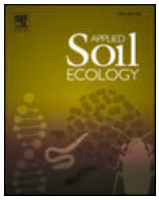
Applied Soil Ecology 214 (2025) 106377

Contents lists available at ScienceDirect

Applied Soil Ecology

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



Research paper

The structure and co-occurrence networks of banana root-associated microbiota are shaped by sampling year and plant developmental stage

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ARTICLE INFO

Keywords:
Musa acuminata
Banana developmental stage
Rhizosphere microbiota, root endophytes
Vegetative propagation
Co-occurrence networks

ABSTRACT

Bananas (*Musa* spp.) are one of the most important food staples worldwide. Although plant-associated microbiota is essential to host health, the effect of banana developmental stage on microbial communities remains poorly understood. Here we studied the differences in the rhizosphere and root endosphere microbiota of banana mother and sucker plants, in two consecutive years and in two different orchards located in Tenerife (Canary Islands). The potential vegetative transmission of belowground microorganisms from mothers to suckers was also assessed by metabarcoding techniques. Sampling year exhibited a greater impact than plants' developmental stage on microbial communities. Few, subtle differences were registered between mother and sucker plants in both orchards. Overall, the rhizosphere microbiota was more diverse in the first sampling campaign than in the second one, whereas endosphere microorganisms displayed the opposite trend. Rhizosphere microbiota of mother plants formed more compartimentalized co-occurrence networks, while suckers exhibited complex networks of root endophytes. We also detected 12 bacterial ASVs that could be vertically transferred from mothers to their offspring in both orchards. Contrariwise, no fungal ASVs were virtually transferred from mothers to their progeny in both orchards. Potentially transferred microorganisms could be employed in the establishment of new banana fields regardless of the environmental or agricultural practices. They could also be employed in *in vitro* mipropagation tasks in order to ensure their presence in axenic conditions. Our results suggest that root-associated microbiota is more profoundly shaped by environmental or plant changes along time than by the developmental stage of the host.

1. Introduction

Bananas (*Musa* spp.) are one of the most widely produced and consumed fruits in the world and they constitute important food staples in many countries (FAO, 2024). It has been estimated that the total export volume reached 19.3 million tons in 2023, with Canary Islands (Spain) supplying 50 % of the bananas produced in the European Union (FAO, 2024). The banana plant is an excellent reservoir for root-associated microorganisms due to the morphology of the mats. They are comprised by the underground rhizome from which "suckers" or new clonal shoots arise from the "mother" plant, the pseudostem (composed by tightly packed overlapping leaf sheaths), the "true" stem, and the leaves (ProMusa, 2021; Simmonds, 1962). Thus, suckers are a form of vegetative reproduction that eventually produce an inflorescence when reaching the adult state. Once fruit bunches are harvested, mother plants are cut since they no longer produce bananas. This cycle is repeated continuously under favorable conditions, so that suckers will grow and become adult/reproductive plants (i.e., next generation of

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https://doi.org/10.1016/j.apsoil.2025.106377
Received 29 May 2025; Received in revised form 28 July 2025; Accepted 8 August 2025
Available online 12 August 2025
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mothers) from which new suckers will arise to produce the next clonal progeny. The development of new clonal shoots from the rhizome could entail the transmission of microbial endophytes to the suckers, which makes these plants a continuing reservoir of microorganisms. In some banana cultivation areas (and especially among smallholders) farmers transplant suckers to establish new fields (Kaushal et al., 2020). By doing so, the planting material is usually cheaper and less prone to physical damages, the longevity of plants is higher, and the preservation of native and rare varieties is favored (Bhende and Kurien, 2015). On the contrary, most commercial bananas are vegetatively propagated *in vitro* in order to diminish the transmission of soil-borne pathogens (Dagnew et al., 2012). Characterizing the microbiota of banana plants could aid in detecting beneficial microorganisms that could even improve the efficiency of both multiplication approaches.

Part of the microbiota of plant holobionts propagated by clonal multiplication has been shown to be transmitted to the progeny (Song et al., 2024; Vannier et al., 2018). Such microorganisms were proposed to help the clonal offspring to grow and adapt to the environment (Vannier et al., 2018). By transferring microbial members from the banana mother plants to the new clonal shoots, the costs associated to the foraging for endophytes may be minimized. It is assumed that microorganisms transferred by sexual or vegetative propagation have coevolved along with their host and have developed efficient mechanisms to colonize the host tissue, thereby improving the fitness of the plant (Johnston-Monje et al., 2021). Hence, in order to implement microbiome-based approaches aimed at increasing agricultural production, the origin, assembly and transmission patterns of plant/soil microorganisms need to be examined carefully (Johnston-Monje et al., 2021).

The microbiota of banana plants has been intensively studied over the last two decades. Most of the works have been devoted to describing the composition of the microbiota associated to healthy banana mats or to plants affected by different pathogens, and/or to evaluating the impact of biocontrol agents effective against specific diseases (Fan et al., 2023; García-Giraldo et al., 2022; Gómez-Lama Cabanás et al., 2022; Kaushal et al., 2020; Köberl et al., 2017; Liu et al., 2019). Yet, basic aspects of the banana microbiota such as the influence of biotic (e.g., the effect of the host phenological stage) and abiotic factors (e.g., rainfall patterns) should be first unraveled before using specific microorganisms in agricultural production.

Within the holobiont conceptual framework, no consensus has so far been reached on the influence exerted by the banana plant host on its microbiota. On the one hand, Birt and co-workers (2022,2023) revealed that the diversity and composition of bacterial and fungal communities do not differ among genotypes. On the other hand, Gómez-Lama Cabanás et al. (2021) documented differences in diversity, structure and co-occurrence patterns of root microbial dwellers, even between clonal mother and sucker plants of the same banana genotype. In addition, many other loose ends regarding the ecology of the microbiota associated to banana plants still remain to be solved. Although the latter authors reported variations in the root microbiota depending on the developmental stage of the banana plants (i.e., mothers *vs*. suckers), these changes were only evaluated during one campaign. Thus, it is still unclear whether it is a one-time phenomenon or sustained over successive growing seasons. Identifying microorganisms enriched in each developmental stage of banana plants could greatly assist in the development of bioformulations aimed to be specifically applied at each growth phase. Moreover, it has been described that a great share of microorganisms is found in different above- and belowground plant compartments of the same host plant (Birt et al., 2022, 2023; Liu et al., 2019). Although in these studies the microbiota is postulated to be able to migrate from different host tissues, the information regarding microbiota transmission among plant generations or clonal progenies is still fragmentary. Hence, deciphering whether microorganisms are transferred from mothers to suckers is of high interest for understanding the origin and dynamics of the banana belowground microbiota.

Based on the current research gaps, the hypotheses to be tested were: i) the diversity, composition and co-occurrence networks of root-associated microbiota differ according to the plant developmental stage and sampling year, and ii) the bacteriome and mycobiome of suckers are partly acquired from mother plants, entitling the description of a vegetatively transmitted banana root microbiota. Moreover, we pursued two specific goals in this work. Firstly, we examined the effect of the plant developmental stage (mothers and suckers) and sampling year on the structure of the microbial communities inhabiting the rhizosphere and root endosphere of banana plants grown in two different orchards in Tenerife (Canary Islands, Spain). In order to infer the potential origin of the banana root microbial community our second goal was to investigate whether the root endosphere microbiota of the suckers was similar to that of mother plants and different from rhizosphere microbial communities.

2. Materials and methods

2.1. Experimental areas

Two banana orchards in Tenerife Island (Canary Islands, Spain) were selected. One orchard was located in the municipal district of Guía de Isora [denominated as “South plot” from here onwards; 28°10′44”N 16°47′02”W, altitude: 293 masl (meters above sea level)] under a protective netting system. The second orchard was placed at the municipal district of La Orotava (named as “North plot” from now on; 28°24′40”N 16°30′56”W, altitude: 170 masl). Plants in this plot were cultivated under an open-field system with spontaneous cover vegetation mainly composed by *Tradescantia* spp. plants. In both cases, the senescent banana plant material is usually left in the soil to protect the roots and avoid water losses due to evaporation. Supplementary Fig. 1 shows the general appearance and location of both plots.

2.2. Sample collection and processing

Roots and rhizosphere soil from healthy (i.e., with no visible symptoms of disease(s) or stress) banana plants (*Musa acuminata* cv. Pequeña Enana synonymous with Dwarf Cavendish) were collected in two consecutive years (November 2022 and 2023). Each sampling campaign consisted of the collection of roots from 12 mats (per orchard) in two different developmental stages: mother (adult plant at full fruiting) and sucker (immature plants but always reaching 1.50–1.70 m high), as summarized in Supplementary Fig. 2. Thus, 48 root samples per plot (i.e., 12 replicates × 2 developmental stages × 2 years) were collected, according to the experimental design shown in Supplementary Fig. 3. In the first sampling campaign (2022), all mats in both orchards (*ad hoc* selected in order to fulfil the criteria previously mentioned but randomly distributed throughout the orchard) showed unripe banana bunches (mothers) and suckers reaching the size indicated above. However, lack of uniformity of the mats was unavoidable in 2023 since the originally selected mats followed their natural growth process. Thus, some mother plants had produced full bunches (92 % and 45.5 % of the plants in the South and North plots, respectively), while others were only at the initial fruiting stage. In spite of this inevitable heterogeneity, all mothers were adult, reproductive plants. All mats in 2023 displayed well-developed suckers, although their size was not as uniform as that of the suckers sampled in 2022 (i.e., the mothers in 2023). In order to guarantee that sample collection from mother and sucker plants was conducted unmistakably, the upper layer of the soil was removed and roots (10–15 cm depth) were taken at the opposite sites of the rhizome, and always checking that roots were connected with its corresponding corm as described in Gómez-Lama Cabanás et al. (2021), and depicted in Supplementary Fig. 3. Root samples were washed in tap water and transferred without delay into polyethylene tubes to avert excessive desiccation and stored at 4 °C until processing (within 6 h). Roots were then surface sterilized according to Gómez-Lama Cabanás and co-

workers (2021). In brief, roots were firstly washed with tap water with the help of a vortex to ensure the removal of soil particles attached to the roots (five times). They were immersed in 96 % ethanol (1 min) and 4.7 % sodium hypochlorite (3 min) afterwards. Subsequently, roots were washed with sterile, distilled water four times, and aliquots of the last rinse were plated onto Nutrient Agar (Oxoid, UK) and Potato Dextrose Agar (Oxoid) media. Plates were incubated at 28 °C for 14 days and microbial growth was periodically evaluated. Once sterilized, roots were air-dried on sterile blotting sheets and preserved submerged in DESS solution (20 % dimethyl sulphoxide, DMSO; 0.25 M diamine tetra-acetic acid, EDTA; saturated with NaCl, pH 8). A detailed protocol for preparation of this solution has been described by Beknazarova et al. (2017), which has been shown to be a valid procedure of preserving samples for DNA-based microbial analysis with the same effectiveness as freezing at −80 °C immediately after collection (Carvalhais et al., 2021).

For rhizosphere soil sampling, collected roots were manually rubbed and the soil firmly adhered to each of the roots (approximately 1 g) was transferred into polyethylene tubes containing 3 ml of LifeGuard™ Soil Preservation Solution (Qiagen, Germany).

2.3. Measurement of edaphic properties and climatic parameters

In order to determine the edaphic parameters, soil samples were also collected in each orchard (just in 2023). For that purpose, two digs were performed at each sampling site, and the soil near the roots of mother and sucker plants was collected and mixed (1 kg). Three composite soil samples were obtained in each plot, which were analyzed by Laboratorio Analítico Bioclínico S.L.U. (Almería, Spain) by standardized procedures.

To register the approximate climatic conditions at each orchard, data of mean, maximum and minimum annual temperatures, mean annual precipitation and solar radiation were obtained from the two weather stations closest to the experimental orchards, namely GUIAISO1 (municipal district of Guía de Isora; 28°11′28.2”N 16°46′15.1”W, close to the South plot) and OROTAV01 (municipal district of La Orotava; 28°24′23.6”N 16°30′51.5”W, close to the North plot). Data were downloaded from the Agriculture and Rural Development Technical Service (AgroCabildo) of Tenerife (https://www.agrocabildo.org/).

2.4. DNA extraction and Illumina sequencing

The tubes with the roots were vortexed to remove any residual DESS solution. The roots were then dried using sterile filter paper and placed into new sterile tubes. Subsequently, the roots were frozen at −80 °C, and after 48 h, they were lyophilized. These root samples were ground to a fine powder in a stainless steel mill jar with two tungsten beads using a MM 301 mixer mill (Retsch GmbH, Haan, Germany). DNA from 0.1 and 0.25 g of ground roots and rhizosphere samples was extracted by means of the Maxwell RSC and the PureFood GMO and Authentication Kit (Promega Corporation; Madison, MI, USA), and DNeasy® PowerSoil® Pro Kit (Qiagen; Hilden, Germany), respectively, following the manufacturers' instructions. DNA yields were determined by using the fluorimeter Qubit 3.0 (Life Technologies; Carlsbad, CA, USA).

DNA from roots and rhizosphere soil was sequenced through Illumina MiSeq platform at the genomics service of the Institute of Parasitology and Biomedicine López-Neyra (CSIC, Granada, Spain). For that purpose, amplicon libraries were prepared by amplifying the hypervariable regions V3-V4 of the bacterial gene *16 rRNA* and the fungal ITS2 region by using the primer pairs Pro341F and Pro805R (Takahashi et al., 2014), and ITS4 (White et al., 1990) and fiTS7 (Ihrmark et al., 2012), respectively. In order to minimize the amplification from host plant mitochondria and plastids, amplicons corresponding to root endosphere bacterial libraries were treated with PNA PCR clamps (Lundberg et al., 2013). Three samples of the mock community ZymoBIOMICS Microbial Community Standard II (ZYMO Research; Irvine, CA, USA) were included in each sequencing run as quality controls. A 2 × 275 PE sequencing strategy was followed, according to the criteria of the genomics service.

2.5. Sequencing data processing

High-throughput sequencing reads were bioinformatically processed by using R software, version 4.4.1 (R Core Team, 2024). The procedure described by Lasa, 2024 and Lasa et al. (2024) was followed, and different functions included in the package DADA2 (Callahan et al., 2016) were employed unless otherwise stated. Processing of bacterial and fungal reads was performed in the same way, with slight modifications. Briefly, the quality of the sequencing reads was checked by means of the function *plotQualityProfile*. For the bacterial dataset, Figaro software (Sasada et al., 2020) was employed to determine the best parameters for the subsequent trimming step. Bacterial reads were trimmed by truncating them at the positions proposed by Figaro, and the maximum expected errors (maxEE) were set accordingly depending on the sequencing run [maxEE ranged from 2 to 3 in both forward (F) and reverse (R) reads]. In the case of the fungal dataset, maxEE values ranged from 3 to 4 and from 4 to 6 for F and R reads, respectively. Furthermore, bacterial and fungal reads shorter than 50 bp and/or with ambiguities were removed from the analyses (function *filterAndTrim*). For both datasets, primers were removed by means of Cutadapt tool (Martin, 2011). Learning of error rates and sample inference (functions *learnErrors* and *dada*) were performed prior to the merging of F and R reads by means of the function *mergePairs*, and Amplicon Sequencing Variants (ASV) were obtained. All the reads from the different runs were merged (function *mergeSequenceTables*) and chimeras were removed by running the function *removeBimeraDenovo*. A second trimming step was carried for the bacterial dataset, selecting those sequences ranging from 401 to 428 bp. High quality bacterial and fungal sequences were taxonomically classified using a modified version of the Ribosomal Database Project RDP-II training set v.19 (Wang and Cole, 2024) and UNITE v.9.0 databases (Abarenkov et al., 2023), respectively (function *assignTaxonomy*). All the ASVs accounting for less than 0.0012 % of total sequences were removed from both datasets, according to the sequencing detection limit established by the mock community and by means of the function *MockCommunity* (package micro4all; Wentzien, 2024). ASVs classified as chloroplasts, mitochondria, Eukaryota (just in the bacterial dataset), *incertae sedis* at phylum level (just in the fungal dataset), and unclassified sequences at kingdom level, were removed from the datasets. Fungal sequences not classified at phylum level were compared by BLASTn against the NCBI GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and sequences classified as *Musa* spp. or as organisms other than fungi were not retained for further analyses.

2.6. Microbial ecology analyses

A detailed description of all the analyses, functions and R packages employed in this work is provided in Supplementary material (technical information). Briefly, we firstly addressed the alpha diversity by calculating the observed ASVs, and Shannon, Inverse of Simpson and Pielou's indices. For beta diversity analyses, sequence counts were normalized by the Trimmed-Means of M-value. Permutational analysis of variance test (PERMANOVA) test based on Weighted UniFrac distances (bacterial dataset) and Bray-Curtis dissimilarities (fungal dataset) was applied to decipher differences in the structure of microbial communities. When necessary, pairwise PERMANOVA tests were applied as multivariate *post-hoc* tests, and the size of effect was measured when significant differences among samples were detected. The dispersion of the data was also calculated by PERMDISP2 tests, and the distribution of the samples in the multivariate space was visualized by Principal Coordinate Analysis (PCoA). Details about all the models tested are found in Supplementary material (technical information).

ANCOM-BC statistical test was applied to address potential differences in the taxonomical profiles among groups of samples. In order to infer the co-occurrence networks, the software Molecular Ecological

Network Analysis Pipeline (MENAp) was applied. Networks were calculated based on Spearman's Rho correlation coefficients as detailed in Supplementary material.

The potential microbial transmission from mothers (2022) to the entire progeny (first and second suckers) was addressed by comparing the root endosphere of mothers (2022) *vs.* suckers (2022). Shared ASVs in the root endosphere were then compared to all the ASVs in the rhizosphere soil of first suckers, and those not detected in rhizosphere were considered as "potentially transferred ASV in 2022". The same comparisons were performed for microbial data of 2023 (and for two orchards). Then, potentially transferred ASVs in 2022 were compared to that virtually transferred in 2023, and ASVs shared among both groups were regarded as "vegetatively transferred ASVs" in mothers, first and second suckers. This procedure was applied to both orchards and virtual vegetatively transferred ASVs detected in each orchard were compared to each other eventually (see Supplementary material).

2.7. Univariate statistics

For univariate statistics, the R functions and packages detailed in the Supplementary material (technical information) were used. Firstly, the normality, homoscedasticity and the presence of extreme outliers was addressed. When the assumptions of normal distribution and homogeneity of variances were met, parametric statistical tests were used, such as Student's *t*-test (for two groups comparisons) or factorial ANOVA (multiple groups comparisons). In the latter case, Tukey's HSD test was applied as *post-hoc* test. When extreme outliers were detected, robust statistics was performed. In the case of two groups comparisons, Yuen's test was applied, while factorial ANOVA on trimmed means was conducted when more than two groups were compared each other. *Post-hoc* tests were applied as described in Supplementary material (technical information). When assumptions of normality and homoscedasticity were not met, Welch's test or ANOVA based on Aligned Rank Transformation were implemented. The confidence level selected for all the hypothesis contrasting tests was >95 %, and in the case of significant differences among groups, the size of the effect was also calculated. The procedures and the models checked are detailed in Supplementary material (technical information).

3. Results

3.1. Soil physicochemical properties and climatic parameters

Statistically significant differences were found in the edaphic properties measured in both orchards. The plot located in the south of the island showed statistically significant higher values of pH, assimilable potassium and phosphorus (Student's *t*-test, $p < 0.046$, Supplementary Table 1). Conversely, the content of organic matter and slime were significantly higher in the northern plot (Student's t-test, $p < 0.042$). Differences found between the two plots were classified as large, as indicated by the size of the effect (Supplementary Table 1). Regarding the climatic parameters, significant higher annual mean, maximum and minimum temperatures and annual solar radiation were registered in the north orchard in 2023 than in 2022 (Student's t-test, $p < 0.035$; Supplementary Table 1).

Consequently, and due to the known influence of edaphic properties and agricultural management practices on the structure of microbial communities (Bulgarelli et al., 2012; Hartman et al., 2018), the two plots were analyzed separately from here onwards.

3.2. General characteristics of high-throughput sequencing data

A total of 16,727,803 bacterial and 19,900,020 fungal raw reads were obtained from the Illumina MiSeq platform. After filtering and trimming steps, 19 endosphere samples were removed from further analyses because they were very similar to rhizosphere samples. This could be a consequence of an ineffective root surface sterilization process (i.e., incomplete removal of rhizosphere microorganisms or their DNA), what could otherwise provide biased data. Two samples (one from the rhizosphere and another one from the root endosphere) were also discarded because they accounted for a low number of sequences (< 8730 sequences). Eventually, 5788 (bacterial dataset) and 1391 (fungal dataset) ASVs were registered. Supplementary Table 2 summarizes the number of replicates and sequences per group of samples.

3.3. Effect of plant developmental stage and sampling year on the banana root microbiota

3.3.1. Effect on alpha and beta diversity

Alpha diversity analysis revealed that the plant developmental stage (mothers and suckers) had the same effect on the alpha indices in both sampling years. Likewise, in case of differences in microbial diversity according to the sampling campaigns, these shifts followed the same trend in mother and sucker plants (two-way ANOVA, $p_{\text{Sampling Year*Plant developmental stage}} > 0.306$; Supplementary Table 4).

Rhizosphere bacterial communities of sucker plants showed significant (albeit subtle) higher values of Pielou index than those of the mothers in the northern plot (two-way ANOVA, $p = 0.016$, $\eta^2 = 0.129$; Fig. 1a). On the contrary, significantly more fungal ASVs were observed for mother plants in the southern plot (two-way ANOVA, $p = 0.036$; Fig. 1c; Supplementary Tables 3 and 4), although this difference was small ($\eta^2 = 0.096$). Interestingly, fungal communities were richer in 2022 than in 2023 in both plots (two-way ANOVA, $p < 0.002$; Fig. 1c; Supplementary Tables 3 and 4).

Bacterial root endophytes showed differences just in the southern plot. In this orchard, diversity was mostly affected by the sampling year, although small significant differences were also detected when the developmental stage of the plants was analyzed (two-way ANOVA, $\eta^2_{\text{Sampling year}} > 0.2$, $\eta^2_{\text{Plant developmental stage}} < 0.1$ (Fig. 1b; Supplementary Tables 3 and 4). No differences were observed between mothers and suckers for fungal endophytes, and only the number of ASVs and Shannon index showed a year effect (Fig. 1, Supplementary Tables 3 and 4).

The structure of bacterial and fungal rhizosphere communities followed a similar trend: it only depended on the sampling year, regardless of the plant developmental stage (Fig. 2). Although statistically significant differences were found between 2022 and 2023, this factor only explained a small percentage of the total variance (PERMANOVA, $R^2 < 0.064$), and the differences detected were minor (PERMANOVA, $\omega^2 < 0.042$; Table 1). Conversely, bacterial and fungal endophytes of mothers and suckers were significantly, albeit slightly, different in the southern plot (PERMANOVA, $p < 0.036$, $\omega^2 < 0.029$; Fig. 2c and g). The sampling year also determined the structure of bacterial and fungal communities in this plot (PERMANOVA, $p < 0.024$). However, due to the different sample size and the heterogeneity in terms of variance (PERMDISP2, $p = 0.003$), the results of PERMANOVA were not reliable, as described by Anderson and Walsh (2013). Interestingly, PERMANOVA test revealed that plants at different developmental stages harbored distinct root endosphere fungal communities (northern plot; PERMANOVA, $p_{\text{Sampling year*Plant developmental stage}} = 0.049$). Significant differences were found in the mycobiome of mother and sucker plants in 2022 but not in 2023 in this orchard (pairwise PERMANOVA, Mothers *vs.* Suckers (2022) $p = 0.006$, Mothers *vs.* Suckers (2023) $p = 0.202$; Supplementary Fig. 2 h).

3.3.2. Effect on the composition of root-associated microbial communities

A total of 23 identified bacterial *phyla* were detected in the rhizosphere of banana plants, *Pseudomonadota*, *Actinomycetota*, *Bacillota* and *Acidobacteriota* being the most abundant and accounting for 86.9–93 % of the total sequences (Supplementary Fig. 5a and b). A similar pattern was observed for the root endosphere, although *Bacteroidota* instead of *Acidobacteriota* was among the most abundant *phyla* in this compartment. Meanwhile, the mycobiome of the rhizosphere and root

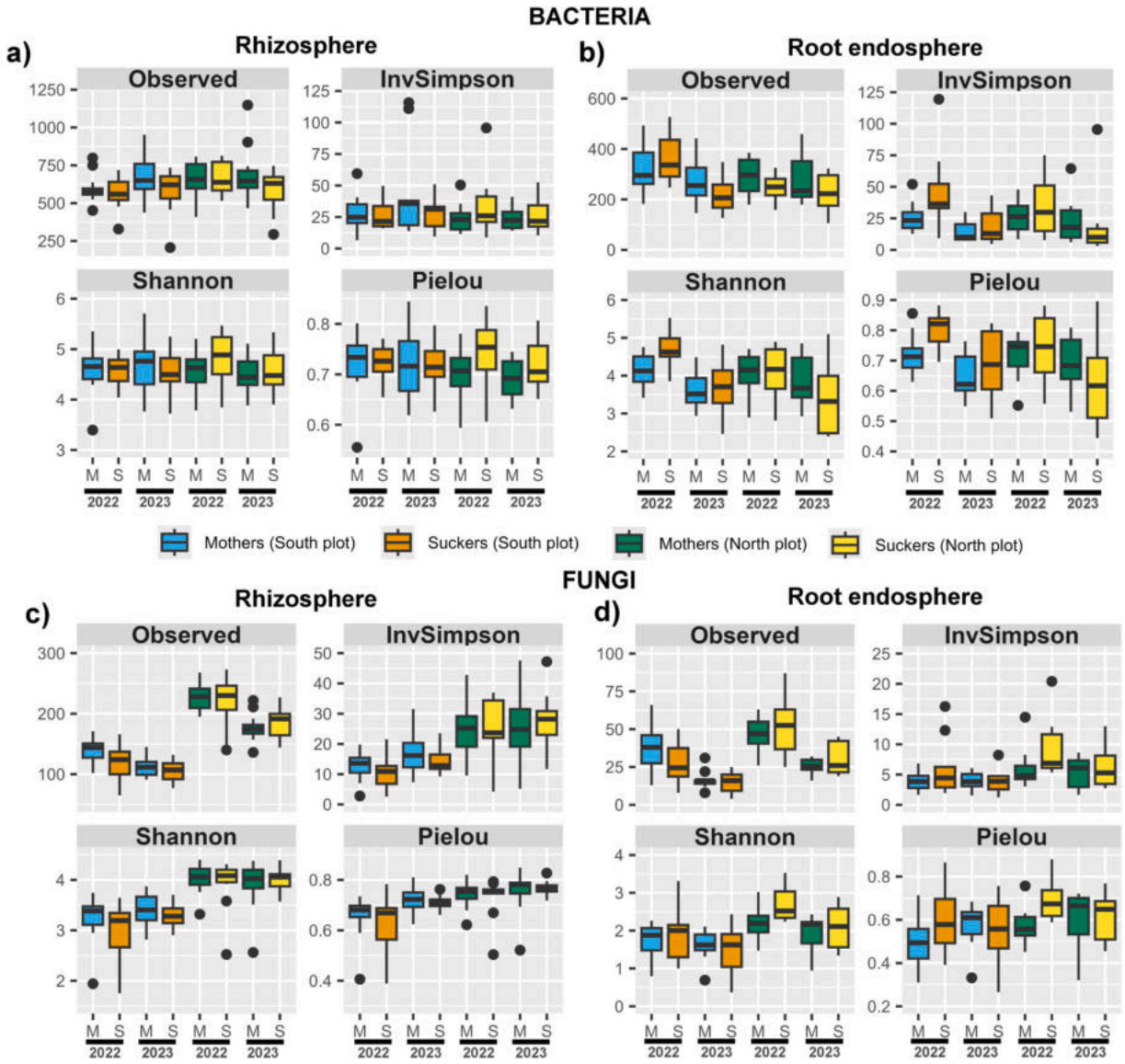

Fig. 1. Alpha diversity of rhizosphere (panel a) and root endosphere (b) bacteriomes and rhizosphere (c) and root endosphere (d) mycobiomes. "M" and "S" letters represent mother and sucker plants, respectively. "Observed" means the number of observed ASVs, while "InvSimpson" refers to the Inverse of Simpson. Results of statistical comparisons are summarized in Supplementary Table 4.

endosphere was composed by only nine and eight identified *phyla*, respectively (Supplementary Fig. 6).

We noted that the rhizosphere bacterial communities of plants located in both plots were mostly dominated by the genera *Citrobacter, Klebsiella* and *Neobacillus*, which together accounted for more than 45 % of the total rhizosphere sequences in some cases (Fig. 3a and b). Few differences were detected in the abundance of the rhizosphere bacteriome between mother plants and suckers in both plots. Moreover, most of the differentially abundant genera were minor taxa (Supplementary Table 5). By contrast, more changes were detected between sampling years (Supplementary Table 5). *Streptomyces* stood out among the bacterial endophytes as the most abundant genus in all root endosphere samples (Fig. 3c and d). Although some changes were detected in the abundance of certain taxa of mother and sucker plants, more differences were found between sampling campaigns at each plant developmental stage (Supplementary Table 5).

The taxonomical profile of the rhizosphere mycobiome strongly depended on the orchard under study (Fig. 3e and f). The southern plot was mainly dominated by the genera *Ovatospora*, *Chrysosporium*, *Acrostalagmus* and *Fusarium*, whereas in the northern plot *Cladosporium*, *Fusarium*, an unclassified genus of the family Wallemiaceae and *Auxarthron* were the most abundant genera (Fig. 3e and f). A striking finding was the high abundance of the genera *Brunneochlamydosporium* and *Fusarium* in the root endosphere of plants located in the southern and northern plots, respectively (Fig. 3g and h). However, no significant differences in the abundance of these genera were found among the plants analyzed. All differences detected between mother and sucker plants, or between 2022 and 2023, corresponded to minor genera (Supplementary Table 5).

3.3.3. Influence of plant developmental stage on microbial co-occurrence networks

Co-occurrence networks corresponding to rhizosphere microbial communities of mother plants (2022, both plots) were more compartmentalized than those of suckers, as showcased by the significantly higher values of Geodesic Distance (GD) and Modularity (M; Fig. 4a, Supplementary Fig. 7 and 8). The same trend was observed for mother plants in the northern plot (2023). On the other hand, co-occurrence networks of suckers located in the northern (2022) and southern (2023) plots were more complex than that of mother plants (more nodes

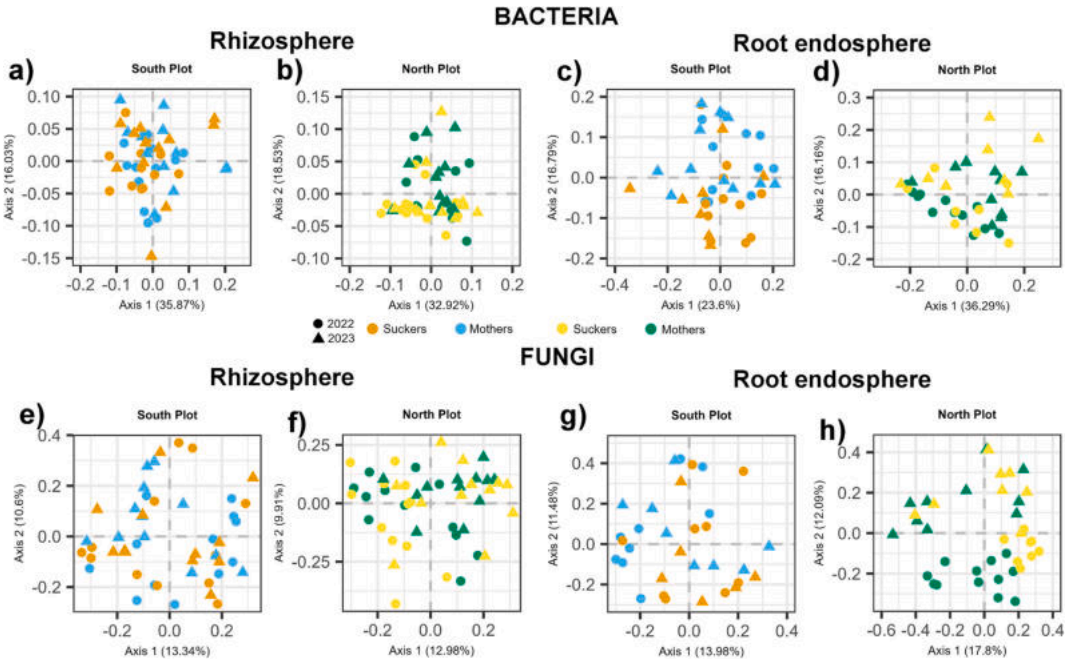

Fig. 2. Beta diversity of rhizosphere (panels a and b) and root endosphere (c and d) bacteriome corresponding to south and north plots respectively, and fungal communities dwelling in the rhizosphere (e and f) and root endosphere (g and h) of banana plots located in the south and north plot, respectively. PCoA plots corresponding to the bacteriome are based on Weighted UniFrac distantes, while those corresponding to the mycobiome are based on Bray-Curtis dissimilarities.

Table 1
Beta diversity analyses of rhizosphere and root endosphere inhabiting microbial communities. Two-way PERMANOVA was employed to test the effect of the plant developmental stage, the sampling year and their interaction on the structure of microbial communities, while the effect of the plant clonal progeny was addressed by one-way PERMANOVA test. PERMDISP2 test was applied to analyze the multivariate homogeneity of groups dispersion.

			BACTERIA						FUNGI					
			PERMANOVA				PERMDISP2		PERMANOVA				PERMDISP2	
	Plot	Factor[a]	R^2	F	*p*	ω^2	F	*p*	R^2	F	*p*	ω^2	F	*p*
Rhizosphere	**South**	**Plant**	0.028	1.375	0.189		0.802	0.377	0.013	0.638	0.957		0.881	0.354
		Year	0.058	2.816	0.013	0.036	1.274	0.261	0.064	3.095	10^{-4}	0.042	0.251	0.626
		Plant*Year	0.015	0.712	0.647				0.018	0.878	0.642			
		Progeny	0.043	1.004	0.412		1.306	0.278	0.059	1.404	0.035	0.0166	3.383	0.052
		M-1S							0.038	1.334	0.260			
		M-2S							0.090	2.176	0.003			
		1S–2S							0.030	1.034	0.368			
	North	**Plant**	0.033	1.56	0.133		4.067	0.052	0.025	1.278	0.136		0.214	0.648
		Year	0.039	1.86	0.076		0.142	0.704	0.092	4.666	10^{-4}	0.071	0.013	0.905
		Plant*Year	0.032	1.54	0.143				0.018	0.891	0.617			
		Progeny	0.049	1.136	0.303		0.311	0.742	0.070	1.703	0.002	0.028	1.735	0.187
		M-1S							0.037	1.324	0.103			
		M-2S							0.109	2.688	0.003			
		1S–2S							0.041	1.469	0.092			
Root endosphere	**South**	**Plant**	0.052	2.208	0.020	0.029	0.360	0.546	0.042	1.610	0.036	0.016	3.816	0.055
		Year	0.062	2.624	0.006	0.038	9.441	0.003	0.044	1.671	0.024	0.017	0.654	0.417
		Plant*Year	0.014	0.603	0.845				0.025	0.959	0.500			
		Progeny	0.073	1.495	0.072		3.740	0.042	0.064	1.190	0.159		20.883	0.13
	North	**Plant**	0.031	1.213	0.257		2.953	0.091	0.059	2.540	0.002	0.039	0.085	0.784
		Year	0.095	3.791	0.004	0.068	8.241	0.006	0.107	4.585	10^{-4}	0.086	0.708	0.4
		Plant*Year	0.026	1.034	0.374				0.038	1.611	0.049	0.016		
		Progeny	0.114	2.255	0.013		6.493	0.003	0.117	2.324	$3{\cdot}10^{-4}$	0.065	1.732	0.192
		M-1S	0.078	2.375	0.096				0.089	2.728	0.006			
		M-2S	0.156	3.20	0.048				0.150	3.182	0.003			
		1S–2S	0.056	1.414	0.162				0.050	1.276	0.179			

[a] Factor to be tested: ***Plant***, plant developmental stage; ***Year***: sampling year; ***Progeny***, plant clonal progeny. In the last case, results of the pairwise comparisons among progenies are shown (***M***, mother plants; ***1S***, first suckers; ***2S***, second suckers).

and links, and higher average degree, avgK).

In the northern plot, endosphere networks corresponding to suckers showed almost the same topology in both sampling campaigns (Supplementary Fig. 10). Indeed, microbial communities were assembled into more complex, compartmentalized and compact networks [higher avgCC values (average Clustering Coefficient)] (Fig. 4b). This pattern was also found in the network of sucker plants located in the southern plot (2023; Fig. 4b). It is worth mentioning that all the networks

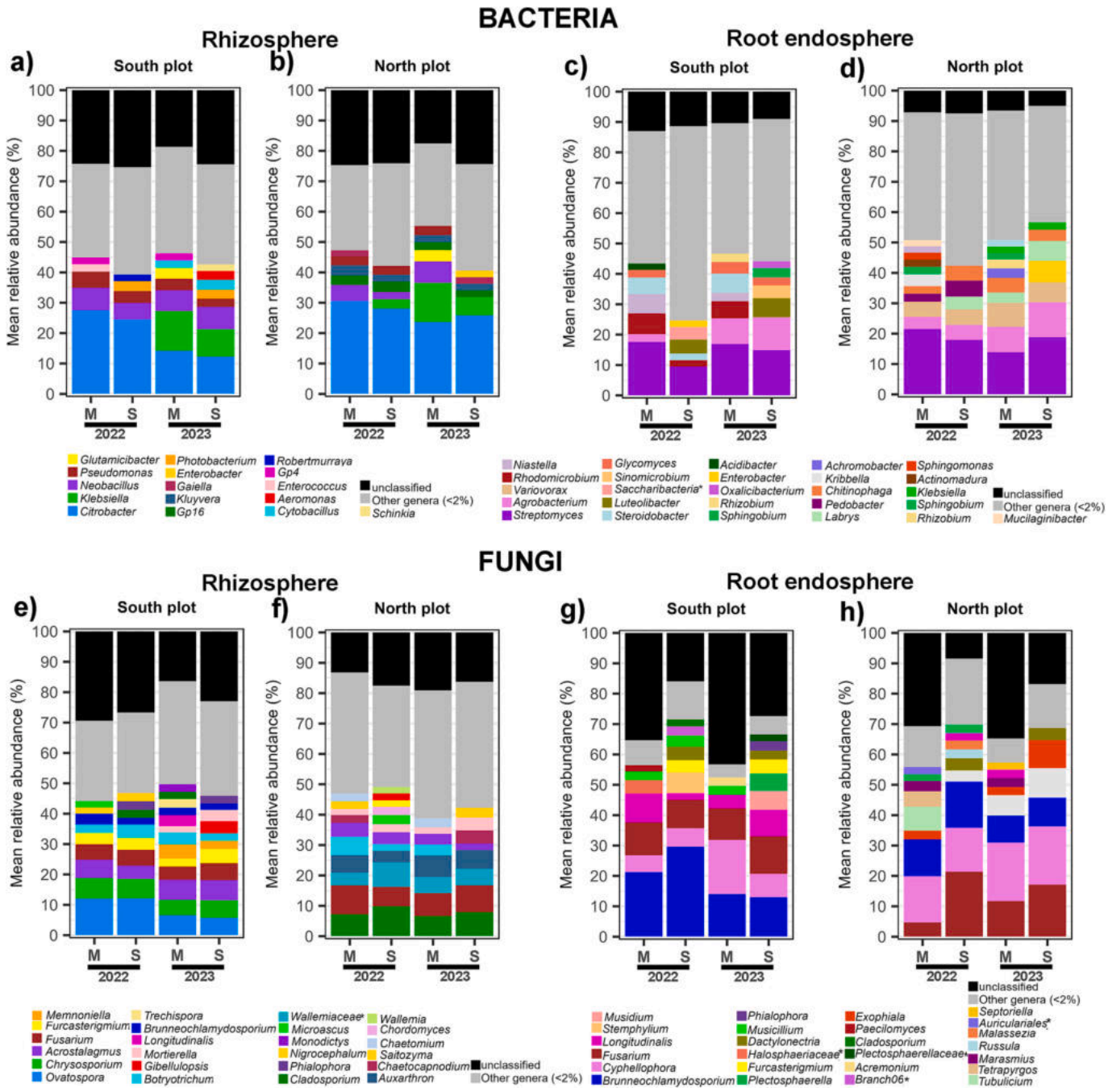

Fig. 3. Relative abundance of the most abundant rhizosphere bacterial genera in the south and north plots (a and b, respectively), root endosphere bacterial genera (c, south plot; d, north plot) and major rhizosphere fungal genera in the south and north plots (e and f), and root endosphere fungal genera (g, south plot; h, north plot). "M" and "S" letters denote mother and sucker plants, respectively. The artificial group "Other genera" emcompassed all the genera that accounted for less than 2 % of total sequences in at least one of the corresponding groups of samples. Asterisks indicate that the corresponding genus was taxonomically classified as *incertae sedis*. Statistical comparisons of the abundance of the bacterial and fungal genera are included in Supplementary Table 5.

displayed an overwhelming percentage of negative links (75.3–95.1 % of the total edges).

It should be noted that ASVs that belong to *Neobacillus* and *Pseudomonas* (genera relatively abundant in the rhizosphere of the north plot) were classified as keystone taxa. Their specific role depended on the co-occurrence network under study (Table 2). In the endosphere networks, clear differences were found between mothers and suckers (both plots). While co-occurrence networks of mother plants were quite prolific in connectors and module hubs (2022), those corresponding to suckers were almost depleted in keystones. We found one module hub belonging to genus *Sphingopyxis* in the network of mother plants located in the north plot (2022; Table 2).

3.4. Unraveling the shifts in the diversity and structure of the root-associated microbiota of three clonal progenies

A comparison of the microbiota among the three plant clonal progenies (mothers, first, and second suckers) failed to detect any differences in bacterial alpha diversity indices in any of the plant compartments and orchards (Fig. 1 and Supplementary Table 3). However, the rhizosphere mycobiome of mother plants was significantly more diverse than that of the first and second suckers in both plots (one-way ANOVA, $p = 0.001$).

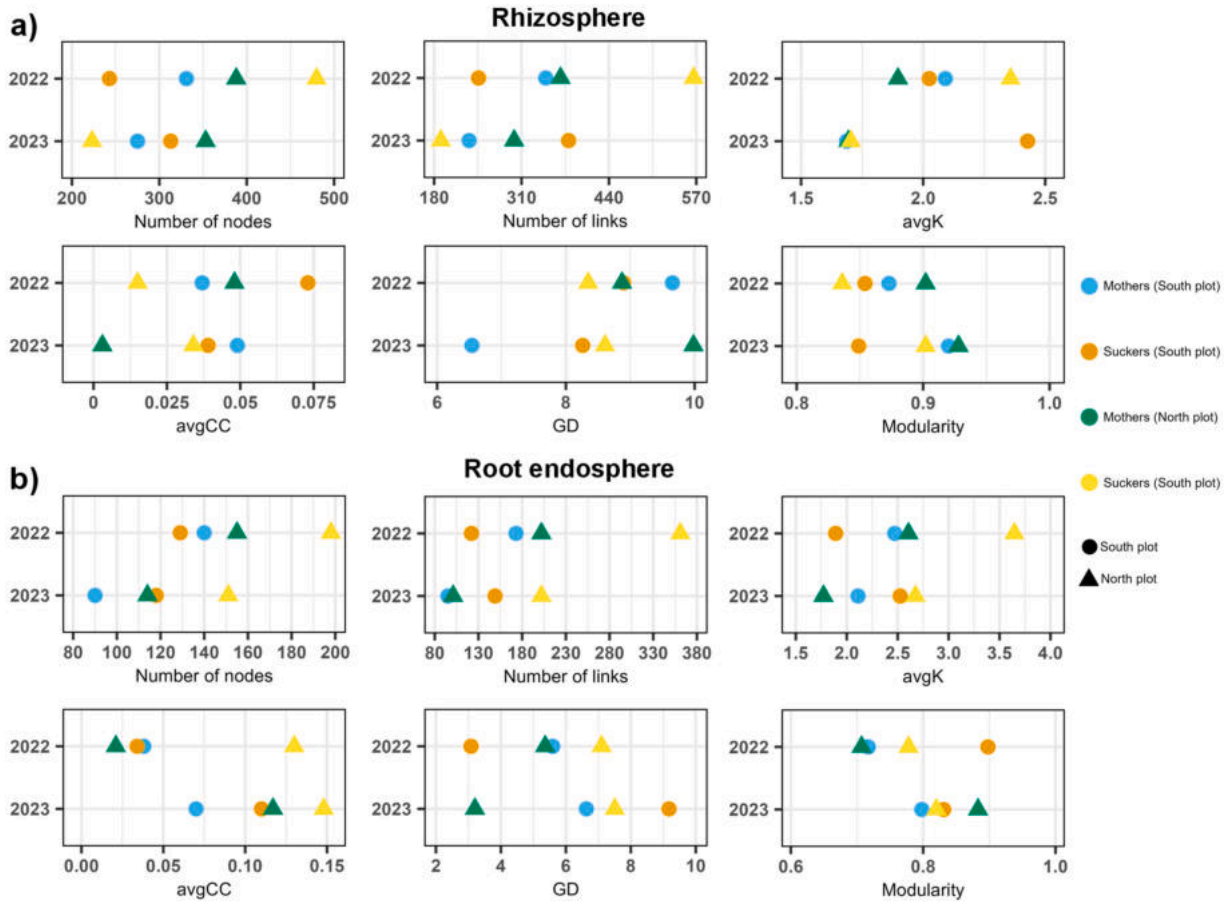

Fig. 4. Main topological properties of the co-occurrence networks corresponding to rhizosphere (a) and root endosphere (b) microbial communities. avgK, avgCC and GD indicate the average degree, the average clustering coefficient, and the Geodesic Distance, respectively. All the topologial parameters showed sinificant differences when networks corresponding to mothers and suckers (same sampling year) were compared each other (Student's t-test, $p < 0.0147$).

The same trend was observed for the fungal community inhabiting the root endosphere of plants located in the southern plot (one-way ANOVA, $p = 9.9{\cdot}10^{-4}$).

3.5. *Dissecting the root microbiota potentially transmitted by vegetative propagation*

Interestingly, 21 % of the bacterial ASVs recorded in the root endosphere of mother plants were also detected in sucker plants but not in the rhizosphere (2022, southern plot; Fig. 5a). These ASVs supposed 18 % of the total ASVs detected in the suckers and they accounted for 19.15 % of the sequences retrieved in the corresponding dataset (Fig. 5b). Similar proportions of ASVs were registered in the northern plot (Fig. 5c and d). When the microorganisms shared among mothers, first, and second suckers in the root endosphere were addressed (southern plot), 93 bacterial ASVs were found in all clonal progenies which otherwise were not detected in the rhizosphere. Among these, ASV00872 (*Xanthomonas*) and ASV02415 (family Micropepsaceae) acted as module hub and connector, respectively, in the root endosphere co-occurrence networks of mother and sucker plants (sampling year 2023, Supplementary Table 5). In the northern plot, 86 ASVs were potentially transmitted from mother plants to the first and second suckers. Remarkably, we found that 12 bacterial ASVs were shared among all the progenies in both orchards, and were undetectable in the rhizosphere soils (Fig. 5i). They belonged to 11 different bacterial genera. As summarized in Fig. 5i, most of them were minor taxa, and just ASV00270 and ASV00093 accounted for about 0.7 % of the total sequences of the root endosphere of mothers and first suckers. Interestingly, 11 out of 12 ASVs acted as peripherals in at least one of the co-occurrence networks. Indeed, ASV00366 was detected as peripheral in five of the networks (Fig. 5i; Supplementary Fig. 9 and 10).

Regarding the mycobiome, only 8.6 % of the ASVs inhabiting the root endosphere of mother plants (accounting for less than 3 % of total sequences) were also detected in the first progeny in the southern plot (but not in the rhizosphere soils). Although similar proportions of ASVs were potentially transferred to the second suckers, they accounted for a high relative abundance (*c*. 40 %; Fig. 5e and f). Their presence in the root endosphere of the second suckers was almost negligible (*c*. 0.9 %, Fig. 5f). None of the fungal endophytic ASVs of mother plants was detected in the second suckers (southern plot). On the contrary, two ASVs belonging to families Ceratobasidiaceae and Tricholomataceae and two ASVs classified as *Serendipita* were shared among mothers and their clonal progenies in the northern plot.

4. Discussion

Microbial communities associated to plants are affected by different biotic and abiotic factors (Bulgarelli et al., 2012; Hartman et al., 2018). In the present study, most of the differences in alpha and beta diversity as well as in taxa abundance were detected between the two successive sampling years. These differences may reflect shifts in environmental conditions along time, such as those observed in annual mean, maximum and minimum temperatures as well as in solar radiation in the northern plot. Notwithstanding, we cannot rule out the influence of biotic drivers (such as the differences in fruit ripeness of mother plants observed between years) on root microbial communities.

Table 2
Keystone taxa comprising the co-occurrence networks and their main topological properties in the networks.

	Plot	Year	Plant	ASV[a]	Phylum	Genus[b]	Z_i[c]	P_i[d]	Role[e]
Rhizosphere	**South**	**2022**	**Mothers**	b_ASV00449	*Pseudomonadota*	*Rhodoplanes*	−0.238	0.720	Connector
				b_ASV02956	*Pseudomonadota*	(Desulfovibrionaceae)	−0.229	0.625	Connector
				f_ASV0102	*Ascomycota*	*Memnoniella*	0.243	0.625	Connector
				b_ASV01388	*Pseudomonadota*	(Azospirillaceae)	3.494	0.000	Module hub
				b_ASV03550	*Pseudomonadota*	*Coxiella*	3.386	0.198	Module hub
				f_ASV0453	*Ascomycota*	*Gymnoascus*	3.316	0.000	Module hub
				f_ASV0480	*Basidiomycota*	*Hemimycena*	2.789	0.245	Module hub
				b_ASV00386	*Pseudomonadota*	*Sphingobium*	2.698	0.000	Module hub
			Suckers	b_ASV00848	unclassified	unclassified	−0.972	0.667	Connector
				f_ASV0329	*Ascomycota*	*Arachniotus*	2.788	0.278	Module hub
				b_ASV00855	unclassified	unclassified	2.623	0.370	Module hub
				b_ASV00636	*Pseudomonadota*	*Stella*	2.623	0.219	Module hub
		2023	**Mothers**	b_ASV01390	*Actinomycetota*	*Gaiella*	−0.106	0.625	Connector
				b_ASV03414	*Acidobacteriota*	*Gp6*	2.958	0.000	Module hub
				b_ASV02349	*Pseudomonadota*	*Bradyrhizobium*	2.680	0.000	Module hub
				b_ASV02525	*Planctomycetota*	*Caulifigura*	2.546	0.000	Module hub
			Suckers	b_ASV00892	*Verrucomicrobiota*	Subdivision3_*gis*	−0.970	0.667	Connector
				b_ASV00503	*Pseudomonadota*	(Rhodospirillaceae)	−0.543	0.625	Connector
				b_ASV04194	*Acidobacteriota*	*Gp18*	−0.543	0.625	Connector
				b_ASV00816	unclassified	unclassified	3.027	0.000	Module hub
				b_ASV03568	*Actinomycetota*	*Actinomarinicola*	2.974	0.320	Module hub
				b_ASV04248	*Actinomycetota*	*Saccharopolyspora*	2.905	0.278	Module hub
				b_ASV00380	*Actinomycetota*	(Actinomycetota)	2.626	0.180	Module hub
	North	**2022**	**Mothers**	b_ASV02705	*Actinomycetota*	*Solirubrobacter*	0.476	0.667	Connector
				f_ASV0610	*Ascomycota*	*Penicillium*	−0.109	0.625	Connector
				b_ASV00813	*Pseudomonadota*	*Acidibacter*	3.841	0.000	Module hub
				b_ASV03582	*Actinomycetota*	*Rhabdothermincola*	3.169	0.000	Module hub
				b_ASV01628	*Actinomycetota*	*Nocardioides*	2.788	0.278	Module hub
				b_ASV01057	*Chloroflexota*	(Ktedonobacterales)	2.673	0.000	Module hub
				f_ASV0216	*Ascomycota*	(Sordariomycetes)	2.664	0.000	Module hub
				b_ASV00607	*Bacteroidota*	*Algoriphagus*	2.646	0.000	Module hub
			Suckers	f_ASV0446	*Basidiomycota*	*Heterogastridium*	1.313	0.688	Connector
				b_ASV01528	*Actinomycetota*	*Mycobacterium*	−0.878	0.667	Connector
				b_ASV01201	*Bacillota*	*Salipaludibacillus*	−0.962	0.667	Connector
				b_ASV02157	*Pseudomonadota*	(Chromatiales)	−0.953	0.667	Connector
				b_ASV00813	*Pseudomonadota*	*Acidibacter*	−0.300	0.625	Connector
				b_ASV00942	*Pseudomonadota*	*Acidibacter*	−0.256	0.625	Connector
				f_ASV0535	*Ascomycota*	*Yunnania*	−0.109	0.625	Connector
				b_ASV04203	*Pseudomonadota*	(Alphaproteobacteria)	3.523	0.320	Module hub
				b_ASV00557	*Bacillota*	*Halocella*	3.001	0.000	Module hub
				f_ASV0234	*Ascomycota*	*Pseudoarthrographis*	2.971	0.000	Module hub
				b_ASV00133	*Verrucomicrobiota*	*Luteolibacter*	2.845	0.245	Module hub
				f_ASV0183	*Basidiomycota*	(Agaricomycetes)	2.752	0.000	Module hub
				b_ASV01722	unclassified	unclassified	2.728	0.000	Module hub
				b_ASV00997	*Pseudomonadota*	*Sphingomonas*	2.626	0.278	Module hub
				b_ASV02988	*Verrucomicrobiota*	Spartobacteria_gis	2.597	0.278	Module hub
				b_ASV00462	*Actinomycetota*	(Actinobacteria)	2.592	0.219	Module hub
		2023	**Mothers**	b_ASV01972	*Bacillota*	*Ureibacillus*	3.341	0.340	Module hub
				f_ASV0914	*Basidiomycota*	*Geastrum*	2.982	0.000	Module hub
				b_ASV02771	*Pseudomonadota*	*Paracoccus*	2.598	0.000	Module hub
				b_ASV01068	*Pseudomonadota*	*Pseudomonas*	2.565	0.000	Module hub
			Suckers	b_ASV02369	*Bacillota*	*Neobacillus*	−0.089	0.625	Connector
				b_ASV04490	*Actinomycetota*	(Micrococcaceae)	3.343	0.406	Module hub
				b_ASV00870	*Bacteroidota*	(Cytophagales)	3.041	0.219	Module hub
Root endosphere	**South**	**2022**	**Mothers**	b_ASV00753	*Bacillota*	*Compostibacillus*	1.000	0.694	Connector
				b_ASV01967	*Bacteroidota*	*Chryseolinea*	−0.289	0.640	Connector
				b_ASV00546	*Bacillota*	*Ammoniibacillus*	−0.503	0.625	Connector
				f_ASV0426	*Basidiomycota*	*Thelephora*	−0.249	0.625	Connector
				b_ASV00923	*Pseudomonadota*	(Alphaproteobacteria)	2.967	0.370	Module hub
				f_ASV0033	*Ascomycota*	(Ascomycota)	2.828	0.000	Module hub
				b_ASV00660	*Pseudomonadota*	(Alphaproteobacteria)	2.696	0.180	Module hub
			Suckers	b_ASV00875	*Actinomycetota*	*Glycomyces*	2.846	0.000	Module hub
		2023	**Mothers**	b_ASV01007	*Bacteroidota*	*Ohtaekwangia*	2.621	0.000	Module hub
				b_ASV00103	*Actinomycetota*	*Promicromonospora*	2.572	0.320	Module hub
			Suckers	b_ASV02415	*Pseudomonadota*	(Micropepsaceae)	−0.439	0.625	Connector
				b_ASV00080	*Pseudomonadota*	*Vitreimonas*	2.525	0.000	Module hub
	North	**2022**	**Mothers**	b_ASV00349	*Pseudomonadota*	*Steroidobacter*	−0.177	0.720	Connector
				b_ASV00883	*Bacteroidota*	*Mucilaginibacter*	1.460	0.667	Connector
				b_ASV00318	*Actinomycetota*	*Streptomyces*	−1.043	0.667	Connector
				f_ASV0309	*Ascomycota*	*Microascales_gis*	0.130	0.640	Connector
				b_ASV01779	*Chloroflexota*	*Dictyobacter*	−0.596	0.625	Connector
				b_ASV02350	*Mycoplasmatota*	(Mollicutes)	−0.211	0.625	Connector
				b_ASV02181	*Planctomycetota*	*Caulifigura*	−0.333	0.625	Connector
				b_ASV00544	*Pseudomonadota*	*Acidibacter*	0.209	0.625	Connector

(*continued on next page*)

Table 2 (*continued*)

	Plot	Year	Plant	ASV[a]	Phylum	Genus[b]	Z_i[c]	P_i[d]	Role[e]
				b_ASV00886	*Pseudomonadota*	*Sphingomonas*	−0.596	0.625	Connector
				b_ASV01534	*Bacillota*	*Romboutsia*	3.059	0.000	Module hub
				b_ASV01784	*Pseudomonadota*	*Sphingopyxis*	2.878	0.180	Module hub
			Suckers	–					
		2023	**Mothers**	b_ASV00026	*Pseudomonadota*	*Escherichia/Shigella*	2.966	0.000	Module hub
			Suckers	b_ASV00694	*Pseudomonadota*	*Mesorhizobium*	3.002	0.000	Module hub

[a] ***b,*** bacterial ASV; ***f***, fungal ASV.
[b] ***gis***, genus *incertae sedis*; names in brackets indicate the lowest taxonomical level at which the corresponding ASV was classified.
[c] Z_i, within module connectivity.
[d] P_i, among module connectivity.
[e] ***Role***, topological role in the corresponding co-occurrence network according to the classification by Olesen et al. (2017).

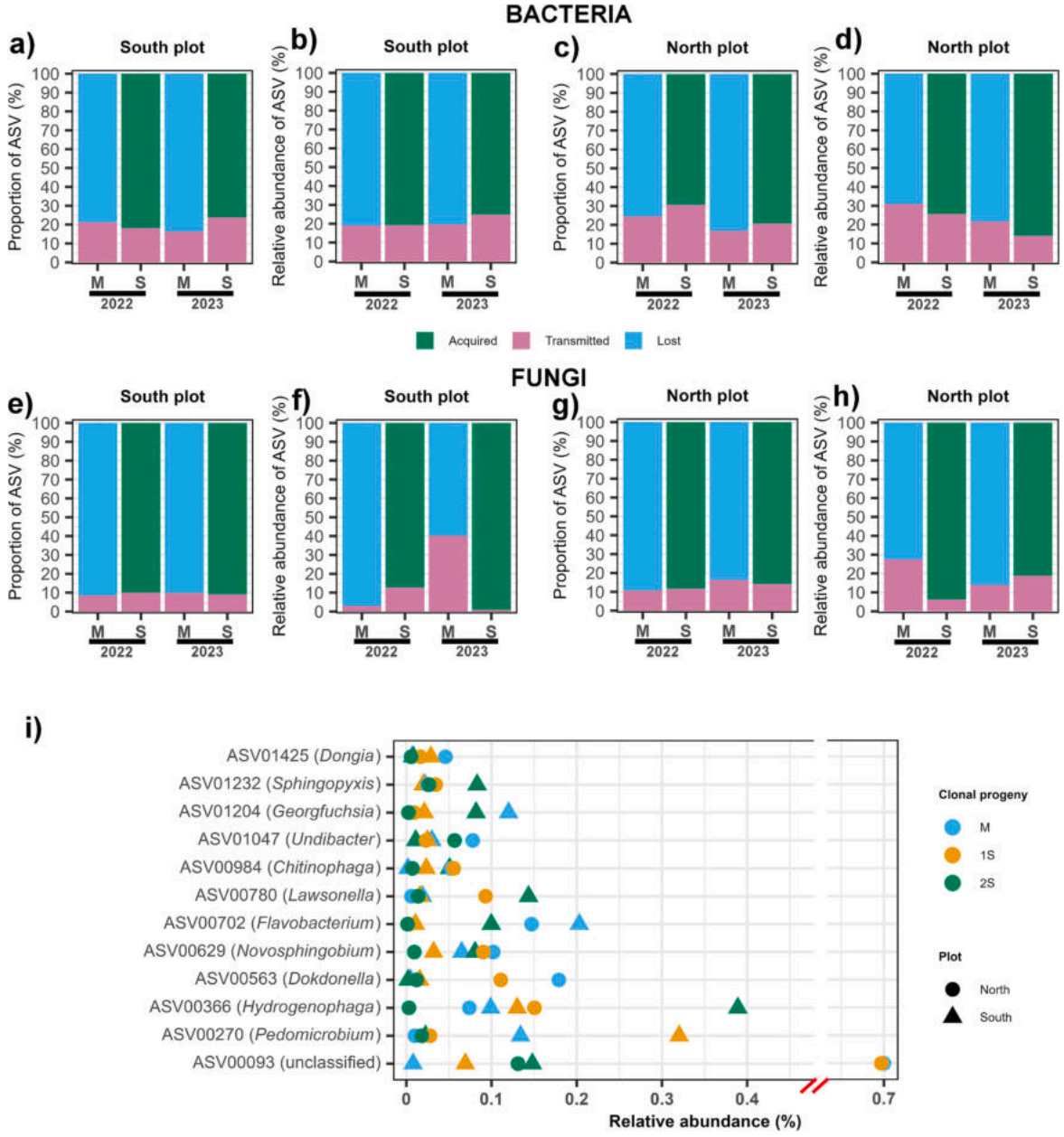

Fig. 5. Proportions (panels a and c) and relative abundances (b and d) of transferred bacterial ASVs from mothers to suckers in the south and north plots, respectively; transmitted fungal ASVs in the south (e, proportion; f, relative abundance); and north (g, proportion; h, relative abundance) plots; and relative abundance of bacterial ASVs transmitted from mothers to first and second suckers in both plots (i). "Acquired", "Transmitted" and "Non-transmitted" refer to the environmentally acquired ASVs in suckers, ASVs transmitted from mothers to suckers, and ASVs that were not transferred from mothers to the suckers, respectively. "M", "S", "1S" and "2S" indicate mothers, suckers, first suckers ("daughters") and second suckers ("granddaughters"), respectively. No common fungal ASVs were transmitted from mothers to first and second suckers in both plots. In panel **i**, the genus to which each ASV belongs is shown in brackets.

In addition, alpha and beta diversity analyses combined with co-occurrence networks demonstrated that the microbial communities were also sensitive to the host developmental stage, especially the root endophytes. It is already well known that the composition of root exudates varies during plant growth, which leads to the recruitment of different rhizosphere microorganisms (Chaparro et al., 2014; Trivedi et al., 2020). However, we also detected shifts in the root endophytome, which is in vein with the results obtained by Xiong et al. (2021). This observation indicates a host-driven selection of microorganisms.

Gómez-Lama Cabanás and co-workers (2021) already reported such an effect in the root endosphere of banana plants, although they focused on a single sampling time-point. It must be underscored that that their study was conducted in different banana orchards than the ones included in our work. Thus, both datasets reflect the effects of the banana developmental stage and of the orchard "context" (i.e., pedological features, crop and soil management, environmental or climatic conditions), thereby confirming the first starting hypothesis of this study. Furthermore, our results show that differences found between mothers and suckers are sustained along successive growing seasons. The ecological significance of these differences still needs to be interpreted with caution. On the one hand, small or moderate significant differences in microbial communities were detected in our study. On the other hand, subtle changes in microbial communities may have important consequences for the ecosystem in which they live. For instance, minor shifts can i) involve microbial taxa with key functional roles, ii) accumulate across generations, iii) become more impactful under stress conditions, or iv) affect networks and lead to cascade effects (Shade et al., 2012).

Plant developmental stage also affected the co-occurrence networks of banana root microbial communities. On the one hand, the root endosphere microbiota of the suckers formed more complex networks than those of the mothers, although this topology was not registered for all the networks. Numerous studies have shown that microbial communities forming complex networks respond better to biotic and abiotic stressors (Fernández-González et al., 2020; Jiemeng et al., 2018; Lasa et al., 2024; Rybakova et al., 2017). Furthermore, more modularized networks are prone to "isolate" external disturbances in specific modules, preventing them from spreading across the network (Fernández-González et al., 2020). On the other hand, a higher compartmentalization was also observed in the rhizosphere network of mother plants (northern plot 2022 and 2023, and southern plot 2022). From a holobiont perspective, this network organization may reflect that mother plants are more resilient against potential stressors by shaping a protective rhizosphere microbiota. Contrariwise, suckers potentially entrust their protection to root microbial dwellers. An ASV of the genus *Sphingopyxis* was classified as module hub in the root endosphere network of mother plants (northern plot, 2022). Interestingly, Gómez-Lama Cabanás et al. (2021) also classified an ASV of this genus as module hub in the network of mother plants. These results suggest that *Sphingopyxis* could have an important role in the arrangement of the microbiota associated to mother plants.

We noticed that mothers, first and second suckers shared certain microorganisms that otherwise were not detected in the rhizosphere. These microbes could have been vegetatively transferred to the offspring. Interestingly, discordant potential transmission patterns were observed for bacterial and fungal communities. This outcome is in agreement with the observations of Vannier and co-workers (2018). These authors concluded that more bacteria than fungi were transferred from mothers to first and second daughters of the vegetatively propagated herb *Glechoma hederacea*. Other studies suggested that the root endosphere mycobiome of banana plants was mostly acquired from the rhizosphere (Birt et al., 2023), which could explain the low percentage of fungal ASVs shared between mother and sucker plants. The lower potential transmission of fungal members is in vein with the loss of richness in the first and second suckers compared to the mother plants in both orchards, although these differences could be partially masked by the effect of the sampling year. Our results suggest that part of the banana root bacteriome may be vegetatively transmitted whereas the root endosphere mycobiome could be mostly acquired from the soil or aboveground plant tissues. Hence, the second hypothesis of this work was partly confirmed. Nevertheless, confirmation of these potential microbial transmission routes would need of appropriate experimental validation in future studies. For instance, potentially transferred microorganisms here found should be isolated, labelled, and traced thereby allowing to determine their movement through the banana rhizome.

Twelve bacterial ASVs appeared to be transferred from mothers even to the second suckers in both orchards. This outcome suggests that, even under diverse environmental and growing conditions, a "core transferred bacteriome" could be defined for banana plants. These ASVs belong to genera *Sphingopyxis*, *Chitinophaga* and *Flavobacterium,* to which important roles in banana plants have already been assigned. For instance, they are keystones in microbial networks (Gómez-Lama Cabanás et al., 2021), abundant taxa in banana roots (Gómez-Lama Cabanás et al., 2022), or members of the core microbiome of banana plants (Gómez-Lama Cabanás et al., 2021). Johnston-Monje et al. (2021) advocated that essential microbial symbionts would be vertically transmitted rather than acquired from a specific soil or ecosystem in which they may not thrive well. Thus, the 12 potentially transmitted ASVs by vegetative propagation could be key members of the banana holobiont due to their potential selection by the plant.

In some banana cultivation areas, suckers are transplanted by farmers to establish new fields, hence, it is assumed that endophytes inhabiting suckers' roots are transferred as well (Kaushal et al., 2020). Since the bacterial ASVs that are potentially transmitted from mothers to first and second suckers in both plots did not seem to be sensitive to edaphic properties, environmental shifts and/or different agricultural managements, they may adapt well to the new environmental conditions when suckers are transferred to the field. Likewise, these microorganisms could also be employed during *in vitro* micropropagation schemes, as micropropagated plants are usually grown in axenic conditions and solely rely on the endophytes inherited from the parental host (García-Giraldo et al., 2022). By inoculating banana plants with the "core transferred bacteriome" in the most suitable phase of the propagation process, their presence could be guaranteed. Taking all the observations into account, here we proposed that the 12 potentially transferred bacterial ASVs should be isolated and studied in depth in further works.

In summary, our results show that banana root microbiota is primarily influenced by the sampling year, which may reflect changes in environmental conditions and/or in the maturity of mother plants. The root microbial communities are also shaped by the developmental stage of the host, although to a lesser extent. Finally, mothers, first and second suckers in both orchards shared bacterial ASVs in the root endosphere that were not detected in the rhizosphere soil, suggesting a vertical transmission between plants independently of environmental conditions and agricultural management practices. By contrast, fungi were seldom shared between mothers and first and second suckers. Thus, our work have identified indigenous microorganisms of the banana belowground microbiota with potential agro-biotechnological interest for the cultivation of this relevant crop. Further studies are needed to verify that shared microorganisms are truly transferred from mothers to all clonal progenies and to uncover the role of these potentially transferred microorganisms in the banana holobiont.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2025.106377.

CRediT authorship contribution statement

Ana V. Lasa: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Carmen Gómez-Lama Cabanás:** Writing – review & editing, Methodology, Investigation. **Antonio J. Fernández-González:** Writing – original draft. **Javier López-Cepero:** Writing – original draft, Methodology.

Pablo J. Villadas: Writing – original draft, Methodology, Investigation. **Antonio Valverde-Corredor:** Methodology. **Cristina López-Díaz:** Methodology. **Lucía Gómez-Gil:** Methodology. **Antonio Di Pietro:** Writing – original draft, Supervision, Funding acquisition. **Manuel Fernández-López:** Writing – original draft, Investigation. **Jesús Mercado-Blanco:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Conceptualization.

Funding

This work is part of the project PLEC2021–00777, supported by the Spanish Ministry of Science, Innovation and Universities, State Investigation Agency, and the European Union "NextGenerationEU/PRTR (Plan de Recuperación, Transformación y Resiliencia)" [MCIN/AEI/10.13039/501100011033]. Ana V. Lasa hired under the Generation D initiative, promoted by Red.es, an organization attached to the Ministry for Digital Transformation and the Civil Service, for the attraction and retention of talent through grants and training contracts, financed by the Recovery, Transformation and Resilience Plan through the European Union's Next Generation funds.

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

The authors are in debt with Prof. Raimundo M. Cabrera Pérez (University of La Laguna, Tenerife, Spain) for granting full access to his laboratory to perform the early manipulation steps of soil/root samples. Authors are grateful to the owners of the two banana orchards for allowing the access to their properties.

Data availability

The datasets generated during the current study are available in the National Center for Biotechnology Information Sequence Read Archive (NCBI SRA) repository, https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1175077, under the BioProject accesion number PRJNA1175077. The code and scripts for the processing of the sequencing reads and for all ecological analyses is publicly available in GitHub (https://anitalasa.github.io/BananaMicrobiota/) and Zenodo repository (Lasa, 2024).

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