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Research Article

Rhizosphere microbiome response to host genetic variability: a trade-off between bacterial and fungal community assembly

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One sentence summary: Host genetic variability shapes the rhizosphere microbiome in vegetatively propagated chrysanthemum cuttings.
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Abstract

Rhizosphere microbial community composition is strongly influenced by plant species and cultivar. However, our understanding of the impact of plant cultivar genetic variability on microbial assembly composition remains limited. Here, we took advantage of vegetatively propagated chrysanthemum (*Chrysanthemum indicum* L.) as a plant model and induced roots in five commercial cultivars: Barolo, Chic, Chic 45, Chic Cream and Haydar. We observed strong rhizosphere selection for the bacterial community but weaker selection for the fungal community. The genetic distance between cultivars explained 42.83% of the total dissimilarity between the bacteria selected by the different cultivars. By contrast, rhizosphere fungal selection was not significantly linked to plant genetic dissimilarity. Each chrysanthemum cultivar selected unique bacterial and fungal genera in the rhizosphere. We also observed a trade-off in the rhizosphere selection of bacteria and fungi in which the cultivar with the strongest selection of fungal communities showed the weakest bacterial selection. Finally, bacterial and fungal family taxonomic groups consistently selected by all cultivars were identified (bacteria Chitinophagaceae, Beijerinckiaceae and Acidobacteriaceae, and fungi Pseudeurotiaceae and Chrysozymaceae). Taken together, our findings suggest that chrysanthemum cultivars select distinct rhizosphere microbiomes and share a common core of microbes partially explained by the genetic dissimilarity between cultivars.

Keywords: chrysanthemum, core microbiome, core selection, host selection, rhizosphere, vegetative propagation

Introduction

The rhizosphere of plants is a hot spot of microbial interactions (Hiltner 1904). The microbial community inhabiting the rhizosphere has been recognized as the plant's 'second genome' (Berendsen *et al.* 2012) and a plant-associated phenotype (Oyserman *et al.* 2021). Conversely, plant genotypes and soil chemistry play a role in rhizosphere microbial assembly (Schlemper *et al.* 2018, Cheng *et al.* 2020). The genetic background of the plant plays a role in microbial assembly by determining the production of exudates and the plant metabolome (Peiffer *et al.* 2013, Schlemper *et al.* 2017, Chang *et al.* 2021a). Such selection has been described in many plant species sexually propagated by seed, including barley (Bulgarelli *et al.* 2015), tomato (Cordovez *et al.* 2021), common bean (Stopnisek and Shade 2021), sorghum (Schlemper *et al.* 2017), and rice (Sun *et al.* 2021); by tubers such as potatoes (Buchholz *et al.* 2019); and by rootstocks such as grapevine (Berlanas *et al.* 2019). However, sexually propagated plants usually present high genetic variability, which makes it challenging to clearly link rhizosphere assembly with plant phylogeny (Chang *et al.* 2021b). This variability can be circumvented by vegetative propagation through cuttings, which allows the production of a large number of plants that retain the genetic content and traits of the stock plant. Many species are propagated vegetatively, either naturally or artificially, especially ornamental plant species (Christiaens *et al.* 2019). However, no study has examined microbial community assemblies attached to roots induced from cuttings.

Here, we used chrysanthemum (*Chrysanthemum indicum* L.) as a model vegetatively propagated plant to study bacterial and fungal rhizosphere community assembly in 14-day-old roots induced from cuttings. We exploited the phylogenetic stability associated with vegetative propagation to implement a statistical approach to determine the microbiome profiles of the rhizospheres of different cultivars and disentangle cultivar-specific selection from 'core' microbiome selection. We examined the microbiome assembly in both rhizosphere and bulk soil to use it as a proxy for plant selection. We defined the core selection as the microbes consistently selected by different cultivars according to model-based approaches (Leite and Kuramae 2020). We hypothesized that (i) the genetic variability of different cultivars impacts rhizosphere bacterial and fungal community assembly and (ii) chrysanthemum cultivars select distinct rhizosphere microbiomes but share a common core of microbes partially explained by cultivar genetic variability. To test these hypotheses, we determined the genetic variability of five commercial chrysanthemum cultivars using random amplified polymorphic DNA (RAPD) markers. We also assessed the bacterial and fungal rhizosphere communities in 14-day-old roots of cuttings using amplicon sequencing of the 16S rRNA partial gene for bacteria and the internal transcribed spacer (ITS) region for fungi. Furthermore, the bacterial and fungal com-

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munity selections were linked to the phylogenetic distances between the different chrysanthemum cultivars.

Materials and methods

Chrysanthemum cultivars and cutting-root induction

Five commercial chrysanthemum (*Chrysanthemum indicum* L.) cultivars were used in this study: Barolo, Chic-042 (Chic), Chic-045 (Chic 45), Chic Cream and Haydar. Chrysanthemum cuttings without any root formation were taken from stock plants provided by the breeding company Royal Van Zanten BV (NL). These specific chrysanthemum cultivars were selected for their different breeding background. Chic, Chic 45 and Chic Cream are genetically related cultivars, while Haydar and Barolo were obtained by distinct breeding selection.

Cuttings of 5 cm length were treated with 0.5% indolylbutyric acid powder to enhance root formation. The cuttings were placed 2 cm deep in a 5-cm commercial block of peat substrate provided by the breeding company Royal Van Zanten (Royal Van Zanten BV, Aalsmeer, The Netherlands) in a PVC container and maintained in a greenhouse at 16/8 h (light/dark) photoperiod, 70% relative humidity for 14 days at 20°C for root development. Prior to the block formation, peat soil was stored at room conditions and then mechanically homogenized (nutrient content and additional information in Table S1 and Fig. S1, Supporting Information). For each cultivar, cuttings were placed in peat soil blocks and kept in separated containers at the same conditions. The first 14-day period is crucial for cuttings' root development and in chrysanthemum production is referred as 'rooting phase'. After 14 days, the root system is developed and the cuttings are ready to be transplanted to agricultural soil for vegetative and flowering stages. Each cutting in peat soil block was considered as single replicate and randomly placed in the greenhouse for a total of four replicates per five cultivars.

Genetic variability of chrysanthemum cultivars

The genetic variability of five chrysanthemum commercial cultivars and a wild relative of chrysanthemum (collected in the Netherlands and used as an outgroup) was determined by RAPD approach. Two leaves of each cultivar were collected from three replicates, and the genomic DNA was extracted using DNeasy Plant Pro Kit (QIAGEN, Inc- Cat. No.69204), following the manufacturer's instructions. The DNA was quantified by Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). Nineteen different primers were used for RAPD reactions (Table S2, Supporting Information).

The RAPD reactions were performed in a total volume of 25 μl with final concentrations of 1× PCR buffer with $MgCl_2$ (1.8 mM), $MgCl_2$ (1.2 mM), dNTP mixture (200 μM), *Taq* DNA polymerase (0.056 U/μl) (Roche FastStart High Fidelity, La Roche Ltd.), Primer (0.5 μM), 5 μl of genomic DNA (10 ng/μl) and remaining sterile distilled water. The amplification was carried out in a DNA thermocycler (C1000 Touch, Bio-Rad Laboratories, Inc) with initial denaturation at 95°C for 4 min, 40 cycles of denaturation at 94°C for 1 min, primer annealing from 37 to 43°C for 1 min depending on the used primer (Table S2, Supporting Information), primer extension at 72°C for 1.30 min and final extension at 72°C for 4 min (Kumar *et al.* 2014). Polymerase chain reaction products were separated on a 1.5% agarose gel. Electrophoresis was carried out at 100 V for 1 h in Tris/Borate/EDTA buffer. The gels were stained with Ethidium bromide and visualized under a UV transilluminator on a gel documentation system (INTAS Science Imaging Instruments, GmbH).

RAPD analysis

DNA bands were scored and treated as a binary unit character ('1' for presence and 'zero' for absence). Cluster analysis was performed with binary distance and unweighted pair group average clustering method (UPGMA) using the package pvclust in R 4.0.1 (Suzuki and Shimodaira 2006). Pvclust package allows to compute the approximately unbiased (AU) probability values (*P*-values), for each cluster based on multiscale bootstrap resampling method with 1000 bootstrap replications. AU test provides a high-order accuracy and controls the selection bias that comes from comparing many trees. AU *P*-values represent percentage support for the branches of the UPGMA tree ranging from 0 to 100, where the higher the value, the higher the confidence in the cluster existence. This information was used to determine the reliability of each one of the formed clusters and estimate the genetic dissimilarity between the five different cultivars of chrysanthemum (Barolo, Chic, Chic 45, Chic Cream and Haydar). The number of bands per primer is provided in Table S2 (Supporting Information).

Rhizosphere and bulk soil DNA extraction and 16S rRNA gene and ITS region amplification and sequencing

Rhizosphere soil was collected from rooted cuttings of each cultivar in four replicates after 14 days. All the cuttings were at the same developmental stage, roots were visible from the surface of the peat soil block. Roots were shaken and the adhering soil was collected using a sterile brush. The soil not in contact with the roots (bulk soil) was collected for every cultivar in four replicates. Rhizosphere and bulk soil were stored at −80°C for further use. Total DNA from rhizosphere and bulk soil was extracted from each replicate with Power Soil Pro kit (QIAGEN, Inc-Cat. No. 47016) following the manufacturer's instructions. The quantity and quality of the DNA were checked by Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). DNA samples were amplified using the primer set 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) for 16S rRNA partial gene (V3–V4 region) for Archaea/bacteria (Caporaso *et al.* 2011), and the primer set ITS1F (CTTGGTCATTTAGAGGAAGT) and ITS2 (GCTGCGTTCTTCATCGATGC) for ITS region for fungi (White *et al.* 1990). Targeted PCR with staggered tagged 16S rRNA gene primers was carried out under the following conditions: an initial denaturation step at 94°C for 2 min, followed by 33 cycles of 94°C for 30 s, 58°C for 30 s, 72°C for 30 s and a final extension step at 72°C for 7 min. Targeted PCR with tagged ITS primers was carried out under the following conditions: an initial denaturation step at 96°C for 15 min, followed by 33 cycles of 96°C for 30 s, 52°C for 30 s, 72°C for 60 s, and a final extension step at 72°C for 7 min. The PCR mix contained 1× of Roche 10× Buffer with 18 mM $MgCl_2$, 5% Roche DMSO, 0.2 mM dNTP mix 10 mM NEB, 0.02 U/μl Roche FastStart High Fi 5 U/μl, 0.6 μM of the 515FP/ITS1, 0.6 μM of the 806RP/ITS2 and sterile Milli-Q water up to the final volume of 20 μl. Verification of amplification was performed on 2% agarose gel. The barcoding step was performed to add an index (or barcode) to each sample and Illumina adapter required for DNA to bind to the flow cell. The PCR was carried out under the following conditions: an initial denaturation step at 95°C for 10 min, followed by 15 cycles of 95°C for 15 s, 60°C for 30 s, 72°C for 60 s, and a final extension step at 72°C for 3 min. The PCR mix contained 2 μl of Roche 10× Buffer without $MgCl_2$, 1.44 μl of Roche $MgCl_2$ 25 mM, 1.00 μl

Roche DMSO, 0.40 μl dNTP mix 10 mM NEB, 0.10 μl Roche FastStart High Fi 5U/μl and sterile Milli-Q water up to the final volume of 20 μl. Verification of barcode incorporation for each sample was performed on 2% agarose gel. Quantification of each amplicon was carried out with Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies Corporation, Thermo Fisher scientific, USA). The library was then generated by pooling the same quantity (ng) of each amplicon. Cleaning-up of the pool (or library) was carried out with sparQ PureMag Beads (Quantabio, USA). Library was quantified using Kapa Illumina GA with Revised Primers-SYBR Fast Universal kit (Kapa Biosystems, UK). Average fragment size was determined using a LabChip GX (PerkinElmer, Inc) instrument. Before sequencing, 15% of PhiX control library was added to the amplicon pools (loaded at a final concentration of 8.5 pM). Sequencing was carried out with the MiSeq 250 bp paired-end Reagent Kit v2 500 cycles from Illumina (Génome Québec Center, Montreal, Quebec).

Role of plant genetic distance in the rhizosphere selection

We described the microbiome selection by the cuttings based on differences in microbial profiles (defined in the section 'Amplicon sequencing and statistical analysis') between rhizosphere and bulk soil. Cuttings were able to grow roots and select rhizosphere communities for 14 days. We evaluated how plant genetic distance explains the microbial selection performed by each of the different cultivars in the rhizosphere using generalized dissimilarity modeling (GDM). GDM statistical approach can be used for community composition turnover analysis. The community composition is given by a dissimilarity index, in our case the Bray–Curtis dissimilarity. The GDM method has the advantage of accounting for the mean–variance relationship in the dissimilarity metric and avoid the bias imposed by the Bray–Curtis dissimilarity (Ferrier *et al.* 2007). GDM was originally designed to model spatial variation in biodiversity between pairs of geographical locations but can also accommodate special types of biological variation, such as genetic distance. Community turnover is measured by the partial ecological distance, which in this study corresponds to the increase in the microbial dissimilarity index (between regression coefficients) following an increase in plant genetic distance. GDM uses l-splines to more accurately estimate the increase in the partial ecological distance and evaluate the increase in community dissimilarity connected to plant genetic distance. Both the genetic distance and the microbiome profile used in the GDM analysis are 5 × 5 matrix (cultivar × cultivar), thus resulting in 25 measurements of dissimilarity and genetic distance. GDM modeled the pairs of cultivars, without repeating combination of cultivars (e.g. BA × CR and CR × BA). From the 5 × 5 dissimilarity matrix, 10 pairs of 'cultivar × cultivar' were fitted in the model.

The microbial genera linked with the genetic dissimilarity of the cultivars were detected following our concept of 'core' selection (described in the section 'Amplicon sequencing and statistical analysis'): different taxa detected by the model coefficients to be significantly positively selected in the rhizosphere of the different chrysanthemum clusters based on genetic variability.

Amplicon sequencing and statistical analysis

The quality of reads was determined using the FASTQC quality control tool version 0.10.0 (Babraham Bioinformatics 2022) for both 16S rRNA gene and ITS amplicon sequences. Subsequently, forward and reverse reads were then truncated using Cutadapt (Martin 2011). The DADA2 v1.16.0 pipeline (Callahan *et al.* 2016) was used to denoise and obtain amplicon sequence variants (ASVs) from demultiplexed reads for all samples. Reads that did not overlap were removed from the rest of the analysis. Taxonomic assignment was performed using the naïve Bayesian classifier (implemented in DADA2) using the 'Silva version 138' database for Archaea/bacteria and the UNITE v8.2 database for fungi. The ASV table was filtered at Genus level and used for further analysis. A threshold of 10 occurrences using the function decostand from Vegan package (Oksanen *et al.* 2018) was applied to merge low occurring microbes that could not be properly modeled in a column named 'Others'. This cutoff was selected because the GJAM model (see later) requires a minimum level of occurrence to produce accurate estimates on microbial abundance. To do that cutoff at ASV is a waste of information since several ASV have low-occurrence but are common at higher taxonomic levels. To not waste this information, we decided to work at Genus levels. A centered log transformation was performed using the package zCompositions (Palarea-Albaladejo and Martín-Fernández 2015) to describe the average abundance of the microbes in the different compartments and cultivars. A total of four replicates per treatment were used for the 16S and ITS sequencing.

Generalized joint attribute modeling (GJAM) was used to obtain the microbiome profile of each chrysanthemum cultivar, extracting the regression coefficients that define the relative abundance of microbes (archaea/bacteria and fungi) for each plant cultivar and to estimate the effects of both plant cultivar and compartment (rhizosphere/bulk soil) in two different models for the bacterial and fungal communities. GJAM package (Clark *et al.* 2017) was selected as a model due to its capacity to analyze compositional count data (Leite and Kuramae 2020). Composition count was selected as data type since the microbiome data is to be considered compositional based on heterogeneous number of reads per sample (Gloor *et al.* 2017). Model diagnosis evaluated the Markov chain Monte Carlo to check when the estimated coefficients reached a stable value. The model was fitted using a non-informative prior for the coefficients and covariance matrix, 10 000 iterations with a burn-in of 4000 iterations. Since the current experiment consists of a two-way factorial design, regression coefficients were compared against the following null hypotheses: Within each plant compartment (rhizosphere/bulk soil) there is no difference between cultivars. The regression coefficients obtained from the GJAM analysis provide the information on the microbial groups (archaea/bacteria and fungi) that were enriched or depleted by each of our treatments (plant compartment and cultivar). This allowed us to consider those regression coefficients as a proxy for the microbiome profile of each chrysanthemum cultivar. The four replicates were used to build the model from where we obtained the coefficients. With that in mind, we looked at the similarities and variances between those different microbiome profiles. For identifying treatments with a similar selection of microbes, we clustered the regression coefficients using WARD distance (Murtagh and Legendre 2014).

For visualizing the variance between the set of regression coefficients (microbiome profiles), we used principal component analysis (PCA), which also allowed us to explore communities' similarities between compartments (rhizosphere vs bulk soil). A between-class analysis (BCA; Kenkel 2006) was used to evaluate the differences between the microbiome profile in rhizosphere and bulk soil. BCA allows assessing the amount of variability that can be explained by the applied treatment. The multivariate distance between microbial profiles of rhizosphere and bulk soil was calculated as a proxy for rhizosphere selection strength. The multivariate distance values were plotted in a bar plot after being ad-

justed according to the percentage of variability explained by each one of the PCA axis.

From the GJAM model, we depicted the positive coefficients representing microbial groups detected to be significantly different and selected in the rhizosphere, and the negative coefficients representing the microbial groups detected to be significantly different and not selected in the rhizosphere. We used the terms 'core' selection to describe the different taxa detected by the model coefficients to be significantly positively selected in the rhizosphere of all the chrysanthemum cultivars; and cultivar-specific selection to describe the different taxa detected by the model coefficients to be significantly positively selected in the rhizosphere of single cultivars. A heat map and dendrogram using WARD distance were generated to visualize the positive and negative coefficients. The bacterial and fungal families shared between cultivars or cultivar specific were shown in a Venn diagram.

The sequences were deposited in the European Nucleotide Archive (ENA; https://www.ebi.ac.uk/ena) under the accession number PROJECT PRJEB48376.

Results

Genetic variability of chrysanthemum cultivars

The genetic variability among the five commercial chrysanthemum cultivars and a wild relative was assessed using RAPD markers. From 19 RAPD primers, 16 generated 246 scorable bands. The UPGMA dendrogram showed that the commercial chrysanthemum cultivars can be divided into two distinct clusters. Cluster I comprises Chic, Chic Cream and Chic 45. In this cluster, Chic and Chic Cream have the closest similarity, with an AU *P*-value of 100; consequently, this cluster was named the 'Chic' cluster. Cluster II comprises Haydar and Barolo with an AU *P*-value of 85 (Fig. 1A). This low AU *P*-value suggests that Haydar and Barolo cultivars are not strongly genetically related.

Microbial profile in the rhizosphere and bulk soil of chrysanthemum cultivars

A total of 15 676 ASVs representing 750 genera were obtained for archaea and bacteria in the rhizosphere and bulk soil of the five chrysanthemum cultivars. A total of 2459 ASVs representing 409 genera were obtained for fungi in the rhizosphere and bulk soil of the five chrysanthemum cultivars (average values of microbial community abundance are represented in Figs S2 and S3, Supporting Information). The total number of archaeal ASVs was 38 from two phyla; given this low number, we will refer to the 16S rRNA community as 'bacteria'. For each cultivar, GJAM regression coefficients allowed us to determine the bacterial and fungal profiles according to plant compartment (rhizosphere and bulk soil). A total of 27 bacterial phyla were present in the rhizosphere. The predominant phyla were Acidobacteriota, Actinobacteriota, Armatimonadota, Bacteroidota, Bdellovibrionota, Cyanobacteria, Dependentiae, Desulfobacterota, Elusimicrobiota, Fibrobacterota, Firmicutes, Gemmatimonadota, Myxococcota, Patescibacteria, Planctomycetota, Proteobacteria and Verrucomicrobiota. A total of five fungal phyla were present in the rhizosphere: Ascomycota, Basidiomycota, Mortierellomycota, Mucoromycota and Rozellomycota. As illustrated in Fig. S2 (Supporting Information), which depicts the changes in bacteria (Fig. S2A, Supporting Information) and fungi (Fig. S2B, Supporting Information) in the rhizosphere microbiome, the chrysanthemum cultivars selected similar microbes. Chic, Chic 45 and Chic Cream exhibited similar rhizosphere microbial assemblies, whereas Barolo and Haydar had very distinct communities. Interestingly, the clusters of chrysanthemum cultivars based on bacterial profile were similar to those based on plant genetics.

PCA plots were constructed to visualize the bacterial (Fig. 1B) and fungal (Fig. 1C) community profiles of the five cultivars in the rhizosphere and bulk soil. Each dot represents the microbial profile of one treatment. The first two axes captured a large portion of the differences between plant cultivars and compartments. Specifically, the first two axes captured 42.9% of the total variability in the microbiome profile of the bacterial community and 40.1% for the fungal community. As a result, we considered the bacterial communities in the bulk soil samples similar regardless of cultivar. By contrast, spreading of the cultivars was evident for the bacterial community in the rhizosphere, suggesting cultivar-specific selection of bacteria. From the BCA, we found that 72% of the variability in the 16S rRNA gene is explained by the compartments ($P < 0.01$). For the fungal community, we did not observe a clear grouping of the bulk soil samples, but we did find a shift between the rhizosphere and bulk soil profiles that also suggested cultivar-specific selection of fungi, albeit weaker than the selection observed for the bacterial communities.

The bacterial and fungal multivariate distances between the rhizosphere and bulk microbiome profiles represent the strength of rhizosphere selection (Fig. 1D and E). For the bacterial community, Chic Cream and Barolo had the highest multivariate distances, with values of 0.593 and 0.426, respectively, while Chic had the highest value for the fungal community, 0.500. Interestingly, the cultivar with the weakest selection of bacteria (Chic) had the strongest influence on the fungal community, whereas the cultivar with the weakest selection of fungi (cultivar Barolo) ranked second in strength of bacterial selection in the rhizosphere. All distances are reported in Table S3 (Supporting Information).

Microbial 'core' selection and cultivar-specific selection

The regression coefficients from the GJAM analysis also permitted the unique rhizosphere selection (cultivar-specific) to be disentangled from the common ('core') selection for the five cultivars. Significantly positive coefficients represent our concept of 'core' selection: bacterial and fungal groups that are always selected in the rhizosphere for all cultivars. Heat maps illustrate the community structure changes based on coefficients for both the bacterial (Fig. S1A, Supporting Information) and fungal (Fig. S1B, Supporting Information) profiles. Table 1 presents the numbers of genera with positive or negative coefficients in the bacterial and fungal profiles.

The percentage of positively selected genera was 23% for bacteria (Fig. 2A) and 25% for fungi (Fig. 2B). No bacterial genus was significantly selected by all five cultivars; instead, the cultivar 'core' selection was observed at the family level. Three bacterial families were significantly selected by all cultivars: Chitinophagaceae, Beijerinckiaceae and Acidobacteriaceae (Fig. 2C). In the fungal community, two families (Pseudeurotiaceae and Chrysozymaceae) were common to all cultivars (Fig. 2D). The numbers of microbial families positively selected and shared by each cultivar are illustrated in Fig. 2C (bacteria) and Fig. 2D (fungi).

In addition to the 'core' selection, unique bacterial genera were selected by specific cultivars: *Pseudarthrobacter*, *Streptomyces*, *Flavobacterium*, *Rhizomicrobium*, *Devosia* and *Acinetobacter* by Barolo; *Mucilaginibacter*, *Asticcacaulis*, *Parvibaculum*, *Pandoraea*, *Methylotenera*, *Massilia* and *Rhodanobacter* by Haydar; *Vampirovibrio*, *Caulobacter*, *Limnobacter*, *Duganella*, *Thermomonas* and *Lacunisphaera* by Chic;

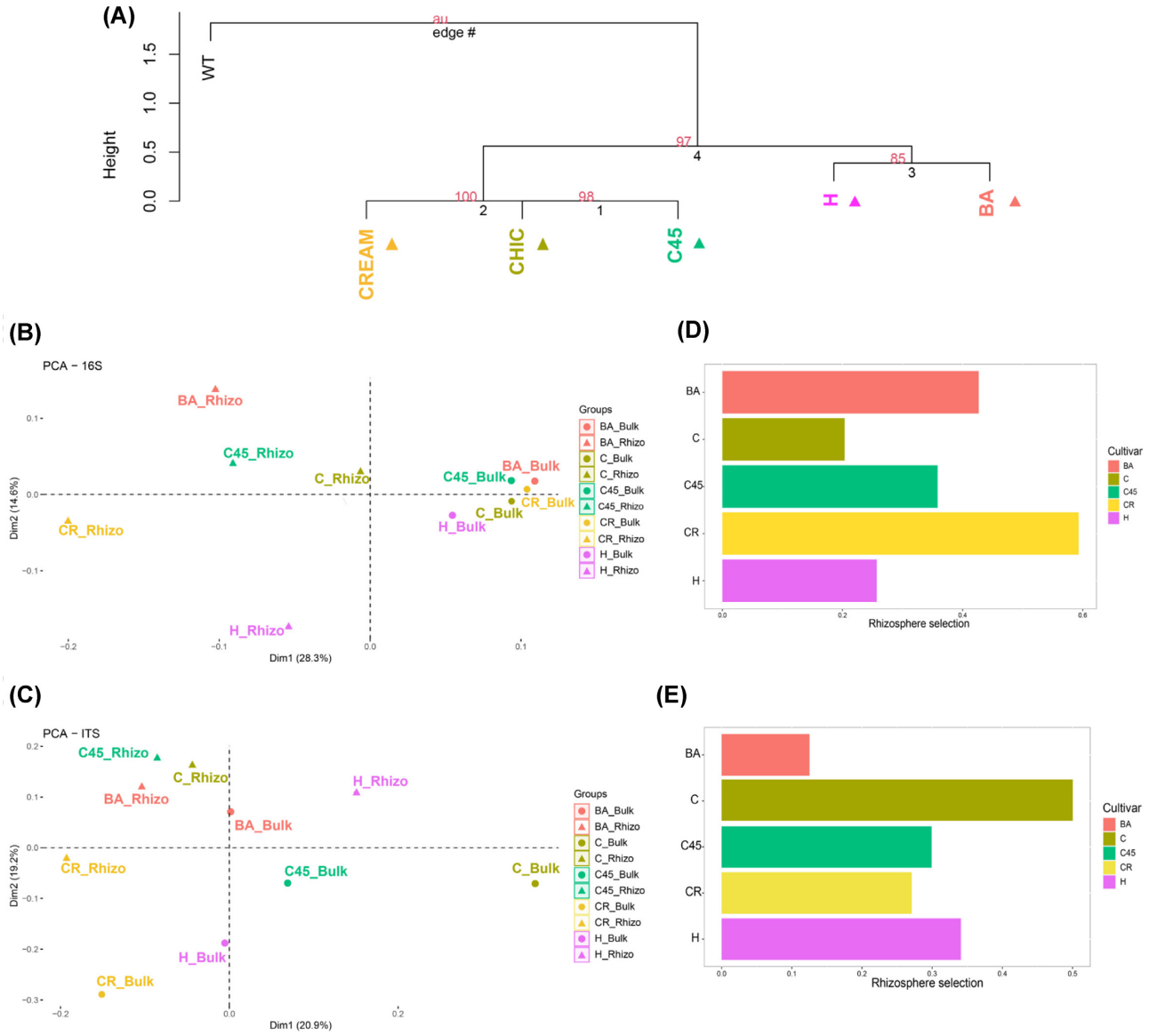

Figure 1. **(A)** Dendrogram based on UPGMA showing the genetic distances between the five chrysanthemum cultivars. AU *P*-values are shown in red for each cluster. WT = wild type, H = Haydar, BA = Barolo, C45 = Chic 045, CR = Chic Cream. PCA showing the differences in the bacterial **(B)** and fungal **(C)** assemblies in the rhizosphere and bulk soil among the cultivars. Bulk samples are represented by circles, and rhizosphere samples are represented by triangles. Each dot represents the microbial profile of four replicates. **(D, E)** Bar plots representing the multivariate distance between the bacterial **(D)** and fungal **(E)** community assemblies in rhizosphere and bulk soil among the different cultivars. On the *y*-axis, H = Haydar, BA = Barolo, C45 = Chic 45 and CR = Chic Cream.

Micropepsis, *Klebsiella* and *Pseudomonas* by Chic 45; and *Mycobacterium* and *Nocardioides* by Chic Cream. Three cultivars selected specific fungal genera: *Oidiodendron*, *Stachybotrys* and *Apiotrichum* by Chic 45; *Coniochaeta*, *Acremonium*, *Phaeotremella*, *Papiliotrema* and *Saitozyma* by Chic Cream; and *Candida* by Haydar. Notably, no specific fungal genera were selected by Barolo and Chic, the cultivars with the weakest and strongest rhizosphere selection, respectively, as measured by multivariate distance (Fig. 1E).

Overall, our model facilitated the identification of microbial groups that were consistently selected regardless of cultivar and represent what we denote as the 'core selection'. The core selection was observed at the family level in both the bacterial and fungal profiles. Cultivar-specific selection was found at the genus level and occurred for all five cultivars in the bacterial profile (Fig. 3A and B).

Relationship of plant genetic distance with the microbial community profile

The selection of microbes by each of the cultivars can be partially explained by genetic variability. GDM revealed an influence of cultivar genetic variability on rhizosphere microbial community selection, as plant genetic distance explained 42.83% of the dissimilarity in bacterial assembly in the rhizosphere. By contrast, the model did not explain the differences in rhizosphere fungal community assembly between the cultivars. In summary, the GDM model applied here successfully measured the range of genetic variability linked with the dissimilarity of rhizosphere microbiome selection (GJAM regression coefficients), at least for the bacterial assembly. The response plot shows that the differences in the rhizosphere bacterial assembly profile among the chrysanthemum cultivars increased with genetic variability. The relation-

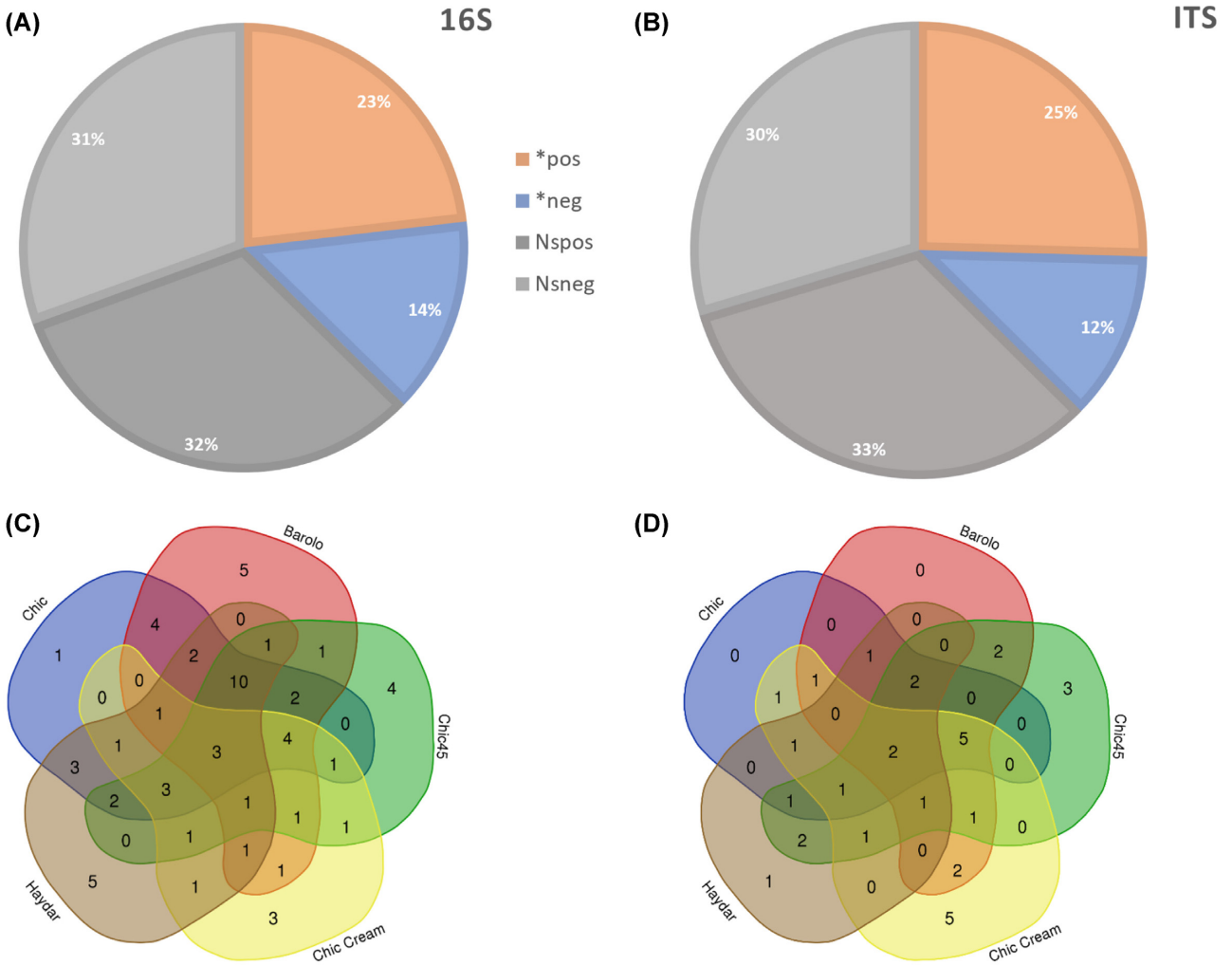

Figure 2. Pie charts showing the percentages of genera with statistically (*) and non-statistically (Ns) different positive (pos) and negative (neg) coefficients in the bacterial profile **(A)** and fungal profile **(B)**. Venn diagrams showing the numbers of bacterial **(C)** and fungal **(D)** families detected exclusively in each chrysanthemum cultivar and the families shared by all cultivars. Color code: red, Barolo; blue, Chic; brown, Haydar; yellow, Chic Cream; and green, Chic 45.

Table 1. Numbers of genera in the rhizosphere bacterial and fungal communities with statistically (*) and non-statistically (Ns) different positive and negative coefficients.

Regression coefficient		Cultivar				
		Barolo	Chic	Chic 45	Chic Cream	Haydar
*Positive	Bacterial	68	70	71	51	67
	Fungal	59	70	58	56	46
*Negative	Bacterial	43	70	26	60	51
	Fungal	13	70	15	27	31
Ns positive	Bacterial	88	105	125	70	81
	Fungal	63	70	71	71	58
Ns negative	Bacterial	93	79	70	111	93
	Fungal	67	59	58	48	67
Cultivar-specific genera	Bacterial	6	70	3	2	7
	Fungal	0	70	3	5	1

ship between plant genetic distance and bacterial assembly in the rhizosphere became less relevant (plateau of the curve in Fig. 4A) when the dissimilarity in the RAPD data was greater than 30%. In total, the dissimilarity between the five cultivars ranged between 1.55% and 41.25%, suggesting a strong impact of plant breeding on the selection of rhizosphere microbes.

The microbial genera linked with the genetic dissimilarity of the cultivars followed our concept of 'core' selection. In the 'Chic' cluster (Clusters 1 and 2), 10 bacterial genera were always selected in the rhizosphere: *Taibaiella*, *Citrifermentans*, *Roseisolibacter*, *Pajaroellobacter*, *Methylovirgula*, *Pseudaminobacter*, *Variovorax*, *Legionella*, *Nevskia* and *Luteolibacter*. In Cluster 3, seven bacterial genera were consistently selected by both cultivars: *Chthonomonas*, *Chitinophaga*, *Haliangium*, *Rhodopirellula*, *Hyphomicrobium*, *Cupriavidus* and *Coxiella* (Fig. 4B). Therefore, the genetically related cultivars selected similar bacterial communities, and the 'Chic' cluster selected a greater number of genera than Cluster 3.

Discussion

In this study, chrysanthemum was used as a model plant to obtain insights on plant host-dependent microbiome assembly in vege-

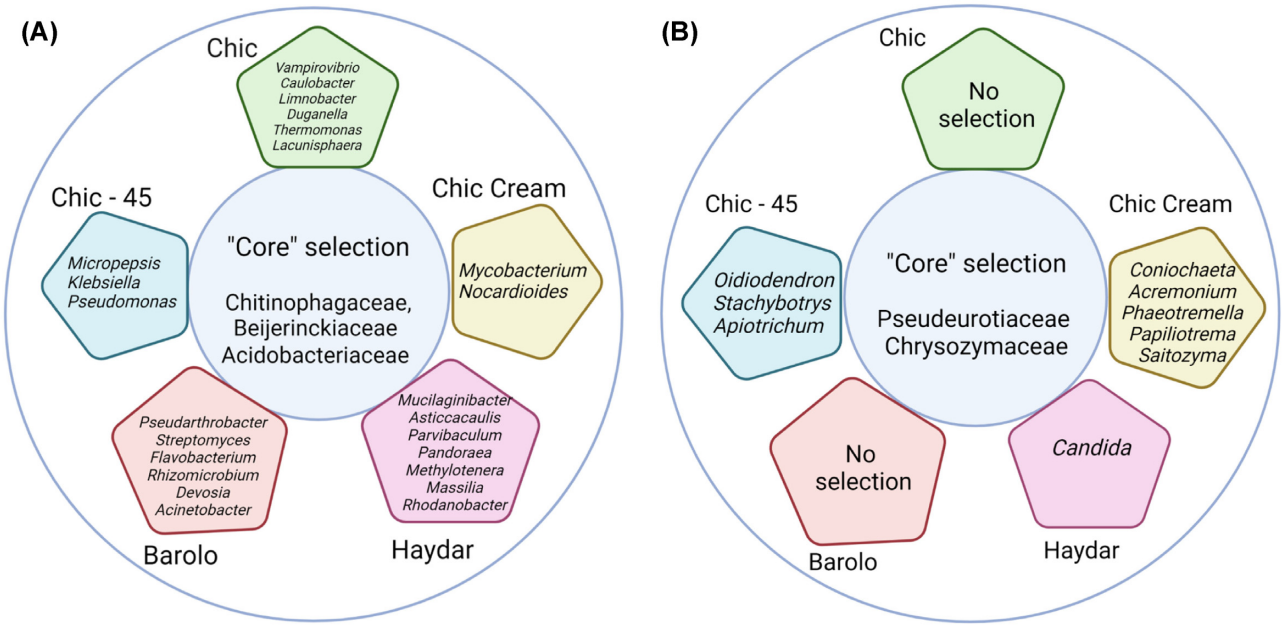

Figure 3. Illustration of the bacterial **(A)** and fungal **(B)** families shared in the 'core' selection (inner circle) and the unique genera that were cultivar specific (each pentagon represents a different cultivar).

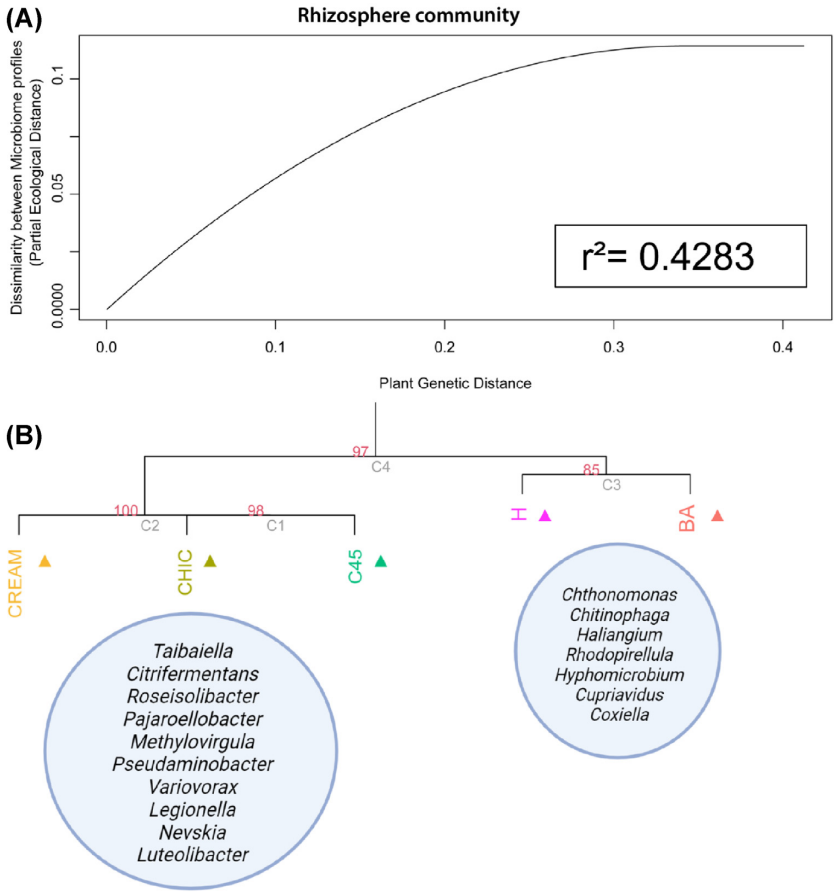

Figure 4. (A) Response plot of GDM connecting the partial ecological distance of the bacterial profile to the plant genetic distance between cultivars. **(B)** Bacterial genera selected in the rhizosphere by plant genetic clusters.

tatively propagated plants. We compared the shift from community assembly between bulk and rhizosphere soil as a proxy for plant selection; however, we have not determined the microbial community of the initial soil sample. The interactions between adventitious roots induced from cuttings and the surrounding soil in rhizosphere assembly have not been described in the literature. The rooting phase is a crucial part of plant development (De Klerk 2002), a proxy for root capacity, and when the first interactions between the soil microbiome and plant roots occur. However, the genetic variability of sexually propagated plants adds another layer of complexity in understanding the microbial assembly process (Barret *et al.* 2015) in the rhizosphere. Therefore, by focusing on the early stage of chrysanthemum development from cuttings, we were able to not only identify the microbiome assembled in the rhizosphere but also link this assemblage with the genetic variability of different cultivars, shedding light on the importance of plant genetic diversity in the selection of rhizosphere microbes.

The analysis of genetic distance using RAPD markers allowed us to separate the five commercial cultivars into two distinct clusters based on genetic variability. The 'Chic' cluster (cluster I) comprises the most genetically related cultivars: Chic, Chic 45 and Chic Cream. In fact, Chic 45 was derived from the original Chic cultivar, and Chic Cream is a (color) mutant of the original Chic cultivar. The second cluster comprised Haydar and Barolo, which are not strongly genetically related to each other. These cultivars are phenotypically different, with different flower colors (pink and red, respectively), and were obtained by distinct breeding selection. Although the RAPD approach is not the most specific method for genetic evaluation (Igor *et al.* 2019), in our analysis, these markers revealed that 42.83% of the total dissimilarity in the bacterial communities assembled by the different cultivar was linked to genetic distance. In sum, our findings suggest a strong link between rhizosphere assembly and genetic variability within the same plant species (in our case, chrysanthemum cultivars).

Our model-based approach allowed us to identify the rhizosphere microbial profile (bacterial and fungal assemblies) of each cultivar. In general, rhizosphere selection was cultivar dependent. For the bacterial community, we found a clear distinction between the assembly in the rhizosphere and that in bulk soil. As demonstrated by PCA (Fig. 1B), the bacterial community in the bulk soil was similar among the cultivars, as indicated by clustering, whereas in the rhizosphere, each cultivar selected a distinct microbiome profile. This result suggests a clear and strong selection of the bacterial community by each of the five chrysanthemum cultivars. However, we did not observe the same pattern for the fungal community. The variability of the fungal community in bulk soil was as great as the variability among the rhizosphere samples. In general, fungi colonize the soil substrate faster than bacteria and act as primary degraders of particulate, predominately terrigenous C (Gessner *et al.* 2007, Fabian *et al.* 2017). In our experiment, the soil substrate was composed of peat soil, a rich source of organic matter and freely available nutrients (Leifeld *et al.* 2020), which might explain the high variability in the fungal community inhabiting the bulk soil. Moreover, bacteria might be faster at reproducing and hence able to respond to plant selection in a shorter period than fungi.

Taken together, our results show that in the early stages of chrysanthemum root growth, rhizosphere selection is stronger for the bacterial community than for the fungal community. Our findings suggest that rhizosphere fungal diversity is more strongly influenced by other factors, such as the organic content in the soil substrate, than rhizosphere bacterial diversity. Another possibility that cannot be ruled out is that in the early stages of root development, the chrysanthemum cultivar selected more strongly for bacteria than for fungi.

Our study only evaluated the microbiomes of 14-day-old chrysanthemum roots, and older plants may show differences in rhizosphere selection. Studies of the dynamics of rhizosphere selection are left as an avenue for future research. Nonetheless, our findings indicate the importance of accounting for both biotic and abiotic factors when investigating the influence of plants on the rhizosphere microbiome, especially for fungi.

We defined the cultivar-specific selection as taxa detected by the model coefficients as significantly positively selected only in the rhizosphere of a single cultivar. Cultivar-specific selection at the genus level was identified for bacteria in all five cultivars and fungi in three cultivars. The cultivars Chic and Barolo did not select specific fungal genera. We connected these results to rhizosphere selection strength by proposing that a trade-off exists between bacterial and fungal selection in the rhizosphere depending on the specific cultivar. The cultivar with the weakest selection of bacteria (Chic) showed the strongest selection for the fungal community, whereas Barolo, which showed strong rhizosphere bacterial selection, had the weakest fungal selection among the cultivars. This pattern is also clear from the analysis of cultivar-specific selection, as Barolo selected six bacterial genera but no fungal ones. Moreover, the strength of rhizosphere selection does not necessarily imply cultivar-specific selection. Chic showed the strongest fungal rhizosphere selection, but no specific fungal genus was found in the rhizosphere of this cultivar. Thus, plant can strongly influence the rhizosphere community by increasing the abundance of specific microbes without selecting unique ones. Another potential explanation for these results is the genetic links between the Chic cultivar and Chic 45 and Chic Cream, as the latter two originated from Chic via breeding and exhibited similar but weaker fungal community selection.

Many studies have focused on the idea of a core microbiome to describe microbial communities with tight relationships with the plant host (Compant *et al.* 2019), but the concept of a core microbiome remains poorly defined. One of the major challenges is statistical support (Shade and Stopnisek 2019, Risely 2020), as the shared microbes suggested as representatives of a core microbiome are often the microbes present in all treatments. These microorganisms might include microbes that are effectively selected by different plants but are not affected by different treatments. To avoid confusion, we propose the concept of 'core selection' to represent microbes that are consistently selected by different plants and are more abundant than other microbes in the rhizosphere. The advantage of this concept is that model-based approaches can be adopted to determine the microbiome profiles of different plants (Leite and Kuramae 2020) and subsequently identify microbes that form the core selection. In the current study, 'core' selections were found at the family level for both bacteria and fungi. Interestingly, the three bacterial families common to all cultivars have been characterized in many studies as beneficial: Chitinophagaceae, Beijerinckiaceae and Acidobacteriaceae. Nitrogen fixation is a notable trait of the family Beijerinckiaceae and enables these bacteria to thrive in habitats in which other potential sources of nitrogen are scarce (Marín and Arahal 2014). Acidobacteriaceae produce extracellular polymeric substances that benefit plants by maintaining moisture in the environment and trapping nutrients (Kielak *et al.* 2016, Costa *et al.* 2018). Further studies considering different cultivars of chrysanthemum and their microbiome selections under different conditions, across several soils, and multiple time points, are needed to define a chrysanthemum 'core' microbiome.

In addition to defining the cultivar-specific and 'core' selections of the five cultivars, in this study we evaluated how plant genetic distance explains microbial selection in the rhizosphere. Using GDM, we found that 42.83% of the dissimilarity in the rhizosphere bacterial community among the different chrysanthemum cultivars was explained by the cultivars' genetic variability. Specific groups of microbes followed the pattern of the chrysanthemum genetic clusters detected by RAPD. Cultivars with a similar genetic background selected a higher number of common bacterial genera in the rhizosphere. In other words, our study shows that genetically related cultivars select similar bacterial communities. A similar phenomenon was not observed for the fungal community, most likely due to the strong influence of the organic content in the soil substrate (peat soil). We suggest further studies to explore the influence of this abiotic factor on rhizosphere fungal assembly.

The effect of plant genetic background on microbial assembly is related to differences in plant exudates and metabolomes (Peiffer *et al.* 2013, Schlemper *et al.* 2017, Chang *et al.* 2021b). Traditionally, plants have been bred by altering their genomic information with little consideration of the interaction of the plants with surrounding organisms (Wei and Jousset 2017). Breeding programs for specific phenotypic traits could also impact rhizosphere selection by cultivars. To confirm and understand the mechanisms of this selection, further studies of the associations of rhizosphere microbiome assembly with the metabolomes of chrysanthemum cultivars are needed.

In summary, this study is the first to evaluate the rhizosphere bacterial and fungal communities in the early stage of vegetative propagation by cuttings. Vegetative propagation allowed us to more clearly examine the influence of plant genetic variability on rhizosphere selection. Additionally, our model-based approach permitted the determination of the specific rhizosphere microbiomes selected by each chrysanthemum cultivar as well as the shared common core selection, which was partially explained by the genetic distance between cultivars. These findings will contribute to the development of a plant genetic baseline to further extend our understanding of the complex plant–microbe interactions in plant breeding programs. Elucidating the influence of genetic variation on rhizosphere selection will allow us to modulate the beneficial associations between microorganisms and plants and engineer specific substrates and optimal inocula.

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Supplementary data

Supplementary data are available at FEMSEC online.

Author contributions

CR, MFAL and EEK conceived and designed the experiments. CR and AP performed the experiments. CR and MFAL performed the data analyses. CR drafted the manuscript. MFAL and EEK revised the manuscript. All authors have read and approved the submitted version.

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