy soil. Environmental Microbiome 18(1), 1-12.

Huang, L., Zhu, Y., Wang, Q., et al., 2021. Assessment of the effects of straw burning bans in China: Emissions, air quality, and health impacts. Science of The Total Environment 789, 147935.

Jin, S., Jin, W., Dong, C., et al., 2020. Effects of rice straw and rice straw ash on rice growth and α-diversity of bacterial community in rare-earth mining soils. Scientific Reports 10(1), 10331.

Jones, D.L., Kielland, K., Sinclair, F.L., et al., 2009. Soil organic nitrogen mineralization across a global latitudinal gradient. Global Biogeochemical Cycles, 23(1).

Kim, H.S., Lee, S.H., Jo, H.Y., et al., 2021. Diversity and composition of soil Acidobacteria and Proteobacteria communities as a bacterial indicator of past land-use change from forest to farmland. Science of the Total Environment 797, 148944.

Koković, N., Saljnikov, E., Eulenstein, F., et al., 2021. Changes in soil labile organic matter as affected by 50 years of fertilization with increasing amounts of nitrogen. Agronomy 11(10), 2026.

Lewis, D.B., Jimenez, K.L., Abd-Elrahman, A., et al., 2021. Carbon and nitrogen pools and mobile fractions in surface soils across a mangrove saltmarsh ecotone. Science of the Total Environment 798, 149328.

Li, H., Yang, S., Semenov, M.V., et al., 2021. Temperature sensitivity of SOM decomposition is linked with a K-selected microbial community. Global Change Biology 27(12), 2763-2779.

Li, S., Hu, M., Shi, J., et al., 2022a. Improving long-term crop productivity and soil quality through integrated straw-return and tillage strategies. Agronomy Journal 114(2), 1500-1511.

Li, X., Yang, T., Hicks, L.C., et al., 2022b. Latitudinal patterns of light and heavy organic matter fractions in arid and semi-arid soils. Catena 215, 106293.

Liu, C., Li, L., Xie, J., et al., 2020. Soil bacterial diversity and potential functions are regulated by long-term conservation tillage and straw mulching. Microorganisms 8(6), 836.

Liu, X., Dong, W., Jia, S., et al., 2021. Transformations of N derived from straw under long-term conventional and no-tillage soils: A 15N labelling study. Science of The Total Environment 786, 147428.

Ma, Q., Wen, Y., Wang, D., et al., 2020. Farmyard manure applications stimulate soil carbon and nitrogen cycling by boosting microbial biomass rather than changing its community composition. Soil Biology and Biochemistry 144, 107760.

Ning, Y., Wang, X., Yang, Y., et al., 2022. Studying the Effect of Straw Returning on the Interspecific Symbiosis of Soil Microbes Based on Carbon Source Utilization. Agriculture 12(7), 1053.

Ozimek, E., Hanaka, A., 2020. Mortierella species as the plant growth-promoting fungi present in the agricultural soils. Agriculture 11(1), 7.

Patle, P.N., Kadu, P.R., Gabhane, A.R., et al., 2019. Consequences provoked due to excess application of agrochemical on soil health deterioration–A review for Sustainable Agriculture. Journal of Pharmacognosy and Phytochemistry 8(2S), 63-66.

Pessi, I.S., Viitamäki, S., Virkkala, A.M., et al., 2022. In-depth characterization of denitrifier communities across different soil ecosystems in the tundra. Environmental microbiome 17(1), 30.

Philippot, L., Chenu, C., Kappler, A., et al., 2023. The interplay between microbial communities and soil properties. Nature Reviews Microbiology, 1-14.

Ren, G., Zhang, X., Xin, X., et al., 2023b. Soil organic carbon and nitrogen fractions as affected by straw and nitrogen management on the North China Plain. Agriculture, Ecosystems & Environment 342, 108248.

Ren, T., Fan, P., Zuo, W., et al., 2023a. Biochar-based fertilizer under drip irrigation: More conducive to improving soil carbon pool and promoting nitrogen utilization. Ecological Indicators 154, 110583.

Semenov, M.V., Krasnov, G.S., Semenov, V.M., et al., 2021. Does fresh farmyard manure introduce surviving microbes into soil or activate soil-borne microbiota? Journal of Environmental Management 294, 113018.

Shan, A., Pan, J., Kang, K.J., et al., 2021. Effects of straw return with N fertilizer reduction on crop yield, plant diseases and pests and potential heavy metal risk in a Chinese rice paddy: A field study of 2 consecutive wheat-rice cycles Environmental Pollution 288, 117741.

Sokol, N.W., Slessarev, E., Marschmann, G.L., et al., 2022. Life and death in the soil microbiome: how ecological processes influence biogeochemistry. Nature Reviews Microbiology 20(7), 415-430.

Srivastava, M., Bansal, S.L., Khan, M.S., et al., 2023. Rice Straw Waste-Based Biogas Production via Microbial Digestion: A Review. Molecular Biotechnology, 1-13.

Tufail, T., Saeed, F., Afzaal, M., et al., 2021. Wheat straw: A natural remedy against different maladies. Food Science & Nutrition 9(4), 2335-2344.

Wang, A., Zhang, Y., Wang, G., et al., 2023a. Soil physicochemical properties and microorganisms jointly regulate the variations of soil carbon and nitrogen cycles along vegetation restoration on the Loess Plateau, China. Plant and Soil 1-24.

Wang, E., Lin, X., Tian, L., et al., 2021c. Effects of short-term rice straw return on the soil microbial community. Agriculture 11(6), 561.

Wang, H., Wu, J., Li, G., et al., 2022a. Effects of Simulated Nitrogen Deposition on Soil Active Carbon Fractions in a Wet Meadow in the Qinghai-Tibet Plateau. Journal of Soil Science and Plant Nutrition 22(3), 2943-2954.

Wang, S., Zhao, S., Yang, B., et al., 2022b. The carbon and nitrogen stoichiometry in litter-soil-microbe continuum rather than plant diversity primarily shapes the changes in bacterial communities along a tropical forest restoration chronosequence. Catena 213, 106202.

Wang, Y., Dong, J., Zheng, X., et al., 2021b. Wheat straw and biochar effect on soil carbon fractions, enzyme activities, and nutrients in a tobacco field. Canadian Journal of Soil Science 101(3), 353-364.

Wang, Y., Li, X., Quan, X., et al., 2023b. Effects of nitrogen stress and nitrogen form ratios on the bacterial community and diversity in the root surface and rhizosphere of Cunninghamia lanceolata and Schima superba. Frontiers in Plant Science, 14.

Wang, Y., Wu, P., Mei, F., et al., 2021a. Does continuous straw returning keep China farmland soil organic carbon continued increase? A meta-analysis. Journal of Environmental Management 288, 112391.

Wolińska, A., Podlewski, J., Słomczewski, A., et al., 2022. Fungal indicators of sensitivity and resistance to long-term maize monoculture: a culture-independent approach. Frontiers in microbiology 12, 799378.

Wu, H., Cai, A., Xing, T., et al., 2021. Fertilization enhances mineralization of soil carbon and nitrogen pools by regulating the bacterial community and biomass. Journal of Soils and Sediments 21, 1633-1643

Wu, J., Ma, W., Li, G., et al., 2020a. Vegetation degradation along water gradient leads to soil active organic carbon loss in Gahai wetland. Ecological Engineering 145, 105666.

Wu, Jinshui., Lin, Qimei., Huang, Qiaoyun., et al., 2006. Methods for the determination of soil microbial biomass and its application. Beijing: Meteorological Press, 117-141(in Chinese).

Wu, L., Ma, H., Zhao, Q., et al., 2020b. Changes in soil bacterial community and enzyme activity under five years straw returning in paddy soil. European Journal of Soil Biology 100, 103215.

Xiao, L., Zhang, W., Hu, P., et al., 2023. Mosses stimulate soil carbon and nitrogen accumulation during vegetation restoration in a humid subtropical area. Soil Biology and Biochemistry 184, 109127.

Yan, C., Yan, S.S., Jia, T.Y., et al., 2019. Decomposition characteristics of rice straw returned to the soil in northeast China. Nutrient Cycling in Agroecosystems 114, 211-224.

Yan, S., Song, J., Fan, J., et al., 2020. Changes in soil organic carbon fractions and microbial community under rice straw return in Northeast China. Global Ecology and Conservation 22, e00962.

Yang, H., Ma, J., Rong, Z., et al., 2019. Wheat straw return influences nitrogen-cycling and pathogen associated soil microbiota in a wheat–soybean rotation system. Frontiers in Microbiology 10, 1811.

Yu, Q., Hu, X., Ma, J., et al., 2020. Effects of long-term organic material applications on soil carbon and nitrogen fractions in paddy fields. Soil and Tillage Research 196, 104483.

Zhang, H., Xu, W., Li, Y., et al., 2017. Changes of soil microbial communities during decomposition of straw residues under different land uses. Journal of Arid Land 9, 666-677.

Zhang, H.L., Bai N.L., Zheng X.Q., et al., 2021. Effects of straw returning and fertilization on soil bacterial and fungal community structures and diversities in rice-wheat rotation soil. Chinese Journal of Eco-Agriculture 29(3), 531-539.

Zhang, W., Wang, Y., Li, C., et al., 2022. Long-term ground cover affects soil bacterial community and carbon metabolism in the Loess Plateau, China. Soil Science Society of America Journal 86(4), 918-931.

Zhang, X., Li, X., Zhao, X., et al., 2020. Bioelectric field accelerates the conversion of carbon and nitrogen in soil bioelectrochemical systems. Journal of hazardous materials. 388, 121790.

Zhao, C., Zhang, Y., Liu, X., et al., 2020a. Comparing the effects of biochar and straw amendment on soil carbon pools and bacterial community struture in degraded soil. Journal of Soil Science and Plant Nutrition 20, 751-760.

Zhao, H., Ning, P., Chen, Y., et al., 2019. Effect of straw amendment modes on soil organic carbon, nitrogen sequestration and crop yield on the North-Central Plain of China. Soil Use and Management 35(3), 511-525.

Zhao, L., Liu, Y., Wang, Z., et al., 2020b. Bacteria and fungi differentially contribute to carbon and nitrogen cycles during biological soil crust succession in arid ecosystems. Plant and Soil 447, 379-392.

Zhao, S., Li, K., Zhou, W., et al., 2016. Changes in soil microbial community, enzyme activities and organic matter fractions under long-term straw return in north-central China. Agriculture, Ecosystems & Environment 216, 82-88.

Zhong, Y., Yan, W., Shangguan, Z., 2015. Soil carbon and nitrogen fractions in the soil profile and their response to long-term nitrogen fertilization in a wheat field. Catena 135, 38-46.

Zhu, H., Gong, L., Ding, Z., et al., 2021. Effects of litter and root manipulations on soil carbon and nitrogen in a Schrenk's spruce (Picea schrenkiana) forest. PLoS One 16(2), e0247725.

Zhu, X., Peng, C., Si, G., et al., 2022. Effects of Straw Returning on Soil Chemical Properties and Microbial Community Diversity under the Rice-Crayfish Integrated System. Sustainability 14(20), 13539.

Table captions:

Table 1 The soil organic carbon and labile organic carbon components under different straw returned.

Table 2. The soil organic nitrogen and labile organic nitrogen components under different straw returned.

Figure captions:

Fig. 1. The alpha-diversity of soil bacteria (A) and fungi (B) under different straw returned.

Fig. 2. The relative abundance of dominant bacterial and fungal communities at phylum and genus levels under different straw returned.

Fig. 3. The bidirectional cluster analysis of 31 carbon sources utilized by soil microorganisms under different straw returned.

Fig. 4. The redundancy analysis (RDA) of soil carbon and nitrogen components and soil microorganisms.

Fig. 5. The heat map analysis of correlation between soil carbon and nitrogen components and soil microorganisms.

Fig. 6. The partial least squares pathway model (PLS-PM) shows the direct and indirect effects of straw return, soil bacteria, fungi and soil carbon metabolism capacity on soil carbon and nitrogen components.

Table 1 The soil organic carbon and labile organic carbon components under different straw returned.

Treatment	SOC ($g \cdot kg^{-1}$)	DOC ($mg \cdot kg^{-1}$)	MBC ($mg \cdot kg^{-1}$)	LFOC ($g \cdot kg^{-1}$)	HFOC ($g \cdot kg^{-1}$)
S0	9.31±0.80b	224.32±2.32b	57.40±3.46d	3.26±0.32b	6.36±0.25a
S1/2	9.90±0.65b	227.49±6.85b	72.48±3.63c	4.97±1.45b	6.14±0.38a
S1	11.05±1.20ab	246.89±16.55b	84.78±2.61b	5.73±1.49ab	5.32±0.79ab
S2	13.01±1.58a	309.41±13.35a	131.63±9.32a	8.35±1.40a	4.66±0.46b

Note: SOC: soil organic carbon; DOC: soil dissolved organic carbon; MBC: microbial biomass carbon; LFOC: light fraction organic carbon; HFOC: heavy organic carbon. The numeric values represent mean ± standard deviation. Different lowercase letters in a column indicate differences among treatments ($P < 0.05$), respectively.**Table 2** The soil organic nitrogen and labile organic nitrogen components under different straw

returned.

Treatment	TN (g·kg^{-1})	DON (mg·kg^{-1})	MBN (mg·kg^{-1})	LFON (g·kg^{-1})	HFON (g·kg^{-1})
S0	0.77±0.02b	20.94±3.43b	16.41±0.51b	0.10±0.01c	0.60±0.04a
S1/2	0.79±0.03ab	25.17±2.46ab	17.18±1.38b	0.17±0.01b	0.61±0.05a
S1	0.82±0.07ab	27.08±0.67a	17.99±1.90b	0.20±0.03b	0.62±0.04a
S2	0.88±0.02a	27.87±0.66a	26.68±1.65a	0.26±0.03a	0.67±0.03a

Note: TN: soil total nitrogen; DON: soil dissolved organic nitrogen; MBN microbial biomass nitrogen; LFON: light fraction organic nitrogen; HFON: heavy organic nitrogen. The numeric values represent mean ± standard deviation. Different lowercase letters in a column indicate differences among treatments ($P < 0.05$), respectively.

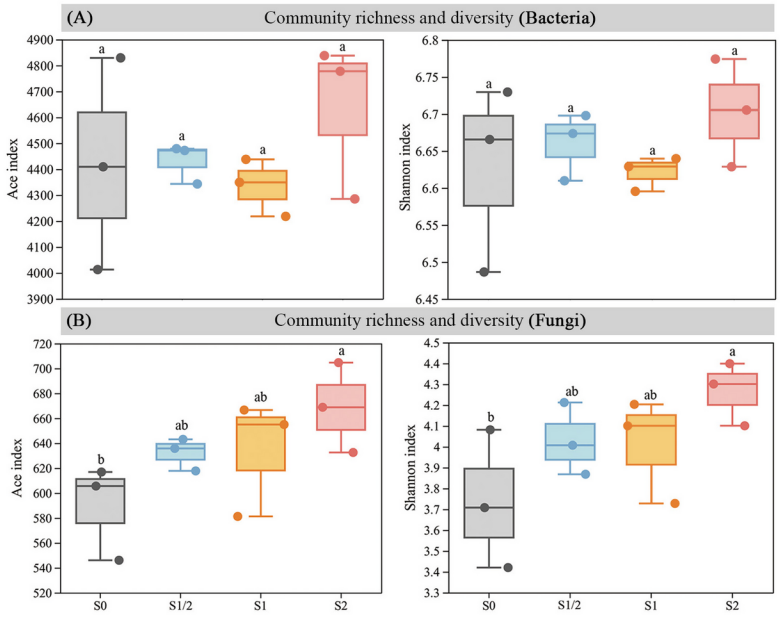


Fig. 1 . The alpha-diversity of soil bacteria (A) and fungi (B) under different straw returned.

Note: Different lowercase letters indicate differences between treatments ($P < 0.05$)

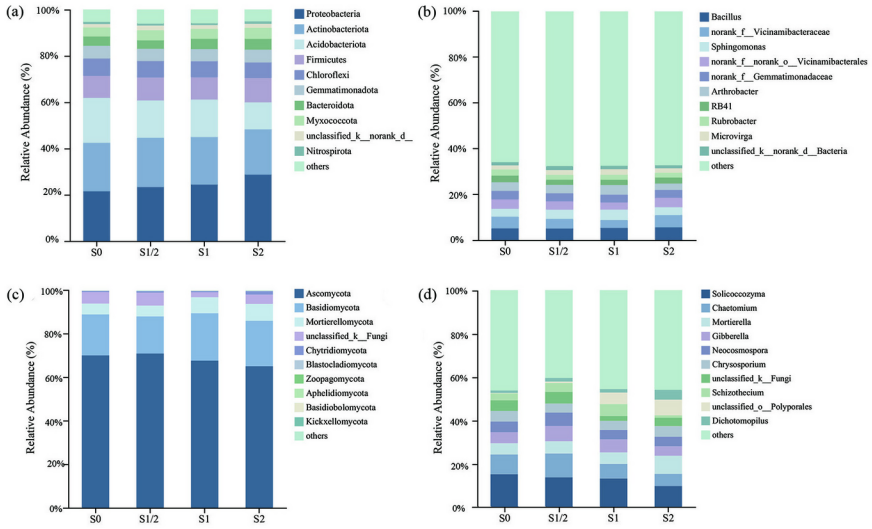


Fig. 2. The relative abundance of dominant bacterial and fungal communities at phylum and genus levels under different straw returned.

Note: (a): phylum of bacteria; (b): genus of bacteria; (c): phylum of fungi; (d): genus of fungi.

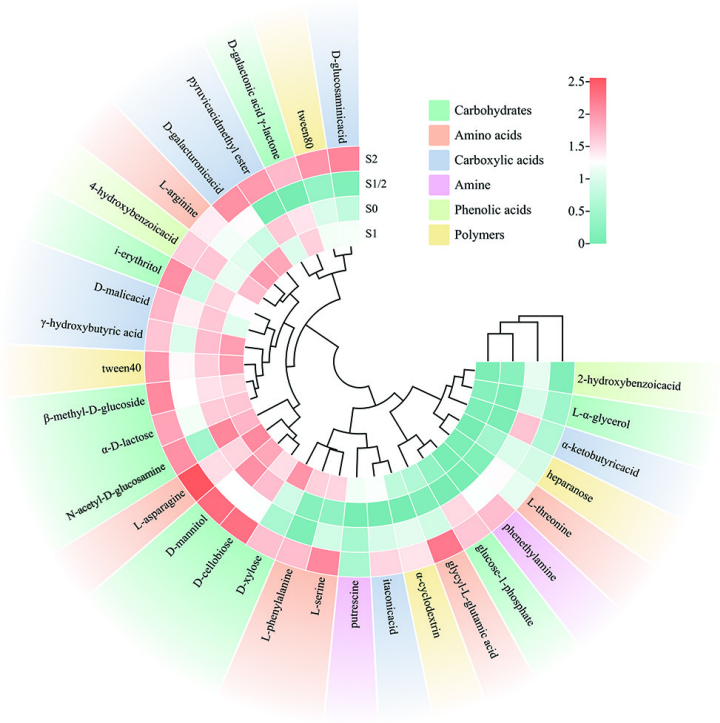


Fig. 3. The bidirectional cluster analysis of 31 carbon sources utilized by soil microorganisms under different straw returned.

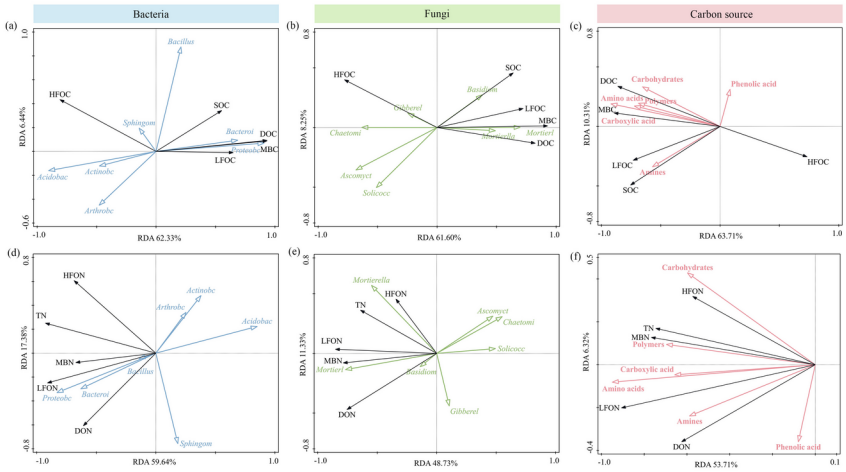


Fig. 4. The redundancy analysis (RDA) of soil carbon and nitrogen components and soil microorganisms.

Note: (a) and (d): Soil bacteria; (b) and (e): Soil fungus; (c) and (f): Carbon sources available to soil microorganisms.

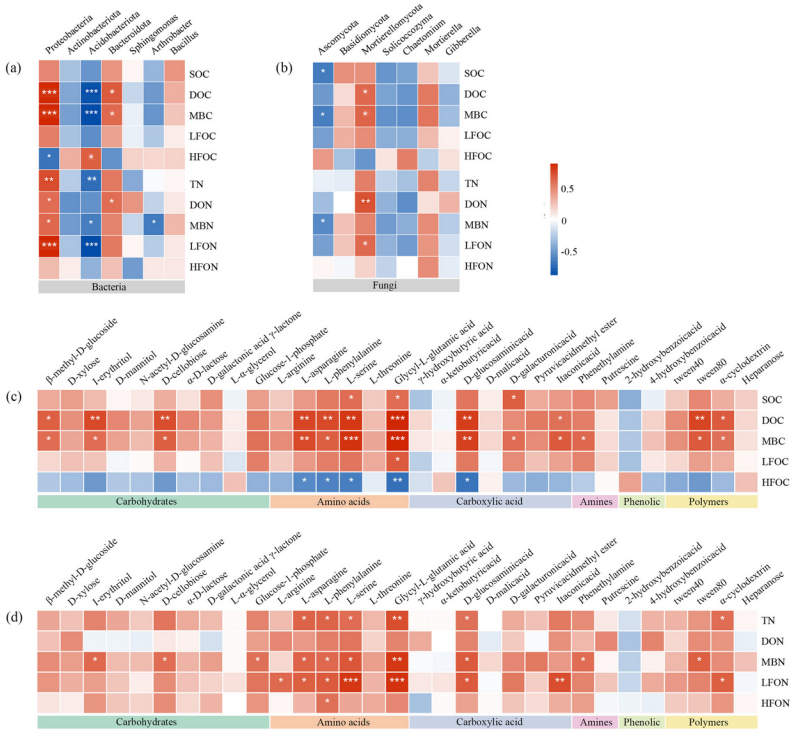


Fig. 5. The heat map analysis of correlation between soil carbon and nitrogen components and soil microorganisms.

Note: (a): Soil bacteria; (b): Soil fungus; (c) and (d): Carbon sources available to soil microorganisms. Stars indicate significant correlation, * indicates $P < 0.05$; ** indicates$P < 0.01$; and *** indicates $P < 0.001$.

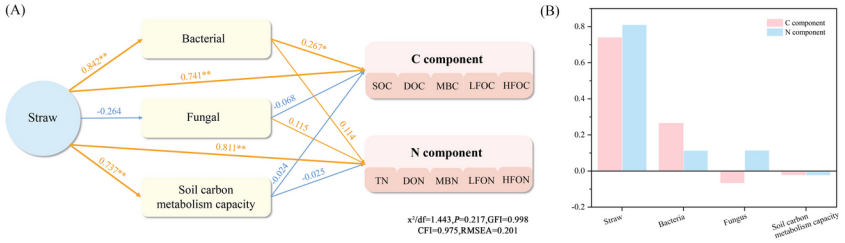


Fig. 6. The partial least squares pathway model (PLS-PM) shows the direct and indirect effects of straw return, soil bacteria, fungi and soil carbon metabolism capacity on soil carbon and nitrogen components.

Note: (A): Partial least squares path modelling; (B): Effect of different factors on soil carbon and nitrogen fractions. Stars denote for significance at $P < 0.05$ and $P < 0.01$ probability levels (* and **, respectively).

Table S1 The basic physical and chemical properties of initial soil(2018yr).

pH	SOC (g·kg^{-1})	TN (g·kg^{-1})	NO_3^--N (mg·kg^{-1})	NH_4^+-N (g·kg^{-1})	AP (g·kg^{-1})	AK (mg·kg^{-1})
8.1	9.85	0.85	22	3.45	23.4	289.6

Note: SOC: soil organic carbon; TN: soil total nitrogen; NO_3^--N: nitrate nitrogen; NH_4^+-N: ammonium nitrogen; AP: available phosphorous; AK: available potassium.

Table S2 Fertilizer application and straw substitution for fertilizer in each treatment.

Year	Treatment	The Fertilization dosage(kg·hm^{-2})	Straw application rates(kg·hm^{-2})	Fertilizer sub
		N	P_2O_5	
2018~2019	S0	82.0	76.0	0.0
	S1/2	72.0	64.0	3250.0
	S1	62.0	52.0	6500.0
	S2	42.0	28.0	13000.0
2019~2020	S0	91.0	35.0	0.0
	S1/2	87.0	31.0	1400.0
	S1	83.0	28.0	2800.0
	S2	75.0	20.0	5600.0
2020~2021	S0	113.0	—	0.0
	S1/2	104.0	—	2826.0
	S1	95.0	—	5653.0
	S2	77.0	—	11306.0
2021~2022	S0	63.0	6.0	0.0
	S1/2	51.4	—	3626.5
	S1	39.8	—	7253.0

Year	Treatment	The Fertilization dosage(kg·hm^{-2})	Straw application rates(kg·hm^{-2})	Fertilizer sub
	S2	16.6	—	14506.0
2022~2023	S0	173.0	67.0	0.0
	S1/2	165.8	63.1	2250.0
	S1	158.6	59.1	4500.0
	S2	144.2	51.2	9000.0
Mean	S0	104.4	59.3	0.0
	S1/2	96.0	52.7	2670.5
	S1	87.7	46.4	5341.2
	S2	71.0	33.1	10682.4

Table S3 The loading factors of 31 carbon sources on PC1, PC2 and PC3.

Type of Carbon Source	Kind of Carbon Source	PC1	PC2	PC3
Carbohydrates	β-methyl-D-glucoside	0.576	0.543	0.349
	D-xylose	-0.227	0.050	0.727
	I-erythritol	-0.261	0.806	0.084
	D-mannitol	0.058	0.917	-0.258
	N-acetyl-D-glucosamine	-0.694	0.671	-0.172
	D-cellobiose	0.094	0.944	-0.009
	α-D-lactose	0.366	0.662	0.127
	D-galactonic acid γ-Lactone	-0.724	0.127	0.393
	L-α-glycerol	0.927	-0.268	0.066
	Glucose-1-phosphate	0.836	0.253	0.359
Amino acids	L-arginine	0.310	0.050	0.535
	L-asparagine	0.106	0.288	0.682
	L-phenylalanine	-0.322	0.441	0.75
	L-serine	0.277	0.428	0.823
	L-threonine	0.946	0.048	0.039
	Glycyl-L-glutamic acid	0.464	0.627	0.527
Carboxylic acid	γ-hydroxybutyric acid	-0.259	0.281	0.003
	α-ketobutyricacid	0.955	-0.262	-0.023
	D-glucosaminicacid	-0.272	0.832	0.426
	D-malicacid	0.512	0.465	-0.452
	D-galacturonicacid	0.275	-0.160	0.778
	Pyruvicacidmethyl ester	-0.620	0.618	0.037
	Itaconicacid	0.542	0.103	0.629
Amines	Phenethylamine	0.862	0.206	0.255
	Putrescine	-0.040	-0.200	0.632
Phenolic acid	2-hydroxybenzoicacid	0.807	-0.456	-0.188
	4-hydroxybenzoicacid	0.517	0.176	0.037
Polymers	Tween40	-0.027	0.695	0.081
	Tween80	-0.117	0.829	0.339
	α-cyclodextrin	0.730	0.147	0.551
	Heparanose	0.653	-0.335	0.168

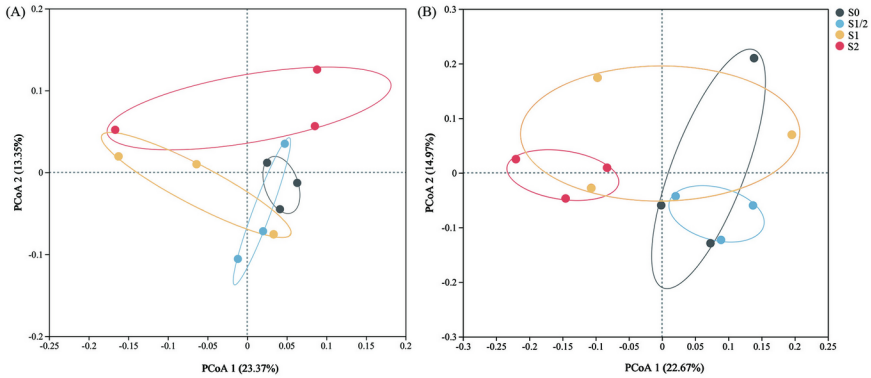
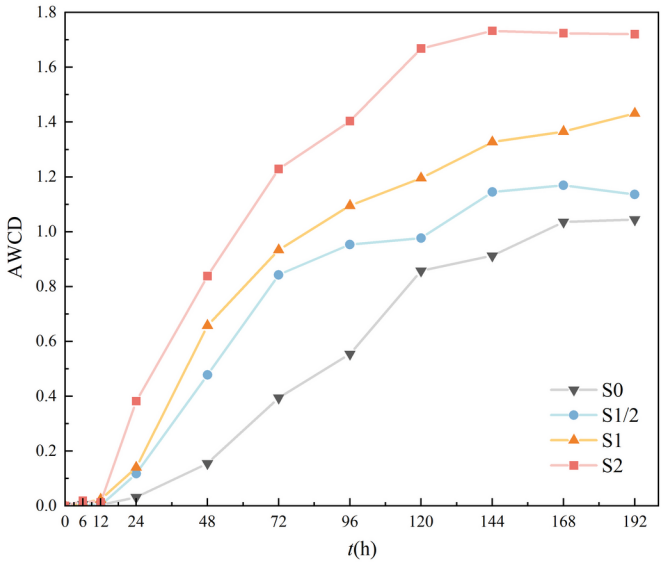


Fig. S1. The beta-diversity of soil bacteria (A) and fungi (B) under different straw returned.



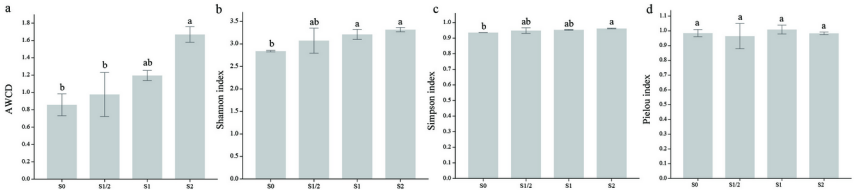


Fig. S2. The average well color development (AWCD) of soil microorganisms under different straw returned.**Fig. S3.** The AWCD values and diversity indices at 120 h of incubation under different straw returned.

Note: **a** : AWCD value; **b** : Shannon index;**c** : Simpson index; **d** : Pielou index. Different lowercase letters indicate significant differences between different treatment ($P < 0.05$).

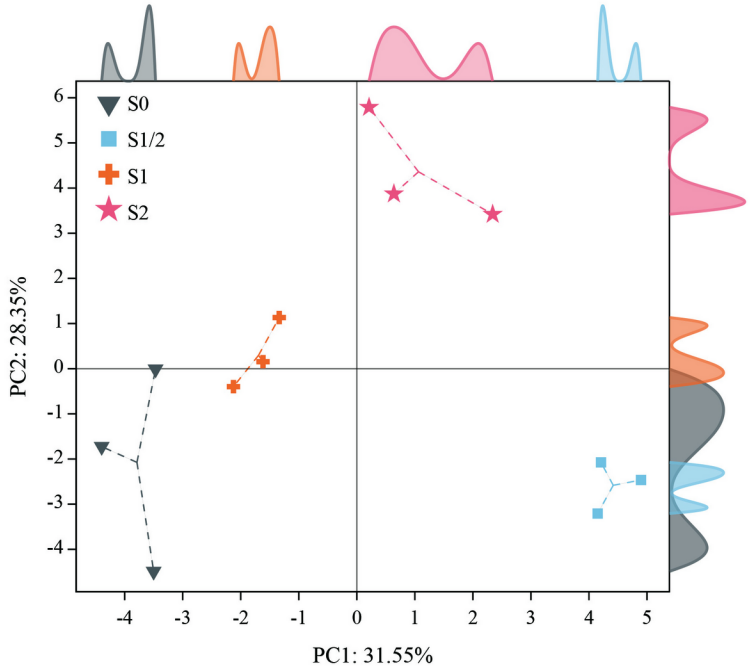


Fig. S4. The principal component analysis (PCA) of soil microbial community carbon utilization under different straw returned.