Complementary pathways of dissolved organic carbon removal pathways in clear-water Amazonian ecosystems: photochemical degradation and bacterial uptake

André M. Amado[1], Vinicius F. Farjalla[1], Francisco de A. Esteves[1], Reinaldo L. Bozelli[1], Fábio Roland[2] & Alex Enrich-Prast[1]

[1]Laboratório de Limnologia, Departamento de Ecologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; and [2]Laboratório de Ecologia Aquática, Departamento de Biologia, Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brazil

Correspondence: Vinicius F. Farjalla, Laboratório de Limnologia, Depto. de Ecologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, PO Box 68020, Zip Code: 21941-590, Ilha do Fundão, Rio de Janeiro, Brazil. Tel./fax: +55 21 22704950; e-mail: farjalla@biologia.ufrj.br

Received 14 April 2005; revised 12 July 2005; accepted 2 August 2005.
First published online 8 February 2006.

doi:10.1111/j.1574-6941.2006.00028.x

Editor: Riks Laanbroek

Keywords
photochemical degradation; planktonic bacteria; dissolved organic carbon; CO_2 production; hydrological variability; Amazon.

Abstract

Dissolved organic carbon (DOC) photochemical reactions establish important links between DOC and planktonic bacteria. We hypothesize that seasonal changes in DOC quality, related to the flood pulse, drive the effects of light–DOC interactions on uptake by planktonic bacteria uptake in clear-water Amazonian ecosystems. Water samples from two ecosystems (one lake and one stream) were incubated in sunlight during different hydrological periods and were then exposed to bacterial degradation. Photochemical and bacterial degradation were driven by seasonal DOC inputs. Bacterial mineralization was the main degradation pathway of autochthonous DOC in the lake, while allochthonous DOC was more available for photochemical oxidation. We suggest that sunlight enhances the bacterial uptake of refractory DOC but does not alter uptake of labile forms. We also observed a positive relationship between sunlight and bacterial degradation of DOC, instead of competition. We conclude that photochemical reactions and bacteria complementarily degrade the different sources of DOC during the flood pulse in Amazonian clear-water aquatic ecosystems.

Introduction

Dissolved organic carbon (DOC) represents one of the largest carbon pools on the planet, and its pathway through bacteria is one of the greatest organic matter fluxes in aquatic systems (Farrington, 1992; Cole, 1999). Photochemical transformations of DOC play an important role in carbon mineralization in aquatic ecosystems (Graneli *et al.*, 1998). The energy from sunlight decomposes DOC molecules, producing dissolved inorganic carbon (DIC), low molecular weight organic compounds, hydrogen peroxide, and other substances (Scully *et al.*, 1996; Kieber *et al.*, 1997; Moran & Zepp, 1997). Among the photochemically produced DOC molecules, low molecular weight compounds, such as carboxylic acids are particularly susceptible to bacterial consumption (Kieber *et al.*, 1989; Bertilsson & Tranvik, 1998). Thus, the energy flux through the bacterial community can be photochemically enhanced (Lindell *et al.*, 1995), but it can also be inhibited by the photoproduction of compounds toxic to bacteria (Lund & Hongve, 1994).

Dissolved organic carbon is classified mainly according to its availability as a bacterial substrate. For instance, algal exudates are considered to be more labile than humic compounds, which are considered to be less labile or more refractory to bacterial growth (Hobbie, 1988). Susceptibility of DOC to photochemical degradation is very dependent on the absorptive characteristics of light energy (Bertilsson & Tranvik, 2000). Humic terrestrial material absorbs significant amounts of sunlight energy, enhancing photochemical oxidation rates (Graneli *et al.*, 1998), but photochemical mineralization is observed even in lakes with high levels of algal DOC (Bertilsson & Tranvik, 2000). It has been suggested that the origin of DOC is the main factor determining its uptake by planktonic bacteria after exposure to sunlight or UV radiation exposure. Bulk DOC with a high proportion of humic compounds is widely consumed by bacteria after sunlight or ultraviolet radiation exposure. This results in a positive correlation between photochemical degradation of DOC and bacterial DOC uptake in humic ecosystems. In bulk DOC, composed mainly of algal exudates, the

light energy reduces DOC uptake by bacteria. In this case, a negative correlation is found between photodegradation of DOC and its uptake by bacteria. Obernosterer *et al.* (2001) suggested that photochemical reactions would reduce DOC availability; in other words, refractory compounds would become more labile after light exposure and thus available for bacterial growth, while labile compounds would become refractory.

The Amazonian aquatic ecosystems are characterized by a large seasonal variation in water level (Sioli, 1984), which results in chemical, physicochemical and biological changes during the year (Bozelli *et al.*, 2000). The flood pulse also changes the morphology of the ecosystem, and determines the intensity of the interaction between aquatic and forest ecosystems. According to Roland & Esteves (1993), the main nutrient sources in Lake Batata, a clear-water Amazonian lake, change during flooding, when nutrients originate mostly from forest leaching. In the low-water period, nutrient release from the sediment stimulates phytoplanktonic production, which increases bacterial DOC availability due to the presence of algal exudates (Anesio *et al.*, 1997; Farjalla *et al.*, in press). Source of DOC and its bioavailability to bacteria thus change according to the variation in water level.

An important question to address is whether seasonal changes in the quality of DOC affect photochemical mineralization and bacterial uptake after photodegradation processes. We hypothesized that the seasonal changes in DOC quality related to the flood pulse would drive the effects of the light–DOC interaction on planktonic bacterial uptake in two clear-water Amazonian aquatic ecosystems. The aims of this research were (1) to evaluate the seasonal photochemical oxidation of DOC; (2) to evaluate the effects of photochemical changes in DOC on bacterial uptake; and (3) to compare the photochemical and bacterial mineralization processes in Amazonian clear-water ecosystems.

Materials and methods

Study sites

This study was performed in Lake Batata and Caranã stream, both located in the Municipality of Oriximiná, State of Pará, Brazil (10°27′S; 56°22′W). These ecosystems are contained within the watershed of the Trombetas River, which is a tributary of the Amazon River. According to the classification of aquatic Amazonian ecosystems proposed by Sioli (1984), Lake Batata and Caranã stream are classified as clear-water Amazonian ecosystems, because of low concentrations of nutrients and suspended material.

Four hydrological periods can be distinguished in Lake Batata based on the flood pulse: drawdown period, between August and October; low-water period, between November and January; filling-up period, between February and April; and high-water period, between May and July. The area of Lake Batata varies from 18.04 to 30.17 km^2, and the mean depth varies from 2.19 to 8.89 m at the low- and high-water periods, respectively (Bozelli *et al.*, 2000). During the high-water period, the lake usually floods the surrounding forest, enhancing the interaction between these ecosystems. During the low-water period, however, the lake-water column retreats, reducing the interaction with the forest.

Caranã stream is a small shallow ecosystem within the tropical forest. Its flood pulse is less marked but is seasonally regulated by rains. We therefore discern two marked hydrological seasons in Caranã stream: the rainy period, between January and June, with the maximum in March; and the dry period, between July and December, with the maximum in September. Thus, the strongest link between the stream and the forest is in March, while the weakest link is in September.

Sampling and experimental design

Sampling was performed in each ecosystem according to the hydrological cycle described in the previous subsection. Water samples were collected from Lake Batata in September 2002 (drawdown period), December 2002 (low-water period), March 2003 (filling-up period) and June 2003 (high-water period). For Caranã stream, water samples were collected in September 2002 (dry season) and March 2003 (rainy season). All samples were collected from the subsurface and put into acid-rinsed (HCl 10%) water-washed polyethylene bottles. In the laboratory, samples were prefiltered through 0.7-μm glass-fiber filters (GF75, Advantec NFS Inc., Dublin, CA). These filters were frozen for further chlorophyll-*a* concentration determination.

The experimental design described below was performed in both ecosystems. Bacteria and other microorganisms were excluded from water samples by filtration through 0.2-μm terile membrane filters (VacuCap®, Gelman Sciences, East Hills, NY) directly into eight sterile UV-transparent culture bags (*c.* 100 mL; Whril Pak, Nasco, Fort Atkinson, WI) and fourteen 45-mL quartz tubes with glass stoppers. Four bags and six tubes were covered with aluminum foil as controls. The culture bags were used only for photochemical experiments, while the quartz tubes were used for incubation of bacteria following photochemical exposure. Half of the tubes (seven for each ecosystem, three being in the dark) were used to follow bacterial growth, while the remainder were used to follow bacterial respiration. All bags and tubes were carefully sealed and incubated in a water bath under sunlight exposure for 6 h around noon (from 9 : 00 h to 15 : 00 h). The temperature of the water bath was kept at *c.* 29 °C. The total radiation during incubation (photosynthetically active radiation (PAR) + UV-A + UV-B; from 280 to 800 nm) was measured each hour using a radiometer (IL 1400) equipped with three different sensors to cover the

wavelengths described above. The total radiation incident on samples was estimated by integrating all values measured during each incubation. Initial DOC samples were poured into dark glass flasks, acidified to pH 2.0, and frozen. Initial samples were also kept refrigerated in plastic vials for absorbance analyses (250, 365 and 430 nm). After the sunlight incubations, aliquots were collected from the bags for analysis of dissolved inorganic carbon (DIC). DIC was fixed with $HgCl_2$ (0.01%, final concentration) and kept refrigerated until analysis. Samples were also kept refrigerated in plastic vials for measurement of absorbance (250, 365 and 430 nm). All analyses were done within 2 weeks of experiments.

Bacterial batch cultures were set up in tubes by inoculating bacteria (10%, final concentration) into 0.2-μm filtered water. Nitrogen and phosphorous were also added (50 μM $N\text{-}NH_4NO_3$ and 5 μM $P\text{-}KH_2PO_4$, final concentrations, respectively). The nutrient addition procedures were taken to avoid the nitrogen or phosphorous limitation previously described the area (Farjalla *et al.*, 2002). Bacteria inocula were prepared by filtering the water sample through 0.7 μm (GF75, Advantec) to remove bacterivores. Two treatments were set up for each ecosystem: (1) bacterial cultures grown in DOC exposed to sunlight (exposed cultures); and (2) bacterial cultures grown in non-exposed DOC (control cultures). The cultures were kept in the dark for 120 h at a constant temperature (*c.* 26 °C). Aliquots for bacterial density and biomass measurements were taken at 0, 48, 72, 96 and 120 h, and fixed with formaline (3.7%, final concentration). Bacterial respiration was estimated by DIC accumulation in the tubes after incubation for 96 h. We assumed bacterial respiration to be linear for calculations of further bacterial parameters (Farjalla *et al.*, unpublished data). The logistics in the Amazon did not allow daily bacterial respiration measurements.

Analytical methods and calculations

DIC and DOC analyses were performed in a Total Carbon Analyzer (TOC 5000, Shimadzu Co., Kyoto, Japan). At least three replicate injections were made for each sample, which resulted in a co-efficient of variation (CV) lower than 2%. CO_2 photoproduction rates were calculated from the difference between DIC concentrations in exposed samples and controls. The absorbances at 250, 365 and 430 nm were used for bulk DOC characterization, and were measured using a Beckman DU 80 spectrophotometer (Fullerton, CA) in a quartz cuvette. The 250/365 nm ratio provides an estimate of the proportion of low-molecular-weight to high-molecular-weight organic compounds in bulk DOC (Strome & Miller, 1978). The absorbance at 430 nm was used as an estimate of the water colour (Strome & Miller, 1978). Finally, the chlorophyll-*a* concentration was determined by the alcohol (70%) extraction method (Nush & Palme, 1975).

Bacterial abundance was measured using a Becton Dickinson FACSort flow-cytometer, as proposed by delGiorgio *et al.* (1996). Syto 13 stain (50 μM, final concentration, Molecular Probes, Carlsbad, CA) and Fluoresbrite™ Carboxy YG Microspheres ($\varnothing = 1.58$ μm, *c.* 3×10^5 mL^{-1} final concentration, Poly-sciences, Warrington, PA) were added to 1 mL sub-samples. The cytometer was controlled by the CellQuest 1.2 software. Bacterial cells and microspheres were separated in a log–log scattergram of green fluorescence intensity (FL1) and side scatter (SSC). Samples were run for 1 min, and bacterial concentration was calculated using the microspheres as an internal standard. Bacterial biomass was estimated using a conversion factor of 35 fg C $cell^{-1}$, as suggested by Theil-Nielsen & Sondergaard (1998).

We calculated the maximum bacterial abundance (MBA) achieved during incubation, the bacterial production rate (BP), bacterial respiration rate (BR), bacterial DOC removal rate (DOC_{REM}), DOC bioavailability (DOC_L) and bacterial growth efficiency (BGE). BP was estimated as the rate of bacterial biomass accumulation during the exponential growth phase. BR was measured by DIC accumulation after incubation for 96 h. DOC_{REM} was calculated as the sum of DOC incorporated into biomass and that respired by bacteria (BP + BR). DOC_L was estimated as the rate of DOC removed (DOC_{REM}) from the total DOC. BGE was calculated as the proportion of DOC_{REM} that was incorporated into the biomass (BP/DOC_{REM}) after incubation for 48 h. We calculated the potential DOC photomineralization rate (DOC_P) during four days to be comparable to DOC_{REM} by bacteria. We consider this to measure the potential rate because it does not take into account the sunlight attenuation in the calculations. We also calculated the total CO_2 production as the sum of the total BR in the water column (per m^2) and the total DOC photomineralization rate (total DOC_P) during four days. For total DOC_P estimations we took into account the sunlight attenuation in the water column from Lake Batata, according to the light extinction equation calculated by Roland & Esteves (1998):

$$\text{depth} = -0.6673\,(\text{Ln}\%\ \text{radiation}) + 3.3401.$$

We assumed that the UV-A radiation profile is similar to the PAR profile in the water column, and did not consider UV-B radiation because of its rapid extinction in the water column (max. 10 cm) (Graneli *et al.*, 1998). We assumed that photo-oxidation occurs for only 6 h per day (incubation time used), although we are almost certainly underestimating the process in the Amazon region.

Statistical analysis

Abiotic changes in exposed samples in relation to the controls were compared by ANOVA and the Tukey *post-hoc*

test. Changes in biological parameters between the two periods were compared by the Student's *t*-test. MBA, BP, BR, DOC_{REM}, DOC_L, BGE and DOC_P were compared between exposed samples and controls using ANOVA and the Tukey *post hoc* test. A significance level of 0.05 was used to determine statistical differences.

Results

Photochemical degradation of DOC in clear-water aquatic ecosystems

Dissolved organic carbon concentration, water colour (absorbance at 430 nm), 250/365 nm ratio, chlorophyll-*a* concentration and bacterial density and biomass changed seasonally in Lake Batata and Caranã stream (Table 1). These parameters were higher in the rainy season than in the dry season in Caranã stream. The highest DOC concentration was observed in the filling-up period, while the highest water colour and 250/365 nm ratio were observed in the high-water period in Lake Batata. Chlorophyll-*a* was not detected in Caranã stream during either rainy or dry periods, while the highest concentrations of chlorophyll-*a* in Lake Batata were observed in the low-water and drawdown periods (Table 1). Bacterial density and biomass were always higher in Lake Batata than in Caranã stream (Table 1). *In situ* bacterial density and biomass were slightly higher in the dry period than in the rainy period in Caranã stream, while in Lake Batata these parameters were at least twice as high in the low-water period than in any other period than the flood pulse (Table 1).

The highest radiation incidence on samples was recorded in September (drawdown and dry periods, in Lake Batata and Caranã stream, respectively), and the lowest radiation incidence was recorded in March, even though this was summertime in the Amazon region (filling period and rainy period in Lake Batata and Caranã stream, respectively; Table 1). DIC concentration in light-exposed samples increased up to 49% (from an initial value of 271 μg DIC L^{-1}) in Caranã stream and up to 26% (from an initial value of 572 μg DIC L^{-1}) in Lake Batata as a result of photochemical degradation. CO_2 photoproduction was detected at low-water and high-water periods in Lake Batata (Table 1). In Caranã stream, CO_2 photoproduction was detected only in the rainy period, even though this was the period that registered the lowest radiation incidence on samples (Table 1). Photochemical changes in absorbance (250, 365 and 430 nm) were detected, but no clear pattern was found (data not shown).

Biological responses to photochemical degradation

Maximum bacterial abundance (MBA) was stimulated by sunlight during drawdown and high-water periods in Lake Batata (Table 2). The increases in bacterial abundance were roughly 51% and 38% during these periods, respectively. MBA was photochemically stimulated by more than 66% in Caranã stream during the dry period (Table 3). Bacterial production (BP) was stimulated only during the drawdown period, while bacterial respiration (BR) was stimulated only during the filling-up period in Lake Batata (Table 2). BP was not stimulated in any period, while BR was stimulated during both dry and rainy periods in Caranã stream (Table 3). In general, bacterial growth efficiency (BGE) was lower in exposed samples than in the controls in both ecosystems (Tables 2 and 3). Photochemical degradation caused reductions in BGE of 46% and 25% during the drawdown and filling-up periods, respectively, in Lake Batata. Photochemical degradation also reduced the BGE by 46% during the rainy period in Caranã stream.

Bacterial DOC removal rates (DOC_{REM}) were photochemically stimulated in both ecosystems during the sampling periods, except during the low-water period in Lake Batata. These stimulations were 42% and 45% during the dry and rainy periods, respectively, in Caranã stream, and 125%, 30% and 33% during the drawdown, filling-up and high-water

Table 1. Dissolved organic carbon (DOC) and chlorophyll-a concentrations, 250/365 nm ratio, water colour (430 nm), bacterial density and biomass, solar radiation and dissolved inorganic carbon (DIC) photoproduction through the sampling period in Lake Batata and Caranã stream

	Lake Batata				Caranã stream	
Period	Drawdown	Low-water	Filling-up	High-water	Dry	Rainy
DOC (mg L^{-1})	4.90	5.04	19.22	5.52	4.05	17.63
Chlorophyll-*a* (μg L^{-1})	11.49	31.60	1.49	3.90	ND	ND
250/365 ratio	4.63	3.79	6.96	7.01	4.11	4.19
Water colour (430 nm)	0.007	0.007	0.003	0.019	0.009	0.021
Bacterial density (10^5 cell L^{-1})	38.38	128.56	41.40	68.89	14.60	13.80
Bacterial biomass (10^{-2} μg C L^{-1})	13.43	45.00	14.49	24.11	5.11	4.83
Radiation (J cm^{-2})	103.3	91.8	62.9	70.0	103.3	62.9
DIC photoproduction (μg L^{-1})	5.0	55.0*	12.8	148.3*	36.0	134.0*

*Significant DIC changes ($p < 0.05$). ND, not detectable.

Table 2. Bacterial parameters estimated for Lake Batata bacterial cultures (exposed and control) at the four sampling periods (drawdown 2002, low-water 2002, filling-up 2003 and high-water 2003)

Lake Batata	MBA 10^8 cells L^{-1} (± SD)	BP μM C h^{-1} (± SD)	BR μM C h^{-1} (± SD)	DOC_{REM} μM C 96 h^{-1} (± SD)	DOC_L (%)	BGE (%)
Drawdown						
Control	5.10 (0.23)^A	0.025 (0.003)^A	0.160 (0.117)	16.78 (9.78)^A	3.99 (2.39)^A	13.35 (11.06)^A
Exposed	7.74 (0.80)^B	0.034 (0.004)^B	0.374 (0.185)	37.85 (15.91)^B	9.01 (3.89)^B	8.42 (4.08)^B
Low-water						
Control	8.43 (1.05)	0.027 (0.003)	0.321 (0.013)	33.31 (1.09)	7.93 (0.26)	7.78 (1.01)
Exposed	9.58 (1.10)	0.024 (0.004)	0.402 (0.183)	40.80 (15.68)	9.71 (3.73)	5.71 (2.09)
Filling-up						
Control	5.25 (0.84)	0.032 (0.005)	0.436 (0.091)^A	42.87 (7.60)^A	2.68 (0.48)^A	6.76 (1.55)^A
Exposed	5.25 (0.47)	0.032 (0.003)	0.581 (0.038)^B	55.95 (3.29)^B	3.49 (0.21)^B	5.18 (0.48)^B
High-water						
Control	5.75 (1.46)^A	0.031 (0.010)	0.058 (0.025)	6.78 (2.11)^A	3.65 (0.46)^A	35.10 (10.07)
Exposed	7.98 (2.35)^B	0.025 (0.007)	0.072 (0.031)	9.03 (2.72)^B	2.41 (0.59)^B	26.05 (10.10)

Capitals (A,B) indicate that control is statistically different from exposed samples. BR was calculated from a 96-h incubation, but BP and BGE are referent to a 48 h incubation.

periods, respectively, in Lake Batata. DOC availability (DOC_L) was also increased by sunlight action during almost all periods for both ecosystems (Tables 2 and 3). Despite being the highest registered in Lake Batata, DOC_L did not change after sunlight exposure only during low-water period (Table 2). It is important to highlight the fact that the sunlight did not cause any changes in bacterial parameters during the low-water period in Lake Batata (Table 2).

DOC removal: bacterial vs. photodegradation

Considering 4 days of photochemical degradation (6 h per day) and BR (24 h per day), the potential CO_2 production (DOC_P+BR) was similar during low- and high-water periods in Lake Batata (Fig. 1a). During the low-water period, however, BR was responsible for more than 54% of the potential CO_2 production, while DOC_P, exclusively, was responsible for less than 46% of the same parameter (Fig. 1a). Conversely, during the high-water period, BR was responsible for less than 10% while DOC_P was responsible for more than 90% of the potential CO_2 production (Fig. 1a).

The potential CO_2 production during the rainy period was more than twice as high as it was during the dry period in Caranã stream (Fig. 1b). During the dry period, the potential CO_2 production in Caranã stream was similar to that in Lake Batata (Figs 1a and b). BR was responsible for about 71% while DOC_P was responsible for about 29% of the potential CO_2 production in that period. During the rainy period, BR was responsible for about 57% and DOC_P for about 43% of the potential CO_2 production in Caranã stream.

The total CO_2 production per square metre estimated in Lake Batata was about 84.3 and 97.2 mm m^{-2} day^{-1} during the low- and high-water periods, respectively (Fig. 2). Therefore, total CO_2 production increased by about 18% between the low- and high-water periods (Fig. 2). The total DOC_P was about 20% of the total CO_2 production, while total BR was responsible for about 80% during the low-water period (Fig. 2). On the other hand, the contribution of total DOC_P to total CO_2 production increased by up to 50%, while the total BR decreased by the same value during the high-water period.

Table 3. Bacterial parameters estimated for Caranã stream bacteria cultures (exposed and control) at the two sampling periods (dry 2002, rainy 2003)

Caranã str.	MBA 10^8 cells L^{-1} (± SD)	BP μM C h^{-1} (± SD)	BR μM C h^{-1} (± SD)	DOC_{REM} μM C 96 h^{-1}	DOC_L (%)	BGE (%)
Dry period						
Control	3.43 (0.31)^A	0.010 (0.001)	0.309 (0.075)^A	30.66 (6.20)^A	9.09 (1.84) ^{A}	3.16 (0.76)
Exposed	5.71 (0.60)^B	0.017 (0.008)	0.438 (0.017)^B	43.70 (1.47)^B	12.95 (0.44) ^{B}	3.77 (1.48)
Rainy period						
Control	3.79 (0.64)	0.023 (0.004)	0.623 (0.073)^A	60.38 (6.03)^A	4.11 (0.41) ^{A}	3.56 (0.59)^A
Exposed	2.99 (0.55)	0.018 (0.003)	0.909 (0.041)^B	87.73 (3.52)^B	5.97 (0.24) ^{B}	1.96 (0.33)^B

BR was calculated during incubation for 96 h, but BP and BGE were calculated during incubation for 48 h.
Capitals (A,B) indicate that control is statistically different from exposed samples.

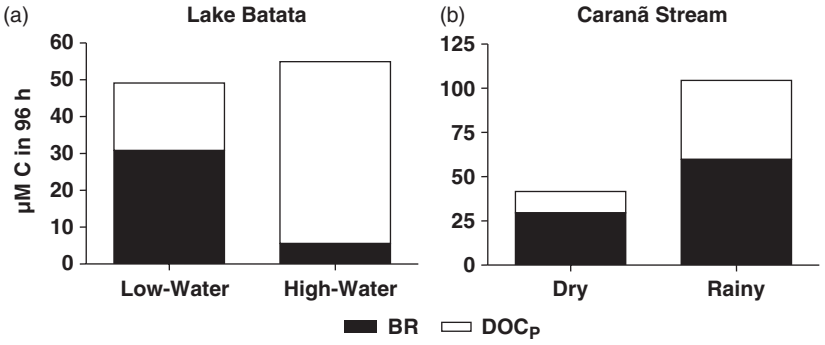

Fig. 1. Potential CO_2 production (BR+DOC_P) in (a) Lake Batata and (b) Caranã stream. All values were estimated for 4 days, 24 h per day for BR and 6 h per day for DOC_P. For this calculation we only considered the subsurface water, where the photochemistry is not altered by attenuation of sunlight in the water. Note the difference in scales.

Discussion

Seasonal DOC dynamics in Amazonian streams and lakes

Most freshwater ecosystems in the world are oversaturated with CO_2. This condition is related to the decomposition of allochthonous material, and shows that aquatic ecosystems are important for terrestrial mineralization of organic matter (Cole *et al.*, 1994; Richey *et al.*, 2002). On the other hand, part of the incoming DOC is considered to be recalcitrant for biological oxidation and might accumulate in aquatic systems (Amon & Benner, 1996). Thus, aquatic ecosystems may also be recognized as sinks for carbon from terrestrial ecosystems. DOC flux from terrestrial to Amazonian aquatic ecosystems occurs through leaching from dead leaves from forest litter during the rainy period. We observed the greatest DOC concentrations in March 2003 during the filling-up and rainy periods in Lake Batata and Caranã stream, respectively (Table 1).

Allochthonous DOC is the only source of DOC source for Caranã stream. Besides presenting no chlorophyll-a during the two periods, DOC concentration in Caranã stream seems to be related to the water colour (Table 1). After input, DOC is carried and degraded throughout the stream, and this concentration declines until the next rainy period. Therefore, the lowest DOC concentration in Caranã stream during the dry period, compared to the rainy period, must be related to the lowest allochthonus DOC input. The dynamics of DOC are, however, different in Lake Batata. During the filling-up and high-water periods, lake water floods the surrounding Amazonian forest and receives large amounts of fresh terrestrial DOC. Autochthonous DOC is also an important source of DOC for Lake Batata, because of phytoplanktonic primary production, mainly during the low-water period. Despite the low variation in DOC concentration among most periods (Table 1), the origin of DOC changes during the year. DOC is mainly autochthonous during the low-water period, and allochthonous during other flood-pulse periods in Lake Batata (Roland & Esteves, 1993; Farjalla *et al.*, in press). The different origins of DOC during different periods should lead to different photochemical and bacterial degradation processes.

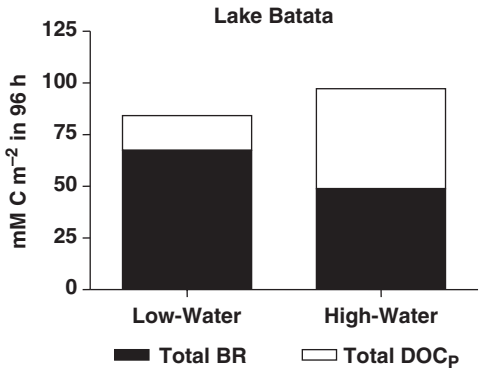

Fig. 2. Total CO_2 production (total BR+total DOC_P) in Lake Batata. These estimates include the effects of sunlight attenuation in the water column in the integration of the total CO_2 photochemical production. BR was considered homogeneous in the entire column. They also include the different mean water column depths in the two periods.

Photochemical changes in bulk DOC and bacterial uptake

Seasonal DOC input in aquatic ecosystems determines susceptibility of DOC to photochemical oxidation (Lindell *et al.*, 2000). We could not detect CO_2 photoproduction in Lake Batata during the drawdown period, despite the highest incidence of sunlight on samples (Table 1). On the other hand, CO_2 photoproduction in Lake Batata during the high-water period is extremely high, despite the fact that these samples were exposed to the lowest incidence of sunlight for the study (Table 1). If we normalize the CO_2 production by the incident radiation on samples (Table 1), CO_2 photoproduction during high-water periods would be even higher, which suggests a greater DOC availability for photochemical oxidation during that period. We suggest that humic DOC is more available to photochemical mineralization than algal DOC. For instance, during the low-water

period, we registered the lowest CO_2 photoproduction, while the highest was recorded for the high-water period in Lake Batata (Table 1).

Photochemical degradation of DOC might stimulate the flow of energy and matter through the aquatic microbial food chain (Lange *et al.*, 2003). Sunlight was able to stimulate bacterial activity through the increase of bacterial DOC removal (DOC_{REM}) rates in all samples, except for the low-water period in Lake Batata. These increases in DOC_{REM} occur as a result of either bacterial production (BP) or respiration (BR) stimulation, or both (Tables 2 and 3). However, the food chain was not stimulated, when BGE was inhibited by the sunlight (Tables 2 and 3). BGE is lower in exposed samples because of a greater increase in BR than in BP. BP changes in aquatic systems are related to N, P and the availability of high-quality DOC, but BR seems to be altered only by changes in DOC quality (Roland & Cole, 1999; Biddanda *et al.*, 2001). Changes in BP related to photochemical alteration in bulk DOC have been broadly reported (Lindell *et al.*, 1995; Anesio *et al.*, 2000, 1999; Farjalla *et al.*, 2001), but few studies have reported changes in BR arising from photochemical processes (Vahatalo *et al.*, 2003). Photochemical reactions can release nutrients (N and P) into the water column (Cotner & Heath, 1990; Jorgensen *et al.*, 1998), but these nutrients occur in very low concentrations in the two ecosystems studied (Farjalla *et al.*, 2002). In the current study we could observe that BR was stimulated by sunlight (Tables 2 and 3), indicating a relationship between BR and quality of DOC.

Finally, besides the fact that the photodegradation stimulates DOC mineralization via direct oxidation, it also stimulates bacterial mineralization through respiration (Vahatalo *et al.*, 2003). This corroborates the idea that the photochemical DOC transformation accelerates carbon cycling in aquatic systems, if it can be removed quickly from the water and not incorporated into bacterial biomass. Thus, despite some differences related to DOC sources and consumption, photochemical degradation accelerates DOC mineralization directly and indirectly through stimulation of bacterial respiration.

Photochemical influence of DOC degradation and CO_2 production

Comparing bacterial DOC removal (DOC_{REM}) with potential photochemical DOC removal (DOC_P), we can suggest an inverse availability of DOC to bacteria and sunlight (Table 2 and 3). During the low-water period in Lake Batata, when DOC is considered easily available to bacteria (Farjalla *et al.*, in press; Anesio *et al.*, 1997), we observed that DOC_{REM} is more effective than DOC_P. On the other hand, during the high-water period, when bulk DOC is mainly composed of allochthonous material, presumably refractory to bacterial (Anesio *et al.*, 1997), we observed an extremely low DOC_{REM}, while DOC_P is comparable to the highest DOC_{REM} rates from Lake Batata (Table 2 and Fig. 1a). CO_2 photoproduction is strongly related to the intensity of incident radiation (Bertilsson & Tranvik, 2000), but incident radiation on samples was higher during the drawdown and low-water periods (Table 1). DOC quality therefore seems to be a stronger factor than seasonal variations in radiation incidence in total DOC removal in clear-water Amazonian ecosystems.

Studies on the chemical composition of bulk DOC should be undertaken for a better understanding of the relationship between photodegradation and biodegradation. According to Tranvik & Bertilsson (2001), DOC bioavailability after photochemical degradation is inversely related to its prior availability. For instance, the DOC photodegradation process would negatively influence bacterial growth where DOC originated predominantly from algae. On the other hand, a positive effect on bacteria would be expected for photo-exposed humic DOC. We did not, however, detect bacterial changes due to DOC photochemical alteration during the low-water period in Lake Batata, when algae are an important source of DOC for the lake (Table 2).

The ability of DOC to absorb light energy is a prerequisite of the photodegradation process (Bertilsson & Tranvik, 2000). We believe that because of its 'less complex' molecular configuration, algal DOC did not interact with radiation at all, and so we did not register great changes in availability. Another factor is the current high availability of algal DOC bacterial consumption. Thus, an eventual increase of DOC availability, arising from sunlight action, would not overstimulate the bacterial activity. We therefore suggest that sunlight does not change bacterial utilization of labile DOC, while sunlight stimulates bacterial uptake of refractory DOC. Tranvik & Bertilsson (2001) proposed that sunlight, even though not altering the amount of labile DOC, can be responsible for the production of toxic compounds, such as peroxides (Scully *et al.*, 1996), that inhibit bacterial growth. Tranvik & Kokalj (1998) also noticed that exposure of algal DOC to UV light could lead to negative effects on bacteria in the presence of dissolved humic matter. The amount of dissolved humic matter in Lake Batata water was almost certainly negligible and unable to inhibit bacterial growth after sunlight exposure.

Miller & Moran (1997) noticed positive degradation interactions between photochemical and bacterial activity with regard to refractory DOC. On the other hand, Cotner & Biddanda (2002) suggested that solar radiation and bacteria could establish competition for DOC degradation. Obernosterer & Benner (2004) showed that sunlight and bacterial degradation present an overlap of 15% in DOC degradation. Based on these results, they concluded that both processes

compete for the same substrate. We should therefore expect a reduction of DOC_{REM} in samples previously exposed to sunlight, once the latter oxidizes part of DOC available to bacteria. However, we observed that DOC_{REM} was always stimulated by photochemical processes (Tables 2 and 3). We suggest that bacteria and sunlight cooperate in DOC degradation in the Amazonian clear-water ecosystems, instead of competing for the same DOC molecules as previously proposed (Cotner & Biddanda, 2002; Obernosterer & Benner, 2004). The inverse availabilities to bacterial and photodegradation processes also suggest that the two processes act mainly on different substrates or sites, which would avoid competition. Thus, we also suggest that aquatic bacteria have evolved not only to compete with other degradation forces, but also to derive benefits from them.

The CO_2 production dynamics are different in the two ecosystems studied. Because we could not observe other DOC sources in Caranã stream, both photochemical and bacterial degradation processes together enhance the potential CO_2 production. In this case, these CO_2 production rates are mainly determined by the DOC diagenetic state (Lindell *et al.*, 2000; Amado *et al.*, 2003). Fresh DOC stimulates both degradation processes during the rainy period, which registers the highest CO_2 photoproduction of the current study in Caranã stream (Table 1 and Fig. 1b). Nevertheless, potential CO_2 production was not altered, despite the different bacterial and photochemical DOC availabilities between the low- and high-water periods in Lake Batata (Fig. 1a). The lowest DOC_P during the low-water period may be compensated by bacterial mineralization. Moreover, the lowest bacterial mineralization (BR) during the high-water period may be compensated by the photochemical mineralization process (Fig. 1a). These findings suggest that sunlight is able to degrade refractory humic DOC as efficiently as bacteria degrade labile algal dissolved organic matter (DOM).

Jonsson *et al.* (2001) argued that photochemical mineralization can account for about 10% of total mineralization in a temperate deep humic lake, even considering a large dark layer of the water column. The current study also highlights photochemical degradation as a significant mineralization process in clear-water Amazonian lakes. Photochemical mineralization that occurs in the euphotic layer of Lake Batata is similar to bacterial mineralization in the whole water column in Lake Batata during the high-water period (Fig. 2). In fact, low total DOC_P during the low-water period is compensated by BR, and low BR is compensated by photochemical mineralization in the high-water period (Fig. 2). Thus, our results support the hypothesis that photochemical and bacterial mineralization present complementary pathways of DOC degradation in the clear-water Amazonian ecosystem.

Conclusions

We conclude that DOC degradation in clear-water Amazonian streams is determined by the DOC diagenetic state, which is controlled by the rains. In Lake Batata, DOC degradation is mainly determined by its seasonal main source to the ecosystem. DOC is more available to bacteria when it originates from algae, and more available to photochemical reactions when it originates from forest. Despite the seasonal changes in DOC origin, a balance or compensation between photochemical and bacterial activities results in similar DOC degradation rates in Lake Batata throughout the year. We also conclude that photochemical and bacterial mineralization establish a cooperative relationship for DOC degradation in clear-water Amazonian ecosystems.

Acknowledgements

The authors are grateful to Mineração Rio do Norte (MRN), CAPES, CNPq, FAPERJ and FINEP for financial support and scholarships. The authors also thank Dr R. Paranhos for methodological help, Dr E. A. Gomes, Dr J. L. Attayde, Dr B. M. Faria and A. L. Suhett for valuable comments on the manuscript, and V. M. Amado for a language review.

References

Amado AM, Farjalla VF, Esteves FA & Bozelli RL (2003) DOC photo-oxidation in clear water Amazonian aquatic ecosystems. *Amazoniana* **17**: 513–523.

Amon RMW & Benner R (1996) Photochemical and microbial consumption of dissolved organic carbon and dissolved oxygen in the Amazon River system. *Geochim Cosmochim Acta* **60**: 1783–1792.

Anesio AM, Abreu PC & Esteves FD (1997) Influence of the hydrological cycle on the bacterioplankton of an impacted clear water Amazonian lake. *Microb Ecol* **34**: 66–73.

Anesio AM, Denward CMT, Tranvik LJ & Graneli W (1999) Decreased bacterial growth on vascular plant detritus due to photochemical modification. *Aquat Microb Ecol* **17**: 159–165.

Anesio AM, Theil-Nielsen J & Graneli W (2000) Bacterial growth on photochemically transformed leachates from aquatic and terrestrial primary producers. *Microb Ecol* **40**: 200–208.

Bertilsson S & Tranvik LJ (1998) Photochemically produced carboxylic acids as substrates for freshwater bacterioplankton. *Limnol Oceanogr* **43**: 885–895.

Bertilsson S & Tranvik L (2000) Photochemical transformation of dissolved organic matter in lakes. *Limnol Oceanogr* **45**: 753–762.

Biddanda B, Ogdahl M & Cotner J (2001) Dominance of bacterial metabolism in oligotrophic relative to eutrophic waters. *Limnol Oceanogr* **46**: 730–739.

Bozelli RL, Esteves FA & Roland F (2000) Lago Batata: impacto e recuperação de um ecosssitema amazônico, IB-UFRJ/SBL, Rio de Janeiro.

Cole JJ (1999) Aquatic microbiology for ecosystem scientists: new and recycled paradigms in ecological microbiology. *Ecosystems* **2**: 215–225.

Cole JJ, Caraco NF, Kling GW & Kratz TK (1994) Carbon-dioxide supersaturation in the surface waters of lakes. *Science* **265**: 1568–1570.

Cotner JB & Biddanda BA (2002) Small players, large role: microbial influence on biogeochemical processes in pelagic aquatic ecosystems. *Ecosystems* **5**: 105–121.

Cotner JB & Heath RT (1990) Iron redox effects on photosensitive phosphorus release from dissolved humic materials. *Limnol Oceanogr* **35**: 1175–1181.

Farjalla VF, Anesio AM, Bertilsson S & Graneli W (2001) Photochemical reactivity of aquatic macrophyte leachates: abiotic transformations and bacterial response. *Aquat Microb Ecol* **24**: 187–195.

Farjalla VF, Azevedo DA, Esteves FA, Bozelli RL, Roland F & Enrich-Prast A (in press) Influence of hydrological cycle on bacterial growth and DOC uptake in a clear-water Amazonian lakes. *Microb Ecol*.

Farjalla VF, Esteves FA, Bozelli RL & Roland F (2002) Nutrient limitation of bacterial production in clear water Amazonian ecosystems. *Hydrobiologia* **489**: 197–205.

Farrington J (1992) Overview and key recommendations – Marine Organic Geochemistry Workshop, January 1990. *Mar Chem* **39**: 5–9.

delGiorgio P, Bird DF, Prairie YT & Planas D (1996) Flow cytometric determination of bacterial abundance in lake plankton with the green nucleic acid stain SYTO 13. *Limnol Oceanogr* **41**: 783–789.

Graneli W, Lindell M, De Faria BM & Esteves FD (1998) Photoproduction of dissolved inorganic carbon in temperate and tropical lakes – dependence on wavelength band and dissolved organic carbon concentration. *Biogeochemistry* **43**: 175–195.

Hobbie JE (1988) A comparison of the ecology of planktonic bacteria in fresh and salt-water. *Limnol Oceanogr* **33**: 750–764.

Jonsson A, Meili M, Bergstrom AK & Jansson M (2001) Whole-lake mineralization of allochthonous and autochthonous organic carbon in a large humic lake (Ortrasket, N. Sweden). *Limnol Oceanogr* **46**: 1691–1700.

Jorgensen NOG, Tranvik L, Edling H, Graneli W & Lindell M (1998) Effects of sunlight on occurrence and bacterial turnover of specific carbon and nitrogen compounds in lake water. *Fems Microbiol Ecol* **25**: 217–227.

Kieber RJ, Hydro LH & Seaton PJ (1997) Photooxidation of triglycerides and fatty acids in seawater: implication toward the formation of marine humic substances. *Limnol Oceanogr* **42**: 1454–1462.

Kieber DJ, McDaniel J & Mopper K (1989) Photochemical source of biological substrates in sea-water – implications for carbon cycling. *Nature* **341**: 637–639.

Lange HJ, Morris DP & Williamsom CE (2003) Solar ultraviolet photodegradation of DOC may stimulate freshwater food webs. *J Plankton Res* **25**: 111–117.

Lindell MJ, Graneli W & Tranvik LJ (1995) Enhanced bacterial-growth in response to photochemical transformation of dissolved organic-matter. *Limnol Oceanogr* **40**: 195–199.

Lindell MJ, Graneli H & Bertilsson S (2000) Seasonal photoreactivity of dissolved organic matter from lakes with contrasting humic content. *Can J Fisheries Aquat Sci* **57**: 875–885.

Lund V & Hongve D (1994) Ultraviolet-irradiated water containing humic substances inhibits bacterial metabolism. *Water Res* **28**: 1111–1116.

Miller WL & Moran MA (1997) Interaction of photochemical and microbial processes in the degradation of refractory dissolved organic matter from a coastal marine environment. *Limnol Oceanogr* **42**: 1317–1324.

Moran MA & Zepp RG (1997) Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. *Limnol Oceanogr* **42**: 1307–1316.

Nush EA & Palme G (1975) Biologische Methoden für die Praxis der Gewässeruntersuchung. *Z Wasser Abwasser Forschung* **116**: 562–565.

Obernosterer I & Benner R (2004) Competition between biological and photochemical processes in the mineralization of dissolved organic carbon. *Limnol Oceanogr* **49**: 117–124.

Obernosterer I, Sempere R & Herndl GJ (2001) Ultraviolet radiation induces reversal of the bioavailability of DOM to marine bacterioplankton. *Aquat Microb Ecol* **24**: 61–68.

Richey JE, Melack JM, Aufdenkampe AK, Ballester VM & Hess LL (2002) Outgassing from Amazonian rivers and wetlands as a large tropical source of atmospheric CO_2. *Nature* **416**: 617–620.

Roland F & Cole JJ (1999) Regulation of bacterial growth efficiency in a large turbid estuary. *Aquat Microb Ecol* **20**: 31–38.

Roland F & Esteves FA (1993) Dynamics of phosphorous, carbon and nitrogen in an Amazonian lake impacted by bauxite tailings (Batata Lake, Pará, Brazil). *Verh Int Vereinigung Theoret Angew Limnol* **25**: 925–930.

Roland F & Esteves FA (1998) Effects of bauxite tailings in PAR attenuation in an Amazonian crystalline water lake. *Hydrobiologia* **377**: 1–7.

Scully NM, McQueen DJ, Lean DRS & Cooper WJ (1996) Hydrogen peroxide formation: the interaction of ultraviolet radiation and dissolved organic carbon in lake waters along a 43–75 degrees N gradient. *Limnol Oceanogr* **41**: 540–548.

Sioli H (1984) The Amazon and its main affluents: hydrography, morphology of the rivers courses, and river types. *The Amazon: Limnology and Landscape of a Mighty Tropical River and its Basin*, *Vol. 1* (Sioli H, ed), pp. 127–165. Dr. W Junk, Dordrecht.

Strome DJ & Miller MC (1978) Photolytic changes in dissolved humic substances. *Verh Int Vereinigung Theoretische Angew Limnol* **20**: 1248–1254.

Theil-Nielsen J & Sondergaard M (1998) Bacterial carbon biomass calculated from biovolumes. *Archiv Hydrobiol* **141**: 195–207.

Tranvik LJ & Bertilsson S (2001) Contrasting effects of solar UV radiation on dissolved organic sources for bacterial growth. *Ecol Lett* **4**: 458–463.

Tranvik L & Kokalj S (1998) Decreased biodegradability of algal DOC due to interactive effects of UV radiation and humic matter. *Aquat Microb Ecol* **14**: 301–307.

Vahatalo AV, Salonen K, Munster U, Jarvinen M & Wetzel RG (2003) Photochemical transformation of allochthonous organic matter provides bioavailable nutrients in a humic lake. *Archiv Hydrobiol* **156**: 287–314.