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Original Research

Short-Term Straw Returning Improves Quality and Bacteria Community of Black Soil in Northeast China

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Abstract

Despite our extensive knowledge on the effects of long-term straw returning on soil, little is known about the effects of short-term straw returning. We identified three study sites and evaluated their soil organic matter (OM), pH, and enzyme activity after two years of straw returning treatment. Bacterial diversity and community were determined by 16s RNA sequencing. We observed elevated OM content and pH after short-term straw returning treatment. The bacterial phyla *Proteobacteria* and *Bacteroidetes* exhibited similar dynamics on straw application. We observed reduced levels of *Actinobacteria* and *Chloroflexi* bacterial phyla after straw returning. Reduced *Actinobacteria* and *Chloroflexi* may be due to competition from dominant bacteria. In general, OM content and enzyme activity had the same trend that closely correlated with the amount and community of microorganisms in the soil. *Proteobacteria* and *Bacteroidetes* were the critical phyla in straw degradation and might improve soil OM content. *Proteobacteria* and *Actinobacteria* were identified as copiotrophic taxa. In summary, straw returning treatment might maintain soil stability and bacterial diversity better. The bacterial phyla *Proteobacteria* and *Bacteroidetes* were dominant over other microbial fractions during straw decomposition. The survival competition might be one of the main reasons for the decrease of *Actinobacteria* and *Chloroflexi*. Short-term straw returning to the field can markedly improve soil quality. However, soils in different locations respond differently to straw return practices and their responses are influenced by the soil's basic parameters and climate.

Keywords: crop straw returning, soil bacteria diversity, bacteria community, soil organic matter

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Introduction

Dubbed the "giant panda of cultivated land", the black soil in Northeast China is one of four major chernozem regions around the world. Covering an area of 1.09 million square kilometers, it is also one of the most fertile regions in the country. Black soil is recognized as the most fertile soil in the world. It takes 200 to 400 years to form a 1-centimeter-thick layer of black soil under natural conditions, according to a white paper released by the Chinese Academy of Sciences last month [1]. However, irrational cultivation and tillage, as well as climate change, have led to the degradation of black soil in Northeast China [2]. And in recent years, this situation has become more and more serious. In order to protect the black soil in Northeast China, a variety of measures have been actively applied to reduce the loss of black soil, among which straw returning to the field is one of the reliable ways.

Crop straw returning to the field may effectively improve soil fertility, soil carbon sequestration and sustain soil productivity [3, 4]. Once returned to the field, straw gradually decomposes into organic matter (OM), which greatly influences soil function and quality. Straw returning is reported to significantly increase soil OM content relative to the application of chemical fertilizers only [5]. High soil OM content can increase soil nutrients supply [6, 7], improve soil physical and biological properties [8], and enhance soil buffering capacity [9]. There is a close correlation between soil microorganisms and soil OM. Soil microorganisms are the main drivers of carbon dynamics and nutrient turnover during biogeochemical cycling [10, 11]. As the main decomposers in soil, bacteria are dominant players in the initial phases of straw decomposition, although some fungi, which decompose more recalcitrant materials dominate later stages [12-15]. Additionally, soil microorganisms are important in soil aggregation and soil structure formation [16]. Thus, microorganisms are regarded as architects of surrounding soil environments [17]. A wide body of evidence shows that soil microorganisms are crucial for soil fertility. Straw degradation products can influence soil microorganisms by modulating stability and diversity of soil microbial structure [18]. This can promote the development of an excellent virtuous circle of soil microorganism-soil OM-straw degradation. Northeast China is an extensive region that generates a lot of crop straw every year. Straw returning may not only save manpower, but also improve soil OM. While multiple studies have shown the long-term effects of straw returning on soil, few have examined the effects of short-term straw returning.

In this study, straw was returned to fields where it had not been previously returned. The study was carried out at 3 sites in Northeast China, located in Songyuan, Dunhua and Jiutai, which are typical black soil regions. We hypothesized that short term straw returning and deep ploughing may improve soil quality. However, whether responses from the different regions are consistent merits further study. Importantly, using high-throughput sequencing technology, we comprehensively examined the effects of short-term straw returning on soil bacteria communities. Our data offer novel insights on short-term straw returning practices.

Experimental

Description of Study Sites and Soil Sampling Strategy

This study was done at 3 locations in Jilin Province, China: Songyuan city (45°17′N, 124°80′E, marked as A), Dunhua city (43°25′N, 128°21′E, marked as B), and Jiutai city (44°8′N, 126º49′E, marked as C). Although these locations belong to different ecological areas, their

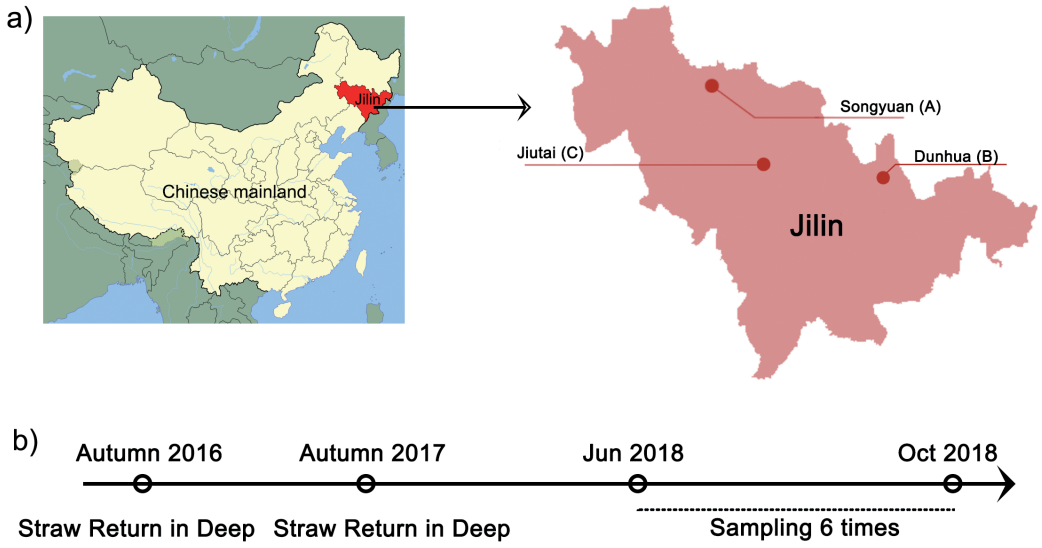

Fig. 1. Location and design. A shows the sampling location, B shows the straw returning treatment and sampling time.

soil types are typical Northeast China black soil. The 3 locations have been treated with inorganic fertilizer for >20 years and straw had not been previously returned to the fields. The study used a randomized complete block design. With or without straw returning were considered random effects. There were 6 blocks at each location, 3 controls and 3 experiment groups. A two years short-term deep tillage with straw returning was done in autumn of 2016 and 2017 (Fig. 1). Maize straw was crushed to 5cm sizes after harvest and evenly sprinkled in the field. In spring of the next year, the straw was ploughed into the soil using a fence hydraulic turning plow (1LYFT-450, Longfeng, China) at a ploughing depth of 35-40cm. Soil samples from fields in Songyuan, Dunhua and Jiutai that underwent short-term straw returning were marked A, B, and C, respectively. Soil samples from fields in Songyuan, Dunhua and Jiutai without short-term straw returning were marked A-CK, B-CK, and C-CK, respectively. All soil samples were collected from June 2018 to October 2018. Sampling was done every 25 days, 6 times in total (Jun. 26, jointing stage; Jul. 21, trumpet stage; Aug. 15, spinning stage; Sep. 9, grouting period; Oct. 4, milk-ripening stage; Oct. 29, full-ripening stage). At each sampling time, 30 samples from each treatment, at each location were collected using the chessboard method and 3 samples randomly selected for 16S sequencing. In total, 108 samples were collected [36 samples for A, B and C, 6 samples (3 treatment and 3 CK) per time per location, 6 times in total]. The other samples were used to analyze soil enzyme activity and organic matter content. All samples were stored at -80ºC until use.

Soil Properties and Soil Enzymes Activity Analysis

Soil enzyme activity is an important index of its biological activity and fertility. To examine this important indicator, we analyzed the soil for the enzymatic activities of invertase, urease, and alkaline phosphatase as described by Yang [19] and Geisseler [20]. Soil organic matter (OM) content is a major parameter in soils and agriculture in general. OM content under various fertilization strategies was determined using the potassium dichromate method as described before [21]. Total nitrogen (TN) was determined by Kjeldahl method; Total phosphorus (TP) was determined using sulfuric acid-perchloric acid digestion method; Total potassium (TK) was determined using the sodium bicarbonate extraction-molybdenum-antimony anti-spectrophotometric method; Additionally, soil water content (SWC, %) at each location and sampling time were measured using the oven-drying method. First, wet soil (W1) was weighed in an aluminum box using an electronic balance and the soil dried for 12 h at 105ºC, until constant weight was reached. Next, the dry soil was weighed in the aluminum box (W2) using an electronic balance. Finally, the aluminum box (W3) was weighed using electronic balance. SWC was calculated using the equation: SWC% = (W1-W2)/(W2-W3). Soil pH was determined using a pH tester (Takemura Electric Works Ltd.).

Isolation of Total Microbial DNA

Soil microbial genomic DNA was extracted using a MOBIO PowerSoil DNA Isolation Kit (Qiagen) following manufacturer instructions. Agarose gel electrophoresis was used to assess DNA integrity. DNA was quantified using Qubit2.0 DNA Assay Kit (Sangon Biotech Co., Ltd). The primers 341f (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC) were used to amplify the V3-V4 region of the 16S rRNA gene [22]. PCR amplification was done using an Eppendorf mastercycler in a 50 µL reaction volume comprised of 10ng genomic DNA, 0.5 µL dNTP (10mM each), 0.5 µL of each PCR primer (50 µM), and 0.5 µL Taq (5 U/µL). Cycling conditions were as follows: initial denaturation for 10 min at 95ºC, followed by 30 cycles at 95ºC for 15 s, annealing at 60ºC for 15 s and extension at 72ºC for 30 s and final extension at 72ºC for 5 min. A gel extraction kit (Axygen) was used to recover desired DNA fragments after electrophoresis. DNA concentration after gel extraction was determined using Qubit2.0 (Life Tech) and DNA quality determined using an Agilent 2100 Bioanalyzer (Agilent). Quantitative PCR (qPCR) was used to test the efficiency of adapters. Based on the efficiency, clone libraries were diluted to a concentration of 1ng/µl for sequencing. Hiseq 2500 (Illumina) was used for pair-end (PE) 250bp sequencing.

Sequence Data Analysis

Sequencing data was separated by barcode annotation and PCR primer sequences, which were then depleted. Data splicing and quality filtering were done using the FLASH (v1.2.7), Qiime (v1.9.1) and UCHIME algorithms (v4.1), respectively. Operational taxonomic units (OTUs) clustering was done using Uparse (version 7.0.1001; http://drive5.com/uparse/) based on 97% identity threshold. OTU abundance (reads number) in each sample was calculated and OTUs with >2 reads used for further analysis. Next, alpha diversity indicators (Chao1, ACE, observed OTUs, Shannon and Simpson) of the sequencing data within each group (n = 3) and beta diversity index (Unweighted UniFrac distance) for each sample were calculated. Principal Co-ordinates Analysis (PCoA) of the samples was done using Unweighted UniFrac distance of beta diversity index. SILVA rRNA database (http://www.arb-silva.de/) on Mothur website (http://www.mothur.org/wiki/RDP_reference_files) was queried for OTU annotations. OTUs relative abundances (phylum ~ species level) was calculated and taxonomy assignment (phylum ~ species level) done using the Ribosomal Database Project (RDP) classifier (80% confidence). A linear model with redundancy analysis (RDA) was used to assess

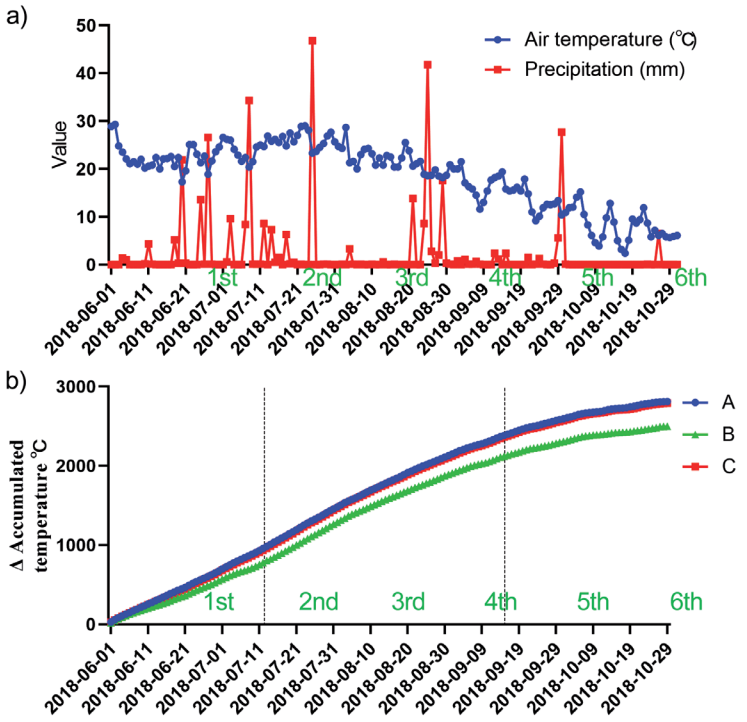

Fig. 2. Temperature and precipitation. a) show the air temperature and precipitation in different months. b) show the accumulated temperature from June 1 to September 30. The accumulated temperature before June 1st defaults to 0. The green arrow indicates the sampling time.

the relationships between environmental factors and species abundance as previously described [23].

Results

Soil Characteristics After Short-Term Straw Returning Treatment

The temperature and precipitation analysis during the experiment revealed gradual temperature increase from June to July, and gradually decrease from August to October. Precipitation was highest in June-August. Temperature gradually plateaued in late September (Fig. 2 a-b).

The short-term straw returning treatment significantly affected soil OM content. As expected, OM content increased after short-term straw returning at all experiment locations and sampling times (Fig. 3 a-c), with OM content peaking at 3rd sampling at A, B, and C. The difference between the control and the treatment group was highest at 3rd sampling at locations A and C, and at 4th sampling at location B (Fig. 3 a-c). Analysis of pH revealed that soil was weakly alkaline at location A and weakly acidic at location B and C. However, soil pH at the 3 locations declined at varying degrees after short-term straw returning (Fig. 3 d-f). Additionally, short-term straw returning significantly increased SWC at location A, B and C at 2nd-6th sampling, indicating the benefit of short-term straw returning (Fig. 3 g-i).

Soil enzyme activity is an important index in the evaluation of soil fertility. Our results show that straw returning enhanced the activity of these four soil enzymes (S-AI, S-AKP, CAT and UE) to varying degrees at study locations (Fig. 4 a-c). Additionally, enzyme activity peaked at the 3rd or 4th sampling time.

Sequence Data Summary

A total of 11,959,033 sequence reads were obtained from the 108 soil samples. After trimming sequence adapters and filtering out low-quality reads, 11,667,501 tags (an average of 108,032 tags per sample) remained (Table S1). Rarefaction curves (Fig. S1a) and species accumulation curves (Fig. S1b) showed that increased OTUs tended to be flat, with increasing sequencing read number and soil samples, respectively. Indicating that the amount of data and sequenced samples were sufficient. Additionally, the PCA and phylogenetic analysis results showed that all the samples in the same group were clustered together, revealing the excellent repeatability in this study (Fig. S2). All the basic statistical results indicated the excellent sequence data quality and consistency of the repetitions.

Effects of Short-Term Straw Returning on Bacterial Diversity

Short-term straw returning significantly influenced soil bacteria diversity, but different regions showed

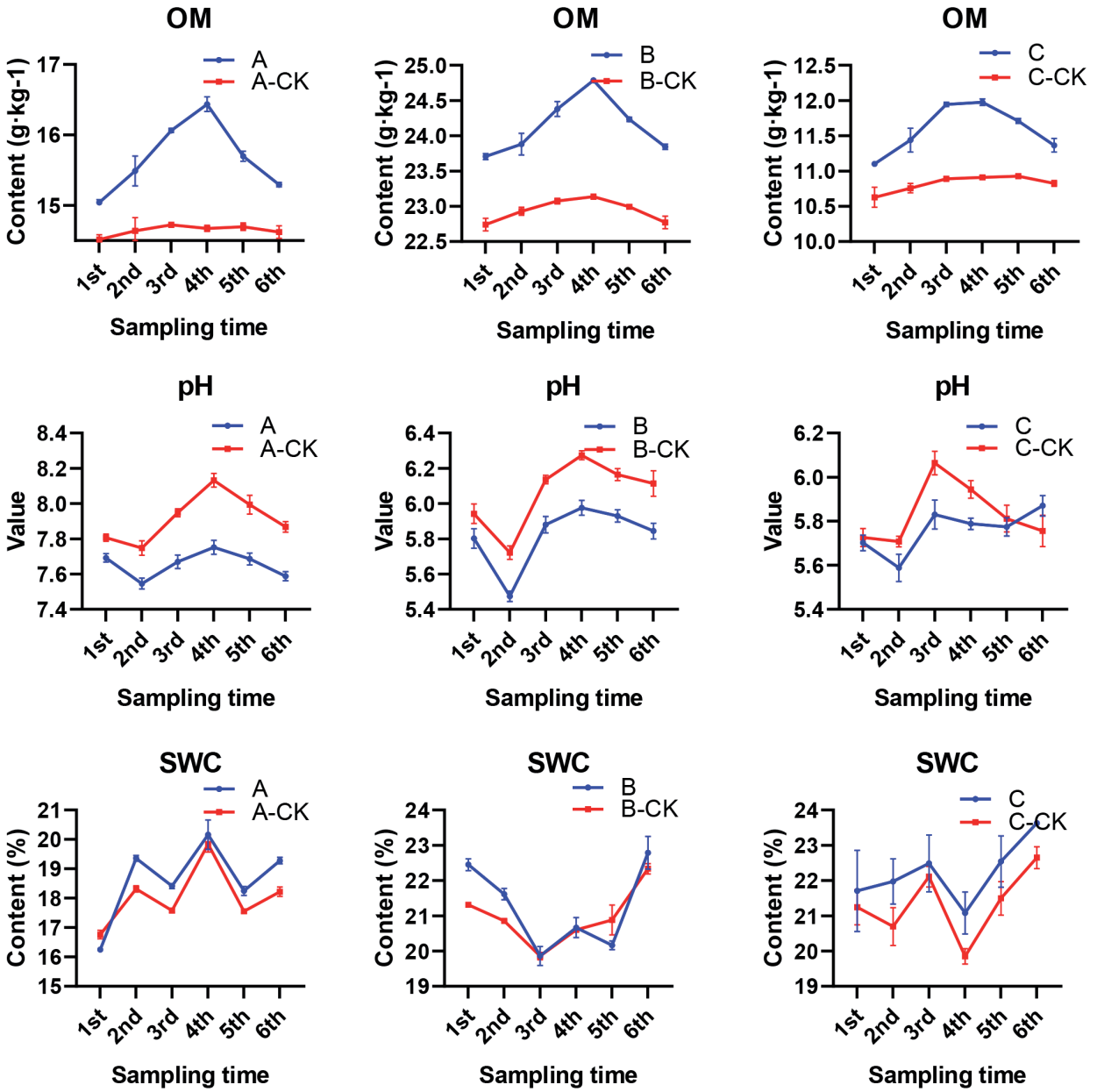

Fig. 3. Soil physical and chemical properties. OM: organic matter, SWC: soil water content. a, b, and c denotes soil samples from Songyuan, Dunhua, and Jiutai, respectively. CK denotes soil samples without straw return.

different patterns. At location A, short-term straw returning significantly reduced the α-diversity indexes, including chao1, observed species, and shannon, especially at the 1st, 2nd, 3rd and 5th sampling (Table 1). At location B and C, the bacteria diversity indexes significantly increased upon short-term straw returning. Additionally, there were great differences between different sampling times. α-diversity indexes, including chao1, goods coverage, observed species, and shannon, at 1st, 2nd, 3rd, 4th and 5th sampling were significantly higher than at 6th sampling in group A, A-CK, B, B-CK, and C-CK (both in control and treatment groups). In group C (treated with short-term straw returning), no significant difference was observed across sampling times (Table 1).

Effects of Short-Term Straw Returning on Bacterial Communities

16s RNA sequencing revealed that bacterial composition changed after short-term straw returning, with some bacteria exhibiting similar changing patterns at the 3 locations. Our data show that *Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, Gemmatimonadetes* and *Chloroflexi* were the dominant bacteria phyla (Fig. 5 a-c).

Short-term straw returning significantly increased *Proteobacteria*, except at the 1st sampling time at location B. Similarly, *Bacteroidetes* and *Gemmatimonadetes* were significantly increased upon short-term straw returning. However, relative abundance

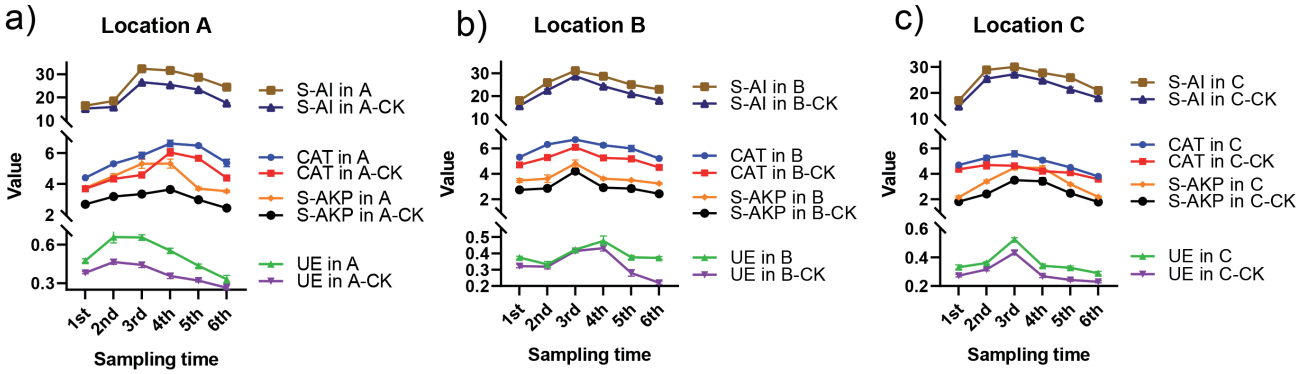

Fig. 4. Soil enzyme activity. S-AI: solid-acid invertase (mg glucose $g^{-1}.24h^{-1}$), S-AKP: soil-alkaline phosphatase (mg phenol $g^{-1}.2h^{-1}$), CAT: catalase (0.1 mol $KMnO_4$ g^{-1} soil.30 mim^{-1}), UE: urease (mg NH_3-N $g^{-1}.3h^{-1}$). a, b, and c notes soil samples from Songyuan, Dunhua and Jiutai, respectively. CK denotes soil samples without straw return.

of *Actinobacteria* and *Chloroflexi* was decreased, and was most obvious at locations A and C. Additionally, we found that the relative abundance of the dominant bacteria varied widely across sampling times in the control, while upon short-term straw returning, relative abundance was stable at various sampling times, indicating the stability of the bacteria communities. Additionally, we calculated the significance of relative abundance of dominant bacteria in different groups (Fig. 6 a-c). For bacteria with low relative abundance, the effect of short-term straw returning treatment was also great. While we did not identify most bacteria at

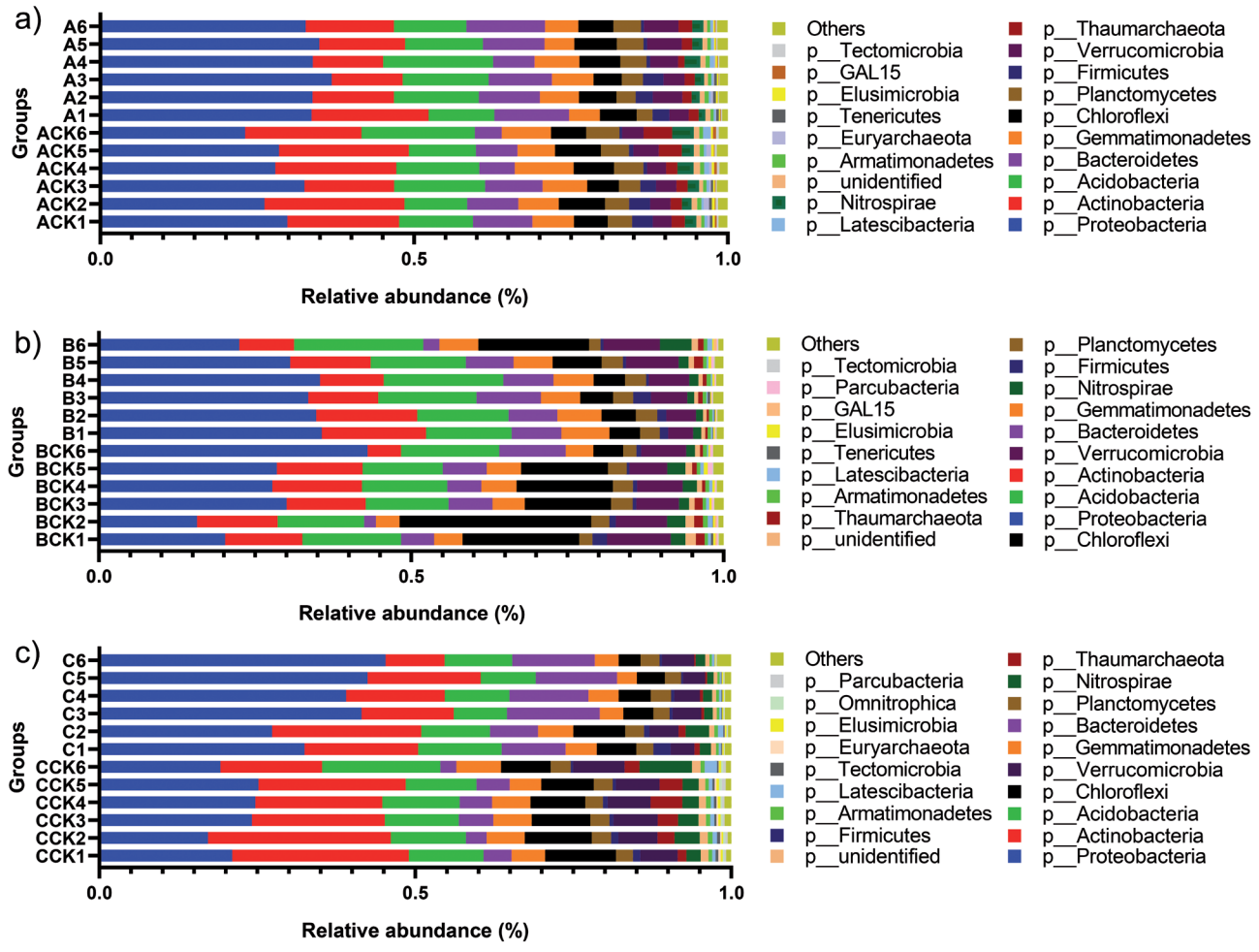

Fig. 5. Effects of short-term straw returning on bacterial communities. a), b), and c) notes soil samples at Songyuan, Dunhua, and Jiutai, respectively. CK denotes soil samples without straw return.

Table 1. Effects of short-term straw returning on bacterial diversity.

			chao1	Goods_coverage	Observed_species	PD_whole_tree	Shannon
Control	A	1st	8093.70±146.35 ab**	0.9165±0.0018 ab	5013.13±178.26 ab*	303.72±12.39 ab	10.7566±0.1024 ab*
		2nd	8323.57±83.07 a**	0.9138±0.0009 a	5168.57±60.31 a**	310.77±5.57 a	10.8622±0.0486 a
		3rd	7765.88±340.56 ab*	0.9201±0.0036 ab	4817.53±205.01 ab*	285.33±9.26 cbd	10.6907±0.1149 ab
		4th	7739.30±598.15 ab	0.9203±0.0060 ab	4797.40±310.99 ab	282.70±12.03 cb	10.6209±0.2275 ab
		5th	7939.45±322.88 ab**	0.9180±0.0025 ab	5004.37±108.32 a**	293.60±7.29 ab*	10.7865±0.0669 a*
		6th	7072.08±564.65 b	0.9278±0.0057 b	4405.70±274.49 b	267.31±6.42 cd	10.3610±0.1964 b
	B	1st	6111.28±193.27 a	0.9428±0.0020 ab	3913.03±188.55 a	222.32±8.42 ab	9.8538±0.2324 ab
		2nd	3536.99±1240.52 b	0.9695±0.0149 bc	2443.43±586.56 b	163.12±28.25 c	8.7413±0.3136 d
		3rd	6436.52±144.76 a	0.9396±0.0014 a	4217.07±196.20 a	228.33±9.15 ab	10.1842±0.2812 ab
		4th	6384.03±124.69 a	0.9401±0.0015 a	4189.13±17.42 a	222.32±2.64 b	10.1199±0.0417 b
		5th	6283.97±252.38 a	0.9412±0.0027 ab	4221.80±168.89 a	237.19±6.53 a	10.1907±0.1179 b
		6th	4878.61±85.16 b	0.9559±0.0008 c	3263.90±50.73 b	193.65±3.81 c	9.6650±0.1614 c
	C	1st	6081.14±127.20 a	0.9298±0.0018 ab	3773.93±117.04 ac	224.61±6.96 ab	10.0965±0.1135 ab
		2nd	5920.38±255.72 ab	0.9318±0.0035 ab	3694.10±217.40 abc	221.50±11.91 ab	9.9567±0.1758 ab
		3rd	6175.58±205.54 a	0.9289±0.0020 a	3825.67±92.81 ac	222.67±3.81 ab	10.0881±0.1443 ab
		4th	6377.73±91.33 a	0.9259±0.0016 a	4026.47±69.46 ab	229.83±4.51 a	10.2048±0.0659 a
		5th	6382.12±182.64 a	0.9254±0.0024 a	4053.87±66.01 b	233.84±8.58 a	10.1088±0.1166 ab
		6th	5296.43±358.53 b	0.9388±0.0045 b	3331.77±247.87 c	202.86±10.93 b	9.6289±0.2638 b
Treatment	A	1st	7036.71±250.29 ab	0.9191±0.0030 ab	4238.83±187.42 ab	279.26±8.34 abc	10.2252±0.2024 ab
		2nd	7097.58±296.53 ab	0.9182±0.0035 ab	4438.20±180.44 ab	285.79±12.44 abc	10.6096±0.1222 ab
		3rd	6970.87±69.04 a	0.9202±0.0008 a	4332.83±18.36 a	281.57±2.71 a	10.4816±0.0813 ab
		4th	6779.91±171.66 ab	0.9226±0.0023 ab	4361.27±75.21 a	272.95±1.00 b	10.5911±0.0405 a
		5th	6785.74±144.98 ab	0.9217±0.0016 ab	4288.40±62.93 a	273.31±6.35 abc	10.5010±0.0804 ab
		6th	6687.07±55.98 b	0.9246±0.0004 b	4141.67±11.93 b	266.27±3.03 c	10.4958±0.0096 b
	B	1st	6216.34±265.52 a	0.9253±0.0031 a**	3854.00±170.92 a	218.99±11.01 ab	10.1094±0.1668 a
		2nd	6172.83±380.08 a*	0.9258±0.0044 a*	3769.70±245.60 a*	218.51±11.04 ab	10.0789±0.2209 a**
		3rd	5995.03±227.41 a	0.9289±0.0030 a*	3693.07±93.42 a*	215.77±1.35 a	10.0762±0.0440 a
		4th	5667.12±274.48 a*	0.9331±0.0037 a	3536.70±173.22 a**	201.59±3.98 b**	10.0396±0.0878 a
		5th	6116.57±332.40 a	0.9275±0.0043 a*	3871.60±210.14 a	218.98±7.72 a	10.2730±0.1283 a
		6th	4476.10±142.60 b*	0.9474±0.0015 b**	2831.57±173.20 b*	174.40±8.45 c*	9.2518±0.3060 b
	C	1st	6569.09±121.24 *	0.9342±0.0012 *	4075.93±124.77	287.46±9.71 **	10.1284±0.1040
		2nd	6019.10±504.64	0.9407±0.0059	3826.73±247.48	268.80±20.43 *	10.0742±0.0861
		3rd	6508.00±278.96	0.9346±0.0029 **	4039.37±158.86	277.71±7.43 **	10.1280±0.0964
		4th	6516.78±100.40	0.9353±0.0015 *	4106.73±134.61	277.30±9.21 **	10.2449±0.0865
		5th	6182.17±598.71	0.9387±0.0060	3852.07±379.64	266.26±15.44	9.9880±0.3112
		6th	5964.58±540.95	0.9414±0.0068	3838.57±341.12	275.25±18.38 **	10.1131±0.1931

the genus level, relative abundance of those identified at genus level was very low.

Co-Occurrence Network Analysis

Co-occurrence network analysis of the complexity of interactions between taxa detected in soils with and without straw return revealed some differences across regions. Higher co-occurrence network complexity was identified at location C, relative to B and A (Fig. 7). Among the taxa in these 3 networks, *Proteobacteria* and *Acidobacteria* showed their key positions in interaction.

Relationships between Species Aabundance and Environmental Factors

RDA results revealed strong correlation between species data and environmental factors, with species-environment correlations on 1st and 2nd axes. Total P content was the dominant environmental variable

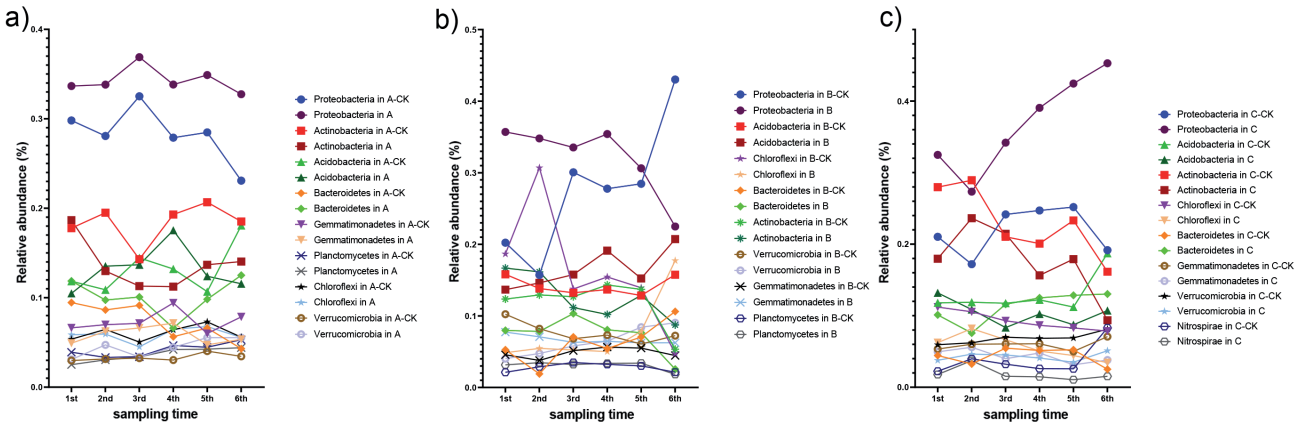

Fig. 6. Relative abundance of dominant bacteria. a), b), and c) notes soil samples in Songyuan, Dunhua and Jiutai, respectively. -CK notes soil samples without straw return treatment. All bacteria were counted at the phylum level.

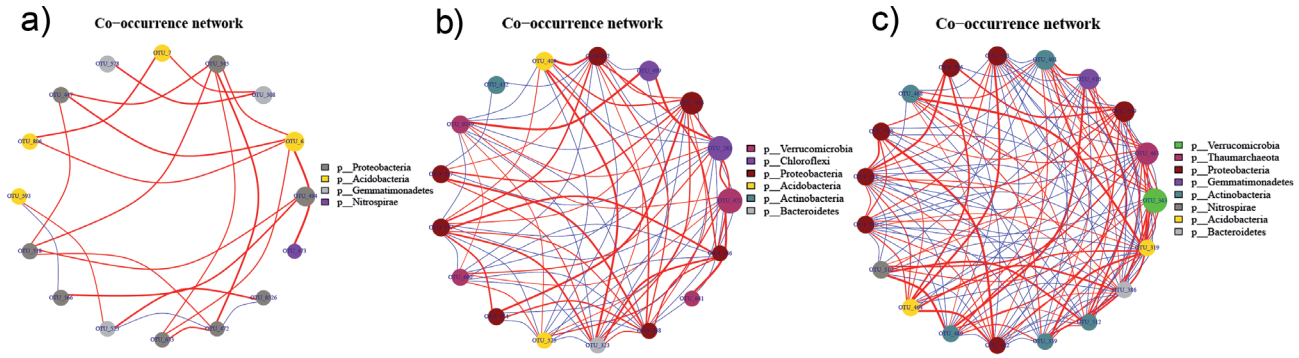

Fig. 7. Co-occurrence network analysis of bacterial OTUs in CK and short-term straw returning treatment samples. a), b), and c) notes the network's location (Songyuan, Dunhua, and Jiutai, respectively).

and mainly correlating with the relative abundance of *Rhodanobacter*, *Burkholderia-Paraburkholderia*, and *Bryobacter_Bradyrhizobium*. PH was the other dominant environmental variable correlating with the relative abundance of *Acidbacter, RB41, Haliangium*. Species like *Acidothermus*, *Candidatus solibacter*, *H16,* and *Nitrospira* negatively correlated with soil enzyme activity. *Sphingomonas, Pedobacter*, *Gemmatimonas,* and *Pseudarthrobacter* positively correlated with soil enzyme activity. However, the total N and soil OM content did not significantly correlate with bacterial abundance (Fig. 8).

Discussion

Crop straw is an important source of organic carbon in Chinese agro-ecosystems [24]. Returning crop straw to soil is a critical means of countering carbon loss due to mineralization in agricultural soils [25, 26]. Here, we find increased OM content after short-term straw returning at all experiment locations and sampling times, which is consistent with Chen's report [27]. It is likely that many nutrients and soluble OM in crop straw are released to soil, resulting in a virtuous circle with soil microorganisms [28]. Additionally, we found that the OM contents peaked at the 3rd (Aug 15) or 4th (Seq 9) time, and troughed at the 1st or 6th time, which may be due to temperature effects and effective accumulated temperature [29, 30]. Higher temperature increased microbial activity and favored soil OM formation. In the experimental area, temperature initially rose before falling, peaking at the end of July. The accumulated temperature reached higher values in September. Since OM change at 3 locations was relatively consistent, we inferred that temperature and accumulated temperature jointly affected OM change. OM is known to be affected by a wide range of soil microorganisms. RDA results did not reveal significant positive correlation between soil OM content and soil microorganisms. Higher temperatures are expected to enhance soil bacteria activity, resulting in higher enzyme activity [31]. Enzyme activity peaked at the 2nd or 3rd sampling, likely due to higher temperature.

Short-term straw returning significantly lowered pH at all locations, although the 3 areas are far away from each other. This was consistent with past findings that pH value decreased (but not significantly) after 3 years of maize straw application [32]. The lower soil pH was an important factor for increased fungal

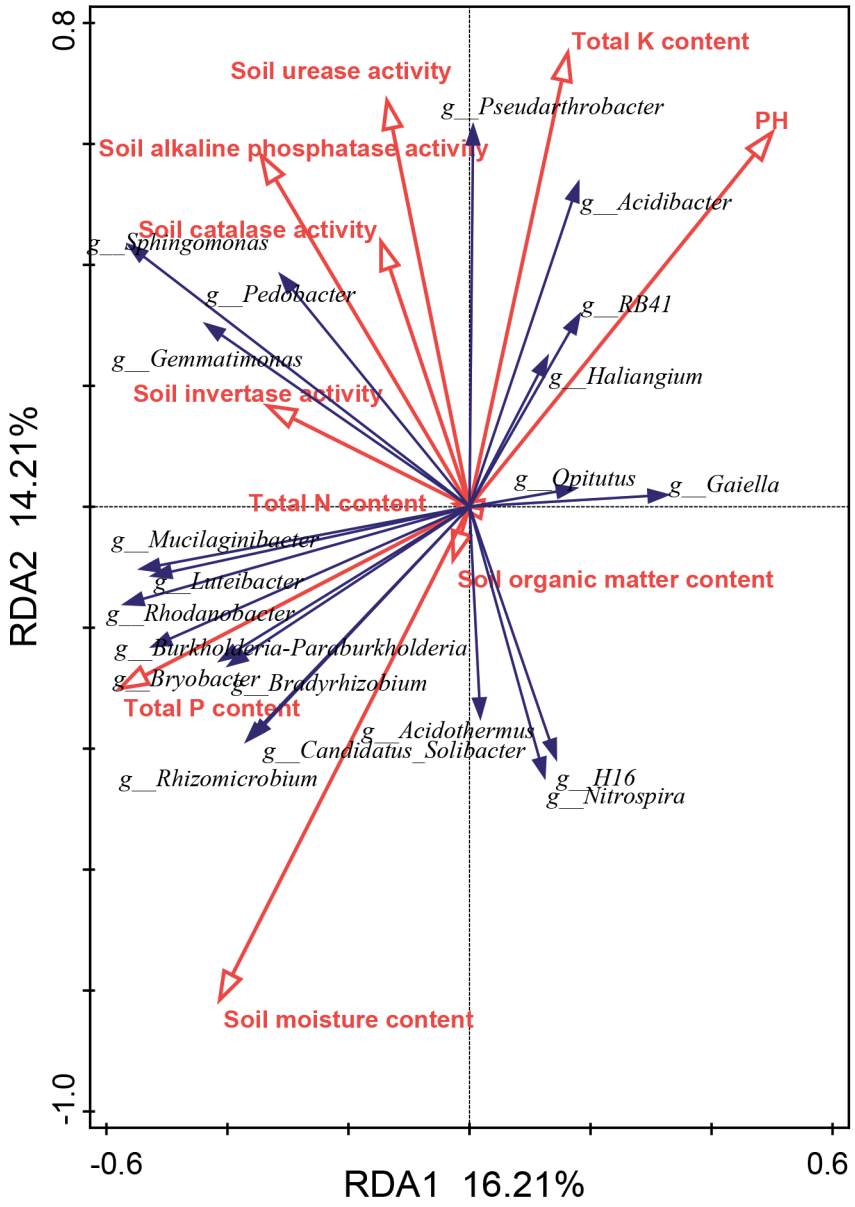

Fig. 8. Ordination diagram showing the results from redundancy analysis of species abundance and bacteria species abundance. The arrow length represents the strength of the correlation between the soil physical and chemical parameters and the bacteria. The longer the arrow length, the stronger the correlation. The perpendicular distance between the soil physical and chemical parameters and the bacteria axes in the plot reflects their correlations. The smaller the distance, the stronger the correlation.

abundance [33] as fungi prefer acidic environments [34]. Interestingly, pH positively correlated with *Acidibacter*, *RB41,* and *Haliangium* (Fig. 7), whose abundance may influence soil pH at different periods. SWC analysis revealed good water retention after soil treatment with short-term straw returning. Soil enzymes are produced and secreted by soil microorganisms, and are proximate agents of OM formation and decomposition [35].

Short-term straw returning also increased enzyme activity. The increased activity of enzyme secreted by soil microorganisms may promote soil OM decomposition, meeting carbon and nitrogen demand for microbial growth [36]. In general, OM contents and enzyme activities had a concerted trend that closely correlated to the amount and structure of soil microorganisms. Indicating that short-term straw returning enriches soil bacterial diversity. The α-diversity of location B at 2nd sampling had the lowest value in the control, but increased upon straw returning. Additionally, short-term straw returning could better maintain the stability of soil bacterial diversity, such as at location C. Different soil environments in the 3 regions might be the main cause of microbial diversity differences. Soil bacteria diversity might be affected by factors like temperature and pH, especially for B and C control groups. However, straw returning significantly alleviated the influence of climate on soil

microbial diversity. The alkalinity of area A soil and the faint acidity of area B and C soil might be one of the main reasons for changes in soil bacteria diversity. However, more evidence is needed to support this view. Short-term straw returning significantly increased the relative abundance of *Proteobacteria* and *Bacteroidetes*. Our data show that the bacterial phyla *Proteobacteria* and *Bacteroidetes* have similar dynamics upon straw application, which indicates that they are dominant in straw decomposition relative to other microbial fractions. A similar role has been reported for *Proteobacteria* [37]. *Proteobacteria* has been shown to consist of many classes that are sensitive to copiotrophic conditions [38]. Furthermore, the abundances of Delta-, Gamma- and Beta-*Proteobacteria,* were significantly improved by OM incorporation [39]. Past studies identified *Bacteroidetes* as the main microbial groups involved in breaking down the chemical components of rice straw, including cellulose, hemicellulose, and chitin [40]. Liu et al found that *Bacteroidetes* played an important role in degradation of the rice straw in paddy soils [41]. Thus, we speculated that *Proteobacteria* and *Bacteroidetes* are the important phyla during straw degradation, and that they promote soil OM accumulation and enhance soil fertility. On the contrary, the dominant phyla, *Actinobacteria* and *Chloroflexi* were reduced by straw returning, indicating that straw returning negatively affects their growth environment. *Chloroflexi* is another major microbial group that breaks down chemical components of crop straw [40]. *Proteobacteria* and *Actinobacteria* are copiotrophic taxa (taxa that thrive in conditions of elevated C and N and that exhibit relatively rapid growth rates) [42-44]. The decomposition process is conceptually separated by a rapid and a slower phase into the early and the late stage, respectively [45]. The organic components of plant residues can be easily degradable, such as long/short chain fatty acids and less-degradable or more persistent to treatment fractions, including cellulose and lignin [46]. Straw input provides C for soil microbial growth, which promotes microbial growth [47]. We speculate that decreased *Actinobacteria* and *Chloroflexi* may be due to competition from dominant bacteria.

The enzyme activity associated with straw residues represents a key biological process that is closely related to nutrient absorption by microorganisms for their own metabolism [48]. In general, some water-soluble compounds and easily decomposed substances like sugar and starch, are important for high microbial activity, because they are N-acetyl-glucosamine kinase and L-leucine aminopeptidase suitable substrates [49]. Here, enzyme activity gradually increased in the early stage. Additionally, *Sphingomonas, Pedobacter*, *Gemmatimonas* and *Pseudarthrobacter,* positively correlated with soil enzyme activity. Therefore, these bacteria may benefit the most from straw decomposition.

Conclusion

Short-term straw returning to the field can markedly improve soil quality, mainly reflected in the increasing of OM content and soil enzymes activity. Straw returning treatment might maintain soil stability and bacterial diversity better. The bacterial phyla *Proteobacteria* and *Bacteroidetes,* exhibited similar dynamics upon straw application, indicating that they are dominant over other microbial fractions during straw decomposition. *Actinobacteria* and *Chloroflexi* decreased after straw returning, indicating that straw returning negatively affects the growth environments of these bacteria. Decreased *Actinobacteria* and *Chloroflexi* may be due to competition from dominant bacteria. However, soil responses to straw returning differ by location, depending on basic soil parameters and climate.

Core Ideas

1. Short-term returning of straw can significantly improve soil quality.
2. The effect of short-term straw return is affected by basic soil conditions and climate.
3. Short-term straw returning has a significant regulatory effect on soil bacteria.
4. *Proteobacteria* and *Bacteroidetes* phyla may be dominant in straw decomposition.

Abbreviations

OM, organic matter;
OTUs, Operational taxonomic units;
PE, pair-end;
PCoA, Principal Co-ordinates Analysis;
qPCR, Quantitative PCR;
RDP, Ribosomal Database Project;
RDA, redundancy analysis;
SWC, Soil water content.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Supplementary Material

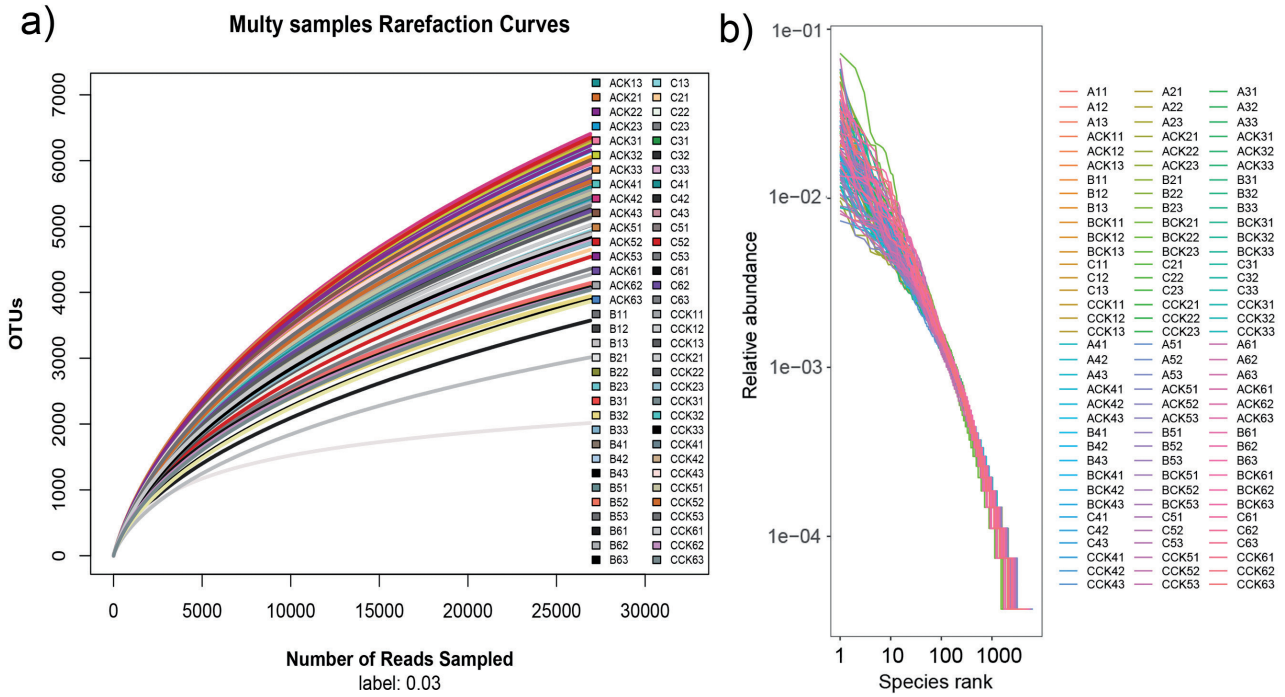

Fig. S1. Rarefaction curves and rank abundance curves of alpha diversity. a) Rarefaction curves plot, X-axis is number of sequencing reads randomly chosen from a certain sample to obtain OTUs. b) Rank abundance curves plot. X-axis shows abundance rank. Y-axis shows relative abundance. Curves for different samples are shown in different colors.

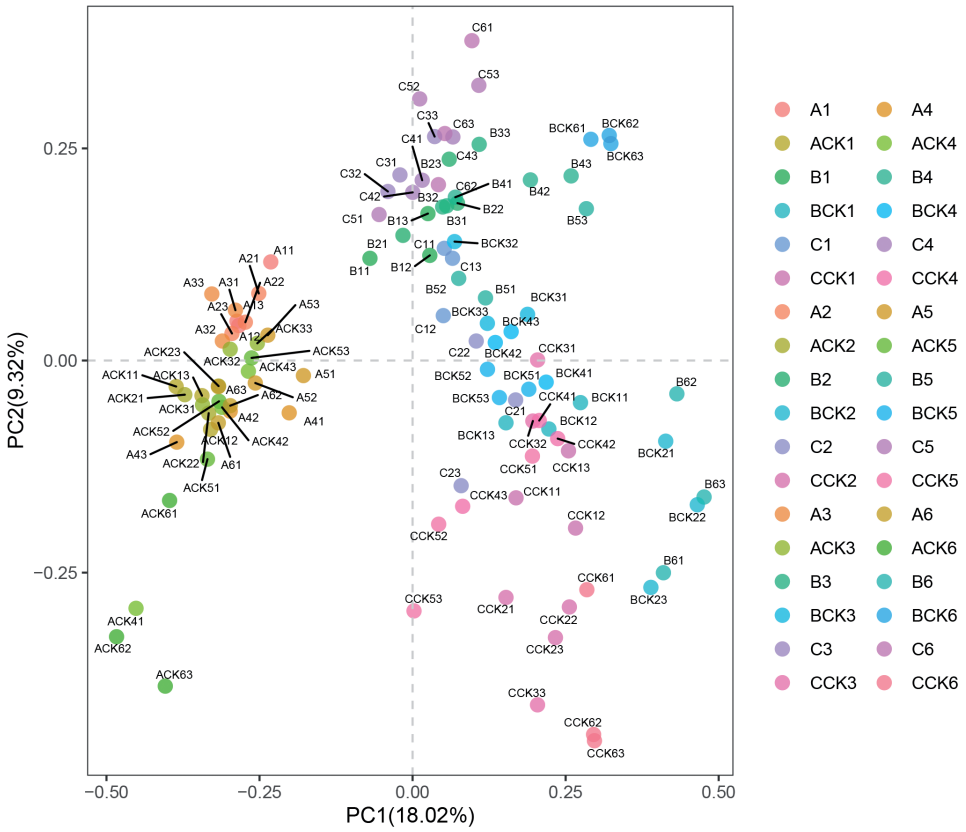

Fig. S2. Principle component analysis (PCA) for bacterial community 16S rRNA gene sequences. A, B, and C denote soil samples from Songyuan, Dunhua, and Jiutai, respectively. CK denotes soil samples without straw return. Different samples are shown in different colors.

Table S1. Sequence data summary. A, B, and C denotes soil samples from Songyuan, Dunhua, and Jiutai, respectively. CK denotes soil samples without straw return.

SampleID	Raw_Tags	Clean_Tags	OTUs
A1_1	78955	76544	5455
A1_2	169550	164681	5877
A1_3	117986	114469	5668
ACK1_1	90273	87849	6328
ACK1_2	65232	63434	6162
ACK1_3	197948	192535	6008
B1_1	168652	164244	5580
B1_2	101581	98573	5384
B1_3	93920	90960	5138
BCK1_1	65012	63686	4876
BCK1_2	152772	149703	5150
BCK1_3	83521	81458	5469
C1_1	78079	76218	5371
C1_2	54844	53433	5353
C1_3	188046	183304	4930
CCK1_1	123663	121033	5546
CCK1_2	95934	94060	5025
CCK1_3	69875	68072	5136
A2_1	67515	65787	5604
A2_2	127991	124601	6056
A2_3	89746	87309	5953
ACK2_1	70980	69350	6372
ACK2_2	50391	49178	6234
ACK2_3	134162	130693	6148
B2_1	122028	119152	5590
B2_2	83333	81260	5224
B2_3	80404	78187	4860
BCK2_1	40451	39689	2016
BCK2_2	36655	35878	3013
BCK2_3	84648	83724	3862
C2_1	59685	57183	4647
C2_2	297317	290762	4566
C2_3	47668	46690	5320
CCK2_1	34963	34391	5480
CCK2_2	29350	28871	4744
CCK2_3	186142	183212	4744
A3_1	119051	115802	5803
A3_2	79472	77289	5663
A3_3	65839	64108	5691

SampleID	Raw_Tags	Clean_Tags	OTUs
ACK3_1	46446	45293	5963
ACK3_2	103532	100150	6288
ACK3_3	112856	110062	5650
B3_1	77763	75736	5353
B3_2	63544	61697	5268
B3_3	70852	69271	4817
BCK3_1	129962	126259	5557
BCK3_2	88420	86014	5174
BCK3_3	75390	73218	5675
C3_1	53710	51692	5232
C3_2	157888	151982	5247
C3_3	124771	120020	4810
CCK3_1	75308	73035	5456
CCK3_2	70081	68052	5471
CCK3_3	168695	166938	4840
A4_1	43210	42201	6228
A4_2	30797	30071	5975
A4_3	97609	96026	5521
ACK4_1	328241	323468	5217
ACK4_2	43636	42341	6407
ACK4_3	34468	33470	6008
B4_1	29398	28640	5360
B4_2	182518	178390	4761
B4_3	56107	54700	4741
BCK4_1	91864	89355	5376
BCK4_2	52668	51010	5597
BCK4_3	58253	56733	5447
C4_1	35312	34032	5430
C4_2	107864	104135	5261
C4_3	86882	83887	4868
CCK4_1	57089	55459	5466
CCK4_2	47785	46502	5503
CCK4_3	68606	66965	5829
A5_1	165494	161328	5839
A5_2	91695	89056	5950
A5_3	89341	86893	5790
ACK5_1	64940	63392	6319
ACK5_2	162482	157884	6349
ACK5_3	119737	116077	6159

Table S1. Continued.

SampleID	Raw_Tags	Clean_Tags	OTUs
B5_1	86066	83807	5835
B5_2	89705	87486	5609
B5_3	76864	74771	5018
BCK5_1	134055	130313	5400
BCK5_2	90210	87717	5683
BCK5_3	61842	60025	5259
C5_1	120113	115047	5681
C5_2	127740	122855	4539
C5_3	93715	90486	4357
CCK5_1	75321	73195	5501
CCK5_2	82367	80600	5687
CCK5_3	172346	168706	5770
A6_1	117008	114025	5652
A6_2	102372	99926	5495
A6_3	64499	62639	5755

SampleID	Raw_Tags	Clean_Tags	OTUs
ACK6_1	214836	210071	5733
ACK6_2	296939	294317	5123
ACK6_3	192603	191013	4766
B6_1	136316	135097	3572
B6_2	147636	144876	4270
B6_3	261378	257343	3901
BCK6_1	228666	222726	3944
BCK6_2	163401	159922	4132
BCK6_3	161480	157543	4141
C6_1	238724	231019	4079
C6_2	272008	263796	5235
C6_3	235502	228663	4906
CCK6_1	214183	209990	4881
CCK6_2	93941	93297	4047
CCK6_3	138349	137424	4038

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