



Preparation of Biofertilizer with Phosphogypsum and Straw: Microbial Community Changes and Plant Growth Effects

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Received: 11 December 2023 / Accepted: 29 April 2024 / Published online: 9 May 2024
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Abstract

Purpose The accumulation of solid phosphogypsum waste poses a great ecological threat. Phosphogypsum is an industrial waste, but the main way of dealing with this waste is still accumulation. This is not only a waste of land but also pollutes the environment. However, environmental problems exist in the treatment of phosphogypsum via physical and chemical methods, and this study aims to present a new environmentally friendly method to treat phosphogypsum and promote its comprehensive utilization. We analyzed the microbial community changes that occur in the phosphogypsum composting process and determined the feasibility of utilizing the biofertilizer prepared in this study through plant experiments.

Methods Biofertilizers were prepared by combining phosphogypsum with waste biomass straw and livestock manure. A composite microbial agent with live bacteria was added to form the finished phosphogypsum biofertilizer after composting. In this study, we simultaneously used high-throughput sequencing and other methods to explore microbial community changes and dominant flora as well as microbial functions. This biofertilizer was applied in plant experiments to test the growth of plants.

Results During the course of the experiments, there was an increase in total nitrogen, exchangeable potassium, and soluble phosphorus in the soil. Among soil physical properties, there was also a significant increase in porosity. During the composting process, the dominant bacteria were Actinobacteria, which accounted for 41.82% of the total. However, over the composting process, this group showed a decreasing trend. The application of this biofertilizer in plant experiments resulted in a significant increase in plant growth; the chlorophyll content of biofertilized pakchoi was more than twice as high as that of unfertilized pakchoi, and the vitamin C content of biofertilized ryegrass was more than twice as high as that of unfertilized ryegrass.

Conclusion Overall, changes in the dominant flora were found during the composting process, and *Actinobacillus* was the most stable flora, which showed a decreasing trend but consistently accounted for the highest percentage of bacteria during the experiment. Additionally, plant growth performance was tested, and the parameters included plant height, length, weight, vitamin C and chlorophyll. Without exception, the growth parameters in the plants grown with phosphogypsum biofertilizer were better than those of the control group. The biofertilized plants had more developed root systems and higher values for all indicators. This indicates the benefits of using phosphogypsum as a biofertilizer.

Keywords Phosphogypsum · Biofertilizer · Soil Characteristics · Plant Growth · Microbial Community Analysis

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1 Introduction

According to statistical analysis, every 1 ton of phosphoric acid produced leads to the generation of at least 4.5 tons of phosphogypsum (Zhang et al. 2019). At this stage, landfills and open storage are still the most popular methods of phosphogypsum disposal. This causes a wastage of land and poses a risk to the environment (Wang et al. 2023). Many scholars believe that phosphogypsum is more prone to soil and water contamination when stockpiled, mainly due to improper stockpiling in most cases (Vyshpolsky et al. 2008). The production of phosphate fertilizers leads to higher phosphogypsum production, and the stockpiling of phosphogypsum places a major pressure on the environments (Didamony et al. 2013). Currently, the global stockpile of phosphogypsum has exceeded 6 billion tons and continues to grow at a rate of approximately 200 million tons per year. However, the comprehensive global utilization rate of phosphogypsum is only approximately 25%. Except for a few countries such as Japan, which have a comprehensive utilization rate of up to 100% because of the lack of gypsum resources, most countries stockpile phosphogypsum. (Cui et al. 2022)

In 2021, the total production of phosphate fertilizer (P_2O_5) in China was 16.844 million tons, an increase of 3.2% from the previous year. The production of phosphogypsum was approximately 80 million tons, an increase of approximately 3.9% from the previous year. (Lu et al. 2021) Phosphate fertilizer production leads to the generation of a large amount of phosphogypsum each year as a byproduct, which currently cannot be properly disposed of and is stockpiled. The stacking of phosphogypsum not only consumes a large amount of land but also pollutes the surrounding ecological environment (Dong et al. 2023). The China Phosphorus Compound Fertilizer industry association estimates that approximately 870 million tons of phosphogypsum will have accumulated in China by 2021 (Cui et al. 2022a). Although the comprehensive utilization rate of phosphogypsum is slowly increasing, it is still less than 50% overall. Phosphogypsum is mostly used in industries, and only a small proportion is used to prepare biofertilizers (Cui et al. 2022b). Phosphogypsum is usually in the form of gray powder, and its particle size generally ranges from 5 μm to 150 μm (El Mouzdahir et al. 2009).

By reducing the acidity of soil and increasing the content and effectiveness of soil nitrogen, phosphorus, and calcium, phosphogypsum can improve soil quality and provide nutrients to plants. Additionally, it can reduce the activity of soil aluminum through chemical reactions such as substitution, complexation, and precipitation of various components, improving acidic yellow soils (Xiao et al. 2008). Phosphogypsum is applied to improve soil fertility and enhance soil

trace elements, which can improve plant growth to some extent.

According to statistics, China is the most productive country in the world in terms of straw production, with production volumes ranging from 600 million to 800 million tons per year (Sun et al. 2019). However, it is still underutilized, and approximately 90% of straw resources are wasted or disposed of by open burning (Fang et al. 2020; Yang et al. 2020). Open burning of rice straw is still the dominant method of straw disposal in China, which results in the emission of large amounts of harmful gases within a short period of time, leading to an increase in ozone concentrations and causing the emission of large amounts of particulate matter (PM2.5), which not only leads to resource wastage but also pollutes the air and soil environment (Huang et al. 2022). Straw can be used to increase the nutrient content of soil and can promote the growth of crops (Xu et al. 2023). Microbial fermentation technology has been used to prepare biofertilizers, mainly through the treatment of straw and benign phosphogypsum. Biofertilizer can be added to soil during crop cultivation to test its effect on crop growth. In testing the effectiveness of biofertilizers, they are applied to the soil to see the changes in soil nutrients and the effect on plant growth. In this way, the effect of biofertilizers on plant growth is explored. (Zhang et al. 2018)

There are a number of studies on the integrated and environmentally friendly utilization of waste resources. A biofertilizer was produced by inoculating *Aspergillus niger* into pressurized mud, and its effect on maize growth was tested. The study showed that it improved the nutrient content of the soil and the yield of corn (Urooj et al. 2022); previously, some scientists studied the industrial application of water hyacinth in biomethane and biofertilizers, providing new ideas for ecologically applied treatments (Fadoua et al. 2024). However, there are no examples of the environmentally sound recycling of phosphogypsum. In this study, phosphogypsum was converted into a biofertilizer containing a microbial agent, and this material was applied in plant experiments for solid phosphogypsum waste treatment, which provides a new method for utilizing phosphogypsum and fertilizing soils.

2 Materials and Methods

2.1 Composting and Sampling

Aerobic composting was conducted in 114°25'48"E, 30°27'32"N. Wuhan, Hubei Province, China. Air-dried rice straw and corn straw were crushed into a 50–100 mesh powder and used as the main carbon source. Air-dried chicken manure was crushed into a 50–100 mesh

powder and used as the main nitrogen source. In this experiment, a 50-mesh sieve was used for air-dried and crushed straw and dried poultry manure, a 100-mesh sieve was used for phosphogypsum, and the sieved powder was collected for the next step of the experiment. The ratio of straw, chicken manure, and phosphogypsum was 3:3:1 (the ratios referred to here are weight ratios). A microbial agent containing yeast, lactic acid bacteria, *Bacillus subtilis*, and filamentous fungi (produced by Beihai Qiangxing Biotechnology Co.) was added to the mixture before it was composted. In particular, *Bacillus subtilis* has been proven to secrete phosphatase, nitrogenase and organic acids, which allow nutrients to be more easily absorbed through chemical transformations, which has a beneficial effect on the availability of essential plant nutrients such as P and N (Kang et al. 2014, 2015). Based on this research, some researchers applied *Bacillus subtilis* bio-fertilizers to not only reduce chemical pollution but also increase the quality of fruits (Qiu et al. 2021). The ratio of straw, chicken manure, and phosphogypsum was 3:3:1 (the ratios referred to here are weight ratios). The total weight of the pile at the time of composting was 50 kg, and an appropriate amount of water and microbial agent (10%) was added to keep the water content between 50% and 60%. A small amount of urea was used to adjust the carbon to nitrogen ratio. Three piles (3 replicates) were set up, and composting was carried out for 50 days. Starting on the third day, the piles were turned every three days for the first three weeks and then once a week thereafter. On Days 0 and 50, samples were collected from a random area at a depth of 25 cm for microbial community analysis using a soil collector. During sample collection, A1 is the pre-reaction sample which contains A1_1; A1_2; A1_3 (3 replicates), A2 is the post-reaction sample which contains A2_1; A2_2; A2_3 (3 replicates). There are 3 parallel random samples in the compost sample replication. The 3 parallel samples will be taken from each random sample during sample collection and combined into one composite sample of compost. Samples were also taken at regular intervals during the composting process for the analysis of water, nitrogen, phosphorus, potassium. Samples taken on Day 0, after the feedstock was mixed, were used as the reference for low microbial activity at the beginning, while samples taken on Day 50, after the completion of fermentation, were used as the reference for maximum microbial activity. This was used to compare microbial community changes and data changes. An uninterrupted sampling and testing program was used to monitor changes in nutrient composition in the pile throughout the composting process. Data are represented as the mean of three replicates \pm standard deviations. $P < 0.05$ and were analyzed by Origin Pro version 2022 SR1 for Windows.

2.2 Analysis of Physicochemical Parameters during Composting

The main parameters analyzed in this study were temperature, water content, pH, total nitrogen, potassium, and effective phosphorus content at different stages of composting. Additionally, ambient temperature is closely related to composting efficiency, and a laboratory thermometer was used to record the ambient temperature at the time of the experiment. Samples collected from the compost were weighed and dried to a constant weight at 105 °C using an Electric Thermostatic Blast Dryer (101-2AB Tianjin Teste Instruments Co., Ltd.) and were then weighed again to determine the moisture content of the samples. Samples collected at a depth of 25 cm were mixed with deionized water at a ratio of 1:2 and shaken at a constant temperature for 45 min. pH and temperature as well as moisture content were monitored daily to ensure timely adjustment of unsuitable conditions for composting. Samples taken were analyzed immediately, and if sequencing was needed, they were placed in an ultralow temperature refrigerator at -80 °C for storage. The samples were dried to a constant weight, ground, and passed through a 100-mesh sieve. They were then mixed with deionized water and centrifuged at 10,000 rpm for 20 min, and 10 mL of supernatant was aspirated. Ammonium molybdate vanadate reagent was added for color development, the sample volume was adjusted to 50 mL, and the sample was analyzed using a UV spectrophotometer (UV-2600 Shimadzu Instruments Co. Ltd.) at a wavelength of 470 nm. The content of effective phosphorus in the sample was then calculated using a standard curve. For the measurement of potassium in fertilizers and soils, the classical method involving sodium tetraphenylboron was used (Xing et al. 1993). Fifteen grams of sodium tetraphenylboron was dissolved in approximately 960 mL of water, 8 mL of sodium hydroxide (200 g L⁻¹) and 20 mL of magnesium chloride hexahydrate (100 g L⁻¹) were added, and the mixture was stirred for 15 min, left undisturbed, filtered, and stored in a brown bottle or plastic bottle. The sodium tetraphenylboron washing solution consisted of a solution of sodium tetraphenylboron diluted with 10 volumes of water. Additionally, 1.0–2.5 g of sample was weighed (to 0.0002 g) in a 250 mL conical flask, approximately 150 mL of water was added to promote dissolution, the sample was transferred to a 250 mL volumetric flask, diluted with water to scale, and used to prepare the sample solution.

Approximately 10 mL of the specimen was placed in a 150 mL beaker, and the sodium tetraphenylboron solution was added dropwise under constant stirring. The amount added was 35 mL. The mixture was stirred for 1 min, left undisturbed for more than 15 min, filtered, and washed with a washing solution 5–7 times. The filter paper with

the precipitate was placed on glassware and dried in a 120 ± 5 °C drying oven for 2.5 h. Then, the precipitate was put in a dryer to cool down. The precipitate was weighed on an analytical balance (the control test was performed at the same time).

The formula is as follows:

$$Kcl (\%) = \frac{(M2 - M1) \times 0.0284}{M0} \times 100\% \quad (1)$$

M0 - mass of the specimen (g).

M2 - mass of filter paper with precipitate (g).

M1 - mass of blank filter paper (g).

We measured the total nitrogen content using a UV spectrophotometer (Xue et al. 2010). In aqueous solutions above 60 °C, potassium persulfate eventually decomposes to produce potassium bisulfate and atomic oxygen. The dissociation of potassium bisulfate in solution results in the formation of hydrogen ions, which undergo complete decomposition in an alkaline medium of sodium hydroxide. The decomposed atomic oxygen can convert the nitrogenous compounds in the sample to nitrate at 120 °C-124 °C, and the organic matter is oxidized and decomposed in the process. Absorbances A₂₂₀ and A₂₇₅ were measured by ultraviolet spectrophotometry at 220 nm and 275 nm, respectively, and the corrected absorbance A: A = A₂₂₀ - 2 A₂₇₅ was obtained for the total nitrogen content.

Soil porosity measurements are necessary; the porosity of the soil reflects to some extent the living space available to microorganisms. Here, we measured soil porosity using the international ring knife method for measuring soil porosity. A standard ring cutter of 100 cm³ (Φ50.46 mm × 50 mm) or 200 cm³ (Φ70 mm × 52 mm) was used, and a ring cutter with a volume of 500 cm³ (Φ100 mm × 63.7 mm) could also be used to obtain more accurate figures. After the soil in its original state was collected, it was soaked in water for a certain period of time to achieve water saturation and then allowed to discharge the excess water from the pores in the soil for different periods of time to calculate the water holding capacity of the soil under different water holding properties. The Data are represented as the mean of three replicates ± standard deviations $P < 0.05$ and were analyzed by Origin Pro version 2022 SR1 for Windows.

2.3 DNA Extraction and PCR Amplification

In this experiment, a kit was used to extract DNA from the samples for subsequent testing. The kit is QIAGEN kit (Cat No./ID: 51,304 QIAamp DNA Mini Kit). The primers used in the experiment were 338 F and 806R, and the related primer sequences were ACTCCTACGGGAGGCA GCAG and GGACTTACHVGGGTWTCTAAT. PCR formal

test was performed using TransGen AP221-02: TransStart FastPfu DNA Polymerase, 20 μl reaction system:

5×FastPfu Buffer	4 μl.
2.5 mM dNTPs	2 μl.
Forward Primer (5μM)	0.8 μl.
Reverse Primer (5μM)	0.8 μl.
FastPfu Polymerase	0.4 μl.
BSA	0.2 μl.
Template DNA	10ng.
Make up ddH ₂ O to	20 μl.

PCR Instrument: ABI GeneAmp® Model 9700. PCR

Reaction Parameters: (a) 1×(3 min at 95 °C), (b) Number of cycles × (30 s at 95 °C; 30 s at annealing temperature °C; 45 s at 72 °C) (c) 10 min at 72 °C, 10 °C until halted by user.

The Data are represented as the mean of three replicates ± standard deviations $P < 0.05$.

2.4 Analysis of Microbial Communities during Composting

The microbial population and related changes that occurred during the experiment were analyzed. All sequencing data are available from the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA), Biological Project Number PRJNA1077631. This allowed us to identify the microbial populations that were most dominant and resistant during the composting process and understand the relationships between these populations. To achieve this, we collected samples randomly from a depth of 20 cm in the compost pile, both before and after composting, with three replicates for each sample (Meng et al. 2022). These samples were then tested and analyzed. The DNA was amplified after extraction. Trans Gen AP221-02: Trans Start Fast pfu DNA Polymerase was used for PCR. Then, the library was constructed and sequenced on an Illumina platform. Using the RDP classifier Bayesian algorithm, representative OTU sequences with 97% similar levels were analyzed and compared with the database.

The 16 S bacterial and archaeal ribosomes were identified using the Silva database (version 138 <http://www.arb-silva.de>). Functional genes: FGR, RDP collated from GeneBank's (Release 7.3 <http://fungene.cme.msu.edu/>) functional gene database. The functional gene database was derived from Fun Gene (version 9.6). Taxonomic level abundance information was generated by the QIIME platform (version 1.9.1) and RDP Classifier (version 2.13). For gene function prediction, PICRUSt in KEGG (version 2.2.0) was used. The OTU (Operational Taxonomic Units) abundance table was first normalized by PICRUSt (the PICRUSt process stores COG information and KO information corresponding to greengene id), i.e., removing the effect of the number of copies of the 16 S marker gene in a species'

genome; and then the abundance of the OTUs was normalized by the PICRUSt process for each OTU corresponding to the greengene id, the COG family information and KEGG Ortholog (KO) information corresponding to the OTU were obtained; and the abundance of each COG and KO abundance were calculated. Based on the information of COG database, the description information of each COG, as well as its functional information, can be parsed from eggNOG database, thus obtaining the functional abundance spectrum; based on the information of KEGG database, the KO, Pathway, and EC information can be obtained and the abundance of each functional category can be calculated based on OTU abundance. In addition, for Pathway, three levels of metabolic pathway information can be obtained by applying PICRUSt, and the abundance table for each level can be obtained separately.

2.5 Plant Experiments

In the plant experiment, ryegrass and pakchoi were selected as the test plants. Ryegrass is easy to grow and can be re-harvested, while pakchoi has a short growth cycle and good growth performance. To ensure optimal germination, the seeds were prepared and moistened for 24 h before planting. In the experiment, soil from the Wuhan suburban area was weighed and sterilized at 121 °C for 30 min. The soil was accurately weighed using an electronic analytical balance (EL204 Mettler-Toledo Instruments Shanghai Co., Ltd.), and a vertical pressure steam sterilizer (BXM-30R Shanghai Boxun Industrial Co.) was used for sterilization. The germinated seeds were then transplanted into the experimental soil, while ensuring that the weight and quality of the soil were consistent. Three rates of fertilization were established: 10%, 20%, and 30% (the weight ratio of fertilizer applied to the soil for the specified amount of planting herein). Additionally, three groups of parallel experiments and blank controls were set up. To test the effect of phosphogypsum microbial fertilizer on plant growth, we conducted a 45-day plant experiment at a constant light intensity and duration and temperature, the light time was 10 h per day and the temperature was kept at 25–30 °C. In the section on analysis of data from all plant experiments, the Data are represented as the mean of three replicates \pm standard deviations $P < 0.05$ and were analyzed by Origin Pro version 2022 SR1 for Windows.

2.6 Biomass of Ryegrass and Pakchoi in Plant Experiments

The biomass of ryegrass and pakchoi was primarily tested at different fertilizer application rates. The main biomass parameters measured were dry matter weight (g plant^{-1}),

fresh weight excluding root weight (g plant^{-1}), plant length (cm), and number of branches. In addition, the vitamin C and chlorophyll contents of the plants were measured to determine their nutrient content. The plant height and root length of plants grown at different fertilizer application rates were measured using a measuring tape and recorded. The number of plant branches was also recorded. The collected plants were weighed for their fresh weight using an analytical balance. Subsequently, they were dried at 105 °C to a constant weight using an Electrothermal Constant Temperature Blast Dryer (101-2AB Tianjin Teste Instruments Co).

2.7 Tests for Chlorophyll and Vitamin C in Ryegrass and Pakchoi in Plant Experiments

To measure the chlorophyll content in ryegrass and pakchoi, samples were collected by cutting the plant leaves into small pieces of approximately 1 mm and placing them in test tubes. The samples were then extracted using 95% ethanol and immersion for 4 h in a constant-temperature water bath at 60 °C. The extract absorbance was then measured at wavelengths of 665 nm and 649 nm using UV spectrophotometry. The chlorophyll concentration in the plants was calculated using Wellbum's formula (Wu et al. 2018).

$$C_a (\mu\text{g mL}^{-1}) = (13.70 \times A_{665} - 5.76 \times A_{649}) \times [V / (1000 \times w)] \quad (2)$$

$$C_b (\mu\text{g mL}^{-1}) = (25.80 \times A_{649} - 7.60 \times A_{665}) \times [V / (1000 \times w)] \quad (3)$$

$$C_{Total} (\mu\text{g mL}^{-1}) = C_a + C_b \quad (4)$$

V represents the volume of the extraction solution, w represents the fresh weight of the leaves, and A₆₆₅ and A₆₄₉ represent the absorbance of the extracts at wavelengths of 665 nm and 649 nm, respectively.

In addition to chlorophyll, we collected samples to measure the vitamin C content of ryegrass and pakchoi. To prepare for the samples for the measurements, we first prepared an acetic acid-sodium acetate buffer and a vitamin C standard solution. The ryegrass and pakchoi to be tested were washed and dried, and 10 g of the plants was weighed on an electronic balance and ground with 0.5 mol L⁻¹ NaCl. The resulting ground plants were loaded into a volumetric flask, and 0.5 mol L⁻¹ NaCl was added in a 10:1 liquid ratio and mixed thoroughly. The grinding solution was then transferred to an EP tube via a pipette and centrifuged at 3000r min⁻¹ for 10 min using a high-speed freezing centrifuge (PF-16R Hunan Ping fan Technology Co). The supernatant was taken in a volumetric flask, and the sample volume to

be tested was 100 mL. This process was repeated to prepare the three batches of samples with the same method. After the samples were prepared, we established a vitamin C standard curve. We added 100 mL, 50 mL, 25 mL, 12.5 mL, 10 mL, 6.25 mL, and 5 mL of 0.5 mol L⁻¹ NaCl to each tube to obtain vitamin C concentrations of 1 µg mL⁻¹, 2 µg mL⁻¹, 4 µg mL⁻¹, 8 µg mL⁻¹, 10 µg mL⁻¹, 16 µg mL⁻¹, 10 µg mL⁻¹, 16 µg mL⁻¹, and 20 µg mL⁻¹. We then added 5 mL of the dilutions to colorimetric tubes, and 5 mL of 0.5 mol L⁻¹ NaCl was added to tube 0 as the control. The absorbance of the solutions was measured at 243 nm using a UV spectrophotometer to establish the standard curve for vitamin C. Once the standard curve was established, we tested the samples. We took 2 mL of the sample to be tested, added 0.5 mL of 100 g mL⁻¹ CuSO₄ to the sample, adjusted the pH with acetic acid-sodium acetate buffer to pH=4.5, and adjusted the volume to 10 mL with 0.5 mol L⁻¹ NaCl. We then added 5 mL of this solution to a colorimetric tube using a pipette and measured the absorbance value of the sample at 243 nm after treatment.

This procedure was repeated three times to obtain three average absorbance values, and the vitamin C content was calculated using the following formula.

$$V_C = \frac{C \times V_{\text{total}} \times V_2 \times 100}{V_1 \times W_{\text{total}} \times 1000} \quad (5)$$

C represents the concentration of vitamin C obtained from the standard curve equation, V_{total} represents the total volume of the volume-adjusted solution, V₂ represents the volume of the sample to be measured, V₁ represents the volume of the curve, and W_{total} represents the total mass of the weighed sample (Liang et al. 2019).

2.8 Rhizosphere Soil Microbial Count

The composition of soil microbial community's changes based on the plant species present. The number of plants associated with the soil as well as changes in the community are critical (Bever et al. 2012). To collect soil samples, the plants were carefully uprooted, and the plants were gently shaken to separate the soil from the plant roots. This soil was collected and put in a conical flask (10 g). Then, 100 mL of sterilized distilled water was added to the flask, and it was shaken well for 2 h at 28 °C and 180r min⁻¹ in a constant temperature shaker. The resulting solution was then diluted.

To measure the bacterial content of the soil, we coated the diluted soil suspensions onto LB agar medium plates using a tenfold gradient dilution method for counting. The LB agar medium consisted of 10 g L⁻¹ peptone, 5 g L⁻¹ yeast extract, 10 g L⁻¹ NaCl, and 1.5% agar, with the pH

adjusted to 6.8-7.0. The plates were then incubated at 28 °C for 48 h, and colony-forming units (CFUs) were counted.

3 Results

3.1 Physicochemical Analysis during the Composting Process

The composting process is typically divided into a medium temperature stage, a high temperature stage, and a cooling stage during the composting process, as shown in Fig. 1a. The temperature tends to rise slowly from room temperature until it reaches a maximum of 50 °C, after which it gradually drops to room temperature again. The reactor temperature began to rise significantly on the third day, reached the high temperature stage (> 45 °C) on the eighth day, reached 50 °C on the tenth day, and maintained a temperature above 50 °C for 22 days. During the composting process, the core temperature of the pile was high (> 45°C) for 29 days, which promoted the growth and propagation of microorganisms. After the compost was fully fermented, the temperature decreased significantly on the 40th day until it reached nearly room temperature.

We chose a stage in which the temperature was approximately 25–30 °C for the experiment, as shown in Fig. 1a. Increased humidity can also enhance bacterial growth at higher temperatures. As shown in Fig. 1b, the water content in the pile decreases and water volatilizes with the growth of microorganisms. Temperature and moisture are very important parameters in compost fermentation, and proper temperature and humidity provide optimal conditions for bacterial colonization. The temperature slowly rose to a peak and then slowly fell, signaling the trend in microbial growth. The water content must be constantly adjusted to control moisture to ensure bacterial growth during the composting process.

In Fig. 2a, it is evident that the total nitrogen, exchangeable phosphorus and potassium content of the soil with biofertilizer in this study were significantly higher than those of the unamended soil. Microorganisms increased the soluble phosphorus and nitrogen and potassium contents during the fermentation and humification of the compost. The addition of this biofertilizer to the soil increased the nutrient content of the soil. Figure 2b shows that the soil porosity and density of the biofertilizer-amended soil were better than those of the unamended soil. This improved plant growth and the density of plant roots and facilitated nutrient transport and water storage while reducing the mechanical resistance of the soil.

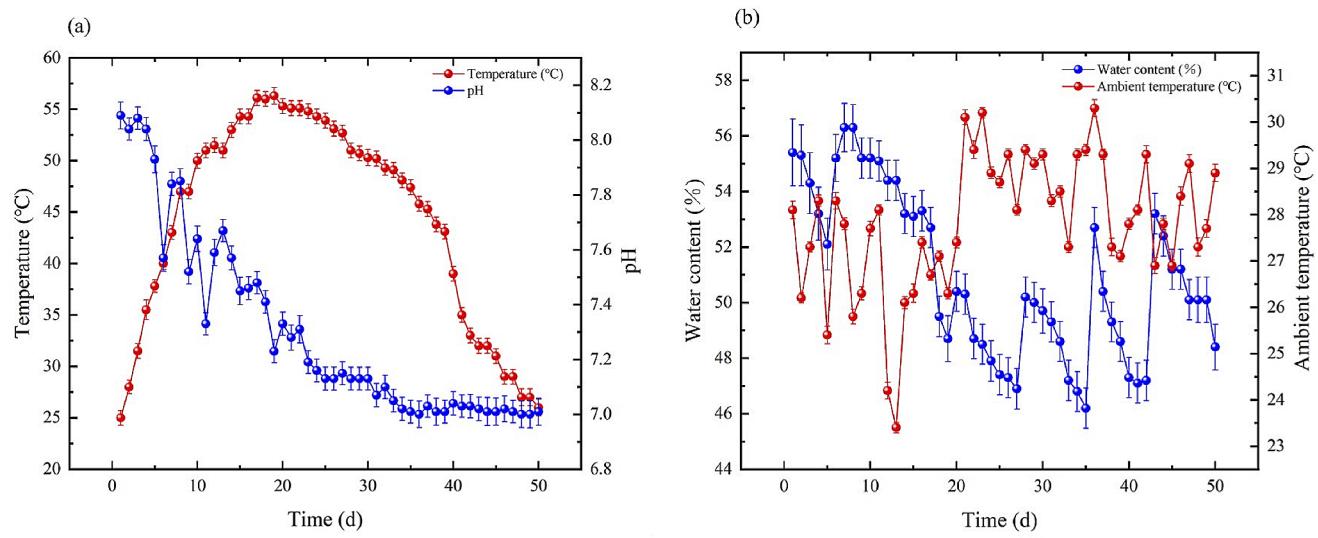


Fig. 1 Changes in physicochemical parameters during the preparation of biofertilizers. **(a)** Compost pile temperature variation and pile pH variation. **(b)** Temperature variation and variation in the water content

of the pile. Data are represented as the mean of three replicates \pm standard deviations. $P < 0.05$

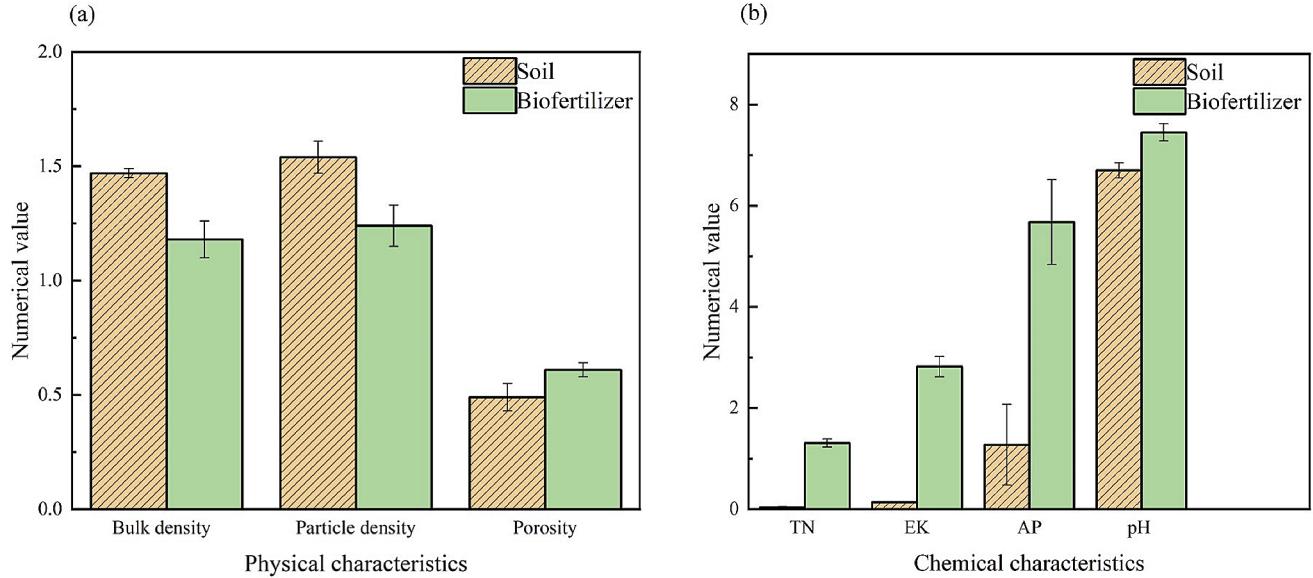


Fig. 2 The physical and chemical properties of the soil and biofertilizer. **(a)** compares physical properties. **(b)** compares chemical properties. TN is total N, EK is exchangeable K, and AP is available P. Data are represented by the mean of three replicates \pm standard deviations. $P < 0.05$

3.2 Changes in Microbial Diversity and Community Composition during the Composting Process

Data analysis was performed on the before and after composting to study the changes that occurred in microbial diversity and community composition during composting. During the total composting process, we obtained 483,395 microbial gene sequences. Bacteria played a much larger role than fungi in the process of pile fermentation, with more than 50 different groups of bacteria identified. The analysis results showed that the most dominant flora was

Actinobacteria both before (A1_1, A1_2, A1_3) and after composting (A2_1, A2_2, A2_3), but *Actinobacteria* abundance showed a tendency to decrease during the fermentation and decomposition process.

Throughout the composting process, the main bacterial taxa at the phylum level were *Actinobacteria* (41.82%), *Firmicutes* (21.95%), *Proteobacteria* (2.29%), *Bacteroidetes* (8.28%), *Chloroflexi* (2.72%), *Deinococcota* (1.68%), *Patescibacteria* (0.56%), *Myxococcota* (0.20%), and others (0.49%), as shown in Fig. 3a and b. Similarly, the abundance of major bacteria at the genus level throughout

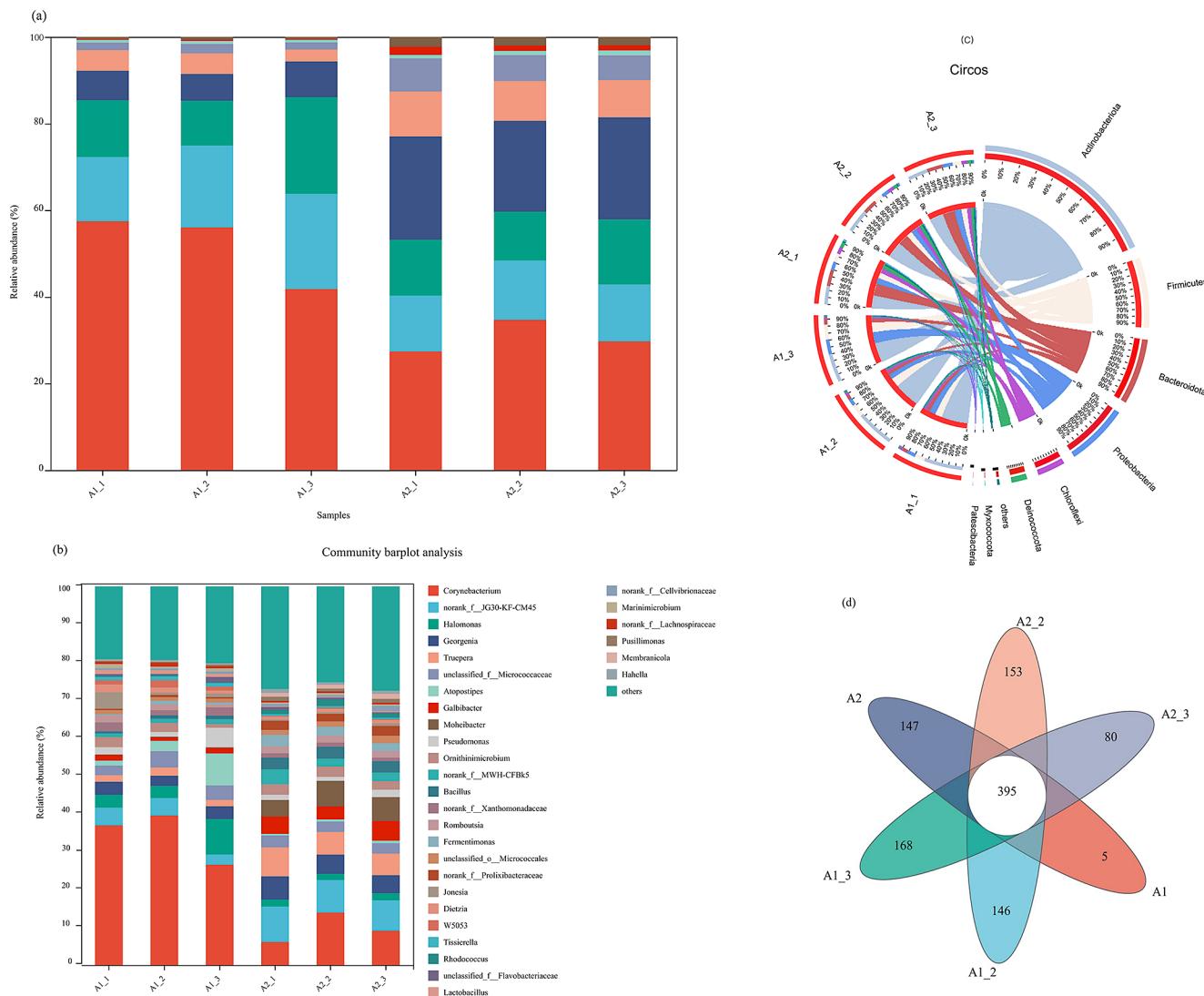


Fig. 3 The relationship between microbial communities, their proportions, and functional prediction. **(a)** Percent community abundance at the phylum level. **(b)** Percent community abundance at the genus level.

(c) Circos sample-to-species diagram, representing the proportion of different species in the sample. **(d)** Venn diagram describing the proportions of species in different samples

the composting process is shown in Fig. 3b and c, with *Corynebacterium* (26.51%) being the most dominant genus, followed by *norank_f_JG30-KF-CM45* (9.40%), *Geogenia* (3.36%), *Atopostipes* (8.45%), *Pseudomonas* (5.15%), and others (20.27%). Figure 3c clearly shows the different proportions of strains in the sample. (Fig. 3d) Different colors in this figure represent different subgroups, and the numbers in the overlapping part represent the number of species common to multiple subgroups, while the numbers in the nonoverlapping part represent the number of species specific to the corresponding subgroup. For all samples, the number of common species reached 395.

3.3 Analysis of the Relationship between Microbial Community Composition and Species

To investigate the differences and similarities in microbial communities in different environments during composting, we conducted a comparative analysis between groups. This was done to identify the most important factors in the data and to reveal the underlying relationships within the complex data. Figure 4a and b clearly show that the three closest points are from sample A2, i.e., after compost stabilization, at which time the microbial community tended to stabilize and no major evolution occurred. However, the points on the left side, which are farther away in Fig. 4a

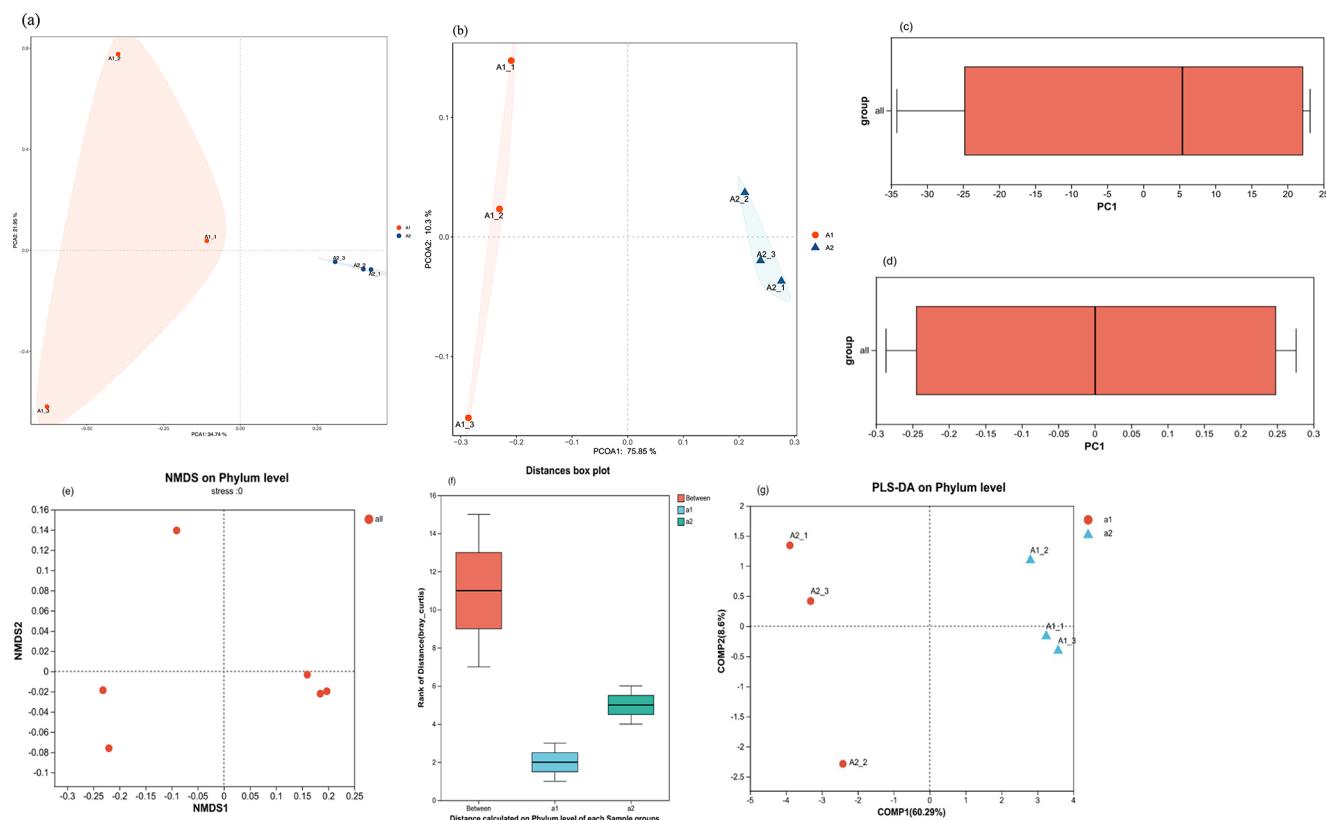


Fig. 4 Sample difference analysis diagram demonstrating differences and similarities between samples. (a) PCA (principal component analysis), i.e., principal component analysis plot. (b) PCoA analysis, i.e., principal coordinate analysis (PCoA) plot showing sample differences. (c) PCA box plot representing the dispersion of the distribution of different groups of samples on the PC1 axis. (d) PCoA box plot representing the dispersion of the distribution of different groups of samples on the PC1 axis. (e) NMDS analysis, i.e., nonmetric multidimensional

scaling (nonmetric multidimensional scaling) is used to replace the original data with new columns in the same order as metric multidimensional scaling analysis to maintain the relationship stress < 0.05 of the original data. (f) Analysis of similarities (analysis of similarities) plot to analyze the degree of explanation of sample differences based on different grouping factors. (g) PLS-DA plots to highlight systematic differences between groups

and b, come from sample A1, indicating that the amounts of microorganisms and the community were evolving at all times before or during the composting process (plots c and d clearly show the distribution of the samples on the PC1 axis.) Plot e preserves the original relationship between the objects while analyzing the categorization, and in this way, we see that the A2 samples are still the closest and the best-sorted when the stress is 0. We also analyzed the distance gap between groups as a way to classify and highlight gaps between groups. The results showed that changes in strain or changes in physicochemical properties greatly affected microbial communities (Fig. 4f and g).

3.4 Comparative Analysis of Bacterial Species, Species Differences and Factor Correlation in the Composting Process

Two groups of samples were randomly selected for comparison. Because the other groups were parallel experiments, we

selected only two groups of samples to analyze the significance of differences between groups. Hypothesis testing was performed based on the microbial community abundance data in the samples. Figure 5a clearly showed that there were six strains of bacteria that underwent the most significant changes. The abundance of *Actinobacteria* decreased significantly after composting. It is possible that the bacterial community was altered during the reaction process, which affected the number of *Actinobacteria*. Additionally, we performed network analysis of microbial communities to show the coexistence between different microorganisms; this included co-occurrence network analysis. The factors influencing microorganisms are clearly shown in Fig. 5b. In the network analysis, the dots represent different species. Lines represent relationships, with red representing positive correlations and green representing negative correlations. The different relationships between the species are clearly shown.

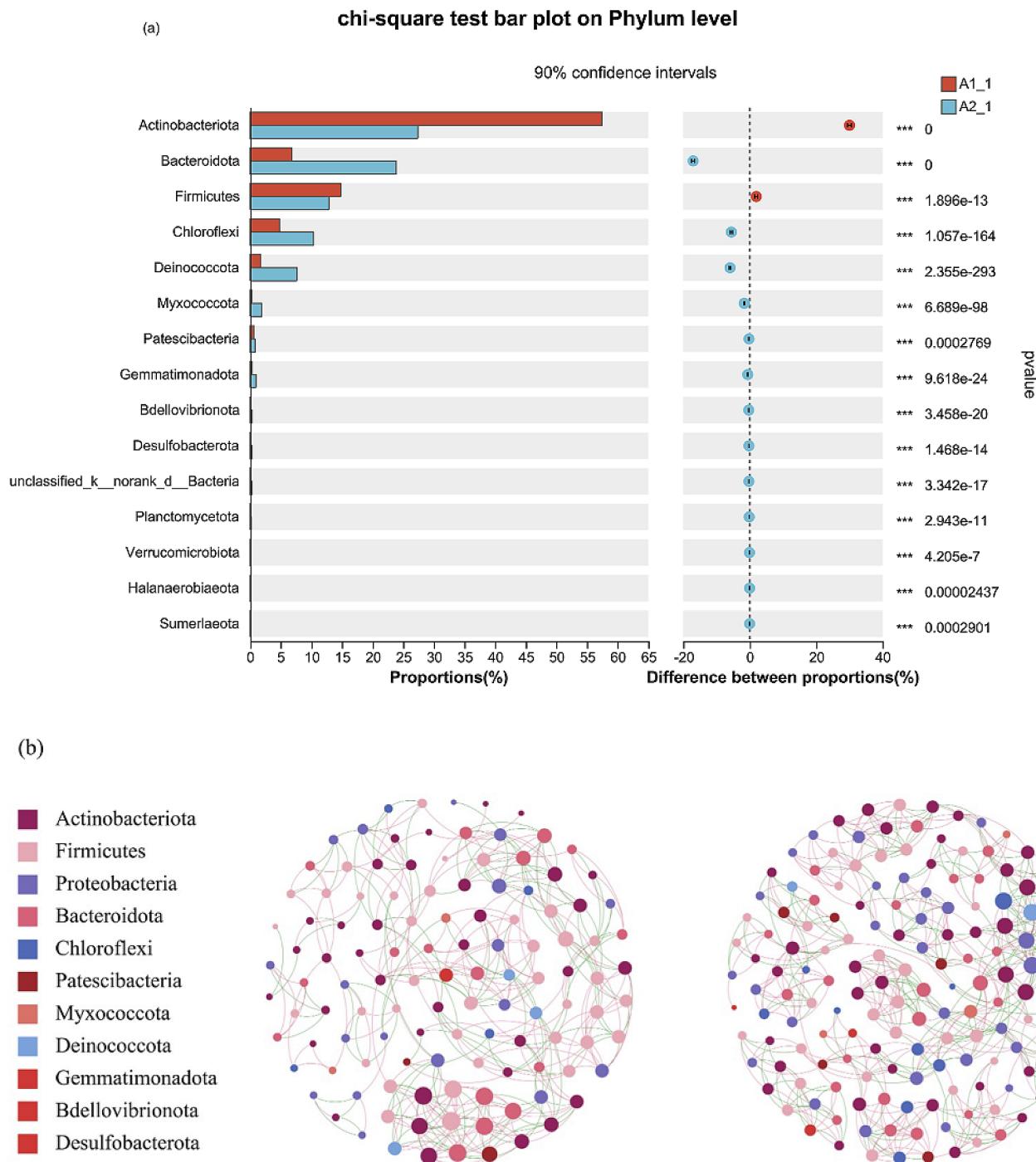


Fig. 5 Single factor analysis of sample differences and microflora. **(a)** The difference analysis diagram between groups, **(b)** The correlation network diagram for the post compost samples, the left network dia-

gram is A1 represents the microbial network correlation before composting, and the right network diagram A2 represents the microbial network correlation after composting

3.5 Prediction and Analysis of Microbial Community Function

PICRUSt was used to predict bacterial function, and annotated information and the functional effects of each bacterial function level were obtained. The data mainly consisted of

24 functions, including amino acid transport and metabolism, general function prediction only, translation, ribosomal structure and biogenesis, inorganic ion transport and metabolism, and energy production and conversion. Figure 6a and b show the box diagram of functional classification and microbial abundance in samples.

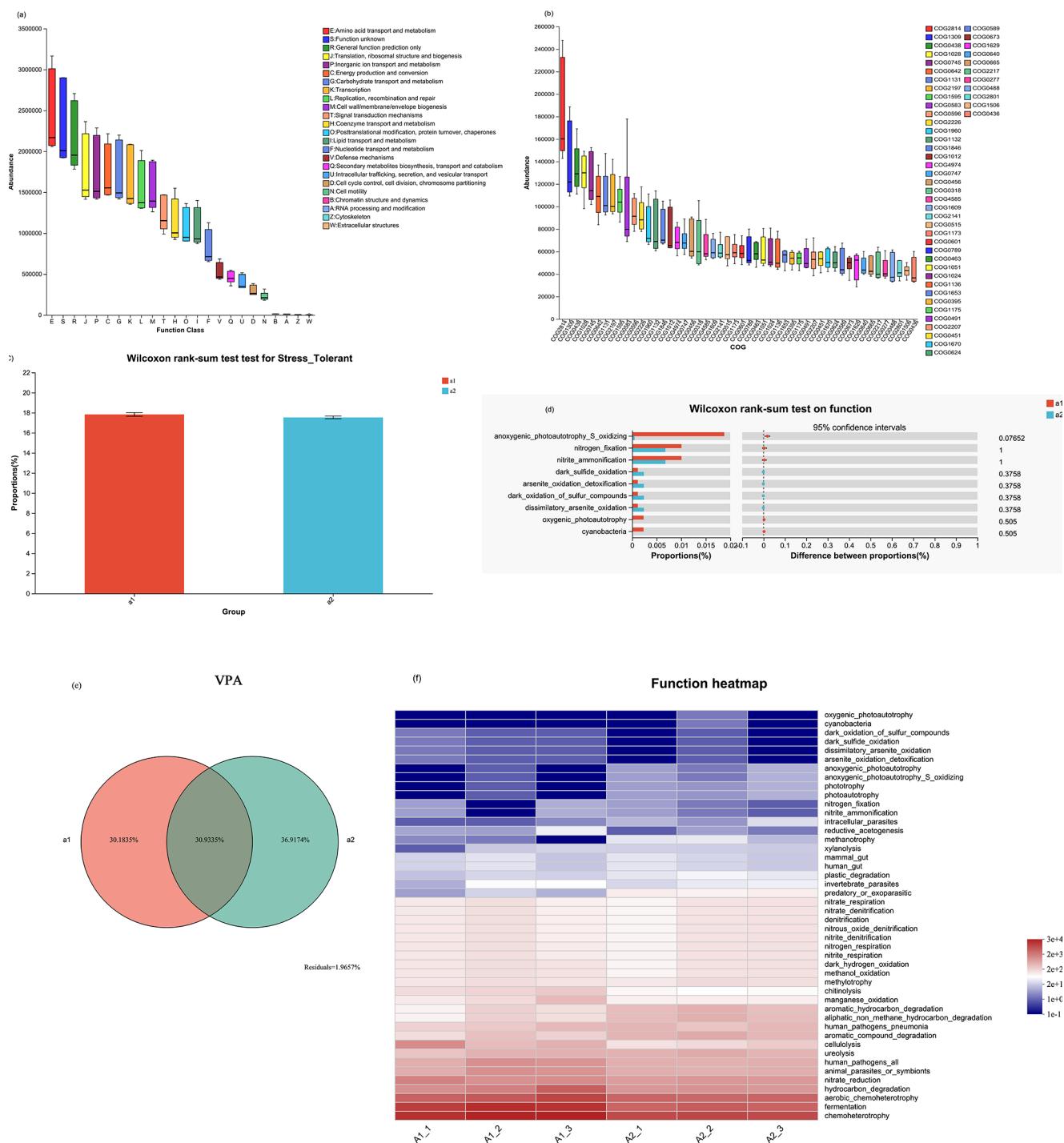


Fig. 6 Functional prediction and abundance differences in bacterial communities. **(a)** COG functional classification box diagram. **(b)** COG sample abundance diagram. **(c)** Phenotypic difference test. **(d)** FAPROTAX functional prediction intergroup difference test diagram.

In our analysis of the two samples that were randomly selected for comparison, it is clear from Fig. 6c that there are no significant phenotypic differences between the two groups. We performed FAPROTAX functional prediction of the samples and found the most significant differences

between the functional groups in the samples (Fig. 6d). Additionally, in Fig. 6f, the FAPROTAX functional prediction heatmap clearly shows the different abundances of species. Figure 6c shows a decrease in anoxygenic-photoautotrophy-S-oxidizing, nitrogen fixation, and nitrite ammonification,

Table 1 Physicochemical characteristics of the soil used in the experiments and biofertilizer

Test item	Soil	Biofertilizer
Total N (g kg^{-1})	0.34 ± 0.02	1.31 ± 0.08
Exchangeable K (mol kg^{-1})	0.14 ± 0.01	2.82 ± 0.20
pH	6.71 ± 0.02	7.49 ± 0.06
Available P (mg kg^{-1})	1.28 ± 0.01	5.73 ± 0.07
Bulk density (g cm^{-3})	1.47 ± 0.03	1.18 ± 0.08
Particle density (g cm^{-3})	1.53 ± 0.07	1.25 ± 0.09
Porosity (%)	48.51 ± 0.63	61.43 ± 0.45

Data are represented as the mean of three replicates \pm standard deviations. $P < 0.05$.

Table 2 Effects of phosphogypsum biofertilizer application on the growth and yield of ryegrass and pakchoi

Treatment	Plant length (cm)	Branches	Dry matter weight (g plant^{-1})	Fresh weight (excluding root weight) (g plant^{-1})
Ryegrass T1 (0%)	17.50 ± 1.01	3.33 ± 0.57	0.15 ± 0.01	2.38 ± 0.20
Ryegrass T2 (10%)	18.30 ± 0.61	5.33 ± 0.57	0.17 ± 0.03	3.59 ± 0.18
Ryegrass T3 (20%)	23.31 ± 2.35	5.67 ± 1.15	0.22 ± 0.01	3.71 ± 0.05
Ryegrass T4 (30%)	27.83 ± 2.92	6.33 ± 1.52	0.26 ± 0.08	3.90 ± 0.03
Pakchoi T1 (0%)	7.50 ± 0.56	4.67 ± 1.15	0.20 ± 0.06	9.52 ± 1.17
Pakchoi T2 (10%)	9.90 ± 0.80	6.67 ± 0.58	0.24 ± 0.02	13.33 ± 0.58
Pakchoi T3 (20%)	12.71 ± 0.96	7.33 ± 0.58	0.27 ± 0.06	19.41 ± 0.65
Pakchoi T4 (30%)	16.10 ± 0.54	7.67 ± 0.58	0.32 ± 0.04	22.52 ± 3.87

Data are represented as the mean of three replicates \pm standard deviations. $P < 0.05$.

while dark sulfide oxidation, arsenite oxidation detoxification, dark oxidation of sulfur compounds, and dissimilatory arsenite oxidation increased significantly. This may have occurred due to microbial community changes and because different flora plays different roles in metabolism when the physicochemical environment changes, which led to community evolution. In Fig. 6e, variance partitioning analysis was used to analyze the differences in the samples, and it is evident that the percentage of species common to A1 and A2 reached 30.9%. This percentage also represents the flora that were most stable before and after composting.

We used different fertilization ratios to test the effectiveness of biofertilizers. We set up a control for ryegrass (no biofertilizer), ryegrass with 10% biofertilizer, ryegrass with 20% biofertilizer, and ryegrass with 30% biofertilizer. Additionally, we set up a control for pakchoi (no biofertilization), pakchoi (10% biofertilizer), pakchoi (20% biofertilizer), and pakchoi (30% biofertilizer). We set up three

Table 3 Chlorophyll and vitamin C content

Sample	Vitamin C (mg g^{-1})	Chlorophyll ($\mu\text{g mL}^{-1}$)
Ryegrass T1 (0%)	23.31 ± 2.93	2269 ± 7.78
Ryegrass T2 (10%)	50.64 ± 2.12	2477 ± 8.48
Ryegrass T3 (20%)	51.92 ± 3.39	2533 ± 14.80
Ryegrass T4 (30%)	57.95 ± 3.52	2666 ± 11.32
Pakchoi T1 (0%)	8.57 ± 0.31	2373 ± 16.40
Pakchoi T2 (10%)	12.92 ± 0.65	6629 ± 4.24
Pakchoi T3 (20%)	16.90 ± 0.55	6696 ± 9.19
Pakchoi T4 (30%)	28.14 ± 1.11	6733 ± 11.33

Data are represented as the mean of three replicates \pm standard deviations. $P < 0.05$.

Table 4 Comparison of microbial growth values in plant root systems

Sample	Bacteria ($\text{CFU g}^{-1}, \times 10^6$)	Fungi ($\text{CFU g}^{-1}, \times 10^6$)
Soil	$5.50 \times 10^6 \pm 0.71$	$7.50 \times 10^6 \pm 0.71$
Biofertilizer	$1.04 \times 10^8 \pm 8.49$	$1.00 \times 10^7 \pm 1.41$
Plant Roots	$1.20 \times 10^8 \pm 5.66$	$1.25 \times 10^7 \pm 2.12$

replicates for each treatment. The data shows that growth, plant height, dry weight, and fresh weight of the fertilized group were higher than those of the unfertilized group. The optimal fertilizer ratio was determined more accurately in the next step.

During microbial fermentation in the reactor, material is decomposed, microbes colonize the material, and organic matter provides nutrients for microbial growth (Table 1). Phosphogypsum biofertilizers, when added to regular soil, have a positive effect on plant growth and improve soil fertility (Table 2).

To further highlight the growth in the fertilization group and the role of phosphogypsum microbial fertilizer, we specifically tested the vitamin C and chlorophyll content of the plants (Table 3). Additionally, the microbial population of the soil, biofertilizer, and plant roots was compared. It is obvious that in the presence of both plant roots and microbial fertilizer, the microbial reproduction capacity increased, exponentially in certain cases, as clearly shown in Table 4. This is one of the reasons for vigorous plant growth.

4 Discussion

In the composting process, different trends in temperature can be seen, the degree of microbial reproduction is related to the temperature of the pile and environmental conditions, and specific microbes are favored to reproduce under specific temperature conditions. Additionally, microbial reproduction has a relationship with compost pH and physical and chemical properties.

pH is also a crucial factor in bacterial growth, as it can affect the activity of urease and the growth and reproduction of bacteria (Li et al. 2021). In our experiment, the pH

changed from neutral to basic, and the pH decreased very slowly until it reached a neutral value and stabilized. This suggests that the bacteria slightly changed the pH value of the reactor environment during their growth and reproduction to achieve the most suitable growth conditions. Proper hydration is also necessary for the growth of microorganisms. In our experiment, we kept the water content of the reactor at approximately 50% and adjusted the water once every 7 to 8 days to maintain the growth and propagation of bacteria. Bacteria are more likely to grow and multiply in a high-humidity and closed environment, and ventilation can reduce their growth (Qiu et al. 2022).

The soil provides a reservoir of water and nutrients for plant growth and uptake, and these processes are strongly related to soil porosity (Robinson et al. 2022). Land use strongly affects change within the Earth system, and soil is one of the basic compartments in the ecosphere involved in biological cycles on Earth. The distribution, size, and morphology of soil pores control the flow of water and gases through the soil environment (Assouline and Or 2013) and microbial and nutrient systems (Or et al. 2007). In the plant experiment, when biofertilizers were introduced into the soil of the experimental plant group, soil porosity changed. In this experiment, we believe that the addition of straw improved the porosity of the soil at the physical level, and at the biological level, the interaction between microbial community activity and plant roots improved the porosity of the soil. It is clear that appropriate porosity and soil capacity are necessary for plant growth. Appropriate amounts of straw addition to the soil has been shown in some studies to improve soil structure and reduce mechanical resistance, which increases the water infiltration rate in the soil and facilitates water uptake by plants (Getahun et al. 2018).

During the preparation of the biofertilizer, the microbial community evolved, and *Actinobacteria* were the most dominant group both before and after composting, although they showed a decreasing trend. This shows that *Actinobacteria* were the dominant strain in the microbial community in this experiment. In the network relationship, *Firmicutes* were more widely connected, and they were included in the relationship of positive and negative correlation. In this experiment, we believe that Actinomycetes and Firmicutes largely determined the synergistic relationship of biofertilizer with microorganisms. The phylum *Actinobacteria* is a fungal phylogenetic branch containing a variety of gram-positive bacteria (Sun et al. 2010) belonging to the phyla *Actinobacteria* and *Thermophile*. Most *Actinobacteria* are aerobic saprophytes, and many can produce bioactive compounds (Eisenlord et al. 2010). *Actinobacteria* are typically the main soil microorganisms (Hill et al. 2011) and are important for the cycling of carbon, nitrogen, phosphorus, potassium, and several other elements in the soil

(Goodfellow et al. 1983). As saprophytes, they produce a range of extracellular hydrolases that degrade plant and animal polymers, including lignin, cellulose, chitin, and other organic compounds (Eisenlord et al. 2010). Cactus spp. are root symbionts that are associated with the rhizomes of various perennial woody dicotyledons (Normand et al. 1996). The most obvious group of bacteria that increased in abundance before and after composting was Bacteroid Ota, which is a very important group of bacteria in the soil. Some studies even suggest that it is related to soil fertility. In the study of soil biodegradation processes, the methanogenic bacterial community can be an important indicator of soil quality, as a decrease in the abundance of these beneficial microorganisms may be associated with a decrease in soil quality, fertility, and ultimately crop yield. These studies also showed that the communities of such bacteria differed in different seasons of the year (Kruczyńska et al. 2023).

Firmicutes is an almost universal phylum, and several genera in this group, such as *Geobacillus*, are recognized for decomposing plant organic matter and producing heat-stable ligninolytic enzymes (Govil et al. 2021). *Firmicutes* are widely distributed in nature, with many of its members being spore-forming gram-positive bacteria that are important components of microbial communities associated with lignocellulosic biomass degradation and carbohydrate polymer breakdown. Therefore, this thick-walled phylum is important when lignin-degrading bacteria and enzymes are needed (Liu et al. 2019). The percentage of *Corynebacterium* was the highest at the genus level, reaching 26.51%, and such strains may be able to degrade organic nitrogen under alkaline conditions. High-throughput sequencing studies have shown that such strains play a bridging and auxiliary microbial community transfer role in alkaline anaerobic fermentation and are important in the process of fermenting biofertilizers (Pang et al. 2020).

After biofertilizer preparation, the apparent nutrient content was enhanced, and we believe that the degradation of straw along with phosphorus solubilization by microorganisms allowed the biofertilizer to provide plants with essential nutrients for growth, such as nitrogen, phosphorus, and potassium (Lu et al. 2020). An orthogonal experimental design was used in a study by Zhang et al. (2023), which was used to optimize the ratio of nitrogen, phosphorus, and potassium fertilizers added for blueberry cultivation. The results showed that fertilizer application improved the growth and fruit quality of blueberries (Zhang et al. 2023). In our plant experiments, we applied the prepared biofertilizer to the experimental group (with phosphogypsum biofertilizer), and plant growth in the experimental group substantially improved compared with that in the control group (no phosphogypsum biofertilizer) at the optimum fertilizer application ratio. In future research, there is a need

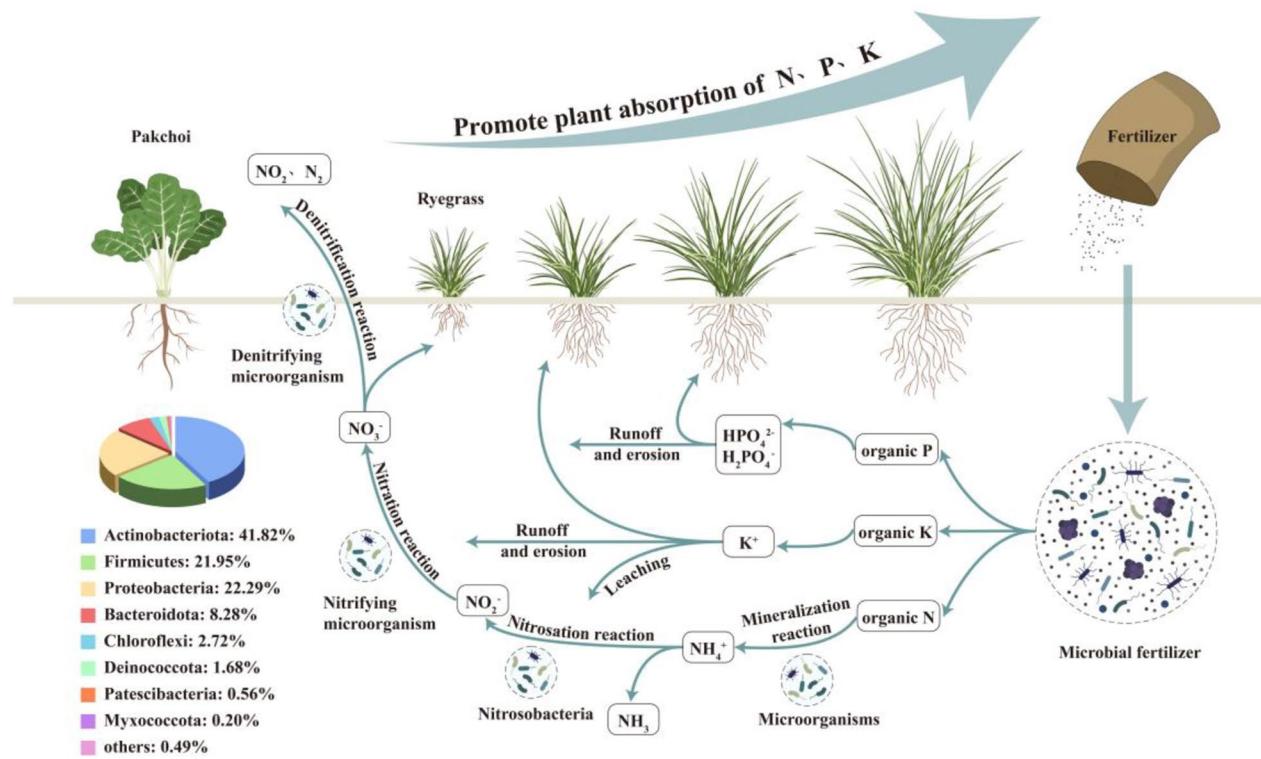


Fig. 7 Effect of biofertilizer production from phosphogypsum and straw via microbial metabolism on plant growth and microbial symbiosis

for greater exploration. During plant growth, we believe that the symbiotic relationship that exists among the microflora in the root systems of plants offers benefits for plant growth, so plant growth improves. The interaction between biofertilizers and microorganisms enhanced the uptake of nitrogen, phosphorus and potassium in plants in the biofertilizer group (Fig. 7). Overall, in this experiment, plant growth improved with changes in soil physicochemical properties and with the fertilizer application ratio, and the number of microorganisms in the root system also increased. In this experiment, the prepared biofertilizer had a positively effect on plant growth and the soil environment.

5 Conclusions

In this study, phosphogypsum was efficiently utilized with straw and chicken manure. It is a good method in the comprehensive utilization of waste resources challenges. The microorganisms present in nature were utilized to solve the problem of phosphogypsum stockpiling in an environmentally friendly, efficient and easy way. During the composting process, its physicochemical properties are also changed due to the action of bacteria. However, actinomycetes and Firmicutes, in particular, dominated the compost community due to their metabolic roles. in the

study, phosphogypsum biofertilizers released into the soil enhanced the nutrients available for plant uptake and also improved soil porosity, making the soil less susceptible to consolidation. Phosphogypsum biofertilizer contains a large number of beneficial microorganisms and when applied to the soil, it simultaneously enhanced the microbial population in the root system of the plant, which is necessary for plant growth. It greatly enhanced plant biomass and increased chlorophyll and vitamin C levels in plant leaves compared to the control. Overall, the environmentally friendly preparation of phosphogypsum and straw as biofertilizers using microbial fermentation technology solves the problem of waste utilization and has a positive effect on plant growth.

Author contribution Yongtong Jin: Design and research, data analysis and writing-original draft. Dan Yang: Research and data analysis. Yuehong Wu: Data analysis. Fang Zhou: Methodology and Software. Junxia Yu: Methodology and funding acquisition. Ruan Chi: Conceptualization, project administration. Chunqiao Xiao: Writing-review and editing, funding acquisition. All authors contributed to the study conceptualization and design.

Funding This work was supported by the Special Project of Central Government for Local Science and Technology Development of Hubei Province (2023EGA028), Chief Scientist Project of Hubei Three Gorges Laboratory (SXCS2204) and Innovation Fund of Hubei Three Gorges Laboratory (SC232005).

Data Availability Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing Interests The authors have no relevant financial or non-financial interests to disclose.

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