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Effects of short-term application of organic manure on the growth of forage maize (*Zea mays* L. cv. Kwangpyeongok) and soil bacterial communities

Su-Yeon Shim[1,5], Junkyung Lee[1,5], Le Tran Yen Linh[1], Soo-Ryang Kim[2], Hyun-Sik Choi[3], Myung-Gyu Lee[4] & Sun-Goo Hwang[4]✉

While chemical fertilizers (CF) ensure rapid crop growth, relying exclusively on them can disrupt natural nutrient cycling and lead to environmental concerns such as nutrient imbalance. In contrast, organic amendments offer a sustainable alternative by promoting resource circulation; however, their efficacy is often variable and difficult to control, depending on complex interactions among application rates, plant types, and field conditions. Therefore, gaining comprehensive insights into their optimal use is essential to maximize agricultural benefits. This study evaluated the effects of composted Hanwoo manure (HM) applied at standard (HM_1x) and quadruple (HM_4x) rates on forage maize growth and soil microbial communities compared with CF and no treatment (NT). Growth parameters indicated that plant length was highest in the CF (219.33 cm) and HM_4× (217.30 cm) groups, followed by HM_1× (176.78 cm) and NT (172.02 cm). Soil analysis indicated that organic matter (OM) and available phosphorus (P_2O_5) were significantly higher in HM-treated soils than in NT. Microbiological analysis revealed distinct shifts in community composition linked to these chemical changes. In HM-treated soils, the relative abundance of Proteobacteria and *Candidatus* Saccharibacteria, known for their roles in OM decomposition and nutrient cycling, significantly increased. Conversely, the CF group showed a higher prevalence of Saprospiraceae, a phosphorus-removing bacterium, which is consistent with the observed reduction in available phosphorus in both soil and plant tissues in the CF treatment. Collectively, this study demonstrates that applying sufficient amounts of composted HM, where appropriate, can result in crop growth comparable to that of CF. Notably, we observed that such growth performance coincided with specific patterns in soil microbial communities related to nutrient availability. By highlighting these co-occurring trends, our research offers valuable insights into the biological dynamics of compost application for sustainable agriculture.

Keywords Microbiome, Bacterial community, Maize, PGPB, Compost

Abbreviations

ABTS	2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic)
ADF	Acid detergent fiber
ASV	Amplicon sequence variant
CCA	Canonical correlation analysis
CF	Chemical fertilizer
DPPH	2,2-diphenyl-1-picrylhydrazyl
EC	Electrical conductivity
HM	Hanwoo manure

[1]Department of Applied Plant Science, Sangji University, Wonju 26339, Korea. [2]Industry-Academic Cooperation Foundation, Sangji University, Wonju 26339, Korea. [3]Hoengseong Agricultural Technology Extension Center, Hoengseong 25208, Korea. [4]Department of Smart Farm Life Science, Sangji University, Wonju 26339, Korea. [5]Su-Yeon Shim and Junkyung Lee contributed equally to this work and share the first authorship. ✉email: sghwang9@sangji.ac.kr

NDF	Neutral detergent fiber
NT	No treatment
OM	Organic matter
ONT	Oxford Nanopore Technology
PCR	Polymerase chain reaction
SOM	Soil organic matter
T-N	Total nitrogen

Chemical fertilizers have been indispensable in modern agriculture, significantly contributing to global food security due to their convenience, immediate nutrient availability, and direct impact on crop productivity[1]. However, dependence on chemical fertilizers, often accompanied by continuous cropping without adequate organic matter management, has raised serious environmental concerns. Indiscriminate application leads to nutrient imbalances in the soil, accumulation of salts and heavy metals, and soil acidification[2–4]. Such degradation disrupts the stability of the soil ecosystem, increasing susceptibility to soil-borne diseases and hindering sustainable agricultural production[5]. Consequently, to alleviate these adverse effects and support soil ecosystem stability, reducing reliance on chemical fertilizers and adopting organic amendments is increasingly recognized as a vital strategy for restoring soil health.

The recycling of livestock manure is a key strategy for sustainable agriculture, converting organic waste into soil amendments and nutrient sources[6]. Since organic manure releases plant-available nutrients through mineralization, its agronomic benefits are often gradual, contributing to long-term soil fertility and ecological balance[7–9]. With rising global meat consumption, manure generation has increased correspondingly[10]. In South Korea, manure production has also expanded, with Hanwoo (Korean native cattle) accounting for the largest proportion, followed by pigs[11–13]. While pig manure is frequently managed as liquid fertilizer due to its high moisture content[14], whereas Hanwoo manure is typically processed into solid compost. Solid cattle manure compost can improve soil organic carbon and physical structure, including aggregate stability, which are important for long-term soil health[15–17]. Therefore, the appropriate use of Hanwoo manure compost represents a practical approach to organic resource recycling and sustained soil productivity.

Many agronomic benefits of organic fertilizers are mediated by soil microorganisms that drive nutrient cycling and promote plant growth. Manure-derived plant growth-promoting bacteria (PGPB), including *Pseudomonas* and Bacillus species, are known to enhance crop performance and nutrient uptake in various cropping systems[18–20]. Furthermore, rhizosphere-associated taxa such as Actinobacteria and *Burkholderiales* have been associated with suppressing soil-borne pathogens, suggesting the disease-suppressive potential of organic amendments[21]. Long-term manure application can also reshape microbial community structure distinct from that under chemical fertilization[22–24]. However, microbial responses to organic amendments are often context-dependent, and evidence remains limited on how short-term compost application influences both crop performance and microbiome shifts.

Therefore, demonstrating the efficacy of compost in the short term is essential for advancing resource-circulating agriculture, in addition to its well-known long-term benefits. However, the slow mineralization of compost often fails to meet immediate nutrient demands compared to chemical fertilizers. To address this, we hypothesized that a heavy application of compost could compensate for this lack of immediate nutrient availability. Using forage maize (*Zea mays* L.), a key crop linking livestock and farming[25], we compared the effects of chemical fertilizers with those of high-dose compost application. We specifically focused on analyzing the correlations among soil properties, crop functional quality, and microbial communities. Given recent advancements in sequencing technology, we used the Oxford Nanopore Technologies (ONT) platform to address the resolution limits of conventional methods. While short-read sequencing (e.g., Illumina) has long been the standard, typically targets only partial 16 S rRNA variable regions (e.g., V3–V4) and often failing to distinguish closely related species. In contrast, recent benchmarking studies have demonstrated that modern full-length 16 S rRNA sequencing (V1–V9) now offers superior taxonomic accuracy and species-level resolution for complex environmental microbiomes[26,27]. By leveraging these recent technical advances, we aimed to capture more robust, high-resolution microbial data. Although optimizing application rates to mitigate environmental loads remains a subject for future research, we anticipate that these biological findings will provide vital insights into overcoming the delayed efficacy of compost in short-term cultivation.

Materials and methods

Experimental design and plant growth survey

Maize seeds (*Zea mays* L. cv. Kwangpyeongok) obtained from the Agricultural Technology Center (Hoengseong-gun, Gangwon-do, Republic of Korea) were grown in a greenhouse for three weeks using 105-cell seedling trays and subsequently transplanted to an experimental field previously maintained as fallow land (37°22′16.2″ N, 127°55′30.1″ E) at Sangji University (Wonju-si, Gangwon-do, Republic of Korea). The experiment was designed using a randomized complete block design[28] with four treatments: chemical fertilizer (CF), no treatment (NT), and Hanwoo cattle manure compost (HM) applied at a standard (HM_1x) or quadruple (HM_4x) rate based on total nitrogen content. The CF treatment supplied N, P_2O_5, and K_2O at 200, 150, and 150 kg ha^{-1}, respectively. Based on the chemical properties of the manure (Table S1), the HM_1x treatment was applied at 13,200 kg ha^{-1} to match the nitrogen standard (approx. 200 kg N ha^{-1}). Consequently, the HM_4x treatment was applied at 53,000 kg ha^{-1} (approx. 800 kg N ha^{-1}). Amendments were applied as a basal dressing two weeks prior to transplanting; following the methods described by Byeon et al.[29] and Lee et al.[30], the amendments were spread evenly on the soil surface and immediately incorporated into the topsoil (approximately 20 cm depth) using a rotary tiller. The HM used in this study was produced at a Hanwoo farm (37°29′9.9″ N, 128°1′46.3″ E) in Hoengseong-gun via a six-month composting process (November 2022 to April 2023). To ensure experimental

reliability, we utilized compost from the same source that was empirically validated for maize cultivation in our previous studies[29,30]. In the current study, the biological maturity of this compost was definitively confirmed using a CoMMe-100 (E-woon, Korea) prior to application, and its physicochemical properties were analyzed (Table 1). The HM had a pH of 9.2, consistent with reports that compost pH increases during maturation[31]. Three weeks after transplanting, plant growth parameters, including plant height, leaf length and width, stem diameter, and chlorophyll content (SPAD), were measured for 12 randomly selected plants per treatment (n = 12) for each experimental group. SPAD values were determined using a SPAD-502 Plus meter (Konica Minolta, Japan). Additionally, the ear emergence rate at 86 days post-transplanting was calculated as the percentage of surviving plants bearing ears in each experimental plot. Fresh weights of the aerial parts were measured, and the samples were dehydrated in a dry oven (FCPO150, Dongseo Science, Korea) at 60 °C for one week. For analyzing feed quality and antioxidant activity, the dried tissues were ground into powder using a grinder (KSP-35, KM, Korea).

Soil chemical components

For surveying the chemical components of the soil, we obtained rhizosphere soil samples from three randomly selected plants per treatment (n = 3). The soil samples were stored in a deep freezer (SBD-215, Alpha Tech, Korea) at − 55 °C before 16 S rRNA sequencing and soil chemical analysis. We determined eight chemical attributes: pH, electrical conductivity (EC), OM, total nitrogen (T-N), available phosphorus pentoxide (P_2O_5), potassium (K), calcium (Ca), and magnesium (Mg). The pH and EC were measured in mixed samples of 10 g rhizosphere soil and 50 mL distilled water using a multiparameter analyzer (Edge HI2020, HANNA instruments, Woonsocket, USA). Total nitrogen was measured in a solution containing 50 g potassium sulfate and 50 g copper (II) sulfate (9:1) using the Kjeldahl method[32,33]. Phenolphthalein solution (500 µL) and sodium hydroxide were added to samples until the color changed to red. For determining the endpoints, 0.01 N sulfuric acid (H_2SO_4) was used to detect the change in color from blue to red. OM was determined in 10 mL of 0.4 N potassium dichromate solution using the Tyurin method[34]. After adding 85% H_3PO_4 and 500 µL diphenylamine, the endpoint was detected using 0.2 N ammonium iron (II) sulfate hexahydrate solution. The available P_2O_5 was analyzed using the Lancaster soil testing method[35] and extracted in 6 L solution with 400 mL acetic acid (CH_3COOH) and 300 mL 10 M lactic acid ($C_3H_6O_3$) after adding 22.2 g ammonium fluoride (NH_4F), 133.3 g ammonium sulfate ($[NH_4]_2SO_4$), and 170 g sodium hydroxide (NaOH). Exchangeable cations (K, Ca, and Mg) were extracted in 1 L of the solution with 77.08 g of ammonium acetate (CH_3COONH_4). The available P_2O_5, K, Ca, and Mg were measured using inductively coupled plasma optical emission spectroscopy (Integra 6000, GBC, Australia). These analyses were carried as described by Lee et al.[30].

Feed quality analysis

We analyzed the crude protein, crude fat, crude ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), and phosphorus (P) content in dried powder samples of the aerial parts of forage maize obtained from three randomly selected plants per treatment (n = 3). The feed quality of forage maize was analyzed following the methods described by Lee et al.[30]. Briefly, crude protein was detected in 7 g of potassium sulfate solution and 10 mL of H_2SO_4 using the Kjeldahl method[32,33]. Hydrochloric acid was used to observe the change in color of the samples after addition of zinc and sodium hydroxide solutions. Crude fat was detected using the ether extraction method[36]. The ether was extracted using a Soxhlet extractor (ANKOM XT15 Extractor, ANKOM Technology, USA). Crude ash was detected using the method of Liu[37]. A porcelain crucible was burned in an electric furnace (Lindberg/Blue M, Thermo Fisher Scientific, USA), and the weight of the samples after removing the porcelain crucible was measured. NDF was measured in 100 mL of neutral detergent solution after adding 2 mL of decahydronaphthalene and 0.5 g sodium sulfite using an automated fiber analyzer (ANKOM A2000, ANKOM Technology, USA). The amount of ADF was determined using the official AOAC method[38] with an acid detergent solution consisting of cetyltrimethylammonium bromide (CTAB), H_2SO_4, and decalin. The P content of the ammonium molybdite solution was determined using the Cavell's method[39].

	pH	EC (dS/m)	OM (g/kg)	T-*N* (%)	P_2O_5 (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)
NT	6.73 (±0.06) c	0.1 (±0) b	13.12 (±0.63) bc	0.08 (±0.01) b	76.67 (±1.53) b	0.07 (±0.01) c	5.3 (±0.17) b	1.73 (±0.06) b
CF	6.1 (±0) d	0.1 (±0) b	12.48 (±0.83) c	0.08 (±0.01) b	81.33 (±11.37) b	0.12 (±0.01) b	4.33 (±0.15) c	2.2 (±0.01) a
HM_1x	7.27 (±0.06) a	0.2 (±0) a	20.83 (±1.24) a	0.13 (±0.01) a	143.33 (±3.21) a	0.15 (±0.02) a	5.93 (±0.12) a	1.7 (±0) b
HM_4x	6.9 (±0.1) b	0.2 (±0) a	15.67 (±1.52) b	0.09 (±0.01) b	138 (±5.57) a	0.14 (±0.01) ab	5.43 (±0.15) b	1.57 (±0.06) b

Table 1. Chemical properties of soil after harvest. Data are presented as mean ± standard deviation (n = 3). Different lowercase letters within the same column indicate statistically significant differences between treatments (P < 0.05) according to Tukey's HSD test. *CF* Chemical fertilizer, *NT* No treatment, *HM_1x* Hanwoo manure 1x, *HM_4x* Hanwoo manure 4x.

Antioxidant activity

Dry powder of the top part of forage maize from three randomly selected plants per treatment (n = 3) was used in the analysis of antioxidant activity, following the method described previously[40]. In brief, the plant extract was prepared in 99% methanol (Daejung, Korea), and the total polyphenol and flavonoid content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic) (ABTS) radical, and nitrite-scavenging activities, and reducing power were analyzed. Gallic acid (Daejung, Korea) was used as a standard for total phenol content and DPPH scavenging activity, and quercetin (Daejung, Korea) was used for total flavonoids. Absorbance was measured using a spectrometer (OPTIZEN POP, KLAB, Korea) at 760 nm for total phenols, at 510 nm for total flavonoids, at 520 nm for nitrite-scavenging activity, at 734 nm for the ABTS assay, and at 700 nm for the reducing power assay. The high R-squared (R^2 >0.99) value of regression line in the measurements was determined using different dilution ratios (Fig. S1).

Soil DNA extraction and 16 S rRNA sequencing analysis

Soil DNA was extracted from approximately 500 mg of soil from each sample using a DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Polymerase chain reaction (PCR) was performed using 16 S primers, 27 F and 1492R. The sequences of primer pairs used for the first amplification were as follows:

27 F: 5′-AGAGTTTGATCMTGGCTCAG-3′.

1492R: 5′-TACGGYTACCTTGTTACGACTT-3′.

The thermocycling involved heat activation for 30 s at 95 °C, followed by 30 cycles of denaturation at 95 °C for 20 s, annealing at 58 °C for 20 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 3 min. Microbiome analysis was conducted using the Oxford Nanopore Technology (ONT) MinION platform to leverage its long-read sequencing capabilities. We selected this platform to sequence the full-length 16 S rRNA gene, which provides higher taxonomic resolution at the species level compared to short-read sequencing methods[41–43]. The PCR products were quantified using 200 fmol of each sample. The concentrations were measured using the Qubit 4.0 fluorometer and Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, each sample was ligated with a sample barcode for identification during sequencing using the Oxford barcoding kit SQK-NBD114.24 (Oxford Nanopore Technologies, Oxford, UK), following the manufacturer's protocol. The library was sequenced using a MinION flow cell R9.4.1 (ONT) on a MinION Nanopore sequencer. The 16 S rRNA gene sequencing data were base-called and demultiplexed using Guppy v7.0.8 (ONT), and adaptors were removed using Porechop v0.2.4[42]. To ensure high data quality and address sequencing errors, raw reads were processed using the Mothur pipeline. We applied strict quality control (QC) by filtering sequences based on length (1,000–1,550 bp) to retain only valid full-length 16 S rRNA genes. Furthermore, a denoising step (pre-clustering) was performed to correct sequencing errors by merging highly similar sequences allowing up to 2 differences (diffs = 2). Taxonomic classification was performed using Emu[44] based on the Emu v3.4.5 database (containing NCBI 16 S RefSeq references). Finally, to standardize sequencing depth across samples while minimizing data loss, we applied Scaling with Ranked Subsampling (SRS) normalization using the R package 'SRS' instead of traditional rarefaction.

Statistical analysis

The sample sizes for the statistical analysis were set to n = 12 for the plant growth survey and n = 3 for the analyses of soil properties, feed quality, and antioxidant activity. The analysis of variance was performed using the R function 'aov' (https://www.rdocumentation.org/packages/stats/versions/3.6.2/topics/aov). The Tukey's test was performed using the R package Agricolae (https://cran.r-project.org/web/packages/agricolae/index.html). The bacterial diversity was analyzed using the R packages ape[45], DESeq2[46], dplyr[47], ggrepel (http://cran.nexr.com/web/packages/ggrepel/index.html), ggsignif (https://cran.r-project.org/web/packages/ggsignif/index.html), ggplot2[48], phyloseq[49], pheatmap (https://www.rdocumentation.org/packages/pheatmap/versions/1.0.12/topics/pheatmap), taxa[50], and vegan (https://cran.r-project.org/ web/packages/vegan/index.html). The Mantel test was conducted using the R package vegan (https://cran.r-project.org/web/packages/vegan/index.html) with 9,999 permutations. The significance of Pearson's correlation coefficient was determined using n-2 degrees of freedom. The canonical correlation analysis (CCA) was performed using the R package CCA (https://cran.r-project.org/web/packages/ CCA/index.html).

Results

Plant growth over short-term cultivation under different fertilizer supplies

To compare the effects of short-term treatment with CF and HM with 1x and 4x nitrogen concentrations on plant growth, we measured plant length, leaf length, leaf width, stem diameter, SPAD value, ear emergence rate, and fresh and dry weights of the aerial parts of forage maize after planting (Fig. 1). The plant length was significantly higher in the CF (219.33 cm) and HM_4 × (217.30 cm) groups after 113 days than in the other groups (176.78 cm for HM_1x and 172.02 cm for NT) (Fig. 1A). Although the HM_1x group showed a slight increase compared with the NT group when livestock manure was supplied, no statistically significant difference was noted until harvest. Similarly, no significant difference was observed between the CF and HM_4x groups, indicating that there was insufficient time for mineralization within the soil organic matter (SOM) to enhance plant growth. The CF and HM_4x groups exhibited increased plant lengths compared to the HM_1x and NT groups. The growth rate of forage maize decreased after 98 d, indicating the transition of the plant's developmental phase from the vegetative to the reproductive stage.

On day 113 after planting, the CF and HM_4x groups (77.03 cm for CF and 75.48 cm for HM_4x) exhibited increased leaf length compared with the HM_1x and NT groups (56.76 cm for HM_1x and 53.13 cm for NT). Leaf length increased until day 77, and the growth rate was maintained after day 77. Consistent tendencies of

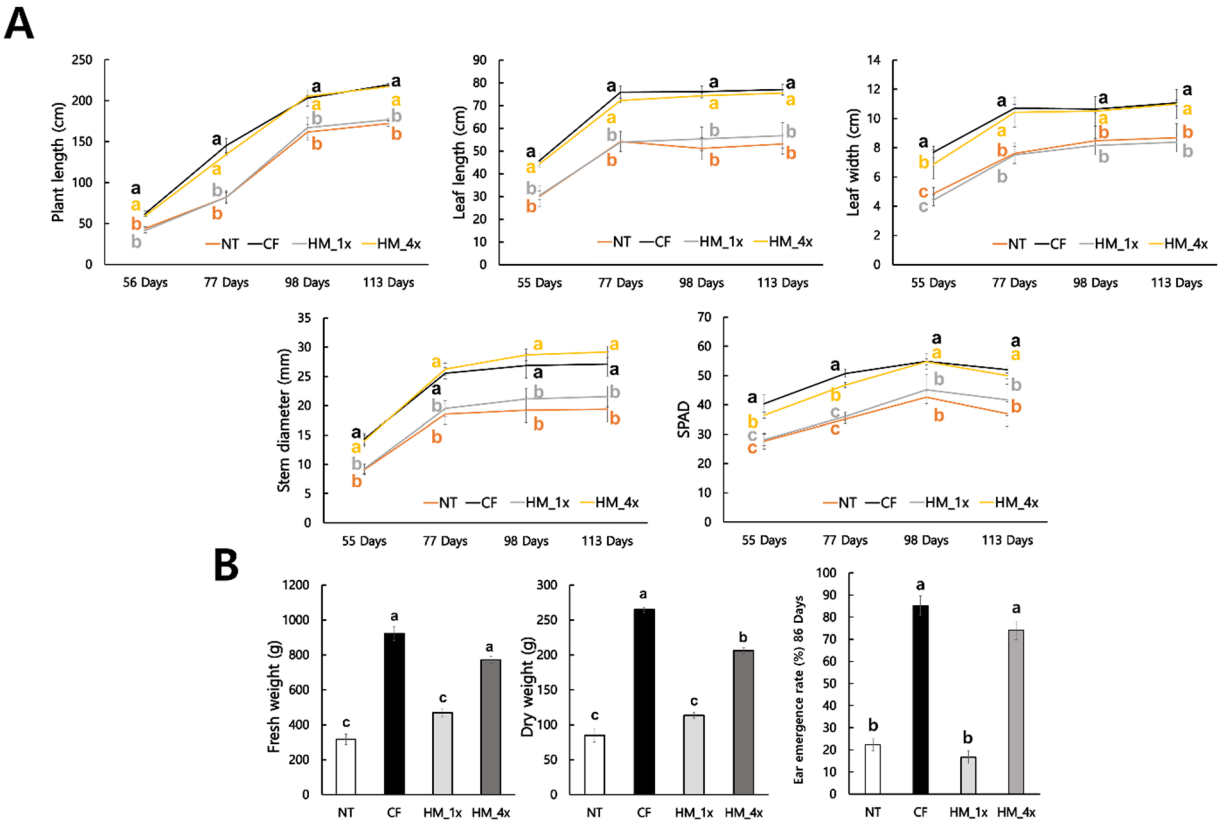

Fig. 1. Growth survey of maize cultivated on soil subjected to different treatments (*CF* chemical fertilizer, *NT* no treatment, *HM_1x* Hanwoo manure 1x, *HM_4x* Hanwoo manure 4x). (**A**) Plant length, leaf length, leaf width, stem diameter, SPAD 113 days after planting. (**B**) Fresh weight, dry weight, ear emergence rate of plant (top part). The values are the mean ± standard deviation ($n = 12$). The lowercase letters represent the significant differences ($P < 0.05$) according to Tukey's HSD test.

plant growth rates between the CF and HM_4x groups, or HM_1x and NT groups, were observed for leaf width, stem diameter, and leaf length. The SPAD unit of plant leaves was higher in the CF group (40.43) after 55 days than in the other groups (36.43 for HM_4x, 28.13 for HM_1x and 27.63 for NT). However, the SPAD units after 98 and 113 days were not significantly different in the CF and HM_4x groups or the HM_1x and NT groups. The SPAD units decreased in forage maize on days 98–113 after various fertilizer treatments, indicating a potential transition in the developmental phase of plants. The ear emergence rates at 86 days post-transplanting were higher in the CF group (85.19%) than in the other groups (74.07% for HM_4x, 22.22% for HM_1x, and 16.67% for NT). Although the CF group had the highest plant length, leaf length, leaf width, SPAD, and stem diameter, no significant differences were noted between the CF and HM_4x groups. However, the fresh and dry weights of the aerial parts were significantly higher in the CF group after 113 days compared with those in the other groups (Fig. 1B). The fresh weight in the CF group was 921.33 g, followed by 772 g in the HM_4x group, 469 g in the HM_1x group, and 316.67 g in the NT group. Similarly, the dry weight in the CF group was 265.67 g, followed by 206 g in the HM_4x group, 113.33 g in the HM_1x group, and 84.67 g in the NT group. These results indicate that the HM_4x supply during short-term cultivation enhances the overall plant growth similarly to that in CF treatment.

Chemical composition of the soil

To determine whether the chemical composition of the soil was altered by the short-term application of different fertilizers, pH, OM, T-N, P_2O_5, K, Ca, Mg, and EC were analyzed for each treatment after harvesting forage maize (Table 1). The group mean differences in soil components highest in the CF group for Mg (2.20 mg/kg), in the HM_1x group for pH (7.27), OM (20.83 g/kg), T-N (0.13%), P_2O_5 (143.33 mg/kg), K (0.15 mg/kg), Ca (5.93 mg/kg), and EC (0.20 dS/m), and in the HM_4x group for P_2O_5 (138.00 mg/kg), K (0.14 mg/kg), and EC (0.20 dS/m). The pH and Ca levels decreased in the CF group, whereas the K and Mg levels increased. The seven components (pH, OM, T-N, P_2O_5, K, Ca, and EC) were higher in the HM_1x group than in the other groups. Additionally, the P_2O_5, K, and EC levels were higher in the HM_4x group. Overall, the HM_1x and HM_4x groups exhibited increased values of pH (7.27 for HM_1x and 6.90 for HM_4x), OM (20.83 g/kg for HM_1x and 15.67 g/kg for HM_4x), P_2O_5 (143.33 mg/kg for HM_1x and 138.00 mg/kg for HM_4x), K (0.15 mg/kg for HM_1x and 0.14 mg/kg for HM_4x), and EC (0.20 dS/m for both HM_1x and HM_4x) in the soil upon application of HM compost, compared with those in the NT group (pH, 6.73; OM, 13.12 g/kg; P_2O_5, 76.67 mg/

kg; K, 0.07 mg/kg; and EC, 0.10 dS/m). Distinct changes in the chemical composition were observed in the rhizosphere soils when CF or HM was applied, despite the short-term cultivation of forage maize.

Feed quality and antioxidant activities

To assess the quality of forage maize, the levels of crude protein, crude fat, crude ash, P, ADF, and NDF in the aerial parts of the plants were analyzed (Fig. 2). Although the crude protein content was high in the HM_1 × (8.71%) and HM_4 × (8.47%) groups, no significant differences were observed among the groups. The HM_4x group (3.3%) had a high crude fat content. The crude ash content in the HM_4x group (5.4%) was significantly increased, whereas that in the CF group was decreased (3.87%). The P content was low in the CF group (0.29%), showing a significant difference from the NT group (0.39%); however, the remaining two groups did not exhibit significant differences, showing values intermediate between the CF and NT groups (0.38% in both HM_1x and HM_4x). Compared with the NT group, the levels of crude ash and P were higher in the HM_1x and/or HM_4x groups and decreased in the CF group. The ADF and NDF contents were not significantly different among the samples.

To analyze the antioxidant activities, we measured the total flavonoid content, ABTS and DPPH radical scavenging activities, and reducing power (Fig. 3). The DPPH assay and total flavonoid content showed no significant differences among the samples. However, the total polyphenol content and ABTS radical scavenging activity were decreased in the CF group (2.24 mg GAE/mL for total polyphenol content and 64.33% for ABTS radical scavenging activity) compared with those in the NT group (2.69 mg GAE/mL for total polyphenol content and 74.76% for ABTS radical scavenging activity). The reducing power was the highest in the HM_1x group (1.38%) and lowest in the CF group (1.14%).

Soil microbial communities

To identify differences in microbial communities in the soil, we conducted a 16 S rRNA sequencing analysis using the MinION nanopore platform (Fig. 4). The rarefaction curves for all samples plateaued at approximately 20,000 reads, indicating that the sequencing depth was sufficient to capture the bacterial diversity and stabilize ASV richness (Fig. 4A). We identified 19 bacterial phyla and one unassigned phylum. The two dominant phyla, Acidobacteria and Proteobacteria, constituted a large percentage of the microbial community in each soil sample

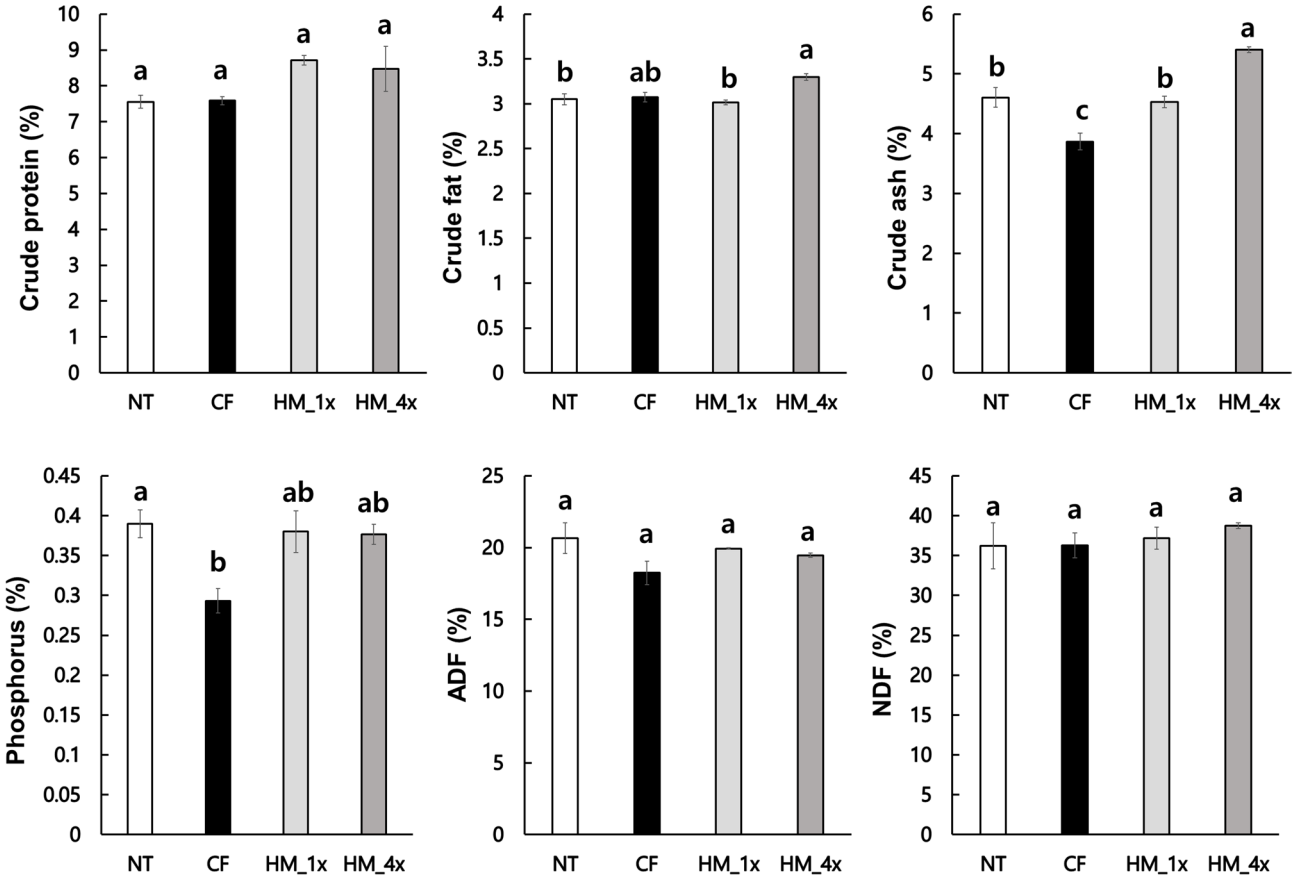

Fig. 2. Feed values differ with different treatments (*CF* chemical fertilizer, *NT* no treatment, *HM_1x* Hanwoo manure 1x, *HM_4x* Hanwoo manure 4x). The values are the mean ± standard deviation ($n = 3$). The lowercase letters represent the significant differences ($P < 0.05$) according to Tukey's HSD test. ADF, acid detergent fiber; NDF, neutral detergent fiber.

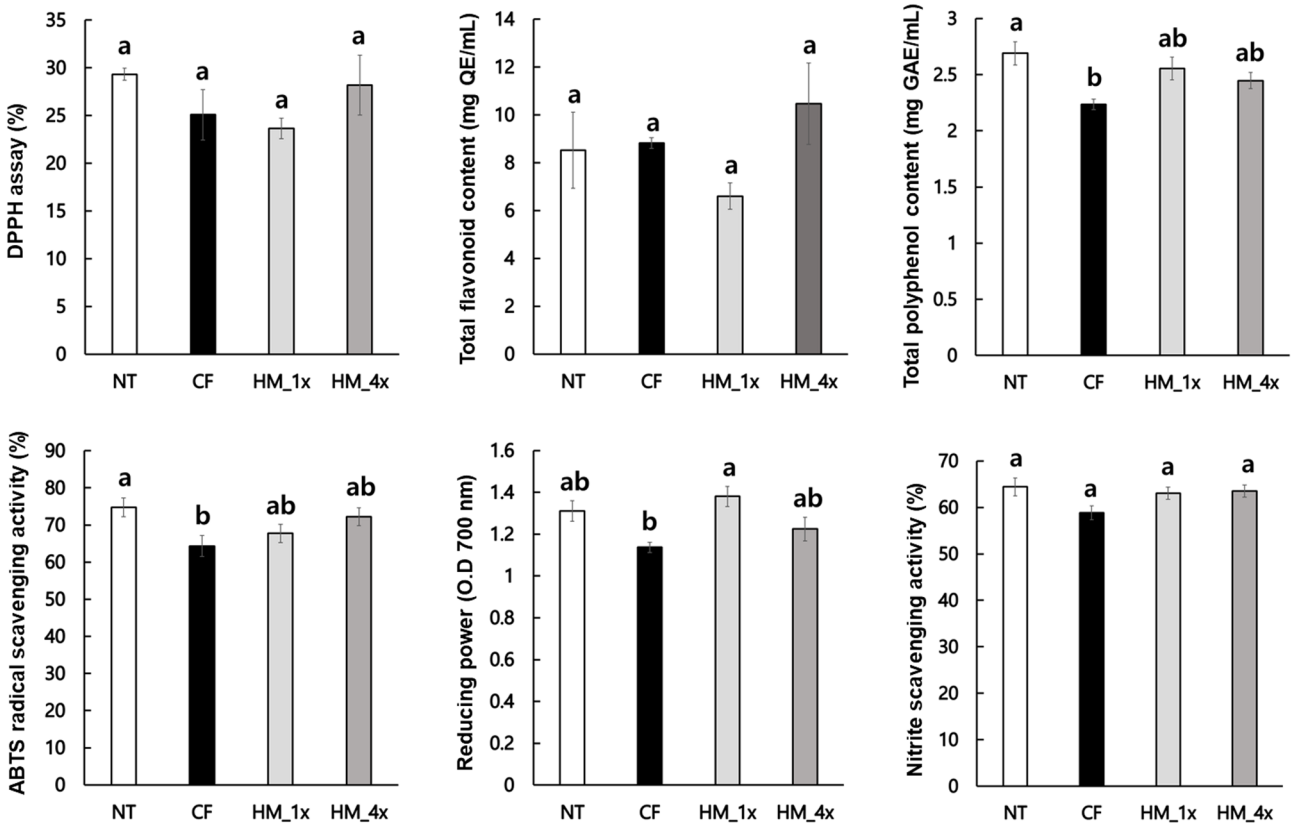

Fig. 3. Antioxidant activity of maize cultivated on soil subjected to different treatments (*CF* chemical fertilizer, *NT* no treatment, *HM_1x* Hanwoo manure 1x, *HM_4x* Hanwoo manure 4x). The values are the mean ± standard deviation ($n = 3$). The lowercase letters represent the significant differences ($P < 0.05$) according to Tukey's HSD test.

(Fig. 4B). Alpha diversity, which refers to the diversity within an area, was the highest in the HM_1x group and lowest in the NT group (Fig. 4C). Overall, the CF and HM groups showed increased alpha diversity in the soils after short-term cultivation. For beta diversity, which refers to the diversity among different areas, the CF and HM_4x groups exhibited similar microbial communities (Fig. 4D). However, the NT group exhibited low similarity in beta diversity among different soil samples. Based on the relatively high abundance of amplicon sequence variants (ASVs), 31 DABs were found in the CF and HM groups compared with those in the NT group (Fig. 4E). The DABs comprised 12 species from 5 phyla between the CF and NT groups, 11 species from 3 phyla between the HM_1x and NT groups, and 17 species from 6 phyla between the HM_4x and NT groups. Proteobacteria were differentially distributed in the CF, HM_1x, and HM_4x groups compared with those in the NT group, whereas *Candidatus* Latescibacteria was common in the HM_1x and HM_4x groups.

Differentially abundant bacteria (DAB) associated with changes in soil chemical composition

The chemical composition of soil directly affects the growth and quality of crops. Therefore, we attempted to identify significant correlation between the DAB and soil chemical composition (Fig. 5A). The seven chemical components (pH, EC, OM, TN, P_2O_5, K, and Ca), except for Mg, showed statistical significance ($P < 0.05$) and exhibited positive correlations ($R > 0$) with the abundance of the differentially abundant ASVs. Particularly, a relatively high significance ($P < 0.01$) was detected for OM ($R = 0.4392$) and P_2O_5 ($R = 0.4372$) (Fig. 5A). Four components (pH, OM, Ca, and T-N) correlated with the HM_1x group, whereas three (K, P, and EC) were situated between the HM_1x and HM_4x groups (Fig. 5B).

The seven chemical properties (pH, EC, OM, T-N, P, K, and Ca) were significantly correlated with the abundance of DABs (Mantel test, $P < 0.05$). Subsequently, Pearson's correlation coefficients were calculated to assess the relationships between the seven chemical properties and the abundance of DABs. The 19 DABs exhibited significant correlations with at least one of the seven chemical properties based on $n - 2$ degrees of freedom (Table 2). The abundance of six bacterial species was lower in the CF-treated soils (Flavobacteria bacterium and Aquificae bacterium), HM_4x-treated soils (Acidobacteriales bacterium, *Acidobacterium* species, and Saprospiraceae bacterium), and HM-treated soils (Latescibacteria bacterium in HM_1x and HM_4x) than in the untreated soil. Two bacterial species, Flavobacteria bacterium and Aquificae bacterium, whose abundance decreased, were positively correlated with several chemical components of the CF-treated soil. In particular, Flavobacteria bacterium was positively correlated with pH, Ca, and EC, whereas Aquificae bacterium showed a positive correlation with pH, OM, T-N, P, Ca, and EC. However, negative correlations were observed for the

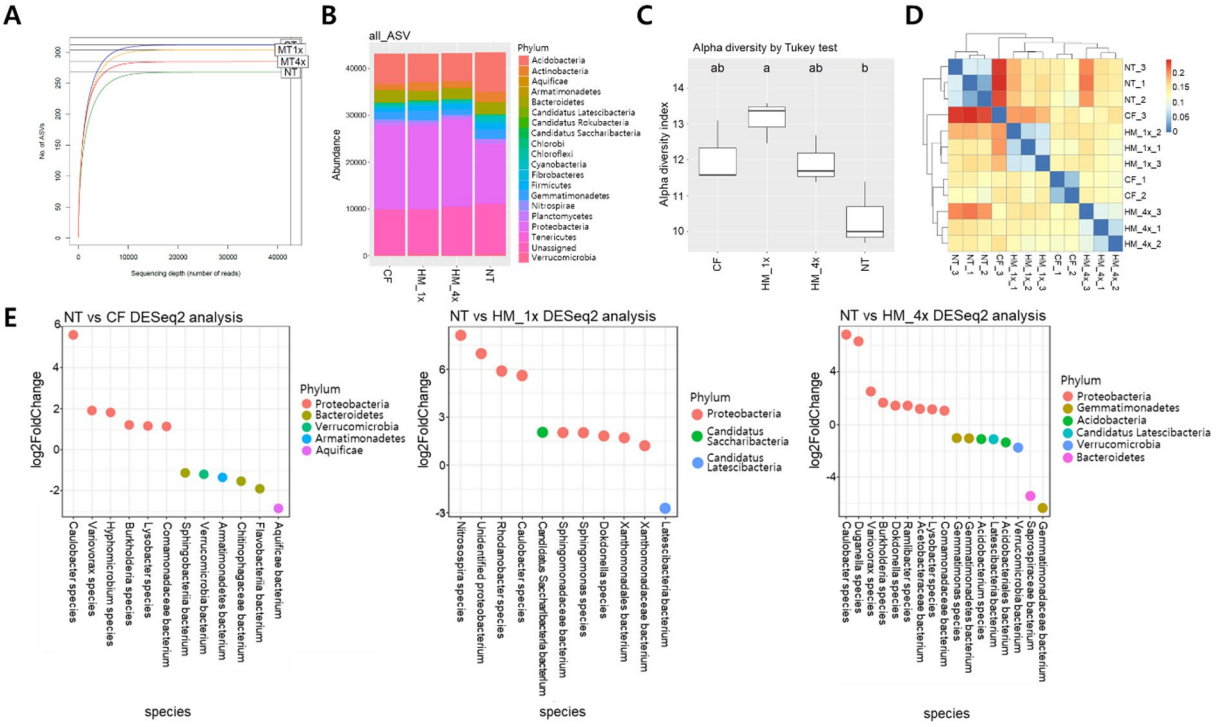

Fig. 4. Amplicon sequence variant (ASV) approach for bacteria in the soils subjected to different treatments (*CF* chemical fertilizer, *NT* no treatment, *HM_1x* Hanwoo manure 1x, *HM_4x* Hanwoo manure 4x). (**A**) Rarefaction curves (**B**) Distribution of the dominant bacteria at the phylum level. (**C**) Alpha diversity of the bacterial population in each sample determined using the inverse Simpson (InvSimpson) method. The lowercase letters represent the significant differences ($P < 0.05$) between groups determined using the Tukey's HSD test. (**D**) Hierarchical clustering of samples. The line colors represent the different levels of beta diversity determined using the Bray–Curtis dissimilarity. (**E**) Bacterial communities for each sample in the phylum level. The box colors represent the phylum of bacteria.

A

	Statistic *R*
pH	0.3061 *
EC	0.361 *
OM	0.4392 **
T-N	0.3844 *
P	0.4372 **
K	0.3586 *
Ca	0.2982 *
Mg	0.06517 nd

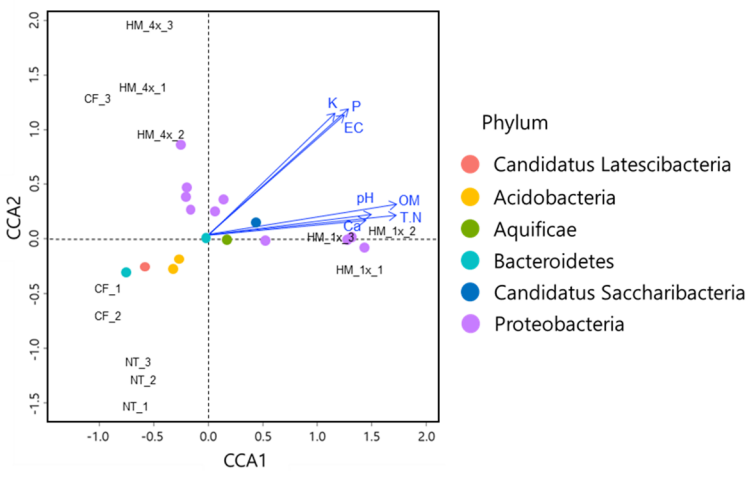

Fig. 5. Correlation analysis between soil properties and bacterial community structure in the soils subjected to different treatments ((*CF* chemical fertilizer, *NT* no treatment, *HM_1x* Hanwoo manure 1x, *HM_4x* Hanwoo manure 4x). (**A**) Mantel test between soil properties on bacterial community composition. Significant results are indicated by * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ (nd, not detected). (**B**) Canonical correspondence analysis of bacteria community composition and soil properties in soils after different treatments.

Species	Features	Change rate	pH	OM	T.*N*	*P*	K	Ca	EC
Flavobacteriia bacterium	CF	–	0.775 **	0.457	0.290	0.491	0.177	0.832 **	0.583 *
Aquificae bacterium	CF	–	0.935***	0.711 **	0.634 *	0.628 *	0.280	0.948 ***	0.672 *
Xanthomonadaceae bacterium	HM_1x	+	0.709 **	0.795 **	0.734 **	0.954 ***	0.848 ***	0.641 *	0.909 ***
Rhodanobacter sp.	HM_1x	+	0.703 *	0.906 ***	0.913 ***	0.623 **	0.612 *	0.668 *	0.588 *
Xanthomonadales bacterium	HM_1x	+	0.718 **	0.858 ***	0.822 **	0.860 ***	0.817 **	0.680 *	0.816 **
Nitrosospira sp.	HM_1x	+	0.703 *	0.893 ***	0.920 ***	0.613 *	0.556	0.669 *	0.577 *
Proteobacterium	HM_1x	+	0.655 *	0.872 ***	0.888 ***	0.564	0.583 *	0.624 *	0.535
Sphingomonas sp.	HM_1x	+	0.767 **	0.962 ***	0.957 ***	0.710 **	0.643 *	0.729 **	0.682 *
Sphingomonadaceae bacterium	HM_1x	+	0.750 **	0.947 ***	0.941 ***	0.668 *	0.628 *	0.709 **	0.648 *
Candidatus Saccharibacteria bacterium	HM_1x	+	0.788 **	0.936 ***	0.909 ***	0.767 *	0.698 *	0.730 **	0.759 **
Acidobacteriales bacterium	HM_4x	–	−0.603 *	−0.518	−0.466	−0.861 ***	−0.621 *	−0.567	−0.844 ***
Acetobacteraceae bacterium	HM_4x	+	0.236	0.097	−0.062	0.587 *	0.506	0.230	0.621 *
Acidobacterium sp.	HM_4x	–	−0.561	−0.374	−0.253	−0.825 ***	−0.615 *	−0.474	−0.816 **
Ramlibacter sp.	HM_4x	+	−0.005	0.099	0.027	0.536	0.646 *	0.039	0.503
Saprospiraceae bacterium	HM_4x	–	−0.868***	−0.560	−0.480	−0.673 *	−0.237	−0.873 **	−0.715 **
Dokdonella sp.	HM_1x, HM_4x	+, +	0.528	0.567	0.487	0.770 **	0.784 **	0.496	0.725 **
Lysobacter sp.	CF, HM_4x	+, +	−0.096	0.174	0.095	0.414 *	0.654 *	−0.170	0.413
Latescibacteria bacterium	HM_1x, HM_4x	–, –	−0.639 *	−0.895 ***	−0.872 ***	−0.871 ***	−0.849 ***	−0.573	−0.819 *
Caulobacter sp.	CF, HM_1x, HM_4x	+, +	0.120	0.163	0.095	0.605 *	0.506	0.066	0.595 *

Table 2. Significant correlation between different chemical components and soil bacteria. The values represent Pearson's correlation coefficients between bacterial abundance and soil chemical properties. Asterisks indicate statistical significance (*$P<0.05$, ** $P<0.01$, *** $P<0.001$). *CF* Chemical fertilizer, *HM_1x* Hanwoo manure 1x, *HM_4x* Hanwoo manure 4x.

three bacterial species, Acidobacteriales bacterium, *Acidobacterium* species, and Saprospiraceae bacterium, in the HM_4x-treated soil. In particular, Acidobacteriales bacterium was negatively correlated with pH, P, K, and EC, whereas *Acidobacterium* species showed negative correlations with P, K, and EC, and Saprospiraceae bacterium showed negative correlations with pH, P, Ca, and EC. Latescibacteria bacterium detected in the HM_1x- and HM_4x-treated soils was negatively correlated with six chemical components (pH, EC, OM, T-N, P, and K), except for Ca. The abundance of eight bacterial species (Xanthomonadaceae bacterium, Rhodanobacter species, Xanthomonadales bacterium, *Nitrosospira* species, Proteobacterium, *Sphingomonas* species, Sphingomonadaceae bacterium, and *Candidatus* Saccharibacteria bacterium) was increased in the HM_1x-treated soil, and their increased abundance was positively correlated with the chemical components (pH, OM, T-N, P, K, Ca, and EC). The two bacterial species (Acetobacteraceae bacterium and *Ramlibacter* species) that increased in abundance showed positive correlations with soil chemical properties in the HM_4x-treated soil. In particular, Acetobacteraceae bacterium was positively correlated with P and EC, whereas *Ramlibacter* species showed positive correlation with K. Furthermore, positive correlations was observed among three bacterial species—*Dokdonella* species in the HM_1x- and HM_4x-treated soil (P, K, and EC); *Lysobacter* species in the CF- and HM_4x-treated soil (P and K), and *Caulobacter* species in all the experimental soils (CF, HM_1x, and HM_4x) (P and EC). Overall, many bacteria with increased abundance showed a positive correlation with various chemical components in each soil sample, particularly in the HM_1x-treated soil.

The alpha diversity of the DABs was higher in the HM_1x and HM_4x groups than in the NT group (Fig. 6A). Although the CF group exhibited lower alpha diversity than the HM_1x and HM_4x groups, the difference was not significant. The beta diversity of the DABs was similar in the NT and CF groups, whereas the HM_1x group exhibited relatively higher diversity than the other groups (Fig. 6B). With regard the distribution of DABs at the phylum level, Aquificae, *Candidatus* Saccharibacteria, and Proteobacteria were more abundant in the HM_1x group than in the other groups (Fig. 6C). Acidobacteria, Bacteroidetes, and *Candidatus* Latescibacteria exhibited relatively higher numbers of ASVs in the CF and NT groups, whereas Verrucomicrobia was more prevalent in the HM_1x and NT groups than in the CF and HM_4x groups. To compare the presence of DABs in each experimental soil with that in the NT group, the abundance of ASVs was examined based on bacterial phylogeny (Fig. 6D). In the phylogenetic tree comparison, five bacteria (Saccharibacteria, Flavobacteriia bacterium, Aquificae, *Sphingomonas* species, and *Rhodanobacter* species) consistently exhibited higher abundance in the HM_1x and HM_4x groups. However, the abundance of Saprospiraceae increased only in the CF group. Two bacteria (*Proteobacterium* and *Nitrosospira* species) showed relatively higher ASV abundance only in the HM_1x group, which exhibited the highest rate of change in soil chemical components before and after the application of different fertilizers. These results indicate that the formation of distinct bacterial communities following the application of HM compost may result in chemical changes in the soil after short-term application.

Discussion

In this study, the HM_4x treatment exhibited crop growth performance comparable to that of the CF group. This trend was observed not only in plant growth but also in the ear emergence rate, where the HM_4x and

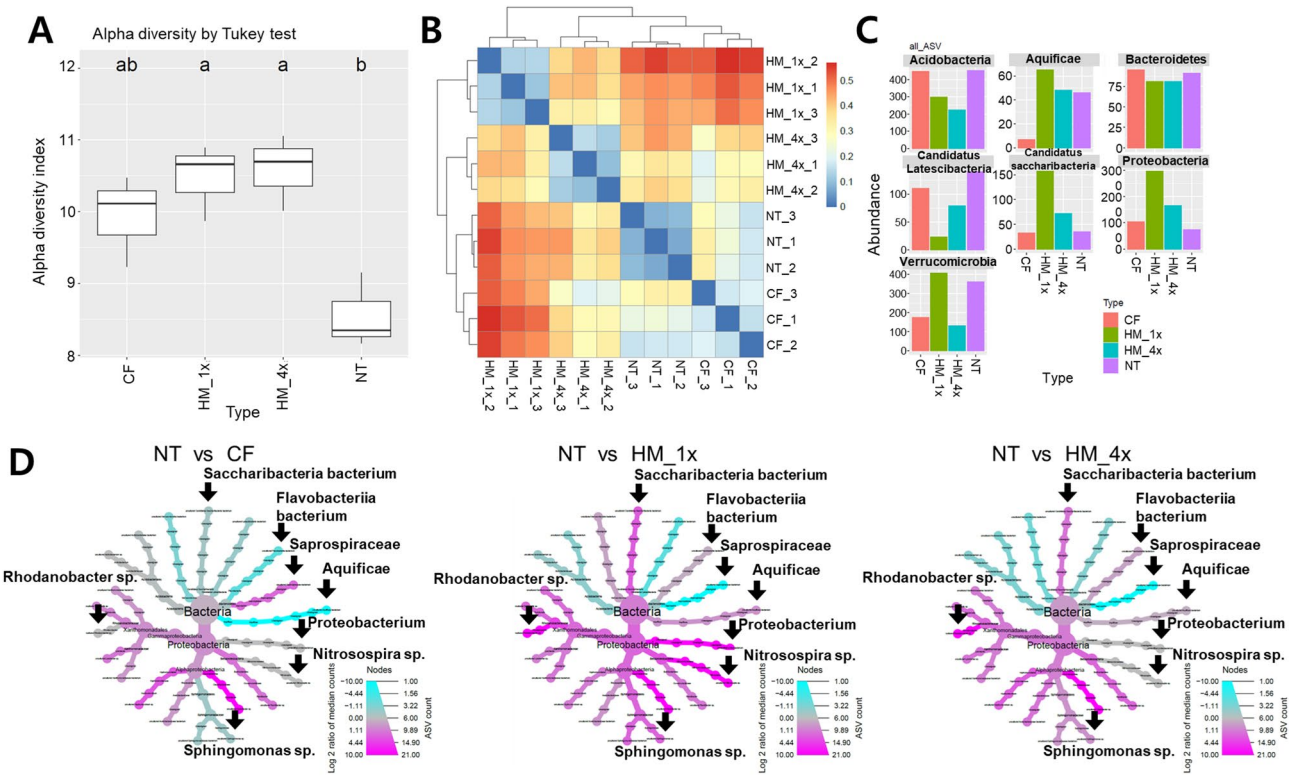

Fig. 6. Taxonomic composition of significant bacteria in the soils subjected to different treatments (*CF* chemical fertilizer, *NT* no treatment, *HM_1x* Hanwoo manure 1x, *HM_4x* Hanwoo manure 4x). (**A**) Alpha diversity of the bacterial population in each sample determined using the inverse Simpson (InvSimpson) method. The lowercase letters represent the significant differences ($P < 0.05$) between groups using the Tukey's HSD test with significant bacteria. (**B**) Hierarchical clustering of sample with significant bacteria. The line colors represent the different levels of beta diversity determined using the Bray–Curtis dissimilarity. (**C**) Distribution of bacterial phyla. The bar colors represent the various samples. (**D**) The relative abundances of significantly correlated bacteria in CF, HM_1x, and HM_4x groups compared with that in the NT groups. Circle size represents the number of bacteria for ASV. The color indicates the log 20 fold change of ASV with *P*-adjust < 0.05. Arrow represents the specific bacteria in each treatment.

CF groups demonstrated superior establishment compared to the HM_1x and NT groups. In contrast, the HM_1x group showed significantly lower growth parameters and emergence rates, and remained similar to the NT group. This difference from our previous study conducted over two years[30], in which cumulative nutrient release allowed standard rates to be effective, implies that in short-term applications without legacy effects, standard compost rates (HM_1x) might be insufficient to meet immediate nutrient demands required for initial crop establishment and subsequent growth due to slow mineralization[51]. Organic composts are generally known to possess high carbon-to-nitrogen (C: N) ratios[7], which often trigger temporary microbial nitrogen immobilization during organic matter decomposition. During the early establishment phase, crops rely heavily on immediately accessible inorganic nitrogen[1,51]. In our study, compost was applied based on total nitrogen, yet only 18.4% existed in an inorganic form (Table S1). Consequently, while the CF treatment supplied 200 kg ha^{-1} of directly available inorganic nitrogen, the HM_1x treatment provided only about 37 kg ha^{-1}. This limited inorganic N pool was likely outpaced by microbially driven immobilization, resulting in restricted crop growth. In contrast, the quadruple application rate (HM_4x) delivered a substantial absolute quantity of inorganic nitrogen (approximately 147 kg ha^{-1}). This ample supply was sufficient to overcome the microbial nitrogen sink and meet the immediate nutritional demands of the crop, resulting in early growth comparable to the CF treatment. These findings emphasize that optimized fertilization strategies should prioritize specific available nitrogen fractions rather than relying solely on total nitrogen content. Regarding soil properties, the residual pH, OM, and EC levels in the HM_4x group were lower than anticipated given the high alkaline compost input. This trend appears to be associated with the vigorous plant growth observed in this group. The secretion of organic acids from active root systems likely neutralized the soil alkalinity, resulting in a pH decrease[52,53]. Furthermore, root exudates might have stimulated microbial activity, potentially accelerating soil organic matter decomposition[54]. These findings suggest that the enhanced crop growth in the HM_4x group likely influenced the soil chemical environment, resulting in chemical profiles distinct from the slower-growing HM_1x group[55].

Regarding feed quality, the CF group showed lower levels of crude ash and phosphorus (P) compared with the compost-treated groups. This observation is consistent with the "dilution effect" in which rapid biomass accumulation outpaces mineral uptake[56], or could be associated with the immobilization of inorganic phosphorus

in the soil[57,58]. Moreover, higher antioxidant activities (e.g., ABTS, reducing power) were observed in the compost-treated groups compared with the CF group. While this aligns with reports that organic amendments may support secondary metabolite enhancement[59–61], it is important to note that these differences could also reflect variations in plant maturity stages or stress responses rather than nutrient sources[62]. Furthermore, given the limited sample size in this study ($n = 3$), these biochemical traits should be interpreted as preliminary trends rather than definitive physiological conclusions.

Regarding soil microbial diversity, our previous study indicated that chemical fertilization lowered alpha diversity compared to the NT group[30]. However, in this short-term study, we did not observe a drastic reduction in the CF group. Notably, the HM_1x group exhibited relatively high bacterial diversity. This observation appears to be associated with the higher residual soil organic matter (OM) in this group, resulting from slower plant growth and reduced nutrient uptake. This aligns with previous studies suggesting that organic amendments provide a diverse substrate pool that supports microbial richness[63,64]. Thus, maintaining microbial diversity via compost application is considered a key component of sustainable agricultural practices.

To understand the interactions between microbial communities and soil properties, we analyzed the correlations between Differentially Abundant Bacteria (DABs) and soil chemical composition. Our analysis revealed a positive correlation between DAB abundance and soil OM and phosphorus (P) levels. Specifically, taxa belonging to Proteobacteria, Flavobacteria, and *Candidatus* Saccharibacteria were enriched in HM treated groups. Previous literature describes these taxa as playing roles in organic matter decomposition and nutrient cycling, including nitrogen and phosphorus turnover[65–68]. For instance, the enrichment of *Nitrosospira* and Saccharibacteria in the HM_1x group corresponded with higher soil nitrogen and phosphorus levels, respectively. The strong correlation observed in this study between these taxa and soil properties implies that the substrate rich environment of the HM groups may have favored their proliferation, consistent with their known ecological traits. Conversely, the CF group was characterized by a relative abundance of Saprospiraceae, a taxon previously linked to phosphorus removal[69], which corresponds to the lower residual P observed in the CF group. However, because amplicon data are compositional, correlation results should be interpreted cautiously. Moreover, as this study relies on 16 S rRNA gene amplicon sequencing, these functional associations are inferred from taxonomic identities; thus, species-level labels should be interpreted as best-hit assignments rather than definitive isolates. Therefore, further research using metagenomics or transcriptomics is required to confirm the specific metabolic activities of these taxa in response to fertilization.

Collectively, while the HM_4x treatment achieved crop growth comparable to chemical fertilizers, the underlying soil-microbial landscapes were fundamentally distinct. Crucially, unlike chemical fertilizers, which tended to limit microbial diversity, compost application expanded the functional microbial pool, favoring taxa closely linked to organic matter decomposition and nutrient cycling. Notably, the variations observed between compost treatments (HM_1x vs. HM_4x) appear to be driven by the dynamic plant-soil feedback in the HM_4x group, where vigorous root activity and nutrient uptake likely altered the soil chemical environment compared to the slower-growing HM_1x group. However, considering potential environmental trade-offs such as nitrate leaching and phosphorus accumulation, the high dose application necessitates future optimization. Therefore, the high-dose application (HM_4x) presented in this study should be interpreted as a proof of concept strategy to compensate for delayed mineralization in short-term cultivation, rather than a blanket recommendation for general agricultural practice. Additionally, soil electrical conductivity (EC) remained low (0.2 dS/m), indicating negligible salinity risks from manure application, future studies should include direct monitoring of residual heavy metals and antibiotics to ensure comprehensive long-term safety. Furthermore, while the generalizability of our results is limited by the single site, short-term design under fallow conditions, this study serves as a critical baseline. Validating these biological correlations through future multi year, multi location trials will ultimately provide the qualitative depth required to establish sustainable alternatives to chemical fertilizers.

Conclusions

The slow mineralization of organic amendments presents a challenge for meeting the immediate nutrient demands of crops in short-term cultivation. This study observed that a high-dose application of Hanwoo manure compost (HM_4x) resulted in agronomic performance in forage maize comparable to that of chemical fertilizers (CF), whereas the standard rate (HM_1x) did not show significant differences from the untreated control. These findings suggest that while standard compost rates may be insufficient for initial growth in fallow soil, adjusting application rates could potentially compensate for delayed nutrient availability. Our analysis highlighted that these agronomic outcomes coincided with distinct shifts in the soil microbiome. Unlike CF treatment, which was associated with specific taxa such as Saprospiraceae, compost application enriched bacterial groups including *Candidatus* Saccharibacteria, Proteobacteria, and Bacteroidetes. Given that these taxa are ecologically linked to organic matter decomposition and nutrient turnover, their enrichment implies that high compost input may support nutrient cycling processes distinct from chemical fertilization. In conclusion, this study provides a baseline for understanding the short-term effects of high-dose compost application. While HM_4x showed potential as a substitute for chemical fertilizers in terms of yield, the environmental trade-offs associated with high nutrient loading, such as potential nitrate leaching or phosphorus accumulation, must be carefully considered. Therefore, future research should focus on optimizing application rates to balance immediate crop productivity with long-term soil health and environmental safety.

Data availability

Raw reads from isolates sequenced in this study are available at the NCBI Short Read Archive (SRA) under Bio-Project accession no. PRJNA1329940 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1329940).

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References

1. Lu, C. & Tian, H. Global nitrogen and phosphorus fertilizer use for agriculture production in the past half century: Shifted hot spots and nutrient imbalance. *Earth Syst. Sci. Data* **9**, 181–192 (2017).
2. Savci, S. An agricultural pollutant: Chemical fertilizer. *Int. J. Environ. Sci. Dev.* **3**, 73 (2012).
3. Atafar, Z. et al. Effect of fertilizer application on soil heavy metal concentration. *Environ. Monit. Assess.* **160**, 83–89 (2010).
4. Guo, J. H. et al. Significant acidification in major Chinese croplands. *Science* **327**, 1008–1010 (2010).
5. Bai, Y. C. et al. Soil chemical and microbiological properties are changed by long-term chemical fertilizers that limit ecosystem functioning. *Microorganisms* **8**, 694 (2020).
6. Lebacq, T., Baret, P. V. & Stilmant, D. Sustainability indicators for livestock farming. A review. *Agron. Sustain. Dev.* **33**, 311–327 (2013).
7. Breza, L. C. & Grandy, A. S. Organic amendments tighten nitrogen cycling in agricultural soils: A meta-analysis on gross nitrogen flux. *Front. Agron.* **7**, 1472749 (2025).
8. Lee, J. Effect of application methods of organic fertilizer on growth, soil chemical properties and microbial densities in organic bulb onion production. *Sci. Hortic.* **124**, 299–305 (2010).
9. Reeve, J. R. et al. Organic farming, soil health, and food quality: considering possible links. *Adv. Agron.* **137**, 319–367 (2016).
10. FAOSTAT. World Food and Agriculture–Statistical Yearbook 2023. FAO, (2023).
11. Jeong, K. H., Kim, J. K., Khan, M. A., Han, D. W. & Kwag, J. H. A study on the characteristics of livestock manure treatment facility in Korea. *J. Korea Org. Resour. Recycl. Assoc.* **22**, 28–44 (2014).
12. Lee, S., Lim, C., Quan, Y., Kim, C. & Im, J. Economic evaluation of manure treatment methods considering manure output and nutrient balance by regions. *Korean J. Agric. Manag. Policy* **49**, 581–599 (2022).
13. Choi, S. H. Treatment and management of the livestock manure. *KCID J.* **14**, 110–120 (2007).
14. Nam, Y., Yong, S. H. & Song, K. K. Evaluating quality of fertilizer manufactured (livestock manure compost) with different sources in Korea. *Korean J. Soil Sci. Fert.* **43**, 800–805 (2010).
15. Das, S., Liptzin, D. & Maharjan, B. Long-term manure application improves soil health and stabilizes carbon in continuous maize production system. *Geoderma* **430**, 116338 (2023).
16. Zhu, Y. et al. Evaluation of the soil aggregate stability under long term manure and chemical fertilizer applications: Insights from organic carbon and humic acid structure in aggregates. *Agric. Ecosyst. Environ.* **376**, 109217 (2024).
17. Abbas, A. et al. The efficacy of organic amendments on maize productivity, soil properties and active fractions of soil carbon in organic-matter deficient soil. *Span. J. Soil Sci.* **14**, 12814 (2024).
18. Esitken, A. et al. Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry. *Sci. Hortic.* **124**, 62–66 (2010).
19. Arikan, Ş & Pirlak, L. Effects of plant growth promoting rhizobacteria (PGPR) on growth, yield and fruit quality of sour cherry (*Prunus cerasus* L). *Erwerbs-Obstbau* **58**, 221–226 (2016).
20. Khan, K. S. et al. Co-application of organic amendments and inorganic P increase maize growth and soil carbon, phosphorus availability in calcareous soil. *Front. Environ. Sci.* **10**, 949371 (2022).
21. Effendi, Y. & Pancoro, A. Revealing the role of plant growth promoting rhizobacteria in suppressive soils against *Fusarium oxysporum* f. sp. cubense based on metagenomic analysis. *Heliyon* **7**, e07826 (2021).
22. Guo, L. et al. Effects of cattle manure compost combined with chemical fertilizer on topsoil organic matter, bulk density and earthworm activity in a wheat–maize rotation system in Eastern China. *Soil Tillage Res.* **156**, 140–147 (2016).
23. Megyes, M., Borsodi, A. K., Árendás, T. & Márialigeti, K. Variations in the diversity of soil bacterial and archaeal communities in response to different long-term fertilization regimes in maize fields. *Appl. Soil Ecol.* **168**, 104120 (2021).
24. Sun, R., Zhang, X. X., Guo, X., Wang, D. & Chu, H. Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. *Soil Biol. Biochem.* **88**, 9–18 (2015).
25. Erenstein, O., Jaleta, M., Sonder, K., Mottaleb, K. & Prasanna, B. M. Global maize production, consumption and trade: Trends and R&D implications. *Food Secur.* **14**, 1295–1319 (2022).
26. Zhang, T. et al. The newest Oxford Nanopore R10.4.1 full-length 16S rRNA sequencing enables the accurate resolution of species-level microbial community profiling. *Appl. Environ. Microbiol.* **89**, e00605–e00623 (2023).
27. Hoffbeck, C., Middleton, D. M., Nelson, N. J. & Taylor, M. W. Benchmarking 16S rRNA gene-based approaches to bacterial taxonomy assignment based on amplicon sequencing with Illumina and Oxford Nanopore. *Int. J. Microbiol.* https://doi.org/10.1155/ijm/7563096 (2025).
28. Shieh, G. & Jan, S. L. The effectiveness of randomized complete block design. *Stat. Neerl.* **58**, 111–124 (2004).
29. Byeon, J. E. et al. Influence of Hanwoo (Korean native cattle) manure compost application in soil on the growth of maize (*Zea mays* L). *Korean J. Crop Sci.* **67**, 164–171 (2022).
30. Lee, J. et al. Effects of Hanwoo (Korean cattle) manure as organic fertilizer on plant growth, feed quality, and soil bacterial community. *Front. Plant. Sci.* **14**, 1135947 (2023).
31. Lee, S., Jeong, G., Lee, D., Lee, D. & Kwang, J. Evaluation of parameters affecting composting of Hanwoo manure. *J. Korea Soc. Waste Manag.* **37**, 124–132 (2020).
32. AOAC. *Official Methods of Analysis* 16th edn (Association of Official Analytical Chemists, 1995).
33. Barbano, D. M., Clark, J. L., Dunham, C. E. & Flemin, R. J. Kjeldahl method for determination of total nitrogen content of milk: collaborative study. *J. Assoc. Off Anal. Chem.* **73**, 849–859 (1990).
34. Schollenberger, C. A rapid approximate method for determining soil organic matter. *Soil Sci.* **24**, 65–68 (1927).
35. Lancaster, J. D. *Mississippi soil test method and interpretation* (Mississippi Agricultural Experiment Station, 1980).
36. Padmore, J. Fat (crude) or ether extract in animal feed. In *Official Methods of Analysis of Association of Official Analytical Chemists* 15th edn, Method 920.39AOAC, (1990).
37. Liu, K. Effects of sample size, dry ashing temperature and duration on determination of ash content in algae and other biomass. *Algal Res.* **40**, 101486 (2019).
38. AOAC. *Official Methods of Analysis* (Association of Official Analytical Chemists, 1990).
39. Cavell, A. J. The colorimetric determination of phosphorus in plant materials. *J. Sci. Food Agric.* **6**, 479–480 (1955).
40. Jo, N. Y. et al. Elevated CO2 concentration induces changes in plant growth, transcriptome, and antioxidant activity in fennel (*Foeniculum vulgare* Mill.) under hydroponics. *Front. Plant. Sci.* **13**, 5113 (2022).
41. Sevim, V. et al. Shotgun metagenome data of a defined mock community using Oxford Nanopore, PacBio and Illumina technologies. *Sci. Data* **6**, 285 (2019).
42. Wick, R. R., Judd, L. M., Gorrie, C. L. & Holt, K. E. Completing bacterial genome assemblies with multiplex MinION sequencing. *Microb. Genom.* **3**, e000132 (2017).
43. Matsuo, Y. et al. Full-length 16S rRNA gene amplicon analysis of human gut microbiota using MinION™ nanopore sequencing confers species-level resolution. *BMC Microbiol.* **21**, 35 (2021).
44. Curry, K. D. et al. Emu: Species-level microbial community profiling of full-length 16S rRNA Oxford Nanopore sequencing data. *Nat. Methods* **19**, 845–853 (2022).

45. Paradis, E., Claude, J. & Strimmer, K. APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289–290 (2004).
46. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550 (2014).
47. Wickham, H., François, R., Henry, L. & Müller, K. *dplyr*: *A Grammar of Data Manipulation*. R package version 0.4.3 (2015).
48. Wickham, H. & Chang, W. Package '*ggplot2*': Create Elegant Data Visualisations Using the Grammar of Graphics Description. (CRAN Repos, (2016).
49. McMurdie, P. J. & Holmes, S. Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* **8**, e61217 (2013).
50. Foster, Z., Chamberlain, S. & Grünwald, N. Taxa: An R package implementing data standards and methods for taxonomic data. *F1000Res* **7**, 272 (2018).
51. Islam, M. R. et al. Mineralization of farm manures and slurries for successive release of carbon and nitrogen in incubated soils varying in moisture status under controlled laboratory conditions. *Agriculture* **11**, 846 (2021).
52. Moritsuka, N., Yanai, J. & Kosaki, T. Effect of plant growth on the distribution and forms of soil nutrients in the rhizosphere. *Soil Sci. Plant Nutr.* **46**, 439–447 (2000).
53. Vives-Peris, V., De Ollas, C., Gómez-Cadenas, A. & Pérez-Clemente, R. M. Root exudates: From plant to rhizosphere and beyond. *Plant Cell Rep.* **39**, 3–17 (2020).
54. Nardi, S. et al. Soil organic matter mobilization by root exudates. *Chemosphere* **41**, 653–658 (2000).
55. Lazcano, C., Gómez-Brandón, M., Revilla, P. & Domínguez, J. Short-term effects of organic and inorganic fertilizers on soil microbial community structure and function: A field study with sweet corn. *Biol. Fertil. Soils* **49**, 723–733 (2013).
56. Davis, D. R. Declining fruit and vegetable nutrient composition: What is the evidence?. *HortScience* **44**, 15–19 (2009).
57. Lee, S. M. Effects of mixed application of chemical fertilizer and liquid swine manure on agronomic characteristics, yield and feed value of corn hybrid for silage in paddy field cultivation. *J. Korean Soc. Grassl. Forage Sci.* **32**, 369–378 (2012).
58. Syers, J. K., Johnston, A. & Curtin, D. Efficiency of soil and fertilizer phosphorus use. *FAO Fertil. Plant Nutr. Bull.* **18**, 5–50 (2008).
59. Oliveira, A. B. et al. The impact of organic farming on quality of tomatoes is associated to increased oxidative stress during fruit development. *PLoS ONE* **8**, e56354 (2013).
60. Faller, A. & Fialho, E. The antioxidant capacity and polyphenol content of organic and conventional retail vegetables after domestic cooking. *Food Res. Int.* **42**, 210–215 (2009).
61. Mie, A. et al. Human health implications of organic food and organic agriculture: A comprehensive review. *Environ. Health* **16**, 111 (2017).
62. Hasanuzzaman, M. et al. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants* **9**, 681 (2020).
63. Shirzad, H. et al. Combined effect of biological and organic fertilizers on agrobiochemical traits of corn (*Zea mays* L.) under wastewater irrigation. *Plants* **13**, 1331 (2024).
64. Salamatpour, S., Niknezhad, Y., Fallah, H. & Tari, D. B. Impacts of chemical and organic fertilizers along with silicon on agronomic traits, yield and nutrient content in rice under plant growth-promoting bacteria. *Cereal Res. Commun.* **53**, 1133–1145 (2025).
65. Duan, C., Liu, Y., Zhang, H., Chen, G. & Song, J. Cadmium pollution impact on the bacterial community of Haplic Cambisols in Northeast China and inference of resistant genera. *J. Soil Sci. Plant Nutr.* **20**, 1156–1170 (2020).
66. Li, X., Sun, M., Zhang, H., Xu, N. & Sun, G. Use of mulberry–soybean intercropping in salt–alkali soil impacts the diversity of the soil bacterial community. *Microb. Biotechnol.* **9**, 293–304 (2016).
67. Adhikari, T. B., Philbrick, A. N. & Louws, F. J. Elucidating the impact of organic amendments on soil bacterial communities and strawberry yield in North Carolina. *Front. Sustain. Food Syst.* **9**, 1550106 (2025).
68. Ma, Y. et al. Organic fertilizer substitution over six years improves the productivity of garlic, bacterial diversity, and microbial communities network complexity. *Appl. Soil Ecol.* **182**, 104718 (2023).
69. Fu, J., Zhang, Z. & Zhu, J. Study on the diversity of denitrification bacteria treating with wastewater by using PPGC filler on SBMBBR at low temperature. *E3S Web Conf.* **194**, 04051 (2020).

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Author contributions

S.-Y. S., J. L., and S.-G. H. conceived and designed the study. S.-Y. S., L. T. Y. L., S.-R. K., H.-S. C., and M.-G. L. conducted the field experiments and collected the samples. S.-Y. S., J. L., and S.-G. H. analyzed the data and interpreted the results. S.-Y. S. drafted the manuscript, and J. L. and S.-G. H. revised and edited the text. All authors reviewed the manuscript, contributed to the revisions, and approved the final version for publication.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to S.-G.H.

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