Science of the Total Environment 766 (2021) 142643

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Impact of flooding on urban soils: Changes in antibiotic resistance and bacterial community after Hurricane Harvey

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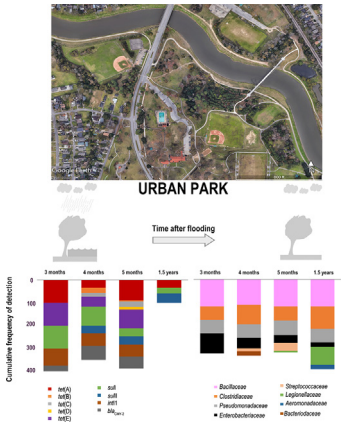
HIGHLIGHTS

- Occurrence of resistance genes was higher short time vs long time after flooding.
- Occurrence of bla_{CMY-2} and *int*I1 was associated to changes in microbial diversity.
- *tet*(E), bla_{CMY-2}, *int*I1 could be markers of soils' exogenous antibiotic resistance.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 August 2020
Received in revised form 21 September 2020
Accepted 23 September 2020
Available online 3 October 2020

Editor: Damia Barcelo

Keywords:
Hurricane Harvey
Microbial community perturbations
Antibiotic-resistance genes
Multiple correspondence analysis
QIIME2
Taxonomy analysis

ABSTRACT

Major perturbations in soil and water quality are factors that can negatively impact human health. In soil environments of urban areas, changes in antibiotic-resistance profiles may represent an increased risk of exposure to antibiotic-resistant bacteria via oral, dermal, or inhalation routes. We studied the perturbation of antibiotic-resistance profiles and microbial communities in soils following a major flooding event in Houston, Texas, caused by Hurricane Harvey. The main objective of this study was to examine the presence of targeted antibiotic-resistance genes and changes in the diversity of microbial communities in soils a short time (3–5 months) and a long time (18 months) after the catastrophic flooding event. Using polymerase chain reaction, we surveyed fourteen antibiotic-resistance elements: *int*I1, *int*I2, *sul*1, *sul*2, *tet*(A) to (E), *tet*(M), *tet*(O), *tet*(W), *tet*(X), and bla_{CMY-2}. The number of antibiotic-resistance genes detected were higher in short-time samples compared to samples taken a long time after flooding. From all the genes surveyed, only *tet*(E), bla_{CMY-2}, and *int*I1 were prevalent in short-time samples but not observed in long-time samples; thus, we propose these genes as indicators of exogenous antibiotic resistance in the soils. Sequencing of the V3-V4 region of the bacterial 16S rRNA gene was used to find that flooding may have affected bacterial community diversity, enhanced differences among bacterial lineages profiles, and affected the relative abundance of *Actinobacteria*, *Verrucomicrobia*, and *Gemmatimonadetes*. A major conclusion of this study is that antibiotic resistance profiles of soil bacteria are impacted by urban flooding events such that they may pose an enhanced risk of exposure for up to three to five months following the hurricane. The occurrence of targeted antibiotic-resistance elements decreased eighteen months after the hurricane indicating a reduction of the risk of exposure long time after Harvey.

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https://doi.org/10.1016/j.scitotenv.2020.142643
0048-9697/

1. Introduction

According to the Centers for Disease Control and Prevention, 2.8 million people in the U.S. are infected with antibiotic-resistant microorganisms, and approximately 1 in 100 of these individuals die of infection each year (USCDC, 2019). Globally, the antimicrobial-resistance surveillance of the World Health Organization reports that in 2018, countries such as Germany, Japan, and the United Kingdom may each have over 10,000 patients infected with high priority antibiotic-resistant pathogens (WHO, 2018). This worldwide public health crisis has been exacerbated due to overuse of antibiotics that have enhanced selection and dissemination of antibiotic-resistance genes among commensal and pathogenic bacteria (Wright, 2019). Dissemination of antibiotic-resistance genes is facilitated by horizontal gene transfer mechanisms that allow bacteria in the same niche to share genetic material (Ochman et al., 2000; Forsberg et al., 2012).

Antibiotic-resistance genes are found naturally in soil bacteria (Allen et al., 2009; Martínez et al., 2015). However, soils that have been perturbed and contain either a high abundance or a high diversity of antibiotic-resistance genes may pose an important risk to public health due to potential oral, dermal and inhalation exposure to these soils. Some studies on extreme flooding events have demonstrated that floodwaters have sewage signatures (Amaral-Zettler et al., 2008) and induce contamination and dissemination of bacterial pathogens, such as fecal coliforms or enteropathogenic bacteria (Divakaran et al., 2019; Gowrisankar et al., 2017; Kapoor et al., 2018; Yu et al., 2018). Consequently, flooding could be a means of soil contamination with exogenous bacteria and could result in higher abundance of antibiotic-resistance genes or genetic diversity compared to natural background levels.

In a four-day period during August 2017, the category 4 Hurricane Harvey delivered the approximate equivalent of one year's rainfall to the Houston metropolitan area, resulting in catastrophic flooding (Blake and Zelinsky, 2018) as well as overflow of hospital, industrial, and domestic wastewater-treatment plants and sanitary sewers (Du et al., 2020; Friedrich, 2017; TCEQ, 2018). Following Harvey, Kapoor et al. (2018) reported that one to four months after the hurricane, fecal indicator bacteria and human-associated fecal genetic markers were prevalent in heavily flooded areas of the Guadalupe River in Texas. Yu et al. (2018) reported that: (a) levels of the fecal indicator *E. coli* and two markers of anthropogenic antibiotic resistance (gene *sul*1 and class 1 integron *int*I1) in flowing water from urban rivers in Houston were higher days after the hurricane compared to months post flood, (b) pathogen gene markers were more abundant in flood-mobilized sediments one month after Harvey compared to surface soils (0–5 cm) and deep-soil cores (15–20 cm) three months post flood, and (c) microbial community composition of floodwater was different than community composition of sediment and soil. An important parameter that has yet to be addressed is how flooding impacts antibiotic resistance and microbial community composition of topsoil over time. The topsoil communities may have different kinetics for antibiotic resistance than flowing water. This represents an important unanswered question because changes in antibiotic-resistance profiles may represent an increased risk of exposure to antibiotic-resistant bacteria in soils.

To address how a major flooding event may impact resistance and microbial community profiles, we examined the presence of targeted antibiotic-resistance genes and changes in the diversity of bacterial communities in soils over a short time (3–5 months) and a long time (18 months) after Hurricane Harvey. We surveyed genes that encode for insertion of antibiotic-resistance genes (*int*I1 and *int*I2) and genes that confer resistance to sulfonamide (*sul*1, and *sul*2), tetracycline (*tet*(A), *tet*(B), *tet*(C), *tet*(D), *tet*(E), *tet*(M), *tet*(O), *tet*(W), and *tet*(X)) and beta lactam (bla_{CMY-2}) antibiotics. We targeted specific antibiotic-resistance genes that include three main mechanisms of antibiotic resistance i.e., efflux of the antibiotic (*tet*(A) to (E)), modification of the susceptible molecular target (*tet*(M), *tet*(O), *tet*(W), *sul*1, and *sul*2), and inactivation of the antibiotic (*tet*(X) and bla_{CMY-2}). We evaluated associations of bacterial community composition with sampling time, location of samples and antibiotic-resistance genes surveyed.

2. Materials and methods

2.1. Sample collection and DNA extraction

Soil samples were collected from six public parks in Houston impacted by Harvey three (T1), four (T2), five (T3), and eighteen (T4) months after the hurricane (See Fig. 1 and Table S1 in the Supporting Information -SI). All the parks are inside the Houston urban area and located in two hydrologically separated units (sub-basins) within the Trinity-San Jacinto river basin. Meyer park is located in the Spring river sub-basin while Addicks, Bear, Cullen, Marron and Mason parks are located in the Buffalo-San Jacinto river sub-basin. Inside the Buffalo-San Jacinto sub-basin, Addicks, Bear, and Cullen are located upstream from Marron and Mason. A sterile, metal hand trowel was used to excavate the superficial layer of vegetation and a sterile 50 mL centrifuge tube was used to core the topsoil at 0–15 cm depth. In all the sampling campaigns, samples were collected in duplicates from two to four different sites in each park. Samples were stored in a cooler at 4 °C, transported to the School of Public Health at Texas A&M University and stored at −20 °C until analysis. For brevity, samples taken 3 to 5 months after the flood, are named “short-term samples” and samples taken 18 months after the flood are named “long-term samples”.

2.2. Detection of antibiotic-resistance genes

Total DNA was extracted from soil samples using DNeasy Powersoil Kit (QIAGEN, Germantown, MD) following the manufacturer's instructions. A spectrophotometer was used to obtain DNA concentration and purity (Nanodrop one, Thermo Scientific). Detection of target resistance genes and integron-integrase genes was done using polymerase chain reaction (PCR) amplification followed by gel electrophoresis detection. Each PCR reaction was composed of Platinum™ Hot Start PCR Master Mix (2×), 200–300 ng DNA template and 10 pmol per μL of forward and reverse primer in a final volume of 12.5 μL. Reactions with positive and negative templates were included in all PCR sets of detection. Details of primers and positive templates are included in Table S2.

We selected the targeted antibiotic-resistance genes in order to: (a) include the three main mechanisms of antibiotic resistance i.e., efflux of the antibiotic (*tet*(A), *tet*(B), *tet*(C), *tet*(D), *tet*(E)), modification of the susceptible molecular target (*tet*(M), *tet*(O), *tet*(W), *sul*1, and *sul*2), and inactivation of the antibiotic (*tet*(X) and bla_{CMY-2}) (Roberts, 2005; Walsh, 2003), (b) include targets that have been used as indicators for anthropogenic pollution (*int*I1), and (c) include genetic elements commonly surveyed in environmental samples, where resistance to tetracycline is the most commonly surveyed, followed by sulfonamide, beta lactam resistance and class 1 integrons.

2.3. Microbial community and taxonomy analysis

After PCR and gel electrophoresis, the extracted DNA was sequenced using the Illumina HiSeq2500 platform with a paired-end method to construct the library and obtained multiplexed samples. The sequenced region was the V3-V4 (338F/806R) of the 16S rRNA gene. The Quantitative Insights into Microbial Ecology (QIIME 2) software was used for diversity and taxonomy analysis (Bolyen et al., 2019). QIIME2 DADA2 was used to obtain a table of frequencies and representative sequences, correct background noise, and remove low quality regions of the reads identified as the first 20 bases in the forward and reverse reads. A rooted phylogenetic tree was constructed from representative sequences using QIIME 2 alignment plug-in and used to calculate microbial community diversity metrics. Alpha diversity metrics included Faith's phylogenetic diversity, Shannon's index and Pielou's evenness while beta diversity metrics included UniFrac and Weighted UniFrac distances. The

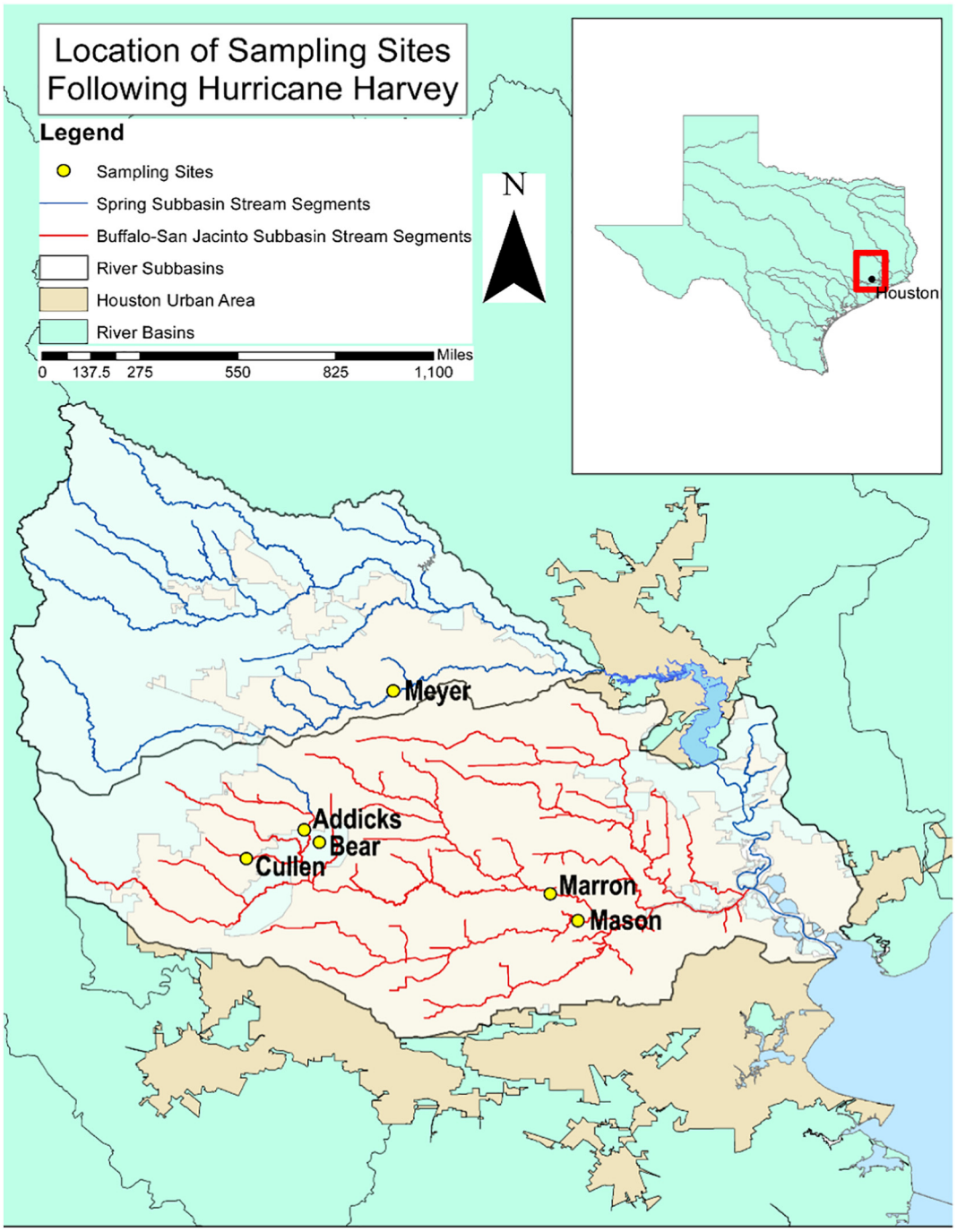

Fig. 1. Location of soil samples collected from six public parks in Houston impacted by Hurricane Harvey three (T1), four (T2), five (T3), and eighteen (T4) months after the event. Parks are located in two hydrologically separated units (sub-basins) within the Trinity-San Jacinto river basin. Meyer park is located in the Spring river sub-basin while the other parks are located in the Buffalo-San Jacinto river sub-basin. All the parks are inside the Houston urban area.

distances between pairs of samples were used to conduct Principal Coordinate Analysis (PCoA) to produce two orthogonal coordinates that explain their variation.

For assigning a taxonomy affiliation to each sequence, QIIME2 taxonomy classifier plug-in was used with a Naïve Bayes classifier trained on the Greengenes full-length sequences (13_8 99% OTUS). In order to identify taxa associated with human contamination, results were filtered to select samples that contained specific bacterial families using the QIIME2 taxa filter-table plug-in (Table S3). Details of the microbial community, alpha and beta diversity, and taxonomy analysis can be found in the SI.

2.4. Statistical analysis

Antibiotic-resistance genes detected in different parks and times were analyzed by multiple correspondence analysis (MCA) (Husson et al., 2010) to obtain similarities between the genes surveyed taking into account all detections simultaneously. MCA is helpful to reveal relationships that are not evident when only the frequencies of each detected gene are obtained. To conduct MCA, we used the results from detection of each antibiotic-resistance gene in each sample and defined each antibiotic-resistance gene as a variable. Additionally, for each antibiotic-resistance gene, each soil sample has a category representing

detection or no detection (yes = 1 or no = 0). Differences of statistical significance of microbial community diversity, richness, evenness and taxonomic abundance at order level in different groups of samples were evaluated using the Kruskal-Wallis test (McDonald, 2014).

3. Results

3.1. Detection of antibiotic-resistance genes

Results show that overall the diversity of resistance genes three (T1), four (T2) and five (T3) months is high compared to eighteen months (T4) after flooding (see Fig. 2 and Table S4). The genes *tet*(E), *int*I1, and bla_{CMY-2} were prevalent shortly after the hurricane (25 to 100% of the samples at T1, T2 and T3) and they were not detected in samples eighteen months later (T4). These results contrast with *tet*(A), *sul*1 and *sul*2, which were present at all times and in all the parks. The genes *tet*(B), *tet*(C), and *tet*(D) were detected at low frequencies in short-term samples (6 to 22% and only at three out of the six parks) and not detected in long-term samples. The *tet*(M), *tet*(O), *tet*(W), *tet*(X) and *int*I2 genetic elements were not detected in any of the samples.

3.2. Relationships among antibiotic-resistance genes surveyed

We used MCA to identify relationships that are not evident when only frequencies are evaluated. The MCA was conducted using Table S4, and plots of antibiotic-resistance genes using the first two dimensions are shown in Fig. 3A. Using the detection of antibiotic-resistance genes in all the samples, we explored relationships among genes detected based on similarities of their detection profile. We observed genes in three clusters: (a) *int*I1 and *sul*1; (b) *tet*(A), *tet*(C), and *tet*(E); and (c) *tet*(B), and *tet*(D). The clustering of *int*I1 and *sul*1 showed that most of the samples that tested positive for *int*I1 were also positive for *sul*1. The second and third clusters were related to main differences among tetracycline resistance profiles. One cluster shows that if the rare *tet*(C) gene is present, then *tet*(A) and *tet*(E) are also present, whereas the other cluster distinguishes that if *tet*(B) is present, *tet*(D) is not.

An additional result from MCA is the relationship between soil samples in terms of antibiotic-resistance genes detected. Using MCA and the presence and absence of detection, we sought to determine which soil samples have similar resistance profiles. Fig. 3B shows clusters of positive ("+") and negative ("-") gene detection. We observed that detection of *tet*(B), *tet*(C) and *tet*(D) cluster to the extreme right of dimension 1, indicating that those categories separate soil samples with unique resistance profiles. Additionally, dimension 2 in Fig. 3B separates positive from negative detection of *int*I1 and *sul*1 indicating that they occurred (or were absent) concurrently in most of the samples. Fig. 3C shows groups of samples with unique characteristics. We observed a group of soil samples that share the rare detection of *tet*(B), *tet*(C) and *tet*(D) (cluster to the right of Fig. 3B and C, mainly Meyer's park samples at T2 and T3). Additionally, we observed that some T4 samples cluster together at the top left of Fig. 3C (Cullen, Marron, and Mason), indicating that these samples have similar profiles uniquely characterized by no detection of the surveyed genes except for *sul*2.

3.3. Association of bacterial community composition with sampling time, location, and antibiotic-resistance genes surveyed

To assess the impact of flooding on soil microbial communities, we obtained microbial diversity metrics and compared values between short-term and long-term samples. Microbial diversity metrics used in this assessment include Faith's phylogenetic diversity, Shannon's

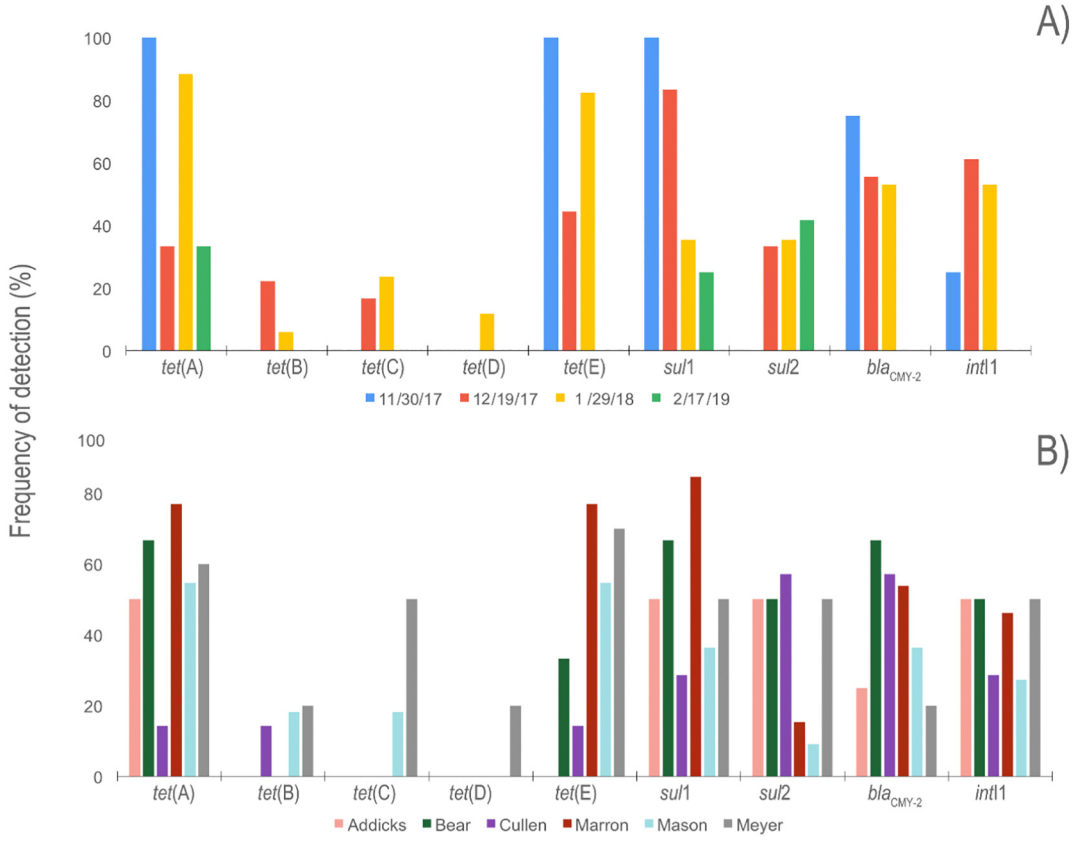

Fig. 2. Diversity of resistance-genes in soil samples taken three (11/30/2017), four (12/19/2017) and five (1/29/2018) and eighteen (2/17/2019) months after flooding in six different parks in Houston (Addicks, Bear, Cullen, Marron, Mason and Meyer). Frequency of genes detected (A) at different times and (B) at different locations. *tet*(M), *tet*(O), *tet*(W), *tet*(X) and *Int*I2 were not detected.

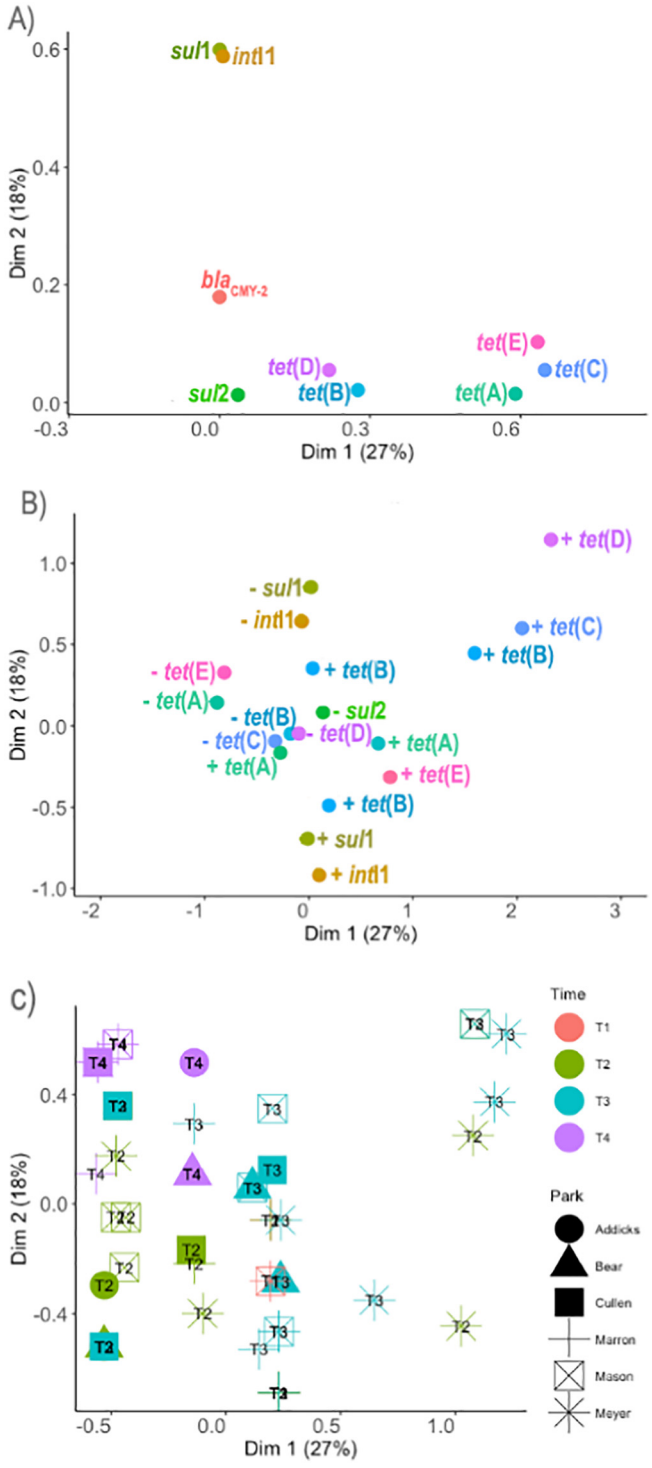

Fig. 3. Multiple correspondence analysis plot for the first two dimensions. Panel (A) reveals relationships between antibiotic-resistance genes. Panel (B) reveals relationships between occurrence or absence of antibiotic-resistance genes, where "+" means positive detection and "−" negative detection. Panel (C) reveals relationships between soil samples with similar resistant profiles, in this panel T1 corresponds to 11/30/217, T2 to 12/19/2017, T3 to 1/29/2018 and T4 to 2/17/2019.

diversity index and Pielou's evenness. Among the three diversity metrics used, statistically significant differences (Kruskal-Wallis test $p < 0.05$) were found only for Faith's values grouped by sampling time, with values higher at later times (Fig. 4A).

Microbial diversity metrics were also used to evaluate if detection of antibiotic-resistance genes was associated to changes in the composition of microbial communities. For this analysis, we compared metric values of samples grouped by positive and negative detection of each antibiotic-resistance gene. We observed that only values grouped by positive and negative detection of *intI*1 and bla_{CMY-2} genes were different (Kruskal-Wallis test $p < 0.05$). Statistical significance was not observed for other metrics grouped by specific genes. Samples with bla_{CMY-2} and *intI*1 genes have higher Shannon values compared to samples that do not contain those genes (Fig. 4B). Additionally, samples containing bla_{CMY-2} have higher Pielou's evenness compared to those that lack the gene (Fig. 4B).

Additionally, we assessed if location of the sample was associated to changes in the microbial communities. Results show that samples from Marron park have higher richness and evenness values compared to Meyer and Cullen, and samples from Mason have higher richness values compared to Meyer (Fig. 4C; Kruskal-Wallis test $p < 0.05$). This means that samples at Marron and Mason have a greater abundance of species and Marron has also a microbial community evenly distributed compared to samples at Meyer and Cullen. Marron, Mason and Cullen are located in the same hydrological sub-basin (Buffalo-San Jacinto, see Fig. 1) with Marron and Mason situated downstream from Cullen. Moreover, Marron and Mason parks are near the Houston ship channel and receive brackish water from the channel and freshwater from upstream urban areas. This might explain the abundance of microbial species and evenly distribution, but more research has to be conducted to test this hypothesis.

3.4. Association of phylogenetic diversity with sampling time, location, and antibiotic-resistance genes surveyed

We obtained UniFrac and Weighted UniFrac distances to assess if there were microbial composition profiles shared among the following sampling features: short-term vs long-term samples, location, and detection of antibiotic-resistance genes. Unweighted UniFrac results show that samples taken eighteen months after the hurricane cluster apart from the samples taken at other times (Fig. 5A). Weighted UniFrac results show that samples taken mainly at Meyer park cluster apart from samples taken at other parks (Fig. 5B). Meyer park is located in a different hydrological subunit than the rest of the parks (Spring subbasin, Fig. 1). Clusters of samples with antibiotic-resistance gene profiles were not observed, thus antibiotic-resistance gene detection was not associated with shared microbial composition profiles.

3.5. Taxonomy analysis

We conducted taxonomy analysis at phylum level to examine taxonomic composition differences among samples. On average, *Actinobacteria*, *Proteobacteria*, *Acidobacteria*, and *Chloroflexi* are characteristic phyla and represent about 80% of the phyla identified in the samples (Fig. S1a and b). Some exceptions are as follows: the four phyla represent about 90% in samples taken three months after flooding (Fig. S1c); and *Cyanobacteria* is more abundant in samples taken from Meyer park compared to other parks (Kruskal-Wallis test $p < 0.05$ with abundance grouped by park and Fig. S1d). In short-term samples (T2 and T3) *Cyanobacteria* contribute approximately 5 to 6% to the total phyla, while its contribution in long-term samples (T4) is below 1% (Fig. S1c). It was also observed that *Verrucomicrobia*, and *Gemmatimonadetes*, abundance increased while *Actinobacteria* decreased in soil samples at later sampling times (Kruskal-Wallis test $p < 0.05$ with abundance of each phylum grouped by time and Fig. S1c).

Focusing on taxonomy at order and family level, we identified samples that contain taxa associated to human contamination. We observed that up to ten different taxonomic orders contribute from 30 to 70% to the total taxonomy (Fig. 6A). On average, soil and aquatic bacteria (*Actinomycetales*, *Rhizobiales*, *Solirubrobacterales*, and *Rhodospirillales*) contributed 30% to the orders identified in the samples (Fig. 6B). All orders known to be associated to human contamination were identified at

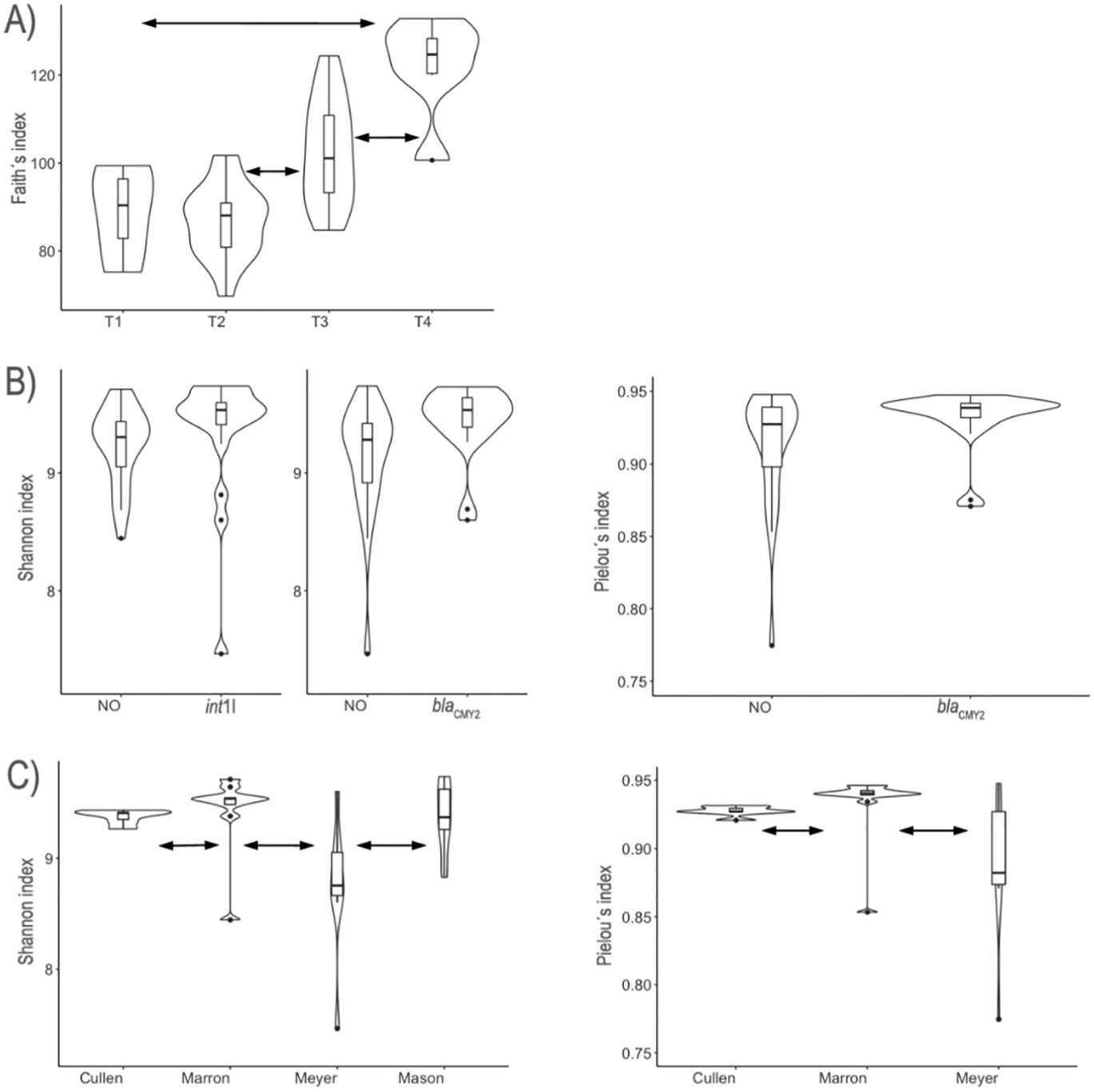

Fig. 4. Statistically significant differences in alpha diversity metrics for different groups of samples (A) Faith's values grouped by sampling time, (B) Shannon and Pielou's index grouped by antibiotic-resistance detected (C) Shannon and Pielou's index grouped by sampling location. T1 corresponds to 11/30/217, T2 to 12/19/2017, T3 to 1/29/2018 and T4 to 2/17/2019. The double arrow in Fig. 3B and C indicate the paired groups for which values are statistically different.

low abundance and, as an ensemble, contributed approximately 1% to 2% to the total orders identified. Although detected at low abundance, their contribution is observed in all samples whether grouped by time or location of sampling (Fig. 6C and D). Results of taxonomy at family level, show that *Bacillaceae* was present in almost all the samples followed by *Clostridiaceae*, and *Pseudomonadaceae* (Fig. 7 and Table S3). When *Enterobacteriaceae* were detected, the frequency of detection decreased over time of sampling. Our results show that all families were observed in short and long-term soil samples except for *Streptococcaceae* and *Bacteroidaceae*. Finally, *Aeromonadaceae* was observed only sporadically (in Addicks park and at time T4 Fig. 7A and B).

4. Discussion

Urban flooding events are natural catastrophes that cause ecological disturbance in microbial communities. Proximity to urban environments increases the probability that microbial niches are affected by infiltration of contaminants such as antibiotics, resistance genes and pathogenic bacteria among others (Garner et al., 2017; Divakaran et al., 2019). It has been demonstrated that hurricanes and other inundations can generate massive contamination influxes that perturb the microbial communities of water bodies and soils, driving processes of mixing of natural soil bacteria with exogenous pathogenic bacteria

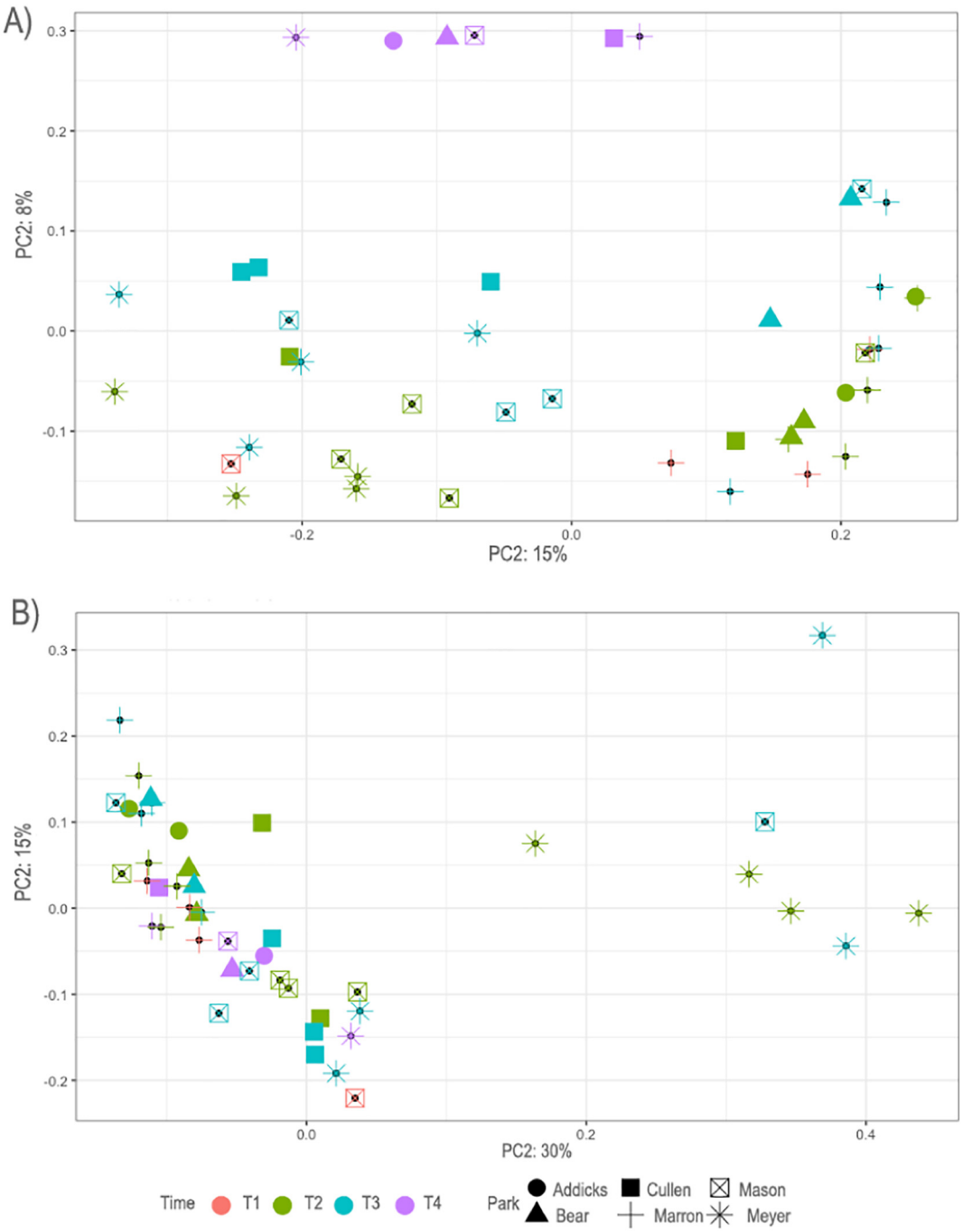

Fig. 5. Principal Coordinate Analysis (PCoA) in two orthogonal coordinates that explain the variation of Unifrac distances between samples (A) Unweighted UniFrac and (B) Weighted UniFrac. T1 corresponds to 11/30/217, T2 to 12/19/2017, T3 to 1/29/2018 and T4 to 2/17/2019.

that could place public health at risk (Chaudhary et al., 2018). Considering that climate change is a current environmental challenge that will cause more frequent hurricanes, flooding, draught and rapid glacial melting among other extreme weather phenomena, it is crucial to evaluate potential consequences associated with perturbations of the microbial populations (Hutchins et al., 2019; Cavicchioli et al., 2019).

4.1. Genetic elements short-term and long-term after flooding

4.1.1. Integrons and intI1-sul1 relationship

The genes *int*I1 and *int*I2 surveyed in this study encode for an integrase and are part of integrons. These elements are commonly linked to the spread and persistence of antibiotic-resistance capable of encoding for mechanisms of incorporation, expression and dissemination of a diverse array of gene cassettes carrying resistance (Partridge et al., 2018; Escudero et al., 2015). These genetic elements are distributed in a number of environmental and clinical bacteria. Commonly detected in bacteria of clinical origin, and observed in mobile genetic elements associated with resistance to antibiotics, disinfectants as well as heavy metals, the class 1 integron has been proposed and used as a marker of anthropogenic contamination (Gillings, 2017; Di Cesare et al., 2020; Stalder et al., 2014; Szekeres et al., 2018; Yu et al., 2018). An important observation in our results is that this gene was detected in 64% of the samples collected during the months following Harvey but was absent in samples obtained one year and a half after the hurricane. It has been postulated that *int*I1-carrying bacteria survive

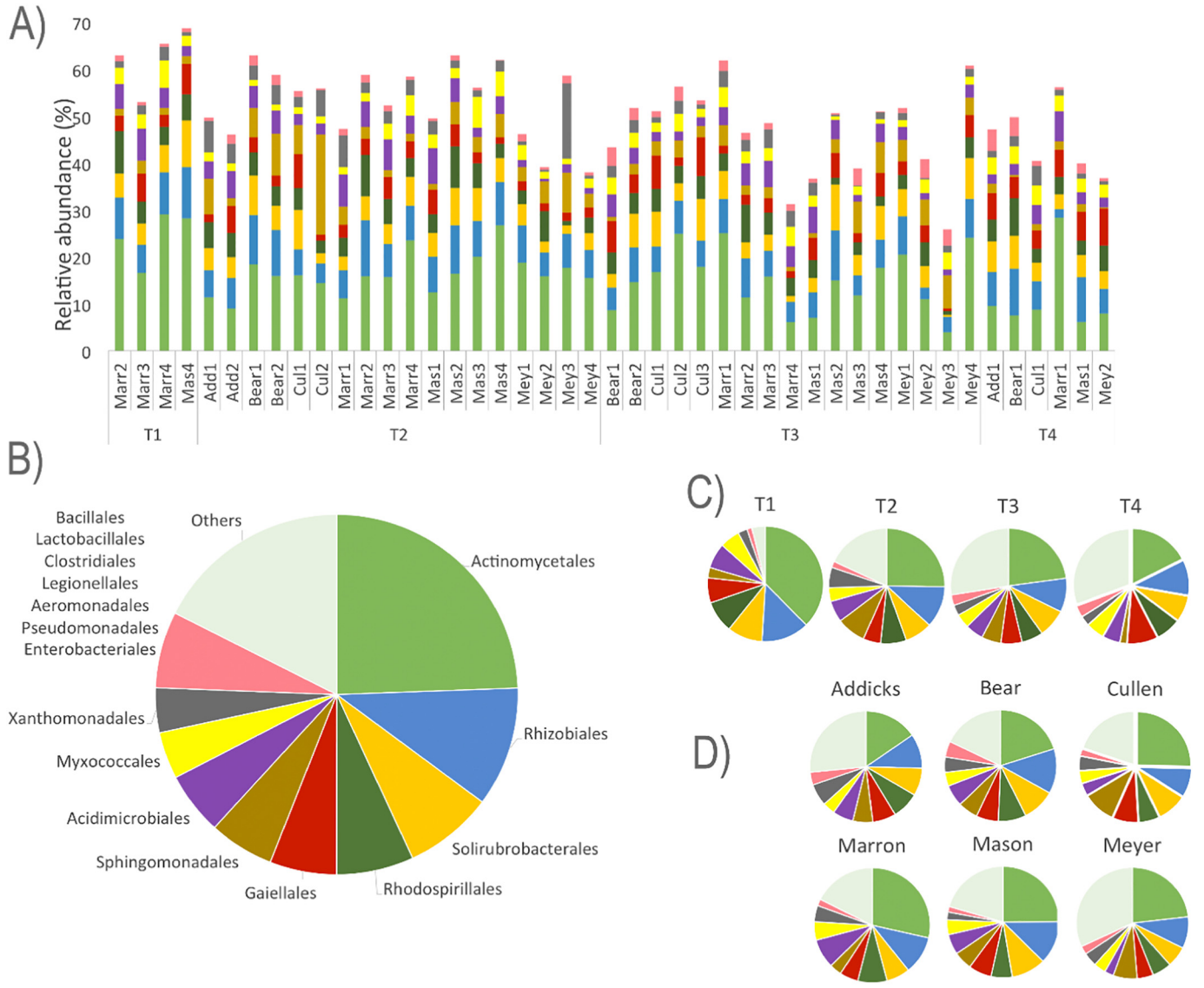

Fig. 6. Taxonomy at order level. The pie represents 60% of the total orders identified. (A) bar plots of abundance for each sample; (B) average of abundance for all the samples, (C) average of abundance for samples grouped by time, T1 corresponds to group of samples taken on 11/30/217, T2 -12/19/2017, T3 - 1/29/2018 and T4 - 2/17/2019; (D) average of abundance for samples grouped by park.

selection imposed by environmental stressors, exhibit slow decay rates, and behave similarly to other persistent pollutants. Additionally, a positive correlation between source of pollution and abundance of *intI*1 has commonly been reported (Gillings et al., 2015).

The class 2 integron was not detected in our samples suggesting that, although *intI*2 is abundant in environmental and clinical samples (Barlow and Gobius, 2006; Kaushik et al., 2018; Buta et al., 2019; Moreira et al., 2019), it may not be a reliable indicator of flooding impact. Negative detection of *intI*2 could be explained by the fact that *intI*2 keeps an inactive or non-functional integrase and displays a limited capacity for horizontal dissemination or it could be due to the detection method used, given that there are reports demonstrating challenges in the detection of *intI*2 (Alonso et al., 2018; Otero-Olarra et al., 2020).

An additional observation in our study is the relationship between *sul*1 and *intI*1. Our results of MCA showed that most of the samples that tested positive for *intI*1 were also positive for *sul*1. This result was expected since previous studies have reported that the class 1 integron is a genetic element with a conserved region linked to *sul*1 (Stalder et al., 2012; Mazel, 2006). Additionally, studies that included detection of *intI*1 and *sul*1 in environmental samples, found positive associations between *intI*1 and *sul*1 (Chen and Zhang, 2013; Miller et al., 2013; Na et al., 2019; Szekeres et al., 2018). MCA results show antibiotic-resistance gene profiles that represent results for all soil samples as a whole. However, when studying the samples individually, we observed that *sul*1 was detected in long-term samples whereas *intI*1 was not detected (Table S4). Our results for long-term samples may be explained by recent demonstrations that not all class 1 integrons contain *sul*1 gene (Gillings, 2014; Gillings, 2017; Ma et al., 2017; Stalder et al., 2014). Similarly to *sul*1 observations, *sul*2 was detected in almost all the short and long-term samples. Both genes encode for mutation of the target of sulfonamides, which are ample spectrum antibiotics and widely disseminated around the world. This antibiotic may exercise a selective pressure for resistant bacteria, which could adapt due to the high prevalence of *sul* genes, and their association to mobile genetic elements (Sköld, 2001; Gnida et al., 2014).

4.1.2. Tetracycline resistance

More than fifty tetracycline-resistance genes that encode for three mechanisms of resistance have been described. In this work, we conducted a search of nine *tet* resistance genes that encode for different

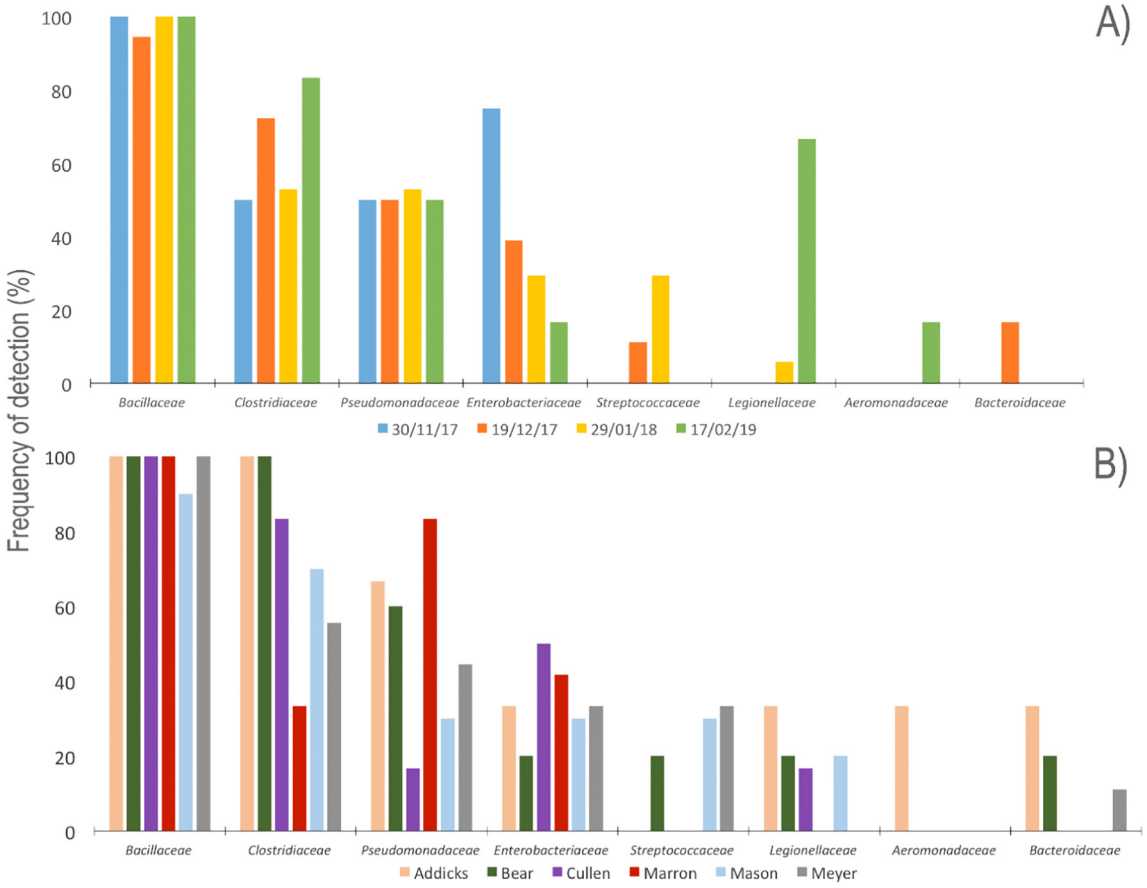

Fig. 7. Diversity of pathogen-associated families in soil samples taken three (11/30/2017), four (12/19/2017) and five (1/29/2018) and eighteen (2/17/2019) months after flooding in six different parks in Houston (Addicks, Bear, Cullen, Marron, Mason and Meyer). Frequency of families detected (A) at different times and (B) at different locations. *Staphylococcaceae*, *Streptococcaceae* and *Bifidobacteriaceae* were not detected.

mechanisms of resistance. *Tet*(A) to (E) encode for efflux pumps, *tet*(M), *tet*(O), and *tet*(W), encode for proteins of ribosomal protection and *tet* (X) encode for an enzyme that catalyzes the degradation of tetracycline (Roberts, 2005). In our study, the *tet* genes encoding for efflux pumps were prevalent and different patterns of detection were observed while *tet* genes encoding for other resistance mechanisms were not detected. This result is consistent with other findings that report that *tet* genes that encode for efflux pumps are common and abundant (Börjesson et al., 2010; Lu et al., 2018). Gibson et al. (2015) described that the mechanisms of resistance to tetracycline are clustered by habitat; in general, soil bacteria carry efflux pumps and human microbiomes carry ribosomal protection. Results in this study suggest that soil-associated tetracycline resistance was widely distributed.

Among efflux-pump *tet* genes, *tet*(A) was detected in all the samples, *tet*(E) was detected in short-term but not detected in long-term samples, and *tet*(B), (C), and (D) were detected at a low frequency. This pattern of detection is in agreement with the common knowledge that *tet* (A) and (E) have a global distribution (Xu et al., 2018), which has not been seen for other *tet* genes. In our MCA results, we observed that: *tet*(C), which occurs rarely, co-occurs with *tet*(A) and *tet*(E); and that detection of *tet*(B) and *tet*(D) was mutually exclusive. The genes *tet* (A) to (E) have only been reported in Gram-negative bacteria and have mainly been found in transmissible plasmids (van Duijkeren et al., 2017). There is little information about main differences within *tet*(A) to (E) genes in the literature thus explanation of the relationship between *tet*(B), (C), and (D) with *tet*(A) and (E) remains unresolved. The observed result that the gene *tet*(E) was detected in short-term, but not detected in long-term samples, suggests that *tet*(E) could be a possible marker of resistance gene dissemination due to Harvey.

4.1.3. Beta lactam resistance

In order to assess resistance to a different class of antibiotic, we surveyed the bla_{CMY-2} gene that confers resistance to beta lactam antibiotics. The bla_{CMY-2} gene is usually plasmid-vectored (named *ampC*) (Hawkey and Jones, 2009) and encodes a beta lactamase that hydrolyzes the antibiotic (Walsh, 2003). The bla_{CMY-2} gene was not found in long-term samples while it was detected 52% and 85% all other times. This gene is commonly associated to multiresistant pathogenic bacteria of the family *Enterobacteriaceae* (Li et al., 2007; Smet et al., 2008; Rodríguez et al., 2009; Sheng et al., 2013; Adenipekun et al., 2019; Koga et al., 2019) and *Aeromonadaceae* (McIntosh et al., 2008). In environmental samples, bla_{CMY-2} has been detected in DNA extracted from contaminated river water, sediments (Xu et al., 2019), and wastewater (Zhang et al., 2019). However, bla_{CMY-2} has been detected at low frequencies in samples from healthy humans (Adenipekun et al., 2016), animals (Dupouy et al., 2019) and samples from areas with low-environmental contamination (Zhang et al., 2019). In a resistome study using functional metagenomics, Gibson et al. (2015) found that class A and C β-lactamase-encoding genes, such as bla_{CMY-2}, were associated to samples of human origin and not to samples from soils. Months after Harvey, we observed human-associated resistance functions suggesting that bla_{CMY-2} could be a powerful indicator of changes in soils driven by environmental impact associated to human waste.

4.2. Bacterial diversity and taxonomy composition short-term and long-term after flooding

Our analysis of different metrics of bacterial community's diversity illustrate important findings explained as follows. The Faith's

phylogenetic diversity index is based on hierarchical relationships among microbial populations and places more emphasis on representativeness of a phylogenetic tree and less on abundance of features (Faith, 1992). Our results indicated that short-term samples had microbial communities with lower diversity compared to the diversity of long-term samples (see Fig. 4A) thus flooding could be a factor that influences the reduction of diversity in soil microbial communities. Shannon's index takes into account the number and abundance of species, i.e. the larger the Shannon index, the higher the number of species (Spellerberg and Fedor, 2003). Pielou's number measures the equal distribution of the number of species in the sample. It represents whether a sample has a small number of species that dominates the community or not (Pielou, 1966). Results of Shannon's and Pielou's metrics indicate that samples that contain *int*I1 and bla_{CMY-2} have high abundance of species and for bla_{CMY-2}-containing samples the abundance is also more diverse compared to samples lacking it. These results lead us to formulate the hypothesis that *int*I1 and bla_{CMY-2} could be markers of flooding impact caused by Harvey given that these genes are the only genes associated to changes in the microbial community and were prevalent in short-term while not observed in long-term samples.

Analysis of samples sharing similar microbial composition profiles using UniFrac and Weighted Unifrac distances may explain drivers of changes in bacterial lineage. Unifrac is a metric of differences of microbial communities calculated by comparing pairs of phylogenetic trees and the fraction of the branches in those trees that have different lineages. In addition to differences in lineages, Weighted UniFrac also takes into account the abundance of species in each branch (Lozupone et al., 2007). The distances between pairs of samples is used to conduct Principal Coordinate Analysis (PCoA) to produce two orthogonal coordinates that explain the variation of the distances. Unweighted UniFrac (Fig. 5A) revealed differences in bacterial lineages at different times and implies that flooding could be a driver for differences in bacterial species. Weighted UniFrac results (Fig. 5B) revealed that differences in the relative abundance of bacterial lineages were associated to location of the samples. For example, samples from Meyer park clustered apart from samples taken at other parks, thus Weighted UniFrac results imply that geographic location seems to be a driver of differences and abundance of bacterial lineages.

Taxonomy analysis was used to identify specific phyla for which changes in abundance were a function of sampling time, i.e. *Cyanobacteria* and *Actinobacteria* had higher abundance in short-term samples than in long-term samples; and *Verrucomicrobia*, and *Gemmatimonadetes* had lower abundance in short-term compared to long-term samples. Our results suggest that changes in abundance of these phyla were associated with the impact of flooding. During the storm Sandy, Ulrich et al. (2016) observed a change in the aquatic bacterial community structure. In their analysis of microbial taxonomy, the phylum *Proteobacteria* was the most abundant followed by *Bacteroidetes*, *Firmicutes*, and *Verrucomicrobia*. Moreover, the phylum *Proteobacteria* abundance decreased during the first 5 days of sampling (Ulrich et al., 2016). Additionally, *Actinobacteria* were found in lower proportions compared to our results. Chaudhary et al. (2018) described that storms disturb the microbial community and observed differences before and after the rainy season in the abundance of the phyla *Actinobacteria*, *Proteobacteria* and *Bacteroidetes*. Other reports indicate that soils contaminated with residual waters contain higher proportions of *Proteobacteria*, *Firmicutes* and *Actinobacteria* (Ibekwe et al., 2018; Garner et al., 2016). Karimi et al. (2018) described that the phyla mentioned above are in general the most abundant in soil samples and small variations depend on factors like pH, soil texture, nutrients and climate. Given that sequencing of the 16S rRNA microbial gene data is integrated, our results suggest that relative abundance differences represent a qualitative change when comparing different times and locations.

Results of the taxonomy analysis at the family level exhibited groups of microbes associated to anthropogenic contamination and wastewater. The families *Streptococcaceae* and *Bacteroidaceae* were identified in short-term samples but not eighteen months after flooding and the *Enterobacteriaceae* frequency decreased over time after the flooding event. Some of these families may contain species of pathogenic bacteria as well as species carrying multiple resistance genes thus exposure to soils short-term after the hurricane may increase the risk of infection or transfer of antibiotic-resistance genes.

We asked whether any connection between antibiotic-resistance and taxa associated to human contamination could be observed in our data. To do so, we looked at results of frequency of detection of antibiotic-resistance genes (Fig. 2) and frequency of detection of specific families (Fig. 7). This analysis revealed that short-term samples from Mason and Meyer parks are diverse in terms of families associated to human contamination and antibiotic-resistance. In contrast, long-term samples contain a lower number of antibiotic-resistance genes detected and human-associated families identified. Although not all the species in the identified families are pathogens, exposure to soils short-term after flooding with a high diversity of both bacteria associated to human contamination and antibiotic-resistance genes may have important implications (for infection or transfer of antibiotic-resistance genes) if exposure to these soils occur.

5. Conclusion

Our results indicate that exposure to topsoil three to five months after the hurricane may have increased the risk of exposure to antibiotic-resistance genes given that a diverse profile of antibiotic-resistance genes were detected and occurrence of some antibiotic-resistance genes were associated to changes in the soil microbial communities. The occurrence of targeted antibiotic-resistance elements decreased eighteen months after the hurricane indicating a reduction of the risk of exposure long time after Harvey. After analyzing results from this study, we propose two main hypotheses regarding the mobilization of resistance genes as well as commensal and pathogenic bacteria in the soil environment, where different factors affect their establishment. The first proposed hypothesis is that integration of exogenous antibiotic-resistance genes among endemic soil bacterial populations may occur when floodwaters, containing exogenous bacteria carrying antibiotic-resistance genes, infiltrate through the soil and deposit in soil particles establishing in localized mixing sites. Another proposed hypothesis is that floodwaters may contain chemicals (which were not analyzed in this study) that, when infiltration occurs, are adsorbed into soils resulting in defined sites that select for resistant bacteria and enhance preservation of resistance genes. More studies are necessary to evaluate those hypotheses proposed here and improve our understanding of the capacity and time scale of soil microbial communities' recovery when affected by extreme weather events. Special focus should be placed in topsoil of urban environments where prevalence of antibiotic-resistance genes and bacteria associated to anthropogenic contamination may increase human exposure risk.

CRediT authorship contribution statement

Abigail Pérez-Valdespino: Conceptualization, Methodology, Investigation, Writing - review & editing. **Ryan Pircher:** Investigation. **Citlali Y. Pérez-Domínguez:** Investigation. **Itza Mendoza-Sanchez:** Conceptualization, Methodology, Investigation, Resources, Formal analysis, Writing - review & editing.

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.

Acknowledgements

Financial support was provided to Abigail Pérez-Valdespino from Instituto Politécnico Nacional SIP grant 20201025, and SNI fellowship; and to Itza Mendoza-Sanchez from Southwest Center for Agricultural Health, Injury Prevention, and Education through Cooperative Agreement # U54-OH007541 from CDC/NIOSH; Center for Translational Environmental Health, Texas A&M Health Science Center; Texas A&M Division of Research; and Texas A&M School of Public Health. Sequencing analysis of this research was conducted with the advanced computing resources provided by Texas A&M High Performance Research Computing.

The authors appreciate the help of: Dr. H. Morgan Scott for early laboratory efforts and guidance on *tet* isolates, Dr. Javier Vinasco-Torres for advice on laboratory methods and approaches, Jorge E. Otero-Olarra for assistance in PCR analysis, John Chris Blazier for advice on sequencing analysis, and Gaston A. Casillas supervised by Dr. Jennifer A. Horney for providing help with initial soil samples. The authors thank Dr. Chris Sales for a thorough and constructive review of a previous version of this paper.

Compliance with ethical standard

This article does not contain any studies with human participants or animals performed by any of the authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.142643.

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