



Compost-driven modulation of rhizosphere bacterial communities during red bell pepper development revealed by long-read 16S metabarcoding

Mateo Córdoba-Agudelo¹ · Maximilian Schmidt² · Maria Serwetnicka³ · Carolyn-Monika Görres⁴ · Jana Zinkernagel³ · Davide Francioli¹

Received: 7 October 2025 / Revised: 27 January 2026 / Accepted: 2 February 2026 / Published online: 16 February 2026
© The Author(s) 2026

Abstract

Organic amendments, such as compost, are widely used to improve soil organic matter content, nutrient retention, and microbial activity; however, their effectiveness depends on multiple factors, including compost type, application rate, soil and crop characteristics, and mode of application. The spatial placement of compost can profoundly influence soil nutrient dynamics and the assembly of plant-beneficial microbial communities, such as microorganisms involved in nutrient cycling, organic matter decomposition, and plant growth promotion. Here, we investigated how two compost application strategies—surface broadcasting and deep banding of green-waste compost—affect soil physicochemical properties and the rhizosphere bacterial community of red bell pepper (*Capsicum annuum* var. *Fritz G740*) across two developmental stages (maturation and ripening). To resolve microbial responses at high taxonomic resolution, we applied Oxford Nanopore long-read sequencing of the full-length 16S rRNA gene, enabling improved taxonomic assignments and more reliable functional predictions compared to short-read approaches. Deep banding resulted in significantly higher organic C, total N, and nitrate concentrations than surface broadcasting, particularly at the ripening stage. These changes were closely associated with pronounced shifts in rhizosphere bacterial community composition, with deep banding selectively enriching N-associated genera, including *Azoarcus*, *Alcaligenes*, and *Ochrobactrum*. Functional predictions further indicated an enhanced potential for N cycle-related pathways, such as nitrate reduction and nitrogen respiration. Overall, our results demonstrate that deep compost banding may enhance soil fertility while promoting a functionally enriched rhizosphere microbiome. By integrating temporal sampling with long-read amplicon sequencing, this study provides a comprehensive framework to evaluate how compost placement modulates soil–plant–microbe interactions in horticultural production systems.

Keywords Compost placement · Rhizosphere microbiome · Oxford nanopore long-read sequencing · N cycling · *Capsicum annuum*

✉ Davide Francioli
davide.francioli@hs-gm.de

¹ Department of Soil Science and Plant Nutrition, Hochschule Geisenheim University, Geisenheim, Germany

² Department of Plant Breeding, Hochschule Geisenheim University, Geisenheim, Germany

³ Department of Vegetable Crops, Hochschule Geisenheim University, Geisenheim, Germany

⁴ Department of Applied Ecology, Hochschule Geisenheim University, Geisenheim, Germany

Introduction

The rhizosphere is a highly dynamic interface shaped by interactions among plant roots, soil physicochemical properties, and complex microbial communities, which together regulate nutrient cycling, plant health, and agroecosystem functioning (Lynch et al. 2001; Trivedi et al. 2020). The composition and functional potential of rhizosphere microbiota are key determinants of plant nutrient acquisition and stress responses, making them central targets for sustainable soil and crop management strategies (Ullah et al. 2025).

Fertilization practices strongly influence these soil–plant–microbe interactions by modifying soil pH, nutrient availability, and moisture regimes, thereby shaping microbial community assembly and activity (Raimi et al. 2023). While mineral fertilizers have substantially increased agricultural productivity, their intensive use has been associated with declines in microbial diversity, disruption of beneficial plant–microbe interactions, and long-term soil degradation (Tsiafouli et al. 2015; Huang et al. 2019; Porter and Sachs 2020). These limitations have stimulated interest in organic amendments, particularly compost, as sustainable complements that can supply nutrients and enhance soil biological functioning, although their ability to fully meet crop nutrient requirements depends on soil nutrient status, compost composition, and application rates (Rosa et al. 2017; Wei et al. 2024).

Compost amendments can improve soil structure, nutrient retention, and microbial activity and may act as biological drivers of rhizosphere community assembly (Martínez-Blanco et al. 2013). However, compost effects are highly context-dependent and influenced by compost characteristics, soil properties, crop species, and—critically—the method of application (Nkebiwe et al. 2016; Gan et al. 2023; Zhu et al. 2024). The spatial placement of organic amendments determines their physical proximity to roots, their exposure to soil moisture and oxygen gradients, and the extent to which nutrients are retained or lost through volatilization, runoff, or surface mineralization. Surface broadcasting is widely used in open-field and perennial cropping systems, as well as in organic and low-input production, due to its operational simplicity and compatibility with reduced soil disturbance. However, it often results in diffuse nutrient distribution and greater exposure of organic matter to environmental losses, potentially limiting sustained root–microbe interactions (Bünemann et al. 2018). In contrast, subsurface placement through deep-banding has been proposed and tested primarily in row crops and horticultural systems, including maize, wheat, and vegetable crops, as a strategy to concentrate organic inputs within the root zone and reduce nutrient losses associated with surface applications (Vido et al. 2024; Wu et al. 2024). By creating localized nutrient hotspots and more stable microenvironments, deep-banding may enhance microbial access to organic substrates, intensify plant–microbe interactions, and promote the establishment of distinct rhizosphere communities compared with surface-applied compost. Despite this potential, the mechanistic effects of compost placement depth on rhizosphere microbial community assembly and functional potential remain poorly understood, particularly in horticultural cropping systems.

Moreover, rhizosphere microbial communities are not static but change dynamically across plant developmental

stages in response to shifts in root exudation patterns and nutrient demand (Nannipieri et al. 2023; Yang et al. 2025). Nevertheless, the majority of studies assessing compost-induced effects on soil and rhizosphere microbiomes rely on sampling at a single time point, thereby overlooking temporal dynamics that may critically influence nutrient cycling and plant performance (Azeem et al. 2020; Liu et al. 2021; Su et al. 2022; Xu et al. 2023). In contrast, only a limited number of studies have incorporated temporal sampling to evaluate how compost amendments influence microbial community succession during crop development (Kraut-Cohen et al. 2023; Gil-Martínez et al. 2025). Consequently, how compost placement interacts with plant developmental stage to shape rhizosphere bacterial communities remains largely unresolved.

Addressing these knowledge gaps requires high-resolution characterization of rhizosphere microbiomes. Short-read amplicon sequencing has been widely used for this purpose, but its limited taxonomic resolution constrains the detection of fine-scale community shifts and hampers reliable functional inference in complex soil systems (Francioli et al. 2021b). Recent advances in Oxford Nanopore long-read sequencing now enable accurate full-length 16S rRNA gene sequencing, providing improved taxonomic resolution down to the species level and enhancing the robustness of taxonomy-based functional predictions (Aja-Macaya et al. 2025; Veselovsky et al. 2025). Despite these advantages, long-read sequencing remains underutilized in studies investigating compost management effects on soil–plant–microbe interactions, particularly across plant developmental stages.

In this study, we investigated how compost application method—no compost (Control), surface-applied compost (Broadcast), and subsurface compost placement (Deep-banding)—influences soil physicochemical properties and rhizosphere bacterial communities of *Capsicum annuum* var. Fritz G740, a widely cultivated horticultural crop, at two key developmental stages (maturation and ripening) that differ markedly in plant nutrient uptake and root exudation patterns. To achieve high-resolution characterization of rhizosphere microbiomes, we employed Oxford Nanopore sequencing of the full-length 16S rRNA gene, enabling species-level taxonomic resolution and improved functional inference compared to conventional short-read approaches. By integrating depth-controlled amendments with temporal sampling and high-resolution sequencing, we provide a comprehensive analysis of the spatial and temporal mechanisms through which compost management shapes the rhizosphere microbiome. This approach offers new insights into the microbial mechanisms underlying compost-driven soil–plant interactions and informs the optimization of compost management practices 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, 1.2% P₂O₅, 2.7% K₂O, 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 properties analysis

Total C (TC) and total N (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 C (Corg). NH₄⁺-N and NO₃[–]-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 NH₄⁺-N and NO₃[–]-N in the clear extracts were determined using a flow injection analyzer (FIAstar 5000, Foss GmbH, Rellingen, Germany). The mineral N pool was dominated by NO₃[–]-N, while the proportion of NH₄⁺-N was negligible and therefore not considered in the study. Soil pH was measured in 0.01 M CaCl₂, and electrical conductivity (EC) was measured using a conductivity meter (WTW, Cond 315i, Germany). Determination of total P (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 0.25 g of soil using the DNeasy PowerLyzer PowerSoil Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The rhizosphere DNA was quantified using a 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 10 ng in 15 µL of nuclease-free water.

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 the 27F and 1492R primers (Weisburg et al. 1991), targeting the full-length V1–V9 region of the 16S rRNA gene. Amplicon library preparation was carried out 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 10 ng 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 purified 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 (Yergaliyev et al. 2025), 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 65,312 sequences per sample, corresponding to the lowest sequencing depth

among all samples (Table S2). 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).

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 composition 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 composition. 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 composition. 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 the maturation stage, compost addition affected only nitrate concentration,

which was significantly higher under deep-banding treatment compared to the control, increasing from 21.2 to 41.4 mg/kg (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 N (TN), organic C (Corg) than the deep-banding treatment, which consistently showed the highest values for these parameters, increasing on average from 121.7 to 181.97 $\mu\text{S cm}^{-1}$ (EC), 0.09 to 0.23% (TN), and 1.25 to 2.79% (Corg), respectively (Table 1). The broadcast treatment differed significantly from the control only in P concentration, presenting the highest values for this macronutrient, increasing from 0.08 to 0.12%, although not significantly different from the deep-banding treatment. Several soil nutrients, 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).

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 N; Corg: organic C.

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. 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 the primary determinants of bacterial community assembly

Factors influencing bacterial community composition in rhizosphere samples were assessed using permutational multivariate analysis of variance (PERMANOVA) based on Bray–Curtis dissimilarities. Across the full dataset, compost addition had a significant effect on bacterial community composition, explaining approximately 30% of the total variation (PERMANOVA, $p<0.001$; Table 2), whereas plant growth stage (PGS) explained 14% of the variation ($p<0.01$). No significant interaction between compost treatment and PGS was detected. These statistically significant

Table 1 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 ($\mu\text{S/cm}$)	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)

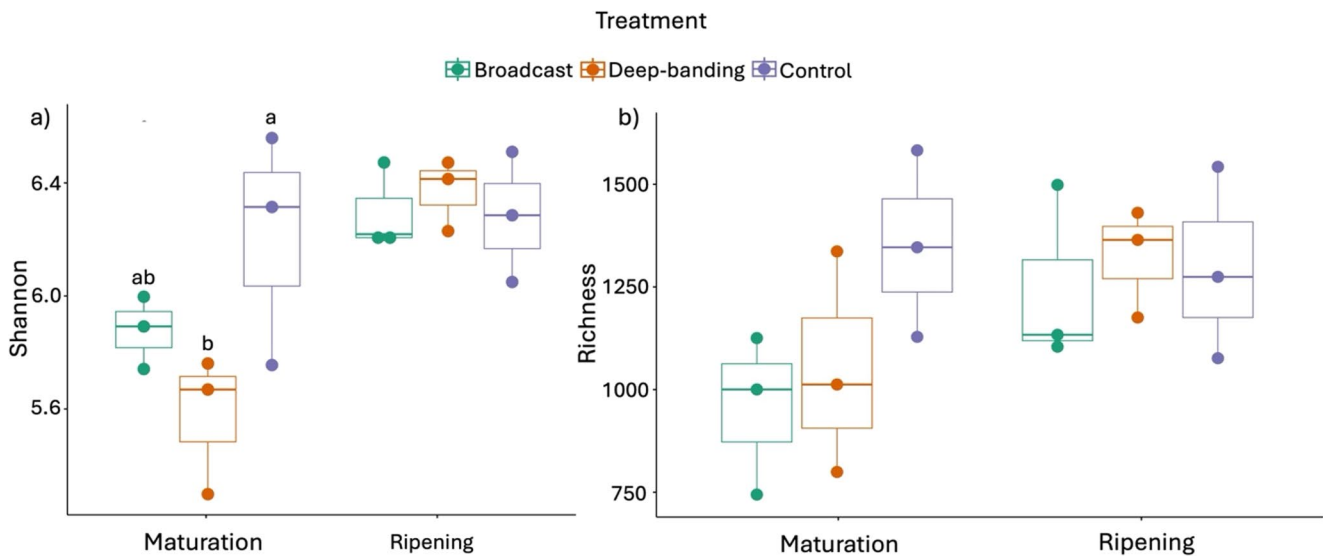


Fig. 1 Boxplots displaying bacterial (a) Shannon index and (b) richness of each sample across the treatments (Control, Broadcast, Deep-banding) at each PGS stage (maturation, ripening). Different

letters indicate statistically significant differences among treatments based on Tukey's HSD test ($p < 0.05$)

treatment effects were supported by principal coordinate analysis (PCoA), in which the first two axes together captured 48.9% of the total variation in community composition (Fig. 3a). The primary separation among samples occurred along the first axis (34.8% of the variation) and was largely attributable to compost application.

To further resolve treatment effects within individual plant growth stages, PERMANOVA was conducted separately for each PGS. Compost application exerted a strong and significant influence on bacterial community composition at both the early stage ($R^2 = 46.2\%$, $p < 0.01$) and the late stage ($R^2 = 46.9\%$, $p < 0.01$; Table 2). Consistent with these results, ordination analyses showed clear clustering by treatment at both PGSs (Fig. 3b, c). Across growth stages, compost-amended samples formed distinct bacterial community assemblages compared with the non-amended control. This separation was most pronounced under deep-banding compost application, which consistently showed the greatest divergence from control samples at both PGSs.

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 C, total N, 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).

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 separately at each PGS. At the early sampling stage, a total of 29 genera exhibited differential abundance among treatments (Fig. 5a), with two genera enriched in the control, four in the broadcast compost treatment, and 23 in the deep-banding treatment. At the late sampling stage, 28 genera differed significantly across treatments, including seven enriched in the control, three in the broadcast, and 18 in the deep-banding treatment (Fig. 5b).

Notably, deep-banding consistently yielded a greater number of significantly enriched taxa than the other treatments at both time points, indicating a strong influence of this application method on bacterial community assembly. Furthermore, the identity of differentially abundant taxa differed markedly 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 shared genera (e.g. *Aquamicrobium*, *Pusillibacter*, *Advenella*, *Cytophaga*, *Oceanobacillus*) across both stages, suggesting a more stable and sustained microbial response to subsurface compost placement.

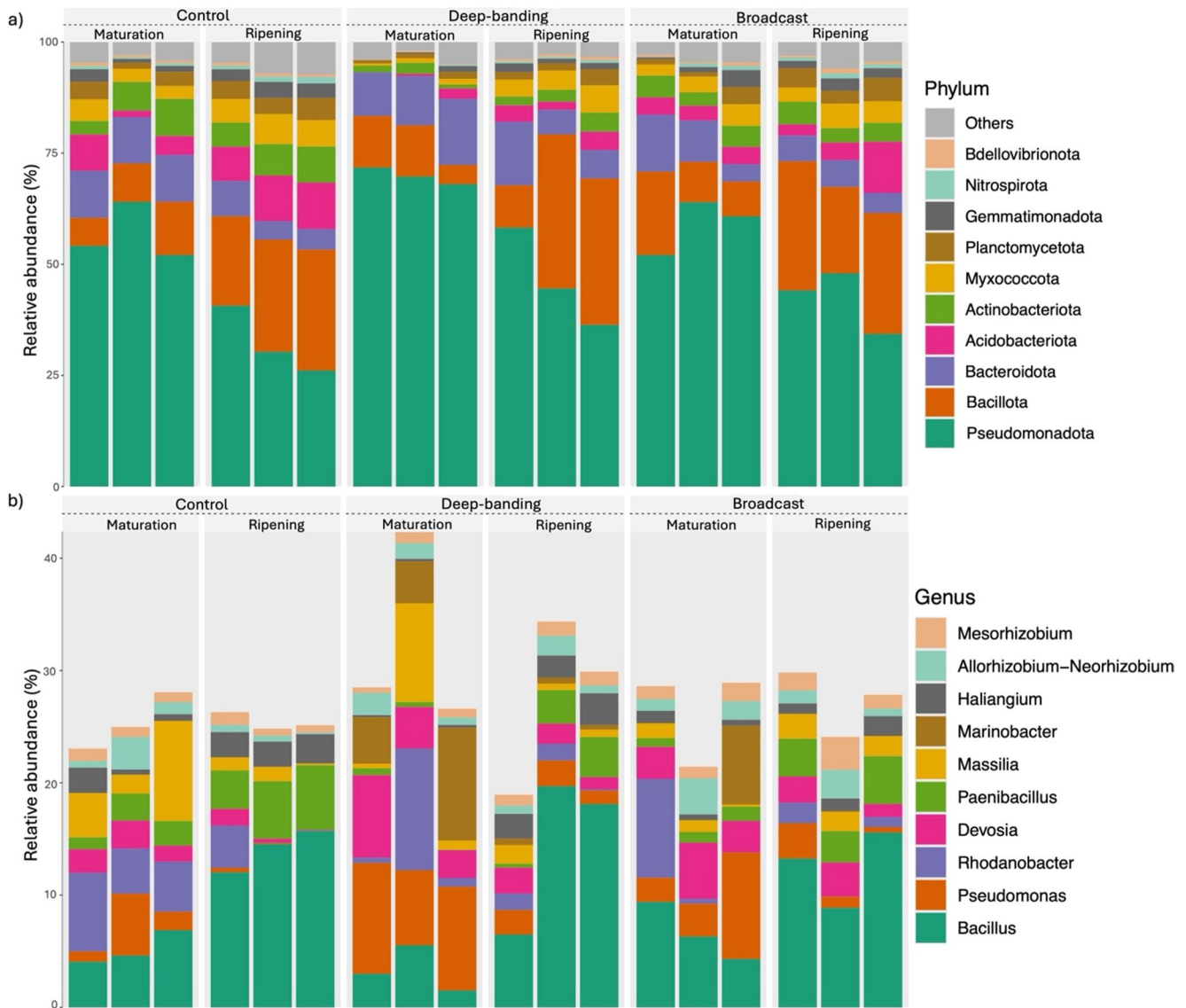


Fig. 2 Bacterial taxonomic composition of each sample across the treatments (Control, Broadcast, Deep-banding) at each PGS stage (maturation, ripening). Only the 10 most abundant (a) phyla and (b) genera are shown; all other taxa are grouped under “Others”

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 ripening, three functions—*nitrogen respiration*, *nitrate respiration*, and *nitrate reduction*—were significantly enriched, again showing the highest relative abundance under the deep-banding treatment. These findings indicate that deep-banding compost application not only alters microbial community composition but may also enhances key microbial functions related to N 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 ($p < 0.05$),

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

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

including 23 that were enriched under the deep-banding compost treatment and 3 associated with the broadcast compost application (Fig. 7a). 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 (Fig. 7b). Among these, three highly expressed N 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 N transformation processes under deep-banding compost application.

Discussion

Organic amendments, particularly compost, are widely promoted as sustainable tools to enhance soil physicochemical properties and stimulate microbial activity, thereby supporting soil health and agroecosystem functioning. Beyond supplying organic matter and nutrients, compost can influence soil microbial community composition and activity, with potential consequences for nutrient cycling and plant performance (Heisey et al. 2022; Edlinger et al. 2022). Despite these recognized advantages, the outcomes of compost application in field systems remain highly variable. This variability reflects differences in compost composition and environmental conditions, but also the spatial placement of organic inputs within the soil profile, which can determine nutrient accessibility and the extent to which microbial communities respond (Nkebiwe et al. 2016; Li et al. 2024). In this context, application strategies such as surface broadcasting and subsurface placement are likely to create contrasting soil microenvironments with distinct implications for nutrient dynamics and rhizosphere microbial assembly. Understanding how compost placement modulates soil–plant–microbe interactions is therefore essential for improving the reliability and sustainability of organic fertilization practices.

Influence of compost placement on edaphic parameters

The distinct effects of compost on soil physicochemical properties observed in this study appear to be driven

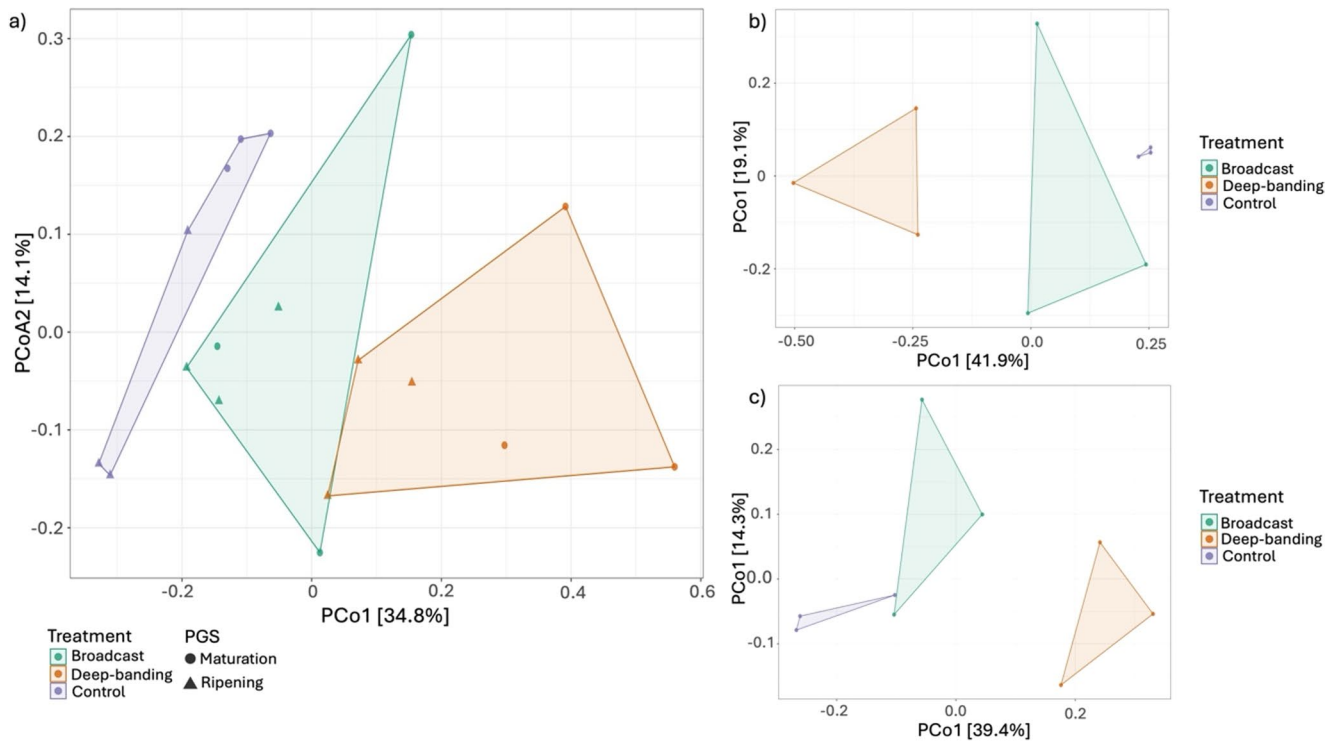


Fig. 3 Principal coordinates analysis (PCoA) of bacterial community composition in the rhizosphere based on (a) the full dataset, (b) samples at maturation, and (c) samples at ripening

primarily by the method of application. Although surface broadcasting is a widely used practice in many cropping systems—particularly in open-field and perennial cropping systems, where soil disturbance is minimized or mechanical incorporation is limited—it exerted only negligible effects on most soil properties throughout the red pepper growth cycle. This limited response may suggest a slow incorporation of surface-applied organic matter into the soil matrix and a delayed release of nutrients, a pattern consistent with observations in other cropping systems (Bünemann et al. 2018). In contrast, deep banding resulted in more pronounced and consistent changes, particularly at the ripening stage, when soil organic carbon, total nitrogen, nitrate, and electrical conductivity were significantly higher than in control soils. These responses align with previous studies reporting enhanced nutrient retention and improved soil quality following subsurface placement of organic amendments compared with surface application (Nkebiwe et al. 2016; Liu et al. 2019). The stronger effects associated with deep banding are likely attributable to the physical integration of compost within the soil matrix, which is expected to reduce nutrient losses and promote a more gradual release of mineral nitrogen. While we did not directly measure gas fluxes or leaching, the increased N availability in the banding zone is consistent with literature suggesting that subsurface placement limits ammonia volatilization by reducing surface exposure (Bünemann et al. 2018; Li et al. 2022).

Similarly, the significant increases in soil organic carbon under deep banding indicate improved carbon retention, potentially driven by enhanced soil aggregation and microbial stabilization processes (Assirelli et al. 2023; Burg et al. 2023). Importantly, deep banding may not be feasible or appropriate for all cropping systems, as it requires specialized equipment, higher energy inputs, and soil conditions that allow subsurface incorporation without excessive disturbance; therefore, its potential benefits should be evaluated within the context of crop type, soil characteristics, and management constraints.

Notably, the persistence of these effects at the ripening stage suggests that deep banding may extend nutrient availability beyond early crop development, thereby influencing soil conditions during later phenological phases when nutrient demand and root activity remain substantial. Such temporal persistence is particularly relevant in horticultural systems, where synchronization between nutrient supply and crop phenology is critical for efficient resource use and environmental sustainability. However, from a horticultural perspective, the increases in nitrogen availability and electrical conductivity observed at ripening warrant careful consideration. Although sustained nutrient availability late in the growing cycle could support continued metabolic activity and fruit development, excessive nitrogen supply or elevated salinity can negatively affect fruit quality and shelf life in pepper production systems. While specific

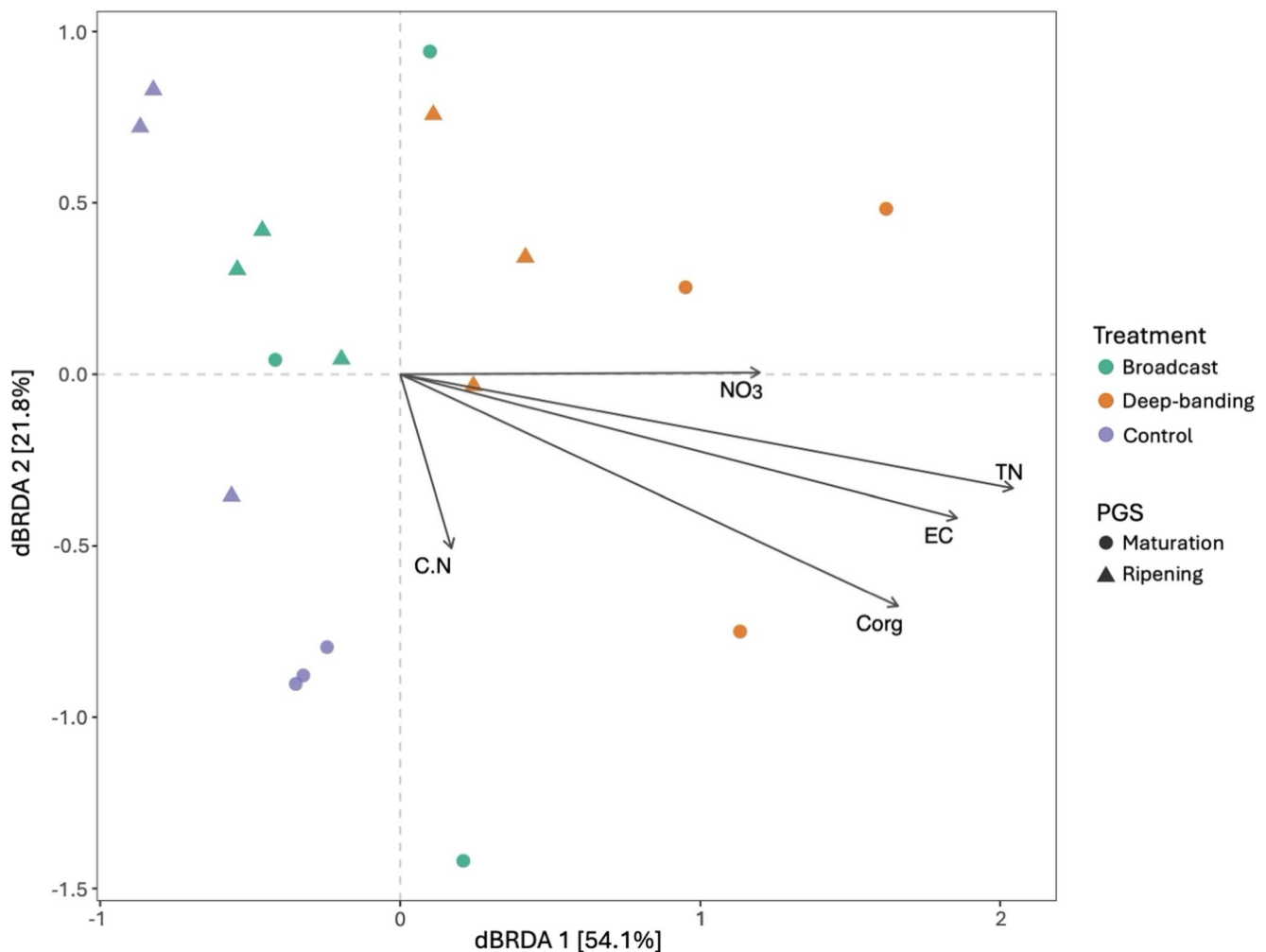


Fig. 4 Distance-based redundancy analysis (db-RDA) illustrating the relationships between soil variables and bacterial community composition across compost treatments (Control, Broadcasted, Deep-banding) in red bell pepper rhizosphere samples

physiological and process-level variables—such as yield, fruit quality, nitrification rates, and root architecture—were not directly quantified, the observed shifts in soil physico-chemical properties provide a robust proxy for altered soil environmental conditions. Nonetheless, the consistency of these findings with previous studies supports the conclusion that subsurface compost placement fosters a soil environment conducive to organic matter stabilization and efficient nutrient cycling, though future targeted measurements are warranted to validate these proposed dynamics.

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

Compost application significantly influenced rhizosphere bacterial community composition, with effects determined not only by compost presence but also by its mode of application. Among treatments, deep banding consistently produced the strongest and most pronounced shifts in

community composition, beta-diversity patterns, and predicted functional traits across both developmental stages of red bell pepper, confirming that subsurface placement can have a stronger effect on microbial communities compared with surface application (Gan et al. 2023; Hsiao et al. 2025). Importantly, our study extends these findings by specifically examining rhizosphere communities in a horticultural crop, capturing temporal dynamics across two key developmental stages (maturation and ripening), and linking community shifts to predicted functional traits, providing a more mechanistic understanding of how compost placement influences plant–microbe interactions over time.

Ordination analyses revealed clear clustering by treatment, with deep-banded samples distinctly separated from the control, underscoring the central role of compost placement depth in shaping rhizosphere bacterial assemblages. Redundancy analysis further demonstrated that soil properties significantly modified by compost application—particularly total nitrogen, nitrate concentration, organic carbon,

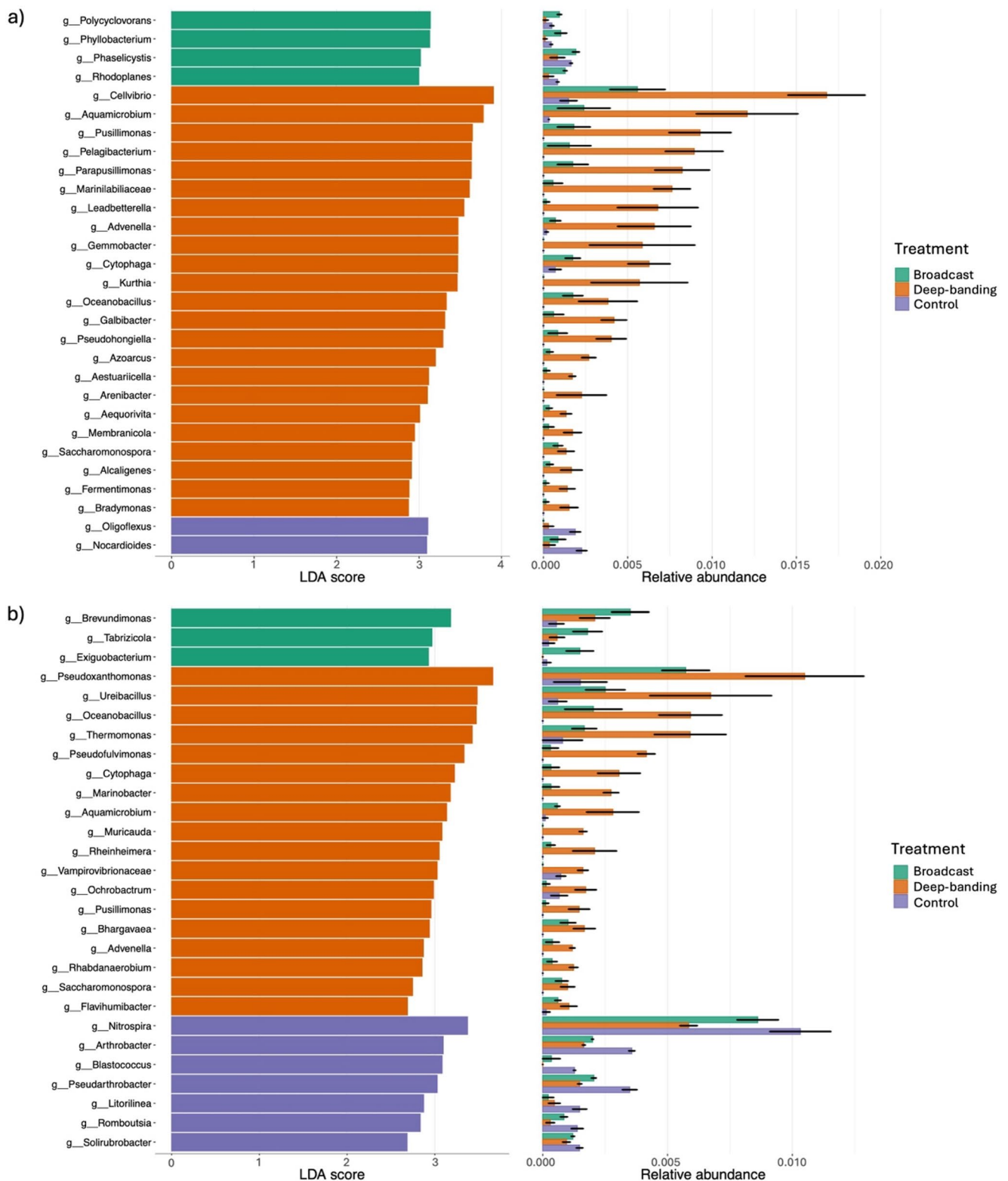


Fig. 5 Linear discriminant analysis (LDA) scores of soil bacterial genera with significant differences ($P < 0.05$) among compost treatments (Control, Broadcasted, Deep-banding) at (a) maturation and (b) ripening. An LDA score threshold of > 2 was applied to identify discrimi-

nant 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

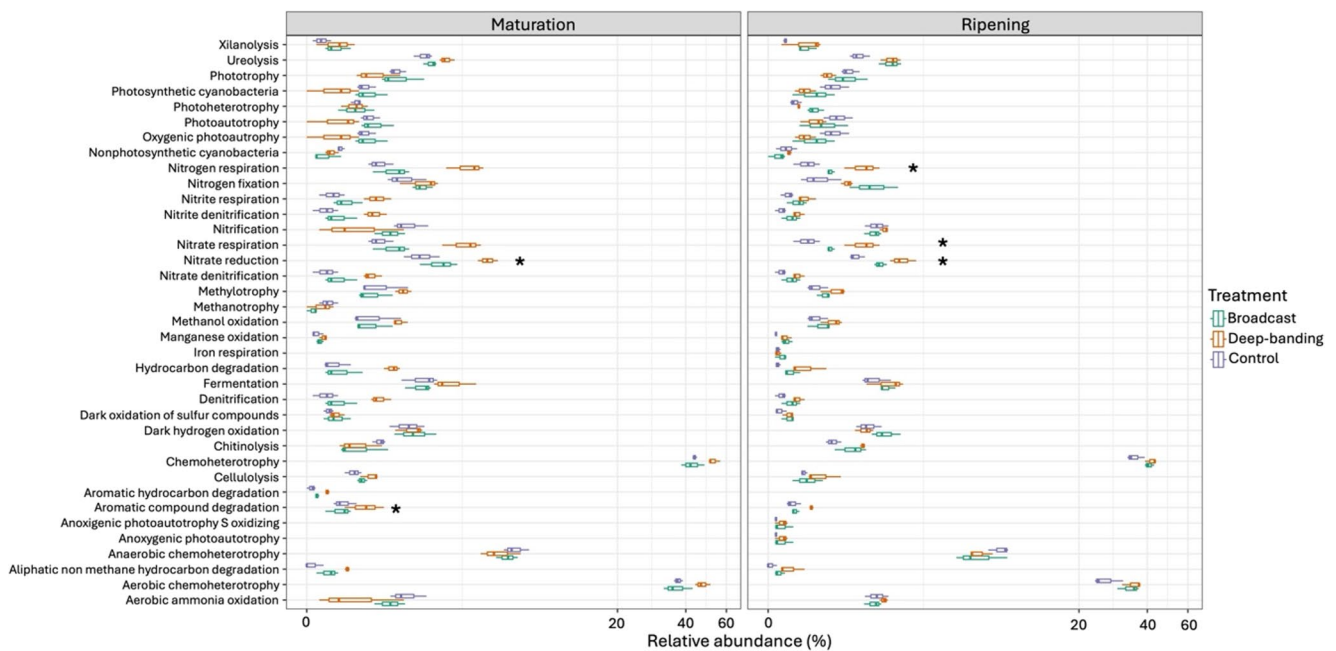


Fig. 6 Differentially abundant predicted functional processes based on FAPROTAX assignments. Boxplots show the relative abundance of predicted soil-related functional groups across compost treatments

C/N ratio, and electrical conductivity—were strongly associated with bacterial community composition. The large proportion of variation explained by the first two RDA axes supports the view that compost-induced changes in soil physicochemical conditions act as primary drivers of microbial community assembly. This finding aligns with growing evidence that soil properties often outweigh plant effects in structuring microbial diversity, whereas plant-driven influences, such as those mediated by root exudation, tend to be more transient and context-dependent (Hannula et al. 2021; Nannipieri et al. 2023). Together, these results reinforce the link between compost placement depth, altered soil conditions, and the recruitment of functionally relevant microbial taxa (Cucu et al. 2019; LeBlanc and Harrigan 2024).

Deep banding exerted the strongest influence on bacterial community assembly, as reflected by the greater number of genera showing significant changes in relative abundance compared with surface broadcasting and control treatments at both growth stages. These taxonomic shifts were particularly pronounced among genera associated with nitrogen cycling. During maturation, *Azoarcus*, *Alcaligenes*, and *Advenella* were enriched, whereas *Thermomonas*, *Ochrobactrum*, and *Marinobacter* increased in relative abundance 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). A higher diversity of nitrogen transformation pathways is widely

(Control, Broadcasted, Deep-banding) at maturation and ripening. Asterisks indicate statistically significant differences between treatments at each time point as determined by LEfSe analysis

regarded as important for stabilizing nitrogen cycling and increasing ecosystem resilience to environmental fluctuations (Philippot et al. 2013). Functional profile prediction via FAPROTAX indicated that deep banding enhanced the potential for key nitrogen cycling pathways, including dissimilatory nitrate reduction and associated anaerobic respiration processes (e.g., using nitrate or other N-oxides as electron acceptors). In this context, the enrichment of predicted nitrate-reducing functions suggests an increased microbial capacity to mediate nitrogen transformations that influence nitrate retention and loss pathways (Liao et al. 2024). Correlation analyses further supported this interpretation, revealing positive associations between enriched taxa (e.g., *Azoarcus*, *Alcaligenes*, *Advenella*, *Arenibacter*) and predicted nitrate reduction pathways, indicating that the observed community shifts are likely functionally meaningful rather than purely compositional.

Beyond treatment effects, our results highlight the importance of temporal dynamics in plant–soil–microbiome interactions. By assessing microbial communities at two key developmental stages, we captured stage-specific responses that are often overlooked in single-time-point studies (Nannipieri et al. 2019). The identity of differentially abundant taxa varied markedly between maturation and ripening, indicating that compost effects on microbial assembly are not static but evolve over the course of plant development. In the deep-banding treatment, a core set of genera persisted across both stages, suggesting a degree of structural stability under sustained subsurface nutrient inputs. During

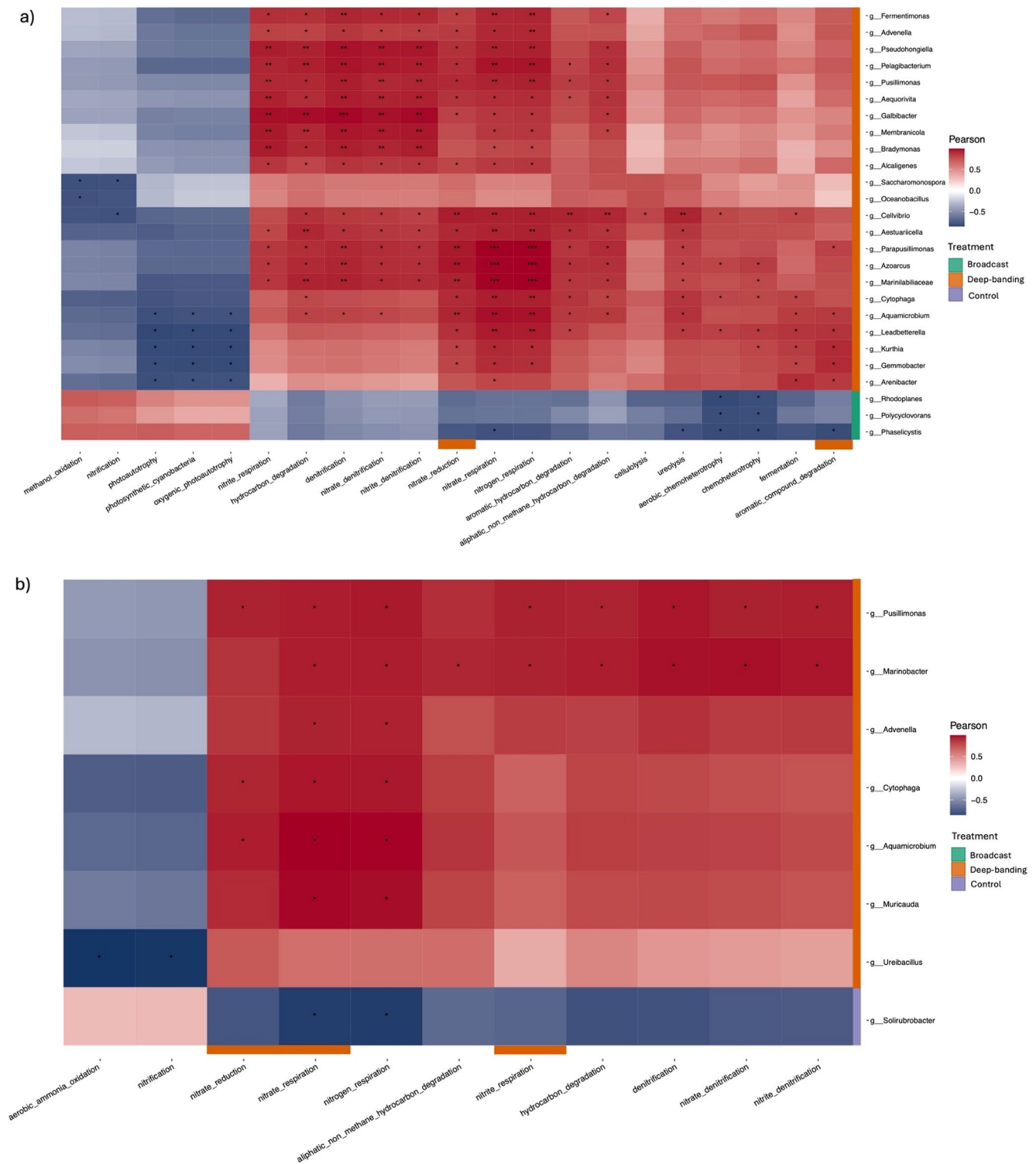


Fig. 7 Correlation between FAPROTAX-predicted functions and differentially abundant genera across treatments (Control, Broadcasted, Deep-banding) at (a) maturation and (b) ripening. The red-to-blue color scale represents positive to negative Pearson correlation values.

Asterisks indicate statistically significant correlations ($p < 0.05$). Edge colors show the treatment in which the predicted function or genus was more abundant

maturation, stronger correlations between taxa and predicted functions were observed, consistent with an early phase in which microbial recruitment is largely driven by soil physicochemical conditions before plant-mediated selection becomes more dominant (Francioli et al. 2018; Xiong et al. 2021). At ripening, these correlations weakened, suggesting the establishment of a more stabilized, plant-shaped microbial community. This pattern aligns with evidence that early-stage rhizosphere communities are more dynamic and responsive to soil conditions, whereas later-stage communities exhibit greater resilience, likely due to tighter microbial interactions and shifts in root exudation profiles (Chaparro et al. 2014; Francioli et al. 2021a; Lewin et al. 2024).

An important strength of this study lies in the integration of temporally resolved sampling with full-length 16S rRNA gene sequencing using Oxford Nanopore technology. Sequencing the entire ~1,500 bp 16S rRNA gene enabled improved taxonomic resolution compared with short-read approaches, allowing a larger fraction of sequences to be classified at the genus and species levels. This enhanced resolution facilitated more confident associations between specific taxa and predicted functional traits across treatments and developmental stages. At the same time, several limitations should be acknowledged. Functional predictions were inferred from taxonomic profiles rather than measured directly, and thus represent potential rather than realized microbial activity. In addition, the experiment was conducted in a single soil type and growing season, which may limit the generalizability of the findings across different pedoclimatic conditions. Despite these constraints, the observed consistency between soil property shifts, microbial community responses, and predicted functional patterns provides a coherent framework for understanding how compost placement influences rhizosphere processes. From an applied perspective, our results suggest that sub-surface compost placement may offer a practical strategy to steer rhizosphere microbial communities toward nitrogen-cycling functions, thereby informing management decisions aimed at improving nutrient use efficiency and soil health in horticultural systems.

Conclusion

This study demonstrates that the spatial placement of compost is a critical determinant of its effectiveness in agroecosystems. While surface broadcasting of green-waste compost induced only minor changes in soil conditions, deep banding consistently improved key edaphic parameters, including soil carbon and nitrogen pools. These changes were accompanied by the establishment of a distinct rhizosphere bacterial community in red bell pepper across

developmental stages, characterized by the selective enrichment of nitrogen-associated taxa and an increased predicted potential for key nitrogen transformation pathways.

Overall, deep compost banding emerges as a strategic soil management practice capable of modulating nutrient availability and shaping rhizosphere microbial community assembly. The combination of temporally resolved sampling with full-length 16S rRNA gene sequencing provides a robust framework for linking compost placement strategies to microbial composition and functional potential with enhanced taxonomic resolution.

To build on these findings, future research should evaluate the long-term persistence and stability of these compost-induced shifts across diverse soil types, cropping seasons, and plant species. Specifically, coupling microbial profiling with direct measurements of nitrogen fluxes, nitrification kinetics, and soil aggregation dynamics is necessary to confirm the mechanistic pathways proposed here. Additionally, integrating root exudate collection and metabolomic profiling would clarify the feedback between compost placement, plant physiology, and rhizosphere assembly. Such integrative approaches are essential to optimize organic amendments for soil health, nutrient-use efficiency, and sustainable horticultural production.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00374-026-01988-8>.

Acknowledgements We wish to thank Viktoria Blumrich, Alina Dietze, Tobias Pfeiffer and Robert Kunz for kindly providing the soil parameters.

Author contributions M.C.A. (Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft), M.S. (Data curation, Formal analysis, Methodology, Validation, Visualization), M.S. (Investigation, Methodology, Validation), C.M.G. (Investigation, Methodology, Project administration), J.Z. (Conceptualization, Investigation, Project administration, Supervision), and D.F. (Conceptualization, Supervision, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review & editing). All authors read and approved the final manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL.

Data availability All sequences have been submitted to the Sequence Read Archive (<https://www.ncbi.nlm.nih.gov/sra>) under the BioProject accession PRJNA1333386.

Declarations

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.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Aja-Macaya P, Conde-Pérez K, Trigo-Tasende N, Buetas E, Nasser-Ali M, Niñón P, Rumbo-Feal S, Ladra S, Bou G, Mira Á, Vallejo JA, Poza M (2025) Nanopore full length 16S rRNA gene sequencing increases species resolution in bacterial biomarker discovery. *Sci Rep* 15(1):26486. <https://doi.org/10.1038/s41598-025-10999-8>
- Assirelli A, Fornasier F, Caputo F, Manici LM (2023) Locally available compost application in organic farms: 2-year effect on biological soil properties. *Renew Agric Food Syst* 38:e16. <https://doi.org/10.1017/S1742170523000078>
- Azeem M, Hale L, Montgomery J, Crowley D, McGiffen ME (2020) Biochar and compost effects on soil microbial communities and nitrogen induced respiration in turfgrass soils. *PLoS ONE* 15(11):e0242209. <https://doi.org/10.1371/journal.pone.0242209>
- Bünemann EK, Bongiorno G, Bai Z, Creamer RE, De Deyn G, De Goede R, Flesskens L, Geissen V, Kuypers TW, Mäder P, Pulleman M, Sukkel W, Van Groenigen JW, Brussaard L (2018) Soil quality – A critical review. *Soil Biol Biochem* 120:105–125. <https://doi.org/10.1016/j.soilbio.2018.01.030>
- Burg P, Badalíková B, Mašán V, Csáki Š, Burgová J, Turan J, Matwijczuk A (2023) Evaluation of the effect of deep compost application in the areas around vineyard tree trunks on selected soil chemical properties and the vegetative growth of the vine. *Processes* 11(2):632. <https://doi.org/10.3390/pr11020632>
- Chaparro JM, Badri DV, Vivanco JM (2014) Rhizosphere Microbiome assemblage is affected by plant development. *ISME J* 8(4):790–803. <https://doi.org/10.1038/ismej.2013.196>
- Cucu MA, Gilardi G, Pugliese M, Matić S, Gisi U, Gullino ML, Garibaldi A (2019) Influence of different biological control agents and compost on total and nitrification-driven microbial communities at rhizosphere and soil level in a lettuce - *Fusarium oxysporum* f. sp. *lactucae* pathosystem. *J Appl Microbiol* 126(3):905–918. <https://doi.org/10.1111/jam.14153>
- De Rosa D, Basso B, Rowlings DW, Scheer C, Biala J, Grace PR (2017) Can organic amendments support sustainable vegetable production? *Agron J* 109(5):1856–1869. <https://doi.org/10.2134/agronj2016.12.0739>
- Doi Y, Takaya N, Takizawa N (2009) Novel denitrifying bacterium *Ochrobactrum anthropi* YD50.2 tolerates high levels of reactive nitrogen oxides. *Appl Environ Microbiol* 75(16):5186–5194. <https://doi.org/10.1128/AEM.00604-09>
- Eidlinger A, Garland G, Hartman K, Banerjee S, Degrune F, García-Palacios P, Hallin S, Valzano-Held A, Herzog C, Jansa J, Kost E, Maestre FT, Pescador DS, Philippot L, Rillig MC, Romdhane S, Saghai A, Spor A, Frossard E, Van Der Heijden MGA (2022) Agricultural management and pesticide use reduce the functioning of beneficial plant symbionts. *Nat Ecol Evol* 6(8):1145–1154. <https://doi.org/10.1038/s41559-022-01799-8>
- Francioli D, Schulz E, Buscot F, Reitz T (2018) Dynamics of soil bacterial communities over a vegetation season relate to both soil nutrient status and plant growth phenology. *Microb Ecol* 75(1):216–227. <https://doi.org/10.1007/s00248-017-1012-0>
- Francioli D, Cid G, Kanukollu S, Ulrich A, Hajirezaei M-R, Kolb S (2021a) Flooding causes dramatic compositional shifts and depletion of putative beneficial bacteria on the spring wheat microbiota. *Front Microbiol* 12:773116. <https://doi.org/10.3389/fmicb.2021.773116>
- Francioli D, Lentendu G, Lewin S, Kolb S (2021b) DNA metabarcoding for the characterization of terrestrial Microbiota—Pitfalls and solutions. *Microorganisms* 9(2):361. <https://doi.org/10.3390/microorganisms9020361>
- Gan J, Zou W, Han X, Chen X, Yan J, Lu X (2023) Effects of organic materials and their incorporation depths on humus substances structure and soil microbial communities' characteristics in a Chinese Mollisol. *Agronomy* 13(8):2169. <https://doi.org/10.3390/agronomy13082169>
- Gil-Martínez M, Madejón P, Madejón E, De Sosa LL (2025) Compost and vegetation cover drive soil fertility, microbial activity, and community in organic farming soils. *Plant Soil* 516(1):299–321. <https://doi.org/10.1007/s11104-025-07720-z>
- Hannula SE, Heinen R, Huberty M, Steinauer K, De Long JR, Jongen R, Bezemer TM (2021) Persistence of plant-mediated microbial soil legacy effects in soil and inside roots. *Nat Commun* 12(1):5686. <https://doi.org/10.1038/s41467-021-25971-z>
- Heisey S, Ryals R, Maaz TM, Nguyen NH (2022) A single application of compost can leave lasting impacts on soil microbial community structure and alter Cross-Domain interaction networks. *Front Soil Sci* 2:749212. <https://doi.org/10.3389/fsoil.2022.749212>
- Hsiao C, Mushtaq M, Sassenrath GF, Zeglin LH, Hettiarachchi GM, Rice CW (2025) Long-Term tillage and compost shape soil microbes under soil organic carbon equilibrium. *Eur J Soil Sci* 76(3):e70125. <https://doi.org/10.1111/ejss.70125>
- Huang R, McGrath SP, Hirsch PR, Clark IM, Storkey J, Wu L, Zhou J, Liang Y (2019) Plant-microbe networks in soil are weakened by century-long use of inorganic fertilizers. *Microb Biotechnol* 12(6):1464–1475. <https://doi.org/10.1111/1751-7915.13487>
- Kraut-Cohen J, Zolti A, Rotbart N, Bar-Tal A, Laor Y, Medina S, Shawahna R, Saadi I, Raviv M, Green SJ, Yermiyahu U, Minz D (2023) Short- and long-term effects of continuous compost amendment on soil Microbiome community. *Comput Struct Biotechnol J* 21:3280–3292. <https://doi.org/10.1016/j.csbj.2023.05.030>
- LeBlanc NR, Harrigan FC (2024) Green waste compost impacts microbial functions related to carbohydrate use and active dispersal in plant Pathogen-Infested soil. *Microb Ecol* 87(1):44. <https://doi.org/10.1007/s00248-024-02361-8>
- Legendre P, Gallagher ED (2001) Ecologically meaningful transformations for ordination of species data. *Oecologia* 129(2):271–280. <https://doi.org/10.1007/s004420100716>
- Lewin S, Schaller J, Kolb S, Francioli D (2024) Amorphous silica fertilization ameliorated soil properties and promoted putative soil beneficial microbial taxa in a wheat field under drought. *Appl Soil Ecol* 196:105286. <https://doi.org/10.1016/j.apsoil.2024.105286>
- Li S, Liu Z, Li J, Liu Z, Gu X, Shi L (2022) Cow manure compost promotes maize growth and ameliorates soil quality in Saline-Alkali soil: role of fertilizer addition rate and application depth. *Sustainability* 14(16):10088. <https://doi.org/10.3390/su141610088>
- Li H, Shang Y, Gao J, Zhang H, Chen H, Wang X, Guo J, Zhang X, Wang J, Li Y (2024) Subsurface manure application enhances soil quality, ecosystem multifunctionality, and crop yield in the North China plain. *Appl Soil Ecol* 203:105674. <https://doi.org/10.1016/j.apsoil.2024.105674>
- Liao X, Tang T, Li J, Wang J, Neher DA, Zhang W, Xiao J, Xiao D, Hu P, Wang K, Zhao J (2024) Nitrogen fertilization increases the

- niche breadth of soil nitrogen-cycling microbes and stabilizes their co-occurrence network in a karst agroecosystem. *Agric Ecosyst Environ* 374:109177. <https://doi.org/10.1016/j.agee.2024.109177>
- Liu W, Xiong Y, Xu X, Xu F, Hussain S, Xiong H, Yuan J (2019) Deep placement of controlled-release Urea effectively enhanced nitrogen use efficiency and fresh ear yield of sweet corn in fluvo-aquic soil. *Sci Rep* 9(1):20307. <https://doi.org/10.1038/s41598-019-56912-y>
- Liu S, Khan MH, Yuan Z, Hussain S, Cao H, Liu Y (2021) Response of soil Microbiome structure and its network profiles to four soil amendments in monocropping strawberry greenhouse. *PLoS ONE* 16(9):e0245180. <https://doi.org/10.1371/journal.pone.0245180>
- Liu Y, Xin J, Wang Y, Yang Z, Liu S, Zheng X (2022) Dual roles of dissolved organic nitrogen in groundwater nitrogen cycling: nitrate precursor and denitrification promoter. *Sci Total Environ* 811:151375. <https://doi.org/10.1016/j.scitotenv.2021.151375>
- Louca S, Parfrey LW, Doebeli M (2016) Decoupling function and taxonomy in the global ocean Microbiome. *Science* 353(6305):1272–1277. <https://doi.org/10.1126/science.aaf4507>
- Lynch JM, Brimecombe MJ, De Leij FA (2001) Rhizosphere. *Encyclopedia of life sciences*, 1st edn. Wiley
- Martínez-Blanco J, Lazcano C, Christensen TH, Muñoz P, Rieradevall J, Möller J, Antón A, Boldrin A (2013) Compost benefits for agriculture evaluated by life cycle assessment. A review. *Agron Sustain Dev* 33(4):721–732. <https://doi.org/10.1007/s13593-013-0148-7>
- Matsuoka M, Park S, An S-Y, Miyahara M, Kim S-W, Kamino K, Fushinobu S, Yokota A, Wakagi T, Shoun H (2012) *Advenella faeciporci* sp. nov., a nitrite-denitrifying bacterium isolated from nitrifying-denitrifying activated sludge collected from a laboratory-scale bioreactor treating piggery wastewater. *Int J Syst Evol Microbiol* 62(Pt12):2986–2990. <https://doi.org/10.1099/ijs.0.037440-0>
- Meng Y, Zhang Z, Li J, Lv Y, Lin X (2019) The complete genome sequence of the denitrifying bacterium *marinobacter* sp. Arc7-DN-1 isolated from Arctic ocean sediment. *Mar Genomics* 45:8–10. <https://doi.org/10.1016/j.margen.2018.10.008>
- Nannipieri P, Penton CR, Purahong W, Schlöter M, Van Elsas JD (2019) Recommendations for soil Microbiome analyses. *Biol Fertil Soils* 55(8):765–766. <https://doi.org/10.1007/s00374-019-01409-z>
- Nannipieri P, Hannula SE, Pietramellara G, Schlöter M, Sizmur T, Pathan SI (2023) Legacy effects of rhizodeposits on soil microbiomes: A perspective. *Soil Biol Biochem* 184:109107. <https://doi.org/10.1016/j.soilbio.2023.109107>
- Nkebiwe PM, Weinmann M, Bar-Tal A, Müller T (2016) Fertilizer placement to improve crop nutrient acquisition and yield: A review and meta-analysis. *Field Crops Res* 196:389–401. <https://doi.org/10.1016/j.fcr.2016.07.018>
- Olberz M, Kahlen K, Zinkernagel J (2018) Assessing the impact of reference evapotranspiration models on decision support systems for irrigation. *Horticulturae* 4(4):49. <https://doi.org/10.3390/horticulturae4040049>
- Philippot L, Spor A, Hénault C, Bru D, Bizouard F, Jones CM, Sarr A, Maron P-A (2013) Loss in microbial diversity affects nitrogen cycling in soil. *ISME J* 7(8):1609–1619. <https://doi.org/10.1038/ismej.2013.34>
- Porter SS, Sachs JL (2020) Agriculture and the disruption of Plant–Microbial symbiosis. *Trends Ecol Evol* 35(5):426–439. <https://doi.org/10.1016/j.tree.2020.01.006>
- Qin Y-L, Liang Z-L, Ai G-M, Liu W-F, Tao Y, Jiang C-Y, Liu S-J, Li D-F (2024) Heterotrophic nitrification by *Alcaligenes faecalis* links organic and inorganic nitrogen metabolism. *ISME J* 18(1):wrae174. <https://doi.org/10.1093/ismej/wrae174>
- Raimi AR, Ezeokoli OT, Adeleke RA (2023) Soil nutrient management influences diversity, community association and functional structure of rhizosphere bacteriome under vegetable crop production. *Front Microbiol* 14:1229873. <https://doi.org/10.3389/fmicb.2023.1229873>
- Rodríguez-Pérez H, Ciuffreda L, Flores C (2021) NanoCLUST: a species-level analysis of 16S rRNA nanopore sequencing data. *Bioinformatics* 37(11):1600–1601. <https://doi.org/10.1093/bioinformatics/btaa900>
- Sakoda M, Mizusawa M, Shiotsu F, Sakagami N, Guo Y, Masutomi Y, Fujii D, Isobe K, Fujiwara T, Senoo K, Nishizawa T (2019) *Azoarcus* sp. strain KH32C affects rice plant growth and the root-associated soil bacterial community in low nitrogen input paddy fields. *Soil Sci Plant Nutr* 65(5):451–459. <https://doi.org/10.1080/00380768.2019.1666648>
- Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C (2011) Metagenomic biomarker discovery and explanation. *Genome Biol* 12(6):R60. <https://doi.org/10.1186/gb-2011-12-6-r60>
- Stock W, Rousseau C, Dierickx G, D'hondt S, Amadei Martínez L, Dittami SM, Van Der Loos LM, De Clerck O (2024) Breaking free from references: a consensus-based approach for community profiling with long amplicon nanopore data. *Brief Bioinform* 26(1):bbae642. <https://doi.org/10.1093/bib/bbae642>
- Su J-Y, Liu C-H, Tampus K, Lin Y-C, Huang C-H (2022) Organic amendment types influence soil Properties, the soil bacterial Microbiome, and tomato growth. *Agronomy* 12(5):1236. <https://doi.org/10.3390/agronomy12051236>
- Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK (2020) Plant–microbiome interactions: from community assembly to plant health. *Nat Rev Microbiol* 18(11):607–621. <https://doi.org/10.1038/s41579-020-0412-1>
- Tsiafouli MA, Thébault E, Sgardelis SP, De Ruiter PC, Van Der Putten WH, Birkhofer K, Hemerik L, De Vries FT, Bardgett RD, Brady MV, Bjornlund L, Jørgensen HB, Christensen S, Hertefeldt TD, Hotes S, Gera Hol WH, Frouz J, Liiri M, Mortimer SR, Setälä H, Tzanopoulos J, Uteseny K, Pižl V, Stary J, Wolters V, Hedlund K (2015) Intensive agriculture reduces soil biodiversity across Europe. *Glob Change Biol* 21(2):973–985. <https://doi.org/10.1111/gcb.12752>
- Ullah F, Ali S, Siraj M, Akhtar MS, Zaman W (2025) Plant microbiomes alleviate abiotic Stress-Associated damage in crops and enhance Climate-Resilient agriculture. *Plants* 14(12):1890. <https://doi.org/10.3390/plants14121890>
- Veselovsky V, Romanov M, Zoruk P, Larin A, Babenko V, Morozov M, Stokach A, Zakharevich N, Khamidova S, Danilova A, Vatlin A, Pavshintsev V, Chenguang F, Tsybizov D, Mitkin N, Galanova O, Zakharenko A, Golokhvast K, Klimina K (2025) Comparative evaluation of sequencing platforms: Pacific Biosciences, Oxford nanopore Technologies, and illumina for 16S rRNA-based soil Microbiome profiling. *Front Microbiol* 16:1633360. <https://doi.org/10.3389/fmicb.2025.1633360>
- Vido JJ, Wang X, Sale PWG, Celestina C, Shindler AE, Hayden HL, Tang C, Wood JL, Franks AE (2024) Bacterial community shifts occur primarily through rhizosphere expansion in response to subsoil amendments. *Environ Microbiol* 26(3):e16587. <https://doi.org/10.1111/1462-2920.16587>
- Wei T-J, Li G, Cui Y-R, Xie J, Teng X, Wang Y-J, Li Z-H, Guan F-C, Liang Z-W (2024) Compost mediates the recruitment of core bacterial communities in alfalfa roots to enhance their productivity potential in saline-sodic soils. *Front Microbiol* 15:1502536. <https://doi.org/10.3389/fmicb.2024.1502536>
- Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 173(2):697–703. <https://doi.org/10.1128/jb.173.2.697-703.1991>
- Wohlfahrt Y, Krüger K, Papsdorf D, Tittmann S, Stoll M (2022) Grapevine leaf physiology and morphological characteristics to elevated CO₂ in the vineyardface (Free air carbon dioxide

- Enrichment) experiment. *Front Plant Sci* 13:1085878. <https://doi.org/10.3389/fpls.2022.1085878>
- Wu P, Wu Q, Huang H, Xie L, An H, Zhao X, Wang F, Gao Z, Zhang R, Bangura K, Xue J, Sun M, Zhang P, Jia Z, Gao Z (2024) Global meta-analysis and three-year field experiment shows that deep placement of fertilizer can enhance crop productivity and decrease gaseous nitrogen losses. *Field Crops Res* 307:109263. <https://doi.org/10.1016/j.fcr.2024.109263>
- Xiong C, Singh BK, He J-Z, Han Y-L, Li P-P, Wan L-H, Meng G-Z, Liu S-Y, Wang J-T, Wu C-F, Ge A-H, Zhang L-M (2021) Plant developmental stage drives the differentiation in ecological role of the maize Microbiome. *Microbiome* 9(1):171. <https://doi.org/10.1186/s40168-021-01118-6>
- Xu D, Yu X, Chen J, Li X, Chen J, Li J (2023) Effects of compost as a soil amendment on bacterial community diversity in saline–alkali soil. *Front Microbiol* 14:1253415. <https://doi.org/10.3389/fmicb.2023.1253415>
- Yang C-X, Chen S-J, Hong X-Y, Wang L-Z, Wu H-M, Tang Y-Y, Gao Y-Y, Hao G-F (2025) Plant exudates-driven Microbiome recruitment and assembly facilitates plant health management. *FEMS Microbiol Rev* 49:fuaf008. <https://doi.org/10.1093/femsre/fuaf008>
- Yergaliyev T, Rios-Galicia B, Camarinha-Silva A (2025) NaMeco - Nanopore full-length 16S rRNA gene reads clustering and annotation. *BMC Genomics* 27(1):62. <https://doi.org/10.1186/s12864-025-12415-x>
- Zhu L, Sun H, Liu L, Zhang K, Zhang Y, Li A, Bai Z, Wang G, Liu X, Dong H, Li C (2024) Optimizing crop yields while minimizing environmental impact through deep placement of nitrogen fertilizer. *J Integr. Agric* <https://doi.org/10.1016/j.jia.2024.05.012>. Agric:S209531192400203X
- Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.