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Theobroma cacao L. agricultural soils with natural low and high cadmium (Cd) in Santander (Colombia), contain a persistent shared bacterial composition shaped by multiple soil variables and bacterial isolates highly resistant to Cd concentrations

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ABSTRACT

Heavy metals can be found in soil as natural components or as product of contaminations events; plants growing in soils are prone to bioaccumulate heavy metals on their biomass. *Theobroma cacao L.* can bioaccumulate cadmium (Cd) in the seed and could be in derived food products, it considered a human health risk; therefore, removal of Cd is desirable but not vet technically and economically feasible; only to avoid Cd in cocoa is by selecting lands plots exhibiting lower Cd concentrations in soils, imposing a serious limitation to farmers and regulators. The study of bacterial communities and isolation bacteria with tolerance and mechanisms to counteract the translocation of Cd to the parts of cocoa plant exhibits high relevance in Colombia economy and especially to companies producing chocolate and derivatives. Here, we explore bacterial communities associated with soils having relatively high natural Cd concentrations in a large agricultural cocoa plot located in the Santander region. We characterized the bacterial communities' compositions by amplicon 16S rRNA sequencing from metagenomics soil DNA and by culturing-based enumeration and isolation approaches.

Culture-dependent techniques allowed the isolation of bacteria tolerant to Cd concentration, complement the information for Colombia, and expand the number of strains characterized with adaptive capacity against Cd with tolerance in a concentration of 120 mg/L, which represents the first capacity for *Exiguobacterium* sp., *Ralstonia* sp., *Serratia* sp., *Dermacoccus* sp., *Klebsiella* sp., *Lactococcus* sp. and *Staphylococcus* sp. In addition to confirming that there is a greater diversity of Cd-tolerant bacteria present in soils of farms cultivated with cocoa in Colombia. As for the results of new generation sequencing, they revealed that, the alpha-diversity in bacterial composition, according to the ANOVA, there are statistically significant differences of the bacterial communities present in the samples. Regarding Pearson correlation analysis, it was found the Shannon Simpson indices, have a positive correlation against OM, C, pH, Mn, C.E.C.I., Ca, P and negatively correlated with S; respect to bacterial community structure, a principal component analysis, which revealed that independent of the concentration of Cd present in soil samples, separates them according to pH value. Phyla to high abundance relative in all samples were Proteobacteria, Acidobacteriota, Actinobacteriota, Verrucomicrobiota, Myxococcota, Chloroflexi, Plactomycetota, Bacteroidota, Gemmatimonadota, Nitrospirota, Firmicutes and NB1_J; the bacteria genera with higher relative abundance (>0.5%) *Nitrospira*, candidatus *Udaeobacter, Haliangium, Cupriavidus*, MND1, *Bacillus, Kitasatospora, Niveibacterium, Acidothermus, Burkholderia, Acidibacter, Terrimonas, Gaiella*, candidatus *Solibacter, Kitasatospora, Sphingomonas, Streptomyces*, this genus with a relationship with the Cd tolerance process. After it, redundancy analysis was performed between the variation of the bacterial communities identified by dependent and independent techniques and edaphic soil variables, where their positive correlation was found against K,

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OM, C, Ca, pH ($p<0.01$) and P, C.E.C.I ($p<0.05$). For soil samples, the bacterial genera that make up the core community were identified, which are present in all samples as Nitrospira sp., *Cupriavidus* sp., *Burkholderia* sp., *Haliangium* sp., candidatus *Udaeobacter*, MND1, *Kitasatospora, Acidothermus, Acidibacter, Streptomyces, Gaiella*, candidatus *Solibacter* and *Terramonas*; the genera identified has a different and fundamental role in ecosystem functioning. The combination of different approaches offers new clues regarding the assessment of bacterial communities in soils cultivated with cocoa in soils with elevated Cd content in Colombia, and the ecological role and interplay of soil components and bacterial communities that contribute to modulate the effect of bioaccumulation in products.

1. Introduction

Heavy metals contamination is a global problem and it represents a threat to both life and the environment due to its high toxicity (Chavez et al., 2015). Cadmium (Cd) is a heavy metal widely spread in nature with good solubility in soil and absorbed by numerous types of crops including cereals, tubers, and fruits.

Cd has been affected by physiological plant processes such as stomatal opening, respiration, and photosynthesis until it accumulates in roots, leaves, and edible parts such as grains (Chavez et al., 2015). In humans, Cd consumption and intoxication symptoms are associated with the development of some types of lung diseases, as well as, nausea, vomiting, abdominal pain, headache, and kidney dysfunction (World Health Organization, 2010).

Theobroma cacao L. is an important industry worldwide; in recent years, Colombia has been increasing cocoa crops (ranking among the top ten producing countries) and production of fine type of export-grade chocolate with Criollo and Trinitarian varieties since agro-ecological conditions are favorable, thus resulting in high-quality organoleptic performance (polyphenols and aromatic composition) and compliance with high-quality standards required in competitive markets (Porras et al., 2019; Chaves-López et al., 2014; An, Y., 2004).

Colombia has a region with the largest cacao growing area; the department of Santander has a planted area of 47,229 hectares, producing 24,890 tons of cocoa. In particular, the municipality of San Vicente de Chucurí, locally known as the capital of cocoa in Colombia, features a greater amount of cacao plantation area worth 17,000 hectares and a total production of 6540 tons (Ministerio de Agricultura, 2019).

In 2018, the European Union announced the incorporation in resolution 1881/2006 of regulation 488/2014, which specifies maximum levels of Cd in chocolate derivatives, coming into force in January 2019 (EU, 2014). The presence of Cd in soils with cocoa crops causes supply problems and limits the areas suitable for cultivation (soils with lower Cd concentrations), which in turn affects the production and processing of chocolate derivatives, as well as local and export sales, complying with regulations for the concentration of Cd in foods (Chavez et al., 2015).

The origin of Cd, as well as other heavy metals present in soils, can be from geogenic and anthropogenic sources. Regarding the geogenic source, the mountain ranges of the eastern part of the Colombian Andes cover the department of Santander. These soils come from Cretaceous geological material formations containing mainly sedimentary and igneous rocks (Mantilla et al., 2013). When comparing the different soil types, soils derived from igneous rock usually contain small amounts of Cd, while soils derived from sedimentary rocks, specifically shales, contain high Cd levels (He et al., 2015).

Regarding anthropogenic sources of Cd contamination in Colombian soils and also specifically in the region of the studied soils (Santander), researchers reported that Cd increases can originate from mining, air pollution, and chemical soil fertilization activities (Bravo et al., 2018). The mobility of trace elements, including Cd, in soil and its absorption by plants depends on factors such as soil texture, pH, Effective Cationic Exchange Capacity (E.C.E.C), organic matter content (OM), Cd concentration, chemical speciation, plant varieties, and agricultural practices (Yang et al., 2016).

Knowledge of soil diversity, particularly of the bacterial fraction, is limited. Only a minor fraction of microbial species have been cultured (Hug et al., 2016) and it is estimated that up to 10,000 genomes and 10^9 bacteria may be present in one gram of soil (Fierer et al., 2017). Thus the characterization of the metabolic potential of microbial communities present in soil and the effect of soil variables and contaminants is a major challenge (Gomez et al., 2011).

Bacteria are important components of soil ecosystems, as they play a fundamental role in biochemical cycles and are widely diverse and abundant depending on the habitat (Fierer et al., 2012), participating in decomposition processes, mineral fixation, and performing maintenance functions of biological activity and soil physicochemical conditions (Bissett et al., 2013). Microorganisms, especially bacteria, exhibit several defense mechanisms to overcome Cd toxicity, which could be used to remediate this heavy metal present in the soil. There is information regarding microbial communities isolated from soils in different Asian and European countries, with special emphasis on their behavior at the level of biosorption and bioaccumulation of Cd (Siripornadulsil and Siripornadulsil, 2013; Mitra et al., 2018; Sun et al., 2020; Shi et al., 2020).

Studies related to the effects of cadmium pollution on microbial communities have used both culture-dependent (Bravo et al., 2018) and culture-independent techniques (Luo et al., 2019). These techniques provide an expanded understanding of the microbial ecosystem in the soil. The selection of media and culture conditions, while contributing key information on the physiology of bacterial isolates and the genes involved, also demonstrates important limitations to achieving a representative picture of the microbial composition.

Culture-independent techniques such as denaturing gel gradient electrophoresis, phospholipid fatty acid analysis and random amplification of polymorphic DNA (Ezekoye et al., 2018), clone libraries, and massive sequencing techniques such as Illumina (Soliman et al., 2017) have been successfully used to describe these complex microbial ecosystems, particularly highlighting the predominance of Proteobacteria, Chloroflexi, Acidobacteria, Actinobacteriota, Gemmatimonadetes, Verrocumicrobia, Thaumarchaeota, Firmicutes and Nitrospirae in soils under metal stress (Ezekoye et al., 2018; Luo et al., 2019).

This work aimed at studying the diversity of bacterial communities associated with cocoa plantation soils and demonstrated the existence of geologically distinct Cd concentrations in land plots located in the Western Colombian Andes (Santander). We examined the taxonomic composition of the microbial community and possible interactions related to a range of physicochemical properties of the soil using conventional culture-dependent methods focused on Cd-resistant bacterial isolates and 16S rRNA gene amplicon sequencing surveys with the aim of finding parameters and microbiome components that are predominant, common, and unique to Cd levels in agricultural cocoa soils.

2. Materials and methods

2.1. Ethics statement

Soil collection for this microbial taxonomy study was conducted on the private cocoa agricultural field with the agreement and permissions

of the farm owners. This project framed within the permit "Marco de Recolección de Especímenes de la Universidad Nacional de Colombia", code project: 44,813, assigned to Microbiodiversity and bioprospecting research group.

2.2. Sites, sampling, and collection of soils samples

Samples were collected from four cocoa farm locations in the municipality of San Vicente de Chucurrí, department of Santander, Colombia. These farms are located in hillside lands between 600 and 900 meters above sea level in the eastern ranges of the Colombian Andes, with an average temperature of 26°C, annual rainfall between 1400 to 2600 mm, Inceptisol-type soils formed by volcanic and sedimentary nature, moderately deep and with a flat to ravine topography (Table 1).

Non-rhizosphere soil samples (horizon A (0–30 cm)) were collected from cocoa farms: Siempre Viva, La Argentina, Los Medios and Yariguies, according to the soil sampling guide by Osorio and Ruiz, 2013. Within each defined batch (seeded with cacao only) for each farm, composite samples were collected during May–June 2017: 12 samples (three independent samples per farm). Each composite sample was obtained from 18 random subsamples of 2 kg, collected in a zigzag path on the area of interest. Chosen points were selected to coincide with cocoa trees in a healthy phytosanitary state. Sampling was performed by clearing the land surface (composite sample) and introducing a borer at the indicated depth. For the collection and final handling of samples, the methodology indicated in national standard NTC 4113–6 was followed. After the homogenization of subsamples, successive quartering, and 2-mm sieve passages, the total sampling unit exhibited an average weight of 3 kg; samples were subsequently deposited in a sterile plastic bag and transported to the laboratory on dry ice where they underwent fractioning in three parts: one for chemical and soil properties analyses, one for culture-dependent assays preserved in refrigeration and one for DNA extractions, stored at – 20°C until processing.

2.3. Physical and chemical analysis of soil properties

The soil properties (exchangeable elements) were measured following the protocols described in the national standard reference norms of the ISO representative in the country (ICONTEC): pH, electrical conductivity (CE), and cationic interchange effective capacity (C.E.C.I) by NTC 5167; texture (bouyoucos method); OM and C (NTC 5403); P (NTC 5530); K, Ca, Mg and Na according to NTC 5349; Cu, Fe, Mn, Al and Zn by NTC 5526; S and B following to NTC 5404 and total Cd (NTC 3934). Table S1 provides detailed information on the soil properties analyses.

2.4. Culture-dependent assays

2.4.1. Isolation of bacteria and colony-forming units

Samples were pooled for each farm: 1 gram (wet weight) was diluted and homogenized in peptoned water and cultured after serial dilutions by surface plating on Nutritive agar (NA) and R_2A (Merck®) for the isolation of heterotrophic aerobic bacteria with or without the additional selective condition of supplemented cadmium chloride (Meyer®) in a concentration of 2500 μM $CdCl_2$ equivalent to 120 mg/L Cd. Cadmium salt was added to the medium, before autoclaving, and medium pH was adjusted to pH 7.0 with NaOH 0.1 N. Plates were incubated aerobically at 30°C for 48 h, for the NA and R_2A one week and bacterial counts of viable Cd tolerant counts were performed. After incubating, colony-forming units per gram (CFU/g) counts were determined for each homogenate, and an analysis of variance (ANOVA) of Log (CFU/g) was performed to determine if any differences were found between growth media and soil samples. Pure colonies with different morphological characteristics (colony size, elevation and pigmentation) were isolated from media plates and sub-cultured to obtain pure bacterial cultures. Then, the isolates selected were characterized microscopically by Gram staining. All isolates were cryopreserved in 20% glycerol at – 20°C.

Table 1
Description of study sites.

Farm+	GPS Locations	Cocoa variety
LA	6° 55N and 73° 28W	ISC-95, CCN-51
SV	6° 53N and 73° 23W	ICS-39, ICS-60, CCN-51
LM	6° 54N and 73° 22W	ICS-95, CCN-51
Y	6° 54N and 73° 44W	ICS-39, ICS-95, CCN-51

+ La Argentina (LA); Siempre Viva (SV); Los Medios (LM); Yariguies (Y).

2.4.2. Ribosomal intergenic spacer analyses (RISA) and 16S rDNA PCR of Cadmium-tolerant bacteria

Colonies were grown in NA medium and three rounds of isolation of single colonies were performed to obtain pure isolates. They were characterized by molecular fingerprinting (RISA) to dereplicate the strain collection of potential identical isolates. Isolates with different RISA patterns were identified through 16S rRNA gene sequencing DNA from bacterial isolates colonies and PCR reactions were performed using the primers L1 (5′-CAA GGC ATC CAC CGT-3′) and G1 (5′-GAA GTC GTA ACA AGG-3′) (Jensen et al., 1993) with the mix and program described in Moreno et al., 2002. RISA patterns were resolved by polyacrylamide gel electrophoresis (PAGE), using Gel Compare Software (Applied Biosystems, Belgium) (García et al., 2016). RISA-PAGE was performed in a Mini-PROTEAN Tetra cell electrophoresis unit with 7% polyacrylamide gels (acrylamide/bis-acrylamide 29:1) for 100 min at 130 v. A cluster analysis was performed using Pearson coefficient and Simple Linkage clustering method. To represent bacterial diversity in the soil isolated strains, a similarity of $\geq$ 90% between RISA patterns was established as a criterion for selecting bacterial isolates for subsequent molecular identification assays (Yim and Ramdeen, 2015). The DNA isolated from the selected colonies was used to amplify the 16S rRNA gene (1.5 kbp), using the primers Eubac 27 F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5′-GGT TAC CTT GTT ACG ACTT-3′) according to Moreno et al., 2002. Positive and negative controls (DNA from pure *Bacillus* sp. cultures and ultrapure sterile water) were routinely included in all PCR reactions. Amplification products were visualized in 1% agarose gel and purified using Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, UK) and the double-stranded DNA was sequenced in both directions using the ABI PRISM 3700 DNA analyzer service of Applied Biosystems.

2.4.3. Sequencing and phylogenetic analysis

Sequences obtained from the PCR products of 16S rRNA gene from pure isolates were edited using BioEdit (http://www.mbio.ncsu.edu/bioedit/bioedit.html), and the presence of chimeric sequences was evaluated with the CHIMERA CHECK software program (http://www.bioinformatics-toolkit.org). The edited sequences were contrasted with submitted sequences at the NCBI database using the Basic alignment search tool (BLAST) (https://blast.ncbi.nlm.nih. gov/Blast.cgi) and SeqMatch search tool from the Ribosomal Database Project (RDP) (http://rdp.cme.msu.edu/seqmatch) and downloaded reference and consensus sequences were aligned using Clustal W with MEGA X (https://www.megasoftware.net) (Thompson et al., 1994). Phylogenetic dendogram analysis of bacterial Cd-tolerant was constructed using the Maximum Likelihood method with Kimura 2-parameter distance (Kimura, M., 1980) with 1000 bootstrap replicates. The sequences were submitted to NCBI Gene Bank databases and accession numbers were obtained.

2.5. Independent-culture techniques

2.5.1. 16S rRNA gene survey and analysis

Total DNA was extracted from 500 mg of each homogenized soil

sample using PowerSoil DNA Isolation Kit (MO BIO) following the manufacturer's protocol. The quality of the purified DNA was assessed using a Nanodrop ND-2000 Spectrophotometer (NanoDrop Technologies Inc, Willington, DE; USA) based on 260/280 nm and 260/230 nm absorbance radios and stored at –20°C for further analyses. PCR amplification of the bacterial 16S rRNA gene fragments were used to determine the soil bacteria diversity and community composition. Hypervariable region V4 of the bacterial 16S rRNA genes present in the soil DNA was amplified using the following forward and reverse primer set: 515F (5-GTGCCAGCMGCCGCGGTAA-3) and 806R (5-GGACTACHVGGGTWTCTAAT-3) with barcode. PCR reactions, containing 25 µl 2 × Premix Taq (Takara Biotechnology, Dalian, China), 1 µl each primer (10 mM) and 3 µl DNA (20 ng/µl) template, were amplified by thermocycling (20 cycles of 30 s denaturation at 94°C, 30 s annealing at 52°C, and 30 s extensión at 72°C) after initialization at 94°C for 5 min, followed by 10 min final elongation at 72°C for 45 s, and final step at 4°C until further processing. The presence and expected size of PCR products were assessed by agarose gel electrophoresis. The amplified products were purified, normalized, and pooled using the SequalPrep Normalization Plate (Thermo Fisher Scientific, Santa Clara, CA, USA) and subjected to 250 bp paired-end Illumina MiSeq (Illumina Inc., San Diego, CA, USA) sequencing.

2.5.2. Processing of sequencing data

The raw sequences data were processed using QIIME 2.0 (v.2018.8) pipeline (Caporaso et al., 2010). After the demultiplexed process, the sequences were subjected to quality control by DADA2 in RStudio; the low-quality raw sequences (average quality score < 30) were discarded; chimeras, singletons and primers were removed from data using the same package (Callahan et al., 2016); This resulted in asembled datasets for each amplicon to detect the counts of each unique Amplicon Sequence Variant (ASV) across all samples at 100% sequence identity and clasified according to SILVA 138 dataset (https://zenodo.org/record/4587955/files/silva_nr99_v138.1_wSpecies_train_set.fa.gz?download=1, access May 2021) (Quast et al., 2013). All generated data were deposited and publicly available at National Center for Biotechnology Information (NCBI) Sequence Read Archive under BioProject PRJNA599345 and BioSample accession number ID SUB6627411.

2.5.3. Statistical analysis

Culture-depended counts were performed ANOVA and Tukey comparision test by RStudio version 1.4.1106. The culture-independent data analysis was performed using MicrobiomeAnalyst (Dhariwal et al., 2017) (https://www.microbiomeanalyst.ca/) and RStudio software. Alpha-diversity estimates to compare bacterial community composition relative abundance using the "Phyloseq" package in R. The alpha-diversity indices were evaluated using Observed, Chao1 (abundance estimator), Shannon (evenness), and Simpson (bacterial diversity). The correlation between soil properties and alpha-diversity was calculated with a two-way analysis of variance (ANOVA) and Pearson's correlation analysis. Likewise, a beta-diversity analysis of bacterial communities was examined using PCoA combined with multivariate PERMANOVA of Bray–Curtis distances based on the relative abundance of phyla were applied to determine the correlations between microbial communities and soil properties with the "vegan" package in RStudio. The features of bacterial communities of genera level were identified using the linear discriminant analysis (LDA) effect size (LEfse) algorithm (Segata et al., 2011) from the top 25 matches (threshold > 4.0). The redundancy analysis (RDA) was performed using the vegan package in R with 999 permutations to present the correlation between soil properties and all bacterial community structure (Oksanen et al., 2016). The relationship between relative abundance ASVs and soil variables was calculated using Spearman's correlation and a heatmap was performed (Pradhan et al., 2017). The core microbiome at the genus level was determined as features present in 100% samples with a mínimum relative abundance of 0.01%.

3. Results

3.1. Variation in soil properties of cocoa farms

After performing Pearson correlation between soil properties and pH, significantly positive correlated with C.E.C.I., C, MO, Ca, Zn ($p<0.01$); Mn, Al and Cd ($p<0.05$); and significantly negative correlated with clay and sand ($p < 0.05$). Organic Matter (OM) showed significantly positive correlation with pH, C.E.C.I., C, Mn, Zn ($p<0.01$); Ca, Fe and Cd ($p<0.05$). Cd was significantly positively correlated with pH, MO, C ($P<0.05$); C.E.C.I., Zn and Al ($p<0.01$). Regarding physical aspects, all cocoa farm soils are inceptisol type, exhibit in common a clay loam texture, a high capacity to retain water and nutrients with fine particle size. Soil pH between 4.5 – 7.0, suitable to cacao crops and Cd levels; about the concentration of Cd, 1.1 mg/Kg is the maximum level allowed to agricultural land according to Europe Union (Ding et al., 2018), LA, SV farms have high concentration of Cd; Y, LM farms have a low concentration of Cd. All soil characteristics see in supplementary materials (Supplementary Table S1)

3.2. Bacterial diversity through culture-dependent assays

3.2.1. Microbiological analyses

Bacterial counts fluctuated between 2.7–2.9 × 10^5 to 4.1–4.3 × 10^5 colony-forming units (CFU/g) across the soils of all the farms samples without differences (Tukey HSD, $p> 0.05$). The abundance of cultivable bacteria varied greatly among the sampling sites, but on average it remained relatively constant over time. 128 Cd tolerant bacteria isolates were obtained in the medium containing 2500 µM $CdCl_2$ for further purification and characterization. 107 (83%) isolates were bacilli, 21 (17%) were cocci, 113 (88%) were Gram-negative and 23 (38%) Gram-positive. Isolates grouped in 16 RISA clusters at 90% similarity, 35 isolates showing distinct patterns representing these 16 clusters were selected for 16S rRNA gene sequence analysis.

3.2.2. Identification of isolates by 16S rRNA gene sequencing

Phylogenetic affiliations and similarities of the Cd resistant isolates selected for sequencing are displayed in Table 2. The isolates are classified as belonging to phyla Proteobacteria, Actinobacteriota and Firmicutes. The sequence examination revealed that *Burkholderia, Exiguobacterium* and *Ralstonia* are found in all samples regardless of the soil Cd levels, while *Bacillus, Cupriavidus* and *Serratia* were found only in soils with high Cd level and *Dermacoccus Enterobacter, Escherichia, Klebsiella, Lactococcus* and *Staphylococcus* in soils with low Cd levels. The phylogenetic relationships among isolates are shown in the dendrogram created by the Maximum Likelihood method (Fig. 1). In figure 2, showed some of the morphological characteristics of isolated colonies; table S3, a summary of the richness of genera for each farm.

3.3. Bacterial diversity through culture-independent assays

3.3.1. Sequencing overview

Across all 12 soil samples, a total of 457,633 reads of partial 16S rRNA gene sequences (region V4) were obtained. After quality filtering, 443,464 good quality reads were obtained, with a mean of 36,955 per sample. After filtration, a total of 5592 different amplicon sequence variants (ASVs) was identified among all samples with a 100% identity threshold. Rarefaction analysis revealed that the sequencing was sufficient to identify the most of the bacteria diversity in all the samples (average 23,869 reads per sample).

3.3.2. Microbiota composition at phyla and genera levels

Overall soil cocoa farms showed the presence of 28 phyla, the most relative abundance phyla were: Proteobacteria (22.87%), Acidobacteriota (18.08%), Actinobacteriota (9.79%), Verrucomicrobiota (9.73%), Myxococcota (6.71%), Chloroflexi (5.12%), Plactomycetota

Table 2
Taxonomic identification of cadmium tolerant bacteria isolated from farm soils, identified by 16S rRNA gene sequencing.

Soil Cadmium level	Isolated code	Culture medium	Phylogenetic Affiliation	NCBI accession number
High	10–2	R2A	*Bacillus toyonensis*	MN587894
	7–1	AN	*Burkholderia anthina*	MT940970
	9–1	AN		MT940971
	18–1	AN		MT940974
	3–2	R2A		MT940975
	5–2	AN		MT940976
	23–2	R2A		MT940979
	8–5	AN		MT940982
	10–1	AN	*Burkholderia ambifaria*	MT900972
	17–2	R2A	*Burkholderia arboris*	MT940978
	17–1	R2A		MN587896
	15–1	R2A	*Cupriavidus necator*	MN587892
	1–2	AN		MT940980
	4–2	AN	*Escherichia fergusonii*	MN587901
	7–2	AN		MT940977
	14–1	R2A	*Exiguobacterium acetylicum*	MT940973
	16–1	R2A	*Ralstonia solanacearum*	MN587895
	6–2	R2A	*Serratia marcescens*	MN587899
	20–2	R2A		MT940981
Low	20–3	AN	*Burkholderia anthina*	MT940990
	21–3	AN	*Burkholderia lata*	MT940984
	7–4	AN		MT940986
	14–5	AN		MT940988
	19–3	AN	*Burkholderia ubonensis*	MT940989
	4–3	R2A	*Dermacoccus barathri*	MN587890
	2–4	AN	*Enterobacter aerogenes*	MT940985
	29–4B	R2A	*Enterobacter tabaci*	MN587891
	11–4A	AN	*Exiguobacterium acetylicum*	MN587893
	9–4	AN		MT940991
	16–4	AN	*Klebsiella variicola*	MT940987
	18–4B	AN		MN587897
	22–4	R2A	*Lactococcus lactis*	MN587898
	21–4	R2A		MT940992
	16–3	AN	*Ralstonia solanacearum*	MT940983
	2–3	R2A	*Staphylococcus capitis*	MN587900

(5.03%), Bacteroidota (3.95%), Gemmatimonadota (3.65%), Nitrospirota (2.93%), Firmicutes (2.23%), NB1_J (2.07%). (Fig. 3A). At the genus level showed 148 genus, the most relative abundance (>0.5%) were: ADurb.Bin063–1 (3.46%), *Nitrospira* (2.90%), candidatus *Udaeobacter* (2.26%), *Haliangium* (2.19%), MND1 (1.99%), *Bacillus* (1.97%), *Kitasatospora* (1.32%), *Niveibacterium* (1.06%), *Acidothermus* (1.05%), *Burkholderia* (0.90%), *Acidibacter* (0.88%), *Terrimonas* (0.79%), *Gaiella* (0.79%), candidatus *Solibacter* (0.78%), *Streptomyces* (0.76%), *Cupriavidus* (0.75%) and *Sphingomonas* (0.59%) (Fig. 3B).

3.3.3. Soil bacterial diversity

Alpha diversity analysis was performed, most of the farm soil (low and high concentration of Cd) showed indices of richness (observed, Chao1) diversity (Shannon and Simpson) and dominance of ASVs represented by values not exceeding 30, 3 and 0.9 respectively (Fig S1), wich are considered low compared to soil without concentration of heavy metals. These results suggested perturbation on the microbiota diversity and the potential dominance of some communities. ANOVA showed significant differences between bacterial soil communities ($p<0.05$). Concerning the Pearson correlation, the concentrations of some soil properties OM, C, pH, Mn, C.E.C.I., Ca, P had a positive correlation and negative correlation with S and the índices on bacterial species diversity ($p<0.05$). The concentration of Cd was not significantly correlated with indices of bacteria species richness and diversity ($p>0.05$). Regarding beta-diversity measure intergroup, showed not divergent ASVs composition (Fig. 4A), whereas intragroup analysis showed statistically significantly differences between the ASVs communities to PERMANOVA test ($F = 14.595$, $p < 0.001$) and PCoA with Bray-Curtis distance separated the samples according to pH values, the first axis (61.8%) samples with neutral pH and second axis (23.4%) samples with acid pH (Fig. 4B). LEfse analysis was performed to identity genus enriched in the differents soil samples. The genus *Nitrospira*, Candidatus *Udaebacter* and MND1 were the most differential taxon with an LDA score > 5.0, while *Haliangium, Crenobacter, Acidothermus*, Candidatus *Solibacter, Cupriavidus, Acidibacter, Gaiella, Streptacidiphilus, Ileronia_Paraburkholderia* with an LDA score > 4.0, all the genus as important taxonomic contributors (Fig. 5).

The RDA analysis was carried out to link the variation of bacterial communities identified by dependent and independent culture technicals with edaphic soil variables. Eleven soil physicochemical properties parameters including S, pH, E.C, Mg, Na, K, P, OM, C, Ca and C.E.C.I, were show on RDA ordination; according to Spearman's correlation, all the bacterial communities were positive correlation with K, MO, C, Ca, pH ($p<0.01$) and P, C.E.C.I ($p<0.05$) and significantly affect the bacterial community in bulk soil (Fig. 6).

Correlations between the relative abundance of the ASVs at the phyla level and soil properties (Fig. 7). The results showed that Plactomycetota, Mixococcota, NB1_J, Nitrospirota, Bacteroidota phyla were a positive correlation with K, MO, C, Ca, pH, P, and C.E.C.I ($p<0.01$) and negative correlation with Actinobacteriota, Proteobacteria, Firmicutes, and Acidobacteriota phyla ($p<0.05$). Cd showed a negative correlation with Firmicutes and a positive correlation with Bacteroidota ($p<0.05$).

Figure 8, showed the core microbiome at genus level; were identify sixteen differents genus found in 100% across of the samples (0.01 to 0.05% relative abundance): ADurb_Bin063_1 (Verrucomicrobiota), *Nitrospira* (Nitrospirota), *Haliangium* (Proteobacteria), Candidatus *Udaebacter* (Actinobacteriota), *Bacillus* (Firmicutes), MND1 (Proteobacteria), *Kitasatospora* (Actinobacteriota), Niveibacterium (Proteobacteria), *Acidothermus* (Actinobacteriota), *Acidibacter* (Proteobacteria), *Cupriavidus* (Proteobacteria), *Gaiella* (Actinobacteriota), Candidatus *Solibacter* (Acidobacteriota), *Burkholderia* (Proteobacteria), *Streptomyces* (Actinobacteriota) and *Terrimonas* (Bacteroidetes); however in the core predominance (Not assigned) was found with more 0.4% abundance in all samples.

4. Discussion

Soil is a highly complex ecosystem inhabited by multiple interacting species of organisms, and knowledge of soil diversity, particularly the bacterial fraction, is limited since only a minor fraction of microbial species have been cultured (Joseph et al., 2003). Soil portrays a great diversity of bacterial communities that contribute to nutrient cycling, health, and structure, key aspects of fertility that contribute to the sustainability of plant species, which in turn, influence bacterial growth, given by root exudation and senescent plant parts that are a source of nutrients for microbial communities (Backer et al., 2018). However, there are still unresolved questions regarding the composition and structure of edaphic bacterial communities since this characterization is a great challenge and requires the integration of multiple strategies offered by microbial ecology (Gómez-Sagasti et al., 2012).

Regarding the physicochemical results and specifically the textural characteristics, which are important when determining crop fertilization plans, nutrient absorption, and avoiding fertilizer residues that could contaminate water sources, the farms used to have a clay loam texture, which retains water and nutrients. All cocoa farms showed a deficiency

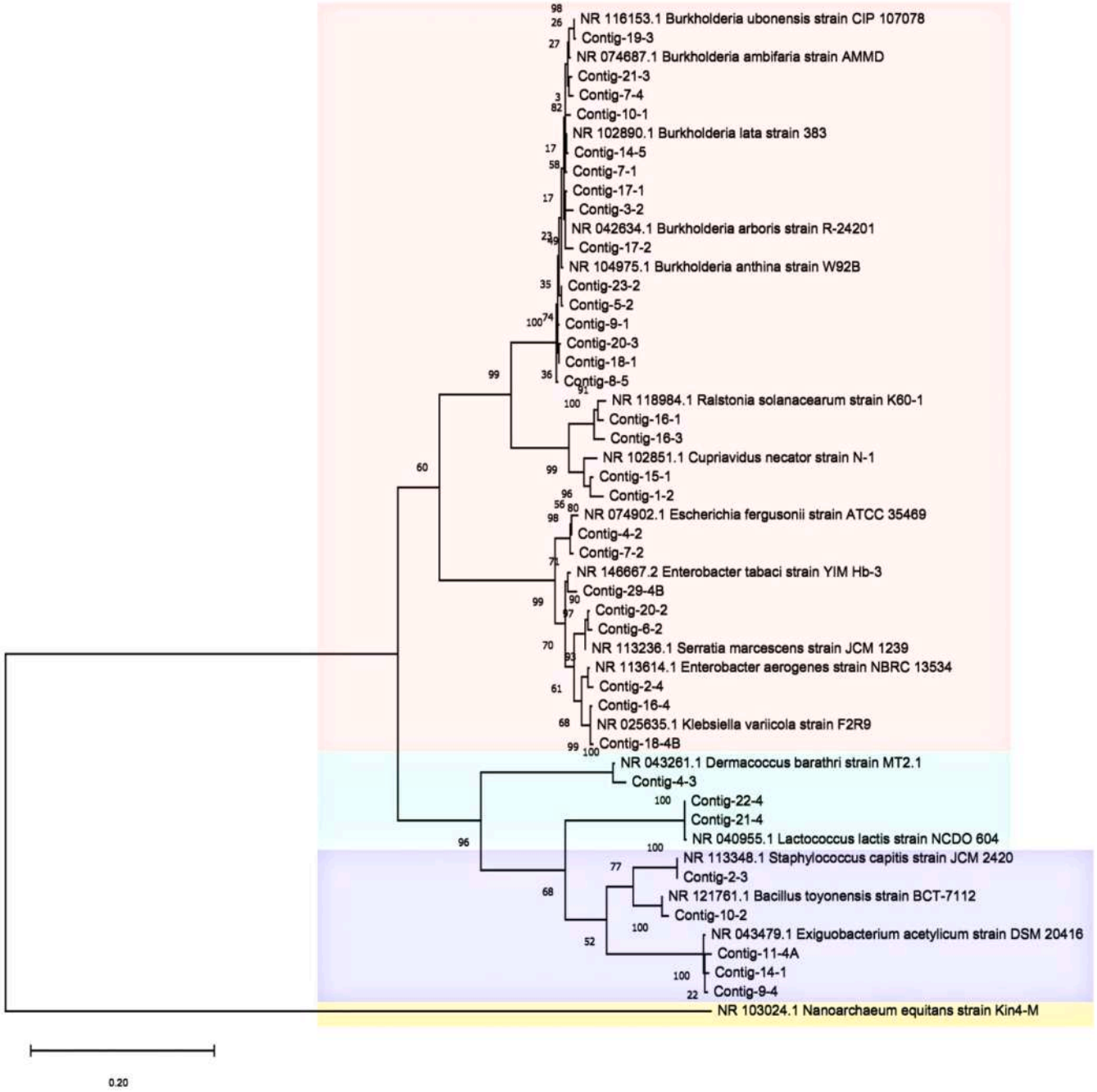

Figure 1. 16S rRNA gene-based dendrogram showing the phylogenetic relationships of Cd tolerant bacterial isolates from *T. cacao* farms soils. The dendrogram was produced in MEGA X, using the Maximum Likelihood method with 1000 bootstrap replicates. The tree is rooted using as outgroup *Nanoarchaeum equitans* 16S rRNA gene sequence (yellow color). Proteobacteria (pink color), Acidobacteriota (green), Firmicutes (blue) phyla.

of OM, P, and K; maybe it is a sign of soil fertility depletion, or in these steep slope soils, erosion by runoff and deforestation, or the establishment of other crops. Other variations in Ca, P, K, and Mg are due to the presence of minerals rich in calcite and calcium phosphates, which fluctuate according to the height of the farms, adding to the antagonism between Ca, Mg, and K, thus causing an imbalance between exchangeable bases, affecting their availability to the crop. In addition, Mg deficiency was detected in all the farms, which affects the yield and commercial quality of the harvested cocoa seed (Puentes-Páramo et al., 2014). The soils were found to be highly heterogeneous, possibly with the presence of microenvironments with low concentrations of nutrients, organic and inorganic compounds, and optimal conditions for the development of oligotrophic microorganisms and the degradation of these compounds affected by nutrient dynamics (Hashimoto et al., 2006).

On the other hand, the concentration of Cd in soils was translocated to the cocoa plants and accumulated in the leaves, cobs, and kernels, which impaired the production and yield of the crop, especially in SV and LA farms (data not shown). Some of the soil factors that influence Cd mobility are pH, OM, and E.C.E.C., physicochemical variables that show a significant positive correlation. According to Shanying et al., (2015), pH is the most important factor that affects the availability and mobility of Cd in soil, with more mobility at pH values of 4.5 - 5.5, while at neutral and alkaline pH, Cd precipitates into insoluble carbonate and phosphate forms. As for organic matter (OM), it is the major source of cation exchange, chelating capacity, and promotion of the microbial

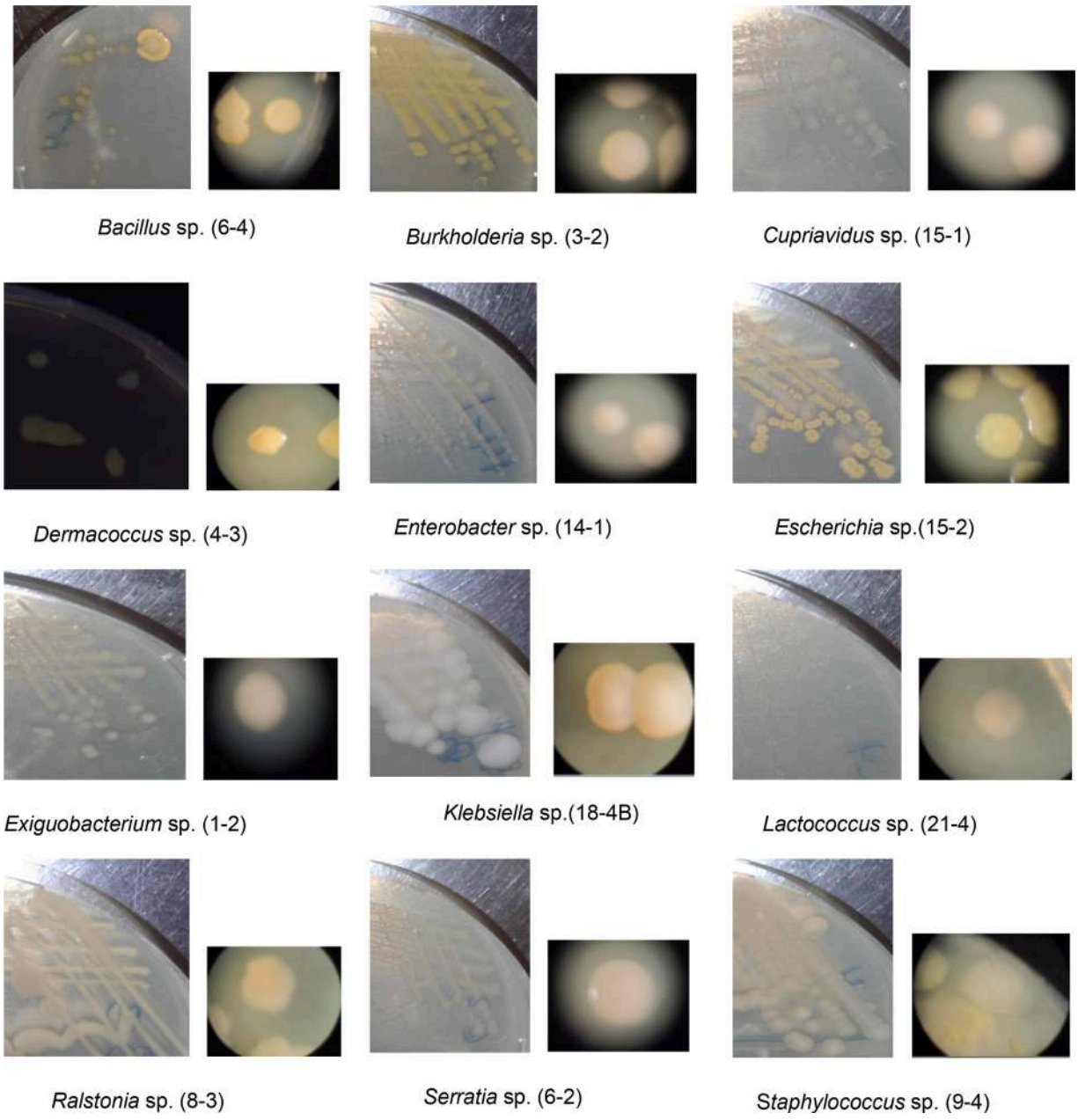

Figure 2. Colony morphology native strains Cd-tolerant isolates in cacao crops soils, amplified by stereoscopy at 20X.

activity. OM absorbs high concentrations of Cd, and an increase in the percentage of OM through amendments or fertilization can reduce the bioavailability of Cd while increasing soil pH (Kirkham, M., 2006).

The characterization of microbial communities is the first step on the way to unraveling the complexity of the soil microbiome. The research set out to study a part of the microbiome such as the composition of the bacterial communities of cocoa soils in the presence of geogenic Cd and the interactions with its physicochemical properties.

Using conventional culture-dependent methods of soil samples, regardless of the culture medium and Cd concentration in the soils, variance analysis showed no significant difference in total counts ($p>0.05$).

The number of Cd-tolerant colonies isolated in the different commercial culture mediums differs considerably (Table 2), given that in nutrient agar a greater number was isolated compared to R2A medium (59% of the colonies were isolated in AN and 41% in R2A agar). This difference is due to the concentration of nutrients, which favors the growth of certain bacterial genera, as is the case of heterotrophic bacteria (Eiler et al., 2003). As shown in Table S1, La Argentina and Yariguies farms, whose soil pH characteristics are acidic, differ in terms of the concentration of total Cd and organic matter, with a greater richness of the genus in La Argentina farm; as for Siempre Viva and Los Medios farms, characterized by a pH close to neutrality but a different concentration of total Cd and organic matter, a difference in genus richness, is more clearly observed in favor of Los Medios farm. This is in agreement with Lazzaro et al., (2006), who indicate the predominance of certain cultivable genera influenced by the concentration of Cd present in the soil.

Concerning the molecular analysis of the different Cd-tolerant isolates, the Proteobacteria phylum was one of the most abundant in the different samples. According to Xiaoqi et al., (2017), who conducted a study of microbial communities in soil with the presence of heavy metals, indicates that this phylum shows a slight increase in genera as a response to the presence of contaminated heavy metal and Bravo et al.,

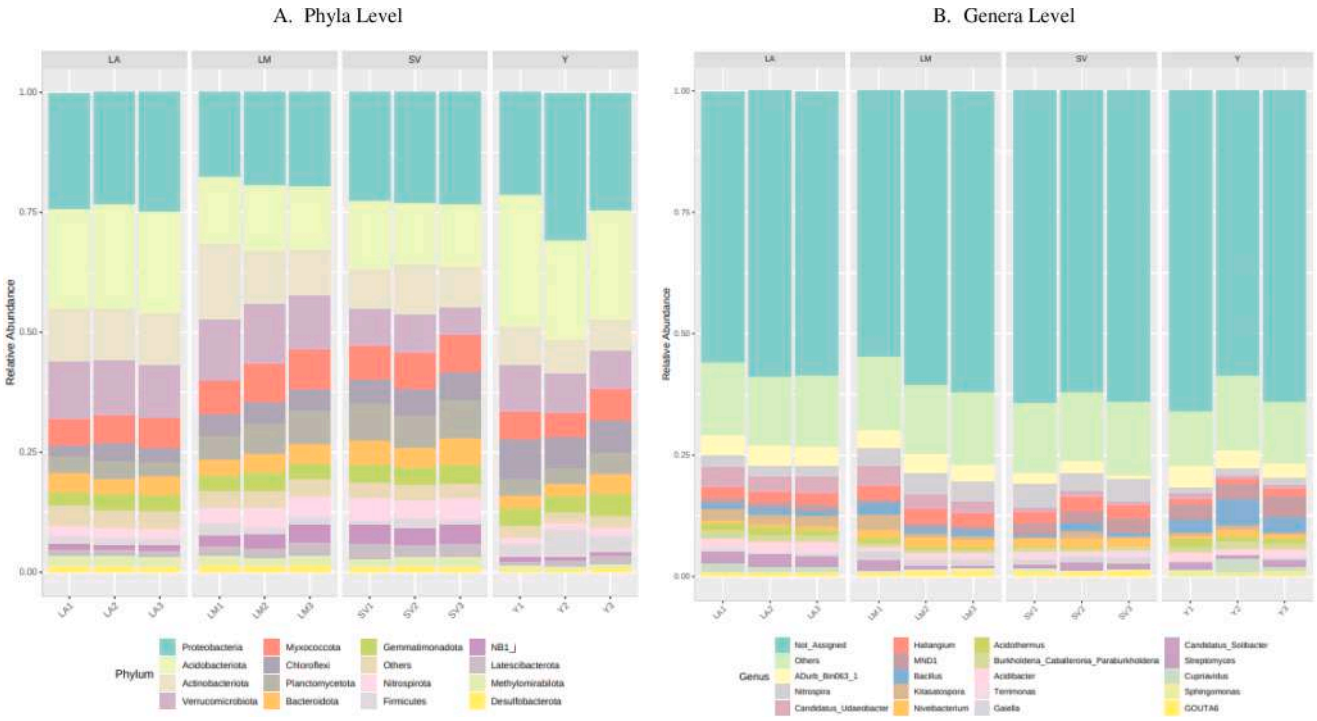

Figure 3. Relative abundance of bacterial communities. A. The average relative abundance histogram of all samples phyla. B. The relative abundance histogram of genera. Argentina (LA); Siempre Viva (SV); Los Medios (LM); Yariguies (Y) farms.

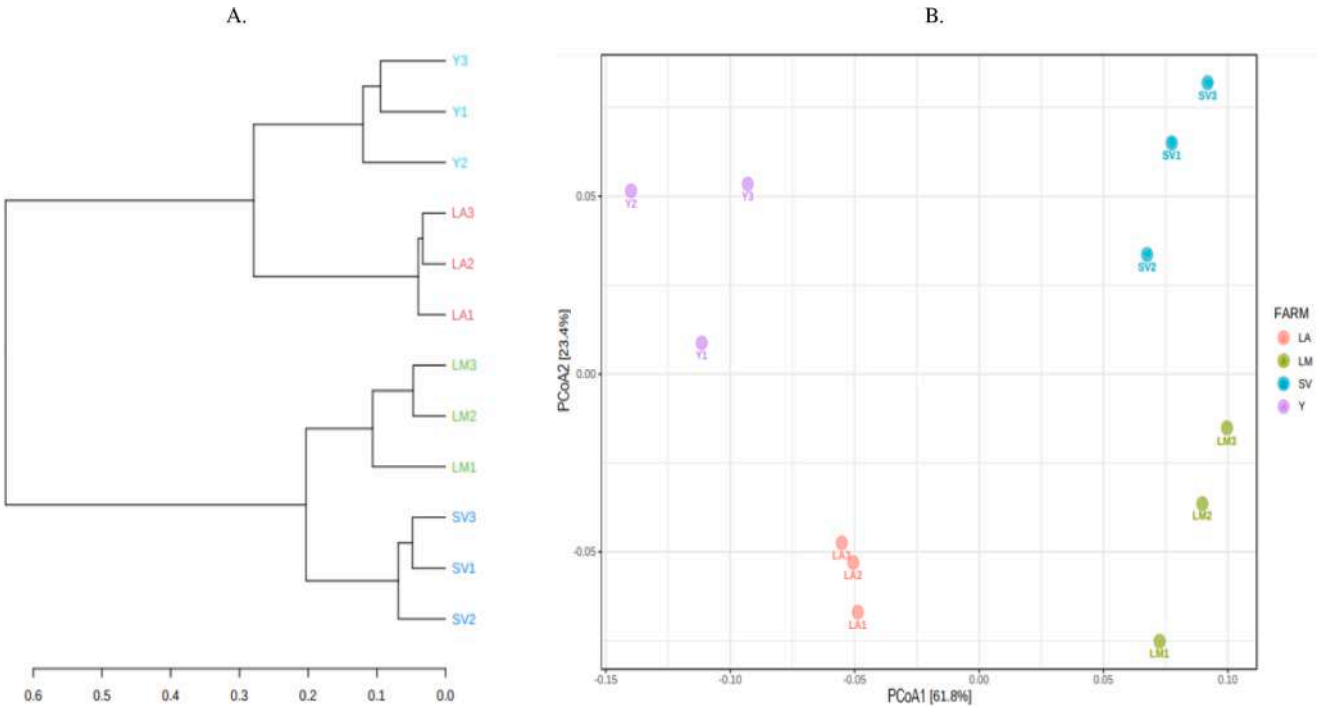

Figure 4. Beta-diversity of bacterial communities. A.Hierarchical clustering analysis of ASVs at phyla level. B. Principal Coordinate Analysis (PCoA) of Bray-Curtis dissimilarities of 16S rRNA filtered ASVs at phyla level. Argentina (LA); Siempre Viva (SV); Los Medios (LM); Yariguies (Y) farms.

(2018) confirmed that the phylum Proteobacteria is the most abundant in the cultivable bacterial community of soils with the presence of Cd.

The results showed the identification of different genera: *Burkholderia* sp., *Cupriavidus* sp., *Enterobacter* sp., *Escherichia* sp., *Klebsiella* sp., *Ralstonia* sp. and *Serratia* sp., (Proteobacteria Phyla). Similar results were reported for soils contaminated with industrial waste and where Proteobacteria increased in response to heavy metal pollution (Xiaoqi et al., 2017). *Burkholderia* sp., has been isolated from non-rhizospheric soils with Cd presence (Jiang et al., 2008); *Cupriavidus* sp. has been isolated from soils contaminated with heavy metals, near zinc mining areas (Zoropogui et al., 2008) and Cd-contaminated soils with rice crops (Siripornadulsil and Siripornadulsil, 2013); and *Enterobacter* sp. isolated from Cd-contaminated soils and the ability to bioremediate the metal, is reported (Xu et al., 2017; Pramanik et al., 2018; Bhattacharya et al.,

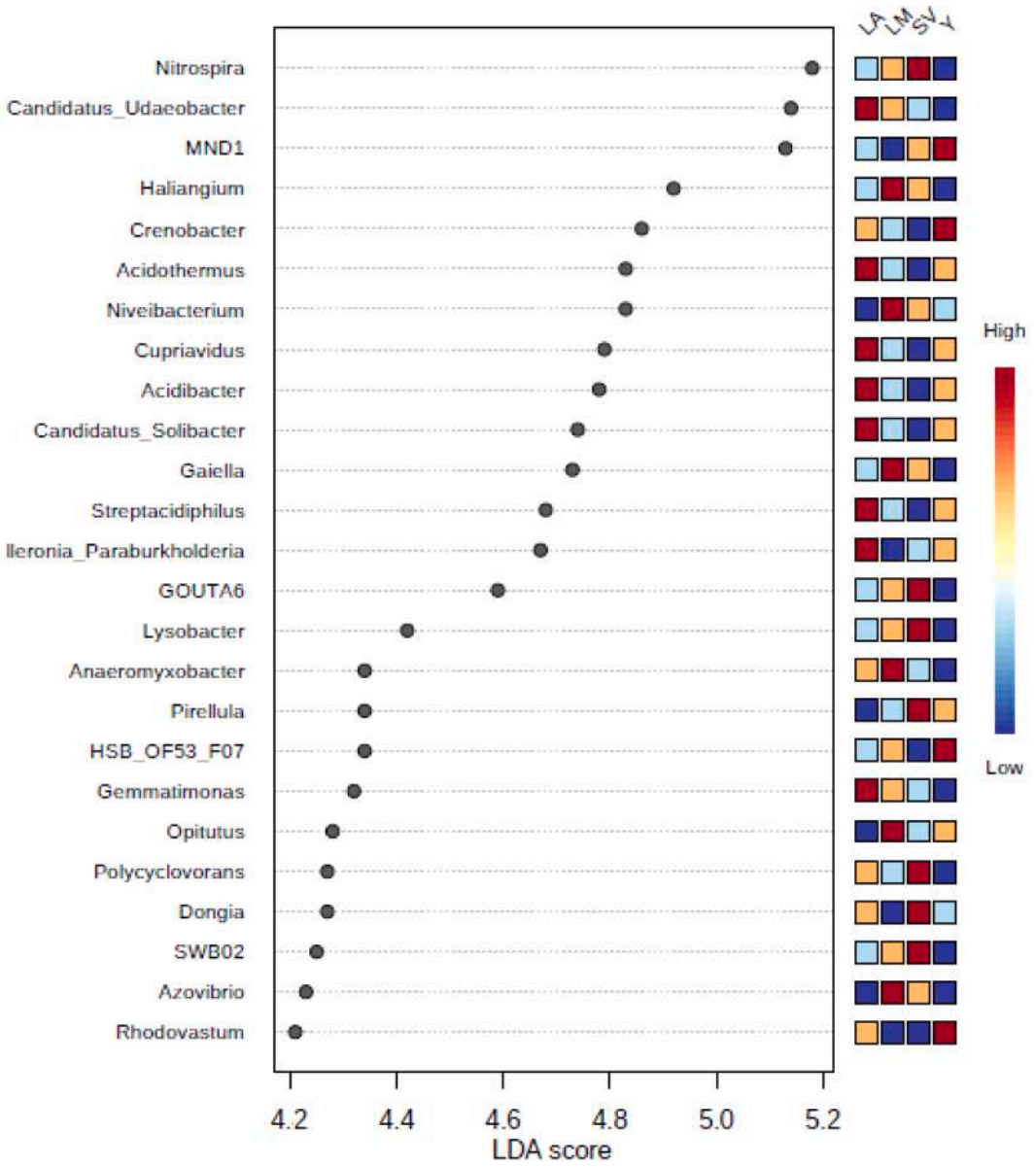

Figure 5. Linear discriminate analysis (LDA) of effect size (LEfSe) to identify differential taxa at the genus level in each cropping system. Argentina (LA); Siempre Viva (SV); Los Medios (LM); Yariguies (Y) farms.

2018). Worden et al., (2009) found that *Escherichia* sp. strains could have increased the ability to tolerate Cd; the genus *Klebsiella* sp., showed tolerance and action against Cd toxicity through biotransformation into inert CdS for *K. pneumoniae* (Holmes et al., 1997). Park et al. (2008) refer to *Ralstonia* sp.HM1 with the ability to transform Cd into CdS. Acording to Becerra-Castro, et al. (2011), they not only isolated a strain of *Dermacoccus* sp. (Actinobacteriota), but also identified this endophytic and rhizospheric bacterium in *Cytisus striatus* with resistance to Zinc.

In the phylum Firmicutes, *Bacillus* sp. were found and some isolated in sediments contaminated with Cd (Kim et al., 2015); Huang et al., (2013) reported the isolation of *Bacillus cereus* RC-1 in soils with Cd and its potential for Cd biosorption in living or dead cells as a bioaccumulation mechanism. *Exiguobacterium* sp. has been reported by Kumari et al., (2014) the capacity of immobilization of Cd in soil by the genus *E. undae* could be converted into $CdCO_3$. Ziagova et al., 2007, isolated *Staphylococcus Xylosus* from soil mining and reported the biosorption capacity of Cd and Cr. In turn, Sheng et al., (2016) reported the characterization of *Lactococcus lactis* subsp. *lactis* isolated from fermented pumpkins in the presence of 10 ppm Cd, with biosorption capacity. 5 of the 12 bacterial genera (*Burkholderia* sp., *Cupriavidus* sp., *Enterobacter* sp., *Escherichia* sp., and *Bacillus* sp.) characterized in this study, which are consistent with findings previously reported in soils cultivated with cocoa in Boyacá, Santander, and Arauca; these isolates were obtained using culture-dependent techniques with a single culture medium (agar layer) at a minimum concentration of 6 mg/L of $CdCl_2$ (Bravo et al., 2018). Our results complement the information for Colombia and expand the number of strains characterized with adaptive capacity against the contaminant with a tolerance of 120 mg/L of Cd, representing the first report with this capacity for *Exiguobacterium* sp., *Ralstonia* sp., *Serratia* sp., *Dermacoccus* sp., *Klebsiella* sp., *Lactococcus* sp. and *Staphylococcus* sp., thus confirming the greater diversity of Cd-tolerant bacteria present in soils of farms cultivated with cocoa. In addition to their ability to act against Cd, the bacterial genera have important functions at the soil level, for *Burkholderia* sp., *Cupriavidus* sp., *Enterobacter* sp., *Klebsiella* sp., and *Lactococcus* sp., the ability to fix N, root nodule formation, and biocontrol ability were reported for some species (Salles et al., 2006; Wolińska et al., 2017; Harindintwali et al., 2021; Higdon et al., 2020); *Enterobacter* sp., *Ralstonia* sp., *Serratia* sp.,

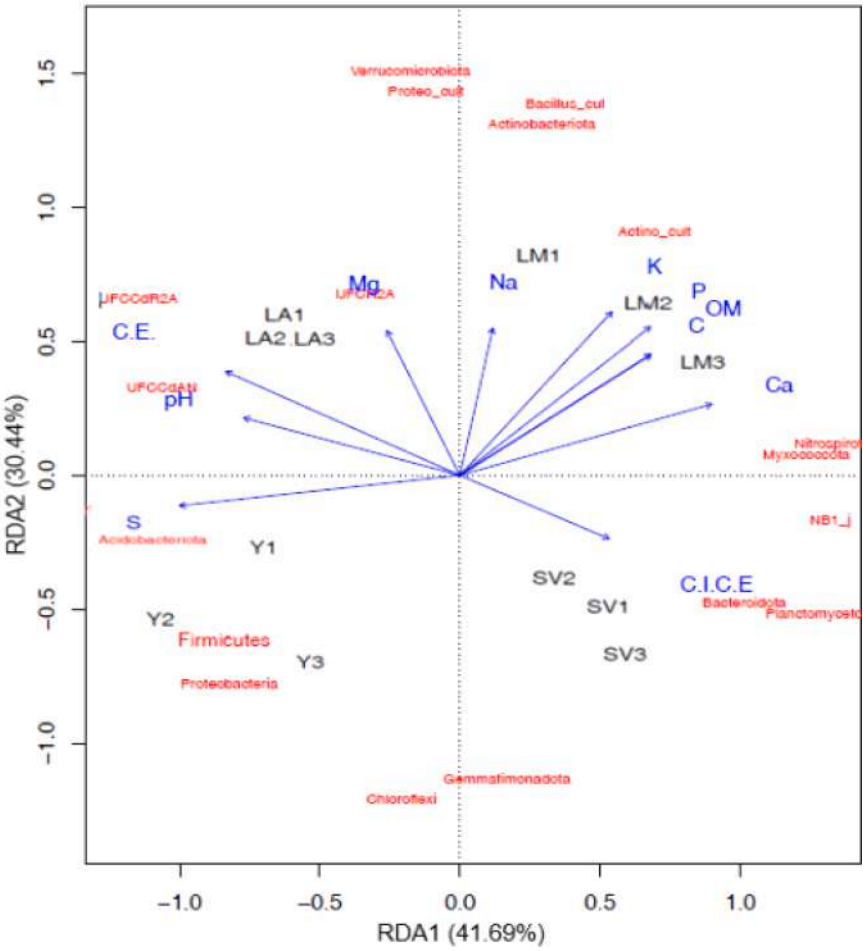

Figure 6. Redundancy analysis (RDA) ordination plots exhibited the relationship between selected soil properties and all soil bacterial communities.

Bacillus sp., *Exiguobacterium* sp., and *Staphylococcus* sp., are considered to have the ability to solubilize phosphates and promote plant growth (Gupta et al., 2019; Hirota et al., 2012; Blanco-Vargas et al., 2020; Sabaté et al., 2020; Pandey and Bhatt, 2016; Zhang et al., 2020), *Escherichia* sp. has the ability to produce polysaccharides that interact with soil and plants (Seo and Matthews, 2014), and *Dermacoccus* sp. can produce anti-protozoan secondary metabolites (Abdel-Magged et al., 2010).

To obtain the bacterial community structure profile, a culture-independent strategy, massive sequencing of the V4 region of the 16S ribosomal RNA gene, and a molecular marker commonly used for this purpose, were used. Cocoa-grown soil samples showed different concentrations of geogenic Cd, which adversely influences the diversity and abundance of microorganisms (Fierer, 2017). In contrast, the diversity indices reflect the composition of soil bacterial communities, and their variation is a response to environmental changes, including heavy metal contamination and related to the endemic level and heterogeneity (O'Brien et al., 2016). In addition to the high number of bacterial genera distributed at a low frequency, they are similar in all samples independent of Cd content (Table S1). According to Yin et al., 2019, the above may be closely related to the different native bacterial communities in soil and their tolerance to Cd, as well as the emergence and disappearance of some of the dominant groups under Cd stress.

The results indicate the degree of adaptation of bacterial communities is such that it is not possible to predict how they respond to this exposure, and no correlation was found between Cd and alpha-diversity indices (Stuart et al., 2010). For diversity indices (Shannon and Simpson), a positive correlation was shown against redox properties (C.E.C.I, pH), organic substances (OM, C), and minerals (Mn, Ca, P), generally associated with biological and bacterial activities (Fierer, 2017; Mann et al., 2019), results according to Liu et al., (2020) and Wu et al., (2017).

Bacterial communities at the phylum level were similar for all soils, even based on the Bray-Curtis distance separated according to the difference in soil pH. The main phyla in their order of relative abundance are: Proteobacteria, Acidobacteriota, Actinobacteriota, Verrucomicrobiota, Myxococota, Chloroflexi, Placntomycetota, and Bacteroidota have been previously reported in Cd contaminated soils; Proteobacteria and Acidobacteriota could resist metal toxicity depending on their complexation and adsorption capabilities (Liu et al., 2020; Luo et al., 2019; Wu et al., 2017; Cao et al., 2020). Not only can Cd concentration influence soil bacterial diversity and abundance, but also physicochemical conditions such as C, pH, C.E.C.I., P, K and microbial predator richness (Jones et al., (2009); Liu et al., (2020)), with the possibility of isolating and identifying native bacteria resistant to the metal.

Wu et al., (2017) and Liu et al., (2020) indicated the lack or weak statistically significant difference between alpha-diversity analysis (Shannon and Simpson) and soil bacterial communities at the phylum level versus soil Cd levels as they showed the option to tolerate different Cd concentrations through different mechanisms such as extracellular precipitation, adsorption at the cell wall level, enzymatic oxidation, and intracellular complexation.

Regarding some edaphic functions of the main phyla identified, the Proteobacteria phylum is widely diverse at the morphological, physiological, and metabolic levels, with paramount participation in the carbon, nitrogen, and sulfur cycles (Spain et al., 2009). In addition, the presence of the order Betaproteobacteriales, Myxococcales, Rhizobiales, NB1-j, and Desulfarcular, has been reported to be associated with an increase in the relative abundance of the phylum Proteobacteria in river

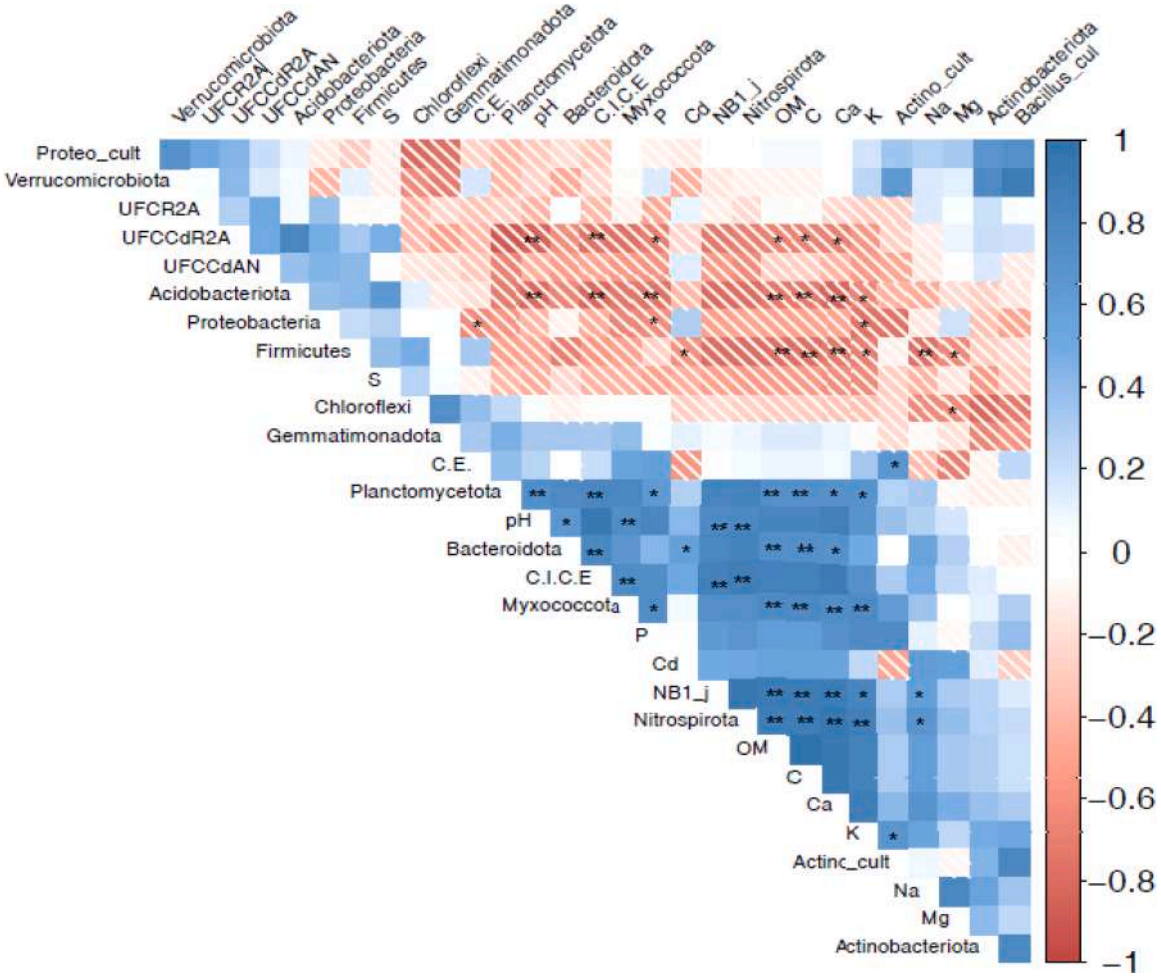

Figure 7. Heatmap between bacterial communities and soil physicochemical properties. The range of Spearman correlation coefficients (r) is indicated in the legend bar on the left side. The asterisks in the figure indicate significant Spearman correlations **p<0.01; *p≤ 0.05.

sediments in the presence of Cd (Xue et al., 2018). In the case of Acidobacteriota, this phylum can use carbon sources such as pectins, starches, and chitin, participating in nitrogen metabolism (Zhang et al., 2014). The phylum Actinobacteriota has been reported to be important in waste degradation, organic matter formation, and secondary metabolite production (Lewin et al., 2016). Verrucomicrobia is a phylum related to the fertility of cultivated soils (Navarrete et al., 2015), and Planctomycetes have distinctive characteristics such as the absence of peptidoglycan in their cell walls and their metabolism is mainly chemoheterotrophic (Chodak et al., 2013).

Bacteria genus with relative abundance (>0.5%) were identified: *Nitrospira*, candidatus *Udaeobacter, Haliangium, Cupriavidus*, MND1, *Bacillus, Kitasatospora, Niveibacterium, Acidothermus, Burkholderia, Acidibacter, Terrimonas, Gaiella*, ADurb.Bin063–1, candidatus *Solibacter, Kitasatospora, Sphingomonas*, and *Streptomyces*; these genera have been related to Cd tolerance processes, for example, Feng et al., (2018), reported the following genera from Cd contaminated soils: *Nitrospira, Bacillus, Burkholderia*, candidatus *Solibacter; Cupriavidus*; as for endophytic genera of Cd-tolerant transgenic tobacco: MND1, *Ralstonia, Sphingomonas, Streptomyces* (Wang et al., 2021), Lazzaro et al., (2006) identified the genera *Streptomyces* and *Kitasatospora* in forest soils with Cd; candidatus genera *Solibacter*, ADurb.Bin063–1 and *Acidibacter* present in soils with the presence of heavy metals (As, Pb, Cd, Zn) around an abandoned copper mine (Chun et al., 2021); Ma et al., (2020) reported *Burkholderia* and *Gaiella* in soils near Sb mines; as for the candidatus genus *Udaeobacter* it was reported as the most abundant genus in soils with the presence of Zn, Cd, Ni and Cu adjacent to an abandoned copper mine (Böhmer et al., 2020); genera such as *Nitrospira* and *Haliangium* were identified in soils with wheat crop contaminated with Cd (Song et al., 2020); Duan et al., (2020) monitored changes in soil bacterial communities in the presence of different Cd concentrations and reported the presence of *Acidothermus* and *Terrimonas*; the genus *Kitasatospora* has tolerance to heavy metals (Ni, Zn, and Cu) as published by Yun et al., (2020).

In context, using the culture-dependent and culture-independent techniques, complementary results were obtained in the characterization of the bacterial community of soils cultivated with cocoa in the presence of geogenic Cd; the culture-dependent technique identified the phyla Proteobacteria, Firmicutes, and Acidobacteriora, while the massive sequencing technique extended the microbial community to Proteobacteria, Acidobacteriota, Actinobacteriota, Verrucomicrobiota, Myxococcota, Chloroflexi, Planctomycetota, Bacteroidota, Gemmatimonadota, Nitrospirota, Firmicutes, NB1_J. In both techniques, Proteobacteria was the dominant phylum in soil samples. At the genus level, *Burkholderia* sp., *Exiguobacterium* sp., *Ralstonia* sp., *Bacillus* sp., *Cupriavidus* sp., *Serratia* sp., *Dermacoccus* sp., *Escherichia* sp., *Klebsiella* sp., *Lactococcus* sp. and *Staphylococcus* sp. By independent culture, 149 genera were identified, 6 of which coincided for both methodologies (% relative abundance) (*Burkholderia* sp. (0.90%), *Ralstonia* sp. (0.04%), *Bacillus* sp. (1.98%), *Cupriavidus* sp. (0.75%), *Serratia* sp. (0.02%), *Klebsiella* sp. (0.05%)).

To identify biomarkers at the genus level for the soils of each of the farms, a linear discriminant analysis together with the effect size (LDA-LEfSe) was performed. According to Figure 5, LA, LM, SV, Y farms had in

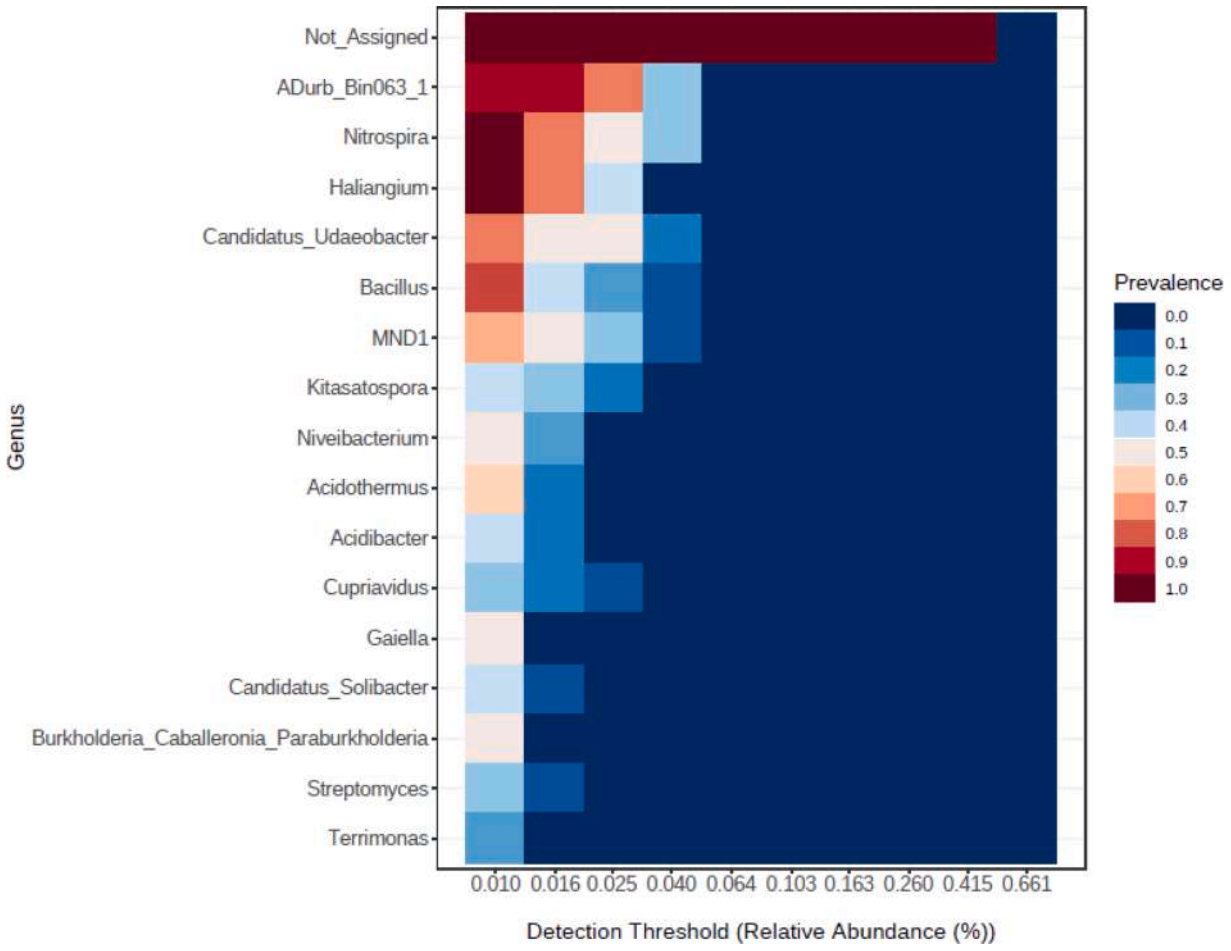

Figure 8. The core microbiome heatmap of genera found in all of samples.

their order 8, 6, 7, and 4 different taxa with an LDA score greater than 4.0. At the genus level for SV, Nitrospira (Nitrospirota) was the taxon with the highest differentiation, followed by GOUTA6 (Proteobacteria), *Lysobacter* (Proteobacteria), *Pirellula* (Planctomycetota), *Polycyclovorans* (Proteobacteria), *Dongia* (Proteobacteria), and SWB02 (Proteobacteria). For LA farm, the differentiating taxa were: candidatus *Udaeobacter* (Verrucomicrobiota), *Acidothermus* (Actinobacteriota), *Cupriavidus* (Proteobacteria), *Acidibacter* (Proteobacteria), candidatus *Solibacter* (Acidobacteriota), *Streptacidiphilus* (Actinobacteriota), *Burkholderia* (Proteobacteria), and *Gemmatimonas* (Gemmatimonadotas). As for LM farm, the highest taxa were *Haliangium* (Myxococcota), *Niveibacterium* (Proteobacteria), *Gaiella* (Actinobacteriota), *Anaeromyxobacter* (Myxococcota), *Opitutus* (Verrucomicrobiota), *Azovibrio* (Proteobacteria) and for Y farm, the highest taxa were MND1 (Proteobacteria) followed by *Crenobacter* (Proteobacteria), HSB-OF53-F07 (Chloroflexi), and *Rhodovastum* (Proteobacteria). These findings indicate that cocoa soils with the presence of geogenic Cd have metal-resistant bacterial genera, which cause reassembly of communities under Cd stress levels, coupled with the loss of metal-sensitive bacteria, which can generate microenvironments.

The effect of soil properties on bacterial communities identified was significantly correlated with variables such as pH, C.E.C.I, OM, C, and minerals P, K, Ca. However, no significant difference was found in the concentration of Cd in cocoa soils, probably due to the permanent presence of the metal in soil, which generates tolerance mechanisms by bacterial genera (Liu et al., 2020). According to Ma et al., (2020) soil pH and nutrients have an indirect effect on bacterial communities, and even the effects of soil properties and heavy metals were greater in soils with neutral pH, while pH had a greater impact in soils with acid pH. The results indicate that, according to the pH of cocoa soils, there is some difference in the relative abundance of the main phyla of the bacterial community of LA and Y farms (acid pH) versus LA and SV farms (neutral pH). This neutrality of the soil probably influenced the proliferation of eutrophic bacterial genera, which caused the decrease in the abundance of other strains (Ma et al., 2020; Mohapatra et al., 2011); moreover, soil nutrients can help microorganisms to resist heavy metal toxicity and increase bacterial metabolism (Gundacker et al., 2010). The above is confirmed by the correlation of the phyla of higher relative abundance of the bacterial community versus soil properties (Fig. 12)

The core community or bacterial communities present in all samples possibly play a fundamental role in the functioning of the ecosystem, being the bacteria indicative of phenomena occurring in the environment (Pereira et al., 2015). In this case, the core community was defined by the ASV's present in all soil samples. The genus *Nitrospira* sp. is ubiquitously distributed in toxic habitats and represents the predominant known nitrite oxidizers in nature, which catalyze the second step of nitrification and are also essential for the biogeochemical nitrogen cycle (Daims, H., 2014). Despite having a low relative abundance, it was possible to identify bacterial genera present in all the samples and that agrees with the identification of genera isolated by dependent culture such as *Cupriavidus* sp. belonging to the phylum Proteobacteria, which produce metabolites such as oxalates, phosphates, and sulfites to immobilize and reduce the accumulation of Cd in rice crops (Shi et al., 2020). Another genus in common among the techniques employed was *Bacillus* sp. reported with activity against pollutants, especially Cd and with carbon metabolic capacities (Chi et al., 2020), and the genus *Burkholderia* sp. is a plant growth promoter resistant to heavy metals including, Cd, which under its influence increases the activity of

antioxidant enzymes such as catalase, and superoxide dismutase (Ma et al., 2020). As for the genus ADurb.Bin063–1 (Verrucomicrobiota), is associated with carbohydrate degradation and *Haliangium* sp. degrades organic compounds and denitrification processes (Wang et al., 2019).

Candidatus *Udaeobacter* genus is a colonizer of oligotrophic soils and can survive and replicate by acquiring amino acids and vitamins from the soil (Venkatachalam et al., 2021).

Genus MND1 (Proteobacteria) is found in soils that are moderately or highly contaminated with heavy metals and is associated with nitrogen cycling (Chun et al., 2021), whereas Kitasatospora is associated with phosphorus solubilization processes (Tchakounté et al., 2018). *Acidothermus* contains genes involved in carbon metabolism (Gosai et al., 2018). Wang et al., (2021) identify *Acidibacter* and *Streptomyces* as endophytic bacteria resistant to Cd; for *Gaiella*, their increased relative abundance is related to their correlation against sucrase, urease, and alkaline phosphatase activity in soils with Cd presence (An et al., 2021). According to Niu et al., (2021) reported candidatus *Solibacter* is a dominant genus in phytoremediation processes in pots using Indian *mustard* and tall fescue grass in the presence of Cd; *Terramonas* not only tolerates Cd but increases its relative abundance, which benefits plant growth (She et al., 2021).

Conclusions

This work adds information to understand aspects of the interaction of bacterial communities associated with cocoa soils at different concentrations of geogenic Cd in the department of Santander, Colombia. The strategies of dependent and independent culture methods complement each other in the identification and increase in the variety of bacterial communities detected and registered so far in this environment. The phyla Proteobacteria, Actinobacteria and Firmicutes were found with predominance in all soil samples by the two strategies. Among the phyla identified, several strains of different genera with tolerance to high levels of Cd (120 mg/L) were characterized. The increase in the number of native strains of *Exiguobacterium* sp., *Ralstonia* sp., *Serratia* sp., *Dermacoccus* sp., *Klebsiella* sp., *Lactococcus* sp. and *Staphylococcus* sp. with the ability to tolerate higher Cd concentrations is reported as a novelty. (Fig. S6) with this capacity and consider its potential in future studies of metal inhibitory capacity.

Bacterial community composition was found to be significantly correlated with soil properties, where pH, OM and total Cd were the major factors of variation. The combination of different techniques in this research offers a new approach to the evaluation of bacterial communities in soils cultivated with cocoa under Cd stress in Colombia, providing information to recognize the ecological role of bacterial communities in future bioprospecting work to study their specific characteristics of tolerance or retention of Cd.

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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2021.100086.

References

An, Y., 2004. Soil ecotoxicity assessment using cadmium sensitive plants. Environ. Pollut. 127 (1), 21–26. https://doi.org/10.1016/S0269-7491(03)00263-X.

An, M., Chang, D., Hong, D., et al., 2021. Metabolic regulation in soil microbial succession and niche differentiation by the polymer amendment under cadmium stress. J. Hazard. Mater. 416, 126094 https://doi.org/10.1016/j.jhazmat.2021.126094.

Backer, R., Rokem, S., Ilangumaran, G., et al., 2018. Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Front. Plant Sci. 9, 1473. https://doi.org/10.3389/fpls.2018.01473.

Becerra-Castro, C., Kidd, P.S., Prieto-Fernández, Á., et al., 2011. Endophytic and rhizoplane bacteria associated with *Cytisus striatus* growing on hexachlorocyclohexane-contaminated soil: isolation and characterisation. Plant Soil 340, 413–433. https://doi.org/10.1007/s11104-010-0613-x.

Bhattacharya, A., Naik, S.N., Khare, S.K., 2018. Harnessing the bio-mineralization ability of urease producing *Serratia marcescens* and *Enterobacter cloacae* EMB19 for remediation of heavy metal cadmium (II). J. Environ. Manage. 215, 143–152. https://doi.org/10.1016/j.jenvman.2018.03.055.

Bissett, A., Brown, M.V., Siciliano, S.D., et al., 2013. Microbial community responses to anthropogenically induced environmental change: towards a systems approach. Ecol. Lett. 16, 128–139. https://doi.org/10.1111/ele.12109.

Blanco-Vargas, A., Rodríguez-Gacha, L., Sánchez-Castro, N., 2020. Phosphate-solubilizing Pseudomonas sp., and Serratia sp., co-culture for Allium cepa L. growth promotion. Heliyon 6 (10), e05218. https://doi.org/10.1016/j.heliyon.2020.e05218.

Böhmer, M., Ozdín, D., Račko, M., et al., 2020. Identification of bacterial and fungal communities in the roots of orchids and surrounding soil in heavy metal contaminated area of mining heaps. Appl. Sci. 10, 7367. https://doi.org/10.3390/app10207367.

Bravo, D., Pardo-Díaz, S., Benavides-Erazo, J., et al., 2018. Cadmium and cadmium-tolerant soil bacteria in cacao crops from northeastern Colombia. J. Appl. Microbiol. 124 (5), 1175–1194. https://doi.org/10.1111/jam.13698.

Callahan, B., McMurdie, P., Rosen, M., et al., 2016. DADA2: high-resolution sample inference fromIllumina amplicon data. Nat. Methods 13 (7), 581–583. https://doi.org/10.1038/nmeth.3869.

Cao, X., Luo, J., Wang, X, et al., 2020. Responses of soil bacterial community and Cd phytoextraction to a Sedum alfredii-oilseed rape (Brassica napus L. and Brassica juncea L.) intercropping system. Sci. Total Environ. 723, 138–152. https://doi.org/10.1016/j.scitotenv.2020.138152.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., et al., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336. https://doi.org/10.1038/nmeth0510-335.

Chavez, E., He, Z.L., Stofella, P.J., et al., 2015. Concentration of cadmium in cacao beans and its relationship with soil cadmium in southern Ecuador. Sci. Total Environ. 533, 205–214. https://doi.org/10.1016/j.scitotenv.2015.06.106.

Chaves-López, C., Serio, A., Grande-Tovar, C.D., et al., 2014. Traditional fermented foods and beverages from a microbiological and nutritional perspective: the colombian heritage. Compreh. Rev. Food Sci. Foos Saf. 13 (5), 1031–1048. https://doi.org/10.1111/1541-4337.12098.

Chodak, M., Gołębiewski, M., Morawska-Płoskonka, J., et al., 2013. Diversity of microorganisms from forest soils differently polluted with heavy metals. Appl. Soil Ecol 64, 7–14. https://doi.org/10.1016/j.apsoil.2012.11.004.

Chun, S., Kim, Y., Cui, Y., et al., 2021. Ecological network analysis reveals distinctive microbial modules associated with heavy metal contamination of abandoned mine soils in Korea. Environ. Pollut. 289, 117851 https://doi.org/10.1016/j.envpol.2021.117851.

Daims, H., 2014. The family nitrospiraceae. In: Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), The Prokaryotes. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-38954-2_126.

Dhariwal, A., Chong, J., Habib, S., et al., 2017. MicrobiomeAnalyst: a web-based tool for comprehensive statistical, visual and metaanalysis of microbiome data. Nucl. Acids. Res. 45, W180–W188. https://doi.org/10.1093/nar/gkx295.

Ding, C., Ma, Y., Li, X., et al., 2018. Determination and validation of soil thresholds for cadmium based on food quality standard and health risk assesment. Sci. Total Environ. 619-620, 700–706. https://doi.org/10.1016/j.scitotenv.2017.11.137.

Duan, C., Liu, Y., Zhang, H., et al., 2020. Cadmium pollution impact on the bacterial community of haplic cambisols in Northeast China and inference of resistant Genera. J. Soil Sci. Plant Nutrit. 20, 1156–1170. https://doi.org/10.1007/s42729-020-00201-5.

Eiler, A., Langenheder, S., Bertilsson, S., et al., 2003. Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations. Appl. Environ. Microbiol. 69 (7), 3701–3709. https://doi.org/10.1128/AEM.69.7.3701-3709.2003.

EU, 2014. COMMISSION REGULATION (EU) No 488/2014 amending regulation (EC) No 1881/2006 as regards maximum levels of cadmium in foodstuffs. Off. J. Eur. Union 138, 75.

Ezekoye, C.C., Chikere, C.B., Okpokwasili, G.C, 2018. Field metagenomics of bacterial community involved in bioremediation of crude oil polluted soil. J. Bioremediat. Biodegradat. 9 (5), 449. https://doi.org/10.4172/2155-6199.1000449.

Feng, G., Xie, T., Wang, X., et al., 2018. Metagenomic analyst of microbial community and fuction involved in Cd Contaminated soil. BMC Microbiol. 18 (11) https://doi.org/10.1186/s12866-018-1152-5.

Fierer, N., Leff, J.W., Adams, B.J., et al., 2012. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. PNAS 109 (52), 21390–21395. https://doi.org/10.1073/pnas.1215210110.
Fierer, N., 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. Nat. Rev. Microbiol. 15, 579–590. https://doi.org/10.1038/nrmicro.2017.87.
García, G., Márquez, G., Moreno, C., 2016. Characterization of bacterial diversity associated with calcareous deposits and drip-waters, and isolation of calcifying bacteria from two Colombian mines. Microbiol. Res. 182, 21–30. http://doi.org/10.1016/j.micres.2015.09.006.
Gomez, A.M., Yannarell, A.C., Sims, G.K., et al., 2011. Characterization of bacterial diversity at different depths in the Moravia Hill landfill site at Medellín, Colombia. Soil Biol. Biochem. 43 (6), 1275–1284. https://doi.org/10.1016/j.soilbio.2011.02.018.
Gómez-Sagasti, M.T., Alkorta, I., Becerril, J.M., et al., 2012. Microbial monitoring of the recovery of soil quality during heavy metal phytoremediation. Water Air Soil Pollut. 223, 3249–3262. https://doi.org/10.1007/s11270-012-1106-8.
Gosai, H., Sachaniya, B., Panseriya, H., et al., 2018. Functional and phylogenetic diversity assessment of microbial communities at Gulf of Kachchh, India: An ecological footprint. Ecol. Indic. 93, 65–75. https://doi.org/10.1016/j.ecolind.2018.04.072.
Gundacker, C., Gencik, M., Hengstschläger, M., 2010. The relevance of the individual genetic background for the toxicokinetics of two significant neurodevelopmental toxicants: mercury and lead. Mutat. Res. 705, 130–140. https://doi.org/10.1016/j.mrrev.2010.06.003.
Gupta, N., Skinner, K., Khan, S., et al., 2019. Draft genome sequence of Enterobacter sp. strain A8, a Carbazole-Degrading bacterium. Microbiol. Resour. Announcement 8, 18. https://doi.org/10.1128/MRA.00301-19 e00301-19.
Harindintwali, J., Zhou, J., Habimana, I., et al., 2021. Biotechnological potential of cellulolytic nitrogen-fixing Klebsiella sp. C-3 isolated from paddy soil. Bioresour. Technol. Rep. 13, 100624 https://doi.org/10.1016/j.biteb.2021.100624.
Hashimoto, T., Whang, K., Nagaoka, K., 2006. A quantitative evaluation and phylogenetic characterization of oligotrophic denitrifying bacteria harbored in subsurface upland soil using improved culturability. Biol. Fertility Soils 42 (3), 179–185.
He, S., He, Z., Yang, X., et al., 2015. Soil biogeochemistry, plant physiology and phytoremediation of cadmium contaminated soils. Adv. Agron. 134, 135–225. https://doi.org/10.1016/bs.agron.2015.06.005.
Higdon, S., Huang, B., Bennett, A., et al., 2020. Identification of Nitrogen Fixation Genes in Lactococcus Isolated from Maize Using Population Genomics and Machine Learning. Microorganisms. 8 (12), 2043. https://doi.org/10.3390/microorganisms8122043.
Hirota, R., Yamane, S., Fujibuchi, T., 2012. Isolation and characterization of a soluble and thermostable phosphite dehydrogenase from Ralstonia sp. strain 4506. J. Biosci. Bioeng. 113 (4), 445–450. https://doi.org/10.1016/j.jbiosc.2011.11.027.
Holmes, J., Richardson, D., Saed, S., et al., 1997. Cadmium-specific formation of metal sulfide 'Q-particles' by *Klebsiella pneumoniae*. Microbiology (Reading) 143 (8), 2521–2530. https://doi.org/10.1099/00221287-143-8-2521.
Huang, F., Dang, Z., Guo, C., et al., 2013. Biosorption of Cd (II) by live and dead cells of *Bacillus cereus* RC-1 isolated from cadmium-contaminated soil. Colloids Surf. B 107, 11–18. https://doi.org/10.1016/j.colsurfb.2013.01.062.
Hug, L.A., Baker, B.J., Anantharaman, K., et al., 2016. A new view of the tree of life. Nature Microbiol. 1 (16048), 1–6. https://doi.org/10.1038/nmicrobiol.2016.48.
Jensen, M.A., Webster, J.A., Straus, N., 1993. Rapid identification of bacteria on the basis of polymerase chain reaction-amplified ribosomal DNA spacer polymorphisms. Appl. Environ. Microbiol. 59 (4), 945–952. https://doi.org/10.1128/AEM.59.4.945-952.1993.
Jiang, C., Sheng, X., Qian, M., et al., 2008. Isolation and characterization of a heavy metal-resistant *Burkholderia* sp. from heavy metal-contaminated paddy field soil and its potential in promoting plant growth and heavy metal accumulation in metal-polluted soil. Chemosphere 72 (2), 157–164. https://doi.org/10.1016/j.chemosphere.2008.02.006.
Jones, R., Robeson, M., Lauber, C., et al., 2009. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. ISME J. 3, 442–453. https://doi.org/10.1038/ismej.2008.127.
Joseph, S., Hugenholz, P., Sangwan, P., et al., 2003. Laboratory cultivation of widespread and previously uncultured soil bacteria. Appl. Environ. Microbiol. 69, 7210–7215. https://doi.org/10.1128/AEM.69.12.7210-7215.2003.
Kim, S., Jin, M., Chung, C., Yun, Y., Jahng, K., Yu, K., 2015. Biosortion of cationic basic dye and cadmium by the novel biosorbent Bacillus catenulatus JB-022 strain. J. Biosci. Bioeng. 119 (4), 433–439. https://doi.org/10.1016/j.jbiosc.2014.09.022.
Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120.
Kirkham, M., 2006. Cadmium in plants on polluted soils: effect of soil factors, hyperaccumulation and amendments. Geoderma 137, 19–32. https://doi.org/10.1016/j.geoderma.2006.08.024.
Kumari, D., Pan, X., Lee, D., et al., 2014. Immobilization of cadmium in soil by microbially induced carbonate precipitation with Exiguobacterium undae at low temperature. Short communication. Int. Biodeterior. Biodegrad. 94, 98–102. https://doi.org/10.1016/j.ibiod.2014.07.007.
Lazzaro, A., Hartmann, M., Blaser, P., et al., 2006. Bacterial community structure and activity in diferent Cd-treated forest soils. FEMS Microbiol. Ecol. 58, 278–292. https://doi.org/10.1111/j.1574-6941.2006.00163.x.
Lewin, G.R., Carlos, C., Chevrette, M.G., et al., 2016. Evolution and ecology of *Actinobacteriota* and their bioenergy applications. Annu. Rev. Microbiol. 70, 235–254. https://doi.org/10.1146/annurev-micro-102215-095748.
Liu, H, Xie, Y, Li, J., et al., 2020. Efect of *Serratia* sp. K3 combined with organic materials on cadmium migration in soil-Vetiveria zizanioides L. system and bacterial community in contaminated soil. Chemosphere 242, 125164. https://doi.org/10.1016/j.chemosphere.2019.125164.
Luo, L.Y., Xie, L.L., Jin, D.C., et al., 2019. Bacterial community response to cadmium contamination of agricultural paddy soil. Appl. Soil Ecol. 139, 100–106. https://doi.org/10.1016/j.apsoil.2019.03.022.
Ma, Y., Wang, Y., Chen, Q., et al., 2020. Assessment of heavy metal pollution and the effect on bacterial community in acidic and neutral soils. Ecol. Indic. 117, 106626 https://doi.org/10.1016/j.ecolind.2020.106626.
Mann, C., Lynch, D., Fillmore, S., et al., 2019. Relationships between field management, soil health, and microbial community composition. Appl. Soil Ecol 144, 12–21. https://doi.org/10.1016/j.apsoil.2019.06.012.
Mantilla, L.C., Bissig, T., Valencia, V., et al., 2013. The magmatic history of the Vetas-California mining district, Santander Massif, Eastern Cordillera, Colombia. J. South Amer. Earth Sci. 45, 235–249. https://doi.org/10.1016/j.jsames.2013.03.006.
Ministerio de Agricultura 2019. Agronet: Reporte: Área, Producción, Rendimiento y Participación Municipal en el Departamento por Cultivo. https://www.agronet.gov.co.
Mitra, S., Pramanik, K., Kumar, P., et al., 2018. Characterization of Cd-resistant *Klebsiella michiganensis* MCC3089 and its potential for rice seedling growth promotion under Cd stress. Microbiol. Res. 210, 12–25. https://doi.org/10.1016/j.micres.2018.03.003.
Mohapatra, B., Gould, W., Dinardo, O., Koren, D., 2011. Tracking the prokaryotic diversity in acid mine drainage-contaminated environments: a review of molecular methods. Minerals Engineer 24, 709–718. https://doi.org/10.1016/j.mineng.2011.03.012.
Moreno, C., Romero, J., Espejo, R.T., 2002. Polymorphism in repeated 16S rRNA genes is a common property of type strains and environmental isolates of the genus *Vibrio*. Microbiology 148 (4), 1233–1239. https://doi.org/10.1099/00221287-148-4-1233.
Navarrete, A.A., Soares, T., Rossetto, R., et al., 2015. Verrucomicrobial community structure and abundance as indicators for changes in chemical factors linked to soil fertility. Antonie Van Leeuwenhoek 108, 741–752. https://doi.org/10.1007/s10482-015-0530-3.
Niu, H., Leng, Y., Li, X., et al., 2021. Behaviors of cadmium in rhizosphere soils and its interaction with microbiome communities in phytoremediation. Chemosphere 269, 128765. https://doi.org/10.1016/j.chemosphere.2020.128765.
O'Brien, S.L., Gibbons, S.M., Owens, S.M., et al., 2016. Spatial scale drives patterns in soil bacterial diversity. Environ. Microbiol. 6, 2039–2051. https://doi.org/10.1111/1462-2920.13231.
Oksanen, J., Blanchet, F., Kindt, R., et al., 2016. Vegan: community ecology package: ordination, diversity and dissimilarities, version 2.3-5. https://cran.r-project.org.
Osorio, W., Ruiz, O., 2013. Guía para el muestreo. Laboratorio de suelos. Universidad Nacional de Colombia, sede Medellín, Colombia. https://ciencias.medellin.unal.edu.co/laboratorios/suelos/.
Pandey, N., Bhatt, R., 2016. Role of soil associated Exiguobacterium in reducing arsenic toxicity and promoting plant growth in Vigna radiata. Eur. J. Soil Biol. 75, 142–150. https://doi.org/10.1016/j.ejsobi.2016.05.007.
Park, Y., Ko, J., Yun, S., et al., 2008. Enhancement of bioremediation by *Ralstonia* sp. HM-1 in sediment polluted by Cd and Zn. Bioresour. Technol. 99, 7458–7463. https://doi.org/10.1016/j.biortech.2008.02.024.
Pereira, L., Vicentini, R., Ottobini, L., 2015. Short Communicarion. Characterization of the core microbiota of the drainage and surrounding soil of Brazilian copper mine. Genet. Mol. Biol. 38 (4), 484–489. https://doi.org/10.1590/S1415-475738420150025.
Porras, L.D., Torres, J.D., Gil, M.A., et al., 2019. Effect of the solar drying process on the sensory and chemical quality of cocoa (*Theobroma cacao* L.) cultivated in Antioquia, Colombia. Food Res. Int. 115, 259–267. https://doi.org/10.1016/j.foodres.2018.08.084.
Pradhan, P., Costa, L., Rybski, D., et al., 2017. A Systematic Study of Sustainable Development Goal (SDG) Interactions. Earth's Future 2017 5, 1169–1179. https://doi.org/10.1002/2017EF000632.
Pramanik, K., Mitra, S., Sarkar, A., et al., 2018. Alleviation of phytotoxic effects of cadmium on rice seedlings by cadmium resistant PGPR strain *Enterobacter aerogenes* MCC 3092. J. Hazard. Mater. 351, 317–329. https://doi.org/10.1016/j.jhazmat.2018.03.009.
Puentes-Páramo, Y., Menjivar-Flores, J., Gómez-Carabalí, A., et al., 2014. Absorción y distribución de nutrientes en clones de cacao y sus efectos en el rendimiento. Acta Agronómica 63 (2), 145–152. https://doi.org/10.15446/acag.v63n2.40041.
Quast, C., Pruesse, E., Yilmaz, P., et al., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, 590–596. https://doi.org/10.1093/nar/gks1219.
Sabaté, D., Petroselli, G., Erra-Balsells, R., et al., 2020. Beneficial effect of *Bacillus* sp. P12 on soil biological activities and pathogen control in common vean. Biol. Control 141, 104131. https://doi.org/10.1016/j.biocontrol.2019.104131.
Salles, J., Samyn, E., Vandamme, P., et al., 2006. Changes in agricultural management drive the diversity of Burkholderia species isolated from soil on PCAT medium. Soil Biol. Biochem. 38 (4), 661–673. https://doi.org/10.1016/j.soilbio.2005.06.018.
Segata, N., Izard, J., Waldron, L., et al., 2011. Metagenomic biomarker discovery and explanation. Genome Biol. 12, R60.
Seo, S., Matthews, K., 2014. Exposure of *Escherichia coli* O157:H7 to soil, manure, or water influences its survival on plants and initiation of plant defense response. Food Microbiol. 38, 87–92. https://doi.org/10.1016/j.fm.2013.08.015.
Shanying, H., Zhenli, H., Xiaoe, Y., et al., 2015. Chapter four - soil biogeochemistry, plant physiology, and phytoremediation of cadmium-contaminated soils. Advances in

agronomy. Academic Press 134, 135–225. https://doi.org/10.1016/bs.agron.2015.06.005.
She, J., Wang, J., Wei, X., et al., 2021. Survival strategies and dominant phylotypes of maize-rhizosphere microorganisms under metal(loid)s contamination. Sci. Total Environ. 774, 145143 https://doi.org/10.1016/j.scitotenv.2021.145143.
Sheng, Y., Wang, Y., Yang, X., et al., 2016. Cadmium tolerant characteristic of a newly isolated Lactococcus lactis subsp. lactis. Environ. Toxicol. Pharmacol. 48, 183–190. https://doi.org/10.1016/j.etap.2016.10.007.
Shi, Z., Zhang, Z., Yuan, M., et al., 2020. Characterization of a high cadmium accumulating soil bacterium. *Cupriavidus sp.* WS2. Chemosphere 247, 125834. https://doi.org/10.1016/j.chemosphere.2020.125834.
Siripornadulsil, S., Siripornadulsil, W., 2013. Cadmium-tolerant bacteria reduce the uptake of cadmium in rice: Potential for microbial bioremediation. Ecotoxicol. Environ. Saf. 94, 94–103. https://doi.org/10.1016/j.ecoenv.2013.05.002.
Song, L, Pan, Z, Dai, Y, et al., 2020. Characterization and comparison of the bacterial communities of rhizosphere and bulk soils from cadmium-polluted wheat fields. PeerJ 8, e10302 http://doi.org/10.7717/peerj.10302.
Spain, A.M., Krumholz, L.R., Elshahed, M.S., 2009. Abundance, composition, diversity and novelty of soil Proteobacteria. ISME J. 3, 992–1000. https://doi.org/10.1038/ismej.2009.43.
Soliman, T., Yang, S., Yamazaki, T., et al., 2017. Profiling soil microbial communities with next-generation sequencing: the influence of DNA kit selection and technician technical expertise. PeerJ 5, e4178. https://doi.org/10.7717/peerj.4178.
Stuart, E., Jones, J., Lennon, T., 2010. Dormancy contributes to the maintenance of microbial diversity. Proc. Natl. Acad. Sci. USA 107 (13), 5881–5886. https://doi.org/10.1073/pnas.0912765107.
Sun, R., Wang, L., Huang, R., et al., 2020. Cadmium resistance mechanisms of a functional strain *Enterobacter* sp. DNB-S2, isolated from black soil in Northeast China. Environ. Pollut. 263, B, 114612 https://doi.org/10.1016/j.envpol.2020.114612.
Tchakounté, G., Berger, B., Patz, S., et al., 2018. Community structure and plant growth-promoting potential of cultivable bacteria isolated from Cameroon soil. Microbiol. Res. 214, 47–59. https://doi.org/10.1016/j.micres.2018.05.008.
Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positionspecific gap penalties and weight matrix choice. Nucl. Acids Res. 22, 4673–4680. https://doi.org/10.1093/nar/22.22.4673.
Wang, X., Ya, T., Zhang, M., et al., 2019. Cadmium (II) alters the microbial community structure and molecular ecological network in activated sludge system. Environ. Pollut. 255, 113225 https://doi.org/10.1016/j.envpol.2019.113225, 0269-7491.
Wang, G., Yang, D., Wang, W., et al., 2021. Endophytic bacteria associated with the enhanced cadmium resistance in NHX1- overexpressing tobacco plants. Environ. Exp. Botany. 188, 104524 https://doi.org/10.1016/j.envexpbot.2021.104524.
Wu, W., Dong, C., Wu, J., et al., 2017. Ecological effects of soil properties and metal concentrations on the composition and diversity of microbial communities associated with land use patterns in an electronic waste recycling region. Sci. Total Environ. 601, 57–65. https://doi.org/10.1016/j.scitotenv.2017.05.165.
Wolińska, A., Kuźniar, A., Zielenkiewicz, U., et al., 2017. Metagenomic analysis of some potential nitrogen-fixing bacteria in arable soils at different formation processes. Microb. Ecol. 73, 162–176. https://doi.org/10.1007/s00248-016-0837-2.
Worden, C., Kovac, W., Dorn, L., et al., 2009. Environmental pH affects transcriptional responses to cadmium toxicity in *Escherichia coli* K-12 (MG1655). FEMS Microbiol. Lett. 293 (1), 58–64. https://doi.org/10.1111/j.1574-6968.2009.01508.x.
World Health Organization, 2010. Exposure to cadmium: a major public health concern. Preventing Disease Through Healthy Environments.
Xiaoqi, L., Delong, M., Juan, L., et al., 2017. Response of soil microbial communities and microbial interactions to long-term heavy metal contamination. Environ. Pollut. 231 (1), 908–917. https://doi.org/10.1016/j.envpol.2017.08.057.
Xu, C., He, S., Liu, Y., et al., 2017. Bioadsorption and biostabilization of cadmium by *Enterobacter cloacae* TU. Chemosphere 173, 622–629. https://doi.org/10.1016/j.chemosphere.2017.01.005.
Xue, W., Peng, Z., Huang, D., et al., 2018. Nanoremediation of cadmium contaminated river sediments: microbial response and organic carbón changes. J. Hazard. Mater. 290–299. https://doi.org/10.1016/j.jhazmat.2018.07.062.
Yang, Y., Chen, W., Wang, M., et al., 2016. Regional accumulation characteristics of cadmium in vegetables: influencing factors, transfer model and indication of soil threshold content. Environ. Pollut. 219, 1036–1043. https://doi.org/10.1016/j.envpol.2016.09.003.
Yim, O., Ramdeen, K., 2015. Hierarchical cluster analysis: comparison of three linkage measures and application to psychological data. Quant. Method. Psychol. 11 (1), 8–21. https://doi.org/10.20982/tqmp.11.1.p008.
Yin, P., Liu, X., Liao, J., et al., 2019. Effects of cadmium stress on microbial community diversity in soil potted with Sasa Argenteastriatus. In: IOP Conference Series Earth and Environmental Science, 300, 052051. https://doi.org/10.1088/1755-1315/300/5/052051.
Yun, B., Malik, A., Kim, S., 2020. Genome based characterization of Kitasatospora sp. MMS16-BH015, a multiple heavy metal resistant soil actinobacterium with high antimicrobial potential. Gene. 733, 144379 https://doi.org/10.1016/j.gene.2020.144379.
Zhang, Y., Zheng, L., Zheng, Y., et al., 2020. Insight into the assembly of root-associated microbiome in the medicinal plant Polygonum cuspidatum. Ind. Crops Prod. 145, 112163 https://doi.org/10.1016/j.indcrop.2020.112163.
Zoropogui, A., Gambarelli, S., Covès, J., 2008. *CzcE* from *Cupriavidus metallidurans* CH34 is a copper-binding protein. Biochem. Biophys. Res. Commun. 365 (4), 735–739. https://doi.org/10.1016/j.bbrc.2007.11.030.