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A first-year melon/cowpea intercropping system improves soil nutrients and changes the soil microbial community

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ABSTRACT

The melon/cowpea intercropping system can be a specific and efficient cropping pattern in a horticultural field. Intercropping systems contribute to the optimization of land use, fostering sustainable and efficient agriculture. This study entails a first-year comparative intercropping assay using cowpea (*Vigna unguiculata*) and melon (*Cucumis melo*) under organic management with different patterns and 30% less organic fertilization than usual in monocrops. We determined the soil nutrients, physicochemical properties, enzyme activities and microbes by high-throughput sequencing. We found that the intercropping system changed the bacterial community structure independently of the intercropping pattern. The bacterial community was characterized by a higher abundance of the phyla Proteobacteria and Bacteroidetes phyla and of the genus *Pseudomonas*, which are related to nutrient cycling, and by greater amounts of other beneficial microorganisms like *Bacillus, Streptomyces* and *Sphingomonas*. The intercropped systems significantly boosted the total nitrogen, available phosphorus and total organic carbon levels in addition to the melon yield. They also enhanced the acid phosphatase and β-glucosidase activity compared to the melon monocrop. Results from this study suggest that melon/cowpea intercropping, starting from the first year, not only provides a stable supply of food and income due to the diversified cropping systems, but is also beneficial for the soil microbial community and environment.

1. Introduction

Intercropping is a practice involving the simultaneous growing of two or more crops on the same land during the same growing season (Zhou et al., 2011). This practice is becoming increasingly important for maintaining and increasing soil quality and subsequently crop productivity (Singh et al., 2016). Intercropping has demonstrated advantages, including efficient nutrient acquisition; reduced pest, disease and weed damage; improved microbial diversity; and improved utilization of land resources (Mousavi and Eskandari, 2011). Different types of intercropping and combined systems have been proposed, but not all intercropping systems constitute improvements, since there must be a balance among the crops used (Gebru, 2015). It is particularly important to not use crops that compete for physical space, nutrients, water, or sunlight, and the environmental conditions in a given area and the crops or varieties available must also be taken into account (Lithourgidis et al., 2011). Maize is one of the predominant intercrops used, often combined with legume crops (Manasa et al., 2018). This combination makes it possible to develop an energy-efficient and sustainable system, as the legumes have an N-fixing capability and more protein-yielding potential in the form of either grain or forage (Maitra et al., 2019). In arid environments, the legume crop cowpea (*Vigna unguiculata L. Walp*) is normally used because of its adaptability and low fertility requirements, and it can improve legume nitrogen uptake by nodulation (Li et al.,

Abbreviations: M, Melon monocrop; MC1, Mixed intercropping, with melon mixed with cowpea in the same row; MC2, Row intercropping at a ratio of 1:1 (melon: cowpea), alternating one melon row and one cowpea row; MC3, Row intercropping at a ratio of 2:1 (melon:cowpea), alternating two melon rows and one cowpea row; C, Cowpea monocrop.

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2007). Therefore, it can be intercropped not only with maize, but also millet, sorghum, and some other crops (Chimonyo et al., 2016; Nelson et al., 2018).

Melon (*Cucumis melo* L.) is the main export crop in the region of Murcia (57%). Intensive melon cultivation can generate soil and water degradation due to the excessive use of pesticides to reduce the impact of pathogens and the necessary application of synthetic fertilizers due to nutrient depletion (Li, 2001). Intercropping melon and cowpea could contribute significantly to overcoming the challenges of developing both productive and environmentally friendly agricultural systems for melon cultivation. In addition, previous studies have reported that the planting pattern could also affect the soil and yield (Raza et al., 2019; Xianhai et al., 2012), so it is necessary to study intercropping as well as plant distribution.

The interactions among microbes, nutrients and enzymes in intercropping systems lead to an increase or decrease in microbe quantity and enzyme activity, contributing to the improvement of the soil microecological environment (Zhou et al., 2019). Soil microorganisms are key drivers of many soil biological, chemical, and physical processes, such as soil structure formation, the nutrient cycle, organic matter turnover, toxin accumulation or removal, and soil-borne pathogen suppression (Bever et al., 2012; Blagodatskaya and Kuzyakov, 2013). Several studies have investigated the changes in the microbial characteristics of soils caused by intercropping (Jin et al., 2020; Li and Wu, 2018). However, changes in the soil microbial community resulting from melon-cowpea intercropping have not been studied in depth. We hypothesized that intercropping would improve crop yield, increase soil bacterial diversity and enzyme activities and change the soil community structure. In this paper, our objective was to investigate physico-chemical properties, nutrient content, enzyme activities and the bacterial community resulting from three different types of melon-cowpea intercropping systems in their first year. We also wished to determine the relationship between these changes and soil chemical properties and crop yield compared to monoculture systems.

2. Materials and methods

2.1. Experimental design and sampling

An intercropping experiment with melon and cowpea was performed under organic conditions in La Palma (Cartagena) (37º 41 18 N 0º 56 60 W), a province of Murcia (S.E. Spain), in May–August 2018. The field trial was conducted in a soil that had been uncultivated for at least the last five years prior to the study; the soil was classified as Haplic Calcisol (Loamic, hypercalcic) (WRB, I.U. of S.S.W.G, 2015). The climate in the area of study is semiarid Mediterranean, with a mean annual temperature of 18 ºC, a mean annual precipitation of 275 mm and an annual potential evapotranspiration of 900 mm.

The assayed treatments were as follows: (*i*) melon (*Cucumis melo)* monocrop (M); (*ii*) cowpea (*Vigna unguiculata)* monocrop (C); (*iii*) mixed intercropping, with melon mixed with cowpea in the same row (MC1); (*iv*) row intercropping at a ratio of 1:1 (melon:cowpea), alternating one melon row and one cowpea row (MC2); and *(v*) row intercropping at a ratio of 2:1 (melon:cowpea), alternating two melon rows and one cowpea row (MC3). The field experiment was a completely randomized design with three plots per treatment, and each plot had a surface area of 120 m^2. Melon seedlings were planted at a density of 0.4 plants per m^{-2}, with a spacing of 200 cm between rows and 120 cm between plants in both the monocropped and intercropped systems. The density of cowpea plants was 2.5 plants per m^{-2} and 1.5 plants per m^{-2} in the 1:1 row (MC2) and 2:1 row (MC3) systems, respectively. In the intercropped row systems, the cowpea rows were spaced 100 cm from the melon rows, and there were 20 cm between cowpea plants in the same row. In the mixed system (MC1), the cowpea density was 0.4 plants per m^{-2} with one cowpea plant between melon plants in each row and spacing of 200 cm between rows and 120 cm between plants. The melon density was thus the same in the different treatments, but the cowpea density changed (Fig. 1).

All crops were drip irrigated and grown under organic management. The melon plot (M) received the equivalent of 3000 kg ha^{-1} of organic fertilizer (N org) (3.2% N and 7% K_2O), and the cowpea plot (C) received the equivalent of 1875 kg ha^{-1} of Norg. The intercropped plots (MC1, MC2 and MC3) received 30% less Norg than the melon monocrop to assess the efficiency of the intercropping in reducing external fertilization needs. The melons and cowpeas were simultaneously harvested twice, on July 31, 2018 and August 6, 2018. The harvest was carried out manually, as is the tradition in the area, to avoid damaging the melon fruits.

Five random soil subsamples (0–10 cm depth) were collected with an auger from the plots on August 10, 2018, just after harvest. Soil samples in MC2 and MC3 were only collected from the melon rows. The samples were taken between two adjacent plants in all cases. The soil samples were separated into two aliquots, one of which was kept at ambient temperature for chemical analyses and the other stored in a cool box with ice for biological analysis. All samples were taken to the lab immediately. The soil was air-dried for one week for chemical analyses and sieved at < 2 mm. Soil for biological analysis was sieved at < 2 mm once in the lab and stored at -20 ºC.

2.2. Soil properties and enzyme activities

The soil pH and electrical conductivity (EC) were measured in deionized water (1:5 w/v). The total organic carbon (TOC) and total nitrogen (TN) were determined using an elemental CHNS-O analyzer (EA-1108, Carlo Erba). Soil NH_4^+ was extracted with 2 M KCl in a 1:10 soil:extractant ratio and measured by colorimetric assay following Kandeler and Gerber (1988) and Keeney and Nelson (1983). Available P (P) was measured using the Olsen method (Olsen, 1954). Available nutrients were measured using ICP-MS (Agilent 7500CE).

Phosphatase and β-glucosidase activities were measured using the a fluorogenic approach according to Marx et al. (2001), and dehydrogenase activity was measured via a colorimetric procedure according to Von Mersi and Schinner (1991).

2.3. Soil DNA extraction, PCR amplification and sequencing

Soil DNA was extracted from 1 g of soil (wet weight) using the DNeasy Power Soil Kit (Qiagen). The quantity and quality of the DNA extracts were quantified using a Qubit 3.0 Fluorometer (Invitrogen, Thermo Fisher Scientific, USA) and a NanoDrop 2000 fluorospectrometer (Thermo Fisher Scientific, Waltham, MA, USA).

The bacterial community was determined via the next-generation sequencing of bacterial 16 S hypervariable regions using an Ion Torrent™ Personal Genome Machine™ (PGM) System. Bacterial 16 S regions were amplified using an Ion 16 S™ Metagenomics Kit (Thermo Fisher Scientific) with two different degenerate primer sets to amplify regions V2–8 and V3–6, V7–9. The amplified 16 S amplicons were then processed using an Ion Xpress™ Plus Fragment Library Kit in combination with an Ion Xpress™ Barcode Adapter 1–96 Kit (Thermo Fisher Scientific). All purification processes between incubation and amplification reactions of the library preparation were processed using DynaMag™– 2 magnetic racks (Thermo Fisher Scientific) and an AMPure XP Purification Kit (Beckman Coulter). After library preparation and barcoding, we determined the size and concentration of the final libraries using an Agilent 2100 Bioanalyzer system and the Agilent High Sensitivity DNA kit. The sequencing templates were prepared using an Ion One Touch 2 System and an Ion PGM™ Hi-Q™ View OT2 Kit (Thermo Fisher Scientific). The sequencing reaction was performed using Ion Torrent PGM with an Ion PGMTM Hi-QTM View Sequencing Kit (Thermo Fisher Scientific).

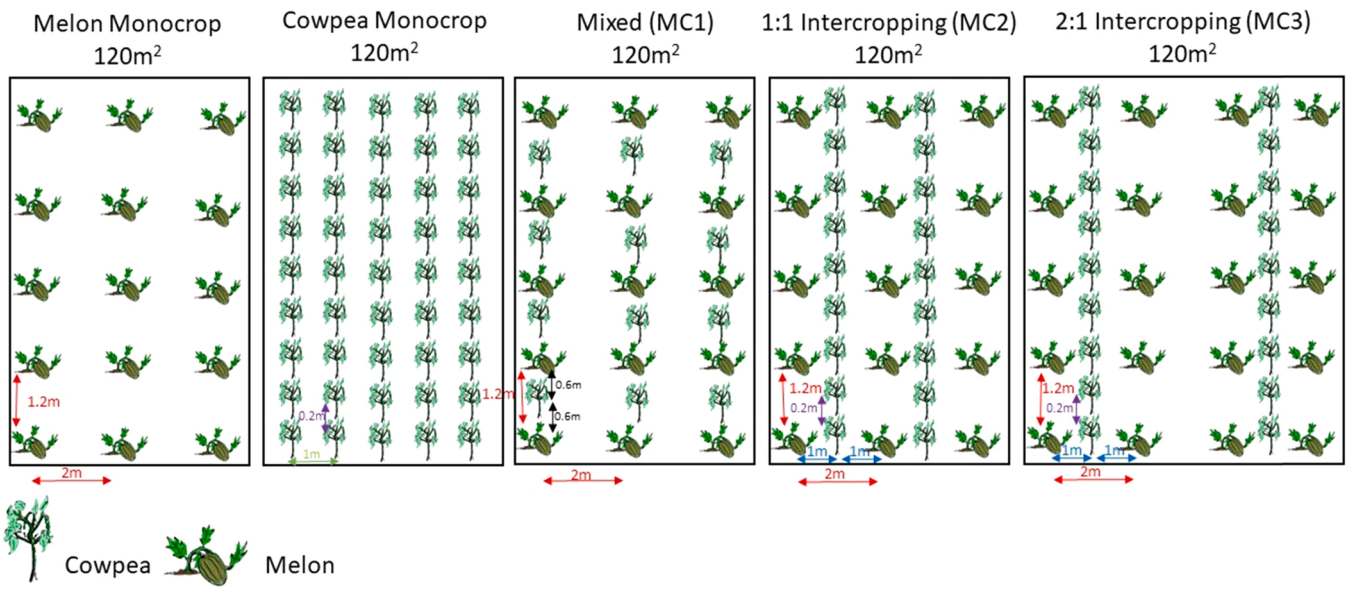

Fig. 1. Planting framework of melon and cowpea intercropping. Distance among rows of melon was 2 m while among melons in line was 1.2 m. In the intercropping (MC2 and MC3), the cowpea plants were arranged between the rows of melon with a separation of one meter between rows. In the MC1, cowpea plants were arranged in the same row, with a separation of 0.6 m among melon plants.

2.4. Sequencing data processing

Bacterial raw sequences, barcodes and primers were trimmed according to the BaseCaller application. The sequences were denoised with ACACIA (Bragg et al., 2012), and low quality sequences were discarded using the Quantitative Insights into Microbial Ecology (QIIME) pipeline (Caporaso et al., 2010) from the Microbiome Helper Virtual Box (Comeau et al., 2017). Briefly, bacterial sequences with a Q < 25 were removed, and the retained sequences were then assigned to Operational Taxonomic Units (OTUs) based on 97% similarity with the SILVA reference database after filtering chimeras using VSEARCH (Rognes et al., 2016) with the ribosomal database project (RDP database). Low-confident OTUs were removed.

The sequences were uploaded to the European Nucleotide Archive (ENA) with the study accession code PRJEB42624.

2.5. Statistical analysis

All tests were performed using R language (Team, 2020). Normality and homogeneity of variance assumptions were assayed by the Shapiro-Wilk and Levene's tests using the car (Fox et al., 2007) package. Mean comparisons were performed with one-way analysis of variance (ANOVA) followed by post-hoc tests, Tukey's honestly significant difference (HSD) for all-pair comparisons and Dunnett's comparisons for the control system. In the cases in which homoscedasticity was not met, Welch's test was performed using the 'pairwise.t.test' function with Bonferroni-Holm corrections for multiple comparisons. The robustness of the estimations was checked by the bootstrapping approach using 100 replicates. When data did not fit a normal distribution, non-parametric Kruskal-Wallis tests were performed, and if the assayed data were significant, a multiple comparison Z-values test was performed using the 'dunnTest' function with Benjamini-Hochberg corrections in the FSA package (Ogle and Ogle, 2017).

Bacterial alpha diversity [Chao1 as richness and Shannon (H') as diversity index] was estimated on rarefied microbial data using the vegan package (Oksanen et al., 2007).

A linear discriminant analysis (LDA) effect size (LEfSe) pipeline (Segata et al., 2011), available at http://huttenhower.sph.harvard.edu/galaxy/, was used with the default parameters at all taxonomic levels to identify genera that were differentially abundant among the cultivation systems. Three different steps were performed using the following algorithm: (i) a nonparametric Kruskal-Wallis test to detect the statistical differences between abundances; (ii) a pairwise test among subclasses using the Wilcoxon rank-sum test to evaluate biological consistency; and (iii) an LDA to estimate the effect size between abundances.

Principal coordinates analysis (PCoA) was used to visualize the variation in community composition by cultivation system based on the Bray-Curtis distance. To evaluate differences between the cropping systems, a Permutational Multivariate Analysis of Variance (PERMANOVA) was conducted using the 'betadisper' and 'adonis' functions with 999 permutations from the vegan package, followed by the 'pairwise.adonis' function with Benjamini-Hochberg corrections for multiple comparisons between specific cultivation systems from the pairwiseAdonis package (Arbizu, 2017) when the homogeneity of variance assumption was met. In the cases in which homoscedasticity was not fulfilled, an Analysis of Similarities (ANOSIM) was carried out instead. Relationships between the bacterial community and the rest of the parameters were determined using the 'bioenv' function from the vegan package to find the best subset of parameters (using Euclidean distance) that had a maximum correlation with the community dissimilarity matrix (Clarke and Ainsworth, 1993). Redundancy analysis (RDA) was performed through the vegan package to visualize the correlation between OTUs and physico-chemical, biological and harvest parameters. The OTU abundance was Hellinger transformed prior to analysis with the retained variables from the bioenv procedure (Legendre and Gallagher, 2001), which was performed via the 'bioenv' function based on Spearman's rank correlation coefficient. To equalize the number of replicates for 'bioenv' and 'rda', the function 'sample_n' in the dplyr package (Wickham et al., 2019) was used.

3. Results

3.1. Effects of intercropping on crop yield

The intercropped melon systems showed a higher melon yield (34%−74%) than the melon monocrop (M), and the yield was significantly higher in MC1 and MC3. We also observed a greater number of melons in the intercrops (MC3 52%, MC1 40% and MC2 33%) than in the monocrop (M) (Table 1). The cowpea yield, on the other hand, was

Table 1
Soil properties and crop yield in the intercropping systems.

Physico-chemical and chemical soil properties							
	C	M	MC1	MC2	MC3	Anova	Kruskal-Wallis
pH	8.4 ± 0.0	8.5 ± 0.0	8.4 ± 0.0	8.3 ± 0.0	8.4 ± 0.0	ns	–
EC (µS cm^{-1})	307 ± 6	290 ± 2	332 ± 30	298 ± 17	299 ± 37	–	ns
TOC (g kg^{-1})	11.8 ± 0.3 a	9.5 ± 0.1 b	11.2 ± 0.4 ab	11.1 ± 0.2 ab	11.9 ± 0.2 a	–	*
TN (mg kg^{-1})	1.3 ± 0.0 a	1.1 ± 0.0 b	1.3 ± 0.0 a	1.3 ± 0.0 a	1.3 ± 0.0 a	–	*
NH_4^+ (mg kg^{-1})	0.53 ± 0.18 b	0.88 ± 0.00 ab	1.83 ± 0.10 ab	3.36 ± 0.63 ab	4.48 ± 0.72 a	–	* *
Ca (mg kg^{-1})	1579 ± 236 a	1540 ± 39 a	1432 ± 297 a	908 ± 77 b* *	951 ± 22 b* *	* *	–
Mg (mg kg^{-1})	360 ± 75 ab	325 ± 62 ab	426 ± 93 a	244 ± 35 b	242 ± 4 b	*	–
K (mg kg^{-1})	325 ± 83	344 ± 70	430 ± 105	263 ± 9	279 ± 36	ns	–
Na (mg kg^{-1})	254 ± 2 ab	268 ± 43 a	271 ± 13 a	159 ± 21 ab	133 ± 14 b	–	*
P (mg kg^{-1})	18 ± 5 b	23 ± 1 b	62 ± 2 a* **	58 ± 3 a* **	49 ± 9 a* **	* **	–
Crop yield							
Melon Yield (kg ha^{-1})	–	15,093 ± 298 b	26,272 ± 3329 a* *	20,287 ± 3038 b	24,759 ± 2050 a* *	* *	–
Number of melons (num ha^{-1})	–	5548 ± 46 b	7752 ± 140 ab	7395 ± 39 ab	8455 ± 547 a	–	*
Cowpea Yield (kg ha^{-1})	2053 ± 59 a	–	106 ± 39 b	871 ± 82 c	463 ± 60 d	* **	–

(mean±sd; n = 5). In each cultivation system (*, **, ***) represent significant differences with respect to the melon monocrop system (control treatment) by Dunnett's test (*P < 0.05; **P < 0.01, **P < 0.001, respectively); missing asterisks denote non-significant differences. Different letters represent significant differences between systems by Tukey's test or Dunn's Kruskal-Wallis Multiple Comparison test; EC, Electrical conductivity; TOC, Total organic carbon; TN, Total nitrogen; NH4 + total ammonium Ca, Mg, K, Na and P; available Ca, Mg, K, Na and P; C, Cowpea monocrop; M, Melon monocrop; MC1, mixed intercropping; MC2, row intercropping 1:1; MC3, row intercropping 2:1.

higher in the monocrop system than in the intercropping systems (Table 1).

3.2. Effects of intercropping on bacterial community diversity and community structure

After filtering, 821,795 reads were yielded and 6676 OTUs were identified with 97% similarity for the bacterial community. No significant differences were found in the Shannon or Chao1 diversity indexes between cropping systems (Figure S1).

Bacterial community structures were distinctly grouped by cropping system on a PCoA plot (Fig. 5). Moreover, the bacterial community structure in the monocrop systems (M and C) differed significantly (F = 2.7262; P = 0.001) from that in the intercropping systems (MC1, MC2 and MC3). This difference was confirmed by pairwise comparison (Table S1).

3.3. Effects of intercropping on soil bacterial composition

Sequence analyses at the phylum and genus taxonomic levels are shown in Fig. 2A and B. Proteobacteria was the most abundant phylum (40%), followed by Actinobacteria (31%). It is noticeable that

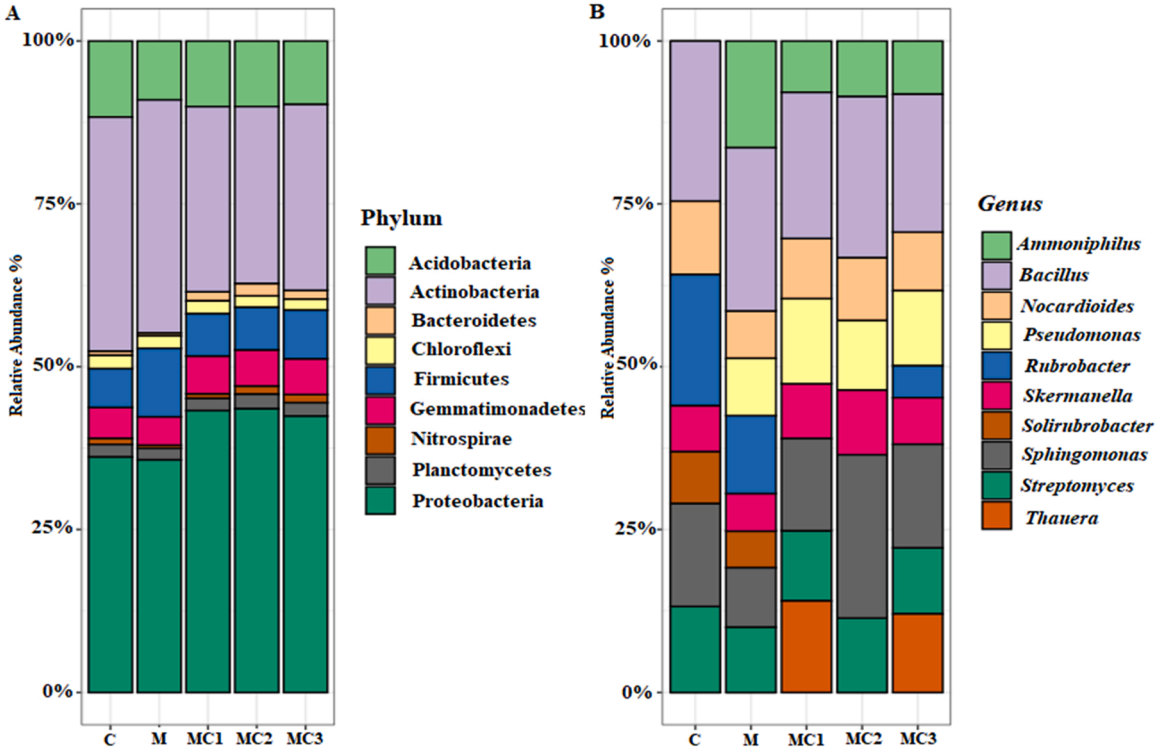

Fig. 2. Relative abundance (>1%) at (A) phylum and (B) genus level of soil bacterial community of intercropping systems. Barplot represents the average of samples for each taxon in each cropping system (n = 5). C, cowpea monocrop; M, melon monocrop; MC1, mixed intercropping; MC2, row intercropping 1:1; MC3, row intercropping 2:1.

Proteobacteria and Bacteroidetes were significantly more abundant and Actinobacteria significantly less abundant in the intercropped soil systems (MC1, MC2 and MC3) than in the monocrop soils (M and C) (Table S2). The other dominant phyla were Acidobacteria (10%), Firmicutes (7%), Gemmatimonadetes (5%), Planctomycetes (2%), Chloroflexi (2%), Bacteroidetes (1%) and Nitrospirae (1%), none of which showed significantly different abundances between the monoculture and intercropping systems (Table S2; Fig. 2A).

The most abundant genera in the different cropping systems were *Bacillus* (23.6%), *Sphingomonas* (17.8%), *Streptomyces* (12.0%), *Nocardioides* (10.1%), *Pseudomonas* (9.0%), *Ammoniphilus* (6.2%), *Rubrobacter* (6.0%), *Skermanella* (5.4%), *Thauera* (4.0%) and *Solirubrobacter* (3.5%) (Fig. 2B; Table S3). *Pseudomonas* was significantly higher in the intercropped systems (MC1, MC2 and MC3) than in the monocrop systems (C and M), whereas *Rubrobacter* and *Solirubrobacter* were significantly lower (Table S3). *Sphingomonas* and *Skermanella* were significantly more abundant in MC2, *Thaurera* in MC1 and *Ammoniphilus* in M.

LDA effect size analysis revealed 20 predominant genera in the melon monocrop (M): *Blastococcus*, *Geofermatophilus*, *Kribella, Kineococcus, Actinoplanes, Micromonospora, Actinophytocola, Saccharomonospora*, *Nonomuraea*, *Actinomadura*, *Rubrobacter*, *Gaiella, Parviterribacter, Solirubacter, Tumebacillus, Gemmatimonas, Microvirda, Rubellimicrobium, Vulcaniibacterium* and *Opitutus*. In the cowpea monocrop (C), on the other hand, only four genera were predominant: *Pseudonocardia*, *Hyphomicrobium*, *Methylotenera* and *Phaselicystis*. In the intercropped systems, five genera were selected as predominant in MC1 (*Peptoclostridium, Turicibacter, Amphiplicatus, Ralstonia* and *Stenotrophomonas*); one genus was predominant in MC2 (*Leptolyngbya*); and one genus was predominant in MC3 (*Piscinibacter*) (Fig. 3; Table S4).

3.4. Effects of intercropping on abundance of genes involved in soil N cycling

Concerning the specific gene community related to N cycles, strong differences were found in AMOA, NARG ($P < 0.05$) and NIRK ($P < 0.01$) genes. In general, the log copies of these three genes were higher in monocropping systems (M and C) than in intercropped systems (MC1, MC2 and MC3). Among the three intercropping patterns, MC2 showed the lowest values (Fig. 4; Table S5).

3.5. Effect of intercropping on the soil properties and enzyme activities

Significant differences were found in some of the physicochemical and chemical soil properties (Table 1). Compared to the melon monocrop (M), TN was significantly ($p < 0.05$) higher in all the intercropped systems, MC1, MC2 and MC3 (with an increase of 18% each compared with monocropping). NH_4^+ was also higher in all the intercropped systems assayed than in the monocrops, but it was only significantly higher for the MC3 treatment ($p < 0.05$). The TOC content was also higher in intercropped systems, MC1, MC2 and MC3 (with an increase of

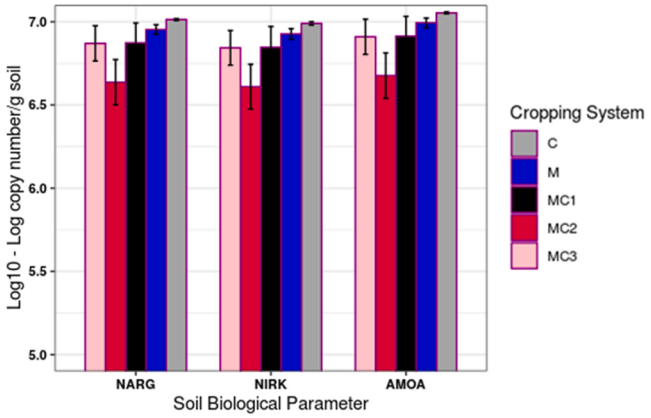

Fig. 4. Influence of intercropping on abundance of NARG, NIRK and AMOA genes belong to soil N cycle. (Bars represent means±sd; n = 5); C, cowpea monocrop; M, melon monocrop; MC1, mixed intercropping; MC2, row intercropping 1:1; MC3, row intercropping 2:1; NARG, narG gene; NIRK, nirK gene; AMOA, amoA gene.

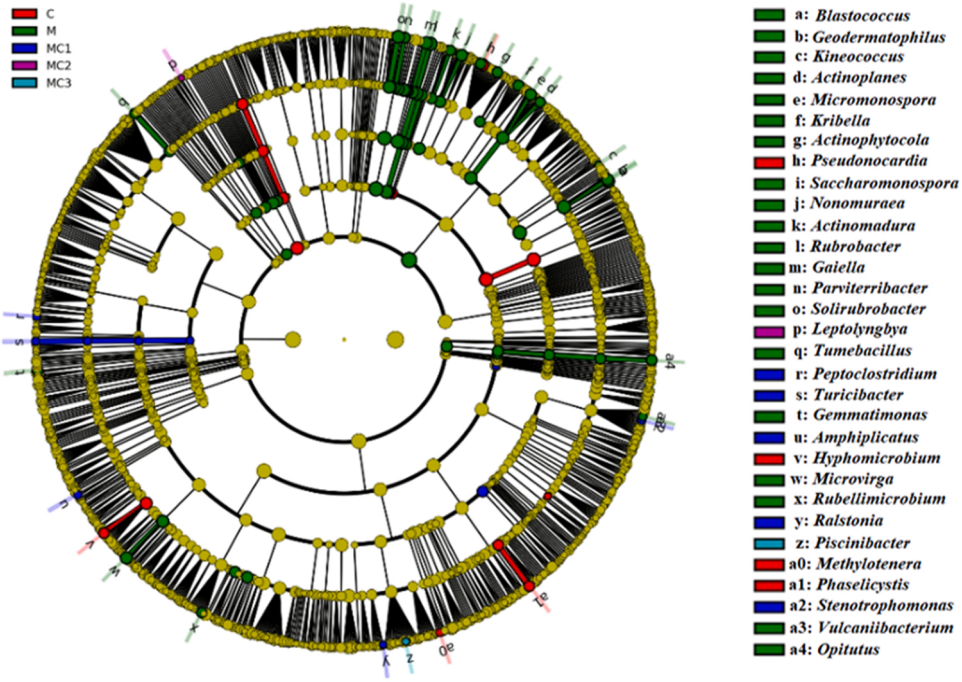

Fig. 3. Cladogram indicating the polygenetic distribution of bacterial lineages at genus level in the intercropping systems as determined by linear discriminant analysis (LDA) effect size (LEfSe). Each circle's diameter is proportional to the taxon's abundance. C, cowpea monocrop; M, melon monocrop; MC1, mixed intercropping; MC2, row intercropping 1:1; MC3, row intercropping 2:1.

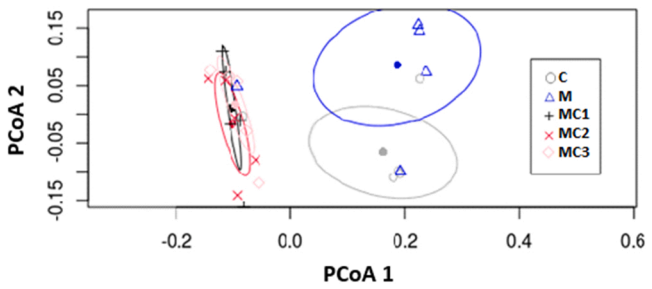

Fig. 5. Principal Coordinate Analysis (PCoA) of bacterial distributions in different intercropping systems. PCoA displays group centroids and dispersions. C, cowpea monocrop; M, melon monocrop; MC1, mixed intercropping; MC2, row intercropping 1:1; MC3, row intercropping 2:1.

18% MC1 and MC2 and 25% MC3 compared with monocropping) compared to the melon monocrop (M). MC3 and C showed the highest TOC content. The available P content was significantly higher ($p < 0.001$) in the intercropped systems [MC1 (169%), MC2 (152%) and MC3 (113%)] than in both monocrops (M and C). Available Mg and Na were significantly higher ($p < 0.05$) in MC1 than in the other treatments, and available Ca was significantly higher in MC1, M and C. No significant differences were observed in available K (Table 1).

Soil enzyme activities after intercropping are shown in Table S6. No significant differences were observed in dehydrogenase activity after one year of intercropping compared to the monocrops. Phosphatase activity, on the other hand, showed a significant increase in MC2 (12%) compared to M, while such differences were not observed in MC1 and MC3. β-glucosidase activity increased in MC1 (50%), MC2 (18%) and MC3 (13%) compared to the melon monocrop (M).

3.6. Relationships between soil properties and the bacterial community

Redundancy analysis (RDA) (Fig. 6) revealed a relationship between the bacterial community structure, soil properties and crop yield. The TN, AmoA, available Na and P and melon yield appeared to be strongly correlated with the bacterial community. Namely, the TN, P content and melon crop yield were correlated with the intercropped systems, while AmoA and Na content were correlated with monocrops. Intercropping systems showed clear divergence from the monocrops (M) and (C), while the latter could not be easily separated.

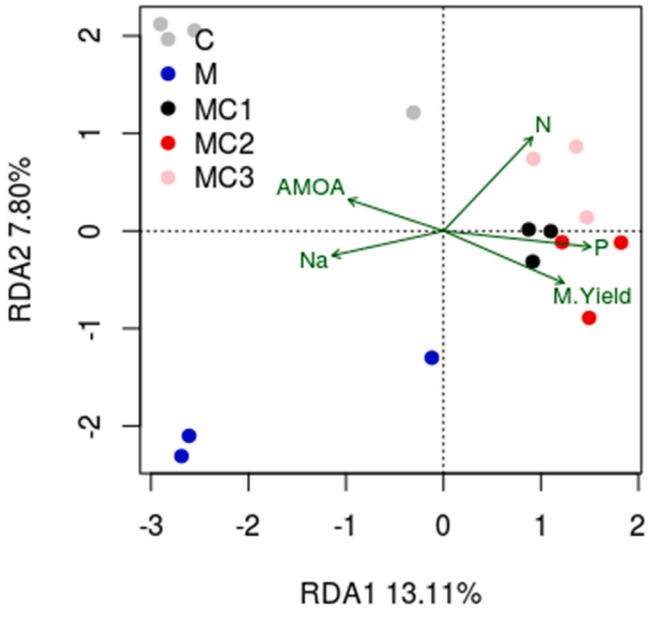

Fig. 6. Redundancy analysis (RDA) based on bacterial community composition of intercropping systems. Sites are coloured by cropping system whereas vectors show the correlation of the chemical, biological and harvest parameters with the community. Na, P, available Na, P; N, total nitrogen; M. Yield, melon yield; C, cowpea monocrop; M, melon monocrop; MC1, mixed intercropping; MC2, row intercropping 1:1; MC3, row intercropping 2:1.

Significant correlation was observed between *Pseudomonas* and the chemical and harvest parameters of the intercropping systems: for P, TN and melon yield, the correlations were r 0.69, $P < 0.01$; r 0.70, $P < 0.01$; and r 0.68, $P < 0.05$, respectively.

4. Discussion

Intercropping is considered to be an environmentally friendly system that can improve crop yield as well as water and nutrient-use efficiency (Chen et al., 2018; Gaiser et al., 2004). Crops have different needs, so it is especially important to combine them in the right way to obtain yield improvements. As far as we know, the melon-cowpea intercropping system and intercropping patterns between these two crops have not been studied in depth. However, this combination could be an important choice for sustainable horticulture management. The cowpea is a legume, which fixes atmospheric nitrogen and thus supplies it to companion plants like watermelon or other melons that at the same time provide soil shading to conserve water moisture (Munisse et al., 2012).

This study indicated that intercropping melon/cowpea in the first year of experimentation changed the microenvironment and altered the soil nutrient content. These changes positively affected soil microbial community growth, soil microbial community structure and crop yield, which ameliorated the problems associated with monocrops. The intercropping systems assayed (MC1, MC2 and MC3) increased melon yield (34%−74%) with respect to the melon monocrop (M), even though 30% less fertilization was used in the intercropped systems. This increase in yield could be due to higher nitrogen disposal from the cowpea rhizosphere, which should be higher in soils with low N fertilization addition (Yu et al., 2018). This fact has previously been observed in other cowpea intercrop relationships, such as cowpea-maize (Latati et al., 2014), cowpea-sorghum (Oseni, 2010) and cowpea-cassava (Sikirou and Wydra, 2008). The cropping patterns and N fertilization rates can alter soil conditions, which subsequently influence the abundance of functional N-cycling genes (Tatti et al., 2014). In our study, we also observed a decreasing trend in nitrification and denitrification processes in the three intercropped systems compared to the monocrops. This decrease in the intercropping systems could allow for sustainable nutrient use, diminishing nitrate loss due to leaching and N oxide emissions (Yang et al., 2018).

The results showed that the intercropped soil improved TN content, available P and phosphatase and β-glucosidase enzyme activities compared to the melon monocrop (M), probably due to the melon/cowpea rhizosphere microorganisms. The normal physiological activities of those microorganisms promote biochemical reactions in the soil microenvironment by secreting extracellular enzymes and releasing intracellular enzymes into the soil (Zeng et al., 2020). In general, legume crops included in intercropping systems improve P availability and soil organic carbon (Ngwira et al., 2012), mostly through root exudates, nodules, and the sloughing off of root cells and root turnover during the growing season (Namatsheve et al., 2020). Roots excrete larger amounts of protons and carboxylates (malonate, malate, and citrate), which would facilitate root-borne phosphatases to hydrolyze organic P (Hinsinger et al., 2011). According to Zhang et al. (2017). Organic P hydrolysis is also likely supported by a high abundance of phosphate-solubilizing bacteria like *Pseudomonas*, which were more abundant in the intercropped soils and correlated with available P, TN and melon yield. Moreover, the presence of several phosphate-solubilizing bacteria like *Bacillus* in both the monocrops and intercropping systems could also influence in this behavior, previously observed by Chen et al. (2006) and Panhwar et al. (2014).

It is important to note that soil microbial community composition is

significantly correlated with changes in soil chemical properties (Campbell et al., 2010; Lauber et al., 2008). In this study, the TN content, available P, AmoA abundance and melon crop yield play important roles in changes in the microbial community structure. Our findings could indicate that nutrient changes subsequently affect the carbon- and nitrogen-use efficiency of bacteria. Generally, an increase in soil microbial diversity is beneficial to soil function and health, but no differences were detected through diversity or richness estimators, indicating that our hypothesis was not validated. To date, there has been no consensus about changes in alpha diversity caused by intercropping systems, since some researchers have reported that some intercropping systems can increase diversity (Zhang et al., 2015; Zhou et al., 2011), while others have found no significant changes (FU et al., 2019; Poggio, 2005).

In our study, we found significant differences in the bacterial community structure between intercropping and monocrop systems, although not between the different intercropping patterns. These differences showed the influence of cowpea on the bacterial structure of the melon crop, suggesting that cowpea could play an important role in maintaining agricultural ecosystem stability and improving crop growth (P. Li et al., 2018). The differences also suggest that interspecies interactions may affect the abundance of some soil microbial populations, but not population diversity (Z.-M. Li et al., 2018; Yu et al., 2019). The dominant taxonomic groups identified in the soils assayed were Proteobacteria, Actinobacteria, Acidobacteria, Firmicutes, Gemmatimonadetes, Planctomycetes, Chloroflexi, Bacteroidetes and Nitrospirae, all depicted as common inhabitants of soil (Zhou et al., 2018). A higher relative abundance of Proteobacteria and Bacteriodetes and a lower abundance of Actinobacteria in the intercropping systems than in the monocrop systems indicated that both plant species and planting patterns can change the abundance of dominant bacterial phyla (FU et al., 2019; Gong et al., 2019; Zhang et al., 2018) due to their adaptability to a new microenvironment. Moreover, Bacteroidetes were associated with N and P soil cycling (Lidbury et al., 2021), and several plant-beneficial microorganisms identified as *Pseudomonas*, *Bacillus, Streptomyces* and *Sphingomonas* (Asaf et al., 2020; Bhattacharyya and Jha, 2012) could reduce the proportion of harmful fungi (Negawo and Beyene, 2017) due to their suppressive activity and their plant promoting growth (Sivasakthi et al., 2014; Tejera-Hernández et al., 2011).

LEfSe analysis indicated which microorganisms are significantly associated with the different cropping systems. The largest number of bacteria were found in the melon monocrop (*Blastococcus, Geodermatophilus, Kineococcus, Actinoplanes, Kribella* or *Gemmatimonas),* and these bacteria have been described as drought-resistant microorganisms (Castro et al., 2018a, 2018b). On the other hand, only five bacteria were associated with the intercropping systems, which indicates that changes are occurring, despite the high resilience of the bacterial community to changes (Griffiths and Philippot, 2013). Moreover, these changes do not depend too much on the specific intercropping pattern. These results indicate that one year of intercropping, which has been studied here, is not enough to result in certain significant microorganisms. It would be expected that long-term intercropping in the same soils would significantly increase the microbial diversity and its function on soils.

5. Conclusion

The intercropping system produced bacterial community structure changes, which correlated with an increase in soil TN and P concentrations and melon crop yield. The intercropped systems were characterized by a higher abundance of beneficial microorganisms such as *Pseudomonas, Bacillus, Streptomyces and Sphingomonas*. In this first-year experiment intercropping cowpea with melon resulted in a sustainable cropping system using less external input and resulting in an increase in melon yield. Starting from the first year, the use of diversified cropping systems thus provides a regular supply of food and income. Further long-term analysis of these intercropping systems will be needed to reinforce findings on the positive interaction between cowpea and melon microbiota and their functions and to study more in depth which intercropping pattern would be the most beneficial for the farmer.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2022.107856.

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