An exploration of the effects of productivity, predators, and nutrient enrichment on metacommunity structure

by

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Abstract

To understand patterns in biodiversity, we first need to understand how it is organized across the landscape. Diversity can be measured in a single patch of habitat as the number of species and number of individuals per species, but patches do not exist in isolation. By organizing the landscape into a collection of patches of habitat, we can define the broader diversity of the metacommunity. By identifying changes to diversity in a single patch (local diversity or alpha diversity) and determining the heterogeneity of diversity among patches (turnover or beta diversity), we can begin to can predict changes in diversity across the landscape (regional diversity or gamma diversity). The aim of this dissertation was to investigate how abiotic and biotic factors influence beta diversity across multiple spatiotemporal scales using a variety of diversity metrics. Using a long-term dataset, I was able to determine that the relationship between primary productivity and species turnover varied across years in part due to annual fluctuations in environmental conditions. To answer questions about how changes to habitat suitability affected community assembly, I conducted experiments in artificial ponds to test the effects of predation and nitrogen enrichment on macroinvertebrates. Fish presence in ponds had a stronger effect on prey diversity than newt or *Anax* presence, reducing overall richness while

increasing abundance of lower-trophic level taxa with some differences across spatial scales. Moderate nitrogen enrichment in ponds increased taxonomic heterogeneity and the heterogeneity of traits present among patches in the metacommunity, but overall, there were signs of functional redundancy across the metacommunity. Together, these results show the breadth of applications of metacommunity ecology for predicting the effects of multiple environmental factors on biodiversity across spatial and temporal scales.

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CHAPTER 1: Introduction

Community ecology, or the study of intraspecific and interspecific interactions in a defined space, is critical to understanding biodiversity. It can inform best practices in conservation (Bush *et al.*, 2016), predict effects of invasive species (Powell, Chase & Knight, 2013), and inform health of individuals (Zaneveld, McMinds & Thurber, 2017). Within a community, niches that are available to species are governed by abiotic factors (e.g., temperature: Mills, Hossie & Murray, 2020; elevation: Khatiwada *et al.*, 2019) and biotic factors (e.g., competition: Connell, 1961; predation: Janzen, 1970). By observing species diversity in a community, we can make inferences about ecosystem quality by quantifying the species richness (number of species) and the relative abundance of each species (Tilman, Isbell & Cowles, 2014). However, these communities of organisms do not exist in a vacuum. Organisms can disperse between patches of habitat from one community to another, resulting in a larger metacommunity (Leibold *et al.*, 2004). These dispersal mechanics add an additional factor to species presence on the landscape, allowing inferior competitors to persist across the larger landscape if they are a superior disperser (Ladouceur *et al.*, 2020).

Four main frameworks have been proposed to guide understanding of how metacommunities are structured: neutral theory, patch dynamics, species sorting, and mass effects. Neutral theory proposes that species traits do not play a role in species assembly, leading to all species having the same competitive ability (Hubbell, 2001). This can result in random species assemblages due to no species being consistently dominant over another. Patch dynamics assumes the environment is separated into identical patches, and species diversity is a trade-off between life-history and dispersal (Lande, 1993). This leads to species randomly becoming locally extirpated within a patch, but dispersal from another patch can lead to their persistence in the larger

metacommunity. Species sorting incorporates resource limitation, treating species diversity as a function of niche availability in any given patch (Steiner & Leibold, 2004). This framework is the basis for much of early metacommunity theory, with species better adapted to certain resource limitations outcompeting within a patch. Lastly, mass effects, also referred to as source-sink patterns, treats dispersal rates as the main driver of species diversity, with source populations supplying individuals to sink populations with available niches (Shmida & Wilson, 1985). In contrast to patch dynamics, this framework assumes constant dispersal saves species from becoming locally extirpated. Most theories surrounding metacommunity ecology can be traced back to these underlying principles based on the weighted importance of resource limitation, competition, and dispersal ability.

While quantifying diversity within a community can be a relatively straight-forward task, scaling up to the metacommunity becomes more complicated. Metacommunity diversity is typically described as a combination of local diversity (alpha diversity), regional diversity (gamma diversity), and the amount of similarity in species composition between communities (species turnover or beta diversity) (Whittaker, 1960). When there is low turnover between communities, the community assemblages are more homogenous, meaning the communities within the metacommunity are made up of the same species (Whittaker, Willis & Field, 2001). A variety of factors are known to affect the magnitude of turnover within and between metacommunities, in large part due to differences in the strength and direction of their effects on local and regional diversity (e.g., parasites: Dallas & Presley, 2014; primary production: Chalcraft *et al.*, 2004; or predation: Chase *et al.*, 2009).

The quantification of biodiversity in metacommunities has evolved rapidly over the last few decades (Chase & Leibold, 2017). When it was first described, beta diversity was calculated as

the quotient of alpha diversity divided by gamma diversity (Whittaker, 1960). However, because of the interdependence of these two terms, there has been a push to find ways to define independent measures of dissimilarity (e.g., Koleff, Gaston & Lennon, 2003; Chase *et al.*, 2018). Additionally, while these concepts are typically applied to taxonomic units (e.g., species diversity), we can also use the collection of traits in a community (i.e., functional diversity) to understand differences in ecosystem function (Loreau *et al.*, 2001; McGill *et al.*, 2006).

Description of Research

This dissertation uses a variety of analytical techniques to investigate patterns in metacommunity structure and community assembly using multiple study systems across multiple spatial and temporal scales. In Chapter 2, I use a long-term dataset from the Jornada Long-Term Ecological Research site in New Mexico to investigate the effects of primary productivity on metacommunity structure. The relationship between productivity and biodiversity is highly variable both within and between spatial scales (Whittaker, 2010), so the goal of this study is to use a long-term dataset to try to understand what factors influence changes in the relationship. Using the Elements of Metacommunity Structure (EMS) method (Leibold & Mikkelson, 2002), I compare patterns of species turnover and boundary clumping across productivity gradients within and among metacommunities over the 33 year dataset in an effort to find environmental characteristics that influence the shape of the relationship between productivity and biodiversity.

The effects of predation on prey communities are highly variable (Morin, 1983; Chase *et al.*, 2009), though there are predicted trends based on predation strategy (Ryberg, Smith & Chase, 2012), which I test in Chapter 3. Generalist predators are expected to lead to loss of species at low abundance within a community, which can lead to more dissimilarity between patches if the identity of rare species differ between communities. In contrast, specialist predators should filter

out specific prey regardless of relative abundance, causing more homogeneous communities as only prey with traits that allow them to coexist with predators will be found. Few studies have tested these predictions (Chase *et al.*, 2009), and fewer have compared predation strategies within a single experiment (Johnston, Pu & Jiang, 2016). I use a mesocosm experiment to test the predicted effects of predation strategy on prey metacommunities using three common aquatic apex predators: sunfish, newts, and *Anax* nymphs. To avoid the interdependence of diversity metrics, I use the Measurement of Biodiversity (MoB) method to break down species richness into three components: abundance, rarefied richness, and evenness.

Finally, in Chapter 4, I explore the effects of nutrient enrichment on the taxonomic and functional diversity of aquatic macroinvertebrate communities. Nitrogen enrichment can have devastating impacts on aquatic ecosystems (Heisler *et al.*, 2008), but it has been shown to have a positive impact on aquatic community assembly (Chase, 2010). To understand the mechanisms that act on changes to metacommunity structure, differences in the traits present in the community (functional diversity) can be incorporated into measures of biodiversity (Mason *et al.*, 2005). In an effort to further understand the effects on community assembly, I establish a mesocosm experiment to determine how nitrogen enrichment affects taxonomic diversity, functional diversity, and intraspecific trait variation within insect orders.

Broader Impacts

We are currently experiencing biodiversity loss up to 100 times greater than the historical background rate (Ceballos *et al.*, 2015). Insects in particular are experiencing declines, stemming from factors like habitat destruction and climate change (Wagner, 2020). These losses can directly result in the loss of ecosystem services like crop failure and maintenance of drinking water (Mace, Norris & Fitter, 2012). If we hope to combat these losses, we first need to have a

greater understanding of how biodiversity is organized across the landscape and the factors that influence the structure of that organization. This dissertation strives to fill in some of these knowledge gaps by applying novel analytical techniques to quantify changes in biodiversity. In doing so, I hope to help inform management practices and provide resources for decision-makers attempting to mitigate species loss across the globe.

References

Bush, A., Harwood, T., Hoskins, A.J., Mokany, K. & Ferrier, S. (2016). Current uses of beta-diversity in biodiversity conservation: A response to Socolar et al. Trends Ecol. Evol. 31, 337–338.

Ceballos, G., Ehrlich, P.R., Barnosky, A.D., García, A., Pringle, R.M. & Palmer, T.M. (2015). Accelerated modern human-induced species losses: Entering the sixth mass extinction. Sci. Adv. 1, 9–13.

Chalcraft, D.R., Williams, J.W., Smith, M.D. & Willig, M.R. (2004). Scale dependence in the relationship between species richness and productivity: the role of spatial and temporal turnover. Ecology 85, 2701–2708.

Chase, J.M. (2010). Stochastic community assembly causes higher biodiversity in more productive environments. Science (80-.). 328, 1388–1392.

Chase, J.M., Biro, E.G., Ryberg, W.A. & Smith, K.G. (2009). Predators temper the relative importance of stochastic processes in the assembly of prey metacommunities. Ecol. Lett. 12, 1210–1218.

Chase, J.M. & Leibold, M.A. (2017). Metacommunity Ecology. Princeton University Press.

Chase, J.M., McGill, B.J., McGlinn, D.J., May, F., Blowes, S.A., Xiao, X., Knight, T.M., Purschke, O. & Gotelli, N.J. (2018). Embracing scale-dependence to achieve a deeper understanding of biodiversity and its change across communities. Ecol. Lett. 21, 1737–1751.

Connell, J.H. (1961). The influence of interspecific competition and other factors on the distribution of the barnacle Chthamalus stellatus. Ecology 42, 710–723.

Dallas, T. & Presley, S.J. (2014). Relative importance of host environment, transmission potential and host phylogeny to the structure of parasite metacommunities. Oikos 123, 866–874.

Heisler, J., Glibert, P.M., Burkholder, J.M., Anderson, D.M., Cochlan, W., Dennison, W.C., Dortch, Q., Gobler, C.J., Heil, C.A., Humphries, E., Lewitus, A., Magnien, R., Marshall, H.G., Sellner, K., Stockwell, D.A., Stoecker, D.K. & Suddleson, M. (2008). Eutrophication and harmful algal blooms: A scientific consensus. Harmful Algae 8, 3–13.

Hubbell, S.P. (2001). The unified neutral theory of biodiversity and biogeography. Princeton University Press.

Janzen, D.H. (1970). Herbivores and the number of tree species in tropical forests. Am. Nat. 104, 501–528.

Johnston, N.K., Pu, Z. & Jiang, L. (2016). Predator identity influences metacommunity assembly. J. Anim. Ecol. 85, 1161–1170.

Khatiwada, J.R., Zhao, T., Chen, Y., Wang, B., Xie, F., Cannatella, D.C. & Jiang, J. (2019). Amphibian community structure along elevation gradients in eastern Nepal Himalaya. BMC Ecol. 19, 1–11.

Koleff, P., Gaston, K.J. & Lennon, J.J. (2003). Measuring beta diversity for presence-absence

data. J. Anim. Ecol. 72, 367–382.

Ladouceur, E., Stanley Harpole, W., Blowes, S.A., Roscher, C., Auge, H., Seabloom, E.W. & Chase, J.M. (2020). Reducing dispersal limitation via seed addition increases species richness but not above-ground biomass. Ecol. Lett. 23, 1442–1450.

Lande, R. (1993). Risks of population extinction from demographic and environmental stochasticity and random catastrophes. Am. Nat. 142, 911–927.

Leibold, M.A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J.M., Hoopes, M.F., Holt, R.D., Shurin, J.B., Law, R., Tilman, D., Loreau, M. & Gonzalez, A. (2004). The metacommunity concept: A framework for multi-scale community ecology. Ecol. Lett. 7, 601–613.

Leibold, M.A. & Mikkelson, G.M. (2002). Coherence, species turnover, and boundary clumping elements of meta-community structure. Oikos 97, 237–250.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U., Huston, M.A., Raffaelli, D., Schmid, B., Tilman, D. & Wardle, D.A. (2001). Biodiversity and ecosystem functioning: Current knowledge and future challenges. Science (80-.). 294, 804–809.

Mace, G.M., Norris, K. & Fitter, A.H. (2012). Biodiversity and ecosystem services: A multilayered relationship. Trends Ecol. Evol. 27, 19–26.

Mason, N.W.H., Mouillot, D., Lee, W.G. & Wilson, J.B. (2005). Functional richness, functional evenness and functional divergence: the primary components of functional diversity. Oikos 111, 112–118.

McGill, B.J., Enquist, B.J., Weiher, E. & Westoby, M. (2006). Rebuilding community ecology from functional traits. Trends Ecol. Evol. 21, 178–185.

Mills, P.B., Hossie, T.J. & Murray, D.L. (2020). Niche determinants in a salamander complex: Does hybridism or reproductive parasitism explain patterns of distribution ? Ecosphere 11, e03265.

Morin, P.J. (1983). Predation, competition, and the composition of larval anuran guilds. Ecol. Monogr. 53, 119–138.

Powell, K.I., Chase, J.M. & Knight, T.M. (2013). Invasive plants have scale-dependent species-area relationships. Science (80-.). 339, 317–319.

Ryberg, W.A., Smith, K.G. & Chase, J.M. (2012). Predators alter the scaling of diversity in prey metacommunities. Oikos 121, 1995–2000.

Shmida, A. & Wilson, M.V. (1985). Biological determinants of species diversity. J. Biogeogr. 12, 1–20.

Steiner, C.F. & Leibold, M.A. (2004). Cyclic assembly trajectories and scale-dependent productivity-diversity relationships. Ecology 85, 107–113.

Tilman, D., Isbell, F. & Cowles, J.M. (2014). Biodiversity and ecosystem functioning. Annu. Rev. Ecol. Evol. Syst. 45, 471–493.

Wagner, D.L. (2020). Insect declines in the anthropocene. Annu. Rev. Entomol. 65, 457–480.

Whittaker, R.H. (1960). Vegetation of the Siskiyou Mountains, Oregon and California. Ecol. Monogr. 30, 279–338.

Whittaker, R.J. (2010). Meta-analyses and mega-mistakes: calling time on meta-analysis of the species richness–productivity relationship. Ecology 91, 2522–2533.

Whittaker, R.J., Willis, K.J. & Field, R. (2001). Scale and species richness: Towards a general, hierarchical theory of species diversity. J. Biogeogr. 28, 453–470.

Zaneveld, J.R., McMinds, R. & Thurber, R.V. (2017). Stress and stability: Applying the Anna Karenina principle to animal microbiomes. Nat. Microbiol. 2.

CHAPTER 2: The relationship between primary productivity and metacommunity structure is mediated by annual variation in the environment

Abstract

Identifying trends in how metacommunities are structured is crucial for understanding the effects of environmental factors on diversity. Turnover and boundary clumping are two particularly important measures of metacommunity structure as they describe how species differ between patches and whether groups of species follow common boundaries across environmental gradients. Productivity is one such gradient that has been repeatedly shown to be important for metacommunity structure, but the exact shape of the relationship between diversity and productivity is variable. Using a meta-analysis approach, we analyzed patterns in grassland and shrubland metacommunity structure across multiple spatial and temporal scales. The goals were to determine if species distributions followed a gradient of primary productivity locally, if regional primary productivity related to observed patterns in turnover and boundary clumping, and if external environmental factors could describe annual differences in the relationship between primary productivity and metacommunity structure. We found that local species distributions were moderately correlated with local primary productivity, though the degree of correlation differed between grasslands and shrublands. On average, turnover had a negative quadratic relationship with regional primary productivity and boundary clumping had a negative linear relationship with productivity. The relationship between regional primary productivity and boundary clumping was variable between years, and the variability was correlated with annual differences in primary productivity and temperature. By analyzing annual variations in the relationship between primary productivity and diversity, we can gain greater insight into how differences in productivity influence diversity in tandem with other environmental features.

Introduction

The collection of species found in a locality varies among locations in a landscape and a fundamental goal in community ecology is to identify generalizable patterns in the distribution of species across the landscape and to understand why those repeatable patterns occur. This is often done by identifying trends in local community structure, defined as the numbers and relative abundances of species within a particular habitat patch. If we reframe these questions in the context of metacommunity ecology, we can identify organization patterns by defining the landscape as a mosaic of patches (Leibold *et al.*, 2004). In doing so, we can compare the community structures across a collection of patches and ascribe differences in the broad metacommunity structure to factors like local variation in environmental parameters (Leibold & Mikkelson, 2002). Niche theory suggests that species will be more likely to occur at their optimal environmental condition and will be less likely to occur farther from that optimum, resulting in the likelihood of a species occurrence along an environmental gradient being best described by a normal probability distribution (Gauch & Whittaker, 1972; Austin, 1985).

To identify the structure of a metacommunity, the order of communities can be reorganized to estimate the distribution of all species across the region by minimizing breaks in both species distributions and the collection of species within each community (Leibold & Mikkelson, 2002). This order of communities is often referred to as the non-specific latent gradient because it assumes the species distributions follow a yet unexplained environmental gradient (Presley, 2020). By examining non-specific latent gradients and comparing them to known environmental gradients, we can determine how the mechanisms that shape metacommunities lead to patterns in diversity among habitat patches (Leibold & Mikkelson, 2002). For example, in some ecosystems, elevation is an important environmental factor in determining species distributions (Presley *et*

al., 2011), while in other ecosystems factors like temperature play a larger role (Stoczynski *et al.*, 2021). Even within an ecosystem type there can be variability in metacommunity structures which have been attributed to differences in environmental factors across the landscape (Henriques-Silva, Lindo & Peres-Neto, 2013). What can be unclear with these studies is whether these metacommunity snapshots are indicative of the general trends in these relationships, or if annual variations in environmental factors can affect these patterns. Species turnover and boundary clumping have been described as important elements of metacommunity structure (Leibold & Mikkelson, 2002), and by measuring changes over large time scales, we can begin to identify possible generalizable trends in structure.

Species turnover is the phenomenon when species are replaced across the landscape by other species due to differences in habitat suitability between patches or random chance (Leibold *et al.*, 2004). As an environmental gradient progresses, species will be lost and gained as the habitat suitability changes. We can therefore quantify species turnover as the frequency of species losses and gains at the edges of each species' range (Leibold & Mikkelson, 2002). By assigning a negative value to species loss and a positive value to species gain, turnover within the metacommunity becomes a continuous range of negative to positive values. Large negative turnover values indicate metacommunities with nested subsets of species, where communities with few species represent a subset of the communities with more species (Patterson & Atmar, 1986). This could result from a situation where a few species can persist at low levels of an environmental gradient, but as the gradient continues more species are able to persist (Hylander *et al.*, 2005). In contrast, metacommunities with positive turnover have high rates of species being replaced across the landscape due to factors like niche availability (López-González *et al.*, 2012) or competitive superiority (Segre *et al.*, 2014).

Boundary clumping is often defined as the tendency of species turnover patterns to follow a Clementsian or Gleasonian distribution. Succession theory often describes community turnover as following a Clementsian distribution, where collections of species within the community occur at sites with similar resource availability (Clements, 1916). Each one of these smaller collections of species share a common range of suitable niches, which means at points along an environmental gradient, multiple species should be replaced simultaneously. Gleason (1926) instead proposed that the edges of each species' range occur independently due to differences in species-specific resource needs. In addition to both of these paradigms, there are cases where there is strong interspecific competition for similar resource requirements, leading to each species range falling within distinct realized niches (Tilman, 1982). This leads to species distribution boundaries occurring at evenly spaced intervals across the landscape. Together, turnover and boundary clumping can provide insights into how community structure within a metacommunity changes along an environmental gradient.

One environmental characteristic that can influence species diversity is net primary productivity (Waide *et al.*, 1999), often measured as the increase in biomass across all primary producing species present within a designated area. The shape of the effect of primary productivity on local community structure has been a topic of debate in the field, with predictions for positive, negative, and hump-shaped responses in species richness to increasing productivity (Mittelbach *et al.*, 2001; Gillman & Wright, 2006; Pärtel, Laanisto & Zobel, 2007). One common theme within this debate has been the importance of incorporating spatial scale, particularly the influence of species turnover within metacommunities on the relationship between diversity and productivity (Whittaker, 2010). However, the shape of the relationship between primary productivity and diversity across the metacommunity can differ even when

explicitly defining the spatial components of diversity. Studies have found increasing effects of productivity at larger scales (Chalcraft *et al.*, 2004), decreasing effects at larger scales (Šímová, Li & Storch, 2013), shifts in the type of response (Chase & Leibold, 2002), and no effect of spatial scale (Lisner *et al.*, 2021). To uncover why the relationship between primary productivity and diversity is so variable, we need to determine how local variation in productivity within a metacommunity affects the patterns we see in metacommunity structure. Once we identify the influence of productivity within a metacommunity, we can then scale up to patterns in metacommunity structure across a range of regional productivity levels. Theory suggests that the variability in the relationship between primary productivity and diversity is due to the relative importance of other factors, like climate and soil fertility, acting in tandem to alter trends in diversity (Loreau *et al.*, 2001; Grace *et al.*, 2016). By using pattern-based metacommunity structure analyses like the Elements of Metacommunity Structure method (Leibold & Mikkelson, 2002), we can determine whether external factors affect the relationship between productivity and diversity, as well as what specific elements of structure are affected.

Using a long-term dataset from a primary productivity study at the Jornada Long-Term Ecological Research (LTER) site in New Mexico, we sought to determine how changes in annual net primary productivity (ANPP) across spatial scales predicted changes in community patterns. We investigated these trends at the local, regional, and landscape scale to disentangle scale-specific differences in the effect of ANPP using 15 metacommunities with 49 communities in each over 33 years (**Figure 2.1**). Within the metacommunity, we predicted the collection of local communities would be structured along a gradient of ANPP. Past work looking at turnover of species occurrences in the Jornada LTER found a hump-shaped relationship with increasing ANPP (Chalcraft *et al.*, 2004), so we expected to see a similar trend for turnover of species

distributions across metacommunities with increasing average ANPP. No work has been done on how productivity influences boundary clumping, but we predicted higher productivity would lead to more individualistic turnover across metacommunities, resulting in a negative linear relationship between ANPP and boundary clumping. Lastly, we predicted heterogeneity in the responses of turnover and clumping within metacommunities across years would be related to other environmental factors, like climate and soil conditions.

Methods

Jornada Dataset

We used 33 years of data (1990-2022) from a long-term vegetation monitoring study at the Jornada Basin LTER site in southern New Mexico, USA to 1) determine whether the metacommunity structure within a region is associated with variation in productivity among local sites, 2) determine if patterns of metacommunity structure are consistent across regions, 3) assess whether variation in metacommunity structure across regions is associated with variation in regional productivity, and 4) determine if heterogeneity in the relationship between metacommunity structure and productivity across years is driven by regional scale differences in environmental conditions (Peters & Huenneke, 2023a; Peters & Huenneke 2023b).

Researchers at the Jornada LTER established three 1-hectare sites to represent each of the five dominant habitat types in the Chihuahuan Desert: creosote bush shrublands, mesquite shrublands, tarbush shrublands, black grama grasslands, and ephemeral lakebeds called playas. Each 1-hectare site represents a metacommunity, resulting in three metacommunities for each of the five habitat types. Sampling within each metacommunity was represented by 49 1 m^2 quadrats (hereafter communities) spaced at 9 m intervals in a 7x7 grid, except one of the playa

sites (College) which had 48 quadrats in a 3x16 grid. Moving forward, we refer to metacommunities as containing 49 communities for the sake of simplicity.

Vegetation surveys in each community were conducted three times per year: fall, spring, and winter. Plants in each community were identified to species and aboveground biomass was calculated using species-specific regressions using volume and dry weight (Huenneke, Clason & Muldavin, 2001). We estimated aboveground net primary productivity in a community (i.e., local ANPP) by calculating the changes in biomass for each species between each timepoint and then summing all positive changes within the community for each year. We used the data from 1990-2022 for the analyses to determine the ANPP gradient within each of the 15 metacommunities in each of the 33 years.

Definition of Spatial Scales

Local scale estimates refer to those that pertain to the community within a single quadrat, providing information about the effect of small-scale differences in the environment on community assembly within a patch. Regional scale estimates encompass the entire collection of communities within a metacommunity which allowed us to determine if metacommunity structures differ based on environmental gradients. The landscape scale included trends across all metacommunities to compare how annual differences altered patterns across the entire study area.

Elements of Metacommunity Structure

We used the Elements of Metacommunity Structure (EMS) analysis outlined in Leibold & Mikkelson (2002) and built-upon by Presley *et al*. (2010) to identify which of six original metacommunity structures (random, checkerboard, nested, evenly-spaced, Clementsian, or Gleasonian) or any non-significant quasi-structures best describe each of the 15

metacommunities that we studied. This method is based on the theory that species distributions follow coherent Gaussian likelihood distributions within a metacommunity without breaks within the range of suitable environmental factors. Using reciprocal averaging, EMS rearranges a site by species presence/absence matrix to reduce the number of absences within the distribution of all species present across the metacommunity. This results in an ordinated matrix where the sites are ordered in a way that should follow one or more environmental gradients. We performed this analysis to produce 33 quadrat by species matrices (one per year) for each of the 15 metacommunities, with each site corresponding to a community in the respective metacommunity.

Each ordinated matrix was tested for three elements: coherence, species turnover, and boundary clumping. This was done by comparing the matrix to a null distribution created by a series of 1000 null matrices. We used the "r0" null model method which produces matrices with fixed row totals to conserve species richness estimates for each community and assigns each species an equiprobable chance of occurrence (Presley *et al.*, 2010). Two of the 495 ordinated matrices had to be removed from the analysis because they did not have enough species present to create the null matrices.

Coherence was assessed by comparing the frequency of embedded absences (species absences within the estimated range of sites) in each ordinated matrix to the frequency estimates across the null distribution to determine if the metacommunities were more structured than random chance. Because the result of reciprocal averaging is a coherent, ordinated matrix, a metacommunity that is not coherent would have a collection of species that were missing from communities within their expected range based on the ordination process. This would likely

mean the metacommunity has a random collection of species that do not follow a discernable environmental gradient.

Turnover was measured as the tendency of species to replace one another between two quadrats in the ordinated matrices by counting the number of times one species replaced another across all possible species pairs. Positive turnover occurred when species replaced each other more frequently than expected based on the null distribution of species replacements across the 1000 null matrices, and negative turnover occurred when species were replaced less frequently than expected. Comparing the number of turnovers in the metacommunity to the null distribution produced a *Z*-score that allowed for a standardized metric of species turnover. This Turnover *Z*-score corresponded to the magnitude of difference between turnover in the observed community and the mean turnover of the null matrices (e.g., a *Z*-score of 2 means there were 2 standard deviations more turnover events than expected by random chance). Boundary clumping was measured using the Morisita's index (*I*) to determine if multiple species exhibited turnover simultaneously more frequently ($I > 1.0$) or less frequently ($I < 1.0$) than the null distribution. This analysis was done using the R package "metacom", which relies heavily on "vegan" (Oksanen *et al.*, 2019; Dallas, 2020). Coherence, turnover, and boundary clumping scores were calculated for each of the 493 ordinated matrices, one for each metacommunity in each year.

Correlation between structure and productivity

To determine whether the latent environmental gradient associated with local community structure within a metacommunity was associated with local variation in primary productivity, we extracted ordination scores associated with the order of communities in the ordinated matrices and calculated the Spearman rank correlations between local ANPP and those community scores for each of the 493 matrices to determine how well latent environmental

gradients matched local ANPP. A strong correlation between these variables suggests that communities are structured across a gradient of local ANPP within the metacommunity, while a weak correlation indicates other environmental gradients are likely a more important factor in the non-random structure of the communities. The direction of the correlation does not provide information about the relative importance of the productivity gradient. In one matrix, community ordination scores could increase with increasing productivity and in another, scores could decrease with increasing productivity. In either case, the metacommunity would be structured along a productivity gradient, but the direction of the correlation coefficient would differ. Because of this, we used the absolute value of the correlation coefficient in our analyses.

Our Spearman rank analyses assessed whether variation in local community structure varied predictably with local ANPP within each of the 15 metacommunities for each of the 33 years of study. Given the variation in the correlation estimates for each year, we performed a meta-analysis to derive an estimate of the average association between ordinated local community scores and local ANPP across all years of study for each metacommunity. This approach assumes there is a single true correlation between these measures and that each correlation coefficient represents a different estimate of the true correlation. We calculated the mean and standard error of the correlation coefficients within each metacommunity across all years. Using these means and standard errors, we obtained a weighted estimate of the true correlation coefficient, weighing each metacommunity based on the relative magnitude of the 95% confidence interval of the correlation coefficient.

When the Jornada LTER was first established, each metacommunity was assigned a habitat type based on differences in biotic and abiotic characteristics. Because we were interested in identifying generalizable trends in the relationship between productivity and structure, we

wanted to account for the range of mean correlation coefficients that would arise from this habitat heterogeneity. To do so, we used a random-effects model with the REML procedure for the meta-analysis of average Spearman rank correlations. A random-effects meta-analysis assumes that there is a distribution of true effect sizes rather than a single true effect and estimates the mean of that distribution (Borenstein *et al.*, 2010). Random-effects meta-analyses also test the heterogeneity of the dataset to determine if the data all come from the same distribution of effects or if there are two or more distributions being compared using the variance of true effect sizes (τ^2) and the amount of variation attributed to between-study heterogeneity (I^2). This was done using the "metagen" function in the R package "meta" (Balduzzi, Rücker & Schwarzer, 2019). All analyses with significant heterogeneity were paired with visualization of the meta-analyses using diagnostic plots to determine which datapoints were driving the heterogeneity. Influence plots were created using the "InfluenceAnalysis" function in the "dmetar" R package (Harrer *et al.*, 2019). Additionally, to test if heterogeneity was due to differences between grassland and shrubland sites, we ran a two-sample t-test using the average correlation estimates for each region.

Productivity effects on metacommunity structure

All of the 493 ordinated matrices were significantly coherent, indicating that there were fewer embedded absences than expected from random chance (*Z*-scores ranged from -15.87 to -40.60 with a positive score indicating lack of coherence). Because of this consistency, we chose to just use the Turnover *Z*-score and the Boundary *I* to identify trends in metacommunity structure patterns across the 15 metacommunities. To do so, we calculated the mean and standard error of Turnover *Z*-score and Boundary *I* for each metacommunity using each year as the replicate, resulting in one mean and standard error estimate for each of the 15 metacommunities.

We then ran meta-analyses on these values for Turnover *Z*-scores and Boundary *I* to determine the average metacommunity structure for the Jornada LTER. The habitat characteristics associated with each habitat type could influence these elements, so we accounted for the heterogeneity in habitat characteristics between the metacommunities using a random-effects model with the REML procedure.

To assess whether variation in turnover or boundary clumping varied predictably among metacommunities differing in regional ANPP, we ran a series of linear models within each year to determine how Turnover *Z*-score and Boundary *I* responded to among metacommunity variation in regional ANPP. Each model used one of the EMS elements as the response variable with regional ANPP as both a linear and a quadratic predictor. We included regional ANPP as both a linear and a quadratic term because literature suggests that turnover (and by association, boundary clumping) can have a hump-shaped relationship with productivity (Chalcraft *et al.*, 2004). Regional ANPP was calculated as the average local ANPP within each site for each year. To avoid multicollinearity of the regional ANPP linear and quadratic terms, we centered regional ANPP values around the mean. This resulted in 33 models for both Turnover *Z*-score and Boundary *I*, each with three parameter estimates: intercept, the linear component of the relationship with regional ANPP, and the quadratic component of the relationship. We then took all of the parameter estimates and standard errors from the 66 models and ran meta-analyses for each collection of estimates, resulting in six meta-analyses each comparing the 33 years. Because we were explicitly interested in how the relationship between regional ANPP and metacommunity structure varied across years, we used random-effects models with the REML procedure.

Impact of environmental characteristics on heterogeneity in metacommunity structures

We were explicitly interested in evaluating the relationship between model parameters that were heterogeneous across years and landscape-scale annual differences in the environment. Doing so would provide insight into how external factors might alter the effect that regional ANPP had on turnover and boundary clumping. For each regression parameter (i.e., intercept, linear, and quadratic) with statistically significant heterogeneity across years, we ran a Spearman rank correlation between the collection of estimates for a parameter (i.e., all 33 years) and landscape ANPP, which was calculated as the average regional ANPP across all metacommunities within a year. Strong correlations meant that the parameter estimate describing how either turnover or boundary clumping changed across metacommunities with different regional ANPP varied with how productive the landscape was in a particular year. For example, a strong negative correlation between landscape ANPP and the slope of a positive linear relationship between boundary clumping and regional ANPP would indicate that the degree to which boundary clumping increases linearly with regional ANPP is lower in years with higher levels of landscape ANPP.

For a subset of the years (2013-2022), researchers at the Jornada LTER have collected a variety of soil and climate measurements at each of the metacommunities, which allowed us to run Spearman rank correlations between these measurements and the parameter estimates that varied cross years according to the tests of heterogeneity in the meta-analyses (see **Supplement** for references to the datasets). Soil temperature and volumetric water content measurements were taken every 30 minutes in each region by a pair of Campbell Scientific CS650 water content reflectometers at three different depths (10 cm, 20 cm, and 30 cm) and were logged by Campbell Scientific Instruments (CSI) CR1000 data loggers. Precipitation, air temperature, and

humidity data were collected in 1-second intervals by a single CSI CR1000 data logger in each region. The average landscape annual value for each environmental characteristic was calculated by first averaging the characteristic per region per year, and then taking the average parameter per year across all regions.

Results

Relationship between ordinated quadrat scores and local productivity

A meta-analysis of Spearman rank correlations between quadrat ordering and local ANPP revealed that in general, communities within a metacommunity are structured along a gradient of local ANPP after removing directionality ($r = 0.309$, $t_{14} = 10.99$, $p < 0.0001$; **Figure 2.2**). There was substantial heterogeneity among the correlation coefficients for the metacommunities (mean $\tau^2 = 0.011$, 95% CI = 0.0055-0.0284; $I^2 = 93.8\%$, 95% CI = 91.2%-95.6%), indicating that structure within some metacommunities more closely followed a productivity gradient than others. On average, the correlation between quadrat scores and local ANPP was higher for shrubland regions than grassland regions ($t_{13} = -3.15$, $p = 0.008$).

Standardized residuals indicated that two metacommunities were potentially influential on the average correlation (GRAV and RABB), which was corroborated by higher Cook's Distance scores and reduction of the τ^2 in a leave-one-out (LOO) test for both regions (**Figure S2.1**). These two regions were those with the strongest correlation between quadrat ordering and local ANPP, but the trend is comparable even with their removal ($r = 0.281$, $t_{12} = 11.44$, $p < 0.0001$).

Metacommunity structure elements

On average, metacommunities within the Jornada LTER have a quasi-Clementsian or Clementsian structure where species replace one another between patches within a metacommunity more often than expected by random chance and do so simultaneously as groups

of species. The pooled Turnover *Z*-score across the 15 metacommunities was significantly positive ($Z = 2.437$, $t_{14} = 9.01$, $p < 0.0001$; **Figure 2.3a**), but there was substantial heterogeneity among metacommunities (mean $\tau^2 = 0.944$, 95% CI = 0.473-2.752; $I^2 = 90.6\%$, 95% CI = 86.1%-93.6%). The prediction interval (*g*) for Turnover *Z*-score indicates that turnover is consistently positive in this system, but it may not always be statistically significant ($g = 0.263$-4.612). The heterogeneity was primarily driven by a single region (CALI), which exhibited non-significant negative turnover on average, indicating possible nested subsets of species (**Figure S2.2**).

Pooled Boundary *I* indicated significant clumping ($I = 2.472$, $t_{14} = 16.56$, $p < 0.0001$; **Figure 2.3b**) with substantial heterogeneity between regions (mean $\tau^2 = 0.262$, 95% CI = 0.129-0.868; $I^2 = 88.9\%$, 95% CI = 83.3%-92.6%). This once again appeared to be caused by the region CALI, which had a much higher Morisita's Index than the other regions (**Figure S2.3**). Regardless, the range edges of species distributions within a metacommunity were consistently clumped across all metacommunities with a Morisita's Index greater than 1 ($g = 1.324$ to 3.619).

Effect of regional productivity on structure elements

Turnover *Z*-score had a quadratic relationship with regional ANPP across years (**Figure 2.4a; Table 2.1**). The intercept estimate was significantly positive ($Z = 2.820$, $t_{32} = 22.40$, $p < 0.0001$) and there was low heterogeneity across years ($I^2 = 21.3\%$, **Table 2.1**, **Figure S2.4**). There was also a significant positive linear component for the quadratic relationship between Turnover *Z*-score and regional ANPP (estimate = 1.536, $t_{32} = 6.77$, $p < 0.0001$) with low heterogeneity between years ($I^2 = 8.3\%$, **Table 2.1**, **Figure S2.5**). The quadratic component for the relationship was significantly negative (estimate = -0.383, $t_{32} = -4.35$, $p = 0.0001$) with low heterogeneity between years ($I^2 = 28.0\%$). The prediction interval for the quadratic estimate did

cross 0 (g = -0.797 to 0.030, **Table 2.1**, **Figure S2.6**), so it is possible that data collected from another year that was not sampled would find a positive linear relationship between regional ANPP and Turnover *Z*-score without a quadratic component.

In contrast, Boundary *I* had a negative linear relationship with regional ANPP with a possible positive quadratic component (**Figure 2.4b; Table 2.1**). The intercept estimate was significantly positive (I = 2.167, t_{32} = 46.45, $p < 0.001$) and had low heterogeneity between years (I^2 = 0.0%, **Table 2.1**, **Figure S2.7**). There was a significant negative linear relationship between Boundary *I* and regional ANPP (estimate = -0.451, t_{32} = -2.72, p = 0.011), but there was moderate heterogeneity between years (I^2 = 47.0%, **Table 2.1**, **Figure S2.8**). Four years had large standardized residuals in the linear relationship and two additional years had a particularly large influence on the heterogeneity (**Figure S2.9**). There is a trend towards a possible positive quadratic relationship between Boundary *I* and regional ANPP (estimate = 0.067, t_{32} = 1.96, p = 0.058) with moderate heterogeneity between years (I^2 = 49.1%, **Table 2.1**, **Figure S2.10**). This is likely because of the larger variance in Boundary *I* values at low regional ANPP causing a steeper slope that declines as regional ANPP increases (**Figure 2.4b**). Two years appeared to be influential according to the Cook's D and τ^2 LOO estimates (2008 and 2010), but the standardized residual plots showed six different outliers (1990, 1997, 2006, 2016, 2017, and 2018) (**Figures S2.11**). The influence of 2008 and 2010 appeared to be driven by the disproportionate weight they were assigned due to their low standard error estimates.

Heterogeneity is influenced by variability in environmental conditions

The linear and quadratic parameter estimates for the relationship between regional ANPP and Boundary *I* had significant heterogeneity across years. Therefore, these were the only two parameter estimates that we tested for a relationship with landscape-scale annual variations in

environmental conditions. There was not a correlation between landscape ANPP and the linear component of the relationship between regional ANPP and Boundary *I* (r = -0.029, p = 0.872), but there was a moderate negative correlation between landscape ANPP and the quadratic component of the relationship between regional ANPP and Boundary *I* (r = -0.574, $p < 0.001$). The only significant correlations between parameter estimates and soil characteristics were between the quadratic component and soil temperature at all three depths (r = 0.817, p = 0.011; **Table 2.2**). Average annual precipitation was negatively correlated with the linear component of regional ANPP and boundary *I* (r = -0.683, p = 0.050), and air temperature was positively correlated with the quadratic component (r = 0.783, p = 0.017).

Discussion

This study supports the hypothesis that primary productivity plays a role in how metacommunities are structured. We found that productivity explains variation in community structure within metacommunities after comparing metacommunity ordination to local variation in ANPP. Our models indicated that regional ANPP explained some of the variation in species turnover and boundary clumping observed in different metacommunities (**Table 1**). Significant heterogeneity across years in the relationship between Boundary *I* and regional ANPP was related to annual differences in environmental factors at the landscape scale (**Table 2**). The variability in species turnover and boundary clumping among metacommunities and across years supports past work showing variable relationships between primary productivity and diversity at multiple spatial scales (Craven *et al.*, 2020), and suggests that this variability is due to changes in other environmental characteristics (Grace *et al.*, 2016).

As predicted, the meta-analysis showed a correlation between local ANPP and the coherent order of quadrats, though it ranged from weak to moderate depending on metacommunity. This

indicates that, on average, communities are structured along a gradient of productivity within metacommunities, but the strength of the relationship is variable between metacommunities. Past work focused on species richness found weak relationships between richness and productivity at the global scale (Adler *et al.*, 2011; Tredennick *et al.*, 2016). One possible reason for the weak relationships in our study could be the importance of the history of a region, including past productivity or diversity. Grace *et al.* (2016) showed through a series of structural equation models that productivity was just a small piece of a larger system of processes that influence diversity, like climatic factors and soil suitability. Soil suitability differences are particularly relevant because studies taking place over large temporal scales that treat years as independent run the risk of missing the effects of prior richness through plant-soil feedbacks (Thakur *et al.*, 2021). However, recent work has shown that historic species richness is a poor predictor of live biomass, indicating that current richness is more important than past richness (Dee *et al.*, 2023). While we did not test the effects of productivity on species richness, there could be an effect of the history of a community on the structure of a given year. Incorporating lagged metrics like prior productivity or richness could help explain differences in the correlation between current productivity and community order.

In our meta-analysis, grasslands tended to have a lower average correlation between local variation in ANPP and community order and shrublands had a higher average correlation. The type of grassland or shrubland did not seem to have an effect, as evidenced by the two influential regions being a creosote bush site and a mesquite site. Work in the field of biodiversity-ecosystem functioning has put an increased emphasis on trait diversity as a predictor for the strength of the relationship between diversity and productivity (Tilman *et al.*, 2014). While similar functional diversity might explain the grouping of shrublands and grasslands, it does not

explain why grasslands had lower correlation coefficients on average. One possible factor is that shrublands consistently had lower productivity and a smaller range of productivity than grasslands. Past work has shown that community structure in higher productivity environments is more stochastic, which would explain lower correlation between community ordering and local ANPP (Chase, 2010).

On average, there was statistically significant positive turnover in each of the 15 metacommunities that occurred during each of 33 years of study, though there was substantial heterogeneity between regions. This indicates that species are replacing one another along the latent environmental gradient within each metacommunity, which moderately follows a productivity gradient. When paired with the consistently positive boundary clumping, the majority of metacommunities can be best described as having either a quasi-Clementsian or a Clementsian distribution. This means that communities within each metacommunity have groups of species that are adapted to similar niches along the latent environmental gradient, which is at least partially related to net primary productivity. The exception to this was the creosote bush shrubland site CALI, which had highly variable turnover depending on the year but on average exhibited non-statistically significant nested subsets. One possible reason for this difference could be because this region had the lowest average regional ANPP. If species richness and productivity have a hump-shaped relationship, then the range of this region's productivity gradient might not reach the point where richness begins to decrease again, making the structure appear nested. This is supported by our finding that within the average year, there was a quadratic relationship between Turnover *Z*-score and regional ANPP. which is consistent with past work done in this system (Chalcraft *et al.*, 2004).

The relationship between Boundary *I* and regional ANPP was highly variable between years but, on average, the Boundary *I* of a metacommunity declined linearly with regional ANPP. This indicates a shift away from Clementsian shared niche boundaries in metacommunities with low ANPP towards Gleasonian individualistic turnover in metacommunities with higher regional productivity, though our metacommunities never reach the point of consistent individualistic turnover. Clementsian distributions are often viewed as a form of deterministic species assembly, while Gleasonian distributions are more stochastic (Måren *et al.*, 2018; Blanco *et al.*, 2021; Costa *et al.*, 2021). If this is the case, then we can interpret the negative relationship between boundary clumping and regional ANPP as metacommunities with higher ANPP being under a greater influence by stochastic processes than metacommunities with lower ANPP, a phenomenon which has been recorded in other systems (Chase, 2010). The linear component of the relationship between Boundary *I* and regional ANPP was moderately negatively correlated with annual precipitation, indicating that years with high precipitation had the largest negative relationship between boundary clumping and regional ANPP. Landscape ANPP was negatively correlated with the quadratic component of these models, implying that years with the highest overall productivity had the weakest quadratic relationship. In contrast, both temperature variables (soil and air) were positively correlated with the quadratic component. In their structural equation models, Craven *et al.* (2020) found that increasing annual temperatures had a negative effect on both biomass and net primary productivity, measured as the sum of daily net photosynthesis minus growth and maintenance. If this relationship is also present in our system, then this trade-off is likely what led to the average quadratic component not being significant in the meta-analysis.

In conclusion, we identified common trends in the relationship between productivity and community structure and the effects of external environmental factors. We have also shown how the individual metrics from the elements of metacommunity structure can be used to gain a better understanding of how structures might differ beyond the prescription of Clementsian or Gleasonian. Simplifying structures to that level can be useful when determining general trends, but given the preponderance of Clementsian or quasi-Clementsian structures identified using this method, more detailed analyses like this study may be warranted (Chase & Leibold, 2017). Doing so could give insights into the degree to which the elements of metacommunity structure differ due to environmental conditions rather than assigning all metacommunity structures into broad categories. Future research should continue to identify trends in species turnover and boundary clumping in this way by applying it to a larger grain to compliment work being done at this scale using richness (Craven *et al.*, 2020). Incorporating this lens will help to determine if changes in biodiversity influence or are influenced by changes in ecosystem function.

References

Adler, P.B., Seabloom, E.W., Borer, E.T., Hillebrand, H., Hautier, Y., Hector, A., Harpole, W.S., Halloran, L.R.O., Grace, J.B., Anderson, T.M., Bakker, J.D., Biederman, L. a, Brown, C.S., Buckley, Y.M., Calabrese, L.B., Chu, C., Cleland, E.E. & Collins, S.L. (2011). Productivity Is a Poor Predictor of Plant Species Richness. Science. 1750, 1750–1754.

Austin, M.P. (1985). Continuum concept, ordination methods, and niche theory. Annu. Rev. Ecol. Syst. 16, 39–61.

Balduzzi, S., Rücker, G. & Schwarzer, G. (2019). How to perform a meta-analysis in R: a practical tuturial. Evid. Based. Ment. Health 22, 153–160.

Blanco, J.A., Lo, Y.H., Kimmins, J.P. & Weber, A. (2021). Highlighting complex long-term succession pathways in mixed forests of the pacific northwest: A markov chain modelling approach. Forests 12.

Borenstein, M., Hedges, L. V., Higgins, J.P.T. & Rothstein, H.R. (2010). A basic introduction to fixed-effect and random-effects models for meta-analysis. Res. Synth. Methods 1, 97–111.

Chalcraft, D.R., Williams, J.W., Smith, M.D. & Willig, M.R. (2004). Scale dependence in the relationship between species richness and productivity: the role of spatial and temporal turnover. Ecology 85, 2701–2708.

Chase, J.M. (2010). Stochastic community assembly causes higher biodiversity in more productive environments. Science. 328, 1388–1392.

Chase, J.M. & Leibold, M.A. (2002). Spatial scale dictates the productivity-biodiversity relationship. Nature 416, 427–430.

Chase, J.M. & Leibold, M.A. (2017). Metacommunity Ecology. Princeton University Press.

Clements, F.E. (1916). Plant Succession, An Analysis of the Development of Vegetation. Carnegie Inst. Washingt.

Costa, A.P.L., Takemoto, R.M., Lizama, M. de los A.P. & Padial, A.A. (2021). Metacommunity of a host metapopulation: explaining patterns and structures of a fish parasite metacommunity in a Neotropical floodplain basin. Hydrobiologia 848, 5103–5118.

Craven, D., van der Sande, M.T., Meyer, C., Gerstner, K., Bennett, J.M., Giling, D.P., Hines, J., Phillips, H.R.P., May, F., Bannar-Martin, K.H., Chase, J.M. & Keil, P. (2020). A cross-scale assessment of productivity–diversity relationships. Glob. Ecol. Biogeogr. 29, 1940–1955.

Dallas, T. (2020). metacom: Analysis of the "Elements of Metacommunity Structure."

Dee, L.E., Ferraro, P.J., Severen, C.N., Kimmel, K.A., Borer, E.T., Byrnes, J.E.K., Clark, A.T., Hautier, Y., Hector, A., Raynaud, X., et al. (2023). Clarifying the effect of biodiversity on productivity in natural ecosystems with longitudinal data and methods for causal inference. Nat. Commun. 14, 1–12.

Gauch, H.G. & Whittaker, R.H. (1972). Coenocline Simulation. Ecology 53, 446–451.

Gillman, L.N. & Wright, S.D. (2006). The influence of productivity on the species richness of plants: A critical assessment. Ecology 87, 1234–1243.

Gleason, H.A. (1926). The Individualistic Concept of the Plant Association. Bull. Torrey Bot. Club 53, 7–26.

Grace, J.B., Anderson, T.M., Seabloom, E.W., Borer, E.T., Adler, P.B., Harpole, W.S., Hautier, Y., Hillebrand, H., Lind, E.M., Pärtel, M., Bakker, J.D., Buckley, Y.M., Crawley, M.J., Damschen, E.I., Davies, K.F., Fay, P.A., Firn, J., Gruner, D.S., Hector, A., Knops, J.M.H., MacDougall, A.S., Melbourne, B.A., Morgan, J.W., Orrock, J.L., Prober, S.M. & Smith, M.D. (2016). Integrative modelling reveals mechanisms linking productivity and plant species richness. Nature 529, 390–393.

Harrer, M., Cuijpers, P., Furukawa, T. & Ebert, D.D. (2019). dmetar: Companion R package for the guide "Doing Meta-Analysis in R."

Henriques-Silva, R., Lindo, Z. & Peres-Neto, P.R. (2013). A community of metacommunities: Exploring patterns in species distributions across large geographical areas. Ecology 94, 627–639.

Huenneke, L.F., Clason, D. & Muldavin, E. (2001). Spatial heterogeneity in Chihuahuan Desert vegetation: Implications for sampling methods in semi-arid ecosystems. J. Arid Environ. 47, 257–270.

Hylander, K., Nilsson, C., Jonsson, B.G. & Göthner, T. (2005). Differences in habitat quality explain nestedness in a land snail meta-community. Oikos 108, 351–361.

Leibold, M.A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J.M., Hoopes, M.F., Holt, R.D., Shurin, J.B., Law, R., Tilman, D., Loreau, M. & Gonzalez, A. (2004). The metacommunity concept: A framework for multi-scale community ecology. Ecol. Lett. 7, 601–613.

Leibold, M.A. & Mikkelson, G.M. (2002). Coherence, species turnover, and boundary clumping elements of meta-community structure. Oikos 97, 237–250.

Lisner, A., Ottavian, G., Klimešová, J., Mudrák, O., Martínková, J. & Lepš, J. (2021). The species richness–productivity relationship varies among regions and productivity estimates, but not with spatial resolution. Oikos 130, 1704–1714.

López-González, C., Presley, S.J., Lozano, A., Stevens, R.D. & Higgins, C.L. (2012). Metacommunity analysis of Mexican bats: Environmentally mediated structure in an area of high geographic and environmental complexity. J. Biogeogr. 39, 177–192.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U., Huston, M.A., Raffaelli, D., Schmid, B., Tilman, D. & Wardle, D.A. (2001). Biodiversity and Ecosystem Functioning: Current Knowledge and Future Challenges. Science (80-.). 294, 804–809.

Måren, I.E., Kapfer, J., Aarrestad, P.A., Grytnes, J.A. & Vandvik, V. (2018). Changing contributions of stochastic and deterministic processes in community assembly over a successional gradient. Ecology 99, 148–157.

Mittelbach, G.G., Steiner, C.F., Scheiner, S.M., Gross, K.L., Reynolds, H.L., Waide, R.B., Willig, M.R., Dodson, S.I. & Gough, L. (2001). What is the observed relationship between species richness and productivity? Ecology 82, 2381–2396.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E. & Wagner, H. (2019). vegan: Community Ecology Package.

Pärtel, M., Laanisto, L. & Zobel, M. (2007). Contrasting plant productivity-diversity relationships across latitude: The role of evolutionary history. Ecology 88, 1091–1097.

Patterson, B.D. & Atmar, W. (1986). Nested subsets and the structure of insular mammalian faunas and archipelagos. Biol. J. Linn. Soc. 28, 65–82.

Peters, D. & Huenneke, L. (2023a). Seasonal aboveground plant biomass estimates at 15 net primary production (NPP) study sites at Jornada Basin LTER from 1989-ongoing ver 4. Environmental Data Initiative. https://doi.org/10.6073/pasta/5507ade25993c2123e1886a545b509c8.

Peters, D. & Huenneke, L. (2023b). Seasonal non-destructive vegetation measurements at 15 net primary production (NPP) study sites at Jornada Basin LTER, 1989-ongoing ver 109. Environmental Data Initiative. https://doi.org/10.6073/pasta/4fb9b7b5ec3d0692b280bd74e8b046fd.

Presley, S.J. (2020). On the detection of metacommunity structure. Community Ecol. 21, 103-106.

Presley, S.J., Higgins, C.L. & Willig, M.R. (2010). A comprehensive framework for the evaluation of metacommunity structure. Oikos 119, 908–917.

Presley, S.J., Willig, M.R., Bloch, C.P., Castro-Arellano, I., Higgins, C.L. & Klingbeil, B.T. (2011). A complex metacommunity structure for gastropods along an elevational gradient. Biotropica 43, 480–488.

Segre, H., Ron, R., De Malach, N., Henkin, Z., Mandel, M. & Kadmon, R. (2014). Competitive exclusion, beta diversity, and deterministic vs. stochastic drivers of community assembly. Ecol. Lett. 17, 1400–1408.

Šímová, I., Li, Y.M. & Storch, D. (2013). Relationship between species richness and productivity in plants : the role of sampling effect , heterogeneity and species pool. J. Ecol. 101, 161–170.

Stoczynski, L., Brown, B.L., Midway, S.R. & Peoples, B.K. (2021). Landscape features and study design affect elements of metacommunity structure for stream fishes across the eastern U.S.A. Freshw. Biol. 66, 1736–1750.

Thakur, M.P., van der Putten, W.H., Wilschut, R.A., Veen, G.F. (Ciska., Kardol, P., van Ruijven, J., Allan, E., Roscher, C., van Kleunen, M. & Bezemer, T.M. (2021). Plant–Soil Feedbacks and Temporal Dynamics of Plant Diversity–Productivity Relationships. Trends Ecol. Evol. 36, 651–661.

Tilman, D. (1982). Resource competition and community structure. Princet. Univ. Press.

Tilman, D., Isbell, F. & Cowles, J.M. (2014). Biodiversity and ecosystem functioning. Annu. Rev. Ecol. Evol. Syst. 45, 471–493.

Tredennick, A.T., Adler, P.B., Grace, J.B., Harpole, W.S., Borer, E.T., Seabloom, E.W., Anderson, T.M., Bakker, J.D., Biederman, L.A., Brown, C.S., et al. (2016). Comment on

"Worldwide evidence of a unimodal relationship between productivity and plant species richness." Science. 351, 457–457.

Waide, R.B., Willig, M.R., Steiner, C.F., Mittelbach, G., Gough, L., Dodson, S.I., Juday, G.P. & Parmenter, R. (1999). The relationship between productivity and species richness. Annu. Rev. Ecol. Syst. 30, 257–300.

Whittaker, R.J. (2010). Meta-analyses and mega-mistakes: calling time on meta-analysis of the species richness–productivity relationship. Ecology 91, 2522–2533.

Tables and Figures

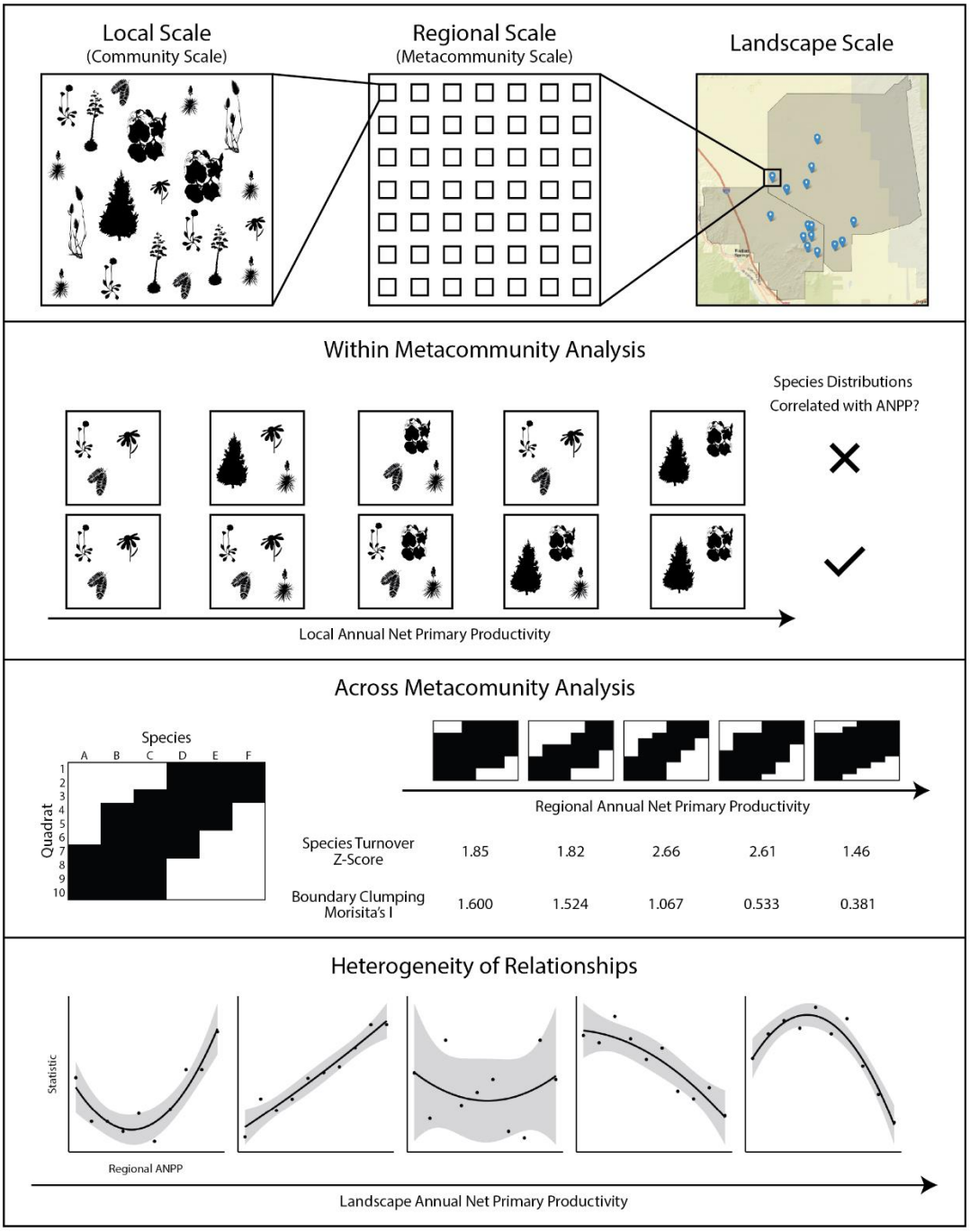

Figure 2.1. Schematic of the questions being asked across the spatial scales of the Jornada LTER dataset. Within metacommunity analyses focus on species assembly in 1-m^2 quadrats and are primarily testing if species distributions of communities follow a gradient of local ANPP. Across metacommunity analyses focus on metacommunity structure in 1-ha regions and compare the relationship between regional ANPP and metrics produced by the EMS method in the 15 metacommunities. Analyses of the heterogeneity of relationships attempts to explain differences in across metacommunity results between years using other environmental variables.

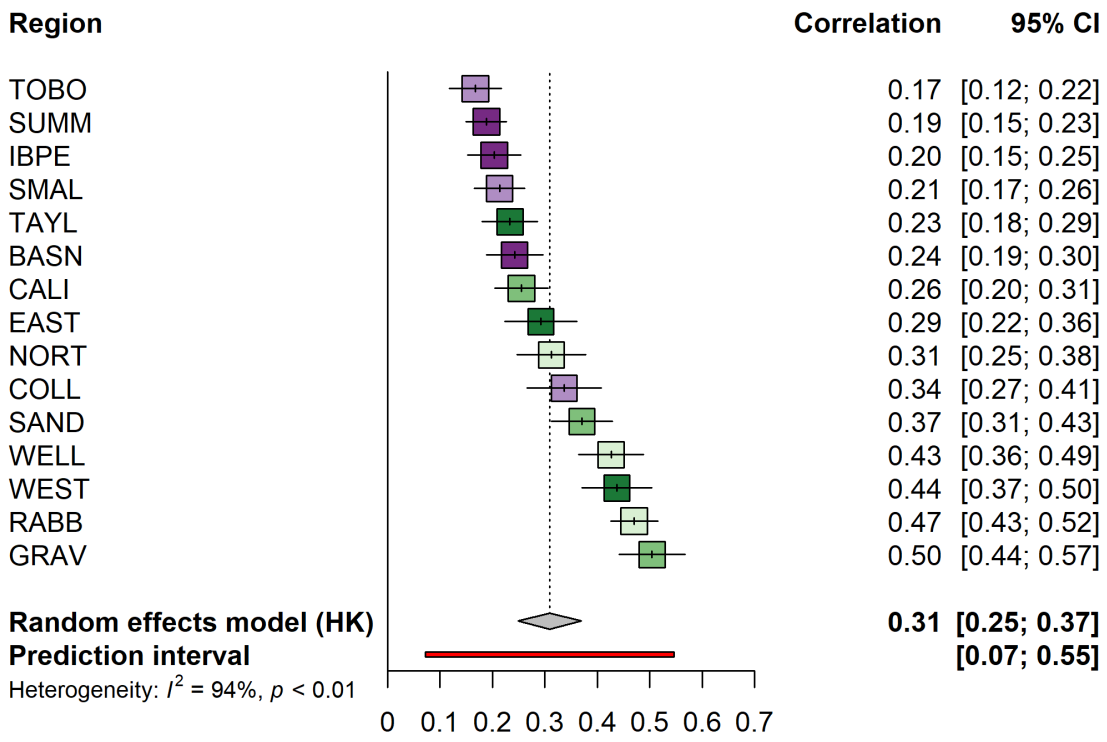

Figure 2.2. Forest plot comparing the average absolute value of the correlation between local annual net primary productivity and quadrat ordering within each region after ordinating a site-species presence-absence matrix. Each region estimate is a collection of 33 correlation estimates, one from each year. The color of each box indicates the habitat type of that region: grasslands are purple (light purple = playa, dark purple = black grama) and shrublands are green (light green = mesquite, green = creosote bush, dark green = tarbush). The relative size of each box is the assigned weight for the analysis and the lines indicate the 95% confidence interval (CI). The width of the diamond at the bottom of the figure gives the 95% CI with the widest point at the average.

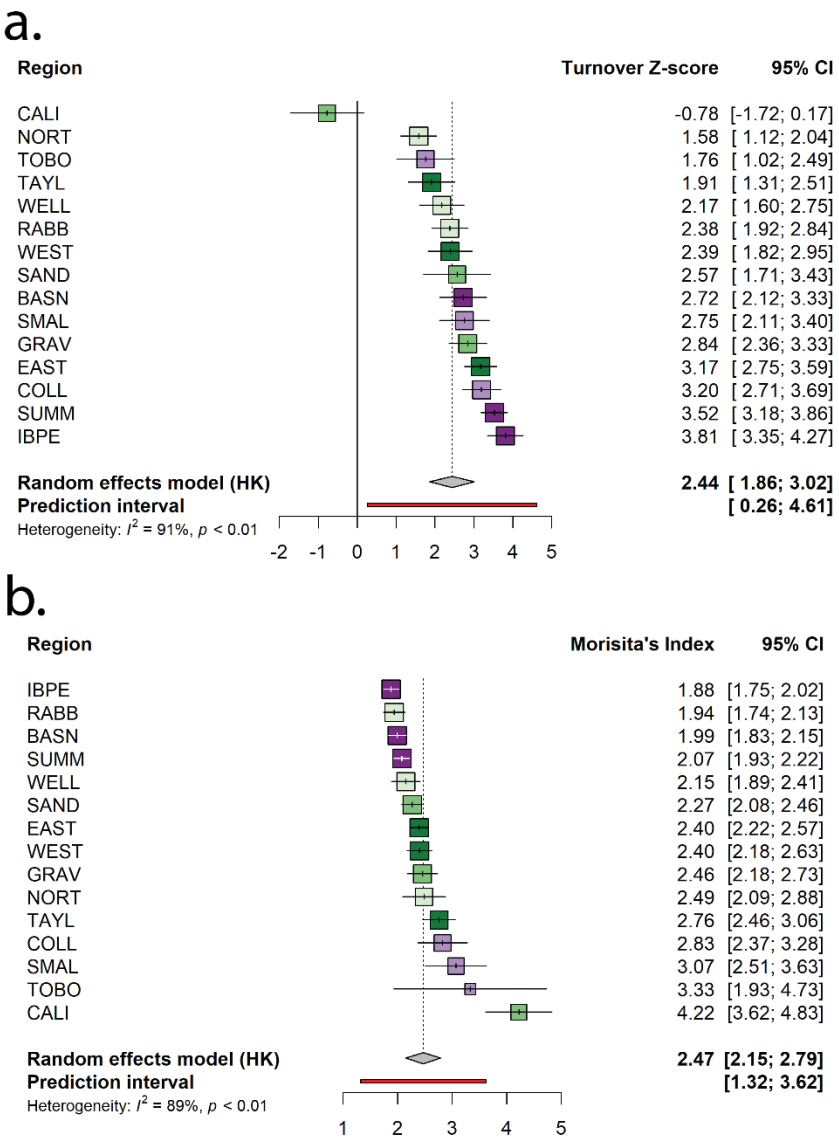

Figure 2.3. Forest plots comparing the average a) Turnover *Z*-score and b) Boundary *I* for each region. Each region estimate is a collection of 33 values, one from each year. The color of each box indicates the habitat type of that region: grasslands are purple (light purple = playa, dark purple = black grama) and shrublands are green (light green = mesquite, green = creosote bush, dark green = tarbush). The relative size of each box is the assigned weight for the analysis and the lines indicate the 95% confidence interval (CI). The width of the diamond at the bottom of the figure gives the 95% CI with the widest point at the average.

Table 2.1. Results from the meta-analyses of the two collections of models testing the effects of regional annual net primary productivity on Turnover *Z*-score and Boundary *I*. Bolded values are significant at $\alpha = 0.05$.

	Main Effect				Test of Heterogeneity	
Parameter Estimate	Pooled Effect	Prediction Interval	*t*	*p*	τ^2 [95% CI]	I^2 [95% CI]
Turnover Z-score ~ Regional ANPP						
Intercept	**2.82**	**1.934-3.708**	**22.40**	**< 0.001**	0.169 [0.000-0.408]	21.3% [0.0%-49.0%]
Linear	**1.536**	**0.184-2.887**	**6.77**	**< 0.001**	0.376 [0.000-1.263]	8.3% [0.0%-39.0%]
Quadratic	**-0.383**	**-0.797-0.03**	**-4.35**	**< 0.001**	0.034 [0.000-0.120]	28.0% [0.0%-53.3%]
Boundary I ~ Regional ANPP						
Intercept	**2.167**	**1.990-2.344**	**46.45**	**< 0.001**	0.005 [0.000-0.030]	0.0% [0.0%-39.2%]
Linear	**-0.451**	**-1.682-0.78**	**-2.72**	**0.011**	**0.338 [0.078-1.278]**	**47.0% [20.3%-64.7%]**
Quadratic	0.067	0.017-0.116	1.96	0.058	**0.000 [0.032-1.194]**	**49.1% [23.8%-66.0%]**

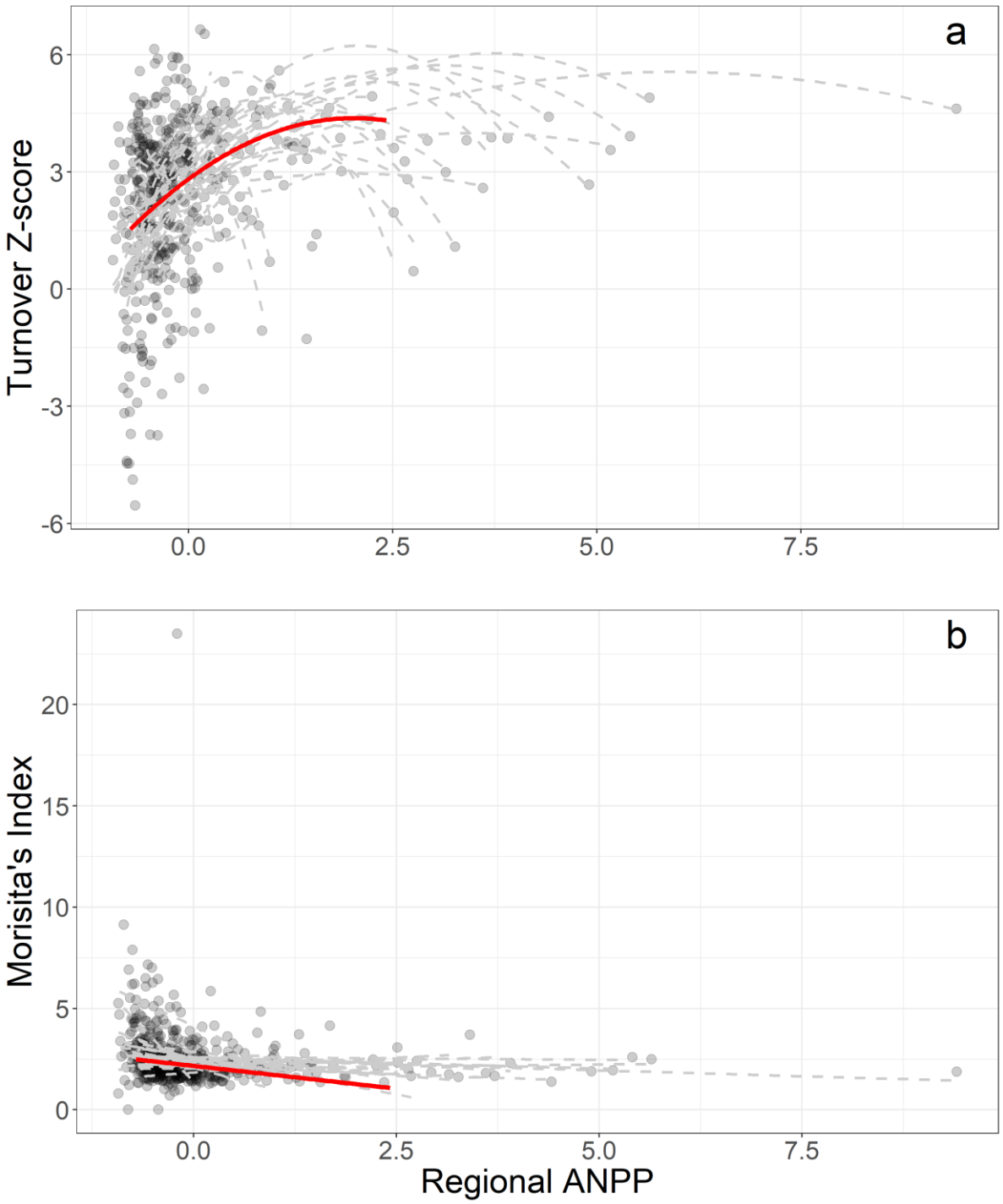

Figure 2.4. Linear regression results from the collections of models comparing regional net primary productivity to a) Turnover *Z*-score and b) Boundary *I*. Each data point is the value for each region in each year. The gray dashed lines are the model results for each year, and the solid red lines are the average parameter estimates based on the meta-analysis results ranging from the average minimum to the average maximum regional ANPP across years. See the supplement for each year plotted individually.

Table 2.2. Correlations between heterogeneous parameter estimates and environmental characteristics. Correlations with landscape ANPP encompass the full spatiotemporal extent of the study, but those with soil temperature and volumetric water content only cover a subset of years (2014-2022). Bolded values are significant at $\alpha = 0.05$.

	Variable	Depth	*r*	*p*
Boundary I ~ Regional ANPP				
Linear	Landscape ANPP		-0.029	0.872
	Soil Temperature	10 cm	0.217	0.581
		20 cm	0.217	0.581
		30 cm	0.217	0.581
	Volumetric Water Content	10 cm	-0.250	0.521
		20 cm	-0.350	0.359
		30 cm	-0.150	0.708
	Precipitation		**-0.683**	**0.050**
	Air Temperature		0.067	0.880
	Relative Humidity		-0.417	0.270
Quadratic	Landscape ANPP		**-0.574**	**< 0.001**
	Soil Temperature	10 cm	**0.817**	**0.011**
		20 cm	**0.817**	**0.011**
		30 cm	**0.817**	**0.011**
	Volumetric Water Content	10 cm	-0.567	0.121
		20 cm	-0.333	0.385
		30 cm	-0.333	0.385
	Precipitation		0.100	0.810
	Air Temperature		**0.783**	**0.017**
	Relative Humidity		-0.650	0.067

Supplemental Information

Climate Data References

Anderson, J. (2024). Jornada Basin LTER: Wireless meteorological station at NPP C-CALI site: 1-hour summary data: 2013 - ongoing ver 41. Environmental Data Initiative. https://doi.org/10.6073/pasta/b4f86ecd6ade922355bd4ccdfbdea25f (Accessed 2024-01-01).

Anderson, J. (2024). Jornada Basin LTER: Wireless meteorological station at NPP C-GRAV site: 1-hour summary data: 2013 - ongoing ver 42. Environmental Data Initiative. https://doi.org/10.6073/pasta/042e453dce86194ecd04bae6654b69c3 (Accessed 2024-01-01).

Anderson, J. (2024). Jornada Basin LTER: Wireless meteorological station at NPP C-SAND site: 1-hour summary data: 2013 - ongoing ver 42. Environmental Data Initiative. https://doi.org/10.6073/pasta/6c49a5dbe3912281d45eb42f37453aa6 (Accessed 2024-01-01).

Anderson, J. (2024). Jornada Basin LTER: Wireless meteorological station at NPP G-BASN site: 1-hour summary data: 2013 - ongoing ver 42. Environmental Data Initiative. https://doi.org/10.6073/pasta/74346d5ce80394808b556ee418362b68 (Accessed 2024-01-01).

Anderson, J. (2024). Jornada Basin LTER: Wireless meteorological station at NPP G-IBPE site: 1-hour summary data: 2013 - ongoing ver 39. Environmental Data Initiative. https://doi.org/10.6073/pasta/74abca5e50a79a6c547b03e128b22e20 (Accessed 2024-01-01).

Anderson, J. (2024). Jornada Basin LTER: Wireless meteorological station at NPP G-SUMM site: 1-hour summary data: 2013 - ongoing ver 41. Environmental Data Initiative. https://doi.org/10.6073/pasta/e71f410c79f567112186a61750efed57 (Accessed 2024-01-01).

Anderson, J. (2024). Jornada Basin LTER: Wireless meteorological station at NPP M-NORT site: 1-hour summary data: 2013 - ongoing ver 41. Environmental Data Initiative. https://doi.org/10.6073/pasta/7a70dea5d5a401d384c9d487eb8715b4 (Accessed 2024-01-01).

Anderson, J. (2024). Jornada Basin LTER: Wireless meteorological station at NPP M-RABB site: 1-hour summary data: 2013 - ongoing ver 40. Environmental Data Initiative. https://doi.org/10.6073/pasta/44a01f9bee594057f4903a60d6985587 (Accessed 2024-01-01).

Anderson, J. (2024). Jornada Basin LTER: Wireless meteorological station at NPP M-WELL site: 1-hour summary data: 2013 - ongoing ver 38. Environmental Data Initiative. https://doi.org/10.6073/pasta/c778b8cef9957ab90ce47f322ffd77a6 (Accessed 2024-01-01).

Anderson, J. (2024). Jornada Basin LTER: Wireless meteorological station at NPP P-COLL site: 1-hour summary data: 2013 - ongoing ver 40. Environmental Data Initiative. https://doi.org/10.6073/pasta/a62cd782dd1ebe775221994074070f5a (Accessed 2024-01-01).

Anderson, J. (2024). Jornada Basin LTER: Wireless meteorological station at NPP P-SMAL site: 1-hour summary data: 2017 - ongoing ver 41. Environmental Data Initiative. https://doi.org/10.6073/pasta/bc5036b362791720afbd79e53adfa909 (Accessed 2024-01-01).

Anderson, J. (2024). Jornada Basin LTER: Wireless meteorological station at NPP P-TOBO site: 1-hour summary data: 2013 - ongoing ver 39. Environmental Data Initiative. https://doi.org/10.6073/pasta/dc98245219920b290fd684839fdcbfe7 (Accessed 2024-01-01).

Anderson, J. (2024). Jornada Basin LTER: Wireless meteorological station at NPP T-EAST site: 1-hour summary data: 2013 - ongoing ver 41. Environmental Data Initiative. https://doi.org/10.6073/pasta/a75db79b9394cc97b21f003e3f95cdbc (Accessed 2024-01-01).

Anderson, J. (2024). Jornada Basin LTER: Wireless meteorological station at NPP T-TAYL site: 1-hour summary data: 2013 - ongoing ver 40. Environmental Data Initiative. https://doi.org/10.6073/pasta/1122644b9c7d6f2e44ee0ebfe0c07384 (Accessed 2024-01-01).

Anderson, J. (2024). Jornada Basin LTER: Wireless meteorological station at NPP T-WEST site: 1-hour summary data: 2013 - ongoing ver 39. Environmental Data Initiative. https://doi.org/10.6073/pasta/8363210787c4db2c090803c7235dfc35 (Accessed 2024-01-01).

Soil Data References

Duniway, M. (2023). Jornada Basin LTER: Wireless meteorological station at NPP C-CALI site: 30-minute soil volumetric water content data: 2013 - ongoing ver 42. Environmental Data Initiative. https://doi.org/10.6073/pasta/370cd78c43042b323297b8bc00f5e7a6 (Accessed 2023-12-29).

Duniway, M. (2023). Jornada Basin LTER: Wireless meteorological station at NPP C-GRAV site: 30-minute soil volumetric water content data: 2013 - ongoing ver 42. Environmental Data Initiative. https://doi.org/10.6073/pasta/39513700ce0d36430e573f21c22bdfa6 (Accessed 2023-12-29).

Duniway, M. (2023). Jornada Basin LTER: Wireless meteorological station at NPP C-SAND site: 30-minute soil volumetric water content data: 2013 - ongoing ver 41. Environmental Data Initiative. https://doi.org/10.6073/pasta/e23f8e8099d9b8561ed489ee1cf8288e (Accessed 2023-12-29).

Duniway, M. (2023). Jornada Basin LTER: Wireless meteorological station at NPP G-BASN site: 30-minute soil volumetric water content data: 2013 - ongoing ver 42. Environmental Data Initiative. https://doi.org/10.6073/pasta/d02be7f0263456dbbe9e9dfc71f59695 (Accessed 2023-12-29).

Duniway, M. (2023). Jornada Basin LTER: Wireless meteorological station at NPP G-IBPE site: 30-minute soil volumetric water content data: 2013 - ongoing ver 40. Environmental Data Initiative. https://doi.org/10.6073/pasta/6b0f39869c54ba3bf1a60cf7ef0f031e (Accessed 2023-12-29).

Duniway, M. (2023). Jornada Basin LTER: Wireless meteorological station at NPP G-SUMM site: 30-minute soil volumetric water content data: 2013 - ongoing ver 40. Environmental Data Initiative. https://doi.org/10.6073/pasta/fc826af59a9ec507f1f3f845f180091a (Accessed 2023-12-29).

Duniway, M. (2023). Jornada Basin LTER: Wireless meteorological station at NPP M-NORT site: 30-minute soil volumetric water content data: 2013 - ongoing ver 39. Environmental Data Initiative. https://doi.org/10.6073/pasta/f7385ff2f9bc266fb1c6516cc48bbd47 (Accessed 2023-12-29).

Duniway, M. (2023). Jornada Basin LTER: Wireless meteorological station at NPP M-RABB site: 30-minute soil volumetric water content data: 2013 - ongoing ver 39. Environmental

Data Initiative. https://doi.org/10.6073/pasta/75a33808cd9c1ad1164836e8cd0a3425 (Accessed 2023-12-29).

Duniway, M. (2023). Jornada Basin LTER: Wireless meteorological station at NPP M-WELL site: 30-minute soil volumetric water content data: 2013 - ongoing ver 40. Environmental Data Initiative. https://doi.org/10.6073/pasta/1076f8c7d20d8c431eb2ee721ff9fd7e (Accessed 2023-12-29).

Duniway, M. (2023). Jornada Basin LTER: Wireless meteorological station at NPP P-COLL site: 30-minute soil volumetric water content data: 2013 - ongoing ver 41. Environmental Data Initiative. https://doi.org/10.6073/pasta/fbebde72065574df7f7a78edd4abfc30 (Accessed 2023-12-29).

Duniway, M. (2023). Jornada Basin LTER: Wireless meteorological station at NPP P-SMAL site: 30-minute soil volumetric water content data: 2017 - ongoing ver 40. Environmental Data Initiative. https://doi.org/10.6073/pasta/0edd71eeaf7efc7dae7c3f32838a3dc7 (Accessed 2023-12-29).

Duniway, M. (2023). Jornada Basin LTER: Wireless meteorological station at NPP P-TOBO site: 30-minute soil volumetric water content data: 2013 - ongoing ver 38. Environmental Data Initiative. https://doi.org/10.6073/pasta/c45c5d6b799aebf71787ae048a643e2e (Accessed 2023-12-29).

Duniway, M. (2023). Jornada Basin LTER: Wireless meteorological station at NPP T-EAST site: 30-minute soil volumetric water content data: 2013 - ongoing ver 40. Environmental Data Initiative. https://doi.org/10.6073/pasta/47abe1d13281fac8f8d420ee59a035d1 (Accessed 2023-12-29).

Duniway, M. (2023). Jornada Basin LTER: Wireless meteorological station at NPP T-TAYL site: 30-minute soil volumetric water content data: 2013 - ongoing ver 40. Environmental Data Initiative. https://doi.org/10.6073/pasta/6ed74b4cc4a819dce1d0f0799ec60cc3 (Accessed 2023-12-29).

Duniway, M. (2023). Jornada Basin LTER: Wireless meteorological station at NPP T-WEST site: 30-minute soil volumetric water content data: 2013 - ongoing ver 40. Environmental Data Initiative. https://doi.org/10.6073/pasta/8c1fbcac5e8f3c1ad579f8debd1da333 (Accessed 2023-12-29).

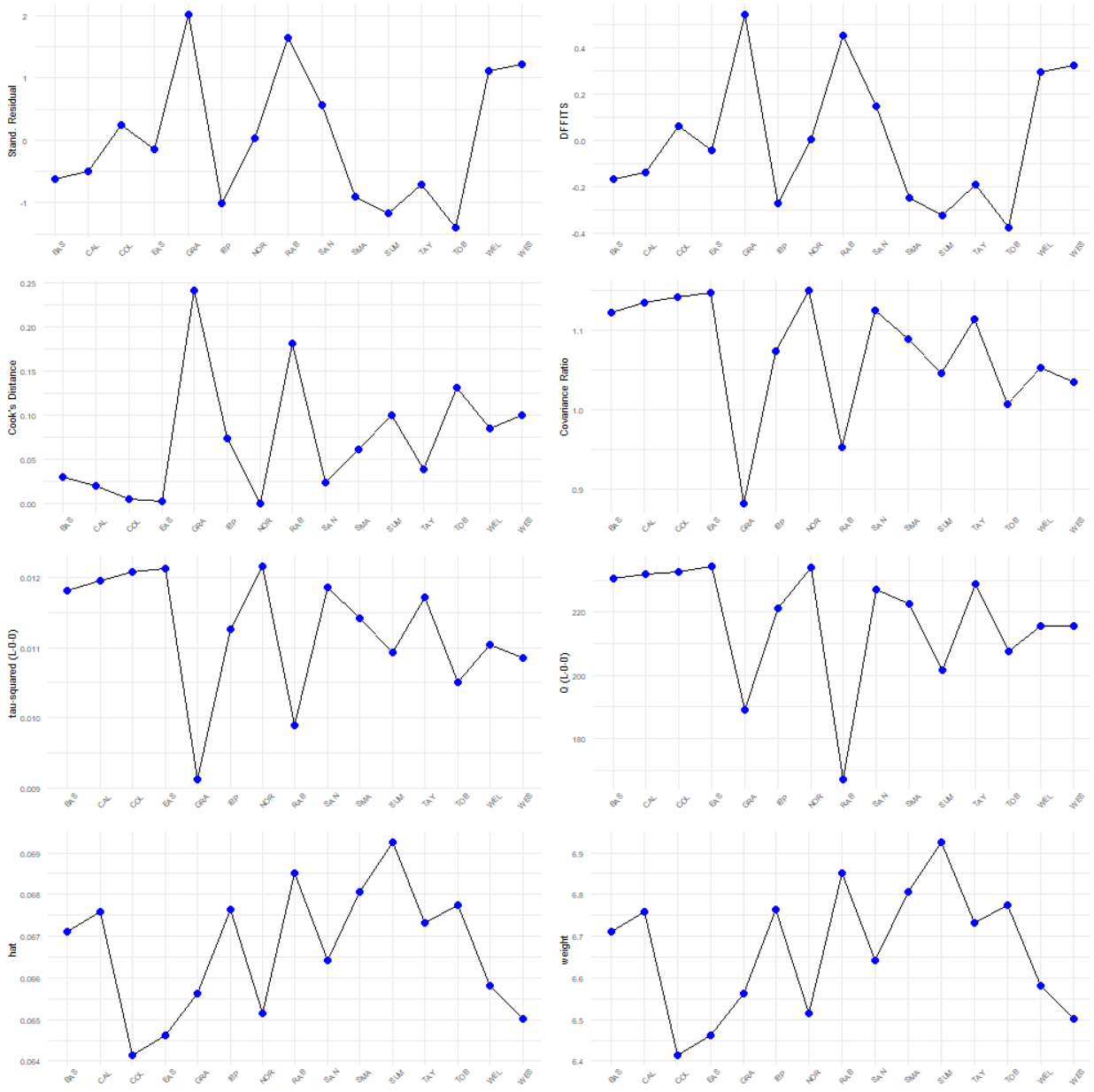

Figure S2.1. Influence plots indicating which regions could cause significant heterogeneity in the meta-analysis of correlation coefficients between local ANPP and quadrat ordering.

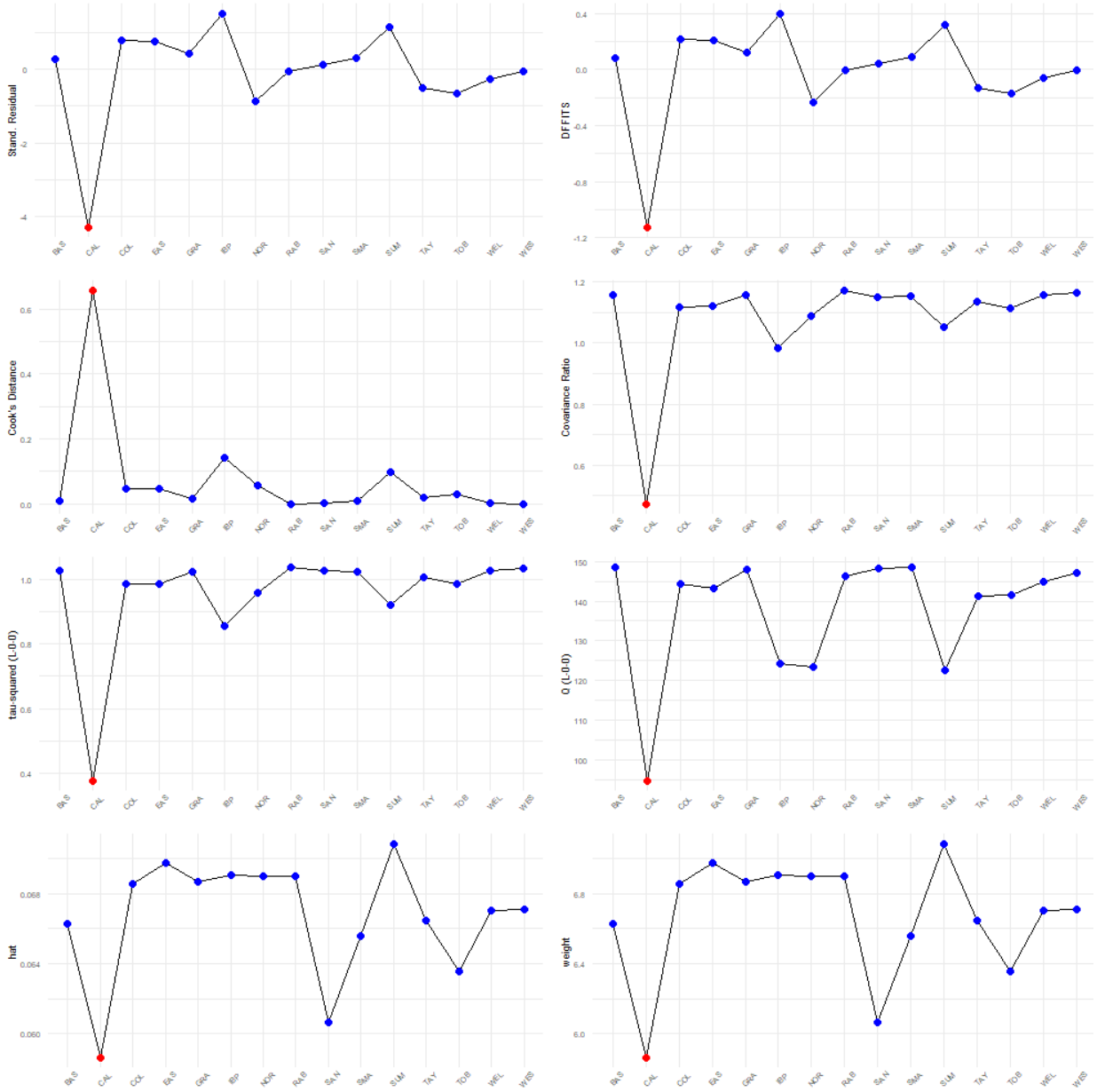

Figure S2.2. Influence plots indicating which regions could cause significant heterogeneity in the meta-analysis of Turnover *Z*-scores for all regions across all years.

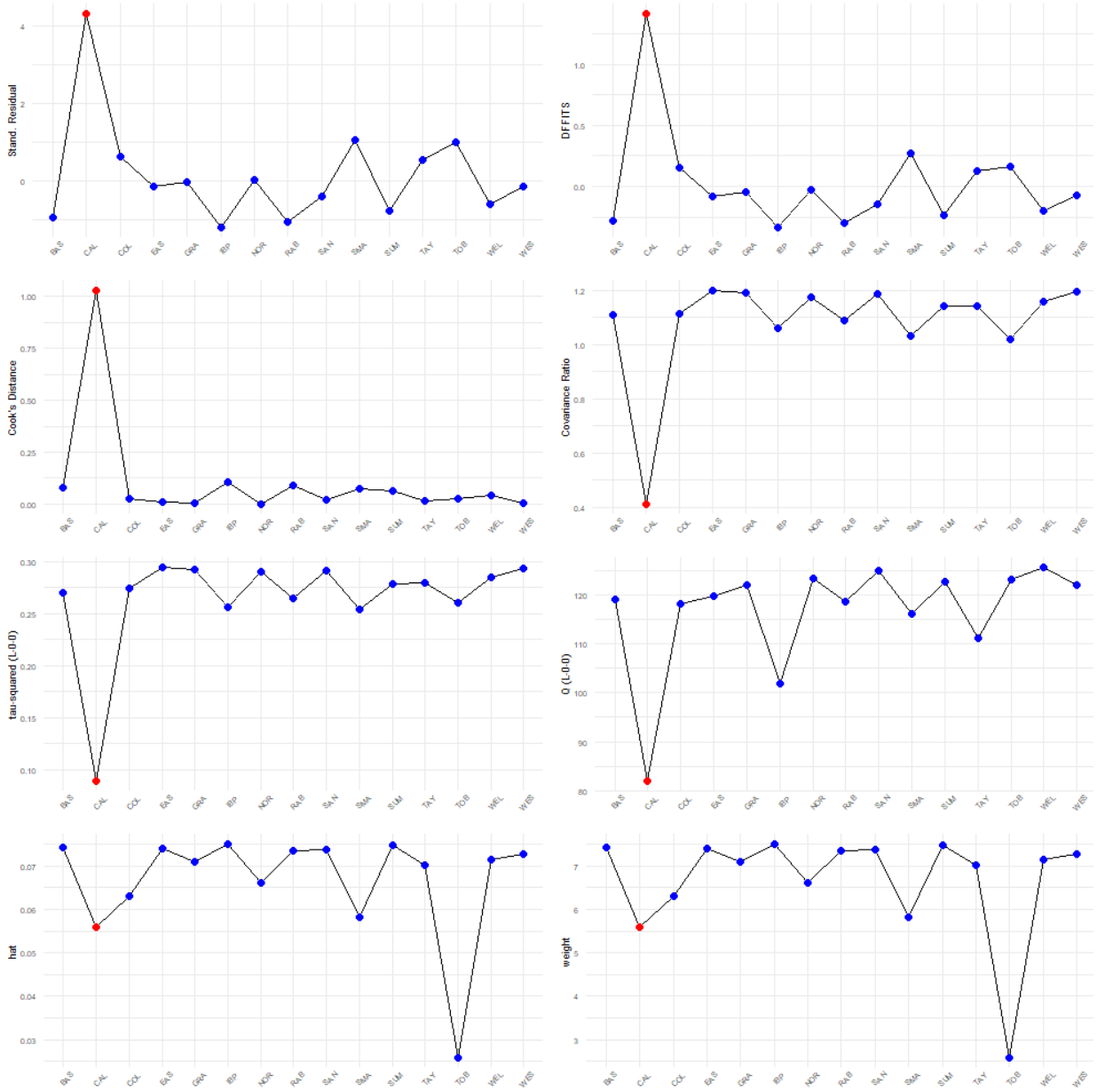

Figure S2.3. Influence plots indicating which regions could cause significant heterogeneity in the meta-analysis of Boundary Clumping *I* for all regions across all years.

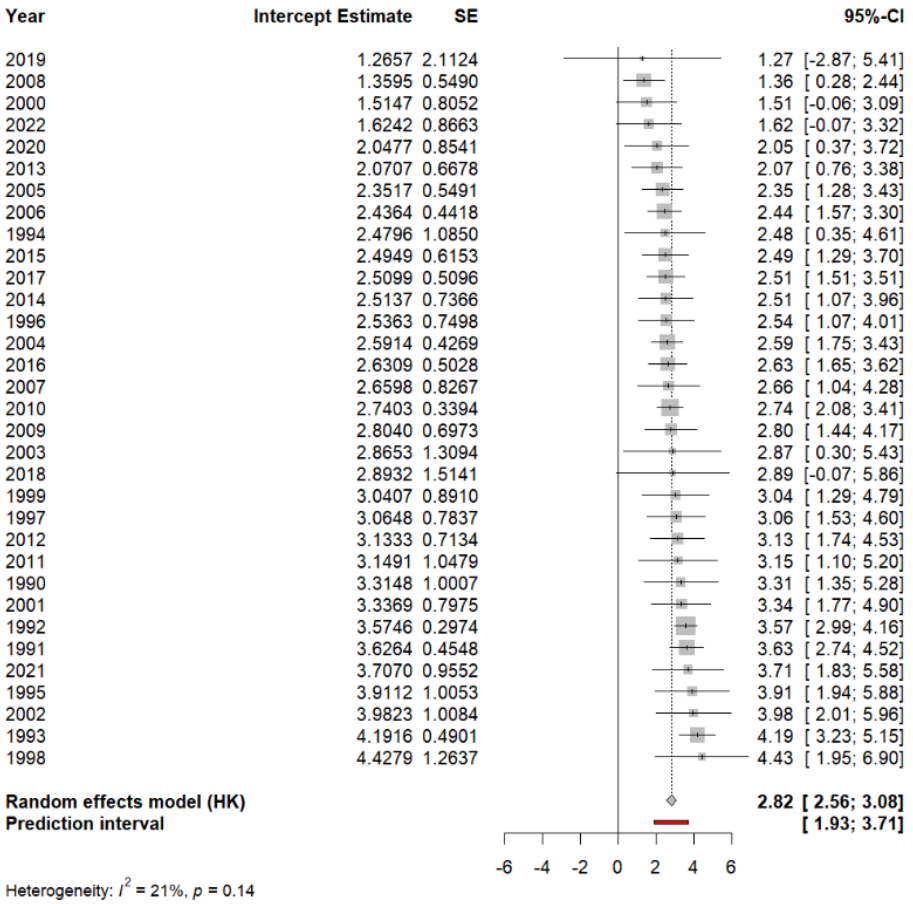

Figure S2.4. Forest plot comparing the intercept estimate of the relationship between regional ANPP and Turnover *Z*-score across regions within a year. The relative size of each box is the assigned weight for the analysis and the lines indicate the 95% confidence interval (CI). The width of the diamond at the bottom of the figure gives the 95% CI with the widest point at the average.

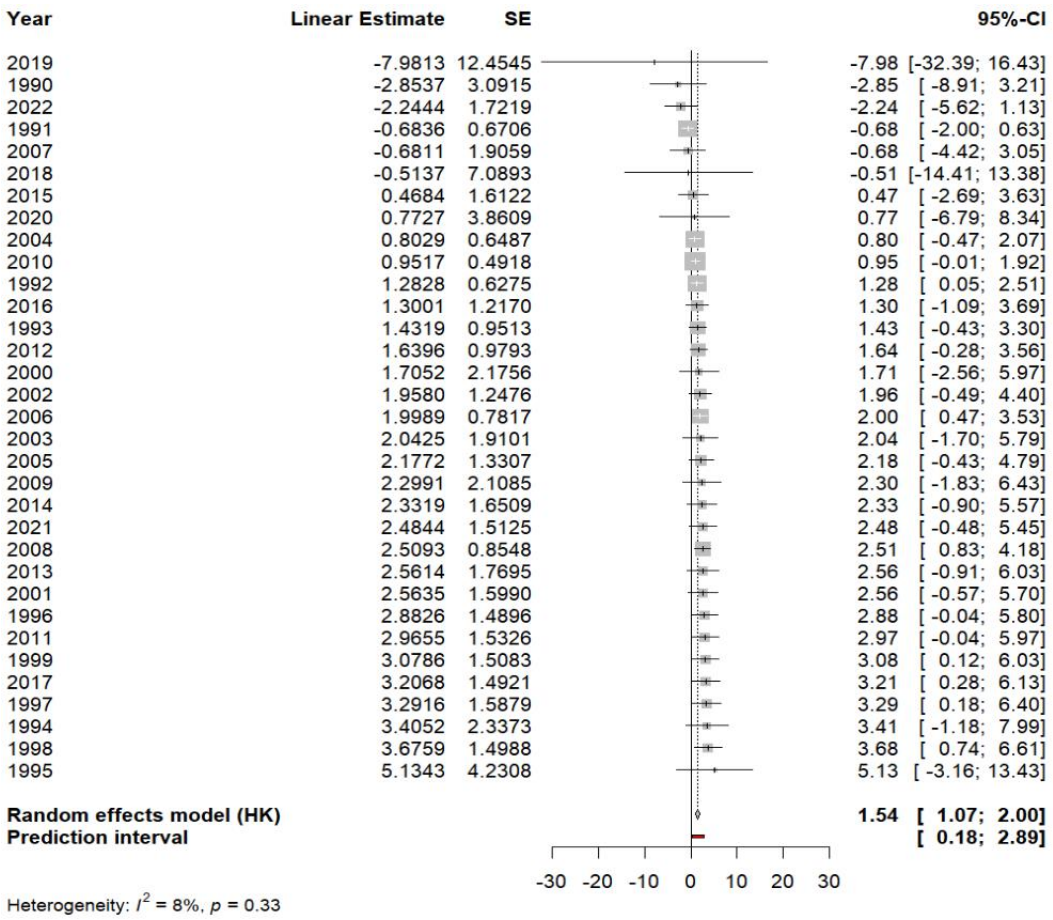

Figure S2.5. Forest plot comparing the linear component estimate of the relationship between regional ANPP and Turnover *Z*-score across regions within a year. The relative size of each box is the assigned weight for the analysis and the lines indicate the 95% confidence interval (CI). The width of the diamond at the bottom of the figure gives the 95% CI with the widest point at the average.

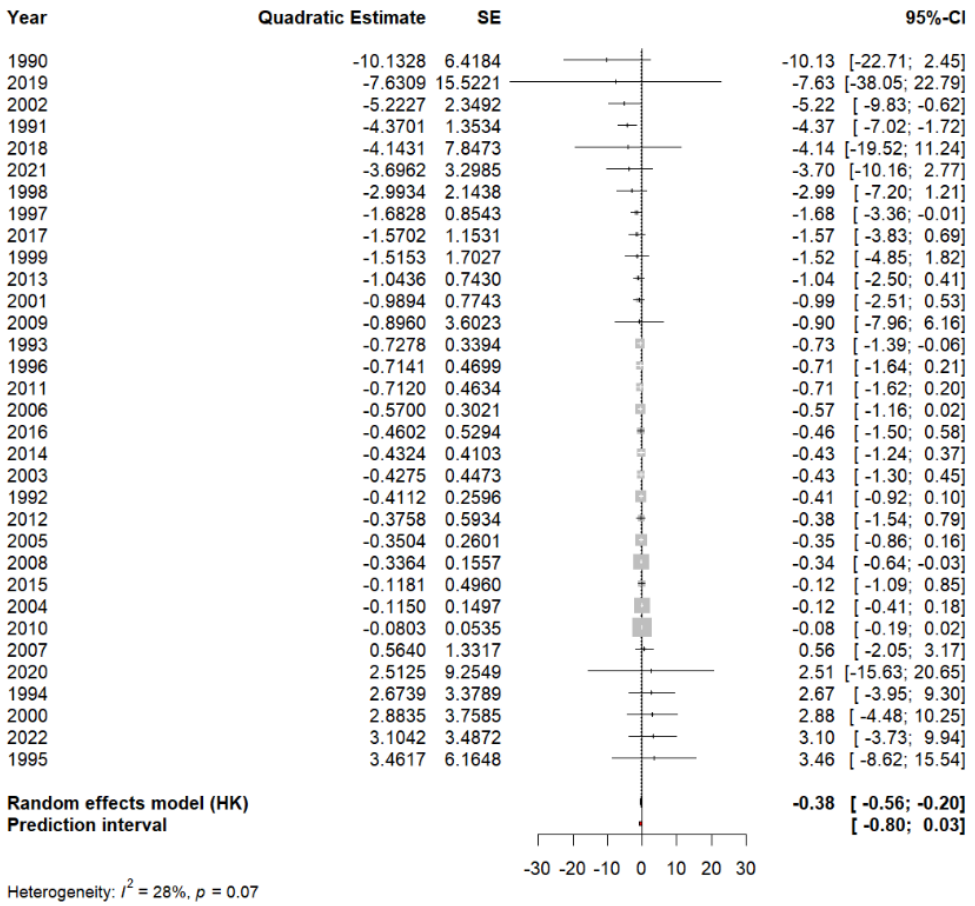

Figure S2.6. Forest plot comparing the quadratic component estimate of the relationship between regional ANPP and Turnover *Z*-score across regions within a year. The relative size of each box is the assigned weight for the analysis and the lines indicate the 95% confidence interval (CI). The width of the diamond at the bottom of the figure gives the 95% CI with the widest point at the average.

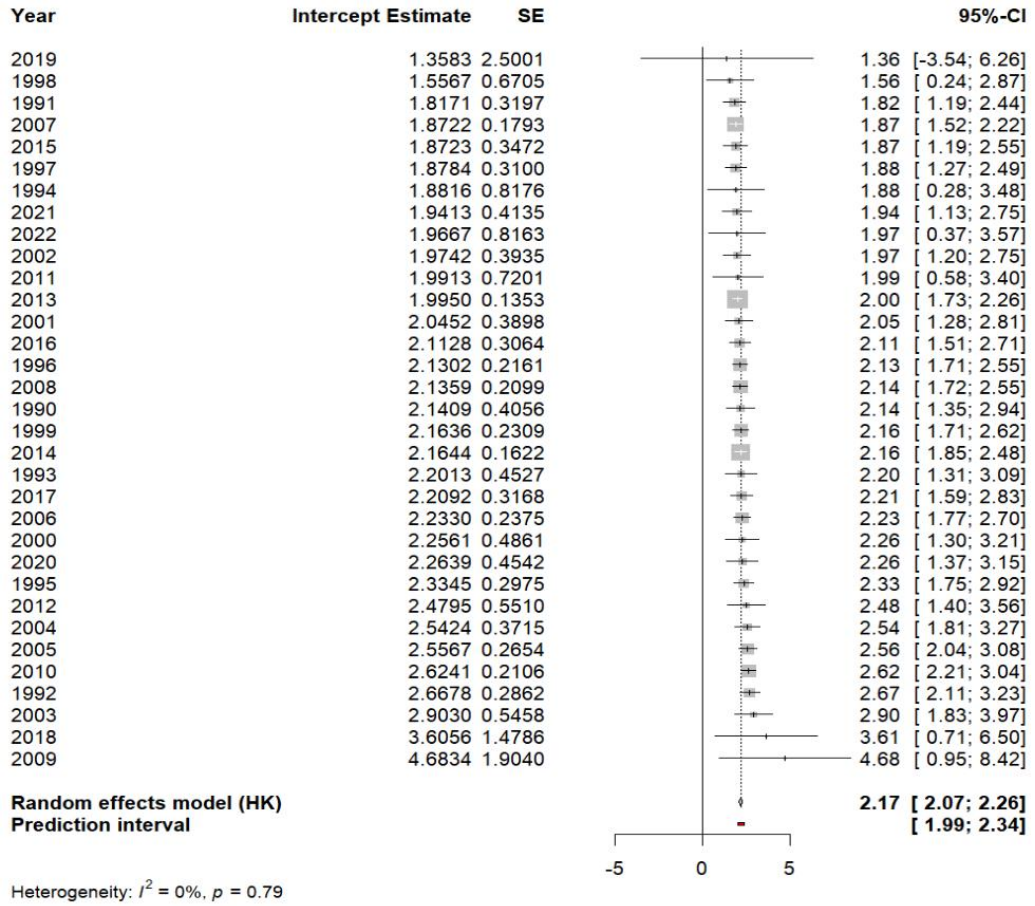

Figure S2.7. Forest plot comparing the intercept estimate of the relationship between regional ANPP and Boundary Clumping *I* across regions within a year. The relative size of each box is the assigned weight for the analysis and the lines indicate the 95% confidence interval (CI). The width of the diamond at the bottom of the figure gives the 95% CI with the widest point at the average.

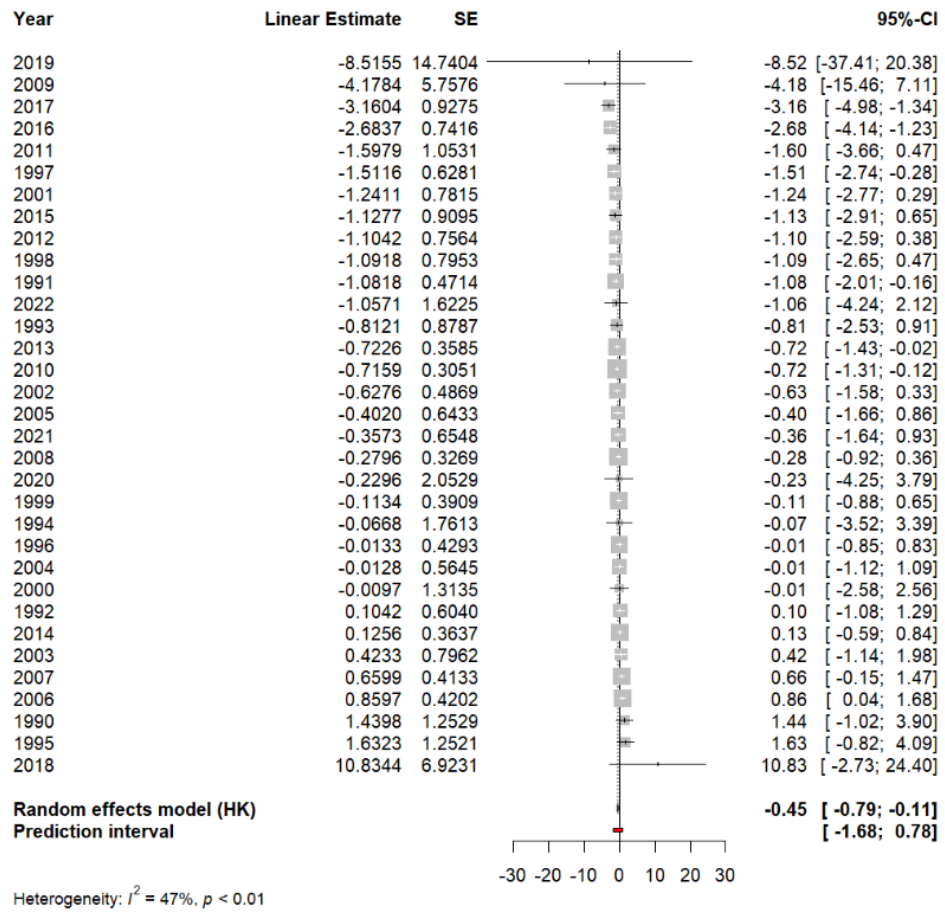

Figure S2.8. Forest plot comparing the linear component estimate of the relationship between regional ANPP and Boundary Clumping *I* across regions within a year. The relative size of each box is the assigned weight for the analysis and the lines indicate the 95% confidence interval (CI). The width of the diamond at the bottom of the figure gives the 95% CI with the widest point at the average.

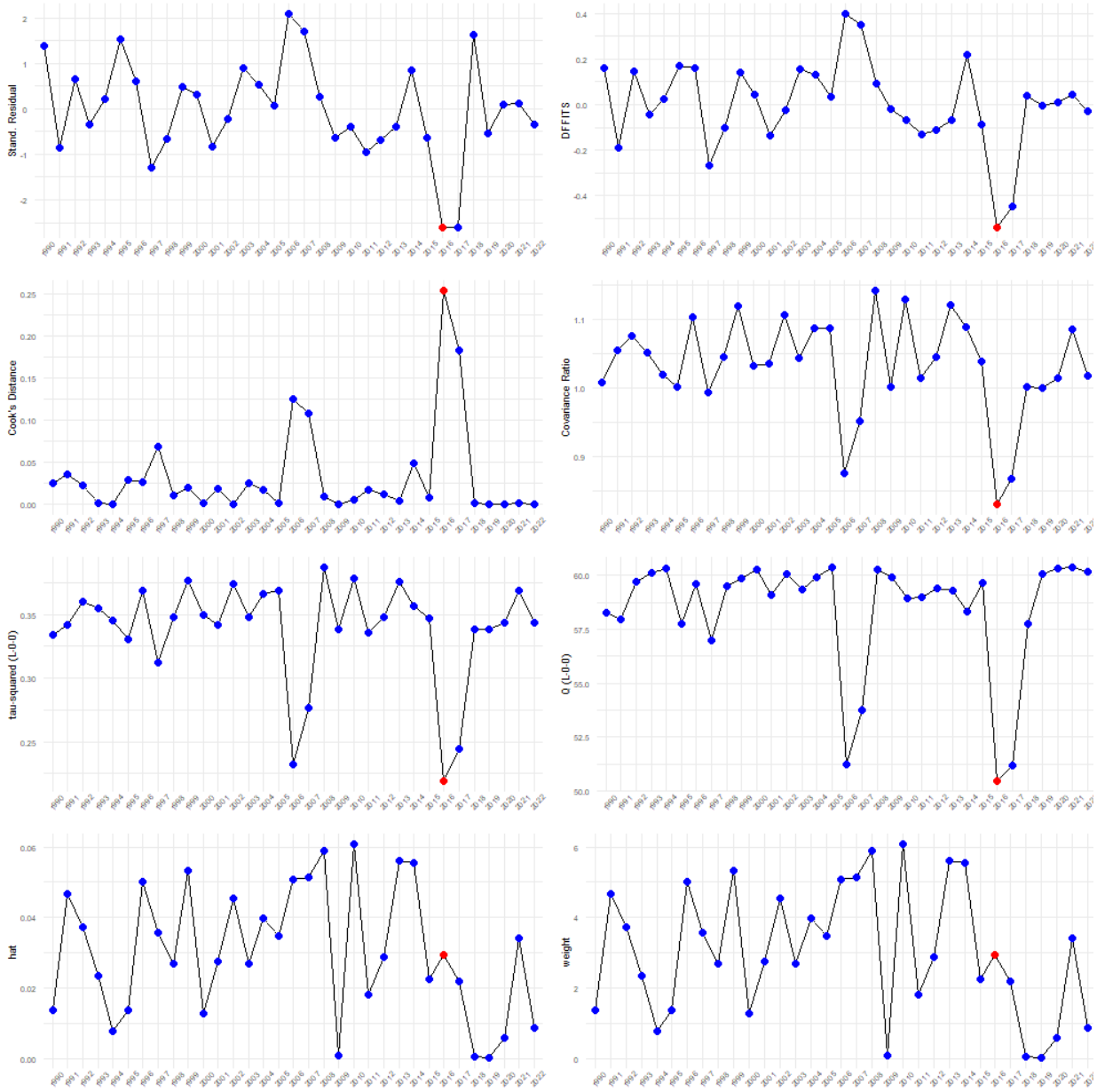

Figure S2.9. Influence plots indicating which years could cause significant heterogeneity in the meta-analysis of the linear component of the relationship between regional ANPP and Boundary Clumping *I*.

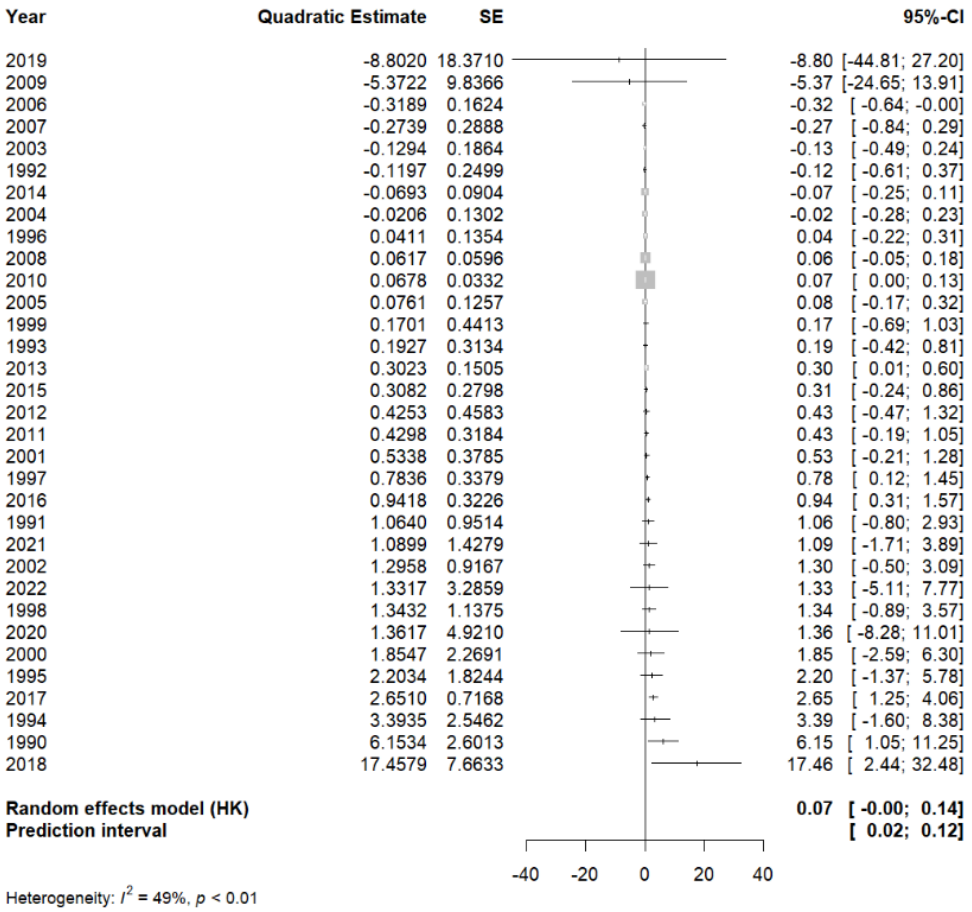

Figure S2.10. Forest plot comparing the quadratic component estimate of the relationship between regional ANPP and Boundary Clumping *I* across regions within a year. The relative size of each box is the assigned weight for the analysis and the lines indicate the 95% confidence interval (CI). The width of the diamond at the bottom of the figure gives the 95% CI with the widest point at the average.

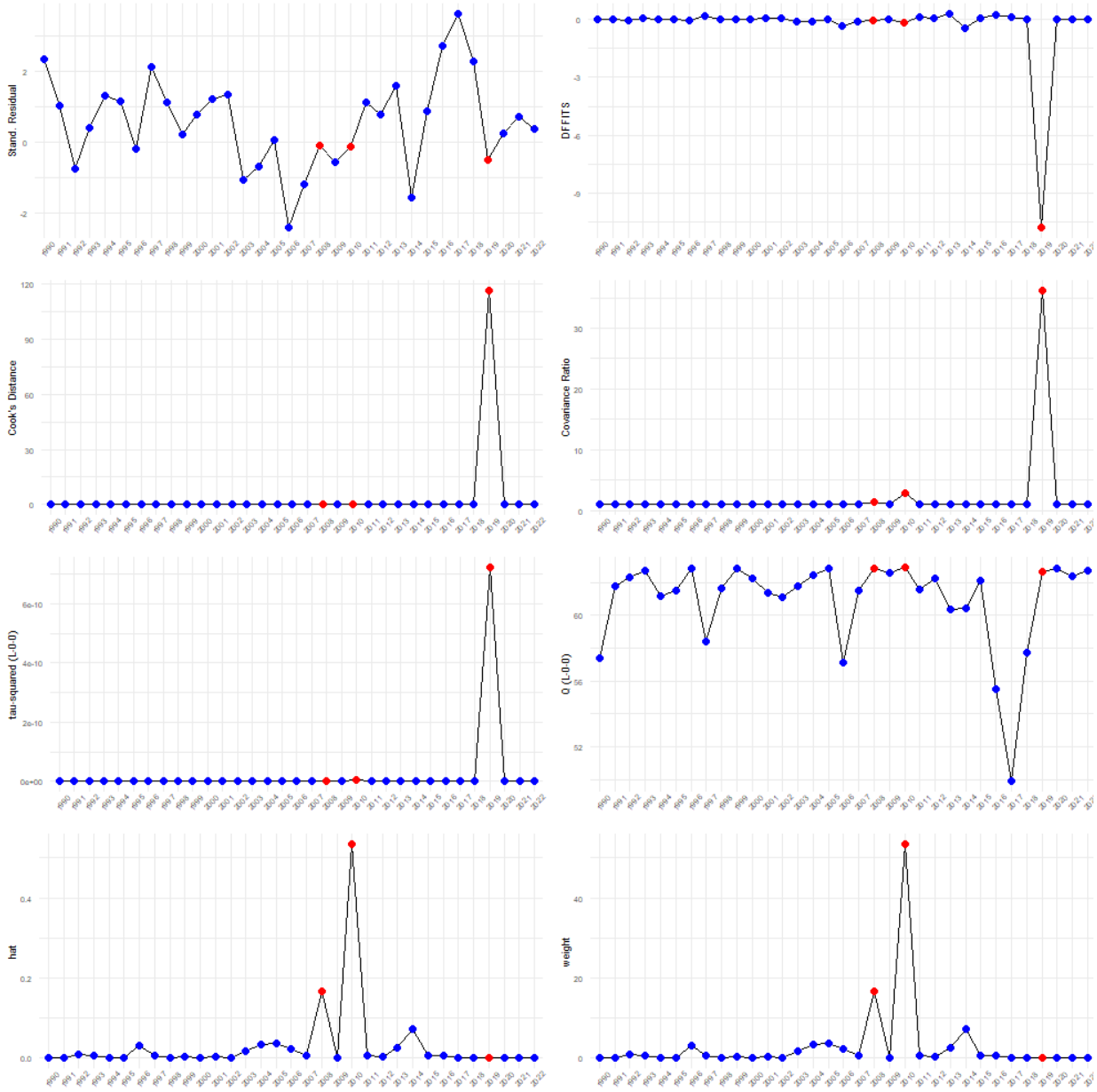

Figure S2.11. Influence plots indicating which years could cause significant heterogeneity in the meta-analysis of the quadratic component of the relationship between regional ANPP and Boundary Clumping *I*.

CHAPTER 3: Predator effects on prey communities differ based on predation strategy and spatial scale

Abstract

The biodiversity of a region (gamma diversity) depends on how many species occur in an average habitat patch within the region (alpha diversity) and how much variation there is in the identities of species present in patches within the region (beta diversity). Models suggest that both generalist and specialist predators should reduce alpha diversity, but generalist predators would enhance beta diversity while specialist predators would reduce beta diversity. Such differences are expected as generalist predators enhance vulnerabilities to extinction in all species by reducing their population numbers while specialist predators enhance vulnerabilities of their preferred prey. We conducted an experiment in artificial ponds to assess the effects of one specialist predator (sunfish) and two generalist predators (newts and *Anax* dragonfly nymphs) on the alpha, beta and gamma species richness, rarefied richness, and evenness of aquatic insects present in ponds. Sunfish presence reduced alpha diversity and gamma diversity compared to ponds that contained no predators, but there was not an effect on beta diversity. Ponds with sunfish also reduced local evenness due to increased relative abundance of Diptera larvae and reduced relative abundance of dragonfly nymphs compared to ponds with no predators added. The presence of either newts or *Anax* did not affect diversity compared to predator free ponds. These results indicate that the effect of predators on prey diversity varies with scale and type of predator present but the particular patterns of change that we observed were not consistent with the patterns predicted by theory, possible due to differences in immigration rates between prey species.

Introduction

An important goal in ecology is to advance our understanding of processes that affect the number of species that co-occur with each other. When multiple species require similar physical conditions or food resources, they will often co-occur in a habitat as a community. In some cases, one species may be the food resource for other species while in other cases multiple species may compete for the same food resource. In this regard, a great deal of work has examined the extent to which predators affect the number of species that occur within a habitat patch (i.e., local diversity or alpha diversity) (Paine, 1966; Sih *et al.*, 1985; Chase *et al.*, 2002). When predators influence prey diversity, they typically have one of two potential effects within a patch. In some cases, predators increase the burden on prey, reducing the likelihood of survival and the overall diversity of prey species in the community (Wilbur, Morin & Harris, 1983; Schoener & Spiller, 1996). Alternatively, predators can preferentially prey upon species that competitively exclude other species, increasing resource availability for a more diverse community (Morin, 1983; Chalcraft & Resetarits, 2003). Because habitat patches do not exist in isolation, it can also be important to look at species diversity in the overarching metacommunity across multiple habitat patches (i.e., regional diversity or gamma diversity). There has been much less work evaluating the impact that predators may have on the number of species that co-occur at scales larger than a habitat patch.

By altering prey habitat suitability, predators can affect the heterogeneity of prey diversity between patches (beta diversity). Theory suggests that specialist and generalist predators will differ in their effects on prey biodiversity and the particular effects can differ at different spatial scales (Ryberg *et al.*, 2012). Generalist predators are expected to depredate all prey species and reduce their abundances to the same degree regardless of identity. Without predators, species

abundances naturally vary between patches, resulting in different species being rare in each patch due to differences in patch history (Hernandez & Chalcraft, 2012) or differences in environmental characteristics like temperature (McNamara, Pintar & Resetarits, 2021) or nutrient enrichment (Young *et al.*, 2014). Therefore, if generalist predators reduce all prey species abundances at similar rates and different species can be rare in each patch, then different prey species should be eliminated from each patch with a generalist predator. In doing so, they will reduce the local diversity in each patch but increase the among patch variation in prey species, increasing beta diversity. At the regional scale, this results in a similar overall species pool across all patches to environments without generalist predators. Generalist predators have been found to reduce local diversity and enhance beta diversity in protist communities (Johnston *et al.*, 2016) and fish communities (Zhang *et al.*, 2023).

In contrast, specialist predators preferentially consume individuals of prey species that possess traits that make them more vulnerable. The vulnerability of prey can vary based on predator foraging strategy (Cooper, Smith & Bence, 1985) or population density (Davenport & Chalcraft, 2014). Because specialists preferentially consume certain species, they should act as a filter for a subset of the metacommunity, removing them from the regional pool regardless of abundance. This will result in lower alpha diversity and greater homogeneity in diversity between patches (i.e., lower beta diversity), leading to a smaller regional species pool. Sunfish have been shown to act as specialist predators for insect communities (Chase *et al.*, 2009), but magnitude of the effect of specialists can be mediated by random dispersal between patches within a metacommunity (Johnston *et al.*, 2016).

One aspect of prey biology that is missing from the theoretical predictions from Ryberg *et al.*, (2012) is the effect predators may have on pre- and post-colonization processes that influence

prey populations. The diversity of a metacommunity can be influenced by both post-colonization interactions and pre-colonization habitat selection, the latter of which can affect the choice to colonize or use the patch as an oviposition site (Sih *et al.*, 1985). In grassland ecosystems, increasing herbivore density reduces prey diversity by filtering out palatable species post-colonization, leaving only a subset of plant species (Germain *et al.*, 2013). The effects of fish presence have been studied extensively, with studies showing their impacts on prey habitat selection (Resetarits, Pintar & Bohenek, 2021a), post-colonization dynamics (McPeek, 1990), and the independent influences of both (Vonesh *et al.*, 2009). There is also evidence that prey can differentiate between the types of predators present when colonizing a site (Resetarits, Pintar & Bohenek, 2021b), which could theoretically be adaptive for prey species regardless of predation strategy. If this is true, then the effects of predator presence can differ from predictions due to changes in prey immigration rates (Ryberg *et al.*, 2012).

To explicitly test the hypotheses presented in Ryberg *et al.* (2012), we will assess how predator species that differ in their degree of prey specialization affects prey diversity at different spatial scales in a metacommunity represented by multiple ponds embedded within a terrestrial matrix. The predators that we will focus on are sunfish, newts, and *Anax* (dragonfly) nymphs, all of which are top predators in lentic aquatic ecosystems in eastern North Carolina. The likelihood of each predator species being found in any given habitat is in part due to hydroperiod: *Anax* are more frequently top predators in temporary ponds, sunfish in permanent ponds, and newts in the intermediate range (Wellborn, Skelly & Werner, 1996). We believe that more species of prey are particularly vulnerable to local extinction by (or avoid habitats containing) fish than either newts or *Anax*. Fish presence in a system is an important predictor for the presence of other aquatic vertebrates and invertebrates, typically leading to exclusion of many species (Chase *et al.*, 2009).

In essence, they are acting as a filter on aquatic ecosystems, filling the role of a specialist in our framework. Both newts and *Anax* are efficient predators, but there is little evidence to suggest that they prevent particular species of insect from persisting in a habitat patch. We predict newts and *Anax* will increase the importance of rare species losses by reducing the abundance of all prey species. This will lead to a reduction in alpha diversity but an increase in beta diversity due to the identity of rare species differing between patches. Fish will act as a filter on certain species, reducing both alpha and beta diversity.

Methods

In May 2021, we established artificial ponds using open-top 1100 L cattle tanks at East Carolina University's West Research Campus. Artificial ponds are an effective way to conduct experiments as they are designed to mimic features of natural ponds but allow for greater environmental control and creation of replicate experimental units than is feasible with the use of natural ponds (Chalcraft, Binckley & Resetarits, 2005). Each pond was filled with 800 L of well water and had 1.25 kg of pine litter added on May 17th – May 20th, 2021. One week later, we added a 750 mL inoculation of zooplankton from a nearby natural pond to each pond. Ponds were arranged in blocks of twelve total ponds. Ponds within a block were spaced 1 m apart, and blocks were spaced 10 m apart to differentiate replicates while also ensuring that all ponds had access to the same pool of colonizing species. In each block, we established four types of homogeneous landscapes that each consisted of three ponds serving as habitat patches. The landscape types differed from one another based on the type of predator present in the patches, mimicking the variety of top predator species found in natural ponds: no predators present, sunfish (*Lepomis macrochirus*) present, newts (*Notophthalmus viridescens)* present, and *Anax* nymphs present. All predator landscapes had the same density of predators (two predators per

pond) that were size matched before being added to the ponds (mean ± 1 SE, sunfish: 3.48 ± 0.25 g, newts: 1.86 ± 0.07 g, *Anax*: 1.49 ± 0.10 g). The resulting array had 60 ponds total (five blocks × four landscape types × three patches).

Predators were collected May 23rd – June 1st, 2021, from various sites across eastern North Carolina and housed according to East Carolina University's IACUC recommendations (AUP D368). Sunfish were collected from the North Carolina Museum of Natural Sciences at Contentnea Creek in Grifton, NC and River Park North in Greenville, NC by dipnetting and seining. Newts were collected from a stream in the Sandhill Gamelands in Hoffman, NC by dipnetting. *Anax* nymphs were collected from a pond in Greenville, NC by dipnetting. After size-matching each pair of predators, we randomly assigned predator pairs to ponds in their respective landscape types on June 1st, 2021.

Ponds were sampled in August-September 2022 using 5-minute visual surveys, stovepipe samples, and rare species sweeps. For the stovepipe samples, we sampled each pond by placing two 0.23 m^2 × 0.81 m stovepipe near the center of each pond and performing 25 sweeps of a fine meshed aquarium net within each stovepipe. Rare species sweeps were conducted one day after the pair of stovepipe samples to allow the pond to return to an undisturbed state. These sweeps consisted of six sweeps from the center of the pond to the southwestern edge and six sweeps along the north-to-east quarter of the edge of the pond with a fine meshed aquarium net. The central sweep direction was chosen randomly and kept consistent in case there were common differences in the microenvironment within a pond (e.g., more algal growth in the south quadrant). The sweeps along the north quarter were chosen to sample the area of the pond least disturbed by the central sweeps. Macroinvertebrates from all sweeps were preserved in 70%

ethanol and identified to the lowest level possible. Vertebrates were identified in the field and returned to the pond they were collected from.

Statistical Analysis

Many of the common measures of species richness across spatial scales are disproportionately affected by differences in alpha diversity (Jost, 2007). Because we expected predator presence to directly affect alpha diversity, we used the Measurement of Biodiversity method (McGlinn *et al.*, 2019). The Measurement of Biodiversity (MoB) method uses individual-based rarefaction curves at both the alpha and gamma spatial scales to partition species richness into three components: total abundance of all individuals that belong to a community, rarefied richness, and evenness (Chase *et al.*, 2018). Abundance measures accounted for all individuals in a tank regardless of species. Rarefied richness (S_n) was calculated as the expected number of species on the rarefaction curve when collecting four individuals, the fewest number of individuals collected from a pond. At the regional scale, we also calculated S_n for the minimum number of individuals multiplied by the number of samples in each treatment (four individuals × 15 ponds) to account for the larger spatial scale. Evenness was calculated as the asymptotic effective number of species using the probability of interspecific encounter (S_{PIE}) (Hurlbert, 1971). S_{PIE} is calculated as the slope of the rarefaction curve between N=1 and N=2, with a shallower slope indicating a lower likelihood of the second individual being a different species. The MoB method calculates beta diversity using Whittaker's multiplicative diversity partition for each component ($\beta = \gamma/\alpha$) (Whittaker, 1960). This resulted in three alpha estimates, one beta, and one gamma estimate for each treatment in each block. To avoid pseudoreplication within blocks, we calculated the average alpha and average beta estimate for each treatment in each block.

Using blocks as replicates, we performed a series of general linear models with richness (S), abundance (N), rarefied richness (S_n), or evenness (S_{PIE}) as response variables and predator treatment as a categorical predictor. Each series of models was run for the average alpha estimate per block, average beta estimate per block, and gamma estimates of each component. The MoB parameters were calculated using the "mobr" package in R (McGlinn *et al.*, 2020) and linear models were done using the *lm* function in base R.

Because S_{PIE} is sensitive to changes in the most common species in the metacommunity, we wanted to identify which species a) were the most abundant across all ponds and b) which of those common species were affected by predator treatments. To do so, we first ranked the taxa by descending relative abundance across the full experiment and chose the taxa whose cumulative relative abundance accounted for 90% of all individuals (excluding predator species). We then correlated the mean relative abundance per pond of each of the identified taxa with the non-metric multidimensional scaling (NMDS) scores for the prey communities using the "metaMDS" and "envfit" functions in the "vegan" package in R (Oksanen *et al.*, 2019). This process uses a permutation-based approach by randomly reassigning each relative abundance value to the collection of NMDS scores to create a null distribution to compare the observed relative abundance value to.

Results

We found 68 taxa in the artificial ponds across all treatments (**Table S3.1**). Species richness differed between predator treatments for both alpha diversity ($F_{3, 16} = 12.75$, $p < 0.001$) and gamma diversity ($F_{3, 16} = 10.90$, $p < 0.001$), but beta diversity did not statistically differ among treatments ($F_{3, 16} = 0.792$, $p = 0.516$). The differences were driven by fish presence, which reduced alpha richness by 5.9 taxa ($t_{16} = -4.64$, $p < 0.001$) and gamma richness by 10.6 taxa (t_{16}

= -4.13, $p < 0.001$) compared to the control (**Figure 3.1**). Ponds with newts or *Anax* did not have statistically different alpha, gamma, or beta richness compared to ponds with no predators added (**Figure 3.1**).

Fish reduced prey abundance by 14.7 individuals at the local scale and 44.2 individuals at the regional scale compared to ponds with no predators, but the differences were not statistically meaningful at either scale (alpha and gamma: $F_{3, 16} = 0.31$, $p = 0.816$; **Figure 3.2**). After controlling for differences in abundance, estimates of S_n were statistically different at the local scale ($F_{3, 16} = 4.62$, $p = 0.016$) but not the regional scale ($F_{3, 16} = 2.53$, $p = 0.094$). These differences were driven by fish presence; compared to controls the presence of fish reduced S_n from 2.78 to 1.86 taxa at the local scale ($t_{16} = -3.45$, $p = 0.003$) and from 2.77 to 1.84 taxa the regional scale ($t_{16} = -2.34$, $p = 0.032$) when sampling four individuals (**Figure 3**). A similar trend was seen when sampling 60 individuals at the regional scale ($F_{3, 16} = 4.74$, p = 0.018), though fish reduced S_n from 10.64 to 4.64 taxa compared to ponds without predators ($t_{16} = -2.89$, $p = 0.011$). There was a non-statistically significant change in S_{PIE} at both the local level ($F_{3, 16} = 2.97$, p = 0.063) and the regional level ($F_{3, 16} = 2.25$, p = 0.122), largely influenced by fish presence at both spatial scales compared to ponds with no predators (alpha: $t_{16} = -2.93$, $p = 0.010$; gamma: $t_{16} = -2.74$, $p = 0.043$; **Figure 3.4**).

Eight taxa made up 90% of the individuals found across mesocosms, regardless of treatment: Orthocladiinae (Diptera), Tanypodinae (Diptera), *Tramea carolina* (Odonata), *Libellula luctuosa* (Odonata), *Pachydiplax longipennis* (Odonata), *Erythemis simplicicollis* (Odonata), *Notonecta indica* (Heteroptera), *Buenoa* (Heteroptera) (**Figure 3.5a**). Of those eight taxa, five were correlated with differences in prey community composition: Orthocladiinae ($r^2 = 0.535$, $p = 0.001$), *T. carolina* ($r^2 = 0.284$, $p = 0.001$), Tanypodinae ($r^2 = 0.226$, $p = 0.002$), *P. longipennis*

(r^2 = 0.114, p = 0.040), and *L. luctuosa* (r^2 = 0.134, p = 0.021). The fitted vectors suggested most taxa were negatively correlated with fish communities, except for the two Diptera taxa (Orthocladiinae and Tanypodinae), which were not correlated with fish communities (**Figure 3.5b**).

Discussion

As we hypothesized, predator identity and spatial scale both influenced the effects of predation on prey communities. Though newt and *Anax* presence did not have a discernible effect on prey communities compared to ponds with no predators added, the presence of fish altered several aspects of prey community structure. Fish presence reduced local and regional richness, including after controlling for the influence of reducing the abundance of prey presence. The effect of fish on reducing rarified estimates of richness was stronger at the local than the regional scale. However, we did not find much evidence of the patterns predicted by theory for how generalist and specialist predators would affect prey communities. Fish predation did not alter any metric of beta diversity but did appear to act as a filter on some of the more common species, which led to decreases in evenness due to higher dominance of Diptera taxa and lower relative abundance of lower trophic level predators (i.e., mesopredators).

While we did see a reduction in local and regional diversity with the presence of our specialist predator, the reduction of beta diversity predicted by theory did not occur (Ryberg *et al.*, 2012). Because the MoB method uses the multiplicative diversity partition, this means that the ratio of regional to local richness was consistent between control and fish treatments. This, combined with the lower prey evenness in ponds with fish, indicates that fish did act as a weak filter, reducing the abundance of a subset of the more common taxa across the regional pool. Rarefied richness was lower in ponds with fish, indicating that rare species were also affected by

fish presence, but there was no evidence of fish filtering out certain rare species based on the rarefied beta diversity. In other words, there were some weak filtration effects on populations of the most common prey species that did not have a strong enough impact to outweigh the natural heterogeneity of prey diversity across ponds.

Three of the common species that were negatively correlated with communities from ponds with fish were predatory dragonfly nymphs (*Pachydiplax longipennis*, *Libellula luctuosa*, and *Tramea carolina*), so this could be a case of fish presence suppressing mesopredator populations. Fish species like *Gambusia affinis* have been used as a biological control for predatory insects like *Tramea* in aquaculture, supporting their role as a mesopredator in aquatic ecosystems (Barr *et al.*, 1978). Work in terrestrial systems has shown that presence of a top predator reduces the perceived predation risk by prey species due to the effect of top predators on mesopredators (Vance-Chalcraft *et al.*, 2007; Gordon *et al.*, 2015), potentially leading to biased habitat selection of lower trophic-level prey towards sites with top predators. While it is not possible to confirm whether this is happening with our dataset, biased oviposition site selection by lower-trophic level species could explain the increase in Diptera nymphs in the ponds with fish. Alternatively, there could have been no differences in site selection by lower-trophic level species and the differences in Diptera relative abundance were simply because of the reduced predation pressure from the missing mesopredators. Further work should be done in a closed system varying the densities of each trophic level to determine if fish reduce mesopredator abundance, therefore reducing pressure on Dipterans, or if colonization is the driver of these differences.

As previously mentioned, we did not see the predicted increase in prey beta diversity in ponds with newts and ponds with *Anax* nymphs. Generalist predators were predicted to reduce abundance of all prey species in the ponds, leading to a loss of rare species. However, we saw no

difference in prey richness or abundance between ponds with generalist predators and ponds with no predators added. Past work found an effect of newt presence on the relative abundance of prey species but not the richness of prey communities (Morin, 1983; Strohmeier, Crowley & Johnson, 1989). In particular, Diptera larvae populations were significantly reduced in treatments with newts present (Strohmeier *et al.*, 1989). Little work has been done with the effects of *Anax* on prey communities, but their presence has been shown to reduce the survivorship of other Odonata species (Robinson & Wellborn, 1987). In general, dragonfly nymph colonization of ponds can reduce both richness and abundance of aquatic insects on a very short timescale (Amoroso & Chalcraft, 2015). The majority of the previously mentioned studies were done in a setting that excluded or reduced the likelihood of predator colonization (Morin, 1983; Robinson & Wellborn, 1987; Amoroso & Chalcraft, 2015) or had predator densities much higher than ours (Strohmeier *et al.*, 1989). By maintaining our ponds as an open system, a likely scenario for the differences between these studies and ours is that newt and *Anax* presence in our ponds did not increase the perceived risk of habitat selection by prey species above the background risk associated with the other predators in the ponds (e.g., *T. carolina* and *P. longipennis*).

In aquatic environments, predator avoidance behavior is driven by multiple sensory cues. One crucial cue for prey are kairomones, an interspecific chemical that informs the receiver often at the detriment of the producer (Brown, Eisner & Whittaker, 1970). The ability to sense predator-associated kairomones can be adaptive for prey species, but avoidance behavior can vary between predator and prey species (Pintar *et al.*, 2018). The impact of newts on mosquito oviposition site selection is mixed and depends on the prey species, even within the same genus of mosquito (Eveland *et al.*, 2016). There is evidence of many prey species responding to *Anax* kairomones (Hossie & Murray, 2012; Barry & Roberts, 2014), but *Anax* presence could have

been masked by the background community of dragonfly species. Alternatively, both newts and *Anax* enter and leave ponds throughout their lifespans, so selection pressures for their avoidance might be weaker than that of fish (Resetarits & Wilbur, 1989).

The predictions from theory by Ryberg *et al.* (2012) were not supported by our results, indicating that the relationship between predators and prey communities is affected by more than just predation strategy. Past research has shown that factors like prey dispersal (Huffaker, 1958; Johnston *et al.*, 2016) and prey identity (Zhang *et al.*, 2023) can affect the resulting prey community dynamics. By testing these questions within a community assembly framework, we can determine not only how predators affect communities through post-colonization interactions, but also how they alter habitat selection processes. This study demonstrates that trophic interactions within the prey community should be taken into consideration when describing the effects of apex predators. Future experiments should manipulate the populations and diversity of mesopredators to determine the relative importance they have on prey site selection by altering potential colonization rates or removing colonization altogether and stocking a closed system.

References

Amoroso, N. & Chalcraft, D.R. (2015). Duration of colonization and interactions between early and late colonists determine the effects of patch colonization history on patch biodiversity. *Oikos* **124**, 1317–1326.

Barr, J.E., Huner, J. V, Klarberg, D.P. & Witzig, J. (1978). The large invertebrate-small vertebrate fauna of several south Louisiana crawfish ponds with emphasis on predacious arthropods. *Proc. Annu. Meet. - World Maric. Soc.* **9**, 683–698.

Barry, M.J. & Roberts, D.M. (2014). Indirect interactions limit the efficiency of odonata as natural control agents for mosquito larvae. *J. Insect Behav.* **27**, 626–638.

Brown, W.L., Eisner, T. & Whittaker, R.H. (1970). Allomones and kairomones: Transspecific chemical messengers. *Bioscience* **20**, 21–22.

Chalcraft, D.R., Binckley, C.A. & Resetarits, W.J. (2005). Experimental venue and estimation of interaction strength: Comment. *Ecology* **86**, 1061–1067.

Chalcraft, D.R. & Resetarits, W.J. (2003). Predator identity and ecological impacts: Functional redundancy or functional diversity? *Ecology* **84**, 2407–2418.

Chase, J.M., Abrams, P.A., Grover, J.P., Diehl, S., Chesson, P., Holt, R.D., Richards, S.A., Nisbet, R.M. & Case, T.J. (2002). The interaction between predation and competition: a review and synthesis. *Ecol. Lett.* **5**, 302–315.

Chase, J.M., Biro, E.G., Ryberg, W.A. & Smith, K.G. (2009). Predators temper the relative importance of stochastic processes in the assembly of prey metacommunities. *Ecol. Lett.* **12**, 1210–1218.

Chase, J.M., McGill, B.J., McGlinn, D.J., May, F., Blowes, S.A., Xiao, X., Knight, T.M., Purschke, O. & Gotelli, N.J. (2018). Embracing scale-dependence to achieve a deeper understanding of biodiversity and its change across communities. *Ecol. Lett.* **21**, 1737–1751.

Cooper, S.D., Smith, D.W. & Bence, J.R. (1985). Prey selection by freshwater predators with different foraging strategies. *Can. J. Fish. Aquat. Sci.* **42**, 1720–1732.

Davenport, J.M. & Chalcraft, D.R. (2014). Increasing conspecific density weakens the ability of intermediate predators to develop induced morphological defences to top predators. *Freshw. Biol.* **59**, 87–99.

Eveland, L.L., Bohenek, J.R., Silberbush, A. & Resetarits, W.J. (2016). Detection of fish and newt kairomones by ovipositing mosquitoes. *Chem. Signals Vertebr. 13* 247–259.

Germain, R.M., Johnson, L., Schneider, S., Cottenie, K., Gillis, E.A. & MacDougall, A.S. (2013). Spatial variability in plant predation determines the strength of stochastic community assembly. *Am. Nat.* **182**, 169–179.

Gordon, C.E., Feit, A., Grüber, J. & Letnic, M. (2015). Mesopredator suppression by an apex predator alleviates the risk of predation perceived by small prey. *Proc. R. Soc. B Biol. Sci.* **282**.

Hernandez, J.P. & Chalcraft, D.R. (2012). Synergistic effects of multiple mechanisms drive

priority effects within a tadpole assemblage. *Oikos* **121**, 259–267.

Hossie, T.J. & Murray, D.L. (2012). Assessing behavioural and morphological responses of frog tadpoles to temporal variability in predation risk. *J. Zool.* **288**, 275–282.

Huffaker, C.B. (1958). Experimental studies on predation: Dispersion factors and predator–prey oscillations. *Hilgardia* **27**, 343–383.

Hurlbert, S.H. (1971). The nonconcept of species diversity: a critique and alternative parameters. *Ecology* **52**, 577–586.

Johnston, N.K., Pu, Z. & Jiang, L. (2016). Predator identity influences metacommunity assembly. *J. Anim. Ecol.* **85**, 1161–1170.

Jost, L. (2007). Partitioning diversity into independent alpha and beta components. *Ecology* **88**, 2427–2439.

McGlinn, D., Xiao, X., McGill, B., May, F., Engel, T., Oliver, C., Blowes, S., Knight, T., Purschke, O., Gotelli, N. & Chase, J. (2020). mobr: Measurement of Biodiversity.

McGlinn, D.J., Xiao, X., May, F., Gotelli, N.J., Engel, T., Blowes, S.A., Knight, T.M., Purschke, O., Chase, J.M. & McGill, B.J. (2019). Measurement of Biodiversity (MoB): A method to separate the scale-dependent effects of species abundance distribution, density, and aggregation on diversity change. *Methods Ecol. Evol.* **10**, 258–269.

McNamara, S.C., Pintar, M.R. & Resetarits, W.J. (2021). Temperature but not nutrient addition affects abundance and assemblage structure of colonizing aquatic insects. *Ecology* **102**, 1–12.

McPeek, M.A. (1990). Behavioral differences between enallagma species (Odonata) influencing differential vulnerability to predators. *Ecology* **71**, 1714–1726.

Morin, P.J. (1983). Predation, Competition, and the Composition of Larval Anuran Guilds. *Ecol. Monogr.* **53**, 119–138.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E. & Wagner, H. (2019). vegan: Community Ecology Package.

Paine, R.T. (1966). Food Web Complexity and Species Diversity. *Am. Nat.* **100**, 65–75.

Pintar, M.R., Bohenek, J.R., Eveland, L.L. & Resetarits, W.J. (2018). Colonization across gradients of risk and reward: Nutrients and predators generate species-specific responses among aquatic insects. *Funct. Ecol.* **32**, 1589–1598.

Resetarits, W.J., Pintar, M.R. & Bohenek, J.R. (2021). Complex multi-predator effects on demographic habitat selection and community assembly in colonizing aquatic insects. *Ecol. Monogr.* **91**, e01474.

Resetarits, W.J. & Wilbur, H.M. (1989). Choice of oviposition site in *Hyla chrysoscelis*: role of predators and competitors. *Ecology* **70**, 220–228.

Robinson, J. V. & Wellborn, G.A. (1987). Mutual predation in assembled communities of odonate species. *Ecology* **68**, 921–927.

Ryberg, W.A., Smith, K.G. & Chase, J.M. (2012). Predators alter the scaling of diversity in prey

metacommunities. *Oikos* **121**, 1995–2000.

Schoener, T.W. & Spiller, D.A. (1996). Devastation of prey diversity by experimentally introduced predators in the field. *Nature* **381**, 691–694.

Sih, A., Crowley, P., Mcpeek, M., Petranka, J. & Strohmeier, K. (1985). Predation, competition, and prey communities: A review of field experiments. *Annu. Rev. Ecol. Syst.* **16**, 269–311.

Strohmeier, K.L., Crowley, P.H. & Johnson, D.M. (1989). Effects of red-spotted newts (*Notophthalmus viridescens*) on the densities of invertebrates in a permanent, fish-free pond: A one-month enclosure experiment. *J. Freshw. Ecol.* **5**, 53–65.

Vance-Chalcraft, H.D., Rosenheim, J.A., Vonesh, J.R., Osenberg, C.W. & Sih, A. (2007). The influence of intraguild predation on prey suppression and prey release: A meta-analysis. *Ecology* **88**, 2689–2696.

Vonesh, J.R., Kraus, J.M., Rosenberg, J.S. & Chase, J.M. (2009). Predator effects on aquatic community assembly: Disentangling the roles of habitat selection and post-colonization processes. *Oikos* **118**, 1219–1229.

Wellborn, G.A., Skelly, D.K. & Werner, E.E. (1996). Mechanisms creating community structure across a freshwater habitat gradient. *Annu. Rev. Ecol. Syst.* **27**, 337–363.

Whittaker, R.H. (1960). Vegetation of the Siskiyou Mountains, Oregon and California. *Ecol. Monogr.* **30**, 279–338.

Wilbur, H.M., Morin, P.J. & Harris, R.N. (1983). Salamander Predation and the Structure of Experimental Communities: Anuran Responses. *Ecology* **64**, 1423–1429.

Young, G.B., Golladay, S., Covich, A. & Blackmore, M. (2014). Nutrient enrichment affects immature mosquito abundance and species composition in field-based mesocosms in the coastal plain of Georgia. *Environ. Entomol.* **43**, 1–8.

Zhang, J., Yang, H., Ma, M., Pu, T. & Yin, X. (2023). Predator-mediated diversity of stream fish assemblages in a boreal river basin, China. *Sci. Rep.* **13**, 1–8.

Tables and Figures

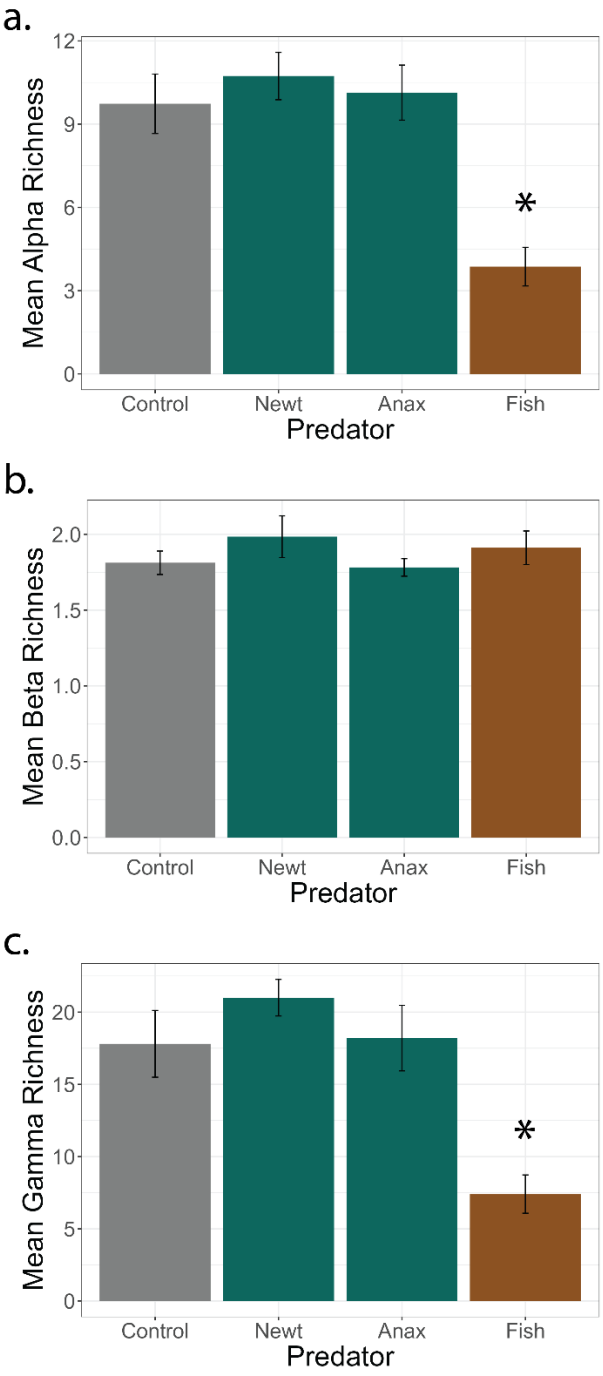

Figure 3.1. Mean (± 1 SE) a) alpha, b) beta, and c) gamma species richness in artificial ponds for the four predator treatments. Color indicates the predicted predation strategy of each predator (grey = no predators added, turquoise = generalist, orange = specialist). * indicates a statistically significant difference from the control treatment at $\alpha = 0.05$. N = 5 in all cases.

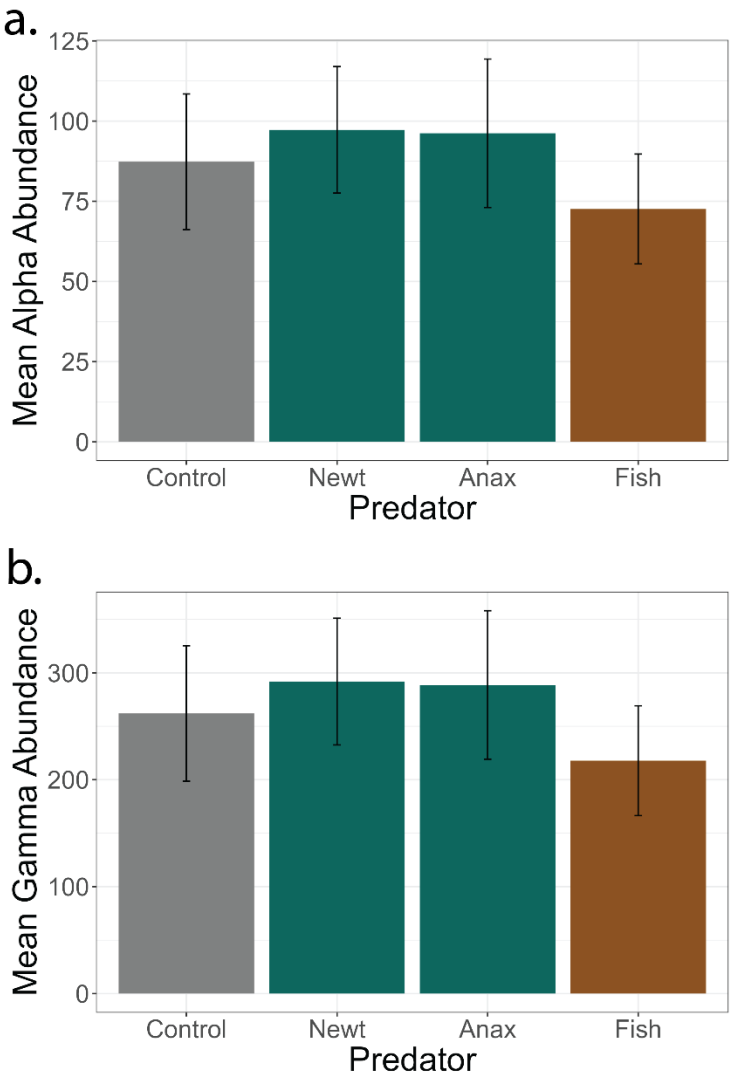

Figure 3.2. Mean (± 1 SE) a) alpha and b) gamma abundance of all individuals in artificial ponds for the four predator treatments. Color indicates the predicted predation strategy of each predator (grey = no predators added, turquoise = generalist, orange = specialist). N = 5 in all cases.

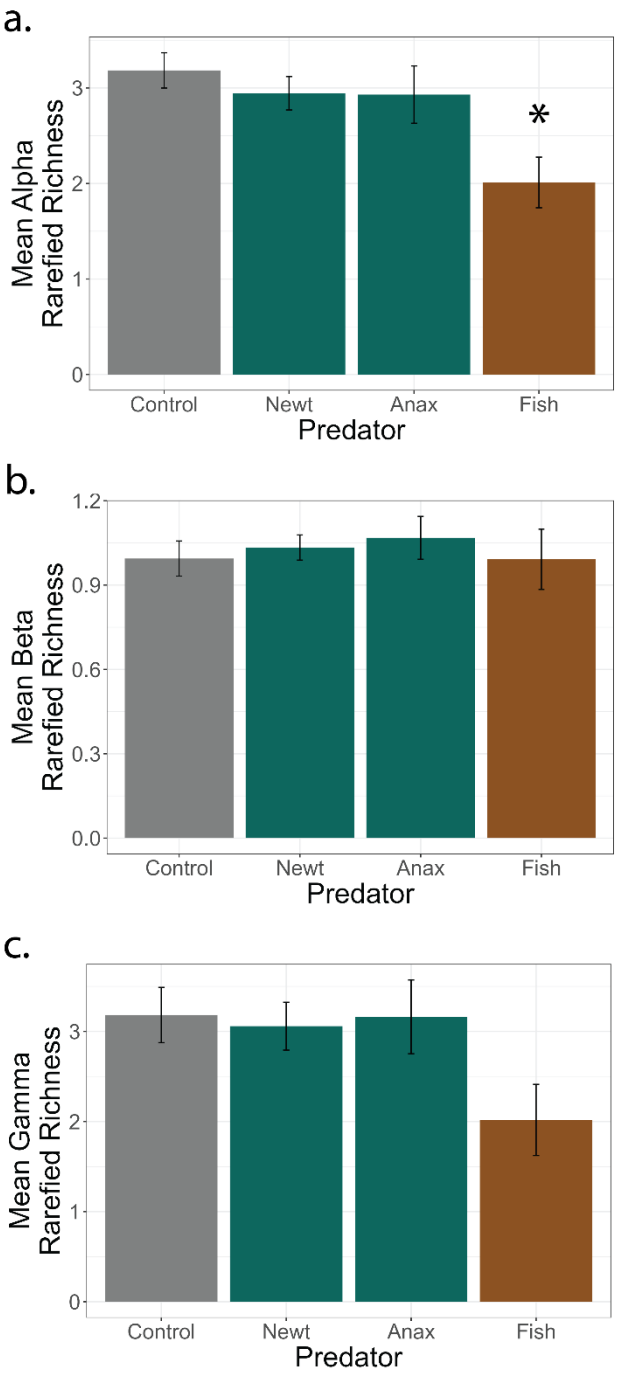

Figure 3.3. Mean (± 1 SE) a) alpha, b) beta, and c) gamma rarefied richness at n = 4 individuals in artificial ponds for the four predator treatments. Color indicates the predicted predation strategy of each predator (grey = no predators added, turquoise = generalist, orange = specialist). * indicates a statistically significant difference from the control treatment at $\alpha = 0.05$. N = 5 in all cases.

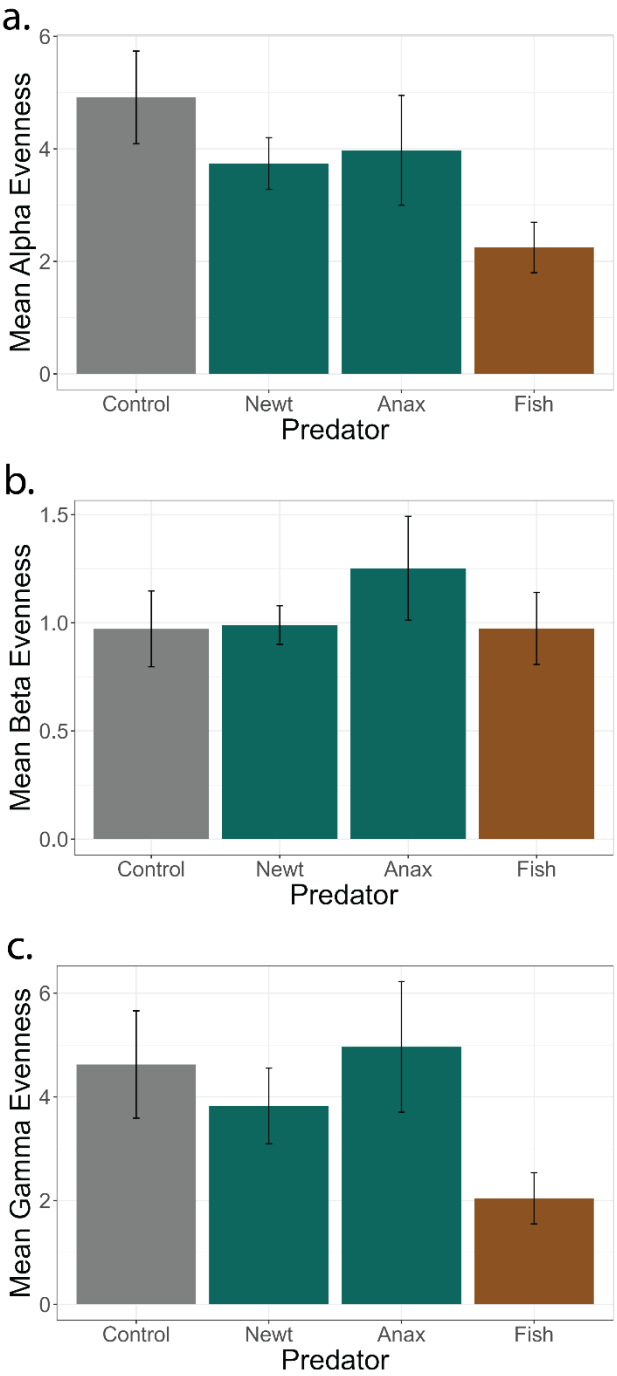

Figure 3.4. Mean (± 1 SE) a) alpha, b) beta, and c) gamma evenness (S_{PIE}) in artificial ponds for the four predator treatments. Color indicates the predicted predation strategy of each predator (grey = no predators added, turquoise = generalist, orange = specialist). N = 5 in all cases.

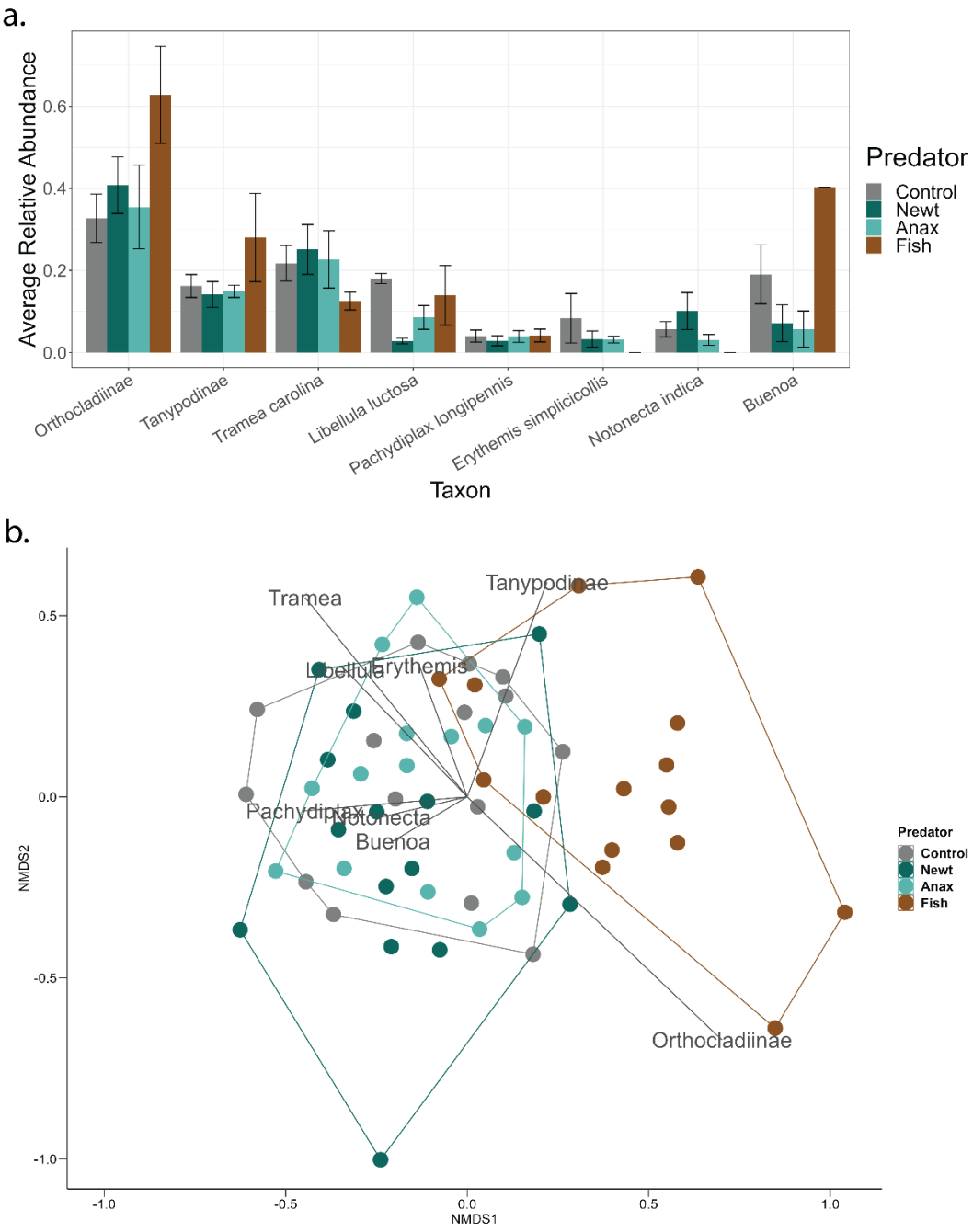

Figure 3.5. a) Mean (± 1 SE) relative abundance per pond for the eight most common taxa across all ponds. Color indicates the predator treatment and hue indicates the predicted predation strategy of each predator (grey = no predators added, turquoise = generalist, orange = specialist). b) Non-metric multidimensional scaling plot indicating the community composition of each pond in the study. Each point is a pond, and the colored lines connect the most extreme points of that color's predator treatment. Black lines indicate the direction of the correlation between community composition and the relative abundance of each of the eight most common taxa.

Supplemental Information

Table S3.1. All prey taxa found in the experiment with what treatments they were found in.

Taxon	**Treatments**
Acilius fraternus	Control
Acris gryllus	Control, *Anax*
Anax junius	Control, Newt, *Anax*
Anthomyiidae	Control, *Anax*
Berosus larva	Control, Newt, Fish
Berosus ordinatus	Newt
Buenoa	Control, Newt, *Anax*, Fish
Caenis	Newt, *Anax*, Fish
Chaoborus	Control, Newt, *Anax*, Fish
Chrysops	Control, Newt
Copelatus chevrolati	Control
Culex	Control, Newt, *Anax*
Culiseta	Control
Dasyhelea	Newt, *Anax*
Dubiraphia bivittata	Newt, *Anax*
Enochrus consors	*Anax*
Enochrus grossi	Control
Erythemis simplicicollis	Control, Newt, *Anax*
Erythrodiplax minuscula	Control
Gerridae nymph	Newt
Hesperocorixa vulgaris	Control, Newt, *Anax*
Hydrochus rugosus	Fish
Hydroporus americanus	Control, Newt
Hydroporus niger	Newt
Hydroporus oblitus	Newt
Hydroporus ruficeps	Control
Hyla femoralis	*Anax*
Hyla squirella	*Anax*
Ischnura posita	Newt
Juvenile *Lepomis*	Fish
Laccophilus maculosus	Newt
Laccophilus proximus	Control, Newt, *Anax*
Libellula axilena	Control, Newt, *Anax*, Fish
Libellula luctuosa	Control, Newt, *Anax*, Fish
Limnoporus	*Anax*, Fish
Lithobates clamitans	Newt
Lithobates sphenocephalus	Newt
Mansonia perturbans	Control, Newt, *Anax*

Microvelia	Control, Newt, *Anax*, Fish
Microvelia americana	Control, Newt, *Anax*
Microvelia paludicola	*Anax*
Microvelia pulchella	Newt, *Anax*
Microvelia with wings	*Anax*
Notonecta indica	Control, Newt, *Anax*
Notonecta irrorata	Control
Notophthalmus larva	Newt
Other Chironomidae	*Anax*
Other Culicidae	Control, Newt, Anax
Other Diptera	Control, Newt
Orthocladiinae	Control, Newt, *Anax*, Fish
Orthopodomyia	Newt
Pachydiplax longipennis	Control, Newt, *Anax*, Fish
Palpomyia	Control, Newt, *Anax*, Fish
Pantala hymenaea	Control, Newt, *Anax*
Pelocoris carolinensis	Control, Newt
Plathemis lydia	Control, *Anax*, Fish
Procloeon rubropictum	Control, Newt
Prodaticus bimarginatus	Control, Newt, *Anax*
Ranatra australis	Control, Newt
Tanypodinae	Control, Newt, *Anax*, Fish
Tetragoneuria spinosa	Control, Newt
Thermonectus basilaris	Newt
Thermonectus basillaris	Control, Newt, *Anax*
Tramea carolina	Control, Newt, *Anax*, Fish
Trichocorixa	Newt
Tropisternus blatchleyi	Control, Newt, *Anax*
Tropisternus collaris	Control, Newt, *Anax*
Tropisternus lateralis	Control, Newt, *Anax*

CHAPTER 4: Nitrogen enrichment affects taxonomic diversity more than functional diversity regardless of spatial scale

Abstract

While studies of taxonomic diversity provide insight into the patterns of species distribution across spatial scales, incorporating functional diversity can improve our understanding of the mechanisms that govern metacommunity structure. By identifying both interspecific trait differences and intraspecific trait variation, we can further develop predictions for how species respond to environmental changes like nitrogen enrichment in aquatic systems. We established a mesocosm array to test the effects of nitrogen enrichment on aquatic insect diversity across spatial scales. For taxonomic diversity, we predicted all enrichment treatments to have similar alpha diversity, but beta and gamma diversity were expected to increase with higher enrichment. We expected the functional composition of ponds to shift from primarily small shredders and collector-gatherers to large predators with increasing nitrogen enrichment. Medium nitrogen enrichment ponds had the highest taxonomic diversity and dissimilarity, as well as the highest functional dissimilarity. Functional diversity did not differ between treatments at the local or regional scale but overlap in mass variation differed between spatial scales. Dragonfly mass variation consistently increased with increasing nitrogen enrichment, but overall Diptera had the highest mass overlap of any insect order and high nitrogen enrichment had the highest mass overlap of any treatment. The changes in taxonomic diversity and lack of change in functional diversity indicate possible functional redundancy in the metacommunity, but more work should be done to identify traits that can more directly impact the function of the broader metacommunity.

Introduction

In metacommunity ecology, biodiversity is often measured by defining the landscape as a mosaic of patches and quantifying the diversity within patches (alpha diversity) and across patches (gamma diversity), and by differences in compositional similarity among patches (beta diversity) (Leibold *et al.*, 2004). In most cases, descriptors of alpha, beta, and gamma diversity focus on the taxonomic identities of species present and their relative abundances. Using taxonomic diversity, ecologists have been able to identify diversity trends associated with environmental conditions like primary productivity (Waide *et al.*, 1999; Mittelbach *et al.*, 2001), elevation (Presley *et al.*, 2011), and disturbance (Hobbs & Huenneke, 1992). However, a singular focus on taxonomic diversity as a descriptor of biodiversity ignores the functional roles that those species play within an ecosystem (McGill *et al.*, 2006).

By incorporating functional diversity into our definition of biodiversity, species can be redefined as the suite of traits that they possess, which can provide a better picture of niche usage and ecosystem function (Mason *et al.*, 2005). Depending on the system, changes to environmental conditions can affect taxonomic and functional diversity in the same direction (Zhang *et al.*, 2019; Dittrich *et al.*, 2023), opposite directions (Fukami *et al.*, 2005), or only affect one and not the other (Spasojevic & Suding, 2012). This is because the rules controlling community assembly differs depending on if one is interested in determining which species are present (i.e., taxonomic diversity) or which collections of traits are represented (i.e., functional diversity) (Fukami *et al.*, 2005; Sutton *et al.*, 2021). Functional diversity is typically lower in environments with more abiotic stressors due to fewer species in the community being able tolerate the stressful conditions (MacArthur & Levins, 1967; Weiher & Keddy, 1995). If the same stressors are present across multiple patches, this can lead to homogenization of functional

diversity across the landscape as fewer niches are available to species (Zhang *et al.*, 2019). In contrast, less stressful environments should have higher functional diversity due to competition limiting similarity in niche usage among species in a community (MacArthur & Levins, 1967). However, depending on what traits are present in the community, environmental stress can instead select for a similarly diverse collection of traits, like traits that confer an advantage for resource acquisition versus traits that give a competitive advantage (Spasojevic & Suding, 2012).

Species coexistence in a community is affected by both the interspecific difference in niche usage (i.e., functional diversity) and the intraspecific variation in niche usage (MacArthur & Levins, 1967). Less stressful environments could lead to higher trait specialization, and therefore lower intraspecific trait variation (Read *et al.*, 2018). If these environments also have greater variety in available niches, then these environments should have higher functional diversity. Alternatively, higher species richness could result in greater intraspecific trait variation if variation helps a species avoid competitive exclusion (e.g., plant height and light competition: Le Bagousse-Pinguet *et al.*, 2014). There has been an increased emphasis on the importance of investigating both interspecific and intraspecific differences in traits to fully understand how communities respond to environmental conditions (Bolnick *et al.*, 2011; Violle *et al.*, 2012).

The effect of nutrient enrichment on taxonomic and functional diversity is highly variable. Enrichment can lead to reductions in taxonomic diversity (Suding *et al.*, 2005; Silvertown *et al.*, 2006), although the magnitude and direction of the effect of nitrogen enrichment can vary based on factors like site productivity (Clark *et al.*, 2007; Chalcraft *et al.*, 2008). In aquatic systems, nutrient enrichment is often associated with human-associated increases in nitrogen and phosphorus leading to eutrophication (Heisler *et al.*, 2008). When the ecosystem is eutrophic, both taxonomic and functional diversity typically decrease (Bini *et al.*, 2014; Zhang *et al.*, 2019).

However, while Zhang *et al.* (2019) found that higher nitrogen and phosphorus levels led to more taxonomically and functionally homogeneous communities (i.e., lower beta diversity), multiple studies have found that taxonomic beta diversity increased with nutrient addition while alpha diversity increased in microcosms (Dittrich *et al.*, 2023) or stayed the same in mesocosms (Chase, 2010).

To test how nutrient enrichment alters community assembly, we investigated changes in taxonomic and functional diversity in aquatic macroinvertebrates with differing levels of nitrogen enrichment in a mesocosm experiment. We expected differences in taxonomic diversity to mirror past mesocosm studies: beta and gamma diversity to increase with increasing nitrogen levels with no change in alpha diversity. In contrast, we predicted increasing nitrogen levels would increase functional alpha, beta, and gamma diversity. Ponds with low nitrogen levels should contain communities primarily containing efficient resource users (e.g., small scrapers and collector-gatherers) at abundances too low to support higher trophic guilds. High nitrogen levels should allow for diverse communities that allow larger carnivores to persist while still containing a subset of the efficient resource users, assuming the ponds do not reach eutrophic levels. Because less stressful environments typically have greater niche availability (MacArthur & Levins, 1967), there should be greater dissimilarity in the traits present in high enrichment ponds than low enrichment ponds. Increased nitrogen availability should also lead to higher trait specialization in macroinvertebrate mass, causing lower intraspecific variation.

Methods

We conducted a field mesocosm experiment between July 2022 to August 2023 at East Carolina University's West Research Campus using open-top 1100 L cattle tanks as artificial ponds. Using artificial ponds in place of natural ponds allows for more control over

environmental conditions and allows replication of experimental units while also allowing for nutrient inputs at a scale that would not be feasible in natural experiments (Chalcraft *et al.*, 2005; Stewart *et al.*, 2013). The ponds were spatially organized in four blocks each containing nine ponds. Each block contained three different sets of ponds with three ponds per set.

with three patches each of three possible enrichment landscapes, resulting in 36 total ponds. We initiated ponds using 1.25 kg of pine litter and filled them with 800 L of water. After one week, we added a 750 mL inoculation of zooplankton from a local natural pond to each pond.

Ponds were randomly assigned to one of three types of homogeneous landscapes: low nutrient enrichment (baseline TDN, 1.63 mg/L), medium nutrient enrichment (2× the baseline TDN, 3.26 mg/L), or high nutrient enrichment (4× the baseline TDN, 6.52 mg/L). Medium and high nutrient enrichment treatment ponds were supplemented with 1.304 g and 3.912 g of $NaNO_3$ respectively one week after inoculation and periodically throughout the experiment to maintain differences in nitrogen levels throughout the year. These nitrogen enrichment treatments were chosen to promote variability in the amount of nitrogen available for the system while staying in the natural range of values according to water quality data across eastern North Carolina (Chescheir *et al.*, 2013). We did not supplement the ponds with phosphorous, another common enrichment nutrient, because the non-enriched TP levels were higher than the published maximum from summer months in eastern North Carolina (0.24 ± 0.015 mg/L vs. 0.12 mg/L) (Chescheir *et al.*, 2013).

The ponds were sampled once in August-September 2023 using 5-minute visual surveys, two stovepipe samples, and rare species sweeps. We placed two 0.23 m^2 × 0.81 m stovepipes near the center of the ponds and performed 25 sweeps of a fine meshed aquarium net within each. For the rare species sweeps, we conducted six sweeps from the center of the pond to the southwestern

edge and six sweeps along the north-to-east quarter of the edge of the pond with a fine meshed aquarium net. The central sweep direction was chosen randomly and kept consistent in case there were common differences in the microenvironment within a pond (e.g., more algal growth in the south quadrant). The sweeps along the north quarter were chosen to sample the area of the pond least disturbed by the central sweeps. Macroinvertebrates from all sweeps were preserved in 70% ethanol and identified to the lowest level possible. Vertebrates were identified in the field and immediately returned to the ponds where they were collected.

Taxonomic Diversity

We used the Measurement of Biodiversity method (MoB) to examine the effects of nutrient enrichment on taxonomic diversity (McGlinn *et al.*, 2019). The MoB method uses individual-based rarefaction curves at both the local and regional scales to calculate species richness and three related components: total individual abundance, rarefied richness, and evenness (Chase *et al.*, 2018). These diversity estimates were calculated for both the local and regional scale. Species richness (S) was the total number of unique species (within a patch for alpha diversity and across all patches within a region for gamma diversity) without correcting for differences in abundance between treatments. Abundance (N) was measured as the total number of individuals in a pond regardless of species. At both spatial scales, rarefied richness (S_n) was calculated as the expected number of species when collecting the minimum number of individuals found per pond (75 individuals). At the regional scale, we also calculated S_n for the expected minimum number of individuals found in a region, which was defined as the minimum number of individuals found in a treatment within a block (538 individuals). Evenness was calculated as the asymptotic effective number of species using the probability of interspecific encounter (S_{PIE}) (Hurlbert, 1971). S_{PIE} is the slope of the rarefaction curve between N=1 and N=2, meaning that a shallower

slope indicates a lower likelihood of the second individual being a different species. The MoB method uses Whittaker's multiplicative diversity partition ($\beta = \gamma/\alpha$) to calculate beta diversity for richness, rarefied richness, and evenness (Whittaker, 1960).

Local diversity was measured as the average alpha estimate of each measure for each treatment in each block, regional diversity was measured as the gamma estimate of each diversity component for each treatment and block, and beta diversity as the average beta estimate for each treatment in each block. We performed general linear models with richness (S), abundance (N), rarefied richness (S_n), or evenness (S_{PIE}) as response variables, enrichment treatment as a categorical predictor, and blocks as replicates. Each series of models was run for each component at the local and regional scale along with beta estimates. To estimate the magnitude of effect size for enrichment treatment, we calculated eta-squared (η^2) for all models (Cohen, 1988). The MoB parameters were calculated using the "mobr" package in R (McGlinn *et al.*, 2020), linear models were done using the *lm* function in base R, and η^2 estimates were calculated using the "effectsize" package in R (Ben-Shachar, Lüdecke & Makowski, 2020).

To test the effect of nitrogen enrichment on dissimilarity of community assemblages, we used permutational analysis of multivariate dispersions (PERMDISP) as an additional test for differences in beta diversity. PERMDISP is the multivariate equivalent to a Levene's test designed to compare the homogeneity of group dispersions from the centroid (Anderson, 2006). This was performed on a Bray-Curtis similarity matrix using the *betadisper* function in the "vegan" package in R (Oksanen *et al.*, 2019). Among-group differences in dispersion were determined using an ANOVA and Tukey test for pairwise differences.

Trait Identification

We measured the masses of individuals and used available databases and primary literature to develop a trait database for macroinvertebrates found in this study (**Supplemental**). The full suite of traits included two numeric variables (average body mass and enrichment tolerance score) and 21 trait values organized into five categories (locomotion habit, functional feeding group, feeding guild, aquatic stage, and respiration mode) (**Table 4.1**). Fuzzy coded traits were organized in a presence-absence matrix, with 0 indicating absence of the trait and 1 indicating the presence of the trait.

We obtained dry mass measures of individuals collected for each taxon to calculate the average body mass for that species. We haphazardly selected up to 9 individuals of each taxon from each pond and dried them in a drying oven for 48 hours at 50^{o}C. After they were dried, they were weighed using a Cahn C-33 microbalance.

Data Imputation

Due to lack of preserved samples and gaps in the literature, we were missing mass data for 11.4% of the community and enrichment tolerance scores for 29.5% of the community. One way to account for missing data is estimating missing values using a variety of predictive techniques through data imputation (Legendre & Legendre, 1998). Recent work has indicated that combating missing trait data with data imputation can help reduce deviations from true functional diversity estimates (Stewart *et al.*, 2023). In particular, incorporating phylogenetic data in the form of phylogenetic eigenvectors improves the accuracy of estimated values from imputation (Penone *et al.*, 2014). To calculate phylogenetic distance between our taxa, we used the R package "rotl" to retrieve a synthetic phylogenetic tree from the Open Tree of Life project for our metacommunity (Michonneau, Brown & Winter, 2016). We then used the "ape" package

to calculate branch lengths and pairwise distances between taxa in our phylogenetic tree (Paradis & Schliep, 2019). To calculate the phylogenetic eigenvectors, we performed a Principal Coordinates Analysis (PCoA) on the phylogenetic pairwise distance matrix and used the first 10 eigenvectors (Penone *et al.*, 2014). The eigenvectors were added to the trait matrix and then we used the "missForest" package in R to impute the missing data using random forest algorithms (Stekhoven & Bühlmann, 2012). Random forests use machine-learning techniques to output the most common clustering of values from a forest of decision trees (Breiman, 2001).

Functional Diversity

In order to calculate the functional diversity of the macroinvertebrate metacommunity, we first needed to map the trait values in two-dimensional space (Villéger, Mason & Mouillot, 2008). To do so, we calculated a Gower dissimilarity matrix to measure the distance between species trait collections, accounting for the fact that we had both numeric and categorical data (Podani & Schmera, 2006). We then performed a PCoA on the dissimilarity matrix and extracted the first two eigenvectors. These eigenvectors represent most of the variation in the multivariate trait space and each represents an independent axis describing variation in the traits of species that are present. The dissimilarity matrix and the PCoA were calculated using the "vegan" package in R (Oksanen *et al.*, 2019).

We measured macroinvertebrate functional diversity in trait space using three metrics: functional richness, functional evenness, and functional divergence (**Figure 4.1**). Functional richness (FRic) is the amount of trait space covered by a community, measured as the volume of a convex hull that connects species with the most extreme points in trait space (Cornwell, Schwilk & Ackerly, 2006) (**Figure 4.1a, b**). Functional evenness (FEve) and functional divergence (FDiv) both measure how abundance is organized in trait space: FEve measures how

evenly species and abundances are spread across trait space (**Figure 4.1c, d**). When all species have the same nearest neighbor distances and the same abundances, then FEve will be 1.FDiv measures where the abundances are organized relative to the center of the convex hull, so if the most abundant taxa are the ones with the most extreme traits, then the FDiv will be 1 (Mason *et al.*, 2005) (**Figure 4.1e, f**).

We performed general linear models for the local scale using the average FRic, FEve, FDiv per block as response variables and enrichment treatment as the predictor. For the regional scale, we pooled all species found in the same treatment and the same block, then derived the same statistics. The measures of functional diversity were calculated using the "fundiversity" package in R (Grenié & Gruson, 2024).

To compare the degree of functional composition variation between enrichment treatments, we performed another PERMDISP analysis on the functional diversity data. We first multiplied the site-species matrix by the original species-trait matrix to obtain a site-trait matrix that was weighted by species abundance. We then performed the previously described PERMDISP analysis using Bray-Curtis similarity, ANOVA, and Tukey test. To determine if increases to species richness were correlated with increases in functional richness, we did a Spearman rank correlation on the two metrics at the local scale.

Intraspecific Trait Variation

We were also interested in incorporating differences in intraspecific trait variation between enrichment treatments. To do so, we measured the degree of overlap in mass measurements between taxa in each enrichment treatment within the four major orders in the metacommunity: Coleoptera, Diptera, Heteroptera, and Odonata. Ephemeroptera were removed for this analysis because only the control treatment had more than one species. We calculated the overlap statistic

by fitting nonparametric kernel density functions to the trait distribution of each taxon with at least two individuals in each treatment and measured the area of overlap (Read *et al.*, 2018). We used the average median pairwise overlap per block for each treatment, which was calculated by determining the overlap of each species pair and taking the median overlap statistic. Values range from 0, indicating no overlap (i.e., high interspecific variation and/or low intraspecific variation), to 1, indicating complete overlap (i.e., low interspecific variation and/or high intraspecific variation). This was done using the "Ostats" package in R (Read *et al.*, 2022). We then performed a general linear model with the overlap statistic as the response variable and enrichment treatment, order, and the interaction between the two as fixed effects.

Results

We found 61 taxa in the artificial ponds across all treatments (**Table S4.1**). Measurement of Biodiversity estimates will be reported as mean ± 1 SE unless otherwise stated. While the differences in the estimates associated with species richness between enrichment treatments were statistically weak (alpha: $F_{2,9} = 1.027$, $p = 0.397$; beta: $F_{2,9} = 0.264$, $p = 0.774$; gamma: $F_{2,9} = 0.538$, $p = 0.602$), there was evidence for an effect of enrichment treatment for both alpha and gamma richness (alpha: $\eta^2 = 0.19$, gamma: $\eta^2 = 0.11$). The medium enrichment treatment had higher alpha (13.5 ± 1.61 taxa) and gamma richness (23.5 ± 3.75 taxa) than both the control (alpha: 11.75 ± 1.06 taxa, gamma: 21.25 ± 2.29 taxa) and high enrichment treatments (alpha: 11.25 ± 0.60 taxa, gamma: 19.75 ± 0.75 taxa; **Figure 4.2**). Ponds with high enrichment tended to have higher abundance of macroinvertebrates (alpha: 367.00 ± 70.50 individuals, gamma: 1101.00 ± 211.50 individuals) than control (alpha: 301.92 ± 53.72 individuals, gamma: 905.75 ± 161.15 individuals) or medium enrichment treatments (alpha: 274.75 ± 49.22 individuals, gamma: 824.25 ± 147.67 individuals, **Figure 4.3**). There was not a statistically significant

difference in abundance between treatments ($F_{2,9}$ = 0.656, p = 0.542), but there was evidence of a treatment effect (η^2 = 0.13). After controlling for differences in abundance, high enrichment ponds had lower rarefied richness across spatial scales (alpha: η^2 = 0.24, beta: η^2 = 0.25, gamma (75 individuals): η^2 = 0.35, gamma (538 individuals): η^2 = 0.23, **Figure 4.4**). This was especially apparent in the gamma estimates when sampling 538 individuals ($F_{2,9}$ = 1.345, p = 0.308). The medium enrichment treatment ponds had the highest rarefied richness (20.54 ± 3.03 taxa), followed by control treatment ponds (18.19 ± 1.37 taxa), and high enrichment treatment ponds had the lowest rarefied richness (15.54 ± 1.71 taxa). Evenness (S_{PIE}) was statistically different between enrichment treatments at the alpha scale ($F_{2,9}$ = 6.047, p = 0.022; η^2 = 0.57; **Figure 4.5**), driven by a higher estimated number of species in the medium enrichment treatment (3.36 ± 0.14 taxa) compared to both the control (2.61 ± 0.22 taxa; t_9 = -2.51, p = 0.033) and the high enrichment treatments (2.37 ± 0.24 taxa; t_9 = -3.34, p = 0.009). There was a similar trend for the gamma estimates for S_{PIE}, though it was not statistically significant ($F_{2,9}$ = 1.984, p = 0.193; η^2 = 0.31).

Functional diversity did not differ between enrichment treatments at either the local or regional scale, likely because the three treatments covered almost identical sections of trait space (**Figure 4.6**). At the local scale, functional richness did not differ between enrichment treatments ($F_{2,9}$ = 0.297, p = 0.750). Because the same taxa (and therefore the same trait combinations) were dominant in all three enrichment treatments, functional evenness and functional divergence also did not differ (FEve: $F_{2,9}$ = 0.673, p = 0.534; FDiv: $F_{2,9}$ = 0.092, p = 0.913). There were also no differences for any of the functional diversity metrics among the different enrichment treatments at the regional scale, likely for the same reasons (FRic: $F_{2,9}$ = 0.259, p = 0.777; FEve: $F_{2,9}$ = 0.107, p = 0.899; FDiv: $F_{2,9}$ = 0.278, p = 0.763).

We found some evidence that nitrogen enrichment impacted taxonomic and functional dissimilarity, though the evidence was statistically week (taxonomic: $F_{2, 33} = 2.500$, $p = 0.097$; functional: $F_{2, 33} = 2.843$, $p = 0.073$). Taxonomic dissimilarity in the medium enrichment treatment (average distance = 0.420) was higher than that observed in either the control (average distance = 0.318, $p = 0.174$) or high enrichment (average distance = 0.307, $p = 0.122$). Differences in functional dissimilarity were primarily driven by higher dissimilarity in medium enrichment (average distance = 0.284) than controls (average distance = 0.160, $p = 0.060$), with high enrichment intermediate between the two (average distance = 0.233). There was no correlation between species richness and functional richness at the local scale ($r = 0.03$, $p = 0.862$).

At the local scale, the interaction between the effects of enrichment treatment and insect order on mass overlap was not statistically important ($F_{6,24} = 1.797$, $p = 0.142$), but each of the main effects were statistically significant (treatment: $p = 0.024$, order: $p = 0.017$; **Figure 4.7a-c**). In general, high enrichment led to more overlap in mass between taxa within an order compared to the control ($t_{24} = 3.041$, $p = 0.006$) and Diptera had the highest overlap statistic of any order regardless of treatment ($t_{24} = 2.870$, $p = 0.008$). At the regional scale, there was a significant interaction between enrichment and insect order, indicating that the amount of mass overlap within orders differed between treatments ($F_{6,26} = 4.252$, $p = 0.004$). This was largely due to Odonata mass overlap in the medium enrichment treatment and Diptera mass overlap in the high enrichment treatment (**Figure 4.7d**). Odonata mass overlap consistently increased with increasing nitrogen enrichment while the other orders had the same or lower overlap in medium treatments compared to low ($t_{26} = 2.018$, $p = 0.054$). Diptera mass overlap increased less than other taxa between medium and high enrichment treatments ($t_{26} = -2.053$, $p = 0.050$). Diptera had

generally higher mass overlap than the other taxa ($t_{26} = 5.627$, $p < 0.001$) and high enrichment ponds had higher overlap than low or medium ($t_{26} = 2.050$, $p = 0.051$).

Discussion

Taxonomic and functional diversity were both influenced by nitrogen enrichment at the local and regional levels. While many of the differences were not statistically significant, there was consistent evidence of an effect of treatment according to the η^2 estimates, so the differences we saw were likely biologically relevant. Ponds with medium nitrogen enrichment had the highest taxonomic diversity and the highest functional dissimilarity compared to ponds with no enrichment and ponds with high enrichment. Ponds with high enrichment had the highest abundance of individuals with low rarefied taxonomic richness, leading to the same richness as ponds with no nitrogen enrichment. High enrichment ponds also had the highest overlap in intraspecific mass variation between species, which could be caused by high intraspecific competition due to higher abundances of individuals.

While there were differences in taxonomic gamma diversity between enrichment treatments, we did not see the expected trends in alpha or Whittaker's beta diversity, nor did we see the expected directionality of gamma diversity effects. There was a trend of ponds with medium nitrogen enrichment hosting the highest alpha diversity, gamma diversity, and taxonomic dissimilarity compared to both low and high enrichment. Past work with macroinvertebrate community assembly in artificial ponds found that enrichment increased beta diversity and had no effect on alpha diversity (Chase, 2010). One possible reason for these contrasting results is the phosphorus content in the ponds, which was at a relatively high concentration for eastern North Carolina regardless of treatment. There is evidence that total phosphorus is a more important limiting nutrient for community structure in aquatic environments (Jeppesen *et al.*,

2000; Zhang *et al.*, 2019), so our manipulations of nitrogen may not have had as strong of an impact as if the phosphorus content was consistently low. Additionally, our ponds were established for only a year, so more time may have been required to see the expected patterns. While Chase (2010) showed that communities were stable over time, manipulations took place over a longer time-scale than our study (3 years versus 1 year), trends in community assembly could fluctuate more soon after establishment and then level off.

There was no evidence for differences in functional richness, evenness, or divergence between nitrogen enrichment treatments at local or regional scales. Communities in each treatment occupied almost identical trait spaces, so there was no evidence of changes to niche availability with increased nitrogen enrichment. Medium enrichment ponds had higher functional dissimilarity than low or high enrichment ponds, and high enrichment ponds had higher dissimilarity than low enrichment ponds. Research focused on the effects of eutrophication on macroinvertebrates found that increasing nutrient enrichment decreased functional dissimilarity between lakes (Zhang *et al.*, 2019) and streams (Schmera, Baur & Erős, 2012).

At the local scale, high nutrient enrichment had the highest amount of overlap in intraspecific mass variation independent of insect order. This would imply that, contrary to our prediction of niche specialization, this is a case of broader niche usage to avoid competitive exclusion (Le Bagousse-Pinguet *et al.*, 2014). At the regional scale, we found an interaction between enrichment treatment and insect taxonomy, primarily driven by enrichment effects on Diptera and Odonata mass variation. Diptera had the highest amount of mass overlap between species across the orders at both the local and regional scale and were the most abundant taxa across the experiment. Given these two factors, it is possible that Diptera exhibited more generalized traits due to competitive pressures in the ponds. Competition among Diptera is well-documented

(Cantrell & McLachlan, 1977; Tokeshi & Townsend, 1987), particularly the effect of larval density on body size (Frouz, Lobinske & Ali, 2009).

When comparing the taxonomic and functional diversity results, two common themes emerge. First, moderate levels of nitrogen enrichment increased dissimilarity in community composition between patches regardless of the type of diversity. In the case of taxonomic diversity, the higher heterogeneity among patches at medium enrichment levels likely had a role in the increased measures of diversity, leading to local communities and regional metacommunities with higher richness and evenness. The difference between the Whittaker's beta diversity measures and the PERMDISP results could be related to interdependence between alpha and beta diversity in multiplicative beta diversity, which is less impactful when using dissimilarity metrics (Jost, 2007). While functional diversity did not change with enrichment, the increase in dissimilarity implies that the composition of traits in each pond was more variable in the medium enrichment treatment than low or high enrichment.

Second, there appeared to be a large degree of functional redundancy in our communities, a phenomenon caused by multiple species exhibiting the same or very similar suites of traits (Loreau, 2004). Given functional richness was not correlated with taxonomic richness, many of the species being added or lost from the metacommunity likely had comparable suites of traits. Nutrient enrichment has been shown to more strongly affect functional diversity than functional redundancy and likely selects against certain combinations of traits (Schmera *et al.*, 2012).

As anthropogenic development continues, aquatic ecosystems will increasingly be impacted by fluctuations in water quality. Nutrient enrichment is of particular importance due to the effect of eutrophication on aquatic communities (Heisler *et al.*, 2008). We found that nitrogen enrichment had a stronger effect on taxonomic diversity than functional diversity, indicating

potential functional redundancy in the metacommunity. Incorporating intraspecific mass variation indicated that increased nitrogen enrichment might also increase competitive pressures between taxa within orders. Measuring functional diversity can fill in gaps in our understanding of how biodiversity is changing across spatial scales. Future studies should further explore these trends by monitoring more dimensions of intraspecific trait variation (Violle *et al.*, 2012) or incorporating ecosystem function traits to describe processes in the community (Streit & Bellwood, 2023).

References

Anderson, M.J. (2006). Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* **62**, 245–253.

Le Bagousse-Pinguet, Y., De Bello, F., Vandewalle, M., Leps, J. & Sykes, M.T. (2014). Species richness of limestone grasslands increases with trait overlap: Evidence from within- and between-species functional diversity partitioning. *J. Ecol.* **102**, 466–474.

Ben-Shachar, M., Lüdecke, D. & Makowski, D. (2020). effectsize: Estimation of Effect Size Indices and Standardized Parameters. *J. Open Source Softw.* **5**, 2815.

Bini, L., Landeiro, V., Padial, A., Siqueira, T., Heino, J., Goia, U.F. De, Federal, U., Grosso, D.M., Ecologia, D. De, Paulista, U.E., Claro, R., Centre, N.E. & Unit, E.C. (2014). Nutrient enrichment is related to two facets of beta diversity of stream invertebrates across the continental US. *Ecology* **95**, 1569–1578.

Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R., Levine, J.M., Novak, M., Rudolf, V.H.W., Schreiber, S.J., Urban, M.C. & Vasseur, D.A. (2011). Why intraspecific trait variation matters in community ecology. *Trends Ecol. Evol.* **26**, 183–192.

Breiman, L. (2001). Random Forests. *Mach. Learn.* **45**, 5–32.

Cantrell, M.A. & McLachlan, A.J. (1977). Competition and Chironomid Distribution Patterns in a Newly Flooded Lake. *Oikos* **29**, 429–433.

Chalcraft, D.R., Binckley, C.A. & Resetarits, W.J. (2005). Experimental venue and estimation of interaction strength: Comment. *Ecology* **86**, 1061–1067.

Chalcraft, D.R., Cox, S.B., Clark, C., Cleland, E.E., Suding, K.N., Weiher, E. & Pennington, D. (2008). Scale-dependent responses of plant biodiversity to nitrogen enrichment. *Ecology* **89**, 2165–2171.

Chase, J.M. (2010). Stochastic community assembly causes higher biodiversity in more productive environments. *Science (80-.).* **328**, 1388–1392.

Chase, J.M., McGill, B.J., McGlinn, D.J., May, F., Blowes, S.A., Xiao, X., Knight, T.M., Purschke, O. & Gotelli, N.J. (2018). Embracing scale-dependence to achieve a deeper understanding of biodiversity and its change across communities. *Ecol. Lett.* **21**, 1737–1751.

Chescheir, G.E., Lebo, M.E., Amatya, D.M., Hughes, J., Gilliam, J.W., Skaggs, R.W. & Herrmann, R.B. (2013). *Hydrology and Water Quality of Forested Lands in Eastern North Carolina*. Raleigh, NC.

Clark, C.M., Cleland, E.E., Collins, S.L., Fargione, J.E., Gough, L., Gross, K.L., Pennings, S.C., Suding, K.N. & Grace, J.B. (2007). Environmental and plant community determinants of species loss following nitrogen enrichment. *Ecol. Lett.* **10**, 596–607.

Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences*. 2nd edition. New York: Academic Press.

Cornwell, W.K., Schwilk, D.W. & Ackerly, D.D. (2006). A trait-based test for habitat filtering: Convex hull volume. *Ecology* **87**, 1465–1471.

Dittrich, J., Dias, J.D., de Paula, A.C.M. & Padial, A.A. (2023). Experimental nutrient enrichment increases plankton taxonomic and functional richness and promotes species dominance overtime. *Hydrobiologia* **850**, 4029–4048.

Frouz, J., Lobinske, R.J. & Ali, A. (2009). Intraspecific competition of *Glyptotendipes paripes* (Diptera: Chironomidae) larvae under laboratory conditions. *Aquat. Ecol.* **43**, 487–500.

Fukami, T., Bezemer, T.M., Mortimer, S.R. & Van Der Putten, W.H. (2005). Species divergence and trait convergence in experimental plant community assembly. *Ecol. Lett.* **8**, 1283–1290.

Grenié, M. & Gruson, H. (2024). fundiversity: Easy computation of functional diversity indices.

Heisler, J., Glibert, P.M., Burkholder, J.M., Anderson, D.M., Cochlan, W., Dennison, W.C., Dortch, Q., Gobler, C.J., Heil, C.A., Humphries, E., Lewitus, A., Magnien, R., Marshall, H.G., Sellner, K., Stockwell, D.A., Stoecker, D.K. & Suddleson, M. (2008). Eutrophication and harmful algal blooms: A scientific consensus. *Harmful Algae* **8**, 3–13.

Hobbs, R.J. & Huenneke, L.F. (1992). Disturbance, diversity, and invasion: Implications for conservation. *Conserv. Biol.* **6**, 324–337.

Hurlbert, S.H. (1971). The nonconcept of species diversity: a critique and alternative parameters. *Ecology* **52**, 577–586.

Jeppesen, E., Jensen, J.P., Sondergaard, M., Lauridsen, T. & Landkildehus, F. (2000). Trophic structure, species richness and biodiversity in Danish lakes: Changes along a phosphorus gradient. *Freshw. Biol.* **45**, 201–218.

Jost, L. (2007). Partitioning diversity into independent alpha and beta components. *Ecology* **88**, 2427–2439.

Legendre, P. & Legendre, L. (1998). *Numerical ecology*. , Developments in Environmental Modelling. 2nd Englis. Amsterdam: Elsevier.

Leibold, M.A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J.M., Hoopes, M.F., Holt, R.D., Shurin, J.B., Law, R., Tilman, D., Loreau, M. & Gonzalez, A. (2004). The metacommunity concept: A framework for multi-scale community ecology. *Ecol. Lett.* **7**, 601–613.

Loreau, M. (2004). Does functional redundancy exist? *Oikos* **104**, 606–611.

MacArthur, R. & Levins, R. (1967). The limiting similarity, convergence, and divergence of coexisting species. *Am. Nat.* **101**, 377–385.

Mason, N.W.H., Mouillot, D., Lee, W.G. & Wilson, J.B. (2005). Functional richness, functional evenness and functional divergence: the primary components of functional diversity. *Oikos* **111**, 112–118.

McGill, B.J., Enquist, B.J., Weiher, E. & Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends Ecol. Evol.* **21**, 178–185.

McGlinn, D., Xiao, X., McGill, B., May, F., Engel, T., Oliver, C., Blowes, S., Knight, T., Purschke, O., Gotelli, N. & Chase, J. (2020). mobr: Measurement of Biodiversity.

McGlinn, D.J., Xiao, X., May, F., Gotelli, N.J., Engel, T., Blowes, S.A., Knight, T.M., Purschke, O., Chase, J.M. & McGill, B.J. (2019). Measurement of Biodiversity (MoB): A method to separate the scale-dependent effects of species abundance distribution, density, and

aggregation on diversity change. *Methods Ecol. Evol.* **10**, 258–269.

Michonneau, F., Brown, J.W. & Winter, D.J. (2016). rotl: an R package to interact with the Open Tree of Life data. *Methods Ecol. Evol.* **7**, 1476–1481.

Mittelbach, G.G., Steiner, C.F., Scheiner, S.M., Gross, K.L., Reynolds, H.L., Waide, R.B., Willig, M.R., Dodson, S.I. & Gough, L. (2001). What is the observed relationship between species richness and productivity? *Ecology* **82**, 2381–2396.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E. & Wagner, H. (2019). vegan: Community Ecology Package.

Paradis, E. & Schliep, K. (2019). Ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* **35**, 526–528.

Penone, C., Davidson, A.D., Shoemaker, K.T., Di Marco, M., Rondinini, C., Brooks, T.M., Young, B.E., Graham, C.H. & Costa, G.C. (2014). Imputation of missing data in life-history trait datasets: which approach performs the best? *Methods Ecol. Evol.* **5**, 961–970.

Podani, J. & Schmera, D. (2006). On dendrogram-based measures of functional diversity. *Oikos* **115**, 179–185.

Presley, S.J., Willig, M.R., Bloch, C.P., Castro-Arellano, I., Higgins, C.L. & Klingbeil, B.T. (2011). A complex metacommunity structure for gastropods along an elevational gradient. *Biotropica* **43**, 480–488.

Read, Q.D., Grady, J.M., Zarnetske, P.L., Record, S., Baiser, B., Belmaker, J., Tuanmu, M.N., Strecker, A., Beaudrot, L. & Thibault, K.M. (2018). Among-species overlap in rodent body size distributions predicts species richness along a temperature gradient. *Ecography (Cop.).* **41**, 1718–1727.

Read, Q.D., Yue, A., Fluck, I.E., Baiser, B., Grady, J.M., Zarnetske, P.L. & Record, S. (2022). Ostats: O-Stats, or pairwise community-level niche overlap statistics.

Schmera, D., Baur, B. & Erős, T. (2012). Does functional redundancy of communities provide insurance against human disturbances? An analysis using regional-scale stream invertebrate data. *Hydrobiologia* **693**, 183–194.

Silvertown, J., Poulton, P., Johnston, E., Edwards, G., Heard, M. & Biss, P.M. (2006). The Park Grass Experiment 1856-2006: Its contribution to ecology. *J. Ecol.* **94**, 801–814.

Spasojevic, M.J. & Suding, K.N. (2012). Inferring community assembly mechanisms from functional diversity patterns: The importance of multiple assembly processes. *J. Ecol.* **100**, 652–661.

Stekhoven, D.J. & Bühlmann, P. (2012). MissForest—non-parametric missing value imputation for mixed-type data. *Bioinformatics* **28**, 112–118.

Stewart, K., Carmona, C.P., Clements, C., Venditti, C., Tobias, J.A. & González-Suárez, M. (2023). Functional diversity metrics can perform well with highly incomplete data sets. *Methods Ecol. Evol.* **14**, 2856–2872.

Stewart, R.I.A., Dossena, M., Bohan, D.A., Jeppesen, E., Kordas, R.L., Ledger, M.E., Meerhoff, M., Moss, B., Mulder, C., Shurin, J.B., Suttle, B., Thompson, R., Trimmer, M. &

Woodward, G. (2013). *Mesocosm Experiments as a Tool for Ecological Climate-Change Research*. *Adv. Ecol. Res.* 1st edition. Elsevier Ltd.

Streit, R.P. & Bellwood, D.R. (2023). To harness traits for ecology, let's abandon 'functionality.' *Trends Ecol. Evol.* **38**, 402–411.

Suding, K.N., Collins, S.L., Gough, L., Clark, C., Cleland, E.E., Gross, K.L., Milchunas, D.G. & Pennings, S. (2005). Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 4387–4392.

Sutton, L., Mueter, F.J., Bluhm, B.A. & Iken, K. (2021). Environmental filtering influences functional community assembly of epibenthic communities. *Front. Mar. Sci.* **8**, 1–19.

Tokeshi, M. & Townsend, C.R. (1987). Random Patch Formation and Weak Competition: Coexistence in an Epiphytic Chironomid Community. *J. Anim. Ecol.* **56**, 833.

Villéger, S., Mason, N.W.H. & Mouillot, D. (2008). New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology* **89**, 2290–2301.

Violle, C., Enquist, B.J., McGill, B.J., Jiang, L., Albert, C.H., Hulshof, C., Jung, V. & Messier, J. (2012). The return of the variance: Intraspecific variability in community ecology. *Trends Ecol. Evol.* **27**, 244–252.

Waide, R.B., Willig, M.R., Steiner, C.F., Mittelbach, G., Gough, L., Dodson, S.I., Juday, G.P. & Parmenter, R. (1999). The relationship between productivity and species richness. *Annu. Rev. Ecol. Syst.* **30**, 257–300.

Weiher, E. & Keddy, P.A. (1995). Assembly rules, null models, and trait dispersion: New questions from old patterns. *Oikos* **74**, 159–164.

Whittaker, R.H. (1960). Vegetation of the Siskiyou Mountains, Oregon and California. *Ecol. Monogr.* **30**, 279–338.

Zhang, Y., Cheng, L., Li, K., Zhang, L., Cai, Y., Wang, X. & Heino, J. (2019). Nutrient enrichment homogenizes taxonomic and functional diversity of benthic macroinvertebrate assemblages in shallow lakes. *Limnol. Oceanogr.* **64**, 1047–1058.

Tables and Figures

Table 4.1. Seven trait categories with 21 trait values and the predicted effect of increased nitrogen enrichment on the functional diversity of each trait category.

Trait Category	Trait Values	Predicted Effect
Mass		Increased mass
Enrichment Tolerance Score		Increased score
Feeding Guild	Herbivore Carnivore Detritivore	Fewer herbivores and detritivores More carnivores
Functional Feeding Group	Deposit Feeder Scraper Filter Gatherer Collector Gatherer Piercer Engulfer	Fewer deposit feeders, scrapers, filter gatherers, and collector gatherers More piercers and engulfers
Locomotion Habit	Swimmer Skater Crawler Burrower Attached	More swimmers
Aquatic Life Stages	Egg Larva Pupa Adult	More adults
Respiration	Gills Tegument Air	Fewer taxa with gills

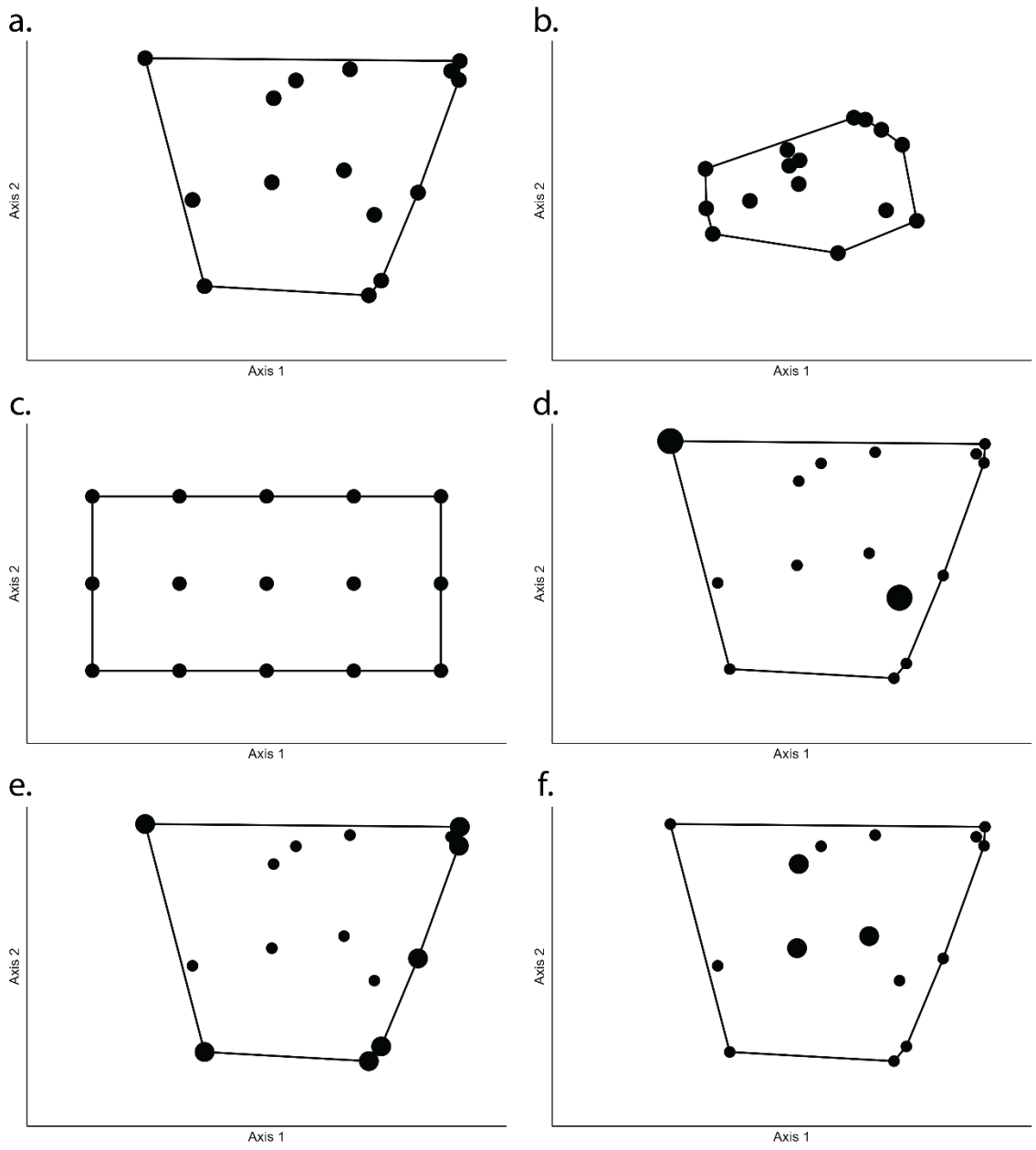

Figure 4.1. Schematic showing the trait space for a theoretical community of organisms to compare high and low values of functional richness (a: high, b: low), functional evenness (c: high, d: low), and divergence (a: high, b: low).

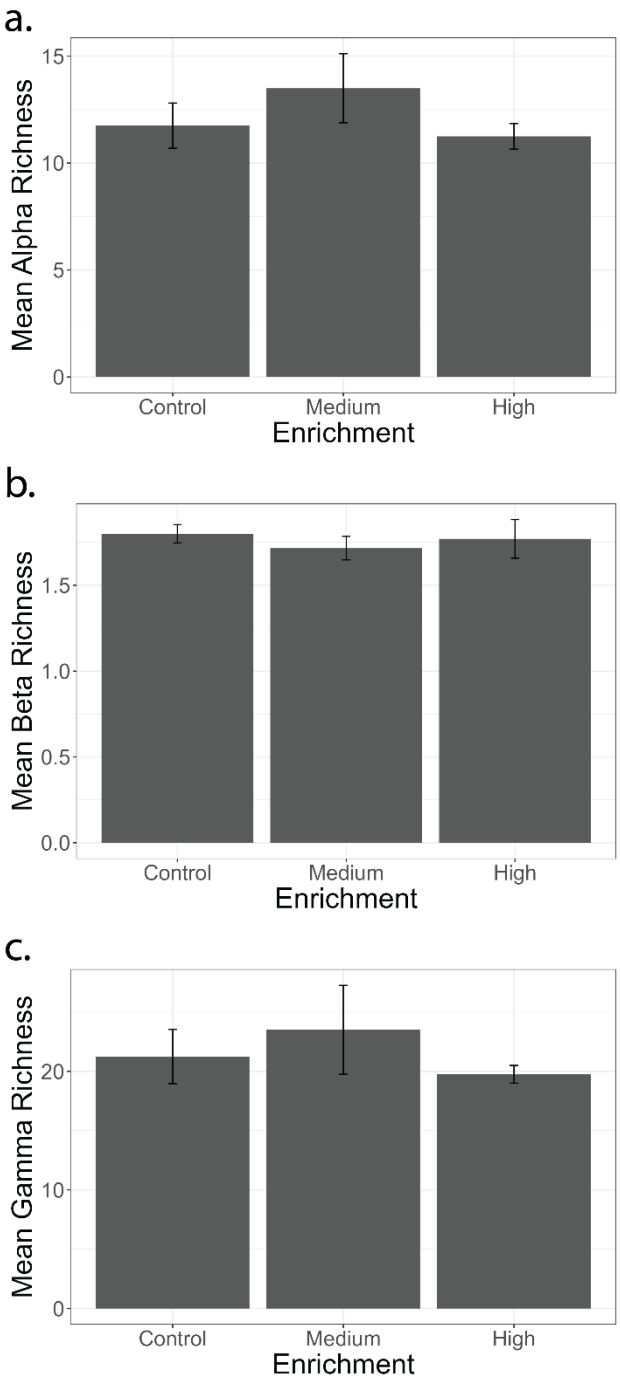

Figure 4.2. Mean (± 1 SE) a) alpha, b) beta, and c) gamma taxonomic richness in artificial ponds for three nitrogen enrichment levels. N = 4 in all cases.

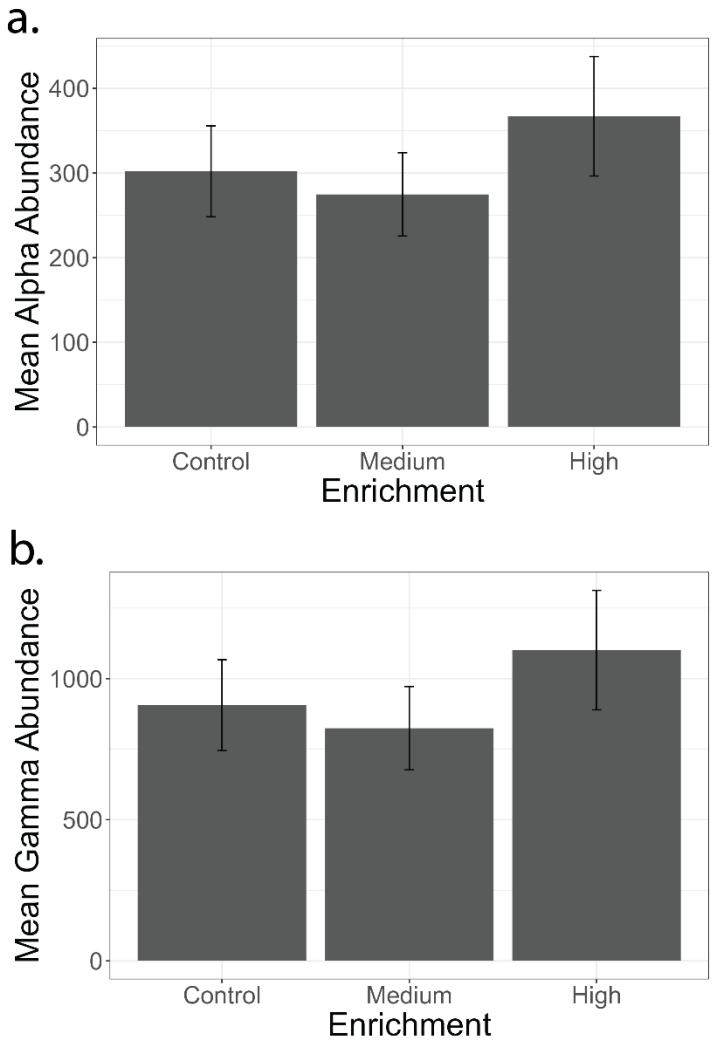

Figure 4.3. Mean (± 1 SE) a) alpha and b) gamma abundance of all individuals in artificial ponds for three nitrogen enrichment levels. N = 4 in all cases.

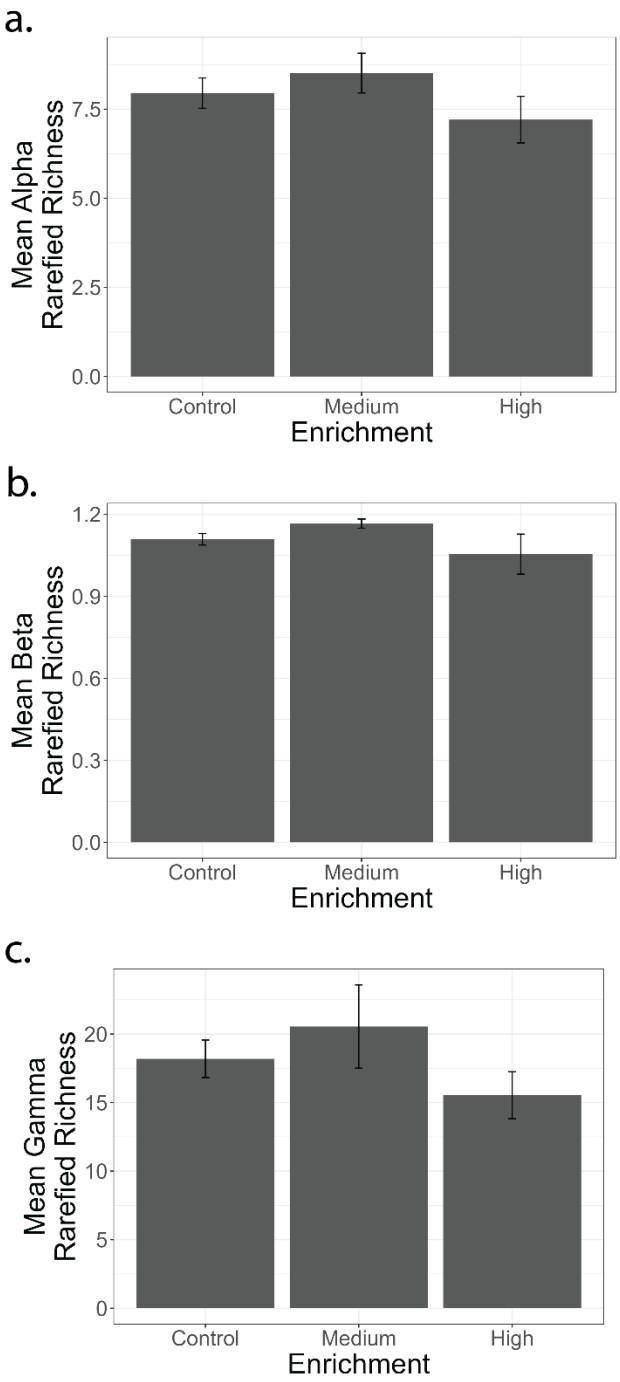

Figure 4.4. Mean (± 1 SE) a) alpha, b) beta, and c) gamma rarefied richness in artificial ponds for three nitrogen enrichment levels. Richness was rarefied to 75 individuals for alpha and beta estimates and 538 individuals for gamma estimates. N = 4 in all cases.

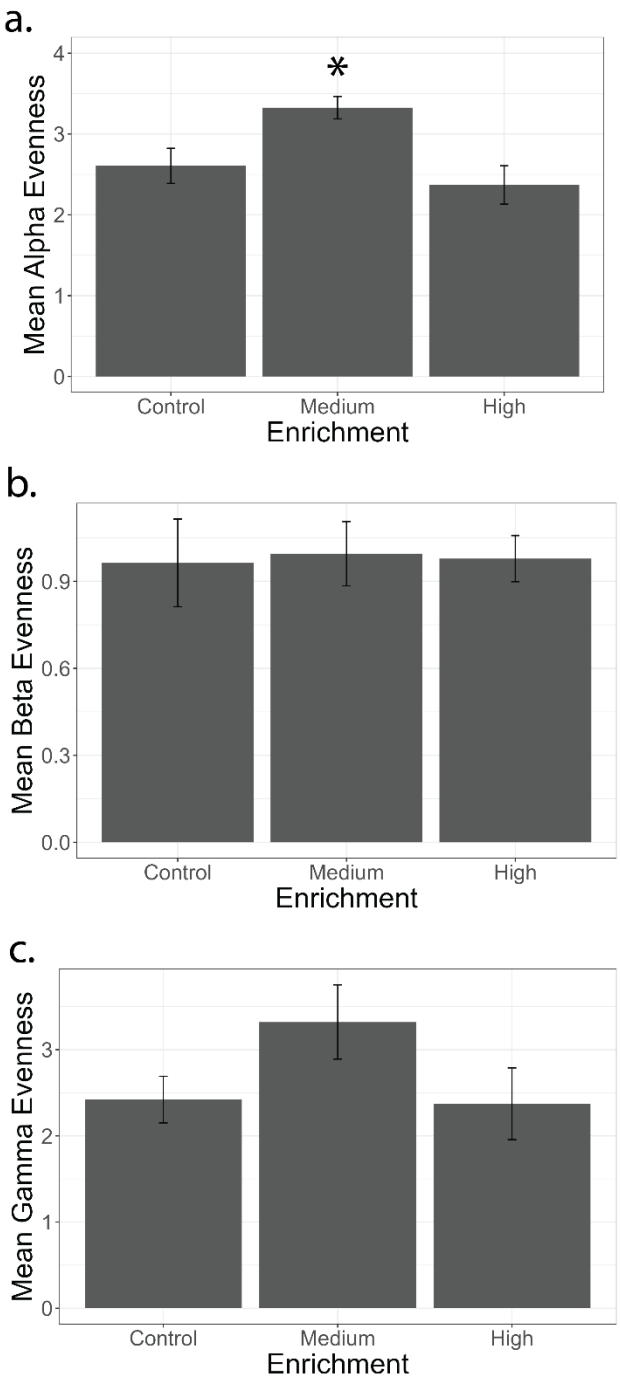

Figure 4.5. Mean (± 1 SE) a) alpha, b) beta, and c) gamma taxonomic evenness (S_{PIE}) in artificial ponds for three nitrogen enrichment levels. * indicates a statistically significant difference from the control treatment at $\alpha = 0.05$. N = 4 in all cases.

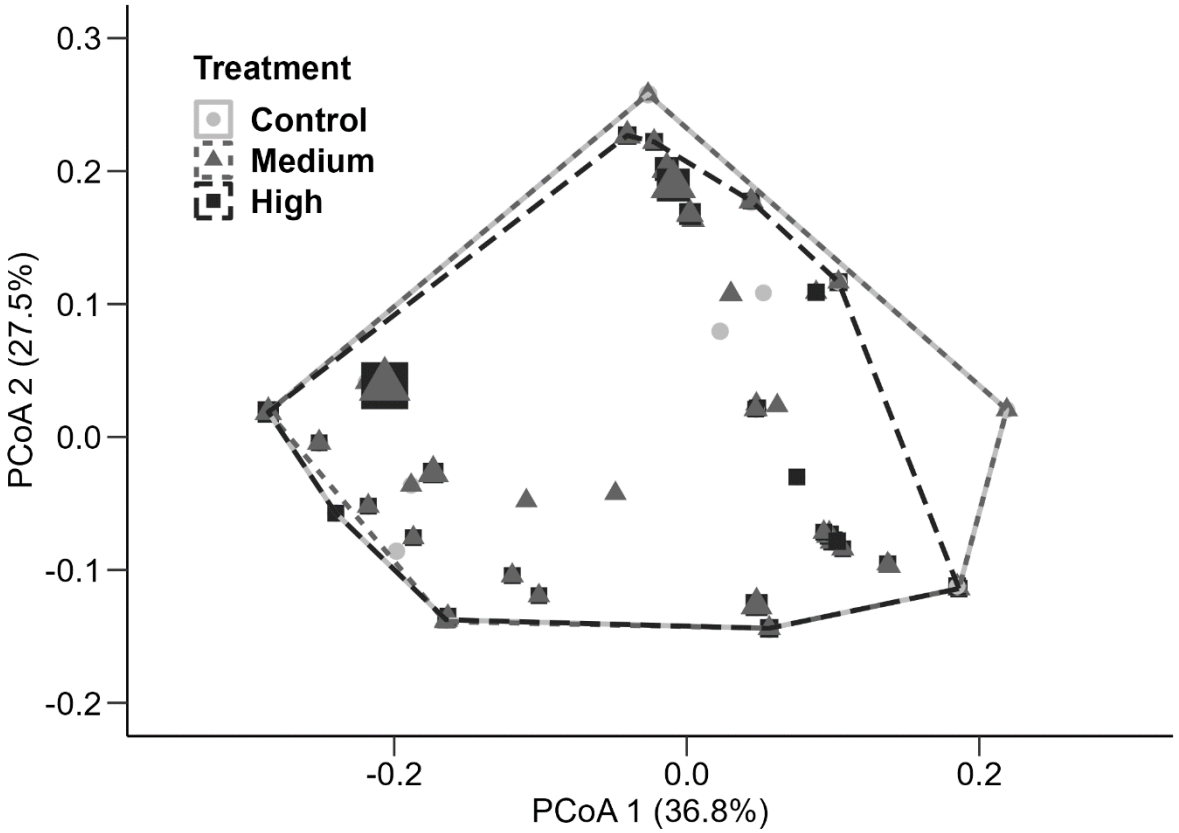

Figure 4.6. Principal Coordinates Analysis (PCoA) plotting trait combinations in trait space for all treatments. Each point is a taxon found in that treatment and the size indicates the abundance across all ponds in each treatment. Shade and shape of the points and color and line type of the convex hull correspond to each treatment (light grey circles with solid lines = low, medium grey triangles with short dashed lines = medium, dark grey squares with long dashed lines = high).

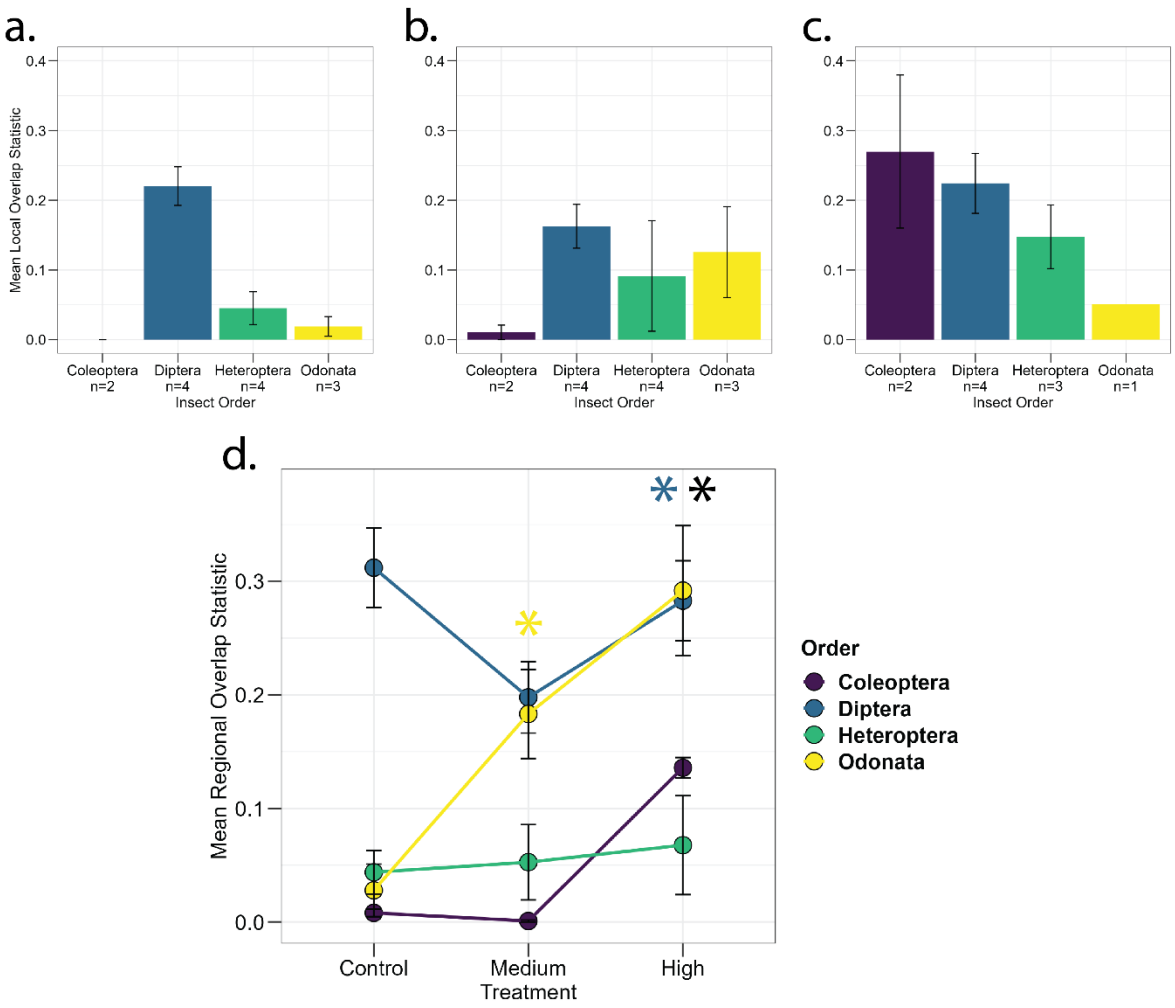

Figure 4.7. Mean (± 1 SE) amount of mass overlap between species within each of the major invertebrate taxa in the experiment in the control (**a**), medium (**b**), and high (**c**) treatment at the local scale and all three treatments at the regional scale (**d**). Each color corresponds to one of the four taxa. * indicates a statistically significant effect at $\alpha = 0.06$. A colored asterisk above a treatment indicates an interaction between that taxon and the treatment. A black asterisk above a treatment indicates the main effect of the treatment is different from the control.

Supplemental Information

Table S4.1. All taxa found in the experiment with what treatments they were found in.

Taxon	**Treatments**
Acilius fraternus	Medium
Aedes	Control
Berosus larva	Control, Medium, High
Berosus ordinatus	Control, High
Buenoa	Control, Medium, High
Buenoa nymph	Control, Medium, High
Caenis	Control, Medium, High
Chaoborus	Control, Medium, High
Copelatus princeps	Medium
Culex	Medium, High
Culicidae	Medium
Dasyhelea	Control, Medium
Dolomedes	Control, Medium
Dubiraphia bivittata	Control, Medium
Erythemis simplicicollis	Control, Medium, High
Erythrodiplax miniscula	Medium
Gerridae nymph	Medium
Hesperocorixa vulgaris	Control, Medium, High
Hydrochus excavatus	Medium, High
Hydroporus americanus	Control, High
Hydroporus ruficeps	Control, Medium
Ischnura posita	Control, Medium
Laccophilus fasciatus	Control, Medium, High
Laccophilus proximus	Control, High
Libellula axilena	Control, Medium, High
Limnoporus	Medium, High
Limnoporus canaliculatus	Medium
Liodessus affinis	Medium
Lithobates sphenocephala	Control
Lumbricidae	Medium
Mansonia perturbans	Control, Medium, High
Matus bicarinatus	High
Matus ovatus	High
Mesovelia amoena	Control, High
Microvelia	Control, Medium, High
Microvelia americana	Control, Medium, High
Microvelia nymph	Control
Microvelia pulchella	Control, Medium, High

Microvelia with wings	Control
Muscidae	Medium
Neoporus lobatus	Control, Medium
Neoporus undulatus	Medium, High
Notonecta indica	Control, Medium, High
Notonecta irrorata	Control, Medium, High
Notonecta nymph	Control, Medium, High
Orthocladiinae	Control, Medium, High
Pachydiplax longipennis	Control, Medium, High
Palpomyia	Control, Medium, High
Pantala hymenaea	Control, Medium, High
Pelocoris carolinensis	Control, Medium, High
Peltodytes	Control, Medium, High
Procloeon rubropictum	Control, Medium, High
Psorophara discolor	Control
Ranatra australis	High
Tanypodinae	Control, Medium, High
Tramea carolina	Control, Medium, High
Trichocorixa	Control, High
Tropisternus blatchleyi	Control, Medium, High
Tropisternus collaris	Control, Medium, High
Tropisternus lateralis	Control, Medium, High
Tropisternus natator	Medium

CHAPTER 5: Conclusion

In this dissertation, I sought to understand metacommunity structure through three lenses. First, I used a long-term dataset to understand the effects of temporal variation in primary productivity on metacommunity structure. Using the Elements of Metacommunity Structure method (Leibold & Mikkelson, 2002), I was able to determine that while metacommunities stayed relatively consistent in their categorical structure (i.e., quasi-Clementsian or Clementsian), primary productivity influenced the rates of turnover and boundary clumping. Additionally, annual variations in environmental conditions like temperature and precipitation played a role in the strength of the relationship between productivity and metacommunity structure.

Next, I established a mesocosm array to investigate how predator identity altered prey metacommunities. My results contradicted predictions established from theory (Ryberg *et al.*, 2012). Generalist predators did not influence prey diversity in the artificial ponds, possibly because the perceived predation risk from the added predators did not increase the risk beyond the background rate from mesopredators in the metacommunity. Specialist predators reduced prey alpha and gamma diversity but did not affect beta diversity. There was evidence that mesopredator abundance was negatively correlated with specialist presence, so specialist predators may have not been a strong enough filter to fully remove prey species from the metacommunity.

Finally, I used a mesocosm array to determine the effects of nitrogen enrichment on macroinvertebrate taxonomic and functional diversity. Taxonomic diversity was highest in medium enrichment treatments regardless of the measure of diversity used at local and regional scales, while functional diversity did not change with increasing nitrogen enrichment. This indicates the potential for functional redundancy within the metacommunity, especially since

there was not a correlation between species richness and functional richness at the local scale. Intraspecific mass had the highest amount of overlap between species at the high enrichment treatment, which was probably caused by higher competitive pressures from the higher abundances in ponds with high nitrogen enrichment.

This dissertation explored a variety of topics and techniques across spatial and temporal scales to understand changes in metacommunity structure. While the included studies may be disparate in their focus, some common themes emerge for next steps to investigate:

1. *Application of trait information to metacommunities*

   One of the biggest struggles of this dissertation was deciding how to incorporate trait information for Chapter 4. Trait selection is far more subjective than taxonomic identification, resulting in choices that can alter the results of analyses (Jeliazkov & Chase, 2024). In plant research, there are well-established databases and study design recommendations (e.g., Kattge *et al.*, 2020), but the same is not yet true for insect traits (though the Status of Insects NSF-funded Research Coordination Network is in the process of developing these tools). Until such tools are readily available, applying trait-based approaches to metacommunity ecology in a generalizable way will be difficult.

2. *Connecting ecosystem function and metacommunity structure*

   Biodiversity is intrinsically linked to ecosystem function, playing a part in features like productivity and stability (Tilman *et al.*, 2014). In turn, ecosystem functions like productivity or nutrient load act as environmental filters that play a role in determining what species can persist in a habitat patch (Mittelbach *et al.*, 2001). Incorporating differences in spatial scale into studies on biodiversity-ecosystem function is a relatively new concept (Leibold, Chase & Ernest, 2017; Gonzalez *et al.*, 2020), and recent studies

have found that processes like dispersal influence how biodiversity alters ecosystem function at larger spatial scales (Ladouceur *et al.*, 2020). While this dissertation focused primarily on the effect of ecosystem functions on biodiversity, the findings can be used to further develop questions about the effect of diversity on ecosystem function. For instance, fish presence likely influences ecosystem function by altering the colonization habits of insects (Weisser & Siemann, 2008), but the effect of this across spatial scales is unclear.

3. *Using metacommunity theory to mitigate biodiversity loss*

   Understanding the dynamics of metacommunity structure is crucial for predicting biodiversity trends across space and time. By quantifying dissimilarity between patches of habitat, conservation practitioners can identify where to focus efforts to maximize diversity (Bush *et al.*, 2016). At an even more basic level, incorporating spatial scale into analyses of biodiversity is crucial to understand context of species loss (Socolar *et al.*, 2016; Chase *et al.*, 2020). Doing this in tandem with functional diversity provides insight into how ecosystem function is affected by changes in diversity (Schmera *et al.*, 2012). By applying the results from this dissertation to questions about anthropogenic change, this work will hopefully help to mitigate biodiversity loss caused by climate change (Chapter 2), introduced species (Chapter 3) or nutrient pollution (Chapter 4).

References

Bush, A., Harwood, T., Hoskins, A.J., Mokany, K. & Ferrier, S. (2016). Current uses of beta-diversity in biodiversity conservation: A response to Socolar et al. Trends Ecol. Evol. 31, 337–338.

Chase, J.M., Jeliazkov, A., Ladouceur, E. & Viana, D.S. (2020). Biodiversity conservation through the lens of metacommunity ecology. Ann. N. Y. Acad. Sci. 1469, 86–104.

Gonzalez, A., Germain, R.M., Srivastava, D.S., Filotas, E., Dee, L.E., Gravel, D., Thompson, P.L., Isbell, F., Wang, S., Kéfi, S., Montoya, J., Zelnik, Y.R. & Loreau, M. (2020). Scaling-up biodiversity-ecosystem functioning research. Ecol. Lett. 23, 757–776.

Jeliazkov, A. & Chase, J.M. (2024). When do traits tell more than species about a metacommunity? A synthesis across ecosystems and scales. Am. Nat. 203, E1–E18.

Kattge, J., Bönisch, G., Díaz, S., Lavorel, S., Prentice, I.C., Leadley, P., Tautenhahn, S., Werner, G.D.A., Aakala, T., Abedi, M., *et al*. (2020). TRY plant trait database – enhanced coverage and open access. Glob. Chang. Biol. 26, 119–188.

Ladouceur, E., Stanley Harpole, W., Blowes, S.A., Roscher, C., Auge, H., Seabloom, E.W. & Chase, J.M. (2020). Reducing dispersal limitation via seed addition increases species richness but not above-ground biomass. Ecol. Lett. 23, 1442–1450.

Leibold, M.A., Chase, J.M. & Ernest, S.K.M. (2017). Community assembly and the functioning of ecosystems: how metacommunity processes alter ecosystems attributes. Ecology 98, 909–919.

Leibold, M.A. & Mikkelson, G.M. (2002). Coherence, species turnover, and boundary clumping elements of meta-community structure. Oikos 97, 237–250.

Mittelbach, G.G., Steiner, C.F., Scheiner, S.M., Gross, K.L., Reynolds, H.L., Waide, R.B., Willig, M.R., Dodson, S.I. & Gough, L. (2001). What is the observed relationship between species richness and productivity? Ecology 82, 2381–2396.

Ryberg, W.A., Smith, K.G. & Chase, J.M. (2012). Predators alter the scaling of diversity in prey metacommunities. Oikos 121, 1995–2000.

Schmera, D., Baur, B. & Erős, T. (2012). Does functional redundancy of communities provide insurance against human disturbances? An analysis using regional-scale stream invertebrate data. Hydrobiologia 693, 183–194.

Socolar, J.B., Gilroy, J.J., Kunin, W.E. & Edwards, D.P. (2016). How Should Beta-Diversity Inform Biodiversity Conservation? Conservation Targets at Multiple Spatial Scales. Trends Ecol. Evol. 31, 67–80.

Tilman, D., Isbell, F. & Cowles, J.M. (2014). Biodiversity and ecosystem functioning. Annu. Rev. Ecol. Evol. Syst. 45, 471–493.

Weisser, W. & Siemann, E. (2008). Insects and Ecosystem Function. , Ecological Studies. Berlin, Heidelberg: Springer Berlin Heidelberg.

APPENDIX: Animal Use Protocol Approvals

Animal Care and Use Committee
003 Ed Warren Life Sciences Building | East Carolina University | Greenville NC 27834 - 4354
252-744-2436 office | 252-744-2355 fax

February 4, 2021

David Chalcraft, Ph.D.
Department of Biology, ECU

Dear Dr. Chalcraft:

Your Animal Use Protocol entitled, " Effect of Differing Predation Strategies on Diversity at Multiple Spatial Scales" (AUP #D368) was reviewed by this institution's Animal Care and Use Committee on 02/3/2021. The following action was taken by the Committee:

"Approved as submitted"

****Please contact Aaron Hinkle prior to any hazard use****

A copy of the protocols is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies. **Please ensure that all personnel associated with this protocol have access to this approved copy of the AUP/Amendment and are familiar with its contents.**

Sincerely yours,

Susan McRae, Ph.D.
Chair, Animal Care and Use Committee

SM/GD

enclosure