

Spatiotemporal Variation in Dispersal as a Driver of Macroinvertebrate  
Metacommunity Dynamics

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## Academic Abstract

Across taxa, community assembly has traditionally been attributed to local processes such as species competition and environmental filtering. Metacommunity theory, however, highlights regional drivers such as dispersal and connectivity as key structuring forces. The mechanisms governing freshwater macroinvertebrates community assemblage have long been studied due these organism's ubiquity, diversity, and sensitivity to environmental stressors. Macroinvertebrates reliance on river networks for their larval stages also makes their dispersal complex, and therefore crucial to frame their assemblages in a metacommunity context. While metacommunity theory has been widely applied to macroinvertebrate community ecology within the last 15 years, the spatial and temporal variability of dispersal across networks is still poorly understood. To address this gap, we conducted a year-long survey of the macroinvertebrate communities in the Little Stoney Creek watershed of Giles County, Virginia. Macroinvertebrates were sampled once a month for 12 months from the benthos and drift at eight sites across a gradient from headwaters to mainstem to discover if there was any difference in community dynamics between poorly connected sites (headwaters) and well connected sites (mainstems) across seasons. Broadly, we hypothesized that mainstem sites would have less temporal variability in community dynamics compared to headwater sites due to mass effects and high dispersal rates. We found high temporal variability in dispersal and the subsequent correlation of dispersal assemblages with the resident benthic community across sites, but only weak evidence to suggest that this variation varies between headwaters and mainstems. However, considering the stochastic and difficult to model nature of macroinvertebrate drift dispersal, we consider the existence of even weak evidence of spatial differences to be promising. Further study of macroinvertebrate dispersal in metacommunities is needed to better understand the complex mechanisms governing macroinvertebrate communities, particularly in the context of biomonitoring applications.

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## **Public Abstract**

The factors that shape the communities of small stream invertebrates have long been of interest to community ecologists. Traditionally, researchers focused on local factors like competition between species or how well suited their habitat is for them. But more recent studies have shown that broader, regional factors—such as how these organisms move between different parts of a stream network—also play a big role in shaping these communities. Freshwater macroinvertebrates are especially important to study in this context because they're common, diverse, and sensitive to changes in their environment. Their life cycles often depend on rivers, making their movement and dispersal patterns complex and worth studying on a larger scale. To explore how these organisms move and settle in different parts of a stream network, we conducted a year-long study of macroinvertebrate communities in Little Stoney Creek, Virginia. We collected samples each month from eight different sites ranging from small headwater streams to larger mainstem areas. Our goal was to see if there were differences in community patterns between isolated headwater sites and well-connected mainstem sites across different seasons. We found that dispersal patterns were highly variable over time and influenced the communities living in different parts of the stream. However, differences between isolated headwaters and connected mainstems were weaker than expected. Given the complexity of these movement patterns, finding even small differences suggests important processes are at play. Understanding how these organisms move through stream networks can help us better manage freshwater systems and improve how we use them to monitor water quality.

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## **CHAPTER 1:**

# **MACROINVERTEBRATE DISPERSAL IN RIVER NETWORKS**

Freshwater macroinvertebrates are an immensely diverse community of invertebrates that include insects, mollusks, crustaceans, and annelids, and that are characterized by their visibility to the naked eye and life histories including at least one stage obligate to an aquatic environment (Thorp & Covich, 2009). In addition to their critical roles in nutrient cycling and energy transfer in stream ecosystems, they also serve as sensitive bioindicators of water quality (Wallace & Webster, 1996). They are also nearly ubiquitous in all aquatic habitats, with even the most degraded systems retaining some limited assemblage of tolerant taxa (Hauer & Lamberti, 2011). Because of macroinvertebrates' ecosystem function, ubiquity, and diversity, study of their assemblages has been a central focus of stream ecology since the inception of the field (Hynes, 1970).

Much of the large body of literature on macroinvertebrates, especially early on, has focused on the complex life histories of insects. In particular, the orders *Ephemeroptera*, *Plecoptera*, *Trichoptera*, *Diptera*, *Megaloptera*, and *Odonata* (Class: Insecta) all have life histories that involve a larval stage in the water, followed by emergence into flying adults that move across the landscape (i.e., not directly constrained by the river network), mate, and deposit eggs back into the water (Thorp & Covich, 2009). While the implications of these complex ecologies are far ranging from population dynamics to aquatic-terrestrial ecosystem subsidies (Sullivan & Manning, 2019), we will constrain the remainder of this review to examining the implications of these complex life histories for the dispersal dynamics of these communities.

Drift is a key mechanism underlying macroinvertebrate dispersal dynamics in riverine systems (Greenwood & Richardot-Coulet, 1996). Macroinvertebrate drift is the process through which macroinvertebrates release themselves into the water column in order to move downstream and is therefore one of the key components to their dispersal and community assembly (Sarremejane *et al.* 2017). Turbulence generated by sediment roughness near the bed creates shear stress, which in turn produces drag and lift forces on invertebrates inhabiting the substrate (Mobes-Hansen & Waringer, 1998; Merigoux & Doleddec, 2004). Once a macroinvertebrate enters the water column, they are flushed downstream, typically returning to the benthos once suitable habitat, such as another riffle, has been identified (Hauer & Lamberti, 2019).

When macroinvertebrate drift was originally described as a phenomenon, it was thought to be entirely incidental, or passive. However, upon further investigation, it has been concluded

that most drift is intentional, or active, and related to foraging and escape from predation (Kohler & McPeck 1989; Forrester, 1994). Broadly speaking, passive drift can be thought of as drift caused by hydraulics (changes in flow), whereas active drift can be thought of as caused by behavior (individuals intentionally release themselves into the water column) (Naman *et al.* 2016). Key evidence for this prevalence of active drift is the strong diel-cycle exhibited in the dynamics. Drift density is almost always markedly highest right after dusk, and many studies have shown that the reduction in light is a trigger for aquatic insects to release themselves and move to new habitat patches (Hauer & Lamberti, 2019), with the darkness likely helping them avoid detection from predators (Kohler & McPeck, 1989, Kohler 1985; Kohler 1983).

There is a rich literature examining the differences between active and passive drift, mainly through the manipulation of predators and resources in controlled environments and observing differences in drift behavior (Peckarsky, 1980; Hernandez & Peckarsky, 2014). While there are clear differences in drift behavioral response to predators and resources, these differences aren't consistent across taxa. For example, mayflies of the genus *Baetis* consistently show strong responses to food and predators (drifting to get more of the former and avoid the latter), yet other taxa, such as caddisflies, show little response (Hernandez & Peckarsky, 2014). In addition to these differences in drift propensity based on minor differences in environmental variables, turbulent flow in streams creates remarkably variable shear stress at a microhabitat scale, resulting in subsequently highly variable passive drift within a single patch of habitat (Hart, Clark, & Jasentuliyana, 1996). All these factors contribute to the difficulty in modeling and interpreting stream drift over larger scales.

With an historical focus on drifting insects in freshwater ecology research, the unidirectional flow of insects moving downstream led to consideration of the “drift paradox” (Hershey *et al.* 1993; Anholt, B. R. 1995). The drift paradox was a term widely used in literature to describe how it was not fully understood how these upstream communities were replenished if there was a constant movement of insects in one direction downstream (Hershey *et al.* 1993). While the drift paradox has largely been empirically and theoretically resolved through the consideration of upstream adult flight and critical thresholds of flow (Lutscher, Pachepsky, & Lewis, 2005; Macneale *et al.*, 2005), it is still central to our understanding of dispersal for macroinvertebrates.

Once macroinvertebrates metamorphose into flying adults, dispersal no longer is constrained entirely by the river network. While dispersal potential is taxa specific and dependent on things like flying strength and energetic demands (Poff *et al.*, 2006), studies have shown movement of over 500 meters throughout the stream channel, as well as between different streams (Macneale *et al.*, 2005; Hershey *et al.*, 1993). This ability for the community to reshuffle is part of what makes dispersal in stream ecosystems unique compared to other systems. Brown & Swan (2010) extensively detailed how a dendritic river network acts to structure lotic macroinvertebrate metacommunities through continual unidirectional flow and the confluence of many habitats into one common outlet.

Ultimately, the main tool that has been used to observe and understand dispersal rates of drift is drift density, or, the volume normalized rate at which organisms drift. While interest in drift density in the context of macroinvertebrate dispersal has existed for years (Dimond, 1967; Allan & Russek, 1985), much of this interest has lain in ecosystem fluxes, such as mass of carbon transported, while the relationship between standing stock, or benthic production, and density has been relatively understudied (Waters, 1966; O'Hop & Wallace, 1983). Faulkner & Copp (2001) propose many shortcomings and assumptions made in studies using drift data, and highlight the need to better understand the underlying mechanisms at play in drift before making large-scale conclusions using drift data.

Aquatic macroinvertebrates exhibit highly intricate life histories and dispersal mechanisms that, despite extensive research, remain incompletely understood. While plenty of work has been done to synthesize existing information on macroinvertebrate drift (e.g., Naman *et al.*, 2016), much work still needs to be done to better understand the ultimate drivers of dispersal and how that may affect assembly mechanics in macroinvertebrate communities.

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## **Chapter 2:**

# **Spatiotemporal Variation in Dispersal as a Driver of Macroinvertebrate Metacommunity Dynamics**

## Introduction

Community ecology is a field of study historically concerned with understanding the spatial distribution, relative abundances, and interactions of co-occurring species (Leibold *et al.* 2004, Lawton 1999, Whittaker 1960). While much effort has been placed on untangling the processes that drive community dynamics at a local scale such as environmental conditions and species interactions, recent research, especially in freshwater ecology, has focused on how local communities exist within a metacommunity framework (Tonkin *et al.* 2018, Heino *et al.* 2015). Simply put, a metacommunity is a set of communities connected by dispersal of species and therefore is viewed as one network-wide community instead of somewhat arbitrarily defined individual communities (Mouquet & Loreau 2003, Logue *et al.* 2011). By shifting the focus of community ecology from discrete local factors such as available habitat and species competition, to regional factors such as movement of individuals across the landscape and heterogeneity of resources, metacommunity theory can describe and predict complex community composition dynamics with higher accuracy (Heino *et al.* 2015; Brown & Swan 2010; Pillai, Gonzalez, & Loreau, 2011). This balance between local and regional factors affecting communities is reminiscent of the debate between deterministic and stochastic processes in community assembly (Chase & Meyer 2011) and thus can be viewed as central to our collective understanding of community ecology.

When considering the mechanisms that control a metacommunity, lotic freshwater macroinvertebrates represent a well-studied model system. Macroinvertebrates encompass an impressive diversity of stream-dwelling insects and non-insect invertebrates and have been studied extensively due to their ecological importance and utility for biomonitoring (Bonada *et al.* 2006). Macroinvertebrates live in the water as larvae and emerge as flying adults that mate and oviposit eggs throughout the river network (Hauer & Resh, 2017). This balance between adherence to the river channel during larval stages and non-adherence during adult stages makes dispersal a crucial yet poorly understood aspect to freshwater macroinvertebrate metacommunity ecology.

Stream ecosystems are notoriously difficult to assign community boundaries, considering the continuous nature of a stream that doesn't provide hard boundaries where one community ends and another begins (Brown *et al.* 2011). In addition, the heavy reliance of this type of

community's structure on dispersal through drifting of larvae downstream with flow, crawling of larvae throughout the river corridor, and aerially dispersing ovipositing adults moving across the landscape between streams makes it an ideal type of community to consider in a metacommunity context (Naman *et al.* 2016), especially considering that the dispersal capacity (flying strength, drift propensity) of different taxa can be highly variable (Poff *et al.* 2006). Considering these communities in a metacommunity context is also imperative to better management, as the incorporation of dispersal into studies of community assemblage aides in biomonitoring (Heino, 2013).

Another key aspect to stream ecosystems is their high levels of disturbance and temporal variability. Stream ecosystems have long been appreciated for the pivotal role that disturbance and temporal change have in shaping their communities (Resh *et al.* 1988; Poff & Ward, 1989). Considering aquatic life is entirely dependent upon flowing water, and that those flows vary widely across seasons and almost constantly at a micro-scale, the disturbance caused by moving water is a foundational driver of any processes in freshwater. In addition to this inherent disturbance, while temporal variation in communities has been appreciated in a life history context with subjects such as relative abundances of certain species or emergence timing, more complex temporal variability such as variation in dispersal rates and variation across trophic levels is less well understood (Siqueira *et al.* 2024; Peniston *et al.* 2024).

While there is a rich set of literature aimed at understanding general temporal flux dynamics of macroinvertebrate drift (e.g., O'Hop & Wallace, 1983), few studies have looked at the assemblage of drifters in the context of the resident benthic community. Siler, Wallace, and Eggert (2001) did compare the benthic and drift assemblages but mainly in an aggregate sense aimed at understanding the density of drifting invertebrates compared to the resident benthic invertebrates. Considering many metacommunity studies use changes in benthic macroinvertebrate assemblages as evidence for differential metacommunity dynamics without directly measuring dispersal (e.g. Brown & Swan, 2010), comparison of drift with benthic macroinvertebrates could aide in our understanding of metacommunity dynamics.

To better understand the factors that influence community assembly, the field of community ecology has developed many ways to measure diversity in and between communities. Beta diversity is a measure of the diversity of species or ecological communities between different habitats or locations within a larger region, quantifying the extent of species

turnover or compositional differences among these distinct assemblages (Anderson *et al.* 2011). Beta diversity has increased in popularity in the last 30 years as a tool to understand the turnover in species diversity along spatial, temporal, or environmental gradients (Anderson *et al.* 2011, Whittaker 1960). At its core, beta diversity is a powerful concept, considering its utility in determining what makes species assemblages more or less similar to each other at different places and times (Vellend, 2010). Especially in the context of metacommunities, beta diversity can inform what broader dynamics exist on a network level scale compared to simple site-level alpha diversity (species richness) measurements.

Metacommunities, as well as communities, have two levels of variation: aggregate and compositional variation (Lamy *et al.* 2021; Micheli *et al.* 1999). Aggregate variation is variation of some totaled value for the community, such as total biomass, contrasting with compositional variation, which relates to the variation in relative abundances of species. Especially considering the importance of dispersal in metacommunity dynamics, studies attempting to document temporal variation in dispersal are a crucial next step to our understanding of community assembly dynamics in macroinvertebrate communities.

Metacommunity theory predicts varying dynamics based on dispersal rates and habitat heterogeneity (Leibold *et al.* 2004). According to Logue *et al.* (2011), metacommunities may align with different paradigms depending on spatial and dispersal dynamics. When dispersal capacity is low, a species sorting perspective may be expected in which differences between patches cause differences in the local demography of species and therefore patch quality and dispersal both affect local community composition. When dispersal is high, a mass effects perspective may be expected, in which high immigration and emigration between patches allows for poorly suited individuals to exist in a community, and therefore spatial dynamics outweigh the effects of patch quality.

These varying perspectives of metacommunities can even be directly applied to different parts of the same system. When considering metacommunity dynamics in river networks, headwaters exhibit low alpha diversity but high beta diversity; this pattern is indicative of dynamics driven by the interactions between species considering their isolation in the network (species sorting perspective) (Finn *et al.* 2011). Mainstem reaches exhibit high alpha diversity, but low beta diversity; this pattern is indicative of dynamics that are driven by high dispersal between sites due to the high connection between sites in the network (mass effects) (Tonkin *et*

*al.* 2018, Anderson *et al.* 2011). These differences in metacommunity dynamics depending on network position are summarized in the Network Position Hypothesis (NPH), which ultimately states that community assembly dynamics should change depending on network position due to differences in dispersal caused by connectivity to the rest of the system (Brown & Swan, 2010; Schmera *et al.* 2019). While intuitively these differences make sense, there is a lack of consensus among empirical evidence and across taxa, necessitating further empirical studies aimed at understanding differences in dispersal.

While the metacommunity dynamics of macroinvertebrates are relatively well studied, the methods that have been used to characterize these dynamics have been relatively limited, with most studies using benthic surveys at single points in time. In broader macroinvertebrate community ecology literature, many different types of sampling strategies have been used to characterize communities, with each method targeting different habitats/life stages depending on the purpose of the community survey (Baxter *et al.* 2017). Benthic surveys are most common for biotic characterization and are composed of samples that disturb the sediment in a stream to dislodge larval macroinvertebrates into a sampling net. These types of samples give a snapshot of the local community at a single place and time and are incredibly useful for assessing the impacts of local environmental factors. However, they lack the representation of dispersal dynamics that is crucial for the characterization of metacommunities (Cañedo-Argüelles *et al.* 2015).

Taxonomic beta diversity has been used extensively to study variation in species composition, but variation in composition through time has received considerably less attention (Lamy *et al.* 2021, Legendre & Gauthier 2014). Benthic macroinvertebrate sampling strategies typically revolve around spring/summer samples to ensure ideal flows and later stage instar larvae (Hauer & Lamberti, 2011), but this targeted sampling obviously reduces the ability to quantify temporal beta diversity, i.e., within-site community composition variation through time. Poor documentation of these dynamics in other metacommunity studies is prevalent, and ripe for exploration considering how dynamic the life history of larval macroinvertebrates is throughout their various instars (Merritt & Cummins 1996, Brown 2003). For example, Göthe *et al.* (2013) use an impressively large spatial array of samples to characterize a boreal macroinvertebrate metacommunity, but all of the sampling occurred within a few weeks in the spring. It is then plausible to suggest that characterizing the metacommunity dynamics at one point in the year

may drastically ignore dynamics that are playing out the rest of the year. Macroinvertebrates are well studied in part due to their complex seasonal life histories, so improving understanding of temporal metacommunity dynamics can aid in better understanding how communities are structured throughout the year.

### *Hypotheses*

Based on our current understanding of spatiotemporal macroinvertebrate metacommunities in river networks, we pose this broad question: How does dispersal, a key mechanism that affects macroinvertebrate metacommunity dynamics in river networks, change across space and time? To answer this question, we posed the following hypotheses:

For compositional patterns, we hypothesized that dispersal would vary widely throughout seasons due to invertebrate life history and changes in stream discharge, but the temporal variation would be larger in headwater sites compared to mainstem sites due to mass effects causing more uniform dispersal across seasons in mainstem sites (Brown & Swan, 2010). We further hypothesized that drift assemblages would most closely resemble resident benthic communities in winter/spring, and in headwater sites, due to increased discharge mobilizing macroinvertebrates into the drift, and headwaters not exhibiting mass effects. Lastly, we hypothesized that drift densities (organisms/L of water) would increase predictably with benthic abundance across seasons and flow patterns if passive drift dominated, with a deviation from this null hypothesis if active drift dominated.

For aggregate patterns, we hypothesized that total drift abundance would be lowest in the fall and highest in late spring, in line with macroinvertebrate life-history dynamics that render larvae larger and more active in late spring. We also expected drift density to follow a similar seasonal trend, with minimal spatial variation due to volume normalization. We also hypothesized that, while environmental variation should drive these seasonal patterns, the variability across seasons should be similar across sites, thus allowing us to focus on network position and season rather than environmental variables governing community assembly.

Overall, these hypotheses are collectively built on the idea that network position should govern community assembly through differential dispersal dynamics.

## Methods

To address our questions on spatiotemporal metacommunity dynamics, we designed a network-wide stream survey encompassing spatial (sites throughout the network) and temporal (samples taken every month) macroinvertebrate sampling. Sites were chosen to represent either headwaters (smaller size and less connected), or mainstems (larger size and more connected to the rest of the network). Benthic and drift samples were taken together to aid in our understanding of how dispersal affects the resident community assemblage.

### *Study System*

We selected Little Stony Creek as our model watershed for this survey. Little Stony Creek is a river network draining 24 square-miles in Giles County, Virginia, USA. At its confluence, the mainstem is a 3<sup>rd</sup> order stream that discharges into the New River—a tenth-order river—in Pembroke, Virginia (Figure 1). The Little Stony Creek watershed is situated predominantly within the Jefferson National Forest, a mountainous, deciduous temperate region characterized by high-gradient streams and marked seasonality (summer: 10–30°C; winter: –10–10°C). Land cover is overwhelmingly forested (>95%), with significant anthropogenic alteration confined to an area within one river mile of the confluence in Pembroke (USGS, 2019). Given the minimal human impact on land cover and channel morphology throughout the remainder of the watershed, anthropogenic influences were considered negligible for this study.

The flow regime in the Little Stony Creek system is typified by low flows in late summer/fall, high flows during spring/early summer, and base flow otherwise. Annual precipitation averages approximately 1,000 mm, with relatively even seasonal distribution; variations in surface flow are primarily driven by seasonal changes in evapotranspiration (Dunn & Mackay, 1995). Little Stony Creek is located within Virginia's Ridge and Valley province, where streambeds composed of various sandstones and quartzites underlie steep ridgelines. Karst processes near the confluence with the New River facilitate high surface–groundwater interchange, which can result in temporary hydrological disconnection to the New River during low-flow periods (late summer and fall). Additionally, Cascade Falls—a 66-foot waterfall situated in the middle of the river network (Figure 2)—constitutes a significant barrier to aquatic

connectivity, effectively isolating the two furthest downstream sites from non-aerial upstream dispersal to the rest of the watershed.

### ***Field Sampling***

To elucidate spatiotemporal shifts in community composition, eight sites spanning the river continuum from 1<sup>st</sup> through 3<sup>rd</sup> order were selected. Four sites (PDRA, LAUC, LSCH, and HIKE) were classified as headwater streams—first- or small second-order systems draining < 3 square miles—while the remaining four sites (ARCH, CASC, 714U, and 714D) were designated as mainstem streams—third-order systems draining >10 square miles (Figure 1 & Table 1). At each site, a 30-meter reach was delineated to standardize sampling between sites. These reaches were sampled monthly over a one-year period (September 2023 to August 2024, excluding January 2024 due to adverse conditions)

During each sampling event, we collected 2 different types of biological samples. Benthic macroinvertebrates were collected using a standard 30 cm x 30 cm (0.09 m<sup>2</sup>) Surber sampler (Hauer and Resh, 2017) within riffle habitats. Riffles are standard stream habitat to focus on for benthic macroinvertebrate sampling due to their wide range of microhabitats coupled with high dissolved oxygen leading to the highest diversity of organisms (Hauer and Resh, 2017). Each benthic sample consisted of 5 replicates, haphazardly taken throughout the reach to avoid sampling bias. Drifting (i.e., actively dispersing in the water column) benthic macroinvertebrates were collected on the same day upstream of the benthic samples by placing a 30 cm x 45 cm drift net placed perpendicular to flow at the top of the reach for roughly 24 hours to encapsulate the full diel cycle of dispersal (Hauer and Resh, 2017).

For the drift nets, average depth and flow were recorded in front of the net at the beginning and end of the 24-hour period to calculate total volume of water (in liters) passing through the net for standardization between samples. Considering the lack of organic debris, clogging was not seen as a significant issue with drift nets, and therefore nets could be set out for 24 hours without clearing (Faulkner & Cobb, 2001). Overall, 160 benthic replicates (8 sites x 5 replicates x 4 months analyzed for this study) and 96 drift samples (8 sites x 11 months) were collected throughout the duration of the study. All invertebrate samples were preserved in 70% ethanol and brought back to the lab for processing and identification down to the lowest possible

taxonomic level (genus for most organisms). Midges of the family *Chironomidae* were identified to tribe (*Tanypodinae* or non-*Tanypodinae*).

In addition to these biological samples, various environmental covariates were collected at the reach or sub-reach scale on a monthly basis as well. Water physicochemical variables (pH, temperature, conductivity), canopy cover (%), substrate characterization, discharge (L/s), and nutrients (total phosphate and nitrate) were the primary environmental variables collected. We also measured wetted width and thalweg depth at the top, middle, and bottom of each reach to further characterize variation in flow. Channel dimension characterizations such as bankfull widths occurred once over the course of the survey (not monthly), as these variables are unlikely to vary on a month-to-month basis.

### ***Statistical Analyses***

A suite of statistical analyses was conducted to explore spatiotemporal patterns in community composition, relationships between benthic and drift assemblages, and the influence of environmental variables on metacommunity dynamics. All analyses were performed using R Statistical Software (v4.2.2; R Core Team 2024).

To examine the responses of aggregated community variables, we explored drift abundances/densities as response variables to non-biological drivers such as drift volume using simple linear regression and boxplots to assess our hypothesis on drift variation in the aggregate. These analyses, in addition to the benthic-drift correlations, were used to aid in post-hoc understanding of variation within the drift samples. In addition to these compositional and aggregate community analyses, site level variation in environmental variables was investigated in two ways. First, coefficient of variation (CV) for each variable at each site was examined to see if there was a similar trend in how environmental variables varied throughout time at each site. CV is defined as the ratio of the standard deviation to the mean and can be used to standardize the variation of a variable between treatments (Abdi, 2010). We then compared CVs to examine if there was wider variation of non-biological drivers at individual sites or across a gradient from headwaters to mainstem. After this CV comparison, we performed a Principal Component Analysis (PCA) using environmental variables (pH, temperature, discharge, wetted width, SRP, TN, and canopy cover) to reduce the dimensionality of environmental variables into

two axes for the purpose of explaining variation across samples considering many environmental variables were highly correlated with each other.

To investigate the effect of network position and season on community composition, community data was first tabulated in a community matrix with each cell representing the total abundance of a specific taxa in that sample. We then conducted dimensionality reduction on this matrix using non-metric multidimensional scaling (NMDS) ordinations with the *metaMDS* function in the *vegan* package in R (Oksanen et al. 2022). These ordinations were mainly used for data visualization. All ordinations and subsequent analyses were conducted twice, first using an abundance-based distance metric (Bray-Curtis) and then using a presence/absence-based distance metric (Jaccard) to investigate their relative contributions on observed patterns. Given that drift data often contain high abundances of a limited number of taxa, reliance solely on Bray-Curtis may obscure subtle compositional patterns that an incidence-based metric such as the Jaccard index can reveal, and therefore both metrics were used.

Compositional variability through space and time with drift assemblages was measured in two different ways. The first method applied beta dispersion with the *betadisper()* function in the *vegan* package in R, where an average centroid of all of the samples in a group in ordination space is calculated and then each sample's deviation from that centroid is calculated (Anderson *et al.*, 2006). The output of this function was then used to assess site level temporal variability. A Levene's test was used to detect differences in univariate dispersion between sites, and a one-way ANOVA was used to test for differences in means of the beta dispersion function (Box, 1954).

The second method used to understand differences in site level temporal variability was second-stage ordination (Clarke et al. 2006). This method calculates Spearman correlations between observations within each site to ultimately ordinate the temporal variability of each site through time. The ordination that is produced has one point representing each site, and their proximity to each other in ordination space indicates how similar the temporal dynamics at each site are to each other. A permutational multivariate analysis of variance (PERMANOVA) using the *adonis2()* function was then used to test for differences between groups.

To test the relatedness of concurrently sampled benthic and drift samples and therefore the predictive power of the benthic community on the drift assemblage, we calculated pairwise Pearson correlation of samples. This correlation compared corresponding rows of benthic and

drift matrices, where each column represented a unique taxon and each cell represented the total number of that taxon found in the sample. Regular multivariate distance metrics (Jaccard and Bray-Curtis) were also used to explore the similarity between drift and benthic samples to see if different patterns emerged with different methods. Ultimately a vector was produced containing a quantitative similarity value for each sampling event which was then analyzed to find potential patterns across sites/time/discharge. Similar to the temporal variability in composition analyses, this analysis was run on both relative abundance matrices and presence/absence matrices. Linear mixed-effects models (LMMs) were fit to the benthic and drift correlations using the *lme4()* package in R to analyze differences across sample periods (Bates *et al.* 2015). The predictive power of the benthic community on the drift assemblage was then analyzed through simple linear regression of concurrently sampled benthic abundances and drift densities to establish a community size pattern.

## Results

### *Environmental Variation*

Environmental variables exhibited highly consistent seasonal variation across sites, indicating pronounced inter-seasonal fluctuations with minimal site-to-site differences. Site level CV's for temperature, wetted width, and pH were similar for each variable between sites (Figure 3). While wetted width did vary differently between sites, the variation was consistent between sample groups (Headwater vs. Mainstem), which was part of the study design. Total Nitrate (TN) and Soluble Reactive Phosphorus (SRP) displayed minimal seasonal variability and remained low overall, consistent with a forested watershed experiencing limited anthropogenic disturbance (Figure 4). Notably, the PDRA site deviated from this trend, with TN and SRP concentrations rising to approximately 800  $\mu\text{g/L}$  and 25  $\mu\text{g/L}$ , respectively.

The PCA of environmental variables explained 63% of the variance across two axes, with the first principal component accounting for 42% and the second for 21% (Figure 5). High loadings of wetted width and discharge on PC1 indicate that these variables primarily drive variation along that axis, whereas nutrient metrics predominantly influence PC2. This PCA is color coded by order and site, thereby elucidating both site-level environmental gradients and the clustering of samples by order.

### *Descriptive Statistics and Aggregate Patterns*

A total of 9,611 individual organisms were collected from 88 drift samples, with a median of 64 individuals per sample (Inner-Quartile Range (IQR) 35–146). For the four months of benthic data (September, December, March, and June), 14,598 individuals were recorded from 160 replicates across 32 samples, with a median of 70 individuals per replicate (IQR = 42–123). Overall, 107 taxa were identified in the drift samples and 117 taxa in the benthic samples, with 96 taxa being common between the two sampling methods, with 11 taxa being unique to the drift and 21 being unique to the benthic. Despite the slight differences in taxa (i.e., drift not being a perfect subset of benthic), the unique taxa represented a very small proportion of the drift. Alpha diversity varied considerably across sites and sampling periods, with a median of 18 taxa per

drift sample and 35 per benthic sample. Some taxa were much more prolific in the drift samples than the benthic samples due to life history related to drift propensity. For example, Dipterans of the family *Simuliidae* were over 10% of the total abundance of drift samples, indicating that their dispersal is incredibly high compared to other more sessile taxa that aren't found in the drift as much, such as Pteronarcyid Stoneflies.

Trends in total abundance and drift density across sites over time revealed distinct patterns (Figure 6). Total abundance varied significantly among sampling periods (ANOVA,  $p = 0.001$ ), and post-hoc Tukey HSD tests indicated significant differences in mean abundance, with notably higher abundances in June and markedly lower abundances in October, March, July, and August. In contrast, drift density did not exhibit statistically significant temporal trends (ANOVA,  $p = 0.223$ ), even after the removal of outliers. Despite this lack of a trend with drift densities, analyzing densities between headwaters and mainstems reveals a distinct trend (Figure 7). Across almost all sample periods, headwaters on average have higher densities, and greater variation in those densities when compared to mainstems.

The relationship between drift density and the log of drift volume showed a linear pattern, and provided further evidence for differences in headwaters and mainstems drift densities (Figure 8). While these two variables are not statistically independent (density is organisms divided by drift volume), the nuances in how density responds to changes in volume, especially in the context of network position, provides interesting ecological insight. There is a clear linear trend with the log of drift volume in which low volumes yield notably higher densities, and high volumes yield notably lower densities, all else being equal.

### ***Spatiotemporal Dynamics of Drift Assemblages***

Using a Bray–Curtis dissimilarity metric, the NMDS of drift assemblages converged on a three-dimensional solution ( $k = 3$ ) with a Stress of 0.19 (Figure 9). To depict compositional changes over time, the ordination was faceted by site, with temporal trajectories of community composition delineated by black lines (Figure 10). Beta dispersion analysis—assessing temporal variability among sites—showed little to no evidence of differences (Levene's test,  $p = 0.85$ ; ANOVA,  $p = 0.68$ ), indicating that the magnitude of seasonal variation was consistent across sites (Figure 11). This finding was corroborated by the second-stage ordination, which converged

on a two-dimensional solution ( $k = 2$ ) with a Stress of 0.10 (Figure 12). Considering the scant evidence of clustering, it could be inferred that temporal dynamics across sites were similar.

When these analyses were repeated using a Jaccard-based (incidence-based) distance metric to observe trends that may have been masked with the Bray-Curtis based analysis, the NMDS of drift assemblages yielded a three-dimensional solution ( $k = 3$ ) with a stress of 0.21 (Figure 13). While a stress of 0.21 is considered high for interpreting fine-scale differences, interpretation of results from this ordination were only considered at a coarse-scale, and therefore the differences were still deemed relevant (Dexter, Rollwagen-Bollens, & Bollens, 2018). From this subsequent faceted ordination (Figure 14) beta dispersion again indicated no significant differences among sites (ANOVA,  $p = 0.54$ ), although the Levene's test ( $p = 0.34$ ) suggested marginal evidence for site-level variability (Figure 15). Notably, the second-stage ordination ( $k = 2$ , stress value = 0.10) in this instance revealed distinct clustering by order (Headwater (HW) vs. Mainstem (MS)) (Figure 16). A subsequent PERMANOVA on group (order: HW versus MS) yielded an  $R^2$  of 0.64 ( $p = 0.346$ ), indicative of strong clustering of temporal dynamics by group with limited data ( $n = 8$  sites).

### ***Benthic-Drift Correlations***

The relationship between concurrently taken benthic and drift samples was analyzed several different ways. In aggregate analyses of benthic vs drift, no strong evidence of a relationship was observed. Drift density vs concurrently sampled benthic abundance revealed no discernible relationship (Figure 17), despite the hypothesis that a linear relationship would exist. This relationship reveals that, at least at the aggregate level, benthic and drift assemblages are only weakly coupled.

Multivariate correlations on abundance-based data normalized by total organism count reveal a clear compositional trend. Specifically, correlations during the intermediate sampling periods (December and March) were significantly higher than those in September or June (Figure 18). This pattern of higher correlation in December and March was confirmed via an LMM incorporating a fixed-effect quadratic term for sample period ( $p < 0.05$ ). Order was also included in the model to discern if there was a spatial trend in correlations, but it was not significant ( $p = 0.6$ ). Regular multivariate distance metrics (Jaccard and Bray-Curtis) were also

used to explore the similarity between drift and benthic samples, but the resulting relationships produced similar results and therefore were not included.

When using an incidence-based matrix for correlations, a clear compositional trend was also found, albeit with subtle differences. Sample period remained significantly associated with correlation values, as evidenced by an LMM ( $p < 0.05$ ), though the modeled relationship was linear rather than quadratic (Figure 19). Additionally, the inclusion of covariates such as order were included in the model, but did not show evidence of an effect. Taken in tandem, abundance-based and incidence-based correlation analyses indicate a robust temporal trend in the relationship between the benthic community and drift assemblage.

## Discussion

Overall, our results lead us to partially reject our initial hypothesis that network position should govern community assembly through differential dispersal dynamics. While we did see some evidence of spatial patterns in the incidence-based drift assemblage beta dispersion and drift densities analyses, our results collectively provided strong evidence towards seasonal temporal variability being the primary driver of differences in dispersal throughout the study.

### *Environmental Variation*

Given our results on environmental variation and considering the relative predictability of flow patterns in this system and this physiographic region at large, biotic instead of abiotic forces should be expected to structure communities heavily (Poff & Ward, 1989). In other words, sudden periods of extreme environmental fluctuation such as a drying event or a catastrophic flood shouldn't be the main drivers of community structure, because environmental variation in this system is consistent compared to, say, a flashy desert stream. Stream ecosystems by their very nature are highly dynamic (Resh *et al.* 1988), but environmental variation beyond a critical threshold not being considered in a study attempting to explain biological variation would very likely be missing an important driver. Therefore, these results support our use of subsequent biological analyses because it lends evidence towards the communities being structured by factors like network position and life history instead of simply being in a certain area because they were stranded by rapidly changing environmental conditions restricting their movement to better habitat. Ultimately, the lack of clustering of sites in the PCA by variables other than those related to network position allows us to focus solely on network position and sample period to describe variation in communities.

Most of the environmental variation throughout our study falls within the predictable variation as outlined above, but it's worth briefly mentioning the nutrient dynamics of PDRA. Nutrient levels at PDRA far exceeded other sites but remained below most biologically relevant thresholds (Miltner & Rankin, 1998). In the absence of a corresponding biological response, these differences are assumed to have had negligible impact on our study. Based on consultation with various Virginia Tech faculty familiar with the broader Mountain Lake Wilderness

ecosystem, we decided the most likely explanation for this phenomenon is the outwash of algae from upstream Mountain Lake, a small natural lake ~1 km upstream of the site (Figure 1). The lake has minimal anthropogenic inputs, but it is hypothesized that simply having a large open canopy was enough to produce a large amount of autochthonous production that was subsequently washed into the stream, resulting in an elevated nutrient signature. Again, it was assumed in this study that this input had minimal effects on the biology of the site, but future studies in this system could benefit from examining this dynamic in comparison to the rest of the system more closely.

### *Descriptive Statistics and Aggregate Patterns*

The observed patterns in drift density have important implications for both abundance-based compositional analyses and our understanding of macroinvertebrate drift behavior. It remains unclear whether the variation in drift density relative to drift volume reflects a nonlinear behavioral response to increasing flows or is simply an artifact of an exponentially increasing denominator. Regardless, these uncertainties warrant further investigation into drift dynamics. Existing studies on drift—particularly the distinctions between active versus passive drift and the role of critical shear stress thresholds—suggest that our aggregate data may be shaped by threshold-dependent environmental conditions (Rosenfeld, 2017). For example, although headwater sites typically experience flows that seldom reach the critical shear stress required to mobilize macroinvertebrates (Lorenz & Wolter, 2019), our data indicate that headwaters nevertheless exhibit higher per-volume dispersal compared to mainstem sites. Together, these findings highlight the need for caution when employing abundance-based metrics for drift compositional analyses and underscore the importance of further consideration and general research into the mechanistic drivers of drift behavior across river networks. Drift behavior is taxa and environment specific, and therefore can be hyper variant on a micro-scale (Kohler, 1983; Kohler, 1985; Kohler & McPeck, 1989). Consideration of what ultimately drives this variation and better ability to model the dynamics is needed for a greater understanding of how this could affect metacommunities.

The first sample of PDRA in September was a remarkable outlier of drift density (and was subsequently removed from drift density analyses for ease of interpretation), at ~3

organisms/L due to a relatively high total abundance and virtually no flow at the site. However, it elucidates the potential mechanisms underlying the elevated drift densities observed in headwaters. When simply looking at total abundances, our results are in line with our hypothesis that the total number of organisms in the drift should be different across seasons due to life histories dictating that they are more or less mobile/active depending on the time of year. But the total abundances seem to shift independent of total drift volume, causing these large spikes in headwater sites that have relatively low flow but a similar number of total organisms. While this result was serendipitous, it may provide intriguing insight moving forward regarding dispersal dynamics throughout river networks, as well as provide another piece of evidence for the disproportionately high ecological impacts of headwaters (Wipfli *et al.* 2007).

A null hypothesis where macroinvertebrates drift in a linear response to flow would expect density to stay more or less constant with increasing drift volumes, resulting in a flat line. However, there is a clear linear trend with the log of drift volume in which low volumes yield notably higher densities, and high volumes yield notably lower densities, all else being equal (Figure 8). Given this and the differences in densities between HW and MS (Figure 7), the data suggests that the density of organisms in headwater sites is markedly different than in mainstem sites, and that the overall trends in how they change are disparate.

### *Spatiotemporal Dynamics of Drift Assemblages*

Macroinvertebrate dispersal in river networks is a fundamentally stochastic and difficult to model process. Werner Heisenberg once remarked on his deathbed, “When I meet God, I am going to ask him two questions: Why relativity? And why turbulence? I really believe he will have an answer for the first,” in reference to the infamous unpredictability of turbulent flows. In stream ecosystems, macroinvertebrate drift is fundamentally shaped by turbulence, which introduces high variability and stochasticity that complicates the quantification of compositional changes (Allan & Russek, 1985; Hart, Clark, & Jasentuliyana, 1996). Therefore, the emergence of statistically significant trends—even if modest—strongly indicates that underlying biological processes are influencing these patterns.

With this stochasticity in mind, the lack of any discernible differences in temporal variability when using a Bray-Curtis based distance metric makes sense when considering the

nature of the dataset being analyzed. Out of the 9611 total organisms in all of the drift samples, 1391 (or 14.5%) were one taxa: Black flies of the genus *Simulium* (Diptera: *Simuliidae*). When analyzing these communities with abundance-based data, the ubiquity of this taxa has a strong effect on results that has the potential to mask the ecological significance of other taxa coming or going, and provides solid justification for using both abundance and incidence-based analyses to better understand shifts in these communities. The “negative” result of no difference in means and variation of beta dispersion between sites is a notable result in its rejection of our hypothesis. In effect, this lack of difference is saying that relative abundances of actively dispersing organisms shift in very similar ways throughout seasons, regardless of position in the network. While no difference in temporal dynamics isn’t necessarily strong counter-evidence to our current understanding of metacommunity dynamics in river networks, it does highlight the importance of life history and season in analyzing these patterns.

While our analyses using an incidence-based metric revealed little evidence of differences in means of variances of beta dispersion ( $p > 0.05$ ), it did provide more evidence compared to the abundance-based metric. Furthermore, the grouping of temporal dynamics by stream order in the second-stage ordination ( $R^2 = 0.64$ ) supports our hypothesis that network position governs community assembly through dispersal dynamics. While the p-value for this PERMANOVA suggested insufficient evidence for differences in groups ( $p = 0.346$ ), we attribute it to limited data and therefore interpret the high  $R^2$  with caution. Again, while there is limited evidence to support these results, a clear pattern does emerge in the data, especially when compared to the abundance-based results. The strong pattern by order is particularly interesting, as it seems to suggest that temporal dynamics are more consistent across headwaters than mainstems, despite environmental variation being relatively similar across all sites. Whether this pattern between network position is caused by connectivity (species sorting metacommunity perspective), differing species pools, or some other more nuanced factor, it suggests that individual communities exhibit different temporal patterns depending on position in the network. This provides us with compelling evidence towards the dynamics that ultimately govern metacommunities in river networks beyond just network position.

### ***Benthic-Drift Correlations***

Relating drift assemblages to resident benthic communities provides valuable context, as drift is predicted to be largely a nested subset of the benthos. Linear mixed-effects models revealed that sample period significantly influences correlation, indicating pronounced seasonal variation in the relationship between these assemblages. In contrast, aggregate analyses of total benthic abundance and drift density showed no significant correlation, suggesting that compositional patterns evident at finer scales are obscured when data are combined. This discrepancy likely reflects a predominance of active drift behavior—organisms may actively enter the drift regardless of benthic composition—implying that benthic–drift correlation could serve as an index of active versus passive drift.

Inclusion of benthic data from all 11 months, rather than a four-month subset, would likely enhance the robustness of these findings. The limited evidence for differences in effects beyond sample period may result from restricted replication (e.g., one drift net per site), despite intensive temporal sampling. Faulkner & Cobb (2001) emphasize the need for replication in drift nets, and provides solid justification for altering sampling protocol in this way. Future studies incorporating increased spatial and observational replication are warranted to further elucidate environmental drivers. While the overall design of this study seems incredibly data rich, much of that richness comes from the intensive temporal sampling, instead of observation specific replication (i.e., 2 drift nets per site instead of 1) or sites. While our multivariate analyses are robust to smaller samples sizes (Buckley, Day, Case, & Lear, 2021), they are inherently data hungry, and therefore an increase in sample size could aid in future interpretations of dispersal dynamics.

There are many different environmental and biotic drivers, beyond discharge and season that could cause invertebrate drift. For example, O'Hop & Wallace (1983) showed that leaf-litter detritus was the primary driver of nymphal *Peltoperla* (Plecoptera: Peltoperlidae) drift, regardless of discharge or season. As for biotic drivers, various studies have shown active drift to be caused by presence of predators and competition for food resources (Peckarsky, 1980; Hernandez & Peckarsky, 2014). Considering this study didn't separate underlying hydraulic gradients and leaf-litter transport, an important next step would be to incorporate taxa specific

dispersal propensities, the differing environmental variables that may initiate their drift, and how this may shift throughout a river network.

## **Conclusions**

Our findings enhance understanding of dispersal's role in shaping macroinvertebrate metacommunities in a few general ways:

Aggregate patterns in macroinvertebrate dispersal seem to indicate different patterns in headwaters vs mainstems. Whether the differences in drift density and total abundances is related to the general noisiness of drift data, or representative of some broader taxon specific trends, further investigation is warranted into the spatial and temporal trends of aggregate dispersal variability.

The general absence of spatial patterns in compositional analyses—aside from the second-stage ordination of presence/absence in drift—highlights the dominance of temporal variation in structuring these communities. While strong spatial differences in variability were not observed, the detected trends suggest a promising avenue for further investigation, particularly at the taxon-specific level.

Lastly, the correlation between benthic and drift assemblages varying between seasons highlights the variability of drift assemblages and its relationship to the resident benthic community. The two different metrics we used showed varying evidence towards changes across seasons, but more data is needed across varying flow levels and seasons to better understand what might change this relationship.

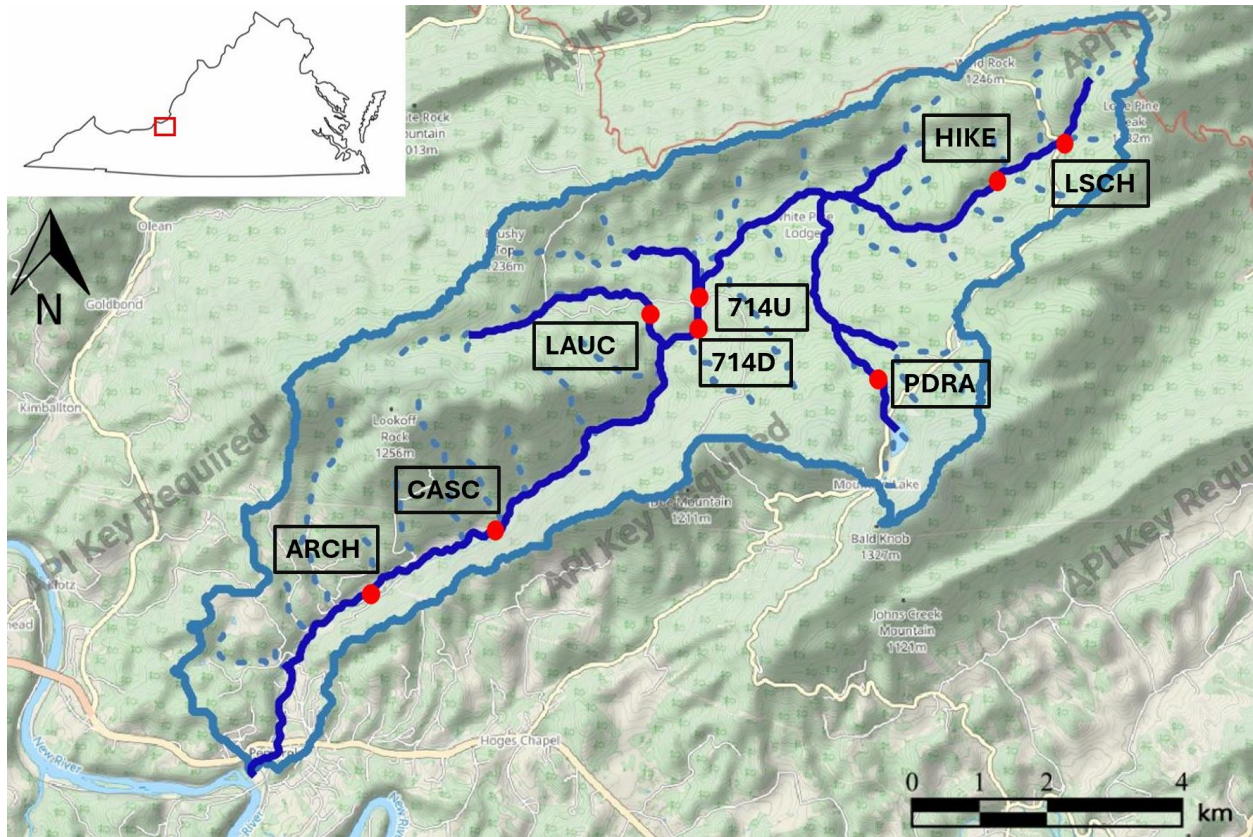
Overall, this study provided strong evidence of temporal variation in flows and life history altering dispersal in macroinvertebrate metacommunities and highlights the need to better understand the ways in which dispersal alters benthic macroinvertebrate communities. Future studies that integrate temporal variation into metacommunity frameworks may better resolve spatial patterns by accounting for these dynamics.

## Table

Site	Drainage Area (mi <sup>2</sup> )	Coordinates	Elevation (m)	Order
Archer Trail (ARCH)	20.1	37.340921, -80.622311	580	3
Cascades (CASC)	18.3	37.350730, -80.600282	660	3
714 Downstream (714D)	11.8	37.378230, -80.568008	939	3
714 Upstream (714U)	11.7	37.380933, -80.568133	946	3
Laurel Creek (LAUC)	1.86	37.380119, -80.576436	960	1
Pond Drain (PDRA)	1.08	37.372912, -80.541925	1150	1
Hike-in Little Stony (HIKE)	2.32	37.396580, -80.517000	1040	2
Little Stony Headwater (LSCH)	0.4	37.405210, -80.509200	1130	1

(Table 1) Metadata for each site within the study

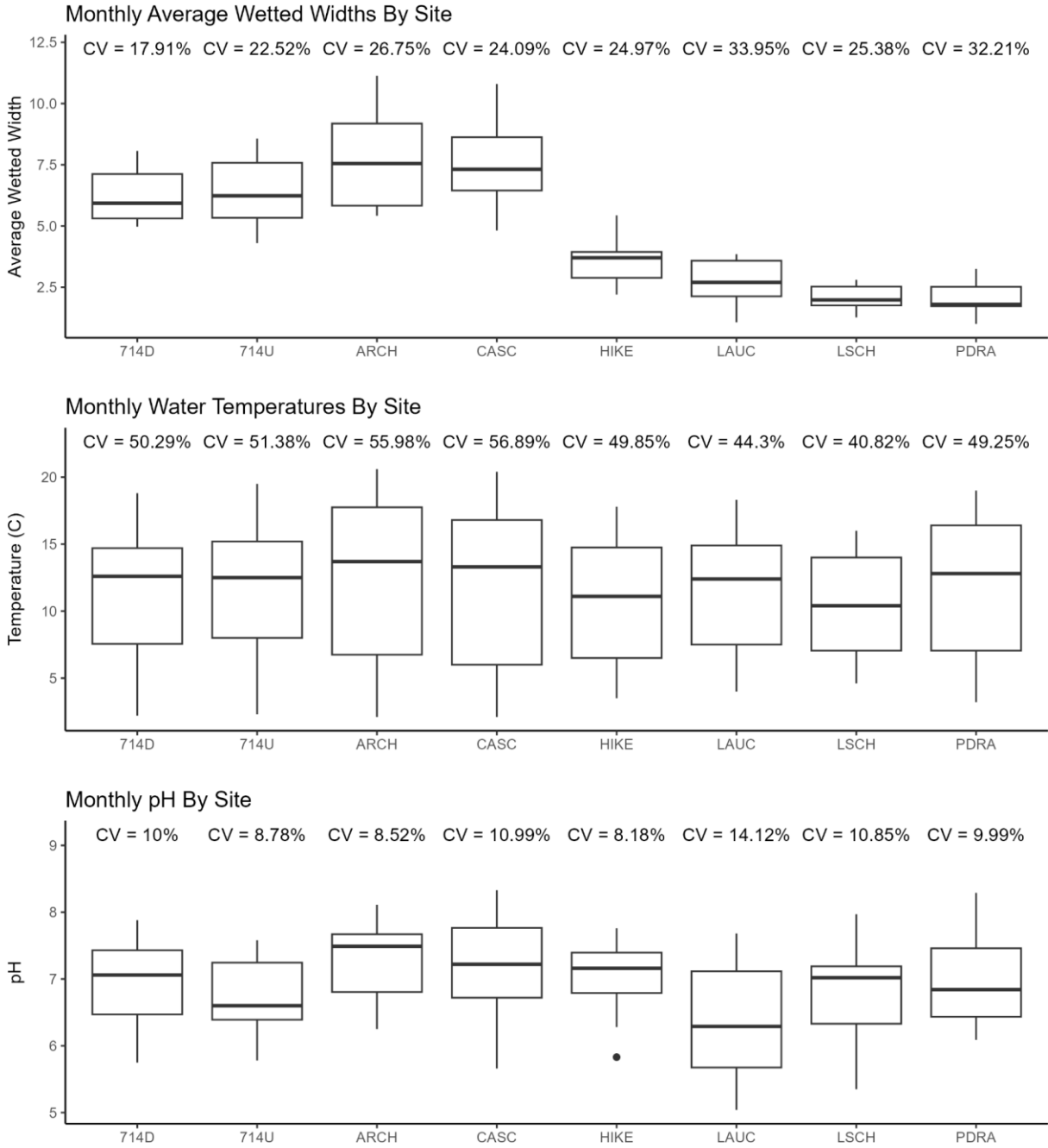
## Figures



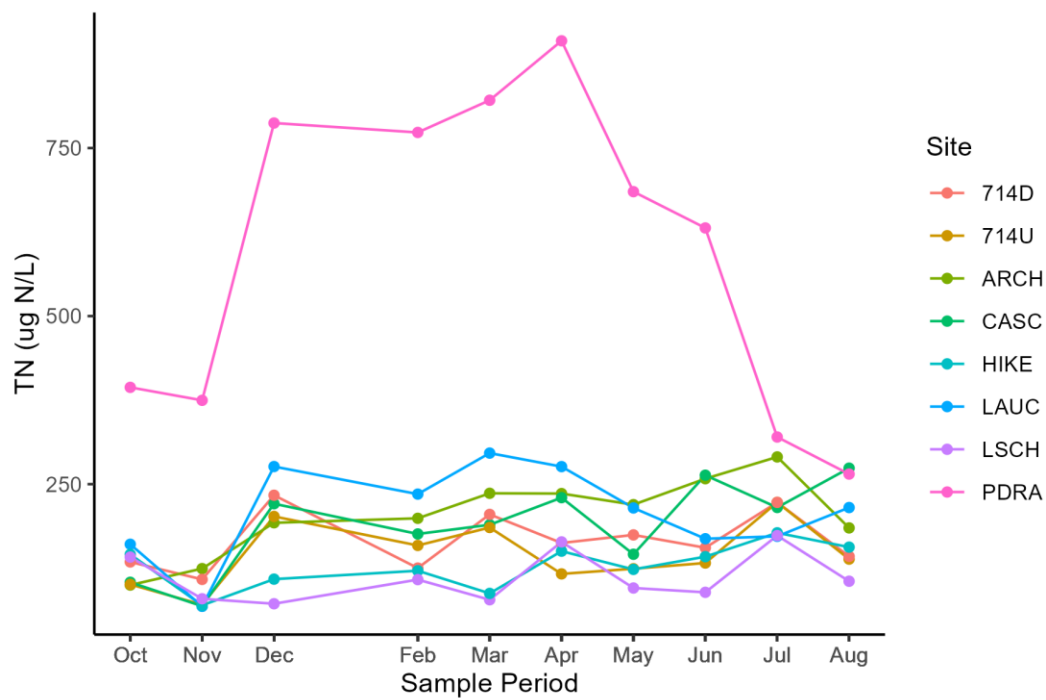
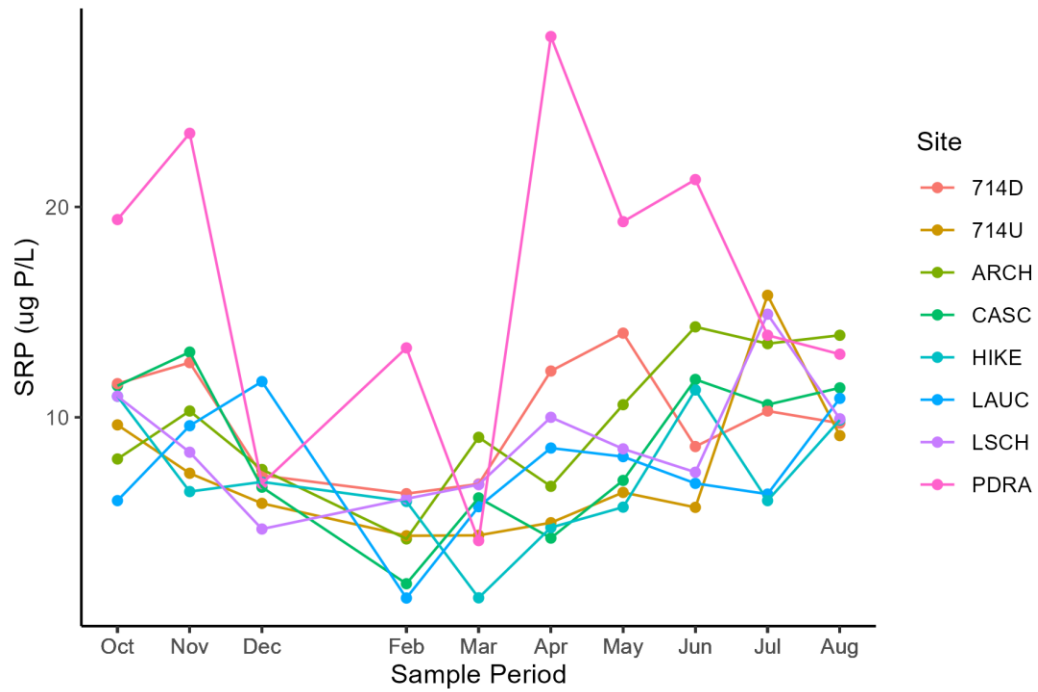
**(Figure 1)** Map of the Little Stony Creek Watershed. Solid light blue lines indicate the watershed boundary, dotted light blue lines indicate ephemeral streams, and dark solid lines indicate perennial streams. Red dots indicate sampling locations.



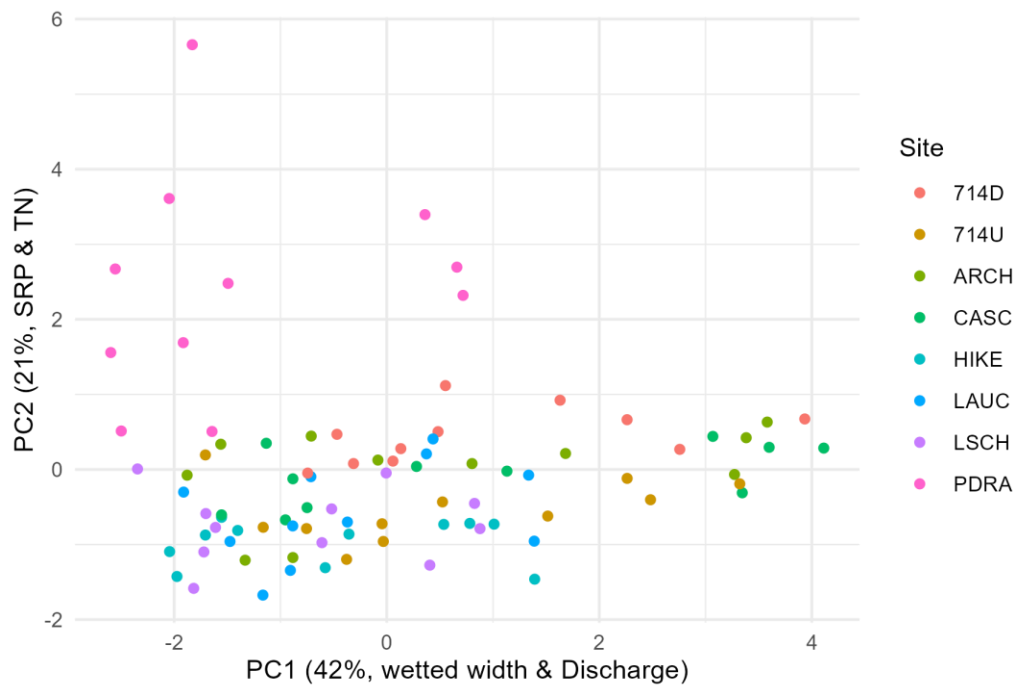
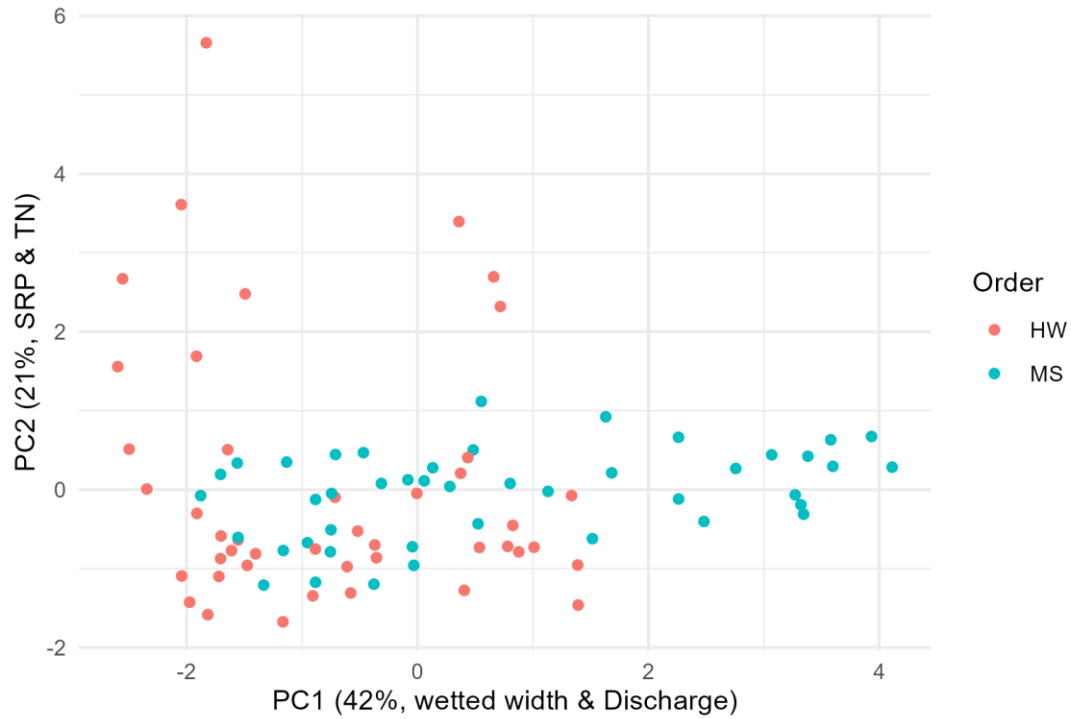
**(Figure 2)** Cascade Falls, a 66-foot waterfall midway through the watershed along the mainstem Little Stoney Creek. It is roughly 2 km upstream of CASC.



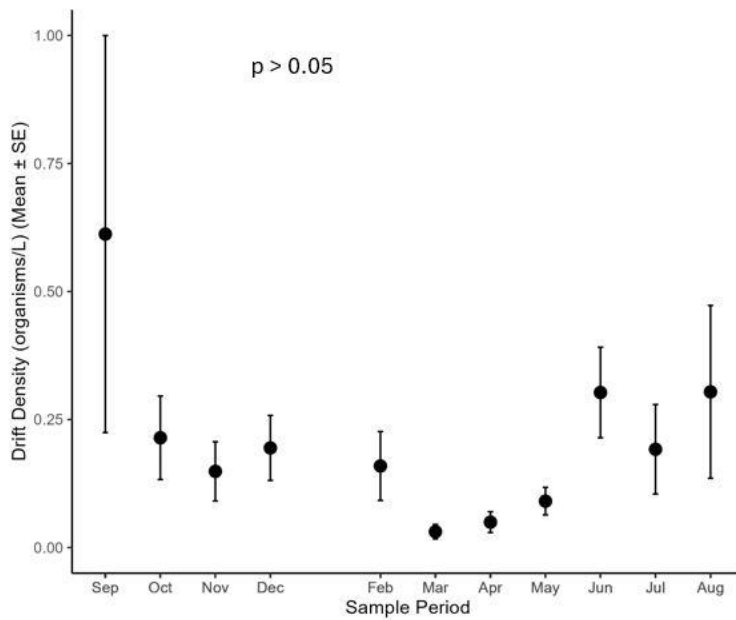
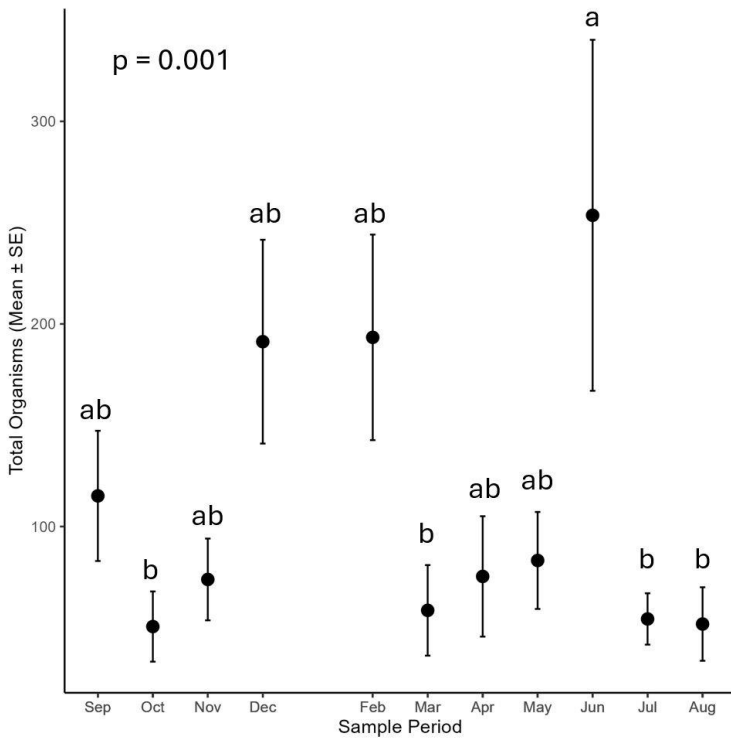
**(Figure 3)** Distribution of selected environmental parameters across sites. CV's are shown at the top of each boxplot to show the similarity of distributions across sites despite variations in the means.



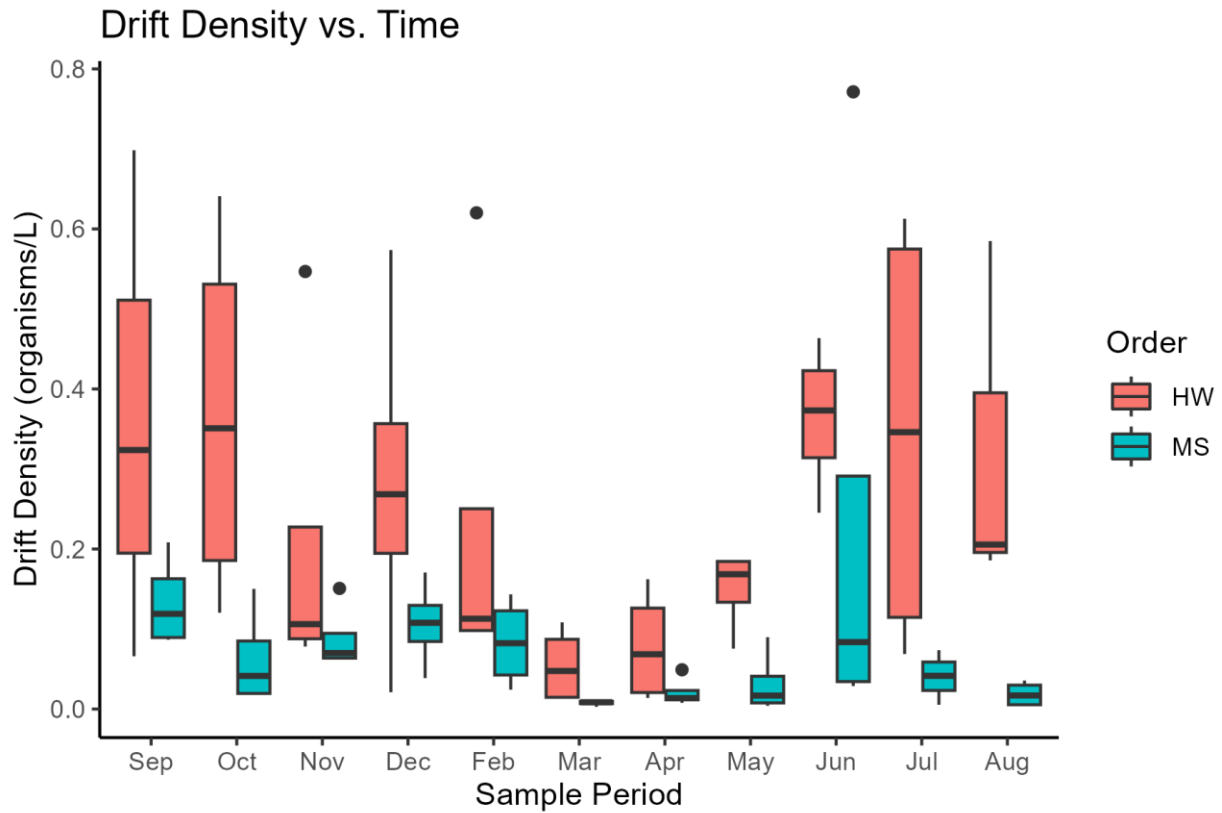
**(Figure 4)** Total Nitrogen (TN) and Soluble Reactive Phosphorous (SRP) for each site across the full 12 months of the study. No data for September and January.



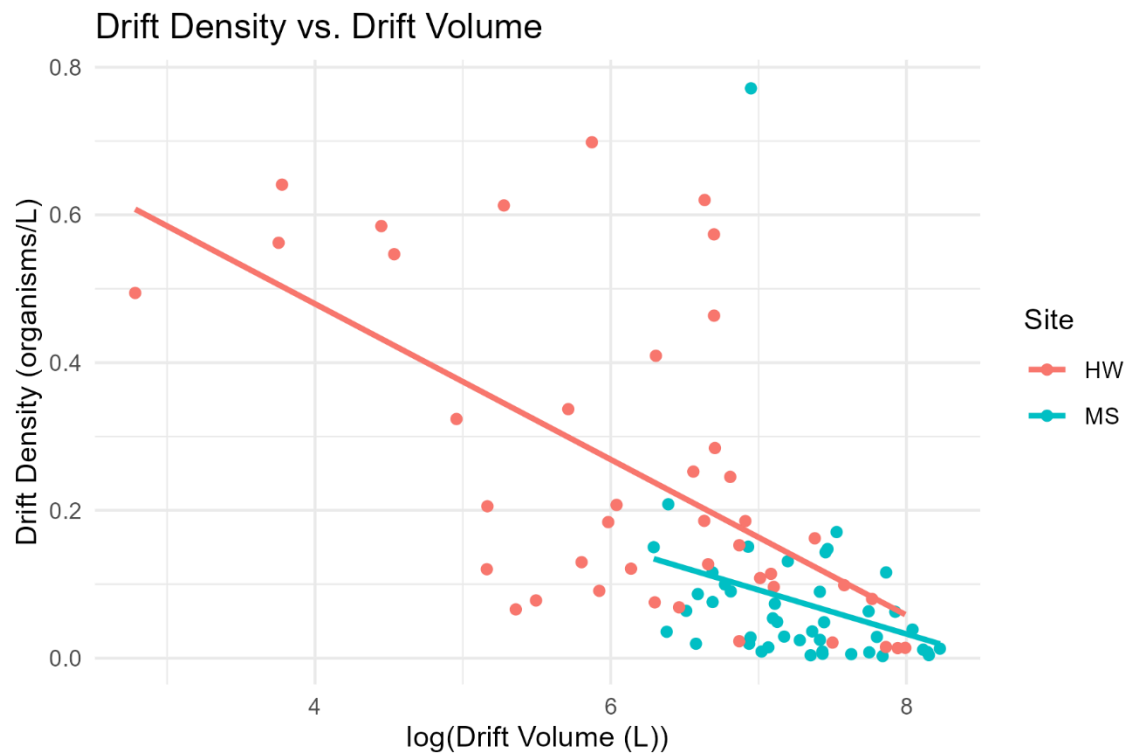
**(Figure 5)** PCA of environmental variables. Top figure colors by order, bottom colors by site. The general lack of clustering by sites based on variables other than those related to discharge allows us to assume that sites environmental variables were similar enough to ignore for subsequent community analyses.



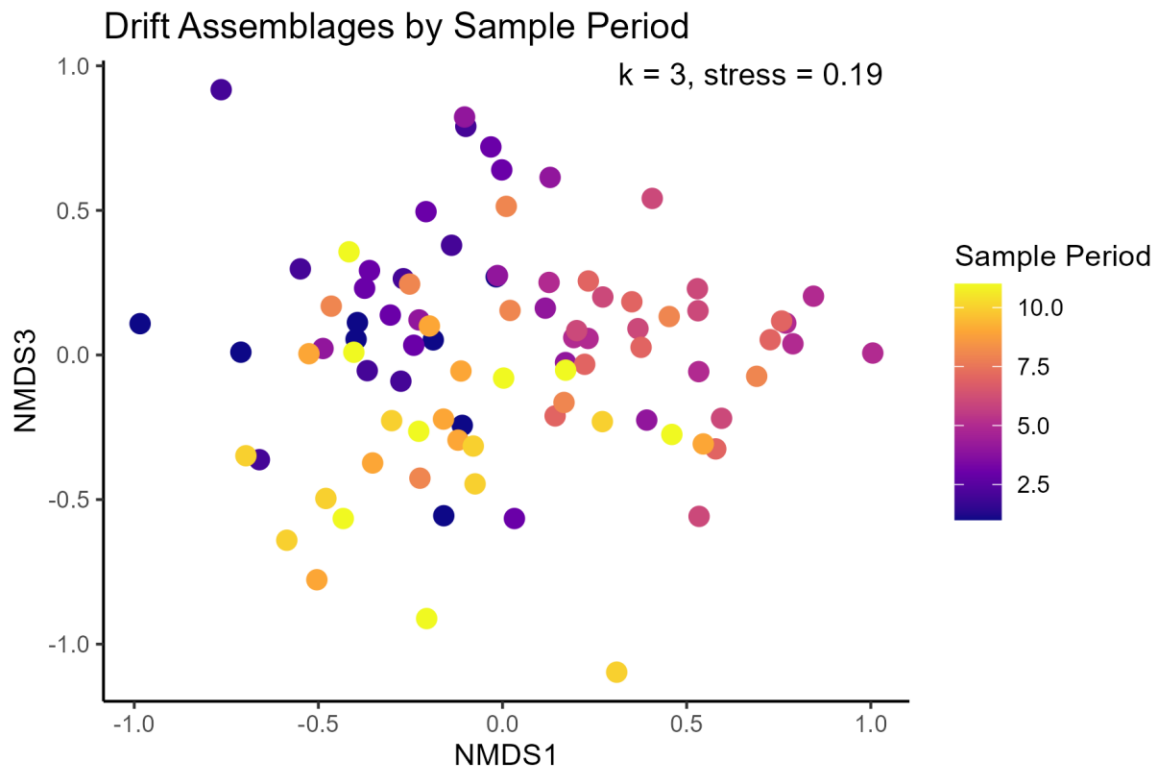
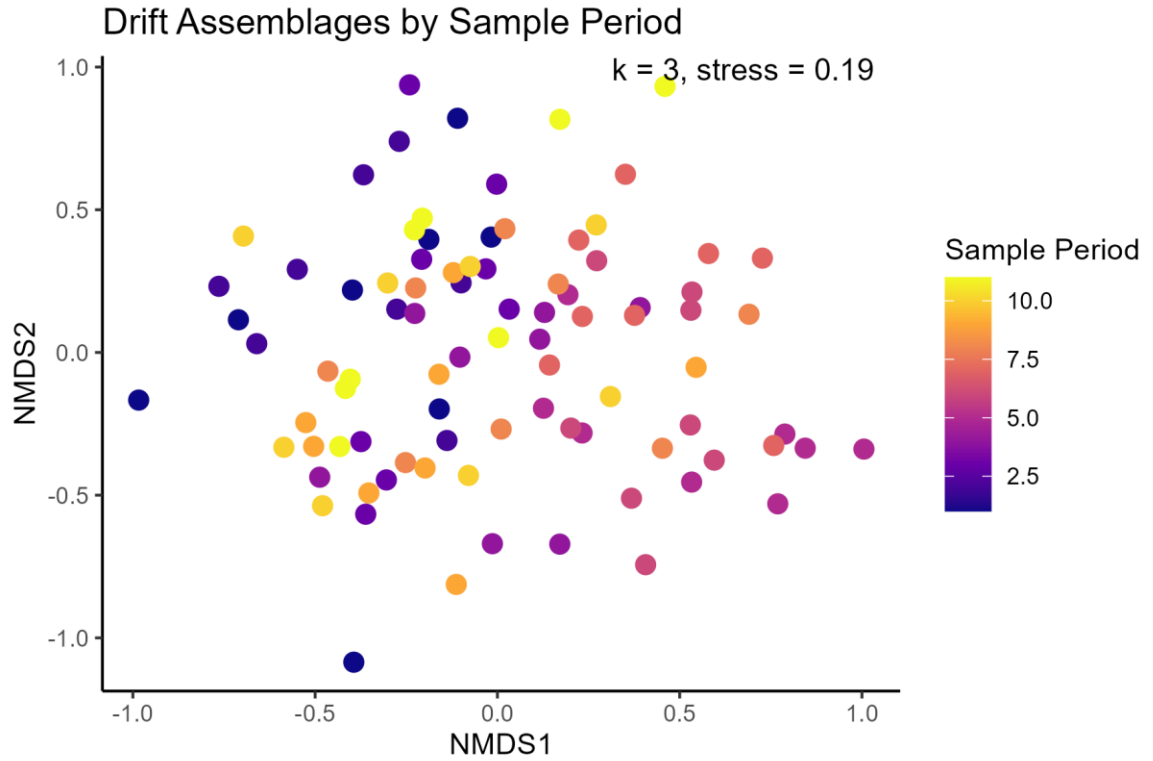
**(Figure 6)** Total organisms and drift density for each sample period (means and standard error error bars plotted). Groups that were found to have statistically significant differences in means are denoted with letters. No difference was found between groups in drift density, even when removing outliers.



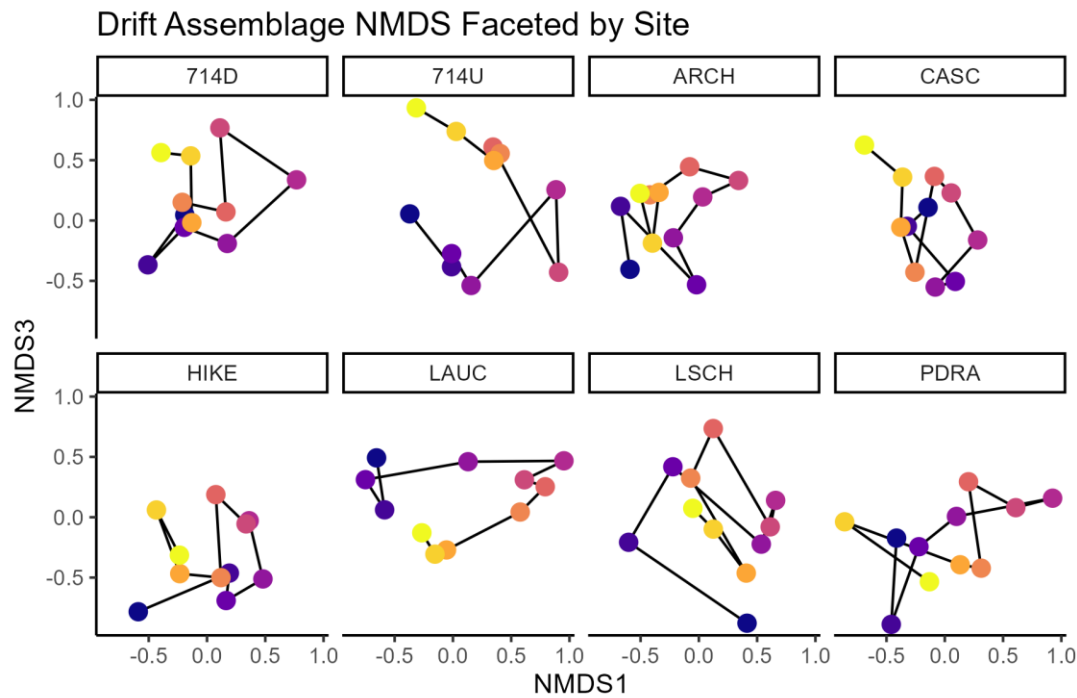
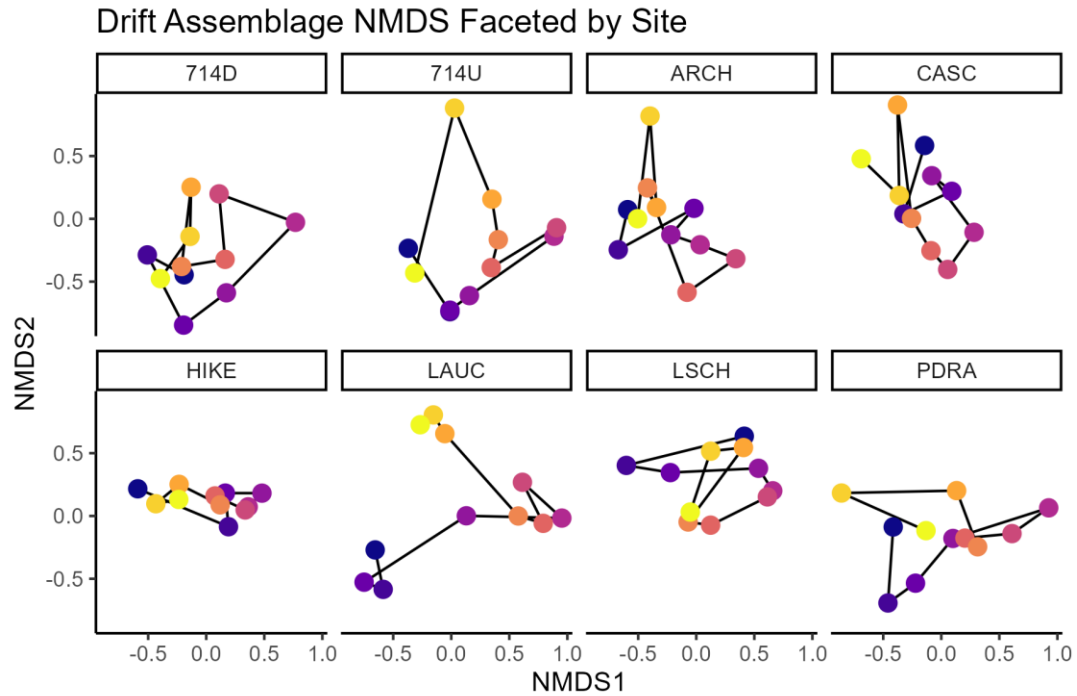
**(Figure 7)** Distribution of drift densities for each sample period for headwaters vs mainstems. Headwaters have consistently higher densities and greater variation between sites.



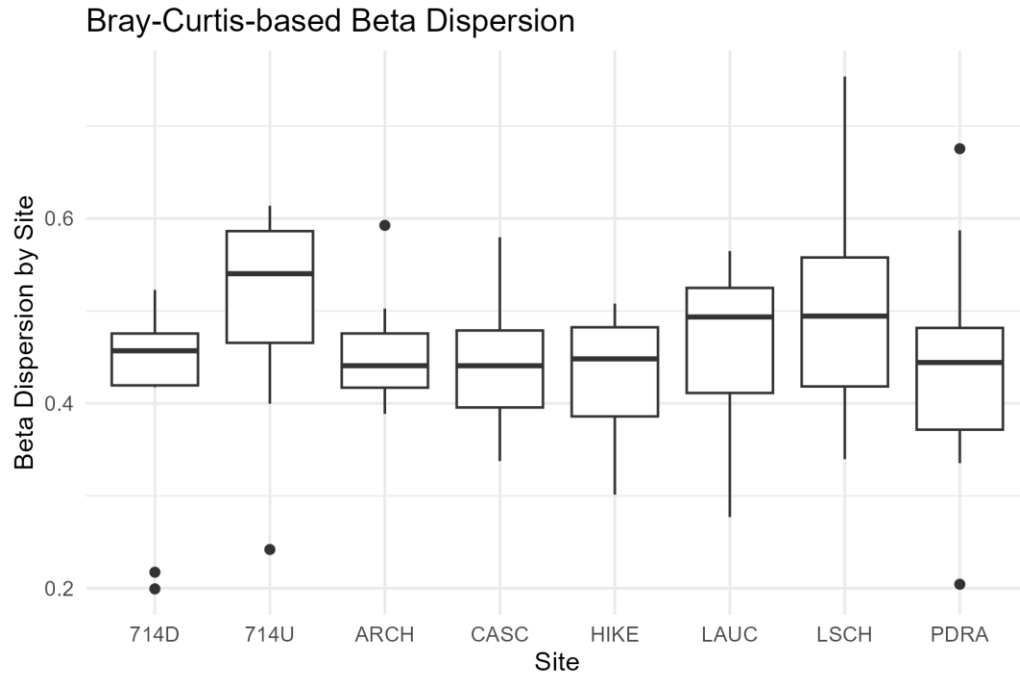
**(Figure 8)** Drift density vs. the log of drift volume, colored by order.  $R^2$  for HW sites was 0.356 and 0.068 for MS, indicating a strong negative relationship with HW and a poor fit for MS. These data suggest a rejection of the null hypothesis of higher drift volumes yielding a linear increase in number of organisms.



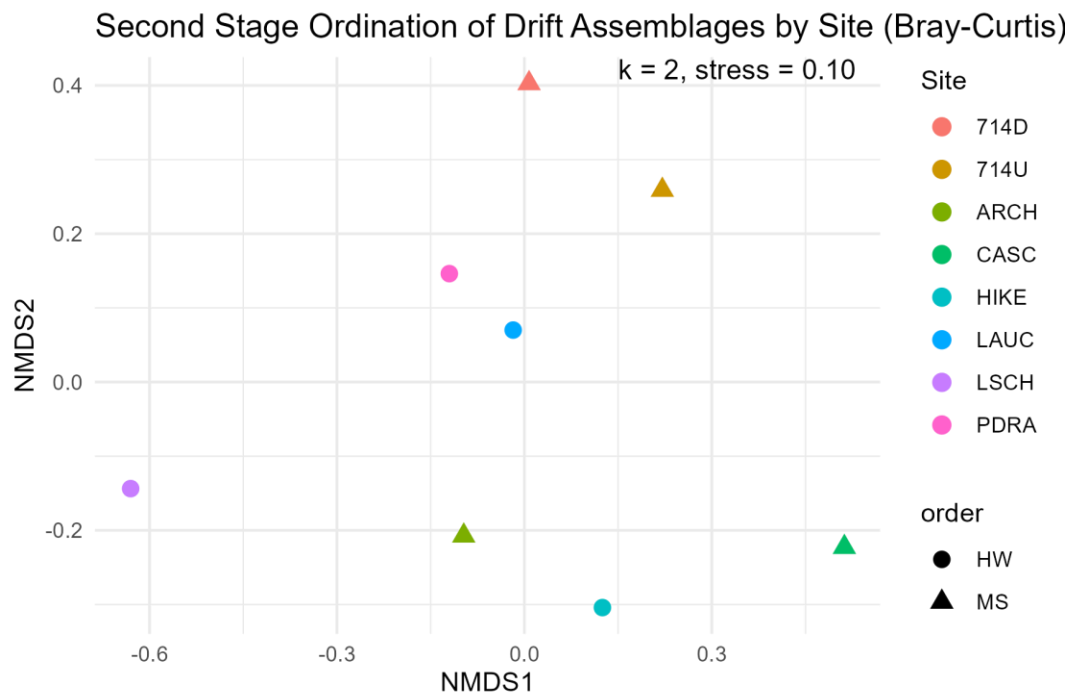
**(Figure 9)** 3 dimensional NMDS of drift assemblages colored by sample period using Bray-Curtis (abundance-based) dissimilarity. Sampling began in September with sample period 1, and ended with August of the following year with sample period 12.



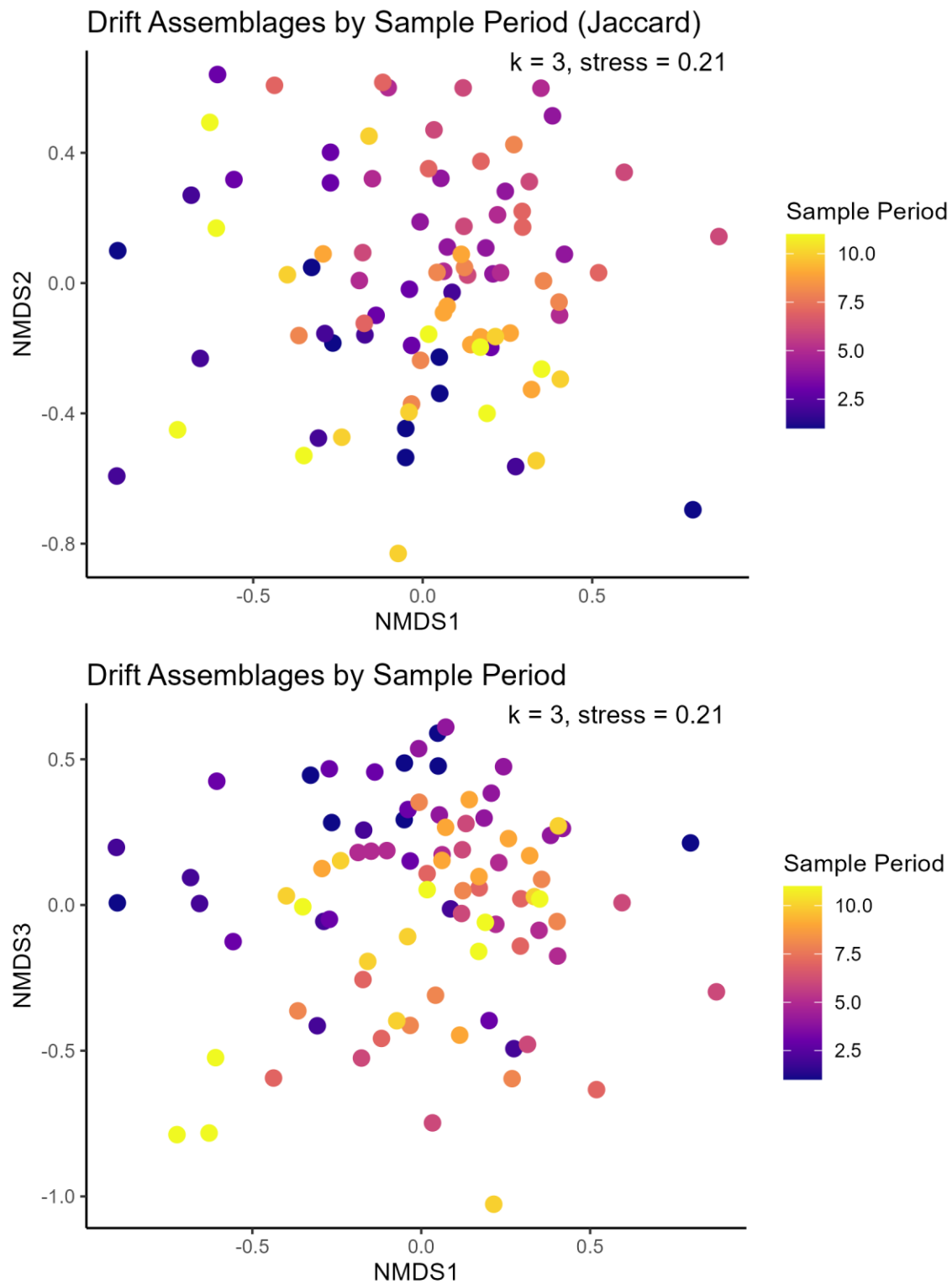
**(Figure 10)** 3 dimensional NMDS of drift assemblages colored by sample period using Bray-Curtis (abundance-based) dissimilarity. Points are faceted by site, and a *geom\_path()* aesthetic is used to show the path the assemblage takes through time. 714D, 714U, ARCH, and CASC are Mainstems (MS), and the rest are Headwaters (HW).



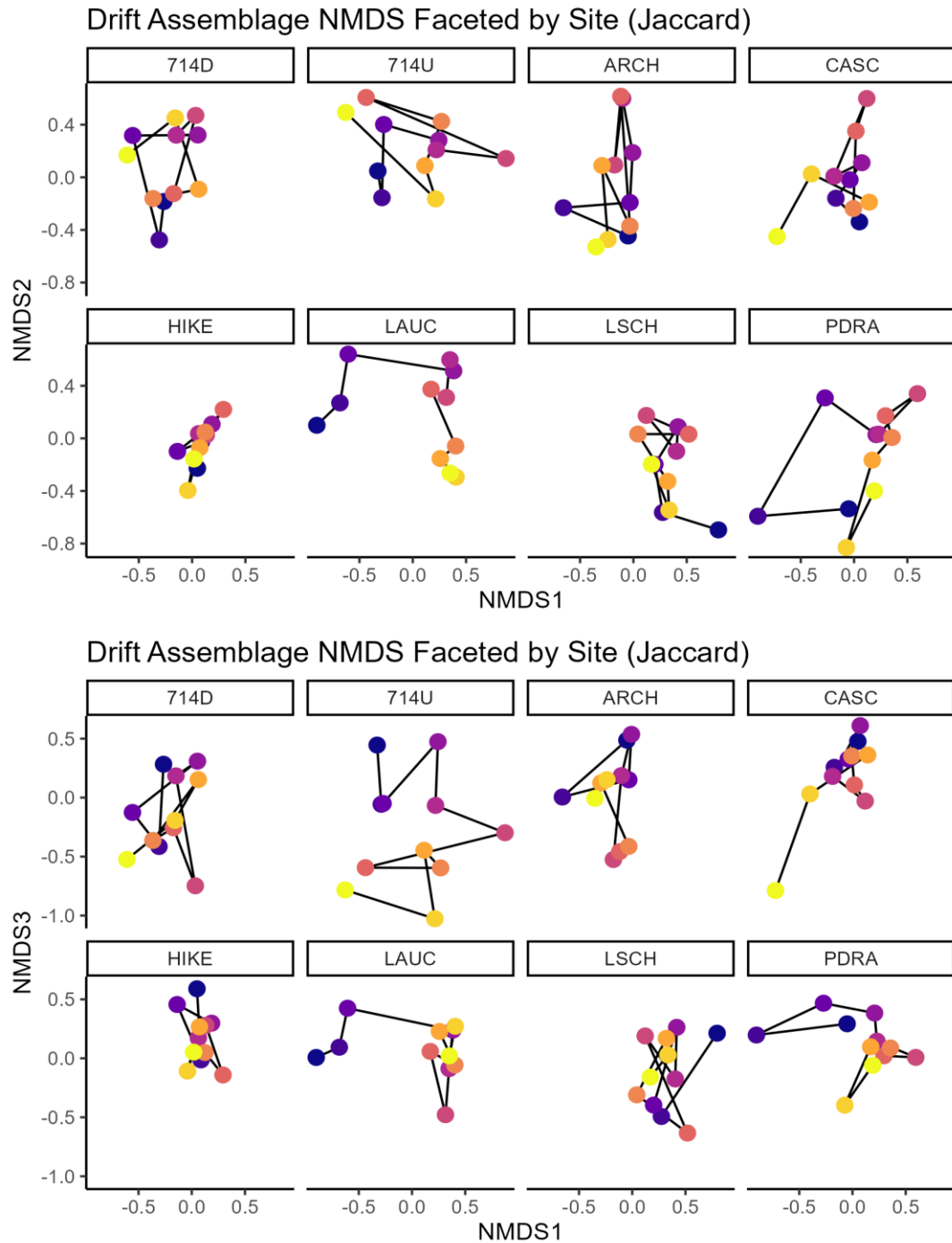
**(Figure 11)** Beta Dispersion results by site using Bray-Curtis distance metric. Applying a Levene’s test to test for univariate dispersion,  $p \gg 0.05$ .



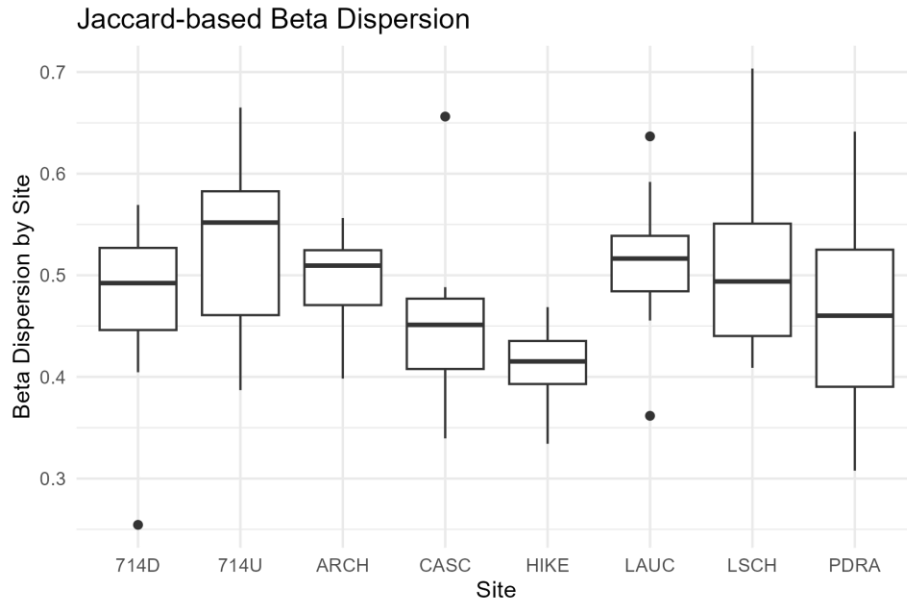
**(Figure 12)** Second stage ordination of Bray-Curtis calculated temporal variability of drift assemblages by site. Proximity in space indicates similarity in temporal variability. Using Bray-Curtis second stage ordination, no clear site level trend was observed.



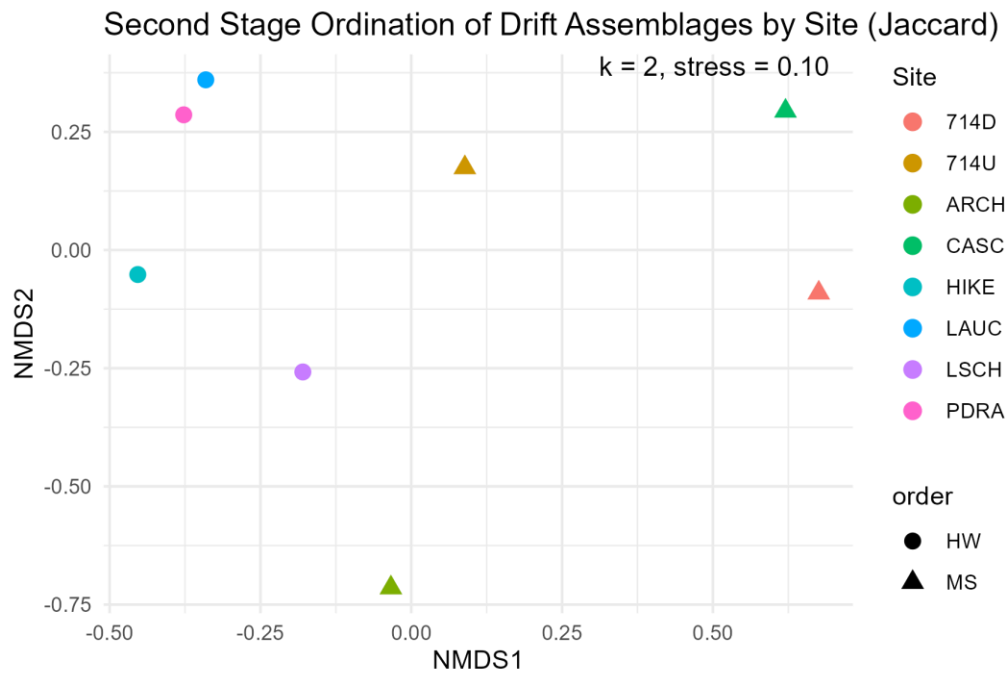
**(Figure 13)** 3 dimensional NMDS of drift assemblages colored by sample period using Jaccard (Incidence-based) dissimilarity. Sampling began in September with sample period 1, and ended with August of the following year with sample period 12.



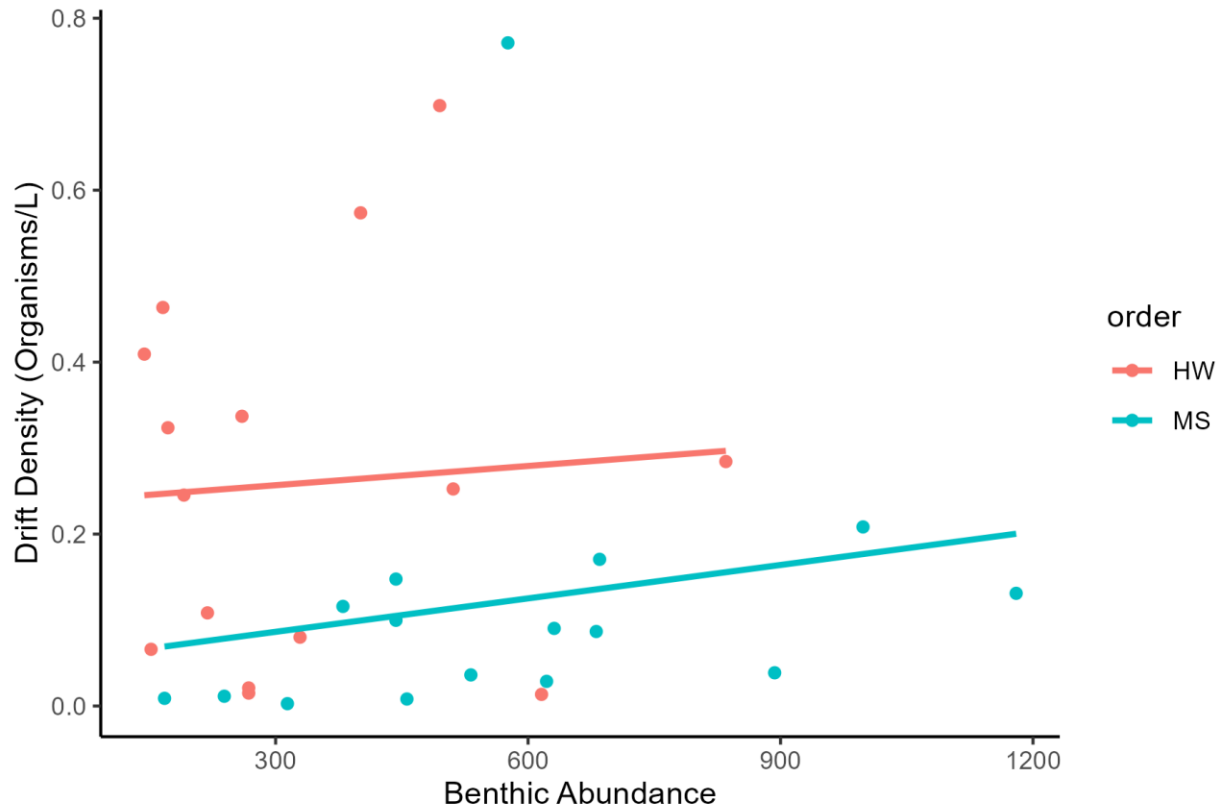
**(Figure 14)** 3 dimensional NMDS of drift assemblages colored by sample period using Jaccard (Incidence-based) dissimilarity. Points are faceted by site, and a *geom\_path()* aesthetic is used to show the path the assemblage takes through time. 714D, 714U, ARCH, and CASC are Mainstems (MS), and the rest are Headwaters (HW).



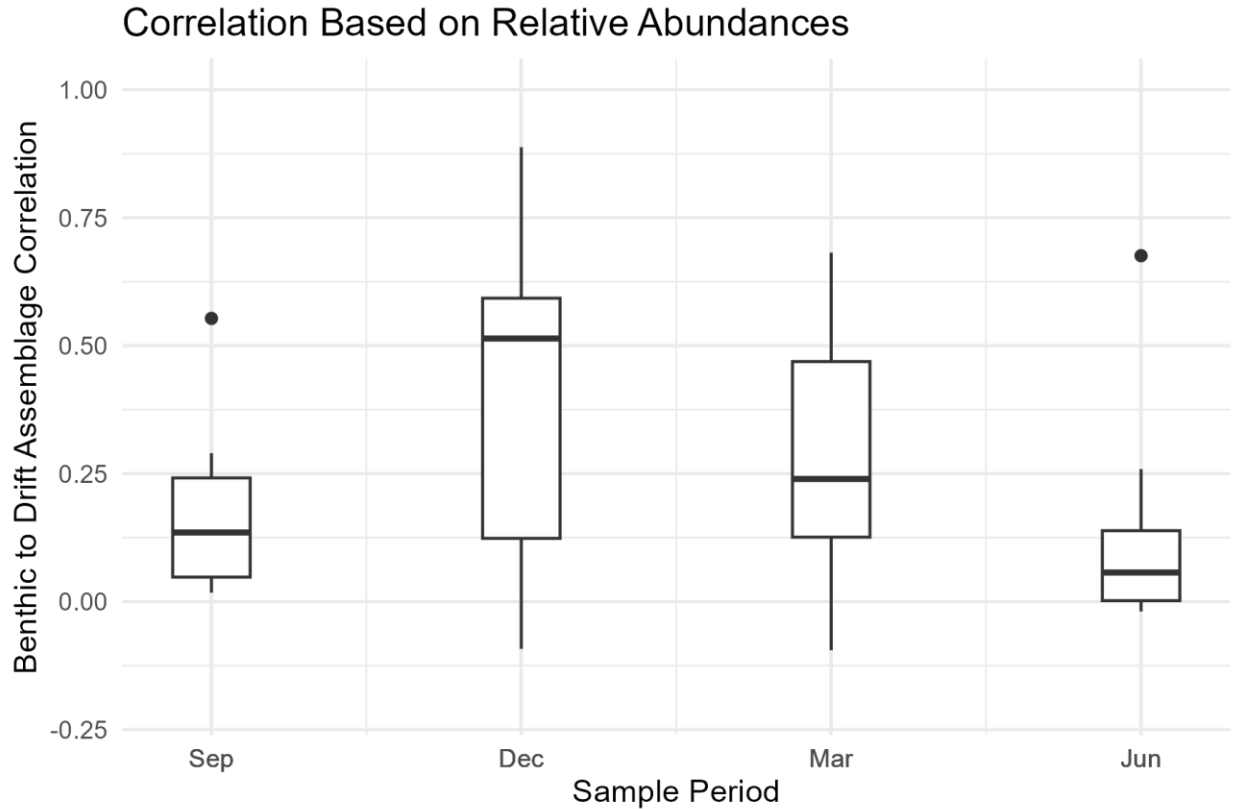
**(Figure 15)** Beta Dispersion results by site using a Jaccard distance metric. Applying a Levene's test to test for univariate dispersion,  $p \gg 0.05$ , but smaller than when run using Bray-Curtis.



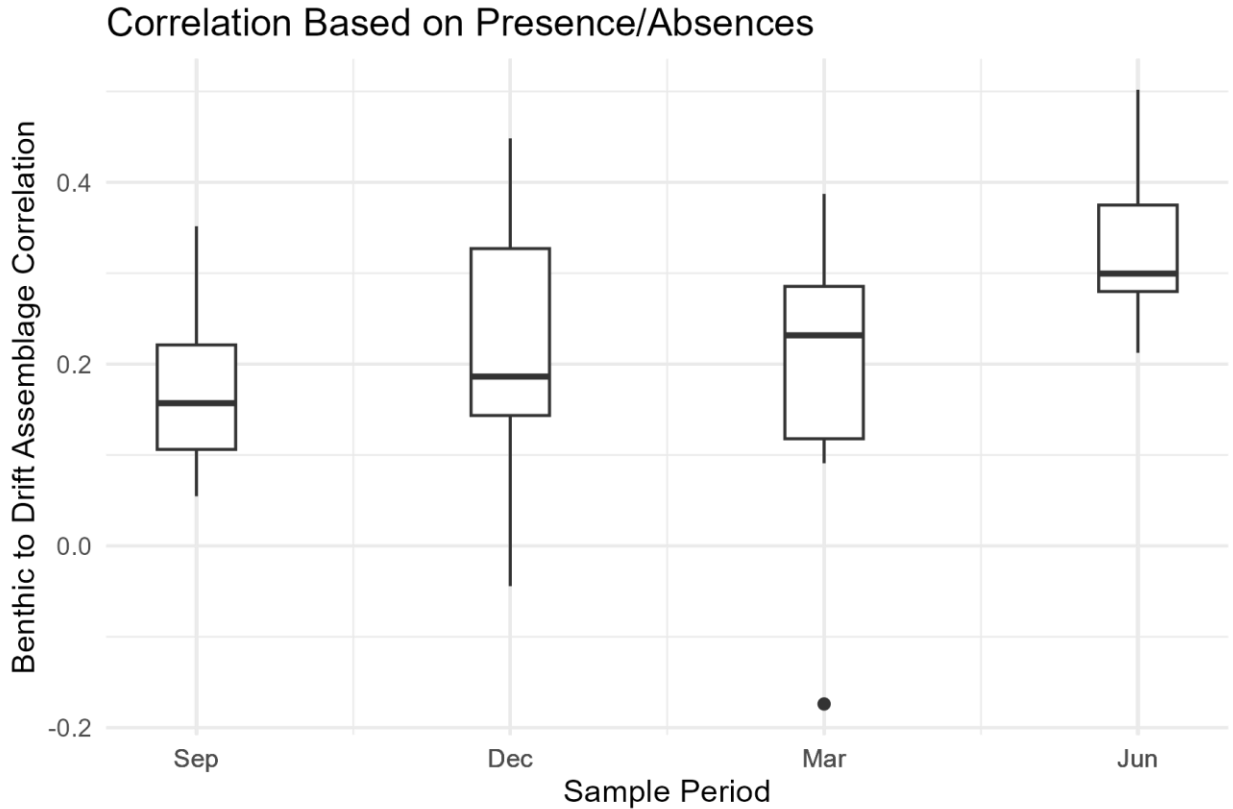
**(Figure 16)** Second stage ordination of Jaccard calculated temporal variability of drift assemblages by site. Proximity in space indicates similarity in temporal variability. Using Jaccard second stage ordination, A clear spatial cluster was observed, with a PERMANOVA run by order indicating an  $R^2$  of 0.64, but a  $p$  value  $\gg 0.05$  due to the lack of points.



**(Figure 17)** Drift density (as measured as number of organisms per liter) vs. total abundance of benthic organisms concurrently sampled. The flat lines and poor correlation for both headwater and mainstem sites indicate no clear relationship between both, despite the hypothesis that increasing benthic abundance should lead to higher drift density. (HW  $R^2 = 0.0001$ , MS =  $0.0365$ )



**(Figure 18)** Pearson correlation between concurrently sampled drift and benthic assemblages using relative abundances. A linear mixed effects model showed a significant ( $p < 0.05$ ) quadratic relationship between sample period (coded as Julian Day) and correlation, with no spatial relationship found. This indicates that the relatedness of drift to the benthic community changes significantly across the year, and is highest in times of higher flows in the winter and spring.



**(Figure 19)** Pearson correlation between concurrently sampled drift and benthic assemblages using presence/absences. A linear mixed effects model showed a significant ( $p < 0.05$ ) linear relationship between sample period (coded as Julian Day) and correlation, with no spatial relationship found. This indicates that the relatedness of drift to the benthic community changes significantly across the year, but in a different way than when considering relative abundances.

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