

Seasonal Differences in Diversity of Macroinvertebrate and Fish Taxa in a Midwestern Stream

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3 1 **Seasonal Differences in Diversity of Macroinvertebrate and Fish Taxa in a Midwestern**
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For Peer Review

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9 **Abstract**

10 Most biomonitoring projects focus on single species groups at time scales either too short
11 or too coarse to detect intra-annual oscillations in biodiversity. Using a multi-faceted approach,
12 we compared diversity indices of larval macroinvertebrate families and fish species in a
13 Midwestern stream during spring and fall of 2009-2013, and discovered contrasting patterns in α
14 and β diversity between the seasons for the two taxa groups. Compared to spring, both α and β
15 diversity were significantly higher during fall for macroinvertebrates; on the contrary, only α
16 diversity differed between the seasons for fishes. For both taxa, we partitioned the overall β
17 diversity to identify contributions of temporal and spatial β diversity on the observed
18 differences. The observed patterns for macroinvertebrates were likely the result of season acting
19 as environmental filter, but sampling effects were likely more important in driving fish diversity
20 patterns. In light of widespread conservation and restoration efforts in the Midwestern streams, it
21 seems prudent to study community composition frequently so that baseline alpha and beta
22 diversity can be obtained for organisms at different taxonomic levels and during different
23 seasons.

24 **Keywords:** Alpha diversity; beta diversity; macroinvertebrates; fishes; seasonal differences;
25 freshwater streams

27 **Introduction**

28 Biodiversity loss or change have important effects on ecosystem functions and services
29 (Cardinale et al., 2006; Worm et al., 2006) and on humanity as a whole (Cardinale et al., 2012),
30 and thus, there is growing interest in understanding the patterns of biodiversity change at local as
31 well as global scales. Globally, biodiversity change has been characterized by a loss in the

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3 32 numbers and/or relative abundances of species in a community (i.e. α diversity), attributed
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5 33 mainly to anthropogenic pressures, climate change, large-scale habitat transformation, though
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7 34 such patterns do not seem to exist at local scales (Dornelas et al., 2014; Vellend et al., 2013). In
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9 35 addition, local communities have also undergone shifts in community composition due to
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11 36 processes such as homogenization, differentiation and local or global extinction (Magurran et al.,
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13 37 2018; Rahel, 2002). Cumulatively, these structural changes have also substantially affected the
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15 38 ecosystem function and services (Frainer et al., 2017; Hillebrand et al., 2018; Spaak et al., 2017).

19 39 Biodiversity loss and change has led to a renewed interest in the study of drivers and
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21 40 patterns of biodiversity in both terrestrial and aquatic systems at various temporal and spatial
22
23 41 scales (e.g. Al-Shami et al., 2013; Anderson et al., 2011; Nekola & White, 1999). The drivers of
24
25 42 such variations in community structure through space and time are likely taxa- and location-
26
27 43 specific, and might include both deterministic and stochastic processes (Korhonen, Soinen, &
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29 44 Hillebrand, 2010; Stegen et al., 2013). Influence of deterministic processes such as competition
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31 45 and environmental filtering leads to specific taxa exploiting specific spatial niche, and thus, to
32
33 46 high spatial and temporal turnover with increasing habitat heterogeneity (Anderson et al., 2011;
34
35 47 Stegen et al., 2013). Stochastic processes such as sampling and priority effects also affect
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37 48 observed biodiversity trends (Stegen et al., 2013). It is increasingly becoming clear that both
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39 49 deterministic and stochastic processes influence the diversity of communities, with the result that
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41 50 α and β components of diversity are inherently linked. In addition, the temporal and spatial β
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43 51 diversity are also related, though the relation may be context dependent (Stegen et al., 2013;
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45 52 Steiner & Leibold, 2004).

51 53 Patterns and drivers of intra-annual variation in biodiversity is gaining increasing
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53 54 attention in the recent years (Bonada & Resh, 2013; Cook, Housley, Back, & King, 2018;
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3 55 Tonkin, Bogan, Bonada, Rios-Touma, & Lytle, 2017), perhaps owing to the realization that
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5 56 intra-annual variability in diversity of aquatic communities is generally much higher than
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7 57 interannual variability (Korhonen et al., 2010), potentially contributing disproportionately to the
8
9 58 overall biodiversity of the ecosystem. Seasonal variations in biodiversity patterns may arise from
10
11 59 different processes for different taxa or trophic levels. For example, evolution has led different
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13 60 species of aquatic macroinvertebrates to mature at different times of a year such that these
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15 61 species are able to exploit different temporal niches corresponding to the seasonal differences in
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17 62 environmental conditions (Bonada & Resh, 2013; Tonkin et al., 2017; Wolda, 1988). Similarly,
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19 63 many species of freshwater fishes perform spawning migrations to headwaters or other streams
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21 64 of lower order during spring, thus occupying different spatial and temporal niches at different
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23 65 times of the year (Jonsson, 1991; Smith, 2002).

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28 66 Research on seasonal patterns of β diversity in the freshwater ecosystems has mostly
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30 67 focused on macroinvertebrate communities (e.g. Costa & Melo, 2008; Finn, Khamis, & Milner,
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32 68 2013; Heino, Muotka, & Paavola, 2003). Few have studied differences in species turnover
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34 69 patterns among taxa at different trophic or taxonomic levels (Heino, Paavola, Virtanen, &
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36 70 Muotka, 2005; Lepori, Palm, Brännäs, & Malmqvist, 2005). This is problematic because
37
38 71 different species or groups may respond differently to environmental factors and anthropogenic
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40 72 stressors. For example, Datry, Moya, Zubieta, & Oberdorff (2016) observed higher β diversity in
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42 73 intermittent streams compared to perennial streams for fishes, but such differences were not
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44 74 observed for macroinvertebrates. Furthermore, most studies focus on a temporal scale either too
45
46 75 fine (weeks-months) or too coarse (repeated annual samples) to detect effects of season (Brown,
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48 76 2003; Mykrä, Heino, Oksanen, & Muotka, 2011). For assemblages of aquatic taxa with active
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50 77 dispersal stages, such approaches would hinder study of metacommunity structure (Hewitt,
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3 78 Thrush, & Ellingsen, 2016; Wolda, 1988) and of intra-annual relationships between the
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5 79 assemblages and the environment (Heino et al., 2015). We studied the overall, temporal and
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7 80 spatial turnover of macroinvertebrate and fish communities in a Midwestern stream, and
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9 81 discovered that the patterns in taxa turnover are different for organisms at the two taxonomic
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11 82 levels.
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17 84 **Methods**

19 85 Kickapoo Creek (Latitude 39°27', Longitude 88°13') is an approximately 15 km long, low
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21 86 gradient, third order Midwestern stream that drains into the Embarras River, Illinois. The
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23 87 drainage (area 262 km²) is mostly agricultural (63.6%) with grasslands (15.0%), forest (10.1%)
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25 88 and urban areas (10.9%) contributing high sediment loading and nitrate concentrations in the
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27 89 water (Keefer, 2004); the substrate is mostly shifting sand and gravel. Much of Kickapoo Creek
28
29 90 displays low geomorphic stability with high rates of bank erosion; shallow channels; low canopy
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31 91 cover; high sediment loads from the adjacent agricultural fields and homogenous raceway
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33 92 habitats with a relatively uniform depth across the length of the reach (Pant, 2014). Four fixed
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35 93 sites selected for this study were 232 m (Site A), 254 m (Site B) and 192 m (Site C) and 183 m
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37 94 (Site D) in stream length (Figure 1). Water temperature, pH and dissolved oxygen concentration
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39 95 were recorded during every sampling period using a YSI-85 water quality meter.
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44 96 Benthic macroinvertebrate assemblages were sampled during base flow twice every year
45
46 97 (May and September) during 2010-2013 using Illinois Environmental Protection Agency's
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48 98 multihabitat 20-jab method (IEPA, 2007). All major habitats within each site were sampled in
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50 99 approximate proportional representation within the site. Semi-quantitative samples were
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52 100 collected from each site using a rectangular dip-net (dimensions 0.5 m * 0.3 m) attached to a
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3 101 long pole, by “jabbing” or “sweeping,” and stored in 75% ethanol. We subsampled ~300 random
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5 102 individuals from each sample to standardize metrics among sites and years (IEPA, 2007).
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7 103 Macroinvertebrates were identified to the family level because a large fraction of individuals
8
9 104 could not be identified below this level. We assigned tolerance values to each individual based
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11 105 on Merritt, Cummins, & Berg (2008).
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14 106 We sampled fishes in all sites during fall (2009-2013) and spring (2010-2012) using an
15
16 107 AC electrofishing seine following the stream sampling guidelines by Illinois Department of
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18 108 Natural Resources (IDNR, 2001). At each reach, we placed block nets (12 m * 1.2 m, 5 mm bar
19
20 109 mesh) at the upstream and downstream ends to form a closed site. For electrofishing, we used an
21
22 110 8 m electric seine with 12 copper electrodes spaced 0.75 m apart and powered by a 2000 watt
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24 111 AC generator. A six person crew made a single pass moving upstream through each reach to
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26 112 deplete the reach of all fishes. Following the electrofishing sample, downstream block nets were
27
28 113 pulled, and all trapped fishes were collected. All fishes in the upstream blocking seine were
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30 114 released without enumeration. Fishes >100 mm in length were identified based on Smith (2002),
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32 115 measured and weighed in the field, and returned back to the water unharmed. All other fishes
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34 116 were euthanized using a lethal dose of MS-222, preserved in 10% formalin, and later processed
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36 117 in lab. Fishes were assigned to tolerance and feeding guilds following Poff & Allan (1995).
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42 118 We took a multifaceted approach to evaluating differences in α and β diversity between
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44 119 fall and spring seasons. As measures of α diversity, we calculated six commonly used metrics
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46 120 related to composition, structure, and function to represent the macroinvertebrate assemblage.
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48 121 These indices included various indices of community composition (richness, % Chironomidae,
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50 122 exponentiated Shannon index and Pielou’s Evenness) and sensitive taxa (proportion of
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52 123 Ephemeroptera-Plecoptera-Trichoptera [EPT] taxa, Hilsenhoff’s Macroinvertebrate Biotic Index
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3 124 [HBI,Hilsenhoff, 1987]). Sample-specific HBI was calculated as the sum of the tolerance values
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5 125 divided by the total number of individuals sampled at the site. For fish assemblage data from
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7 126 each site, we calculated the following six metrics: rarefied species richness, exponentiated
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10 127 Shannon index, Pielou's Evenness, fish Index of Biotic Integrity (IBI, Karr, Fausch, Angermeier,
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12 128 Yant, & Schlosser, 1986), proportion of Cyprinids and proportion of intolerant species. Rarefied
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14 129 species richness was calculated for each sample as the expected species richness in a random
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16 130 subsample of 1000 individuals. Fish IBI was calculated using the Illinois IBI calculator
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18 131 (<http://dnr.illinois.gov/IBICalculation/NewSampleForm.aspx>), a software designed specifically
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20 132 for the state of Illinois to calculate the integrity scores based on fish species abundance and river
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22 133 attributes such as stream width, slope and region. We also calculated the relative density of
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24 134 fishes (catch per unit effort [CPUE]) in each sample as number of fish captured per hour of effort
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26 135 per 100 m² area.

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31 136 We assessed two separate components of β diversity: spatial and temporal β diversity.
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33 137 Spatial β diversity corresponded with the differences in assemblages among all sites during each
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35 138 sampling period (i.e., one beta per sampling trip), and temporal β diversity corresponded with
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37 139 the differences in assemblages among all sampling periods for each site (i.e., one beta per site-
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39 140 season combination). Each of these components of β diversity were calculated using two
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41 141 measures: First, we calculated β diversity as the multivariate dispersion (MVD) around group
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43 142 centroids in multivariate ordination space (Anderson, Ellingsen, & McArdle, 2006). In this
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45 143 method, taxa assemblage abundance data is used to calculate pairwise dissimilarities among
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47 144 different samples using a dissimilarity index of choice. Here, we used Bray-Curtis dissimilarity
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49 145 index to calculate the dissimilarity matrix, and used it to calculate the mean distances to group
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53 146 centroids in multivariate space. To accomplish this, we used the function `betadisper` in R package

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4 147 vegan (version 2.4-6, Oksanen et al., 2018), with the $\sqrt{\frac{n}{n-1}}$ correction to adjust for the small-
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7 148 sample bias in the estimation of dispersion (Stier, Geange, Hanson, & Bolker, 2013). Second, we
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9 149 used the R package betapart to calculate β diversity as the multiple-site community dissimilarity
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11 150 (β_{MS}), an extension of the Bray-Curtis pairwise dissimilarity index (Baselga, 2017; Baselga &
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13 151 Orme, 2012). β_{MS} is considered a better methodological approach when quantifying the overall
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15 152 dissimilarity among more than two sites (Baselga, 2017). For both MVD and MS approaches,
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17 153 we chose Bray-Curtis dissimilarity index because of its common use in ecological literature,
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19 154 owing to its ideal statistical properties and ability to account for patterns of variations in
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21 155 abundances of species along ecological gradients. In addition, we visualized the differences in β
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23 156 diversity of fish or macroinvertebrate assemblages during spring and fall using two-dimensional
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25 157 non-metric multidimensional scaling (NMDS) ordination plots based on the Bray-Curtis
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27 158 dissimilarity matrices. Community metrics significantly correlated to the ordination axes were
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29 159 superimposed on the plot. We also used indicator species analysis (function indval in package
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31 160 labdsv version 1.8-0; Roberts, 2016) to compute the indicator values of each species within each
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33 161 group, and to find significant indicator species for each group (Dufrene & Legendre, 1997). This
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35 162 approach tries to find species that have high specificity and high fidelity. For all analyses based
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37 163 on MVD, the assemblage data were standardized and transformed to reduce the undue influence
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39 164 of highly abundant species relative to uncommon species. Specifically, macroinvertebrate
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41 165 assemblage data were standardized by dividing by the total count for each sample, and fish
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43 166 community assemblage data were log transformed as suggested by Anderson et al. (2006); for
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45 167 each dataset, these standardizations minimized the multivariate stress when fitting NMDS.

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53 168 We compared site- and sample-specific temperature and dissolved oxygen between
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55 169 seasons using two-sample t-tests. We also compared CPUE of fishes between seasons using a

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3 170 generalized least squares model, correcting for the temporal autocorrelation with an
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5 171 autoregressive (order 1) correlation structure. To test for differences in diversity metrics between
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7 172 seasons, we used separate exact two-sample Fisher-Pitman permutation tests for each index.
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10 173 Because these tests do not directly account for the autocorrelated nature of the data, it was
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12 174 necessary to assess whether the observed changes exceeded the baseline expectations. Thus, we
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14 175 used a null model approach (Gotelli & Graves, 1996), based on a toroidal shift permutation
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16 176 scheme. In this scheme, the start time for each taxon in an assemblage is randomized, such that
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18 177 species abundances vary independently, but within-species temporal-autocorrelation is
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20 178 preserved. For each toroidal shift permutation, we ran an exact two-sample Fisher-Pitman test as
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22 179 described above. We ran the permutations 1500 times to construct the null distribution of Z
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24 180 statistics, and calculated P value as proportion of times when the original Z statistic exceeded the
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26 181 Z statistics from the null distribution (Manly, 2006). To assess the effect of taxonomic resolution
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28 182 on the observed trends, we ran the analyses for fishes at both species and family level.
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184 **Results**

185 Water temperatures during spring and fall samples were not statistically different from
186 each other (mean spring: 16.6 °C; mean fall: 18.2 °C; $t = 1.64$; $P = 0.11$). Macroinvertebrates
187 from 56 families were collected from Kickapoo Creek from 2010 to 2013. The most abundant
188 families were Caenidae (Order Ephemeroptera; mean 32.0%), Chironomidae (Order Diptera;
189 30.1%) and Hydropsychidae (Order Trichoptera; 16.9%) in fall, and Chironomidae (64.0%),
190 Hydropsychidae (16.2%) and Simuliidae (Order Diptera; 4.1%) in spring. Plecoptera were not
191 collected during the four year period; therefore, % EPT only includes Ephemeroptera and
192 Trichoptera.

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3 193 Richness and Shannon diversity of macroinvertebrate families were significantly higher
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5 194 during fall compared to spring (Table 1; Figure 2). Both spatial and temporal β diversities
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8 195 assessed using the MVD approach were significantly higher during fall compared to spring
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10 196 (Table 1; Figure 3). In addition, temporal β diversity based on MS dissimilarity was significantly
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12 197 higher during fall compared to spring, but the corresponding spatial β diversity did not differ
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15 198 between seasons. NMDS ordination showed that season was structured along axis 1 of the
16
17 199 NMDS and that samples collected during spring were markedly more similar to one another
18
19 200 compositionally than fall samples (Figure 4). Five diversity metrics were significantly correlated
20
21 201 to the NMDS ordination; of these, Hilsonhoff's Biotic Index and % Chironomidae were higher in
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23
24 202 the spring samples, and % EPT taxa was higher in fall samples. A total of 7 macroinvertebrate
25
26 203 families were identified as indicator species for either spring or fall samples (Table 2). Taxa
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28 204 typically considered to be tolerant of disturbance or pollution, such as Chironomidae and
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31 205 Simuliidae, were indicative of fall, and taxa typically considered indicative of good stream
32
33 206 health, such as members of Caenidae and Coenagrionidae, were indicative of spring samples
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35 207 (Table 2).

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38 208 Within Kickapoo Creek electrofishing samples, 98,938 fish from 11 families (49 species)
39
40 209 were collected, with Cyprinidae the most common family (88.8%), followed by Centrarchidae
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42 210 (3.8%) and Percidae (2.7%). All of the five most common species were Cyprinids: sand shiner
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44 211 (*Notropis stramineus*, 29.1%), spotfin shiner (*Cyprinella spiloptera*, 17.7%), silverjaw minnow
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46 212 (*Notropis buccatus*, 15.4%), bluntnose minnow (*Pimephales notatus*, 12.7%) and central
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49 213 stoneroller (*Campostoma anomalum*, 7.0%).

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51 214 Compared to spring samples of fishes, fall samples had significantly higher CPUE ($F =$
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53 215 33.19, $P < 0.001$; Figure 5), exponentiated Shannon Index and species richness (Table 1). In

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3 216 contrast, there were no seasonal differences in spatial or temporal β diversity indices (Table 1;
4
5 217 Figure 3). The results were similar at both taxonomic resolutions of the fish assemblage, except
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8 218 for family richness, which was not significantly different between spring and fall samples (Table
9
10 219 1). Similar to macroinvertebrates, season was largely structured along axis 1 for fishes as well
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12 220 (Figure 4). Of the four diversity metrics that were significantly correlated to the NMDS
13
14 221 ordination, fish IBI was higher in the fall samples, and percent intolerant species was higher in
15
16 222 spring samples (Figure 4). A total of 13 fish species were identified as indicator species for either
17
18 223 spring or fall samples (Table 2). We did not observe any distinct differences in the functional
19
20 224 form or morphology of the indicator species for the spring and fall seasons. However, it should
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22 225 be noted that species typically associated with smaller streams, such as silverjaw minnow and
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24 226 central stoneroller, were identified as indicator species for fall but not spring season (Table 2).
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31 **Discussion**

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33 229 Our research showed that seasonal β diversity patterns in Kickapoo Creek are uncoupled
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35 230 from β diversity patterns, and that these differences are taxa-specific. We also showed that these
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37 231 patterns are robust to taxonomic resolution (species versus families in fishes).
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39

40 232 There were few analogous results between the macroinvertebrate and fish communities
41
42 233 regarding α and β diversity, suggesting different drivers at these two taxonomic/trophic levels.
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44 234 High instability and stress at the reach and stream levels likely affect macroinvertebrate and fish
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46 235 communities differently in Kickapoo Creek. Because landscape structures often influence
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48 236 diversity at population-genetic and community levels similarly in terrestrial, freshwater as well
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50 237 as marine systems (Chust et al., 2016; Finn et al., 2013; Finn & Poff, 2011; Vellend, 2005), we
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52 238 suspect that differences may occur at population-genetic levels of macroinvertebrates and fishes
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3 239 as well. Seasonal differences in α and β diversity has been shown to be affected greatly by
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5 240 temperature (Cook et al., 2018; Magurran, Dornelas, Moyes, Gotelli, & McGill, 2015); in
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7 241 Kickapoo Creek, the observed differences could not be attributed to temperature differences
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9 242 because spring and fall samples did not differ in water temperature. This suggests that several
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11 243 factors other than temperature also play a crucial role in seasonal differences in diversity and
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13 244 need to be studied more.
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17 245 There is growing consensus that interannual changes in α diversity is fairly low and
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19 246 stable for freshwater phytoplankton, macroinvertebrates and fishes (Goheen, White, Ernest, &
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21 247 Brown, 2005; Gotelli et al., 2017; Hillebrand et al., 2018; Magurran et al., 2018), suggesting that
22
23 248 community regulation is a general feature across taxa and ecosystems. However, major
24
25 249 disturbances or natural shifts in environmental conditions may act as ecological filters and cause
26
27 250 a major change in α diversity over time or space (Anderson et al., 2011; J. M. Chase, 2007).
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29 251 Such severe changes may result from long-term, predictable changes in environment (e.g. global
30
31 252 temperature rise, bleaching of the coral reefs, etc.) or abrupt changes (e.g. oil spill, drought, etc.).
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33 253 In temperate regions of Midwestern United States, different seasons may present drastically
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35 254 different environmental conditions. Life history adaptations of local species to such differences
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37 255 have led local species to exploit different temporal (e.g. intra- or inter-annual differences) and
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39 256 spatial niches (e.g. headwater streams versus higher-order streams, Chase, Kraft, Smith, Vellend,
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41 257 & Inouye, 2011; Cook et al., 2018; Vannote, Minshall, Cummins, Sedell, & Cushing, 1980).
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43 258 Thus, season may directly affect α and β diversities through deterministic or random ecological
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45 259 filtering (Chase, Kraft, Smith, Vellend, & Inouye, 2011).
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51 260 Season change seemed to act as a systematic ecological filter for macroinvertebrates in
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53 261 Kickapoo Creek, such that specific families of macroinvertebrates were affected. Therefore,
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3 262 taxa-specific differences in life history strategies may be an important factor in explaining
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5 263 observed differences. Chironomidae larvae generally emerge as adults during spring and
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7 264 summer, and thus, may not be present in the stream during fall. On the contrary, *Caenis* sp.
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10 265 demonstrate large variations in their life history patterns (e.g. *C. luctuosa* [Cayrou & Céréghino,
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12 266 2003]; *C. horaria* [Menetrey, Oertli, Sartori, Wagner, & Lachavanne, 2008]) with individuals of
13
14 267 these species present in the water throughout the year. This suggests that Ephemeropterans may
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16 268 fill the niche left open by the absence of Chironomidae during spring. Another possible
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18 269 explanation may be provided by the homogenizing effect of the massive spawning of
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20 270 macroinvertebrates during spring (Thorp & Covich, 2009). Stress due to extreme temperature,
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22 271 desiccation and low foraging opportunities during summer may cause differential mortality
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24 272 among taxa, and thus, community composition could be highly heterogeneous during fall. The
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26 273 indicator macroinvertebrate taxa identified by indicator species analysis also reflect seasonal
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28 274 differences in life cycle, with families such as Coenagrionidae and Calopterygidae, indicative of
29
30 275 spring, occurring as adults during much of summer and fall (Thorp & Covich, 2009).

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33 276 Ephemeroptera are typically associated with higher-quality conditions. However,
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35 277 Ephemeroptera species observed in Kickapoo Creek (most importantly *Caenis* sp. and *Baetis* sp.)
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37 278 are relatively tolerant taxa that are commonly found in degraded streams (e.g., Barbour,
38
39 279 Gerritsen, Snyder, & Stribling, 1999; Hilsenhoff, 1987). Thus, the high proportions of
40
41 280 Ephemeroptera, and particularly, *Caenis* spp., in our samples do not necessarily indicate that
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43 281 Kickapoo Creek is a pristine and high-quality ecosystem. This also explains why the Biotic
44
45 282 Index was a strong predictor for spring compared to fall, even though (1) EPT taxa were
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47 283 proportionately more abundant in fall, and (2) Chironomidae, a taxa typically associated with
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49 284 polluted waters, were proportionately more abundant in spring.
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3 285 Observed seasonal differences in fish diversity suggest random sampling effects of
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5 286 season such that entire sampling locations may be affected by seasons. Unlike
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7 287 macroinvertebrates, upstream fish movement and migration can be directly hindered by harsh
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9 288 conditions in the downstream locations. For example, low flow during summer may prevent the
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11 289 upstream movement of migrating fish towards the upstream sites, and thus, all fish species in the
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13 290 upstream sites are affected. As a further example from Kickapoo Creek, during summer 2013, a
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15 291 beaver dam had formed at the downstream end of the site C, thus dramatically increasing the
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17 292 mean depth, obliterating the riffles, and decreasing the stream flow in the entire site. The fall
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19 293 2013 sample for site C included a greatly reduced number of minnows (which are most common
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21 294 in shallow raceway habitats) and darters (which prefer riffles, Smith, 2002). Such dramatic
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23 295 change in species composition was clear in the NMDS plot as well. It should be noted, however,
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25 296 that even though season seemed to affect macroinvertebrates deterministically and fishes via
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27 297 random, reach-specific processes, the actual effect is likely due to a combination of the two
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29 298 processes.

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31 299 Our results may also inform the sampling strategies to be employed when assessing the
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33 300 biodiversity of fishes and macroinvertebrates in Midwestern streams. Significant differences in
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35 301 alpha diversity of macroinvertebrates and fishes in Kickapoo Creek means that long-term
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37 302 monitoring projects, often conducted only once a year, need to be conducted during the same
38
39 303 season for valid comparison. The choice of the season may depend on beta diversity differences
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41 304 between seasons. Lower temporal and spatial β diversity of macroinvertebrates during spring
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43 305 suggests that sampling during spring would allow assessing true changes in assemblages with the
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45 306 need to sample few sites. On the contrary, owing to similar β diversity between spring and fall,
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47 307 choice of season for sampling fishes is not clear. However, NMDS suggested higher overall
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3 308 homogeneity of samples during fall, suggesting that samplings should be conducted during fall.
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5 309 Similar results were also obtained in the Vermillion River, Illinois, where Hastings, Meiners,
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7 310 Colombo, & Thomas (2016) observed more homogenous composition of fishes during fall
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9 311 compared to spring. These results suggest that fish sampling during spring should be conducted
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11 312 in either large reaches or several smaller reaches at multiple occasions (i.e. multiple years).
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13 313 Furthermore, % intolerant fish species was correlated with the NMDS such that higher
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15 314 proportion of intolerant species were collected in spring samples. Also, some large-river-
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17 315 associated fishes, such as the longnose gar (*Lepisosteus osseus*) were only observed in—and
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19 316 identified as indicator species for—the spring samples. Longnose gar and other large-river
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21 317 associated-fishes often migrate upstream into the creek from the Embarras River during spring
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23 318 for spawning (Johnson & Noltie, 1996); some of these species/individuals may remain in the
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25 319 creek during the fall if deeper habitats are available (Schlosser, 1987). Similar life history
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27 320 differences may also explain the observed high density of fishes in fall compared to spring
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29 321 samples. Fall samples likely included young of year fish that were larval stage earlier in the
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31 322 summer. Winter mortality may also contribute to fewer fish in the spring. Finally, fishes tend to
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33 323 stay in one general area during warmer periods (Barbour et al., 1999); therefore, the catchability
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35 324 might have increased during fall samples.
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42 325 We have shown that season has a different effect on species richness and turnover of
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44 326 macroinvertebrates compared to fishes in Kickapoo Creek. We suspect similar patterns exist in
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46 327 other streams as well because most third- to fifth-order streams in the Midwestern United States
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48 328 are disturbed and face issues similar to Kickapoo Creek (e.g. siltation, agricultural runoff,
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50 329 effluent from wastewater treatment plant, etc.). However, stochastic interannual and reach-
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52 330 specific factors also play important role in determining species turnover among
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3 331 macroinvertebrates and fishes. Therefore, frequent and rigorous assessment of animals need to be
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5 332 conducted to understand stream conditions and beta diversity to inform conservation decisions
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8 333 (Socolar, Gilroy, Kunin, & Edwards, 2016).
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11 335 **Acknowledgments**

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13
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15
16
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23
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25
26 341 Center and the U.S. Geological Survey.
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29 343 **Conflict of Interest**

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33 344 The Author declare no conflict of interest.
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For Peer Review

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3 507 Table 1: P-values associated with randomization tests comparing the observed differences in α
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5 508 and β diversity of macroinvertebrates and fish communities during spring and fall with a null
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8 509 distribution generated by a toroidal shift permutation. See text for details.
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Taxa	α diversity		β diversity			
	Shannon Index	Richness	Spatial		Temporal	
			MVD	MS	MVD	MS
Macroinvertebrates	0.027	0.048	0.034	0.168	0.029	0.014
Fish (Species)	0.001	0.006	0.907	0.717	0.482	0.443
Fish (Family)	0.035	0.061	0.901	0.707	0.536	0.229

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3 511 Table 2: Indicator species for fishes and macroinvertebrates collected from Kickapoo Creek,
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5 512 Illinois during spring and fall 2009-2013.
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Season	Indicator species (taxa)	Indicator value
Macroinvertebrates (Family)		
Fall	Chironomidae	0.72
Fall	Simuliidae	0.59
Spring	Caenidae	0.95
Spring	Coenagrionidae	0.88
Spring	Calopterygidae	0.83
Spring	Tricorythidae	0.47
Spring	Corydalidae	0.31
Fishes (Species)		
Fall	Johnny darter	0.85
Fall	Longear sunfish	0.84
Fall	Orangethroat darter	0.83
Fall	Central stoneroller	0.81
Fall	Rainbow darter	0.75
Fall	Silverjaw minnow	0.75
Fall	Golden redhorse	0.73
Fall	Redfin shiner	0.7
Fall	Largemouth bass	0.68
Fall	Mosquitofish	0.4
Spring	Golden shiner	0.33
Spring	Dusky darter	0.25
Spring	Longnose gar	0.25

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3 514 **Figure Legends**
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5 515 Figure 1: Sampling locations in Kickapoo Creek southwest of Charleston, Illinois.
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10 517 Figure 2: Rarefied taxa richness and exponentiated Shannon Index for macroinvertebrates and
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12 518 fishes collected from Kickapoo Creek during spring and fall of 2009-2013. See table 1 and text
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14 519 for details on statistical differences.
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19 521 Figure 3: Spatial and temporal β diversity indices based on multiple-site dissimilarity (MS) and
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21 522 multivariate dispersion (MVD) approaches for macroinvertebrates and fishes collected from
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23 523 Kickapoo Creek during spring and fall of 2009-2013. See table 1 and text for details on statistical
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25 524 differences.
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30 526 Figure 4: Non-metric Multidimensional Scaling (NMDS) plot of macroinvertebrate (a) and fish
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32 527 (B) communities sampled from Kickapoo Creek during spring (\circ) and fall (\blacksquare) of 2009-2013.
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34 528 Arrows represent the strength and direction of community metrics that are significantly
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36 529 correlated to the ordination axes. Labels for each sample denote site and sample year.
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41 531 Figure 5: Mean total fish CPUE (catch/(hr * 100 m²)) was significantly higher during fall
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43 532 compared to spring in Kickapoo Creek (2009-2013).
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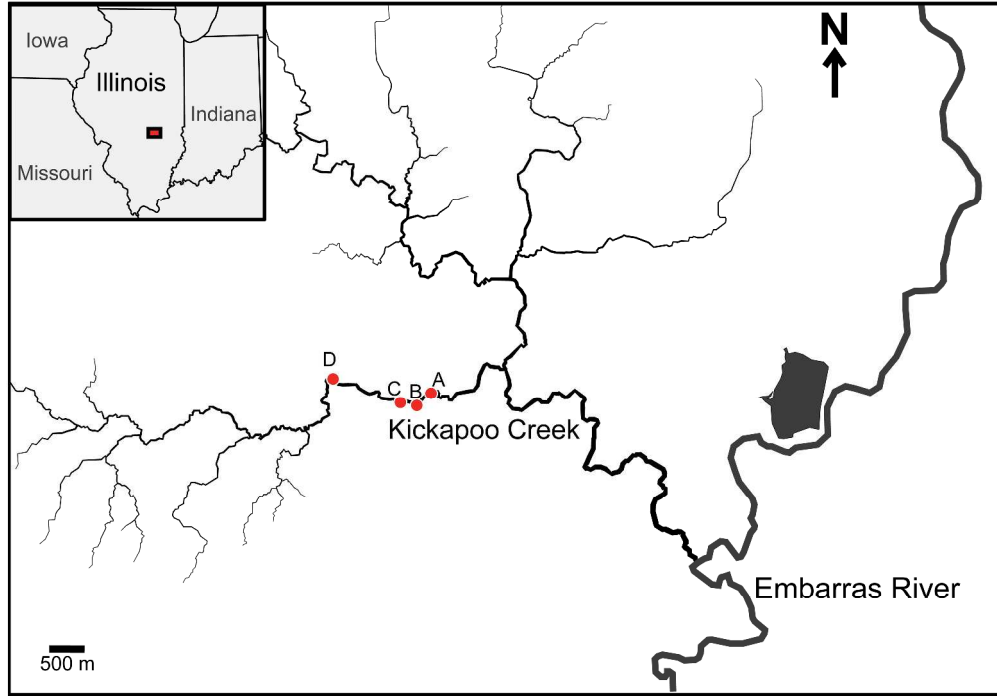


Figure 1. Sampling locations in Kickapoo Creek southwest of Charleston, Illinois.

571x396mm (300 x 300 DPI)

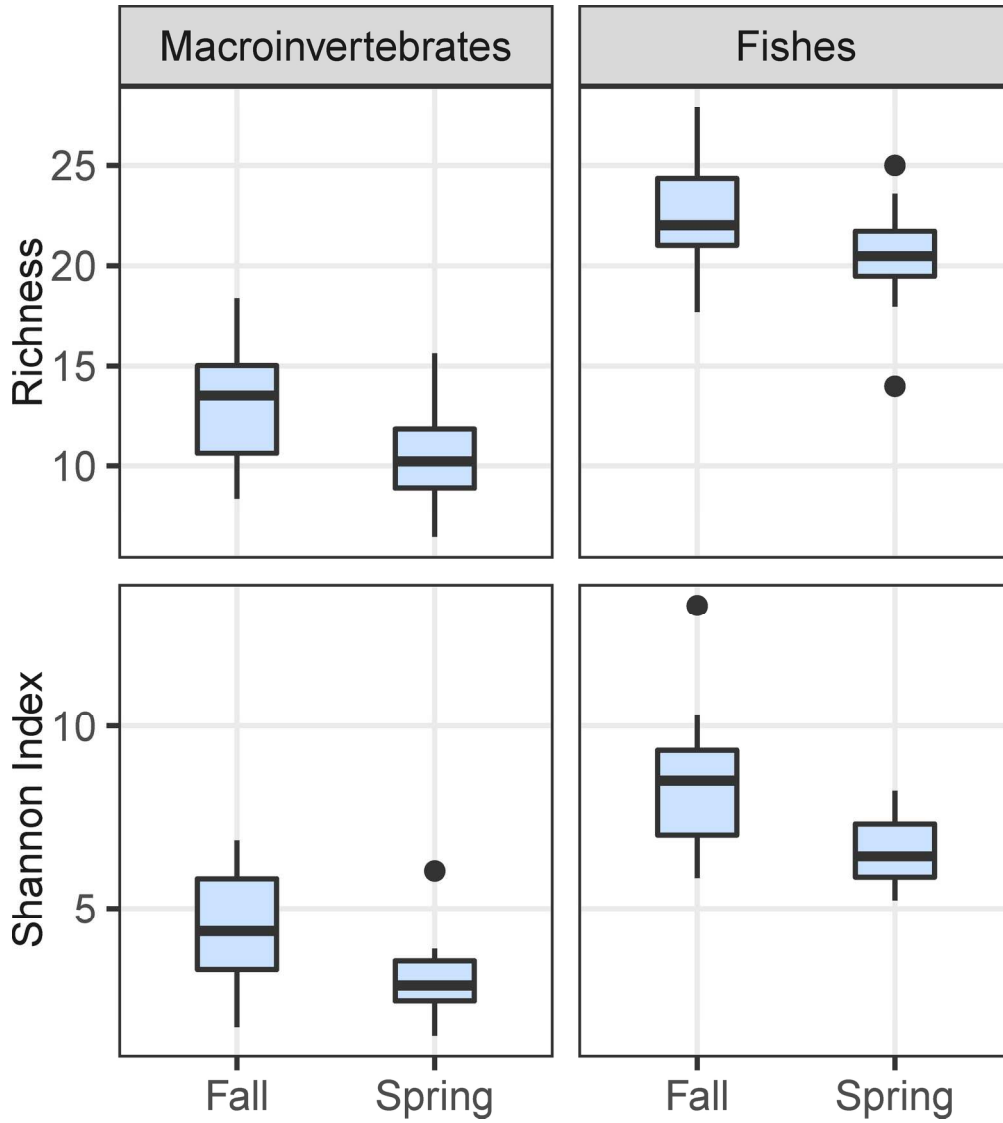
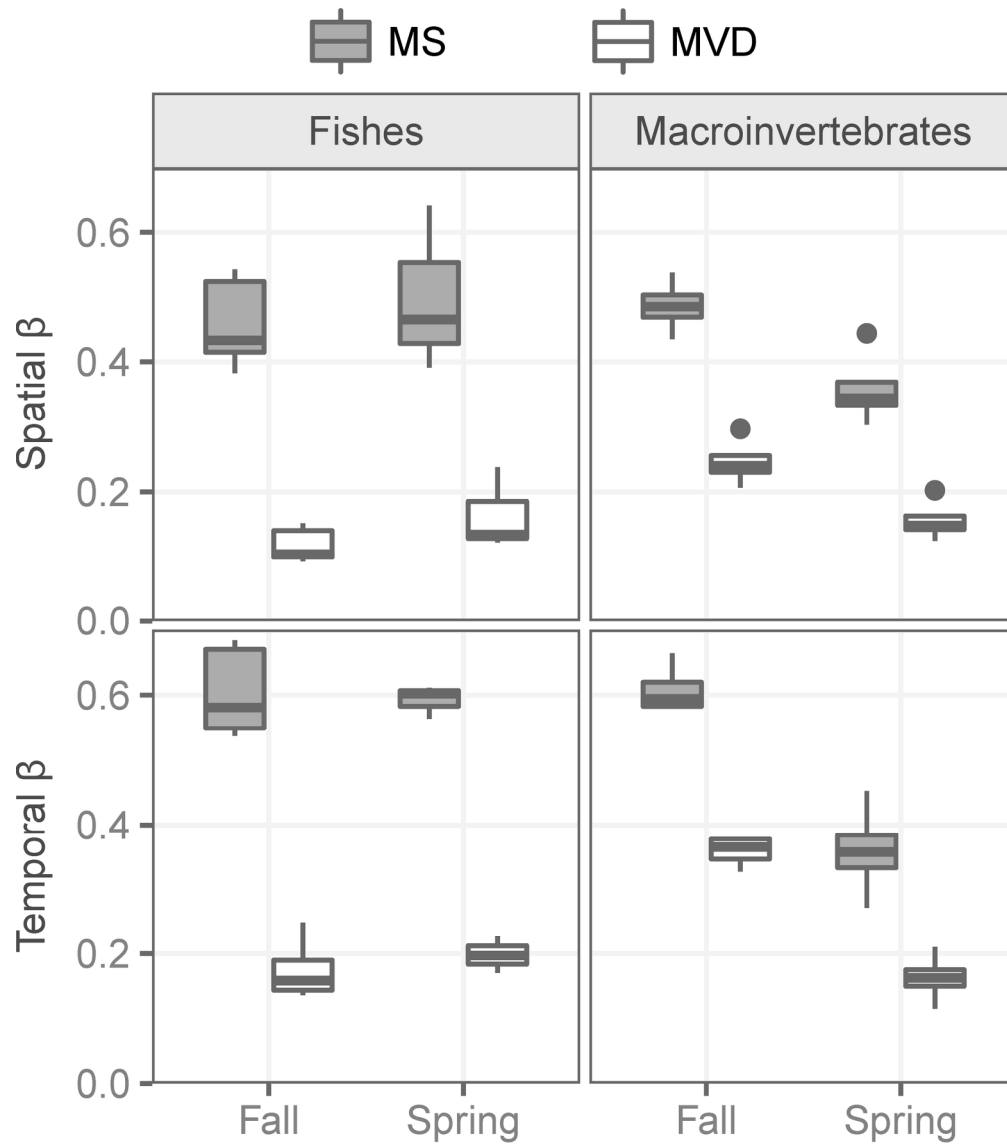


Figure 2. Rarefied taxa richness and exponentiated Shannon Index for macroinvertebrates and fishes collected from Kickapoo Creek during spring and fall of 2009-2013. See table 1 and text for details on statistical differences.

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Spatial and temporal β diversity indices based on multiple-site dissimilarity (MS) and multivariate dispersion (MVD) approaches for macroinvertebrates and fishes collected from Kickapoo Creek during spring and fall of 2009-2013. See table 1 and text for details on statistical differences.

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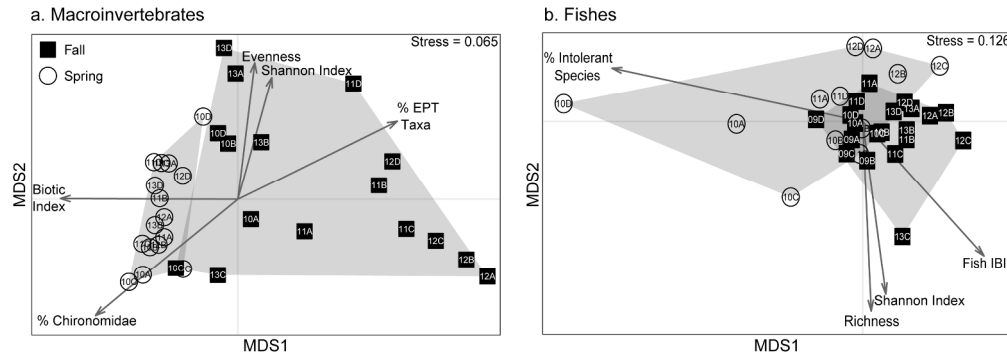


Figure 4. Non-metric Multidimensional Scaling (NMDS) plot of macroinvertebrate (a) and fish (B) communities sampled from Kickapoo Creek during spring (○) and fall (■) of 2009-2013. Arrows represent the strength and direction of community metrics that are significantly correlated to the ordination axes. Labels for each sample denote site and sample year.

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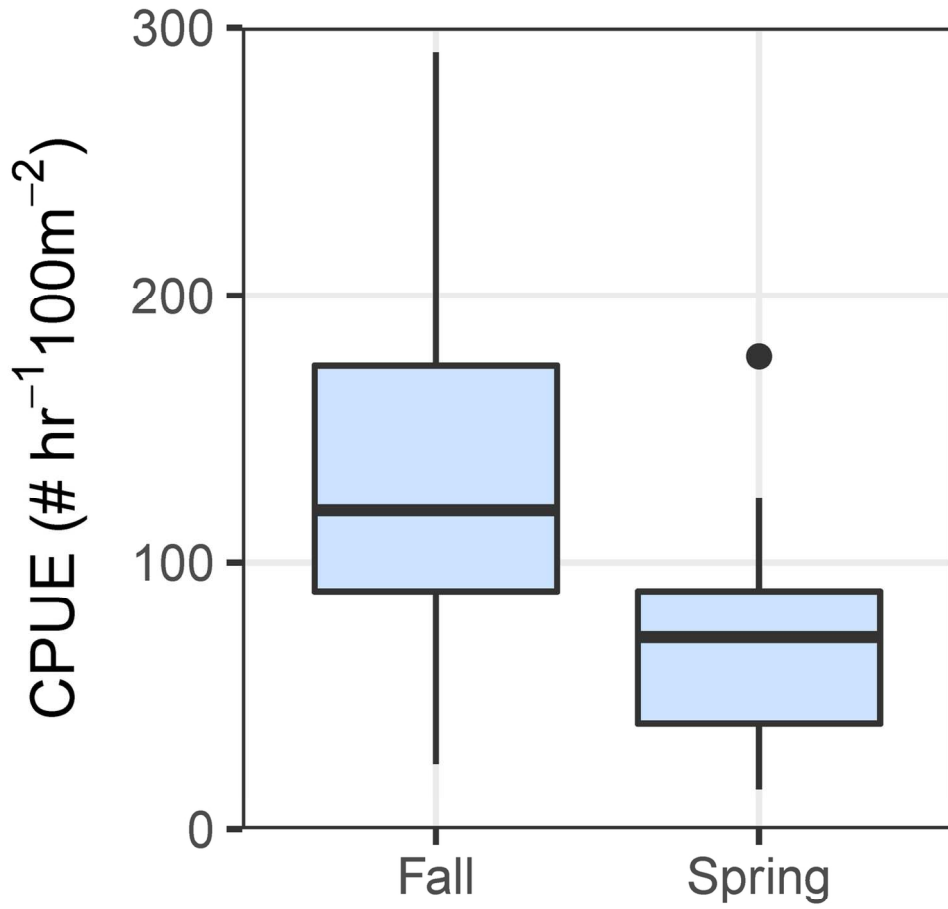


Figure 5. Mean total fish CPUE (catch/(hr * 100 m²)) was significantly higher during fall compared to spring in Kickapoo Creek (2009-2013).

63x63mm (600 x 600 DPI)