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Mushroom residue and sheep manure fermentation with *Bacillus* promoted tomato growth via nutrient release and favorable microbial conditions

Yaoke Duan[1], Min Wang[1], Lei Wang[2], Guofang Wu[2], Ting Mao[1], Hao Sun[1,3], Huili Pang[1], Miao Zhang[1], Zhen Jiao[1,3], Yanping Wang[1], Xiaoping Kong[4], Yimin Cai[1*] and Zhongfang Tan[1*]

Abstract

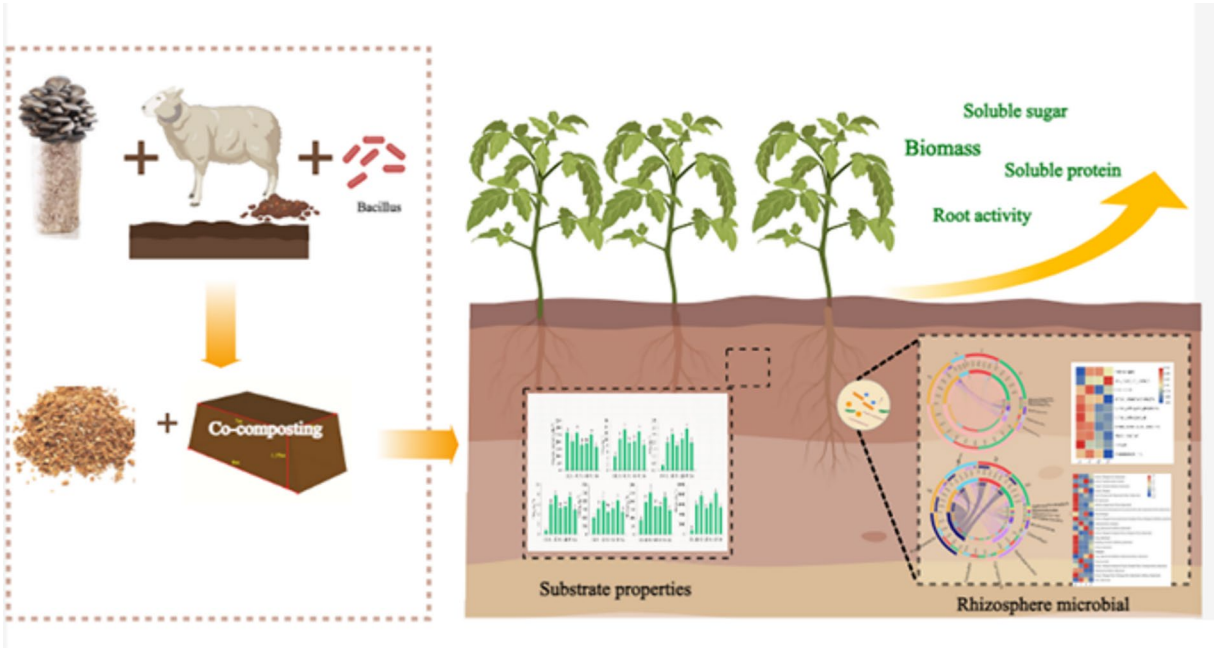
Background Sheep manure and mushroom residue are common agricultural waste which threaten environment but rich in mineral elements and organic matter. Even though fermentation and adding it to soil for crop growth is a commonly used approach, there are concerns about how efficient the fermentation process is and whether the microbial community remains safe for both the crops and those working in agriculture. We have discovered a composite microbial agent, previously known as CMA, that demonstrates significant efficacy in the fermentation of mushroom residue and sheep manure. Despite its high activity, the impact of this microbial agent on soil nutrient release, soil microbial composition, and plant growth remains still uncertain.

Results After fermenting sheep manure and mushroom residue with *Bacillus* CMA, this study investigated the fermentation products mixed with vermiculite and perlite for the cultivation of tomato. The results demonstrate that the composite substrate align closely within the ideal range for seedling substrates. Notably, compounded with CMA compost products and vermiculite in a 2:1 ratio, yields the most favorable growth for tomato, which may be attributed to the increased nutrient release and most favorable microbial conditions. Moreover, it significantly decreased the abundance of pathogenic bacteria harmful to human and animal health, thereby reducing the risk to individuals engaged in field labor, and mitigating the threat of plant pathogenic bacteria.

Conclusions Sheep manure and mushroom residue fermentation with CMA added significantly promoted tomatoes growth and reduced the risk of diseases in crops, animals, and people. These findings hold significant implications also for the reuse of agricultural biowaste and residues, besides the crop growth and safety of humans and animals in agricultural environments.

*Correspondence:
Yimin Cai
cai@affrc.go.jp
Zhongfang Tan
tzhongfang@zzu.edu.cn
Full list of author information is available at the end of the article

Graphical Abstract



Keywords Mushroom residue, Sheep manure, Microbiome, High-throughput sequencing, *Bacillus*, Environment security

Background

Agricultural biowaste refers to the residual material remaining after the cultivation and processing of agricultural products. For example, more than 50 million tons mushrooms were produced annually on the world, but five times mushroom residue were generated as byproduct [1, 2]. Mushroom residue is a by-product of mushroom cultivation containing high levels of organic matter, carbohydrates, and minerals [3, 4]. The improper disposal of mushroom waste can result in environmental issues, including soil contamination, air and water pollution caused by the dispersal of pathogenic bacteria and fungi, as well as the seepage of decomposed substances into surface and groundwater [5, 6]. Though mushroom residue is often classified as agricultural waste and discarded, it is a potential energy and nutrition supplier [7–9]. In addition, the growth of the animal husbandry industry has led to a rapid increase in the production of livestock, which result in an increase of feces. However, the comprehensive utilization rate was less than 60% [8]. It is forecasted that by 2030, the Earth will produce over 5 billion tons of feces annually [10], of which approximately thirty percent of livestock waste is attributed to sheep manure [8]. The improper handling of animal manure can lead to spatial and public health issues as microorganisms in animal feces could cause food contamination and diseases [11]. In addition, animal manure can contaminate water sources, release odors and gases, and harm plant and animal life [12]. However, authors found that sheep manure holds 0.75%, 0.50%, and 0.45% of nitrogen, phosphorus, and potassium, respectively [13], much higher than their concentration in soil [14], making it a potential candidate culture substrate for plants growth.

The management of agricultural waste can be achieved through composting fermentation, which can convert the waste into products possibly used as plant culture substrates [15–19]. Composting with biocontrol bacteria can optimize microbial populations, enhance plant growth, and improve nutrient transformation and metabolism in agricultural waste [20–25]. Though the types and compositional properties of agricultural waste in different regions were distinctive, application research has revealed its potential in plant substrates due to its varied composition and nutrient-richness [26]. For instance, the introduction of thermophilic *Bacillus* fermentation compost enhanced the yield of carrots and influenced rhizosphere microorganism [27]. Similarly, Liu et al. demonstrated that the mixture of mushroom waste, sawdust, catalyst and vermiculite (8:2:5:5) could be employed as a seedling substrate for Chinese cabbage [28]. Meng et al. demonstrated that compost products from biogas production residues, mushroom residue and pig manure

could replace peat as the substrate for tomato seedling cultivation, providing nutrition comparable to chemical fertilizers, but potentially increasing *Fusarium* pathogens [29].

Tomatoes are worldwide vegetable that necessitates the planting of high-quality seedlings [30, 31], leading to an increased demand for seedling cultivation media, which are generally sourced from peat that, however, is a non-renewable resource with a high production cost. Previously we identified two composite microbial agents (CMA), *Bacillus cereus* QS7 and *Bacillus pumilus* QM6 which demonstrated outstanding activity during fermentation [32]. In this study, we investigated the effects of composted mushroom residue and sheep manure on soil nutrient, rhizosphere microbial communities, and tomato growth. The nitrogen, phosphorus, and potassium content and physicochemical properties of different substrates compounded with peat and vermiculite were determined. In addition, the impact on the growth of tomato seedlings and rhizosphere microorganisms were analyzed. This study provides a scientific basis for the utilization of mushroom residue and sheep manure in horticultural soilless substrate production, thus reducing the environmental burden of agricultural waste, as well as the input of peat and chemical fertilizers in agricultural production.

Materials and methods

Preparation and treatment of sheep manure and mushroom residue

Sheep manure and mushroom residue were used as raw materials during a composting process. Sheep manure was obtained from Erhai Animal Husbandry Ecological Breeding professional cooperative (Qinghai, China) and it was mixed to soil (Kastanozems in Harmonized World Soil Database) at the proportion of 1:10 (v/v). Mushroom residue was provided by Huitian Ecological Co., ltd. (Qinghai, China). The raw materials were pulverized to 80-mesh by a grinder after collection. The CMA composed of *Bacillus cereus* QS7 and *Bacillus pumilus* QM6 was prepared in nutrient agar medium (0.5% beef extract, 1.0% peptone, 0.5% NaCl and 1.7% agar at pH 7.0) at 37 °C for 24 h at the Key Laboratory of Ion Beam Bioengineering at Zhengzhou University [32]. 0.1% bacterial solution containing 1×10^9 CFU/mL CMA (consisting of 50% QS7 and 50% QM6) was added before composting. The basic physicochemical properties for composting raw materials, the size of each compost pile and the moisture content were as described previously [32]. The compost products of T1 and T2 groups, which were produced after the completion of fermentation (as published by Wang et al. [32],) were produced by two treatments: T1 (mushroom residue:sheep manure, volume ratio = 9:1, with CMA) and T2 (mushroom residue:sheep manure, volume ratio = 9:1, without CMA). The final products of fermentation were then mixed with peat and vermiculite (peat:vermiculite = 2:1) and used as the control. Finally, a total of seven substrate treatments, including control, were set up: CK (peat:vermiculite = 2:1), S1 (T1:vermiculite = 1:1), S2 (T1:vermiculite = 2:1), S3 (T1:peat:vermiculite = 1:1:1), S4 (T2:vermiculite = 1:1), S5 (T2:vermiculite = 2:1), and S6 (T2:peat:vermiculite = 1:1:1).

Plant seedling preparation

Tomato seeds ('Ailsa Craig') were sterilized in 55 °C distilled water for 15 min and then germinated on two layers of moist filter paper (Whatman, UK) at 28 °C for 2 days. After two days of growing in the dark, the germinated seeds were placed in plastic pots (20 cm in inner diameter and 20 cm in height) filled with the substrate and let it grow in a growth chamber (LRX-1400D, Prandt, Ningbo, China) under a day/night photoperiod of 14 h/10 h and temperature regime of 25 °C/18 °C.

Determination of biometric parameters of the plant

The plant height and stem diameter were measured 35 and 55 d after treatment, the leaf area of the second fully expanded leaf of the seedlings was measured using ImageJ software. Fresh weight (FW) of plants shoot and root were measured, and after being dried in an oven for 3 d the plant dry weight (DW) was weighed. The seedling index is calculated as Liu et al., described: Seedling index = (Stem diameter/Height + Root DW/Shoot DW) × Total DW [33]. The effect of various composite substrates on tomatoes growth was evaluated as $\frac{1}{10}\sum_1^{10} R(Xi)$, of which R(Xi) refer to (Xi-Xmin)/(Xmax-Xmin) and Xi is any biometric parameter of the plant [34].

The extraction and determination of chlorophyll was assayed according to Feng et al. [35], about 0.10–0.20 g leaves were extracted in 25 mL 95% ethanol and the absorbance at OD_{649} and OD_{665} was determined. Root activity was assessed through the utilization of the 2, 3, 5-triphenyltetrazolium chloride (TTC) [36]. Following grinding, the root was extracted in 10 ml of ethyl acetate, and the optical density at 485 nm of the extracted solution was used to assess root activity through the reduction of TTC by root dehydrogenase; The soluble proteins was stained with Coomassie Brilliant Blue G-250 after extracted with phosphate buffer (pH = 7.8) and the optical density at 595 nm was recorded. The soluble sugar in 0.2 g tomato leaves was extracted in boiling water, 0.5 mL extract was added with 1.5 mL distilled water, 0.5 mL 0.2% anthrone and 5 mL H_2SO_4, and the optical density at 630 nm was measured [37]. We have performed six

replicates for each parameter with six plants for each replicate.

Determination of physical parameters of the substrates

The pH and electrical conductivity (EC) of the substrate were assessed with a pH meter (PHS-3C, Yueping, Suzhou, China) and conductivity meter after being diluted with distilled water in a ratio of 1:9 [32]. Total porosity, aeration porosity (AP), and water-holding porosity (WHP) were determined by the method proposed by Wang et al. [4]. Briefly, a 25 mL beaker with a mass m1 was filled with substrate, then the weight was determined as m2. The beaker was covered with gauze and completely submerged in water for 24 h to allow the sample to absorb water fully. After removing the gauze, the beaker was weighed to determine mass m3. The beaker was then wrapped in gauze, inverted, and left to drain for 6 h to ensure no water seeped out. Finally, the beaker was weighed to determine the total mass m4. Total porosity was calculated as $(m_3\text{-}m_2)/V*100\%$, the aeration porosity was calculated as (m3-m4)/V*100%, and the water-holding porosity was calculated as (m4–m2)/V*100%, of which V represent the volume of the beaker. The ratio of compost weight to initial compost volume was used to calculate the bulk density.

Determination of the organic matter, nitrogen, phosphorus and potassium content of the substrates

The chemical properties of the substrates can be determined according to the methods of Bao [38], the organic matter content is measured with the potassium dichromate sulfuric acid oxidation method, the total nitrogen content is calculated using the Kjeldahl method. The total phosphorus content is determined using the molybdenum antimony colorimetric method after being melted with sodium hydroxide. The total potassium content is determined by the flame photometric method after being melted with sodium hydroxide. The alkali hydrolyzed nitrogen content is determined by the alkali hydrolyzation diffusion absorption method, and the effective phosphorus content is calculated with the sodium bicarbonate molybdenum antimony colorimetric method. The effective potassium content is extracted with ammonium acetate and measured with flame photometry.

Sequencing of the microbial communities in tomato rhizosphere

Genomic DNA was isolated from the tomato rhizosphere substrates using The FastDNA® Spin Kit for Soil (MP Biomedicals, Solon, OH, USA) following the manufacturer's instruction, the quality and concentration of the DNA was assessed using 1% agarose gel electrophoresis and the NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Subsequently, PCR was conducted to amplify the V3-V4 region of the bacterial 16S rRNA gene and the ITS1 region of the fungal ITS gene. The V3-V4 region was amplified with primer pairs F (5′- CCTAYGGGRBGCASCAG -3′) and R (5′-GGACTACNNGGGTATCTAAT-3′), as well as the ITS1 region using primers F (5′-CTTGGTCATTTAGAG GAAGTAA-3′) and R (5′-GCTGCGTTCTTCATCGAT GC-3′). The PCR amplification was performed as follows: 98 °C for 1 min, then 30 cycles of 98 oC for 10 s, 50 oC for 30 s, and 72 oC for 30 s, with a final extension of 72 oC for 5 min. Each PCR reaction mixture (30 μL) contained 2 × Phusion Master Mix (15 μL), 1 μM PrimerF (0.2 μL), 1 μM PrimerR (0.2 μL) and 1 ng/μL of GDNA (10 μL), with the rest of the volume filled with ddH_2O. Three replicates of each sample were performed. PCR amplifications were conducted three times for each sample and the replicate products were pooled as a representative sample. High-throughput sequencing was carried out on an Illumina NovaSeq PE250 platform at Novogene Biotechnology Co. ltd (Beijing, China).

Sequence data processing and analysis

Clean tags were obtained by filtering and concatenating raw reads using FASTP and FLASH version 1.2.11 software. The UPARSE algorithm of USEARCH software was then used to cluster the clean tags, the chimeric tags were removed using the UCHIME algorithm of USEARCH software, and the bacteria effective tags were collected; Vsearch software in Fungl analysis was employed to eliminate chimeric tags, and the DADA2 module in QIIME2 software was utilized for noise reduction and to filter out sequences with abundance lower than 5. BLASTN was introduced to detect bacterial and fungal representative sequences from the SILVA and UNITE databases. To evaluate the taxonomic assignment of each ASV, RDP Classifier and QIIME2 were employed, respectively, with a cut-off of 0.8 to eliminate uncertain or unmatched assignments.

The Shannon, Simpson and Chao indices of microbial communities were calculated using the Qiime version 1.9.1. Venn diagram was calculated using R language Venn Diagram package with version 1.6.16. Circos version 0.69–3 software is utilized to create a dynamic Circos map that displays the top 10 species with abundance rankings and tags greater than 2000 in all groups. FAPROTAX ecological function prediction of bacterial communities was performed based on the SILVA annotated abundance; FunGuild that provides a functional classification of the ecological roles was employed to investigate the functional characteristics of fungal communities. The correlation of the soil properties, microbial composition and tomato growth was calculated, and the

multiple regression model (lm function in "stats" package in R) with variance decomposition analysis (calc.relimp function in the "relaimpo" package in R) was employed [30, 39].

Results and discussion

Physical properties of the growing media

The physical characteristics of the growing media were showed in Table 1. The bulk density of the growth substrate is indicative of its structure, which is determined by the total volume of soil and the volume of pore space [40]. The bulk density of all treatments varied between 0.18 and0.41 g/cm^3, with the S1–S6 (T1, mushroom residue:sheep manure, volume ratio = 9:1, with CMA; T2, mushroom residue:sheep manure, volume ratio = 9:1, without CMA; CK, peat:vermiculite = 2:1; S1, T1:vermiculite = 1:1; S2, T1: vermiculite = 2:1; S3, T1:peat:vermiculite = 1:1:1; S4, T2:vermiculite = 1:1; S5, T2:vermiculite = 2:1; S6, T2:peat:vermiculite = 1:1:1.) treatments displaying a higher value than the control group and the highest being 0.41 g/cm^3 in the S5 treatment. The ideal bulk density for seedling growth is disputed, several research suggested that a suitable bulk density for seedling substrate was 0.19–0.7 g/cm^3 [41, 42], while Abad et al., and Garcia-Gomez et al., claimed that the suitable bulk density should not exceed 0.4 g/cm^3 [43, 44]. Despite some controversy, the bulk density of all treatment is roughly accepted by both ranges in this study. The total porosity of S1, S2, S4, and S5 treatments (66.18–69.26%) was found to be lower than that of the CK (76.54%), with no significant difference between S3, S6 treatments and CK. All groups' total porosity was within the optimal range of 50–95% [41], the aeration porosity indicates the air permeability of the growing media [45], when compared to CK, S2 (9.93%) and S5 (10.63%), demonstrated a significantly higher aeration porosity, which is like that of a solid substrate for cornflower [40]. The water holding capacity of the S1-S6 group (55.55–66.51%) were lower than the control (69.67%), yet all of them were within the ideal range of 30–76% [40]. The electrical conductivity (EC) indicates the strength of the overall salinity of substrate, the EC values of the six treatments were significantly higher than the CK, yet all were below 2.00 mS/cm, within a suitable range for plant growth [46, 47].

Organic matter, nitrogen, phosphorus and potassium content of the growing media

The concentration of organic matter and macronutrient including nitrogen, phosphorus and potassium of the substrate was shown (Fig. 1). In comparison to the control (CK, peat:vermiculite = 2:1), the organic matter content of S1 (T1, mushroom residue:sheep manure, volume ratio = 9:1, with CMA; T2, mushroom residue:sheep manure, volume ratio = 9:1, without CMA; S1, T1:vermiculite = 1:1), S3 (T1:peat:vermiculite = 1:1:1), S4 (T2:vermiculite = 1:1), and S6 (T2:peat:vermiculite = 1:1:1) was significantly lower, while that of S2 (T1: vermiculite = 2:1) and S5 (T2:vermiculite = 2:1) was comparable, it indicated that the organic matter content in the fermentation products of sheep manure and mushroom residue is equivalent to or less than that of peat, and the addition of CMA during the fermentation process does not affect the organic matter content in the final product. All treatments (S1–-S6) had significantly higher levels of total nitrogen, total phosphorus, total potassium, alkali hydrolyzed nitrogen, available phosphorus, and available potassium than control (CK). Particularly, the growing medium of S2 and S5 contained higher levels of these nutrient than the others media. The addition of decomposed sheep manure and mushroom residue led to significantly higher nitrogen, phosphorus, and potassium content compared to the control group. By and large, the element contents of S1–S6 increased with increasing concentration of

Table 1 Physical characteristics of compound substrates

Treatment	Bulk density (g/cm3)	Total porosity (%)	Aeration porosity (%)	Water-holding porosity (%)	AP/WHP	EC (mS/cm)
CK	0.18 ± 0.00^{e}	76.54 ± 3.48^{a}	6.87 ± 1.34^{b}	69.67 ± 2.29^{a}	0.10 ± 0.017^{b}	0.53 ± 0.04^{f}
S1	0.37 ± 0.01^{b}	67.65 ± 0.60^{b}	8.63 ± 0.77^{ab}	59.02 ± 1.34^{c}	0.15 ± 0.017^{ab}	1.39 ± 0.03^{d}
S2	0.38 ± 0.01^{b}	66.74 ± 0.83^{b}	9.93 ± 1.15^{a}	56.81 ± 1.23^{c}	0.18 ± 0.023^{a}	1.58 ± 0.01^{c}
S3	0.28 ± 0.02^{d}	72.84 ± 2.36^{ab}	9.22 ± 3.09^{ab}	63.62 ± 4.53^{b}	0.15 ± 0.057^{ab}	1.07 ± 0.03^{e}
S4	0.35 ± 0.01^{c}	69.26 ± 1.24^{b}	6.42 ± 0.79^{b}	62.85 ± 0.57^{b}	0.10 ± 0.012^{b}	1.69 ± 0.06^{b}
S5	0.41 ± 0.01^{a}	66.18 ± 1.91^{b}	10.63 ± 0.48^{a}	55.55 ± 1.45^{c}	0.19 ± 0.004^{a}	1.88 ± 0.02^{a}
S6	0.27 ± 0.00^{d}	75.95 ± 3.10^{a}	9.44 ± 2.48^{ab}	66.51 ± 0.64^{ab}	0.14 ± 0.036^{ab}	1.42 ± 0.02^{d}

AP denotes Aeration Porosity, WHP stands for Water-Holding Porosity, and EC represents Electrical Conductivity. CK (control, peat moss:vermiculite = 2:1), S1 (T1:vermiculite = 1:1), S2 (T1:vermiculite = 2:1), S3 (T1:peat moss:vermiculite = 1:1:1), S4 (T2:vermiculite = 1:1), S5 (T2: ermiculite = 2:1), S6 (T2:peat moss: vermiculite = 1:1:1). Data are means ± SD (n = 3). For each column, the same letter indicates no significant difference at $P \leq 0.05$ level

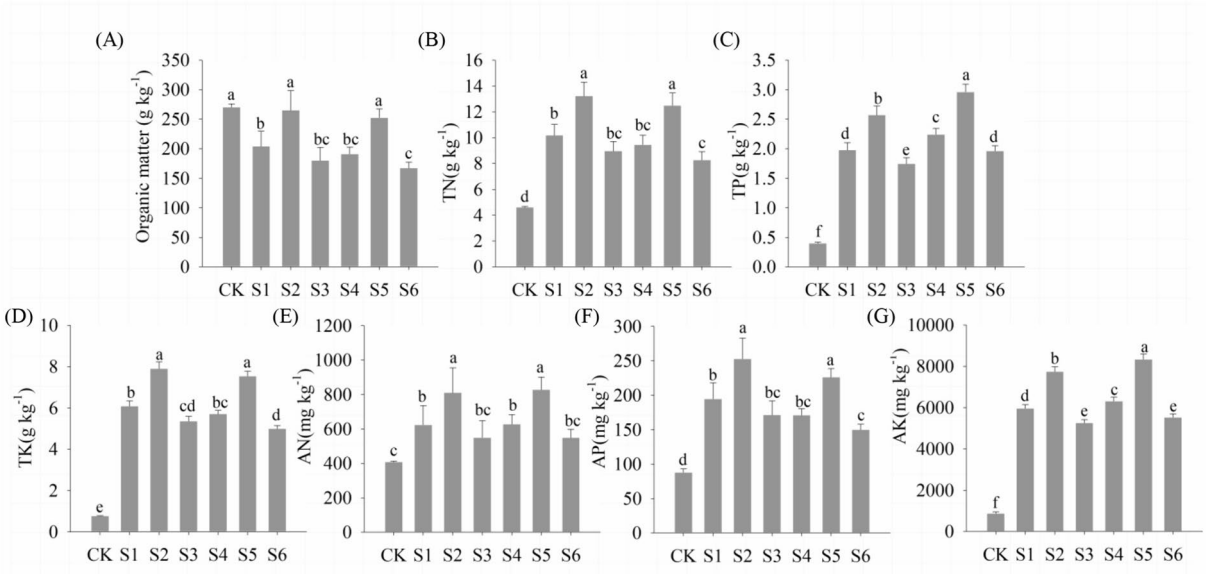


Fig. 1 Organic matter, nitrogen, phosphorus and potassium content in the compound substrates. Organic matter (OM), total nitrogen (TN), total phosphorus (TP), total potassium (TK), alkali-hydrolyzable nitrogen (AN), available phosphorus (AP), available potassium (AK) are shown in **A–G**, respectively. Data are means ± SD ($n = 3$). The same letter above bars indicates no significant differences at $P \leq 0.05$ level

fermentation product, it suggested that the nitrogen, phosphorus, and potassium content in the fermentation products of sheep manure and mushroom residue is higher than that of peat. The utilization of a greater proportion of compost in the substrate can effectively increase the organic matter and nutrient content, which will beneficially plant growth [48–50]. In addition, CMA did not influence the levels of alkali hydrolyzed nitrogen, available phosphorus, and available potassium in the fermentation products (Fig. 1E–G), it suggested that CMA may not be effective in converting nitrogen, phosphorus, and potassium in sheep manure and mushroom residue into forms that are easily absorbed by plants.

Effects of the substrates on the tomato seedlings growth

Investigation of tomato seedlings after 35 days growth suggested that the biometric indicators values in different group of substrates were similar although groups like S2 (T1, mushroom residue:sheep manure, volume ratio = 9:1, with CMA; T2, mushroom residue:sheep manure, volume ratio = 9:1, without CMA; S2, T1: vermiculite = 2:1), S3 (T1:peat:vermiculite = 1:1:1), S4 (T2:vermiculite = 1:1) and S6 (T2:peat:vermiculite = 1:1:1) showed a positive trend than others in certain growth indicators like plant height, leaf area, total weight, and shoot fresh weight (Fig. 2). When tomato seedlings were cultured to 55 days, all tomato seedlings in the treatment groups showed significant improvements in plant height, stem diameter, shoot dry, fresh weight, root dry, fresh weight and total plant dry weight compared to the control group, which may be associate with the increased nutrient element by mushroom residue: sheep manure.

Of all the treatment, the growth indicators of the S2 (T1, mushroom residue:sheep manure, volume ratio = 9:1, with CMA; T2, mushroom residue:sheep manure, volume ratio = 9:1, without CMA; S2, T1: vermiculite = 2:1) group were notably superior to those of the other groups (Fig. 2). For example, the plant height of the S2 group was 80.0% greater than CK (peat:vermiculite = 2:1) and higher than other treatment. In comparison to the control, the S2 group exhibited a substantial increase in total dry weight (2.3 times) and shoot fresh weight (3.22 times), followed by the S1 (T1:vermiculite = 1:1) and S4 (T2:vermiculite = 1:1) treatments. Al though the shoot dry weight, root fresh, dry weights and sound seedlings index of the S2 group was similar with group like S4, S2 still remains one of the top-performing treatments. The comprehensive evaluation of tomato seedling growth indicators at 55 d (Table 2) showed that S2 had the highest value, followed by S1, S4, S5 (T2: ermiculite = 2:1), S3, S6 (T2:peat moss:vermiculite = 1:1:1), and CK. Given that the matrices of groups S2 and S5 contain the same amount of sheep manure and mushroom residue, it indicates that diverse fermentation products could potentially influence the growth of tomato seedlings through pathways beyond nutrient elements (Fig. 3).

The root to shoot ratio of the treatments S1–S6 (T1, mushroom residue:sheep manure, volume ratio = 9:1,

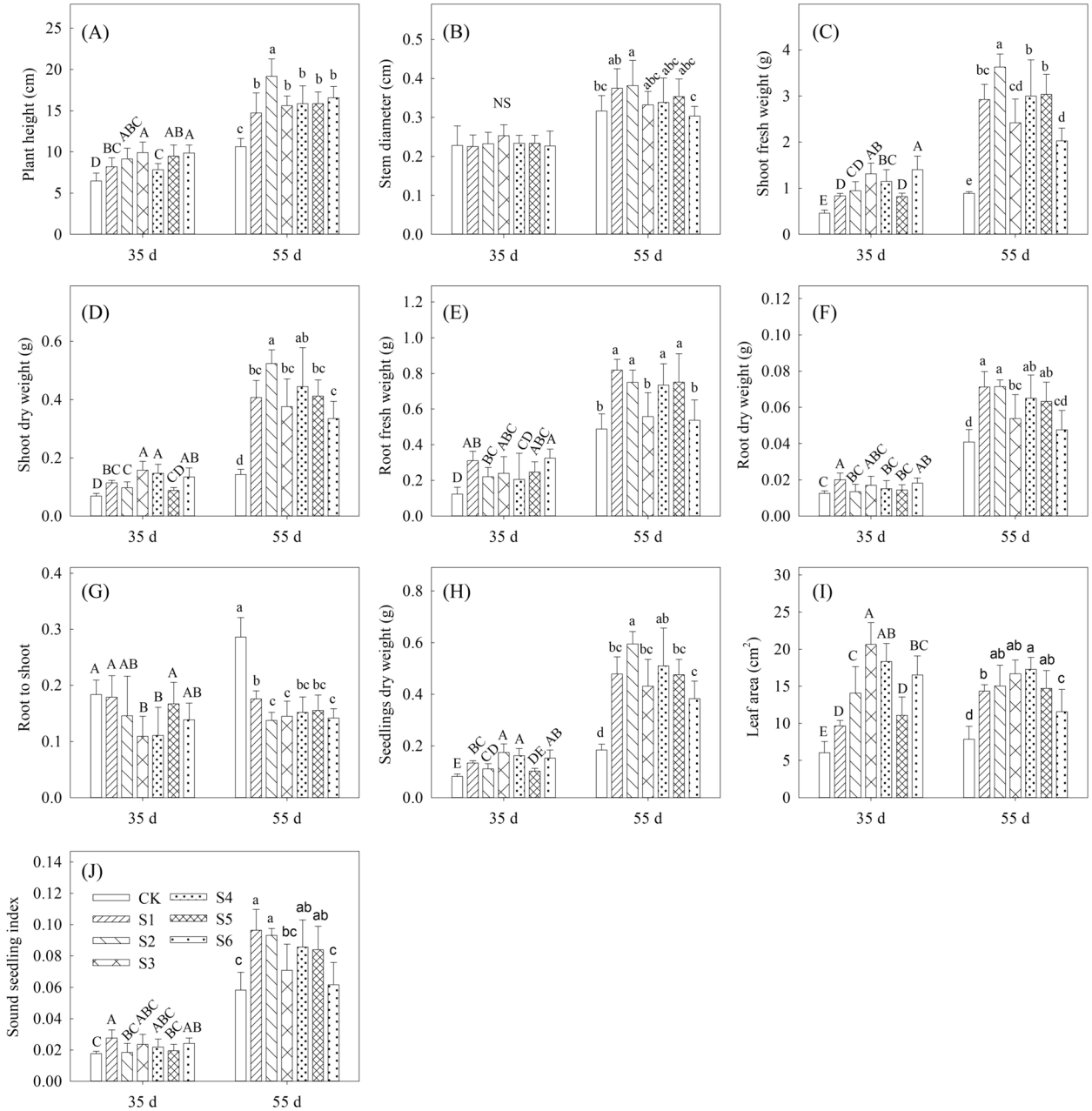


Fig. 2 Effects of compound substrates on the growth of tomato seedings. Plant height (**A**), stem diameter (**B**), shoot fresh weight (**C**), shoot dry weight (**D**), root fresh weight (**E**), root dry weight (**F**), root to shoot (**G**), seedings dry weight (**H**), leaf area (**I**), seeding index (**J**) are measured at 35d and 55d, respectively. Data are means ± SD ($n = 6$). The same letter above bars indicates no significant differences at $P \leq 0.05$ level

with CMA; T2, mushroom residue:sheep manure, volume ratio = 9:1, without CMA; CK, peat:vermiculite = 2:1; S1, T1 vermiculite = 1:1; S2, T1:vermiculite = 2:1; S3, T1:peat:vermiculite = 1:1:1; S4, T2:vermiculite = 1:1; S5, T2:vermiculite = 2:1; S6, T2:peat:vermiculite = 1:1:1.) was significantly lower than that of the control group (CK), especially for S2, S3 and S6. This might be attributed to the fact that the increase in biomass of the roots in the treatment group is significantly lower compared to the aboveground growth, possibly due to variations in nutrient levels in different substrates. For instance, the lower N content in the control group could potentially stimulate the growth of tomato roots [51].

Investigation of the effects of the substrates on the physiological indicators of tomato seedlings at 55 d revealed that in comparison to the control (CK,

Table 2 Comprehensive evaluation of growth indexes of tomato seedlings in different compound substrates

Treatment	Plant height	Thick stem	Leaf area	Shoot fresh weigh	Root fresh weigh	Shoot dry weigh	Root dry weigh	Dry weigh of seeding	Root-to-shoot ratio	Seedling index	Comprehensive evaluation	Sort
CK	0^{c}	0.16^{b}	0^{b}	0^{c}	0^{b}	0^{c}	0^{b}	0^{c}	1.00^{a}	0^{b}	0.12	7
S1	0.48^{b}	0.90^{a}	0.69^{ab}	0.75^{ab}	1.00^{a}	0.71^{b}	0.97^{a}	0.72^{ab}	0.27^{b}	1.00^{a}	0.75	2
S2	1.00^{a}	1.00^{a}	0.76^{ab}	1.00^{a}	0.79^{a}	1.00^{a}	1.00^{a}	1.00^{a}	0^{c}	0.92^{ab}	0.85	1
S3	0.58^{b}	0.37^{ab}	0.94^{a}	0.56^{b}	0.21^{b}	0.63^{b}	0.42^{b}	0.6^{b}	0^{c}	0.34^{b}	0.47	5
S4	0.61^{b}	0.44^{ab}	1.00^{a}	0.77^{ab}	0.76^{a}	0.79^{ab}	0.77^{ab}	0.79^{ab}	0.07^{bc}	0.74^{ab}	0.67	3
S5	0.61^{b}	0.65^{ab}	0.73^{ab}	0.78^{ab}	0.79^{a}	0.71^{ab}	0.71^{ab}	0.71^{ab}	0.13^{bc}	0.68^{ab}	0.65	4
S6	0.70^{b}	0^{b}	0.39^{b}	0.42^{b}	0.15^{b}	0.50^{b}	0.23^{b}	0.48^{b}	0^{c}	0.11^{b}	0.30	6

For each column, the same letter indicates no significant difference at $P \leq 0.05$ level

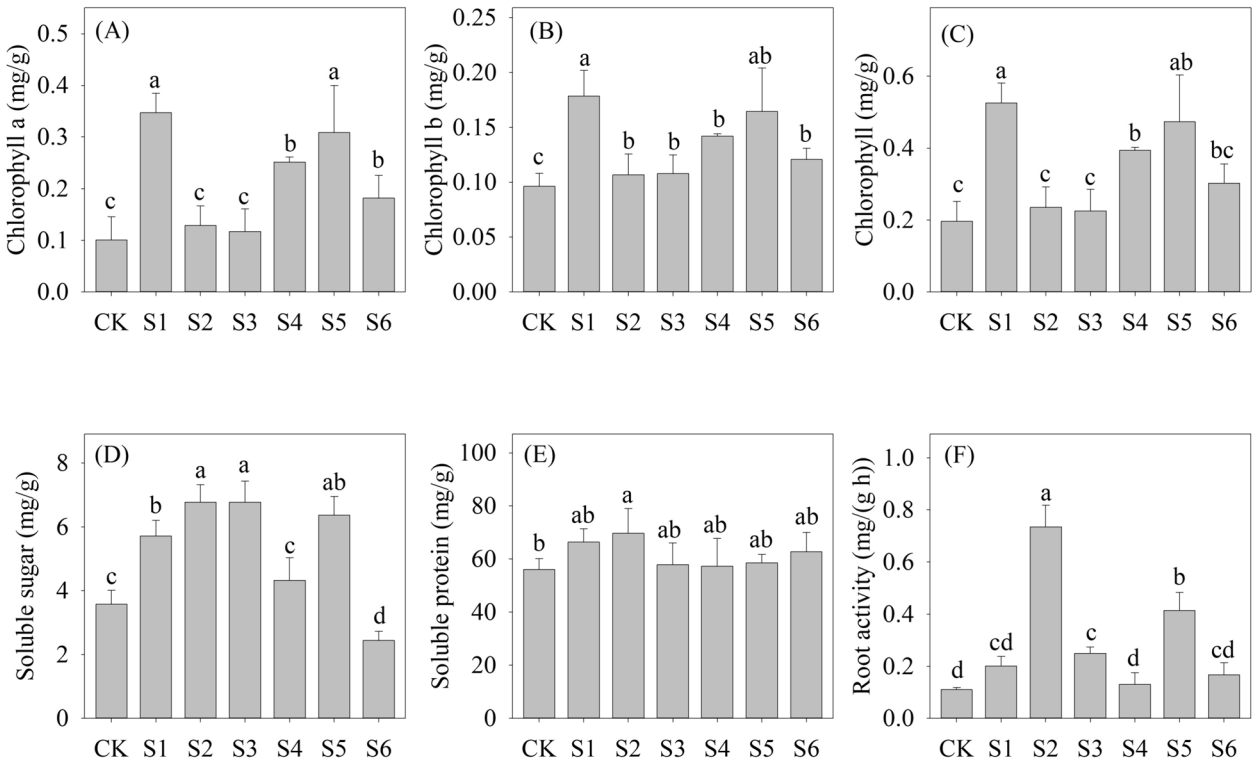


Fig. 3 Effects of compound substrates on the nutrient accumulation and root development of tomato seedings. Effect of compound substrates on the chlorophyll-a (**A**), chlorophyll-b (**B**), chlorophyll (**C**), soluble sugar (**D**), soluble protein (**E**) and root development (**F**) of tomato at 55d, respectively. Data are means ± SD ($n = 6$). The same letter above bars indicates no significant differences at $P \leq 0.05$ level

peat:vermiculite = 2:1), the accumulation of chlorophyll, soluble sugars, soluble proteins, and root vitality in tomato seedlings in each treatment group was either equivalent to or significantly higher than the control (Fig. 3). This indicates that the increased nutrient elements in the different substrates may promote the accumulation of chlorophyll, soluble sugars, soluble proteins, and root vitality in tomato seedlings [6, 52–55]. Correlation analysis between plant physiological indicators and substrate nutrient parameters revealed a significant correlation between root vitality and total nitrogen, available nitrogen, and available phosphorus in the substrate, while no significant correlations were observed between other plant physiological indicators and soil nutrients (Figure S1). This indicates that the accumulation of elements in the substrate significantly influences the vitality of tomato roots. However, besides this, the substrate may impact other tomato physiological indicators in different ways.

Effects of the substrates on the microbial α-diversity

To further investigate the mechanism which have induced tomato growth enhancement, the microbial community in the rhizosphere was investigated. By utilizing Venn diagrams, we presented the shared and distinct ASVs in the different substrates at 55 days. For bacterial communities, CK (peat:vermiculite = 2:), S1 (T1, mushroom residue:sheep manure, volume ratio = 9:1, with CMA; S1, T1:vermiculite = 1:1), S2 (T1:vermiculite = 2:1), and S3 (T1:peat:vermiculite = 1:1:1) shared a total of 195 ASVs, making up 7.04% of the total ASVs (2768). The unique ASVs of the four treatments were 675, 410, 337 and 232, respectively. For fungal communities, CK, S1, S2 and S3 shared a total of 17 ASVs, accounting for 2.79% of the total ASVs (609). The unique ASVs of the four treatments were 164, 131, 81 and 65, respectively (Fig. 4A, B), which may be associated with to the differences observed in the growth and physiological features of tomato seedlings among the different treatment groups.

The Alpha diversity analysis of bacterial and fungal communities in S1-S3 (T1, mushroom residue:sheep manure, volume ratio = 9:1, with CMA; CK, peat:vermiculite = 2:1; S1, T1:vermiculite = 1:1; S2, T1:vermiculite = 2:1; S3, T1:peat:vermiculite = 1:1:1) treatments (Table 3) revealed that the average coverage rate of the four composite matrices was higher than 99%, indicating that the sequencing results accurately reflected the microbial community in the sample. The Shannon and

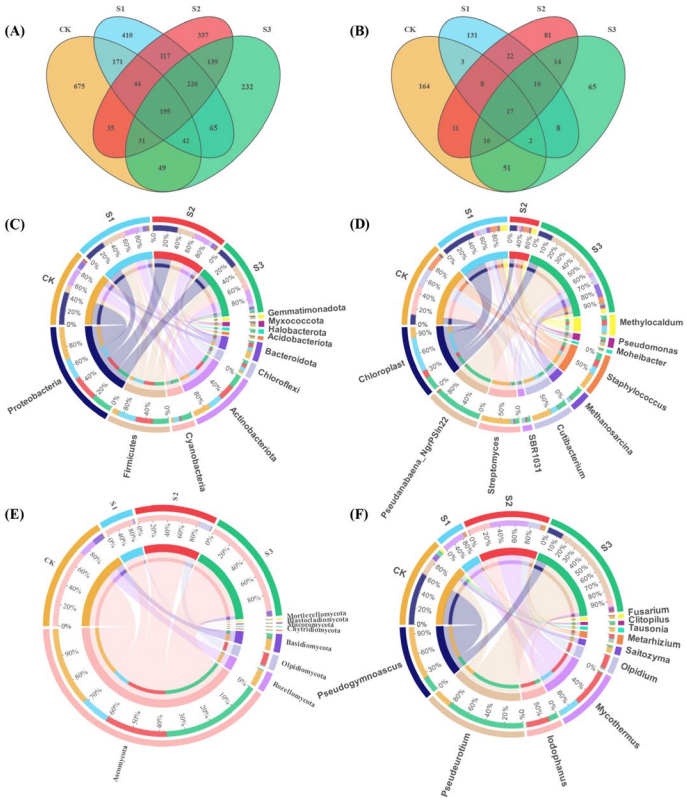


Fig. 4 Bacterial and fungal composition and diversity in tomato rhizosphere. Bacterial and fungal composition and diversity in tomato rhizosphere after amendment with different compound substrate: **A** and **B** are the Venn's diagram of bacterial and fungal communities; bacterial composition at the phylum (**C**) and genus (**D**) level; fungal composition at the phylum (**E**) and genus (**F**) level

Table 3 Alpha-diversity indices of bacterial and fungal communities in rhizosphere

Treatments	Bacteria			Fungi		
	Shannon	Simpson	Chao	Shannon	Simpson	Chao
CK	8.58^{b}	0.99^{ab}	1228.37^{a}	4.30^{a}	0.87^{a}	272.00^{a}
S1	8.75^{ab}	0.99^{ab}	1263.04^{a}	3.24^{bc}	0.71^{b}	205.00^{b}
S2	8.93^{a}	1.00^{a}	1124.24^{b}	3.50^{b}	0.85^{a}	185.00^{c}
S3	8.01^{c}	0.98^{b}	972.09^{c}	3.03^{c}	0.69^{b}	188.50^{c}

T1, mushroom residue:sheep manure, volume ratio = 9:1, with CMA; CK, peat:vermiculite = 2:1; S1, T1:vermiculite = 1:1; S2, T1: vermiculite = 2:1; S3, T1:peat:vermiculite = 1:1:1. Data are means ± SD ($n = 3$). For each column, the same letter indicates no significant difference at $P \leq 0.05$ level

Simpson indices of the S1 and S2 treatments showed an increase compared to the control group, suggesting that the addition of sheep manure, mushroom residue, and CMA notably improved the bacterial Shannon and Simpson indices in the tomato rhizosphere, thereby indicating an increase in bacterial abundance and diversity. The rise in the relative abundance of bacteria in the treatment group may be associated with the addition of fermentation products and the growth of tomatoes. Microorganisms from sheep manure, mushroom residue, the introduction of CMA, the new nutrients from substrate and nutrient uptake and secretion release by tomatoes roots could all contribute to the observed increase in bacterial diversity [22, 32].

Effects of the substrates on the microbial composition

After 55 days from the sowing of tomato seeds, the relative abundance of bacterial community at the phylum level was determined (Fig. 4C). The top 6 dominant bacterial phyla belonged to *Proteobacteria*, *Firmicutes*, *Cyanobacteria*, *Actinobacteria*, *Chloroflexi*, and *Bacteroidota*, collectively constituting 83.06–93.85% of the total

bacterial population. Similar composition of such dominant bacterial phyla was reported by other authors [56, 57]. At the bacterial genera level (Fig. 4D), the predominant genera in each treatment (including the control) was *Chloroplast*, *Pseudanabaena_NgrPSln22*, *Streptomyces*, *SBR1031*, and *Cutibacterium*, it indicates that the addition of sheep manure and mushroom residue fermentation products, as well as CMA, did not alter the dominant bacteria at the phylum and genera level in the substrate.

In comparison with the control group (CK, peat:vermiculite = 2:1), the treatment group showed a decrease in the relative abundance of *Proteobacteria, Actinobacteria* and *Bacteroidota*, while the relative abundance of *Firmicutes* increased at the phylum. At genera level, the relative abundance of *Chloroplast* (of Cyanobacteria at the phylum), *SBR1031* (of Chloroflexi at the phylum), *Metanosarcina* (of Archaea at the Kingdom), and *Methylocaldum* (of Proteobacteria at the phylum) was increased while *Streptomyces* and *Cutibacterium* were reduced. The results indicated that the addition of sheep manure, mushroom residue, and CMA has led to intricate changes in microbial diversity within the substrate. These alterations may be directly attributed to the presence of sheep manure or mushroom residue, as Metanosarcina is commonly found in animal feces [58–61], suggesting that the increase of Metanosarcina in the substrate could be a direct result of sheep manure addition. The complex variations in microbial diversity could also be indirectly influenced by the incorporation of sheep manure, mushroom residue, and CMA.

At the fungal phyla level (Fig. 4E), *Ascomycota* dominated both the treatment and control groups, comprising over 50% in CK (peat:vermiculite = 2:1), S2 (T1, mushroom residue:sheep manure, volume ratio = 9:1, with CMA; S2, T1: vermiculite = 2:1), and S3 (S3, T1:peat:vermiculite = 1:1:1). The relative abundance of *Rozellomycota, Basidiomycota, Chytidiomycota*, *Mucoromycota*, and *Mortierellomycota* in the treatment group decreased, while the relative abundance of *Blastocladiomycota and Olpidiomycota* increased. It indicates that the influence of sheep manure, mushroom residue and CMA fermentation products on fungal community composition might surpass its effect on bacterial populations.

The distribution of fungal genera in tomato rhizosphere under different substrates is distinctive (Fig. 4F). *Pseudogymnoascus* and *Pseudoeurotium* are the predominant fungal genera in S3 (T1, mushroom residue:sheep manure, volume ratio = 9:1, with CMA; S3, T1:peat:vermiculite = 1:1:1) groups and the control (peat:vermiculite = 2:1). In contrast, the dominant fungal genus in the S1 (T1:vermiculite = 1:1) and S2 (T1: vermiculite = 2:1) groups is represented by *Mycothermus*, followed by *Iodophanus* and *Olpidium*. The *Mycothermus* genus, known for its thermophilic nature, is typically found in high-temperature environments such as compost, haystacks, and wood chips, contributing to the degradation of organic matter [62, 63]. A previous study revealed that the presence of *Mycothermus* in soil was related to *lisianthus* cultivation, being effectively protected by the growth of *Fusarium oxysporum* f. sp. *eustomae*, the causative agents of lisianthus Fusarium wilt disease [64]. This indicated that decomposed sheep manure and mushroom residue can enhance the proliferation of the *Mycothermus* genus more than peat, thereby aiding in the suppression of plant pathogens. The relative abundance of *Iodophanus* in the treatment group increased to 0.43%-18.7%, higher than that in the control group (0.07%), indicating a potential link to the maturation and fermentation of sheep plate feces in the treatment group substrate. The relative abundance of the *Olpidium* genus followed a similar trend, with lower levels in the CK and S3 groups and higher levels in the S1 and S2 groups. This suggested a positive correlation between the increase in the *Olpidium* genus and the proportion of sheep manure and mushroom residue in the composting substrate.

The application of sheep manure and mushroom residue to the soil led to an increase in the prevalence of *Olpidium*, specifically *Olpidium Brassicae*. However, it is noteworthy that *Olpidium Brassicae* has minimal detrimental effects on plant health, as indicated by previous studies [65–67]. The findings suggested that the addition of sheep manure and mushroom residue does not elevate the abundance of harmful *Olpidium* fungi in the soil.

Relationships among soil properties and rhizosphere microbial composition to tomato growth

The Spearman' correlation matrix suggested that the enhancement of tomato seedling growth may be primarily attributed to the improved nutrient release in the substrate that could be facilitated by microbial fermentation (Fig. 5A). By using species annotation and Operational Taxonomic Unit (OTU) information from SILVA, we can predict the FAPROTAX, revealing the ecological function and functional analysis of bacteria in tomato rhizosphere in various substrates (Fig. 6A). The carbon metabolism related methylotrophy function were more enriched in the treatment group compared to the control, which can utilize single carbon compounds, secrete enzymes and hormones to enhance crop nutrition. This suggested that the bacterial community in the treatment group may enhance plant growth by improving available nutrient [68]. Furthermore, the Dark Hydrogen Oxidation pathway, which can utilize hydrogen gas and enhance soil fertility [69], was significantly more active in the treatment group. In the S1 (T1, mushroom

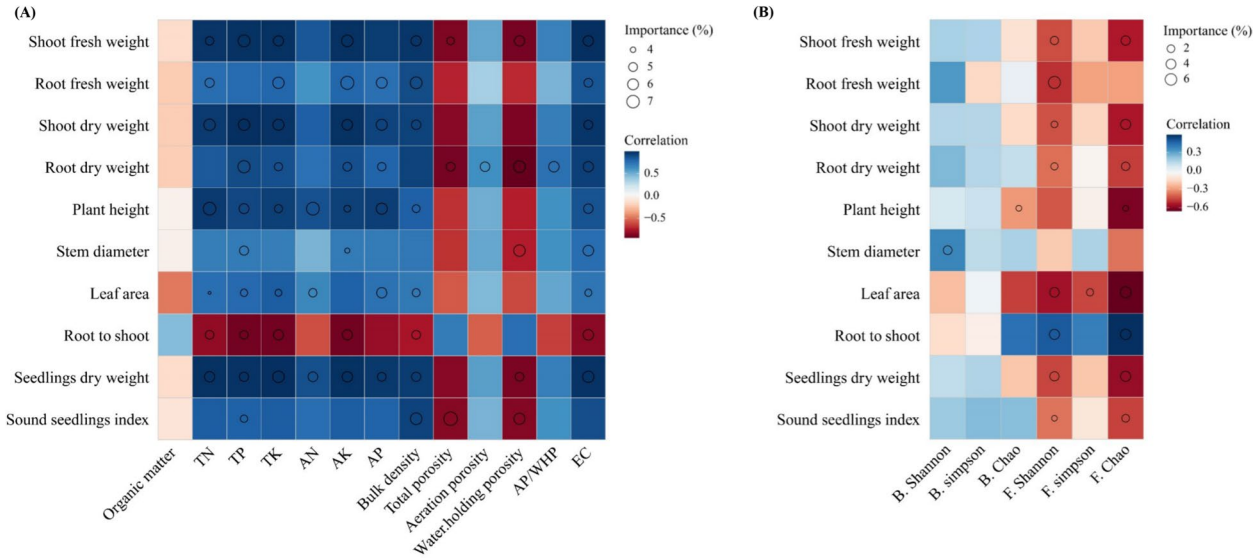


Fig. 5 Contributions of soil properties and microbial composition to tomato growth. Correlation of the soil properties and biometric parameters of tomato growth as **A** well as correlation of microbial composition and biometric parameters of tomato growth **B** were demonstrated. Circle size represents the variable importance. Colors represent Spearman correlations

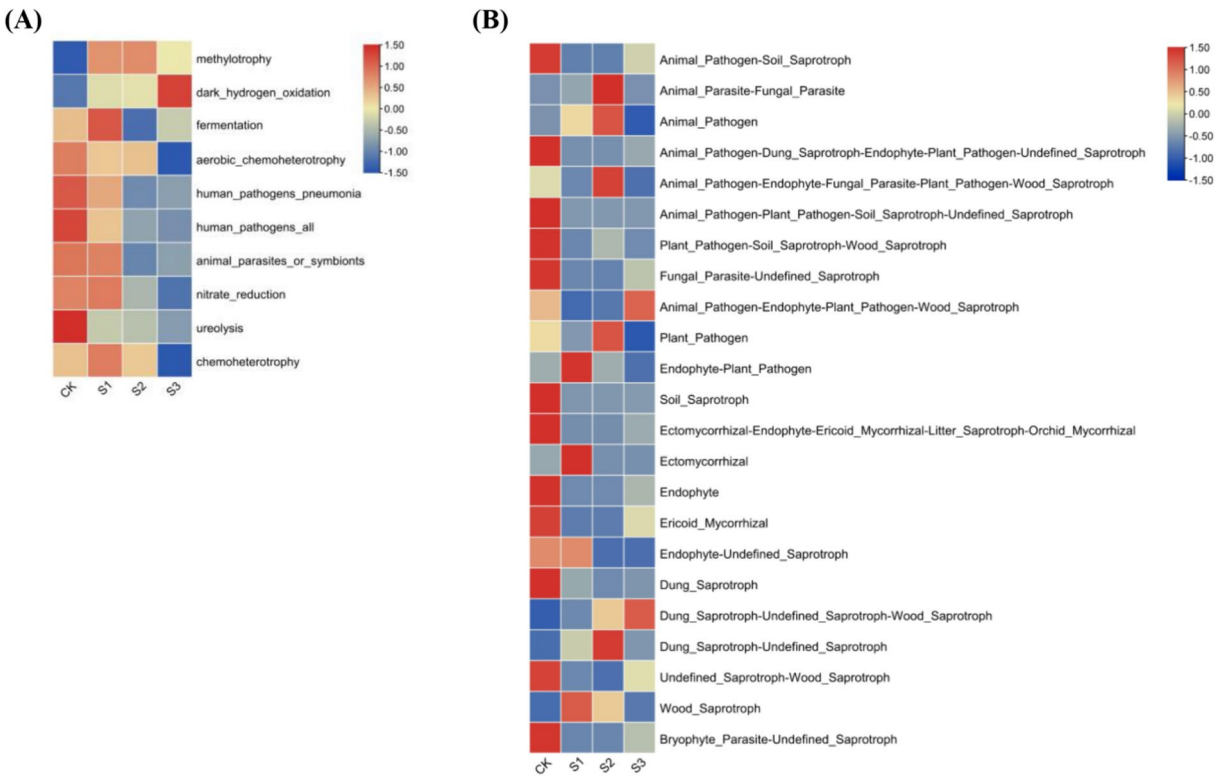


Fig. 6 Functional analysis of bacteria and fungi in tomato rhizosphere. Functional analysis of bacteria and fungi in tomato rhizosphere under different compound substrate treatments. **A** and **B** showed the functional analysis of bacteria and fungi in tomato rhizosphere under compound substrate treatments

residue:sheep manure, volume ratio = 9:1, with CMA; S1, T1:vermiculite = 1:1) treatment group, the nitrate reduction function exhibited minimal change compared to the control, while that in the S2 (T1: vermiculite = 2:1) and S3 (T1:peat:vermiculite = 1:1:1) groups was lower than the control (peat:vermiculite = 2:1). The ureolysis pathway, responsible for decomposing urea into ammonia for plant absorption and utilization, also demonstrated lower activity in the treatment groups than the control. In addition, the aerobic chemoheterotrophy function, involved in utilizing organic carbon sources for carbon cycling, showed lower activity in the rhizosphere microbial community of most treatment groups compared to the control group. Although these trends in function changes may have adverse effects on plant growth, tomato seedlings growth suggested in this study that their impact may be limited.

The analysis of the correlation and significance between tomato seedling growth and microbial diversity revealed that a rise in bacterial diversity exerted a modest enhancing influence on tomato growth, whereas reduced fungal diversity could support the growth of tomato seedlings (Figs. 5B, 6A). This phenomenon may be attributed to the influence of the substrate and tomato seedling roots on the rhizosphere microbial environment, culminating in a microbial milieu that is intricately linked to the growth of tomato roots, which is evidenced by the functional analysis. For example, the addition of sheep manure and mushroom residue to the substrate led to a notable increase in a microbiota closely associated with methylotrophy (Fig. 6A), potentially enhancing the soil microenvironment by boosting the availability of soil organic matter [70]. These microorganisms are known to secrete enzymes and hormones, thereby stimulating plant growth [71–74]. Specifically, the abundance of *Methylocaldum*, a methane-oxidizing microorganism, significantly rose in the treatment group receiving sheep manure and mushroom residue (Fig. 4D). Methane oxidation was linked to a beneficial impact on soil organic matter accumulation and nitrate nitrogen content, consequently improving soil fertility [70, 75]. This suggested that *Methylocaldum* in the rhizosphere microbial environment may enhance plant growth and development by influencing nitrate nitrogen content and soil organic matter levels.

Furthermore, the addition of sheep manure and mushroom residue resulted in a significant increase in the relative abundance of a microbiota associated with dark_hydrogen_oxidation (Fig. 6A), and particularly the abundance of the *Mthanosarcina* genus which can utilize H_2 for reduction reactions (Fig. 4D). Previous research on soybean, barley, wheat, canola, clover, alfalfa, and lentils demonstrated that bacterial hydrogen oxidation function plays a crucial role in promoting plant growth [76–79]. These microorganisms have the potential to enhance soil fertility by secreting plant hormones like auxin, thereby increasing nutrient availability in the rhizosphere and promoting plant growth [80–82]. In the case of *Leguminosae* plants, hydrogen oxidation bacteria can be recruited by nodules, facilitating nodule formation and subsequently promoting plant nitrogen absorption [80, 83, 84]. The microorganisms involved in nitrate reduction in the treatment group (T1, mushroom residue:sheep manure, volume ratio = 9:1, with CMA; S1, T1:vermiculite = 1:1; S2, T1: vermiculite = 2:1; S3, T1:peat:vermiculite = 1:1:1) exhibited a declining trend (Fig. 6A), indicating that the addition of decomposed sheep manure and mushroom residue can delay nitrate reduction in the soil, thereby reducing nitrate ion (NO^{3-}) loss, increasing available nitrogen for plants, and promoting plant growth [85]. This phenomenon was also observed by An et al., in their investigation of soil microorganisms under long-term organic fertilizer application [86]. In conclusion, these findings suggested that the nutrient release from substrate due to microbial decomposition notably enhanced tomato seedling growth, while the interplay between tomato roots and substrate composition contributed to create a favorable microbial environment for promoting tomato growth.

The composite substrates may offer benefits for promoting a sustainable agricultural environment

The combination of mushroom residue and sheep manure, along with CMA, can serve as a profitable plant substrate, facilitating the virtuous reuse of agricultural biowaste from the perspective of a rising agri-based circular bioeconomy, expediting nutrient and energy recirculation, mitigating environmental contamination, and freeing up usable space [87]. This composite substrate diminishes reliance on peat in agricultural production, fosters plant growth, and conserves non-renewable environmental resources. Its high organic matter, nitrogen, phosphorus, and potassium content reduces the need for chemical fertilizers in agriculture, thereby lessening their impact on soil, water sources, and environmental microorganisms. The study determined that the appropriate ratio of CMA-enhanced fermentation products to vermiculite effectively reduces the prevalence of disease-causing microorganisms in the rhizosphere, benefiting the health of humans and animals in the environment. As human activities increasingly encroach upon the ecological environment, the composite substrates not only mitigate the environmental impact of human production activities but also enhance environmental safety by diminishing the pathogenicity of soil microorganisms.

This contributes to the safety of the environment and humans by impacting the microbial community.

Conclusion

The composite substrate here tested exhibited physical properties within the optimal range for seedling growth, with high levels of nitrogen, phosphorus, potassium, and organic matter. The S2 group obviously promoted tomato seedlings growth, that can be attributed to the increased nutrient release and favorable microbial conditions. Furthermore, there is a reduction in the risk of soil microorganisms being pathogenic to humans, animals, and plants. Fermentation of agricultural waste like sheep manure and mushroom residue with *Bacillus*-based microbial inoculants may serve as an efficient way to effectively repurpose agricultural, promote crop growth and safeguard the well-being of agricultural personnel and livestock.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-024-00618-5.

Additional file 1.

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Not applicable.

Author contributions
Yaoke Duan: investigation, data curation, writing—original draft. Min Wang: formal analysis, investigation, methodology, writing. Lei Wang: methodology, writing. Guofang Wu: methodology, writing. Ting Mao: investigation, methodology. Hao Sun: conceptualization, writing. Huili Pang: writing—review and editing. Miao Zhang: writing—review and editing. Zhen Jiao: writing—review and editing. Yanping Wang: writing—review and editing. Xiaoping Kong: writing—review and editing. Yimin Cai: writing—review and editing, supervision. Zhongfang Tan: conceptualization, writing—review and editing, supervision.

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Availability of data and materials
The data sets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Henan Key Laboratory of Ion-beam Green Agriculture Bioengineering, School of Agricultural Sciences, Zhengzhou University, Zhengzhou 450052, China. [2]Plateau Livestock Genetic Resources Protection and Innovative Utilization Key Laboratory of Qinghai Province, Key Laboratory of Animal Genetics and Breeding on Tibetan Plateau, Ministry of Agriculture and Rural Affairs, Qinghai Academy of Animal and Veterinary Medicine, Qinghai University, Xining 810016, China. [3]State Key Laboratory of Cotton Bio-breeding and Integrated Utilization, School of Agricultural Sciences, Zhengzhou University, Zhengzhou 450001, China. [4]Xining Vegetable Technical Service Center, Xining 810000, People's Republic of China.

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