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# How do Stream Confluences Influence Aquatic Invertebrate Taxonomic and Functional Diversity?

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## Introduction:

Since the colonization of North America, humans have changed the inherent structure and function of riverine communities. From changing natural flow and sedimentation rates (Kondolf 1997) to altering ecosystem habitats and damaging water quality (Moore et al. 1991), the physical changes to aquatic systems have echoed throughout the biotic communities (Barnett et al. 2005). In response, biologists have begun to reverse these changes and restore ecosystem habitats. Yet they have had limited success due to political and social pressures as well as an incomplete understanding of the drivers of aquatic community composition and structure (Roni et al. 2002).

Predictions for natural ecosystems are guided by our understanding and development of ecological theories. One of the more influential theories is the River Continuum Concept (RCC) (Vannote, 1980). The RCC proposes that physical characteristics of streams change longitudinally (upstream to downstream) in a gradual and consistent rate and the biological communities follow suit. Vannote et al. (1980) intended this concept to be applied to streams with forested headwaters that lead into wide river valleys. The RCC highlights that headwater streams are often narrow and dominated by riparian canopy cover, leading to an increase in allochthonous input and coarse particulate organic matter that support aquatic invertebrates shredding leaves and collecting smaller particles. Conversely, aquatic invertebrates that rely on scraping algae are not supported in headwaters due to decreased sun penetration and autochthonous production. Vannote et al. (1980) uses these predictions to establish expected communities ranging from a river's headwaters to its valley bottoms (Figure 1). The RCC predicts a gradual continuum across river communities that has proved useful in developing landscape scale predictions for aquatic community shifts. Yet, more recent studies have argued that stream dynamics such as flood and fire disturbance and tributary confluences instead create a 'discontinuum' between aquatic communities (Benda et al. 2004).

Streams are dendritic (Figure 1) in structure rather than the simplified channel depicted by the RCC. The intersection of these stream confluences represent a mixing of the physical habitats and biological communities. At these confluences, the smaller tributary introduces more water, fine and coarse particulate organic matter, sediment, a different thermal regime, and a new biological community to the larger stream (Benda et al. 2004). This represents a sudden and localized change to the physical habitat and, in response, the biological community of the main channel. Some ecologists suggest this would result in a strong step-wise change in the biological community rather than a gradual change (Bruns et al. 1984, Benda et al. 2004, Rice et al. 2001), while others claim that the confluence's effect is merely an adjustment of the community along the RCC spectrum, with the continuum being maintained (Minshall et al. 1985). Rice et al. (2001) found that rivers with a complex dendritic network rarely experience the gradual change suggested by the RCC. Instead, they proposed the link discontinuity concept, which claims that streams and their communities are dictated by the arrangement of hydrologic and sediment networks (Rice et al. 2001). They postulated that small scale changes in velocity, sediment load, and substrate availability will dictate changes in the biological community. For example, a variety of studies have looked at how velocity and Reynolds number, a measure of turbulence, can affect aquatic macroinvertebrate assemblages (Voelz and Ward 1996, Quinn and Hickey 1994). Stream confluences are often associated with increased turbulence, suggesting they could

be a mechanism for changes in the community. Additionally, Bruns et al. (1984) found that macroinvertebrate collectors, who feed on fine and coarse particulate organic matter, were more abundant below confluences, suggesting that they were benefitting from the increased nutrient load. There have been few studies that investigate the community shifts associated with river confluences across a landscape.

Macroinvertebrate communities offer an ideal metric for studying community shifts as they provide powerful insight into the health and function of stream ecosystems (Heino 2005, Péru et al. 2010). They are sensitive to a variety of environmental changes due to their limited ability to move away from stressors and their strong dependence on local nutrient sources (Gayraud et al. 2003). In addition, macroinvertebrate communities exist in multiple functional groups including; scrapers, who eat algae growing on substrate, shredders, who eat leaves and macrophytes, filterers, who filter fine and coarse particulate organic matter, and predators, who eat other invertebrates and small fish (Pavluk et al. 2000). Thus, macroinvertebrates are ideal for studying how river confluences may influence riverine communities.

**Question:** How do macroinvertebrate community taxonomic and functional diversity consistently change as associated with stream confluences?

**Hypothesis 1:** River confluences provide more heterogeneous habitat and nutrients that can support a more diverse and different macroinvertebrate community.

**Prediction:** Macroinvertebrate communities will be more diverse (increased number of genera and evenness) below stream confluences than above.

**Prediction:** Pairwise comparisons of samples with the effect of river confluences will have more dissimilarity than pairwise samples without the effect of river confluences.

**Hypothesis 3:** River confluences provide more invertebrate prey for macroinvertebrate predators.

**Prediction:** There is more invertebrate prey downstream of confluences because of the increased habitat heterogeneity and increased watershed area.

**Hypothesis 4:** River confluences provide organic matter for macroinvertebrate collectors.

**Prediction:** Macroinvertebrate communities will have higher proportions of collectors below river confluences than above

**Hypothesis 5:** River confluences will change aquatic communities enough to significantly alter decomposition rate, a vital stream process.

**Prediction:** River reaches below river confluences will have higher decomposition rates than river reaches above.

## Methods

My study area was the Absaroka Beartooth Wilderness Area near the Boulder River and Big Timber, MT (Figure 2). I selected two creeks, Fourmile and Bridge Creeks, and designated four confluences on each creek (Figures 3 and 4). When I chose these creeks and their tributaries, I controlled for stream order based on Strahler (1957), designating each confluence a joining of a first order tributary with a second order stream (Figure 5). At each confluence I sampled macroinvertebrates in five places around the confluence (Figure 6) in order to establish pairwise comparisons between sites affected and unaffected by the confluence. I sampled macroinvertebrates twice across the summer of 2016, once in late May and again August. I used a surber net to control for area sampled ( $.2 \text{ m}^2$ ), sampled for two minutes each sample, and always sampled the nearest riffle zone to control for habitat type. Samples were placed into sealed bags with 60-70% ethanol to kill and preserve macroinvertebrates for later identification.

At each confluence I recorded canopy cover using a densitometer and visually estimated average substrate size for above the confluence on the main channel and tributary and below the confluence. In late May, I placed HOBO temperature loggers (logging at half hour intervals) and leaf-litter bags at the highest and lowest sites in each watershed. I made fine and medium mesh decomposition bags with loofas (10-15 mm mesh) and fine netting (1 mm mesh) and placed one of each size at the highest and lowest sites. I added 10 grams of dried leaves into each bag in order to measure change in weight over the study period (2 months), a proxy for decomposition rate (Gessner et al. 2002). Both HOBOS and leaf-litter bags were anchored to rocks and logs within the stream.

In the fall of 2016 I began picking and identifying bugs. First, I randomly selected 300 individuals from each sample by randomly scooping the sample into a tray and picking all of them with a large magnifying lens. Undergraduate volunteers helped in the process and were trained to use the random protocol. I quality controlled all samples picked by volunteers and had 10% of the samples I picked quality controlled by a volunteer. Once the samples of 300 were picked, I used a dissecting scope and a variety of taxonomic guides to identify samples down to genus (Voshell 2003). After the first round of identifying I sent the three most diverse samples to Rhithron Associates for quality control. They identified the samples to genera and provided vouchers of each taxa collected. I re-identified my samples to increase accuracy. Once samples were identified to genus, I used a functional feeding guilds guide (Poff et al. 2006) to give each genus present in a sample a functional feeding value for the categories collector, shredder, predator, or grazer. This allowed me to identify the proportion of each functional group in each sample.

To evaluate my hypotheses, I focused analyses of diversity and functional feeding groups around the three Orders: Ephemeroptera, Plecoptera, and Trichoptera (EPT) due to the difficulty in identifying genera of Diptera. To analyze taxonomic diversity, I used Simpson's Diversity Index, which takes into account both number of genera present and evenness within a sample. I visually compared macroinvertebrate functional groups between samples with pie charts. In addition, I used Bray Curtis Dissimilarity to evaluate the dissimilarity of functional feeding groups between paired samples. A dissimilarity values of 0 indicates that the two samples are the same and a dissimilarity value of 1 means that the two samples are completely different. A pairwise comparison with a higher dissimilarity value indicates the communities changed more than the pairwise comparison with a lower dissimilarity value.

Due to time restrictions and the large amount of effort required to identify invertebrate samples down to genus, only samples collected in August from Fourmile Creek were analyzed. August samples were chosen because the invertebrates would be larger than in late May and more readily identifiable. In addition, the two lower sites (1 and 2) were dewatered in August, thus restricting the analysis to Fourmile Creek Sites 3 and 4 (Figure 3). Bridge Creek was not selected for analysis because sites 1 and 2 were considerably steeper, flowing down a treacherous talus slope that made sampling dangerous and provided a very different physical habitat type than the other sites.

## **Results**

### *Diversity:*

I predicted that diversity would consistently increase below the confluence, however I found no consistent trends in diversity around confluences. At Fourmile Creek site 4, The Simpsons diversity index was higher below the confluence (10.9) than above the confluence on the main channel (8.9) and the tributary (7.4) (Figure 7). At Fourmile Creek site 3, diversity was lower below the confluence (5.3) than above the confluence on the main channel (9.8) and the tributary (10) (Figure 7).

### *Dissimilarity:*

I predicted that pairwise comparisons of communities with a confluence between them would be more dissimilar than communities without, however this prediction was not consistent across sites. At Fourmile Creek site 4, the pairwise dissimilarity was higher in the main-main communities (0.22) than the main-below communities (0.10) and the tributary-tributary communities (0.14) was just as dissimilar as the tributary-below communities (0.14) (Figure 8). At Fourmile Creek site 3, the pairwise dissimilarity was higher in the main-main communities (.18) than the main-below communities (0.11) and the tributary-tributary communities were less dissimilar (0.12) than the tributary-below communities (0.36) (Figure 8).

### *Community Composition:*

I predicted that predators and collectors would consistently be in higher proportions below the confluences than above, however I found there was no consistent changes in their proportions. At Fourmile Creek Site 4, the main channel proportion of predators was insignificantly higher (25%) than below the confluence (24%) and the main channel proportion of collectors was insignificantly lower (32%) than below the confluence (36%) (Figure 9). At Fourmile Creek Site 3, the main channel proportion of predators was equivalent (24%) to below the confluence (24%) and the main channel proportion of collectors was insignificantly lower (26%) than below the confluence (29%) (Figure 9).

### *Stream Decomposition Rate:*

Unfortunately, most leaf litter bags placed were swept away by the spring floods. The few that remained either tore, releasing their material, or were partially buried by the sediment. No analysis was completed for stream decomposition.

## **Discussion**

These data suggest that there are no consistent trends in how stream confluences affect taxonomic and functional diversity. I observed diversity both increasing and decreasing below the confluence and dissimilarity between communities was often smallest between communities with a confluence. I also observed no consistent shifts in community composition. Ultimately, my predictions that the confluence effect alone would shape communities were not supported with my data, suggesting that either confounding factors or my hypothesis needs revising.

The strongest limiting factor of my study is my small sample size. While the original study design would have allowed for a stronger comparison, the difficulties with collecting data in the field and the enormous amount of labor required to sort and identify the samples restricted the amount of data available to me. Perhaps with a larger sample size the variation in the trends I observed would have been lessened and something consistent would have emerged. Additionally, though I had thought my study sites to be unaffected by human development within the wilderness, Rhithron Associates found that some *chironomidae* had deformed heads, suggesting there was some metal pollution. However, due to the large number of observe EPT I observed, I am not convinced that the community was drastically altered as a result. Also, while the habitat data I collected was focused on variables known to influence macroinvertebrates, my analysis was limited by sample size and the scale at which I collected the data. To determine if habitat was the primary predictor of communities, I would suggest looking for changes in habitat heterogeneity between sites and whether stream confluences consistently shifted the habitat type. When I collected my habitat data, I expected average substrate size to increase below confluences due to increased velocity and for canopy cover to decrease from tributaries to the main channel. Instead, I found that there were no predictable habitat changes due to confluences (Table 1). If my habitat predictions had proved true, I would have expected the diversity at these sites to increase as well as the food available to predators and collectors. However, a recent comprehensive review of the success of restoring stream habitat heterogeneity suggests that habitat may not be the primary driver of stream aquatic macroinvertebrate diversity and communities (Palmer et al. 2010).

Palmer et al. (2010) reviewed 78 different stream restoration projects to search for evidence supporting the assumption that habitat heterogeneity increases biodiversity. Consequently, they found little evidence that increasing habitat heterogeneity had any effect on macroinvertebrate diversity. As we try to predict aquatic invertebrate communities we might expect variation on the landscape driven by a multitude of contributing factors such as food sources, habitat heterogeneity, and confluences. If we better understand how natural systems function, we may be able to better concentrate our efforts to restore the disturbed systems.

Table 1. Habitat variables (average substrate size, canopy cover, and slope) for Fourmile Creek Site 4 and Site 3.

Site	Average Substrate Size	Canopy Cover	Slope
Site 4_Below Confluence	400-600 mm	40%	7%
Site 4_Above Confluence	100-400mm	40%	7%
Site 4_Above Tributary	10-50 mm	60%	9%
Site 3_Below Confluence	10-50 mm	90%	3%
Site 3_Above Confluence	50-200 mm	60%	3%
Site 3_Above Tributary	20-100 mm	20%	7%

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Figures

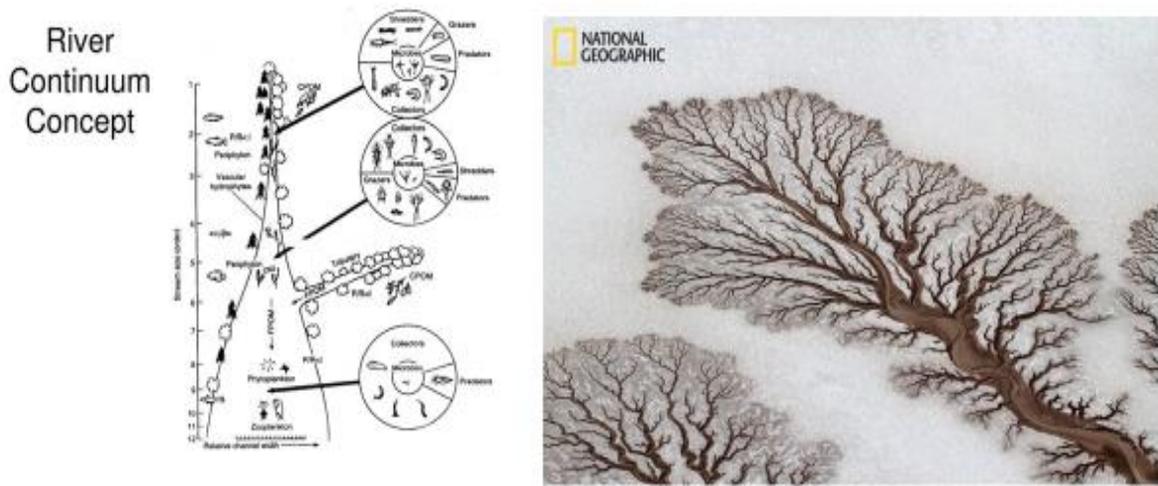


Figure 1: The River Continuum Concept as proposed by Vannote et al. (1980) and a dendritic river network showing the complex connectivity of rivers (National Geographic).

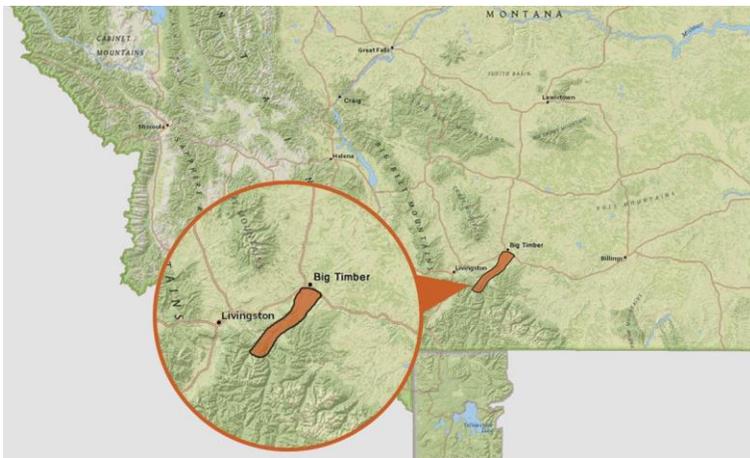


Figure 2. Map of the MT with the study area on the Boulder River zoomed in.

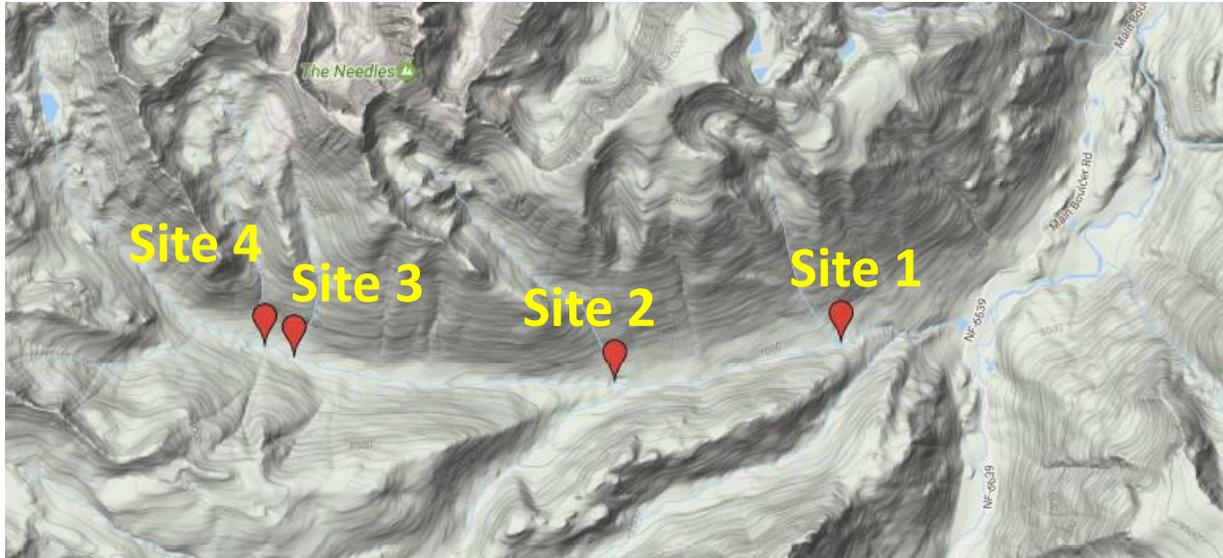


Figure 3. Topographic map of Fourmile Creek with confluences/sampling sites pin pointed in red. Sites 3 and 4 were analyzed for this study.



Figure 4. Topographic map of Bridge Creek with confluences/sampling sites pin pointed in red.

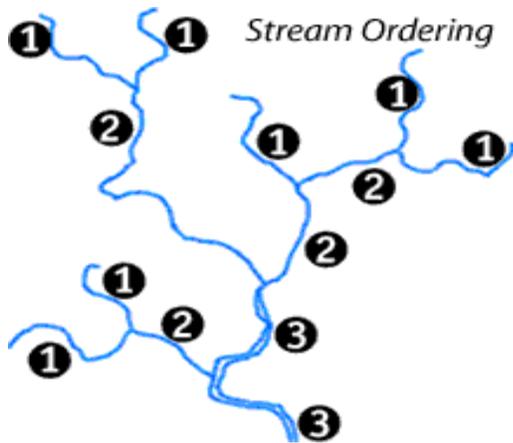


Figure 5. Strahler's Theory of Stream Order (Strahler 1957)

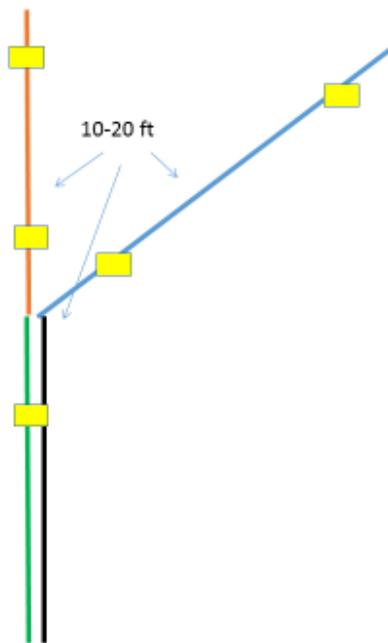


Figure 6. An example of sampling area lay-out. The blue-black line represents the smaller tributary and the orange-green line represents the main channel. The yellow boxes indicate the areas sampled.

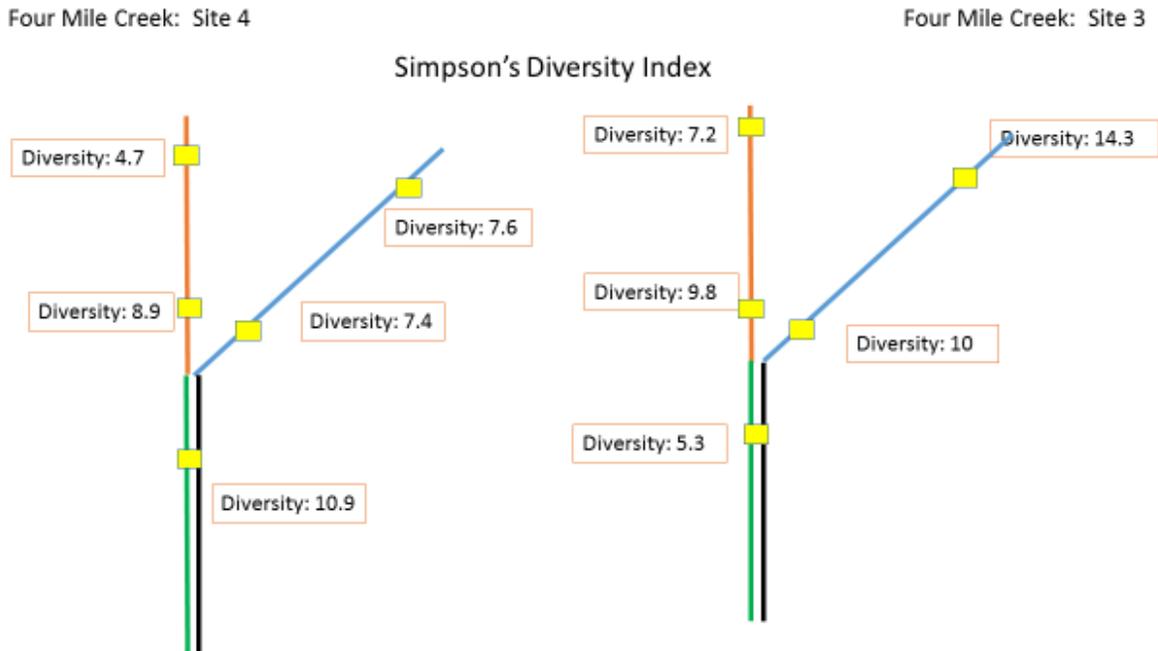


Figure 7. Simpson's Diversity Index values for Fourmile Creek sites 3 and 4.

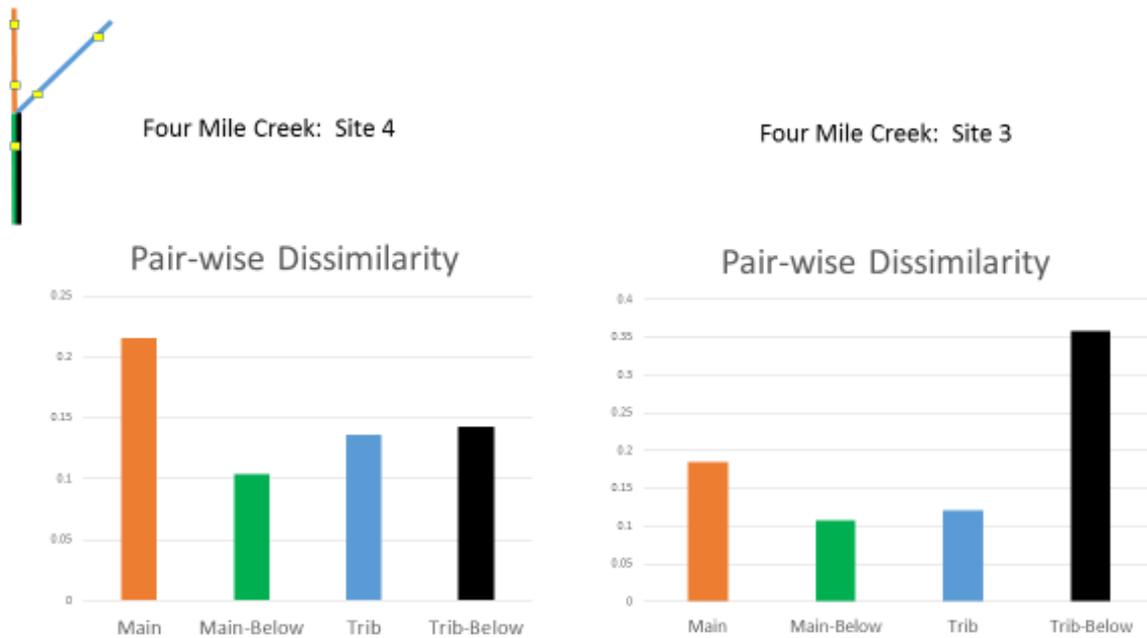


Figure 8. Bray Curtis Dissimilarity values for pairwise comparisons main-main, main-below, trib-trib, and trib-below for Fourmile Creek Site 4 and 3.

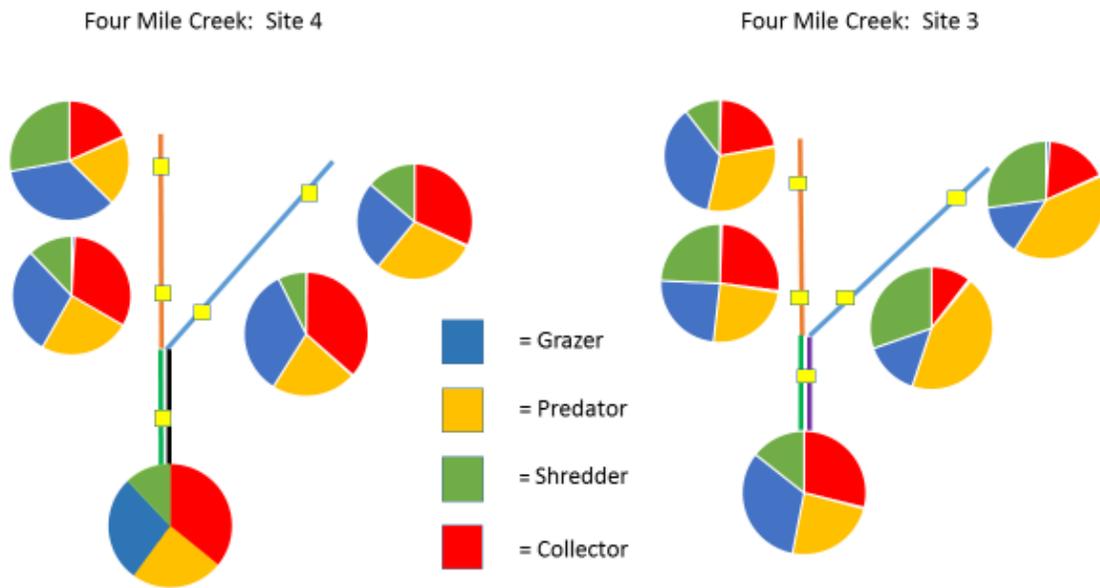


Figure 9. Macroinvertebrate community proportions of functional feed groups (grazers, predators, shredders, and collectors) for Fourmile Creek Site 4 and Site 3 for each sample.