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Summer 2024

The Relationship Between Changing Land Cover and Benthic Macroinvertebrate Diversity in Choccolocco Creek, Northeast Alabama

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Recommended Citation

The Relationship Between Changing Land Cover and Benthic Macroinvertebrate Diversity in Choccolocco Creek, Northeast Alabama

A Thesis Submitted to the
Graduate Faculty of Jacksonville State University
in Partial Fulfillment of the
Requirements for the Degree of
Master of Science
with a Major in Biology

By

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Jacksonville, Alabama
August 2, 2024

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Abstract

Land cover changes, driven by urbanization, agriculture, and deforestation, significantly affect the physical and chemical characteristics of water bodies at both the watershed and riparian levels. At the larger spatial scale, a stream's surrounding watershed facilitates lateral inputs into the stream that are essential for biodiversity support, hydrological stability, and water quality. When a stream becomes disconnected from the lateral inputs due to urban or agricultural development, the morphology of the stream is significantly altered. On a smaller spatial scale, the riparian zone can act as a buffer to pollutants. The vegetation surrounding the stream bank promotes stability to the instream morphology. Riparian zones trap sediments, preventing excessive sedimentation in the stream. In aquatic systems, the sensitivity of organisms to degraded rivers is most apparent in benthic (stream bottom-dwelling) macroinvertebrates such as insects, crustaceans, mollusks, and annelids. Benthic macroinvertebrates, key indicators of water quality and ecosystem health, are highly sensitive to alterations in their habitats. This, in turn, reduces macroinvertebrate diversity by favoring tolerant species over sensitive ones, leading to homogenized communities. Our research analyzed LULC from three time periods in the Choccolocco Creek watershed in Northeast Alabama at two spatial scales (watershed and riparian) to assess their impacts on benthic macroinvertebrate diversity over time. This study design was based on two previous sample periods, 2000 and 2012, where macroinvertebrate samples were collected at four sites in Choccolocco Creek. LULC data was made available from the NLCD Landsat imagery database for all years sampled (2000,2012,2022). Urban, agriculture, and forest percent changes in LULC were determined among the three study intervals for both watershed and riparian zones. Over time, changes in the biodiversity of benthic macroinvertebrates were crucial in determining the resilience of a stream system to changes in land use and land cover (LULC) in the Choccolocco Creek watershed in Northeast Alabama. All

three studies used standardized EPA biomonitoring benthic invertebrate collection protocols in riffles. Four riffles in the watershed were sampled during all 3 decades that occurred in April, July, and October. All macroinvertebrates collected were pooled for each event and were identified at the family level for all three studies. Invertebrate diversity statistics of richness, evenness as the Shannon-Wiener index, were determined for each of the four sites across the three sampling events. These values were correlated to changes in LULC across sites and sampling events. Differences in invertebrate community structure among sites and sampling events were with non-metric multi-dimensional scaling analysis. In addition, comparisons of beta diversity (community stability) across sites were used to assess change in communities through time. Results showed that all sites had forest as the most abundant land cover in both watershed and riparian zones across all sampling locations and events (WS- 45 to 80%, Riparian 40 to 65%), agriculture was second (WS 15 to 37%, Riparian 32 to 47%) and urban had the smallest cover (WS 3 to 25%, Riparian 0 to 10%). However, at both the watershed and riparian buffer scales, the urban land cover had the highest percentage increase at each site 5 to 115%), except site one found in the national forest exhibited little change in land cover at either watershed or riparian scales. Results also showed that declines in macroinvertebrate richness and evenness from 2000 to 2022 were correlated to increasing agriculture and urban land cover and decreasing forest cover and that these relationships were similar at both the riparian and watershed scales. NMDS showed that differences in community composition differed across sites although communities did not vary across time. Still, beta diversity estimates were highest in site 3 which also had the largest percent change in land use. This study highlights the need for continued monitoring on multiple spatial scales to maintain biological integrity within a stream.

Acknowledgments

I would like to extend my deepest gratitude to the Jacksonville State University Biology Department for their many resources and support during my thesis process. My experience during this program is something I will hold dear to me forever.

I would also like to thank my thesis advisor, Dr. Lori Tolley-Jordan. Throughout this process, she has not only been the best mentor I could have asked for, but she has become a lifelong friend who has cared for me and guided me academically and, most importantly, emotionally. She is an incredible person for whom I will forever be thankful. My thesis committee, Dr. Sean Chenoweth, Dr. Tenzing Ingty, and Dr. Grover Brown, have also spent so much of their time and attention teaching and guiding me with their expertise in their respective fields. Without them, I would not be where I am today.

To my mom and sister, thank you from the bottom of my heart for helping me with fieldwork and lab work and for listening to my presentations and papers over and over. Thank you for pushing me and believing in me when I did not believe in myself. Your love and unconditional support have made me who I am today. Both of you inspire me, and with you in my corner, I feel like every goal I set is attainable.

It takes a village, and I am so thankful for mine.

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Abbreviations

1. LULC – Land use land cover

2. ADEM- Alabama Department of Environmental Monitoring

3. GIS- Geographic Information System

4. USGS- United States Geological Survey

5. US EPA- United States Environmental Protection Agency

6. ESRI- Environmental Systems Research Institute

7. HUC- Hydrological Unit Code

8. NMDS- Non-metric Multidimensional Scaling

9. ANOSIM- Analysis of Similarity

Introduction

Freshwater habitats are among the most intensively human-influenced on Earth, linked to regional and global extinctions of freshwater fauna (Dudgeon et al., 2006). Specifically, habitat loss from changes to the natural landscape to agricultural or urban environments significantly impacts freshwater systems' physical (geomorphology), chemical, and biological features (Darwall et al., 2018). Physical features, including geology, topography, and water movement through the stream, constrain processes such as stream bed and bank erosion and subsequent downstream transport and deposition of dislodged sediments.

When the landscape within the watershed is altered due to urban or agricultural development, altered morphology due to channel straightening and deepening (Marchand et al., 2022) leads to markedly altered water movement patterns in the stream channel. Specifically, water flow's magnitude, timing, and frequency during storm events are changed significantly. As most streams have sediments of varying particle sizes, altered flow regimes result in modifications of sediment transport that can cause channel incision, meaning the vertical erosion of a stream bed deepens; as a result, the stream is disconnected from the lateral inputs of water needed for maintaining water in a channel during times of drought (Graf et al., 2016).

Riparian zones are considered stream buffers in that the vegetation surrounding the stream bank promotes stability to the instream morphology (Allen et al., 2021). As streams are strongly connected to their valleys, changes in stream morphology cause a disconnect between the surrounding landscape to the stream. When landscapes are altered by land use or land cover (LULC) changes because of agriculture and urban development, the riparian zones are reduced or eliminated. Without vegetation to prevent erosion and reduce the rate of water entry into the stream, the banks become steep, and water quality decreases due to the input of pollutants from farming, crop cultivation, and deforestation, such as pesticides or herbicides, fertilizers, gravel,

and overloading of nutrient deposits (Cranswick & Crook, 2015). Soil erosion leads to downstream sedimentation because heavy layers of silt/clay that are less permeable are deposited, causing embeddedness and flooding. As a result, downstream waterways can become clogged, blocking the connectivity of the upstream and downstream habitats, leading to pollution and degradation of downstream habitats and their taxa (Clark & Wilcock, 2000). The combined effects of altered in-stream morphology and loss of riparian zones lead to significant declines in water quality. These changes generally result in a more substantial likelihood of input of pollutants such as pesticides or herbicides, fertilizers, gravel, and overloading of nutrient deposits (Cranswick & Crook, 2015).

Changes in morphology and water chemistry from LULC from natural environments to urban or agriculture cause significant alterations in the biodiversity of organisms in streams so that those that are tolerant to changes remain. In contrast, intolerant organisms are lost (Bauer, 2019). This pattern is so pervasive and consistent that a stream ecology discipline known as biomonitoring was developed to evaluate stream health. Biomonitoring is a methodology that systematically uses biological responses to assess environmental conditions, particularly the presence and impact of pollutants (Rosenburg & Resh, 1993). By observing and analyzing the health, behavior, and population dynamics of specific organisms, scientists can detect and quantify the levels of contaminants in ecosystems. This approach is crucial because it provides direct evidence of the effects of pollution on living organisms, offering a more comprehensive and realistic assessment of environmental health than chemical or physical monitoring alone (McKie, 2018). In aquatic systems, the most significant gradient in the sensitivity of organisms to degraded rivers is found in macroinvertebrates, including insects, crustaceans, mollusks, and annelids. This is due, in large part, to the high abundances, numbers of species, and habitat use

by macroinvertebrates as compared to other groups so that well-defined metrics of stream health for all regions of the U.S. are defined based on macroinvertebrate assemblages (USEPA 1997).

Freshwater macroinvertebrates' response to land-use changes yields striking patterns in regions of exceptional aquatic biodiversity like Alabama (Elkiens et al., 2019). Here, long-term patterns in LULC due to agriculture are well documented. Agricultural land development, land clearing, and wood harvest resulted in a forest loss of 36.12 Mha in the Southeast region of the United States over the last four centuries, with rapid forest loss of 30.28 Mha from 1850-1920 because of the conversion of forest land to cropland and pasture (Xiaoyong et al., 2022). After extensive agricultural exploitation in the 19th century, land use began to convert from agricultural to pine plantations in the 20th century (Drummond & Loveland, 2010). An estimation of 10 million acres of agricultural land to commercial forest land was predicted in Alabama between 1992-2020 and has proven to be accurate as there are currently about 9.7 million acres of commercial forests (Wear and Greis, 2002; Alabama et al., 2021). Forest extraction and agricultural development in Alabama's watersheds have a direct correlation to stream degradation resulting in erosion of banks, increased baseflow due to reduced soil retention, increased nutrient loading and suspended solids leading to hypoxia, and an increase in bacterial growth reducing water quality (Schilling et al., 2008). Currently, changing LULC in Alabama is primarily due to urban development. Shrestha et al. (2023) showed that for ten metropolitan areas in Alabama, urban cover increased for all sites from a minimum of 20% to a maximum of 54%, with corresponding losses of vegetation cover. Therefore, macroinvertebrates sensitive to changes in stream morphology and water chemistry due to landscape degradation often do not recover from legacy land-use changes (Harding et al., 1998). Historical land use changes combined with current throughout the state can give significant insight into the issues that each type of land use causes, both long and short-term (Shrestha et al., 2023). As such, in systems

where invertebrate diversity is monitored through decades, the cumulative losses in diversity can be used to show the significance of landscape degradation through time. In Northeast Alabama, Choccolocco Creek is an ideal setting to study the relationship between LULC and invertebrate biodiversity. Invertebrate biodiversity along the creek has been documented during the past 20 years, and LULC data is available for changes among urban, agricultural, and forested (natural areas) across the watershed. In addition, riparian cover data has also been available across the 20 years. As such, in this study, we evaluated the impact of percent changes in forest, agriculture, and urban LULC across the watershed and determined the degree of effects changing riparian zones have on invertebrate diversity over time.

Site Descriptions

Choccolocco Creek is a major tributary of the Coosa River in Northeast Alabama. The basin is 97,408 hectares and flows 113.6 km from its headwaters near Piedmont, Alabama, to its confluence with the Coosa River near Pell City, Alabama. The Choccolocco Watershed occurs within the Appalachian Physiographic Province and is characterized by a humid, warm climate characteristic of the southeastern United States (Soil Conservation Service 1979).

The sample sites included in this study site are on the Talladega Upland and Southern Limestone/Dolomite Valley and Low-Rolling Hills (Griffith et al., 2001; O'Neil & Chandler, 2001) (Figure 1). Site 1, in the Talladega National Forest (N 33o49'22.3 W 85o34'45.7), is in the Talladega Upland eco-region and is characterized by a heavily forested area underlain with strongly deformed metamorphic rock, Site 3 located in Munford, AL (N 33o33'42.3 W 85o56'25.8), and Site 4 located at Jackson Shoals in Lincoln, AL (N 33o32'49.5 W 85o05'33.7) are part of the Southern Limestone/Dolomite Valley and Low Rolling Hills eco-region characterized by low rolling hills and valleys with limestone and cherty dolomite below (Bailey et al., 1994;

Griffith et al. 2001, and USEPA 2000). Site 2 in Oxford, AL (N 33o36'09.6 W 85o47'15.7) was sampled in 2000 and the repeated survey year in 2011(Figure 1). However, Site 2 was excluded from this 2022 study due to unsafe conditions that would not permit access. Therefore, the sample sites in the survey remain referred to as Site 1, Site 3, Site 4, and Site 5.
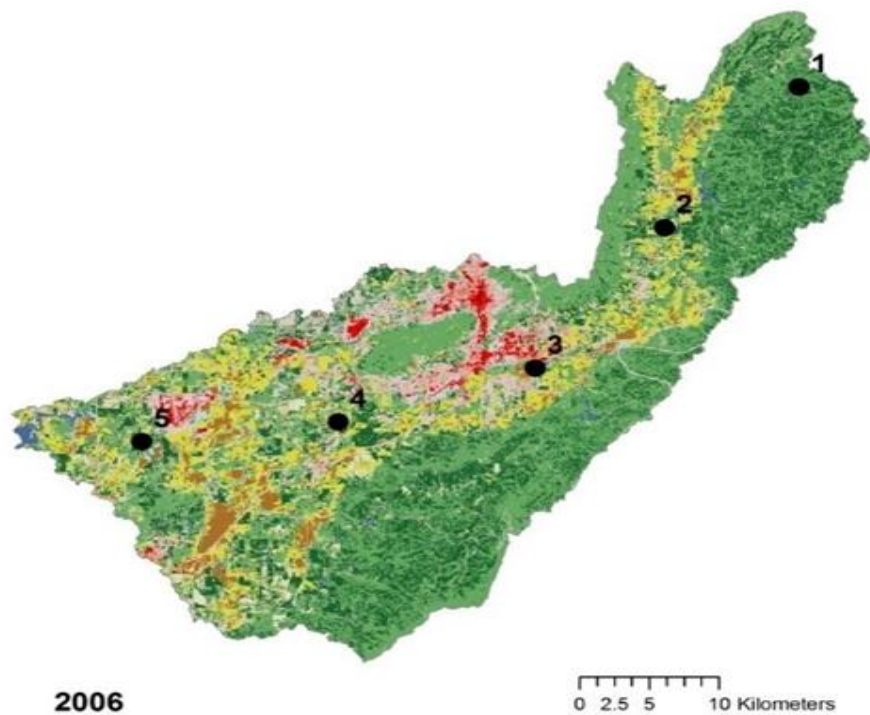
Figure 1. Choccolocco Creek Watershed with land use cover is designated as green (Forest), brown/tan (crop), and red/pink (urban) land cover. Numbers represent five sites sampled in 2000 (Carver, 2000) and 2011 (Allen, 2012), and Sites 1, 3, 4, and 5 were sampled in 2022 (this study). Site 2 was excluded from this study due to lack of access. Map used with permission by authors L. Tolley-Jordan and H. Allen

Sub-Watersheds with Sites	Area of sub-watershed (km^2)
Cottaquilla (Site 1)	140.90
Tuskegee (Site 3)	152.40
Emorai (Site 4)	65.40
Jackson Shoals (Site 5)	102.50
Choccolocco Watershed	615.90

Table 1. The area of each sub-watershed sampled and the total of the entire watershed in km^2. Sites are listed from most upstream to downstream with a corresponding increase in drainage area.

METHODS

Determining Land use/Landcover (LULC) in 2000, 2011, and 2022

Sub-Watersheds

Conversion of forested parcels of the Choccolocco Creek watershed to agriculture and urban development corresponding to the 2000, 2011, and 2022 collections was obtained from the United States Geological Service (USGS) from National Land Cover Data Sets (NLDS-Vogelmann *et al.,* 2001; Homer *et al.,* 2004). The National Land Cover Database (NLCD) provided nationwide data on land cover and change at a 30m resolution with a 16-class legend based on a modified Anderson Level II classification system (ArcGIS, 2016). Determined by the USGS, a hierarchical hydrologic unit code (HUC) consisting of two additional digits for each level in the hydrologic unit system was used to identify the hydrologic areas studied. The

assigned codes used in this study were HUC-10 for the entire watershed and HUC-12 for the

sub-watersheds within the entire Choccolocco Creek watershed (USGS, 2016).

The percentage of forest, urban, and agricultural land cover (as defined by Anderson *et

al.,* 1976) for the Choccolocco Creek watershed was calculated using ArcGIS ™ v.9.2 (ESRI,

2006). The land class code legend was obtained from the USGS to determine the land cover type

(Table 1). Land cover percentages were calculated for the entire Choccolocco Creek watershed

delineated by the USGS 12-Digit Hydrologic Unit Codes (HUC-12).

Riparian Corridor

There are five sub-watersheds in the Choccolocco Creek basin where the LULC

percentages for the four sample sites were obtained (Table 2). According to Pilotto et al. (2015),

LULC most influenced macroinvertebrate communities at the most local scale, the 200-meter

segment riparian corridor. The study segment length is defined using the orthoimage to delineate

the channel centerline upstream from the sampling site for a distance, x, in kilometers, which is

the logarithm of the basin area (Ad) in square kilometers, using the following equation:

$$x = \log 10 \ (Ad) \ (1)$$

The purpose is to derive a segment length value to standardize segment length to the

basin area. The segment-level riparian zone was characterized and defined by the maximum

buffer width, 200 m, extending outward from the stream centerline (USGS, 2007).Using the

delineated riparian buffer, the LULC data for the entire watershed is obtained using the

abovementioned methods. Land cover percentages were calculated for the 200 m riparian buffer

delineated by the USGS 10-Digit Hydrologic Unit Codes (HUC-10). Percent change in land

cover between each time interval: 2000 to 2011, 2011 to 2022 was calculated as: To assess the

relationship between change in land cover and catchment richness, the following calculations

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were made based on Poole and Downing (2004), % change in land cover for each of the watershed scale and riparian scales surrounding the 4 sites.

% change in land cover = ((year of land cover area/ catchment area)/ (year of land cover area/catchment area)) X 100. This was conducted for 2000-2011, 2000-2022, and 2011-2022 for both the watershed and riparian buffer zones.

Table 2. USGS NLCD land class codes (NLDS-Vogelmann *et al.,* 2001). Developed (1) land cover was used for the Urban land cover class, Cropland (2) and Grass/Shrub (3) were combined to make up the Agricultural land cover class, and Tree Cover (4) was used for the Forest land cover class in this study. These primary land classes were the only ones calculated to determine LULC data in this study.

Class	Land Cover	Description
1	Developed	Areas of intensive use with much of the land covered with structures (e.g., high-density residential, commercial, industrial, mining, or transportation), or less intensive uses where the land cover matrix includes vegetation, bare ground, and structures (e.g., low-density residential, recreational facilities, cemeteries, transportation and utility corridors, etc.), including any land functionally related to the developed or built-up activity.
2	Cropland	Land in either a vegetated or unvegetated state used for the production of food, fiber, and fuels. This includes cultivated and uncultivated croplands, hay lands, orchards, vineyards, and confined livestock operations. Forest plantations are considered as forests or woodlands (Tree Cover class) regardless of the use of the wood products.
3	Grass/Shrub	Land predominantly covered with shrubs and perennial or annual natural and domesticated grasses (e.g., pasture), forbs, or other forms of herbaceous vegetation. The grass and shrub cover must comprise at least 10% of the area and tree cover is less than 10% of the area.
4	Tree Cover	Tree-covered land where the tree cover density is greater than 10%. Cleared or harvested trees (i.e., clearcuts) will be mapped according to current cover (e.g., Barren, Grass/Shrub).
5	Water	Areas covered with water, such as streams, canals, lakes, reservoirs, bays, or oceans.
6	Wetland	Lands where water saturation is the determining factor in soil characteristics, vegetation types, and animal communities. Wetlands are comprised of mosaics of water, bare soil, and herbaceous or wooded vegetated cover.
7	Ice/Snow	Land where accumulated snow and ice does not completely melt during the summer period (i.e., perennial ice/snow).
8	Barren	Land comprised of natural occurrences of soils, sand, or rocks where less than 10% of the area is vegetated.

Determining Macroinvertebrate Diversity at four sites in 2000, 2011, and 2022

Field and Laboratory Procedures

All studies collected benthic macroinvertebrates from the stream substrate with a D-framed kick net (mesh size 500 microns) from the same sampling area (riffle) during April, July, and October. Using standard biomonitoring protocols, invertebrates were preserved in 70% ethanol and transported to the laboratory for identification and enumeration following the design described in Carver (2000) and Allen (2012). According to Merritt and Cummins (4th edition), all benthic macroinvertebrates were identified to family. As all samples from Carver (2000) and Allen (2012) are housed at JSU (Jacksonville State University), any taxonomic discrepancies were reviewed by comparing specimens across the three surveys.

Diversity Metrics: Family Richness, Evenness, Community composition and Beta Diversity

The measures of diversity including taxa richness, evenness, community composition and beta diversity were based on the total numbers of individuals collected of all sampling dates (April, July, and October) for each survey (2000, 2012, and 2022, respectively). Diversity estimates included taxa richness (number of species per site) and evenness. These measurements are standard for biomonitoring.

Evenness was calculated using the following formula Where **n** is the number of individuals of each family, and **N** is the total number of individuals of all families.

$$(H=\sum[(pi)*\ln(pi)]),$$

Differences in community composition across the three surveys and sites were determined by Non-metric Multidimensional scaling (NMDS) using a Bray-Curtis dissimilarity matrix for community composition. The Bray-Curtis indices provide a reliable summary of the NMDS plots. The NMDS represents a visual representation of similarity by the distances from each site to another. Therefore, the axis does not have meaning. The greater the distance between the ellipses the more dissimilarity in community composition. High overlap indicates more significant similarity in the communities by sampling year. An Analysis of Similarity (ANOSIM) was performed on the NMDS output to determine if significant differences occurred across the sites and decades. In addition, Beta diversity, a measure of spatial overlap of communities, was calculated using the following formula: Where **α** is the average number of species at each site for pooled sampling years, and **γ** is the total number of species of the pooled data from each site.

$$\beta = \gamma/\alpha,$$

All analyses were performed in RStudio.

Relationships between changing LULC, Taxa Richness, and Evenness

To assess the relationship between change in land cover and catchment richness, the following calculations were made based on Poole and Downing (2004): the percentage change in land cover, taxa richness, and evenness were calculated.

% change in land cover = ((year of land cover area/ catchment area)/ (year of land cover area/catchment area)) X 100. This was conducted for 2000-2011, 2000-2022, and 2011-2022 for both the watershed and riparian buffer zones.

The % change in watershed richness (ΔR_w, following) and ΔE (evenness as Shannon-Wiener index) was calculated as follows:

$$\Delta R_{2000-2022} = (R_{2022} - R_{2000})/R_{2000}), \quad \text{and} \quad \Delta E_{2000-2022} = (E_{2022} - E_{2000})/E_{2000},$$

RESULTS

Land Use Land Cover

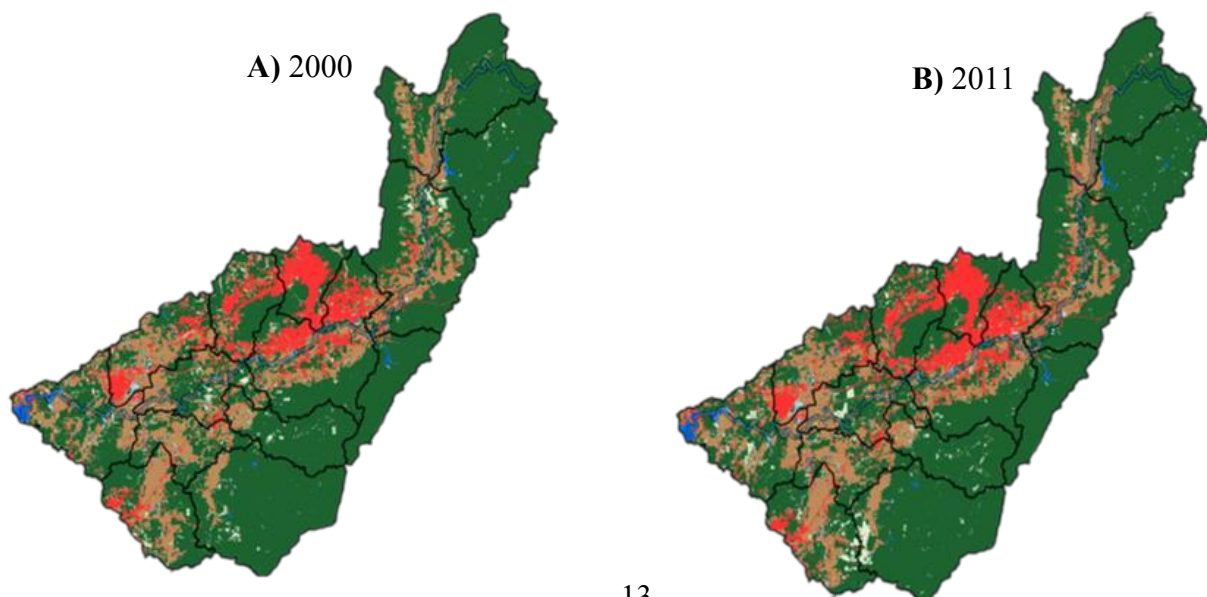*Land Use Land Cover-Watershed scale*

The primary land cover type for all sites and years in the sub-watersheds was forest cover (Figure 2 and Figure 3). However, urban land cover showed the most considerable percent change over the sampling years (Figure 4). Site One had little change in LULC at the sub-watershed level over the three sampling years, as this is the reference site. The 2000 sampling

year was excluded from the percent change figures because it was the baseline for % change in 2011 and 2022. From 2011 to 2022, sites 3 and 4 showed the most extensive loss of forest cover and the highest conversion from forest to urban land cover. Site 5 had the highest percentage of agricultural land cover, with Site 4 being remarkably close in terms of agricultural land cover percentage (Figure 4). However, sites 3 and 4 had the highest increases in agriculture. In 2022, urban land cover increased at all sites, with the most significant increases at sites 4 and 5, and forest land cover decreased at all sites, with the most significant losses at sites 3 and 4 (Figure 4). In all years, Site 3 had the lowest percent forest cover and the highest percentage of urban and agricultural land usage (Figure 3).

Land Use Land Cover-Riparian Buffer Zone Scale

The 200 m riparian buffer zone followed similar percentage patterns of each land use type at the watershed level for all years and sites (Figure 2 and Figure 5). For the 2011 and 2022 sampling periods, urban land cover increased significantly at Site 3 than at Sites 4 and 5. However, urban land cover increased at all sites, and agricultural land cover increased except at Site 1 (Figure 6).

A) 2000

B) 2011

0 2.5 5   10 Kilometers
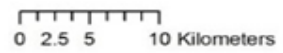
Figure 2. 2000 (A), 2011(B), and 2022 (C) land cover data of Choccolocco Creek at the riparian corridor (entire watershed scale) and sub-watersheds that contained each sampling site where red represents urban, brown-agriculture, green-forest, grey-barren, and blue-water.
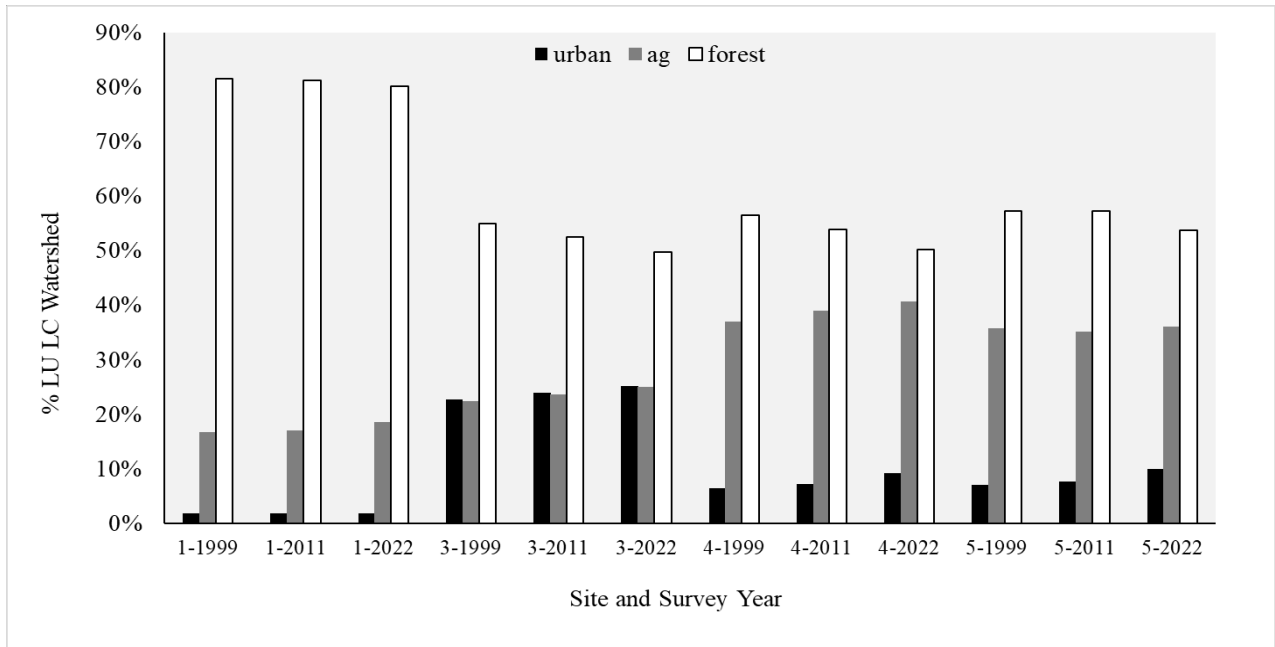
Figure 3. Percentage of LULC for Forest, Urban, and Agriculture in 2000, 2011, and 2022 in the four HUC 12 sub-watersheds surrounding the four sites based on USGS: NLDS LULC layers.
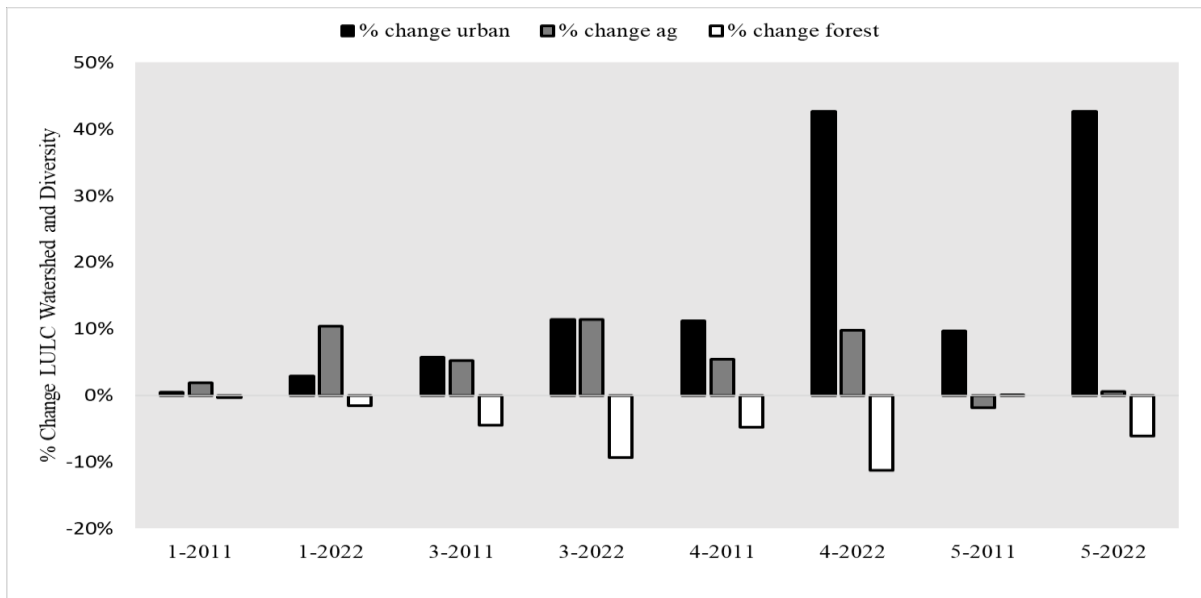
Figure 4. Percent change of LULC for each sub-watershed surrounding the four sites excluding the reference year, 2000. % change was based on differences between 2000 and 2011 and 2000-2022.

15
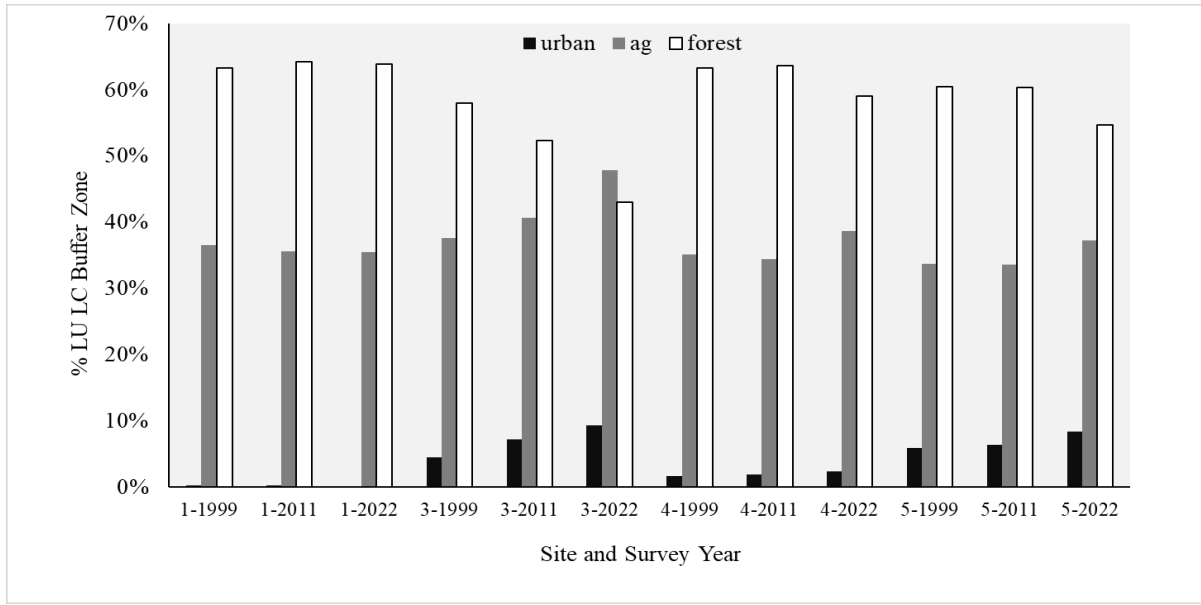
Figure 5. Percentage of LULC for Forest, Urban, and Agriculture in 2000, 2011, and 2022 in the riparian buffer zones of each sub-watershed based on USGS: NLDS LULC layers.
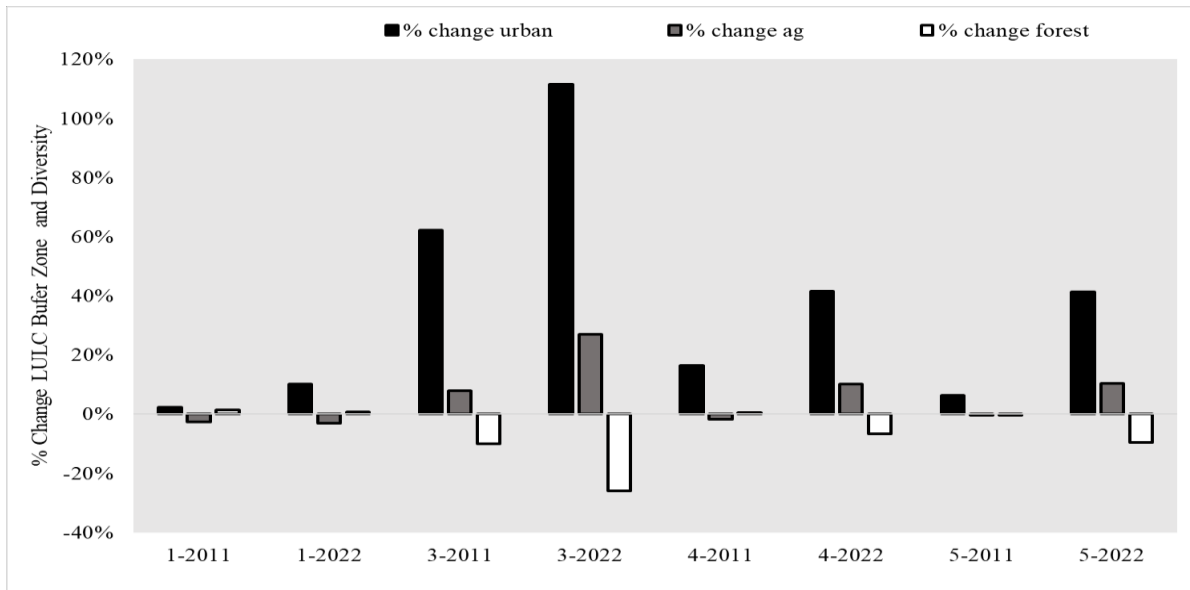
Figure 6. Percent change of LULC for each riparian buffer in each sub-watershed surrounding the four sites excluding the reference year, 2000. % change was based on differences between 2000 and 2011 and 2000-2022.

Changes in Macroinvertebrate Diversity across sites and time

In 2022, 2331 individuals from 36 families were meticulously collected in kick samples at all four sites over three sampling seasons, providing a comprehensive dataset. This data revealed a significant increase in individual taxa from 2815 in 2000 to 3262 in 2011 (Table 3). However, the number of families present has fluctuated, rising from 46 in 2000 to 44 in 2011 and then decreasing to 36 families and 2331 individuals in 2022 (Table 3).

A consistent trend of decreasing overall taxa richness and evenness from 2000 to 2022 was shown (Table 4). The reference site (Site One) saw a decrease in taxa richness in 2011 but remained consistent in 2000 and 2022. The Shannon-Weiner values for Sites 3 and 4 were consistently lower for the sampling years 2011 and 2022 compared to Sites 1 and 5 in their respective years. The values at all sites, except for the reference site, demonstrated a consistent decrease from 2000 to 2011 to 2022, indicating a long-term loss in taxa abundance, taxa richness, and evenness at all sites over time (Table 4).

Community assemblages differed significantly among the 4-sites based on the NDS analyses (Stress value = 0.15) of the Bray-Curtis dissimilarity index (Table 5). The 2-D plot showed similar compositions of Sites 3 and 4 while sites 1 and 5 had very dissimilar communities to each other and sites 3 and 4/ Analysis of Similarity of the NDS values showed that the separation in communities was significant ($p = 0.001$, R-value of 0.759, Figure 8).

The results for the NMDS for the three sampling years indicate a similarity in community composition found in all three years where there is overlap. However, there was high variability, particularly in 2011 and 2022, as depicted by the distance of the dots representing the four sites sampled that do not overlap compared to the sampling period in 2000, where there is almost complete overlap (Figure 9). However, the ANOSIM analysis showed no significant difference in community composition across sampling years (Figure 10). The P-value was greater than 0.05 (0.592), indicating no statistically significant difference in macroinvertebrate communities across

years. The R-value of -0.037 reflects no differences between the inter- and intra-groups, meaning there was no dissimilarity among years (Figure 10).

Beta diversity for each site showed the highest turnover at site 3, although all sites exhibited relatively high turnover ranging from 0.41 to 0.72 (Table 6)

Table 3. Summarizes the macroinvertebrate families found in Choccolocco Creek in the 2022, 2012, and 2000 surveys. It indicates the presence of taxa with (1) and their absence with (0).

Order	Family	1999	2011	2022
Coleoptera	Dryopidae	1	1	0
	Dytiscidae	0	0	1
	Elmidae	1	1	1
	Gyrinidae	1	1	1
	Haliplidae	0	1	0
	Psephenidae	1	1	1
Isopoda	Asellidae	1	1	1
Amphipoda	Gammaridae	1	0	1
Decapoda	Astacidae	1	0	1
Diptera	Athericidae	0	0	0
	Blepharceridae	0	1	0
	Ceratopogonidae	0	1	1
	Chironomidae	1	1	1
	Culicidae	0	1	0
	Dixidae	1	1	1
	Empididae	1	1	1
	Simuliidae	1	1	1
	Tabanidae	0	1	0
	Tipuliidae	1	1	1
Ephemeroptera	Baetidae	1	1	1
	Baetiscidae	0	1	0
	Caenidae	0	1	1
	Ephemerellidae	1	1	1
	Heptageniidae	1	1	1
	Leptohyphidae	1	1	1
	Leptophlebiidae	1	1	1
	Neoephemeridae	1	0	0
	Isonychiidae	1	1	1
Hemiptera	Gerridae	1	0	0
	Veliidae	0	1	0
Lepidoptera	Pyralidae	1	0	0
Megaloptera	Corydalidae	1	1	1
Odonata	Aeshnidae	1	0	0
	Calopterygidae	1	0	0
	Coenagrionidae	1	0	1
	Cordulidae	1	0	0
	Gomphidae	1	1	1
	Libelluidae	1	1	0
Plecoptera	Capniidae	1	1	1
	Leuctridae	0	1	0
	Perlidae	1	1	1
	Perlodidae	1	1	0
	Pteronarcyidae	1	1	1
	Taeniopterygidae	1	1	0
Trichoptera	Branchycentridae	1	1	1
	Glossosomatidae	1	1	1
	Helicopsychidae	1	0	0
	Hydrophilidae	1	1	0
	Hydropsychidae	1	1	1
	Leptoceridae	1	1	1
	Limnephilidae	1	0	1
	Philopotamidae	1	1	1
	Polycentropodidae	1	1	1
Oligochaeta	Tubificidae	1	1	1
Coenogastropoda	Pleuroceridae	1	1	1
Veneroidea	Corbiculidae	1	1	1

Table 4. Diversity estimates (Species Richness and Evenness as Shannon-Weiner Index across

sites and years. The higher values indicate higher richness, evenness, and diversity. Values were

based on data pooled from all sampling events (July, October, and April) at each site (1,3,4 and 5) in 2000, 2011, and 2022.

Year	Site	Species richness	Shannon Index
1999	1	39	2.865189
1999	3	34	2.510012
1999	4	28	2.676204
1999	5	27	2.517888
2011	1	31	2.508485
2011	3	20	2.457499
2011	4	16	2.03986
2011	5	42	2.58875
2022	1	32	2.636581
2022	3	12	1.590583
2022	4	12	2.217651
2022	5	22	2.468567

Table 5 The Bray-Curtis dissimilarity matrix: This table presents pair-wise comparisons of community composition at each site (1,3,4 and 5) for 2000, 2011, and 2022 surveys. The values of 1 and 0 indicate complete overlap and no overlap in community composition, respectively.

|	1999	1999	1999	1999	2011	2011	2011	2011	2022	2022	2022	2022
1999	1	0.600149	0.641196	0.573957	0.685927	0.526611	0.518577	0.423216	0.656203	0.346789	0.459152	0.544872
1999	0.600149	1	0.648824	0.57604	0.560799	0.630909	0.472952	0.456574	0.484272	0.54513	0.563948	0.494651
1999	0.641196	0.648824	1	0.657609	0.556594	0.69719	0.711688	0.442851	0.552117	0.493878	0.69697	0.653793
1999	0.573957	0.57604	0.657609	1	0.472782	0.48245	0.528376	0.604583	0.488169	0.332842	0.445521	0.691466
2011	0.685927	0.560799	0.556594	0.472782	1	0.513253	0.494141	0.352679	0.587056	0.407538	0.490411	0.462472
2011	0.526611	0.630909	0.69719	0.48245	0.513253	1	0.547945	0.36193	0.456621	0.632822	0.78022	0.501139
2011	0.518577	0.472952	0.711688	0.528376	0.494141	0.547945	1	0.5	0.451513	0.463016	0.572668	0.637988
2011	0.423216	0.456574	0.442851	0.604583	0.352679	0.36193	0.5	1	0.435928	0.383695	0.29387	0.613861
2022	0.656203	0.484272	0.552117	0.488169	0.587056	0.456621	0.451513	0.435928	1	0.253142	0.375	0.604798
2022	0.346789	0.54513	0.493878	0.332842	0.407538	0.632822	0.463016	0.383695	0.253142	1	0.5917	0.386228
2022	0.459152	0.563948	0.69697	0.445521	0.490411	0.78022	0.572668	0.29387	0.375	0.5917	1	0.4514
2022	0.544872	0.494651	0.653793	0.691466	0.462472	0.501139	0.637988	0.613861	0.604798	0.386228	0.4514	1
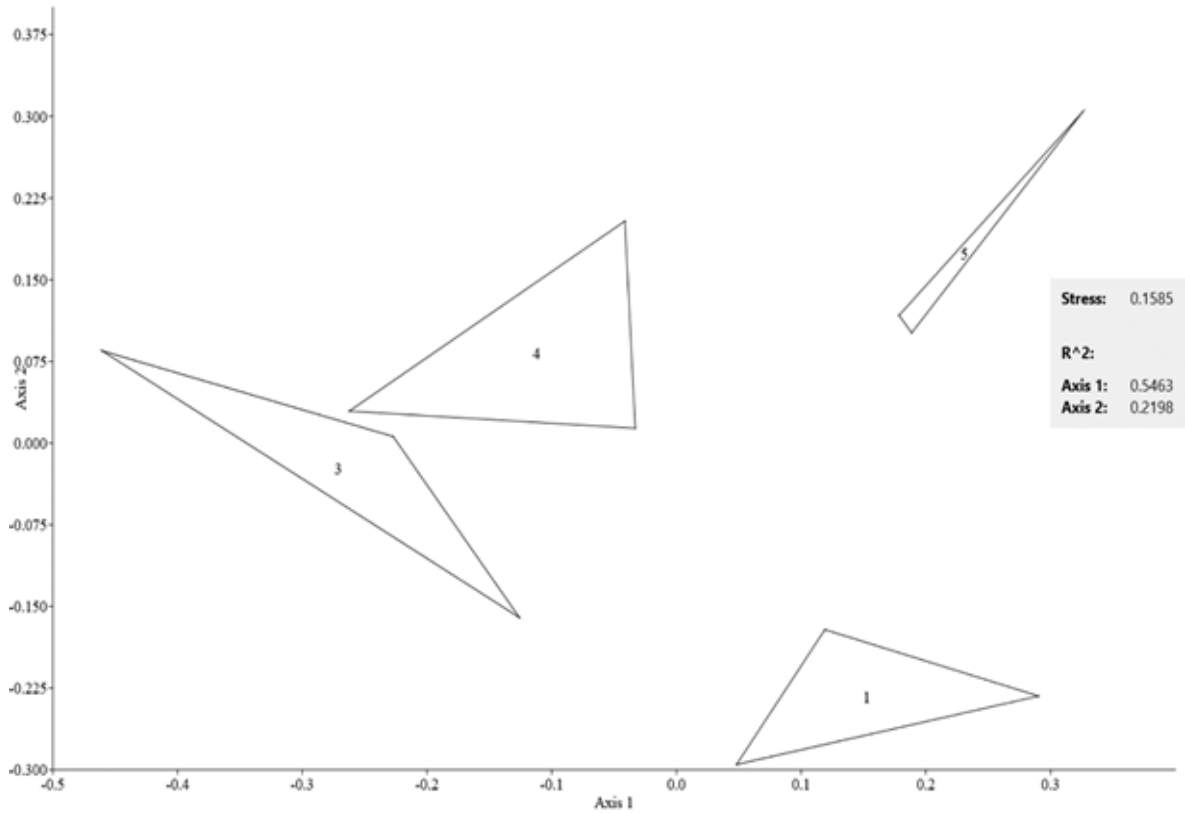
Figure 7. Non-metric multidimensional scaling (NMDS) of Bray–Curtis dissimilarity matrix of the benthic macroinvertebrate assemblages of five sites sampled in Choccolocco Creek by years (2000, 2011, and 2022). A stress level of 0.1572 indicates a good-moderate model fit. Each dot represents one sampling year (2000, 2012, and 2022), and ellipses represent the sampling sites.
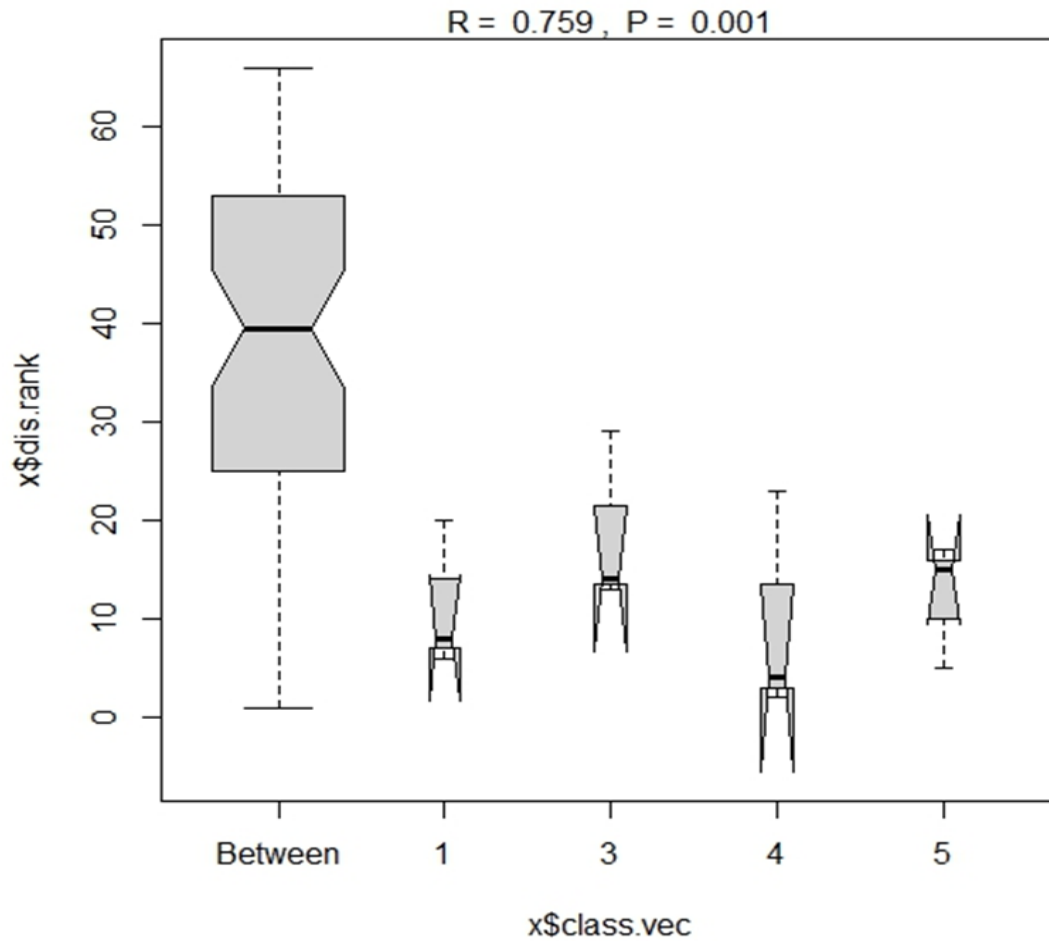
Figure 8. ANOSIM analysis: R-value range (–1, 1). An R-value close to 0 represents no significant differences in inter-group and intra-group. An R-value close to 1 that inter-group differences are greater than intra-group differences. The y-axis represents the distance rank among sampling sites, and the x-axis represents the results among each site. Intra-group results are shown for each site. $p < 0.05$ indicates a significant difference among the years sampled but does not show pair-wise differences.

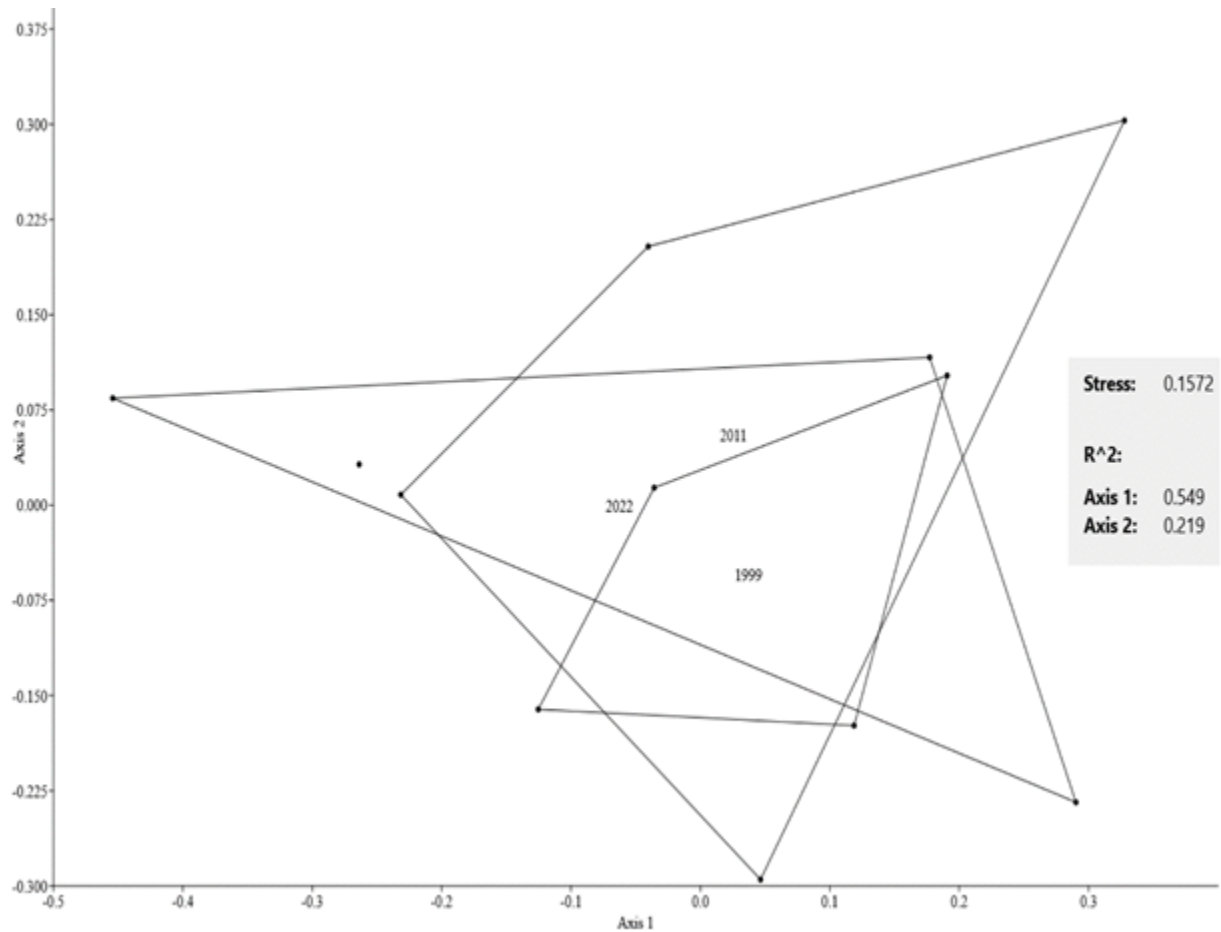**Figure 9.** Non-metric multidimensional scaling (NMDS) of the Bray–Curtis dissimilarity matrix of the benthic macroinvertebrate assemblages of four sites sampled in Choccolocco Creek by years (2000, 2011, and 2022). A stress level of 0.1572 indicates a good-moderate model fit. Each dot represents one of the year's four sampling sites (1,3,4, and 5), and the shapes represent the sampling year.
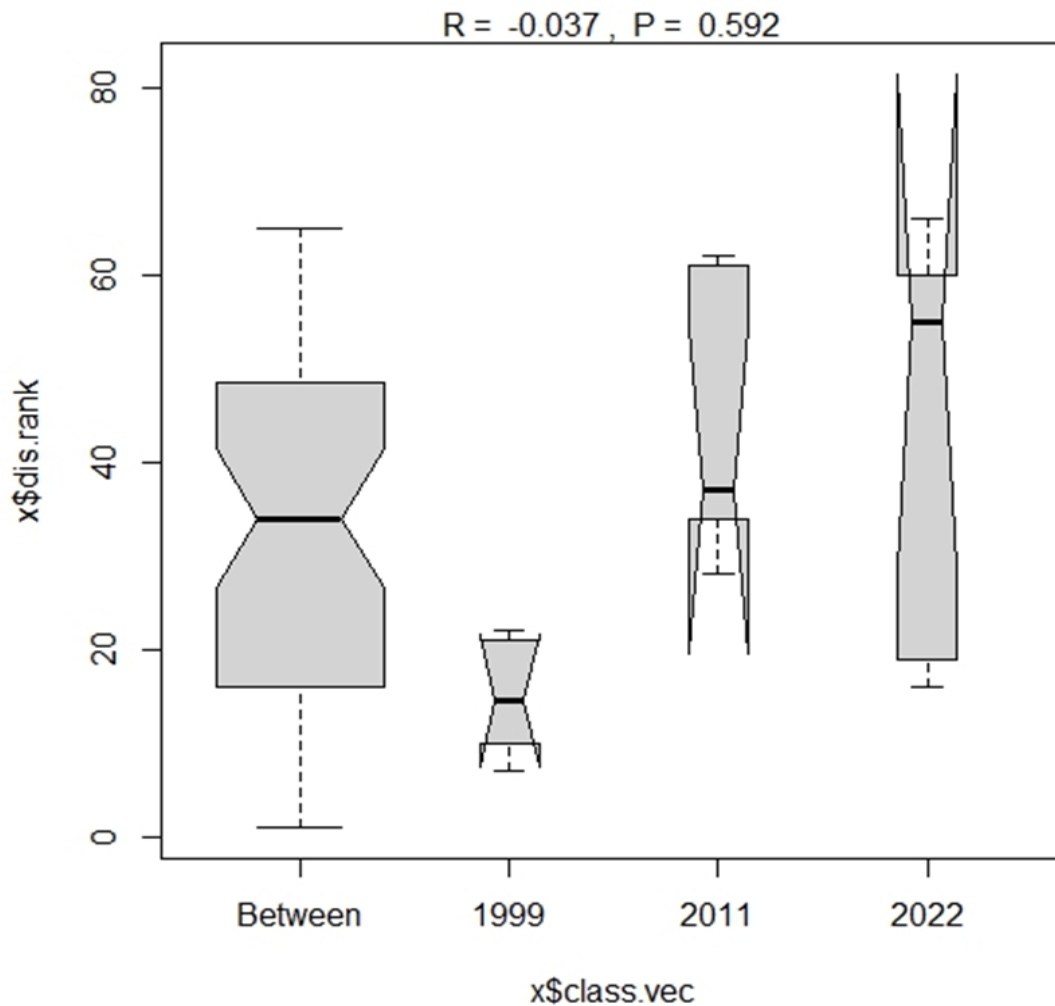
Figure 10. ANOSIM analysis: R-value range (–1, 1). An R-value close to 0 represents no significant differences in inter-group and intra-group. An R-value close to 1 indicates that inter-group differences are greater than intra-group differences. The y-axis represents the distance rank among all sampling years, and the x-axis represents the results among each year sampled. Intra-group results are shown for each site. $p < 0.05$ indicates a significant difference among all sites but does not show differences among sites.

Table 6. The measure of the difference in species composition among sites. Higher beta diversity values indicate a low level of community similarity, while a low beta diversity index shows a high level of community similarity.

Site	alpha=average number of species	gamma=total number of species	beta= (gamma/alpha)-1
1	34	48	0.4117647
3	22	38	0.7272727
4	18.66667	29	0.5535714
5	30.33333	51	0.6813187

Relationships Between LULC and Macroinvertebrate Diversity

At the watershed and riparian scales, richness and evenness declined with increasing urban and agricultural land cover and decreasing forest land cover (Figures 11 and 12). The R^2 values for the watershed scale ranged from 0.23 to 0.72 (Figure 11); at the riparian buffer zone, R^2 values ranged from 0.46 to 0.61 (Figure 12). The average R^2 values at the watershed level (0.56) and the riparian level (0.52) were similar and above 0.50, indicating that LULC has a high correlation to declining family richness and evenness at both scales. Within the watershed level, percent change in urban land cover had the lowest R^2 values for taxa richness (0.29) and evenness (0.23). Agriculture and forest percent change highly correlate to taxa richness and evenness declines. In contrast, at the riparian scale, percent change in urban land showed the most substantial relationship between taxa richness (0.60) and evenness (0.61). However, all R^2 values at the riparian scale were at least 0.46 n or higher, indicating a strong correlation among percent change in urban, agriculture, and forest loss within the riparian buffer and declining taxa richness and evenness. The effects of the watershed and riparian land change on the family richness and evenness showed similar trends. There was a negative relationship between urban and agricultural land changes on family richness and evenness. Alternatively, there was a positive relationship between forest cover and family richness and evenness at both the watershed and riparian scales.

Figure 11. Relationships between % change in land use (urban, agriculture, and forest) from 2000 to 2011 and 2000 to 2022 and family richness- Panels A through C and Evenness Panels D-F for all sites sampled in the corresponding sub-watersheds. Higher R^2 values indicate a stronger correlation between variables.
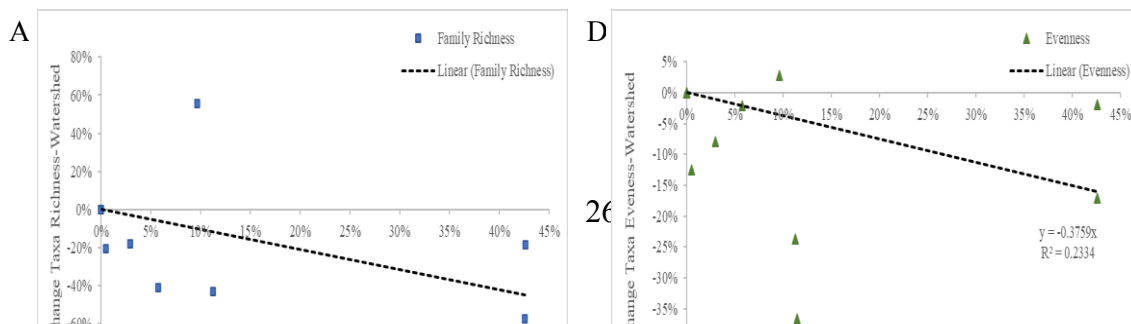

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Figure 12. Relationships between % change in land cover (urban, agriculture, and forest) from 2000 to 2011 and 2000 to 2022 and family richness- Panels A through C and Evenness Panels D-F for all sites sampled (1,3,4, and 5) in the 200 m riparian buffer zones of each of the sub-watersheds. Higher R^2 values indicate a stronger correlation among variables.

Discussion

Our results suggest macroinvertebrate diversity is controlled by land use changes at both scales, with the watershed scale having a slightly higher effect. This finding, however, does not decline the importance of riparian buffer factors, which also produce considerable variation in

macroinvertebrate communities. Our study has unveiled the significant influence of local habitat and biological diversity of streams and rivers by landforms and land use within the surrounding valley at multiple scales, which follows a standard hierarchical paradigm of stream structure and function (Allen, 2004).

Forest cover dominated the land use type at both the watershed and riparian levels, with agriculture being the second highest land cover type. However, the most active land use conversion was urban at both scales through the three sampling periods. Similar trends were shown in a study conducted in 2022 to determine land use changes in the Southeast United States from 1945 to 2012 (Nedd & Anandhi, 2022). Forest land and agriculture had most of the land cover (55%). Forest land accounted for 12% of the change and agricultural land for 20%. Those changes were in response to the growing population in the region, which has increased by 2.59% on average annually. The land type changes were shown to increase and decrease for each time frame in the study period, and those changes were related to various land use change drivers. As agricultural land usage increased, so did urban land development at a higher rate (Nedd & Anandhi, 2022).

The effect of LULC on macroinvertebrates at the watershed scale is far more documented than at the riparian scale. However, studies on both spatial scales concluded that macroinvertebrate diversity is significantly impacted by changing land cover over time (Vaughn, 1997). This is shown in the results of this study by the high correlations of land change percentage to declining macroinvertebrate communities on both scales. A prior study done in 2015 found significant effects of land use within a 200 m zone from the riparian corridor on benthic macroinvertebrates. The study attributed this to morphological alterations to the riparian zone and the riparian buffer land use changes (Pilotto et al., 2015). However, in a separate study, results found that watershed LULC changes have a more significant impact on macroinvertebrate communities. Additional literature emphasized that watershed characteristics have been strongly linked to stream biota,

and some studies cite land use/land cover (LULC) at the watershed scale as having the most significant impact on aquatic macroinvertebrate diversity and abundance (Kyriakeas & Watzin, 2006; Dovciak & Perry, 2002). The results of this study show a strong relationship among land conversion and the richness and evenness of macroinvertebrate communities at both scales. These findings can be confirmed by a similar study that compared long-term land usage data and macroinvertebrate samples collected in multiple watersheds. The results showed that the macroinvertebrate taxonomic richness was much higher in forested streams at both the watershed and riparian scales than that of agricultural or urban (Harding et al., 1998). The most dynamic changing land use in this system was urban cover. Although these changes are registered at the watershed and buffer zone scales, evaluating changes in in-stream habitat and chemical processes is needed to determine more localized community scale changes. Numerous studies have reported flashier hydrographs, smaller sediment sizes, elevated levels of suspended solids, and altered channel morphology that are measured at in-stream scales reflect reduced biotic richness and increased dominance of tolerant species as forested land is lost (Kennen et al., 2012; Cuffney et al., 2010). Faster-moving water dislodges and displaces animals, reduces sediment pore space, which is a critical habitat component for many taxa, and increases turbidity, which interferes with respiration and feeding processes (Cuffney et al., 2010; Kennen et al., 2012). These effects can differentially affect habitats within a watershed, altering the habitat and changing the community composition, such as evenness, individual abundance, and taxa richness among sites and sampling years

The community composition of macroinvertebrates was significantly different among sampling sites. This was expected due to Alabama's high diversity, geologically diverse terrain, climate stability, and the substantial amount of water flowing through the state (Duncan & Wil-

son, 2013). In contrast, our results showed that there was not a significant difference in community composition among years. However, high amounts of variability shown in 2011 and 2022 could reflect community composition differences through time, but the sampling size of this study was not adequate to test the interaction among sampling events and locations. This high variability and effect of time on community structure was reflected in the higher beta values at sites with the greatest changes in LULC and lowest at the reference site. Community composition results based only on landscape-level factors may not accurately reflect drivers of differences as in-stream properties of streams at smaller scales strongly contribute to community composition (Agra et al., 2021). Given the limited sample numbers and lack of in-stream measurements, these results did not reveal the strongest indicators of changing diversity, highlighting the need for detailed surveys through time.

Studies such as this can be included in efforts to effectively mitigate the effects of changing land use and ensure the health of stream biota in the Southeastern US (Reid et al., 2018), particularly in Alabama. Freshwaters in Alabama are the epicenter for the biodiversity of aquatic invertebrates such as snails, mussels, and crayfishes, along with aquatic insect groups of stoneflies (Grubbs, 2011), mayflies (McCafferty, 2010) and caddisflies (Harris et al., 1991). These groups are strong indicators of healthy stream systems due to their low tolerance to pollutants and are an extensive part of biomonitoring in the state (ADEM, 2010). Understanding the diversity of invertebrates in Alabama is vital. The highest extinction rates of snails and mussels in North America occurred over the past 100 years due to stream degradation, primarily impoundments (GSA, 2009). Monitoring such studies is critical in understanding diversity changes to document sensitive taxa losses.

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