

EFFECT OF BIOTURBATION BY *CHIRONOMUS* ON NUTRIENT FLUXES IN AN URBAN EUTROPHIC RESERVOIR

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ABSTRACT

In the process of colonizing sediment benthic macroinvertebrates carry out bioturbation promoting the transport of solutes and particles at the sediment-water interface. The objective of the present study was to evaluate the influence of *Chironomus* larva, by way of bioturbation, on the flow of dissolved oxygen, carbon dioxide, phosphate, ammonia and nitrate at the sediment-water interface of the Ibirité reservoir (Brazil). For this, we recreated microcosms in 24 plexiglass cylinders ("core") (20 cm long, 4.5 cm inner diameter) containing the contents for the sediment-water interface of the Ibirité reservoir, being that 12 cores had sandy sediment and 12 had muddy sediment. The cores were subjected to different treatments in triplicate: controls (without *Chironomus* larvae) and incubated cores with 2, 4 and 6 *Chironomus* larvae (1250, 2500, 3750 ind.m⁻²). Significant correlations were observed between the fluxes of O₂, PO₄, NH₄⁺ and NO₃ and the density of *Chironomus* (Pearson, p<0.05). In both types of sediment we observed the influence of *Chironomus* in the release of PO₄ and NH₄⁺, as well as in the consumption of O₂ and the reduction in the concentration of NO₃ in the overlying water. The current study revealed that the largest fluxes occurred in the muddy sediment in relation to the sandy sediment (ANCOVA, p<0.05) except for CO₂ release. The data presented here demonstrates the importance that *Chironomus* larvae and sediment characteristics have on nutrient flows at the sediment-water interface of eutrophic reservoir.

Keywords: bioturbation, nutrient fluxes, sediment-water interface, dissolved oxygen, carbon dioxide.

INTRODUCTION

The continuous sedimentation of particulate and dissolved matter originating from the autochthonous and/or allochthonous origins characterize the benthic layer as a location of intense nutrient cycling. In sediment, aerobic or anaerobic metabolism frees up nutrients for aquatic biota demonstrating the important connectivity between sediment and the water column (Fukuhara & Sakamoto, 1988; Schindler & Scheuerell, 2002).

The colonization by benthic fauna of sediment in aquatic environments promotes the active transport of solutes and particulate matter along the sediment-water

interface (bioturbation) (Aller, 1982). The re-suspension of solutes and particles, ingestion, digestion and liberation of excreted products, as well as changes in the characteristics of sediments caused by benthic macroinvertebrates have been regarded as important steps in the cycling and remobilization of nutrients (e.g., nitrogen, phosphorus and carbon) from sediment to the water column within aquatic ecosystems (Leal *et al.*, 2003). Conversely, the intrinsic characteristics of the sediment such as granulometry, porosity, amount of interstitial water, concentration and quality of organic matter and the microbial community, represent variables of heightened importance in nutrient flow dynamics (Boudreau & Jørgensen, 2001).

Considering that there is a lack of information regarding the role of benthic macroinvertebrates in nutrient cycling in tropical reservoirs, the main goal of this study was to test the predictions of the hypothesis that bioturbation by *Chironomus* is important in increasing fluxes in the sediment-water interface. We predict that: (i) nutrient fluxes are high in muddy sediment with a higher content of organic matter and interstitial water, when high chironomid oxygen consumption and CO₂ liberation is observed; (ii) the retention and/or release of the different forms of PO₄ are dependent on the density of invertebrates; (iii) mechanical bioturbation of the sediment by fauna when construction burrows stimulate the N-NH₄⁺ and N-NO₃⁻ fluxes in the water column.

MATERIAL AND METHODS

The Ibirité Reservoir (19°07'00''- 20°02'30'' S; 44°07'30''- 44°05'00'' W) belongs to the Paraopeba river watershed, affluent of the São Francisco River in Minas Gerais State, southeastern Brazil. The watershed is composed by the sub-basins of the Pintados, Retiro and Do Onça streams forming the Ibirité River. The Ibirité River dam was constructed in the 60's giving rise to the Ibirité Reservoir. The Ibirité River is a direct affluent of the Paraopeba River. The surrounding areas are densely inhabited with slums and there is no domestic sewage treatment, which is released *in natura* into the rivers of the Ibirité reservoir watershed.

The sediment samples were collected at two distinct sampling stations taking into consideration the predominate type of sediment at each station: sandy (near the mouth of the Ibirité River) and muddy (near the dam of the reservoir). The sampling stations considered in the present study correspond to stations number 8 and 11, (sand and muddy, respectively) in the study conducted by Moreno & Callisto (2006).

Sediment sampling and procedures

At station 1, sediment was collected using a core sampler. Following collection, the sample was taken to the laboratory and sieved with water from the reservoir over a 1.0 mm mesh, thereby excluding invertebrate organisms. This procedure was necessary due to the elevated density of benthic macroinvertebrates present in the sediment samples. After being sieved, the sediment was placed in an aquarium of approximately 20 liters until the sediment layer reached a height of 30 cm. Filtered reservoir water (GFC membrane, 1.7 μm mesh) was added to the aquarium after which the aquarium was allowed to settle for 10 days, as proposed by Svensson (1998). At station 2, the sediment (muddy sediment) was also collected with the aid of a core sampler. The sample was also

taken to the laboratory yet was not sieved over a 1.0 mm mesh because it did not present any signs of benthic macroinvertebrates colonization.

Nearly 200 *Chironomus* larvae were collected using hand nets and placed in polystyrene foam containers with reservoir water. In the laboratory, 72 individuals in 3^o - 4^o instars were selected for use in the study.

Bioturbation experiment preparation

From the sandy sediment, which was sieved and placed in an aquarium for 10 days, 12 plexiglass cores were removed (internal diameter = 4 cm and length = 20 cm) containing the sediment-water interface without causing the re-suspension or disturbance of the sediment. Following removal, cores incubated with sand and mud sediments were submitted to an acclimatization period. Cores containing the interface from both stations were incubated with a layer of 9.0 - 11.5 cm of sediment and 8.5 - 10.6 cm of water (overlying water), following the methodology of Leal et al. (2003).

During the 24 hr acclimatization period the cores were maintained open and submersed in reservoir water, previously filtered (GFC membrane) to remove phytoplankton, in a thermic box (70 liters). Each box was fitted with aeration pumps favoring the oxygenation of the water both inside and outside of the cores. In order to allow constant oxygenation and homogenization of the core interiors magnets, propelled by a motor to rotate at 45 rpm, were used in accordance with Svensson (1997). After 24 hr all cores displayed a saturation of 98 ± 1.6 % of dissolved oxygen (Winkler method) and a mean temperature of 24.0 ± 1.4 °C.

Following the acclimation period, mature *Chironomus* larva were added, in triplicate, in the following densities: 2, 4 and 6 larva/core, totaling 9 cores with organisms for each type of sediment (sandy and muddy). The densities utilized represented, respectively, values of 1250, 2500 and 3750 ind.m⁻². These densities are the same as those found for Chironomidae larva in the Ibirité reservoir during the study period (Moreno & Callisto, 2006). Three cores were maintained as controls (without *Chironomus* larva). After the addition of larva the cores were still kept open for a period of 4 hr, allowing for the acclimation of the organisms in the interior of the sediment. Afterwards, the cores were covered, interrupting the exchange of water between the internal section of each core to the external water in the thermic box. The cores were maintained in the dark and with the motor propelled magnets during the entire time of this experiment (12 hours).

Nutrient Analysis

The nutrients released were estimated in the control cores and in the cores with *Chironomus* larva, by determining the initial and final concentrations after 12 hours of incubation. To determine the dissolved fractions, samples of water (20-50 ml) were filtered in GFF membrane (previously incinerated at 550°C) and frozen afterwards. In the laboratory NH₄⁺ concentrations were determined using the indophenol blue method, orthophosphate (PO₄), and dissolved organic carbon (DOC) via analysis in a TOC Analyzer (SHIMADZU - 5000). The concentrations of NO₃ were determined by cadmium reduction method. In order to determine the concentrations of dissolved oxygen

in the water column samples of 15 ml were taken from each core, with the aid of a syringe and transferred to glass flasks of 15 ml and measured with the Winkler method. The concentrations of CO₂ were estimated using the pH - alkalinity method.

The release rates were estimated by using the equation previously described by Dalsgaard *et al.* (2000): Flux = (Fc - Ic).v/A.t; where: Fc = Final Concentration, Ic = Initial concentration, v = volume of overlying water in the cores (liters), A= core area (m²) and t = Incubation time (h).

Sediment Analysis

Sediment at each sampling station was collected using a corer sampler. The organic matter content of the samples was determined by incineration of the first 10 cm of the samples at 550°C during 4h, while granulometric composition, and the interstitial water content was determined by oven drying at 60°C during 72 hr.

Statistical procedures

To evaluate the influence of *Chironomus* on O₂, CO₂, PO₄, NH₄⁺ and NO₃⁻ fluxes, we used correlation analysis (r-Pearson, p<0.05). The ANCOVA test was used to compare the O₂, CO₂, PO₄, NH₄⁺ and NO₃⁻ fluxes in sandy and muddy sediments. To compare the control and treatment groups with *Chironomus* we used the non parametric analyses of variance Kruskal-Wallis test (p<0.05). All statistical tests were performed by GraphPad Prism®, version 3.0 by Software, Inc.

RESULTS

Sediment characteristics

Sediment collected in the two sampling stations (sandy and muddy), used in the incubation treatments was distinct with respect to their physical and chemical characteristics (Table 1). In relation to the granulometric composition, the sandy sediment, presented as the main class sizes: the medium, coarse and fine sands, with silt and clay class sizes less than 4.9%. On the other hand, the muddy sediment had as its main granulometric class size silt and clay (62.0 %) followed by sand; coarse (20.0%) and fine (18.0%).

With regards to the interstitial water, both the sediments displayed rather high levels (>70.0%), with values being slightly greater for the muddy sediment. Values of organic material were significantly greater (Kruskal-Wallis, p<0.05) for muddy sediment (2.7 times greater) in relation to the sandy sediment (Table 1).

Dissolved Oxygen consumption and CO₂ release

The incubated cores with muddy sediment displayed rates of O₂ consumption significantly greater than those observed for cores with sandy (ANCOVA, p<0.05). The consumption of oxygen was significantly greater (Kruskal-Wallis, p<0.05) in the treatments with *Chironomus* in relation to the control, in both treatments (Table 3). Significant correlations were observed between the rates of O₂ consumption and the density of *Chironomus* larvae in both sediments (r-Pearson, p<0.05) (Table 2; Figure 1a).

In the sandy sediment cores, an increase in the release of CO₂ was observed when they were incubated with *Chironomus* larva, reflecting a significant linear correlation between the flux of CO₂ and the *Chironomus* density (r-Pearson, p<0.05)

(Table 2; Figure 1b). In the cores incubated with muddy sediment, the presence of larva did not reflect on the effects of the fluxes of CO₂ (r-Pearson, p>0.05), given that the values were significantly lower than those observed for the cores with sandy sediment (ANCOVA, p<0.05).

PO₄, N-NH₄⁺ and N- NO₃ release

In both types of sediment the release of orthophosphates from the sediment to the water column was dependant on the density of the larva, a significant correlation was observed (r-Pearson, p<0.05) (Figure 1c), although, this was observed only in treatments that had densities equal to or higher than 2500 ind.m⁻². In the sandy sediment cores, release was observed in the treatments with 6 individuals per core (3750 ind.m⁻²) and for the muddy sediment the release of PO₄ was observed in the treatments with 4 and 6 *Chironomus* larvae (2500 and 3750 ind.m⁻²) (Table 3). In the cores with 2 individuals, a precipitation and/or consumption PO₄ from the water column to the sediment was observed, resulting in negative fluxes. Comparing the two types of sediment, the ANCOVA analysis revealed significant differences between the regressions for the treatments with sandy and muddy sediments, with higher rates of release observed in the muddy sediments.

The N-NH₄⁺ release (Figure 1d) revealed elevated correlation between the density of *Chironomus* larva incubated in the sandy (r²=0.76, p<0.05) and muddy sediments (r²=0.86, p<0.05). However, we point out the release of N-NH₄⁺ in the muddy sediment, which was significantly greater than that observed in the sandy sediment (ANCOVA, p=0.001).

The results obtained reveal values up to two times greater in the cores with Chironomidae in relation to the control cores. The NH₄⁺ release for the sediment to the water column was 4 times greater in the treatments with larva in relation to the controls in the cores incubated with muddy sediment, and 2 times greater in the cores with sandy sediment (Table 3).

Significant linear correlations were observed between the density of *Chironomus* larva and the flux of N-NO₃⁻ both for sandy (r²=0.66, p<0.05) as well as muddy sediments (r²=0.97, p<0.05). Yet, the observed patterns for this variable were quite distinct between both types of sediment. In the cores with sandy sediment, the fluxes were positive and the values were significantly greater in comparison to the incubations in muddy sediment, where the fluxes were always negative (ANCOVA, p=0.005, Figure 1e).

Table 1: Physical and chemical characteristics of water and sediments used in incubation experiments.

	Sandy sediment	Muddy sediment
Water characteristics		
pH	6.80 ± 0.22	6.65 ± 0.26
Conductivity (uS/cm)	13.35 ± 15.06	13.10 ± 14.80
Turbidity (NTU)	72.58 ± 48.60	39.85 ± 25.93
Dissolved oxygen (mg/L)	2.25 ± 1.17	2.45 ± 0.24
Temperature (°C)	23.98 ± 1.47	24.08 ± 1.54
TDS (mg/L)	0.09 ± 0.10	0.17 ± 0.01
Redox (mV)	-26.50 ± 6.36	18.00 ± 46.67
Sediment characteristics		
Organic Matter (% DW)	6.72 ± 0.36	18.20 (± 6.44)
Interstitial water content (%)	70.76 ± 8.38	72.3 (± 0.91)
Granulometric composition	23.5 % coarse sand	20.0% coarse Sand
	34.4 % middle sand	18.0% fine Sand
	27.2 % fine sand	62.0% Silt + clay
	4.9 % silt + clay	

Table 2: Correlation matrix (r-Pearson), concerning the different variables quantified during the study. Values obtained for cores with sandy sediment, above diagonal line (**bold font**), and the values obtained for cores with muddy sediment, below diagonal line (*italic font*). The values labeled with (*) represent significant correlations ($p < 0.05$, r-Pearson).

<i>Variables</i>	Density	O ₂ consump	CO ₂ flux	PO ₄ flux	NO ₃ flux	NH ₄ flux
Density		0.66*	0.73*	0.78*	-0.66*	0.75*
O ₂ consump.	<i>0.61*</i>		0.49	0.35	-0.19	0.42
CO ₂ flux	<i>-0.09</i>	<i>0.54</i>		0.46	-0.45	0.74*
PO ₄ flux	<i>0.58*</i>	<i>0.17</i>	<i>-0.37</i>		-0.79*	0.73*
NO ₃ flux	<i>-0.82*</i>	<i>-0.86*</i>	<i>-0.26</i>	<i>-0.47</i>		-0.83*
NH ₄ flux	<i>0.87*</i>	<i>0.78*</i>	<i>-0.02</i>	<i>0.49</i>	<i>-0.85*</i>	

In the cores incubated with sandy sediment, we observed positive fluxes in all treatments (control and with *Chironomus* larva). On the other hand, in the muddy sediment incubations, the negative fluxes indicated a reduction in the concentrations of N-NO₃ in the water column. Although, in both treatments the presence of *Chironomus* larva caused a reduction of N-NO₃ in the overlying water, or rather, it stimulated the consumption and/or transformation of this element (Table 3). The decrease of N-NO₃ in overlying water was significantly greater in the treatments with *Chironomus* larva when compared to the control (Kruskal-Wallis, $p < 0.05$). No significant differences were observed between the treatments with larva and control in the incubation with sandy sediment.

Table 3: Mean and standard deviation (\pm) of O₂ consumption and CO₂, PO₄, NO₃, NH₄ flux in microcosmic incubations of some treatments: control (0 ind/m²) and different *Chironomus* sp. larval density (1250, 2500 and 3750 ind/m²) in sandy and muddy sediments of the Ibirité reservoir.

Sediment treatments		Larval Density			
		Control (0 ind.m ⁻²)	1250 (ind.m ⁻²)	2500 (ind.m ⁻²)	3750 (ind.m ⁻²)
O ₂ consump. (mmol.m ⁻² .h ⁻¹)	Sand	1.33 ± 0.10	1.48 ± 0.03	1.44 ± 0.06	1.54 ± 0.10
	Mud	1.40 ± 0.24	1.80 ± 0.12	1.80 ± 0.17	1.90 ± 0.38
CO ₂ flux (mmol.m ⁻² .h ⁻¹)	Sand	2.12 ± 0.34	3.58 ± 0.57	5.06 ± 0.06	4.07 ± 0.60
	Mud	1.23 ± 0.22	1.76 ± 0.31	1.78 ± 0.39	1.10 ± 0.75
PO ₄ flux (μmol.m ⁻² .h ⁻¹)	Sand	-0.50 ± 0.05	-0.46 ± 0.20	-0.01 ± 0.01	0.36 ± 0.30
	Mud	-0.45 ± 0.31	-0.18 ± 0.25	-0.05 ± 1.66	0.86 ± 0.57
NO ₃ flux (μmol.m ⁻² .h ⁻¹)	Sand	134.10 ± 21.75	155.74 ± 2.87	74.3 ± 44.64	72.30 ± 40.36
	Mud	-464.84 ± 41.43	-599.80 ± 83.35	-653.53 ± 109.57	-800.21 ± 134.42
NH ₄ flux (μmol.m ⁻² .h ⁻¹)	Sand	51.92 ± 9.57	77.64 ± 4.07	123.56 ± 30.60	109.42 ± 25.83
	Mud	106.72 ± 58.19	281.66 ± 35.13	263.59 ± 29.45	430.65 ± 77.51

DISCUSSION

The higher consumption rates of O₂ in the incubations with muddy sediment can be explained by the physicochemical characteristics of the sediment (Table 1). Muddy (organic) sediment may present greater rates of mineralization of organic material causing greater oxygen consumption. The larger quantity of interstitial water observed in this type of sediment allows for a better diffusion of oxygen into the lower, normally anoxic, deeper layers. In this case, the chemical oxidation of the substrates encountered in reduced forms (such as Fe⁺³ and Mn⁺⁴) or by processes like nitrification (NH₄⁺ + 2O₂ - > NO₃⁻ + H₂O + 2H⁺), intensify the consumption of O₂. Furthermore, muddy sediments favor the colonization and better displacement of benthic organisms facilitating even more the diffusion of O₂ and increased the depth distribution of the oxidised surface layer of the sediment (to expand the oxic benthic boundary layer). Thus, the colonization of aerobic microorganisms increases and the consumption of O₂ is intensified as a result of aerobic decomposition (Svenson, 1998).

The increase in oxygen consumption in the cores incubated with *Chironomus* larva in both sediments, compared to the control cores, was directly related to the activity of these organisms in the sediment. As *Chironomus* larva construct U-shaped permanent burrows tubes for living inside the sediment, and promote a continuous water flux across the burrows, thereby expanding the sediment oxidation layer. Bioturbation by benthonic organisms increases O₂ consumption through their respiration, bacterial community, and through the re-oxidation of chemical compounds (Svensson, 1998).

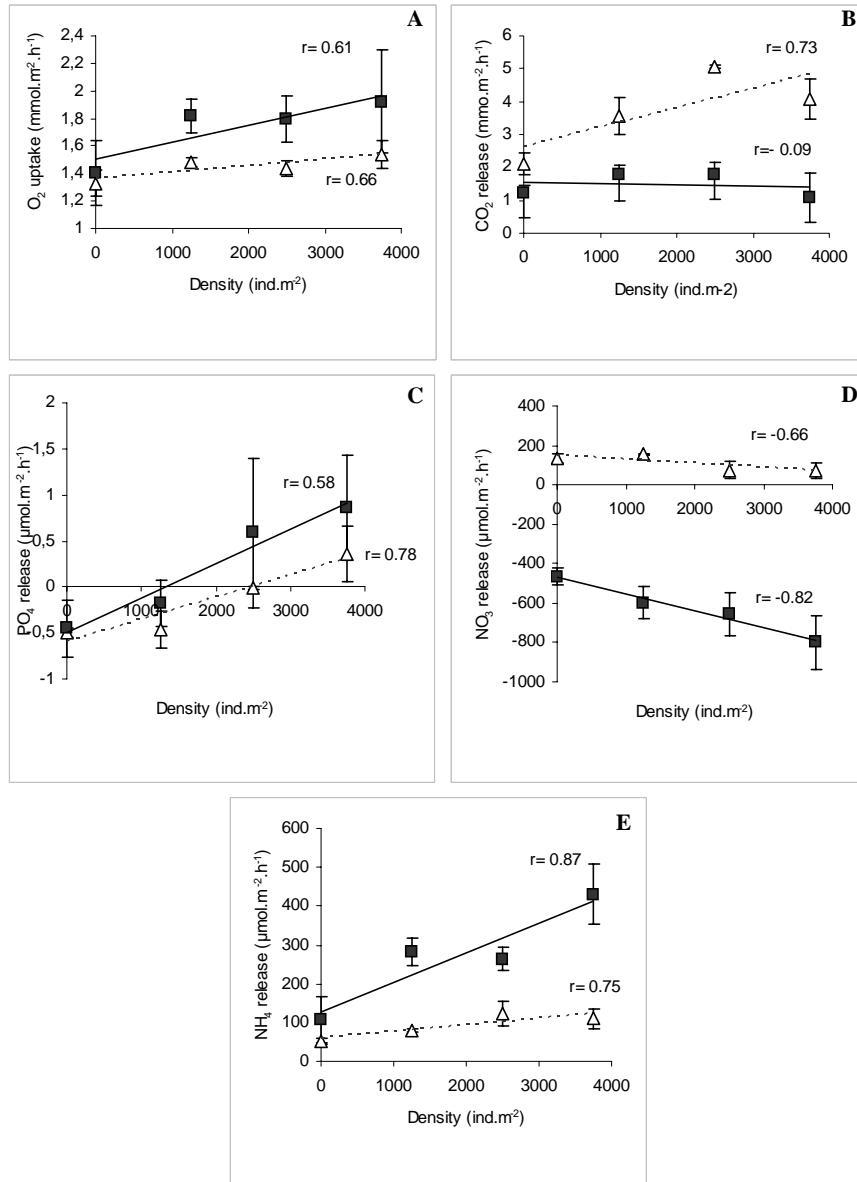


Figure 1: Fluxes of O₂ (A), CO₂ (B), PO₄ (C), NO₃ (D) and NH₄ (E), for both types of sediments (Δ – Sand sediment and \blacksquare - Mud sediment) in different densities of *Chironomus* larva. The error bars represent standard deviation (n=3) and the dotted and continuous lines the linear regressions.

In the present study the activity of *Chironomus* larva in the sandy sediment intensified the mineralization of organic matter, given that concurrent to O₂ consumption; we observed an increase in the release of CO₂. However, in the muddy sediment this stimulus did not occur based on the fact that we did not observe an increase in the release of CO₂. In this case the oxygen consumption was related to the redox changes in the sediment, to favor the oxidation of different inorganic substrates, like oxidation sensitive metals such as Fe³⁺, along burrow walls, to Fe₂O₃. In this case the oxygen consumption did not generate CO₂ as a final product. This process was very common in the organic-anoxic sediments (Boudreau and Jørgensen, 2001).

The *Chironomus* larva density was a determining factor in the release PO₄ from the sediment to the water column. Apparently the release and stimulation of the phosphate forms from the sediment to the water column can be seen as negative effects in the management of eutrophic aquatic ecosystems. Processes that seek to deposit and stock phosphate forms in the benthic compartment do reflect the removal of this element. The slow and steady remobilization of phosphate forms is favored by the bioturbation carried out by benthic organisms thereby making this nutrient available to diverse communities such as aquatic macrophytes. In this case, by incorporating this nutrient in plant biomass and through the management of these plants elevated loads of these nutrients can be removed from eutrophic ecosystems (Kadlec & Knight, 1996).

The reduction of NO₃⁻ release in the sandy sediment, as well as the induction of greater consumption in the muddy sediment, stimulated by the *Chironomus* larva, reflect the strong influence that bioturbation has on the dynamic of this element. Several authors have investigated the role of benthic macrofauna in such as ammonification, nitrification e denitrification (Svensson, 1997; 1998). In the present study the rise in the rates of NH₄⁺ release, the reduction in the rates of NO₃⁻ release (sandy sediment), and the intensification of the consumption rates of NO₃⁻ (muddy sediment) demonstrate the distinct behavior of nitrogen in sediments with different characteristics.

The stimulation of NH₄⁺ release, by the bioturbation may be caused by at least three processes: 1) mechanical bioturbation of the sediment, i.e. increased transport for invertebrates, 2) animal metabolic excretion, and 3) enhanced bacterial metabolic activity in or close to the burrow walls (Svensson, 1998). These same authors suggested that there are many indications that the excretion by the fauna is of subordinate importance. Both the physical activity exerted by the infauna when constructing burrows and the intermittent pumping of water are suggested to be of more importance for the release of ammonium.

We conclude that the presence of *Chironomus* larva in both sediment types intensified the flux of nutrients at the sediment-water interface in the Ibirité reservoir. The (i) prediction was partially confirmed for the O₂, PO₄, NH₄ and NO₃ fluxes which were all intensified by the larvae of *Chironomus* in muddy sediments, the only exception was the CO₂ flux. The predictions (ii) and (iii) were confirmed, since we observed that the density of *Chironomus* determined the release of PO₄ from the sediment to the water column and the fluxes of NH₄ and NO₃ were intensified by *Chironomus* bioturbation in

both the sediments. Given that the predictions were confirmed, our hypothesis was corroborated.

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