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Spatio-temporal variability of epilithic diatoms in a watershed in southern Peru and their relationship with physicochemical variables

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ABSTRACT

This study analyzes the spatio-temporal variability in the abundance and diversity of epilithic diatoms and their relationship with physical and chemical water variables in a key hydrological system in southern Peru. Samples were collected at 12 sites (lower, middle, and upper reaches of the river) during four campaigns (May, August, November, and December 2019). Physicochemical parameters and community metrics of diatoms were measured, employing multivariate analyses to assess the relationships between environmental and biological variables. Sixty-three species were identified, including *Surirella brebissonii, Nitzschia clausii, Ulnaria acus, Achnanthidium minutissimum,* and *Nitzschia palea*. The highest species richness was recorded in the lower reaches. The Shannon index ranged from 1.9 (upper reaches) to 3.59 (middle reaches), while the Simpson index showed greater dominance (0.64) in the upper reaches. Evenness (Pielou index) indicated a homogeneous distribution. Electrical conductivity was significantly correlated with *S. brebissonii and N. clausii.* Species richness showed spatial differences, and variables such as conductivity and major cations significantly influenced community structure, showing a moderate correlation with the relative abundance of species in the Tambo River basin.

Keywords: lotic waters, periphyton, aquatic communities, diversity indices, Tambo River.

INTRODUCTION

Rivers represent the most dynamic, essential, and biodiverse freshwater aquatic environments. Unfortunately, they are also among the most modified and threatened ecosystems worldwide [Best, 2019]. Agricultural activities significantly impact river water quality and aquatic ecosystems [Quinteros et al., 2017; Zhang et al., 2022]. Diatoms are a key group of organisms recommended by the Water Framework Directive introduced by the European Union in 2000 for assessing ecological gradients of water quality in rivers [Lobo et al., 2019]. They are among the first communities affected by agricultural pollution and respond quickly to anthropogenic disturbances in freshwater ecosystems due to their cosmopolitan nature and rapid cell cycle [Dalu et al., 2017; Nicolosi Gelis et al., 2024]. Epilithic diatoms have also been studied as bioindicators to assess the conservation status of ephemeral wetlands at a global scale, as shown by Taurozzi et al. [2022], who highlighted their effectiveness in detecting ecological changes in these vulnerable aquatic systems. Among periphytic diatoms, epilithic diatoms are one of the most important components of microalgal communities in aquatic habitats in terms of diversity, biomass, and ecosystem metabolism [Blanco et al., 2020]

Numerous studies have demonstrated that diatom communities respond to environmental variations across different aquatic ecosystems [Jannel et al., 2024; Rusanov et al., 2024; Schultz et al., 2024]. Across continents, studies have consistently shown that epilithic diatoms respond to gradients in conductivity, pH, and nutrient levels, regardless of climatic zone. For example, they are influenced by seasonal chemical variations in water across different geographic regions [Costa et al., 2022; Kaddeche et al., 2022], serve as indicators of river pollution (Shen et al., 2018; Andrade-Servín and Israde-Alcántara, 2021], and are widely used as bioindicators for water quality monitoring [Nicolosi Gelis et al., 2024]. To assess anthropogenic influences on diatom communities, multimetric indices have been developed [Carlisle et al., 2022]. Some of the most widely used indices for evaluating water quality in rivers and streams include biotic indices, diversity indices, and multivariate analysis [Lobo et al., 2019; Kaddeche et al., 2022].

In Peru, recent diatom research has primarily focused on paleontological studies [Grana and Prieto, 2021; King et al., 2021; Gariboldi et al., 2023]. However, studies focusing on botanical, ecological, and bioindicator aspects remain scarce [Torres-Franco et al., 2019; Sala et al., 2021; Motta and Ranilla, 2024].

The Tambo River watershed is located on the southwestern flank of the Andean Cordillera and is a large and socially significant basin due to the extensive agricultural activity occurring in its lower reaches. Andean mountain rivers and their basins represent a vast network with diverse geologic and geomorphologic patterns united by a surrounding environment of hydraulic and nutrient regimes, land-use, and altitudinal gradients with similar temperatures. This characteristic, along with the biodiversity of Andean fluvial systems, must be the basis for developing regional plans for identifying critical areas in need of conservation [Donato-R et al., 2022]. Given the ongoing degradation of water quality in Europe, there is a clear need to develop more precise and tailored approaches for biomonitoring environmental quality. A study conducted by Masouras et al. [2021] reviewed the implementation of benthic diatoms in river biomonitoring, emphasizing their critical ecological role in aquatic ecosystems. This global perspective complements our research in the Tambo River basin, reinforcing the importance of diatoms as bioindicators and highlighting the need for effective biomonitoring strategies on a worldwide scale. It is hypothesized that spatio-temporal variations in the physicochemical properties of water, associated with differences among sectors of the watershed and natural seasonal dynamics, are reflected in the structure and composition of epilithic diatom communities. Due to their high sensitivity to environmental changes, these communities represent effective biological indicators of aquatic ecosystem conditions. In this context, the aim of this study was to analyze the spatio-temporal variation in the abundance and diversity of these communities and their relationship with physicochemical variables in the Tambo River basin. This study is among the first to determine diatom species variability in this basin, where similar research has not yet been conducted, establishing a baseline for bioindication studies in Peruvian rivers.

MATERIALS AND METHODS

Study area

The study was conducted in the Tambo River watershed, located in southern Peru. This watershed is part of the Pacific slope hydrographic system, flowing from northeast to southwest before emptying into the Pacific Ocean [INGEMMET, 2020]. The watershed covers an area of 12,454 km², with 8.149 km² characterized by an elongated shape, deep and rugged terrain, steep slopes, and narrow canyons intersected by deep ravines. It is primarily a desert region with minimal precipitation throughout most of the year, experiencing a rainy season from December to March, which is influenced by climatic variability associated with the "El Niño" phenomenon. The region exhibits significant thermal variations, with high temperatures during the summer months (December–March) and low temperatures during winter (June–August), representing the most notable climatic contrasts in the area [INGEMMET, 2020].

The waters of the Tambo River basin support multiple activities, including agriculture, human consumption, livestock farming, and hydroelectric power generation. A total of 12 sampling sites were established along the main river course of the watershed (Figure 1).

Sampling periods

Sampling was conducted in May (autumn), August (winter), November (spring), and December (summer) of 2019. The study covered the lower (E1-E6), middle (E7-E10), and upper (E11-E12) sections of the river (Table 1).

Sample collection

For epilithic diatom collection, a 10-meter river segment was selected at each sampling site. A composite sample was formed by collecting five rocks (approximately 10–20 cm in diameter), from which an area of approximately 20 cm² was

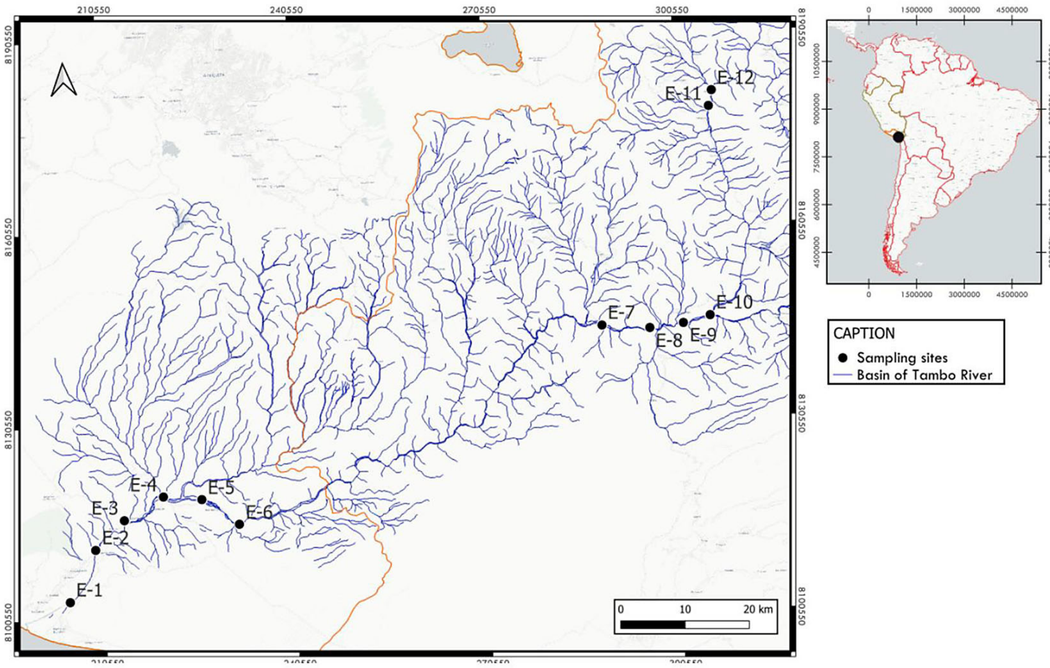

Figure 1. Map of sampling sites in the Tambo River watershed

Table 1. Coordinates and geographical characteristics of the sampling sites

Section of the basin	Sites	Latitude	Longitude	Altitude m s. n. m.	Agricultural activity ha
Low	E-1	17°08'0264" S	71°46'2491" W	37	982371
	E-2	17°03'4240" S	71°44'0178" W	106	
	E-3	17°01'4001" S	71°41'2514" W	157	
	E-4	16°59'2450" S	71°37'5564" W	227	
	E-5	16°59'4401" S	71°34'3371" W	288	
	E-6	17°01'5569" S	71°31'2075" W	369	
Middle	E-7	16°46'0838" S	70°58'5981" W	1325	2097
	E-8	16°46'2839" S	70°54'4903" W	1499	
	E-9	16°46'0854" S	70°51'5199" W	1584	
	E-10	16°45'3301" S	70°49'3017" W	1670	
High	E-11	16°27'5481" S	70°49'0502" W	2497	No agricultural activity, raising of South America
	E-12	16°26'3503" S	70°48'4699" W	2555	

scraped using a hard-bristled toothbrush [Kelly et al., 2001; Castillejo et al., 2022]. The collected material was stored in 100 mL hermetically sealed polyethylene bottles and preserved in 4% formalin at neutral pH.

Simultaneously, water samples were collected to analyze physical and chemical parameters, including water temperature, pH, electrical conductivity, total dissolved solids (TDS), dissolved oxygen (DS), and turbidity. Additional 1000 mL and 300 mL polyethylene bottles were used to collect samples for analyzing suspended solids (TSS), ammonium (NH_4^+), nitrate (NO_3-N), total phosphorus (TF), and total metals (TSS, TF, Al, As, B, Ca, Fe, Li, Mg, K, Si, Na, Zn).

All samples were stored in refrigerated containers (< 4 °C) and transported to Laboratorio Certificaciones del Perú S.A. (Arequipa, Perú) for analysis following APHA (2017) and ISO 17294-2:2016 standards.

Field measurements

Water temperature (T), pH, dissolved oxygen (DO), electrical conductivity (EC), TDS, and turbidity (Turb) were measured in situ using an Aquared AP2000 multiparameter probe (Kent, England).

Diatom sample processing

In the laboratory, diatom samples underwent treatment, and after 48 hours of natural sedimentation, the frustules were cleaned following the hot peroxide method [Kelly et al., 2001]. Permanent slides were prepared using Naphrax mounting medium (RI = 1.74). For diatom taxon counting, a minimum of 400 valves were examined at 1000X magnification using a Motic BA310E optical microscope (Barcelona, Spain) equipped with a Moticam S12 microphotographic system [Kelly et al., 2001; Quevedo et al., 2018; Tibby et al., 2019]. Counts were converted to relative abundance (%) where 100% represents the total number of individuals across all species. Taxon identification was based on specialized keys, illustrations, and atlases by Krammer and Lange-Bertalot [1986, 1991], Round et al. [1990], Blanco et al. [2010, 2011], and Wehr et al. [2015], complemented by the Diatoms.org online database https://diatoms.or [Spaulding et al., 2021].

Data analysis

Descriptive and inferential statistical analyses were applied to both biological and physicochemical variables. To verify distribution assumptions, data were subjected to normality tests (Shapiro-Wilk) and homogeneity of variances tests (Levene's statistic). Subsequently, to determine differences in relative abundance and ecological indices across river sections and seasonal periods, either parametric (ANOVA and Tukey's test) or non-parametric (Kruskal-Wallis test) statistical methods were applied, with a significance level of 0.05. The most abundant species were analyzed using a boxplot diagram based on their relative abundances across river sections and seasonal periods. Diversity indices were calculated for each sampling site, including species richness, Shannon diversity index (H'), Simpson's dominance index (1-D), and Pielou's evenness index (J').

Physicochemical variables were compared against environmental quality standards (ECA) to assess compliance with Peruvian water quality regulations. To evaluate the variation in diatom species composition explained by environmental variables, canonical correspondence analysis (CCA) was performed [Lobo et al., 1995]. The analysis included biological variables and 13 physicochemical parameters (pH, T, EC, TDS, DO, NH_4^+, TF, As, B, Ca, Mg, Na, and Si). Only species with relative abundances exceeding 5% in at least one sample were considered for the multivariate analysis [Milićević et al., 2024; Schultz et al., 2024]. A global CCA was conducted for the entire dataset. To quantify relationships between physicochemical and biological variables, Spearman's correlation coefficient (r_s) was applied. All data were log-transformed (log (x+1)) before statistical analysis [Kaddeche et al., 2022]. The statistical analyses were conducted using Excel and PAST 3.23 software [Hammer et al., 2001].

RESULTS

Spatio-temporal variation of diatoms

A total of 20 families were recorded, distributed across 30 genera, with *Nitzschia* and *Navicula* exhibiting the highest number of species (Table 2). The most abundant species were: *Surirella brebissonii* (59.5%), *Nitzschia clausii* (45.2%), *Ulnaria acus* (47.6%), *Denticula tenuis* (39.9%), *Achnanthidium minutissimum* (38.9%) and *Nitzschia palea* (12.3%), based on their relative abundance within their respective genera. Percentages indicate the proportion of each species relative to the total number of individuals recorded for that genus. A total of 15 species exhibited a relative abundance greater than 5% in at least one sample, being the most abundant and ecologically significant species (Figure 2).

In the lower section of the watershed, *Nitzschia clausii* and *Ulnaria acus* were the most abundant species, whereas in the middle and upper sections, the most abundant species were *Surirella brebissonii* and *Achnanthidium minutissimum*. During winter, *Denticula tenuis* was abundant in the lower section, while in spring, it was most prevalent in the upper section. In summer, it was abundant in both the middle and upper sections of the watershed. In autumn, *Nitzschia palea* exhibited the highest abundances across the entire watershed. The analysis revealed that the highest average abundance of *A. minutissimum* was recorded in the upper section (26.12 ± 16.21) and the lowest in the lower section (10.05 ± 7.21). *A. minutissimum* and *S. brebissonii*

Table 2. List of families and species recorded in the water of the Tambo River basin, Peru, in 2019

N°	Family	Species
1	Achnanthidiaceae	*Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot 1999
2		*Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot 1999
3	Achnanthaceae	*Achnanthes coarctata* (Brébisson ex WSmith) Grunow
4		*Achnanthes sp*
5		*Achnanthidium minutissimum* (Kützing) Czarnecki 1994
6	Bacillariaceae	*Denticula tenuis Kützing* 1844
7		*Nitzschia fonticola* (Grunow) Grunow 1881
8		*Nitzschia amphibia* Grunow 1862
9		*Nitzschia clausii* Hantzsch 1860
10		*Nitzschia recta* Hantzsch 1862
11		*Nitzschia media* Hantzsch 1860
12		*Nitzschia palea* (Kützing) WSmith 1856
13		*Nitzschia linearis* WSmith 1853
14		*Nitzschia inconspicua* Grunow 1862
15		*Nitzschia dissipata* (Kützing) Rabenhorst 1860
16		*Tryblionella apiculata* WGregory 1857
17		*Tryblionella calida* (Grunow) DGMann 1990
18	Catenulaceae	*Amphora ovalis* (Kützing) Kützing 1844
19		*Amphora sp*
20	Cocconeidaceae	*Cocconeis placentula* Ehrenberg 1838
21		*Cocconeis pediculus* Ehrenberg 1838
22	Cymbellaceae	*Cymbella compacta* Østrup 1910
23		*Cymbella turgidula* Grunow 1875
24		*Cymbella excisa* Kützing 1844
25	Diploneidaceae	*Diploneis ovalis* (Hilse) Cleve 1891
26	Eupodiscaceae	*Pleurosira laevis* (Ehrenberg) Compère 1982
27	Gomphonemataceae	*Encyonema minutum* (Hilse) DGMann 1990
28		*Encyonema mesianum* (Cholnoky) DGMann 1990
29		*Gomphonema lagenula* Kützing 1844
30		*Gomphonema parvulum* Kützing 1844
31		*Gomphonema truncatum* Ehrenberg 1832
32		*Gyrosigma acuminatum* (Kützing) Rabenhorst 1853
33	Fragilariaceae	*Fragilaria rumpens* (Kützing) GWFCarlson 1913
34		*Fragilaria vaucheriae* (Kützing) JBPetersen 1938
35		*Fragilaria tenera* (WSmith) Lange-Bertalot 1980
36		*Odontidium mesodon* (Ehrenberg) Kützing 1849
37	Naviculaceae	*Navicula rhynchocephala* Kützing 1844
38		*Navicula lanceolata* Ehrenberg 1838
39		*Navicula tripunctata* (OFMüller) Bory 1822
40		*Navicula salinarum* Grunow 1880
41		*Caloneis bacillum* (Grunow) Cleve 1894
42	Pinnulariaceae	*Pinnularia microstauron* (Ehrenberg) Cleve 1891
43		*Pinnularia lata* (Brébisson) WSmith 1853
44	Rhoicospheniaceae	*Rhoicosphenia abbreviata* (CAgardh) Lange-Bertalot 1980

45	Rhopalodiaceae	*Epithemia argus* (Ehrenberg) Kützing 1844
46		*Epithemia sorex* Kützing 1844
47		*Rhopalodia musculus* (Kützing) OMüller 1900
48		*Rhopalodia gibba* (Ehrenberg) OMüller 1895
49	Sellaphoraceae	*Sellaphora pupula* (Kützing) Mereschkovsky 1902
50	Surirellaceae	*Surirella brebissonii* Krammer & Lange-Bertalot 1987
51		*Surirella spiralis* Kützing 1844
52		*Surirella ovalis* Brébisson 1838
53		*Surirella striatula* Turpin 1828
54	Staurosiraceae	*Pseudostaurosira polonica* (MWitak & Lange-Bertalot) EAMorales & MBEdlund 2003
55		*Pseudostaurosira brevistriata* (Grunow) DMWilliams & Round 1988
56		*Staurosira construens* Ehrenberg 1843
57	Tabellariaceae	*Diatoma vulgaris* Bory 1824
58		*Diatoma moniliformis*
59		*Meridion lineare* DMWilliams 1985
60	Stephanodiscaceae	*Stephanocyclus meneghinianus* (Kützing) Kulikovskiy, Genkal & Kociolek 2022
61		*Cyclotella atomus* Hustedt 1937
62	Ulnariaceae	*Ulnaria ulna* (Nitzsch) Compère 2001
63		*Ulnaria acus* (Kützing) Aboal 2003

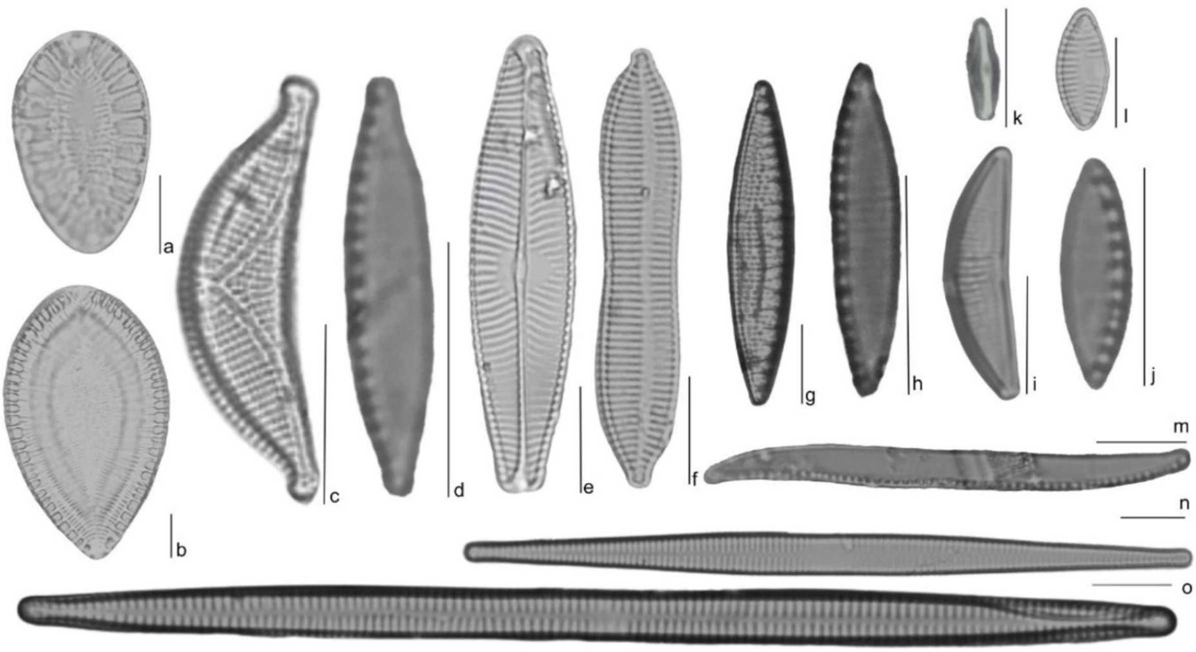

Figure 2. Microphotographs of the most abundant and ecologically relevant diatoms in the Tambo River watershed. (a) *Surirella brebissonii.* (b) *Surirella ovalis.* (c) *Epithemia sorex*. (d) *Nitzschia palea.* (e) *Navicula lanceolata* (f) *Tryblionella apiculata.* (g) *Denticula tenuis*. (h) *Nitzschia amphibia*. (i) *Amphora sp*. (j) *Nitzschia inconspicua*. (k) *Achnantidium minutissimum*. (l) *Planothidium frequentissimum.* (m) *Nitzschia clausii* (n) *Ulnaria acus*. (o) *Ulnaria ulna.*
The scale attached to each species represents 10 microns

exhibited a similar abundance pattern, with their abundance increasing at higher elevations within the watershed. On the other hand, *D. tenuis*, *U. acus*, and *N. clausii* were present throughout the entire watershed, with their highest abundances recorded in the lower section (Figure 3).

In Figure 4, the relative abundance of *A. minutissimum* was lowest in autumn, differing significantly from the other months of the year ($p < 0.05$). Conversely, the population of *N. palea* exhibited its highest abundance in autumn ($p < 0.05$). Meanwhile, *S. brebissonii* showed the highest relative abundance in autumn and the lowest in summer ($p < 0.05$). In contrast, *D. tenuis* displayed the lowest relative abundance in winter and the highest in summer ($p < 0.05$). Spatially, the Kruskal-Wallis test (Fig. 4) did not reveal significant differences between sections of the watershed, but it did show temporal differences ($p > 0.05$). The species *A. minutissimum*, *N. palea*, *S. brebissonii*, and *D. tenuis* exhibited significant differences in relative abundances across seasons.

The diatom community in this watershed was characterized by a species richness ranging from 27 to 46 species, with higher average values in the lower section ($\bar{X}$ = 40.8, SD = 4.79) and the lowest in the upper section ($\bar{X}$ = 29, SD = 2.45). Regarding diversity metrics (Figure 5), the highest diversity according to the Shannon index (3.59) and Pielou's evenness index (0.92) was recorded during the spring sampling in the middle section of the watershed. In contrast, the highest dominance (0.64) was observed in autumn in the upper section, primarily represented by the species *S. brebissonii*. No temporal differences were observed in the diversity metrics. Spatially, only species richness showed significant differences ($p > 0.05$) (Figure 6).

Physicochemical variables

The physicochemical variables are summarized in Tables 3 and 4, presenting the average values calculated for the three watershed sections and the four seasons of the year. The highest average values for temperature (T) and pH were recorded in summer and spring, with 24.76 ± 2.68 °C and 8.58 ± 0.26, respectively. Regarding electrical conductivity (EC), the highest values were recorded in summer (3777.9 ± 1250.60 µS cm^{-1}), while the lowest were found in winter (1909.07 ± 206.73 µS cm^{-1}). Dissolved oxygen (DO) showed the highest average values in the lower section of the watershed (8.42 ± 2.16 mg L^{-1}), increasing as altitude decreased. Particularly, the highest values were recorded in spring (9.53 ± 0.88 mg L^{-1}), while the lowest average was observed in summer (4.63 ± 2.23 mg L^{-1}). Turbidity reached its highest level in summer (74.61 ± 1.61 NTU) and its lowest in spring (23.16 ± 8.83 NTU). The variables EC, TDS, turbidity, and DO exhibit significant spatial and temporal differences ($p < 0.05$).

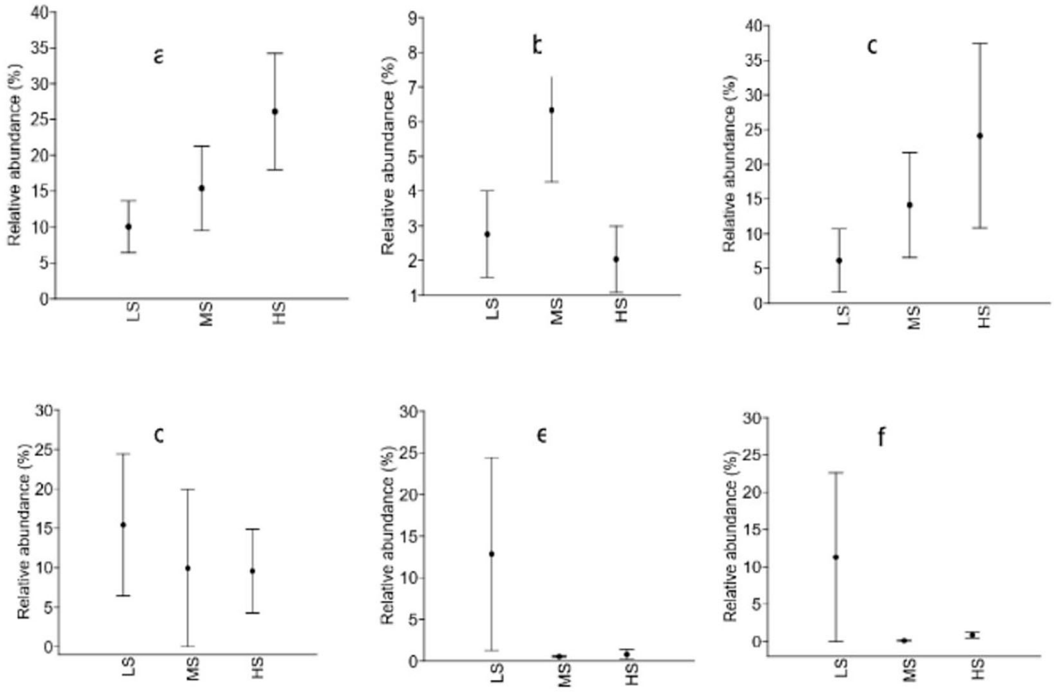

Figure 3. Spatial variation in the relative abundance of the most abundant diatoms.
(a) *A. minutissimum*, (b) *N. palea*, (c) *S. brebissonii*, (d) *D. tenuis*, (e) *U. acus*, (f) *N. clausii*.
TB = Lower Section, TM = Middle Section, TA = Upper Section

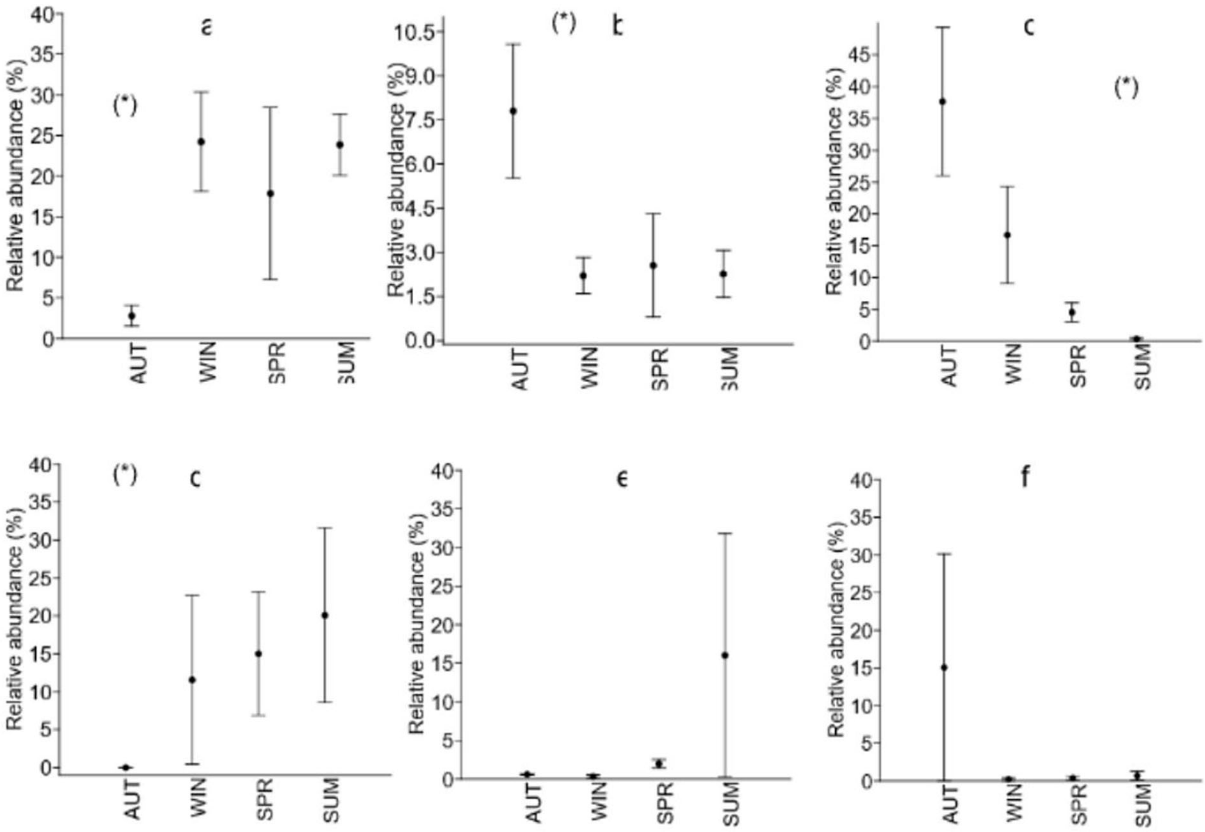

Figure 4. Temporal variation in the relative abundance of the most abundant diatoms (a) *A. minutissimum*. (b) *N. palea*. (c) *S. brebissonii*. (d) *D. tenuis*. (e) *U. acus*. (f) *N. clausii*. Kruskal-Wallis test was used (*). AUT= autumn, WIN = winter, SPR = spring, SUM = summer

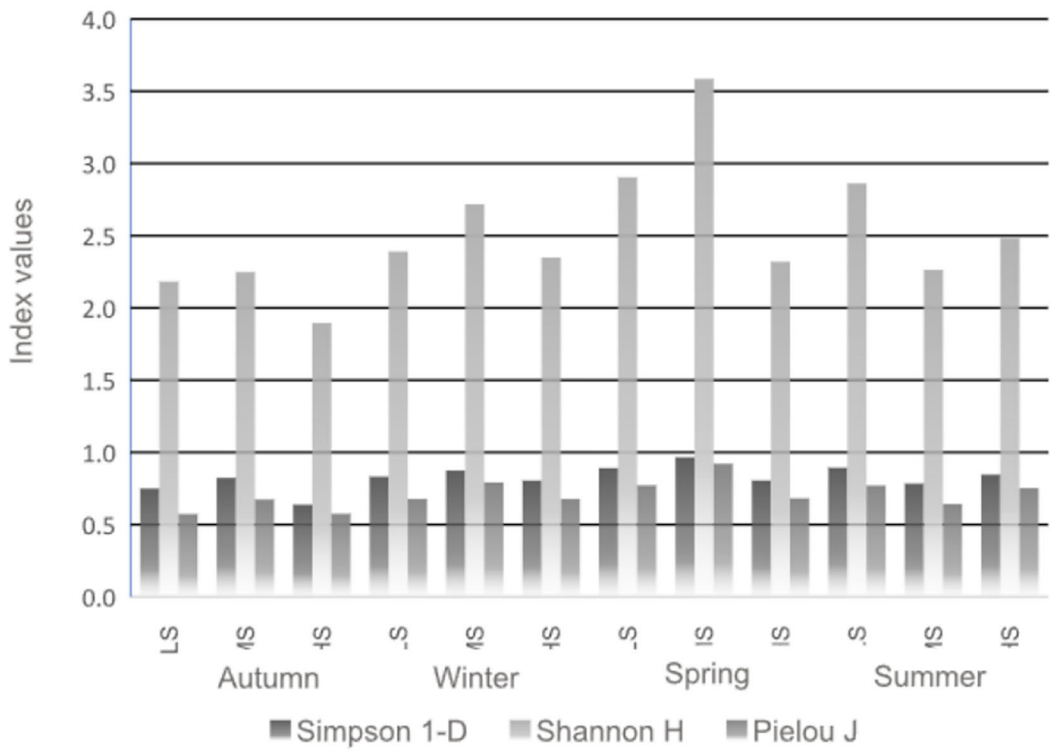

Figure 5. Diversity indices in the lower, middle, and upper sections of the Tambo River watershed, Peru, During Autumn, Winter, Spring, and Summer of 2019

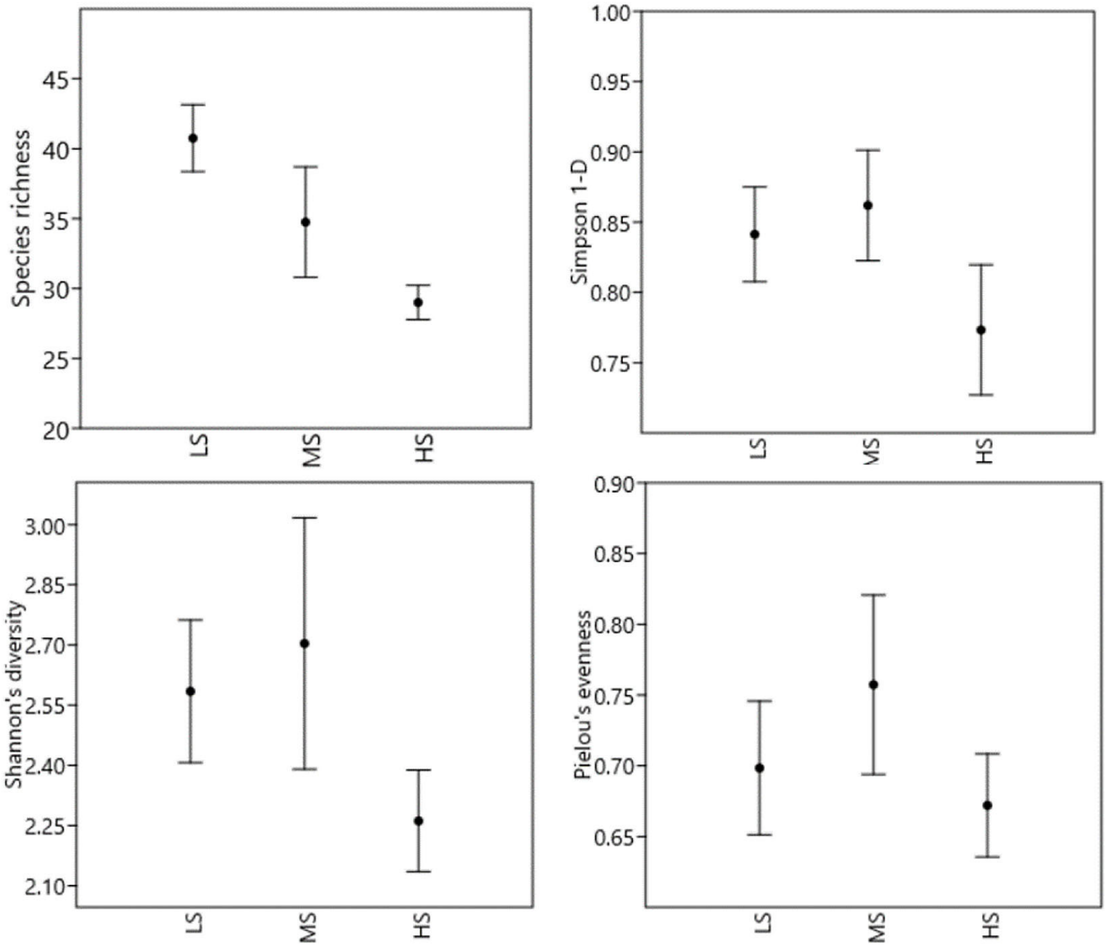

Figure 6. Variation in diversity metrics across the lower (LS), middle (MS), and upper (HS) sections of the Tambo River watershed. ANOVA was used to compare the means of the diversity metrics. (*) Significant differences p < 0.05

In the upper section of the watershed, total phosphorus (TF) had the lowest recorded values (86 ± 26 µg L^{-1}). In the middle and lower sections, arsenic (As) and boron (B) had the highest concentrations, with 0.2113 ± 0.0473 mg L^{-1} and 5.83 ± 1.18 mg L^{-1}, respectively (Table 3). Nitrate (NO_3^-) levels remained constant across all four seasons, whereas ammonium (NH_4^+) showed significant seasonal differences ($p < 0.05$), with the lowest values recorded in autumn (0.89 ± 0.15 mg L^{-1}). Silicon (Si) had its lowest value in autumn (0.21 mg L^{-1}), while sodium (Na) had the highest average concentration in summer (517.67 ± 109.47 mg L^{-1}) (Table 4). Some physicochemical variables exceeded the Peruvian environmental quality standards (ECA) for water intended for aquatic environment conservation and irrigation, including temperature variation (>3 °C), pH (6.5–8.5), electrical conductivity (EC > 1000 µS cm^{-1}), dissolved oxygen (DO < 5 mg L^{-1}), total phosphorus (TF > 50 µg L^{-1}), boron (B > 1 mg L^{-1}), and arsenic (As > 0.15 mg L^{-1}).

Epilithic diatom community and its relationship with physicochemical variables

The CCA results, which illustrate the ordination of diatom species and environmental variables, are shown in Figure 7. The eigenvalues of the first two axes were 0.19451 and 0.15145, explaining 55.33% of the total variance in the correlation between diatom species and environmental factors. Along axis 1, autumn sampling points were clustered, while spring and summer samples were positioned on the left-negative side of the axis. Winter samples were primarily located in the center of the triplot (Figure 7).

According to the sampled sections of the Tambo River watershed, the lower section was positioned in the negative side of the first axis,

Table 3. Average values and standard deviation of the variables in the lower, middle, and upper sections of the Tambo River basin water, 2019. p significance level

Physicochemical variable	Units	Low section	Middle section	High section	p
T	°C	21.29 ± 3.26	19.01 ± 2.43	15.73 ± 5.84	*
pH		7.93 ± 0.47	8.45 ± 0.10	8.57 ± 0.38	*
EC	$\mu S\ cm^{-1}$	3084.01 ± 1287.64	2320.69 ± 660.11	2883.29 ± 1293.72	*
TDS	$mg\ L^{-1}$	1444.53 ± 494.19	1123 ± 398.06	1419.92 ± 653.75	*
DO	$mg\ L^{-1}$	8.42 ± 2.16	7.43 ± 2.91	7.75 ± 1.41	*
Turb	FNU	39.93 ± 8.13	59.44 ± 11.22	33.47 ± 5.23	*
NH^{+4}	$mg\ L^{-1}$	1.12 ± 0.33	1.95 ± 0.34	1.33 ± 0.48	
NO_3-N	$mg\ L^{-1}$	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	
TSS	$mg\ L^{-1}$	31.08 ± 24.63	40.24 ± 21.27	43.07 ± 20.74	*
TF	$\mu g\ L^{-1}$	148 ± 28	141 ± 24	86 ± 26	*
Al	$mg\ L^{-1}$	1.06 ± 0.74	1.56 ± 0.880	2.00 ± 1.27	
As	$mg\ L^{-1}$	0.2079 ± 0.1261	0.2113 ± 0.0473	0.0621 ± 0.0071	*
B	$mg\ L^{-1}$	5.83 ± 1.18	5.04 ± 1.24	3.77 ± 1.36	*
Ca	$mg\ L^{-1}$	116.33 ± 9.38	109.71 ± 11.89	99.13 ± 6.85	*
Co	$mg\ L^{-1}$	0.00148 ± 0.0012	0.00424 ± 0.00192	0.00751 ± 0.00425	*
Cu	$mg\ L^{-1}$	0.03219 ± 0.02646	0.04162 ± 0.02456	0.09117 ± 0.06324	*
Sr	$mg\ L^{-1}$	1.96 ± 0.26	1.98 ± 0.34	2.42 ± 0.63	
P	$mg\ L^{-1}$	0.09 ± 0.014	0.07 ± 0.043	0.06 ± 0.05	
Fe	$mg\ L^{-1}$	0.91 ± 0.71	1.15 ± 0.55	1.88 ± 1.09	
Li	$mg\ L^{-1}$	3.268 ± 4.909	3.49 ± 4.95	3.51 ± 4.65	
Mg	$mg\ L^{-1}$	29.33 ± 4.24	28.14 ± 5.12	27.31 ± 5.62	
Pb	$mg\ L^{-1}$	0.00081 ± 0.00058	0.00077 ± 0.00019	0.00098 ± 0.00048	
K	$mg\ L^{-1}$	20.46 ± 3.34	20.09 ± 4.33	21.34 ± 6.32	
Si	$mg\ L^{-1}$	13.36 ± 8.44	18.23 ± 8.11	13.5 ± 3.79	
Na	$mg\ L^{-1}$	358.11 ± 55.79	376.25 ± 82.44	467.28 ± 141.19	
Ti	$mg\ L^{-1}$	0.0127 ± 0.01111	0.0148 ± 0.0074	0.0135 ± 0.0095	
Zn	$mg\ L^{-1}$	0.02872 ± 0.1978	0.04771 ± 0.02349	1.8792 06220	*

Note: * Significance level: 0.05.

the middle section in the negative side of the second axis, and the upper section in the center of the CCA plot. The autumn samples along the first axis were positively associated with the highest DO values and negatively associated with temperature (T), ammonium ($NH^{+}4$), silicon (Si), and major cations (B, Ca, Mg, and Na). The species associated with the first axis included *Nitzschia palea*, *Navicula lanceolata*, *Surirella brebissonii*, *Surirella ovalis*, and *Tryblionella apiculate*.

Nitzschia palea showed a negative correlation with sodium (Na) (r_s = -0.61, p = 0.035). On the other hand, *Surirella brebissonii* was correlated with temperature (T), boron (B), magnesium (Mg), sodium (Na), electrical conductivity (EC), and total dissolved solids (TDS) (r_s > -0.55, p < 0.05). *Surirella ovalis* correlated with dissolved oxygen (DO) (r_s = 0.59, p = 0.046). *Navicula lanceolata* and *Tryblionella apiculata* were associated with higher silicon (Si) concentrations (r_s = 0.59 and r_s = 0.81, respectively; p < 0.05) and calcium (Ca) concentrations (r_s = 0.54 and r_s = 0.71, respectively; p < 0.05). Additionally, *Navicula lanceolata* correlated with total phosphorus (TF) (r_s = 0.59, p = 0.043).

In the second axis, electrical conductivity (EC) and total dissolved solids (TDS) were positively associated, while total phosphorus (TF) was negatively associated. The species related to this axis were *Nitzschia clausii* and *Epithemia sorex*. Among them, *Nitzschia clausii* correlated with electrical conductivity (EC) (r_s = 0.67, p = 0.017) and total dissolved solids (TDS) (r_s = 0.58, p = 0.049).

Table 4. Average values and standard deviation of physicochemical variables in autumn, winter, spring, and summer in the water of the Tambo River basin, 2019

Physicochemical variable	Units	Autum	Winter	Spring	Summer	p
T	°C	18.61 ± 2.93	16.37 ± 3.43	18.78 ± 3.49	24.76 ± 2.68	*
pH		8.34 ± 0.51	8.55 ± 0.2	8.58 ± 0.26	8.31 ± 0.19	
EC	μS cm^{-1}	2889.06 ± 1161.02	1909.07 ± 206.73	2324.57 ± 244.75	3777.9 ± 1250.60	*
TDS	mg L^{-1}	1394.69 ± 630.06	952.96 ± 105.16	1146.06 ± 110.33	1719.1 ± 451.38	*
DO	mg L^{-1}	8.61 ± 0.94	9.03 ± 0.72	9.53 ± 0.88	4.63 ± 2.23	*
Turb	FNU	41.92 ± 10.6	42.16 ± 5.6	23.16 ± 8.83	74.61 ± 1.61	*
NH^+4	mg L^{-1}	0.89 ± 0.15	1.47 ± 0.31	1.29 ± 0.29	-	*
NO3-N	mg L^{-1}	0.02	0.02	0.02	0.02	
TSS	mg L^{-1}	46.48 ± 4.96	65.62 ±10.54	22.13 ± 12.72	16.3 ± 9.15	*
TF	µg L^{-1}	125 ± 28	152 ± 10	129 ± 30	104 ± 46	
Al	mg L^{-1}	2.30 ± 0.33	2.15 ± 0.73	0.69 ± 0.19	0.36 ± 0.13	*
As	mg L^{-1}	0.1550 ± 0.0856	0.1776 ± 0.0967	0.1117 ± 0.0745	0.2218 ± 0.1428	
B	mg L^{-1}	3.52 ± 0.82	4.14 ± 0.99	5.72 ± 0.91	6.49 ± 0.84	*
Ca	mg L^{-1}	100.12 ± 10.02	112 ± 9.77	121.33 ± 8.26	102.98 ± 5.81	*
Co	mg L^{-1}	0.00601 ± 0.00252	0.00544 ± 0.00349	0.00402 ± 0.00469	0.001173333	
Cu	mg L^{-1}	0.07934 ± 0.011	0.07717 ± 0.05531	0.04579 ± 0.05063	0.00786 ± 0.00211	*
Sr	mg L^{-1}	1.72 ± 0.14	1.86 ± 0.15	2.10 ± 0.21	2.72 ± 0.46	*
P	mg L^{-1}	<0.02	0.11 ± 0.002	0.04 ± 0.01	<0.02	
Fe	mg L^{-1}	1.89 ± 0.13	1.91 ± 0.96	0.87 ± 0.64	0.40 ± 0.12	*
Li	mg L^{-1}	0.483 ± 0.042	11.233 ± 0.877	0.901 ± 0.066	1.035 ± 0.094	*
Mg	mg L^{-1}	22.87 ± 1.47	25.52 ± 1.22	30.08 ± 1.43	34.92 ± 0.66	*
Pb	mg L^{-1}	0.00124 ± 0.00046	0.00110 ± 0.00024	0.00062 ± 0.00027	0.00042 ± 0.00015	
K	mg L^{-1}	16.5 ± 0.69	17.67 ± 0.69	20.82 ± 0.82	27.38 ± 2.44	*
Si	mg L^{-1}	< LD - 0.21	19.75 ± 2.69	19.37 ± 2.64	13.43 ± 5.08	*
Na	mg L^{-1}	300 ± 24.69	348.83 ± 37.15	417.5 ± 52.13	517.67 ± 109.47	*
Ti	mg L^{-1}	0.0198 ± 0.0086	0.0221 ± 0.0057	0.0080 ± 0.0032	0.0046 ± 0.0019	*
Zn	mg L^{-1}	0.07264 ± 0.1386	0.070634 ± 0.04251	0.05399 ± 0.06308	0.01199 ± 0.00402	*

Note: * Significance level: 0.05.

DISCUSSION

Spatio-temporal variation of the epilithic diatom community

Results revealed that the genera *Navicula* and *Nitzschia* had the highest number of species. Similar findings have been reported for others rivers in the region, such as the Chili River [Torres-Franco et al., 2019] and the Ocoña River [Motta and Ranilla, 2024], both of which originate in the Andes Mountains and flow into the Pacific Ocean, just like the Tambo River. In the Tohma Stream (Malatya, Turkey), these genera were also represented by the most abundant species [Yildirim and Baran, 2019] even though these watercourses differ in flow rate and fluvial morphology. Among the identified diatom species, *N. lanceolata*, *N. inconspicua, N. palea*, *Cocconeis placentula*, and *Rhoicosphenia abbreviata* have been previously documented in studies conducted in hydrographic basins of southern Peru [Torres-Franco et al., 2019; Motta and Ranilla, 2024].

Among the most abundant species, *Achnanthidium minutissimum* was recorded as a common species in lentic and lotic environments, showing low sensitivity to chemical variables [Rusanov et al., 2024]. In a study conducted in the Ying River, China [Shen et al., 2018], the species was also identified as pollution-tolerant due to its dominance as a taxon at various sampling points along the East Zhejiang Canal, China contributing to the assessment of the canal's eutrophication [Jin et al., 2024]. *A. minutissimum* was previously identified as an indicator of low nutrient content in water.

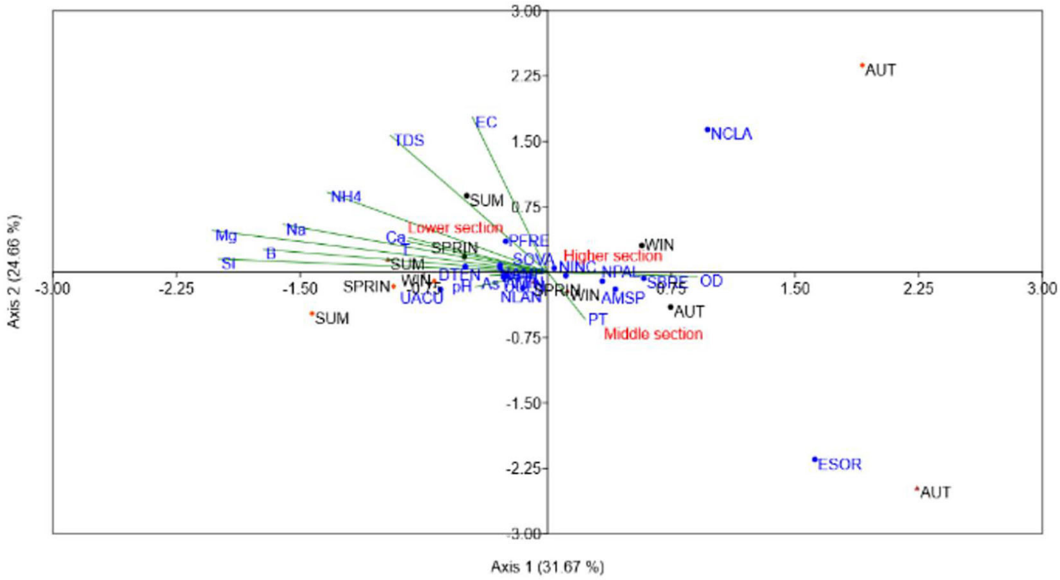

Figure 7. Canonical correspondence analysis (CCA) illustrating the ordination of diatom species across the lower, middle, and upper sections of the Tambo River watershed, along with 13 environmental variables. Environmental variables are represented by green arrows, while the seasons of the year are labeled in black uppercase letters: AUT = Autumn, WIN = Winter, SPR = Spring, and SUM = Summer. Diatom species are identified using the following codes: AMIN = *Achnanthidium minutissimum*, AMSP = *Amphora sp*, ESOR = *Epithemia sorex*, NLAN = *Navicula lanceolata*, DTEN = *Denticula tenuis*, NAMP = *Nitzschia amphibia*, NCLA = *Nitzschia clausii*, NPAL = *Nitzschia palea*, NINC = *Nitzschia inconspicua*, PFRE = *Planothidium frequentissimum*, SBRE = *Surirella brebissonii*, SOVA = *Surirella ovalis*, TAPI = *Tryblionella apiculata*, UULN = *Ulnaria ulna*, UACU = *Ulnaria acus*

However, in our study, this species exhibited its highest abundance in the upper section of the watershed, an area characterized by eutrophic conditions and elevated pH values. This observation contrasts with findings from Costa [2021], where *A. minutissimum* was the dominant species in mesotrophic waters and in waters with high nutrient enrichment. The discrepancy between these studies may reflect regional variations in environmental factors that influence species distribution linked to their tolerance to nutrient levels. As documented by Donato-R et al, [2022] *Achnanthidium minutissimum* and *Epithemia sor*ex, another frequently occurring species in our study. *Nitzschia clausii* exhibited the highest abundance in the lower section of the watershed during autumn (sites E2 and E4), a period when electrical conductivity values exceeded the Environmental Quality Standards (ECA) (2500 µS cm^{-1}). According to the literature, this species has been reported in eutrophic rivers near agricultural lands where runoff contains high concentrations of phosphates and nitrates. Although in our study nitrate levels were recorded below 0.2 mg L^{-1}, the total phosphorus (TF) levels classified this section as hyper-eutrophic.

According to Torres-Franco et al. [2019], in the Chili River, the Shannon diversity index (H') ranged from 1.1 to 2.4, with the highest values observed in the lower section. This pattern may be influenced by hydrological dynamics specific to Andean rivers, where flow regimes are largely driven by steep altitudinal gradients rather than seasonal variations, as is typical in temperate regions [Donato-R et al., 2022]. Such gradients regulate water velocity and sediment transport, which in turn affect habitat heterogeneity and therefore the distribution of biological assemblages. Consequently, the observed increase in the middle section of the river could reflect the cumulative effects of these geomorphological and ecological processes. In the Ocoña River, another river located in southern Peru, H' varied between 0.51 and 2.45 [Motta and Ranilla, 2024], with the lowest values observed in autumn. In our study, H' ranged from 1.9 to 3.59, with sites exhibiting higher diversity in spring in the lower section of the watershed. Compared to rivers impacted by wastewater and agricultural activities, the lower section of the Tambo River showed higher species richness and higher H' and Pielou's evenness index (J'). This higher diversity may be due to species contributions from the upper watershed, resulting in a richer and more diverse diatom assemblage [Marino et al., 2024],

Lobo et al. [1995], in their study conducted in 28 rivers in Tokyo (Japan), reported that Shannon and Pielou indices reached their highest values in moderately polluted sites, although Pielou's evenness index (J') showed a weak trend. While the Shannon index is considered a good indicator of environmental impact on diatoms, it has been criticized for not considering important aspects such as species tolerance, sensitivity, and adaptability to environmental changes [Lobo and Kobayashi, 1990]. Similarly, species diversity and richness varied over time but did not reflect differences in water quality across study sites in a river in Argentina [Nicolosi Gelis et al., 2024]. When evaluating Pielou's evenness index (J'), we observed that the middle and upper sections of the Tambo River showed a uniform trend in the diatom community (> 67%), whereas in the lower section, values were < 67%.

Physicochemical variables and their relationship with the epilithic diatom community

According to Peruvian environmental legislation, which establishes environmental quality standards (ECA) for aquatic ecosystem conservation, electrical conductivity (EC) exceeded the established limit (1000 µS cm^{-1}) at all sampled sites in the watershed. Additionally, the ECA threshold for irrigation water (2500 µS cm^{-1}) was exceeded in the lower section. The pH limit (8.5) was exceeded in the upper section during spring and winter 2019. The entire watershed exhibited an alkaline pH trend, primarily due to its natural hydrogeological characteristics [ANA, 2015; INGEMMET, 2020].

The diatom community in freshwater ecosystems is strongly influenced by dissolved oxygen (DO) levels [Jannel et al., 2024]. DO levels can serve as an indicator of organic pollution, as adequate DO concentrations are essential for water quality, but excessively high levels may negatively affect aquatic life [Latif et al., 2024]. Several studies have reported high DO values (> 6–11.7 mg L^{-1}) in eutrophic environments [Schuch et al., 2015; Andrade-Servín and Israde-Alcántara, 2021]. Similar conditions were found in the Tambo River, where, despite being eutrophic, the altitudinal gradient of this river, could influence DO solubility, combined with the effects of temperature and atmospheric pressure, likely leading to higher DO concentrations in the lower section. Conversely, low DO levels recorded during summer across the entire watershed could be attributed to increased runoff from rainfall, which elevates turbidity and decreases DO due to suspended sediments and organic matter transport [Shields and Knight, 2012]. Also attributable to temperature, in the Sava River, Slovenia, it was observed that during winter sampling, there were smaller temperature changes downstream than in summer and significantly more oxygen was dissolved in the water [Zelnik and Sušin, 2020]. Literature suggests that diatom species richness increases as DO levels rise [Teittinen et al., 2016; Dalu et al., 2017], a trend confirmed in our study, as the highest species richness was recorded in the lower section, where DO levels averaged 8.42 ± 2.16 mg L^{-1}.

The entire watershed recorded electrical conductivity (EC) values ranging from 1647.27 to 4905.41 µS cm^{-1}, exceeding the reference value in the lower section of the watershed ($p < 0.05$). This situation is likely due to two main factors: first, natural factors related to the hydrogeological characteristics of the watershed, which is composed primarily of rocks rich in major cations [ANA, 2015; INGEMMET, 2020]; and second, anthropogenic factors, such as the use of fertilizers and agrochemicals resulting from intensive agricultural activity, which are transported via runoff facilitated by the steep slope from the upper to the lower watershed. Nitrate and ammonium levels did not show significant differences ($p > 0.05$) across watershed sections, contrary to expectations, whereas TF exhibited significant differences ($p < 0.05$). According to Environment Canada (2004), which categorizes trophic states of water bodies based on TF, the middle and lower sections of the watershed were classified as hypereutrophic (TF > 100 µg L^{-1}), while the upper section was classified as eutrophic (35 < TF < 100 µg L^{-1}). Studies in Andean rivers of Ecuador identified *Nitzschia palea*, *Nitzschia clausii*, and *Navicula lanceolata* as dominant taxa in eutrophic conditions [Castillejo et al., 2023], mirroring their abundance in our findings from southern Peru, which suggests a consistent ecological response to nutrient enrichment across Andean regions.

An experimental study conducted in streams within the Pescado River Basin in Buenos Aires, Argentina, which are heavily impacted by agricultural activities, found that the most abundant species included *Nitzschia palea, Nitzschia amphibia, Nitzschia clausii,* and *Nitzschia filiformis* [Nicolosi Gelis et al., 2024]. *Nitzschia palea* is recognized as a tolerant species that can thrive in high-nutrient environments, organic pollution, and agrochemical contamination [Debenest et al., 2010; Lobo et al., 2015; Shibabaw et al., 2021]. Under experimental conditions, this species has been observed to dominate environments with natural light, alkaline pH (8–9), and high temperatures ranging between 25 to 30 °C [Hao et al., 2021]. In our study, *Nitzschia palea* showed the highest abundances in autumn, the season in which the lowest sodium (Na) concentrations were recorded.

Chemical variables such as total phosphorus and electrical conductivity showed significant relationships ($p < 0.05$) and contributed to variations in the structure of benthic diatom communities in lacustrine environments [Rusanov et al., 2024]. Similarly, several studies conducted in the Safsaf, Kebir, and Guebli river basins in Algeria, as well as the Gilgel Gibe basin in Ethiopia [Shibabaw et al., 2021; Kaddeche et al., 2022], reported that conductivity was one of the key variables controlling diatom distribution. *Nitzschia clausii* was one of the species that positively correlated with EC and TDS. It has been reported in environments with conductivity around 13.4 µS cm^{-1}, pH between 5.7 and 5.9, and dissolved oxygen (DO) levels ranging from 4.5 to 7 mg L^{-1} [Sala et al., 2015]. However, in our study, we recorded its highest abundance in the lower section of the watershed during autumn, where EC (3084.01 µS cm^{-1}) and TDS (1444.53 mg L^{-1}) reached some of their highest concentrations, along with an alkaline pH tendency (8.4) and high DO levels.

Castillejo et al. [2024] reported *Achnanthidium minutissimum* as being abundant exclusively in non-urban sites in Andean rivers in Ecuador. It has also been reported as dominant and frequent in sampling sites without agricultural activity or human settlements in the Sakarya River in Turkey and the Ljubovića River in Serbia [Sevindik et al., 2023; Milićević et al., 2024]. According to Lange-Bertalot et al. [2017] it is one of the most widespread and early-colonizing species in aquatic environments. *Achnantidium minutissimum* was classified as indicators of anthropopressure, especially with regard to high nutrient contents as a codominant species in the Sava River located in Slovenia [Zelnik and Sušin, 2020]. In our study, this species was very frequent, with its highest abundance recorded in the upper section of the watershed, which is characterized as a non-urban zone. However, when establishing relationships with environmental variables, we observed only a weak correlation with pH.

A genus of great importance due to its high relative abundance was *Surirella*, considered an indicator of the transition between freshwater and marine environments, in addition to its preference for brackish waters; it has also been reported in freshwater ecosystems (Solak et al., 2023). In our study, we recorded two species of *Surirella*: *Surirella brebissonii* and *Surirella ovalis*. Despite their differences in environmental preference, *S. brebissonii* prefers environments with the lowest concentrations of positive ions such as boron, magnesium, and sodium, as well as lower EC, TDS, and temperature values. Instead, *S. ovalis* preferred more oxygenated environments ($r_s > 0.5$). In freshwater systems of the Eastern Mediterranean region and the Western Middle East, this genus is found across a wide range of habitats (Solak et al., 2023). It appears that EC and TDS, are associated with the use of fertilizers and agrochemicals, and are responsible for changes in the abundance of diatom species such as *Nitzschia clausii* and *Surirella brebissonii*. However, these interpretations require further field and laboratory studies to validate the findings. Silicon, the chemical element that forms silica, was found in lower concentrations during autumn ($p < 0.05$) compared to other seasons. Since silica is the primary component of diatom cell walls (Round et al., 1990), we expected to find a strong positive correlation with the highest abundance of all large taxa. However, we only observed moderate correlations with *Tryblionella apiculata* ($r_s > 0.81$) and *Navicula lanceolata* ($r_s > 0.58$).

Contrary to our expectations, we did not find strong spatiotemporal correlations between relative abundance of diatoms and environmental variables. Instead, we observed moderate correlations that influenced the seasonal variation in diatom assemblages. This suggests that other predictive variables may directly influence the structuring of this aquatic community.

CONCLUSIONS

We conclude that the spatio-temporal variation in the relative abundance of epilithic diatom species was influenced more by temporal than spatial changes. In contrast, species richness was influenced by spatial variations, with the highest richness observed in the lower section of the basin. The Tambo River basin is exposed to significant local agricultural pressure, likely shaping its physicochemical characteristics, leading to eutrophic and hypereutrophic conditions. A spatio-temporal variation in physicochemical variables was determined, which likely influenced the variability in diatom community structure. A moderate linear relationship was found between the relative abundance of certain species and environmental variables such as conductivity, temperature, and major cations, which have potential as bioindicators of water quality.

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