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ANALYSES OF THE RHIZOSPHERE MICROBIOTA IN THREE DIFFERENT CROP SYSTEMS (CONVENTIONAL, ORGANIC AND SYNTROPIC AGRICULTURE), USING A PORTUGUESE MAIZE POPULATION AND CCP ('PIGARRO' AND 'SINPRE').

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Abstract: Maize is one of the most important crops in the world for feed and food, which makes its contribution to organic farming crucial. The adaptation to organic agriculture can depend on the interaction between the microbiota present in the rhizosphere, allowing a more efficient extraction of nutrients from the soil for growth and development.

The aim of our study was to understand how different production systems (conventional, organic) and different open-pollinated maize populations ('SinPre' and 'Pigarro') can influence the rhizosphere microbiota.

The data collected from the maize trial comprehends phenological data plus the structural diversity of the bacterial and fungal communities from the maize rhizosphere.

Three replicates of three plants by two maize populations were collected for each cultivation system, at a depth of approximately 15 cm, forming a total of 15 composite samples. The bacterial microbiota was determined from DNA extracted from maize rhizosphere samples based on the V3-V4 region of the bacterial 16S rRNA and from ITS2 region of the fungal ITS gene using Illumina's MiSeq sequencing.

From our results, we can conclude that the farming system has an impact on fungal diversity since a higher diversity was found in organic farming systems when compared with the conventional. In addition, the fungal microbiota was more diverse in 'Pigarro' rhizosphere in comparison with 'SinPre'.

Comparing the diversity between 'Pigarro and 'SinPre' bacterial populations, the first presented always the highest number of genera despite the farming system. Contrarily to what we observed for the fungal diversity, the number of shared bacteria was similar in both farming systems.

The main conclusion was that the farming systems have significant impact in maize rhizosphere microbiota. In addition, the maize rhizosphere microbiota is population specific.

Introduction: Maize is one of the most important cereal crops in human and animal nutrition worldwide representing 325 million Mg in an area of 129,87 million hectares (FAOSTAT, 2017). Maize for grain is the second most important cereal, representing 14% of the approximately 4.5 million ha of organic global area of cereals (Lernoud & Willer, 2019). This situation indicates opportunities for improvement on both agronomic practices and breeding related with microorganism interactions (e.g. control of root diseases) and organic management, for which rhizosphere microbial communities are critically important for soil nitrogen cycling and plant productivity (Schmidt et al., 2016; Emmett et al., 2017; Wille 2018). Indeed, rhizosphere is a critical interface that supports the exchange of resources between plants and their related soil environment. Rhizosphere microbial diversity is influenced by the physical and chemical properties of the rhizosphere, some of which are genetically determined by the host plant as well as the soil type (Peiffer et al., 2013). In addition, life history traits associated with plant resource acquisition (e.g., longer lifespan, high nitrogen use efficiency, and larger seed size) showed correlations with variation in bacterial community composition and enzyme activity. These results indicate that plant evolutionary history and life history strategy influence rhizosphere bacterial community composition and activity (Emmett et al., 2017; Walters et al. 2018). This work aims to understand how different production systems (conventional, organic and syntropic) and different populations ('SinPre' and 'Pigarro') can influence the microbiota of the rhizosphere.

Material and methods: The open pollinated maize population 'Pigarro' (Mendes-Moreira et al, 2017) and the CCP 'SinPre' (Composite cross population CCP derived from 10 Portuguese and two American populations, using the same methodology described in Mendes-Moreira et al., 2009) were used as germplasm. These two populations were tested in conventional and organic agriculture, plus the agroforestry system-syntropic (AF), that is included in the organic area, for 'SinPre'. The test locations belong to IPC-ESAC and the distance between organic and conventional is 2.4 km. The organic matter content and pH were respectively for agroforestry (4%; 7.1), organic (1.8%; 6.4) and conventional farming (0.8%; 6.7). The stand was 60000 plants/ha for 'SinPre' in organic and agroforestry; and 75000 plants/ha for the other cases.

The phenotyping samples used two to three plots of 9.6 m2 randomly distributed per production system. Data included HUNTERS descriptors (H - Plant Height; H1E - 1st ears' height, U - uniformity, N – Leaf angle; T-Tassel; R-Root and S-Stalk Lodging) and yield (Mendes-Moreira et al, 2017). IBM SPSS® statistics program was used for data analyses.

The maize microbiota study consisted in the harvest of the maize rhizosphere at flowering stage at a depth of approximately 15cm. For each population and location, 3 samples composed of 3 random plants were selected. The root samples were stored in cool temperature and moved rapidly to the laboratory (FITOLAB) where they were washed and cut into smaller portions (Peiffer et al., 2013). The samples of the maize rhizosphere were sent to Genoinseq (Cantanhede, Portugal) facilities for analysis of the microbiota. The bacterial and fungal microbiome community were determined from DNA extracted from the maize rhizosphere. Bacterial diversity was determined from the V3-V4 region of the bacterial 16S rRNA gene and fungal communities were performed from ITS2 region of the fungal ITS gene using Illumina's MiSeq sequencing. Raw reads were extracted from Illumina MiSeq® System in fastq format and quality-filtered with PRINSEQ version 0.20.4 to remove sequencing adapters, reads with less than 100 bases for the ITS2 region and 150 bases for the 16S rRNA gene, and trim bases with an average quality lower than Q25 in a window of 5 bases. The forward and reverse reads were merged by overlapping paired-end reads with AdapterRemoval version 2.1.5 using default parameters. After sequencing the bacterial and fungal communities were analyzed using the QIIME software package. Chimeric sequences were removed using the consensus method and clustered in operational taxonomic units

(OTUs) at 99% using a closed-reference. Taxonomy was assigned to bacterial and fungal OTU sequences using SILVA database (release 132) and UNITE v.7.2 respectively.

Results: The phenotypic characterization indicates that 'SinPre' for plant height and first ear height was higher for conventional (236.4 cm; 174.3 cm respectively) than for organic (206.2 cm; 114.6 cm respectively). The same tendency was observed for 'Pigarro', however, the differences between conventional and organic were smaller (262.3 cm versus 250.4 cm for plant height and 159.85 cm versus 152.6 cm for ear height). 'Pigarro' showed a higher root lodging under conventional than under organic farming (4.06% versus 0.5%) as well as for stalk lodging (15.95% versus 10.53%). 'SinPre' was not affected by root lodging in both systems, but showed 17.06% stalk lodging for conventional versus 3% for organic.
The first year of agroforestry CCP 'SinPre' revealed a very low germination rate that was probably due to lower soil temperature and presence of manure on the installation of the agroforestry system. Therefore, results of these samples have to be taken with caution.
Microbial analysis of the maize rhizosphere revealed a larger number of fungal OTUs in organic than in conventional: 47% were in common, while 40% OTUs were only in organic and 13% OTUs only in conventional. 'Pigarro' showed larger number of genera compared to 'SinPre', in both farming systems, with 75% and 50% exclusive OTUs in organic and conventional farming, respectively. It was also observed that in the organic farming system, there was a notable higher presence of mycorrhizae (Glomeromycota), especially for 'SinPre', in contrast to the conventional farming system.
The bacterial composition showed similar pattern with higher diversity under organic farming, however, a much higher share (77%) of common bacterial OTUs. Comparing the diversity between 'Pigarro and 'SinPre' populations, the first presented always the highest number of genera in both farming systems. Contrarily to what we observed for the fungal diversity, the number of shared bacteria was similar in the different farming systems. Based on the indices of Shannon (H), Equitability (J), and Simpson (D) the fungal and bacterial diversity were more uniformly distributed in organic compared to conventional farming systems.

Discussion: The results indicate that the farming system has a significant impact on fungal diversity, and higher diversity was always found in organic farming systems versus conventional. In addition, the fungal microbiota indicates a higher diversity in 'Pigarro' rhizosphere versus 'SinPre'. In parallel, there was a significant different plant and ear height measurements between farming systems.
The bacterial composition of both 'Pigarro' and 'SinPre' rhizosphere was mostly shared among the tested production systems. Comparing the diversity between 'Pigarro' and 'SinPre' populations, the first presented always the highest number of genera despite the farming system. While fungal diversity showed a clear distinction between 'Pigarro' and 'SinPre' and on average of 53% distinct OTUs between farming systems, the percentage of bacterial OTUs that occurred only in one population or one farming system was much lower.
The main conclusion was that the farming systems have significant impact in maize rhizosphere microbiota. In addition, the maize rhizosphere microbiota is population specific.

Disclosure of Interest: None Declared

Keywords: Conventional farming, Organic Farming, Maize, Microbiota; Fungi; Bacteria

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