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## Article

# Relationships between Physico-Chemical Parameters and Taxonomic Structure of Benthic Macroinvertebrate Assemblages in Streams of West Cameroon

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**Abstract:** Tropical rivers are strongly influenced by stormwaters, pollutants and agro-pastoral activities. These systems are no longer able to maintain their native biota. Therefore, it seems important to understand how biological assemblages are driven by environmental gradients at different spatial scales. However, information on the relationships between the distribution of aquatic macroinvertebrates and environmental variables remains scarce in Cameroon. To improve our knowledge on the drivers of such distributions, a study was carried out at 11 contrasted sites from three catchments located in West Cameroon. This study aimed at understanding the spatio-temporal variations of 19 taxonomic metrics calculated for the benthic macroinvertebrate assemblages of these sites sampled during 13 months, concurrently with physico-chemical analyses of water quality. Two hundred and twelve taxa were caught. Diptera(rf-S), Shannon-Wiener diversity, 1–GOLD, total richness, Pielou evenness and Heteroptera(rf-S) revealed their high ability to respond to anthropogenic pressures or disturbances. Conductivity, water temperature, dissolved oxygen, nitrates, total hardness and alkalinity were identified as the main physico-chemical drivers of the taxonomic structure of benthic assemblages. These results will allow further exploration of the implementation of a tool for monitoring the ecological quality of West Cameroon rivers.

**Keywords:** urbanization; macroinvertebrate community; spatial distribution; metrics; environmental variables; bioassessment



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## 1. Introduction

Nowadays, running waters are impaired by multiple stressors acting simultaneously [1–4] or sequentially [5]. Climate change, increasing urbanization and intensive agro-pastoral activities, which have adverse effects on stream biota [6,7], are the most important stressors of small tropical streams. The physico-chemical and hydromorphological stream impairments that specifically occur in urbanized watersheds have been labeled the “urban stream syndrome” [8,9]. These human-induced environmental changes cause local habitat degradation and fragmentation as well as longitudinal connectivity loss, which can profoundly alter the structure and composition of aquatic assemblages and lead to the decline of biodiversity in many rivers of developing countries [10,11]. Water pollution assessment and control are also major and widespread challenges in developing countries because water can indirectly favor the transmission of many diseases [12,13]. Thus, various methods have been developed to analyze water quality impairment. Water temperature, dissolved oxygen, pH, conductivity, suspended matter, nutrients (including

ammonium, nitrites, nitrates and orthophosphates (e.g., [14,15]) and sometimes micropollutants (e.g., [16,17]) have been routinely measured in several tropical streams. Unfortunately, such analyses are expensive and routine surveys of pollutant concentrations (which may greatly vary both temporally and spatially) in tropical areas are scarce and cannot allow efficient detection of diffuse and/or episodic contaminations when based only on one-off measures [18,19].

Adding biological parameters related to the structure and the composition of aquatic assemblages to the physico-chemical assessment of rivers has proven its efficiency in fully assessing the impact of anthropogenic stressors [20,21]. Benthic macroinvertebrates are the most frequently used biological indicators of river conditions because they are diverse, long-lived, rather sedentary, and they react rapidly and often predictably to anthropogenic pressures [22,23]. Benthic macroinvertebrates also play an important role in freshwater ecosystem functioning. They contribute to carbon and nitrogen cycles by (i) feeding on algae, coarse detritus or fine particulate organic matter [24,25] and (ii) providing food to higher trophic levels. They are also taxonomically diverse and relatively easy to identify (at least at the genus or family level). Invertebrate assemblages integrate environmental changes over several months [26], and examining the structure and composition of such assemblages has the potential to relevantly inform on the ecological status of water bodies [19,27–31] and to demonstrate the effects of past and present environmental degradation [31,32].

Several studies in sub-Saharan Africa have demonstrated that stream biomonitoring based on benthic macroinvertebrates is clearly in progress (e.g., [33–38]). In southeastern Ivory Coast, Edia et al. (2013, [39]) randomly sampled the aquatic invertebrates of eight sites on eight occasions (including the rainy and dry seasons) and related the presence of several genera of Diptera and Ephemeroptera to the most and weakly mineralized sites, respectively. Kaboré et al. (2016, [40]) related the taxonomic and functional structure of macroinvertebrate assemblages to watershed habitat use in 29 semi-arid stream reaches of Burkina Faso. They found (i) a gradual decline of collector-filterers and habitat-sensitive Ephemeroptera and Trichoptera (mainly the Baetidae and Hydropsychidae families) and (ii) a gradual increase in the relative abundance of tolerant Diptera (Chironomidae, Culicidae, Psychodidae and Syrphidae) from the least impaired river reaches to reaches subjected to intensive agriculture or urban pressure. However, taxonomic richness, the Shannon-Wiener index, or the proportion of the dominant tolerant Diptera did not distinguish invertebrate assemblages of “protected” or “extensive agriculture” areas from those of “intensive agriculture” because high organic matter and fertilizer inputs in the second group of reaches had increased both macrophytic and periphytic production, enhancing habitat heterogeneity and the development of molluscs and coleopterans [40]. Those species have compensated for the loss of water quality sensitive taxa [40]. In Cameroon, Menbohan et al. (2013, [41]) and Tchakonté et al. (2015, [42]) related the high abundance of EPT taxa to very low anthropogenic pressures in the peri-urban streams of Yaoundé and in the forest streams of Douala, respectively. Tchakonté et al. (2015, [42]) considered (i) the absence of EPT species and some coleopteran and hemipteran families and (ii) the decrease of both shredders (indicators of riparian vegetation health) and predators as good predictors of severe anthropogenic pressures.

In Cameroon, aquatic environment management and protection have taken place slowly due to a lack of cooperation and synergy between the various stakeholders from governmental and non-governmental institutions. Anthropogenic pressures on rivers remain high. There is still direct dumping of solid wastes and liquid wastewaters from households, agriculture and industries into streams. In the Bafoussam region, agro-pastoral activities have accelerated this degradation of natural environments, favoring the leaching and drainage of fertilizers in streams during intense rain events [43,44]. In addition, the lack of wastewater treatment plants often converts streams into dumping grounds of various kinds of wastes, described by Parent-Raoult and Boisson (2007, [45]) as “urban discharges in rainy weather” and representing a certain health risk for local popula-

tions (e.g., proliferation of mosquitoes, epidemics and parasitic diseases) and ecosystems (e.g., biodiversity loss).

In this context, understanding how watershed anthropogenic level can influence water quality and macroinvertebrate diversity in a tropical stream and identifying the major environmental drivers of invertebrate community structure could therefore be a major challenge for freshwater managers in Cameroon and other tropical regions.

The aim of our study was to (i) diagnose the water quality in the different river sub-catchments of the Bafoussam region, (ii) highlight the main relationships between water quality descriptors and macroinvertebrate community-based metrics, (iii) identify which metrics could be the best candidates for a regional bioassessment tool of streams in this area, and (iv) rank such metrics according to their ability to discriminate “least impacted” conditions from conditions “significantly impacted” by anthropogenic activities, despite the low knowledge level on life history traits and ecological preferences of local macroinvertebrate species.

## 2. Material and Methods

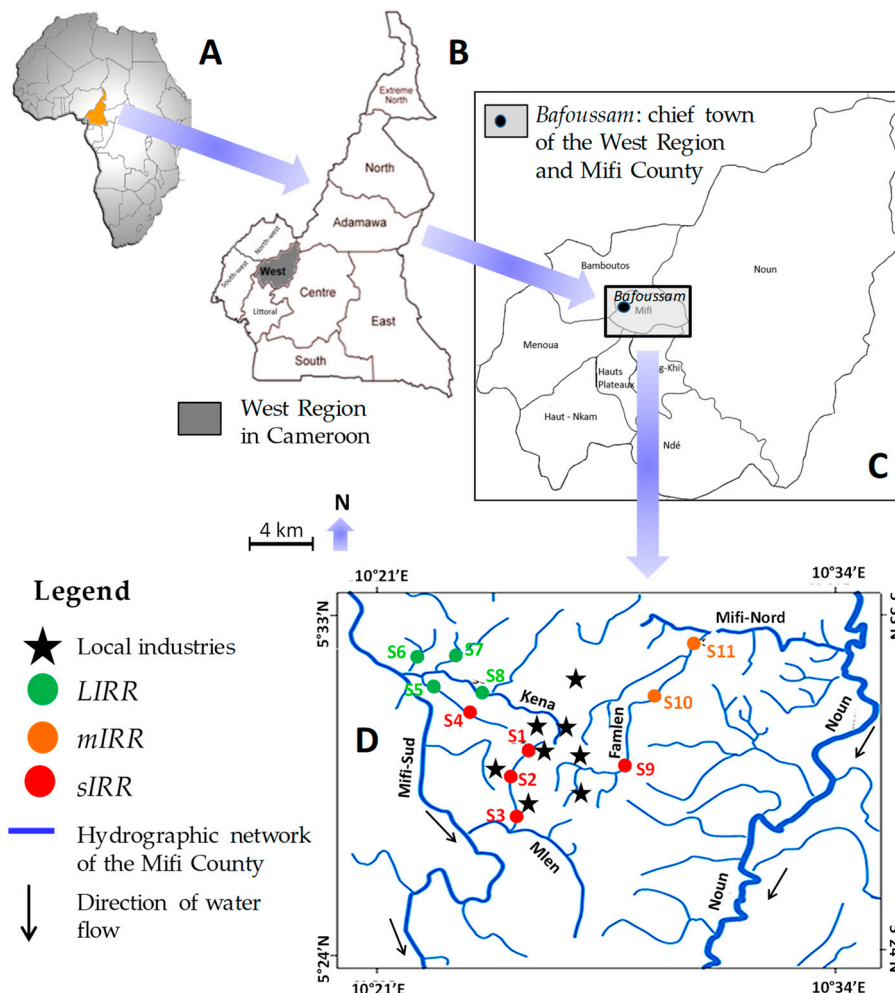
### 2.1. Study Area and Human Pressure-Based Typology of Sites

The western region, whose chief town is Bafoussam (Mifi County), covers about 13,892 km<sup>2</sup>. Roughly, this area is between the latitudes 5°24′–5°33′ N and the longitudes 10°21′–10°34′ E, with an average altitude of 1450 m (Figure 1). This region benefits mainly from (i) agriculture involving the utilization of natural (compost) and chemical (industrial) fertilizers, (ii) livestock farming and (iii) primary trade of local products. Urban and industrial areas are also developing rapidly at the expense of natural vegetation cover. This region is located in a tropical climate zone [46], characterized by a long and abundant wet season from March to October (rainfall ranging from 84.8 to 271.4 mm month<sup>-1</sup>), and a dry season from November to February (6.7–50.1 mm month<sup>-1</sup>). The average annual rainfall and temperature (over the 1995–2005 period) were 1741.8 mm and 21.0 °C, respectively (meteorological station in Bafoussam).

Eleven sites were selected in three sub-catchments of Mifi County to optimally illustrate gradients of various anthropogenic pressures occurring in this region (e.g., domestic, industrial, physical and agro-pastoral contaminations), from Least Impacted River Reaches (LIRRs) often located in rural zones with low population density to severely Impacted River Reaches (sIRRs) often located in more urbanized areas (Table 1; Figure 1). Three sampling sites (S01, S02 and S03), expected as severely impaired by anthropogenic pressures, were chosen in the sub-catchment Nlem. They were situated in an urban zone gathering breweries, a soap factory and an agricultural cooperative. These industries, located upstream from riparian residential areas and city drainage services, were directly discharging their wastewaters in the river. Five sampling sites (S04 to S08; Table 1) were located on tributaries of the Kena River, in a rural zone where farming and traditional breeding are the main activities. Except for site S04 draining an intensively cropped area (coffee and vegetables that require fertilizers) with cattle breeding, domestic discharge and boulder extraction, all these sites were subjected to limited anthropogenic pressures (Table 1). Three sampling sites were located on the Famlem stream, which takes its source in an urban zone (S09) before flowing in a least impaired rural area (S10 and S11). Food crop cultivation and local sand extraction (leading to substrate clogging) are the main activities along this stream (Table 1). All the study sites were situated between an altitude of 1117 m (S11) and 1466 m (S01).

Because no precise information was locally available for quantifying the intensity of the different sources of anthropogenic pressures on sites, we decided to describe semi-quantitatively the potential impact of anthropogenic pressures using three (low/moderate/high) impact levels for eight pressure categories (Table S1 in Supplementary Material). These pressure categories were related to the importance of “urban” areas, “food crops”, “cash crops”, “cattle and poultry” breeding, untreated “domestic, municipal and agricultural” or “industrial” wastewater effluents, riverine “factories” and “civil engineering and mining” at reach scale. The corresponding array (11 sites × 24 modalities from eight

pressure categories) was analyzed by Multiple Correspondence Analysis (MCA, [47]). Then, sampling sites were hierarchically clustered based on Euclidean distances calculated between site locations on the MCA first factorial plane and applying the complete linkage method [48].



**Figure 1.** Locations of the sampling area (A–C) and the sampling sites ((D); S01–S11) in Mifi County of the West Region, Cameroon in Africa (A), the West Region in Cameroon (B), and Mifi County in the West Region (C). LIRR = Least Impacted River Reach, mIRR = moderately Impacted River Reach, sIRR = severely Impacted River Reach. Black stars are the main local industries close to the streams.

## 2.2. Benthic Macroinvertebrate Sampling and Community Structure Description

Invertebrate sampling was performed by kick sampling using a hand net (30 × 30 cm opening, conical thread, mesh size: 400 μm), applying the multi-habitat approach described by Stark et al. (2001, [49]). Site sampling was performed in a 100 m stretch, for an approximate total sampled surface of 3 m<sup>2</sup>.

All the available habitats within each study site were sampled, including boulders, gravel, sand, mud, macrophyte beds, leaf packs, branches and rafts. Substrate samples were sieved (mesh size: 400 μm). Large invertebrates were sorted by the naked eye, whereas the smaller ones were sorted under a dissecting microscope. The sorted organisms were fixed with 10% formalin. They were identified to the genus or species level (when possible), based on Durand and Lévêque (1981, [51]), Day et al. (2002, [52]), De Moor and Day (2002, [53]), De Moor et al. (2003a&b, [54,55]) and Tachet et al. (2010, [56]). Invertebrate assemblages were sampled monthly from December 2015 to December 2016 (i.e., 13 sampling campaigns).

**Table 1.** Description of the 11 sampling sites. The expected ecological status was evaluated based on a multivariate analysis taking into account anthropogenic pressures at the reach scale (see Section 2.1 for further details). LIRR = Least Impacted River Reach; mIRR = moderately Impacted River Reach; sIRR = severely Impacted River Reach. «High» population density corresponds to “>200 inhabitants km<sup>-2</sup>” and «Low» to “≤200 inhabitants km<sup>-2</sup>” (adapted from [50]). Types of waste deposits: “a” = agricultural, “d” = domestic, “i” = industrial and “m” = municipal.

Catchment Water Courses	Sampling Sites	GPS Coordinates (Latitude/Longitude)	Population Density	Anthropogenic Activities in the Watershed and/or Directly in the Stream	Nature of the Stream Bed	Expected Ecological Status
Nlem	S01	05°28'10"85 N 10°24'47"91 E	high	food crops, agricultural cooperative of planters, waste deposits (d, m, i) and cattle farming	waste and mud	sIRR
	S02	05°27'58"16 N 10°24'13"80 E	high	soap factory, brewery of Cameroon, waste deposits (d, m, i)	boulders, sand and waste	sIRR
	S03	05°27'17"08 N 10°24'08"08 E	high	construction of a retaining wall, food crop and society of coffee company	mud and sand	sIRR
Kena	S04	05°30'38"03 N 10°23'10"60 E	low	rock extraction, vegetable farming, waste deposits (d, a)	gravel and sand	sIRR
	S05	05°30'43"85 N 10°22'16"62 E	low	food crops	root mats and sand	LIRR
	S06	05°31'33"01 N 10°21'52"79 E	low	swampy area	mud and sand	LIRR
	S07	05°31'33"69 N 10°23'08"77 E	low	food crops	sand	LIRR
	S08	05°30'54"46 N 10°24'23"54 E	low	food crops	sand	LIRR
Famlem	S09	05°28'43"38 N 10°26'40"62 E	high	waste deposits (d, m) and food crops	waste deposits and mud	sIRR
	S10	05°29'20"31 N 10°28'00"46 N	low	sand mining activities and food crops	sand	mIRR
	S11	05°30'40"15 N 10°28'43"38 E	low	sand mining activities	sand and boulders	mIRR

Because of the lack of precise information on the biological traits and ecological preferences of the Cameroonian macrobenthic fauna, only 19 metrics describing the taxonomic structure and composition of benthic macroinvertebrate assemblages were selected (Table 2). These taxonomy-based metrics included total richness, the relative richness of thirteen taxonomic groups (e.g., Coleoptera, Ephemeroptera, Odonata, Heteroptera), the richness (EPT) or the abundance (log<sub>10</sub>(EPTD + 1), 1-GOLD) of combinations of these groups, and the Shannon-Wiener diversity and Pielou evenness indices.

**Table 2.** Taxonomy-based metrics calculated on benthic macroinvertebrate assemblages of three river catchments of the West Cameroon Region. rf-S = relative frequency of the taxon in the assemblage in terms of taxonomic richness. The variation of individual metrics was predicted along an increasing anthropogenic pressure gradient (right column). D = Diptera, E = Ephemeroptera, P = Plecoptera and T = Trichoptera.

Metric Name	Short Description	Reference	Predicted Variation
Total richness	The number of taxa within an assemblage	Barbour et al., 1999 [57]	decrease
Shannon-Wiener diversity (H)	H accounts for taxonomic richness and relative taxon abundance distribution	Shannon, 1948 [58]	decrease
Pielou evenness (J)	$J = H/H_{max}$ (Feld et al., 2014, [59])	Pielou, 1966 [60]	decrease
Bivalvia(rf-S)	Relative richness of this taxonomic group in the invertebrate assemblage (relative frequency)		increase
Coleoptera(rf-S)	idem		decrease
Dictyoptera(rf-S)	idem		increase
Diptera(rf-S)	idem		increase
Ephemeroptera(rf-S)	idem	[57]	decrease
Gastropoda(rf-S)	idem		increase
Heteroptera(rf-S)	idem		decrease
Hirudinea(rf-S)	idem		increase
Odonata(rf-S)	idem		decrease
Oligochaeta(rf-S)	idem		increase
Plecoptera(rf-S)	idem	[57]	decrease
Trichoptera(rf-S)	idem	[57]	decrease
Turbellaria(rf-S)	idem		decrease
EPT Family richness	Number of Ephemeroptera, Plecoptera and Trichoptera families	[57]	decrease
$\log_{10}(EPTD + 1)$	Log-transformed sum of the abundances of a selection of insect families: Heptageniidae, Ephemeridae, Leptophlebiidae (E), Brachycentridae, Goeridae, Polycentropodidae, Limnephilidae, Odontoceridae (T), Dolichopodidae, Stratiomyidae, Dixidae, Empididae, Athericidae (D) and Nemouridae (P)	Buffagni et al., 2006 [61]	decrease
1-GOLD	1—relative abundance of Gastropoda, Oligochaeta and Diptera	[61]	decrease

### 2.3. Physico-Chemical Parameters

Seventeen physico-chemical variables were measured in the field (i.e., during the 13 invertebrate sampling campaigns) or at the laboratory with standard methods for water (cf. [62,63] for protocols). Dissolved oxygen and conductivity (HACH multiparameter probe, HACH, Dubai, United Arab Emirates), pH (pH-meter CG 818 model SCHOTT Geräte GmbH, SCHOTT France SAS, Colombes, France) and water temperature (hand-held thermometer;  $\pm 0.1$  °C) were measured in situ. If daytime variation in dissolved oxygen concentration (DO) was not controlled during the sampling design, the study sites nearest to Bafoussam (e.g., S01 to S03), which belong to sIRRs, were the last sites sampled in the afternoon (i.e., in the most favorable DO measurement conditions) to avoid a confounding effect of daytime variation if a DO deficit was observed. Water samples were collected in polyethylene bottles (1000 mL) and were stored in refrigerated enclosures. Back to the laboratory, concentrations of nitrates, nitrites, ammonium and orthophosphates

were measured using a HACH DR/2800 spectrophotometer, as well as suspended matter, water color and turbidity. The Biological Oxygen Demand (BOD5) was measured with a Liebherr BOD analyzer, whereas alkalinity, total hardness, oxidizability (per permanganate method), chlorophyll a and dissolved CO<sub>2</sub> were measured by volumetric methods. The concentrations of micropollutants were not measured during the study.

#### 2.4. Environmental and Faunal Data Analyses

Non-parametric Friedman tests with blocks were applied to evaluate the statistical significance of differences among sites for physico-chemical parameters and invertebrate-based metrics. Season was the repeated measure defining block and sites were regarded as different “treatments” within each block. Significant Friedman tests were followed by pairwise comparisons using Nemenyi post-hoc tests for unreplicated blocked data [64].

A normalized Principal Component Analysis (nPCA) was applied to the array [143 samples (i.e., 11 sites × 13 campaigns) × 10 environmental variables with significant between-sites differences based on the Friedman test results] to compare the abiotic characteristics of sampling sites. Five samples were excluded from the final analysis due to extreme values (S02 and S03 in February and May, and S02 in December 2016). Using the same matrix, sampling sites were hierarchically clustered based on Euclidean distances calculated between site locations on the nPCA first factorial plane and applying the complete linkage method [48].

An nPCA was applied also to the array [143 samples × 17 taxonomic metrics with significant between-sites differences based on the Friedman test results]. Preliminary “within-site” and “within-season” nPCA analyses [65] were performed on both datasets. They demonstrated a low effect of “season” on (i) the typology of sites based on taxonomic metrics and (ii) physico-chemical parameter variations (Figures S1 and S2 in Supplementary Material). Sampling sites were also clustered based on the taxonomic structure of their assemblages using the same procedure as above (i.e., applying the complete linkage method on a matrix of Euclidean distances between site locations on the first factorial plane of the taxonomy-based nPCA).

Finally, the relationships between abiotic parameters and taxonomy-based metrics were analyzed with redundancy analysis (RDA; [66]), and sampling sites were clustered based on their locations on the RDA first factorial plane (similarly as above). The Discrimination Efficiency (DE) of each metric was also calculated. The DE of a given metric is the proportion of IRRs that exhibited values (i) lower than the first quartile of the LIRR value distribution (for metrics decreasing in impaired conditions) or (ii) higher than the third quartile of the LIRR value distribution (for metrics increasing in impaired conditions).

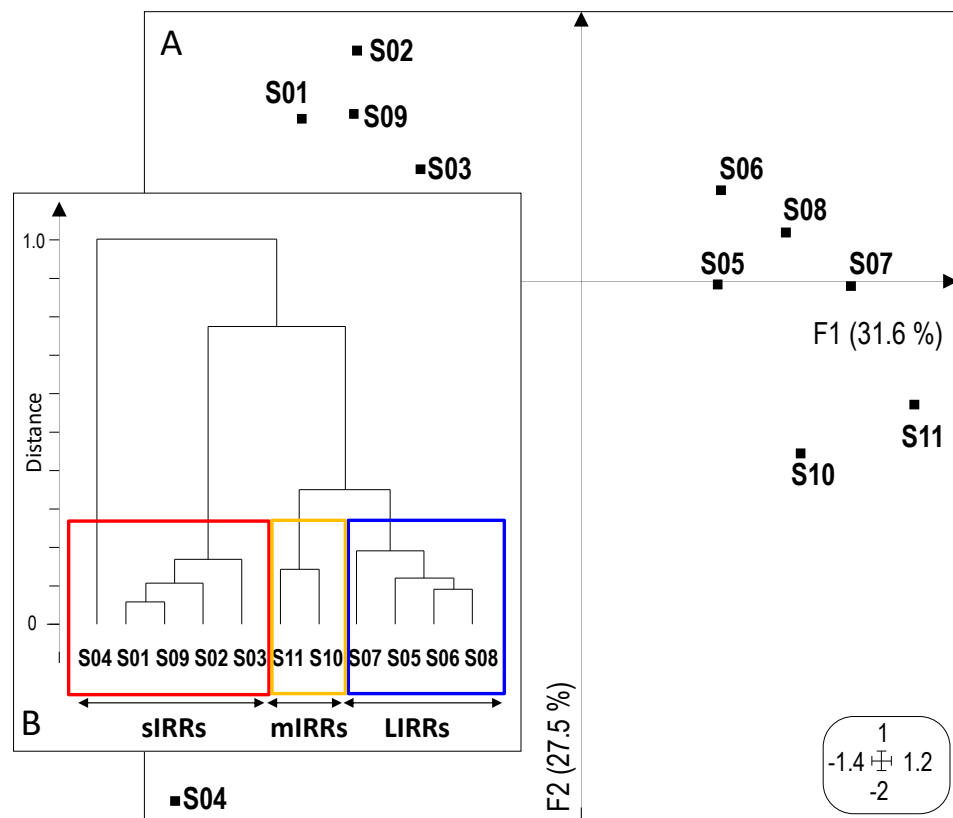
Analyses were conducted with the packages *ade4* [67] and *vegan* [68] using the software R (version 3.4.1 [69]).

### 3. Results

#### 3.1. Human Pressure-Based Typology of Sites

The MCA provided a typology of sampling sites based on the description of anthropogenic pressures potentially impairing water quality and habitat integrity (Figure 2). The first factorial plane (F1-F2; Figure 2A) explained 59.1% of the total inertia. Axis F1 discriminated S01-S03, S09 (F1 < 0; F2 > 0) and S04 (F1 and F2 < 0) by high population density and moderate to high level of pressure for most of the anthropogenic pressure categories (see Figure S3), S05 to S08 (F1 and F2 > 0) by low population density and low level of pressure for most of the pressure categories, and S10 and S11 (F1 > 0; F2 < 0) by only highly impaired by sediment mining and civil engineering. According to the correlations ratios (*cr*; [70]), factories (*cr* = 0.948), industrial and domestic/municipal/agricultural (*cr* = 0.909) or industrial (*cr* = 0.908) wastewater effluents and cattle/poultry breeding (*cr* = 0.763) best discriminated the sites along the F1 axis. Riverine factories (*cr* = 0.870), cash crop (*cr* = 0.842), civil engineering and sediment mining (*cr* = 0.692) and urban areas (*cr* = 0.621) best discriminated the sites along the F2 axis.





**Figure 2.** Ordination of sites by Multiple Correspondence Analysis (MCA) based on eight anthropogenic pressure categories. (A) Projection of 11 sites (S01 to S11) on the first factorial plane (F1-F2). (B) Dendrogram, based on the Euclidean distance (calculated between sites according to their coordinates on the first factorial plane of the MCA) and the complete linkage method, highlighting the similarity of the 11 study sites considering the intensity of anthropogenic pressure categories. LIRRs = Least Impacted River Reaches, mIRRs = moderately Impacted River Reaches, sIRRs = severely Impacted River Reaches. See Table S1, Figure S3 and the text for further details.

The cluster analysis (Figure 2B) highlighted three main groups of sites. The first group gathered sites weakly urbanized and located in rural zones (S05 to S08), which could be considered as “Least Impacted River Reaches” (LIRRs). The second group consisted of two sites (S10 and S11) only moderately impacted by anthropogenic pressures (mIRRs). The third group included five sites (S01 to S04 and S09) more severely impaired by different combinations of anthropogenic pressures (sIRRs). S04, the most impacted site, was included in this third group.

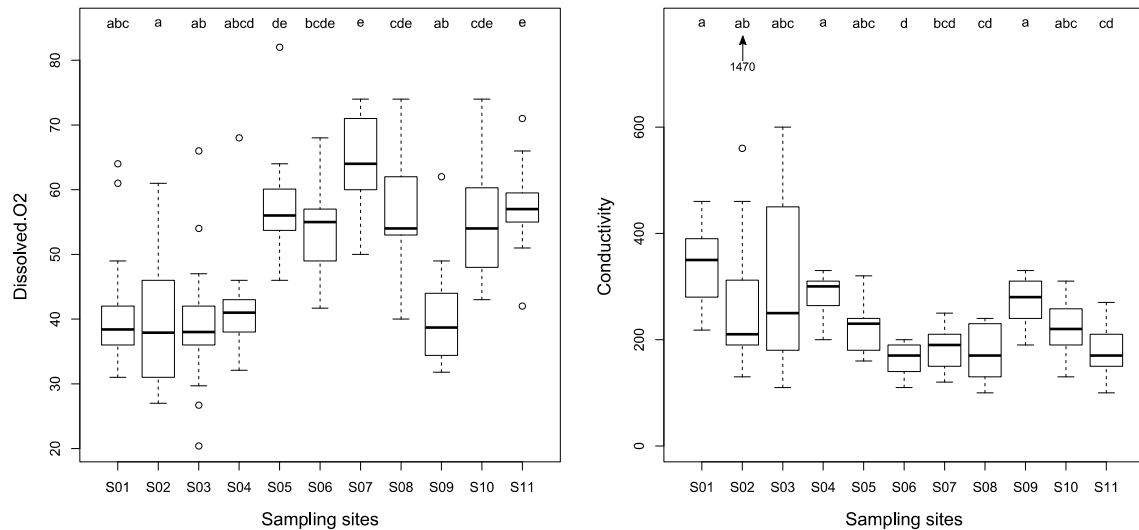
### 3.2. Physico-Chemical Characteristics of Streams

The highest electrical conductivity values were obtained in the tributary of the Nlem stream, the site S02 exhibiting values up to  $1470 \mu\text{S cm}^{-1}$  in December 2016. Similarly, the pH values in this sub-catchment reached 11.3 at site S02 and 10.8 at site S03, reflecting the basic character of the effluents received by this stream. Dissolved oxygen concentration was low in this sub-catchment (<50%; Table 3). In contrast, the Kena tributaries (S05 to S08) exhibited the highest dissolved oxygen concentrations (>50%). Significantly higher dissolved oxygen concentration, and lower temperature, conductivity and total hardness were observed in sites with the LIRR status (Figure 3, Table 3). It was more difficult to relate significant variation of other parameters to the LIRR vs. IRR status based on Nemenyi post-hoc test results (Figure S4 in Supplementary Material). No significant between-sites differences in turbidity, suspended matter, dissolved  $\text{CO}_2$ , orthophosphates, chlorophyll a and BOD5 were identified.

**Table 3.** Mean values ( $\pm$ standard deviation) of physico-chemical parameters for the 11 sampling sites, over the study period. Asterisks indicate significant differences among sites, based on Friedman’s tests (\*\* $p < 0.001$ ; \* $p < 0.01$ ; \* $p < 0.05$ ). Parameters exhibiting significant differences are those included in the nPCA (see Section 3.2) and RDA (see Section 3.5). See text for further details.

Sampling Sites	Nlem Stream				Kena Stream				Famlem Stream		
	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11
Physico-chemical parameters											
T (°C) ***	22.8 $\pm$ 1.9	21.9 $\pm$ 2.3	22.3 $\pm$ 1.8	20.8 $\pm$ 1.9	19.6 $\pm$ 1.5	20.1 $\pm$ 1.5	21.3 $\pm$ 1.2	21.0 $\pm$ 2.2	22.7 $\pm$ 0.8	22.8 $\pm$ 1.3	21.9 $\pm$ 1.2
pH ***	7.0 $\pm$ 0.5	8.0 $\pm$ 1.7	7.9 $\pm$ 1.4	7.3 $\pm$ 0.6	7.0 $\pm$ 0.6	6.9 $\pm$ 0.6	7.4 $\pm$ 0.4	7.3 $\pm$ 0.3	6.5 $\pm$ 0.6	7.0 $\pm$ 0.6	7.6 $\pm$ 0.5
Dissolved O <sub>2</sub> (%) ***	41.9 $\pm$ 10.2	38.8 $\pm$ 9.9	39.4 $\pm$ 11.6	42.2 $\pm$ 8.7	58.0 $\pm$ 8.7	53.6 $\pm$ 6.9	64.0 $\pm$ 7.8	57.0 $\pm$ 8.8	40.7 $\pm$ 8.4	55.6 $\pm$ 9.2	57.1 $\pm$ 6.9
Conductivity ( $\mu$ S/cm) ***	342.3 $\pm$ 75.7	358.3 $\pm$ 356.3	299.7 $\pm$ 168.9	281.9 $\pm$ 41.7	222.8 $\pm$ 45.6	164.8 $\pm$ 31.1	180.8 $\pm$ 40.7	176.9 $\pm$ 50.9	272.4 $\pm$ 43.6	225.0 $\pm$ 55.4	179.2 $\pm$ 54.7
Total hardness (mg/L) ***	76.1 $\pm$ 16.6	57.0 $\pm$ 19.2	56.4 $\pm$ 33.4	58.1 $\pm$ 17.7	51.4 $\pm$ 14.4	34.0 $\pm$ 9.9	42.5 $\pm$ 16.9	34.9 $\pm$ 15.2	47.9 $\pm$ 16.0	46.6 $\pm$ 9.8	40.4 $\pm$ 11.9
Color (mg/L)	100.8 $\pm$ 62.1	210.7 $\pm$ 394.7	167.5 $\pm$ 232.1	74.8 $\pm$ 85.9	102.4 $\pm$ 83.4	60.5 $\pm$ 41.0	84.1 $\pm$ 93.7	108.1 $\pm$ 131.4	92.5 $\pm$ 113.7	138.1 $\pm$ 142.5	190.0 $\pm$ 195.3
Dissolved CO <sub>2</sub> (mg/L)	10.7 $\pm$ 7.8	9.3 $\pm$ 9.6	10.0 $\pm$ 8.3	10.0 $\pm$ 8.0	11.2 $\pm$ 7.6	10.4 $\pm$ 7.6	10.0 $\pm$ 7.3	9.9 $\pm$ 6.4	11.5 $\pm$ 6.2	8.7 $\pm$ 5.6	8.7 $\pm$ 5.7
Alkalinity (mg/L) *	32.2 $\pm$ 38.3	34.8 $\pm$ 43.5	30.5 $\pm$ 37.2	32.8 $\pm$ 42.8	30.2 $\pm$ 37.4	28.9 $\pm$ 37.5	24.3 $\pm$ 34.3	25.4 $\pm$ 33.6	20.2 $\pm$ 23.6	22.3 $\pm$ 27.7	23.1 $\pm$ 30.8
Suspended matter (mg/L)	24.2 $\pm$ 34.7	47.8 $\pm$ 44.2	41.3 $\pm$ 61.5	28.4 $\pm$ 36.6	34.3 $\pm$ 45.8	30.7 $\pm$ 46.7	39.3 $\pm$ 60.6	29.5 $\pm$ 44.2	30.4 $\pm$ 64.7	75.4 $\pm$ 103.0	51.1 $\pm$ 68.4
Turbidity (mg/L)	28.1 $\pm$ 26.0	60.3 $\pm$ 113.3	32.0 $\pm$ 26.9	26.3 $\pm$ 23.9	30.2 $\pm$ 38.3	27.5 $\pm$ 23.4	38.7 $\pm$ 53.5	41.6 $\pm$ 66.9	26.4 $\pm$ 37.2	39.2 $\pm$ 50.6	57.4 $\pm$ 71.0
Nitrate (mg/L NO <sub>3</sub> <sup>-</sup> ) **	2.3 $\pm$ 1.2	5.2 $\pm$ 8.4	3.2 $\pm$ 3.3	2.3 $\pm$ 1.2	1.8 $\pm$ 1.3	1.5 $\pm$ 1.1	2.5 $\pm$ 1.9	1.7 $\pm$ 0.9	3.0 $\pm$ 1.9	3.0 $\pm$ 1.6	3.0 $\pm$ 2.3
Orthophosphate (mg/L PO <sub>4</sub> <sup>3-</sup> )	1.9 $\pm$ 2.2	2.7 $\pm$ 3.6	2.5 $\pm$ 4.3	0.8 $\pm$ 1.0	1.1 $\pm$ 1.3	0.5 $\pm$ 0.8	0.5 $\pm$ 0.5	0.7 $\pm$ 1.2	0.6 $\pm$ 1.0	1.6 $\pm$ 2.0	1.0 $\pm$ 1.4
Nitrite (mg/L NO <sub>2</sub> <sup>-</sup> ) **	1.2 $\pm$ 1.5	4.5 $\pm$ 7.7	5.7 $\pm$ 8.8	1.9 $\pm$ 2.5	1.6 $\pm$ 2.2	2.5 $\pm$ 4.3	1.2 $\pm$ 2.3	1.3 $\pm$ 3.3	1.0 $\pm$ 1.3	3.8 $\pm$ 4.9	3.0 $\pm$ 4.7
Ammonium (mg/L NH <sub>4</sub> <sup>+</sup> ) ***	1.3 $\pm$ 1.4	0.7 $\pm$ 0.7	1.0 $\pm$ 1.2	1.3 $\pm$ 1.0	1.0 $\pm$ 0.8	0.6 $\pm$ 1.0	0.5 $\pm$ 0.4	0.5 $\pm$ 0.6	0.4 $\pm$ 0.4	1.1 $\pm$ 1.4	1.0 $\pm$ 1.3
BOD5 (mg/L O <sub>2</sub> )	39.2 $\pm$ 33.8	40.4 $\pm$ 27.8	32.4 $\pm$ 24.3	44.6 $\pm$ 32.5	51.9 $\pm$ 30.09	48.3 $\pm$ 23.0	26.2 $\pm$ 19.5	35.4 $\pm$ 23.9	53.8 $\pm$ 40.6	38.1 $\pm$ 21.4	57.3 $\pm$ 67.2
Chlorophyll a ( $\mu$ g/L)	0.2 $\pm$ 0.2	0.2 $\pm$ 0.2	0.2 $\pm$ 0.3	0.1 $\pm$ 0.3	0.2 $\pm$ 0.3	0.1 $\pm$ 0.2	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.1 $\pm$ 0.2	0.2 $\pm$ 0.2	0.1 $\pm$ 0.2
Oxidizability (mg/L) *	4.6 $\pm$ 3.6	5.1 $\pm$ 3.6	6.4 $\pm$ 6.0	6.5 $\pm$ 3.1	5.4 $\pm$ 3.7	4.6 $\pm$ 2.9	4.3 $\pm$ 2.7	4.7 $\pm$ 2.6	3.4 $\pm$ 1.9	4.0 $\pm$ 4.1	3.5 $\pm$ 2.5

For alkalinity and nitrites, even if the Friedman test identified a significant heterogeneity among sites, Nemenyi post-hoc tests were unable to identify precisely the corresponding significant differences (probably due to beta errors in post-hoc test statistical decisions).



**Figure 3.** Box-plots (Min/Q25/Median/Q75/Max) describing the distribution of values of “dissolved oxygen” (in % saturation) and conductivity (in  $\mu\text{S cm}^{-1}$ ) over a period of thirteen months, for 11 study sites (S01 to S11). «a», «b», «c», «d» and «e» are distinct groups identified by post-hoc Nemenyi tests applied after identifying, for a given parameter, a significant difference among sites by a non-parametric Friedman test. Outliers (open circles) are out of the 1.5 interquartile range. See text for further details.

### 3.3. Abiotic Typology of Sampling Sites

The nPCA provided a typology of sampling sites based on their physico-chemical characteristics (Figure 4). The first factorial plane (F1-F2) explained 38.5% of the total inertia. Axis F1 discriminated sites exhibiting high percentage of dissolved oxygen (S05, S07 and S08;  $F1 > 0$ ) from sites with high conductivity and total hardness (S01 and S04;  $F1 < 0$ ). Axis F2 mainly opposed S09 ( $F2 > 0$ ) sites with rather low pH and oxidizability values to the other sites.

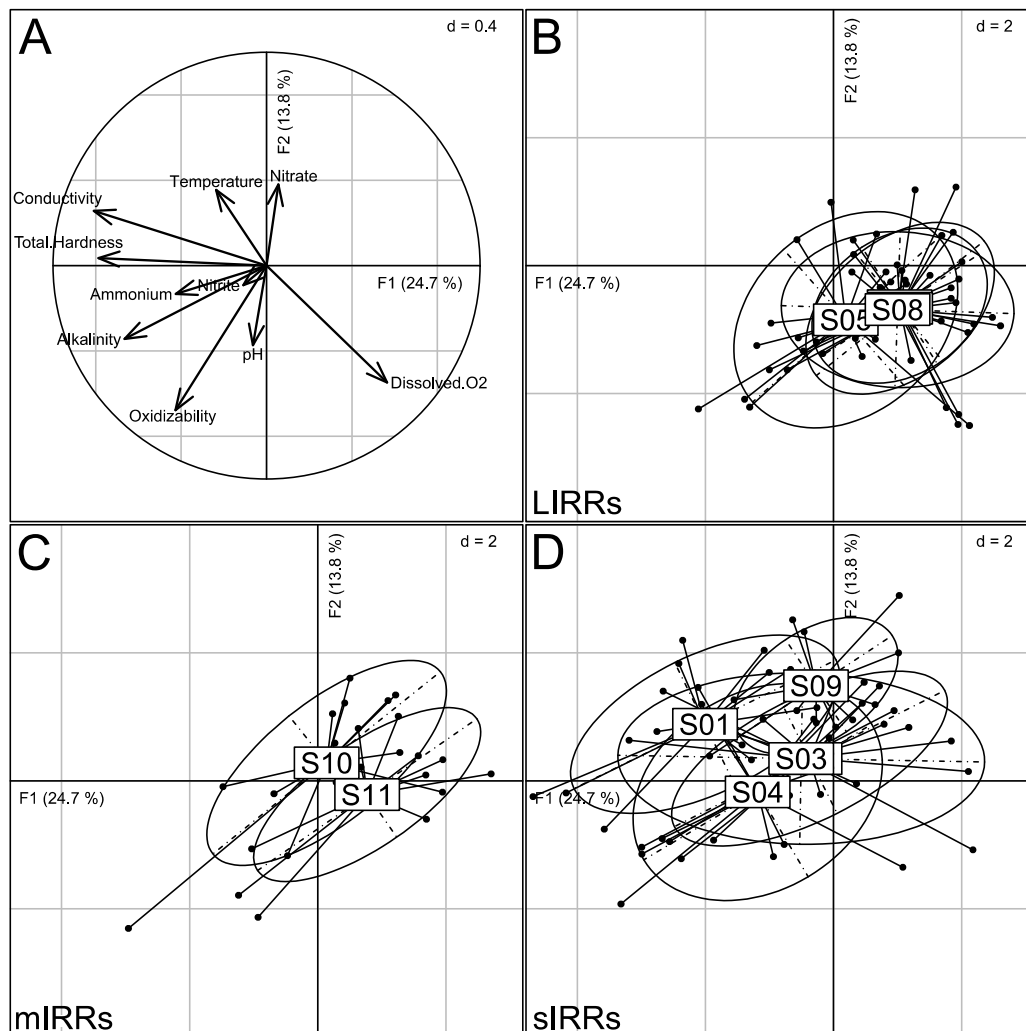
The cluster analysis (Figure S5) highlighted two main groups of sites. The first group gathered sites weakly urbanized and located in rural zones, i.e., S05 to S08 (LIRRs) and S11 (mIRR). The second group consisted of sites located in more urbanized areas, with two sub-groups: S01 and S04 (sIRRs) on the one hand, and S02 and S03 (sIRRs) with S09 and S10 (mIRRs) on the other hand.

### 3.4. Taxonomic Structure of Stream Benthic Macroinvertebrate Assemblages

A total of 31,099 organisms were sorted and identified, distributed in 5 phyla, 8 classes, 25 orders and 98 families. Insects, the most diversified group (11 orders, 76 families and 177 taxa), represented 86.5% of the total abundance.

Among the most representative taxa, the genus *Chironomus* (Diptera) was found in almost all the sampling sites (except S05, S07 and S08), with a global occurrence frequency (OF) of 76%. The genus *Coenagrion* (Odonata) was also highly common (57%), and mainly found in S05 to S08, and S11. The genera *Physa* (Gastropoda), *Caenis*, *Baetis* (Ephemeroptera), *Polymorphanicus* (Trichoptera), *Eurymetra*, *Rhagovelia*, *Limnogonus* (Heteroptera), *Orthetrum*, *Pseudagrion* (Odonata), *Dineutus* (Coleoptera), and the families Lumbriculidae (Oligochaeta) and Simuliidae (Diptera) were rather commonly found ( $25\% < \text{OF} < 50\%$ ). Significantly higher values of taxonomic richness and diversity (Shannon-Wiener index), evenness (Pielou index), EPT richness (mainly due to Ephemeroptera), 1-GOLD index and sig-

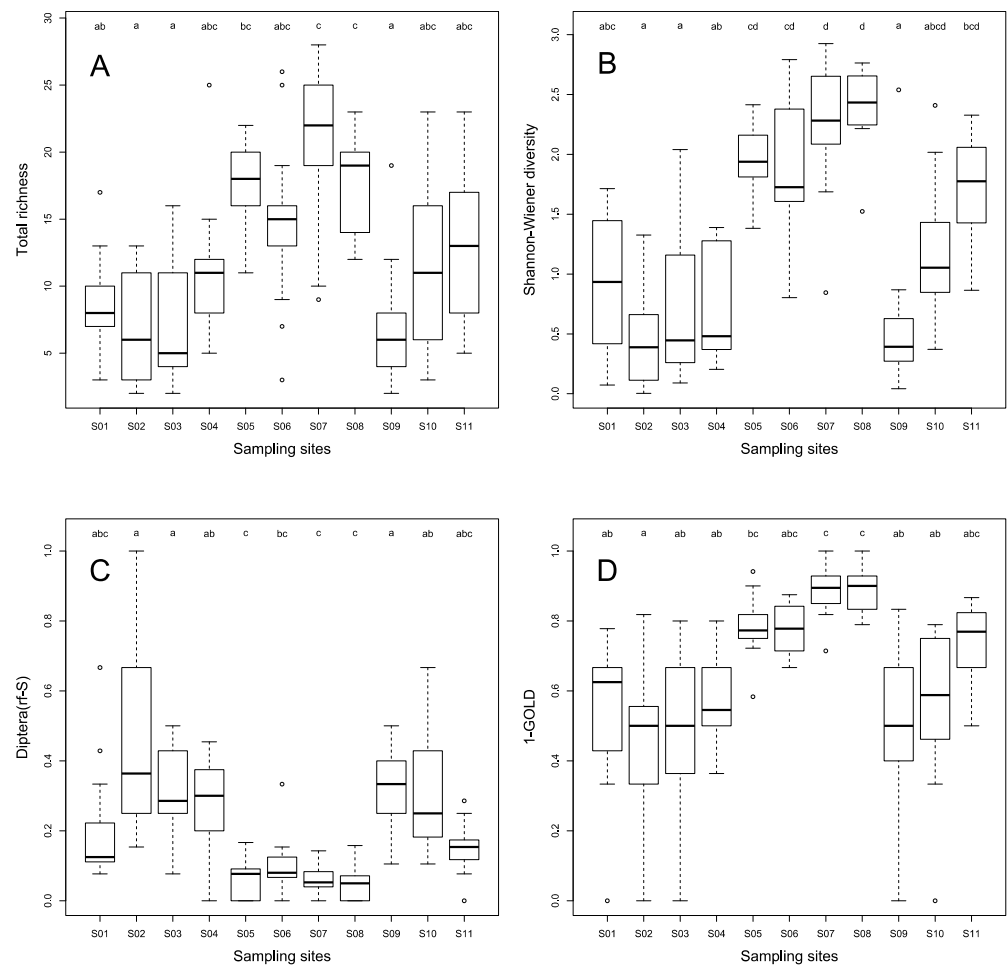
nificantly lower values of Diptera richness seemed related to the LIRR status of sites (Table 4; Figure 5 and Figure S6 in Supplementary Material). Even if taxonomic richness of Ephemeroptera, Odonata, Heteroptera, Hirudinea and Gastropoda significantly varied among sites, it was much more difficult to relate these variations to clear differences in site status (Figure S6 in Supplementary Material).



**Figure 4.** Ordination of sites by nPCA based on 10 physico-chemical parameters. (A) Projection of physico-chemical parameters on the first correlation circle. (B–D) Locations of sites (S01 to S11) on the first factorial plane at the weighted average of the locations of corresponding monthly sampling events (solid points). Each sampling event is linked by a line to the mean location of the corresponding site. Ellipses of inertia (95%) are provided by site. Sites are gathered according to anthropogenic pressure level: LIRRs = Least Impacted River Reaches; mIRRs = moderately Impacted River Reaches; sIRRs = severely Impacted River Reaches. See Table 1 and Figure 1 for the description and location of sites.

**Table 4.** Mean values ( $\pm$ standard deviation) of invertebrate-based metrics for the 11 sampling sites over the study period. Asterisks indicate significant differences among sites based on Friedman tests (\*\* $p < 0.001$ ; \* $p < 0.01$ ; \* $p < 0.05$ ). Metrics exhibiting significant differences are those included in the taxonomy-based nPCA (see Section 3.4) and RDA (see Section 3.5). Richnesses were expressed in terms of relative frequencies (rf-S). See text for further details.

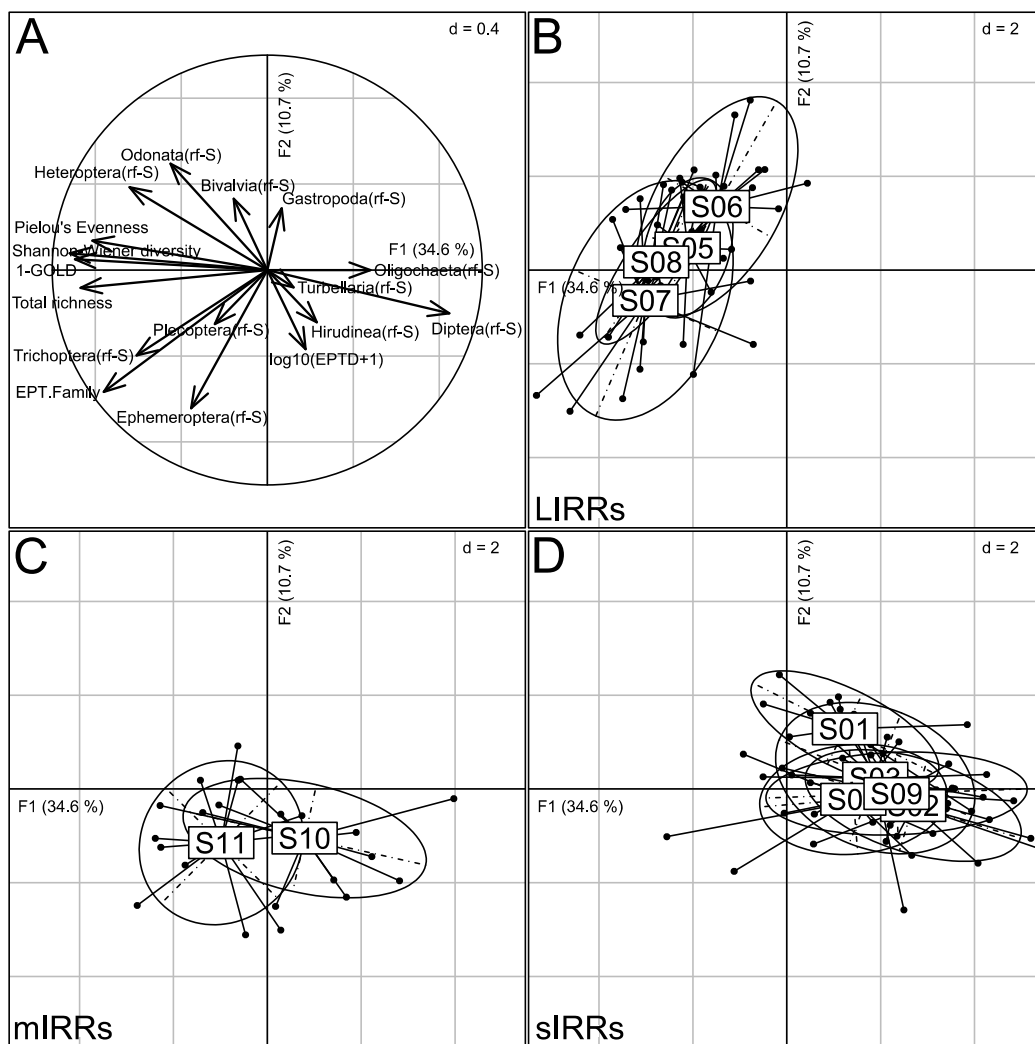
Sampling Sites	Nlem Stream				Kena Stream				Famlem Stream		
	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11
Metrics											
Total richness ***	8.62 $\pm$ 3.75	6.69 $\pm$ 4.01	7.38 $\pm$ 4.75	11.31 $\pm$ 5.07	17.46 $\pm$ 3.71	14.69 $\pm$ 6.42	20.69 $\pm$ 6.36	17.38 $\pm$ 3.84	6.69 $\pm$ 4.55	11.23 $\pm$ 6.26	13.31 $\pm$ 5.41
Shannon diversity ***	0.92 $\pm$ 0.60	0.48 $\pm$ 0.44	0.69 $\pm$ 0.62	0.71 $\pm$ 0.47	1.94 $\pm$ 0.29	1.94 $\pm$ 0.61	2.27 $\pm$ 0.56	2.41 $\pm$ 0.33	0.58 $\pm$ 0.63	1.23 $\pm$ 0.59	1.72 $\pm$ 0.42
Evenness (Pielou) ***	0.42 $\pm$ 0.25	0.23 $\pm$ 0.17	0.35 $\pm$ 0.26	0.29 $\pm$ 0.16	0.68 $\pm$ 0.08	0.76 $\pm$ 0.15	0.76 $\pm$ 0.15	0.85 $\pm$ 0.08	0.30 $\pm$ 0.20	0.54 $\pm$ 0.17	0.69 $\pm$ 0.12
Bivalvia(rf-S) *	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.01 $\pm$ 0.02	0.01 $\pm$ 0.03	0.00 $\pm$ 0.01	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Coleoptera(rf-S)	0.13 $\pm$ 0.11	0.10 $\pm$ 0.16	0.09 $\pm$ 0.10	0.07 $\pm$ 0.06	0.07 $\pm$ 0.06	0.18 $\pm$ 0.12	0.14 $\pm$ 0.08	0.15 $\pm$ 0.09	0.19 $\pm$ 0.18	0.10 $\pm$ 0.12	0.10 $\pm$ 0.07
Dictyoptera(rf-S)	0.01 $\pm$ 0.03	0.00 $\pm$ 0.00	0.02 $\pm$ 0.03	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.01 $\pm$ 0.01	0.00 $\pm$ 0.01	0.00 $\pm$ 0.01	0.00 $\pm$ 0.02	0.00 $\pm$ 0.02
Diptera(rf-S) ***	0.21 $\pm$ 0.17	0.46 $\pm$ 0.26	0.32 $\pm$ 0.14	0.28 $\pm$ 0.12	0.06 $\pm$ 0.06	0.11 $\pm$ 0.08	0.06 $\pm$ 0.04	0.05 $\pm$ 0.05	0.34 $\pm$ 0.12	0.31 $\pm$ 0.17	0.15 $\pm$ 0.07
Ephemeroptera(rf-S) ***	0.01 $\pm$ 0.04	0.03 $\pm$ 0.06	0.06 $\pm$ 0.14	0.07 $\pm$ 0.08	0.11 $\pm$ 0.05	0.05 $\pm$ 0.08	0.16 $\pm$ 0.10	0.14 $\pm$ 0.11	0.04 $\pm$ 0.07	0.20 $\pm$ 0.11	0.25 $\pm$ 0.16
Gastropoda(rf-S) ***	0.17 $\pm$ 0.17	0.03 $\pm$ 0.05	0.12 $\pm$ 0.11	0.12 $\pm$ 0.05	0.13 $\pm$ 0.05	0.09 $\pm$ 0.06	0.04 $\pm$ 0.04	0.06 $\pm$ 0.05	0.03 $\pm$ 0.07	0.06 $\pm$ 0.06	0.06 $\pm$ 0.08
Heteroptera(rf-S) ***	0.14 $\pm$ 0.12	0.05 $\pm$ 0.08	0.10 $\pm$ 0.11	0.14 $\pm$ 0.09	0.24 $\pm$ 0.08	0.23 $\pm$ 0.12	0.26 $\pm$ 0.08	0.29 $\pm$ 0.09	0.09 $\pm$ 0.12	0.05 $\pm$ 0.08	0.12 $\pm$ 0.10
Hirudinea(rf-S) ***	0.01 $\pm$ 0.03	0.08 $\pm$ 0.14	0.06 $\pm$ 0.07	0.13 $\pm$ 0.07	0.03 $\pm$ 0.03	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.01 $\pm$ 0.03	0.01 $\pm$ 0.03	0.06 $\pm$ 0.07	0.04 $\pm$ 0.06
Odonata(rf-S) ***	0.23 $\pm$ 0.15	0.18 $\pm$ 0.14	0.16 $\pm$ 0.18	0.15 $\pm$ 0.09	0.27 $\pm$ 0.09	0.26 $\pm$ 0.10	0.23 $\pm$ 0.13	0.23 $\pm$ 0.08	0.07 $\pm$ 0.11	0.10 $\pm$ 0.10	0.13 $\pm$ 0.11
Oligochaeta(rf-S) **	0.08 $\pm$ 0.09	0.07 $\pm$ 0.09	0.07 $\pm$ 0.12	0.03 $\pm$ 0.05	0.01 $\pm$ 0.03	0.02 $\pm$ 0.04	0.01 $\pm$ 0.02	0.01 $\pm$ 0.03	0.15 $\pm$ 0.15	0.07 $\pm$ 0.11	0.06 $\pm$ 0.05
Plecoptera(rf-S) *	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.01 $\pm$ 0.01	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Trichoptera(rf-S) ***	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.05 $\pm$ 0.05	0.03 $\pm$ 0.05	0.05 $\pm$ 0.05	0.04 $\pm$ 0.04	0.00 $\pm$ 0.01	0.00 $\pm$ 0.00	0.03 $\pm$ 0.04
Turbellaria(rf-S) ***	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.01 $\pm$ 0.04	0.00 $\pm$ 0.02	0.00 $\pm$ 0.01	0.02 $\pm$ 0.03	0.01 $\pm$ 0.03	0.07 $\pm$ 0.10	0.00 $\pm$ 0.01	0.05 $\pm$ 0.06
EPT Family ***	0.08 $\pm$ 0.28	0.31 $\pm$ 0.63	0.38 $\pm$ 0.65	0.92 $\pm$ 1.19	2.31 $\pm$ 0.85	1.00 $\pm$ 1.29	3.08 $\pm$ 1.44	2.62 $\pm$ 1.71	0.46 $\pm$ 1.13	1.69 $\pm$ 0.95	2.62 $\pm$ 1.12
log([EPTD + 1]) ***	0.02 $\pm$ 0.08	0.25 $\pm$ 0.24	0.11 $\pm$ 0.23	0.18 $\pm$ 0.25	0.00 $\pm$ 0.00	0.02 $\pm$ 0.08	0.13 $\pm$ 0.21	0.00 $\pm$ 0.00	0.04 $\pm$ 0.13	0.11 $\pm$ 0.23	0.02 $\pm$ 0.08
1-GOLD ***	0.54 $\pm$ 0.21	0.44 $\pm$ 0.24	0.49 $\pm$ 0.24	0.56 $\pm$ 0.14	0.79 $\pm$ 0.09	0.78 $\pm$ 0.07	0.89 $\pm$ 0.08	0.89 $\pm$ 0.06	0.48 $\pm$ 0.23	0.56 $\pm$ 0.23	0.74 $\pm$ 0.12



**Figure 5.** Box-plots (Min/Q25/Median/Q75/Max) describing the distribution of values of taxonomic richness (A), Shannon-Wiener index (B), Diptera richness (Diptera(rf-S); in proportion of the total richness) (C) and 1-GOLD index (D) over a period of thirteen months, for 11 study sites (S01 to S11). «a», «b», «c» and «d» are distinct groups identified by post-hoc Nemenyi tests applied after identifying, for a given metric, a significant difference among sites by a non-parametric Friedman test. Outliers (open circles) are out of the 1.5 interquartile range. See text for further details.

The nPCA first factorial plane providing the ordination of site assemblages according to 17 taxonomic metrics (Table 2) explained 45.3% of the total inertia (Figure 6). Three groups of sites were identified. Along the first axis, S05-S08 and S11 ( $F1 < 0$ ), exhibiting the highest values of Shannon-Wiener diversity, Pielou evenness, total and EPT Family richness, 1-GOLD, Heteroptera(rf-S) and Trichoptera(rf-S), were opposed to S01-S04 and S09 ( $F1 > 0$ ), having the highest values of Diptera(rf-S) and Oligochaeta(rf-S). Axis F2 mainly opposed S10-S11 ( $F2 < 0$ ), with the highest values of Ephemeroptera(rf-S), to S06 ( $F2 > 0$ ), which exhibited a high proportion of Odonata.

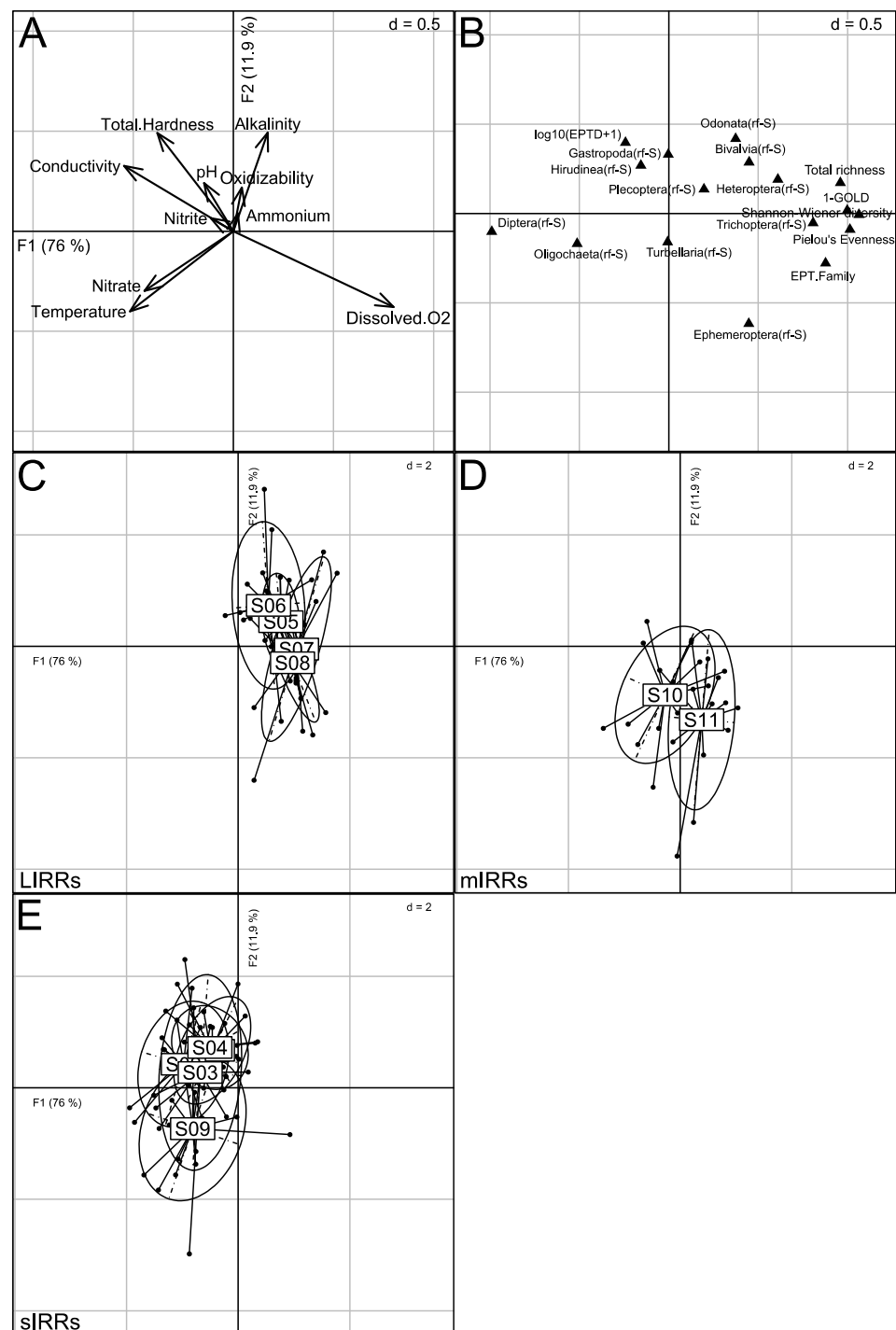
The cluster analysis based on taxonomic criteria (Figure S7) clearly separated sites with an expected LIRR status (i.e., S05 to S08) from severely (sLIRRs; S01-S04 and S09) or moderately (mLIRRs; S10-S11) impaired sites.



**Figure 6.** Ordination of sites by nPCA, based on 17 taxonomy-based metrics. (A) Projection of taxonomic metrics on the first correlation circle; (B–D) Locations of sites (large labels, S01 to S11) on the first factorial plane at the weighted average of the locations of corresponding monthly sampling events (solid points). Each sampling event is linked by a line to the mean location of the corresponding site. Ellipses of inertia (95%) are provided by site. Sites are gathered according to anthropogenic pressure level: LIRRs = Least Impacted River Reaches; mIRRs = moderately Impacted River Reaches; sIRRs = severely Impacted River Reaches. See Table 1 and Figure 1 for the description and location of sites.

### 3.5. Relationships between Physico-Chemical Parameters and the Taxonomic Metrics of Benthic Macroinvertebrate Assemblages

The influence of physico-chemical parameters on the taxonomic metrics describing stream invertebrate assemblages was examined using redundancy analysis (RDA; Figure 7). The first two factorial axes accounted for 87.9% of total inertia, and ANOVA-like permutation tests showed that both the model and the first axis were significant ( $p$ -value(model) < 0.001;  $p$ -value(F1) < 0.001). The model explained 20.8% of the variance in taxonomic metric values (adjusted  $R^2$ ).



**Figure 7.** Ordination of sites by Redundancy Analysis (RDA) based on 17 taxonomic metrics. Factorial axes are linear combinations of 10 physico-chemical parameters. **(A)** Canonical weights of physico-chemical parameters. Only the main parameters are labeled; **(B)** location of metrics on the first factorial plane; **(C–E)** locations of sites (large labels) on the first factorial plane at the weighted average of the locations of corresponding monthly sampling events (solid points). Each event is linked by a line to the mean location of the corresponding site. Labels corresponding to the mean locations of S01 and S02 are partially masked by S03 and S04 labels, respectively. Ellipses of inertia (95%) are provided by site. Sites are gathered according to anthropogenic pressure level: LIRRs = Least Impacted River Reaches; mIRRs = moderately Impacted River Reaches; sIRRs = severely Impacted River Reaches. See Table 1 and Figure 1 for the description and location of sites.

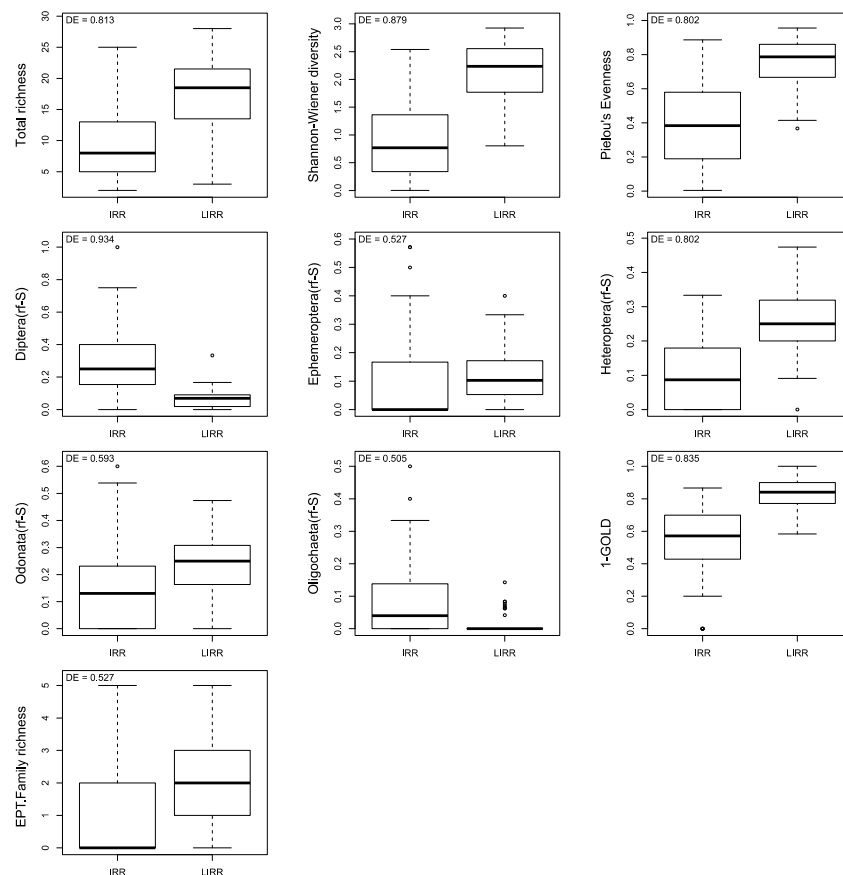


Three groups of sites were distinguished on the first factorial plane. Axis 1 opposed S05–S08 ( $F1 > 0$ ) to S01–S04 ( $F1 < 0$ ). S05–S08 exhibited mainly high water oxygenation. They were associated with the highest values of the Shannon-Wiener and Pielou indices, total richness, 1-GOLD index, Trichoptera(rf-S), Heteroptera(rf-S) and EPT Family richness (see also Table S2 in Supplementary Material). In contrast, S01–S04 were characterized by high mineralization and high nitrate load, and exhibited the highest values of Diptera(rf-S). Along the F2 axis, S09–S11 ( $F2 < 0$ ) were characterized by the highest levels of temperature, nitrate concentration and dissolved oxygen concentration. S10 and S11 were associated with the highest proportion of Ephemeroptera(rf-S) and opposed mainly to S01, S04 and S06.

The cluster analysis based on the RDA results (Figure S8) clearly separated S01–S04 expected as severely impacted sites from other sites subdivided in two sub-groups. The first sub-group gathered sites expected as least impacted sites (i.e., S05–S08) and the second sub-group gathering sites expected as only moderately (S10–S11) or more severely impacted (S09) by anthropogenic activities.

### 3.6. The Discrimination Efficiency (DE) of the Metrics Describing Benthic Invertebrate Assemblages

The DE of the 19 calculated metrics ranged from 0.000 for Bivalvia(rf-S) and Plecoptera(rf-S) to 0.934 for Diptera(rf-S). Only 10 out of 19 metrics exhibited a DE value greater than 0.5, which are (in decreasing order): Diptera(rf-S) (0.934), Shannon-Wiener diversity (0.879), 1-GOLD (0.835), total richness (0.813), Heteroptera(rf-S) and Pielou evenness (0.802), Odonata(rf-S) (0.593), EPT Family and Ephemeroptera(rf-S) (0.527) and Oligochaeta(rf-S) (0.505). Among these 10 metrics, only Diptera(rf-S) and Oligochaeta(rf-S) increased with site degradation (Figure 8), as expected in Table 2.



**Figure 8.** Box-plots (Min/Q25/Median/Q75/Max) of the most discriminant ( $DE > 0.5$ ) macroinvertebrate-based metrics in the LIRRs (Least Impacted River Reaches) and IRRs (moderately and severely Impacted River Reaches). Discrimination Efficiency (DE) values are given in the top-left part of each subplot. Outliers are out of the 1.5 interquartile range.

## 4. Discussion

### 4.1. Physico-Chemical Characteristics of Streams

Some river reaches of the streams in Mifi County (western region of Cameroon) are clearly impacted by anthropogenic activities occurring in or near the city of Bafoussam. The studied sites were mainly discriminated according to their degree of contamination by factory effluents and waste waters (both domestic/municipal/agricultural and industrial), cattle/poultry breeding and their location in either densely or sparsely populated areas.

Based on the pressure description (MCA in Section 3.1) and physico-chemical criteria (nPCA in Section 3.2), sites located on the Kena stream and its tributaries (S04 to S08) were globally distinguished from the other sites by better water quality, illustrated by significantly higher oxygen concentration and lower mineralization. In this catchment, only S04 exhibited poor water quality. Thus, S05 to S08 represented the less polluted and impacted reaches within the sampling design, and seemed to be good candidates for defining reference conditions for wadeable rivers in this area.

The upper reaches of the Famlem stream (S09), located in an urban area, were subjected to fertilized agriculture and various forms of domestic and commercial activities. In S10 and mainly S11, located in a rural area, the water quality increased due to decantation or sedimentation of matter on the stream bed and dilution of watershed inputs along the stream. The Nlem tributary (S01 to S03) was subjected to high anthropogenic pressure, mainly related to industrial and domestic effluents, which resulted in high values of conductivity and nutrient concentrations. These sites were located in the densely populated surroundings of Bafoussam City, where several industries were known to release wastewaters in the stream. Moreover, many domestic effluents (e.g., latrines in the riparian area [50]) were directly discharged into the stream, enriching it with nitrogen and organic matter. The IRR vs. LIRR status allocated to sites based on MCA results was confirmed by physico-chemical characterization for most of the sites. Only S11, considered as moderately impaired (mIRR) based on potential anthropogenic pressures, was gathered with LIRRs based on physico-chemical criteria (Figure S5).

The accuracy of this physico-chemical characterization indeed relies on the availability of data used to characterize anthropogenic pressures. One of the caveats of our characterization is that micropollutant concentrations were not measured due to the expensive cost of analyses. Data on water concentrations of pesticides are extremely rare in Cameroon. However, Galani et al. (2018, [71]) measured the residues of 99 pesticides in samples of 12 local agricultural products collected in the western highlands of Cameroon, including the Bafoussam region. They found in the Bafoussam region 18.2% of measured pesticide residue concentrations above the Maximum Residue Levels fixed by the European Union, including three fungicides (captan, hexachlorobenzene and matalaxyl), three herbicides (alachlor, chlorotoluron and terbuthylazine) and seven insecticides (aldrin, cypermethrin, dieldrin, malathion, methoxychlor, p,p'-DDT and  $\gamma$ -HCH), indicating that such micropollutants were used in the agricultural areas of the studied watersheds. They could therefore be present in some sections of the rivers of the Bafoussam region and should be taken into account in further studies.

### 4.2. Invertebrate-Based Metrics and Their Relationships with Physico-Chemical Parameters

Overall, the benthic fauna present in the different sites during this study exhibited a composition rather similar to that of invertebrate communities found in other African rivers [72,73]. Insects had the highest diversity in the three catchments, due to their high capacity to colonize various ecological niches with specific mesohabitat conditions [74–76]. Diptera, Coleoptera, Heteroptera, Ephemeroptera, Odonata (Insecta) and gastropods were the most abundant groups.

Site typologies provided by multivariate analyses based on physico-chemical parameters (nPCA, Figure S5), taxonomy-based metrics (nPCA, Figure S7) and taxonomy-based metrics related to physico-chemical parameters (RDA, Figure S8), were not similar. Sites moderately impacted by anthropogenic activities (mIRRs) were indeed gathered with least

impacted (S11 based on physico-chemical criteria, Figure S5) or severely impacted (S11 based on taxonomic criteria, Figure S7) river reaches. However, relating taxonomy-based metrics to the physico-chemical parameters by RDA highlighted three groups of sites highly coherent with their expected ecological status (Figure S8).

The first group gathered weakly mineralized and well oxygenated sites (S05 to S08) from the Kena stream, located in rural areas, where anthropogenic pressures were low (e.g., low nutrient concentrations and conductivity). These sites hosted pollution-sensitive organisms (high EPT family richness, Shannon-Wiener diversity, Heteroptera(rf-S) and 1-GOLD values), highlighting their good water quality. Similarly, Qu et al. (2010, [77]), Rawson et al. (2010, [78]), Arimoro et al. (2010, [79]), Shelly et al. (2011, [80]), Myers et al. (2011, [81]) and Kaboré et al. (2016, [82]) identified higher abundance of pollution-sensitive insects in reaches with predominantly preserved habitats. Moreover, Duka et al. (2017, [83]) already observed a drastic decrease in the taxonomic richness of sensitive organisms with increasing watershed urbanization. In Cameroon, Menbohan et al. (2013, [41]) and Tchatcho et al. (2014, [84]) reported similar results in the peri-urban forested rivers of Yaoundé.

The second group, aggregated to the first one, was represented by the sites on the Famlem stream (S09–S11), with increasing water quality along the upstream/downstream longitudinal gradient explained by both the self-purifying capacity of the river and the dilution of anthropogenic inputs along the longitudinal gradient. Diptera was the most abundant group of organisms upstream (in S09, situated in the urban zone), while a higher taxonomic diversity was observed downstream due to partial recolonization of the lower reaches by more pollution-sensitive taxa. This pattern was confirmed by the high richness of Ephemeroptera at S11.

The third group included the sites located on the tributaries of the Nlem (S01 to S03) and Kena (S04) streams. These sites hosted mainly pollution-tolerant taxa (e.g., high Diptera(rf-S)), and can be considered as disturbed reaches with poor water quality, due to domestic and industrial waste contamination. Solid and liquid wastes were dumped into the streams, impairing water quality and favoring resistant organism proliferation (Diptera). Such pollution has already been associated with macroinvertebrate local extinctions and/or biodiversity decline [85]. Rawson et al. (2010, [78]) and Dar and Reshi (2014, [86]) also considered that urbanization-related variations in water physico-chemical characteristics reduce the abundance of sensitive taxa and promote the development of more tolerant organisms, including Oligochaeta (Lumbriculidae), Diptera and Gastropoda. Moreover, habitat degradation contributes in many cases to benthic mosaic homogenization. Mud was the dominant substrate in most of these stream sites, offering a very low diversity of potential ecological niches for benthic invertebrates. Similarly, Doretto et al. (2018, [87]) observed a reduction in taxonomic richness and abundance of macroinvertebrates (especially EPT) when a high level of siltation occurred in a stream reach.

#### 4.3. Metrics of Interest for Future Biomonitoring Tool

The total taxonomic richness, EPT family richness, Shannon-Wiener diversity, Pielou evenness, 1-GOLD, Ephemeroptera(rf-S), Heteroptera(rf-S), Odonata(rf-S), Diptera(rf-S) and Oligochaeta(rf-S) were the ten metrics best responding to anthropogenic pressures commonly acting on streams of the West Cameroon region, based on their efficiency in the discrimination of LIRRs from IRRs (DE; Figure 8). Six of these metrics exhibited a DE value greater than 0.8, indicating their especially high capacity to discriminate IRRs from LIRRs. They can be ranked along a decreasing order of discrimination efficiency: Diptera(rf-S) > Shannon-Wiener diversity > 1-GOLD > total richness > Pielou evenness = Heteroptera(rf-S). These metrics should provide the best overview of the water quality of sampled sites, due also to their high relationship with some of the physico-chemical variables measured (Table S2). They could correspond to the best set of candidate taxonomic metrics for inclusion in an invertebrate-based multimetric bioassessment tool for the wadeable streams of the West Cameroon region. Following Mondy et al. (2012; [88]), reference values (i.e., values expected in reference conditions) and four ecological qual-

ity class boundaries (delimiting 'high', 'good', 'moderate', 'poor' and 'bad' classes) were defined for each of these metrics (the detailed procedure and corresponding values are provided in Table S3 in the Supplementary Material). Such values will help in the ecological diagnostics of river reaches in the Bafoussam region based on the taxonomic structure of macroinvertebrate assemblages, taking into account the original ecological information provided by each of these metrics.

The total taxonomic richness, Shannon–Wiener diversity and Pielou evenness describe the taxonomic characteristics of an assemblage driven by both habitat complexity and stability [88–91]. As expected (Table 2), the sites less exposed to anthropogenic pressures (S05 to S08) had far higher values of these three metrics than sites moderately and severely exposed (S10–S11 and S01–S04 + S09, respectively).

Based on EPT family richness, Tchatcho et al. (2014, [84]) and Kaboré et al. (2016, [82]) already efficiently monitored forest streams in the peri-urban area of Yaoundé (Cameroon) and streams in protected zones of Burkina Faso, respectively. This metric has been used worldwide for assessing the biological condition of streams [57] in North America [92], South America [93], Europe [94–96], Asia [97] and Australia [98], often in combination with Ephemeroptera(rf-S) [99,100]. However, this metric exhibited a rather low discrimination efficiency ( $DE = 0.527$ ) in the Bafoussam region, probably due to the low family richness of the EPT assemblage (zero to five families), even in reference conditions.

Dias-Silva et al. (2010, [101]) and Vieira et al. (2014, [102]) already considered Heteroptera as indicators of good water quality in Brazilian streams because they are sensitive to the anthropogenic alteration of the riparian aquatic vegetation that would provide them food and offer shelter against predators or during heavy rains. Tchakonté et al. (2015, [42]) concluded similarly based on the invertebrate assemblages of forest streams of Douala (Cameroon). We confirmed the high efficiency of this metric in the discrimination of LIRRs from IRRs in the Bafoussam region ( $DE > 0.8$ ).

1-GOLD and Diptera(rf-S) were two metrics best reacting to stream degradation ( $DE = 0.835$  and  $0.934$ , respectively). The 1-GOLD metric gathers information from Gastropoda, Oligochaeta and Diptera: three groups of rather pollution-tolerant organisms. Gastropoda and Oligochaeta were involved in the multimetric index evaluating the ecological quality of drainage ditch systems in the Netherlands [103] or siliceous river systems in Portugal [104], and in the identification of metal contamination of soft sediments in French and Swiss rivers [105–107]. Diptera, dominated by Chironomidae (especially *Chironomus*), were found in almost all the sites. This wide distribution can be explained by their ubiquitous traits and their capacity to rapidly colonize new environments. Moreover, *Chironomus*'s ability to capture in water and carry dissolved oxygen with hemolymphic hemoglobin gives it a high physiological tolerance even in environments with very low oxygen concentration [28,108,109]. This collector-filterer is already considered as highly tolerant to pollution in many African tropical streams [110,111], as are the Oligochaeta [41].

Odonata, generally exhibiting a long period of larval development, are good witnesses of river habitat quality and stability, and are extremely diverse in tropical areas [112]. Odonata(rf-S) can provide a reliable measure of anthropogenic impact. In particular, disturbance of the riparian vegetation has a direct effect on the structure of the adult dragonfly community, potentially impairing larval populations [113]. Miguel et al. (2017, [114]) and De Oliveira-Junior et al. (2017, [115]) noticed their high sensitivity to the degradation of riparian vegetation, potentially altered by the local use of streams by riverine human populations.

#### 4.4. Seasonal Effect on Macroinvertebrate-Based Metrics

Our results demonstrate only a moderate influence of “season” on invertebrate-based metric variations in the studied streams of West Cameroon. Even though Tonkin et al. (2017, [116]) and Dalu et al. (2017, [117]) pointed out the driving force of seasonality on stream community dynamics, its effects on invertebrate assemblages of West Cameroon rivers seem to be modulated by the high predictability of the successive hydrological events

(rainy season vs. dry season). However, such events can lead to some structural and compositional changes within benthic communities (e.g., a decrease in taxonomic richness and total abundance) via the physical disturbance of bottom substrates due to faster current velocity and higher water level in the rainy season [118]. This rather moderate effect of season on aquatic invertebrate community structure may be explained also by the absence of strong between-season variation in the physico-chemical characteristics of water. If Masese et al. (2014, [111]) found a high influence of season on the structural and functional organization of macroinvertebrate assemblages in Kenyan highland streams (due to the exacerbation of differences in water quality and habitat characteristics), a moderate effect of season was already pointed out by Tonkin et al. (2016, [119]) and Dalu et al. (2017, [120]) in Afrotropical Nigerian streams and in an austral South African stream, respectively. This rather low influence of seasonality on the taxonomic structure of invertebrate assemblages should facilitate the development of a future biotic index for West Cameroon rivers.

## 5. Conclusions

In conclusion, human activities in and around streams (farming, vegetation clearance, sand extraction, bathing, laundry, riparian culture, and effluent discharge from households and industries) drastically reduce the within-stream invertebrate biodiversity in the West Region of Cameroon. An urgent need for the conservation and wise use of the watercourses in this area was highlighted. We identified the main relationships between pressure-sensitive invertebrate-based metrics and physico-chemical characteristics of streams. This identification will facilitate the selection of the scale at which efforts for protection and/or rehabilitation should be directed to best improve and/or maintain sustainable ecological conditions in West Cameroon streams. Invertebrate-based biomonitoring of these streams seems very promising, several taxonomic metrics clearly varying according to the intensity of human impacts. Diptera(rf-S), Shannon-Wiener diversity, 1-GOLD, total richness, Pielou evenness and Heteroptera(rf-S) seem the most promising metrics for identifying a significant anthropogenic impairment. The selection of these best candidate metrics is the first significant step towards the construction of an invertebrate-based multimetric index efficiently assessing the ecological status of streams in this region.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w14091490/s1>, Figure S1: Ordination of sites by a “within-season” nPCA based on 17 taxonomy-based metrics. (A) Projection of taxonomic metrics on the first correlation circle (F1-F2); (B, C and D) Locations of sites (large labels) on the first factorial plane F1-F2 at the weighted average of the locations of corresponding monthly sampling events (solid points). Each event is linked by a line to the mean position of the corresponding site. Ellipses of inertia (95%) are provided by site. Sites are gathered according to anthropogenic pressure level; Figure S2: Ordination of sites by a «within-season» nPCA, based on 10 physico-chemical parameters. (A) Projection of physico-chemical parameters on the first correlation circle (F1-F2); (B, C and D) Locations of sites (large labels) on the first factorial plane at the weighted average of the locations of corresponding monthly sampling events (solid points); Figure S3: Ordination of impact levels (three categories: low/moderate/high) of eight pressure categories by Multiple Correspondence Analysis (MCA) based on the description of 11 study sites; Figure S4: Box-plots (Min/Q25/Median/Q75/Max) describing the distribution of values of the remaining physico-chemical parameters exhibiting significant differences among sites, over a period of thirteen months, for 11 study sites (S01 to S11). « a », « b », « c » and « d » are distinct groups identified by post-hoc Nemenyi tests applied after identifying, for a given parameter, a significant difference among sites by a non-parametric Friedman test; Figure S5: Dendrogram, based on the Euclidean distance (calculated between sites according to their coordinates on the first factorial plane of the nPCA) and the complete linkage method, highlighting the similarity of the 11 study sites (S01 to S11) considering their physico-chemical characteristics during the study period; Figure S6: Box-plots (Min/Q25/Median/Q75/Max) describing the distribution of values of the remaining taxonomy-based metrics exhibiting significant differences among sites, over a period of thirteen months, for 11 study sites (S01 to S11). “a”, “b”, “c” and “d” are distinct groups identified by post-hoc Nemenyi tests applied after identifying, for a given parameter, a significant difference

among sites by a non-parametric Friedman test; Figure S7: Dendrogram, based on the Euclidean distance (calculated between sites according to their coordinates on the first factorial plane of the nPCA) and the complete linkage method, highlighting the similarity of the 11 study sites (S01 to S11) considering the structure of their macroinvertebrate assemblage described by 17 taxonomy-based metrics; Figure S8: Dendrogram, based on the Euclidean distance and the complete linkage method applied to sampling events coordinates on the first factorial plane of the RDA, highlighting the similarity of the 11 study sites (S01 to S11) based on the structure of their macroinvertebrate assemblage described by 17 taxonomy-based metrics explained by 10 physico-chemical variables; Table S1: Semi-quantitative description of eight categories of anthropogenic pressures for 11 sampling sites (S01-S11) in three catchments of the Bafoussam region (West Cameroon). The potential intensity of each pressure category has been described using three modalities: 1 = low, 2 = moderate and 3 = high. The eight pressure categories are related to the importance of “urban” areas, “food crop”, “cash crop”, “cattle and poultry” breeding, untreated “domestic, municipal and agricultural” or “industrial” wastewater effluents, riverine “factories” and “civil engineering and mining” at reach scale; Table S2: Correlations (Spearman’s rho coefficient) between the 19 tested macroinvertebrate-based metrics and the environmental parameters; Table S3: Proposed reference values and ecological quality class boundaries for the ten metrics exhibiting significantly different ranges of values between LIRRs and IRRs in wadeable rivers of the Bafoussam Region. Ecological class boundaries have been defined following Mondy et al. (2012; [88]). As recommended by the WFD, ecological quality class boundaries (i.e. delimiting ‘high’, ‘good’, ‘moderate’, ‘poor’ and ‘bad’ classes) have been defined based on the distribution of each metric values from the LIRR data set. For each metric decreasing with increasing anthropogenic pressures (i.e., all the metrics except Diptera(rf-S) and Oligochaeta(rf-S); cf. Figure 8), the 95th, 75th and 25th percentiles of the metric value distribution in the LIRRs were calculated. These values have been considered as the reference value, the ‘high–good’ boundary and the ‘good–moderate’ boundary, respectively. For the two metrics increasing with increasing anthropogenic pressures (i.e. Diptera(rf-S) and Oligochaeta(rf-S)), the 5th, 25th and 75th percentiles of the metric value distribution in the LIRRs were considered as the reference value, the ‘high–good’ boundary and the ‘good–moderate’ boundary, respectively. For defining the ‘moderate–poor’ and the ‘poor–bad’ boundaries, we divided the metric scoring range between the minimal possible value (decreasing metric) or the maximal possible value (increasing metric) and the ‘good–moderate’ boundary in three equal classes.

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