ABSTRACT

MEZA SALAZAR, ANA MARIA. Surface-groundwater Interactions in a Changing Climate: Impacts of Acidification and Phosphorus Enrichment on Tropical Stream Macroinvertebrate Assemblages. (Under the direction of Dr. Alonso Ramírez).

Tropical freshwater ecosystems are increasingly vulnerable to climate change, yet the effects of natural stressors, such as episodic acidification and nutrient dynamics, remain underexplored. This dissertation investigates these processes to understand their influence on macroinvertebrate assemblages, functional roles, and physiological responses in undisturbed tropical streams within a volcanic landscape in the Caribbean basin. By addressing critical gaps, this work establishes a valuable baseline for assessing the impacts of natural stressors and their interactions with climate change. The first objective was to synthesize existing research on climate change impacts on Caribbean freshwater ecosystems through a comprehensive literature review. The review revealed significant progress in understanding diversity, ecology, and hydrology in the region, but highlighted gaps in the study of indirect effects, such as shifts in water chemistry driven by climate change. It emphasized the need for research focusing on natural drivers, including acidification and nutrient gradients, to inform conservation efforts and support the management of tropical freshwater ecosystems under changing environmental conditions. The second objective examined the effects of episodic acidification events on macroinvertebrate assemblages and their physiology. In unbuffered streams, pH declines during the rainy season significantly disrupted community composition, reducing biodiversity and altering the distribution of functional feeding groups. Physiological measurements revealed that macroinvertebrates exposed to low pH conditions exhibited reduced respiration and growth rates, indicating metabolic stress with potential consequences for ecosystem functions. The third objective investigated how a natural phosphorus gradient influences macroinvertebrate

communities and their body condition. Streams enriched by geothermal groundwater inputs exhibited elevated phosphorus concentrations, shaping community structure, functional feeding group distribution, and physiological condition. Differences in macromolecule content (proteins, lipids, and carbohydrates) among macroinvertebrates reflected variations in resource availability and metabolic demands, demonstrating how nutrient gradients drive both community-level and taxon-specific responses with implications for nutrient cycling and energy flow.

Together, these findings underscore the interconnectedness of community structure and physiological responses, revealing how natural stressors influence biodiversity and ecosystem processes. By identifying sensitive taxa and their functional responses to environmental changes, this study provides valuable insights into the resilience of tropical freshwater ecosystems. The integration of community-level and physiological assessments highlights the importance of understanding how these processes affect ecosystem stability and function. This research contributes to general and applied ecology by establishing a baseline for natural processes in relatively undisturbed systems. It offers practical insights for conservation and adaptive management strategies to protect biodiversity and maintain ecosystem function in tropical streams facing the challenges of climate change and human activities.

Surface-groundwater Interactions in a Changing Climate: Impacts of Acidification and Phosphorus Enrichment on Tropical Stream Macroinvertebrate Assemblages.

by
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APPROVED BY:

Alonso Ramírez Chair of Advisory Committee	Marcelo Ardón
Adam Terando	Brad Taylor

DEDICATION

To my family and friends, whose constant support has been a cornerstone in my life. To my extended family—my uncles, aunts, and cousins—thank you for always believing in me and celebrating my achievements, no matter the distance.

To my dear friends, who patiently listened to me talk endlessly about my research for the past five years. Thank you for your unwavering support, encouragement, and understanding. Your friendship has been invaluable throughout this journey.

To my father, José Luis Meza García, my mother, Luz Stella Salazar Arbeláez, and my siblings, Juan Pablo Meza Salazar and Marcela Meza Salazar. You have been my foundation, my inspiration, and my greatest source of strength. Everything I have accomplished has been possible because of your love, support, and the values you have instilled in me.

Especially, to my mother, Luz Stella, who taught me to dream big and gave me the courage to pursue those dreams. It was through her example that I found my passion for studying life and understanding the world around me. This achievement is because of her and for her.

BIOGRAPHY

Ana Meza-Salazar was born in Manizales, Colombia, and is a biologist with over a decade of experience studying rivers and streams, focusing on the effects of land use and climate change on aquatic ecosystems. She completed her Bachelor's degree in Biological Sciences at the University of Caldas, where her undergraduate research titled *"Composition and trophic structure of aquatic macroinvertebrates in the upper Chinchiná river basin"* highlighted the importance of riparian vegetation in maintaining aquatic biodiversity.

Ana pursued her Master's degree in Biological Sciences at the same institution, earning cum laude honors for her thesis, *"Structure and function of aquatic macroinvertebrates in areas impacted by gold mining, agricultural and livestock activities in the upper Chinchiná river."* Her work provided valuable insights into how anthropogenic activities disrupt aquatic communities and ecosystem functioning.

Recently, Ana completed her PhD in Applied Ecology at North Carolina State University under the mentorship of Dr. Alonso Ramírez. Her dissertation, titled *"Surface-groundwater interactions in a changing climate: impacts of acidification and phosphorus enrichment on tropical stream macroinvertebrate assemblages,"* examined how episodic acidification events and geothermal nutrient inputs affect aquatic macroinvertebrates in tropical streams. Her research combined field sampling and mesocosm experiments to explore the effects of changing physicochemical conditions on aquatic communities.

Throughout her career, Ana has participated in multidisciplinary research projects involving the study of periphyton, macroinvertebrates, and fish as indicators of ecosystem health. She has led and collaborated on projects to monitor and evaluate aquatic ecosystems in various contexts, including the impact of mining activities, agricultural runoff, and hydropower

developments. Her work has included field sampling, taxonomic identification, data analysis, and the preparation of scientific reports to inform environmental management and conservation strategies.

In addition to her research, Ana has extensive teaching experience, having served as a lecturer at the University of Caldas, where she taught courses in Limnology and Stream Ecology, Project Formulation, Cell Biology Lab, and General Biology. She has also mentored undergraduate students, guiding them through research projects and theses. Ana is passionate about advancing freshwater science and applying her findings to support conservation and sustainable resource management in tropical ecosystems.

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I would also like to express my gratitude to the members of my committee, Dr. Adam Terando, Dr. Brad Taylor, and Dr. Marcelo Ardón, for their insightful feedback, constructive criticism, and continuous support. Their guidance and expertise have significantly contributed to the success of this research.

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Chapter 1: Introduction

Climate change, through shifts in rainfall patterns and rising temperatures, poses substantial threats to stream ecosystems, impacting both biodiversity and ecosystem function (Sabater et al. 2023). Some of the impacts of climate change that have received greater attention in stream ecosystems include changes in flow patterns, water temperature, and water availability (Buisson et al. 2008, Davis et al. 2013, Jarić et al. 2019). However, other effects, such as changes in the physicochemical properties of water, receive less attention as potential impacts of climate change, despite their capacity to significantly alter ecosystem dynamics (Pinheiro et al. 2021, Singh et al. 2021). These less direct impacts include episodic acidification events and fluctuations in phosphorus concentrations, which can modify community structure and function in stream ecosystems (Dodds 2003, Schöpp et al. 2003, Moiseenko 2005, Simon et al. 2009, Zhang et al. 2019). While much research has focused on acidification and phosphorus dynamics resulting from anthropogenic activities (Psenner 1994, Smil 2000, Hasler et al. 2018, Hu et al. 2020), there is a critical knowledge gap concerning how natural streams respond to these variables in the absence of human impacts.

Stream ecosystems in the Caribbean are particularly vulnerable to climate change due to unique climatic and geographic factors (Scatena et al. 2005, 2012). Unlike regions with marked seasonal variations, the Caribbean has relatively stable temperatures year-round because of its tropical oceanic environment, with precipitation as the primary climatic driver (Taylor and Alfaro 2005). However, this stability is increasingly threatened by climate change, as projections indicate a decrease in average annual precipitation and intensified extreme events, including more severe droughts, floods, and stronger hurricanes (Aguilar et al. 2005, Campbell et al. 2011, Hall et al. 2013, Knutson et al. 2021). Given these projections, understanding how Caribbean

ecosystems respond to such shifts is essential. In this context, there is a need to summarize existing knowledge on climate change impacts and the state of research on tropical freshwater systems. By consolidating and evaluating past studies, we would better understand what has already been explored and where gaps remain (Linnenluecke et al. 2020), which is vital for advancing our knowledge and addressing the specific challenges that climate change poses to the Caribbean.

Natural episodic acidification in tropical streams is closely linked to precipitation patterns (Small et al. 2012). These acidification events can have significant impacts on aquatic biodiversity and disrupt essential ecosystem functions (Ardón et al. 2013, Klem and Gutiérrez-Fonseca 2017, Ganong et al. 2021). Volcanic landscapes in Costa Rica offer ideal conditions for studying these effects within forested ecosystems, where some streams undergo episodic acidification while others maintain stable pH levels due to natural buffering capacity (Pringle et al. 1993, Small et al. 2012). Macroinvertebrates serve as indicators for examining the impacts of acidification in tropical streams (Klem and Gutiérrez-Fonseca 2017, Ganong et al. 2021), allowing assessment of both community-level changes and physiological responses. This dual approach provides insights into how acidification influences ecosystem functioning in tropical settings, offering valuable information for understanding ecosystem resilience and guiding conservation strategies in the face of climate change (Helmuth 2009, Bozinovic and Pörtner 2015, Pérez-Ruzafa et al. 2018, Ullah et al. 2024).

Another significant effect of climate change in volcanic landscapes is the alteration of the balance between groundwater and surface water, an interaction that determines phosphorus concentrations in streams draining volcanic landscapes (Triska et al. 2006). In these environments, inputs of phosphorus-rich groundwater are common and play a crucial role in

nutrient availability (Rosemond et al. 2002, Ramírez et al. 2003). However, with the anticipated reduction in precipitation, there could be shifts in the proportion of groundwater entering these streams, potentially leading to changes in phosphorus levels (Triska et al. 2006). Aquatic organisms in these ecosystems have developed various mechanisms to manage and utilize available phosphorus, which is essential for carrying out their physiological and metabolic processes (Small et al. 2011). While responses to phosphorus scarcity or enrichment have been extensively studied in systems impacted by human activities (Carpenter et al. 1998, Smil 2000, Zhang et al. 2019, Hu et al. 2020), much less is known about how organisms respond to natural phosphorus fluctuations. This study addresses this gap, using macroinvertebrates as indicators to examine ecosystem responses to natural variations in phosphorus, providing insights into the functional implications of nutrient dynamics in tropical streams.

Given the potential impacts of climate change on tropical stream ecosystems, my study aims to investigate how less-studied effects, such as episodic acidification and natural variations in phosphorus availability, influence ecosystem dynamics in Caribbean landscapes. Using macroinvertebrates as response organisms, this research assesses changes in community structure and ecosystem function under these natural stressors. The study integrates a synthesis of existing knowledge on climate change impacts in freshwater ecosystems with empirical analyses that explore pH and nutrient fluctuations, providing critical insights for conservation in naturally functioning ecosystems.

The objective of Chapter 2 is to provide a comprehensive review of the current state of knowledge on climate change impacts in freshwater ecosystems, focusing specifically on streams in the Caribbean. This review synthesizes existing research to identify knowledge gaps and highlight underexplored areas related to climate-driven changes in water chemistry, biodiversity,

and ecosystem functioning. This review guides my research and establishes a basis for identifying the vulnerabilities and response patterns of Caribbean stream ecosystems to climate change.

Chapter 3 aims to evaluate the impact of natural episodic acidification events on macroinvertebrate assemblages and their physiological responses in tropical streams within a volcanic landscape. By analyzing assemblage composition and insect physiological adaptations, this chapter seeks to understand how episodic acidification may influence ecosystem resilience and adaptability in these naturally dynamic environments.

The objective of Chapter 4 is to assess how a natural phosphorus gradient affects macroinvertebrate assemblages, functional feeding group composition, and body condition, measured through macromolecule content. This chapter explores how variations in phosphorus availability shape community structure and influence the metabolic and nutritional dynamics of stream organisms, providing insights into nutrient-driven ecological processes in tropical freshwater ecosystems.

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Chapter 2: From hurricanes to droughts and rising temperatures: current knowledge on the impact of climate change on Caribbean streams

2.1 Abstract

Climate change impacts freshwater ecosystems by altering rainfall patterns, elevating temperatures, and intensifying extreme events, resulting in significant effects in ecosystem structure and function. Although research has been increasing, most of our understanding comes from temperate regions. This study explores this knowledge gap by presenting current climate projections and analyzing the state of knowledge to elucidate potential responses of Caribbean stream ecosystems to climate change. The Caribbean basin, renowned for its biodiversity and unique interconnectivity between ecosystems, is a biodiversity hotspot. Climate projections anticipate reduced precipitation, increased frequency of extreme weather events, and major hurricanes. Those expected impacts will alter stream biodiversity and ecosystem function. We conducted a literature search spanning the period from 2000 to 2022, with the goal of assessing our knowledge of the effects of climate change on Caribbean rivers and streams. We identified 497 papers related to climate change, with ecology, hydrology, and disturbances among the most frequently studied topics. Puerto Rico, Costa Rica, and Colombia were leaders in studies concerning climate change and extreme weather events. Increased water temperatures favor growth for some organisms, but raise mortality in invertebrates with lower thermal thresholds, while certain tropical fish adapt to fluctuating conditions. Extreme events will affect habitat characteristics and aquatic diversity, with droughts impacting water physicochemistry and habitat availability. Hurricanes contribute to canopy openings and detrital inputs, impacting water nutrients and insect populations. Our understanding of Caribbean stream ecosystem

responses to climate change is limited; however, the potential effects of altered precipitation and intensified extreme weather events could profoundly reshape ecosystem dynamics and functionality.

2.2 Introduction

Climate change and its consequences, such as altered rainfall patterns and increased temperatures, significantly impact ecosystems, particularly streams (Sabater et al. 2023). These effects can manifest gradually, through rising surface water temperatures over time, or suddenly, with increased occurrences of extreme events like heat waves, forest fires, droughts, or floods (IPCC 2022, Rose et al. 2023). The increase of greenhouse gas concentrations in the atmosphere alters the global water cycle, leading to significant changes in ecosystem hydrology (Prudhomme et al. 2014). Land and sea warming resulting from anthropogenic activities leads to measurable changes in global annual precipitation patterns, including increased variability and shifts in precipitation distributions, which subsequently impact both terrestrial and aquatic environments (Dore 2005, He and Soden 2017).

Aquatic communities and ecosystem functions are directly affected by alterations in the hydrological regime and increased temperatures due to climate change (Buisson et al. 2008, Jarić et al. 2019). Understanding and predicting these climate change effects on freshwater ecosystems is essential, as they constantly interact with other factors that impact aquatic biota and the environment. Some of the major impacts associated with climate change include changes in stream flow patterns, water temperature, and water availability (Davis et al. 2013). These impacts have significant implications for aquatic communities, altering phenology, distribution, and biotic interactions (Thackeray et al. 2010, Woodward et al. 2010, Wenger et al. 2011).

However, climate change does not occur in isolation; it interacts simultaneously with a multitude of stressors, such as alterations in water nutrient concentrations and changes in land use derived from human activities (Maxwell et al. 2018, Ríos-Touma and Ramírez 2019). These combined stressors exacerbate the impacts on aquatic ecosystems, creating complex interactions at multiple scales (Grimm et al. 2008).

Climate change effects on Caribbean stream ecosystems are complex due to interactions with various factors associated with its geographic location. The Caribbean is in close proximity to both the Pacific and Atlantic oceans. Both oceans play a role in shaping the climate of the basin, along with the effects of the annual cycle of the Intertropical Convergence Zone, easterly trade winds, and tropical storms and hurricanes (Taylor et al. 2012, Hall et al. 2013). Large-scale oscillations, El Niño-Southern Oscillation (ENSO), and North Atlantic Oscillation (NAO) are also important drivers of climate variability in the Caribbean basin (Scatena et al. 2005, 2012). ENSO is related to ocean surface temperature anomalies and often results in increased hurricane activity in the Caribbean (Spence et al. 2004, Scatena et al. 2012). In contrast, the influence of NAO in the Caribbean is variable due to increased moisture absorption in the tropics by the extra heat flux from the ocean to the atmosphere (Jury et al. 2007). Along with the intensification of human activities, these characteristics make Caribbean streams highly susceptible to climate change.

Climate change impacts are now evident in many regions within the Caribbean, and projections indicate further intensification in the coming years (IPCC 2022). However, our understanding of those effects is limited and fragmented, and there is a need to bring the information together and assess the current state of knowledge. To improve our understanding of climate change impacts on the basin, here, we (1) summarize current information on climate

change projections for the Caribbean basin, and (2) analyze the state of knowledge of climate change effects on freshwater ecosystems in the last two decades for the Caribbean. We conducted a literature review of Caribbean climate projections that influence river and stream ecosystems to assess potential responses to future climate change.

2.3 The Caribbean basin

The Caribbean Sea is a suboceanic basin situated in the western Atlantic Ocean, spanning latitudes 9° to 22° N and longitudes 89° to 60° W. It covers an area of approximately 2,753,000 km^2. This region is bounded by Venezuela, Colombia, and Panama to the south; Costa Rica, Nicaragua, Honduras, Guatemala, and Belize to the west; the Greater Antilles islands of Cuba, Hispaniola, Jamaica, and Puerto Rico to the north; and the Lesser Antilles arc, extending from the Virgin Islands in the northeast to Trinidad in the southeast, to the east (Figure 2.1). Compared to main ocean basins worldwide, the Caribbean has a larger ratio between the total ocean surface and coastal margin, which modulates physical, geological, and biological processes (Miloslavich et al. 2010). The basin represents a connection between North and South America, and it is considered a biodiversity hotspot (Brooks et al. 2002), with many endemic species due to the heterogeneity of ecosystems (McHugh et al. 2014, Devenish-Nelson et al. 2019, Votýpka et al. 2019, Crews and Esposito 2020).

The tropical oceanic environment provides the Caribbean with relatively stable temperatures throughout the year, making precipitation the most influential climatic component in the basin (Taylor and Alfaro 2005). The annual precipitation cycle creates a dry season from December to April (Gamble and Curtis 2008). However, in some areas, the dry season can best be defined as not strictly dry season (Jones et al. 2016). The wet season has a bimodal pattern

with an early rainy period in May-July, a short dry period during July, and a late rainy period in August-November (Taylor et al. 2002, Moron et al. 2016). The Caribbean basin is drier between September and October during El Niño events and wetter between April and July when ENSO effects often decrease (Taylor et al. 2002, Scatena et al. 2012). Therefore, precipitation changes are modulated by Caribbean moisture transport and ENSO effects (Durán-Quesada et al. 2017, 2020).

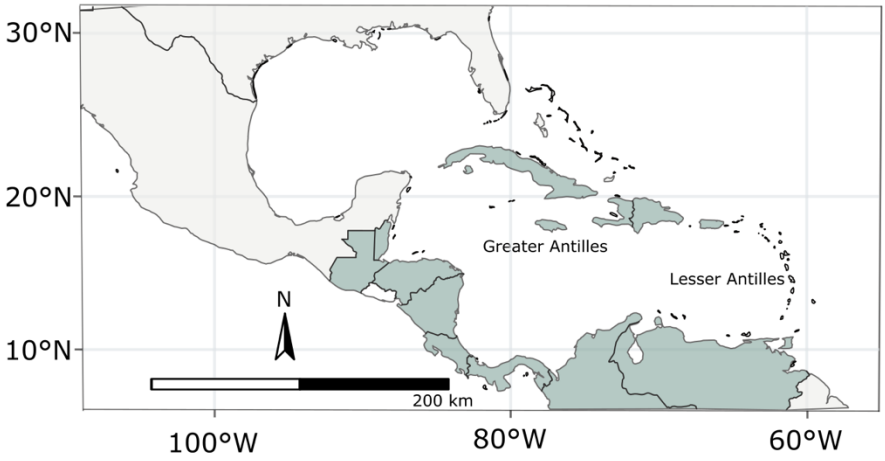

Figure 2.1 The Caribbean basin. Countries and territories considered part of the Caribbean region are highlighted in green.

2.4 Methods

Our literature search was conducted using the Web of Science database. We collected studies from 2000 to 2022 and included articles published in English and Spanish. The search was performed on November 6, 2023. To refine our search, we utilized the following Web of Science categories: Environmental Sciences, Marine Freshwater Biology, Ecology, Zoology, Biodiversity Conservation, Water resources, Multidisciplinary Science, Veterinary Sciences,

Physical Chemistry, Biology, Evolutionary Biology, Fisheries, Entomology, Genetics Heredity, Biochemistry Molecular Biology, Environmental Studies, Limnology, Microbiology, Physiology, Environmental Engineering, Behavioral Sciences, Toxicology, Biotechnology Applied Microbiology, Multidisciplinary Chemistry, Cell Biology, Forestry, Urban Studies, and Biochemical Research Methods.

To ensure comprehensive coverage of publications in the Caribbean, we conducted four searches by location, encompassing the Lesser Antilles, the Greater Antilles, Central America, and the South American Caribbean (Table 1). To narrow our search to lotic ecosystems, we added the phrase "stream*" OR "river*" OR "creek*" to each of these searches. Additionally, to refine our search and focus specifically on studies related to climate change or its effects, we conducted five additional separate searches. For each of these searches, we combined the location and ecosystem parameters with the following phrases: 1) "climate change" or "global warming", 2) "hurricane*", 3) "drought*", 4) "flow" or "discharge" or "precipitation" or "rainfall", and 5) "temperature". These searches formed the basis for our analysis throughout the paper.

To filter out articles unrelated to freshwater or solely focused on social issues, or those not pertaining to the Caribbean, we conducted manual curation and utilized the R libraries dplyr (Wickham et al. 2021) and stringi (Gagolewski 2021) to refine the information. Subsequently, each article meeting our search criteria underwent individual review, from which we extracted information regarding the country or locality where the study was conducted, the study topic, and for studies involving living organisms, the taxonomic group to which they belonged.

Table 2.1 Geographic locations covered in searches for Caribbean publications. Search terms were conducted across the Caribbean, encompassing the Lesser Antilles, Greater Antilles, Central America, and South American Caribbean.

Geographic Location	Search Terms
Lesser Antilles	"Anguilla" OR "Antigua and Barbuda" OR "Dominica" OR "Guadeloupe" OR "British Virgin Islands" OR "U.S. Virgin Islands" OR "Martinique" OR "Montserrat" OR "Saint Kitts and Nevis" OR "Saint Lucia" OR "St. Martin" OR "Saint Vincent and the Grenadines" OR "Aruba" OR "Barbados" OR "Bonaire" OR "Curaçao" OR "Grenada" OR "Saba" OR "Sint Eustatius" OR "Trinidad and Tobago"
Greater Antilles	"Cuba" OR "Hispaniola" OR "Dominican Republic" OR "Haiti" OR "Jamaica" OR "Puerto Rico" OR "Cayman Islands"
Central America	"Caribbean" OR "Central America" OR "Panama" OR "Costa Rica" OR "Nicaragua" OR "Honduras" OR "Guatemala" OR "Belize"
South America	“Colombia” OR “Venezuela”

2.5 Results and discussion

2.5.1 State of knowledge in the Caribbean basin

We obtained 1,022 results after conducting our searches based on location, lotic ecosystems, and climate change-related terms. Following the filtration process, we included a total of 497 papers in our study. Of these, 16% (78 papers) addressed climate change or global warming, 9% (45 papers) focused on hurricanes, 9% (43 papers) centered on droughts, 81% (402 papers) examined flow, discharge, precipitation, or rainfall, and 18% (91 papers) focused on temperature (Suppl.). Across various combinations of our criteria, our five search terms overlapped in 123 papers (Figure 2.2), which accounts for why the sum of the proportions per search term exceeds 100%.

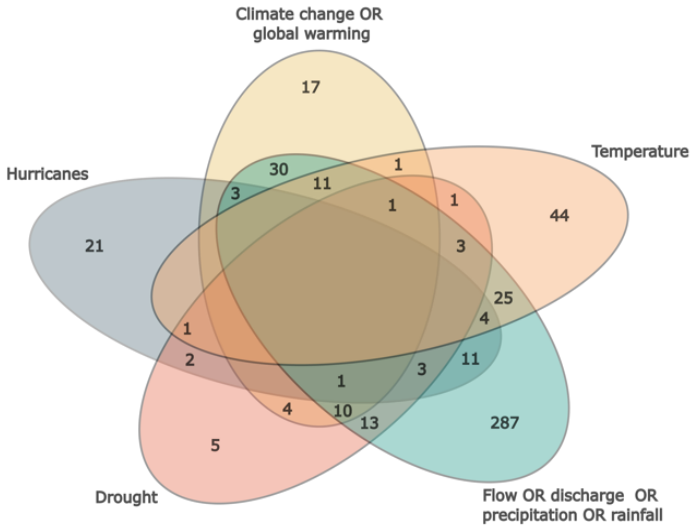

Figure 2.2 Venn diagram showing the number of publications by search term and combinations of terms. The study topics include "climate change" or "global warming," "hurricane*," "drought*," "flow" or "discharge" or "precipitation" or "rainfall," and "temperature."

In the Caribbean basin, studies focusing on lotic ecosystems have seen a noticeable increase over the past decade (Figure 2.3). Climate change-related research has been a consistent area of interest since 2009. It is likely driven by the growing concern of understanding the consequences of accelerated global warming on rivers and streams and the ecosystem services they provide. Moreover, we observed a rise in publications related to climatic events such as droughts and hurricanes. These publications delve into studying the impacts of these events on lotic ecosystems, addressing various aspects such as hydrology or effects on aquatic organisms. This trend reflects a post-event surge in publications aimed at disseminating the specific impacts of these climatic events on freshwater ecosystems.

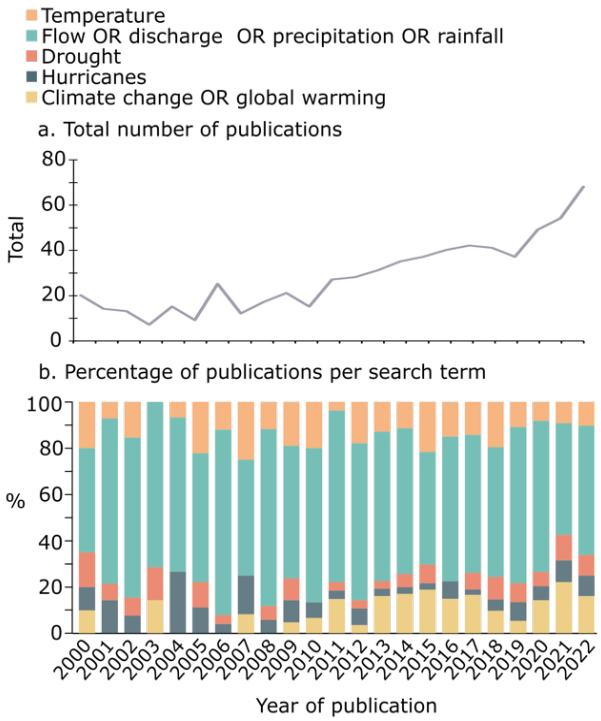

Figure 2.3 Number of publications per search term: "climate change" or "global warming," "hurricane*," "drought*," "flow" or "discharge" or "precipitation" or "rainfall," and "temperature" per year, shown as total number (a) and as percentage (b).

Puerto Rico, Costa Rica, and Colombia stand out as the locations with the highest number of publications in studies related to climate change or global warming, droughts, flow, discharge, precipitation, rainfall, and temperature (Figure 2.4). In addition, Puerto Rico accounted for the highest number of papers specifically related to hurricanes, comprising 69% of the registered studies. Although the Greater Antilles are vulnerable to hurricanes due to their location, we found a low number of publications in these islands, except for Puerto Rico, which has the smallest area among this group of islands. Excluding Puerto Rico, these islands contributed to

only 8% of the total registered publications related to hurricanes. Utilizing the five terms of our search, 30% (186 articles) were studies related to living organisms. Of these, 48% were conducted with aquatic macroinvertebrates, 23% with fish, 10% with microbes and the remaining 19% were studies related to aquatic plants, plankton, periphyton and vertebrates (e.g. tadpoles, turtles, alligators).

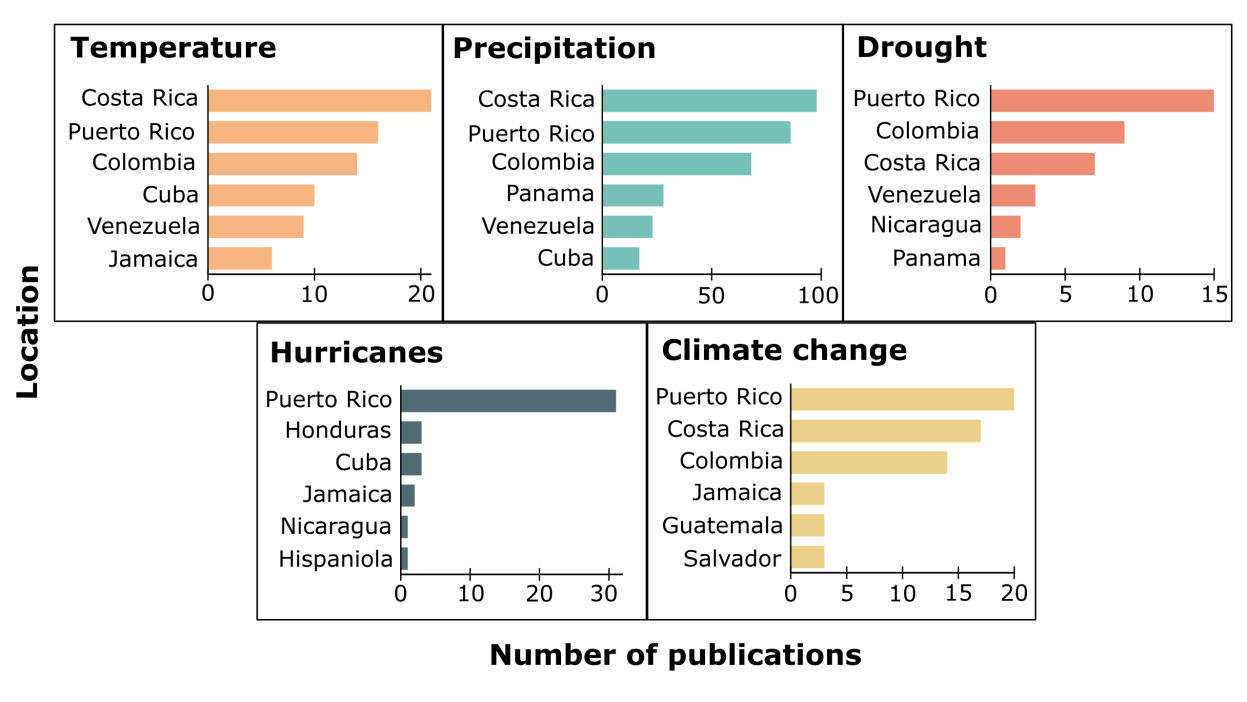

Figure 2.4 Number of publications per study location in the Caribbean basin per search term: "climate change" or "global warming," "hurricane*," "drought*," "flow" or "discharge" or "precipitation" or "rainfall," and "temperature."

Between 45% and 51% of studies related to climate change or global warming, droughts, and hurricanes primarily focused on the study of hydrology, hydrogeology, and disturbances

(Schaefer et al. 2000, Goyal et al. 2015, Betanzos-Vega et al. 2020, Salgado et al. 2022). Hydrology and hydrogeology, comprising 37% of the articles (Figure 2.5), were also a focal point in publications related to flow, discharge, precipitation, and rainfall (Post and Jones 2000, Ogden et al. 2013, Hund et al. 2021). Regarding temperature, almost 50% of the studies were related to biomonitoring, diversity, and ecology (Anger et al. 2007, Pérez-Reyes et al. 2015, Alvarez et al. 2017). Some of these studies have focused on the relationship between environmental variables, including water temperature, and the structure of aquatic organism communities (Alvarez et al. 2017, Rondón and Aragón 2018, Mariani-Ríos et al. 2022).

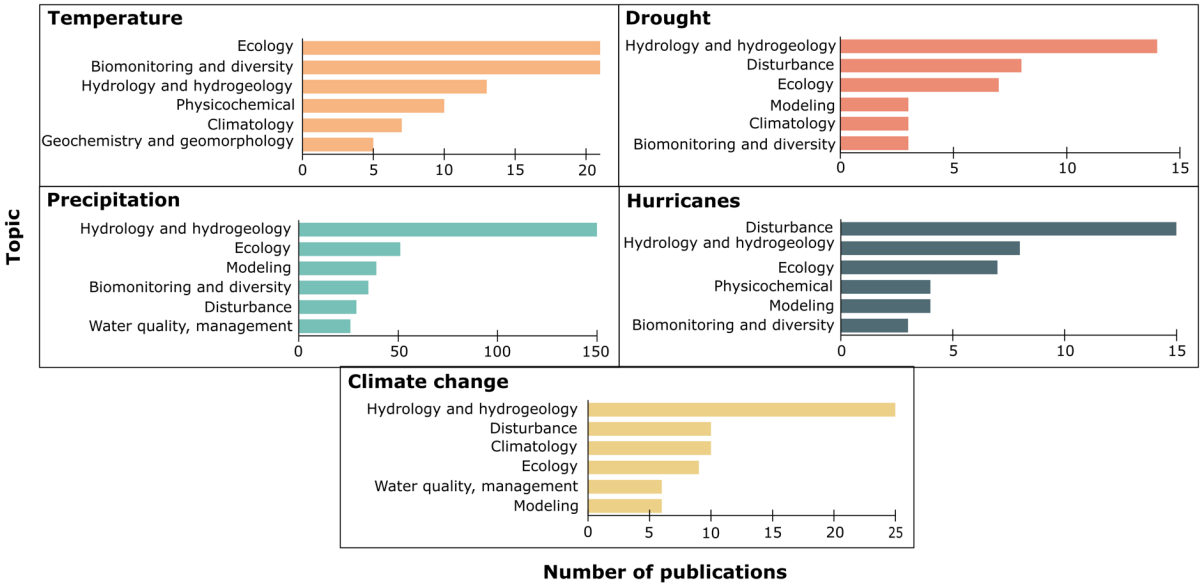

Figure 2.5 Number of publications per study topic per search term: "climate change" or "global warming," "hurricane*," "drought*," "flow" or "discharge" or "precipitation" or "rainfall," and "temperature."

2.5.2 How does climate change impact Caribbean rivers and streams?

In the Caribbean region, climate change poses significant challenges, directly and indirectly impacting rivers and streams by altering their natural flow regimes and associated physicochemical conditions (Covich et al. 2000, Truitt et al. 2020). For example, rising temperatures favor the growth of organisms such as mayflies, shrimp (Pérez-Reyes et al. 2015, Moody et al. 2017) and vertebrates like turtles (Páez et al. 2009), while more temperature-sensitive species, such as certain caddisflies, may become restricted to cooler areas (Rogowski and Stewart 2016). Droughts negatively impact shrimp populations, and floods can affect the abundance of aquatic insects, and fish (Gutiérrez-Fonseca et al. 2018, Reyes-Torres and Ramírez 2018). Additionally, extreme events like hurricanes can lead to increases in herbivorous species, while shrimp abundance tends to decrease (Covich and McDowell 1996, Gutiérrez-Fonseca et al. 2020) (Fig 2.6).

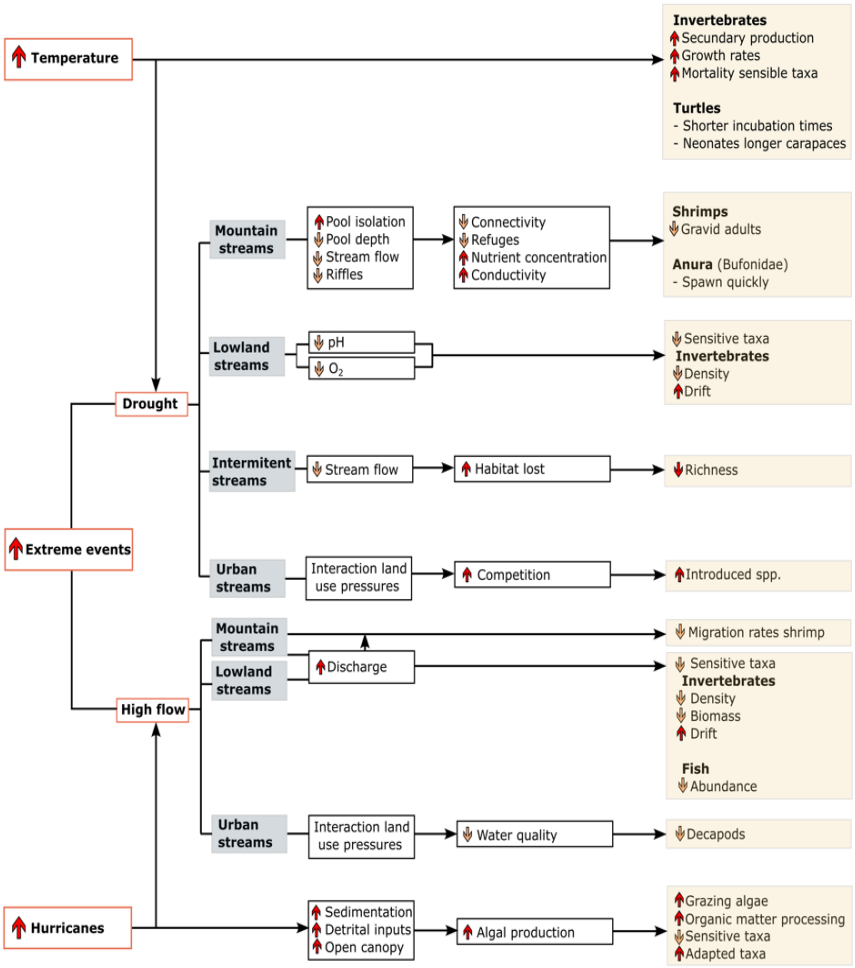

Figure 2.6 Diagram illustrating the effects of climate change on Caribbean streams related to temperature, extreme events (drought and high flow), and hurricanes.

Temperature. Global warming is increasing average air temperatures (Campbell et al. 2011, Stephenson et al. 2014, Diaz et al. 2022, IPCC 2022). Models project temperature will continue to increase by 2.0-3.0°C by the end of the century throughout the Caribbean, regardless of the season (Hall et al. 2013). This warming trend leads to changes in temperature extremes, with notable increases in extreme highs compared to extreme lows (Aguilar et al. 2005, IPCC 2022). Consequently, the frequency of warm days and nights is expected to rise while cold days and

nights decrease (Peterson et al. 2002). These temperature shifts could have significant implications for the basin. Even if temperatures stabilize in the future, the effects of warming, such as ocean warming and sea level rise, will continue to alter ecosystems for centuries or millennia (IPCC 2022).

The seasonality in Caribbean streams is primarily driven by precipitation patterns. Water temperature varies seasonally and is influenced mainly by factors such as rainfall and dry seasons, along with wind intensity, insolation, proximity to the ocean, and river channelization (Martínez et al. 2001, Truitt et al. 2020). These factors can affect the relationship between water temperature and salinity in coastal areas, resulting in specific patterns depending on the time of year and local conditions (Martínez et al. 2001). During the dry season, an increase in stream salinity is likely due to reduced freshwater input and higher evaporation rates, combined with higher temperatures (Truitt et al. 2020). Conversely, during the rainy season, salinity tends to decrease due to increased freshwater flow, while temperatures may drop slightly due to the higher cloud cover and precipitation (Truitt et al. 2020). However, a different pattern was observed in a Venezuelan watershed during the dry season, where water temperature and salinity had an inverse relationship (Martínez et al. 2001). Reduced river flow decreased freshwater mixing with seawater, increasing water residence time and warming the river more due to prolonged solar exposure (Martínez et al. 2001).

The influence of temperature is pivotal in shaping the structure of aquatic communities in diverse environments, including volcanic landscapes (Rojas-Gätjens et al. 2021), and lowland and mountain streams (Goldschmidt 2004, Serna et al. 2015, Mena-Rivera et al. 2018). In Caribbean volcanic landscapes, temperature was one of the factors that regulated the microbial community in rivers and streams, and it was related to lower oxygen values, favoring the growth

of specialized thermophilic bacteria with low oxygen requirements (Rojas-Gätjens et al. 2021). In Costa Rica, such conditions have been observed in rivers near to the Poás Volcano crater, where warm underground water emerges from volcanic rocks, creating high-temperature, extremely acidic environments that promote the development of thermophilic microorganisms (Rojas-Gätjens et al. 2021). In Caribbean lowland lotic ecosystems, the structure of macroinvertebrate assemblages was influenced by the interaction of temperature with other physicochemical variables such as nutrients and suspended solids (Mena-Rivera et al. 2018), as well as characteristics like habitat type and elevation (Goldschmidt 2004). In mountainous areas, lower temperature values were associated with higher dissolved oxygen levels, which favored the greatest abundance of taxa sensitive to environmental changes (Serna et al. 2015).

Temperature plays a critical role in determining the developmental responses of Caribbean aquatic organisms to environmental changes, leading to distinct adaptations observed across various species and life stages. The increase in water temperature, driven by rising air temperatures, directly influences the secondary production of invertebrates in streams (Patrick et al. 2019). This temperature increases favors higher growth rates among mayflies in lowland streams (Moody et al. 2017) and shrimp in mountain streams (Pérez-Reyes et al. 2015). However, increased temperature can elevate mortality rates among invertebrates with lower thermal thresholds, confining them to cooler forested streams (Rogowski and Stewart 2016). Conversely, certain tropical fish species demonstrate remarkable adaptability to fluctuating tropical waters, enduring lethal temperature spikes during hot periods by rapidly enhancing thermal resistance while experiencing slow heat tolerance loss (Chung 2000). Nevertheless, some species (e.g., Bigmouth Sleeper larvae) exhibit greater hatch success and longer survival at low temperatures (23 °C) (Olivieri-Velázquez and Neal 2018). Other vertebrates like turtles

exhibit shorter incubation times and neonates with longer carapaces at higher temperatures, with a pivotal temperature near 33.4°C (Páez et al. 2009), whereas low temperatures (<28°C) increase mortality rates (Correa-H et al. 2010).

Precipitation. Models project a decrease in average annual precipitation (Cashman et al. 2010, Campbell et al. 2011, Hall et al. 2013; Antuña-Marrero et al. 2016, Taylor et al. 2018, IPCC 2022) and slightly lower amounts of rainfall over tropical oceans (Adler et al. 2003). This decrease is expected to affect the entire Caribbean basin during the entire year, particularly during the wet season (Imbach et al. 2018). Despite the predicted decrease in precipitation, projections suggest a rising trend in extreme precipitation events, encompassing both drought conditions and heavy precipitation with extreme values per event (Peterson et al. 2002, Aguilar et al. 2005, IPCC 2022). These projections align with future climate scenarios indicating drier conditions, particularly in the eastern Caribbean basin, which includes the Lesser Antilles (Jones et al. 2016).

The intensity of extreme hydrological and climatic events, such as droughts and high flows, impact the biodiversity and functioning of lotic ecosystems (Sabater et al. 2023). The density and biomass of aquatic invertebrates decrease during high flow (Rincón and Cressa 2000, Tamaris-Turizo et al. 2013, Reyes-Torres and Ramírez 2018), resulting in the loss of taxa sensitive to environmental changes (Quesada-Alvarado et al. 2020). Additionally, high stream discharge can negatively impact the migration rates of shrimp species *Xiphocaris elongata* and *Macrobrachium* spp., as their morphological traits may make them more susceptible to being displaced by strong water currents (Kikkert et al. 2009). High flow conditions also negatively affect fish species, particularly cichlids, in mountain streams of Honduras (McMahan et al.

2020). In these environments, increased precipitation influences water depths and flow rates, creating conditions that are unfavorable for cichlids, which prefer habitats characterized by narrower channel widths, shallower depths, and substrates dominated by large boulders and rocks (McMahan et al. 2020). In urban streams, the interaction between land use pressures and the disproportionate increase in water discharge contributes to the reduction of decapod abundance (Pérez-Reyes et al. 2016).

Climate change also leads to drought events, resulting in a decrease in stream flow (IPCC 2022) and changes in the physicochemical characteristics of water. The period between 2013 and 2016 underwent the most extreme and extensive drought in recent decades in the Caribbean, caused by reduced rainfall due to El Niño and increased potential evapotranspiration (Herrera and Ault 2017). Evaluating the hydrological and ecological effects of dry periods on lotic ecosystems became a focal point in publications following the drought event (Hoyos et al. 2019; Correa et al. 2020; Gutiérrez-Fonseca et al. 2020). Mountain stream habitats in Puerto Rico experience increased isolation between pools, reduced channel connectivity, and the formation of rapids (Gutiérrez-Fonseca et al. 2020). Furthermore, their response to prolonged droughts will be conditioned by the finite water storage potential in this mountainous landscape (Hall et al. 2022). In the lower reaches of watersheds, during the dry season, pH and dissolved oxygen decrease due to heterotrophic processes dominating in water bodies and mixed areas, while ammonium concentrations increase due to the oxidation of organic matter transported in the river (Martínez et al. 2001).

Decreased rainfall can result in increased concentration of nutrients, which leads to excessive algae growth and deteriorating water quality (Pringle and Triska 1991). These changes in water quality and reduced water flow significantly impact aquatic biodiversity in Caribbean

streams (Covich et al. 2000). Certain aquatic species, particularly those sensitive to drought-induced changes, face challenges to their survival and persistence in freshwater ecosystems (Collier et al. 1990; Ramírez et al. 2006; Gutiérrez-Fonseca et al. 2018; Ganong et al. 2021). The algal growth, coupled with reduced flow, can elevate the density of herbivorous macroinvertebrates, though not necessarily enhancing their richness (Gutiérrez-Fonseca et al. 2020). This shift potentially leads to habitat reduction and declines in other organisms (Covich and Crowl 2002; Gutiérrez-Fonseca et al. 2020). In mountainous streams, reduced habitat availability can intensify crowding and resource competition, resulting in prolonged declines in the reproductive output of aquatic invertebrates (Covich et al. 2003). Additionally, during dry periods, shrimp populations exhibit lower abundances and fewer gravid adults (Covich et al. 2003; 2006). However, in years with lower precipitation levels, female frogs (Bufonidae) spawn quickly, resulting in a more extended breeding period that benefits male survival and reproduction throughout the year (Rocha Usuga et al. 2017).

Reduction in precipitation increase the proportion of inter-basin geothermally modified groundwater (IMG; Pringle et al. 1990) in lowland streams (Genereux et al. 2002), affecting buffer capacity and ion concentration (Small et al. 2012). In many areas of the Caribbean, large aquifers are influenced by solute inputs from basaltic rocks in tectonically and volcanically active regions (Pringle et al. 1990). IMG acquires solutes from volcanic rocks over thousands of years (Genereux et al. 2002). In contrast, local aquifers without volcanic influence depend entirely on solute-poor local precipitation. When IMG enters lowland streams, it contains high concentrations of solutes and ions, including carbonates, bicarbonates, phosphorus (P), chloride (Cl), magnesium (Mg), and calcium (Ca), which alter stream water chemistry (Pringle et al. 1986, 1990). Therefore, streams with significant IMG contributions are solute-rich, while those

with mostly local groundwater are solute-poor. Consequently, the expected decrease in rainfall might result in an increase of water acidification events by altering the stream buffering capacity (Small et al. 2012).

Research in La Selva in Costa Rica has found, that after prolonged droughts, leaf litter accumulated in the forest floor is decomposed at the beginning of the rains, increasing CO_2 concentrations (Small et al. 2012). When CO_2 dissolves with water and moves into streams, it produces carbonic acid (H_2CO_3), which causes a decrease in stream pH (Small et al. 2012, Klem and Gutiérrez-Fonseca 2017). The magnitude and duration of these acidification events can vary; a greater decrease in precipitation in the dry season may lead to a greater magnitude of seasonal acidification (Small et al. 2012). High carbonate concentrations in solute-rich streams buffer them against changes in stream pH. In contrast, solute-poor streams lack carbonates and periods of acidification occur (Ramírez et al. 2006, Small et al. 2012, Marzolf et al. 2022). Therefore, solute-rich streams present a more stable pH while solute-poor streams often have periods of prolonged pH declines (Small et al. 2012, Ardon et al. 2013, Marzolf et al. 2022). For this reason, the responses of stream ecosystems to climate change may be more severe on solute-poor streams (Ganong et al. 2021).

Low rainfall and droughts negatively impact aquatic macroinvertebrates (Ramírez et al. 2006). In some regions, natural acidification has long acted as an environmental stressor in freshwater ecosystems, promoting adaptations in aquatic organisms (Collier et al. 1990). For example, Chironomids exhibit higher tolerance to naturally low pH levels, whereas mayflies and shrimp have low tolerance to acidification, particularly when pH drops below 4 (Ganong et al. 2021). Acidification experiments confirm the low pH tolerance threshold in macroinvertebrates, which drift in search of better conditions when water acidifies (Ardón et al. 2013). Additionally,

it has been observed that aquatic macroinvertebrate densities tend to be higher in the dry season compared to the wet season, this variation is attributed to changes in pH and stream discharge between seasons (Ramírez et al. 2006, Gutiérrez-Fonseca et al. 2018).

However, droughts can benefit certain vertebrate species. In urban rivers, resource competition during droughts can lead to increased dominance of introduced fish species over native species (Ramírez et al. 2018). Drought events also affect the size of native species, with small individuals present during drought periods (Ramírez et al. 2018). In intermittent streams in Venezuela, macroinvertebrate densities typically decreased during both drought conditions and periods of high flow (Rincón and Cressa 2000). Low flow conditions often result in the lowest species richness but the highest density of macroinvertebrates (Rincón and Cressa 2000). Extreme fluctuations in flow are likely to significantly influence the structure of macroinvertebrate communities, while intermediate flow periods may allow other biotic processes to shape community composition (Rincón and Cressa 2000).

Tropical hurricanes. It is anticipated that tropical cyclones and hurricanes will increase in intensity with rising global temperatures (Diaz et al. 2022). Warm ocean temperatures will provide more thermal energy and foster tropical cyclone development (Knutson et al. 2021; IPCC 2022). The combination of reduced mean precipitation intensified extreme events, and factors such as increased El Niño events will create favorable conditions for hurricane development in the Caribbean (IPCC 2022; Klotzbach 2011; Knutson et al. 2021). While the total number of tropical cyclones may remain relatively stable, the frequency and intensity of hurricanes and cyclones category 3 to 5 are projected to increase, and storm development will intensify over hours to days (Knutson et al. 2021, IPCC 2022).

High flows and canopy opening resulting from hurricanes cause the input of sediments and detritus from the forest floor into streams (McDowell and Liptzin 2014). These high flows lead to increased sedimentation in streams, carried from steep hillsides, with the strength of this effect depending on the land use along the riverbanks and the characteristics of the vegetation (Haigh et al. 2004). Furthermore, hurricane storms damage the canopy, and this disturbance pressure and environmental changes lead to reduced production of flowers and fruits of riparian trees for at least 10 months following the storm (Zimmerman and Covich 2007). Therefore, hurricanes cause canopy opening and detrital inputs from the forest floor into streams, resulting in nitrogen outputs from the water due to interactions between canopy vegetation damage and recovery, and decomposition of detrital contributions (McDowell and Liptzin 2014).

Nutrient concentrations in streams can take several months to recover following hurricane disturbances (Schaefer et al. 2000; McDowell et al. 2013; McDowell and Liptzin 2014), but this recovery may differ in ecosystems with dams, where altered flow patterns and sediment retention can impact ecosystems dynamics (Johnson 2021). Hurricanes significantly alter biological processes that regulate nitrogen and potassium levels in mountain streams, often resulting in persistent increases in these nutrients for months or even years after the event (Schaefer et al. 2000; McDowell et al. 2013). Those disturbances disrupt forest canopies, causing elevated stream water concentrations of nutrients like potassium and nitrate due to reduced plant uptake (Douglas et al. 2000). These changes, however, are generally temporary, as the ecosystem's biological control mechanisms recover with the regrowth of the plant canopy (Douglas et al. 2000). While nutrient cycles gradually recover as the forest canopy regrows, in ecosystems with dams, hurricanes present additional challenges. Intense rainfall accelerates erosion and sediment accumulation in reservoirs, reducing their storage capacity and

compromising their efficiency, as observed in Puerto Rico (Johnson 2021). These impacts, combined with droughts, increase the vulnerability of both water infrastructure and surrounding ecosystems, emphasizing the broader environmental consequences of extreme weather events (Johnson 2021).

Hurricanes contribute to increases in litter inputs and grazing invertebrates, as these organisms respond to the high abundance of algae. As an effect of hurricanes, there is an increase in leaf and wood inputs into the streams in the short term (McDowell and Liptzin 2014). However, when the leaves disappear, these contributions are reduced, and the entry of light increases due to the opening of the canopy (Bellingham et al. 1996). These characteristics contribute to an increase in the densities of aquatic insects and, in particular, of herbivorous individuals that find more food (Masteller and Buzby 1993). More decomposing leaves and algae favor the presence of generalized consumers such as shrimp, whose density increases rapidly with the availability of these unusually abundant food resources (Covich et al. 1991).

However, the effects of hurricanes may vary among streams along elevation gradients, in the headwaters, shrimp densities (e.g., *Atya*) may suffer reductions due to floods (Covich et al. 1991, Covich and McDowell 1996). Changes in invertebrate density, such as shrimp, can have significant impacts on these ecosystems, particularly by disrupting the recycling of organic matter and altering the flow of energy and nutrients through the food web (Bobeldyk and Ramírez 2007). In contrast, studies on the effects of hurricanes in lowland streams are scarce, but it has been documented that hurricanes can lead to increased discharge in watersheds, causing significant floods (Mandal et al. 2016).

In summary, climate change will strongly affect streams in the Caribbean through increased temperatures, extreme weather events, and major hurricanes. The interaction between

temperature and other physicochemical and habitat variables plays a crucial role in shaping the structure and function of aquatic communities in lowland and mountain streams. This influence is reflected in higher growth rates among invertebrates and changes in vertebrate life cycles (e.g., fish). Additionally, extreme climate events such as droughts, high flows, and hurricanes significantly alter habitat characteristics, affecting the diversity of aquatic organisms. Droughts lead to changes in water physicochemistry in lowland streams, while intermittent rivers experience a reduction in available habitats, both impacting organism abundance and richness. In mountain streams, hydrology is the primary driver of impacts from droughts and hurricane disturbances, causing fluctuations in flow that result in environmental changes affecting aquatic ecosystems, their communities, and interactions (Figure 6).

2.5.3 Future directions

Interest in the study of climate change has grown significantly in recent years, and while there is a growing body of research on various aspects of Caribbean freshwater ecosystems, there is still a need for more comprehensive and long-term studies. Existing studies have explored the effects of water flow, discharge, and temperature in the Caribbean basin, but they are often limited to short-term observations (less than two years). This temporal limitation hinders our ability to fully attribute changes in freshwater ecosystems to climate change and its consequences. To address this knowledge gap and better understand the long-term impacts of climate change, it is crucial to conduct extended studies that can capture and demonstrate the evolving dynamics in streams and aquatic organisms over time.

While there has been considerable research on diversity, ecology, and hydrology in the Caribbean basin, there remains a notable opportunity to advance our understanding of how

climate change affects freshwater ecosystem processes, food web dynamics, and trophic interactions, as well as the physiological and behavioral responses of species to environmental changes. Although many studies have provided valuable insights, more in-depth research is needed to fully understand these mechanisms. Controlled experiments represent a promising avenue for addressing these gaps (e.g., Ganong et al. 2021), as they can isolate and replicate specific climate change factors, offering a clearer picture of how Caribbean ecosystems and their inhabitants respond to environmental stressors.

Historically, research has predominantly focused on analyzing the effects of extreme climatic events such as droughts and hurricanes after they occur (e.g., Rincón and Cressa 2000, Correa et al. 2020). While these studies are valuable, there is a significant opportunity to advance the field by simulating or experimentally predicting the potential impacts of climatic events on aquatic ecosystems and their communities before they happen. Prospective studies could involve manipulating variables such as temperature, precipitation, and storm frequency in controlled settings to explore how ecosystems might respond to these changes in advance. Such experimental approaches could help forecast shifts in species composition, nutrient cycling, and ecosystem resilience, providing a proactive framework for managing and mitigating future climate impacts. Furthermore, integrating modeling techniques with empirical data could enhance predictive capabilities, offering a comprehensive understanding of how future climate scenarios may influence freshwater ecosystems.

Although significant research is available on the effects of reduced precipitation, droughts, and hurricanes on stream ecosystems, there remains a critical knowledge gap concerning the impacts of increased temperatures, one of the primary drivers of climate change, on these environments. It is essential to understand how water temperature has changed over

time and its potential short and long-term consequences. Temperature affects freshwater organisms at various levels, from genes to communities, and understanding these macroecological effects is crucial for developing effective conservation strategies (Bonacina et al. 2022). It is essential to investigate how rising temperatures have altered water temperatures over time and their potential short- and long-term consequences. Factors such as locomotion, growth, and reproduction are known to be strongly influenced by environmental temperature (Deutsch et al. 2008).

Furthermore, while much of the existing research on hurricanes has been concentrated in Puerto Rico, there is a pressing need to extend these studies to other Caribbean regions, particularly the Antilles, which are highly vulnerable and frequently in the path of hurricanes (Cécé et al. 2017). Exploring these areas will provide a more comprehensive understanding of how climate change impacts different types of freshwater ecosystems, such as lowland and coastal systems, and the aquatic organisms associated with them. This broader perspective is essential.

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Chapter 3: Effects of episodic acidification events on tropical stream communities: insights from a volcanic landscape

3.1 Abstract

Natural episodic acidification events, although underexplored in tropical streams, can significantly impact aquatic communities, especially in areas with low buffering capacity. This study aimed to evaluate the effects of these events on macroinvertebrate assemblages and their physiological responses in a volcanic landscape. We conducted a year-long comparison between buffered and unbuffered streams in a tropical volcanic region, integrating monthly macroinvertebrate sampling with measurements of water physicochemical variables (pH, conductivity, temperature, dissolved oxygen, water level, and nutrients). Laboratory mesocosm experiments further assessed the impact of acidification on macroinvertebrate growth and respiration. No acidification events (defined as pH levels below 5) were recorded in the buffered stream, while 371 acidification events occurred in the unbuffered stream during the rainy season. Significant differences in macroinvertebrate assemblages were found across streams, with higher taxa diversity in the buffered stream, where Ephemeroptera dominated. In contrast, the unbuffered stream was dominated by Diptera, particularly Chironomidae. Physiological responses to acidification varied among taxa: Plecoptera showed minimal changes in growth and respiration, while others exhibited stress responses. These results highlight how natural acidification events shape community structure and drive physiological adaptations, with implications for biodiversity and ecosystem function. Our findings emphasize the importance of considering both community and taxa-specific physiological responses when assessing the impact of acidification in tropical stream ecosystems.

3.2 Introduction

Freshwater acidification is a globally recognized environmental stressor driven by both natural and anthropogenic factors, with significant consequences for ecosystem structure and function (Psenner 1994, Petrin et al. 2008). Natural sources of acidification are influenced by a combination of geological, geochemical, biological, and climatic factors (Henriksen et al. 1988, Gałuszka and Migaszewski 2020), including processes such as organic matter decomposition and local geological influences (Petrin et al. 2008, Cai et al. 2021). In contrast, anthropogenic acidification is primarily caused by human activities such as acid rain, CO_2 emissions, and industrial pollution, all of which contribute to lowering pH levels in freshwater systems (Paces 1985, Moiseenko et al. 2017, Wu et al. 2021). These processes, whether natural or human-induced, disrupt ecosystem processes (Schöpp et al. 2003), leading to declines in biodiversity (Muniz 1990), shifts in species composition (Muniz 1990, Dangles and Guerold 2000), and impaired ecological functions such as nutrient cycling and energy flow (Moiseenko 2005, Simon et al. 2009).

Natural acidification in freshwater ecosystems is a common phenomenon, resulting from processes such as mineral weathering, the presence of organic acids in humid regions and the release of acids from biological respiration (Petrin et al. 2008, Cai et al. 2021). Many streams have natural buffering mechanisms that help regulate pH fluctuations, including the protonation of bicarbonate and organic acids, along with ion exchange from streambed substrates, which release base cations and stabilize pH levels (Henriksen et al. 1988, Hruska et al. 1999, Ardón et al. 2013). In many naturally acidic freshwaters, these acidic conditions have prevailed over

evolutionary timescales, leading to communities that are more adapted to these conditions (Petrin et al. 2008).

Some tropical streams experience significant events of natural episodic acidification. In lowland rainforest stream in Costa Rica, temporal fluctuations in pH are associated with changes in precipitation, with levels typically dropping during the wet season (Small et al. 2012). This pattern is exacerbated after extremely dry periods, such as during El Niño events, when pH levels may fall below 4.0 in poorly buffered streams (Small et al. 2012, Ganong et al. 2021). The pH in many tropical streams is primarily controlled by the balance of carbonate systems, the availability of base cations in soils, and the influence of organic acids (Markewitz et al. 2001). In tropical volcanic landscapes in Costa Rica, streams receive groundwater inputs enriched with nutrients and bicarbonate from the dissolution of basaltic rock, enhancing their buffering capacity and maintaining more stable pH levels (Pringle et al. 1993, Small et al. 2012). In contrast, streams lacking those groundwater inputs are more susceptible to episodic acidification, with pH levels declining to around 5.0 (Small et al. 2012). These acidification events can significantly impact aquatic communities by reducing organism survival rates, altering community composition, and decreasing overall biodiversity (Ardón et al. 2013, Klem and Gutiérrez-Fonseca 2017, Ganong et al. 2021). Understanding the dynamics of episodic acidification is particularly important, as it is not commonly recognized as a direct consequence of climate change; however, it is associated with precipitation patterns (Small et al. 2012) and can have profound consequences for freshwater ecosystems.

The study of acidification requires an integrative approach that combines ecological and physiological perspectives to fully understand its impacts on the resistance and resilience of freshwater biota (Helmuth 2009, Bozinovic and Pörtner 2015, Pérez-Ruzafa et al. 2018, Ullah et

al. 2024). For example, biophysical ecology, which examines how the physical environment influences the physiological performance of organisms, improves predictions of climate change impacts on biological communities when physiological data are incorporated (Helmuth et al. 2005). Similarly, (Bozinovic and Pörtner 2015) propose a unified framework that integrates physiological, ecological, and evolutionary approaches to better predict how populations and ecosystems respond to environmental stressors. This integrative framework supports the idea that physiological research provides a mechanistic link between environmental changes and broader ecological patterns, which is critical for identifying vulnerable species or populations in conservation efforts (Seebacher and Franklin 2012). These frameworks underscore the importance of physiological variation among species in shaping interactions and community responses to environmental changes (Kroeker and Sanford 2022). By linking physiological processes to population-level changes, this approach improves long-term ecological predictions and helps identify vulnerable populations for targeted conservation (Calow and Sibly 1990).

In this study, our goal was to evaluate the impact of natural episodic acidification events on macroinvertebrate assemblages and their physiological responses in tropical streams within a volcanic landscape. By focusing on these streams, we aimed to provide insights into the ecological consequences of acidification in tropical freshwater ecosystems. We expected to find differences in macroinvertebrate assemblages between streams that experience acidification events with those that do not. Additionally, we expected that acidification would lead to changes in physiological responses, such as respiration rates and growth, indicating a sensitivity to pH fluctuations across the macroinvertebrate community. Costa Rica provides an ideal natural laboratory for addressing these questions, given the spatial variability in solute-rich buffered groundwater inputs in volcanic regions. This natural variability allowed us to explore how

natural episodic acidification affects macroinvertebrate communities and their physiological responses. Ultimately, our study provides valuable insights into the resilience and adaptability of tropical freshwater ecosystems, which can inform future conservation and management efforts.

3.3 Methods

3.3.1 Study Site

This study was conducted in the Arboleda, Taconazo, and Sura streams within the La Selva Research Station (LSRS) in Costa Rica. LSRS is situated on the Caribbean slope of Costa Rica, at the transition between the Cordillera Central and the coastal plain (10°26'N, 84°01'W). The reserve spans 1,563 hectares and is part of a larger biological corridor, ranging in elevation from approximately 35 meters to 2,900 meters above sea level, encompassing mature and secondary tropical rainforests (Holdrige 1967). The climate at LSRS is characterized by a bimodal precipitation distribution, with peak rainfall occurring in June-July and November-December (Sanford et al., 1994), and a dry season from February to April with average precipitation of 551 mm (1986-2016). LSRS registered an average annual precipitation of 4,207 mm and an annual air temperature averaging 25.8°C from 1986 to 2016, with slight variations observed throughout the year (available at https://anetium.ots.ac.cr/meteoro/default.php?pestacion=1=).

Stream hydrology is characterized by natural interbasin transfers of solute-rich groundwater flowing from volcanic bedrock in the Cordillera Central (Pringle and Triska 1991, Pringle et al. 1993, Genereux et al. 2009). This groundwater originates from high elevations, where it absorbs volatile gases such as carbon dioxide (CO_2) and hydrogen sulfide (H_2S) from a

deep magma source beneath nearby dormant Barva Volcano (Pringle et al. 1993, Genereux et al. 2009). Consequently, water emerging in seeps at the base of Pleistocene lava flows exhibits high concentrations of solutes, including phosphorus (P), sodium (Na^+), chloride (Cl^-), and bicarbonate (HCO^{3-}), with solute concentrations 13 to 29 times higher than those in local solute-poor groundwater (Pringle et al. 1993, Genereux et al. 2002). These solutes play a significant role in the ecological dynamics of the streams, buffering against seasonal changes in pH (Small et al. 2012, Ganong et al. 2021). However, not all streams in the area exhibit these characteristics; some receive only solute-poor local groundwater and experience episodic acidification events (Small et al. 2012).

Arboleda and Taconazo (Figure 3.1) were the main focal study streams for this study. Both have small watersheds (Arboleda: 46.1 ha, Taconazo: 27.9 ha) and similar environmental characteristics, but differ in their water sources (Ganong et al. 2015). Arboleda receives a substantial portion of its stream flow from regional interbasin groundwater, resulting in well-buffered water and a constant pH over time (Genereux et al. 2005, Small et al. 2012). In contrast, Taconazo has entirely locally generated stream flow from precipitation within its boundaries, resulting in poorly buffered water and pronounced pH fluctuations, with episodes of acidification reaching values below 4.5 (Small et al. 2012, Ardón et al. 2013).

An additional stream, the Sura was included in this study specifically for the collection of aquatic macroinvertebrates for laboratory experiments. The Sura is one of the major streams draining La Selva, covering an area of 120 ha (IGNCR 1985, Genereux et al. 2002). Two sampling sites were selected along Sura. Sura-60 (60 refers to elevation) is a solute-poor reach, prior to the influence of regional interbasin groundwater inputs and is characterized by fluctuating water pH (4.2-6.5). The second, Sura-30, is solute-rich as it receives regional

groundwater inputs, resulting in a stable pH (5.5-6.6). Henceforth, in this paper, we will refer to Arboleda and Sura-30 as "buffered streams" and Taconazo and Sura-60 as "unbuffered streams".

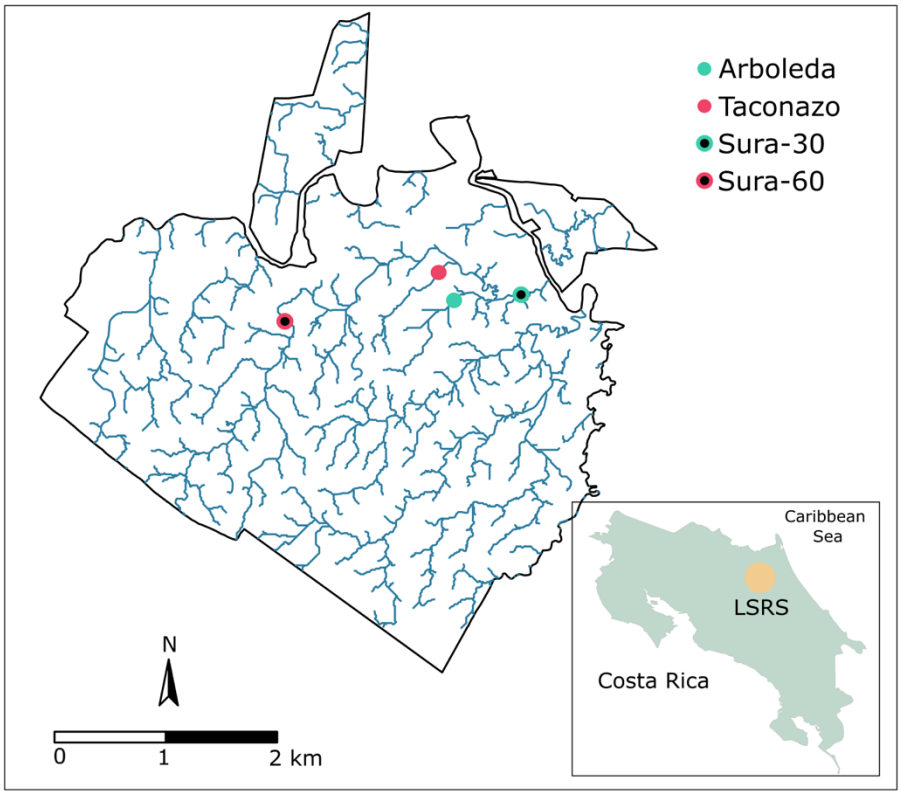

Figure 3.1 Location of the study streams at La Selva Research Station, Costa Rica (LSRS). The green color represents the buffered streams (Arboleda and Sura-30), and the pink color represents the unbuffered streams (Taconazo and Sura-60). The black dot indicates the streams used specifically for the collection of aquatic macroinvertebrates for laboratory experiments. In the bottom left corner, the location of LSRS is marked by a yellow circle on the map of Costa Rica.

3.3.2 Physicochemical water variables

We recorded temperature and pH at 15-minute intervals in both the Arboleda and Taconazo streams during 2021, using an YSI EXO1 multiprobe sonde (Xylem Inc., Yellow Springs, OH). The sonde was permanently stationed within each stream, and data was downloaded, and the sonde recalibrated every 20 days, ensuring precision in measurements. Water level was recorded concurrently using water level gauges installed in both streams to monitor fluctuations over time. We also measured conductivity, total suspended solids, dissolved oxygen, and water temperature using a YSI 600xlm multiprobe. Additionally, water samples were collected to determine NO_3 and Soluble Reactive Phosphorus (SRP) using 60 mL bottles. Samples were filtered in the field (0.45 μm) and maintained frozen until analyzed at North Carolina State University. SRP underwent analysis using an AA3 Segmented Flow Analyzer (Seal Analytical, Mequon, WI), while NO_3 was examined utilizing a Metrohm 930 ion chromatograph (Metrohm, Ionenstrasse, Switzerland).

3.3.3 Macroinvertebrate sampling

To characterize macroinvertebrate assemblages and their temporal dynamics, five plastic boxes measuring 0.35 x 0.20 x 0.07 m were positioned in Arboleda (buffered) and Taconazo (unbuffered). Boxes were elevated to avoid direct contact with the streambed using ribbed bars to prevent direct contact with the streambed, minimizing sediment buildup and low oxygen conditions. Each box was divided into two compartments, each with an area of 0.089 m^2. One of the compartments contained six fallen leaves of Guacimo Colorado (*Luehea seemannii* Triana and Planch), a prevalent tree species in the region with intermediate decomposition rates (91 days to 95% mass loss, Ardon and Pringle 2008), while the other compartment contained five

medium-sized rocks. Monthly collections were conducted during 2021 (January to December). We washed the materials using water from the stream and a 500-micron sieve to separate the macroinvertebrates from the leaves and rocks. Invertebrates were placed in plastic bags in 80% ethanol. To maintain consistency, fresh leaves of *L. seemannii* were added to the boxes each month, while the same set of rocks was used to collect macroinvertebrates during the entire sampling period.

Organisms were identified at the lowest possible taxonomic level using available taxonomic keys (Springer et al. 2010, Hamada et al. 2018). Biomass was calculated employing the length-mass model: $M = aL^b$, where M is organism mass (mg), L is individual head width (mm), and *a* and *b* are constants (Benke et al. 1999).

3.3.4 Study taxa

For the mesocosm experiments, we collected recently fallen leaves from *Ficus insipida* Willd., a dominant riparian tree species in Central American rivers with rapid decomposition rates (Rosemond et al. 1998). This characteristic makes it suitable for assessing microbial activity associated with the leaves. We also included four macroinvertebrate taxa based on their abundance and functional feeding group (Merritt and Cummins 1996, Ramírez and Gutiérrez-Fonseca 2014).

- Grazers/scrapers: *Traverella holzenthali* Lugo-Ortiz and McCafferty (Ephemeroptera: Leptophlebiidae)
- Collectors: *Ulmeritoides* sp. (Ephemeroptera: Leptophlebiidae)
- Shredders*: Phylloicus* spp. (Calamoceratidae: Trichoptera)
- Predators: *Anacroneuria* spp. (Perlidae: Plecoptera)

3.3.5 Mesocosm experiments

Organisms were collected in September-October 2021 from buffered streams (Arboleda and Sura-30) and unbuffered streams (Taconazo and Sura-60). We collected macroinvertebrates manually, transported them to the laboratory alive and maintained them for 24-hour to acclimate before experiments.

Mesocosms were set up using plastic containers (0.15 x 0.60 m). The containers were filled with water from the Taconazo stream, chosen for being an unbuffered stream, which lacks carbonates that stabilize pH compared to buffered streams. This characteristic allowed for greater manipulation of pH levels in the laboratory. Inside each container, 4 to 8 cups (5 cm in diameter) were placed, with each cup accommodating an individual from the selected taxa. The largest individuals were chosen to ensure they were in advanced larval stages, and Ephemeroptera nymphs were collected with developed wing pads. Continuous aeration was maintained using permanent aeration pumps, and the cups were perforated to facilitate water exchange and maintain optimal oxygen levels within the mesocosms.

pH manipulation was achieved by bubbling carbon dioxide (CO_2) directly into the containers. We employed three treatments: 1) Control, maintaining a stream water pH between 5.4 and 5.6; 2) Pulses, alternating 8-hour intervals of low pH (between 4.6 and 4.9) and natural stream pH (5.4-5.6) throughout the experiment's duration; 3) Continuous low pH (between 4.6 and 4.9) for the entire experiment. Every three hours, we measured pH, dissolved oxygen, and water temperature in the containers. To maintain experimental conditions, we replaced 30% of the mesocosm water with fresh stream water every other day throughout the experiment.

3.3.6 Growth rates

We assessed growth rates of *Ulmeritoides*, *Traverella*, and *Anacroneuria* individuals across the three treatments. The experiment duration for each taxon was determined through preliminary tests conducted in the laboratory, observing the time each nymph could be exposed before undergoing maturation into the adult stage. The experiment extended over a period of 4.33 days for *Ulmeritoides* and *Anacroneuria*, and 5.33 days for *Traverella* to observe an appreciable change in size. For substrate and food source, conditioned leaf discs of *Ficus insipida* were used, obtained from leaves previously incubated in stream water for five days. Additionally, for *Anacroneuria*, which is a predator, two chironomid larvae (approximately 2 to 3 mm in size) were provided during a 24-hour acclimatization period, after which any uneaten individuals or remains were removed before starting the growth experiments. We took measurements of the total length and head width of each individual at the beginning and the end of the experiment using calibrated photographs. Biomass was calculated following Benke et al. (1999), and growth rates for each taxon were determined using the equation (Olson and Hawkins 2017):

Growth rate = (Ln final biomass in mg - Ln initial biomass in mg) / time interval in days

3.3.7 Respiration rates

Respiration rates of *Ulmeritoides*, *Traverella*, *Phylloicus*, *Anacroneria*, and conditioned leaves of *Ficus insipida*, were measured after 4 to 5-day of incubation in our three water treatments. For airtight conditions, we employed 125 mL BOD glass bottles fitted with stoppers. Within each bottle, an individual organism was introduced to ascertain its respiration rate within each treatment. Prior to introducing the organisms into the bottles, we measured the dissolved

oxygen content, repeating the measurement 24 hours thereafter. To calculate respiration rates, we use the modified equation of the one proposed by Benfield et al. (2017):

$$R = ((DO_{start} - DO_{end}/t) - (DO_{control\ start} - DO_{control\ end}/t_{control})) * \text{Volume } H_2O \text{ Chamber}/\text{Mass}$$

Where DO= dissolved oxygen (mg/L), R = O_2 consumption in units of mgO_2* g AFDM of the sample (in g), and t and $t_{Control}$= respective incubation period (in h).

3.3.8 Data Analysis

Environmental variables. To explore the relationship between environmental variables and streams, we performed a Principal Component Analysis (PCA) using monthly averages of conductivity, SRP, pH, water level, temperature, dissolved oxygen, and nitrate (NO_3) for both the buffered and unbuffered streams in 2021. Prior to the analysis, we applied z-score normalization, transforming each variable to have a mean of 0 and a standard deviation of 1. This standardization ensures that all variables contribute equally to the PCA, regardless of their original scales. The PCA was conducted using the 'prcomp' function from the *stats* package. Data visualizations were created using the *ggplot2* and *ggbiplot* packages. Additionally, the *factoextra* package was utilized to visualize variable contributions and generate biplots.

Frequency, Duration, and Magnitude of Acidification Events. To analyze the frequency, duration, and magnitude of acidification events (defined as pH levels below 5, (Ganong et al. 2021), we used the *dplyr* library. For frequency, we counted the total number of acidification events and calculated the average number of events per month (lasting more than 15 minutes each). For duration, we measured the length of each acidification event and averaged the

duration monthly. For magnitude, we defined it as the difference between the monthly maximum pH and the minimum pH value recorded in the same month. This difference represents the maximum magnitude of change in pH for each month. All values were calculated monthly for the year 2021.

Macroinvertebrate Assemblage. To analyze the density, richness, and biomass of macroinvertebrates across streams while accounting for temporal variation, we conducted an ANCOVA using month as a covariate. The ANCOVA was performed using the *stats* package. Diagnostic plots of the model were created to assess the assumptions of ANCOVA, including normality and homoscedasticity. Diversity was estimated as the effective number of species or morphotypes (^{q}D; Jost, 2006). When $q \approx 1$, typical diversity is obtained, which is equivalent to the exponential of the Shannon entropy index and is not biased by the presence of rare or abundant species in the sample. When $q = 2$, the diversity value reflects the effective number of more abundant species or morphotypes, equivalent to the inverse of Simpson's entropy index (Moreno et al. 2011). Diversity (^{q}D) was compared under the same sample completeness or coverage ($\hat{C}m$), which indicates the proportion of the community represented by the captured species (or morphospecies) and ranges from 0% (low completeness) to 100% (high completeness (Chao and Jost 2012). The 95% confidence intervals (CIs) for each diversity expression (^{1}D, ^{2}D) were used as the statistical criteria for comparing ^{q}D. The estimation of $^{q}D \pm 95\%$ CI and the respective sample coverage ($\hat{C}m$) was conducted using the *iNEXT* package (Hsieh et al. 2016).

We analyzed the macroinvertebrate assemblages using Non-metric Multidimensional Scaling (NMDS) to visualize the differences in community composition between streams. The NMDS was performed with three dimensions (K=3) and Bray-Curtis as the distance metric. To

test for significant differences in macroinvertebrate communities between streams, we conducted a Permutational Multivariate Analysis of Variance (PERMANOVA) with 9,999 permutations using Bray-Curtis. Additionally, a Similarity Percentage Analysis (SIMPER) was applied to identify the specific taxa that contributed most to the dissimilarities between streams. SIMPER calculates the average contribution of each taxon to the overall Bray-Curtis dissimilarity, allowing us to determine which species were primarily responsible for the observed differences in community composition. We performed the NMDS, PERMANOVA, and SIMPER using the *vegan* package. Boxplots and the NMDS ordination plot were generated using the *ggplot2* package. All analyses were conducted separately for each habitat type (rocks and leaves).

Effects of Physicochemical Variables. We evaluated the effects of environmental factors, particularly pH, on response variables including density, richness, and biomass. Analyses were conducted separately for each stream (buffered and unbuffered) and habitat (leaves and rocks). Generalized linear mixed models (GLMMs) were applied for abundance (count data), using a negative binomial distribution to account for overdispersion, implemented with the *glmmTMB* package (Brooks et al. 2017). Biomass data (continuous data) was analyzed using Gaussian models after log transformation (log(x+1)) to meet normality requirements, also executed using the *glmmTMB* package. For richness, which met normality assumptions, linear mixed models (LMMs) were employed, using the *lme4* package (Bates et al. 2003). The month of sampling was included as a random effect in all models to account for temporal variability. Temporal correlation was evaluated using the Mann-Kendall test from the Kendall package (McLeod 2022) and Sen's slope from the trend package (Pohlert 2023), with no significant evidence of temporal autocorrelation detected.

Fixed effects for the buffered stream included minimum pH and water level, while for the unbuffered stream, the same variables were considered alongside the frequency of acidification episodes. Water level was included as a fixed effect in both streams due to evidence suggesting it influences macroinvertebrate assemblages (Ramírez and Pringle 2006). However, the frequency of acidification was only included in the unbuffered stream, as this stream experiences periodic acidification events, whereas the buffered stream does not. Minimum pH was included as a predictor for both streams, given the focus of the study on its effects.

To standardize the scales of the independent variables and address convergence issues, all predictors were transformed to z-scores (mean = 0, standard deviation = 1). Model selection was performed using the Akaike Information Criterion corrected for small sample sizes (AICc), with models ranked using the *AICcmodavg* package (Mazerolle 2023). Models with a ΔAICc < 2 were retained, and ANOVAs were performed on these selected models to assess the significance of fixed effects using 'Anova' function in the *car* library (Fox and Weisberg 2018). Model validation was conducted through residual analysis and quantile-quantile plots (qqplots) using the *DHARMa* package (Hartig, 2022), with additional tests for uniformity (Kolmogorov-Smirnov test), dispersion, and outlier detection to evaluate model fit. Multicollinearity was assessed by calculating the Variance Inflation Factor (VIF) for the main terms in each model, ensuring that VIF values remained below 10, using the *performance* package (Lüdecke et al. 2021).

Growth and Respiration Rates. To assess the effects of pH treatments (control, continuous, and pulse) on individuals collected from buffered and unbuffered streams, we used Generalized Linear Models (GLM). We applied Gamma, Gaussian, and inverse Gaussian distributions. For the respiration rate data of *Phylloicus*, *Ulmeritoides*, and *Traverella*, a log transformation

(log(x+1)) was used to meet normality requirements. The fixed effect for all taxa was treatment, with three levels (Control, Continuous, and Pulse). For *Anacroneuria*, *Phylloicus*, and microorganisms associated with *Ficus insipida*, both treatment and stream type (buffered vs. unbuffered) were included as fixed effects. Similar to the approach described for the GLMMs previously, model selection was performed using the Akaike Information Criterion corrected for small sample sizes (AICc) with the *AICcmodavg* package. Models with $\Delta AICc < 2$ were retained, and ANOVAs were conducted on these selected models to assess the significance of fixed effects using the 'Anova' function from the *car* library. The gamma and Gaussian distributions were fitted using the *glmmTMB* package, while the inverse Gaussian models were fitted using the *stats* package.

All statistical analyses were performed using R (version 4.3.1, R Core Team 2023) and RStudio (version 2023.6.1.524, RStudio Team 2023).

3.4 Results

The primary environmental gradients distinguishing the buffered and unbuffered streams are related to pH, SRP, and conductivity. The PCA revealed distinct clustering patterns between streams (Figure 3.2). Water physicochemistry in the buffered stream was associated with high values of pH, SRP, and conductivity; these variables showed strong correlations with the first principal component (PC1), which explained 74.1% of the total variance. In contrast, water physicochemistry in the unbuffered stream was characterized by low values for these variables along the first component. Average pH, SRP, and conductivity in the buffered stream were 6.56, 147.3 µg/L, and 353.4 µS/cm, respectively, while in the unbuffered stream the averages were 5.36, 6.7 µg/L, and 20.7 µS/cm, respectively.

The second principal component (PC2), which explained 9.2% of the variance, was mainly influenced by variations in water level, temperature, and O_2. Average water level was 0.4 m in the buffered stream and 0.3 m in the unbuffered stream. Temperature remained relatively constant across sampling events for both stream types, ranging between 23.6 and 24.8 °C. O_2 concentration was similar between streams, with an average of 6.7 mg/L in the buffered stream and 7.3 mg/L in the unbuffered stream. NO_3 did not play a role differentiating between streams but ranged between 0.058 and 0.412 mg/L in both streams.

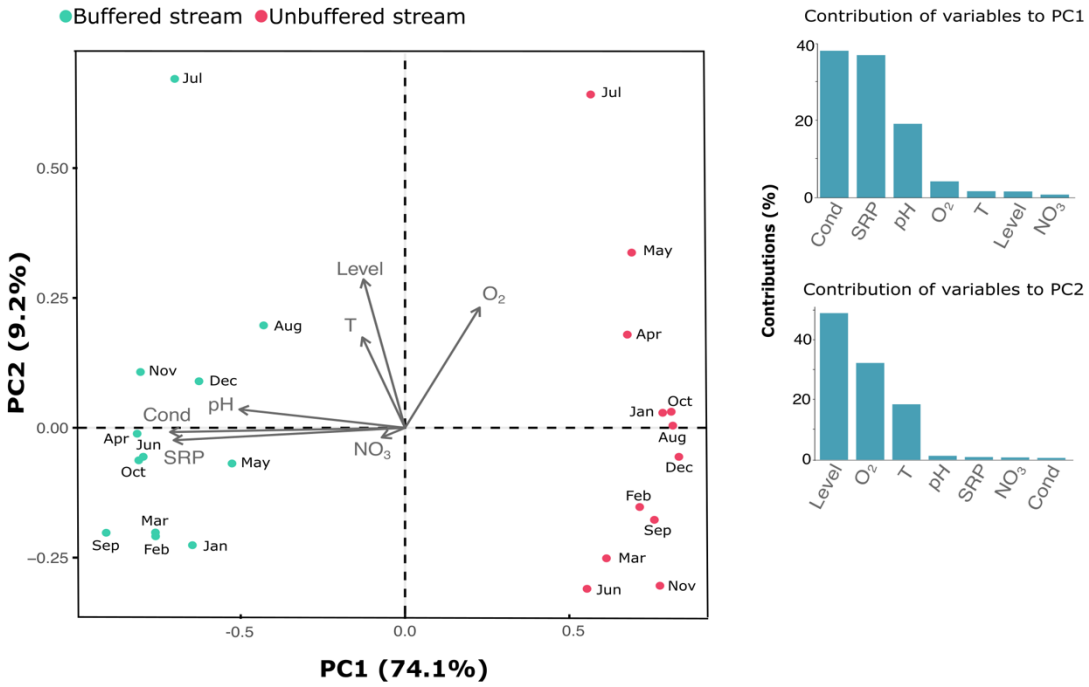

Figure 3.2 Principal Component Analysis (PCA) of buffered (pink dots) and unbuffered streams (green dots). Principal component (PC1) explains 74.1% of the variance, is strongly associated with conductivity (Cond), soluble reactive phosphorus (SRP), and pH, distinguishing buffered stream samples from unbuffered stream samples. The second principal component (PC2),

accounting for 9.2% of the variance, is influenced by water level (Level), temperature (T), and dissolved oxygen (O_2). Nitrate (NO_3) had a minimal impact on the variance.

Regarding acidification events, the minimum pH observed in the buffered stream was 5.95, compared to 3.77 in the unbuffered stream. During 2021, the unbuffered stream experienced 371 acidification events (lasting more than 15 minutes each), with an average duration of 2 hours. The magnitude of these events, calculated as the difference between the maximum pH and the minimum pH for each month, ranged from 0.75 to 2.53 (ΔpH) (Figure 3). Acidification events were not observed between January and April and occurred only from June to December 2021. During this period, both the frequency and duration of these events increased, showing a general upward trend from June to December. However, the magnitude of acidification events was higher between July and September. Due to equipment malfunction, we lack data for May regarding the frequency, duration, and magnitude of acidification events. In contrast, the buffered stream did not exhibit any acidification events throughout the year.

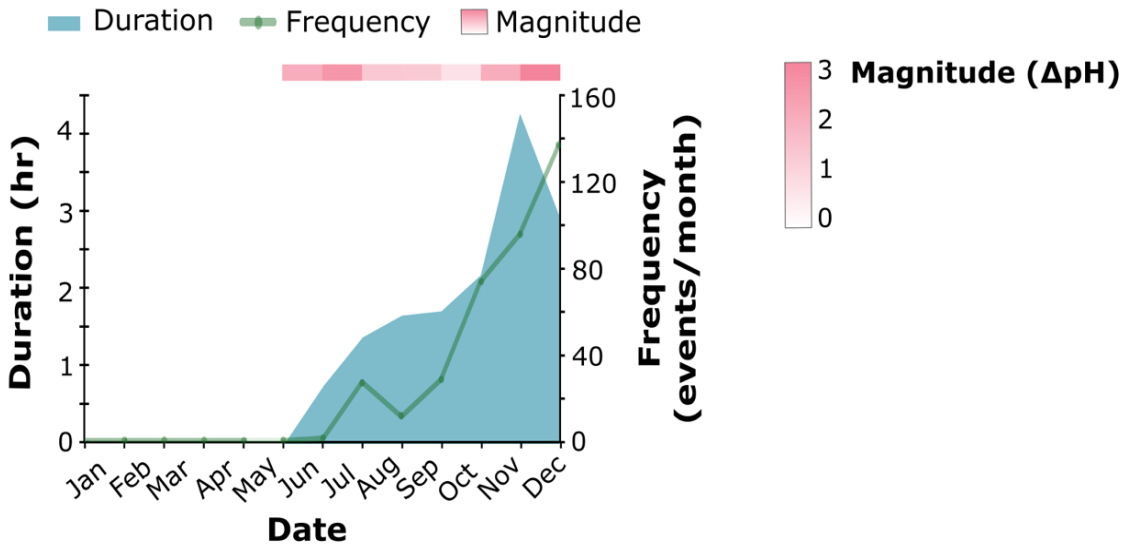

Figure 3.3 Frequency, duration, and magnitude of acidification events in the unbuffered stream. Acidification events occurred between June and December 2021. In contrast, the buffered stream did not exhibit any acidification events during 2021. Data on the frequency, duration, and magnitude of acidification events were unavailable for May. Frequency represents the number of acidification events per month. Duration is the average time (hours) per acidification event each month. Magnitude is defined as the difference between the maximum and minimum pH values recorded for each month with acidification events (ΔpH).

3.4.1 Macroinvertebrate assemblages

Richness and diversity of aquatic macroinvertebrates colonizing our rock and leaf substrates were higher in the buffered stream, while abundance was higher in the unbuffered stream. Richness was significantly greater in the buffered stream compared to the unbuffered stream for both substrates (ANCOVA, p-value < 0.001, df = 11, F = 4.263 for rocks; p-value < 0.001, df = 11, F = 2.644 for leaves). Additionally, the unbuffered stream exhibited higher

densities than the buffered stream (ANCOVA, p-value < 0.001, df = 11, F = 4.23 for rocks; p-value = 0.012, df = 11, F = 2.367 for leaves). Biomass did not show significant differences between streams for either substrate (ANCOVA, p-value = 0.114 for rocks; p-value = 0.3348 for leaves). Analysis of diversity expressions ${}^{q1}D$ and ${}^{q2}D$, with a 95% confidence interval, revealed higher values in the buffered stream. The buffered stream exhibited between 1.5 (leaves) and 1.6 (rocks) times more diversity in terms of effective number of equally common taxa (${}^{1}D$) compared to the unbuffered stream. Similarly, the effective number of the most abundant taxa (${}^{2}D$) differed from 1.5 to 1.6 for leaves and rocks, respectively (Appendix A).

Model selection criteria indicated that minimum pH was a variable explaining the patterns in macroinvertebrate assemblages across all top models from both the buffered and unbuffered streams (Appendix B). Of these top models, minimum pH significantly affected abundance, biomass, and richness in the unbuffered stream (ANOVA, p-value < 0.05, Table 3.1, 3.2). Additionally, the interaction between minimum pH and the frequency of acidification events had a significant effect on the richness of macroinvertebrates in the unbuffered stream (ANOVA, leaves: $\chi2=11.931$, p−value<0.001; rocks: $\chi2=9.379$, p−value=0.002). Similarly, the interaction between minimum pH and average level also significantly influenced macroinvertebrate richness in this stream (ANOVA, leaves: $\chi2=6.959$, p−value=0.008; rocks: $\chi2=7.605$, p−value=0.006). In contrast, in the buffered stream, minimum pH and average level did not have a significant effect on abundance, biomass, or richness.

Table 3.1 Generalized Linear Mixed Model (GLMM) results for macroinvertebrate abundance and biomass in buffered and unbuffered streams. These models were selected from the top-ranked models in the AICc selection process (ΔAIC < 2). Degrees of freedom (df) are shown for the Chi² tests. Significant p-values from ANOVA ($p < 0.05$) highlight predictors with a significant effect on the response variables across different habitats. The variable pH_Min refers to the minimum pH of the water recorded each month and Level_Av represents the average monthly water level.

Stream	Response Variable	Habitat	Model predictor	Estimate	Std. Error	z value	p value	Chi²	df	p-value ANOVA
Unbuffered (Taconazo)	Abundance	Leaves	Intercept	4.085	0.074	55.050	<2e-16			
			pH_Min	0.167	0.074	2.250	0.024	5.070	1	0.024
		Rocks	Intercept	3.460	0.080	43.230	<2e-16			
			pH_Min	0.181	0.080	2.270	0.023	5.140	1	0.023
	Biomass	Leaves	Intercept	5.030	0.089	56.620	<2e-16			
			pH_Min	0.259	0.089	2.900	0.004	8.190	1	0.004
			Level_Av	0.308	0.108	2.860	0.004	8.910	1	0.003
			pH_Min: Level_Av	0.067	0.097	0.690	0.493	0.470	1	0.493
		Rocks	Intercept	4.189	0.123	34.160	<2e-16			
			pH_Min	0.244	0.123	1.980	0.047	3.930	1	0.047
Buffered (Arboleda)	Abundance	Leaves	Intercept	3.528	0.102	34.570	<2e-16			
			pH_Min	-0.140	0.100	-1.400	0.162	1.950	1	0.162
			Intercept	3.528	0.109	32.370	<2e-16			
			Level_Av	0.016	0.106	0.150	0.878	0.020	1	0.878
		Rocks	Intercept	2.752	0.178	15.431	<2e-16			
			pH_Min	-0.202	0.176	-1.148	0.251	1.320	1	0.251
			Intercept	2.752	0.186	14.800	<2e-16			
			Level_Av	0.077	0.180	0.426	0.670	0.020	1	0.878

Table 3.1 (Continued)

	Biomass	Leaves	Intercept	4.938	0.128	38.640	<2e-16		
			Level_Av	-0.130	0.128	-1.010	0.311	1.030	1	0.311
			Intercept	4.938	0.132	37.380	<2e-16		
			pH_Min	-0.058	0.133	-0.430	0.665	0.190	1	0.665
			Intercept	4.703	0.174	27.044	<2e-16		
			pH_Min	0.029	0.151	0.194	0.846	0.930	1	0.335
			Level_Av	-0.828	0.379	-2.188	0.029	1.920	1	0.166
			pH_Min: Level_Av	-0.646	0.353	-1.831	0.067	3.350	1	0.067
		Rocks	Intercept	3.772	0.162	23.300	<2e-16		
			pH_Min	-0.046	0.163	-0.280	0.779	0.080	1	0.780
			Intercept	3.772	0.162	23.292	<2e-16		
			Level_Av	-0.043	0.163	-0.267	0.789	0.070	1	0.789

Table 3.2 Linear Mixed Model (LMM) results for macroinvertebrate richness in buffered and unbuffered streams. These models were selected from the top-ranked models in the AICc selection process ($\Delta AIC < 2$). Degrees of freedom (df) are shown for the Chi^2 tests. Significant p-values from ANOVA ($p < 0.05$) indicate predictors with a significant effect on the response variables across different habitats. The variable pH_Min refers to the minimum pH of the water recorded each month, Level_Av represents the average monthly water level, and Frequency denotes the number of acidification events per month.

Stream	**Response Variable**	**Habitat**	**Predictor**	**Estimate**	**Std. Error**	**t value**	**Chi^2**	**df**	**p value ANOVA**
Unbuffered (Taconazo)	Richness	Leaves	Intercept	7.2	0.28	25.32			
			Level_Av	0.33	0.29	1.16	1.36	1	0.24
			Intercept	8	0.42	19.1			
			pH_Min	1.14	0.44	2.61	0.01	1	0.92
			Level_Av	-1.64	0.75	-2.18	2.26	1	0.13

Table 3.2 (Continued)

		Frequency	1.48	0.59	2.53	1.07	1	0.3
		pH_Min: Level_Av	-2.09	0.9	-2.31	1.52	1	0.22
		pH_Min: Frequency	1.41	0.99	1.43	11.93	1	0
		Level_Av: Frequency	-2.12	0.81	-2.62	6.96	1	0.01
		pH_Min: Level_Av: Frequency	-3.89	1.93	-2.02	4.07	1	0.04
		Intercept	7.2	0.3	24.26			
		Frequency	-0.15	0.3	-0.52	0.27	1	0.61
		Intercept	7.2	0.3	24.02			
		pH_Min	0.04	0.3	0.13	0.02	1	0.9
		Intercept	7.46	0.28	26.76			
		pH_Min	0.38	0.32	1.21	0	1	0.99
		Frequency	-0.07	0.27	-0.28	0.35	1	0.56
		pH_Min: Frequency	0.97	0.45	2.17	4.71	1	0.03
	Rocks	Intercept	1.72	0.06	30.17			
		pH_Min	-0.08	0.06	-1.44	2.08	1	0.15
		Intercept	1.85	0.07	25.41			
		pH_Min	0.1	0.08	1.29	3.67	1	0.06
		Level_Av	-0.51	0.13	-3.94	0.66	1	0.42
		Frequency	0.36	0.1	3.59	0.01	1	0.91
		pH_Min: Level_Av	-0.52	0.16	-3.34	7.61	1	0.01
		pH_Min: Frequency	0.13	0.17	0.79	9.38	1	0
		Level_Av: Frequency	-0.49	0.14	-3.51	12.42	1	0
		pH_Min: Level_Av: Frequency	-0.86	0.33	-2.57	6.58	1	0.01
		Intercept	7.2	0.3	24.26			
		Frequency	-0.15	0.3	-0.52	0.27	1	0.61
		Intercept	7.46	0.28	26.76			
		pH_Min	0.38	0.32	1.21	0	1	0.99

Table 3.2 (Continued)

			Frequency	-0.07	0.27	-0.28	0.35	1	0.56
			pH_Min: Frequency	0.97	0.45	2.17	4.71	1	0.03
Buffered (Arboleda)	Richness	Leaves	Intercept	7.62	0.36	20.92			
			pH_Min	-0.64	0.37	-1.75	3.07	1	0.08
		Rocks	Intercept	5.53	0.49	11.3			
			pH_Min	-0.53	0.49	-1.08	1.16	1	0.28

The composition and structure of macroinvertebrate assemblages differed between the two stream types, with Diptera, Ephemeroptera, and Odonata being the primary contributors to these differences. NMDS analysis revealed a clear separation in macroinvertebrate composition between the streams for both rock and leaf substrates (Figure 3.4A). These differences were statistically supported in terms of density (PERMANOVA, p-value < 0.01, df = 1, F = 10.621 for rocks; p-value < 0.01, df = 1, F = 14.303 for leaves) and biomass (PERMANOVA, p-value < 0.01, df = 1, F = 9.559 for rocks; p-value < 0.01, df = 1, F = 8.794 for leaves). Flies from the family Chironomidae (Diptera), including Chironominae (28%), Tanypodinae (15%), and Tanytarsini (6%), significantly contributed to these differences, showing higher densities in the unbuffered stream. In contrast, the mayfly genera *Ulmeritoides* and *Caenis* exhibited higher densities in the buffered stream, each reaching 4% on rock substrates, while *Ulmeritoides* reached 7% and *Caenis* 5% on leaf substrates. In terms of biomass, dragonflies from the family Gomphidae (Odonata) (16% for rocks; 13% for leaves), along with flies from Chironominae (14% for rocks; 13% for leaves) and Tanypodinae (2.2% for rocks; 2.6% for leaves), displayed significant variations between streams, with higher biomass generally observed in the unbuffered stream. Similar to the density pattern, the mayflies *Ulmeritoides* (13.4% for rocks; 17% for

leaves) and *Caenis* (1.6% for rocks; 1% for leaves) exhibited higher biomass values in the buffered stream (Figure 3.4B).

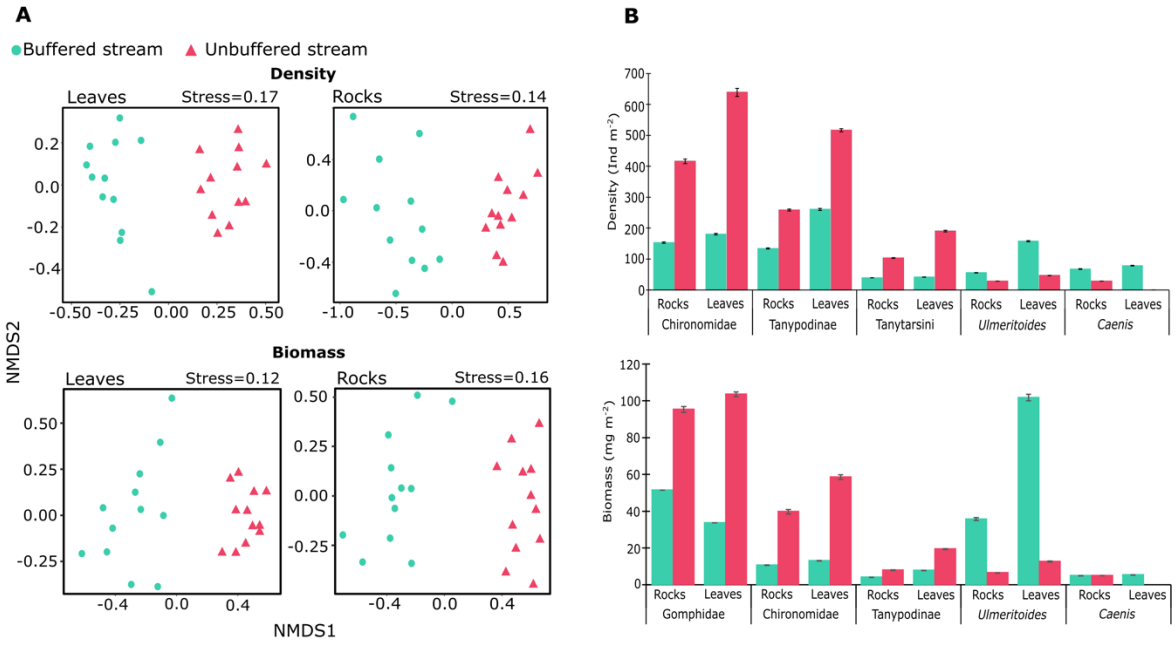

Figure 3.4 (A) Non-Metric Multidimensional Scaling (NMDS) of macroinvertebrate density (Ind m^{-2}) and biomass (mg m^{-2}) in rocks and leaves substrates in the buffered (green dots) and the unbuffered stream (pink triangles). Stress values for the analysis ranged between 0.12 and 0.17. (B) Taxa with the greatest contribution to the SIMPER (Similarity Percentage) analysis of macroinvertebrate density and biomass in rocks and leaves substrates for the buffered (pink) and the unbuffered stream (green). Error bars represent standard errors of the mean.

3.4.2 Experimental acidification. Among the top-selected models (Appendix B), we found significant differences in both respiration and growth rates between treatments, but not between individuals collected from different streams (Table 3.3). Respiration rates of microorganisms associated with *Ficus insipida* leaves were statistically different between treatments (ANOVA,

$\chi 2$=11.931, p−value<0.001), with higher respiration rates observed in the continuous and pulse treatments compared to the control. The responses of aquatic insect respiration to experimental water acidification varied among taxa (Figure 5). *Phylloicus* exhibited a similar pattern to microorganisms, showing significant differences between treatments (ANOVA, $\chi 2$=6.03461, p−value=0.048), with higher respiration rates in the continuous and pulse treatments compared to the control. However, while *Ulmeritoides* and *Traverella* also showed significant differences between treatments (ANOVA, $\chi 2$=8.815, p−value=0.012), individuals of these taxa had lower respiration rates in the continuous and pulse treatments compared to the control. In contrast, respiration rates for *Anacroneuria* were similar across treatments and did not differ significantly (Figure 3.5).

Table 3.3 Generalized Linear Model (GLM) results for the respiration and growth rates of microorganisms associated with *Ficus insipida* leaves and aquatic insects in controlled lab experiments. The models presented were selected from the top-ranked models in the AICc selection process (ΔAIC < 2). Degrees of freedom (df) are provided for the Chi² tests. Significant p-values from ANOVA (p < 0.05) indicate predictors with a significant effect on the response variables.

Response Variable		**Model predictor**	**Chi²**	**df**	**p value ANOVA**
Respiration rate	*Anacroneuria*	Treatment	3.55	2	0.17
		Stream	<0.01	1	0.96
	Phylloicus	Treatment	6.04	2	0.05
		Stream	0.04	1	0.84
	Ulmeritoides	Treatment	8.82	2	0.01

Table 3.3 (Continued)

	Traverella	Treatment	23.35	2	0
	Microorganisms	Treatment	51.34	2	0
Growth rate	*Anacroneuria*	Stream	0.25	1	0.61
	Ulmeritoides	Treatment	15.34	2	<0.01
	Traverella	Treatment	9.75	2	0.01

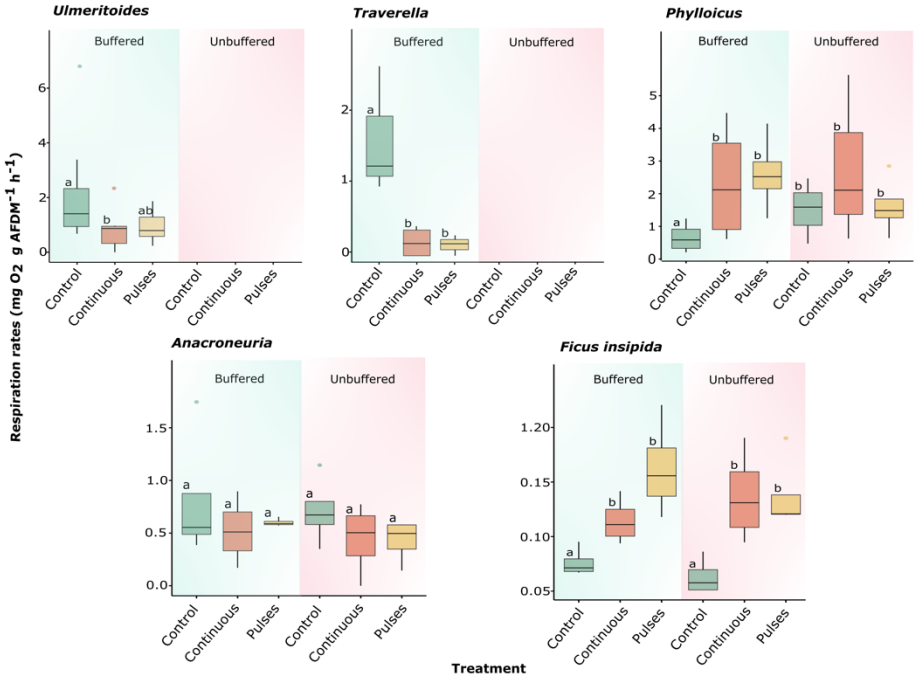

Figure 3.5 Respiration rates (mg O_2 g $AFDM^{-1}$ h^{-1}) for *Ulmeritoides*, *Traverella*, *Phylloicus*, *Anacroneuria* and microorganisms associated with *Ficus insipida* leaves under control (green), continuous (red), and pulses (yellow) treatments in buffered (light green) and unbuffered (light pink) streams. *Ulmeritoides* and *Traverella* were not present in sufficient numbers to measure respiration rates in unbuffered streams. Error bars represent standard errors of the mean. Different letters indicate significant differences (generalized linear models, GLMs).

Growth rates showed significant differences between treatments for *Traverella* (ANOVA, $\chi2$=9.749, p−value=0.008) and *Ulmeritoides* (ANOVA, $\chi2$=15.342, p−value<0.001). *Traverella* exhibited higher growth rates in the continuous (0.052 mg mg^{-1} day^{-1}+/- 0.032) and pulses (0.048 mg mg^{-1} day^{-1}+/- 0.031) treatments compared to the control (0.013 mg mg^{-1} day^{-1} +/- 0.008). *Ulmeritoides* showed the highest growth rates in the continuous treatment (0.010 mg mg^{-1} day^{-1}+/- 0.048), followed by the control (0.062 mg mg^{-1} day^{-1} ± 0.047), with the lowest rates observed in the pulses treatment (0.016 mg mg^{-1} day^{-1} +/- 0.013). Growth rates of *Anacroneuria* were similar across treatments and did not differ between buffered and unbuffered streams (Figure 3.6).

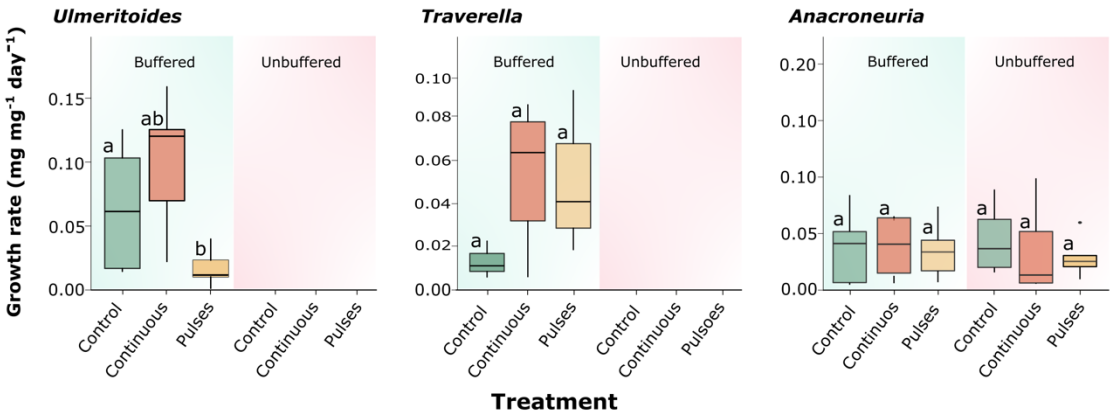

Figure 3.6 Growth rates (mg mg^{-1} day^{-1}) for *Ulmeritoides*, *Traverella* and *Anacroneuria* under control (green), continuous (red), and pulse (yellow) treatments in buffered (light green) and unbuffered (light pink) streams. *Ulmeritoides* and *Traverella* were not present in sufficient numbers to measure growth rates in unbuffered streams. Error bars represent standard errors of the mean. Different letters indicate significant differences (generalized linear models, GLMs).

3.5 Discussion

Natural acidification events have complex effects on stream macroinvertebrate assemblages. Our study shows differences in macroinvertebrate assemblages between buffered and unbuffered streams, consistent with the expected impacts of episodic acidification. However, the responses of individual taxa physiology were diverse; while some exhibited increased respiration rates and reduced growth under acidification stress, others showed decreased respiration rates or remained unaffected. This variability highlights the necessity of integrating community structure and physiological data to comprehensively evaluate responses to natural acidification events, as it allows us to identify which species are more vulnerable to acidification and understand how these physiological responses at the individual level contribute to community resilience or shifts in assemblage composition. Ultimately, understanding these dynamics is crucial for predicting how macroinvertebrate assemblages will respond to changing environmental conditions.

Our study confirms the role of carbonate-rich groundwater inputs in stabilizing pH levels in the streams we studied. Streams receiving carbonate and nutrient-rich groundwater maintained relatively stable pH values (between 6.0 and 7.0), effectively preventing fluctuations and acidification events. In contrast, streams lacking these groundwater inputs exhibited greater pH variability (between 3.8 and 7.1), underscoring the buffering capacity of carbonates in mitigating pH changes (Ramírez and Pringle 2006, Small et al. 2012, Marzolf et al. 2022). The ability of carbonates to stabilize pH has been well-documented in other systems, where they neutralize acidic inputs and minimize pH fluctuations. For example, carbonates play a key role in sulfuric acid neutralization in shallow groundwater (Ali and Atekwana 2011), and in volcanic regions,

carbonate precipitation in aquifers helps to mitigate the impact of heavy metals (Olsson et al. 2014). In addition, carbonate-rich waters have been shown to buffer arsenic-laden groundwater, reducing potential health risks (Bandara et al. 2018). These examples further support our findings that carbonate-rich groundwater inputs are essential for maintaining stable pH conditions in freshwater ecosystems

Acidification events in our unbuffered stream were limited to the rainy season. Following the dry season, the accumulation of leaf litter on the forest floor undergoes decomposition at the onset of the rainy season, leading to elevated CO_2 inputs to streams (Small et al. 2012). This CO_2, when dissolved in water and transported into streams, forms carbonic acid (H_2CO_3), resulting in acidification events (Small et al. 2012, Klem and Gutiérrez-Fonseca 2017). Although the frequency and duration of acidification events increased steadily from June to December, the highest magnitudes were observed during the shorter events between July and September, and November and December. Brief and intense acidification events impose significant stress on biological communities, leading to fish mortality and altered macroinvertebrate assemblages (Wright et al. 1988, Muniz 1990, Lepori et al. 2003). Short periods of acidic conditions can reduce survival rates and metabolic activity in zooplankton and macroinvertebrates, with varying sensitivity among species (Felten and Guérold 2006, Gao et al. 2022).

Macroinvertebrate assemblage composition and structure reflected the contrasting pH conditions in our study streams. The higher density of invertebrates in the unbuffered stream is primarily due to a high abundance of Diptera, particularly Chironomidae, which accounted for over 50% of the organisms collected. Fujii et al. (2021) found that chironomids exhibit a high degree of environmental adaptability, with some species (e.g., *Chironomus sulfurosus*), developing mechanisms for acid tolerance, including the ability to maintain near-neutral body

fluid pH even under acidic conditions. However, tolerance can vary among species, with some being more resilient than others (Havas and Hutchinson 1983, Orendt 1999).

Ephemeroptera taxa had lower abundances in the unbuffered stream, highlighting their role as indicators of pH variations. This is consistent with findings from various geographic regions and stream types, where these taxa generally decrease significantly in streams with lower pH levels (Smith et al. 1990, Rosemond et al. 1992, Petrin et al. 2007, Ganong et al. 2021). Some Ephemeroptera species are known to persist in acidified streams, but normally do so in reduced numbers (Feldman and Connor 1992, Rosemond et al. 1992), and are generally absent when pH levels fall below 4.8 (Mackay and Kersey 1985). Similarly, in our study, *Ulmeritoides* exhibited lower abundance in the unbuffered stream, while *Caenis* was entirely absent, further supporting the sensitivity of certain Ephemeroptera species to acidic conditions.

Water pH stability is crucial for maintaining biodiversity and ecosystem function across various environments. In our study, the highest richness and diversity of macroinvertebrates were found in the buffered stream, which experienced minimal pH fluctuations. Acidification has been shown to reduce species diversity in freshwater plants, microbes, and fish in temperate (Muniz 1990) zones. Acidification broadly affects aquatic ecosystems by reducing biodiversity through the loss of sensitive species and trophic structure alterations (Moiseenko 2005). These effects extend beyond freshwater systems. In marine environments, ocean acidification can decrease species diversity in coral reefs and mussel beds, although it may enhance growth in seagrass and some macroalgal habitats (Sunday et al. 2017). Terrestrial ecosystems also face similar challenges, with declining soil pH linked to reduced species richness (Gade et al. 2021). Tropical and subtropical moist forests are especially sensitive to soil pH drops below 4.1, potentially leading to significant losses in vascular plant species richness (Azevedo et al. 2013). Overall, soil

acidification represents a major threat to global terrestrial biodiversity and ecosystem functions (Chen et al. 2023).

Physiological responses to acidification among microorganisms and aquatic insects vary across taxa. In our study, the differing responses observed in leaves-associated microorganisms and aquatic insects such as *Phylloicus*, *Ulmeritoides*, and *Traverella* likely reflect their varying adaptations or tolerances to environmental changes. We found that microorganism respiration increased under acidification stress, which contrasts with findings in temperate streams, where respiration of leaf-associated microorganisms was reduced or inhibited as acidity increased (Dangles et al. 2004, Simon et al. 2009). Different species of aquatic biota, including microorganisms, zooplankton, benthos, and fishes, exhibit distinct physiological responses to acidification, impacting processes such as respiration, metabolism, and growth (Havas and Rosseland 1995, Dangles et al. 2004). The magnitude of these responses varies across taxonomic groups, with some predictable trait-based differences in sensitivity (Kroeker et al. 2013). However, the generally similar response of taxa to both continuous and pulse acidification treatments in our study suggests that it is the acidification itself, rather than the duration of exposure, that represents a significant stressor for these organisms. This indicates that the taxa are similarly affected by acidification, regardless of its temporal pattern.

Physiological responses to acidification highlight potential costs and adaptive mechanisms required to cope with pH changes, which can affect growth and survival. Changes in respiration rates (either increased or decreased compared to the control) and growth rates observed in aquatic insects in our study may reflect species-specific metabolic adjustments to acid stress. Acidification can impair the gill functions of invertebrates, which are essential for respiration, osmoregulation, ion regulation, and maintaining acid-base balance (Havas and

Rosseland 1995). Disruption of these physiological functions may compromise homeostasis, ultimately affecting survival and performance (Havas and Rosseland 1995, Moiseenko 2005). The reduced growth observed in *Ulmeritoides* in the continuous treatment may have resulted from a combination of increased metabolic demands due to acidification and a general decline in appetite and food conversion efficiency, as has been reported in fish (Havas and Rosseland 1995). Although sensitivity to acidification has been documented in Plecoptera (Doherty and Hummon 1980, Tixier and Guérold 2005), we did not observe significant effects on respiration or growth rates in stoneflies under acidification stress. However, it is possible that these insects are responding in ways not captured by the parameters we measured, potentially exhibiting other physiological changes.

In our study, we observed physiological changes in organisms, such as altered respiration and growth, combined with a reduction in taxa diversity due to acidification. These changes can significantly impact ecosystem processes and dynamics. For example, in our unbuffered stream, the decline of pH-sensitive taxa, such as Ephemeroptera (which function as detritivores), may be associated with disruptions in key ecological roles, including nutrient cycling, primary production, and energy flow (Dangles et al. 2004, Simon et al. 2009). This loss of biodiversity can create imbalances in food webs, affecting predator-prey relationships, resource availability, and community composition (Moiseenko 2005, Ullah et al. 2024). Furthermore, the reduction in functional diversity increases the vulnerability of the ecosystem to other stressors, potentially making recovery from disturbances more difficult (Teixidó et al. 2018, Ullah et al. 2024). These findings are particularly important because, although our study site experiences natural acidification, other studies have shown that anthropogenic acidification can exacerbate these

effects, leading to even greater losses in taxa diversity and ecosystem functioning (Petrin et al. 2008, Laurent et al. 2017).

Combining macroinvertebrate assemblage information with physiological responses in our study provided a more comprehensive understanding of stream ecological conditions, as it allowed us to capture both community structure and organisms' adaptations to fluctuating environmental factors. The composition and diversity of macroinvertebrate assemblages in our streams served as indicators of ecological conditions, reflecting not only the presence or absence of specific taxa, but also how these communities responded to environmental stressors, such as acidification. Additionally, the changes observed in respiration and growth rates under acidification in our study highlight that physiological responses are effective indicators of environmental stress, offering valuable insights into how organisms cope with changing conditions. Monitoring the physiological responses of organisms is crucial for detecting the effects of stressors early, providing important information on how these stressors influence ecosystem function (Fränzle 2006). This approach is especially important in ecosystems where environmental conditions can shift rapidly, for example, due to rainfall or in regions where climate change is expected to have a significant impact (Helmuth et al. 2009). In our study, Ephemeroptera showed high susceptibility to acidification, suggesting that these organisms could be effectively targeted in studies assessing water quality and pH effects. They may also serve as potential biomarkers in bioassays aimed at monitoring environmental changes

Acidification, driven by both natural processes and anthropogenic activities, has significant negative effects on ecosystems, that might get enhanced by climate change. In the Caribbean region, climate projections suggest reduced rainfall and more frequent dry periods (IPCC 2022), likely weakening the natural buffering capacity of streams and leading to more

intense and prolonged acidification events (Small et al. 2012). These events of low pH can disrupt key ecosystem functions, affecting biota, energy flow, and essential ecosystem services (Muniz 1990, Kroeker et al. 2010, Meng et al. 2019, Zunino et al. 2021). While both natural and human-induced acidification alter ecological processes, anthropogenic sources tend to be more severe, causing prolonged disruptions (Petrin et al. 2008). Climate change and land-use alterations, along with increased emissions of acids from anthropogenic sources, could accelerate acidification by raising dissolved CO_2 levels in freshwater ecosystems (Psenner 1994, Hasler et al. 2018). Furthermore, acidification interacts with other contaminants, exacerbating its effects on biota and making restoration efforts more challenging (Steinberg and Wright 1994). Ultimately, the rapid pace of these environmental changes may surpass the ability of many species to acclimate, resulting in significant biodiversity loss and lasting changes to ecosystem structure and function (Nagelkerken and Connell 2015).

As climate change and anthropogenic pressures intensify, tropical ecosystems will likely experience more frequent and severe acidification events. By improving our understanding of how natural acidification affects aquatic communities, this study offers a foundation for predicting and managing the potential impacts of anthropogenic acidification in tropical systems. Effective management strategies will need to prioritize the conservation of biodiversity and the protection of ecosystem services, particularly in regions where acidification may interact with other environmental stressors to further destabilize aquatic ecosystems.

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Chapter 4: The role of phosphorus-rich groundwater inputs in shaping stream macroinvertebrate assemblages and insect body condition in a tropical volcanic landscape

4.1 Abstract

Nutrient dynamics play a crucial role in shaping stream ecosystems, yet much of the existing research focuses on anthropogenic influences on phosphorus inputs, leaving a gap in understanding natural nutrient gradients. This study aims to assess how a natural phosphorus gradient affects macroinvertebrate assemblages, functional feeding group composition, and insect body condition (macromolecule content) in streams draining a tropical volcanic landscape. We conducted our research at La Selva Research Station, Costa Rica, where we sampled aquatic macroinvertebrates and measured water physicochemical variables (conductivity, temperature, dissolved oxygen, pH, discharge) across four streams differing in phosphorus concentrations due to geothermal inputs. Additionally, we macromolecule extraction from selected aquatic insects to evaluate their protein, lipid, and carbohydrate content. Our results demonstrate that phosphorus-rich streams support greater diversity and distinct functional group compositions compared to phosphorus-poor streams, with Ephemeroptera being the most abundant in the phosphorus-rich stream. In contrast, the phosphorus-poor stream exhibited the highest total density, along with greater densities of collectors and predators, which were predominantly represented by Diptera. Detritivores showed minimal changes in macromolecule content across the phosphorus gradient, while predators exhibited increased protein and lipid accumulation in phosphorus-enriched environments. However, not all taxa responded similarly, likely due to specific life-history traits. These findings highlight the importance of understanding natural nutrient gradients in shaping biodiversity and ecosystem processes. Understanding biotic responses to natural nutrient

gradients provides a valuable baseline for assessing the potential impacts of future anthropogenic nutrient loading on tropical stream ecosystems.

4.2 Introduction

Nutrients are fundamental in structuring stream ecosystems, where they support primary production and drive various ecological processes (Dodds 2003). Phosphorus, often a limiting nutrient, plays a crucial role in shaping community composition and ecosystem functioning, as its scarcity exerts selective pressures that influence nutrient uptake mechanisms (Elser et al. 1990, Dodds 2002). In natural waters, phosphate, the primary form of inorganic phosphorus, is efficiently assimilated even at low concentrations due to evolutionary adaptations that optimize uptake under phosphorus-limited conditions (Dodds 2002). However, when phosphorus levels exceed natural limits, they can disrupt the balance of stream ecosystems (Dodds 2003, Zhang et al. 2019), accelerating photosynthetic and heterotrophic productivity and causing profound alterations in aquatic food webs (Ensign and Doyle 2006, Ardón et al. 2021). Nevertheless, much of the research on phosphorus impacts has focused on ecosystems affected by anthropogenic activities (Smil 2000, Zhang et al. 2019, Hu et al. 2020), leaving a limited understanding of how phosphorus influences stream ecosystems in environments with naturally occurring nutrient inputs.

In natural phosphorus gradients, differences in phosphorus concentrations may create resource gradients for basal trophic levels, with potential effects on the secondary production of primary consumers (Ramírez and Pringle 2006). Studies have shown that higher phosphorus concentrations increase microbial biomass and activity on leaf litter, to which insect biomass also responds positively (Rosemond et al. 2002, Ramírez et al. 2003). In high-phosphorus

environments, detritivores, which primarily consume phosphorus-poor detritus, may struggle to regulate excess nutrients, potentially resulting in lower assimilation efficiency and imbalances in internal macromolecule composition (Sperfeld et al. 2017, Raubenheimer and Simpson 2018). In contrast, predators that consume phosphorus-enriched prey may utilize this additional resource to increase their protein and lipid reserves, thereby optimizing growth and reproduction (Anderson et al. 2004).

La Selva Research Station in Costa Rica provides an ideal setting to examine the effects of a natural phosphorus gradient in detritus-based streams. Streams at La Selva exhibit natural variability in phosphorus concentrations due to differential inputs of geothermally modified groundwater (Pringle 1991). Previous studies at La Selva have demonstrated that primary consumers in phosphorus-rich streams, enriched over centuries by geothermal inputs, contain significantly higher phosphorus levels than those in phosphorus-poor streams, suggesting a deviation from strict stoichiometric homeostasis (Small and Pringle 2010, Small et al. 2011). In this context, adaptation and nutrient regulation strategies may vary across taxa; while some organisms excrete excess phosphorus, others store it in macromolecules, adjusting their internal composition based on trophic role and the phosphorus gradient they experience over time (Boersma and Elser 2006, Sperfeld et al. 2017). However, it remains unclear whether increased phosphorus availability consistently leads to greater accumulation of macromolecules, such as proteins, lipids, and carbohydrates, or to what extent macromolecule content varies among different functional groups and trophic levels.

The aim of this study was to assess how a natural phosphorus gradient affects macroinvertebrate assemblages, functional feeding group composition, and body condition, measured through macromolecule content. We hypothesized that streams at the high end of the

phosphorus gradient would exhibit distinct assemblages and functional feeding group composition relative to phosphorus-poor streams. Regarding body condition, we anticipated that detritivores would show minimal changes in protein, lipid and carbohydrate content across the phosphorus gradient, as they may have limited capacity to assimilate excess phosphorus. Conversely, we expected that predators, with greater nutrient assimilation, would display increased macromolecule content, particularly proteins and lipids, in phosphorus-rich streams.

4.3 Methods

4.3.1 Study Site

This study was conducted at the La Selva Research Station (LSRS) in Costa Rica, located on the Caribbean slope at the transition between the Central Mountain Range and the coastal plain (10°26'N, 84°01'W). The reserve spans 1,563 hectares and is part of a broader biological corridor, encompassing mature and secondary tropical rainforests (Holdrige 1967) at elevations ranging from approximately 35 to 2,900 meters above sea level. The LSRS climate is tropical wet, with an average annual rainfall of around 4,207 mm and a mean annual air temperature of 25.8°C (available at https://anetium.ots.ac.cr/meteoro/default.php). Rainfall is distributed aseasonal, but peaks can be delimited during June-July and November-December, with lower precipitation from February to April (Sanford et al. 1994).

Stream hydrology in our study area is influenced by natural flows of solute-rich groundwater from volcanic bedrock in the Central Mountain Range (Pringle and Triska 1991, Pringle et al. 1993, Genereux et al. 2009). This groundwater, enriched as it passes through volcanic substrates, eventually emerges in lowland streams with elevated concentrations of

phosphorus, sodium, and chloride among other solutes (Pringle 1991). For this study, we selected four streams with varying levels of solute-rich groundwater inputs, creating a gradient of soluble reactive phosphorus (SRP) from 6.35 to 152.19 µg SRP/L (Table 1). Among these, Arboleda had the highest phosphorus concentration (phosphorus-rich), Taconazo was the lowest in phosphorus (phosphorus-poor), and Saltito-60 and Pantano had intermediate levels (Table 1). All streams are third-order and drain primary forests within LSRS (Figure 4.1).

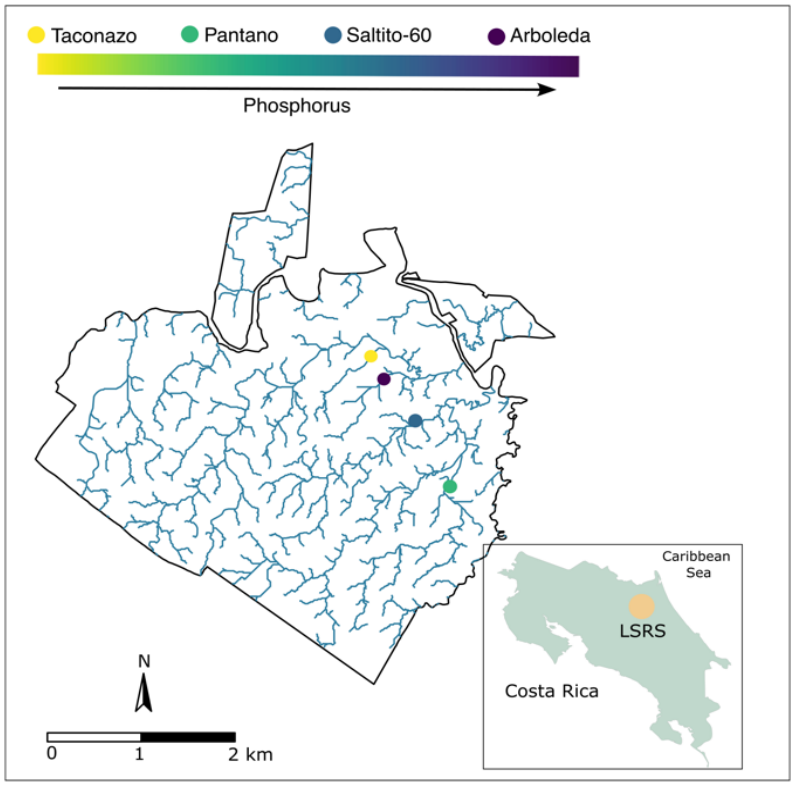

Figure 4.1 Study streams at La Selva Research Station (LSRS), Costa Rica. The colors represent streams along the phosphorus gradient, increasing from left to right. In the bottom right corner, the location of LSRS is marked by an orange circle on the map of Costa Rica.

4.3.2 Stream Physicochemical Variables

We sampled our four studied streams once during August 2023 and again during February 2024. Using a YSI 600XLM multiprobe, we measured conductivity, total suspended solids, dissolved oxygen, and water temperature. We also measured stream discharge at each stream once during each sampling as an indicator of stream size. Our ongoing research project at LSRS monitors water solute concentrations, including SRP and conductivity, monthly (Ardón and Ramirez 2024), and we used them to characterize our phosphorus gradient.

4.3.3 Macroinvertebrate sampling

Macroinvertebrate assemblages were characterized using standard substrates, we placed five plastic boxes (0.35 x 0.20 x 0.07 m) in each of the four streams along the phosphorus gradient. These boxes were elevated with ribbed bars to prevent direct contact with the streambed, minimizing sediment buildup and low oxygen conditions. Each box was divided into two compartments, each covering 0.089 m^2. One compartment held six senescence leaves of *Guacimo Colorado* (*Luehea seemannii* Triana and Planch), a common tree species in the area known for its intermediate decomposition rate (91 days to 95% mass loss, Ardón and Pringle 2008). The other compartment contained five medium-sized rocks. Collections took place in August 2023 (wet season) and February 2024 (dry season). After leaving the boxes in the streams for 30 days, we rinsed the leaves and rocks with stream water over a 250-micron sieve to separate the macroinvertebrates, which we then preserved in 80% ethanol in plastic bags.

Organisms were identified to the lowest feasible taxonomic level using available keys (Springer et al. 2010, Hamada et al. 2018). Biomass was estimated using a length-mass model, M

= aL^b, where M is organism mass (mg), L is individual body length (mm), and *a* and *b* are constants (Benke et al. 1999).

4.3.4 Macromolecular Analysis

Focal Taxa. To assess macromolecular content (proteins, lipids, and carbohydrates) in aquatic insects, we selected four abundant taxa from different dominant functional feeding groups (Merritt and Cummins 1996, Ramírez and Gutiérrez-Fonseca 2014).

- Collectors: *Ulmeritoides* sp. (Ephemeroptera: Leptophlebiidae)
- Shredders: *Phylloicus* spp. (Trichoptera: Calamoceratidae)
- Predators: *Anacroneuria* spp. (Plecoptera: Perlidae)
- Predators: *Phyllogomphoides* spp. (Odonata: Gomphidae)

Focal taxa were collected manually during a single sampling effort between May and June 2023 from each stream along the phosphorus gradient. We collected individuals of similar sizes and selected the largest individuals within each taxon for analysis. For *Ulmeritoides* (Ephemeroptera), nymphs with developed wing pads were chosen after sorting them by sex.

Organisms were transported alive to the laboratory, where they were allowed to empty their gut contents over a 24-hour period. Continuous aeration was provided using permanent aeration pumps during this period. Following gut evacuation, individuals were grouped by taxon: 11 to 22 *Ulmeritoides* individuals, 9 to 11 *Phylloicus*, 9 to 16 *Anacroneuria*, and 1 to 4 *Phyllogomphoides* per group.

Organisms were frozen at -20°C and lyophilized (freeze-dried) using a vacuum freeze-dryer (Labconco, Model 75035, Series 805318), a process that removes water under low temperature and pressure to preserve macromolecular integrity (Meryman 1960, Rockinger et al.

2021). Samples were maintained frozen until analysis at North Carolina State University. For analysis, samples were ground and homogenized, and 5 mg subsamples were taken from each group for macromolecular extraction.

The number of individuals used in extractions varied among streams due to differences in sampling success. The collector *Ulmeritoides* was sorted by sex and analyzed in both the phosphorus-rich stream, Arboleda (females: n=10, males: n=3), and the phosphorus-poor stream, Taconazo (females: n=7 for proteins and carbohydrates, n=6 for lipids; males: n=3). Shredders (*Phylloicus*) and predators (*Anacroneuria*) were examined in both the phosphorus-rich stream (Phylloicus: n=10 for proteins and lipids, n=9 for carbohydrates; *Anacroneuria*: n=10) and the phosphorus-poor stream (*Phylloicus*: n=10 for proteins and lipids, n=9 for carbohydrates; *Anacroneuria*: n=3), as well as the intermediate phosphorus gradient stream, Pantano. In Pantano, *Phylloicus* samples consisted of n=10 for proteins and n=9 for both lipids and carbohydrates, while *Anacroneuria* samples were composed of n=7 for all analyses. Finally, *Phyllogomphoides*, a predator, was collected from all four streams along the phosphorus gradient, with n=10 individuals analyzed in each stream.

Macromolecule Extraction. To determine the lipid, protein, and carbohydrate content in macroinvertebrate samples, we followed an adapted version of the MEDI protocol (Cuff et al. 2021). We used approximately 5 mg subsamples from each macroinvertebrate group. Lipid extraction was performed by immersing each sample in a 1:12 chloroform: methanol solution for several minutes, after which the supernatant was removed to ensure thorough lipid extraction. Following lipid extraction, the remaining tissue was solubilized in 0.1 M NaOH to prepare for protein and carbohydrate analyses. Protein content was measured using the BCA assay (Pierce

BCA Protein Assay Kit, Thermo Scientific), and lipid content was quantified with a colorimetric lipid assay kit (Abcam Inc.), both following the instructions of the manufacturers for 96-well microplate use. Carbohydrate content was measured using the anthrone method, which produces a colorimetric response when reacting with simple sugars. To ensure accuracy, we conducted three technical replicates per sample for each macromolecule assay.

4.3.5 Data Analysis

All statistical analyses were performed using R (version 4.3.1, R Core Team 2023) and RStudio (version 2023.6.1.524, RStudio Team 2023).

Physicochemical variables. We found a strong correlation between monthly values of conductivity and phosphorus concentrations in our studied streams using data collected between January 2020 and December 2022 (Ardón and Ramirez 2024). The Spearman's correlation coefficient was 0.81 (p-value <0.001), indicating a strong positive association. Based on this correlation, we used conductivity as a proxy for phosphorus levels during our study as we did not collect samples associated with our aquatic insect sampling.

Temporal Variation. To assess differences between sampling months for each response variable (density, richness, biomass, and the density and biomass of collectors and predators) across streams and substrate, we first evaluated the normality of each variable using the Shapiro-Wilk test and tested for homoscedasticity with Levene's test. For normally distributed and homoscedastic data, we conducted paired t-tests, while for non-normally distributed or heteroscedastic variables, we used the Wilcoxon signed-rank test.

Effects of Physicochemical Variables. We evaluated environmental factor effects (Table 1) on the density, richness, and biomass of the assemblage and the density and biomass of collectors and predators. Analyses were conducted separately for each substrate (leaves and rocks). Generalized linear mixed models (GLMMs) were applied to count data (richness) using a negative binomial distribution to account for overdispersion, implemented in the glmmTMB package (Brooks et al. 2017). For biomass and density, Gaussian or Gamma models were used, applying a log transformation (log(x+1)) to meet normality requirements when necessary. Richness, which met normality assumptions, was analyzed with linear mixed models (LMMs) using the lme4 package (Bates et al. 2003). Fixed effects included conductivity and pH, while sampling month and discharge were included as random effects in all models to account for temporal variability and stream size. Temporal correlation was assessed with the Mann-Kendall test (Kendall package, McLeod 2022) and Sen's slope estimator (trend package, Pohlert 2023), revealing no strong evidence of temporal autocorrelation. To standardize the scales of the predictors and address potential convergence issues, all independent variables were transformed to z-scores (mean = 0, standard deviation = 1).

Model selection was performed using the Akaike Information Criterion corrected for small sample sizes (AICc), with models ranked and compared using the AICcmodavg package (Mazerolle 2023). Models with $\Delta AICc < 2$ were retained, and ANOVAs were conducted on these selected models to assess the significance of fixed effects using the ANOVA function from the car package (Fox and Weisberg 2018). Model validation included residual analysis and quantile-quantile plots (qq-plots) with the DHARMa package (Hartig 2022), complemented by tests for uniformity (Kolmogorov-Smirnov test), dispersion, and outlier detection to ensure

model fit. Multicollinearity was assessed by calculating the Variance Inflation Factor (VIF) for key predictors, ensuring VIF values remained below 10 using the performance package (Lüdecke et al. 2021).

Macroinvertebrate Assemblage. Diversity was estimated using the effective number of taxa, expressed as diversity order q (^{q}D, Jost 2006). This approach calculates diversity based on the relative abundances of taxa, where q-values reflect different sensitivity levels to taxa rarity or abundance. For example, when $q \approx 1$ diversity represents the effective number of equally common genera, aligning with the exponential form of Shannon entropy and providing an unbiased measure across rare and abundant taxa. When q=2, diversity reflects the effective number of more abundant taxa, corresponding to the inverse of the Simpson entropy index (Moreno et al. 2011).

Since density was used as the abundance measure, sample coverage estimation (see Chao and Jost 2012) per stream was not necessary prior to diversity comparisons. Observed values of ^{q}D were directly compared, given a completeness level of 99% (absence of singletons). The CI 95% (confidence intervals) were calculated for each diversity expression (^{1}D, ^{2}D), with non-overlapping CIs indicating significant differences (Cumming et al. 2007, Chao et al. 2020). Calculations of $^{q}D \pm$ 95% CI were performed using the iNEXT package in R (Hsieh et al. 2016). By standardizing diversity estimates under the same and maximum sample coverage (99%), we adhered to the replication principle, allowing us to quantify the diversity ratio (MD = sampling stream 2 / sampling stream 1) and determine how many times one stream is more or less diverse than another (Jost 2006, Moreno et al. 2011). The diversity was calculated by combining both sampling events to provide a comprehensive measure of the total diversity for each stream. This

approach is consistent with the use of sample coverage to estimate diversity accurately, as combining data from multiple sampling events reduces the impact of singletons and rare taxa that may appear sporadically across individual samples. By consolidating the two sampling events, we aimed to represent the cumulative diversity of each stream along the phosphorus gradient.

Macroinvertebrate assemblages were analyzed to assess differences in community composition along the phosphorus gradient. A Permutational Multivariate Analysis of Variance (PERMANOVA) with 9,999 permutations, based on Bray-Curtis dissimilarity, was used to test for significant differences among streams. To identify the specific taxa contributing most to the dissimilarity between streams, we conducted a Similarity Percentage Analysis (SIMPER), which calculates the average contribution of each taxon to the overall Bray-Curtis dissimilarity, highlighting the primary drivers of community composition differences. Both PERMANOVA and SIMPER analyses were performed with the vegan package, while figures were generated with the ggplot2 package. Analyses were conducted separately for each substrate (rocks and leaves).

Macromolecules. We used Generalized Linear Models (GLMs) to assess the effects of the phosphorus gradient on the content of proteins, lipids, and carbohydrates in insects. Depending on the characteristics of each response variable, we applied Gamma, Gaussian, and inverse Gaussian distributions, and the stream was considered a fixed effect. Following the approach used in previous GLMM analyses, model selection was based on the Akaike Information Criterion corrected for small sample sizes (AICc) using the AICcmodavg package. Models with $\Delta AICc < 2$ were retained, and ANOVAs were conducted on these selected models to assess the significance of fixed effects using the Anova function from the car package. Gamma and

Gaussian distributions were fitted using the glmmTMB package, while inverse Gaussian models were fitted with the stats package.

4.4 Results

4.4.1 Environmental Variables

We observed that conductivity followed the expected phosphorus gradient, with the highest values recorded in the phosphorus-rich stream, Arboleda (290.0 µS/cm and 328.0 µS/cm), and the lowest values in the phosphorus-poor stream, Taconazo (22.8 µS/cm and 18.3 µS/cm). In contrast, water temperature varied little across the streams, ranging from 23.8°C to 25.4°C (Table 4.1). The phosphorus-rich stream and those in the middle of the gradient, Saltito-60 and Pantano, displayed similar pH values, between 5.81 and 6.92. The phosphorus-poor stream had the lowest pH values, 5.64 and 4.81, a difference of more than 0.8 units between the two sampling dates.

Table 4.1 Physicochemical parameters in our four studied streams. SRP concentrations (*, +/- standard deviation) are monthly averages from 2020 to 2022. All other variables were measured in August 2023 and February 2024.

Stream	Sampling dates	*SRP (ug/L)	Conductivity (µS/cm)	Temperature (°C)	pH	Discharge (L/s)
Arboleda	Aug-23	152.19 (±0.21)	290.00	25.00	6.14	187.20
	Feb-24		328.00	25.40	5.81	171.30
Saltito-60	Aug-23	46.68 (±0.63)	131.80	24.90	6.18	267.20
	Feb-24		171.50	24.60	5.93	292.10
Pantano	Aug-23	6.57 (±0.11)	39.60	24.90	6.92	18.50

Table 4.1 (Continued)

	Feb-24		111.90	23.80	6.21	16.90
Taconazo	Aug-23	6.35 (±0.02)	22.80	25.20	5.64	37.70
	Feb-24		18.30	24.70	4.81	28.90

4.4.2 Macroinvertebrates Assemblages

There was variability among sampling months for several response variables across streams, though not all streams showed significant differences for every variable (Appendix D). This variability was accounted for by including sampling month as a random effect in subsequent models. Model selection criteria indicated that conductivity (our indicator of stream P) was included in 60% of the top models explaining macroinvertebrate assemblage patterns across the streams (Appendix E). In these models, conductivity was negatively related with density in both leaves and rocks substrates (leaves: χ^2=6.424, p-value=0.011; rocks: χ^2=15.641, p-value<0.001). The phosphorus-poor stream, Taconazo, had a higher density than the phosphorus-rich stream, Arboleda, in both substrates (Figure 4.2). No variable had a significant effect on biomass and richness (Table 4.2, 4.3).

Diversity followed the phosphorus gradient, with the phosphorus-rich stream being the most diverse (^{q1}D and ^{q2}D), followed by the streams in the middle of the gradient, and finally, the phosphorus-poor stream. According to the 95% confidence intervals, the phosphorus-rich stream was 1.5 times (rocks) and 1.8 times (leaves) more diverse than phosphorus-poor stream in terms of the effective number of equally common taxa (^{1}D) and the effective number of the most abundant taxa (^{2}D). The streams in the middle of the gradient (Saltito-60 and Pantano) did not show significant differences from each other. However, these two streams were significantly

different from the extremes of the gradient in the leaf substrate, although this pattern was not observed in the rock substrate (Figure 4.3).

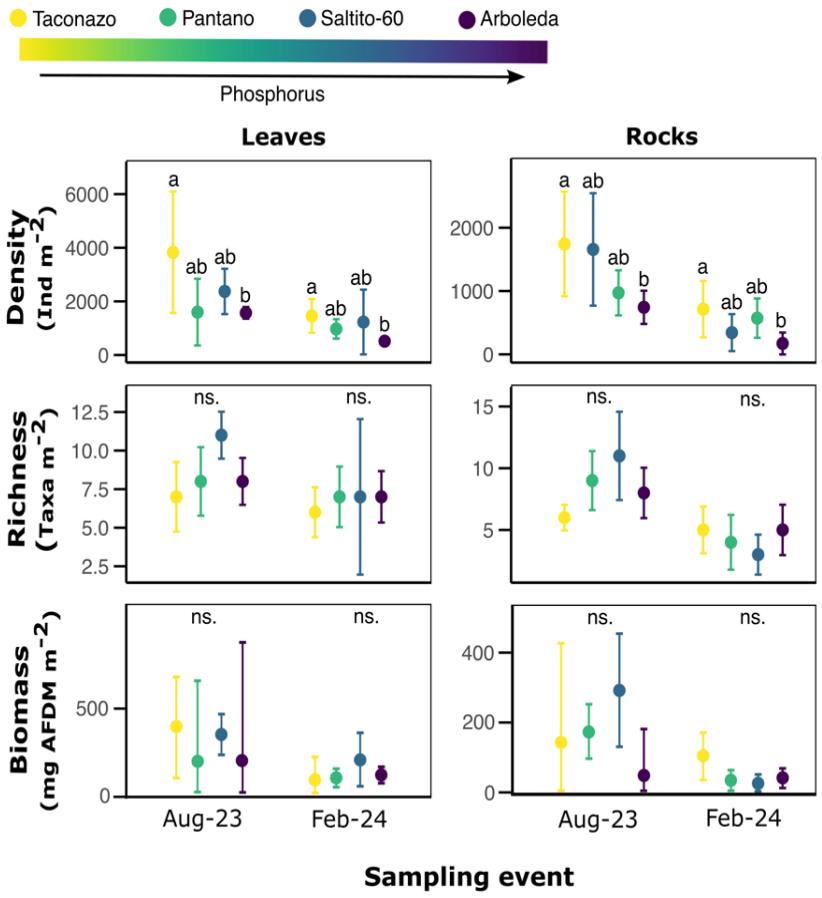

Figure 4.2 Density (Ind m^{-2}), richness (taxa m^{-2}), and biomass (mg AFDM m^{-2}) of macroinvertebrates on leaf and rock substrates during the wet and dry seasons. Error bars represent standard errors of the mean. The colors represent streams along the phosphorus gradient, increasing from left to right.

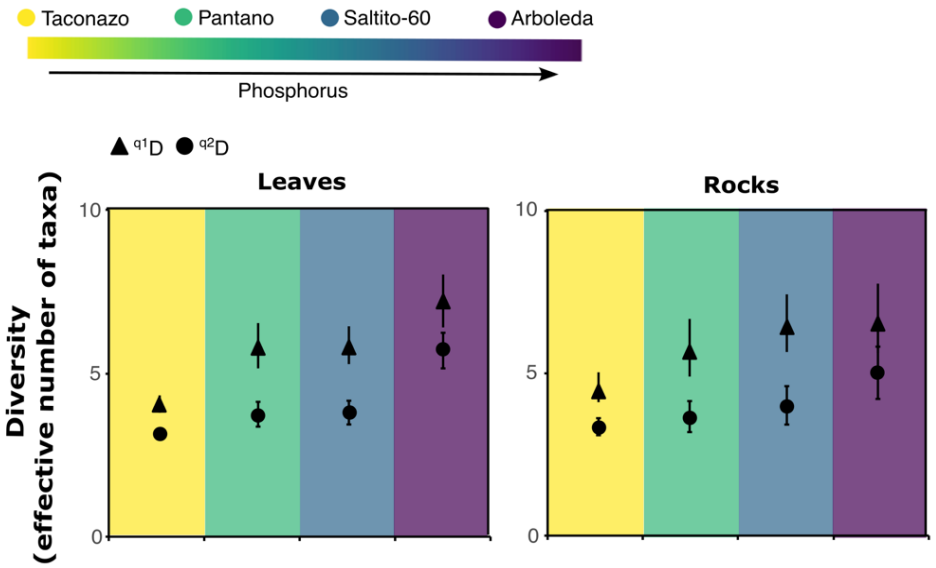

Figure 4.3 Macroinvertebrate diversity ^{q1}D and ^{q2}D on leaves and rocks substrates. Error bars represent 95% confidence intervals.

Table 4.2 Generalized Linear Mixed Model (GLMM) results for macroinvertebrate density, biomass and richness (rocks) in streams along the phosphorus gradient. These models were selected from the top-ranked models in the AICc selection process (ΔAICc < 2). Degrees of freedom (df) are shown for the Chi² tests. Significant p-values from ANOVA ($p < 0.05$) indicate predictors with a significant effect on the response variables across different substrates. Conductivity (as an indicator of stream phosphorus) and pH were included as fixed effects, while sampling event and discharge were included as random effects in the models.

Response Variable	Substrate	Model predictor	Estimate	Std. Error	z value	p value	Chi²	df	p value ANOVA

Table 4.2 (Continued)

Abundance	Leaves	Intercept	3.98	0.23	17.28	<0.001			
		Conductivity	-0.29	0.11	-2.53	0.01	6.42	1	0.01
	Rocks	Intercept	3.26	0.36	9.10	<0.001			
		Conductivity	-0.37	0.09	-3.96	0.00	15.64	1	<0.001
Biomass	Leaves	Intercept	5.12	0.29	17.51	<0.001			
		Conductivity	0.05	0.13	0.40	0.69	0.16	1	0.69
		Intercept	5.12	0.29	17.50	<0.001			
		pH	-0.02	0.15	-0.12	0.90	0.02	1	0.90
	Rocks	Intercept	4.27	0.56	7.69	0.00			
		pH	-0.14	0.18	-0.80	0.42	0.64	1	0.42
		Intercept	4.27	0.50	8.62	<0.001			
		Conductivity	-0.11	0.16	-0.70	0.48	0.50	1	0.48
Richness	Rocks	Intercept	1.77	0.22	8.03	0.00			
		Conductivity	-0.05	0.08	-0.62	0.54	0.38	1	0.54
		Intercept	1.78	0.22	8.06	0.00			
		pH	0.02	0.10	0.18	0.86	0.03	1	0.86

Table 4.3 Linear Mixed Model (LMM) results for macroinvertebrate richness (leaves) along the phosphorus gradient. This model was selected based on AICc (ΔAICc < 2). Degrees of freedom (df) are shown for the *t* tests. Significant *p*-values from ANOVA ($p < 0.05$) indicate predictors with a significant effect on richness. Conductivity and pH were included as fixed effects, while sampling event and discharge were included as random effects in the models.

Response Variable	**Substrates**	**Predictor**	**Estimate**	**Std. Error**	**t value**	**Chi²**	**df**	**p value ANOVA**
Richness	Leaves	Intercept	7.68	0.56	13.79			
		pH	0.51	0.43	1.19	1.40	1	0.24

The composition and structure of macroinvertebrate assemblages differed significantly between streams, with *Ulmeritoides* and *Caenis* (Ephemeroptera), Tanypodinae, Chironominae, and Ceratopogonidae (Diptera), and *Macronema* (Trichoptera) being the primary taxa contributing to these differences. These differences were statistically supported in terms of both density (PERMANOVA, df = 3, F = 5.3879, p-value < 0.01 for leaves; F = 5.2655, p-value < 0.001 for rocks) and biomass (PERMANOVA, df = 3, F = 4.3197, p-value < 0.001 for leaves; F = 2.9284, p-value < 0.001 for rocks). The taxa contributing most to the differences between streams were *Ulmeritoides* (Leptophlebiidae, Ephemeroptera), which contributed between 9% and 47%, and *Caenis* (Caenidae, Ephemeroptera), with a contribution of 10% to 62%. Among Diptera, Tanypodinae (Chironomidae) contributed 31% to 36%, Chironominae (Chironomidae) contributed 32% to 58%, and Ceratopogonidae contributed 9% to 20%. *Macronema* (Hydrosychidae, Trichoptera) also contributed to biomass differences, ranging from 5% to 21% (Figure 5). The greatest number of taxa contributing to these differences was observed between the phosphorus-rich stream and the phosphorus-poor stream. In contrast, fewer families contributed to the differences between streams at the extremes of the phosphorus gradient and those in the middle of the gradient (Figure 4.4).

Ephemeroptera and Diptera exhibited contrasting responses to the phosphorus gradient, with Ephemeroptera thriving in the phosphorus-rich stream and Diptera in the phosphorus-poor stream. Ephemeroptera density was highest in the phosphorus-rich stream, and their biomass followed the phosphorus gradient, showing the highest values in the phosphorus-rich stream (4342.9 ind m^2, 702.1 mg AFDM m^2) and the lowest values in the phosphorus-poor stream (742.9 ind m^2, 126.7 mg AFDM m^2). Streams in the middle of the gradient, Saltito-60 (4485.7 ind m^2, 527.3 mg AFDM m^2) and Pantano (1742.9 ind m^2, 194.7 mg AFDM m^2), had

intermediate values. In contrast, Diptera density and biomass were highest in the phosphorus-poor stream (22971.4 ind m², 1604.0 mg AFDM m²) and lowest in the phosphorus-rich stream (5428.6 ind m², 413.7 mg AFDM m²), with intermediate values in Pantano (10857.1 ind m², 720.0 mg AFDM m²) and Saltito-60 (13771.4 ind m², 1395.4 mg AFDM m²)

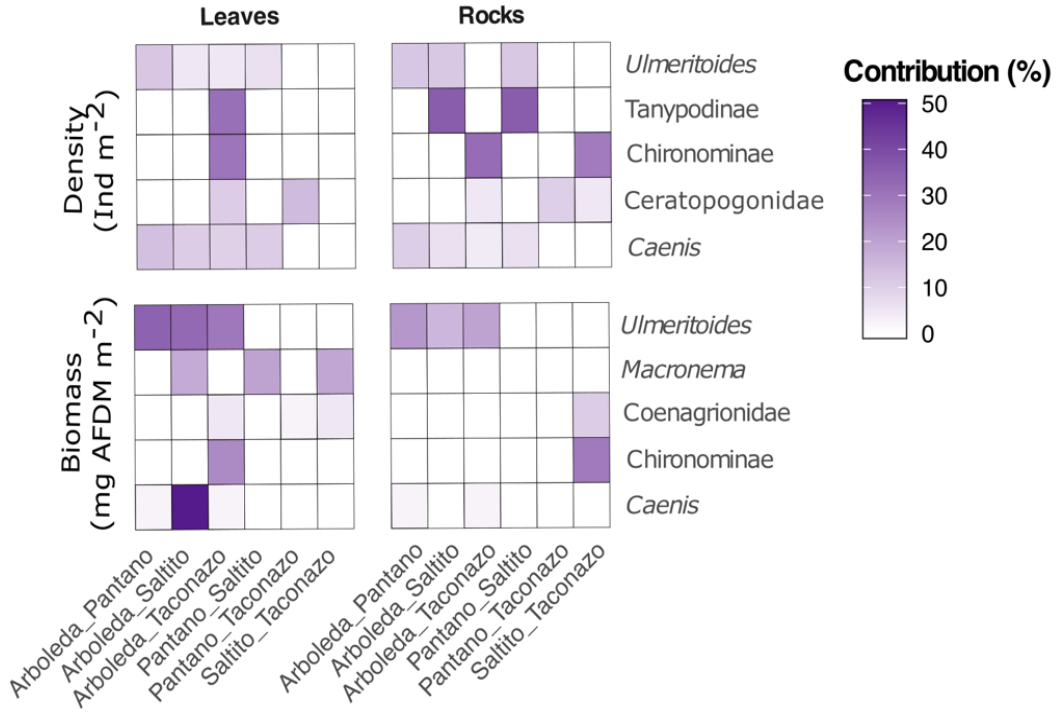

Figure 4.4 Taxa with the greatest contribution to differences among streams (SIMPER analysis). Only taxa contributing more than 10% to pairwise differences between streams are shown.

Collectors and predators were the most abundant functional feeding groups across streams, with densities ranging from 20% to 54% for collectors and 38% to 70% for predators, while filters (0–28%) and shredders (0–15%) showed lower densities (Figure 4.5). In contrast, filter biomass was higher in the phosphorus-rich stream and the stream just below it on the

gradient, Arboleda and Saltito-60, where it contributed up to 69% and 73% of the total biomass, respectively, primarily due to the dominance of Hydropsychidae (Trichoptera). Shredder biomass was significantly higher in one of the mid-gradient streams, Pantano, where it contributed between 0% and 89% of the total biomass, with *Nectopsyche* (Leptoceridae, Trichoptera) being the main contributor.

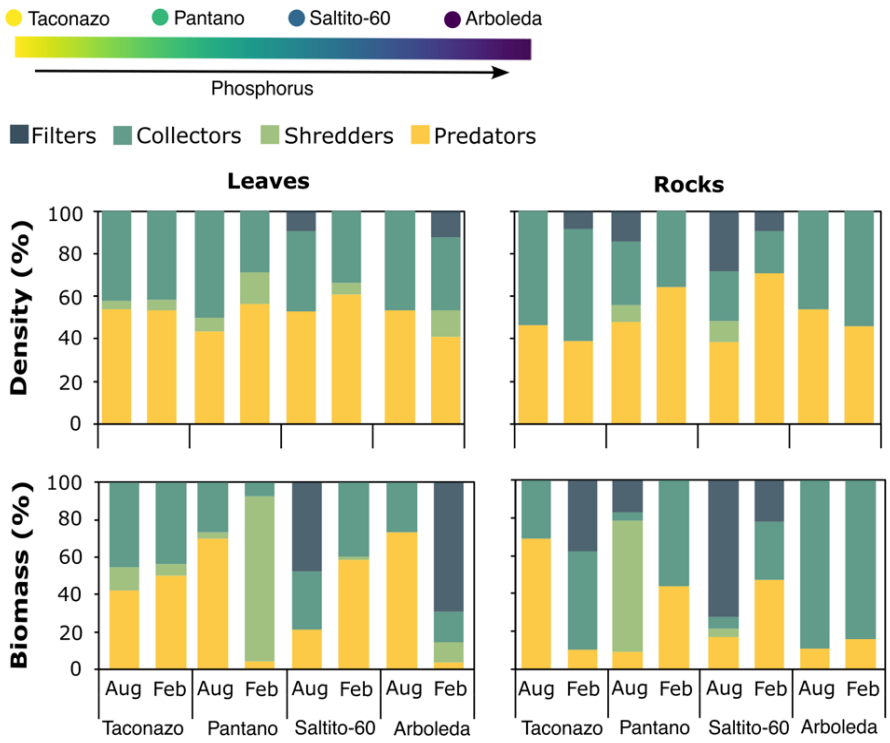

Figure 4.5 Percentage of macroinvertebrate density and biomass for functional feeding groups in leaves and rocks among streams.

Given their high abundance and consistent presence across all streams, our analysis of the effects of physicochemical variables focused on the density and biomass of collectors and predators. Conductivity, present in 75% of the top models (Appendix D), had a significant

negative effect on the density of collectors on rocks (χ^2=6.563, p=0.010) and on the density of predators in both substrates (leaves: χ^2=7.258, p=0.007; rocks: χ^2=7.547, p=0.006), as well as on the biomass of predators on rocks (χ^2=5.799, p=0.016). The highest values of density and biomass were observed in the phosphorus-poor stream, while no significant effects of either conductivity or pH were detected on the density on leaves, biomass of collectors across streams, or biomass of predators on leaves (Figure 4.6, Table 4.4).

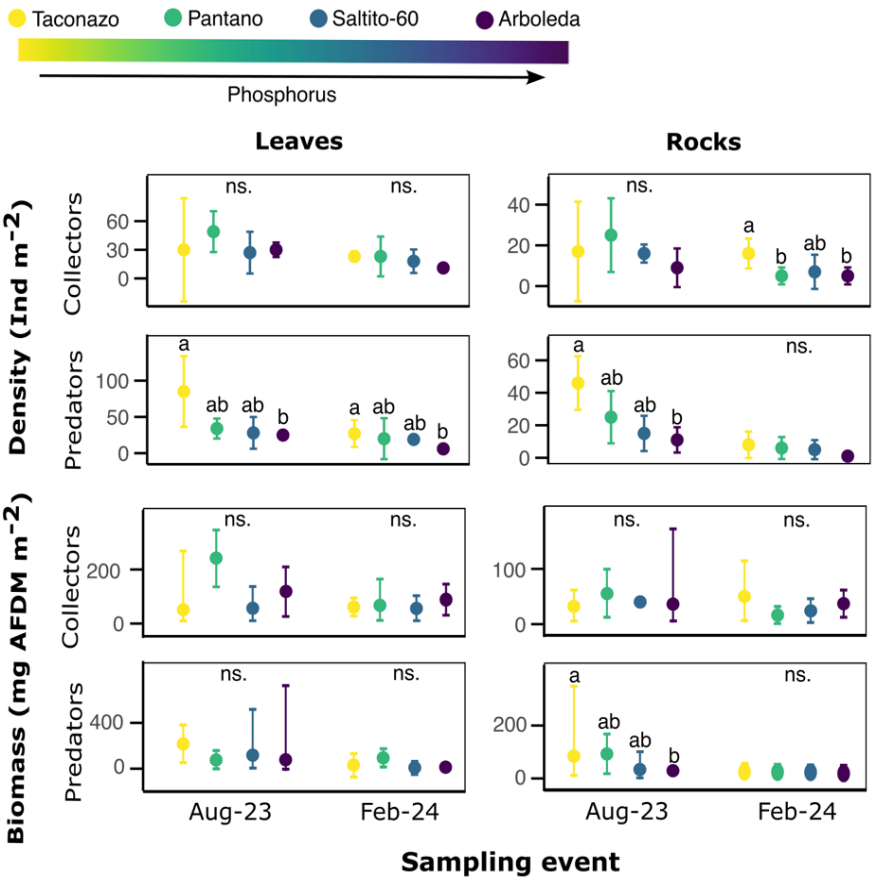

Figure 4.6 Density (Ind m^{-2}) and biomass (mg AFDM m^{-2}) of collectors and predators across sampling dates in rocks and leaves substrates in streams along the phosphorus gradient. Error

bars represent the standard error of the median. The colors represent streams along the phosphorus gradient, increasing from left to right.

Table 4.4 Generalized Linear Mixed Model (GLMM) results for the density and biomass of collectors and predators in streams along the phosphorus gradient. Models were selected from the top-ranked models in the AICc selection process (ΔAICc < 2). Degrees of freedom (df) are shown for the Chi² tests. Significant ANOVA *p*-values ($p < 0.05$) indicate predictors with a significant effect on response variables across substrates. Conductivity (as an indicator of stream phosphorus) and pH were included as fixed effects, while sampling event and discharge were included as random effects.

Response Variable	Substrates	Model predictor	Estimate	Std. Error	z value	p value	Chi²	df	p value ANOVA
Collectors' density	Leaves	Intercept	3.26	0.25	13.16	<2e-16			
		Conductivity	-0.19	0.1	-1.91	0.06	3.65	1	0.06
	Rocks	Intercept	2.54	0.27	9.56	<2e-16			
		Conductivity	-0.28	0.11	-2.56	0.01	6.56	1	0.01
Predators' density	Leaves	Intercept	3.28	0.21	15.7	<2e-16			
		Conductivity	-0.39	0.15	-2.69	0.01	7.26	1	0.01
	Rocks	Intercept	2.51	0.42	5.95	0			
		Conductivity	-0.42	0.15	-2.75	0.01	7.55	1	0.01
Collectors' biomass	Leaves	Intercept	4.67	0.27	17.15	<2e-16			
		Conductivity	0.09	0.12	0.79	0.43	0.62	1	0.43
		Intercept	4.68	0.28	16.74	<2e-16			
		pH	-0.06	0.14	-0.44	0.66	0.19	1	0.66
	Rocks	Intercept	3.81	0.18	21.09	<2e-16			
		Conductivity	0.15	0.17	0.86	0.39	0.73	1	0.39
		Intercept	3.81	0.19	20.19	<2e-16			
		pH	-0.09	0.18	-0.48	0.63	0.23	1	0.63
Predators' biomass	Leaves	Intercept	4.49	0.56	8.04	0			

Table 4.4 (Continued)

	Conductivity	-0.22	0.27	-0.81	0.42	0.65	1	0.42
	Intercept	4.5	0.59	7.63	0			
	pH	-0.04	0.31	-0.13	0.9	0.02	1	0.9
Rocks	Intercept	2.78	0.61	4.58	0			
	Conductivity	-0.58	0.24	-2.41	0.02	5.8	1	0.02

4.4.3 Macromolecules in Aquatic Insects

In terms of macromolecule content across taxa, the collector *Ulmeritoides* (Ephemeroptera) had lower levels of proteins, lipids, and carbohydrates compared to the other taxa (the shredder *Phylloicus* (Trichoptera) and the predators *Anacroneuria* (Plecoptera) and *Phyllogomphoides* (Odonata). The shredder *Phylloicus* displayed the highest protein levels compared to *Anacroneuria* and *Ulmeritoides* (χ^2=49.151, p<0.001). For lipid content, *Ulmeritoides* had lower values than the predator *Anacroneuria* (χ^2=23.352, p<0.001), though no significant differences were found when compared with *Phylloicus* and the predator *Phyllogomphoides*. *Anacroneuria* and *Ulmeritoides* had the lowest carbohydrate values, which were significantly lower than those of *Phylloicus* and *Phyllogomphoides* (χ^2=74.361, p<0.001). Overall, *Phyllogomphoides* exhibited the greatest variability in macromolecule content among the taxa studied (Figure 4.7).

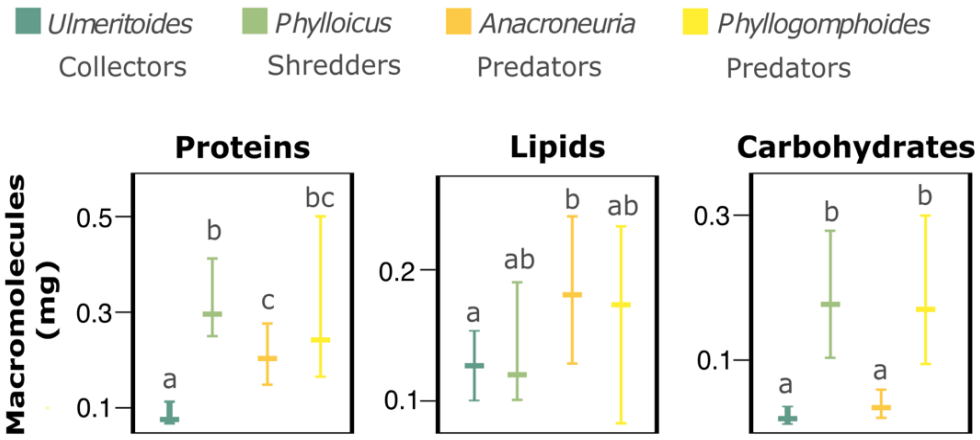

Figure 4.7 Protein (mg), lipid (mg), and carbohydrate (mg) content in aquatic insects in streams in the phosphorus gradient. The taxa represented include *Ulmeritoides* (collector, with males and females), *Phylloicus* (shredder), *Anacroneuria* (predator), and *Phyllogomphoides* (predator). Error bars represent the standard error of the median. The colors represent different taxa and functional groups.

The collector *Ulmeritoides* exhibited greater variability in protein, lipid, and carbohydrate concentrations in the phosphorus-poor stream compared to the phosphorus-rich stream, with no significant effect of the phosphorus gradient across streams (Table 4.5). The shredder *Phylloicus* showed a significant effect of the phosphorus gradient on protein levels, with higher values in the phosphorus-poor stream than in Pantano, a mid-gradient stream (χ^2=9.526, p<0.001), though this effect was not significant compared to the phosphorus-rich stream. In contrast, the predator *Anacroneuria* showed a significant effect of the phosphorus gradient on protein and lipid content, with higher levels in the phosphorus-rich stream compared to the phosphorus-poor stream (Protein: χ^2=6.648, p=0.006; Lipids: χ^2=7.178, p=0.003). *Anacroneuria* was also present

in Pantano, but macromolecule concentrations there did not differ significantly from the streams at the extremes of the phosphorus gradient. For *Phyllogomphoides*, macromolecule content varied widely in Saltito-60, with no clear pattern of differences across the phosphorus gradient (Figure 4.8).

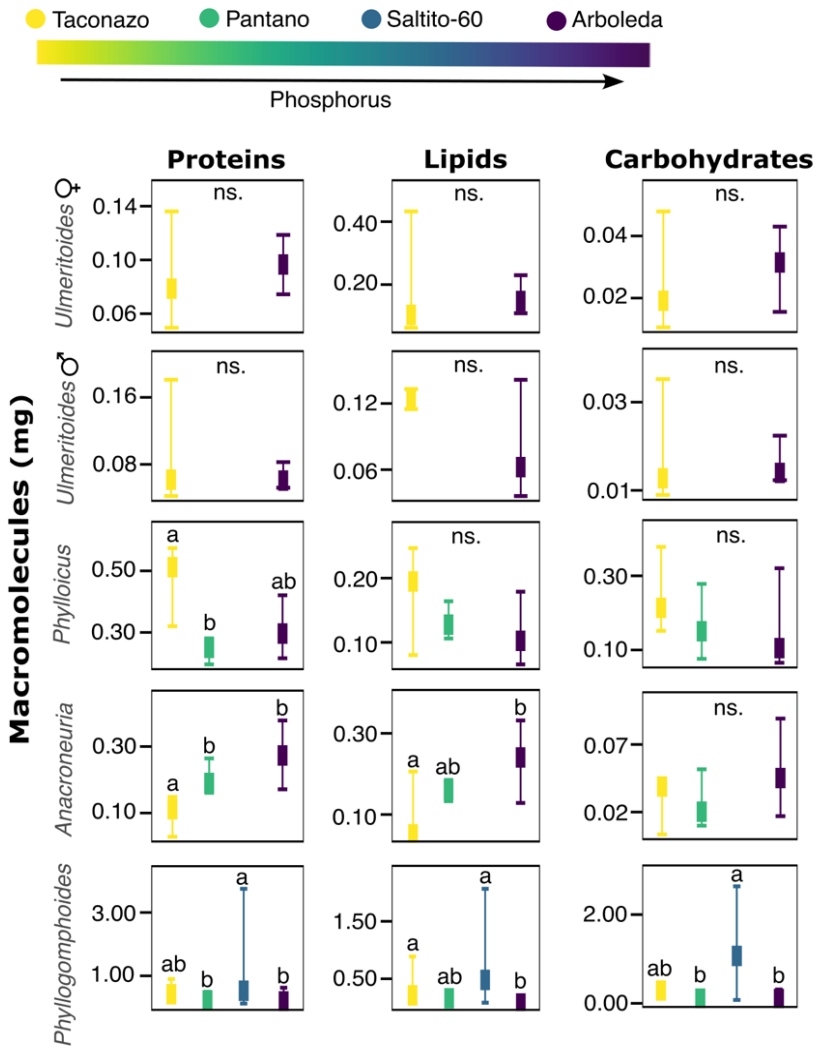

Figure 4.8 Protein (mg), lipid (mg), and carbohydrate (mg) content in aquatic insects in our four studied streams along the phosphorus gradient. The taxa represented include *Ulmeritoides* (collector, with males and females), *Phylloicus* (shredder), *Anacroneuria* (predator), and

Phyllogomphoides (predator). Error bars represent the standard error of the median. The colors represent streams along the phosphorus gradient, increasing from left to right.

Table 4.5 Generalized Linear Model (GLM) results for proteins (mg), lipids (mg) and carbohydrates (mg) in streams along the phosphorus gradient. These models were selected from the top-ranked models in the AICc selection process (ΔAIC < 2). Degrees of freedom (df) are shown for the Chi² tests. Significant p-values from ANOVA ($p < 0.05$) highlight the predictor with a significant effect on the response variables.

Taxa	Response Variable	Sex	Model predictor	Chi²	df	p-value (ANOVA)
Ulmeritoides	Proteins	Male	Stream	0.962	1	>0.001
		Female	Stream	0.331	1	>0.001
	Lipids	Male	Stream	3.104	1	>0.001
		Female	Stream	0.007	1	>0.001
	Carbohydrates	Male	Stream	0.229	1	>0.001
		Female	Stream	0.219	1	>0.001
		Male*Female Phosphorus-rich stream				
	Proteins		Sex	4.490	1	0.034
	Lipids		Sex	5.076	1	0.024
	Carbohydrates		Sex	3.564	1	0.059
		Male*Female Phosphorus poor stream				
	Proteins		Sex	0.0350	1	0.852
	Lipids		Sex	0.403	1	0.525
	Carbohydrates		Sex	0.379	1	0.538
Phylloicus	Proteins	NA	Stream	9.526	2	>0.001
	Lipids		Stream	1.108	2	>0.001
	Carbohydrates		Stream	1.487	2	0.475
Anacroneuria	Proteins	NA	Stream	6.648	2	0.036
	Lipids		Stream	7.178	2	>0.001
	Carbohydrates		Stream	3.253	2	>0.001

Table 4.5 (Continued)

Phyllogomphoides	Proteins	NA	Stream	20.695	3	>0.001
	Lipids		Stream	22.287	3	>0.001
	Carbohydrates		Stream	21.463	3	>0.001
All taxa	Proteins	NA	Taxa	49.151	3	>0.001
	Lipids		Taxa	23.352	3	>0.001
	Carbohydrates		Taxa	74.361	3	>0.001

In terms of sexual dimorphism within *Ulmeritoides*, we found a significant effect of sex in the phosphorus-rich stream, where females had higher protein and lipid content than males (Proteins: χ^2=4.490, p=0.034; Lipids: χ^2=5.076, p=0.024). In contrast, there was no significant effect of sex in the phosphorus-poor stream; however, females showed greater variability in lipid and carbohydrate content, while males exhibited higher variability in protein levels (Figure 4.9).

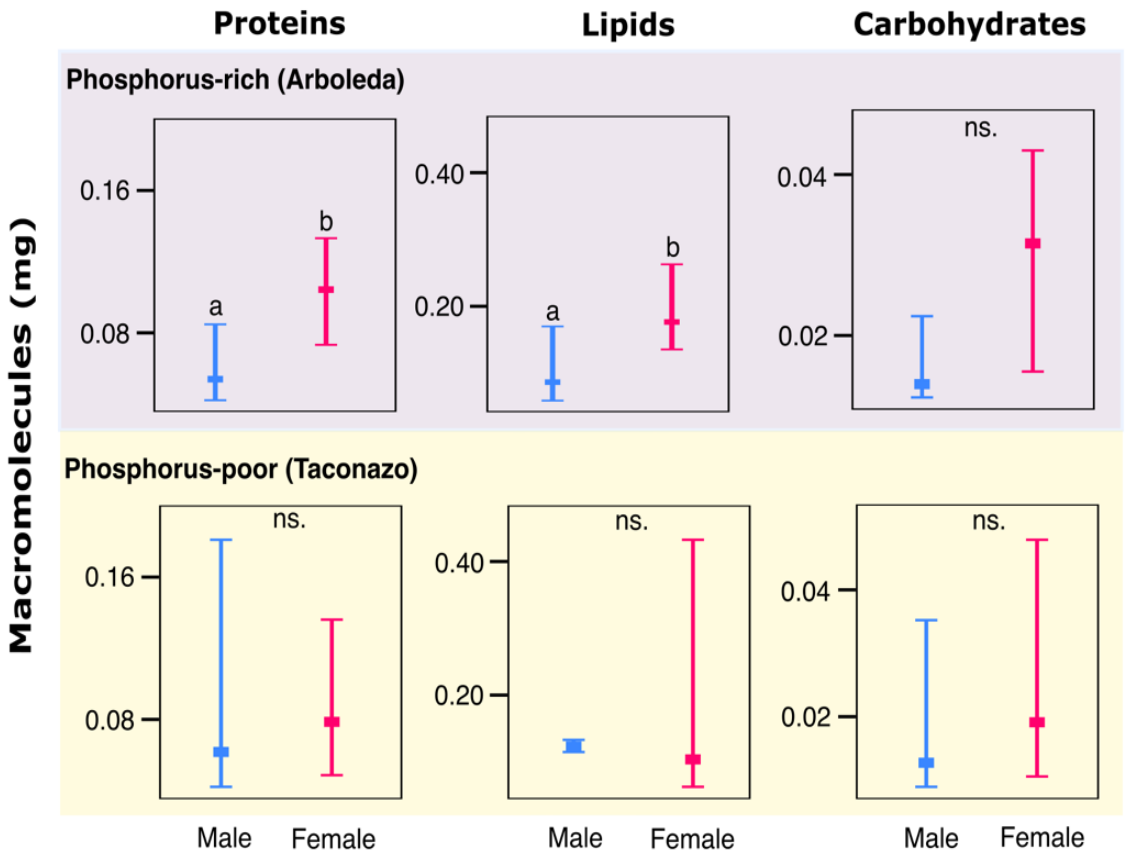

Figure 4.9 Protein (mg), lipid (mg), and carbohydrate (mg) content in the collector *Ulmeritoides* (Ephemeroptera) females and males in the phosphorus-rich stream (Arboleda, upper panel) and the phosphorus-poor stream (Taconazo, lower panel). Error bars represent the standard error of the median.

4.5 Discussion

Our findings underscore the complex and multifaceted responses of macroinvertebrates to nutrient concentrations in tropical stream ecosystems. The phosphorus gradient emerged as a key driver, influencing community composition, functional group distribution, and the body

condition of individual taxa. While diversity and density exhibited contrasting patterns along the gradient, individual taxa showed distinct variations in macromolecule content, reflecting unique adaptations to nutrient availability. The observed patterns demonstrate how phosphorus concentrations affect multiple levels of ecological organization, from community structure and functional roles to the biochemical condition of organisms. This comprehensive perspective on phosphorus inputs reveals their impact on tropical stream ecosystems, highlighting the significance of natural nutrient gradients in shaping biodiversity and ecosystem processes.

The phosphorus gradient influenced macroinvertebrate assemblages, creating contrasting patterns in density and diversity between streams. Phosphorus-poor streams were dominated by a few taxa, particularly Chironomidae, which comprised up to 76% of the assemblage. In contrast, the phosphorus-rich stream exhibited the highest diversity (^{q1}D and ^{q2}D), likely due to a more even distribution among dominant taxa. While Chironomidae remained dominant in the phosphorus-rich stream (37%), other taxa, such as Caenidae and Leptophlebiidae (21% each), contributed to a more balanced assemblage structure, resulting in higher overall diversity compared to the phosphorus-poor stream. Chironomids in phosphorus-poor environments may have evolved adaptations to manage nutrient scarcity, including reduced phosphorus demand and strategies to balance elemental intake (Small et al. 2011). These adaptations likely involve selective feeding on nutrient-rich leaf patches (Arsuffi and Suberkropp 1985, Demi et al. 2022), compensatory feeding, enhanced phosphorus assimilation (He and Wang 2007), and behaviors that regulate intake based on phosphorus concentration (Tordoff 2017, Demi et al. 2021). These strategies may enable chironomids to thrive and maintain essential roles in low-phosphorus streams, supporting ecosystem processes (Small et al. 2011).

Richness and biomass along the phosphorus gradient reflect shifts in dominant taxa, with overall values appearing similar among streams but differing in taxonomic composition and biomass distribution. Total taxa richness was comparable across streams; however, this similarity was driven by contrasting groups. In the phosphorus-rich stream, richness was characterized by a high number of Ephemeroptera taxa, whereas the phosphorus-poor stream exhibited greater richness within Diptera and Odonata. Biomass showed a similar pattern, while total biomass across streams was similar, the phosphorus-rich stream had a more even distribution across multiple taxa, unlike the phosphorus-poor stream, where biomass was concentrated mainly in Diptera.

Previous studies in La Selva have reported high macroinvertebrate biomass in phosphorus-rich streams (Rosemond et al. 2002). This discrepancy may be partly due to the leaf species used as substrates in each study. Rosemond et al. (2002) used *Ficus insipida* Willd. leaves, which decompose rapidly (Rosemond et al. 1998) and contain lower lignin and cellulose levels than the *Luehea seemannii* leaves used in our study (Ardón et al. 2006). Because high levels of lignin and cellulose in leaf litter can affect nutrient content changes during decomposition under high phosphorus conditions, litter quality likely mediates the effect of ambient phosphorus on microbial and insect processing (Ardón et al. 2006). Thus, the high quality of *F. insipida* may enhance macroinvertebrate density and biomass under elevated phosphorus, while *L. seemannii* with its low quality, may dampen this response. Our results suggest that the leaves in our collection boxes may have served primarily as substrates rather than as food for macroinvertebrates, supported by the similar assemblage patterns observed when we used rocks as substrate.

The influence of phosphorus is strongest at the gradient extremes, with some variations linked to temporal shifts. Streams with intermediate phosphorus levels had similar macroinvertebrate assemblages (density, diversity, richness, biomass, and composition), blending patterns and taxa from both extremes. Previous studies have shown that phosphorus enrichment can reduce invertebrate abundance in terrestrial and aquatic ecosystems (Nessel et al. 2021), cause ecological damage by decreasing macroinvertebrate genera (Struijs et al. 2011), alter community composition by increasing abundance but reducing ecosystem integrity (McCormick et al. 2004), and negatively correlate with richness and diversity (Dou et al. 2022). However, most research has focused on anthropogenic phosphorus inputs, often interacting with other stressors (Nessel et al. 2021), leaving the effects of natural phosphorus inputs largely unexplored. Additionally, patterns observed in ecological attributes, both in transitional zones and gradient extremes, may reflect temporal variability typical of stream ecosystems. These natural fluctuations, driven by various biotic and abiotic factors (Gutiérrez-Fonseca et al. 2018, Guo et al. 2019, Liu et al. 2021), tend to be more pronounced under extreme drying or wet flow conditions (Pearson 2014). However, our study did not specifically address seasonality.

The phosphorus gradient influenced functional feeding group density and biomass, with collectors and predators as the most abundant across streams, while shredders and filterers had high biomass. The high density of collectors underscores their essential role in nutrient cycling and energy flow in tropical streams (Masese et al. 2014), a trend observed across other tropical studies, where collectors dominate and shredders are scarce (Ardón and Pringle 2008, Masese et al. 2014, Akamagwuna et al. 2022). Shredders are typically less abundant in tropical than temperate streams, likely due to higher microbial activity and lower leaf palatability in tropical regions (Dobson et al. 2002, Li and Dudgeon 2009, Bruder et al. 2014), with taxa like decapods

sometimes filling the shredding role (Coughlan et al. 2010). Although filters and shredders contributed less to density, their substantial biomass highlights their importance in energy transfer and nutrient dynamics (Cheshire et al. 2005). The negative relation between phosphorus and collector and predator density may explain the dominance of Chironomidae (collectors) and certain predators, such as Tanypodinae and Ceratopogonidae, in the phosphorus-poor stream, where low phosphorus levels favor Diptera taxa. In contrast, larger predators in phosphorus-rich streams, like fish, may contribute to a more balanced community structure by limiting the density of collectors and smaller predators (Nieoczym et al. 2020, Pelinson et al. 2021).

The observed differences in macromolecule content among the studied taxa likely reflect their distinct nutritional requirements, feeding strategies, and physiological adaptations. Detritivores like *Ulmeritoides* and *Phylloicus* may have a limited capacity to benefit from excess phosphorus, especially in environments where phosphorus levels exceed the typical nutrient composition of detritus (Sperfeld et al. 2017, Raubenheimer and Simpson 2018). Adapted to low-phosphorus diets, these detritivores may excrete excess phosphorus rather than incorporate it into macromolecules (Anderson et al. 2004). In contrast, the predator *Anacroneuria* appears to benefit from additional phosphorus, incorporating it into proteins and lipids for storage and growth, consistent with stoichiometric theory, which predicts higher nutrient retention in organisms at higher trophic levels under enriched conditions (Boersma and Elser 2006). However, *Phyllogomphoides* did not follow this pattern, showing high variability in macromolecule content without a clear response to the phosphorus gradient. These differences may relate to specific life-history traits; *Anacroneuria* has a shorter life cycle and associates with leaf litter, whereas *Phyllogomphoides* has a longer life cycle and inhabits sediment-rich substrates (Novelo-Gutiérrez 1993, Jackson and Sweeney 1995). Therefore, responses to

phosphorus demand are likely taxon-specific and may be predicted by traits such as growth rate and life history (Boersma and Elser 2006).

In *Ulmeritoides*, the observed sexual dimorphism in macromolecule content, particularly higher protein and lipid levels in females from the phosphorus-rich stream, could be related to physiological adaptations linked to reproductive demands. Proteins and lipids are essential for oogenesis (Leyria et al. 2024, Adams and Tariq 2024). Proteins are particularly important in vitellogenesis, as they form the primary component of yolk and supply key nutrients for early embryo development (Adams and Tariq 2024). Lipids, on the other hand, provide energy reserves necessary for egg maturation and support offspring until they are self-sufficient (Leyria et al. 2024). Given that adult *Ulmeritoides* do not feed (Salles et al. 2018), females may capitalize on the phosphorus-rich aquatic environment to build and store proteins and lipids, optimizing their reproductive output. These reserves would be critical for egg formation and the initial stages of embryonic development (Beenakkers et al. 1988, Van Handel 1993, Leyria et al. 2024). This storage strategy may also explain why sexual dimorphism in macromolecule content was only evident in the high-P stream, where the nutrient-rich conditions may enable females to accumulate reserves essential for successful reproduction. In phosphorus-poor environments, such storage may be constrained by limited nutrient availability, aligning with stoichiometric predictions that reproductive strategies adjust to resource limitations (Boersma and Elser 2006, Small and Pringle 2010).

Our study focuses on natural phosphorus inputs in natural tropical streams, addressing a significant gap, as most research on nutrient enrichment emphasizes anthropogenic sources or experimental additions. By evaluating responses at both the assemblage and taxon levels, particularly in terms of body condition (i.e., macromolecular content), we highlight the value of

understanding differences among taxa, which may have implications for ecological roles and nutrient dynamics. Conducting research within these natural nutrient gradients in tropical streams provides a valuable baseline for assessing the impacts of future anthropogenic nutrient loading. Future experimental work could further clarify the specific roles of individual taxa or functional groups under varying nutrient conditions, deepening our understanding of phosphorus dynamics in tropical ecosystems.

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Chapter 5: Conclusions

This study provides evidence that climate change influences Caribbean stream ecosystems, with episodic acidification and phosphorus inputs emerging as key drivers of ecological change. These factors, which are closely tied to precipitation patterns and natural groundwater inputs, affect not only community structure but also reveal different response patterns among taxa, influencing biodiversity and ecosystem function in distinct ways. The findings underscore the need to pay closer attention to these processes as potential climate-driven stressors in tropical freshwater systems. By highlighting how acidification and nutrient dynamics shape macroinvertebrate assemblages, this work expands our understanding of ecological resilience and adaptability under natural stressors, offering insights that are relevant not only for tropical ecosystems but also for stream systems in general.

The literature review in Chapter 2 summarizes our current understanding of climate change impacts on Caribbean freshwater ecosystems, drawing attention to critical knowledge gaps and highlighting areas that require further exploration. This synthesis shows that climate-driven changes, including shifts in hydrology, extreme weather events, and other environmental stressors, can fundamentally reshape aquatic biodiversity and disrupt essential ecosystem functions. By focusing on the unique climatic and ecological characteristics of Caribbean streams, this review provides a framework for understanding how these ecosystems may respond to ongoing environmental changes. While research on tropical ecosystems, especially in the Caribbean, has advanced in recent years, there remain significant opportunities for further study, particularly through experiments and simulations that anticipate the effects of extreme events (e.g., droughts and hurricanes). This chapter lays the groundwork for targeted conservation

efforts, supporting a deeper understanding of the vulnerabilities and resilience of freshwater ecosystems in tropical regions.

The findings on episodic acidification in tropical streams (Chapter 3) underscore the ecological importance of naturally occurring pH fluctuations in shaping community dynamics and ecosystem functions. Acidification events linked to precipitation patterns reveal how periodic decreases in pH can disrupt macroinvertebrate assemblages, affecting biodiversity and altering community composition. This research highlights the unique vulnerabilities of unbuffered streams to acidification, with implications for taxa persistence and ecosystem stability in tropical freshwater systems. Understanding how these naturally acidified ecosystems respond to climate-driven changes provides valuable insights for managing streams under future environmental stress. The results suggest that prioritizing taxa and communities sensitive to pH fluctuations can strengthen adaptive management strategies aimed at preserving biodiversity and ecosystem function in the face of climate change.

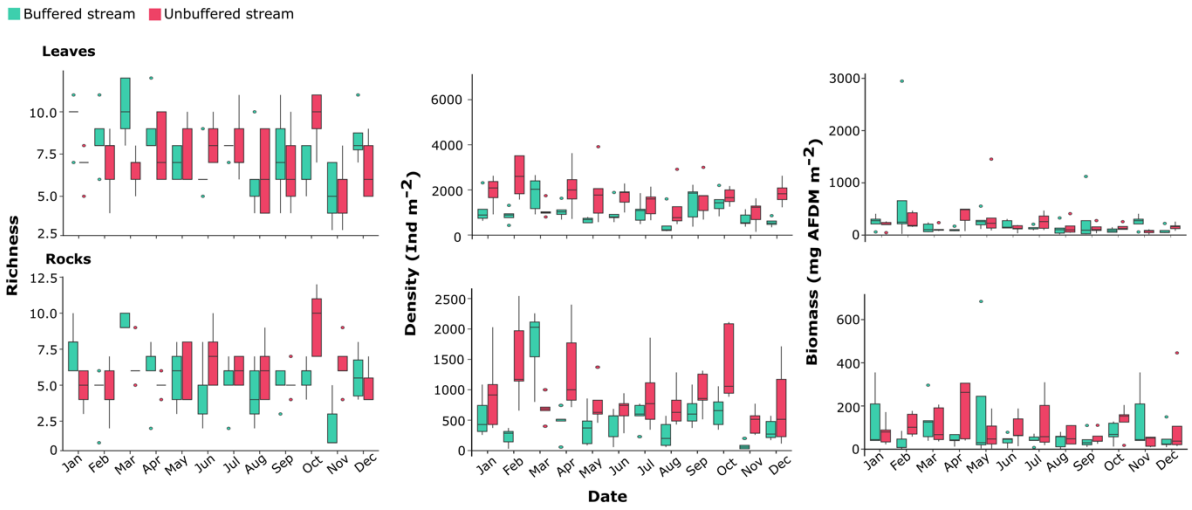
The findings on natural phosphorus gradients in tropical streams (Chapter 4) highlight the substantial role of nutrient availability in shaping macroinvertebrate assemblages and influencing individual body condition. Variations in phosphorus concentrations, driven by groundwater inputs, reveal distinct community structures and functional responses among macroinvertebrates, underscoring the importance of nutrient dynamics in maintaining biodiversity. These results emphasize that, even in natural, unimpacted ecosystems, nutrient availability can be a critical factor in determining community composition and potentially affect ecosystem function. From an applied perspective, understanding these natural nutrient gradients can guide conservation and management strategies that support ecosystem stability in the face of environmental changes. Prioritizing nutrient-sensitive communities and maintaining the natural nutrient balance in

tropical streams may enhance their resilience, offering a foundation for conservation actions that mitigate the impacts of climate change on tropical freshwater ecosystems.

Overall, this study serves as a valuable baseline for understanding how natural stressors associated with climate change, such as episodic acidification and phosphorus gradients, influence tropical freshwater ecosystems. By examining these effects in undisturbed Caribbean streams within volcanic landscapes, this research provides insights applicable to ecosystems impacted by human activities, where nutrient fluctuations and acidification may have even greater effects when interacting with climate change. The study highlights the importance of assessing both community assemblage and taxon-specific roles, as changes in assemblage structure and functional roles can alter key ecosystem processes, such as nutrient cycling and energy flow. Additionally, this work helps identify taxa most sensitive to environmental stressors, offering a foundation for future research, monitoring, and conservation efforts. Altogether, these findings contribute to our understanding of ecosystem responses to disturbance and nutrient dynamics, while also providing practical insights to support biodiversity conservation and ecosystem management under shifting environmental conditions.

APPENDICES

Appendix A



Richness (taxa m^{-2}), density (Ind m^{-2}), and biomass (mg AFDM m^{-2}) of aquatic macroinvertebrates during the sampling months for rocks and leaves substrate in buffered (green) and unbuffered (pink) streams. Error bars represent standard errors of the mean.

Appendix B

Akaike Information Criterion corrected (AICc) model selection results for the composition and structure of macroinvertebrate assemblages in buffered and unbuffered streams. Models were ranked according to ΔAICc (difference in AICc between the current model and the best model). K represents the number of parameters in each model, and the cumulative weight (Cum. weight) reflects the total AICc weight for the ranked models.

Stream	Response Variable	Habitat	Model Parameters	K	AICc	Delta AICc	AICc Weight	Cum. Wt	Log-Likelihood
Unbuffered (Taconazo)	Abundance	Leaves	pH_Min	4	581.06	0.00	0.55	0.55	-286.17
			pH_Min * Frequency	6	583.54	2.48	0.16	0.71	-284.98
			Frequency	4	584.42	3.35	0.10	0.82	-287.84
			pH_Min * Level_Av	6	584.84	3.77	0.08	0.90	-285.63
			Level_Av	4	584.90	3.84	0.08	0.98	-288.09
			Level_Av * Frequency	6	588.15	7.09	0.02	1.00	-287.28
			pH_Min * Level_Av * Frequency	10	593.40	12.34	0.00	1.00	-284.46
		Rocks	pH_Min	4	511.50	0.00	0.57	0.57	-251.39
			Frequency	4	514.65	3.15	0.12	0.69	-252.96
			pH_Min * Level_Av	6	514.78	3.27	0.11	0.80	-250.60
			pH_Min * Frequency	6	515.01	3.51	0.10	0.90	-250.71
			Level_Av	4	516.05	4.55	0.06	0.96	-253.66
			pH_Min * Level_Av * Frequency	10	518.19	6.69	0.02	0.98	-246.85

Appendix B (Continued)

			Level_Av * Frequency	6	518.57	7.07	0.02	1.00	-252.49
	Richness	Leaves	Level_Av	4	258.31	0.00	0.29	0.29	-124.79
			pH_Min * Level_Av * Frequency	10	259.01	0.69	0.21	0.50	-117.26
			Frequency	4	259.33	1.02	0.17	0.67	-125.30
			pH_Min	4	259.58	1.27	0.15	0.82	-125.43
			pH_Min * Frequency	6	260.22	1.91	0.11	0.94	-123.32
			Level_Av * Frequency	6	262.48	4.16	0.04	0.97	-124.45
			pH_Min * Level_Av	6	262.97	4.66	0.03	1.00	-124.69
		Rocks	pH_Min	4	48.10	0.00	0.35	0.35	-19.69
			pH_Min * Level_Av * Frequency	10	48.71	0.61	0.26	0.61	-12.11
			Level_Av	4	49.77	1.67	0.15	0.76	-20.52
			Frequency	4	50.00	1.90	0.14	0.90	-20.64
			pH_Min * Level_Av	6	52.27	4.17	0.04	0.94	-19.34
			pH_Min * Frequency	6	52.89	4.79	0.03	0.97	-19.65
			Level_Av * Frequency	6	53.29	5.19	0.03	1.00	-19.85
	Biomass	Leaves	pH_Min * Level_Av	6	138.93	0.00	0.35	0.35	-62.67
			Level_Av * Frequency	6	139.17	0.24	0.31	0.65	-62.79
			Level_Av	4	141.47	2.54	0.10	0.75	-66.37
			pH_Min * Level_Av * Frequency	10	141.59	2.66	0.09	0.84	-58.55
			pH_Min	4	141.93	3.00	0.08	0.92	-66.60

Appendix B (Continued)

			pH_Min * Frequency	6	142.75	3.82	0.05	0.97	-64.58
			Frequency	4	143.74	4.81	0.03	1.00	-67.51
		Rocks	pH_Min	4	172.83	0.00	0.55	0.55	-82.05
			Frequency	4	175.50	2.66	0.14	0.69	-83.39
			Level_Av	4	176.15	3.31	0.10	0.80	-83.71
			pH_Min * Frequency	6	176.18	3.35	0.10	0.90	-81.30
			pH_Min * Frequency	6	177.05	4.21	0.07	0.97	-81.73
			Level_Av * Frequency	6	178.87	6.03	0.03	0.99	-82.64
			pH_Min * Level_Av * Frequency	10	181.50	8.66	0.01	1.00	-78.50
Buffered (Arboleda)	Abundance	Leaves	pH_Min	4	520.91	0.00	0.67	0.67	-256.09
			Level_Av	4	522.72	1.82	0.27	0.94	-257.00
			pH_Min * Level_Av	6	525.57	4.66	0.06	1.00	-255.99
		Rocks	pH_Min	4	456.51	0.00	0.59	0.59	-223.89
			Level_Av	4	457.58	1.08	0.35	0.94	-224.43
			pH_Min * Level_Av	6	461.12	4.62	0.06	1.00	-223.77
	Richness	Leaves	pH_Min	4	260.58	0.00	0.70	0.70	-125.92
			Level_Av	4	263.25	2.67	0.18	0.88	-127.26
			pH_Min * Level_Av	6	264.11	3.53	0.12	1.00	-125.26
		Rocks	pH_Min	4	268.67	0.00	0.56	0.56	-129.97
			Level_Av	4	269.59	0.92	0.35	0.91	-130.43
			pH_Min * Level_Av	6	272.44	3.77	0.09	1.00	-129.43
	Biomass	Leaves	Level_Av	4	171.20	0.00	0.43	0.43	-81.24
			pH_Min	4	172.00	0.80	0.29	0.71	-81.64

Appendix B (Continued)

		pH_Min * Level_Av	6	172.01	0.81	0.29	1.00	-79.21
	Rocks	pH_Min	4	199.00	0.00	0.45	0.45	-95.14
		Level_Av	4	199.01	0.01	0.45	0.90	-95.14
		pH_Min * Level_Av	6	201.94	2.94	0.10	1.00	-94.18

Appendix C

Akaike Information Criterion corrected (AICc) model selection results for for the respiration and growth rates of microorganisms associated with *Ficus insipida* leaves and aquatic insects in controlled lab experiments. Models were ranked according to ΔAICc (difference in AICc between the current model and the best model). K represents the number of parameters in each model, and the cumulative weight (Cum. weight) reflects the total AICc weight for the ranked models.

Response Variable		Model Parameters	K	AICc	Delta AICc	AICc Weight	Cum. Weight	Log-Likelihood
Respiration rates	*Anacroneuria*	Treatment	4	40.4	0	0.53	0.53	-15.14
		Stream	3	40.8	0.4	0.44	0.97	-16.8
		Treatment *Stream	7	46.16	5.77	0.03	1	-12.58
	Phylloicus	Treatment	4	33.47	0	0.72	0.72	-11.56
		Stream	3	35.44	1.98	0.27	0.99	-14.05
		Treatment *Stream	7	41.29	7.82	0.01	1	-9.64
	Microorganisms	Treatment	4	-117.28	0	0.95	0.95	63.69
		Treatment *Stream	7	-111.19	6.09	0.05	1	66.1
		Stream	3	-94.3	22.98	0	1	50.75
Growth rates	*Anacroneuria*	Stream	3	-208.52	0	0.73	0.73	107.54
		Treatment	4	-206.48	2.04	0.26	0.99	107.72
		Treatment *Stream	7	-199.29	9.23	0.01	1	108.08

Appendix D

Results of statistical comparisons between sampling months for each response variable across different streams and substrates. The table shows the type of test used (t-test or Wilcoxon) and the corresponding p-values for each combination of stream, habitat, and response variable. Variables that met the assumption of normality were analyzed using a t-test, while variables that did not meet this assumption were analyzed using a Wilcoxon test. Significant p-values ($p < 0.05$) indicate significant differences between sampling months.

Stream	Substrate	Response variable	Test	p-value
Arboleda	Leaves	Density	t-test	<0.001
Arboleda	Leaves	Biomass	Wilcoxon	0.222
Arboleda	Leaves	Richness	t-test	0.085
Arboleda	Leaves	Collectors' density	t-test	0.098
Arboleda	Leaves	Predators' density	t-test	0.208
Arboleda	Leaves	Collectors' biomass	t-test	0.188
Arboleda	Leaves	Predators' biomass	Wilcoxon	0.032
Arboleda	Rocks	Density	t-test	0.002
Arboleda	Rocks	Biomass	Wilcoxon	0.310
Arboleda	Rocks	Richness	t-test	0.020
Arboleda	Rocks	Collectors' density	t-test	0.208
Arboleda	Rocks	Predators' density	t-test	0.002
Arboleda	Rocks	Collectors' biomass	Wilcoxon	1.000
Arboleda	Rocks	Predators' biomass	t-test	0.041
Pantano	Leaves	Density	Wilcoxon	0.421
Pantano	Leaves	Biomass	Wilcoxon	0.548
Pantano	Leaves	Richness	t-test	0.294
Pantano	Leaves	Collectors' density	t-test	0.698
Pantano	Leaves	Predators' density	Wilcoxon	0.101
Pantano	Leaves	Collectors' biomass	t-test	0.337
Pantano	Leaves	Predators' biomass	Wilcoxon	0.548
Pantano	Rocks	Density	t-test	0.008
Pantano	Rocks	Biomass	t-test	0.008
Pantano	Rocks	Richness	t-test	0.004

Appendix D (Continued)

Pantano	Rocks	Collectors' density	t-test	0.105
Pantano	Rocks	Predators' density	Wilcoxon	0.101
Pantano	Rocks	Collectors' biomass	t-test	0.135
Pantano	Rocks	Predators' biomass	Wilcoxon	0.151
Saltito-60	Leaves	Density	t-test	0.180
Saltito-60	Leaves	Biomass	t-test	0.039
Saltito-60	Leaves	Richness	t-test	0.092
Saltito-60	Leaves	Collectors' density	Wilcoxon	0.044
Saltito-60	Leaves	Predators' density	Wilcoxon	0.194
Saltito-60	Leaves	Collectors' biomass	t-test	0.039
Saltito-60	Leaves	Predators' biomass	t-test	0.970
Saltito-60	Rocks	Density	t-test	0.014
Saltito-60	Rocks	Biomass	t-test	0.010
Saltito-60	Rocks	Richness	t-test	0.003
Saltito-60	Rocks	Collectors' density	t-test	0.013
Saltito-60	Rocks	Predators' density	t-test	0.001
Saltito-60	Rocks	Collectors' biomass	t-test	0.069
Saltito-60	Rocks	Predators' biomass	t-test	0.020
Taconazo	Leaves	Density	t-test	0.053
Taconazo	Leaves	Biomass	Wilcoxon	0.151
Taconazo	Leaves	Richness	t-test	0.203
Taconazo	Leaves	Collectors' biomass	Wilcoxon	1.000
Taconazo	Leaves	Predators' biomass	Wilcoxon	0.222
Taconazo	Leaves	Collectors' density	t-test	0.437
Taconazo	Leaves	Predators' density	Wilcoxon	0.230
Taconazo	Rocks	Density	t-test	0.010
Taconazo	Rocks	Biomass	Wilcoxon	0.421
Taconazo	Rocks	Richness	t-test	0.467
Taconazo	Rocks	Collectors' density	Wilcoxon	0.123
Taconazo	Rocks	Predators' density	t-test	0.002
Taconazo	Rocks	Collectors' biomass	t-test	0.270
Taconazo	Rocks	Predators' biomass	Wilcoxon	0.008

Appendix E

Akaike Information Criterion corrected (AICc) model selection results for the composition and structure of macroinvertebrate assemblages in streams along the phosphorus gradient. Models were ranked according to ΔAICc (difference in AICc between the current model and the best model). K represents the number of parameters in each model, and the cumulative weight (Cum. weight) reflects the total AICc weight for the ranked models. Conductivity (our indicator of stream P) and pH were included as fixed effects, while sampling event and discharge were included as random effects in the models.

Response Variable	Substrates	Model Parameters	K	AICc	Delta AICc	AICc Weight	Cum Wt	Log Likelihood
Density	Leaves	Conductivity	5	387.62	0	0.82	0.82	-187.93
		pH	5	391.6	3.98	0.11	0.93	-189.92
		pH* Conductivity	7	392.48	4.86	0.07	1	-187.49
	Rocks	Conductivity	5	330.3	0	0.73	0.73	-159.27
		pH* Conductivity	7	332.61	2.32	0.23	0.96	-157.56
		pH	5	336.33	6.03	0.04	1	-162.28
Biomass	Leaves	Conductivity	5	112.33	0	0.5	0.5	-50.28
		pH	5	112.48	0.14	0.47	0.97	-50.36
		pH* Conductivity	7	117.96	5.63	0.03	1	-50.23
	Rocks	pH	5	123.8	0	0.48	0.48	-56.02
		Conductivity	5	123.89	0.09	0.46	0.94	-56.06
		pH* Conductivity	7	128.1	4.3	0.06	1	-55.3
Richness	Leaves	pH	5	185.86	0	0.59	0.59	-87.05
		Conductivity	5	186.92	1.06	0.35	0.94	-87.58
		pH* Conductivity	7	190.32	4.46	0.06	1	-86.41
	Rocks	Conductivity	5	187.35	0	0.52	0.52	-87.79
		pH	5	187.7	0.35	0.44	0.96	-87.97
		pH* Conductivity	7	192.35	5	0.04	1	-87.43
Collectors' density	Leaves	Conductivity	5	341.51	0	0.71	0.71	-164.87
		pH	5	343.96	2.45	0.21	0.92	-166.1

Appendix E (Continued)

		pH* Conductivity	7	345.95	4.45	0.08	1	-164.23
	Rocks	Conductivity	5	291.03	0	0.67	0.67	-139.63
		pH	5	293.31	2.28	0.21	0.88	-140.77
		pH* Conductivity	7	294.47	3.44	0.12	1	-138.49
Predator' density	Leaves	Conductivity	5	338.56	0	0.84	0.84	-163.4
		pH	7	343.17	4.62	0.08	0.93	-162.84
		pH* Conductivity	5	343.46	4.9	0.07	1	-165.85
	Rocks	Conductivity	5	282.04	0	0.86	0.86	-135.14
		pH* Conductivity	7	287.08	5.04	0.07	0.93	-134.79
		pH	5	287.19	5.15	0.07	1	-137.71
Collectors' biomass	Leaves	Conductivity	6	464.28	0	0.53	0.53	-224.87
		pH	6	464.69	0.42	0.43	0.97	-225.07
		pH* Conductivity	8	469.79	5.52	0.03	1	-224.57
	Rocks	Conductivity	6	403.26	0	0.44	0.44	-194.36
		pH	6	403.71	0.45	0.35	0.79	-194.58
		pH* Conductivity	8	404.79	1.53	0.21	1	-192.07
Predators' biomass	Leaves	Conductivity	6	462.71	0	0.53	0.53	-224.08
		pH	6	463.35	0.64	0.39	0.92	-224.4
		pH* Conductivity	8	466.56	3.85	0.08	1	-222.96
	Rocks	Conductivity	5	133.87	0	0.84	0.84	-61.05
		pH	5	137.99	4.12	0.11	0.95	-63.11
		pH* Conductivity	7	139.45	5.59	0.05	1	-60.98