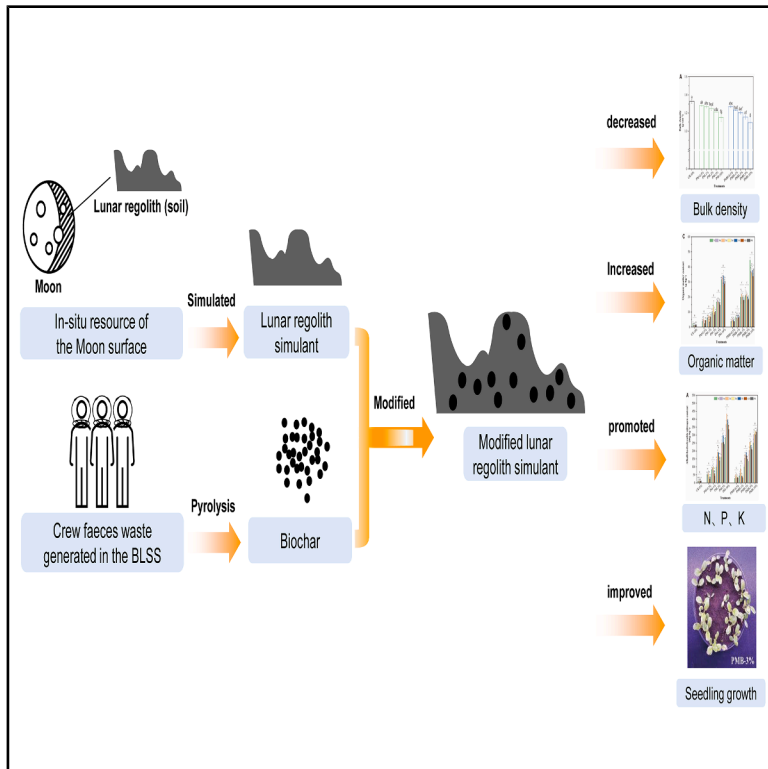


Biochar improves lettuce seedling growth by influencing the nutrient content of simulated lunar soil

Graphical abstract



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In brief

Agricultural science; Plant Biology; Soil biology; Soil science; Space sciences

Highlights

- Biochar mainly improved the physico-chemical properties of lunar regolith simulant
- pH, AN, and TN might serve as critical factors influencing lettuce growth
- The optimal amount of pig manure biochar for amending lunar regolith simulant is 3%
- Pyrolysis shows great potential for treating solid waste in BLSS

Article

Biochar improves lettuce seedling growth by influencing the nutrient content of simulated lunar soil

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SUMMARY

The utilization of native lunar soil and recycled organic waste as primary resources will reduce the reliance on terrestrial inputs and improve the self-sufficiency of bioregenerative life support systems (BLSSs) in future lunar bases. A major question to address is the suitability of lunar soil for plant growth and how soil agronomic performance is affected by biochar. Biochar treatments increased the organic matter content and promoted greater availability of N, P, and K in the simulated lunar soil. It also reduced the bulk density and facilitated the optimal distribution of plant roots. Redundancy analysis indicated that the physical and chemical properties of soil pH, alkali-hydrolyzable nitrogen (AN), and total nitrogen (TN) content might be critical factors influencing lettuce growth. The aforementioned analysis suggested that the optimum rate of biochar addition to improve simulated lunar soil is 3%. The results showed that the utilization of pyrolysis for treating solid waste in BLSSs has significant potential, with biochar enhancing the properties of the simulated lunar soil.

INTRODUCTION

In recent years, there has been an increase in interest in space exploration and the potential establishment of colonies on extra-terrestrial bodies such as the moon. Leading space agencies such as National Aeronautics and Space Administration (NASA),¹ European Space Agency (ESA),² and China National Space Administration (CNSA)³ have been joined by private companies such as Space X⁴ in their exploration of deep space. To support crewed missions to the moon, space research is currently focused on developing bioregenerative life support systems (BLSSs) that can produce food crops using *in situ* resource utilization (ISRU) while also reducing inputs and recycling organic wastes.⁵ A well-designed BLSS would efficiently utilize limited materials, and if extraterrestrial planetary resources could be used, the need for material transport from Earth would be further reduced, increasing the robustness of the BLSS and the feasibility of manned space exploration.⁶ An efficient and sustainable BLSS can be developed on the basis of the ISRU concept, which requires the use of native materials such as extraterrestrial soil and waste as primary resources.⁷ In this context, important questions arise regarding the suitability of native lunar soil for plant growth and how its agronomic performance is affected by the addition of organic matter from crew waste. To improve the closure coefficient of BLSS for reducing stowage and resupply of life support materials, solid waste, including inedible plant biomass and human feces, should be

treated and reused as a soil-like substrate for greater plant cultivation in space.^{8,9}

Lunar soil, as the most abundant and available resource on the lunar surface, has the potential to be used as a substrate for growing plants in lunar BLSSs.¹⁰ Elemental analysis has shown that lunar soil does not contain any toxic elements for plants,¹¹ but it does contain the essential basic macroelements (C, H, O, N, P, S, K, Mg, Na, and Ca) and minor elements (Mn, Cr, Ni, Mo, Cu, Fe, and Zn) necessary for life.^{12–14} However, the main factors limiting the use of lunar soil as a substrate for plant cultivation are low nutrient bioavailability and poor water-holding capacity due to the absence of organic carbon.^{15,16} Additionally, characteristics such as small particle size, low porosity, high bulk density (BD),¹⁷ and a lack of organic matter make lunar soil not conducive to plant growth.¹⁸ Plants grown in real lunar soil have stunted roots similar to those grown in simulated lunar soil, even though the seeds germinated in different substrates.¹⁹ Therefore, a sustainable ISRU system for crop production using simulated lunar soil would require exogenous inputs of inorganic fertilizer or organic matter.²⁰ This finding indicates a need to improve the bioavailability of both simulated lunar soil and real lunar soil for plant cultivation. It may be difficult for plants to grow without the modification of lunar soil.^{7,21} Therefore, incorporating organic matter into lunar soil can increase the bioavailability of nutrients in the soil. In space missions, human excreta such as urine and feces are valuable resources that can be recycled in BLSSs to recover compounds for use as

fertilizer or compost to support plant growth.¹⁴ However, few attempts have been made to prepare and reuse fermented residues from crop biomass and human feces as substrates for plant growth in space.^{22,23} Adding stable organic materials such as compost or cocopeat to simulated lunar soil is beneficial for improving soil particle aggregation, water holding capacity, biological activity, and the physicochemical properties of the plant growth environment.^{24–26} In addition, studies have analyzed the adaptability of black soldier flies, earthworms, and microorganisms in simulated lunar soil. The results show that these organisms have a good survival status and can adapt well to the simulated lunar soil environment, which may play an important role in improving lunar soil in the future.^{27–29}

Currently, the primary approach for the *in situ* utilization of human feces in BLSSs is compost fermentation. Monogastric manure was chosen for use as organic material to improve the physicochemical characteristics of the regolith because of its similarity to human waste.^{30–32} Caporale et al.³³ incorporated a commercially available monogastric organic manure into simulated lunar soil as a potential substitute for an on-board produced organic amendment. As anticipated, there was an approximately linear increase in the total and bioavailable levels of macronutrients (N, S, P, Ca, K, and Mg) and micronutrients (Fe, Mn, Cu, and Zn) with the addition of manure to the simulated lunar soil. Additionally, the use of organic manure increased the suitability of simulated lunar soil for lettuce cultivation. The addition of manure, a promising amendment, resulted in increased biomass in plants grown in simulated extraterrestrial soil, and the chemical composition of the plants was differentially affected based on the rate of manure addition to the stimulated lunar soil.⁷ Caporale et al.⁵ investigated the impact of a mixture of simulated lunar soil and commercial horse/swine monogastric manure on plant growth and physicochemical properties. They reported that the addition of monogastric manure significantly increased both above- and belowground plant biomass and improved plant nutrient uptake and translocation. Liu et al.²² utilized fermented residues as substrates and examined the effects of different ratios of these residues on wheat seed germination, growth, photosynthetic properties, and antioxidant capacity. The results indicated that the optimal ratio of fermented residue significantly increased seed germination, root length, shoot length, and biomass production. These studies focused mostly on the effects of different ratios of amendments on plant growth in simulated lunar soil without considering the effects of the different treatment times of the amendments on the physicochemical properties of the simulated lunar soil, especially the contents of nutrient elements that affect plant growth. Moreover, potentially toxic elements (PTEs), pathogenic microorganisms, antibiotic residues, biocides, and other micropollutants in manure or compost threaten animals, human, and environmental health and pose certain risks for subsequent application, especially when used to improve simulated lunar soil.^{34–36} Furthermore, the processes of organic fertilizer production, such as composting, often involve long cycles and numerous factors that control fermentation, which requires a long treatment cycle, high moisture content, and careful control of temperature, pH, and oxygen levels and involves the production of odors.³⁷ In addition to composting, direct pyrolysis treatment offers a

shorter treatment cycle, reduces water consumption, and does not require control of the treatment conditions.³⁸ Pyrolysis, the thermochemical conversion under oxygen-limited conditions, has potential as an alternative treatment for animal manure.³⁹ Pyrolysis reduces the bioavailability of PTEs and eliminates pathogenic microorganisms and organic micropollutants.³⁹ The Boeing Company has conducted a comprehensive assessment of various waste treatment methods for a six-person manned exploration mission, assuming a biological regeneration rate of 75%.⁴⁰ Initial findings suggest that pyrolysis is the preferred option, as it allows for water recovery at a food closure rate of 75%. Pyrolysis is a process by which waste materials are thermally degraded in the absence of or under a limited supply of oxygen to generate energy products such as bio-oil, biochar, and syngas.⁴¹ The reactor, which serves as the core component of the pyrolysis system, is heated to high temperatures via either an external heater or an internal electrical heating element. In addition, the combustion or electric heating process makes the reactor system more complex.⁴¹ Pyrolysis can be integrated with other technologies to facilitate the rapid degradation and conversion of challenging-to-degrade wastes as controlled life-support systems on lunar bases expand and food closure rates increase.⁴² The integration of a solar heating system into a pyrolysis system can effectively address the heating challenge of pyrolysis by providing a sustainable and cost-efficient energy source.⁴¹ Solar pyrolysis utilizes concentrated solar energy as the primary heat source driving biomass pyrolysis reactions. The economic analysis of solar pyrolysis indicates approximately 25% energy savings when solar-assisted pyrolysis is applied compared with conventional pyrolysis.⁴³ The bio-oil and syngas produced during pyrolysis can be purified and separated for use as energy fuels.⁴⁴ In the future, the lunar base can efficiently achieve waste treatment and reduce energy consumption through solar thermal decomposition technology. Additionally, biochar, the solid product of pyrolysis, can be utilized to increase the lunar soil's ability to support plant growth and increase crop yield and quality. This comprehensive use of *in situ* resources also offers substantial potential for sequestering carbon and improving soil fertility and physical properties.^{45–47} Owing to its unique pore structure and large surface area, biochar improves soil porosity, aeration, water retention, and fertility to increase crop root growth. Additionally, it retains nutrients and minerals in the soil, reduces nutrient loss, and ultimately increases crop yield.⁴⁸ To our knowledge, in previous experiments, researchers have utilized simulated lunar soil compost mixtures as plant growth media. However, the impact of pig manure (PM)-derived biochar on improving the physicochemical properties of lunar soil was not examined in these studies. The phosphorus, potassium, and other nutrient contents of manure/waste biochars are greater than those of crop residue and woody biochars.⁴⁹ The structure and function of the swine gastrointestinal tract and the morphology and pharmacokinetics of the pancreas are similar to those of humans.⁵⁰ Anatomical and physiological similarities to humans make swine an excellent large animal model for human health and disease control.⁵¹ Finally, the conditions in space are extremely complex and diverse. For example, the moon features high vacuum, wide temperature differences, high radiation and low gravity, which inevitably affect the

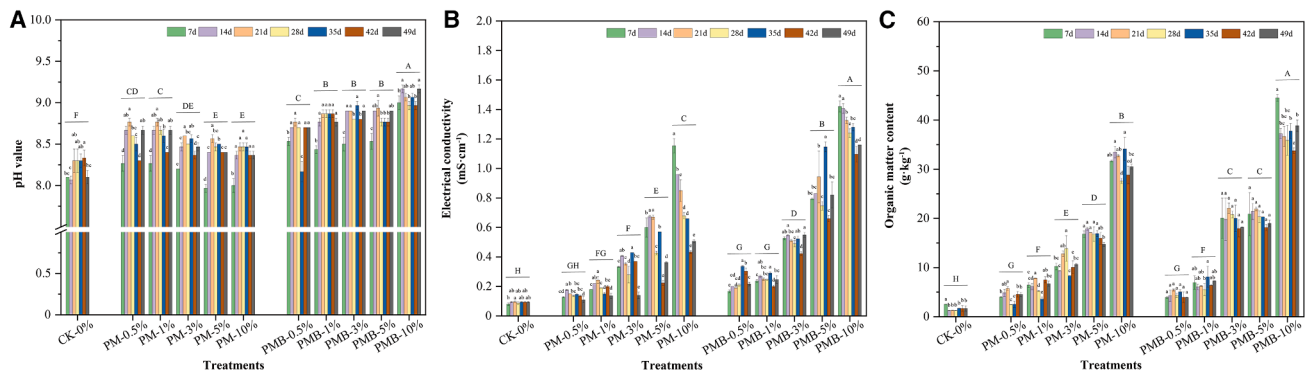


Figure 1. Chemical properties changes of simulated lunar soil after treatments

Changes over time in lunar regolith simulant pH (A), electrical conductivity (B), and organic matter (SOM) content (C).

CK, PM (–0.5%, –1%, 3%, 5%, and 10%), and PMB (–0.5%, –1%, 3%, 5%, and 10%) denote the treatments. Means \pm SD. Significant differences between treatments and doses were indicated using different lowercase and uppercase letters (Duncan's test, $p < 0.05$).

experimental results.⁵² Conducting relevant research is highly valuable and necessary. However, owing to the limitations of experimental conditions, it is still currently impossible to carry out simulation tests of related space conditions. The focus of this study is on whether PM biochar can improve the physical and chemical properties of simulated lunar soil and be used as a planting substrate for crops. Therefore, in this study, PM was selected to simulate human feces because of the similarity in the physiological functions of humans and pigs.⁵³

In the absence of real lunar soil samples on Earth, scientific experiments on space agriculture for human missions can be conducted using simulated crushed terrestrial rock that replicates the geotechnical and compositional features of lunar soil. The hypothesis was that PM biochar has significant potential for enhancing the crop cultivation performance of lunar soil. In this study, the objectives were (1) to investigate the effects of adding varying amounts of PM-derived biochar on the physicochemical properties of lunar soil; (2) to evaluate the effects of different biochar treatment times on these properties; and (3) to explore the key factors through which biochar influences the physicochemical properties of lunar soils and its effects on seed germination and root length in amended lunar soil. For experimental purposes, PM, which simulates human feces produced in a BLSS, was applied to prepare PM-based biochar.

RESULTS

Changes in the chemical properties of the simulated lunar soil

pH, electrical conductivity, and organic matter

The pH fluctuated over time, depending on the treatment (Figure 1A). Compared with the control, biochar treatment of the simulated lunar soil resulted in an increase in pH, while the pH of the feedstock treatment group decreased. When the amount of biochar and feedstock added exceeded 1% and 3%, respectively, there was no significant difference in pH compared with that of CK. In addition, there was no significant variation in the pH of the simulated lunar soil in the biochar and feedstock groups over 21 and 28 days, respectively. Compared

with the control, the pH of the simulated lunar soil changed by approximately 1.0 units with the addition of 3% biochar. The changes in pH not only affects the availability of soil nutrients and soil structure but also influences the growth of the plant roots and nutrient uptake.^{54,55} Based on the results of this study, it is highly probable that biochar can increase the pH of simulated lunar soil, and enhance the availability of simulated lunar soil nutrients.

The addition of pig manure biochar (PMB) and PM increased the electrical conductivity (EC) (Figure 1B) of the simulated lunar soil. Furthermore, the effects of biochar on the EC of the simulated lunar soil were greater than those of the biochar feedstocks. Specifically, when biochar and its feedstocks were added at rates of 3%, the EC of the simulated lunar soil increased by 83.04% (from 0.09 ± 0 to 0.55 ± 0.02 mS/cm) and 33.36%, respectively. After the treatment time exceeded 28 days, the changes in EC were not significant. Soil EC serves as an indicator of the concentration of soluble salts in the soil solution. The presence of these soluble ions facilitates their direct absorption and utilization by plants, thereby promoting the development of root systems.⁵⁶

The application of biochar to simulated lunar soil significantly increased the organic matter content (Figure 1C). When the added amount exceeds 3%, the effects of biochar on organic matter content of the simulated lunar soil were greater than those of feedstocks. The organic matter content increased by 90.70% (from 1.69 ± 0.02 to 18.12 ± 0.10 g/kg) and 84.19%, respectively. Besides, when the processing time reaches approximately 28 days, the organic matter content tends to stabilize and exhibits no statistically significant variation. Soil organic matter (SOM) promotes the formation of soil aggregate structure, enhances soil porosity, reduces soil BD, and improves aeration.⁵⁷ Upon decomposition, it releases essential nutrients such as nitrogen, phosphorus, and potassium.⁵⁸ Biochars can maintain their particulate form over extended periods of time.⁵⁹ This phenomenon may also occur following the application of biochar to the simulated lunar soil. This is consistent with the trend of reduced BD and increased nutrient content in simulated lunar soil after the application of biochar.

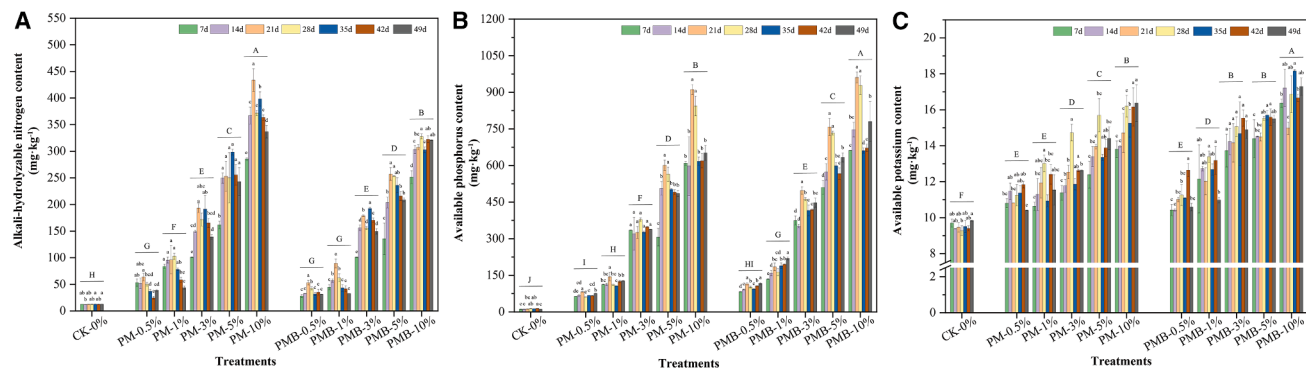


Figure 2. Changes in the content of available nutrients in simulated lunar soil after treatments

Alkali-hydrolyzable nitrogen content (A), available phosphorus content (B), and available potassium content (C) of lunar regolith simulant during the different treatments. CK, PM, and PMB denote the treatments and statistical levels are indicated by lower-case letters and capitals as in Figure 1.

Available N, P, and K

The availability of nutrients in the soil can significantly influence their absorption and utilization by plants, especially when the simulated lunar soil itself is deficient in nutrients. The addition of various amounts of biochar and its feedstocks significantly increased the levels of alkali-hydrolyzable nitrogen (AN) (Figure 2A), available phosphorus (AP) (Figure 2B), and available potassium (AK) (Figure 2C) in the simulated lunar soil. The concentrations of AN in the soils treated with PMB and PM were significantly greater than those in the control, with increases of 91.54% and 90.91%, respectively, at the 3% treatment rate. However, when biochar was added to the simulated lunar soil at rates of 3% and 5%, there was no significant difference in the AK content. Compared with the control, the PMB and PM treatments greatly increased the available P in the soil by 97.65% and 96.89%, respectively. Compared with the control, in the simulated lunar soil, the AK content, an important indicator of the soil nutrient status for crop plant growth, also increased by 33.92% and 22.09%, respectively for the PMB and PM treatments. Among the treatments, the change in AP was most pronounced at approximately 21 days. In addition, after the application of biochar and its feedstocks to the simulated lunar soil, the AN, AP, and AK contents of the simulated lunar soil all tended to first increase but then decreased with increasing treatment time.

Total N, P, and K

The impacts of the different treatments on the total nutrient content of the simulated lunar soil were analyzed because of their crucial role in plant growth. The results indicated a significant increase in the total nitrogen (TN) (Figure 3A), total phosphorus (TP) (Figure 3B), and total potassium (TK) (Figure 3C) contents of the simulated lunar soil with the addition of biochar and its feedstocks. Compared with the control, there were significant increases in TN (93.94% from 0.0068 ± 0 to 0.7151 ± 0.01 g/kg), TP (84.15% from 0.0068 ± 0 to 1.1282 ± 0.01 g/kg), and TK (84.78% from 0.31 ± 0.02 to 2.03 ± 0.13 g/kg), whereas the changes in TK content were nonsignificant (9.27% and 12.68%). As expected, with increasing amounts of biochar added to the simulated lunar

soil, there were significant increases in the EC (by up to one order of magnitude, reaching values that can adversely affect plant growth), organic matter and total P contents (by up to two orders of magnitude), and exchangeable K content.

Changes in the physical properties of the simulated lunar soil

Soil BD and aeration directly affect the growth and distribution of plant roots. Unimproved simulated lunar soil is not conducive to the growth of plant roots. The addition of biochar and its feedstocks significantly affected the physical properties of the simulated lunar soil. As the amount of biochar and its feedstocks increased, the BD (Figure 4A) of the simulated lunar soil decreased notably. Furthermore, when the biochar addition rate was 10%, the BD decreased by 18.05%. Additionally, both the total porosity (Figure 4B) and the air porosity (Figure 4C) of the simulated lunar soil significantly increased with increasing rates of biochar and feedstock addition. In brief, the impact of biochar on the physical properties of simulated lunar soil is greater than that of its feedstocks.

Changes in seedling growth

Seedling growth morphology

Lettuce seedlings were grown directly in the simulated lunar soil. When lettuce was grown in the unamended simulated lunar soil (which served as the control treatment), the growth of the lettuce seedlings was markedly inhibited (Figures 5A and 5B), with a reduced germination percentage (GP) and shortened root length (Table 1). Furthermore, as the rate of addition of biochar and its feedstocks increased, the GP and root length of lettuce seedlings in the improved simulated lunar soil significantly increased, with a more pronounced effect observed in the PMB treatment. This improvement may be attributed to the enhanced physical properties and increased nutrient availability of the soil resulting from the application of biochar. However, when the addition of biochar exceeded 5%, there was a significant decrease in both the GP and root length of the lettuce seedlings in the simulated lunar soil. The growth of the seedlings was significantly inhibited when PM was added at a rate of more than 5%, but this effect did not occur when

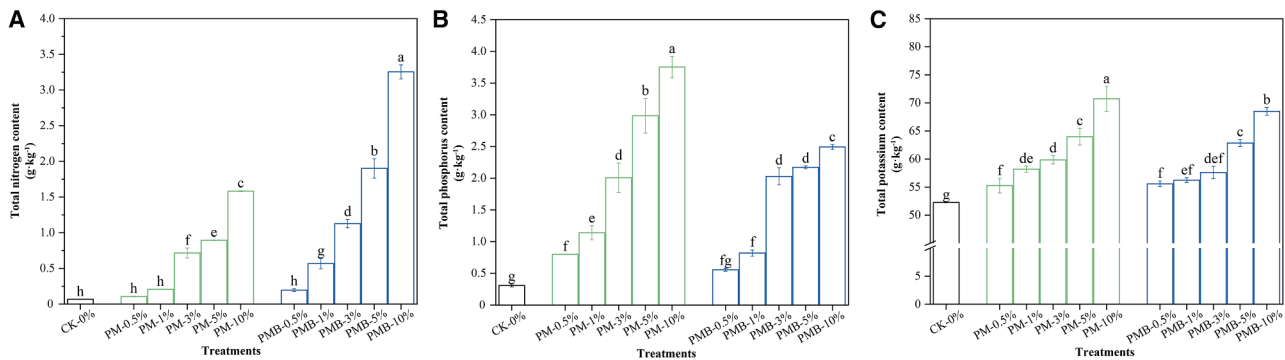


Figure 3. Changes in total nutrient content of simulated lunar soil after treatments

Total nitrogen content (A), total phosphorus content (B), and total potassium content (C) of lunar regolith simulant during the different treatments. CK, PM, and PMB denote the treatments and statistical levels are indicated by lower-case letters and capitals as in Figure 1

the plants were treated with biochar. The results show that biochar is more suitable than feedstocks for improving simulated lunar soil.

Seedling biomass

Compared with the control, the fresh and dry weights of the lettuce seedlings in the simulated lunar soil significantly increased after treatment with PMB and PM (Figure 6). Additionally, with increasing PMB and PM addition, the biomass of the lettuce seedlings tended to first increase but then decreased (when the rate of addition exceeded 5%). Specifically, compared with the control, the fresh weight (FW) of the lettuce seedlings in the simulated lunar soil increased by 25.39%, whereas the dry weight (DW) increased by 20.98% when 3% biochar was added.

Key factors driving the effects of simulated lunar soil physicochemical properties on seedling growth

The final agronomic performance of the growth media may not be related to a single factor, such as water availability, porosity, BD or nutrient concentrations, because of the simultaneous variation and interactive or offsetting effects of these factors. A redundancy analysis (RDA) (Figure 7) of the physicochemical properties of the modified simulated lunar soil and the growth indicators of the lettuce seedlings revealed that the GP, DW, and FW of lettuce were negatively associated with the AN, TK, and TP contents but positively associated with the soil air porosity (SAP), BD and pH. Among these factors, AN, pH, and TN accounted for 64.5%, 11.1%, and 3.6%, respectively, of the variation in the growth indicators of the lettuce seedlings ($p < 0.05$) (Table 2). These findings indicate that AN, pH, and TN are the primary factors influencing the growth of lettuce seedlings in simulated lunar soil containing biochar. A significance-based heatmap was created using Spearman's correlation analysis ($p < 0.05$) to further explore the relationships among the simulated lunar soil properties and the lettuce seedling growth indicators (Figure 8). The results of the heatmap analysis revealed significant positive correlations among the lettuce RL, DW, and FW, and BD in the simulated lunar soil. Interestingly, there was no significant correlation between the GP and any of the physicochemical properties of the simulated lunar soil. Therefore, the extraterrestrial soil, if not improved with external organic or inorganic

inputs, may not adequately sustain crop growth in an ISRU-based BLSS.

DISCUSSION

Currently, there is a widespread belief that lunar soil has enormous potential as a substrate in BLSSs as it contains the nutrient elements necessary for plant growth. For example, the apatite crystals abundant in lunar soil can provide calcium and phosphorus, but they also contain potassium, iron, sulfur, and other elements essential for plant growth.¹¹ In previous studies, researchers have utilized lunar soil brought back from the Apollo 11 and Apollo 12 missions to cultivate plants under sterile conditions, examining seed germination, plant growth, and disease occurrence. Their findings indicate that lunar soil did not induce plant diseases and promoted seed germination by supplying mineral nutrients for plant growth, and variations in the synthesis of secondary metabolites and the accumulation of lunar mineral elements among different plant species were revealed.^{60–63} The Apollo era provided valuable insights into the biological effects of the lunar environment on terrestrial life forms on the basis of plant experiments with lunar samples, which are beneficial for future research supporting lunar exploration. With the rapid development of modern omics methods, researchers have utilized transcriptomics to analyze the physiological and metabolic strategies adopted by plants during growth in real lunar soil. Paul et al.¹⁹ employed omics approaches to investigate the germination and growth of *Arabidopsis* in different Apollo 11, 12, and 17 lunar soils. Plant growth and development were slow, suggesting specific stresses and variations in gene expression related to ionic stress. Although lunar soil can potentially support plant growth in the short term, it is not a suitable substrate because of its lack of essential nutrients such as N and P, lack of organic matter, high pH values, high Na^+ content, and poor water retention capacity.⁷ The pH, EC, and organic matter content play important roles in regulating nutrient availability in a plant growth medium.⁶⁴ Studies have shown that simulated lunar soil typically has a pH greater than 6 and may exhibit alkaline properties in some cases.¹⁶ The main obstacle to using lunar soil as a growth substrate is its high pH, which is due to the predominance of soluble alkaline minerals and the absence of acidic components.

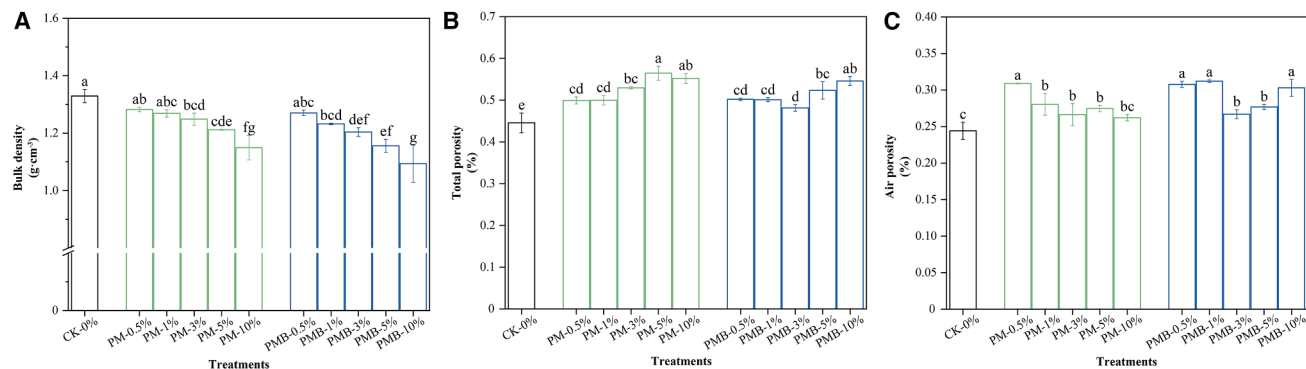


Figure 4. Physical properties changes of simulated lunar soil after treatments

Bulk density (A), total porosity (B), and air porosity (C) of lunar regolith simulant during the different treatments. CK, PM, and PMB denote the treatments and statistical levels are indicated by lower-case letters and capitals as in Figure 1.

While a high pH is generally considered detrimental to plant growth, it can also be beneficial for plant cultivation through certain mechanisms. SOM plays a crucial role in promoting the sustainability and resilience of agricultural production by facilitating the uptake and release of essential nutrients and enhancing the soil structure.^{65,66} Furthermore, the moon lacks an atmosphere and the associated weathering processes affecting Earth's soils, causing lunar soil to have characteristics such as small particle size, low porosity, high BD, and poor water retention capacity.^{16,67} Additionally, lunar soil contains sharp particles that can potentially damage plant roots.⁶⁸ In summary, despite certain limitations in the physicochemical properties of lunar soil, it holds considerable potential as a planting substrate within the BLSS of a lunar base.

To address the limitations of lunar soil as a planting substrate, effectively utilizing the solid waste generated within the BLSS of a lunar base is recommended. First, this approach can facilitate material recycling within the BLSS, enhancing system closure. Second, it can substantially reduce payload transportation costs and decrease dependence on Earth-based resources. In one study, researchers developed a controlled ecological life support system waste model and estimated solid waste generation.⁴² Their findings indicated that, under the assumption of 75% food closure and a crew size of six individuals, the anticipated daily solid waste production (FW) was 35.90 kg/d. This primarily comprises 4.32 kg per day of human metabolic waste and 27.00 kg per day of inedible plant biomass. By recycling the solid waste generated in the BLSS and applying it to lunar soil, the nutrient element and organic matter contents can be increased, improving the physical and chemical properties of lunar soil to enhance the soil's plant growth performance, meeting the needs for plant growth, and forming a suitable cultivation substrate for crop production. The addition of organic amendments derived from the solid waste of BLSS (e.g., compost or manure) or mineral fertilizer to simulated lunar soil could increase the availability of essential nutrients and supply vital elements such as nitrogen, phosphorus, and organic carbon, affecting nutrient availability and dynamics in a growth medium.⁷ Caporale et al.⁶⁹ conducted a study on the mixing of organic compost with simulated extra-terrestrial soil and revealed that the soil BD decreased progres-

sively as the amount of compost in the mixture increased, ranging from 1.39 g/cm³ (pure simulant) to 0.60 g/cm³ (pure compost). The average particle diameter of 0.5 mm in the simulated lunar soil was consistent with the value reported by Li et al.⁷⁰ for various growth systems. Increased porosity is expected to be beneficial for plant growth.^{71–73} Moreover, the addition of solid waste significantly reduced the BD of the substrate, and this reduction was further enhanced by fermentation. Furthermore, plants typically absorb only bioavailable forms of elements, such as those in readily soluble and exchangeable forms. The availability of nutrients to plants in simulated lunar soil is determined by the equilibrium state between the water and solid phases rather than by the total nutrient content.⁷ Biochar, a solid product obtained through the pyrolysis of plant waste or animal manure under O₂-limited conditions, is an environmentally friendly soil amendment with advantages such as a large specific surface area, complex pore structure, abundant surface functional groups, and stable physicochemical properties.⁷⁴ Additionally, it can absorb soil nutrients and minerals.⁴⁸ Biochar has a unique pore structure and large specific surface area; therefore, it can increase soil porosity, air permeability, and water retention.⁷⁴ Wu et al.⁷⁵ reported that the addition of biochar enhanced soil quality and function by increasing the concentrations of available soil N and P. This finding is similar to the results of this study, which revealed that biochar has beneficial effects on simulated lunar soil, resulting in increased contents of SOM, AN, AP, and AK. The total amount of major elements in the simulated lunar soil may exceed the requirements for plant growth. Additionally, biochar significantly improved the organic matter content, pH, and EC of the simulated lunar soil in this study. The results of this study revealed that the germination rate, root length, and fresh and dry weights of lettuce seedlings in the simulated lunar soil improved significantly more with biochar amendment than with the CK treatment (Table 1 and Figure 6). Similarly, these results also show that the addition of organic matter or functional bacterial strains to simulated lunar soil can significantly promote the growth of plant seedlings. Lunar regolith simulant (TLS-01) amended with coconut coir significantly increased sunflower seedling development and GP.⁷⁶ Hosamani et al. reported that in simulated lunar soil

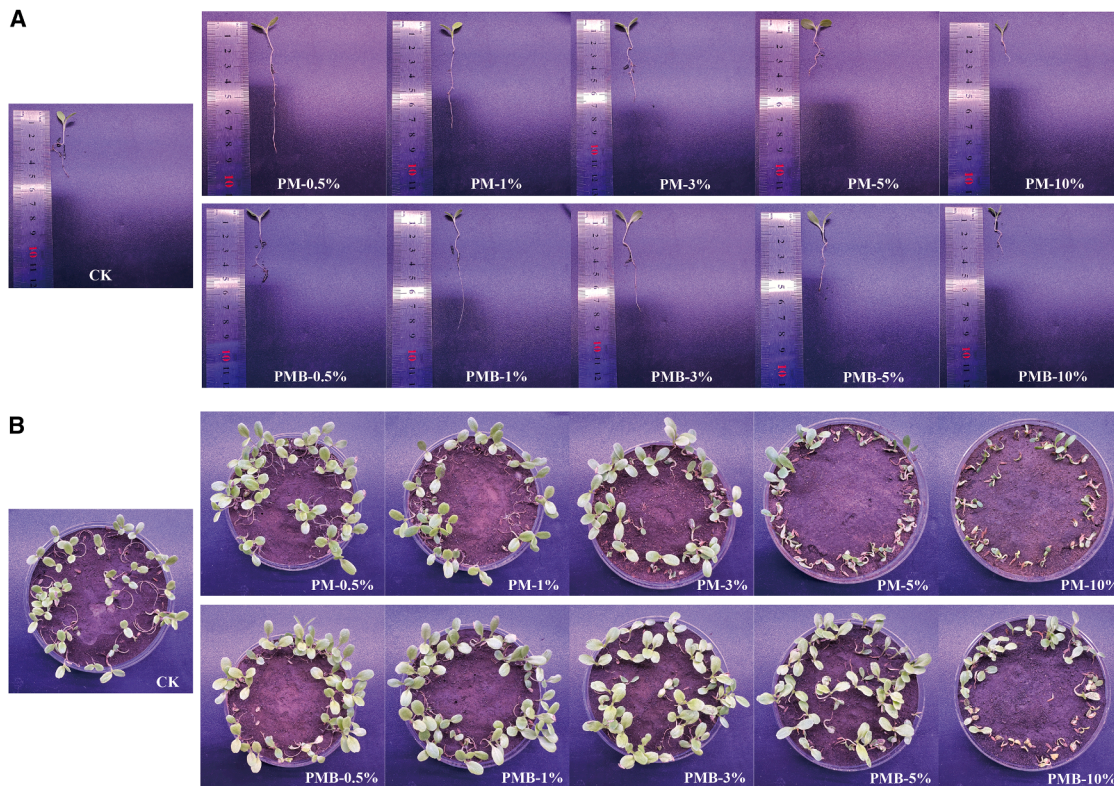


Figure 5. Seedlings root and growth morphology in lunar regolith simulant during the different treatments

(A) The root morphology of lettuce seedlings.
(B) The growth changes of lettuce seedlings.

(LSS), the germination rate, root length and FW of four crops (wheat, tomato, groundnut, and cotton) significantly improved with increasing cocopeat coir addition.²⁶ Xia et al. inoculated simulated lunar soil with phosphorus-solubilizing bacterial strains, which released inorganic phosphorus directly available to plants, promoting plant growth.²⁹

Table 1. Effects of PMB biochar and its feedstock on lettuce seedling growth

Treatment	GP (%)	RL (mm)
CK	78.67 ± 0.01b	32.38 ± 5.16de
PM-0.5%	91.33 ± 0.02a	50.23 ± 12.62a
PM-1%	81.33 ± 0.03b	45.39 ± 6.59 ab
PM-3%	90.00 ± 0.03a	26.19 ± 8.55ef
PM-5%	87.33 ± 0.02a	19.64 ± 3.58 fg
PM-10%	82.00 ± 0.03b	14.65 ± 0.91g
PMB-0.5%	88.67 ± 0.02a	38.91 ± 4.23bcd
PMB-1%	88.67 ± 0.02a	44.57 ± 8.80abc
PMB-3%	92.00 ± 0.02a	35.38 ± 5.41cde
PMB-5%	92.67 ± 0.001a	30.10 ± 5.04de
PMB-10%	82.00 ± 0.03b	16.63 ± 8.28g

Mean ± SD values followed by different superscripts within the same column are significantly different (Duncan's test, $p < 0.05$). GP, germination percentage; RL, root length.

Biochar is rich in elements such as C, O, H, N and P. Typically, the carbon content of biochar exceeds 60%, with easily decomposable carbon readily absorbed and utilized by microorganisms through biomineralization. The oxygen content in biochar ranks second only to that of carbon, at approximately 10%–20%. The hydrogen content remains relatively stable, fluctuating between 2% and 5%. The O/C and H/C ratios are often used as important indicators of the properties of biochar. Nitrogen is abundant in biochar, ranking third in content behind carbon and oxygen; it can provide the nutrients necessary for plant growth, improving soil fertility.^{48,77,78} Biochar has the potential to significantly improve soil properties, increase soil fertility, and increase crop yields. The primary benefits of using biochar for soil improvement include improved physical and chemical properties and increased soil fertility, plant growth and crop yield, and microbial activity. Biochar, as a soil amendment, contains abundant nutrients from various biomass materials. Once applied to soil, biochar continues to release nutrients through interactions with soil biotic or abiotic constituents, thereby improving the physical and chemical properties of the soil. The unique porous structure and various functional groups of biochar enable it to effectively retain the nutrients present in the soil and prevent the loss of various nutrients, such as nitrogen and phosphorus, thereby increasing soil fertility, promoting plant growth, and increasing crop yield.^{79,80} Biochar enhances plant growth and crop yield primarily by impacting soil fertility and

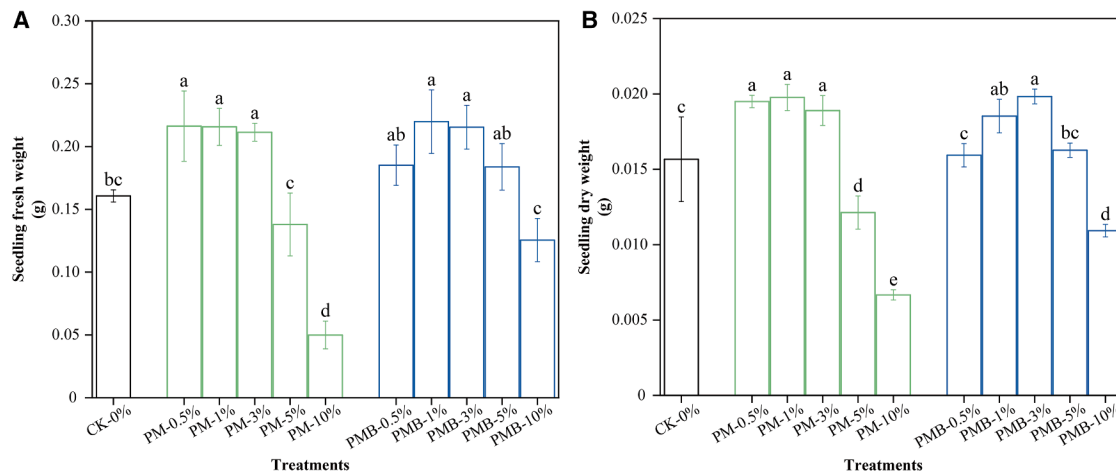


Figure 6. The biomass changes of lettuce seedlings in the improved simulated lunar soil

Fresh weight (A) and dry weight (B) of individual lettuce under different treatments. CK, PM, and PMB denote the treatments and statistical levels are indicated by lower-case letters and capitals as in Figure 1

physicochemical properties.⁸¹ The application of biochar to soil can decrease soil BD, increase soil porosity and soil aggregate stability, and improve soil physical properties.⁸² Biochar can alter soil ion exchangeability by increasing the soil pH, increasing the availability of nutrients for plants.⁸³ However, while biochar effectively enhances the biological availability of N, P, and K in soil, the addition of biochar to alkaline soil may reduce the biological availability of metal components such as Fe, Zn, Cu, and Mn.⁸³ Adding biochar to simulated lunar soil has the potential to increase mineral weathering rates, improve nutrient release and availability, facilitate particle aggregation to achieve a more porous system, and contribute to overall soil improvement.

In this study, the greenhouse device was designed based on the environmental conditions of the Earth and did not consider the special environmental conditions on the lunar surface, such as low gravity, high vacuum and strong radiation. However, all these lunar environmental conditions affect plant growth. Gravity is a key environmental element that influences plant growth and development via gravitropism.⁸⁴ The absence of buoyancy-driven convection around plant leaves under microgravity conditions in space can lead to the accumulation of stagnant and nonuniform gas mixtures, potentially hindering normal metabolic processes.⁸⁵ Previous space-based plant cultivation experiments have highlighted the detrimental effects of insufficient ventilation and inadequate control over the CO₂ concentration, both of which can significantly impair plant growth.⁸⁶ In addition, plants grown in closed systems under microgravity environments produce elevated levels of ethylene, which can adversely affect plant development by inhibiting cell growth, accelerating leaf senescence, and reducing blooming and fruit-bearing capabilities.⁸⁷ Plants cultivated under microgravity conditions exhibit a significantly reduced growth rate compared to those grown under Earth's gravity. Both simulated microgravity and simulated moon gravity treatments led to moderately decreased growth rates relative to those of the control treatment.^{88,89} The lunar environment features ultrahigh vacuum, which presents great

differences from growth condition on Earth. For the normal growth and metabolism of crops under reduced atmospheric pressure, the O₂ concentration should be maintained above ~15 kPa. Under reduced pressure, the plant transpiration rate increases due to higher diffusion rates, such as in the case of lettuce and wheat.⁹⁰ In the space environment, ionizing radiation constitutes one of the primary constraints on plant growth. Plant responses to ionizing radiation are dose-dependent, with high doses typically causing permanent damage, intermediate levels leading to harmful effects, and low doses potentially inducing stimulatory effects.⁹¹ Low doses of gamma rays (2, 4, and 8 Gy), applied at the seed stage, have been reported to increase photosynthesis, respiration, and the electron transport rate.^{92,93} Conversely, high doses of ionizing radiation negatively influence photosynthesis by inhibiting chlorophyll and light-harvesting complex synthesis.^{94,95} The water permeability of lunar regolith is one of the key physical properties critical for enhancing our understanding of lunar surface processes and agricultural applications of regolith utilization.⁹⁶ Due to the microgravity environment, condensed liquid does not flow as it would under normal gravity conditions but instead adheres to the sidewall owing to surface tension effects. To increase the recycling and utilization of water, a designated capillary tube connects the refrigeration module with the soil. Through capillary action, the liquid accumulated around the refrigeration module is absorbed into the soil.⁹⁷

Are there obvious differences between real lunar soil and simulated lunar soil, and are they comparable? Can the findings of this study provide theoretical support for future lunar soil cultivation within a BLSS on the moon? It is crucial to acknowledge the scarcity of real lunar soil. Since the inception of human lunar exploration, 381.7, 0.32, and 1.731 kg of lunar soil samples have been collected during the Apollo, Luna, and Chang'e-5 missions, respectively. However, owing to the limited quantity and extreme value of real lunar soil, the use of lunar soils as experimental materials in lunar soil studies on Earth is impractical. Simulated lunar soil has a mineral composition and chemical components similar to those of lunar samples and similar particle sizes,

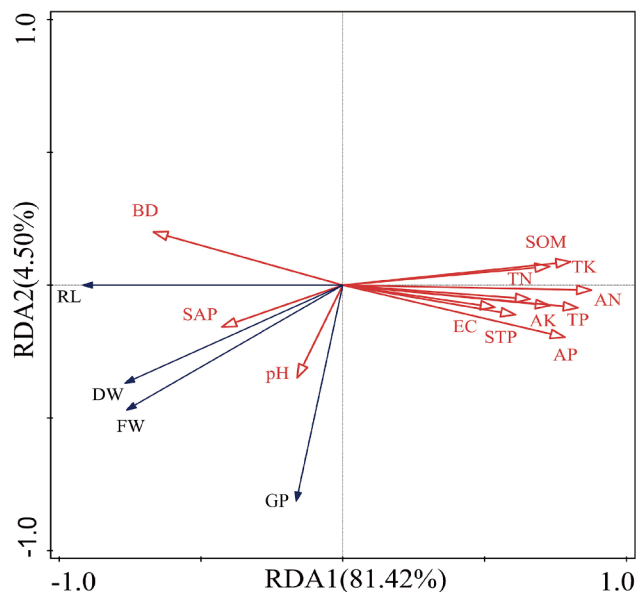


Figure 7. Redundancy analysis between seedlings growth changes and physicochemical properties

AK, available potassium; AN, alkali-hydrolyzable nitrogen; AP, available phosphorus; BD, bulk density; DW, dry weight; EC, electrical conductivity; FW, fresh weight; GP, germination percentage; RL, root length; SAP, soil air porosity; SOM, soil organic matter; STP, soil total porosity; TK, total potassium; TN, total nitrogen; TP, total phosphorus.

mechanical strength, porosity, density, and electrical properties. It is a geochemical replica of lunar samples on Earth.⁹⁸ The JSC-1 lunar soil simulant was developed under the leadership of the Johnson Space Center, a division of the NASA. Its chemical composition, mineralogical characteristics, particle size distribution, and physical-mechanical properties closely resemble those of the lunar soil collected from the Apollo 14 landing site.⁹⁹ The seeds produced by the three species were tested for germination (radish, rye, and cress), and the degree of germination in the moon soil simulant was significantly lower than in the Earth control soil.¹⁰⁰ The LHS-1 simulant was purchased from the Center for Lunar and Asteroid Surface Science Exolith Lab, University of Central Florida (Orlando, FL, USA). The LHS-1 simulant does not have a single terrestrial lithology but simulates the lunar regolith texture faithfully by combining rock and mineral fragments in accurate proportions. The bio-fertilization of lunar LHS-1 regolith simulants with commercial horse/swine monogastric manure increased the potential of these substrates as effective plant growth media in BLSSs.¹⁰¹ The CAS-1 simulated lunar soil has a similar chemical composition, mineral composition, and physical and mechanical properties to the lunar samples collected by Apollo14. It is an ideal low-titanium basalt simulated lunar soil.¹⁰² Phosphorus-solubilizing bacteria can effectively improve the fertility of lunar regolith simulants, making them good cultivation substrates for higher plants.¹⁰³ Therefore, it is imperative to develop a simulated lunar soil that closely mimics the characteristics of real lunar soil, with a focus on enhancing its planting performance to promote crop growth, increase biomass and nutritional components, and pro-

Table 2. Explanation of the influence of each index on lettuce seedling growth

Index	Explains (%)	Pseudo-F	p value
AN	64.5	56.4	0.002
pH	11.1	13.6	0.002
TN	3.6	4.9	0.04
AP	1.8	2.7	0.118
BD	1	1.6	0.248
AK	0.8	1.1	0.304
SOM	0.5	0.7	0.432
SAP	0.2	0.4	0.522
EC	0.3	0.5	0.478
TP	0.2	0.3	0.61
STP	0.1	0.1	0.714
TK	<0.1	<0.1	0.91

AK, available potassium; AN, alkali-hydrolyzable nitrogen; AP, available phosphorus; BD, bulk density; DW, dry weight; EC, electrical conductivity; FW, fresh weight; GP, germination percentage; RL, root length; SAP, soil air porosity; SOM, soil organic matter; STP, soil total porosity; TK, total potassium; TN, total nitrogen; TP, total phosphorus.

vide stable and reliable food sources for astronauts. Research on improving the crop cultivation performance of simulated lunar soil is currently limited, and the results must be rigorously verified and tested to ensure the accuracy of the experimental outcomes and provide a solid theoretical foundation for real lunar soil cultivation. In this study, the growth of lettuce seedlings in improved simulated lunar soil was the focus. Subsequent research will further analyze the effects on the growth and physiological and biochemical indicators of mature lettuce. Furthermore, the long-term effects of biochar-amended simulated lunar soil, especially the response to the environmental conditions on the lunar surface, and whether biochar-amended simulated lunar soil further affects plant growth are worth further consideration. Once applied to soil, biochar does not remain static but interacts dynamically with the surrounding environment, which has implications for its persistence, stability, and traceability in soil.¹⁰³ Based on current research on terrestrial soils, the benefits of biochar application extend beyond short-term increases in biomass production and crop yield. Biochar applications in agricultural lands, monitored over several years, have demonstrated numerous short and long-term positive effects, including a liming effect, enhanced water-holding capacity of the soil, and improved availability of nutrients for crops.^{104–106}

Conclusions

A thorough understanding of the physicochemical properties of simulated lunar soil is essential for developing extraterrestrial BLSSs via the ISRU approach, wherein modified substrates serve as plant growth media. Pyrolysis can substantially increase the amount of solid waste generated from bioregenerative life-support systems by converting waste into biochar. In this study, this process not only improved the physicochemical properties of the simulated lunar soil but also promoted the growth of lettuce seedlings and increased their biomass. Specifically, the addition of biochar to the simulated lunar soil markedly



increased the soil physicochemical properties by increasing the organic matter content and improving the availability of nitrogen, phosphorus, and potassium. This study demonstrated that biochar derived from the inedible parts of plants via pyrolysis significantly improved simulated lunar soil by enhancing its physicochemical properties and promoting plant growth and biomass accumulation. This approach holds considerable promise for the future improvement of simulated lunar soil for BLSSs. Given the chemical similarity between real lunar soil and simulated lunar soil, these findings can be reasonably extrapolated to actual lunar soil, providing a solid foundation for future lunar missions in which lunar soil is utilized.

The next phase of research will focus on the entire growth cycle of lettuce cultivated in biochar-amended simulated lunar soil. This will encompass a detailed analysis of the trends in lettuce physiological and biochemical parameters, including biomass accumulation, nutrient composition, and antioxidant mechanisms. Moreover, in previous studies, researchers have explored the inoculation of functional microbial strains into simulated lunar soil to increase soil quality and stimulate plant growth; however, inoculated single strains are frequently susceptible to inactivation. In the future, research should focus on introducing a complex microbial community into simulated lunar soil to achieve more effective soil improvement, increased crop biomass, and greater soil resilience to adverse conditions. This is also a key research objective that our team is currently pursuing.

Limitations of the study

There are some limitations. First, the growth of lettuce seedlings in improved simulated lunar soil was examined in this study. Future research will further investigate the effects on the physiological and biochemical indicators of mature lettuce. Second, the long-term effects of biochar amended simulated lunar soil, especially for plant biomass, as well as whether such soil influences the growth of different plant species, warrant further investigation. Finally, in this study, the greenhouse device was designed based on the environmental conditions of the Earth and did not consider the special environmental conditions on the lunar surface, such as low gravity, high vacuum, and strong radiation. It should be recognized that, all these lunar environmental conditions affect plant growth. However, realizing these experimental conditions on Earth is highly challenging and necessitates collaborative efforts from researchers worldwide to advance the progress of such studies.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Gengxin Xie (xiegengxin@vip.sina.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The data supporting the findings of this study are availability within the manuscript and the [supplemental information](#).
- This paper does not report the original code.
- Any additional information required is available upon reasonable request to the [lead contact](#).

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AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. C.M., data curation, formal analysis, investigation, methodology, visualization, writing – original draft preparation, and writing – review & editing. G.X., conceptualization, funding acquisition, project administration, resources, supervision, validation, and writing – review & editing. Y.H., investigation, methodology, and visualization. M.P., data curation, investigation, methodology.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **METHOD DETAILS**
 - Preparation of simulated lunar soil and biochar
 - Simulated lunar soil improvement and seedling growth experiments
 - Simulated lunar soil physicochemical property analysis
 - Analysis of plant biomass characteristics
- **QUANTIFICATION AND STATISTICAL ANALYSIS**

SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
$K_2Cr_2O_7$	Chronchem	7778-50-9
H_2SO_4	Chronchem	7664-93-9
$FeSO_4$	Chronchem	7720-78-7
NaOH	Chronchem	1310-73-2
$NaHCO_3$	Chronchem	144-55-8
NH_4OAc	Chronchem	631-61-8
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	Chronchem	13106-76-8
$C_8H_4K_2O_{12}Sb_2$	Chronchem	11071-15-1

METHOD DETAILS

Preparation of simulated lunar soil and biochar

The simulated lunar soil (named Chongqing University 1, CQU-1) used in this study was prepared in detail as described by Han et al.¹⁰⁷ The rocks were pretreated by natural air drying, crushing, grinding, and screening.¹⁰⁷ Basalt-type volcanic rocks from the western foot of Changbai Mountain (near Huinan County, Jilin Province, PRC) were crushed into particles 1 mm in diameter or smaller with a rock pulverizer at the Center of Space Exploration, Ministry of Education, Chongqing University, and then used as simulated lunar soil. The particle size distribution of the simulated lunar soil matched that of Apollo soils, with the particle size of all lunar samples being < 1 mm.¹⁹ The mineral composition of the simulated lunar soil was analyzed using petrographic methods and X-ray diffraction, with the detailed findings reported by Han et al.¹⁰⁸ The percentages of SiO_2 , Al_2O_3 , Fe_2O_3 , CaO, K_2O , MgO, MnO, Na_2O , P_2O_5 , and TiO_2 in each pallet were subsequently determined using a Supermini 200 (Rigaku Corporation, Tokyo, Japan) wavelength-dispersive X-ray fluorescence spectrometer with a Pd tube (50 kV, 4 mA, 200 W). The mineral content and characteristics of CQU-1 were analyzed, and the results were comparable to lunar soil samples collected during the Apollo missions. The chemical composition and mineralogical and physical properties of the CQU-1 sample are similar to those of the lunar soil samples from Apollo 14, making CQU-1 an ideal simulant for soil from lunar mares. However, the simulated lunar soil lacks the nitrogen and organic matter essential for plant growth, and has a high bulk density, which is not conducive to the growth of plant roots. The soil chemical composition (XRF) was similar to that of real lunar soil retrieved by the Apollo missions (Table S1), and it contained mainly alkaline minerals, indicating an insufficient bioavailability of nutrients, which prevents its use as a plant cultivation substrate (Table S2).

Biochar (PMB) was produced from pig manure (PM), and its specific physicochemical properties were described by Mei et al.¹⁰⁹ The details of the biochar preparation process have been previously described.¹¹⁰ Briefly, the pig manure was dried at 80°C for 48 hours, ground using a stainless steel grinding machine, and sieved to obtain particles smaller than 1.0 mm. The resulting powder was then placed in a stainless-steel reactor and pyrolyzed in a muffle furnace under a N_2 atmosphere (99.99%, flow rate of 100 mL/min) at various temperatures (200, 300, 400, 500, 600, 700, 800°C) for a duration of 4 hours (heating rate of 10°C/min for 60 min). A comparison of biomass pyrolysis at different temperatures was conducted to determine the optimal temperature for preparing biochar for efficient soil improvement as determined by the characteristics of lunar soil, which is known for its extreme pH, EC, organic matter content, and low nutrient element content. The specific determination methods are described in the supplemental information. A biomass pyrolysis temperature of 300°C was chosen as the thermal decomposition temperature for the biochar in subsequent experiments because of the high content of organic matter and nutrient elements in the produced biochar (Table S3). At this point, the Brunauer-Emmett-Teller (BET) surface area of biochar is 2.24 m²/g, the total porosity is 0.012 cm³/g, and the average pore diameter is 10.75 nm. These properties are conducive to improving the physical and chemical properties of the simulated lunar soil.

Simulated lunar soil improvement and seedling growth experiments

The study was conducted in a typical greenhouse at Chongqing University (29.3347°N, 106.2812°E) under an Earth-like atmosphere, gravity, and light conditions. To assess the impact of biochar on the physicochemical characteristics of the simulated lunar soil, tests were carried out in a temperature-controlled chamber at 201°C for 49 days. Analyses revealed the following physicochemical properties of the simulated lunar soil: bulk density, 1.30±0.06 g/cm³; pH, 8.07±0.05; electrical conductivity (EC), 0.08±0 cmol/kg; organic matter (OM), 1.19±0.03 g/kg; total N, 0.07±0 g/kg; total P, 0.31±0.01 g/kg; and total K, 52.06±0.87 g/kg. The simulated lunar soil improvement experiments were conducted by mixing 100 g of simulated lunar soil with different amounts (0.5%, 1.0%, 3.0%, 5.0% and 10.0%) of biochar (PMB-0.5%, PMB-1.0%, PMB-3.0%, PMB-5.0%, and PMB-10.0%) and its feedstocks (PM-0.5%,

PM-1.0%, PM-3.0%, PM-5.0%, and PM-10.0%). A control treatment with no soil amendments was included (CK). The experiments were conducted in triplicate. Throughout the treatment period, the soil moisture level was maintained at 60% of the water-holding capacity (as determined in a preliminary experiment). Soil samples were collected every 7 days for a total of 49 days. These samples were used to determine the changes in the physicochemical properties of the soil after air drying.

Trials were conducted to observe the growth of seeds in simulated lunar soil that had been treated with PMB and PM. A Petri dish bioassay for lettuce seeds was performed *in vitro*, following a methodology reported by Sánchez et al.¹¹¹ and Zabaleta et al.¹¹² A Petri dish, measuring 8.5 cm in diameter and 1 cm in height, was filled with improved simulated lunar soil. Three replicates were included for each treatment. Lettuce was selected as the plant species for this study, and 50 seeds were sown in each Petri dish. Lettuce is considered a potential crop for BLSSs because of its short life cycle and nutritional value; it can contribute vitamins, minerals, and fiber to the diet of astronauts.¹¹³ The Petri dishes were sprayed with deionized water once a day to keep the soil moist, and the plants grew normally without the addition of nutrient solution. During the experimental period, the average temperature was $20 \pm 1.0^\circ\text{C}$, and the air humidity was $60 \pm 5.0\%$. Lamps yielding $80 \mu\text{mol}/\text{m}^2/\text{s}$ were used to create 12-hour periods of light to simulate day and night. Ambient air was used, and no CO_2 was added. The percentage of seed germination was recorded. The plants were subsequently allowed to grow for 7 days prior to harvest, with the aim of observing differences in root growth and measuring biomass and root length.¹¹⁴

Simulated lunar soil physicochemical property analysis

The physical characteristics of the soil, such as bulk density, total porosity, and air porosity, were measured using a method similar to that previously described, with slight adjustments involving the use of ring knives instead of nylon bags.^{115–117} Briefly, the cylinder method was used to determine the soil bulk density in g/cm^3 , total porosity and air porosity were calculated using the estimated bulk density, and the air porosity was calculated using the estimated bulk density and water ratio as determined using a glass electrode (PHB-3, SANXIN, Shanghai, China).¹¹⁸ The soil solution EC (mS/cm) was determined with a microprocessor conductivity meter (DDS-307, YUEPING, Shanghai, China) with soil and distilled water (w/v) at a 1:5 ratio.¹¹⁸ The soil organic matter (SOM) content was analyzed using a $\text{K}_2\text{Cr}_2\text{O}_7\text{-H}_2\text{SO}_4$ solution with FeSO_4 titration.¹¹⁹ Alkali-hydrolyzable N was determined with 1.0 M NaOH extraction according to the methods of Lu et al.¹²⁰ as an indicator of N availability. Available P was extracted using a 0.5 M NaHCO_3 solution following the method of Olsen et al.¹²¹ Available K was extracted using a 1 M NH_4OAc (pH 7) solution and analyzed using an atomic absorption spectrophotometer (novAA350, Analytik Jena, Jena, Germany). The total N content was determined using the Kjeldahl digestion-distillation method. Total P was extracted using molten sodium hydroxide and measured using the molybdenum-stibium-antichlor method. Total K was extracted using molten sodium hydroxide and measured using flame emission spectrometry. These methods were detailed in Sparks et al.¹²²

Analysis of plant biomass characteristics

All Petri dishes were incubated at 25°C for 48 hours in the dark, after which the seed germination percentage and root length were assessed. The rate of seed germination was assessed by counting all the seeds in each Petri dish and calculating the percentage of germinated seeds out of the total number of seeds. After 7 days, five plants were randomly chosen from each experimental unit, and their root lengths were measured (mm) from the tip of the root to the apex of the stem. The fresh weights of the seedlings were obtained by washing and weighing. The dry weights were determined by placing the samples in an oven at 105°C for 30 minutes and drying them at 60°C until they reached a constant weight.

QUANTIFICATION AND STATISTICAL ANALYSIS

The data are reported as the means and standard deviations. Statistical analyses were conducted using SPSS 22.0, and statistical calculations were performed using Origin 2024b software (OriginLab, United States). The influences of PMB and PM on soil total N, total P, total K, bulk density, total porosity, and air porosity and on seedling germination percentage, root length, fresh weight and dry weight were tested using one-way analysis of variance (ANOVA), and the effects of the experimental period and doses of PMB and PM were tested using two-way ANOVA. Duncan's multiple-range test was used to assess the impact of various amendment treatments on the measured parameters. Redundancy analysis (RDA) and correlation heatmap analysis were conducted using the 'vegan' package in R (v.4.1.0). Statistical significance was set to $p < 0.05$.