



# Macroinvertebrate community structure and feeding interactions along a pollution gradient in Gilgel Gibe watershed, Ethiopia: Implications for biomonitoring



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## ABSTRACT

Feeding interactions among functional feeding groups (FFGs) of macroinvertebrates are robust indicators of aquatic ecosystem interactions. They provide information regarding organic matter processing, habitat condition and trophic dynamics. In tropical rivers with pronounced wet and dry seasons, macroinvertebrate based ecological monitoring tools are explicitly focused on metrics and indices, while ignoring interactions of FFGs. Therefore, the objective of this study was to investigate the functional feeding type metrics, diversity indices and feeding interactions among FFGs of macroinvertebrates along the water pollution gradient in Gilgel Gibe watershed, Ethiopia. Water quality parameters and macroinvertebrate community attributes were assessed for samples collected from upstream sites (15 sites), urban-impacted stretches (12 sites) and wetland-affected river zones (7 sites) of the watershed during the rainy (July) and dry (February) seasons. To understand the effect of pollution on the feeding interactions, stable carbon and nitrogen isotopes were analyzed. Macroinvertebrate-based diversity indices and functional feeding type metric showed deterioration of ecological integrity at the urban-impacted sites and substantial recovery in the wetland-affected downstream sites. Omnivorous feeding behavior of macroinvertebrates was noted for the upstream sites, whereas clear trophic guilds of FFGs were suggested for the wetland-affected river zones by the stable isotope results. The results of pollution gradient analysis and feeding interactions among FFGs revealed that the urban-impacted sites showed weaker interactions when compared to upstream and wetland influenced sites. This affirms the potential importance of feeding interactions among FFGs of macroinvertebrates in water quality monitoring.

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## 1. Introduction

The settings of river systems are usually ideal for human settlement, which makes them prone to high human pressure. In the absence of water resources protection, rivers collect pollutants as they flow through urban areas and agricultural landscape (Devi et al., 2008; Xu et al., 2014). Recently, river pollution has become an escalating environmental problem in developing countries (Awoke et al., 2016; Ayenew, 2007; Nyenje et al., 2010). It is mainly due to unlimited population growth, which led to

urbanization, intensification of agricultural activities and deforestation of river watershed, which in turn trigger the direct release of pollutants (Awoke et al., 2016; Devi et al., 2008). Several case studies reported that rivers in the developing world are being stressed and losing their natural state (Kloos and Legesse, 2010; Ndiritu et al., 2006) primarily due to anthropogenic disturbances (Tejerina-Garro et al., 2005). Therefore, monitoring and detection of the impact of environmental stressors on aquatic systems are crucial for the protection and restoration of river ecosystems through appropriate planning and implementation of integrated watershed management (Friberg et al., 2011; Iliopoulou-Georgudaki et al., 2003).

The increased production of sewage associated with the expansion of urban centers and the widespread utilization of fertilizers in agriculture within watersheds leads to the overloading of

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receiving water bodies with allochthonous nutrients, mainly nitrate and phosphate (Bannon and Roman, 2008). Such eutrophication of water bodies has been directly linked to the loss of aquatic biodiversity, functional feeding groups, and alteration of benthic food web structures and functions (Vander Zanden et al., 2005; Xu et al., 2014). Thus, aquatic ecosystems biomonitoring is very important to establish the causal relationships between stressors and ecologically substantial responses (Clements et al., 2002).

The status of aquatic life is considered as the most direct and effective measure of a water body's overall ecosystem health (i.e., chemical, physical, and biological integrity) (Nadushan and Ramezani, 2011). However, water monitoring schemes that consider the presence/absence and abundance of an indicator organism may overlook important components of the food web. Therefore, besides the composition and diversity of aquatic biota along the pollution gradient, quantitative studies of feeding relationships among FFGs of macroinvertebrates are essential to detect allochthonous material and nutrient load (Vander Zanden et al., 2005; Zah et al., 2001). In these regard, stable isotopes provide a way to trace and identify details of anthropogenic nitrogen input and pathway in water bodies (Bannon and Roman, 2008; Vander Zanden et al., 2005). The analysis of stable isotopes, which does not depend on species relative abundances, can also help us overcome sampling limitations associated with sampling efforts in river health monitoring programs (di Lascio et al., 2013). Furthermore, stable carbon and nitrogen isotopes have proven their utility to serve as a useful tool for the identification of trophic relationships among consumers and their food sources (Roussel et al., 2014; Vander Zanden et al., 2005).

Due to stepwise trophic level enrichment of isotopic signatures, stable nitrogen isotope ( $\delta^{15}\text{N}$ ) is used to infer the trophic position, while stable carbon isotope ( $\delta^{13}\text{C}$ ) provides information regarding the sources of energy to higher trophic level consumers (Vander Zanden et al., 2005). The nitrogen isotopic signature ( $\delta^{15}\text{N}$ ) of aquatic primary producers, which indeed cascades to higher trophic level consumers through fractionation, is a function of concentration of dissolved inorganic nitrogen in the water (Hoefs, 2008; Loomer et al., 2015) and types of nitrogen sources (Bannon and Roman, 2008). For instance, higher inputs of inorganic nitrogen, including industrially produced ammonia, into eutrophic systems leads to more fractionation against  $^{15}\text{N}$ , which in turn lowers the trophic position ( $\delta^{15}\text{N}$ ) of consumers. On the other hand, low fractionation against  $^{15}\text{N}$  in the nitrate-depleted systems leads to high nitrogen isotopic signature of aquatic consumers (Somes et al., 2010).

Macroinvertebrates are among the most common bioindicators of water pollution in tropical river systems (Aschalew and Moog, 2015; Beyene et al., 2009a, 2009b). They also occupy a central position in aquatic food web through linking food sources with top trophic level consumers (Resh, 2008). The distributions of FFGs of macroinvertebrates provide scientific information regarding organic matter processing, habitat condition, trophic dynamics (Xu et al., 2014) and ecological quality of rivers and river-associated wetlands (Ambelu et al., 2010; Mereta et al., 2013). Although a number of studies have addressed the feeding relationships among the FFGs of macroinvertebrates in the temperate region (Bunn et al., 2003; Imberger et al., 2014; Zah et al., 2001), little is known about how feeding interactions are altered by differing river water quality influenced by human activities in tropical Africa. Therefore, investigating feeding interactions among macroinvertebrates, their distribution and diversity can improve our understanding of nutrient loading and ecological integrity in aquatic ecosystems. This paper aims at assessing the macroinvertebrate community structure and feeding interactions among FFGs along a pollution gradient.

## 2. Materials and methods

### 2.1. Sampling sites

The study was conducted in the Gilgel Gibe catchment of the Omo-Gibe river basin, southwest of Ethiopia (Fig. 1). Jimma town is the largest urban and administrative center in the catchment. The small rivers, which cross Jimma town and flowing to Gilgel Gibe dam, serve as a natural sewerage lines for domestic waste (Devi et al., 2008). In the present study, a total of 34 sampling sites that permit upstream and downstream comparisons were selected from three different parts of the catchment (Reynoldson et al., 1997): upstream (Up1–Up15), urban-impacted (U1–U12) and Boye wetland/dam-affected (W1–W7) sites. The study was undertaken during the representative months of rainy (July, 2013) and dry (February, 2014) seasons.

Upstream sites (Up1–Up15) - The Rivers are relatively fast-flowing with no/little urban influence. They are partly covered with riparian vegetation.

Urban-impacted sites (U1–U12) - The sampling sites are subjected to direct urban influence from Jimma town. Domestic liquid and solid waste is directly discharged into the river from different sources. There is no/little riparian vegetation.

Boye wetland/dam-affected sites (W1–W7) - The sampling sites are downstream from Jimma town past the Boye wetland/dam. The rivers are slow-flowing.

### 2.2. Water sampling, processing and analysis

American Public Health Association et al. (2005) recommends in-situ measurements for the parameters that changes over time due to chemical reactions or biological changes. The levels of dissolved oxygen (DO), water temperature and electrical conductivity (EC) were measured using HACH-hd401 multi-parameter (HACH, Loveland, USA). Turbidity and current velocity (Velo) were measured using Wag-WT3020 turbidity meter (Halma, Amersham, UK) and a flow meter, respectively.

In order to collect sufficiently mixed and representative water samples across the width of rivers, inclusion of three to five points is recommended (Bartram and Ballance, 1996). Accordingly, water samples were collected from three sampling points across the width of the rivers and mixed proportionally. Unfiltered water samples were collected using 250 ml polyethylene bottles for total nitrogen (TN), total phosphorous (TP), total suspended solids (TSS) and total dissolved solids (TDS) analysis. Water samples were also filtered onsite using a filtration apparatus and Whatman glass microfiber filters (GF/F) and kept in 100 ml polyethylene bottles for the analysis of dissolved nutrients (i.e. soluble reactive phosphorus (SRP) and nitrate-nitrogen ( $\text{NO}_3\text{-N}$ )). Within six hours after sampling, samples were transported in an ice-box to the laboratory of Environmental Health Sciences and Technology, Jimma University, Ethiopia and immediately kept in a deep freezer until the analyses were made. All the aforementioned parameters were determined following the standard methods described in American Public Health Association et al. (2005). SRP and TP (after digestion with persulfate) were measured by the Ascorbic acid method.  $\text{NO}_3\text{-N}$  and TN were determined by the sodium salicylate and Kjeldahl methods, respectively. TDS and TSS were measured gravimetrically.

### 2.3. Macroinvertebrate sampling and identification

A rectangular frame kick net with a 250  $\mu\text{m}$  mesh size on a 50  $\times$  33 cm frame was used to collect macroinvertebrates. The river bed was disturbed with the feet of a person, facing the water current, for three minutes to dislodge macroinvertebrates found within a ten-meter stretch. After three minutes of sampling, the

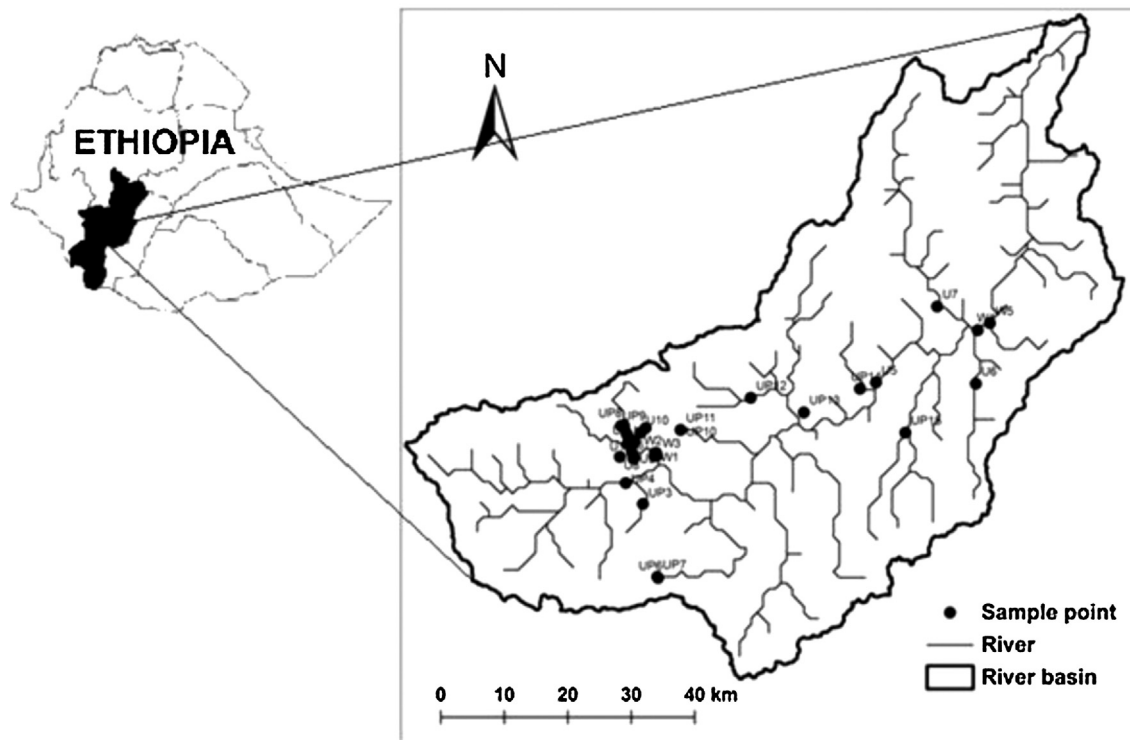


Fig. 1. Map of sampling sites in Gilgel Gibe watershed, Ethiopia (Up = upstream, U = urban-impacted, W = wetland-affected).

content of the net was transferred into a white tray, partly sorted onsite and kept in vials. The remaining unsorted content was transferred to a collecting jar containing 70% ethanol. This procedure was performed three times in different microhabitats of a sampling site to be able to calculate the pooled value (Beyene et al., 2009a).

All the samples in the vials and jars were transported to the laboratory of Environmental Health Science and Technology Department, Jimma University, for sorting (samples in the jars) and identification. All macroinvertebrates were sorted, identified to the family level using standard systematic identification keys and counted using a binocular dissecting microscope (RW, 2004; Tachet et al., 2000).

#### 2.4. Collection, processing and analysis of macroinvertebrate samples for stable isotopes

Macroinvertebrate samples for isotopic analysis were collected from upstream, urban-impacted, and wetland-affected sites during the dry season. In order to avoid the effect of preservatives on the isotopic signatures (Hershey et al., 2006), samples for stable carbon and nitrogen isotopic analysis were not preserved. The macroinvertebrate samples were categorized into their functional feeding groups as predators, collector-gatherers, collector-filterers, grazers and shredders, based on dietary descriptions (Mandaville, 2002). The samples were dried at 50 °C for 48 h in a drying oven and ground into fine powder using mortar and pestle. For larger macroinvertebrates, each replicate sample consisted of a single individual, whereas for smaller invertebrates, samples consisted of a composite of many individuals to obtain sufficient biomass for the analysis (Vander Zanden et al., 2005). To prevent possible contamination from non-dietary carbonates, carapaces of crustaceans and shells of large-sized mollusks were removed manually (Finlay et al., 2002). About one mg sub-samples were transferred into tin capsules (5 × 9 mm) and analyzed using Delta V isotope ratio mass spectrometer connected to an elemental analyzer (Thermo

Fisher Scientific company, Massachusetts, USA) at the Analytical and Environmental Chemistry laboratory of Vrije Universiteit Brussel, Belgium. Carbon and nitrogen isotopic ratios were expressed as  $\delta^{13}\text{C}$  (‰) and  $\delta^{15}\text{N}$  (‰) in relation to PDB limestone and atmospheric nitrogen, respectively (Finlay et al., 2002). Due to financial constraints, samples from the rainy season were not analyzed.

$$\delta^{13}\text{C}(\text{‰}) \text{ or } \delta^{15}\text{N}(\text{‰}) = \left[ \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] * 1000$$

where  $R = {}^{13}\text{C}/{}^{12}\text{C}$  or  ${}^{15}\text{N}/{}^{14}\text{N}$ . We used IAEA-CH<sub>6</sub> ( $\delta^{13}\text{C} = -10.4 \pm 0.1\text{‰}$ ) and IAEA-N<sub>1</sub> ( $\delta^{15}\text{N} = 0.43 \pm 0.7\text{‰}$ ) as working standards.

#### 2.5. Data analysis

Ambelu et al. (2010) and Mereta et al. (2013) suggested the use of highly sensitive macroinvertebrate metrics to indicate ecological quality of rivers and riverine wetlands in Ethiopia. Following the recommendation, Shannon ( $H'$ ) and Simpson (1-D) diversity indices were computed using Biodiversity Professional 2 (The Natural History Museum, London, UK) (McAleece et al., 1997). Shannon diversity index was corrected for macroinvertebrate abundance to provide a bio-community index (B) (Pan et al., 2013). The biological monitoring working party (BMWP) score, which indicates the level of organic pollution, was also calculated and the water quality classes were determined (Mustow, 2002). Community attributes and environmental variables of the two sampling seasons and three site groups were compared using STATISTICA 8.0 (StatSoft company, Hamburg, Germany) (StatSoft, 2008). Differences at  $p < 0.05$  level were considered statistically significant.

Relationships between environmental variables and macroinvertebrate abundance were assessed using canonical multivariate analysis with the package CANOCO 4.5 (Wageningen University and Research Centre, Gelderland, Netherlands). The length of gradient determined by detrended correspondence analysis (DCA) is

an estimate of species heterogeneity. Most commonly, when the length of gradient from DCA is less than 3 standard deviations (SD), linear models (PCA, RDA) are most recommended. When it is greater than 4 SD, unimodal methods (DCA, CA, CCA) are appropriate. When the length of gradient lies between 3 and 4 SD, either of the ordination methods that explained better can be used (Leps and Smilauer, 2007). Besides, the constrained or unconstrained nature of the ordination determines the ordination technique (Ter Braak and Smilauer, 2002).

To explore the response of macroinvertebrates to the environmental variables, we performed a DCA using the most abundant taxa (>5%). Since taxa abundances exhibited linear responses (with gradient lengths of 2.285 and 2.099 standard deviations for the rainy and dry seasons, respectively) to environmental gradients, we performed a Redundancy analysis (RDA) with automatic forward selection using 999 permutations. Prior to ordination analysis, the log ( $X + 1$ ) and Hellinger transformations (Legendre and Gallagher, 2001) were applied for the environmental variables and macroinvertebrate data, respectively. Principal component analysis (PCA) was also employed using the Hellinger transformed macroinvertebrate abundance data to detect seasonal patterns of sampling sites. The stable carbon ( $\delta^{13}\text{C} \pm \text{SD}$ ) and nitrogen ( $\delta^{15}\text{N} \pm \text{SD}$ ) isotopic ratios of different FFGs were computed using Microsoft Excel 2010 (Microsoft Company, Redmond, Washington, USA) and the results are presented in a biplot.

### 3. Results

#### 3.1. Physicochemical parameters

During the dry season, the concentrations of total nitrogen (TN), nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ), total phosphorus (TP) and total dissolved solid (TDS) were significantly higher (Kruskal-Wallis test,  $p < 0.05$ ) at the urban-impacted sites compared to upstream and wetland sites. Conversely, dissolved oxygen was more depleted to an average level of  $4.05 \text{ mg L}^{-1}$  at the urban-impacted sites (Table 1). However, nutrient concentrations were not significantly different across the spatial gradient during the rainy period (Kruskal-Wallis test,  $p > 0.05$ ). TP, TN and current velocity were significantly higher during the rainy season compared to the dry season (Wilcoxon signed rank test,  $p < 0.01$ ) (Table 1). Higher sediment deposition was also noted during the rainy season.

#### 3.2. Macroinvertebrate functional feeding types and diversity indices

The families Baetidae (Ephemeroptera) and Chironomidae (Diptera) were common at each site during both seasons, while Caenidae (Ephemeroptera), Hydropsychidae (Trichoptera) and Heptageniidae (Ephemeroptera) were frequently encountered at the upstream sites. During the rainy season, collector-gatherers (CG) were the dominant FFGs, while the percentage abundances of predators (Pr) and grazers (Gr) increased during the dry season (Fig. 2). Consistent decreases in the abundance of collector-filterers (CF) were observed at the polluted sites during the dry season. Shredder macroinvertebrates were rare at all locations (not represented in the figure).

The dry season data indicated that macroinvertebrate abundance and diversity indices ( $H'$ , 1-D and B) were significantly lower (Kruskal-Wallis test,  $p < 0.05$ ) at the urban-impacted sites compared to the upstream river stretches (Table 2). The diversity was also found to have recovered at the wetland-affected sampling sites. According to BMWP score, the urban-impacted rivers belonged to rivers of poor water quality status, while upstream and wetland-affected sites correspond to rivers of good/fair water

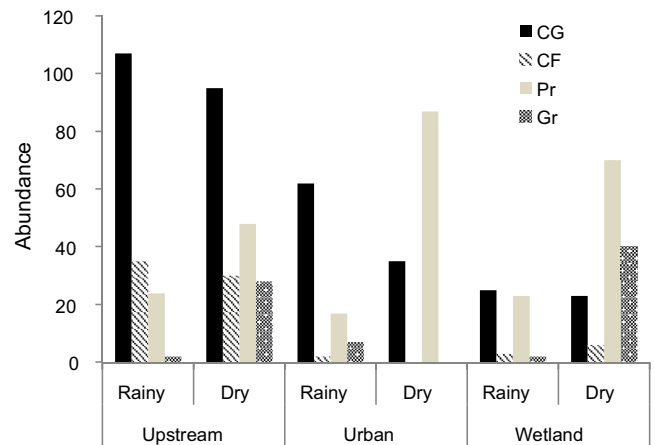


Fig. 2. Macroinvertebrates functional feeding group (FFG) composition; CG = Collector-gatherers, CF = Collector-filterers, Pr = Predators and Gr = Grazers.

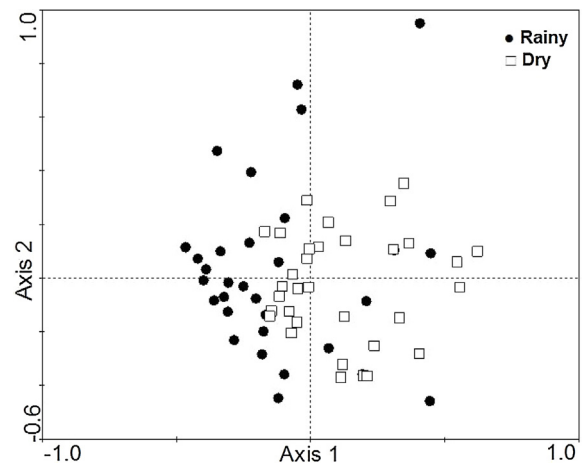


Fig. 3. Principal component analysis (PCA) scatter plot of sampling sites during the two seasons.

quality level (Table 2). The abundance and diversity ( $H'$ , 1-D and B) were also relatively lower at each site during the rainy season compared to those of the dry season (Table 2).

#### 3.3. Ordination analysis

Principal component analysis (PCA) of macroinvertebrate data showed a clear clustering of sampling sites based on the two seasons (Fig. 3). Current velocity and TN significantly ( $p = 0.029$  and  $0.01$ , respectively) affected the distribution of macroinvertebrates during the rainy season (Fig. 4a). However, the analysis did not show a clear grouping of sampling sites into upstream, urban-impacted and wetland-affected sites. The results rather implied indifferent homogenization of both physicochemical variables and macroinvertebrates (Kruskal-Wallis test,  $p > 0.05$ ; Fig. 4a). When considering RDA of data of the dry season (Fig. 4b), the most important environmental variables influencing macroinvertebrate distribution were TP, EC ( $p = 0.001$ ) and TN ( $p = 0.003$ ). The second axis of this RDA separated urban-impacted sites (U), which were strongly correlated with TP, TN, EC and TSS from all other sites. Oligochaeta, Culicidae (Diptera), Helodidae (Coleoptera) and Notonectidae (Hemiptera) were dominant at the urban-impacted sites. Upstream sites (Up) were characterized by higher concentration of DO and current velocity, while wetland-affected sites (W) were negatively correlated with TP and TSS (Fig. 4b).



**Table 1**  
Physicochemical parameters (mean  $\pm$  SE) at the three river groups during the rainy and dry seasons.

Parameters	Upstream sites		Urban-impacted sites		Wetland and dam-affected sites	
	Rainy (n = 14)	Dry (n = 15)	Rainy (n = 11)	Dry (n = 12)	Rainy (n = 7)	Dry (n = 6)
TP (mg L <sup>-1</sup> )**	0.14 (0.05)	0.11 (0.01)	0.12 (0.02)	0.30 (0.08) <sup>a</sup>	0.10 (0.01)	0.06 (0.01)
TN (mg L <sup>-1</sup> )**	3.40 (0.67)	1.53 (0.20)	3.51 (0.86)	4.92 (1.15) <sup>a</sup>	2.50 (0.23)	0.71 (0.12)
NO <sub>3</sub> -N (mg L <sup>-1</sup> )	0.84 (0.16)	0.32 (0.08)	0.90 (0.19)	0.80 (0.16) <sup>a</sup>	0.72 (0.24)	0.07 (0.02)
TDS (mg L <sup>-1</sup> )	153.3 (14.5)	121.9 (20.6)	184.8 (12.4)	216.5 (19.5) <sup>a</sup>	117.7 (12.6)	114.6 (24.1)
DO (mg L <sup>-1</sup> )	6.95 (0.18)	6.96 (0.14)	4.80 (0.60)	4.05 (0.65)	4.66 (0.94)	6.31 (1.68)
EC ( $\mu$ S cm <sup>-1</sup> )	78.4 (5.6)	100.2 (9.8)	120.2 (14.5)	208.1 (12.4)	127.4 (19.8)	240.3 (22.5)
Velo (m s <sup>-1</sup> )***	1.80 (0.12)	0.26 (0.04)	0.45 (0.14)	0.09 (0.01)	0.52 (0.11)	0.07 (0.01)

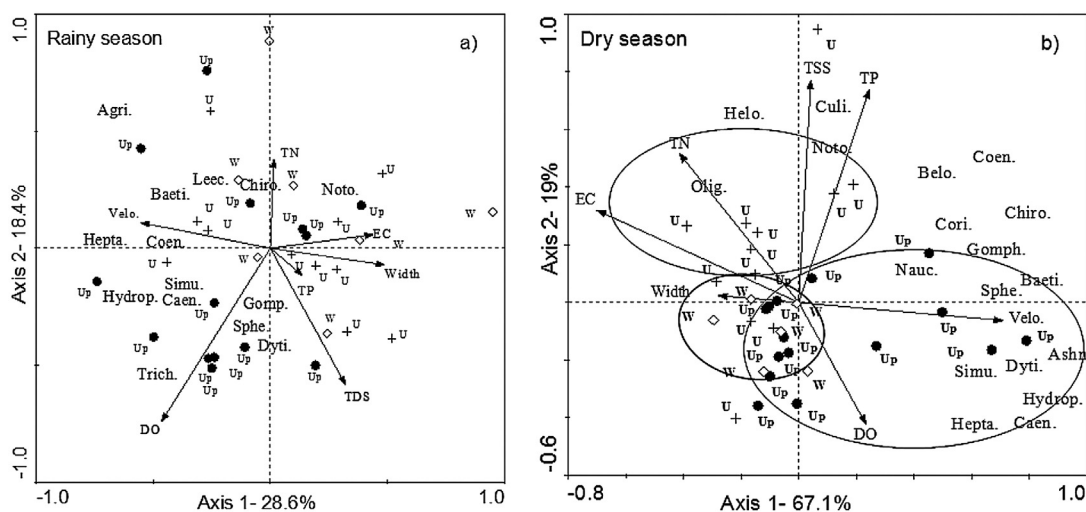
<sup>a</sup> Indicates significantly higher values at the urban-impacted sites (dry season only); (Kruskal-Wallis test). 'n' represents the number of sample sites.

\*\*\* Indicates significantly higher values during the rainy season compared to the dry season; \*\* p < 0.01, \*\*\* p < 0.001 (Wilcoxon signed rank test).

**Table 2**  
Macroinvertebrate community attributes (mean  $\pm$  SE) at the three river groups during the rainy and dry seasons.

Indices	Upstream sites		Urban-impacted sites		Wetland and dam-affected sites	
	Rainy	Dry	Rainy	Dry	Rainy	Dry
BMWP*	57.4 (12.1)	96.2 (7.1)	36.0 (24.2)	41.0 (15.1)	54.0 (6.5)	64.0 (7.2)
H**	1.60 (0.15)	1.80 (0.22)	1.20 (0.17)	1.30 (0.20)	1.60 (0.24)	1.70 (0.14)
B**	6.90 (0.91)	8.02 (0.73)	4.80 (1.16)	5.20 (1.20)	5.02 (0.72)	7.90 (0.54)
1-D*	0.70 (0.05)	0.80 (0.02)	0.60 (0.04)	0.70 (0.04)	0.77 (0.14)	0.82 (0.13)
Taxa richness	12.9 (1.3)	16.4 (1.1)	9.4 (1.1)	11.0 (1.5)	9.7 (0.9)	14.0 (1.5)
Abundance*	168 (25)	198 (20)	89 (18)	122 (19)	52 (18)	140 (22)
Collector-gatherers (%)	63 (2.7)	47 (3.2)	72 (4.7)	27 (4.5)	47 (2.6)	17 (3.1)
Collector-filterers (%)**	21 (3.2)	15 (1.7)	2 (0.5)	0 (0)	6 (0.5)	4 (0.3)
Grazers (%)*	2 (0.6)	14 (2.8)	8 (4.1)	1 (0.5)	4 (1.9)	29 (4.5)

\* Indicates significantly lower values at the urban-impacted sites during the dry season; \*p < 0.05, \*\*p < 0.01 (Kruskal-Wallis test).



**Fig. 4.** Redundancy analysis (RDA) triplot of samples, macroinvertebrate taxa and environmental variables for the rainy (a) and dry (b) seasons. Up, U and W represents upstream, urban-impacted and wetland-affected sites, respectively (see full names of species codes in Appendix A).

### 3.4. Functional feeding groups (FFGs) interactions

Higher values of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotopic ratios (‰) were recorded for predator macroinvertebrates than other functional groups at the wetland-affected (W) and upstream (Up) sites (Fig. 5). This shows that predators were primarily feeding on other functional groups, especially collector-gatherers. Macroinvertebrates at the wetland-affected sites showed clear trophic guilds of functional feeding groups (connecting lines in Fig. 5), while narrower range of nitrogen signature was noted for the upstream sites. The lowest nitrogen signature of macroinvertebrates was recorded for the urban-impacted sites (Fig. 5).

## 4. Discussion

Many rivers in Ethiopia, flowing through or located in proximity of urban centers, are locally used for domestic, commercial and industrial purposes and are thus vulnerable to pollution (Alemayehu, 2004; Beyene et al., 2009a). Agricultural pollution is also rampant in Ethiopia (Ayenew, 2007; Beyene et al., 2012; Devi et al., 2008). The higher concentrations of nutrients and lower level of DO observed at the urban-impacted sites of the present study can be attributed to urban discharge of waste from Jimma town and the subsequent decomposition of organic matter. Other studies conducted in Ethiopian rivers also reported high organic pollution of streams crossing urban centers that significantly amplifies nutri-

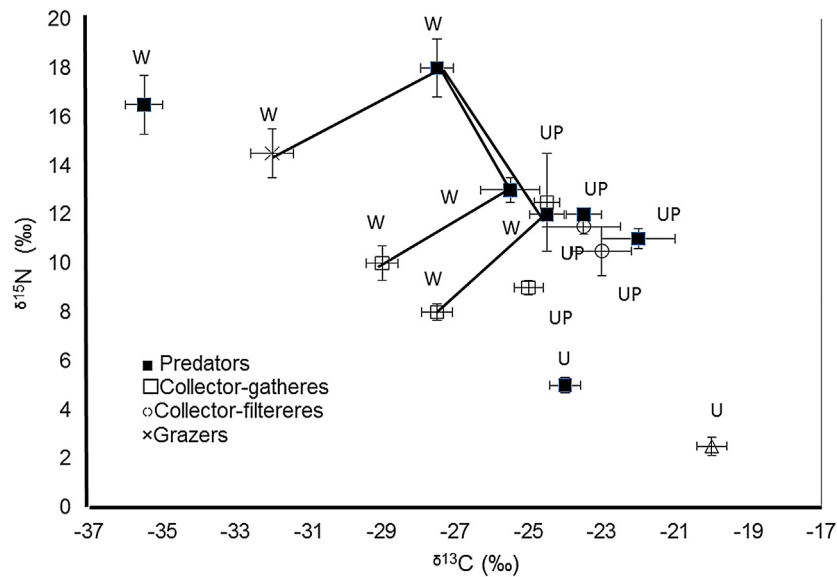


Fig. 5. Macroinvertebrates stable carbon and nitrogen isotopic compositions (mean  $\pm$  SD).

ent concentrations and results in deplete dissolved oxygen levels (Awoke et al., 2016; Beyene et al., 2009a).

Wetlands have the capacity to retain nutrients and reduce soil erosion (Meyer, 2007; Pan et al., 2013). Besides, wetland plants can absorb nutrients such as nitrate (Meyer, 2007). Our finding also indicated substantial improvement of the water quality of urban-impacted rivers after flowing through the natural Boye wetland.

#### 4.1. Macroinvertebrate composition and diversity indices

The composition of benthic macroinvertebrate communities usually provides useful insights into the ecological quality of rivers, as these organisms are sensitive to organic pollution (Ambelu et al., 2010; Mereta et al., 2013). In the present study, higher abundance and diversity of total macroinvertebrates were recorded at the upstream sites. Some of these upstream sites were found in a forested area characterized by reduced organic load. However, the organic load from the discharge of domestic wastes from urban settlements had severe effect on the pollution-sensitive aquatic life forms resident in the rivers. Oligochaetes, which are highly tolerant of depletion of DO and enrichment of nutrients (Lagauzere et al., 2009), were common at the urban-impacted sites. The macroinvertebrate diversity indices also showed a deterioration of the ecological quality of Gilgel Gibe river catchment downstream from Jimma town during both seasons. RDA of the dry season data also separated urban sites, which were characterized by lower levels of DO and higher concentration of TN, from other sites. This finding is in agreement with the results reported for the Borkena River (Beyene et al., 2009a) and other river basins of Ethiopia (Awoke et al., 2016) and Niger Delta, Nigeria (Arimoro and Ikomi, 2009).

Besides improving the chemical quality of water (Meyer, 2007), wetlands support plants which in turn provide attachment sites and materials to build protective retreats to macroinvertebrates (Shah et al., 2011). This may explain the improved macroinvertebrate diversity observed in the wetland-affected sites in the Gilgel Gibe catchment. Studies also stated that wetlands are important sites that support rich biodiversity and high productivity (Maltchik et al., 2010; Mitsch and Gosselink, 2000).

Hydrological variability also plays a significant role in maintaining the ecological integrity and community structure of aquatic ecosystems (Beyene et al., 2009a). It was reported that the sheet

erosion that results mainly from poor agricultural practices and associated runoff of Gilgel gibe catchment area was estimated at 2210 ton km<sup>-2</sup> (Devi et al., 2008). In the present study, current velocity was significantly higher during the rainy season, and can possibly homogenize different habitats. Consequently, the differences in the macroinvertebrate community structure were not clearly observed along the pollution gradient during the rainy season. Rather few families, mainly Baetidae (Ephemeroptera) and Chironomidae (Diptera), constituted most of the macroinvertebrate communities.

Macroinvertebrate abundance and diversity indices were lower during the rainy season possibly due to the runoff that destabilized substrates and swept away macroinvertebrates. Furthermore, the heavy runoff and the consequent deposition of sediment particles interfere with the growth of primary producers. This in turn leads to shortage of food for the primary consumers and top predators (Allan and Castillo, 2007). On the contrary, substrate stability during low flow period may have enabled gradual recolonization of the habitats by the various families of macroinvertebrates (Arimoro and Ikomi, 2009). Similar observation was reported by Arimoro and Ikomi (2009) for the Warri River of the Niger Delta, Nigeria.

#### 4.2. Macroinvertebrates functional feeding groups (FFGs) metric and feeding interactions

Feeding strategies of macroinvertebrates could reflect the adaptation of species to stressors and form part of a unified measure across communities differing in taxonomic composition (Tomanova et al., 2006). The distributions of FFGs provide information regarding organic matter processing (Xu et al., 2014), habitat condition and quality of rivers and river-associated wetlands (Ambelu et al., 2010; Mereta et al., 2013). The present study showed that collector-gatherers were dominant across all sites during the rainy season, while predators and grazers accounted for higher proportions of macroinvertebrates during the dry season. Grazer and predator macroinvertebrates usually move actively and visit unstable substrates while they are in search of food (Tomanova et al., 2006). Consequently, grazers and predators are at higher risk of drift during the heavy rainy period, while collector-gatherers tolerate (avoid) the effects of fast currents. Our findings are in

agreement with the observations made by [Nadushan and Ramezani \(2011\)](#) for Kordan stream, Iran.

Though some studies indicated variable response of functional feeding metrics to perturbation ([Barbour et al., 1999](#); [Tomanova et al., 2006](#)), the results of the present study showed a decline in the proportion of collector-filterers with increasing load of organic pollution. This suggests the potential use of the group (% CF) as a monitoring metric. [Mereta et al. \(2013\)](#) also successfully utilized the same FFG to monitor the status of natural wetlands.

Functional feeding classification of macroinvertebrates also enhances our knowledge of trophic dynamics in streams by simplifying the benthic community into trophic guilds ([Mandaville, 2002](#)), suggested by the  $\delta^{15}\text{N}$  signatures. The isotopic composition of higher level consumers (e.g. predators) reflects that of their diet. Consequently, predators show higher values of  $\delta^{15}\text{N}$  signatures than those of their potential prey ([Vander Zanden et al., 2005](#); [Zah et al., 2001](#)). In streams with continuous flow, the supply and persistence of a particular food item is highly variable ([Tomanova et al., 2006](#)). As a result, macroinvertebrates adapt to an increased degree of omnivory associated with current velocity and continuous inputs of allochthonous materials ([Faria and da Silveira Costa, 2010](#)). The present study also showed narrower range of  $\delta^{15}\text{N}$  values of macroinvertebrates, which suggested an omnivorous feeding habit at the sites characterized by higher current velocity. This diet flexibility might contribute to an increase in their survival ability, and may have facilitated the spatial colonization of macroinvertebrates in fast-flowing rivers.

On the contrary, habitat heterogeneity and stability associated with larger ecosystem size and lower water current velocity, respectively, contribute to a higher dietary specialization, compartmentalized food webs and lower omnivory ([Post et al., 2000](#)). Our study also revealed more defined trophic guilds of FFGs at the wetland-affected sites, which were characterized by larger river width and riparian vegetation. The result indicates predator macroinvertebrates (e.g. Belostomatidae and Notonectidae) were feeding on collector-gatherers (e.g. Baetidae and Chironomidae). The feeding interactions were not, however, clearly observed at the urban-impacted sites characterized by eutrophication and lower macroinvertebrate diversity. This is due to the low interspecific competition among few varieties of species at the urban-impacted sites. The opportunistic species monopolize the available resources, grow and multiply in the absence of their natural predators ([Miserendino and Pizzolon, 2000](#)). The results seem to provide valuable insight into the adverse effects of organic pollution on aquatic communities' interactions.

Anthropogenic activities have been shown to affect the stable isotopic compositions of aquatic organisms ([Dube et al., 2005](#); [Loomer et al., 2015](#)). In the present study, the results of the stable isotope analysis demonstrated the effect of level of water pollution on the isotopic signatures of the inhabiting macroinvertebrates. Lower nitrogen signature of macroinvertebrates was recorded at the urban-impacted sites, which was consistent with the higher concentration of nitrate in the water. Primary producers, which

are directly dependent on nitrate supply from the environment, and the animals they support at the top of the food web are typically  $\delta^{15}\text{N}$  depleted in the nitrate-replete environments than in nitrate-deplete environments ([Somes et al., 2010](#)). These data seem to suggest that isotopic signals can be used as functional indicators of organic pollution and its impact on river communities. Our findings are in agreement with the study made by [di Lascio et al. \(2013\)](#), which recorded a lower nitrogen signature ( $\delta^{15}\text{N}$ ) of macroinvertebrates at a highly-impacted part of Tiber River, Italy.

## 5. Conclusion

Physicochemical parameters, macroinvertebrate diversity and feeding interactions among functional feeding groups (FFGs) were investigated in the Gilgel Gibe watershed, Ethiopia. Human-induced physicochemical stress in urban-impacted sites strongly affected the macroinvertebrate community structure and FFG interactions. For instance, the concentrations of TN and TP were about three-folds higher at the urban-impacted sites compared to the upstream sites during the dry season, while the natural Boye wetland significantly reduced the level of nutrients observed at the urban polluted streams. Hence, this wetland, with further optimization, can be considered as a nature-based solution to the problems of restoring impacted rivers. The use of Shannon, Simpson and biocommunity indices, and abundance of collector-filterers were effective in discriminating sites with different levels of organic pollution. The differences in human-induced perturbation and associated organic pollution among the sites also affected the interactions among FFGs. Based on the results of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of macroinvertebrates, wetland-affected sites seem to have exhibited clear trophic guilds, while no/weak feeding interactions were observed at the urban-impacted sites. Therefore, stable isotope analysis coupled with community structure of macroinvertebrates could improve our understanding of the river ecosystem health in a more integrated manner and help determine the adverse effects of organic pollution on aquatic communities. However, further research is required on the feeding interactions among different consumers and their basal food sources.

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## Appendix A. Mean densities of the most abundant macroinvertebrate taxa in each category of sites during the rainy and dry seasons in Gilgel Gibe catchment.

Order/higher level	Family	Upstream		Urban-impacted		Wetland-affected	
		Rainy	Dry	Rainy	Dry	Rainy	Dry
Ephemeroptera	Baetidae (Baeti.)	67	44	16	0	12	13
	Heptageniidae (Hepta.)	2	28	0	0	0	0
	Caenidae (Caen.)	14	23	4	0	2	5
	Trichorhytidae (Trich.)	4	0	0	0	0	0
Trichoptera	Hydropsychidae (Hydrop.)	24	27	0	0	0	0
Odonata	Gomphidae (Gomph.)	1	2	0	0	1	0
	Ashnidae (Ashn.)	0	6	0	2	1	2
	Coenagrionidae (Coen.)	13	18	0	6	8	13
Hemiptera	Agrionidae (Agri.)	7	3	1	0	0	4
	Notonectidae (Noto.)	0	3	2	9	2	5
	Naucoridae (Nauc.)	0	6	0	4	3	8
Coleoptera	Corixidae (Cori.)	0	4	2	10	0	5
	Belostomatidae (Belo.)	0	4	0	20	0	4
	Dytiscidae (Dyti.)	2	8	2	6	0	5
Diptera	Helodidae (Helo.)	0	0	5	17	6	10
	Chironomidae (Chiro.)	22	26	42	35	11	4
	Simuliidae (Simu.)	10	1	0	0	2	0
Oligocheata	Culicidae (Culi.)	0	0	2	5	1	2
	Oligochaeta (Olig.)	0	0	3	17	0	0
Leech	Leech (Leec.)	0	0	8	0	0	0
Mollusca	Spheriidae (Sphe.)	1	2	3	0	0	0

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