Microbial Ecology

Influence of Hydrological Pulse on Bacterial Growth and DOC Uptake in a Clear-Water Amazonian Lake

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Abstract

This study was conducted to evaluate: (1) the bacterial growth and the dissolved organic carbon (DOC) uptake in an Amazonian lake (Lake Batata) at high-water and low-water periods of the flood pulse; (2) the influence of nitrogen and phosphorus (NP) additions on bacterial growth and DOC uptake in Lake Batata at two flood pulse periods; and (3) the bioavailability of the main DOC sources in Lake Batata. Lake Batata is a typical clear-water Amazonian lake, located in the watershed of Trombetas River, Central Amazon, Brazil. Bacterial batch cultures were set up with 90% 0.2-μm filtered water and 10% inoculum from Lake Batata. N-NH_4NO_3 and P-KH_2PO_4, with final concentrations of 50 and 5 μM, respectively, were added to the cultures, except for controls. Extra sources of DOC (e.g., algal lysate, plant leachates) were added to constitute six distinct treatments. Bacterial response was measured by maximum bacterial abundance and rates of bacterial production, respiration, DOC uptake, and bacterial growth efficiency (BGE). Bacterial growth and DOC uptake were higher in NP treatments than in controls, indicating a consistent nutrient limitation in Lake Batata. The composition of DOC also seems to be an important regulating factor of bacterial growth in Lake Batata. Seasonally, bacterial growth and DOC bioavailability were higher at low-water period, when the phytoplankton is a significant extra source of DOC, than at high-water period, when the forest is the main source of DOC. DOC bioavailability was better estimated based on the diversity and the diagenetic stage of carbon compounds than on single classes of labile compounds. Changes in BGE were better related to CNP stoichiometry in the water, and the "excess" of organic substrates was oxidized in catabolism, despite the quality of these compounds for bacterial growth. Finally, we conclude that bacterial growth and DOC uptake vary throughout the flood pulse in clear-water Amazonian ecosystems as a result of changes in nutrient concentration and in DOC composition.

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Introduction

Dissolved organic carbon (DOC) in aquatic ecosystems is one of the largest carbon pools in the biosphere. The role of DOC in aquatic ecosystems remained elusive for a long time, associated with metal binding and stability of aquatic ecosystems [23]. DOC importance as the main substrate and energy source for the growth of planktonic bacteria was enhanced after the introduction of the microbial loop concept [5]. Planktonic bacteria oxidize DOC into CO_2, through respiration, or convert DOC into bacterial biomass, providing an important link to higher trophic levels through predation. Bacteria may be considered the basis of planktonic food chains in several aquatic ecosystems, and may also have an important role in CO_2 flux in the biosphere [13, 43]. Therefore, the possible interactions between the DOC pool and planktonic bacteria have a central role in carbon cycling or flux in aquatic ecosystems.

DOC uptake by bacteria is mainly related to the composition of DOC pool, the degree of diagenesis, and the availability of other nutrients, such as N and P [3, 28, 30, 48]. For instance, DOC uptake by planktonic bacteria is directly linked to the C/N and C/O ratios, which indicate the degree of DOC diagenesis, and to the C/H ratio, which is related to the ratio of aliphatic and

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aromatic moieties in the DOC pool [48]. In addition, some sources of DOC, such as the leachate from aquatic macrophytes and the exudates of planktonic algae, are more easily consumed by planktonic bacteria than other sources of DOC, such as the leachate of pine and cypress leaves [48].

The Amazon floodplain—composed of the Amazon River; large secondary rivers such as the Madeira, the Tapajós, and the Trombetas; floodplain lakes; and small streams—is the greatest drainage basin on Earth. The flood pulse, based on filling and drawdown periods, is the main structural force in Amazonian aquatic ecosystems [32]. In the event of flooding, the connection between terrestrial and aquatic ecosystems increases and the DOC in aquatic ecosystems is mostly allochthonous [28, 33]. During the drawdown period, the influence of tropical forest on aquatic ecosystems is lower, and the importance of autochthonous DOC increases [33].

Recently, substantial efforts have been made to determine the role of Amazonian aquatic ecosystems as sinks or sources of carbon on a global basis [27, 43]. According to Richey *et al.* [43], the net sink of carbon fixed in the Amazonian rain forest is oxidized in Amazonian aquatic ecosystems (large rivers, lakes, and small streams) and the carbon balance between fixed and released CO_2 in the Amazon biome might be close to zero. However, a better understanding of the mechanisms of CO_2 production and DOC fate in Amazonian aquatic ecosystems is still necessary. In general, there are few studies focusing on DOC fate in Amazonian aquatic ecosystems; all studies were performed in humic and turbid Amazonian aquatic ecosystems [3, 6, 53]. There are no studies related to the hydrological cycle effects on DOC consumption by planktonic bacteria in clear-water Amazonian ecosystems.

The goals of this study were to evaluate: (1) the bacterial growth and DOC uptake in natural water samples of a clear-water Amazonian lake (Lake Batata) during two flood pulse periods (high- and low-water periods); (2) the influence of NP additions on bacterial growth and DOC uptake in the water samples from Lake Batata during two flood pulse periods; and (3) the influence and bioavailability of the main DOC sources on bacterial growth.

Methods

Study Area. Lake Batata (1°30′N and 56°20′W) is a typical large Amazonian floodplain lake, located on the western floodplain of the Trombetas River near Porto Trombetas, in the municipality of Oriximiná, state of Pará, Brazil. A detailed map can be found in Anesio *et al.* [4]. According to the typology of Amazonian aquatic ecosystems proposed by Sioli [46], Lake Batata is a clear-water ecosystem, characterized by slightly acidic waters and low concentrations of suspended material and nutrients (average of 40 μM total N and 0.5 μM total P) [21].

Amazonian rivers and floodplain lakes are subject to seasonal changes in water level [32]. The high-water period starts from June and ends in August, and the low-water period from November to January in Lake Batata. The annual fluctuation in water level ranges from 7 to 8 m [40]. During the high-water period, the lake floods the surrounding forest and the interconnection between the lake and the Trombetas River increases, whereas during the low-water period, the water retreats, and the interconnection with the Trombetas River decreases.

Limnological Parameters. We measured pH, water temperature, depth, light penetration in the water column (Secchi disk depth), concentrations of nutrients (C, N, and P) and chlorophyll-*a*, and bacterial abundance and secondary production in June 2001 and December 2002 (the high- and low-water periods, respectively). It is important to note that there were no significant differences in water quality and chlorophyll-*a* concentration between December 2001 and December 2002 (data not shown). Thus, June 2001 and December 2002 could be compared as contrasting periods of flood pulse although they do not belong to the same hydrological year.

Water samples were filtered through 0.7-μm pore size filters (filter GF/F, Whatman), and the filters were kept frozen in the dark for the chlorophyll-*a* analysis. Water samples were acidified to pH 1.0 with H_2SO_4 for further analyses of initial concentrations of DOC, total nitrogen (TN), and total phosphorus (TP). Bacterial production was estimated less than 3 h after sampling. Samples of initial bacterial abundance and biomass were fixed with buffered formaline (final concentration, 3.7%).

Bacterial Uptake of Natural DOC. Water samples were collected in acid-washed and deionized-water-rinsed polyethylene flasks in June 2001 and December 2002 from Lake Batata. Water samples were maintained at a constant temperature (25°C in June, and 30°C in December) until the start of the experiment (~2 h). Samples were filtered through 0.2-μm pore size filters (VacuCap filters, Gelman Science) to remove bacteria and larger organisms. An inoculum was prepared by filtering the lake water through 0.7-μm pore size filters (GF/F filter, Whatman). We previously observed that 80% of bacterial cells pass through 0.7-μm pore size filters. The filter apparatus was flushed with approximately 500 mL of lake water before filtering to minimize organic and inorganic contamination. The bacteria were grown in cultures composed of 90% of 0.2-μm filtered lake water and 10% of inoculum. Next, the cultures were poured into acid-rinsed (HCl 10%), heat-sterilized (120°C, 1 atm

autoclavation) 200-mL glass flasks and into 60-mL BOD bottles. The entire setup took 2–3 h.

Two sets of experiments ($n = 4$) were performed at high- and low-water periods: Control (cultures without nutrient addition) and NP treatment [cultures enriched with 50 μM N-NH_4NO_3 and 5 μM P-KH_2PO_4 (final concentrations)]. These concentrations were determined to avoid N or P limitation of bacterial growth in accordance with the N/P molar ratio of 5:1 proposed by Fagerbakke *et al.* [19] and in accordance with other experiments [20]. The cultures were incubated at a constant temperature (~22°C) in water bath in a dark-acclimated room. Bacterial growth was followed through changes in abundance, biomass, and respiration until the stationary phase was reached. Bacterial abundance was sampled at 0, 24, 48, 72, 96, and 120 h of growth in 200-mL glass flasks. Bacterial biomass was calculated by bacterial abundance/bacterial biomass conversion factor of 35 fg C $cell^{-1}$ proposed by Theil-Nielsen and Søndergaard [51] for bacterial growth in batch cultures. Bacterial respiration was estimated via increase in dissolved inorganic carbon (DIC) concentration between 0 and 96 h of incubation in BOD bottles. These sampling times were established in other experiments with nutrient additions in which bacteria achieved the stationary phase of growth at 96 h and DIC concentration increased linearly between 0 and 96 h of incubation (Farjalla *et al.*, manuscript in preparation).

We also estimated bacterial growth efficiency (BGE), DOC uptake rate, and DOC bioavailability in the cultures. BGE was calculated during the exponential phase as BP / (BP + BR), where BP is the bacterial production rate, estimated from the increase in bacterial biomass, and BR is the bacterial respiration rate, estimated from the increase in DIC concentration [49]. The DOC uptake rate was calculated as BP + BR. DOC bioavailability was calculated as $DOC_L = (DOC_B + DOC_R) / DOC_T$, where DOC_L is the labile DOC to bacteria, DOC_B is the DOC converted into bacterial biomass, DOC_R is the dissolved organic carbon respired by bacteria, and DOC_T is the initial DOC concentration.

Bacterial Uptake of Different DOC Sources. A10-L water sample was collected in an acid-washed and deionized-water-rinsed polyethylene flask from the subsurface (~0.5 m) of Lake Batata in June 2001. The water sample was maintained at a constant temperature (~25°C) until the start of the experiment (~2 h). The experiment was set up in seven treatments and one control ($n = 4$). N-NH_4NO_3 and P-KH_2PO_4 (final concentrations of 50 and 5 μM, respectively) were added to all cultures, except the control. Six extra sources of DOC were added to set up each treatment: extract and leachate of *Oryza glumaepatula*, extract and leachate of a litter sample from the tropical rainforest, algal lysate, and natural humic substances (see below). These were considered to be the main sources of DOC in Lake Batata during the flood pulse. The concentration of carbohydrates and other organic compounds in each extra DOC source were used as indicators of the quality of DOC source to bacterial growth.

The control and the NP treatment were set up as described above. The cultures of the other treatments were composed of the water sample from Lake Batata and of stock solutions of the extra DOC sources, both filtered through 0.2-μm pore size filters (VacuCap filters, Gelman Science). The final concentration of extra DOC added to each treatment was approximately 10 mg C L^{-1}. An inoculum was added to each culture. The cultures, composed of 90% of 0.2 μm filtrate and 10% of inoculum, were poured into 200-mL flasks and into 60-mL BOD flasks as described above. The whole process took 2–3 h to complete.

Bacterial growth was accomplished in all cultures through changes in bacterial abundance, biomass, and respiration. Bacterial abundance and biomass were sampled at 0, 24, 48, 72, 96, and 120 h of growth. Bacterial respiration was estimated after 96 h of incubation. BGE and DOC bioavailability are described above.

DOC Extractions. Leaves and stems of *O. glumaepatula* Steud (wild rice) were collected from Lake Batata in March 1999. A litter sample of the surrounding Amazonian rainforest was collected in March 2001. Both samples were washed with tap water, to remove periphyton, mud, and associated macrofauna, oven-dried at 40°C, and stocked at room temperature until the experiment.

The DOC of *O. glumepatula* and forest litter samples were (1) leached into Milli-Q water and (2) extracted in alcohol. In the leaching process, the samples were soaked in Milli-Q water in the dark, at approximately 4°C, for 48 h. Although the leaching process was not sterile, bacterial numbers were less than 5×10^7 cells L^{-1}, which we assume had minor impact on the amount and quality of plant leachates. DOC leached from the samples was prefiltered through a 0.7-μm pore size filter (GF/F filter, Whatman) and diluted in Milli-Q water to a 100 mg C L^{-1} stock solution. During alcohol extraction, the samples were ground in a mill (10 g, final weight) and the DOC was four-times extracted with 50 mL of a 9:1 dichloromethane/methanol solution in an ultrasound bath. The combined extracts of each sample were concentrated by rotary evaporation, transferred to vials, and derivatized with diazomethane to esterify the alkanoic acids.

The algal lysate was prepared from cultures of *Chlorella* sp. and *Ankistodesmus* sp. grown in Z8 medium until stationary growth. The cultures were placed in 15-mL centrifugation tubes and spun at 1000 rpm for 20 min. The overwater was discarded and the pellets were

Table 1. Some abiotic and bacterial parameters in high-water period (June 2001) and low-water period (December 2002) at Lake Batata

Period	Temp. (°C)	pH	Depth (m)	Secchi (m)	DOC (mM)	Total N (μM)	Total P (μM)	Chlor-a (μg L^{-1})	Bacterial abundance (10^9 cells L^{-1})	Bacterial production (μM C h^{-1})
High water	28.1	6.19	8.5	1.1	0.71	43.6	0.05	1.72	1.31	0.026
Low water	32.3	6.36	1.9	0.5	0.42	40.0	1.46	31.60	2.61	0.107

resuspended in 10 mL NaCl 0.9%. This procedure was repeated four times to prevent possible contamination with nutrients from the cultures. The pellets were resuspended in Milli-Q water and the cells were thermically fractured (freezing at −20°C and quick heating to ~36°C). This procedure was repeated three times. Finally, the tubes were centrifuged at 8000 rpm for 30 min; the overwater was removed and combined in a tube, and considered to be algal lysate. The algal lysate was filtered through 0.7-μm pore size filters (GF/F filter, Whatman) and diluted to 100 mg C L^{-1} (stock concentration).

The humic substances were extracted from a water sample of a highly humic lagoon located in Rio de Janeiro State, Brazil (Comprida lagoon). For further information about Comprida lagoon, see Farjalla *et al.* [20]. The humic substances were extracted in XAD-8 resin, according to Bussmann [10]. The XAD-8 resin was intensively washed with methanol before packing in an ion exchange column. Approximately 5 L of water was collected in a plastic flask, previously washed with HCl 10%. The water sample was acidified to pH 2.0 with 6 N HCl. Extraction was performed by passing the acidified water sample from Comprida lagoon through the column. The column was washed with 0.01 N HCl to remove the salts, and humic substances were eluted in 200 mL of a 0.1 M NaOH solution. Finally, pH was adjusted to 7.0. The humic substances solution was filtered through 0.7-μm pore size filters (GF/F filter, Whatman) and diluted in 100 mg C L^{-1} (stock concentration).

Analytical Procedures. Total nitrogen concentrations were measured by digestion at 320°C and distillation, according to the procedure described Mackereth *et al.* [37]. Total phosphorus measurements were carried out by autoclaving the water samples with the subsequent formation of ammonium molybdate through phosphorus reaction [26]. Chlorophyll-*a* was extracted with ethanol 90% and measured at 665 nm in a spectrophotometer [39].

Bacterial density was ascertained via the method proposed by Hobbie *et al.* [29]. Samples stained with acridine orange (final concentration, 0.005%) were filtered through black polycarbonate filters (0.2 μm Nucleopore® filter), and bacteria were counted in a epifluorescence microscope (Axiovert Zeiss Universal) at 1600-fold magnification. At least 300 bacteria or 30 fields were counted in each filter. The controls were prepared with sterilized water.

Bacterial respiration was evaluated from the increase in DIC concentration in the cultures. DIC samples were fixed with $ZnCL_2$ (final concentration, 0.01%) and maintained at 4°C. DOC samples were fixed with 6 N HCl (final pH = 2.0) and maintained at −20°C. DOC and DIC measurements were performed in a Shimadzu TOC-5000 Carbon Analyzer. Total carbon is measured as CO_2, via high-temperature oxidation in a platinum catalyzer. DOC is estimated by subtracting DIC from total carbon. DIC was measured through phosphoric acid (25%) addition in an infrared sensor. At least three measurements of DOC and DIC were made for each sample, and the coefficient of variation was always less than 2%.

Polymeric carbohydrates of each extra DOC source were analyzed after hydrolysis [34] by high-performance liquid chromatography coupled to pulse amperometric detection (PAD-HPLC). PAD-HPLC analysis was performed in a Dionex™ DX500. PA-10 (Dionex™) anion-exchange analytical column (4 × 250 mm), fitted with a corresponding guard column (4 × 50 mm), was used to separate the monosaccharides. Solutions of 18 mM NaOH and 200 mM NaOH were used to separate and recover carbohydrates, respectively. All the samples were desalted in Bio-Rad™ ionic exchange resin (AG2X8, anion exchange; AG50W, cation exchange).

Carbon compounds extracted from *O. glumaepatula* and forest litter samples in alcohol were analyzed by gas chromatography-mass spectrometry (GC/MS). Analyses were performed on a Hewlett-Packard Model 5890A gas

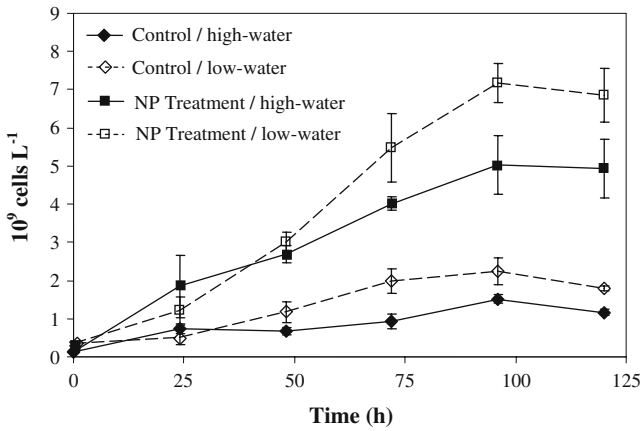

Figure 1. Bacterial growth in unamended (control) and NP-enriched (NP treatment) cultures at high- and low-water periods of the flood pulse in Lake Batata (n = 4, bars = SD).

Table 2. Bacterial abundance and ratios of bacterial production, bacterial respiration, bacterial growth efficiency (BGE), DOC removal rate, and DOC bioavailability in batch cultures in high-water and low-water periods at Lake Batata [n = 4, mean (±SD)]

	Maximum bacterial abundance (10^9 cells L^{-1})	*Bacterial production rate (μM C h^{-1})*	*Bacterial respiration rate (μM C h^{-1})*	*DOC removal rate (μM C h^{-1})*	*DOC bioavailability (%)*	*BGE (%)*
High water						
Control	1.52 (0.11)	0.042 (0.003)	0.165 (0.026)	0.207 (0.008)	2.8 (0.0)	20.3 (0.6)
NP treatment	5.03 (0.76)	0.149 (0.024)	0.269 (0.043)	0.418 (0.020)	5.7 (0.3)	35.4 (1.1)
Low water						
Control	2.23 (0.37)	0.053 (0.007)	0.144 (0.047)	0.197 (0.010)	4.5 (0.1)	26.7 (0.9)
NP treatment	7.17 (0.50)	0.160 (0.028)	0.235 (0.099)	0.395 (0.026)	8.9 (0.6)	40.2 (1.5)

chromatograph, coupled to a Hewlett-Packard HP5972 atomic mass selective detector. Electron ionization of 70 eV and linear scanning over the mass range of 50–600 Da were used. Helium was used as carrier gas. Gas chromatographic (GC) analyses were carried out using a DB-5 fused silica capillary column (30 m × 0.25 mm i.d., d_f = 0.25 μm; J&W Scientific, Folsom, CA, USA), splitless injection. Oven temperature varied from 60 to 300°C at 6°C min^{-1}, held at 300°C for 20 min. Structural assignments were obtained by analyzing each spectrum in the Wiley 275 standard reference of mass spectral and in other specific references [1, 8, 41, 45].

Statistical Analyses. Differences in bacterial growth and in rates of bacterial production, respiration, BGE, and DOC bioavailability in the first experiment were tested with a two-way ANOVA in which flood pulse period and nutrient addition were the independent variables. In the second experiment, differences in bacterial growth curves and in rates of bacterial production, respiration, BGE, and DOC bioavailability among all six treatments with extra DOC additions, NP treatment, and control were analyzed with ANOVA and t tests. We used STATISTICA 5.0 software to perform all tests. A probability level of $\alpha = 0.05$ was used throughout to determine statistical significance.

Results

Bacterial Growth and DOC Uptake. The flood pulse influenced bacterial density and secondary production in Lake Batata. The highest depth, light penetration, and DOC concentration were recorded at high-water period, and the highest water temperature and phosphorus and chlorophyll-*a* concentrations were recorded at low-water period (Table 1). *In situ* bacterial abundance and production were also higher at low-water period than at high-water period (Table 1).

All cultures showed a logarithmic phase based on a fast increase in bacterial numbers, and a stationary phase based on a period of stability in bacterial abundance (Fig. 1). Initial bacterial abundances were 0.13 × 10^9 cell L^{-1} at high-water period and 0.34 × 10^9 cell L^{-1} at low-water period. NP enrichment significantly stimulated bacterial growth and DOC uptake at both periods of flood pulse (Table 2, Fig. 1).

Bacterial abundance and production were higher at low-water period than at high-water period (Table 2, Fig. 1). Maximum abundance reached in both control and NP treatment cultures at low-water period was significantly higher than its counterpart at high-water period. The highest bacterial production rates were also observed in low-water period cultures, although differences were not significant. On the other hand, the highest bacterial respiration rates were observed in high-water period cultures, and differences were also not significant. BGE was higher in control and NP treatment cultures from the low-water period compared to those at the high-water period (Table 2).

DOC uptake rates were slightly higher in high-water period bacterial cultures compared to low-water period ones, mainly due to higher bacterial respiration rates in high-water period cultures (Table 2). However, DOC

Table 3. Carbohydrates concentration (mg carbohydrates/100 mg total C) from the main DOC sources to Lake Batata

	O. glumaepatula extract	*Forest extract*	*Humic substances*	*O. glumaepatula leachate*	*Forest leachate*	*Algal lysate*
Fucose	0.01	0.01	0.07	0.12	0.12	0.12
Ramnose	0.01	0.06	0.22	0.50	1.55	0.57
Arabinose	0.01	0.06	0.20	0.65	0.86	1.10
Galactose	0.02	0.12	0.30	6.26	2.73	9.50
Glucose	0.09	0.40	0.64	20.02	6.43	22.30
Manose/Xylose	0.04	0.12	0.35	2.51	2.21	4.11
Fructose	0.12	0.23	0.25	7.68	4.43	11.80
Total	0.30	0.99	2.04	37.73	18.34	49.40

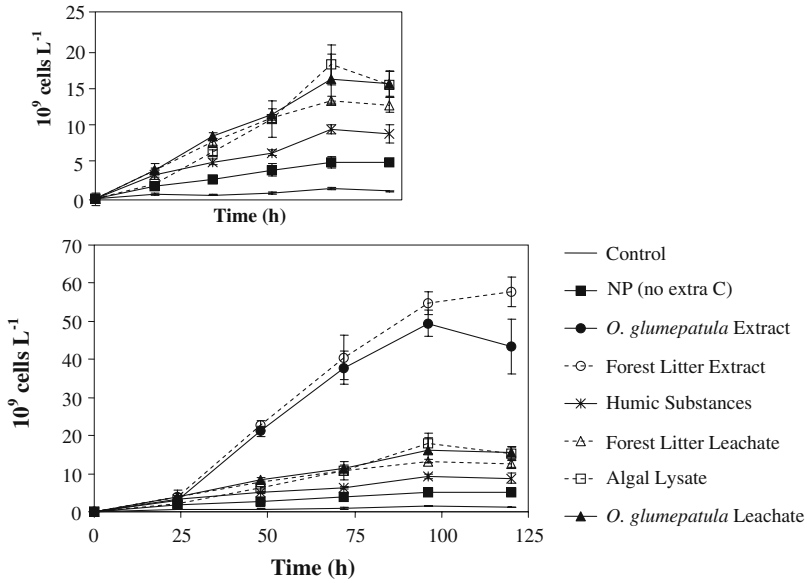

Figure 2. Bacterial growth in unamended (control), NP-enriched (NP treatment), and NP-DOC-enriched cultures at high-water period in Lake Batata. The upper graphic refers to an amplification of the bottom part of the lower graphic (n = 4, bars = SD).

bioavailability was higher in low-water period bacterial cultures than in high-water period ones. DOC bioavailability varied from 2.8 to 5.7% at high-water period to 4.5 to 8.9% at low-water period.

Bacterial Growth and DOC Uptake of Different DOC Sources. The highest concentration of carbohydrates was found in the algal lysate, followed by *O. glumaepatula* leachate, forest litter leachate, humic substances solution, and extracts of forest litter and *O. glumaepatula* (Table 3). Of the analyzed carbohydrates, glucose showed the highest concentration of all DOC sources, followed by fructose (except in the humic substances solution) and galactose, in algal lysate and leachates, and manose/xylose, in extracts.

Some less hydrophilic compounds were obtained in the alcohol extracts. These compounds were analyzed by GC/MS and represent about 5% of the total DOC in extracts of forest litter and *O. glumaepatula*. The terpenes (cyclic compounds), such as α-copaene, danielic acid, stigmasterol, and ursenol, were especially present in the forest litter extract. We also found acyclic isoprenoids, from 12 to 32 atoms of carbon ($C_{12}-C_{32}$), in both extracts. The acyclic isoprenoids of lower molecular weight ($C_{14}-C_{18}$) were the main compounds observed in the *O. glumaepatula* extract.

All bacterial cultures showed a logarithmic growth phase up to 72–96 h and a stationary growth phase up to 120 h (Fig. 2). Treatments with additions of extra sources of DOC showed higher bacterial abundances and production rates than the control and NP treatment. Bacterial abundances and production rates were significantly higher in treatments with additions of forest litter and *O. glumaepatula* extracts than in other treatments (Table 4). Maximum bacterial abundance and production rate observed in the treatment with addition of algal lysate were similar to maximum bacterial abundance and production rate observed in the treatment with addition of *O. glumaepatula* leachate, and those were significantly higher than their counterparts in other treatments. The maximum bacterial abundance and production rate observed in the treatment with addition of forest litter

Table 4. Bacterial abundance, bacterial production rate, bacterial respiration rate, bacterial growth efficiency (BGE), and DOC bioavailability in the control and in the treatments with NP additions and several DOC sources [n = 4, mean (±SD)]

	Maximum bacterial abundance (10^9 cells L^{-1})	*Bacterial production rate (μM C h^{-1})*	*Bacterial respiration rate (μM C h^{-1})*	*DOC bioavailability (%)*	*BGE (%)*
Control	1.52 (0.11)	0.04 (0.00)	0.17 (0.03)	2.8 (0.0)	20.3 (0.6)
NP treatment	5.03 (0.76)	0.15 (0.02)	0.27 (0.04)	5.7 (0.3)	35.4 (1.1)
O. glumaepatula extract	49.46 (3.43)	1.50 (0.11)	3.28 (0.37)	29.7 (2.4)	31.4 (2.3)
Forest extract	57.77 (3.82)	1.66 (0.09)	3.34 (0.30)	31.1 (2.1)	33.0 (2.0)
Humic substances	9.38 (0.63)	0.28 (0.02)	1.21 (0.12)	9.3 (0.7)	18.9 (0.9)
Forest leachate	13.24 (0.53)	0.40 (0.02)	1.16 (0.09)	9.7 (0.6)	25.5 (1.3)
Algal lysate	17.95 (2.67)	0.54 (0.08)	1.64 (0.21)	15.2 (1.6)	24.8 (1.9)
O. glumeapatula leachate	16.13 (3.22)	0.49 (0.10)	1.33 (0.12)	11.3 (1.0)	26.8 (1.6)

leachate was significantly higher than those in the treatment involving addition of humic substances.

Bacterial respiration rate was also significantly higher in treatments with the addition of extra sources of carbon than in the control and NP treatment (Table 4). Respiration rates in treatments with addition of *O. glumaepatula* and forest litter extracts were significantly higher than those in treatments with algal lysate, leachates from *O. glumaepatula* and forest litter, and humic substances.

BGE and DOC bioavailability were estimated based on the rates of bacterial production and respiration. The highest BGE was observed in the NP treatment, followed by treatments with addition of extracts of *O. glumaepatula* and forest litter. Treatments with addition of algal lysate and leachates of *O. glumaepatula* and forest litter showed similar BGE rates, but higher than that in the treatment with addition of humic substances (Table 4). DOC bioavailabilty was also higher in treatments with addition of extracts of forest litter and *O. glumaepatula*, followed by treatments with addition of algal lysate, leachates from *O. glumaepatula* and forest litter, and humic substances (Table 4).

Discussion

Microcosms are small ecosystems often used to manipulate an individual environmental factor and to explore the role that factor plays in structuring ecosystems [25]. Advantages of using microcosms include control over environmental variables, ease replication, and power to manipulate the parameters and treatments under investigation [17, 25]. Limitations and disadvantages include restricted space, uncoupling between input and output process, and oversimplification [11]. We used bacterial microcosms to test the bacterial growth and DOC uptake at a clear-water Amazonian lake. Because microcosms were set up with diluted bacterial cultures, the bacteria were grown in the absence of predators, and the rates of bacterial growth and DOC removal were obtained during the exponential bacterial growth phase. Conclusions of this study must be regarded as the potential rates of bacterial growth and DOC uptake on DOC pool at Lake Batata or on natural DOC sources to Lake Batata. Therefore, we may be overestimating the rates of bacterial growth and DOC uptake under natural conditions. However, comparison among treatments and controls is valid because we used the same approach in all experiments.

In microcosms without predators, both abundance and activity of bacteria are regulated by bottom-up factors, such as the availability of nutrients, or environmental characteristics, such as temperature and pH. All cultures were maintained at constant temperature and, when necessary, the pH of extra carbon stocks was regulated to 7.0. Therefore, we assumed that differences in bacterial abundance and activity are exclusively related to nutrient availability.

To increase in size and divide, bacteria must assimilate organic carbon and other macronutrients, mainly nitrogen and phosphorus. Low concentrations of nitrogen and phosphorus are considered to be the main limiting factors of bacterial growth and DOC uptake in several aquatic ecosystems [20, 38, 47, 52]. Concentrations of phosphorus were low at both flood pulse periods in Lake Batata, and there was an imbalance between natural concentrations of carbon, nitrogen, and phosphorus in the water column (Table 1) and the demand of C, N, and P for bacterial growth (bacterial molar ratio of 50:10:1, according to Fagerbakke *et al.* [19]). Therefore, as there was no nutrient addition to the controls and the bacteria grew under natural conditions found in the lake, the lowest rates of bacterial production and DOC uptake in the controls, in relation to NP cultures, must be related to the lowest phosphorus concentrations. Furthermore, the highest bacterial production and DOC uptake in low-water period control cultures in relation to high-water period control cultures may be related to higher phosphorus concentrations during the low-water period in Lake Batata.

To all the other treatments, we added 50 and 5 μM of N and P, respectively, and we added approximately 10 mg C L^{-1} to treatments with extra sources of DOC. The C/N/P ratio in the cultures was switched from 14,200:852:1, in the water from Lake Batata at high-water period (control), to 141:19:1 in the NP treatment and to 306:19:1 in other treatments. Assuming a C/N/P ratio in a bacterial cell of 50:10:1 [19] and an average growth efficiency of 30% (Tables 2–4), bacteria might be phosphorus-limited in all the cultures with extra additions of dissolved organic matter (DOM), despite the NP additions. However, based on the rates of DOC uptake by bacteria in the cultures (Tables 2–4) and assuming the 50:10:1 ratio, we found that only 0.3–3.2 μM of P was incorporated into bacterial biomass, representing 6–63% of the P found in the cultures. We conclude that in these growth cultures, characterized by quick bacterial growth in a short period of time, bacterial abundance and activity are limited by the quality of the DOC.

DOC is supplied to aquatic ecosystems from both internal and external sources, and differences in origin lead to significant differences in bacterial metabolism [23]. Large rivers and lakes of the Amazonian drainage basin are subject to annual changes in the water level that result in considerable changes in chemical, physical–chemical, and biological characteristics [33]. During periods of flooding, the water column of Amazonian aquatic ecosystems advances on the surrounding tropical forest, receiving allochthonous nutrients and organic matter and creating new habitats for aquatic species. According to Roland and Esteves [44], the area of Lake

Batata increases from 18.04 km^2 at low-water period to 30.17 km^2 at high-water period, mainly attributable to an increase in the water volume of the lake due to the flood. Phytoplanktonic primary production is low and the large stands of aquatic macrophytes are absent during the high-water period in Lake Batata [7]. Even though exchanges between aquatic and terrestrial ecosystems are intensified during the high-water period, there is a decrease in the role of cycling processes within the aquatic ecosystem. We suggest that most DOC is composed of allochthonous humic substances derived from the decomposition of dead organic matter deposited on the forest floor during the dry period.

Nutrient concentration and organism density are higher in the low-water period when nutrient exchange between the sediment and the water column is also intensified. During the low-water period, there is an increase in chlorophyll-*a* concentration as well as the establishment and expansion of stands of aquatic macrophytes, basically wild rice (*O. glumaepatula*), in Lake Batata (Table 1, [7]). We suggest that the autochthonous primary producers significantly contribute to the concentration and composition of DOC during the low-water period in Lake Batata.

DOC bioavailability is directly related to the diagenetic state of carbon compounds. DOC previously degraded by microbial activity would be less susceptible to further bacterial attack, whereas younger molecules, diagenetically unaltered, would be more available for bacterial growth [3]. Allochthonous humic substances are usually characterized by high C/N molar ratio related to the lignin component and to the intense diagenetic processes during transport from the forest soil to aquatic ecosystems [2, 28]. On the other hand, both phytoplankton and aquatic macrophytes are important sources of autochthonous carbon compounds, such as sugars and amino acids, that are labile compounds readily assimilated by bacteria for growth [9, 54]. Therefore, the highest bacterial growth observed at the low-water period is probably related to the extra influx of fresh, diageneticaly unaltered organic compounds from autochthonous sources to the pelagic zone.

The highest bacterial growth on autochthonous DOC in relation to allochthonous DOC was observed in other temperate and tropical freshwater ecosystems. Jonsson *et al.* [31] observed that more than 95% of DOC bulk in a Swedish temperate lake is composed of allochthonous humic substances, but less than 10% is consumed by planktonic bacteria and the small amount of autochthonous DOC is the main substrate for bacterial growth. Almost 90% of the carbon demand of aquatic bacteria is supported by aquatic macrophytes in Lake Calado, a small turbid lake in the Amazon flooplain, despite the higher contribution of allochthonous DOC (~85%) to DOC bulk [53]. However, Benner *et al.* [6] observed the highest bacterial growth rates at the high-water period in the Amazon River, basically related to the great inflow of allochthonous DOC. The Amazon River is characterized by short residence time and by low phytoplanktonic productivity throughout the year [24]. Moreover, bacterial growth is carbon-limited in the Amazon River [6], and the inflow of allochthonous humic DOC at the high-water period would result in an increase in bacterial activity in this river. Therefore, we postulate that the processes of production, uptake, and export of DOC vary throughout the year in the different Amazonian aquatic ecosystems, and this fact leads to differences in bacterial growth and DOC uptake.

DOC availability is usually estimated indirectly based on bacterial uptake of DOC [16, 50]. Here, we also tried to qualify the main DOC sources of Lake Batata based on the concentration of sugars. Carbohydrates are polar organic compounds that often provide a labile source of carbon to bacteria [35]. Carbohydrate concentration appears to be coupled with bacterial activity [9, 42, 54].

We did not observe a positive correlation between the rates of bacterial production, respiration, and DOC uptake by bacteria, and carbohydrate concentration from different sources of DOC. The highest rates of bacterial growth and DOC uptake were observed in cultures with additions of *O. glumaepatula* and forest litter extracts, which showed the lowest carbohydrate concentrations (Tables 3 and 4). The extracted DOM from *O. glumaepatula* and forest litter, however, showed a greater diversity of terpenes and other relatively complex compounds, which we hypothesized to be less labile to bacterial growth.

DOC found in aquatic ecosystems is a mixture of several organic molecules, each one usually present in low concentrations. Bacteria in aquatic ecosystems must be able to, simultaneously, uptake several substrates for growth or as an energy source [22]. According to Egli [18], the growth kinetics in a mixture of substrates is greater than when only one substrate is available, because different compounds are related to different nutritional (physiological) needs of the microorganisms. Therefore, a greater diversity of relatively labile substrates, each one present in low concentrations, may stimulate bacterial growth in cultures with additions of *O. glumaepatula* and forest litter extracts. We conclude that the diversity of organic substrates might be a better indicator of DOC bulk bioavailability than the concentration of a single class of organic compounds, despite the higher bioavailability of the latter.

Humic substances are also composed of a great diversity of organic compounds, and we could also expect greater bacterial growth in the treatment involving the addition of humic substances. However, the diagenetic processes during transport from the forest soil and from aquatic ecosystems strongly decrease the bioavailability of humic substances. These processes were

negligible in the case of the freshly extracted samples from *O. glumaepatula* and forest litter. Thus, the degree of diagenesis of organic compounds is, together with the diversity, a determinant factor for DOC uptake by planktonic bacteria in natural systems. Other factors not measured here, such as microbial community composition, could also be significant for DOC uptake by planktonic bacteria.

BGE is a useful indicator of metabolic activity of bacterial cells, the fate of incorporated DOC, and ultimately of the role of planktonic bacteria in aquatic ecosystems [14, 49]. BGE was greater at the low-water period of flood pulse in Lake Batata (Table 2), when the concentration of N and P was higher, and there was probably a greater amount of labile DOC. Addition of N and P in the cultures also stimulated the bacterial production more than the bacterial respiration, resulting in an increase in BGE rates (Table 2). However, despite the increase in bacterial production, addition of other sources of DOC also strongly stimulated bacterial respiration and a substantial amount of incorporated DOC was converted into CO_2, thereby lowering BGE rates (Table 4). Therefore, the availability of inorganic nutrients and organic substrates seems to act differently on catabolism and anabolism and, consequently, on BGE rates.

In cultures where bacterial growth is constrained by the supply of inorganic nutrients or organic substrates, an uncoupling between catabolism and anabolism is usually observed [14]. In oxic conditions, bacteria utilize O_2 as the main electron acceptor in catabolic substrate consumption, whereas the incorporation of inorganic nutrients is basically related to the anabolic process. On the other hand, organic substrates are used in both catabolic and anabolic consumption. According to Kirchman [36], bacteria regulate the catabolism of organic substrates to attain the correct intracellular stoichiometry.

Under the natural conditions of Lake Batata, a considerable amount of incorporated DOC is oxidized through respiration, and the energy generated is used in the maintenance of bacterial cells, due to the lower availability of inorganic nutrients in relation to organic substrates (high CNP ratios). By increasing the NP concentrations, bacteria change the fate of DOC incorporated in the cell and there is an enhancement of biosynthetic processes, and consequently an increase in BGE rates. Therefore, at the high-water period, a large amount of allochthonous DOC incorporated by bacteria is oxidized into CO_2, and bacteria seem to be basically a sink of DOC. At the low-water period, higher concentrations of N and P stimulate bacterial production, a greater amount of DOC is incorporated in the bacterial cell, and DOC may flux more efficiently into the microbial food chain.

The regulation of BGE by CNP stoichiometry in the water was also observed in other studies (see review in [15]). Cimbleris and Kalff [12] observed a positive correlation between bacterial respiration rates and the C/N and C/P ratios across 14 temperate lakes, suggesting lower BGE rates in oligotrophic ecosystems. In addition, Farjalla *et al.* [20] observed a substantial increase in BGE in cultures from oligotrophic coastal lagoons enriched with N and P. In cultures with extra addition of organic substrates, and consequently an increase in CNP ratios, we observed a decrease in BGE rates, mainly as a result of high bacterial respiration rates, indicating that bacteria consumed the extra DOC "luxuriously" in the catabolism, independently of the quality of the extra DOC source.

We conclude that bacterial growth and DOC uptake vary throughout the flood pulse in clear-water Amazonian ecosystems, due to changes in nutrient concentration and in DOC composition. Nutrient concentration and DOC composition reflect directly on the fate of DOC in the bacterial cell in clear-water Amazonian ecosystems.

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