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Intra- and Inter-Watershed Variability in Benthic Macroinvertebrate Community Diversity, Taxa Richness, and Biotic Integrity: Citizen Scientist Sampling Within a Minnesota USA Region Dominated by Agriculture

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ABSTRACT: Volunteer citizen scientists collected benthic macroinvertebrate samples from 35 streams throughout multiple watersheds in southeastern Minnesota, USA, during the period 1999–2013 to assess community diversity, taxa richness, and biotic integrity as indicators of water quality and general habitat conditions. In total, 452 invertebrate samples containing >46,000 organisms were collected, processed, and analyzed. Only 45% of the citizen scientists completed their 5-year sampling commitment. However, their samples generally demonstrated significant differences in total taxa richness, Ephemeroptera-Plecoptera-Trichoptera (EPT) taxa richness, Simpson and Shannon diversities, and a regional benthic index of biotic integrity (BIBI) within and/or among the watersheds examined. Streams in the two larger watersheds averaged significantly higher taxa richness and BIBI scores than those in smaller watersheds. Overall, streams in this region exhibited mostly poor or very poor biotic integrity based on their macroinvertebrate communities, indicating continued impacts from environmental stressors within these agricultural watersheds.

Keywords: Benthic macroinvertebrates; Taxa richness; Diversity; Biotic integrity; Citizen scientists

1. Introduction

Aquatic benthic macroinvertebrates are an important part of stream and river food webs, linking basal resources to higher level consumers [1–3]. Macroinvertebrates typically occur in lotic systems ranging from headwater spring creeks to great rivers [1]. Because they frequently exhibit high abundance and biodiversity [2,3] and possess a wide range of sensitivities to habitat disturbance, impaired water quality, and unnatural temperature changes [4,5], benthic macroinvertebrates have been used extensively to monitor conditions in flowing water systems [6–10]. Aquatic invertebrate communities normally respond to a variety of

environmental stressors in consistent and predictable ways [6–9,11], allowing investigators to determine the type and intensity of the stressor causing the response [8,11].

Volunteer citizen scientists from around the world have monitored stream and river quality by sampling and analyzing benthic macroinvertebrate communities [12–21]. Citizen scientist collections may be used to supplement limited sampling efforts by professional agency scientists, to save money for other conservation projects or activities, and to increase awareness and enthusiasm among the general public for protecting surface waters from potentially harmful human activities [15,16,18]. Some citizen science invertebrate monitoring programs may be extensive, spanning state- or nation-wide and involving hundreds to thousands of volunteer monitors [14,17,18,22,23], whereas others may be more regional and involve far fewer citizen scientists [12,13,16]. Regardless of program size, the contributions of citizen scientists monitoring benthic macroinvertebrates in streams and rivers have been proven to be of high value [13,15,16,20,21]. Even when monitoring protocols are greatly simplified to accommodate volunteers' skill levels, results can be as useful as those obtained by professionals using much more complex protocols [20].

Macroinvertebrate communities have been used for over a century to assess water quality impairments in streams and rivers in southeastern Minnesota, USA [24–27]. However, macroinvertebrate surveys within this region usually have been intermittent, short-term, and highly localized academic research efforts, with no coordinated efforts to survey large numbers of streams throughout multiple watersheds using consistent protocols over an extended time period. Even the Minnesota Pollution Control Agency, which is tasked with systematically monitoring fish and macroinvertebrate communities in streams and rivers throughout the state to assess compliance with state and national water quality standards, is limited (by time, personnel, and funding) to surveying randomly selected sites within a given watershed only once every 10 years [28]. Clearly, additional stream macroinvertebrate monitoring, covering multiple watersheds more frequently than once each decade and using standardized protocols, would positively supplement the region's assessments of water quality.

This study was undertaken to expand and enhance the monitoring of lotic macroinvertebrate communities in streams and rivers within southeastern Minnesota, adding to the efforts of state agencies, academic professionals, and non-profit groups who have been using this biotic group for water quality assessment. This region was selected for this effort due to strong public interest in regional stream water quality and the high value of local stream fisheries, especially for trout (L. Gates, Minnesota Department of Natural Resources–Fisheries, retired). Local citizen scientists committed to a multi-year monitoring program paired with organism identification by professionals produced a large data set allowing for region-wide comparisons of macroinvertebrate biotic integrity, diversity, and taxa richness across regional watersheds. It was hypothesized that these comparisons would reveal significant differences in macroinvertebrate community metrics both within and among watersheds influenced by karst geology and past and present land use practices.

2. Study Area

Southeastern Minnesota USA, is predominantly an agricultural region underlain by karst geology and drained by an extensive network (>1500 km) of streams and rivers [29,30]. Many of these are sustained by groundwater spring discharges and are categorized as coldwater systems (summer maximum water temperatures <20 °C), others are warmwater systems (summer maximum water temperatures >25 °C) supported primarily by surface runoff, and finally some are intermediate coolwater streams and rivers (summer maximum water temperatures 20–25 °C) [29]. All lotic systems within this region drain to the Mississippi River, many formed originally as outlet streams from the melting Des Moines lobe of the late Wisconsin glaciation approximately 10,000 years ago [30]. This study examined benthic macroinvertebrate communities in three of the five largest drainages in the region (Zumbro River, Whitewater River, and Root

River watersheds) as well as several smaller watersheds lying between the larger systems (Figure 1). All study watersheds are dominated by agricultural land uses (e.g., croplands, pasture/haylands; Table 1).

Table 1. Percentage land uses within each of the study watersheds in southeastern Minnesota, USA.

Watersheds	Land Use Categories (%)				
	Croplands	Pasture/Haylands	Forests	Wetland/Water	Developed
Whitewater	58	8	13	14	7
Root	48	20	26	1	5
Zumbro	56	23	10	9	2
Others	50	12	25	8	5

This region has a history of poor agricultural land use practices during the late-1800s and early-1900s that resulted in severe soil erosion and catastrophic consequences for the streams and rivers that flowed through the impacted watersheds [29,31–33]. Destruction and recovery (via stocking and other management activities) of lotic fish communities have been documented [33], but the effects of past land abuse followed by more recent conservation practices have not been specifically examined or on a broad regional scale [25–27,34].
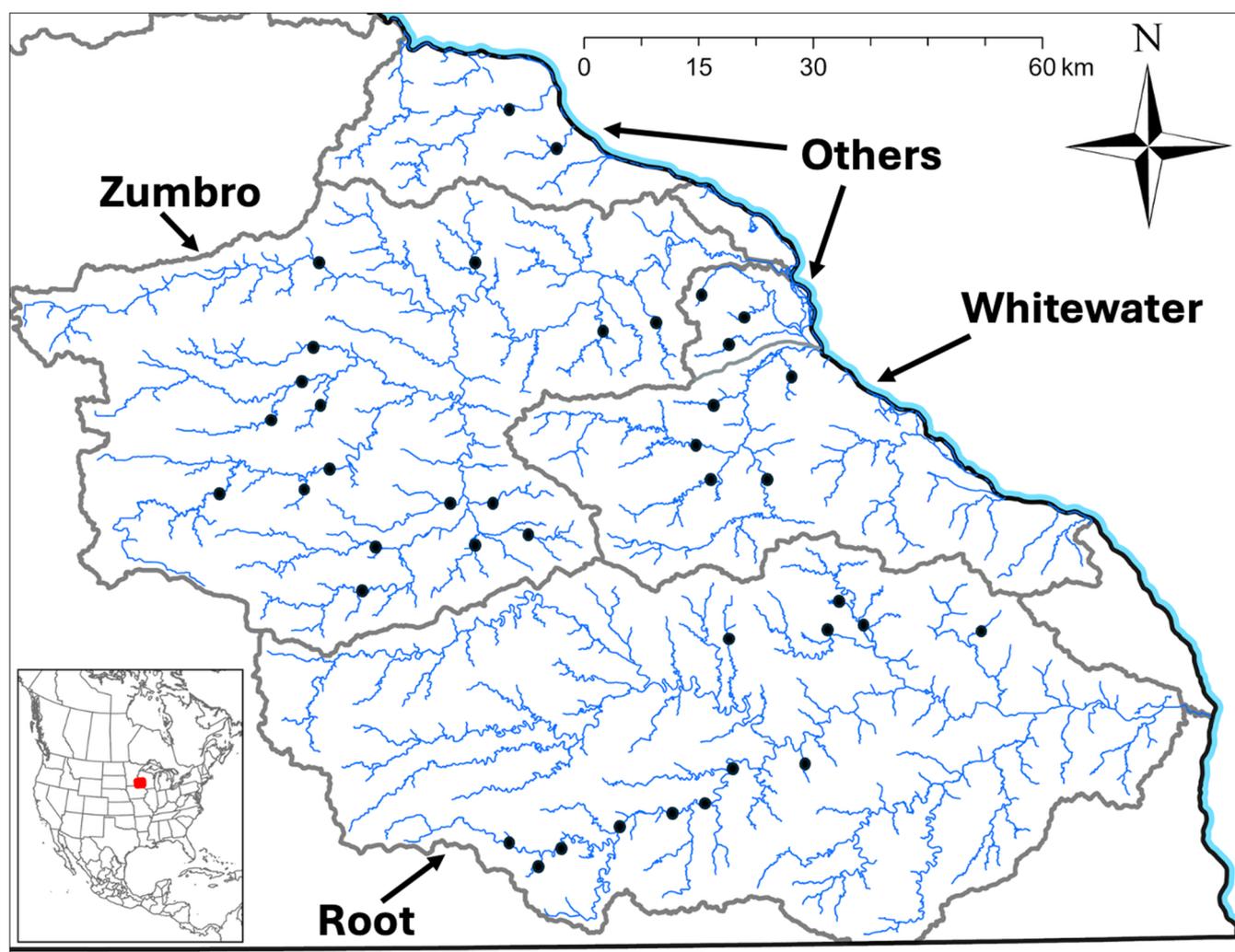
Figure 1. Map depicting 40 stream sites (black dots) in three major watersheds (Zumbro, Whitewater, Root) and other small watersheds (Others) in southeastern Minnesota, USA, where volunteer citizen scientists monitored benthic macroinvertebrates during 1999–2013. The inset maps show the location of the study area (red square) in North America. Gray lines depict watershed boundaries.

3. Methods

Project sampling was coordinated by a collaboration between the Minnesota Department of Natural Resources-Fisheries, Trout Unlimited members, university scientists, and volunteer citizen scientists. Volunteers (e.g., private landowners, anglers, state park personnel, county water planners, high school teachers/students) were recruited to participate based on interest in a specific stream, willingness to follow specific sampling protocols, and ability to continue annual sampling for a period of five years. Samples were collected, sorted, and preserved by volunteers, transferred to a single experienced individual (Trout Unlimited member with professional experience in benthic macroinvertebrate identification) for a quality assurance check (see description in Section 3.2 below), and submitted to university personnel for identification and enumeration. The program continued for 15 years, from 1999–2013.

3.1. Field Collecting Protocol

Volunteers were asked to make benthic macroinvertebrate collections each year between mid-July (late summer) and mid-October (mid-autumn), preferably in September or early October during the period of maximum invertebrate abundance in streams within the region. Benthic invertebrates are also large enough at this time to be easily noticed and picked from samples by volunteers. It was recommended that volunteers collect samples from their stream during approximately the same one- or two-week time period each year that they participated.

After choosing which stream they would sample, participants were asked to select and make their collections from within a 50–75-m reach of their stream. The reach chosen was meant to be representative or typical of the stream overall to prevent targeted and potentially biased sampling [35,36]. An experienced collector (a Trout Unlimited member with a professional background in benthic macroinvertebrate collection and identification) accompanied new volunteers on their first sampling effort to evaluate the selected sampling site, oversee general procedures, highlight potential errors or biases that could occur, and help volunteers to recognize typical macroinvertebrates [22,35,36]. Within each stream reach, separate benthic macroinvertebrate samples were collected from each of three separate riffles (shallow water, faster current velocity with disturbed surfaces, coarse substrates). Riffle-only sampling was chosen to simplify procedures and eliminate possible oversampling of specific habitat types when multihabitat protocols are used [36]. Samples were collected with a D-frame aquatic dip net (0.5-mm mesh) by placing the net against the stream bottom (open end facing upstream) and disturbing the coarse sediments upstream from the net by kicking for 30 s, causing dislodged invertebrates to be washed downstream into the net. Two, 30-s kicks were conducted in each riffle (one in a fast portion, one in a slow portion), and all invertebrates collected were combined into a single riffle sample.

Invertebrates were either picked from debris in the field and preserved in 90% isopropyl alcohol, or the entire sample with debris was preserved prior to later sorting indoors. Volunteers were allowed to use low-magnification (under 5×) hand lenses to aid in sample sorting. Field or indoor sample sorting used a shallow gridded pan. Volunteers were instructed to spread the sample evenly throughout the pan, then randomly select grid squares to remove invertebrates, continuing to sort out all invertebrates (all sizes from large to small to avoid bias) one grid square at a time until approximately 100 organisms had been sorted. If samples contained fewer than 90 organisms, volunteers were encouraged (but not required) to conduct another 30-s kick sample to collect additional organisms. Volunteers were discouraged from collecting >120 organisms/sample or spending >30 min sorting each sample to prevent bias [37]. It is acknowledged that a fixed-count approach likely underestimates any taxa richness metrics assessed [38], but it should not affect comparisons when all samples are collected using this same protocol.

Each year, prior to making collections, citizen scientists checked in with the same experienced collector who assisted them with their initial collection. This gave the volunteers an opportunity to ask questions

about sampling and sorting or to work through any problems that may have arisen previously. Submitting their sorted samples to the same experienced taxonomist each year (see below) provided another opportunity for volunteers to ask questions or seek advice. These types of regular connections have been suggested to improve retention rates of volunteers in citizen scientist programs [39,40].

3.2. Quality Assurance Check, Laboratory Identifications, and Data Returns

All invertebrate samples were subjected to a brief quality assurance check by an experienced invertebrate taxonomist. Each sample was inspected for: proper labelling (stream, date of collection, collector name or collector identification code number, sample number), adequate preservation, lack of debris (*i.e.*, clean sort), and general sample size (to ensure an adequate number of organisms per sample). When a sample passed these checks, it was forwarded for identification.

In the laboratory, all invertebrates in each sample were counted and identified (typically to genus level) [41–47] with the aid of a dissecting microscope (8 to 50× magnification; Nikon SMZ645, Nikon Instruments Inc., Melville, NY, USA). However, midge larvae and pupae (Chironomidae) were identified only to the family level.

After sample identifications were completed, data (list of taxa and counts for each sample, scores and ratings for each sample based on a regional biotic integrity index; see below) were summarized in tabular and graphical formats for return to individual volunteers. Each year, new data were added to information from previous years so volunteers could visualize changes (or lack of them) in macroinvertebrate communities in their chosen streams. This regular feedback was intended to keep volunteers engaged to continue with the monitoring program by demonstrating how their data could be used and what it meant for their stream.

3.3. Data Analyses

Several diversity, taxa richness, and biotic integrity metrics were calculated for all benthic macroinvertebrate samples collected by citizen scientist volunteers, to determine if the various metrics performed similarly in their ratings of the benthic macroinvertebrate communities. The Simpson diversity index (range from 0 [no diversity] to a maximum of 1) and the Shannon diversity index (base 10; range from 0 [no diversity] to a maximum of $\log_{10}[X]$ where X = number of taxa) were calculated for each individual sample using Quantan Quantitative Analysis in Ecology software (May 1997 version) [48]. The total number of taxa (total taxa richness; genus for all organisms except for Chironomidae midges [family]) and the number of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) taxa (EPT taxa richness; based on identification to genus) were also tabulated for each sample. Previous research in southeastern Minnesota (using fixed-count [100 organism] sampling) indicated that impaired stream sites averaged less than eight total taxa (based on genus-level identification) and three EPT taxa per sample, whereas high-quality reference sites averaged >15 total taxa and eight EPT taxa [49]. Finally, a regional, multimetric benthic invertebrate biotic integrity index (BIBI) [49,50] was calculated for each macroinvertebrate sample as an indicator of benthic biological integrity at the stream sites. This specific BIBI was developed and validated using data from 100-organism samples collected by citizen scientists and other researchers [49]. Scoring for the BIBI (possible scores from 0 to 100) was based on values for 10 different invertebrate community metrics that included various forms of taxa richness, tolerance to environmental stressors, feeding strategies, and longevity measures [49] (Table 2). Each metric received a score of 0, 5, or 10 based on the value for that metric, and collectively they produced ratings such as: excellent (total BIBI scores ranging from 65–100), good (50–60), fair (30–45), poor (10–25), and very poor (0–5) [49].

Table 2. Benthic index of biotic integrity (BIBI) metrics and scoring (0, 5, or 10) based on numbers of taxa (mostly genera except for Chironomidae midges) or percentages of organisms in individual samples. Sample BIBI score was the sum of all 10 metric scores (possible BIBI score range between 0 and 100). Table adapted from [49].

	Score		
Metric	**10**	**5**	**0**
Percent Plecoptera	>12	6–12	<6
Percent long-lived	>12	6–12	<6
Percent predators	>13	6.5–13	<6.5
Number of total taxa	>12	7–12	0–6
Number of Plecoptera taxa	>1	1	0
Number of Trichoptera taxa	>3	2–3	0–1
Number of long-lived taxa	>1	1	0
Number of Diptera taxa	>4	3–4	0–2
Number of intolerant taxa	>3	2–3	0–1
Number of filterer taxa	>4	3–4	0–2

Simpson and Shannon diversities, total taxa and EPT taxa richness, and BIBI scores for all years were grouped by watershed, summarized, and compared among the four watershed groupings surveyed (Whitewater, Root, Zumbro, Others). Each community measure was compared among watersheds in two ways. First, watershed values were compared with separate Kruskal-Wallis tests, and if significant differences were detected, Tukey's honest significant difference (HSD) tests were used to detect which watersheds differed from the others (VassarStats: Website for Statistical Computation; vassarstats.net; accessed on 10 January 2026). Second, variable values within each watershed were grouped into five or six bins or categories, and distributions of the counts within bins were compared among watersheds both visually and with Chi-square contingency tables [51]. Bins were used here to better align and compare all measures with the BIBI rating categories (five bins). Six bins were used for total taxa richness to balance category ranges and simplify break points between categories (*i.e.*, groupings of three taxa).

To determine if BIBI scores changed from year to year at the various stream sites, BIBI scores at individual stream sites were compared among years using separate Kruskal-Wallis tests for each site. Only sites with three or more years of samples were compared in this way.

Relationships among the various community measures (combined across all watersheds) were also assessed in several different ways. First, multivariate analysis was used to produce a Spearman's rank correlation coefficient table and scatterplot matrix comparing the strength of the relationships among the five macroinvertebrate community measures (JMP Pro 18.0.1; JMP Statistical Discovery LLC, Cary, NC, USA). Next, analysis of covariance (ANCOVA) was used to compare the relationships between each of the two taxa richness measures (total taxa, EPT taxa) and BIBI scores across the various watersheds to determine if the relationships varied among watersheds. Finally, the proportion of total taxa in each sample comprised of EPT taxa was compared among watersheds with Kruskal-Wallis and Tukey's HSD tests to determine if EPT taxa were more important to overall community structure in some watersheds compared to others.

4. Results

Across the 15 years of sampling within the watersheds of southeastern Minnesota, 42 volunteer citizen scientists (individuals or teams) collected 452 samples of benthic macroinvertebrates from 35 different streams and rivers (including six sites on one stream [South Branch Root River]; Table 1). For various reasons, only 19 of the 42 collectors (45%) fulfilled the requested five years of sampling at their chosen stream. Six of the 23 volunteers who did not make five annual collections missed sampling their stream site during only a single year in the middle of their five-year sampling period, citing high water conditions or

family issues that prevented them from sampling. Six of the 19 collectors who completed five years of sampling continued sampling for one or more years beyond their original commitment. Across all watersheds, volunteers averaged 3.8 years of sampling (SD = 1.8 years, range = 1 to 7 years) and collected an average of 11.3 samples (SD = 5.7 samples, range = 1 to 19 samples). In some instances, multiple collectors chose to sample the same stream for their collections (e.g., six different collectors in the South Branch Root River), but this was allowed by monitoring program coordinators only on a few of the larger (longer and higher order) streams to better spread out the monitoring efforts. Overall, samples were collected from five streams in the Whitewater River watershed; eight streams in the Root River watershed; 17 streams in the Zumbro River watershed; and five streams each in their own small watersheds (Table 3).

In total, 46,763 individual organisms were collected and identified during this study, an average of 104 organisms/sample (95% CI = 4). These organisms represented 82 taxa, including insects, non-insect arthropods, molluscs, segmented worms, and flatworms. EPT taxa comprised 53 (65%) of the 82 taxa collected (Table 4). Although communities were highly variable among streams and years, benthic samples often were dominated by one or more of the following: amphipods (*Gammarus*), baetid (*Baetis*) mayflies, midges (Chironomidae), net-spinning caddisflies (*Hydropsyche*, *Cheumatopsyche*), brachycentrid (*Brachycentrus*) caddisflies, and riffle beetles (*Optioservus*).

Table 3. Summary data for streams monitored by citizen scientists in four watersheds in southeastern Minnesota, USA, during the period 1999–2013. Values are means with standard deviations in parentheses. EPT = Ephemeroptera, Plecoptera, Trichoptera; BIBI = Benthic Index of Biotic Integrity. Numbers after stream names are stream orders. BIBI ratings are based on the mean BIBI score (see text for explanation of ratings based on scores).

Watershed/Site	Years Sampled	Number of Samples	Simpson Diversity	Shannon Diversity	Taxa Richness	EPT Taxa Richness	BIBI Score	BIBI Rating
Whitewater River Watershed								
North Fork Whitewater River (4)	2001–2004	12	0.695 (0.088)	0.673 (0.091)	9.9 (1.6)	4.8 (0.8)	18.8 (5.7)	Poor
Middle Fork Whitewater River (4)	2000, 2005	9	0.659 (0.121)	0.605 (0.120)	8.3 (1.8)	4.6 (1.0)	16.1 (8.6)	Poor
South Fork Whitewater River (4)	2000–2004	15	0.752 (0.090)	0.736 (0.114)	9.9 (1.6)	5.6 (1.2)	19.7 (7.4)	Poor
Beaver Creek (2)	2000–2005	19	0.676 (0.102)	0.670 (0.137)	10.8 (2.8)	5.1 (2.1)	21.8 (9.9)	Poor
Trout Valley Creek (2)	2001, 2002, 2005	9	0.614 (0.121)	0.524 (0.128)	6.9 (2.4)	3.7 (1.0)	15.0 (12.0)	Poor
Whitewater watershed averages			*0.686 (0.108)*	*0.657 (0.135)*	*9.5 (2.5)*	*4.9 (1.5)*	*19.0 (8.9)*	*Poor*
Root River Watershed								
South Branch Root River #1 (5)	2000–2004	15	0.679 (0.089)	0.627 (0.100)	9.0 (2.7)	6.0 (1.4)	29.3 (16.0)	Poor/Fair
South Branch Root River #2 (5)	2001–2004	11	0.652 (0.102)	0.578 (0.128)	8.1 (2.6)	4.9 (2.0)	21.8 (17.9)	Poor
South Branch Root River #3 (2)	2003–2007	15	0.742 (0.106)	0.759 (0.133)	11.2 (2.1)	7.0 (1.1)	44.7 (12.2)	Fair/Good
South Branch Root River #4 (3)	2003–2006	12	0.795 (0.070)	0.829 (0.097)	12.2 (1.9)	8.0 (1.1)	67.5 (7.8)	Excellent
South Branch Root River #5 (3)	2003–2006	12	0.731 (0.160)	0.759 (0.225)	12.4 (3.7)	8.3 (2.3)	54.6 (25.0)	Good
South Branch Root River #6 (4)	2003–2006	12	0.707 (0.113)	0.698 (0.163)	9.6 (2.5)	5.3 (1.2)	22.5 (8.1)	Poor
Ferguson Creek (2)	2000–2004	15	0.763 (0.085)	0.789 (0.120)	12.3 (2.2)	6.3 (1.3)	47.7 (8.2)	Fair/Good
Rush Creek (4)	2000–2004	15	0.684 (0.098)	0.619 (0.121)	8.5 (2.2)	3.6 (0.9)	12.7 (6.2)	Poor
Trout Run Creek (3)	2000–2004	18	0.282 (0.134)	0.243 (0.091)	4.1 (1.1)	2.4 (0.8)	3.3 (5.1)	Very Poor
Corey Creek (2)	2002	3	0.607 (0.171)	0.575 (0.251)	8.3 (4.9)	3.0 (2.6)	16.7 (7.6)	Poor
Etna Creek (2)	2003–2007	15	0.708 (0.067)	0.639 (0.119)	7.7 (2.4)	4.9 (1.4)	19.7 (9.3)	Poor
Gribben Creek (2)	2004, 2005, 2007	9	0.593 (0.107)	0.511 (0.116)	7.2 (3.1)	3.3 (1.1)	16.1 (14.5)	Poor
Coolridge Creek (1)	2007	3	0.664 (0.12)	0.606 (0.151)	9.0 (1.0)	4.0 (0.0)	28.3 (7.6)	Poor/Fair
Roor watershed averages			*0.657 (0.178)*	*0.630 (0.209)*	*9.2 (3.5)*	*5.3 (2.3)*	*29.8 (22.3)*	*Poor/Fair*
Zumbro River Watershed								
North Fork Zumbro River (5)	2000–2002	9	0.747 (0.112)	0.757 (0.179)	10.9 (2.3)	5.7 (1.8)	36.7 (18.0)	Fair
Middle Fork Zumbro River (5)	1999	2	0.693 (0.165)	0.720 (0.238)	12.5 (4.9)	6.0 (1.4)	40.0 (14.1)	Fair
Middle Fork South Branch Zumbro River (4)	2001–2005, 2013	18	0.792 (0.086)	0.845 (0.145)	13.2 (2.1)	6.7 (2.0)	41.7 (17.1) *	Fair
South Fork Zumbro River (4)	1999, 2013	4	0.840 (0.047)	0.925 (0.075)	16.8 (2.1)	7.5 (1.3)	32.5 (8.7)	Fair

Cold Spring (2)	1999–2004	19	0.409 (0.154)	0.327 (0.116)	4.6 (1.5)	1.4 (0.6)	4.2 (7.1)	Very Poor
Long Creek (3)	2001–2005	15	0.374 (0.202)	0.333 (0.174)	5.5 (2.3)	2.3 (1.2)	5.7 (6.5)	Very Poor/Poor
West Indian Creek (3)	2001–2005	15	0.567 (0.173)	0.529 (0.166)	8.1 (1.8)	2.7 (0.9)	12.7 (6.2)	Poor
Silver Creek (2)	1999–2000	4	0.519 (0.211)	0.529 (0.230)	9.5 (3.1)	4.3 (1.3)	20.0 (16.8)	Poor
Bear Creek (2)	2000–2001	9	0.596 (0.145)	0.551 (0.129)	8.2 (1.5)	3.1 (1.9)	18.9 (12.4)	Poor
Cascade Creek (3)	1999–2000	5	0.761 (0.062)	0.713 (0.115)	10.0 (3.7)	4.6 (2.5)	25.0 (8.7)	Poor
Milliken Creek (2)	2001–2005, 2013	17	0.822 (0.073)	0.881 (0.124)	13.2 (3.4)	5.8 (1.8)	25.3 (13.5)	Poor/Fair
Masten Creek (2)	2001–2005, 2013	18	0.820 (0.040)	0.825 (0.086)	10.8 (2.9)	5.3 (1.4)	46.1 (12.9) *	Fair/Good
Salem Creek (3)	1999, 2001–2005, 2013	19	0.728 (0.112)	0.758 (0.138)	11.7 (3.3)	6.8 (1.6)	38.4 (13.5)	Fair
North Branch Salem Creek (2)	2013	3	0.812 (0.058)	0.843 (0.075)	13.3 (0.6)	5.0 (1.0)	30.0 (8.7)	Fair
Harkcom Creek (2)	2013	3	0.591 (0.167)	0.598 (0.182)	13.0 (2.6)	4.3 (1.2)	30.0 (8.7)	Fair
Dodge Center Creek (2)	2013	2	0.744 (0.005)	0.769 (0.013)	16.0 (1.4)	7.5 (0.7)	35.0 (14.1)	Fair
Willow Creek (2)	1999	1	0.508	0.499	11	5	15	Poor
Zumbro watershed averages			*0.660 (0.204)*	*0.660 (0.245)*	*10.1 (4.0)*	*4.6 (2.4)*	*26.2 (18.8)*	*Poor/Fair*
Other Watersheds								
East Indian Creek (2)	2000–2002, 2004	12	0.581 (0.159)	0.517 (0.160)	7.5 (2.6)	2.46(0.9)	12.1 (9.4)	Poor
Wells Creek (2)	2000–2004	18	0.775 (0.084)	0.757 (0.123)	9.7 (2.4)	4.3 (1.5)	24.7 (13.8)	Poor
Gorman Creek (2)	1999–2004	13	0.651 (0.175)	0.588 (0.171)	7.0 (1.6)	3.1 (0.6)	17.3 (9.7)	Poor
Snake Creek (2)	2001, 2003–2005	12	0.416 (0.179)	0.397 (0.169)	7.4 (2.1)	2.8 (0.5)	12.9 (5.8)	Poor
Gilbert Creek (2)	2001–2005	15	0.723 (0.110)	0.674 (0.140)	8.5 (2.5)	3.7 (1.7)	17.7 (11.3) *	Poor
Other watersheds averages			*0.639 (0.186)*	*0.599 (0.195)*	*8.2 (2.5)*	*3.4 (1.4)*	*17.7 (11.7)*	*Poor*

* BIBI scores differed significantly among years.

Total taxa richness in samples averaged approximately nine taxa across all watersheds and years (Table 4), although some streams or individual samples had much lower (four or fewer) or much higher (12 or more) averages (Table 4, Figure 2A). Root River and Zumbro River watersheds both had some of the poorest and best taxa richness values (Table 4, Figure 2A), with five to 10 percent of samples from those two watersheds having 16 or more taxa (Figure 2A). Total taxa richness differed significantly (Kruskal-Wallis $H = 15$, df = 3, $p = 0.0018$) among the four watersheds, with richness lowest in the group of "Other watersheds". Distributions of samples among the various total taxa richness groupings also differed significantly (Chi-square = 46.6, df = 15, $p < 0.001$) among watersheds (Figure 2A).

Table 4. Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa collected from four watersheds in southeastern Minnesota, USA, by volunteer citizen scientists during 1999–2013. X = taxon collected within watershed.

	Watersheds			
Ephemeroptera (Mayfly) Genera	**Whitewater**	**Root**	**Zumbro**	**Others**
Baetis	X	X	X	X
Baetisca		X	X	
Caenis		X	X	
Ephemera		X		
Ephemerella	X	X	X	X
Ephoron			X	
Habrophleboides	X			
Heptagenia		X	X	
Heterocloeon		X	X	
Hexagenia			X	
Isonychia		X	X	X
Leptophlebia	X	X	X	
Paraleptophlebia	X	X	X	X
Pseudocloeon			X	
Rhithrogena			X	
Stenonema	X	X	X	X
Tricorythodes	X	X	X	
Number of mayfly taxa	7	12	15	5
Plecoptera (stonefly) genera				
Acroneuria		X	X	
Allocapnia		X		
Amphinemura	X	X		X
Hastaperla				X
Isogenoides		X		
Isoperla	X	X		X
Nemoura		X		
Neoperla	X		X	
Paragnetina		X	X	
Phasganophora			X	
Pteronarcys	X	X	X	
Strophopteryx		X		
Taeniopteryx		X	X	
Number of stonefly taxa	4	10	6	3
Trichoptera (caddisfly) genera				
Brachycentrus	X	X	X	X
Cheumatopsyche	X	X	X	X
Chimarra		X	X	
Glossosoma	X	X	X	X

Helicopsyche		X	X	
Hesperophylax		X	X	X
Hydatophylax			X	X
Hydropsyche	X	X	X	X
Hydroptila	X	X	X	
Lepidostoma				X
Limnephilus	X	X	X	X
Micrasema	X	X		
Neophylax		X		
Nyctiophylax			X	X
Oecetis		X	X	
Onocosmoecus	X			
Platycentropus	X	X		X
Polycentropus			X	
Pseudostenophylax		X		
Psychomyia		X	X	
Ptilostomis	X			X
Pycnopsyche	X	X		
Rhyacophila		X		
Number of caddisfly taxa	11	17	14	11
Total number of EPT taxa	22	39	35	19

The overall variety of EPT taxa was much higher in the Root (39 taxa) and Zumbro River (35 taxa) watershed samples than in samples from the other watersheds (22 taxa in Whitewater, 19 taxa in "Others") (Table 4). Individual samples across all watersheds and years averaged approximately five EPT taxa, although the "Other watersheds" samples averaged significantly (Kruskal-Wallis $H = 39.8$, df = 3, $p < 0.0001$) fewer EPT taxa than those collected elsewhere (Table 4). Distributions of samples among the various EPT taxa richness groupings also differed significantly (Chi-square = 64.7, df = 12, $p < 0.001$) among watersheds (Figure 2B). Across all watersheds and years, total taxa richness and EPT taxa richness of samples were significantly correlated with one another (Figure 3, Table 5). When sample EPT taxa richness was expressed as a percentage of sample total taxa richness and compared among watersheds, significant differences (Kruskal-Wallis $H = 93.13$, df = 3, $p < 0.0001$) were detected among watersheds (Figure 4), with EPT taxa representing nearly 60% of taxa collected in Root River samples but 52% or less in samples from other watersheds.

Neither Simpson (Kruskal-Wallis $H = 1.84$, df = 3, $p = 0.606$) nor Shannon (Kruskal-Wallis $H = 5.13$, df = 3, $p = 0.163$) diversity indices of the macroinvertebrate samples displayed significant differences among the watersheds monitored, although several individual streams within most watersheds exhibited relatively low and relatively high diversities (Table 3). However, distributions of samples among the various Simpson and Shannon diversity groupings both differed significantly (Simpson: Chi-square = 35.2, df = 12, $p < 0.001$; Shannon: Chi-square = 40.0, df = 12, $p < 0.001$) among watersheds (Figure 5A,B). For both indices, the Zumbro River watershed samples skewed more toward higher values compared to samples from the other watersheds (Figure 5A,B). The two diversity indices were significantly correlated with one another, as well as with both total taxa richness and EPT taxa richness of the samples (Figure 3, Table 3), with Shannon diversities exhibiting slightly stronger correlations with both taxa richness variables compared to Simpson diversity correlations with those same variables.
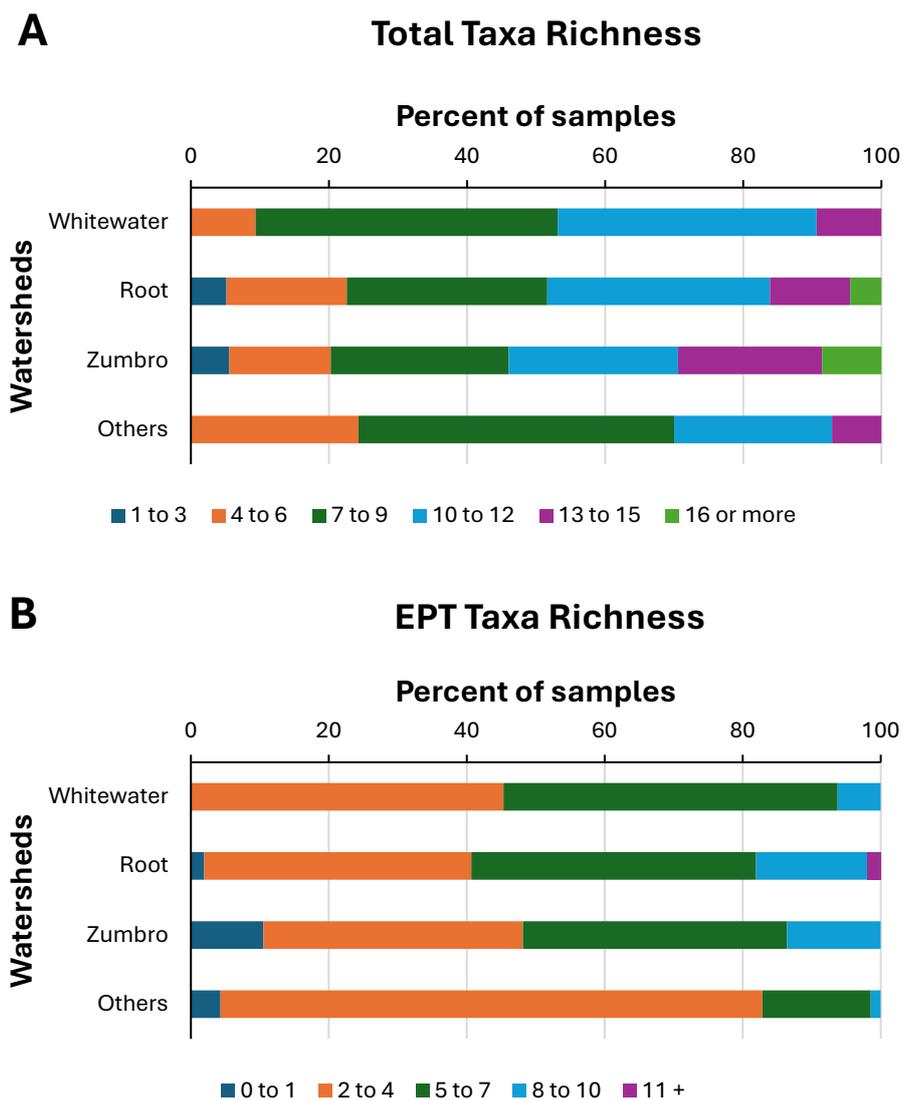
Figure 2. Distributions of benthic macroinvertebrate samples among total taxa richness categories (**A**) and Ephemeroptera-Plecoptera-Trichoptera (EPT) taxa richness categories (**B**) for four watersheds in southeastern Minnesota, USA. Citizen scientists collected samples from 1999–2013.
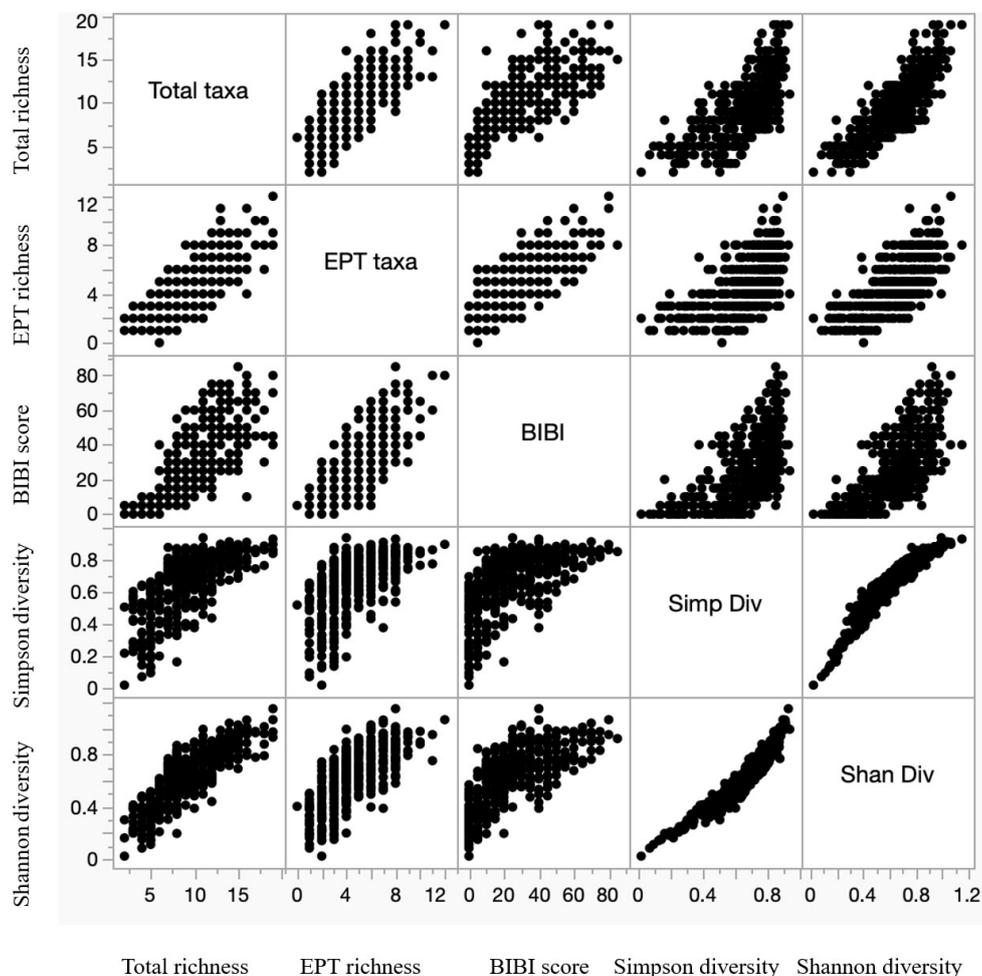
Figure 3. Scatterplot matrix displaying relationships among taxa richness, diversity, and biotic integrity variables for 452 samples of benthic macroinvertebrates collected by citizen scientists from four watersheds in southeastern Minnesota, USA, during 1999–2013. EPT = Ephemeroptera, Plecoptera, Trichoptera; BIBI = Benthic Index of Biotic Integrity.
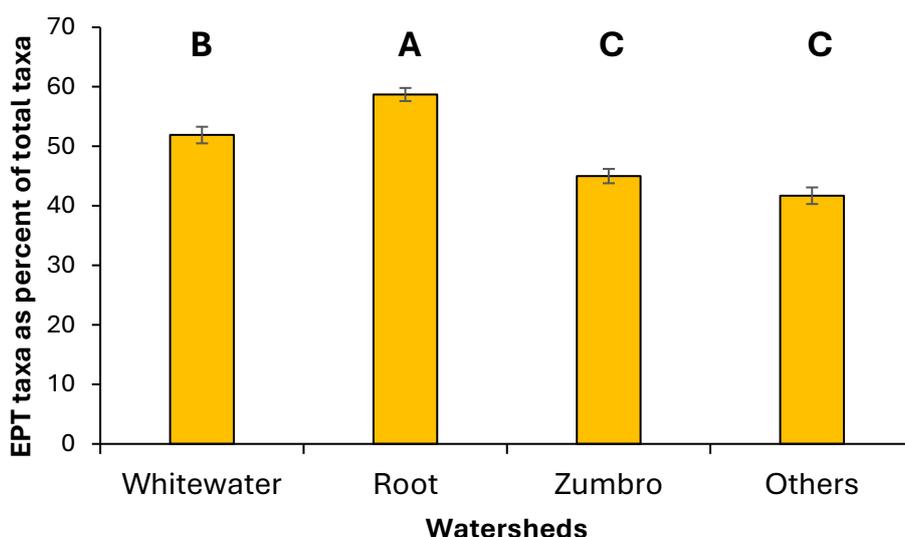
Figure 4. Percentages of total taxa richness of benthic macroinvertebrate samples comprised of Ephemeroptera-Plecoptera-Trichoptera (EPT) taxa. Values are means (± 1 standard error) of all samples from each of four watersheds in southeastern Minnesota, USA. Bars with different letters above them are significantly different from one another (Tukey's HSD test, $p < 0.05$).

Table 5. Spearman rank correlation coefficient matrix depicting strengths of relationships among taxa richness, diversity, and biotic integrity variables for 452 samples of benthic macroinvertebrates collected by citizen scientists from streams within four watersheds in southeastern Minnesota, USA, 1999–2013. EPT = Ephemeroptera, Plecoptera, Trichoptera; BIBI = Benthic Index of Biotic Integrity.

|	EPT Taxa	BIBI Score	Simpson Diversity	Shannon Diversity
Total taxa	0.805 *	0.811 *	0.688 *	0.821 *
EPT taxa		0.820 *	0.633 *	0.733 *
BIBI score			0.646 *	0.740 *
Simpson diversity				0.969 *

*$p < 0.0001$.
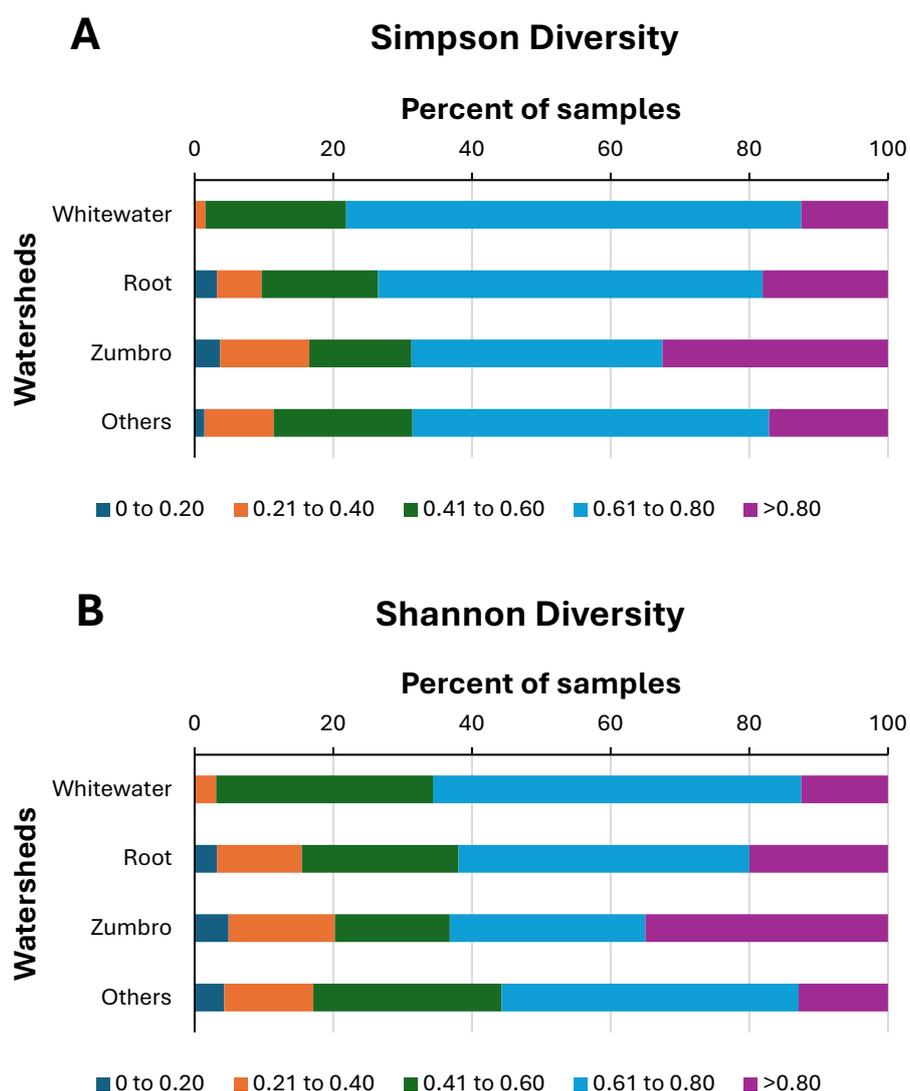
Figure 5. Distributions of 452 benthic macroinvertebrate samples among Simpson diversity categories (**A**) and Shannon diversity categories (**B**) for four watersheds in southeastern Minnesota, USA. Citizen scientists collected samples during 1999–2013.

BIBI scores and ratings were highly variable across both streams and watersheds (Table 3). BIBI scores differed significantly (Kruskal-Wallis $H = 18.23$, df = 3, $p = 0.0004$) among watersheds, higher in the Root and Zumbro systems compared to the others (Figure 6A). However, BIBI scores differed significantly among years at only three of the 30 stream sites where three or more years of samples were collected (Table 3). Distributions of samples among the various BIBI rating categories also differed significantly (Chi-

square = 73.8, df = 12, $p < 0.001$) among watersheds (Figure 6B), with Root and Zumbro watersheds having 14% or more of their samples rated good or excellent, whereas no single sample from the other systems ever rated higher than fair. Overall, a majority (63.3%) of all samples were rated as poor or very poor, whereas only a small proportion (13.5%) was rated as good or excellent. BIBI sample scores were significantly correlated with both taxa richness and measures, and both diversity indices, although correlations were higher with the taxa richness variables than with the diversity indices (Figure 3, Table 5).
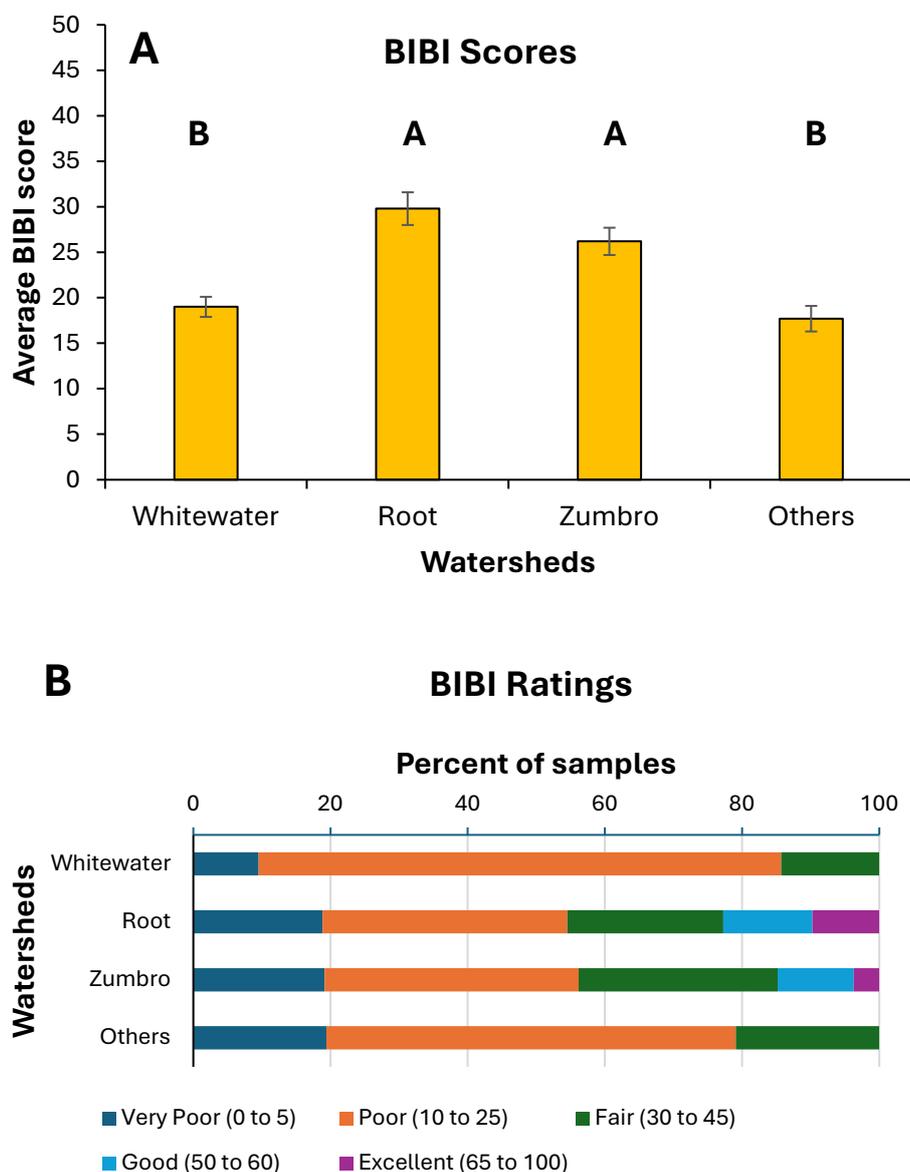
Figure 6. Mean (±1 standard error) benthic index of biotic integrity (BIBI) scores of benthic macroinvertebrate samples (**A**) and distribution of samples among BIBI rating categories (**B**) for 452 collections made by citizen scientists in four watersheds in southeastern Minnesota during 1999–2013. Mean BIBI score bars with different letters above them are significantly different from one another (Tukey's HSD test, $p < 0.05$). Watershed sample sizes were: Whitewater = 64, Root = 155, Zumbro = 163, Others = 67.

When relationships between BIBI scores and the taxa richness variables were examined within each watershed, ANCOVA indicated that there were significant differences in both total taxa richness-BIBI and EPT taxa richness-BIBI regression variables (Y intercept, slope) among watersheds (Table 6, Figure 7A,B). For Root River watershed samples, BIBI scores increased more quickly (*i.e.*, steeper regression slope) with

increasing taxa richness (both total and EPT), whereas for Whitewater River watershed samples, BIBI scores increased more slowly (*i.e.*, shallower regression slope) with increasing richness values (Figure 7A,B). Regressions for the Zumbro River and "Other" watersheds were similar and intermediate between the Root and Whitewater regressions.
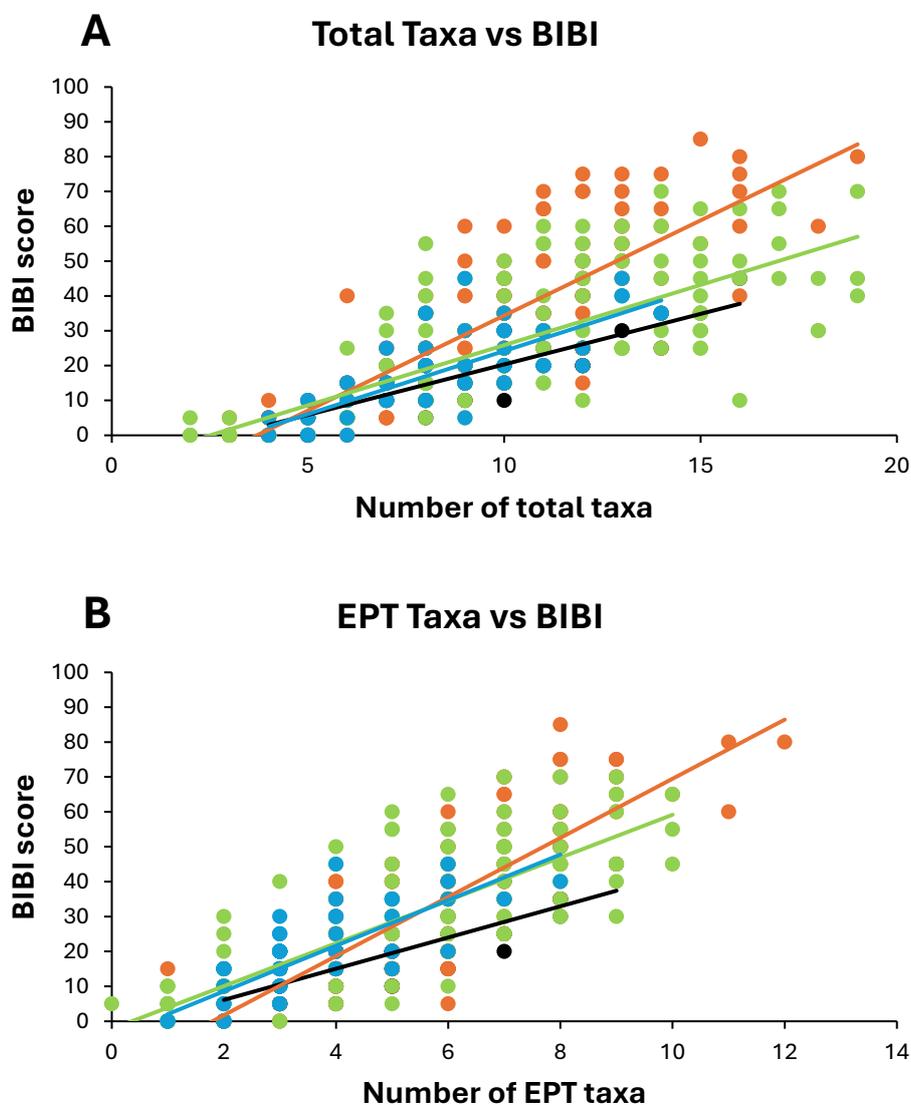
Figure 7. Linear regression relationships between total taxa richness and benthic index of biotic integrity (BIBI) scores (**A**) and Ephemeroptera-Plecoptera-Trichoptera (EPT) taxa richness and BIBI scores (**B**) for benthic macroinvertebrate samples collected from four watersheds in southeastern Minnesota, 1999–2013. Watershed sample sizes were: Whitewater = 64, Root = 155, Zumbro = 163, Others = 67. Not all points from each watershed are visible due to value overlays. For both points and lines, orange = Root watershed, green = Zumbro, black = Whitewater, and blue = Other. See Table 4 for ANCOVA statistics.

Table 6. Analysis of covariance statistics of sample taxa richness variables versus biotic integrity scores for 452 samples of benthic macroinvertebrates collected by citizen scientists from streams within four watersheds in southeastern Minnesota, USA, 1999–2013. EPT = Ephemeroptera, Plecoptera, Trichoptera; BIBI = Benthic Index of Biotic Integrity.

Comparison/Regression Component	Degrees of Freedom	*F*	*p*
Total taxa richness versus BIBI score			
Y intercept	3, 444	22.47	<0.0001
Slope	3, 441	14.37	<0.0001
EPT taxa richness versus BIBI score			

Y intercept	3, 444	11.78	<0.0001
Slope	3, 441	10.42	<0.0001

5. Discussion

This study revealed several important findings about the usefulness of macroinvertebrate community data gathered by citizen scientists from streams and rivers in southeastern Minnesota. First, these collections produced sample-based diversity indices, taxa richness metrics, and BIBI scores that were all significantly correlated with each other and generally displayed differences within and among watersheds. Second, streams in the two largest watersheds (Root, Zumbro) with the highest proportions (>20%) of pasture and haylands frequently had higher invertebrate taxa richness, diversity, and biotic integrity than the smaller systems. Finally, based on samples collected by citizen scientists during a 15-year period, a majority (nearly two-thirds) of stream macroinvertebrate communities surveyed in southeastern Minnesota have poor or very poor biotic integrity, indicative of continuing environmental stressors impacting stream systems.

All measures of community structure (diversities, taxa richness metrics, BIBI) calculated from macroinvertebrate samples in this study were significantly correlated with one another. Single-metric diversity and taxa richness values have a long history of use in assessing communities [52], whereas multi-metric biotic integrity scores represent a more recent approach to understanding how communities can be affected by environmental disturbances [6]. Multi-metric approaches like the BIBI have been favored over single metric indices because they combine several different community characteristics (often including both diversity and taxa richness) that may respond slightly differently to environmental stressors [6,7,53]. The BIBI used here included total taxa richness as one of 10 metrics included when deriving a total BIBI score. It also included separate metrics for stonefly and caddisfly taxa richness along with other taxa richness metrics for longevity, tolerance, and feeding guilds, but did not include any specific diversity measure as a metric [49]. This may partially explain why BIBI scores in this study were more strongly correlated to taxa richness measures than to diversity indices.

Of the community indices or metrics determined for each macroinvertebrate sample, only the BIBI determines a condition rating (range from excellent to very poor). The fixed-count protocol (*i.e.*, limiting sample size to approximately 100 organisms; see Section 3) used by volunteers probably underestimated total taxa richness and EPT taxa richness values and overall community diversity levels [38], so direct comparisons of data from this study to taxa richness and diversity values from other studies where entire samples were used would be inappropriate. However, the BIBI was developed for use specifically on 100-count samples [49], so sample, stream, and watershed ratings should be realistic and comparable to other studies using similar multi-metric indices [6,7,53]. Other regional studies e.g., [25–27,34,54] conducted using multi-metric benthic macroinvertebrate indices before, during, and after the period of the present study produced stream ratings similar to those presented in this study (see below).

Although macroinvertebrate community metrics differed considerably among streams sampled within each of the watersheds surveyed in southeastern Minnesota, the two largest watersheds (Root, Zumbro) with the highest proportions of pasture and haylands had higher average taxa richness and BIBI scores and marginally higher average diversities than the streams in the smaller watersheds. All watersheds contained a mix of coldwater, coolwater, and warmwater streams, so macroinvertebrate community differences among watersheds likely cannot be attributed to differing thermal conditions. All watersheds also have similar underlying geology, climate, and topography. Land use differed somewhat among watersheds, with the Zumbro and Root systems having considerably more pasture/haylands (>20% in each watershed) than the other watersheds (12% or less) examined. Greater proportions of grass on the landscape may serve to reduce soil erosion and thereby maintain better stream habitat that can support macroinvertebrate communities with greater diversity and biotic integrity [26,34,50]. Another possible explanation for higher taxa richness and BIBI scores in the Root and Zumbro watersheds may arise from watershed size and the

degree of isolation from potential colonizers that may have survived a period of significant disturbance. Larger watersheds may be more likely than smaller systems to have one or more subwatersheds that served as macroinvertebrate refugia [55] during major historical disturbances [29,32,33] that likely extirpated sensitive invertebrates from much of the remainder of the watershed. Recolonization within a watershed from these few refugia subwatersheds theoretically could occur over the course of many decades via downstream drift [56–58] or slow upstream migration of winged adults [55]. However, smaller watersheds within the region may not have any pockets of surviving sensitive species from which to initiate recolonization. Also, recolonizers may be less likely to arrive from larger neighboring watersheds because aquatic invertebrates are generally poor lateral dispersers [59] and streams located in narrow valleys in different watersheds may be separated by many km (e.g., 10 km or more) of high ridges (typical in the landscape of southeastern Minnesota) that can pose significant dispersal barriers.

During the 15-year monitoring period, streams in the southeastern Minnesota region mostly exhibited poor to very poor biotic integrity based on the macroinvertebrate collections made by citizen scientists. These findings generally are supported by more localized studies of stream invertebrates conducted within the region by professional scientists before [25], during [26,54], and after [27] the time period of this study. An earlier study in the Root watershed by University of Minnesota (U of MN) scientists [25] reported mostly poor, pollution-tolerant macroinvertebrate communities in areas dominated by agriculture. During the same period when citizen scientists were collecting samples for this study, scientists from the U of MN [26] and Winona State University (WSU) [54] reported mostly poor and very poor macroinvertebrate communities in streams within the Whitewater and Root watersheds, respectively. After citizen scientist collections ended, WSU scientists reported poor macroinvertebrate communities throughout the Whitewater watershed [27]. In addition, 19 of the 35 streams monitored by citizen scientists are currently listed as impaired for benthic macroinvertebrates by the Minnesota Pollution Control Agency [28], and all 19 of those streams were rated as fair, poor, or very poor by citizen scientists.

Despite widespread implementation of soil conservation practices, runoff control structures, and livestock manure management systems beginning in the mid-1900s [29,32,33], stream systems in southeastern Minnesota were still being impacted by environmental stressors during the study period. Legacy deposits of eroded soils still blanket river valleys and form steep eroding banks along many regional streams [32], contributing to elevated suspended sediment loads during high flow events [54] and embedding coarse stream bottom substrates with fine-grained silts and clays [27]. Fecal coliform bacteria levels also were high in regional streams after heavy rain events during the study period [54], suggesting continuing problems with controlling livestock waste runoff from pastures and/or feedlots.

6. Conclusions

This study demonstrated that volunteer citizen scientists were able to collect samples of benthic macroinvertebrates that were used to assess and compare stream water quality among several watersheds in southeastern Minnesota, USA. These samples allowed for the calculation of several community measures (taxa richness, diversity, biotic integrity) that were correlated significantly with one another and demonstrated differences in benthic community structure within and among the four watersheds examined. Communities within the two largest watersheds exhibited significantly higher taxa richness and better biotic integrity than those in smaller watersheds, suggestive of protective refugia potentially existing in larger watersheds during past disturbance events and subsequently providing a source of within-watershed recolonizers. Unfortunately, streams in southeastern Minnesota were characterized by mostly poor and very poor biotic integrity based on the macroinvertebrate communities surveyed by citizen scientists, indicative of continued environmental stressors likely acting to reduce water quality and instream habitat conditions.

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Ethics Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data on which this paper is based can be obtained from the author upon reasonable request.

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Declaration of Competing Interest

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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