Response of Rice and Bacterial Communities to the Incorporation of Rice Straw in Areas Mined for Heavy Rare Earth Elements

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Experiments were conducted in pots to study the effects of 2.5% rice straw and 1% rice straw ash on rice growth and bacterial community abundance in areas mined for heavy rare earth elements. The results showed that the incorporation of rice straw improved the pH value of soil, reduced the α-diversity of the soil bacterial community, improved the abundance of Proteobacteria and Firmicutes; reduced the abundance of Acidobacteria, Nitrospirae, *etc.*; reduced the abundance of *Candidatus Solibacter*, *Syntrophobacter*, *Haliangium*, *Candidatus Koribacter*; and increased the abundance of *Ideonella*, *Anaeromyxobacter*, *Roseomonas*, *Clostridium sensu stricto 10*, and *Geobacter*. The decrease in the abundance of beneficial bacteria, Acidobacteria and Nitrospirae, inhibited the growth of the rice; reduced the dry weight of the rice roots, the shoots, and the grains, and increased the concentration of rare earth elements in the rice. Returning 1% rice straw ash to the field had little effect on the diversity and richness of the bacterial community in areas mined for heavy rare earth elements. Also the returned rice straw had little effect on the rice growth, the dry weight of the roots, the shoots, the grains, and the concentration of rare earth elements in these parts of the rice.

Keywords: Heavy rare earth mined areas; Rice; Bacterial communities; Rice straw; Rice straw ash

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INTRODUCTION

Rare earth elements (REEs) are located in the third subgroup, the sixth cycle of the Periodic Table of Elements, and include lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), lutetium (Lu), scandium (Sc), and yttrium (Y) (Wang *et al.* 2014; Zhao and Wilkinson 2015). Depending on slight differences in the atomic mass and the chemical properties of REEs, they can be divided into light REEs and heavy REEs. China is a large country, rich in rare earth resources, especially ionic rare earth elements, and accounts for approximately 90% of the world's REE resources (Jin *et al.* 2019). Ionic rare earth elements are a type of REE which are adsorbed onto clay minerals *via* hydrated ions or hydroxyl hydrated ions and are insoluble in water and do not hydrolyze. However, they follow the law of ion exchange and can be extracted *via* chemical methods. Southern China is the most abundant area for ion-type REEs in the world, especially the heavy ion-type REEs, which are mainly distributed in Longnan County, Ganzhou City, Jiangxi Province. The known ion-type rare earth reserves in Longnan county account for 70% of the total reserves in the world, ranking first in quantity in the world. Thus, Longnan is known as the “home of heavy rare earth” (Jin *et al.* 2014).

The large-scale mineral mining and smelting operations have led to a major increase in the mining of REEs. Jin *et al.* (2014) determined that the distribution of REEs are primarily concentrated in the eastern part of Longnan county. The research also showed that the REEs concentration in the soil ranged from 538 mg x kg^{-1} to 1.62 g x kg^{-1}, with an average value of 976 mg x kg^{-1}, which is 4.5 times and 5.1 times the background values of REEs in Jiangxi Province and the whole country, respectively. Rare earth elements are not essential nutrients for the growth of various organisms, and a small amount can promote the growth of crops. However, a large amount can prove toxic to the growth of organisms, although their toxicity is not as strong as other heavy metals (Hu *et al.* 2006). The toxicity effect is closely related to the concentration and properties of the REEs in the soil, the physical and chemical properties of soil, the types of crops found in the soil, and the management measures (Jin *et al.* 2016, 2018; Martinez *et al.* 2018). Ionic rare earth mining areas in southern China overlap with important rice production areas, which produce over 100 million tons of rice straw every year. Because the farmers in southern Jiangxi seldom use rice straw as fuel or forage, most rice straw has been directly returned to the field or changed into rice straw ash, and then returned to the field. The incorporation of rice straw can increase the soil organic matter, the levels of nitrogen (N), phosphorus (P), potassium (K) and other nutrients, the electric potential (Eh), the bioavailability of heavy metals, the growth of crops and the bacterial community, and affect the soil pH, which can affect the plant-soil ecosystem (Guo *et al.* 2016; Zhao *et al.* 2017; Wang *et al.* 2019). The effects of rice straw on the plant-soil ecosystem is related to the application method, the amount, as well as the time and the season of the rice straw incorporation (Ding *et al.* 2018; Li *et al.* 2019). Some studies have shown that partial incorporation of rice straw has a greater effect on rice growth than the total incorporation of rice straw, and that the incorporation of rice straw later in the growth cycle can promote greater rice growth than earlier growth cycle incorporation (Jin *et al.* 2018). In June and July, the rapid rise of temperature in southern China promotes the decomposition of rice straw, which forms large amounts of dissolved organic matter (DOM) and organic acids, which accumulate in paddy fields and harm the root systems of the crops when the concentrations become too high (Luo *et al.* 2016; Ding *et al.* 2018). Studies have shown that an increase in DOM results in a significant increase in the concentration of water-soluble REEs, which affect the soil microorganisms and the crop growth (Wang *et al.* 2011, 2017; Jin *et al.* 2018). The DOM composition was significantly correlated with the bacterial community structure (Blanchet *et al.* 2015; Li *et al.* 2018). The DOM composition also affects bacterial metabolic pathways, such as anaerobic carbon sequestration, glycolysis, decomposition, and the fermentation of lignins and methanogenesis (Li *et al.* 2018). The distribution of DOM was significantly correlated with the function of the rice microbial community (Traving *et al.* 2017). Rapid decomposition of straw led to soil eutrophication, inhibited the growth of beneficial bacteria, promoted the growth of harmful bacteria, and endangered the growth of crops. Under certain reduction conditions, such as being submerged in acidic soils, Fe^{3+} (iron) can be reduced to Fe^{2+} [$Fe(OH)_3 + 3H^+ + e^- = Fe^{2+} + 3H_2O$]. This process consumes the H^+ ion, which results in a rise of pH in the soil solution (Sun *et al.* 2019). The pH is considered a key factor that affects the structure and function of bacterial communities, *e.g.*, Gammaproteobacteria prefers acidic environments, while Betaproteobacteria prefers alkaline environments (Chen *et al.* 2016). The variation in pH affects the abundance of *Acidobacteria*, and it was reported that *Acidobacteria* can promote plant growth and can be regarded as beneficial bacteria in terms of plant growth (Kielak *et al.* 2016).

Rice straw ash (RSA) is alkaline and contains more than 70% potassium oxide, as well as silicon dioxide (Duan *et al.* 2016). Silicate ions react with REEs to form rare earth silicate precipitate compounds, which can affect crop growth (Jin *et al.* 2018). Silicon is beneficial to crop growth, which can enhance the resistance of crops to disease and increase the activity of soil enzymes (Töpper *et al.* 2010; Wang *et al.* 2013; Samaddar *et al.* 2019). Other studies have shown that silicon (Si) inhibits the uptake of manganese (Mn) and Fe by roots of crops. Since Mn and Fe can reduce the uptake of heavy metals and REEs, the uptake mechanism of rare earth elements *via* silicon-containing materials by crops is particularly complex (Tubaña and Heckman 2015). When silicon-containing materials are applied to soils, the soil pH value will increase (Jin *et al.* 2019). The pH value is one of the factors affecting soil microorganisms (Wang *et al.* 2019). Microorganisms play an important role in the regulation of the energy and nutrient fluxes in the soil, thus affecting the function and productivity of the soil. The most abundant microorganisms in soil are bacteria, which includes Proteobacteria, Chloroflexi, Bacteroidetes, Actinobacteria, Acidobacteria, *etc.*, and each a play different role in soil. With the changes in physical and chemical properties of the soil, the functional structure of these dominant bacteria also changed, which affects the growth of rice, thus affecting the rice-soil ecosystem.

Currently, the effects of the incorporation of rice straw on the soil-plant ecosystem in REE mining areas are still unclear. Pot experiments were conducted to study the effects of the incorporation of rice straw on rice growth and bacterial community in areas mined for heavy REEs, which could provide a useful reference for the rational utilization of biological resources, ecological environment protection, and the food safety of residents in the mining areas.

EXPERIMENTAL

Materials

The tested soil (at depths of 0 cm to 20 cm) was collected from abandoned paddy fields around the Fukeng heavy REE mining area (Dujiang town, Longnan, Jiangxi Province) (114°46′8″E, 24°51′14″N). Soil samples were prepared *via* natural air-drying, grinding, and sieving (2 mm). The tested rice straw (RS) was collected from the Guangfeng District in Shangrao City, Jiangxi Province, where there was no rare earth mineral distribution. The RS was crushed into powder and burned to convert it into RSA for the reserve. The physical and chemical properties of the soil samples, the RS, the RSA, and their REE concentrations are shown in Table 1.

Treatment Procedure

A pot experiment was conducted to study the effects of different methods of rice straw incorporation on the growth of rice in heavy REE mining areas. Three treatment methods were set up, control check (CK) with no addition of RS, the addition of 2.5% RS, and the addition of 1.0% RS (the weight was reduced when the RS was burned into RSA). Each treatment was repeated three times. Each pot contained 5 kg soil, 5.4 g urea (containing 46.6% N), and 0.6 g of potassium chloride (containing 62.9% of K_2O). According to the design, the different additives, soils, and fertilizers were evenly stirred, and the flooded water was balanced for one week.

Table 1. Physicochemical Properties and REE Concentrations of the Soil, RS, and RSA

Properties	Soil	RS	RSA
pH	4.90	5.87	11.94
Cation exchange capacity (c mg/kg)	7.00	-	-
Organic matter (g/kg)	1.71	-	-
Total N (g/kg)	1.30	-	-
Total C (g/kg)	14.51	-	-
Total S (g/kg)	0.45	-	-
Total P (g/kg)	0.70	-	-
Clay soil particle size (%)	11.79	-	-
Silt soil particle size (%)	77.30	-	-
Sand soil particle size (%)	10.92	-	-
Y (mg/kg)	166.36	0.62	1.78
La (mg/kg)	58.71	0.92	3.22
Ce (mg/kg)	91.35	0.77	2.67
Pr (mg/kg)	34.86	0.27	0.81
Nd (mg/kg)	128.87	0.68	2.42
Sm (mg/kg)	41.59	0.19	0.63
Eu (mg/kg)	2.58	0.12	0.23
Gd (mg/kg)	124.92	0.19	0.55
Tb (mg/kg)	6.94	0.08	0.15
Dy (mg/kg)	21.46	0.11	0.37
Ho (mg/kg)	8.64	0.05	0.13
Er (mg/kg)	20.62	0.08	0.27
Tm (mg/kg)	2.91	0.05	0.12
Yb (mg/kg)	18.66	0.08	0.23
Lu (mg/kg)	2.57	0.05	0.08
Total REEs (mg/kg)	731.03	4.03	13.32

The rice variety used in the experiment was Jiangzao 361, which was sourced from the Institute of Grain and Oil Crops, Nanchang Academy of Agricultural Sciences (Nanchang, Jiangxi Province, China). The average growth period for this variety of rice is 110.2 days, with a moderate plant type, which was suitable for planting in the area of Jiangxi province with a low occurrence of rice blast fungus. On April 8, 2016, the seeds were sterilized with a 30% H_2O_2 solution for 15 min, then they were sowed in soil without REE pollution for seedling cultivation after germination. On May 6, the rice seedlings were transplanted into culture pots with two plants per pot. The pot experiment was conducted in the greenhouse. The light intensity was 4000 lx, the illumination duration was 12 $h \cdot d^{-1}$, the temperature range was from 20 °C to 28 °C and the relative humidity(RH) was 80%. According to the characteristics of rice growth, watering and management were carried until the rice matured. Then the rice roots, shoots, and grains were harvested. The seeds, shoots, and roots of the rice were dried to a constant weight and ground into powder, which was stored at room temperature for analysis. When the rice was harvested, soil was collected from the culture pots and placed in a refrigerator at -80 °C. The soil was later used for molecular experiments.

Chemical Analytical Methods

The soil pH, the organic matter content, and the cation exchange capacity (CEC) were tested by referring to the methods recommended and compiled by Lu (2000), and the carbon (C), the nitrogen (N), the sulfur (S), and the texture composition of the soil were tested *via* the methods by Jin *et al.* (2018). Soil samples were digested with aqua regia (HNO_3:HCl = 3:1) and plant samples were digested with HNO_3. Then the digested samples were cooled, the acid was removed, the volume was stabilized, and then the specimens were filtered. The samples were determined *via* inductively coupled plasma mass spectrometry (ICP-MS). The digestion and determination process of the samples were in reference to methods by Jin *et al.* (2018).

Amplification and Sequencing of 16S r RNA gene

A soil DNA extraction kit (Qiangen, PowerSoil DNA Isolation Kit, Hilden, Germany) was used in this experiment. The genomic DNA of the soil microorganisms was extracted according to its operating manual, then the purity and concentration of the DNA was detected *via* agarose gel electrophoresis. Samples were placed in centrifuge tube and diluted with sterile water to 1 ng/µL. The diluted genomic DNA was used as a template, and specific primers with barcodes and enzymes for high-efficiency and high-fidelity, were used for PCR in accordance to the selection of the sequencing regions, which ensured the efficiency and accuracy of the amplification. The 16S rRNA V4 region of the bacteria was amplified by primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The PCR amplification procedure was as follows: the samples were pre-denatured at 95 °C for 2 min, denatured at 95 °C for 30 s, annealed at 55 °C for 30 s, extended at 72 °C for 30 s, then these steps were repeated 30 times, and finally extended at 72 °C for 10 s. The PCR products were purified; then the purified products were equally mixed and submitted to Beijing Xinke Open Source Bioinformatics Technology Co., Ltd. (Beijing, China) for sequencing, which used MiSeq (Illumina, San Diego, CA) for processing. The original sequence obtained *via* sequencing was submitted to the NCBI database and the obtained sequence number was SRP190111.

The experiment was conducted *via* pair-end sequencing. First, the quality control was carried out on the original data. Flash (McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, version 1.2.11, Baltimore, USA) was used to connect the sequence that had undergone quality control, the sequence that could not be connected was discarded, and the sequence used for analysis was obtained. Quantitative insights into microbial ecology (Qiime) (Center for Applied Microbiome Science, Pathogen and Microbiome Institute, Northern Arizona University, version 1.7.0, Flagstaff, AZ, USA) was used to classify the sequence into several operational taxonomic units (OTU) according to their similarity. After the output of OTUs, the number of samples containing OTUs and the number of sequences that contained each OTU were counted. Uparse (Drive5, version 7.1, Tiburon, USA) was used for OTU clustering, the sequence similarity in the OTUs was set to 97%, then the representative sequence of OTUs was obtained. In order to obtain the corresponding species classification information of each OTU, the RDP classifier Bayesian algorithm was used to classify and analyze the representation sequences of each OTU with a 97% similarity level, and the community composition of each sample was calculated at each level (phylum, class, genus). The relative abundance of the dominant population was stipulated to be more than 10% at the phylum and the class levels, and more than 1% at the genus level. The OTUs with a 97% similarity level were selected to generate the expected dilution curve, then Mothur (Mothur software development team in the Department of Microbiology and Immunology, University of Michigan, version v.1.41.1, Michigan, USA) was used to calculate the indexes of richness, Chao1, Ace and diversity.

Data Analysis

Sequencing data processing and OTU clustering and species annotation methods were defined by Jin *et al.* (2019). Qiime (Version 1.7.0, Boulder, CO, USA) was used to calculate the sample complexity and make a comparative analysis of various samples and R (R Foundation, version 2.15.3, Vienna, Austria) was used to draw a dilution curve and NMDS diagram, respectively. Excel 2010 and SPSS (IBM, version 17.0, Armonk, NY, USA) were used for the statistical analysis of all basic data. Statistical significance was conducted *via* a one-way ANOVA. The correlation tests between α-diversities and environmental factors were determined using Pearson correlation, and the heatmaps were drawn using the software of Origin (Originlab, version 9.0., MA, USA).

RESULTS AND DISCUSSION

Effects of Different Rice Straw Incorporation Methods on the Biomass, REE Content in Rice, and pH Value of the Soil

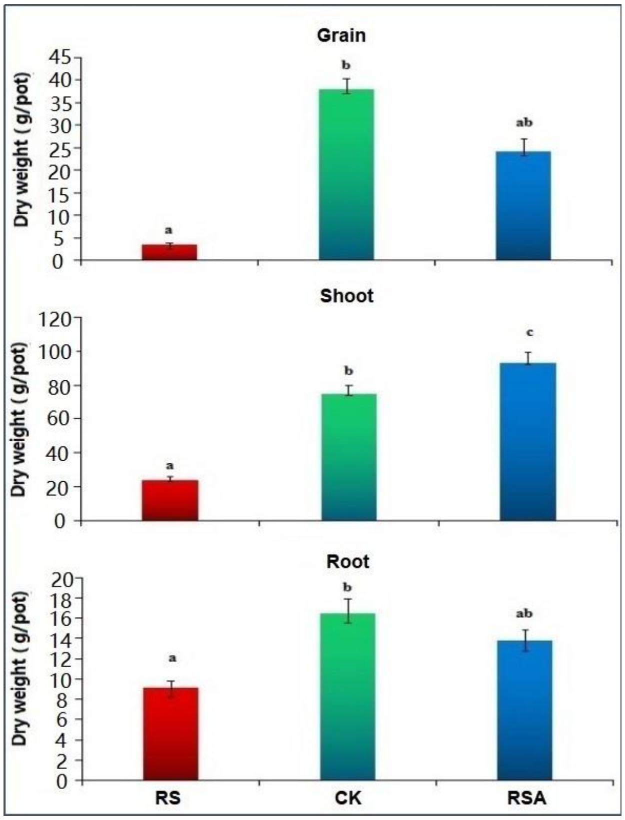

Fig. 1. Effects of different rice straw incorporation methods on the biomass of rice
Note: The data were averaged (n = 3), and the different lower-case letters in the same part of the rice showed significant difference among the different treatments (p-value was less than 0.05).

As shown in Fig. 1, rice straw returned directly to the field caused the dry weight of the rice roots, the shoots, and the grains to be reduced by 44.2%, 68.1%, and 90.8%, respectively. Rice straw ash returned to the field also had an impact on rice growth, which reduced the dry weight of the rice roots and the grains by 16.4% and 36.6%, respectively, and improved the dry weight of the shoots by 24.5%.

As shown in Table 2, rice straw returned directly to the field increased the REE concentration in the rice roots, the shoots, and grains by 210.8%, 14.3%, and 11.4%, respectively. The promotional effect of rice straw on the absorption of heavy rare earth elements (Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Sc, and Y) by different parts of rice was higher than that of light rare earth elements (La, Ce, Pr, Nd, Sm, and Eu).

Table 2. Effects of Different Rice Straw Incorporation Methods on the Total REE Concentration in Rice Roots, Shoots, and Grains

Element	Grain			Shoot			Root		
	RS	CK	RSA	RS	CK	RSA	RS	CK	RSA
Y	18.92 (b)	11.43 (a)	12.17 (a)	2.53 (b)	2.43 (b)	1.34 (a)	222.36 (b)	82.45 (a)	101.72 (a)
La	29.64 (b)	25.22 (a)	25.61 (a)	1.07 (b)	1.06 (b)	0.61 (a)	59.65 (b)	22.31 (a)	27.04 (a)
Ce	52.84 (b)	44.92 (a)	45.26 (a)	1.55 (b)	1.75 (b)	0.98 (a)	87.16 (b)	46.67 (a)	52.95 (a)
Pr	39.92 (c)	37.62 (a)	38.32 (b)	0.93 (c)	0.68 (b)	0.42 (a)	43.85 (b)	6.35 (a)	6.84 (a)
Nd	50.83 (b)	40.8 (a)	43.68 (a)	3.29 (c)	2.43 (b)	1.33 (a)	247.38 (b)	78.35 (a)	85.59 (a)
Sm	34.55 (c)	33.48 (b)	32.25 (a)	0.42 (b)	0.42 (b)	0.25 (a)	77.7 (b)	15.38 (a)	18.91 (a)
Eu	30.72 (a)	31.07 (b)	31.04 (b)	0.09 (b)	0.1 (c)	0.08 (a)	5.05 (b)	3.34 (a)	3.5 (a)
Gd	29.34 (b)	22.6 (a)	22.57 (a)	2.78	1.85	1.04	275.93 (b)	73.28 (a)	82.62 (a)
Tb	20.05 (b)	19.95 (a)	19.94 (a)	0.11 (b)	0.11 (b)	0.08 (a)	10.42 (b)	4.18 (a)	4.75 (a)
Dy	22.65	22.49	22.69	0.43 (b)	0.42 (b)	0.28 (a)	26.74 (b)	1.23 (a)	3.15 (a)
Ho	14.08 (b)	13.77 (a)	13.72 (a)	0.12 (b)	0.11 (b)	0.073 (a)	13.57 (b)	5.212 (a)	6.054 (a)
Fr	21.07 (c)	19.66 (a)	20.63 (b)	0.24 (b)	0.23 (b)	0.14 (a)	27.38 (b)	11.59 (a)	13.51 (a)
Tm	14.74	14.64	14.57	0.06 (c)	0.05 (b)	0.04 (a)	3.72 (b)	1.56 (a)	1.8 (a)
Yb	18.34 (b)	18.39 (b)	17.22 (a)	0.2 (b)	0.19 (b)	0.12 (a)	22.57 (b)	9.25 (a)	10.72 (a)
Lu	11.72	11.5	11.5	0.04	0.04	0.04	2.87 (b)	1.22 (a)	1.42 (a)
ΣREE	409.42 (b)	367.54 (a)	371.15 (a)	13.87 (b)	11.89 (ab)	6.78 (a)	1126.34 (b)	362.37 (a)	420.58 (a)

Note: The data were averaged (n = 3), and the different lower-case letters in the same part of the rice showed significant difference among the different treatments (p-value was less than 0.05).

The heavy REE concentration in the rice roots and the shoots increased by 218.8%, 19.6%, respectively; while the light REE concentration in the rice roots and the shoots increased by 202.1% and 14.2%, respectively. The Y concentration found in grains increased by 65.5%, which was the greatest increase.

As shown in Fig. 2, rice straw directly returned to the field and rice straw ash returned to the field raised soil pH by 12.8% and 5.7%, respectively.

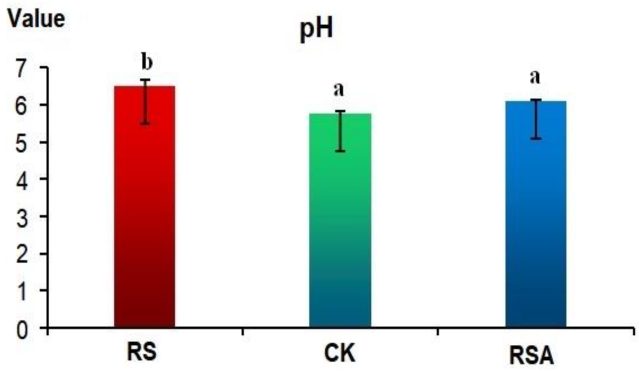

Fig. 2. Effects of different rice straw incorporation methods on the pH value of the soil.
Note: The data was averaged (n = 3), and the different lower-case letters in the same part of the rice showed significant difference among the different treatments (p-value was less than 0.05).

Effects of Different Rice Straw Incorporation Methods on the α-Diversity of Soil Bacterial Communities

The diversity index and species richness were calculated by using the MOTHUR algorithm. Diversity was expressed by indices of Shannon and Simpson by calculating the number of observed OTUs; species richness is expressed by index of Chao1.

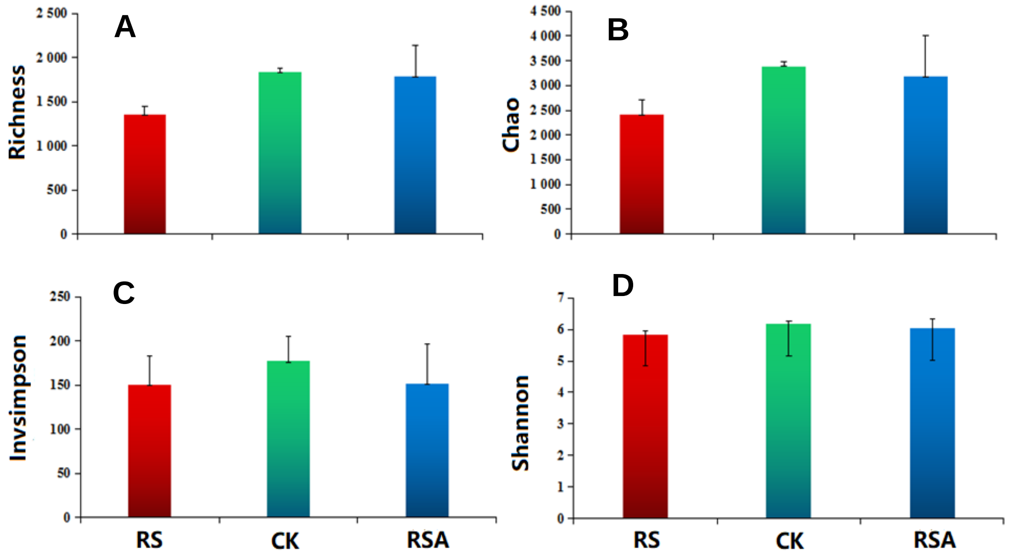

Fig. 3. Effects of different rice straw incorporation methods on the α-diversity of soil bacterial communities

As shown in Fig. 3, rice straw that was directly returned to the field reduced the α-diversity of the soil bacterial community and had a greater impact compared to other treatments on the richness index, the Chao index, and the inv-Simpson index, which decreased by 26.3%, 29.0%, and 15.0% respectively. Rice straw ash returned to field slightly reduced the inv-Simpson index of soil bacterial community, but had a lesser impact on the richness index, the Chao index, and the Shannon index.

The dilution curve reflected the sampling depth of the samples and can be used to evaluate whether the sequencing quantity was sufficient to cover all the groups. Figure 4 shows the dilution curve of all samples in this experiment under a similarity condition of 0.97. As shown in Fig. 4, the dilution curves of all the soil samples were flat, which meant the sampling was reasonable, and the confidence value of the bacterial community structure in a real environment was high, which more truly reflected the bacterial community situation of the soil samples.

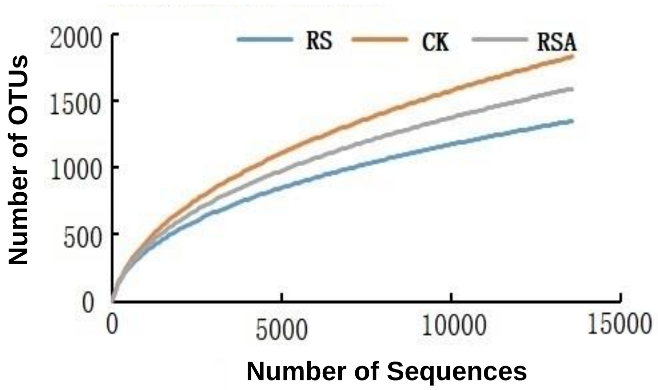

Fig. 4. Rarefaction curves of bacterial 16S gene sequences

Effects of Different Rice Straw Incorporation Methods on the Structure of the Soil Bacterial Community

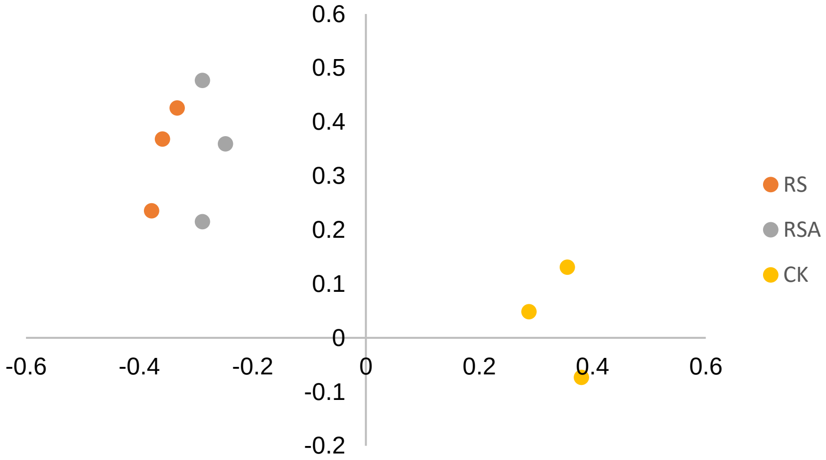

Fig. 5. NMDS analysis of the effect of different methods of rice straw incorporation on bacterial community

NMDS (non-metric multidimensional scaling) was used to compare the differences of samples with different treatments. Figure 5 shows that the distance among points was obvious, which indicated that different treatments of RS, RSA, and CK had significant effects on bacterial community. It can be seen from the Fig. 5 that the difference among the points treated with RS and those treated with CK was the greatest, and the points treated with RSA lay between the points treated with RS and those treated with CK.

As shown in Fig. 6, rice straw directly returned to the field had a major effect on the abundance of different phylum of bacteria. The abundance of Proteobacteria, Bacteroidetes, Gemmatimonadetes, Firmicutes, and Spirochaetae increased by 22.1%, 162.3%, 47.7%, 174.1%, and 158.5%, respectively. The abundance of Acidobacteria, Actinobacteria, Chloroflexi, Verruicrobia, and Nitrospirae decreased by 62.4%, 32.8%, 60.2%, 59.8%, 54.9%, and 96.7%, respectively. The effects of returned rice straw ash were similar to the CK treatment and had little effect on the bacterial community. The abundance of Proteobacteria, Bacteroidetes, Gemmatimonadetes, Chloroflexi, and Verrucomicrobia slightly increased, while the abundance of Firmicutes, Spirochaetae, Actinobacteria, Chloroflexi, and Nitrospirae slightly decreased.

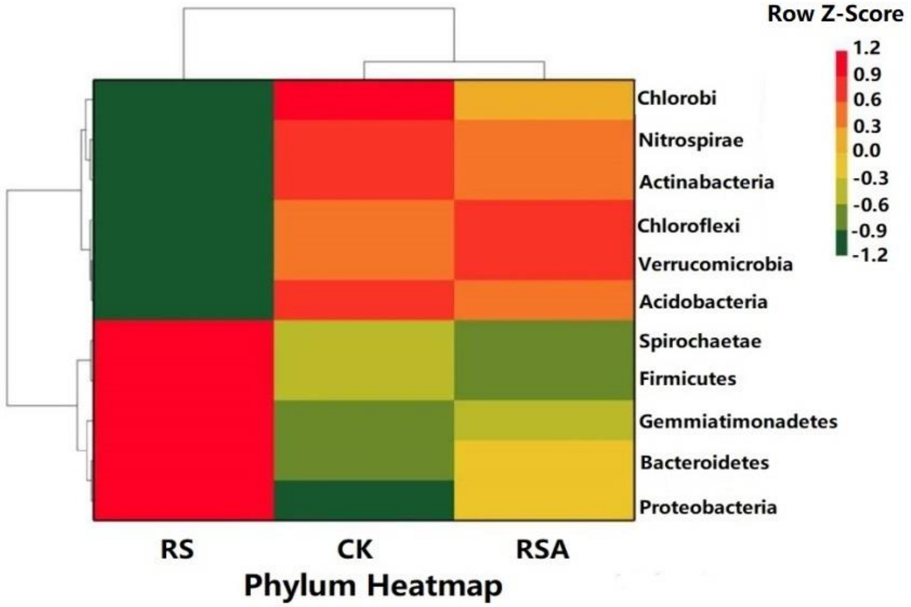

Fig. 6. Effects of RS and RSA at the phylum level in the bacterial community

As shown in Fig.7, rice straw directly returned to the field had a major effect on the community structure of soil bacteria in areas mined for heavy REEs. The percent abundance of *Ideonella*, *Anaeromyxobacter*, *Roseomonas*, *Clostridium sensu stricto 10*, and *Geobacter* was drastically increased by 1070%, 261%, 1196%, 163%, and 66%, respectively. The abundance of *Candidatus solibacter*, *Syntrophoter*, *Haliangium*, and *Candidatus koribacter* was drastically reduced by 38.3%, 61.8%, 40.8%, and 59.7%, respectively. Rice straw ash returned to the field had little effect on the community structure of soil bacteria in areas mined for heavy REEs, which was similar to the CK treatment. The abundance of *Ideonella*, *Anaeromyxobacter*, *Syntrophobacter*, and *Candidatus koribacter* was slightly increased, while the abundance of *Clostridium sensu stricto 10*, *Geobacter*, *Haliangium*, and *Candidatus koribacter* was slightly decreased.

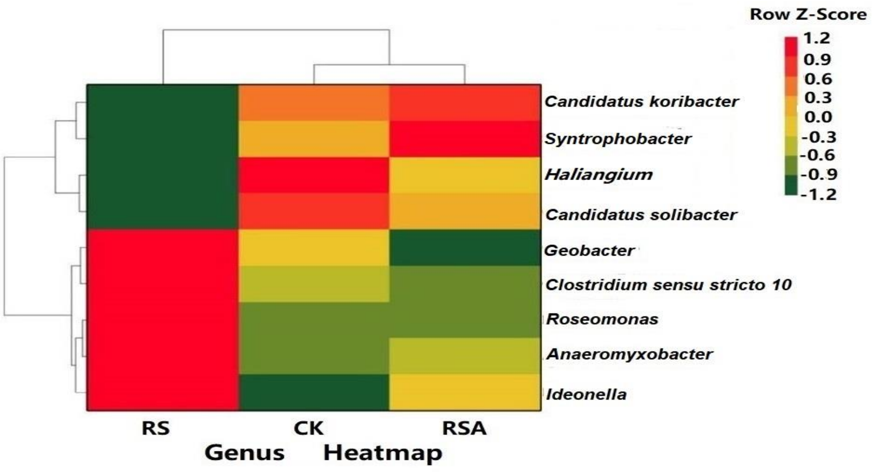

Fig. 7. Effects of RS and RSA at the genus level in the bacterial community

Direct and Indirect Effects of Environmental Factors on Soil Bacterial Communities

As shown in Table 3, the sobs index, the Chao index, and the Shannon index were negatively correlated with the concentrations of REEs in the rice roots, the shoots, and the grains. The rice roots and the grains were more strongly correlated, and the correlation coefficient of the sobs index for the roots and the grains was -1, which showed complete correlation. The inv-Simpson index was negatively correlated with the REE concentration in the rice roots and the grains but had little correlation with the REE concentration in the shoots. The REE concentration in the rice roots and the grains were negatively correlated with their dry weight and positively correlated with their pH. The dry weight of the rice root, the shoots, and the grain were strongly negatively correlated with the pH.

Discussion

Rice growth rates, the bacterial community structure, and the physical and chemical properties of the soil are closely related to environmental factors. Rice straw directly returned to the field and RSA returned to the field had an obvious effect on the soil pH and the amount of organic matter, P and Si, found in the soil (Zhang *et al.* 2019). The tested soil was located in an area mined for heavy REEs, and its soil was red paddy soil with a pH value of 4.9, which made it acidic soil. It was reported that the pH value of the red paddy soil with the addition of RS was higher than without the addition of RS in a submerged state. The reasons for the increase may be as follows: 1) the degradation and ammoniation of organic nitrogen during the decomposition of the RS; 2) the generation of various organic anions during the decomposition of the RS, and the decarboxylation of these organic anions consumed protons, thus, raised the pH value; or 3) the submerged state caused a decrease of the redox potential of the soil, the decomposition of the organic matter produced organic reductive substances, which caused the reduction of high-valent Fe and Mn in the soil, the consumption of protons, and rise of pH value (Huang 2013). In this research, the incorporation of rice straw caused the soil pH to rise by 12.8%., and the soil pH value was raised by 5.7%, since RSA is an alkaline substance.

Table 3. Correlation between the Dry Weight of Rice, REE Concentration of the Rice, Soil pH, and α-Diversity of the Bacteria

	Sobs	Chao	Inv-Simpson	Shannon	GR	SR	RR	GW	SW	RW	pH
Sobs	1	0.993	0.622	0.949	-1.000**	-0.647	-1.000*	0.779	0.241	0.477	-0.856
Chao	-	1	0.707	0.979	-0.992	-0.556	-0.99	0.845	0.35	0.574	-0.909
Inv-Simpson	-	-	1	0.838	-0.61	0.194	-0.602	0.976	0.91	0.985	-0.937
Shannon	-	-	-	1	-0.944	-0.373	-0.94	0.937	0.536	0.731	-0.976
GR	-	-	-	-	1	0.659	1.000**	-0.769	-0.226	-0.463	0.848
SR	-	-	-	-	-	1	0.666	-0.026	0.584	0.361	0.16
RR	-	-	-	-	-	-	1	-0.763	-0.216	-0.455	0.843
GW	-	-	-	-	-	-	-	1	0.796	0.923	-0.991
SW	-	-	-	-	-	-	-	-	1	0.968	-0.708
RW	-	-	-	-	-	-	-	-	-	1	-0.863
pH	-	-	-	-	-	-	-	-	-	-	1

* The correlation was significant at the 0.05 level (double tails, n = 3). GW, SW, and RW represents the dry weight of the rice grains, the shoots, and the roots, respectively. GR, SR, and RR represents the REE concentrations of the rice grains, shoots, and roots, respectively.

Environmental factors, *e.g.* physical and chemical soil properties, are an important reason for a functional structure change in the soil bacterial community. It was reported that the diversity and richness of bacterial communities increased in acidic environments The indexes of the soil bacteria richness, Chao, and inv-Simpson of the soil bacteria decreased, and the pH value increased, due to the direct return of rice straw to the field; the abundance of Acidobacteria, Actinobacteria, Chloroflexi, Verrucomicrobia, and Nitrospirae significantly decreased. Since Nitrospirae is suitable for growing in an environment of low carbon (C) and low nitrogen (N), its abundance decreased with the addition of RS (Zhou *et al.* 2015; Luo *et al.* 2016). Acidobacteria and Nitrospirae are both beneficial bacteria, their reduction can inhibit rice growth and reduce the dry weight of the rice roots, shoots, and grains (Kielak *et al.* 2016; Luo *et al.* 2016). When rice straw was incorporated, heavy REEs had a stronger migration ability than light REEs, which promoted the rice to absorb and accumulate REEs, which increased the REE concentration in the rice, and had adverse effects on the plant-soil ecosystem in the mining area.

The incorporation of rice straw increased the total nitrogen in the soil, the total organic carbon in the soil, and the soil moisture content, which resulted in a significant increase in the abundance of Proteobacteria (Zhao *et al.* 2017). It was reported that Firmicutes were related to the REE concentration, and their abundance increased when the environment was polluted (Jin *et al.* 2018). The high temperatures during summer promotes the rapid decomposition of the RS, which led to soil eutrophication and inhibited the growth of beneficial bacteria. DOM formed from the rice straw decomposition is a negatively charged colloid, which can promote the dissolution and desorption of REEs in the soil, increase the soluble REEs in the soil, and pollute the soil environment, so the abundance of Firmicutes was significantly increased. DOM is divided into peptides, carbohydrates, and unsaturated fats; an increase in these materials can result in the increase of Spirochaetae. The incorporation of rice straw increased the levels of cellulose, hemicellulose, lignin, *etc.* in the soil. Bacteroidetes can degrade cellulose, which is suitable for metabolizing DOM with a high molecular weight. Its abundance was positively correlated with the pH; therefore, the abundance of Bacteroidetes increased significantly with the addition of RS in the polluted soils (Lauber *et al.* 2009; Nacke *et al.* 2011; Leff *et al.* 2015). The increase in DOM contributed to the increase in the proportion of Alphaproteobacteria and Gemmatimonades in the whole microbial communities (Blanchet *et al.* 2015; Traving *et al.* 2017). Studies have shown that the microbial diversity decreased significantly and that the proportion of Chloroflexi decreased due to biofertilizer treatments (Cahyadi *et al.* 2017; Masrukhin *et al.* 2017). The microbial diversity after the bio-fertilizer treatment decreased significantly, and the proportion of Chloroflexi decreased as well (Masrukhin *et al.* 2017), and Verrucomicrobia is closely related to the type, quality and quantity of organic matter.

Genus *Ideonella* belongs to the class Betaproteobacteria, and the amount of Betaproteobacteria increased with the rise in pH (Chen *et al.* 2016). *Proteobacteria* account for the largest proportion of bacterial communities in the soil, and the soil pH value increased after the addition of RS. The amount of *Ideonella* increased in both soils and the changes in the relative abundance of *Ideonella* were related to the changes in the physical and chemical properties of the soil, as well as the organic matter content (Jones *et al.* 2009). The incorporation of rice straw and rice straw ash can promote the transformation of mineral nutrients, and increase the inorganic nutrients in the soil, *e.g*, P and K (Tarasova *et al.* 2015; Geelhoed *et al.* 2016). These inorganic nutrients are beneficial to Deltaproteobacteria, so its proportion also increased in the whole soil microbial community.

Thus, the abundance of *Geobacter* and *Anaeromyxobacter*, which belong to Deltaproteobacteria, also increased. Because the abundance of Alphaproteobacteria increased after the incorporation of rice straw, the abundance of the genus *Roseomonas* belong to this phylum also increased. The increased abundance of *Roseomonas* was due to the improvement of the soil nutrients (Ahn *et al.* 2012). *Clostridium sensu stricto 10* belongs to the phylum Firmicutes, and the increase in its abundance was caused by the increase in the concentration of REEs in the soil (Jin *et al.* 2019). Both *Candidatus Solibacter* and *Andidatus Koribacter* belong to the phylum Acidobacteria, and their abundance decreased with the increase in pH after the incorporation of rice straw (Bulgarelli *et al.* 2013; Chhabra *et al.* 2013).

The addition of 2.5% RS to the soil of areas mined for medium and light REEs significantly promoted rice growth and improved the dry weight of the rice roots, the shoots, and the grains (Jin *et al.* 2018). In this research, the incorporation of rice straw had little effect on the diversity and richness of the bacterial community in areas mined for heavy REEs, and it also had little effect on the growth of the rice, the dry weight, or the REE concentration of the rice roots, the shoots, or the grain. This could be caused by two reasons: 1) the differences between medium and light REEs, and heavy REEs; or 2) that a 1% RSA treatment had very little impact on the plant-soil ecosystem in the mining area due to its low concentration.

CONCLUSIONS

1. The incorporation of rice straw in areas mined for heavy REEs raised the soil pH value and reduced the α-diversity of the soil bacterial community; which had a major impact on the richness, Chao, and inv-Simpson indexes. In addition, it inhibited the growth of beneficial bacteria, promoted the migration of REEs in the soil, especially heavy REEs, inhibited the growth of the rice, reduced the dry weight of the rice, and increased the REE concentration in the rice.
2. The incorporation of rice straw in areas mined for heavy REEs significantly reduced the abundance of Acidobacteria, Actinobacteria, Chloroflexi, Verrucomicrobia, and Nitrospirae, while it significantly increased the abundance of Proteobacteria, Bacteroidetes, Gemmatimonadetes, Firmicutes, and Spirochaetae. It also reduced the abundance of *Candidatus Solibacter*, *Syntrophobacter*, *Haliangium*, and *Candidus Koribacter* while it significantly increased the abundance of *Ideonella*, *Anaeromyxobacter*, *Roseomonas*, *Clostridium sensu stricto 10*, and *Geobacter*.
3. Rice straw ash returned to the field had little effect on the diversity and richness of the bacterial community in areas mined for heavy REEs. In addition, the effects on the rice growth, the dry weight of the roots, the shoots, and the grains of rice, the REE concentration, and its overall effects on the plant-soil ecosystem of REE mining area were also slight.

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