Effects of resources and food web structure on bacterioplankton production in a tropical humic lagoon

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The majority of studies of mechanisms regulating bacterioplankton processes have focused on assessing the isolated effects of nutrients and predation. However, in natural ecosystems, microorganisms may interact strongly with larger organisms in an array of complex, direct and indirect interdependencies. In this work, we report the results of a field mesocosm experiment in which, over 7 weeks, we evaluated the individual and interactive effects of resource availability (N and P addition) and indirect effects originating from the presence of an omnivorous fish species (*Hyphessobrycon bifasciatus*) on bacterioplankton production (BP). Nutrient addition and fish presence both had individual positive effects on BP, but bottom-up control effects were stronger than indirect top-down control effects. The positive effect of nutrients on BP was mainly direct, through increasing the availability of inorganic N and P. There was no significant interaction between fish and nutrients. The positive indirect top-down effects of omnivorous fish on BP were probably related to both fish-mediated changes in the zooplankton community structure and fish-mediated cross-habitat nutrient regeneration. Our results show that changes in food web structure, due to the presence or absence of vertebrate macroconsumers, can also affect heterotrophic microbial processes.

KEYWORDS: trophic interactions; inorganic nutrients; omnivorous fish; microbial food web; enclosures

INTRODUCTION

Heterotrophic bacteria are key components driving biogeochemical processes in aquatic ecosystems (see Fenchel et al., 1998 and references therein). They are among the most numerous planktonic organisms in aquatic systems and also play an important role in organic carbon (C) and nutrient mineralization (Sigge, 2005). The process of bacterial production in aquatic ecosystems has receive much interest in recent decades (Fenchel, 2008), since it has been considered a link between dissolved organic carbon (DOC) and organisms at higher trophic levels (Azam et al., 1983). This perception of the importance of bacterioplankton in aquatic systems, both as recyclers of organic matter and as a trophic link to top consumers, has spurred considerable efforts to understand the factors that regulate


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bacterioplankton production in aquatic systems (Fenchel, 2008).

Resource-mediated changes in microbial activity have the potential to alter ecosystem processes, such as biomass production and respiration (Lennon and Cottingham, 2008). It is known that the abundance and productivity of bacterioplankton are influenced by the availability of inorganic nutrients (Toolan et al., 1991; Elser et al., 1995; Rivkin and Anderson, 1997; Smith and Prairie, 2004). Moreover, the availability and quality of C can exert a substantial influence on bacterial activity level and metabolism. The production of labile organic matter by phytoplankton is considered the most important source of C substrates for bacteria (Cole, 1982). However, bacterial metabolism may be uncoupled from local primary production (del Giorgio et al., 1982). In this case, bacterial growth may be limited by nutrient availability and labile organic matter (Lennon and Pfaff, 2005).

Although bottom-up control is considered to be the most important factor governing bacterioplankton production, studies have shown that bacterial predators may also exert substantial top-down control on bacterioplankton communities (Sherr and Sherr, 2002), especially in eutrophic systems (Thelaus et al., 2008). Many authors cite protozoa, mostly ciliated and flagellated protists, as the main consumers of bacteria (Weisse and Frahm, 2001; Sherr and Sherr, 2002; Samuelsson and Andersson, 2003; Agasild and Noges, 2005; Sigge, 2005; Zingel et al., 2007). However, protozoan predation pressure on bacteria can be attenuated, or even nullified by cascades of indirect interactions. Copepod consumption of protozoans and small metazoans can affect bacterial and phytoplankton by minimizing grazing by such communities (Adrian and Schneider-Olt, 1999; Hansen, 2000). Besides this positive indirect effect, some direct negative effects of zooplankton grazing on bacterioplankton have also been reported (Hessen and Andersen, 1990; Pace and Cole, 1996; Jürgens and Jeppesen, 1998; Thouvenot et al., 1999).

The important role of fish in structuring the pelagic and littoral compartments of shallow lakes through both top-down and bottom-up trophic cascades is well documented in the literature (Jeppesen et al., 1992; Vanni and Layne, 1997; Carpenter et al., 2001); however, most of our knowledge about the effects of fish on pelagic food webs comes from cascading interactions involving phytoplankton and zooplankton communities (Bell et al., 2003). Relatively little is known about the impacts of fish-mediated trophic cascades on bacterioplankton community processes (Pace and Funke, 1991). Some studies have shown that the presence of planktivorous fish changed the biomass and composition of zooplankton (Rieman, 1985; Christoffersen et al., 1993; Jeppesen et al., 1996), and this, in turn, affected bacterial density and production (Markosova and Jezek, 1993; Riemann, 1985) by altering the grazing pressure on bacteria. Even if omnivorous species can have profound effects on the trophic dynamics of communities and ecosystem processes, the effects of omnivorous fish, the most common feeding strategy of fish in warm-water lakes (Fernando, 1991; Kolding, 1993; Starling et al., 2002), on microbial processes remain largely uninvestigated. Therefore, our knowledge of the individual and interactive effects of top-down and bottom-up mechanisms on the regulation of bacterial processes in food webs, which are naturally composed by both macro- and microorganisms, remains limited.

In this study, we used large within-lake enclosures, which included most components of the natural planktonic food web of a tropical humic coastal lagoon. Our main objective was to evaluate the individual and interactive effects of nutrient addition, and the presence of omnivorous fish on bacterioplankton production, over 7 weeks. This approach allowed us to evaluate the long-term effects (regarding microbial generation time) of trophic complexity (originating from omnivorous behavior) and resource availability on a key microbial process in an aquatic ecosystem.

METHOD

Experimental site

This study was carried out in Cabiuñas Lagoon, located at Restinga de Jurubatiba National Park, Rio de Janeiro, Brazil (22°15’S, 41°40’W). Cabiuñas is a distrophic coastal lagoon with a surface area of 0.35 km² and a mean depth of 2.5 m. The water is humic (13.06 mg C L⁻¹ of DOC and 0.044 of color) and slightly acidic (pH 6.3), with an average annual temperature of 23.6°C (Guarento et al., 2009). The mean phytoplankton biomass estimated by chlorophyll a (Chl a) was 30 µg L⁻¹, and the mean Secchi disk depth was 0.5 m. The enclosures were placed close to the lagoon’s littoral region, which is colonized by dense stands of the broad-leaved cattail Typha domingensis (Pers.), but no macrophytes were included in the enclosures.

Enclosures

Enclosures consisted of cylindrical transparent plastic containers that were 2.0 m in diameter and 2.4 m tall, equipped with iron rings at the top and bottom to facilitate structural stability, to attach floats (top ring) and to
anchor the enclosure to the sediment (bottom ring). The enclosures were open at the bottom and pushed down 0.1 m into the sediment to prevent fish escape and allow access to benthic food. All enclosures were placed at a depth of ~2 m, so that the top ring of the enclosures reached 0.3 m above the water surface to protect the enclosures against lagoon water ingestion (Guariento et al., 2010). This depth was chosen, because it is closer to the average water depth of Cabiñas Lagoon. At this depth, each enclosure’s volume was ~6300 L.

**Experimental design**

Our experiment followed a 2 × 2 factorial design (± nutrients and ± fish): control (NF0), nutrients (N+), fish (F+) and fish + nutrients (FN+)]. The treatments were replicated four times for each possible combination, resulting in 16 enclosures. To evaluate potential influences of spatial heterogeneity, whole factor treatments were distributed into four experimental spatial blocks.

**Experimental setup**

The treatments were established 1 week after installation of the enclosures. This period allowed limnological conditions to stabilize after enclosure installation (i.e. first week) and guarantee that aquatic communities inside the enclosures had enough time to respond to experimental manipulation (i.e. second week). After this period, depth-integrated water samples from each enclosure were collected weekly over the five subsequent weeks for assessment of pelagic concentration of Chl a (a proxy for phytoplankton biomass), inorganic N (NH4 and NO3), inorganic P (PO4) and DOC. Water color and bacterial production were also assessed. To control for the “cage effect”, we conducted parallel sampling of the variables listed above in Cabiñas Lagoon close to the experimental enclosures.

Zooplankton sampling was carried out by vertical hauls from near bottom to the surface, using 50 μm mesh plankton nets, and the samples were immediately fixed in 4% buffered formaldehyde. Densities of all zooplankton species were estimated by two subsample quantification in open chambers under a stereomicroscope. Rare species were counted in the entire sample. For further analysis, zooplankton was divided into functional groups of rotifers, cladocerans, cyclopoid and calanoid copepods. Chl a was first estimated in the field through a semi-quantitative spectrofluorimetric determination (Turner® 3A) with a subsequent transformation of fluorescence values to mass concentrations of Chl a. For chemical analysis of nutrients, DOC and water color, water from the enclosures was filtered through a filter (GF/F Whatman—0.7 μm pore size) before analysis. Ammonia-N determination was made manually
using the phenol-hypochlorite technique (Solorzano, 1969). Nitrate-N concentration was determined by flow injection analysis according to Golterman et al. (Golterman et al., 1978). Inorganic nitrogen was then calculated by summation of Ammonia-N and Nitrate-N concentrations. Orthophosphate-P was determined by using the molybdenum blue technique according to Golterman et al. (Golterman et al., 1978). The DOC concentration was determined using a Pt-catalyzed high-temperature combustion method with a TOC-5000 Shimadzu Carbon Analyzer. The water color was determined based on light absorbance at 430 nm using a Beckman Du® spectrophotometer and 1 cm quartz cuvettes. We used color/DOC ratios to represent the humic fraction of DOC, as suggested by Suhett et al. (Suhett et al., 2007).

Bacterial production (BP) was estimated based on 3H-Leucine incorporation into DNA (Smith and Azam, 1992). BP values were obtained right after sampling by incubation of 1.3 mL water samples in the dark for 45 min with 0.1 mL of 3H-Leucine (5-fold diluted solution, 159 Ci mmol−1, Amersham) with a final concentration of 10 nM. After incubation, 90 μL of 100% Trichloroacetic acid (TCA) was added to halt the reaction. Each tube was washed sequentially with 5% TCA and 80% ethanol, and 500 μL of Scintillation Cocktail (Aquasol 2, Dupont) was added to each tube. The activity (disintegration per minute, DPM) was measured in a Beckman LS-5600 Liquid Scintillation System. Bacterial production was calculated assuming an intracellular leucine dilution factor of 2 and a cellular carbon-to-protein ratio of 0.36 (Simon and Azam, 1989).

**Statistical analysis**

Log_{10}-transformed variables were used to reduce the heterogeneity of the variances. We used a repeated-measures two-way analysis of variance (RM-ANOVA) to test individual and interactive effects of fish and nutrients on bacterial production over time. Nutrients and fish were treated as the categorical variables (between-subjects factors), and the sampling time (i.e. 5 weeks, not counting the first 2 weeks of stabilization) was treated as a repeated factor (within effects). The values of BP were considered the dependent variable. Data sampled outside the enclosures in Cabo Frio Lagoon were not formally included in the analysis; instead of they were plotted together with data from experimental treatments to permit visual comparisons. A principal components analysis (PCA) based in a correlation matrix was performed to elucidate interrelationships among abiotic (DOC, Color/DOC ratio) and biotic variables (zooplankton groups density, BP and Chl a) with experimental factors over the time. Both the analysis of variance and the PCA were performed using STATISTICA 6.0 software for Windows® (StatSoft, 2001).

We also calculated the standard mean differences (D) as effect sizes, based on Hedges’ d (Gurevitch and Hedges, 1993), to compare the effects of fish and nutrients on the magnitude of bacterial production. D is given by the formula:

$$D = \frac{\bar{X}_T - \bar{X}_C}{S} \times J$$  

(1)

For D, the difference between two means is standardized by pooled standard deviation of both treatments, where \(\bar{X}_C\) is the mean of the control, \(\bar{X}_T\) the mean of the treatment for each manipulated factor, S the pooled standard deviation and \(J\) corrects for bias due to small sample size (see calculations below). The number of replicates is \(n\) and SD is the standard deviation of treatments.

$$S = \sqrt{\frac{(n_T - 1)(SD_T)^2 + (n_C - 1)(SD_C)^2}{n_T + n_C - 2}}$$  

(2)

$$J = 1 - \left(\frac{3}{4(n_T + n_C - 2)} - 1\right)$$  

(3)

The effect sizes of fish presence (\(D_{fish}\)) and nutrient addition (\(D_{nutrient}\)) were calculated for the five sampling weeks. \(D_{nutrient}\) was calculated both in the absence of fish, when \(\bar{X}_T\) was N+ treatment and \(\bar{X}_C\) was the NF0 treatment, and in the presence of fish, when \(\bar{X}_T\) was NF+ treatment and \(\bar{X}_C\) was the F+ treatment. The same procedure was used to evaluate \(D_{fish}\), when it was calculated both without nutrient addition (\(\bar{X}_T = F+\) and \(\bar{X}_C = NF0\)) and with nutrient addition (\(\bar{X}_T = NF+\) and \(\bar{X}_C = N+\)). To test the significance of \(D_{fish}\) and \(D_{nutrient}\) values, one-sample t-tests were performed comparing each value against the hypothetical value of zero, expected in the absence of interaction between factors (Dang et al., 2005).

**RESULTS**

The experimental manipulation of inorganic N concentration reached values of almost 50 μM of inorganic N just in the first weeks of the experiment; however, during the last weeks, these values started to decline irrespective of the continuous nutrient addition (Fig. 1A). On the other hand, the experimental manipulation of inorganic
P concentration was successful throughout the experiment, as the intended final concentrations of 10 μM of P were achieved and maintained over the experiment duration (Fig. 1B). On average, inorganic N and P concentration was 5× and 50× higher, respectively, in treatments that included nutrients compared with those that did not. The NID:PID ratio also remained constant and near the desired value (5:1) in the treatments that received nutrient addition (Fig. 1C). Inorganic N and P concentrations of Cabiuñas Lagoon were similar (4.3 ± 1.8 μM of N and 0.2 ± 0.2 μM of P) to those of the control (9.4 ± 4.0 μM of N and 0.1 ± 0.1 μM of P) and fish (4.8 ± 1.3 μM of N and 0.1 ± 0.04 μM of P) treatment (Fig. 1A and B). The N:P ratio of Cabiuñas Lagoon was similar to the control treatment throughout most of the experiment, and became lower than the control treatment just in the end of the experiment (Fig. 1C). Although no additions of carbon were performed, the color/DOC ratio showed a little decline during the experiment, principally during the last 2 weeks (Fig. 1D). However, the color/DOC ratio was very similar between treatments and with the Cabiuñas Lagoon, except for the seventh week, when the lagoon had a color/DOC ratio lower than the treatments (Fig. 1D).

The main effects of nutrient addition (treatments N+ and NF+) and fish presence (treatments F+ and NF+) were statistically significant (Table I), and both variables had overall positive effects on BP over the experimental period (Fig. 2). Treatments without nutrient addition (NF0 and F+) had the lowest values of BP (Fig. 2). The interaction of fish and nutrients (NF+) was not significant with BP (Table I; Fig. 3). Time had an effect, but no significant interactions between time and the other two factors were observed (Table I). The BP values of Cabiuñas Lagoon were similar to those of the control treatment during all the experiment (Fig. 2).

Effect sizes showed that nutrients had significant positive effects on BP, both in the absence and in the presence of fish, as the lack of interception of the 95% CI with the x-axis for both effect sizes (Fig. 3). $D_{\text{nutrient}}$ in the absence of fish was not significantly different from $D_{\text{nutrient}}$ calculated in the presence of fish. Moreover, fish effect sizes on BP, $D_{\text{fish}}$, were significantly independent of nutrient additions (Fig. 3). Such a result indicates that the presence of fish has an overall positive effect on BP. However, the effect of nutrient addition on BP was more intense than that of fish presence (Fig. 3).
Table I: Summary of results from RM-ANOVA

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>Df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish (T)</td>
<td>0.152</td>
<td>1</td>
<td>0.152</td>
<td>11.81</td>
<td>0.0049†</td>
</tr>
<tr>
<td>Nutrient (N)</td>
<td>1.191</td>
<td>1</td>
<td>1.191</td>
<td>148.17</td>
<td>0.0000‡</td>
</tr>
<tr>
<td>F × N</td>
<td>0.003</td>
<td>1</td>
<td>0.003</td>
<td>0.24</td>
<td>0.6278</td>
</tr>
<tr>
<td>Time (T)</td>
<td>0.782</td>
<td>4</td>
<td>0.195</td>
<td>16.32</td>
<td>0.0000‡</td>
</tr>
<tr>
<td>T × F</td>
<td>0.017</td>
<td>4</td>
<td>0.004</td>
<td>0.36</td>
<td>0.8326</td>
</tr>
<tr>
<td>T × N</td>
<td>0.026</td>
<td>4</td>
<td>0.006</td>
<td>0.55</td>
<td>0.7000</td>
</tr>
<tr>
<td>T × F × N</td>
<td>0.042</td>
<td>4</td>
<td>0.010</td>
<td>0.88</td>
<td>0.4793</td>
</tr>
</tbody>
</table>

The table gives the individual and interactive effects of nutrient addition and fish presence on bacterial production. Nutrients, fish and their interaction were treated as main factors and time was treated as a repeated factor. Bolded P-values indicate statistically significant effects (P < 0.05). Arrows indicate the direction of effect. SS, sum of squares; Df, degrees of freedom; MS, mean of squares.

Fig. 2. Temporal variation of bacterioplankton production in response to nutrient addition and the presence of omnivorous fish. The symbols represent means (n = 4) and error bars are the standard deviation. Treatment abbreviations are as in Fig. 1. The first 2 weeks were not included in the analysis.

The first two axes of the PCA explained 67.06% of the cumulative percentage of variance in the data (Fig. 4). The first axis explained 42.46% of the total variance and was negatively correlated with BP (r = −0.88), Cyclopoideas density (r = −0.79), DOC concentration (r = −0.87) and Chl a (r = −0.79). The second component explained 24.59% of the total variance and exhibited a negative correlation with Cladocera and Calanoida density (r = −0.82 and −0.81, respectively) and color/DOC ratio (r = −0.25), and a positive correlation with Rotifera density (r = 0.65). Throughout the experiment, most of the replicates that received nutrient addition (NF+ and N+) were located on the negative side of the first axis, while the replicates of the treatments that did not receive nutrients (F+ and NF0) were positioned on the positive side of this axis. On the other hand, fish presence (NF+ and F+) had a positive relation with the second axis of the PCA (Fig. 4).

**DISCUSSION**

Over 5 weeks, we evaluated the direct effects of resource availability (N and P addition) and the indirect effects of omnivorous fish on BP in a humic coastal freshwater lagoon. The utilization of within-lake mesocosms was adequate to evaluate the effects of nutrients and food web structure on BP over the temporal and spatial scales which encompassed much of the biotic and abiotic components of the Cabiúnas Lagoon. Fish and inorganic P manipulations were effective, since fish density and inorganic P concentration were stable during the 7 weeks of the experiment. Although we could not demonstrate that inorganic N manipulation remained constant, its concentration in the enriched treatments was kept significantly higher than those observed in unenriched treatments. Furthermore, N:P ratio remained relatively constant during the experiment. One possible reason for the observed NID concentration depletion could be attributed to the fact that our nutrients analyses were done 2 days after the nutrient additions, in this way, NID could have been consumed by the organisms, absorbed in sediment or depleted from the system by denitrification, and as consequence, we could have underestimated its initial concentration. In general, the nutrient concentration and the BP magnitude of Cabiúnas Lagoon were very similar to the control, indicating that the patterns observed in this experiment were not due to a “cage effect” resulting from the enclosures.

Nutrient addition and fish presence both stimulated BP, but the nutrient effect was stronger than the fish effect, corroborating the results of a recent meta-analysis that showed significant asymmetries between bottom-up and top-down regulation on the magnitude of
community processes (Borer et al., 2006). Fish and nutrients had no significant interactive effect on BP. Moreover, inspections of effect sizes showed that the positive effect of fish on BP also exists when the background nutrient concentration was high. Our results corroborate previous studies that have shown that BP is highly dependent on nutrient availability (del Giorgio and Cole, 1998; Farjalla et al., 2002a), but adds to the indirect effects of an omnivorous fish species on microbial processes in naturally complex food webs, an important aspect that has been largely neglected in the literature.

Bottom-up effects of nutrient addition on BP may have operated through different mechanisms. Many studies have already reported that nutrient limitation is an important aspect limiting bacterial growth in many aquatic systems (Toolan et al., 1991; Rivkin and Anderson, 1997). In fact, Farjalla et al. (Farjalla et al., 2002b), using batch culture experiments, showed that P was the principal nutrient limiting bacterioplankton production and abundance in the Cabiúnas Lagoon. However, in the same study, these authors showed that the quality of organic carbon is also an important aspect in determining bacterioplankton growth rates (Farjalla et al., 2002b). In general, labile organic carbon originating from phytoplankton has a greater ability to stimulate BP than more refractory carbon originating from terrestrial sources (Cole, 1982; Kritzberg et al., 2005; Farjalla et al., 2006). We observed that BP had a positive relationship with DOC and Chl a concentrations (Fig. 4). Therefore, a first hypothesis could be that the increase in nutrient concentration was stimulating BP both directly, through an increase in N and P availability, and/or indirectly, through enhancing phytoplankton growth, thereby increasing the availability of labile carbon for microbial consumption.

The relative importance of direct or indirect effects of nutrient additions on BP is difficult to evaluate. The observed decrease in color/DOC ratio during the experiment indicates a greater contribution of autochthonous-labile carbon (Fig. 1D), which would sustain a greater importance of the indirect effects of nutrient addition over direct effects, but the values of color/DOC ratios were similar between all treatments and with the Cabiúnas Lagoon, and not related to nutrient additions (Fig. 1D). In addition, color/DOC ratio did not show a strong relationship with BP, which probably indicates that the positive relationships of Chl a with BP were paired responses of such variables to increasing nutrient availability rather than resulting from BP limitation for labile carbon. Therefore, our results indicate that the greater availability of inorganic

![Fig. 4.](image-url) Scatter plot of the two first axes resulting from the PCA, elucidating the relationships between the abiotic and biotic variables of each treatment during the experiment. Index values in brackets show the cumulative percentage of variance explained by the respective axis. Treatment abbreviations are NF0 (control), N+ (nutrient addition), F+ (fish presence) and NF+ (nutrient addition plus fish presence) and correspond to the centroid of each categorical variables. The number that follows treatment abbreviations corresponds to the sampling week. The explanatory variables are bacterial production (BP), chlorophyll a concentration (Chl a), DOC concentration, color/DOC ratio (col/DOC) and Rotifera (Rot), Cyclopoida (Cyc), Cladocera (Cla) and Calanoida (Cal) density.
N and P was the most important direct bottom-up factor determining BP in our experiment. An observation that supports this hypothesis is the significant values of $D_{nutrient}$ on BP, both in the presence and absence of fish, which indicates that effects of nutrient availability on BP were consistent and independent of food web manipulations.

In our study, the presence of fish tended to structure the zooplankton community in the mesocosms, principally in the first weeks, since it seems to selectively remove cladocerans and calanoids, and as a consequence indirectly increased rotifer density (Fig. 4). In this way, fish could exert top-down control on bacterioplankton indirectly by changing the structure of the microbial food web. Indeed, it has already been demonstrated that predation by fish on zooplankton alters the size and structure of the community, generally favoring the dominance of smaller zooplankton, such as rotifers (Vanni, 1986). Small zooplankton, on average, excrete nutrients in a lower N:P stoichiometric ratio than do larger zooplankton and therefore contribute to higher production at lower trophic levels (Vanni, 1987; Vanni and Layne, 1997). However, fish may also directly mediate nutrient recycling through their own excretion. Although we did not detect any increase in nutrient values in treatments where fish were added, it is possible that inorganic nutrients available through fish excretion were readily taken up by bacterioplankton. Empirical and theoretical studies have demonstrated that omnivorous fish have a great capacity to improve nutrient regeneration (i.e. recycling and translocation) in aquatic systems, since they can forage in different aquatic compartments, such as littoral regions and sediment; and through their mobility they regenerate nutrients through excretion to subsidize new production in pelagic compartments (Schindler and Scheuerell, 2002; Vadeboncoeur et al., 2002; Vander Zanden and Vadeboncoeur, 2002; Vanni et al., 2004). Such multi-level omnivorous behavior (sensu, Vadeboncoeur et al., 2005) might have enhanced nutrient translocation from the benthic systems to the pelagic compartment in our study, since H. bifasciatus consistently foraged on benthic invertebrates and periphyton that grew up on the enclosure’s wall (visual observation). Therefore, top-down-mediated nutrient recycling by fish was probably the main mechanism to explain the significant positive effect size of fish on BP.

We observed that both resource availability and fish presence had significant individual effects on BP. Although nutrient availability was the most important factor in increasing BP in our experiment, fish presence was also important for mediating BP. With respect to the functioning of humic tropical systems where the primary production by phytoplankton may be substantially limited by light availability and where omnivorous fish are abundant (Esteves et al., 2008), it is important to understand the factors that mediate microbial heterotrophic processes, since upward transfer of energy via the microbial loop is potentially important in these systems (Cotner and Biddanda, 2002; Kritzberg et al., 2006).

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