



## Productivity gradient affects the temporal dynamics of testate amoebae in a neotropical floodplain



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### ABSTRACT

Testate amoeba communities are influenced by temporal variation in the productivity levels in the environment, and may be used as an indicator group for these changing conditions. Here, we analysed the effect of temporal variation in the levels of productivity variables on testate amoeba community of the upper Paraná River floodplain. We evaluated the hypothesis that the frequency and abundance of the testate amoeba community change along an environmental gradient, with different taxa establishing at different points along the gradient in response to changes in the levels of productivity variables. We predicted that the number of species would increase and decrease at points associated with higher and lower levels of productivity variables, respectively. Testate amoeba species were sampled quarterly between 2000 and 2012 from six lakes in the upper Paraná River floodplain, Brazil. We recorded 110 species belonging to 11 families. Threshold Indicator Taxa Analysis identified positive and negative significant shift points in response to the concentration of chlorophyll-*a*, total nitrogen, and total phosphorus on the frequency and abundance of the testate amoeba community. Our results indicated that change intervals in the levels of productivity variables were associated with the establishment of different taxa. The main bioindicator species of productivity were *Diffugia acuminata*, *D. amphoralis*, *D. helvetica multilobata*, *D. kempny*, *D. lobostoma multilobata*, *D. parva*, *D. schurmanni*, *D. ventricosa*, and *Lesquereusia ovalis*. These species were linked to the increase and decrease in the levels of productivity, confirming the ecological importance of the role of these organisms as bioindicators in aquatic ecosystems.

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### 1. Introduction

Organisms in floodplain ecosystems respond directly to changes in the chemical and physical composition of water, particularly concentrations of chlorophyll-*a*, phosphorus, and nitrogen (Pagiore et al., 2005). These environmental variables potentially limit the establishment of species in aquatic environments (Wall et al., 2001), due to their ecological importance to productivity. These productivity variables are directly linked to the availability of food resources for the microbial food chain, and are indirectly linked to other aquatic organisms (Auer et al., 2004). Thus, differences

in the levels of productivity variables may affect the availability of resources (Matsumura-Tundisi et al., 1986), altering species interactions and promoting changes to the structure of aquatic communities (Cotner and Biddanda, 2002).

Previous studies have indicated that variation in the structure of communities is favoured or limited by the productivity of ecosystems (Schmid, 2002; Worm and Duffy, 2003; Cardinale et al., 2009). Thus, temporal variation in the levels of productivity variables might act as environmental filters on aquatic communities (Armynot du Châtelet et al., 2004). Consequently, these environmental variables may restrict the establishment of certain species at any given point in space or time, thereby species that exhibit greater tolerance get established in the new environmental conditions (Ferraro et al., 2006).

A group of species may be used as an indicator (or substitute) of a given environment, reflecting temporal variation in the levels of productivity variables (Heino, 2010; Padial et al., 2012). The responses of these species to the changes in the environmental facilitate the assessment of the degree of environmental variation

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(Simões et al., 2012). Thus, such organisms may be used as tools to implement rational environmental management activities, assisting in the implementation of planning strategies and biodiversity conservation (Palmer et al., 2013).

Testate amoeba communities are influenced by variation in the productivity of the environments they inhabit, resulting in their being an indicator group (or substitute) of these conditions. These organisms are widely distributed, with high species richness and abundance in floodplain ecosystems (Lansac-Tôha et al., 2007; Alves et al., 2010). Furthermore, testate amoeba species are small in size and have short generation times and high dispersion rate. These characteristics are ecologically advantageous for scientific research at both spatial and temporal scales (Schwind et al., 2013).

Therefore, the present study aimed to assess how temporal variation in productivity variables affected the testate amoeba community of the upper Paraná River floodplain. We hypothesised that the frequency and abundance of testate amoebae would change along an environmental gradient, with different taxa establishing in association with changes to productivity variables. We also predicted that indicator species would be influenced by increases and decreases in productivity variables.

## 2. Materials and methods

### 2.1. Study area

This study was performed at six lakes in the Upper Paraná River basin, Brazil. These lake environments are part of the Environmental Protection Area of the Floodplain Islands of the Paraná River, Brazil (22°40'–22°50' S and 53°10'–53°40' W), and belong to three sub-basins formed by the major river (Paraná River sub-basin) and two major tributaries (Baía River and Ivinhema River sub-basins) (Fig. 1).

The Paraná River sub-basin is formed by the Paraná River, lakes, channels, islands, and backwaters, and has an average depth of 4 m. The Baía River sub-basin includes several lakes and the Baía River that have an average depth of 3.2 m, and that connect with the Paraná River in the lower region. The Ivinhema sub-basin contains several lakes and the Ivinhema River, which is one of major tributaries of the Paraná River. It has an average depth of 3.9 m, and is connected to Paraná River through the Ipoitã Channel (Thomaz et al., 1992).

### 2.2. Sampling design

Testate amoeba samples were collected quarterly between 2000 and 2012 on the subsurface of the pelagic zone, resulting in the collection of 312 samples (six lakes \* two periods \* 13 years). Using a motorised pump, 6 L of water was filtered per sample through a plankton net (68 µm). Organisms were transferred to polyethylene-labelled vials and fixed with 4% formaldehyde solution buffered with calcium carbonate. The samples were stained with Rose Bengal. Only living testate amoebae with a cytoplasm stained by the dye were counted.

Testate amoebae abundance was determined using a Sedgewick–Rafter counting chamber under an optical microscope at a magnification of 400× (Olympus CX31). Counting was performed using sets of three sequential sub-samples obtained by a Hensen–Stempel pipette. At least 50 individuals were counted per sample, with 7.5 mL being counted from each sample in total. Samples were fully quantified when the minimum number of individuals per sample was not attained (Bottrell et al., 1976). Total abundance was expressed in individuals per cubic metre (ind m<sup>-3</sup>).

The taxonomic classification was based on Adl et al. (2012) proposed guidelines. In the sample, only the organisms that had an identified stained protoplasm were stained with rose bengal, assuming that the samples were living during collection. We sorted the individuals from each sample and prepared glycerine slides for further identification. Species identification was performed using selected literature (Deflandre, 1928, 1929; Gauthier-Lièvre and Thomas, 1958, 1960; Velho and Lansac-Tôha, 1996; Velho et al., 1996; Alves et al., 2007; Lansac-Tôha et al., 2014).

We measured total nitrogen (µg L<sup>-1</sup>), total phosphorus (µg L<sup>-1</sup>), and chlorophyll-*a* (µg L<sup>-1</sup>) in each sample. We analysed chlorophyll-*a* and total phosphorus in the laboratory according to the method of Golterman et al. (1969), and we analysed total nitrogen according to the method of Mackereth et al. (1978).

### 2.3. Data analysis

The taxon-specific and community response of testate amoebae to the environmental gradients of nitrogen, phosphorus, and chlorophyll-*a* were evaluated using Threshold Indicator Taxa Analysis (TITAN) (Baker and King, 2010). This statistical analysis was conducted using the “mvpart” package (De’ath, 2014) from free software R, version 3.2.2 (R Core Team, 2015).

TITAN allows the identification of limits and points of changes for each taxon and all communities along the environmental gradient, detecting changes in species distribution. The response of individual species is indicated by the percent of a positive or negative change in the environmental gradient. This response is then computed with Value Indicator (IndVal score). A significant response is related to a positive ( $z^+$ ) or negative ( $z^-$ ) change when  $\text{IndVal} < 0.05$ , with purity and reliability cut offs at 0.95. Abundance data were square root transformed and only species that were detected at three or more sites were included in analysis. Rare species that occurred in less than three sites (or sampled units) were excluded to remove the effect of outliers. We used 500 permutations to determine species specific  $z$ -scores, as our calculations were based on a small data set, and we aimed for high precision in the individual taxa  $z$ -scores.

## 3. Results

### 3.1. Composition of the testate amoeba community

We identified 110 species of testate amoebae belonging to 11 families. The genus with the greatest number of species was Diffugiidae (56 species), followed by Arcellidae (22 species), Centropyxidae (13 species), and Lesquereusiidae (9 species). The others species belonged to Trigonopyxidae (three species), Plagiopyxidae (two species), Cyphoderiidae (one species), Euglyphidae (one species), Heleoperidae (one species), Hyalospheniidae (one species), and Phryganellidae (one species) (Supplemental material 1).

### 3.2. Characterisation and variation of environmental conditions

The floodplain lakes showed clear variation in the environmental variables that influence productivity. The coefficient of variation showed that chlorophyll-*a* had the highest temporal variation (128.5%), followed by total nitrogen (72.9%) and total phosphorus (69.7%) (Table 1).

### 3.3. Temporal variability of the testate amoeba community

TITAN identified a significant change point threshold ( $p < 0.05$ ) for the frequency and abundance of the testate amoeba community in response to chlorophyll-*a*, phosphorus, and nitrogen (Table 2).

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