Photochemical and microbial consumption of dissolved organic carbon and dissolved oxygen in the Amazon River system

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Abstract—Bacterial and photochemical mineralization of dissolved organic matter were investigated in the Amazon River system. Dissolved oxygen, dissolved organic carbon (DOC), and bacterial growth were measured during incubations conducted under natural sunlight and in the dark. Substrate addition experiments indicated that the relatively low rates of bacterial activity in Amazon River water were caused by C limitation. Experiments to determine the photoreactivity of this biologically refractory DOC revealed unusually high rates of photochemical consumption of DOC (～4.0 µM C h⁻¹) and dissolved oxygen (～3.6 µM O₂ h⁻¹) in Rio Negro surface waters. In additional experiments we observed that bacterial growth and respiration were not significantly stimulated or inhibited during periods of sunlight exposure. The molar ratio of DOC to O₂ consumed during photochemical processes was close to one (1.11–1.14) in all phototovision experiments. Sunlight exposure over 27 h showed that at least 15% of Rio Negro DOM was photoreactive. The rate of photochemical consumption of DOC was approximately sevenfold greater than bacterial DOC utilization in Rio Negro surface waters; however, integrated over the entire water column microbial remineralization was the dominant process for oxygen and DOC consumption. Photomineralization of biologically refractory riverine DOM appears to be more important than previously believed and could be a major removal mechanism for terrestrially-derived DOM in the coastal ocean.

1. INTRODUCTION

The anthropogenically triggered decrease in stratospheric ozone concentrations and the subsequent increase in solar ultraviolet radiation has heightened interest in the effects of UV-light on aquatic ecology and biogeochemistry (Gracel, 1989). Numerous studies investigated the photochemical production of reactive transients and their involvement in aquatic chemistry (Blough and Zepp, 1990 and references therein). More specifically, the production of labile low-molecular-weight organic compounds from humic substances and the linkages between photochemical and heterotrophic processes have been investigated (Amador et al., 1989; Kieber et al., 1990; Mopper et al., 1991; Lindell et al., 1995), and deleterious effects of increased UV-radiation on aquatic biota have been elucidated (Blough and Zepp, 1990; Suttle and Chen, 1992; Herndl et al., 1993; Karentz et al., 1994). Although many details of the reaction mechanisms and interactions have yet to be illuminated, many of these processes involve dissolved organic matter (DOM) as a source, a sensitizer, or product of photochemical reactions. DOM is known to be an important light absorbing component of natural waters, and hence plays an important role in aquatic photochemical processes (Zepp, 1988).

Photochemical transformations of DOM occur upon direct absorption of UV and visible light by organic chromophores in aquatic environments. These organic chromophores can react either directly or indirectly as photosensitizers in reactions with other substances (Zafiriou et al., 1984; Cooper et al., 1989; Frimmel, 1994). The direct or primary reactions are restricted to compounds which can be excited by direct absorption of light. The photochemical transformation of chromophores to an excited state can lead to intramolecular reactions, such as dissociation into radicals, photoisomerization, and intramolecular decomposition, rearrangement, and electron transfer (Zika, 1981). Indirect or secondary reactions involve photochemically produced free radicals that react with other organic compounds in the water (Zika, 1981). Most photochemical reactions involve dissolved oxygen which plays a key role due to its universal importance as an electron acceptor and a participant in secondary reactions (Zafiriou et al., 1984). The role of dissolved oxygen in the photochemical mineralization of DOM is still obscure. Photochemical oxygen consumption has been observed during the oxidation of photochemically produced organic radicals and the photooxidation of metal-organic complexes (Langford et al., 1973; Zafiriou et al., 1984). The consumption ratio of oxygen and DOC during photochemical processes likely depends on the type of reaction (direct or indirect reactions) as well as on the chemical composition of the reactive organic compound.

In this study we investigated the consumption of dissolved oxygen and DOC in Amazon River waters and the relative importance of photochemical and biological mineralization processes. The few existing reports of naturally-occurring photochemical processes involving dissolved oxygen and DOC consumption indicate high variability in rates of processes depending on environmental conditions (Laane et al., 1985; Kieber et al., 1990). Light conditions and DOM quantity and quality are two of the major controlling factors for these processes. Reported values for photochemical oxygen consumption in surface waters vary from about 17 µM O₂ h⁻¹ in humic-colored fresh waters (Lindell and Rai, 1994) to 0.2 µM O₂ h⁻¹ in clear Caribbean waters (Laane et al.,

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2.2. Experimental Design

Substrate addition experiments were designed to investigate both the rates of microbial degradation of DOM and the chemical factors influencing degradation rates. These experiments were performed in four different types of water, the Rio Negro (blackwater river), the Rio Solimões (white-water river), a white-water FPL, and a clear-water FPL. In order to determine the potential limiting factor for the growth of heterotrophic bacteria in these environments, we amended parallel samples with inorganic nutrients and labile organic carbon in the form of glucose. Samples were processed within 2 hours in sampling after transport to the laboratory at the Instituto Nacional de Pesquisas da Amazônia (INPA) in Manaus. Five treatments with substrate additions of phosphate (KH$_2$PO$_4$; 3 µM final concentration), nitrate (NaNO$_3$; 50 µM final concentration), glucose (50 µM final concentration), phosphate and nitrate, phosphate and nitrate and glucose, and one untreated control, were prepared using water from the different environments. The different treatments were mixed in polycarbonate bottles and transferred to acid-rinsed BOD bottles (60 ml) which were incubated in the dark for 14–24 h. Triplicate bottles were used for each determination of dissolved oxygen, and one bottle served for the determination of bacterial abundance and leucine incorporation (see below). Measurements were taken at the beginning and the end of the incubations.

The photochemical consumption of dissolved oxygen and DOC was investigated in Rio Negro water and in filtered (0.2 µm) water from the sediment-laden Rio Solimões. Samples were placed in BOD bottles (Pyrex glass) and incubated under natural sunlight in a flow-through water bath under constant temperature (~28°C). The UV (UV-A and UV-B) transmittance of Pyrex bottles was determined using a radiometer/photometer (International Light Inc.) with sensors for UV-B (290–320 nm) and UV-A (320–400 nm) radiation. On average 20% of the UV-A and 50% of the UV-B irradiance was adsorbed by the Pyrex glass, indicating that all reported rates should be considered conservative since part of the high energy radiation was adsorbed by the bottles. The bottles were submerged with about 2 cm of Rio Negro water covering the bottles at all times. After carefully filling the bottles, the initial treatments of dissolved oxygen was measured and samples were saved for the initial DOC concentration. Samples were exposed to sunlight between 4 and 27 hours. For dark controls BOD bottles were wrapped in aluminum foil and kept in the same water bath during incubation. During the long-term (75 h) incubation, subsamples were taken during the early evening of each day. Triplicate or duplicate bottles were analyzed for dissolved oxygen and, if available, duplicate bottles were analyzed for DOC. In three of the four experiments bacterial leucine incorporation was also measured in the dark controls.

A separate set of experiments was conducted to investigate if photochemical processes stimulate bacterial activity by the production of labile DOM from the more refractory fraction of DOM. To test this possibility, Rio Negro water or Rio Negro DOM which was concentrated by ultrafiltration with a 1 kDa or 30 kDa filter (Amon and Benner, 1996) was exposed to sunlight for at least 3 hours. Water samples were passed through a 0.2 µm pore-size Nuclepore polycarbonate cartridge filter to remove bacteria prior to ultrafiltration. Dark controls were wrapped in aluminum foil. After exposure to sunlight the samples and dark controls were inoculated with a natural bacterial community. Sunlight exposed samples and dark controls were placed in BOD bottles and incubated in the dark to determine if microbial activity was affected by prior exposure of water samples to solar radiation. Incubations lasted between 3 and 24 hours and oxygen consumption was measured along with bacterial leucine incorporation. Bacterial respiration and leucine incorporation rates in sunlight pretreated samples were compared to those in the dark controls.

Additional experiments were conducted to investigate the direct influence of sunlight exposure on bacterial production. Two different approaches were taken to address this question. First, unfiltered water samples and corresponding dark controls were incubated under natural sunlight for at least 3 h. Samples were then returned to the laboratory and bacterial leucine incorporation was measured within 15 minutes. In the second approach, samples were spiked with [H]$^3$-leucine (specific activity of 60 Ci mmole$^{-1}$) or $^{15}$N-thymidine (specific activity of 60 Ci mmole$^{-1}$) to provide a source of labeled carbon and nitrogen for any potential new growth.
activity of 81.3 CI mmol⁻¹, to a final concentration of 10 nM for both. Spiked samples, including dark controls, were incubated under natural sunlight for 3 h. After incubation, samples were immediately filtered onto 0.22 μm Nuclepore membrane filters and processed as described below.

2.3. Measurements

Bacterial abundances were determined by epifluorescence microscopy using a Zeiss Universal microscope. Samples (5 ml) were preserved in Borax buffered formaldehyde (4% final concentration) and counted within 3–5 months of sampling. The best results were achieved by staining samples for 15 minutes with DAPI (4',6-
diamidino-2-phenylindole; Porter and Feig, 1980) at an elevated concentration of 1 μg DAPI ml⁻¹. Sediment-laden samples were diluted (200 μl of sample up to 2 ml with 0.2 μm filtered sample water) prior to staining in order to minimize background interference. At least 300 cells were counted per sample.

Bacterial production was estimated from rates of DNA and protein synthesis as measured by rates of radiolabeled thymidine (Chin-Leo and Benner, 1991) and leucine incorporation (Kirchman et al., 1985), respectively. Triplicate water samples (10 ml) and one killed control were incubated with 10 nM (final concentration) of [4,5⁻³H]leucine or [³H]thymidine for 1 h at ambient temperature in the dark. Time course experiments showed that incorporation of leucine was linear for at least 1 h. For comparative purposes some estimates of bacterial carbon production were made by converting bacterial leucine incorporation rates using the conversion factor of 3.1 kg C produced mol⁻¹ leucine incorporated (Simone and Azam, 1989).

DOC concentrations were determined by high-temperature combustion using a Shimadzu TOC-5000 analyzer (Benner and Strom, 1993). Samples were preserved by acidification to pH 2 with H₂PO₄. Samples were sonicated for 5 minutes immediately prior to analysis in order to disperse floculates that formed during storage (Tranvik, 1994).

Dissolved oxygen concentrations were determined by the Winkler method using a Mettler DL 21 autotitrator with potentiometric endpoint detection (Granelli and Granelli, 1991). Duplicate or triplicate samples were incubated in acid-rinsed 60 ml BOD bottles. Dissolved oxygen consumption rates were calculated from the slope of a linear regression line through multiple data points.

3. RESULTS

3.1. Microbial Consumption of DOC and Dissolved Oxygen

In substrate addition experiments, rates of respiration, leucine incorporation, and bacterial abundances in control experiments as well as the response to substrate additions demonstrated distinct differences in the four environments. The clear-water FPL, which was sampled during a phytoplankton bloom, supported the highest rates of community respiration (1.30 μM O₂ h⁻¹), and leucine incorporation (1.75 nM leucine h⁻¹), and had the highest bacterial abundance (6.28 × 10⁶ cells ml⁻¹; see Fig. 1a, b, and c). These values are 1.4–40 times higher than the values measured in the other three environments. Neither glucose additions nor the addition of glucose plus nitrate and phosphate increased rates of respiration (Fig. 1a) or leucine incorporation (Fig. 1b) relative to the control. The high rates of microbial activity and the lack of enhanced activity with C and nutrient addition suggested that the naturally occurring DOM was of high quality.

In contrast, the Rio Solimões as well as the white-water FPL exhibited very different responses to substrate additions. In the Rio Solimões, respiration rates increased from 0.49 μM O₂ h⁻¹ in the control to 1.76 μM O₂ h⁻¹ with glucose addition and to 2.52 μM O₂ h⁻¹ with glucose, nitrate, and phosphate (Fig. 1a). Leucine incorporation showed a similar response, increasing sixfold from 0.47 nM leucine h⁻¹ in the control to 2.37 nM leucine h⁻¹ with glucose addition and to 2.96 nM leucine h⁻¹ in the treatment with added glucose, nitrate, and phosphate (Fig. 1b). Bacterial abundances were also highest in those treatments (Fig. 1c). The white-water FPL showed a similar pattern with respiration rates increasing threefold relative to the control in response to glucose addition and to glucose plus nitrate and phosphate additions. Leucine incorporation approximately doubled in the treatment with glucose and the treatment amended with

![Fig. 1. Natural water samples from different environments were amended with phosphate (+P), nitrate (+N), glucose (+G), and combinations of the three. Untreated controls received no substrate additions. Respiration (a), leucine incorporation (b), and bacteria abundance (c) were measured in dark incubations. Incubation times varied among the different environments with 16 h in the Rio Negro, 24 h in the Rio Solimões, 18 h in the white-water FPL, and 14 h in the clear-water FPL. Abbreviation: WW FPL = white-water floodplain lake, CW FPL = clear-water floodplain lake.](image-url)
all three substrates, and bacterial abundance increased slightly in all treatments relative to the untreated control.

The Rio Negro was the environment with the lowest rates of respiration (0.22 μM O₂ h⁻¹), leucine incorporation (0.034 nM leucine h⁻¹), and bacterial abundance (Fig. 1a,b, and c). Responses to substrate additions in the Rio Negro did not exhibit a significant trend, but bacterial growth and respiration were slightly greater in the treatment with added glucose, phosphate, and nitrate. Glucose additions increased respiration rates slightly from 0.22 μM O₂ h⁻¹ in the control to 0.27 μM O₂ h⁻¹ in the treatment with glucose addition and to 0.35 μM O₂ h⁻¹ in the treatment amended with glucose, nitrate, and phosphate.

The results of the substrate addition experiments in the Rio Solimões and Rio Negro agree well with previously reported measurements (Benner et al., 1995). Typical respiration and leucine incorporation rates for the Rio Solimões are between 0.21–0.71 μM O₂ h⁻¹ and 0.22–0.86 nM leucine h⁻¹, respectively. Information on the Rio Negro is scarce, but rates of respiration and leucine incorporation appear to be somewhat lower than in the Rio Solimões and the Amazon mainstem with respiration rates ranging from 0–0.70 μM O₂ h⁻¹ and leucine incorporation rates between 0.05 and 0.17 nM leucine h⁻¹ (Benner et al., 1995; R. M. W. Amon, unpubl. data).

3.2. Photochemical Consumption of DOC and Dissolved Oxygen

The relative importance of photochemical processes for the fate of DOC was investigated in three experiments with Rio Negro water and one experiment with 0.2 μm filtered water from the Rio Solimões. Oxygen and DOC consumption during a short-term (4 h) incubation with Rio Negro water (Fig. 2a and b) indicate a pronounced difference in oxygen consumption between light and dark treatments. The oxygen consumption rate in the light was high (3.9 μM O₂ h⁻¹) while no significant oxygen consumption occurred in the dark control (Fig. 2a). The consumption rate of DOC in the light was 4.4 μM C h⁻¹, and there was no significant DOC consumption in the dark.

A second experiment with Rio Negro water was conducted with 10 h of sunlight exposure and yielded similar results (Fig. 2c and d). The oxygen consumption rate in the light was 3.8 μM O₂ h⁻¹. Dark consumption of oxygen (0.21 μM O₂ h⁻¹) was 5% of the consumption rate in the light. The rate of DOC consumption in the light (4.5 μM C h⁻¹) was also similar to the rate in the first short-term experiment. The rate of dark consumption of DOC was 0.4 μM C h⁻¹.

A third experiment with Rio Negro water was conducted over three consecutive days with samples collected for dissolved oxygen and DOC at sunset of each day. The data presented in Fig. 2e and 2f include incubation during the day and night. During 75 h of incubation, samples were exposed to sunlight for 27 h. The oxygen consumption rate in the light was 3.68 μM O₂ h⁻¹. This rate is more than twice times higher than the rate of oxygen consumption in the dark (0.30 μM O₂ h⁻¹). The rate of DOC consumption in the light was 4.70 μM C h⁻¹ and was eight times higher than the dark consumption rate (0.58 μM C h⁻¹).

In order to investigate the potential photochemical reactivity of Rio Solimões DOC, water was filtered to remove suspended sediments as well as bacteria. During a 6 h exposure to sunlight the rates of consumption of oxygen and DOC in the light were 0.98 μM O₂ h⁻¹ and 1.10 μM C h⁻¹, respectively (Fig. 2g and h). These rates are considerably lower than those in the Rio Negro. By pooling the experimental data of photochemical oxygen consumption and relating them to initial DOC concentration in the different experiments we found a strong correlation (P < 0.002) between photochemical oxygen consumption and DOC concentration (Fig. 3a). It appears that the concentration of photoreactive DOC is related to the overall concentration of DOC in these environments.

Rates of photochemical oxygen consumption were similar in the three Rio Negro experiments but indicate a trend of decreasing rates as incubation time increases. In the Rio Negro the rates of photochemical oxygen and DOC consumption were about threefold higher than rates in the Rio Solimões samples (Table 1). On a molar basis, photochemical DOC consumption was slightly higher than photochemical oxygen consumption. The ratio of DOC:O₂ consumed ranged from 1.11–1.14. Respiration rates were at least eight times lower than photochemical oxygen consumption rates in the Rio Negro experiments, whereas in the Rio Solimões respiration and photochemical oxygen consumption rates were similar. Total heterotrophic carbon consumption (respiration plus bacterial production) was less than 17% of the photochemical carbon consumption in the Rio Negro surface samples. In the Rio Solimões experiment total biological carbon consumption in unfiltered dark controls was slightly greater (1.4 times) than photochemical carbon consumption in 0.2 μm filtered samples.

3.3. Interactions between Photochemical and Biological Processes

Several of the above described experiments utilized unfiltered Rio Negro water, so it is possible that the observed consumption of dissolved oxygen and DOC in light incubations resulted from a combination of photochemical and biological processes. Photochemical processes could transform refractory DOM to more labile substrates that are readily utilized by heterotrophic bacteria. However, the addition of labile substrates to Rio Negro water only increased the rate of oxygen consumption in the dark to 0.35 μM h⁻¹ (Fig. 1), so it seems unlikely that the observed tenfold higher oxygen consumption rates during incubations under natural sunlight (Table 1) could result from microbial utilization of labile substrates produced during photodegradation of DOM. A series of experiments was designed to further address interactions between photochemical and biological processes by exposing riverine DOM to sunlight prior to incubation with riverine bacteria. If photochemical processes produced labile substrates, we expected to observe enhanced rates of bacterial activity in samples previously exposed to sunlight.

All samples of DOM from the Rio Negro were very photoreactive as indicated by the high rates of photochemical oxygen consumption during sunlight exposure (Table 2). In some experiments (B, C, F) rates of bacterial respiration
were higher in incubations with photooxidized DOM, but in two of these experiments (C, F) the differences in the rates were not found to be statistically significant as demonstrated by the overlapping standard errors and two-tailed t-test analyses (Table 2). In experiment A, the respiration rate was significantly higher in the control than in the sunlight exposed sample (Table 2). In the two experiments in which leucine incorporation rates were measured, the rates in the controls were slightly higher than the rates in the sunlight exposed sample (Table 2). These results were equivocal and did not provide convincing evidence for the stimulation of bacterial activity by the production of labile substrates by photochemical processes.

Additional experiments further investigated whether sunlight significantly inhibited or stimulated bacterial production. Unfiltered Rio Negro and Rio Solimões water was incubated in light and dark controls, and leucine incorporation rates were measured immediately after incubation (Experiments A–F). The results of these experiments were also equivocal and showed no consistent trends (Table 3). To determine if photochemical processes and bacterial activity are closely coupled processes we did additional experiments (G, H) in which Rio Negro water was spiked with radiotracer before incubation (light vs. dark). Neither leucine (G) nor thymidine (H) incorporation rates differed significantly between light and dark incubated samples (Table 3). These results indicated that there were no measurable direct effects of photochemical processes on the activity of the heterotrophic bacterial community.

4. DISCUSSION
The substrate addition experiments provided valuable information on substrate quality in the different riverine and
lake environments. The clear-water floodplain lake supported an ongoing phytoplankton bloom and very high rates of respiration and bacterial growth. Glucose and nutrient additions did not further stimulate biological activity suggesting that the naturally occurring DOM is of high quality. In contrast, DOM from the Rio Negro and Rio Solimões was more resistant to degradation as indicated by the lower rates of microbial activity in the untreated controls and elevated rates in response to glucose additions. Long-term batch culture incubations (>100 h) of Amazon River water demonstrated that only 1.4–7.5% of the total DOC pool was mineralized by bacteria (Benner et al., 1995; R. M. W. Amon, unpubl. data), indicating that most of the C transported in the Amazon River system is of refractory nature. Similarly, Richey et al. (1990) and Quay et al. (1995) suggested that the organic matter pool in the mainstem Amazon is dominated by refractory compounds. The reason for the refractory nature of most Amazon River DOM is likely its highly degraded diagenetic state (Hedges et al., 1994).

The net heterotrophic character of the Amazon River and the refractory nature of its DOM led us to the hypothesis that photochemical processes partially break down natural DOM into biologically labile compounds which in turn could be respired by bacteria. Results presented in this study, however, did not provide unequivocal evidence that bacterial activity is stimulated by the photochemical production of labile compounds. There have been recent reports of stimulatory (Amador et al., 1989; Lindell et al., 1995) and inhibitory (Herndl et al., 1993; Lund and Hongsve, 1994) effects of UV radiation on microbial activity in natural environments. Inhibition of bacterial activity can be the result of direct UV damage to the organism (Karentz et al., 1994) or of the inhibitory effect of toxic intermediates produced during photodegradation of natural DOM (Lund and Hongsve, 1994). The simultaneous occurrence of stimulatory and inhibitory effects could have masked each other and would, therefore, not be detected by the experimental design in the present study.

Another potential cause for undetectable stimulation of microbial activity could be the use of glass during light incubations. The Pyrex bottles attenuated a major fraction of UV-B light, and Kieber et al. (1990) reported that there was no production of low molecular weight carbonyl compounds during radiation with light above 320 nm. We measured the %-transmittance of UV-light through a Pyrex-bottle wall and found that ~20% of UV-A and more than 50% of UV-B were attenuated by the bottle. This agrees with another study reporting that more than 50% of light with a wavelength below ~350 nm did not penetrate through a glass bottle wall (Sunda and Hutsman, 1994). This means that our measurements of photochemical DOC consumption on the surface could be underestimated. The magnitude of underestimation is difficult to determine, but in a study of photochemical oxygen consumption, Laane et al. (1985) reported a 75% reduction of oxygen consumption rates due to glass bottles as compared to quartz containers.

Comparison of the photochemical consumption rates of DOC and dissolved oxygen demonstrated that Rio Negro

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Sunlight exposure time (h)</th>
<th>photochemical oxygen consumption (µM O₂ h⁻¹)</th>
<th>photochemical DOC consumption (µM C h⁻¹)</th>
<th>DOC/O₂ ratio (µM O₂/µM C)</th>
<th>respiration (µM O₂ h⁻¹)</th>
<th>bacterial production (µM C h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rio Negro 1</td>
<td>4</td>
<td>3.91</td>
<td>4.42</td>
<td>1.13</td>
<td>BD</td>
<td>0.16</td>
</tr>
<tr>
<td>Rio Negro 2</td>
<td>10</td>
<td>3.61</td>
<td>4.10</td>
<td>1.14</td>
<td>0.50</td>
<td>0.18</td>
</tr>
<tr>
<td>Rio Negro 3</td>
<td>27</td>
<td>3.27</td>
<td>3.62</td>
<td>1.11</td>
<td>0.11</td>
<td>ND</td>
</tr>
<tr>
<td>Rio Solimões</td>
<td>6</td>
<td>0.98</td>
<td>1.10</td>
<td>1.12</td>
<td>1.40*</td>
<td>0.18*</td>
</tr>
</tbody>
</table>

*These were measured in unfiltered dark controls. Abbreviations: BD = below detection limit; ND = not determined.
Table 2. Photochemical - biological interactions in Rio Negro water. Ultrfiltered DOM concentrations and whole water from the Rio Negro were exposed to sunlight for 3-10 h. After exposure, sunlight-exposed samples and dark controls received either a bacterial inoculum (experiments A - D), mixed with untreated sample water (experiment E) or were not manipulated at all (experiment F), and were incubated in the dark. Experiments were performed to determine if bacterial activity (respiration and leucine incorporation) was stimulated by the photo-production of labile organic compounds. Incubation times were 4 h for experiments A and B, 5 h for C and D, 3 h for E, and 24 h for experiment F. Respiration rates represent the slopes of a linear regression and the leucine incorporation rates are integrated over time.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Exposure time (h)</th>
<th>Photochemical O2 consumption (µM O2 h⁻¹)</th>
<th>Respiration rate (µM O2 g⁻¹ h⁻¹) ± SE</th>
<th>Leucine incorp. rate (nmol h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: RN DOM (&gt;30 kDa)</td>
<td>3</td>
<td>10.55</td>
<td>0.11 ± 0.27</td>
<td>1.55 ± 0.23</td>
</tr>
<tr>
<td>B: RN DOM (&gt;1 kDa &lt;30 kDa)</td>
<td>3</td>
<td>8.53</td>
<td>0.10 ± 0.28</td>
<td>0.53 ± 0.34</td>
</tr>
<tr>
<td>C: RN DOM (&gt;30 kDa)</td>
<td>4</td>
<td>8.11</td>
<td>0.10 ± 0.14</td>
<td>0.81 ± 0.44</td>
</tr>
<tr>
<td>D: RN DOM (&gt;1 kDa &lt;30 kDa)</td>
<td>4</td>
<td>7.00</td>
<td>BD</td>
<td>BD</td>
</tr>
<tr>
<td>E: RN DOM (&gt;1 kDa)</td>
<td>3</td>
<td>4.01</td>
<td>BD</td>
<td>BD</td>
</tr>
<tr>
<td>F: Rio Negro water</td>
<td>4</td>
<td>3.61</td>
<td>0.39 ± 0.15</td>
<td>0.23 ± 0.03</td>
</tr>
</tbody>
</table>

*Two-tailed t-test analyses (Zar, 1984) showed no significant difference between the two slopes, the p-values for experiments C, and F were p<0.5, and p>0.1, respectively; Abbreviations: RN = Rio Negro; BD = below detection limit; ND = not determined.

Water supports threefold greater rates than Rio Solimões water. It appears that the higher photooxidation rates in the Rio Negro result from the higher concentrations of DOC and humic substances than those in the Rio Solimões. Relationships between DOC concentration and rates of photochemical processes (Fig. 3a) were also shown in other studies (Miles and Brezonik, 1981; Laane et al., 1985; Lindell and Raj, 1994). Rates of photochemical oxygen consumption in the literature are typically between 0.6 and 2.8 µM O₂ h⁻¹ for humic freshwater systems with DOC concentrations ranging from 2–68 mg C l⁻¹ (Miles and Brezonik, 1981; Lindell and Raj, 1994). Carbon normalized oxygen consumption rates in the present study are more than twice as high.

On a molar basis, the ratio of DOC:O₂ consumed in our experiment ranged from 1.11–1.14. If and how photochemical oxygen and DOC consumption are coupled remains a matter of further research. It is known that molecular oxygen is involved in a variety of photochemical reactions. The photochemical production of hydrogen peroxide consumes molecular oxygen, however, the fate of this radical was shown to be biological decomposition (Moffett and Zafiriou, 1990) without DOM oxidation. Other radicals that are photochemically produced and involve oxygen consumption could be oxidizing agents for natural DOM. Some investigators measured sunlight-induced decreases of absorbance and specific organic compounds that were not associated with decreases in DOC (see review by Zepp, 1988; Ertel, 1990). Other studies reported the rapid loss of DOC during UV-radiation in acidic environments and with the addition of H₂O₂ (Zepp, 1988; Backlund, 1992). This indicates that at least in some cases the photochemical mineralization of DOC could be mediated by secondary photo-reactions.

The photochemical generation of dissolved inorganic carbon (DIC) from DOM was reported in recent studies which investigated photochemical DOM degradation in a salt marsh, river (Miller and Zepp, 1995), and humic lake (Graueli et al., 1995). The photochemical production of CO₂ was also demonstrated by Miles and Brezonik (1981) who conducted photooxidation studies with humic-colored waters. They reported a ratio of ~2 mol of CO₂ produced for every mole of O₂ consumed and suggested that carboxyl groups were the major photoreactive functional groups producing CO₂. Miles and Brezonik (1981) reported that at least part of the photochemical oxygen consumption was due to photochemical decarboxylation. Photochemical decarboxylation has been reported in several studies (Langford et al., 1973; Miles and Brezonik, 1981; Miller and Zepp, 1995) and appears to be one of the major reactions involved in photochemical mineralization of natural DOM.

Natural DOM potentially consists of a variety of photoreactive compounds, however, little is known about their chemical structure. More is known about the products of photooxidation and it appears that CO₂ is the major end product (Miller and Zepp, 1995). Most recent studies of photochemical alterations of DOM stressed the importance of photoproduction of labile organic substrates for bacterial growth (Geller, 1986; Hwang et al., 1986; Amador et al., 1989; Kieber et al., 1989; Mopper et al., 1991). These studies demonstrated that biologically-labile compounds can be produced during the photodegradation of natural DOM. These low-molecular-weight compounds include formald-
hyde, acetaldehyde, glyoxylate, and pyruvate. Photoconversion rates of these carbonyl compounds vary from \(~500\) nM C h\(^{-1}\) in the Florida Everglades to \(~2-4\) nM C h\(^{-1}\) in the Sargasso Sea (Kieber et al., 1990; Mopper et al., 1991).

The photoconversion of carbon monoxide has also been demonstrated in several aquatic environments with rates varying from \(~80\) nM C h\(^{-1}\) in the Orinoco River plume near the river mouth (Jones and Amador, 1993) to \(~20\) nM C h\(^{-1}\) in Sargasso Sea surface waters (Mopper et al., 1991). This would yield a maximal combined photo-production rate of \(~580\) nM C h\(^{-1}\) for carbonyl compounds and carbon monoxide in the freshwater examples. Photochemical DOC consumption rates observed in our study averaged \(~4,000\) nM C h\(^{-1}\) in the Rio Negro. Miller and Zepp (1995) reported even higher rates (\(~1,000-20,000\) nM DIC h\(^{-1}\)) of photooxidation in a water sample from the Suwannee River, using an artificial light source (solar simulator).

In relation to rates of bacterial respiration, photochemical mineralization of DOC appears to be one order of magnitude higher in Rio Negro surface waters and of similar magnitude in the Rio Solimões. Comparing the total carbon mineralization by the two different processes over a 75 h period shows that \(~15\)% of the initial DOC was consumed by photochemical processes while only between 1.2 and 2.7% were consumed by bacteria. This suggests that photochemical processes degrade part of the refractory pool of DOM that is not readily available for bacteria. The long-term sunlight exposure of Rio Negro water indicates that rates of photochemical consumption remained high over an extended time span (3 days) and that at least 15% of the Rio Negro DOM is very photoactive. However, rates of photooxidation decreased with increasing incubation time indicating that not all DOM components exhibit the same photoactivity.

In order to estimate the relative importance of photochemical and biological mineralization processes throughout the entire water column several assumptions have to be made. Depth-integrated estimates of microbial and photochemical oxidation were derived assuming a river width of \(~5,500\) m and an average depth of \(~50\) m at the sampling station during the high water season (J. E. Richey, pers. comm.). We assumed that the reported rates of photochemical DOC consumption were representative for the upper \(~10\) cm in the Rio Negro. This assumption seems reasonable because the water depth in the bottles varied from \(~2-7\) cm in the photodegradation experiments.

Using the assumptions above and an average photochemical DOC mineralization rate of \(~4.05\) \(\mu\)M C h\(^{-1}\), the depth-integrated photochemical DOC consumption rate is \(~0.41\) mmolasses C m\(^{-2}\) h\(^{-1}\) as compared to a biological DOC consumption rate of \(~13.0\) mmolasses C m\(^{-2}\) h\(^{-1}\). The biological DOC consumption rate was estimated using a respiration rate of \(~0.26\) \(\mu\)M O\(_2\) h\(^{-1}\) and assuming a respiratory quotient of \(~1\). These depth-integrated rates correspond to the consumption of \(~0.01\) and \(~0.75\)% of the DOC per day, respectively. These estimates indicate that photochemical processes account for a relatively small fraction of carbon mineralization in the river. Added together the two mineralization processes account only for \(~0.76\)% of DOC degradation per day. The average residence time of DOC in the river (from Manaus to the ocean) is about \(~2\) weeks, indicating that only \(~10\)% of the DOC reservoir is mineralized prior to discharge into the coastal ocean.

The biologically refractory nature of Amazon River DOM raises many questions about the fate of riverine DOC in the ocean. The global input of riverine DOC would replace the entire oceanic carbon pool in less than \(~3,000\) years (Deuser, 1988), however, only a small fraction of oceanic DOM has been identified as terrestrial-derived DOM. Investigations of the chemical (Meyers-Schulte and Hedges, 1986) and stable isotope compositions (Eadie et al., 1978) of marine DOM indicate only a minimal contribution of riverine organic matter to the marine DOC pool. Furthermore, conservative behavior of riverine DOM during estuarine mixing indicates that coagulation and precipitation are not responsible for the removal of terrestrial-derived DOM (Mantoura and Woodward, 1983).

Estimates of the potential photochemical consumption of DOC in the Amazon River plume are very dependent on light conditions and the penetration of effective irradiance. It has been argued (Zika et al., 1993) that photochemical processes in the coastal ocean are sensitive to varying inputs of riverine DOM. In extensive river plumes of large tropical rivers like the Amazon, photochemical carbon mineralization could be a major removal process for riverine DOC. Several studies report wide aerial extensions of the Amazon River plume (Ryther et al., 1967; Moore, 1986; Muller-Karger et al., 1988), sometimes reaching more than \(~3 \times 10^7\) km\(^2\). The freshwater lens in the Amazon River plume is largely devoid of suspended solids, and it still shows the characteristic brownish-black coloration typical for the Amazon River DOM (Ryther et al., 1967). The lack of suspended solids allows for deeper penetration of light in the water column and a greater potential for photochemical processes. The underwater light field of a similar environment, the Orinoco River plume, was investigated by Farmer et al. (1993) who reported light penetration depths of \(~0.1-5\) m for UV-B and from \(~0.2-15\) m for UV-A. Special emphasis should be put on the importance of UV-A radiation in photochemical DOC degradation since it has the potential to penetrate much further into the water column than UV-B (Blough et al., 1993; Piazera and Håder, 1994). The potential underestimation of the photochemical significance of UV-A has been pointed out in several recent publications (Sikorski and Zika, 1993; Valentine and Zepp, 1993; Sunda and Hutsman, 1994).

The daily DOC discharge of the Amazon River into the coastal ocean equals about \(~61.3\) Gg (Richey et al., 1990). A rough estimate of the potential photochemical mineralization of riverine DOM in the Amazon River plume can be calculated using the conservative assumptions of a \(~1.06 \times 10^4\) km\(^2\) plume area, an average penetration depth of \(~1\) m for effective irradiation, and an average reactive DOC concentration of \(~35\) \(\mu\)M C (assuming that \(~25\)% of riverine DOC is photoactive) in the plume area. The area of the Amazon River plume was estimated from salinity contours reported by Lentz (1995). The weighted average for the concentration of riverine DOC in the plume \(~140 \mu\)M C) was estimated by stepwise calculation of DOC values depending on salinity changes, assuming conservative mixing. An approximate rate of photochemical carbon mineralization was estimated from the relationship between reactive DOC concentration...
(35 μM) and photochemical oxygen and DOC consumption (Fig. 3). Due to the low number of observations for photochemical DOC consumption at different initial DOC concentrations we are not able to demonstrate a strong relation between the two. The relation between DOC concentration and photochemical oxygen consumption (Fig. 3a), which appears to be a strong indicator of photochemical C mineralization (Fig. 3b), was therefore, used to estimate a photooxidation rate of 0.15 μM C 1−1 h −1. This rate falls at the low end of photochemical C mineralization rates reported from the Mississippi River plume by Miller and Zepf (1995).

Based on those assumptions, 1.91 Gg DOC or ~3% of the daily DOC discharge of the Amazon River could be photooxidized on a daily basis. The residence time of riverine DOM in the river plume area determines how much of the terrestrially-derived DOM is lost in the coastal transition zone. For our model plume it would take ~7 days to fill a water parcel the size of 1.06 km² (1 m deep), during which more than 20% of riverine DOC could be lost due to photooxidation. A recent study by Miller and Zepf (1995) estimated a half-life of 1.5 years for riverine DOC in the ocean. It appears that photooxidation is an important sink for riverine DOM in the coastal ocean, but additional studies should focus on the structural identification of photoreactive DOM components and measurement of in situ rates of photo-mineralization to provide a better estimate of the quantitative significance of the process.

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