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16S Long-Read Metabarcoding in Field Conditions Uncovers Compost-Driven Modulation of Rhizosphere Bacterial Communities During Red Bell Pepper Development

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Abstract

Organic amendments are a sustainable alternative to mineral fertilizers, but their effectiveness depends strongly on the mode of application. The spatial placement of compost can shape soil nutrient dynamics and the assembly of beneficial microbial communities. Here, we investigated how two strategies—surface broadcasting versus deep banding of green-waste compost—affect soil physicochemical properties and the rhizosphere bacterial community of red bell pepper (*Capsicum annuum* L.) across two developmental stages (maturation and ripening). To capture these dynamics with high resolution, we applied Nanopore long-read sequencing of the full-length 16S rRNA gene, enabling precise taxonomic assignments and improved functional predictions compared to short-read approaches.

Deep banding consistently outperformed surface broadcasting, significantly improving organic carbon, total nitrogen, and nitrate content, particularly at ripening. These soil changes were tightly linked to shifts in rhizosphere microbiota, with deep banding inducing a distinct community composition and selectively enriching nitrogen-associated genera such as *Azoarcus*, *Alcaligenes*, and *Ochrobactrum*. Functional predictions revealed an enhanced potential for nitrogen cycling pathways, including nitrate reduction and nitrogen respiration.

Our findings demonstrate that deep compost banding not only enhances soil fertility but also engineers a functionally enriched rhizosphere. By integrating temporal sampling with long-read sequencing, this study provides a novel framework to assess how compost placement influences soil–plant–microbe interactions, offering a promising strategy for sustainable crop production.

Introduction

The rhizosphere represents a dynamic and complex interface shaped by multifaceted interactions among plant roots, soil physicochemical properties, and microbial communities (Francioli et al., 2018). These soil–plant–microbe interactions play a central role in regulating nutrient cycling, root physiology, and microbial community assembly, thereby exerting a profound influence on ecosystem functioning and agricultural productivity (Trivedi et al., 2020). The taxonomic composition and functional capacities of these microbial assemblages are critical determinants of plant nutrient acquisition, growth dynamics, and resilience to both biotic and abiotic stressors (Ullah et al., 2025). Environmental conditions and disturbances, including climatic variability and anthropogenic pressures, further modulate these interactions, reshaping microbial community structure and their functional contributions to ecosystem stability and plant performance (Jiao et al., 2019; Philipp et al., 2025). Among land management practices, fertilization strategies are particularly influential, as fertilizer inputs strongly affect edaphic parameters such as pH, nutrient availability, and soil moisture—factors that are closely linked to microbial community assembly processes within agroecosystems (Raimi et al., 2023).

Mineral fertilizers have played a pivotal role in boosting agricultural productivity and ensuring global food security. However, their overuse and mismanagement has contributed to environmental degradation and can disrupt soil–plant–microbe interactions that are fundamental to rhizosphere functioning (Porter & Sachs, 2020; Edlinger et al., 2022). Excessive inputs can reduce microbial diversity, alter community assembly processes, and weaken beneficial symbioses, thereby compromising soil health and long-term plant resilience (Tsiafouli et al., 2015; Huang et al., 2019; Waqas et al., 2025). This underscores the need for complementary and sustainable alternatives that sustain crop productivity while maintaining the ecological balance of soil–plant systems. In this context, the application of organic amendments, particularly compost, has garnered increasing attention as a sustainable alternative to synthetic fertilizers (de Rosa et al., 2017).

Compost enhances soil fertility by contributing organic matter and essential nutrients, and may additionally function as a biological inoculant that modulates microbial diversity and activity in the rhizosphere (Martínez-Blanco et al., 2013; Wei et al., 2024). However, the efficacy of compost in shaping microbial communities is contingent not only upon its chemical characteristics but also on the mode of its application. While surface broadcasting remains a common practice, subsurface placement via deep-banding has the potential to improve nutrient availability at the root–soil interface and promote targeted microbial colonization (Vido et al., 2024). Despite these promising attributes, comparative studies evaluating the influence of different compost application techniques on soil properties and rhizosphere microbiomes remain scarce.

Capsicum species rank among one of the world's most economically and nutritionally important vegetable crops (Bosland & Votava, 2012). Cultivated under a wide range of conditions—from open-field agriculture to highly controlled greenhouse systems—*Capsicum* is frequently subject to intensive management involving regular inputs of synthetic or organic fertilizers and drip irrigation. The

crop's high nutrient demand and sensitivity to soil conditions render it an exemplary model for investigating the impacts of compost amendments on rhizosphere microbial community structure and function (Li et al., 2019; You et al., 2025). A comprehensive understanding of how compost application modulates soil properties and rhizosphere bacterial communities in *C. annuum* is essential for devising sustainable management practices that optimize crop productivity. Furthermore, given that rhizosphere microbiota undergo dynamic shifts throughout plant development, it is imperative to assess microbial responses at multiple growth stages (Lewin et al., 2024). Existing literature predominantly focuses on single time-point analyses, thereby neglecting the temporal dynamics inherent in plant–microbe interactions. Nonetheless, variations in root exudate composition and nutrient requirements across developmental stages are well-documented drivers of microbial community composition and functional shifts (Nannipieri et al 2023; Yang et al., 2025).

Recent advancements in sequencing technologies, particularly long-read platforms such as Oxford Nanopore, show potential to improve the characterization of soil microbial communities. Enhanced accuracy, supported by improved basecalling algorithms and updated sequencing chemistries, now enables reliable full-length sequencing of 16S rRNA genes, overcoming the limitations of short-read approaches that capture only partial regions and offer limited phylogenetic signal (Aja-Macaya et al., 2025; Veselovsky et al., 2025). Full-length sequencing refines taxonomic resolution, allowing more precise identification of bacterial taxa down to the species level. This is especially critical in soils, where closely related species may differ in ecological function and many taxa remain poorly described. By reducing ambiguities in database matching, long-read sequencing strengthens both taxonomic and functional inference, providing a robust framework for linking microbial community composition to ecosystem processes. Despite these advances, its application to soil–plant–microbe interactions remains limited, underscoring the need for broader adoption (Francioli et al., 2021).

The present study examines the effects of three compost treatments—no compost (Control), surface-applied compost (Broadcast), and subsurface compost (Deep-banding)—on the rhizosphere bacterial communities of *C. annuum* var. Fritz G740 (red bell pepper) across two developmental stages: maturation and ripening. Oxford Nanopore sequencing of the full-length 16S rRNA gene was employed to characterize the bacterial communities in the collected samples. In parallel, we analyzed the influence of compost application and delivery method on several soil physicochemical properties. We hypothesized that (i) compost amendments and their mode of application will differentially modify soil physicochemical properties; (ii) these changes in soil conditions will, in turn, shape bacterial community assembly and functional potential in the rhizosphere, with the magnitude and direction of the effects depending on the application method; and (iii) the influence of compost on both soil properties and microbiome dynamics will vary over time in accordance with plant developmental stage. Elucidating these interactions is essential for optimizing compost utilization in sustainable horticultural production systems.

Materials and Methods

Plant material and field experimental design

Seeds of *Capsicum annuum* var. Fritz G740 (red bell pepper) were obtained from Bingenheimer Saatgut AG (Germany). They were initially sown in a nutrient substrate under greenhouse conditions, and seedlings were transplanted to the experimental field at the eight-leaf stage. The field trial was conducted at Hochschule Geisenheim University, Geisenheim, Germany (49.9834° N, 7.9605° E). The site is characterized by a sandy loam soil, a mean annual precipitation of 527 mm, and a mean annual temperature of 11.0°C (long-term average from 1991 to 2020) (Wohlfahrt et al., 2022). The experiment was arranged in a randomized block design with three replicate plots per treatment. Treatments included: (i) surface compost application (30 t/ha; Broadcast), (ii) deep-banded compost application (30 t/ha; Deep-banding), and (iii) no compost application (Control). Each plot measured 12 m² (10 m × 1.2 m) and contained 44 red bell pepper plants, with 45 cm spacing between rows and plants. The compost, which consisted entirely (100%) of plant-based materials derived from gardening and landscaping activities (Table S1), was applied a few days before transplanting at a rate of 36 kg per plot (equivalent to 30 t/ha or 3 kg/m²). In the broadcast treatment, compost was evenly distributed over the soil surface and manually incorporated using a rake. In the deep-banding treatment, holes (15–20 cm depth) were dug with a spade, filled with the respective compost volume, and seedlings were transplanted directly on top of the compost-filled holes. Transplanting took place in the first week of May 2023. An organic slow-release fertilizer (Beckmann Phytoperls®N, 12% N total nitrogen, of which 8.1% NH4, 1.2% P_2O_5, 2.7% K_2O, 5.5% MgO, 14% S and 2.3% Na) was applied as a top dressing after transplanting. All plots received the same total fertilizer input, corresponding to 50 kg N/ha (504 g per plot) at transplanting, to cover the initial nutrient demand. A second top dressing with the same fertilizer was applied on 12 June 2023 at a rate of 80 kg N/ha.

Irrigation was managed based on a tensiometer threshold of − 200 hPa, following irrigation planning according to the Geisenheim irrigation model (Olberz et al., 2018). Rhizosphere soil was sampled at two key plant growth stages (PGS) of *C. annuum*: maturation (25 May 2023) and ripening (6 July 2023). From each plot, six randomly distributed soil cores were collected near the *C. annuum* stems (from 10 to 30 cm soil depth and 1.5 cm in diameter) using a sampling probe. The cores were then pooled to create one rhizosphere soil replicate. Soil samples were sieved through a 2 mm mesh and stored at 4°C for chemical analyses and at − 80°C for molecular analyses.

Soil parameters analysis

Total carbon (TC) and total nitrogen (TN) contents were determined in triplicate by dry combustion using an elemental analyzer (Vario MAX Cube, Elementar Analysensysteme, Germany). Given the negligible carbonate concentration (< 2%), TC was considered equivalent to organic carbon (Corg). NH_4^+-N and NO_3^--N were extracted from 10 g of fresh soil with 1 M KCl (1:4 w/v) by horizontal shaking for 1.5 h. After filtration of the suspension (Whatman Schleicher & Schuell 595 1/5, Ø 270 mm), the concentrations of NH_4^+-N and NO_3^--N in the clear extracts were determined using a flow injection analyzer (FIAstar 5000, Foss GmbH, Rellingen, Germany). The mineral nitrogen pool was dominated by NO_3^--N, while the proportion of NH_4^+-N was negligible and therefore not considered in the study. Soil pH was measured in 0.01 M $CaCl_2$, and electrical conductivity (EC) was measured using a conductivity meter (WTW, Cond 315i, Germany). Determination of total phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu) and Zinc (Zn) in the rhizosphere soil was performed via microwave digestion followed by analysis by Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES) iCAP 6300 Duo (Thermo Fisher Scientific).

DNA extraction, amplicon library preparation and sequencing

Total rhizosphere DNA was extracted from 0.30 g of soil using the DNeasy PowerLyzer PowerSoil Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The rhizosphere DNA was quantified using Qubit fluorimeter and Qubit dsDNA High Sensitivity kit (Invitrogen, 15860210). DNA purity was measured using the NanoPhotometer® NP80 (Implen, Westlake Village, CA, USA), with an acceptable 260/280 ratio ≥ 1.7. DNA extracts were normalized before amplification to a concentration of 10ng in 15µL of nuclease free water.

Bacterial community characterization was conducted as described by Francioli et al. (2021), and library preparation was performed using the Oxford Nanopore Technologies (ONT) 16S Barcoding All-in-One Kit (16S Barcoding Kit 24 V14; SQK-16S114.24). This kit employs primers targeting the full-length V1–V9 region of the 16S rRNA gene (primer sequences not disclosed by manufacturer). Amplicon library preparation was performed according to the ONT protocol (Rapid Sequencing DNA 16S Barcoding Kit V14, SQK-16S114.24; protocol available at: https://nanoporetech.com/document/rapid-sequencing-DNA-16s-barcoding-kit-v14-sqk-16114-24). In brief, PCR amplifications were performed in a total volume of 50 µL reaction mix containing 15 µL of soil DNA template solution containing 10ng input DNA, 25 µL of LongAmp Hot Start Taq 2X Master Mix (New England Biolab, MA, USA) and 10 µL of each 16S-Barcode. The thermal profile used for preparation of bacterial rDNA amplicon libraries was as follows: initial denaturation at 95°C for 1 min, 30 cycles of denaturation at 95°C for 20 s, annealing at 55°C for 30 s, and extension at 65°C for 120 s, followed by a final extension period at 72°C for 5 min. The amplicons produced were puriefied using AMPure XP Beads (Beckman Coulter, Brea, CA, United States), and processed according to the Rapid Sequencing DNA 16S Barcoding Kit V14 protocol to finalize the amplicon library production for each sample. Then, equimolar amounts of individual libraries were pooled, and the final library was loaded onto an R10.4.1 flow cell (FLO-MIN114). Sequencing was performed on a MinION device (Oxford Nanopore Technologies, Oxford, UK) available at the Department of Plant Breeding, Hochschule Geisenheim University. The sequencing run was conducted for 26 h 27 min, generating a total of 5.14 million reads and 7.94 gigabases. All sequences have been submitted to the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) under the BioProject accession PRJNA1333386.

Bioinformatic analysis

Oxford Nanopore Technologies (ONT) long reads were live-basecalled within MinKNOW version 24.11.10 using super-high accuracy basecalling for the ONT 16S sequencing kit (SQK-16S114-24). Using MinKNOW's default settings, reads with an error rate higher than 10% (Q-score < 10) were discarded and not considered for further analysis. After this filtering and sample assignment, we obtained 4.74 million reads totaling 7.23 gigabases. Reads were processed using the Nameco pipeline

(https://github.com/timyerg/NaMeco), which performs quality filtering, clusters highly similar reads, and generates a consensus sequence for each cluster to reduce sequencing errors inherent to Nanopore technology. These consensus sequences, hereafter referred to as Consensus Cluster Sequences (CSCs), represent full-length 16S rRNA gene sequences (covering V1–V9 regions) and serve as reliable taxonomic units for downstream analyses, including taxonomic classification and diversity analysis (Rodríguez-Pérez et al., 2021; Stock et al., 2024). Taxonomic assignment was conducted in QIIME2 (version 2024.5). Within QIIME2, the *scikit-learn* classifier and the pre-trained SILVA 138 (99% OTUs full-length sequences) database were used. All downstream data analyses were conducted using the microeco R package (version 1.10.0). First, low-abundance CSCs (frequency < 2) and CSCs not assigned to the kingdom *k__Bacteria* were removed. To ensure sequence uniformity for alpha diversity, rarefaction was performed at a standardized depth of 65312 sequences per sample, corresponding to the lowest sequencing depth among all samples (Table S2). This approach was validated using accumulation curves (Figure S1).

Data analysis and statistics

All statistical analyses were performed in R (version 4.3.3). To test differences in soil conditions among compost treatments, ANOVA assumptions were first verified, followed by ANOVA and Tukey's post hoc test ($p < 0.05$) for each plant growth stage (PGS). Normality was assessed using the Shapiro–Wilk and Jarque–Bera tests, while homogeneity of variance was examined with Levene's test. Variables that did not meet parametric assumptions were log10-transformed.

Bacterial alpha diversity was evaluated based on CSC richness and the Shannon–Wiener index. Differences in bacterial CSC richness and diversity were tested using ANOVA followed by Tukey's HSD post hoc test. Bacterial community structure was assessed across PGSs and compost treatments using Bray–Curtis dissimilarities, calculated after applying a Hellinger transformation (square-root transformation of relative abundances; Legendre and Gallagher, 2001). Permutational multivariate analysis of variance (PERMANOVA) based on Bray–Curtis dissimilarity was conducted with 999 permutations to test the effects of the experimental factors on bacterial community structure. A model of multivariate analysis of variance was constructed using distance-based redundancy analysis (db-RDA) based on the Bray–Curtis distance to determine the environmental variables that were most influential on the bacterial community structure. Linear Discriminant Analysis Effect Size (LEfSe; (Segata et al., 2011)) was applied to identify genera with differential abundance between compost treatments at each PGS. Functional potential related to each CSC was inferred using FAPROTAX (Louca et al., 2016). Finally, Pearson correlation analyses were performed between FAPROTAX-predicted functions and differentially abundant genera at both PGSs (maturation and ripening). These correlations were adjusted using FDR correction to account for multiple testing.

Results

Deep-banding compost application significantly altered edaphic properties

Compost addition, as well as the method of application (broadcast vs. deep-banding), significantly influenced specific soil physicochemical parameters. At maturation stage, compost addition affected only nitrate concentration, which was significantly higher under deep-banding treatment compared to the control (Table 1). By the ripening stage, however, the effects of compost application on soil properties became more pronounced. The control treatment exhibited significantly lower values of electrical conductivity (EC), total nitrogen (N), organic carbon (Corg) than the deep-banding treatment, which consistently showed the highest values for these parameters (Table 1). The broadcast treatment differed significantly from the control only in phosphorus concentration, presenting the highest values for this macronutrient, although not significantly different from the deep-banding treatment. Several variables, including nitrate, Ca, Mn, Fe, and Zn, displayed increasing trends at ripening with both compost application methods. In contrast, soil pH, the C/N ratio, and Cu did not exhibit consistent patterns associated with compost application at either plant growth stage (PGS) (Table 1).

Table 1
Edaphic properties of soil samples across compost treatments (Control, Broadcasted, Deep-banding) at red bell pepper maturation and ripening.

	Maturation			Ripening		
	Control	Broadcast	Deep-banding	Control	Broadcast	Deep-banding
pH	7.20 (0.18)	7.33 (0.14)	7.33 (0.15)	7.49 (0.09)	7.41 (0.08)	7.47 (0.12)
EC (µS/cm)	209.4 (39.03)	209.45 (15.13)	298.5 (59.12)	121.7 (10.23)^{b}	156.98 (14.52)^{ab}	181.97 (12.85)^{a}
Corg (%)	2.37 (0.65)	2.02 (0.43)	3.51 (0.85)	1.25 (0.19)^{b}	1.56 (0.16)^{ab}	2.79 (0.64)^{a}
TN (%)	0.13 (0.03)	0.13 (0.02)	0.23 (0.05)	0.09 (0.01)^{b}	0.12 (0.01)^{b}	0.23 (0.03)^{a}
NO_3^--N (mg/kg)	21.2 (1.35)^{b}	24.1 (5.99)^{ab}	41.4 (4.35)^{a}	9.07 (1.09)	13.1 (0.473)	16.5 (4.9)
C/N ratio	17 (1.62)	14.92 (1.64)	14.61 (0.89)	13.35 (1.20)	13.48 (0.75)	11.83 (1.06)
P (%)	0.08 (0.01)	0.08 (0.01)	0.10 (0.01)	0.08 (0.01)^{b}	0.12 (0.01)^{a}	0.11 (0.01)^{ab}
K (%)	0.26 (0.00)	0.26 (0.01)	0.28 (0.01)	0.26 (0.01)	0.27 (0.01)	0.24 (0.02)
Ca (%)	0.8 (0.08)	0.84 (0.1)	1.13 (0.14)	0.82 (0.15)	1.11 (0.17)	1.06 (0.12)
Mg (%)	0.30 (0.02)	0.27 (0.01)	0.31 (0.01)	0.30 (0.01)	0.30 (0.02)	0.31 (0.02)
Mn (ppm)	499.23 (46.05)	565.8 (6.27)	533.1 (4.27)	512.40 (42.04)	524.17 (15.16)	567.87 (51.96)
Fe (ppm)	14506.67 (878.15)	14400.00 (432.47)	14850.00 (397.37)	14306.67 (384.89)	14896.67 (1111.61)	15023.33 (192.38)
Cu (ppm)	21.67 (0.45)	22.60 (0.39)	25.03 (2.76)	24.32 (1.11)	22.60 (0.39)	24.38 (1.80)
Zn (ppm)	58.04 (3.30)	59.03 (1.92)	63.54 (3.17)	62.10 (4.27)	64.29 (3.83)	66.39 (5.55)

Values represent means, with standard errors in parentheses (n = 3). Different letters indicate statistically significant differences among treatments based on Tukey's HSD test ($p < 0.05$). EC: electrical conductivity; TN: total nitrogen; Corg: organic carbon.

Bacterial diversity patterns and taxonomic composition

A total of 3,117,034 high-quality bacterial 16S rRNA gene reads were obtained from the 18 samples, averaging 173,169 reads per sample (Table S2). These clustered into 6,225 Consensus Cluster Sequences (CSCs) with a mean sequence length of 1,445 bp. The bacterial accumulation curves approached a plateau for all the samples, indicating sufficient sequencing depth to capture most of the bacterial diversity within the samples investigated (Figure S1). Alpha diversity analysis, based on the Shannon index, revealed significant differences between treatments only at maturation, with the control treatment exhibiting significantly higher diversity than the deep-banding compost application (Fig. 1a). Observed bacterial richness followed a similar trend; however, no statistically significant differences were detected between treatments at either PGS (Fig. 1b).

Overall, bacterial sequences were assigned to 39 bacterial phyla (99.71% of total reads; 6218 CSCs), 102 classes (99.4% reads; 6154 CSCs), 236 orders (98.7% reads; 6070 CSCs), 376 families (94.78% reads; 5676 CSCs), 737 genera (81.60% reads; 4698 CSCs), and 426 species (21.41% reads; 837 CSCs). Pseudomonadota was the most prevalent phylum across all treatments, accounting for 51.7% of the total bacterial reads, followed by Bacillota with 17.79% (Fig. 2a). At the class level, the most abundant groups were Gammaproteobacteria (32.09%), Alphaproteobacteria (19.49%), and Bacilli (16.29%). At the genus level, *Bacillus*, *Pseudomonas*, and *Rhodanobacter* accounted for 9.57%, 3.33%, and 2.60% of the total reads, respectively (Fig. 2b).

Compost application and delivery method are primary determinants of bacterial community assembly

Factors influencing the structure of bacterial communities in rhizosphere samples were first investigated using permutational multivariate analysis of variances (PERMANOVA) based on the Bray—Curtis dissimilarity index. Analysis of the complete dataset revealed that fertilization treatment accounted for approximately 30% of the variation in bacterial community structure, while plant growth stage (PGS) explained 14% (Table 2). No significant interaction between fertilization treatment and PGS was observed. These results were corroborated by Principal Coordinate Analysis (PCoA), where the first two axes together explained 48.9% of the total variation in bacterial community structure across all samples (Fig. 3a). The primary separation among samples occurred along the first axis (34.8% of variation), which was largely attributable to differences due compost application. To further assess the impact of compost application on bacterial community assembly, separate PERMANOVA analyses were conducted for each PGS. At the early stage, fertilization treatment explained 46.2% of the variation, and at the late stage, it accounted for 46.9% (Table 2). These findings were consistent with the corresponding ordination analyses, underscoring the significant influence of compost application on shaping bacterial communities. Compost-amended samples consistently diverged from the control samples forming distinct community assemblages. This divergence was especially pronounced under the deep-banding compost application, where treated samples consistently showed the greatest separation from control samples—a trend observed at both growth stages (Fig. 3b and 3c).

Table 2
Contribution of plant growth stage (PGS; maturation and ripening) and compost treatments to the bacterial community structure associated with rhizosphere samples, as revealed by PERMANOVA. Results are shown for the full dataset as well as separately for each PGS.

	Full model				Maturation PGS				Ripening PGS			
Parameter	**df**	**Pseudo-F**	**R^2**	**P-value**	**df**	**Pseudo-F**	**R^2**	**P-value**	**df**	**Pseudo-F**	**R^2**	**P-value**
Treatment	2	3.8828	0.29523	0.001	2	2.5849	0.46283	0.009	2	2.6525	0.46926	0.009
PGS	1	3.8073	0.14474	0.002								
Treatment*PGS	2	1.3653	0.10381	0.157								

Next, we evaluated whether the evident shifts in microbiota composition between the different compost treatments were correlated with the alterations in the soil properties promoted by compost addition to the soil. A significant effect ($P < 0.05$) of various soil properties on the microbiota was revealed by partial db-RDA (Fig. 4). Soil organic carbon, total nitrogen, nitrate concentration, C/N ratio and EC were found to be significantly ($P < 0.05$) correlated with the bacterial community assembly in the treatments investigated (Fig. 4). These results confirmed the strong relationship between soil properties and bacterial assemblage dynamics.

Changes in bacterial taxa abundance induced by compost application

To identify bacterial genera with significantly different relative abundances among treatments, Linear Discriminant Analysis Effect Size (LEfSe) was performed at each PGS. In total, 29 genera were differentially abundant at the early sampling stage (Fig. 5a). Among these, two genera were enriched in the control treatment, four in the broadcast treatment, and 23 in the deep-banding compost treatment. At the late sampling stage, 28 genera exhibited significant differences across treatments, with seven enriched in the control, three in the broadcast, and 18 in the deep-banding treatment (Fig. 5b). Notably, the deep-banding application consistently yielded a greater number of significantly enriched taxa compared to the other treatments at both time points, indicating a pronounced effect of this application method in shaping bacterial community assembly. Moreover, the composition of differentially abundant taxa varied considerably between the early and late stages. In the control and broadcast treatments, no genera were consistently enriched across both sampling points. In contrast, the deep-banding treatment retained a core set of ten genera (e.g., *Aquamicrobium*, *Pusillibacter*, *Advenella*, *Cytophaga*, *Oceanobacillus*) that were shared between the two stages, suggesting a more stable and sustained microbial response to deep-banded compost application.

Compost application, particularly through deep-banding, altered the functional potential of bacterial communities

Functional predictions based on taxonomic assignments were conducted using FAPROTAX, resulting in the identification of 37 soil-related functional groups. At the early PGS, 34 functions were detected; *iron respiration*, *anoxygenic photoautotrophy S-oxidizing*, and *anoxygenic photoautotrophy* were not observed (Fig. 6a). By the ripening stage, 36 functions were present, with *aromatic hydrocarbon degradation* being the only function absent (Fig. 6b). LEfSe analysis revealed that during maturation of red bell pepper, two functions—*nitrate reduction* and *aromatic hydrocarbon degradation*—were significantly different among treatments, both being more abundant in the deep-banding compost application and least abundant in the control. At maturation, three functions—*nitrogen respiration*, *nitrate respiration*, and *nitrate reduction*—were significantly enriched, again showing the highest relative abundance under the deep-banding treatment. In contrast, *nitrite ammonification* and *nitrate ammonification* were more prevalent in the control treatment and reduced in the deep-banding application (Fig. 6). These findings indicate that deep-banding compost application not only alters microbial community composition but also enhances key microbial functions related to nitrogen cycling.

To assess the potential functional implications of compositional shifts in the bacterial community, Pearson correlation analysis was conducted between FAPROTAX-predicted functions and genera identified as differentially abundant across treatments during the early and late PGS. In the early PGS, 26 genera exhibited significant correlations ($p < 0.05$), including 23 that were enriched under the deep-banding compost treatment and 3 associated with the broadcast compost application. Notably, 18 genera enriched in the deep-banding treatment were positively correlated with the *nitrate reduction* pathway, which also exhibited high functional abundance in this treatment. Likewise, six genera associated with the broadcast treatment were positively correlated with the *aromatic compound degradation* pathway, which was predominantly expressed under broadcast compost conditions. In the late PGS, only eight differentially abundant genera were significantly correlated with predicted functional pathways. Among these, three highly expressed nitrogen cycling pathways—*nitrate reduction*, *nitrate respiration*, and *nitrite respiration*—under the deep-banding treatment were positively associated with 3, 6, and 2 bacterial genera, respectively. These results highlight a treatment-specific enrichment of microbial taxa linked to key soil functions, particularly nitrogen transformation processes under deep-banding compost application.

Discussion

Conventional agriculture relies heavily on mineral fertilizers to sustain yields, but intensive use has been linked to soil degradation, nutrient leaching, biodiversity loss, and greenhouse gas emissions (Gomiero, 2016; Ortiz et al., 2021; Clark et al., 2020). Organic fertilization offers a sustainable alternative, improving soil health and productivity (Diacono and Montemurro, 2010). Pre-processed amendments such as composts can increase soil carbon, enhance nutrient availability and structure, and improve water retention, supporting root growth and yield (Möller and Müller, 2012; Lucchetta et al., 2023; Edlinger et al., 2024). However, their effectiveness under field conditions varies with composition, environment, and application method. In this study, we compared two compost application strategies—broadcasting and deep banding of green-waste compost—on soil properties and the rhizosphere microbiota of red bell pepper at maturation and ripening, aiming to clarify how spatial placement influences nutrient dynamics, soil health, and microbial assembly.

Deep-banding compost application improved key edaphic parameters

The effectiveness of compost as a nutrient supplier in agroecosystems has been well documented for different soils, climate conditions and for several source materials (Nendel et al., 2007; Edlinger et al., 2025). For pepper cultivation, compost application is widely recognized as a beneficial practice due to its ability to enhance nutrient availability, soil structure, and organic matter content (Selvakumar et al., 2018; Wang et al., 2020; Kebede et al., 2023). However, its effectiveness depends not only on presence but also on the method of application (Nkebiwe et al., 2016; Zhu et al., 2025). Our findings support the initial hypothesis that compost amendments, and particularly their mode of application, differentially influence soil physicochemical properties. In our study, surface broadcasting of compost had limited effects on most soil properties compared with the control at both maturation and ripening stages. At ripening, phosphorus exhibited a significant increase, whereas nitrate and total nitrogen showed upward trends that did not reach statistical significance relative to the control. This limited response likely reflects the slower incorporation of surface-applied compost into the soil matrix and the delayed release of nutrients, a pattern also reported in other cropping systems

(Bünemann et al., 2018). In contrast, deep banding of compost exerted a stronger and broader influence on soil properties. During red bell pepper maturation, only nitrate concentration was significantly higher in deep-banded plots compared with the control. At ripening, multiple parameters—including organic carbon, total nitrogen and electrical conductivity—were significantly elevated. These findings align with previous studies demonstrating that deep placement of organic amendments enhances soil health and crop productivity more effectively than surface application (Liu et al., 2019; Wu et al., 2024; Ray et al., 2025). Deep banding improves soil nitrogen pools by reducing losses from ammonia volatilization and nitrate leaching, as limited surface exposure allows greater retention of total nitrogen, mineral nitrogen, and nitrate within the soil profile (Nkebiwe et al., 2016; Bünemann et al., 2018). This is facilitated by better integration of compost with soil particles at deeper depths (10–30 cm), which enhances microbial stabilization of organic matter and promotes a more gradual and sustained nutrient mineralization process, while potentially limiting rapid nitrification and gaseous losses (N_2O, NH_3) (Li et al., 2022; Burg et al., 2023). For soil carbon pools, deep placement slows the overall rate of organic matter decomposition compared with surface application, allowing more carbon to be retained in the soil over time while still supporting a steady release of nutrients (Assirelli et al., 2023; Sweet et al., 2025). Mechanisms include enhanced soil aggregation that physically protects carbon, increased humification (e.g., higher humic acid content and degree of humification), and elevated microbial biomass, which stabilize carbon compounds, improve organic matter quality, and support long-term sequestration by converting applied organic matter into more resistant forms (Bünemann et al., 2018; Burg et al., 2023; Edlinger et al., 2024). These processes are further supported by rhizosphere changes, such as stimulated root growth around nutrient depots, which enhance nutrient cycling, carbon retention, and root–soil contact (Wang et al., 2020; Wang et al., 2022). Deep banding also fosters macroaggregate formation, improving soil structure, facilitating nutrient uptake, supporting root development, and creating a favorable environment for microbial activity, thereby increasing organic matter accumulation (Wang et al., 2020; Chen et al., 2022; Udding et al., 2025).

Compost application, especially via deep-banding, drives bacterial assembly and functional potential

Compost addition significantly affected the structure of bacterial communities, with outcomes determined not only by the presence of compost but also by the mode of its application. Among treatments, deep banding produced the most pronounced and consistent effects, resulting in distinctive rhizosphere community composition, beta diversity patterns, and predicted functional traits across both developmental stages of red bell pepper. This outcome aligns with recent studies reporting that compost placement depth significantly alters microbial diversity, community composition, and structural patterns (Gan et al., 2023; Hsiao et al., 2025). Ordination analysis confirmed clear clustering by treatment, with deep-banding communities strongly separated from the control, highlighting the decisive role of compost placement depth in structuring rhizosphere bacterial communities (Fig. 3). These findings validate our second hypothesis that compost-induced changes in soil conditions shape bacterial community assembly and functional potential, with the magnitude of effects determined by the application method. Redundancy analysis (RDA) further showed that soil properties significantly altered by compost application—particularly total nitrogen, nitrate concentration, organic carbon, C/N ratio and electrical conductivity—were strongly associated with bacterial community composition (Fig. 4). The large proportion of variation explained by the first two axes corroborates the structuring effect of compost-modified soil conditions on microbial assemblages. Overall, deep compost placement shapes the soil microbiome primarily through treatment-induced changes in nutrient availability and chemical balance, which regulate microbial recruitment and activity. This reinforces the link between green-compost application depth, altered soil properties, and the enrichment of functionally relevant microbial taxa (Cucu et al., 2019; LeBlanc et al., 2024).

Deep banding consistently exhibited the strongest impact on bacterial community assembly, as reflected not only in overall structural differences but also in the higher number of genera with significant changes in relative abundance compared to the other treatments at both growth stages. These taxonomic shifts were particularly notable for nitrogen-associated genera: *Azoarcus*, *Alcaligenes*, and *Advenella* were enriched during maturation, whereas *Thermomonas*, *Ochrobactrum*, and *Marinobacter* became more abundant at ripening. Members of these taxa are known to participate in key nitrogen transformations, including biological nitrogen fixation, nitrate reduction, and denitrification (Doi et al., 2009; Matsuoka et al., 2012; Meng et al., 2019; Sakoda et al., 2019; Liu et al., 2022; Qin et al., 2024).

In parallel, functional profile prediction indicated that deep banding enhanced the potential for key nitrogen cycling pathways, including nitrogen respiration, nitrate respiration, and nitrate reduction. These functional traits suggest an increased microbial capacity to convert atmospheric or inorganic nitrogen forms into bioavailable compounds, as well as to regulate nitrogen losses via

denitrification (Liao et al., 2024). Moreover, correlation analysis between enriched taxa and predicted functions revealed strong positive associations with nitrogen cycling processes, indicating that the observed community shifts are not merely taxonomic but also functionally meaningful. For instance, the relative abundance of *Azoarcus*, *Alcaligenes*, *Advenella*, and *Arenibacter* was positively correlated with nitrate reduction potential, highlighting their likely role as active contributors to nitrogen turnover in the root zone. Taken together, these findings suggest that deep compost banding creates a favorable microenvironment for nitrogen-cycling microbes, promoting both the structural enrichment of beneficial taxa and the functional enhancement of specific nitrogen transformation pathways.

Importantly, our study goes beyond confirming the efficacy of deep compost placement: it also demonstrates the value of considering temporal dynamics in plant–soil–microbiome interactions. Most studies on soil microbial diversity rely on a single sampling time, which can obscure the stage-specific effects of management interventions. By following community dynamics across two key developmental stages, our results validate our third hypothesis that the influence of compost on both soil properties and microbiome dynamics varies over time in accordance with plant development. The composition of differentially abundant taxa varied considerably between maturation and ripening: while no genera were consistently enriched across both sampling points in the control and broadcast treatments, the deep-banding treatment retained a core set of 10 genera throughout plant development. During maturation, shifts in rhizosphere bacterial diversity and stronger correlations between taxa and predicted functions were observed, reflecting an early phase in which microbial recruitment is largely soil-driven before the plant exerts stronger selective pressure on its microbiome (Francioli et al., 2018; Xiong et al., 2021). At ripening, correlations between taxa and functions decreased, consistent with a more stabilized, plant-shaped community. These findings align with evidence that the microbiota of early growth stages undergoes dynamic establishment, during which community assembly is less resilient to physicochemical stresses, while later stages show greater stability due to tighter microbial interactions (Francioli et al., 2021; Lewin et al., 2024). This transition may also be driven by temporal shifts in root exudation, as both the quality and quantity of plant-derived metabolites change with development, mediating plant–microbe interactions and shaping community structure (Chaparro et al., 2014). Such shifts can actively recruit functionally relevant taxa, including nitrogen-transforming microorganisms, linking stage-dependent plant inputs with the observed enrichment of N-related pathways under deep-banding conditions. Overall, these temporal patterns highlight the dynamic nature of plant–microbiome–soil interactions and underscore the importance of considering plant developmental stage when evaluating the functional outcomes of soil management.

Finally, a major innovative aspect of our study is methodological. To achieve high-resolution taxonomic profiling of the rhizosphere bacterial community, we employed Nanopore long-read sequencing, which remains underutilized in agricultural microbiome research. This approach yielded high-quality, full-length 16S rRNA gene data, averaging 171,000 reads per sample. Sequencing the entire ~ 1,500 bp gene, covering all nine hypervariable regions, provided exceptional taxonomic resolution: over 95% of sequences were classified at the family level, 82% at the genus level, and 21.5% at the species level. This surpasses the limitations of short-read amplicon sequencing, which typically captures only a subset of variable regions. Such resolution is critical not only for linking compositional shifts to treatments but also for improving functional characterization. While the 16S rRNA gene is primarily a phylogenetic marker, species-level assignments form a robust foundation for predictive functional profiling with tools such as FAPROTAX. By providing more precise taxonomic inputs, this approach reduces uncertainty associated with broader classifications and allows a more comprehensive understanding of how treatments modulate both the structure and potential function of the rhizosphere microbiome. Thus, the combination of a temporal approach and high-resolution long-read sequencing represents a novel framework for assessing how soil management practices influence plant-associated microbial communities.

Conclusion

In this study, we demonstrated that the spatial placement of compost is a critical factor governing its effectiveness in agroecosystems. While surface broadcasting yielded minimal benefits, deep banding of green-waste compost significantly improved key soil physicochemical properties, enhancing soil carbon and nitrogen pools. These edaphic improvements created a distinct and functionally specialized rhizosphere bacterial community in red bell pepper. Leveraging high-resolution, long-read sequencing, we showed that deep banding selectively enriched for key nitrogen-cycling taxa and increased the genetic potential for crucial nitrogen transformation pathways. These findings highlight deep banding not merely as an application technique but as a strategic management practice to simultaneously boost soil health, optimize nutrient dynamics, and steer the rhizosphere microbiome toward beneficial functions, offering a promising path for sustainable agriculture.

Declarations

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Ethics declarations

The authors have nothing to declare.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

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Figures

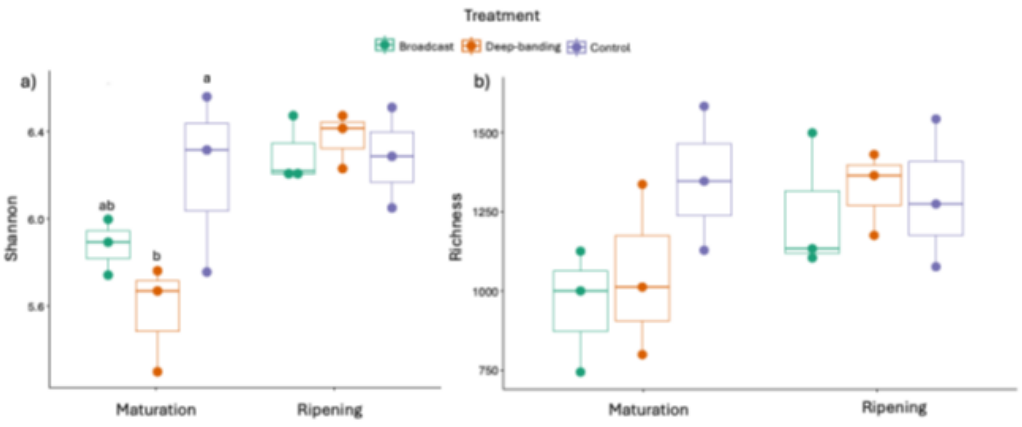


Figure 1

Boxplots displaying bacterial (a) Shannon index and (b) richness of each sample across the treatments (Control, Broadcasted, Deep-banding) at each PGS stage (maturation, ripening). Different letters indicate statistically significant differences among treatments

based on Tukey's HSD test ($p < 0.05$).

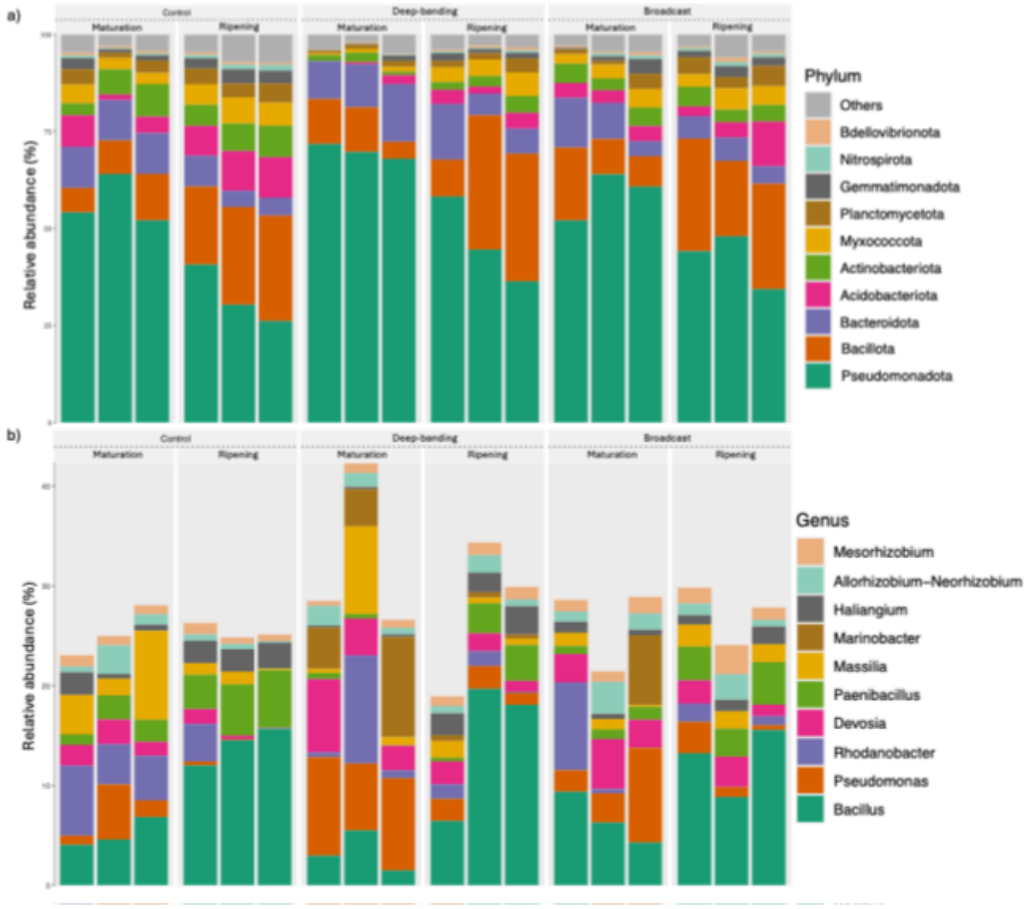


Figure 2

Bacterial taxonomic composition of each sample across the treatments (Control, Broadcasted, Deep-banding) at each PGS stage (maturation, ripening). Only the 10 most abundant (a) phyla and (b) genera are shown; all other taxa are grouped under "Others."

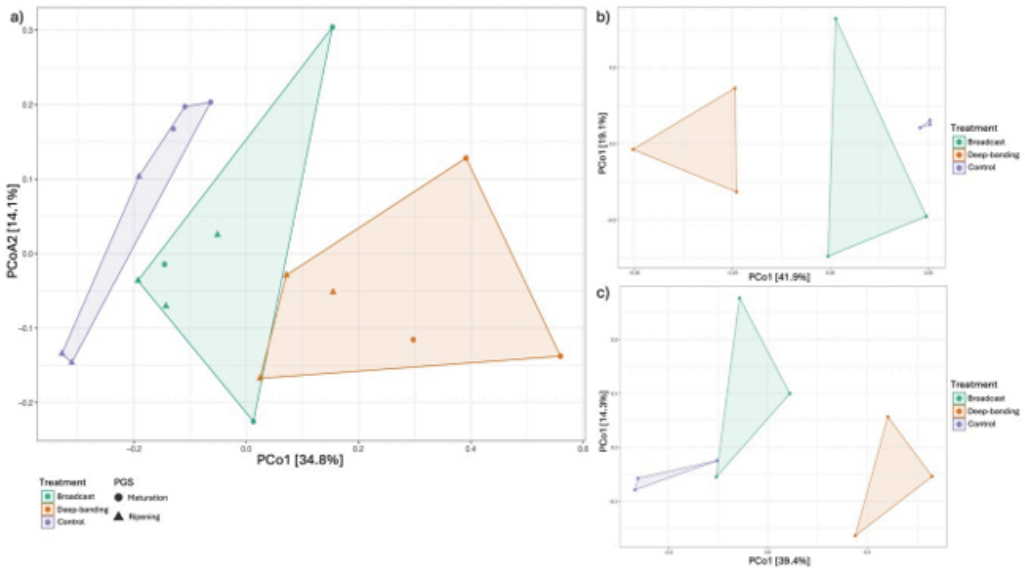


Figure 3

Principal coordinates analysis (PCoA) of bacterial community structure in the rhizosphere based on (a) the full dataset, (b) samples at maturation, and (c) samples at ripening.

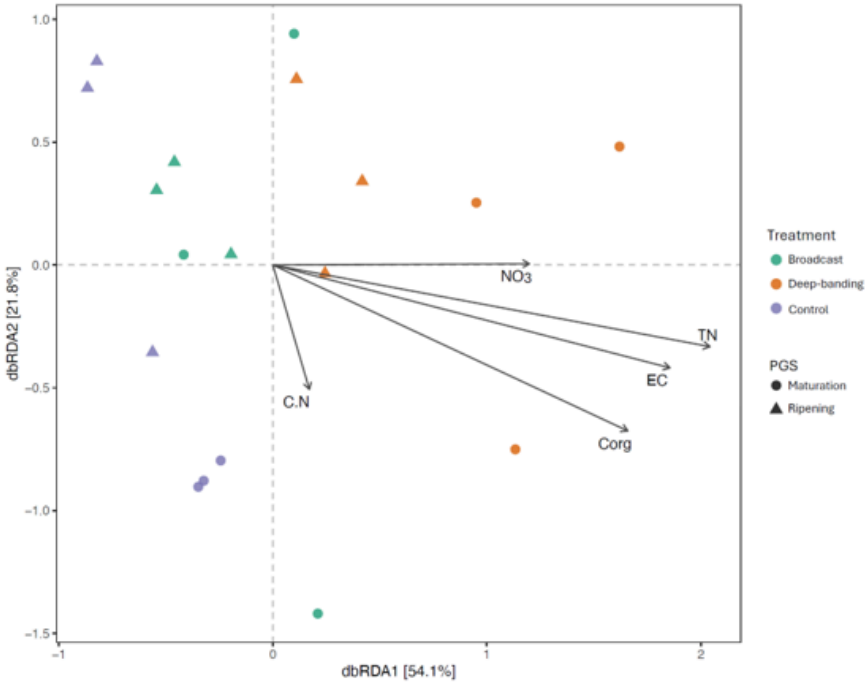


Figure 4

Distance-based redundancy analysis (db-RDA) illustrating the relationships between soil variables and bacterial community structure across compost treatments (Control, Broadcasted, Deep-banding) in red bell pepper rhizosphere samples.

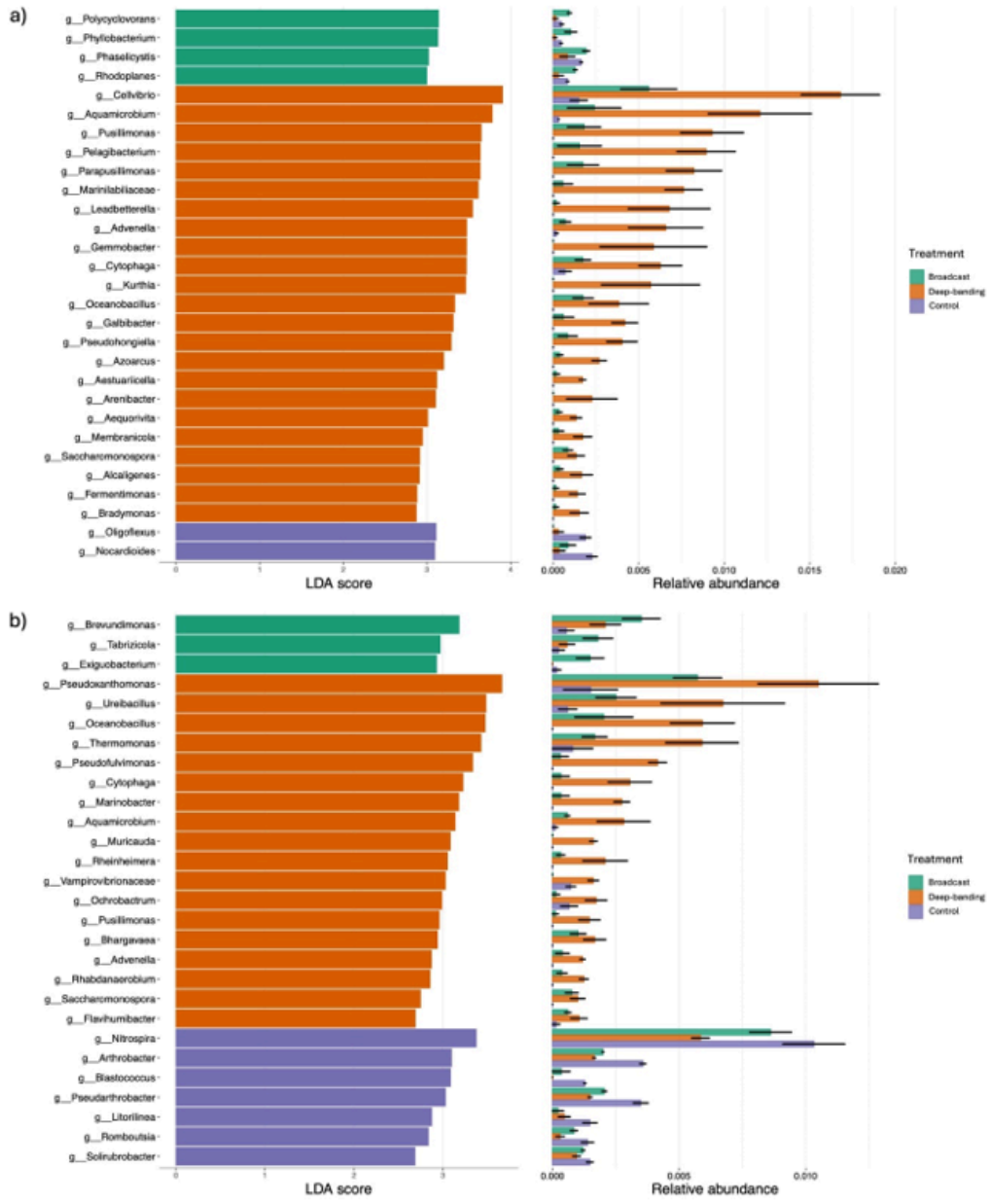


Figure 5

Linear discriminant analysis (LDA) scores of soil bacterial genera with significant differences ($P < 0.05$) among compost treatments (Control, Broadcasted, Deep-banding) at (a) maturation and (b) ripening. An LDA score threshold of >2 was applied to identify discriminant taxa. The right panels display the relative abundances of these genera, ranked in descending order according to their contribution to the model's classification accuracy. Bars represent mean relative abundances, and error bars denote the standard error of the mean.

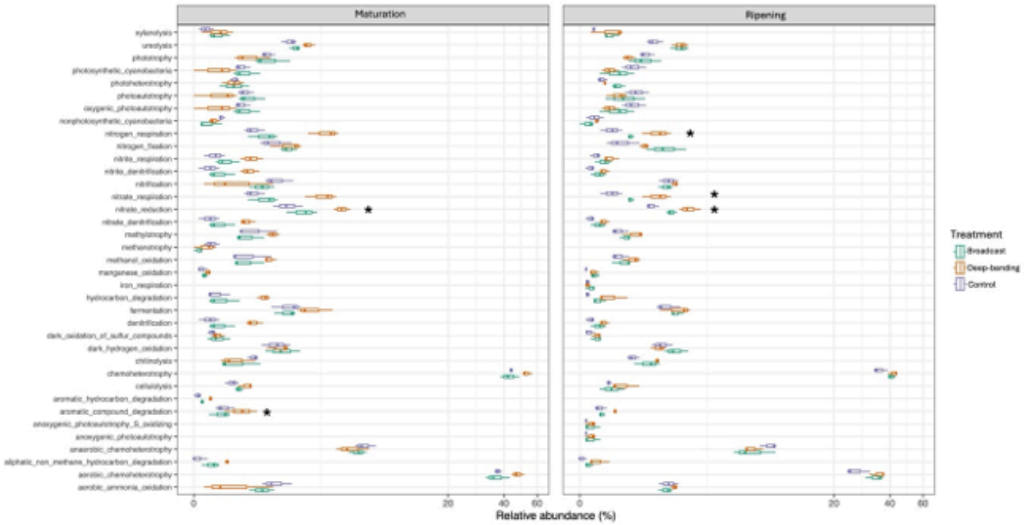


Figure 6

Differentially abundant predicted functional processes based on FAPROTAX assignments. Boxplots show the relative abundance of predicted soil-related functional groups across compost treatments (Control, Broadcasted, Deep-banding) at maturation and ripening. Asterisks indicate statistically significant differences between treatments at each time point as determined by LEfSe analysis.

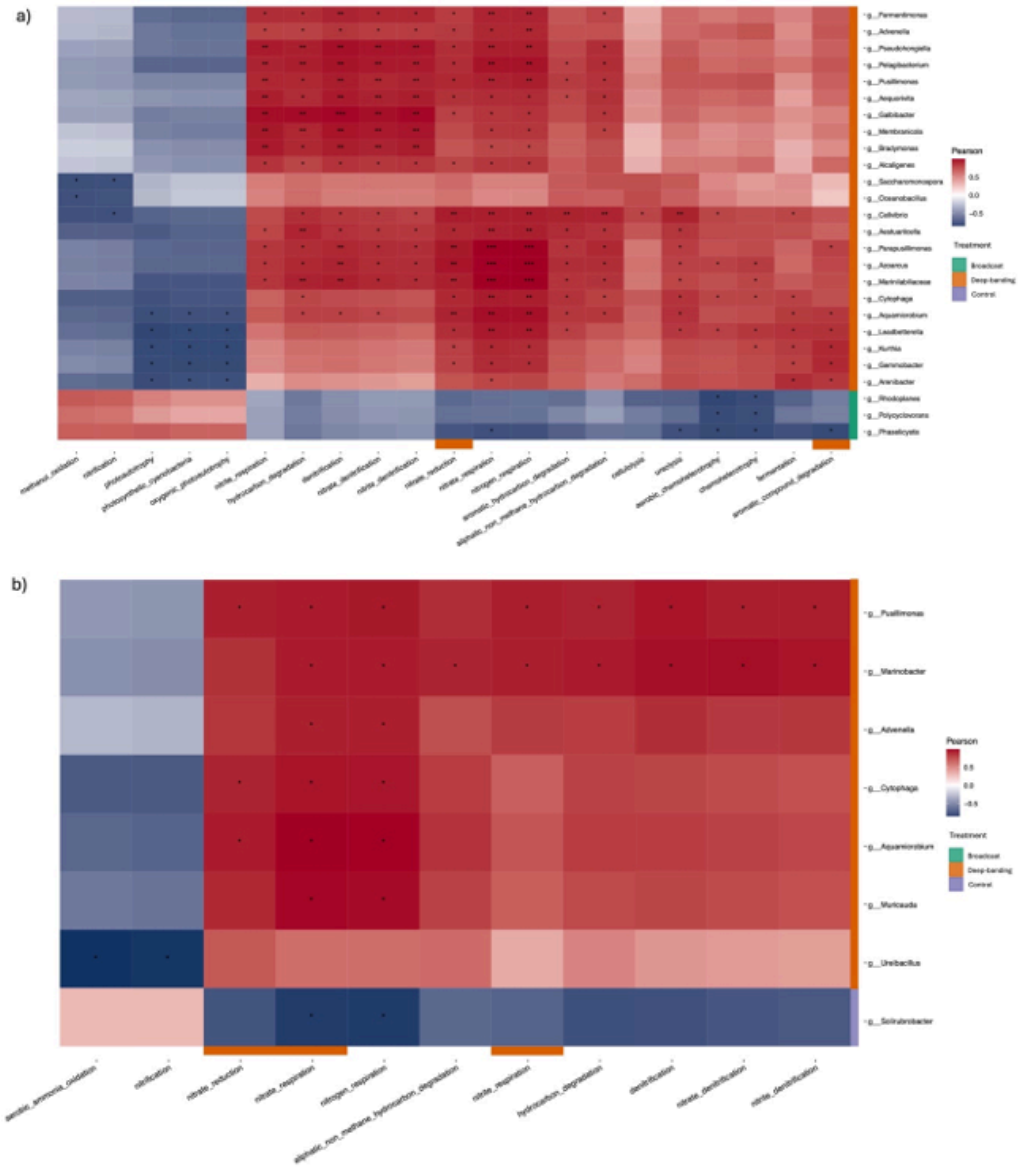


Figure 7

Correlation between FAPROTAX-predicted functions and differentially abundant genera across treatments (Control, Broadcasted, Deep-banding) at (a) maturation and (b) ripening. The red-to-blue color scale represents positive to negative Pearson correlation values. Asterisks indicate statistically significant correlations ($p < 0.05$). Edge colors show the treatment in which the predicted function or genus was more abundant.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial.docx