# Effects of Biogas Slurry Application Years on Remediation of *Pennisetum × sinese* on Soil Physical and Chemical Properties and Microorganisms of Rare Earth Tailings

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**Abstract** [**Objectives**] This study was conducted to analyze the effects of continuous application of biogas slurry for many years on soil ecosystem restoration of rare earth tailings by planting *Pennisetum × sinese*, in order to provide basis for scientific application of biogas slurry. [**Methods**] The fields with different years of continuous application of biogas slurry in Dingnan Rare Earth Tailings Ecological Restoration Demonstration Park were selected as the research object, and the differences in soil physical and chemical properties and microbial community structure after application of biogas slurry for different years (0, 3 and 5 years) were studied. [**Results**] The bulk density of soil with continuous application of biogas slurry showed a downward trend, while the maximum water holding capacity, capillary water holding capacity, porosity, aeration, pH, organic matter, nitrogen, phosphorus and potassium, alkali-hydrolyzable nitrogen and available phosphorus showed an upward trend. Moreover, the effects achieved by application for 5 years were better than those by application for 3 years. Continuous application of biogas slurry could significantly improve the activity of soil urease, acid phosphatase, sucrase and cellulase, and it effects increased with the application year increasing. Continuous application of biogas slurry could significantly improve the abundance of dominant bacteria in soil, and with the increase of application years, the abundances of dominant bacteria also increased. [**Conclusions**] Continuous application of biogas slurry effectively improved soil physical and chemical properties and soil fertility in rare earth tailings areas where *Pennisetum × sinese* was planted to restore rare earth tailings. This study provides a theoretical support for establishing key ecological restoration technoiques.

**Key words** Biogas slurry; Application period; Rare earth tailings; Soil physical and chemical properties; Soil microorganism

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China's ionic rare earth minerals are one of the main sources of heavy rare earth minerals in the world, and most of them are located in Ganzhou City, Jiangxi Province. Because of their special mining techniques, long-term disorderly mining and lagging environmental protection, ionic rare earth tailings face ecological and environmental problems such as soil acidification, high bulk density, decreased fertility, destruction of surface vegetation and serious soil erosion[1]. Phytoremediation can not only effectively control soil erosion and prevent soil degradation, but also has the advantages of less investment, easy management, no secondary pollution and good effect, and it is one of the most effective measures to prevent soil degradation[2]. *Pennisetum × sinese* is a perennial herb of *Pennisetum* in Gramineae with developed root system and strong resistance, which is widely used in ecological restoration of mines[3]. Studies have shown that in the process of phytoremediation, reasonable fertilization can effectively improve soil, promote plant growth and shorten remediation period[4]. Biogas slurry, as a by-product of biogas production by anaerobic fermentation of livestock and poultry excreta, contains a lot of nutrients needed by plant growth, and is often used as a high-quality organic liquid fertilizer and improver in farmland system[5-6]. The balanced nutrient of biogas slurry can be quickly absorbed by plants, which not only improves crop yield and quality, but also facilitates the improvement of soil fertility[7].

Soil physical and chemical properties and soil biochemical properties are important indicators for evaluating phytoremediation effect[8]. Soil enzymes are produced by the metabolism of soil microorganisms or the decomposition of soil animal and plant residues[9], and their activity can serve as an important indicator for evaluating soil environmental quality and soil remediation effectiveness[10]. Studies have shown that soil enzyme activity is closely related to soil physicochemical indicators, but the effects of different regions, vegetation types and remediation treatments on soil enzyme activity vary. Relevant research results show that returning biogas slurry to the field can improve nitrogen absorption, microbial enzyme activity and soil microbial community structure[8].

Therefore, studying the effects of continuous application of biogas slurry on soil physicochemical properties and microbial communities after *Pennisetum × Chinese* remediation of ionic type rare earth tailings in southern Jiangxi and evaluating the ecological effects of this plant remediation model are of great significance for exploring the optimal soil remediation model for ionic rare earth tailings in southern Jiangxi. Previous studies on remediation of rare earth tailings are mainly focused on the screening of remediation agents, remediation plants and models, and the effects of different remediation models on soil physicochemical properties[11-13], while few studies have been conducted on the changes in soil physicochemical properties and microorganisms after plant

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remediation of rare earth tailings. Therefore, with plots continuously applied with biogas slurry for different years as the research object, the differences in soil physical and chemical properties and microbial community structure between different application years (0 year, 3 years, 5 years) of biogas slurry were studied, aiming to provide a theoretical basis for exploring soil remediation modes for ionic rare earth tailings in southern Jiangxi.

## Materials and Methods

### General situation of experimental area

The experimental field was located in Lingbei Green Eco-agriculture Circular Park, Dingnan County, Ganzhou City (25°01′27.47″ N, 115°04′47.53″ E, altitude 458 m). The surface of the experimental mining area was exposed before restoration, and there was almost no vegetation coverage. The soil texture was sandy loam.

### Experimental materials

The experimental planting variety was *P. purpureum* × *P. americanum* cv. Reyan No. 4, and the biogas slurry was provided by Dingnan Green Eco-agriculture Circular Park. The nutrient contents of the biogas slurry were as follows: organic matter 0.81%, total nitrogen 0.65%, total phosphorus 0.11% and total potassium 0.09%.

### Experimental design

Three treatments were set in the experimental field, namely, no application of biogas slurry (CK), continuous application for 3 years (T20) and continuous application for 5 years (T18). Specific treatment of T18: From November to December, 2018, biogas slurry was applied as base fertilizer at a rate of 400 t/hm$^2$ to the abandoned mine, and *P. purpureum* × *P. americanum* cv. Reyan No. 4 was planted in the period from late March to early April, 2019. Specific treatment of T20: From November to December, 2020, biogas slurry was applied as base fertilizer at a rate of 400 t/hm$^2$ to the abandoned mine, and *P. purpureum* × *P. americanum* cv. Reyan No. 4 was planted in the period from late March to early April, 2021. *P. purpureum* × *P. americanum* cv. Reyan No. 4 was planted at a density of 1.0 m × 1.0 m, and biogas slurry was applied after each mowing. After mowing in mid-June, 100 t/hm$^2$ of biogas slurry was applied as topdressing. After mowing in mid and late July, 120 t/hm$^2$ of biogas slurry was applied. After mowing in early September, 150 t/hm$^2$ of biogas slurry was applied. At the end of November, 100 t/hm$^2$ of biogas slurry was applied after mowing.

### Soil sample collection

Soil samples were collected in March 2023 (before fertilization), and surface soil samples of 0 – 20 cm were collected by the 5-point sampling method. Three undisturbed soil samples taken by cutting rings were collected from each sampling point for the determination of physical properties, and another 1 kg of soil sample was taken from the same sampling point and mixed evenly. After removing stones, plant roots and other impurities, part of the original soil samples were stored in a refrigerator for the determination of soil enzyme activity and soil microorganisms, and the other part was placed indoors for air drying, and then ground and sieved with 2 and 0.25 mm sieves for the determination of chemical properties.

### Determination indexes and methods

Soil bulk density, maximum moisture capacity, capillary moisture capacity, field moisture capacity, soil porosity and aeration were all measured by the cutting ring method, and the calculation methods referred to the industry standard *Determination of forest soil water-physical properties* (LY/T 1215-1999)[14].

The determination of soil chemical properties referred to Bao Shidan's *Soil Agrochemical Analysis*[15]. In specific, soil pH was determined by potentiometry. The determination of soil organic matter adopted the method of potassium dichromate external heating. Soil alkali-hydrolyzable nitrogen was determined by alkali-hydrolyzable diffusion neutralization titration. Available phosphorus was determined by molybdenum-antimony colorimetry. And available potassium was determined using a flame photometer.

The determination of soil enzyme activity referred to Guan Songyin's *Soil Enzymes and their Research Methods*[16]. In specific, urease activity was determined by indophenol blue colorimetry. The determination of acid phosphatase activity adopted p-nitrophenylphosphate disodium salt. Sucrase and cellulase activity were determined by 3,5-dinitrosalicylic acid colorimetry.

Extraction of soil total DNA and sequencing of 16S rRNA gene: After collecting soil samples, they were immediately sent to Shanghai Majorbio Bio-pharm Technology Co., Ltd. for sequencing of the V4-V5 region of 16S rRNA gene, using 515F (5-gtgycagcmgcgcggta-3′) and 806R (5′-ggactachvgggtwtctaat-3′) as primers respectively. Product recovery and purification were performed using the AxyPep DNA Gel Extraction Kit (AxygenBiosciences, Union City, CA, USA), and the recovered products were quantitatively determined by Quantus analysis Fluorometer (Promega, USA). Library construction was conducted using NEXTflex™ Rapid DNA Seq Kit (BiooScientific, USA), and Illumina Miseq platform was used for sequencing.

### Data analysis and comprehensive evaluation

Microsoft Excel 2013 was used for data statistical analysis and plotting. SPSS 24.0 was used for one-way ANOVA and significance analysis. Correlation analysis charts was drawn by OriginPro 2021. The 16S rRNA sequencing data of microorganisms were calculated and analyzed by R studio software.

## Results and Analysis

### Differences in soil physical properties after application of biogas slurry for different years

It can be seen from Table 1 that continuous application of biogas slurry could significantly improve the physical structure of soil, and with the increase of application years, soil structure was continuously improved. After continuous application for 3 years and 5 years, soil bulk density was significantly lower than that of the CK ($P < 0.05$), and the value decreased by 10.6% and

23.0% respectively. The maximum moisture capacity after 3 and 5 years of continuous biogas slurry application was significantly higher than that of the CK group ($P<0.05$), and the value increased by 22.4% and 35.4% respectively. Meanwhile, the maximum water holding capacity after 5 years of continuous application was significantly higher than that after 3 years of application. Capillary water holding capacity increased with biogas slurry application years increasing, and after 3 and 5 years of continuous application, it increased significantly by 79.7% and 91.9% respectively compared with the CK group. Field water holding capacity increased significantly with the increase of biogas slurry application years ($P<0.05$). Compared with the CK group, the field water holding capacity of soil increased by 12.8% and 20.7% after 3 and 5 years of biogas slurry application, respectively. Continuous application of biogas slurry could significantly improve soil porosity, and it increased with the application years increasing. After 3 and 5 years of continuous application, soil porosity increased by 28.3% and 47.7% compared with the CK group. Soil aeration increased significantly with the increase of biogas slurry application years ($P<0.05$), and after 3 and 5 years of continuous application, it was 21.2% and 47.4% higher than that of the CK group, respectively.

**Table 1 Effects of different biogas slurry application years on soil physical properties**

| | Bulk density g/cm$^3$ | Maximum moisture capacity//g/kg | Capillary moisture capacity//g/kg | Field moisture capacity//g/kg | Soil porosity//% | Soil aeration//% |
|---|---|---|---|---|---|---|
| T18 | 1.13±0.054 3 a | 392.13±20.13 a | 288.33±19.23 a | 192.26±8.981 a | 52.37±8.231 a | 29.82±2.368 a |
| T20 | 1.25±0.042 8 b | 354.34±23.69 b | 270.00±20.12 a | 171.96±12.13 b | 45.47±5.342 a | 25.67±3.289 b |
| CK | 1.39±0.061 1 c | 289.45±30.21 c | 150.23±23.98 b | 152.45±7.346 c | 35.45±4.234 b | 20.23±1.348 c |

Different lowercase letters following data in the same column indicate a significant difference between different application years ($P<0.05$). The same below.

**Differences in soil pH value and nutrient contents after application of biogas slurry for different years**

As shown in Table 2, continuous application of biogas slurry could significantly improve soil pH and increase soil nutrient contents ($P<0.05$). With the increase of application years, soil pH value increased significantly. Compared with the CK group, soil pH value increased by 0.40 and 0.41 units after application of biogas slurry for 3 and 5 years, respectively. The content of soil organic matter increased significantly with the application years of biogas slurry increasing ($P<0.05$). Compared with the CK group, the content of soil organic matter increased to 275.4% and 424.8% respectively after 3 and 5 years of continuous application. The application of biogas slurry could significantly increase alkali-hydrolyzable nitrogen content ($P<0.05$), and it increased with the application years of biogas slurry increasing. Compared with the CK group without biogas slurry application, the content of soil alkali-hydrolyzable nitrogen increased by 426.1% and 461.7% after 3 and 5 years of continuous application, respectively. Also, the application of biogas slurry could significantly increase the content of available potassium in soil ($P<0.05$), and it increased significantly with the application years of biogas slurry increasing. Compared with the CK group, the content of soil available potassium increased by 149.2% and 180.7%, respectively, after 3 and 5 years of continuous application. The application of biogas slurry could significantly increase the content of soil available phosphorus ($P<0.05$), but its content did not change significantly with the increase of application years.

**Table 2 Nutrient contents and pH of soil after application of biogas slurry for different years**

| | pH | Organic matter//g/kg | Alkali-hydrolyzable nitrogen//mg/kg | Available potassium//mg/kg | Available phosphorus//mg/kg |
|---|---|---|---|---|---|
| T18 | 6.15±0.052 9 a | 17.53±2.448 0 a | 288.33±28.39 a | 127.26±6.722 a | 169.65±4.330 a |
| T20 | 6.14±0.043 5 a | 12.53±0.816 2 b | 270.00±11.83 a | 112.96±2.352 b | 169.32±11.34 a |
| CK | 5.74±0.032 9 b | 3.34±0.609 8 c | 51.33±9.375 b | 45.33±3.225 c | 0.98±0.337 9 b |

**Differences in soil enzyme activity after application of biogas slurry for different years**

It can be seen from Table 3 that the application of biogas slurry could significantly improve the activity of soil acid phosphatase, urease, sucrase and cellulase ($P<0.05$). With the increase of application years, the activity of soil acid phosphatase also increased. Compared with the CK group, the activity of acid phosphatase increased by 87.47% and 226.79% after continuous application of biogas slurry for 3 and 5 years, respectively. Compared with the treatment of no biogas slurry, soil urease activity increased by 366.67% and 516.67%, respectively, after application of biogas slurry for 3 and 5 years ($P<0.05$). Soil sucrase activity increased with the application years of biogas slurry increasing. Compared with the CK group, soil sucrase activity increased by 632.82% and 764.88% after 3 and 5 years of continuous biogas slurry application, respectively. Also, the application period of biogas slurry significantly affected soil cellulase activity. Compared with the CK group, soil cellulase activity increased by 158.13% and 249.18% after continuous application of biogas slurry for 3 and 5 years, respectively.

**Difference in soil microbial community diversity after application of biogas slurry for different years**

**Difference in relative abundance of soil microbial community after application of biogas slurry for different years** As shown in Fig. 1, the relative abundance of microbial communities in different soil samples was analyzed. It could be known that after different years of biogas slurry application, soil microorganisms were similar at the phylum level, but there were certain differences in relative abundance. The dominant bacterial groups in the soil samples of the CK group were Proteobactercia, Acidobacteria, Chloroflexi, Planctomycetes and GAL15, with relative abundances of 21.08%, 30.14%, 12.25%, 6.35%, and 7.39%, respectively, accounting for 77.21% of the total bacterial count.

**Table 3 Enzyme activity of soil after application of biogas slurry for different years**

| | Acid phosphatase // μg/(d·g) | Urease // mg/(d·g) | Sucrase // mg/(d·g) | Cellulase // mg/(d·g) |
|---|---|---|---|---|
| T18 | 61.83 ±1.580 0 a | 0.74 ±0.019 76 a | 11.33 ±0.045 09 a | 8.59 ±0.114 60 a |
| T20 | 35.47 ±0.957 8 b | 0.56 ±0.011 0 b | 9.60 ±0.270 50 b | 6.35 ±0.079 63 b |
| CK | 18.92 ±0.590 8 c | 0.12 ±0.007 0 c | 1.31 ±0.041 58 c | 2.46 ±0.098 02 c |

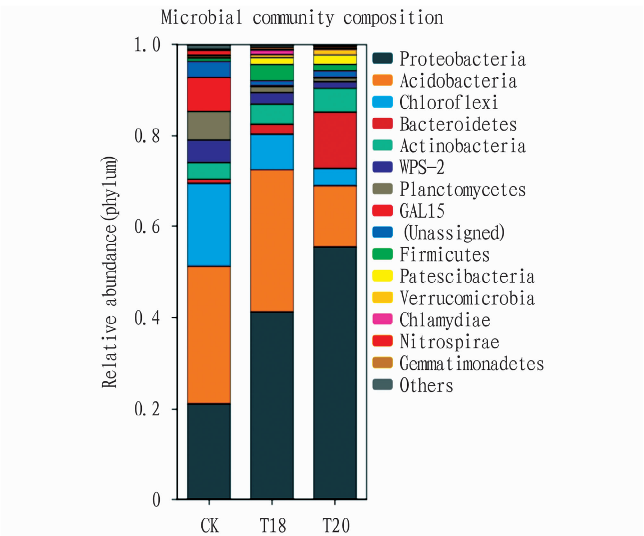


**Fig. 1 Relative abundance of soil microbial community**

The dominant bacterial groups in the soil samples of the treatment groups were Proteobactercia, Acidobacteriaceae, Chloroflexi, Bacteroidota and Acidobacteria, and the content of these bacterial groups accounted for more than 80% of the total bacteria. In treatment T20 with 3 years of biogas slurry application, the relative abundances of Proteobacteria, Acidobacteria, Chloroflexi, Bacteroidota and Actinobacteria were 41.22%, 31.27%, 7.79%, 2.21% and 4.41%, respectively, accounting for 86.9% of the total bacteria. In treatment T18 with 5 years of biogas slurry application, the relative abundances of Proteobacteria, Acidobacteria, Chloroflexi, Bacteroidota and Actinobacteria were 55.50%, 13.47%, 3.82%, 12.33% and 5.25%, respectively, accounting for 90.37% of the total bacteria. Compared with the CK treatment, the application of biogas slurry mechanism obviously improved the relative abundance of Proteobacteria, but the relative abundance of Chloroflexi decreased. Meanwhile, with the application years increasing, the relative abundance of dominant microorganisms also increased.

**Difference in soil microbial diversity indexes after application of biogas slurry for different years** As shown in Fig. 2, in order to further study the response of flora diversity to different treatments, the α diversity index of each sample was calculated and the differences of α diversity among groups were analyzed. The results showed that there were significant differences in α diversity among groups. In the evaluation of richness, Chao1 index identified by T20 had no significant difference from the CK group. Compared with the CK group, T20 treatment showed a significant difference ($P<0.05$) in the Simpson index based on the comprehensive uniformity and richness. T18 and the CK group had no significant difference in uniformity and richness.

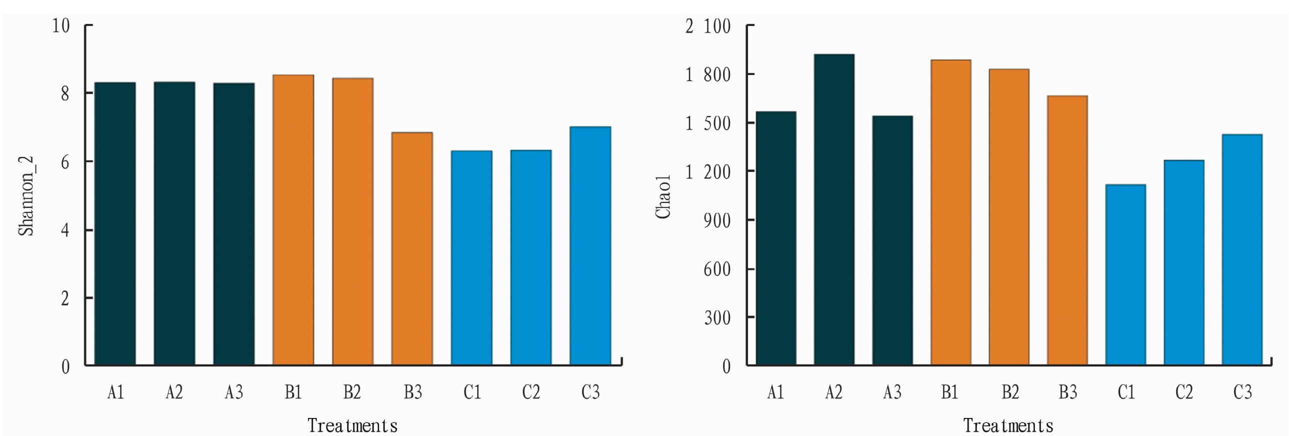


**Fig. 2 Statistical chart of α diversity index**

As shown in Fig. 3, the sample relationship β diversity analysis conducted on soil microbial communities among different treatments was more intuitive. The results showed that compared with the CK group, the intra-group distances of various groups were closer, while the distances from the control group were farther, indicating that there were differences in community structure. Compared with the CK, treatments T18 and T20 were different in the composition of soil microorganisms. PCoA analysis and NMDS analysis mutually confirmed this point.

As shown in Fig. 4, in order to explore how different treatments affected soil microorganisms, Lefse analysis was adopted. The threshold $LDA = 2$ was set to study the indicator species with significant differences between the CK group and various treatment groups. The results showed that there were many microbial species with significant differences between treatments T18 and T20 and the CK group. There was obvious biological correlation between differential microorganisms.

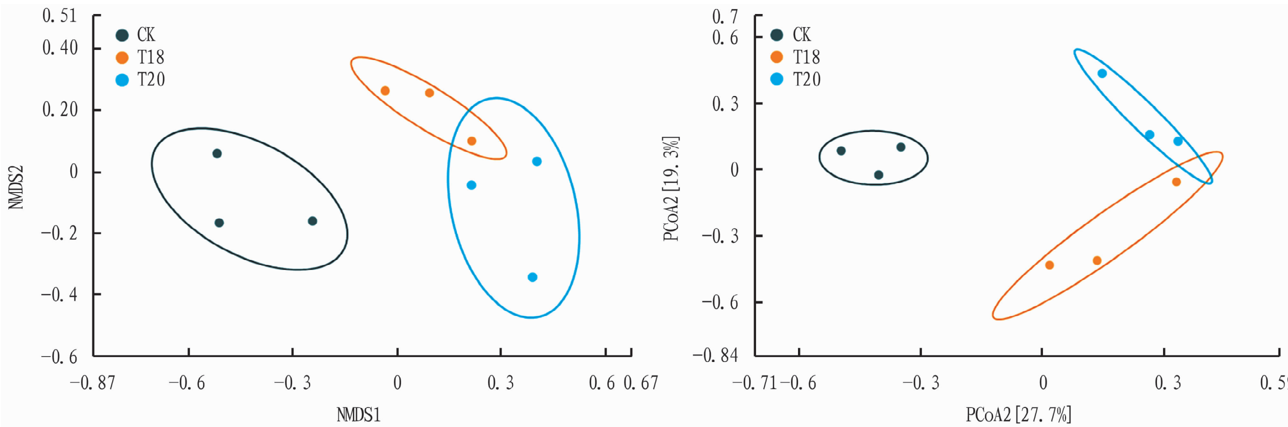


**Fig. 3 β diversity analysis**

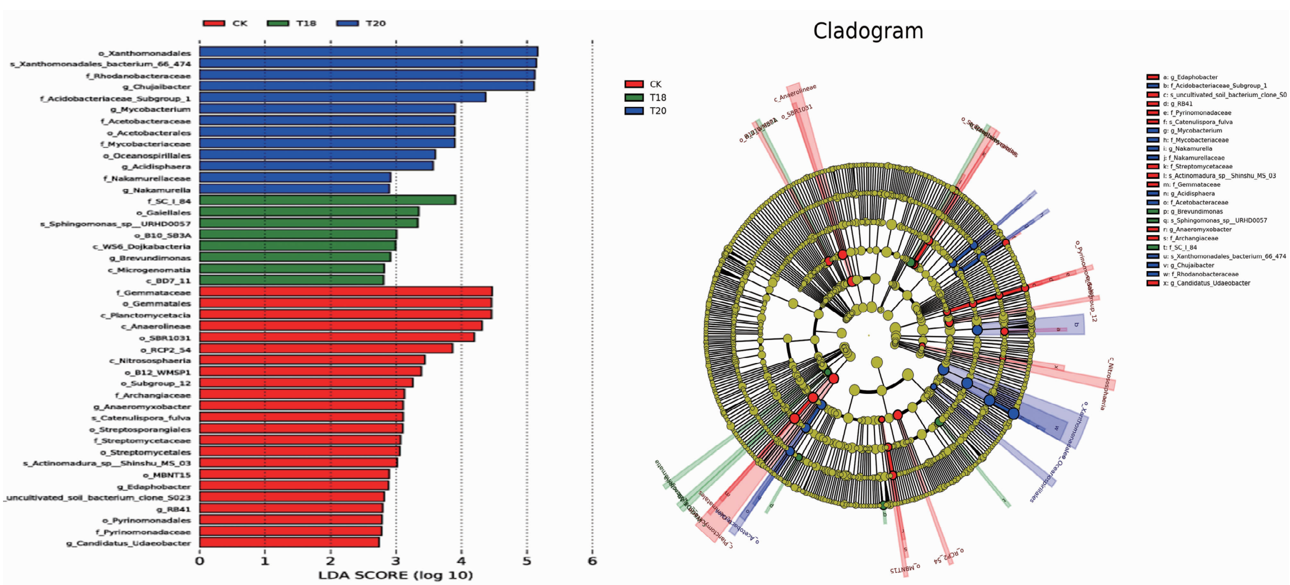


**Fig. 4 Lefse analysis**

# Conclusions and Discussion

## Effects of biogas slurry application on soil physical and chemical properties

Studies have shown that the application of biogas slurry can significantly improve the physical and chemical properties of soil[18]. Biogas slurry, as an organic fertilizer, can increase the contents of total nitrogen, total potassium, total phosphorus, available nitrogen, available phosphorus and available potassium in soil, and improve soil fertility[19-20]. The application of biogas slurry is helpful to the formation of soil aggregate structure, the increase of soil water retention and the reduction soil bulk density, thus improving soil structure[21]. The pH value of biogas slurry is mainly distributed in the neutral range, so the application of biogas slurry is helpful to the adjustment of the pH value of soil that is over-acidic or over-alkaline, and make it tend to be neutral gradually[22-24]. Biogas slurry can activate trace elements such as copper, zinc and iron in soil to meet the nutritional needs of soil microorganisms[25-26]. In this study, the application of biogas slurry could significantly improve soil nutrient contents and significantly increase pH value of acidic soil, which is consistent with previous studies.

## Effects of biogas slurry application on soil enzyme activity

Soil enzymes play multiple important roles in the formation and transformation of soil humus, nutrient cycling and driving of biochemical reactions, so they are important indicators for evaluating soil health. The results showed that the application of biogas slurry had significant effects on soil enzyme activity[27]. The application of biogas slurry could improve the activity of soil enzymes, including sucrase, urease, catalase, and alkaline phosphatase[28-29]. Specifically, the application of biogas slurry can activate some enzymes in soil and establish a good soil environment[30]. After long-term application of biogas slurry, soil hydrolase and oxidoreductase activity will increase significantly, such as protease, sucrase, urease, acid phosphatase, cellulase, nitrate reductase, nitrite reductase, dehydrogenase and catalase[17]. In addition, the application of biogas slurry can also improve soil urease activity and catalase activity, which has a positive impact on soil biochemical process[31]. In this study, the application of biogas slurry significantly improved soil enzyme activity, which is consistent with previous research results.

**Effects of biogas slurry application on soil microbial community**

Soil microorganisms are very important to soil health, and they maintain soil ecological balance through multiple functions[32]. Soil microorganisms can decompose organic matter, recycle nutrients, and increase soil fertility, thus providing a rich nutritional basis for plant growth[33]. Meanwhile, they can maintain the stability of soil structure, enhance the ability of soil to maintain water and nutrients, and reduce the impact of soil-borne diseases, thereby protecting the healthy growth of crops. In addition, microorganisms as biological control agents can inhibit the growth of pathogenic microorganisms, and reduce the occurrence of diseases and the use of chemical pesticides. The diversity of soil microorganisms is also an important indicator of agricultural ecosystem health. Rational utilization and management of microbial resources can promote ecological balance and maintain and increase biodiversity[32-33]. The application of biogas slurry has a significant effect on soil microbial community structure. Biogas slurry from different sources (such as chicken manure, pig manure and cow manure) has different effects on soil microbial carbon metabolism, including increasing inorganic nitrogen, total nitrogen, available phosphorus, available potassium, pH and conductivity[34]. The application of biogas slurry can also increase the richness and diversity of soil microorganisms, and increase soil probiotics such as *Sphingomonas* and *Lysobacter*[35]. Moreover, the application of biogas slurry is beneficial to balanced growth and population distribution of various microorganisms in the soil, but it may also reduce fungal diversity in soil aggregates of some particle sizes[36]. Under the experimental conditions of this study, continuous application of biogas slurry significantly improved the abundance of dominant bacterial communities in soil and increased the uniformity of microorganisms, which is basically consistent with previous studies.

In a word, continuous application of biogas slurry in rare earth tailings areas where *Pennisetum* × *sinese* is planted can achieve the effects of activating soil dominant microorganisms, improving soil enzyme activity, soil structure and soil fertility, facilitating the growth of green crops, and shortening bioremediation cycle.

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treatment $T_3$ (high-concentration inorganic fertilizer). Compared with treatment $T_1$ (low-concentration inorganic fertilizer), the partial factor productivity of nitrogen and potassium fertilizers increased by 6.2% and 13.78%, respectively, while that of phosphate fertilizer decreased by 11.5%. Meanwhile, the agronomy fertilizer use efficiency of nitrogen (phosphorus, potassium) fertilizer was significantly improved.

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