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Exploring the diversity of the root-associated microbiome of *Ilex paraguariensis* St. Hil. (Yerba Mate)

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ABSTRACT

Ilex paraguariensis St. Hil. (Yerba Mate) is an important crop for which a decrease in yields associated to unsustainable agricultural practices is well documented. The aim of this study is to investigate the diversity of bacteria and fungi inhabiting roots of Yerba Mate. This is an important pre-requisite for the use of microorganisms inhabiting roots to modulate plant nutrition and health as an ecologically friendly agricultural alternative for this crop. The diversity of the root-associated microbiome from eleven plantations with different agricultural practices was analyzed by high throughput sequencing of the 16S rRNA gene as a bacterial marker, whereas the fungal communities were targeted by amplifying the ITS region of the ribosomal RNA gene cluster. A comparison of the bacterial and fungal communities between plantation sites and cultivation practices was made to address the major factors contributing to the structure of the root microbiome of this crop. Operational taxonomic units (OTUs) related to well-known plant growth promoting bacteria such as *Burkholderia, Bradyrhizobium, Weissella, Enterobacter* and *Rhizobium* were detected. Those might constitute targets for future enrichment efforts of plant growth promoting clades. The analysis of the fungal community composition demonstrated that arbuscular mycorrhizae colonize Yerba Mate roots, and that the frequency of this group is favored in degraded soils. The detection of other groups harboring potential phytopathogens might help to broaden the understanding of the ailments affecting this crop. This study provides the first description of the root-associated microbiome of Yerba Mate and constitutes a stepping-stone towards harnessing the role of microbes in the sustainable cultivation of this crop.

1. Introduction

Degradation of agricultural soils is known to significantly impact crop productivity around the globe (Bindraban et al., 2012). Land degradation affects seriously the productivity of *Ilex paraguariensis* St. Hil. (Yerba Mate, Aquifoliaceae). Yerba Mate is a native tree from Northeastern Argentina, Paraguay, South Brazil and part of Uruguay (Grondona, 1954). The leaves of Yerba Mate are used to produce an energizing beverage widely consumed as an alternative to coffee. Aside South America, beverages derived from Yerba Mate are becoming increasingly popular in others regions, including the Middle East, Europe, and the United States, where it is appreciated as a natural energizing drink due to its high content of antioxidants and its nutritional benefits (Heck and De Mejia, 2007). Currently, soil degradation due to inadequate agricultural practices maintained over time is affecting a considerable fraction of the area of Yerba Mate production (INYM-INTA, 2008). This is the case in Misiones (INYM-INTA, 2008), the leading productive region of this crop in Argentina. In order to maintain and improve soil quality, more sustainable agricultural practices have been implemented for Yerba Mate including zero-tillage, use of green covers and of associative trees (Burtnik 2003; Day et al., 2011; Ilany et al., 2010).

In agricultural soils, the microbiome associated to plant roots play a key role in regulating plant growth through the production of phytohormones (Spaepen and Vanderleyden, 2011) or facilitating access to mineral nutrients (Rodrigues et al., 2008). In other cases, the soil microbiome provides plant protection by exerting antagonistic effects against phytopathogens (Compant et al., 2005). Currently, high-throughput DNA sequencing approaches

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are used to analyze the root-associated microbiome of different plant species in order to reveal the taxonomic and functional diversity of microbes involved in plant growth promotion (Hacquard et al., 2015). Such culture-independent methods can help to discover potentially beneficial microbial taxa that could be subsequently isolated. For instance, the isolation and selection of diazotrophic bacteria associated to sugarcane was based on the enriched taxa present in the roots of this crop (Paungfoo-Lonhienne et al., 2014).

The root-associated microbiome of Yerba Mate remains unexplored and its analysis might constitute a guide for the isolation and selection of particular microbial taxa as potential bio-inoculants contributing to plant nutrition and protection in low-input soils. In a previous study, it has been shown that bio-inoculation with plant growth promoting rhizobacteria (PGPR) isolated from Yerba Mate roots increased the biomass yields of seedlings in nursery (Bergottini et al., 2015). In addition, endomycorrhizal associations in this crop have been reported in native trees of the Atlantic forest (Andrade et al., 2000) and in seedlings cultivated in nurseries (Gaiad and Lopes, 1986). We hypothesized that the root-associated microbiome of Yerba Mate may present microbial taxa involved in functions of plant growth promotion and protection. According to this, the aim of this study was to analyze the root-associated microbiome of Yerba Mate in plantations with different agricultural management practices and located at different sites within the province of Misiones, the main productive area of this crop in South America. The bacterial and fungal communities of the root microbiome were compared between plantation sites and cultivation practices to address the major factors contributing to structure the root microbiome in this crop.

2. Material and methods

2.1. Roots collection and soil sample analysis

Eleven plantations located across the principal region of Yerba Mate production in Northeast Argentina were selected to analyze the root-associated microbiome of Yerba Mate. The selected plantations presented different historical agricultural management practices and contrasting productivity yields. A detailed description of each plantation is given in Table 1. In each plantation, seven plants were randomly selected to collect three subsamples of roots (up to a depth of 10 cm) per plant and pooled to constitute one sample per plantation. Bulk soil samples were collected to determine extractable phosphorus (Bray and Kurtz, 1945), nitrogen (by the semi-micro-Kjeldahl method) (Kjeldahl, 1883), potassium (K), sodium (Na), magnesium (Mg), and calcium (Ca) (measured in an ammonium acetate extract) concentrations. The soil chemical properties are described in Supplementary Table 1. The sampling was held in the winter season of July 2013. Mean annual temperature (MAT) values were 19.7 °C in Santo Pipó, 14.3 °C in Jardín América, and 19.7 °C in Andresito. Mean annual precipitation (MAP) values were 1720.5 mm in Santo Pipó, 1857.4 mm in Jardín América, and 2341.0 mm in Andresito.

2.2. DNA extraction, PCR and pyrosequencing

To access the root-associated microbiome, roots were washed with sterile distillated water (under a laminar flow cabinet) in order to remove the rhizospheric soil fraction not closely attached to Yerba Mate roots. One gram of washed roots per sample was processed for DNA extraction with the FastDNA Spin Kit for Soil (MP Biomedicals, California) according to the manufacturer's instructions. Amplicon generation and further 454-pyrosequencing analyses were performed by Eurofins Genomics GmbH (Switzerland). To target the bacterial communities the 16S rRNA gene was amplified with the primer pair 27F (Lane, 1991) and 1492R (Stackebrandt and Liesack, 1993) whereas the fungal communities were targeted by amplifying the ITS region with the primer pair ITS1-F/ITS4 (White et al., 1990; Gardes and Bruns 1993). The sequencing of the 16S rRNA gene and the ITS region was performed unidirectionally using the forward primers.

2.3. Sequence processing

Bacterial and fungal amplicon sequences were analyzed independently, using the mothur software version 1.34.4 (Schloss et al., 2009). Bacterial reads were processed largely following the Schloss standard operating procedure (Schloss et al., 2011). First, sequencing errors were reduced by implementation of the AmpliconNoise algorithm (minflows = 360 and maxflows = 720) and low-quality sequences were removed (minimum length of 360 bp, allowing 1 mismatch to the barcode, 2 mismatches to the primer, and homopolymers no longer than 8 bp). Barcode and primer sequences were removed. Subsequently sequences were

Table 1
Agricultural management of the plantations selected to investigate the diversity of the root-associated bacterial and fungal communities of Yerba Mate (*Ilex paraguariensis* St. Hil.).

Plantation location[b]	Latitude	Longitude	Productivity[a]	Agricultural practices			Age of plants
				P fertilization (kg/ha)	N fertilization (kg/ha)	Type of plantation	
AI1	25°49′58.80″S	53°55′56.32″W	Medium	15	72	Monoculture, tillage-zero, green covers	25
AI2	25°50′1.28″S	53°.55′56.00″W	High	25	125–150	Monoculture, tillage-zero, green covers	25
AI3	25°50′4.25″S	53°55′54.76″W	High	25	125–150	Monoculture, tillage-zero, green covers	25
AL	25°47′40.15″S	53°58′26.69″W	Low	0	0	Monoculture, conventional tillage, no fertilization, no green covers	25
AM	25°50′1.21″S	53°56′8.87″W	Medium	15	72	Rainforest converted into an agroforestry system with native tree, tillage-zero, green covers	25
AA	25°50′0.76″S	53°55′51.11″W	Medium	15	72	Co-cultivated with few tree species, native green covers	25
JA1	26°59′32.67″S	55°14′01.68″W	Medium	15	72	Monoculture, tillage-zero, green covers	25
JA2	26°59′35.43″S	55°14′2.15″W	High	25	125–150	Monoculture, tillage-zero, green covers	25
JA3	26°59′34.75″S	55°14′0.96″W	High	25	125–150	Monoculture, tillage-zero, green covers	25
SPB	27°8′18.07″S	55°25′27.11 ″W	Low	10	50	Monoculture, conventional tillage, fertilization, no green covers	30
SPA	27°6′10.96″S	55°18′55.26″W	Medium	10	75	Co-cultivated with few tree species, native green covers	30

[a] High: 16.000-18.000 kg/ha, Medium: 13.000 kg/ha, Low: 7.000 kg/ha.
[b] Plantation location: Andresito (A), Jardín América (JA), Santo Pipó (SP).

aligned to the SILVA reference database release 119 (Quast et al., 2013) and preclustered (pre.cluster, diffs = 1). Chimeras were removed using the chimera.uchime mothur command and singletons were excluded. Finally, sequences were classified using the naïve Bayesian classifier (Wang et al., 2007) implemented in mothur with the SILVA reference database release 119 (Quast et al., 2013). Sequences identified as chloroplasts or mitochondria were removed. Operational taxonomic units (OTUs) were generated using the average neighbor algorithm. An OTU was defined at the 97% sequence similarity level.

Regarding the fungal sequences, reads were quality processed with the same parameters as described above except that the minimum length was set to 370 bp. After removing chimera and singletons, the presence of fungal ITS was checked using ITSx version 1.0.11 (Bengtsson-Palme et al., 2013) and non-fungal ITS sequences were discarded. Subsequently ITS sequences were pairwise aligned to generate a distance matrix using the pairwise.seqs command. Finally, sequences were classified using the naïve Bayesian classifier (Wang et al., 2007) implemented in mothur with the UNITE v6_sh_dynamic database (Kõljalg et al., 2013). Operational taxonomic units (OTUs) were generated using the average neighbor algorithm. An OTU was defined at the 97% sequence similarity level. The amplicon sequences have been submitted to the GenBank database under accession number SRP069065.

2.4. Diversity and statistical analysis

Rarefaction curves (calculated from 10^4 iterations), diversity indices and relative abundances of OTUs were estimated using mothur (Schloss et al., 2009). All statistical analyses were computed using R software version 3.1.3 (R Development Core Team, 2015). Comparisons of both the alpha diversities and soil chemical properties according to geographical location or the plant productivity were performed using non-parametric Kruskal-Wallis tests. After normalization by random subsampling (1376 and 4785 sequences in each sample for the bacterial and fungal community, respectively, corresponding to the lowest number of sequences in a sample), OTU matrices were log (1 + x) transformed and Bray-Curtis dissimilarity matrices were computed for both bacterial and fungal communities using the *vegan* package (Oksanen et al., 2012). Global Non-Metric MultiDimensional Scaling (GNMDS) based on Bray-Curtis distances were computed using the same package. The effects of soil chemical properties and geographical location on both the bacterial and fungal community composition were estimated by a distance-based permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) using "adonis" function of the *vegan* package with Bray-Curtis distance matrix and 10^5 random permutations. To test the relationship between the bacterial and fungal community matrices, the Mantel test was performed using the *ecodist* package (Goslee and Urban, 2007), with Bray-Curtis dissimilarity matrices, Spearman's rank correlation coefficient and 10^6 random permutations.

The genetic diversity between the different locations was evaluated using Analysis of Molecular Variance (AMOVA) and Homogeneity of Molecular Variance (HOMOVA), both based on 10^6 iterations and computed with mothur. For the bacterial communities, the effect of the location on the phylogenetic diversity was investigated based on weighted UniFrac distances (Lozupone and Knight, 2005). Bacterial 16S rRNA gene sequences aligned in Mothur were used to compute a neighbor joining phylogenetic tree with Clearcut (Sheneman et al., 2006). Subsequently, this tree was used to compute a phylogenetic distance matrix based on both the unweighted and weighted UniFrac algorithms implemented in mothur (Lozupone and Knight, 2005).

The taxonomic composition of different core microbiomes was also investigated. Core microbiome was defined here as the set of OTUs present within all samples of a defined group. To evaluate the co-occurrence probability between the OTUs of the root-associated microbiome, the Veech probabilist model of species co-occurrence (Veech, 2013) was applied using the *cooccur* package (Griffith et al., 2016) in R.

3. Results

3.1. Soil properties of the plantations

Eleven plantations with different historical management practices and productivity yields located in two regions of the state of Misiones were selected to collect samples of Yerba Mate roots and soil for chemical analysis (Table 1). It is worth mentioning that the age of the plants was comparable among the different plantations, thus ruling out an effect of plant age on the comparison of the associated microbiomes. The soil chemical properties were analyzed in each plantation (Supplementary Table 1) in order to reveal the nutrient content of soils long-term managed under different agricultural practices. Soil properties were compared between the Northern and Southern regions, but no significant differences were observed between the edaphic variables such as pH, organic matter content, C, N and P of the two regions (Kruskal-Wallis rank sum test, $p > 0.05$).

3.2. Pyrosequencing data and OTUs assembling

A total of 106620 raw pyrotag reads were obtained from eleven samples for the bacterial diversity analysis. After quality control (chimera, singletons, and chloroplast/mitochondrial sequences removal), 29470 reads were retained with an average length of 354 bp. Sequences were clustered into 1566 bacterial operational taxonomic units (OTUs) at a sequence similarity of 97%. To compare the diversity among samples with different read counts, we normalized the data by selecting the lowest number of sequences in a sample ($n = 1376$) to rarefy the data set. A total of 15136 sequences were obtained corresponding to 1228 OTUs. For the fungal diversity analysis, a total of 247048 raw pyrotag reads were obtained from the same samples. After chimera, singletons and non-fungal sequences removal, 172583 reads remained with an average length of 398 bp. This corresponded to 1061 fungal OTUs at 97% similarity level. After normalization by random subsampling (4785 sequences by sample), 52420 sequences corresponding to 907 OTUs were obtained. For both data sets, the Good's coverage estimation (Good, 1953) (Table 2) and the rarefaction curves (Supplementary Fig. 1) showed that the sequencing depth was sufficient to estimate and compare the microbial diversity of the samples.

3.3. Alpha-diversity and taxonomic composition

We analyzed whether plantations with different geographical locations (Northern versus Southern plantations) or different productivity yields impacted the bacterial and fungal *alpha* diversity. No significant effects of the location or of the productivity yields were observed in the number of OTUs (after normalization), Chao1 richness estimator, Shannon diversity and Shannon evenness indices (Kruskal-Wallis rank sum test, $p > 0.05$) (Table 2). Most of the samples presented a similar *alpha* diversity for both bacterial and fungal communities, except the bacterial community of AM that presented a higher number of OTUs (Supplementary Fig. 1a) supported by a higher Chao1 estimation of richness (Table 2).

Table 2
Values of alpha diversity calculated for both the bacterial and fungal communities associated to the roots of Yerba Mate (*Ilex paraguariensis* St. Hil.).

Plantation	Good's Coverage	Bacterial α-diversity					Fungal α-diversity			
		Observed richness (♯ OTUs)	Chao1 Richness	Shannon Diversity	Shannon Evenness	Good's Coverage	Observed richness (♯ OTUs)	Chao1 Richness	Shannon Diversity	Shannon Evenness
AI1	0.92	276	361	4.45	0.79	0.99	116	157	2.33	0.49
AI2	0.95	224	260	4.42	0.82	0.99	108	143	2.83	0.61
AI3	0.96	203	225	4.30	0.81	0.99	165	197	3.28	0.64
AL	0.98	159	165	3.78	0.75	1.00	142	153	3.22	0.65
AA	0.95	140	214	2.12	0.43	0.99	216	229	3.75	0.70
AM	0.90	326	443	4.68	0.81	0.99	249	268	4.29	0.78
JA1	0.94	231	283	4.23	0.78	0.99	187	219	3.52	0.67
JA2	0.97	215	233	4.35	0.81	0.99	189	236	3.22	0.61
JA3	0.93	241	317	4.32	0.79	0.99	139	186	2.76	0.56
SPA	0.97	179	193	4.03	0.78	0.99	186	237	3.45	0.66
SPB	0.98	168	179	4.00	0.78	1.00	158	158	3.79	0.75

Regarding the taxonomic composition, at the phylum level, *Proteobacteria* (57%), *Acidobacteria* (13%), *Firmicutes* (8%), *Actinobacteria* (7%) and *Plantomycetes* (5%), composed the rarefied bacterial root-associated microbiome of Yerba Mate. Other phyla including *Chloroflexi*, *Bacteriodetes* and *Verrumicrobia* were represented at a lower relative abundance (2%), while the rest of the phyla represented 1% or less of the taxonomic community composition. In the AA plantation, *Firmicutes* represented 63% of the total relative abundance thus breaking the stable pattern of community composition at phylum level (Fig. 1a).

At the genus level, the root-associated bacterial microbiome was mainly dominated by unclassified bacterial sequences, representing approximately 21% of the sequences. Among the unclassified OTUs at the genus level, uncultured bacteria from the phylum *Acidobacteria* were the most abundant. For those OTUs that could be classified, we observed among the most abundant, the following genera: *Burkholderia* (17%), *Acidibacter*-like (8%), *Weissella* (6%), *Bradyrhizobium* (6%), *Phenylobacterium* (3%), *Enterobacter* (3%), *Gemmata* (2%), *Bryobacter* (2%), *Mycobacterium* (2%) and *Rhizobium* (2%). The bacterial OTU *Acidibacter*-like could not be accurately classified with the Silva database, therefore we used EzTaxon (Chun et al., 2007) to obtain a better identification. The results indicated 95% of similarity with *Acidibacter ferrireducens* strain MCF85, an obligate heterophic strain isolated from a metal mine, which is able to tolerate high concentrations of various metals (Falagan and Johnson, 2014).

Ascomycota (72%), followed by *Basidiomycota* (13%), *Zygomycota* (9%) and *Glomeromycota* (5%) dominated the root-associated fungal microbiome at the phylum-level. *Chytridiomycota* and unclassified genera occurred at less than 1%. When comparing among plantations, *Ascomycota* remained dominant in all samples, while *Glomeromycota* was differentially enriched in the microbiome of the sample from the AL plantation (Fig. 1b). A total of 83 OTUs assigned to the *Glomeromycota* phylum, and corresponding to arbuscular mycorrhizal fungi (AMF), were found among the eleven samples. Members of this phylum were present in each sample. AMF richness varied from 4 to 25 OTUs depending of the plantation, with a relative abundance ranging from 0.13 to up to 33.77% of the total fungal community (Fig. 1B). Approximately 81% of the *Glomeromycota* OTUs belonged to unclassified genera of the family *Acaulosporeacea*, including also the most abundant arbuscular mycorrhizal OTU (48% of the relative abundance of *Glomeromycota OTUs). Glomus* spp. (9%), a group formed by unclassified genera of *Glomeraceae* (6%), and *Rhizophagus* (2%) were also identified among these *Glomeromycota* OTUs. *Gigaspora*, *Entrophospora* and *Paraglomus* represented less than 1% of the relative abundance of AMF. Approximately 2% of the OTUs could be only identified at the order level (*Glomeromycetes*).

The most abundant fungal genus corresponded to a group of unclassified OTUs of the order *Hysteriales* (12%). In a decreasing order of abundance we identified a group of unclassified OTUs belonging to the *Myriangiales* (9%), *Ilyonectria* (6%), *Fusarium* (5%), *Atractiellales* (5%), *Mortierella* (5%), *Acaulospora* (4%), *Cladophialophora* (4%), *Schizangiella* (4%) and *Flagellospora* (4%). One common fungal OTU assigned to *Fusarium oxysporum* (93% identity) was identified in all samples representing approximately 5% of the total relative abundance. The consensus sequence of this OTU was classified with the Fusarium-ID database (Geiser et al., 2004) and assigned to *Fusarium concolor* (97.36% similarity).

3.4. Factors affecting the microbial community structure

The bacterial community structure was represented in a GNMDS plot using Bray-Curtis distances. This showed that the root-associated bacteria of some plantations grouped by proximity distances (Fig. 2a). In the Southern region, the contiguous plots from the site Jardin América (JA1, JA2, JA3) and those of more distant plantations in the site Santo Pipó (SPL, SPA) formed a defined group in the ordination plot (Fig. 2a). However this was not the case for the North-located plantations of the site Andresito (AA, AI1, AI2, AI3, AL, AM) notably dispersed in the GNMDS plot. In order to evaluate the hypothesis of a location effect on the bacterial community structure, PERMANOVA analysis was performed. These results confirmed that the bacterial community composition was significantly different between the two geographical locations (Northern versus Southern sites) ($R^2 = 0.142$, $p < 0.05$). Additionally, both genetic (HOMOVA, $B = 0.43$, $p < 0.01$; AMOVA, $Fs = 1.76$, $p < 0.05$) and phylogenetic diversity (Weighted UniFrac test, $W = 0.23$, $p < 0.001$; Unweighted UniFrac test, $W = 0.77$, $p < 0.001$) differed significantly between these two locations, suggesting a geographical differentiation of the root-associated bacterial community. Subsequently, the relationship between community dissimilarity (weighted UniFrac distances) and geographical distance was computed (Supplementary Fig. 2) and revealed a significant distance-decay relationship ($R^2 = 0.07$, $p < 0.05$). This confirmed that distant bacterial communities tend to be more dissimilar than geographically close communities.

Regarding Yerba Mate root fungal communities, unconstrained ordination of the Bray-Curtis distances indicated that contiguous plots from the site Jardin América (JA1, JA2, JA3) were grouped together while plantations from the other sites did not (Fig. 2b). The hypotheses of geographical location and local environmental conditions effects (pH, nutrients availability, mean annual temperature and mean annual precipitations) were evaluated by PERMANOVA analyses. These analyses revealed a significant difference in community composition between the two

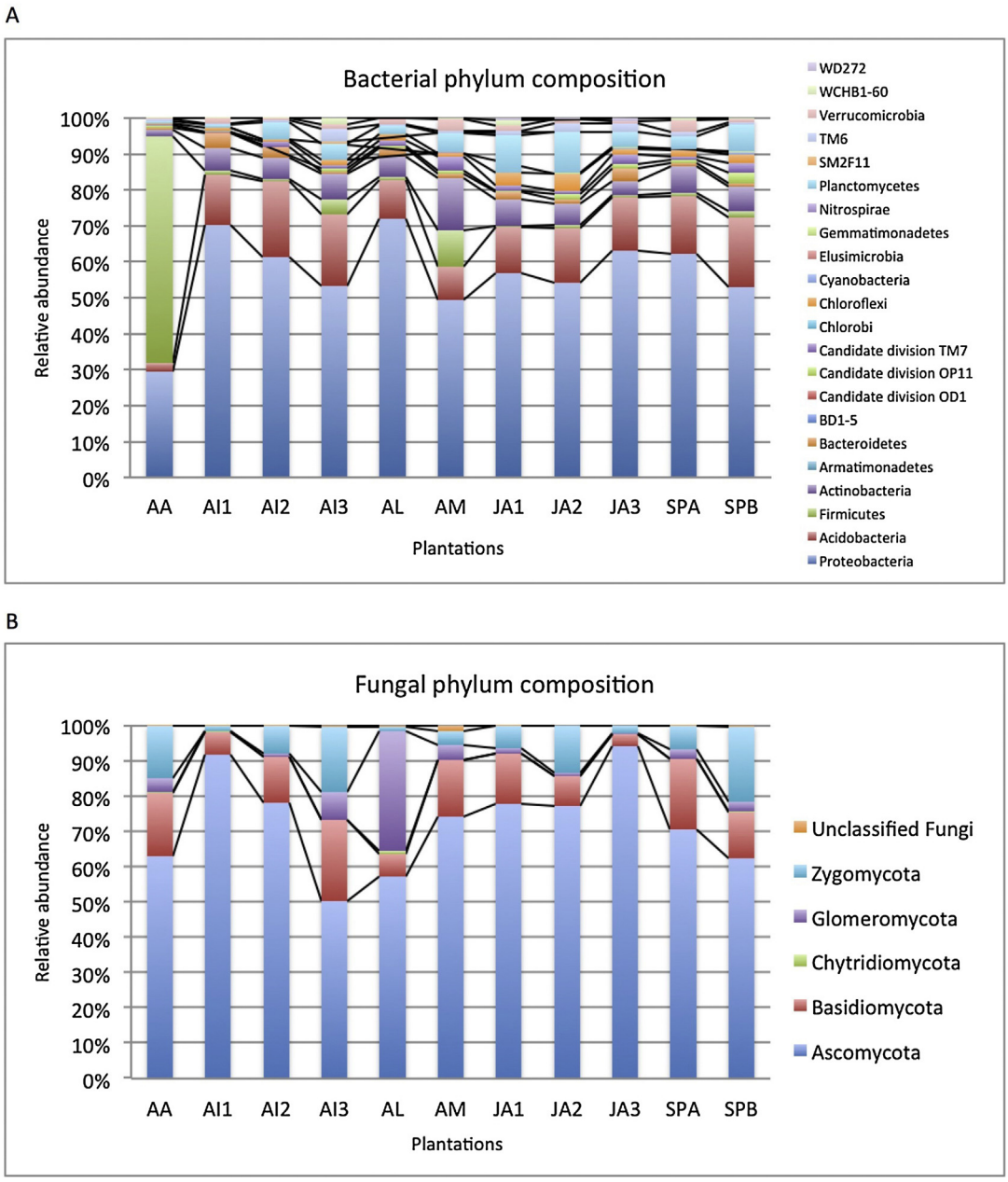

Fig. 1. Bacterial (a) and fungal (b) community composition at phylum level of the Yerba Mate (*Ilex paraguariensis* St. Hil.) root-associated microbiome from eleven plantations.

geographical locations (Northern versus Southern sites) (PERMANOVA, $R^2 = 0.152$, $p < 0.05$). Additionally, the genetic diversity was significantly different between the two plantation locations (Northern versus Southern sites) (AMOVA, Fs = 1.83, $p < 0.05$). Soil pH (PERMANOVA, $R^2 = 0.164$, $p < 0.005$) and mean annual temperature (PERMANOVA, $R^2 = 0.18$, $p < 0.01$) also significantly influenced the fungal community composition of Yerba Mate microbiome.

3.5. Interactions among the root-associated microbiome

Besides the effect of geographical location and edaphic variables, the analysis of the root-associated microbiome of *Ilex paraguariensis* St. Hil. revealed similarities between the different samples. Considering the bacterial and fungal OTUs present in all the samples, a core microbiome was defined. The root-associated core microbiome contained nine bacterial OTUs and five fungal OTUs, representing 29.50% and 13.22% of the relative abundance of the bacterial and fungal sequences, respectively. These OTUs were among the 22 and 19 most abundant bacterial and fungal OTUs, respectively.

Among the root-associated microbiome, pairwise patterns of OTU co-occurrence were investigated using a probabilistic model (Veech, 2014). Considering the 1128 bacterial OTUs found across the eleven sites, 1.2% of non-random co-occurrence were detected including 297 positive cases of co-occurrence. Regarding the 907 fungal OTUs, 1.6% of non-random co-occurrence was detected including 338 positive cases of co-occurrence. Additionally, co-occurrences were also detected between the bacterial and fungal OTUs (1.3% of non-random co-occurrence). Moreover, a significant relationship between the bacterial and fungal community dissimilarity matrices indicated that bacterial and fungal community compositions were correlated independently of the sampling site (Mantel test, $r_s = 0.43$, $p < 0.05$).

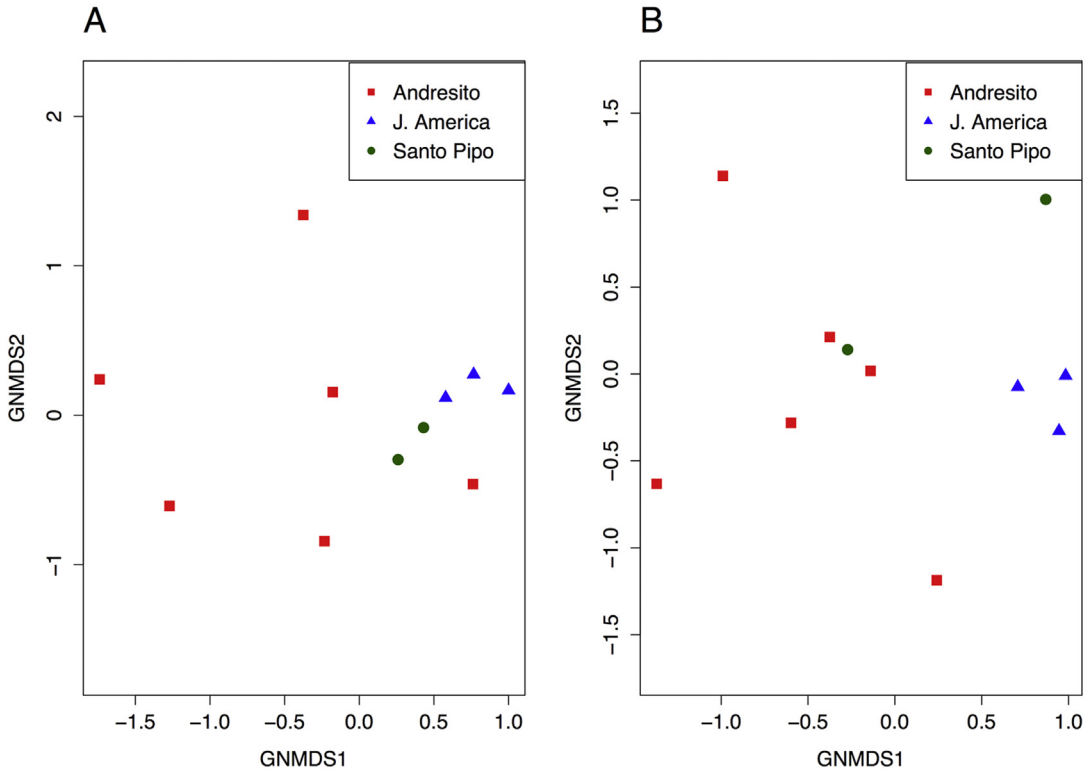

Fig. 2. Global Nonmetric multidimensional scaling (GNMDS) plots based on Bray-Curtis distances among (a) the bacterial communities (two dimensions, stress: 10.20%) and, (b) fungal communities (two dimensions, stress: 12.08%) associated to Yerba Mate roots in the state of Misiones, Argentina. Symbols represent the location sites of the plantations.

4. Discussion

In this study the composition of the root-associated microbiome of Yerba Mate was studied as a gateway for the selection and isolation of particular microbial taxa that can constitute potential bio-inoculants to ameliorate nutrition and protection of this crop in low-input soils. Regarding the taxonomic composition at the phylum level, the bacterial community was dominated by the phyla *Proteobacteria* followed by *Acidobacteria* in almost all samples regardless of the productivity yields and location sites. Our results are consistent with the characterization of the root-associated microbiome of some woody species such as poplar (Gottel et al., 2011) and oak (Uroz et al., 2010) in which *Proteobacteria and Acidobacteria* were also the dominant phyla. In the case of Yerba Mate, uncultured bacteria from the phylum *Acidobacteria* were the most abundant among the unclassified OTUs at the genus level. The functional and ecological role of members of this phylum within the rhizosphere remains unclear (Nunes da Rocha et al., 2009), but selection of particular groups of *Acidobacteria* in the rhizosphere has been suggested (Nunes da Rocha et al., 2013). Our results for Yerba Mate would support this selection and moreover suggest that this bacterial phylum is enriched not only in the rhizosphere but also within the roots. For example, the bacterial OTU *Acidibacter*-like, which had 95% of similarity with *Acidibacter ferrireducens* strain MCF85, occurred in all samples representing the most abundant OTU in the dataset. Although future studies should include bulk soil samples to confirm if there is a specific association of this *Acidibacter*-like OTU with Yerba Mate roots, the abundance of this OTU regardless of the origin of the samples or the agricultural practices of the plantation suggests a particular association with Yerba Mate roots.

Considering the different agricultural practices analyzed here, only a few concrete examples of the influence of those in the composition of the root-associated microbiome could be defined. For example, the only exception to the dominance of *Acidobacteria* and *Proteobacteria* in the root-associated bacterial microbiome of Yerba Mate was the dominance of *Firmicutes* (63% of the community) in plants from the AA plantation (Fig. 1a). We expect this shift in *Firmicutes* to be related to a potential problem of soil compaction in this plantation since a previous study has shown some members of this phylum to be favored in compacted soils due to their anaerobic respiration (Hartmann et al., 2014), and accordingly, the most abundant OTU was assigned to *Weissella,* a facultative anaerobe. The higher alpha diversity of the bacterial community in the plantation AM constitutes another example of the effect of agricultural practices on the structure of the root-associated microbiome of Yerba Mate. This plantation was established in a logged-rainforest field and has been managed from its creation as an agroforestry system with native trees, which might contribute to a higher bacterial richness in the AM plantation. This is supported by a previous study showing an increase in the microbial *alpha* diversity in forest-soils converted into agricultural fields (Rodrigues et al., 2013). Likewise, when comparing the composition of the fungal microbiome between plantations, *Ascomycota* remained dominant in all samples while *Glomeromycota* was differentially enriched in the AL plantation. SPB and AL plantations are two fields historically managed with conventional practices, which resulted nowadays in low-productivity yields. However, when comparing the soil P content between these two fields, a much lower P content was measured in the AL plantation. Therefore, our results suggest that this shift in *Glomeromycota* in AL plantation might be related to a higher

selective pressure by the plant for mycorrhizal associations as expected for soils with low P content (Mäder et al., 2000). In its natural habitat, Yerba Mate is a mycorrhizal colonized tree (Andrade et al., 2000) and the taxonomical identification of the mycorrhizal species could allow future biotechnological applications. Previously, Gaiad and Lopes (1986) reported that *Acaulospora* and *Glomus* were the dominant genera colonizing Yerba Mate seedlings in different nurseries in Brazil. Since that study, there are no further reports in the literature about the mycorrhizal fungi associated to this crop. Our study provides the first extensive taxonomic description of Yerba Mate associated mycorrhizae and the first molecular evidence of the presence of AMF associated to the roots of Yerba mate, in all the samples analyzed.

The presence of potentially beneficial microorganisms in the root-associated microbiome is highlighted by the enrichment of OTUs related to specific bacterial genera. The enrichment of many species of the genus *Burkholderia* has been reported for the rhizosphere of a wide range of plant species, including sugarcane (Paungfoo-Lonhienne et al., 2014), oak (Uroz et al., 2010) and pine (Timonen and Hurek, 2006). This genus is known to present a particular wide tolerance to acidic soils (Stopnisek et al., 2013) and many species have been reported as plant growth promoting bacteria (Suárez-Moreno et al., 2012). Among the other abundant genera that could play a role in plant growth and protection are the lactic acid bacteria *Weissella* (Fhoula et al., 2013), *Bradyrhizobium* and *Rhizobium* reported as potential PGPRs for non-legume plants (Antoun et al., 1998), and *Enterobacter* reported as phosphate-solubilizing rhizobacteria associated with Yerba Mate (Collavino et al., 2010). In contrast, the dominance of the fungal families *Hysteriaceae* (*Hysteriales*) and *Elsinoacea* (*Myriangiales*), which are known to harbor phytopathogenic strains (Alexopoulos et al., 1996; Jayawardena et al., 2014), suggests that some root-associated microorganisms could be considered as potential pathogens for this crop. One common fungal OTU assigned to *Fusarium concolor* (97.36% similarity) was identified in all samples representing approximately 5% of the total relative abundance. In agricultural soils, some *Fusarium* species (e.g. *Fusarium oxysporum*) are responsible of causing wilt diseases in many crops (Armstrong and Armstrong, 1981), however there are also non-pathogenic strains used nowadays as bio-control agents (Postma and Rattink, 1992). This is potentially the case of the OTU found in association with Yerba Mate, which was described as a saprobe and its co-inoculation with AMF has been shown to enhance shoot dry weight of *Eucalyptus globulus* in heavy-metal contaminated soils (Arriagada et al., 2007). Thus the identification of this fungus in the root-associated microbiome of Yerba Mate could contribute to a broader understanding of the role of the *Fusarium* species associated to this crop.

In our study neither the agricultural practices nor soil properties accounted as sources of variation to explain the structure of the bacterial communities, therefore other unmeasured biotic factors such as plant genotype (Aira et al., 2010), plant immune system and microbe-microbe interactions (Hacquard et al., 2015), might be important to shape the bacterial community composition of the root-associated microbiome of Yerba Mate. This has been suggested in other plant-microbe models. For example, the plant was shown to have a stronger effect on shaping the overall microbial community patterns in both the rhizosphere and the endosphere in poplar trees, compared to other factors such as soil properties (Gottel et al., 2011).

Overall, geographic distance impacted the bacterial community structure of the root-associated microbiome (significant distance-decay relationship). Although, local and regional scale effects explained the similarities on the bacterial microbiomes from the South-located plantations, this was not the case for the plantations located in the Northern region (Andresito). In the latter, microbiomes of contiguous plots were markedly different and thus it is likely that unmeasured factors contributed to shape the microbiome more significantly. Similarly, the analysis of the root microbiome of maize has shown that a geographical pattern (two distinct climatic regions) was insufficient to explain the differences among microbiomes (Peiffer et al., 2013). In contrast, another study has shown that field location and cultivation practices governed the root microbiome composition of rice (Edwards et al., 2015).

In contrast to the bacterial communities, abiotic factors (soil pH and mean annual temperature), as well as geographical patterns, shaped significantly the root-associated fungal microbiome of Yerba Mate. This was the case for the contiguous plots in the site Jardín América where local environmental conditions can explain the similarities among fungal communities. Previously, it has been shown that the biogeography of *Agave* species determines the fungal community of the root-associated microbiome (Coleman-Derr et al., 2016), whereas soil properties (Ca, Mn, and moisture content) and geographic distances explained the differences among the fungal rhizosphere communities of poplar trees (Shakya et al., 2013). In addition, soil pH has been reported to have an effect on the soil fungal community composition at a field scale (Rousk et al., 2010), which was consistent with a global scale survey (Tedersoo et al., 2014).

Although the proportion of non-random associations is low, those reflect a structure in the root-associated microbiome. The existence of a core microbiome suggests the selection by the plant of a subset of specific microbes, regardless of the plant location or soil characteristics. Additionally, it highlights the significant importance of this core microbiome in terms of relative abundance. Although we did not included in our analysis bulk soil samples to confirm this selective enrichment, a marked rhizosphere effect has been observed for other crops such as barley (*Hordeum vulgare*) (Bulgarelli et al., 2015) and rice (Edwards et al., 2015), highlighting the importance of the plant host for the root-associated microbiome. Moreover, previous studies have shown the presence of core microbiomes, represented mainly by limited dominating taxa in *Arabidopsis thaliana* (Bulgarelli et al., 2012, Lundberg et al., 2012) and in barley (Bulgarelli et al., 2015). Additionally, co-occurrence was also detected between bacterial and fungal OTUs (1.3% of non-random co-occurrence), underlying the importance of bacterial-fungal interactions in the rhizosphere (Frey-Klett et al., 2011). Moreover, a significant relationship between the bacterial and fungal community dissimilarity matrices indicated that bacterial and fungal community compositions were correlated independently of the sampling site, which is in agreement with a recent study on soil microbial ecology (Menezes et al., 2015).

In conclusion this study describes for the first time the bacterial and fungal communities associated to Yerba Mate roots in plantations with different historical management practices. Our analysis revealed that the roots of this crop harbor microbial taxa potentially involved in plant growth promotion and pathogenesis. Our results suggest that future efforts should be concentrated in the isolation and selection of endophytic or PGPRs strains from the genera *Burkholderia* and *Enterobacter*. The analysis of the fungal communities revealed a diversified community of AMF associated with this crop, with species of *Acaulospora* as particularly enriched in plantations studied here. Further analysis using specific primers to target the *Glomeromycota* phylum will provide higher resolution for the identification at the species-level. Many causal agents of Yerba Mate diseases are still unknown and the predominance of unclassified genera of the orders *Hysteriales* and *Marangiales* might point at those groups as potential targets to investigate further phytopathogens. The root-associated microbiome is gaining importance as a key element to understand plant health. This

first insight into the taxonomic diversity of the microbiome of Yerba Mate constitutes a stepping-stone towards building a more sustainable strategy for the cultivation of this important crop.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2016.09.013.

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