

Article

Freshwater Diatom Assemblage as Bioindicators of Land-Use Changes in Tropical Andean Streams

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Abstract

Water pollution can compromise the ecological integrity of freshwater habitats, thereby exposing freshwater communities to sources of pollution such as wastewater and agricultural and industrial discharges. Diatoms are key organisms because of their role as primary producers; thus, they have been regularly used as bioindicators to assess water quality. The diatom assemblages were collected from the surface of small stones in four rivers (El Carmen, Jipiro, San Simón, and Curitroje) across three zones defined according to a land-use gradient (high, medium, and low). A total of 54 diatom species were recorded. The El Carmen stream showed the highest total richness (29 species), followed by Curitroje (21), Jipiro (19), and San Simón (11). The results revealed significant changes in diatom richness, abundance, diversity indices, trophic index (IT) and community structure associated with both stream and zone. Following a similar pattern, temperature, conductivity, TDS (total dissolved solids), and pH also strongly influenced community composition. *Achnanthes subatomus*, *Sellaphora lanceolata*, *Odontidium mesodon* were indicators of the high zone and show a general preference for zones characterized by conserved riparian vegetation, fast, clear and oxygenated water. On the other hand, *Gomphonema reichardtii*, *Rhopalodia musculus*, *Gomphonema clavatum*, *Gomphonema subclavatum*, *Gomphonema variostriatum*, *Eunotia incisa* were indicators of a low zone with heavy organic pollution, water pollution, and environmental changes in temperature, conductivity, TDS and pH.

Keywords: community structure; diatoms; indicator species; stream; trophic index

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1. Introduction

Land-use changes have been strongly associated with the degradation of water quality in freshwater ecosystems worldwide [1–4]. For example, urbanization, industrialization, grazing and agricultural processes have negative impacts on water quality at all scales and in tropical and temperate zones [5–7]. These activities alter the hydrological cycle and increase pollutant inputs in water quality in streams [8,9], with adverse effects on ecological functions and diversity of several aquatic organisms [10–12], including diatoms, which often constitute a dominant group in benthic habitats [13].

Stream pollution can compromise the ecological integrity of freshwater habitats, thus freshwater communities in urban areas are increasingly exposed to sources of pollution such as wastewater and agricultural and industrial discharges [14,15]. Diatoms are key organisms because of their role as primary producers and their ability to transform inorganic compounds into organic forms usable by other organisms [16–19]. Thus, diatoms are key bioindicators of environmental changes related to water quality in freshwater habitats [20–23]. Diatoms offer several advantages over other aquatic organisms in the environmental biomonitoring of water quality, as they respond rapidly and sensitively to changes in physicochemical characteristics such as pH, temperature, dissolved oxygen, among others [24]. Their use in monitoring has been adopted by the European Water Framework Directive 2000/60/EC (WFD) [25].

Diversity metrics, including species richness, diversity, species composition, and trophic indices, have been widely applied in diatom studies across temperate and tropical regions worldwide [26–28]. Therefore, these metrics provide sensitive indicators of anthropogenic impacts and environmental degradation in freshwater ecosystems [22]. Previous studies have shown that diatom diversity and community composition change in rivers and streams exposed to high levels of disturbance in various cities. For instance, zones affected by agricultural, grazing, and urban activities tend to have low richness and diversity compared to undisturbed streams with low levels of water pollution [2,29–33]. However, few studies have addressed the use of diatoms as bioindicators of water quality in the Neotropical region [34,35].

In Ecuador most previous studies have focused on physicochemical parameters [36,37], aquatic macroinvertebrate communities [38–40], and aquatic bryophytes [41,42] as bioindicators of water pollution in rivers and streams. Although only a limited number of studies are available for this region, they have demonstrated that diatoms are effective bioindicators of water pollution [28,43–46]. Castillejo et al. [28] demonstrated that the trophic tolerance values of diatom species to nutrient enrichment (nitrogen and phosphorus) associated with eutrophication are not directly applicable to local sites. Following this pattern, diatom assemblages in the most degraded sites were markedly distinct from those in conserved areas, underscoring their sensitivity and value as bioindicators of ecological change [47]. Therefore, it is necessary to establish a diatom trophic index specifically for the Andean region of Ecuador.

However, no studies have examined their performance in South Andean streams. Therefore, this study was conducted in the city of Loja with the following objectives: (1) analyze the relationship of environmental factors with the diversity, trophic index and species composition of diatoms at rivers within zones (high, medium and low) with different land use in South Ecuador, and (2) establish a baseline of indicator species for the different zones as a tool for monitoring ecological water quality processes. We hypothesize that differences in water properties across zones will determine changes in diatom diversity and composition in streams of South Ecuador.

2. Materials and Methods

2.1. Study Area

The study was conducted in the canton of Loja, province of Loja, in southern Ecuador. Monitoring took place during the dry season, between March and May 2024, in an Andean territory characterized by irregular topography and an altitudinal gradient ranging from 120 to 3880 m a.s.l. The predominance of steep slopes (25–50% and 50–100%) directly influences hydrological dynamics, land use, and anthropogenic intervention processes. The study encompassed the El Carmen, Jipiro, San Simón, and Curitroje streams (Figure 1).

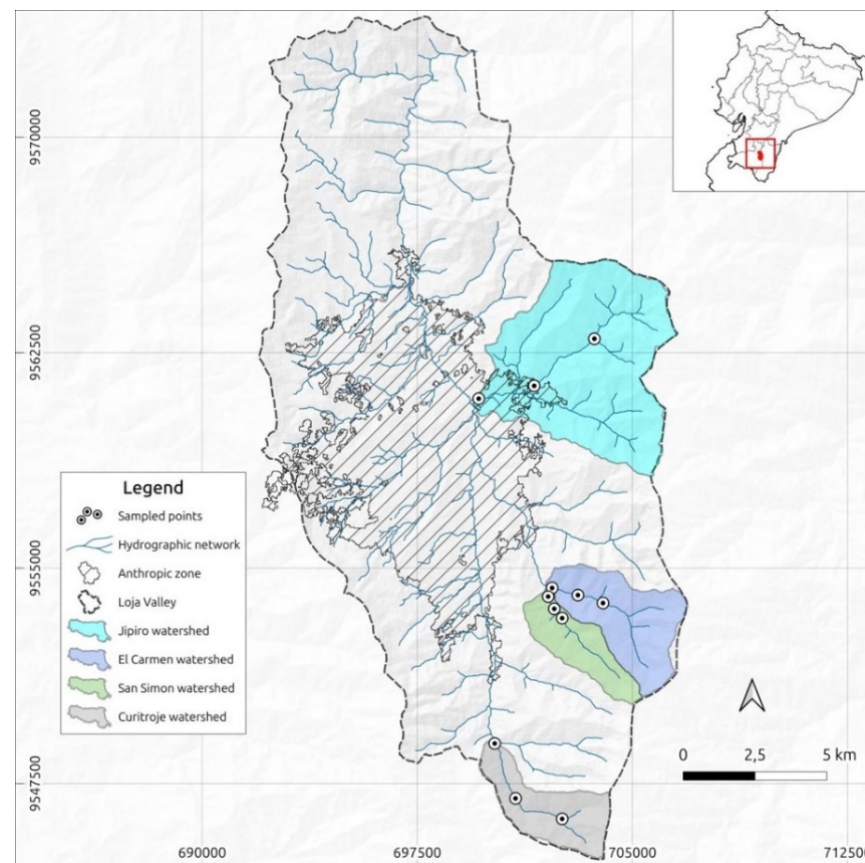


Figure 1. Location map of the Jipiro, El Carmen, San Simón, and Curitroje streams in the city of Loja, Ecuador. The red box on the right-hand map indicates the locations of the study streams in Ecuador.

The spatial analysis using Geographic Information Systems (GIS), together with land use and riparian vegetation cover along the extent of each stream, enabled the definition of three conservation gradients: high, middle, and low (Figure 2). The upper zone is characterized by hosting the main water intake points and by constituting priority areas for water conservation, with the presence of native vegetation cover in good conservation condition and minimal anthropogenic disturbance, particularly in the surroundings of the intake sources and along stream riparian zones. The middle zone comprises landscapes influenced by agricultural and livestock activities (mainly dairy cattle farming), where anthropogenic interventions have altered vegetation cover, particularly in riparian areas, which exhibit varying levels of disturbance due to productive land use and the presence of farming (including agroecological practices, corn cultivation, family farming the production of legumes, vegetables and fruits) and grazing activities. The lower zone is predominantly influenced by urban expansion and receives the cumulative impacts of the watershed, exhibiting alterations in environmental quality due to domestic wastewater

discharges. Additionally, agricultural and livestock activities are present, contributing to increased pressure on natural resources and to changes in environmental conditions.

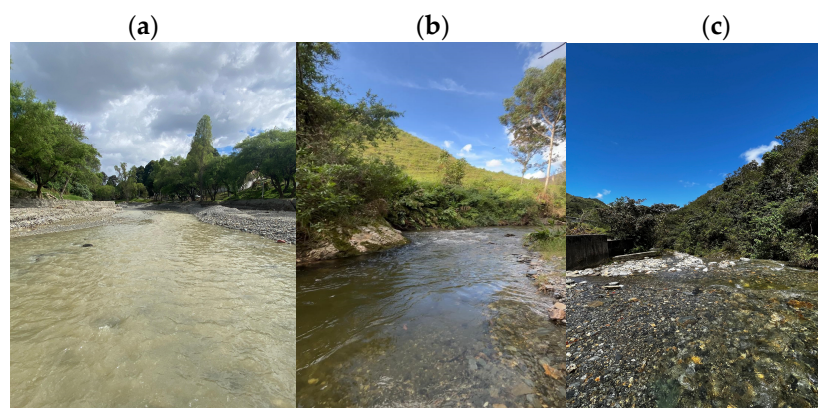


Figure 2. Riparian conservation status gradients: (a) lower zone; (b) middle zone; (c) upper zone.

2.2. Monitoring of Diatom Communities and Physicochemical Parameters

In each stream zone, 80 m transects were established, defining a monitoring point every 20 m, for a total of five replicates per zone [47]. For diatom sampling, five natural substrates were selected (small stones approximately 20 cm in diameter). Each substrate was washed and scraped with 20 mL of distilled water until the total volume reached 100 mL. The collected samples were labeled correctly, and 1 mL of Lugol's solution was added for preservation under cold-chain conditions [48]. Additionally, in situ physicochemical parameters were measured at each monitoring point using a Hanna HI9819 multiparameter probe, which was calibrated in the field using certified standard solutions, including NIST-traceable pH buffer solutions (pH 4.01, 7.00, and 10.01), potassium chloride (KCl) conductivity standards, and sodium sulfite (Na_2SO_3) solution for zero-oxygen calibration, complemented with air-saturation calibration for dissolved oxygen. The parameters recorded included dissolved oxygen (mg L^{-1}), temperature ($^{\circ}\text{C}$), electrical conductivity ($\mu\text{S cm}^{-1}$), total dissolved solids (TDS) (mg L^{-1}) and pH.

2.3. Identification of Diatom Taxa

The diatom community was identified at the Aquatic Ecology Laboratory of the Center for Research and Services in Chemical Analysis (CISAQ) at the National University of Loja. For the cleaning process, 2–3 mL of the bottom layer of each sample were transferred to 15 mL centrifuge tubes, where 5.5% sodium hypochlorite was added to remove residual organic material [49]. The digestion process was carefully monitored to ensure the removal of organic matter and preserve the integrity of the frustules and their structures.

Once the cleaned material was obtained, slides were prepared for microscopic observation. Photomicrographs were taken at 40 $\times$ and 100 $\times$ magnification (Axiolab 5 microscope, Carl Zeiss AG, Oberkochen, Germany), using TopView software (ToupTek Photonics Co., Ltd., Hangzhou, China) to support morphometric analysis and taxonomic verification. Individual counts were performed in 30 random microscopic fields using a Sedgwick–Rafter chamber, with a sampling intensity sufficient to identify between 90% and 95% of the species present. Final taxonomic determination was based on detailed examination of valve morphology and morphometric characteristics, following taxonomic keys [48,50–58].

2.4. Trophic Index

The Water Quality Trophic Index (TWQI) was calculated based on the trophic value of diatom species. The trophic value for each species was assigned according to the list proposed by Lobo et al. [56,57]. For taxa not included in the list, a default trophic value of one ($tv = 1$) was assigned. The Water Quality Trophic Index was then determined using the following formula (Equation (1)).

$$TWQI = \frac{\sum(vt_i \times h_i)}{\sum h_i} \quad (1)$$

where

TWQI: Water Quality Trophic Index;

vt_i: Trophic value assigned to the species;

h_i: Relative abundance of the species.

The *TWQI* values range from 1 to 4 in aquatic environments, according to the values shown in Table 1 [56].

Table 1. Trophic Water Quality Index values.

TWQI	Pollution Levels
1–1.5	Oligotrophic (negligible contamination)
1.5–2.5	β-mesotrophic (moderate contamination)
2.5–3.5	α-mesotrophic (high contamination)
3.5–4	Eutrophic (excessive contamination)

2.5. Data Analysis

We calculated species richness, abundance, diversity and trophic index in each stream. The Shannon–Weaver index is based on the assumption that individuals are randomly selected and that all species are represented in the sample, while the Simpson index is considered as a measure of species dominance whereas [59]. The effects of environmental variables, such as stream, zone, DO, conductivity, TDS, temperature, and pH, on richness, abundance, diversity, and trophic index were modeled using Generalized Linear Models (GLMs) [60]. The richness and abundance models for diatoms were fitted with Poisson errors, and the diversity indices and trophic index were fitted with Gamma errors. The minimal adequate model was selected based on Akaike’s Information Criterion (AIC).

We computed a non-metric multidimensional scaling (NMDS) ordination of the species cover matrix to reveal the degree of similarity among zones and streams. To detect the effects of environmental variables (stream, zone, DO, conductivity, TDS, temperature and pH), we performed a permutational multivariate analysis of variance (PERMANOVA) on the cover data [61]. We used the Bray–Curtis distance measure and 999 Monte Carlo permutations. NMDS and PERMANOVA were conducted using the R package ‘vegan’ [62]. Finally, to determine which diatom species were associated with a specific zone, we used indicator species analysis [63] implemented in the ‘labdsv’ package in R (R version 4.2.2) [64].

3. Results

A total of 54 diatom species were recorded. The El Carmen stream showed the highest total richness (29 species), followed by Curitroje (21), Jipiro (19), and San Simón (11). A similar pattern was observed with the Chao 2 richness estimator, confirming a high number of species estimated in the El Carmen stream (41), followed by Curitroje, Jipiro, and San Simón with 24, 19, and 11 estimated species, respectively.

3.1. El Carmen Stream

In the El Carmen stream, 14 diatom taxa were identified in the lower zone, with a total of 1362 individuals classified as abundant. The community was dominated by *Gomphonema subclavatum* (69.35%; 945 individuals) and *Gomphonema variostratum* (15.94%; 217 individuals), which represented the most characteristic taxa. In the middle zone, 18 diatom taxa were recorded, comprising 1988 abundant individuals. The assemblage was mainly composed of *G. pumilum* (28.33%; 563 individuals), *G. subclavatum* (21.02%; 418 individuals), and *G. parvulum* (18.66%; 371 individuals). In the upper zone, 13 taxa were observed, with *Hannaea arcus* being the predominant species, accounting for 60.59% of the total abundance, indicating a community structure associated with well-preserved environmental conditions.

3.2. Jipiro Stream

In Jipiro Stream, 24 diatom taxa were identified in the lower zone, where 1039 individuals were classified as abundant. The dominant species were *Gomphonema pumilum* (37%; 384 individuals) and *G. lagenula* (15%; 156 individuals). In the middle zone, 24 taxa and 1306 abundant individuals were recorded, with *G. pumilum* (48.65%; 635 individuals), *Mayamaea atomus* (10.18%; 133 individuals), and *Fragilaria rumpens* (8.98%; 117 individuals) being the most representative. The upper zone also exhibited 24 taxa, dominated by *Mayamaea atomus* (13.74%; 228 individuals), *G. pumilum* (20.92%; 347 individuals), and *G. minutum* (7.41%; 123 individuals), reflecting a gradual transition in community structure along the altitudinal gradient.

3.3. Curitroje Stream

In Curitroje Stream, the lower zone presented a richness of 10 diatom taxa, with 806 individuals classified as abundant. The dominant taxa were *G. parvulum* (35%; 282 individuals), *F. rumpens* (24.62%; 198 individuals), and *G. clavatum* (16.42%; 132 individuals). In the middle zone, 12 taxa and 639 abundant individuals were recorded, with *G. soprophilum* (14.10%; 90 individuals), *Navicula simulata* (13.35%; 85 individuals), and *Navicula erifuga* (12.97%; 83 individuals) as the most representative species. In the upper zone, eight taxa were identified, totaling 550 abundant individuals. *G. subclavatum* (18.88%; 104 individuals), *Geissleria punctifera* (18.40%; 101 individuals), and *G. minutum* (15.16%; 83 individuals) exhibited the highest relative abundances, suggesting assemblages adapted to low-disturbance conditions.

3.4. San Simón Stream

In the San Simón stream, five diatom taxa were identified in the lower zone, with *G. subclavatum* as the overwhelmingly dominant species, accounting for 84.73% of total abundance (1714 individuals). In the middle zone, nine taxa were recorded, with *G. subclavatum* again prevailing (66.76%; 1233 individuals). In the upper zone, ten taxa were identified, and *G. subclavatum* remained the most abundant species (80.33%; 1315 individuals). This pattern highlights *G. subclavatum*'s broad ecological tolerance across environmental gradients, demonstrating its capacity to persist under varying degrees of anthropogenic disturbance.

The violin plot indicated the highest richness, trophic index, abundance, and diversity in the Jipiro stream, followed by the El Carmen and Curitroje streams. In contrast, the San Simón stream exhibited the lowest values (Figure 3).

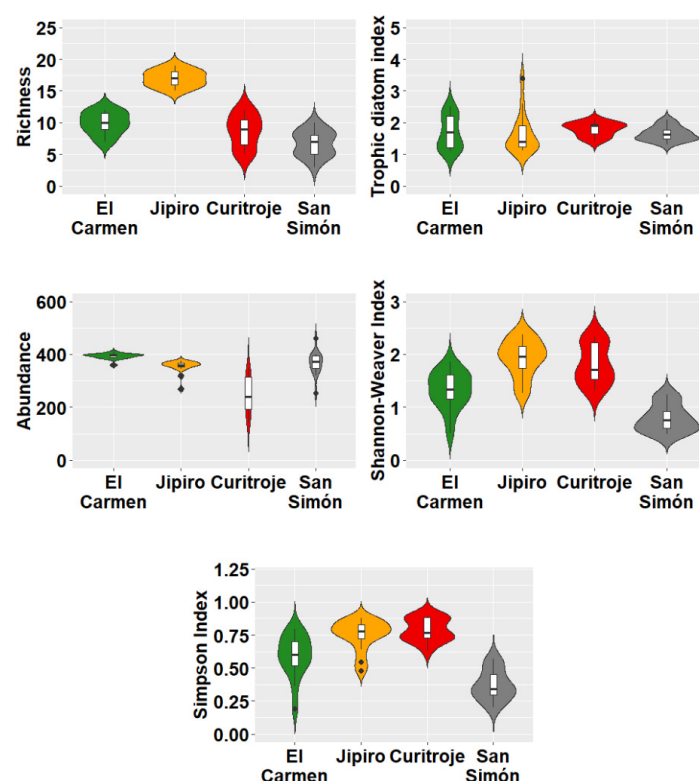


Figure 3. Violin plot of diatoms species richness, trophic index, abundance and diversity at different streams. Violin plots show the kernel density distribution of the data. The box indicates the interquartile range (IQR), the horizontal line within the box represents the median, and the whiskers extend to $1.5 \times \text{IQR}$.

The medium and high zone streams showed the highest total richness, with 43 and 40 species, respectively, followed by the low-zone stream with 35 species. A similar pattern was observed with the Chao 2 richness estimator, confirming a high number of species estimated in the medium zone (47), followed by the high and low zones streams (45 and 38, respectively). The violin plot indicated the highest richness, trophic index, abundance, and diversity in the high and medium streams, whereas the low stream exhibited the lowest values (Figure A1).

Generalized Linear Models showed that the most relevant predictors for diatom richness, trophic index, abundance, and diversity indices were stream and zone (Table 2). Also, conductivity, DO and pH have significant influence in richness and IT. On the other hand, temperature influenced the abundance and diversity indices of diatoms.

Table 2. Results of the Generalized Linear Model (GLM) of richness, trophic index, abundance and diversity indices (Shannon and Simpson) related with environmental variables as such as stream, zone, DO, conductivity, TDS, temperature and pH.

Factor	Df	Richness		Trophic Index		Abundance		Shannon Index		Simpson Index	
		Chi-Test	<i>p</i>	Chi-Test	<i>p</i>	Chi-Test	<i>p</i>	Chi-Test	<i>p</i>	Chi-Test	<i>p</i>
Conductivity	1	14.94	0.1605	0.2639	0.0135	1.829	0.1763	1.57	<0.001	0.6749	<0.001
DO	1	30.42	0.0452	0.1697	0.0477	15.381	<0.001	0.0319	0.3905	0.0305	0.3867
Location	2	389.3	<0.001	0.1449	0.1876	43.984	<0.001	1.164	<0.001	0.7879	<0.001
pH	1	54.4	0.0074	0.0978	0.1328	61.711	<0.001	0.5258	<0.001	0.5528	<0.001
TDS	1	19.64	0.1076	0.1299	0.0832	58.557	<0.001	0.0425	0.3213	0.0008	0.8898
Temperature	1	1.24	0.6866	0.0546	0.2615	285.489	<0.001	1.5273	<0.001	1.1187	<0.001
Streams	3	236.92	<0.001	0.7428	<0.001	203.137	<0.001	3.4644	<0.001	2.7514	<0.001

A total of 54 diatom species were recorded. The El Carmen stream showed the highest total richness (30 species), followed by the Curitroje (20 species), the Jipiro (19 species), and the San Simón (11 species). A similar pattern was observed with the Chao 2 richness estimator, confirming a high number of species estimated in the El Carmen stream (41), followed by Curitroje, Jipiro, and San Simón with 24, 19, and 11 estimated species, respectively.

3.5. Diatoms Community Structure

The NMDS analysis revealed strong clustering of samples based on diatom assemblage composition between streams (Figure 4A). However, the NMDS ordination showed only slight clustering of samples by stream zones (Figure 4B).

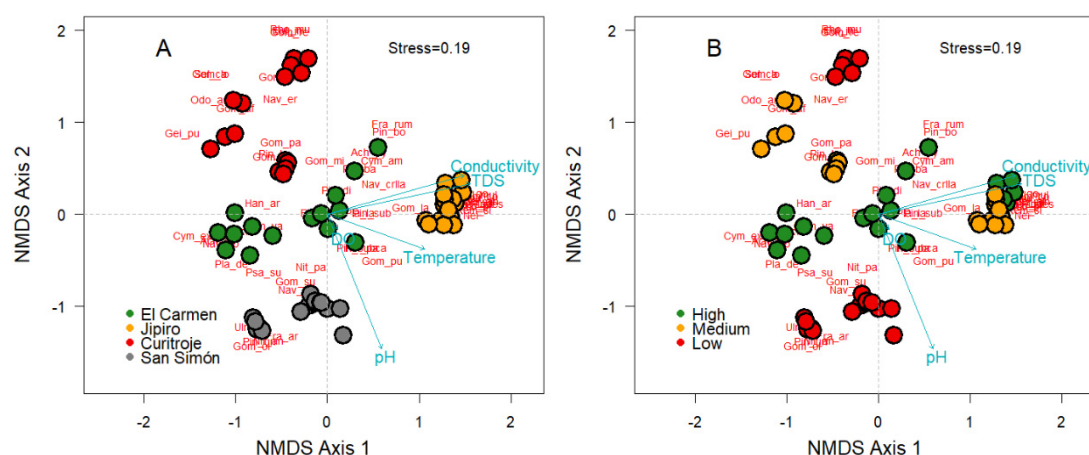


Figure 4. NMDS plot of epilithic diatom assemblages classified by stream (A) zones (B).

PERMANOVA showed that diatom communities varied significantly both within streams and zones, explaining 27% and 22% of the variation, respectively (Table 3). TDS (correlated with conductivity), pH and temperature explained 16%, 11% and 10% of the variance in diatom communities, respectively. We identified eight indicator species in the El Carmen stream, 18 in the Jipiro stream, 10 in the Curitroje stream, and seven in the San Simón stream (Figure A2). On the other hand, we registered six indicator species in the high stream zone, 12 in the middle stream zone, and seven in the lower stream zone (Table A1).

Table 3. Results of PERMANOVA analysis of species composition by environmental factors gradient. *p*-value < 0.05 are highlighted in bold and considered significant. Df = degrees of freedom; SS = sum of squares; F = statistical; R² = coefficient of variation.

Factor	Df	SS	R ²	F	<i>p</i> -Value
TDS	1	0.333	0.017	3.0122	0.006
Conductivity	1	0.344	0.01756	3.1116	0.014
Temperature	1	2.2974	0.11729	20.7785	0.001
pH	1	0.7101	0.03625	6.4223	0.001
Streams	3	8.7042	0.44437	26.2416	0.001
Zone	2	0.7699	0.03931	3.4817	0.001
DO	1	1.0114	0.05164	9.1479	0.001
Residual	49	5.4177	0.27658		
Total	59	19.5878	1		

4. Discussion

Our results revealed significant changes in diatom richness, abundance, diversity, trophic diatom index (IT) and community structure associated with both stream and zone. Temperature, DO, TDS, and pH also strongly influenced alpha and beta diversity. Similarly, sensitive diatom species (*Achnanthes subatomus*, *Sellaphora lanceolata*, *Odontidium mesodon*) were rarely observed in the lower stream zones, which were characterized by water pollution and environmental changes environmental factors (e.g., temperature, conductivity, TDS, and pH).

The richness, abundance, diversity, and IT were high in the conserved zones (high and medium zones) of the stream, which showed a well-preserved condition of riparian vegetation. Similarly, we documented greater diatom species diversity in the upper areas of river basins, attributed to the well-preserved condition of riparian vegetation in these zones with less urbanization [2]. The vegetation plays a key role in protecting water resources; for instance, it functions as a physicochemical barrier that traps contaminants and nutrients (such as nitrates and phosphates) originating from agricultural and livestock areas [65]. Conversely, in the lower zones of the stream, diatom richness, abundance, diversity, and the trophic diatom index (IT) declined, related to changes in riparian vegetation and changes in environmental factors. A study conducted in the Mónica River (Ecuador) reported that reductions in riparian vegetation were associated with declines in diatom diversity, likely driven by increasing anthropogenic activities, particularly in the middle and lower reaches influenced by agriculture, livestock, and nearby settlements [66].

Community structure was associated with changes in temperature, conductivity, TDS, and pH at the stream and zone levels. In accordance, previous studies showed that community composition of diatom species is closely linked to the presence of anthropogenic disturbances, exhibiting different tolerance ranges to environmental variables such as land use and the physicochemical characteristics of the water, including pH, temperature, and conductivity [67,68]. Streams lacking forest cover in their catchment areas and subjected to intensive livestock grazing negatively affect the integrity of aquatic ecosystems [40]. Following a similar pattern, Castillejo et al. [33] showed that diatom assemblages were clearly related to environmental variables and agricultural land use.

In the same line, temperature, TDS, and pH showed significant effects on diatom species composition. Previous studies have reported that temperature reflects variations in thermal regimes, while TDS is closely associated with salinity gradients and nutrient availability [69]. pH is a key environmental driver that influences both the physiological performance of diatoms and the chemical characteristics of aquatic ecosystems, thereby shaping species distribution and community composition [70]. In addition, conductivity is generally correlated with dissolved nutrient concentrations in streams and is widely used as a conservative proxy for nutrient enrichment in freshwater systems [71]. Regarding dissolved oxygen (DO), several studies have demonstrated its relevance in structuring diatom assemblages, as it reflects organic pollution and ecosystem metabolic conditions [31,69].

In the high zones of streams, the species *Achnanthes subatomus* and *Sellaphora lanceolata* are indicators of environments unaffected by anthropogenic activities with well-preserved riparian vegetation (in the Carmen and Jipiro streams, respectively). According to Tan et al. [72], nutrient-sensitive species such as *Achnanthes subatomus* show a strong preference for clear, unpolluted water. Similarly, most species of the genus *Navicula* are typically associated with oligotrophic conditions and are considered sensitive to pollution [73]. In our study, *Navicula cryptotenelloides* was recorded in the upper zones of the stream [73]. In contrast, *Navicula cryptotenella*, which can tolerate moderate pollution and eutrophic conditions, was found in the middle zone. Finally, previous studies have reported that other sensitive diatom species (e.g., *Hannea arcus*) are typically associated with

oligotrophic environments characterized by low concentrations of nitrogen and phosphorus, and are therefore considered reliable bioindicators of good water quality [46,74]. This finding supports the role of well-preserved riparian vegetation in maintaining the ecological quality of freshwater systems.

In contrast, in the lower zones of the stream, species of the genus *Gomphonema* (*Gomphonema variostriatum*, *Gomphonema subclavatum*, *Gomphonema clavatum*, *Gomphonema reichardtii*) were dominant and identified as bioindicator species associated with anthropogenic disturbances. These species are generally recognized as indicators of poor water quality; however, they are also found in clean streams, which may be related to their tolerance to nutrient enrichment [75]. For instance, Ivorra et al. [76] showed that *Gomphonema parvulum* is a common species in both clean and metal-contaminated rivers. Thus, Salomoni et al. [77] classified diatom species into three groups (species more tolerant to strong organic pollution and eutrophication, tolerant and widely distributed species, and species less tolerant to pollution), noting that species from similar genera may be included in different groups [74]. This pattern may occur because diatoms respond rapidly to short-term hydrological changes compared with other aquatic organisms. However, diatom community composition may also exhibit a delayed ecological response when recent land-use disturbances affect water quality [46].

Finally, the absence of measurements of major ions (cations and anions), key nutrients (e.g., nitrate and phosphate), and metals which have been identified as important limiting factors for diatom assemblages in freshwater ecosystems [69,78–80] may constrain a more comprehensive interpretation of the environmental drivers of diatom communities. Therefore, future research is necessary to evaluate the response capacity of diatom communities to environmental changes over time. Although Ecuador currently lacks fully implemented diatom-based regulatory frameworks, in several European countries, diatom-based indices are already incorporated into official water quality monitoring programs under the Water Framework Directive, demonstrating their applicability for routine ecological assessment [81,82]. In this context, our study provides baseline evidence from tropical Andean streams that supports the feasibility of adopting similar diatom-based approaches in Ecuador, contributing to the development of future biomonitoring frameworks for river conservation and restoration.

5. Conclusions

The results of this study indicate that both alpha and beta diversity of diatom communities respond to land-use changes driven by anthropogenic activities at stream and zone levels. These changes were associated with variation in key physicochemical parameters, including temperature (13.7–16.9 °C), dissolved oxygen (8.2–9.5 mg L⁻¹), TDS (4–16 mg L⁻¹), conductivity (7–32 µS cm⁻¹) and pH (5.1–8.2), which influenced diatom diversity and community composition. In fact, the identification of sensitive indicator species (*Achnanthes subatomus*, *Navicula cryptotenelloides* and *Odontidium mesodon*) highlights the importance of considering the influence of land use on aquatic diversity. Therefore, there is a clear need to establish diatom-based protocols and indices for water-quality assessment, aimed at developing strategies to protect riparian zones. Thus, the results of this study highlight the usefulness of diatom assemblages as sensitive indicators of anthropogenic disturbance. The observed relationships with conductivity, temperature, pH, DO and TDS variables suggest that diatom-based metrics can be effectively applied for monitoring water quality and supporting management actions aimed at reducing land-use impacts and improving stream ecological condition.

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Data Availability Statement: Data included in this study will be available from the Dryad Digital Repository upon acceptance.

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Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

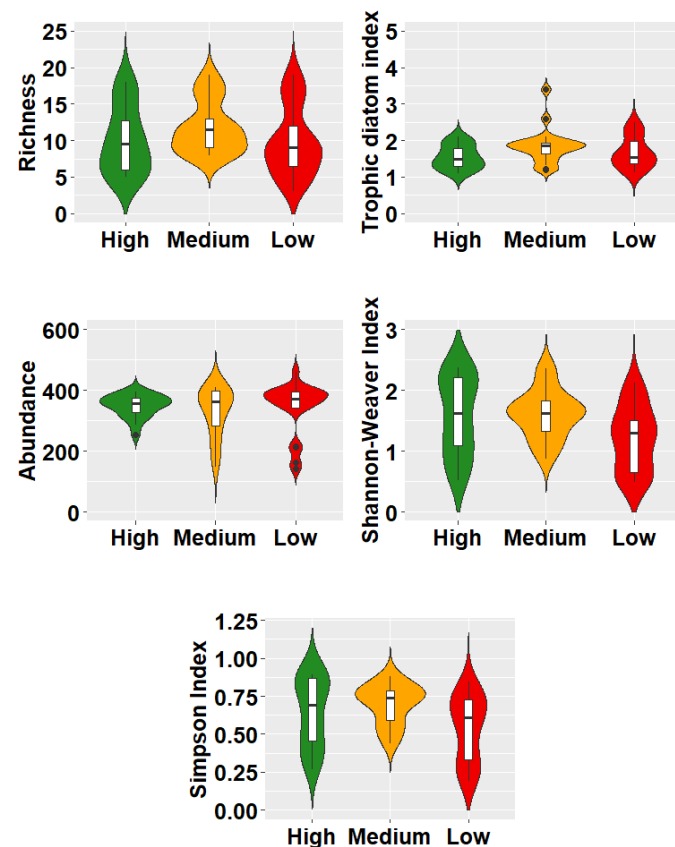


Figure A1. Violin plot of diatoms species richness, trophic index, abundance and diversity at different zones. Violin plots show the kernel density distribution of the data. The box indicates the interquartile range (IQR), the horizontal line within the box represents the median, and the whiskers extend to $1.5 \times \text{IQR}$.

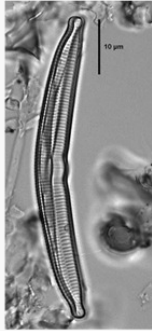
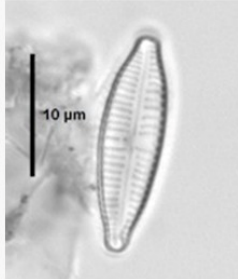
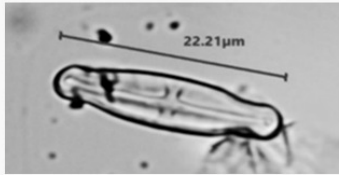
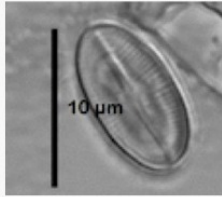
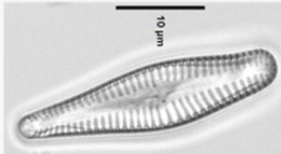
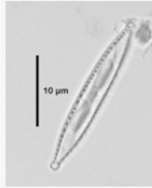
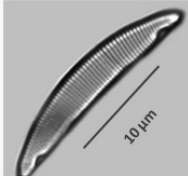
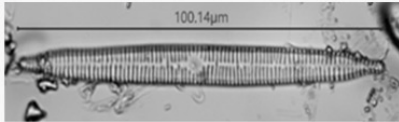
El Carmen stream	
	
<i>Hannea arcus</i>	<i>Gomphonema parvulum</i>
Jipiro stream	
	
<i>Sellaphora lanceolata</i>	<i>Psammothidium subatomoides</i>
San Simón stream	
	
<i>Gomphonema subclavatum</i>	<i>Nitzschia angustata</i>
Curitroje stream	
	
<i>Eunotia incisa</i>	<i>Ulnaria ulna</i>

Figure A2. Indicator species of diatoms in streams.

Appendix B

Table A1. Indicator values (IV) for diatom species. *p*-values < 0.05 are highlighted in bold and considered significant. Species with high indicator value are considered as the best indicators.

Species	Streams	Indicator Value	<i>p</i> -Value	Zone	Indicator Value	<i>p</i> -Value
<i>Achnanthidium pyrenaicum</i>	El Carmen	13.33	0.0638	Medium	10	0.1053
<i>Achnanthidium subatomus</i>	El Carmen	33.33	0.0006	High	25	0.0033
<i>Adlafia multomahii</i>	Jipiro	93.33	0.0001	High	19.01	0.0852
<i>Cymboplectra amphicephala</i>	El Carmen	13.33	0.0616	Medium	10	0.1054
<i>Cocconeis placentula</i>	Jipiro	100	0.0001	Low	14.87	0.1727
<i>Cymbella excisa</i>	El Carmen	6.667	0.2466	Medium	6.209	0.6218
<i>Fragilaria rumpens</i>	Jipiro	59.83	0.0001	Low	20.43	0.0979
<i>Encyonema minutum</i>	Jipiro	66.67	0.0001	High	6.061	0.4342
<i>Eunotia incisa</i>	Curitroje	26.67	0.0028	Low	20	0.0103
<i>Frustulia amphipleuroides</i>	El Carmen	6.667	0.2527	Low	5	0.3379
<i>Fragilaria arcus</i>	San Simón	53.33	0.0001	Medium	10.43	0.1496

<i>Geissleria punctifera</i>	Curitroje	6.667	0.2517	High	5	0.3271
<i>Gomphonema variostriatum</i>	El Carmen	66.67	0.0001	Low	22.88	0.0204
<i>Gomphonema minutum</i>	Jipiro	49.56	0.0003	High	24.69	0.1277
<i>Gomphonema lagenula</i>	Jipiro	67.9	0.0001	Low	23.69	0.1033
<i>Gomphonema pumilum</i>	Jipiro	67.31	0.0001	Medium	22.49	0.3335
<i>Gomphonema subclavatum</i>	San Simón	67.64	0.0001	Low	37.01	0.0334
<i>Gomphonema clavatum</i>	Curitroje	58.01	0.0001	Low	30.02	0.0039
<i>Gomphonema parvulum</i>	El Carmen	50.79	0.0002	Low	20.28	0.2764
<i>Gomphonema reichardtii</i>	Curitroje	33.33	0.0009	Low	25	0.0029
<i>Gomphonema soprophilum</i>	Curitroje	33.33	0.0003	Medium	25	0.0035
<i>Gomphonema affine</i>	Curitroje	86.67	0.0001	Medium	11.68	0.2189
<i>Gomphonema longiceps</i>	Curitroje	6.667	0.2534	High	5	0.3341
<i>Gomphonema olivaceum</i>	San Simón	53.33	0.0001	Medium	24.63	0.0064
<i>Hannea arcus</i>	El Carmen	94.49	0.0001	High	22.98	0.1436
<i>Humidophila contenta</i>	Jipiro	60	0.0001	Medium	7.101	0.3262
<i>Luticula goeppertiana</i>	Jipiro	84.37	0.0001	High	19.71	0.0681
<i>Mayamaea atomus</i>	Jipiro	100	0.0001	High	10.16	0.3697
<i>Luticula mutica</i>	Jipiro	66.28	0.0001	High	6.538	0.471
<i>Neidiomorpha</i>	Jipiro	73.33	0.0001	Low	18.5	0.0498
<i>Nitzschia palea</i>	El Carmen	16.28	0.5307	Medium	38.4	0.037
<i>Nitzschia angustata</i>	San Simón	60	0.0001	Medium	20.09	0.02
<i>Navicula cryptotenelloides</i>	El Carmen	33.33	0.0004	High	18.73	0.0112
<i>Navicula simulata</i>	San Simón	38.15	0.0016	Medium	48.23	0.0005
<i>Navicula cryptotenella</i>	Jipiro	36.29	0.002	Medium	34.76	0.0042
<i>Navicula erifuga</i>	Curitroje	66.67	0.0001	Medium	19.05	0.028
<i>Odontidium mesodon</i>	Curitroje	33.33	0.0005	High	25	0.0035
<i>Pinnularia parvulissima</i>	El Carmen	6.667	0.2485	Medium	5	0.3318
<i>Sellaphora lanceolata</i>	Jipiro	45.4	0.0001	High	28.57	0.0104
<i>Pinnularia divergentissima</i>	El Carmen	6.667	0.2529	Medium	5	0.3346
<i>Pinnularia subanglica</i>	El Carmen	6.667	0.2485	Medium	5	0.3318
<i>Pinnularia borealis</i>	El Carmen	6.667	0.2587	Medium	5	0.328
<i>Pinnularia latarea</i>	Curitroje	26.67	0.0029	Medium	20	0.0113
<i>Pinnularia undula</i>	San Simón	33.33	0.0008	Medium	15.2	0.0296
<i>Psammothidium subatomus</i>	El Carmen	25.92	0.0531	Medium	19.69	0.2445
<i>Psammothidium subatomoides</i>	Jipiro	100	0.0001	High	12.04	0.2678
<i>Planothidium bagualense</i>	El Carmen	26.67	0.0035	Medium	20	0.0105
<i>Planothidium delicatulum</i>	El Carmen	6.667	0.2442	Low	5	0.3309
<i>Planothidium lanceolatum</i>	El Carmen	55.28	0.0002	Medium	34.12	0.0405
<i>Remeira sinuata</i>	Jipiro	100	0.0001	Low	14.49	0.2057
<i>Rhopodia musculus</i>	Curitroje	26.67	0.0033	Low	20	0.01
<i>Sellaphora atomoides</i>	Jipiro	100	0.0001	High	17.37	0.1622
<i>Sellaphora capitata</i>	Curitroje	6.667	0.2534	High	5	0.3341
<i>Ulnaria ulna</i>	San Simón	21.96	0.0061	Medium	22.06	0.0066

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