

Simulated Lunar Soil: Can It Be Organically Modified through Compost Cultivation?

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Abstract: This study aimed to explore the possibility of improving the fertility of lunar soil through the reuse of resources by composting household waste and collecting composting fermentation broth. The fermentation broth was used to culture a simulated lunar soil at different concentration gradients for 30 days under aerobic and anaerobic conditions. Microbial biomass carbon and nitrogen content, typical mineral elements, and the microbial community were tested to determine whether the fertility of the lunar soil had improved. Results showed that the microorganisms in the simulated lunar soil samples successfully adhered and grew under both aerobic and anaerobic experimental conditions. The simulated lunar soil samples cultured in the anaerobic environment outperformed those in the aerobic environment regarding microbial biomass growth and water-soluble mineral elements. The study results create opportunities for the future reuse of domestic garbage on the lunar base, providing a technical basis for the in situ reuse of lunar soil resources for plant cultivation.

Keywords: simulated lunar soil; compost fermentation broth; aerobic; anaerobic; microbial biomass; microbial community structure



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1. Introduction

Based on the current development status of the aerospace industry, humans plan to establish a lunar base using the Controlled Ecological Life Support System (CELSS) to explore and develop lunar resources. Under the prospect that mankind will live in the lunar base for a long time, two problems cannot be ignored: the disposal of domestic garbage and the source of production and living materials. Some scholars have proposed the idea of directly utilizing lunar soil, such as preparing low valent oxides of silicon (Si) and oxygen (O₂) from lunar soil by vacuum thermal decomposition method [1], extracting O₂ and volatile matter from lunar soil [2], recovering O₂ through the lava electrolysis metallurgical process [3], or sintering lunar soil to manufacture building materials [4]. Other scholars have tried to improve lunar soil to give it life-sustaining characteristics. For example, Ming and Henninger [5] and Kozyrovska et al. [6] proposed that lunar soil could be biodegraded to release mineral nutrients using pioneer plants' root systems combined with microorganisms. Some researchers proposed that nitrogen-fixing cyanobacteria could be directly used to improve the fertility of lunar soil biologically [7]. In recent years, studies have also found that lunar soil simulants can be improved through short-term biological weathering (fermentation with organic solid waste for 10–20 days) to better support plant cultivation [8]. In addition, directly adding phosphorus-solubilizing bacteria can effectively improve the fertility of lunar weathering layer simulants, making them a good cultivation substrate for higher plants [9]. There was also a study reporting on the fertility change of lunar and Martian simulated soils

based on commercial horse/swine monogastric manure, which improved the potential of these substrates as plant growth media [10]. However, acquiring massive quantities of livestock excreta is impossible on the lunar base. The readily available human-induced daily food or garbage waste could be an alternative organic modifier. Therefore, the objectives of the study were to investigate whether composting fermentation broth could make lifeless lunar soil microbially sustainable and to compare the effects of anaerobic and aerobic conditions on soil fertility changes. This study can not only effectively treat household waste but also transform the waste into lunar soil amendments, which is beneficial for the sustainable development of the base.

2. Methods and Materials

2.1. Obtaining Compost Fermentation Broth

The experiment first obtained compost fermentation broth under facultative anaerobic conditions. The raw material for the fermentation broth came from household waste (such as fruit peels and vegetable leaves), which will also be produced in large quantities in lunar bases. We collected waste fruit peels (oranges, mangoes, pineapples, and citrus) and vegetable leaves (common green vegetables) from markets and fruit shops to simulate the raw materials of household waste in the lunar base. Then, the collected materials were mixed and crushed, transferred to a 20 L composting and fermentation tank, and an appropriate amount of straw was added to adjust the moisture content to about 60%. The mixture was stirred evenly and, finally, a layer of EM composting bacteria (Shandong Junde Biotechnology Co., Ltd., Shandong Sheng, China) was evenly sprinkled on the top layer of the materials. The fermentation tank lid was closed and kept at room temperature.

During composting, it was found that white mycelium began to grow on the compost material on the third day. Meanwhile, the volume of the material began to decrease and the temperature increased. Afterward, the temperature remained at 40–55 °C and the mycelium gradually covered the surface of the material, while the color of the vegetables and fruit peels significantly deepened. After one week, the temperature gradually decreased to room temperature and the volume of the material significantly decreased, appearing as brownish clumps. At the same time, a large amount of brownish-yellow fermentation broth was produced, indicating that the compost had entered the maturation stage and could be collected for fermentation broth. The fermentation liquid was then collected and filtered with filter paper. The final clear brownish liquid was stored at 4 °C before subsequent experiments.

2.2. Simulated Lunar Soil Cultivation

The simulated lunar soil came from the Beijing Institute of Spacecraft Environmental Engineering. The sterilized simulated lunar soil (No. TYII-2, median diameter 43.25 µm, bulk density 1.27 g/cm³, moisture ratio 0.38%, and porosity 53.63%) was cultivated at four concentration gradients, including the blank control group (CK) and 20%, 30%, and 40% *w/w* fermentation broth addition. Each group contained the same amount of simulated lunar soil (7 g) in an opaque vessel and the total moisture was kept at 60%. Each treatment group had three replicates. The above soil samples were incubated in an aerobic or anaerobic environment for 30 days. The experiment under the aerobic condition was in a cool ventilated laboratory and the experiment under the anaerobic condition was performed in a glove box filled with nitrogen (the air pressure inside the box was maintained at 0.4 MPa). The technology roadmap is shown in Figure 1.

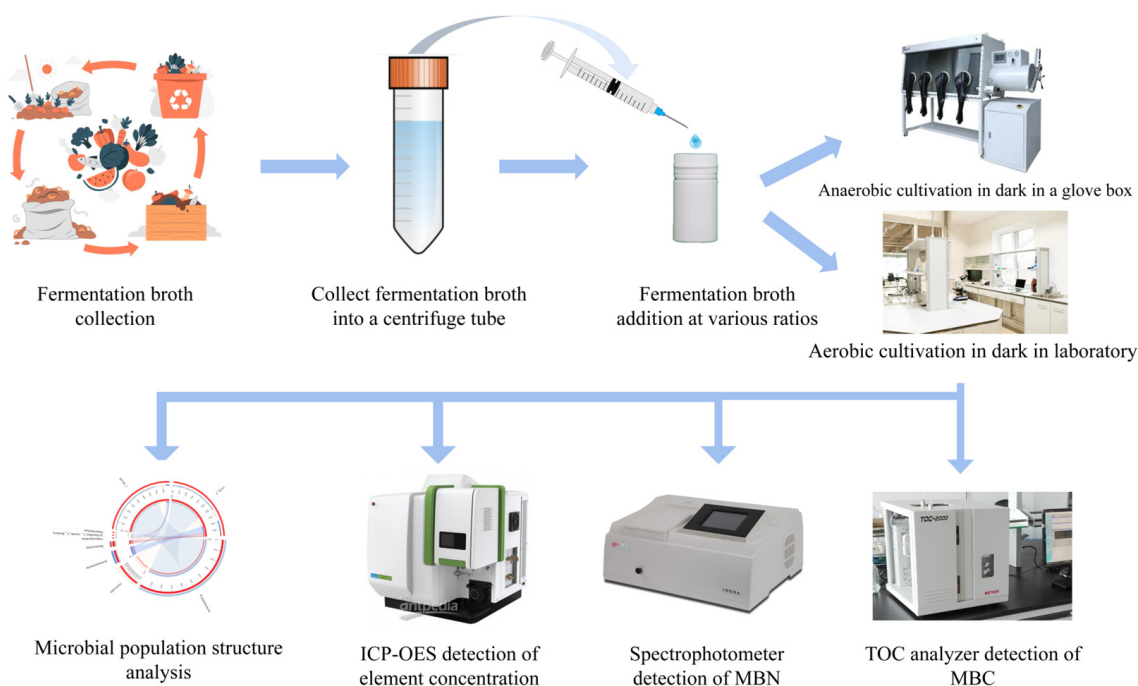


Figure 1. The schematic flow of the study.

2.3. Indicator Detection

Two grams of the simulated lunar soil samples were taken from each experimental group every 10 days for microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) detection using the chloroform fumigation method and the C and N content were detected using TOC (Shimadzu, Nakagyo-ku, Kyoto, Japan) and ninhydrin colorimetric methods, respectively. The water-soluble mineral elements (Fe Mn, Cu, Zn, Ca, and Mg) were extracted using DI water at a soil/water ratio of 1:10 on the 30th day. The content of the above elements was measured on inductively coupled plasma optical emission spectrometry (ICP-OES, Avio 560 Max, Platinum Elmer GmbH, Shelton, CT, USA) at a corresponding test wavelength (238.204 nm for Fe, 257.610 nm for Mn, 324.752 nm for Cu, 206.200 nm for Zn, 317.933 nm for Ca, and 285.213 nm for Mg, respectively). Finally, high-throughput sequencing was used to detect the community composition of soil samples with good microbial growth trends in the aerobic and anaerobic environments (PCR was performed using TransGen AP221-02: TransStart Fastpfu DNA Polymerase; PCR instrument: ABI GeneAmp®-9700 type, Waltham, MA, USA).

3. Results and Discussion

3.1. Microbial Biomass Carbon and Nitrogen Content

The MBC content of the simulated lunar soil cultured with different concentrations of fermentation broth is shown in Figure 2a. It can be seen that the overall growth of microorganisms in the simulated lunar soil cultivated under anaerobic conditions was better than that under aerobic conditions. In addition, the number of microorganisms under aerobic conditions decreased except for the initial attachment growth, which was related to the fact that the microorganisms in the fermentation broth were cultivated through anaerobic facultative fermentation. Meanwhile, due to the lack of nutrient supplementation during the cultivation period and the lack of sufficient nutrient sources in the simulated lunar soil itself, each group eventually showed a decreasing trend in quantity after one month. This indicated that the content of the fermentation broth itself was a limiting factor for microbial growth in whatever aerobic or anaerobic condition. Different from a gradual decrease in the MBC in the aerobic condition, the MBC content in the anaerobic condition went through an increase for the 20% and 40% concentration groups on the 20th

day. The treatment group with better microbial growth was the fermentation broth with a concentration of 20% in the aerobic culture group and the one with a 40% concentration fermentation broth in the anaerobic culture group. Compared with the control group without any addition of the fermentation broth, the microbial biomass in the above two treatments increased by 4.81 times and 23.67 times, respectively. It suggested that the anaerobic environment was more suitable for the growth of bacterial strains from the fermentation broth. This further indicates that using garbage leachate for cultivating and improving microbial sustainability of lunar soil in the lunar environment is feasible.

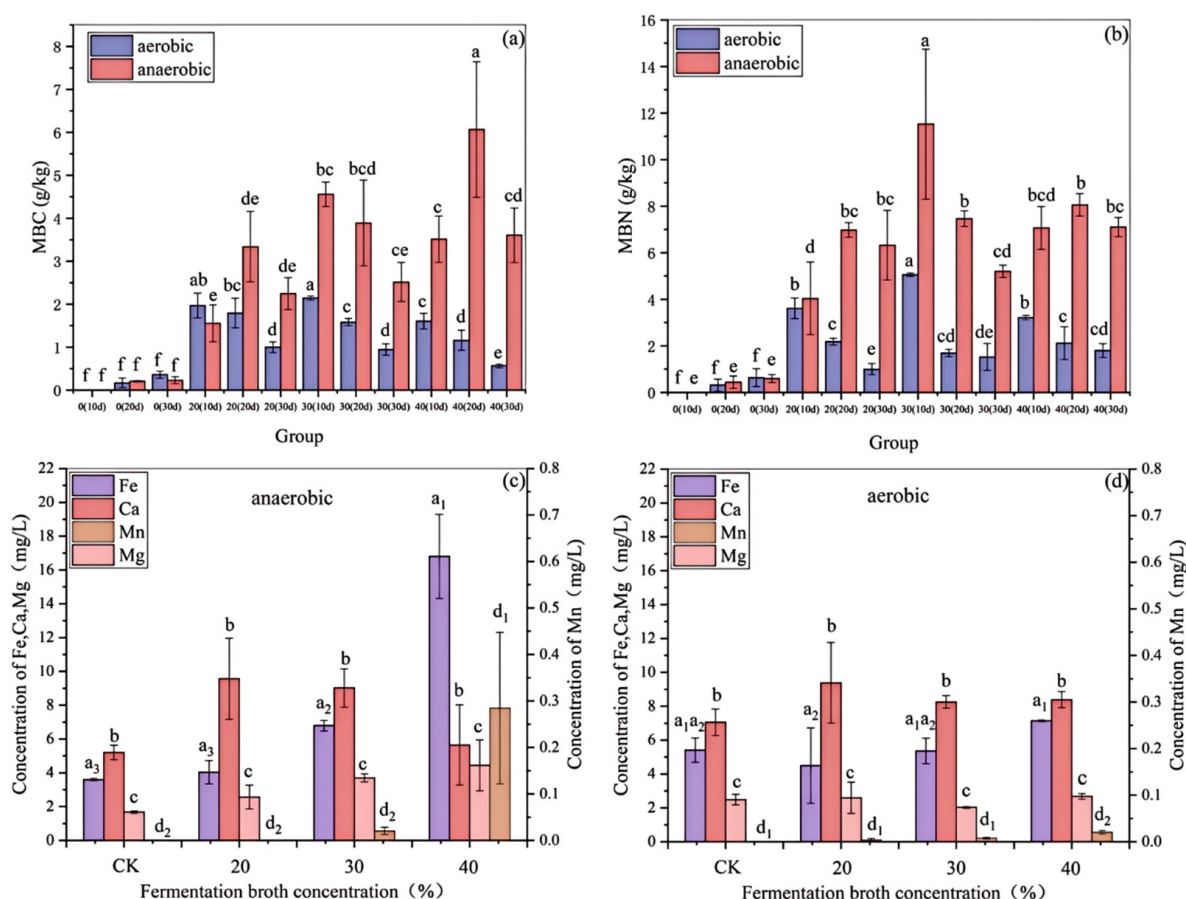


Figure 2. Experimental data. (a) MBC content under aerobic and anaerobic conditions; (b) MBN content under aerobic and anaerobic conditions; (c) Content of water-soluble mineral elements under anaerobic conditions; (d) Water soluble mineral element content in aerobic conditions. Note: The letters in the figure indicate significant differences ($p < 0.05$) between various indicators at different times and fermentation broth concentrations. The subtitles in (a,b) show the difference analysis results under aerobic and anaerobic conditions, respectively; In (c,d), each letter displays the corresponding metal element, with different subscripts representing the statistical differences. Conversely, the same letter represents no differences.

The trend of changes in MBN content is shown in Figure 2b and it can be observed that the trend of changes in MBN was basically consistent with that of the MBC content. Compared to the aerobic condition, the MBN of simulated lunar soil samples in the anaerobic environment was higher. Therefore, the above conclusion can be further confirmed. It is interesting to note that there was a small amount of MBC and MBN content in the control group after one month under both aerobic and anaerobic conditions, implying exogenous environmental input of microorganisms during the cultivation process. At the same time, the MBN in the aerobic environment showed a significant downward trend.

3.2. Dynamic Changes of Water-Soluble Mineral Elements

This experiment explored the karst characteristics of bacteria growing in simulated lunar soil by detecting changes in water-soluble mineral element content. The measurement results are shown in Figure 2c,d. Taking the control group under the anaerobic condition as an example, the simulated lunar soil itself had the highest content of Ca ions, about 5.20 mg/L, followed by Fe and Mg ions with higher content, about 3.60 mg/L and 1.68 mg/L, respectively. The trace element Mn ion content was very low and the Cu and Zn contents were almost below the detection limits, so it was not involved in the analysis. The concentrations of the above mineral elements indicated that these elements originally existed mainly in an insoluble state in the simulated lunar soil, making it difficult to directly carry out vegetation planting.

As the fermentation broth was added, the concentrations of the soluble mineral elements increased. Under the anaerobic condition (Figure 2c), the metal concentrations showed a significant increase compared to the control group, indicating that the increased water-soluble mineral elements were either from the added garbage fermentation broth or from the dissolution effect of the bacteria growing in this condition. The water-soluble Fe, Ca, and Mg ion contents in the lunar soil with 40% fermentation broth increased by 3.98, 1.72, and 2.65 times compared to the control group, respectively. Under the anaerobic condition, the lunar soil samples cultured with 30% concentration fermentation broth began to dissolve Mn ions.

Under the aerobic condition, the water-soluble Fe ion content in the lunar soil with a 40% concentration of fermentation broth increased by 1.32 times compared to the control group. Mn can be leached out from the lunar soil sample cultured in a 40% concentration fermentation broth. The contents of Mg and Ca were not changed much across various treatments. Among the six elements measured in total, the fermentation broth only significantly increased the concentrations of Fe and Mn in the 40% concentration group, indicating that the microorganisms growing in it had a limited dissolution effect on these two components and had almost no dissolution effect on Ca, Mg, Cu, or Zn.

In addition, by comparing data from different concentrations of garbage fermentation broth, it was found that there were differences in the dissolution content of mineral elements under the anaerobic and aerobic conditions. The contents of water-soluble mineral elements in the lunar soil samples under the anaerobic condition increased with the increase in fermentation broth concentration while those under the aerobic condition did not fluctuate significantly, which was consistent with the trend of microbial biomass. The more microbial growth, the more mineral elements were dissolved, indicating that the fermentation broth culture positively impacted the lunar soil sample.

Similar conclusions have been reached in previous studies [10]. Researchers found that the total content and bioavailability of macro (N, S, P, Ca, K, and Mg) and trace (Fe, Mn, Cu, and Zn) nutrients increased approximately linearly as the amount of fertilizer added to lunar and Martian soil simulants increased. This was an important discovery because Lunar and Martian regolith simulant-based soils are basically unable to provide macro and micronutrients coming from exclusively (N), mostly (P and S), or partly (K, Ca, Mg, Fe, Zn, Cu, Mn, B, Cl, and Ni) from organic matter degradation. Therefore, without the help of external organic or inorganic inputs, simulated weathering layers cannot maintain sufficient crop growth.

3.3. Microbial Diversity

The community composition of two soil samples with good microbial growth trends in aerobic and anaerobic environments was tested using high-throughput sequencing, namely the aerobic sample with 20% fermentation broth (YY20_3) and the anaerobic sample with 40% fermentation broth (WY40_1). The results are shown in Figure 3. Each sample was subjected to sample sequence flattening according to the minimum number of sample sequences. The effective number of sequences of 136,103, and 7 phyla, 10 genera, and 325 OTUs were obtained. It can be seen from the species abundance ratio of each species

at the phyla level that the bacterial microorganisms in the two sample soils were mainly composed of *Proteobacteria*, *Firmicutes*, and *Actinobacteriota*, accounting for more than 90% of the total abundance.

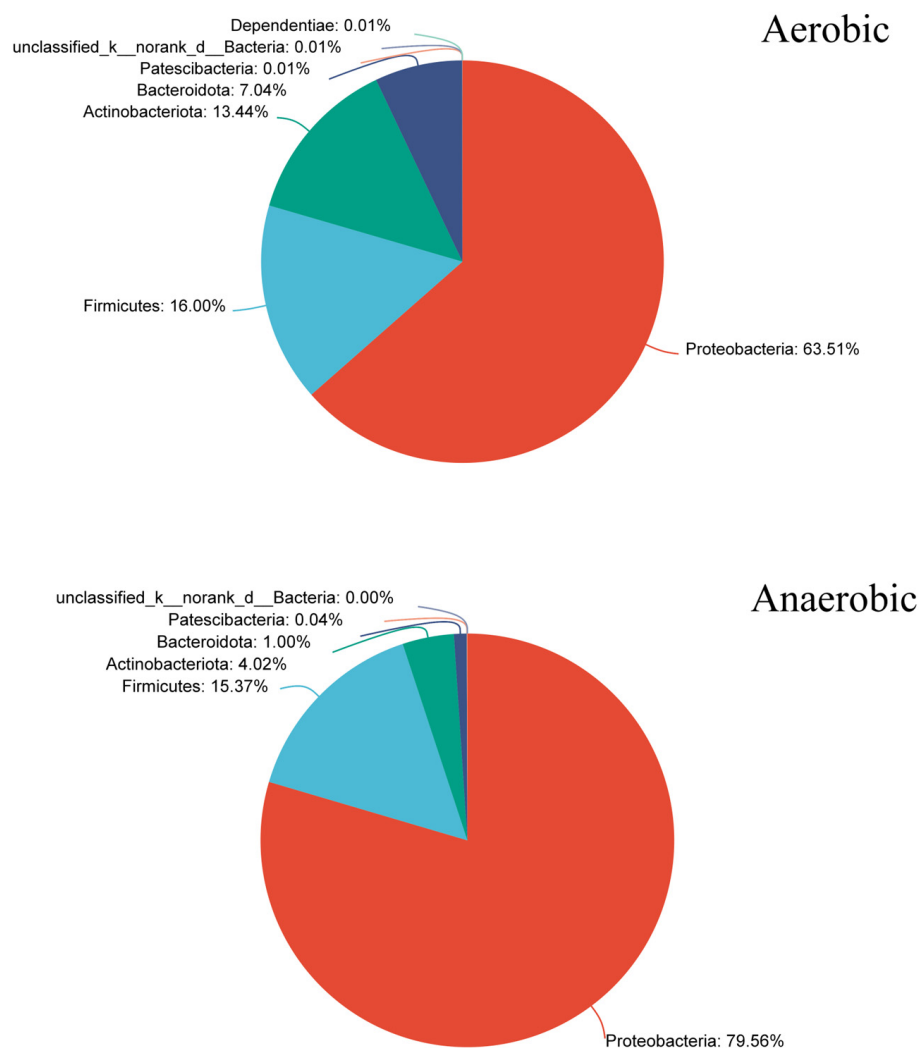


Figure 3. Microbial population pie chart. Horizontal community composition of the two samples in the phylum level.

The similarity and difference in community composition at the genus level of different samples can be reflected by the color change and similarity on the community heatmap. The darker the red color in the figure, the higher the abundance (Figure 4). Due to the large differences in viable microbial species caused by aerobic and anaerobic conditions, aerobic, anaerobic, and facultative bacteria could be clearly distinguished from the figures. Similar to what Yao et al. [8] found in their research, the composition of bacterial communities in solid waste mixed with lunar soil simulants underwent significant changes after fermentation. Among them, *Acetobacter* (41.67%) dominates the sample WY40_1, accounting for nearly half of the content. In sample YY20_3, the genera *Novosphingobium* (12.73%), *Microbacterium* (10.35%), *denitrifying bacteria* (10.23%), *Stenotrophomonas* (8.87%), and *Lactobacillus* (8.50%) have the main advantages, accounting for about half of all genera. Further analysis of advantageous species can lay the foundation for future targeted cultivation and seed selection.

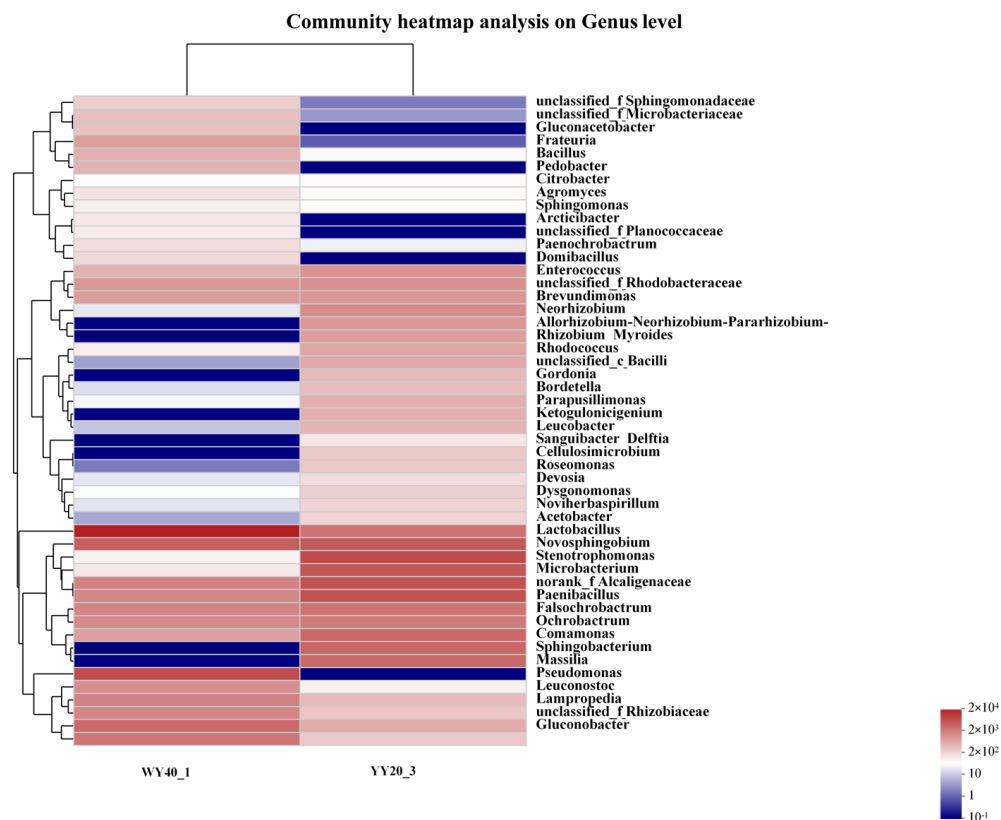


Figure 4. Microbial community heatmap diagram. Horizontal community composition of the two samples in the genus level.

4. Conclusions

In summary, this study explored the effect of facultative anaerobic fermentation of household waste on the fertility improvement in simulated lunar soil under aerobic and anaerobic conditions, providing a scientific basis for further soil modification in a lunar base. The experimental results indicated that under aerobic and anaerobic conditions, microorganisms in the simulated lunar soil samples had successfully adhered and grown, providing favorable conditions for achieving root microbial symbiosis. At the same time, the growth and reproduction of microorganisms in the simulated lunar soil could affect the physicochemical properties of the soil itself and the improvement in soil nutrient levels also provided promising prospects for subsequent plant planting in the simulated lunar soil. In particular, the simulated lunar soil samples cultured in the anaerobic environment were superior to the aerobic environment in terms of microbial biomass growth and water-soluble mineral elements. The result is inspiring because it is more feasible to conduct a large-scale application of garbage fermentation broth for lunar soil improvement in a lunar anaerobic atmosphere. The current study is only a preliminary exploration of the organic modification of lunar soil. Further research can be conducted on screening specific microbial species in the lunar soil improvement and utilization process. Other efforts simulating the lunar environment to the closest (including temperature, atmosphere, etc.) are also necessary to evaluate the strategy of waste utilization and lunar soil modification, providing sufficient technical references for the final construction of lunar bases.

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