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The use of biological indices and morphology of diatoms for the environmental assessment of streams

O uso de índices biológicos e da morfologia de diatomáceas na avaliação ambiental de riachos

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Abstract: Aim: Our aim was to verify whether morphological diatom metrics performed as effectively as diversity metrics and diatom indices for ecological assessments in streams in the Brazilian Pampa biome. **Methods:** We sampled physical and chemical variables along with benthic diatom communities from 15 Pampean streams. These streams are inserted along a gradient of agricultural land use. We calculated species richness, and three common diatom indices used for stream ecological assessment. In addition, we quantified the percentage of deformed valves in the communities and analyzed the shape of *Achnanthidium minutissimum* s.l. through geometric morphometrics. All biological responses were evaluated in relation to physical and chemical variables summarized through Principal Component Analysis (PCA). **Results:** We found that the Pampean Diatom Index was positively related to turbidity and velocity and negatively related to total nitrogen and pH. On the other hand, the percentage of deformed diatom valves and the different shapes of *A. minutissimum* s.l. were associated with total phosphorus, depth and total dissolved solids. **Conclusions:** Our study is one of the first to investigate morphological biomarkers in diatoms for ecological assessments in the Brazilian Pampa biome. We found that morphological diatom metrics were more effective in assessing the effects of the land use gradient is streams compared to taxonomic metrics. However, we highlight the importance of replicating this study with a larger sample size and across lotic environments influenced by diverse impacts. This will improve the understanding of the utility of different metrics for ecological assessments and biomonitoring in streams.

Keywords: diatom index; teratology; *Achnanthidium minutissimum*; geometric morphometrics; Pampa biome.

Resumo: Objetivo: Nosso objetivo foi verificar se as métricas morfológicas de diatomáceas teriam um desempenho tão eficaz quanto as métricas de diversidade e os índices de diatomáceas para avaliações ecológicas em riachos no bioma Pampa brasileiro. **Métodos:** Amostramos variáveis físicas e químicas, juntamente com comunidades de diatomáceas bentônicas de 15 riachos do bioma Pampa. Esses riachos estão inseridos ao longo de um gradiente de uso agrícola. Calculamos a riqueza de espécies e três índices de diatomáceas comumente utilizados na avaliação ecológica dos riachos. Além disso, quantificamos a porcentagem de valvas deformadas nas comunidades e analisamos a forma de *Achnanthidium minutissimum* s.l. por meio de morfometria geométrica. Todas as respostas biológicas foram avaliadas em relação às variáveis físicas e químicas, sumarizadas em uma Análise de Componentes Principais (PCA). **Resultados:** Verificamos que o Índice de Diatomáceas Pampeanas apresentou correlação positiva com a turbidez e a velocidade, e negativa com o nitrogênio total e o pH. Por outro lado, a porcentagem de valvas de diatomáceas deformadas e diferentes formas de *A. minutissimum* s.l. apresentaram associação com o fósforo total, profundidade e sólidos totais dissolvidos. **Conclusões:** Nosso estudo é um dos primeiros a investigar biomarcadores morfológicos em diatomáceas para avaliações ecológicas no bioma Pampa brasileiro. Constatamos que as métricas morfológicas para diatomáceas foram mais eficazes na avaliação dos efeitos do gradiente de uso da terra em riachos em comparação com as métricas taxonômicas. No entanto, destacamos a importância de replicar este estudo com uma amostra maior e em ambientes lóticos influenciados por impactos diversos. Isso aumentará a compreensão sobre a utilidade de diferentes métricas para avaliações ecológicas e biomonitoramento em riachos.

Palavras-chave: índice de diatomáceas; teratologia; *Achnanthidium minutissimum*; morfometria geométrica; bioma Pampa.

1. Introduction

In recent decades, anthropogenic activities have been responsible for the ecological imbalance in several natural ecosystems, especially in freshwater environments (Dudgeon, 2019; Antonielli et al., 2024). Freshwater ecosystems support high biodiversity and provide essential ecosystem services to humans (e.g., Newton et al., 2018). However, these ecosystems have been destroyed by human activities at a rapid pace through impacts such as hydrological changes (e.g., water withdrawal), water pollution, habitat degradation, eutrophication, and alteration of the natural landscape for purposes like livestock and monoculture activities (Dudgeon, 2019; He et al., 2019).

The detection of anthropogenic impacts on freshwater depends on ecological assessments and further biomonitoring of ecosystems (e.g., Friberg et al., 2011). However, due to the accelerated process of environmental degradation, strategies for efficient ecological assessment and biomonitoring need to consider the cost and time required to process the sampled biological material in the laboratory (Bennett et al., 2017). Studies that seek such approaches commonly evaluate the use of taxonomic surrogates (Landeiro et al., 2012), reduced taxonomic resolution (Oliveira Junior et al., 2020) or reduced counting effort (Castro et al., 2023). However, the use of non-taxonomic approaches has been less evaluated, despite their potential to effectively reduce the cost and duration of sample processing (Costa & Schneck, 2022). Among these approaches, we highlight the use of biomarkers such as lipid body measurements in diatom cells or the presence of deformed valves, which have been shown to be efficient metrics for assessing water quality (Pandey et al., 2018).

Intensified land use is a major factor driving shifts in biological communities and contributing to biodiversity loss (Reid et al., 2019; Petsch et al., 2021). In river catchments, the replacement of native vegetation with agricultural or urban landscapes leads to marked changes in stream ecosystems, including increased nutrient concentrations and sedimentation, and changes in hydrological dynamics (Allan, 2004). For example, an excessive increase in nutrients, especially in low-order streams, causes a decrease in the species richness of periphytic diatom communities (Torres-Franco et al., 2019; Costa et al., 2022) and leads to changes in the structure of these communities (e.g., Soininen et al., 2004). Furthermore, increased sedimentation in the streambed reduces the biomass available to the periphytic community, as evidenced in experimental streams (Maciel et al., 2025).

The periphytic community is commonly found in shallow lotic environments with little or no riparian vegetation (Biggs, 1996). This is a complex community made up of various organisms such as bacteria, fungi, small invertebrates and microalgae (Wetzel, 1983). Pennate diatoms usually predominate in stream periphyton due to various substrate adhesion strategies that enable their successful colonization, making this community important for autochthonous primary production in these ecosystems (Allan & Flecker, 1993). In addition, diatoms commonly stand out in biomonitoring and ecological assessment studies due to their specific characteristics, such as a fast life cycle, wide distribution, and rapid response to environmental changes (Stevenson et al., 2010).

Assessments of alpha diversity (e.g., species richness or Shannon and Simpson indices) (e.g., Gokce & Gulbenk, 2019) or variation in community structure, as well as diatom indices (e.g., Pillsbury et al., 2019), are frequently used to evaluate anthropogenic impacts on freshwater ecosystems (Costa & Schneck, 2022). Moreover, diatom indices are commonly based on the frequency and abundance of species and are widely used to determine the trophic state of ecosystems, such as the Trophic Diatom Index (TDI; Kelly & Whitton, 1995). However, these metrics tend to be time-consuming and costly due to the need for a specialist in diatom taxonomy to identify all organisms at the species level. On the other hand, alternative metrics, such as morphological assessments, have been highlighted in literature as potential biomarkers of environmental stressors. These assessments include the percentage of teratological (or deformed) valves, which are already linked to industrial effluents (Pandey et al., 2018), and valve shape alterations, associated with acid mine drainage (Olenici et al., 2017). Morphological alterations in diatom valves are easily observed in response to environmental stress. In addition, teratological diatom valves are more frequently observed in environments with increased organic matter and nutrients (Dziengo-Czaja et al., 2008).

Here, we investigated the efficiency of different taxonomic metrics (e.g., diatom metrics that require the identification of entire communities) and morphological metrics in assessing streams along an environmental gradient in the Brazilian Pampa. We expected that morphological metrics (percentage of deformed valves and shape of *Achnanthidium minutissimum* s.l.) would be as efficient as taxonomic metrics (diversity and diatom indices) for the ecological assessment of Pampa streams.

2. Material and Methods

2.1. Study area

We sampled 15 independent low-order streams in the municipalities of São Gabriel, Rosário do Sul, and Alegrete in the state of Rio Grande do Sul, Brazil (Figure 1), in January 2020. The geographical distance between stream pairs ranged from 0.40 km (between streams 3 and 4) to 110 km (between streams 1 and 6). The climate of the region is subtropical Cfa according to the Köppen classification (Alvares et al., 2013). The sites are in the Brazilian Pampa biome, which is characterized by the predominance of grasslands (Overbeck et al., 2007). The Brazilian Pampa region has undergone accelerated conversion of its natural landscape into agricultural and silvicultural lands (Overbeck et al., 2007; Oliveira et al., 2017). All 15 streams studied are characterized as mesotrophic, according to the Trophic State Index of Environmental Company of the State of São Paulo (CETESB) based on total phosphorus concentration.

2.2. Environmental variables

We used a multiparameter probe to measure the pH, dissolved oxygen (DO; mg L^{-1}), electrical conductivity (EC; µS cm^{-1}), turbidity (NTU)

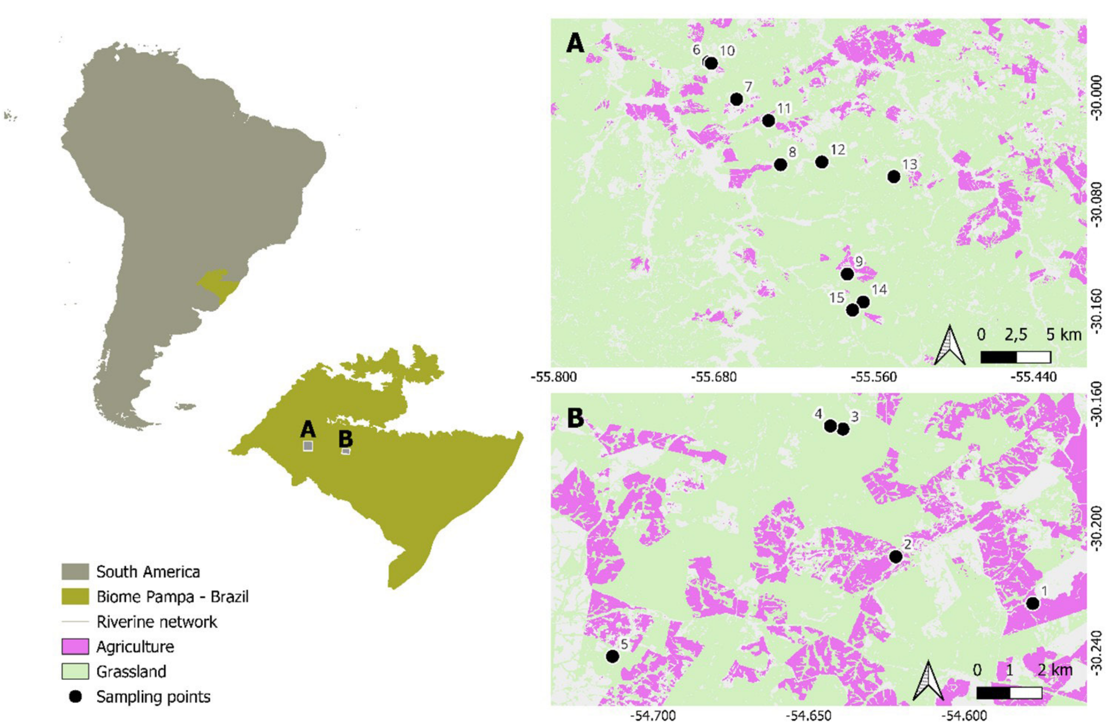

Figure 1. Fifteen streams sampled in the Brazilian Pampa biome.

and total dissolved solids (TDS; g L^{-1}). We made 10 measurements for each variable. We used a measuring tape to obtain the width and depth at five points in each stream. We also measured the flow water at five points using a flowmeter. Moreover, we sampled 500 ml of surface water from each stream for analyses of total nitrogen (TN; Allen et al., 1974) and total phosphorus (TP; Valderrama, 1981; Baumgarten et al., 1996) (Table 1).

2.3. Diatom sampling

We sampled benthic diatoms from the surface of streambed soft sediment using a syringe. For stones we use a brush. After sampling, we preserved the material in 4% formalin. In the laboratory, the biological material was oxidized according to Simonsen (1974), adapted by Moreira-Filho & Valente-Moreira (1981). Finally, we prepared permanent slides using Naphrax® (Brunel Microscopes Ltd., Chippenham, UK). For the community quantitative analyses (500 valves counted for each stream) we only used the communities from sediment because it was the substrate that occurred in all the 15 streams. The percentage of deformed valves was obtained simultaneously with the quantification of the community. We consider four categories of deformities: raphe alteration, deformed striations, altered valve outline or mixed alterations (Pandey et al., 2014). For geometric morphometrics analyses, we used populations of *Achnanthidium minutissimum* s.l. (represented by 50 specimens from each stream) (Figure 2) from sediment and stones. We chose to use both substrates for the *A. minutissimum* s.l. evaluations to increase the number of individuals measured within the populations. Moreover, we chose this species because it occurred abundantly in the streams. For diatoms identification and counting, we used a Zeiss optical microscope with a magnification of 1000×. We identified taxa at the lowest possible taxonomic level using specialized bibliography. We found 229 diatom taxa distributed among the 15 studied streams (the complete list of taxa can be found in Costa & Schneck, 2024).

2.4. Geometric morphometrics analyses

Each of the 750 specimens of *Achnanthidium minutissimum* s.l. (50 per stream) were previously identified and photographed using an optical microscope with a camera at a magnification of 1000×. Next, we identified the images and converted them into .tps files using tpsUtil software version 1.78 (Rohlf, 2013). For each specimen, two landmarks were recorded, and valve morphology was modeled using two configurations of curves, each containing 25 semi-landmarks – totaling 50 semi-landmarks (Figure 2). The landmarks and semi-landmarks were recorded using the tpsDIG version 2.31 (Rohlf, 2015). To determine *A. minutissimum* s.l. valve shape through geometric morphometrics, we used coordinates from generalized Procrustes analyses (GPA; Rohlf & Slice, 1990). This allows to obtain shape information with the effects of translation, scaling, and rotation minimized. We ran Principal Component Analysis (PCA) to summarize the information about *A. minutissimum* s.l. shape and

Table 1. Mean of environmental variables from 15 streams sampled on Brazilian Pampa.

Stream	pH	DO (mg L^{-1})	Cond (µS cm^{-1})	TDS (g L^{-1})	Turbidity (NTU)	TP (µg L^{-1})	TN (mg L^{-1})	Vel (cm s^{-1})	Width (m)	Depth (cm)
1	7.8	11.1	0.22	0.14	24.14	72.6	0.77	<1.0	5.2	49.1
2	7.4	5.6	0.10	0.07	41.32	52.1	0.60	<1.0	5.9	49.8
3	6.9	7.3	0.04	0.02	29.16	94.1	0.84	<1.0	2.5	23.9
4	7.2	7.9	0.06	0.03	20.98	38.8	0.63	<1.0	1.9	25.5
5	7.5	7.8	0.03	0.02	6.38	134.4	0.92	<1.0	1.2	20.2
6	7.3	6.6	0.15	0.09	21.75	43.0	0.44	21.0	1.1	17.5
7	7.9	7.9	0.07	0.04	18.79	47.1	0.60	23.9	3.0	14.6
8	7.1	8.0	0.04	0.02	32.20	30.6	0.52	66.4	2.2	21.9
9	7.5	6.2	0.08	0.06	29.28	49.6	0.74	<1.0	1.0	9.5
10	7.6	7.6	0.04	0.03	18.20	11.4	0.56	<1.0	5.5	5.4
11	7.4	7.0	0.06	0.04	18.27	25.7	0.60	9.7	4.1	16.8
12	8.4	7.8	0.12	0.08	9.14	29.0	0.81	<1.0	1.3	9.8
13	7.5	6.4	0.11	0.07	19.94	27.3	0.81	<1.0	1.9	39.8
14	7.5	8.0	0.07	0.05	23.67	33.1	0.74	30.5	4.1	7.3
15	7.3	6.7	0.07	0.04	32.72	96.5	0.60	38.5	4.7	11.1

Cond = electrical conductivity; DO = dissolved oxygen; TDS = total dissolved solids; TP = total phosphorus; TN = total nitrogen; Vel = current velocity.

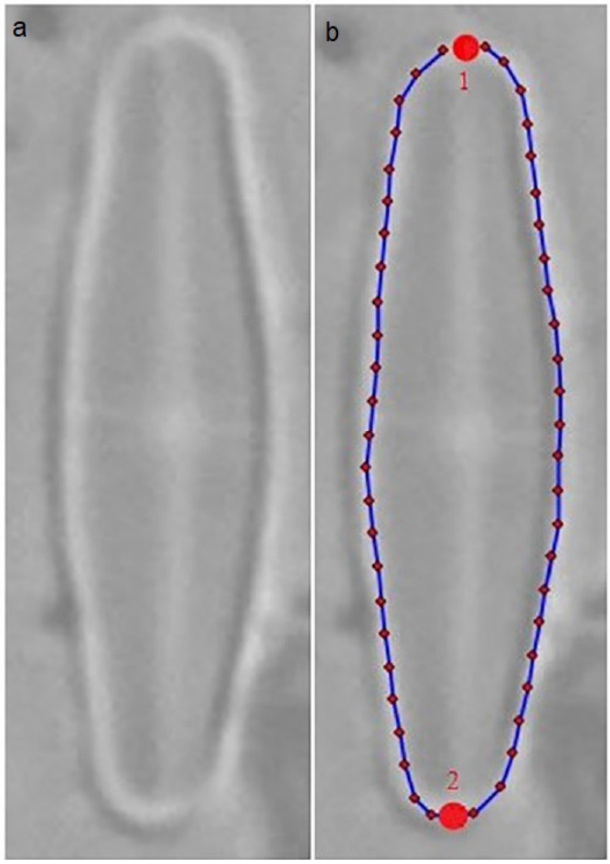

Figure 2. *Achnanthidium minutissimum* sensu lato; the species of diatom used for geometric morphometrics analysis (a), and position of landmarks (1 and 2) and 50 semi-landmarks forming two curves along the valve outline (b).

identify the axes of maximal and minimal shape variance among all specimens. These analyses were performed with the symmetrical component.

2.5. Data analysis

For each stream, we obtained the species richness and calculated three diatom indices: the *l'Indice de Polluosensibilité Spécifique* (IPS; Cemagref, 1983), the Trophic Diatom Index (TDI; Kelly & Whitton, 1995; Kelly, 1998), and the Pampean Diatom Index (IDP; Gómez & Licursi, 2001). These diatom indices are calculated based on the occurrence and abundance of each taxon, each of which is assigned to a sensitivity value. TDI is more specific for detecting eutrophication impacts, whereas IDP and IPS are sensitive to both eutrophication and organic pollution. Non-taxonomic metrics included the percentage of deformed frustules within 500 valves and the shape of *A. minutissimum* s.l.

We evaluated the correlation among environmental variables, and those with correlation coefficients greater than 0.8 (conductivity and oxygen saturation) were excluded from further analyses. We performed a Principal Component Analysis (PCA) to reduce and summarize the dataset of environmental variables. Finally, we used linear regression to examine the influence of environmental variables (represented by the first two principal components from the PCA) on richness, diatom indices, and morphological characteristics of diatoms.

We used the R environment (R Core Team, 2022) to perform all analyses. For diatom indices we used the *DiaThor* package (Gelis et al., 2022), for geometric morphometrics analysis we used *Geomorph* (Adams & Otárola-Castillo, 2013) and *MASS* (Venables & Ripley, 2002), and for graphic illustrations we used *ggplot2* (Wickham, 2016).

3. Results

The streams exhibited variation in their limnological characteristics (Figure 3). The first two PCA axes explained 50.1% of the total variation in environmental variables and were retained for further analysis. The first principal component accounted for 27.5% of the data variation, showing positive associations with turbidity and velocity and negative associations with pH and total nitrogen (Figure 3). The second principal component

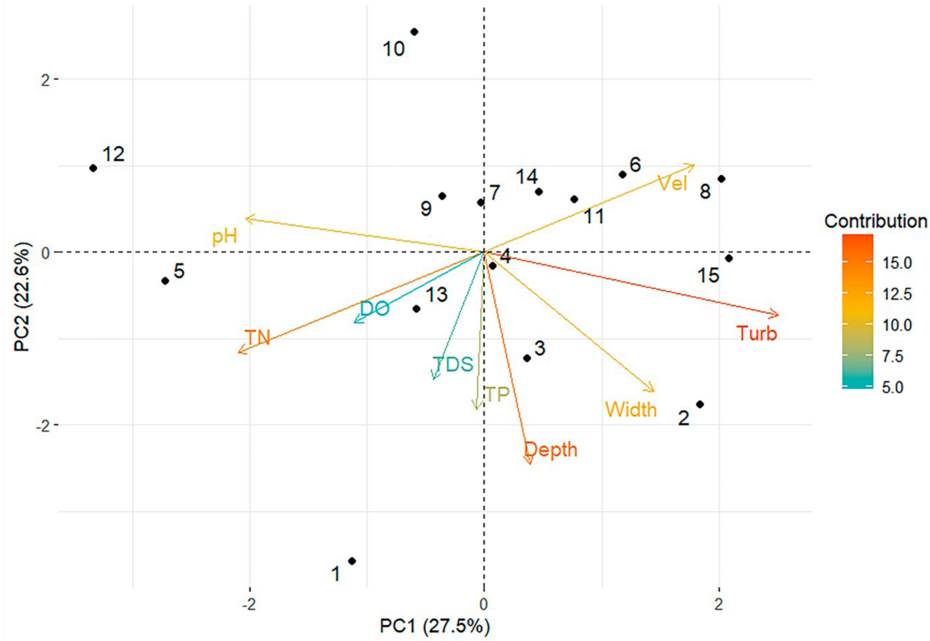

Figure 3. Principal Component Analysis (PCA) using limnological variables from 15 streams in the Brazilian Pampa. Numbers represent the streams sampled; TP = total phosphorus; TN = total nitrogen; TDS = total dissolved solids; DO = dissolved oxygen; Vel = velocity; Turb = turbidity.

accounted for 22.6% of the data variation, showing negative associations with water depth, total phosphorus and total dissolved solids (Figure 3).

Diatom species richness ranged from 37 to 81 species per stream (Table 2). Values of the diatom indices varied across streams, with IDP ranging from 1.48 to 2.99, IPS from 10.77 to 17.40, and TDI from 31.08 to 67.30 (Table 2). The percentage of deformed frustules per stream varied from 0 to 2.8% (Table 2).

Regarding valve shape, the first principal component (85.15% of total variability) mostly described changes at the apices of the *A. minutissimum* s.l. (Figure 4a), so that the valve outline at apices changed from elliptic (Figure 4b) to capitate (Figure 4c).

There was no relationship between species richness, TDI and IPS with environmental conditions of the streams (Table 3). On the other hand, the IDP index was positively correlated with turbidity and negatively correlated with pH and total nitrogen (Table 3; Figure 5a). In addition, an increase in the percentage of deformed diatom valves was positively correlated with depth, total dissolved solids and total phosphorus (Table 3; Figure 5b). Similarly, the shape of *A. minutissimum* shifted from an elliptic to a capitate outline at the apices as stream depth, total dissolved solids, and total phosphorus concentrations increased (Table 3; Figure 5c).

4. Discussion

Among the taxonomic metrics, only the Pampean Diatom Index (IDP) responded significantly to environmental gradients, showing a positive relationship with turbidity and velocity, and negative associations with pH and total nitrogen. In addition, the percentage of deformed diatom valves was positively correlated with total phosphorus concentrations, total dissolved solids, and depth. Furthermore, increases in total phosphorus, total dissolved solids and stream depth influenced changes from elliptic to capitate apices outline of *Achnanthidium minutissimum* s.l.

Elevated levels of nutrients, dissolved solids, and water turbidity are frequently used as proxy indicators of agricultural land use in streams. These conditions are commonly associated with lower diatom species richness, whereas reference environments typically exhibit higher diatom species richness than agriculturally impacted sites (Gabel et al., 2012). In the last years, diatom diversity indices have been commonly used in biomonitoring or ecological assessment of lotic environments (Costa & Schneck, 2022). However, diversity metrics may differ in their sensitivity to the effects of agricultural land use surrounding streams. For instance, although species richness tended to be higher in reference sites, the Simpson index failed to distinguish significantly between agricultural

Table 2. Evaluated metrics per stream.

Stream	*S*	IDP	IPS	TDI	DVD (%)
1	76	2.16	12.63	63.18	2.2
2	69	1.99	14.42	48.99	2.8
3	67	2.52	14.10	32.49	1.0
4	48	2.03	14.13	37.24	2.6
5	37	1.48	17.40	31.08	2.0
6	81	2.58	11.76	49.86	0.6
7	79	2.55	11.13	67.30	1.4
8	66	2.29	14.60	46.01	0.6
9	78	2.22	12.10	51.30	1.6
10	54	2.07	15.63	44.34	0.2
11	67	2.41	10.77	64.88	0.2
12	71	1.97	14.63	51.16	0
13	55	1.69	16.44	44.23	0.2
14	70	2.59	13.57	59.87	0
15	72	2.99	12.37	65.13	0

S = diatom species richness; IDP = Pampean Diatom Index; IPS = *l'Indice de Polluosensibilité Spécifique*; TDI = Trophic Diatom Index; DVD = diatom valve deformation.

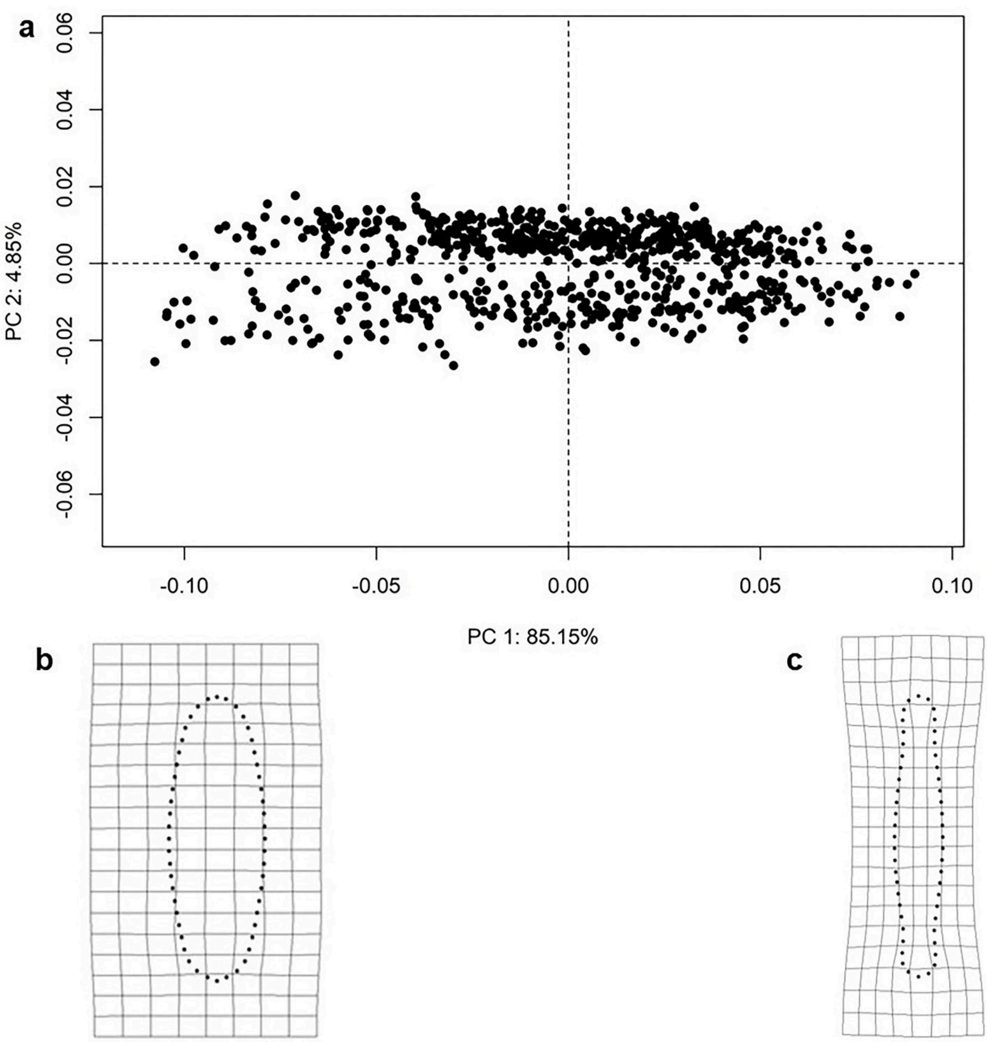

Figure 4. Principal Component Analysis (PCA) of total valve shape variation in *A. minutissimum* s.l. populations (a); negative extreme position in morphospace in the first principal component (b); and positive position in morphospace in the first principal component (c).

Table 3. Results of the linear regressions for diatom richness.

	Estimate	SE	t-value	P-value	adjusted R²
Richness					
Intercept	65.067	3.128	20.799	<0.001	
PCA axis 1	2.834	2.058	1.377	0.192	0.06
Intercept	65.066	3.342	19.47	<0.001	
PCA axis 2	-0.557	2.426	-0.23	0.822	-0.07
TDI					
Intercept	50.477	3.034	16.639	<0.001	
PCA axis 1	1.981	1.995	0.993	0.339	-0.001
Intercept	50.476	3.146	16.044	<0.001	
PCA axis 2	-0.104	2.283	-0.046	0.964	-0.07
IPS					
Intercept	13.715	0.466	29.383	<0.001	
PCA axis 1	-0.521	0.307	-1.699	0.113	0.11
Intercept	13.715	0.515	26.581	<0.001	
PCA axis 2	-0.015	0.374	-0.041	0.968	-0.07
IDP					
Intercept	2.241	0.079	28.122	<0.001	
PCA axis 1	0.157	0.052	3.003	**0.010**	0.36
Intercept	2.241	0.102	21.842	<0.001	
PCA axis 2	0.039	0.074	0.532	0.604	-0.05
Deformities					
Intercept	1.026	0.267	3.837	<0.001	
PCA axis 1	-0.030	1.795	-0.172	0.866	-0.07
Intercept	1.026	0.216	4.733	<0.001	
PCA axis 2	-0.411	0.0157	-2.611	**0.020**	0.29
***A. minutissimum* shape**					
Intercept	0.0007	0.003	0.205	0.841	
PCA axis 1	0.003	0.002	1.310	0.213	0.04
Intercept	-0.001	0.003	-0.369	0.718	
PCA axis 2	-0.006	0.002	2.530	**0.020**	0.27

TDI = Trophic Diatom Index; IPS = *l'Indice de Polluosensibilité Spécifique*; IDP = Pampean Diatom Index; percentage of deformities in valve diatoms and *A. minutissimum* shape. Separate regressions were performed using each PCA axis as a predictor. For each regression, the intercept, estimate (slope), standard error (SE), *t*-value, *p*-value, and adjusted R^2 are reported. Significant *p*-values ($p < 0.05$) are shown in bold.

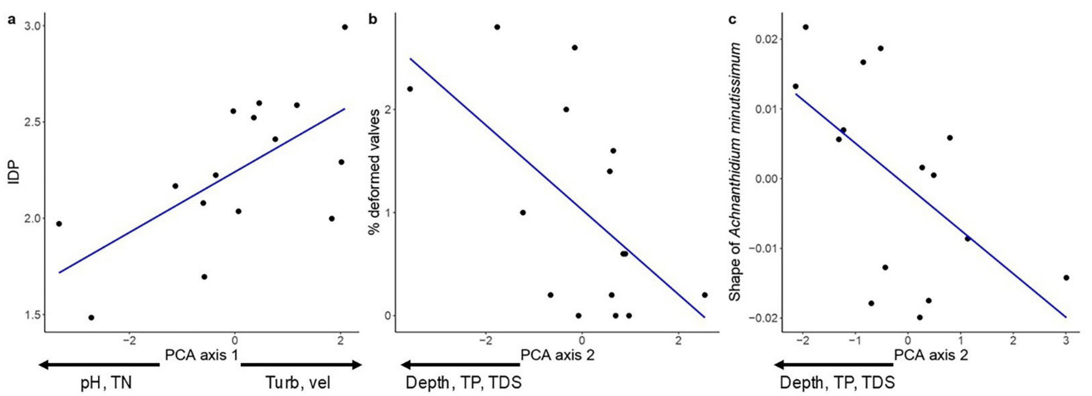

Figure 5. Relationships between diatom metrics and predictor variables derived from Principal Component Analysis (PCA): (a) Pampean Diatom Index (IDP); (b) percentage of deformed diatom valves; (c) variation in the shape of *A. minutissimum*. TN = total nitrogen; Turb = turbidity; Vel = velocity; TP= total phosphorus; TDS = total dissolved solids.

and reference streams (Gabel et al., 2012). In fact, algal diversity can be influenced not only by local factors, such as physical and chemical characteristics but also by factors acting at regional scales, such as climate or landscape (Chase & Leibold, 2002). In this sense, using only diatom diversity indices for stream ecological assessments is commonly discouraged (Blanco et al., 2012).

In general, diatom indices are based on values assigned to species depending on their sensitivity and frequency (e.g., Stoermer & Smol, 1999; Rey et al., 2008) and are widely used to assess the ecological status of freshwater ecosystems (Costa & Schneck, 2022). Here, only the regionally applied IDP index showed a significant relationship with the environmental gradient. The values of this index typically increase as water quality declines due to organic pollution, eutrophication, and agricultural activities; however, in our results, the lowest index values were associated with higher concentrations of total nitrogen. This lack of effective response of traditional diatom indices is not a new finding, as they can be influenced by factors such as taxonomic uncertainty or regional differentiation, such as ecoregion parameters (Besse-Lototskaya et al., 2011). Moreover, the initial effects of eutrophication in lentic environments were also not detected by traditional diatom indices but by morphological biomarker metrics (Vilmi et al., 2015).

Non-taxonomic metrics proved to be more effective in capturing environmental variation in the studied streams. The positive relationship between the percentage of deformed valves and total phosphorus concentrations highlights the efficacy of this metric, indicating its potential for application in streams affected by land use intensification. The percentage of diatom-deformed valves is easy to measure when compared to community assessment approaches at the species level, and this metric has been used to assess anthropogenic impacts such as heavy metals (Pandey et al., 2014), pesticides (Lavoie et al., 2017), and eutrophication (Falasco et al., 2009). Here, the highest percentage of deformed frustules was 2.6%, which was within the expected percentage for preserved environments (<5%, according to Lavoie et al., 2017). Nevertheless, even such low deformation rates have been shown to effectively indicate increased nutrient levels in the studied low-order streams.

We performed prior taxonomic identification of the entire quantified community (including *A. minutissimum* s.l.). Subsequently carried out morphometric analyses of *A. minutissimum* s.l. because it was abundant in several streams. In addition, previous studies had demonstrated changes in valve shape of *A. minutissimum* in response to heavy metals (Cerisier et al., 2019) and water acidification (Olenici et al., 2017). Our results on valve shape of *A. minutissimum* s.l. indicate that geometric morphometry is a tool that could also be potentially used to identify freshwater ecosystems differing in their environmental characteristics (e.g., Olenici et al., 2017). Despite the complexity of the analysis, measuring the valve shape of diatom populations can be a less time-consuming and cheaper alternative than identifying communities at the species level to apply traditional taxonomic metrics.

In conclusion, our study is a first step to investigate the use of biomarkers for ecological assessments in streams of the Brazilian Pampa biome. The non-taxonomic metrics responded better to agricultural land use than traditional diatom metrics. We thus emphasize that studies that include greater replication in space and time are necessary to determine the most effective metrics to ecological assessments of streams from the Brazilian Pampa.

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Data availability

Data supporting the results of this study are available upon request from the corresponding author.

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