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The effects of straw return on soil bacterial diversity and functional profiles: A meta-analysis

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Abstract

Returning straw directly to agricultural fields increases soil carbon sequestration and influences soil microbial activities. However, the precise impacts of straw return on soil bacterial diversity, composition, and function profiles remain unclear, necessitating a comprehensive synthesis and standardized analysis. In this study, we conducted a meta-analysis of 729 original bacterial sequencing samples from fields with and without straw return to elucidate how soil bacteria respond to different experimental conditions after straw return (e.g., straw and fertilizer management practices and return duration). Our analysis revealed that overall straw return slightly increased the Chao 1 index while the Shannon index remained unaffected. However, we found strong responses depending on the experimental conditions of straw return. Particularly, bacterial community diversity increased significantly, particularly under high nitrogen, low straw addition, or continuous straw return. Furthermore, straw return altered bacterial community composition, elevating the abundance of *Proteobacteria* and *Bacteroidetes*, while decreasing *Chloroflexi*, *Firmicutes*, and *Nitrospirae*. Straw return also increased modularity in bacterial co-occurrence networks. Functional annotation analysis highlighted that straw return substantially enriched microbes involved in chemoheterotrophy, nitrogen fixation, and functional groups related to straw decomposition (chitinolysis, cellulolysis, xylanolysis), while inhibiting microbes associated with nitrification and nitrate reduction. Surprisingly, straw return did not impact the relative abundance of plant pathogens. Overall, our findings offer an in-depth understanding the effects of straw return on soil bacterial communities, including increased diversity, modified structure, and enhanced functional profiles related to straw decomposition.

Keywords: Straw return; Soil bacterial community; Diversity index; Community composition; Functional annotation.

1. Introduction

Excessive application of mineral fertilizers has decreased soil organic matter, reduced microbial diversity in farmland soils at an alarming rate (Delgado-Baquerizo et al., 2018; Xu et al., 2020a; Xu et al., 2020b; Zhou et al., 2020). Straw, being rich in nutrients, offers a promising solution; its return to fields can replace some chemical fertilizers and provide readily available nutrients and energy for microbial metabolism (Liu et al., 2022a). Moreover, this strategy could contribute to enhance soil organic carbon, crop productivity, soil fertility, and soil microbial biomass and associated activities in farmland (Li et al., 2021a; Li et al., 2021b; Zhang et al., 2022). However, the effects of straw return on soil microbial diversity, community composition, and diversity are complex and influenced by factors such as variations in straw and nitrogen application rates (Cui et al., 2023; Shu et al., 2022; Wang et al., 2023).

Microbial diversity arises from the coexistence of numerous adjacent microhabitats (Young et al., 2008), which is important for mediating various ecosystem functions and impacting plant productivity (Bastida et al., 2016; Delgado-Baquerizo et al., 2016; van der Heijden et al., 2008), and metrics like the Shannon and Chao1 indexes are used to assess bacterial alpha diversity, reflecting soil health (Wagg et al., 2019). The Chao1 index is directly related to species richness, for which it provides a higher estimate compatible with the sample abundance data, and the Shannon index is a species diversity metric (Haegeman et al., 2013). The advent of high-throughput sequencing methods has revolutionized our ability to delineate microbial species, enhancing our understanding of microbial diversity and richness (Wang et al., 2023). Despite numerous studies investigating the effects of straw return on soil microbial diversity and richness, the results remain conflicting. While some studies have reported positive impacts of straw return on soil microbial communities, leading to enhanced soil bacterial diversity (Luo et al., 2020; Zhang et al., 2023), others have found no significant effect (Sun et al., 2015) or even a decrease on soil microbial diversity (Dong et al., 2018; Jin et al., 2020; Jin et al., 2019). Variations in environmental factors like water and temperature can influence straw decomposition

and soil bacterial communities (Li et al., 2021b; Wahdan et al., 2023), contributing to the lack of consensus regarding bacterial characteristics under straw return conditions. The varying outcomes observed in studies investigating the impact of straw return on soil microbial communities may also be attributed to differences in straw application rates, durations, fertilizer management, and other factors (Bastian et al., 2009; Govaerts et al., 2007; Liu et al., 2022b; Wang et al., 2012). Consequently, further analysis is needed to understand how soil microbial diversity responds to different straw and nitrogen application rates and which factors influence soil microbial diversity indexes.

Microorganisms are the main driving force for straw decomposition and turnover in soil (Marschner et al., 2011). Straw return can significantly increase carbon inputs and alter elemental cycling within soils (Schimel and Schaeffer, 2012). Individual soil microorganisms exhibit preferences for specific substrates and employ distinct nutrient acquisition strategies (Gui et al., 2023). Studies have reported inconsistent findings regarding the impact of straw return on soil microbial phyla composition (Li et al., 2022; Li et al., 2021b; Lian et al., 2019), and it remains unclear which genera are predominantly affected by straw return. Bridging these knowledge gaps by synthesizing the results from high-throughput sequencing studies is imperative. Changes in soil microbial community resulting from straw return can further influence microbial functional traits (Guan et al., 2023; Wahdan et al., 2023), including their roles in maintaining soil carbon and nitrogen cycles (de Vries et al., 2018; Mäder et al., 2002; Sokol and Bradford, 2019), and their involvement in functional processes associated with straw decomposition (Liu et al., 2023a; Wahdan et al., 2023). Soil microbiome composition and functional capacity directly affect agricultural productivity by shaping key ecosystem functions like nutrient cycling and plant pathogen resistance (de Vries et al., 2018; Hawkes et al., 2005; Yuan et al., 2018). Previous studies using high-throughput amplicon sequencing methods have primarily focused on assessing soil microbial community performance after straw return during single sampling periods or experiments (Fu et al., 2019; Huang et al.,

2019; Xu et al., 2023). Another question that has not been clearly addressed by sufficient sequencing data is whether straw return affects the abundance of plant pathogens. Hence, a comprehensive analysis of existing high-throughput amplicon sequencing studies is warranted to address this knowledge gap. Furthermore, co-occurrence network analyses offer a valuable approach for investigating microbial interactions among microorganisms (Gui et al., 2023) and understanding microbial community structure (Ma et al., 2016). Several ecological processes generate modules—clusters in the co-occurrence network—including similarities in environmental preferences, i.e. environmental niches, and strong species interactions (Eiler et al., 2012; Ling et al., 2022; Olesen et al., 2007). Yet, there is limited research on co-occurrence network changes following straw return under varying straw application rates and durations. Understanding how straw return shapes co-occurrence networks remains a challenging but crucial endeavor.

Meta-analyses employ quantitative and comprehensive methods to assess the overall effects of studies conducted under different conditions (Koricheva et al., 2013). In recent years, an increasing number of studies have used meta-analysis to analyze microbial responses to environmental and management variables (Cui et al., 2023; Le Geay et al., 2024; Liu et al., 2023b; Wang et al., 2023). In addition, some researchers have explored the effects of organic amendments on soil microbial diversity through meta-analysis (Cui et al., 2023; Shu et al., 2022). However, no separate meta-analysis has focused on the effects of straw return on soil bacterial diversity and functional profiles or how these effects vary with management factors. Hence, a meta-analysis addressing soil microbial diversity and functional profiles using high-throughput sequencing methods and exploring how soil bacteria respond to different experimental conditions following straw return is essential.

In this study, we collected data for 729 original bacterial sequencing samples from fields with and without straw return and conducted a comprehensive evaluation of bacterial community responses to various experimental conditions after straw return by combining and analyzing microbial data. We aimed to (1) determine the

main factors influencing soil microbial diversity indicators and variations in soil microbial diversity with return duration and straw and nitrogen application rates, (2) investigate changes at phyla and genera level after straw return, (3) elucidate shifts in the functional profiles of soil bacterial microbial genomes and assess the change in abundance of plant pathogens, and (4) identify shifts in soil bacterial co-occurrence networks after straw return. We hypothesized that (1) changes in soil bacterial community diversity are related to climate, straw and fertilizer management, and duration and straw return alters soil bacterial community composition, (2) straw return increases the abundance of straw decomposition-related microorganisms and pathogenic bacteria, enhances bacterial network modularity, and alters the dominant keystone taxa.

2. Materials and methods

2.1 Data collection

We conducted an extensive literature search using the China National Knowledge Infrastructure (<https://www.cnki.net>), Web of Science (<https://www.webofscience.com/>), and Google Scholar (<http://scholar.google.com>) databases up to April 2022. Keywords were “soil bacterium” OR “soil bacteria” OR “soil microbial community” OR “soil microbial communities” AND “residue,” OR “straw return,” OR “straw incorporation”. Two sets of selection criteria were applied to identify relevant studies. For the analysis of straw return’s impact on soil bacterial community diversity, studies were required to meet the following criteria: (1) straw return and corresponding no straw treatments included, encompassing various straw types and reporting Chao 1 and Shannon diversity indexes; (2) field or greenhouse experiments conducted in agroecosystems. For a more detailed analysis of straw return’s effects on soil bacterial community composition and network structure, studies had to meet the following criteria: (1) straw return and corresponding no straw treatment included, encompassing various straw types; (2) sequencing metadata and

sample names available from public repositories; (3) field or greenhouse studies and experiments conducted in agroecosystems; (4) supplied samples with > 10,000 reads in bacteria. Supplementary Table 1 presents metadata from the original studies examining straw return effects on soil bacterial raw sequences. Supplementary Table 2 and the Reference list present the publications with soil bacterial community diversity data and soil bacterial raw sequences used in this synthesis.

Data from published figures were extracted using GetData software (<http://getdata-graph-digitizer.com>). In addition to original sequencing data, Chao 1 and Shannon diversity indexes, we collected information on annual average temperature (AAT), annual average precipitation (AAP), initial soil properties (initial soil organic carbon (SOC_i), initial pH (pH_i)), nitrogen application rate (NAR), straw application rate (SAR), returning depth, duration, geographical location (country, latitude, and longitude), altitude, soil properties (soil organic carbon (SOC), pH, total nitrogen (TN)), and the average values, repeated values, standard deviations or standard errors of each indicator. In cases where AAT and AAP were not reported, we estimated this information using data from relevant meteorological stations (Chinese Meteorological Data Network, <http://data.cma.cn/>). We obtained 362 pairs of diversity index metadata and 150 pairs of original sequencing data for straw return versus no straw (729 microbial samples) from 91 studies (Supplementary Figure 2).

2.2 Data analysis

2.2.1 Bioinformatics analysis

We downloaded raw sequence data with FASTQ files from the National Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov>) according to the accession numbers in each study and checked them using FastQC v0.11.7 (Brown et al., 2017). Primers and barcodes of sequence data were screened and removed with cutadapt v1.14. The paired-end reads were merged, discarding reads with expected errors per base > 1% and Phred scores < 20 in VSEARCH v2.21.1 (Rognes et al., 2016). Since the datasets included sequences targeting different regions of the 16S

rRNA gene, a closed-reference workflow was used to map the fragments to the full-length 16S rRNA gene sequences with the “closed_ref” function in USEARCH v10.0.240 (Edgar, 2017). Using the bacterial Greengenes database 13.8, the sequences were clustered into OTUs with 97% identity, with the reference sequences assigned taxonomy in the closed-reference workflow. The OTU table in each study was converted to BIOM format and merged using QIIME2 (Bolyen et al., 2019). Singleton OTUs (consisting of only one read) and OTUs present in only one sample were removed. Finally, after removing samples with < 10,000 reads, we rarefied all samples to the same sequencing depth of 10,000 reads.

2.2.2 Soil bacterial community composition

Beta diversity of bacterial samples in no straw and straw return treatments was estimated and visualized using Bray–Curtis dissimilarity matrixes and principal coordinates analysis with the *vegan* package. Permutational multivariate analysis of variance was used to analyze beta diversity. The top ten phyla of bacterial communities were selected to determine changes in bacterial composition. Pearson’s correlations between soil properties (SOC, TN, pH) and the top ten phyla of bacteria communities and between climate, initial soil properties, duration, and fertilizer management were determined using the *corrplot* package. Random forest analysis generated a classification model of no straw and straw return to detect changes in bacterial communities at the genus level using the *randomForest* and *rfPermute* packages in R. Cross-validation with five replicates was performed to identify important genera in no straw and straw return. Random forest analysis was also used to determine the relative importance of factors influencing the effect size of the top ten phyla of bacteria communities in response to straw return.

2.2.3 Network construction and analysis

Soil bacterial co-occurrence networks were constructed using the Molecular Ecological Network Analysis Pipeline (<http://ieg4.rccc.ou.edu/mena>), including only OTUs appearing in $\geq 50\%$ of the samples. Correlations were determined using the

network enhancement method, with p-values adjusted using the Benjamini–Hochberg false discovery rate control procedure. Correlation thresholds were determined using the random matrix theory method. Data with p-values < 0.05 and correlation coefficients > correlation threshold were retained. Network visualization was performed using Cytoscape 3.7.272 and Gephi 0.9.5. The network modularity of each network was characterized. For each node its topological role in the network was determined based on its within- and among-module connectivity. All nodes were categorized into four subcategories: network hubs (within-module connectivity ≥ 2.5 and among-module connectivity ≥ 0.62), module hubs (within-module connectivity ≥ 2.5 and among-module connectivity < 0.62), peripherals (within-module connectivity < 2.5 and among-module connectivity < 0.62), and connectors (within-module connectivity < 2.5 and among-module connectivity ≥ 0.62).

2.2.4 Prediction of functions

Soil bacterial genomes were functional annotated using the Functional Annotation of Prokaryotic Taxa pipeline (<http://www.loucalab.com/archive/FAPROTAX/>). Pearson's correlations between functional profiles of soil bacterial genomes and soil properties (SOC, TN, pH) were determined using the *corrplot* package. Random forest analysis was used to determine the relative importance of factors influencing the effect size of functional profiles of soil bacterial genomes in response to straw return.

2.2.5 Response ratio

The effect sizes of straw return on bacterial community characteristics (including Chao 1 and Shannon diversity indexes and functional profiles of soil bacterial genomes) were estimated using a natural log-transformed response ratio (*lnRR*) metric.

$$\ln RR = \ln(X_t) - \ln(X_c) \quad (1)$$

where X_c and X_t are mean values of the given variable under no straw and straw return, respectively. The variances of each *lnRR* were calculated as:

$$\text{Variances} = \frac{(S_t)^2}{n_t(X_t)^2} + \frac{(S_c)^2}{n_c(X_c)^2} \quad (2)$$

where S_c and S_t are the corresponding standard deviations of the means under no straw and straw return, and n_c and n_t are the sample sizes, respectively. For studies reporting means without standard deviations, each observed value's mean coefficient of variation was calculated, with the mean value multiplied by the mean coefficient of variation to estimate the missing standard deviations (van Groenigen et al., 2011). $\ln RR$ was converted into percentage change to explain the result better:

$$\text{Percentage change} = (e^{\ln RR} - 1) \times 100 \% \quad (3)$$

We used a random effects model with Metawin 2.1 to determine the effect size of straw return on the variable. The 95% confidence interval was estimated using bootstrapping with 999 iterations. When the 95% confidence interval for the effect size of a variable did not overlap zero, we considered the variable to be significantly different between no straw and straw return. For uniformity of data volume between different categorical groups and more significant results, we divided AAT, AAP, SOC_i, pH_i, NAR, SAR, returning depth, and duration into different groups. In the categorical group analysis, significant between-group heterogeneity means that the mean effect size between groups in each category significantly differed. Relative importance of variables for Chao 1 and Shannon diversity indexes in response to straw return was evaluated using random forest analysis. All statistical analyses and figures were conducted in R 4.1.1, RStudio, and OriginPro 2022 (OriginLab Corporation, Northampton, MA, USA).

3. Results

3.1 Effect of straw return on soil bacterial alpha diversity

Compared with no straw, straw return significantly increased the bacterial Chao 1 diversity index by 3.5% but did not significantly affect the Shannon diversity index (Fig. 1a, b). Various factors were significantly correlated with the soil bacterial Chao

1 diversity index. The most influential variable was SAR, followed by AAT, pH_i, SOC_i, NAR, returning depth, AAP, and duration (Supplementary Fig. S3a). Subgroup analysis showed that AAT ≤ 14°C, AAP ≤ 800 mm, and SOC_i ≤ 14 g·kg⁻¹ had significantly higher promotion effects on the Chao 1 index than AAT > 14°C, AAP > 800 mm, and SOC_i > 14 g·kg⁻¹, respectively. In addition, acidic and neutral soils significantly increased the Chao 1 index compared with alkaline soil. Nitrogen fertilizer and return measures significantly increased the Chao 1 index. Subgroup analysis showed that NAR > 200 kg·ha⁻¹, SAR > 8,000 kg·ha⁻¹, and 20–40 cm returning depth had significantly higher promotion effects on the Chao 1 diversity index than NAR ≤ 200 kg·ha⁻¹, SAR ≤ 8,000 kg·ha⁻¹, and 0–20 cm returning depth, respectively (Fig. 1a). SAR also emerged as the most important variable affecting the Shannon diversity index under straw return, followed by duration, SOC_i, pH_i, AAT, NAR, AAP, and returning depth (Supplementary Fig. S3b). pH_i, SAR, and duration significantly increased the Shannon diversity index, except for duration < 1 year and SAR > 8,000 kg·ha⁻¹. The effect of straw return on the Shannon index was strongest in neutral soil and weakest in alkaline soil (Fig. 1b). Partial correlations were also observed among AA, AAP, and other factors (Supplementary Fig. S4).

3.2 Effect of straw return on soil bacterial community composition

Straw return significantly changed soil bacterial community composition (Fig. 2a), as did duration (duration < 1 year, 1 year ≤ duration ≤ 5 years, duration > 5 years), returning depth [straw mulching (SM) and straw burying (SB)], and SAR (SAR ≤ 8,000 kg·ha⁻¹ and SAR > 8,000 kg·ha⁻¹) (Fig. 2b–d). Compared to no straw, straw return produced more *Proteobacteria* (+3.2%) and *Bacteroidetes* (+10.3%), fewer *Actinobacteria* (−5.9%), *Chloroflexi* (−7.2%), *Gemmatimonadetes* (−8.0%), *Firmicutes* (−14.7%), *Crenarchaeota* (−12.1%), and *Nitrospirae* (−10.0%), and had no significant effect on *Acidobacteria* and *Planctomycetes* (Fig. 3a–j). In addition, through 10-fold cross-validation, random forest analysis identified 99 important genera as biomarkers, primarily belonging to *Proteobacteria* (46%), followed by *Actinobacteria* (23.4%) and *Bacteroidetes* (8.1%) (Fig. 4).

Various soil, environment, and agronomic management factors significantly were correlated with the effect of straw return on the top ten bacterial phyla. For instance, *Proteobacteria* were notably sensitive to AAT, AAP, SOC_i, pH_i, NAR, returning depth, and duration (Fig. 3a), with duration the most influential, followed by AAT, AAP, pH_i, SOC_i, NAR, and returning depth (Supplementary Table 6). *Acidobacteria* were susceptible to the environment and returning measures but were not correlated with initial soil properties (SOC_i, pH_i) (Fig. 3b), with returning depth and duration emerging as the most important variables (Supplementary Table 6). *Bacteroidetes* were significantly correlated with AAT, AAP, SOC_i, pH_i, SAR, and duration (Fig. 3d), with AAP, SOC_i, duration, pH_i, and AAT being the most influential (Supplementary Table 6). *Firmicutes* were correlated with all factors except SOC_i and returning depth (Fig. 3g), with AAT and duration as the most important variables (Supplementary Table 6). *Chloroflexi*, *Gemmatimonadetes*, *Actinobacteria*, *Planctomycetes*, *Crenarchaeota*, and *Nitrospirae* were relatively unresponsive to environmental changes (Fig. 3c–j). The effect size of SOC positively was correlated with the effect size of *Proteobacteria* and *Bacteroidetes* was negatively correlated with the effect size of *Actinobacteria*, *Chloroflexi*, and *Gemmatimonadetes*. The effect size of TN positively was correlated with the effect size of *Proteobacteria* and negatively correlated with the effect size of *Chloroflexi*, *Gemmatimonadetes*, and *Crenarchaeota* (Fig. 3k).

3.3 Effect of straw return on soil bacterial co-occurrence network structure

Molecular ecology networks were generated to evaluate differences in microbial interactions between no straw (Fig. 5a) and straw return (Fig. 5b). The networks of no straw and straw return could be divided into 35 and 38 functional modules, with 12 and 13 modules comprising more than six OTUs, respectively. The network of no straw had 303 nodes and 520 edges, while the network of straw return had 279 nodes and 412 edges. The modularity of bacterial networks increased from no straw to straw return. The keystone OTUs connecting modules differed between the networks of no straw and straw return (Fig. 5e). Both under no straw and under straw return, one

keystone OTU acted as a connector, belonging to the *Betaproteobacteria* and *Acidobacteria-6* classes, respectively. The keystone OTUs in the no straw module hubs belonged to *Nitrospira*, *Actinobacteria*, and *Acidimicrobia*, while those in the straw return module hubs mainly belonged to *Alphaproteobacteria*, *Ellin6529*, and *Gammaproteobacteria*. Thus, the dominant keystone OTUs were *Actinobacteria* in the no straw network and *Proteobacteria* in the straw return network. (Fig. 5d, e).

We evaluated the co-occurrence network based on duration (duration < 1 year, 1 year \leq duration \leq 5 years, and duration > 5 years), returning depth (SM and SB), and SAR (SAR \leq 8,000 kg·ha⁻¹ and SAR > 8,000 kg·ha⁻¹) to characterize the impact of straw return measures on the bacterial network (Supplementary Fig. S5–7). Under different duration networks, most keystone OTUs belonged to *Proteobacteria*, *Actinobacteria*, and *Acidobacteria*. The modularity of 1 year \leq duration \leq 5 years was the highest, followed by duration > 5 years (Supplementary Fig. S5). The keystone OTUs under the SM and SB networks were only in module hubs, with *Proteobacteria* as the dominant keystone OTU under SM. The SB network had higher modularity than the SM network (Supplementary Fig. S6). Networks with duration > 5 years and SM had more links, greater average degree, higher average clustering coefficient, and smaller average path distance than other networks (Supplementary Fig. S5, 6). The keystone OTUs in the SAR \leq 8,000 kg·ha⁻¹ network belonged to *Planctomycetes*, *Proteobacteria*, and *Acidobacteria*, while SAR > 8,000 kg·ha⁻¹ belonged to *Proteobacteria*, *Actinobacteria*, and *Acidobacteria*. The bacterial network under SAR > 8,000 kg·ha⁻¹ had higher modularity, nodes and links, average degree, and average clustering coefficient than the other networks (Supplementary Fig. S7).

3.4 Effect of straw return on functional profiles of soil bacterial genomes

Relative to no straw, it was predicted that straw return significantly increased chemoheterotrophy (+4.7%), chitinolysis (+10.0%), cellulolysis (+23.2%), xylanolysis (+41.5%), nitrogen fixation (+19.3%), aromatic compound degradation (+10.0%), ureolysis (+15.3%), methylotrophy (+26.1%), and methanol oxidation (+37.4%), decreased nitrification (-14.3%), nitrate reduction (-4.6%), aerobic

ammonia oxidation (−20.9%), and aerobic nitrite oxidation (−9.9%), and had no significant effect on plant pathogen, denitrification, fermentation, nitrate denitrification, or respiration of sulfur compounds (Fig. 6 and Supplementary Fig. S8).

Remarkably, it was predicted that some environmental and management factors were significantly correlated with the effects on functional profiles of soil bacterial genomes. For instance, ureolysis were correlated with duration, AAT, and pH_i (Supplementary Table 7), significantly increasing under conditions of high temperature, high humidity, alkalinity, and short addition duration (Fig. 6a). Chemoheterotrophy was favored under low temperature and low humidity, SM, and large nitrogen application rate (Fig. 6b), with returning depth the most influential variable (Supplementary Table 7). Chitinolysis were correlated with duration and SAR, and was enriched at SAR > 8,000 kg·ha⁻¹ and 1 year ≤ duration ≤ 5 years (Fig. 6c). Duration was the most important variable affecting xylanolysis (Supplementary Table 7), significantly affecting this process (Fig. 6e). Notably, plant pathogens were significantly correlated with duration, AAT, and AAP (Supplementary Table 7), and were enriched at AAT ≤ 14°C, AAP ≤ 800 mm, NAR > 200 kg·ha⁻¹, duration > 5 years, and SM (Fig. 6f). SAR > 8000 kg·ha⁻¹, 0–20 cm returning depth, and 1 year ≤ duration ≤ 5 years did not significantly affect nitrification (Fig. 6h). Nitrate reduction responded differently under different AAT, AAP, returning depth, and duration (Fig. 6i), and nitrogen fixation were significantly correlated with duration (Fig. 6g).

4. Discussion

4.1 Soil bacterial alpha diversity with and without straw return

Partly in line with our first hypothesis, our meta-analysis revealed that straw return significantly increased the Chao1 index by 3.5%, with no significant effect on the Shannon index (Fig. 1). This discrepancy may be related to our finding that the Chao1 index was significantly correlated with various factors under straw return (Fig. 1a), while the Shannon index was only significantly correlated with pH_i, return duration,

and SAR under straw return (Fig. 1b). Among the factors measured, SAR emerged as the most influential variable for both diversity indexes (Supplementary Fig. S3), probably because SAR directly affects carbon input amounts into the soil. But excessive SAR, leading to a higher soil carbon-to-nitrogen ratio, decreased the Shannon diversity index (Fig. 1b). Increasing nitrogen fertilizer under such conditions may mitigate this negative effect by addressing nitrogen deficiency in the soil. Continuous straw return over time significantly increased both Shannon and Chao1 indexes, indicating its positive impact on bacterial community diversity. However, short-term duration (< 1 year) did not affect Chao 1 and decreased the Shannon diversity index (Fig. 1), highlighting the importance of sustained straw return for enhancing bacterial community diversity in farmland ecosystems.

4.2 Soil bacterial community composition with and without straw return

Supporting our first hypothesis, straw return significantly altered soil bacterial community composition (Fig. 2a), with certain phyla responding differently to the input of exogenous organic matter. Microbial succession can change specific bacterial phyla during plant residue decomposition (Geisseler and Scow, 2014; Prewitt et al., 2014). For instance, straw return significantly increased *Proteobacteria* and *Bacteroidetes*, and the effect size of SOC positively correlated with the effect sizes of *Proteobacteria* and *Bacteroidetes* (Fig. 3). This might be explained by noting that most *Proteobacteria* and *Bacteroidetes* are copiotrophic populations, which use labile forms of carbon to grow and metabolize, thriving under high carbon availability conditions, with high metabolic activity and rapid growth and reproduction characteristics (Eilers et al., 2010; Fierer et al., 2007; Ramirez et al., 2010a; Ramirez et al., 2010b). In contrast, straw return significantly decreased *Chloroflexi*, *Firmicutes*, and *Nitrospirae*, and the effect size of SOC negatively correlated with the effect sizes of *Actinobacteria*, *Chloroflexi*, and *Gemmatimonadetes* (Fig. 3). This is probably related to the fact that most *Chloroflexi*, *Nitrospirae*, and *Firmicutes* are oligotrophic populations, which grow slowly, mineralize recalcitrant SOC and dominate nutrient-poor environments (Eilers et al., 2010; Fierer et al., 2007; Ramirez

et al., 2010a; Ramirez et al., 2010b). Thus, increased soil nutrients from straw return stimulate the growth of copiotrophic populations while inhibiting oligotrophic bacteria (Fierer et al., 2012; Ramirez et al., 2012).

Straw return provides soil microorganisms with a stable carbon source and other nutrients (Wang et al., 2020). *Proteobacteria*, the most abundant soil microbial phylum, contains diverse bacteria in terms of ecological and nutritional characteristics (Madigan and Martinko, 2006), involved in straw and plant residue decomposition, crucial for carbon and nitrogen cycling (Fan et al., 2014). High-throughput sequencing technology makes it possible to understand the changes at the genus level due to straw return. and the random forest analysis revealed that genera belong to *Proteobacteria* and *Actinobacteria* dominated these changes, with 46.0% and 23.4%, respectively (Fig. 4). Nine genera (e.g., *Agrobacterium* and *Bradyrhizobium*) within *Rhizobiales*, five genera (e.g., *Sphingobium* and *Sphingomonas*) within *Sphingomonadales*, and nine genera (e.g., *Thermomonas* and *Lysobacter*) within *Xanthomonadales* play important roles in plant decomposition (de Vries et al., 2015; Eichorst and Kuske, 2012). *Arthrobacter*, a saprophytic organism known for degrading complex residue debris (Barka et al., 2016), may become more abundant with the addition of unstable SOC (Goldfarb et al., 2011), synthesizing extracellular enzymes to degrade complex carbon source organic matter (Fan et al., 2014). Thus, straw return measures stimulate nutrient cycling and selectively enrich some bacterial species capable of decomposing plant residues, contributing to complex organic matter decomposition (Degruene et al., 2017; Wang et al., 2020) and potentially increasing the Chao1 index, reflecting increased species richness.

4.3 Soil bacterial co-occurrence network structure with and without straw return

In this study, bacterial network modularity increased after straw return in 25 environments (Fig. 5c), supporting our second hypothesis. Modularity is considered a key feature of biological networks (Zhou et al., 2010), with module eigenvalues related to ecosystem versatility (Lurgi et al., 2019; Zhou et al., 2021). The straw return bacterial network indicated enhanced niche differentiation and a more complex

topological structure compared to no straw. The duration, returning depth, and SAR influenced network modularity (Supplementary Fig. S5–7), highlighting the importance of these factors in shaping microbial community interactions. Keystone taxa, which frequently interact with other taxa (Yuan et al., 2021) and are crucial in controlling microbial community structure and function (Hartman et al., 2018), shifted from *Actinobacteria* to *Proteobacteria* in the network after straw return (Fig. 5d, e), further supporting our second hypothesis. Members of *Actinobacteria*, prominent in the no straw network, secrete spores to resist environmental disturbances (Ventura et al., 2007), while *Nitrospirae* oxidize nitrite to nitrate. In the straw return network, two keystone taxa within *Proteobacteria*—*Sphingomonadales* and *Xanthomonadales*—are associated with cellulose degradation (de Vries et al., 2015; Eichorst and Kuske, 2012). These shifts suggest that bacteria involved in straw degradation occupy key positions in the network structure following straw return, indicating changes in community composition and ecological roles in response to external organic matter inputs.

4.4 Functional profiles of soil bacterial genomes with and without straw return

Straw return significantly enhanced functional groups related to organic matter decomposition and transformation, including chitinolysis, cellulolysis, and xylanolysis (Fig. 6), supporting our second hypothesis. This finding aligns with increased enzyme activity observed under straw return conditions (Liu et al., 2022b; Liu et al., 2023a), reflecting higher bacterial abundance and activity in decomposing organic matter and enriched bacterial functional groups related to straw decomposition. Additionally, straw return enriched chemoheterotrophic and methylotrophic microorganisms (methylotrophy and methanol oxidation) (Fig. 6 and Supplementary Fig. S8), which are involved in consuming various natural organic compounds, synthetic organic polymers, and non-C-C bond low-carbon compounds. Straw return increases the carbon-to-nitrogen ratio in soil (Zhang et al., 2022), changing the functional bacteria related to nitrogen transformation (Fig. 6 and Supplementary Fig. S8). Straw return also increases the nitrogen fixation and

ureolysis functional groups related to the nitrogen cycle, enhancing the nitrogen fixation ability of soil microorganisms, with their effect sizes related to soil pH_i, NAR, and duration (Fig. 6 and Supplementary Fig. S8). Moreover, straw addition produces some acidic compounds in the cellulose degradation process, reducing soil pH (Kato et al., 2005; Sun et al., 2015), which may lead to nitrification and nitrate reduction under straw return (Fig. 6) as neutral or alkaline soils are most suitable for nitrification. However, reduced nitrification and nitrate reduction prevent topsoil available nitrogen loss and N₂O production for continuous nutrient supply to crops. The shift in functional groups related to nitrogen cycle observed in this study partly explains why straw return improved nitrogen use efficiency and crop yield.

Surprisingly, contradicting our second hypothesis, straw return did not increase soil bacterial plant pathogens. Possible explanations include (1) the increase in beneficial microorganisms, such as *Azotobacter* and growth-promoting microorganisms, which can enhance plant defenses and nutrient absorption capacity (Fan et al., 2021), (2) the increase in microbial diversity protecting against soil-borne diseases and inhibiting plant pathogens (Lupatini et al., 2017; Vida et al., 2020; Wang et al., 2021), and (3) only a few groups of pathogenic bacteria being soil-borne, as non-spore-forming bacteria do not survive long in non-rhizosphere soil (Ling et al., 2022). However, longer durations of straw return were associated with an increase in plant pathogens (Fig. 6f and Supplementary Table 7), indicating the need for further investigations into the impact of straw return on plant pathogenesis, focusing on enhanced nutrient cycling and soil health through microbial activities. It is essential to note that current tools for functional gene annotation are rather coarse, and more accurate methods would be useful to improve the precision of functional profiles in the future.

5. Conclusions

Our synthesis of original sequencing data from various published studies revealed significant changes in soil bacterial community diversity, composition, co-

occurrence network, and functional profiles following straw return. Straw return significantly increased the Chao1 and Shannon indexes, except under specific conditions (duration < 1 year and SAR > 8,000 kg·ha⁻¹). Straw return significantly enriched copiotrophic populations (e.g., *Proteobacteria* and *Bacteroidetes*) but decreased oligotrophic populations (e.g., *Chloroflexi*, *Nitrospirae*, and *Firmicutes*). Moreover, straw return increased bacterial network modularity more than no straw, indicating a more complex network structure. With new organic compounds added to the soil under straw return, it was predicted that the rapidly growing bacteria had many functional profiles rich in chitinolysis, cellulolysis, and xylanolysis and others genes related to organic matter decomposition and transformation, chemoheterotrophy and methylotrophy microorganisms. Surprisingly, straw return did not significantly affect soil bacterial plant pathogens, suggesting a need for further investigation into the dynamics of pathogenic microorganisms under straw return conditions. This study summarizes and expands the understanding bacterial community shifts following plant straw return. Future research should focus on conducting standardized field trials on the effect of straw return at a global scale to validate these findings and reduce biases arising from methodological variations in extraction and sequencing protocols. This approach will facilitate more accurate assessments of bacterial community diversity, structure, and function om response to straw return.

Declaration of competing interest

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

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Data availability

Source data supporting the results of this study are provided in supplementary materials. Other data supporting this study's findings are available from the corresponding author upon request.

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Figure 1

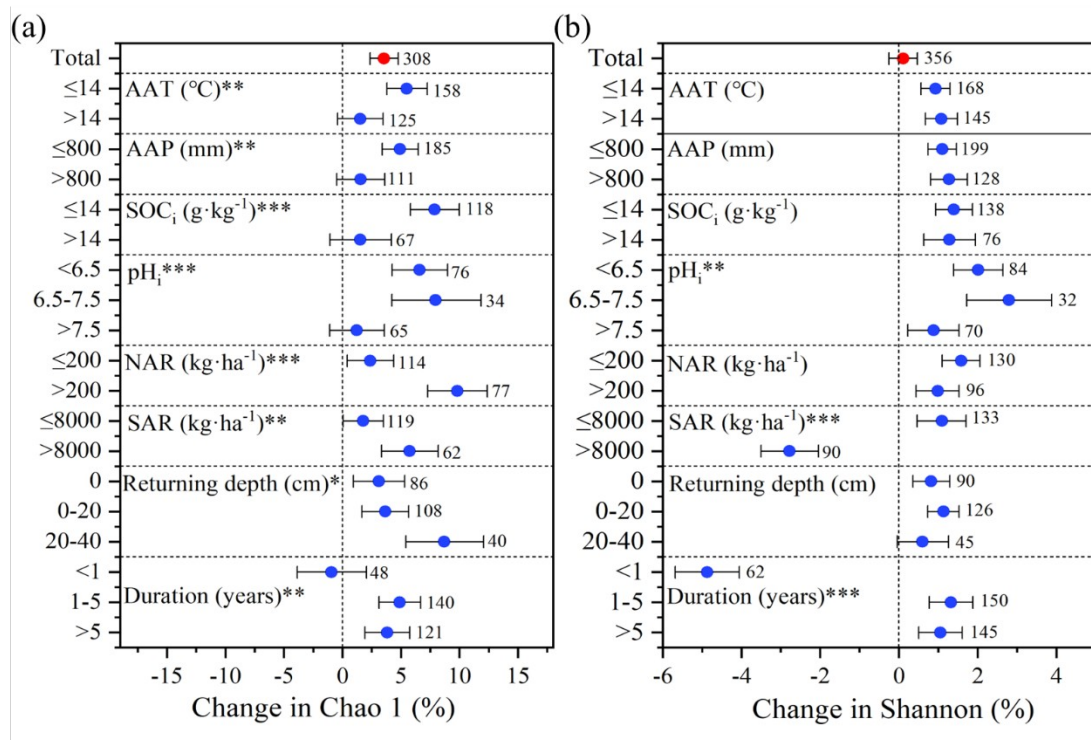


Figure 1. Effect of straw return on the (a) Chao 1 diversity index and (b) Shannon diversity index. Points and error bars represent effect size means and 95% confidence intervals, respectively. *, **, and *** denote significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. AAT: annual average temperature, AAP: annual average precipitation, SOC_i: initial soil organic carbon, pH_i: initial pH, NAR: nitrogen application rate, SAR: straw application rate.

Figure 2

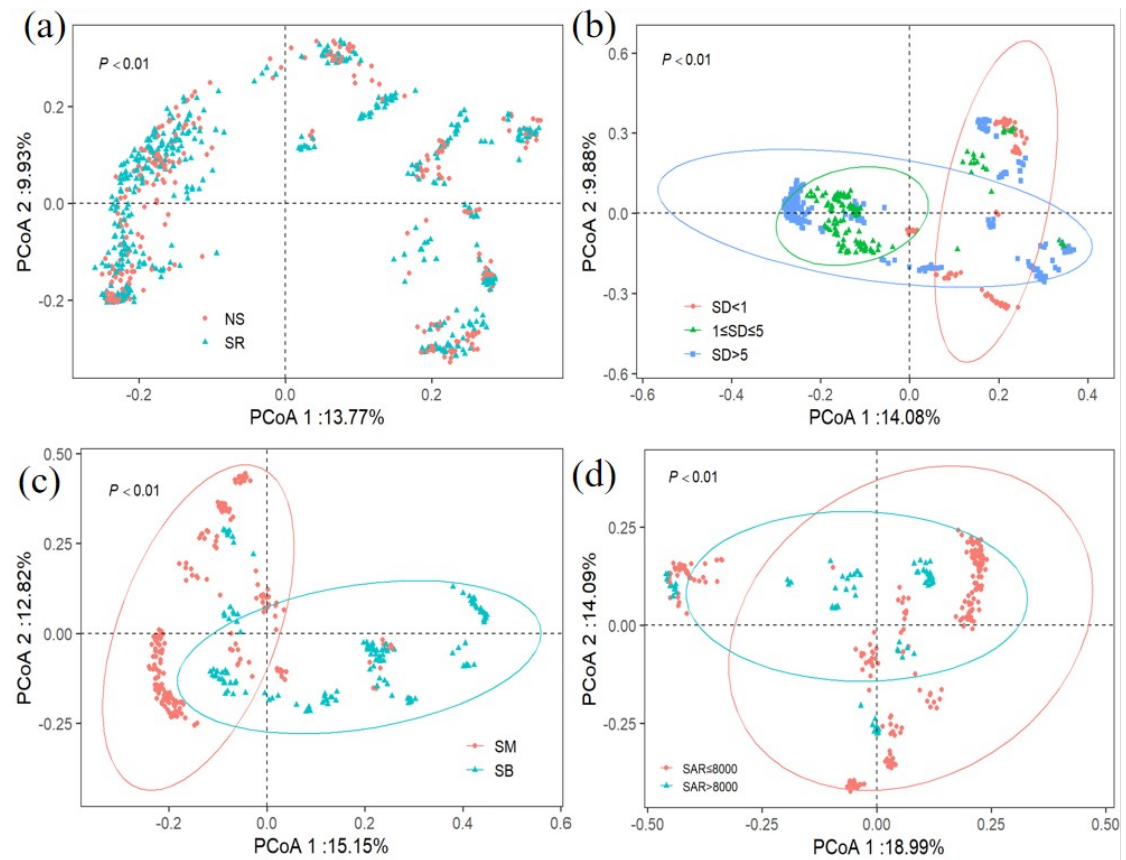


Figure 2. Differences in principal coordinates analysis (PCoA) for bacterial communities in relation to (a) straw return (SR) versus no straw (NS); (b) straw return duration (SD, years); (c) returning depth: straw mulching (SM) and straw burying (SB); (d) straw application rate (SAR, $\text{kg}\cdot\text{ha}^{-1}$). P-value determined using the Adonis test with 9,999 permutations.

Figure 3

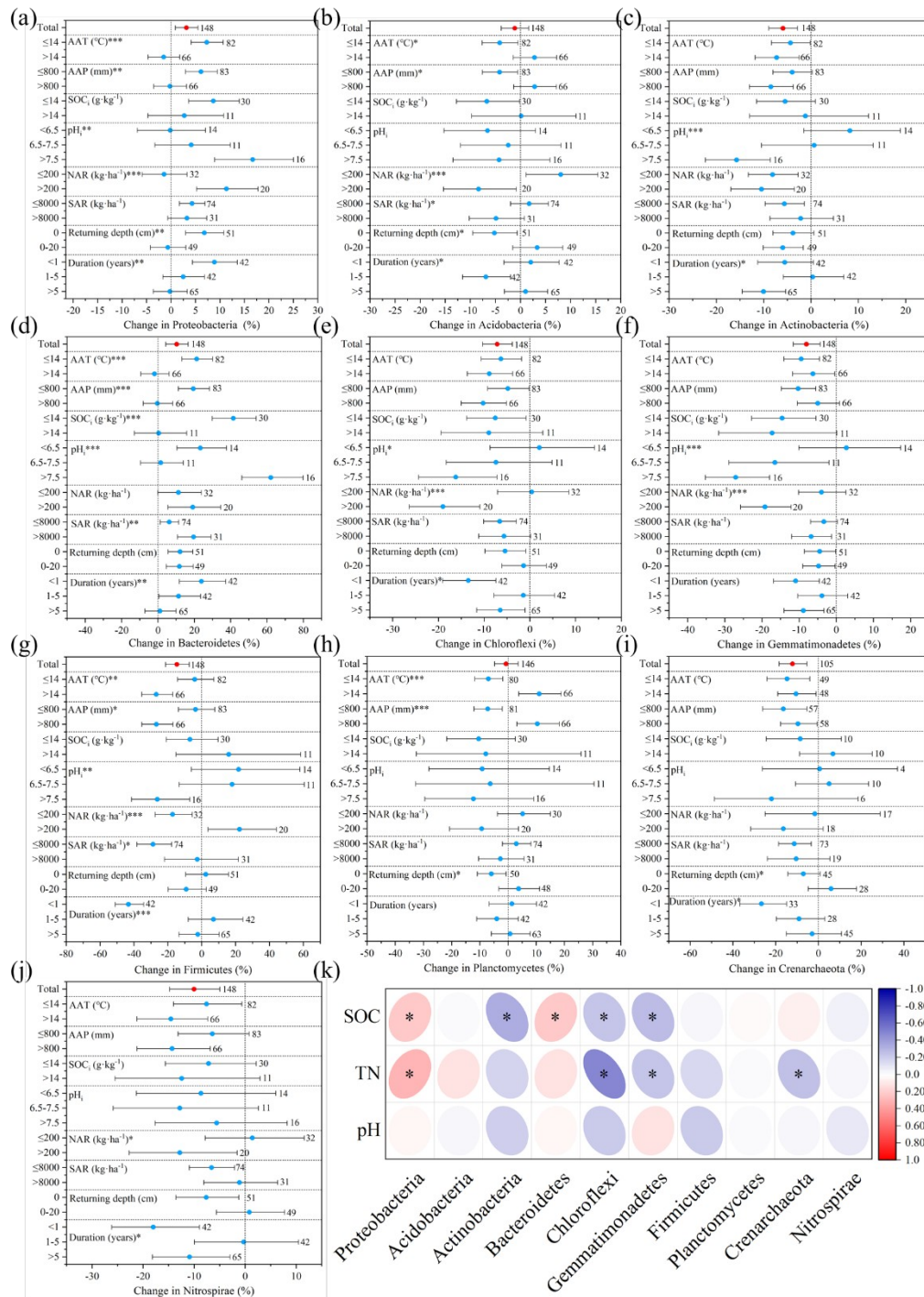


Figure 3. Effect of straw return on the top 10 bacterial phyla for (a) *Proteobacteria*, (b) *Acidobacteria*, (c) *Actinobacteria*, (d) *Bacteroidetes*, (e) *Chloroflexi*, (f) *Gemmatimonadetes*, (g) *Firmicutes*, (h) *Planctomycetes*, (i) *Crenarchaeota*, and (j) *Nitrospirae*. Points and error bars represent effect size means and 95% confidence intervals, respectively. *, **, and *** denote significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

0.01, and $p < 0.001$, respectively. AAT: annual average temperature, AAP: annual average precipitation, SOC_i : initial soil organic carbon, pH_i : initial pH, NAR: nitrogen application rate, SAR: straw application rate. (k) Relationships between the top 10 bacterial phyla and soil physiochemical properties [soil organic carbon (SOC), pH, total nitrogen (TN)], with the models' R^2 and p-values noted.

Figure 4

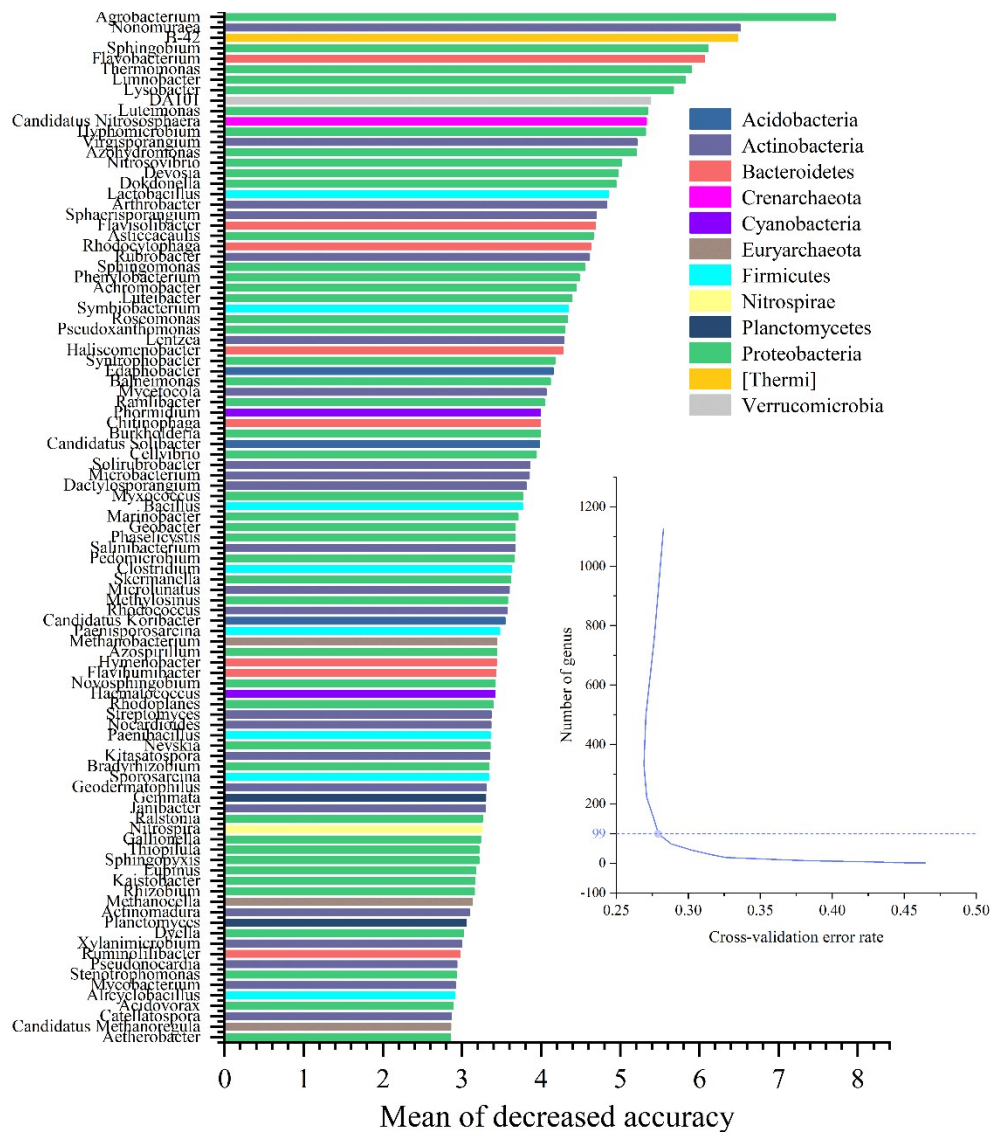


Figure 4. Differences in bacterial taxa between straw return and no straw at the genera level from random forests analysis. Genera are ranked in descending order of importance to the model's accuracy. Insert is the 10-fold cross-validation error as a function of identifying the number of important genera.

Figure 5

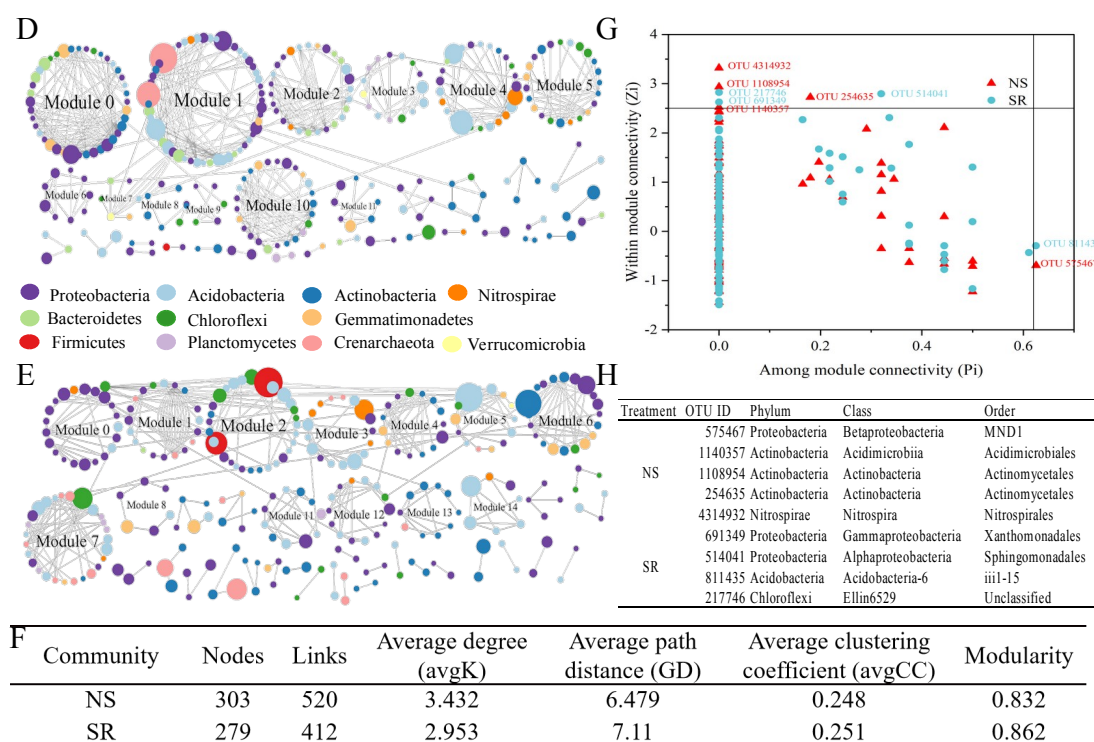


Figure 5. Microbial ecology networks and functional modules of soil bacteria under straw return (SR) and no straw (NS): (a) co-occurrence in NS soil; (b) co-occurrence in SR soil. Node colors indicate different major phyla; (c) topological properties of bacterial communities under NS and SR; (d) Zi–Pi plot showing the node classifications for identifying keystone OTUs in the networks; (e) species classification information corresponding to the keystone OTUs. Node sizes indicated the richness of OTUs.

Figure 6

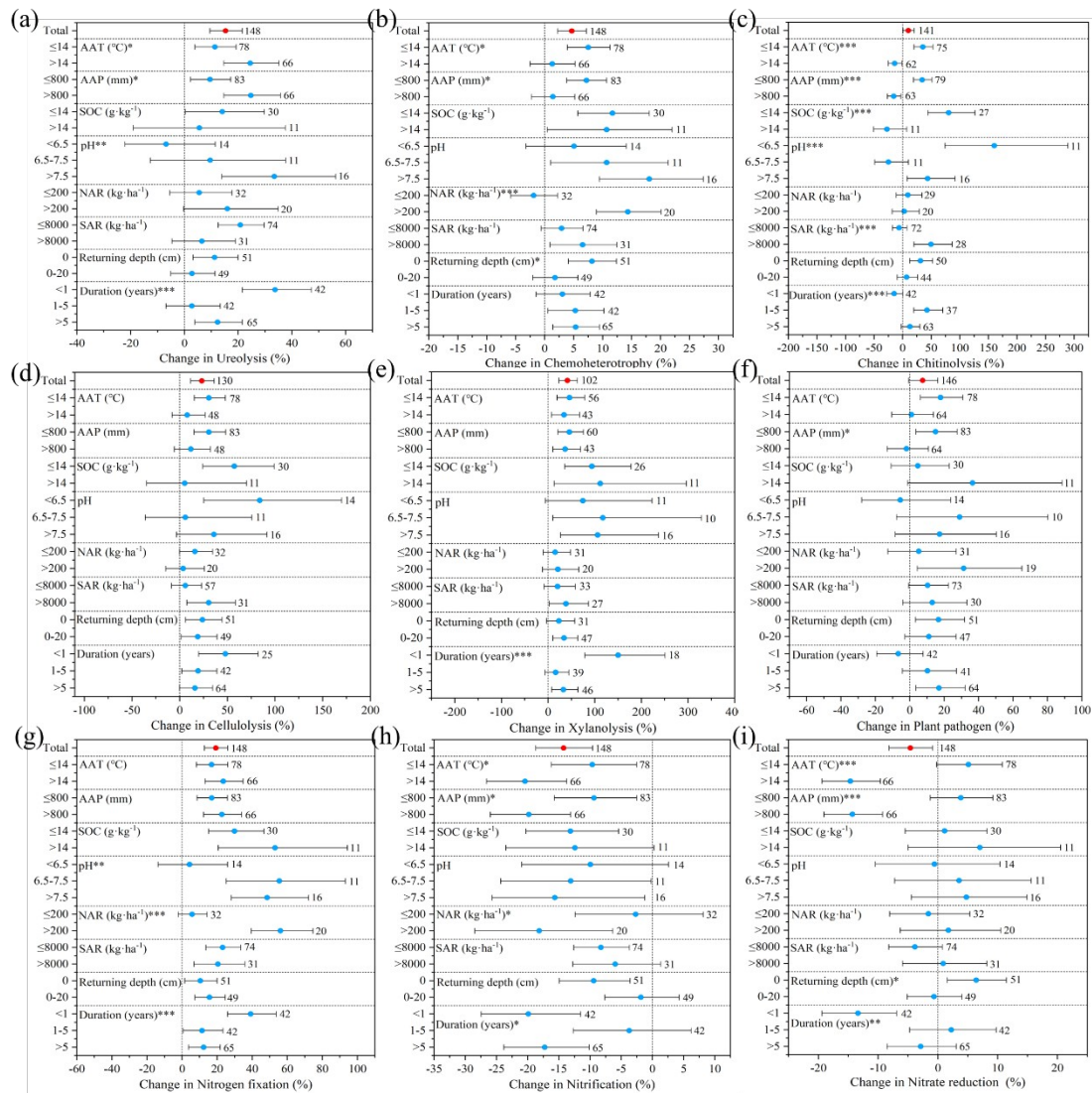


Figure 6. Functional profiles of soil bacterial communities under no straw and straw return, with differences related to (a) ureolysis, (b) chemoheterotrophy, (c) chitinolysis, (d) cellulolysis, (e) xylanolysis, (f) plant pathogen, (g) nitrogen fixation, (h) nitrification, and (i) nitrate reduction. Points and error bars represent effect size means and 95% confidence intervals, respectively. *, **, and *** denote significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. AAT: annual average temperature, AAP: annual average precipitation, SOC_i: initial soil organic carbon, pH_i: initial pH, NAR: nitrogen application rate, SAR: straw application rate.