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Ecological quality of Alibori river (In northern Benin) based on macroinvertebrates community by multivariate statistical approach

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Abstract
Disturbances in aquatic ecosystems are studied with several methods based on macroinvertebrates. This study aims to characterize the ecological status of the Alibori River through the use of macroinvertebrates using multivariate statistical analyzes. The data collection was conducted from June 2015 to May 2016. Measures of physico-chemical parameters were carried out *in situ* and the nutrients analysed by the ICS 1000 chromatographic method. Macroinvertebrate sampling was conducted monthly using an Eckman grab. Multivariate analyzes were used to illustrate the distribution patterns of organisms and to identify environmental factors determining the community distribution. The results show a clustering of taxa according to their susceptibility to pollution and upstream-downstream and seasonal distribution. Mineralization parameters were the main variables that governed the distribution of benthic macrofauna. The mineralization parameters were the main variables that governed the distribution of benthic macrofauna. The differences in structure and community organization indicated by multivariate analysis are related to the environmental conditions of stations appearing less stable downstream and highlighting a stress gradient on macroinvertebrates.

Keywords: Macroinvertebrates, fresh water, pollution gradient, multivariate analyzes

Introduction
Disturbances in aquatic ecosystems have deleterious effects on the macroinvertebrate community that can be revealed by studying community structure through diversity and similarity indices. The differences between community structures or species can then be the subject of classification and ordination analyzes to characterize the spatio-temporal variations of the macroinvertebrate community [1]. Generally more effective than univariate methods, multivariate methods are based on similarities between stations groups or species, and on the relation of these characteristics to environmental parameters [2, 3]. Concomitant use of several types of multivariate analyzes in water quality assessment with macroinvertebrates may provide a better understanding of structure of the data and relate it to environmental parameters [4, 5, 6]. These techniques also make it possible to assign objective values to some parameters that are difficult to quantify, such as the sensitivity of species to pollution [7].
Thus, multivariate analyzes indicate changes in macroinvertebrate assemblages related to environmental factors and predict changes that may occur due to anthropogenic pressures on aquatic ecosystems [8, 9, 10].
In Benin, chemical aggression of the environment is a serious threat to aquatic biodiversity, particularly in the sections of north Benin, where the most cotton is produced (up to 90% of national production). Cotton production requires the use of large quantities of pesticides and fertilizers [11]. Thus, these products pollute the aquatic ecosystems of the Benin cotton basin and affect their biological integrity [12]. The Alibori River is an ecosystem located in the Benin cotton basin where it is subjected to intense pressure of agricultural origin. This study aims to use multivariate analyzes based on macroinvertebrates of the Alibori River to highlight ecological variations, changes in macroinvertebrate assemblage patterns along the river and the condition of macroinvertebrate assemblages in response to human impacts.

Materials and Methods
Study area
The Alibori River is an ecosystem located in the Sudanian zone between 10° 30 ' and 12° north latitude and 1° 32' and 3° 50 'east longitude (Fig 1). The climate of this region corresponds to a dry semi-arid Sudanian zone with a rainy season and a dry season. The area is watered with a pluviometry which fluctuates between 700 mm and 1200 mm per year. The Alibori River (length 338 km) is the most important freshwater river crossing in the six largest cotton-producing (Malanville, Karimama, Banikoara, Kandi, Gogounou, Sinendé), with Banikoara being the greatest cotton-producing municipality at 40% of national production [13]. Therefore, the Alibori River collects the drainage from most of the agricultural areas in the cotton-producing basin [11]. The river originates in the central plateau of Benin and moves northwards into Niger River.

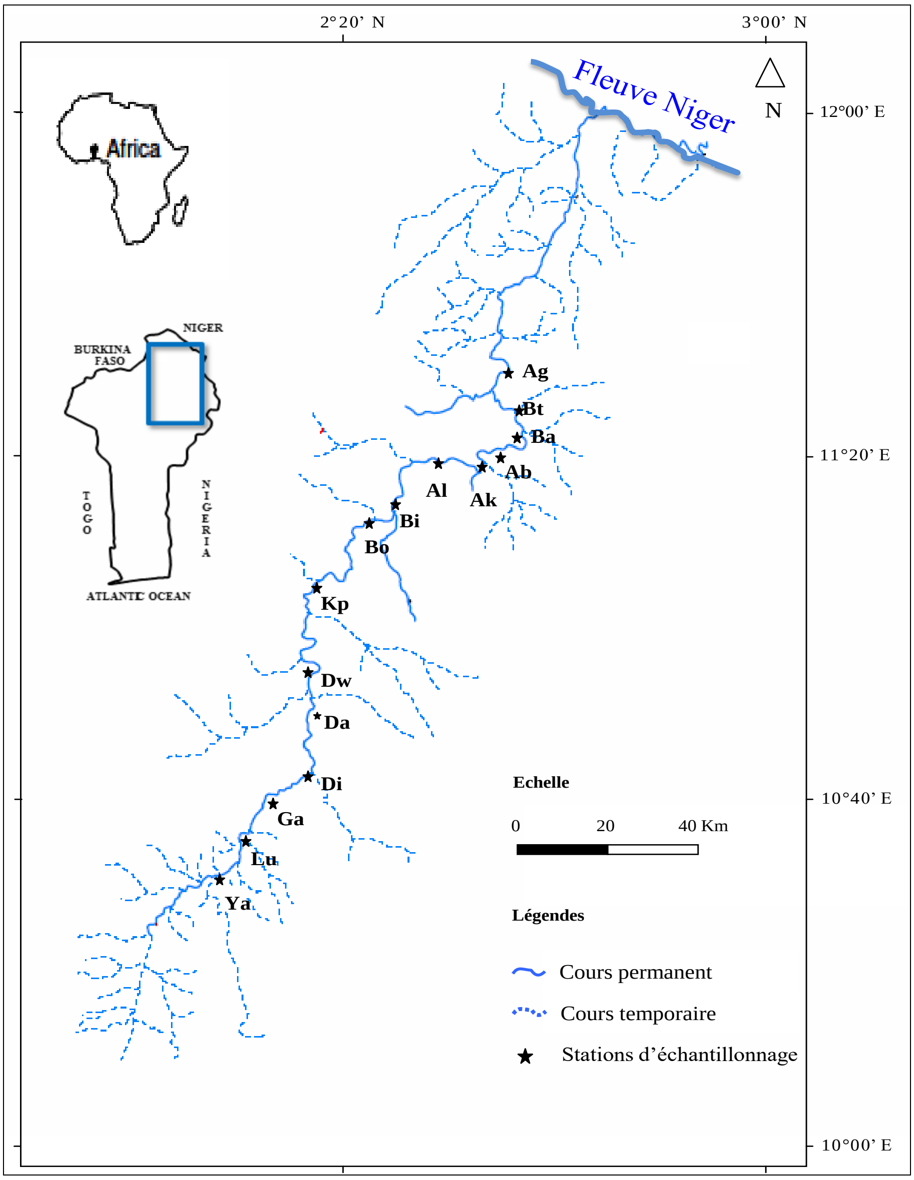

Fig 1: Map of Albori River Showing sampling sites

Collection of physicochemical parameters
Samples were collected monthly from the June, 2015 to, May 2016 at 15 different sites usually between 6:00 am and 10:00 moon. The water transparency and water depth were determined using a Secchi disk. The pH, conductivity, temperature, dissolved oxygen (DO) and Total Dissolved Solids (TDS) were measured using a multiprocessor conductometer. The surface velocity (Vs) of the water was measured using a float and a decametre. A stopwatch was used to measure the time taken by a float to travel a distance of 1 m measured with decametre. The current velocity (Vc) is obtained according to the relation: Vc = 0.80 × Vs [14]. It is expressed in cm / s. Water sampling was collected at each station for the determination of dissolved salts. At the laboratory, the nitrites, nitrates and total phosphorus concentration were measured using a SHIMADZU UV-1205 spectrophotometer.

Sampling of benthic macroinvertebrates
Benthic samples were collected from 15 sampling sites of the study area (Fig 1) using an Ekman grab sampler of 225 cm² at a depth of approximately 10 cm. For each sampling site, 10 hauls were made by sending the grab down into the bottom. Invertebrates were kept in 4% formaldehyde solution. Once in the laboratory, the invertebrate specimens from each site were sorted out into different groups and preserved in 70% alcohol. They were counted and identified under microscope using appropriate identification guides.

Statistical analyzes

Factorial Correspondence Analysis (CFA) was used to determine the distribution of macroinvertebrate communities along the Alibori River. The AFC was performed on the "ln-transformed" abundance table, whose rare taxa were excluded to avoid overestimating the weight of marginal contributors in the analysis. Before performing the factor analysis, the data were transformed into their natural logarithm to ensure a normalized and standardized distribution for a centered distribution. STATISTICA software version 4.5 was used.

The spatial variations of the benthic assemblages were determined using the artificial non supervised neuron networks, the "Self Organizing Maps (SOM)" or Kohonen maps [15, 16]. The SOM algorithm calculates the connection intensities between input and output layers by using an unsupervised competitive learning procedure [17]. The relevant groups or sample clusters which characterize the sampling sites assemblages were determined by performing a hierarchical classification analysis (Ward's linkage and the Euclidean distance method). The analysis was carried out with the SOM toolbox for Matlab (version 2).

The discriminant factor analysis (AFD) was carried out to identify the abiotic variables that discriminate the groups defined by the typology obtained with SOM. Then, the significance of the AFD was verified by a Monte-Carlo permutation test (999 permutations). In addition, the "leave-one-out" test was performed to estimate the ability of the model to predict the membership of each sample to each defined group [18]. The Kruskal-Wallis and Mann-Whitney tests were used to compare the taxonomic richness between the groups defined by SOM. STATISTICA software version 4.5 was used.

The multivariate technique redundancy analysis (RDA) was used to assess the correlations between abiotic parameters and main species (abundance > 5%) of the macrofauna of the River [19]. The analysis relevance was verified by a Monte-Carlo permutation test performed on 499 random permutations. This analysis was carried out with CANOCO 4.5 (CANOnical Community Ordination version 4.5).

The Kruskal-Wallis test was used to assess the overall variability of taxonomic richness of SOM groups. The Mann-Whitney test was used to compare the taxonomic richness between SOM groups.

Results

Affinity group of main taxa of macroinvertebrates

The analysis shows that first two axes explain 60.8% of the total variability of the information. At axis F1, there is a strong positive correlation *of Hydrochus elongatus*, *Clivina fossor*, *Haematopota* sp., *Critocopus* sp., *Chironomus* sp. (R^2 > 0.7). Two taxa of gastropod class, *Melanoides tuberculata* and *Lymnaea* sp., are well represented on the same axis with a high positive correlations ($R^2 > 0.9$) (Fig 2).

On the axis F2, taxa such as *Libellula pulchella* and *Lanistes variscus* showed a high positive correlation. The sensitivity of taxa to pollution has defined the taxa distribution along the river. Thus, two groups were distinguished according to the factorial design. The F1 axis grouped pollutant-resistant and opportunist taxa and the F2 axis was characterized by sensitive taxa.

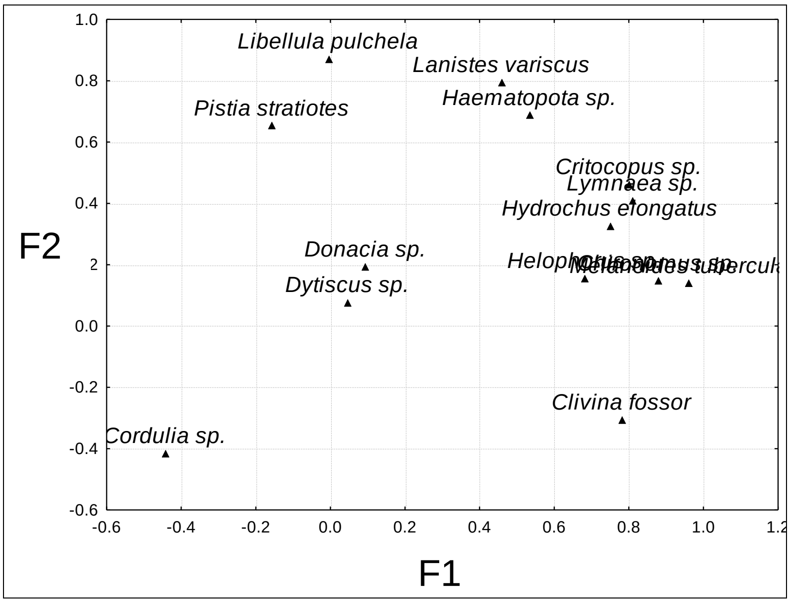

Fig 2: Distribution of main macroinvertebrates of Alibori River in map of factorial analysis.

Classification and distribution patterns of macroinvertebrates

The Kohonen's Self-Organizing Map (SOM) analysis carried out on the presence-absence of species in stations, allowed to classify samples according to the organism's distributions of and their occurrence probability. Samples are grouped in the SOM according to their faunistic similarities. Based on the quantification and topography errors, a 30-cell Kohonen map (5 rows × 6 columns) was chosen to project the samples (15 stations × 12 months) (Fig 3A). The result of hierarchical classification analysis (Fig.3B.) allowed grouping 30 cells of the Kohonen map into four groups (I to IV). Each group identified on the map (Fig 4) with the same pattern, was constituted of samples having similar taxonomic compositions.

The analysis revealed a zonal distribution upstream-downstream and a seasonal distribution. Groups I and II, poor in taxon, are isolated from groups III and IV, which are rich in taxa. Group I consists of 20 samples from only downstream stations, 19 of which are from the dry season. Group II consists of 54 samples (85.18% of downstream stations), 40 of which are from the rainy season. Group III comprised 32 samples from only upstream stations, 30 of which belong to the rainy season. Group IV comprised 74 samples (59.45% of upstream stations), 59 of which are from the dry season. The groups dominated by dry season samples, are poor in taxa and those characterized by rainy season samples are very rich in taxa. The season has influenced the diversity and composition of macroinvertebrate communities in the Alibori River.

Taxa number ranged from 12 taxa for group I to 17 for group II. Group III and Group IV contain 29 and 19 taxa respectively (Fig 5) Overall, the number of taxa differs from one group to another (Kruskal-Wallis, p <0.05). The richness of group I is significantly different from those of groups III and IV (Mann-Whitney, *p*<0.05). The taxa number of group II is different from that of groups III and IV (Mann-Whitney, p <0.05). Also, the richness of group III is significantly different from that of group IV (Mann-Whitney, *p*<0.05). Between groups I and II, the difference is not significant (Mann-Whitney, p > 0.05).

The discriminant factor analysis carried out on different groups of the SOM and the abiotic parameters made it possible to identify the factors which contribute to the discrimination of these groups. Monte Carlo permutation test (n = 999 permutations) shows that the groups are correctly predicted (*p*<0.05). The cross validation leave-one-out test revealed that the best prediction was obtained in group II (92.59%) while group I (85%) recorded the lowest prediction (Table 1). The AFD results indicate that group I is characterized by high nitrite values, total nitrogen and TDS. Group II is distinguished by parameters such as nitrates, phosphates and depth. Group III discriminates by the high values of water transparency, dissolved oxygen and current velocity. Finally, group IV is distinguished by pH.

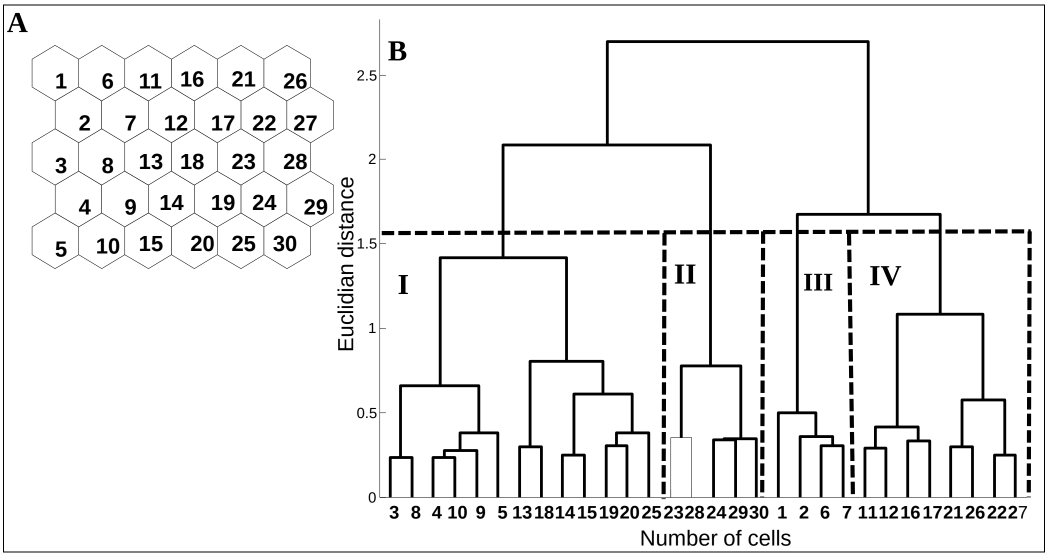

Fig 3: Kohonen map (A) and hierarchical classification of Kohonen map cells (B) based on taxonomic richness with Ward method and Euclidean distance as assembly distance.

The numbers (1 to 30) correspond to the cell numbers of the Kohonen map; Roman numerals (I to IV) represent the groups selected.

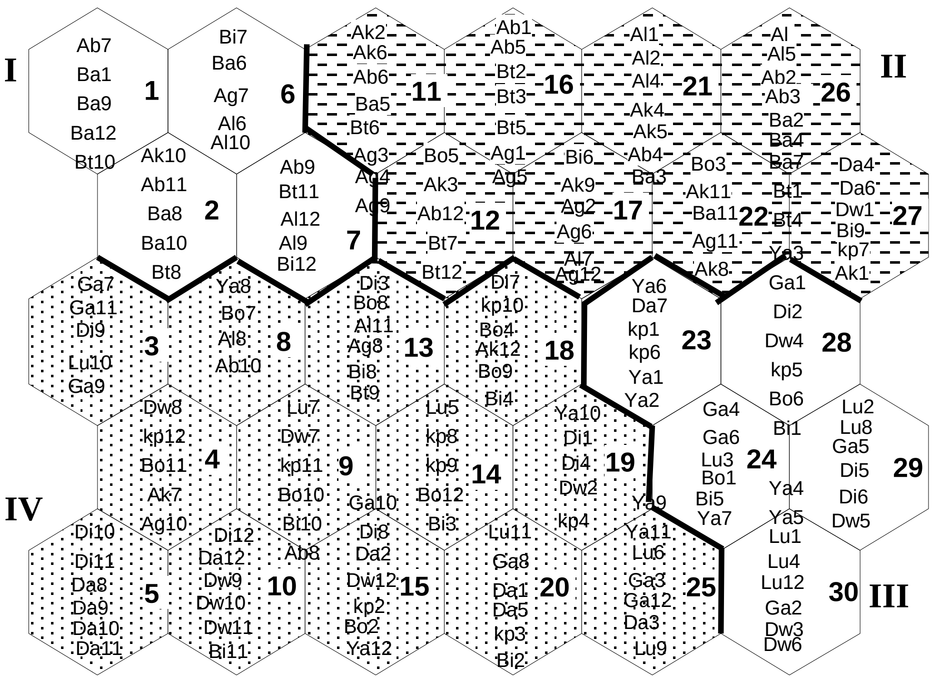

Fig 4: Distribution of Alibori River samples on the Kohonen map based on taxonomic composition.

The groups are numbered from I to IV; Ya, Lu, Ga. are the site codes and the numbers next to them (1 to 12) indicate the sampling months; the numbers from 1 to 30 in bold represent the numbers of the cells.

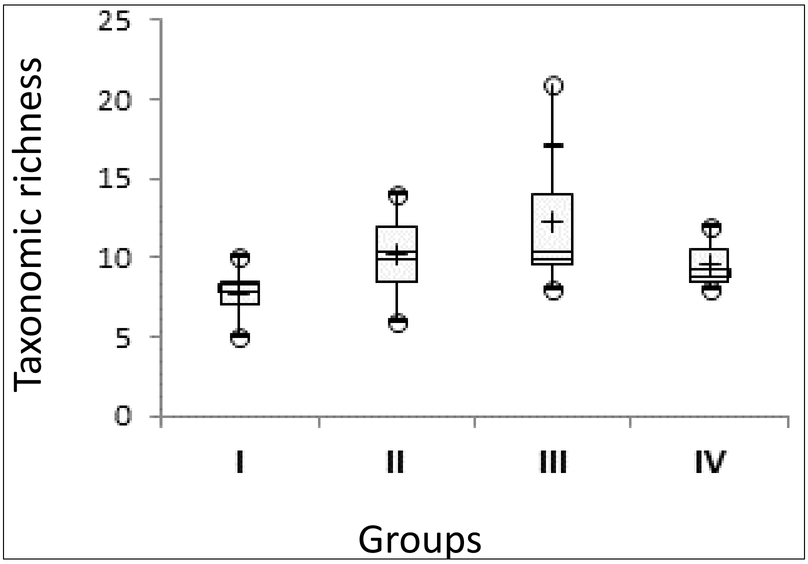

Fig 5: Taxonomic richness of the groups defined by the SOM at Alibori River.

Table 1: Results of AFD classification and cross-validation "leave-one-out" test. Numbers of well-predicted samples are in bold

Groups	Samples Number of predicted groups					
	Samples	**I**	**II**	**III**	**IV**	**% of prediction**
I	20	17	3	0	0	85
II	54	2	50	2	0	92,59
III	32	0	0	28	4	90,62
IV	74	1	0	4	68	91,89
Total	180	20	54	34	72	90,02

Influence of environmental variables on taxa distribution

The results of the redundancy analysis performed between environmental variables and macroinvertebrates taxa at the different sites are presented in Fig 6. The Monte-Carlo permutation test showed that the result of this analysis is significant (p = 0.004 and total variance = 1). Moreover, this analysis reveals that the first two axes expressed 89.2% of the information. Axis 1 is highly and positively correlated with dissolved oxygen concentration, transparency and current velocity. On the other hand, the depth, the conductivity, the TDS, the contents in phosphates, nitrites, nitrates and total nitrogen were negatively and highly correlated with the axis 1. These mineralization parameter (axis 1) influence the distribution of benthic macrofauna taxa.

Libellula pulchella and *Cordulia* sp. correlated with axis 1, are determined by dissolved oxygen and water transparency at upstream stations (Ya, Lu, Di, Da, Kp Dw, Bi) during the rainy season. *Chironomus* sp. and gastropod taxa such as *Melanoides tuberculata*, *Lanistes variscus*, *Lymnaea* sp. correlated with axis 1, are highly present at downstream stations (Ak, Al, Ab, Ba, Ag) during the rainy season. The group consisting of these taxa, are determined by depth and high levels of nitrates and phosphates. *Helophorus* sp., *Hydrochus elongatus*, *Donacia* sp., *Haematopota* sp. and *Critocopus* sp. which are highly correlated with TDS, conductivity, and nitrite and total nitrogen, are mainly found downstream during the dry season.

Axis 1 identifies indicator taxa of water status and expresses the evolution of water quality according to the presence of indicator taxa and environmental variables. The presence of Odonate taxa such as *Libellula pulchela*, *Cordulia* sp. and *Pistia stratiotes* associated with a high concentration of dissolved oxygen, a high water transparency and a low current velocity, indicate good water quality, especially during the dry season. *Chironomus* sp. (Chironomidae) and *Melanoides tuberculata*, *Lanistes variscus*, *Lymnaea* sp., (Gastropods) are indicators of water pollution by nitrates and phosphates in the rainy season. On the other hand, in the dry season, *Helophorus* sp. (Helophoridae beetles), *Hydrochus elongates* (Coleoptera Hydrochidae), *Donacia* sp. (Coleoptera Chrysomelidae), *Haematopota* sp. (Tabanidae), and *Critocopus* sp. (Chironomidae) indicate mineralized water and high suspended solids content.

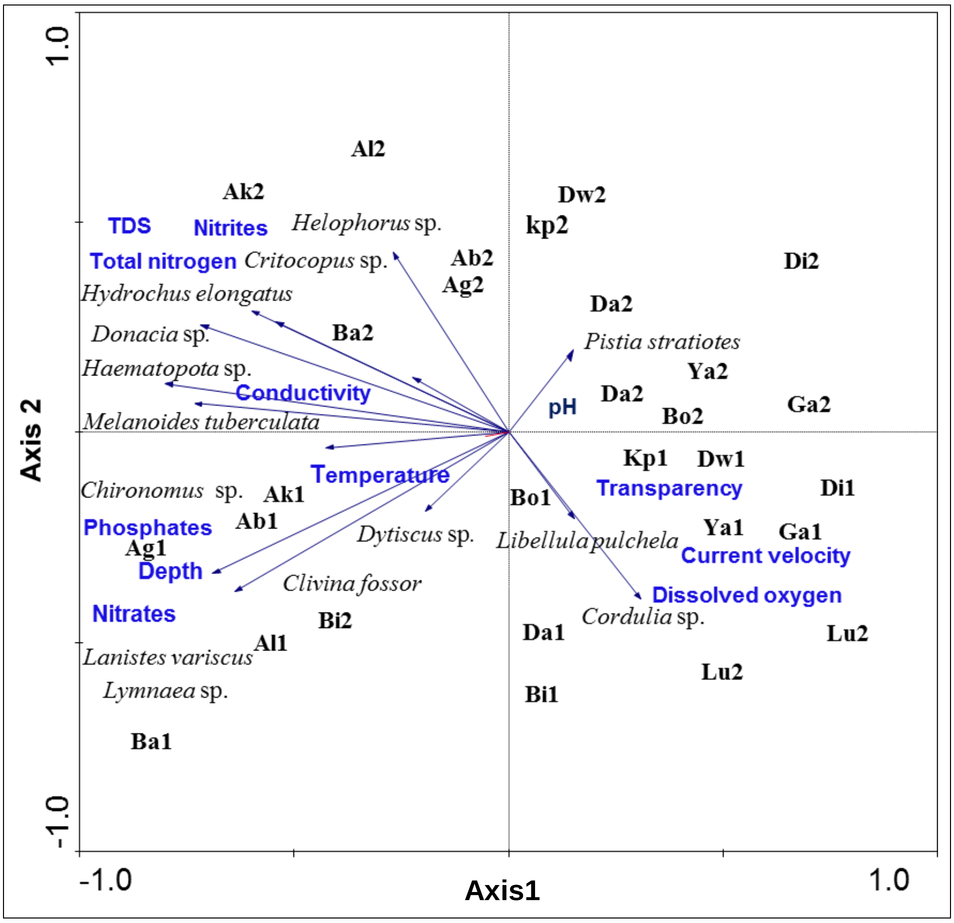

Fig 6: Redundancy analysis biplot showing the correlation between environmental variables and macroinvertebrates taxa at the different sites, 1= rainy season, 2 = dry season

Discussion

Spatio-temporal variation of composition

The composition of the benthic macrofauna of the Alibori River varies significantly depending on the season and the longitudinal profile of the river. The macroinvertebrate community is affected by the two seasons of the study area. The hydrological conditions of the rainy season influence the macroinvertebrates of the Alibori River and determine the temporal variation of the community [20]. This dynamic is related to rainfall and water volume, which during periods of flood accelerate the productivity of aquatic ecosystems and a dense vegetation cover of habitats [14, 21]. The flow and volume of water in the rainy season provide a great availability of food resources during this period, as opposed to the dry season [22]. Dry seasons with high temperatures cause mortality of taxa mostly insects, increase predation and create conditions of anoxia for some organisms [23]. Thus, rains favour the larvae recruitment of invertebrates, especially insects, and their development [24, 25]. The seasonal distribution of macrofauna in the Alibori River could be explained by the phenomenon of macroinvertebrates re-colonization of aquatic habitats in the rainy season, as this season offers new ideal conditions for these environments subject to natural and anthropogenic disturbances.

The spatial distribution of macroinvertebrates reveals an upstream-downstream zonal distribution and indicates that the different stations have been differentiated by a number of taxa that are characteristic of each station. This distribution highlights two large communities. The community of groups III and IV formed from samples of stations located upstream and remote from agricultural areas, presents a high richness taxonomic. This community consists of less polluted stations samples that display a diversified variety of macroinvertebrates, especially during the rainy season. Indeed, upstream is characterized by a good water renewal and good oxygenation [18, 2, 26]. In contrast, the community of groups I and II composed of samples from downstream stations of low diversity. These stations are the most disturbed and are located in areas of intense agricultural activities. The fauna of this community rich in pollution-tolerant taxa, justifies the AFC results which indicate taxa assembling according to their sensitivity to pollution. The decline in the diversity of this community is logically consistent with the discrimination of its samples by the mineralization parameters. On the other hand, the high transparency and the high concentration of dissolved oxygen are associated with a high richness upstream.

Pollution gradient

The structure of macroinvertebrate community in the Alibori River is influenced by environmental factors, the most important of which are the longitudinal gradient, water transparency, dissolved oxygen, and mineralization parameters. The results of the canonical redundancy analysis show that taxa such as *Helophorus* sp., *Hydrochus elongatus*, Donacia sp., *Haematopota* sp., and *Critocopus* sp. prefer mineralized stations downstream. *Chironomus* sp. and gastropod taxa (*Melanoides tuberculata*, *Lanistes variscus*, *Lymnaea* sp.) also showed a strong correlation with phosphates and nitrates in the same zone.

This group of taxa prefer organic matter and nutrients. The strong correlation between the mineralization parameters, Chironomidae, Chrysomelidae and Thiaridae indicates a proliferation of pollutant-resistant taxa at sites enriched with organic matter and nutrients. Indeed, agricultural land use pollutes water, disturbs benthic communities and contributes to the spatial distribution of species [27, 28]. These observations are identical with those obtained in similar studies, which showed that young larvae of Chironomidae and Chrysomelidae proliferate in nutrient-enriched environments [29]. The high mineralization of downstream waters is due to the drainage of agricultural soils rich in suspended matter [26, 30]. Also, the large correlation of dissolved salts, especially phosphates, with downstream stations during rainy season, reinforces hypothesis of drainage phenomenon. In fact, streams in agricultural areas are often affected by multiple stressors such as nutrient inputs, suspended solids, pesticides, and changes in hydrology and geomorphology [31, 28]. These results confirm those already obtained in this basin, which revealed that streams located downstream of Alibori River are disturbed by the cotton growing activities and are full of pollutant resistant taxa [26, 32, 33]. In contrast, Odonate taxa including *Libellula pulchella* and *Cordulia* sp. seem to prefer highly oxygenated and transparent waters. Also, according to axis1 of the GDR, these taxa favor the stations situated upstream of the river and indicate the good state of the habitats of the zone.

Conclusion

The spatio-temporal variations of the macroinvertebrate community are influenced by environmental factors of Alibori River. The structuration of river taxa is determined by longitudinal and seasonal gradients. The multivariate analyses indicate taxa assembling with a sensitivity affinity to pollution. The qualitative and quantitative structuring of community reveals the impact of human activities on the river. Analyzes indicate large disturbances along the river in areas of intense agricultural activity.

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