ZANCO Journal of Pure and Applied Sciences

TYPE Original Article
DOI http://dx.doi.org/10.21271/ZJPAS.38.2.11

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RECEIVED :25 /02 /2025
ACCEPTED :29/10/ 2025
PUBLISHED :30/ 04/ 2026

KEYWORDS:
Organic amendments, Microbial community analysis, Spinach growth, Sandy loam soil, Water hyacinth compost, Chicken manure, Soil fertility.

From Degradation to Productivity: Organic Strategies for Sandy Loam Restoration, Microbial Enrichment, and Spinach Growth

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ABSTRACT

This research evaluates how organic amendments, particularly water hyacinth compost (WHC) and chicken manure (CM), can rehabilitate the degraded sandy loam soil at the J.K. College Premises (CP), Purulia, West Bengal, India. Soil quality and plant growth had been severely impacted due to construction activities. Spinach was used as an indicator crop because of its rapid foliar growth, making it a sensitive marker for amendments' effects. Treatment with WHC and CM significantly improved several growth parameters, including root length, root diameter, shoot height, and leaf area. The application of WHC and CM (organic compost and manure, referred to as OCM or amended soil) increased soil fertility, evidenced by reductions in bulk density from 1.194 g/cm³ to 0.825 g/cm³ and an increase in porosity from 45.28% to 68.87%. Total nitrogen rose from 357.8 to 1120 mg/kg, with notable gains in phosphorus and potassium as well. Statistical analysis using Two-Way ANOVA confirmed significant treatment effects. Results were visualized through matrix-based heatmaps in R 4.3.2, showing clear biometric trends. Microbial diversity, assessed via 16S rRNA and ITS amplicon sequencing, was markedly higher in OCM (2,962 bacterial and 937 fungal species) compared to CP (427 and 899 species, respectively). This elevated diversity in OCM soil reflects the effect of nutrient-rich inputs like WHC and CM, which support beneficial taxa such as *Actinomadura keratinolytica*. In contrast, the lower diversity in CP soil suggests nutrient limitations that may weaken ecological resilience and hinder crop productivity, with dominated by *Bacillus mannanilyticus*. Fungal community shifts were less pronounced. Visualization tools, including stacked bar charts at various taxonomic levels and Sankey diagrams for dominant taxa, effectively illustrated microbial community changes. Overall, this study shows that WHC and CM amendments significantly restore soil health, boost microbial diversity, and improve crop productivity in degraded semi-urban soils.

1. Introduction

Soil degradation is a significant global threat to agricultural productivity and environmental sustainability. It is driven by a combination of physical, chemical, biological, and socioeconomic factors (Abebaw, 2019). This degradation results in reduced crop yields, nutrient depletion, and the loss of vital ecosystem services. Soil erosion alone contributes to an estimated global loss of $400 billion annually (Eswaran *et al*., 2019).

Sustainable soil management practices offer solutions to these challenges by preserving soil health and minimizing environmental impacts (Tahat *et al*., 2020). Key strategies include minimizing soil disturbance, enhancing organic matter, managing and cycling nutrients, optimizing water use, integrating pest management, and promoting beneficial microorganisms (White *et al*., 2014; De Corato, 2020).

While many studies have explored individual soil amendments, limited research has examined how combining specific organic amendments can synergistically restore highly degraded sandy loam soils in agricultural regions. Enhancing organic matter improves a range of soil properties and supports microbial diversity (Al-Shammary *et al*., 2024). Organic matter amendments improve soil physicochemical characteristics, including soil structure, water retention, aeration, and nutrient content. Such amendments boost total organic carbon and overall nutrient availability (Ozores-Hampton *et al*., 2011).

Additionally, organic amendments reduce bulk density and improve soil structure, making the soil more favorable for plant growth. They also stimulate microbial activity and diversity, strengthen ecosystem resilience, and enhance crop productivity (Shu *et al*., 2022).

This research evaluates the effectiveness of water hyacinth compost (WHC) and chicken manure (CM) in restoring degraded sandy loam soils by enhancing both soil health and crop productivity. Specifically, the study addresses three key research questions:

1. What soil physicochemical properties change in response to these amendments?
2. How do these amendments influence spinach growth parameters such as leaf area, root length, and plant height?
3. What impact do they have on soil microbial diversity and community composition?

The study applies a soil health framework to explore the interconnectedness of physical structure, chemical composition, and biological communities within the soil system. Controlled experiments were conducted using various combinations of WHC and CM treatments, compared against an untreated control, referred to as CP soil (from the college premises as baseline soil). Based on statistical analysis, the most effective combination—referred to as OCM soil (baseline soil amended with WHC and CM)—was selected for physicochemical and amplicon sequencing studies. These results demonstrate that organic amendments such as WHC and CM can serve as effective, low-cost tools for restoring soil health and enhancing plant productivity. By advancing our understanding of these organic inputs, the research offers practical strategies that farmers and land managers, particularly those in semi-urban and resource-limited regions, can adopt to rehabilitate degraded agricultural land. The findings highlight the potential of WHC and CM to improve soil fertility, boost microbial health, and ultimately support sustainable crop yields.

2 Materials and methods

2.1 Organic Amendment Preparation

2.1.1 Compost preparation

This study utilized water hyacinth (*Eichhornia crassipes* (Mart.) Solms), an invasive aquatic plant collected from a local lake in Purulia (23.3395° N, 86.3586° E), for composting. According to Sarika *et al*., 2014, water hyacinth is rich in organic matter, moisture, optimal carbon-nitrogen (C:N) ratio as well as essential macronutrients such as potassium and phosphorus, while being low in recalcitrant organics—making it an ideal substrate for microbial activity and decomposition. The Composting of this species reduces its environmental impact and also produces a valuable soil amendment. The composting process involved shredding the water hyacinth into ½-inch pieces and placing them in galvanized wire cages (3 ft diameter × 4 ft height, ¼-inch mesh) set up outdoors from June 25 to October, 2023. A base layer of fractured bricks, gravel, stones, and sand ensured proper drainage and aeration. To support microbial activity and maintain optimal conditions, the shredded biomass—initially containing 84.2% moisture—was regularly wetted using a hose-end sprayer and turned approximately 40 times at three- to four-day intervals to preserve aerobic conditions. Temperature and pH were monitored twice daily, with the temperature peaking at 57°C and facilitating organic matter breakdown and pathogen reduction. After the active composting phase, the material was cured for 30 days to stabilize nutrients, then screened through a 2 mm mesh, to produce a fine, uniform compost with a black, crumbly texture and earthy odor, indicating maturity and agricultural readiness. The procedure followed composting guidelines provided by the Tamil Nadu Agricultural University (TNAU) Agritech Portal (https://agritech.tnau.ac.in/org_farm/orgfarm_composting.html), with practices such as proper layering, moisture control, and turning frequency used as benchmarks to evaluate compost maturity and microbial activity. Although separate control piles were not maintained in this experiment, adherence to these standardized protocols was considered sufficient to ensure consistent compost quality. The mature compost was then mixed with CM and soil in various combinations, and plant growth was monitored across treatments. The combination showing the highest plant growth was selected for further analysis, and the physicochemical properties of soil include—bulk density, porosity, pH, nitrogen, phosphorus, and potassium were assessed and are presented in Table 1 of the Results section.

2.1.2 Chicken Manure Preparation

CM was collected from a local farm with an initial moisture content of 30%. It was air-dried for 4 days, followed by sterilization in an autoclave at 121°C, 15 psi for 15 minutes. After sterilization, it was air-dried again for 3 days to stabilize moisture at 8%. Moisture content was confirmed by applying the oven-drying method at 105°C for 24 hours in accordance with standard protocol for manure moisture determination). Once dried, the manure was sieved through a 2.00-mm mesh to remove large particles and achieve a uniform texture, suitable for blending with WHC.

2.1.3 Final Organic Amendment Mixtures

Nine treatments were prepared using varying proportions of WHC and CM, with the remaining weight comprised of soil to achieve a total of 6000 g per mixture. The WHC:CM: Soil combinations (in grams) were as follows: 600:12:5388, 600:24:5376, 600:48:5352, 1200:12:4788, 1200:24:4776, 1200:48:4752, 1800:12:4188, 1800:24:4176, and 1800:48:4152. CM were sterilized at 121°C for 15 minutes to eliminate pathogens. The sterilized amendments were then hand-mixed with sandy loam soil in the above specified ratios to produce organic fertilizer mixtures with uniform nutrient distribution. These formulations were subsequently applied to spinach cultivation to assess their effectiveness in improving soil health and promoting crop growth.

2.2. Site Description and Experimental Setup

The experiment was conducted at the Department of Botany, Jagannath Kishore College, Purulia, West Bengal, India (23.3173° N, 86.3690° E), to evaluate the effects of WHC and CM on spinach cultivation. Baseline soil parameters (CP)—including pH, organic matter content, and nutrient content were recorded prior to treatment implementation, as presented in the Results section.

Water-holding capacity was determined using a standard gravimetric method (funnel, filter paper, and drainage), following the Maximum Water-Holding Capacity and Field Fresh Plant Density protocol. Containers made of durable polyethylene (10-inch diameter × 8-inch height) were used to ensure adequate root development and aeration. Each container was filled with 6 kg of soil and amended with different levels of WHC (600 g, 1200 g, or 1800 g) and CM (12 g, 24 g, or 48 g), resulting in the following treatment designations:

- E1: Pure soil (CP soil – unamended control from college premises)
- E2: Pure compost (WHC only)
- E3: 600 g WHC + 12 g CM
- E4: 600 g WHC + 24 g CM
- E5: 600 g WHC + 48 g CM
- E6: 1200 g WHC + 12 g CM
- E7: 1200 g WHC + 24 g CM
- E8: 1200 g WHC + 48 g CM
- E9: 1800 g WHC + 12 g CM
- E10: 1800 g WHC + 24 g CM
- E11: 1800 g WHC + 48 g CM

The amendment mixtures were homogenized thoroughly and allowed to stabilize for seven days before planting. This design enabled a systematic assessment of how different combinations of WHC and CM influenced soil characteristics and plant growth. E2 (pure compost/WHC) was included to isolate the effect of the compost alone, while E1 (CP soil) served as the true unamended control.

Each treatment was repeated four times to make sure the results are reliable and to reduce differences caused by chance. Using four repeats is common practice in small-scale or pot experiments because it provides enough data for meaningful analysis while still being easy to manage. Although the study used shade nets and controlled conditions for the containers, some outside factors like changes in humidity, occasional insects, and microbes from the air or water might have affected the microbes in the soil. These minor influences could not be avoided in semi-controlled experiments but were minimized as much as possible.

The experiment was conducted from October 2023 to February 2024. Relative humidity inside the containers was maintained at approximately 60%. Daytime temperatures ranged from 30°C to 34°C, while nighttime temperatures ranged from 23°C to 25°C. All containers were arranged systematically under the shade net to ensure uniform environmental exposure across treatments, thereby supporting a fair comparison of plant growth and soil properties under simulated natural conditions.

2.3 Growth Study of Spinach

The study evaluated four growth parameters of spinach—root length, root diameter, above-ground height, and leaf area—to assess responses under soil degradation and rehabilitation conditions. Measurements were recorded 50 days after sowing (after that reproductive growth phase began), with four replicates per treatment. Pots were irrigated daily to maintain soil moisture at 60% field capacity, and soil pH was monitored concurrently. Root length, an indicator of soil structure and nutrient distribution, was measured using a ruler after gently uprooting the plants and cleaning the roots. Root diameter, which reflects nutrient absorption efficiency and anchorage strength, was measured at the midpoint of the primary root using a digital caliper. Above-ground height was measured from the soil surface to the shoot apex using a measuring tape. Leaf area was calculated by tracing the largest fully expanded leaf onto graph paper.

A two-way ANOVA (performed in R v 4.3.2) was used to test whether individual treatments or their interaction significantly affected growth parameters. This method is appropriate when testing two independent factors at multiple levels, improving the robustness of interpretation compared to single-factor analysis.

Biometric results were visualized using matrix-based heatmaps generated with the “pheatmap (v1.0.12)” package in R. Color gradients (dark to light shades) represented variable magnitudes and patterns across treatments. These visualizations aided interpretation of multiple variable interactions in spinach growth.

2.4 Sample collections, preparation and physiochemical characterization of soil

To assess the impact of organic amendments on soil health, samples were collected from the top 0–15 cm layer, representing the spinach root zone, which plays a vital role in nutrient absorption and plant vitality. For each treatment, four replicates (n = 4) were collected using a clean auger and homogenized to form composite samples. The collected soils were air-dried, sieved through both 2 mm and 0.2 mm mesh, and stored in polyethylene bags until analysis. The coarse fraction (≤2 mm) was used for physicochemical characterization, while the fine fraction (≤0.2 mm) was preserved at −20°C for microbial isolation and amplicon sequencing.

All treatments were applied uniformly, and sampling was conducted for physicochemical and microbial analyses was done 50 days after sowing to assess amendment-induced changes during spinach growth. This approach aimed to reveal how WHC and CM amendments influence microbial diversity and soil fertility for improved crop productivity and sustainability. Measurements were performed in quadruplicate (depending on the assay), and values were calculated as mean ± standard error (SE). Calibration standards and blanks were included to ensure analytical precision and accuracy, and all reagents used were of analytical grade.

Soil quality was assessed through analysis of physical and chemical properties. Bulk density and porosity were measured following Sobek (1978) to evaluate soil compaction and water-holding capacity. Soil pH was determined at a 1:2

soil-to-water ratio using a calibrated digital Hanna HI5522 meter, based on Thomas,1996. Nutrient availability was assessed by measuring total nitrogen (Bremner, 1996), available phosphorus (Olsen, 1954), and potassium (Chapman and Goldsmith, 1982), indicating soil fertility status.

WHC (600–1800 g) and CM (12–48 g) application rates were optimized using Response Surface Methodology (RSM) in Design-Expert software, version13. A Central Composite Design (CCD) was employed to model the interaction between WHC and CM through a quadratic polynomial equation. Among the tested combinations, Treatment E8 was identified as optimal, corresponding to 1184.17 g WHC and 41.72 g CM—closely matching the 1200:48 (WHC:CM) ratio. This combination yielded a predicted maximum leaf area of approximately 225 cm², which was validated under controlled container conditions (Parveen and Ghosh, 2024). This method effectively evaluated the influence of WHC and CM on soil physicochemical properties and spinach growth to guide optimization of amendment.

E1 (CP soil, i.e., untreated control from the college premises) was used to establish baseline soil properties, while E8 (treated with 1200 g WHC + 48 g CM) represented the optimized treatment for leaf area response, selected based on RSM and CCD results.

2.5 DNA Extraction and Sequence Analysis

2.5.1 DNA Extraction

DNA was extracted from CP and OCM (optimized amended) soil samples using the DNeasy PowerSoil Kit (Qiagen), following the manufacturer's May 2017 protocol. For each treatment, four replicate samples were processed individually, with 0.25 g of soil per replicate added to separate PowerBead Tubes. Mechanical lysis was performed via bead-beating at 6.5 m/s for 10 minutes to disrupt microbial cells. After centrifugation at 10,000 × g for 1 minute, the supernatant was transferred to a clean tube. Reagents C2 and C3 were added sequentially, followed by vertexing and incubation at 4°C to precipitate inhibitors and non-DNA materials. A second centrifugation at 10,000 × g clarified the solution.

The resulting supernatant was mixed with binding solution C4 and transferred to a spin filter for DNA binding. This was centrifuged at 10,000 × g for 1 minute. The DNA bound filter was washed twice with ethanol-based washes using solution C5. DNA was eluted by adding 100 µL of buffer C6, followed by a 1-minute incubation and a final centrifugation step.

Following extraction, DNA from the four replicates per treatment was pooled to ensure sufficient quantity and to represent the treatment as a composite sample. Separate pooled DNA samples were prepared for bacterial 16S rRNA and fungal ITS sequencing analyses. The purified DNA was stored under -20°C for further downstream sequencing.

2.5.2. 16S rRNA and Fungal ITS Gene Sequencing and Analysis

2.5.2.1 Bacterial Community Analysis

The bacterial community was profiled by targeting the V3–V4 region of the 16S rRNA gene using primers 341F and 805R. Sequencing was performed on the Illumina platform (QBiogen Lab, Chennai), ensuring high-throughput and accuracy. To maintain data integrity, technical replicates and negative controls (blank DNA extractions) were included for quality assurance. Sequence identification and taxonomic assignment were performed using SILVA reference alignment and taxonomy within the QIIME pipeline. Only those sequences with ≥97% similarity to known type strains were retained for further analysis.

2.5.2.2 Fungal Community Analysis

Fungal diversity was assessed by amplifying the Internal Transcribed Spacer (ITS) region using primers ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS-2043R (GCTGCGTTCTTCATCGATGC).

Amplicons were sequenced on the Illumina platform, consistent with bacterial community profiling. Taxonomic assignments were made at the genus or species level by comparing representative OTU sequences against the UNITE database (v8.3) (Abarenkov *et al.*, 2020) using QIIME's Mothur method with a confidence threshold of 0.8 to 1. The UNITE database was selected for its comprehensive, curated fungal ITS reference sequences, optimized for fungal taxonomy.

Alpha diversity metrics (Shannon and Simpson indices) and beta diversity (Bray-Curtis dissimilarity) were calculated for both bacterial and fungal communities.

2.6. Bioinformatics and Data Analysis

Sequencing data were processed using QIIME (v1.7.0), a widely used platform for microbial community analysis. Initial preprocessing—including feature table construction, quality filtering, and error correction—was carried out using the DADA2 pipeline, which denoises raw reads to produce high-quality, biologically informative sequences. To investigate microbial community composition, Principal Coordinate Analysis (PCoA) was performed. This multivariate technique reduced dimensionality to visualize microbial clustering across treatments, revealing compositional differences between CP and OCM soils. Additionally, a phylogenetic tree was constructed to explore evolutionary relationships among the identified taxa, offering deeper insight into diversity patterns. Sankey diagrams were generated in R using the dplyr (v 1.1.4)**,** networkD3 (v 0.4.1)**,** webshot (v 0.5.5)**, and** htmlwidgets (v 1.6.4) packages to illustrate taxonomic transitions from phylum to genus level. The widths of the connecting bands represented relative taxon abundance, highlighting dominant microbial groups and ecological shifts across treatments. Together, this analytical workflow offered a comprehensive view of microbial diversity, community dynamics, and functional roles, effectively linking taxonomic changes to soil health outcomes.

3 Results

3.1 Soil Physicochemical Properties

The application of compost and manure significantly improved the physical and chemical properties of the soil (OCM) compared to the CP soil. The results are presented in Table 1. Statistical analysis (ANOVA) showed significant differences ($p < 0.001$) between CP and OCM soils, with highly significant improvements in all parameters except pH.

Table 1: Soil physicochemical properties of CP and OCM soil.

Soil Type	**Bulk Density** ($g\ cm^{-3}$)	**Porosity (%)**	**pH**	**N (mg/kg)**	**P (mg/kg)**	**K (mg/kg)**
CP	1.194[a]	45.28[a]	7.2[b]	357.8[a]	23.60[a]	110[a]
OCM	0.825[a]	68.87[a]	7.2[b]	1120[a]	106[a]	143[a]

[a] difference between CP and OCM soil for each parameter (p<0.001), and [b] indicate non-significant differences.

3.2 Investigation of Biometric parameters in spinach

Heatmap analysis revealed distinct growth patterns in leaf area, root length, root breadth, and plant height across treatments (Fig. 1). Treatments with higher WHC levels (1200 g–1800 g) consistently outperformed others, with E8 (1200 g WHC: 48 g CM) and E10 (1800 g WHC: 24 g CM) achieving the highest leaf area and root length values. Root breadth also improved significantly under these treatments, reflecting the synergistic effect of WHC and CM on lateral root expansion. The tallest plants (36.00 cm) were observed in E8, emphasizing the importance of balanced organic amendments for optimal growth. Two-way ANOVA revealed that WHC had a highly significant effect ($p < 0.001$) on all spinach growth parameters, while CM and their interaction had variable significance. Tukey's post hoc test confirmed that higher WHC levels led to significant pairwise differences, reinforcing WHC's dominant effect. Root breadth showed limited sensitivity to CM, with fewer significant pairwise contrasts.

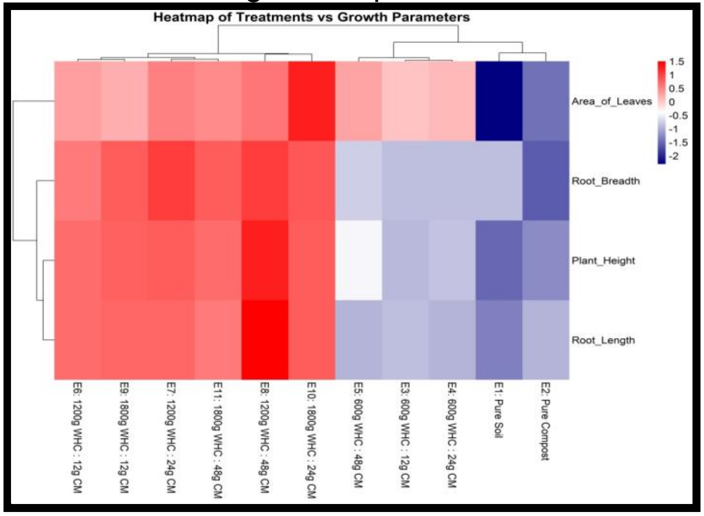

Figure.1: Heatmap showing the performance of various treatments across the growth parameters. Darker shades in the heatmap correspond to higher biometric values, highlighting E8 and E10 as top-performing treatments.

3.3 Comparative Analysis of Soil Microbial Diversity: Differences in Bacterial and Fungal Diversity Metrics

3.3.1 Amplicon Sequencing and Data Quality

The sequencing data from both CP and OCM soil samples were analyzed for quality metrics. After quality filtering and chimera removal, 130,339 and 76,432 high-quality bacterial reads and 182,631 and 164,760 high-quality fungal reads were retained for CP and OCM samples, respectively (Table 2). The Q30 scores exceeded 93% for all samples, indicating excellent base-calling accuracy and minimal sequencing errors. High Q30 values (>95%) ensure confidence in downstream taxonomic classification. GC content ranged from 54.5% to 57.6%, which is typical for soil microbial communities and supports sequence stability. These quality metrics ensure the robustness of downstream diversity comparisons between OCM and CP soils, thereby supporting reliable interpretation of WHC/CM effects on microbial diversity.

Table 2: Sequencing quality metrics for bacterial and fungal soil samples from CP and OCM soils.

Soil Sample	Raw PE (paired-end reads)	Combined (Number of reads merged and passed initial filtering)	Qualified (Number of high-quality reads after filtering (ambiguous bases, length)	Nochime (Number of chimera-free reads)	Base (nt) Total nucleotides in all qualified reads	Avg Length (nt) Average read length of qualified sequences	Q20%	Q30%	GC%
Bacterial sample									
CP	152979	151786	146365	130339	55438116	425.34	97.83	93.27	54.77
OCM	204661	202631	199316	76432	31736937	415.23	98.67	95.30	57.62
Fungal sample									
CP	217131	191978	189927	182631	51079231	279.69	98.84	95.95	54.53
OCM	205299	203805	202832	164760	52335314	317.65	99.23	96.95	55.50

3.3.2 Alpha and Beta Diversity of Microbial Communities in CP and OCM Soils

Alpha diversity analysis (Table 3) revealed marked differences between CP and OCM soils. Bacterial communities in OCM showed elevated values with Observed Species number 2,962, Shannon index of 9.411, Simpson index of 0.995, Chao1, ACE, and PD Whole Tree, indicating greater diversity and evenness than in CP. Conversely, fungal alpha diversity was slightly higher in CP, based on Shannon and PD Whole Tree values, suggesting domain-specific variation in community complexity. These indices provided a comprehensive view of species richness and evenness, and Good's Coverage values near 1.0 confirmed sufficient sequencing depth across samples. Beta diversity (Table 4), assessed using Bray–Curtis (abundance-based) and Jaccard (presence–absence) indices, further demonstrated distinct microbial compositions between CP and OCM soils. For bacteria, dissimilarity was notably high (Bray–Curtis: 0.9877; Jaccard: 0.9679), reflecting major shifts in abundance and composition due to amendments. Fungal communities also differed considerably (Bray–Curtis: 0.8038; Jaccard: 0.811), indicating that WHC and CM reshaped both bacterial and fungal populations.

Table 3: Alpha diversity metrics for bacterial and fungal soil samples from CP and OCM soils*.

Soil Sample	Observed Species	Shannon	Simpson	Chao1	ACE	Good's Coverage	PD Whole Tree
Bacteria sample							
CP	427	4.068	0.800	436.130	433.431	1.000	65.239
OCM	2962	9.411	0.995	3103.923	3141.060	0.994	236.345
Fungal sample							
CP	899	6.855	0.964	899.000	899.000	1.000	355.056
OCM	937	5.889	0.944	970.693	972.701	0.999	226.092

*Alpha diversity indices were calculated from a composite sample of pooled DNA (from four replicates per treatment); therefore, statistical comparisons were not included.

Table 4: Pairwise dissimilarity among bacterial & fungal communities based on Bray–Curtis (abundance-based) and Jaccard (presence–absence) indices.

	CP	**OCM**
Bacteria sample		
CP	**0** / *0*	**0.9877** / *0.9679*
OCM	**0.9877** / *0.9679*	**0** / *0*
Fungal sample		
CP	**0** /0	**0.8038** /0.811/
OCM	**0.8038** /0.811	**0** /0

*Note: **Bray-Curtis** in bold / *Jaccard* dissimilarity in italics.

3.4 Microbial Composition at different taxonomic Level

3.4.1 Phylum Level Shifts:

Microbial community composition at the phylum level (Figures. 2 and 3) revealed significant shifts between CP and OCM soils. Among bacteria, Actinobacteriota increased markedly from 6.13% in CP to 36.07% in OCM, becoming the dominant phylum in amended soil. Meanwhile, Proteobacteria and Firmicutes, initially dominant in CP (50.2% and 35.49%, respectively), declined substantially in OCM to 30.49% and 4.32%. Other enriched phyla in OCM included Acidobacteriota (0.08% to 6.11%) and Myxococcota (0.17% to 5.23%).

In the fungal community, Ascomycota remained dominant but increased from 78.19% in CP to 83.39% in OCM. In contrast, Basidiomycota decreased sharply from 13.14% to 3.31%. Notably, Rozellomycota and Mortierellomycota also increased in abundance—rising from 3.75% to 6.61% and 0.63% to 4.64%, respectively. These taxonomic changes suggest that the application of organic amendments enhanced soil microbial diversity and shifted community structure toward more functionally diverse taxa.

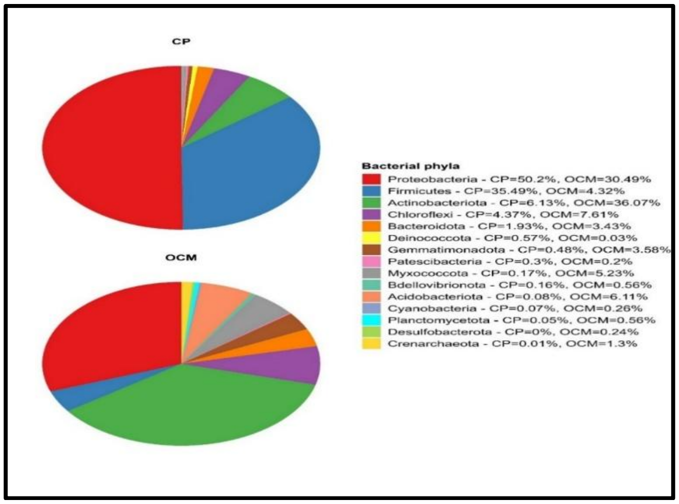

Figure 2: Distribution of bacterial phyla in CP and OCM soils.

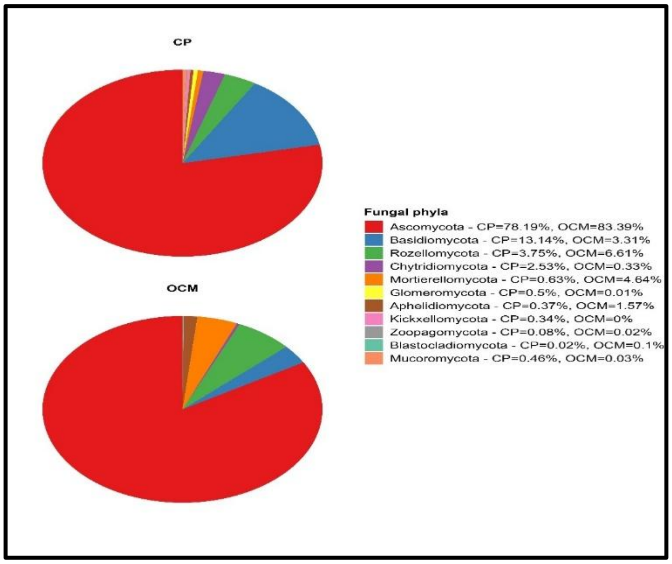

Figure 3: Distribution of fungal phyla in CP and OCM soils.

3.4.2 Order Level Shifts:

At the order level (Figures 4 and 5), Enterobacterales (43.62%) and Bacillales (30.36%) dominated in CP soil but were nearly absent in OCM at 0% and 1.80%, respectively. In contrast, OCM soils showed strong enrichment of Rhizobiales (10.51%) and Solirubrobacterales (5.58%), which were negligible or undetected in CP (0.36% and 0.03%, respectively). This major shift suggests that organic amendments substantially restructured the dominant bacterial orders, favouring beneficial soil bacteria.

Eurotiales and Sordariales were the most abundant fungal orders in both samples but showed higher representation in OCM (Eurotiales: 29.39%, Sordariales: 20.62%) compared to CP (24.37% and 10.35%, respectively). Additionally, Glomerellales increased from 1.22% in CP to 7.68% in OCM. These shifts reflect a transition toward a more functionally diverse and possibly more symbiotic fungal community following amendment application.

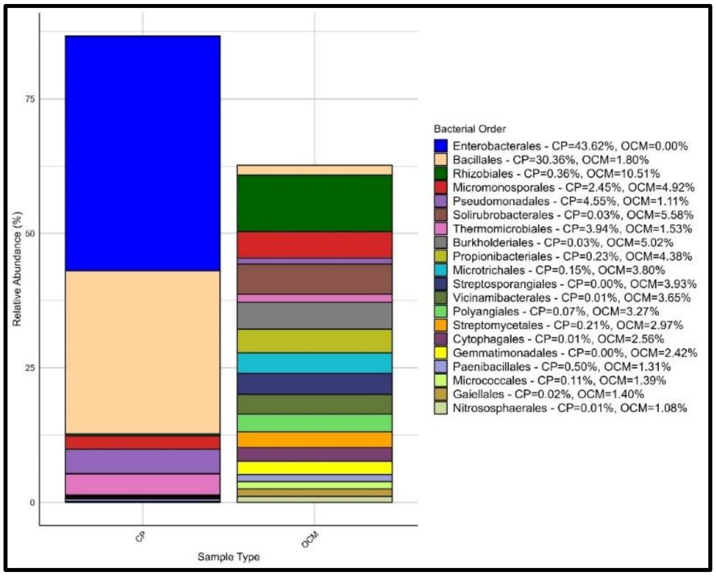

Figure 4: Distribution of bacterial orders in CP and OCM soils.

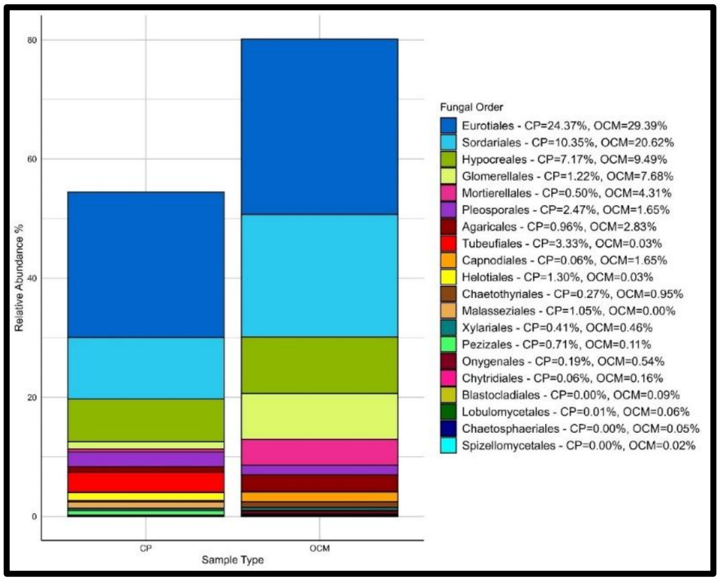

Figure 5: Distribution of fungal orders in CP and OCM soils.

3.4.3 Family Level Shifts:

At the bacterial family level (Figure 6), Bacillaceae dominated in CP soils (16.72%) but sharply declined in OCM (1.73%), while families like Nocardioidaceae (0.02% to 3.85%) and Nitrosomonadaceae (0% to 2.77%) showed remarkable enrichment in OCM. Micromonosporaceae increased from 2.45% in CP to 4.92% in OCM, indicating a shift toward Actinobacteria-associated families following amendment. Families such as Solirubrobacteraceae, Gemmatimonadaceae absent in CP, appeared prominently in OCM, reflecting enhanced microbial diversity.

In the fungal community (Figure 7), Aspergillaceae was dominant in both soils but increased from 24.37% in CP to 27.01% in OCM. Notably, Plectosphaerellaceae showed a substantial rise from 0.96% to 7.56%, while Mortierellaceae also increased (0.50% to 4.30%), highlighting a favorable fungal shift in amended soils. Families like Trichocomaceae and Bionectriaceae, absent in CP, were uniquely present in OCM, indicating amendment-induced changes in fungal composition that likely support improved soil health and soil ecological function.

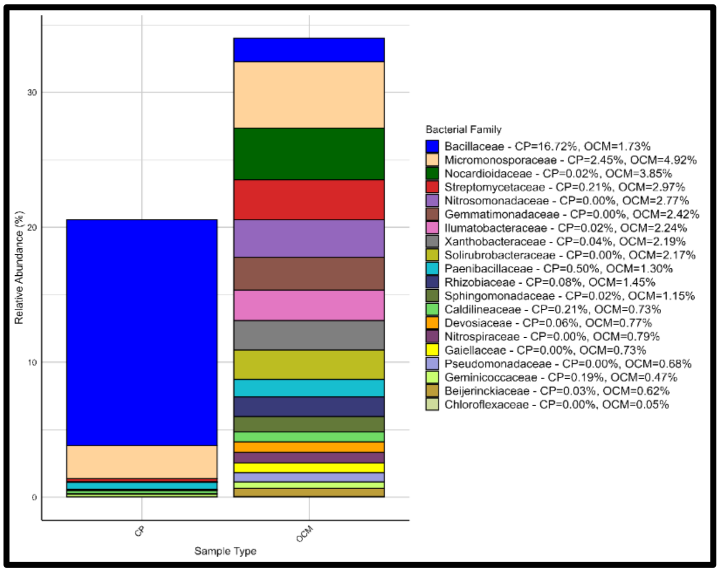

Figure 6: Distribution of bacterial families in CP and OCM soils.

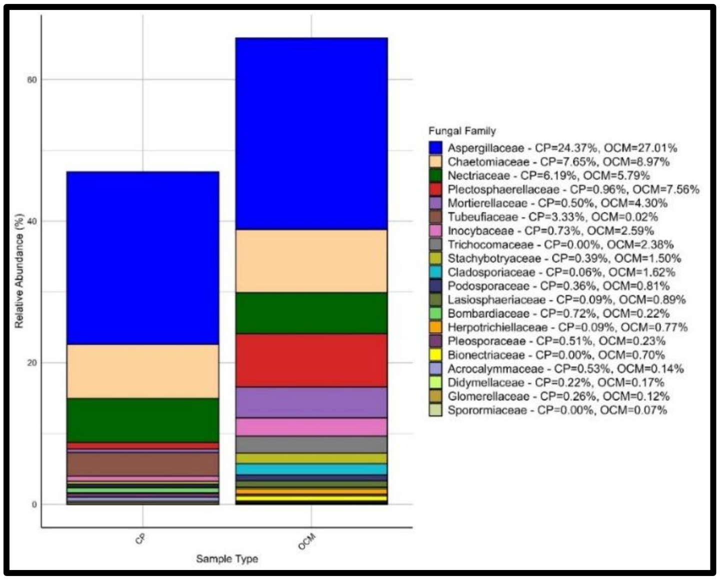

Figure7: Distribution of fungal families in CP and OCM soils.

3.4.4 Genus-Species Level Shifts:

Among the identified bacterial species in CP, *Bacillus mannanilyticus* (1.62%) and *Lysinibacillus* sp. YS11 (1.59%) were the most abundant, both derived from soil environments, indicating the dominance of native soil microbiota. *Micromonosporaceae* bacterium, also soil-associated, ranked third in abundance (1.12%), reinforcing the prevalence of soil-derived bacterial taxa. Among the fungal taxa, *Penicillium* sp. was most dominant (16.58%), followed by *Amphinema* sp. (4.21%) and *Aspergillus piperis* (3.84%).

In OCM, *Actinomadura keratinilytica* dominated the bacterial community (34.45%), followed by *Bacillus cellulasensis* (7.00%) and *Archangium gephyra* (5.57%). Presence of these taxa were reflecting shifts in bacterial composition under amended conditions. The fungal community was led by *Aspergillus* sp. (21.48%), followed by *Sordariales* sp. (10.45%) and *Plectosphaerellaceae* sp. (7.48%). These shifts indicate clear changes in both bacterial and fungal assemblages between CP and OCM soils.

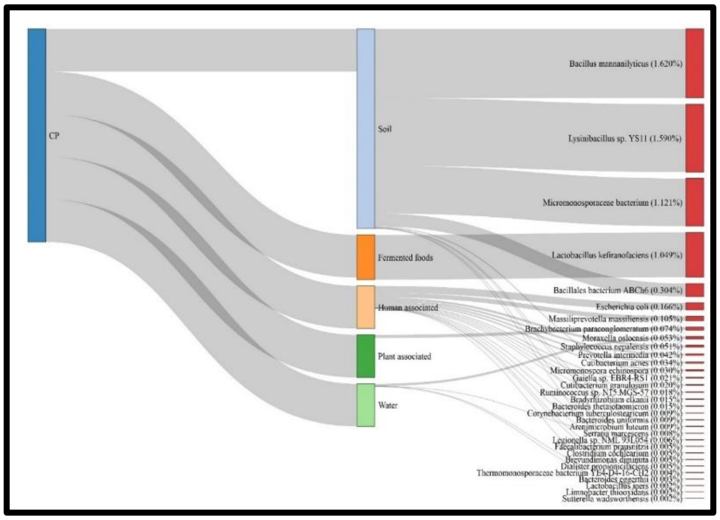

Figure 8: Sankey diagram of highly abundant bacterial species (≥0.1% relative abundance) in CP soil, highlighting the most prominent species.

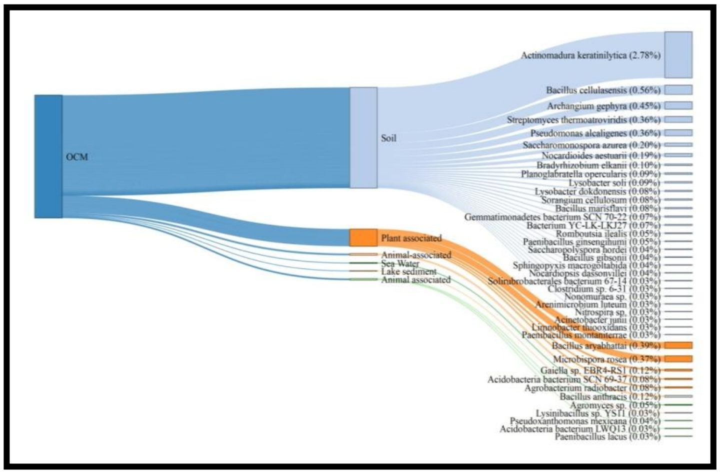

Figure 9: Sankey diagram of highly abundant bacterial species (≥0.1% relative abundance) in OCM soil, highlighting the most prominent species.

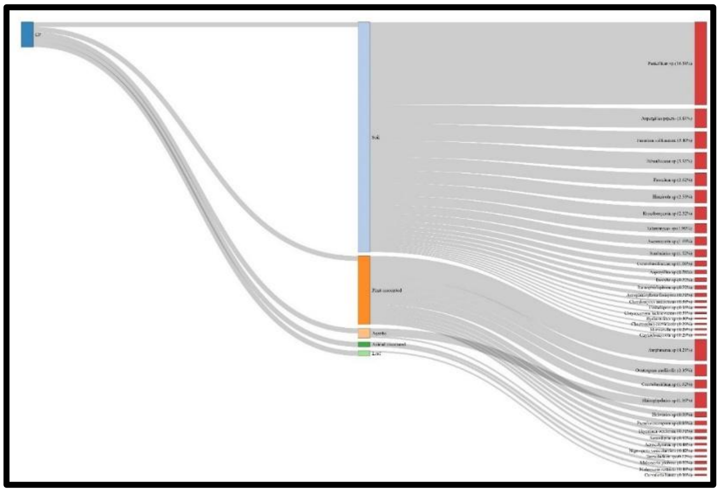

Figure 10: Sankey diagram depicting the highly abundant fungal species (≥0.2% relative abundance), representing the most prominent fungal species in CP soil samples.

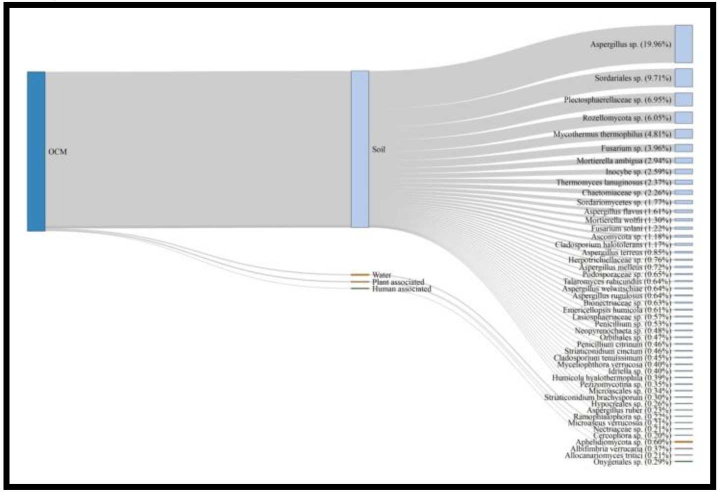

Figure 11: Sankey diagram depicting the highly abundant fungal species (≥0.2% relative abundance) in OCM soil, highlighting the most prominent species.

4.Discussion

The application of compost in OCM soil significantly reduced bulk density and increased porosity and nutrient levels compared to CP soil (Table 1), reflecting improved soil structure and enhanced nutrient availability—conditions favorable for microbial activity and plant growth. This indicates that compost amendments help alleviate compaction and improve the quality of degraded sandy loam soils. These observations are consistent with prior studies (Lal, 2021; Smith *et al*., 2015; Six *et al*., 2002), which reported that organic inputs enhance aeration and nutrient dynamics through slow nutrient release and stimulation of microbial communities. The improvements are likely driven by presence of labile organic matter from compost and manure, promoting aggregation and supporting microbially mediated nutrient cycling. Overall, these findings underscore the value of compost and manure in restoring soil fertility, structure, and resilience, thus advancing plant health and supporting global efforts to combat land degradation through sustainable soil management (Ozores-Hampton *et al*., 2011).

Heatmap analysis (Fig. 1) showed that the combined application of water hyacinth compost (WHC) and chicken manure (CM), particularly under the E8 treatment (1200 g WHC + 48 g CM), significantly improved spinach biometric traits, including leaf area, root length, root width, and plant height. The superior performance under E8 suggests a synergistic effect of balanced organic amendments in enhancing vegetative growth and biomass accumulation. These results support the findings of Hammam *et al*. (2022), who observed enhanced barley growth in sandy and clayey loam soils following application of water hyacinth-derived biochar and poultry manure, reinforcing the efficacy of aquatic plant-based composts and poultry manure across soil and crop types. The observed improvements likely result from enhanced nutrient availability, better aeration, and stimulated microbial activity under optimal WHC-CM ratios, promoting root development, nutrient uptake, and foliar expansion. Agronomically, these results highlight the potential of integrated organic inputs to boost crop productivity and soil health in degraded soils. Future research should validate these outcomes in field conditions and assess long-term effects on soil–plant–microbe interactions.

While the experiment was conducted under semi-controlled conditions using container-based trials, the consistent and statistically significant improvements observed in plant growth and soil health metrics suggest strong potential for field-scale application. However, field conditions introduce additional variables such as rainfall, soil heterogeneity, pest dynamics, and fluctuating temperatures that were minimized in this study.

Therefore, further field-based validation is essential to assess the scalability and stability of WHC and CM effects under real agricultural settings. The findings offer a promising foundation for integrated nutrient management strategies aimed at rehabilitating degraded soils.

Amplicon sequencing revealed that organic amendments significantly influenced microbial community dynamics, with higher bacterial abundance and diversity in OCM soils compared to CP, indicated by increased raw and qualified reads and elevated GC content. This suggests that bacterial communities responded positively to nutrient enrichment from compost, shifting toward more complex or stress-tolerant taxa. These findings align with Shen *et al*. (2023), who reported deterministic restructuring of bacterial communities following organic inputs, and support Siedt *et al*. (2024), who observed limited fungal responses due to stochastic assembly and substrate specificity. The modest fungal changes observed here are consistent with the relative resilience or selectivity of fungal communities under compost influence. These results highlight the utility of amplicon sequencing for detecting taxonomic shifts induced by soil amendments and suggest the need to integrate this with functional gene and enzymatic assays to better link microbial composition to ecosystem services such as nutrient cycling and plant health.

Organic amendments substantially increased bacterial diversity, with higher species richness, Shannon diversity, and phylogenetic diversity in OCM soils (Table 3). In contrast, fungal communities showed only a modest increase in species richness, with decreases in Shannon and phylogenetic diversity, indicating reduced evenness and evolutionary breadth. This implies that while bacterial communities became more diverse and evenly distributed, fungi experienced dominance by a few taxa. The increase in bacterial α-diversity supports previous findings by Cesarano *et al*. (2017), while the decline in fungal evenness mirrors observations by Chen *et al*. (2022), suggesting niche specialization or competitive exclusion. Enhanced nutrient availability and improved microhabitats likely stimulated bacterial proliferation, whereas fungi underwent selective filtering. These findings stress the importance of recognizing taxon-specific responses to amendments when designing strategies to support microbial diversity and agroecosystem resilience.

Alpha diversity analysis showed that compost and manure amendments (OCM) notably increased the diversity and evenness of bacterial communities compared to untreated soil (CP), while fungal diversity was slightly higher in CP, indicating domain-specific responses to organic inputs. These findings align with previous studies demonstrating that organic amendments promote bacterial richness by enhancing nutrient availability, though fungal communities may be less responsive or undergo competitive shifts (Pantelides *et al*., 2023). Beta diversity analysis, using Bray–Curtis and Jaccard indices, revealed substantial differences in both bacterial and fungal community composition between treatments, confirming that organic amendments strongly reshape soil microbiomes. These results highlight that targeted compost and manure applications can enhance microbial diversity and reconfigure community structure, supporting improved soil health and ecosystem resilience.

Organic amendments also induced notable shifts in bacterial taxonomic composition (Figures 2). The addition of organic amendments alters soil nutrient availability and environmental conditions, leading to changes in bacterial community structure. The results indicate a reduction in the abundance of Proteobacteria and Firmicutes, both fast-growing copiotrophs that thrive in environments rich in simple organic substrates. In contrast, there is an increased abundance of Acidobacteriota and Chloroflexi, which are oligotrophs adapted to utilize low nutrient levels and more complex substrates over longer periods. Some copiotrophs, such as Actinobacteriota, may also increase due to their ability to degrade complex organic matter. These shifts suggest that the supplied organic amendments provide nutrients slowly and contain complex organic compounds. Overall, these changes promote microbial communities that enhance long-term nutrient retention and support plant growth. These trends align with findings by Dincă *et al*. (2022)

and Lazcano *et al*. (2013), who reported increased abundance of nutrient-cycling taxa and decreased copiotroph dominance following compost application, supporting the beneficial role of compost in improving soil microbial ecology and health. The enrichment of Rhizobiales, Burkholderiales, and Nitrososphaerales in OCM indicates improved nitrogen fixation, phosphate solubilization, and nitrification capacity, corroborating findings by Compant *et al*. (2010) and Tourna *et al*. (2011). These changes are likely driven by improved substrate quality and nutrient input from chicken manure. Collectively, the restructuring of microbial communities enhances nutrient cycling, pathogen suppression, and soil structure—essential for sustainable crop production and soil health.

Significant taxonomic restructuring of Bacillales and Actinobacteria was also observed. CP soil was dominated by Bacillales (30.36%), particularly Bacillaceae (16.72%), whereas OCM supported a more taxonomically diverse but less abundant Bacillales population. Additionally, Actinobacterial families like Nocardioidaceae (3.85% in OCM vs. 0.02% in CP), Streptomycetaceae, and Micromonosporaceae were enriched in OCM. This suggests a transition from dominance by a few taxa to a more functionally redundant and ecologically stable microbial community under organic amendments. These findings are consistent with Yu and Whalen, 2020 and Shu *et al*. (2022), who reported enhanced microbial richness and niche saturation due to increased resource availability. Improved nutrient status and reduced compaction in OCM likely favored a broader set of decomposer taxa, supporting organic matter degradation and nutrient cycling (Yeager *et al*., 2017). Enrichment of taxa such as Nocardioides, Streptomyces, and Saccharomonospora suggests enhanced soil functional capacity and microbial-mediated fertility restoration.

Organic amendments also encouraged the growth of beneficial microbes in OCM soil, such as Pseudomonas alcaligenes, while suppressing potentially harmful, human-associated pathogenic microbes. These changes were associated with disease-free spinach growth and a reduced or absent presence of human pathogens. This indicates that organic inputs support microbial assemblies that enhance plant health. The suppression of *Enterobacterales* (43.62% in CP but absent in OCM), alongside enrichment of *Actinobacteriota* (36.07%) and *Rhizobiales* (10.51%), is in agreement with Trivedi *et al*. (2016) and Wang *et al*. (2018), who observed similar beneficial shifts in microbiomes under organic management. The presence of *Pseudomonas alcaligenes* supports findings by Akhtar and Siddiqui, 2010 regarding its roles in pathogen suppression and plant growth promotion. These shifts likely arise from competitive displacement and altered resource dynamics induced by organic amendments. Scientifically, these results highlight how compost and manure enhance nutrient cycling, strengthen plant–microbe interactions, and reduce potential health risks, thereby improving soil health and supporting long-term agricultural sustainability (Renaut *et al*., 2019).

Despite shifts in abundance, a subset of microbial species—including *Lysinibacillus* YS11, *Bradyrhizobium elkanii*, *Gaiella* sp. EBR4-RS1, and *Limnobacter thiooxidans*—was consistently detected in both CP and OCM soils (Figure 8-9). This indicates the presence of a resilient core microbiome that persists across different soil management practices. These findings align with Pershina *et al*. (2018), who reported that soil microbiomes have conserved core microbes across different soil types. Similarly, Pantoja-Guerra *et al*. (2023) described plant-growth-promoting *Lysinibacillus*, and Wang *et al*. (2018) reported nitrogen-fixing *Bradyrhizobium* in agricultural soils. They are still present in amendment soil because they can survive in different environments and are capable of adjusting to changes in nutrients and competition. Scientifically, the persistence of core taxa with key functional traits suggests that vital ecosystem services such as nitrogen and sulfur cycling can be sustained or enhanced under organic amendments, contributing to soil microbiome resilience and long-term soil health.

Fungal communities also exhibited substantial compositional shifts in response to organic amendments (Figure 3). *Ascomycota* and *Mortierellomycota* were enriched in OCM soils, while *Basidiomycota* and *Chytridiomycota* declined, reflecting altered niche availability and substrate preferences. This trend suggests a shift toward decomposer-dominated communities that enhance organic matter degradation and nutrient cycling. These results are consistent with Ma *et al*. (2013) and Ozimek & Hanaka (2020), who reported similar fungal responses to organic inputs. The increased presence of functional fungi such as *Aspergillus*, *Mortierella*, *Rozellomycota*, and thermophiles like *Mycothermus thermophilus* and *Thermomyces lanuginosus* aligns with their known roles in biomass decomposition and nutrient mineralization (Charria-Girón *et al*., 2022; Singh *et al*., 2000; Pan *et al*., 2024). These changes are likely driven by enhanced carbon availability and thermal niches in OCM soils, which favor decomposers and symbionts over lignin-degrading or aquatic fungi. The increased abundance of beneficial fungi, including mycorrhizal species such as *Inocybe* and *Ceratobasidium*, suggests improved nutrient acquisition and soil fertility under organic management (Durán-López *et al.,* 2019). Collectively, these findings reinforce the role of organic amendments in fostering diverse and ecologically functional fungal communities that support sustainable soil health.

5. Conclusions

This study demonstrates that organic amendments like WHC and CM enhance soil fertility. By reducing bulk density and increasing porosity, these amendments create a more favorable environment for root growth, enhancing the transport of nutrients and water. Organic inputs enhance the levels of plant macronutrient such as nitrogen, phosphorus, and potassium, which are essential for optimal plant development. This research specifies that the WHC (1200 g) and CM (48 g) in 6 kg of soil per container as ideal formulation of organic inputs. An effective combination of organic inputs improves spinach biometric traits and promotes greater microbial diversity, particularly within the bacterial community. These microorganisms play a critical role in supporting soil health and enhancing spinach productivity by facilitating nutrient cycling and decomposing organic matter. This study focuses exclusively on sandy loam soil and spinach. Further research across different soil types and crops is necessary to optimize the use of these amendments in diverse farming systems. These findings suggest that such amendments may support food production and improve soil resilience in sustainable systems.

Conflict of interest:

The authors declare no conflict of interest.

Funding:

This work did not receive any funding.

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