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Organic Amendment Types Influence Soil Properties, the Soil Bacterial Microbiome, and Tomato Growth

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Abstract: The overuse of chemical fertilizers deteriorates soil quality, and the application of organic amendments has been proposed as an alternative to mineral fertilizers. This study aimed to investigate the effects of organic amendment types on soil properties, the soil microbiome, and tomato growth. Tomato seedlings were grown in soils applied with ten fertilizer treatments, including a no fertilization control, a chemical fertilization control, and eight organic amendment treatments. Compared with the chemical fertilization treatment, the application of manure compost significantly increased the shoot dry weight of tomato plants. In addition, manure compost and tea seed meal remarkably increased soil organic matter (SOM) in comparison with the no fertilization and chemical fertilization treatments. Moreover, manure compost significantly increased soil-exchangeable K and Mg. The application of neem cake and manure compost significantly increased both bacterial diversity and richness. The relative abundance of *Lysinibacillus* was significantly positively related to the shoot and total dry weights of tomato plants, and its relative abundance was positively influenced by SOM and soil-exchangeable K. Overall, the manure compost used in this study can increase SOM, soil-exchangeable K and Mg, and the relative abundance of *Lysinibacillus*, consequently promoting tomato growth.

Keywords: cake fertilizer; compost; organic matter fertilizer; soil fertility; soil organic matter

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1. Introduction

Soil organic matter (SOM) is an important soil component that influences the activity of soil microorganisms, crop productivity, and soil quality [1,2]. In addition, SOM is a vital factor affecting the physical, chemical, and biological properties of the soil [3,4]. However, intensive agricultural practices, such as conventional tillage, generally result in a substantial loss of SOM, and changing from conventional tillage to conservation tillage systems increases the content of SOM in the topsoil layer in a relatively short time [5]. In addition, it is possible to increase SOM by C inputs to soil [6]. The use of organic amendments such as composts, organic matter fertilizers, and organic wastes has been proposed as an effective practice to increase SOM [7,8]. Moreover, increasing SOM may sequester more C and consequently reduce the level of atmospheric CO_2 and the greenhouse effect [6,9].

Soil microorganisms play a vital role in plant growth and health, nutrient cycling, and soil functions [10,11]. It is important to investigate how fertilizers influence the soil microbiome since soil microbial community composition and diversity are indicative of soil quality. Soil acidification caused by the application of inorganic fertilizers usually leads to a decrease in bacterial diversity and abundance and changes in the bacterial community [12].

In contrast, the combined use of chemical and organic fertilizers increases soil microbial biomass, community richness, and diversity, thus promoting crop yields [13,14]. It is generally recognized that the type and application rate of fertilizers are important factors influencing soil microbial communities [10]. However, it is not well known how different types of organic fertilizers, such as cake fertilizers and manure composts, influence soil bacterial abundance and diversity compared with chemical fertilization.

The application of different types of organic amendments may affect soil fertility and plant health. It has been suggested that the use of organic fertilizers such as organic wastes and composts not only increases the content of SOM, the availability of soil nutrients, and the stability of soil structure, but also helps maintain soil pH stability [7,15]. In addition, increased microbial activity and induction of disease suppression have also been proposed to explain the positive effects of organic fertilizers on soil fertility and health [16,17]. Soil nutrient availability, such as soil salinity and available P and K, plays a vital role in shaping the soil bacterial community, while SOM and pH also influence the soil microbiome [15]. On the other hand, the increase in soil bacterial community composition and diversity may improve soil nutrient availability, which in turn enhances nutrient uptake by plants [16]. However, little is known about how the soil microbiome responds to the exogenous application of different types of organic amendments in the soil used to grow tomato plants. In addition, it is necessary to elucidate the relationship between the soil microbiome, soil nutrient availability, and tomato growth, which is of great importance for the suitable use of fertilizers for tomato plants.

Tomato is an important vegetable crop in the world, and the total global tomato production area has been increasing due to a continual escalation in tomato consumption [18,19]. Chemical fertilizer is important for tomato production, but its excessive use and long-term application may decrease soil quality and productivity [20]. Therefore, it is imperative to develop environmentally friendly fertilizers such as organic fertilizers and biofertilizers to partly substitute chemical fertilizers [21,22]. The application of organic fertilizers can increase SOM and improve soil health and plant growth [15,23]. Of these organic fertilizers, composts and cake fertilizers have been used to improve soil properties and crop growth [22,24]. Cake fertilizers or oilseed meals provided by industrial chains, such as oilless seed coproducts from the biodiesel industry, are usually used as organic fertilizers, but they are still underexplored for their potential to increase disease suppression of soil and plant health [25]. In addition, it is unclear how composts and cake fertilizers influence soil properties, the soil microbiome, and tomato growth.

This study aimed to investigate the effects of organic amendments (cake fertilizers and manure compost) on soil properties, the soil microbiome, and tomato growth. We hypothesized that different organic amendments affected bacterial abundance and diversity and soil properties such as soil pH, SOM, and nutrient availability, resulting in changes in tomato growth. In addition, shifts in the bacterial community structure would be closely related to changes in soil properties caused by the application of these fertilization treatments.

2. Materials and Methods

2.1. Soil Sampling

The soil was collected from a field located in a productive area of tomato growing in central Taiwan (24°2′2″ N, 120°28′28″ E). The field soil was sampled from the first 20 cm layer, air-dried, sieved (2 mm), and subjected to a determination of physical and chemical properties. The soil had a silt loam texture with a pH of 7.55 (1:1, w/v), electrical conductivity (EC) of 0.38 dS m^{-1} (1:5, w/v), available N of 32.7 mg kg^{-1}, available P of 22.1 mg kg^{-1}, exchangeable K of 132 mg kg^{-1}, exchangeable Ca of 5610 mg kg^{-1}, exchangeable Mg of 351 mg kg^{-1}, and soil organic matter of 26.3 g kg^{-1}. The analysis of soil properties is described below.

2.2. Pot Experiment Setup

Each pot contained 1 kg of air-dried soil. The experiment consisted of a no fertilization control, a chemical fertilization control, and eight organic amendments: castor cake; manure compost (made from cow dung, chicken manure, swine manure, and spent mushroom waste at a weight ratio of 1:2:2:5) applied at 5, 10, and 20 g kg^{-1} soil; neem cake; rapeseed meal; soybean meal; and tea seed meal. Each pot of the chemical fertilization treatment contained 0.22 g urea, 0.42 g calcium superphosphate, and 0.10 g potassium chloride, which were equivalent to the recommended rate of 200 kg ha^{-1} for N, 150 kg ha^{-1} for P_2O_5, and 120 kg ha^{-1} for K_2O. The application rate of castor cake (1.67 g pot^{-1}), neem cake (2.01 g pot^{-1}), rapeseed meal (1.43 g pot^{-1}), tea seed meal (5.01 g pot^{-1}), and soybean meal (1.39 g pot^{-1}) was calculated based on their nitrogen contents that provided the same N amount of the chemical fertilization treatment. Each treatment consisted of six replicates. After the application of the fertilizer treatments, the soil water content was adjusted to 70% of the maximum water-holding capacity (WHC) for two weeks before the transplantation of tomato seedlings.

For surface sterilization, tomato seeds of the cultivar "Farmers 301" were soaked in 0.6% NaClO (*v*/*v*) for 10 min and then washed three times with sterilized water. The sterilized seeds were planted in seed trays with a commercial growth medium (Known-You Co., Ltd., Kaohsiung City, Taiwan). Two weeks after sowing, seedlings at the cotyledon stage were transplanted into pots, each including one seedling. The seedlings were placed in a growth chamber held at 28 ± 2 °C with a 12 h light photoperiod. At 30 days after transplanting, tomato plants were harvested, and plant height and shoot and root dry weights (oven-dried at 70 °C for 72 h) were measured. Two soil samples were (each ~100 g) collected from each pot. One of the soil samples was air-dried for soil property analyses, and the other was stored at −80 °C for microbiological community analysis as described below.

2.3. Soil Property Analysis

The pH of rhizosphere soils was determined in 1:1 (*w*/*v*) soil: H_2O extracts, and the soil EC was analyzed in 1:5 (*w*/*v*) soil: H_2O extracts [26]. Total organic carbon was determined by the Walkley–Black method [27]. Soil-available nitrogen was extracted by 2 N KCl and analyzed using the steam distillation method [28]. Soil-available phosphorus was extracted and measured by using the Bray-1 method [29]. In addition, soil samples were extracted for exchangeable K, Ca, and Mg using 1 M NH_4OAc [30] and determined with inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Perkin Elmer, Waltham, MA, USA).

2.4. DNA Extraction and Nanopore Sequencing of the 16S rRNA Gene

Total DNA extraction from soil samples (0.30 g) was carried out by using the PowerSoil DNA Isolation Kit (Mo Bio, Carlsbad, CA, USA). The quantity of DNA was measured using a Qubit 3.0 Fluorometer and Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, Waltham, MA, USA), and the quality of DNA was determined using a NanoDrop ND-2000c UV–Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Ten nanograms of each soil DNA sample were used to amplify the nearly full length (V1–V9 region) of the bacterial 16S rRNA gene using the primers fD1 and rD1 and PCR conditions as previously described [31]. Amplifications were performed in an Analytik Jena AG FlexCycler2 thermocycler (Analytik Jena AG, Göttingen, Germany). Amplified DNA was purified using a Gene-Spin 1-4-3 DNA Purification Kit (Protech Technology Enterprise Co., Taipei, Taiwan) and quantified using a Qubit 3.0 Fluorometer.

Amplicons (30 ng) from each sample were end-repaired and dA-tailed using the KAPA Hyper Prep Kit (Roche, Branchburg, NJ, USA) according to the manufacturer's instructions. Barcodes were ligated to the dA-tailed DNA using the Blunt/TA Ligase Master Mix of the KAPA Hyper Prep Kit. Adapters were ligated to the pooled barcoded reads using the sequencing kit 1D SQK-LSK109, R9 version (Oxford Nanopore Technologies, Oxford, UK)

to complete the library building. The prepared DNA library (13 µL) was mixed with 37.5 µL of sequencing buffer and 25.5 µL of loading beads and consequently loaded onto the FLO-MIN106 R9.4 flow cell (Oxford Nanopore Technologies). MINKNOW software ver. 1.11.5 (Oxford Nanopore Technologies) was used for data acquisition. Fast5 files generated by MINKNOW were base called using Guppy ver. 3.15 (Oxford Nanopore Technologies), and output DNA sequence reads were saved as fastq files. Taxonomic classification of each fastq file was performed using Centrifuge ver. 1.0.4 [32] and TaxonKit ver. 0.8.0 [33].

2.5. Bioinformatic Data Analysis

Microbial community analyses were performed using R (www.r-project.org, accessed on 8 April 2022). The phyloseq package in R was used to estimate the relative abundances of different taxa and microbial diversity in each sample [34]. A minimum relative abundance cutoff of 0.02 was used to select the most abundant Phyla and 0.01 was used to choose the most abundant genera in each sample. Those below the cutoff values were collapsed into the other phyla or genera categories because merging minor taxa helps to better visualize notable taxonomic patterns in the data [35]. The pheatmap package in R was used to draw heatmaps of the relative abundance of the 30 most abundant genera in each sample. Pearson correlation coefficients between the relative abundance of the 30 most abundant genera and soil properties and tomato growth characteristics were calculated using the stats package in R. In addition, the relationship between dominant genera, soil properties, and organic amendment treatments was shown by redundancy analysis (RDA) using the vegan package.

2.6. Statistical Analysis

The generalized linear mixed models in PROC GLIMMIX of SAS (version 9.4; SAS Institute, Gary, NC, USA) were used to analyze the effects of the treatments on the response variables. The least squared means (LSMEANS) function of the GLIMMIX procedure in SAS was used to compare the treatment means at a 5% level of significance according to Fisher's least significant difference (Fisher's LSD) tests. Pearson's correlation analysis was performed to determine the relationships between soil properties and tomato growth characteristics, microbial richness and diversity, and dominant bacterial phyla using SAS.

3. Results

3.1. Effects of Organic Amendments on Tomato Growth

Types of organic amendments significantly influenced tomato plant heights. Compared with the no fertilization and chemical fertilization treatments, castor cake, tea seed meal, and manure compost significantly enhanced plant heights. Of these organic amendments, the manure compost applied at 20 g kg^{-1} resulted in the highest plant height, with increases of 77.9 and 150% compared with the no fertilization and chemical fertilization treatments, respectively (Table 1). Although castor cake, tea seed meal, and manure composts applied at 5, 10, and 20 g kg^{-1} showed a significantly greater shoot dry weight than no fertilization, only manure composts applied at 10 and 20 g kg^{-1} significantly increased the shoot dry weight by 54.0 and 168%, respectively, compared with the chemical fertilization treatment. In addition, only the application rate of manure compost at 20 g kg^{-1} significantly increased the root dry weight in comparison with the chemical fertilization treatment.

3.2. Effects of Organic Amendments on Soil Properties

There was no significant difference in soil pH between the no fertilization treatment and soil applications of chemical fertilization, manure compost, neem cake, and rapeseed meal, but castor cake, soybean meal, and tea seed meal resulted in a significantly greater pH than the no fertilization treatment (Table 2). Although soil applications of manure compost, rapeseed meal, soybean meal, and tea seed meal did not significantly increase soil EC compared with the no fertilization treatment, chemical fertilization, and neem

cake treatments significantly increased soil salinity. Of these fertilizers, tea seed meal significantly increased SOM by 13.6% compared with the no fertilization treatment. In addition, manure compost applied at 10 and 20 g kg^{-1} remarkably enhanced SOM by 15.5 and 35.2%, respectively. Except for the manure compost and tea seed meal treatments, the other fertilization treatments significantly increased soil-available N by 24.5–92.2%. Chemical fertilization and soybean meal showed the greatest level of soil-available N compared with the other treatments. There was no significant difference in soil-available P between the no fertilization and rapeseed meal treatments, but the soil application of the other fertilization treatments significantly increased soil-available P. The chemical fertilization treatment showed the greatest soil-available P, with a significant increase of 159% compared with the no fertilization control. Except for castor cake and rapeseed meal, the other fertilization treatments significantly enhanced soil-exchangeable K. Of these treatments, the application of manure compost applied at 20 g kg^{-1} had the greatest soil-exchangeable K (233 mg kg^{-1}). Although the manure compost applied at 5, 10, and 20 g kg^{-1} did not significantly increase soil-exchangeable Ca, the other treatments significantly increased Ca by 5.61–10.7% in comparison with the no fertilization control. Except for the manure compost applied at 5 g kg^{-1}, the other treatments significantly increased the soil-exchangeable Mg by 6.89–13.0%.

Table 1. Effect of organic amendment types on tomato growth.

Treatment	Plant Height (cm)	Shoot Dry Weight (g $Plant^{-1}$)	Root Dry Weight (g $Plant^{-1}$)
Castor cake	14.3 ± 1.96 bc [a]	0.56 ± 0.15 c	0.11 ± 0.03 b
Chemical fertilization	11.3 ± 1.87 ef	0.50 ± 0.18 cd	0.09 ± 0.03 b–d
Manure compost (5 g kg^{-1})	14.0 ± 2.03 c	0.65 ± 0.22 bc	0.10 ± 0.02 bc
Manure compost (10 g kg^{-1})	15.9 ± 1.98 b	0.77 ± 0.12 b	0.11 ± 0.03 b
Manure compost (20 g kg^{-1})	20.1 ± 1.13 a	1.34 ± 0.19 a	0.17 ± 0.03 a
Neem cake	11.7 ± 1.27 de	0.34 ± 0.10 de	0.07 ± 0.02 cd
No fertilization	8.05 ± 0.67 g	0.25 ± 0.11 e	0.04 ± 0.01 e
Rapeseed meal	9.38 ± 2.37 fg	0.32 ± 0.17 e	0.06 ± 0.03 de
Soybean meal	11.1 ± 1.17 ef	0.30 ± 0.08 e	0.06 ± 0.02 de
Tea seed meal	13.6 ± 1.86 cd	0.52 ± 0.13 cd	0.10 ± 0.02 bc

[a] Values are mean ± standard deviation. Different letters within the same column show significant differences at the 5% level of significance according to Fisher's least significant difference (Fisher's LSD) test.

Table 2. Effects of organic amendment types on soil properties.

Treatment	pH	EC [a]	OM [b]	Avail. N [c]	Bray-1P	Exch. K	Exch. Ca	Exch. Mg
		(dS m^{-1})	(g kg^{-1})			(mg kg^{-1})		
Castor cake	7.47 ± 0.41 ab [d]	0.32 ± 0.03 d	26.8 ± 0.62 d	73.8 ± 10.3 c	25.1 ± 3.81 b–d	165 ± 16.3 d–f	6491 ± 154 a	431 ± 9.32 a–c
Chemical fertilization	6.88 ± 0.04 c	0.52 ± 0.01 a	27.8 ± 0.89 cd	114 ± 13.3 a	38.8 ± 4.21 a	185 ± 12.9 b–d	6192 ± 152 a–d	423 ± 11.5 b–d
Manure compost (5 g kg^{-1})	7.09 ± 0.35 bc	0.36 ± 0.04 cd	27.6 ± 0.53 cd	45.6 ± 10.4 ef	25.2 ± 8.98 b–d	178 ± 21.5 c–e	5877 ± 105 c–e	406 ± 11.7 de
Manure compost (10 g kg^{-1})	6.94 ± 0.66 c	0.36 ± 0.03 cd	30.5 ± 1.38 b	33.1 ± 5.85 fg	26.0 ± 5.65 bc	191 ± 11.9 bc	6025 ± 381 b–e	421 ± 17.8 b–d
Manure compost (20 g kg^{-1})	7.03 ± 0.45 c	0.40 ± 0.08 bc	35.7 ± 1.96 a	30.7 ± 10.1 g	31.3 ± 6.40 b	233 ± 18.1 a	5723 ± 89.7 e	443 ± 14.9 a
Neem cake	7.00 ± 0.09 c	0.56 ± 0.07 a	29.1 ± 1.86 cd	79.0 ± 17.0 bc	21.9 ± 9.94 cd	174 ± 10.8 c–e	6226 ± 244 a–c	431 ± 24.2 a–c
No fertilization	6.94 ± 0.06 c	0.40 ± 0.06 bc	26.4 ± 0.68 d	59.3 ± 8.04 de	15.0 ± 2.80 e	150 ± 17.2 f	5863 ± 96.3 de	392 ± 6.48 e
Rapeseed meal	7.09 ± 0.23 bc	0.45 ± 0.08 b	26.8 ± 3.18 d	92.6 ± 11.7 b	18.6 ± 2.56 de	161 ± 8.53 ef	6299 ± 98.2 ab	437 ± 11.0 ab
Soybean meal	7.63 ± 0.23 a	0.41 ± 0.05 bc	27.7 ± 0.88 cd	108 ± 18.7 a	24.5 ± 3.41 cd	174 ± 14.5 c–e	6271 ± 98.3 ab	426 ± 13.0 a–c
Tea seed meal	7.48 ± 0.27 ab	0.44 ± 0.03 b	30.1 ± 1.55 b	71.7 ± 14.9 cd	25.4 ± 4.99 bc	202 ± 35.1 b	6210 ± 686 a–d	419 ± 20.9 cd

[a] EC—electrical conductivity; [b] OM—organic matter; [c] available N extracted by 2 N KCl; available P extracted by the Bray-1 P method; exchangeable K, Ca, and Mg extracted by 1 M NH4OAC buffered at pH 7. [d] Values are mean ± standard deviation. Different letters within the same column show significant differences at the 5% level of significance according to Fisher's least significant difference (Fisher's LSD) test.

3.3. Microbial Community Composition

Chao's coverage indices for all treatments were not significantly different and were greater than 0.964 (Table 3), suggesting that the nanopore sequencing used in this study efficiently captured the majority of the diversity for each soil sample [36].

Table 3. Estimated number of observed Chao1 and ACE richness indices, diversity, and coverage for different fertilizer treatments.

Treatment	Chao1	ACE	Shannon	Chao's Coverage
Castor cake	2152 ± 98.7 a–d [a]	2344 ± 82.5 ab	5.03 ± 0.07 de	0.970 ± 0.003
Chemical fertilization	2089 ± 24.5 cd	2296 ± 25.0 ab	5.03 ± 0.05 de	0.967 ± 0.005
Manure compost (5 g kg^{-1})	2251 ± 54.6 a	2397 ± 51.8 a	5.18 ± 0.07 bc	0.981 ± 0.003
Manure compost (10 g kg^{-1})	2175 ± 42.5 a–d	2372 ± 24.1 a	5.36 ± 0.02 a	0.977 ± 0.004
Manure compost (20 g kg^{-1})	2065 ± 112 d	2256 ± 83.9 b	5.26 ± 0.05 ab	0.964 ± 0.006
Neem cake	2218 ± 83.4 ab	2366 ± 68.6 a	5.39 ± 0.10 a	0.968 ± 0.008
No fertilization	2084 ± 79.2 cd	2255 ± 54.5 b	4.90 ± 0.11 e	0.972 ± 0.005
Rapeseed meal	2207 ± 74.5 a–c	2358 ± 70.5 ab	5.30 ± 0.13 ab	0.970 ± 0.006
Soybean meal	2117 ± 36.5 b–d	2308 ± 15.5 ab	5.10 ± 0.03 cd	0.967 ± 0.004
Tea seed meal	2164 ± 99.5 a–d	2346 ± 83.7 ab	5.29 ± 0.07 ab	0.966 ± 0.003

[a] Values are mean ± standard deviation. Different letters within the same column show significant differences at the 5% level of significance according to Fisher's least significant difference (Fisher's LSD) test.

The Chao1 and ACE richness indices showed that the no fertilization control exhibited the lowest estimated number of bacterial operational taxonomic units (OTUs). The application of neem cake or manure compost at 5 g kg^{-1} showed a significantly higher Chao1 index than that of the chemical fertilization treatment, while there was no significant difference in the Chao1 index between the other organic amendments and the chemical fertilization treatment. In addition, the application of neem cake and manure compost at 5 and 10 g kg^{-1} resulted in a significantly higher ACE index than the no fertilization treatment. The soil applied with neem cake resulted in the highest Shannon diversity index, whereas the no fertilization control had the lowest Shannon index. Compared with the chemical fertilization treatment, the application of manure compost, neem cake, rapeseed meal, and tea seed meal showed a significantly higher Shannon index.

Considering the bacterial composition at the phylum level, a total of 39 phyla were observed in all samples. Although the relative abundance varied across fertilization treatments, the 7 most dominant phyla accounted for 94.6–95.5% including *Acidobacteria* (15.0–24.8%), *Actinobacteria* (2.58–5.51%), *Chloroflexi* (2.98–4.87%), *Firmicutes* (15.2–19.4%), *Gemmatimonadetes* (2.04–3.35%), *Planctomycetes* (2.40–3.31%), and *Proteobacteria* (40.1–50.0%) (Figure 1). No fertilization had the lowest abundance of *Proteobacteria* (40.1%), but the soil applied with neem cake resulted in the greatest abundance of this phylum (50.0%). The application of soybean meal resulted in the highest abundance of *Actinobacteria* (5.51%), whereas the application of manure compost at 20g kg^{-1} had the lowest abundance of this phylum (2.58%). Although the application of neem cake showed the lowest abundance of *Chloroflexi* (3.76%), the greatest abundance of this phylum was observed under the chemical fertilization treatment (4.87%). No fertilization showed the greatest abundance of *Acidobacteria* (24.8%), but the application of neem cake resulted in the lowest abundance of this phylum (14.9%). Although the application of chemical fertilization had the lowest abundance of *Firmicutes* (15.2%), the highest abundance of this phylum was observed in the manure compost applied at 20 g kg^{-1} (19.4%). The soil applied with castor cake had the greatest abundance of *Gemmatimonadetes* (3.35%), whereas manure compost at 10 g kg^{-1} resulted in the lowest abundance of this phylum (2.05%). Although the application of

manure compost at 20 g kg^{-1} had the lowest abundance of *Planctomycetes* (2.41%), the highest abundance of this phylum was observed in the soybean meal treatment (3.31%).

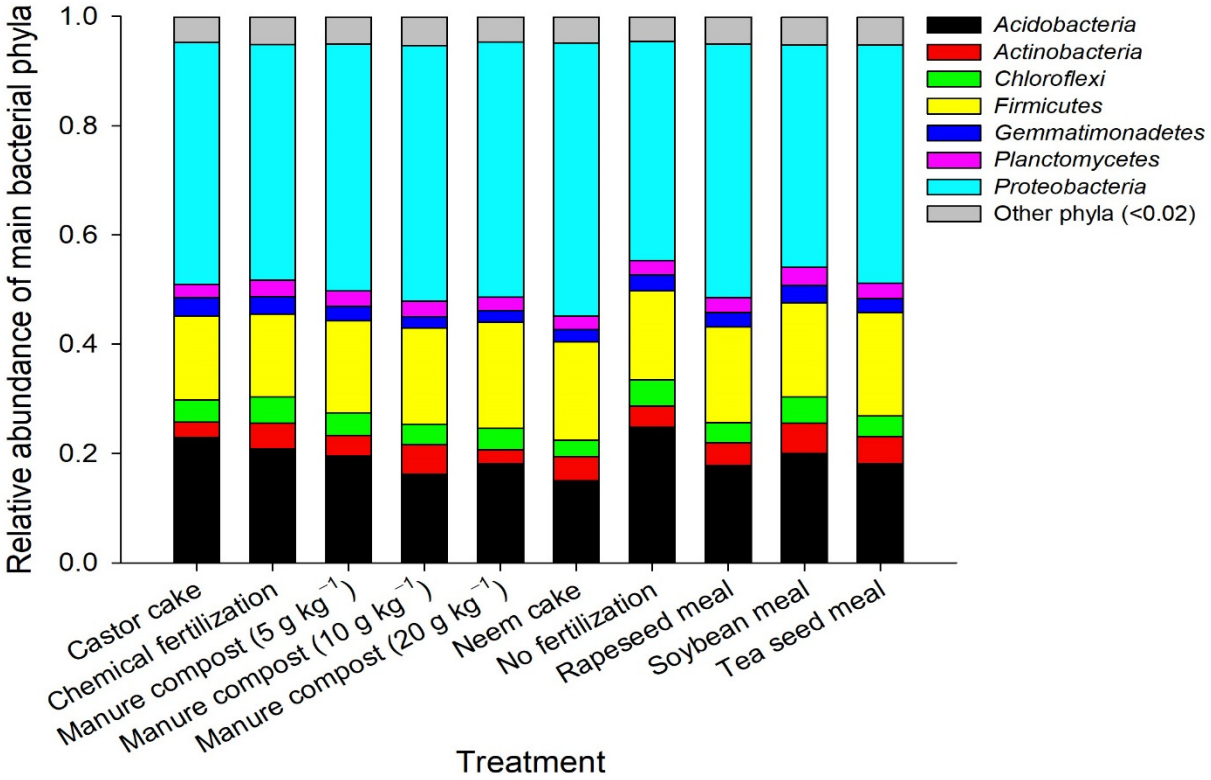

Figure 1. Effect of organic amendment types on the dominant bacterial phyla in the tomato-grown soil. Each treatment consisted of three replicates for nanopore sequencing of 16S rRNA gene amplicons.

Although a total of 696 genera were detected in all samples, the 12 most abundant genera consisted of *Acidobacterium*, *Bacillus*, *Bradyrhizobium*, *Clostridium*, *Gemmatimonas*, *Lysobacter*, *Massilia*, *Pseudomonas*, *Rhizobium*, *Sphingomonas*, *Stenotrophomonas*, and *Xanthomonas*, accounting for 39.5–45.4% (Figure 2). The other genera with a relative abundance of less than 1% accounted for 54.6–60.5%. Of these 12 dominant genera, the relative abundance of *Bacillus* was the highest in all samples. The soil applied with neem cake had the highest abundance of *Bacillus* (21.1%), whereas the application of castor cake showed the lowest abundance of this genus (17.2%). Although the application of neem cake also had the highest abundance of *Massilia*, *Pseudomonas*, *Rhizobium*, and *Sphingomonas*, it resulted in the lowest abundance of *Acidobacterium*, *Clostridium*, and *Gemmatimonas*. In contrast, the soil applied with soybean meal resulted in the lowest abundance of *Massilia* and *Pseudomonas*. Moreover, chemical fertilization and castor cake treatments had the lowest abundance of *Rhizobium* and *Sphingomonas*, respectively. The application of manure compost at 20 g kg^{-1} showed the greatest abundance of *Lysobacter*, whereas the soil applied with tea seed meal had the lowest abundance of this genus. In addition, the application of tea seed meal resulted in the lowest abundance of *Bradyrhizobium* and *Xanthomonas*. However, the no fertilization control showed the greatest abundance of *Bradyrhizobium* and the soil applied with castor cake resulted in the highest abundance of *Xanthomonas*.

3.4. Relationship between Soil Properties, Tomato Growth, and Soil Microbiome

EC and soil-available N were negatively related to plant height and shoot and root dry weights of tomato plants (Table 4). In addition, the shoot dry weight of tomato plants was significantly negatively correlated with soil-exchangeable Ca. In contrast, plant height and shoot and root dry weights showed a significant positive correlation with SOM and exchangeable K. Soil-exchangeable Mg and SOM were positively related to the Shan-

non diversity index, but the Shannon index was significantly negatively correlated with soil-available N. Soil pH was positively correlated with *Actinobacteria* and *Planctomycetes*. Although *Acidobacteria* and *Gemmatimonadetes* were negatively affected by SOM, a significantly positive correlation between SOM and *Chloroflexi*, *Firmicutes*, and *Proteobacteria* was observed. Soil-available N was positively related to *Chloroflexi* and *Gemmatimonadetes*, but it was significantly negatively correlated with *Firmicutes* and *Proteobacteria*. In addition, *Planctomycetes* was positively affected by soil-available P, and exchangeable K was positively related to *Firmicutes*.

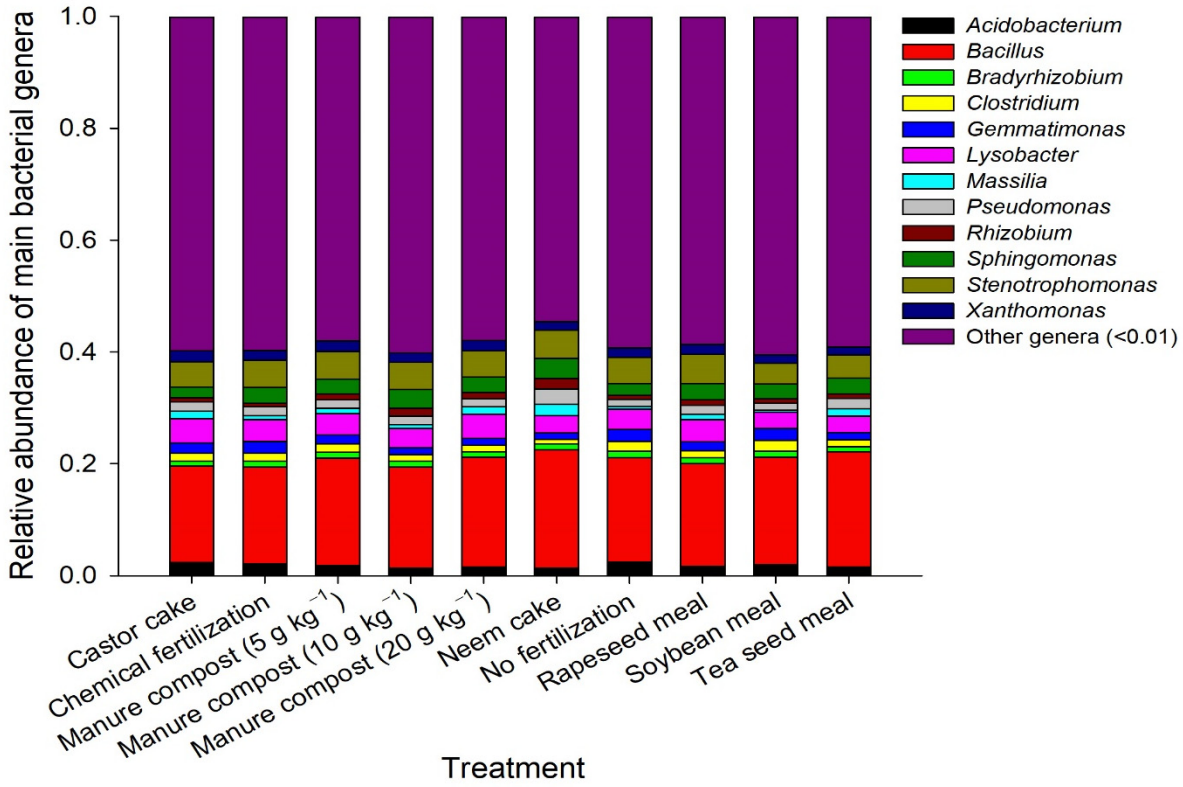

Figure 2. Effect of organic amendment types on the dominant bacterial genera in tomato-grown soil. Each treatment consisted of three replicates for nanopore sequencing of 16S rRNA gene amplicons.

Table 4. Pearson's correlations between soil properties and tomato growth characteristics, microbial richness and diversity, and dominant bacterial phyla.

	pH	EC [a]	SOM [b]	Avail. N [c]	Bray-1P	Exch. K	Exch. Ca	Exch. Mg
Plant height	−0.11 [c]	−0.57 ***	0.64 ***	−0.66 ***	0.17	0.53 **	−0.24	0.31
Shoot dry weight	−0.19	−0.56 **	0.60 ***	−0.71 ***	0.18	0.46 **	−0.38 *	0.15
Root dry weight	−0.05	−0.46 **	0.52 ***	−0.49 **	0.27	0.48 **	−0.29	0.17
Chao1 richness	0.05	0.10	−0.22	−0.12	−0.20	−0.18	0.13	0.03
ACE	0.11	0.04	−0.21	−0.10	−0.11	−0.09	0.21	0.12
Shannon	0.05	0.20	0.56 **	−0.36 *	−0.07	0.31	0.08	0.37*
Acidobacteria	−0.11	−0.26	−0.52 **	0.27	−0.06	−0.31	−0.03	−0.30
Actinobacteria	0.48 **	0.30	−0.16	0.22	0.23	0.10	0.29	0.03
Chloroflexi	0.14	−0.17	0.43 *	0.38 *	0.29	−0.09	−0.09	−0.22
Firmicutes	−0.02	−0.06	0.71 ***	−0.37 *	−0.21	0.44 *	−0.05	0.27
Gemmatimonadetes	0.03	0.02	−0.61 ***	0.62 *	0.10	−0.32	0.13	−0.11
Planctomycetes	0.49 **	0.08	−0.26	0.34	0.40 *	0.06	0.06	0.02
Proteobacteria	−0.21	0.18	0.45*	−0.39 *	−0.07	0.10	−0.08	0.19

[a] EC—electrical conductivity; [b] SOM—soil organic matter. [c] Values are Pearson coefficients. Significant differences at $p < 0.05$ (*), <0.01 (**), and <0.001 (***) are reported.

The results of correlation analysis between soil properties and the 30 most abundant genera showed that soil-exchangeable Mg was significantly positively related to the relative abundance of *Cytobacillus* but negatively correlated with *Bradyrhizobium* (Figure 3). In addition, soil-exchangeable Ca was significantly positively correlated with *Pontibacter*. EC was negatively correlated with *Acidovorax* and *Granulicella* but positively related to *Achromobacter*, *Pseudomonas*, and *Ralstonia*. A significantly negative correlation was observed between soil-available P and *Granulicella*. Although *Pointibacter* and *Nocardioides* were positively affected by soil pH, *Lysinibacillus* was negatively influenced by soil pH. *Acidobacterium*, *Fictibacillus*, *Gemmatimonas*, and *Nitrospira* were positively affected by soil-available N, but *Mesorhizobium* and *Rhizobium* were negatively influenced by soil-available N. A significantly positive correlation was observed between soil-exchangeable K and *Lysinibacillus*. Although *Acidobacterium*, *Bradyrhizobium*, *Clostridium*, *Geobacter*, *Gemmatimonas*, and *Nitrospira* were negatively affected by SOM, *Massilia* and *Lysinibacillus* were positively correlated with SOM.

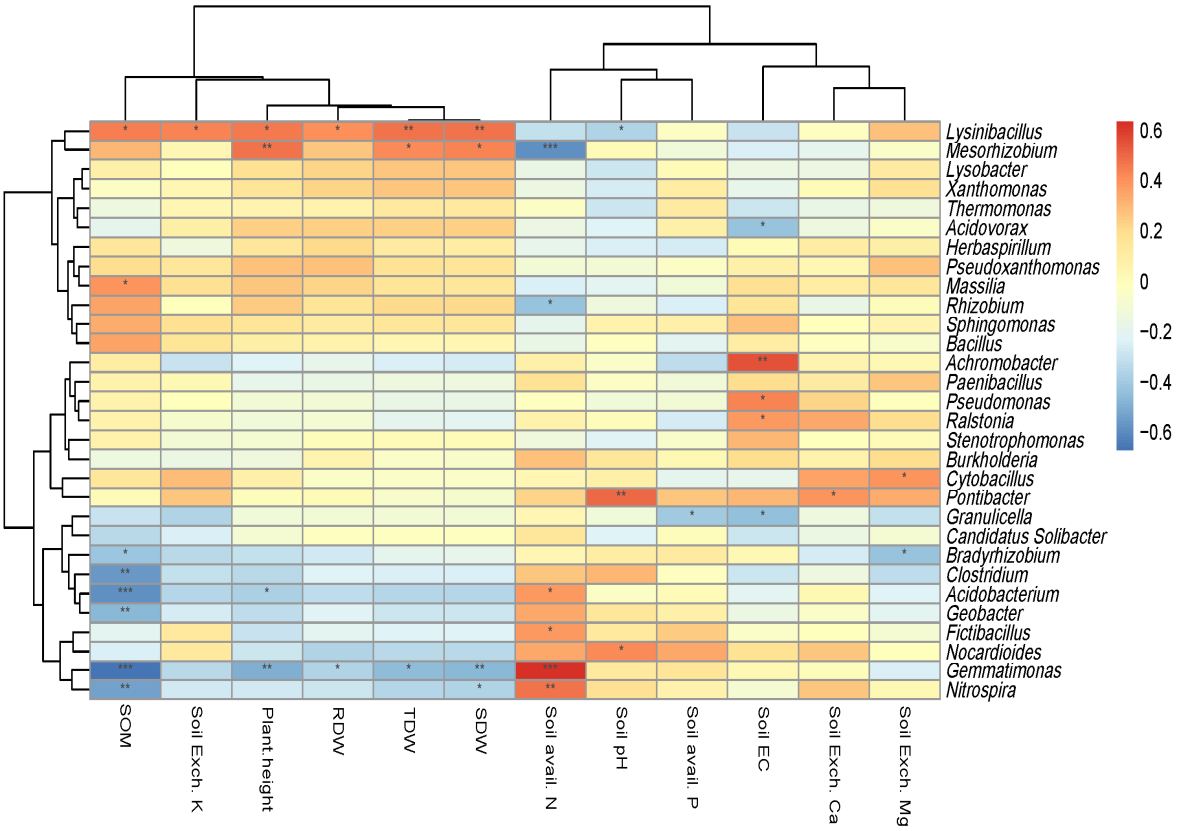

Figure 3. Pearson's correlations between soil properties, tomato growth, and the 30 most abundant genera. Positive and negative correlations are represented by shades between red and dark blue, respectively. Statistically significant correlations at the level of $p < 0.05$ are marked with an asterisk, $p < 0.01$ is marked with two asterisks, and $p < 0.001$ is marked with three asterisks. Soil EC—soil electrical conductivity; SDW—shoot dry weight; TDW—total dry weight; RDW—root dry weight; SOM—soil organic matter.

The plant height of tomato plants was negatively affected by *Acidobacterium* and *Gemmatimonas* but positively related to *Mesorhizobium* and *Lysinibacillus*. Shoot and total dry weights were positively influenced by *Mesorhizobium* and *Lysinibacillus* but negatively related to *Gemmatimonas* and *Nitrospira*. In addition, *Lysinibacillus* was positively correlated with the root dry weight, whereas a significantly negative correlation was observed between *Gemmatimonas* and the root dry weight.

3.5. Relationship between Organic Amendments and Selected Dominant Genera, Soil Properties, and Tomato Growth

There was a clear differentiation in the five genera considerably related to tomato growth between organic amendment treatments at harvest (Figure 4). The plant height and shoot and root dry weights of tomato plants were positively associated with the application of manure compost. In addition, the application of manure compost was positively related to SOM and soil-exchangeable K. The relative abundance of *Lysinibacillus*, *Massilia*, and *Mesorhizobium* was positively influenced by the manure compost treatments. Moreover, the plant height and shoot and root dry weights of tomato plants were positively related to the relative abundance of these genera. In contrast, the relative abundance of *Gemmatimonas* and *Nitrospira* was negatively correlated with tomato growth, and neem cake, rapeseed meal, and soybean meal considerably increased their abundance.

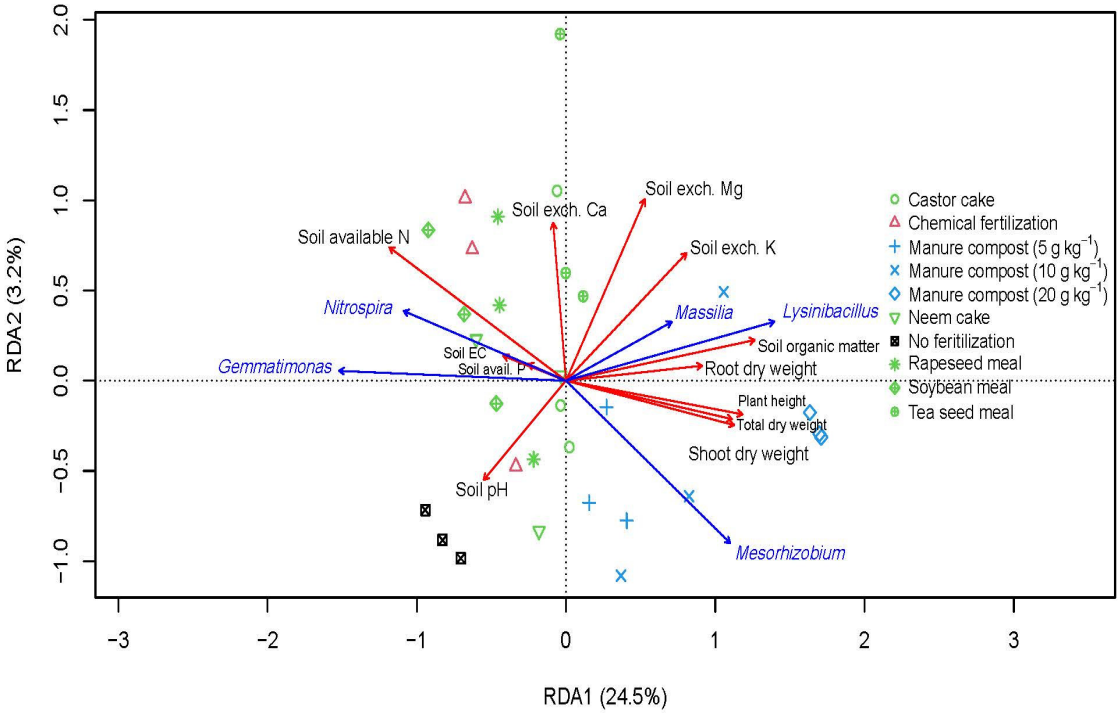

Figure 4. Redundancy analysis (RDA) of soil properties, selected dominant bacterial genera, and tomato growth under different fertilization treatments. Soil EC—soil electrical conductivity.

4. Discussion

The main question addressed by this study was whether organic amendment types influenced tomato growth by changing soil properties and the soil bacterial microbiome. We hypothesized that organic amendments affected bacterial abundance and diversity and soil properties such as soil pH, SOM, and nutrient availability, contributing to changes in tomato growth. Several lines of evidence support the hypothesis. First, our study showed that manure compost and tea seed meal significantly increased SOM in comparison with the no fertilization and chemical fertilization treatments. In addition, manure compost significantly increased soil-exchangeable K. Interestingly, tomato growth was positively correlated with SOM and soil-exchangeable K. Moreover, the application of neem cake and manure compost significantly increased the Chao1 richness and Shannon diversity indices in comparison with the chemical fertilization treatment. The relative abundance of *Lysinibacillus* was significantly positively correlated with the shoot and total dry weights of tomato plants, and its relative abundance was positively influenced by SOM and soil-exchangeable K. These findings suggest that the application of organic amendments may result in shifts in the bacterial community structure that is closely related to changes in soil properties. Of these organic amendments used in this study, manure compost can

significantly increase SOM, soil-exchangeable K, and the relative abundance of *Lysinibacillus*, consequently promoting tomato growth.

The application of mineral and organic matter fertilizers significantly influences soil properties. Our study showed that castor cake, soybean meal, and tea seed meal resulted in a significantly greater pH than the no fertilization control partly because the mineralization of these organic amendments releases NH_4^+ and OH^- [37]. In this study, chemical fertilization used urea as the N source, and urea may increase soil pH upon hydrolysis to NH_4^+ and HCO_3^- [38]. However, the chemical fertilization treatment did not significantly affect soil pH, probably because a portion of NH_4^+ is converted to NO_3^- and H^+ through nitrification [37]. Excess use of chemical fertilizers may cause a salinization effect, consequently reducing crop yield [39,40]. In this study, manure compost, rapeseed meal, soybean meal, and tea seed meal did not significantly increase soil EC compared to the no fertilization treatment. In contrast, the chemical fertilization treatment significantly increased soil salinity. The addition of organic amendments increases soil organic matter [7,8]. Our results suggested that tea seed meal and manure compost applied at 10 and 20 g kg^{-1} remarkably increased SOM by 13.6–35.2%, whereas the other treatments did not significantly increase SOM. Therefore, an increase in SOM depends on the type of organic amendment. Our study showed that the soil applied with the manure compost had a lower soil-available N than the other organic amendments, suggesting that the mineralization rate of the manure compost was lower than that of the oilseed meals used in this study. In contrast, chemical fertilization and soybean meal showed the greatest level of soil-available N compared with the other treatments, suggesting that they can rapidly release available mineral N into the soil. Although the manure compost had a lower mineralization rate, it significantly increased SOM, Bray-1P, and exchangeable K and Mg, consequently promoting tomato growth in this study.

Organic amendments can support plant growth differently, partly due to their various mineralization rates in soil. The application of organic amendments slowly releases mineral N, probably resulting in short-term N deficiency for plants [41]. In addition, the long-term use of composts alone may reduce corn yield compared with composts plus mineral N fertilizers [42]. Our study showed that manure compost applied at 10 and 20 g kg^{-1} significantly increased shoot dry weight in comparison to the chemical fertilization treatment, whereas the pot experiment was performed for a short-term period of 30 days after transplanting. It is necessary to further evaluate whether this growth-promoting effect can be reflected in tomato yield.

The use of organic matter fertilizers is known to influence the diversity and richness of soil microorganisms and, therefore, crop growth and yield [8,10]. The repeated application of manure and sewage sludge increases soil microbial biomass and changes microbial community structure compared with chemical fertilizer treatment [43]. In comparison with chemical fertilization, the application of alfalfa straw increases microbial community functioning, abundance, and diversity, but reduces the yield of rocket (*Eruca sativa*). The lower yield may be attributed to soil N depression after the application of alfalfa straw [7]. Our results showed that fertilizer types significantly affected bacterial richness and diversity. Although the no fertilization control exhibited the lowest bacterial richness and diversity, the application of neem cake or manure compost showed a significantly higher richness index than that of the chemical fertilization treatment. In addition, the application of manure compost, neem cake, rapeseed meal, and tea seed meal showed a significantly higher Shannon diversity index than the chemical fertilization treatment. These results suggested that the availability of organic C provided by these organic amendments may enhance bacterial richness and diversity [7,44]. In addition to the bacterial richness and diversity influenced by organic amendments, nutrients in or mineralized from organic amendments may also affect plant growth.

It has been suggested that the increase in bacterial community composition and diversity improves soil nutrient availability [16]. Although there was no significant difference in the Chao1 and ACE indices between manure treatments applied at 5 and 10 g kg^{-1}, the

application of manure compost at 10 g kg^{-1} resulted in a significantly higher Shannon index than that at 5 g kg^{-1}. In addition, our study showed that manure compost applied at 10 g kg^{-1} rather than 5 g kg^{-1} significantly increased the shoot dry weight of tomato plants compared with the mineral fertilization treatment. Therefore, nutrients provided by and mineralized from manure compost by diverse microorganisms may affect tomato growth. In addition, soil N limitation was not observed after application of the manure compost partly because net N mineralization occurred or the soil used in this study had sufficient available N for tomato growth, or both. Although the quality of organic amendments may affect soil community structure and functioning, soil fertility also contributes to the responses of tomato growth to different organic amendments.

Types of organic amendments significantly influence the soil bacterial community. Our results showed that *Proteobacteria* was the most prevalent taxon in all samples, and the application of neem cake resulted in the greatest abundance of this phylum. In contrast, the no fertilization control showed the lowest abundance of this phylum. The relative abundance of *Firmicutes* was higher in the manure compost applied at 20 g kg^{-1}, whereas the chemical fertilization treatment had the lowest abundance of this phylum. Since bacteria in the phyla *Proteobacteria* and *Firmicutes* prosper in soil with high carbon availability [45], the application of these organic amendments may increase the abundance of these two copiotrophic phyla. On the other hand, the application of soybean meal resulted in the highest abundance of *Actinobacteria*, which are also considered as copiotrophic microorganisms [46]. In addition, the relative abundance of copiotrophic *Gemmatimonadetes* was observed in the soil applied with castor cake. These organic amendments provide carbon availability to bacteria in these phyla, thereby increasing their abundance. However, the no fertilization control had the greatest abundance of *Acidobacteria*, which is consistent with them being generally considered as oligotrophs [47]. *Chloroflexi* is an oligotrophic-associated phylum [48], and our study showed that the soil applied with chemical fertilization showed the greatest abundance of this phylum due to the lack of carbon availability to bacteria. The abundance of *Planctomycetes* was unexpectedly the greatest in the soil applied with soybean meal partly because this phylum exhibits both copiotrophic and oligotrophic genomic features [48].

This study suggested that fertilizer treatments influenced the abundance of bacterial genera that were significantly related to tomato growth. The relative abundance of *Mesorhizobium* and *Lysinibacillus* was significantly positively correlated with the shoot and total dry weights of tomato plants. Of these organic amendments, manure compost significantly increased the abundance of these two genera in comparison with the chemical fertilization treatment. *Lysinibacillus* was previously classified as *Bacillus* [49], and this newly classified genus is ubiquitous [50]. Several *Lysinibacillus* species possess plant growth-promoting traits such as auxin and antibiotic production, phosphate solubilization, siderophore production, and nitrogen fixation [50–53]. Therefore, some species in this genus have been reported as biological control agents and plant growth-promoting bacteria. Our study suggests that *Lysinibacillus* may be used to improve tomato growth. Although *Mesorhizobium* species are widely known as nitrogen-fixing bacteria with legumes, their plant growth-promoting traits in addition to symbiotic N fixation have been proven to promote tomato growth [54]. Thus, increased abundances of *Lysinibacillus* and *Mesorhizobium* may be involved in promoting tomato growth.

Since very early tomato cultivars need to complete their growth and reproductive cycle in less than 60–80 days after transplanting, a question that may be asked is why this study was performed only 30 days in pots with a 1 kg of field soil. In addition, the effect of organic amendments on tomato growth may be different between 30 and 60 days after transplanting. To avoid fertilizer loss and provide a better estimation of the effect of organic amendments on soil properties, the soil microbiome, and tomato growth, our study used pots with the soil collected from a tomato-grown field to grow tomato plants under controlled conditions including irrigation, temperature, and photoperiod. Moreover, the correlation analysis between the microbial community composition, soil properties,

and tomato growth could be performed using the same pots. In addition, it has been suggested that the vegetative dry weight of tomato plants is highly correlated with the fruit dry weight [55,56]. Although our study merely evaluated the short-term effect of organic amendments on the shoot and root dry weights of tomato plants, the vegetative dry weight might reflect the distribution amount of photosynthesis assimilates to tomato fruits. Indeed, it is necessary to further evaluate the effects of these organic amendments on tomato yield under field conditions.

5. Conclusions

The results presented herein show that organic amendment types significantly affect soil properties, soil microbiota, and tomato growth. In this study, the application of manure compost remarkably increased soil organic matter, and chemical fertilization and neem cake treatments significantly enhanced soil salinity. No fertilization control exhibited the lowest estimated number of bacterial OTUs, but the application of neem cake or manure compost showed significantly higher Chao1 and ACE richness indices. Moreover, the application of neem cake and manure compost showed a significantly higher Shannon index than the chemical fertilization treatment. *Proteobacteria* was the dominant phylum in all fertilizer treatments, and the relative abundance of *Bacillus* was the highest in all samples. The relative abundance of *Mesorhizobium* and *Lysinibacillus* was significantly positively correlated with the shoot and total dry weights of tomato plants. Manure compost significantly increased the abundance of these two genera in comparison with the chemical fertilization treatment, suggesting that types of organic amendments influenced the abundance of the bacterial genera related to tomato growth. The relative abundance of *Lysinibacillus* was positively influenced by SOM and soil-exchangeable K, suggesting that the application of organic amendments may increase the abundance of this genus with several plant-growth-promoting traits, and in turn promote tomato growth. Overall, the manure compost used in this study can significantly increase SOM, soil-exchangeable K, and the relative abundance of *Lysinibacillus*, consequently promoting tomato growth. To promote tomato health and yield, further studies may need to evaluate how to use organic amendments to increase the relative abundance of *Lysinibacillus* in soil under field conditions.

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