

# Multivariate analyses of the effect of an urban wastewater treatment plant on spatial and temporal variation of water quality and nutrient distribution of a tropical mid-order river

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**Abstract** Freshwater resources are increasingly scarce due to human activities, and the understanding of water quality variations at different spatial and temporal scales is necessary for adequate management. Here, we analyze the hypotheses that (1) the presence of a wastewater treatment plant (WWTP) and (2) a polluted tributary that drains downstream from the WWTP change the spatial patterns of physicochemical variables (pH, turbidity, dissolved oxygen, and electrical conductivity) and nutrient concentrations (reactive soluble phosphorus, total phosphorus, nitrogen series, total nitrogen, and total dissolved carbon) along a mid-order river in SE Brazil and that these effects depend on rainfall regime. Six study sites were sampled along almost 4 years to evaluate the impacts of human activities, including sites upstream (1–3) and downstream (5–6) from the WWTP. The impacts were observed presenting an increasing trend from the source (site 1) towards Água Quente stream (site 4, the polluted tributary), with signs of attenuation at site 5 (downstream from both WWTP

and site 4) and the river mouth (site 6). Input of nutrients by rural and urban runoff was observed mainly at sites 2 and 3, respectively. At sites 4 and 5, the inputs of both untreated and treated wastewaters increased nutrient concentrations and changed physicochemical variables, with significant impacts to Monjolinho River. Seasonal variations in the measured values were also observed, in agreement with the pluviometric indexes of the region. Univariate analyses suggested no effect of the WWTP for most variables, with continued impacts at sites downstream, but non-parametric multivariate analysis indicated that these sites were recovering to chemical characteristics similar to upstream sites, apparently due to autodepuration. Therefore, multivariate methods that allow rigorous tests of multifactor hypotheses can greatly contribute to determine effects of both point and non-point sources in river systems, thus contributing to freshwater monitoring and management.

**Keywords** Monjolinho River · Surface waters · Phosphorus · Nitrogen · Carbon

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## Introduction

Anthropogenic activities due to rapid urbanization have been putting in risk the water quality and, consequently, endangering the aquatic life of different riverine ecosystem worldwide. Pollution of water bodies is a problem at the global scale (Wang et al. 2010) and requires regular evaluations. Recently, several studies have evaluated the

water quality and nutrient concentrations, mainly of phosphorus- and nitrogen-based compounds (Lim and Choi 2015; Lobo et al. 2015; Ross et al. 2015; Lee et al. 2015). Several studies reported in the literature have shown the relationship between growing urban and agricultural expansion and a consequent decline in the water quality of freshwater and groundwater, in addition to their effects on aquatic ecosystem food chains (Gall et al. 2013; Geist 2011; Hoffman et al. 2009; McCullough et al. 2012; Shrestha et al. 2012; Varekar et al. 2015). The eutrophication caused by the presence of excessive amounts of nutrients, particularly phosphorous and nitrogen, is one of the most evident consequences of the decreased water quality (Dodds et al. 2008; Li et al. 2012; Paerl 2009; Prepas and Charette 2003).

The quality of the wastewater treatment plants (WWTPs) is a key component in the management of water resources in urban areas (Carey and Migliaccio 2009). However, the effects of WWTPs on surface water quality are variable and may constitute point sources of pollution to receiving waters (Carey and Migliaccio 2009; Drury et al. 2013; Gücker et al. 2006; Marti et al. 2004; Naidoo and Olaniran 2014). In fact, WWTP effluents may increase nutrient loads to streams (Carey and Migliaccio 2009; Marti et al. 2004), primary production, respiration, and macrophyte and benthic biomasses (Gücker et al. 2006); reduce abundance and diversity of benthic microbial communities (Drury et al. 2013); increase harmful microbial populations (Naidoo and Olaniran 2014); or even subsidize autotrophic and heterotrophic organisms (Aristi et al. 2015). Nutrient concentrations downstream from WWTPs can be higher or lower than upstream sites, with differentiated effects of the WWTP effluents on water quality. For example, Gücker et al. (2006) studied the effects of an urban tertiary WWTP and a smaller rural secondary WWTP in two streams and found significantly higher concentrations of  $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N, dissolved organic carbon (DOC), and soluble reactive phosphorous (SRP) downstream from the WWTP in the larger urban stream, but found no differences in the smaller rural stream. As pointed by Gücker et al. (2006), different nutrient uptake patterns occur when reference and impacted sites are compared, considering that additional differences must result from seasonality (Gücker et al. 2006). On the other hand, Marti et al. (2004) found higher nutrient loads and longer nutrient uptake lengths downstream from the WWTPs, suggesting that they may have both

local and regional impacts due to the transport of nutrient loads kilometers away from the source. WWTPs can influence not only chemical but also hydrological characteristics of receiving waters, since effluent discharge can be important for stream water volume, mainly during dry times (Carey and Migliaccio 2009). Aristi et al. (2015) carried out a study in the Segre River, a tributary of the Ebro River in Oriental Pyrenees (NE Iberian Peninsula), and assessed the effects of WWTP effluents on river biofilms and ecosystem metabolism (in one river segment upstream from a WWTP and three segments downstream from the WWTP). In conclusion, they found ample evidence of WWTP effluents acting as a subsidy, but more limited evidence them acting as a stressor (Aristi et al. 2015).

Figueroa-Nieves et al. (2014) examined the effects of WWTPs on water quality in several streams in Puerto Rico on the Caribbean island. Nutrient concentrations, discharge, dissolved oxygen (DO), carbonaceous biochemical oxygen demand (CBOD), and specific UV absorbance (SUVA) were measured in the effluent and also upstream and downstream from the WWTP. Some of the water quality parameters, particularly nutrient concentrations, exceeded the maximum values that can be discharged to a water body. The effect of WWTPs on nutrient loads was significantly stronger for a treatment plant where the change in flow is larger, suggesting that when there is relatively high wastewater flow, a greater effect on stream nutrient loads is expected. Management activities that focus on developing nutrient criteria and their removal before being released should be a top priority in these tropical streams to reduce effluent nutrient loads in streams (Figueroa-Nieves et al. 2014). Therefore, understanding the impacts of WWTPs is important to understand stream ecosystem dynamics and responses to altered nutrient concentrations.

In this study was evaluated the spatial and temporal variation of water quality parameters along the entire length of the Monjolinho River, a tropical mid-order river in SE Brazil, in relation to an urban WWTP implanted in 2008. The river headwaters are an important water source for the city of São Carlos with 241,000 inhabitants, but also receives its effluents downstream, which are collected by an urban sewage system and conducted to the WWTP. Therefore, six sites were sampled along the Monjolinho River, including upstream and downstream WWTP, to evaluate the effects of treated wastewater discharge. The hypothesis was that the implementation of the WWTP would change spatial

patterns in stream water quality, mainly by reducing nutrients, and that this effect would be stronger during high-flow periods (e.g., during the wet season). We later found that downstream from the WWTP, another polluted tributary flows into the river, which receives untreated sewage from a nearby district of nearly 50,000 inhabitants. Therefore, we included this tributary in our sampling design for a further 13-month period, to test the hypothesis that sites downstream the WWTP were more influenced by this tributary than by the WWTP. To test both hypotheses, we sampled surface water of both watercourses and used a combination of univariate and multivariate analyses to verify changes in spatial and temporal patterns in water chemical parameters. Furthermore, we compared our results to studies in the literature evaluating spatial patterns in nutrient concentrations along the Monjolinho River that were carried out before the implementation of the WWTP, to evaluate if this plant influenced chemical parameters at larger temporal scales.

## Materials and methods

### Study area

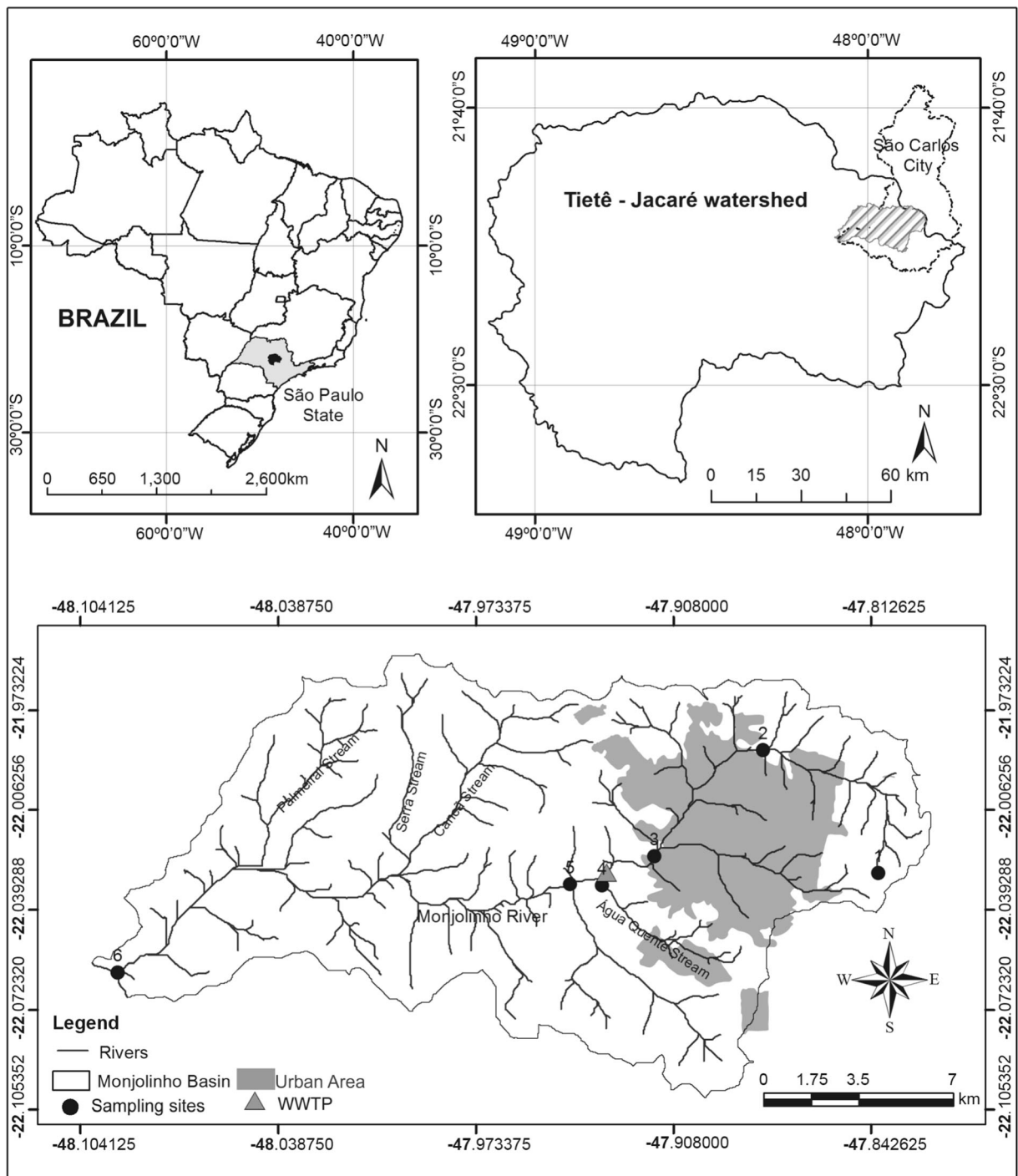
The Monjolinho River basin is located in the municipality of São Carlos, central region of São Paulo state, within the Tietê River catchment as part of the Tietê–Jacaré Water Resources Management Unit (UGRHI-13), SE Brazil (Fig. 1). The city has approximately 241,000 inhabitants (IBGE—Instituto Brasileiro de Geografia e Estatística 2014) and has an industrial complex which covers industries in the automotive, refrigeration, paper and paperboard, cosmetics, textiles, and school material sectors. The Monjolinho River basin occupies an approximate area of 275 km<sup>2</sup>, with headwaters at about 900-m amsl; after flowing along 43.25 km, the Monjolinho River reaches the Jacaré-Guaçu River, at about 543-m amsl (Espíndola 2000). Land use/cover in the Monjolinho River basin in 1998 (Crisuolo et al. 2000) was mainly for sugarcane cultivation (35.7%), pasture (20.0%), native vegetation (24.3%), and urban development (15.5%). Climate in the region is Cwb following Köppen's classification, with well-defined dry (April to September) and wet seasons (October to March), mean monthly temperatures varying between 16.8 °C in July and 22.5 °C in

February, and mean annual rainfall between 1200 and 1500 mm (Espíndola 2000).

The Monjolinho WWTP started to operate in December 2008 and is being implanted in three phases (according to the estimation of the population growth of São Carlos-SP). In this first phase (until 2015), the WWTP will process 100% of the wastewater with nominal flow rate of 636 L s<sup>-1</sup>, produced by the 213,000 inhabitants. The domestic sewage treatment consists of a primary treatment (screening, gritting, and removal of floating materials, oils, and greases); biological treatment with upflow anaerobic sludge blanket (UASB) reactors; dissolved air flotation to remove suspended solids generated in biological process; flocculation with metal coagulants and polymers (for phosphorus removal); effluent disinfection with ultraviolet (UV) radiation; and post-aeration in cascade aerator (SAAE 2015). The wastewater treatment processes efficiency reaches the average removal values of 92% for BOD, 84% for DOC, 71% for total phosphorus, and 80% for suspended solids, in a raw sewage average of 460 L per second (SAAE 2015). According to CETESB (São Paulo State Environmental Agency 2012), 99.6% of the wastewater produced in the city is collected, but only 85% of it is treated by the local WWTP, so that the Monjolinho River receives both treated and untreated sewage discharge. The other 15% of untreated sewage is discharged directly on the Água Quente stream. Therefore, it will be possible to verify the effects of WWTP effluents and the untreated sewage on some water quality parameters of the Monjolinho River.

### Sampling

For the present study, six sampling sites were selected, according to the major drivers of impacts on the water body. Site 1 is situated in an agricultural area corresponding to the vicinity of the river source, being an area dominated by forested land and with few rural activities in the surroundings. This region is subject to low anthropogenic impacts and is therefore considered as a reference site. Sampling site 2 is located at the beginning of the urban region, where the water body starts getting influenced by both rural and urban activities. Site 3 is located upstream from the wastewater treatment plant (WWTP), where the river undergoes impacts from urban surface runoff and possible sewage discharges. Site 4 is the only site that is not located in the main river body, but within the Água Quente stream



**Fig. 1** Location of sampling sites along the Monjolinho River and Água Quente stream, SE Brazil

which is a tributary of the Monjolinho River. This sampling site was added in the monitoring studies from November 2013 onwards, due to the input of untreated effluents into this water body coming from a neighborhood of São Carlos city. Site 5 is located downstream

from the WWTP, where the river receives high treated (WWTP) and untreated (Água Quente stream) effluent discharges. Finally, site 6 corresponds to the river mouth (where it discharges to the Jacaré-Guaçu River), with decreased urban cover but predominant rural activities.

All surface water samplings were done monthly from March 2011 to July 2012, and bimonthly from September 2012 to November 2014, while the sediment samples were collected every 4 months from March 2011 to November 2014 (due to logistic reasons), summing up, approximately, 4 years of monitoring period.

Surface water samples were collected and stored in pre-rinsed PET (polyethylene terephthalate) bottles. All samples were kept refrigerated at 4 °C during the transport to the laboratory. Samples for total phosphorus (TP) and total nitrogen (TN) determinations were maintained at -6 °C with no pre-treatment, while samples destined for analysis of soluble reactive phosphorus (SRP) and nitrogen series concentrations were filtered in cellulose acetate membranes of 0.45 µm (Sartorius, Goettingen, Germany).

The sediment samples were collected and stored in polyethylene recipients and also kept refrigerated at 4 °C during transport and stored at -6 °C. For the analysis, they were dried at 45 °C in an air recirculation oven, crushed and sieved through a 350-mesh sieve, and subsequently stored at -20 °C.

#### Analytical determinations of nutrients in surface waters

The physicochemical parameters (pH, Turbidity, DO, and EC) were determined in situ with a multiparametric probe YSI 6820 V2-2 (Yellow Springs, Ohio, USA).

The SRP concentrations in surface water samples were determined according to the ascorbic acid method (method 4500-P E, Baird 2017), using a Femto 600 (Femto, São Paulo, Brazil) spectrophotometer equipped with a glass cuvette with 10 cm of optical path. The limit of quantification (LOQ) of this method was 2.50 µg L<sup>-1</sup>. For the TP determinations, the samples passed through a previous digestion step with potassium persulfate in acid (method 4500-P B3, Baird 2017), in order to convert all the phosphoric species in solution into the PO<sub>4</sub><sup>3-</sup> form. After this step, the quantification was done according to the ascorbic acid method; the LOQ was also 2.50 µg L<sup>-1</sup>.

The NO<sub>3</sub><sup>-</sup>-N and the NO<sub>2</sub><sup>-</sup>-N analyses were done by ionic chromatography, using an ion chromatograph Metrohm 881 Compact IC Pro (Metrohm, Herisau, Swiss) with conductimetric detection. For this purpose, a polyvinyl alcohol column containing quaternary groups of ammonium was used, with particle sizes of 5 µm. The eluent used was a 4.0-mmol L<sup>-1</sup> solution of sodium carbonate and the suppression was performed with a 100-mmol L<sup>-1</sup> solution of H<sub>2</sub>SO<sub>4</sub>. The limit of quantification of this method was 10 µg L<sup>-1</sup> for NO<sub>3</sub><sup>-</sup> and

8 µg L<sup>-1</sup> for NO<sub>2</sub><sup>-</sup>. The determination of NH<sub>4</sub><sup>+</sup>-N was conducted in the same equipment, but with the use of a silica gel column containing carboxylic groups, with particles sizes of 5 µm. The eluent used was composed of a 0.70-mmol L<sup>-1</sup> solution of pyridinecarboxylic acid and a 1.7-mmol L<sup>-1</sup> solution of HNO<sub>3</sub>, without the need of chemical suppression. The quantification limit of this method was 23 µg L<sup>-1</sup>. For the TN determinations, a previous digestion step with potassium persulfate in basic medium was carried out (method 4500-N C, Baird 2017). The eluent and the column used were the same used for the NO<sub>3</sub><sup>-</sup>-N and NO<sub>2</sub><sup>-</sup>-N determinations, but for the suppression, a 0.5-mol L<sup>-1</sup> solution of H<sub>2</sub>SO<sub>4</sub>, a 25.2-g L<sup>-1</sup> solution of oxalic acid, and acetone 5% were used. The LOQ of this method was 5 µg L<sup>-1</sup>.

TOC concentrations were obtained by a Shimadzu TOC-L CPN/CPN (Tokyo, Japan) analyzer.

#### Analytical determination of nutrients in sediment

The digestion of the sediment samples for determination of TP was done as described by Andersen (1976), followed by quantification by the ascorbic acid method with colorimetric detection, as done for SRP and TP in surface waters. Iron was determined according to the 3050B method (EPA 1996) and detected by a Varian SpectraAA-640 spectrometer (Agilent Technologies, CA). Total nitrogen (TN<sub>(sed)</sub>) and total carbon (TC<sub>(sed)</sub>) were determined by elemental analyses in a Fisons equipment, model EA 1108 (Thermo Scientific, MA, USA), LOQ of 0.05 w.%.

#### Data analysis

To evaluate temporal patterns (wet vs. dry season) in the physical and chemical characteristics of the Monjolinho River, we included, in the wet season, data obtained between October and March and in the dry season, data obtained between April and September, independent of the year. To test hypothesis 1 (spatial and temporal variation among sites in the Monjolinho River), we used data obtained between March 2011 and September 2013. To test hypothesis 2 (effects of Água Quente Stream on spatial and temporal variation among sites in the Monjolinho River), we used data obtained between November 2013 and November 2014. We used a two-way ANOVA with fixed factors (sampling sites vs. seasons) to test both hypotheses. Data were log-transformed to obtain normality and homoscedasticity, except pH values. When significant differences were



found, we used Tukey's Honestly Significance Difference (HSD) a posteriori test to determine pairwise differences. We also evaluated multivariate patterns of spatial and temporal variation in water quality parameters, as suggested by Alberto et al. (2001) and Singh et al. (2004). We first calculated a matrix of Euclidian distances based on log-transformed values; variables were standardized for zero means and one unit of standard deviations. Then we carried out a non-parametric permutational multivariate analysis of variance (PERMANOVA) test using the same ANOVA model described above (Anderson 2001). There are two main advantages to using PERMANOVA on multivariate data (Anderson 2001): (1) there is no requirement of a multivariate normal distribution, contrary to MANOVA; (2) PERMANOVA allows an adequate partition of the variation across more than one factor (similarly to multifactorial ANOVA), contrary to other non-parametric multivariate methods; calculation of  $P$  tests are carried out by permutation to obtain the distribution of the statistic under the null hypothesis. Pairwise a posteriori tests can be constructed using the multivariate analogue of the  $t$  test, and exact  $P$  values are obtained using the sets of permutations across the pair of groups being compared (Anderson 2001). Since these calculated  $P$  values are not corrected for experiment-wise error rates, we used the Bonferroni method for adjusting error rates (Anderson 2001). In the present study, comparisons were carried out by permutation of residuals under a reduced model, with 4999 permutations. To evaluate distribution patterns, we carried out a principal component analysis (PCA) on the distance matrix. Nutrient concentrations in the sediment were analyzed only with univariate analyses, because only two variables were recorded (see Results). All analyses were performed with the software Systat 13.0 and PRIMER/PERMANOVA 6.0 (Anderson et al. 2008; Clarke and Gorley 2006). Significance values considered in this study were  $P < 0.05$ .

## Results

### Physico-chemical parameters of water samples

In the first period analyzed (March 2011–September 2013), there was no significant interaction between season and sampling sites, and no significant effects of season on EC and DO values, although significantly

higher pH and turbidity values were observed in the dry season (Table 1, Fig. 2). Significant differences among sampling sites were found for all variables, with values generally increasing from sites 1 to 3 and higher values at sites 4 and 5 (Fig. 2). Turbidity and pH presented less variation among the sampling sites, and EC was higher at site 5, intermediate at site 6, and lower at site 3 (Fig. 2). DO concentrations were significantly lower at site 5, with similar values at sites 1, 3, and 6.

We included the Água Quente stream in the second period analyzed (November 2013–November 2014) and the patterns differed. There was no significant interaction between sites and seasons for pH, EC, and turbidity, and only the latter presented seasonal effects, with significantly higher values in the wet season (Table 1, Fig. 2). Spatial patterns depended on the variable analyzed: although differences in pH were significant at  $\alpha = 0.05$ , the  $P$  value recorded (0.046) resulted in no significant differences among sites following Tukey's HSD test (Fig. 2). EC was significantly higher at sites 4–6, when compared to sites 1–3, whereas turbidity presented higher values at sites 4–6, intermediate at site 2, and lower values at sites 1 and 3 (Fig. 2). DO presented significant interaction between sites and seasons with lower values at sites 4 and 5 when compared to other sites, but with lower values in the dry season when compared to the wet season (Fig. 2).

### Nutrient concentrations in surface waters

Nutrient concentrations along the Monjolinho River were generally higher at sites 5 and 6 (downstream from the WWTP) than at sites 1–3 (upstream from the WWTP). However, analyses in different periods indicate that this pattern largely reflects the effects of the Água Quente stream for most variables.

Between March 2011 and September 2013, TN concentrations varied depending on the season considered, with a significant interaction between sites and seasons. In the dry season, values at sites 5 and 6 were similarly higher than upstream from the WWTP, which presented an increase in values from sites 1 to 3 (Table 1, Fig. 3). In the wet season, concentrations were higher at site 5 than at site 6, which presented values similar to site 3, whereas sites 1 and 2 presented similar values. Regarding the N species,  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  presented similar patterns, with higher values at sites 5 and 6 and significantly lower values at sites 1–3 (Fig. 3). Although  $\text{NO}_3^-\text{-N}$  presented a similar pattern, site 3 presented

**Table 1** Physico-chemical variables in surface water of six sampling sites at Monjolinho River. Values are means (standard deviation) [range]

Variables	Sampling sites					
	1	2	3	4	5	6
pH	6.34 (0.70) [4.22–7.49]	6.57 (0.58) [4.30–7.27]	7.13 (0.86) [4.75–8.57]	7.02 (0.46) [6.21–7.41]	6.90 (0.64) [4.45–7.51]	7.06 (0.57) [5.09–7.76]
EC ( $\mu\text{S cm}^{-1}$ )	28 (46) [8–168]	47 (14) [31–90]	106 (34) [41–236]	292 (122) [36–378]	228 (89) [3–395]	191 (56) [94–367]
Turbidity (NTU)	9.4 (19.4) [0.01–101.2]	16.1 (15.8) [0.02–68.9]	30.2 (77.1) [0.06–388]	88.8 (128.4) [23.2–378]	48.9 (119.1) [0.06–626]	38.7 (28.6) [0.1–133]
DO ( $\text{mg L}^{-1}$ )	6.70 (0.72) [4.96–7.90]	5.85 (1.38) [1.83–9.68]	8.03 (1.43) [3.10–9.68]	2.39 (1.38) [0.82–4.06]	3.91 (1.51) [1.40–8.83]	7.37 (0.52) [6.44–8.19]
TOC ( $\text{mg L}^{-1}$ )	1.10 (0.80) [0.16–3.68]	3.35 (1.95) [1.09–7.71]	4.00 (2.45) [1.10–12.3]	7.49 (5.69) [16.2–47.5]	31.2 (13.1) [0.52–25.0]	6.02 (4.07) [2.13–20.6]
SRP ( $\text{mg L}^{-1}$ )	7.09 (7.98) [<LOQ–40.3]	8.67 (6.91) [<LOQ–28.9]	49.4 (33.0) [<LOQ–157.9]	337.0 (227.5) [113.0–660.2]	93.2 (64.0) [4.91–256.4]	38.5 (23.7) [8.79–125.8]
TP ( $\text{mg L}^{-1}$ )	13.48 (18.76) [<LOQ–96.4]	102.82 (142.7) [6.54–606.39]	185.9 (159.4) [38.52–748.30]	3188 (2316.1) [201.0–7393]	698.4 (635.1) [117.9–2367]	338.2 (287.8) [97.8–1228]
N– $\text{NH}_4^+$ ( $\text{mg L}^{-1}$ )	0.25 (0.43) [<LOQ–1.20]	0.68 (0.77) [<LOQ–2.47]	0.76 (0.72) [<LOQ–2.47]	14.77 (3.03) [9.29–18.82]	4.72 (3.08) [0.67–10.66]	3.64 (2.82) [0.34–12.00]
N– $\text{NO}_2^-$ ( $\text{mg L}^{-1}$ )	0.05 (0.11) [<LOQ–0.35]	0.14 (0.16) [<LOQ–0.45]	0.31 (0.38) [<LOQ–1.19]	0.35 (0.22) [<LOQ–0.74]	0.23 (0.21) [<LOQ–0.60]	0.33 (0.33) [<LOQ–0.82]
N– $\text{NO}_3^-$ ( $\text{mg L}^{-1}$ )	0.29 (0.33) [<LOQ–0.97]	0.44 (0.57) [<LOQ–1.88]	5.74 (5.33) [<LOQ–13.75]	1.08 (1.05) [0.21–3.34]	1.83 (4.07) [<LOQ–21.13]	1.97 (4.07) [<LOQ–19.45]
TN ( $\text{mg L}^{-1}$ )	1.23 (0.70) [0.32–3.26]	4.61 (4.55) [0.50–18.44]	14.88 (4.23) [6.69–22.98]	77.74 (39.57) [8.47–122.3]	38.79 (15.23) [15.36–66.75]	30.15 (16.89) [1.24–74.79]

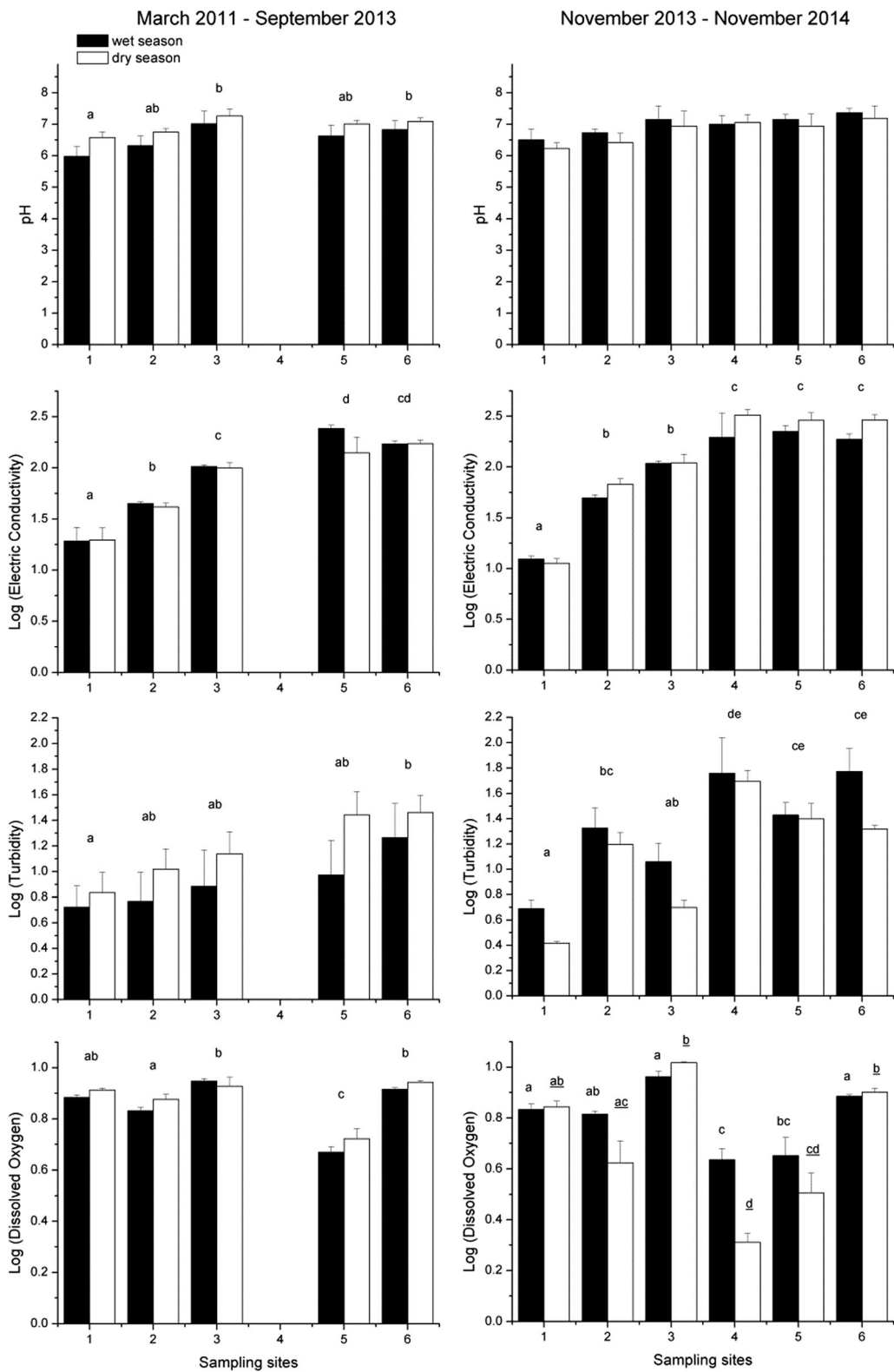
LOQ limit of quantification

significantly higher values than all other sites (Fig. 3). SRP and TP values were lower at sites 1 and 2, intermediate at sites 3 and 6, and higher at site 5, whereas TOC was lowest at site 1 and presented similar values at all other sites (Fig. 3).

Nutrient concentrations in the Água Quente stream (site 4) reflected the input of untreated sewage during the second sampling period (November 2013–November 2014), with a significantly higher load than the other sampling sites for  $\text{NH}_4^+$ -N, SRP, and TOC, and similar values of TN,  $\text{NO}_3^-$ -N and  $\text{NO}_2^-$ -N among downstream sites (Fig. 3). Comparing with site 3 (upstream the WWTP), SRP values were almost 7 × higher, TP values were 17 × higher,  $\text{NH}_4^+$ -N values were more than 19 × higher, TN values were 5 × higher, and TOC concentrations were almost 2 × higher (Table 1). On the other hand, sampling sites 1 and 2 tended to have significantly lower nutrient concentrations (Fig. 3). No significant interactions were found between sampling sites and seasons ( $P > 0.05$ ), except for  $\text{NO}_3^-$ -N ( $P = 0.048$ ): as in the previous study period (March 2011–September

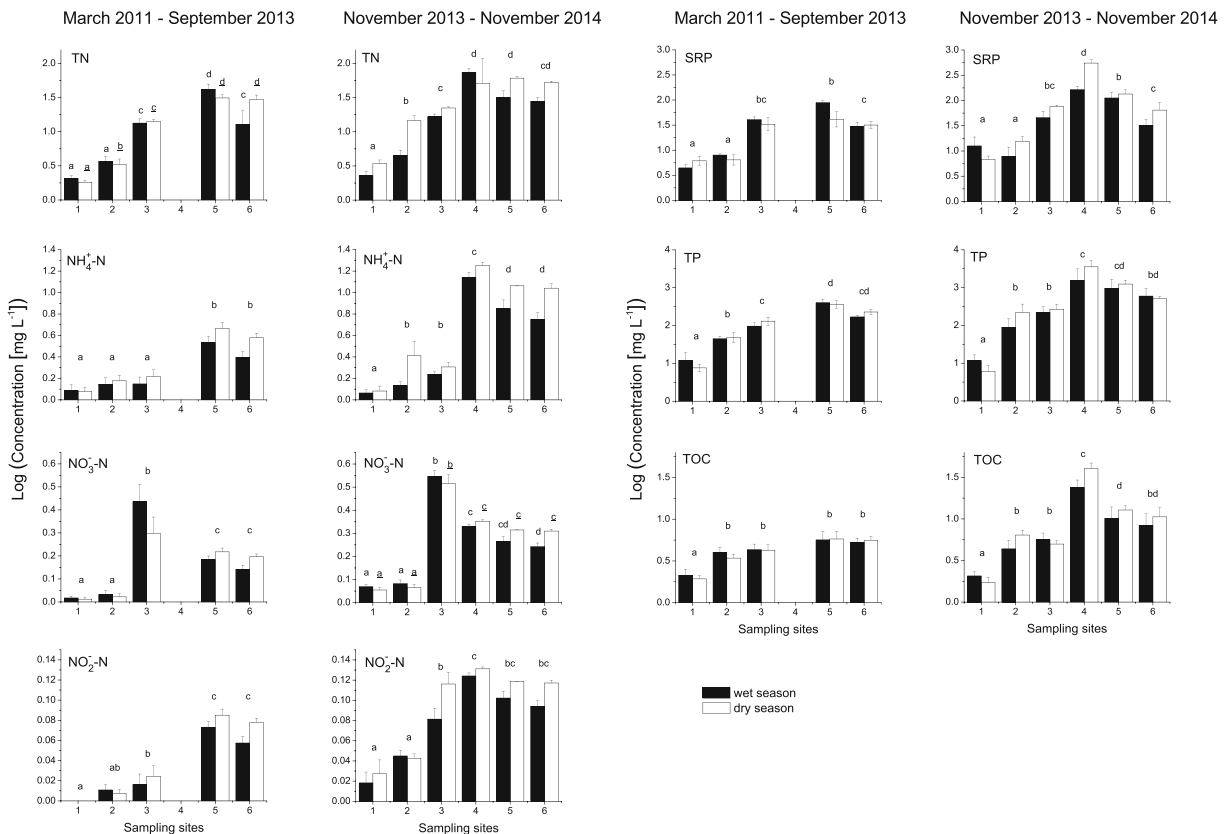
2013), a high value was recorded at site 3, which gradually decreased along sites 4, 5, and 6, whereas in the dry season, these three sites presented similar values; sites 1 and 2 presented similar low values in both seasons (Fig. 3). Significant seasonal effects were verified for TN,  $\text{NH}_4^+$ -N,  $\text{NO}_2^-$ -N, and SRP, with lower values in the wet season (Fig. 3). Spatial patterns along the Monjolinho River were roughly similar to those recorded in the previous study period, mainly among sites 3, 5, and 6, the exceptions being  $\text{NO}_2^-$ -N, with similar values among these sites, whereas in the previous period, site 3 had significantly lower concentrations (Fig. 3), and TOC, which presented a higher value at site 5 when compared to the other sites.

Multivariate analysis confirmed the general patterns presented earlier, and it was more effective in determining the actual situation of sites downstream from the WWTP and the Água Quente stream, by considering the whole dataset. There was no significant interaction between sampling sites and seasons (Table 2), but main effects were significant, both for seasons ( $F_{1,130} = 2.70$ ,



**Fig. 2** Mean values (+ SE) of water physical and chemical characteristics along the sampling sites in the wet and dry seasons for both periods of the study. Similar letters connect means that did not differ significantly (Tukey’s HSD test,  $P > 0.05$ )





**Fig. 3** Mean (+ SE) nutrient concentrations along the sampling sites in the wet and dry seasons for both periods of the study. Similar letters connect means that did not differ significantly (Tukey’s HSD test,  $P > 0.05$ )

$P = 0.039$ ) and sampling sites for both periods of the study (Table 2). The pairwise tests indicated that all sampling sites were significantly different from each other, even when considering Bonferroni correction ( $P < 0.05$ ).

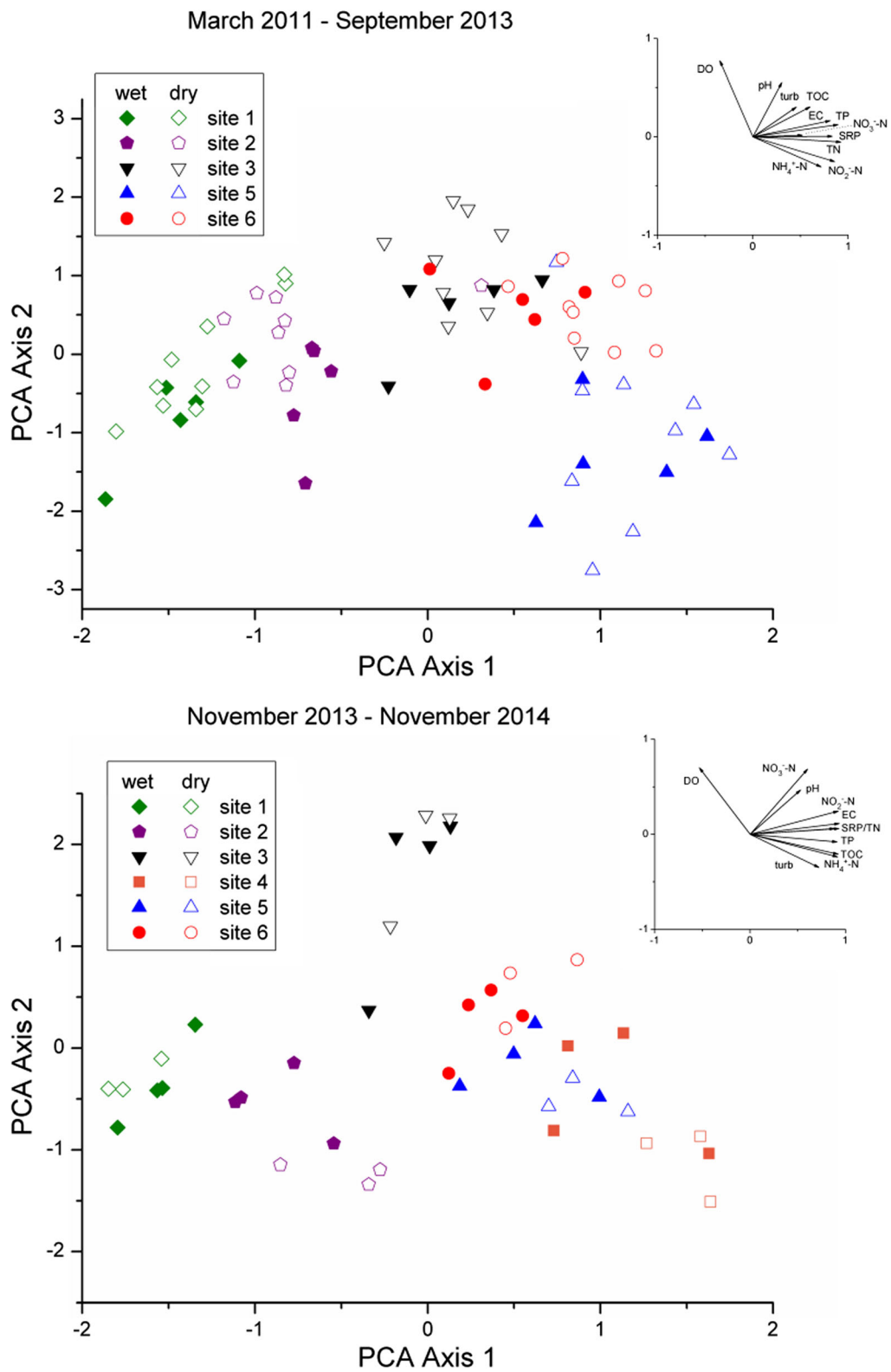
The PCA analysis confirmed there was a gradient in river water quality among sampling points. In the first study period (March 2011–September 2013), the first three axes explained 70.0% of the total variance. The first axis (eigenvalue = 5.305) explained 48.2% of the variance and represented a gradient from sites with lower nutrient and higher DO concentrations (sites 1 and 2) to sites with high nutrient concentrations and EC values (Fig. 4). The second axis (eigenvalue = 1.273) explained 11.6% of the total variance and ordinated samples with higher  $\text{NH}_4^+-\text{N}$  concentrations to samples with higher turbidity, pH, DO, and TOC concentrations (Fig. 4), mainly representing seasonal variation patterns indicated in the univariate analyses. Both axes combined resulted in a separation of sites 3, 5,

and 6: site 5 had the highest values, site 6 intermediate between sites 3 and 5, and site 3 was closer to the origin of the graph (Fig. 4). The third axis (eigenvalue = 1.120) explained 10.2% of the variation, much due to the higher

**Table 2** Average (standard deviation) values of nutrients and total iron in sediments of the sampling sites at Monjolinho River. Similar letters connect means that did not differ significantly (Tukey’s HSD test,  $P > 0.05$ )

Sampling site	TC <sub>(sed)</sub> (mg g <sup>-1</sup> )	TP <sub>(sed)</sub> (mg g <sup>-1</sup> )	Total-Fe <sub>(sed)</sub> (mg g <sup>-1</sup> )*
1	0.50 (0.59) <sup>a</sup>	0.43 (0.29) <sup>a</sup>	46.39 (1.11) <sup>a</sup>
2	6.70 (5.21) <sup>b</sup>	13.53 (3.74) <sup>b</sup>	199.84 (25.56) <sup>b</sup>
3	1.34 (0.81) <sup>ab</sup>	1.52 (0.45) <sup>c</sup>	79.17 (7.69) <sup>c</sup>
4	1.03 (1.10) <sup>ab</sup>	1.22 (0.59) <sup>ac</sup>	31.58 (1.56) <sup>d</sup>
5	0.76 (0.65) <sup>a</sup>	2.52 (1.06) <sup>cd</sup>	50.59 (5.35) <sup>ae</sup>
6	1.02 (0.76) <sup>ab</sup>	3.72 (2.65) <sup>d</sup>	63.95 (5.18) <sup>ce</sup>

\*Determined for samples of August 2014 only



**Fig. 4** Ordination of sampling sites by principal components analysis and vectors related to component loadings for both periods of the study. Open symbols indicate samples collected in the dry season, and closed symbols indicate samples from the wet season

concentrations of  $\text{NO}_3^-$ -N recorded at site 3 (Fig. 3), resulting in the highest loading associated with this axis (0.644). In the second study period (November 2013–November 2014), including the Água Quente stream, the first two axes explained 80.7% of the variation, with first axis (eigenvalue = 7.345) explaining 66.8% of total variation. High values of nutrient concentrations in the Água Quente stream ordinated samples from this site in the opposite gradient of sites 1 and 2 (Fig. 4). The second axis (eigenvalue = 1.535) explained 13.9% of the variation and was strongly influenced by high DO concentrations at sites 1, 2, and 3 and high values of  $\text{NO}_3^-$ -N at site 3 (Fig. 4). When both axes were combined, there was a clear gradient of pollution, from the least polluted sites 1 and 3 to the more polluted 3, 6, 5, and 4, suggesting that site 6 (at the end of the Monjolinho River) is approaching chemical characteristics of site 3 (which is upstream from the WWTP and the Água Quente stream), whereas site 5 (downstream from both the WWTP and the Água Quente stream) is intermediate between sites 4 (Água Quente stream) and 6.

Determination of nutrients in sediment

Nutrients in sediments presented some variation in concentrations, with the highest values of both  $\text{TC}_{(\text{sed})}$  and  $\text{TP}_{(\text{sed})}$  at sampling site 2 (Table 3), especially for  $\text{TP}_{(\text{sed})}$  ( $P < 0.001$ ). There were no significant effects of seasons or sampling sites  $\times$  seasons ( $P > 0.20$ ).  $\text{TN}_{(\text{sed})}$  values were below the limit of quantification. Considering that the biogeochemical cycle of phosphorus in sediment is deeply bound to the presence of Fe (Allan and Castillo 2007), the determination of total  $\text{Fe}_{(\text{sed})}$  was done in a random sample collected in August 2014. We also found higher total  $\text{Fe}_{(\text{sed})}$  concentrations at site 2 ( $P < 0.001$ ), when compared with the other sampling sites (Table 3).

Discussion

Surface waters

The Monjolinho River presented large spatial and temporal variation in physical and chemical characteristics, as found for other rivers worldwide, such as the Gomti River in India, which showed a large variation of physico-chemical variables during the years of 1994–1998, mainly due to effects of seasons (dry, wet, and monsoons) and the different impacts to which the river is subject (Singh et al. 2004). Zhou et al. (2007) found a similar scenario at the Northwestern New Territories region in Hong Kong during the monitoring time of 2000–2004, being possible to observe both spatial and temporal variations. The water quality assessment of the Melen River, in Turkey, during the period 1995–2006, showed a high variation of the river water quality between the wet-dry seasons, mainly due to urban and industrial activities (Koklu et al. 2010). However, in our study, we found that an important tributary which receives untreated sewage, the Água Quente stream, presents a strong effect on spatial variation of nutrient and DO concentrations, so that sampling sites downstream from the municipal WWTP are largely influenced by this stream. Also, urban and rural land uses may have significant effects on nutrient concentrations, even before the Monjolinho River enters the main urban patch in São Carlos city and downstream from the urban area (possible effects of sugarcane plantation and ethanol industry presence in the river mouth area).

The lowest values for all nutrients and EC were found only at the headwaters site, with increasing concentrations at downstream sites. However, when analyzing other studies that sampled in nearby regions (site 1 = headwaters, site 2 = upstream from urban patch), we

**Table 3** Average (standard deviation) values of nutrients and total iron in sediments of the sampling sites at Monjolinho River. Similar letters connect means that did not differ significantly (Tukey’s HSD test,  $P > 0.05$ )

Sampling site	$\text{TC}_{(\text{sed})}$ ( $\text{g}^{-1}$ )	$\text{TP}_{(\text{sed})}$ ( $\text{mg g}^{-1}$ )	$\text{TN}_{(\text{sed})}$ ( $\text{mg g}^{-1}$ )	Total- $\text{Fe}_{(\text{sed})}$ ( $\text{mg g}^{-1}$ )*
1	0.50 (0.59) <sup>a</sup>	0.43 (0.29) <sup>a</sup>	< 0.50	46.39 (1.11) <sup>a</sup>
2	6.70 (5.21) <sup>b</sup>	13.53 (3.74) <sup>b</sup>	< 0.50	199.84 (25.56) <sup>b</sup>
3	1.34 (0.81) <sup>ab</sup>	1.52 (0.45) <sup>c</sup>	< 0.50	79.17 (7.69) <sup>c</sup>
4	1.03 (1.10) <sup>ab</sup>	1.22 (0.59) <sup>ac</sup>	< 0.50	31.58 (1.56) <sup>d</sup>
5	0.76 (0.65) <sup>a</sup>	2.52 (1.06) <sup>cd</sup>	< 0.50	50.59 (5.35) <sup>ac</sup>
6	1.02 (0.76) <sup>ab</sup>	3.72 (2.65) <sup>d</sup>	< 0.50	63.95 (5.18) <sup>ce</sup>

\*Determined for samples of August 2014 only

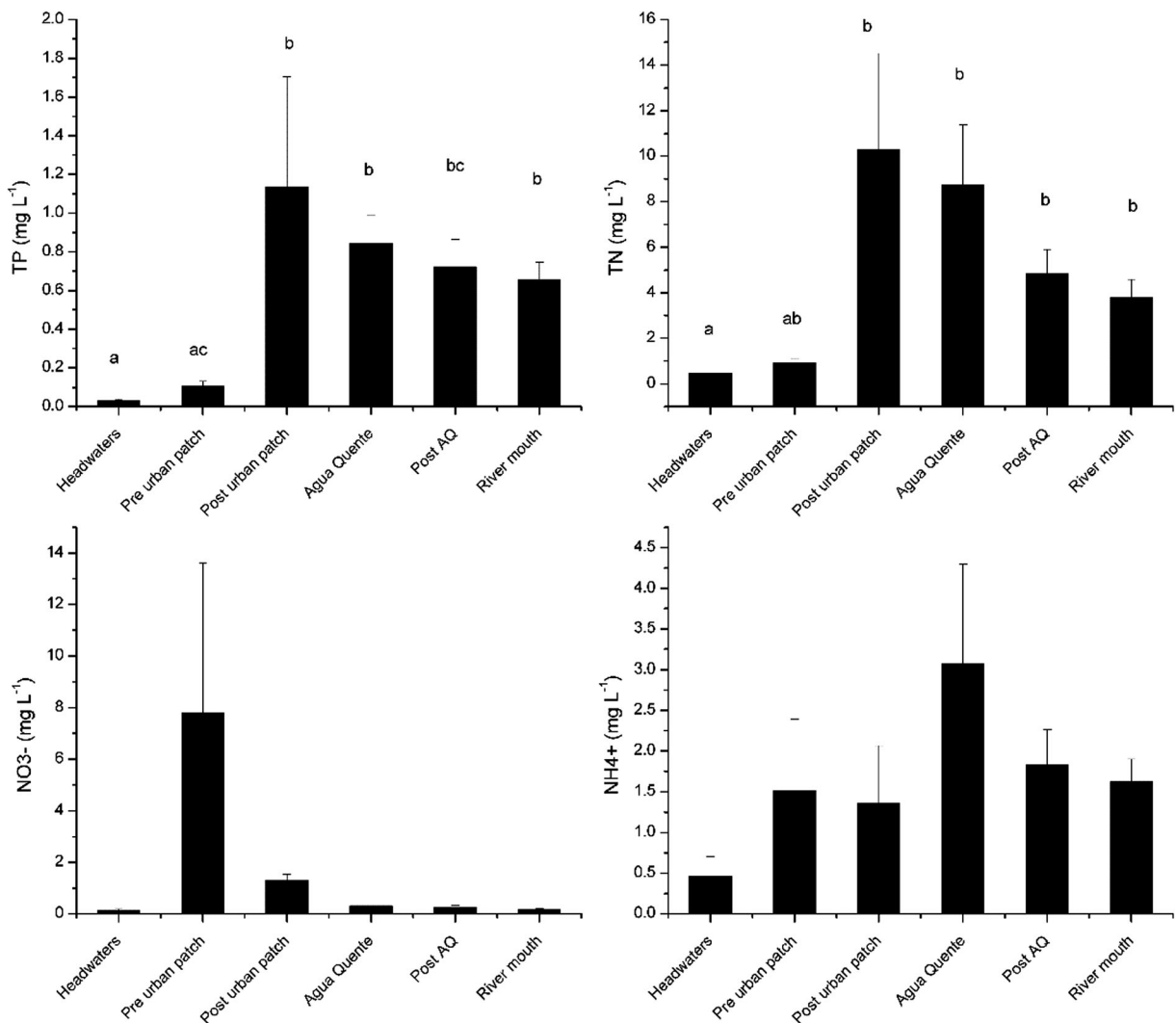
found no significant differences between these sites (Fig. 4; see also Bere and Tundisi 2011a), suggesting that the site 2 is being progressively influenced by local changes in land use and consequent effects in water quality. At site 3, downstream from the urban patch, increased nutrient concentrations and EC due to urban runoff are noted. Higher DO values are observed due to a waterfall upstream from this point, which possibly favors oxidation of N species to  $\text{NO}_3^-$ -N. At downstream sites, with lower DO concentrations,  $\text{NH}_4^+$ -N predominates.

The Água Quente stream, and the downstream sites in the Monjolinho River, shows the effects of untreated sewage. We found higher EC, DOC, SRP, TP, TN, and  $\text{NH}_4^+$ -N values and lower DO concentrations at these sites, with the highest DOC and EC values in the Água Quente stream due to higher concentrations of organic matter from the untreated effluent, and significantly lower DO concentrations, since it is consumed in the organic matter oxidation. The mean values obtained for SRP and TP at the Água Quente stream and downstream from the WWTP are similar to those found in Mae Tao Stream area, region where there are intense agriculture and mining activities (Weerapran et al. 2015). However, they are inferior to those found in the Zomar stream (Palestine), where there is input of untreated sewage from cities and industries along its pathway (Suleiman et al. 2012). Lower concentrations of TP were found in the Doce River (Minas Gerais State, Brazil), with mean values of  $200 \mu\text{g L}^{-1}$  (Jardim et al. 2014). Therefore, the main source of phosphorus input to the Monjolinho River is the discharge of treated and untreated sewage at sites 4 and 5. According to Kim et al. (1996), a factor that can influence the TP concentrations is household sewage and urban runoff from the surrounding cities, which corroborates the data presented in this paper.

The nitrogen series concentration values show that each of the nitrogen forms has a different pattern.  $\text{NH}_4^+$ -N concentrations were lower at sites 1–3, higher at site 4, and intermediate at sites 5 and 6, due to the intense effluent discharges (Fig. 3) and consumption of almost all DO for the ammonium oxidation in solution. Therefore, almost all DO present is consumed in the oxidation of ammonium nitrogen in solution, resulting in a significant negative correlation between DO and  $\text{NH}_4^+$ -N concentrations ( $r = -0.568$ ,  $P < 0.001$ ). On the other hand, the  $\text{NO}_3^-$ -N values presented different patterns depending on the season, due to a significant interaction

effect when including the Água Quente stream; in the first study period, there was no interaction, with the highest values at site 3. This might be due to the combination of two main factors: the high DO concentrations and the trend towards low nitrogen species concentrations at site 3. For these reasons, most nitrogen species in solution at site 3 (derived from urban runoff) are oxidized to nitrate, while the low availability of DO at the downstream sites favor the  $\text{NH}_4^+$ -N species. During the wet seasons, the increase of the river flow due to precipitation allows the solubilization of oxygen, increasing its availability mainly at site 3. The effect of urban runoff is also higher at this site, justifying the elevated values of  $\text{NO}_3^-$ -N in wet seasons. At sites where  $\text{NH}_4^+$ -N occurs due to discharge of effluents (sites 4, 5 and 6), higher values tend to occur during the dry season. By being an intermediate form between  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N,  $\text{NO}_2^-$ -N does not present a well-defined spatial or temporal distribution pattern, with significantly low concentrations at sites 1 and 2 and similar values among the other sampling sites (Fig. 4). TN concentrations increased from headwaters to the WWTP, with a decrease at the river mouth. In Maringá stream (Southern Brazil), Freire et al. (2013) found a similar scenario compared to the data obtained from the Monjolinho River, with  $\text{NH}_4^+$ -N concentrations close to  $13 \text{ mg L}^{-1}$ , coming from the discharge of effluents in the water body. The average  $\text{NO}_3^-$ -N values were even higher at some regions, with values above  $7.5 \text{ mg L}^{-1}$ . Ternus et al. (2011) studied the relation between urbanization and water quality in the upper Uruguay River basin, relating the increase of nitrate concentrations to the urban runoff, use of nitrogen additives and ammonium for fertilizers to be used for rural agricultural activities, and an industrial sewage dump.

Some studies described in the literature (Peres 2002, Novelli 2005, Viana 2005, Dornfeld 2006, Baio 2009, Bere and Tundisi 2011a, b) have revealed that before the WWTP started its operations, no significant differences in TP and TN were seen from downstream from the urban patch to the river mouth, including the Água Quente stream (Fig. 5). The authors attributed such an observation to the input of all untreated sewage which is now directed to the WWTP, so there was no sign of nutrient depuration. Also, no significant differences were found among sites for both nitrate ( $2.13 \pm 9.01 \text{ mg L}^{-1}$ , mean  $\pm$  SD) and ammonium ( $1.48 \pm 1.81 \text{ mg L}^{-1}$ ), although values ranged between 0 and  $57.62 \text{ mg L}^{-1}$  and  $0.002$ – $7.81 \text{ mg L}^{-1}$ , respectively



**Fig. 5** Mean (+ SE) TP and TN concentrations from samples collected before the WWTP implantation, between 2003 and 2009. Data are from Peres (2002), Novelli (2005), Viana (2005), Dornfeld (2006), Baio (2009), and Bere and Tundisi (2011a, b)

(Appendix 1). On the other hand, our results show a trend for the decreasing nutrient concentrations at the river mouth (site 6) when compared to sites 4 and 5, suggesting that although the Monjolinho River now receives both untreated (Água Quente stream) and treated (WWTP) sewage, it now presents an autodepuration capacity.

We found significant seasonal effects for some of the nutrients, with higher values in the dry season, suggesting a dilution effect due to rainfall, which directly influenced multivariate patterns, contributing to variance, mainly in the second PCA axis (Fig. 4). Other studies found seasonal effects, mainly related to rainfall, on stream water quality parameters (Singh et al. 2004;

Townsend-Small et al. 2011). However, we also found a large variation in nutrient concentrations along the studied period (Table 1), suggesting that both stochastic and among-year variations in concentrations may influence temporal water quality patterns.

Nutrients in sediments

The data in Table 4 show that the composition of the Monjolinho River sediment is predominantly sandy. Table 3, site 2, presents a higher value of total carbon (6.7 mg g<sup>-1</sup>) that can possibly be attributed to the presence of clay in the sediment composition. The Fe<sub>(sed)</sub> analysis found higher Fe values at this site,



**Table 4** Granulometric characterization of the sediment of the Monjolinho River sampling sites

Site	Clay (%)	Silt (%)	Sand (%)
1	4.0	0.2	95.7
2	14.0	1.3	70.6
3	4.1	0.2	95.8
4	2.5	0.7	96.7
5	2.7	0.8	96.4
6	3.8	0.7	95.5

suggesting that (1) most phosphate discharged at site 2 is retained to the sediment by the Fe in solution (Jensen et al. 1992) or (2) the samples from that site correspond to fractions of eroded soil by the riverside at this reach. Due to the biogeochemical interaction between Fe and P, at low DO concentrations, the release of sediment-bound P to the water column may occur, increasing its concentrations mainly during the dry season at site 2. Such patterns were not observed at the other sampling sites, so that sediment characteristics probably favor higher TP values at this site.

## Conclusions

The long-term data survey in this study demonstrates an increasing trend of the impacts caused by anthropic activities on the Monjolinho River, from its headwaters until downstream from the WWTP, and signs of slight recovery near the mouth. Site 1 presents characteristics of an aquatic body with little influence of human activities, similar to those bodies non-impacted. The first signs of rural runoff are noticed at site 2, even though to a lesser extent, while the effects of urban runoff seem to be mainly responsible for the values found at site 3. At sites 4 and 5, the worst conditions of the Monjolinho River are observed, which receives the discharge of non-treated and treated effluents. Site 6 is located after a long stretch with little human activities, allowing the river to naturally attenuate the effects of previous regions. By comparing the data obtained in this study with those published prior to the implementation of the WWTP, it is possible to observe that the presence of this facility increases the water quality in the stream, corroborating the initial hypothesis of this work in relation to the effect of the WWTP.

However, this effect was not detected in most univariate analyses, and only multivariate analyses enabled a clear distinction of this pattern, with highly significant differences among sampling sites detected by PERMANOVA and the indication of site 6 as an intermediate between sites 5 and 3 in the PCA ordination. Multivariate techniques allow a more complete analysis of water quality parameters, incorporating all the available information. Although ordination techniques are well established to visualize patterns in multivariate space, rigorous hypothesis testing on multiple factors has been limited by the premises of the analyses, mainly MANOVA. Non-parametric techniques such as PERMANOVA now allow not only testing multiple factors, but also allow multiple comparisons tests by using adequate permutation tests, being robust in relation to differences in sample sizes, correlation structure, error distribution, and number of variables (Anderson and Walsh 2013).

Considering the present situation of freshwater scarcity for consumption, and the large-scale collection of water quality data on spatial and temporal scales from streams and rivers worldwide, the use of robust statistical techniques can greatly contribute to implement policies for the recovery and maintenance of surface water quality.

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