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Taxonomic and functional diversity of aquatic macroinvertebrate from natural forest as reference for streams health indicators in Lasolo Watershed, Southeast Sulawesi, Indonesia

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Abstract. *Nasaruddin N, Sabaruddin L, Analuddin K, Sudia LB. 2023. Taxonomic and functional diversity of aquatic macroinvertebrate from natural forest as reference for streams health indicators in Lasolo Watershed, Southeast Sulawesi, Indonesia. Biodiversitas 24: 5523-5538.* Macroinvertebrates are known to play a crucial role in nutrient recycling and serve as valuable stream degradation indicators. Therefore, this study aimed to examine the structural and functional diversity of macroinvertebrate communities as a reference for stream health indicators in tropical Sulawesi, Indonesia. Spatial sampling using a multi-habitat method was conducted between September 2021 and March 2022 in five streams connected to natural forest and three from drainage areas in dryland farming. Data on forest cover, physical-chemical variables, and macroinvertebrate samples were collected, while the species richness and diversity trends across streams were analyzed using individual rarefaction curves. In addition, the composition of macroinvertebrate functional group was also evaluated. The results showed that 2474 individuals belonging to 77 genera were recorded throughout the area. We found a reduction in taxa composition by comparing the reference and impacted stream at the genus, family, and order levels with approximate values of 83.1%, 74.07%, and 55.6%, respectively. At least seven significantly different potential metrics that differentiated between reference and the impacted ecosystem were found, including the number of family taxa (#family), the number of insect taxa (#insect), the number of Ephemeroptera-Plecoptera-Trichoptera taxa (#EPT), the number of scraper taxa (#scraper), as well as Shannon-Wiener diversity, Simpson Evenness, and Margalef Richness index. These metrics offer a strong method for assessing land use change and their impact on freshwater biodiversity, emphasizing the importance of conservation efforts in the ecosystem.

Keywords: Aquatic insect, bioassessment, EPT, functional feeding group, land use/land cover

INTRODUCTION

Exploring biological benchmarks to determine aquatic ecosystem integrity in a particular region is a complex task (Karr et al. 2022). Meanwhile, biological reference refers to standards commonly developed from specific conditions (Clapcott et al. 2017), serving as a baseline against which any alteration to the current biological conditions is measured using indicators (Hawkins and Carlisle 2022). Biological indicators are instruments for sustainably managing aquatic ecosystems and water resources both in river systems (Dobriyal et al. 2017; Singh et al. 2020; Santos et al. 2021; Weerasooriya et al. 2021) and coastal areas (Analuddin et al. 2015; Gracia et al. 2018). In several previous studies, biological indicators of beta diversity were used as an important tool in environmental or conservation-based censuses and the establishment of nature reserves (Cleary 2003; Koleff et al. 2003; Tuomisto et al. 2003; Purvis and Hector 2000). Meanwhile, other studies provided systematic environmental mitigation by offering an appropriate water quality classification, including physical parameters (Hou et al. 2020; Feio et al. 2021).

The assessment of physical conditions is essential in evaluating the overall health of ecosystems and determining their ecological integrity. This can be achieved by measuring the ability to maintain physical features, species composition, diversity, and functional structure similar to those found in reference conditions (Kuehne et al. 2017; Zhang et al. 2020). The ability of aquatic animals to survive and reproduce in nature is related to their biological and physiological characteristics and the influence of environmental stressors (Hess et al. 2020). These species traits are linked to physiological and behavioral processes that determine how a community responds to environmental changes, commonly determined using the term biotic index (Pinsky et al. 2020; Kroeker and Sanford 2022). A biotic index is a quantitative measure that refers to differences in water quality.

Tropical aquatic ecosystems are at risk of habitat fragmentation, sedimentation, flow control, biological invasion, and water pollution (de Mello et al. 2018; Hansen et al. 2020). Consequently, it is necessary to assess the health of water bodies through biological evaluations that use both macroinvertebrate taxonomic and functional metrics. These assessments directly quantify natural conditions that incorporate pressures across scales of anthropogenic disturbances compared to evaluations of the physical or chemical properties of the water (Agra et al. 2021; Green et al. 2022). The presence of native forest cover in the catchment area plays a crucial role in regulating the amount of surface runoff, nutrients, and organic matter absorbed by the stream. This, in turn, improves the physical-chemical conditions of the habitat and water quality, further supporting the diversity of stream macroinvertebrates (Luiza-Andrade et al. 2020; Espinoza-Toledo et al. 2021). The combination of taxonomic and functional diversity has been extensively studied as an ecological indicator to determine anthropogenic impacts on ecosystem function (Laini et al. 2019; Luiza-Andrade et al. 2020). Functional diversity using bioassessment in different ecological conditions of aquatic ecosystems with an environmental gradient cannot be explained using traditional indicators (Malacarne et al. 2023).

Numerous community attributes of aquatic organisms (e.g., macroinvertebrates and fish) have been used by scientists since the development of the biotic index (Herman et al. 2015). For example, the macroinvertebrate group is commonly used as a bioindicator for freshwater ecosystem assessment. Various perspectives have been explored in different studies to improve accuracy, including the application of taxonomic and functional feeding groups (Chen et al. 2017; Pallottini et al. 2017), alpha diversity and biotic indices (Etemi et al. 2020), the effect of forest losses and agricultural land use (Gerth and Giannico 2017; Brito et al. 2020), as well as the impact of single and multiple stressors (Fierro et al. 2019; Leitner et al. 2021).

According to a previous study conducted using a biotic index metric (Family Biotic Index) with minimum sample size, the performance was higher in streams with good environmental quality (Nasaruddin et al. 2023). This is consistent with the results obtained from studies conducted in the tropics (e.g., Aazami et al. 2015) but differs from others (Ghani et al. 2018; Hui and Fikri 2021). Further investigation using multiple samples on this metric is needed to increase the sensitivity of the tools used in a particular ecological region, such as Sulawesi. It is also important to understand the significance of reference ecosystems as a benchmark for establishing regional bioassessment based on macroinvertebrate communities in the Lasolo River basins. Therefore, this study aimed to (1) identify the taxonomic and functional structure of various reference stream ecosystems using aquatic macroinvertebrates and (2) develop potential metrics in the category of taxonomic diversity, functional feeding groups, and biological indices as a benchmark for stream health assessment in the Lasolo watershed, Sulawesi.

MATERIALS AND METHODS

Study area and stream selection

This study was conducted in the Lasolo watershed located at 02º45'93"S-3º31'96"S-121º47'73"E-122º08'91"E, Southeast Sulawesi, Indonesia. The total area of the watershed is 600,191.03 hectares, of which 544,763.89 hectares are forest (Damarraya et al. 2021). The topography of the region was typically steep, undulating, and mountainous, between 100 and 1500 meters above sea level (Adnan et al. 2017). The average annual rainfall (1990-2021) of the regions of the Asera, Langgikima, and Routa was 2522 mm, 1958 mm, and 2792 mm, respectively (available at: https://power.larc.nasa.gov/data-access-viewer/, accessed May 26, 2022). The sampling site was selected based on the land use/cover criteria, with streams having >60% of natural forest cover categorized as reference, while those with <60% were considered impacted. Based on these criteria, five and three stations were selected for reference and impacted streams respectively (Figure 1). Data were collected in first- to second Strahler streams order (Hughes et al. 2011), with a natural forest cover of reference streams ranging from 65% to 100%. The other three were in dryland farming-impacted streams, as depicted in Table 1 below.

Table 1. The areas and landscape category of stream sampled in the Lasolo watershed, Southeast Sulawesi, Indonesia

Stream code	Area name	Streams order	Latitude/Longitude	Landscape pressures	Category
Ref.1	Andowia	2^{nd}	3°34'0.88"S, 122° 8'11.47"E	Minimal, natural forest	Reference
Ref.2	Oheo	2^{nd}	3°25'22.22"S, 122° 4'24.85"E	Least disturb forest, farming	Reference
Ref.3	Langgikima	1^{st}	3°18'59.98"S, 122°11'48.59"E	Minimal, natural forest	Reference
Ref.4	Oheo	2^{nd}	3°19'8.62"S, 122° 9'34.52"E	Minimal, natural forest	Reference
Ref.5	Wiwirano	2^{nd}	3° 7'12.18"S, 122° 3'53.35"E	Minimal, natural forest	Reference
Imp.1	Landawe	1^{st}	3°17'11.06"S, 122°10'41.78"E	Dryland farming, mainly oil palm	Impacted
Imp.2	Langgikima	2^{nd}	3°17'21.34"S, 122°12'50.94"E	Dryland farming, mainly oil palm	Impacted
Imp.3	Langgikima	2^{nd}	3°17'38.72"S, 122°14'4.52"E	Dryland farming, mainly oil palm	Impacted

Note: Ref.: Reference stream, Imp: Impacted stream. The number following each stream code indicates the number of the stream surveyed

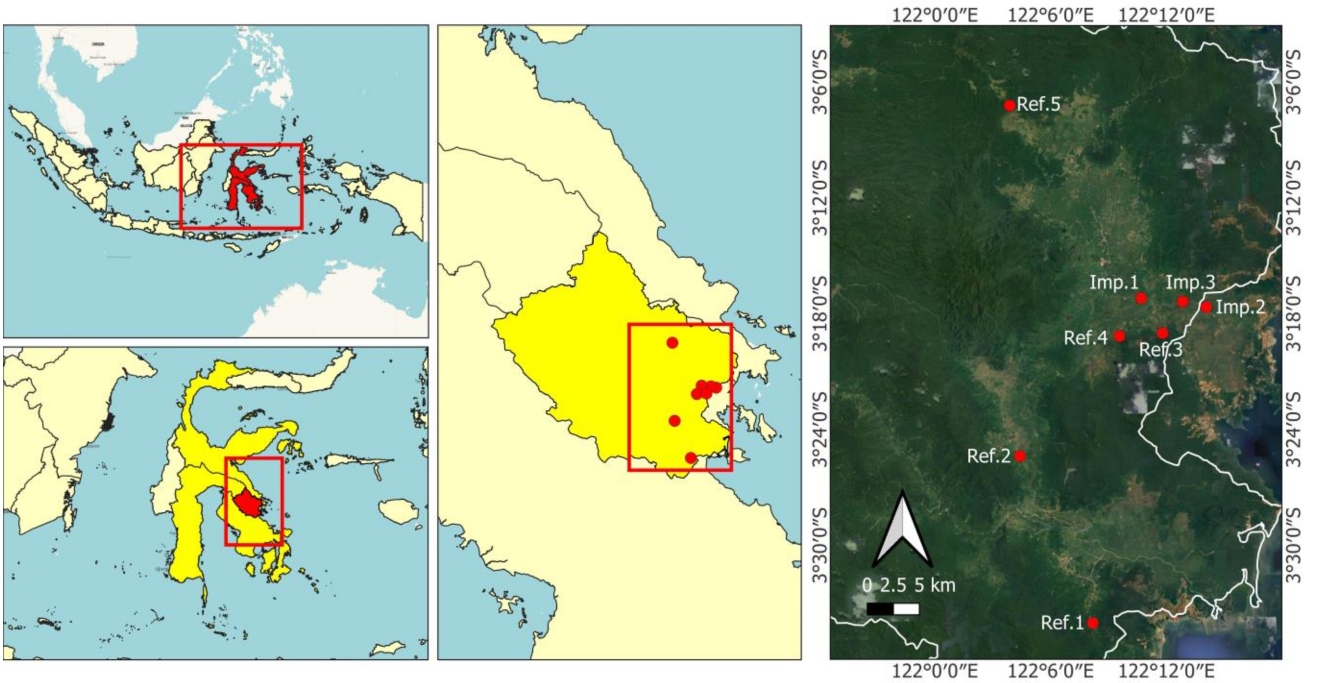

Figure 1. The study map shows stream location in the Lasolo watershed, Southeast Sulawesi, Indonesia. The circle with Ref code indicates reference streams (n = 5), and the circle with Imp code represents impacted streams (n = 3). The point coordinate is presented in Table 1.

Environmental data collection

From September 2021 to March 2022, spatial samples of macroinvertebrates were collected from five streams connected to a primary forested catchment and three with a predominance of agricultural land. These samples were designed in a zigzag pattern every 15 meters for 150-meter stream segments representing the three different types of microhabitats (riffles, run, and pool) for a total area of 1 m^2 at each location. From downstream to upstream, we divided each stream segment into ten longitudinal sections and 11 transects representing sub-samples of the left, center, and right stream sections (USEPA 2017). We conducted sampling with five replications (location) in reference streams and three replications in impacted streams (as shown in Table 1). Each location is visited one time.

In each stream segment, ten water physical-chemical parameters were measured, including pH, dissolved oxygen, total dissolved solids, conductivity, alkalinity, hardness, nitrate, phosphate, turbidity, and velocity. The ten physicochemical parameters were measured off-site after 3 to no more than 24 hours from ± 1-liter water sample at each location. The dissolved oxygen content (mg L^{-1}), conductivity (μS cm^{-1}), pH, and total dissolved solids (mg L^{-1}) were analyzed using a portable Water Quality Meter (AZ 86031, S/N 1048249), while the alkalinity, hardness, nitrate, and phosphate values were assessed with the LaMotte AM12 Testtabs Water Investigation Kit (Code 5849). Turbidity Meter Lutron TU-2016 was used to test the turbidity of water, while the difference between the initial and 5-day oxygen content in dark bottles after incubation at room temperature was used to determine the biological oxygen demand (BOD_5 mg L^{-1}). The current velocity was analyzed using flowmeter 4.3 Geopacks 2018 with a 7% accuracy and a range of 0.05 m/s to 8.0 m/s. Within each stream site, the physical characteristics of the channel, including the average depth and wetted width, were evaluated with a tape measure. Furthermore, the average depth was estimated from the measurements of 3 - 5 stream sections across the locations. At each location, this study assessed (1) the pressure of stream catchment (including the percentage of natural forest and land farming cover), (2) the particle size distribution of the substrate category/class according to (Cummins and Lauff 1969; Lorenz and Wolter 2019). These categories included silt (<0.05 mm), very fine (class 11): 0.05-0.1 mm, fine (class 10): 0.1-0.25 mm, medium (class 9): 0.25-0.5 mm, coarse (class 8): 0.5-1 mm, and very coarse sand (class 7): 1-2 mm, as well as gravel (class 4 to 6): 2-16 mm, and pebble (class 3): >16 mm), taken from various microhabitat types sediment with the volume range of 449.4 g-961.3 g per stream. Substrates were composited into two classes, namely gravel (2 mm to 19 mm) and sand (<2 mm), for statistical analyses. Stream discharge was calculated using the standard method (USEPA 2017), while the total organic carbon in the sediment was assessed with the wet oxidation method (Oduor et al. 2018).

Macroinvertebrate data collection and processing

D-shaped nets with a 30 cm aperture and 250 μm mesh size, along with surber nets featuring a 30 cm^2 aperture and 500μm opening size, were used to collect stream benthic macroinvertebrates. To achieve a representative sample, 11 sampling points were performed with at least 4 (four) replicates on the left and center side of the stream and at least 3 (three) replicate samples on the right side. Sub-samples were collected at the station, combined with a total of 1 m^2 per site, stored in plastic bottles, treated with 10% formalin preservation, and then transported to the laboratory for further analysis using a stereo microscope (USEPA 2017). All samples were identified to the lowest taxon level, with a focus on at least the genus, based on available identification guidelines (Winterbourn et al. 1989; Cummins et al. 2005; Narumon and Boonsoong 2006; Zettel et al. 2011; Dobson et al. 2012; Polhemus and Polhemus 2013; Agouridis et al. 2015; Damborenea et al. 2020). To obtain a broader understanding of biotic responses in determining potential indicators, macroinvertebrate collection included the richness and structural composition of taxa (taxonomy). Additionally, the behavioral groups and feeding habits in the ecosystem (functional feeding groups) were considered. The classification of functional feeding groups was based on established sources (Bouchard et al. 2004; Cummins et al. 2005; Edwards 2014; Merritt et al. 2017). The classes include (1) Scraper (Scr): Grazes on organic films (algae) growing on cobbles and other substrates, (2) Shredder (Shr): Feeds mainly on macrophytes, including macroalgae and coarse particle organic matter, primarily allochthonous detritus, (3) Gathering-collector (GCo): Feeds on tiny organic particulates, (4) Filtering-collector (FCo): Capture and ingest suspended organic particles for food, and (5) Predator (Pre): Chews or pierces other invertebrates.

Data analysis

Paleontological Statistics (PAST) software version 4.11 was used for all statistical analyses of abiotic and biotic data (Hammer et al. 2001).

Environmental data analysis

The Kruskal Wallis was used to test the environmental variables differences between reference and impacted streams.

Taxonomic, diversity indices, biotic indices, and functional feeding group data analyses

The rarefaction curve served as a valuable tool for illustrating taxonomic richness at the genus level and the Shannon-Wiener index in watershed scale. Several biological indicators, often referred to as metrics, were tested in this study. A metric is defined as a measurement index that refers to differences in water quality. The value of macroinvertebrate metrics was calculated to characterize biotic communities across streams in the region. The assessment included taxonomic-based metrics, comprising (1) taxa composition and richness which consisted of the total abundance, the number of genera taxa (#genera), the number of family taxa (#family), the number of order taxa (#order), the number of insect taxa (#insect), and the number of Ephemeroptera-Plecoptera-Trichoptera taxa (#EPT) (2) Alpha diversity indices including the Simpson Evenness, Shannon-Wiener, Margalef Richness, and Pielou Evenness index. Additionally, metrics related to functional feeding groups (FFGs) were evaluated, including (1) taxa richness comprising (#): the number of scraper taxa (#scraper), the number of shredder taxa (#shredder), the number of gathering-collector taxa (#gathering-collector), the number of filtering-collector taxa (#filtering-collector), and the number of predator taxa (#predator), as well as (2) FFG abundance (%) consisting of the number of scraper individuals (%scraper), the number of shredder individuals (%shredder), the number of gathering-collector individuals (%gathering-collector), the number of filtering-collector individuals (%filtering-collector), and the number of predator individuals (%predator). Biotic indices were also used, namely Family Biotic Index (FBI) (Hilsenhoff 1988), Biological Monitoring Working Party (BMWP) (Armitage et al. 1983), Average Score Per Taxon (ASPT), Biological Monitoring Working Party Vietnam (BMWP-Viet) (Nguyen et al. 2014), and Average Score Per Taxon Vietnam (ASPT-Viet) index. After verifying the normality (Shapiro-Wilk test), student-t and the Mann-Whitney were used to test for differences in macroinvertebrate metrics between reference and impacted streams. Metrics that exhibited significant differences were then visualized in the box plots, while Principal Component Analysis (PCA) was used to determine the relationship between abiotic and biotic variables in the two types of ecosystems. Furthermore, streams were clustered using the paired group UPGMA method based on Euclidean distance. Before running the analysis, the data were first transformed.

The formula for biological metrics calculation is as follows:

Diversity indices:

The Simpson Evenness index (1 - D), where D is the dominance index (Hammer et al. 2001; Magurran 2004), is calculated by the equation:

$$D = \sum_{i=1}^{s} p_i^2$$

Where:
S : Number of genera
p_i : Proportion of the total sample represented by the ith genus

Shannon-Wiener index was estimated by the equation (Hammer et al. 2001; Magurran 2004):

$$H' = -\sum_{i=1}^{s} \rho_i \ln \rho_i$$

Where:
H' : Index of taxa diversity (represented as genera)
ρ_i : Proportion of the total number of individuals in the ith genus

Margalef richness index (Hammer et al. 2001; Magurran 2004) D_{Mg}:

$$D_{Mg} = \frac{(S-1)}{\ln N}$$

Pielou evennes index (Hammer et al. 2001, Magurran 2004):

$$J = H' / \ln S$$

N and S are the total number of individuals and taxa within the site.

Biotic Indices:

The family biotic index: FBI was calculated by the formula (Hilsenhoff 1988):

$$FBI = \frac{\sum_{i=1}^{s} n_i * t_i}{\sum_{i=1}^{s} n_i}$$

Where:

S : Number of families included in the analysis

n_i and t_i : Number of individuals and the tolerance value of the *i*th family, respectively.

The Biological Monitoring Working Party Index: BMWP was determined by adding all the families' sensitivity scores in a sample.

The Average Score per Taxon index: ASPT was calculated as the ratio of the BMWP index value to the number of families found in a sample. The list of macroinvertebrate families and its sensitivity scores for BMWP-ASPT referred to (Armitage et al. 1983), while BMWP-Viet and ASPT-Viet referred to (Nguyen et al. 2014).

RESULTS AND DISCUSSION

Characteristics of reference streams

The measurement results of environmental variables from five and three streams at reference and impacted locations respectively, including land cover/use aspects, riverbed substrate (sediment) composition, velocity, discharge, and water quality parameters, are presented in Table 2. Figure 2 illustrates the distribution of eight particle size classes within river substrate ranging from pebble, gravel, very coarse, coarse, medium, fine, and very fine sand to silt, as the sediment characteristics for the two stream groups. Based on the results, the particle size within the two main classes (gravel and sand), showed significant differences between reference and impacted streams (Table 2).

In reference streams, the percentage of gravel ranged from 23.9 to 55.4 (mean of±SD 34.4±12.65), while in the impacted streams, the value was between 4.2 and 25 (mean of±SD 12.4±4.05). Sand in reference and impacted streams ranged from 18.8 to 53.9 (mean±SD 32.0±14.50) and 87.7 to 95.9 (mean±SD 78.5±4.10) respectively. Scatter plots (n-MDS results) showed the distribution of the environmental variables gradient across stream sites (Figure 3). Stream distribution in the n-MDS analysis was plotted based on the PC1 and PC2 axis scores derived from the selected environmental data. The ordinance results with principal component analysis indicated that PC1 explained 82.9% of the data variance, while PC2 accounted for 8.6% (Figure 3).

Table 2 shows significant differences among the sampling groups for the assessed environmental variables, including forest, farmland, pH, TDS, conductivity, TSS, gravel, and sand (Kruskal-Wallis test, p-value <0.05). However, there were no differences in the DO, BOD_5, nitrate, turbidity, organic, or velocity variables (Kruskal-Wallis test, p-value >0.05).

Table 2. Mean and standard deviation of landscape and environmental variables for reference and impacted stream sites in Lasolo Watershed, Southeast Sulawesi, Indonesia. The number of streams in each habitat group is in parentheses

Environmental variables	Reference streams (n=5)		Impacted streams (n=3)			n-MDS scores	
	Mean±SD	Range	Mean±SD	Range	p-value	Axis-1	Axis-2
SCA (ha)	151.2±133.92	39.3 to 384.2	457.4±596.62	77.2 to 1145.1	-	-	-
Nat (%)	85±13.33	65.2 to 100	4±7.50	0 to 13	0.024	-	-
Far (%)	10±12.05	0 to 25	74±23.23	56 to 100	0.024	0.97	0.25
TSS (mg L^{-1})	28±1.87	26 to 30	41±11.59	33 to 54	0.024	0.64	-0.30
TDS (mg L^{-1})	143.9±36.50	104.8 to 204.0	9.7±9.30	0.1 to 18.7	0.024	-	-
Turb (NTU)	2.2±1.47	0.6 to 3.8	8.3±5.96	3.4 to 15.0	0.101	0.73	-0.29
Cond (µS cm^{-1})	285±73.36	211 to 408	20±18.70	0 to 38	0.025	-	-
pH	8.1±0.19	7.8 to 8.3	6.8±0.67	6.4 to 7.6	0.021	-0.78	0.42
DO (mg L^{-1})	7.6±0.37	7 to 7.9	7.1±0.38	6.8 to 7.5	0.095	-0.46	0.42
BOD_5 (mg L^{-1})	0.8±0.40	0.4 to 1.4	0.6±0.44	0.2 to 1.0	0.651	-0.14	0.29
Hard (mg L^{-1})	164±29.66	120 to 200	40±0.00	40 to 40	0.024	-0.92	0.28
Alk (mg L^{-1})	244±35.78	200 to 280	40±0.00	40 to 40	0.024	-0.91	0.36
N (mg L^{-1})	4±1.10	3 to 5	5±0.00	5 to 5	0.606	0.21	-0.50
P (mg L^{-1})	4±0.89	2 to 4	2±0.00	2 to 2	0.085	-0.85	-0.07
Organic (%)	2.34±0.42	1.70 to 2.80	2.30±0.87	1.80 to 3.30	0.880	-0.14	-0.40
Wid (m)	2.2±0.90	0.9 to 3.2	4.1±2.05	2.1 to 6.2	0.099	-	-
Dep (m)	0.1±0.04	0.1 to 0.2	0.4±0.17	0.2 to 0.5	0.024	0.74	-0.22
Vel (m sec^{-1})	0.45±0.51	0.16 to 1.35	0.28±0.16	0.10 to 0.41	0.881	0.02	0.52
Disc-(m^3 sec^{-1})	0.17±0.29	0.01 to 0.69	0.35±0.20	0.16 to 0.56	0.177	0.58	0.31
Gravel (%)	34.38±12.65	23.90 to 55.40	8.20±4.05	4.20 to 12.30	0.024	-0.86	0.44
Sand (%)	32.00±14.50	18.80 to 53.90	91.80±4.10	87.70 to 95.90	0.025	0.94	-0.09

Note: SCA: Total area of streams catchment, Nat: % of natural forest cover, Far: % of farming land use, TSS: Total Suspended Solids, TDS: Total Dissolved Solids, Turb: Turbidity, Cond: Electrical conductivity, pH: Negative log of hydrogen ion concentration, DO: Dissolved Oxygen, BOD_5: Biochemical oxygen demand, Hard: Hardness, Alk: Alkalinity, N: Total nitrogen, P: Total phosphorus, Organic: Total organic carbon, wid: Average width, dep: Average depth, vel: Average velocity, disc: Discharge, gravel, and sand; (-): Do not include in the analysis

Taxonomic and functional composition, species richness, and diversity on the watershed scale

A total of 2474 individuals from 54 families and 19 orders of macroinvertebrates were identified across the eight streams (Table 3). All streams habitat were found to have 15 genera or 19.5% of singleton species including *Hirudinaria, Opalia, Bellamnya, Gordius, Teleogryllus, Nauphoeta, Phryganistria, Anisops, Antipodochlora, Notoaeschna, Tetracanthagyna, Libellula, Similium, Eukiefferiella*, and *Dineutus*, as well as five genera or 6.5% of doubleton species namely *Lymnaea, Naucoris, Pseudolestes*, *Crocothemis*, and *Calliarcys*. Taxonomic richness was higher in reference streams than in the impacted.

A comparison of the abundance in the order level between reference and impacted streams is presented in Figure 5. In the taxonomic order category, the group with the highest abundance was the Decapoda, with a total of 687 individuals, while the abundance ratio at the two locations was 1.40 and 4.7 respectively. The Odonata order had a total abundance of 498 individuals with an abundance ratio of 2.11 at reference and 1.90 at the impacted streams. The group with the third highest abundance (253) was the Ephemeroptera order, with a ratio of 1.01 and 1.27 respectively. However, the results differed in terms of species richness. Odonata had the highest species richness (33 species) with a ratio of 1.3 at reference and 4.7 at the impacted streams. This was followed by Decapods, which had 14 species with a ratio of 1.20 and 7.0 respectively. Ephemeroptera consisted of 13 species with a ratio of 1.10 at reference and 13 at impacted streams.

At the genus level, approximately 64 (or 83.1%) of the 77 streams macroinvertebrate genera were not found at the impacted streams. On the contrary, three new genera appeared at the impacted streams but were not found at reference, namely *Calliarcys*, *Antipodochlora*, and *Hypolestes*. At the family level, about 40 (or 74.07%) of 54 families in reference streams were not found at the impact. Three new families appeared in the impacted streams and were not found in reference, including Leptophlebiidae, Corduliidae, and Hypolestidae. Regarding the order level, about 10 (or 55.6%) of the total 18 orders did not appear in impacted streams, namely *Plecoptera, Phasmatidea, Blattodea, Orthoptera, Araneae, Gordioidea, Mesogastropoda, Sorbeoconcha, Caenogastropoda,* and *Arhynchobdellida*.

A total of 62 genera or 80.5% of the total, were found only in reference streams, while three (3.9%) were discovered in the impacted, and 12 (15.6%) in both. All streams were found to contain 65 insect genera (84.4%) with 62.4% abundance. The total abundance in reference and impacted streams were 1934 and 540 individuals respectively. The most abundant genera included *Paratya* (350), *Caridina* (226), and *Diplacodes* (202) as depicted in Table 3.

Among the total individuals, 1933 (78.2%) functional abundance was found in reference streams and only 540 (21.8%) were impacted. The results also showed that there was a shift in the distribution pattern of functional groups between the two locations. In reference streams, the predominant functional group was shredders and predators, with a relative abundance of 42.1% and 24.5%. Meanwhile, in the impacted site, predators and shredders dominated with a value of 52.4% and 36.9%, respectively (Figure 5).

Based on the results, the observed species richness in the region or all stream habitats was estimated at 77. However, more or larger samples are needed to represent absolute species richness accurately. This was evident from the rarefaction curve, indicating that species accumulation in reference habitat was still rising and did not reach a horizontal asymptote (Figure 4A). Additionally, the species diversity trend, as measured by the Shannon-Wiener diversity index (Figure 4B) showed distinct values, with the accumulation reaching 1.61 and 3.40 in impacted and reference streams, respectively.

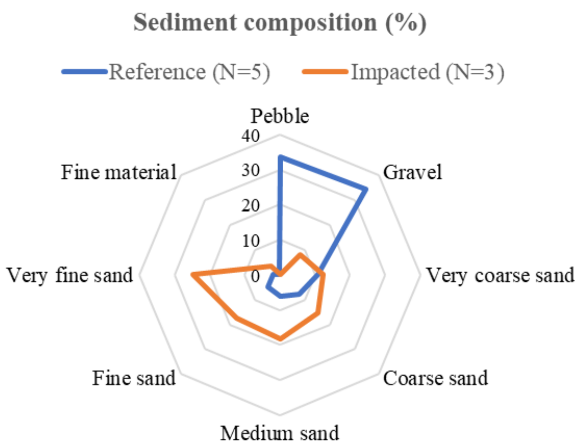

Figure 2. The difference in the sediment composition of reference (N=5) and impacted Streams (N = 3) in the Lasolo Watershed, Indonesia. Note: The grain size and substrate classes; >16 mm = Pebble, 2-16 mm = Gravel, 1-2 mm = Very coarse sand, 0.5-1 mm = Coarse sand, 0.25-0.5 mm = Medium sand, 0.1-0.25 mm = Fine sand, 0.05-0.1 mm = Very fine sand, and <0.05 mm = Silt

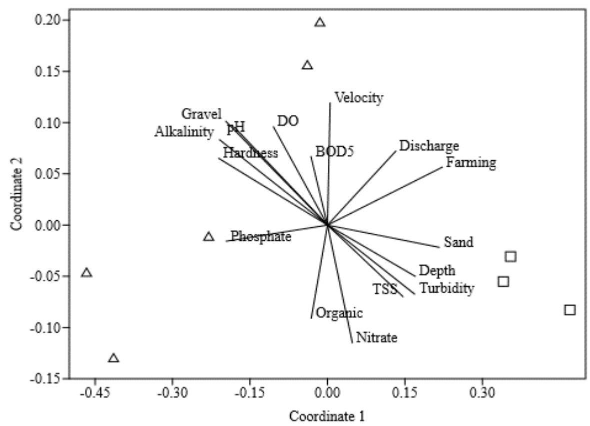

Figure 3. The non-metric Multidimensional Scale (n-MDS) of Euclidean distance shows the distribution of selected environmental variables. Sites were plotted based on the axis PC1 and PC2 variance-covariance scores, PC1 (82.9%) and PC2 (8.6%). The triangle (∆) indicates reference streams, while squares (□) represent the impacted streams. Data were transformed using log (x+1)

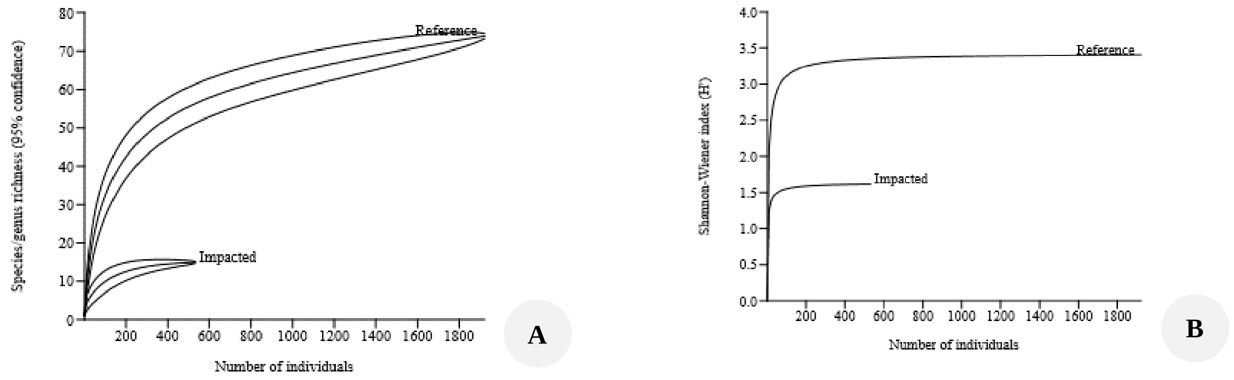

Figure 4. A. The rarefaction curves represent the trend of taxa richness (genus level), and B. The Shannon-Wiener diversity index of streams macroinvertebrates in reference and impacted sites in the Lasolo watershed, Southeast Sulawesi, Indonesia

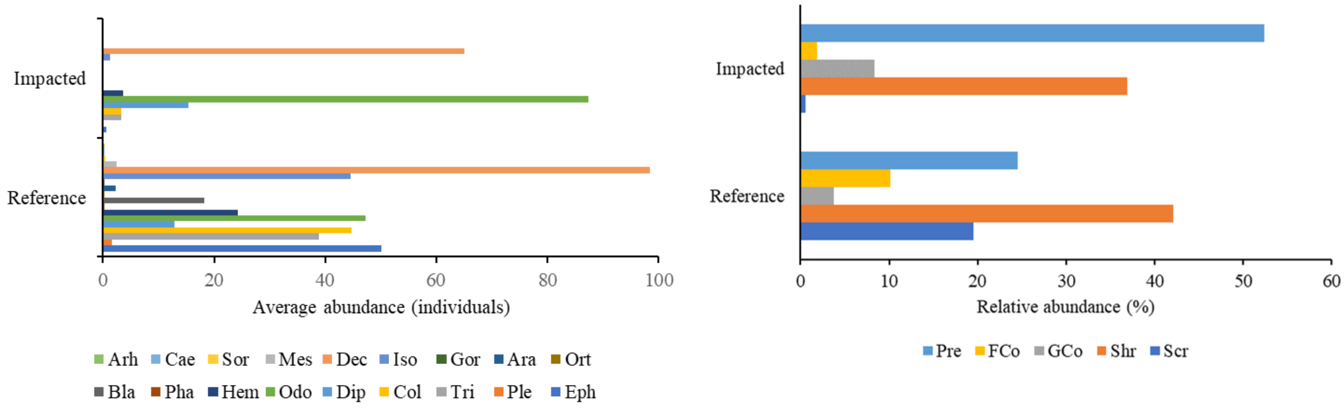

Figure 5. The average abundance of taxonomic groups (order level) and functional feeding groups relative abundance of macroinvertebrate assemblages in the five references and three impacted streams in the Lasolo Watershed, Southeast Sulawesi, Indonesia. Note: Abbreviation for taxonomic group (order): Ephemeroptera (=Eph), Plecoptera (=Ple), Trichoptera (=Tri), Coleoptera (=Col), Diptera (=Dip), Odonata (=Odo), Hemiptera (=Hem), Phasmatidea (=Pha), Blattodea (=Bla), Orthoptera (=Ort), Araneae (=Ara), Gordioidea (=Gor), Isopoda (=Iso), Decapoda (=Dec), Mesogastropoda (=Mes), Sorbeoconcha (=Sor), Caenogastropoda (=Cae), Arhynchobdellida (=Arh); and the acronym for FFG: scraper (Scr), shredder (Shr), gathering-collector (GCo), filtering-collector (FCo), and predator (Pre)

Taxonomic, functional, alpha diversity, and biotic index response in reference and impacted streams

Table 4 presents a summary of 13 biological metrics, including their range, mean, standard deviation, and p-value. These metrics were derived from the analysis of significantly different macroinvertebrates, along with six metrics that did not exhibit significant differences between reference and impacted streams. In total, 19 metrics were tested, and strong evidence of differences was found in taxa richness between both streams (t = 1.44, p<0.01). However, there were no significant differences in the relative abundance of FFG metrics, including scraper, shredder, gathering-collector, filtering-collector, and predator (p-values>0.05). The taxa richness metrics, on the other hand, exhibited significant differences, as shown in Table 4. The range of Shannon-Wiener diversity in reference and impacted streams ranged from 2.22 to 2.82 and 1.44 to 1.77, respectively.

Figure 6 presents a box plot of 15 metrics to confirm the statistical test results of the t-test and Mann-Whitney. Among these metrics, the Family Biotic Index (FBI) did not show a significant difference (t = 1.441, p = 0.200) as indicated by the overlapping interquartile areas between reference and impacted streams. The other fourteen metrics consisting of genera (t = 0.001, p = 0.001), #family (z = 2.099, p = 0.036), #order (t = 5.415, p = 0.002), #insect (t = 7.859, p = 0.000), and #Ephemeroptera-Plecoptera-Trichoptera (#EPT) (z = 2.013, p = 0.034), Shannon-Wiener index (t = 5.345, p = 0.002), Simpson Evenness (z = 2.099, p = 0.036), Margalef Richness (t = 7.848, p < 0.001), BMWP (t = 4.493, p = 0.004), and BMWP-Viet index (t = 5.303, p = 0.001), #scraper (z = 2.152, p = 0.031), #shredder (t = 6.574, p = <0.001), #filtering-collector (z = 1.974, p = 0.048), and #predator (t = 6.625, p = 0.001) showed non-overlapping interquartile values (the 25th-75th percentiles). Metrics that were significantly different from one another were included in the PCA analysis (Figure 7). The analysis produced seven potential metrics, including #family, #insect, #EPT, Shannon-Wiener, Simpson Evenness, and Margalef Richness index, as well as #Scraper. The first axis of the PCA explained 82.8% of the data variability, while the second axis accounted for 13.5%. Furthermore, using environmental and biotic data (Figure 7), a dendrogram was developed showing the degree of similarity between stream sites of the habitat group. The cluster analysis, based on the UPGMA Euclidean distance similarity index, effectively separated reference and impacted streams (Figure 8).

Table 3. The taxa list, Functional Feeding Groups (FFG), and abundance of macroinvertebrates in five natural forests (reference) and three dry agricultural (impacted) streams in the Lasolo watershed, Southeast Sulawesi, Indonesia

Group	Order/Family/Genus	FFG (sources)	Ref.1	Ref.2	Ref.3	Ref.4	Ref.5	Imp.1	Imp.2	Imp.3	∑	%
Insect												
	Ephemeroptera											
	Heptageniidae											
	Heptagenia	Scr (Edwards 2014)	0	0	41	0	0	0	0	0	41	1.65
	Stenacrom	Scr (Edwards 2014)	92	34	0	7	0	0	0	0	133	5.37
	Nexi	Scr (Edwards 2014)	0	0	2	5	0	0	0	0	7	0.28
	Baetidae											
	Baetis	GCo (Bouchard et al. 2004)	0	6	3	3	0	0	0	0	12	0.48
	Baetodes	GCo (Bouchard et al. 2004)	0	27	2	0	0	0	0	0	29	1.17
	Tenuibaetis	GCo (Bouchard et al. 2004)	29	0	0	0	0	0	0	0	29	1.17
	Leptophlebiidae											
	Calliarcys	GCo (Merritt et al. 2017)	0	0	0	0	0	2	0	0	2	0.08
	Plecoptera											
	Perlodidae											
	Alloperla	Pre (Edwards 2014)	0	8	0	0	0	0	0	0	8	0.32
	Trichoptera											
	Hydropsychidae											
	Chaumatopsyche	FCo (Cummins et al. 2005)	0	26	32	0	0	0	0	0	58	2.34
	Hydropsyche	FCo (Cummins et al. 2005)	69	0	0	33	1	0	0	0	105	4.24
	Polycentropididae											
	Plectrocnemia	FCo (Cummins et al. 2005)	9	0	0	0	0	10	0	0	19	0.77
	Philopotamidae											
	Chimarra	FCo (Merritt et al. 2017)	0	0	0	10	0	0	0	0	10	0.40
	Brachycentridae											
	Brachycentrus	FCo (Merritt et al. 2017)	1	0	2	0	3	0	0	0	5	0.20
	Limnephilidae											
	Limnephilus	Shr (Cummins et al. 2005)	0	0	0	7	1	0	0	0	8	0.32
	Coleoptera											
	Scirtidae											
	Scirtes	Scr (Edwards 2014)	16	4	63	37	9	3	0	0	132	5.33
	Psephenidae											
	Psephenus	Scr (Cummins et al. 2005)	0	0	5	2	1	0	0	0	8	0.32
	Hydraenidae											
	Hydraena	Scr (Cummins et al. 2005)	0	0	0	44	0	0	0	0	44	1.79
	Gyrinidae											
	Dineutus	Pre (Cummins et al. 2005)	0	0	0	1	0	0	0	0	1	0.04
	Gyrinus 1	Pre (Cummins et al. 2005)	0	0	0	1	0	0	0	7	8	0.32
	Noteridae											
	Hydrocanthus	Pre (Cummins et al. 2005)	0	0	0	5	0	0	0	0	5	0.20
	Dytiscidae											
	Hydrovatus	Pre (Bouchard et al. 2004)	0	0	0	0	21	0	0	0	21	0.85
	Hydrophiliidae											
	Hydrobiomorpha	Pre (Bouchard et al. 2004)	0	0	0	0	15	0	0	0	15	0.61
	Odonata											
	Libellulidae											
	Trithemis	Pre (Bouchard et al. 2004)	1	0	10	0	0	0	0	0	11	0.44
	Crocothemis	Pre (Bouchard et al. 2004)	0	2	0	0	0	0	0	0	2	0.08
	Diplacodes	Pre (Bouchard et al. 2004)	0	0	17	0	0	163	0	22	202	8.16
	Libellula	Pre (Bouchard et al. 2004)	0	0	1	0	0	0	0	0	1	0.04
	Pachydiplax	Pre (Bouchard et al. 2004)	0	0	0	3	0	0	0	0	3	0.12
	Zyxomma	Pre (Bouchard et al. 2004)	0	1	0	0	9	0	0	0	10	0.40
	Scapanea	Pre (Bouchard et al. 2004)	0	3	0	0	0	0	0	0	3	0.12
	Aeshnidae											
	Anax	Pre (Bouchard et al. 2004)	0	0	8	0	44	0	0	0	52	2.10
	Tetracanthagyna	Pre (Bouchard et al. 2004)	0	0	0	0	1	0	0	0	1	0.04
	Argiolestidae											
	Austroargiolestes	Pre (Bouchard et al. 2004)	0	0	0	0	9	0	0	0	9	0.36
	Telephlebiidae											
	Notoaeschna	Pre (Bouchard et al. 2004)	0	0	0	0	1	0	0	0	1	0.04
	Chlorocyphidae											
	Arstocypha	Pre (Bouchard et al. 2004)	2	1	6	1	0	0	0	0	10	0.40

Corduliidae											
Antipodochlora	Pre (Bouchard et al. 2004)	0	0	0	0	0	1	0	0	1	0.04
Coenagrionidae											
Ischnura	Pre (Edwards 2014)	0	1	0	3	0	56	4	9	73	2.95
Platystictidae											
Palaemnema	Pre (Bouchard et al. 2004)	78	0	12	0	0	0	0	0	90	3.64
Protosticta	Pre (Bouchard et al. 2004)	0	0	9	4	0	0	0	0	13	0.52
Pseudolestidae											
Pseudolestes	Pre (Bouchard et al. 2004)	0	0	2	0	0	0	0	0	2	0.08
Gomphidae											
Austrogomphus	Pre (Bouchard et al. 2004)	0	7	0	0	0	0	0	0	7	0.28
Hypolestidae											
Hypolestes	Pre (Bouchard et al. 2004)	0	0	0	0	0	7	0	0	7	0.28
Diptera											
Chironomidae											
Chironomus	Pre (Bouchard et al. 2004)	1	0	0	0	0	32	0	11	44	1.78
Eukiefferiella	Pre (Bouchard et al. 2004)	0	0	0	1	0	0	0	0	1	0.04
Simulildae											
Similium	Pre (Bouchard et al. 2004)	0	0	0	1	0	0	0	0	1	0.04
Prosimulium	Pre (Bouchard et al. 2004)	8	0	0	0	0	0	0	0	8	0.32
Tipulidae											
Tipula	Pre (Bouchard et al. 2004)	0	5	12	0	0	0	0	0	17	0.68
Limoniidea											
Hexatoma	Pre (Bouchard et al. 2004)	1	20	3	0	3	0	3	0	30	1.21
Tabanidae											
Crysops	Pre (Bouchard et al. 2004)	0	0	3	2	0	0	0	0	5	0.20
Athericidae											
Aetherix	Pre (Bouchard et al. 2004)	1	1	0	0	2	0	0	0	4	0.16
Hemiptera (Hem)											
Gerridae											
Tenagogonus	Pre (Bouchard et al. 2004)	0	21	7	1	0	0	0	0	29	1.17
Ptilomera	Pre (Bouchard et al. 2004)	0	8	4	1	0	0	4	0	17	0.68
Aquarius	Pre (Bouchard et al. 2004)	5	0	0	1	12	0	0	2	20	0.81
Neogerris	Pre (Bouchard et al. 2004)	4	0	0	0	0	0	0	0	4	0.16
Nepidae											
Ranatra	Pre (Bouchard et al. 2004)	0	0	0	1	0	0	1	4	5	0.20
Naucoridae											
Naucoris	Pre (Bouchard et al. 2004)	0	0	0	2	0	0	0	0	2	0.08
Notonectidae											
Notonecta sp.1	Pre (Bouchard et al. 2004)	0	0	0	0	10	0	0	0	10	0.40
Notonecta sp.2	Pre (Bouchard et al. 2004)	0	1	0	0	4	0	0	0	5	0.20
Anisops	Pre (Bouchard et al. 2004)	0	0	0	1	0	0	0	0	1	0.04
Veliidae											
Rhagovelia	Pre (Bouchard et al. 2004)	0	34	0	0	4	0	0	0	38	1.53
Blattodea											
Blaberidae											
Gromphadorhina	Shr (Cummins et al. 2005)	0	6	78	0	6	0	0	0	90	3.64
Nauphoeta	Shr (Cummins et al. 2005)	1	0	0	0	0	0	0	0	1	0.04
Orthoptera											
Gryllidae											
Teleogryllus	GCo (Cummins et al. 2005)	0	0	0	1	0	0	0	0	1	0.04
Phasmatidea											
Phasmatidae											
Phryganistria	Pre (Bouchard et al. 2004)	1	0	0	0	0	0	0	0	1	0.04
Araneae											
Pisauridae											
Dolomedes	Pre (Bouchard et al. 2004)	0	8	0	0	3	0	0	0	11	0.44
Non-insect											
Isopoda (Iso)											
Cirolanidae											
Cirolana	Shr (Cummins et al. 2005)	0	2	51	100	0	3	1	0	157	6.34
Philosciidae											
Philoscia	Shr (Cummins et al. 2005)	0	10	0	11	1	0	0	0	22	0.89
Eubelidae											
Koweitoniscus	Shr (Cummins et al. 2005)	48	0	0	0	0	0	0	0	48	1.94

Decapoda											
Gecarcinucidae											
Parathelphusa	Shr (Cummins et al. 2005)	0	6	52	16	10	0	0	0	78	3.15
Potamidae											
Potamon	Shr (Cummins et al. 2005)	27	0	0	0	0	0	0	0	27	1.09
Atydae											
Paratya	Shr (Cummins et al. 2005)	0	13	37	93	12	177	0	18	350	14.15
Caridina	Shr (Cummins et al. 2005)	9	0	0	215	2	0	0	0	226	9.13
Mesogastropoda											
Viviparidae											
Bellamya	Scr (Merritt et al. 2017)	0	0	0	1	0	0	0	0	1	0.04
Thiaridae											
Melanoides	Scr (Merritt et al. 2017)	10	0	0	1	0	0	0	0	11	0.44
Sorbeoconcha											
Lymnaeidae											
Lymnaea	Scr (Merritt et al. 2017)	0	0	0	2	0	0	0	0	2	0.08
Caenogastropoda											
Epitoniidae											
Opalia	Scr (Merritt et al. 2017)	0	1	0	0	0	0	0	0	1	0.04
Arhynchobdellida											
Hirudinidae											
Hirudinaria	Pre (Cummins et al. 2005)	0	0	1	0	0	0	0	0	1	0.04
Gordioidea (Gor)											
Gordiidae											
Gordius	Par (Bouchard et al. 2004)	1	0	0	0	0	0	0	0	1	0.04

Note: The abbreviation: Ref.: Reference stream, Imp: Impacted stream, FFG: Scraper (Scr), Shredder (Shr), Gathering-collector (GCo), Filtering-collector (FCo), Predator (Pre), and Parasite (Par)

Table 4. The range, mean, standard deviation, and p-value of potential macroinvertebrate metrics in the Lasolo Watershed, Southeast Sulawesi

Metrics category	**Reference streams (n=5)**		**Impacted streams (n=3)**		**Statistical test**	***p-value***
	Range	**Mean ± SD**	**Range**	**Mean± SD**		
Taxonomic based						
Total Abundance	184 to 617	386.8 ± 171.59	13 to 454	180 ± 239.18	t = 1.440	0.200
#Genera	22 to 34	26.4 ± 4.56	5 to 10	7.3 ± 2.52	t = 6.532	0.001
#Family	20 to 29	22.6 ± 3.65	5 to 10	7.3 ± 2.52	z = 2.099	0.036
#Order	9 to 12	10.8±1.304	4 to 7	5.3±1.53	t = 5.415	0.002
#Insect	18 to 26	21.4 ± 2.97	4 to 8	6 ± 2.00	t = 7.859	0.000
#EPT	3 to 6.0	5 ± 1.22	0 to 2	0.7 ± 1.15	z = 2.013	0.034
Shannon-Wiener index	2.22 to 2.82	2.54 ± 0.27	1.44 to 1.77	1.7 ± 0.17	t = 5.345	0.002
Simpson Evenness index	0.82 to 0.92	0.9 ± 0.04	0.7 to 0.81	0.8 ± 0.06	z = 2.099	0.036
Margalef Richness index	3.49 to 5.14	4.3 ± 0.61	1.40 to 1.56	1.5 ± 0.08	t = 7.848	<0.001
Pielou Evenness index	0.63 to 0.87	0.8 ± 0.10	0.63 to 1.00	0.8 ± 0.20	t = 0.621	0.557
Functional feeding group						
#Scraper	2 to 5	3 ± 1.41	0 to 1	0.3 ± 0.58	z = 2.152	0.031
#Shredder	4 to 6	5 ± 1.00	1 to 2	1.3 ± 0.58	t = 6.574	<0.001
#Gathering-collector	0 to 3	1.8 ± 1.10	0 to 2	1 ± 1.00	z = 0.959	0.337
#Filtering-collector	1 to 4	2.4 ± 1.14	0 to 1	0.3 ± 0.58	z = 1.974	0.048
#Predator	8 to 15	13 ± 2.83	4 to 5	4.3 ± 0.58	t = 6.625	0.001
%Scraper	10 to 118	75.4 ± 48.07	0 to 3	1.0 ± 1.73	z = 2.100	0.036
%Shredder	32 to 442	162 ± 173.2	1 to 180	66.3 ± 98.8	t = 0.866	0.420
%Gathering-collector	0 to 33	14.6 ± 15.6	0 to 34	15.0 ± 17.35	t = 0.034	0.974
%Filtering-collector	4 to 87	39 ± 30.61	0 to 10	3.3 ± 5.77	z = 1.800	0.072
%Predator	27 to 138	98.4 ± 42.29	12 to 227	94.3 ± 116	t = 0.008	0.994
Biotic Index-based						
BMWP index	66 to 113	91.4 ± 19.89	16 to 46	31 ± 15.00	t = 4.493	0.004
BMWP-Viet index	80 to 135	99.4 ± 21.17	14 to 37	27.3 ± 11.93	t = 5.303	0.001
ASPT index	3 to 4.1	3.5 ± 0.43	3.2 to 4.6	4.1 ± 0.76	t = 1.512	0.181
ASPT-Viet index	3.64 to 4.1	3.8 ± 0.19	2.8 to 4.43	3.6 ± 0.82	t = 0.355	0.735
Family Biotic Index	0.36 to 1.9	1.3 ± 0.62	1.2 to 2.3	1.9 ± 0.60	t = 1.441	0.200

Note: #scraper: number of scraper taxa, #shredder: number of shredder taxa, #gathering-collector: number of gathering-collector taxa, #filtering-collector: number of filtering-collector taxa, #predator: number of predator taxa, %scraper: number of scraper individuals, %shredder: number of shredder individuals, %gathering-collector: number of gathering-collector individuals, %filtering-collector: number of filtering-collector individuals, %predator: number of predator individuals

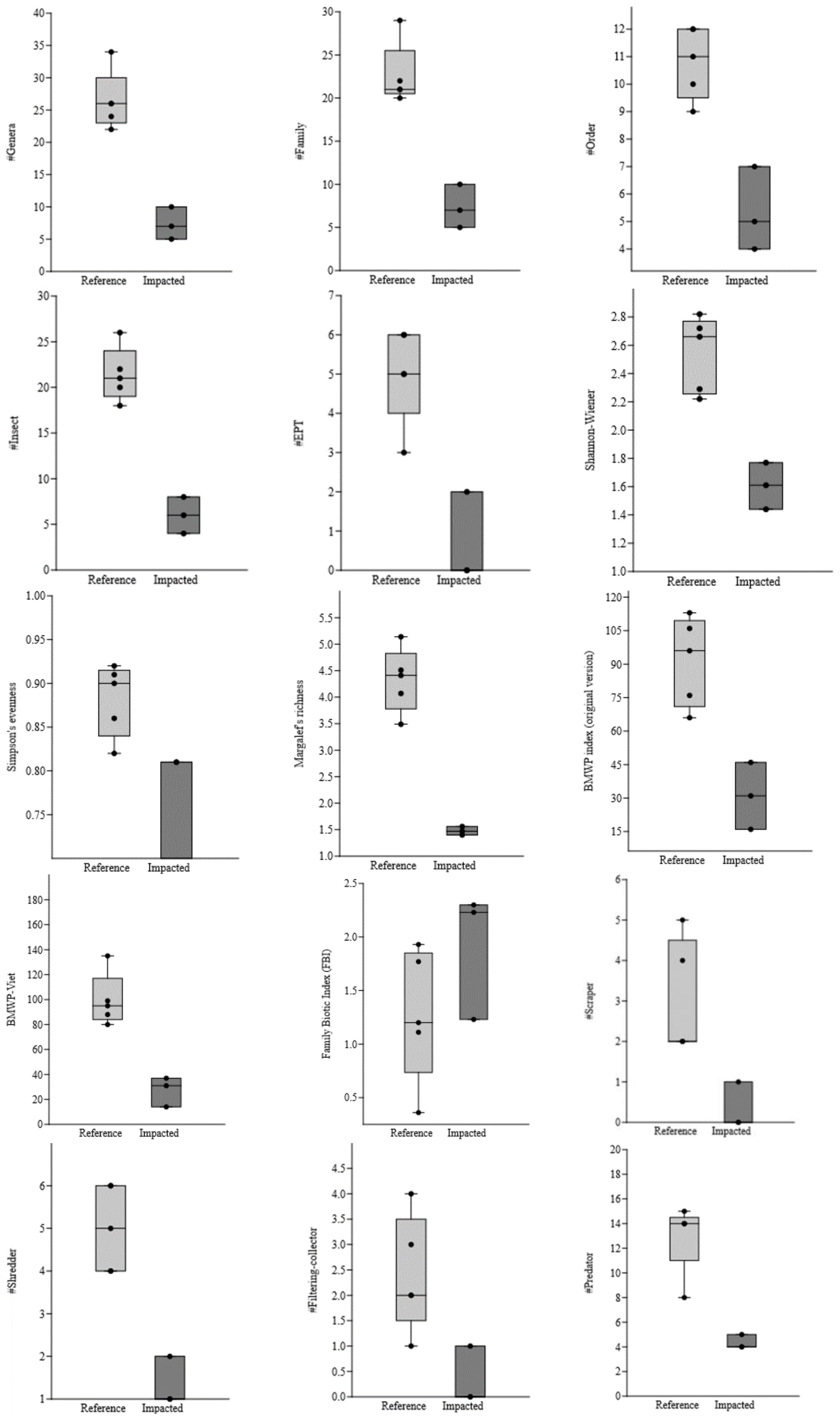

Figure 6. Box plot of fifteen selected metrics used to differentiate between reference and impacted streams in the Lasolo Watershed, Southeast Sulawesi, Indonesia. For each metric, the box shows the 25th-75th percentiles, the horizontal line inside the box shows the median and the short horizontal line shows the range (min-max) value. The metric value of each stream sample is plotted as a dot

Discussion

Biological monitoring integrates the physical, chemical, and biological conditions of aquatic ecosystems (Forio and Goethals 2020; Sumudumali and Jayawardana 2021). In the last five years, the development of benthic invertebrates as a tool for environmental assessment has been widely applied to stream/river ecosystems in various countries, including Indonesia (Kahirun et al. 2019; Hamid et al. 2021; Harahap et al. 2021; Wakhid et al. 2021; Badjoeri and Samir 2022; Ilmi et al. 2023; Prakoso et al. 2023; Retnaningdyah et al. 2023). However, to determine an index or metric suitable for bioassessment in a particular climatic region, it is necessary to validate its performance. This can be achieved by comparing how well the metric performs in ecosystems exposed to single or multiple stresses with those exposed to little or no stress (McDaniel and Pascoe 2017; Hawkins and Carlisle 2022). In addition, the resulting metrics must be independent, for example, in developing a multimetric index (Macedo et al. 2016).

The microhabitat structure in stream/river changes due to anthropogenic disturbances and natural factors. These alterations have consequences for macroinvertebrate richness, density, and diversity taxonomically, as well as functionally (Bhandari et al. 2018; Guareschi et al. 2021; Li et al. 2021; Marques et al. 2021; Godoy et al. 2022; Calderon et al. 2023). Changes in macroinvertebrate composition were also found in response to agricultural land use and urbanization, accompanied by increasing total phosphate content, conductivity, and water temperature. These changes are often associated with modifications in-stream hydro morphologic elements such as sediment composition and flow rate (Zhang et al. 2018; Lorenz and Wolter 2019; Shuman et al. 2020).

Reference stream characteristics that played an essential role in the stability and health of the river flow included the riparian buffer and canopy cover, as well as the complexity of the microhabitat. The riparian forest buffer and chemical pollution in the agricultural landscape affect the functional diversity of macroinvertebrates. For example, forested watersheds tend to have an abundance of shredders, while plantation areas with and without riparian buffers predominantly consist of scrapers and collectors-filterers respectively (Pallottini et al. 2017; Marques et al. 2021). Changes in habitat, water quality, and macroinvertebrate communities are associated with the loss of riparian vegetation (Espinoza-Toledo et al. 2021). In addition, riparian buffers and canopy cover, for example, in oil palm plantations, play a role indirectly by increasing physical barriers, reducing surface runoff, and preventing bank erosion, thereby enhancing the ability of river banks to preserve sediment and create microhabitats. These microhabitats offer shelter, food, and a breeding ground for aquatic organisms (Chellaiah and Yule 2018).

The EPT metric, which stands for the insect orders Ephemeroptera (=mayflies), Plecoptera (=stoneflies), and Trichoptera (=caddisflies), found higher species richness in reference compared to impacted streams, with a statistically significant difference ($z = 2.013$, $p = 0.034$). Previous studies have shown EPT as indicators metric for macroinvertebrate groups highly sensitive to various sources or types of environmental stress, including changes in land use (Gomes et al. 2022), river bed sediment structure (Katano et al. 2021), discharge of wastewater from point sources (Kimmel and Argent 2019), and enrichment nutrition (Zhang et al. 2018). The EPT, specific to Hydropsychidae, Perlidae, and Baetidae, are typically only found in forest-covered stream stations and are categorized as indicators of clean water species. These organisms tend to reside, forage, and seek shelter between or beneath rocks, particularly in gravel-rich environments resembling those preferred by baetid. According to observations, rocks with fast currents and a stable substrate had Hydropsychidae adhering to their surfaces. Physical modifications to the substrate can impact the variety of aquatic insects (Masese et al. 2021).

This study showed that the accumulation value of the Shannon-Wiener index (3.42) was higher in reference streams, with 2.82 as the highest value. Previous studies found that the Shannon-Wiener Index varied geographically, for example, it was 2.68 in forested headwater streams of Ciliwung River (Wakhid et al. 2021), 3.10 in Menala River, Sumbawa (Sany et al. 2023), and 3.3 in waterfall ecosystem, Bawean island (Retnaningdyah et al. 2023). In several multimetric studies conducted in the tropics, this index was not found as the primary indicator for differentiating between reference and impacted streams (Nguyen et al. 2014; de Carvalho et al. 2017). This differed from the results obtained in the Zio River basin in Togo (Tampo et al. 2020). Furthermore, the analysis results of the Family Biotic Index (FBI or HBI) differ from several other studies, including within the Cau River basin in Vietnam (Nguyen et al. 2014) and in Malaysia (Arman et al. 2019). The core metrics observed in this study including #insects, #EPT, Shannon-Wiener index, and Margalef index were similar to the 23 metrics produced from macroinvertebrate-based multimetric index studies in the tropics (Nguyen et al. 2014; Macedo et al. 2016; Arman et al. 2019; Tampo et al. 2020).

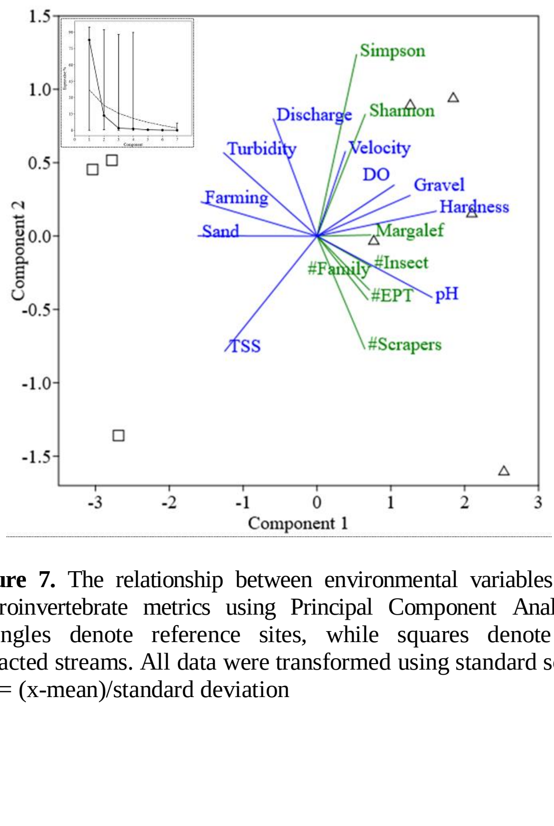

Figure 7. The relationship between environmental variables and macroinvertebrate metrics using Principal Component Analysis. Triangles denote reference sites, while squares denote the impacted streams. All data were transformed using standard scaler (x') = (x-mean)/standard deviation

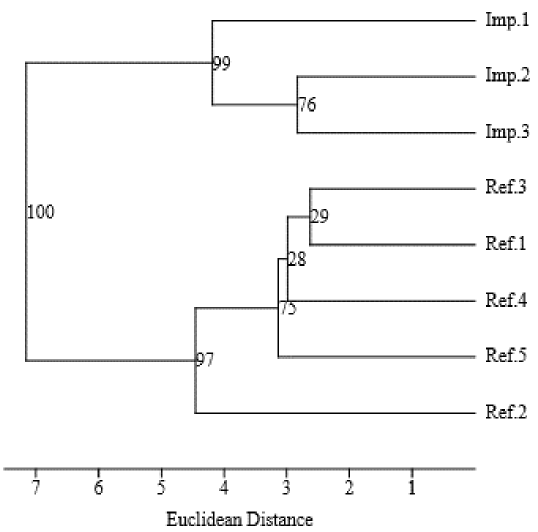

Figure 8. Cluster tree of reference and impacted streams in the Lasolo Watershed Sulawesi, based on UPGMA Euclidean Distance similarity index of 10 environmental parameters and seven biological metrics by Paired Group (UPGMA) method, bootstrap 999x. The corresponding number of each node indicates the bootstrap percentage. Note: Ref.: Reference stream, Imp.: Impacted stream

In conclusion, the total number of species found in eight stream networks within the Lasolo watershed, Southeast Sulawesi, was 77, totaling 2474 individuals. The results provided a basis for comparing the number and composition of taxa (aquatic macroinvertebrate biodiversity) between reference and impacted streams. These changes were evident through an 83.1%, 74.07%, and 55.6% decrease at the genus, family, and order level. Functionally, there was also a shift in the distribution pattern of the relative abundance. The predominant functional groups were shredders and predators in reference (42.1% and 24.5%) and impacted (52.4% and 36.9%) streams. A total of fourteen metrics were found in the category of taxonomic and functional richness, alpha diversity, and biotic index, which effectively differentiated between reference and impacted streams. These metrics included genera (#genera), family (#family), order (#order), insect (#insect), and Ephemeroptera-Plecoptera-Trichoptera taxa (#EPT), Shannon-Wiener, Simpson Evenness, Margalef Richness index, BMWP, BMWP-Viet, number of scraper (#scraper), shredder (#shredder), filtering-collectors (#filtering-collector), and predator taxa (#predator). Based on the redundancy analysis, there was a significant correlation between these metrics, and the visualization results from the PCA and Cluster analysis produced seven "potential" metrics, which effectively differentiated between reference and the impacted ecosystems, namely: #family, #insect, #EPT, #scraper, Shannon-Wiener diversity index, the Simpson Evenness index, and the Margalef Richness index. The top limit that indicated good condition was the 75th percentile of every seven positive metrics from reference sites, while the lower limit representing poor condition in the impacted sites was the 25th percentile. Adopting a multimetric method, using reference ecosystem metrics to assess land use change is crucial for the conservation of freshwater biodiversity.

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