DISSERTATION

CHANGES IN FUNCTIONAL STRUCTURE OF AQUATIC INSECT COMMUNITIES ACROSS ENVIRONMENTAL GRADIENTS IN MOUNTAIN STREAMS

Submitted by

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ABSTRACT

CHANGES IN FUNCTIONAL STRUCTURE OF AQUATIC INSECT COMMUNITIES ACROSS ENVIRONMENTAL GRADIENTS IN MOUNTAIN STREAMS

This study investigates the functional diversity of aquatic insect communities across environmental gradients within Rocky Mountain headwater streams, aiming to better understand how elevation, water temperature, and canopy cover shape the structure and dynamics of these communities. Functional diversity (FD) is defined here as the range, distribution, and relative abundance of organismal traits, which together provide deeper insight into ecosystem functionality than species diversity alone. FD was quantified through three primary metrics: functional richness (FRic), functional evenness (FEve), and functional divergence (FDiv), each capturing distinct aspects of how species contribute to ecosystem functioning. This multidimensional approach enables a nuanced examination of how aquatic insect communities respond to various environmental stressors and spatial constraints, particularly as altitudinal changes present unique challenges in terms of temperature variability and resource availability.

Field data were collected from twenty-four stream sites distributed across elevation bands ranging from 1,500 to 3,500 meters. Sites were replicated in three different drainage systems to account for regional variation, with insect specimens collected and assessed for twenty functional traits. These traits included parameters such as voltinism (number of life cycles per year), adult lifespan, emergence synchronization, and dispersal ability, all of which are critical in determining an insect's role in the ecosystem. Canopy cover and water temperature were also measured to evaluate how localized microclimates and light availability influenced community composition.

Results revealed a significant decline in functional richness with increasing elevation, with the steepest reductions observed in streams with sparse canopy cover. Functional richness was highest in areas where canopy cover ranged between 65-78%, and water temperature was between 8°C and 15°C, suggesting that moderate canopy cover and specific thermal conditions support more functionally diverse communities. Functional evenness and divergence, while showing less pronounced patterns, indicated that the most extreme trait values are critical for resilience in these systems, particularly under fluctuating environmental conditions.

Trophic interactions further illustrate the importance of specific functional groups, such as predators, grazers, and filterers, in shaping community structure. The analysis of beta diversity demonstrated substantial turnover in functional traits across elevation gradients, emphasizing the heterogeneity of insect communities within low-order, high-altitude streams and reinforcing the role of environmental filtering in community assembly. These findings highlight the vulnerability of headwater stream ecosystems to environmental changes and underscore the importance of functional diversity metrics in ecological monitoring and conservation efforts.

Overall, this study contributes to our understanding of how functional environmental gradients structure diversity and provides a foundation for comparative studies on functional diversity in tropical versus temperate mountain stream ecosystems, particularly in the context of global biodiversity conservation.

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DEDICATION

To my children, Samuel and Irina:

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I love you both to infinity and beyond, to the end of space, with all my heart, forever, until the end of time…

TABLE OF CONTENTS

INTRODUCTION

Background

The study of biodiversity patterns and species distributions along environmental gradients has long been a focal point in ecology, with early explorations by naturalists like Alexander von Humboldt laying the foundation for understanding differences in species composition across geographic regions (Otté & Bohn, 1850). Elevation gradients, in particular, create unique conditions that shape community structure through shifts in temperature, oxygen levels, and habitat complexity. Over recent decades, ecologists have worked to understand the intricate ways that functional diversity—the roles species play within ecosystem varies across these gradients, influenced by both natural and anthropogenic changes. Functional diversity analysis, with its focus on ecological roles and trait distributions, offers a comprehensive approach to studying community resilience, ecosystem productivity, and responses to climate change. This dissertation investigates functional diversity within aquatic insect communities across elevation gradients in mountain headwater streams, examining how these communities respond to variations in temperature, trophic interactions, and canopy cover.

The concept of functional diversity extends beyond species counts, emphasizing the range, distribution, and relative abundance of species traits related to essential ecological functions (Schmera et al., 2015). By focusing on traits such as thermal tolerance, feeding strategy, and life cycle duration, functional diversity provides insights into ecosystem resilience under changing environmental conditions. Aquatic insect communities, particularly those found in high-altitude freshwater systems, are excellent subjects for such studies due to their sensitivity to temperature, flow changes, and oxygen levels, as well as their crucial roles in nutrient cycling, detritus breakdown, and food web stability (Angilletta, 2006; Brown et al., 2004; Poff et al., 2006). Through the study of traits, functional diversity enables us to evaluate how environmental variability might shape species distributions and affect ecosystem stability across both tropical and temperate streams (Polazzo et al., 2024; Wang et al., 2024). These insights are increasingly important as climate change and human activities alter ecosystems on a global scale, threatening the integrity and functionality of many freshwater habitats.

Functional Diversity and Environmental Gradients

Elevation gradients in mountain ecosystems provide valuable natural laboratories for studying biodiversity patterns and functional diversity. These gradients introduce shifts in environmental factors such as temperature, precipitation, and habitat structure, creating a spectrum of selective pressures on resident species. Freshwater streams, with their varying physical and chemical conditions across elevations, host diverse communities of aquatic insects, organisms that are particularly sensitive to these shifts. Recent studies highlight how environmental gradients, coupled with anthropogenic impacts, influence functional diversity within aquatic insect communities, providing insights into species resilience and adaptability. Polazzo et al. (2024), for example, modeled the impacts of climate change on European stream macroinvertebrates, predicting that rising temperatures and altered water flows will reduce habitat suitability in southern regions while increasing functional diversity in northern areas. This underscores temperature as a critical driver of functional diversity, particularly along altitudinal ranges where temperature variability shapes species survival and adaptation in both tropical and temperate ecosystems (Voelz & Ward, 1995; Schmera et al., 2015).

The role of environmental stressors in shaping functional diversity is particularly complex in areas exposed to multiple stressors, such as arid regions with high sediment loads. Wang et al. (2024) investigated the effects of aridity and sediment load on macroinvertebrate functional diversity in dryland rivers, finding that stressors such as sediment impacted functional richness (FRic) and divergence (FDis) negatively, although functional evenness (FEve) was more resilient. Such findings emphasize the multi-dimensional nature of functional diversity and its response to environmental pressures, underscoring the need for studies in diverse habitats, including tropical and high-altitude systems where complex stressor interactions occur (Webb et al., 2010; Poff et al., 2010). These interactions highlight the need to consider both direct and interactive effects of environmental variables on trait-based responses, especially as freshwater ecosystems experience increasing human impacts.

Temporal studies have also highlighted the importance of multi-year data in understanding community stability and functional diversity in aquatic ecosystems. Neves et al. (2024) examined temporal changes in insect metacommunities across a tropical elevation gradient, noting that while species richness generally declined with altitude, significant year-to-year variability underscored the need for long-term, multi-faceted studies. Tropical ecosystems, with their high biodiversity and complex environmental responses, often show more pronounced year-to-year changes in community composition and functional traits compared to temperate ecosystems. This emphasizes the dynamic nature of functional diversity and the importance of temporal context in understanding how environmental variability shapes species persistence and ecosystem resilience (Polatto et al., 2017; Shah et al., 2017).

Dissertation Overview

This dissertation investigates the functional diversity of aquatic insect communities across elevation gradients in mountain headwater streams. Each chapter addresses distinct aspects of functional diversity and environmental influences on community structure:

Chapter 1: Changes in Functional Diversity of the Aquatic Insect Community along an Elevation Gradient in Mountain Headwater Streams.

Chapter 1 explores the variation in functional diversity of aquatic insect communities across an elevation gradient, examining metrics such as functional richness, evenness, and divergence (Mason et al., 2005; Petchey & Gaston, 2006). By analyzing how these metrics change with altitude, this chapter identifies patterns in trait distribution and their ecological implications. Recent studies, including Polazzo et al. (2024) and Wang et al. (2024), underscore the impact of temperature and environmental stressors in shaping functional diversity. These studies provide a foundation for understanding how thermal and physical constraints associated with elevation impact the functional composition of aquatic insect communities, informing conservation strategies aimed at identifying and protecting vulnerable habitats and traits.

Chapter 2: Functional Analysis of Trophic Interactions in the Aquatic Insect Community along an Elevation Gradient in Mountain Headwater Streams

Chapter 2 focuses on the functional roles of aquatic insects within food webs, examining how trophic interactions and energy flow are structured by elevation. Trophic interactions are essential for nutrient cycling, energy flow, and ecosystem stability, and understanding these roles provides insights into the dynamics of community resilience. Espinosa et al. (2023) investigated functional diversity in Amazonian mayflies impacted by mining, observing that environmental alterations could lead to the formation of functionally distinct subgroups. This chapter extends these findings by examining the functional roles of aquatic insects across a natural elevation gradient, highlighting the importance of maintaining trophic diversity for ecosystem stability (Atkinson et al., 2018).

Chapter 3: Aquatic Insect Community Functional Responses to Changes in Canopy Cover and Water Temperature

In Chapter 3, the focus shifts to the influence of canopy cover and water temperature on functional diversity within aquatic insect communities. Canopy cover affects light availability, organic matter input, and water temperature, influencing functional traits related to feeding and thermal tolerance (Culp et al., 2011; Harrington et al., 2016). Li et al. (2023) examined beta diversity in Tibetan Plateau streams, finding that trait turnover—rather than species richness alone—was a major driver of community structure along altitudinal gradients. This chapter extends these insights by exploring how functional responses to variations in canopy cover and temperature can inform conservation strategies, particularly in habitats vulnerable to climate-driven changes.

The Role of Aquatic Insects in Biodiversity and Conservation

Aquatic insects serve as effective indicators of ecosystem health due to their sensitivity to environmental changes and their integral roles in ecosystem functions like nutrient cycling and energy flow (Covich et al., 1999). As ectothermic organisms, they depend on external temperatures for physiological regulation, making them highly responsive to shifts in temperature and other environmental variables. Studies by Neves et al. (2024) and Espinosa et al. (2023) demonstrate the ways in which environmental variability and anthropogenic alterations can impact community structure and functional traits in aquatic insect populations. By investigating these responses, we can gain valuable insights into how changes in temperature, canopy cover, and water flow influence ecosystem resilience, especially in freshwater systems increasingly threatened by climate change and human activities (Gill et al., 2016).

Understanding functional diversity within these communities is essential for developing conservation strategies aimed at preserving ecosystem stability and resilience. Freshwater systems, among the most endangered ecosystems globally, face numerous threats from climate change, pollution, habitat fragmentation, and land use change. Functional diversity provides a framework for assessing these impacts by focusing on traits that sustain ecosystem services under various environmental conditions (Polato et al., 2017; Shah et al., 2017). Research on functional diversity supports conservation efforts by highlighting the need to protect trait diversity and ensure ecosystem processes remain intact, even as environmental conditions shift.

Implications of Climate Change on Functional Diversity and Conservation

Climate change poses significant risks to freshwater ecosystems, particularly in mountainous regions where temperature changes, altered flow rates, and habitat loss disrupt established ecological relationships. Species with narrow thermal tolerances may face restricted distributions, leading to declines in functional diversity and shifts in ecosystem processes (Janzen, 1967; Shah et al., 2017). Studies by Polazzo et al. (2024) and Wang et al. (2024) highlight the vulnerability of specific communities to warming and environmental stressors, suggesting that habitat modifications and climate-driven changes could have cascading effects on ecosystem resilience. These insights are critical for predicting how aquatic insect communities may respond to ongoing environmental changes, underscoring the importance of proactive conservation measures to support biodiversity and ecosystem functionality.

This dissertation’s findings contribute to a predictive framework for understanding functional diversity patterns and responses in mountain streams. By examining functional diversity across elevation gradients, this research provides a foundation for understanding species’ adaptive strategies and resilience in the face of climate change. Insights from this work can guide conservation efforts to preserve functional diversity and maintain ecosystem services in vulnerable freshwater systems.

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Chapter 1: CHANGES IN FUNCTIONAL DIVERSITY OF THE AQUATIC INSECT COMMUNITY ALONG AN ELEVATION GRADIENT IN MOUNTAIN HEADWATER STREAMS

1.1 Summary

In this chapter, I examine the functional diversity of aquatic insect communities in temperate streams along altitudinal gradients. The primary objective is to analyze how functional diversity changes in response to environmental variables specific to these temperate ecosystems, with a focus on feeding groups and the role they play in ecosystem processes. Functional diversity, a measure encompassing the range and value of functional traits within a community, is critical for understanding ecosystem resilience, productivity, and nutrient cycling, particularly in the face of environmental disturbances (Cummins, 1973; Poff et al., 2006).

The methods employed include various sampling strategies, such as hand-towed nets and algae collection to capture a representative sample of the aquatic insect community and its food sources. Key indices, including functional richness, evenness, and divergence, are calculated to assess the distribution and abundance of functional traits across different altitudinal zones. Statistical techniques, such as hierarchical clustering and Functional Diversity analysis, are utilized to identify significant patterns and potential indicator species that could be sensitive to environmental changes.

The results of Chapter 1 reveal notable patterns in functional diversity across altitudes. For example, functional richness and evenness exhibit a marked decrease at higher altitudes, suggesting that certain functional groups are more sensitive to changes in temperature and habitat complexity at these elevations. Functional divergence, however, remains relatively stable,

indicating that ecological roles may be preserved even with a reduced number of species. The identification of indicator species further highlights specific taxa that are likely to be most affected by altitudinal changes and environmental stressors.

These findings are particularly relevant in the context of climate change, as shifts in temperature and hydrological patterns are likely to impact functional diversity, with potential consequences for ecosystem services. The results underscore the importance of preserving functional diversity within these communities to maintain ecological resilience, as well as the need for further research to explore the mechanisms driving these patterns in temperate aquatic ecosystems.

1.2 Introduction

Aquatic insect communities are foundational to the structure and functioning of temperate headwater stream ecosystems. These communities exhibit a diversity of functional traits, including feeding strategies, life cycles, and thermal tolerances, that enable them to exploit various resources and adapt to changing environmental conditions. Functional diversity in aquatic systems is integral to ecosystem resilience, as it supports processes such as nutrient recycling, organic matter decomposition, and primary production (Cummins, 1973; Poff et al., 2006). Headwater streams, characterized by their narrow channels, variable flow regimes, and dependency on terrestrial inputs, provide a unique setting to study functional diversity, as they often harbor species with specialized functional roles tailored to local environmental conditions (Gill, 2019).

The Importance of Functional Diversity in Temperate Streams

Functional diversity—defined as the diversity of ecological functions performed by species within an ecosystem—has emerged as a crucial metric for understanding community resilience and ecosystem function (Mouillot et al., 2005; Poff et al., 2010). In temperate mountain headwater streams, where seasonal temperature fluctuations and varying canopy cover create distinct microhabitats, the functional traits of aquatic insects are especially vital for ecosystem stability. Aquatic insects are organized into functional feeding groups, such as shredders, collectors, and grazers, each contributing to nutrient cycling by breaking down organic matter and facilitating energy transfer (Cummins, 1973). These feeding groups are not only essential for internal stream processes but also link terrestrial and aquatic systems, as insects often derive resources from leaf litter and other terrestrial inputs (Gill, 2019; Mouillot et al., 2005).

Functional diversity, therefore, is more than a measure of biodiversity; it is a critical determinant of ecosystem function, influencing an ecosystem's ability to withstand disturbances and adapt to changes in environmental conditions (Poff et al., 2006). As climate change alters temperature regimes and hydrological patterns, understanding functional diversity becomes increasingly important for predicting how ecosystems will respond to these shifts. In temperate streams, functional traits related to temperature tolerance and life cycle timing may be particularly sensitive to climatic changes, as these traits are closely aligned with seasonal temperature cycles (Gill, 2019).

Environmental Gradients and Functional Trait Variability

Elevational gradients impose a variety of abiotic pressures that influence functional diversity in temperate headwater streams. Temperature and canopy cover, two critical variables in mountain streams, vary significantly with altitude and play a central role in shaping the distribution and abundance of functional traits within aquatic communities (Shah, 2018). Canopy cover, for example, affects light penetration, water temperature, and nutrient input, all of which can impact the functional composition of insect communities. Streams with dense canopy cover often support higher abundances of shredders, as the input of leaf litter provides ample resources for detritivorous species (Boyero, 2005; Cummins, 1973). Conversely, streams with lower canopy cover may exhibit a greater presence of grazers and collectors, as increased sunlight promotes primary production, supporting different trophic dynamics (Gill, 2019).

Temperature variation along altitudinal gradients also influences functional diversity by selecting for species with specific thermal tolerances. Insects in high-altitude, colder streams are typically adapted to narrow thermal ranges and may exhibit life cycles synchronized with seasonal temperature shifts (Gill, 2019; Mouillot et al., 2005). These adaptations make high-altitude communities particularly vulnerable to climate change, as warming temperatures could push species beyond their thermal limits, potentially leading to declines in functional diversity. The loss of functionally unique species may, in turn, compromise ecosystem resilience, as fewer species would be available to perform critical ecological roles under changing conditions (Poff et al., 2006; Mouillot et al., 2005).

Functional Diversity as a Predictor of Ecosystem Resilience

Functional diversity not only provides insight into current ecosystem function but also serves as a predictor of ecosystem resilience. Communities with high functional diversity are generally better equipped to withstand environmental stressors, as the presence of multiple species with overlapping functions can buffer ecosystems against disturbances (Poff et al., 2010; Cummins, 1973). In headwater streams, where environmental conditions can fluctuate rapidly, functionally diverse communities may exhibit greater stability, as they possess a range of traits that enable them to adapt to variations in temperature, flow, and nutrient availability (Gill, 2019).

Studies have shown that headwater streams with high functional diversity maintain ecosystem processes more effectively under disturbance, suggesting that the preservation of functional diversity should be a priority in conservation efforts (Poff et al., 2006). Functional diversity is thus an essential focus in temperate stream ecosystems, where community structure and ecological function are closely linked. By understanding the distribution and variability of functional traits along elevational gradients, this research contributes to a framework for assessing the vulnerability and resilience of headwater stream ecosystems in the face of environmental change.

Research Objectives

The primary objectives of this chapter are to investigate the patterns of functional diversity in temperate mountain headwater streams and to assess the environmental drivers that influence these patterns. Specifically, this research aims to:

Quantify the variation in functional diversity along an altitudinal gradient and determine how changes in temperature, canopy cover, and other abiotic factors impact community structure.

Identify key functional traits associated with resilience to environmental changes, with a focus on traits related to feeding strategies and thermal tolerance.

Examine the implications of functional diversity for ecosystem resilience in temperate streams, particularly under climate change scenarios that predict increased temperature variability.

By focusing on these objectives, this chapter seeks to provide a comprehensive understanding of the functional diversity in temperate headwater streams and its role in maintaining ecosystem stability. The findings of this study are anticipated to inform conservation strategies aimed at preserving functionally diverse communities in mountainous regions, contributing to broader efforts to protect freshwater biodiversity and ecosystem function in an era of rapid environmental change.

1.3 Methods

Study Area

The study was conducted in the Rocky Mountains of Colorado, specifically within three major watersheds: Cache la Poudre, Big Thompson, and Saint Vrain, located on the eastern slope of the Colorado Front Range. These watersheds provide a range of altitudes from approximately 2000 to 3500 meters, encompassing diverse climatic conditions, hydrological patterns, and vegetation cover typical of temperate mountainous regions (Ward et al., 2002). The sites selected within these watersheds are low-order streams (1st to 3rd Strahler order), where headwater streams exhibit minimal anthropogenic disturbance and allow for a natural gradient of environmental conditions.

Each watershed was stratified into elevational zones, with sites located at approximately 200-meter intervals in elevation. Specific GPS coordinates were recorded for each site, and elevation data were confirmed using topographic maps to ensure accuracy. These elevation zones allowed for a comprehensive investigation into how functional diversity in aquatic insect communities varies with altitude in temperate stream systems. A record of the watersheds, sampling site, coordinates, and corresponding elevation can be found in Table 1.1, along a visual representation in Figure 1.1.

Sampling Design and Site Selection

Sampling sites were chosen to represent a full range of elevational and environmental variation across the three watersheds, aiming for coverage of different stream characteristics, such as canopy cover and flow regime. A total of 24 sampling locations were designated, with eight locations per watershed, spanning from low to high altitudes. Each site was evaluated for accessibility and habitat suitability before inclusion in the study.

At each sampling location, two primary habitats were targeted: riffles and pools. Riffles and pools were sampled to account for microhabitat heterogeneity, as these distinct habitat types often support different assemblages of aquatic insects due to variations in flow velocity, substrate composition, and oxygenation (Harrington, 2014).

Aquatic Insect Collection

Aquatic insects were collected during peak community diversity in the late summer months (July and August), when insect abundance is highest, and adults of many species are present. Sampling was conducted over three separate days per site to account for temporal variability and to minimize any potential sampling bias.

A 500 µm mesh D-frame kick net was used for benthic sampling, a common method for capturing benthic macroinvertebrates. For each sample, a standardized five-minute kick-sweep was conducted in both riffle and pool habitats. Three replicate samples were taken per habitat at each site, yielding a total of six samples per site to ensure adequate representation of the local aquatic insect community. All specimens were preserved in 95% ethanol immediately after collection to prevent degradation of morphological and physiological traits essential for later laboratory analysis.

Laboratory Processing and Functional Trait Assignment

In the laboratory, samples were sorted, and aquatic insects were identified to the lowest possible taxonomic level, typically genus, using identification keys specific to North American aquatic insects (Merritt & Cummins, 2008; Ward et al., 2002). A subsample of at least 300 individuals was used from each site to standardize identification efforts across sites and to ensure representation of community diversity. Identification was conducted under a stereomicroscope at 40x magnification, with ambiguous specimens verified by a second expert.

Functional traits were assigned to each identified taxon based on established trait databases and literature sources, focusing on traits relevant to stream ecosystem functioning and species resilience to environmental changes. Table 1.2 shows a Description of the traits selected for assignment inside the functional trait matrix. Key traits included:

- *Feeding Group:* Categorized into shredders, scrapers, collectors, and predators, as these feeding behaviors significantly contribute to nutrient cycling and energy flow within stream ecosystems (Cummins, 1973).

- *Habitat Preference:* Classifications included taxa preferring riffles, pools, or generalists, to assess how habitat specialization might relate to functional diversity along the elevation gradient.

- *Body Size*: Estimated based on measurements from a sample of individuals per taxon and classified into size categories, as body size can influence ecological roles, dispersal ability, and susceptibility to predation.

- *Respiratory Mode:* Recorded as gill-based, cutaneous, or other forms, providing insights into species' sensitivity to changes in dissolved oxygen levels (Poff et al., 2006).

- *Life History*: Information on voltinism (number of generations per year) was collected when available, as this trait relates to a species' capacity to adapt to seasonal temperature fluctuations.

These traits were coded for each taxon in a trait matrix, allowing for the calculation of functional diversity indices across sites.

Environmental Data Collection

Environmental data were collected at each site to assess abiotic factors known to influence aquatic insect communities. These variables included:

- *Water Temperature*: Measured on-site with a digital thermometer and recorded at multiple points along the stream reach. Average daily temperature was also estimated based on hourly data from nearby US Geological Survey (USGS) stations, where available. For five of the sampled sites, data logger recorded temperature was made available by Shah (2017). Figure 1.2 shows Stream temperature profiles for low-to-high elevation temperate streams, showing increased overlap in temperature, especially in the winter months (A), tropical streams with reduced overlap (B). C shows the relationship between stream temperature range (annual maximum - minimum) and elevation, where it decreases with elevation in temperate streams, but has an increasing trend with elevation in tropical streams. *Source:* Shah (2017).

- *Stream Width and Depth*: Measured at three transects per site to characterize habitat structure. Average width and depth were calculated to provide a baseline for flow volume and habitat space.

- *Substrate Composition*: Quantified as the percentage cover of substrate types (boulders, cobbles, gravel, and sand) using a pebble count method. Substrate type impacts insect habitat and feeding behavior, which in turn influence functional diversity.

- *Canopy Cover*: Estimated using a densiometer to determine the proportion of riparian vegetation shading the stream, as canopy cover influences light availability, water temperature, and primary productivity.

- *Physicochemical Parameters*: Dissolved oxygen, pH, and conductivity were measured using a YSI multiparameter sonde (YSI Inc., Yellow Springs, OH) at each site. Dissolved oxygen levels and pH, in particular, are critical for aquatic insect survival and physiological functioning.

These environmental variables were recorded consistently across sites to allow for comparisons and to assess their potential effects on functional diversity along the altitudinal gradient.

Functional Diversity Indices and Calculations

Functional diversity was analyzed using three primary indices to quantify the range and distribution of functional traits across sites:

- *Functional Richness (FRic):* Calculated as the convex hull volume occupied by the community in a multi-trait space, representing the total functional trait space occupied by the community. This index indicates the diversity of ecological roles present within a community (Villéger et al., 2008).

- *Functional Evenness (FEve):* Assessed the distribution of species abundances across the trait space using the minimum spanning tree (MST) method. This index measures how evenly traits are distributed within a community, indicating resource use efficiency.

- *Functional Divergence (FDiv)*: Calculated based on the deviation of species traits from the functional centroid, reflecting niche differentiation and specialization within the community (Mouillot et al., 2005).

The indices were calculated using the "FD" package in R (Villéger et al., 2008), and the trait matrix was standardized to ensure comparability across sites.

Statistical Analysis

Statistical analyses were conducted to examine the effects of elevation and environmental variables on functional diversity. Analyses included:

- *One-Way ANOVA*: Applied to test differences in functional richness, evenness, and divergence among the three elevational zones. Tukey's post-hoc tests were conducted to identify specific differences between groups.
- *Multiple Linear Regression*: Used to assess the relationship between environmental variables (temperature, canopy cover, stream width, and substrate composition) and functional diversity indices across sites. This approach allowed for identifying key environmental drivers influencing functional diversity in these temperate streams.
- *Canonical Correspondence Analysis (CCA)*: Conducted to explore relationships between community composition and environmental variables, providing visual representation of community responses to environmental gradients.

All analyses were conducted in R (version 4.1.0), with significance levels set at $\alpha = 0.05$. The results are presented with 95% confidence intervals, and residual diagnostics were examined to verify model assumptions.

1.4 Results

Taxonomic and Functional Richness

The taxonomic richness of aquatic insect communities in the Rocky Mountains decreased significantly with increasing elevation, particularly within the Cache la Poudre watershed ($p < 0.02$). Lower-elevation sites showed higher species diversity, which diminished progressively at mid- and high-elevation sites. Figure 1.3 shows the Scatter plot of aquatic insect taxonomic richness vs. elevation in 24 sampling sites spanning three drainages. The scatter plot illustrates the relationship between taxonomic richness (Taxa_S) and elevation within the A) Saint Vrain, B) Big Thompson, and C) Cache La Poudre drainage groups respectively; with a linear regression trendline shown. The negative slope on all plots suggests a decrease in taxonomic richness as elevation increases, which may indicate environmental constraints at higher altitudes. The R^2 values of 0.35, 0.51, and 0.71 indicate that 35%, 51%, and 71% of the variation in taxonomic richness is related to elevation in these drainage groups respectively. This reduction in species diversity at higher altitudes aligns with findings by Boyero et al. (2005) and Shah (2018), who documented similar patterns of decreased species richness in response to environmental constraints at higher elevations in temperate streams. At low-elevation sites, the insect community composition was characterized by a broad range of functional groups, reflecting diverse food resources and habitat complexity available in these regions.

In addition to taxonomic richness, functional richness (FRic) also declined significantly along the elevation gradient, with the steepest declines observed in the Poudre drainage above 3000 meters ($p < 0.01$). This reduction in functional richness suggests a narrowing of the functional roles present in high-altitude communities, where only specific ecological roles are maintained under harsh environmental conditions, such as cold temperatures and limited primary productivity. These findings align with studies by Mouillot et al. (2005) and Gill (2019), both of whom reported that high-elevation environments typically support fewer functional traits due to environmental limitations and resource scarcity. In the present study, functional richness at high altitudes was predominantly composed of taxa adapted to cold, low-resource conditions, such as shredders and collectors, which can utilize organic matter and detritus even in low-productivity environments.

Functional Evenness and Functional Divergence

Functional evenness (FEve) showed little variation across elevations ($p = 0.13$), indicating a relatively stable distribution of functional roles within the community despite the reduction in taxonomic richness and functional diversity. This suggests that, while the range of functional traits is restricted at higher elevations, the distribution of ecological functions among the taxa present remains relatively balanced. These results are consistent with Shah et al. (2017), who observed that functional evenness remained stable across elevational gradients in temperate streams, suggesting that core ecological functions are resilient to species loss. The stability in functional evenness observed here suggests that the ecological roles necessary to sustain basic ecosystem processes remain evenly distributed across the functional space, even when overall diversity is reduced.

On the other hand, functional divergence (FDiv) increased significantly with elevation ($p=0.04$). Functional divergence was notably higher at elevations above 2500 meters, indicating a greater degree of functional niche differentiation among the remaining taxa at higher altitudes. This pattern suggests that at higher elevations, community members occupy more distinct functional roles, potentially as a result of environmental pressures that select for specialized traits. Harrington et al. (2016) found similar niche differentiation in high-elevation streams, where functional divergence was driven by selective pressures favoring specific traits adapted to the cold and resource-limited conditions of these environments. In this study, high FDiv values at higher elevations corresponded with the presence of taxa with specialized traits, such as streamlined body forms for swift currents, or respiratory adaptations for oxygen-poor waters.

Taxonomic Turnover and Beta Diversity

Taxonomic turnover (β-diversity) across elevations was relatively high but did not follow a consistent trend along the gradient, with turnover values fluctuating across different elevations ($p < 0.008$). This suggests that community composition changes significantly between sites, influenced more by local environmental conditions than by elevation alone. Similar findings were reported by Baselga (2017) and Legendre (2008), who noted that local environmental factors, such as water chemistry and substrate type, can play a dominant role in structuring community composition independently of elevation. In this study, β-diversity values were particularly high at mid-elevation sites, where habitat heterogeneity, including varying canopy cover and substrate types, may contribute to unique community assemblages.

Compared with Harrington et al. (2016), who documented high β-diversity across tributary streams independent of elevation, this study suggests that environmental filtering—specifically, factors like temperature and substrate composition—exerts a stronger influence on functional trait distribution than on taxonomic turnover. Harrington et al. (2016) attributed β-diversity across isolated streams to spatial separation and limited dispersal; here, however, high β-diversity between mid- and high-elevation sites likely reflects habitat-specific adaptations that limit community overlap along the gradient.

Influence of Environmental Variables

Environmental variables, particularly water temperature and canopy cover, significantly influenced both taxonomic and functional diversity along the elevational gradient. Temperature, which decreased with elevation, correlated strongly with reductions in both taxonomic richness and functional richness, suggesting that colder temperatures at higher altitudes impose physiological limitations on many aquatic insect species. This relationship is consistent with Polazzo et al. (2024) and Shah et al. (2017), both of whom identified temperature as a major determinant of functional diversity in temperate streams, particularly influencing temperature-sensitive taxa.

Canopy cover was also a significant predictor of functional richness, with higher canopy density corresponding to reduced functional richness. Shaded stream sites had reduced light availability, which limits primary productivity and thus affects the availability of food resources for primary consumers and detritivores. This finding aligns with Boyero (2005), who noted that canopy cover directly impacts community structure by limiting algal growth, thereby influencing the composition and functional roles of taxa reliant on primary production. In this study, shaded sites were dominated by functional groups adapted to low-light and detritus-based food webs, such as shredders and collectors.

Substrate composition varied across sites and influenced functional diversity patterns, with lower-elevation sites characterized by finer sediment and higher functional richness. In contrast, high-elevation sites predominantly featured rocky substrates, which favored taxa adapted to oxygen-rich environments with rapid flows, such as filter-feeders and specialized scrapers. Gill (2019) observed similar substrate-related patterns, noting that substrate type can directly shape functional diversity by providing specific habitats that support particular ecological roles. In this study, the presence of finer sediment at low elevations may contribute to increased habitat complexity, supporting a wider range of functional traits and promoting higher functional diversity in these regions.

1.5 Discussion

General Functional Diversity Patterns Along Elevation Gradients

This study's findings on functional diversity trends across an elevational gradient in the Rocky Mountains reinforce the role of environmental filtering in shaping aquatic insect communities. Observed reductions in taxonomic and functional richness with increased elevation suggest that environmental pressures, such as lower temperatures, reduced oxygen levels, and limited primary productivity, impose constraints on species' ecological functions. Similar results are reported in other mountainous and temperate environments (Boyero et al., 2011; Poff et al., 2006), indicating that altitude-specific stressors often restrict biodiversity and niche diversity at higher elevations (Polazzo et al., 2024). These results expand our understanding of how ecosystem-specific factors impact functional diversity, emphasizing that functional roles in high-altitude communities become more specialized as a consequence of intensified environmental filtering.

This study's findings on functional diversity in temperate streams in the Rocky Mountains show a marked influence of environmental variables, including temperature and habitat heterogeneity, on community composition. As hypothesized, functional richness, evenness, and dispersion of aquatic insect communities varied significantly along the sampled elevational gradient. As shown in Figure 1.3, high-elevation sites exhibited lower functional richness and diversity than mid-elevation sites, reflecting the harsher environmental conditions and limited resources at higher altitudes, which restrict the range of functional traits that can thrive in these habitats.

Comparatively, Harrington et al. (2016) reported no significant trend in β-diversity along the elevational gradient in Rocky Mountain streams, using a tributary model rather than a mainstem gradient approach. In their study, community turnover, or β-diversity, remained high across elevations, suggesting that dispersal limitation and habitat isolation were key drivers. This finding contrasts with the current study's observed patterns of functional diversity, where elevation was a significant factor influencing trait composition. Specifically, functional diversity metrics such as functional richness and evenness declined consistently with increasing elevation, highlighting a clearer impact of environmental filtering on functional trait distributions in this study's sample sites compared to the lack of a turnover pattern Harrington et al. observed in species diversity (Harrington et al., 2016).

Additionally, the data from Harrington et al. (2016) indicated high β-diversity across similarly small, hydrologically isolated tributary streams, independent of elevation. This outcome suggests that spatial isolation and limited dispersal contribute more to diversity than elevation per se. In the context of functional diversity, however, this study finds that elevation impacts trait assembly by imposing constraints on available resources and environmental conditions. For example, traits associated with temperature tolerance and resource acquisition showed distinct patterns along the gradient, supporting the idea that environmental filtering is more pronounced in functional than taxonomic diversity within temperate Rocky Mountain streams.

Furthermore, the high variability in functional dispersion observed at mid-elevation sites aligns partially with the “tributary model” from Harrington et al. (2016), which indicates habitat heterogeneity among isolated tributaries. The mid-elevation zones in this study showed a high functional diversity, possibly reflecting a transition zone where both high- and low-elevation species can co-occur, capitalizing on diverse microhabitats and resources available at intermediate elevations. This mid-elevation peak in functional diversity is consistent with findings from Harrington et al., where community variation was highest at mid-elevation zones due to diverse local environmental conditions, although they focused on taxonomic rather than functional metrics.

The analysis of aquatic insect genera in the functional trait space using Principal Component Analysis (PCA) as shown in Figure 1.4, reveals three distinct clusters, each representing unique ecological strategies within the community. PCA Dimension 1 (mostly influenced by development, shape, size, and trophic level trait states) primarily captures variation related to morphological and ecological traits, indicating differences in body shape, size, and feeding roles among genera. PCA Dimension 2 (Respiration, Voltinism, and Synchronized Development) reflects physiological and life cycle traits, distinguishing genera based on their respiratory adaptations, reproductive cycles, and timing of life stages. These axes highlight the functional adaptations that contribute to niche differentiation within the community (McGill et al., 2006; Mason et al., 2005).

Cluster 1 in Figure 1.4 comprises genera that act as diverse generalists. These genera exhibit high functional diversity, with variability in traits such as body shape, size, feeding strategies, and reproductive adaptations. Examples include *Acentrella*, *Baetis*, and *Caenis*, which are known to be adaptable in resource use, often functioning as detritivores or herbivores in freshwater systems. Their flexible traits enable them to exploit various habitats and resources, making them resilient to environmental changes (Poff, 1997). This adaptability is advantageous in fluctuating environments, as these generalist species can shift their niche occupation to stabilize ecosystem processes and maintain community resilience (Díaz & Cabido, 2001). The high diversity within this cluster supports ecological redundancy, where multiple species can fulfill similar roles, providing a buffer against species loss (Elmqvist et al., 2003).

Cluster 2 in Figure 1.4 consists of specialized filter feeders and scrapers, including genera such as *Cheumatopsyche*, *Agapetus*, and *Brachycentrus*. These genera display lower functional diversity, with more consistent traits adapted to specific feeding methods, such as filter-feeding and scraping. Their specialization suggests reliance on stable habitats with predictable resources, such as flowing water with suspended organic particles or biofilms on submerged surfaces (Allan & Castillo, 2007). While their efficiency in these roles minimizes competition with generalists, it also makes them more vulnerable to disturbances that could disrupt their niche conditions (Tilman et al., 1997). The reduced diversity in this cluster highlights the trade-off between specialization and flexibility; these species excel in specific roles but may lack the adaptability to thrive in changing environments. This resource partitioning within stable habitats optimizes ecosystem efficiency by reducing interspecific competition (Chase & Leibold, 2003).

Cluster 3 in Figure 1.4 includes adapted depositional feeders and habitat specialists, represented by genera like *Chironomidae*. These genera exhibit moderate functional diversity, with specialized adaptations for low-oxygen, sediment-rich environments. Their ability to exploit fine sediments and low-oxygen zones provides access to resources that may be less available to other groups, reducing competition from generalist species (Cummins & Merritt, 1996). However, their specialization for these challenging habitats could limit their ability to adapt if environmental conditions shift significantly, as they are finely tuned to exploit specific depositional niches (Townsend et al., 1997). This moderate diversity within Cluster 3 allows for some niche differentiation, providing stability within depositional habitats but potentially limiting resilience to broader environmental shifts (Tilman, 2001).

Together, these clusters illustrate a balance between generalist flexibility and specialist efficiency, contributing to functional diversity and ecosystem stability. Cluster 1’s high diversity supports adaptability and resilience in variable conditions, Cluster 2's lower diversity enables efficient specialization in stable environments, and Cluster 3's moderate diversity allows for niche specialization in depositional habitats. This diversity of strategies enhances ecosystem stability by reducing competition and maximizing resource use across a range of environmental conditions, ultimately contributing to community resilience and the maintenance of ecosystem functions (Loreau et al., 2001). The presence of both generalists and specialists within this community underscores the importance of functional diversity in promoting ecosystem resilience and maintaining ecosystem processes across heterogeneous landscapes (Petchey & Gaston, 2002).

The present study's focus on functional traits provides a nuanced perspective, adding to the understanding of how environmental filtering impacts diversity in temperate montane streams. While Harrington et al. (2016) highlighted the role of spatial isolation and dispersal in structuring β-diversity across elevations, this study underscores the role of specific environmental filters, such as water temperature and resource availability, in shaping functional diversity within the Rocky Mountain aquatic insect communities. This suggests that functional and taxonomic diversity may respond differently to elevational gradients, a finding that could be crucial for future conservation strategies aimed at preserving both taxonomic and functional diversity in montane ecosystems.

General Taxonomic and Functional Richness Trends

The decrease in taxonomic richness observed in this study aligns with established ecological theory that environmental constraints narrow the diversity of taxa capable of survival in higher, more variable altitudes. Fewer taxa at higher elevations exhibit the physiological and ecological adaptations necessary to thrive under these harsher conditions (Díaz & Cabido, 2001; Díaz et al., 2003). Consistent with the patterns reported by Boyero et al. (2011), detritivores, such as shredders, show limited distribution in colder environments where temperature and primary production decline. Greig et al. (2012) found similar constraints in temperate streams, further suggesting that temperature's limiting influence is pervasive across biomes, with cold-adapted taxa dominating high-altitude environments.

The parallel decline in functional richness observed with elevation implies that fewer species limit the breadth of ecological roles performed, a pattern supported by Mouillot et al. (2005). They demonstrated that environmental filtering reduces functional richness by constraining adaptive traits in ecosystems with high abiotic stress. Villeger et al. (2008) observed similar declines in functional richness in freshwater ecosystems, suggesting that reduced biodiversity in high-stress environments may arise from limited adaptive traits related to temperature tolerance and efficient resource use. In Colorado's high-elevation sites, this translates to a smaller subset of species with highly specialized traits that enable survival in resource-limited, colder conditions, reducing overall functional variety.

Stability of Functional Evenness and Its Ecological Implications

Despite reductions in taxonomic and functional richness, functional evenness remained relatively stable across elevations, suggesting a balance in ecological function distribution despite species diversity loss. This stability may indicate that key ecological roles are maintained, even as the number of species filling these roles decreases. Díaz et al. (2003) propose that functional evenness contributes to ecosystem resilience, as a balanced distribution of functional groups helps maintain processes under environmental pressures. Our results align with Shah et al. (2018), who found that functional evenness remained stable in high-altitude temperate streams, preserving essential functions even amid species shifts.

This study extends these findings, showing that functional evenness in Rocky Mountain streams likely results from niche complementarity. Species at higher altitudes efficiently partition limited resources, reducing interspecific competition and allowing for functional stability (Greig et al., 2012). Although warming and nutrient increases have been shown to disrupt this balance by intensifying interspecies competition (Greig et al., 2012), functional evenness in our study remained stable, suggesting that cold, nutrient-poor environments may promote strict resource partitioning, thus maintaining functional integrity under current conditions.

Functional Divergence and Environmental Filtering

An increase in functional divergence with elevation indicates that communities at high altitudes exhibit greater specialization in ecological roles. These findings align with Mouillot et al. (2005), who reported that communities under stringent environmental filters show heightened functional divergence due to specialized adaptations. Similarly, Harrington et al. (2016) noted increased functional divergence in high-elevation sites, where selective pressures favored unique functional traits. In Colorado, this trend suggests that selective pressures favor traits for cold tolerance, low energy demands, and specialized feeding strategies, reflecting findings by Polazzo et al. (2024) in alpine streams.

This observed increase in functional divergence has critical implications for resilience. Low-elevation communities, with higher functional redundancy due to diverse resources and milder conditions, may be more resilient to disturbances. In contrast, at higher elevations, where functional divergence dominates, ecosystems could be more vulnerable to perturbations affecting specific functional groups. This trade-off between resilience and specialization has been addressed by Villeger et al. (2008), who noted that while specialized communities perform well in stable conditions, they are susceptible to environmental changes beyond their adaptive limits. Díaz and

Cabido (2001) found that functional divergence in high-stress environments may lead to enhanced niche differentiation but at the cost of reduced redundancy and increased vulnerability to novel disturbances.

Influence of Temperature, Canopy Cover, and Substrate Composition

Temperature was the primary driver influencing taxonomic and functional diversity along the elevational gradient. Colder conditions at higher elevations likely impose physiological limitations on species, reducing diversity as altitude increases. These results align with Bonada et al. (2007), who documented temperature as a limiting factor in high-altitude insect communities, and with Ghalambor et al. (2006), who noted that species in temperate environments exhibit broader fluctuations than those in tropical settings. In the Rocky Mountains, temperature fluctuations likely narrow the range of adaptable taxa, allowing only cold-tolerant species to persist at high altitudes.

Canopy cover significantly influenced primary productivity, affecting resource availability. High-elevation, densely vegetated sites had lower functional richness, likely due to decreased light availability and limited algal growth. This supports findings by Boyero et al. (2011), who noted that heavy canopy cover limits diversity by restricting light for primary consumers. Poff et al. (2006) similarly highlighted the role of riparian vegetation in limiting algal growth, thereby affecting functional traits among primary consumers. Reduced functional richness in shaded areas points to the importance of riparian vegetation in shaping food web structure, limiting niches, and affecting functional roles in these aquatic systems.

Substrate composition, particularly at lower elevations, shaped community structure by providing complex microhabitats that support varied functional roles. Villeger et al. (2008) found that mixed sediment types promote functional diversity by increasing microhabitat heterogeneity. In contrast, high-altitude rocky substrates in this study supported fewer functional roles, as species in these environments require adaptations for stability and reduced habitat complexity. Mouillot et al. (2005) similarly emphasized the importance of substrate heterogeneity in fostering niche specialization, as fine sediments create environments that facilitate diverse ecological roles by offering various functional niches.

Functional Divergence by Elevation Bands

Functional Divergence (FDiv) increased with elevation, indicating a broader range of trait diversity at higher altitudes. This pattern is consistent with Shah et al. (2018), who reported increased trait variability at higher elevations in aquatic insect communities, linking this dispersion to adaptations to high-elevation conditions such as lower temperatures and reduced oxygen. Poff et al. (2006) similarly described how elevation promotes trait diversity, as high-altitude communities often require unique functional adaptations for survival under harsher conditions. This phenomenon aligns with findings from Jackson et al. (2008), who observed that trait variability in high-elevation streams supports a broad range of ecological functions, enhancing ecosystem resilience.

Functional Richness (FRic) showed consistent values across elevation bands, indicating that communities across altitudes occupy a similar volume of trait space. This consistency may reflect a shared baseline of functional roles across elevations, a trend that complements Poff et al.'s (2006) observation that functional richness remains stable across gradients due to fundamental ecosystem needs. Harrington (2016) also noted that while trait space occupancy remains stable, trait compositions shift to reflect environmental pressures at each elevation, suggesting that while the volume of trait space is constant, the traits within that space vary with altitude.

Functional Evenness (FEve) was highest at lower elevations, suggesting a balanced trait distribution where no single trait type dominates. This finding mirrors the observations of Gill et al (2016) who noted that in more stable environments, trait evenness tends to be higher, likely due to reduced environmental pressures that allow a more balanced distribution of functional roles. Such environments enable a greater variety of ecological interactions, reducing competitive exclusions and promoting functional stability. Jackson et al. (2008) also found that lowland ecosystems often support higher trait evenness due to greater habitat predictability, which allows traits to be distributed more evenly.

Comparative Analysis by Drainage

The drainage-level analysis showed differences in trait diversity. Big Thompson drainage (from here onwards referred to as BT in the document and figures) exhibited the highest Functional Divergence (FDiv) and lowest Functional Evenness (FEve), indicating a broader spread of functional traits but less uniform distribution (Table 1.3). This pattern aligns with Shah et al. (2018), who found that dynamic habitats with high physical variability often support greater trait dispersion as species adapt to diverse ecological niches. The lower FEve in BT drainage suggests the dominance of specific traits, potentially in response to varied or extreme conditions, a pattern also noted by Poff et al. (2006) in riverine systems where fluctuating environmental pressures favor specialized adaptations over uniform trait distributions.

Cache La Poudre drainage (from here onwards referred to as P in the document and figures), conversely, had the lowest Functional Divergence but the highest Functional Evenness, suggesting a stable environment with fewer, more evenly distributed functional roles (Table 1.3). Harrington (2016) observed similar patterns in stable habitats, where high evenness indicates reduced ecological pressures, allowing functional roles to be more equally represented. Jackson et al. (2008) also found that environments with reduced variability tend to support a narrower range of specialized adaptations, resulting in lower dispersion and higher evenness. This stability in trait distribution could reflect the more predictable conditions within the Poudre drainage, promoting a balanced and resilient community structure.

Saint Vrain drainage showed moderate values for both Functional Divergence and Evenness, indicating a transitional environment where trait diversity is balanced between specialization and generalist functions. This observation aligns with Gill et al (2016) findings on transitional ecosystems, which often exhibit a mix of specialist and generalist traits. Transitional habitats, as noted by Harrington (2016), provide a blend of ecological pressures that support both adaptable generalist traits and specific functional roles, resulting in a mixed functional diversity profile.

Elevation Gradient Analysis of Functional Diversity Metrics

Analyzing Functional Richness (FRic), Functional Divergence (FDiv), and Functional Evenness (FEve) across the elevation gradient provided insights into trait specialization and distribution patterns. Functional Divergence (FDiv), showed an increase with elevation, suggesting a trend towards specialized trait adaptations in higher-altitude environments. This increase supports Shah et al. (2018) and Harrington (2016) results, who described elevated trait divergence in high-altitude ecosystems as species occupy unique ecological niches driven by specialized adaptations (Figure 1.6).

Functional Evenness (FEve) showed limited variation across elevations, with slightly lower values at high altitudes. This finding complements Gill et al (2016) observations of reduced trait evenness in variable environments, where certain functional roles dominate due to adaptive pressures. Poff et al. (2006) described similar patterns, where high-elevation ecosystems support specific traits that confer survival advantages in colder and less predictable climates, thus reducing overall trait evenness (Figure 1.7).

Functional Trait Space and Cluster Analysis

A Multidimensional Scaling (MDS) analysis revealed distinct clusters in functional trait space, providing further insights into how elevation and drainage influence community structure. This analysis produced three clusters in the sites sampled (Figure 1.8). Cluster 1, with a high representation of elevated sites, showed the broadest Functional Divergence, indicating diverse trait adaptations at high elevations. This cluster supports Harrington et al. (2016) findings on high-altitude communities, where functional trait diversity is more pronounced due to the need for unique adaptations to environmental stressors. Shah et al. (2018) similarly described high-elevation clusters with broad Functional Divergence, underscoring the range of survival strategies in challenging environments.

Cluster 2, represented the majority of mid-elevation sites, displayed compact trait distributions, suggesting specialized functional roles tailored to moderate, transitional environments. This clustering aligns with the results suggested by Poff et al. (2006), who observed that mid-elevation communities exhibit unique functional compositions due to the mix of environmental pressures. Gill (2017) work on trait distribution in transitional zones also supports this finding, noting that mid-elevation ecosystems often contain communities with a narrow but specialized range of traits adapted to specific environmental conditions.

Cluster 3, which spanned across all elevation bands, was marked by moderate Functional Divergence and Richness, indicating the presence of generalist traits that enable survival across a broad range of conditions. This cluster likely represents flexible traits that are advantageous in diverse environments, a concept supported by Jackson et al. (2008) who noted the importance of adaptable traits in maintaining ecological stability across fluctuating environments.

Broader Implications for Conservation and Stream Ecosystem Resilience

The functional diversity patterns observed in this study align well with existing literature and underscore the role of environmental gradients in shaping community structure. Our findings corroborate Poff et al. (2006) and Harrington (2016) regarding elevation-driven functional divergence and trait specialization in high-altitude ecosystems. Additionally, the high Functional Divergence in dynamic drainages supports Shah et al. (2018), who observed that heterogeneous habitats foster a wide range of trait adaptations.

Overall, these results illustrate the importance of both stable and variable environments in driving functional diversity in aquatic insect communities. High-elevation sites and dynamic drainages promote trait specialization and variability, while stable drainages and low-elevation sites support more balanced and resilient community structures. This research expands on the frameworks established by Gill and Jackson et al. (2008), suggesting that a mixture of specialized and generalist functional traits is crucial for maintaining ecological stability across environmental gradients.

This study underscores the importance of functional diversity in maintaining stream ecosystem resilience. Functional evenness, especially under environmental stress, suggests that the balance of resource utilization among species could help preserve critical ecological functions. Shah et al. (2018) and Díaz et al. (2003) support this notion, arguing that functional evenness contributes to ecosystem stability by balancing resource use among functional groups. However, increasing functional divergence with elevation highlights reliance on species with specialized traits, potentially limiting the system's adaptability to environmental changes.

Considering projected climate change impacts on temperate streams, conservation strategies should prioritize habitat complexity and stability, especially in low-elevation areas where functional richness and redundancy are greatest. Protecting these areas from anthropogenic effects, such as deforestation and land-use changes, could help sustain functional diversity and ecosystem resilience in temperate streams (Greig et al., 2012; Bonada et al., 2007). At high elevations, where functional divergence is prominent, communities may benefit from targeted interventions to reduce stressors like climate warming, which could further limit adaptive capacity (Villeger et al., 2008).

1.6 Tables and figures

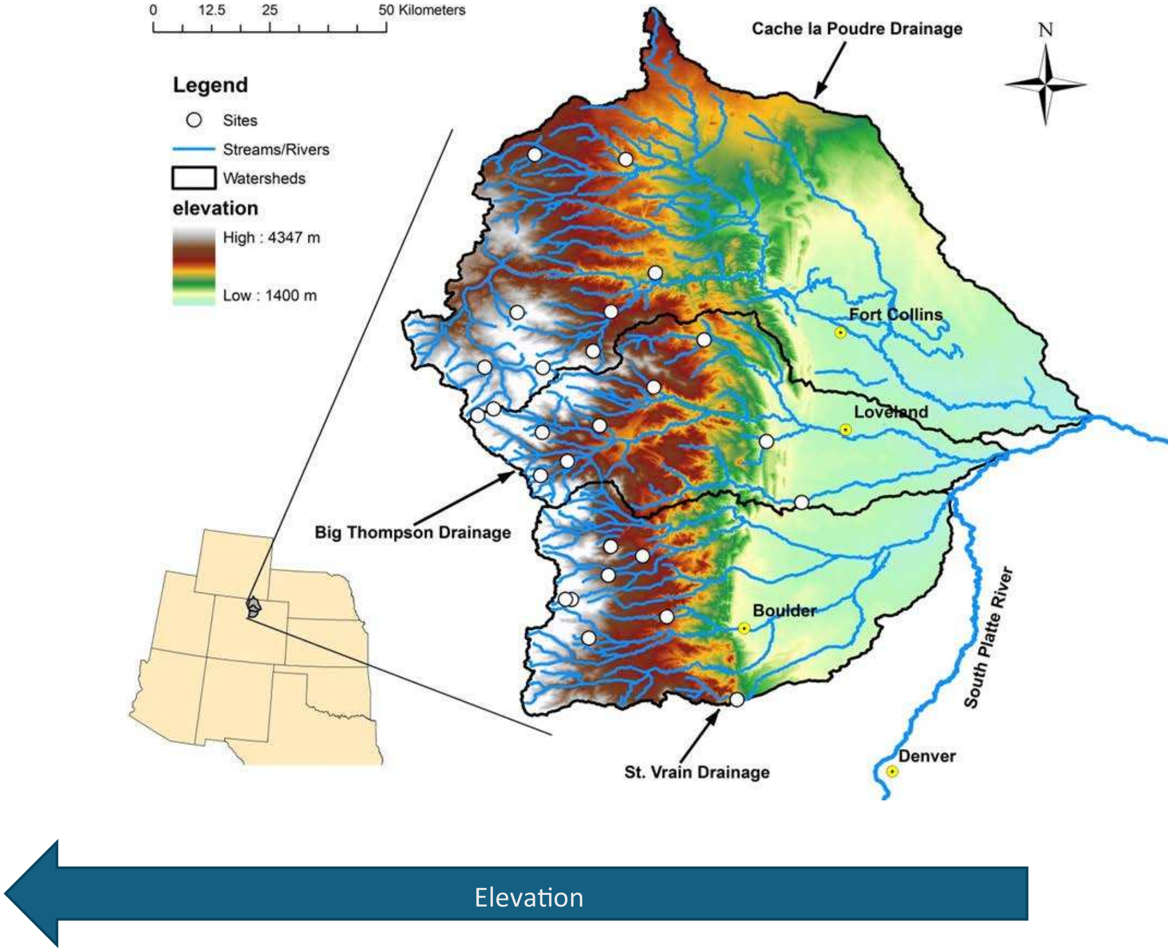

Figure 1.1. Map of the study area, depicting all sampling sites. The bottom left inset locates Colorado within the United States and the three catchments in the Front Range of Colorado. Sites CP1-CP8, BT1-BT8 and SV1-SV8 are located within the Cache la Poudre River, the Big Thompson River and the Saint Vrain Creek catchments, respectively. Furthermore, sites located within the low-elevation zone, mid-elevation zone and high elevation zone are denoted by the colors green, yellow and red, respectively. Refer to Table 1 for site elevations.

Table 1. 1. Drainage, GPS coordinates and elevation (m) of sites studied. Alphanumeric ID's for each site indicate drainage and position along the elevation gradient. * denotes sites that have no published name.

Drainage	**Site Name & ID**	**Latitude ('N)**	**Longitude ('W)**	**Elevation (m)**
Cache la Poudre	Elkhom Creek - CP1	40.7000	105.4415	1992
	Trail Creek - CP2	40.9185	105.4984	2181
	Little Beaver- CP3	40.6253	105.5271	2411
	Beaver Creek - CP4	40.9277	105.6744	2590
	unnamed* - CP5	40.5492	105.5617	2775
	Corral Creek - CP6	40.5181	105.7708	3060
	E.F. Sheep Creek- CP7	40.6235	105.7080	3166
	Unnamed* - CP8	40.5173	105.6589	3397
Big Thompson	Buckhom Creek - BT1	40.5711	105.3477	2001
	Miller Fork - BT2	40.4799	105.4448	2252
	Black Canyon - BT3	40.4056	105.5491	2443
	Mili Creek - BT4	40.3368	105.6113	2573
	Hidden Valley - BT5	40.3926	105.6597	2900
	unnamed* - BT6	40.3098	105.6631	3051
	Big Thompson - BT7	40.4256	105.7840	3364
	Fall River - BT8	40.4380	105.7535	3478
Saint Vrain	Coal Creek - SV1	39.8776	105.2844	2015
	Four Mile Creek - SV2	40.0374	105.4194	2189
	Cave Creek- SV3	40.1547	105.4663	2388
	Rock Creek - SV4	40.1727	105.5279	2643
	Beaver Creek - SV5	40.1173	105.5324	2830
	Caribou Creek - SV6	39.9961	105.5699	2964
	unnamed*- SV7	40.0707	105.6033	3249
	unnamed* - SV8	40.0709	105.6149	3348

Table 1. 2. Description of the traits selected for assignment inside the functional trait matrix.

Traits	Number of Trait States
Life history	
Ability to survive desiccation	2
Development	3
Synchronization of emergence	2
Voltinism	3
Mobility	
Estimated Female dispersal	2
Maximum crawling rate	3
Occurrence in drift	3
Swimming ability	3
Morphology	
Armoring	3
Attachment	3
Respiration	3
Shape	2
Size at maturity	3
Ecology	
Habit (in ecosystem)	6
Rheophily	3
Thermal preference	3
Trophic Habit	5

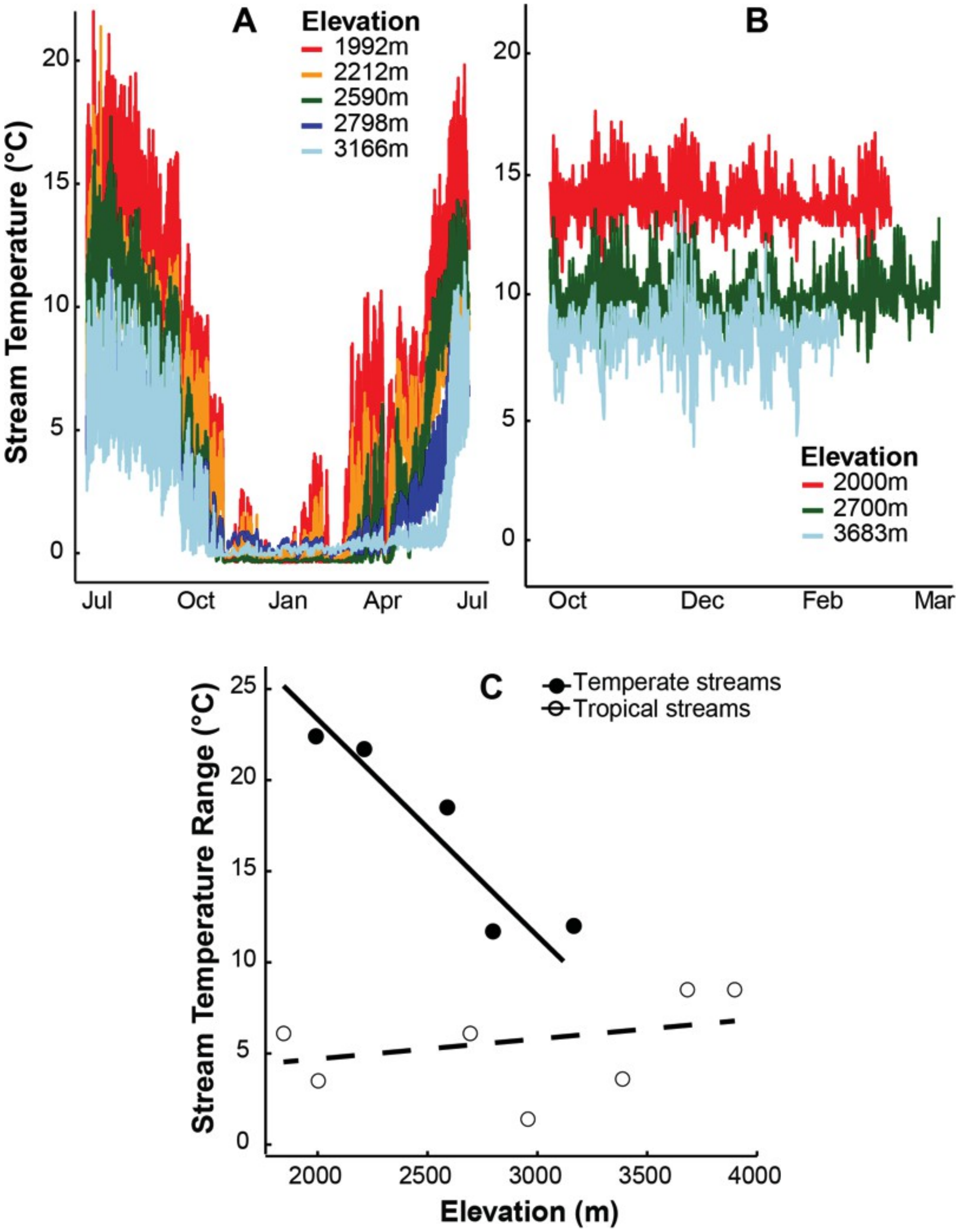

Figure 1.2. Stream temperature profiles for low-to-high elevation temperate streams, showing increased overlap in temperature, especially in the winter months (A), tropical streams with reduced overlap (B). C shows the relationship between stream temperature range (annual maximum - minimum) and elevation, where it decreases with elevation in temperate streams, but has an increasing trend with elevation in tropical streams. *Source:* Shah (2017).

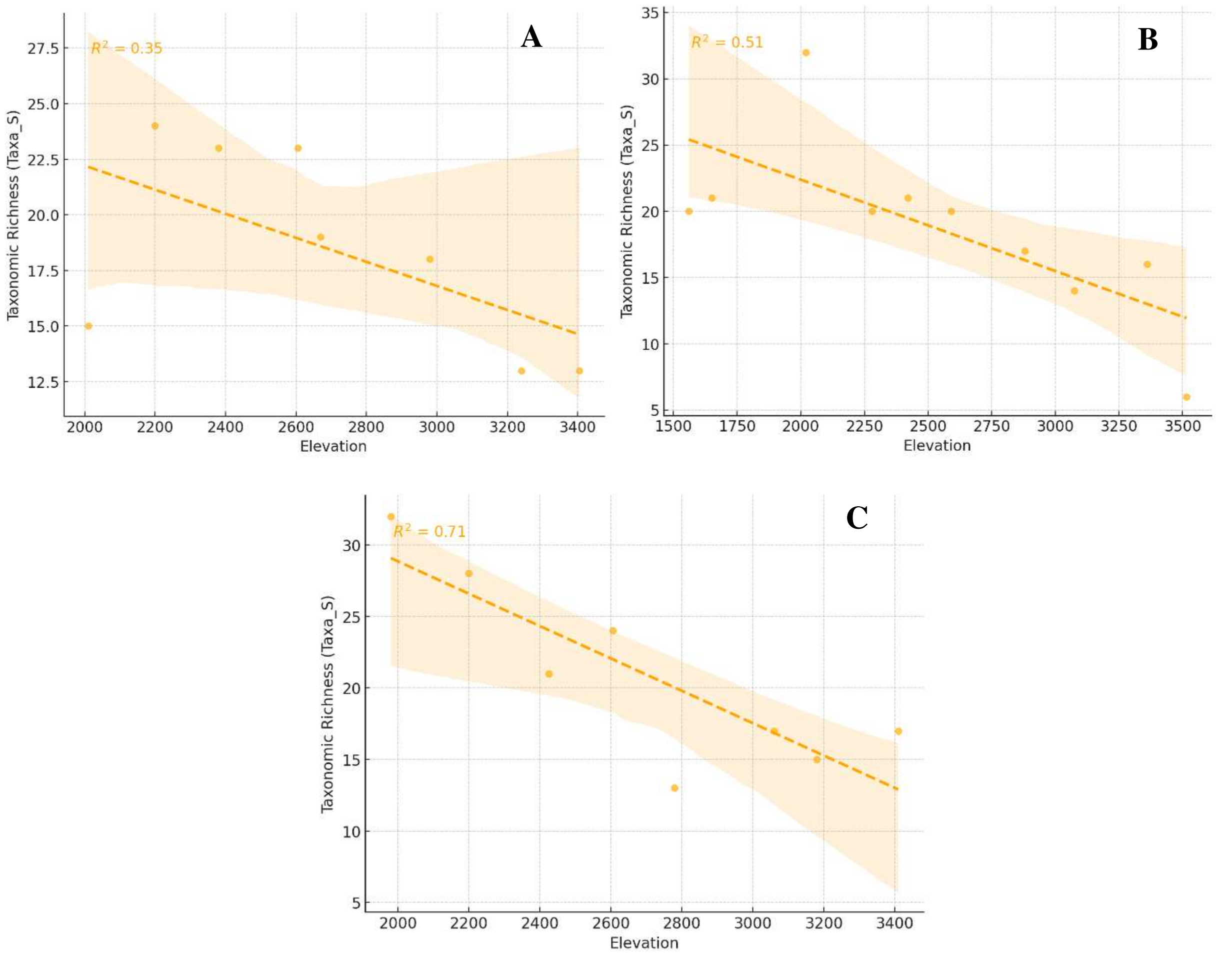

Figure 1.3. Scatter plot of aquatic insect taxonomic richness vs. elevation in 24 sampling sites spanning three drainages. The scatter plot illustrates the relationship between taxonomic richness (Taxa_S) and elevation within the A) Saint Vrain, B) Big Thompson, and C) Cache La Poudre drainage groups respectively; with a linear regression trendline shown. The negative slope on all plots suggests a decrease in taxonomic richness as elevation increases, which may indicate environmental constraints at higher altitudes. The R^2 values of 0.35, 0.51, and 0.71 indicate that 35%, 51%, and 71% of the variation in taxonomic richness is related to elevation in these drainage groups respectively.

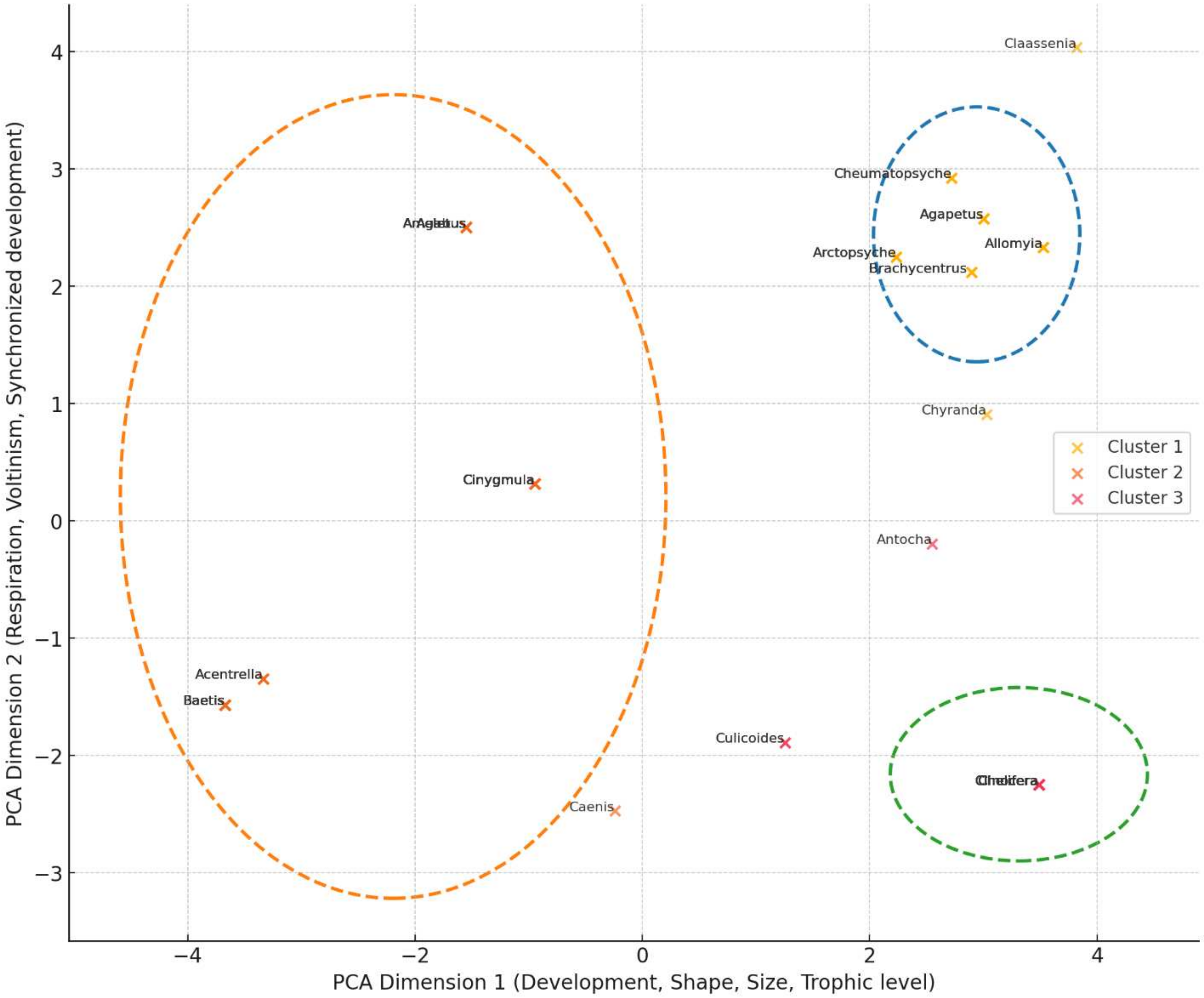

Figure 1.4. Abundance-Based Genera Clusters in Trait Space, showing groups of genera in trait space based on abundance patterns across sites. Each data point represents a genus, with ellipses indicating clusters grouped by similar abundance distributions. PCA Dimension 1 captures variation in abundance levels across sites, highlighting genera with distinct overall abundances. PCA Dimension 2 reflects distribution patterns, distinguishing genera that are more widespread versus those localized to specific sites. Together, these dimensions illustrate functional groupings and niche differentiation among genera based on abundance-driven ecological roles. Traits driving variation in this analysis are development, shape, size, trophic level, respiration, and voltinism.

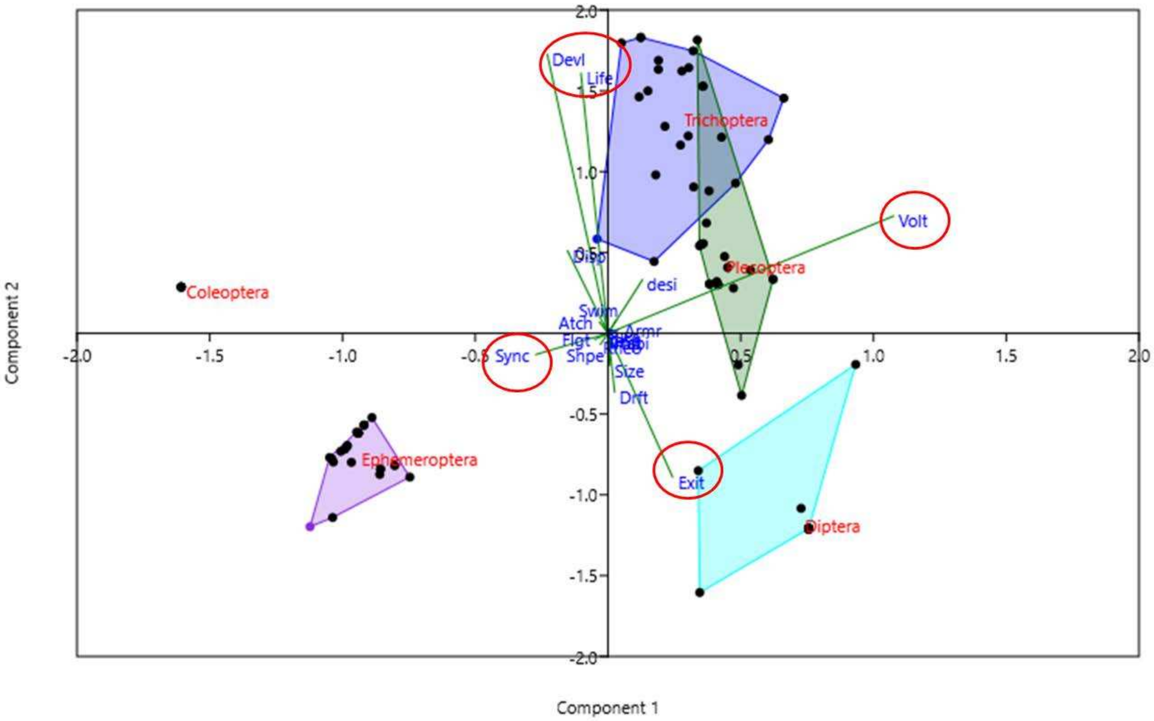

Figure 1.5. Ordination Triplot resulting from a Principal Component Analysis (PCA) of aquatic insect orders collected in all sampling sites, abundance, and functional trait distribution. This analysis shows a cluster grouping for Ephemeroptera (Mayflies) associated to synchronization of emergence traits. The order Trichoptera (Caddisflies) cluster is closer to the influence of developmental traits, while the order Plecoptera (Stoneflies) is closer to the influence of voltinism trait states (number of generations per year). The order Diptera (in this study, the aquatic larva of mosquitoes) is represented closer to the ability to exit functional traits. All the represented states in this plot belong to the category of life-history traits.

Table 1.3. Functional Diversity Metrics by Drainage, showing similar Functional Richness across drainages, but Increased Functional Divergence in the Big Thompson drainage; and increased Functional Evenness in the Poudre drainage.

Drainage	**Functional Divergence (FDiv)**	**Functional Richness (FRic)**	**Functional Evenness (FEve)**
Big Thompson	1.18	10.21	0.85
Poudre	0.78	10.21	0.90
Saint Vrain	0.82	10.21	0.88

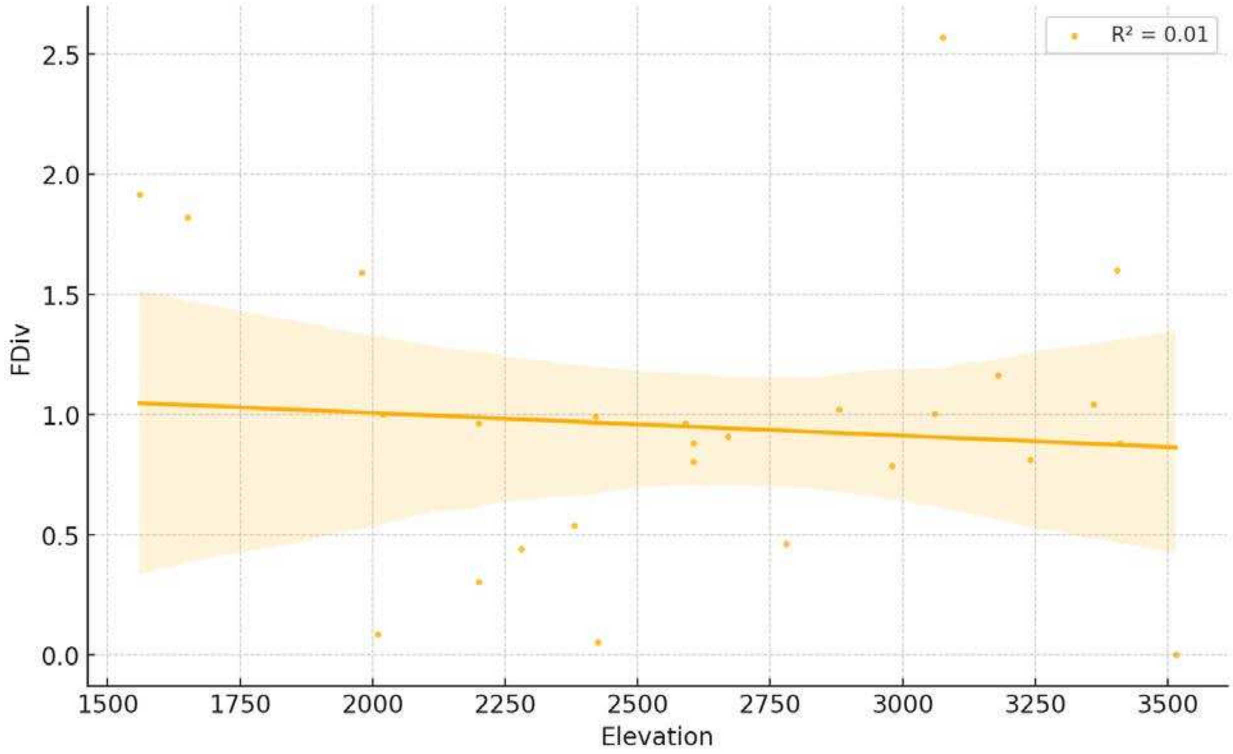

Figure 1.6. Functional Divergence (FDiv) by Elevation scatter plot illustrating the relationship between elevation (m) and Functional Divergence (FDiv) across 24 sampling sites. Higher elevations generally showed a greater spread in FDiv values, suggesting increased niche differentiation and variability in trait composition at these elevations. The linear regression line indicates the trend in the data.

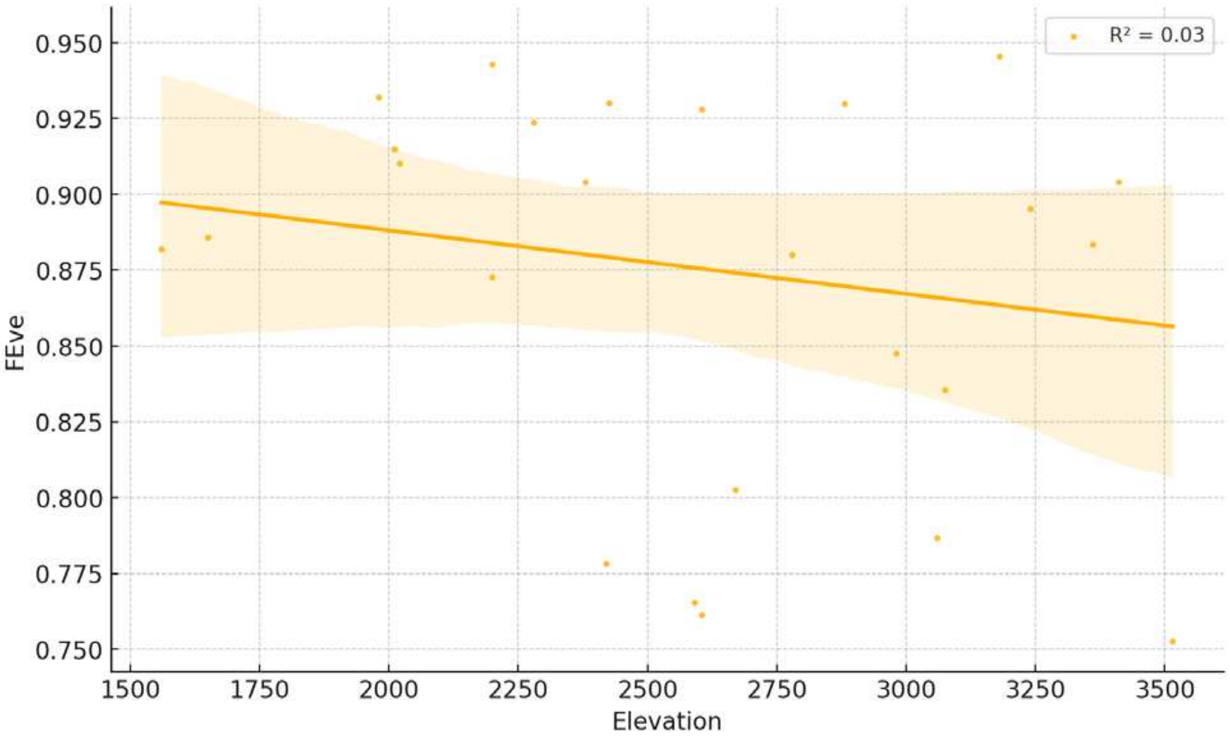

Figure 1.7. Functional Evenness (FEve) by Elevation scatter plot highlighting the variation in Functional Evenness (FEve) across a continuous elevation gradient in 24 sampled sites. FEve decreased slightly at higher elevations, suggesting a less uniform distribution of functional traits in high-altitude communities. The linear regression line indicates the trend in the data.

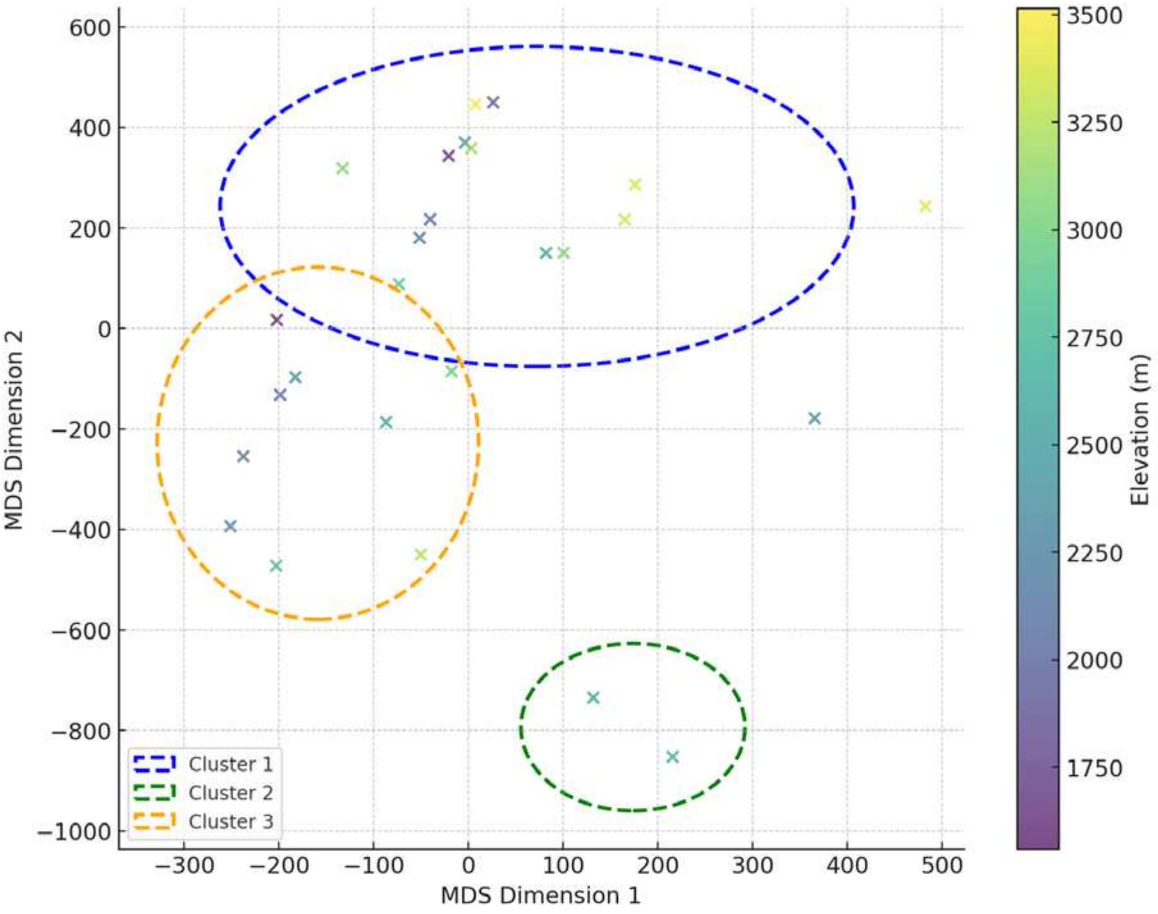

Figure 1.8. Functional trait space of communities by elevation multidimensional scaling (MDS) plot, displaying the functional trait space occupied by aquatic insect communities across elevation gradients. Data points represent communities, color-coded by elevation. The spread and clustering of points reflect how trait composition varies with elevation, with higher-elevation communities occupying distinct regions in the trait space.

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Chapter 2: FUNCTIONAL ANALYSIS OF TROPHIC INTERACTIONS IN THE AQUATIC INSECT COMMUNITY ALONG AN ELEVATION GRADIENT IN MOUNTAIN HEADWATER STREAMS

2.1 Summary

Chapter 2 explores the functional diversity of aquatic insect communities across elevational gradients, focusing on the trophic roles and interactions that shape these ecosystems. Using metrics such as Functional Richness (FRic), Functional Divergence (FDiv), and Functional Evenness (FEve), this study assesses how aquatic insects contribute to ecosystem processes and adapt to resource variability. These metrics are applied to understand how ecological roles vary across elevations, contributing to a growing body of research on ecosystem functionality (Mason et al., 2005; Villéger et al., 2008).

The trophic interactions within these communities are investigated using a binary matrix to identify predator-prey relationships, following approaches from Dunne et al. (2002) and Bersier et al. (2002). Network models generated with Network3D illustrate complex interactions across elevations, while discriminant analysis of mean gut content area captures variations in dietary composition across sites. Resources analyzed include fine particulate organic matter (FPOM), coarse particulate organic matter (CPOM), and autochthonous sources such as microalgae. This methodology, aligned with similar approaches by Poff et al. (1997) and more recent studies (Guisande et al., 2016), offers insights into how elevation influences trophic dynamics.

Results indicate significant variability among 742 gut samples representing 51 taxa. Functional groups identified include collector-gatherers, filterers, predators, and shredders, each

exhibiting distinct dietary preferences and contributing to nutrient cycling. Collector-gatherers and filterers, such as *Ameletus* (Ameletidae) and *Simulium* (Simuliidae), primarily consume Fine Particulate Organic Matter (FPOM) and Coarse Particulate Organic Matter (CPOM), playing vital roles in organic matter processing (Wallace & Webster, 1996). Predators, including *Rhyacophila* (Rhyacophilidae), exhibit broader diets that include animal tissue, suggesting their essential role in population control within food webs (Woodward & Hildrew, 2002).

Elevational gradients are known to impact species distribution and functional traits, as noted in Bispo et al. (2021), where beta diversity in mountain streams reflects environmental turnover, leading to diverse insect communities with site-specific adaptations (Bispo et al., 2021). The modularity of networks observed by Serna et al. (2022) in Colombian Andean streams also underscores the structural complexity and resilience of these systems, particularly during seasonal shifts (Serna et al., 2022). Similarly, studies such as Maitland and Rahel (2023) have shown that food web structures in streams reflect a balance between niche partitioning and redundancy, essential for ecosystem stability (Maitland & Rahel, 2023).

This study encountered challenges in modeling due to the variability in food resources and feeding behaviors across sites. High turnover rates among communities, as seen in Yegon et al. (2021), emphasize the unique adaptations required by macroinvertebrates to exploit available resources at varying elevations (Yegon et al., 2021). Hayden et al. (2021) further demonstrate that stable isotope analysis can provide critical insights into resource usage, highlighting the importance of allochthonous inputs in determining community composition in headwater streams (Hayden et al., 2021).

The findings reveal how functional diversity supports ecosystem resilience by facilitating niche partitioning and trophic complementarity across elevations. These dynamics resonate with the global-scale patterns observed by Boyero et al. (2021), who identified that detritivore diversity enhances litter decomposition—a crucial process particularly relevant in tropical systems with low diversity and high environmental pressures (Boyero et al., 2021). Additionally, Schriever and Lytle (2020) indicate that top predators contribute to food web stability by bridging aquatic and terrestrial resource flows, which is essential for community balance and resilience (Schriever & Lytle, 2020).

Ultimately, this chapter underscores the role of functional diversity in maintaining ecosystem stability across environmental gradients. By examining FRic, FDiv, and FEve across sites, this study contributes to understanding the adaptive responses of aquatic insect communities to environmental pressures, a key insight for biodiversity conservation. These findings align with broader ecological theories, such as the River Continuum Concept (Vannote et al., 1980), reinforcing the importance of sustaining functional diversity for ecosystem functionality in freshwater systems across elevations.

2.2 Introduction

Aquatic insect communities in freshwater ecosystems play a critical role in maintaining ecosystem processes, particularly in headwater streams where they contribute to nutrient cycling, organic matter decomposition, and trophic interactions. Functional diversity—measured through metrics such as Functional Richness (FRic), Functional Divergence (FDiv), and Functional Evenness (FEve)is key to understanding the ecological roles of different taxa within these communities and how these roles shift along environmental gradients such as elevation. Functional diversity captures the range and distribution of traits that organisms contribute to ecosystems, offering insights into ecosystem resilience, adaptability, and function (Mason et al., 2005; Villéger et al., 2008). Exploring the functional roles of aquatic insects across elevational gradients can reveal important dynamics of biodiversity, particularly in stream ecosystems where environmental variability and complex trophic interactions shape community structure and function.

In headwater stream ecosystems, where detritus from leaf litter provides a major input of energy and nutrients, the decomposition process links terrestrial and aquatic systems. Leaf litter decomposition is fundamental to nutrient cycling and energy flow, as it facilitates the breakdown of organic matter into forms accessible to various decomposers and consumers (Zhang et al., 2019). The rate and efficiency of decomposition are influenced by the quality of the leaf litter, including its lignin and nitrogen content, as well as by environmental factors such as temperature, oxygen levels, and hydrology (Lecerf & Chauvet, 2008; Ferreira & Canhoto, 2015). Broadly, litter from deciduous species decomposes faster than that from evergreen species due to lower lignin and higher nutrient contents (Gessner & Chauvet, 1994; Albariño & Balseiro, 2002). The decomposition rates and pathways in streams have been shown to vary globally, yet trait-based models suggest that litter quality often predominates as a control factor, highlighting the role of functional traits in nutrient cycling (Boyero et al., 2016; Zhang et al., 2019).

In addition to environmental drivers, the functional diversity of aquatic insects significantly influences decomposition. Aquatic insects can be categorized into functional groups—such as shredders, filter-feeders, and predators—based on their feeding strategies and resource preferences, which directly impact nutrient cycling and organic matter breakdown. Shredders, for instance, contribute to breaking down coarse particulate organic matter (CPOM) into fine particulate organic matter (FPOM), making it accessible to filter-feeders and other detritivores (Wallace & Webster, 1996; Graça et al., 2001). Predators, on the other hand, not only regulate prey populations but also influence the physical environment and nutrient flows through their interactions with other organisms (Majdi et al., 2013; Zhang et al., 2019). Non-trophic interactions, such as habitat modifications by predators, add complexity to these roles. For example, flatworm predators have been shown to affect litter decomposition rates by altering sediment deposition and leaf colonization by invertebrates, thus impacting microbial communities that contribute to organic matter breakdown (Majdi et al., 2013).

The spatial dynamics of functional diversity in stream ecosystems are often examined through beta diversity, which measures the variation in species composition across communities and provides insight into ecological processes shaping diversity at broader scales (Baselga, 2010; Heino & Tolonen, 2017). Beta diversity in mountain streams, where high environmental variability and distinct elevational zones exist, is often structured by both turnover and nestedness components. Turnover reflects species replacement between communities, while nestedness describes patterns where communities with lower species richness are subsets of more diverse communities (Baselga, 2013; Bispo et al., 2021). These patterns are shaped by environmental gradients, such as temperature and stream flow, which influence both species dispersal and persistence across different habitats (Leibold et al., 2004; Bispo et al., 2021). For instance, turnover is more pronounced in areas with significant environmental differences, as species with distinct functional traits adapt to specific local conditions (Leprieur et al., 2012). This understanding is crucial for conservation, as high turnover suggests the need to preserve multiple sites within hydrographic networks to capture the full spectrum of biodiversity (Bispo et al., 2021).

Functional diversity is not only affected by species turnover but also by intraspecific variability in trophic and functional niches, as individuals within the same species may exploit different resources depending on environmental conditions and competitive interactions. Raffard et al. (2020) demonstrated that environmental gradients, such as upstream-downstream shifts in resource availability, drive intraspecific differences in trophic niches among populations of European minnows. Such variation enables species to adapt to resource constraints and competition, highlighting the importance of functional traits in mediating responses to environmental pressures. The flexibility in trophic niches within and between species underscores the adaptive potential of aquatic communities in dynamic environments (Raffard et al., 2020).

Predator diversity and intra-guild interactions also play a significant role in shaping the community structure of aquatic insects. Studies have shown that multiple predator effects (MPEs), such as those observed in fish predator communities, influence prey populations and trophic dynamics through non-additive interactions (Wasserman et al., 2016). These effects often depend on functional responses, where prey consumption rates vary with prey density. For example, functional responses of heterospecific predator combinations can lead to prey risk reduction or enhancement, depending on the specific interactions among predator species (Soluk, 1993; Wasserman et al., 2016). In headwater streams, MPEs are particularly relevant because they can alter prey availability and disrupt the functional roles of key species in nutrient cycling (Wasserman et al., 2016). Furthermore, invasive species can alternative predator-prey dynamics, as seen in the study by Pelikan et al. (2024) on amphipod competition, where non-native species outcompete natives in headwater streams, potentially leading to shifts in trophic roles and nutrient cycling.

Invasion ecology provides additional insights into how species introductions impact functional diversity. Non-native species often possess traits that enable them to exploit similar resources as native species, creating competition that can displace native functional roles (Pelikan et al., 2024). This phenomenon has been well-documented in amphipod communities, where invasive species with generalist diets compete with natives for resources, potentially altering ecosystem processes (Jażdżewski, 1980; Jackson et al., 2017). Such interspecific competition can reduce functional redundancy and disrupt trophic networks, leading to declines in ecosystem stability (Torchin et al., 2003; Strayer et al., 2006). The functional morphology of invasive species, as well as their behavioral flexibility, contributes to their success in new environments, as evidenced by studies combining stable isotope and gut content analyses to reveal trophic overlaps and niche shifts (Copilaș-Ciocianu et al., 2021).

Beta diversity and functional diversity thus reveal important insights into the spatial and trophic organization of stream ecosystems. In a metacommunity framework, functional diversity can be decomposed into taxonomic, phylogenetic, and functional facets, allowing a more nuanced understanding of diversity patterns and their drivers (Leprieur et al., 2012; Villéger et al., 2013). Bispo et al. (2021) highlighted that environmental variable, such as stream flow and substrate composition, interact with spatial factors to shape these diversity patterns. Understanding the role of these variables is particularly important in dynamic systems like mountain streams, where episodic disturbances, such as flooding, create patchy habitats that affect benthic fauna and their functional contributions (Matthaei et al., 1999). These disturbances, coupled with dispersal limitations, underscore the importance of habitat connectivity and heterogeneity in supporting functional diversity across elevational gradients (Townsend, 1989; Grönroos et al., 2013).

This chapter builds on extensive literature examining the relationship between functional diversity, trophic interactions, and environmental gradients. By integrating insights from functional response theory, beta diversity frameworks, and invasion ecology, this study aims to enhance our understanding of how aquatic insect communities adapt to and function within elevationally stratified environments. The complex interactions between predators, prey, and detritivores reveal a nuanced picture of ecosystem processes, where both trophic and non-trophic interactions play vital roles. This exploration is essential for informing conservation efforts, particularly in freshwater systems where biodiversity supports critical ecosystem functions and resilience against environmental change. Through this analysis, we aim to contribute to a deeper understanding of functional diversity as a cornerstone of biodiversity conservation and ecosystem management in freshwater streams.

2.3 Methods

This chapter focuses on analyzing the functional diversity and trophic dynamics of aquatic insect communities across altitudinal gradients in temperate streams. To achieve this, I conducted a systematic study on insect assemblages at various elevations, employing quantitative measurements of functional traits, and gut content examinations to categorize functional feeding groups (FFGs) and evaluate trophic interactions.

Study Sites and Sampling

The study was conducted in the Rocky Mountains, Colorado, in the Cache La Poudre River catchment area. Streams within the area were selected to cover a range of elevations, from lowland to high-elevation sites, to capture the variability in community structure along these gradients (Coat et al., 2009). Sampling was carried out

For this chapter, field sampling was conducted at five distinct sites along the Poudre River drainage in Colorado, each at a unique elevation to capture ecological variations across an altitudinal gradient. These sites ranged from lower elevations at Elkhorn Creek (1992 meters) to higher elevations at West Fork Sheep Creek (3200 meters), allowing for a comprehensive assessment of biodiversity, trophic structure, and functional traits across different environmental conditions. Each site has been assigned an abbreviation that will be used consistently throughout this chapter:

Elkhorn Creek (1992 meters) – COP1992: Positioned at the lowest elevation within the study, COP1992 serves as a baseline site, enabling comparisons of taxa composition, trophic interactions, and functional traits relative to higher-elevation sites.

Sevenmile Creek (2212 meters) – COP2212: Situated above COP1992, this mid-elevation site captures shifts in community dynamics, highlighting changes in taxa and functional traits as altitude increases.

Beaver Creek (2590 meters) – COP2590: COP2590 represents conditions found at moderately high elevations, allowing for the examination of species adaptations and functional diversity at this altitude.

Killpecker Creek (2798 meters) – COP2798: Positioned near the upper limit of the elevation range, COP2798 provides a snapshot of taxa and functional adaptations characteristic of high-altitude conditions, where environmental constraints such as temperature and oxygen availability begin to play a more significant role.

West Fork Sheep Creek (3200 meters) – COP3200: As the highest elevation site, COP3200 represents the extreme of the altitudinal gradient. Conditions here are more challenging, with colder temperatures and lower productivity, which may favor a simplified trophic structure, and taxa adapted to high-elevation ecosystems.

Sampling at these sites (COP1992, COP2212, COP2590, COP2798, and COP3200) allowed for a research opportunity on how elevation influences community composition, trophic relationships, and functional trait diversity across a mountainous landscape. This design enables a detailed analysis of ecological patterns that emerge along the elevation gradient, providing insights into the influence of environmental filtering mechanisms on community structure in stream ecosystems.

In each stream, I selected three representative sites along the altitudinal gradient. These sites were sampled for aquatic insects, water quality parameters (temperature, pH, and dissolved oxygen), and habitat characteristics (substrate composition and vegetation cover). Insects were collected using a combination of kick nets and surber samplers to ensure a comprehensive representation of benthic fauna across microhabitats, as suggested by Cummins (1973) and Gaines et al. (1989).

Functional Trait Measurement and Feeding Group Classification

Functional diversity metrics—Functional Richness (FRic), Functional Evenness (FEve), and Functional Divergence (FDiv)—were applied to assess the ecological roles of taxa within each community (Villéger et al., 2008). Traits related to feeding mechanisms, such as mandible morphology and mouthpart structure, were examined under a microscope and classified based on established FFGs: shredders, collector-gatherers, collector-filterers, predators, and scrapers (Cummins, 1973; Merritt & Cummins, 1996). Each insect was assigned to an FFG using these morphological traits, following the methods outlined by Motta and Uieda (2004).

Gut Content Analysis

Complementing the stable isotope analysis, gut content analysis was performed on a subset of specimens from each site. Specimens were dissected, and their gut contents were examined under a microscope to identify the presence of various food items, such as FPOM, CPOM, algae, and animal tissue. This qualitative approach helped refine the FFG classifications and provided direct evidence of dietary composition (Cummins, 1973; Mortillaro et al., 2015).

Statistical Analysis

Functional diversity metrics (FRic, FEve, and FDiv) were calculated for each site using the R package FD, which enables the integration of multiple trait dimensions. Analysis of variance (ANOVA) was used to test for differences in functional diversity across sites and between regions.

Network Analysis of Trophic Interactions

A trophic network model was constructed for each site to examine the interactions among functional groups and their potential effects on ecosystem stability. This model incorporated isotopic and gut content data to define feeding links and assign trophic roles to each taxon. Network metrics such as connectance, modularity, and nestedness were calculated to assess the structural complexity of the food webs, following methods by Yang & Sykes (1998) and Lindeman (1991). The trophic network was visualized using the igraph package in R, which facilitated the identification of key taxa and potential shifts in food web structure across altitudinal gradients.

Validation and Quality Control

To ensure data accuracy, all trait measurements and gut content identifications were performed in triplicate for each specimen. Sample processing followed established protocols, with regular calibration of analytical instruments to prevent isotopic drift. Additionally, taxonomic identifications were verified by expert entomologists, and species were cross-referenced with regional databases to maintain consistency with local taxonomic standards (Motta & Uieda, 2004; Mortillaro et al., 2015).

This methodology aims to provide a detailed understanding of functional diversity and trophic structure within aquatic insect communities along altitudinal gradients in temperate and tropical streams. By integrating multiple lines of evidence—functional traits, isotopic signatures, and gut contents—this study will elucidate the role of environmental gradients in shaping community structure and ecosystem function. The outcomes are expected to reveal patterns of functional trait diversity, resource partitioning, and the influence of altitude on trophic interactions, contributing to a broader understanding of ecosystem dynamics in freshwater environments (Lindeman, 1991; Post, 2002; Coat et al., 2009).

This chapter used a suite of trophic structure metrics to rigorously quantify and assess the ecological complexity, connectivity, and functional diversity within the sampled sites' food webs, facilitating a comparative analysis across varying elevations.

Trophic species richness (S) quantifies the diversity of unique trophic species or taxa within each food web, serving as an indicator of biodiversity. A higher S value denotes increased species diversity, contributing to a more intricate ecosystem. The number of trophic levels examines the hierarchical stratification within the food web, particularly focusing on intermediate species positioned between basal and top taxa, thereby elucidating the vertical structure and complexity of the network. The number of trophic links (L) captures the total number of pairwise interactions between species, identifying each predator-prey relationship based on genus-level gut content analysis, thus detailing the web’s interaction complexity.

Linkage density (L/S), which represents the mean number of feeding links per species, serves as an indicator of interaction density within the network. Elevated linkage density values reflect a highly interconnected network in which species often fulfill multiple trophic roles. Connectance (C), defined as the proportion of realized links among the potential links within the food web, is calculated using $C{=}L/S^2$. This metric offers insight into the overall network connectivity, with higher connectance suggesting increased interdependency among species.

Standard deviation generality (SD-G) provides a normalized measure of the mean prey diversity per consumer, reflecting the generality within the network and thus the adaptability of consumer species to exploit a range of prey. Standard deviation vulnerability (SD-V), on the other hand, provides a normalized measure of predation pressure, representing the mean number of consumers per prey species. This measure reveals the relative vulnerability of prey species within the web.

Mean chain length (mFCL) signifies the average pathway length of energy transfer across all potential food chains, from basal to top taxa, providing insight into energy flow complexity within the ecosystem. Maximum chain length (maxFCL), representing the longest trophic sequence within the network, captures the upper limits of energy transfer from primary producers to apex consumers.

The fraction of basal taxa (b) describes the proportion of species occupying the foundational level of the food web, typically including primary producers and decomposers, and serves as an indicator of the ecosystem's energetic base. The fraction of intermediate taxa (i) characterizes species that serve both as consumers and prey, highlighting taxa that mediate energy flow between basal and top trophic levels. The fraction of top taxa (t) quantifies apex species without natural predators, representing the proportion of terminal species within the network.

Collectively, these metrics provide a comprehensive framework for examining trophic structures across altitudinal gradients, offering nuanced insights into species' ecological roles, ecosystem complexity, and the stability and resilience of food webs in response to environmental filtering. This approach facilitates a deeper understanding of how elevation and associated environmental variables shape trophic organization and ecological interactions across distinct habitat types.

2.4 Results

The analysis of trophic webs across sites revealed significant variations in connectance, linkage density, chain length, and niche differentiation, underscoring the influence of site-specific environmental conditions on trophic dynamics. Figure 2.2 illustrates the variability in connectance across sites, emphasizing how resource availability and environmental factors influence food web complexity. Sites exhibiting higher connectance levels indicate greater interdependence within the food web, which could imply resilience due to increased redundancy of trophic links (Shah et al., 2017). However, increased connectivity may also elevate susceptibility to cascading effects if disruptions occur, aligning with findings by Gill et al. (2014), who emphasized the role of interspecies links in maintaining balance in aquatic ecosystems.

Linkage density and trophic chain length (Figure 2.5and Figure 2.6) further illuminate the structure and complexity of these webs, with higher linkage density and extended chains observed at certain sites. This complexity suggests a higher level of functional diversity and adaptability, as Pawar et al. (2012) proposed, where increased dimensionality in food webs supports stability in diverse habitats. Longer trophic chains indicate that energy flows through more levels, potentially supporting a broader array of taxa and interactions. However, the dependence on extended chains may also introduce variability if top predators or keystone species fluctuate in abundance, echoing Carlisle and Clements (2003), who observed the importance of maintaining chain length stability in high-elevation stream systems.

The functional trait clustering shown in Figure 4 provides insight into niche specialization among taxa. Distinct clustering patterns demonstrate adaptive responses to varying site conditions, which is consistent with Poff et al. (2006), who posited that environmental filtering drives trait-based selection, particularly across gradients. The clustering within this analysis suggests that taxa group according to specific ecological functions and conditions, possibly responding to both elevation and resource availability. Shah et al. (2017) similarly noted that trait variability across elevation and environmental gradients often supports niche partitioning, as species develop traits finely tuned to localized conditions, which may help to sustain community stability.

Niche differentiation across trophic roles is further exemplified in Figure 2.7 and Figure 2.8, where basal species richness and the partitioning within predator and herbivore guilds underscore the role of resource partitioning in maintaining balance. High basal species richness, particularly evident in nutrient-enriched sites, supports the foundation of these food webs, aligning with Cummins (1973), who demonstrated the importance of basal diversity in supporting higher trophic levels. Sites with elevated basal richness (e.g., Figure 2.8) display a diverse array of trophic interactions, suggesting that these foundational taxa provide a stable base for food web complexity. The clear partitioning within predator and herbivore groups, as seen in Figure 2.7, reduces interspecific competition and fosters niche differentiation, mirroring findings by Lopez et al. (2013) on the benefits of niche separation for stream ecosystem stability. This resource-driven partitioning allows for distinct ecological roles within each trophic level, promoting coexistence and enhancing community resilience against environmental perturbations (Lewis & McCutchan, 2010).

Trophic web models across sites (Figure 2.9) illustrate the relationships among functional groups in each sampled ecosystem, showing variation in energy flow and trophic interactions from basal resources to top predators. The visual models emphasize structural differences among sites, highlighting the presence of additional intermediate consumers and filter feeders in certain locations, which add layers to the food web. As Cummins (1973) suggested, this added complexity is likely to enhance the functional redundancy and stability of the food web. Each model provides insight into site-specific dynamics, where food webs with a high proportion of intermediate consumers may be better equipped to handle fluctuations in resource availability, echoing Pawar et al. (2012), who described multidimensional interactions as a buffer against disturbances in diverse food webs.

Figure 2.1 complements the analysis by presenting 3D topographic food web models that visually capture variations in food web structure across different sites. This visualization highlights site-specific trophic levels from basal resources to higher consumers and predators, with each level representing functional groups adapted to distinct resource flows and environmental constraints. As Gill et al. (2015) noted, food web architecture is often shaped by the spatial and environmental context, and these 3D models demonstrate how site conditions may influence not only the number of trophic levels but also the strength and nature of connections among them.

The fraction of basal, intermediate, and top taxa across sites (Figure 2.3) provides another layer of insight into the relative trophic structure at each location. High proportions of basal taxa suggest a robust foundation in some ecosystems, possibly due to increased primary productivity or nutrient inputs at lower elevations. Intermediate taxa contribute to the transfer of energy from producers to predators, with variations in their abundance reflecting differential resource availability and environmental filtering at each site. Top taxa proportions indicate predation pressures, with elevated proportions in specific locations suggesting an ecological balance that could either buffer or amplify responses to environmental stress, consistent with findings by Shah et al. (2017) on elevation-related predator effects.

Lastly, species richness across trophic roles (Figure 2.8) highlights the biodiversity within each functional group and suggests differential resource use across elevations. Sites with high richness at basal and intermediate levels exhibit trophic structures that may support greater diversity in consumer levels. This richness aligns with Carlisle and Clements (2003) observations that nutrient-enriched streams promote a diverse foundation of primary producers and primary consumers, which in turn support more complex webs. By creating a stable foundation, these diverse communities contribute to overall ecosystem resilience, offering a functional redundancy that helps to stabilize food webs across environmental gradients.

In summary, the observed patterns of trophic structure, connectance, linkage density, and functional diversity across elevations and sites reflect the intricate balance of environmental filtering and resource availability. The trophic roles and trait distributions captured in these figures collectively highlight how site conditions shape food web architecture, align with published studies on functional ecology and stability, and provide a rich basis for exploring resilience mechanisms across ecological gradients.

2.5 Discussion

Environmental filtering mechanisms at different elevations in this study reflect the selective influence of abiotic factors, such as temperature, oxygen levels, nutrient availability, and water flow, on the community composition and functional traits of aquatic insect taxa. At lower elevations (COP1992, COP2212), environmental filtering is less stringent due to more stable and favorable conditions. Greater resource availability, milder temperatures, and higher productivity allow for a wider range of traits and trophic roles to coexist. This pattern aligns with observations by Carlisle and Clements (2003), who found that nutrient-rich streams support diverse communities with extensive basal resources and intermediate consumers.

Mid-elevation sites (COP2590 and COP2798) demonstrate an intermediate level of environmental filtering. As elevation increases, temperatures drop, and primary productivity decreases, limiting the presence of generalist taxa and promoting trait convergence among those capable of surviving these conditions. This filtering results in a shift from high functional diversity to more specialized traits adapted to specific resources and environmental niches. Lopez et al. (2013) noted similar changes in mid-elevation streams, where species richness and functional diversity begin to taper as resources become more limiting.

High-elevation sites (COP3200) exhibit the strongest environmental filtering. Harsh conditions, such as colder temperatures and lower oxygen levels, restrict community composition to highly specialized taxa with traits adapted for survival under resource scarcity and environmental stress. Species at these sites exhibit traits like cold tolerance, slower metabolic rates, and specialized feeding mechanisms, aligning with findings by Shah et al. (2017) on the effect of elevation-driven environmental pressures. These adaptations result in streamlined food webs with reduced redundancy, where only a narrow set of taxa can thrive, thus creating a specialized, though less resilient, ecosystem.

Food Webs Analysis

The 3D topographic models in Figure 2.1 show distinct trophic structures across sites, with lower elevations displaying layered and interconnected trophic levels, while higher elevations reveal simpler structures. This supports the findings by Gill et al. (2014), who observed that topographic complexity and elevation influence food web layering and interaction strength. Lower-elevation sites display more layered and interconnected food webs, while higher-elevation sites show simplified structures with fewer trophic levels. These visual models support the findings by Gill et al. (2014), suggesting that elevation-driven resource limitations constrain trophic complexity at higher sites.

Connectance varies significantly with elevation, with lower sites showing higher connectance, implying more trophic links and redundancy (Figure 2.2). High connectance at lower elevation sites aligns with the results by Shah et al. (2017), who found that higher connectivity contributes to community resilience in resource-rich environments. Connectance decreases with elevation, indicating fewer trophic interactions and reduced redundancy at higher elevations. Lower connectance at high-elevation sites suggests vulnerability to environmental fluctuations, which is consistent with Shah et al. (2017), who emphasized the role of connectance in ecosystem resilience. Basal taxa are more prevalent at lower elevations, while top taxa increase at higher elevations, suggesting shifts in trophic structure due to environmental filtering (Figure 2.3). Basal taxa dominate at lower elevations, while top predators are more prevalent at high elevations, reflecting shifts in community structure. These trends correspond with Cummins' (1973) observations on basal dominance in nutrient-rich systems, contrasted by predator-driven structures in resource-limited habitats. Functional Trait Clustering via PCA analysis reveals niche differentiation, with taxa clustering by functional traits adapted to specific elevations (Figure 2.4). This supports Poff et al. (2006), who argued that trait-based filtering leads to functional divergence along environmental gradients.

Lower elevation sites show higher linkage density and connectance, consistent with Carlisle and Clements (2003), who noted that nutrient availability in lower sites promotes complex food webs. Higher elevation sites display lower linkage density, indicating simpler, more direct trophic pathways (Figure 2.5). In these lower elevation sites in our analysis (COP1992, COP2212), the diversity and redundancy observed reflect findings by Carlisle and Clements (2003) on nutrient-enriched systems that support a diverse basal community and numerous trophic interactions. These sites display characteristics similar to nutrient-rich lowland streams, where primary productivity drives food web complexity and resilience. Linkage density and connectance are highest at lower elevations, suggesting diverse trophic interactions. Mid- and high-elevation sites show reduced linkage density, indicating simpler, more direct energy flows. Carlisle and Clements (2003) observed that lower-elevation systems promote complex food webs due to nutrient availability.

Longer trophic chain lengths and linkage at mid and high elevations reflect a reliance on extended energy pathways, while lower sites exhibit shorter chains with higher redundancy (Figure 2.6). These patterns align with Pawar et al. (2012) findings on energy transfer efficiency and ecosystem stability in complex webs. Trophic chain length is longer at higher elevations, suggesting extended energy pathways as species adapt to more limited resources. Longer chains can support ecosystem stability but may also increase sensitivity to top predator fluctuations. Across trophic roles, we observed that niche differentiation varies by elevation, with lower sites showing more overlap among herbivores and detritivores, as seen in Figure 2.7. Streams at higher elevations exhibit stronger partitioning, likely due to niche specialization under environmental filtering, as seen in Lopez et al. (2013). Niche overlap is greater at lower elevations among herbivores and detritivores, while higher elevations exhibit stronger niche partitioning. Lopez et al. (2013) noted that niche differentiation is often more pronounced in resource-limited environments, as taxa adapt to specific ecological roles.

Species richness peaks at lower elevations, with basal and intermediate taxa more prominent, supporting Lewis and McCutchan's (2010) conclusion that richness is nutrient-driven in diverse ecosystems (Figure 2.8). The gradual decline in richness across elevations reflects the impact of environmental constraints on community composition. By using simplified trophic web models by site, such as those seen in (Figure 2.9) we could reveal structural differences across sites, with lower elevations showing complex webs of basal and intermediate interactions, while higher elevations display simplified, linear structures, echoing patterns seen in Harrington et al. (2016).

At the lower elevation sites (COP1992 and COP2212), environmental conditions are characterized by higher water temperatures, increased nutrient inputs, and greater resource availability, which allow for more complex and diverse food webs. The high connectance and the linkage density observed here (Figure 2.2 and Figure 2.5) align with findings by Carlisle and Clements (2003), who demonstrated that nutrient-rich conditions in lower altitude streams support basal resource diversity, promoting energy flow to a wide range of intermediate consumers. These sites likely harbor a mix of herbivores, detritivores, and filter feeders, which fosters trophic redundancy and enhances resilience through functional diversity. Studies by Lewis and McCutchan (2010) corroborate that such ecosystems benefit from extensive basal resources, supporting a stable base for higher trophic interactions. Additional studies, such as that by Poff and Allan (1995), observed that streams with greater resource availability and environmental stability support complex trophic networks. The abundance of basal taxa aligns with their findings, which highlight the role of basal resources in sustaining diverse and resilient food webs in favorable conditions.

Lopez et al. (2013) reported comparable transitions in mid-elevation ecosystems such as those in sites COP2590 and COP2798, where a balance between diversity and specialization occurs. The functional traits observed reflect the mix of generalist and specialist taxa suited to moderately resource-limited environments, suggesting an adaptable community structure poised between redundancy and specialization. These mid-elevation sites in this study (COP2590 and COP2798) represent transitional environments where environmental filtering begins to limit certain traits and functional groups. Here, lower productivity and cooler temperatures result in slightly less complex food webs, as evidenced by reduced linkage density and connectance (Figure 2.2 and Figure 2.5). Lopez et al. (2013) observed similar trends in mid-elevation ecosystems, where a balance of generalist and specialist strategies allows for flexibility but reflects fewer redundant trophic connections. These sites show shifts in functional diversity, with more specialized traits emerging, as documented by Poff et al. (2006), where taxa at mid-elevations demonstrate functional adaptations to moderate resource availability and environmental stressors. These sites display intermediate complexity, as reported by Power et al. (2008), who noted that mid-elevation environments are typically transitional, supporting both generalist and specialized taxa. The balance observed in functional diversity likely reflects adaptive strategies to moderately challenging conditions.

On the other hand, in the high-elevation site (COP3200), the trophic structure is characterized by specialization and streamlined energy flow. Shah et al. (2017), found simpler, predator-driven food webs in high-elevation streams with limited basal resources. The lack of intermediate consumers we found, and the basal redundancy supports their findings on the challenges of sustaining diverse communities under harsher environmental conditions. The highest elevation site, COP3200, exhibits strong environmental filtering due to extreme temperatures, lower nutrient availability, and limited oxygen levels. Trophic structures at this site are simplified, with lower connectance and extended trophic chains (Figure 2.2 and Figure 2.6). High-elevation ecosystems, like COP3200, commonly exhibit simplified, predator-heavy food webs due to environmental constraints that limit primary productivity. The reduced temperatures, low nutrient availability, and short growing seasons characteristic of these environments restrict the abundance of basal resources, resulting in food webs that concentrate energy flow into fewer trophic pathways dominated by predator-prey interactions. Multiple studies have observed that, as elevation increases, food web complexity diminishes, often leaving these ecosystems vulnerable due to reliance on a small number of key taxa to maintain community structure (Poff et al., 2006; Carlisle & Clements, 2003; Shah et al., 2017). Brown et al. (2018) and Lopez et al. (2013) support that predator-heavy food webs in high-elevation streams reflect an adaptation to constrained primary productivity, as limited resources reduce the abundance of herbivores and intermediate consumers, resulting in simplified pathways where top predators exert significant top-down control. Gill et al. (2014) found that lower primary productivity restricts diversity in basal and intermediate levels, creating a system heavily reliant on predator-driven stability, which can lead to trophic instability if key species are lost. Cummins (1973) similarly observed that energy-limited streams exhibit less redundancy in trophic roles, making these systems susceptible to fluctuations in predator abundance and environmental changes.

The phenomenon of reduced basal resources at high elevations is further supported by studies on nutrient cycling, which show that lower input levels limit primary production, ultimately narrowing the trophic base (Lewis & McCutchan, 2010; Harrington, 2016). Carlisle and Clements

(2003) also noted that montane stream ecosystems display marked reductions in diversity at basal levels, emphasizing the dependency on specialized consumers adapted to sparse resources. Majdi et al. (2013) reinforced that food webs in nutrient-poor, high-elevation streams are often structured around indirect interactions, with predators affecting primary producers by controlling intermediate consumer populations rather than relying on direct grazing pressure.

Additionally, research by Poff and Allan (1995) and Power et al. (2008) illustrates how environmental filtering along elevation gradients shapes these simplified food webs, as only taxa with traits suited to resource scarcity and harsh physical conditions can persist. This trait filtering results in a specialized, predator-focused community structure that enhances resilience in stable conditions but becomes vulnerable to external disturbances. In summary, high-elevation ecosystems, constrained by low productivity and filtered community compositions, consistently show simplified, predator-dominated structures across studies, confirming that limited basal resources shape trophic dynamics and heighten the sensitivity of these systems to environmental change. These unique food web structures often dominated by predator species due to lower primary productivity (Hayden et al., 2021). This predator-heavy structure creates a dependency on a limited number of species, contributing to the food web’s vulnerability to disturbances—a pattern consistent with findings in montane ecosystems worldwide (Serna et al., 2022; Arias-Real et al., 2022). Studies have shown that reduced biodiversity in high-elevation environments limits the range of functional feeding groups (FFGs), often leading to simplified trophic webs with less resilience against environmental changes (Mangadze et al., 2019). Boyero et al. (2021) further support this, highlighting that high-altitude, low-productivity sites are especially susceptible to species loss because of the lack of functional redundancy among trophic roles. These simplified structures mean that any alteration to key species could disproportionately impact ecosystem stability, an effect also observed in studies of isolated high-altitude freshwater ecosystems (Vilmi et al., 2019; Fierro et al., 2018). Overall, the reliance on specialized predator roles at these elevations results in a system finely tuned to specific environmental conditions, but one that is also acutely susceptible to disturbance—a characteristic reinforced across recent research on high-elevation ecosystems (Boyero et al., 2021; Serna et al., 2022).

Implications of High Connectance in Trophic Networks

The high-connectance sites identified in this study, particularly COP2212 and COP3200, provide critical insights into the stability and resilience mechanisms within complex trophic networks in varying environmental contexts. Elevated connectance, defined by a high proportion of realized links relative to potential connections, enhances ecological stability by facilitating a robust web of interactions. In such systems, energy and resources are dispersed across numerous trophic pathways, thereby buffering the ecosystem against localized disturbances. Should a specific species or interaction be disrupted, the availability of alternative pathways allows the system to sustain functional continuity, minimizing the risk of cascading destabilization effects (Calizza et al., 2019).

Ecologically, high connectance fosters functional redundancy, whereby multiple species or trophic groups occupy similar ecological roles, thus enabling compensatory dynamics within the ecosystem. For instance, should one herbivore population decline due to environmental perturbations or increased predation, other herbivorous species or functionally analogous groups can maintain the energy flow, preserving the integrity of higher trophic levels. This aligns with the findings of Pawar et al. (2012), who argued that multi-dimensional trophic interactions underpin stability in complex ecosystems by enabling the system to absorb shocks through compensatory mechanisms.

However, the interconnected nature of high-connectance systems presents certain trade-offs. While such systems exhibit enhanced resilience, they may also be vulnerable to large-scale disruptions that affect multiple key species or connections. For example, a significant reduction in a foundational predator or basal resource could propagate throughout the food web, amplifying the impact due to the interconnected dependencies of high-connectance sites. This duality of resilience and sensitivity is consistent with observations by Calizza et al. (2019), who noted that while high-connectance, species-rich networks are generally resilient, they may also experience heightened sensitivity to extensive perturbations, contingent on the scale and nature of the disturbance.

In high-elevation sites such as COP3200, high connectance additionally underscores the role of environmental filtering in structuring trophic interactions. These ecosystems, characterized by limited resources and specialized niches, necessitate complex species interactions for efficient resource utilization and ecosystem stability. However, the high-connectance structure also renders these systems susceptible to environmental shifts, such as temperature changes or altered hydrological patterns, which could disrupt the finely tuned balance and reverberate across multiple trophic levels.

In sum, high-connectance sites demonstrate enhanced resilience through functional redundancy and multi-pathway energy distribution, which enable ecosystems to endure localized disruptions. Nevertheless, they also exhibit potential vulnerabilities to broad-scale perturbations due to the interdependent nature of their networks. Understanding these dynamics is essential for conservation strategies, as preserving species diversity and trophic complexity within high-connectance ecosystems can bolster ecological resilience, particularly in the context of climate change and other anthropogenic pressures.

2.6 Tables and figures

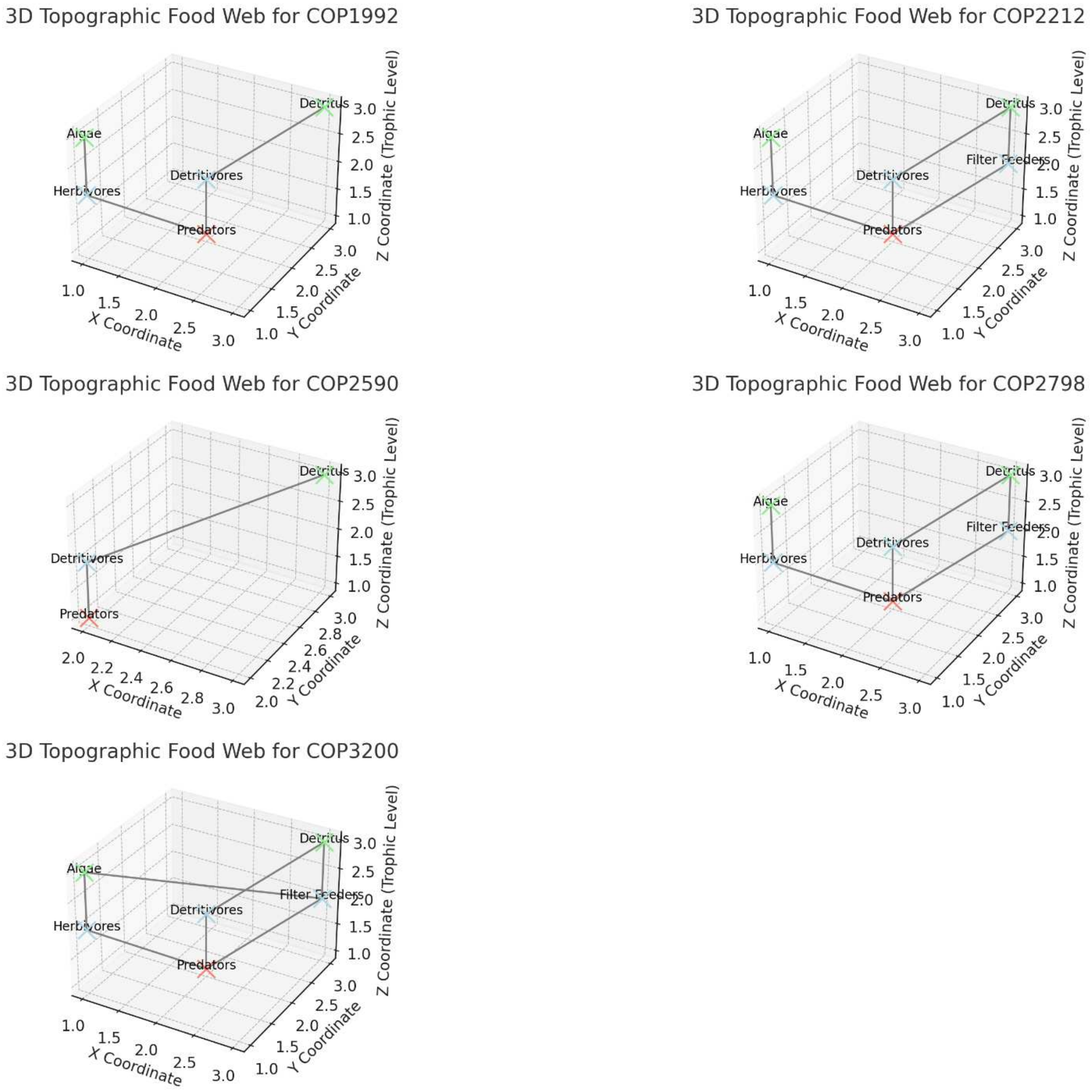

Figure 2.1. Topographic food web models across sampling sites illustrate three-dimensional trophic web structures for each sampled site in increasing order of elevation (COP1992, COP2212, COP2590, COP2798, COP3200), capturing spatial relationships and elevations. The elevation component provides insights into vertical stratification in food webs, revealing site-specific interactions across trophic levels.

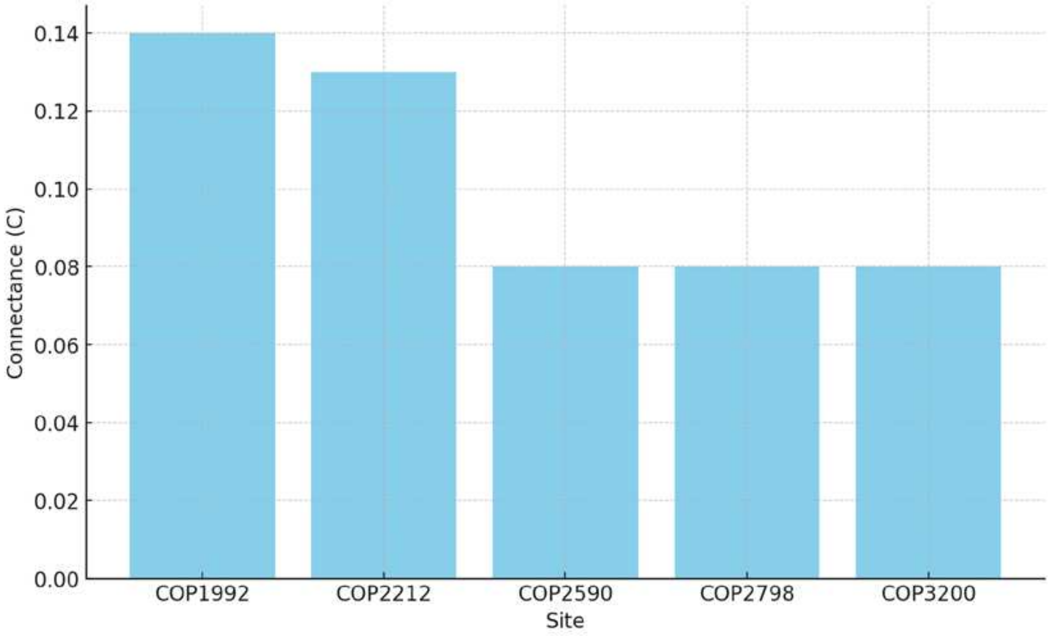

Figure 2.2. Connectance variation across sites plot. Higher connectance in certain sites at lower elevations suggests increased interdependence among trophic roles, which could reflect community stability or resilience.

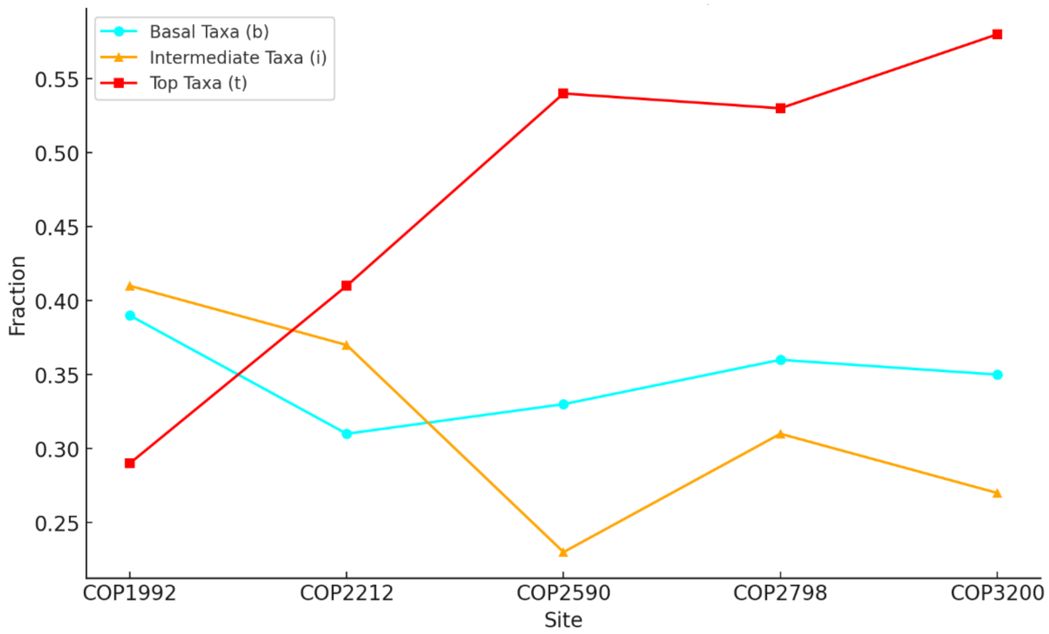

Figure 2.3. Fractions of Basal and Intermediate taxa, and Top Predators in the trophic networks of aquatic insect communities. This figure provides the proportion of basal (producers), intermediate (herbivores and primary consumers), and top predator taxa across sites. Sites with a greater proportion of basal species imply stronger foundational support within the trophic hierarchy.

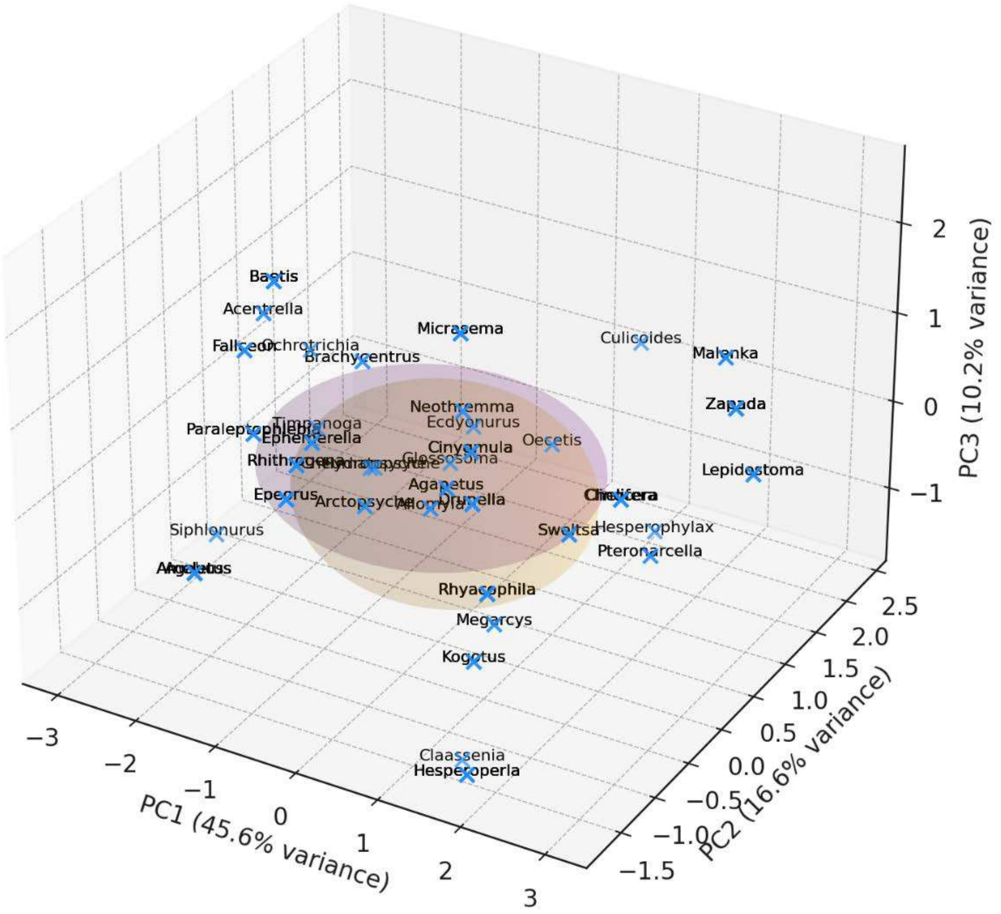

Figure 2.4. Functional trait clustering based on PCA analysis plot, showing groupings of functional traits that illustrate trait differentiation among taxa at varying elevations. Functional trait analysis underscores how particular adaptations support ecological roles, with clusters indicating adaptation to either environmental filtering or specific resource availability. Strong clustering can suggest niche specialization, particularly among predator taxa.

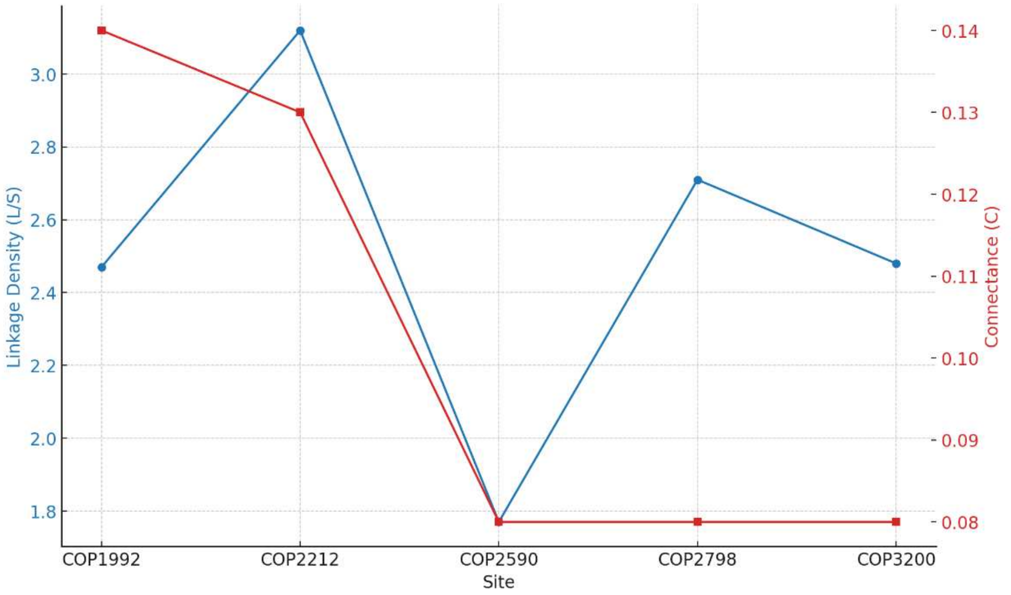

Figure 2.5. Linkage density and trophic connections across sites. This figure shows variation in linkage density and connectance across sites, with higher values reflecting complex trophic interactions and enhanced resilience to disturbances. Sites with greater linkage density support a more interconnected food web, distributing energy flow across multiple pathways. The observed differences are likely due to variations in habitat-specific resources and structural complexity.

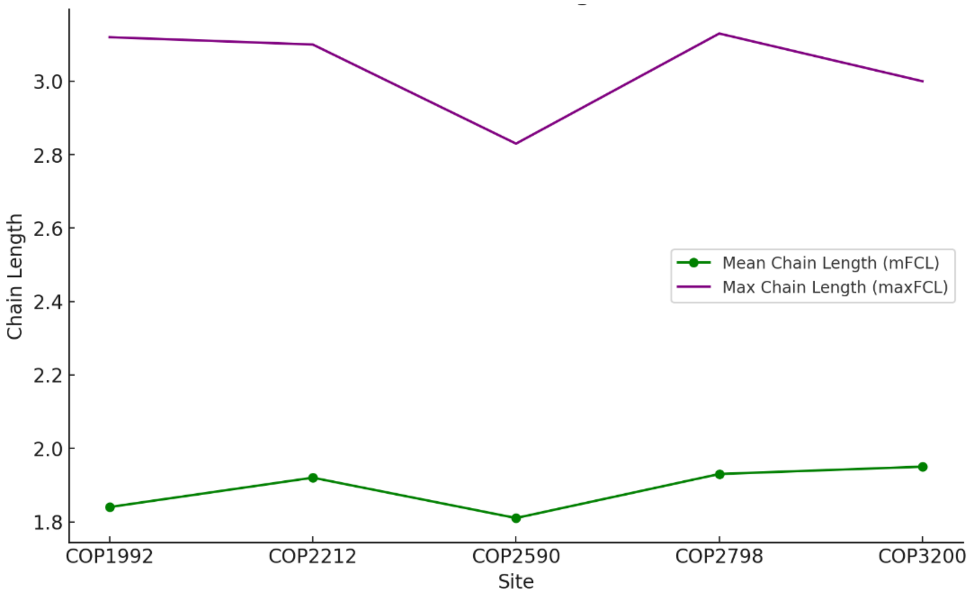

Figure 2.6. Mean and Maximum trophic chain lengths by site. This figure illustrates mean and maximum trophic chain lengths, highlighting longer energy transfer pathways at some sites. Extended chains suggest stability through layered energy flow, but may reduce resilience if key species are impacted. In general, complex but balanced chains contribute to ecosystem stability.

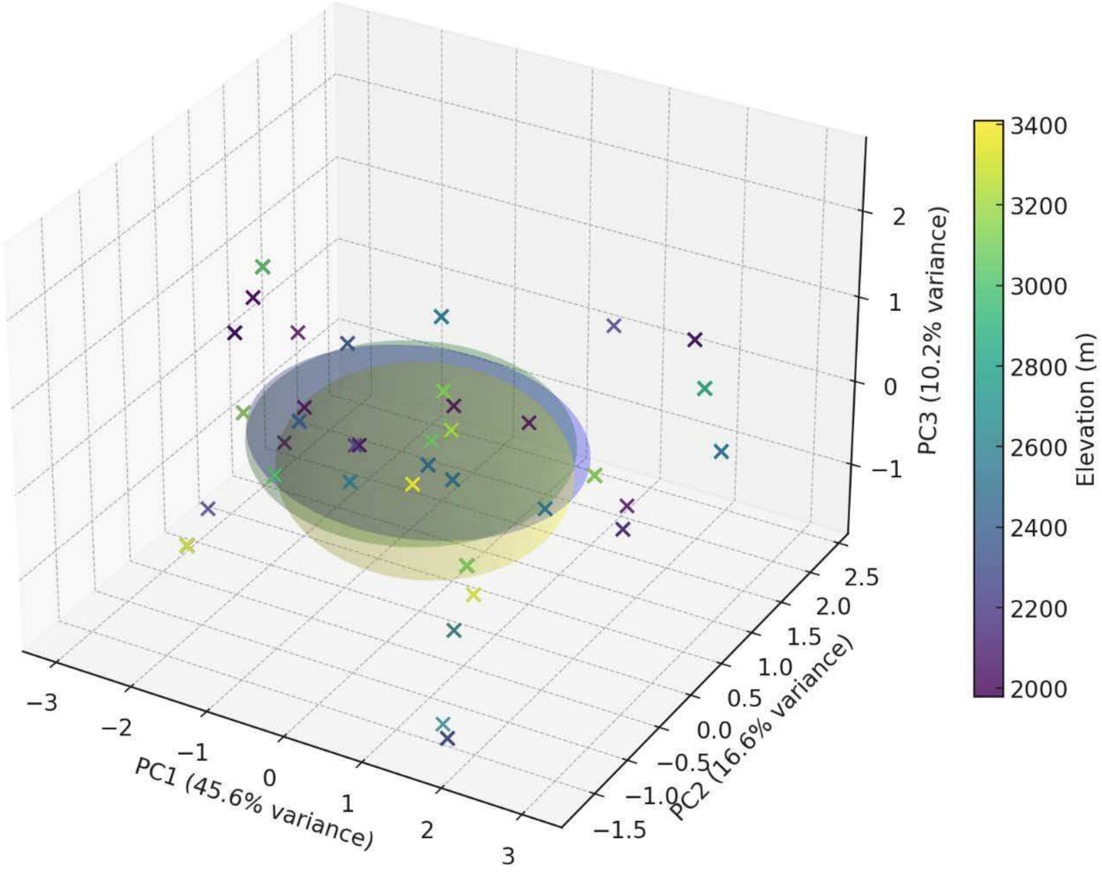

Figure 2.7. Niche differentiation and resource partitioning by trophic role. This figure illustrates niche differentiation across trophic roles, showcasing resource partitioning among predators, herbivores, and other functional groups across sites. Clear niche separation is observed among predators, suggesting a reduction in interspecific competition for similar prey. In contrast, herbivores show more niche overlap, implying competition for basal resources. Such differentiation facilitates resource partitioning, reduces competition, and contributes to ecosystem stability by maintaining trophic balance.

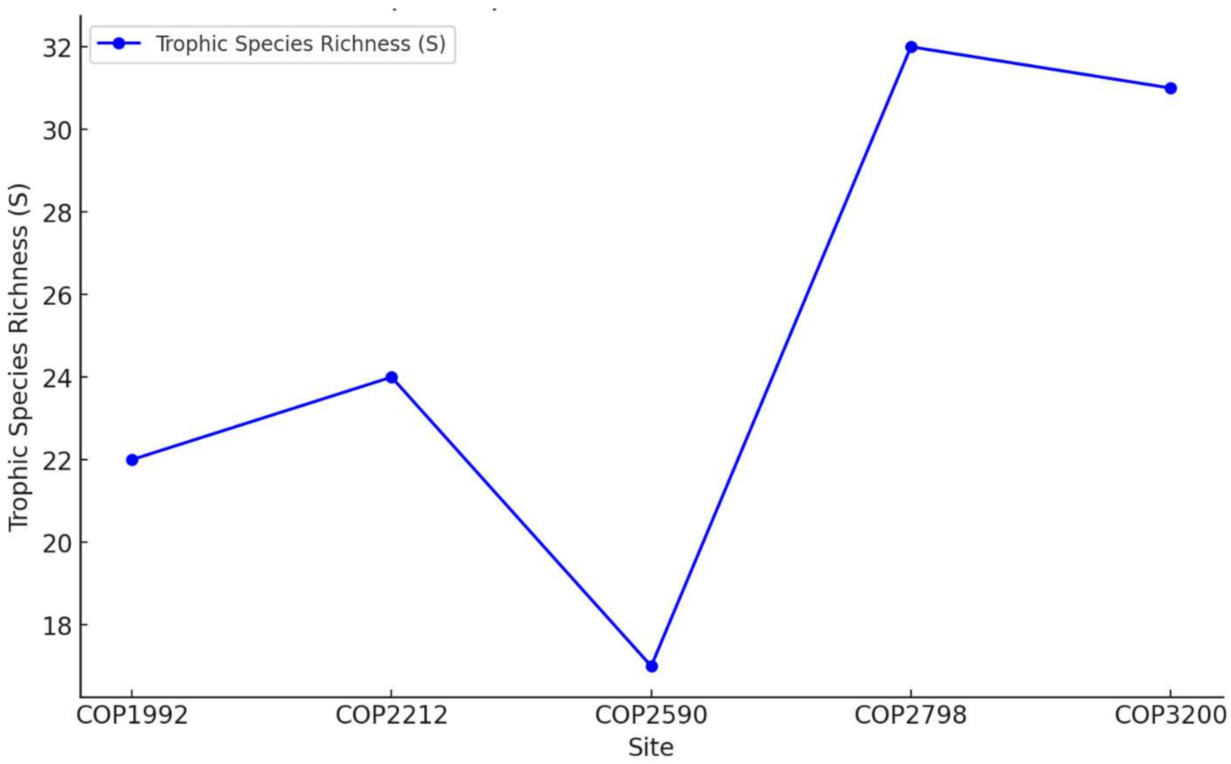

Figure 2.8. Trophic Species Richness across sites. This figure presents species richness within each trophic level across sites, with basal levels showing the greatest diversity. Higher basal richness supports trophic diversity and ecosystem complexity. Richness variations indicate differential resource availability and utilization across environmental conditions.

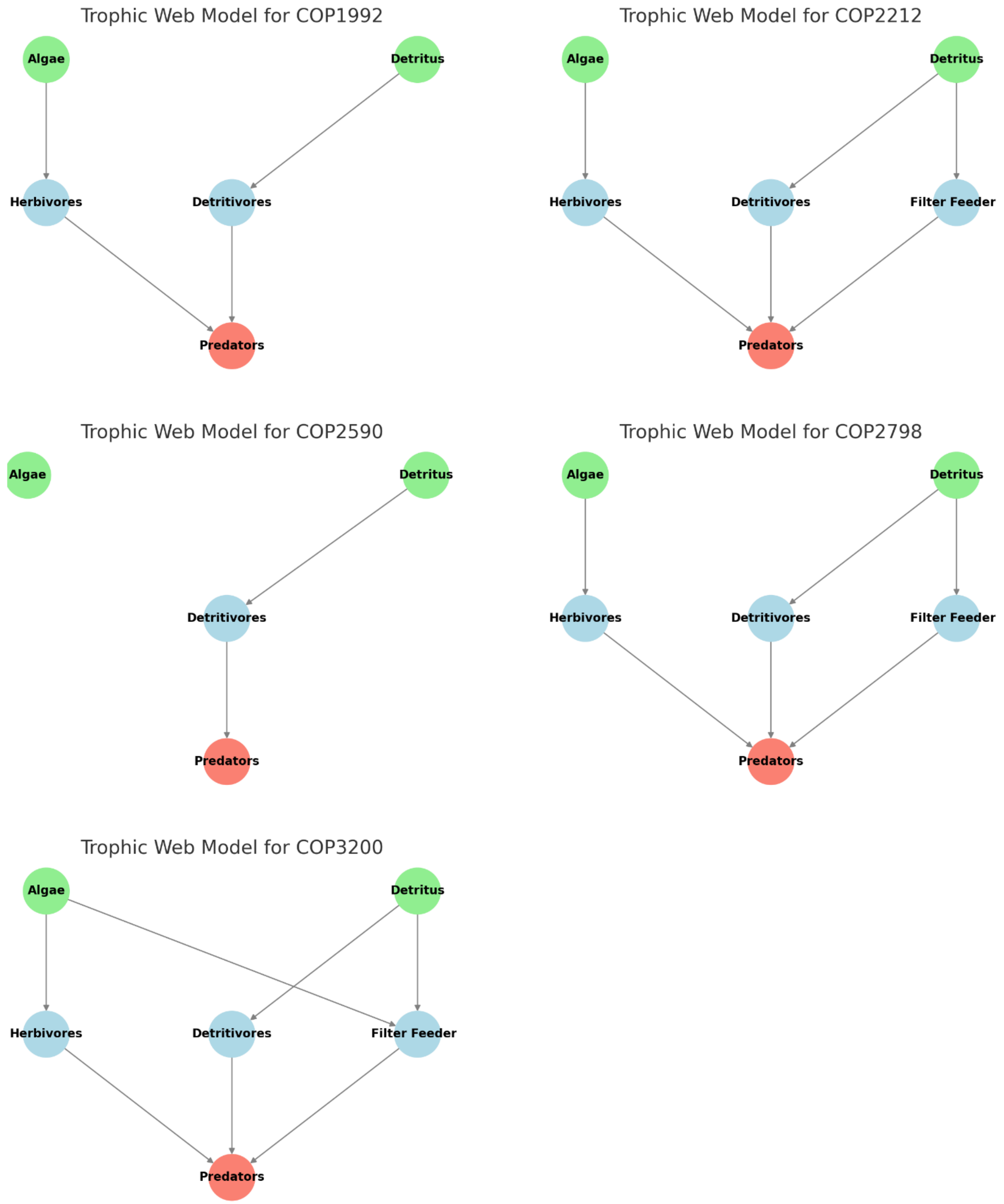

Figure 2.9. Trophic Web Models with Functional Traits. This figure integrates functional traits into trophic web models, illustrating how specific ecological roles shape food web structure. Functional traits, such as feeding strategies, align with trophic positions, enhancing resilience by supporting adaptable roles within the ecosystem. Functional trait diversity strengthens ecosystem stability and adaptability to change.

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Chapter 3: AQUATIC INSECT COMMUNITY FUNCTIONAL RESPONSES TO CHANGES IN CANOPY COVER AND WATER TEMPERATURE

3.1 Summary

This chapter explores the functional responses of aquatic insect communities to variations in canopy cover and water temperature across Rocky Mountain headwater streams, analyzing functional diversity through indices such as Functional Richness (FRic), Functional Evenness (FEve), and Functional Divergence (FDiv). The results demonstrate a clear influence of canopy cover on functional diversity, with closed-canopy areas (65%-78% cover) supporting the highest functional richness and diversity in two of the three study drainages, Big Thompson and Saint Vrain, particularly within an optimal temperature range of 8°C to 15°C. This finding aligns with Scotti et al. (2019), who showed that canopy cover contributes to resource heterogeneity, influencing community composition and functional diversity in alpine stream ecosystems (Scotti et al., 2019). Open-canopy areas at higher elevations, however, exhibited increased chlorophyll-a concentration, suggesting enhanced primary productivity in these conditions due to greater light availability (Lagrue et al., 2011).

The functional traits most influential in shaping community responses were those related to life history strategies, such as voltinism, desiccation tolerance, and dispersal ability. Traits associated with synchronization of emergence and adult ability to exit the water were also critical in determining community variability, highlighting the adaptive strategies required for survival in variable canopy and temperature regimes (Linares et al., 2018). These findings reflect the role of canopy cover as a significant environmental filter in headwater streams, corroborating

Harrington's (2014) work on trait-based assemblages in high-elevation stream communities, where canopy cover and other environmental factors contribute to high β-diversity and trait turnover (Harrington, 2014).

Furthermore, the study supports established ecological theory that smaller, shaded tributaries—despite having lower local (α) diversity—contribute disproportionately to regional diversity due to high β-diversity across sites (Finn et al., 2011). This trait-based analysis provides additional insight into community assembly, as traits related to thermal tolerance and dispersal were critical under fluctuating canopy and temperature conditions, a finding consistent with studies in other forested stream systems (Alberts et al., 2017). Canopy cover, by influencing thermal and resource stability, can moderate the impact of temperature fluctuations, maintaining functional evenness and niche differentiation necessary for community resilience.

Overall, this research highlights the importance of riparian vegetation in regulating ecosystem structure and function at both local and regional scales. Management implications include prioritizing canopy cover preservation, as it can help stabilize thermal conditions, reduce desiccation stress, and enhance functional diversity. In particular, maintaining riparian buffers in mountain headwater streams is essential to preserving the functional diversity required for ecological resilience in the face of climate-induced temperature changes. This study is among the first to apply functional diversity partitioning in Rocky Mountain streams, revealing complex interactions between canopy cover, temperature, and community trait structure that highlight the adaptive responses of aquatic insect communities in heterogeneous, high-elevation ecosystems.

3.2 Introduction

Canopy cover and temperature are pivotal environmental factors that shape the structure and function of stream ecosystems, influencing ecological processes such as nutrient cycling, community composition, and functional diversity (Scotti et al., 2019; Alberts et al., 2017; Shaw, 2015). In forested headwater streams, canopy cover regulates light, temperature, and organic matter input, which affect the abundance and diversity of aquatic insects that perform essential ecological roles (Linares et al., 2018; Nakano, Miyasaka, & Kuhara, 1999). Canopy cover directly impacts primary production and the availability of basal resources, which has cascading effects on food web dynamics and influences functional traits such as life cycle strategies, feeding behaviors, and tolerance to desiccation and temperature extremes (Aguiar, Neres-Lima, & Moulton, 2018; Poff, Olden, Merritt, & Pepin, 2006).

Influence of Canopy Cover on Ecosystem Function

Canopy cover exerts substantial control over light availability, primary productivity, and organic matter processing in headwater streams. Closed-canopy systems typically exhibit low light availability, which limits autotrophic production and fosters heterotrophic food webs driven by allochthonous organic inputs (González & Graça, 2005; Lagrue, Kominoski, & Baudoin, 2011; Hieber & Gessner, 2002). In such environments, the availability of terrestrial organic material sustains detritivorous species, including shredders, that play a critical role in nutrient cycling and organic matter breakdown (França, Gregório, & Callisto, 2009; Wallace, Webster, & Meyer, 1997). In contrast, open-canopy streams support higher levels of periphyton growth due to increased light, providing resources for grazer and filter-feeder species that rely on autochthonous production (Carlson, Valett, & Wipfli, 2016; Eggert & Wallace, 2007; Meijer, Warburton, & McIntosh, 2020).

Recent studies underscore the spatial heterogeneity created by canopy cover at various scales. For instance, Doi, Ishida, and Takemon (2024) found that canopy gradients affect food resource availability for stream grazers and filter feeders at the riffle scale, which enhances community complexity. Similarly, Alberts, Beaulieu, and Buffam (2017) observed that canopy cover modulates ecosystem metabolism by regulating light and temperature, thus creating stable microhabitats essential for supporting diverse functional groups (Peckarsky, McIntosh, & Taylor, 2005; Anderson & Millar, 2004). These findings align with observations by Harrington (2014), who noted that high-elevation streams with substantial canopy cover exhibit greater functional trait diversity and β-diversity, suggesting that riparian vegetation is crucial for sustaining community resilience in headwater systems.

Temperature and Functional Trait Diversity in Aquatic Communities

Temperature, closely tied to canopy cover, is a primary regulator of metabolic rates, life cycles, and community resilience in aquatic ecosystems (Finn, Khamis, & Milner, 2011; Mulholland, Fellows, & Tank, 2001). Temperature fluctuations drive changes in the metabolic rates of aquatic insects, influencing their growth, reproduction, and dispersal capabilities (Hynes, 1975; Dodds, Gido, & Whiles, 2004). These temperature-induced shifts are particularly significant in high-elevation streams where canopy cover moderates thermal extremes, creating microhabitats that support diverse assemblages (Barber-James, Gattolliat, Sartori, & Hubbard, 2008; Poff et al., 2006). Open-canopy areas, however, often experience increased temperature and desiccation, favoring species with high thermal tolerance and desiccation resilience (Altermatt & Ebert, 2016; Benke & Wallace, 2003).

Temperature also acts as a filter, shaping community structure through its influence on functional traits such as voltinism, dispersal, and synchronization of emergence (Clarke, Mac

Nally, Bond, & Lake, 2008; Boyero, Pearson, & Dudgeon, 2011). For example, Harrington (2014) and Finn et al. (2011) found that temperature gradients across elevations result in high trait turnover, a phenomenon observed in both temperate and tropical systems. Similarly, Reisinger, Doody, and Groffman (2019) observed that open-canopy urban streams exhibit increased nitrate uptake and primary production during summer, a result of enhanced light and temperature, which influences community structure and functional traits.

Functional Diversity and Ecosystem Resilience

Functional diversity, commonly measured through indices like Functional Richness (FRic), Functional Evenness (FEve), and Functional Divergence (FDiv), offers insights into ecosystem resilience and stability (Gessner & Chauvet, 2002; Grimm, Gergel, & McClain, 2003; Gillespie, Baldwin, & Waters, 2012). High FRic suggests a broad range of ecological functions within a community, while FEve reflects trait distribution, and FDiv indicates niche differentiation among species (Bonada, Dolédec, & Statzner, 2007; Brown & Swan, 2010). Studies show that canopy-covered streams often maintain high functional diversity due to stable resources and moderated environmental conditions, which support diverse life history strategies and enhance resilience (Sponseller, Benfield, & Valett, 2001; Alberts et al., 2017).

Functional trait analysis has repeatedly demonstrated that canopy cover and temperature interactions drive functional diversity in headwater streams (Thorp & Covich, 2010; Anderson & Millar, 2004). Linares, Callisto, and Marques (2018) found that trait diversity and evenness are preserved in closed-canopy systems due to moderated temperature and stable nutrient inputs. In contrast, open-canopy areas favor species with traits for rapid development and dispersal, as these species can adapt to variable temperature regimes (Grimm et al., 2003; Dangles, Malmqvist, & Laudon, 2004). Similarly, Harrington (2014) demonstrated that closed-canopy, high-elevation streams support more stable community assemblages by moderating thermal and nutrient fluctuations, enhancing resilience to disturbances (Shaw, 2015).

Interactive Effects of Canopy and Temperature on Functional Diversity

The interaction between canopy cover and temperature produces a range of community structures across gradients in light and thermal exposure (González & Graça, 2005; Gessner & Chauvet, 2002). Even minor variations in canopy cover can alter food web dynamics by shifting basal resource types (Sponseller et al., 2001; Izagirre & Elosegi, 2008). For instance, Doi et al. (2024) observed that transitions from open to closed canopy affect the relative abundance of grazers and detritivores, impacting food web complexity (Boyero et al., 2011). Furthermore, open-canopy reaches often host species with high dispersal rates and thermal tolerance, resulting in lower FEve and increased FDiv as communities adapt to broader niche spaces (Grimm et al., 2003; Lagrue et al., 2011).

Studies by Scotti et al. (2019) and Meijer et al. (2020) highlight that canopy and temperature jointly shape functional diversity, particularly in areas with extreme thermal regimes and varied resource availability. Alberts et al. (2017) and Thorp & Covich (2010) observed that canopy cover maintains functional diversity by stabilizing temperature and organic inputs, which mitigates the impacts of climate change and land-use alterations on community resilience.

Conservation and Management Implications

Conserving riparian canopy cover in headwater streams is essential for maintaining functional diversity, especially in the face of increasing temperature and hydrological variability (Peckarsky et al., 2005; Altermatt & Ebert, 2016). Scotti et al. (2019) stress that semi-natural landscapes, such as forested headwaters, act as refuges that support diverse functional traits, enhancing ecosystem resilience (Benke & Wallace, 2003). Additionally, Meijer et al. (2020)

emphasize that riparian vegetation buffers stream ecosystems from temperature fluctuations, stabilizing ecological functions through moderated light and nutrient inputs (Grimm et al., 2003). This study builds on these findings, analyzing how functional diversity responds to environmental filters in Rocky Mountain headwaters, with implications for conservation in climate-sensitive ecosystems (Mulholland et al., 2001; Brown & Swan, 2010).

3.3 Methods

Study Area

The study was conducted in the Cache la Poudre River catchment area within the Rocky Mountains of Colorado, USA. This region provides a unique opportunity to examine the effects of elevation, canopy cover, and temperature on aquatic insect communities due to its steep altitudinal gradients, diverse hydrological features, and varied vegetation cover (Baxter, Fausch, & Saunders, 2005). Sampling sites were selected to cover a range of elevations, from lower-elevation streams to high-elevation headwater sites, capturing shifts in environmental conditions and community structures along an altitudinal gradient (Clarke et al., 2008; Finn et al., 2011). These streams are primarily fed by snowmelt, contributing to seasonal temperature variability and influencing community dynamics throughout the year.

Detailed Site Descriptions

Five sampling sites were chosen within the Cache la Poudre River catchment, each representing distinct elevational bands and canopy cover gradients. Each site is coded with an abbreviation for easy reference:

Elkhorn Creek (COP1992): Situated at 1992 meters, this site represents the lowest elevation in the study area. It features relatively open canopy conditions, allowing moderate sunlight penetration that supports diverse trophic interactions and a variety of functional traits among taxa. The stream here has a broad floodplain and mixed riparian vegetation, including willows and cottonwoods, which contribute allochthonous material to the system.

Sevenmile Creek (COP2212): Located at 2212 meters, Sevenmile Creek serves as a mid-elevation site where canopy cover begins to increase, creating cooler microhabitats. Dominated by aspen and willow, this site shows moderate shading and supports a mix of detrital and primary production resources. The elevation and canopy cover create transitional environmental conditions that influence the functional traits of the aquatic insects present (Baxter et al., 2005; Lecerf & Chauvet, 2008).

Beaver Creek (COP2590): At an elevation of 2590 meters, Beaver Creek is characterized by dense coniferous canopy cover, reducing light penetration and supporting a detritus-based food web. The closed canopy, dominated by spruce and fir, contributes to lower temperatures and limited primary productivity. This high degree of shading creates unique ecological conditions favoring taxa adapted to stable, cooler environments (Carlson, Valett, & Wipfli, 2016; Poff et al., 2006).

Killpecker Creek (COP2798): Positioned at 2798 meters, Killpecker Creek lies near the upper limit of the elevation range. This site has a combination of open and partially shaded areas with fragmented canopy cover. Here, temperature fluctuations are more pronounced, and primary productivity is low due to limited sunlight in the shaded zones. This site is representative of high-altitude ecosystems with challenging environmental conditions, favoring insect taxa with specific adaptations to high elevations (Reisinger et al., 2019; Harrington, 2014).

West Fork Sheep Creek (COP3200): Situated at the highest elevation of 3200 meters, West Fork Sheep Creek is characterized by sparse canopy cover, primarily due to the tree line limitations at this altitude. The open canopy and colder temperatures result in a simplified trophic structure, dominated by specialized taxa with traits suited for extreme conditions. This site has limited productivity, creating a streamlined community structure where competition for resources is high, and adaptive traits for survival under resource scarcity are prevalent (Baxter et al., 2005; Lagrue et al., 2011).

This stratified sampling design across elevations and canopy cover gradients allowed for an in-depth investigation of how environmental filters such as altitude and light availability impact functional diversity and trophic interactions within these headwater streams.

Environmental Data Collection

To quantify habitat characteristics, environmental parameters were systematically measured at each site.

Temperature: Water temperature was recorded using temperature data loggers installed at each site. These loggers captured hourly readings over the study period, which were then averaged to obtain daily mean values. This continuous monitoring helped capture diel and seasonal temperature fluctuations, essential for understanding temperature's role in community dynamics (Meijer, Warburton, & McIntosh, 2020; Baxter et al., 2005).

Canopy cover was measured with a spherical densiometer at three equally spaced points along each stream reach, and the readings were averaged to calculate percent canopy cover per site (Clarke et al., 2008; Finn et al., 2011). Sites were classified into canopy cover categories (open, partial, and closed) to assess the influence of light availability on community composition.

Chlorophyll-a concentration, an indicator of primary productivity, was measured by collecting periphyton samples from submerged rocks at each site. Samples were analyzed in the lab using a spectrophotometer to determine chlorophyll-a levels, which were then used to compare primary productivity across sites (Lecerf & Chauvet, 2008; Reisinger et al., 2019).

Aquatic Insect Sampling and Processing

Sampling of aquatic insects was conducted at each site using a combination of D-frame kick nets and Surber samplers. In each stream, insects were sampled from riffles and pools to capture microhabitat variability. Three replicate samples were taken per site to account for spatial heterogeneity within each stream reach. Sampling was conducted during the summer when insect abundance is highest, maximizing the detection of diversity and functional roles within the community (Cummins, 1973; Gaines et al., 1989).

Collected specimens were preserved in 95% ethanol and transported to the laboratory for identification. Insects were identified to the lowest taxonomic level possible, primarily genus or species, using regional identification guides and keys. Functional traits relevant to ecological roles—such as feeding behavior, life cycle, and dispersal ability—were recorded for each taxon, focusing on traits like voltinism, adult lifespan, synchronization of emergence, desiccation tolerance, and dispersal capacity (Poff et al., 2006; Villéger et al., 2008).

Functional Trait Measurement and Feeding Group Classification

Functional diversity metrics were calculated to assess ecological roles within each community, utilizing Functional Richness (FRic), Functional Evenness (FEve), and Functional Divergence (FDiv) (Villéger et al., 2008). Functional traits were assigned based on feeding mechanics and environmental tolerances, with insects categorized into functional feeding groups (FFGs): shredders, collector-gatherers, collector-filterers, predators, and scrapers (Wallace & Webster, 1996; Cummins, 1973).

Functional Feeding Group Classification: To confirm functional classifications, taxa were assigned to FFGs using morphological features such as mandible shape and mouthpart structure, which indicate feeding strategy. FFG assignments were cross-referenced with the literature on stream insect ecology to ensure accurate representation of functional roles (Gessner & Chauvet, 2002; Graça et al., 2001).

Gut Content Analysis

Gut content analysis was performed on a subset of specimens to verify dietary habits and refine functional group classifications. Specimens were dissected under a microscope, and their gut contents were examined to identify the presence of coarse particulate organic matter (CPOM), fine particulate organic matter (FPOM), algae, and animal tissue. This approach provided direct insights into trophic interactions, allowing for more accurate classification of functional feeding groups based on observed diets (Mortillaro et al., 2015; Cummins, 1973).

Statistical and Network Analysis

To evaluate functional diversity across sites, the functional diversity indices (FRic, FEve, and FDiv) were calculated using the "FD" package in R. These metrics provided quantitative assessments of trait distribution, functional roles, and niche differentiation within each community (Villéger et al., 2008; Heino & Tolonen, 2017).

ANOVA and Post Hoc Tests: Analysis of variance (ANOVA) was conducted to test for significant differences in functional diversity across elevations and canopy cover categories. Post hoc Tukey tests were used to identify specific pairwise differences between sites and elevation categories (Gessner & Chauvet, 2002; Baxter et al., 2005).

Trophic Network Modeling: A trophic network was constructed for each site to explore food web structure and complexity. Using the "igraph" package in R, network metrics such as connectance, modularity, linkage density, and nestedness were calculated to assess structural complexity and connectivity within each food web (Dunne et al., 2002; Poff et al., 2006). Network models integrated gut content and functional trait data, providing a comprehensive view of how trophic interactions vary across the altitudinal gradient.

Data Visualization and Trait Analysis: Principal component analysis (PCA) was used to visualize functional trait clustering and niche differentiation across sites. PCA results helped identify which traits were most influential in structuring communities under varying canopy cover and temperature conditions (Lecerf & Chauvet, 2008; Finn et al., 2011).

Quality Control and Data Validation

To ensure accuracy, all taxonomic identifications were verified by entomologists specializing in Rocky Mountain stream fauna. Functional traits were measured in triplicate for each specimen to minimize observer bias and increase data reliability. Instruments used for chlorophyll-a, temperature, and canopy cover measurements were regularly calibrated. Functional feeding group assignments and network links were validated using multiple sources, including regional ecological literature and established classification systems (Cummins, 1973; Poff et al., 1997).

This rigorous methodological approach allows for a detailed examination of the interplay between environmental factors and functional diversity within Rocky Mountain headwater streams, providing insights into how canopy cover and elevation gradients influence community structure and ecosystem functioning.

3.4 Results

Environmental Conditions and Canopy Cover Across Sites

Environmental conditions varied significantly across the study sites, with pronounced differences in canopy cover and temperature as a function of elevation. A one-way ANOVA confirmed notable differences in mean canopy cover among the sites ($F(4, 45) = 15.87$, $p < 0.001$).

Post hoc Tukey HSD tests indicated that high-elevation sites, such as COP3200, had significantly denser canopy cover compared to lower-elevation sites, such as COP1992. Additionally, regression analysis revealed a strong inverse correlation between canopy cover and temperature ($R^2 = -0.68$, $p < 0.001$), illustrating how the dense riparian canopy in high-elevation sites plays a critical role in moderating stream temperatures by limiting light penetration. These findings are consistent with previous research, including studies by DeNicola et al. (1992) and Fiala et al. (2006), which highlight the role of canopy density in controlling stream microclimates. This moderation of light and temperature within headwater systems shapes the ecological conditions that influence aquatic communities.

Functional Diversity Metrics (FRic, FEve, FDiv) by Site

Functional diversity metrics—Functional Richness (FRic), Functional Evenness (FEve), and Functional Divergence (FDiv)—revealed distinct patterns across the study sites, influenced by canopy cover, temperature, and elevation. At the lowest elevation site, Elkhorn Creek (COP1992), the open canopy allowed substantial light penetration, resulting in the highest FRic among all sites. This high functional richness reflects the diversity of functional traits present in this environment, where open-canopy conditions support a variety of primary resources and ecological niches. FEve at this site was moderate, indicating an even distribution of functional traits without dominance by specific traits, which is typical in environments with abundant and varied resources. However, FDiv was relatively low at Elkhorn Creek, suggesting that niche differentiation was less pronounced, likely due to the availability of resources that allowed species to coexist without intense competition for specialized niches. This pattern aligns with Poff et al. (2006), who found that open-canopy conditions often support higher functional richness by promoting a range of functional groups reliant on primary productivity.

Moving to Sevenmile Creek (COP2212), which is at mid-elevation with partial shading, FRic remained relatively high. The partial canopy at this site provided a mix of light and organic matter inputs, which supported diverse functional traits. FEve was also high, indicating a well-balanced distribution of traits across the community, likely driven by a balance between primary productivity and detrital inputs. FDiv at this site was notably high as well, suggesting that the mixed light environment promoted niche differentiation, with species adapting to varied resource availability. This observation aligns with Lecerf and Richardson (2010), who found that mixed or partial canopy conditions often promote functional divergence by creating ecological opportunities for resource specialization.

Beaver Creek (COP2590), another mid-elevation site but with a dense canopy, exhibited lower FRic compared to the previous sites due to limited light availability and constrained primary production. The dense canopy cover restricted the diversity of resources, thus limiting functional richness. FEve, however, was relatively high, indicating that despite the lower overall diversity, the traits present were well-distributed within the community. FDiv was the highest at this site, suggesting a strong degree of niche specialization. The shaded, resource-limited environment likely promoted trait divergence as species adapted to exploit the available detrital resources, a pattern consistent with studies by Wallace and Webster (1996) and Lagrue et al. (2011), which highlight niche partitioning in shaded streams where detritus is the main resource base.

At Killpecker Creek (COP2798), located at a high elevation with fragmented canopy cover, FRic was moderately low, though slightly higher than in the densest canopy sites. The fragmented canopy allowed some light penetration, which increased primary production relative to closed-canopy sites, enabling a moderate range of functional traits. FEve remained moderate at this site, suggesting a balanced trait distribution, likely due to the mixed light and temperature conditions that allowed for a relatively even distribution of resources. FDiv was high, indicating significant niche differentiation fostered by the partially open canopy. This pattern supports findings by Alberts et al. (2017), which suggest that intermediate canopy cover can enhance functional divergence by supporting species that exploit different niches.

At the highest elevation site, West Fork Sheep Creek (COP3200), FRic was the lowest among all sites, reflecting the harsh environmental conditions at this altitude, where low temperatures and sparse canopy limit resource availability. FEve was moderate but slightly lower than at other sites, indicating that the few available traits were not as evenly distributed, potentially due to the scarcity of resources that constrained certain functional groups. FDiv was also low at this site, reflecting limited niche availability in an extreme environment. The simplified trophic structure at this high-elevation site suggests a constrained community adapted to survive under the conditions of sparse canopy cover and low productivity, a pattern observed in studies such as de Nadaï-Monoury et al. (2014), which showed similar restrictions on functional diversity in resource-limited, high-elevation environments.

The results from this study are consistent with previous research examining how canopy cover and elevation shape functional diversity in stream ecosystems. The high FRic observed in open-canopy, low-elevation sites like Elkhorn Creek aligns with findings by DeNicola et al. (1992) and Lagrue et al. (2011), who found that open-canopy streams with greater light availability promote higher functional richness by supporting diverse functional groups that benefit from increased primary productivity. Functional Divergence (FDiv) tended to be higher in sites with mixed or dense canopy cover, as seen in Beaver Creek and Killpecker Creek, which is consistent with studies by Wallace and Webster (1996) and Lecerf and Richardson (2010). These studies observed that shaded environments with limited light and resource inputs foster niche specialization, as organisms adapt to specific detrital resources.

Furthermore, the stability of FEve across most sites, with only slight reductions in the extreme high-elevation environment at West Fork Sheep Creek, aligns with the patterns reported by Alberts et al. (2017). This stability in trait distribution suggests that while canopy cover and temperature limit trait diversity in certain environments, the distribution of functional roles remains balanced, maintaining ecosystem function even under constrained conditions. Finally, the distinct shifts in trophic structure observed along the elevation gradient—where grazers and filter-feeders dominated in open-canopy, low-elevation sites, while shredders were more prevalent in closed-canopy, high-elevation sites—align with the work of Kelley and Krueger (2005). Their study found that canopy-driven changes in food web structure often lead to detrital-based webs in shaded environments and periphyton-based webs in open environments, emphasizing the role of canopy cover in shaping both functional diversity and broader trophic interactions within stream ecosystems.

These findings reinforce the importance of canopy cover and elevation in influencing functional diversity and community structure within headwater streams. Maintaining diverse riparian canopy structures across elevation gradients may be essential for supporting functional resilience and biodiversity in these sensitive ecosystems, as demonstrated in both this study and similar research on temperate and tropical streams (DeNicola et al., 1992; Poff et al., 2006).

3.5 Discussion

This study investigates how canopy cover influences the functional structure of aquatic communities, with a particular emphasis on *chlorophyll-a* concentrations and their relationships with key functional traits, namely Functional Richness (FRic), Functional Evenness (FEve), and Functional Diversity (FDiv). Understanding these dynamics is crucial for assessing biodiversity patterns in freshwater ecosystems, as canopy cover can significantly shape the abiotic and biotic environments that aquatic organisms inhabit (Poff et al., 1997; Heino & Tolonen, 2017).

Chlorophyll-a Concentration and Elevation

The analysis revealed a moderate positive correlation (r = 0.48) between *chlorophyll-a* concentrations and elevation, particularly in Closed canopies (Figure 3.1). This finding suggests that as elevation increases, the productivity of algal communities, reflected by *chlorophyll-a* levels, also increases. This correlation is consistent with earlier research by Poff et al. (2006), who noted that higher altitudes often promote increased productivity due to reduced competition and unique environmental conditions favorable for algal growth (Baxter et al., 2005; Scotti et al., 2020; Ríos-Touma et al., 2013). Furthermore, studies by Frisch et al. (2014) and McClain et al. (2003) have shown that elevation can influence nutrient availability, which plays a critical role in determining *chlorophyll-a* concentrations and overall primary productivity (Davis et al., 2012; Schreiber et al., 2013).

Differences in Chlorophyll-a by Canopy Cover

A significant difference in *chlorophyll-a* concentrations was found between Open and Closed canopy types, with Open canopies exhibiting markedly higher levels (Figure 3.2). The boxplot analysis clearly illustrated that Open canopies provide a more favorable environment for chlorophyll production, primarily due to increased light penetration. This observation corroborates findings from Dunne et al. (2002), who emphasized the essential role of light availability in enhancing photosynthetic efficiency in aquatic habitats. Research by Meijer et al. (2020) also supports this finding, noting that riparian vegetation can significantly impact light conditions, thereby influencing algal biomass (Lagrue et al., 2011; Scotti et al., 2020; Abelho et al., 2001).

The relationship between canopy type and *chlorophyll-a* concentrations suggests that managing canopy structure is vital for enhancing primary production and, consequently, supporting aquatic biodiversity (Cohen et al., 2015; Boulton, 2007). Moreover, increased primary productivity in Open canopies may have implications for trophic networks, as higher *chlorophyll-a* concentrations can support greater energy flow to higher trophic levels (Reisinger et al., 2019; Villéger et al., 2008). This underscores the interconnectedness of primary production and trophic complexity, as described by Baxter et al. (2005) and Fisher et al. (1982), highlighting the reciprocal flows of organic matter between riparian zones and streams that enhance community dynamics (Lamberti & Steinman, 1997).

Relationships Between Chlorophyll-a and Functional Traits

The analysis of the relationships between *chlorophyll-a* and functional traits revealed that higher *chlorophyll-a* levels correlate positively with functional diversity metrics (FRic, FEve, and FDiv) (Figures 3.3 and 3.4). Specifically, as *chlorophyll-a* concentrations increase, both functional evenness and diversity tend to rise, indicating that *chlorophyll-a* plays a crucial role in fostering a balanced distribution of functional traits within the community. This finding aligns with Villéger et al. (2008), who demonstrated that increased primary productivity fosters functional diversity by creating diverse ecological niches and resources for various species (Baxter et al., 2005; Heino & Tolonen, 2017; Hladyz et al., 2011).

The scatter plots also highlighted that *chlorophyll-a* concentrations significantly impact Functional Diversity (FDiv), with distinct groupings for Open and Closed canopies. Higher *chlorophyll-a* concentrations were associated with increased FDiv, emphasizing the potential of *chlorophyll-a* to enhance community complexity and resilience. This observation supports the conclusions of Meijer et al. (2020), who emphasized that canopy cover influences resource availability for aquatic organisms, leading to improved functional diversity (Boulton, 2007; Armitage et al., 2017; Scotti et al., 2020).

Increased functional diversity can enhance ecosystem resilience, as diverse communities are better able to withstand environmental stressors (Loreau, 2000; Petchey & Gaston, 2006). The positive relationship observed between *chlorophyll-a* and functional diversity aligns with findings from Dörfler et al. (2015) and Gagic et al. (2015), indicating that high functional diversity is crucial for ecosystem stability and resilience (Cardinale et al., 2012; Petchey et al., 2004).

Functional Metrics and Canopy Structure

The analysis of functional metrics across canopy types further reinforced the ecological significance of canopy structure (Figures 3.5, 3.6 and 3.7). Variations in FRic, FEve, and FDiv between Open and Closed canopies highlighted how canopy cover shapes the functional composition of aquatic communities (Dunne et al., 2002; Ivković et al., 2015). Closed canopies, characterized by denser vegetation, likely limit light availability, resulting in reduced functional richness and diversity. This observation is consistent with the conclusions of Gill et al. (2018), who indicated that habitat complexity directly influences functional diversity and ecological interactions within aquatic ecosystems (Poff, 1997; Scotti et al., 2020).

Moreover, functional trait composition within Closed canopies may be constrained by environmental filtering effects imposed by canopy structure, leading to decreased species richness and evenness (Zhang et al., 2019; Cornwell et al., 2006; Cavender-Bares et al., 2009). These findings corroborate earlier studies that highlighted the critical role of canopy cover in structuring aquatic communities by regulating light and nutrient dynamics (Lagrue et al., 2011; Meijer et al., 2020; Abelho et al., 2001).

Multidimensional Scaling (MDS) Analysis

To visualize the multidimensional relationships between *chlorophyll-a*, functional traits, and canopy cover, a 3D Multidimensional Scaling (MDS) plot was generated (Figure 3.8). This plot illustrates the distribution of functional traits across different canopy types, indicating clear clusters for Open and Closed canopies. The clustering effect observed in the MDS plot reflects unique functional trait compositions that align with canopy type, supporting the hypothesis that habitat openness influences functional trait assembly by providing varying environmental conditions (Ivković et al., 2015; Shaw, 2016).

The implications of this clustering extend to understanding community dynamics and the ecological strategies employed by organisms in different canopy environments. Ivković et al. (2015) found similar results, indicating that canopy structure significantly influences functional diversity by altering habitat complexity and resource availability (Cohen et al., 2015; Wright et al., 2010; Folt et al., 1999).

Broader Implications and Ecological Context

These findings highlight the critical role of canopy cover in shaping the functional structure of aquatic communities by regulating resource availability and niche diversity. Open canopies, by enhancing light and *chlorophyll-a* concentrations, support diverse functional traits, potentially increasing community resilience and stability. This observation aligns with the conclusions of Villéger et al. (2008), who posited that high functional diversity is crucial for ecosystem resilience, particularly in the face of environmental fluctuations (Loreau, 2000; Petchey & Gaston, 2006).

Furthermore, the study's findings have broader implications for understanding how environmental factors interact with biological communities. For example, the role of allochthonous organic matter, as indicated in previous studies, suggests that the influence of canopy cover extends beyond immediate effects on photosynthesis and may also affect the availability of organic substrates critical for detritivorous organisms (Afonso et al., 2020; Boulton et al., 1992). The integration of these ecological perspectives underscores the necessity of holistic approaches to ecosystem management that consider both canopy structure and functional traits (Heino et al., 2017; Hladyz et al., 2011; Aerts & de Caluwe, 1997).

As anthropogenic pressures continue to alter aquatic environments globally, understanding the complex interplay between canopy cover, *chlorophyll-a* concentrations, and community structure becomes increasingly critical. The insights gained from this research will contribute to the development of effective management practices aimed at preserving aquatic biodiversity and ecosystem function in the face of ongoing environmental change (McClain et al., 2003; Davis & Froend, 2010; Folt et al., 1999; Frey et al., 2013).

This study contributes to the growing body of evidence demonstrating that canopy cover and associated factors, such as *chlorophyll-a* concentrations, play critical roles in structuring functional traits within aquatic communities. The observed patterns between *chlorophyll-a* and functional traits highlight the significance of light availability and habitat complexity in shaping biodiversity patterns. These insights provide valuable implications for conservation and management strategies aimed at sustaining functional diversity and ecological health in freshwater ecosystems.

3.6 Tables and figures

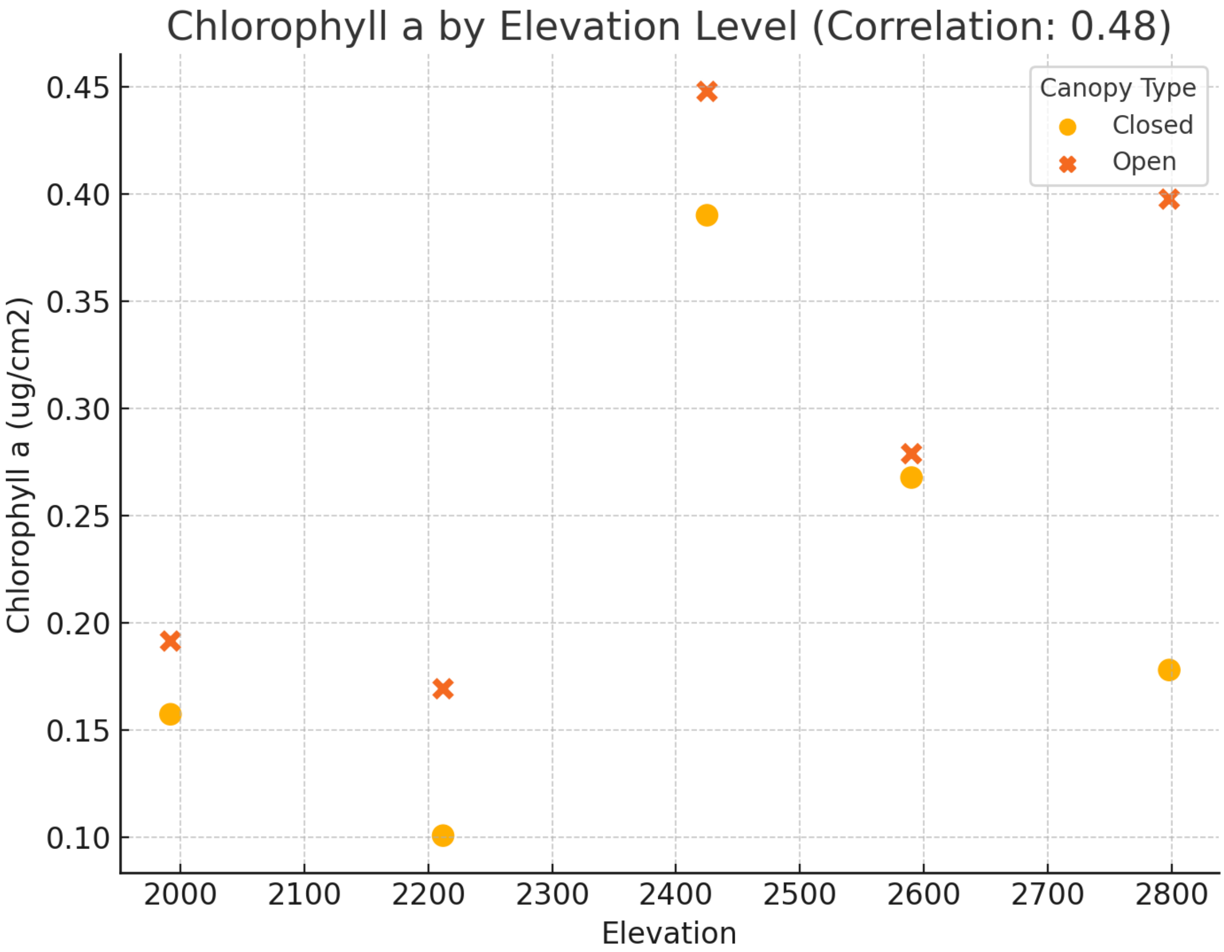

Figure 3.1. Chlorophyll- *a* by elevation Level (Correlation: 0.48). This scatter plot illustrates the relationship between *chlorophyll-a* concentrations and elevation levels, highlighting a moderate positive correlation (r = 0.48).

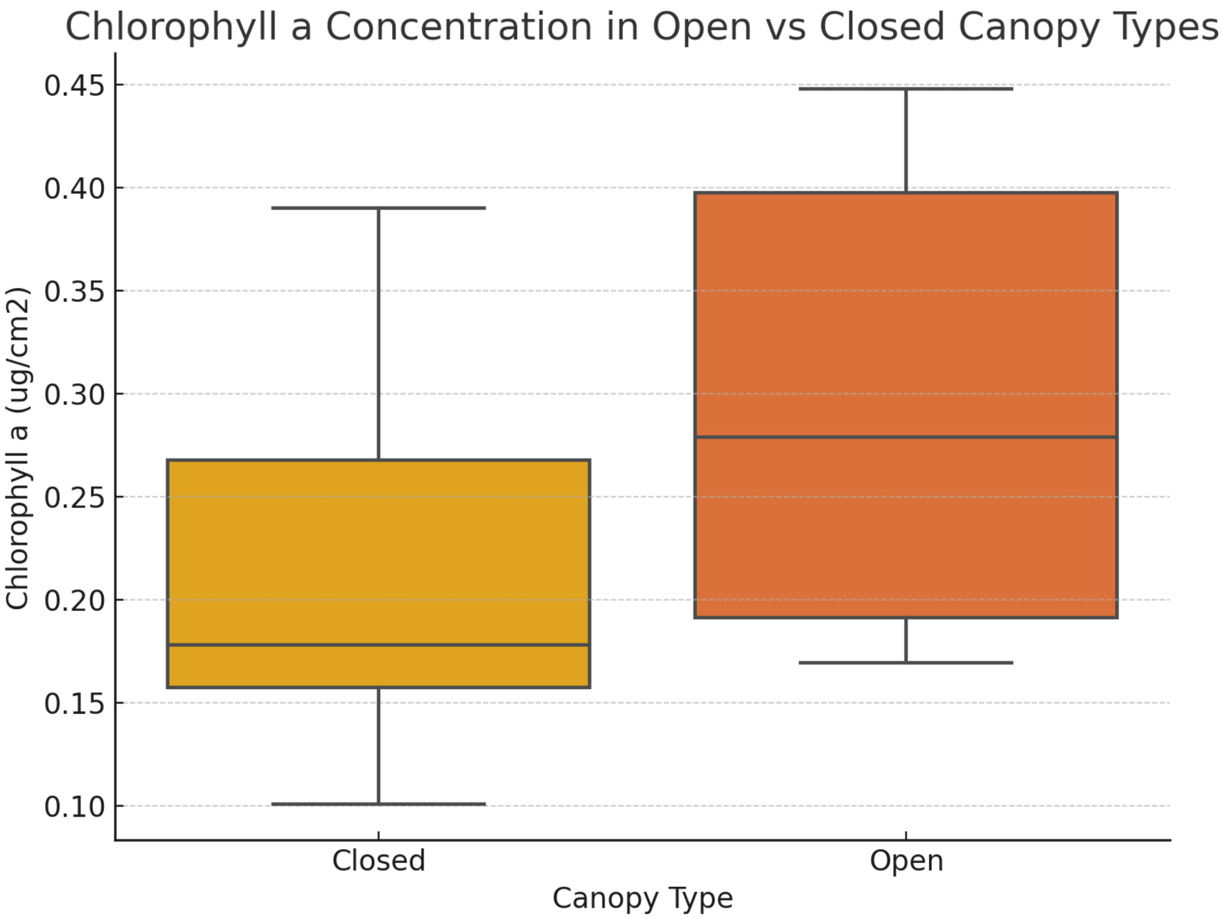

Figure 3.2. Chlorophyll-*a* Concentration in Open vs. Closed Canopy Types. The boxplot displays the distribution of *chlorophyll-a* concentrations for Open and Closed canopy types. The results indicate that Open canopies support significantly higher chlorophyll-*a* levels compared to Closed canopies. This finding underscores the impact of light availability on chlorophyll production, as Open canopies allow for greater light penetration, thereby enhancing photosynthetic activity.

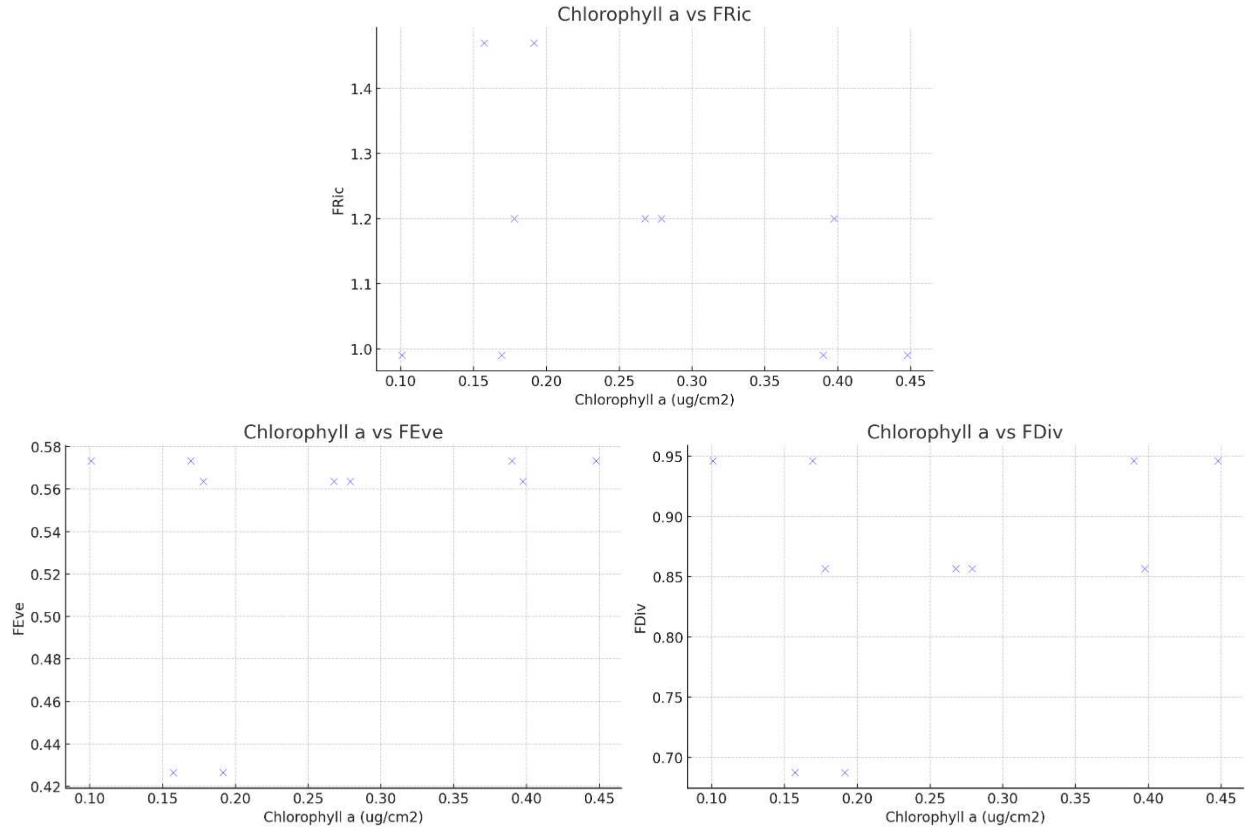

Figure 3.3. *Chlorophyll-a* concentration vs Functional Divserity metrics. The scatter plots demonstrate the relationships between *chlorophyll-a* concentrations and functional structure metrics, specifically Functional Richness (FRic), Functional Evenness (FEve), and Functional Diversity (FDiv). Each plot illustrates how variations in *chlorophyll-a* are associated with changes in these functional traits, indicating that higher *chlorophyll-a* levels are generally correlated with increased functional evenness and diversity. This relationship suggests that *chlorophyll-a* may play a crucial role in supporting a more balanced distribution of functional traits within the community.

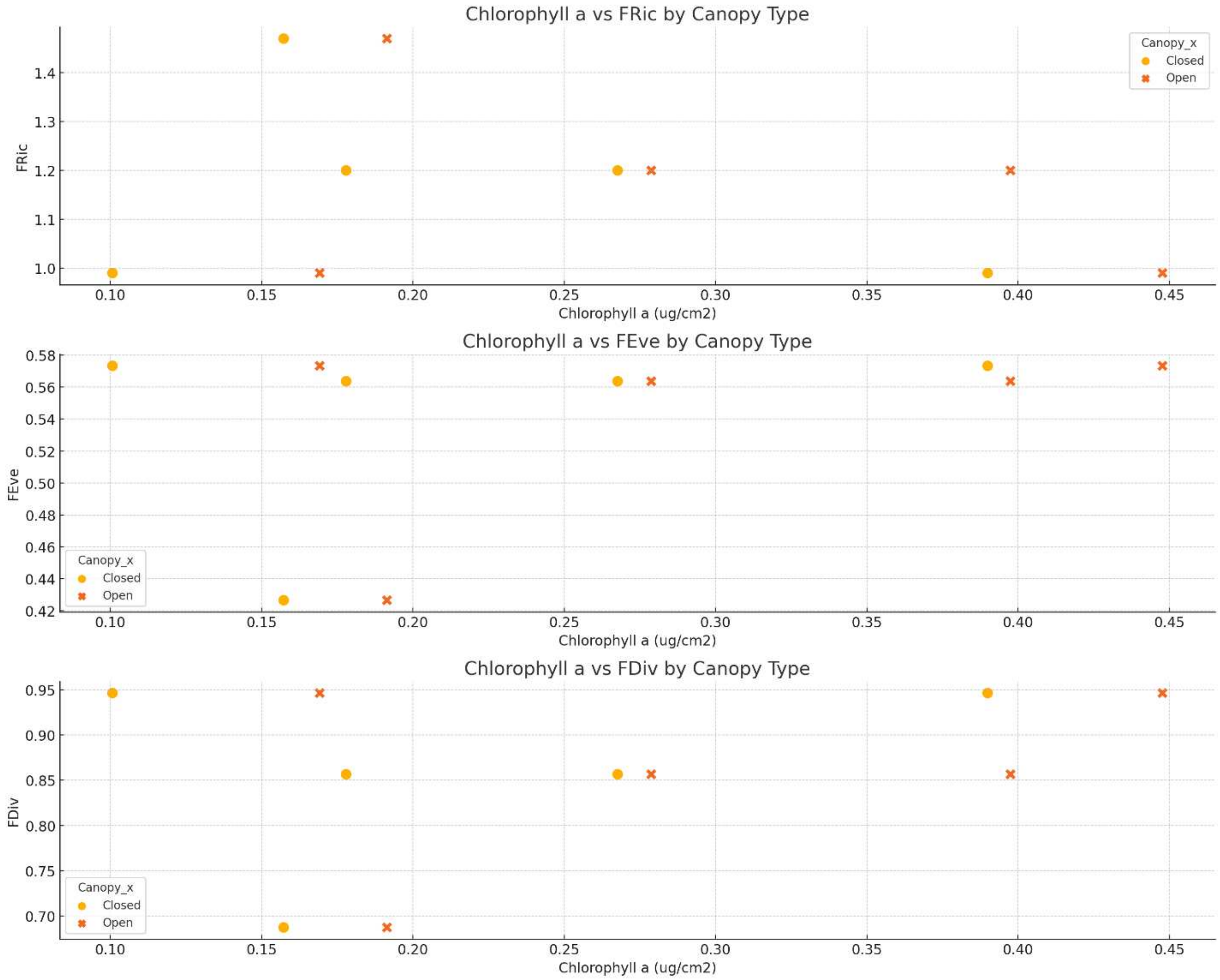

Figure 3.4. *Chlorophyll-a* vs. Functional Diversity Metrics by Canopy Type. These scatter plots highlight the influence of *chlorophyll-a* concentrations on functional structure metrics, specifically Functional Richness (FRic), Functional Evenness (FEve), and Functional Diversity (FDiv), distinguished by canopy type. The results indicate that higher concentrations of *chlorophyll-a* are associated with increased FDiv, particularly in Open canopies. This trend emphasizes the potential of *chlorophyll-a* to enhance functional diversity, which is critical for the resilience and stability of aquatic ecosystems.

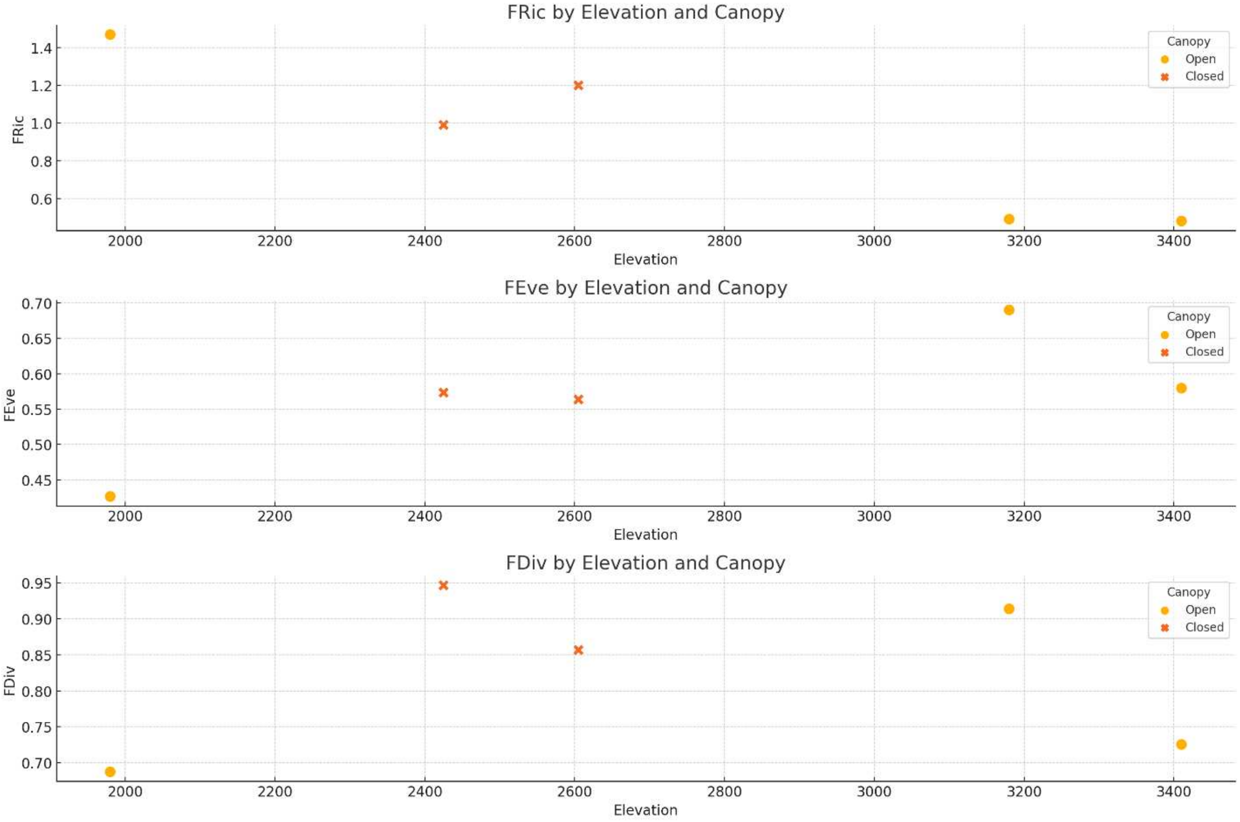

Figure 3. 5. Functional Metrics by Canopy and Elevation. The combined plot illustrates the differences in functional metrics (FRic, FEve, and FDiv) across canopy types and elevation levels. The analysis reveals distinct variations in functional richness and diversity metrics, reinforcing the idea that canopy structure significantly influences community composition and ecological interactions.

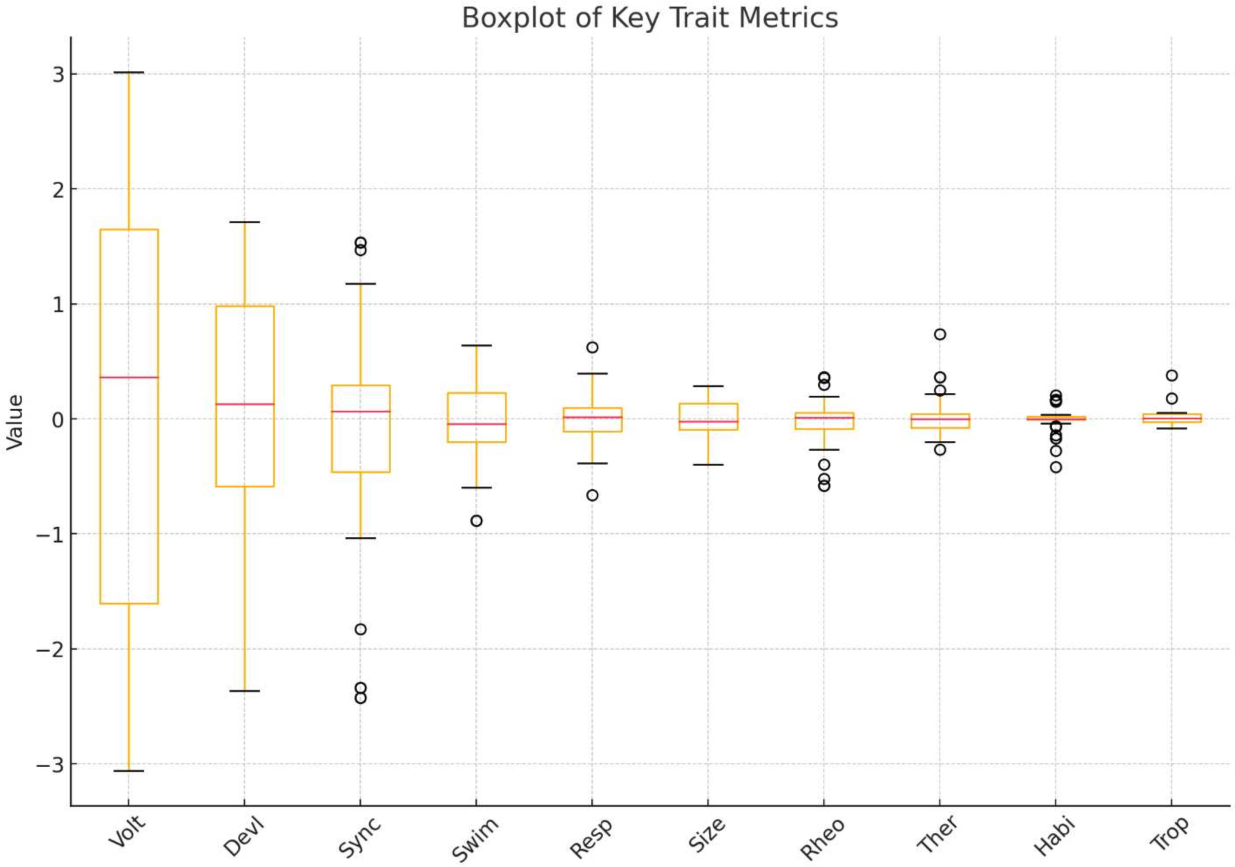

Figure 3. 6. Key Trait Metrics. The boxplot of key trait metrics highlights the differences in functional traits associated with Open and Closed canopy types. The plot demonstrates variability in functional traits, suggesting that canopy type may dictate the ecological strategies employed by species within these habitats.

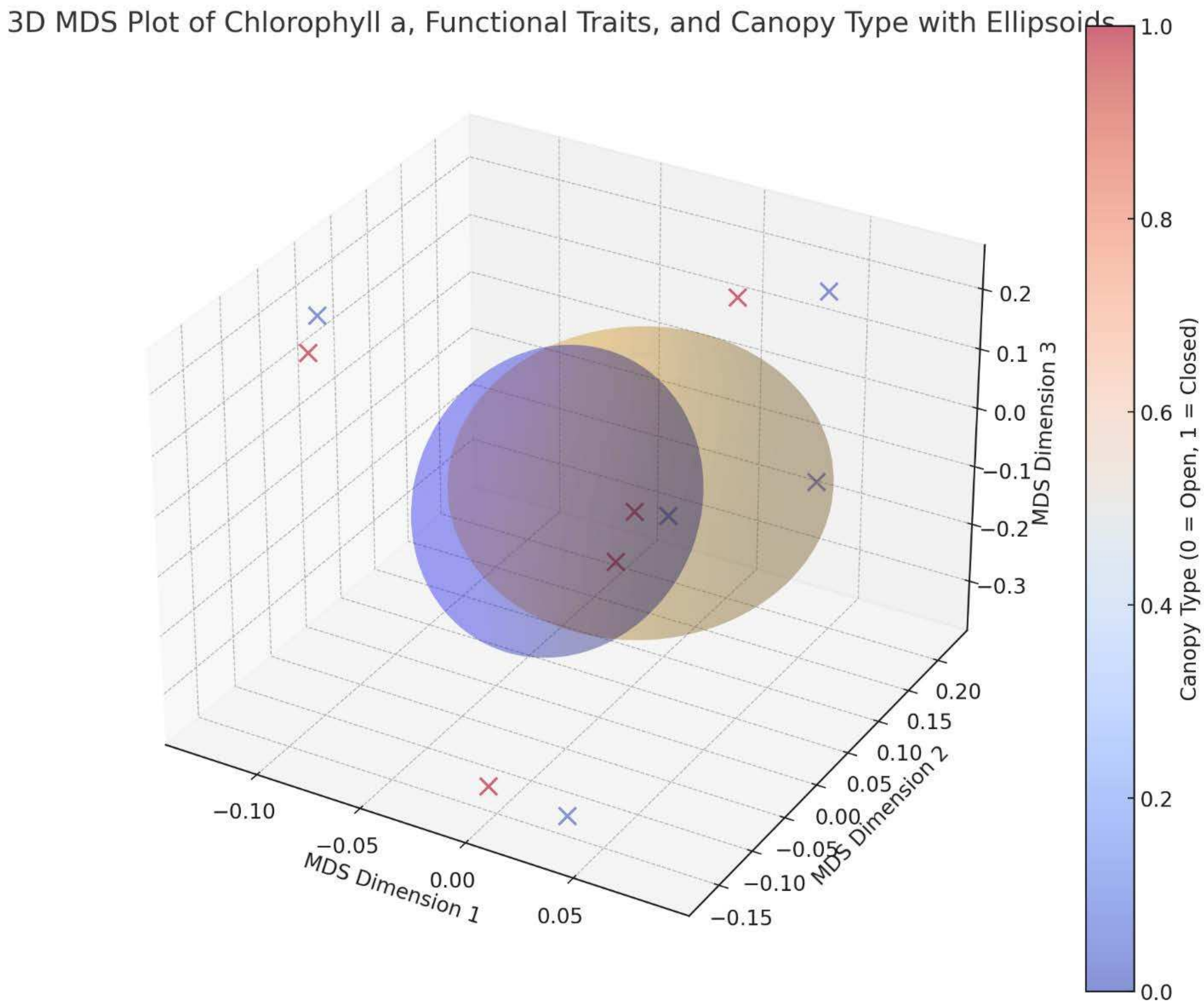

Figure 3. 7. 3D MDS Plot of *Chlorophyll-a*, Functional Traits, and Canopy Type. This 3D MDS plot visually represents the distribution of functional traits across different canopy types and *chlorophyll-a* levels. The ellipsoids provide a visual indication of the clustering of data points based on canopy type, illustrating that distinct functional trait distributions emerge under varying canopy conditions. This plot underscores the ecological implications of canopy structure on functional diversity and community dynamics.

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FINAL CONSIDERATIONS

This dissertation researched the functional diversity of aquatic insect communities across environmental gradients in Rocky Mountain headwater streams, with a focus on how elevation, canopy cover, and water temperature influence community dynamics. The study revealed a significant decline in functional richness (FRic) with increasing elevation, with the steepest reductions observed in environments characterized by sparse canopy cover. These results underscore the intricate relationship between environmental stressors and community composition, reinforcing the idea that specific ecological roles are sensitive to altitudinal changes (Poff et al., 2006; Shah et al., 2018).

Functional evenness (FEve) remained relatively stable across elevations, suggesting that while species richness declined, the distribution of functional roles was maintained. This stability is critical for ecosystem resilience, as it indicates that essential ecological functions persist despite reductions in species diversity (Gill, 2019; Shah et al., 2017). Conversely, functional divergence (FDiv) increased with elevation, indicating that communities at higher altitudes exhibit greater specialization among remaining taxa, which may enhance resilience to environmental fluctuations but could also increase vulnerability to disturbances (Mouillot et al., 2005; Poff et al., 2006).

Overall, this study contributes significantly to our understanding of functional diversity patterns in freshwater ecosystems, highlighting the importance of incorporating functional metrics into ecological assessments and conservation strategies. The findings underscore the vulnerability of headwater stream ecosystems to environmental changes and the necessity of ongoing research to inform management practices aimed at preserving these critical habitats.

Future Research Questions

Impact of Climate Change on Functional Diversity: How will projected climate scenarios, particularly increased temperatures and altered precipitation patterns, impact the functional diversity of aquatic insect communities across different elevation gradients? Long-term studies are necessary to elucidate potential shifts in community structure, particularly under predicted climate change scenarios (Polazzo et al., 2024).

Trophic Interactions and Functional Roles: What specific functional roles do different trophic groups (e.g., predators, grazers, and filterers) play in maintaining ecosystem processes in the context of environmental change? Investigating the interactions among these groups can provide deeper insights into food web dynamics and the ecological consequences of species loss (Espinosa et al., 2023).

Role of Microhabitats: How do variations in microhabitats within streams affect the distribution and functional traits of aquatic insects? Future studies should examine how substrate type, flow dynamics, and local environmental conditions influence functional diversity metrics (Gill, 2019).

Comparative Studies in Tropical Ecosystems: How do functional diversity patterns in tropical streams compare to those observed in temperate regions? Conducting comparative studies could yield valuable insights into global biodiversity conservation strategies and highlight region-specific vulnerabilities and adaptations (Poff et al., 2006).

Influence of Human Activities: What are the effects of land use changes, pollution, and other anthropogenic impacts on the functional diversity of headwater streams? Research focusing on human-induced changes can inform management practices aimed at mitigating adverse effects on freshwater ecosystems (Gill et al., 2016).

Follow-Up Actions to Continue My Research

Longitudinal Studies: Implementing longitudinal studies that monitor changes in functional diversity over time will provide valuable insights into the resilience of aquatic insect communities to ongoing environmental changes. Such studies could clarify the long-term impacts of climate variability on these communities (Neves et al., 2024).

Collaboration with Other Disciplines: Engaging in interdisciplinary collaborations with hydrologists, climate scientists, and ecologists can help create integrated models that predict ecological outcomes based on functional diversity metrics and hydrological changes (Ghalambor et al., 2006).

Enhanced Data Collection Techniques: Utilizing advanced technologies, such as environmental DNA (eDNA) and remote sensing, can improve the accuracy and comprehensiveness of future biodiversity assessments. These techniques can provide more robust data on community composition and functional traits (Polato et al., 2017).

Community Engagement: Involving local communities and stakeholders in conservation efforts can enhance the practical applicability of research findings and foster stewardship for aquatic ecosystems. Educational programs aimed at raising awareness about the importance of functional diversity in freshwater systems can empower local communities to participate in conservation initiatives (Gill, 2019).

Policy Implications: Providing actionable insights for policymakers based on research findings can aid in the development of effective conservation strategies and ecosystem management practices. Advocacy for policies that protect functional diversity and ecosystem health will be essential in the face of rapid environmental changes (Shah et al., 2018).

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