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Effects of DOM photochemistry on bacterial metabolism and CO_2 evasion during falling water in a humic and a whitewater river in the Brazilian Amazon

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Abstract In the Amazon river system, the source of the large quantity of CO_2 evading from river surfaces remains unidentified. Photochemical transformation of dissolved organic matter (DOM) into dissolved inorganic carbon (DIC) and low molecular weight organic acids (LMWOAs) is a promising candidate. Few studies in the Amazon river system, and river systems in general, have attempted to quantify the contribution of these specific photoproducts to CO_2 evasion. We conducted photochemical degradation and ^{14}C addition experiments to measure the rate of production and the impact on bacterial metabolism, respectively, in the black water Rio Negro and in the white water Rio Solimões during low water. We found statistically significant production of both photoproducts in the Rio Negro and none in Rio Solimões. We also found that two photochemically produced LMWOAs—acetic and formic acid—may play a significant role in bacterial metabolism in both rivers. Based on our experimental results, we estimate that photochemically produced CO_2, acetic acid and formic acid alone contribute to only 0.5% of the CO_2 evading from the Rio Negro. Due to our experimental set-up, analytical methods and time of sampling, we caution that our estimate is very conservative. More extensive research is needed before drawing conclusions on the contribution of photochemistry to CO_2 evasion from river surfaces of the Amazon basin.

Keywords Dissolved organic matter · Photochemical degradation · Photomineralization

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Introduction

A common thread among almost all river systems is that river water is supersaturated with CO_2 (Cole and Caraco 2001). High CO_2 concentrations result from the following inputs: (1) CO_2 from soil respiration and mineral weathering imported into river channels from terrestrial environments by subsurface waters, (2) CO_2 derived from in situ respiration of organic matter (OM) within the river channel, (3) CO_2 from respiration of macrophytes growing in river channels, or (4) CO_2 produced as a result of the interaction of sunlight with riverine OM in the river channel. For a large river system such as the Amazon, the relative strength of each source will vary spatially with river sizes ranging from small streams to kilometer-wide

river channels (River Continuum concept, Vannote et al. 1980) and through time over the hydrologic year as water levels rise, inundate floodplains and drain back into channels (Flood Pulse concept, Junk et al. 1989).

The Amazon river releases more than 10 times the amount of carbon by CO_2 evasion from water surfaces than is exported to the ocean as TOC and DIC combined (Richey et al. 2002). In the Amazon (Mayorga et al. 2005; Richey et al. 2002) and in other river systems (Cole and Caraco 2001; Mulholland et al. 2001), the high CO_2 concentrations driving this evasive flux have been attributed to in situ respiration of organic carbon. In the Amazon, Ellis et al. (in review) recently demonstrated that respiration of in situ OM fuels 15–35% of the CO_2 evasive flux in the Rio Negro and 80–100% in the Rio Solimões. However, the organic substrates fueling respiration in these rivers remains unresolved.

Basin-wide surveys of the lignin (Hedges et al. 1986; Ertel et al. 1986) and carbohydrate and amino acid (Hedges et al. 1994) compositions of riverine OM show that a large component of the OM pool in Amazonian waters is highly degraded and resistant to biological mineralization. This precludes in situ respiration of a large component of the OM pool in these rivers. However, collection methods used in these OM compositional studies (ultrafiltration and adsorption onto XAD resins) exclude 25–40% of mostly hydrophilic, low molecular weight DOM from compositional analyses. Thus, the bioavailability of a significant fraction of the total dissolved OM pool is unknown. There are clearly unmeasured carbon substrates fueling respiration within these river channels (Richey et al. 1990; Mayorga et al. 2005).

Photochemistry is a promising candidate for addressing both the questions of unmeasured dissolved carbon substrates fueling in situ respiration and of unresolved CO_2 sources to these rivers. The role of photochemistry in producing biologically labile substrates from refractory dissolved organic matter (DOM) became widely recognized with the work of Geller (1986). Many different classes of photoproducts have been measured over the past two decades, with DIC and low molecular weight organic acids (LMWOAs) recognized as most dominant (see review by Moran and Zepp 1997). About 60% of identified biolabile organic photoproducts are LMWOAs, with low molecular weight keto-acids making up the remainder (Zepp et al. 1995). In terrestrial aquatic systems, LMWOAs are a rapidly cycling, yet poorly studied, pool of organic compounds composing about 75% of the total biolabile pool (Kaplan and Newbold 2003). In the Amazon river system, photochemically produced LMWOAs may be part of the unmeasured carbon substrates fueling in situ respiration. In addition to the production of biologically labile DOM, direct photochemical production of DIC from DOM has also been recognized (Granéli et al. 1996) and may contribute directly to CO_2 evasion from Amazonian waters.

Most studies that quantify photoproduction rates of DIC and LMWOAs, and subsequent effects on bacterial metabolism, have been carried out in estuaries and lakes. In estuaries, photochemistry is thought to play a role in the loss of terrestrial OM from the ocean (Mopper and Kieber 2002; Miller and Zepp 1995; Mopper et al. 1991) and its products as a substrate for bacteria (Miller and Moran 1997). In lakes, the interaction of sunlight with terrestrially derived, humic DOC has been recognized as major source of both DIC (Prairie 2008) and biologically labile substrates for bacteria (Bertilsson and Tranvik 1998; Bertilsson and Tranvik 2000). Granéli et al. (1998) measured DIC photoproduction rates ranging from 1,300 to 24,000 nM C/h in Swedish and Brazilian lake waters varying from clear to humic-rich. In a survey of 38 Swedish lakes covering a large range of DOC concentrations, Bertilsson and Tranvik (2000) measured DIC photoproduction rates of 40–13,000 nM C/h.

Quantitative studies of DIC and LMWOA photoproduction are less common in rivers. Bertilsson et al. (1999) measured the photoproduction rates of LMWOAs (100–2,200 nM C/h) and DIC (500–1,000 nM C/h) in groundwater and stream water from two small boreal catchments. Data from a study by Pullin et al. (2004) on the Parker River in the northeastern US yield LMWOA photoproduction rates of about 500 nM C/h. Gao and Zepp (1998) measured direct CO_2 photoproduction rates of 22,000 nM C/h in the humic-rich Satilla river in Georgia, USA. In a clear water lake and stream with a relatively low humic content, Amado et al. (2006) found DIC photoproduction rates ranging from 200 to 500 nM C/h at various stages of the hydrograph. Miller et al. (2002) found that production rates of biologically labile photoproducts in a humic river in

the southeastern US ranged from about 1,000 nM C/h for UV-B wavelengths to about 100 nM C/h for visible light.

Other studies of DOM photochemistry in rivers have measured decreases in DOC or TOC concentrations as a whole. In the humic-rich waters of the Rio Negro, Amon and Benner (1996) found DOC loss rates of 4,000 nM C/h due to photochemical consumption and that at least 15% of Rio Negro DOC is photoreactive. In a humic-rich boreal stream in northern Sweden, where 95% of the total organic carbon (TOC) pool is DOC, Köhler et al. (2002) found a mean TOC loss rate of 3,700 nM C/h due to photochemical processes. Data from the Parker River study by Pullin et al. (2004) yield DOC loss rates of about 2,000 nM C/h.

The diverse rivers of the Amazon basin provide an ideal environment for answering major questions regarding the impact of photochemistry on aquatic ecosystem metabolism and CO_2 outgassing. The large river channels of the Amazon represent an array of chemically diverse water types. In addition, compared to other biomes, the ecological significance of photochemistry may be most apparent in the tropics. At these latitudes, solar zenith angles are small year-round and the percent contribution of UV light to the solar spectrum is relatively high (Whitehead et al. 2000). It is a well-known that DOM interacts strongly with the UV portion of the sunlight spectrum (Miller et al. 2002; Kirk 1994; Keiber et al. 1990).

Despite this, the Amazon and tropical river systems in general are understudied with respect to DOM photochemistry and there has been little to no focus on the specific contribution of the two dominant photoproducts—DIC and LMWOAs—to CO_2 evasion. Photochemistry may directly contribute to CO_2 evasion from rivers through mineralization of DOM to DIC. Photochemical production of LMWOAs can contribute indirectly to CO_2 evading from these rivers and this will depend on the relative bacterial utilization of these compounds as substrates for in situ respiration versus incorporation into biomass.

In this study, we estimate the contribution of DOM photochemistry to CO_2 evasion in two diverse water types of the Amazon basin by performing photochemical experiments using filtered water from the Rio Negro (blackwater) and the Rio Solimões (whitewater). We hypothesize that DOM photochemistry is a significant CO_2 production mechanism that operates in these river channels that results in both the direct addition of CO_2 (photomineralization) and the addition of biologically labile photoproducts (photodegradation), which are subsequently respired to CO_2. To evaluate this hypothesis, we (1) measured the production rates of LMWOAs and DIC during in situ photochemical experiments and (2) measured the bacterial utilization rates (respiration versus bacterial production) of ^{14}C-labeled LMWOAs during dark incubations. We then used these experimental rates to estimate the relative contribution of DOM photochemistry to overall published CO_2 evasion rates measured in these rivers.

Methods

Field sites

Samples for all photochemical and bacterial uptake experiments were collected from October 1, 2007 to October 7, 2007 at main channel field sites on the Rio Negro (03°05′S, 60°06′W) and the Rio Solimões (03°15′S, 60°00′W) during the falling water stage of the hydrograph. With its low suspended sediment and dissolved ion concentrations, and high concentrations of tea-colored dissolved humic substances, the Rio Negro is classified as a blackwater river (Sioli 1950, 1951, 1956) and drains primarily the Caatinga forests of northern Brazil (Sioli 1984). According to the same Sioli classification system, the Rio Solimões is a whitewater river. This sediment-laden river is fed by numerous tributaries flowing from the Andean Cordillera. As a result, it has high dissolved ion concentrations and moderate concentrations of dissolved humic substances.

Photochemical experiments

In order to examine photodegradation and photomineralization of DOM, a total of four in situ photochemical experiments were carried out during the first week of October in 2007. Two identical experiments were conducted on the Rio Negro with Rio Negro river water on two different days: N1 (October 2, 2007) and N2 (October 6, 2007). A similar experiment was conducted on the Rio Solimões with Rio Solimões water: S1 (October 5, 2007). In order to compare the

photodegradation and photomineralization of Rio Negro and Rio Solimões DOM under a similar light regime, one experiment was conducted in which Rio Solimões DOM was incubated under the Rio Negro light regime: NS2 (October 7, 2007). For each experiment, a fresh sample of river water was collected using a submersible pump from a depth of 2 m. Samples were stored on ice and in the dark until the start of each experiment.

In preparation for each photochemical experiment, river water was filtered through two stacked glass fiber filters (Whatman® GF/F, 0.7 μm pore size, pre-combusted at 500°C for 4 h) to remove particulate material and bacteria. Removal of bacteria was to insure that photodegradation and photomineralization, rather than bacterial processes, were the processes breaking down DOM during the course of the photochemical experiments. Troussellier et al. (1997) found that two stacked GF/F filters removed 99% of bacteria from marine and freshwaters. However, it should be noted that at the average temperatures of these Amazonian waters (27°C), the 1% of remaining bacteria may have multiplied rapidly enough to significantly impact water chemistry during the incubations. Therefore, our acetic and formic acid photoproduction rates are likely underestimates.

Immediately following filtration, the filtered river water was transferred to a 90 ml quartz test tube (Quartz Scientific Inc., Vancouver, WA, USA) and sealed with an acid-washed silicone stopper. Each test tube was then positioned horizontally in a floating test tube rack and secured with a single zip tie. Six river water replicates and three dark controls covered in tin foil were placed in the floating test tube rack. Four HOBO® Temperature/Light data loggers (Onset Computer Corporation, Bourne, Massachusetts, USA) were secured along side the quartz test tubes in the floating test tube rack. A sample for initial LMWOA and DIC concentrations was collected at the beginning of each experiment. The rack was deployed approximately 2.5 cm beneath the water surface under natural sunlight. Incubation time was dependent on travel conditions on the rivers and ranged from 7 to 22 daylight hours. For example, during the S1 experiment there was a violent storm at the end of the sampling day such that samples could not be retrieved until early afternoon of the next day. However, mean total sunlight received at 2.5 cm depth during each experiment was calculated from data collected by four ($n = 4$) HOBO data loggers placed in each corner of the floating test tube rack.

At the end of each experiment, river water in each test tube was sub-sampled for LMWOAs and DIC for the determination of photodegradation and photomineralization rates, respectively. Immediately after opening each test tube, 20 ml of water was withdrawn with a 60 cc plastic syringe for DIC analysis. Samples were stored in the syringes and kept cold until arrival in the laboratory at the Instituto Nacional de Pesquisas da Amazônia (INPA) in Manaus. Water for LMWOA analyses was poured into a 20 ml NALGENE Sterile PETG media bottle (Thermo Fisher Scientific, Rochester, NY, USA), preserved with thymol (Gillett and Ayers 1991) in the field and placed in a freezer upon return to the laboratory. Prior to collecting the samples, bottles were washed once with carbon-free NaOH detergent soap, once with 1 N HCl and three times with UV-treated Nanopure water. Samples were kept frozen until analysis.

To determine DIC concentration, samples were injected directly from the collection syringe into a Shimadzu TOC Analyzer (Model V-CHP) within 24 h of field collection. After injection into the instrument, the sample was automatically acidified and the CO_2 was measured in a non-dispersive infrared detector. Concentrations were obtained by comparison with external Shimadzu $NaHCO_3$ standards, which were cross-checked with Trois-96 freshwater standards (Canadian Water Research Institute). For both standards and samples, 3–5 injections were made per sample resulting in a coefficient of variation of less than 0.5%.

LMWOA concentrations were analyzed by a reverse-phase high pressure liquid chromatography method adapted from Albert and Martens (1997). The pyridine buffer, N_2 bubbling and KOH addition steps used by Albert and Martens (1997) for marine sediments were omitted. Because of high background concentrations of acetic acid, the 2-nitrophenylhydrazine (NPH) used in the derivatization step was purified by recrystallization. The solid NPH was dissolved in 1 l of Nanopure water, heated to 95°C and filtered through a pre-combusted GF/F filter. The filtrate was cooled in an ice bath and then vacuum-filtered through a pre-combusted GF/F filter for 8 h to remove as much water as possible. After derivatization, we ran our samples on Shimadzu SCL-10A with a Gilson 231 Autosampler, a Shimadzu SPD-10A

UV–VIS detector, a Spheri-5 RP-8 analytical column (Alltech), a NewGuard RP-8 guard column (Alltech) and a PRP-1 Guard column (Hamilton) as the concentrator column in place of a sample loop in a Rheodyne 6-port injection valve. For both standards and samples, five injections were made per sample resulting in an average peak area coefficient of variation of 1.5% for acetic acid and 2.0% for formic acid.

^{14}C bacterial utilization experiments

Bacterial utilization rates and bacterial growth efficiencies (BGEs) for acetic and formic acids were determined for both water types using ^{14}C-labeled compounds. Samples for these experiments were collected once from each water type during two of the four photochemical experiments (N1 and S1). Samples were collected from 2 m depth using a submersible pump and transported back to the laboratory at INPA in a 10 l, acid-washed carboy. Experiments were initiated within 4 h immediately upon return to the laboratory.

The experimental set up based on the work of Yager and Deming (1999) which was adapted from the original closed-system method developed by Hobbie and Crawford (1969). Prior to the addition of river water, each vial was pre-loaded with ^{14}C-labeled acetic or formic acid so that, when the 20 ml of river water was added, the ^{14}C-labeled acetic acid concentration in each vial would be 500, 1,000 and 1,800 nM C and the formic acid concentration 250, 500 or 900 nM C. The radiolabeled acetic acid (specific activity: 56 mCi/mmol; radioactive concentration: 1.0 mCi/ml) and formic acid (specific activity: 53 mCi/mmol; radioactive concentration: 1.0 mCi/ml) were purchased from MP Biomedicals (Irvine, CA, USA). Incubations for acetic and formic acid were run in separate vials. For each acid, three replicate incubations were analyzed. One formaldehyde-killed control was incubated for each type of sample. Accurate formic acid ambient concentrations in the river waters studied here were known beforehand by preliminary HPLC analyses. However, accurate acetic acid concentrations were not know prior to the ^{14}C utilization experiments due to high acetic acid background concentrations at the time of preliminary HPLC analyses (see previous section). Therefore, the acetic acid concentrations were purposefully set up to cover a larger range. Units were converted to nM C for both acids in order to calculate the BGE, defined as BP/(BP + BR) where BP, *bacterial production*, is nmol of carbon incorporated into biomass and BR, *bacterial respiration*, is nmol of carbon respired. The denominator, BP + BR, is the *total utilization*.

For each sample, 20 ml of unfiltered river water was added to a 40 ml amber EPA glass vial (Cole-Parmer, Vernon Hills, IL, USA). The vials are certified as pre-cleaned to meet the United States Environmental Protection Agency (EPA) standards for more than 60 volatile organic acids, including acetic and formic acids. After the water was added, the vial was immediately capped with a rubber septa. Samples were incubated for 1 h in the dark in a water bath kept at river water temperature (27°C) on an orbital shaker at 90–95 rpm. After 1 h, samples were killed by adding formaldehyde through the septa of each vial to a final concentration of 2% using a 10 cc syringe and needle. Next, 1 ml of 1 N NaOH (CO_2 scrubber) was added directly into a 10 × 75 mm open glass test tube already in the vial through the septa of each 40 ml vial using a 1 cc syringe and needle. The NaOH-filled open test tube served as a CO_2 collector. The CO_2 added to the vial due to respiration occurring during the incubation is trapped in the test tube when it dissolves in the NaOH and is converted to carbonate (CO_3^-), preventing CO_2 outgassing from the vial. Recoveries of CO_2 in blanks averaged 85 ± 3%. Then, 0.4 ml of 4 N H_2SO_4 was added through the septum of the vial with a 1 ml syringe and needle. The acid was added gently against the side of the vial to prevent splashing. The H_2SO_4 served to drive the CO_2 out of solution and into the headspace of the vial, where it was collected in the NaOH-filled test tube. The vials were allowed to sit for 2 weeks to give time for respired CO_2 to be driven out of solution and captured by the NaOH.

After 2 weeks, each vial was opened and all of the NaOH from the test tube was transferred to a scintillation vial containing 10 ml of Ecolume Scintillation Cocktail. This sample was used to calculate the respiration rate of each acid. The remaining 20 ml sample in each vial was filtered onto a separate 0.2 μm cellulose-acetate membrane filter. Each filter was added to a separate scintillation vial along with 10 ml of Ecolume. This sample was used to calculate the incorporation rate of each acid. Radioactivity of

the respired and incorporated ^{14}C-labeled compounds was estimated by liquid scintillation counting.

Respiration and incorporation rates determined from these experiments for both LMWOAs were adjusted to account for the quantity of formic and acetic acid already in river water. The ^{14}C-incubations were run on river water collected during the N1 and the S1 photochemistry experiments; therefore, the initial concentrations for the N1 and S1 photochemistry experiments were used (Table 1) to calculate the experimental concentration (^{14}C-labeled + non-labeled) of acetic and formic acid for each incubation. In this type of ^{14}C-incubation experiment, where the quantity of ^{14}C-labeled compounds added is the same order of magnitude as the non-labeled compounds present in the natural water being tested, it is assumed that the uptake of ^{14}C-labeled and non-labeled compounds is nondiscriminate (i.e. ^{14}C-labeled acetic acid and non-labeled acetic acid are utilized and processed at the same rate). As a result of this assumption, the respiration and incorporation rates obtained from liquid scintillation counting of ^{14}C-labeled compounds were doubled to account for the equivalent uptake of non-labeled compounds.

Statistical analyses

For the purposes of all statistical analyses, outliers were excluded using the Q-test, which compares the difference between the suspected outlier and its nearest neighbor relative to the overall data range. Data were assessed for normality using the Kolmogorov–Smirnov test. Differences among sample types in the photochemical experiments were tested using ANOVA ($\alpha = 0.05$), with post-hoc Scheffe tests to determine which pair-wise comparisons were significant when the ANOVA indicated that differences existed among groups. In order to determine photodegradation and photomineralization rates, we first determined if there was a statistically significant difference among the initial, the irradiated sample and the dark control concentrations. If there was a statistically significant difference between the irradiated and initial concentrations, and between the irradiated and dark controls, then photoproduction was statistically significant. The change in concentration due to photodegradation or photomineralization was calculated by subtracting the concentration in the dark controls from the concentration in the irradiated samples.

Depth-integrated photodegradation and photomineralization rates

UV-B wavelengths are thought to be the dominant wavelength responsible for DOM photochemistry (Keiber et al. 1990; Miller et al. 2002). We did not have the instrumentation (i.e. a radiometer) required

Table 1 Concentrations and rates from photodegradation and photomineralization experiments for Rio Negro (N1, N2) and Rio Solimões (S1, NS2) waters

	N1 (6.6 h[a])	N2 (6.8 h[a])	S1 (44.3 h[a])	NS2 (23.3 h[a])
Initial (nM C)				
Acetic	1,970 ± 3,000 ($n = 2$)	1,800 ± 100 ($n = 3$)	2,200 ± 100 ($n = 3$)	1,200 ± 100 ($n = 3$)
Formic	790 ± 130 ($n = 3$)	690 ± 80 ($n = 3$)	930 ± 30 ($n = 3$)	820 ± 120 ($n = 3$)
DIC	10,000 ± 2,000 ($n = 3$)	n.d.	n.d.	n.d.
Change (nM C)				
Acetic	900 ± 400	500 ± 540*	−400 ± 2,500*	700 ± 780*
Formic	890 ± 480	1,190 ± 320	−20 ± 140*	280 ± 450*
DIC	16,000 ± 7,000	15,000 ± 3,000	3,000 ± 10,000*	1,000 ± 3,000*
Hourly rate (nM C/h)				
Acetic	140 ± 60	78 ± 80*	−9 ± 60*	30 ± 30*
Formic	135 ± 70	175 ± 50	0.5 ± 3*	12 ± 30*
DIC	2,400 ± 1,000	2,200 ± 400	70 ± 200*	40 ± 130*

Initial is the concentration in the river water during the time of sampling and at the start of each experiment. *Change* is the difference in concentration between the dark control and the irradiated sample. Numbers for each sample type are the mean ± standard deviation of *n* samples. [a] Time of exposure to sunlight. * Not statistically significant

to directly measure the UV–Visible spectra of our two water types. Therefore, we estimated the diffuse attenuation coefficients (k) for UV-B light in these waters using measured Secchi disc depth (z_{SD}) and empirically derived relationships between k and z_{SD} (Calkins 1975). Secchi disc depth was recorded on each day that a fresh sample was collected.

Photochemical production of LMWOAs and DIC occur at the surface, whereas bacterial metabolism of these compounds occurs throughout the water column. Thus, in order to estimate the contribution of photodegradation and photomineralization to bacterial metabolism and overall CO_2 evasion rates in these rivers, we used our experimental rates to calculate mean daily depth-integrated rates for 03°S latitude over the course of a typical year. Depth-integrated rates were estimated for the Rio Negro only because there was no detectable photochemical production of LMWOAs or DIC during the Rio Solimões experiments (see Results).

For a range of natural waters, Calkins (1975) derived the following relationship between k and z_{SD}: $k = 9/z_{SD}$. Using the Beer–Lambert law (Kirk 1977) to model the vertical attenuation of light in water with depth and our measured Secchi disc depth for the Rio Negro (52.5 ± 3.5 cm), this k corresponds to a 90% attenuation depth of about 15 cm for UV-B light. Therefore, we integrated over a depth of 20 cm. In order to integrate over this depth, we needed to know the amount of light incident on the water surface (I_0) and this was not directly measured during our field experiments. However, we did measure light intensity at 2.5 cm ($I_{2.5}$) depth using the HOBO Temperature/Light data loggers. Thus, to get an estimate of I_0 during the course of our experiments, we used the mean $I_{2.5}$ measured during each Rio Negro experiment.

For our overall production estimate, we calculated a daily production rate for each experiment by multiplying hourly rates by 12 h of daylight and then normalized to light intensity using the calculated I_0. The daily production rate for an average day at this latitude was then calculated by multiplying this value by a mean daily irradiance of 4.21 ± 1.46 Mlux/day, which was obtained from multi-year, daily irradiance data collected at an LBA tower site in Santarem, Ceara State, Brazil (5°10′S, 39°40′W) from July 2000 to March 2004. The daily mean value of 4.21 ± 1.46 Mlux was determined by transforming tower irradiance data in W/m^2 to Mlux based on the spectral distribution of sunlight. We integrated our volumetric daily production rates over the top 20 cm of the water column to obtain depth-integrated, areal daily production rates.

We conducted a single-factor sensitivity analysis of our calculation by varying three input values that are not well-constrained. We determined the effect of varying these three values on the mean sum of the acetic acid, formic acid and DIC production rates for the two Rio Negro photochemical experiments. As noted above, Calkins (1975) found a mean coefficient of 9 for a range of natural waters for the following relationship between z_{SD} and k: $k = 9/z_{SD}$. This coefficient ranged from values as high as 25 for humic waters to as low as 2 for turbid waters. Therefore, the k coefficients values bounding this range were used to calculate k. The mean daily irradiance was also varied in the calculations because the amount of light, and especially UV-B light, incident at the water surface varies with atmospheric conditions such as solar angle and cloud cover. We varied daily irradiance using the 4-year maximum (6.91 Mlux) and minimum (0.02 Mlux) irradiance values from the Santarem tower site data set. Finally, the depth over which the experimental rates were integrated was also varied because the k values used in the calculations are for UV-B light only. UV-A and some visible wavelengths of light also contribute to DOM photochemistry (Granéli et al. 1998). UV-B light has the shortest wavelength of all three types and will be attenuated at the shallowest depths, whereas visible light has the longest wavelength and will penetrate the deepest. Poole and Atkins (1929) derived a mean slope of 1.7 for a plot of z_{SD} versus $1/k$ for visible light in a range of natural waters, which corresponds to 90% attenuation of visible light at a depth of about 70 cm. The highest slope of 25 found by Calkins (1975) for UV-B light corresponds to a 90% UV-B light attenuation depth of 5 cm. Therefore, the depth of integration was varied from 5 to 70 cm to cover all depths to which wavelengths important to DOM photochemistry may penetrate.

Results

Photochemical degradation and photomineralization experiments

With the exception of acetic acid in the Solimões 1 sample set, all data sets passed the Kolmogorov–Smirnov test for normality with significance values

greater than 0.05, indicating a non-significant result and evidence for a normal distribution. For both acetic and formic acid in the Rio Negro, there was a statistically significant difference in concentration between the irradiated samples and the initial samples, and between the irradiated samples and the dark controls (Table 1). For the S1 and NS2 experiments, there was no statistically significant difference among the three sample types (Table 1).

There was a statistically significant difference in concentration between the irradiated samples and the initial samples, and the irradiated samples and the dark controls, for the N1 photomineralization experiment (Table 1). No initial sample was taken for the N2 experiment, but there was a statistically significant difference between the irradiate samples and the dark controls. There was no statistically significant production of DIC in either the S1 or NS2 experiments. However, due to the high background DIC concentrations in the Rio Solimões, photochemical DIC production that may have occurred may not have been detectable by our methods.

Bacterial utilization of acetic and formic acids

Across all experimental concentrations and water types, formic acid utilization rates were almost always less than acetic acid (Fig. 1). In the Rio Negro incubations, the formic acid incorporation rate ($I_{Neg,FA}$) reached saturation at 12 nM C/h and at a formic acid concentration of approximately 1,200 nM C (Fig. 1b). The formic acid respiration rate ($R_{Neg,FA}$) did not reach saturation over the range of experimental concentrations, but it began to level off (Fig. 1b). For all experimental concentrations, more formic acid was respired than incorporated (Fig. 1b). At ambient formic acid concentrations in the Rio Negro (Table 1), $R_{Neg,FA}$ is four times that of $I_{Neg,FA}$ (Table 2). Neither Rio Negro acetic acid incorporation ($I_{Neg,AA}$) nor respiration ($R_{Neg,AA}$) rates reached saturation over the range of experimental concentrations (Fig. 1a). For all concentrations, much more acetic acid was incorporated into biomass than was respired. At ambient acetic acid concentrations in the Rio Negro (Table 1), $I_{Neg,AA}$ is four times that of $R_{Neg,AA}$ (Table 2).

In the Rio Solimões incubations, formic acid uptake rates did not reach saturation within the range of experimental concentrations and the formic acid respiration rate ($R_{Sol,FA}$) was higher than the incorporation rate ($I_{Sol,FA}$) (Fig. 1d). As in the Rio Negro, at ambient formic acid concentrations in the Rio Solimões, $R_{Sol,FA}$ is four times $I_{Sol,FA}$ (Table 2). The acetic acid uptake rates also did not reach saturation in the Rio Solimões incubations over the range of experimental concentrations (Fig. 1c). The acetic acid incorporation rate ($I_{Sol,AA}$) was higher than the respiration rate ($R_{Sol,AA}$). At ambient acetic acid concentrations, $I_{Sol,AA}$ is about 2.5 times $R_{Sol,AA}$ (Table 2).

We estimated the contribution of formic and acetic acid respiration to the total CO_2 evading from the two water types studied here using the BGE calculated from our experimental data (Table 2). In the Rio Negro incubations, the formic acid BGE increased with concentration until formic acid uptake neared saturation at about 1,200 nM C/h and there was a slight decrease between the second and third experimental concentration (Fig. 1b). At ambient concentrations, BGE for the Rio Negro was about 0.20 (Table 2). This implies that 80% of utilized formic acid in the Rio Negro is respired. For acetic acid in this river, there was a slight increase in BGE with concentration (Fig. 1a) and at ambient concentrations BGE was 0.80 (Table 2). This implies that about 20% of acetic acid in the Rio Negro is respired. Acetic acid BGE was four times higher in this river than formic acid BGE (Table 2).

For Rio Solimões formic acid, BGE increased with experimental concentration (Fig. 1d) and at ambient concentrations was 0.20 (Table 2). Acetic acid BGE in the Rio Solimões also increased with increasing experimental concentration (Fig. 1c). At ambient concentrations, the acetic acid BGE was 0.73 (Table 2), implying that 27% of acetic acid is respired in this river. Acetic acid BGE in the Rio Solimões was 3.5 times higher than formic acid BGE (Table 2).

Depth-integrated photodegradation and photomineralization rates

The depth-integrated acetic acid, formic acid and DIC photoproduction rates are shown in Table 3. The results of the sensitivity analysis are shown in Fig. 2. The mean sum of the acetic acid, formic acid and

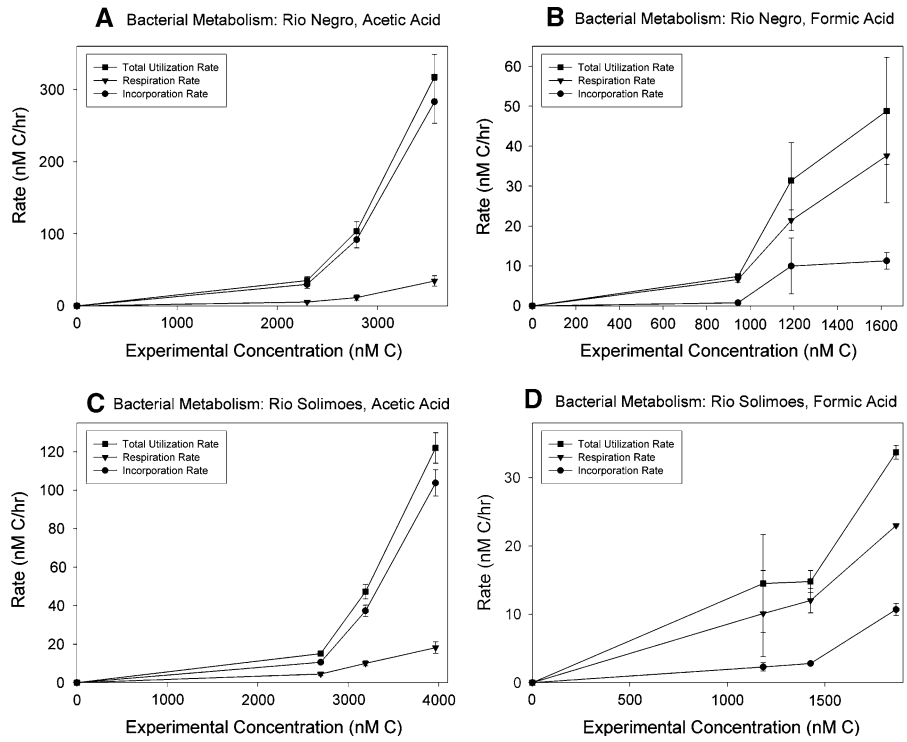

Fig. 1 Results of bacterial uptake experiments for the Rio Negro (**a**, **b**) and the Rio Solimões (**c**, **d**). *Error bars* are the standard deviation of four replicate incubations

Table 2 Respiration (BR) and incorporation rates (BP), and BGEs, for both acids at ambient concentrations in both rivers

	BP (nM C/h)	BR (nM C/h)	Total utilization (nM C/h)	BGE
Negro				
Acetic acid	20 ± 3	5 ± 1	25 ± 3	0.80
Formic acid	1 ± 0.03	4 ± 1	5 ± 1	0.20
Solimões				
Acetic acid	8 ± 1	3 ± 0.5	11 ± 1	0.73
Formic acid	2 ± 0.1	8 ± 1	10 ± 1	0.20

DIC production rates (ΣNeg) for the two Rio Negro photochemical experiments for the original depth integrated calculations was 260 ± 140. At higher k coefficients than that used in our calculation (k coefficient = 9), ΣNeg is up to 2.5 times lower than the value that we calculated based on our assumptions. Over the range of possible daily irradiance values used in the sensitivity analysis, ΣNeg changes linearly and over two orders of magnitude. Given the k coefficient value that we used in our calculation, ΣNeg is most sensitive to shallow integration depths of about 20 cm or less.

Discussion

Photochemical degradation and photomineralization experiments

Given the diversity of aquatic ecosystems composing the 700,000 km^2 basin drained by the Rio Negro, DOM used in our photochemical experiments is likely a mixture of DOM derived from lakes, stream, wetlands and macrophytes. This is supported by the fact the our acetic and formic acid relative production rates (Table 1), which are equivalent within the

Table 3 Depth-integrated photoproduction rates calculated from Rio Negro photodegradation and photomineralization experimental data

	N1 (6.6 h) acetic	N1 (6.6 h) formic	N1 (6.6 h) DIC	N2 (6.8 h) acetic	N2 (6.8 h) formic	N2 (6.8 h) DIC
Production rate (nM C/h)	140 ± 60	135 ± 70	2,400 ± 1,000	78 ± 80*	175 ± 50	2,200 ± 400
Irradiance at water surface, I_0 (Mlux)[1]	3.1 ± 0.3	3.1 ± 0.3	3.1 ± 0.3	3.0 ± 0.3	3.0 ± 0.3	3.0 ± 0.3
Daily production rate (nM C/day)	2,200 ± 1,200	2,200 ± 1,400	39,000 ± 21,000	1,200 ± 1,400*	3,000 ± 1,600	37,000 ± 15,000
Depth-integrated production rates (nmol C/cm^2 day)	10 ± 5	10 ± 5	200 ± 100	10 ± 5*	20 ± 10	200 ± 100

* Not statistically significant

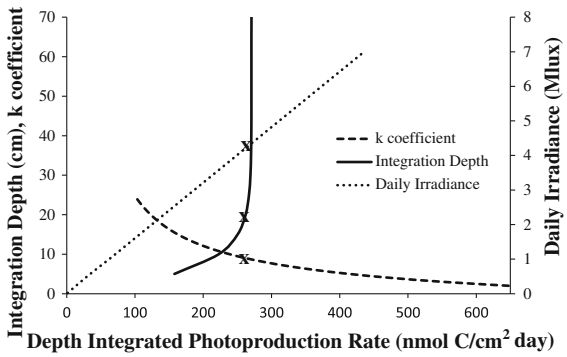

Fig. 2 Results of the single-factor sensivity analysis

calculated error, fall within range of the relative rates of several studies in which OM from specific aquatic environments was photochemically degraded. Irradiation studies of water from humic-rich lakes, streams, and wetlands and from macrophyte leachate demonstrate formic acid:acetic acid photoproduction rate ratios that range from 2 to 4 times higher (Bertilsson and Tranvik 1998; Bertilsson et al. 1999; Dahlén et al. 1996) to five times lower (Wetzel et al. 1995), and the ratio has been found to vary in sign depending on the species of macrophyte (Farjalla et al. 2001).

Photo-oxidation rates are correlated with DOC concentration (Granéli et al. 1996). Thus, in order to compare our absolute photoproduction rates for the Rio Negro to other studies of similarly Fe-rich, low pH, humic waters, we normalized our hourly production rates to the DOC concentration of the Rio Negro (10 mg/l, Ertel et al. 1986). This yielded DOC-normalized production rates of 11 nM C/h for acetic acid, 16 nM C/h for formic acid and 230 nM C/h for DIC. Slightly higher DOC-normalized photodegradation rates for acetic acid (30 nM C/h) and formic acid (60 nM C/h) were found by Bertilsson and Tranvik (1998) in a humic lake in Sweden. At a similar latitude in humic streams, Bertilsson et al. (1999) found a range of DOC-normalized photoproduction rates of acetic (6–40 nM C/h) and formic (7–50 nM C/h) acids. Our values fall within these ranges. Dahlén et al. (1996) also find photoproduction rates of acetic (3 nM C/h) and formic (17 nM C/h) acid in a humic lake that are comparable to our Rio Negro rates. A key different between these studies and ours is that, due to the more turbulent nature of water in river channels, we incubated our samples at 2.5 cm depth and these other studies incubated their samples at the water surface. In humic waters, UV-B light is attenuated to 10% of the surface irradiance values within the first 0.5–2.5 cm (Granéli et al. 1998). Therefore, very little UV-B reached our samples at 2.5 cm depth. In a survey of 38 lakes, Bertilsson and Tranvik (2000) found that 80% of the variability in photoproduction rates among four carboxylic acids could be explained by the total absorbed radiation energy. Thus, due to our deeper incubation depths, our rates are likely underestimates of the actual photoproduction of acetic and formic acid in the Rio Negro.

Our Rio Negro DIC production rates are lower than rates determined for similar waters. Rates determined by Granéli et al. (1998) for the Rio Negro (980 nM C/h, DOC-normalized) are almost an order of magnitude greater than the DOC-normalized DIC production rates determined in our experiment. Bertilsson and Tranvik (2000) found DOC-normalized DIC production rates ranging from 2 to 900 nM C/h for 38 chemically diverse lakes. The lower rate in our experiment may be

due to our incubation depth of 2.5 cm compared to the water surface for these studies.

In addition to being underestimates of actual photoproduction rates at the water surface, the relative amounts of LMWOAs versus DIC produced may also be inaccurate in our study. In the Rio Negro, the LMWOA production rate was about 10% of the DIC production rate. In other studies of humic waters, LWMOA photoproduction rates are a much larger percentage. In a survey of 38 lakes, Bertilsson and Tranvik (2000) found that LMWOA production was about 34% of the median DIC production rate. The higher values reported in this study may be another consequence of our incubation depth (2.5 cm versus the water surface). Keiber et al. (1990) found no production of LMW carbonyl compounds when UV-B light was excluded. For DIC photoproduction, UV-A and PAR wavelengths are more important than UV-B (Granéli et al. 1998). Therefore, it may be that the lower percentage of LMWOAs produced at 2.5 cm depth in our study, relative to DIC, may be due to the exclusion of most UV-B light at this depth combined with a higher proportion of wavelengths important in DIC production.

DOM photochemistry comparison between the Rio Negro and Rio Solimões

The lack of detectable LMWOA and DIC production in the Rio Solimões may be a result of water chemistry and the availability of photolabile DOM. DOM is more photochemically reactive in lakes with lower pH, alkalinity and conductivity (Bertilsson and Tranvik 2000). The Rio Negro has a low pH (4.0), zero alkalinity and low conductivity (15 μS) relative to the Rio Solimões (7.0, 400–900 μeq/l, and 70 μS, respectively) (Devol et al. 1995). Another difference is the dissolved Fe concentrations in the Rio Negro (3 μM) and in the Solimões (1 μM) (Aucour et al. 2003). Photoproduction rates of DIC and other DOM photoproducts increase in the presence of Fe (Gao and Zepp 1998; Miles and Brezonik 1981). Bertilsson and Tranvik (2000) found a positive correlation between DIC photoproduction and dissolved Fe concentrations.

Differences in the photolability of DOM itself may also explain the differences in DOM photochemistry between the Rio Negro and the Rio Solimões. The concentration of dissolved humic substances (DHSs) in the Solimões (1.5 mg C/l) is lower than that of the Rio Negro (5.8 mg C/l) (Ertel et al. 1986). Lindell et al. (2000) found that both DIC and LMWOA photoproduction rates were eight times higher in humic lakes compared to clear water lakes with low concentrations of DHSs. In addition to lower DHS concentrations, DOM in the sediment-laden Rio Solimões may be protected from photodegradation by mineral sorption. Tietjen et al. (2005) found that DHSs are preferentially sorbed by clay minerals.

It is important to note that our study considered only the dissolved OM pools. The removal of particulate organic matter (POM) during filter-sterilization of Rio Solimões water prior to our photochemical experiments may have removed a source of photoreactive organic matter. Mayer et al. (2006) demonstrated the loss of 2 mg OC/l of POM from rivers in the lower Mississippi basin during irradiation. This corresponds to a loss rate of almost 2,000 nM C/h and is comparable to our DIC production rates in the Rio Negro. Mayer et al. (2006) suggest photodissolution, or the desorption of OM from clay mineral surfaces, as the major mechanism of photochemical POM loss. Therefore, in addition to releasing bioavailable DOM (Baldock and Skjemstad 2000; Keil et al. 1994), photodissolution may also release DOM that can be further degraded by sunlight since photolabile DOM is also preferentially sorbed (Tietjen et al. 2005).

Bacterial utilization of acetic and formic acids

Depending on experimental concentration, total utilization rates of acetic acid were 3–7 times faster than formic acid for the Rio Negro and 2–3 times faster for the Rio Solimões. These results are consistent with a similar ^{14}C-labeled substrate experiment by Bertilsson and Tranvik (1998). Acetic acid is used by bacteria in many metabolic reactions (Gottschalk 1986), possibly due to the different molecular thermodynamic energy contents (Eiler et al. 2003), while formic acid is not.

One major difference in formic acid total utilization between the two water types is that the formic acid total utilization rate reached or approached saturation within the range of experimental concentrations for the Rio Negro, whereas total utilization rates did not reach a maximum in the Rio Solimões. These trends may be a function of a positive

correlation between bacterial abundance and pH (Ellis et al. in review). In the Rio Negro (mean pH of 4), formic acid total utilization rates may be limited by the number of bacterial cells present. The Solimões mean pH is 7.

In both rivers, R_{FA} was 2–3 times higher than the I_{FA} and this has been observed in other studies (Bertilsson and Tranvik 1998). It may be that the bacteria are not actually using formic acid during respiration. Rather, they may be converting the formic acid to CO_2 by some other means, such as mineralization to CO_2 by the extracellular enzyme formadehydrogenase (Gottschalk 1986).

In both water types and for both LMWOAs, except for formic acid in the Rio Negro where incorporation rate leveled off (Fig. 1b), BGE increased with experimental concentration. This increase with increasing concentration may be a result of increased bacterial growth with increased bioavailable substrate concentrations (acetic and formic acid). Eiler et al. (2003) found that BGE increased with increasing bulk DOC concentration. The higher BGE found for acetic acid at ambient concentrations in both rivers is consistent with other studies of the uptake of ^{14}C-labeled compounds (Bertilsson and Tranvik 1998). Higher acetic acid BGEs relative to formic acid might be explained by the frequent incorporation of acetic acid into metabolic reaction pathways (Gottschalk 1986).

BGE for formic acid at ambient concentrations was similar in the Rio Negro and the Rio Solimões; however, acetic acid BGE was slightly higher in the Rio Negro. Bacteria need a source of reduced organic or inorganic nitrogen for growth. Compared to the Rio Solimões, dissolved NO_3^- is lower in the Negro and NH_4^+ is low in both rivers (Richey et al. 1990). This contradicts other studies in which a neutral response or a negative response of BGE was observed with the addition of biolabile organic substrates (Abboudi et al. 2008; Reche et al. 1998). Rio Solimões bacteria may be using fine particulate organic carbon (FPOC) as a substrate and, therefore, do not need to rely as much on acetic acid (Ellis et al. in review). FPOC concentrations are twice as high in the Solimões as in the Negro (Hedges et al. 1994). Bulk BGEs range from 0.04 to 0.55 for a variety of whitewater and blackwater rivers in the Amazon basin (Benner et al. 1995). Our acetic acid BGEs are much higher, whereas our formic acid BGEs fall on the low end of this range. However, our compound-specific BGE values cannot be directly compared to this range, which is for a mixture of 1000s of molecules composing the carbon pool of these rivers.

Using data collected from previous studies of bacterial metabolism in these rivers, we can assess the role of these compounds in bacterial metabolism. Mean bacterial production for total organic carbon (DOM + POM) is about 100 nM C/h for both the Negro and Solimões (Benner et al. 1995). Using our total utilization rates at ambient concentrations (Table 2), bacterial incorporation of acetic and formic acid accounts for about 20% of the total BP in the Rio Negro and 10% in the Solimões. The lower contribution of acetic acid to total BP in the Solimões may be a result of the use of other substrates (FPOC, Ellis et al. in review). Despite the nutrient limitation in the Rio Negro (Benner et al. 1995), more growth may be fueled by acetic acid compared to a wider range of substrates in the Solimões (i.e. acetic acid and FPOC). Based on our measured rates, formic and acetic acid respiration rates make up a significant percentage of bulk respiration rates: Rio Negro = 100 nM, Rio Solimões = 300 nM C/h (Ellis et al. in review). Thus, acetic and formic acid respiration together account for 10% of total respiration in the Rio Negro and 5% in the Rio Solimões.

Effects of DOM photochemistry on bacterial metabolism

Using our depth-integrated acetic and formic acid production rates from the Rio Negro, we assessed the role of photochemically produced acetic and formic acid in bacterial metabolism. For most of its course, the depth of the Rio Negro is about 10 m during low water (Richey, J.E., unpublished data). Integrating our bacterial utilization rates over this depth, we obtain depth-integrated daily rates for total utilization (720 nmol C/cm^2 day), respiration (144 nmol C/cm^2 day) and incorporation (528 nmol C/cm^2 day) of acetic and formic acid combined. Comparing these rates to our depth-integrated photodegradation rates, we find that photoproduction of these acids in the Rio Negro may fuel as much as 3% of total utilization, 17% of respiration and 5% of incorporation of these compounds. The Rio Negro can reach up to 50 m depth and, assuming similar bacterial uptake rates of

these acids during high water, photochemically produced acetic and formic acids may play a much smaller role in bacterial metabolism at that stage of the hydrograph.

Other studies in the Amazon river basin have looked at the effect of photochemistry on bacterial metabolism at various stages of the hydrograph. During high water and at the same locations as our study, Amon and Benner (1996) used leucine and thymidine incorporation rates to measure the effect of photochemically produced substrates on bacterial metabolism. However, they suggest that their results are inconclusive due to a faulty experimental design. Amado et al. (2006) used measured bacterial production and respiration in a clear water lake and stream located in the Brazilian Amazon basin. They found varying degrees of photochemical stimulation of bacterial production and respiration at different stages of the hydrograph. Thus, seasonal differences in the impact of DOM photochemistry on bacterial metabolism may have been missed in our study (low water) and by Amon and Benner (high water).

Effect of DOM photochemistry on CO_2 evasion in the Rio Negro

The overall contribution of DOM photochemistry to CO_2 evading from water surfaces in the Rio Negro is a combination of the direct production of CO_2 by photomineralization of DOM and the addition of biolabile substrates produced by photodegradation that are subsequently respired to CO_2. An average of 45 μmol C/cm^2 day as CO_2 evades from the Rio Negro (Alin et al. in review). Comparing this to our depth-integrated photomineralization rates (Table 3), photochemically produced CO_2 contributes 0.5% to CO_2 evasion from this river. We used the average depth-integrated acetic (10 nmol C/cm^2 day) and formic (15 nmol C/cm^2 day) acid photoproduction rates and BGEs of 0.80 and 0.20, respectively, to calculate the percent contribution of the respiration and found that photochemically produced acetic and formic acid collectively contribute <0.05% to CO_2 evasion.

Accounting for direct (photomineralization) and indirect (photodegradation) addition of CO_2 resulting from the interaction of sunlight with DOM, our measurements show that photochemically produced DIC, acetic acid and formic acid collectively contribute about 0.5% of CO_2 evasion in the Rio Negro. Amon and Benner (1996) found a DOC loss rate due to photochemical degradation of 4,000 nM C/h. Integrating this rate over the top 20 cm of the water column, we find a depth-integrated DOC loss rate due to photochemistry of about 2,000 nmol C/cm^2 day. This value is about 10 times our combined depth-integrated rates for DIC and LMWOA photoproduction (200 ± 80 nmol C/cm^2 day). Our compound-specific production rates are consistent with, although an order of magnitude lower than, the DOC loss rate measured by Amon and Benner (1996). Amon and Benner (1996) caution that their DOC loss rates should be considered conservative due to the use of Pyrex glass, which absorbed 20% of UV-A and 50% of UV-B irradiance, rather than quartz containers during their photochemical degradation experiments.

Because of the assumptions made in our calculation, these numbers should be viewed with caution. The results of the sensitivity analysis show that depth-integrated rates would be up to 2.5 times lower if the k coefficient used in the calculation were higher. The highest k coefficient used as the upper bound for this analysis (25) was that found for humic waters (Calkins 1975). In addition, the calculation is very sensitive to integration depths <20 cm, below which production rates decrease exponentially. If the k coefficient of 25 is more accurate for the Rio Negro, then integration depth would be much shallower because a k coefficient of 25 corresponds to a 90% UV-B attenuation depth of about 5 cm. However, UV-B light is not the only wavelength of importance, especially for DIC photoproduction (Granéli et al. 1998).

Impact of photochemical processes on CO_2 evasion from Amazonian waters

Conclusions drawn from this study regarding the overall contribution of photochemistry to CO_2 evasion in the Amazon river system should be tentative. There are several reasons why our DIC and LMWOA photochemical production rates may be conservative. First, samples were incubated under natural sunlight at 2.5 cm depth and this incubation depth may exclude photochemically important wavelengths (i.e. UV-B). Second, we measured only two of the many possible biolabile photoproducts formed from

the degradation of DOM. Sixteen specific compounds and two major compound classes (amino acids and carbohydrates) have been measured as photoproducts in a range of water types and using analytical methods (Moran and Covert 2003). Also, the bulk DOM remaining after photochemical interaction may be rendered more biolabile (Moran and Zepp 1997) and this was not assessed here. Third, the importance of photochemistry in the Rio Solimões is not conclusive without the assessment of POM photochemistry, as demonstrated by Mayer et al. (2006) in the Mississippi river. Finally, our photochemical experiments were conducted during falling water, but rivers may be loaded with more photochemically reactive OM during rising and high water (Smith et al. 1997; Suhett et al. 2007; Lindell et al. 2000; Rodriguea-Zuniga et al. 2008). During identical trial experiments conducted on the Rio Negro in April 2007 (rising water), a combined acetic and formic acid photoproduction rate of about 600 nM C/h was measured. This hourly photodegradation rate is twice the hourly photodegradation rate measured in October 2007 during falling water (Table 1). During the April 2007 experiments, DIC production was not measured and light sensors were not deployed; however, assuming a similar increase in DIC production and a comparable light regime, DOM photochemistry may contribute up to 20% of CO_2 evasion in this river during rising water from photochemical production of DIC, acetic acid and formic acid alone. Future studies of DOM photochemistry in the Amazon should be seasonal.

Conclusions

Our results suggest that photochemical degradation of OM to DIC, acetic acid and formic acid contributes a small (0.5%) fraction of the CO_2 evading from water surfaces in the Rio Negro during falling water. However, our estimate of photochemical contribution to CO_2 evasion is uncertain and likely an underestimate due to the extinction of UV wavelengths at our incubation depth, other photochemically produced compounds that may have been present but were not measured, and the exclusion of POM and seasonal dynamics. In the Rio Negro, there are likely significant CO_2 sources other than respiration and photochemistry. In the future other sources of CO_2 and biolabile substrates to this river, such as anaerobic "hotspots" (hyporheic zones, mid-channel sandbars and macrophyte beds) and precipitation (from photodegradation of isoprenes in the atmosphere emitted by trees), should be investigated. The percent contribution of respiration to CO_2 evasion in the Rio Solimões (80–100%, Ellis et al. in review) and POM loss rates determined by Mayer et al. (2006) suggest that water column respiration and photochemistry may be able to account for all of the CO_2 evading from water surfaces in this river. The accurate quantification of photochemical and other processes adding CO_2 to these rivers will be necessary to better constrain the sources of the CO_2 evading from river surfaces in the Amazon basin.

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