


# Plankton community interactions in an Amazonian floodplain lake, from bacteria to zooplankton

I. B. Feitosa · V. L. M. Huszar  · C. D. Domingues · E. Appel ·  
R. Paranhos · R. M. Almeida · C. W. C. Branco · W. R. Bastos ·  
H. Sarmiento

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**Abstract** The simple view of the classical phytoplankton–zooplankton–fish food chain (CFC) has been replaced by a more complex framework, integrating microbial compartments (microbial food web, MFW). Few studies considered all components of the pelagic MFW in freshwaters and mostly are from temperate regions. We investigated carbon partitioning in the CFC and the MFW in an Amazonian floodplain system and analyzed the strength of interactions among components through structure equation

modeling. We hypothesized that (i) MFW contributes highly to total plankton biomass throughout the year; and (ii) all plankton communities increase in biomass during low water, increasing the role of trophic interactions. We collected 30 subsurface samples (nutrients and plankton communities). MFW predominated over CFC in carbon biomass, and plankton components and their interactions changed according to the contrasting water level. Because phosphorus can be a potentially limiting resource for strict primary producers, higher biomass and a more complex MFW occurred during low water. We concluded that hydrology is a key factor shaping biotic interactions during low-water periods, and that MFW plays a key

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I. B. Feitosa · W. R. Bastos  
Laboratory of Environmental Biogeochemistry, Federal University of Rondônia, Pôrto Velho, RO 76800-500, Brazil

V. L. M. Huszar (✉) · C. D. Domingues · E. Appel  
Laboratory of Phycology, Department of Botany, National Museum, Federal University of Rio de Janeiro, Rio de Janeiro, RJ 20940-040, Brazil  
e-mail: vhuszar@gbl.com.br

R. Paranhos  
Laboratory of Hydrobiology, Department of Marine Biology, Institute of Biology, Federal University of Rio de Janeiro, Rio de Janeiro, RJ 21941-901, Brazil

R. M. Almeida  
Laboratory of Aquatic Ecology, Department of Biology, Institute of Biology, Federal University of Juiz de Fora, Juiz de Fora, MG 36036-900, Brazil

R. M. Almeida  
Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853, USA

C. W. C. Branco  
Biosciences Institute, Federal University of the Rio de Janeiro State, Rio de Janeiro, RJ 22290-240, Brazil

H. Sarmiento  
Laboratory of Microbial Processes and Biodiversity, Department of Hydrobiology, Federal University of São Carlos, São Carlos, SP 13565-905, Brazil

role in floodplain lakes, being potential mixotrophy an important strategy for phytoplankton.

**Keywords** Microbial food web · Classical food chain · Seasonal interactions · Mixotrophy · Structural equation modeling

## Introduction

The seminal microbial-loop concept originally proposed for marine systems (Azam et al., 1983) has paved the way for a paradigm shift, in which the simple view of the phytoplankton–zooplankton–fish food chain was complemented by a more complex framework of a food web, integrating previously neglected microbial compartments. Since then, many efforts have been made to understand how matter and energy flow through the tiny phototrophs, bacteria, and heterotrophic protists (Fenchel, 2008). Besides the transfer of matter and energy to higher trophic levels, the food web structure plays an important role in carbon emissions to the atmosphere (Müller-Navarra, 2008; Aufdenkampe et al., 2011). The mechanisms that lead to these processes are complex, and many of them are mediated by the structure of the microbial food web (MFW) (Schindler et al., 1997; Atwood et al., 2013). However, only a few studies have considered all components of the pelagic MFW in freshwater ecosystems (Özen et al., 2018), and most of these were conducted in temperate regions (see Table S1).

Although the importance of microbial components of plankton food webs in tropical lakes has been hypothesized repeatedly, relatively few studies have quantified carbon partitioning in these regions (for a review, see Sarmento, 2012). The importance of evaluating the entire size spectrum of the pelagic food web to capture the complex carbon dynamics of tropical ecosystems has been highlighted (e.g., Domingues et al., 2016). The use of a single currency in studies of carbon partitioning of pelagic communities, from bacteria to zooplankton, has been increasing. In a rapid literature search, we found eight studies encompassing 149 ecosystems, of which only one reported data for tropical reservoirs (see Supplementary Material, Table S1). On average, the carbon contents of phytoplankton (21–76%) and zooplankton

(8–65%) are the dominant fractions in these systems, followed by ciliates (0.1–24%), heterotrophic bacteria (1.3–30%), heterotrophic nanoflagellates (0.3–24.5%), and picophytoplankton (0.7–8.4%).

Considerable effort has been expended to clarify the main factors regulating microbial communities, particularly the combined effects of top-down and bottom-up controls (Fenchel, 2008). For warm (tropical and subtropical) lakes, small-bodied metazooplankton are typically dominant due to the high rates of fish predation, and it is expected that the metazooplankton would have fewer cascading effects on the microbial community than in colder systems (Fernando, 1994; Jeppesen et al., 2005; Domingues et al., 2016; Özen et al., 2018), favoring control by protozooplankton (ciliates and heterotrophic nanoflagellates) on pelagic heterotrophic bacteria (Meira et al., 2018; Segovia et al., 2018). A recent comprehensive study testing the coupling between the abundance of heterotrophic bacteria and heterotrophic nanoflagellates on a latitudinal gradient indicated that grazing by ciliates and cladocerans plus higher temperatures may explain the high rates of bacterial loss in the tropics (Segovia et al., 2016).

In tropical regions, floodplain lakes occur widely along large river basins (e.g., Paraná, Amazon), and their ecological dynamics are strongly modulated by the seasonal oscillation of water level. Floods promote the exchange of water, sediments, organic matter, nutrients, and biota between the main river and the floodplain units, i.e., shallow lakes, secondary channels, and wetlands (Junk et al., 1989, 2011). For example, the hydrodynamics exert a strong influence on the community structure of bacterioplankton (Anésio et al., 1997; Carvalho et al., 2003; Vidal et al., 2015; Doherty et al., 2017), phytoplankton (Huszar & Reynolds, 1997; Loverde-Oliveira et al., 2009; Stević et al., 2013), and zooplankton (Bozelli, 1994; Aoyagui & Bonecker, 2004; Balkić et al. 2017). These communities commonly increase in abundance and/or biomass during low water, ultimately affecting community metabolism and CO<sub>2</sub> emissions (Amaral et al., 2018). The seasonal oscillation also plays a key role in regulating the plankton viral and bacterial communities (Barros et al., 2010; Almeida et al., 2015a). Furthermore, considering the entire plankton community, a higher contribution of MFW to the total plankton carbon was reported in nutrient-poor Amazonian reservoirs compared to reservoirs in the

Cerrado (savanna) and Atlantic Forest biomes (Domingues et al., 2016). Seasonal changes in the flow regime influence trophic interactions in floodplain systems, with higher contributions of allochthonous organic matter and MFW in high-water periods, and stronger phyto-zooplankton relationships in low water (Carvalho et al., 2003; Segóvia et al., 2015). As far as we know, the entire aquatic MFW in Amazonian floodplain lakes has been studied in only one system, in the Bolivian part of the Madeira River basin (Rejas et al., 2005). This experimental study found a positive overall effect of microzooplankton on bacterial abundance, since bacterial growth under phosphorus-limited conditions and in the absence of grazers was lower than in the presence of heterotrophic nanoflagellates, ciliates, and rotifers.

Here, we investigated carbon partitioning in the MFW and CFC in an Amazonian floodplain system with contrasting seasonal conditions (high- and low-water levels), and then analyzed the strength of interactions among plankton components through structure equation modeling of the carbon content of each compartment, using the carbon content as a common currency. We hypothesized that (i) MFW compartments make a high contribution to total plankton biomass throughout the year; and (ii) in the low-water period, with longer water residence time, all plankton communities increase (as previously observed in other floodplain lakes) increasing the potential for trophic interactions. Therefore, we expect a high contribution of the MFW throughout the year in the studied ecosystem, as suggested in literature for tropical systems, and more complex models (with more interactions) in the low-water period.

## Methods

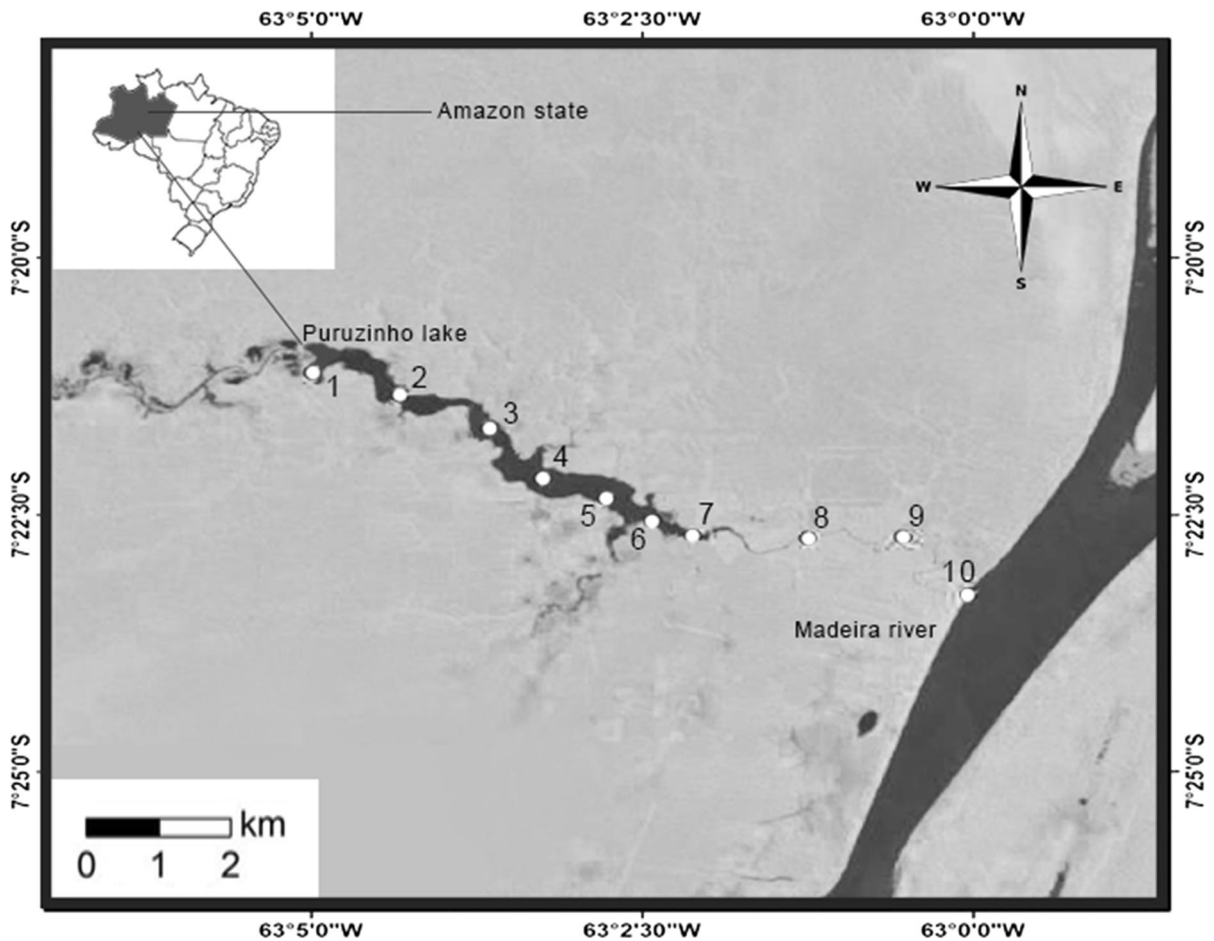
### Study ecosystem

Puruzinho is a floodplain system (07°21′09.6 S; 63°04′52.8 W, Amazonas state, Brazil), composed of a long lake (8.6 km<sup>2</sup>) and an 8-km-long channel that connects the lake with the Madeira River (Fig. 1). The Madeira River is the largest tributary on the right bank of the Amazon River, which undergoes a predictable monomodal flood pulse (~ 11 m) (Fig. 2). The regional climate is humid equatorial rainforest (Am in the Köppen classification, updated by Alvares

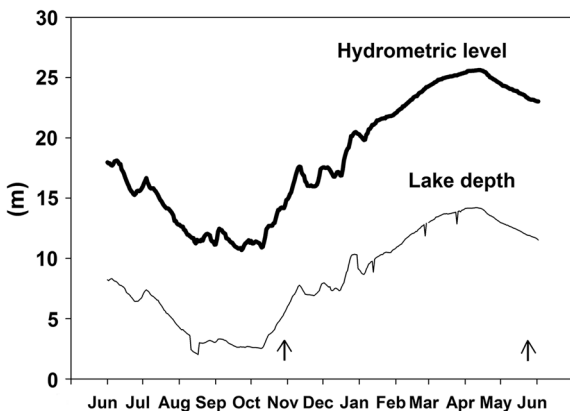
et al., 2014), with a mean temperature of 26°C (minimum 19°C and maximum 39°C). During the low-water period (LW), the lake depth decreases and sediment-rich waters (*sensu* Sioli, 1984 and Junk et al., 2011) from the Madeira River may reach the lake through the channel. The Madeira River is rich in nutrients such as phosphorus (mean concentration of total phosphorus = 278 µg l<sup>-1</sup> at Porto Velho), although the high phosphorus contents are not necessarily indicative of eutrophication because this element occurs predominantly as inorganic phosphorus adsorbed to fine suspended particles (Almeida et al., 2015b). Even though phosphorus is mostly in particulate form, it may be released from particles and become bioavailable (Chase & Sayles, 1980). For instance, a study in the sediment-rich Amazon River demonstrated that about 25% of the algal-available phosphorus is in the particulate form (Engle & Sarnelle, 1990). During the high-water period (HW), the lake water, which is “black” (tea-colored) because of the high concentration of dissolved allochthonous organic matter from the floodplain, flows into the river. Puruzinho Lake is also a CO<sub>2</sub> source to the atmosphere (heterotrophic metabolism, Menezes, 2010) and is dominated mainly by detritivorous and omnivorous fishes and to a lesser extent by planktivorous fishes (unpublished data).

### Sampling and data collection

A total of 30 subsurface (0.5 m) sample units in each hydrological period (LW, 30/10/2013 and HW, 22/05/2014) for nutrients (nitrogen, phosphorus, and carbon), plankton communities (heterotrophic bacteria, HB; picophytoplankton, PPP; protozooplankton—heterotrophic nanoflagellates, HNF + ciliates, CIL; and phytoplankton, PHY) were taken with a van Dorn bottle in random triplicates at 10 sampling stations along the longitudinal axis of the lake (1–7) and in the connecting channel to the Madeira River (8–10) (Fig. 1). Metazooplankton (ZOO) was sampled at the subsurface with the same sampling design used for chemicals and the other plankton communities, by collecting 50 L water using a vessel and filtering in a 50-µm sieve. Water transparency was measured with a Secchi disk (SD); the euphotic zone ( $z_{eu}$ ) was considered as three times the SD depth (Cole, 1994). For analyses of total phosphorus (TP), total nitrogen (TN) and total carbon (TC) concentrations, non-filtered



**Fig. 1** Map and location of the Puruzinho system, showing sampling stations in the lake (1–7) and in the channel (8–10)



**Fig. 2** Daily values of the hydrometric level of the Madeira River (m a.s.l.) and depth (m) of the Puruzinho Lake from June 2013 to June 2014, showing the sampling dates (arrows)

samples were used. For dissolved-nutrient concentrations (ammonium,  $\text{N-NH}_4^+$ ; nitrite,  $\text{N-NO}_2^-$ ; nitrate,  $\text{N-NO}_3^-$ ; soluble reactive phosphorus, SRP; dissolved organic carbon, DOC), water samples were filtered through Whatman GF/C filters. Samples for total and dissolved nutrients were kept frozen till analysis, and DOC samples were acidified with  $\text{H}_2\text{PO}_4$ . Samples for HB quantification were preserved in paraformaldehyde (PFA) 10% + glutaraldehyde (GLU) 0.5%; for PPP, in PFA at 1% final concentration; HNF in GLU 25% at 1% final concentration; CIL in acetic Lugol solution at 2% final concentration; PHY in neutral Lugol solution; and ZOO in 4% formalin final concentration.

Daily lake depth and water color were obtained by local residents, from a permanent gauge located near the margin ( $07^\circ 21' 09.6\text{S}$ ;  $63^\circ 04' 52.8\text{W}$ ). Daily hydrometric level of the Madeira River was retrieved from

the ANA – Agência Nacional de Águas, 2014 ([www.ana.gov.br/telemetria](http://www.ana.gov.br/telemetria), station 15630000, Humaitá, Amazonas State, accessed on 13/11/2014).

### Laboratory analyses

TP was analyzed by sodium persulfate digestion (Mackereth et al., 1978); SRP by the ascorbic acid method;  $\text{N-NO}_3^-$  and  $\text{N-NO}_2^-$  through cadmium reduction of the samples; and  $\text{N-NH}_4^+$  through the phenol–hypochlorite method (Wetzel & Likens, 2000). Dissolved inorganic nitrogen (DIN) was defined as the sum of  $\text{N-NO}_3^-$ ,  $\text{N-NH}_4^+$ , and  $\text{N-NO}_2^-$ . TN was analyzed by the colorimetric method, using sulfuric acid (Crompton et al., 1992); and DOC through UV persulfate oxidation in a total carbon analyzer (Tekmar–Dohrmann Phoenix 8000, Japan). Nutrient limitation for phytoplankton growth was assessed through the DIN and SRP concentrations. Concentrations were compared to those that have roughly been considered suitable for phytoplankton growth, based on the half-saturation constants for most of the microalgal species (phosphorus was considered limiting  $< 10 \mu\text{g P l}^{-1}$ , SAS, 1989; and nitrogen  $< 100 \mu\text{g N l}^{-1}$ , Reynolds, 1997).

PPP and HB abundances ( $\text{cells ml}^{-1}$ ) were estimated by flow cytometer (FACS Calibur, BD, USA), equipped with 15-mW, 488 nm, and air-cooled argon laser. PPP were detected based on the autofluorescence and HB were stained with Syto-13 (Molecular Probes) and evaluated using FL1 (530 nm), FL2 (585 nm), and FL3 ( $> 650 \text{ nm}$ ) fluorescence sets. Samples were run three times at a low flow rate for 30 s (further details provided by Sarmento et al., 2008). PPP and HB biomass were estimated based on conversion factors (C-content per cell): HB = 20 fg C per cell and PPP = 103 fg C per cell (picoprokaryotes) + 467 fg C per cell (picoeukaryotes) (Zubkov et al., 1998).

For HNF abundance ( $\text{cells ml}^{-1}$ ), 10 ml was filtered through 0.8- $\mu\text{m}$  black polycarbonate filters (Nuclepore) previously stained with DAPI (4,6-diamidino-2-phenylindole) at 0.1% (Porter & Feig, 1980). Cells were counted under an epifluorescence microscope (Olympus BX-51, USA) at 1000 $\times$  magnification using the UV filter set; the photosynthetic flagellates were distinguished using the blue and green filter sets. HNF cells were measured directly under the fluorescence microscope. Biovolume was calculated

from geometric formulas, using a conversion factor of  $220 \text{ fg C } \mu\text{m}^{-3}$  (Borsheim & Bratbak, 1987) for carbon content.

CIL abundance ( $\text{cells ml}^{-1}$ ) was estimated through the settling technique (Utermöhl, 1958); morphotypes were enumerated in the entire chamber under an inverted microscope (Olympus, CKX4, Japan) at 400 $\times$  magnification. Mean volume of each morphotype ( $\mu\text{m}^3$ ) was estimated according to the approximate geometric formula. In general, 30 cells of each morphotype were measured under the microscope. Carbon content of CIL was estimated through a conversion factor of  $140 \text{ fg } \mu\text{m}^{-3}$  (Putt & Stoecker, 1989) after a correction factor of 1.4 (Müller & Geller, 1993).

Phytoplankton (PHY) populations (individuals  $\text{ml}^{-1}$ ) were estimated by the settling technique (Utermöhl, 1958) under inverted microscope (Zeiss Oberkochen Axiovert 10, Germany). Phytoplankton units (cells, colonies and filaments) were enumerated in random fields (Uehlinger, 1964) to at least 100 specimens of the most frequent species (Lund et al., 1958). PHY was sorted into potentially mixotrophic phytoplankton, PMP (cryptophyceans + dinoflagellates + chrysophyceans + raphidophyceans, Olrik, 1998; Ward & Follows, 2016) and non-potentially mixotrophic phytoplankton, non-PMP (chlorophyceans + diatoms + euglenoids + zygmatophyceans), following the description of this nutrition mode by Flynn et al. (2013). Most euglenoids found in our samples were pigmented and were not included in PMP, as most photoautotrophic euglenoids have a reduced feeding apparatus and lost the predatory ability (Leander et al., 2017).

In general, 30 individuals of each species were measured under the microscope. Specific phytoplankton biovolume ( $\text{mm}^3 \text{ l}^{-1}$ ) was estimated according to Hillebrand et al. (1999), and population volume through multiplication of population abundance (organisms  $\text{ml}^{-1}$ ) of each species by the mean volume of organisms ( $\mu\text{m}^3$ ). Carbon content of each species was estimated from the biovolume, using the conversion formula:  $\text{C} = aV^b$ , where  $a = 0.1204$ ,  $b = 1.051$ , and  $V = \text{algae volume}$  (Rocha & Duncan, 1985). The carbon content of PHY was estimated by multiplying the population abundance and mean carbon content of each taxon ( $\mu\text{g C l}^{-1}$ ).

ZOO populations were enumerated in a Sedgewick-Rafter counting chamber. Aliquots for counting were

removed from a well-mixed sample using a Hensen–Stempel pipette. At least 200 individuals were counted in each of five sequential subsamples. The entire sample was inspected for rare species. ZOO was sorted into small-sized filter-feeders, SFF (rotifers + copepodites + nauplii), medium-sized filter-feeders, MFF (cladocerans + calanoid copepod) and omnivores, OMN (cyclopoid copepods) (Loverde-Oliveira et al., 2009). ZOO biovolume was considered as equal to the fresh weight. Rotifer biovolume was estimated from the geometric shapes (Ruttner-Kolisko, 1977). The dry weight was calculated as a percentage of the fresh weight (Pauli, 1989), for each major group. The dry weight of microcrustaceans was evaluated in an analytic microbalance (Mettler Toledo, MX-5, United States), after drying for 24 h, except for nauplii. Nauplii dry weight was calculated according to Manca & Comoli (1999), assuming dry weight as equivalent to 10% of the biovolume. ZOO biomass was estimated assuming the carbon content as 50% of dry weight (Latja & Salonen, 1978).

Here, we considered the MFW as HB + PPP + HNF + CIL + PMP and the classical food chain as non-PMP + ZOO. Because components of the MFW are more frequently analyzed as abundance, we show the data in both units (abundance and carbon content) in Table 1. Carbon content is used here as the main unit because it allows better comparisons among entire plankton communities.

## Statistical analyses

To test if the contribution of the MFW is higher than the MFC (hypothesis i), and to evaluate the effect of season (LW versus HW) on biological and chemical variables (hypothesis ii), we used linear mixed-effects models through the ‘lmer’ function of the package lme4 (Bates et al., 2015) in the R Statistical Software version 3.3.2 (R Development Core Team, 2016). To meet normality and homoscedasticity assumptions, response variables were log-transformed. This type of model was used because it is more suitable when there is non-independence in the data due to random factors (Bolker et al., 2009), as is the case for sampling station. We thus considered sampling station as a random effect to assess differences between LW and HW.

To estimate direct and indirect effects of bottom-up and top-down controls for plankton components during high- and low-water periods, we used Structural Equation Model analysis (SEM). The relationships were defined *a priori* in two models based on theoretical predictions: bottom-up and top-down (Figure S1) for each hydrological period (LW and HW). The abiotic variables included in these models were  $z_{\text{cup}}$ , DIN, SRP, and DOC. Plankton components were HB, PPP, HNF, CIL, PHY (PMP, non-PMP), and ZOO (SFF, MFF, OMN) (Figure S1). We tested the goodness of fit of the model using a Chi-square test and Bollen–Stine bootstrap, due to the small sample

**Table 1** Minimum (Min), maximum (Max), mean values and standard deviations (SD) of physical and chemical variables and coefficient of variation (CV) in the Puruzinho system during high water ( $n = 30$ ) and low water ( $n = 30$ )

	Low water					High water				
	Min	Max	Mean	SD	CV	Min	Max	Mean	SD	CV
Euphotic zone (m)	0.2	1.5	0.8	0.5	0.64	0.6	3.8	3.2	0.7	0.24
Secchi depth (m)	0.1	0.5	0.3	0.2	0.64	0.2	1.3	1.1	0.2	0.24
Maximum depth (m)	4.4	15.0	6.2	3.0	0.49	13.0	23.6	14.8	3.0	0.21
Total nitrogen ( $\mu\text{g l}^{-1}$ )	853.5	3093.0	1638.1	504.0	0.31	1296.9	2304.3	1599.6	227.2	0.14
Total phosphorus ( $\mu\text{g l}^{-1}$ )	32.6	309.4	103.5	89.4	0.86	27.6	168.0	60.0	35.5	0.50
Nitrate ( $\mu\text{g l}^{-1}$ )	731.5	1245.9	796.1	94.6	0.12	826.2	1229.0	905.0	90.9	0.10
Ammonium ( $\mu\text{g l}^{-1}$ )	5.9	80.3	24.4	15.8	0.65	40.6	251.2	109.5	58.9	0.54
Dissolved inorganic nitrogen ( $\mu\text{g l}^{-1}$ )	756.1	1267.7	825.4	97.7	0.12	894.3	1339.0	1018.2	113.1	0.11
Soluble reactive phosphorus ( $\mu\text{g l}^{-1}$ )	6.7	63.7	16.4	12.1	0.74	7.7	32.9	15.4	6.6	0.43
Dissolved organic carbon ( $\text{mg l}^{-1}$ )	2.0	3.1	2.7	0.3	0.10	3.9	6.0	5.0	0.6	0.12

size ( $n = 60$ , Bollen & Stine, 1992). The non-significant Chi-square test indicates a suitable relationship between the observed covariance matrix and the proposed model, and the model can be accepted. We used four indexes to determine the goodness of fit of each model as Root Mean Square Error of Approximation (RMSEA) equal to or higher than 0.07 (Steiger, 2007); Standardized Root Mean Square Residual (SRMR) less than 0.05; Comparative Fit Index (CFI) equal to or higher than 0.96; and Tucker–Lewis Index (TLI) equal to or lower than 0.96 (Hu & Bentler, 1999). The most parsimonious model was determined by Akaike’s Information Criterion (AIC). We assumed marginally significant  $p$  values between 0.05 and 0.07. The SEM graphs were constructed using CytoScape Software. SEM analyses were performed with the “lavaan” package (Rosseel, 2014, <http://www.jstatsoft.org/v48/i02/>) in R 3.0.3 (R Development Core Team).

## Results

### The abiotic environment

Puruzinho Lake and the Madeira River are permanently connected through a channel, resulting in increased system depth (2.0–14.2 m) following the increase of the Madeira River water level (10.9–25.6 m a.s.l.) during the study period (June 2013–June 2014). According to the observations by local residents, the water was white (high in suspended sediments) during LW and black during HW.

Because of the marked hydrologic variation, high annual variability was observed, particularly for physical variables as shown by the variation coefficient, mostly during LW (Table 1). The system was more transparent during HW, when  $z_{eu}$  was fourfold greater than in LW ( $F = 186$ ,  $P < 0.001$ ) (Table 1, Fig. 2). The water was moderately enriched in TN ( $1619 \mu\text{g l}^{-1}$ ), with no significant difference between LW and HW. DIN, particularly nitrate concentrations, also varied little. On average, DIN was significantly higher during HW ( $F = 74$ ,  $P < 0.001$ ) and was about 50% of the TN. All samples showed DIN concentrations more than sevenfold higher than the algal requirements ( $> 100 \mu\text{g l}^{-1}$ , see Methods section). We observed TP enrichment, with significantly higher values in LW ( $103 \mu\text{g l}^{-1}$ ) than in HW ( $82 \mu\text{g l}^{-1}$ )

( $F = 9$ ,  $P < 0.001$ ). SRP did not differ between periods and was about 20% of TP, although the data varied widely in each hydrologic period (see coefficient of variation in Table 1). Lower concentrations than SRP algal requirements ( $< 10 \mu\text{g l}^{-1}$ , see Methods section) were found during LW, particularly in the lake (60% of the samples). Mean DOC concentrations were higher during HW ( $5.0 \text{ mg l}^{-1}$ ) than during LW ( $2.1 \text{ mg l}^{-1}$ ) ( $F = 455$ ,  $P < 0.001$ ).

### Components of the plankton food web

The plankton components of the food web, in terms of biomass, were much more variable than the physical and chemical variables ( $P < 0.0001$ , Mann–Whitney test) in the Puruzinho system (see coefficient of variation in Tables 1 and 2). The MFW ( $245.5 \pm 155.5 \mu\text{gC l}^{-1}$ ) was, on average, one order of magnitude higher than the CFC ( $24.4 \pm 22.8 \mu\text{gC l}^{-1}$ ) ( $F = 13.4$ ;  $P < 0.001$ ). The mean biomass of the entire plankton community was  $269.6 \pm 152.3 \mu\text{gC l}^{-1}$ , with a significant difference between LW and HW (Table 2). However, the biomass of the MFW was significantly higher during LW than during HW ( $F = 6.5$ ,  $P < 0.05$ ); it was composed of PMP, PPP, HB, HNF, and CIL, which encompassed about 90% of the total plankton in each hydrologic period. For all samples combined, the mean HB biomass ( $80.3 \pm 39.6 \mu\text{gC l}^{-1}$ ) was significantly higher in HW than in LW ( $F = 23.2$ ,  $P < 0.001$ ). HNF biomass ( $5.1 \pm 4.0 \mu\text{gC l}^{-1}$ ) was very low, with no significant difference between hydrologic periods. Mean PPP biomass was  $26.7 \pm 18.4 \mu\text{gC l}^{-1}$  and was significantly higher during LW ( $F = 4.08$ ,  $P < 0.05$ ), with prokaryote PPP biomass one order of magnitude higher than the biomass of eukaryote PPP. The mean CIL biomass was  $53.7 \pm 53.8 \mu\text{gC l}^{-1}$  and was significantly higher during LW ( $F = 11.3$ ,  $P < 0.01$ ) (Fig. 3).

The mean total phytoplankton (PHY) biomass was  $103.5 \pm 96.8 \mu\text{gC l}^{-1}$  and was significantly higher during LW ( $F = 6.0$ ,  $P < 0.05$ ). In this study, we sorted PHY into potentially mixotrophic phytoplankton (PMP) and non-potentially mixotrophic phytoplankton (non-PMP) (see Methods section). The mean PMP biomass was  $84.5 \pm 85.8 \mu\text{gC l}^{-1}$  and comprised 81% of PHY, showing the potential importance of mixotrophy in the Puruzinho system. PMP biomass was similar during LW and HW, but non-PMP

**Table 2** Minimum (Min), maximum (Max), mean values and standard deviations (SD) of plankton biomasses and coefficient of variation (CV) in the Puruzinho system during high water ( $n = 30$ ) and low water ( $n = 30$ )

	High water					Low water				
	Min	Max	Mean	SD	CV	Min	Max	Mean	SD	CV
Heterotrophic bacteria ( $\mu\text{gC l}^{-1}$ ), HB	42.2	148.7	98.9	26.1	0.26	0.0	107.3	61.7	42.4	0.69
Picophytoplankton ( $\mu\text{gC l}^{-1}$ ), PPP	0.3	35.0	12.7	7.7	0.61	0.0	58.9	32.6	22.8	0.70
Prokariota picophytoplankton ( $\mu\text{gC l}^{-1}$ )	0.0	34.5	11.8	7.8	0.66	0.0	52.3	28.6	20.0	0.70
Eukariota picophytoplankton ( $\mu\text{gC l}^{-1}$ )	0.2	1.7	0.9	0.5	0.55	0.0	8.0	4.0	2.9	0.73
Heterotrophic nanoflagellates ( $\mu\text{gC l}^{-1}$ ), HNF	0.0	13.6	3.1	3.2	1.02	0.0	14.7	5.5	4.8	0.88
Ciliates ( $\mu\text{gC l}^{-1}$ ), CIL	0.2	162.5	43.7	44.7	1.02	0.0	187.2	63.8	60.6	0.95
Phytoplankton ( $\mu\text{gC l}^{-1}$ ), PHY	0.0	320.0	108.6	85.3	0.79	0.0	411.5	98.3	108.2	1.10
Potentially mixotroph phytoplankton ( $\mu\text{gC l}^{-1}$ ), PMP	0.0	272.5	85.3	74.6	0.87	0.0	348.1	83.7	97.0	1.16
Non-potentially mixotroph phytoplankton ( $\mu\text{gC l}^{-1}$ ), non-PMP	0.0	105.8	23.7	21.3	0.93	0.0	103.7	14.6	21.3	1.46
Zooplankton ( $\mu\text{gC l}^{-1}$ ), ZOO	0.0	12.7	2.6	3.0	1.15	0.1	22.7	7.9	6.5	0.82
Rotifers ( $\mu\text{gC l}^{-1}$ )	0.0	12.7	0.9	2.3	2.45	0.1	7.9	2.4	2.1	0.88
Cladocera ( $\mu\text{gC l}^{-1}$ )	0.0	1.0	0.2	0.2	1.41	0.0	4.5	0.6	0.9	1.59
Copepods ( $\mu\text{gC l}^{-1}$ )	0.0	5.9	1.5	1.9	1.23	0.0	20.2	4.9	5.3	1.09
Small filter-feeders ( $\mu\text{gC l}^{-1}$ ), SFF	0.0	12.7	1.9	2.6	1.37	0.1	17.5	5.7	4.7	0.83
Medium-sized filter-feeders ( $\mu\text{gC l}^{-1}$ ), MFF	0.0	4.6	0.6	0.9	1.61	0.0	4.7	0.8	1.0	1.29
Omnivorous/carnivorous ( $\mu\text{gC l}^{-1}$ ), OMN	0.0	0.7	0.1	0.2	1.52	0.0	9.5	1.5	2.7	1.85
Total planktonic biota ( $\mu\text{gC l}^{-1}$ )	55.5	493.7	270.0	116.7	0.43	0.1	744.0	269.6	209.9	0.78
Microbial food web ( $\mu\text{gC l}^{-1}$ ), MFW	52.6	462.1	243.7	107.8	0.44	0.0	667.4	247.2	193.9	0.78
Classical food chain ( $\mu\text{gC l}^{-1}$ ), CFC	1.0	109.8	26.3	22.2	0.84	0.1	109.7	22.4	23.6	1.05
Autotrophic plankton ( $\mu\text{gC l}^{-1}$ )	3.9	320.3	121.3	882.9	0.68	0.0	460.6	130.9	123.1	0.94
Heterotrophic plankton ( $\mu\text{gC l}^{-1}$ )	48.6	268.0	148.3	56.8	0.38	0.1	283.4	138.8	102.2	0.74
Heterotrophic plankton:Autotrophic plankton	0.5	13.1	2.4	2.9	1.23	0.5	3.9	1.4	0.9	0.67
Heterotrophic bacteria:Autotrophic plankton	0.4	12.3	1.7	2.5	1.44	0.0	1.4	0.52	0.37	0.72
Heterotrophic bacteria:Heterotrophic nanoflagellates	5.3	446.8	67.6	86.7	1.28	4.8	65.0	16.1	13.3	0.82
Heterotrophic nanoflagellates:Heterotrophic bacteria	0.0	0.2	0.03	0.04	1.25	0.02	0.2	0.09	0.05	0.57
Copepods:Cladocera	0.3	109.5	16.0	24.4	1.53	0.8	47.9	16.6	14.4	0.87
Grazing	0.0	0.26	0.04	0.05	1.47	0.01	3.87	0.36	0.80	2.19

Abbreviation as in the text are included

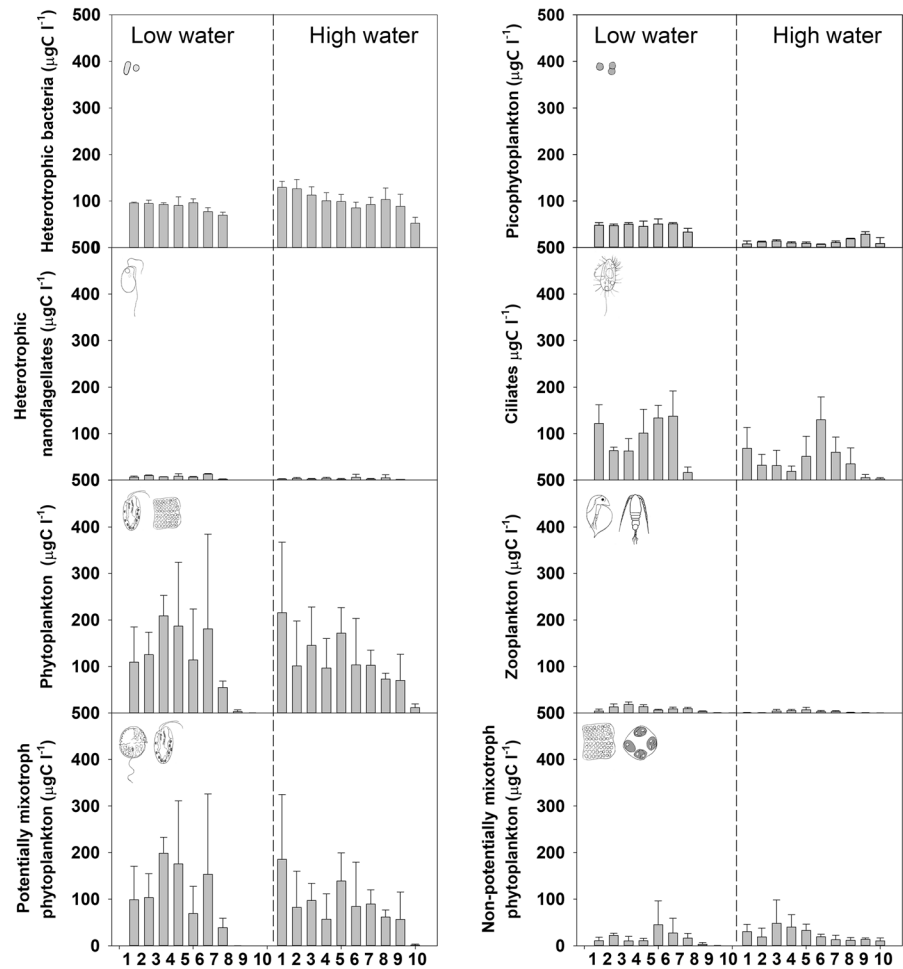
biomass was higher during HW ( $F = 8.1$ ,  $P < 0.01$ ). As for HNF, total zooplankton biomass (ZOO) was remarkably low ( $5.2 \pm 5.7 \mu\text{gC l}^{-1}$ ) and small-sized filter-feeders (SFF, rotifers + nauplii + copepodites) were the main group. ZOO ( $F = 17.9$ ,  $P < 0.01$ ), SFF ( $F = 14.4$ ,  $P < 0.01$ ) and omnivorous (OMN) zooplankton ( $F = 10.3$ ,  $P < 0.01$ ) were significantly higher in LW, but medium-sized filter-feeders

(MFF) were not significantly different between hydrologic periods (Fig. 3).

Therefore, the main components of plankton communities in the Puruzinho system were PHY, particularly PMP, followed by HB and CIL. Most of the plankton components showed a higher biomass during LW (PPP, CIL, PHY, ZOO) than during HW. However, HB was higher during HW, and non-significant differences between periods were observed for HNF

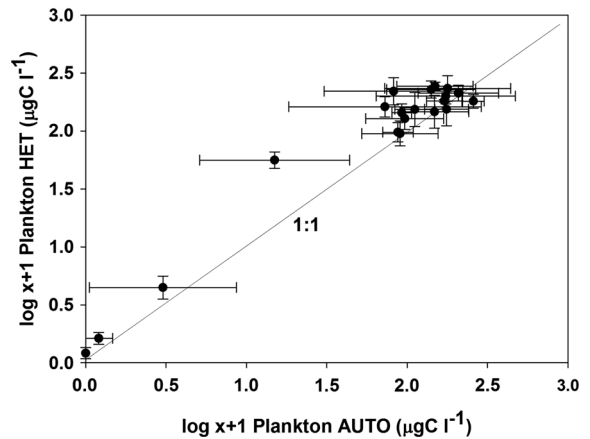


**Fig. 3** Mean values and standard deviation ( $n = 3$ ) of the plankton components along the longitudinal axis of the Puruzinho system (1–7, lake; 8–10, channel), during low and high water, showing potentially mixotrophic phytoplankton, heterotrophic bacteria, and ciliates as the main components. Note the Y axes are on the same scale

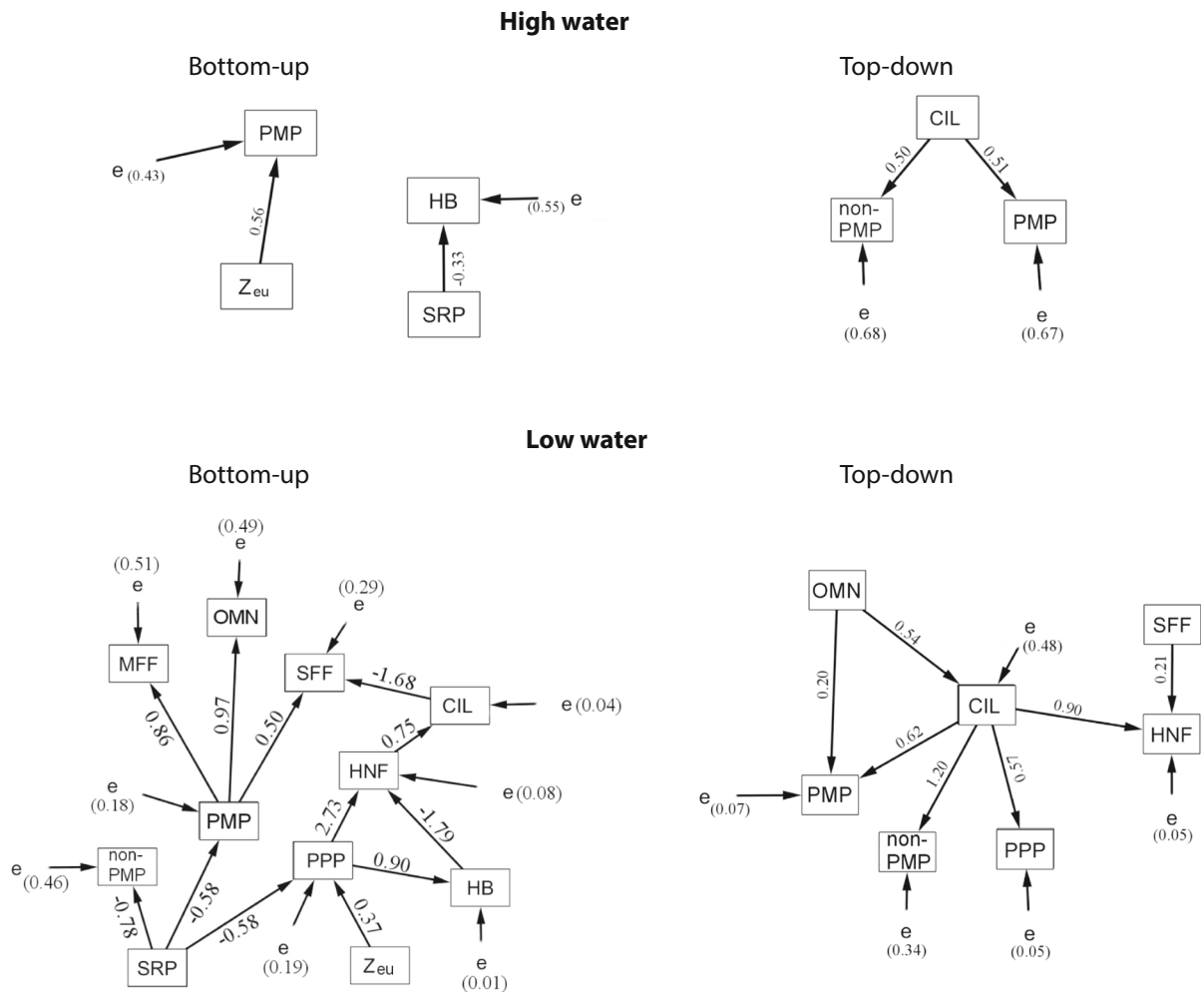


and PMP. Because these components are more frequently analyzed as abundance, in Table 1 we also show the results in both units, abundance and carbon content.

Biomass of heterotrophic plankton, HET (HB + HNF + CIL + ZOO) was higher than biomass of autotrophic plankton, AUTO (PPP + PHY), leading to a HET:AUTO ratio always  $> 1$ , which was twofold higher during HW than during LW ( $F = 8.3$ ,  $P < 0.01$ ) (Fig. 4, Table 2). The mean ratio between the biomass of heterotrophic bacteria (HB) and total autotrophic plankton (AUTO = PHY + PPP) was  $1.49 \pm 3.24$  and was four times higher during HW. HB:HNF (mean  $45.5 \pm 70.4$ ) was also higher during HW, but copepods:cladocerans (mean  $16.3 \pm 19.6$ ) and grazing pressure (mean  $0.19 \pm 0.56$ ) were higher in LW than in HW (Table 2).



**Fig. 4** Relationship between biomasses of autotrophs (AUTO) and heterotrophs (HET) ( $\log x + 1$ ); Dashed line shows equal biomass (1:1)



**Fig. 5** Final structure equation models (SEM) for hydrological periods (high and low water), including bottom-up and top-down and abiotic and biotic variables. Arrows indicate significant effects ( $P < 0.05$ ); numbers on the arrows are standardized coefficient estimates; direction of the arrows goes from the independent to dependent variables. *HB* heterotrophic bacteria; *PPP* picophytoplankton; *non-PMP* non-potentially mixotrophic phytoplankton = chlorophyceans, diatoms,

euglenoids, zygmatophyceans; *PMP* potentially mixotrophic phytoplankton = cryptophyceans, dinoflagellates, chrysophyceans, raphidophyceans; *HNF* heterotrophic nanoflagellates; *CIL* ciliates; *SFF* small-sized filter-feeders = rotifers, copepodites, and nauplii; *MFF* medium-sized filter-feeders = cladocerans and calanoid copepods; *OMN* omnivores = cyclopoid copepods; *e* standard errors

### Effects of bottom-up and top-down controls

The SEM analysis for bottom-up and top-down relationships in the HW and LW periods indicated no significant deviation between the observed covariance matrix and the predicted model. The two LW (top-down and bottom-up) model indexes showed higher values for goodness of fit and lower AIC, as well as higher significance values for interactions compared to the HW period. Most of the bottom-up

and top-down models showed positive interactions during both LW and HW; no significant indirect effects were found, except for top-down control during LW (Fig. 5).

### HW period

The model for bottom-up control ( $\chi^2 = 80.95$ ,  $df = 31$ ,  $P = 0.28$ ,  $AIC = -285.92$ ; goodness of fit indices:  $CFI = 0.60$ ,  $TLI = 0.08$ ,  $RMSEA = 0.23$ ,  $RMR =$

0.13) indicated a positive effect of  $z_{eu}$  on PMP ( $P = 0.025$ ) and a negative effect of SRP on HB ( $P = 0.045$ ) during HW. The model for top-down effects ( $\chi^2 = 12.49$ ,  $df = 9$ ,  $P = 0.45$ ,  $AIC = -39.16$ ; goodness of fit indices:  $CFI = 0.88$ ,  $TLI = 0.50$ ,  $RMSEA = 0.11$ ,  $RMR = 0.06$ ) indicated positive direct effects of CIL on non-PMP ( $P = 0.008$ ), PMP ( $P = 0.029$ ), and HNF ( $P = 0.039$ ).

### LW period

The bottom-up model ( $\chi^2 = 71.68$ ,  $df = 31$ ,  $P = 0.37$ ,  $AIC = -324.5$ ; goodness of fit indices:  $CFI = 0.92$ ,  $TLI = 0.82$ ,  $RMSEA = 0.21$ ,  $RMR = 0.10$ ) indicated a positive effect of  $z_{eu}$  on PPP ( $P = 0.02$ ) and a strong positive effect of PPP on HB ( $P < 0.001$ ). However, we found a negative effect of SRP on the plankton primary producers, i.e., non-PMP ( $P = 0.003$ ), PPP ( $P = 0.001$ ), and PMP ( $P = 0.01$ ). The resources for zooplankton from the MFW indicated a positive effect of PPP ( $P < 0.001$ ) and a negative effect of HB ( $P = 0.004$ ) on HNF, which in turn had a positive effect on CIL ( $P = 0.004$ ). PMP positively affected SFF ( $P = 0.04$ ), MFF ( $P = 0.03$ ), and OMN ( $P < 0.001$ ), while CIL indicated a negative effect only on SFF ( $P = 0.04$ ). For top-down relationships ( $\chi^2 = 21.67$ ,  $df = 9$ ,  $P = 0.44$ ,  $AIC = -42.68$ ; goodness of fit indices:  $CFI = 0.97$ ,  $TLI = 0.88$ ,  $RMSEA = 0.22$ ,  $RMR = 0.03$ ), CIL was the main plankton component, which indicated positive direct effects on non-PMP ( $P = 0.06$ ), PPP ( $P = 0.001$ ), PMP ( $P = 0.007$ ), HB ( $P < 0.001$ ), and HNF ( $P < 0.001$ ). OMN positively affected PMP ( $P = 0.01$ ) and CIL ( $P = 0.04$ ). HNF was affected positively by SFF ( $P = 0.002$ ) and negatively by MFF ( $P = 0.06$ ). HB was affected marginally significantly by SFF ( $P < 0.06$ ) and PNF ( $P < 0.07$ ).

Indirect effects were observed only during LW for top-down control. SFF positively affected PPP (standard coefficient = 0.17;  $P = 0.04$ ) and PMP (standard coefficient = 0.19;  $P = 0.065$ ) through HNF; as well as HB through HNF (standard coefficient = 0.23;  $P = 0.03$ ). Also, CIL positively affected PPP (standard coefficient = 0.62;  $P < 0.001$ ) and PMP (standard coefficient = 0.67;  $P < 0.001$ ) through HNF. Despite the lack of a direct effect of CIL on HB, we also observed a positive indirect effect of CIL on HB (standard coefficient = 0.57;  $P < 0.001$ ) through HNF.

## Discussion

In this study, we investigated the carbon partitioning in the microbial food web (MFW) and classical food chain (CFC) in an Amazonian floodplain system with contrasting seasonal water levels, and we found a predominance of the MFW over the CFC in terms of carbon biomass. Moreover, the seasonality of the plankton components and their interactions changed dramatically in the contrasting water levels, with higher biomass and a more complex food web configuration during the LW period.

### Plankton carbon partitioning

The MFW, composed of heterotrophic bacteria (HB), heterotrophic nanoflagellates (HNF), ciliates (CIL), phototrophic picoplankton (PPP), and potentially mixotroph phytoplankton (PMP) accounted for approximately 90% of the total plankton biomass and was significantly higher during LW. This is an expected pattern in floodplain lakes, because nutrients and biota become more concentrated when the water level recedes (Forsberg et al., 1988; Huszar & Reynolds, 1997; Forsberg et al., 2017). The most plankton components (PHY, PPP, CIL, and ZOO) were higher in LW, while the HB was higher in HW. While HB can be strongly top-down-controlled (Segovia et al., 2018), the effects of predation on HB can be buffered by high nutrient availability and consequently by rapid growth of the prominent bacterial strains (Šimek et al., 2003, 2005). For these reasons, HB abundance is more stable than other components of the plankton (Gasol et al., 2002). The variation of abundance in Puruzinho was within the expected range for floodplain lakes ( $0.06$  and  $13.4 \times 10^6$  cells  $ml^{-1}$ , Roland et al., 2010), but it was close to the upper limit for tropical systems (for a review, see Segovia et al., 2016) and higher than the mean abundance values for 847 Brazilian systems (Roland et al., 2010). However, in terms of biomass, HB levels were close to those found in temperate deep mesotrophic reservoirs (Burns & Galbraith, 2007) and temperate hypertrophic lakes (Auer et al., 2004). In contrast to HB, HNF abundance (Segovia et al., 2016) and biomass were very low in our study system, with levels close to low nutrient-enriched temperate, Mediterranean, and other tropical systems (Callieri et al., 1999; Auer et al., 2004; Conty & Becares, 2013; Domingues et al.,

2016). CIL biomass was within a similar range to low nutrient-enriched Mediterranean shallow lakes (Conty & Becares, 2013), but also to a more nutrient-rich tropical system (Esquivel et al., 2016). PPP biomass had relatively high levels in the Puruzinho system, being 2–20-fold higher than in several temperate systems (Burns & Galbraith, 2007; Sarmiento et al., 2008) and 2–4-fold higher than in tropical oligomesotrophic and mesotrophic reservoirs (Domingues et al., 2016). Prokaryotic PPP biomass was one order of magnitude higher than eukaryotic PPP, as usually observed in many systems (Stockner & Shortreed, 1991; Callieri & Stockner, 2002).

Total PHY biomass, including PMP and non-PMP, was within the range of mesotrophic lakes and reservoirs in both tropical (Silva et al., 2014; Domingues et al., 2016) and temperate regions (Auer et al., 2004; Burns & Galbraith, 2007). PMP (cryptophyceans, dinoflagellates, chrysophyceans, and raphidophyceans) comprised 81% of the total PHY biomass, which highlights the importance of potentially mixotrophs in the Puruzinho system. ZOO biomass was remarkably low and within a similar order of magnitude to a moderately nutrient-rich Mediterranean shallow lake (Özen et al., 2018) and a mesotrophic Amazonian deep reservoir (Domingues et al., 2016). Fish intensively control the ZOO community structure, mainly the metazooplankton, alleviating the pressure on the CIL (Jeppesen et al., 1996; Fermani et al., 2013; Karus et al., 2014), and may contribute to a more heterotrophic state of the system (Cremona et al., 2014). Because the Puruzinho system is dominated mainly by omnivorous and detritivorous fishes and to a lesser extent by planktivorous fishes (unpublished data), there may be high predation pressure on ZOO and a subsequently reduced contribution of ZOO carbon and of predation pressure on PHY (Carpenter & Kitchell, 1993). Indeed, PMP dominate this plankton fraction, also due to their adaptive mechanisms to growth-limiting concentrations of soluble reactive phosphorus, especially during LW. This could also be one reason for the high contribution of CIL in this system.

#### Effects of bottom-up and top-down controls

Our results for the SEM clearly indicate a higher number of interactions among plankton components during LW for both top-down and bottom-up controls.

When the water level recedes, nutrients and biota become more concentrated. Most of the bottom-up and top-down models showed positive interactions during both LW and HW; indirect effects were non-significant, except for top-down control during LW.

During HW, the bottom-up model indicated that the increase in water transparency promotes the growth of PMP, which contributes almost half of the total PHY biomass in this period. We also found a negative effect of dissolved inorganic phosphorus on HB, indicating possible competition between HB, PPP, and PHY (Drakare et al., 2002). The top-down model during HW revealed only positive relationships between CIL and PHY, indicating that both communities benefit from nutrient inputs during this period. CIL have been recognized for their crucial role in nutrient cycling in the water column (e.g., Segovia et al., 2016), favoring an increase of potentially and non-potentially mixotrophs.

A more complex network appeared during LW, when nutrient bottom-up control was more intense for PHY growth, particularly in the upstream (lake) sampling points. Therefore, the negative relationship between all fractions of primary producers (PPP, PMP, non-PMP) indicated nutrient depletion by increasing biomass. Among the phytoplankton fractions considered here, only PMP was linked to all zooplankton fractions (SFF, MFF, ONI). Besides being a high-quality food (Carpenter & Kitchell, 1993), PMP was the main component of the phytoplankton community in this period. PMP, CIL, and HB were the main contributors in terms of biomass in the system, and they are all components of the MFW. Despite the lower biomass compared to PMP, CIL, and HB, PPP occurred in high biomass to other systems (Burns & Galbraith, 2007; Domingues et al., 2016). Therefore, PPP is probably a source of labile dissolved organic carbon to HB via excretion (Morana et al., 2014). However, we did not observe a bottom-up control of HB by DOC. Most of the DOC in Amazonian floodplain systems is of terrestrial origin (Moreira-Turcq et al., 2013), especially in HW (Vidal et al., 2015), accounting in different ways for HB variation. Indeed, the HET:AUTO was higher than 1:1 in the Puruzinho system, suggesting an energy subsidy from the watershed. Moreover, HNF seems to exert a strong control on HB, as evidenced by the negative effect between these two compartments, which has been extensively reported elsewhere (Gasol et al., 2002;

Karus et al., 2014). However, a comprehensive literature review found lower abundances of both HB and HNF in tropical than in temperate regions, and no difference between HNF-HB coupling, indicating that not only HNF but also CIL and small zooplankton may be controlling HB abundance in tropical systems (Segovia et al., 2016).

On the other hand, HNF appeared as a resource for CIL, which in turn was negatively related to SFF, indicating possible competition between these two components because they may share prey preferences (Berninger et al., 1991; Segovia et al., 2015). Resources for CIL are thought to be mainly HB, PPP, HNF, and small phytoplankton (Šimek et al., 1996; Galbraith & Burns, 2010; Posch et al., 2015; Segovia et al., 2015), and they are usually grazed by metazoans and fish larvae (Esquivel et al., 2016).

The top-down model during LW indicated a key role of CIL, which had several positive relationships with all fractions of the primary producers and with the other components of the MFW. A metazooplankton community mainly composed of copepods (as in our study, termed OMN) may favor small-sized CIL and flagellates (Karus et al., 2014) and plankton energy transfer through MFW (Meira et al., 2018). Positive relationships between CIL and HNF have been reported in previous studies (Segovia et al., 2015; Domingues et al., 2016). A possible explanation for this observation is niche overlap, when both CIL and HNF compete for the same resources (PPP, HB, small particles of abioseston). When resources are available, both CIL and HNF can increase at the same time, or CIL have a wider spectrum of prey preference, which releases predation pressure on PPP and HB, which are the main prey of HNF (Auer et al., 2004; Galbraith & Burns, 2010). Although CIL are able to consume HNF, HB, and PHY, microzooplankton can contribute highly to nutrient remineralization, which may affect phytoplankton and bacterial dynamics (Hambright et al., 2007).

In summary, our data provided evidence that the microbial food web carries most of the carbon fluxes in this Amazonian floodplain system (our first hypothesis), because P is a limiting resource for the strict primary producers. Potential mixotrophy is an important strategy for phytoplankton in this turbid system. We also found that hydrology is a key factor increasing biomass (our second hypothesis) shaping more complex interactions during the low-water period. In

addition, most of the bottom-up and top-down models indicated positive interactions during both LW and HW, with no significant indirect effects found, except for top-down control during LW. The use of a single currency in studies of the biotic and abiotic drivers of each part of the entire pelagic community may enable more consistent findings.

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