

# Effect of Straw And Wood Ash On Soil Carbon Sequestration and Bacterial Community in a Calcareous Soil

**Huili Zhao**

Ludong Univ. College of Resources & Environ.

**Ying Zhao**

Ludong Univ. College of Resources & Environ.

**Xiaohong Guo**

Ludong Univ. College of Resources & Environ.

**Nan Wu** (✉ [wunan@ldu.edu.cn](mailto:wunan@ldu.edu.cn))

Ludong University

**Xiaohong Tian**

Ludong Univ. College of Resources & Environ.

**Shiwei Zhou**

Ludong Univ. College of Resources & Environ.

**Shakeel Ahmad**

Ludong Univ. College of Resources & Environ.

---

## Research Article

**Keywords:** Wood ash, Bacterial community, Carbon sequestration, Straw incorporation

**Posted Date:** November 17th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-1052311/v1>

**License:**   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

*Background and aims* The effective utilization of agricultural waste can improve soil fertility. Straw provides energy for the soil, and wood ash supplies nutrients to enrich the soil. However, few studies have examined the effects of wood ash and straw on the sequestration of soil carbon and the soil bacterial community, particularly in calcareous soils.

*Methods* The goal for this study was to quantify the impact of a combination of wood ash and straw on the indicators described above using stable  $\delta^{13}\text{C}$  isotope analyses by applying wheat straw (a  $\text{C}_3$  plant) to a calcareous soil under a long-term  $\text{C}_4$  crop rotation. The incubation experiment included four treatments as follows: (i) no amendment (Control); (ii) amendment with wood ash (W); (iii) amendment with straw (S); and (iv) a combined amendment of straw and wood ash (SW).

*Results* The results showed that sequestration of soil inorganic carbon (SIC) in the SW and W treatments was obviously higher than that in S and Control treatments. The sequestered soil organic carbon (SOC) in the SW treatment was 1.25-fold greater than that in the S treatment, while there was no evident effect on the SOC content compared with straw alone. The microbial biomass carbon and dissolved organic carbon derived from microbial biomass in the four treatments was distributed as  $\text{SW} > \text{S} > \text{W} > \text{Control}$ . The pH and electrical conductivity were obviously higher in the W and SW treatments than in the S treatment and the Control. The SW was conducive to the maintenance of soil enzymatic activities and bacterial diversity. Bacteroidetes and Actinobacteriota dominated in SW, while the Acidobacteria phyla dominated in S treatment. The diversity of bacteria in the soil and community composition of the bacteria were predominantly assessed by the levels of soil labile carbon, pH, and electrical conductivity.

*Conclusions* The incorporation of straw and wood ash is probably more effective at improving SIC and SOC sequestration and ameliorate the soil microhabitat.

## Introduction

It is very common to use agricultural wastes to improve soil fertility (Talukdar et al. 2018; Li et al. 2021). The principal source of SOC in farming systems is organic manure and crop residues (Liang et al. 2021). In some areas, organic manure is primarily reserved for cash crops, and straw return is effective at improving the sequestration of SOC in farmland systems. The wood ash industry produces substantial amounts of wood ash that is increasing costly to store, leading stakeholders to find alternative uses (Juárez et al. 2013). Recycling biomass ash in The agricultural industry can provide a use for biomass ash by recycling it, which could settle the issues of disposal and reduce the need for commercial fertilizer to use on crops.

Zhao et al. (2017, 2018) reported that comprehensively adding wood ash and straw could reduce the emissions of  $\text{CO}_2$  that are partly owing to the formed inorganic carbon. Nevertheless, the effect of integrating crop straw with wood ash on SOC turnover has not been assessed thoroughly in calcareous soil. Previous studies found that using mineral amendments, such as wood ash, to apply to the soil

resulted in carbon with a highly specific surface area and high contents of metal oxides that helps organic residues to raise the amount of C in the soil (Reed et al. 2017). Lei et al. (2019) found that the use of fly ash composite and organic fertiliser was a suitable amendment to improve reclaimed soil. It is not clear whether wood ash promotes or inhibits C mineralization in native SOC, particularly when combined with straw, and also little is known regarding the combination on straw-derived C sequestered in calcareous soil.

In addition, it is highly important to investigate the modifications induced by addition of straw and wood ash on bacterial community of soil. Bacterial diversity and abundance are the foundations for soil productivity in agricultural soil (Bhat et al. 2013). Most of the studies reported so far only address the addition of wood ash to acidic agricultural and/or forest soils. In addition, some of the studies that analyzed the soil biological characteristics produced conflicting reports on the usage of wood ash on the components of soil biology. Peltoniemi et al. (2016) showed that mycorrhizae and wood-decomposing fungi appeared owing to the higher pH and additional nutrients caused by wood ash fertilization in a soil that is acidic. Noyce et al. (2016) observed that applying wood ash to two different global forest biomes was much less like to disrupt the composition of soil microbial communities. Straw provides energy for the soil, and wood ash provides nutrients for the soil. However, few studies have explored the combination on microbial communities, especially in calcareous soils.

An incubation experiment was conducted to investigate the chemical properties, soil carbon sequestration and bacterial community in calcareous agricultural soil after amendments with straw and wood ash. Our goals were to (i) assess variations in the soil carbon sequestration after adding a combination of wood ash and straw, (ii) measure the enzyme activities and soil bacterial communities after the adjunction of a combination of straw and wood ash, and (iii) illustrate the interactions that take place between soil bacterial communities and carbon sequestration. We hypothesized that the adjunction of straw and wood ash would enhance the nutrient supply and alter the soil pH, consequently increasing the activities of soil enzymes and the relative diversity and abundance of the soil bacteria, resulting in greater amounts of soil carbon sequestration.

## **Materials And Methods**

### **Characterization of wood ash, wheat straw and the soil**

The soil for this experiment was collected from several sampling points of the top 20-cm layer (collected from several sampling points) in the Changwu Agricultural and Ecological Experimental Station (35.14° N, 107.40° E; 1,152 m a.s.l.) on the Loess Plateau in northwestern China. This field had been cultivated under a monocropped planting of maize, a C<sub>4</sub> crop, for at least 12 years. The soil was air-dried, well mixed and dislodged crop residues were passed through a sieve of 2 mm. The soil with a  $\delta^{13}\text{C}$  value of -19.5‰ was classified as a Cumuli-Usticlsolumosol by the USDA classification system and had a texture of silty loam. The soil samples contained 8.9 g kg<sup>-1</sup> SOC, 1.2 g kg<sup>-1</sup> total nitrogen (TN), 163.0 mg kg<sup>-1</sup> MBC, 32.4 mg

kg<sup>-1</sup> DOC as determined in the methods below, 18.4 mg kg<sup>-1</sup> of available phosphorus, and 152.3 mg kg<sup>-1</sup> of available potassium, pH (H<sub>2</sub>O) 7.9 (Gong et al. 2007).

Wheat straw (a C<sub>3</sub> plant) harvested from the Doukou Experimental Station of Northwest A&F University (Shaanxi, China) was dried in a 60°C oven and cut into pieces that were approximately 2 cm long before they were mixed with the soil. The straw had total C and N contents of 456.1 and 6.8 g kg<sup>-1</sup> (66 C:N), respectively. The wheat straw had a δ<sup>13</sup>C value of -27.5‰.

The wood ash was the ash of kiwifruit branches after burning. The ash was then dried for 24 h in a 60°C oven and mixed before its use and analysis. The δ<sup>13</sup>C value of the wood ash was -26.4‰ (SE). The total carbon from the wood ash (as described in the method below) and the organic C were 39.3 g kg<sup>-1</sup> and 3.2 g kg<sup>-1</sup>, respectively, and the SIC added from wood ash was 432 mg C kg<sup>-1</sup> soil. This study comparatively assessed the composition of the chemicals in the wood ash used in these experiments in relation to the wood ash used in other studies to evaluate to what extent the results of this study could be extrapolated to other wood ash and soils (Table 1).

Table 1  
Elemental composition of the wood ash used in this study and those from other studies

Parameters	Wood ash <sup>δ</sup>	Delgado-Baquerizo et al. (2013)	Klemedtsson et al. (2010)	Reed et al. (2017)
pH	12	13	-	11
EC*	25	4	-	17
Ca <sup>#</sup>	310	250	209	194
K <sup>#</sup>	125	26	35	79
Si <sup>#</sup>	58	57	43	-
Mg <sup>#</sup>	59	27	24	-
P <sup>#</sup>	49	7	13	17
Al <sup>#</sup>	24	-	19	-
S <sup>#</sup>	16	87	6	6
Fe <sup>#</sup>	17	10	10	-
Na <sup>#</sup>	8	-	5	-
Cl <sup>#</sup>	7	-	-	3
Ti <sup>#</sup>	2	-	1	-
Sr <sup>#</sup>	1	-	-	-
Cr <sup>□</sup>	400	38	-	-
Zn <sup>□</sup>	400	410	-	1369
Cu <sup>□</sup>	200	120	-	197
Rb <sup>□</sup>	200	-	-	-

<sup>δ</sup> The wood ash that was used in this study. \* The unit was dS m<sup>-1</sup>. # The unit was g kg<sup>-1</sup>. & The unit was mg kg<sup>-1</sup>

## Experimental design

The incubation experiment was conducted at 25°C in a dark laboratory, which included four treatments with three replicates: (1) no amendment (Control), (2) amendment with wood ash (W, 12 g kg<sup>-1</sup> soil), (3)

amendment with wheat straw (S, 10 g kg<sup>-1</sup> soil), and (4) amendment with combined wheat straw and wood ash (SW, 10 g straw kg<sup>-1</sup> and 12 g wood ash kg<sup>-1</sup> soil). The soils (250 g dry weight equivalent) were mixed with straw and/or wood and then incubated in 12 plastic jars that were 15 cm high and 9 cm in diameter. Diammonium phosphate and urea were suspended in deionized water before addition as a solution (159 mg N kg<sup>-1</sup> dry soil, which corresponded to 357 kg<sup>-1</sup> ha<sup>-1</sup> in field conditions, and 185 mg P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup> dry soil, which corresponded to 416 kg<sup>-1</sup> ha<sup>-1</sup> in field conditions). The moisture of the soil (or soil-straw and/or wood ash mixture) in the jars was adjusted to 70% of field water holding capacity and maintained throughout the experiment.

## Measurement of soil CO<sub>2</sub> effluxes and soil characteristics

The entire process for the incubation and determination of CO<sub>2</sub> and its calculations was conducted as described by Zhao et al. (2017) and Zhao et al. (2018). Soil CO<sub>2</sub> emissions were determined on days 2, 3, 4, 5, 7, 10, 15, 20, 25, 35, 45, 65, 95, and 118 after incubation. All the samples pass through 2 mm sieve, mixed evenly, and separated into three subsamples at the end of incubation. One portion was stored at 4°C for analysis; one was dried in the open air for soil analysis, and the other was stored at -80°C to extract the DNA extraction and subsequent molecular analyses. The SOC was determined using the wet-oxidation-redox titration method (Lu et al. 2000). We measured the δ<sup>13</sup>C natural abundance of the wood ash, SOC, and wheat straw as described by Zhao et al. (2017) and Zhao et al. (2018). One gram of soil was preconditioned for 12 hours with 10 mL of 1 M HCl to extricate the carbonate. We quantified the amount of straw-derived C (newly formed SOC) using a two end-member mixing model (Balesdent et al. 1987).

$$f_{\text{new}} (\%) = (\delta^{13}\text{C}_{\text{SOC-a}} - \delta^{13}\text{C}_{\text{SOC-b}}) / (\delta^{13}\text{C}_{\text{material}} - \delta^{13}\text{C}_{\text{SOC-b}}) \quad (1)$$

Where  $f_{\text{new}} (\%)$  is the ratio of SOC from wheat straw to total SOC;  $\delta^{13}\text{C}_{\text{SOC-a}}$  is the  $\delta^{13}\text{C}$  of SOC in the amended soils following incubation;  $\delta^{13}\text{C}_{\text{SOC-b}}$  is the  $\delta^{13}\text{C}$  of SOC in non-amended soils before incubation, and the  $\delta^{13}\text{C}_{\text{material}}$  is  $\delta^{13}\text{C}$  of straw and straw plus the wood ash mixture.

$$\text{Newly SOC formed} = f_{\text{new}}(\%) \times \text{Total SOC} \quad (2)$$

The amount of sequestered SOC was calculated as follows (Kirkby et al. 2014):

$$\text{Sequestered SOC (mg kg}^{-1}\text{)} = \text{SOC}_a - \text{SOC}_b \quad (3)$$

Where  $\text{SOC}_a$  and  $\text{SOC}_b$  are the amounts of SOC with straw amendments that were added after the incubation and those without straw before the incubation, respectively.

$$\text{Native SOC mineralization} = \text{Newly SOC formed} - \text{Sequestered SOC}$$

The soil MBC was measured using a chloroform-fumigation as an extractant (Vance et al. 1987). The DOC was extracted from 10 g of moist soil at 25.8°C using a ratio of 1:2.5 soil to water (Jiang et al. 2006). The soil TN was determined as described by Huang et al. (2007). The soil pH and EC were determined in a 1:5 slurry of soil:water (w/w). Mineral nitrogen (Min-N) included ammonium nitrogen (AN) and nitrate nitrogen (NN). The soil KCl-extractable AN and NN were extracted with 2 M of KCl, steam distillation, and titration for analysis. The SIC was determined as described by Bao et al. (2008). The and total carbon of wood ash

and soil organic C were measured using an elemental analyzer (Vario MAX; Elementar, Germany). The activity of catalase was determined by titrating with  $\text{KMnO}_4$  using  $\text{H}_2\text{O}_2$  as the substrate (Guan et al. 1986). The activity of invertase was assayed by titration with sodium thiosulfate as described by Guan et al. (1986). The activity of dehydrogenase (EC 1.1) was estimated by reducing 2,3,5-triphenylterazolium chloride (Casida et al. 1984). The activities of three hydrolytic enzymes,  $\beta$ -glucosidase,  $\beta$ -1,4-xylosidase, and cellobiohydrolase were measured with some buffer modifications using a fluorescent microplate enzyme assay (DeForest et al. 2009).

## **Soil total community DNA extraction and 16S rRNA sequencing**

Samples of the total genomic DNA were extracted using an OMEGA Soil DNA Kit (D5625-01) (Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's instructions and then stored at  $-20^\circ\text{C}$  for additional analysis. The quantity and quality of the extracted DNAs were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively. High-throughput sequencing of the bacterial 16S rRNA genes from the soil samples was performed by the Xuan Chen Biological Technology Co., Ltd. (Shaanxi, China). The V3–V4 region of the bacterial 16S rRNA genes were amplified by PCR using the forward primer 338F (5'-ACTCCTACGGGAGGCAGCA-3') and the reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3').

## **Statistical analysis**

ASV-level alpha diversity indices, such as the Chao1 richness estimator, Shannon diversity index, Observed species, Good's coverage, and the Abundance-Based Coverage estimator (ACE), were calculated using the ASV table in QIIME2 and visualized as box plots. Graphs were prepared using SigmaPlot 12.5 (SYSTAT, San Jose, CA, USA). A correlation analysis was conducted using Hemi. Distance-based redundancy analysis with CANOCO 5.0 was used to analyze the correlations between the physicochemical and biological properties of the soil and bacterial community structure. Variance partition analysis (VPA) was used to quantify the contribution rates of the major bacterial communities to SOC using the "Vegan" program in R 3.5.1 statistical software. All the statistical differences were calculated using an analysis of variance (ANOVA), and the means were segregated by an LSD multiple comparison test at  $P < 0.05$  using SPSS v. 19 (IBM, Inc., Armonk, NY, USA).

## **Results**

### **Soil C mineralization, soil chemical characteristics and enzyme activities**

The cumulative  $\text{CO}_2$ -C emissions (namely all of the  $\text{CO}_2$  measurements) decreased by 11.1% in the treatment with wood ash compared with that of soil alone. Moreover, the cumulative  $\text{CO}_2$ -C emissions decreased by 6.0% in straw plus wood ash treatment relative to the addition of straw alone (Table 2). The SOC between straw alone and straw plus wood ash treatments did not differ significantly. However, the

contents of newly and sequestered SOC increased by 8.8% and 54.7% in the straw plus wood ash relative to straw treatment, respectively (Figure 1). On average, SIC content (removed the SIC in wood ash itself) showed an increase of 218 mg C kg<sup>-1</sup> soil under the wood ash treatments with or without straw relative to those under the control and straw treatment. The DOC and MBC levels for the straw plus wood ash, straw, and wood ash treatments increased by 112%, 66%, and 37%, and 143%, 102%, and 14%, respectively, with significant differences compared with the Control treatment (Figure 1; Table 2).

Table 2  
Effects of wood ash, maize straw residue and their interaction on soil chemical characteristics and enzyme activity after 118 days of incubation

	Control	W	S	SW	S	W	S×W
Cumulative CO <sub>2</sub> -C (g kg <sup>-1</sup> )	0.9±5.13 c	0.8±31.19 d	5.0±7.09 a	4.7±6.08 b	<0.001	<0.001	<0.001
DOC (mg kg <sup>-1</sup> )	50.9±2.8 d	69.9±3.0 c	84.8±5.0 b	107.9±3.7 a	<0.001	<0.001	0.362
Soil moisture	0.246±0.0 b	0.247±0.0 b	0.259±0.0 a	0.258±0.0 a	<0.001	0.922	0.514
Water soluble Ca (mg kg <sup>-1</sup> )	286±5.1 ab	318±31.9 a	282±40.2 ab	249±16.5 b	0.047	0.963	0.070
Water soluble K (mg kg <sup>-1</sup> )	0.3±0.1 b	20.3±0.6 a	23.6±2.4 a	22.5±4.1 a	<0.001	<0.001	<0.001
Water soluble Mg (mg kg <sup>-1</sup> )	21.6±8.2 b	28.9±6.7 ab	27.5±17.0 ab	44.5±13.0 a	0.159	0.116	0.495
EC (us/cm)	180±1.5 b	619±34.9 a	379±12.8 c	608±33.5 a	<0.001	<0.001	<0.001
TN (g kg <sup>-1</sup> )	1.2±0.0 b	1.2±0.0 b	1.3±0.0 a	1.3±0.0 a	<0.001	0.130	0.614
Min-N (mg kg <sup>-1</sup> )	79.8±0.5 a	80.1±0.1 a	61.9±1.3 b	58.6±3.3 c	<0.001	0.037	0.030
Enzyme activity							
β-1,4-xylosidase (nmol h <sup>-1</sup> g <sup>-1</sup> )	7.0±0.1 b	5.8±0.6 b	10.8±2.0 a	9.7±1.6 a	<0.001	0.144	0.953
β-glucosidase (nmol h <sup>-1</sup> g <sup>-1</sup> )	18.3±0.5 b	16.1±1.0 b	23.6±2.1 a	22.4±0.3 a	<0.001	0.040	0.466
Cellobiohydrolase (nmol h <sup>-1</sup> g <sup>-1</sup> )	6.7±0.4 b	4.6±0.4 c	8.8±0.2 a	7.4±0.5 b	<0.001	<0.001	0.120
Invertase (mg g <sup>-1</sup> d <sup>-1</sup> )	52.1±5.2 b	53.5±10.6 b	59.7±3.4 ab	69.1±1.2 a	0.012	0.172	0.298

DOC, dissolved organic carbon; TN, total nitrogen; EC, electrical conductivity; Min-N, mineral nitrogen

	Control	W	S	SW	S	W	S×W
Catalase (mg g <sup>-1</sup> d <sup>-1</sup> )	1.2±0.0 a	0.9±0.1 b	1.1±0.0 a	1.2±0.1 a	0.008	0.017	<0.001
Dehydrogenase (mg kg <sup>-1</sup> h <sup>-1</sup> )	7.2±0.8 c	5.6±0.6 d	16.8±1.3 b	20.7±0.6 a	<0.001	0.042	<0.001
DOC, dissolved organic carbon; TN, total nitrogen; EC, electrical conductivity; Min-N, mineral nitrogen							

The highest water soluble K was found in the soil amended with straw, followed by treatment with wood ash, indicating that the availability of potassium from the wood ash was relatively high and was even equivalent to soil in which mineral K fertilizer was applied. The data showed that the soil pH differed significantly among the treatments. The application of wood ash brought about an average augment of pH from 7.9 in the Control and straw treatments to 8.2 in wood ash and straw plus wood ash treatments (Figure 2). The EC in wood ash, and straw plus wood ash treatments was 33.7% and 30.4% higher than that of the Control, respectively.

The effects of addition of straw and the interaction of application of straw × wood ash on catalase and dehydrogenase activity were significant (Table 2). Only adding wood ash decreased the activities of cellobiohydrolase, catalase and dehydrogenase. However, integrating straw with wood ash maintained them at the original level. Surprisingly, adding wood ash alone increased the activity of invertase, and integrating straw with wood ash further enhanced their activities.

## Species richness and diversity of bacteria

The Ace and Chao1 indices did not identify any significant differences among the treatments, and the Shannon index of the wood ash, straw and straw plus wood ash treatments were higher than those of the Control (Figure 3). In contrast, the Shannon index significantly positively correlated with the DOC ( $r = 0.67$ ,  $P < 0.05$ ), MBC ( $r = 0.59$ ,  $P < 0.05$ ), TN ( $r = 0.60$ ,  $P < 0.05$ ), pH ( $r = 0.80$ ,  $P < 0.01$ ), and EC ( $r = 0.82$ ,  $P < 0.01$ ) (Figure 4), suggesting that the diversity of soil bacteria was affected by soil labile carbon and pH among the addition of exogenous substances.

## Bacterial community structure

The Illumina platform analysis was adopted to clarify the bacterial community structure and composition in soil. *Proteobacteria*, *Chloroflexi* and *Gemmatimonadetes* were the three dominant phyla, and the abundances of phyla of *Acidobacteriota*, *Actinobacteriota*, and *Bacteroidetes* differed significantly between the wood ash, straw plus wood ash and straw alone treatments, thus indicating that the change of soil pH changed the relative abundance of bacteria (Fig. 5).

Distance-based redundancy analysis (db-RDA; Fig. 6a) and the Pearson's correlation coefficient (Supplementary Fig. S1) were performed to examine the relationship between chemical characteristics, enzyme activities, and bacterial community composition. The bacterial communities shifted with addition

of straw and wood ash along RDA1 and RDA2, respectively. The study also showed that the six soil samples treated by wood ash and straw plus wood ash were closely related and differed significantly from the soil samples treated with straw alone and those from the Control (Fig. 6a). Environmental factors significantly correlated with the composition of bacterial community. These factors explained 85.5% of the bacterial community variation (Fig. 6a). Among all the environmental variables examined, the water soluble K ( $F = 9.3$ ,  $P = 0.002$ ), soil EC ( $F = 5.4$ ,  $P = 0.008$ ), pH ( $F = 4.6$ ,  $P = 0.01$ ), and soil moisture ( $F = 3.8$ ,  $P = 0.016$ ) explained 48.1%, 35.3%, 31.4% and 27.7% of the variation of communities, respectively, indicating that these variables have an important role in altering the bacterial communities (Fig. 6a). The correlations between the bacterial communities and enzyme activity, newly formed SOC and sequestered SOC showed that treatment with straw plus wood ash is conducive to the sequestration of SOC and production of newly formed SOC (Fig. 6a).

A VPA analysis indicated that the relative abundances of Acidobacteria, Actinobacteria and Bacteroidetes contributed 14 %, 10% and 5% to the variation in the content of SOC, respectively (Fig. 6b). The total rate of contribution of each variable and its interaction to the variation in content of SOC was 54%.

Furthermore, the relationship between the relative abundance (>1%) of the soil characteristics and phyla is shown in Supplementary Fig. S1. The sub-phylum *Alphaproteobacteria* negatively correlated with the contents of DOC, MBC content, pH and EC. The *Gemmatimonadetes* had a significantly negative relationship with the cumulative CO<sub>2</sub>-C ( $P < 0.05$ ),  $\beta$ -1,4-xylosidase ( $r = 0.81$ ,  $P < 0.01$ ),  $\beta$ -glucosidase ( $r = 0.84$ ,  $P < 0.01$ ), and cellobiohydrolase ( $r = 0.68$ ,  $P < 0.05$ ). In addition, the *Acidobacteriota* had a clear opposite trend of a positive correlation. Furthermore, *Actinobacteriota*, *Bacteroidetes*, and *Myxococcota* positively correlated with the soil pH and EC to a remarkable extent (Supplementary Fig. S1).

## Discussion

Effects of the application of wood ash and straw on the mineralization of soil C, soil chemical properties, and enzyme activities

Previous studies that examined the effects of wood ash on the emissions of CO<sub>2</sub> have primarily concentrated on forest soils, and the results are contradictory. In some studies, long-term data on the effects of wood ash on the CO<sub>2</sub> emissions of forest peatland increased owing to an increase in the pH and nutrient contents of the soil (Moilanen et al. 2012). In contrast, Klemetsson et al. (2010) found that amendment with wood ash could be an appropriate mitigation measure for CO<sub>2</sub> emissions from a spruce forest. This study found that the application of wood ash significantly decreased the total emissions of soil CO<sub>2</sub> in the order of straw > straw plus wood ash > Control > wood ash (Table 2). The decrease in CO<sub>2</sub> emissions in straw plus wood ash treatment was much higher than the amount of SIC generated, which could be partly owing to the formation of SIC and partly owing to the conversion of more straw to newly formed SOC (e.g., the labile organic C fraction). All of those results indicated that the addition of wood ash did reduce CO<sub>2</sub> emissions in acidic and alkaline soils. In addition, the data showed that the addition of wood ash promoted the formation of new organic carbon and the retention of net organic carbon, but did

not affect the mineralization of native SOC. This result suggests that when wood ash was present, the increase in the content of SOC might be owing to the progressive breakdown and transformation of straw by the enhanced enzyme activities. However, this result was inconsistent with that of Reed et al. (2017), who noted that wood ash brought about a lasting adverse impact on SOM turnover owing to the CEC and the ability of the specific surface area of wood ash to chemically stabilize the pH of SOM.

The addition of straw and wood ash obviously enhanced the contents of MBC and DOC relative to treatment with the addition of straw (Table 2; Fig. 2), which may be directly affected by the enhanced enzyme activity and a change in the pH of soil. The application of wood ash apparently increased the pH, which was influenced largely by the dissolution of several oxides, hydroxides, carbonates, and bicarbonates contained in wood ash (Vassilev et al. 2013). Consistent with the change in pH, the increase in soil EC after wood ash was added was owing to the dissolution of wood ash during cultivation that contained high amounts of alkaline ions (Table 2). The addition of straw and wood ash had an effect on mineral nitrogen with the order: straw plus wood ash < straw < wood ash  $\approx$  Control (Table 2). This indicated the combination increased the immobilization of mineral nitrogen, which may further promote the formation of soil organic nitrogen, improving the inorganic nitrogen sequestration, reducing the leaching loss of nitrogen.

The fact that catalase and dehydrogenase were only reduced by wood ash could be attributed to at least two reasons. First, the wood ash adsorbed the substrate used by catalase and dehydrogenase and inhibited the progress of enzyme reaction. Second, the high pH of the wood ash itself could also produce changes in the microbial community, resulting in a decrease in the secretion of catalase and dehydrogenase. The promotion of enzyme activity by straw plus wood ash was owing to the high pH caused by added wood ash to promote the hydrolysis of straw to produce more catalase and dehydrogenase reaction matrix (Gömöryová et al. 2016). Interestingly, the invertase activity was enhanced under wood ash compared with the Control. This was probably caused by the increase in pH owing to wood ash, which aided in the release of more sucrose from the straw or soil (Table 2).

## **Effects of the application of wood ash and straw on soil bacterial diversity**

There were no differences of bacterial species richness among the treatments. Toke et al. (2017) recently found that the EC and pH had an obviously adverse impact on bacterial diversity, while our results are contrary to theirs, we found that the labile carbon, pH and EC have an important role in the increase in bacterial diversity based on Pearson's correlation coefficients (Fig. 4). Indeed, the decomposition of C provides energy for most soil microorganisms, and recent studies found that soil bacterial diversity is driven by soil C storage (Delgado-Baquerizo et al. 2013; Maestre et al. 2013). However, the long-term experiment combining straw with wood ash used in the field merits further study.

## **Effects of the application of wood ash and straw on the soil bacterial community structure**

Figure 5 shows that some specific bacterial groups obviously varied among different treatments. Soil pH is a primary factor regulating microbial community structure. High pH values were favourable to *Bacteroidetes* and *Actinobacteriota*, while low pH values could be better suited for *Acidobacteriota* (Lauber et al. 2009). *Bacteroidota* and *Actinobacteriota* increased in relative abundance following the straw plus wood ash and wood ash alone relative to the straw alone (Fig. 5). Noyce et al. (2016) indicated that applying wood ash could increase the abundance of *Bacteroidota*. The possible increase in DOC and the more neutral pH at applications of 22 t ha<sup>-1</sup> of ash improves the conditions for the growth of the copiotrophic *Bacteroidota*. *Acidobacteriota* was enhanced by the straw alone and is a heterotroph that can utilize extensive sources of carbon (Ward et al. 2009), thus, displaying a crucial role in the carbon cycle. This result was comparable to those of Rousk et al. (2010), who found that *Acidobacteriota* predominate in conditions of relatively lower pH values. The Control treatment in concert with the lack of effects of the application of degradable C can help to explain the lower bacterial numbers and the increased abundance of *Firmicutes* in this treatment that lacked amendments. The dominant phylum *Proteobacteria* maintains a high relative abundance (32.4–36.5%) throughout all the treatments (Fig. 5), which might be explained by the general resistance of *Proteobacteria* to environmental changes (Barnard et al. 2013). This study found that the highest percentage of *Alphaproteobacteria* was identified in the treatment that lacked amendments (lower pH), which is consistent with those of Ding et al. (2016), who found that the populations of *Alphaproteobacteria* negatively correlated with the soil pH. In addition, soil moisture is a critical factor in governing soil community composition. In this study, the relative abundance of *Myxococcota* was significantly lower in the Control treatment compared with those of the other three treatments, which had a strongly positively correlated with the soil moisture.

Previous studies indicated that changes that wood ash caused in the EC and pH were important determinants of the changes observed in the community composition of bacteria (Toke et al. 2017; Fierer et al. 2006; Fierer et al. 2007). This study found that soil properties, such as water soluble K, pH, EC, and soil moisture, obviously contributed to the variation in bacterial community structure (Fig. 6a). The relationship among the enzymatic activity, SOC, and bacterial community composition was studied. For example, the newly formed and sequestered SOC were obviously bound up with dehydrogenase, invertase and hydrolase activities based on the Distance-based redundancy analysis (Fig. 6b).

## Conclusions

This study showed that the addition of wood ash to agricultural soil can result in obvious variations to its chemical and microbial characteristics. Treatments with wood ash (straw plus wood ash and wood ash) increased the content of SIC relative to other treatments. Interestingly, the amounts of newly formed and sequestered SOC in the straw plus wood ash treatment were greater than those in the straw treatment. Our results confirmed that the addition of exogenous substances enhanced the bacterial diversity compared with that of the Control. Furthermore, a redundancy analysis showed that the EC and water soluble K are the most influential factors that determine the structure of soil bacterial communities. In conclusion, integrating straw with wood ash had the same effect on bacterial diversity relative to the addition of straw alone. However, the former might be conducive to SOC and SIC sequestration.

## Declarations

## Acknowledgment

This work was supported by the Natural Science Foundation of Shandong Province, Grant/Award (ZR2020QD115, ZR2019JQ12, ZR2018PC004), the National Natural Sciences Foundation of China (no. 32101262&41977039), the Taishan Scholars Program of Shandong Province (no.tsqn201812097). We thank the Xuan Chen Biological Technology Co., Ltd. (Shaanxi, China) for their help with the analysis of data.

## References

1. Balesdent J, Mariotti A, Guillet B (1987) Natural  $^{13}\text{C}$  abundance as a tracer for studies of soil organic matter dynamics. *Soil Biol Biochem* 19:25–30
2. Bao SD (2008) *Soil Agrochemistry Analysis* Chinese Agriculture Press. (in Chinese)
3. Barnard RL, Osborne CA, Firestone MK (2013) Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. *ISME J* 7:2229–2241
4. Bhat AK (2013) Preserving microbial diversity of soil ecosystem: a key to sustainable productivity. *International Journal of Current Microbiology and Applied Sciences* 2:85–101
5. Casida LE, Kklein DA, Santoro T (1964) Soil dehydrogenase activity. *Soil Sci* 98:371–376
6. DeForest J (2009) The influence of time. storage temperature. and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and L-DOPA. *Soil Biol Biochem* 41:1180–1186
7. Delgado-Baquerizo M, Maestre FT, Gallardo, Bowker MA, Wallenstein MD, Quero JL, Ochoa V, Gozalo B, García-Gomez M, Soliveres S, Garcia-Palacios P (2013) Decoupling of soil nutrient cycles as a function of aridity in global drylands. *Nature* 502:672–676
8. Ding JL, Jiang X, Ma MC, Zhou BK, Guan DW, Zhao BS, Zhou J, Cao FM, Li L, Li J (2016) Effect of 35 years inorganic fertilizer and manure amendment on structure of bacterial and archaeal communities in black soil of northeast china. *Appl Soil Ecol* 105:187–195
9. Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. *Ecology* 88:1354–1364
10. Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. *proceedings of the national academy of sciences of the united states of America* 103: 626–631
11. Gömöryová E, Pichler V, Tóthová S, Gömöry D (2016) Changes of chemical and biological properties of distinct forest floor layers after wood ash application in a norway spruce stand. *Forests* 7:108
12. Gong ZT, Zhang GL, Chen ZC (2007) *Pedogenesis and soil taxonomy*. Science Press Publishing. (in Chinese)
13. Guan S (1986) *Soil Enzymes and Its Methodology*. Agricultural Press, Beijing pp, pp 274–340

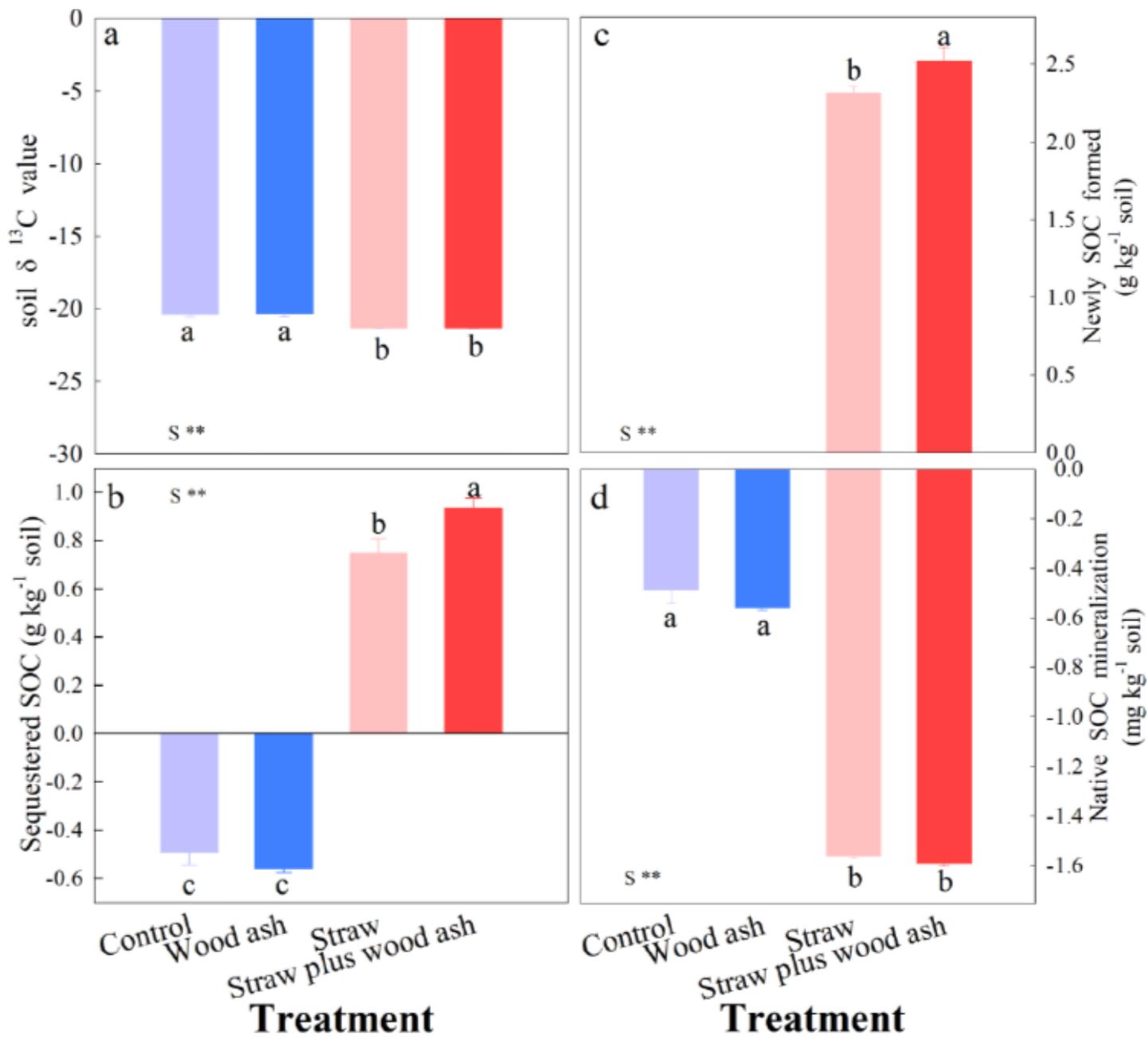
14. Huang B, Sun W, Zhao Y, Zhu J, Yang R, Zou Z, Ding F, Su J (2007) Temporal and spatial variability of soil organic matter and total nitrogen in an agricultural ecosystem as affected by farming practices. *Geoderma* 139:336–345
15. Jiang PK, Xu QF, Xu ZH, Cao ZH (2006) Seasonal changes in soil labile organic carbon pools within a *Phyllostachys praecox* stand under high rate fertilization and winter mulch in subtropical China. *Forest Ecol Manag* 236:30–36
16. Juárez MFD, Waldhuber S, Knapp A, Partl C, Gómez-Brandón M, Insam H (2013) Wood Ash Effects on Chemical and Microbiological Properties of Digestate- and Manure-Amended Soils. *Biol Fert Soils* 49:575–585
17. Kirkby CA, Richardson AE, Wade LJ, Passioura JB, Batten GD, Blanchard C, Kirkegaard JA (2014) Nutrient Availability Limits Carbon Sequestration in Arable Soils. *Soil Biol Biochem* 68:402–409
18. Klemedtsson L, Ernfors M, Björk RG, Weslien P, Rütting T, Crill P, Sikström U (2010) Reduction of Greenhouse Gas Emissions by Wood Ash Application to a *Picea Abies* (L.) Karst. Forest on a Drained Organic Soil. *Eur J Soil Biol* 61:734–744
19. Lauber CL, Hamady M, Knight R, Fierer N (2009) Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl Environ Microb* 75:5111–5120
20. Lei N, Han JC, Mu XM, Sun ZH, Wang HY (2019) Effects of improved materials on reclamation of soil properties and crop yield in hollow villages in china. *J Soil Sediment* 19:2374–2380
21. Li YY, Feng G, Tewolde H, Zhang FB, Yan C, Yang MY (2021) Soil aggregation and water holding capacity of soil amended with agro-industrial byproducts and poultry litter. *J Soil Sediment* 21:1127–1135
22. Liang XM, Chen Q, Rana MS, Dong ZH, Liu XD, Hu CX, Tan QL, Zhao XH, Sun XC, Wu SW (2021) Effects of soil amendments on soil fertility and fruit yield through alterations in soil carbon fractions. *J Soil Sediment* 21:2628–2638
23. Lu RK (2000) *Methods for Soil Agrochemistry Analysis*. China Agricultural Science and Technology Press, Beijing pp, pp 106–310
24. Maestre FT, Escolar C, De Guevara ML, Quero JL, Lázaro R, Delgado-Baquerizo M, Ochoa V, Berdugo M, Gozalo B, Gallardo A (2013) Changes in biocrust cover drive carbon cycle responses to climate change in drylands. *Global Change Biol* 19:3835–3847
25. Moilanen M, Hytönen J, Leppälä M (2012) Application of wood ash accelerates soil respiration and tree growth on drained peatland. *Eur J Soil Sci* 63:467–475
26. Noyce GL, Fulthorpe R, Gorgolewski A, Hazlett P, Tran H, Basiliko N (2016) Soil microbial responses to wood ash addition and forest fire in managed Ontario forests. *Appl Soil Ecol* 107:368–380
27. Peltoniemi K, Pyrhönen M, Laiho R, Moilanen M, Fritze H (2016) Microbial communities after wood ash fertilization in a boreal drained peatland forest. *Eur J Soil Biol* 76:95–102
28. Reed EY, Chadwick DR, Hill PW, Jone DL (2017) Critical Comparison of the Impact of Biochar and Wood Ash on Soil Organic Matter Cycling and Grassland Productivity. *Soil Biol Biochem* 110:134–142

29. Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Knight R, Fierer N (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J* 4:1340–1351
30. Talukdar P, Baruah M, Saikia P (2018) Waste management for sustainable agricultural production - A brief review. *Asian Journal of Home Science* 13:657–659
31. Bang-Andreasen T, Nielsen JT, Voriskova J, Heise J, Ronn R, Kjoller R, Hansen HCB, Jacobsen CS (2017) Wood ash induced pH changes strongly affect soil bacterial numbers and community composition. *Front Microbiol* 8:1400
32. Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass-C. *Soil Biol Biochem* 19:703–707
33. Vassilev SV, Baxter D, Andersen LK, Vassileva CG (2013) An overview of the composition and application of biomass ash. Part 1. Phase-mineral and chemical composition and classification. *Fuel* 105:40–76
34. Ward NL, Challacombe JF, Janssen PH, Henrissat B, Coutinho PM, Wu M, Xie G, Haft DH, Sait M, Badger J, Barabote RD, Bradley B, Brettin TS, Brinkac LM, Bruce D, Creasy T, Daugherty SC, Davidsen TM, DeBoy RT, Detter JC, Dodson RJ, Durkin AS, Ganapathy A, Gwinn-Giglio M, Han CS, Khouri H, Kiss H, Kothari SP, Madupu R, Nelson KE, Nelson WC, Paulsen I, Penn K, Ren Q, Rosovitz MJ, Selengut JD, Shrivastava S, Sullivan SA, Tapia R, Thompson LS, Watkins KL, Yang Q, Yu C, Zafar N, Zhou L, Kuske CR (2009) Three genomes from the phylum acidobacteria provide insight into the lifestyles of these microorganisms in soils. *Appl Environ Microb* 75:2046–2056
35. Zhao HL, Tian XH, Chen YL, Dong JJ, Shi JL (2017) Effect of exogenous substances on soil organic and inorganic carbon sequestration under maize stover addition. *Soil Sci Plant Nutr* 63:591–598
36. Zhao HL, Zhang HJ, Shar AG, Liu JF, Chen YL, Chu SJ, Tian XH (2018) Enhancing Organic and Inorganic Carbon Sequestration in Calcareous Soil by the Combination of Wheat Straw and Wood Ash and/or Lime. *PLoS ONE* 13:e0205361

## Supplemental Data

Supplementary Figure 1 is not available with this version.

## Figures



**Figure 1**

Effects of wood ash and straw treatment s(addition or no addition) on the value of soil  $\delta^{13}\text{C}$  (a), on the net change in SOC sequestration (b), on newly SOC formed (c) and native SOC mineralization (d) at the end of incubation

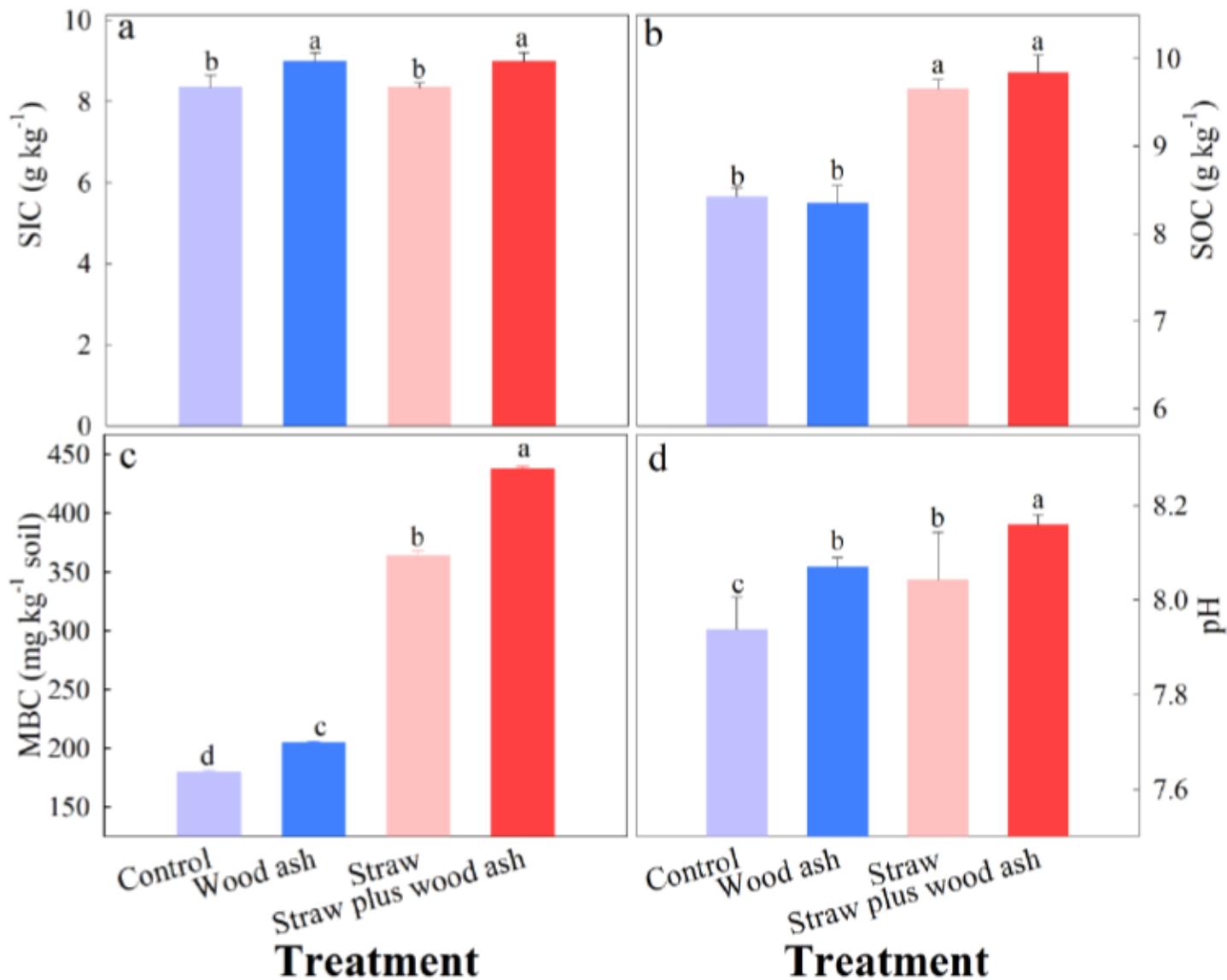
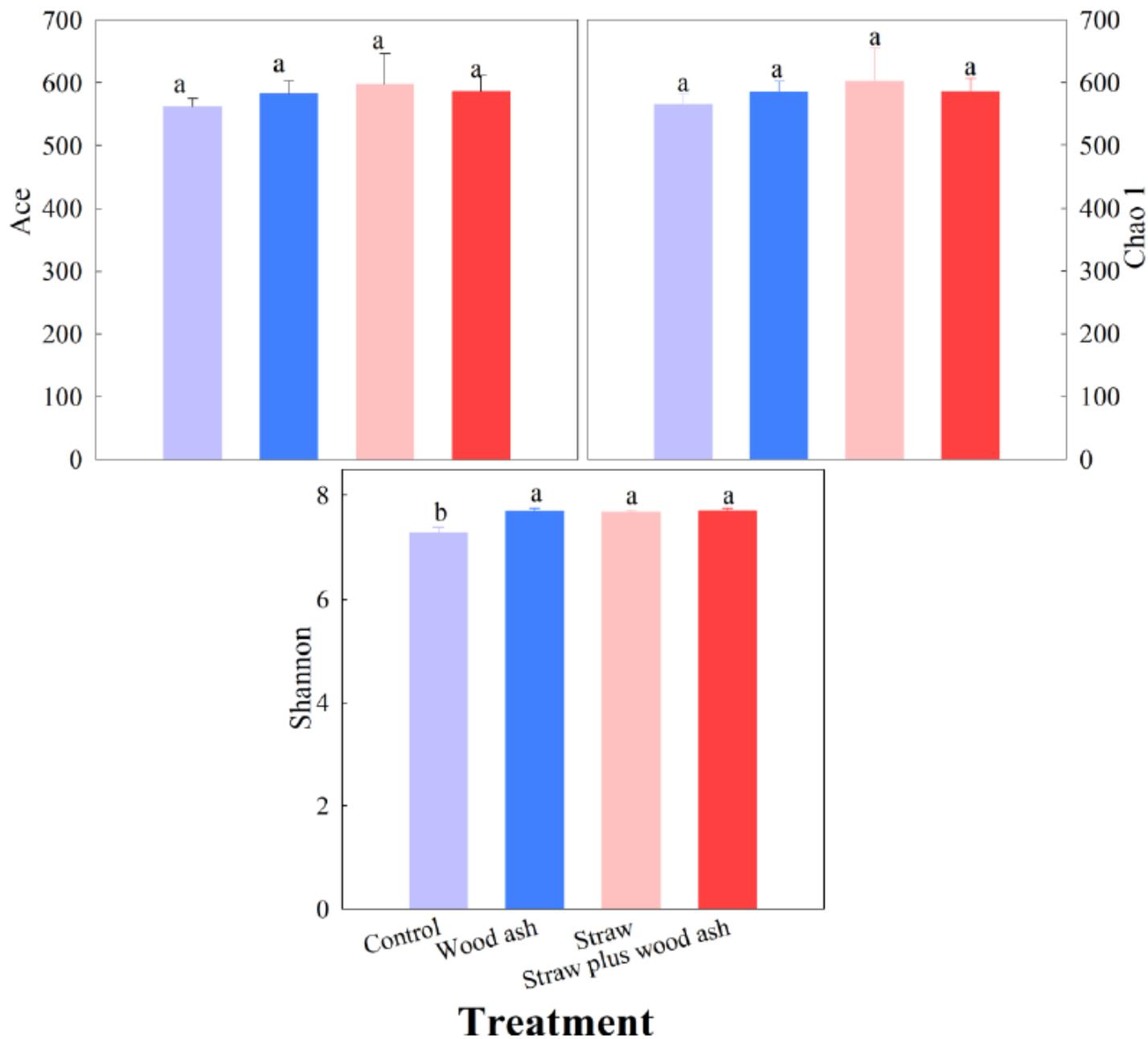


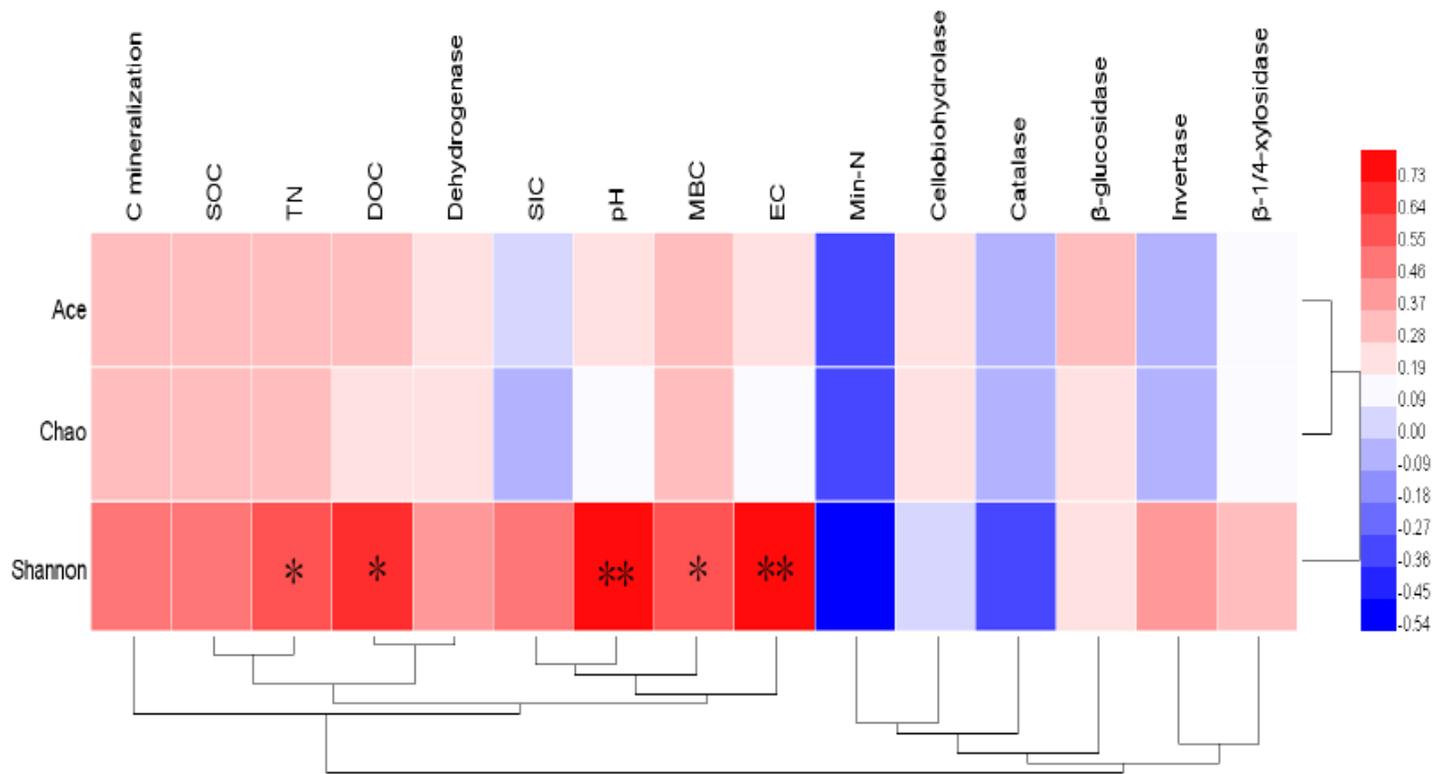
Figure 2

Effects of wood ash and maize straw residue and their interaction on soil chemical characteristics after 118 days of incubation. SOC, the content for soil with any partially degraded straw removed; SIC, soil inorganic carbon; MBC, microbial biomass carbon



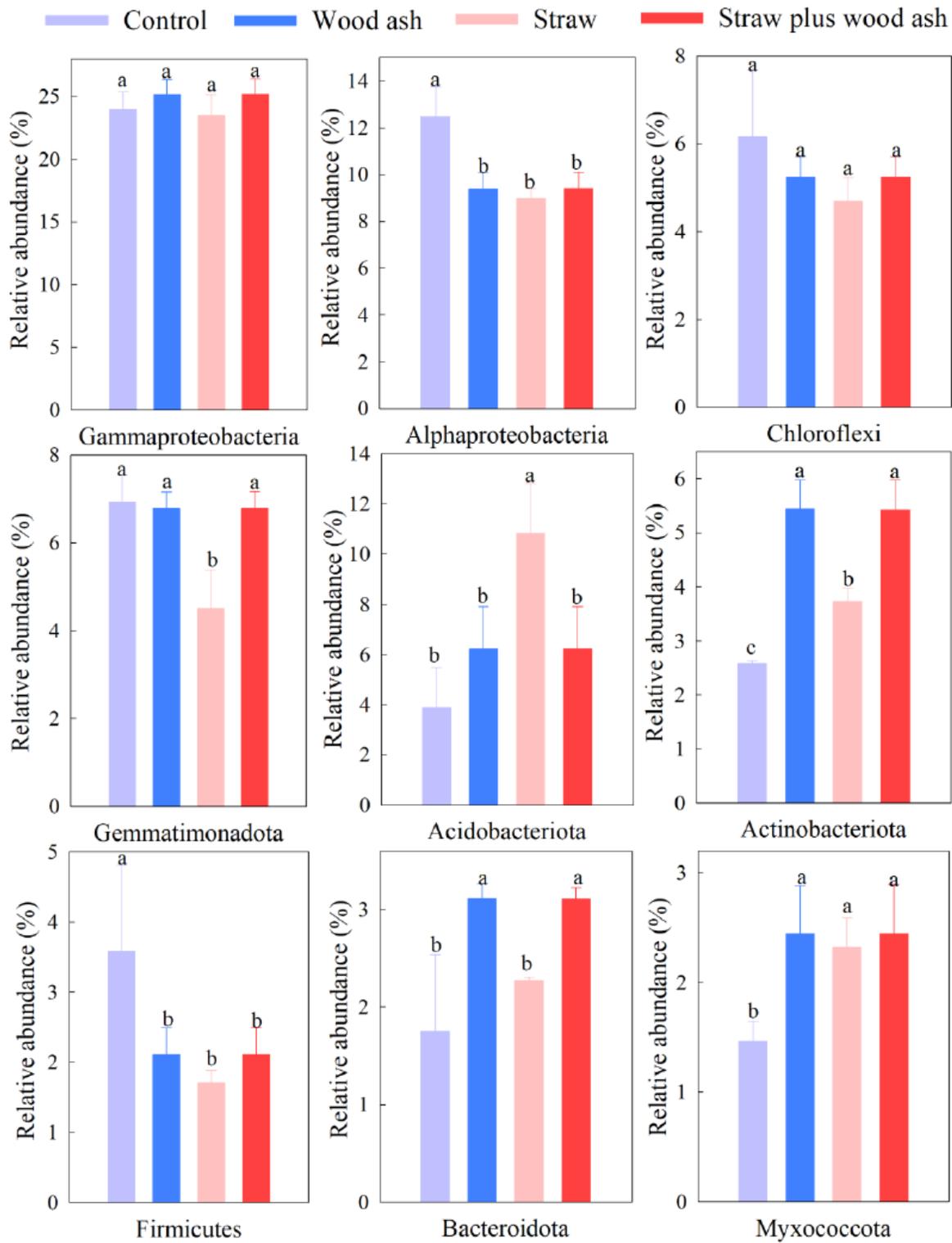
**Figure 3**

The richness of soil bacterial community and the diversity of soil for each treatment after incubation for 118 d



**Figure 4**

Pearson's correlation coefficients between alpha diversity and the properties of soil. \* $P < 0.05$ ; \*\* $P < 0.01$



**Figure 5**

The relative abundance of levels of different phyla in this study

