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Spatiotemporal Variation of Benthic Macroinvertebrates in Some Tropical Forest Streams of the Nyong Catchment (Cameroon)

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Abstract

With the aim of assessing the benthic macroinvertebrates' diversity, a study was carried out in some tropical forest streams of the Nyong River catchment in Cameroon from February 2019 to February 2020. A total of 167 samples were carried out from 13 stations during 13 months. Some environmental variables were measured. These parameters varied more or less from station to station but significantly at the temporal level. In this study, 13,690 benthic macroinvertebrates belonging to 4 phyla, 7 classes, 16 orders and 93 families were collected. The benthic macroinvertebrates were more abundant and more diversified in the stations whose waters are well oxygenated and present a moderate current compared to the stations with a very weak current. The diversity varied significantly from 1.33 ± 0.14 bits/ind to 2.00 ± 0.35 bits/ind and the high values were found in stations with multiple substrates and well-oxygenated waters. Temporally, the diversity varied significantly from 1.10 ± 0.16 bits/ind in NM sampling station in February 2020 to 1.87 ± 0.1 bits/ind in the OB sampling station in September. In addition, the settlement was more abundant during the short dry season, more precisely during the month of August (1471 individuals) but richer during the long dry season during the month of February (54 families). The distribution of the abundances of the benthic fauna in the different stations made it possible to identify five typological groups using the rarefaction curves, the ascending hierarchical classification and the principal component analyses. Each of these five groups is characterized by a specific taxonomic richness, composition and abundance.

Keywords

Benthic Macroinvertebrates, Environmental Variables, Faunistic Diversity,

Taxonomic Richness, Biotypology, Tropical Forest Streams, Cameroon

1. Introduction

Since the Earth Summit in Rio in 1992, international conventions and protocols have been established around biodiversity [1] [2]. Biodiversity offers irreplaceable and essential goods for our daily lives by playing a very important role in ecosystem services [3] [4]. Benthic invertebrates are an important component of biodiversity which can be assessed by taking into account the diversity of ecosystems, species or genes in space and time, as well as the interactions within these levels of the organization and between them [5] [6]. To assess the diversity of benthic invertebrates in rivers, the various elements of the lists of species and ecosystems must be taken into account. Many authors use multiple methods to evaluate the biological diversity of streams. This involves counting the number of individuals, taxonomic richness, dominance, and taxonomic diversity among others [7] [8]. In Cameroon, the streams are explored but knowledge of benthic macroinvertebrates is in its infancy despite some work already carried out [9]-[17]. These studies have shown that Cameroonian streams shelter numerous benthic macroinvertebrates taxa depending on the habitats and seasons. The present work focuses on the taxonomic diversity of benthic macroinvertebrates in tropical forest streams of the Nyong watershed. In other words, it is a contribution to the knowledge of the diversity of benthic macroinvertebrates in some forest watercourses in Cameroon. More precisely, it aims to evaluate the physic-chemical parameters of water (1), the spatiotemporal richness and diversity of the benthic macroinvertebrates in these streams (2) and highlight the biological characteristics of the stations (3).

2. Material and Methods

2.1. Material

2.1.1. Study Site

The study area is located between 3˚20' - 3˚37' North latitude and 11˚26' - 11˚34' East longitude, in the Nyong-and-So'o division. The region has a Guinean equatorial climate with four seasons: a long dry season from mid-November to mid-March, a short rainy season from mid-March to the end of June, a short dry season that extends from July to August and a long rainy season that goes from September to mid-November [18]. The precipitations vary between 1500 and 2000 mm and the hydrographic network is dense. The average annual temperature is around 24.6˚C with an annual amplitude average of 4.9˚C [18]. Ferralitic soils are found at the top of interfluves and at the bottom of slopes, hydromorphic soils are found in marshy valleys and poorly evolved soils are located on steep mountainous reliefs [19]. The vegetation is dense, humid evergreen forest type with medium and high altitudes [19].

2.1.2. Sampling Stations Descriptions

The sampling stations are all located in the Nyong River catchment (**Figure 1**) and their characteristics (altitudes, coordinates, order level, vegetation and substrates) are listed in **Table 1** below. The Strahler classification method was used to characterize the position of each stream in the drainage catchment [20].

2.2. Methods

At each site, environmental parameters and macroinvertebrates samples were collected monthly from February 2019 to February 2020.

Table 1. Characteristics of the sampling stations.

Streams	Stations codes	Altitude (m)	N latitude	E longitude	Description
Kongolo watershed	K1	645	03˚36'41.3"	011˚29'37.1"	Secondary dense vegetation, sandy substrates, dead leaves, woods, 3.9 km from the spring, Strahler order 3.
	K2	638	03˚32'28.7"	011˚30'30.9"	Degraded vegetation, sandy substrates, 7.35 km from the source, Strahler order 3.
	K3	634	03˚31'14.3"	011˚31'00"	Degraded vegetation, muddy substrates, 9.65 km from the source, Strahler order 3.
Nloumou watershed	AN1	681	03˚33'46.2"	011˚30'02.8"	Secondary dense vegetation, sandy/rocky substrates, dead leaves, 1.85 km from the source, Strahler order 1.
	AN2	645	03˚32'02.6"	011˚31'59.4"	Secondary dense vegetation, sandy substrate, order 1 of the Nloumou stream, 3.4 km from the source,.
	N	643	03˚32'09.0"	011˚32'43.9"	Secondary dense vegetation, sandy/rocky substrates, 8.35 km from the source, Strahler order 3.
Ibe-Mfeme watershed	IM	644	03˚24'02.1"	011˚28'01.1"	Secondary dense vegetation, bedrock, dead leaves, 0.9 km from its source, Strahler order 1.
Nsoe-Mekok watershed	NM	647	03˚23'44.5"	011˚28'02.9"	Secondary dense vegetation, muddy/rocky substrates, dead leaves, woods, 1.3 km from the source, Strahler order 1.
Akoumbegue watershed	A	643	03˚24'26.0"	011˚29'20.3"	Secondary dense vegetation, sandy substrate, wood, 5.85 km from its source, Strahler order 2.
	C	641	03˚24'30.5"	011˚28'24.5"	Secondary dense vegetation, muddy substrates, dead leaves, woods, 0.7 km from the source, order 1 of the Akoumbegue stream.
Zoetoupsi watershed	Z	653	03˚26'10.0"	03˚26'10.0"	Secondary dense vegetation, muddy substrates, wood, 0.9 km from its source, Strahler order 1.
	OB	651	03˚26'10.0"	011˚30'43.7"	Degraded vegetation, fish farming activities, muddy/rocky substrate, 0.9 km from its source, order 1 of the Zoetoupsi stream.
Ossoe-Nkoro watershed	ON	645	03˚28'40.6"	03˚28'40.6"	Degraded vegetation, fish farming activities, muddy/rocky substrate, dead leaves, 1.5 km from its source, order 1 of the Zoetoupsi stream.

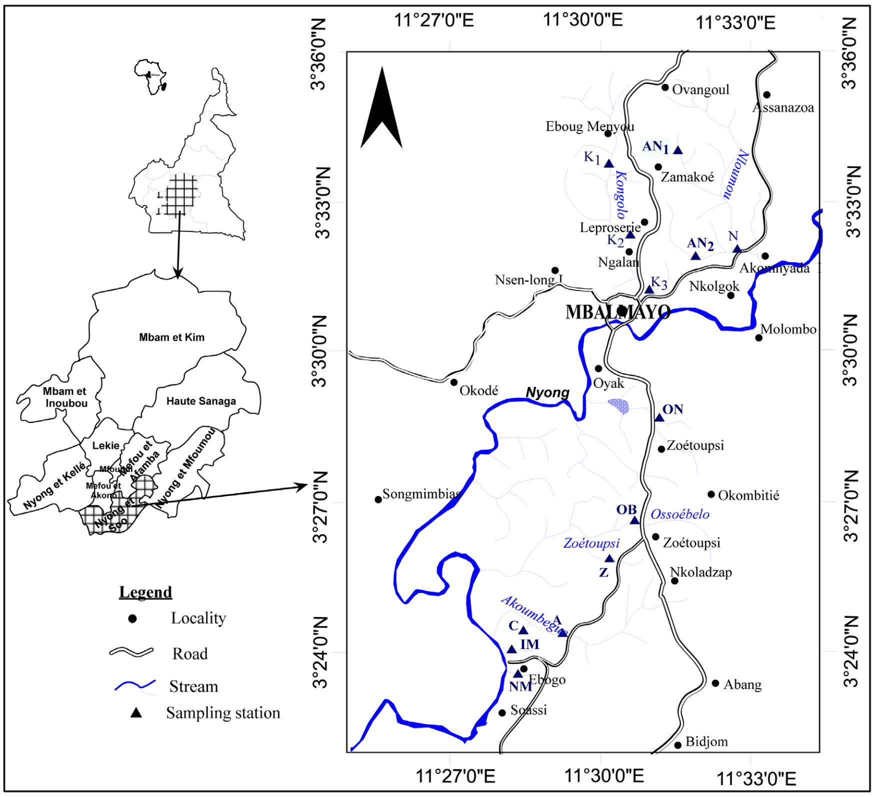


Figure 1. Study area showing streams with sampling stations.

2.2.1. Measurement of Environmental Variables Analysis

At each sampling date, meteorological parameters were measured in the field using a Testo® 610 thermo-hygrometer for air temperature and humidity, and a Testo® 540 lux meter for luminosity. The hydrological parameters such as width, depth and current velocity were measured at each station once every two months. The width of the water column was measured using a graduated string stretched horizontally from the end of one bank to the other. The depth was measured using a graduated stake. The current velocity of water was determined by measuring the distance that goes about one minute by methylene (neutral and non-toxic colorant). The physicochemical analyzes were performed both in the field and in the laboratory following standard protocols [21] [22]. Temperature, dissolved oxygen, pH and electrical conductivity were measured *in situ* using a Combo® Water Quality Meter 86,031 multimeter. For laboratory analyses, water samples were taken in each station using sterilized 1000 mL polyethylene bottles and transported to the laboratory in a refrigerated cooler. Turbidity, alkalinity, nitrates, ammonium and phosphates were measured in the laboratory using the HydroTest® HT 1000 spectrophotometer.

2.2.2. Sampling and Identification of Benthic Macroinvertebrates

The collection of benthic macroinvertebrates was made using a kick net (square-shaped stirrup of 30 cm side) equipped with a conical net of 500 µm mesh size and 50 cm depth, following the multihabitat approach [23] [24]. At each station, twenty landing net tows were made each approximately 50 cm in length, equivalent to a surface area of 3 m^2, in different habitats characterized by the substrate/velocity. The organisms retained in the net were collected on the field using a pair of fine forceps and fixed in 10% formalin for 24 hours. In the laboratory, the specimens were washed in tap water to remove formaldehyde, and then stored in 70˚ ethanol. The organisms were then introduced into Petri dishes and grouped according to their size and morphology, then identified at least to the rank of family, under a Bresser® Science ETD-101 binocular magnifying glass using keys and identification books [25]-[33].

2.2.3. Data Analysis

The Kruskal Wallis test for no pairing data and Friedman test for pairing data were used to compare the means of environmental and biological data between the stations on the one hand and between the months on the other hand. These analyzes are performed by GraphPad Prism 8 software. Analyzes of the benthic macroinvertebrate diversity were carried out using biocenotic indices such as abundance (N), taxonomic richness index (S), the Shannon and Weaver index (H'), the Pielou equitability index (J) and the rarefaction curves. Hierarchical ascending classification (HAC) based on euclidean distance and principal component analyzes (PCA) was performed to search the affinity links between biological variables and sampling stations and to make biotypology of stations using XL Stat 2007. Biocenotic indices were realized using the PAST® Software version 1.0.0.0 [34].

3. Results and Discussion

3.1. Results of Environmental Variables

Table 2 presents the annual data of the extrema, the mean and the standard deviation of the environmental variables at the level of each sampling station. On average, water velocity was higher at stations OB, ON and N with large temporal variations ($p < 0.001$) compared to stations K3, IM, C and Z. The deepest waters were observed at stations K3 and AN2. Station K3 was the sunniest with a luminosity reaching 70752.92 ± 38820.74 lux. The value of the standard deviation on the mean indicates a large variability in luminosity throughout the year at this station. The stations that received very little light were NM, AN1, K1 and C. Statistical tests ($\alpha = 5\%$) showed significant differences between incident light values ($p < 0.001$). The waters were well saturated with oxygen at all stations. Nevertheless, the waters of the K3 station presented values ranging from 25.4% to 91.9% and were statistically different from the values of the other stations ($p = 0.003$). Overall, the waters were slightly acidic with values fluctuating from 4.59 UC (station A) to 7.9 UC (station K9) and nutrients (nitrates, ammonium and phosphates) were low at all stations. Notwithstanding these low values, the ammonium contents differ from one station to another ($p = 0.030$). The turbidity of the waters of station Z fluctuated a lot with values ranging from 5.0 FNU to 6138

Table 2. Mean, standard deviation and ranges values of environmental parameters evaluated at each sampling station during the study period.

Variables		Sampling stations												
		K1	K2	K3	AN1	AN2	N	IM	NM	C	A	Z	OB	ON
Current velocity (m/s) [CV]	Mean	3.48	2.07	0.34	2.81	3.97	4.19	0.38	1.17	1.93	3.36	1.65	5.49	4.63
	±SD	8.13	4.59	0.44	6.45	9.14	9.72	0.54	2.45	4.87	8.32	3.45	14.03	11.67
	Ranges	0.056 - 20.08	0.081 - 11.44	0.0122 - 0.94	0.138 - 15.97	0.164 - 22.62	0.125 - 24.03	0.015 - 1.16	0.044 - 6.71	0.02 - 12.98	0.002 - 22.22	0.027 - 9.42	0.029 - 37.31	0.047 - 31.1
Water width (m) [WW]	Mean	3.88	2.96	7.52	2.68	2.03	3.56	7.13	2.80	2.89	2.93	2.11	1.40	3.34
	±SD	0.70	0.49	0.43	0.37	0.53	0.46	8.62	3.70	1.02	0.83	0.50	0.35	1.95
	Ranges	2.93 - 4.71	2.48 - 3.82	6.78 - 7.98	2.13 - 3.17	1.32 - 2.49	2.92 - 4.00	1.91 - 26.3	1.12 - 11.16	1.4 - 3.82	1.54 - 3.95	1.4 - 2.702	0.97 - 2.03	1.32 - 5.61
Water depth (m) [WD]	Mean	0.29	0.32	0.68	0.17	0.40	0.24	0.32	0.15	0.25	0.31	0.32	0.25	0.22
	±SD	0.06	0.13	0.30	0.05	0.09	0.06	0.16	0.09	0.11	0.09	0.12	0.08	0.06
	Ranges	0.2 - 0.35	0.064 - 0.4	0.131 - 0.96	0.09 - 0.239	0.24 - 0.5	0.16 - 0.33	0.009 - 0.44	0.019 - 0.29	0.014 - 0.36	0.19 - 0.42	0.094 - 0.48	0.11 - 0.362	0.12 - 0.306
Luminosity (lux) [L]	Mean	517.46	12541.23	70752.92	494.85	13750.85	4824.92	7259.08	358.85	1043.17	13958.92	4609.54	4615.54	9066.54
	±SD	123.79	2174.63	38820.74	81.18	4088.33	1976.16	2515.32	53.78	172.56	3543.55	1361.19	1019.50	1015.51
	Ranges	81.00 - 1577.00	3264.00 - 30900.00	8054.00 - 419700.00	219.00 - 1271.00	1108.00 - 53920.00	468.00 - 26810.00	908.00 - 36543.00	185.00 - 872.00	256.00 - 2167.00	987.00 - 45620.00	16.00 - 12832.00	1015.00 - 13722.00	5594.00 - 17879.00
Humidity (%) [H]	Mean	66.29	71.90	71.95	75.44	70.88	69.65	75.53	73.71	70.43	77.59	76.24	77.51	76.06
	±SD	2.94	2.77	2.62	2.22	3.53	2.84	2.69	1.56	2.89	2.55	1.49	1.68	3.12
	Ranges	47.10 - 78.30	57.10 - 87.60	46.70 - 79.50	63.90 - 86.30	41.10 - 83.00	41.60 - 77.80	63.40 - 93.30	64.00 - 83.40	55.60 - 89.20	68.00 - 98.20	66.50 - 85.20	64.90 - 87.10	53.20 - 93.40
Air temperature (˚C) [AT]	Mean	30.99	29.64	27.90	28.99	30.70	29.67	28.53	29.19	29.95	29.13	28.07	28.31	27.89
	±SD	1.07	0.77	0.78	0.66	1.21	0.99	0.40	0.39	0.86	0.57	0.55	0.71	1.19
	Ranges	27.20 - 38.70	25.20 - 35.00	25.10 - 35.20	25.20 - 33.00	26.30 - 39.40	26.20 - 38.50	25.10 - 30.40	27.00 - 31.40	25.70 - 34.60	24.50 - 31.00	24.10 - 32.00	24.30 - 33.80	23.80 - 37.20
Water Temperature (˚C) [WT]	Mean	24.04	24.98	24.01	23.62	24.28	23.62	24.78	23.62	23.39	23.08	23.02	24.72	23.58
	±SD	1.39	1.29	0.90	1.20	1.22	0.95	1.71	0.93	1.28	0.98	0.95	1.71	1.50
	Ranges	22.2 - 26.4	22.5 - 27.7	22.7 - 25.4	22 - 26.8	22.6 - 26.8	22.4 - 25.6	21.9 - 28.9	22.3 - 25.4	21.9 - 25.9	21.8 - 24.5	21.6 - 24.4	22.9 - 27.5	22.3 - 28

Continued

pH (UC) [pH]	Mean	5.92	6.03	6.52	6.04	5.92	6.35	5.53	5.92	5.46	5.42	5.19	5.78	5.91
	±SD	0.53	0.40	0.70	0.35	0.56	0.60	0.59	0.48	0.55	0.49	0.47	0.72	0.55
	Ranges	5.2 - 6.66	5.42 - 6.72	5.63 - 7.9	5.39 - 6.6	5.12 - 6.72	5.47 - 7.17	4.71 - 6.72	5.3 - 6.84	4.63 - 6.32	4.59 - 6.27	4.6 - 6.43	4.99 - 7.77	5.07 - 6.86
Electrical conductivity (uS/Cm) [EC]	Mean	22.03	26.65	62.65	23.12	21.18	20.25	17.28	20.91	19.33	22.28	22.96	22.16	26.70
	±SD	5.49	4.64	9.34	1.08	3.40	3.15	4.83	4.90	3.37	10.04	12.17	14.56	11.31
	Ranges	16.1 - 32.3	21.2 - 36.9	46.5 - 77.4	21.7 - 25.9	16.5 - 28.6	15.7 - 27.1	13.5 - 28.6	8.5 - 27.9	15.7 - 26.3	16.7 - 53.8	15.3 - 62.4	8.2 - 68.2	14.5 - 59.3
O_2 (%) [% DO]	Mean	80.34	74.39	54.57	77.27	73.64	80.16	71.61	81.49	65.35	76.16	57.58	74.85	59.07
	±SD	11.66	10.26	18.98	10.88	12.25	7.78	15.74	9.44	17.88	10.80	16.86	9.58	14.22
	Ranges	48.6 - 96.3	56.1 - 93	25.4 - 91.9	45.8 - 89	42 - 86.9	66.6 - 89.1	30.4 - 88.9	61.5 - 98.7	24.6 - 87.1	56 - 91.3	12.2 - 79.2	59.7 - 87.9	24 - 86.1
Turbidity (FNU) [Turb]	Mean	51.23	44.46	48.92	46.92	44.85	44.85	50.31	68.54	77.58	61.58	522.00	81.85	71.46
	±SD	40.09	38.44	35.55	36.85	36.79	34.41	44.09	55.79	59.74	58.56	1688.10	107.93	57.22
	Ranges	6 - 126	2 - 118	8 - 134	7 - 114	1 - 133	4 - 123	5.0 - 123	1.0 - 186	6.0 - 198	8.0 - 167	5.0 - 6138	7.0 - 406	7.0 - 160
Alkalinity (mg/L $CaCO_3^-$) [Alka]	Mean	55.62	47.69	56.62	53.00	54.00	49.62	48.00	66.54	67.83	65.17	139.62	64.77	58.77
	±SD	23.16	22.32	22.40	23.26	24.66	25.38	21.58	37.84	40.84	41.71	275.96	30.47	30.24
	Ranges	25 - 89	11 - 89.0	19 - 94	23 - 90	6.0 - 85.0	11.0 - 87.0	17 - 85	22 - 165	26 - 178	19 - 175	9.0 - 1048	22 - 121	11.0 - 105
Nitrates (mg/L NO_3^-) [Nitra]	Mean	1.01	1.59	2.08	1.78	1.63	1.96	1.52	1.45	1.65	1.42	1.71	1.86	1.77
	±SD	0.63	1.10	1.06	1.19	0.97	1.40	0.77	1.00	1.07	1.16	1.22	1.36	0.98
	Ranges	0.36 - 2.33	0.36 - 3.94	0.66 - 3.57	0.54 - 3.84	0.57 - 3.25	0.74 - 4.41	0.73 - 3.06	0.4 - 3.53	0.42 - 3.48	0.4 - 4.25	0.59 - 4.86	0.48 - 4.15	0.65 - 3.61
Ammonia (mg/L NH_4^+) [Amm]	Mean	0.17	0.26	0.23	0.20	0.18	0.22	0.19	0.23	0.33	0.31	0.39	0.32	0.38
	±SD	0.17	0.44	0.23	0.19	0.19	0.23	0.18	0.20	0.25	0.19	0.27	0.23	0.27
	Ranges	0.04 - 0.62	0.04 - 1.66	0.03 - 0.81	0.04 - 0.57	0.02 - 0.67	0.04 - 0.72	0.03 - 0.6	0.04 - 0.62	0.03 - 0.79	0.04 - 0.57	0.03 - 0.84	0.03 - 0.65	0.03 - 0.86
Phosphates (mg/L PO_4^-) [Phos]	Mean	0.67	0.64	0.91	0.58	0.58	0.48	0.57	0.82	0.81	0.73	0.84	0.93	0.93
	±SD	0.69	0.62	0.99	0.59	0.70	0.68	0.66	0.65	0.71	0.66	0.78	0.72	0.71
	Ranges	0.07 - 2.6	0.09 - 2.51	0.05 - 3.45	0.06 - 2.39	0.05 - 2.72	0.06 - 2.56	0.05 - 2.62	0.14 - 2.51	0.09 - 2.76	0.06 - 2.54	0.06 - 2.65	0.12 - 2.8	0.13 - 2.86

FNU with an average of 522.00 ± 1688.10 FNU. However, no significant difference was observed between the different stations.

3.2. Results of Benthic Macroinvertebrates

Table 3 shows the biological data recorded during this study. 13,690 individuals belonging to 4 phyla, 7 classes, 16 orders and 93 families were collected. The Arthropoda phylum dominated with 97.82% relative abundance followed by Molluscs (1.73%). Annelids and Platyhelminthes barely reached 0.5% relative abundance (see also the supplementary Figure S1). The class of insects dominated with (58.20%) relative abundance followed by the class of Crustaceans (38.35%). The other classes (Turbellarians, Achaetes, Oligochaetes and Gastropods) represent less than 1% of the relative abundance see also the supplementary Figure S2). Table 3 presents the abundances and richness of benthic macroinvertebrates per order. Decapods are the most abundant (38.35% of individuals) followed by Coleoptera (22.30%) and Heteroptera (12.40%) (see also the supplementary Figure S3). The order Diptera has 17 families, followed by Coleoptera (15 families), Trichoptera (12 families) and Heteroptera (11 families). Plecoptera, Blattoptera, Basommatophora, Triclada, Arhynchobdellida and Rhynchobdellida are represented by only one family. Although more abundant, Decapods (3 families) are less rich than Diptera and Trichoptera.

Table 3. Total abundance, percentage and number of families by order of benthic macroinvertebrates collected during the study period.

Orders	Abundances	Abundances (%)	Number of families
Triclada	1	0.01	1
Haplotaxida	47	0.34	3
Rhynchobdellida	48	0.35	1
Arhynchobdellida	2	0.02	1
Eulamelibrancha	340	2.48	2
Basommatophora	17	0.12	1
Mesogastropoda	17	0.12	4
Decapoda	5250	38.35	3
Coleoptera	3053	22.30	15
Diptera	755	5.52	17
Heteroptera	1697	12.40	11
Odonata	1147	8.38	10
Ephemeroptera	532	3.89	10
Plecoptera	235	1.71	1
Trichoptera	419	3.06	12
Blattoptera	130	0.95	1
Total	13,690	100	93

3.3. Spatiotemporal Variation of Abundances, Richness and Diversity of Benthic Macroinvertebrates

Figure 2 presents the spatial and temporal variation of abundances and taxonomic richness of benthic invertebrates. The stations presenting the high abundances were K1 (50 - 183 individuals), K3 (10 - 315 individuals), AN1 (44 - 285 individuals), AN2 (35 - 181 individuals), NM (24 - 213 individuals), A (41 - 223 individuals), and ON (33 - 129 individuals) with more than 1000 individuals collected. The lowest abundances were recorded at stations C (16 - 79 individuals), Z (4 - 79 individuals) and OB (8 - 112 individuals) during this study (Figure 2(a)). The least rich stations with 28 families were the sampling stations C (7 - 13 families) and Z (3 - 13 families) (Figure 2(c)). The abundance and richness are significantly different between sampling stations ($p < 0.0001$). Indeed, a high abundance of benthic invertebrates was observed in August (34 - 227 individuals), June (12 - 315 individuals) and September (17 - 261 individuals). Low abundances were observed in October (10 - 141 individuals) and March (12 - 133 individuals) (Figure 2(b)). Furthermore, On the other hand, the richest sampling dates were observed in February 2019 (8 - 19 families), March (3 - 16 families) and May (6 - 22 families) (Figure 2(d)). The abundance and taxonomic richness are significantly different between monthly sampling ($p < 0.0001$).

The Shannon & Weaver and Pielou indices showed that the benthic macroinvertebrates of K1 (1.10 - 2.44 bits/ind), AN1 (1.24 - 2.26 bits/ind), N (0.81 - 2.37 bits/ind), IM (1.38 - 2.46 bits/ind), C (1.22 - 2.35 bits/ind), Z (0.92 - 2.31 bits/ind),

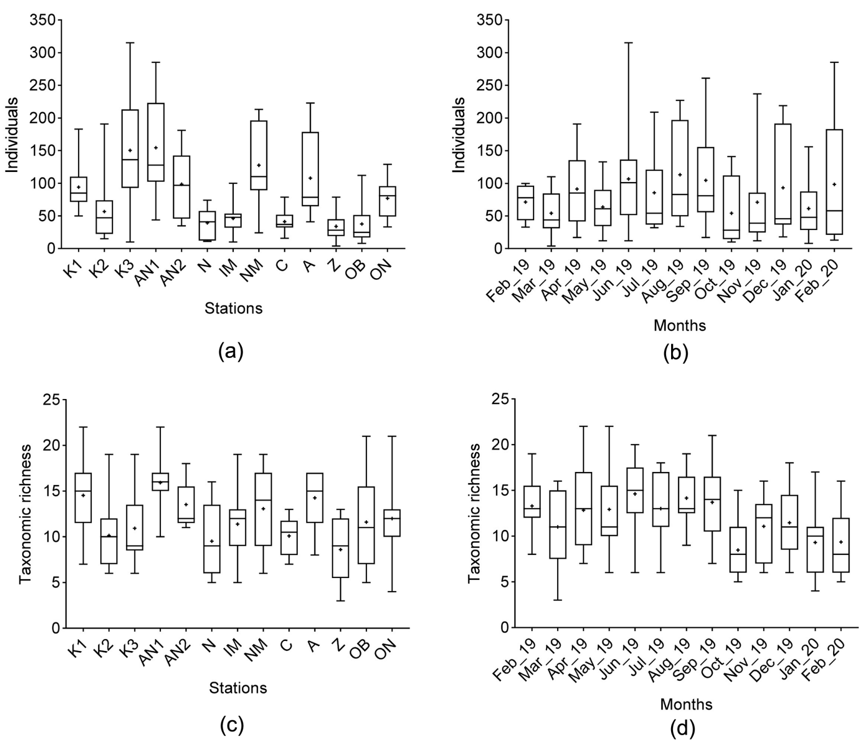


Figure 2. Spatial and temporal variation of abundance and taxonomic richness of benthic invertebrates during the study period.

OB (1.49 - 2.59 bits/ind) and ON (0.95 - 2.70 bits/ind) are more diversified (p = 0.0307) and evenly (J > 0.5) distributed among the taxa present (**Figure 3(a)** and **Figure 3(c)**). The month of September (1.87 ± 0.09 bits/ind) and May (1.86 ± 0.13 bits/ind) presented significantly diversified communities and were evenly distributed compared to the other sampling periods (p = 0.0064). Nevertheless, the mean of Shannon & Weaver's indices varied between 1.33 ± 0.14 bits/ind. At the sampling station NM and 2.00 ± 0.35 bits/ind. to the sampling station OB (**Figure 3(b)** and **Figure 3(d)**). Overall, the biological communities were weakly diversified compared to the theoretical diversity expressed by $\log_2$ (S) (6.54 bits/ind).

3.4. Rarefaction Curves on Benthic Macroinvertebrates

The rarefaction curves (**Figure 4**) present the spatial and temporal evolutions of the taxonomic richness compared to the total abundance. Spatially, **Figure 4(a)** distinguishes five groups of stations, the first of which is characterized by a community that is both less rich and less abundant (Z and C), the second by a community that is less diversified and relatively abundant (ON), the third by a diversified but less abundant (IM, N, K2 and OB), the fourth by a diversified and relatively abundant (AN2, K1 and A) and the last by a relatively diversified but very abundant (NM, AN1 and K3). The sampling effort seems low at all the stations, with no curve having reached the asymptote; that is to say, the theoretical

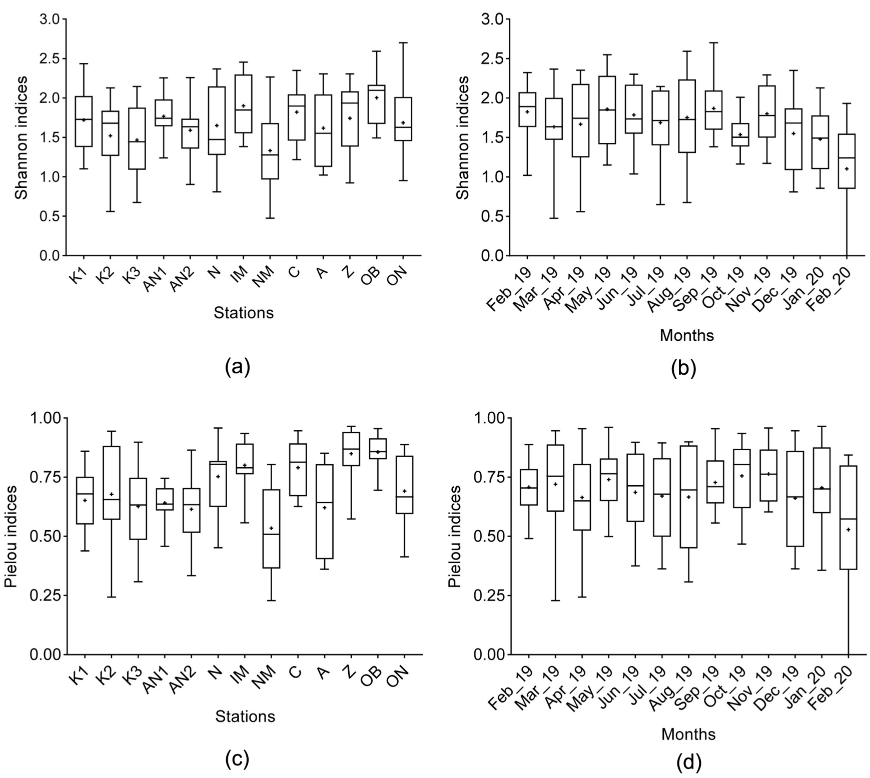
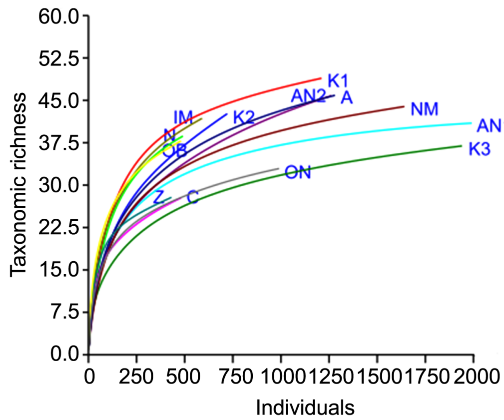
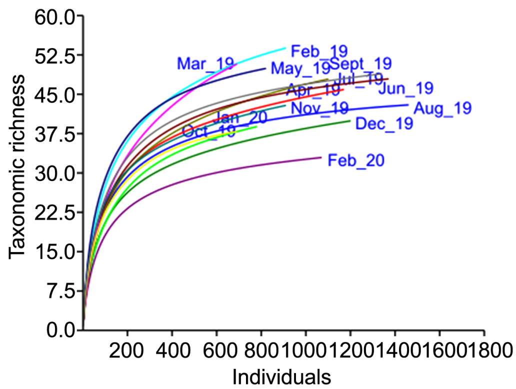


Figure 3. Spatial and temporal variation of Shannon & Weaver and Pielou indices of benthic invertebrates during the study period.



(a)



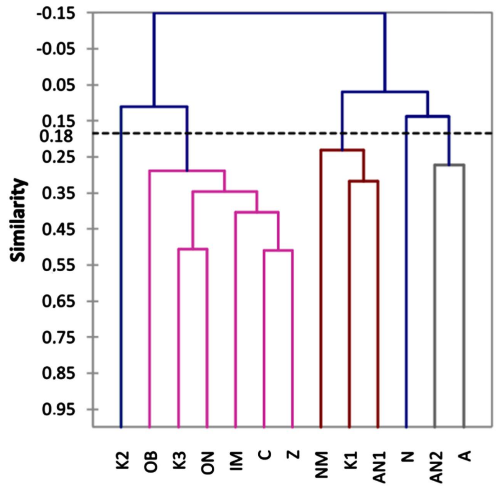
(b)

Figure 4. Spatial (a) and temporal (b) evolution of taxonomic richness in relation to the total abundance of benthic macroinvertebrates during the study period.

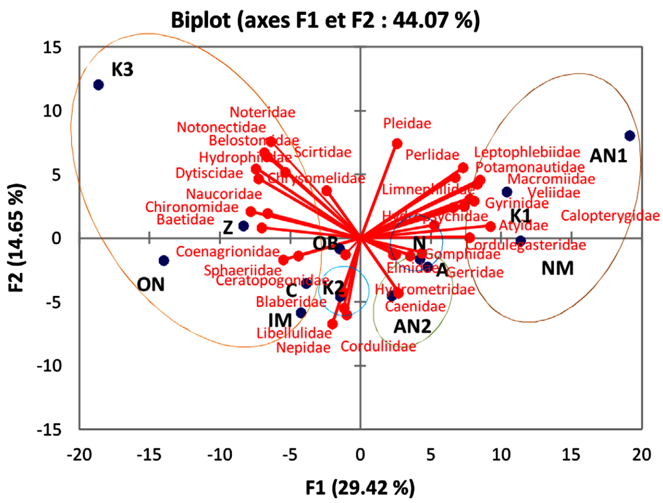
richness is not approached. However, the AN1 curve tends towards an asymptote indicating its tendency towards theoretical diversity (S = 41; Chao-1 = 42) (see also the supplementary **Table S1**). Monthly, **Figure 4(b)** distinguishes four groups of sampling periods. The first extending to the month of February 2020 is characterized by a less diversified and relatively abundant community, the second concerns the months of October 2019, November 2019 and January 2020 with a relatively diversified and less abundant community, and the third covers the months' March 2019, May 2019 and February 2019 with a diversified and less abundant community and the last comprising the months of April 2019, June 2019, July 2019, August 2019, September 2019 and December 2019 with a relatively diversified and very abundant community (see also the supplementary **Table S1**).

3.5. Biological Characteristics of the Sampling Stations

The ascending hierarchical classification distinguishes 5 groups (GI, GII, GIII, GIV and GV) of stations (S = 0.18) (**Figure 5(a)**). Group I includes stations K1, AN1 and NM where Atyidae, Potamonautidae, Perlidae, Leptophlebiidae, Hydroscaphidae, Calopterygidae, Macromiidae, Gyrinidae, Hydrophilidae and Dryopidae proliferated. Group II includes station K2, which is suitable for the



(a)



(b)

Figure 5. Dendrogram of hierarchical clustering (a) and biotypology of stations (b) based on the abundances of taxa.

Libellulidae and Coenagrionidae development. Group III includes stations K3, IM, C, Z, OB and ON where Dytiscidae, Noteridae, Scirtidae, Chrysomelidae, Naucoridae, Chironomidae and Sphaeriidae emerged. Group IV consists of AN2 and A stations where Caenidae, Gomphidae, Hydrometridae, Elmidae and Gerridae predominated. While group V which contains station N which is characterized by the Veliidae, the Mesoveliidae and the Corduliidae.

4. Discussion

4.1. Environmental Variables

The little or non-anthropic character of these forest streams and their intense canopy due to the presence of forests in most of the surveyed sites would be the cause of the high saturation of the water with dissolved oxygen. References [17] [35] [36] estimate that the waters of forest streams are rich in oxygen. Nevertheless, some low values of dissolved oxygen recorded at certain periods would be related to the high demand for oxygen in the process of decomposition of organic matter. Indeed, Reference [12] has noted these low oxygen values in a stream with organic matter. The high turbidity and alkalinity values during the long rainy season would be linked to water loaded with solid particles and sludge rich in calcium carbonate which can make the water turbid and colored [37]. The high ammonium value obtained at station K2 would reflect a process of incomplete degradation of organic matter. The same is true for phosphates, the high levels of which testify to the phosphate nature of the terrain crossed [21]. All of these overall high nutrient levels could be attributed to the decomposition of organic matter and in particular litter in streams [38] [39].

4.2. Diversity of Benthic Macroinvertebrates

The supremacy of the insect class (58.20% relative abundance) would be linked to the little or non-anthropic character of these forest watercourses. In addition, the habitats are diversified in these rivers. Indeed, References [16] and [40] noticed that insects dominate in forest waters. In addition, the high taxonomic richness of the class of insects (77 families) would be linked to the several microhabitats within streams seem to present and to testify the low degradation of the environment by anthropogenic activities. The very remarkable presence of Decapods in these environments can be explained by the high oxygenation of the waters and the presence of rocky and sandy substrates. References [41] and [35] claim that Decapods like unpolluted and highly oxygenated environments. The classes of Achaetes, Oligochaetes and Gastropods very weakly represented here would prefer rivers rich in organic matter because they were collected mainly in the stations where the signs of organic pollution are present. These same observations have been made in the anthropized watersheds of Mfoundi and Wouri in Cameroon on Achaeta and Gastropoda [10] [42].

4.3. Biological Characteristics of Sampling Stations

Benthic macroinvertebrates preferred certain habitats over others. Indeed, specimens were more abundant at stations with moderate water velocity. On the other hand, the abundances were low in slow-moving streams and with muddy or muddy substrates. The current velocity would therefore have a potential influence on the variability of the microhabitats capable of hosting a diversified community. The slow current would allow the deposition of fine particles and the homogenization of the bottom of the water. The authors [4] [16] [43] mention that benthic invertebrates have a preference for certain microhabitats over others and taxonomic diversity increases with microhabitat diversity. In the case of stations with diversified microhabitats, the community seems to be more diversified and in balance. The change in abundance according to the seasons would be linked to the increase in water flow and the contribution of matter by runoff water, thus leading to the modification of microhabitats and the community. This is the case of the results observed at the K2 and NM stations whose Shannon & Weaver diversity, Pielou equitability and Simpson dominance indices were the lowest. Indeed, [44] [45] stipulate that the modification of the banks as well as the bottom of the streams leads to the change in the structure of the community. In these works, the highest abundances and taxonomic richness were recorded during periods of the dry season or low rainfall. This would be linked to the stability of the environments presenting more varied habitats than during periods of flooding. The references [46] [47] believe that environmental stability is one of the conditions for the multiplication of benthic macroinvertebrates and the diversification of community. Additionally, [48] and [49] say that the distribution of benthic macroinvertebrates is conditioned by water depth, dissolved oxygen and water flow. This may be the reason why the diversity of benthic macroinvertebrates was higher in these streams such as the Mabounié river than in this present work [36]. It was observed that during periods of flooding, communities seemed to be even less diversified and less abundant. These low abundance and richness of the community would be the consequence of the increase in speed and water depth. Reference [50] go further by saying that the increase in water flow leads to a reduction in taxonomic richness. Both spatially and temporally, no community has reached maximum diversity due to the constant renewal of taxa. This would be related to the low sampling effort. Sampling efforts help maximize diversity in diversity studies. They therefore show a weakness in the sampling effort [51] [52] [53]. Sampling efforts should be scaled up to reach Chao estimators and recover all rare taxa. The principal component analyses, although explaining only 44.07% of the information, expose the affinities of taxa at certain stations. These environments would present important elements in the proliferation of taxa. For example, stations NM, AN1 and K3 seem to host a community whose taxonomic richness obtained is closer to the theoretical richness estimated by the Chao-1 index. These communities were less rich and more abundant than the community from stations K1, AN2 and A where the Chao estimator would predict a net increase in taxonomic diversity if the sampling effort is increased. Our observations corroborate to this effect those made by many authors [54] [55]. The curves of the sub-samples for the months of December, June and August increase rapidly to stabilize indicating a decrease in new taxa and a fairly constant presence of taxa in all the sampling stations. This stabilization would be linked to the stability of the environment during these periods. The authors [9] said that the population is stable during low water periods when the waters are calm. Additionally, [53] says that diversity is strongly related to the number of frequent species and their abundances. On the other hand, those of March and May were very rich with a constant renewal of taxa but less abundant. These temporal variations of the rarefaction curves could be conditioned by seasonal successions of the community structure. References [56] [57] [58] believe that abiotic gradients and other community assemblage rules also influence the evolution of stand structure.

5. Conclusions

A brief environmental analysis made it possible to make an abiotic characterization of the streams. Thus, the air brightness and temperature variables were low at most streams, but the dissolved oxygen was higher. The waters were generally shallow, very well-oxygenated, weakly mineralized with low levels of nitrogenous elements and slightly acidic. Using standard diversity descriptors and biotypology analysis, we identified rich and diversified benthic macroinvertebrates communities. These results indicate a dominance of crustaceans followed by insects. Of the 7 classes identified, insects were the most diverse. As a result, the rivers studied present favorable conditions for the development of insects and freshwater Atyidae. So, this study showed that:

1) Taxonomic diversity is higher in forest streams with diversified microhabitats, moderate current velocity, and therefore well-oxygenated waters.

2) The taxonomic richness obtained is much lower than the theoretical richness estimated from the abundance of the specimens collected.

3) The Caenidae, Heptagenidae and Leptophlebiidae (Ephemeroptera), (Trichoptera), Perlidae (Plecoptera) and the Macromiidae, Calopterygidae, Corduliidae and Gomphidae (Odonata); the Gerridae, Hydrophilidae, Gyrinidae, Dryopidae and Hydrometridae; the Nepidae, Veliidae and Mesoveliidae (Hemiptera) and; the Atyidae and Potamonautidae (Decapods) were mainly collected in very well-oxygenated waters with sandy substrates.

4) Chironomidae (Diptera), Noteridae, Scirtidae, Dytiscidae (Coleoptera), the Sphaeridae, Limnaeidae and Physidae (Molluscs); the Naucorides, Belostamatidae and Notonectidae (Hemiptera) prefer slow and turbid waters with organic matters.

Further studies on the taxonomy of these groups are essential to better understand the species ecology.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Supplementary Data

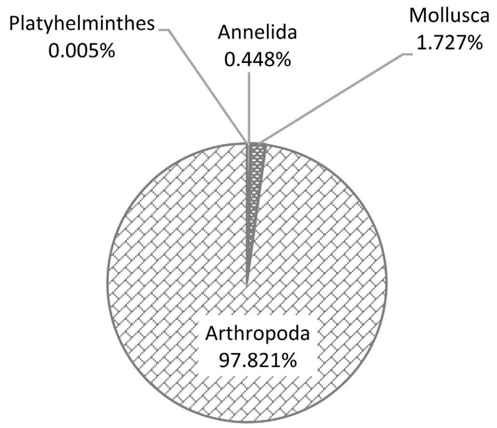

Figure S1. Relative abundances of benthic invertebrates by phyla.

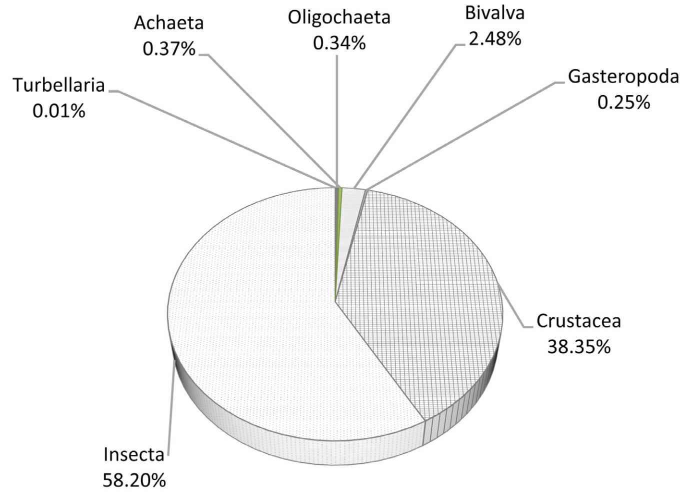

Figure S2. Relative abundances of benthic invertebrates by classes.

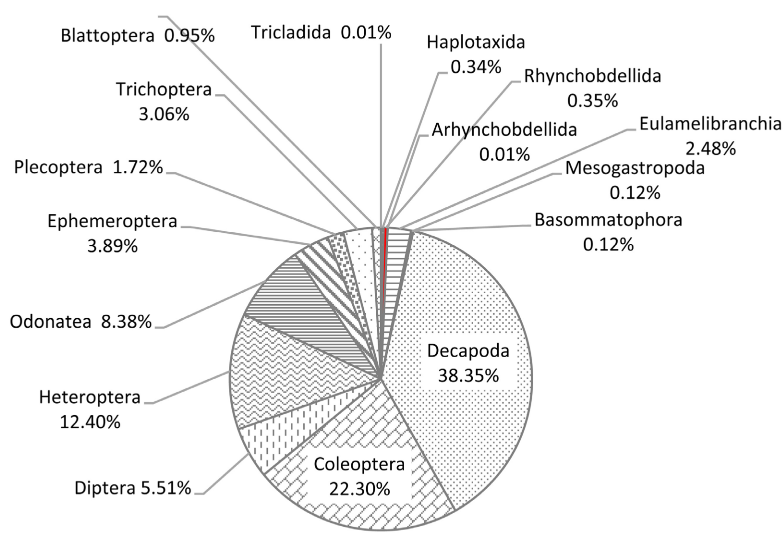

Figure S3. Relative abundances of benthic invertebrates by orders.

Table S1. Summary of spatial faunal abundance, richness and diversity of benthic invertebrates during the study period.

	K1	K2	K3	AN1	AN2	N	IM	NM	C	A	Z	OB	ON	Total
Individuals	1223	737	1956	2006	1278	509	601	1657	497	1294	441	491	1000	13,690
Taxa_S	49	43	37	41	45	39	42	44	28	46	28	38	33	93
Chao-1	58	77	44	42	56.14	43.5	55.75	56	37.33	55.17	49	50	36.5	120.1
Simpson_1-D	0.73	0.62	0.71	0.71	0.72	0.79	0.89	0.56	0.83	0.64	0.88	0.93	0.83	0.84
Log2 (S)	5.61	5.43	5.21	5.36	5.49	5.29	5.39	5.46	4.81	5.52	4.81	5.25	5.04	6.54
Shannon_H	2.29	1.86	1.92	2.05	2.07	2.28	2.76	1.69	2.37	1.93	2.58	2.96	2.32	2.78
Equitability_J	0.59	0.49	0.53	0.55	0.54	0.62	0.74	0.45	0.71	0.50	0.78	0.81	0.66	0.61

Table S2. Summary of temporal faunal abundance, richness and diversity of benthic invertebrates during the study period.

	Feb_19	Mar_19	Apr_19	May_19	Jun_19	Jul_19	Aug_19	Sept_19	Oct_19	Nov_19	Dec_19	Jan_20	Fev_20
Individuals	928	703	1183	830	1387	1111	1471	1359	702	925	1212	797	1082
Taxa_S	54	51	46	50	48	48	42	49	38	42	40	39	33
Chao-1	59	59.27	53.2	53.5	51	54.43	43.2	56	40	45.5	47	46	38
Simpson_1-D	0.88	0.73	0.80	0.91	0.87	0.74	0.79	0.88	0.85	0.90	0.78	0.85	0.68
Log2 (S)	5.75	5.67	5.52	5.64	5.58	5.58	5.39	5.61	5.25	5.39	5.32	5.29	5.04
Shannon_H	2.87	2.34	2.49	3.07	2.71	2.31	2.43	2.80	2.57	2.84	2.32	2.46	1.96
Equitability_J	0.72	0.59	0.65	0.78	0.70	0.60	0.65	0.72	0.71	0.76	0.63	0.67	0.56