Research Square

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The Core Bacterial Microbiome of Banana (Musa spp.)

Henry W. G. Birt
The University of Queensland
Anthony B. Pattison
Queensland Government
Adam Skarshewski
The University of Queensland
Jeff Daniells
Queensland Government
Anil Raghavendra
The University of Queensland
Paul G. Dennis (p.dennis@uq.edu.au)
The University of Queensland

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Abstract

Background

Bananas (*Musa* spp.) are a globally significant crop and are severely afflicted by diseases for which there are no effective chemical controls. Banana microbiomes may provide novel solutions to these constraints but are difficult to manage due to their high diversity and variability between locations. Hence 'common core' taxa, which are a subset of the microbiome that frequent all, or most, individuals of a host species, represent logical targets for the development of microbiome management approaches.

Results

To identify the common core bacterial microbiome of banana, we first performed a pot experiment to characterise the effects of two factors that are likely to differ between farms (*viz*. edaphic conditions and host genotype) on bacterial diversity in bulk soil and seven plant compartments. As diversity was found to differ between plant compartments and soils, but not genotypes, we identified populations that were frequent across most plants irrespective of the soil in which they were grown. Shortlisted 'candidates' were then refined according to whether they were also frequent in a survey of 52 field-grown *Musa* spp. This led to the selection of 36 'common core' bacteria, that represented 65–95% of the dominant taxa in field-grown plants and were identified as highly interconnected 'hubs' using networks – a characteristic shown to be indicative of microbes that influence host fitness in studies of other plants. Lastly, by comparing the sequences of our core taxa with those reported in 22 previous studies, we demonstrated that they are closely related to banana-associated bacteria observed on five other continents.

Conclusions

Our study provides a robust list of common core bacterial taxa for *Musa* spp. Further research may now focus on how changes in the frequencies and activities of these most persistent taxa influence host fitness. Notably, for several of our core taxa highly similar populations have already been isolated in previous studies and may be amenable to such experimentation. This contribution should help to accelerate the development of effective *Musa* spp. microbiome management practices.

Background

Bananas (*Musa* spp.) are among the world's most-produced crops and represent an important food staple in many countries [1]. Abiotic and biotic stresses undermine banana production and include diseases that can persist in soils for long periods, and for which there are no effective chemical controls [2, 3]. Like other macro-organisms, *Musa* spp. host diverse microbial communities that influence their health and nutrition. Management of these communities may offer novel solutions to production constraints but is challenging due to the complexity of plant-microbe interactions and their dependency

on environmental conditions. Hence, an intervention that works on one farm may be ineffective on another due to differences in the microbiome. Despite this variability, evidence indicates that all plants harbour a subset of 'core taxa' with which they are persistently associated across diverse environmental gradients [4–6]. The pervasive nature of these organisms, suggests that they play important roles in the regulation of host function [7–9], and presents an opportunity to focus research efforts on a relatively small, impactful and frequently encountered subset of taxa. This approach may increase the likelihood of microbiome management interventions being effective on farms with different abiotic and biotic characteristics.

To identify a 'core bacterial microbiome' of banana, *viz*. a shortlist of bacterial taxa that are shared by all or most *Musa* spp., it is necessary to characterise the microbiome in as many settings as possible. While Cavendish cultivars dominate commercial banana production, many genotypes are grown around the world, on land with differing edaphic conditions [10]. Hence, the extent to which these factors influence *Musa* spp. microbiomes must be assessed and considered appropriately when designating core taxa. For *Musa* spp., the effects of edaphic conditions and genotype on bacterial diversity are poorly understood. Nonetheless, results from a wide-range of other plant species indicate large differences between soils, and significant but much smaller effects of genotype that are positively associated with host phylogenetic distance [6].

Another important host-associated factor to consider when defining core taxa, is that microbial communities differ between plant compartments [11]. In general, the composition of root microbiomes is most strongly determined by edaphic conditions, as soil is the primary source of root-colonising microbes [12, 13]. In contrast, leaf microbiomes, are distinct from belowground microbial communities [14, 15] and comprise a larger proportion of taxa derived from aerial sources [16]. Understanding these spatial patterns is likely to be important for the design of effective microbiome management approaches. For example, *Fusarium oxysporum* f. sp. *cubense* (*Foc*), the causal agent of Fusarium wilt of banana (FWB), tends to infect at root apices, and then migrate to the leaves via the xylem, rhizome, and outer leaf sheaths of the pseudostem [17]. Hence, determining the core taxa associated with these compartments may help to identify where relevant *Foc* suppressive populations occur and contribute to the development of biological control strategies.

Here, we define the common core bacterial microbiome of *Musa* spp. This process began with pot experiments to determine whether *Musa* spp. microbiomes vary with edaphic conditions and between host genotypes. From these experiments, we characterised the microbiomes of 560 samples from multiple above and belowground plant compartments and generated a list of 'candidate core' bacterial taxa. We then performed a survey of 52 distinct field-grown *Musa* spp., comprising a further 700 samples, to validate the importance of candidate core taxa in mature plants. Finally, we compared the sequences of our refined core bacterial taxa with the SILVA database, and data from 22 previous studies of *Musa* spp.-associated bacteria. This meta-analysis demonstrated that our core taxa are closely related to bacterial populations that have previously been observed in association with *Musa* spp. worldwide.

Methods

Pot experiments

The aims of our pot experiments were to: 1) characterise the bacterial communities associated with different *Musa* spp. plant compartments; 2) determine whether *Musa* spp. microbiomes vary with edaphic conditions and between host genotypes; and 3) identify a shortlist of 'candidate core' bacterial taxa that are shared by all *Musa* spp. irrespective of differences in edaphic conditions and host genotype.

Experimental setup: Soil was collected (0–30 cm depth) from five *Musa* (AAA Group, Cavendish Subgroup) 'Williams' production sites in The Wet Tropics of North Queensland – the primary banana-producing area of Australia (Table S1). Within this region, soils are grouped into series, which have horizons derived from similar parent material, and with similar properties and arrangement in the profile [18–20]. Each soil in our study represents one of the dominant soil series of the region (Innisfail, In; Liverpool, Li; Pin Gin, Pg; Tully, Tu; and Tolga, To), and has distinct edaphic properties (Table S1). All soils were sieved to < 8 mm and then placed in 15 cm diameter pots, with 1 kg of soil (fresh weight) per pot. Ten pots of each soil were directly planted with sterile 'Williams' tissue culture plantlets (Kool Bananas tissue culture facility, Mission Beach). These plants were used to assess the impact of edaphic properties on the bacterial microbiome of *Musa* spp. An additional 20 pots were then filled with Innisfail soil: 10 for *Musa* (AAB Group, Pome Subgroup) 'Lady Finger', and 10 for *Musa* (AAAB Group, Prata Anã x SH-3142) 'Goldfinger' plantlets (Department of Agriculture and Fisheries, Nambour tissue culture facility). Together with the 10 pots of 'Williams' in Innisfail soil, these plants were analysed as a separate experiment to assess the impact of host genotype on the bacterial microbiome of *Musa* spp. 'Williams' and 'Lady Finger' represent c. 97% and 3% of banana production in Australia, respectively [21]. 'Goldfinger' is not widely grown but has a tetraploid hybrid genome and is more resistant to Fusarium Wilt [22]. All plants were grown in a glasshouse in a randomised design under controlled conditions. After three months of growth, the plants were harvested.

Sample collection: For each plant we collected samples from eight compartments: 1) bulk soil (BS), 2) apical ectorhizosphere (AER), 3) basal ectorhizosphere (BER), 4) apical endorhizosphere (AEnR), 5) basal endorhizosphere (BEnR), 6) rhizome (R; also known as the corm), 7) pseudostem (PS), and 8) leaves (L), as previously described [23]. This design yielded 560 samples (70 plants × 8 compartments). Briefly, the first three most recently emerged leaves were removed and finely chopped. Plants were then removed from pots and shaken to separate and collect bulk soil. Roots were cut 50 mm from the base and apex, placed in separate sterile 50 ml falcon tubes, and retained at 4°C in a refrigerator for later processing on the same day. After washing in deionised water, the rhizome and pseudostem were separated, surface sterilised in 4% NaOCl for 5 min, and washed three times in sterile deionised water [24]. All samples were then stored at -20°C after collection. Tubes containing roots were removed from the refrigerator, filled with 40 ml sterile 1X phosphate buffer saline (PBS) solution, and then vortexed for 1 min, sonicated for 1 min, and vortexed for another 1 min. Roots were then transferred to a fresh tube using sterile forceps, and the resulting slurry was centrifuged for 15 min at 27 RCF. The clear supernatant was removed, and the soil

pellet was retained as the ectorhizosphere sample. Roots were washed in sterile distilled water by repeatedly vortexing, then removing and replacing water until it appeared clear after vortexing (c. 5–8 times). Roots were then sonicated in 50 ml of water for 1 min to remove remaining rhizoplane organisms, surface sterilised in 4% NaOCl for 5 min, and then washed three times in sterilised distilled water. All samples were then stored at -20°C until further processing.

Field experiment

The aims of our field experiment were to determine whether the 'candidate core' taxa identified in our pot experiment were: 1) important in mature field-grown plants; and 2) present in a much larger range of genotypes.

Experimental design and sample collection

Bulk soil, root, pseudostem, and leaf samples were collected from 55 mature field-grown plants, representing 52 genotypes from the Australian Banana Germplasm Collection (17.60667ºS, 145.9983ºE), which is maintained at the Centre for Wet Tropics Agriculture, South Johnstone, Queensland (Table S2). These genotypes included dessert bananas, cooking bananas, and wild *Musa* spp. (Table S2). While the soil at the site is of Innisfail series, it is from a different location to that used in the pot experiment. For each plant we collected samples in triplicate. Bulk soil and a mixture of apical and basal roots were collected from the base of three pseudostems at different stages of flowering (emergent, immature, and mature). A sample of each pseudostem was then collected using a sterile corer, and the central section of the second most recently emerged leaf was collected with sterile cutting tools. All samples were stored on ice in the field, and then transferred to -20ºC in the lab, except for root samples. Roots were processed as described above to obtain ectorhizosphere and endorhizosphere samples, which were then frozen at -20ºC.

DNA extraction, and PCR and sequencing of 16S rDNA

DNA extraction

Samples were transferred from frozen storage to a freeze drier, lyophilised, and then ground using a TissueLyser II (Qiagen). For each sample, DNA was extracted from 150 mg of fine powder using DNeasy PowerSoil HTP kits (Qiagen) according to the manufacturer's instructions, except for an extra 400 µl of PowerBead solution, which ensured sufficient moisture within each well.

PCR: Universal bacterial 16S rRNA genes were amplified by polymerase chain reaction (PCR) using the primers 799F (5'- AAC MGG ATT AGA TAC CCK G-3') and 1193R (5'- ACG TCA TCC CCA CCT TCC-3'), each modified on the 5' end to contain the Illumina overhang adapter for compatibility with the P5 and i7 Nextera XT indices, respectively. PCR reactions contained: 2 µl of DNA sample in 5X Phire Green Reaction Buffer (Thermo Fisher), 100 µM of each of the dNTPs (Invitrogen), 0.4 µl of Phire Green Hot Start II DNA Polymerase (Thermo Fisher), and 10 mM of each primer. This reaction was made up to a total volume of 20 µl with molecular biology grade water. Thermocycling conditions were as follows: 98°C for 45 sec,

then 35 cycles of 98°C for 5 sec, 56°C for 5 sec, 72°C for 6 sec, and 72°C for 1 min. Amplifications were performed using a SimpliAmp® 96-well Thermocycler (Applied Biosystems). Blank DNA extraction controls, and no-template PCR controls were verified to be negative using gel electrophoresis.

Sequencing

Amplicons were purified using magnetic beads [25] and subjected to dual indexing using the Nextera XT Index Kit (Illumina) according to the manufacturer's instructions. Indexed amplicons were purified using magnetic beads and then quantified using a PicoGreen dsDNA Quantification Kit (Invitrogen). Equal concentrations of each sample were pooled and sequenced on an Illumina MiSeq using 30% PhiX Control v3 (Illumina) and a MiSeq Reagent Kit v3 (600 cycles; Illumina) according to the manufacturer's instructions.

Processing of sequence data

Sequence data from the pot experiments and field survey were processed together using a modified UPARSE workflow [26]. Briefly, demultiplexing and primer removal was performed using cutadapt in QIIME2 (v2017.9.0) [27]. Then in USEARCH (v10.0.240) [28], fastx_truncate was used to trim (250 bp) forward reads, which were quality filtered using fastq_filter (– fastq_maxee = 1.0), and then mapped against representative sequences, generated using fastx_uniques and cluster_otus (sequence similarity = 0.97), to create an operational taxonomic unit (OTU) table using otutab. OTUs were assigned SILVA 138 [29] taxonomy using BLASTN (v2.3.0+) [30] in QIIME2, and those classified as chloroplasts, mitochondria, archaea or eukaryotes were removed from the OTU table using BIOM [31]. Representative bacterial sequences were aligned using MAFFT (v7.221) [32] and masked using QIIME2 to calculate phylogenetic distance, and generate a midpoint-rooted phylogenetic tree using FastTree (v2.1.9) [33]. Samples were rarefied to 1000 reads, and the mean numbers of observed (Sobs) and predicted (Chao1) [34] OTUs, as well as Shannon's Diversity Index [35], and Faith's Phylogenetic Diversity Index (Faith's PD) [36] were calculated using QIIME2. Variation in the composition of microbial communities between samples was investigated from a taxonomic (Hellinger transformed relative OTU abundances) [37] and phylogenetic (weighted UniFrac distances) perspective [38].

Data analyses for the pot experiments

Effects of compartment and soil/genotype

The effects of soil and genotype were analysed as separate experiments – one focussing on a single genotype in five soils, and the other focussing on three genotypes in one soil. The main and interactive effects of compartment (bulk soil, and plant compartments) and soil/genotype on bacterial alpha diversity (Sobs, Chao1, Shannon, and Faith's PD) were assessed using analysis of variance (ANOVA) with Tukey's HSD *post hoc* analyses as implemented using the R (v4.0.3) [39] packages *base* and *agricolae* [40]. The main and interactive effects of compartment and soil/genotype on the composition of bacterial communities (*i.e.* beta diversity as represented by Hellinger transformed OTU relative abundances and

weighted UniFrac distances) were assessed using Permutational Multivariate Analysis of Variance (PERMANOVA) [41] as implemented in *vegan* [42]. The extent to which the microbiomes associated with different compartments resembled one another was visualised using detrended correspondence analysis (DCA). In addition we used SourceTracker, a Bayesian approach implemented through QIIME [43], to determine the proportion of shared taxa between compartments. In this analysis, each compartment was analysed as a source and compared to all others as a sink.

Defining 'candidate core' bacterial taxa

For each level of a significant factor (*i.e.* soil or genotype), we selected candidate-core OTUs on the basis that they were in ≥ 50% of replicates within one or more compartments, at a mean relative abundance of ≥ 0.5% where present.

Data analyses for the field survey

By combining all pot and field data in a single bioinformatics analysis, it was possible to investigate the same OTUs in both settings. As the candidate-core OTUs were identified using the pot experiment only, this approach enabled us to use the field survey as an independent dataset for validation purposes.

Assessing the importance of candidate core taxa using networks: The importance (*i.e.* centrality) of candidate-core relative to non-core OTUs in community interactions were first assessed using network analysis. A network summarising community interactions for the field dataset was inferred using the R package *SpiecEasi* [44]. The network was inferred using OTUs with > 15% prevalence, and at least one occurrence where its relative abundance was ≥ 1% to reduce computational load and spurious connections. Network centrality metrics were calculated for each node (OTU) using the *igraph* and *centiserve* R packages [45, 46] and included: 1) degree, 2) weighted degree, 3) betweenness centrality, 4) closeness centrality, 5) Markov centrality, and 6) PageRank score. Wilcoxon rank-sum tests were used to assess whether these 'importance' metrics differed significantly between 'candidate core' and non-core OTUs using the *base* R package. Gephi [47] was used for network projection and visualisation.

Assessing the importance of candidate core taxa based on their abundance and prevalence in field-grown plants

Next, we determined whether the candidate-core OTUs were found within the 'key constituents' of field-grown plants, which we defined as OTUs that were present in at least one plant compartment, in ≥ 50% of plants, at ≥ 0.5% relative abundance. Candidate core OTUs that were listed among the 'key constituents' of field-grown plants were elevated to full core status, while those that were found only in potted plants were dismissed.

Comparing core OTUs with sequences from 22 previous studies

Finally, we sought to evaluate whether close relatives of our core OTUs have been observed in previous studies of *Musa* spp. bacterial communities. Accordingly, we downloaded bacterial 16S rRNA gene amplicon sequences from 22 previous studies covering five continents (Table S3; Fig. S1). All Sanger datasets had been quality filtered prior to being made publicly available; therefore, no further processing was necessary. For Illumina and 454 datasets, primer and barcode sequences were removed in QIIME v1.9.1 [48] using multiple_extract_barcodes.py. Each 454 dataset was homopolymer-error corrected using Acacia version 1.52 [49]. Illumina and 454 datasets were then quality filtered (qual score = 25) using multiple_split_libraries_fastq.py in QIIME v1.9.1.

These data were generated using different sequencing platforms (Sanger, 454, and Illumina) and by amplifying different regions of 16S rRNA genes, hence, direct blast comparisons with our core OTUs were not possible. For this reason, our core OTU sequences were first queried against full length sequences in SILVA 138 using BLASTN (v2.3.0+). The full-length sequences with the best matches to our core taxa were then extracted and, for each core taxa, converted into a blast database. The quality filtered sequences from previous studies were then queried against each core taxa blast database. The highest percentage sequence similarity from each study to each core taxa blast database was then reported for all hits over > 95% of the query sequence length.

Results

Effects of compartment and soil/genotype

Compartment

Bacterial diversity and community composition differed significantly between compartments, and this effect was considerably stronger than those associated with soil or host-genotype (Tables 1, S4, and S5). The alpha diversity of bulk soil and ectorhizosphere communities was more extensive than that of endophytic communities and declined towards the leaves (Fig. 1). The composition of bacterial communities was more similar in more proximal compartments, with bulk soil and ectorhizosphere communities being most dissimilar to those associated with the pseudostem and leaves (Fig. 2). This finding was also supported by SourceTracker [43] – a tool used to generate Bayesian estimates of the proportions of each community derived from other compartments (Table S6). For example, this analysis indicated that 96% of OTUs associated with leaves were derived from the pseudostem, while only 7% were derived from bulk soil (Table S6).

Table 1 The impacts of soil, genotype, and plant compartment on observed bacterial OTUs and community composition (Hellinger transformed OTUs) using ANOVA and PERMANOVA, respectively.

Predictor variable		Number of observed OTUs		Community composition		
	d.f.	*F* value	*P* value	*F* value	R^2 (%)	*P* value
Compartment	7	268.6	<0.001 ***	26.1	29.3	<0.001 ***
Soil	4	1.6	0.167	6.2	4.0	<0.001 ***
Compartment: Soil	28	4.1	<0.001 ***	2.2	10.0	<0.001 ***
Compartment	7	113.2	<0.001 ***	16.2	33.3	<0.001 ***
Genotype	2	0.6	0.569	1.3	0.8	0.110
Compartment: Genotype	14	0.6	0.845	1.1	4.6	0.115

Musa spp. bacterial communities were dominated by representatives of the Acidobacteriota, Actinobacteriota, Bacteroidota, Firmicutes, Gemmatimonadota, Nitrospirota, Proteobacteria, and Verrucomicrobiota (Fig. 3). Acidobacteriota were frequent in soil and ectorhizosphere compartments, but not in endophytic compartments, which were strongly associated with members of the Bacilli (Fig. 3). Representatives of the actinobacteriotal classes Actinobacteria and Thermoleophilia were found throughout the plant, although members of the latter were not frequent on leaves (Fig. 3). By far the most frequent bacteria were members of the Alpha- and Gamma-proteobacteria (Fig. 3), which represented 14–85% and 6–43% mean relative abundance within each compartment, respectively (Fig. 3).

Soil/genotype

In addition to compartment, bacterial diversity and community composition differed significantly between soils, but not host genotypes (Tables 1, S4, and S5). Between soils, differences in alpha diversity were generally apparent in belowground (bulk soil and rhizosphere) compartments but not elsewhere (Tables 2 and S7). Differences in bacterial community composition between soils were not detected in leaves but were apparent in all other compartments (Tables 2 and S8). In summary, our results demonstrate that the diversity and composition of bacterial communities associated with *Musa* spp. differs between the various compartments of a plant and the soil in which it is grown, but not between the genotypes assessed.

Table 2 The influence of soil on the numbers of observed bacterial OTUs and community composition (Hellinger transformed OTUs) within each compartment, as assessed by ANOVA and PERMANOVA, respectively.

Compartment	Number of observed OTUs			Community composition			
	F value	*P* value		*F* value	R^2 (%)	*P* value	
Bulk soil	3.2	0.022	*	3.8	25.2	<0.001	***
Apical ectorhizosphere	3.5	0.014	*	3.1	21.8	<0.001	***
Apical endorhizosphere	7.0	<0.001	***	2.5	19.5	<0.001	***
Basal ectorhizosphere	6.9	<0.001	***	4.2	27.0	<0.001	***
Basal endorhizosphere	2.7	0.045	*	2.5	18.3	<0.001	***
Rhizome	1.1	0.385		1.7	13.3	<0.001	***
Pseudostem	2.2	0.085		3.1	22.9	<0.001	***
Leaf	1.0	0.394		1.2	9.6	0.226	

Defining ‘candidate core’ bacterial taxa

Based on our findings, we sought to define a core microbiome of banana comprising taxa found in all five soils within the plant compartments characterised. For each soil, we selected candidate-core OTUs on the basis that they were in at least five of ten replicates within one or more compartments, at a mean relative abundance of ≥ 0.5% where present (Fig. S2). This approach yielded a total of 228 candidate-core OTUs (Table S9). Of these, 47 were shared between all soils within at least one compartment and hence maintained candidate-core status (Fig. S3 and S4). The remainder lost candidate-core status and were mostly specific to a particular soil (Fig. S2; Table S9).

Confirming ‘core status’ in mature field-grown Musa spp. plants

Differences between pot and field microbiomes

While our pot experiments enabled the effects of genotype and edaphic conditions to be studied in isolation of other potential drivers of bacterial diversity, we were conscious of the need to consider our results under more realistic conditions. Hence, we performed a survey of mature field-grown *Musa* spp. to facilitate comparisons of pot and field associated plant microbiomes. Field-grown *Musa* spp. microbiomes were dominated by representatives of the same phyla as pot plants (Figs. 3 and S5). As for pot plants, compartment was a strong predictor of bacterial diversity in the field (Table 3). Nonetheless, depending on the compartment studied, there were relatively small but significant differences attributable to setting (field or pot; Table 3). Relative to pot plants, field microbiomes were less diverse in belowground compartments and more diverse in aboveground compartments (Fig. S6). In terms of community composition, differences between settings were most pronounced in the bulk soil and ectorhizosphere (Fig. S7).

Table 3 The impact of the setting plants were grown in (field vs. pot) and plant compartment on observed bacterial OTUs and community composition (Hellinger transformed OTUs) as assessed using ANOVA and PERMANOVA, respectively.

Predictor variable		Number of Observed OTUs		Community composition (Hellinger distance)		
	d.f.	*F* value	*P* value	*F* value	R^2 (%)	*P* value
Setting	1	5.3	0.022 *	60.2	3.6	<0.001 ***
Compartment	4	860.7	<0.001 ***	85.2	20.5	<0.001 ***
Setting: Compartment	4	45.2	<0.001 ***	22.5	5.2	<0.001 ***

Assessing the importance of candidate-core OTUs using networks

In light of our findings, we used network analysis to infer whether the candidate-core OTUs identified in our pot experiments were important members of field-grown *Musa* spp. microbiomes. Interactions between bacterial populations for the field dataset were inferred using a SPIEC-EASI network. Centrality metrics, representing the relative importance of each population, were then calculated for each node (OTU). All metrics indicated that candidate-core OTUs were significantly more important than non-core OTUs (Tables 4 and S10; Figs. 4 and S8).

Table 4 Wilcoxon rank-sum test results highlighting differences in SPIEC-EASI network metrics for candidate-core and non-core taxa in field grown Musa spp.

Metric	Candidate-core (median, 1st quartile, 3rd quartile)	Non-core (median, 1st quartile, 3rd quartile)	*W*	*P*
Betweenness centrality	411 (116, 1572)	49 (0, 298)	13085	<0.001
Weighted degree	0.57 (0.3, 1.3)	0.29 (0.1, 0.6)	12620	<0.001
PageRank	$2.95*10^{-3}$ ($1.8*10^{-3}$, $5.2*10^{-3}$)	$2.71*10^{-3}$ ($8.2*10^{-4}$, $2.7*10^{-3}$)	12717	<0.001
Closeness centrality	$4.29*10^{-5}$ ($4.3*10^{-5}$, $4.3*10^{-5}$)	$4.27*10^{-5}$ ($4.2*10^{-5}$, $4.3*10^{-5}$)	12608	<0.001
Degree	8 (5, 17)	5 (2, 9)	11920	<0.001
Markov centrality	$4.93*10^{-4}$ ($4.5*10^{-4}$, $5.7*10^{-4}$)	$4.37*10^{-4}$ ($3.0*10^{-4}$, $5.1*10^{-4}$)	11800	<0.001

Representation of candidate-core OTUs in field microbiomes

In total, 90 OTUs were classified as 'key constituents' of field-grown *Musa* spp. microbiomes (i.e. OTUs present in at least one plant compartment, in ≥ 50% of plants, at ≥ 0.5% relative abundance), and while representing only 1.3% of total OTUs (6,775), they accounted for 42% of total sequences. Among the key constituents, 77% (36/47) of the 'candidate-core' taxa were present as the same OTU, or less frequently, as a close relative (i.e. an OTU with identical taxonomy; Fig. 5). These candidates were elevated to full 'core' status (Fig. 6 and S9), while those that were not found were dropped.

The core Musa spp. bacterial microbiome

The 36 core taxa are associated with 17 distinct genera. They include members of the Actinobacteriota, Firmicutes, Gemmatimonadota, Nitrospirota, and Proteobacteria, with notably diverse representation among the proteobacterial orders: Rhizobiales, Sphingomonadales and Burkholderiales (Fig. 6 and S9; Table S11). Of a total of 6,775 OTUs in field-grown plants, 62.4% were singletons or doubletons and

represented only 3.2% of total sequences. The 36 core OTUs, on the other hand, represented < 0.5% of total OTUs in field-grown plants but 24.5% of total sequences, with close relatives (OTUs with the same taxonomy) representing an additional 4.0% of total OTUs and 7.4% of total sequences (Fig. S10).

Within compartments, core taxa represented a larger proportion of the key constituents of field microbiomes than non-core taxa (*viz*. ectorhizosphere = 65%, endorhizosphere = 95%, pseudostem = 67% and leaves = 71%; Fig. 5), as well as being some of the most abundant key constituents (Figs. S11-14). Furthermore, except for OTU 61, which was absent in the genotype, Sugar (AAB), all core taxa were present in all 52 genotypes, either as the same OTU or a close relative (i.e. an OTU with identical taxonomy; Figs. S10-13). In other words, 100% of core taxa were present in 98% of genotypes, while Sugar harboured 97% of the core taxa. The representative sequences for the core and candidate-core OTUs are provided in Table S11.

Have our core OTUs been detected in other banana-related studies?

To infer the potential geographical range of our core OTUs, we compared their sequences with those reported in 22 previous studies of banana-associated bacterial communities (Fig. 7; Table S3). For the 10 culture-independent studies that characterised the whole domain, highly similar matches were observed for all populations (Fig. 7). On average, for example, 100%, ≥ 99% and ≥ 97% matches were observed 72.5%, 82.5%, and 95% of these 10 studies, respectively (Fig. 7). In contrast, fewer close matches were detected in the nine culture-dependent studies, with 13 and 15 of our core OTUs having a ≥ 97% match in zero or just one study, respectively (Fig. 7).

Discussion

Common core bacterial taxa represent the bacterial component of a microbiome that are relatively frequent across all, or most, individuals of a host species [50]. Hence, in the context of learning how to manage crop microbiomes, they represent logical candidates for further study because they are the most likely organisms to be present. While we acknowledge that rare microbes also contribute to host function [51], we posit that it is important to first identify the most common taxa and then develop an understanding of their ecological preferences and functional traits. Here we focussed on identifying the common core bacterial taxa associated with *Musa* spp. To this end we considered it necessary to: 1) characterise bacterial communities using methods that encompass all, or most, lineages within the domain, 2) account for variation throughout the plant, and 3) consider variation associated with factors that are likely to differ between farms (e.g. edaphic conditions and cultivar selection). Hence, we sequenced ‘universal’ bacterial 16S rRNA genes amplified from the microbiomes of multiple plant compartments, soils and genotypes, in pot and field experiments.

Musa spp. are associated with diverse bacterial communities

Our results, encompassing variation throughout the plant, highlight that banana microbiomes comprise diverse bacterial lineages, with most frequent representation within the Actinobacteriota, Firmicutes, and Proteobacteria (Fig. 3). This is in broad agreement with other studies that have identified *Musa* spp.-associated bacteria using methods that target the 'whole' domain, albeit these tend to have either focussed exclusively on bulk soil [52–55], or characterised single plant compartments, such as the pseudostem [56], endorhizosphere [57, 58], shoot tips [59], or ectorhizosphere [60]. In contrast, comparisons of bacterial diversity between multiple banana plant compartments tend to have focussed on specific lineages, such as the Gammaproteobacteria [24, 61, 62], shown here to represent 6–43% mean relative abundance depending on plant compartment. In the sole exception to this, where bacterial communities were characterised using 'universal' methods in multiple banana plant compartments (rhizosphere, roots, and corm), statistical comparisons of diversity were not possible due to a lack of replication [58]. Hence, our results greatly expand existing knowledge of the spatial distribution and structure of bacterial communities throughout banana plants.

Bacterial diversity differs between banana plant compartments

We observed large differences in the alpha diversity (Figs. 1 and S5) and composition (Figs. 2 and S6) of bacterial communities between plant compartments. These differences were larger than those attributable to genotype and edaphic conditions and were remarkably similar in the pot and field experiments, which focussed on three-month old and mature plants of varying ages, respectively. Hence, *Musa* spp. appear to establish compartment-specific microbiomes from an early age that persist through to maturity. This information is useful from a management perspective and is not the case in all plants [63].

Relative to bulk soil, the compositional similarity of bacterial communities in both experiments followed the order: ectorhizosphere > endorhizosphere > pseudostem > leaves (Figs. 2 and S6), but the magnitude of these changes was not even. For example, there were larger shifts in membership between ecto- and endo-rhizosphere communities, than between the ectorhizosphere and surrounding bulk soil (Fig. S6). This transition, from outside to inside the roots, was also associated with the largest reductions in alpha diversity (Figs. 1 and S5). These observations are consistent with studies of other plants [64–67] and likely reflect the need for bacteria to have a range of traits that enable them to enter roots and persist [68, 69]. A meta-analysis of 25 previous studies found that the endophytic bacterial communities of various plant species were dominated by Proteobacteria, Actinobacteriota, Firmicutes and Bacteroidota, while members of the Acidobacteriota and Gemmatimonadota were rare despite being common in bulk and ectorhizosphere soil [66]. These observations are commersurate with our findings for *Musa* spp. endophytes (Figs. 3 and S4). Other relatively large shifts in community composition included those between the endorhizosphere, rhizome, pseudostem and leaves (Figs. 2 and S6), perhaps reflecting substantial differences in environmental conditions between these habitats.

In terms of dispersal, roots are typically considered to be colonised by microorganisms from soil, while leaf communities are thought to comprise a larger proportion of taxa from aerial sources [16, 70, 71]. Our SourceTracker analyses indicated that while 81–90% of the ectorhizosphere community may be sourced from bulk soil, only 11–21% was potentially sourced from leaves (Table S6). In the endorhizosphere, however, the likely contributions from leaves (37–40%) were almost as large as those from bulk soil (48–54%), suggesting that within the plant, microorganisms are relatively easily dispersed between above and belowground compartments (Table S6). That said, the estimated contributions to the rhizome and pseudostem from leaves (63%) were larger than those from bulk soil (36–55%; Table S6), suggesting that the rhizome, particularly towards the roots, limits microbial dispersal. Knowledge of the transmission of endophytes within banana would be useful to support the development of microbiome management approaches. For example, if core taxa could be isolated it may be possible to increase their abundances inside roots, or other compartments, via pseudostem injection – a common method for herbicide treatment on commercial banana farms.

Musa spp. microbiomes differ between soils but not genotypes

Many previous studies have demonstrated that, at least belowground, soil is the main allogenic factor influencing plant microbiomes [72–74]. Hence, it was important to determine whether differences in edaphic properties influenced *Musa* spp. microbiomes, and if so, to consider only taxa present in all soils tested for designation as members of the common core. As such, we grew bananas in five distinct soils from Australia's main production area and profiled their microbiomes. We found that, with the exception of leaves, bacterial communities in all plant compartments differed significantly between soils. This highlights that the dominant bacteria associated with *Musa* spp. in one area may not be present in another due to differences in edaphic conditions. For this reason, we only considered bacteria that were strongly represented in all soils tested when selecting candidate core taxa.

Given that a wide variety of bananas are grown around the world [10], it was also important to consider whether differences in genotype influence *Musa* spp. microbiomes. Hence, we compared the microbiomes of *Musa* (AAA Group, Cavendish Subgroup) 'Williams', *Musa* (AAB Group, Pome Subgroup) 'Lady Finger', and *Musa* (AAAB Group, Prata Anã x SH-3142) 'Goldfinger'. Despite, differences in ploidy and genome composition between these cultivars [75], the diversity of their bacterial communities did not differ significantly irrespective of plant compartment. In some plant species, different genotypes have been shown to harbour distinct bacterial communities [76–78]. However, this is not always the case [79], and as a rule of thumb, host phylogenetic distance tends to be negatively associated with the similarity of root microbiomes [6]. Hence, our observation that bacterial diversity did not differ significantly between *Musa* spp. genotypes, may reflect the fact that commercial *Musa* spp. are clonally propagated and closely related to their wild relatives [75, 80].

Defining and refining the core bacterial microbiome of Musa spp.

Based on the findings of our pot experiments, we identified 47 OTUs that were relatively frequent in at least one compartment across most of the plants grown in each soil. As microbiomes can differ between pot and field-grown plants [81, 82], we considered these OTUs as 'candidates' rather than immediately designating them as common core taxa. Full core status was assigned only after confirming that they were relatively frequent in at least one compartment across most of the field-grown plants characterised in our survey of the Australian Banana Germplasm Collection. Of the 47 candidates, 36 OTUs were confirmed to be frequent in most plants and were elevated to full core status (Figs. S10-13). In the endorhizosphere, for example, 95% of dominant OTUs were listed as members of the common core (Fig. 5), and of the 52 *Musa* spp. genotypes surveyed, 51 contained all core OTUs, with only one missing in the other genotype.

Core OTUs have close banana-associated relatives around the world

By comparing the sequences of our core OTUs with those detected in 22 previous studies of banana-associated bacteria we found that they have close relatives in Brazil, China, Costa Rica, the Canary Islands, India, Malaysia, Mexico, Nicaragua, Tanzania, and Uganda. Hence, these findings indicate that our core taxa are pervasively associated with *Musa* spp. across steep environmental gradients spanning different time points, continents, genotypes, plant ages, climates, and edaphic factors. This raises the possibility that they play important roles in the regulation of host function, as has been suggested in other plant microbiome studies [7–9]. To test this hypothesis empirically, the functional responses of *Musa* spp. to changes in the frequencies and/or activities of core taxa need to be measured. This is beyond the scope of our current study; however, in the following discussion we present evidence from network analyses and inferences from the literature that support the notion that our core taxa influence host fitness.

Evidence that core OTUs are important 'hub species'

Network analyses indicated that common core OTUs were significantly more interconnected than non-core OTUs and could be considered as 'microbial hubs' [83] (Table 4). While these apparent interactions may be indirect, current evidence indicates that 'hub species' are important for host fitness and can mediate interactions between the plant and the microbiome [83]. Hence, their loss may render the host more susceptible to disease and compromise resource acquisition [84]. However, there are examples where hub species negatively impact their host. For example, while enhancing plant nutrient uptake, mycorrhizal fungi can inadvertently promote plant-parasitic nematodes by disarming plant defences [85]. Similarly, while facilitating efficient energy harvest, certain gut microbes can promote obesity in humans [86].

Associations of core taxa with host fitness

As all plants in our study lacked symptoms of disease or signs of abnormal pest pressure, the common core OTUs may be considered representative of 'healthy' banana plants. Interestingly, circumstantial evidence from the literature supports that changes in the relative abundances of populations closely

related to our common core influence plant health (Table S12). For example, in a comparison of the pseudostems of healthy and bacterial wilt affected bananas, all of our core pseudostem taxa were found in healthy plants, but only three were detected in sick plants [56]. Furthermore, rotating banana production with pineapple [53], or chilli [87] has been found to reduce the severity of Fusarium Wilt of Banana (FWB) while increasing the relative abundances of genera that contain close relatives of our common core: i.e. *Bradyrhizobium*, or *Gemmatimonas*, *Pseudomonas*, *Sphingobium*, and *Sphingomonas*, respectively. Direct addition of isolates that are closely related to the core has also been observed to protect bananas against diseases. For example, *Pseudomonas* spp. have been shown to lessen the severity of FWB [57, 88–90] and Banana bunchy top disease [91]. Similarly, the addition of *Bacillus* spp. to soil has been shown to reduce the severity of FWB [55, 92–95], with concomitant increases in the relative abundances of *Gemmatimonas*, *Sphingomonas*, *Rhizobium* and/or *Pseudolabrys* populations [96]. Lastly, many studies have demonstrated suppression of *Foc* in culture by close relatives of the common core isolated from banana [97], including members of the *Bacillus* [98, 99], *Pseudomonas* [57, 100, 101] *Rhizobium* [100], and *Streptomycetes* [102].

Despite being representative of 'healthy' banana plants, some members of the common core are closely related to lineages that harbour devastating *Musa* spp. pathogens. For example, OTU10 is a member of the genus *Ralstonia*, which includes the causal agents of Moko/Bugtok disease (*Ralstonia solanacearum*) and banana blood disease (*Ralstonia syzygii* ssp. *celebensis*). In addition, OTU8 and OTU20 are members of the *Enterobacteriaceae* and *Xanthomonadales*, which include the causal agents of *Erwinia*-associated diseases (i.e. *Erwinia carotovora* ssp. *carotovora*; *E. chrysanthemi*, and *Dickeya paradisiaca*) and Xanthomonas wilt (*Xanthomonas campestris* pv. *musacearum*) [103]. While these populations may be latent pathogens [104], bacterial genera frequently contain species with non-pathogenic and pathogenic strains [105–107] – differentiated by only a small number of virulence genes [104]. Hence, given the persistently strong associations between common core taxa and their host, it is logical that cooperative symbioses will be undermined, from time-to-time, by 'cheaters', such as parasites and pathogens [108].

Evidence suggests that common core taxa may also influence other aspects of banana plant fitness. Plant growth promotion, for example, has frequently been observed in bananas inoculated with close relatives of the common core originally isolated from *Musa* spp., such as *Bacillus* spp. [109–111], *Enterobacter* spp. [112], *Pseudomonas* spp. [113] and *Rhizobium* spp. [114, 115]. Furthermore, genome sequences and *in vitro* lab assays have revealed that these, and other close relatives of the common core, harbour genes and exhibit phenotypes that are associated with multiple approaches to promote plant growth, enhance plant stress tolerance, and interact with other organisms [57, 69, 101, 115–118]. *Rhizobium* spp. isolated from banana, for example, have been reported to produce the auxin phytohormone indole-3-acetic acid, fix nitrogen, and solubilise phosphate [115].

Conclusion

Our findings have meaningful implications for the development of strategies to manage *Musa* spp. microbiomes. We have demonstrated that while the bacterial communities associated with banana are highly similar across genotypes, they can vary greatly between locations due to differences in edaphic conditions. Hence, our findings support the notion that research efforts to maximise plant productivity by controlling the abundances and activities of their symbionts, are best focussed on core microbes, as they are most likely to be present. Critically, we have identified 36 common core OTUs that share 100% sequence similarity with bacteria detected in bananas grown in other parts of the world. These core bacteria were found in pot and field grown banana plants, irrespective of soil and genotype, and were identified as highly interconnected 'hubs' within networks – a characteristic shown to be indicative of microbes that influence host fitness in studies of other plants. Circumstantial evidence from the literature demonstrates that close relatives of the common core help to reduce banana diseases and promote growth. However, to justify a focus of research efforts on this small but frequently encountered subset of taxa, it is critical to experimentally verify these associations. To do this, it is necessary to measure the functional responses of *Musa* spp. to changes in the frequencies of core taxa. Such changes may be achieved by manipulating soil properties (e.g. pH [119]), or on-farm practices (e.g. rotation [87]). However, while these approaches may represent useful levers for future microbiome managers, current knowledge of their effects on the abundances of core taxa is sparse, and their lack of specificity represents a major obstacle to establishing the causality of plant-microbe interactions. Another approach may be to manipulate the frequencies of core taxa through their cultivation and subsequent addition to *Musa* spp. in gnotobiotic experiments, either alone, or as synthetic communities (SynComs [120, 121]). The controlled nature of these studies would help to establish causality and to identify the mechanisms of interaction between banana and their core bacteria. In this regard, we would like to point out that highly similar matches to some of our core OTUs have already been isolated in previous studies and may be suitable for experimentation (Fig. 8). Having identified a robust list of banana-associated core bacteria, we hope that this contribution will accelerate the acquisition of knowledge that is most relevant to the development of effective *Musa* spp. microbiome management practices.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The 16S rRNA gene amplicon sequences associated with this study have been deposited in the NCBI SRA under accession: PRJNA729168.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

PGD and ABP secured funding and designed the study. HB, PGD and ABP collected samples and performed experiments. HB and AR performed marker gene sequencing. HB, PGD and AS analysed data. PGD and HB wrote the paper with input from all authors.

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References

1. Food and Agriculture Organization of the United Nations. FAOSTAT statistics database. http://www.fao.org/faostat/en/. Accessed 14 January 2022.
2. Dita M, Barquero M, Heck D, Mizubuti ESG, Staver CP. Fusarium Wilt of Banana: Current Knowledge on Epidemiology and Research Needs Toward Sustainable Disease Management. Front Plant Sci. 2018;9:1468.
3. Ploetz RC, Evans EA. The future of global banana production. In: Janick J, editor. Horticultural Reviews Volume 43. Hoboken, New Jersey: Wiley; 2015. p. 311–52.
4. Lay C, Bell TH, Hamel C, Harker KN, Mohr R, Greer CW, et al. Canola Root – Associated Microbiomes in the Canadian Prairies. Front Microbiol. 2018;9:1188.
5. Chen L, Xin X, Zhang J, Redmile-Gordon M, Nie G, Wang Q. Soil Characteristics Overwhelm Cultivar Effects on the Structure and Assembly of Root-Associated Microbiomes of Modern Maize. Pedosphere. 2019;29(3):360–73.
6. Yeoh YK, Dennis PG, Paungfoo-Lonhienne C, Weber L, Brackin R, Ragan MA, et al. Evolutionary conservation of a core root microbiome across plant phyla along a tropical soil chronosequence. Nat Commun.2017;8:215.
7. Shade A, Handelsman J. Beyond the Venn diagram: The hunt for a core microbiome. Environ Microbiol. 2012;14(1):4–12.

8. Toju H, Sugiura R, Schlaeppi K, Yamamichi M, Fukuda S, Kiers ET, et al. Core microbiomes for sustainable agroecosystems. Nat Plants. 2018;4:247–57.
9. Shade A, Stopnisek N. Abundance-occupancy distributions to prioritize plant core microbiome membership. Curr Opin Microbiol. 2019;49:50–8.
10. Stover RH, Simmonds NW. Bananas. 3rd ed. London: Longman; 1987.
11. Rossmann B, Müller H, Smalla K, Mpiira S, Tumuhairwe JB, Staver C, et al. Banana-associated microbial communities in Uganda are highly diverse but dominated by Enterobacteriaceae. Appl Environ Microbiol. 2012;78(14):4933–41.
12. Dennis PG, Miller AJ, Hirsch PR. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiol Ecol. 2010;72:313–27.
13. Zarraonaindia I, Owens SM, Weisenhorn P, West K, Hampton-marcell J, Lax S, et al. The Soil Microbiome Influences Grapevine-Associated Microbiota. 2015;6(2):e02527-14.
14. Blaustein RA, Lorca GL, Meyer JL, Gonzalez CF, Teplitski M. Defining the core citrus leaf- and root-associated microbiota: Factors associated with community structure and implications for managing huanglongbing (citrus greening) disease. Appl Environ Microbiol. 2017;83:e00210-17.
15. Wagner MR, Lundberg DS, Rio TG, Tringe SG, Dangl JL, Mitchell-olds T. Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. Nat Commun. 2016;7:12151.
16. Dastogeer KMG, Tumpa FH, Sultana A, Akter MA, Chakraborty A. Plant microbiome–an account of the factors that shape community composition and diversity. Curr Plant Biol. 2020;23:100161.
17. Warman NM, Aitken EAB. The Movement of Fusarium oxysporum f. sp. cubense (sub-tropical race 4) in Susceptible Cultivars of Banana. Front Plant Sci. 2018;9:1748.
18. Enderlin NG, Barry E V, Phillip SR. Soils of the Mareeba-Dimbulah Irrigation Area (MDIA). Queensland Department of Natural Resources. Mareeba, Queensland. 1997.
19. Cannon MG, Smith CD, Murtha GG. Soils of the Cardwell-Tully area, North Queensland. Division of Soils, CSIRO;1992.
20. Murtha GG. Soils of the Tully-Innisfail area, North Queensland. CSIRO; 1986.
21. Australian Banana Growers Council. Key Facts. https://abgc.org.au/our-industry/key-facts/. Accessed 13 May 2021.
22. De Ascensao AR, Dubery IA. Panama Disease: Cell Wall Reinforcement in Banana Roots in Response to Elicitors from Fusarium oxysporum f. sp. cubense Race Four. Phytopathology. 2000;90(10):1173–80.
23. Birt HWG, Dennis PG. Appropriate sampling of plants for use in culture independent microbiome studies. In: Carvalhais LC, Dennis PG, editors. Plant Microbiome: Methods and Protocols. New York: Springer;2021.
24. Köberl M, Dita M, Martinuz A, Staver C, Berg G. Agroforestry leads to shifts within the gammaproteobacterial microbiome of banana plants cultivated in Central America. Front Microbiol.

2015;6:91.
25. Rohland N, Reich D. Cost-effective, high-throughput DNA sequencing. Genome Res. 2011;22:939–46.
26. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods. 2013;10:996–8.
27. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37:852–7.
28. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010;26:2460–1.
29. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2012;41:D590–6.
30. Zhang Z, Schwartz S, Wagner L, Miller W. A greedy algorithm for aligning DNA sequences. J Comput Biol. 2000;7:203–14.
31. McDonald D, Clemente JC, Kuczynski J, Rideout JR, Stombaugh J, Wendel D, et al. The Biological Observation Matrix (BIOM) format or: how I learned to stop worrying and love the ome-ome. Gigascience. 2012;1(1):7.
32. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30:772–80.
33. Price MN, Dehal PS, Arkin AP. FastTree 2–approximately maximum-likelihood trees for large alignments. PLoS One. 2010;5(3):e9490.
34. Chao A. Nonparametric estimation of the number of classes in a population. Scand J Stat. 1984;265–70.
35. Shannon CE. A mathematical theory of communication. Bell Syst Tech J. 1948;27:379–423.
36. Faith DP. Conservation evaluation and phylogenetic diversity. Biol Conserv. 1992;61(1):1–10.
37. Legendre P, Gallagher ED. Ecologically meaningful transformations for ordination of species data. Oecologia. 2001;129:271–80.
38. Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol. 2005;71:8228–35.
39. R Core Team. A Language and Environment for Statistical Computing. www.R-project.org. Accessed 10 October 2020.
40. de Mendiburu F. Statistical Procedures for Agricultural Research. R Package. 2019.
41. Anderson MJ. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 2001;26:32–46.
42. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. vegan: Community Ecology Package. R package. 2017.
43. Knights D, Kuczynski J, Charlson ES, Zaneveld J, Mozer MC, Collman RG, et al. Bayesian community-wide culture-independent microbial source tracking. Nat Methods. 2011;8:761–5.

44. Kurtz ZD, Müller CL, Miraldi ER, Littman DR, Blaser MJ, Bonneau RA. Sparse and Compositionally Robust Inference of Microbial Ecological Networks. PLoS Comput Biol. 2015;11(5):e1004226.
45. Jalili M. centiserve: Find Graph Centrality Indices. R package. 2017
46. Csardi G, Nepusz T. The igraph software package for complex network research. InterJournal. 2006;1695(9):1–9.
47. Bastian M, Heymann S, Jacomy M. Gephi: an open source software for exploring and manipulating networks. Third international AAAI conference on weblogs and social media. 2009.
48. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. Nature Publishing Group; 2010;7:335–6.
49. Bragg L, Stone G, Imelfort M, Hugenholtz P, Tyson GW. Fast, accurate error-correction of amplicon pyrosequences using Acacia. Nat Methods. 2012;9:425–6.
50. Risely A. Applying the core microbiome to understand host–microbe systems. J Anim Ecol. 2020;89:1549–58.
51. Jousset A, Bienhold C, Chatzinotas A, Gallien L, Gobet A, Kurm V, et al. Where less may be more: How the rare biosphere pulls ecosystems strings. ISME J. 2017;11:853–62.
52. Shen Z, Ruan Y, Chao X, Zhang J, Li R, Shen Q. Rhizosphere microbial community manipulated by 2 years of consecutive biofertilizer application associated with banana Fusarium wilt disease suppression. Biol Fertil Soils. 2015;51:553–62.
53. Wang B, Li R, Ruan Y, Ou Y, Zhao Y, Shen Q. Pineapple-banana rotation reduced the amount of Fusarium oxysporum more than maize-banana rotation mainly through modulating fungal communities. Soil Biol Biochem. 2015;86:77–86.
54. Shen Z, Ruan Y, Wang B, Zhong S, Su L, Li R, et al. Effect of biofertilizer for suppressing Fusarium wilt disease of banana as well as enhancing microbial and chemical properties of soil under greenhouse trial. Appl Soil Ecol. 2015;93:111–9.
55. Shen Z, Xue C, Penton CR, Thomashow LS, Zhang N, Wang B, et al. Suppression of banana Panama disease induced by soil microbiome reconstruction through an integrated agricultural strategy. Soil Biol Biochem. 2019;128:164–74.
56. Suhaimi NSM, Goh SY, Ajam N, Othman RY, Chan KG, Thong KL. Diversity of microbiota associated with symptomatic and non-symptomatic bacterial wilt-diseased banana plants determined using 16S rRNA metagenome sequencing. World J Microbiol Biotechnol. 2017;33:168.
57. Gómez-Lama Cabanás C, Fernández-González AJ, Cardoni M, Valverde-Corredor A, López-Cepero J, Fernández-López M, et al. The Banana Root Endophytome: Differences between Mother Plants and Suckers and Evaluation of Selected Bacteria to Control Fusarium oxysporum f.sp. cubense. J Fungi. 2021;7:194.
58. Kaushal M, Swennen R, Mahuku G. Unlocking the microbiome communities of banana (Musa spp.) under disease stressed (fusarium wilt) and non-stressed conditions. Microorganisms. 2020;8:443.

59. Thomas P, Sekhar AC. Cultivation Versus Molecular Analysis of Banana (Musa sp.) Shoot-Tip Tissue Reveals Enormous Diversity of Normally Uncultivable Endophytic Bacteria. Microb Ecol. 2017;73:885–99.
60. De la Torre-González FJ, Fernández-Castillo E, Azaharez-Llorente D, Lara J, Avendaño E, Castañeda A, et al. Response to Edaphoclimatic Conditions and Crop Management of the Bacterial Microbiome of Musa acuminata Rhizosphere Profiled by 16S rRNA Gene Amplicon Sequencing. Microbiol Resour Announc. 2021;10:e01437-20.
61. Nimusiima J, Köberl M, Tumuhairwe JB, Kubiriba J, Staver C, Berg G. Transgenic banana plants expressing Xanthomonas wilt resistance genes revealed a stable non-target bacterial colonization structure. Sci Rep. 2015;5:18078.
62. Köberl M, Dita M, Martinuz A, Staver C, Berg G. Members of Gammaproteobacteria as indicator species of healthy banana plants on Fusarium wilt-infested fields in Central America. Sci Rep. 2017;7:45318.
63. Doherty JR, Crouch JA, Roberts JA. Plant age influences microbiome communities more than plant compartment in greenhouse-grown creeping bentgrass. Phytobiomes J. 2021;5(4):373–81.
64. Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, et al. Defining the core Arabidopsis thaliana root microbiome. Nature. 2012;488:86–90.
65. Coleman-derr D, Desgarennes D, Fonseca-garcia C, Gross S, Clingenpeel S, Woyke T, et al. Plant compartment and biogeography affect microbiome composition in cultivated and native Agave species. New Phytol. 2016;209:798–811.
66. Liu H, Carvalhais LC, Schenk PM, Dennis PG. Effects of jasmonic acid signalling on the wheat microbiome differ between body sites. Sci Rep. 2017;7:41766.
67. Hamonts K, Trivedi P, Garg A, Janitz C, Grinyer J, Holford P, et al. Field study reveals core plant microbiota and relative importance of their drivers. Environ Microbiol. 2018;20:124–40.
68. Reinhold-Hurek B, Hurek T. Living inside plants: Bacterial endophytes. Curr Opin Plant Biol. 2011;14:435–43.
69. Sessitsch A, Hardoim P, Döring J, Weilharter A, Krause A, Woyke T, et al. Functional Characteristics of an Endophyte Community Colonizing Rice Roots as Revealed by Metagenomic Analysis. Mol Plant-Microbe Interact. 2012;25:28–36.
70. Frank AC, Guzmán JPS, Shay JE. Transmission of bacterial endophytes. Microorganisms. 2017;5:70.
71. Llontop MEM, Tian L, Sharma P, Heflin L, Bernal-Galeano V, Haak DC, et al. Experimental evidence pointing to rain as a reservoir of tomato phyllosphere microbiota. Phytobiomes J. 2021;5:382–99.
72. Peiffer JA, Spor A, Koren O, Jin Z, Tringe SG, Dangl JL, et al. Diversity and heritability of the maize rhizosphere microbiome under field conditions. Proc Natl Acad Sci USA. 2013;110:6548–53.
73. Shakya M, Gottel N, Castro H, Yang ZK, Gunter L, Labbé J, et al. A Multifactor Analysis of Fungal and Bacterial Community Structure in the Root Microbiome of Mature Populus deltoides Trees. PLoS One. 2013;8(10):e76382.

74. Walters WA, Jin Z, Youngblut N, Wallace JG, Sutter J, Zhang W, et al. Large-scale replicated field study of maize rhizosphere identifies heritable microbes. Proc Natl Acad Sci USA. 2018;115:7368–73.
75. Christelová P, De Langhe E, Hřibová E, Čížková J, Sardos J, Hušáková M, et al. Molecular and cytological characterization of the global Musa germplasm collection provides insights into the treasure of banana diversity. Biodivers Conserv. 2017;26:801–24.
76. Edwards J, Johnson C, Santos-Medellín C, Lurie E, Podishetty NK, Bhatnagar S, et al. Structure, variation, and assembly of the root-associated microbiomes of rice. Proc Natl Acad Sci USA. 2015;112:E911–20.
77. Bulgarelli D, Garrido-Oter R, Münch PC, Weiman A, Dröge J, Pan Y, et al. Structure and function of the bacterial root microbiota in wild and domesticated barley. Cell Host Microbe. 2015;17:392–403.
78. Cregger MA, Veach AM, Yang ZK, Crouch MJ, Vilgalys R, Tuskan GA, et al. The Populus holobiont: Dissecting the effects of plant niches and genotype on the microbiome. Microbiome. 2018;6:31.
79. Rochefort A, Simonin M, Marais C, Guillerm-erckelboudt A, Barret M. Transmission of Seed and Soil Microbiota to Seedling. Msystems. 2021;6:e00446-21.
80. Price NS. The origin and development of banana and plantain cultivation. In: Gowen S, editor. Bananas and Plantains. Dordrecht: Springer; 1995. p. 1–12.
81. Poorter H, Fiorani F, Pieruschka R, Wojciechowski T, van der Putten WH, Kleyer M, et al. Pampered inside, pestered outside? Differences and similarities between plants growing in controlled conditions and in the field. New Phytol. 2016;212:838–55.
82. Kecskés ML, Choudhury A, Casteriano A V, Deaker R, Roughley RJ, Lewin L, et al. Effects of bacterial inoculant biofertilizers on growth, yield and nutrition of rice in Australia. J Plant Nutr. 2016;39:377–88.
83. Agler MT, Ruhe J, Kroll S, Morhenn C, Kim ST, Weigel D, et al. Microbial Hub Taxa Link Host and Abiotic Factors to Plant Microbiome Variation. PLoS Biol. 2016;14(1): e1002352.
84. van der Heijden MGA, Hartmann M. Networking in the Plant Microbiome. PLoS Biol. 2016;14(2):e1002378.
85. Frew A, Powell JR, Glauser G, Bennett AE, Johnson SN. Mycorrhizal fungi enhance nutrient uptake but disarm defences in plant roots, promoting plant-parasitic nematode populations. Soil Biol Biochem. 2018;126:123–32.
86. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444:1027–31.
87. Hong S, Jv H, Lu M, Wang B, Zhao Y, Ruan Y. Significant decline in banana Fusarium wilt disease is associated with soil microbiome reconstruction under chilli pepper-banana rotation. Eur J Soil Biol. 2020;97:103154.
88. Thangavelu R, Palaniswami A, Doraiswamy S, Velazhahan R. The effect of Pseudomonas fluorescens and Fusarium oxysporum f.sp. cubense on induction of defense enzymes and phenolics in banana. Biol Plant. 2003;46:107–12.

89. Ayyadurai N, Ravindra Naik P, Sreehari Rao M, Sunish Kumar R, Samrat SK, Manohar M, et al. Isolation and characterization of a novel banana rhizosphere bacterium as fungal antagonist and microbial adjuvant in micropropagation of banana. J Appl Microbiol. 2006;100:926–37.
90. Akila R, Rajendran L, Harish S, Saveetha K, Raguchander T, Samiyappan R. Combined application of botanical formulations and biocontrol agents for the management of Fusarium oxysporum f. sp. cubense (Foc) causing Fusarium wilt in banana. Biol Control. 2011;57:175–83.
91. Kavino M, Harish S, Kumar N, Saravanakumar D, Samiyappan R. Induction of systemic resistance in banana (Musa spp.) against Banana bunchy top virus (BBTV) by combining chitin with root-colonizing Pseudomonas fluorescens strain CHA0. Eur J Plant Pathol. Springer; 2008;120:353–62.
92. Zhang N, He X, Zhang J, Raza W, Yang XM, Ruan YZ, et al. Suppression of Fusarium Wilt of Banana with Application of Bio-Organic Fertilizers. Pedosphere. Soil Science Society of China; 2014;24:613–24.
93. Shen Z, Wang B, Lv N, Sun Y, Jiang X, Li R, et al. Effect of the combination of bio-organic fertiliser with Bacillus amyloliquefaciens NJN-6 on the control of banana Fusarium wilt disease, crop production and banana rhizosphere culturable microflora. Biocontrol Sci Technol. Taylor & Francis; 2015;25:716–31.
94. Fu L, Penton CR, Ruan Y, Shen Z, Xue C, Li R, et al. Inducing the rhizosphere microbiome by biofertilizer application to suppress banana Fusarium wilt disease. Soil Biol Biochem. 2017;104:39–48.
95. Shen Z, Xue C, Taylor PWJ, Ou Y, Wang B, Zhao Y, et al. Soil pre-fumigation could effectively improve the disease suppressiveness of biofertilizer to banana Fusarium wilt disease by reshaping the soil microbiome. Biol Fertil Soils. Biology and Fertility of Soils; 2018;54:793–806.
96. Shen Z, Wang D, Ruan Y, Xue C, Zhang J, Li R, et al. Deep 16S rRNA pyrosequencing reveals a bacterial community associated with banana Fusarium wilt disease suppression induced by bio-organic fertilizer application. PLoS One. 2014;9(5):e98420.
97. Mon YY, Bidabadi SS, Oo KS, Zheng S-J. The antagonistic mechanism of rhizosphere microbes and endophytes on the interaction between banana and Fusarium oxysporum f. sp. cubense. Physiol Mol Plant Pathol. 2021;116:101733.
98. Souza A, Cruz JC, Sousa NR, Procópio ARL, Silva GF. Endophytic bacteria from banana cultivars and their antifungal activity. Genet Mol Res. 2014;13:8661–70.
99. Souza SA, Xavier AA, Costa MR, Cardoso AMS, Pereira MCT, Nietsche S. Endophytic bacterial diversity in banana “Prata Anã” (Musa spp.) roots. Genet Mol Biol. 2013;36:252–64.
100. Thangavelu R, Gopi M. Field suppression of Fusarium wilt disease in banana by the combined application of native endophytic and rhizospheric bacterial isolates possessing multiple functions. Phytopathol Mediterr. 2015;54:241–52.
101. Bubici G, Kaushal M, Prigigallo MI, Gómez-Lama Cabanás C, Mercado-Blanco J. Biological Control Agents Against Fusarium Wilt of Banana. Front Microbiol. 2019;10:616.

102. Cao L, Qiu Z, You J, Tan H, Zhou S. Isolation and characterization of endophytic streptomycete antagonists of Fusarium wilt pathogen from surface-sterilized banana roots. FEMS Microbiol Lett. 2005;247:147–52.
103. Blomme G, Dita M, Jacobsen KS, Pérez Vicente L, Molina A, Ocimati W, et al. Bacterial diseases of bananas and enset: Current state of knowledge and integrated approaches toward sustainable management. Front Plant Sci. Frontiers; 2017;8:1290.
104. Ehrlich GD, Hiller NL, Hu FZ. What makes pathogens pathogenic. Genome Biol. 2008;9:225.
105. Cadot C, Tran SL, Vignaud ML, De Buyser ML, Kolstø AB, Brisabois A, et al. InhA1, NprA, and HlyII as candidates for markers to differentiate pathogenic from nonpathogenic Bacillus cereus strains. J Clin Microbiol. 2010;48:1358–65.
106. Groisman EA, Ochman H. How Salmonella became a pathogen. Trends Microbiol. 1997;5:343–9.
107. Fouts DE, Matthias MA, Adhikarla H, Adler B, Amorim-Santos L, Berg DE, et al. What Makes a Bacterial Species Pathogenic?:Comparative Genomic Analysis of the Genus Leptospira. PLoS Negl Trop Dis. 2016;10(2):e0004403.
108. Foster KR, Kokko H. Cheating can stabilize cooperation in mutualisms. Proc R Soc B Biol Sci. 2006;273:2233–9.
109. Yuan J, Ruan Y, Wang B, Zhang J, Waseem R, Huang Q, et al. Plant growth-promoting rhizobacteria strain bacillus amyloliquefaciens njn-6-enriched bio-organic fertilizer suppressed fusarium wilt and promoted the growth of banana plants. J Agric Food Chem. 2013;61:3774–80.
110. Xue C, Penton CR, Shen Z, Zhang R, Huang Q, Li R, et al. Manipulating the banana rhizosphere microbiome for biological control of Panama disease. Sci Rep. 2015;5:11124.
111. Wang B, Shen Z, FengGe Z, Razam W, Yuan J, Huang R, et al. Bacillus amyloliquefaciens strain W19 can promote growth and yield and suppress Fusarium wilt in banana under greenhouse and field conditions. Pedosphere. 2016;26:733–44.
112. Macedo-Raygoza GM, Valdez-Salas B, Prado FM, Prieto KR, Yamaguchi LF, Kato MJ, et al. Enterobacter cloacae, an Endophyte That Establishes a Nutrient-Transfer Symbiosis With Banana Plants and Protects Against the Black Sigatoka Pathogen. Front Microbiol. 2019;10:804.
113. Gamez R, Cardinale M, Montes M, Ramirez S, Schnell S, Rodriguez F. Screening, plant growth promotion and root colonization pattern of two rhizobacteria (Pseudomonas fluorescens Ps006 and Bacillus amyloliquefaciens Bs006) on banana cv. Williams (Musa acuminata Colla). Microbiol Res. 2019;220:12–20.
114. Martínez L, Caballero-Mellado J, Orozco J, Martínez-Romero E. Diazotrophic bacteria associated with banana (Musa spp.). Plant Soil. 2003;257:35–47.
115. Salvador-Figueroa M, Ruíz-Valdiviezo VM, Rogel-Hernandez MA, Gutiérrez-Miceli FA, Rincón-Molina CI, Dendoveen L, et al. Rhizobium strain, a banana (Musa spp.)-associated bacterium with a high potential as biofertilizer. J Plant Nutr. 2016;39:1449–59.
116. Straub D, Rothballer M, Hartmann A, Ludewig U. The genome of the endophytic bacterium H. frisingense GSF30T identifies diverse strategies in the Herbaspirillum genus to interact with plants.

Front Microbiol. 2013;4:168.
117. Karthik M, Pushpakanth P, Krishnamoorthy R, Senthilkumar M. Endophytic bacteria associated with banana cultivars and their inoculation effect on plant growth. J Hortic Sci Biotechnol. 2017;92:568–76.
118. Sheibani-Tezerji R, Rattei T, Sessitsch A, Trognitz F, Mitter B. Transcriptome profiling of the endophyte burkholderia phytofirmans psjn indicates sensing of the plant environment and drought stress. MBio. 2015;6(5):e00621-15.
119. Zhang J, Bei S, Li B, Zhang J, Christie P, Li X. Organic fertilizer, but not heavy liming, enhances banana biomass, increases soil organic carbon and modifies soil microbiota. Appl Soil Ecol. 2019;136:67–79.
120. Vorholt JA, Vogel C, Carlström CI, Müller DB. Establishing Causality: Opportunities of Synthetic Communities for Plant Microbiome Research. Cell Host Microbe. 2017;22:142–55.
121. Liu YX, Qin Y, Bai Y. Reductionist synthetic community approaches in root microbiome research. Curr Opin Microbiol. 2019;49:97–102.

Figures

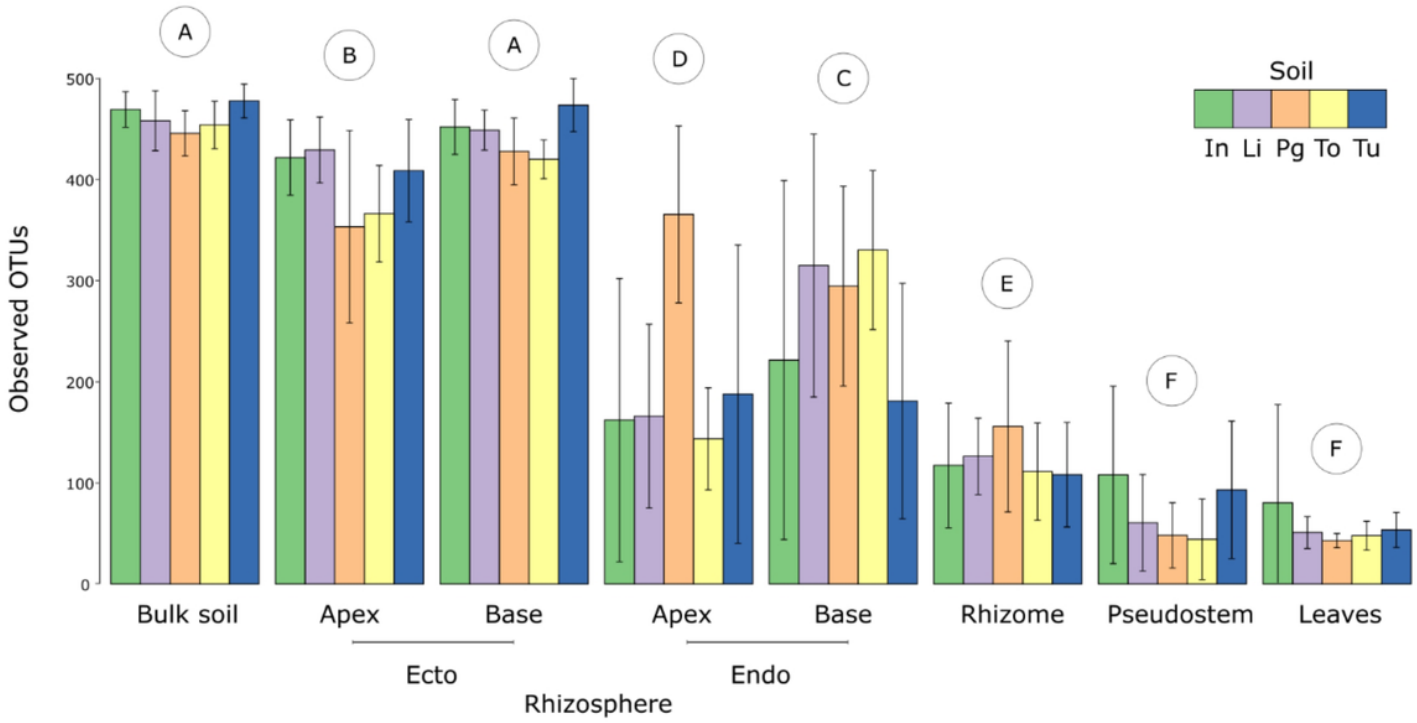

Figure 1

Numbers of observed bacterial OTUs associated with different compartments and soils. Error bars represent standard errors of the means. The letters in circles indicate compartments that differ across soils according to Tukey post hoc tests. Members of the same groupings share the same letter.

Abbreviations for soils are as follows: In – Innisfail, Li – Liverpool, Pg – Pin Gin, To – Tolga, and Tu – Tully.

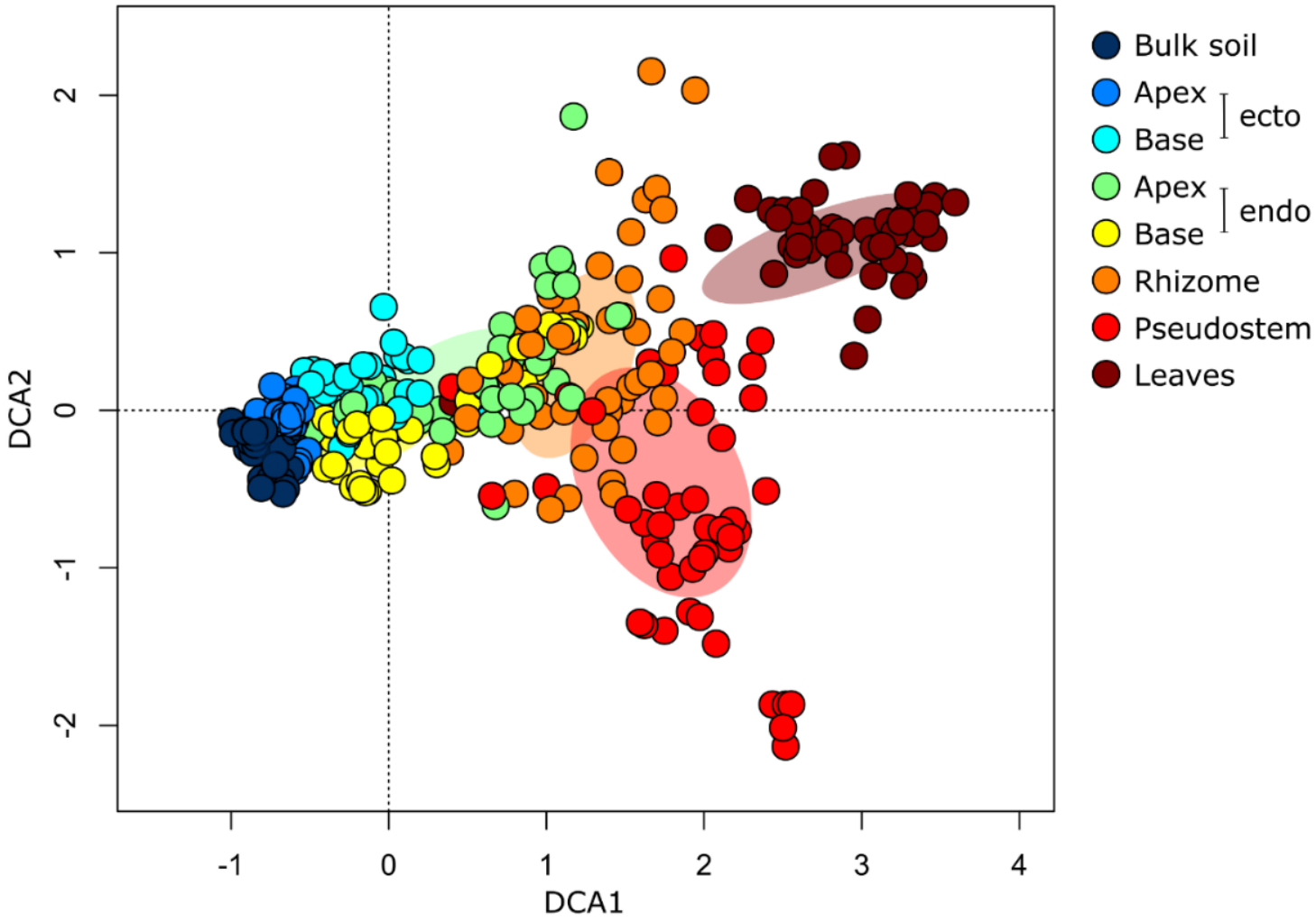

Figure 2

Detrended Correspondence Analysis (DCA) ordination highlighting differences in the composition of bacterial communities (Hellinger transformed OTUs) associated with Williams Cavendish in different plant compartments. Each compartment is represented by 50 replicates comprising 10 samples from each soil. The ellipses represent standard deviations of the group centroids.

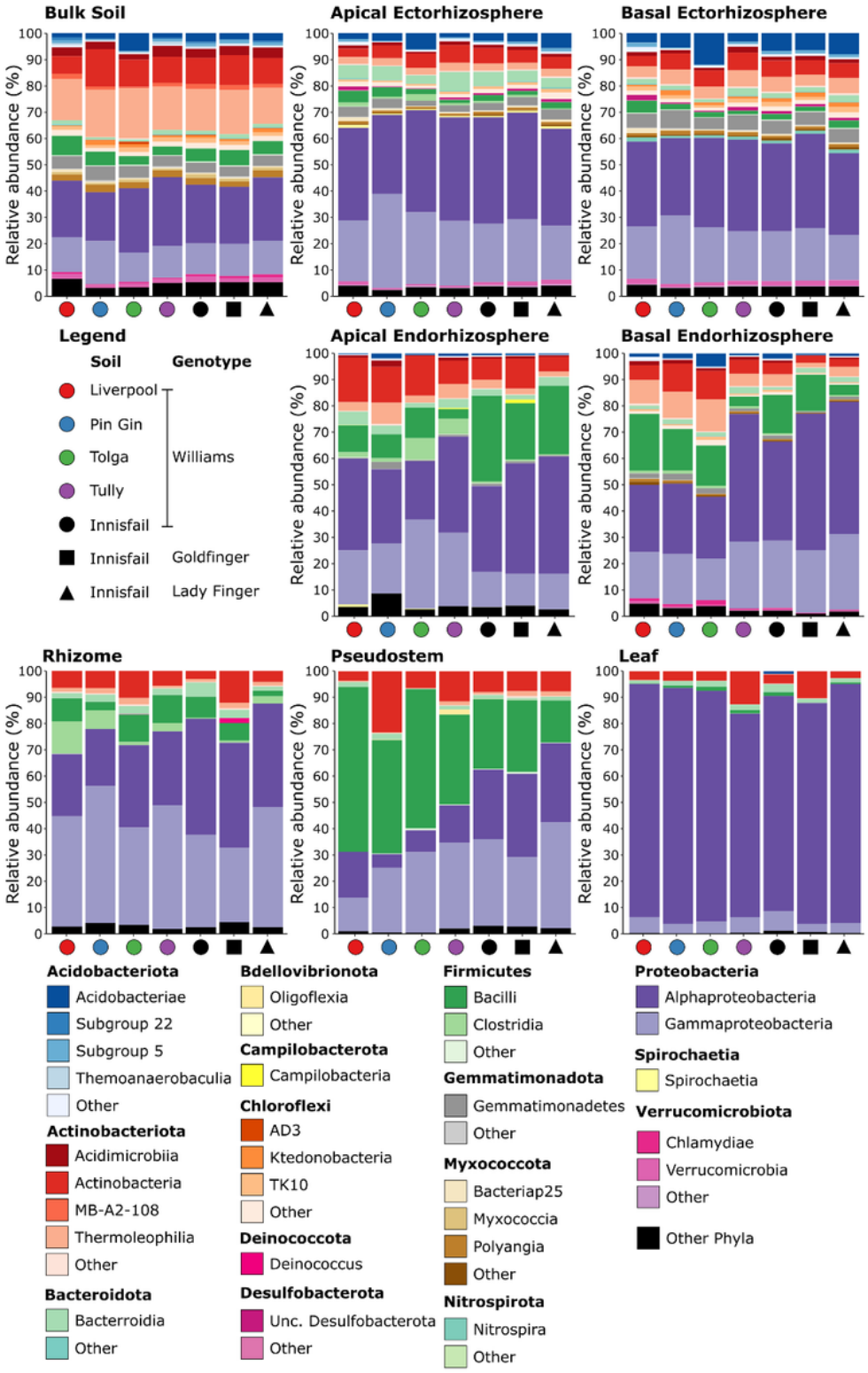

Figure 3

The mean relative frequencies of bacterial classes within each compartment across soils and genotypes. Within each phylum, classes represented at <1% mean relative abundance are grouped as other.

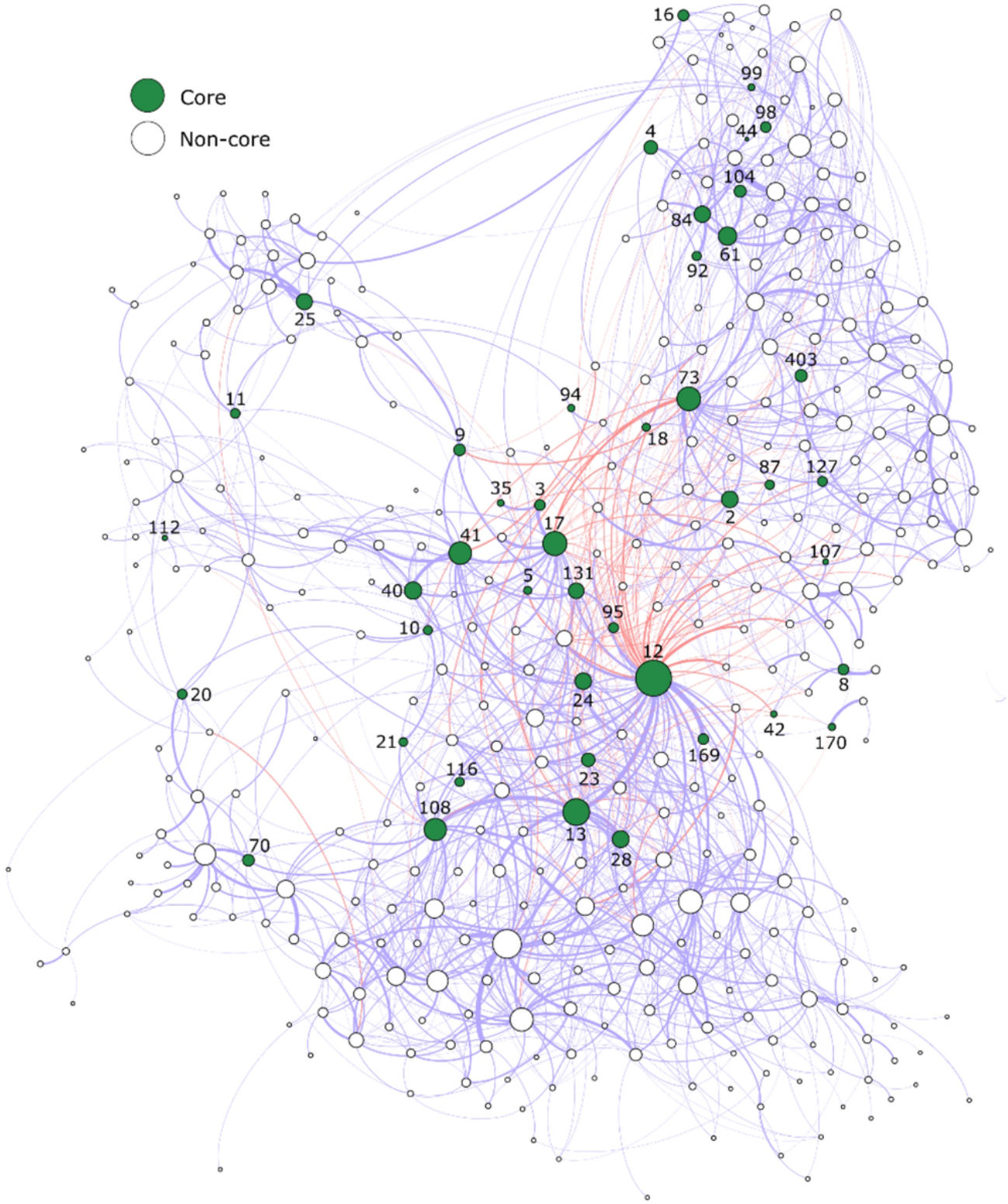

Figure 4

A SPIEC-EASI network graph showing the co-occurrences of core and non-core bacteria in field-grown Musa spp. Nodes are coloured by core status and size is positively associated with weighted degree. Edge colours represent positive (blue) and negative (red) associations between taxa. Edge width is positively associated with the coefficient for the co-occurrence between the taxa. The numbers next to the nodes represent OTU IDs.

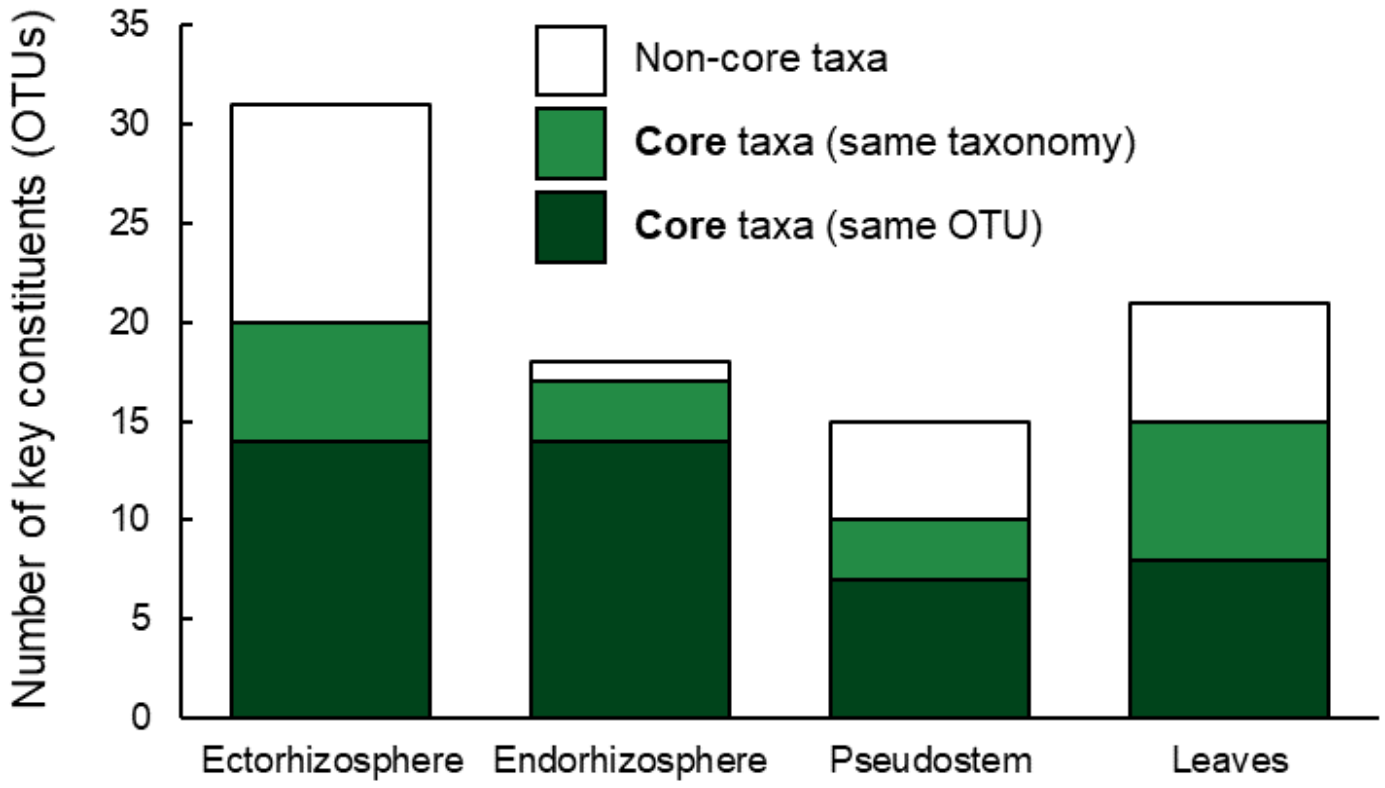

Figure 5

The total numbers of 'key constituent' OTUs in field-grown plants (i.e. OTUs in ≥50% of field-grown plants at ≥0.5% relative abundance) and the numbers that match the core taxa.

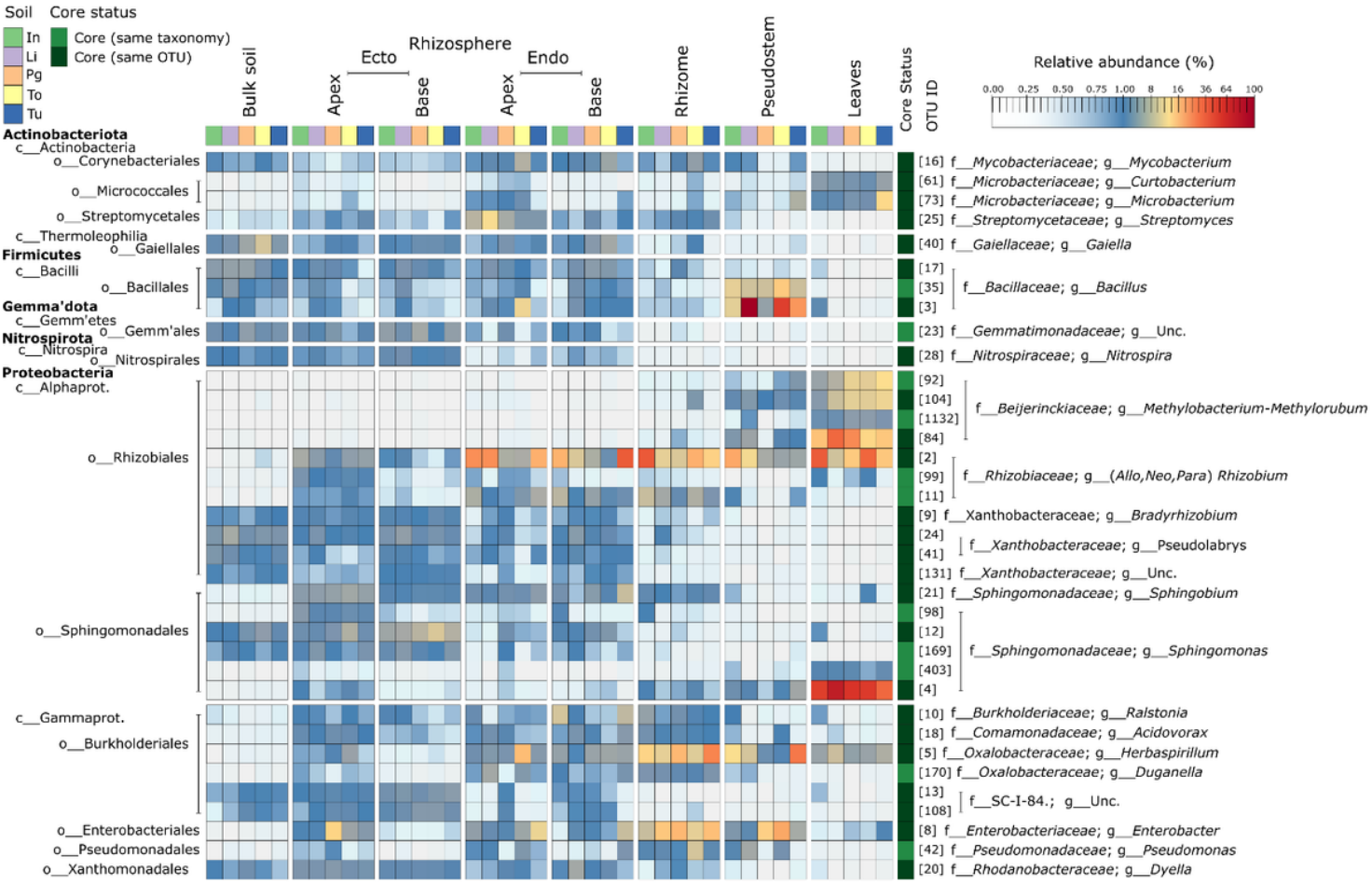

Figure 6

The core bacterial microbiome of Musa spp. The heatmap highlights the relative abundances of core OTUs in each compartment and soil. Each cell represents the mean of the replicates for that treatment.

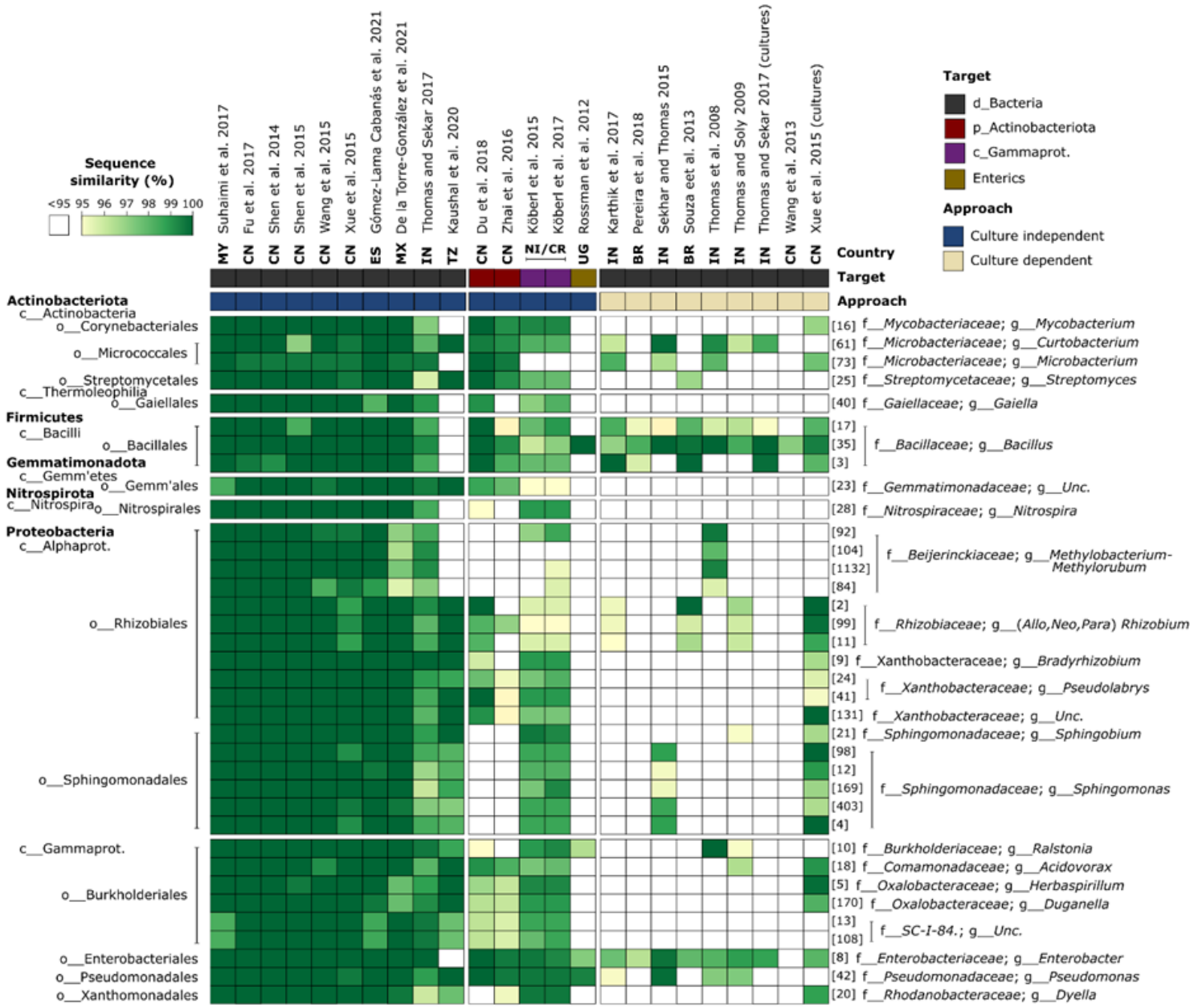

Figure 7

Similarity of bacterial sequences detected in 22 previous studies of banana-associated bacteria to those of the common core OTUs presented in the present study. Countries of origin are shown as two letter codes as described in the ISO 3166 international standard. Note that for two studies (Xue et al., 2015; Thomas and Sekar, 2017), data were generated and are shown for both culture independent and dependent methods.

Supplementary Files

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- Birtetal.2022.SupplementaryInformation.pdf