Rhizosphere 40 (2026) 101517

Contents lists available at ScienceDirect

Rhizosphere

journal homepage: www.elsevier.com/locate/rhisph

Groundcover is associated with compositional turnover in banana microbiomes, including shifts in multiple core taxa

Sutrisno [a,b], Rebecca Lyons [a], Hazel R. Lapis-Gaza [d], Jiarui Sun [a], Luciasih Agustini [c], Anthony B. Pattison [d], Paul G. Dennis [a,*]

[a] School of the Environment, The University of Queensland, Brisbane, QLD, 4072, Australia
[b] Department of Biology, Walisongo State Islamic University of Semarang, Semarang, 50185, Indonesia
[c] Research Center for Applied Microbiology, National Research and Innovation Agency, Cibinong, 16911, Indonesia
[d] Department of Primary Industries, Centre for Wet Tropics Agriculture, 24 Experimental Station Road, South Johnstone, QLD, 4859, Australia

ARTICLE INFO

Keywords:
Microbiome engineering
Agroecological management
Banana microbiome
Core microbiome
Sustainable agriculture
Food security

ABSTRACT

Banana production is constrained by Fusarium wilt and other soil-borne stresses, driving interest in the banana soil microbiome because of its potential roles in pathogen suppression, nutrient cycling, and crop resilience. Here, we used existing variation in groundcover density among commercial banana farms to test its association with soil bacterial communities, potential extracellular enzyme activities, and the relative abundance of previously defined banana core taxa. Bacterial abundance, estimated by quantitative PCR, and bacterial alpha diversity were not significantly associated with groundcover. Of the measured potential extracellular enzyme activities, only chitinase and acid phosphatase were significantly associated with groundcover after accounting for measured edaphic variation. Metabarcoding revealed significant differences in bacterial community composition across groundcover classes, and this association remained significant after accounting for differences in measured soil properties. Of the 48 dominant bacterial OTUs, 32 (66.7%) matched previously defined banana core taxa. Although the summed relative abundance of core-matching OTUs was not associated with groundcover, eight of the 15 relatively abundant OTUs that differed significantly among groundcover classes matched previously defined core taxa. Together, these results indicate that variation in groundcover density was associated with differences in bacterial community composition, including the relative abundances of individual core taxa. If experimentally validated, these findings could provide a basis for exploring groundcover management as an agroecological approach to shaping banana-associated soil microbiomes.

1. Introduction

Bananas (*Musa* spp.) are among the world's most important fruit crops and make major contributions to food security, livelihoods, and export economies across the tropics and subtropics (FAO, 2026). Their production, however, is constrained by a range of biotic stresses, among which Fusarium wilt is one of the most damaging because effective and durable control remains difficult (Dita et al., 2018). These challenges have driven growing interest in the banana microbiome as a target for improving plant health, soil functioning, and production resilience (Birt et al., 2022; Noviana et al., 2025; Xue et al., 2015). In principle, microbiome-informed management could contribute to disease suppression, nutrient cycling, and more stable crop performance, but translating this potential into practical field strategies remains challenging (Toju et al., 2018).

One major obstacle is the sheer complexity of banana-associated microbiomes. Soil and plant-associated bacterial communities are highly diverse and can vary strongly across compartments, farms, soils, and environmental conditions (Toju et al., 2018; Birt et al., 2022). This compositional heterogeneity makes it difficult to distinguish taxa that are persistent and ecologically important from those that are transient or context-dependent. Focusing on the core microbiome provides one way to reduce this complexity (Risely, 2020). In banana, previous work identified 36 core bacterial OTUs consistently associated with Australian banana systems (Birt et al., 2022), and this concept has since been extended across multiple countries to define a broader international core (Clarke et al., 2026). These persistent taxa provide a tractable set of organisms for moving beyond descriptive community analysis towards

* Corresponding author.
E-mail address: p.dennis@uq.edu.au (P.G. Dennis).

https://doi.org/10.1016/j.rhisph.2026.101517
Received 7 July 2026; Received in revised form 21 October 2026; Accepted 21 October 2026
Available online 24 October 2026
2452-2198/© 2026 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

ecological and functional understanding of the banana microbiome. Because they recur across banana systems, they represent persistent members of the microbial assemblages associated with banana plants and may play important ecological roles. They provide, therefore, a practical focus for biobanking, genome-based characterisation, functional screening, and the development of microbiome-informed strategies for banana production (Paterson, 2024; Zhang et al., 2022).

To translate this ecological knowledge into practical benefit for banana production, it is necessary to understand not only which taxa persist, but also how their abundance and activity can be influenced. One strategy is direct intervention through the isolation and reintroduction of individual strains or synthetic communities (Lv et al., 2023; Prigigallo et al., 2022). In this regard, core taxa may be particularly promising because their repeated association with banana suggests strong host compatibility (Zhou et al., 2024); however, successful establishment and consistent field performance cannot be assumed (Fadiji et al., 2024; Henry and Bergelson, 2025). A complementary strategy is agroecological management: identifying the agronomic practices and environmental conditions that favour or suppress desirable members of the microbiome already present in plantations (Zhou and Fong, 2021). This approach is especially attractive because it connects microbiome management to practices already used on farms, while also creating scope to develop new practices specifically aimed at shaping the microbiome (Delitte et al., 2021). Understanding how routine farm management influences the microbiome may therefore help steer banana microbiomes in situ and guide the design of management practices intended to shape them.

Groundcover management is one such candidate practice (Rames et al., 2018). In banana plantations, the amount of living groundcover varies markedly among farms and can influence several aspects of the below-ground environment, including light penetration to the soil surface, litter inputs, root exudation, soil protection, and potentially soil moisture and nutrient dynamics (Koudahe et al., 2022; Xu et al., 2024). Previous studies in banana and other perennial cropping systems suggest that groundcover can alter soil biological communities and, in some cases, improve ecosystem services (Blanco-Canqui et al., 2015; Vukicevich et al., 2016). Groundcover has been associated with reduced Fusarium wilt incidence in banana, and studies in other orchard and plantation systems have reported associations between groundcover vegetation, microbial community composition, and taxa linked with biological regulation or soil functioning (Ma et al., 2021; Pattison et al., 2014; Rames et al., 2018; Santiago et al., 2023). Together, these findings highlight groundcover as a potentially important factor associated with below-ground ecological variation. However, it remains unclear whether variation in groundcover is associated primarily with broad shifts in banana-associated bacterial community structure, with changes in previously defined core taxa, or with both, and to what extent any such patterns are shared with edaphic differences among farms.

A further complication is that groundcover in commercial systems is rarely independent of other farm-to-farm differences. Farms with contrasting groundcover may also differ in edaphic properties, and those soil differences may represent either confounding background variation or potential mediating pathways linking groundcover to the microbiome. For example, some variables, such as total organic carbon or nitrogen, may plausibly increase under greater vegetative cover through enhanced primary production, litter return, and rhizodeposition (Badri and Vivanco, 2009; Koudahe et al., 2022). In contrast, other properties, such as aluminium and sodium content, may more strongly reflect background site conditions not directly generated by groundcover itself (Yan and Arthur, 2025). As a result, observational comparisons across farms cannot cleanly separate all direct and indirect pathways. Nevertheless, comparing unadjusted associations with models accounting for measured edaphic variation allows us to assess whether associations between groundcover and microbial communities persist after accounting for differences in soil properties. However, these analyses cannot establish an independent causal effect of groundcover, as some soil properties may lie on pathways linking groundcover to microbial communities.

In this observational study, we used existing variation in groundcover density among commercial banana farms to examine associations between groundcover classes and plant-associated soil bacterial communities. We assessed bacterial abundance by quantitative PCR, characterised bacterial diversity and composition using 16S rRNA gene amplicon sequencing, quantified several potential extracellular enzyme activities as indicators of microbial functional potential, and evaluated whether the relative abundances of individual OTUs matching previously defined Australian (Birt et al., 2022) and international (Clarke et al., 2026) banana core taxa, as well as their summed relative abundances, varied among groundcover classes. To address the possibility that farms with different groundcover densities also differed in soil chemistry, we analysed the data using both unadjusted models and complementary edaphic-adjusted approaches based on forward selection of soil variables. We hypothesised that groundcover would be associated with differences in soil bacterial community composition, and further examined whether bacterial abundance, alpha diversity, potential extracellular enzyme activities, and the summed relative abundance of previously defined banana core taxa also varied among groundcover classes. Finally, we assessed whether these associations persisted after accounting for measured edaphic variation.

2. Materials and methods

2.1. Soil collection and site descriptions

Soil samples were collected from nine commercial banana farms across Far North Queensland, Australia (Table S1). Farms were assigned to one of three groundcover classes based on vegetative groundcover density: low ($0 \leq$ groundcover $< 33.3\%$), medium ($33.3\% \leq$ groundcover $< 66.7\%$), and high (groundcover $\geq 66.7\%$). Groundcover density was assessed along three line transects per farm, with each transect extending from the interrow to the base of the banana plants. Assessments were conducted during a single season by a single assessor using the same procedure across all farms. Groundcover vegetation was not systematically characterised but included grasses (including buffalo grass and signal grass), legumes, creepers, and ruderal species, with patches of bare soil also present in some transects. Soil samples were collected 5 cm from the base of the banana plants. For each farm, three replicate soil samples were collected to a depth of 10 cm using a sterile trowel. Samples were kept on ice during transport to the laboratory and stored at $-20^{\circ}C$ prior to DNA extraction, potential enzyme activity assays, and soil chemical analysis.

2.2. Soil chemical and biochemical analysis

Soil samples were analysed for physicochemical characteristics by Nutrient Advantage Laboratory (Werribee, Victoria) using methods described by Rayment and Lyons (2010). Analyses included measurements of pH and electrical conductivity (EC) in water (1:5 w/v), total organic carbon (combustion), total nitrogen (combustion), Colwell phosphorus (P), exchangeable cations (K, and Na), and chloride (Cl). Potential activities of extracellular enzymes were determined as described by Lapis-Gaza and Pattison (2021). These included alpha-glucosidase, beta-glucosidase, chitinase, acid phosphatase, arylsulphatase, and xylosidase.

2.3. DNA extraction and quantitative PCR (qPCR)

DNA was extracted from all soil samples using the DNeasy PowerSoil Pro kit (Qiagen) according to the manufacturer's protocol, with the addition of a plasmid containing an artificial 16S rRNA gene sequence (pMUT16S; Daniell et al., 2012; Lyons et al., 2025) to facilitate estimation of DNA extraction efficiency and PCR inhibition. For preparation

of the plasmid standard, *Escherichia coli* cultures transformed with pMUT16S were grown in Luria Broth (LB) supplemented with ampicillin (100 μg mL^{-1}) at 37°C with shaking for 15 h. Plasmid DNA was extracted using the PureYield Plasmid Miniprep System (Promega) and quantified using a Qubit fluorometer (Thermo Fisher Scientific), following the manufacturers' protocols.

Quantitative PCR was used to quantify bacterial 16S rRNA gene copy number and the pMUT16S spike-in control. Primer pairs MUT4-F (CCTACGGGAGGCACGTC) and MUT4-R (ATTACCGCGGCTGGACC) were used for pMUT16S rRNA genes, (Lyons et al., 2025; Tim Daniell, pers. comm.), and Eub338 (ACTCCTACGGGAGGCAGCAG) and Eub518 (ATTACCGCGGCTGCTGG) were used for bacterial 16S rRNA genes (Fierer and Jackson, 2006). Reactions were performed using ABI SYBR Green PCR Master Mix (Thermo Fisher Scientific) for bacterial 16S and Bioline Sensi-Fast Sybr Lo-Rox kit (Bioline) for MUT16S. Each 10 μL reaction contained 2 μL template DNA, 1× Master Mix, and primers at final concentrations of 0.3 μM for pMUT16S or 0.5 μM for bacterial 16S, with molecular biology grade water added to volume. Thermal cycling was conducted on a QuantStudio 1 qPCR machine (Applied Biosystems). For pMUT16S, conditions were 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 66°C for 20 s, followed by melt curve analysis. For bacterial 16S, conditions were 95°C for 15 min, followed by 40 cycles of 95°C for 1 min, 60°C for 30 s, and 72°C for 1 min, followed by melt curve analysis. Standard curves were generated using 10-fold serial dilutions ranging from 10^{10} to 10^{1} copies of the pMUT16S rRNA gene and from 10^{8} to 10^{1} copies of a plasmid containing the bacterial 16S rRNA gene amplicon. Each assay was run in triplicate, and quantification was performed using QuantStudio Design and Analysis Software v1.2. Amplicon specificity was verified from melt curves. Bacterial 16S rRNA gene copy numbers were converted to copies g^{-1} dry soil and corrected for DNA recovery according to Lyons et al. (2025), where:

DNA recovery = pMUT16S rRNA gene copies in the DNA extract/ pMUT16S rRNA gene copies added as a spike.

Corrected bacterial 16S rRNA gene copies = Bacterial 16S rRNA gene copies g^{-1} dry soil/DNA recovery.

2.4. PCR amplification and sequencing of 16S rRNA gene

Polymerase chain reaction (PCR) was used to amplify 16S rRNA genes with the primers (AACMGGATTAGATACCCKG) and 1193R (ACGTCATCCCCACCTTCC) (Redford et al., 2010). The end of primer sequence was modified to have adapter sequence overhang on the 5′ end ensuring compatibility with the Illumina flow cell and allow indexing for multiplexing. The following condition was used to amplify the 16S rRNA genes: 95°C for 10 min followed by 35 cycles of 95°C for 15 s, 54°C for 15 s, 72°C for 30 s, and 72°C for 5 min for final extension. Following purification and concentration normalization, amplicon samples were pooled in equal volumes. The pooled library was then subjected to sequencing on an Illumina MiSeq system following the protocol provided by the manufacturer. Indexed amplicons were then quantified using a PicoGreen dsDNA Quantification Kit (Invitrogen, Scoresby, VIC, Australia). Equal concentrations of each sample were pooled and sequenced on an Illumina MiSeq using 30% PhiX Control v3 (Illumina, Melbourne, VIC, Australia) and a MiSeq Reagent Kit v3 (600 cycles; Illumina) according to the manufacturer's instructions.

2.5. Bioinformatic analyses and matching sequences to the core banana microbiome

Sequence data were processed using a modified UPARSE pipeline (Edgar, 2013), with sequences clustered into operational taxonomic units (OTUs) at 97% similarity. This approach was selected to maintain methodological consistency with previous studies defining the Australian (Birt et al., 2022) and international (Clarke et al., 2026) banana core microbiomes, allowing direct comparison of OTU sequences with these reference datasets. Forward reads were demultiplexed using cutadapt within QIIME2 v2024.10 (Bolyen et al., 2019; Martin, 2011). Using USEARCH v10.0.240 (Edgar, 2010), we performed trimming, quality filtering, dereplicating, and OTU clustering with functions fastq_truncate, fastq_filter, fastx_uniques, and cluster_otus (97% similarity), respectively. Taxonomy was assigned to OTUs using BLASTN v2.16.0+ within QIIME2 against Greengenes2 (release 2024.09; McDonald et al., 2024). Non-bacterial sequences were removed based on their taxonomy using BIOM (McDonald et al., 2012). The 16S rRNA gene count dataset was rarefied to the lowest sequencing depth across samples (1350 reads per sample) and thereby retaining all samples in the analysis. Although this sequencing depth may limit detection of rare taxa, previous research indicates that broad patterns in bacterial beta diversity can be recovered at similar sequencing depth (Lundin et al., 2012). Alpha diversity, represented by the numbers of observed OTUs and Shannon's Diversity Index, was calculated using QIIME2. Representative OTU sequences from the present study were then compared to the sequences of the Australian 36 core bacteria reported by Birt et al. (2022) and the 74 international core bacterial taxa reported by Clarke et al. (2026) using USEARCH (Edgar, 2010). Sequences that were ≥97% similar to a core OTU sequence were considered potential matches to previously defined banana core taxa. For each sample, the relative abundances of OTUs matching the Australian and international core taxa were summed separately.

2.6. Statistical analyses

Differences in measured edaphic properties among low-, medium-, and high-groundcover farms were first assessed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) tests where appropriate. Effect sizes for univariate models are reported as η^2 and were calculated using the eta_squared function from the R package effect size (Ben-Shachar et al., 2020). Multivariate differences in edaphic properties among groundcover classes were assessed using permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) implemented using the adonis2 function from the R package vegan (Oksanen et al., 2026).

For bacterial abundance estimated by 16S rRNA gene qPCR, bacterial alpha-diversity metrics, summed relative abundance of OTUs matching previously defined banana core sets, and potential extracellular enzyme activities, we used a three-step modelling approach: 1) testing the association with groundcover alone in unadjusted linear models, 2) identifying a minimally adequate set of soil properties by forward selection of measured edaphic variables, and 3) testing whether the association with groundcover remained significant after inclusion of the retained soil properties. For Step 1, Tukey's HSD tests were used for *post hoc* comparisons among groundcover classes for unadjusted ANOVA models where the omnibus test was significant. For Step 2, candidate predictors were considered individually and the variable providing the strongest significant improvement in model fit was added. The procedure was repeated iteratively with the remaining variables until no further term improved the model without causing the reduced model to lose overall significance, yielding a final minimally adequate soil model. In Step 3, groundcover was then added to this model to test whether its association with the response variable remained significant after accounting for the retained soil properties.

Differences in bacterial community composition were assessed using PERMANOVA (veganadonis2) based on Hellinger-transformed OTU relative abundances (Legendre and Gallagher, 2001). As for univariate responses, we used a three-step modelling approach: 1) unadjusted models tested the association with groundcover alone; 2) a minimally adequate set of soil properties was identified by forward selection of measured edaphic variables, with candidate predictors considered individually at each step and the variable explaining the greatest additional proportion of variation (largest R^2) added to the model; and 3) groundcover was added to the resulting soil model to test whether its association with community composition remained significant after

accounting for the retained edaphic predictors. For Step 2, terms were evaluated sequentially using *by* = *"terms"* so that each candidate variable was assessed according to the additional variation it explained at the point of entry into the model. For Step 3, the final Soil + Groundcover model was evaluated using *by* = *"margin"*, so that the association with groundcover was tested while accounting for the retained soil variables and the other terms in the model. To assess whether significant PERMANOVA results reflected shifts in community centroids rather than differences in within-group dispersion, multivariate homogeneity of dispersion was evaluated using the function betadisper from the vegan R package (Oksanen et al., 2026). Community patterns were visualised using redundancy analysis (RDA) constrained by groundcover.

Differential-abundance analysis was performed using DESeq2 on pairwise contrasts among groundcover classes. OTU-level models were interpreted without inclusion of edaphic covariates because some measured soil properties, particularly TOC and TN, may plausibly lie on pathways linking groundcover to the soil microbiome. Including such variables in OTU-level models could therefore obscure associations involving potential mediating pathways, rather than isolating only background edaphic confounding. For interpretation and display, particular attention was given to OTUs that were both significant and had mean relative abundance ≥0.5% in at least one groundcover class.

3. Results

3.1. Groundcover classes differed in edaphic properties

Edaphic properties differed significantly among groundcover classes when analysed as a multivariate dataset (PERMANOVA, F = 3.01, R^2 = 20.1%, P = 0.013). In univariate analyses, TOC, available K, EC, and Cl differed significantly among groundcover classes, whereas pH, TN, Colwell P, and Na did not (Table 1; Fig. S1). TOC was generally higher in farms with greater groundcover, while EC, Cl, and available K showed less monotonic patterns, with medium-groundcover farms often distinct from both low- and high-groundcover farms. TN also tended to be higher in farms with greater groundcover, but this trend was not significant (P = 0.092).

3.2. Bacterial abundance

Bacterial abundance, estimated by 16S rRNA gene qPCR, was not significantly associated with groundcover in the unadjusted model (Table 2; Fig. S2). Although bacterial abundance varied among farms within each groundcover class, no consistent difference among low, medium, and high groundcover was evident (Fig. S2). Forward selection of edaphic variables identified total nitrogen (TN) and pH as the strongest predictors of bacterial abundance (Table 2). When groundcover was added after these retained edaphic predictors, its association with bacterial abundance remained non-significant (Table 2). These results indicate that variation in bacterial abundance was more strongly associated with differences in soil properties among farms, particularly TN and pH, than with groundcover class.

Table 2
Unadjusted and edaphic-adjusted linear models for bacterial abundance across groundcover classes. Bacterial abundance was estimated by 16S rRNA gene qPCR. The unadjusted model tested the effect of groundcover alone. The selected soil model shows the edaphic variables retained by forward selection, and the Soil + Groundcover model shows the effect of groundcover after accounting for those retained soil predictors. Effect sizes are reported as η^2 (%), with corresponding *F* and *P* values for each term. Asterisks denote significant *P* values ($P < 0.05$*, $P < 0.010$**, $P < 0.001$***).

Model	Term	η^2 (%)	F	P
Unadjusted	**Groundcover**	**3.0**	**0.4**	**0.69**
Selected soil model	TN (%)	64.8	34.4	<0.001***
	pH	18.2	5.3	0.030*
Soil + Groundcover	TN (%)	64.6	40.1	<0.001***
	pH	23.8	6.9	0.016*
	Groundcover	**14.7**	**1.9**	**0.175**

3.3. Bacterial alpha diversity

Bacterial alpha diversity was not significantly associated with groundcover class, whether assessed as observed richness (Sobs) or Shannon diversity (Fig. S3). Edaphic variables were likewise not retained as significant predictors of either alpha-diversity metric in the fitted models. Together, these results indicate that groundcover was not associated with broad changes in bacterial alpha diversity across farms.

3.4. Bacterial community composition

Bacterial communities were dominated by members of the Acidobacteriota, Actinomycetota, Bacillota, Chloroflexota, Gemmatimonadota, Nitrospirota, Pseudomonadota, and Verrucomicrobiota (Fig. 1). Of the 48 OTUs with a mean relative abundance of at least 0.5% in one or more groundcover class, 32 (66.7%) matched previously defined banana core taxa, indicating that recurrent banana-associated lineages were well represented among the dominant members of these communities (Fig. 1; Supplementary file 1).

In contrast to bacterial abundance and alpha diversity, bacterial community composition showed a stronger association with groundcover (Fig. 2; Table 3). Ordination showed clear separation among low, medium, and high groundcover communities with multiple core taxa contributing strongly to this pattern (Fig. 2). Groundcover explained 11.0% of variation in the unadjusted model (Table 3), and tests of multivariate dispersion were non-significant, indicating that this pattern reflected shifts in community centroids rather than unequal within-group dispersion ($P = 0.843$). Forward selection of edaphic variables retained Colwell P, EC, TOC, pH, Na, and Cl in the soil model (Table 3). When groundcover was added to the model after accounting for the retained edaphic predictors, its association with bacterial community composition remained significant, explaining an additional 7.9% of variation (Table 3). These results indicate that, although part of the

Table 1
Edaphic properties of soils from banana farms across the groundcover gradient. Values are means ± standard deviations for farms with low, medium, or high groundcover. Differences among groundcover classes were tested using one-way ANOVA. Effect sizes are reported as η^2 (%), together with the corresponding *F* and *P* values for the groundcover term. Asterisks indicate significant overall effects of groundcover ($P < 0.05$*, $P < 0.010$**, $P < 0.001$***), and different superscript letters indicate significant pairwise differences among groundcover classes according to Tukey's HSD *post hoc* tests.

Variable	Low	Medium	High	η^2 (%)	*F*	*P*
pH	6.23 ± 0.66	6.40 ± 0.71	6.53 ± 0.28	4.8	0.60	0.557
TOC (%)	2.07 ± 0.48^{a}	2.87 ± 0.84^{ab}	3.50 ± 1.66^{b}	23.9	3.78	0.037 *
TN (%)	0.13 ± 0.03	0.20 ± 0.05	0.25 ± 0.18	18.0	2.64	0.092
Colwell P (mg/kg)	277 ± 164	278 ± 158	232 ± 209	1.6	0.20	0.822
Available K (mg/kg)	430 ± 241^{a}	810 ± 570^{a}	397 ± 95^{a}	23.3	3.64	0.042 *
EC (dS/m)	0.11 ± 0.04^{a}	0.27 ± 0.16^{b}	0.11 ± 0.05^{a}	42.9	9.02	≤0.001 ***
Cl^- (mg/kg)	10.0 ± 0.0^{a}	144 ± 137^{b}	27.0 ± 19.2^{a}	38.5	7.52	0.003 **
Na (%)	0.79 ± 0.04	0.82 ± 0.34	0.93 ± 0.19	7.5	0.98	0.391

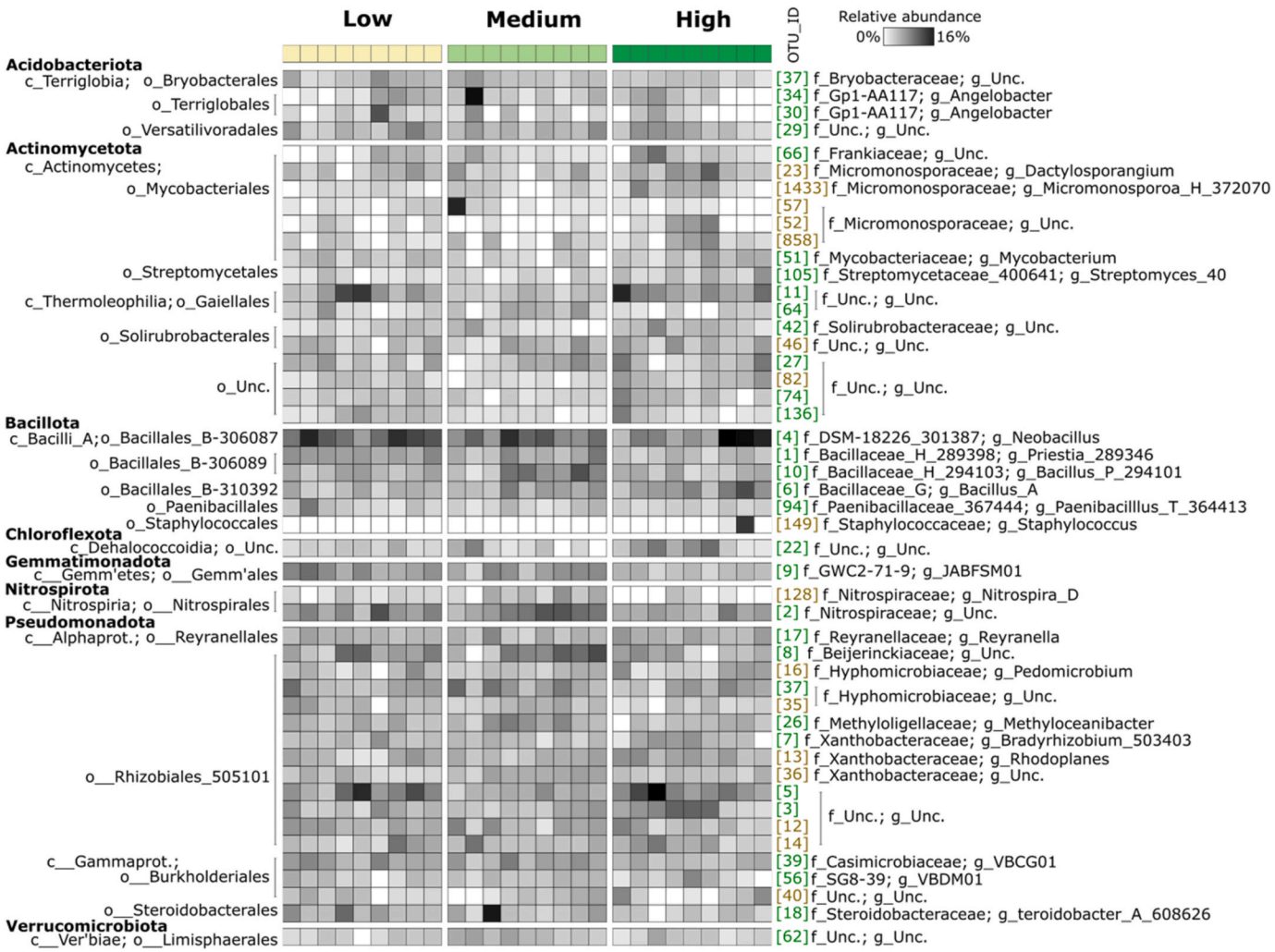


Fig. 1. Heatmap showing the composition of soil bacterial communities across banana farms with low, medium, and high groundcover. OTUs shown are those with mean relative abundance ≥0.5% in at least one groundcover class. Cell shading represents Hellinger-transformed relative abundance from zero (white) to the maximum relative abundance in any sample (16%, black). Rows are ordered by taxonomy and grouped by phylum. Green OTU IDs indicate taxa matching previously defined banana core microbiome members, whereas brown IDs indicate non-core taxa. OTU IDs are consistent across all figures.

compositional signal was shared with edaphic differences among farms, bacterial beta diversity retained a residual association with groundcover after accounting for the measured soil properties.

3.5. OTUs differing in relative abundance among groundcover classes

DESeq2 identified numerous OTUs whose relative abundances differed among groundcover classes (Fig. 3). A total of 26, 17, and 62 OTUs differed significantly in the low vs medium, low vs high, and medium vs high contrasts, respectively (Supplementary file 2). When attention was restricted to OTUs that were both significant ($P \leq 0.05$) and relatively abundant (mean relative abundance ≥0.5% in at least one groundcover class), 15 taxa remained, including 8 OTUs matching previously defined banana core taxa (Fig. 3; Supplementary file 2). These results suggest that the relative abundances of some, but not all, core taxa differed among groundcover classes.

3.6. Summed relative abundance of previously defined banana core taxa

A total of 75 OTUs in the present dataset matched 69 of the 74 core OTUs reported by Clarke et al. (2026) including all but 3 of the 50 OTUs they identified as bulk soil core taxa (Supplementary file 1). In addition, 21 OTUs in the present dataset matched 20 of the 36 core OTUs reported by Birt et al. (2022), including all 7 of the OTUs they identified as bulk-soil core taxa (Supplementary file 1). Of the 16 core OTUs identified by Birt et al. (2022) that were not detected in the present study, 12 were associated with leaves or endophytic compartments, and the remaining 4 were associated with rhizosphere soil (Supplementary file 1). All OTUs in the present dataset that matched the Australian banana core (Birt et al., 2022) also matched the broader international banana core (Clarke et al., 2026), consistent with the substantial overlap previously reported between these core sets.

Despite clear turnover in overall community composition (Table 3), and in the relative abundances of eight differentially abundant OTUs matching previously defined banana core taxa (Fig. 3; Supplementary file 2), groundcover was not significantly associated with the summed relative abundance of OTUs matching previously defined banana core taxa (Table S2). For OTUs matching core taxa from the international core microbiome of Clarke et al. (2026), the mean summed relative abundance was 35.39 ± 2.77%, 33.47 ± 4.69%, and 34.39 ± 4.81% under low, medium, and high groundcover, respectively. Corresponding values for OTUs matching core taxa from the Australian core banana microbiome of Birt et al. (2022) were 18.67 ± 3.17%, 15.85 ± 5.60%, and 15.47 ± 4.23%. In forward-selection models, pH was retained for both core metrics, whereas Colwell P and Na were additionally retained for the Australian core metric (Table S2). Groundcover remained

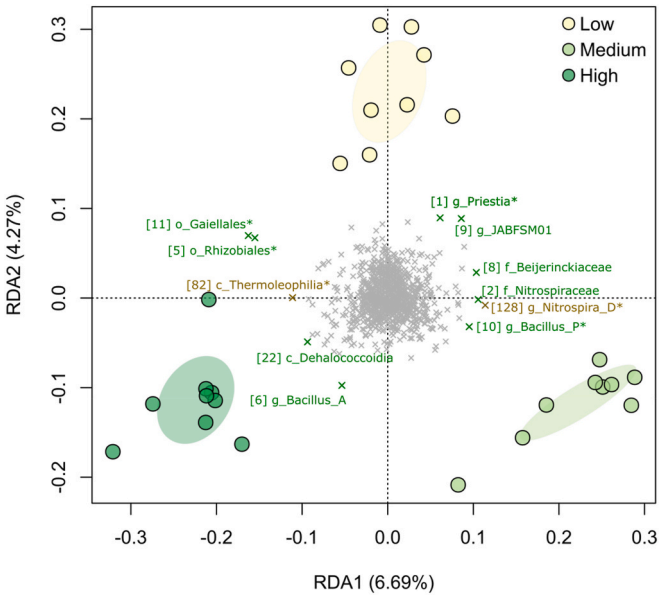


Fig. 2. Redundancy analysis (RDA) of bacterial community composition across groundcover classes based on Hellinger-transformed OTU relative abundances. Points represent soil samples from farms with low, medium, and high groundcover. Ellipses represent one standard deviation of sample scores within each groundcover class. Crosses indicate OTUs, with labels identifying those with the most extreme ordination scores. Green labels indicate taxa matching previously defined banana core taxa, brown labels indicate non-core taxa, and asterisks identify significantly differentially abundant OTUs with a mean relative abundance ≥0.5% in at least one groundcover class (DESeq2). The remaining OTUs are shown as unlabelled grey crosses to illustrate their distribution in ordination space.

Table 3
Unadjusted and edaphic-adjusted PERMANOVA models for bacterial community composition based on Hellinger-transformed OTU relative abundances across groundcover classes. The unadjusted model tested the effect of groundcover alone. The selected soil model shows the edaphic variables retained by forward selection, with terms evaluated sequentially. The Soil + Groundcover model shows the effect of groundcover after accounting for those retained edaphic predictors, with each term tested while accounting for the others in the model. Effect sizes are reported as R^2 (%), together with the corresponding pseudo-*F* and *P* values for each term. Asterisks denote significant *P* values ($P < 0.05$*, $P < 0.01$**, $P < 0.001$***).

Model	Term	R^2 (%)	F	P
Unadjusted	**Groundcover**	**11.0**	**1.5**	**0.005****
Selected soil model	Colwell P (mg/kg)	9.2	2.9	<0.001***
	EC (dS/m)	5.7	1.8	0.002**
	TOC (%)	5.6	1.7	0.003**
	pH	5.4	1.7	0.005**
	Na (%)	4.9	1.5	0.012*
	Cl^- (mg/kg)	5.1	1.6	0.004**
Soil + Groundcover	Colwell P (mg/kg)	4.8	1.5	0.017*
	EC (dS/m)	4.4	1.4	0.032*
	TOC (%)	4.4	1.4	0.034*
	pH	4.4	1.4	0.028*
	Na (%)	4.6	1.5	0.023*
	Cl^- (mg/kg)	4.4	1.4	0.029*
	Groundcover	**7.9**	**1.3**	**0.034***

non-significant after inclusion of these retained edaphic predictors (Table S2). These results indicate that, although groundcover was associated with marked community turnover and with shifts in the relative abundances of individual core taxa, it was not significantly associated with the summed relative abundance of OTUs matching the previously identified banana core microbiome.

3.7. Potential extracellular enzyme activity

Associations between potential enzyme activities and groundcover differed among enzymes. In unadjusted analyses, chitinase and acid phosphatase differed significantly among groundcover classes, whereas beta-glucosidase, arylsulphatase, alpha-glucosidase, and xylosidase did not (Fig. 4; Table S3). Chitinase showed the clearest increase from low to medium and high groundcover, while acid phosphatase also tended to be higher in farms with greater groundcover (Fig. 4). Forward selection models indicated that potential enzyme activities were strongly associated with edaphic variation, although the retained predictors differed among enzymes (Table S3). Enzymes involved in carbon acquisition did not show a single common edaphic pattern: beta-glucosidase was associated with Na, alpha-glucosidase with TN and pH, and xylosidase with TN and available K. Chitinase, which is involved in nitrogen cycling and targets chitin-rich substrates such as fungal cell walls, was associated with TOC, TN, Na, and Cl. In contrast, acid phosphatase, which is involved in phosphorus cycling, was associated with Colwell P, available K, Cl, pH, and TOC. Lastly, arylsulphatase was associated with Cl, available K, and Colwell P, which may reflect the use of potassium sulphate or potassium chloride within the region (Table S3). After accounting for the retained soil variables, chitinase and acid phosphatase remained significantly associated with groundcover, whereas beta-glucosidase, arylsulphatase, alpha-glucosidase, and xylosidase did not. Overall, these results indicate that groundcover-associated differences in bacterial community composition were accompanied by differences in some potential extracellular enzyme activities, but not others.

4. Discussion

Groundcover was associated primarily with differences in banana-associated soil bacterial communities rather than bacterial abundance or alpha diversity. Across farms, groundcover was linked to clear differences in community composition, including the relative abundances of several taxa matching previously defined banana core microbiome members, whereas total bacterial abundance, alpha diversity, and the summed relative abundance of the core microbiome did not differ significantly among groundcover classes. Together, these findings indicate that groundcover was more strongly associated with changes in community composition than with overall bacterial abundance or diversity.

4.1. Groundcover was associated with compositional turnover

This study shows that groundcover was associated with clear shifts in bacterial community composition across banana farms, even though bacterial abundance and alpha diversity showed little evidence of corresponding change. In unadjusted multivariate analyses, groundcover accounted for a substantial proportion of compositional variation, and this association remained significant after accounting for measured edaphic covariation. Thus, although part of the microbiome pattern was shared with soil differences among farms, bacterial beta diversity retained a residual association with groundcover, although this does not establish an independent causal effect. Together, these findings indicate that groundcover was associated more strongly with turnover in community composition than with broad changes in bacterial abundance or alpha diversity, consistent with previous studies (Prober et al., 2015; Wang et al., 2021; Yi et al., 2025).

This distinction is important because management practices do not necessarily influence microbial communities in the same way across different levels of biological organisation (Camacho-Sanchez et al., 2023; Hartman et al., 2018). In the present study, 16S rRNA gene abundance did not differ significantly among groundcover classes, and bacterial alpha diversity was likewise unrelated to groundcover, whereas the compositional signal was stronger and more persistent. This pattern suggests that groundcover was associated primarily with

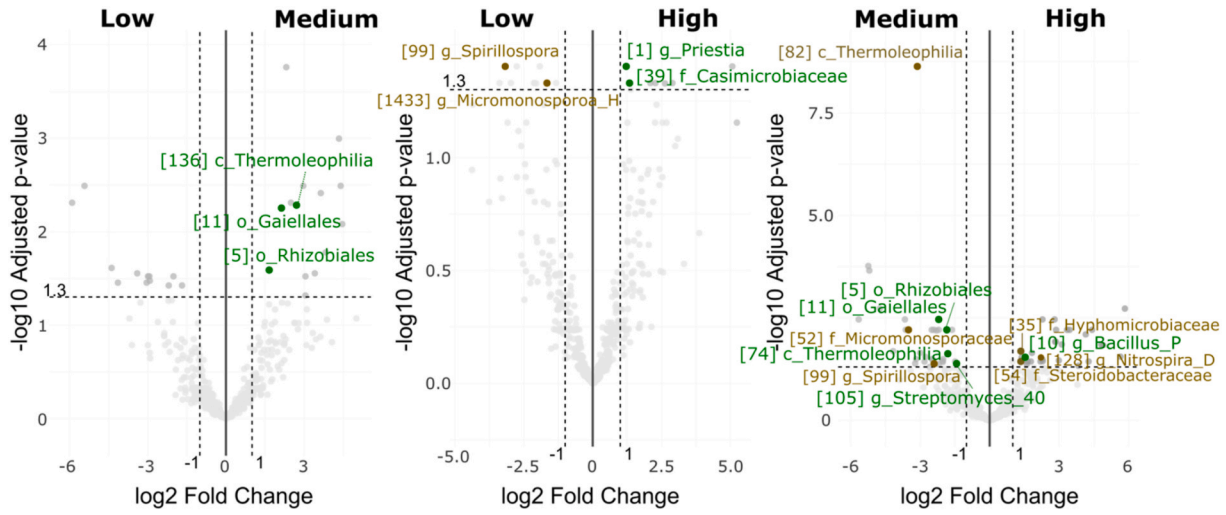


Fig. 3. DESeq2 volcano plots showing differentially abundant OTUs in pairwise comparisons among low, medium, and high groundcover classes. Each point represents an OTU, plotted by log2 fold change and adjusted significance, with higher y-values indicating smaller adjusted p-values. Vertical dashed lines indicate log2 fold change thresholds of ±1, and the horizontal dashed line indicates the adjusted significance threshold ($P = 0.05$). Labelled OTUs are those that were significantly differentially abundant and had mean relative abundance ≥0.5% in at least one groundcover class. Green labels indicate taxa matching previously defined banana core taxa, whereas brown labels indicate non-core taxa.

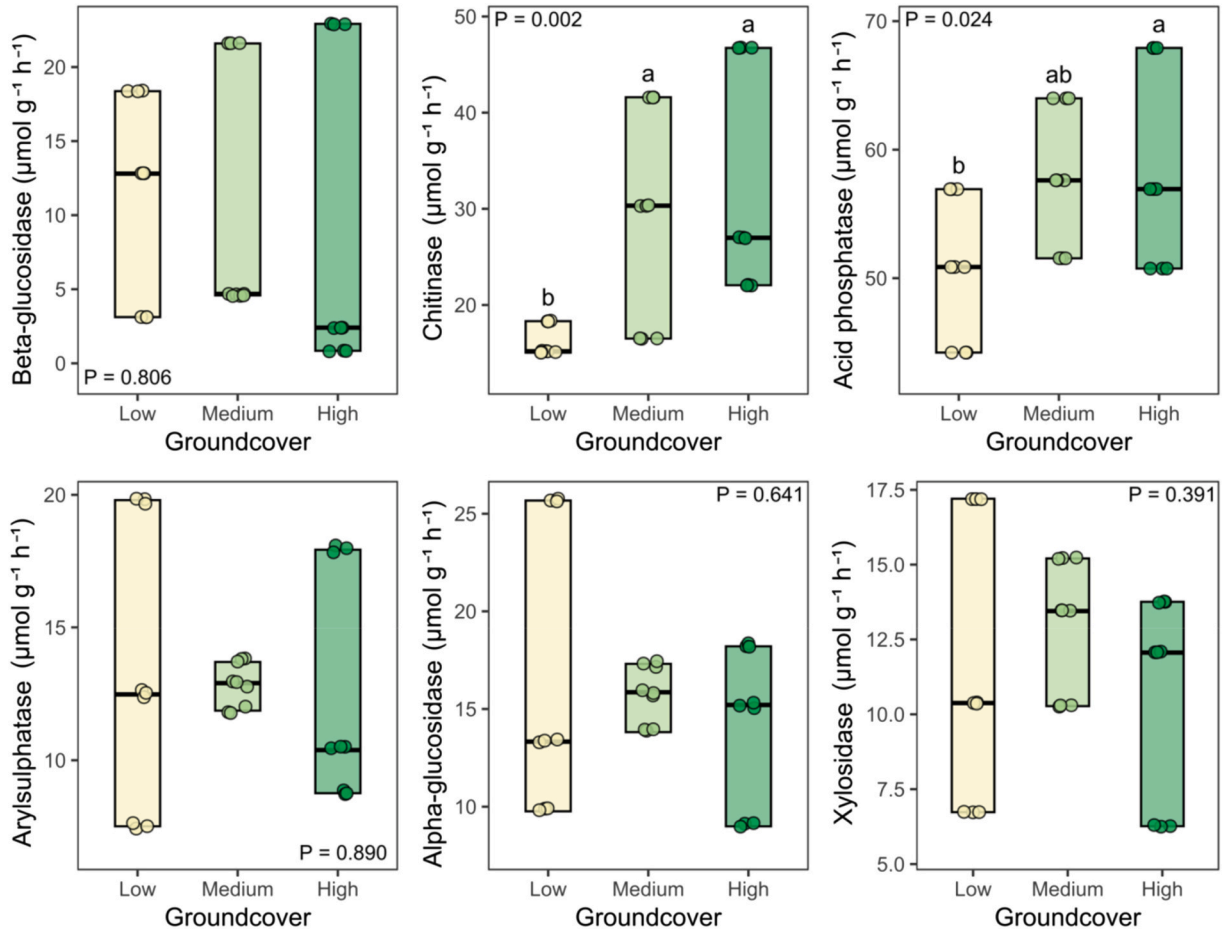


Fig. 4. Potential extracellular enzyme activities across banana farms with low, medium, and high groundcover. Points represent individual samples. Horizontal lines within boxes indicate medians, boxes indicate interquartile ranges, and whiskers extend to 1.5 times the interquartile range. The *P* values within each panel are from one-way ANOVA testing differences among groundcover classes, and different letters indicate significant pairwise differences based on Tukey's HSD *post hoc* tests.

community turnover rather than with a simple increase or decrease in bacterial biomass or overall diversity. Such turnover is plausible because groundcover may alter the quantity and quality of organic inputs, the physical environment at the soil surface, and the timing and spatial

distribution of rhizosphere derived resources, potentially favouring some bacterial groups over others rather than uniformly stimulating the community (Abdalla et al., 2019; Badri and Vivanco, 2009; Koudahe et al., 2022; Vukicevich et al., 2016; Yan and Arthur, 2025).

The ordination and differential abundance analyses support this interpretation. The RDA showed clear separation among groundcover classes, and DESeq2 identified numerous OTUs whose relative abundances differed among pairwise contrasts. Notably, the largest number of differentially abundant OTUs occurred between medium and high groundcover, indicating that microbiome patterns were not simply a monotonic contrast between sparse and dense vegetation cover. Instead, the results are more consistent with compositional differences in bacterial assemblages across groundcover classes, potentially reflecting variation in resource availability, microhabitats, and plant–soil interactions (Yang et al., 2026).

4.2. Groundcover was associated with variation in individual core-associated taxa, but not their summed relative abundance

A central aim of this study was to test whether groundcover was associated not only with overall bacterial community composition, but also with the previously defined banana core microbiome. The results support a nuanced interpretation. Many OTUs in the present dataset matched taxa previously identified as core members of banana-associated microbiomes, including most of the international bulk-soil core and all Australian bulk-soil core taxa represented in the reference datasets. In addition, 8 of the 15 OTUs that were both differentially abundant and relatively abundant matched previously defined banana core taxa. Hence, groundcover was clearly associated with differences in the relative abundances of individual members of the banana core microbiome.

However, these differences in the relative abundances of individual OTUs were not accompanied by significant differences in the summed relative abundance of OTUs matching the Australian or international banana core sets. Mean summed abundance of core-matching OTUs remained broadly similar across low, medium, and high groundcover classes, and groundcover was not significant in the edaphic adjusted models for either core metric. These results therefore do not provide evidence that the summed relative abundance of the banana core microbiome differed among groundcover classes. Instead, they suggest that groundcover was associated with differences in the relative abundances of individual core taxa, with some taxa becoming relatively more abundant and others relatively less abundant, while the aggregate contribution of core-matching taxa to the community remained broadly stable. However, the observed changes in relative abundance cannot be interpreted as changes in absolute abundance.

This is an important conceptual point for microbiome management. A management practice may influence taxa of interest without necessarily shifting a broad summary metric such as total core abundance (Camacho-Sanchez et al., 2023). In banana systems, that distinction may be especially relevant because the core microbiome is unlikely to be functionally uniform (Chakraborty and Kumar, 2025). Different core taxa are likely to contribute in different ways to nutrient transformations, rhizosphere interactions, pathogen suppression, or plant colonisation (Bulgarelli et al., 2013; Ye et al., 2022). From that perspective, selective changes in particular core taxa may still be biologically meaningful even when the summed abundance of the whole core set is unchanged. The present study therefore identifies groundcover management as a potential approach for modifying the relative abundances of individual core taxa, rather than broadly enriching the banana core microbiome, although experimental validation is required to establish whether these associations are causal.

4.3. Interpreting the groundcover signal in the context of edaphic variation

Groundcover classes also differed in several edaphic properties, including variables related to soil organic matter and nutrient status. This is biologically plausible, because vegetation cover can influence litter inputs, root turnover, nutrient capture, and microclimatic conditions at the soil surface (Koudahe et al., 2022). At the same time, the observational design means that groundcover and edaphic variation cannot be treated as fully independent. Some soil properties may lie on causal pathways linking groundcover to microbial communities, whereas others may reflect broader differences among farms unrelated to groundcover itself; however, these alternative explanations cannot be distinguished using the present observational data.

In this context, it is notable that the main multivariate compositional pattern remained significant after accounting for retained soil variables. This indicates that the association between groundcover and bacterial community composition persisted after accounting for measured edaphic variation but does not establish an independent causal effect of groundcover. At the same time, the reduction in explained variation after soil adjustment suggests that part of the groundcover signal was shared with soil properties. Rather than viewing these interpretations as competing, the most reasonable conclusion is that the association between groundcover and microbiome structure partly overlapped with edaphic variation. This also supports the decision to present unadjusted ordination and OTU level analyses in the main narrative, because some soil variables may lie on causal pathways linking groundcover to microbiome composition, and adjusting for them could obscure associations operating through these potential pathways.

4.4. Potential enzyme activity responses were selective rather than general

The potential extracellular enzyme activity data add a functional dimension to the community results, but the pattern was selective rather than general. In unadjusted analyses, potential chitinase and acid phosphatase activities differed significantly among groundcover classes, whereas the other measured potential enzyme activities did not. After accounting for retained edaphic predictors, potential activities of these two enzymes remained significantly associated with groundcover, while beta-glucosidase, arylsulphatase, alpha-glucosidase, and xylosidase remained non-significant. Thus, the results do not indicate a broad, uniform shift in potential extracellular enzyme activity across groundcover classes. Instead, they suggest that groundcover-associated differences in bacterial community composition coincided with differences in some, but not all, measured potential extracellular enzyme activities.

Chitinase is particularly notable because its association with groundcover remained significant after adjusting for measured edaphic variation. This is consistent with the study of Papp et al. (2018), who also found potential chitinase activity to be sensitive to groundcover management. As chitinase contributes to nitrogen (N) cycling by degrading chitin-rich substrates such as fungal cell walls, variation in its potential activity may reflect differences in N cycling, the availability of chitin-rich substrates, and/or the abundance or activity of organisms involved in chitin turnover (Hamid et al., 2013). However, potential chitinase activity cannot be attributed specifically to bacterial activity, as soil chitinases may originate from bacteria, fungi, or plants. Potential activity of acid phosphatase also remained associated with groundcover after accounting for soil variables, with activity tending to be higher from low to high groundcover. Because acid phosphatase is involved in the mobilisation of organically bound phosphorus, this result may indicate greater potential for phosphorus mobilisation in farms with higher groundcover than in those with sparse cover. Even so, the overall potential enzyme activity results were more limited than the compositional results, reinforcing the conclusion that groundcover was associated most clearly with bacterial community structure, while differences in potential enzyme activity were enzyme-specific rather than general.

The potential enzyme activity was measured *in vitro* under standardized assay conditions and may not reflect in situ ecosystem function. The relationships among groundcover, bacterial community composition, and potential enzyme activities should be interpreted as associative rather than causal, as these variables were measured concurrently and causal pathways were not experimentally tested.

4.5. Implications for banana microbiome management

Taken together, these findings provide a basis for future experimental studies testing whether groundcover management can serve as a plausible lever for influencing banana-associated soil microbiomes, while also highlighting the limits of what can be inferred. Groundcover was associated with differences in bacterial community composition, including changes in relatively abundant taxa and several OTUs matching previously defined banana core taxa. If experimentally validated, these associations could provide opportunities to explore groundcover management as an agroecological approach to shaping banana associated microbiomes, particularly because groundcover is already a practical and farm-relevant management dimension.

At the same time, the present results do not establish whether groundcover improves banana soil health or promotes beneficial soil functions. The observed associations involved differences in the relative abundances of individual core taxa rather than their summed relative abundance, and we did not assess disease suppression, plant performance, yield, or Fusarium wilt outcomes. Hence, whether the observed groundcover-associated differences in bacterial community composition are linked to soil health or agronomic outcomes remains untested. Accordingly, the main contribution of this work is to demonstrate associations between groundcover and bacterial community composition, including differences in the relative abundances of individual core taxa, rather than to demonstrate direct agronomic or plant-protective benefits. The limited replication of three farms per groundcover class may also have reduced statistical power to detect subtle groundcover-associated differences. Groundcover was assessed in a single season without species-level vegetation identification, limiting assessment of temporal and species-specific variation. Future work should therefore move beyond broad community summaries and test whether taxa that were relatively more abundant under different groundcover classes contribute to functions relevant to banana production, such as pathogen suppression, nutrient mobilisation, or resilience to edaphic stress. Experimental approaches combining vegetation management with targeted measurements of pathogen pressure, plant performance, potential enzyme activity, and taxon-specific functional assays would be especially valuable for evaluating causal relationships between microbial community composition and ecosystem functioning.

5. Conclusions

Groundcover was associated with turnover in banana-associated soil bacterial community composition, and this association remained detectable after accounting for measured edaphic covariation. In contrast, bacterial abundance and alpha diversity were not significantly associated with groundcover. Groundcover was also associated with differences in the relative abundances of several individual OTUs matching previously defined banana core taxa, but not the summed relative abundance of the Australian or international banana core sets. Potential extracellular enzyme activities showed selective associations with groundcover, with the clearest and most consistent signals observed for chitinase and acid phosphatase. Overall, these findings indicate that groundcover was associated with differences in banana soil bacterial community composition, including individual core taxa, rather than with broad changes in the summed relative abundance of the core microbiome. If experimentally validated, these findings could provide a basis for exploring groundcover management as an agroecological approach to shaping banana-associated microbiomes.

CRediT authorship contribution statement

Sutrisno: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Rebecca Lyons:** Writing – review & editing, Methodology. **Hazel R. Lapis-Gaza:** Writing – review & editing, Methodology. **Jiarui Sun:** Writing – review & editing, Formal analysis. **Luciasih Agustini:** Writing – review & editing, Methodology. **Anthony B. Pattison:** Writing – review & editing, Funding acquisition. **Paul G. Dennis:** Writing – review & editing, Writing – original draft, Funding acquisition, Formal analysis.

Funding

This publication has been funded by the Australian Government through the Australian Centre for International Agricultural Research (project HORT/2018/192). The views expressed in this publication are the author's alone and are not necessarily the views of the Australian Government.

Declaration of competing 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 paper.

Acknowledgements

We gratefully acknowledge all banana growers and landholders for allowing us access to their properties. We thank Mr Harry Cosgrove for technical assistance in collecting samples and determining groundcover on farms. We gratefully acknowledge Tim Daniell, University of Sheffield, for providing the artificial reference plasmid pMUT16S.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rhisph.2026.101517.

Data availability

All sequence data are available on the Sequence Read Archive (SRA) portal of NCBI under BioProject: PRJNA1455193.

References

Abdalla, M., Hastings, A., Cheng, K., Yue, Q., Chadwick, D., Espenberg, M., Truu, J., Rees, R.M., Smith, P., 2019. A critical review of the impacts of cover crops on nitrogen leaching, net greenhouse gas balance and crop productivity. Glob. Change Biol. 8, 2530–2543. https://doi.org/10.1111/gcb.14644.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46 pp.x.

Badri, D.V., Vivanco, J.M., 2009. Regulation and function of root exudates. Plant Cell Environ. 32, 666–681. https://doi.org/10.1111/j.1365-3040.2009.01926.x.

Ben-Shachar, M., Lüdecke, D., Makowski, D., 2020. effectsize: estimation of effect size indices and standardized parameters. J. Open Source Softw. 5, 2815. https://doi.org/10.21105/joss.02815.

Birt, H.W.G., Pattison, A.B., Skarshewski, A., Daniells, J., Raghavendra, A., Dennis, P.G., 2022. The core bacterial microbiome of banana (Musa spp.). Environmental Microbiomes 17, 46. https://doi.org/10.1186/s40793-022-00442-0.

Blanco-Canqui, H., Shaver, T.M., Lindquist, J.L., Shapiro, C.A., Elmore, R.W., Francis, C. A., Hergert, G.W., 2015. Cover crops and ecosystem services: insights from studies in temperate soils. Agron. J. 107, 2449–2474. https://doi.org/10.2134/agronj15.0086.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K. Bin, Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G. I., Lee, J., Ley, R., Liu, Y.X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C.,

Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K.C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., Van Themaat, E.V.L., Schulze-Lefert, P., 2013. Structure and functions of the bacterial microbiota of plants. Annu. Rev. Plant Biol. 64, 807–838. https://doi.org/10.1146/annurev-arplant-050312-120106.

Camacho-Sanchez, M., Herencia, J.F., Arroyo, F.T., Capote, N., 2023. Soil microbial community responses to different management strategies in almond crop. J. Fungi 9, 95. https://doi.org/10.3390/jof9010095.

Chakraborty, S., Kumar, M., 2025. The core plant microbiome: roles, functions, and importance in plant health. In: Renu, Gupta, S., Sharma, T.R. (Eds.), Meta-Omics in Crop Improvement. Springer, Singapore, pp. 175–191. https://doi.org/10.1007/978-981-95-2294-1_9.

Clarke, A.B.C., Ansale, C.R., Banayag, L., Bayang, S., Bothma, S., Chittarath, K., Juruena, M., Karamura, G., Lapis-Gaza, H.R., Limbaga Jr., C., Lyons, R., Sun, J., Viljoen, A., Mostert, D., Saidi, N.B., Mohd Suhaimi, N.S., Pattison, A.B., Dennis, P.G., 2026. Core rhizosphere bacteria associated with banana (*Musa* spp.) across multiple continents. Appl. Soil Ecol. 223, 107107.

Daniell, T.J., Davidson, J., Alexander, C.J., Caul, S., Roberts, D.M., 2012. Improved real-time PCR estimation of gene copy number in soil extracts using an artificial reference. J. Microbiol. Methods 91, 38–44. https://doi.org/10.1016/j.mimet.2012.07.010.

Delitte, M., Caulier, S., Bragard, C., Desoignies, N., 2021. Plant microbiota beyond farming practices: a review. Front. Sustain. Food Syst. 5, 624203. https://doi.org/10.3389/fsufs.2021.624203.

Dita, M., Barquero, M., Heck, D., Mizubuti, E.S.G., Staver, C.P., 2018. Fusarium wilt of banana: current knowledge on epidemiology and research needs toward sustainable disease management. Front. Plant Sci. 9, 1468. https://doi.org/10.3389/fpls.2018.01468.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996–998. https://doi.org/10.1038/nmeth.2604.

Fadiji, A.E., Xiong, C., Egidi, E., Singh, B.K., 2024. Formulation challenges associated with microbial biofertilizers in sustainable agriculture and paths forward. J. Sustain. Agric. Environ. 3, e70006. https://doi.org/10.1002/sae2.70006.

FAO, 2026. Bananas: commodity in focus. Food and Agriculture Organization of the United Nations. https://www.fao.org/markets-and-trade/commodities-overview/bananas-tropical-fruits/bananas/1/en. (Accessed 14 April 2026).

Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proc. Natl. Acad. Sci. USA 103, 626–631. https://doi.org/10.1073/pnas.0507535103.

Hamid, R., Khan, M.A., Ahmad, M., Ahmad, M.M., Abdin, M.Z., Musarrat, J., Javed, S., 2013. Chitinases: an update. J. Pharm. BioAllied Sci. 5, 21–29. https://doi.org/10.4103/0975-7406.106559.

Hartman, K., van der Heijden, M.G.A., Wittwer, R.A., Banerjee, S., Walser, J.C., Schlaeppi, K., 2018. Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. Microbiome 6, 14. https://doi.org/10.1186/s40168-017-0389-9.

Henry, L.P., Bergelson, J., 2025. Applying ecological principles to microbiome engineering. Nat. Microbiol. 10, 2111–2121. https://doi.org/10.1038/s41564-025-02076-7.

Koudahe, K., Allen, S.C., Djaman, K., 2022. Critical review of the impact of cover crops on soil properties. Int. Soil Water Conserv. Res. 10, 343–354. https://doi.org/10.1016/j.iswcr.2022.03.003.

Lapis-Gaza, H.R., Pattison, A.B., 2021. Functional soil biological measurements to support healthy soils. In: Carvalhais, L.C., Dennis, P.G. (Eds.), The Plant Microbiome. Methods in Molecular Biology. Humana, New York, pp. 265–281. https://doi.org/10.1007/978-1-0716-1040-4_20.

Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129, 271–280. https://doi.org/10.1007/s004420100716.

Lundin, D., Severin, I., Logue, J.B., Östman, Ö., Andersson, A.F., Lindström, E.S., 2012. Which sequencing depth is sufficient to describe patterns in bacterial α- and β-diversity? Environ. Microbiol. Rep. 4, 367–372. https://doi.org/10.1111/j.1758-2229.2012.00345.

Lv, N., Tao, C., Ou, Y., Wang, J., Deng, X., Liu, H., Shen, Z., Li, R., Shen, Q., 2023. Root-associated antagonistic Pseudomonas spp. contribute to soil suppressiveness against Banana fusarium wilt disease of banana. Microbiol. Spectr. 11. https://doi.org/10.1128/spectrum.03525-22.

Lyons, R., Clarke, A.B.C., Lapis-Gaza, H.R., Sun, J., Birt, H.W.G., Pattison, A.B., Dennis, P.G., 2025. Landuse affects the likelihood of soil colonization by a key plant pathogen. Agric. Ecosyst. Environ. 393, 109814. https://doi.org/10.1016/j.agee.2025.109814.

Ma, W., Yang, Z., Hou, S., Ma, Q., Liang, L., Wang, G., Liang, C., Zhao, T., 2021. Effects of living cover on the soil microbial communities and ecosystem functions of hazelnut orchards. Front. Plant Sci. 12, 652493. https://doi.org/10.3389/fpls.2021.652493.

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J. 17. https://doi.org/10.14806/ej.17.1.200.

McDonald, D., Clemente, J.C., Kuczynski, J., Rideout, J.R., Stombaugh, J., Wendel, D., Wilke, A., Huse, S., Hufnagle, J., Meyer, F., Knight, R., Caporaso, J.G., 2012. The biological Observation Matrix (BIOM) format or: how I learned to stop worrying and love the ome-ome. GigaScience 1, 2047–217X–1–7. https://doi.org/10.1186/2047-217X-1-7.

McDonald, D., Jiang, Y., Balaban, M., Cantrell, K., Zhu, Q., Gonzalez, A., Morton, J.T., Nicolaou, G., Parks, D.H., Karst, S.M., Albertsen, M., Hugenholtz, P., DeSantis, T., Song, S.J., Bartko, A., Havulinna, A.S., Jousilahti, P., Cheng, S., Inouye, M., Niiranen, T., Jain, M., Salomaa, V., Lahti, L., Mirarab, S., Knight, R., 2024. Greengenes2 unifies microbial data in a single reference tree. Nat. Biotechnol. 42, 715–718. https://doi.org/10.1038/s41587-023-01845-1.

Noviana, Z., Kurniawati, W., Dewi, T.K., Ruhimat, R., Nugroho, A.A., Sutisna, E., Ratdiana, Agustiyani, D., Santosa, D.A., Antonius, S., 2025. Rhizosphere bacterial communities differ across banana generations and health status under Fusarium wilt pressure. Rhizosphere 36, 101207. https://doi.org/10.1016/j.rhisph.2025.101207.

Oksanen, J., et al., 2026. Vegan: Community Ecology package (R package version 2.8-0). https://cran.r-project.org/web/packages/vegan/index.html. (Accessed 22 April 2026).

Papp, R., Marinari, S., Moscatelli, M.C., van der Heijden, M.G.A., Wittwer, R., Campiglia, E., Radicetti, E., Mancinelli, R., Fradgley, N., Pearce, B., Bergkvist, G., Finckh, M.R., 2018. Short-term changes in soil biochemical properties as affected by subsidiary crop cultivation in four European pedo-climatic zones. Soil Tillage Res. 180, 126–136. https://doi.org/10.1016/j.still.2018.02.019.

Paterson, O., 2024. Assigning Function to the Core Bacterial Microbiome of Musa spp. School of the Environment, The University of Queensland. https://doi.org/10.14264/cc2eb78. PhD Thesis.

Pattison, A.B., Wright, C.L., Kukulies, T.L., Molina, A.B., 2014. Ground cover management alters development of fusarium wilt symptoms in ducasse bananas. Australas. Plant Pathol. 43, 465–476. https://doi.org/10.1007/s13313-014-0296-5.

Prigigallo, M.I., Gómez-Lama Cabanás, C., Mercado-Blanco, J., Bubici, G., 2022. Designing a synthetic microbial community devoted to biological control: the case study of fusarium wilt of banana. Front. Microbiol. 13, 967885. https://doi.org/10.3389/fmicb.2022.967885.

Prober, S.M., Leff, J.W., Bates, S.T., Borer, E.T., Firn, J., Harpole, W.S., Lind, E.M., Seabloom, E.W., Adler, P.B., Bakker, J.D., Cleland, E.E., Decrappeo, N.M., Delorenze, E., Hagenah, N., Hautier, Y., Hofmockel, K.S., Kirkman, K.P., Knops, J.M. H., La Pierre, K.J., Macdougall, A.S., Mcculley, R.L., Mitchell, C.E., Risch, A.C., Schuetz, M., Stevens, C.J., Williams, R.J., Fierer, N., 2015. Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. Ecol. Lett. 18, 85–95. https://doi.org/10.1111/ele.12381.

Rames, E.K., Pattison, A.B., Czislowski, E., Smith, M.K., 2018. Soil microbial community changes associated with ground cover management in cultivation of ducasse banana (Musa sp. ABB, Pisang Awak subgroup) and suppression of Fusarium oxysporum. Australasian Plant Pathol 47, 449–462. https://doi.org/10.1007/s13313-018-0578-4.

Rayment, G.E., Lyons, D.J., 2010. Soil Chemical Methods – Australasia. CSIRO Publishing, Collingwood. https://doi.org/10.1071/9780643101364.

Redford, A.J., Bowers, R.M., Knight, R., Linhart, Y., Fierer, N., 2010. Ecology of the phyllosphere: geographic and phylogenetic variability in the distribution of bacteria on tree leaves. Environ. Microbiol. 12, 2885–2893. https://doi.org/10.1111/j.1462-2920.2010.02258.x.

Risely, A., 2020. Applying the core microbiome to understand host–microbe systems. J. Anim. Ecol. 89, 1549–1558. https://doi.org/10.1111/1365-2656.13229.

Santiago, J.M., Fox, J.P., Guzmán, S.M., Rossi, L., 2023. Effect of fabric mulch ground covers on lemon trees rhizosphere microbiome in Florida flatwood soils. Front. Soil Sci. 3, 1110370. https://doi.org/10.3389/fsoil.2023.1110370.

Toju, H., Peay, K.G., Yamamichi, M., Narisawa, K., Hiruma, K., Naito, K., Fukuda, S., Ushio, M., Nakaoka, S., Onoda, Y., Yoshida, K., Schlaeppi, K., Bai, Y., Sugiura, R., Ichihashi, Y., Minamisawa, K., Kiers, E.T., 2018. Core microbiomes for sustainable agroecosystems. Nat. Plants 4, 247–257. https://doi.org/10.1038/s41477-018-0139-4.

Vukicevich, E., Lowery, T., Bowen, P., Úrbez-Torres, J.R., Hart, M., 2016. Cover crops to increase soil microbial diversity and mitigate decline in perennial agriculture. A review. Agron. Sustain. Dev. 36, 48. https://doi.org/10.1007/s13593-016-0385-7.

Wang, Y., Li, C., Tu, B., Kou, Y., Li, X., 2021. Species pool and local ecological assembly processes shape the β-diversity of diazotrophs in grassland soils. Soil Biol. Biochem. 160, 108338. https://doi.org/10.1016/j.soilbio.2021.108338.

Xu, S., Zhang, W., Goodwin, P.H., Wang, Y., Zheng, S.J., Li, X., 2024. Effect of cover crop on soil fertility and bacterial diversity in a banana plantation in southwestern China. Soil Tillage Res. 240, 106092. https://doi.org/10.1016/j.still.2024.106092.

Xue, C., Penton, C.R., Shen, Z., Zhang, R., Huang, Q., Li, R., Ruan, Y., Shen, Q., 2015. Manipulating the banana rhizosphere microbiome for biological control of Panama disease. Sci. Rep. 5, 11124. https://doi.org/10.1038/srep11124.

Yan, F., Arthur, E., 2025. Cover crops alter soil physicochemical properties: a global meta-analysis. Geoderma 460, 117436. https://doi.org/10.1016/j.geoderma.2025.117436.

Yang, G., Wen, F., Wang, D., Zhao, D., Liu, Q., Ren, M., Zheng, J., Zhang, R., Xia, Z., Zhang, J., Zhang, L., Wan, C., Luo, X., 2026. Environmental quality affects the formation of generalist and specialist taxa in microbial communities. ISME Commun. 6, ycag057. https://doi.org/10.1093/ismeco/ycag057.

Ye, Z., Wang, J., Li, J., Liu, G., Dong, Q., Zou, Y., Chau, H.W., Zhang, C., 2022. Different roles of core and noncore bacterial taxa in maintaining soil multinutrient cycling and microbial network stability in arid fertigation agroecosystems. J. Appl. Ecol. 59, 2154–2165. https://doi.org/10.1111/1365-2664.14228.

Yi, M., Cong, P., Zhang, D., You, J., Zhang, Y., Jing, W., Shang, L., 2025. Plant diversity exerts a stronger influence than short-term climate manipulations on the structure of soil bacterial communities. Microorganisms 13, 2844. https://doi.org/10.3390/microorganisms13122844.

Zhang, Liyu, Zhang, M., Huang, Shuyu, Li, L., Gao, Q., Wang, Y., Zhang, S., Huang, Shaomin, Yuan, L., Wen, Y., Liu, K., Yu, X., Li, D., Zhang, Lu, Xu, X., Wei, H., He, P., Zhou, W., Philippot, L., Ai, C., 2022. A highly conserved core bacterial microbiota with nitrogen-fixation capacity inhabits the xylem sap in maize plants. Nat. Commun. 13, 3361. https://doi.org/10.1038/s41467-022-31113-w.

Zhou, J., Fong, J.J., 2021. Strong agricultural management effects on soil microbial community in a non-experimental agroecosystem. Appl. Soil Ecol. 165, 103970. https://doi.org/10.1016/j.apsoil.2021.103970.

Zhou, Y., Liu, D., Li, F., Dong, Y., Jin, Z., Liao, Y., Li, Xiaohui, Peng, S., Delgado-Baquerizo, M., Li, Xiaogang, 2024. Superiority of native soil core microbiomes in supporting plant growth. Nat. Commun. 15, 6599. https://doi.org/10.1038/s41467-024-50685-3.