



SPATIAL AND TEMPORAL DYNAMICS OF PHYTOPLANKTON IN A HIGHLY IMPACTED TROPICAL MONTANE RIVER

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Abstract: Rivers, as most bodies of water, are subject to eutrophication and water contamination, mainly from domestic sewage and industrial discharges from urban centers and agricultural areas. These growing human impacts result in a gradual loss of water quality and changes in biotic communities, including phytoplankton. Analyzing the dynamics of phytoplankton is important to understand how, in addition to hydrography and the physical, chemical and biological variables, human impact can be a controlling factor in this dynamic. In this study, we analyzed abiotic variables and zooplankton abundance to identify the factors controlling different phytoplankton attributes at six sampling points, in two climatic periods and three years, in the Piabanha River, a slowly flowing stream with to strong human impact beginning in its upper course, in a mountain region in southeastern Brazil. The results showed homogeneous environmental variables and different phytoplankton attributes along most of the stretch analyzed, except for sampling point 1, located in a preserved forest area but with nutrient concentrations limiting phytoplankton growth. The difference in flow between the two climatic periods (dry and wet) was not sufficient to change the total phytoplankton biovolume. Temperature seemed to strongly affect the phytoplankton response, with larger relative contributions from cyanobacteria in the wet period, with higher temperatures, and from phytoflagellates in the dry period, with lower temperatures. As expected, zooplankton showed no influence on phytoplankton dynamics, with a large contribution from small rotifers. In this tropical montane river, with slow flow and intense human impacts, nutrient concentrations and temperature were the main drivers of the spatial and temporal dynamics of phytoplankton, respectively.

Keywords: eutrophication; flow regime; Piabanha River; Potamoplankton; water temperature.

INTRODUCTION

For survival in rivers, phytoplankters require adaptations to compensate for the hydrological characteristics of these systems, with the ability to live in turbulent systems with large fluctuations in light availability. The phytoplankton species tend to have high surface-to-volume ratios, silica exoskeletons (Reynolds 2000), and high growth rates (Reynolds *et al.* 2002, Reynolds 2006, Soares *et al.* 2007). In addition, phytoplankters depend strongly on lentic zones, which are generally confined areas with a longer residence time and higher water temperature, which support higher biomass and exchange their water with the main flow, thus contributing inocula to the system (Reynolds 2006, Descy *et al.* 2017). The contributions of benthic forms brought into suspension (tychoplankton) and inocula of true planktonic taxa from tributaries are especially important in these environments (Descy *et al.* 2017).

In temperate rivers, diatoms comprise the majority of phytoplankton species. In tropical rivers, diatoms can also be important (Graco-Roza *et al.* 2020), although relatively larger contributions of desmids and coccal green algae, both unicellular and colonial, are common (Rojo *et al.* 1994, Zalocar de Domitrovic *et al.* 2014, Descy *et al.* 2017). In terms of biovolume or abundance, in tropical rivers the phytoplankton may be dominated by diatoms and green algae (Abony *et al.* 2012, Descy *et al.* 2017). Although nutrient concentration is not a determining factor in phytoplankton composition, cyanobacteria tend to be favored in rivers with high nutrient concentration and low flow (Soares *et al.* 2007, Devercelli & O'Farrell 2013, Okogwu & Ugwumba 2013). Phytoflagellates, represented mainly by euglenoids and cryptomonads, can also form large populations in nutrient-enriched rivers (Devercelli & O'Farrell 2013, Santana *et al.* 2016). Despite the growing problem of eutrophication in rivers, knowledge of the responses of these environments to eutrophication is still limited, due mainly to their dynamic nature, *i.e.*, the effects of turbidity on light availability, the short water residence times, and the phytoplankton response to this dynamic (Hilton *et al.* 2006, Okogwu & Ugwumba 2013). Some studies have

indicated that high concentrations of N and P select for certain species, mainly filamentous cyanobacteria (Cardoso *et al.* 2017), and may lead to greater homogenization of phytoplankton in eutrophic rivers (Wengrat *et al.* 2018).

In Brazil, despite the extensive hydrographic network, studies of the composition and dynamics of riverine phytoplankton are still incipient. The Piabanha River, located in a high-altitude region of the State of Rio de Janeiro, in the Atlantic Forest biome, drains into the middle valley of the Paraíba do Sul River. The Piabanha basin contains growing, unregulated domestic settlements, industries with high pollution potential, and agricultural activities (Cesar *et al.* 2011, Villas-Boas *et al.* 2017). The river has a mean annual discharge of 40 m³/s, oscillating between 80 m³/s (wet period) and 15 m³/s (dry period) (Silveira *et al.* 2016), which may vary widely along its course, with lower mean discharge in the upper stretch (Da Silva *et al.* 2017). For phytoplankton, Graco-Roza *et al.* (2020), based on monthly sampling for one year in different stretches of the river, found a relationship between phytoplankton variation and environmental heterogeneity, highlighting the importance of river dynamics in shaping phytoplankton communities, with a smaller effect during the rainy period. The results showed that downstream transport processes and nutrient enrichment modified the relationship between phytoplankton and environmental heterogeneity (Graco-Roza *et al.* 2020). The authors also emphasized the importance of studies with sampling over a wide spatial reach and the long term for a better understanding of the phytoplankton community dynamics.

In this study, we analyzed abiotic variables and zooplankton abundance to identify the factors controlling different phytoplankton attributes at six sampling points in the Piabanha River, in the dry and wet seasons for three years. We hypothesized that: 1) the lowest biovolumes and diversity of phytoplankton would occur in the wet period, due to greater water discharge (flow); 2) the less-impacted area, with a steeper slope, would have different phytoplankton attributes than at the other points; and 3) the phytoplankton would respond to variations in flow, nutrient concentrations, and water temperature, since

the river is located in a mountain region with marked climatic seasonality.

MATERIAL AND METHODS

Study area

Located in the mountain region of Rio de Janeiro State along the border with Minas Gerais State (22.11–22.54° S and 43.38–42.71° W), the Piabanha River basin has an area of approximately 2,059.83 km². The basin covers the municipality of Teresópolis and part of the municipalities of Areal, Paraíba do Sul, Paty do Alferes, Petrópolis, São José do Vale do Rio Preto, and Três Rios (Figure 1). This region lies within the Atlantic Forest biome. The Piabanha River is about 82 km long and flows through the cities of Petrópolis, Areal, and Três Rios as well as agricultural and dense urban and industrial areas. The increasing pressure on the Piabanha River basin are causing degradation of natural riverine habitats, including the Piabanha, and their biota as well as heightened risks for the

human population (Villas-Boas *et al.* 2017, De Mello *et al.* 2018).

Based on the Köppen-Geiger climate classification, the region is wet temperate with a dry winter and rainy summer (CWb). The Piabanha Basin is located in the Tropical Central Brazil climatic zone (IBGE 2002). Rainfall is most frequent from December through March (monthly mean 150 to 450 mm). The dry period extends from May through August (monthly mean 15 to 90 mm) (Marques *et al.* 2017).

Sampling

Water samples and phytoplankton and zooplankton communities were collected at 6 locations during the wet and dry periods of 2012, 2013 and 2014 (Figure 1). The sampling points were located in the downstream and upstream sections of the Piabanha River (Figure 2). This river longitudinal profile was extracted from a 30-m resolution radar-derived digital elevation model (DEM), which was imported

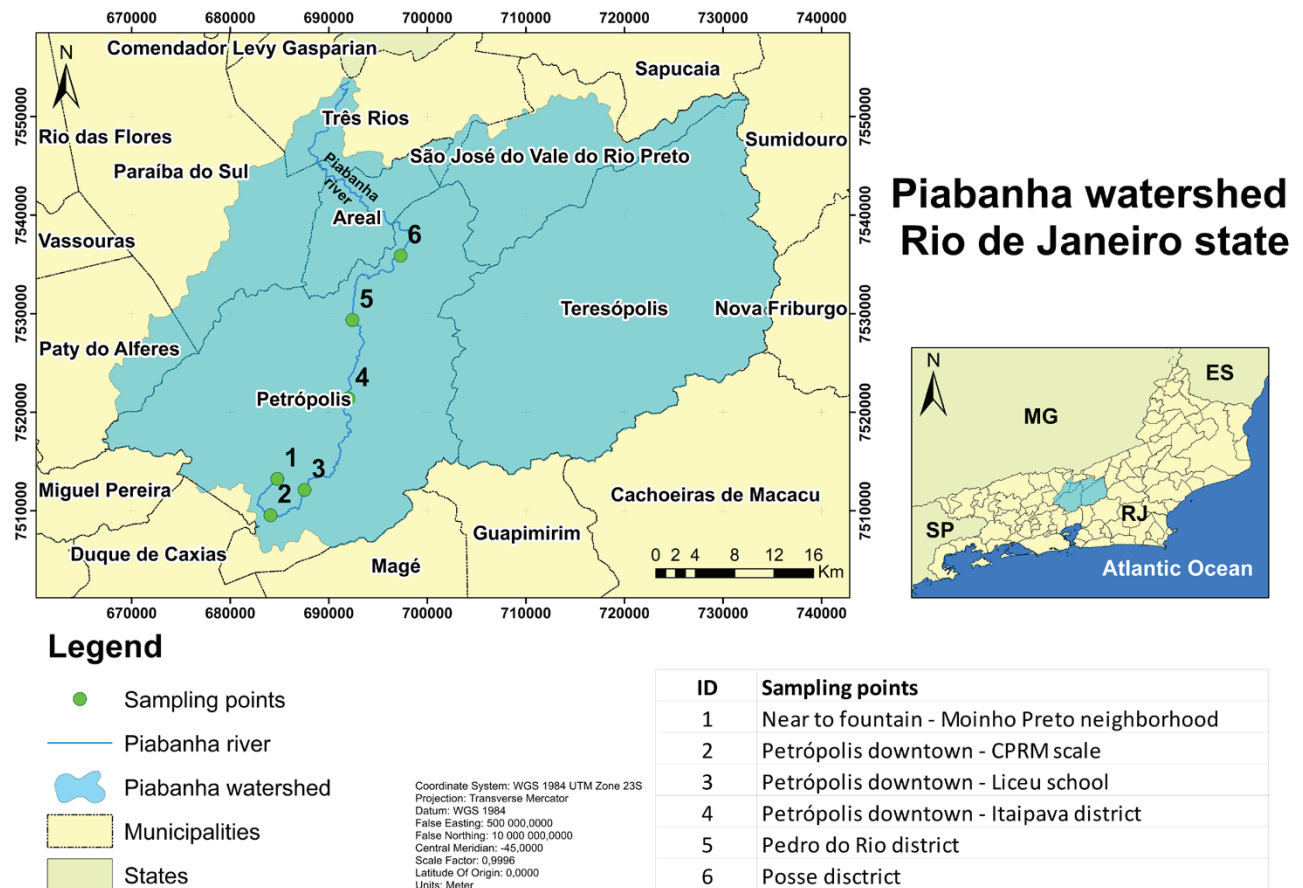


Figure 1. Map of the study area in Rio de Janeiro State. The Piabanha watershed and the spatial distribution of the sampling points in the Piabanha River. Samples were collected in the dry and wet periods of 2012, 2013, and 2014.

into ArcGis Desktop 10.7.1 software for flow line and elevation processing, using “extract values to points toolbox”. Sampling points 1, 2 (located at a knickpoint) and 3 were located in the upper course, where erosion processes predominate due to the high relief, which gives the river more potential energy. The elevation varies between 850 and 1,300 m. The channel then flows downward along a steep gradient. Sampling points 4 and 5 were located in the middle course. This area has lower relief (> 500 m and < 800 m) and the river has increased discharge, and this gives the water the (kinetic) energy to further erode the riverbed and banks. The last sampling point was situated at the beginning of the lower course (< 600 m; Figure 2).

Climatic and hydrological data

The temporal variability of the precipitation and temperature in the Piabanha River region was provided by remote-sensing technology. Precipitation data were acquired from the website of the National Institute for Space Research (INPE) for January 2000 through December 2016. The data were measured by the Tropical Rainfall Measuring Mission (TRMM) satellite, with a spatial resolution of 0.25° (approximately 28 km) (Kummerow *et al.* 1998). Minimum and maximum temperatures were estimated by the Terra satellite

(NASA Program Earth Observing System – EOS) and the data were downloaded from the EOS data gateway.

Because of the rapid generation time of phytoplankters, to analyze the influence of rainfall and flow on phytoplankton in the sampling periods, we used the cumulative total precipitation and the mean flow data from the 7 days prior to each sampling. Precipitation data were obtained from a weather station located near sampling point 2 (station code 2243268), and the flow data from a hydrological station located near point 5 (station code 58405000) of the information system of the National Water Agency (ANA). For this reason, we used these data only for seasonal comparisons.

Field parameters

Depth, turbidity, temperature, electrical conductivity, and dissolved oxygen (DO) were measured at each sampling point. Depth, temperature, and electrical conductivity were determined with a Thermosalinometer (YSI, model 30); turbidity with a turbidimeter (Lamotte, model 2010); and DO concentrations with a digital oximeter (YSI, model 550A).

At each sampling point we collected 1 L of water for nutrient analysis and 100 mL for phytoplankton

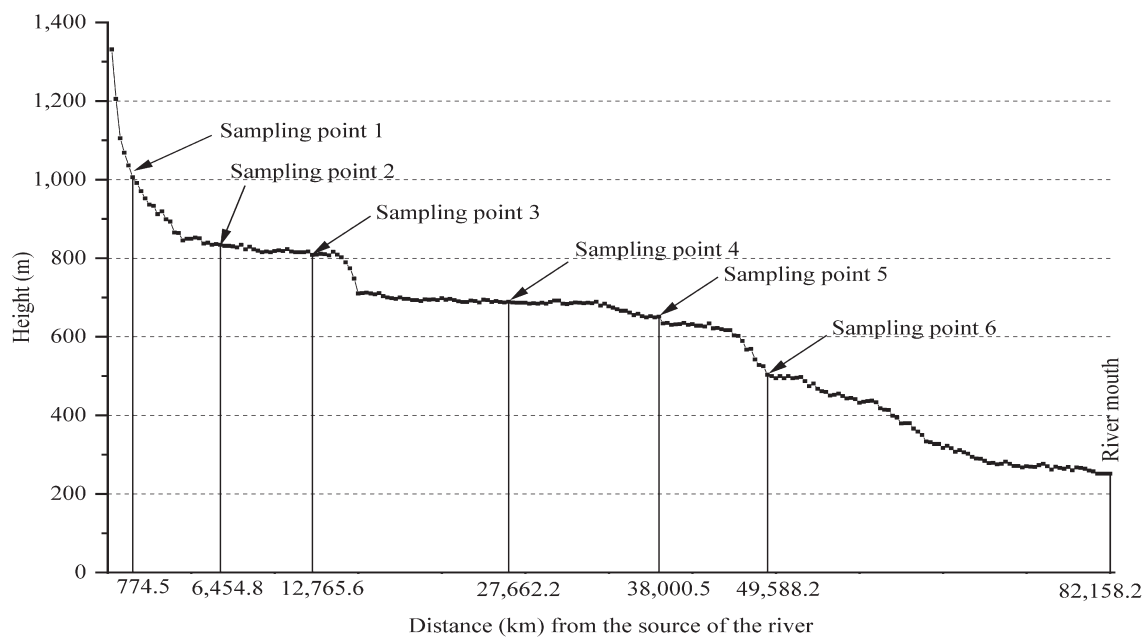


Figure 2. Piabanha River topographic profile. The horizontal axis represents the distance between the river source and its mouth, and the elevation is shown on the y-axis. The profile was generated from the NASA Shuttle Radar Topography Mission (SRTM). Sampling points 1, 2, and 3 = upper course, 4 and 5 = middle course, and 6 = lower course.

analysis. Zooplankton was sampled by collecting the subsurface water with a calibrated bucket and filtering 100 L in a conical net with 60 μm mesh. Water samples for nutrient analyses were stored in polyethylene bottles until taken to the laboratory for pH measurements (pH meter; Analion[®], model PM608). Part of each sample was filtered in glass-fiber filters ($\text{Ø} = 47$ mm, ~ 0.7 μm pore size) for dissolved-nutrient analysis. Samples for total and dissolved-nutrient fractions were stored in acid-washed vials and immediately frozen for subsequent analysis. Phytoplankton samples were immediately fixed with neutral Lugol solution and zooplankton samples were fixed in 4% formaldehyde solution.

Analytical methods in the laboratory

The following analyses were conducted in the laboratory: total phosphorus (TP), orthophosphate (P-PO_4^-), total nitrogen (TN), nitrate (N-NO_3^-), and ammonium (N-NH_4^+). We used the molybdenum blue complex reaction method for analysis of TP and P-PO_4^- (Golterman *et al.* 1978). TP samples were previously oxidized with persulfate. TN and N-NO_3^- samples were analyzed by nitrate reduction in a cadmium column with post-nitrite (N-NO_2^-) determination in a flow injection analysis (FIA) in the nutrient analyzer (Asia, Ismatec). TN samples were previously oxidized with alkali persulfate solution. N-NH_4^+ was determined using the salicylate-hypochlorite method (Bower & Holm-Hansen 1980). Dissolved inorganic nitrogen (DIN) was defined as the sum of N-NH_4^+ and N-NO_3^- . Possible nutrient limitation to phytoplankton growth was evaluated in the following manner: (i) DIN:SRP ratio (atomic), if the median is below 13, the system is considered limited by N; and above 50, limited by P (Morris & Lewis 1988). (ii) DIN and SRP were compared to half-saturation constants for phytoplankton growth: if $\text{P/L} < 10$ μg (~ 0.3 $\mu\text{mol P/L}$), P was considered limiting (Sas 1989), and if $\text{N/L} < 100$ μg ($\sim 6\text{--}7$ $\mu\text{mol N/L}$), N was considered limiting (Reynolds 1997).

Phytoplankton was quantified by the Utermöhl (1958) sedimentation method in a Zeiss Axiovert 10 inverted microscope (400 \times magnification; Oberkochen, Germany) and individuals (colonies, filaments, or single cells) were counted in random fields. The phytoplankton biovolume (mm^3/L) was estimated by multiplying the density of each

species by the mean volume of its cells (Hillebrand *et al.* 1999). Phytoplankters were identified and separated into the following groups: cyanobacteria, diatoms, phytoflagellates (chrysophyceans + cryptophyceans + dinoflagellates + euglenoids + flagellated green algae), and green algae without flagella (chlorophyceans + trebouxioephyceans + klebsormidiophyceans + zygmatophyceans) (Bicudo & Menezes 2017). Species diversity (H') was estimated by the Shannon-Wiener index (Shannon & Weaver 1963), based on the density and expressed in bits/ind.

Qualitative and quantitative zooplankton analyses were performed by subsampling 1 mL in a Sedgwick-Rafter chamber observed with an optical microscope, or 5 mL in an open chamber observed with a stereoscopic microscope. Taxonomic identification was carried out to the lowest possible level, considering the three main groups, Rotifera, Cladocera, and Copepoda, and using taxonomic works by Koste (1978), Fernando (2002), and Elmoor-Loureiro (1997). The group "Others" was also included in the sample analysis, *i.e.*, organisms that pass part of their life cycle in the water column, such as larval insects.

Statistical Analyses

Two different approaches were used for analyses of climate data. In the first, we analyzed the annual precipitation and temperature over 17 years (2000–2016). One of the commonly used tools for detecting changes in a climatic time series is the non-parametric Mann-Kendall (MK) trend test (Gilbert 1987). The purpose of analyzing trends by the MK test is to assess whether or not a statistically significant trend exists in a series. Deterministic seasonality, *i.e.*, a regular and stable seasonal behavior over time of precipitation and temperature, was tested using the non-parametric Kruskal-Wallis test (Kruskal & Wallis 1952). In this case, the null hypothesis states that annual rainfall series exhibit a regular trend.

Normal distribution was not observed, and non-parametric tests were used. A Kruskal-Wallis test was performed to elucidate the spatial pattern during the study period. For this, the 6 sampling points were tested against each other, followed by the pairwise Dunn's test when a significant difference was observed. We used a Mann-Whitney test to compare the parameters analyzed

between the climatic periods. In this case, sample values were grouped and used as replicates of dry and wet seasons. The statistical analyses were performed in SigmaPlot 12.5 software (Systat Software Inc., California, USA) and the results considered statistically significant if $p < 0.05$.

Constrained data ordination was performed in order to assess the proportion of the variance of phytoplankton groups explained by the environmental variables. Detrended correspondence analysis (DCA) indicated gradient length ≤ 3.0 with a linear method more appropriate. Therefore, we performed a redundancy analysis (RDA; Šmilauer and Lepš 2014). Variables with a high variance inflation factor ($VIF > 10$; Ter Braak & Šmilauer 2012) were excluded from the analysis. The explanatory variables used in the analysis were: precipitation, flow, depth, water temperature, turbidity, electrical conductivity, pH, DO, DIN, SRP, and the biomass of total zooplankton, cladocerans, copepods, and rotifers. Abiotic data and biovolume of phytoplankton groups were $\log_{10}(x + 1)$ transformed. The importance of each variable in the model was assessed through the progressive selection (forward-selection) method, and only the significant variables ($p < 0.05$) were represented in the model. The significance of the environmental variables was evaluated by Monte Carlo test, with 999 permutations. The analysis was performed in CANOCO 5.0 software for Windows.

RESULTS

Climate

To assess trend detection in the climatic-variable time series, the rainfall data obtained in the 2000–2016 period showed that the annual precipitation varied from 913.3 mm in 2014 to 2,066.7 mm in 2008. In 2012 and 2013, annual precipitation measured by satellite was 1,322.5 and 1,635.3 mm, respectively. The Mann-Kendall test indicated a no positive or negative monotonic trend over time ($S = -0.16$; $p = 0.387$).

The rainfall regime showed a regular interannual pattern in magnitude, timing, and duration. The lowest monthly precipitation occurred in July 2001 (0 mm) and the highest in January 2007 (data not shown); monthly precipitation increased during summer and

decreased in winter ($S = 158.2$; $p < 0.0001$). The dry period began in April and ended in August, and in general the total monthly rainfall was less than 100 mm. The wet period started in November and rainfall reached its maximum in the next two or three months (up to 300 mm monthly).

During 2001–2016 the mean temperature was near 20 °C, with no positive or negative monotonic trend over time ($S = 0.037$; $p = 0.451$). The minimum temperature was measured in July 2016 (10.4 °C) and the maximum in October 2002 (31.5 °C). Similarly, to the precipitation, the interannual seasonal temperature pattern was quite regular, with lower temperatures during winter months and higher in summer ($S = 154.6$; $p < 0.0001$).

During the sampling period (Figure 3), the mean air temperature was usually above 15 °C. The results showed greater temperature range, predominantly in autumn; the lowest temperatures occurred in May, June, July, and August, *i.e.*, the end of autumn and winter, when solar radiation in the southern hemisphere is lowest. At the end of winter, atmospheric heating increases gradually and mean temperatures up to 22 °C were measured. In summer months, maximum temperature reached 26 °C or more. The total precipitation in the 7 days prior to sampling periods was significantly higher in the wet period (median = 126 mm, $p = 0.004$; Table S1).

Hydrological, physical, and chemical features

In the wet period, water temperature (median = 21 °C) and flow (13.8 m³/s) were significantly higher ($p < 0.001$), and DO concentrations were lower (median = 5.8 mg/L; $p = 0.013$). Depth, turbidity, electrical conductivity, and pH did not differ between periods (Table S1). The median depth ranged from 0.5 (dry period) to 0.6 m (wet period; Table S1). Electrical conductivity ranged from 119 (wet period) to 118.0 $\mu\text{S}/\text{cm}$ (Table S1). Median values of pH were circumneutral, ranging from 7.1 (dry period) to 7.2 (wet period). DIN and TN concentrations did not differ between periods, with median values from 26 and 271 μM (wet period) to 40 and 350 μM (dry period), respectively (Figure 4a, Table S1). SRP and TP concentrations were significantly higher in the dry period (median = 9 and 21 μM ; $p = 0.021$ and $p = 0.010$, respectively; Figure 4c, Table S1). The DIN:SRP molar ratio was

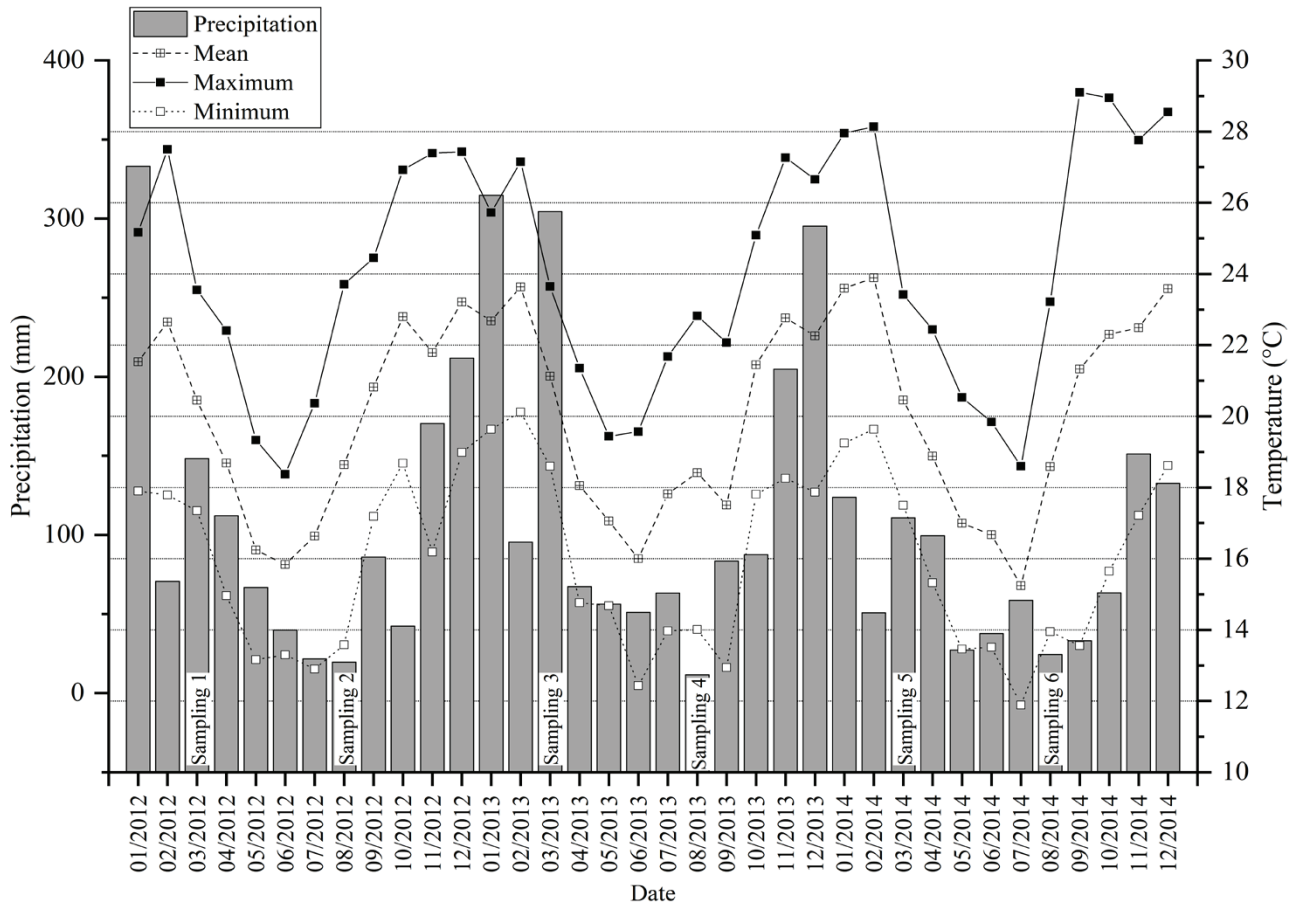


Figure 3. Monthly total precipitation (mm) estimates for the Piabanha River region, based on data from the Tropical Rainfall Measuring Mission (TRMM) satellite; and mean, minimum and maximum monthly temperature ($^{\circ}\text{C}$) estimates based on data from the Terra satellite (NASA Program Earth Observing System – EOS), over a 3-year period (2012–2014).

higher in the wet period (median = 12; $p = 0.010$; Figure 4e).

Among sampling points, the largest differences in the Piabanha River were observed at sampling point 1. This point was significantly shallower than point 6 (median = 0.3 and 1.0 m, respectively; $p < 0.001$; Table S2), with colder water than at point 3 (median = 17 and 21 $^{\circ}\text{C}$, respectively; $p = 0.021$; Table S2). Point 1 was significantly less turbid (median = 1.2 NTU) than at points 5 and 6 (median = 15.8 and 24.9 NTU; $p < 0.001$), with lower electrical conductivity (median = 16 $\mu\text{S}/\text{cm}$) than at points 2, 3, and 4 (median = 183, 212, and 189 $\mu\text{S}/\text{cm}$, respectively; $p < 0.001$), pH values were significantly lower at point 2 than at point 6 ($p = 0.009$), and no significant differences in DO concentrations were observed (Table S2). High concentrations of nutrients were consistently observed, except at sampling point 1 (Figure 4). DIN concentrations were higher at points 2

(median = 61.5 μM) and 3 (median = 57.3 μM) than at point 1 (median = 2.3 μM ; $p = 0.003$; Figure 4b). TN concentrations were higher at points 3 (median = 493.1 μM), 4 (median = 352.5 μM), and 5 (median = 350.9 μM) than at point 1 (median = 14.1 μM ; $p = 0.002$; Table S2). SRP concentrations were lower at point 1 (median = 0.1 μM) than at point 3 (median = 7.4 μM ; $p = 0.004$; Figure 4d), and smaller concentrations of TP were observed at point 1 (median = 1.0 μM) in relation to point 3 and 6 (median = 14.9 and 14.0 μM , respectively; $p = 0.007$; Table S2). No significant difference was observed among the sampling points in relation to DIN:SRP molar ratio ($p = 0.130$), with a tendency of limitation by SRP (Figure 4e).

Zooplankton

A total of 70 species were identified during the study period, in the main zooplankton groups: Rotifera, Cladocera, and Copepoda. The total

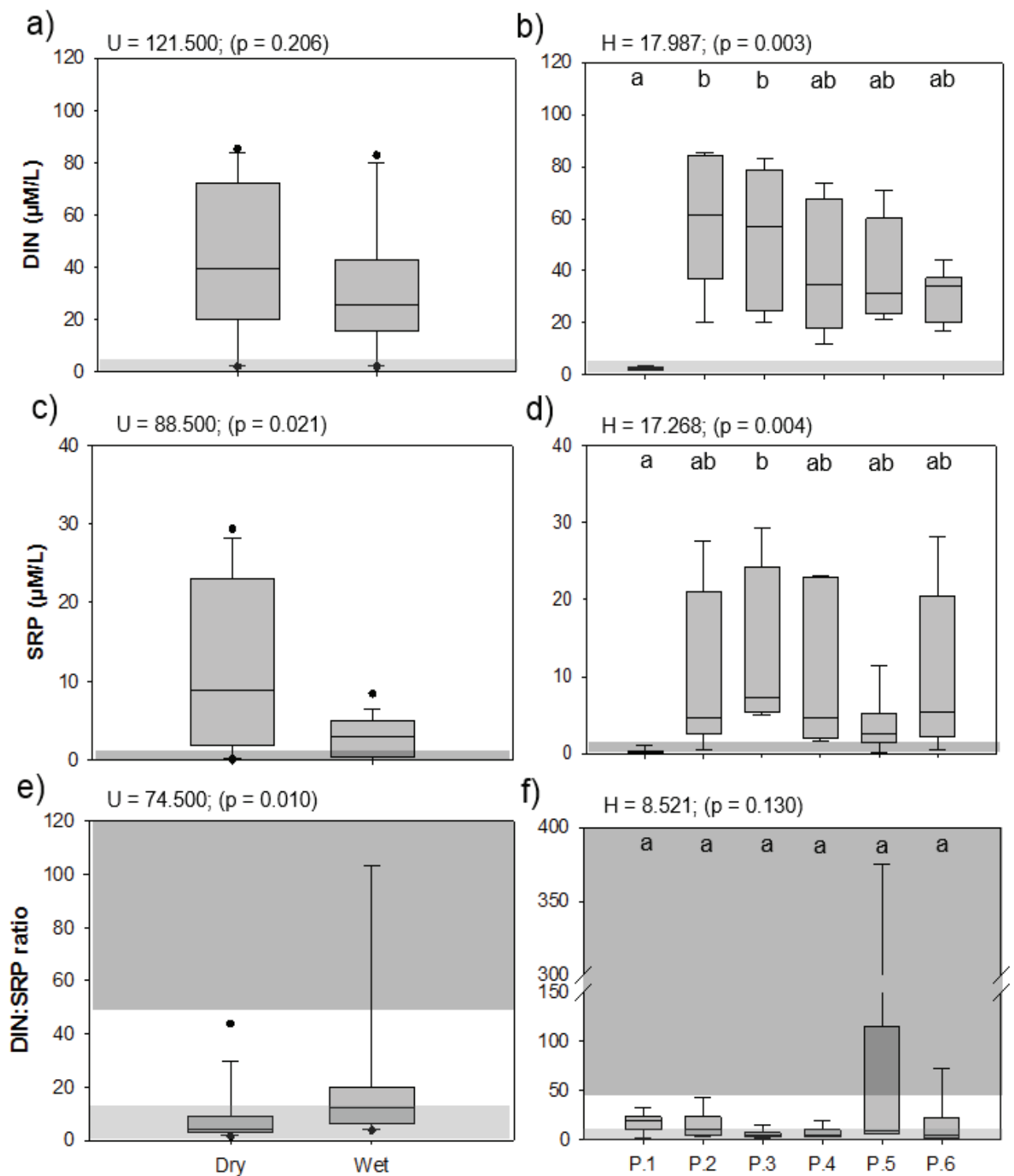


Figure 4. Climatic period variations (a, c, e) and sampling points (b, d, f) of DIN = Dissolved inorganic nitrogen; SRP = Soluble reactive phosphorus; DIN:SRP = molar ratio. The variations are expressed as box-whisker plots, where the line within boxes is the median, and the boundaries of the box plots indicate the 25th and 75th percentiles. Whiskers above and below indicate the 90th and 10th percentiles. Dots are outlying points. Uppercase letters above box plots indicate groups with significantly similar medians. Dark-gray areas represent potentially P-limited conditions, and light-gray areas represent potentially N-limited conditions. Points 1, 2 and 3 = upper course, 4 and 5 = middle course, and 6 = lower course.

zooplankton abundance did not differ between periods (median = 1752 ind/L, dry period and 2365 ind/L, wet season; Table S1) and among sampling points (Table S3). Similarly, no seasonal differences were observed for the zooplankton groups (Table S1), nor among sampling points (Table S3). The major contribution to this abundance was from Rotifera (84%), mainly *Bdelloidea* and *Habrotrocha* sp., followed by copepods (14%).

Phytoplankton

One hundred and eighty-four taxa were identified during the study. Green algae was the most species-rich group (74 taxa), followed by diatoms (42 taxa), cyanobacteria (35 taxa), and phytoflagellates (33 taxa), with no differences between the total

number of taxa or the different groups in the wet and dry periods. About 50% of the cyanobacteria consisted of filamentous forms, and 83% of the diatoms were pennate. Phytoflagellates were represented mainly by chrysophyceans (28%), euglenophyceans (26%), and cryptophyceans (22%); 46% of the green algae were members of Chlorophyceae (data not shown).

Comparing the wet and dry periods, no difference was observed for the total biovolume (median = 0.214 mm³/L, wet period and 0.144 mm³/L, dry period; Figure 5a; Table S1). Cyanobacteria ranged from 0.0002 (dry period) to 0.281 mm³/L (dry period; Table S1) and was the only group that showed significantly higher biovolume in the wet period ($p = 0.041$; Table S1).

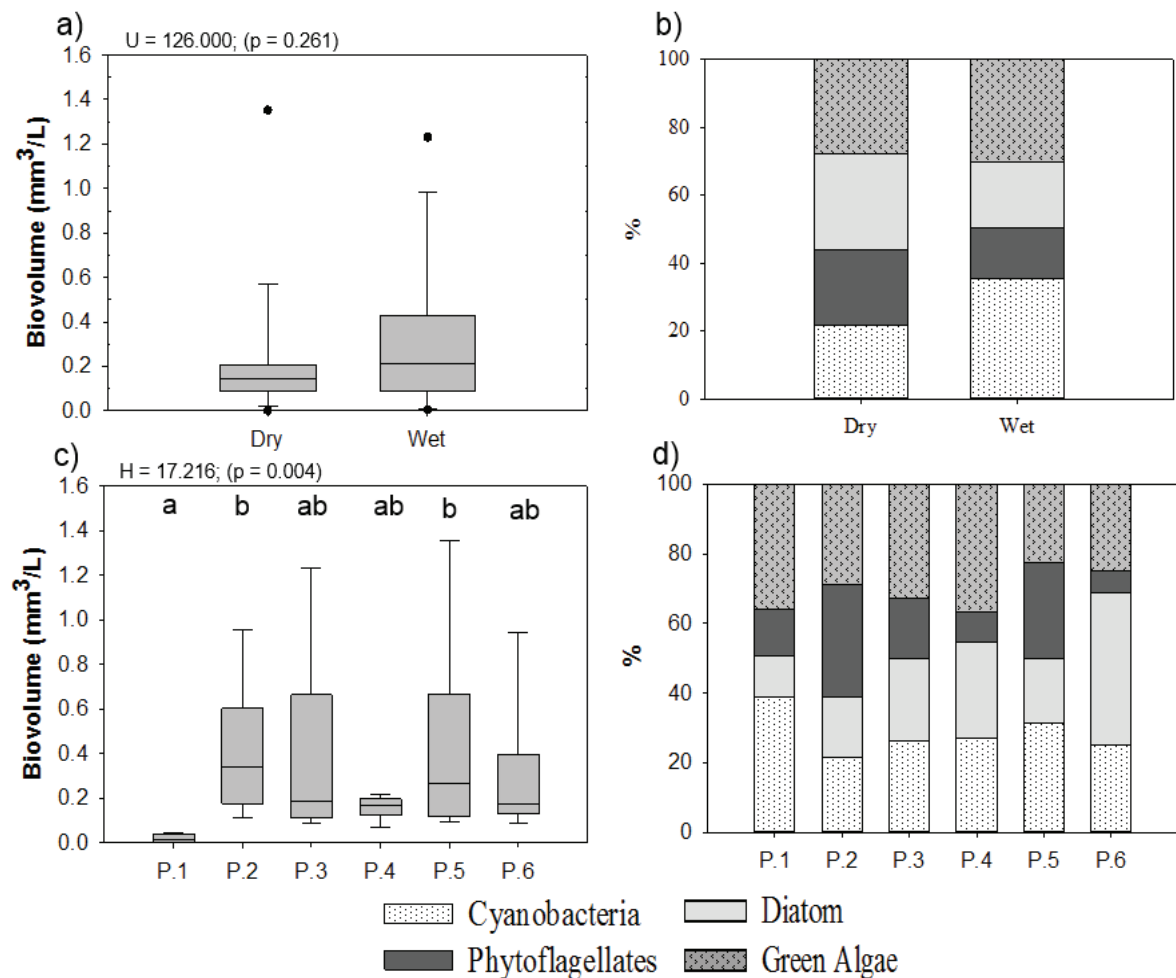


Figure 5. Phytoplankton biovolume variation in climatic periods (a) and at sampling points (c). Relative percentage of phytoplankton class in climatic periods (b) and at sampling points (d) in the Piabanha River. The variations are expressed as box-whisker plots, where the line within boxes is the median, and the boundaries of the box plots indicate the 25th and 75th percentiles. Whiskers above and below indicate the 90th and 10th percentiles. Dots are outlying points. Uppercase letters above box plots indicate groups with significantly similar medians. Relative contribution (%) of phytoplankton groups in climatic periods (c) and at sampling points (d). Points 1, 2 and 3 = upper course, 4 and 5 = middle course, and 6 = lower course.

The contributions of the different phytoplankton groups were similar, with relatively larger contributions from cyanobacteria and green algae in the wet period and from phytoflagellates and diatoms in the dry period (Figure 5b).

The total biovolume of the phytoplankton was consistently low during the study period, mainly at sampling point 1 (median = 0.013 mm³/L), which had lower biovolumes than at points 2 (median = 0.340 mm³/L) and 5 (median = 0.263 mm³/L; $p = 0.004$; Figure 5c; Table S3). Of the phytoplankton groups, only Cyanobacteria showed a significantly higher biovolume in the wet period (median = 0.043 mm³/L; $p = 0.041$; Table S2). In relation to the sampling points, cyanobacteria was important at all sampling points and significantly lower at point 1 (median = 0.004 mm³/L) than point 5 (median = 0.066 mm³/L; $p = 0.030$; Table S3); diatoms were lower at point 1 (median = 0.001 mm³/L) than at point 6 (median = 0.075 mm³/L; $p = 0.042$; Table S3); green algae were lower at point 1 (median = 0.005 mm³/L) than at points 2 and 3 (median = 0.055 and 0.075 mm³/L, respectively; $p = 0.018$; Table S3); and phytoflagellates did not differ among sampling points. Regarding the contributions of the different phytoplankton groups (%), cyanobacteria was important at all sampling points, especially at point 1; green algae, especially at points 1, 3, and 4; diatoms, especially at point 6; and phytoflagellates, especially at points 2 and 5 (Figure 5d).

Species richness and diversity showed neither significant seasonal (Table S1) nor spatial variability (Table S3). The species richness ranged from 19 (dry period) to 21 taxa/sample (wet period; Table S1) and from 15 (point 1) to 25 taxa/sample (point 2; Table S3). Diversity ranged from 0.7 (point 6) to 3.0 bits/ind (point 6) in the dry period (Table S1) and from 1.6 (points 2 and 5) to 3.4 bits/ind (point 6) in the wet period (Table S2), with medians higher than 2 bits/ind in the two climatic periods (Table S3). The diversity showed medians higher than 2 bits/ind in the two climatic periods and all sampling points (Tables S1 and S3).

RDA analysis explained 38% of the phytoplankton-environment relationship. Axes 1 and 2 together explained 43% of the data variability (Axis 1: 37% and Axis 2: 6%; Figure 6). The forward selection indicated that DIN concentrations (Pseudo-F = 12.9; $p = 0.001$), water

temperature (Pseudo-F = 5.8; $p = 0.002$), and SRP concentrations (Pseudo-F = 2.7; $p = 0.047$) were most significantly related to the phytoplankton groups. Green algae, cyanobacteria, and diatoms were negatively related to Axis 1, with higher DIN and SRP concentrations and higher water temperatures. Phytoflagellates, on the other hand, did not show a clear relationship to the selected variables, and only a tendency toward a higher contribution in the dry period, related positively to axis 2. We observed important spatial differences between sampling point 1 and the other points, with smaller contributions from all phytoplankton groups at point 1 (Figure 6). Temporal differences were mainly related to the variation in water temperature between the two seasonal periods, with a cold and more homogeneous environment in the dry period that seems to have favored phytoflagellates in relation to the other groups.

DISCUSSION

In this study, we aimed to evaluate the dynamics of the phytoplankton in a tropical montane river in response to climatic, hydrological, and limnological variables and zooplankton herbivory. Contrary to our first hypothesis, the total phytoplankton biovolume and diversity were not significantly different between the wet and dry periods, despite the higher flow in the wet period. Confirming our second hypothesis, at point 1, in a stretch with a steeper slope and less impacted, the total phytoplankton biovolume was lower, as were the cyanobacteria, diatoms, and green algae biovolume. Nutrient concentration and temperature were the determining variables in the phytoplankton dynamics in the Piabanha River, located in a mountain region with marked climatic seasonality.

In rivers, plankton organisms are subject to unidirectional transport and are affected mainly by the physical and geographical characteristics of the watershed (Reynolds & Descy 1996). Therefore, the potamoplankton is highly influenced by the seasonal patterns of hydrometeorological events (Abony *et al.* 2012). Water discharge, channel retention, light penetration, and temperature have been identified as the main selective mechanisms for potamoplankton (Reynolds

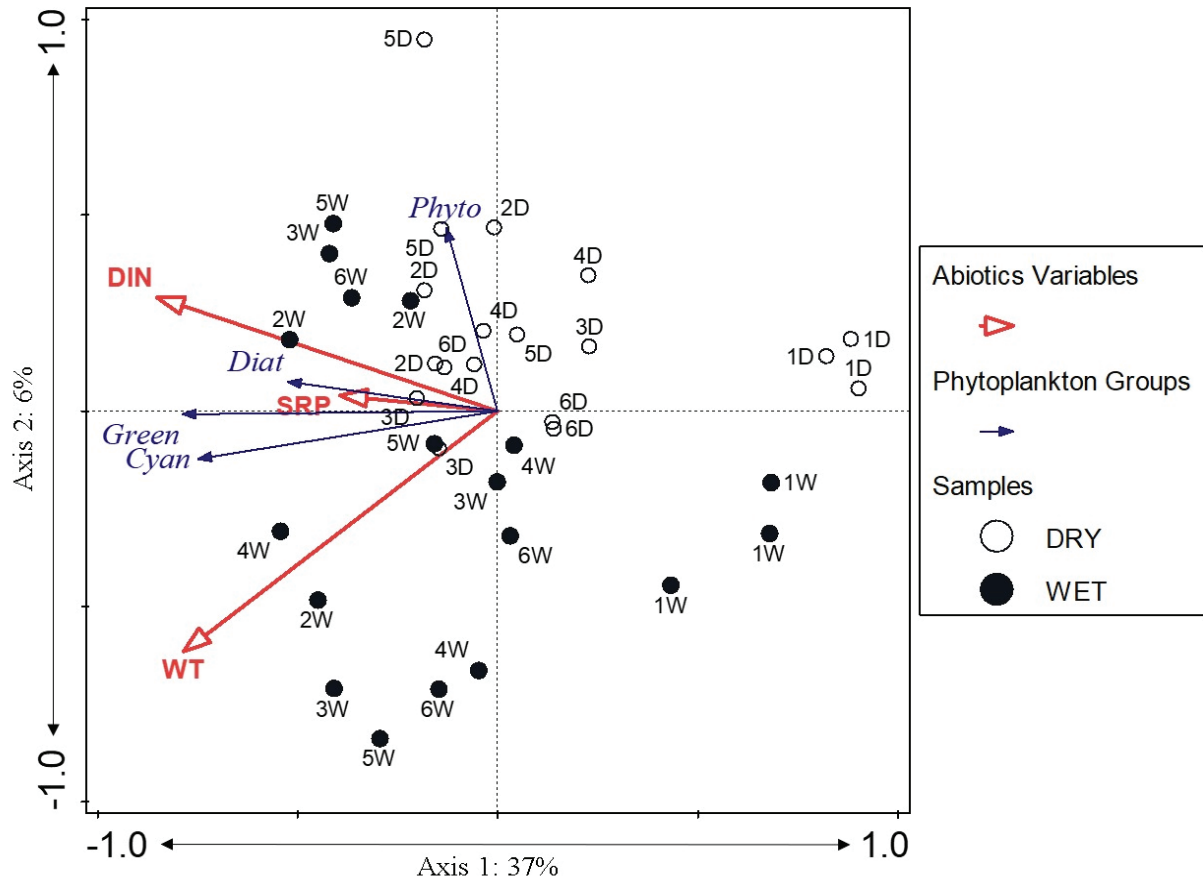


Figure 6. RDA ordination diagram of phytoplankton groups, environmental variables, and sampling points in the Piabanha River in the dry and wet periods. WT = water temperature, DIN = dissolved inorganic nitrogen, SRP = soluble reactive phosphorus, Cyan = cyanobacteria, Green = green algae, diat = diatom, Phyto = phytoplankton. Points 1, 2, and 3 = upper course, 4 and 5 = middle course, and 6 = lower course.

& Descy 1996, Reynolds 2000, Burdis & Hirsch 2017). Sediment loads suspended in small tropical streams are highly variable because they are influenced by local precipitation (Jacobsen 2008). In the Piabanha River, a seventh-order stream, the development of a true plankton is possible, as expected for rivers of the third or fourth order and higher, due to the slowing of the current and the increases in luminosity and the different sources of inoculum (Reynolds 2006). However, the Piabanha River receives releases of inadequately treated domestic sewage from point 2 downward, still in its upper course (Alvim *et al.* 2014, De Mello *et al.* 2018), and the water current is increased by the steep slopes, heavy rainfall, and the growing urban settlement in the region. These factors, acting in conjunction with channelization along the river, are changing the hydrological pattern, causing silting and flooding in urban areas (Silva *et al.* 2012, Marques *et al.* 2017), as

well as increasing turbidity and reducing light availability. Therefore, the phytoplankton in the Piabanha River is represented mainly by species originating in the river itself, in addition to varying contributions of benthic forms in suspension (tychoplankton) due to discharge, for example filamentous cyanobacteria and small pennate diatoms. These organisms survive the conditions imposed by the unidirectional flow, even though it is low and responds to local precipitation, partly because they are adapted to the eutrophic conditions resulting from the intense human impacts beginning in the upper course (from sampling point 2), which reduce light availability.

In the Piabanha River, only point 1 showed limiting nutrient concentrations for phytoplankton growth, both for DIN (Reynolds 1997) and for SRP (Sas 1989). A possible limitation of DIN on phytoplankton growth was observed principally in the dry period, since the median

DIN:SRP ratio was < 13 (Morris & Lewis 1988, Rangel *et al.* 2012), due to significantly higher SRP concentrations at that time. Despite this indication of DIN limitation to phytoplankton growth, high concentrations were observed throughout the study (except at point 1). Such changes in the nutrient ratios may indicate natural or anthropogenic influences and can stimulate changes in the phytoplankton community (Reynolds 2006, Naselli-Flores & Barone 2011), although a possible nitrogen limitation to algae growth has been noted in studies in high-altitude tropical streams (Jacobsen 2008). In the wet period, the increase in water level seemed to dilute the nutrient concentrations in wastewater (Graco-Roza *et al.* 2020), which was reflected in a higher DIN:SRP and which did not indicate any limitation on phytoplankton growth. Furthermore, among the sampling points, the DIN:SRP fraction did not differ significantly and showed only a tendency toward limitation by SRP.

Lower discharges with longer water retention times favor phytoplankton growth, while the reverse leads to a decline in biovolume due to dilution (Devercelli & O'Farrell 2013, Descy *et al.* 2017). The phytoplankton biovolume did not increase during the dry period in the study area, probably due to the lower temperature (< 15 °C). This reduced temperature may have limited the phytoplankton growth, although during this period a significantly lower flow rate and an increase in the SRP concentration were observed. The importance of water temperature, which directly affects metabolic processes and the growth of plankton communities, has been documented in different studies, mainly in lentic environments (Bonecker *et al.* 2007, Roland *et al.* 2010, Silva *et al.* 2014). In rivers, high temperatures can potentiate the effects of hydrology and may have a positive effect on phytoplankton (Reynolds 2000, Devercelli & O'Farrell 2013). In the Piabanha, the higher temperatures in the wet period may have led to the large cyanobacteria biovolume at that time. As yet, little can be concluded regarding the factors that limit or regulate algal biomass and production in tropical high-altitude streams, although low temperatures and possible nitrogen limitation may regulate growth (Jacobsen 2008).

Zooplankton in rivers is strongly influenced

by hydrodynamics, mainly related to flow and spatial heterogeneity (Pace *et al.* 1992, Reynolds & Glaister 1993, Burdis & Hirsch 2017). This control is even more important for zooplankton than for phytoplankton (Gosselain *et al.* 1998a, 1998b), since organisms with short generation times, such as rotifers, are favored in riverine conditions. Food availability may also be a necessary condition for development of an abundant zooplankton (Burdis & Hirsch 2017). In the Piabanha River, despite the higher relative contribution of rotifers, zooplankton did not seem to have a controlling effect on phytoplankton, as previously documented for large rivers in situations of low flow (Gosselain *et al.* 1998a, 1998b). In the study environment, despite the slow current, the intense human impacts and low phytoplankton biovolume did not seem to favor zooplankton development.

For the phytoplankton structure, green algae (desmids and coccal green algae, both unicellular and colonial) comprised the largest number of taxa, followed by diatoms, which are often relatively more numerous in tropical rivers (Rojo *et al.* 1994, Soares *et al.* 2007, Okogwu & Ugwumba 2013). The total taxonomic richness, the species richness, and the diversity of the phytoplankton of the Piabanha River are comparable to other eutrophic tropical rivers (Soares *et al.* 2007, Okogwu & Ugwumba 2013).

Cyanobacteria and green algae contributed most to the phytoplankton biovolume in the wet period, and phytoflagellates in the dry period when minimum temperatures are lower. Some studies have shown that high concentrations of P and N generally select for certain species, such as filamentous cyanobacteria (Cardoso *et al.* 2017, Graco-Roza *et al.* 2020), and can lead to greater biotic homogenization (Wengrat *et al.* 2018) as observed in a previous study in the Piabanha River, mainly in its upper section (Graco-Roza *et al.* 2020). In other studies, diatoms and green algae were the most important groups (Soares *et al.* 2007, Abony *et al.* 2012, Descy *et al.* 2017), but cyanobacteria dominated in rivers with high concentrations of nutrients and low discharge (Soares *et al.* 2007, Devercelli & O'Farrell 2013, Okogwu & Ugwumba 2013). In the Piabanha River, green algae were mainly important at points in the upper stretch, except at point 1. In the case

of diatoms, the high phosphorus availability may also have contributed to the increase of small pennate and centric species, which are associated with shallow eutrophic systems and turbid waters, and also appear to be tolerant to flow (Reynolds *et al.* 2002, Soares *et al.* 2007, Santana *et al.* 2016). In the Piabanha River, diatoms contributed significantly to the phytoplankton biovolume at point 6 (lower course), where turbidity was higher. Phytoflagellates, able to form large populations in nutrient-enriched rivers (Devercelli & O'Farrell 2013, Okogwu & Ugwumba 2013, Santana *et al.* 2016), also comprised a significant part of the phytoplankton biovolume, mainly in the lower temperatures during the dry period; this group was comprised mainly of euglenoids and cryptomonads. Lower temperatures and reduced light availability have been identified as favorable conditions for phytoflagellates (Kruk & Segura 2012). The growth of Euglenophyceae can be favored by a decrease in the flow rate (Reynolds & Descy 1996) and some species can respond positively to an increase in DIN concentration (Rosowski 2003, Santana *et al.* 2016). For cryptomonads, the low light availability may have favored their growth, due to their mixotrophic potential (Medeiros *et al.* 2015); this is a generalist strategy and can be advantageous under low light or nutrient conditions (Troost *et al.* 2005a, 2005b).

In summary, the results indicate more-homogeneous environmental variables and different phytoplankton attributes along this stretch of the river. The intense human impacts from point 2 in the upper stretch downward were important in separating sampling point 1, located in an area of preserved forest and with better water quality, although with possible nutrient limitation to phytoplankton growth. Although the Piabanha River has relatively slow flow, as its channel passes through valleys the resulting rapids make it difficult to form lentic zones. In addition, because of the location in a high-altitude region, in the rainy period when temperatures are more suitable for phytoplankton growth, the concurrent increase in flow may have prevented an increase in phytoplankton biomass, in which only cyanobacteria had a higher biovolume. In contrast, during the dry period, despite the lower flow, the cooler temperatures may have inhibited phytoplankton growth.

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SUPPLEMENTARY MATERIAL

Table S1. Median values and range (in parentheses) of limnological variables in the dry and wet periods in the Piabanha River. Seasonal periods were compared by Mann-Whitney test. Significant values are in bold (N = 18; p < 0.05).

Table S2. Median values and range (in parentheses) of abiotic variables at different sampling points of the Piabanha River. Points 1, 2 and 3 = upper course, 4 and 5 = middle course, and 6 = lower course. Sampling points were compared by Kruskal-Wallis test. Significant values are in bold and different letters indicate significant differences among sampling points (N = 6; p < 0.05).

Table S3. Median values and ranges (in parentheses) of biotic variables at different sampling points in the Piabanha River. Points 1, 2 and 3 = upper course, 4 and 5 = middle course, and 6 = lower course. Sampling points were compared by Kruskal-Wallis test. Significant values are in bold and different letters indicate significant differences among sampling points (N = 6; p < 0.05). Tphyto = total biovolume of phytoplankton (mm³/L), Cyano = cyanobacteria (mm³/L), Diat = diatoms (mm³/L), Phyto = phytoflagellates (mm³/L), Green = Green algae (mm³/L), (Richn = phytoplankton richness (taxa/sample), Diver = phytoplankton diversity (bits/ind), Tzoo = total abundance of zooplankton (ind/L), Roti = Rotifers (ind/L), Clad = Cladocerans (ind/L), Cope = Copepods (ind/L).

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