Prokaryotic Community Composition and Diversity in Sediments from Choctafaula, Uphapee, and Cubahatchee Creeks in the Lower Tallapoosa River Basin, Macon County, Alabama: An Observational Study

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ABSTRACT: Sedimentary prokaryotes are primarily responsible for metabolic activity in rivers. They are subjected to a variety of natural and anthropogenic pressures, allowing us to use the composition and diversity of their communities as indicators of ecological health. For our investigation, we looked at sediments from three ecologically critical streams in the Lower Tallapoosa River Basin (Choctafaula, Uphapee, and Cubahatchee Creeks). The prokaryotic community was characterized using molecular approaches, and elemental concentrations were determined by inductively coupled plasma atomic emission spectrometry. This research demonstrated that Proteobacteria (45.02–80.73%), Bacteroidetes (1.98–26.52%), Firmicutes (1.36–50.67%), Actinobacteria (1.55–16.81%), and Acidobacteria (0.13–8.77%) were the most prevalent phyla. Key physicochemical parameters, core communities at multiple taxonomic levels, and several pathogenic genera shifted radically between streams. Weighted and unweighted unifrac distance metrics based PCoA plots indicated the structural and membership similarity of samples from specific creek ecosystems. Based on our research findings, it is evident that the composition and diversity of prokaryotic communities in sediment could serve as significant indicators in stream ecosystems. Further investigation and application of these indicators could prove valuable in assessing the health of streams, particularly in light of the accelerated changes resulting from climate change within a condensed timeframe.

KEYWORDS: Freshwater sediments, physicochemical properties, prokaryotic community composition, diversity, pathogenic genera

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Introduction

The presence of aquatic organisms in rivers and streams plays a crucial role in sustaining human existence due to the abundant availability of food and medicinal resources they offer. Drinking, cooking, swimming, fishing, and other leisure activities can also be done using river and stream water. In turn, the deterioration of these systems presents major issues. Rivers' biomarkers are challenging to identify because of the complexity of their ecosystems. Several institutions presently use benthic macroinvertebrates (Kerans & Karr, 1994), diatoms (Y. K. Wang et al., 2005), and fish (Karr, 1981) for biomonitoring. Macroinvertebrates are most likely to be reliant on them since they are easy to get and identify, are numerous in permanent stream ecosystems, and include several species that are sensitive to pollution and are significant markers of stream health (Rosenberg & Resh, 1993; Wright & Ryan, 2016). On the other hand, the collection, evaluation, and identification of macroinvertebrate data as bioindicators of stream quality requires time.

Microbes play an important role in biogeochemical cycles and processes. They're essential for biodiversity and ecosystem function, and understanding what drives microbial diversity and dispersion might help us better understand ecosystems across natural and human-influenced gradients. Molecular techniques have been applied to environmental samples, resulting in a major improvement in our understanding of microbial biodiversity (Hug et al., 2016) and important implications for environmental research. Microbe-based ecosystem monitoring, and evaluation has a lot of potential for assessing the health of freshwater streams because a small, easy-to-get sample could help or even replace the huge amount of work required by traditional eukaryote-based methods (Stranko et al., 2019). Unlike surface water, sediment provides a consistent and predictable substrate for dynamic biogeochemical processes. Therefore, tracking prokaryotic populations in sediment can be a reliable way to keep track of how organisms respond to different types of pollution. Recently, Numerous factors, such as microplastics (Yin et al., 2023), antibiotic resistance (Y. Feng et al., 2023), salinity profile (L. Feng et al., 2023), pollution originating from construction activities in waterbodies (Shao et al., 2023), herbicides (Zhao et al., 2023), grain size distribution (J. Lin et al., 2023), nutrient removal (M. Zhang et al., 2023), petroleum hydrocarbon pollution (Hamdan et al., 2023), the impact of offshore wind farms (T. Wang et al., 2023), thallium spill (Chen et al., 2023), and granulated coal ash (Patil et al., 2023), have all been extensively investigated in terms of their impact on the microbial community structure within sedimentary environments.

Elemental pollution, specifically heavy metal pollution in aquatic ecosystems, requires a great deal of public attention due to its persistence and eventual fate in food chains (Q. Lin et al.,

2016; Z. Zhang et al., 2016). Massive amounts of anthropogenic metal-containing residues are now rapidly deposited in aquatic sediments, where they strongly bind with various sediment particle types (Simpson & Spadaro, 2016; Tessier & Campbell, 1987). However, little is known about how they alter prokaryotic communities there.

According to the Environmental Protection Agency's "National Rivers and Streams Assessment 2008/2009," around 46% of American rivers are in a poor biological state (U.S. Environmental Protection Agency (EPA), 2016). Alabama's rivers are in a similar precarious situation. According to the National Water Quality Inventory study from 2000, 73% of Alabama's 2628 miles of rivers are likewise in poor condition (U.S. Environmental Protection Agency (EPA), 2002). Several creeks in the Tallapoosa River Basin have been declared impaired waterbodies (Alabama Department of Environmental Management (ADEM), 2016). Choctafaula and Uphapee Creeks are categorized as "Fish and Wildlife," while Cubahatchee Creek is designated as "Fish, Wildlife, and Swimming."Therefore, these streams are important for aquatic life and recreational activities such as fishing and swimming for residents of Macon County, Alabama. However, relatively little study has been done on the ecological function of these vital streams. Considering the following circumstances, it is critical to study the ecological profile of this watershed. The objectives of this study were to analyze the prokaryotic community structure and diversity in three different creeks using massively parallel sequencing techniques based on amplification of the 16S rRNA gene, as well as to explore the variables that impact the structure of communities using well-known scientific concepts and supporting evidence.

Study Area and Sample Collection

The confluence of Chewacla and Opintlocco Creeks creates Uphapee Creek (Markewich & Christopher, 1982). Both Uphapee and Choctafaula Creeks go through the Tuskegee National Forest, which features pine-hardwood, lowland hardwood, and wetland habitats. Outside of Tuskegee National Forest, Choctafaula Creek joins Uphapee Creek before entering the Tallapoosa River. Cubahatchee Creek originates in Bullock County, north of Union Springs, and flows into the Tallapoosa River. Pine, deciduous, and mixed forests cover the majority of the Cubahatchee watershed. Within this watershed, there are additional pastures and hay fields. Within each creek, three remote locations were selected for the collection of sediment samples (Table 1 and Figure 1). Samples were collected at the interface between the water level and the stream bed. A total of 24 sediment samples were analyzed from each creek: 12 for a study of the prokaryotic community and diversity and 12 more for the analysis of physicochemical characteristics. The Tuskegee National Forest owned all the samples taken from Choctafaula and Uphapee Creeks. The samples were collected during the day in March and April of 2019.

Table 1. Geographic Coordinates of Sampling Stations.

CREEK	LATITUDE	LONGITUDE
Choctafaula Creek		
	32.50757	−85.57853
	32.49802	−85.59164
	32.46356	−85.65777
Uphapee Creek		
	32.43728	−85.63587
Cubahatchee Creek		
	32.44479	−85.64805
	32.45191	−85.65625
	32.34646	−85.89071
	32.35662	−85.92514
	32.39499	−85.97242

Note. Nine stations were set up in the waterways of Choctafaula, Uphapee, and Cubahatchee Creek to collect sediment samples.

Samples were collected from the stream bed and placed in plastic bottles, stored on ice, and transported to Tuskegee University's Water Quality Laboratory, where they were refrigerated until examination.

Materials and Methods

Measurement of pH, organic matter (%), and elemental concentrations

The pH, organic matter (%), and elemental concentrations of samples were measured in the Soil Testing Laboratory, Auburn University, Auburn, AL 36849-5411. Lignin pHugly: Type ST 6 Channel pH Tester was used to determine the pH of the samples. The loss on ignition method was used to determine the organic matter (%) (Schulte & Hopkins, 1996). Elemental concentrations were determined simultaneously by inductively coupled plasma atomic emission spectrometry using a Varian Vista-MPX radial spectrometer (Martin et al., 1994).

DNA extraction, amplification, and sequencing

The DNeasy PowerSoil Kit (Qiagen, USA) was used to extract DNA from 36 sediment samples with a few modifications to the manufacturer's protocol in order to achieve a high quantity of DNA. Before being combined in a single spin filter, sediments were replicated three times according to the instructions. The manufacturer's protocol was followed, except for eluting DNA with 60 µL of sterile elution buffer instead of 100 µL. The Nanodrop ND-2000 spectrophotometer (Nanodrop Technologies, Wilmington, DE) was used to quantify the extracted DNA samples (1 µL). The samples were then placed in the freezer until they were sent to MR DNA in Shallowater,

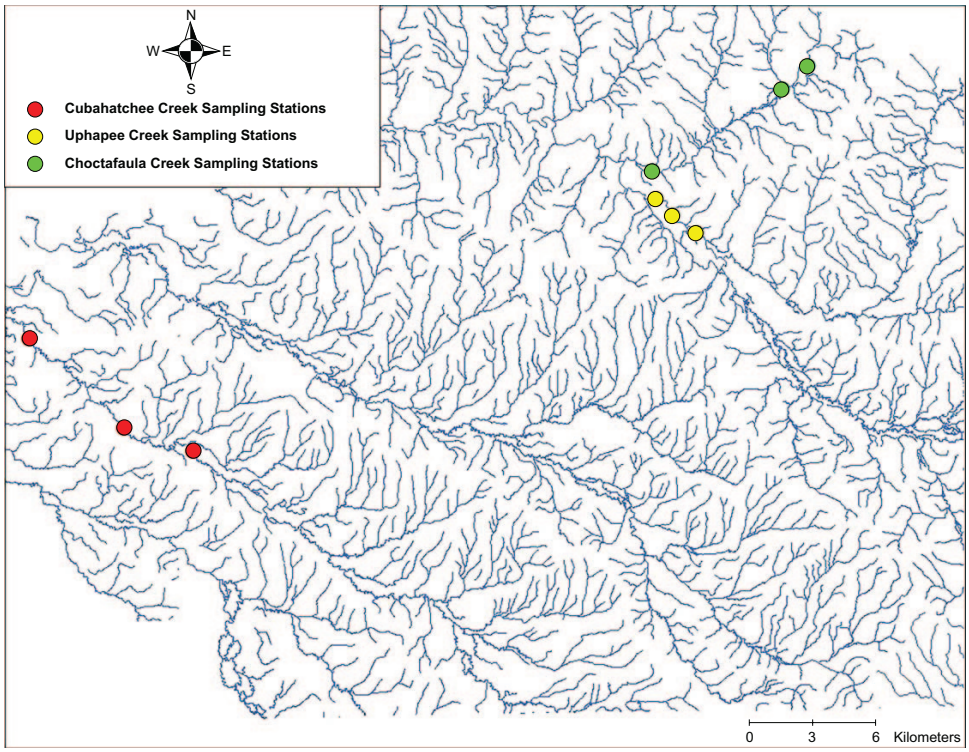

Figure 1. Locations of nine sampling sites across the Choctafaula, Uphapee, and Cubahatchee Creek areas. ArcGIS 10.7.1 (ESRI [Environmental Systems Research Institute]) was used to create the map.

Texas, USA, for amplification and sequencing. The 16S rRNA gene V4 variable region PCR primers 515F (5′-GTGYCA GCMGCCGCGGTAA-3′) and 806 R (5′-GGACTACNV GGGTWTCTAAT-3′) were used using the HotStarTaq Plus Master Mix Kit (Qiagen, USA). The following conditions were used: 94°C for 3 min, then 30 to 35 cycles of 94°C for 30 s, 53°C for 40 s, and 72°C for 1 min, followed by a final elongation step at 72°C for 5 min.

After amplification, PCR products were examined on a 2% agarose gel to measure amplification success and relative band intensity. Based on their molecular weights and DNA concentrations, multiple samples (e.g. 100 samples) are pooled together in equal proportions. Using calibrated Ampure XP beads, pooled samples were purified. The Illumina DNA library was then created using the pooled and purified PCR product. Sequencing was done on a MiSeq at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) according to the manufacturer's instructions. The MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA) was used to process the sequence data. In summary, sequences were joined, depleted of barcodes then sequences <150 bp removed, sequences with ambiguous base calls removed. Sequences were denoised, OTUs generated, and chimeras removed. Operational taxonomic units (OTUs) were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLASTn against a curated database derived from RDPII and NCBI (www.ncbi.nlm.nih.gov, http://rdp.cme.msu.edu). Bioinformatic processing was then carried out using Qiime (Quantitative insights into microbial ecology).

Mathematical and Statistical Analyses

To compare means of relative abundance, diversity, pH, organic matter (%), and elemental concentrations (ppm), a one-way ANOVA was used, followed by Tukey's HSD test for pairwise comparisons. However, prior to undertaking ANOVA and Tukey's HSD analysis, raw data of relative abundance (%) and organic matter (%) were arcsine transformed, and elemental concentrations were converted into $\log_{10}(x)$ or $\log_{10}(x+1)$, depending on the situation. The means and standard errors of untransformed values are shown here, but significances are presented from the statistical analysis of transformed values. All mathematical and statistical analyses were performed using Microsoft Excel and IBM SPSS Statistics 27 software. Weighted and unweighted unifrac, as well as bray-curtis and jaccard distance metrics, were utilized to generate Principal Coordinate Analysis (PCoA) plots. These plots were employed to assess the structural and membership similarities among samples obtained from distinct stream ecosystems. The visualization of PCoA results was conducted using Emperor plots in QIIME2.

Results and Discussions

In observational studies, researchers use known scientific concepts and corroborating evidence to try to explain natural phenomena, even if the explanation is simply supposition. The following conversation is structured in the same way to discuss the current study's findings.

Physicochemical Properties

To quantify physicochemical properties and their differences in creek sediments, two of the most important parameters,

Table 2. Physicochemical Properties and Prokaryotic Alpha Diversity Indices.

SEDIMENT PROPERTIES	CHC	UPC	CUC
Physicochemical properties			
pH	7.54 ± 0.12a	7.16 ± 0.29	6.50 ± 0.27b
OM (%)	0.18 ± 0.05b	0.83 ± 0.33	0.99 ± 0.16a
Aluminum	653.82 ± 114.12b	1854.72 ± 717.62	2820.36 ± 575.42a
Boron	1.90 ± 0.23b	5.02 ± 1.52a	4.02 ± 0.54a
Barium	154.29 ± 21.56	117.05 ± 27.03	152.28 ± 29.39
Calcium	126.52 ± 39.96b	674.97 ± 287.98a	713.77 ± 149.43a
Chromium	3.18 ± 0.50	3.83 ± 0.98	5.64 ± 1.15
Copper	3.10 ± 0.20	4.55 ± 0.85a	2.55 ± 0.26b
Iron	1912.23 ± 261.63	4351.67 ± 1150.63	3422.20 ± 626.03
Potassium	77.36 ± 7.42b	256.85 ± 81.64a	196.86 ± 27.69a
Magnesium	143.85 ± 27.76b	732.18 ± 332.84	433.05 ± 88.33a
Manganese	156.85 ± 8.28a	109.07 ± 17.20a	49.60 ± 7.59b
Sodium	11.39 ± 1.31b	22.77 ± 4.44a	23.92 ± 3.13a
Alpha diversity indices			
Observed OTUs	610.50 ± 40.25	464.42 ± 64.37	744.17 ± 123.27
Shannon	7.08 ± 0.20	5.80 ± 0.45	6.86 ± 0.45
Faith PD	69.64 ± 3.95	62.55 ± 8.59	86.52 ± 12.38

Note. Estimates of the sediment physicochemical properties and prokaryotic alpha diversity indices are presented for Choctafaula, Uphapee, and Cubahatchee Creeks (mean ± standard error). CHC, UPC, and CUC represent Choctafaula, Uphapee, and Cubahatchee Creek, respectively. Significant differences are designated by different lower-case letters (the mean difference is significant at the .05 level).

pH and organic matter (%), as well as 17 elemental concentrations (ppm), were measured. However, arsenic, cadmium, and nickel were found to be < 0.1 ppm in all 36 samples, and the data sets for phosphorus, lead, and zinc all have one or more values of <0.1 ppm (see Appendix Table A1). Therefore, they were excluded from the analysis. The remaining physicochemical variables were then mathematically and statistically studied (Table 2). Our findings revealed that the organic matter (%) in Cubahatchee Creek sediments was significantly greater than that in Choctafaula Creek sediments. Several factors can be attributed to the increase in organic matter (%). One of them is siltation (Kamp-Nielsen et al., 2002), which can be caused by erosion of the land or activities in the water. Since Cubahatchee Creek was declared an impaired water body due to siltation generated by agricultural operations and surface mining (Alabama Department of Environmental Management (ADEM), 2016), an increase in organic matter content in sediments due to siltation could be a possibility.

Organic matter is known to emit H+ ions, which acidify the soil (McCauley et al., 2009). We discovered in our sediment study that samples from Cubahatchee Creek have significantly higher organic matter (%) than samples from Choctafaula Creek but have a significantly lower pH. Based on our research, most of the elements were more concentrated in the Cubahatchee and Uphapee samples. Higher levels of organic materials in sediments may be the cause of this phenomenon since organic matters have negative functional groups that strongly attract positively ionized metallic elements (Evans, 1989) and hence increase their amounts in those sediments. Manganese, on the other hand, behaved differently than other elements. Cubahatchee Creek sediments, which are higher in organic matter (%), have a lower manganese concentration than two others less organic-matter rich creek sediments. Elemental pollution due to surface mining and other anthropogenic activities near creeks could be another contributing factor to the overall increased concentrations of elements in Cubahatchee and Uphapee Creeks.

Diversity

According to most scientific organizations, more biological diversity leads to greater ecosystem stability, predictability, and reliability (Loreau et al., 2001; McGrady-Steed et al., 1997; Naeem & Li, 1997). However, owing to natural and manmade factors, not all ecosystems throughout the world are equally prosperous. Because the earth's biodiversity is vanishing at an alarming rate in the modern world (Barnosky et al., 2011), several organizations are attempting to develop mathematical systems to assess diversity. Prokaryotes are vital for biogeochemical cycling, so determining their diversity may be crucial for determining the health of an ecosystem. In this study, we assess alpha diversity by means of Observed Otus, Shannon Diversity, and Faith PD (Table 2). Observed Otus is a qualitative indicator for alpha diversity that can be quantified. The Shannon Index is a metric that may be used to determine both diversity and evenness. Faith's Phylogenetic Diversity (Faith PD) measures the amount of the phylogenetic tree covered by communities. Although the three indices demonstrated no significant differences between our creeks, Uphapee Creek samples revealed less diversity than two other creek samples. Regarding beta diversity, PCoA plots based on the weighted and unweighted unifrac distance metrics are also presented, which indicate a clear pattern of clustering of community structure and membership by specific creek samples with a few outliers (Figures 2 and 3).

These plots strongly demonstrated that each of these streams has unique characteristics (physicochemical properties and topography) that affect how prokaryotic communities are built structurally and in terms of membership, even though they are all in Macon County and close to one another. These plots also strongly suggested that the composition and diversity of prokaryotic communities could be used as key indicators to detect changes in stream ecosystems.

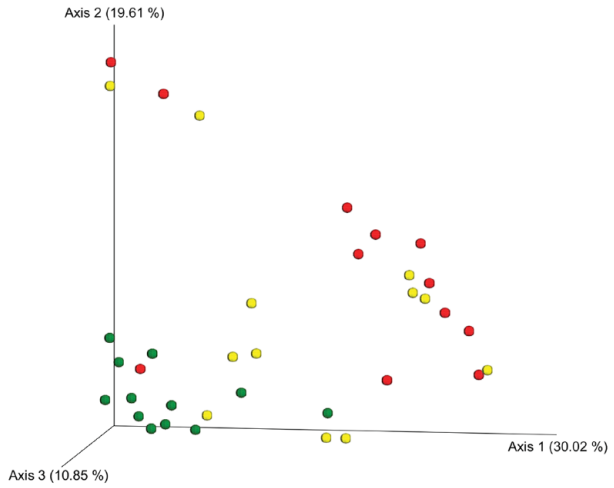

Figure 2. PCoA plot based on weighted unifrac distances for 36 sediment samples. Green, yellow, and red spheres represent Choctafaula, Uphapee, and Cubahatchee samples, respectively.

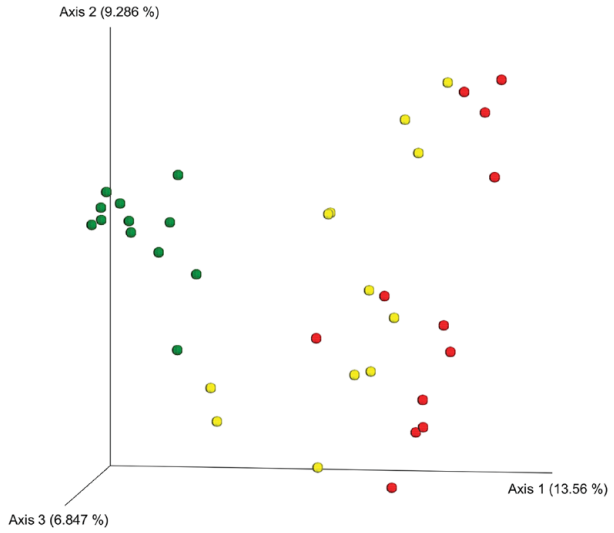

Figure 3. PCoA plot based on unweighted unifrac distances for 36 sediment samples. Green, yellow, and red spheres represent Choctafaula, Uphapee, and Cubahatchee samples, respectively.

A General Overview of Prokaryotic Community Structure

30 phyla, 68 classes, 143 orders, 321 families, 1,051 genera, and 2,094 species of bacteria and 4 phyla, 11 classes, 18 orders, 25 families, 43 genera, and 64 species of archaea were identified in 36 samples. Proteobacteria were previously reported to be the most dominant phylum in freshwater sediments (Tamaki et al., 2005b; J. Zhang et al., 2015). Except for one sample in Uphapee Creek, this phylum was the most prevalent in our study, ranging from 45.02% to 80.73%. Other significant phyla were Bacteroidetes (1.98%–26.52%), Actinobacteria (1.55%–16.81%), Firmicutes (1.36%–50.67%), and Acidobacteria (0.13%–8.77%). When coupled with the previous phyla, Verrucomicrobia (0.24%–6.68%), Planctomycetes (0.19%–3.75%), Chloroflexi (0.09%–4.09%), Cyanobacteria (0.05%–11.68%), Nitrospirae (0.02%–1.72%), Euryarchaeota (0.01%–1.01%), and Thaumarchaeota (0.00%–2.06%) accounted for 95.93%–99.94% of all prokaryotic phyla detected in our samples. Alphaproteobacteria (4.02%–20.24%), Betaproteobacteria (5.87%–48.47%), Gammaproteobacteria (6.74%–58.11%), Sphingobacteriia (0.35%–18.33%), and Bacilli (0.53%–50.46%) were the most notable classes discovered in our study. *Massilia* (0.51%–38.89%), *Acinetobacter* (0.34–50.86%), *Pseudomonas* (0.69%–23.55%), *Exiguobacterium* (0.15%–50.07%), and *Arthrobacter* (0.17%–11.25%)-all these genera had high prevalence across the streams. The frequently observed species were *Massilia timonae* (0.33%–29.20%), *Pseudomonas putida* (0.35%–18.34%), *Acinetobacter piperi* (0.22%–35.76%), *Exiguobacterium undae* (0.09%–17.54%), *Arthrobacter globiformis* (0.04%–4.52%), and *Duganella zoogloeoides* (0.04%–3.20%).

Relative Abundances of Prokaryotic Communities

In this study (Table 3), the mean relative abundances of notable prokaryotic communities at various taxonomic levels (phyla,

Table 3. Mean Relative Abundances of Prokaryotic Communities in Sediments from Choctafaula, Uphapee, and Cubahatchee Creeks.

PROKARYOTES	CHC	UPC	CUC
Phyla			
Proteobacteria	61.24 ± 1.38	62.39 ± 3.60	56.82 ± 2.14
Bacteroidetes	15.87 ± 1.26a	6.52 ± 1.28b	6.38 ± 1.67b
Firmicutes	4.04 ± 0.97b	13.54 ± 4.50	12.71 ± 2.17a
Actinobacteria	6.37 ± 0.62	5.45 ± 0.93	7.93 ± 1.21
Acidobacteria	3.34 ± 0.43	2.31 ± 0.48	3.52 ± 0.68
Verrucomicrobia	3.18 ± 0.36	3.08 ± 0.47	3.95 ± 0.55
Planctomycetes	2.46 ± 0.24a	0.94 ± 0.13c	1.52 ± 0.17b
Chloroflexi	1.39 ± 0.19b	0.97 ± 0.13b	2.86 ± 0.28a
Cyanobacteria	0.72 ± 0.12b	3.51 ± 1.08a	1.33 ± 0.45
Nitrospirae	0.27 ± 0.03b	0.29 ± 0.07b	0.82 ± 0.14a
Euryarchaeota	0.02 ± 0.00b	0.06 ± 0.02b	0.35 ± 0.09a
Thaumarchaeota	0.03 ± 0.00b	0.08 ± 0.03	0.25 ± 0.17a
Classes			
Alphaproteobacteria	12.57 ± 1.28	10.15 ± 0.87	9.48 ± 1.02
Betaproteobacteria	27.14 ± 1.91a	23.30 ± 3.60	15.04 ± 1.99b
Gammaproteobacteria	17.98 ± 2.40	25.44 ± 5.12	25.69 ± 3.78
Sphingobacteriia	9.68 ± 0.96a	2.57 ± 0.50b	2.73 ± 0.68b
Bacilli	3.10 ± 0.99	11.62 ± 4.74	7.52 ± 2.15
Actinobacteria	6.29 ± 0.61	5.38 ± 0.92	7.86 ± 1.21
Acidobacteriia	2.29 ± 0.26a	1.18 ± 0.17b	1.96 ± 0.32
Deltaproteobacteria	3.39 ± 0.21	3.17 ± 0.89b	5.76 ± 1.24a
Verrucomicrobiae	2.61 ± 0.28	2.81 ± 0.43	3.62 ± 0.50
Flavobacteriia	2.88 ± 0.43a	0.77 ± 0.10b	0.65 ± 0.16b
Methanomicrobia	0.01 ± 0.00b	0.02 ± 0.01b	0.20 ± 0.07a
Thaumarchaeota	0.03 ± 0.00b	0.08 ± 0.03	0.25 ± 0.17a
Genera			
Massilia	17.11 ± 1.56a	13.77 ± 3.55	6.04 ± 2.30b
Pseudomonas	9.49 ± 1.35	5.72 ± 1.51	11.07 ± 2.25
Acinetobacter	5.90 ± 1.81	16.08 ± 5.61	10.17 ± 2.83
Exiguobacterium	1.98 ± 0.67	9.77 ± 4.76	6.27 ± 2.12
Arthrobacter	2.32 ± 0.27	2.17 ± 0.63	3.02 ± 0.94
Acidobacterium	1.95 ± 0.18a	0.95 ± 0.12b	1.48 ± 0.19

(Continued)

Table 3. (Continued)

PROKARYOTES	CHC	UPC	CUC
Novosphingobium	2.03 ± 0.27a	1.97 ± 0.36	0.99 ± 0.25b
Duganella	1.98 ± 0.30a	0.59 ± 0.20b	1.04 ± 0.29b
Flavobacterium	2.00 ± 0.34a	0.45 ± 0.10b	0.43 ± 0.12b
Janthinobacterium	1.15 ± 0.18	1.48 ± 0.93	1.46 ± 0.38
Methanosaeta	0.00 ± 0.00b	0.01 ± 0.01b	0.15 ± 0.05a
Thermogymnomonas	0.00 ± 0.00b	0.01 ± 0.01b	0.03 ± 0.01a
Species			
Massilia timonae	12.79 ± 1.15a	9.82 ± 2.70	4.70 ± 2.03b
Pseudomonas putida	6.96 ± 1.10	3.81 ± 1.06	8.54 ± 1.91
Acinetobacter piperi	3.76 ± 1.03	12.28 ± 4.18	5.42 ± 1.79
Exiguobacterium undae	1.08 ± 0.37	4.30 ± 1.99	3.38 ± 1.15
Arthrobacter globiformis	0.89 ± 0.10	0.81 ± 0.24	1.23 ± 0.38
Duganella zoogloeoides	1.46 ± 0.22a	0.48 ± 0.16b	0.91 ± 0.25
Bradyrhizobium liaoningense	0.53 ± 0.16	0.21 ± 0.04	0.38 ± 0.06
Janthinobacterium lividum	0.70 ± 0.15	1.41 ± 0.91	1.40 ± 0.38
Pseudomonas syringae	0.31 ± 0.06	0.24 ± 0.07	0.69 ± 0.20
Rhodoferax ferrireducens	0.14 ± 0.02	0.49 ± 0.18	0.14 ± 0.04
Candidatus nitrososphaera	0.00 ± 0.00	0.00 ± 0.00b	0.01 ± 0.00a
Methanobacterium flexile	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Note. Estimates of the relative abundances at four taxonomic levels (phylum, class, genus, and species) of notable prokaryote communities are presented for Choctafaula, Uphapee, and Cubahatchee Creek samples (mean ± standard error). CHC, UPC, and CUC represent Choctafaula, Uphapee, and Cubahatchee Creeks, respectively. Significant differences are designated by different lower-case letters (the mean difference is significant at the 0.05 level).

classes, genera, and species) were compared between creek ecosystems. A graphical illustration is also presented so that the differences can be quickly visualized (Figure 4). Several environmental factors linked to separate creeks appeared to have little effect on the communities of Proteobacteria, Actinobacteria, Acidobacteria, and Verrucomicrobia. Other major phyla, on the other hand, have been shown to develop in a variety of ways in diverse stream environments.

One of the most important physicochemical variables influencing the bacterial community is pH (Krause et al., 2012; Wu et al., 2017; Y. Zhang et al., 2017). Many bacteria are affected by the amount of hydrogen ions in their environment. Each bacterial taxon requires a certain pH range in order to survive and grow. In 88 soils from North and South America, a previous study revealed a positive association between Bacteroidetes and pH (Lauber et al., 2009). Furthermore, several Bacteroidetes species have been reported to be hydrocarbon-sensitive (Timmis et al., 2010). Hydrocarbon levels in urban aquatic sediments have risen over time (Van Metre et al., 2000), indicating greater discharges related to industry and urbanization, such as increased automobile use. This study validated the idea that higher Bacteroidetes growth occurs in basic environments by observing a significant expansion of above phylum development in Choctafaula Creek sediments as compared to the other two stream samples. Due to Cubahatchee Creek's location outside of Tuskegee National Forest, it may experience more human-caused pollution. Even though Uphapee Creek samples were collected in the Tuskegee National Forest, they may contain more pollution from its tributary, the heavily urbanized Chewacla Creek. The poor development of Bacteroidetes in those creeks may also be a result of these elevated pollution levels. The classes Sphingobacteriia and Flavobacteriia as well as the genus *Flavobacterium* all belong to the phylum Bacteroidetes and exhibit similar patterns. Firmicutes differed substantially between Choctafaula and Cubahatchee Creeks. Many members of the Firmicutes that can generate spores can

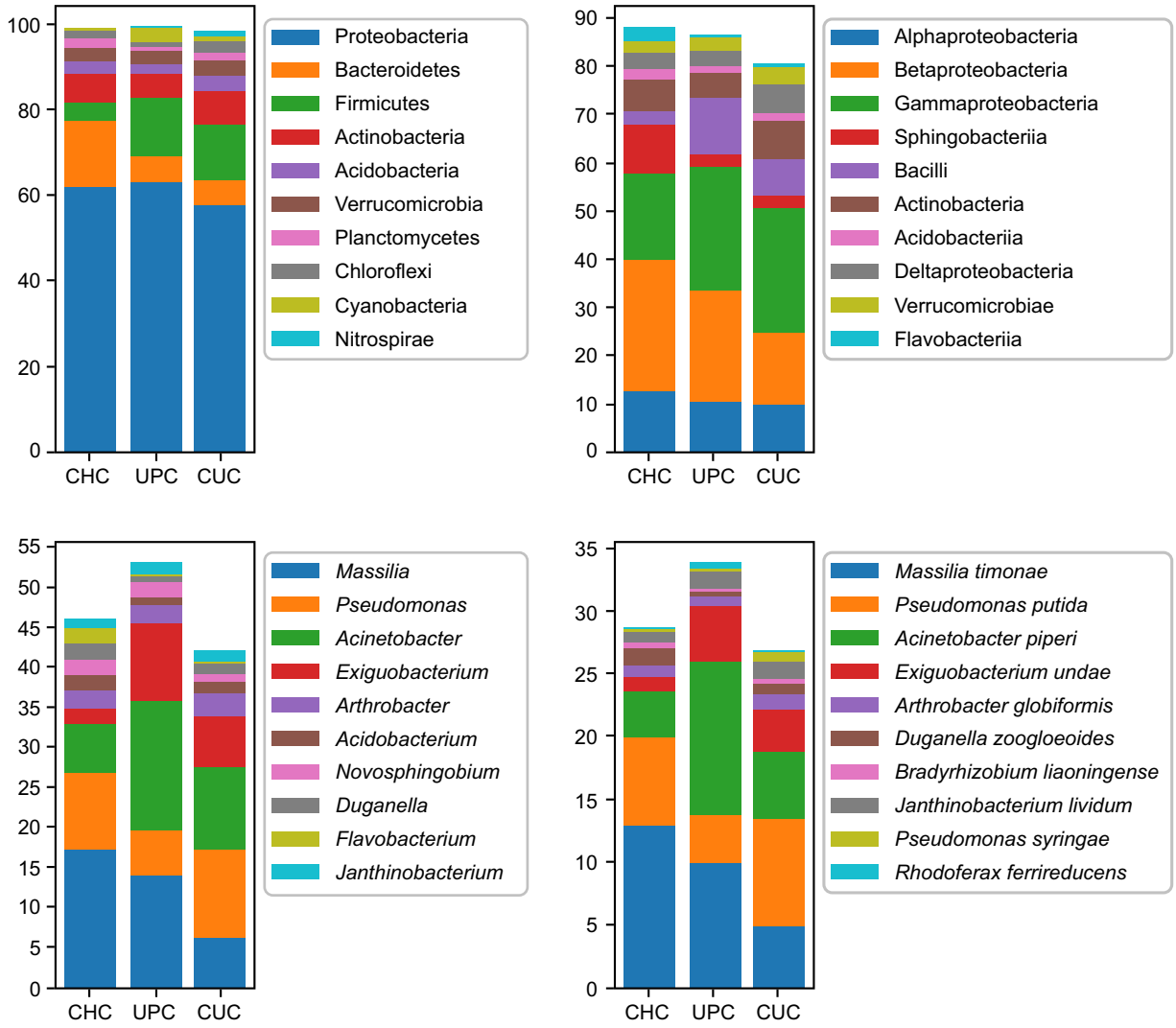

Figure 4. The mean relative abundances of 10 notable prokaryotes at 4 taxonomic levels [phyla (upper left), classes (upper right), genera (lower left), and species (lower right)] are presented for Choctafaula, Uphapee, and Cubahatchee Creek samples. CHC, UPC, and CUC denote Choctafaula, Uphapee, and Cubahatchee Creeks, respectively.

withstand severe environments (Zhuang et al., 2010), and the poor development of Bacteroidetes and other phyla in Cubahatchee samples due to the acidic climate and pollution may provide enough habitat for Firmicutes to thrive there.

Chloroflexi and Nitrospirae are two of the most common phyla in wastewater treatment plants (Shu et al., 2015; Xie et al., 2021) and are recognized to play a crucial role in nutrient transformation. Their abundance in Cubahatchee Creek was much higher than in two other streams, which suggests that there may be concentrated waste in the area. Planctomycetes can grow at a wide range of pH levels, ranging from 4.2 to 11.6 (Schlesner, 1994). They were much lower in Uphapee Creek than in both Choctafaula and Cubahatchee Creeks. This phylum is also significantly more abundant in Choctafaula sediments compared to those of Cubahatchee Creek, according to our research. Another noticeable difference at the phylum level was the percentage of Cyanobacteria in Uphapee Creek, which was significantly higher than in Choctafaula Creek. Cyanobacteria thrive in nutrient-rich stagnant water (Paerl et al., 2011; Schindler et al., 2008; Smith & Schindler, 2009), but they may also grow in slowly flowing sections of flowing streams, implying that some areas of Uphapee Creek have low flow, providing ideal conditions for Cyanobacteria to develop and, as a result, a high relative abundance in sediments. Cyanobacterial blooms have been observed in all 50 states of the United States (Anderson et al., 2021). Additionally, instances of disease outbreaks related to recreational water activities resulting from exposure to cyanobacterial toxins have been observed (Carmichael et al., 1985). Hence, it is imperative to implement measures to proactively manage cyanobacterial blooms prior to their onset.

The ecology and metabolism of the newly found phylum Acidobacteria are poorly understood, and the vast majority have not been cultured (Quaiser et al., 2003). Even though this

phylum did not differ much, the class Acidobacteriia and genus *Acidobacterium* did differ significantly in our streams. The Archaea phylum Euryarchaeota is known to have adapted to highly acidic climates (Korzhenkov et al., 2019). Although the Cubahatchee Creek sediments are only somewhat acidic, Euryarchaeota has a competitive advantage over other prokaryote phyla that are less successful in acidic circumstances, resulting in a greater relative abundance in those sediments. Thaumarchaeota, another archaea phylum with numerous members that are ammonia-oxidizing archaea (AOA), is known to be sensitive to pH and organic matters (Oton et al., 2016). Thaumarchaeota, like Euryarchaeota, has a high relative abundance in the low pH and high organic matter rich Cubahatchee Creek, according to our research.

Because Archaea are extremophiles, their high relative abundance in harsh environments may imply competitive advantages over other phyla that are sensitive to extreme environments. In the case of adverse conditions such as pollution and climate change, an analysis of the entire prokaryote community may therefore provide much better ecological indications than an analysis of only the bacterial community. The class Betaproteobacteria, however, did differ significantly between the streams, even though Proteobacteria did not.

Our research demonstrates Betaproteobacteria and its genus *Massilia*, as well as the species *Massilia timonae*, were much more common in the lower organic content rich Choctafaula sediments than in the higher organic matter rich Cubahatchee Creek. *Duganella*, another notable genus of Betaproteobacteria, showed significant differences between Choctafaula and both other two streams. But its species, *Duganella zoogloeoides*, only differed between Choctafaula and Uphapee Creeks.

Previous research indicates that Alphaproteobacteria are oligotrophic groups in aquatic habitats that are competitive in low-nutrient environments (Pinhassi & Berman, 2003). Although Alphaproteobacteria did not differ significantly between stream conditions in our study, one of its prominent genera, *Novosphingobium*, did differ significantly between Choctafaula and Cubahatchee Creeks. Several species of Deltaproteobacteria are known to exhibit predatory behavior (Strauch et al., 2007), and this class's high abundance in Cubahatchee Creek suggests that there are more prey species present. In this study, several prokaryotes from various taxonomic groups were found to be significantly more abundant in organic matter-rich sediments. Most of the elements were also concentrated in organic matter-rich environments. As a result, it is difficult to determine whether specific elements shift a specific community, regardless of organic matter. Carefully controlled studies are therefore required to determine the independent impact of elements or heavy metals on the prokaryotic community.

Pathogenic Genera

Several potentially pathogenic genera that can cause various illnesses in humans and animals were compared across creek environments (Table 4). *Aeromonas* has been linked to gastroenteritis and wound infections. In most cases, gastroenteritis is caused by contaminated water or food, while wound infections are caused by exposure to contaminated water. Necrotizing fasciitis caused by *Aeromonas* spp. can be life-threatening and necessitate antibiotic treatment and even amputation in the most severe cases (Minnaganti et al., 2000). Compared to Choctafaula Creek, Cubahatchee Creek had a significantly higher abundance of this genus in terms of relative abundance. In Cubahatchee samples compared to other streams, *Bacillus* that cause anthrax (Boutiba-Ben Boubaker & Ben Redjeb, 2001; Jedrzejas, 2002; Mock & Fouet, 2001) and food poisoning (Bergdoll et al., 1973; Hauge, 1955) were substantially more prevalent. In contrast to Uphapee samples, it was also higher in Choctafaula samples. *Clostridium*, a genus associated with traumatic gas gangrene (McClane & Rood, 2001), foodborne disease (Grass et al., 2013), and antibiotic-related diarrhea (Carman, 1997; Rupnik et al., 2009), was found to be significantly more prevalent in Cubahatchee Creek than in the other two streams.

Leptospira also showed substantial differences between Choctafaula and Cubahatchee Creeks. This genus can cause leptospirosis in humans, and each year, 1.03 million cases of leptospirosis are reported, resulting in 58,900 deaths (Costa et al., 2015). People are known to become infected after encountering *Leptospira* that has been shed in the urine of diseased animals in contaminated water or soil (Waitkins, 1986). In addition, leptospirosis infection in livestock costs money in industrial farming (Ellis, 2015). In contrast to the other two streams, Choctafaula Creek displayed a high relative abundance of *Brucella*, a genus that causes brucellosis in humans and livestock. Like leptospirosis, brucellosis results in significant economic losses for the livestock industry (Wareth et al., 2022). *Burkholderia*, *Campylobacter*, *Chlamydia*, *Helicobacter*, *Legionella*, and *Serratia* are some of the other prominent genera that revealed significant differences between creek ecosystems. According to our research, the total mean relative abundance of selected potentially pathogenic genera was highest in Cubahatchee Creek (19.28%), moderate in Choctafaula Creek (11.13%), and lowest in Uphapee Creek (8.78%).

This study provided compelling evidence that siltation and other anthropogenic pollution not only alter prokaryotic community structure but also increase the relative abundances of potentially pathogenic genera in freshwater creeks. The presence of potentially pathogenic genera in greater abundance in Cubahatchee Creek is especially concerning because this creek was designated as "Fish, Wildlife, and Swimming." Therefore, people may use this creek for wading and swimming, increasing their chances of being infected by several bacteria- related diseases. To guarantee that such a designated watercourse serves its intended purpose, it is necessary to conduct regular testing for pathogens.

Table 4. Mean Relative Abundances of Potentially Pathogenic Genera.

POTENTIALLY PATHOGENIC GENERA	CHC	UPC	CUC
Aeromonas	0.02 ± 0.01b	0.12 ± 0.05	1.09 ± 0.61a
Arcobacter	0.01 ± 0.00	0.23 ± 0.11	0.73 ± 0.58
Bacillus	0.21 ± 0.03b	0.10 ± 0.01 c	0.37 ± 0.06a
Bordetella	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01
Brucella	0.02 ± 0.00a	0.00 ± 0.00b	0.01 ± 0.00b
Burkholderia	0.43 ± 0.07	0.43 ± 0.06	0.72 ± 0.11
Campylobacter	0.12 ± 0.06a	0.03 ± 0.02	0.01 ± 0.00b
Clostridium	0.41 ± 0.05b	1.04 ± 0.24b	4.19 ± 1.21a
Chlamydia	0.03 ± 0.00b	0.03 ± 0.01b	0.09 ± 0.02a
Corynebacterium	0.01 ± 0.00	0.03 ± 0.02	0.03 ± 0.01
Enterobacter	0.04 ± 0.01	0.15 ± 0.08	0.14 ± 0.05
Enterococcus	0.18 ± 0.11	0.44 ± 0.38	0.12 ± 0.03
Helicobacter	0.00 ± 0.00b	0.00 ± 0.00b	0.01 ± 0.00a
Klebsiella	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01
Listeria	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Legionella	0.02 ± 0.00	0.06 ± 0.02	0.05 ± 0.01
Leptospira	0.00 ± 0.00b	0.02 ± 0.01	0.05 ± 0.02a
Mycobacterium	0.05 ± 0.01	0.04 ± 0.01	0.08 ± 0.02
Mycoplasma	0.03 ± 0.01	0.11 ± 0.04	0.11 ± 0.05
Neisseria	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Nocardia	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Pseudomonas	9.49 ± 1.35	5.72 ± 1.51	11.07 ± 2.25
Rickettsia	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
Serratia	0.01 ± 0.00b	0.14 ± 0.10	0.30 ± 0.14a
Staphylococcus	0.02 ± 0.00	0.03 ± 0.01	0.04 ± 0.01
Streptococcus	0.00 ± 0.00	0.01 ± 0.00	0.02 ± 0.01
Total	**11.13**	**8.78**	**19.28**

Note. Estimates of the relative abundances of potentially pathogenic genera in prokaryote communities are presented for Choctafaula, Uphapee, and Cubahatchee Creek samples (mean ± standard error). CHC, UPC, and CUC represent Choctafaula, Uphapee, and Cubahatchee Creek, respectively. Significant differences are designated by different lower-case letters (the mean difference is significant at the .05 level).

Conclusion

Freshwater creeks are essential components of ecosystems, and their deterioration due to agriculture and industrialization has prompted laws to protect them and a desire to restore creek habitats. Despite all attempts to maintain watersheds, there is no consensus on how to evaluate the usefulness of streams. In addition, little is known about the ecology of the baseline species (prokaryotes) that regulate the watershed's fundamental metabolic activities. Therefore, beginning a study in a region with varied levels of land management can provide pertinent prokaryotic community ecology markers. Although these findings are intriguing, further controlled research is required to

fully comprehend the concepts of river sediment prokaryotic ecology. Choctafaula, Uphapee, and Cubahatchee Creeks are vital habitats for threatened species. Therefore, continuous ecological profile monitoring is required. This study's findings will also benefit future scientists who wish to do research in these environments.

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Author Contributions

Conceived and designed the experiments: TH, RS, RA. Analyzed the data: TH, RS, and WM. Wrote the first draft of the manuscript: TH. Contributed to the writing of the manuscript: TH, RS, RA, SF, WM, JQ, TS, and JA. Agree with the manuscript results and conclusions: TH, RS, RA, SF, WM, JQ, TS, and JA. Jointly developed the structure and arguments for the paper: TH and RS. Made critical revisions and approved the final versions: RS and SF. All authors reviewed and approved the final manuscript.

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Appendix

Table A1. Concentrations (ppm) of Arsenic, Cadmium, Nickel, Phosphorus, Lead, and Zinc.

CREEK	AS	CD	NI	P	PB	ZN
Choctafaula Creek						
	<0.1	<0.1	<0.1	55.27	9.81	5.36
	<0.1	<0.1	<0.1	80.16	9.56	5.38
	<0.1	<0.1	<0.1	62.62	13.57	7.10
	<0.1	<0.1	<0.1	58.31	13.39	6.11
	<0.1	<0.1	<0.1	16.13	3.92	6.34
	<0.1	<0.1	<0.1	46.95	9.50	2.71
	<0.1	<0.1	<0.1	33.99	8.64	4.46
	<0.1	<0.1	<0.1	63.09	2.55	3.28
	<0.1	<0.1	<0.1	40.22	4.75	4.59
	<0.1	<0.1	<0.1	46.04	<0.1	<0.1
	<0.1	<0.1	<0.1	21.29	8.55	4.84
	<0.1	<0.1	<0.1	9.24	5.14	5.67
Uphapee Creek						
	<0.1	<0.1	<0.1	77.01	10.52	6.57
	<0.1	<0.1	<0.1	82.58	6.00	2.58
	<0.1	<0.1	<0.1	48.13	6.79	5.19
	<0.1	<0.1	<0.1	73.09	9.10	2.57
	<0.1	<0.1	<0.1	53.95	2.86	4.41
	<0.1	<0.1	<0.1	25.32	4.45	3.32
	<0.1	<0.1	<0.1	<0.1	1.91	2.35
	<0.1	<0.1	<0.1	<0.1	17.47	5.08
	<0.1	<0.1	<0.1	26.36	4.02	8.28
	<0.1	<0.1	<0.1	29.87	5.71	18.72
	<0.1	<0.1	<0.1	59.77	14.59	19.36
	<0.1	<0.1	<0.1	<0.1	20.22	6.09
Cubahatchee Creek						
	<0.1	<0.1	<0.1	190.96	12.46	10.28
	<0.1	<0.1	<0.1	34.53	6.44	2.87
	<0.1	<0.1	<0.1	182.09	7.89	6.32
	<0.1	<0.1	<0.1	70.48	13.39	6.28
	<0.1	<0.1	<0.1	22.36	3.39	4.53

(Continued)

Table A1. (Continued)

CREEK	AS	CD	NI	P	PB	ZN
	<0.1	<0.1	<0.1	<.1	14.06	6.12
	<0.1	<0.1	<0.1	40.96	4.41	5.20
	<0.1	<0.1	<0.1	78.50	15.45	7.65
	<0.1	<0.1	<0.1	<0.1	2.44	0.65
	<0.1	<0.1	<0.1	92.77	16.93	<0.1
	<0.1	<0.1	<0.1	44.86	23.31	2.17
	<0.1	<0.1	<0.1	31.68	19.20	0.87

Note. Concentrations (ppm) of arsenic (As), cadmium (Cd), nickel (Ni), phosphorus (P), lead (Pb), and zinc (Zn) are presented for sediment samples from the Choctafaula, Uphapee, and Cubahatchee Creek ecosystems.

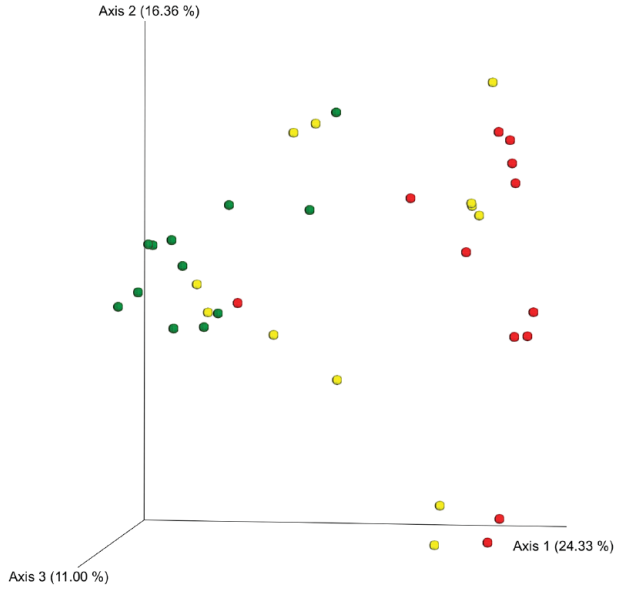

Figure A1. PCoA plot based on bray-curtis distances for 36 sediment samples. Green, yellow, and red spheres represent Choctafaula, Uphapee, and Cubahatchee samples, respectively.

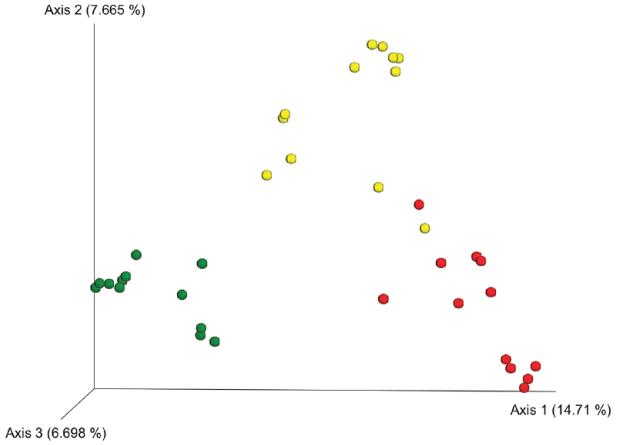

Figure A2. PCoA plot based on jaccard distances for 36 sediment samples. Green, yellow, and red spheres represent Choctafaula, Uphapee, and Cubahatchee samples, respectively.