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Contrasting interactions mediate dissolved organic matter decomposition in tropical aquatic ecosystems

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ABSTRACT: The interaction between photochemical and microbial degradation processes can have important effects on dissolved organic matter (DOM) decomposition in aquatic systems. Photochemical processes can stimulate or inhibit biological DOM degradation while biological processes often stimulate photochemical degradation. The aim of the present study was to evaluate whether these 2 degradation processes compete for the same organic substrates or use complementary components in 2 tropical systems with contrasting DOM sources (one dominated by humic, mostly terrigenous DOM and the other dominated by autochthonous phytoplankton production). We performed sequential exposures of DOM from both systems to photochemical or biological degradation. We then measured bacterial growth and respiration and photochemical oxygen consumption in addition to changes in DOM optical properties. In the humic lagoon, photochemical degradation stimulated bacterial degradation up to 500% with little complementary photochemical stimulation by bacterial degradation (an increase of only 13%). In the eutrophic lagoon, we found that photochemical degradation inhibited bacterial degradation 17%, suggesting competition with microbial substrates, while bacterial degradation had no effect on photochemical degradation. The net effect of these eutrophic lagoon interactions was a ca. 2% reduction in dissolved organic carbon degradation. Thus, we conclude that there was net complementary behavior between photochemical and bacterial processes in the humic lagoon in DOM degradation, while we observed little net competitive behavior in the eutrophic lagoon.

KEY WORDS: Dissolved organic matter · Photochemical degradation · Bacterial degradation · Dissolved organic carbon · Decomposition · Tropics

INTRODUCTION

Freshwater aquatic systems are recognized as important carbon dioxide (CO_2) sources to the atmosphere through decomposition of terrigenous organic matter (Cole et al. 1994). Bacterial respiration and photochemical degradation are two of the most relevant processes in mineralization of dissolved organic matter (DOM) in the water column, together representing up to 70% of total pelagic mineralization in temperate lakes (Jonsson et al. 2001). It has been shown that photochemical transformation processes affect bacterial

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degradation by either stimulating or inhibiting metabolism through changes in DOM bioavailability (i.e. Tranvik & Bertilsson 2001). Additionally, bacterial metabolism may affect the light-absorbing properties of DOM by releasing colored DOM (CDOM) molecules, which results in increased photochemical degradation (Kramer & Herndl 2004, Nieto-Cid et al. 2006). Thus, photochemical and bacterial DOM degradation have potentially coupled interactions that could play important roles in either carbon mineralization or energy/matter flow through aquatic food webs (microbial loop). For instance, photochemical degradation can increase bacterial respiration up to 100% and bacterial, heterotrophic flagellate and metazooplankton biomass up to 40, 55 and 500%, respectively (Amado et al. 2006, Daniel et al. 2006).

The reactivity and the fate of DOM are both related to its composition, sources and chemical characteristics (see Moran & Covert 2003). A previous study suggested that both bacterial and photochemical degradation could compete for DOM decomposition in the water column because some fraction of DOM may be both photo- and bioavailable (Obernosterer & Benner 2004). However, due to different structural characteristics, some DOM molecules (e.g. algal-originated DOM) are more readily available to bacterial consumption and refractory to photochemical degradation, while others (e.g. aromatic humic substances) present the opposite availability pattern (for review see Moran & Covert 2003). Furthermore, planktonic bacteria have been shown to preferentially consume non-colored and/or non-fluorescent DOM (Saadi et al. 2006). Thus, as the DOM pool in aquatic ecosystems is very heterogeneous and comprises a great variety of different organic compounds that differ spatially and seasonally in origin, concentration, composition and chemical and optical properties (Benner 2002), the degree of overlap between microbial and photochemical substrates in DOM degradation in aquatic ecosystems needs to be addressed. Specifically, we need to understand whether these 2 processes compete or complement each other regarding the substrates that they consume.

Aquatic ecosystems in the tropics mediate large fluxes of carbon from land to the atmosphere (up to 1 Mg C ha^{-1} yr^{-1} in the Amazon basin), and bacterial and photochemical degradation can be equally relevant to this process (Richey et al. 2002, Amado et al. 2006). Photochemical degradation can reach CO_2 production rates in the order of 400 µM C d^{-1} in tropical humic systems (Suhett et al. 2007), which is at least 1 order of magnitude higher than in temperate systems (e.g. Vahatalo et al. 2003). We are aware of only 2 studies that have investigated the photochemical effects on bacterial metabolism in tropical systems, but neither of these reported the biological effects on photochemical degradation in the same region (Amon & Benner 1996, Amado et al. 2006).

The aim of the present study was to examine the relationship between photochemical and bacterial DOM transformations in 2 tropical freshwater aquatic ecosystems. We performed sequential exposures of DOM to sunlight and bacterial degradation in 2 different tropical coastal lagoons with very distinct DOM photochemical and light absorption characteristics: a high-humic dystrophic lagoon and a eutrophic lagoon with high chlorophyll *a* (chl *a*) concentration. Our results suggested strong complementary effects of photochemical processes on biological decomposition in the humic-dominated system but little effects of microbes on photochemical degradation. In contrast, bacterial degradation complemented photochemical processes in the eutrophic lagoon, with little negative effect of photochemical processes on bacterial degradation.

MATERIALS AND METHODS

Study area. Water samples were collected in 2 tropical coastal lagoons (Imboassica Lagoon and Comprida Lagoon), both located in the northern part of Rio de Janeiro State, Brazil. Imboassica Lagoon, is near an urban area and receives non-treated sewage; concentrations of chl *a*, dissolved nitrogen (N) and dissolved phosphorus (P) in this lagoon are high (Table 1). Phytoplankton is considered one of the most important DOM sources to this ecosystem. Comprida Lagoon is a high-humic, dark-water and dystrophic ecosystem located in a conservation unit area (Restinga de Jurubatiba National Park). The main DOM source in this lagoon is the surrounding vegetation ('restinga' vegetation), and humic substances represent more than 90% of total dissolved organic carbon (DOC) concentration. This

Table 1. Dissolved organic carbon (DOC), dissolved nitrogen (N), dissolved phosphorus (P) and chlorophyll *a* (chl *a*) concentrations and optical characteristics (a_{430} [absorbance coefficient at 430 nm] and a_{250}:a_{365} [ratio between absorbance coefficient at 250 and 365]) in the 2 lagoons studied. Data extracted from Brazilian Long-Term Ecological Research (LTER) program (Site -5) monthly monitoring (1 yr average before the experiments)

	DOC (mM)	N (µM)	P (µM)	Chl *a* (µg l^{-1})	a_{430} (m^{-1})	a_{250}:a_{365}
Comprida Lagoon	3.90	47.44	0.19	1.08	32.70	4.38
Imboassica Lagoon	1.04	63.43	0.80	28.22	3.22	7.55

ecosystem has low chl *a* and nutrient concentrations and high DOC concentration and water color (a_{430} [absorbance coefficient at 430 nm]; Table 1) due to the large contribution of allochthonous humic compounds.

Sample collection, preparation and experimental set up. Water samples were collected from a central point in each lagoon during spring 2003 (November) into acid-washed (10% HCl) and deionized water rinsed 20 l carboys. The carboys were also rinsed with lagoon water before samplings. In the laboratory, a portion of the water samples was immediately filtered through 0.7 µm glass fiber filters (GF-75; Advantec MFS) and subsequently filtered through 0.2 µm Supor filter (SuporCap 100; Pall Corporation), to remove microorganisms. The filtered samples were put into acid-washed (10% HCl) borosilicate bottles, rinsed with Milli-Q water and autoclaved. Filtered samples were kept in a refrigerator until the beginning of experiments (within 24 h).

We performed 4 different treatments in each lagoon: (1) DOM exposed to sunlight degradation (Treatment L); (2) DOM exposed to bacterial degradation (Treatment B); (3) DOM exposed to bacterial degradation after exposure to sunlight degradation (Treatment LB); and (4) DOM exposed to sunlight degradation after exposure to bacterial degradation (Treatment BL). Treatments L and B worked as controls to Treatments BL and LB, respectively. All tubes and flasks used in the experiments were acid-washed (10% HCl), rinsed with Milli-Q water- and autoclaved.

To set up Treatment L, sub-samples of the previous 0.2 µm filtered water samples were placed into ten 120 ml quartz tubes (diameter = 0.03 m and length = 0.18 m), 5 of which were wrapped with aluminum foil (control to photochemical exposure). The water was also poured into eight 40 ml quartz tubes (diameter = 0.02 m and length = 0.12 m), 4 of which were wrapped with aluminum foil (control to photochemical exposure). All tubes were exposed to sunlight for 6 h d^{-1} for 2 sequential days (12 h total) in water baths with circulating water. The temperature in the water bath was continuously monitored and kept constant near ambient conditions (26 ± 1 °C). Total radiation (PAR + UV-A + UV-B; from 280 to 700 nm) was measured each hour during incubations using a radiometer (IL 1400, International Light) equipped with 3 different sensors to cover the wavebands described above. The oxygen concentration, fluorescence (excitation [ex.]: 360 nm; emission [em.]: 460 nm) and absorbance spectra of all samples in the 120 ml tubes were measured before and immediately after the incubations, to evaluate the photochemical transformations in the DOM. Details of these analyses are provided in 'Analytical procedures and calculations' below. The remaining water in the 120 ml tubes (ca. 117 ml) was kept to set up bacterial dilution cultures to bacterial abundance measurements in Treatment LB (see below). The 40 ml tubes were used to set up the bacterial dilution cultures for bacterial respiration measurements in Treatment LB.

For Treatment B, 16 BOD bottles (150 ml) were filled with 0.2 µm filtered water samples and bacteria inocula in a 9:1 ratio. The bacteria inocula were prepared by filtering total water sample through pre-combusted (550°C, 4 h) 0.7 µm glass fiber filters (GF-75; Advantec MFS) to remove bacterivores. Nitrogen (N) and phosphorus (P) were also added (50 µM N-NH_4NO_3 and 5 µM P-KH_2PO_4 final concentrations, respectively) into the cultures to minimize nutrient limitation. The cultures were incubated in the dark for 120 h at constant temperature (25°C). In order to detect the exponential growth phase of the cultures, bacterial abundance over time from 4 BOD bottles were measured. Samples for bacterial abundance were taken at 0, 24, 48, 72, 96 and 120 h and fixed with buffered formalin (3.7%, final concentration). We also measured the initial and final fluorescence (ex.: 360 nm; em.: 460 nm) and absorbance spectra of all samples from these BOD bottles. Four other BOD bottles were used to monitor bacterial respiration during the incubation; oxygen concentration (non-destructive method) in each bottle was measured at 0, 48 and 120 h. Any lost volume was replaced with the 0.2 µm filtered lagoon water after the 48 h sampling, leaving no headspace. The dilution in the samples was disregarded because it represented less than 1% of the volume. Before closing the bottles, we measured new initial oxygen concentrations to estimate the respiration in the following incubation interval (from 48 to 120 h). The other 8 BOD bottles were used to set up Treatment BL, subsequent to Treatment B.

Treatment LB was set up by adding bacterial inocula and N and P (50 µM N-NH_4NO_3 and 5 µM P-KH_2PO_4 final concentrations, respectively) to the samples (exposed and control) after exposure to sunlight (right after Treatment L); thus, bacterial dilution cultures were prepared in quartz tubes (the ten 120 ml and eight 40 ml from Treatment L; procedures similar to Treatment B, but in quartz tubes instead of BOD bottles). The same measurements were taken as in Treatment B. The 120 ml tubes were used to monitor bacterial growth and the 40 ml tubes were used to measure bacterial respiration.

Treatment BL was set up by filtering (0.2 µm, SuporCap 100; Pall Corporation) the water previously exposed to bacterial degradation (the Treatment B water) and then pouring into 4 quartz tubes and into 4 control tubes (quartz tubes wrapped with aluminum foil). All the tubes were then exposed to sunlight similarly to Treatment L. The same measurements were taken as in Treatment L. Before and after the sunlight

incubations in Treatment BL, we fixed aliquots for bacterial abundance determination (formalin 3.7%, final concentration) as a control of the previous filtration and to ensure that there was no significant bacterial growth during this incubation (data not shown).

Analytical procedures and calculations. We characterized the optical changes in 2 different ways: (1) measuring DOM fluorescence intensity as a humic substance indicator (Coble et al. 1993) with a Cary Eclipse fluorometer (Varian) at an excitation wavelength of 360 nm and an emission wavelength of 460 nm; (2) measuring the spectrum of absorbance from 250 to 450 nm (every 5 nm) with a spectrophotometer (Beckman DU 80), before and after all incubations. In both analyses we used a 1 cm pathlength quartz cuvette and Milli-Q water blanks were used as the reference. Based on the spectrum of absorbance, we calculated the spectral slope coefficient (S) between 300 and 450 nm with a non-linear regression of the following equation (according to Stedmon et al. 2000):

$$a_\lambda = a_{\lambda 0}{}^{S(\lambda 0 - \lambda)} \quad (1)$$

where a_λ is the absorbance coefficient at a certain wavelength, λ, and $a_{\lambda 0}$ is the absorbance coefficient at a reference wavelength, λ0, chosen to be 400 nm (Stedmon et al. 2000). S is a measure of how the absorbances decrease with respect to wavelength. The absorbance coefficient used was calculated as (according to Hu et al. 2002):

$$a_\lambda = (A_\lambda \times 2.303)/L \quad (2)$$

where A_λ is the absorbance at a certain wavelength, λ, and L is the optical pathlength (cuvette) in meters. We used the absorbance coefficient at 430 nm (a_{430}) as an estimate of water color. The ratio between absorbance coefficient at 250 and 365 nm (a_{250}:a_{365}) was used as an estimate of relative DOM molecular size. An increase in the ratio indicates an increase in the proportion of smaller molecules and vice versa (see Granéli et al. 1998). We also calculated the amount of solar energy absorbed by CDOM (Q) in the quartz tubes (according to Hu et al. 2002). The total radiation incident on samples was estimated by integrating all measured values during incubations. We estimated the amount of sunlight energy that reached the samples ($E(0)$) from 280 to 700 nm (every 5 nm) based on the sunlight energy contribution to each interval (IL-1400 Calibration certificates). The amount of energy absorbed at each wavelength (Qa) was then estimated by the following formula:

$$Qa = E(0) \times (a^{gm}/a_t) \times \delta \times [1 - \exp(a_t \times L)] \times t \quad (3)$$

where a^{gm} is the geometric average of DOM light absorbance coefficients (in a fixed wavelength, a) before and after the exposure to the sunlight, a_t is the total light absorption by DOM, δ is the cross section of the illuminated area of the quartz tubes, L is the pathlength (quartz tube), and t is the time of sunlight exposure (in seconds). Q was then calculated by the integration of the Qa at all wavelengths.

Bacterial abundance was measured using a CyAn ADP (Dako) flow cytometer. Syto 13 stain (2.5 µmol l^{-1} final concentration, Molecular Probes) and Fluoresbrite™ Carboxy YG Microspheres (∅ = 1.58 µm, ca. 3×10^5 ml^{-1} final concentration, Poly-sciences) were added to 1 ml sub-samples (del Giorgio et al. 1996). The cytometer was controlled by Summit software (Dako). Bacterial cells and microspheres were separated in a log-log scattergram of green fluorescence intensity (FL1) and side scatter (SSC). Triplicate counts were made at low flow speed (34%) and up to 10 000 events and bacteria concentration was calculated by 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) for bacterial batch cultures.

The oxygen concentration in all treatments was measured with a micro-oxygen probe connected to a picoamperemeter (Unisense©). Bacterial respiration rates in Treatments B and LB were estimated by the difference in oxygen concentration between time points (0 to 48 and 48 to 120 h). Bacterial respiration was converted to a carbon basis assuming that all oxygen consumed was transformed into CO_2 (transformation quotient of 1).

We calculated the bacterial production rates, bacterial DOC removal rates (DOC_{REM}), DOC lability (DOC_L) and bacterial growth efficiency (BGE). Bacterial production was estimated by bacterial biomass accumulation during the exponential growth phase (48 h for all cultures, data not shown). DOC_{REM} was calculated by the sum of bacterial production and bacterial respiration. DOC_L was estimated as the percentage of DOC that was biologically taken up from the DOC pool, i.e. {[(bacterial respiration + bacterial production) × 48 h] /DOC} × 100. BGE was calculated as the proportion of DOC_{REM} that was incorporated into biomass, i.e. (bacterial production/DOC_{REM}) × 100, during the exponential growth phase.

Photochemical oxygen consumption (photo-oxidation) in Treatments L and BL was calculated as the difference between oxygen concentration in exposed samples and controls after the sunlight exposure, thus removing all oxygen consumption other than the photochemical.

Statistical analysis. Photo-oxidation rates (photoconsumption of oxygen), bacterial production, bacterial respiration, DOC_{REM}, DOC_L, BGE, a_{430}, a_{250}:a_{365} and S data were compared between initial and final, sunlight exposed and control and among treatments (e.g.

Treatment L vs. Treatment BL) by *t*-test using the software GraphPad Prism 4.0. A significance level of 0.05 was used to determine statistical differences.

RESULTS

Biological and photochemical changes in DOM

The photochemical effects on DOM optical properties were consistent in both Comprida Lagoon and Imboassica Lagoon. There was decreased fluorescence, a_{430} and S and increased a_{250}:a_{365}, indicating CDOM breakdown (Table 2). Both systems also experienced decreased absorbance over the spectrum (from 300 to 450 nm), especially between 300 and 370 nm wavelengths (Fig. 1a,d,e,h).

The bacterial degradation effect on the DOM optical properties were also consistent, as it increased fluorescence, a_{430} and S and decreased a_{250}:a_{365} in both Comprida Lagoon and Imboassica Lagoon (Table 2). One exception was in Imboassica Lagoon, where there was no change in S in Treatment LB (Table 2). The absorbances over the spectrum increased due to biological action in Treatment LB in Comprida Lagoon and in Treatment B in Imboassica lagoon (Fig. 1c,f).

Photochemical and bacterial DOM degradation

The DOM in Treatments L and BL was exposed to similar amounts of solar radiation during the 12 h incubation (ca. 1.20×10^{-2} mol photons). The DOM light absorption coefficient (Q) was higher in Comprida Lagoon than in Imboassica Lagoon (Table 2). In agreement, the highest photo-oxidation rates in the present study were observed in Comprida Lagoon treatments. Photo-oxidation rates in both Treatments L and BL were more than 10 times higher in Comprida Lagoon ($p < 0.05$) than in Imboassica Lagoon (e.g. 20.05 and 1.61 µM C h^{-1} in Comprida and Imboassica, respectively, in Treatment L; Table 3). Photo-oxidation was 13% higher in Treatment BL than in Treatment L ($p < 0.05$) in Comprida Lagoon, indicating that previous bacterial degradation stimulated photo-oxidation; however, these 2 treatments showed similar photo-oxidation rates in Imboassica Lagoon (Table 3).

The bacterial density in the growth cultures for all treatments and controls increased exponentially, reaching a stationary phase at 48 h incubation (Table 4). Bacterial respiration rates (in µM h^{-1}) in all treatments and controls reached a maximum between 0 and 48 h incubation, being from 3- to 6-fold higher than the rates between 48 and 120 h incubation (Table 3). As bacterial metabolism was much reduced after 48 h incubation, we calculated bacterial production between 0 and 48 h incubation (maximum rates). For the same reason, we hereafter refer to bacterial respiration as the rate between 0 and 48 h incubation.

Bacterial respiration in Treatment B in Imboassica Lagoon was twice as high as in Comprida Lagoon ($p < 0.05$), indicating higher bacterial activity and available substrates. However, the effects of sunlight on

Table 2. Optical properties (fluorescence, a_{430}, a_{250}:a_{365} and S [spectral slope coefficient]) of each treatment (B: bacterial degradation; L: photochemical degradation; LB: biological degradation after photochemical degradation; BL: photochemical degradation after bacterial degradation) and dissolved organic matter light absorption coefficient (Q) in Comprida Lagoon and Imboassica Lagoon. Control of Treatment BL has the same value as Treatment B final, and the Initial of Treatment LB has the same value as Treatment L exposed. SD in parentheses. Different superscript letters indicate statistical differences among initial and final or control and exposed in single treatments (Treatments L or B, upper-case) and sequential treatments (Treatments BL or LB, lower-case)

Treatment		Fluorescence	a_{430} (m^{-1})	a_{250}:a_{365}	S	Q (µmol photons)
Comprida Lagoon						
B	Initial	2745.2^{A} (45.4)	67.89^{A} (0.1)	4.59^{A} (0.01)	15.80^{A} (0.01)	
	Final	$2796.5^{B,a}$ (16.6)	$72.45^{B,a}$ (1.6)	$4.55^{B,a}$ (0.02)	$17.96^{B,a}$ (0.50)	
BL	Exposed	2419.0^{b} (25.5)	53.78^{b} (0.8)	4.87^{b} (0.01)	16.06^{b} (0.10)	2.57
L	Control	2755.1^{A} (39.7)	72.66^{A} (0.8)	4.54^{A} (0.01)	15.34^{A} (0.05)	
	Exposed	$2369.2^{B,a}$ (41.7)	$62.58^{B,a}$ (0.5)	$4.72^{B,a}$ (0.21)	$15.02^{B,a}$ (0.03)	2.58
LB	Final	2305.6^{b} (14.5)	67.46^{b} (0.2)	4.51^{b} (0.01)	14.66^{b} (0.02)	
Imboassica Lagoon						
B	Initial	631.1^{A} (2.9)	4.77^{A} (0.1)	8.49^{A} (0.01)	21.00^{A} (0.01)	
	Final	$666.5^{B,a}$ (10.4)	$8.91^{B,a}$ (0.8)	$7.81^{B,a}$ (0.24)	$17.96^{B,a}$ (0.50)	
BL	Exposed	415.9^{b} (4.5)	2.33^{b} (0.8)	11.79^{b} (0.54)	23.33^{b} (0.22)	0.17
L	Control	649.8^{A} (11.6)	8.38^{A} (2.0)	7.82^{A} (0.25)	18.46^{A} (1.43)	
	Exposed	$378.4^{B,a}$ (8.9)	$4,88^{B,a}$ (0.7)	$10.85^{B,a}$ (0.48)	$20.22^{B,a}$ (0.63)	0.17
LB	Final	448.1^{b} (8.0)	4.14^{a} (0.4)	9.60^{b} (0.45)	20.82^{a} (0.72)	

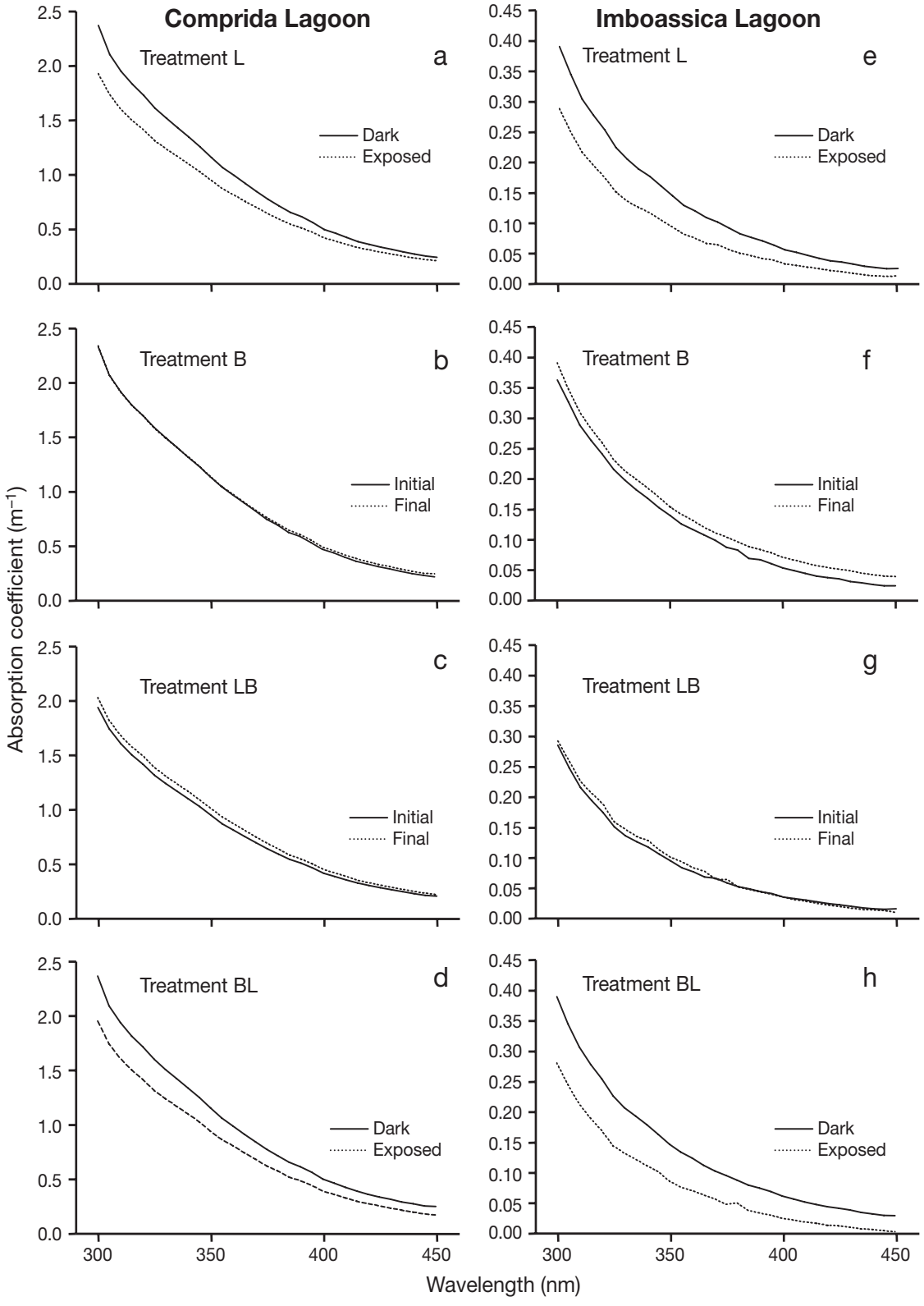

Fig. 1. Spectrum of absorbance between 300 and 450 nm in (a–d) Comprida Lagoon and (e–h) Imboassica Lagoon, before and after bacterial incubations and after exposure to sunlight and dark control in 4 treatments — L: photochemical degradation; B: bacterial degradation; LB: biological degradation after photochemical degradation; BL: photochemical degradation after bacterial degradation. Note different scales on the *y*-axes

Table 3. Mean photo-oxidation rates and bacterial parameters in the different treatments (Treat.) (B, L, LB and BL; see Table 2 for definitions) in Comprida Lagoon and Imboassica Lagoon. SD in parentheses. Different superscript letters indicate statistical differences among treatments in the same system. DOC_L was calculated based on the DOC concentration presented in Table 1

Treat.	Photo-oxidation ($\mu M\ O_2\ h^{-1}$)	Treat.	Bacterial respiration ($\mu M\ C\ h^{-1}$) 0–48 h	48–120 h	Bacterial production ($\mu M\ C\ h^{-1}$)	DOC_{REM} ($\mu M\ C\ h^{-1}$)	DOC_L (%)	BGE (%)
Comprida Lagoon								
L	20.05^A (0.7)	B	0.49^A (0.1)	0.11^A (< 0.1)	0.01^A (<0.1)	0.51^A (0.1)	0.60^A (0.1)	2.30^A (1.0)
BL	22.74^B (0.5)	LB	1.37^B (0.1)	0.45^B (0.1)	0.03^B (<0.1)	1.41^B (<0.1)	1.74^B (0.1)	2.56^A (0.5)
Imboassica Lagoon								
L	1.61^A (0.5)	B	1.06^A (<0.1)	0.16^A (0.1)	0.40^A (0.2)	1.46^A (0.1)	6.80^A (0.6)	26.60^A (8.1)
BL	2.42^A (1.9)	LB	0.92^B (0.1)	0.33^A (0.1)	0.33^A (0.1)	1.22^B (0.1)	5.60^B (0.5)	25.80^A (1.7)

Table 4. Mean bacterial densities over time in Treatments B and LB in Comprida Lagoon and Imboassica Lagoon. SD in parentheses

Treatment	0 h	24 h	48 h	72 h	96 h	120 h
Comprida Lagoon ($\times 10^8$ cells l^{-1})						
B	0.06 (0.02)	1.10 (0.17)	1.96 (0.98)	0.65 (0.26)	0.88 (0.19)	0.68 (0.12)
LB	0.38 (0.28)	1.77 (1.15)	6.17 (1.14)	2.97 (0.94)	2.67 (0.92)	2.16 (0.61)
Imboassica Lagoon ($\times 10^9$ cells l^{-1})						
B	0.72 (<0.01)	5.11 (1.63)	7.36 (2.56)	7.51 (3.01)	6.50 (1.74)	7.20 (2.26)
LB	0.71 (0.01)	3.60 (0.67)	6.21 (0.92)	3.06 (1.15)	3.81 (0.48)	5.94 (1.75)

bacterial activity were highest in Comprida Lagoon. Bacterial respiration in Treatment LB was almost 3-fold higher than Treatment B ($p < 0.05$) in Comprida Lagoon, while in Imboassica Lagoon Treatment LB exhibited a 13% inhibition relative to Treatment B (Table 3).

Similarly, bacterial production rates were 1 order of magnitude higher in Imboassica Lagoon than Comprida Lagoon, while the greatest effects of sunlight occurred in Comprida Lagoon. Bacterial production rates were 3-fold higher ($p < 0.05$) in Treatment LB than in Treatment B in Comprida lagoon (Table 3), while there was no difference between Treatments B and LB in Imboassica Lagoon (Table 3). Both biological DOC_{REM} and DOC_L were higher in Treatment LB than Treatment B in Comprida Lagoon and lower in Treatment LB than Treatment B in Imboassica Lagoon (Table 3). Previous photochemical degradation increased both DOC_{REM} and DOC_L by almost a factor of 3 in Comprida Lagoon, while both of these parameters decreased by 17% in Imboassica Lagoon (Table 3). BGE was at least 10 times higher in Imboassica lagoon than in Comprida Lagoon, consistent with other measures showing higher bacterial activity in Imboassica Lagoon. BGE was similar between Treatments B and LB in both Comprida Lagoon and Imboassica Lagoon (Table 3).

DISCUSSION

The results presented here are relevant to the 2 most important processes in DOM degradation in surface waters: photochemical and microbially mediated losses. Our primary observation was that both processes increased degradation rates via complementary action, i.e. photochemical processes stimulated biochemical degradation and vice versa. However, the importance of this complementary behavior varied depending on the characteristics of the system studied. Specifically, there was strong evidence for complementary behavior from both processes in the humic-dominated system (Comprida Lagoon). In contrast, in the algal-dominated system (Imboassica Lagoon), there was some evidence that bacterial degradation complemented photochemical degradation but there was stronger evidence that photochemical degradation inhibited bacterial degradation, suggesting competition for similar substrates. Although others have observed this competitive behavior before (Obernosterer & Benner 2004), this is the first observational study of this phenomenon in tropical systems; here the potential effect of sunlight on bioavailability is much higher than in temperate regions.

In addition, the differences we observed in comparing Comprida Lagoon and Imboassica Lagoon were

similar to what others have also reported regarding the sources of DOM and its degradation dynamics in aquatic systems (Moran & Covert 2003, Amado et al. 2006). Algal-derived DOM was mainly degraded by biological processes, while allochthonous organic matter was preferentially degraded by photochemical processes (Tables 2 & 3). Despite the fact that photochemical degradation affects bacterial DOC uptake, it did not affect BGE, which is contrary to previous studies (Table 3; e.g. Amado et al. 2006).

In addition to DOM degradation, bacterial and photochemical degradation play different roles with respect to the characteristics of DOM remaining in aquatic systems. Photochemical degradation causes CDOM breakdown into smaller molecules, causing losses of absorbance and total fluorescence with concomitant spectral slope increases, while microbial degradation tends to increase absorbance and fluorescence and decrease the spectral slope (Moran et al. 2000, Fu et al. 2006). In agreement, we observed that photochemical degradation (Treatments L and BL) reduced fluorescence and the absorbance over the UV-visible range of the spectrum and increased a_{250}:a_{365} as well as S (Fig. 1, Table 2). In general, microbial degradation had the opposite effect (with the exception of S in Treatment LB in Imboassica Lagoon; Fig. 1, Table 2). Nonetheless, photochemical transformations can stimulate bacterial degradation due to formation of more bio-available compounds (Amado et al. 2006). Furthermore, other studies have shown that the microbes stimulate photochemical degradation in seawater (Kramer & Herndl 2004, Nieto-Cid et al. 2006, Saadi et al. 2006). These facts suggest that both photochemical and bacterial degradation complement each other through formation of substrates available to the other process.

Nevertheless, only one study has actually demonstrated that competition for the same substrates was the dominant interaction between photochemical and bacterial DOC degradation (Obernosterer & Benner 2004). Based on differences in photochemical DOC degradation prior to and following extensive biomineralization, Obernosterer & Benner (2004) estimated that there was a 15% overlap in substrates used by either bacterial or photochemical processes in an allochthonous-DOM-dominated system. This result suggests that both degradation processes act, in part, on the same group of molecules. However, it was also shown that photochemical processes stimulated bacterial degradation by 22%. The net effect of these competitive and complementary interactions is that DOC degradation would have been stimulated by 7%, which indicates overall complementary interactions, instead of net competition.

Our results in the humic system (Comprida Lagoon) agreed with this complementary pattern. Exposure of DOM to sunlight stimulated bacterial degradation, and DOM exposure to bacterial metabolism, despite low overall rates, stimulated the photochemical degradation due to bacterial CDOM generation (Table 3; Nieto-Cid et al. 2006). Based on the optical properties and transformations by bacterial and photochemical processes (Fig. 1; Moran et al. 2000), the preference of bacteria to act on non-fluorescent DOM (Saadi et al. 2006) and the preference of photochemical degradation to act on fluorescent and/or CDOM (Bertilsson & Tranvik 2000), complementary interactions are not surprising. However, we found evidence for competitive interactions in Imboassica Lagoon, once photochemical degradation inhibited bacterial degradation by 17% and there was no bacterial stimulation to photochemical degradation (Table 3).

In order to estimate the degree of overlap among DOM compounds consumed through photo-chemical degradation and microbial degradation processes in Imboassica Lagoon, we assumed that DOC_{REM} represented bacterial degradation and that photo-oxidation rates represented the photochemical degradation in each treatment over 2 d. We also assumed that bacterial degradation occured 24 h d^{-1}, while photochemical degradation occured only 6 h d^{-1} (which is conservative for tropical regions) and converted the photo-consumption of oxygen to photo-reaction with carbon using a transformation quotient of 1. It has been shown that not all oxygen consumed oxidizes DOC into inorganic carbon, but previous experiments in coastal lagoons (including Comprida Lagoon) showed that more than 80% of the O_2 photo-consumed was changed into DIC (A. M. Amado unpubl. data) and also that DIC photo-production was strongly related to photobleaching (r^2 = 0.86; Granéli et al. 1998). Furthermore, Amon & Benner (1996) verified that the sunlight exposure length similar to our experiment led to DOC photo-consumption and was strongly correlated to the oxygen consumption in a rate close to 1 in tropical systems. Thus, this rate might be estimated with little error, because the partial oxidation reactions can lead to photo-humification, which were not recorded in our experiments. Nonetheless, in this study we considered the oxygen photo-consumption as an index of photochemical reaction with DOM (including total and partial oxidation). It is important to mention that in Treatment BL, where DOM was previously exposed to bacterial and then to photochemical degradation, we assumed that bacterial degradation was effective for only 48 h considering that bacterial CDOM release occurs during active growth (Kramer & Herndl 2004).

Over 2 d, 340.5 and 99.2 µM of C was removed by combined photochemical and bacterial degradation processes from Comprida Lagoon and Imboassica Lagoons, respectively (Fig. 2). In Comprida Lagoon,

the DOC was degraded by 7% biological degradation, 71% photo-oxidation, 9% photo-oxidation due to biological transformations and 13% biological degradation due to photo-transformations (Fig. 2). In Imboassica Lagoon, DOC was degraded by 59% biological degradation, 19% photo-oxidation, 10% photo-oxidation due to biological transformations and 12% due to photo-oxidation or biological degradation processes (Fig. 2). Thus, the net effect of these interactions suggest that photochemical and bacterial degradation complement each other, stimulating DOM degradation in about 75.50 µM of DOC in Comprida Lagoon. In contrast, these interactions suggest net competition between photochemical and bacterial degradation for about 1.87 µM of DOC in Imboassica Lagoon.

As the bulk DOM is an assemblage of compounds with different origins and physical, chemical and biological properties (Benner 2002), it is likely that the competition is a consequence of specific compounds that are labile for both bacterial and photochemical degradation. Fluorescence in the present study (ex. 360 nm; em: 460 nm) increased with microbial activity and decreased with photochemical degradation (Table 2), suggesting that bacteria produced organic substrates that were subsequently degraded photochemically. Additionally, bacterial degradation did not affect the spectral slope subsequent to photochemical degradation (Treatment LB) in Imboassica Lagoon, indicating that the photochemical degradation may have depleted at least those bio-available substrates that most strongly affect absorbance in this region of the spectrum (Table 2).

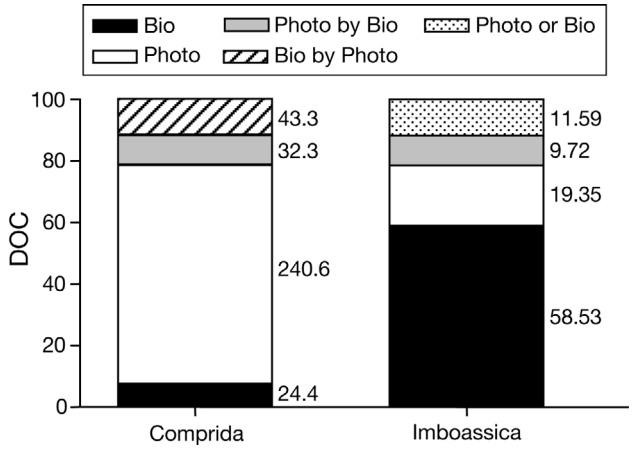

Fig. 2. Estimates of the dissolved organic carbon (DOC) fraction biologically reactive (Bio), photochemically reactive (Photo), biologically reactive due to previous photochemical degradation (complementary) (Photo by Bio), photochemically reactive due to previous biological action (complementary) (Bio by Photo) and photochemically or biologically reactive (where the 2 processes competed for substrates) (Photo or Bio) in Comprida Lagoon and Imboassica Lagoon. Degradation rates (µM C in 48 h) are placed close to the bars and the respective processes

Important molecules to bacterial metabolism that are also photo-available, such as some amino acids (tryptophan, tyrosine, histidine, methionine and cysteine), which are actually extremely reactive to photo-produced singlet oxygen (Michaeli & Feitelson 1994), could be the aim of the competition between photochemical and biological degradation to DOM degradation. Reactive oxygen species, such as the singlet oxygen, promote the oxidation of bioavailable substrates, resulting in reduced bacterial growth (Scully et al. 2003). Assuming that the amino acids (free and combined to DOM) contribute from 2 to 5.5% to the carbon (DOC) in the bulk DOM (Cammack et al. 2004), and that the photo-available amino acids contribute up to 7.5% of the amino acids pool in natural waters (Volk et al. 1997), we estimate that the contribution to bulk DOC of these photo-labile amino acids (which is 1.04 mM in Imboassica lagoon, Table 1) is from 1.56 to 4.29 µM, covering the concentration of 'competitive DOC' (1.87 µM) that we estimated in Imboassica lagoon.

Finally, we suggest that photochemical and bacterial degradation complement each other through formation of substrates available to the other process in tropical humic systems. In contrast, both processes might compete for DOM degradation in eutrophic systems, usually rich in compounds available to both processes, such as amino acid-like fluorophores (i.e. tryptophan-like and tyrosine-like fluorofores; Cory & McKnight 2005). In addition, this relationship might be stronger in tropical than in temperate systems since the radiation dose that degrades the DOM is stronger in that region (Granéli et al. 1998).

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