The fate of C₄ and C₃ macrophyte carbon in central Amazon floodplain waters: Insights from a batch experiment

J.M. Mortillaro a,1,*, C. Passarelli a, G. Abri b,c, C. Hubas a, P. Alberic d, L.F. Artigas e, M.F. Benedetti f, N. Thiney a, P. Moreira-Turcq g, M.A.P. Perez c,f, L.O. Vidal h, T. Meziane a

a Unité Mixte de Recherche Biologie des organismes et écosystèmes aquatiques (BOREA UMR 7208), Sorbonne Université, Muséum national d'Histoire naturelle, Université Pierre et Marie Curie, Institut de Recherche pour le Développement, Université de Caen Basse-Normandie, Université des Antilles, Paris, France
b Laboratoire Environnements et Paléoenvironments Océaniques (EPOC) UMR-CNRS 5805, Université de Bordeaux, Allée Geoffroy Saint-Hilaire 33615 Pessac, France
c Universidade Federal Fluminense, Department of Geochemistry, Niterói, Rio de Janeiro, Brazil
d Institut des Sciences de la Terre d’Orléans, 1A rue de la Férolerie, 45071 Orléans Cedex 2, France
e Laboratoire d’Océanologie et Géosciences (LOG), UMR-CNRS-UlCO-UL1 8187, Université du Littoral Côte d’Opale (ULCO), 32 avenue Foch, 62930 Wimereux, France
f Equipe Géochimie des Eaux, Institut de Physique du Globe de Paris, Université Paris Diderot, Sorbonne Paris Cité, 35 rue Hélène Brion, 75205 Paris Cedex 13, France
g Institut de Recherche pour le Développement, (IRD)–GEO, Lima, Peru
h Laboratório de Ecologia Aquática, Departamento de Biologia, Universidade Federal de Juiz de Fora, Rua José Lourenço Kelmer, MG 36036-900 Juiz de Fora, Brazil
i CIRAD, UMR 116 ISEM, Institut de Recherche pour le Développement, TA B116/16, Montpellier, France

ARTICLE INFO

Article history:
Received 15 May 2015
Received in revised form 1 March 2016
Accepted 6 March 2016
Available online 17 May 2016

SUMMARY

The central Amazon floodplains are particularly productive ecosystems, where a large diversity of organic carbon sources are available for aquatic organisms. Despite the fact that C₄ macrophytes generally produce larger biomasses than C₃ macrophytes, food webs in the central Amazon floodplains appear dominantly based on a C₃ carbon source.

In order to investigate the respective fate and degradation patterns of C₄ and C₃ aquatic plant-derived material in central Amazon floodplains, we developed a 23-days batch experiment. Fatty acid and carbon concentrations as well as stable isotope compositions were monitored over time in 60L tanks. These tanks contained Amazon water, with different biomasses of C₄ and C₃ macrophyte, representative of in situ densities occurring in central Amazon floodplains.

In the C₄ Paspalum repens treatments, organic (POC, DOC) and inorganic carbon (DIC) got rapidly enriched in ¹³C, whereas in the C₃ Salvinia auriculata treatments, POC and DOC showed little change in concentration and isotopic composition, and DIC got depleted in ¹³C. The contribution of P. repens to POC and DOC was estimated to reach up to 94.2 and 70.7%, respectively. In contrast, no differences were reported between the C₃ S. auriculata and control treatments, an observation attributed to the lower C₃ biomass encountered in the field, to a slower degradation rate of C₃ compared to C₄ compounds, and to similar isotopic compositions for river POC and DOC, and C₃ compounds.

The ¹³C enrichments of POC, DOC, and DIC from P. repens treatments were attributed to an enhanced bacterially-mediated hydrolysis and mineralization of C₄ material. Evolutions of bacterial abundance and branched fatty acid concentrations confirmed the role of heterotrophic microbial communities in the high P. repens decomposition rate. Our experiment highlights the predominant role of C₃ aquatic plants, as a large source of almost entirely biodegradable organic matter available for heterotrophic activity and CO₂ outgassing to the atmosphere.

© 2016 Elsevier GmbH. All rights reserved.

1. Introduction

One of the largest sources of organic carbon in Amazon floodplains is derived from aquatic macrophytes, which contribute up
to half of the ecosystem primary production (Melack and Forsberg, 2001). These macrophytes grow emerged, submerged or floating, with 388 species described in a várzea (i.e. white-water nutrient rich floodplain) located near the city of Manaus, in central Amazon (Junk and Piedade, 1993b). Among the most abundant species, the floating grasses Echinocloa polystachya and Paspalum fasciculatum (Poaceae family) can reach biomasses of 80 and 60 t ha⁻¹, respectively (Junk and Piedade, 1993a; Piedade et al., 1991). Another macrophyte largely represented in the várzea is P. repens (up to 22 t ha⁻¹. Fig. 1, Junk and Piedade, 1993a). These aerial species, which convert atmospheric carbon dioxide into biomass through a C₃ pathway, constitute floating meadows that can extend over large areas of floodplains (Junk and Howard-Williams, 1984; Hess et al., 2003; Silva et al., 2013). Amazon floating meadows may also be composed of macrophytes using the C₄ photosynthetic pathway such as Eichhornia sp., Pistia stratiotes and Salvinia auriculata (Fig. 1). However, the biomass of all these C₄ macrophytes add up to 3–15 t ha⁻¹ only (Furch and Junk, 1992).

The ecology of C₃ and C₄ macrophytes, and particularly their biomass and production rates, have been thoroughly examined (e.g. Morison et al., 2000; Engle et al., 2008; Silva et al., 2013). Despite their large abundance, C₄ macrophytes constitute a minor source of energy for Amazon aquatic food webs (Hamilton et al., 1992; Forsberg et al., 1993; Mortillaro et al., 2015) and modest contributors to particulate organic carbon (POC, Hedges et al., 1986; Mortillaro et al., 2011; Moreira-Turcq et al., 2013). Indeed, C₄ macrophytes are largely ¹³C enriched (−12‰, Hedges et al., 1986; Mortillaro et al., 2011) compared to POC in rivers and várzea of the Amazon (−30‰, Quay et al., 1992; Hedges et al., 1994). The almost constant isotopic composition of dissolved organic carbon (DOC), at around −29‰ in the Amazon River, suggests a dominant C₃ source such as terrestrial plants and/or macrophytes. In contrast, dissolved inorganic carbon (DIC) is much heavier (−17.7 to −11.5‰, Quay et al., 1992), i.e. closer to C₄ macrophytes signature, but also closer to the signature of atmospheric CO₂. This isotopic composition results from isotopic equilibration induced by CO₂ gas exchange (Quay et al., 1992; Hedges et al., 1994; Mayorga et al., 2005; Abril et al., 2014), and/or due to carbonate mineral weathering (e.g. chemical or mechanical decay of rocks, Mayorga et al., 2005).

Previous macrophyte (C₃ and C₄) degradation experiments in litterbags, exposed to natural weathering, found an initially rapid loss of weight and a decrease in nutrients (Howard-Williams and Junk, 1976; Furch and Junk, 1992). However, the contribution of macrophytes to the Amazon aquatic food webs was not verified by Fellerhoff et al. (2003) in their degradation experiment. Therefore, in order to examine the apparent discrepancy between the high biomass of C₄ macrophytes in the floodplains and their modest contribution to the organic matter (OM) pool in the Amazon river-floodplain ecosystem, a degradation experiment was designed to investigate the fate of carbon from C₄ and C₃ macrophytes in microcosms. Particulate and dissolved carbon concentrations, stable isotope compositions and fatty acids (FA) concentrations, were analyzed in large volumes of Amazon waters incubated with variable amounts of a C₄ and a C₃ macrophyte. Bacterial abundance and nutrient concentrations complemented the description of macrophyte degradation throughout a 23-day experiment. Our work hypothesis was that very fast decomposition and mineralization of C₄ macrophytes explain their low contribution to central Amazon aquatic food webs.

2. Material and methods

2.1. Sampling

In order to follow the fate of OM during the degradation of two aquatic macrophytes and the influence of degradation products on the quality of POM, samples of P. repens (C₄) and S. auriculata (C₃, Fig. 1) were collected in the Camaleão Lake (várzea), located by the Solimões River. These two macrophytes were selected for their widespread distribution in central Amazon floodplains and their large FA concentration compared to other macrophyte species (i.e. Eichhornia sp. and Pistia stratiotes), as characterized in a previous study (Mortillaro et al., 2011).

Macrophyte samples were sorted in order to eliminate dead leaves and washed to remove sediment and invertebrates. About 900 L of water were also sampled from the lake and distributed into 15 microcosms of 60 L each. Three water samples and three portions of each macrophyte were collected in order to get their initial composition (i.e. stable isotopes and FA).

2.2. Experimental setup

The experiment was implemented on the Yane José IV boat in August-September 2010. Five experimental treatments were used: high and low biomasses of P. repens (PR-HB and PR-LB, respectively) and S. auriculata (SA-HB and SA-LB, respectively), as well as a control without macrophyte (Ctrl). These five treatments were selected...
to distinguish species effect from biomass effect on water characterization. For each treatment, triplicate microcosms were used. In PR-HB microcosms, 250 g of fresh P. repens leaves (25.6 gC) were used. This amount was estimated from a maximum dry biomass of 22 t ha⁻¹ (recorded in the Januári Lake near Camaléo Lake, Junk and Piedade, 1993a), with less than one fifth of emerged P. repens biomass (Silva et al., 2009), an estimated water proportion of 75.9% and a microcosms surface of 0.13 m². Similarly, the mean S. auriculata dry biomass was estimated to be 1 ha⁻¹ (Junk and Piedade, 1997) giving a theoretical wet weight of 200 g (93.5% of water). However, low biomasses of S. auriculata were available during sampling, so that only 100 g (2.3 gC) per microcosm were used (SA-HB). Low biomass treatments contained 10% of the high biomass amount for each species (25 g of macrophytes for PR-LB and 10 g for SA-LB). Macrophyte leaves were introduced into microcosms filled with floodplain water, and were kept in the dark under constant temperature conditions (28°C). Permanent stirring with a water pump ensured oxygenation of the microcosms (from 40 to 100% air-saturation) and avoided anaerobic conditions to occur. Although low dissolved oxygen is common under floating macrophytes in várzea (~50% saturation, Engle and Melack, 1993), permanent oxygenation was maintained in order to match natural conditions within floating meadows in open waters, where wind and water currents are sufficient to maintain aerated to hypoxic waters (30% min) in 2–6 m depth water column (Vidal et al., 2015).

In each treatment, samples were collected after 3, 6, 12, 18 and 23 days of experiment. At each sampling time, around 3.5 L of water were collected and pre-filtered on 200 μm to avoid heterogeneity between samples due to contamination with macrophyte fragments. Consequently, POC concentrations reported here concern the fraction smaller than 200 μm. Microcosms volume (60 L) was chosen so that at the end of the experiment, 70% of the initial water volume was still available, to avoid any concentration bias in the course of the experiment.

2.3. Sample analyses

The FA compositions of POM and macrophytes were analyzed with a gas chromatograph (Varian CP-3800 with flame ionization detector) after extraction, following a modified method of Bligh and Dyer (1959) as described in Mortillaro et al. (2011). POM samples were obtained after immediate on-board filtration (GF/F, 0.7 μm, pre-combusted 12 h at 450°C) using a vacuum system under low pressure, after which filters were freeze dried and stored at −20°C until analysis. The carbon and nitrogen compositions, and isotopic ratio (13C/12C or 15N/14N), of dried POM and macrophyte samples were determined at the UC Davis Stable Isotope Facility and reported in standard delta notation (δ13C or δ15N), defined as parts per thousand (‰) deviation from a standard (Vienna PeeDee Belemnite for δ13C and atmospheric N2 for δ15N, Peterson and Fry, 1987). The DOC concentrations and isotopic compositions (δ13C-DOC) were determined using an EA-IRMS analyzer following the protocol of Alberici (2011). The DIC isotopic compositions (δ13C-DIC) were also determined using an EA-IRMS analyzer following the protocol of Bouillon et al. (2007). However, DIC concentrations were not measured, as microcosms were continuously aerated with pumps. The concentration of nitrates (NO₃⁻), nitrates (NO₂⁻) and ammonium (NH₄⁺) dissolved fractions, summarized as DIN (dissolved inorganic nitrogen), as well as orthophosphate concentrations (PO₄³⁻) were determined by spectrophotometry following a modified method of Grasshoff et al. (1999). Total bacterial abundances were determined by direct epifluorescence microscopy counting, using 4,6-diamidino-2-phenyl-indole (DAPI) up to a final concentration of 1 μg mL⁻¹ after 15 min of incubation (Porter and Feig, 1980). Direct counts were performed at 1250x magnification, under an epifluorescence microscope (Leica Leitz DMR; 365 nm). In turbid samples (high suspended matter content), subsamples were pre-treated (before staining) by addition of 150 μl of Tween, sonicated at 35 kHz for 5 min, and centrifuged at 3000g during 10 min at 4°C (Chevaldonna and Godfroy, 1997; Hubas et al., 2007a,b).

2.4. Data analysis

The data obtained for each microcosm were compared, to evidence variations between treatments. All FA of POM (up to 40%) were used in these analyzes without transformation and were represented by their relative proportions. A dissimilarity matrix between each pair of samples was calculated using the Bray-Curtis index. Dissimilarities between samples were then graphically represented using an nMDS (non-metric MultiDimensional Scaling, Zuur et al., 2007). Differences between groups were tested using analyzes of similarities tests (ANOSIM, Oksanen et al., 2010), without considering temporal variability. When differences were significant, a similarity percentages analysis (SIMPER, Oksanen et al., 2010) was used to determine the relative contribution of each FA to differences between two groups.

The differences in the isotopic compositions (δ13C-DOC, δ13C-POM, δ13N-PON and δ13C-DIC) as well as differences in POC, DOC, DIN, PO₄³⁻ concentrations and bacterial abundance between treatments (n = 5) were tested using a non-parametric Kruskal-Wallis test (KW). Non-parametric Mann-Whitney-Wilcoxon (MWV) Post-Hoc tests were then used to identify the differences between individual treatments, after correction following a Benjamini and Hochberg (BH) procedure for multiple comparisons (Benjamini and Hochberg, 1995).

The relative contribution of C₄ macrophytes to POM was calculated using a two end member mixing model (Forsberg et al., 1993):

\[
\%C_4 = \frac{1 - \delta^{13}C_{\text{source}} - \delta^{13}C_{C_4}}{\delta^{13}C_{C_4} - \delta^{13}C_{C_0}} \times 100
\]

where \(\delta^{13}C_{\text{source}}\) was the δ¹³C-POM compositions for each time and treatment, \(\delta^{13}C_{C_4}\) was the mean P. repens composition, \(\delta^{13}C_{C_0}\) was the mean POM composition at the beginning of the experiment and \(\%C_4\) the P. repens contribution to the isotopic compositions of OM in each sample.

All statistical analyzes were implemented within the R programming environment (R Development Core Team 2010, package Vegan, Oksanen et al., 2010), with the probability \(\alpha\) set at 0.05.

3. Results

Among macrophyte samples, 41 FA were identified (Table 1) with an intragroup similarity of 92.5 and 93.2% within P. repens and S. auriculata, respectively, at the beginning of the experiment. P. repens was 13C and 15N enriched compared to S. auriculata (Table 1). The water collected in the várzea was characterized by 37 FA (Table 1), where saturated 14:0, 15:0, 16:0 and 18:0 accounted for 70% of the total FA composition of POM. The POC and DOC concentrations were respectively of 1.3 ± 0.1 and 2.8 ± 0.3 mg L⁻¹ (Fig. 3), with a carbon isotopic composition of −30.1 ± 0.4% for POC and −28.7 ± 0.4% for DOC (Fig. 4). DIC was 13C enriched relative to POC and DOC (δ¹³C-DIC of −11.9 ± 0.2‰, Fig. 4).

Water from the five treatments showed significant differences in their global FA compositions (ANOSIM, R = 0.35, p < 0.001, Fig. 2). Samples from CTRL, SA-LB, SA-HB, and PR-LB had a similar FA composition but differed from samples of PR-HB (Table 2). Similarities in the FA composition within each treatment were higher than 77% (Table 3). A higher proportion of branched FA (mainly 15:0:iso and 15:0:anteiso) and a lower proportion of 18:0 were observed in PR-HB compared to other treatments (Table 3). The concentrations of
Table 1

FA concentrations and stable isotope compositions ($\delta^{13}$C and $\delta^{15}$N) of POM (FA: µg L$^{-1}$) and macrophytes (FA: mg g$^{-1}$) collected in Camaleão várzea. In bold are the proportion of saturated (SFA), branched (BFA), monounsaturated (MUFA), polyunsaturated (PUFA) and long chain FA (LCFA).

<table>
<thead>
<tr>
<th>Group</th>
<th>POM (µg L$^{-1}$)</th>
<th>P. repens (mg g$^{-1}$)</th>
<th>S. auriculata (mg g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR-LB</td>
<td>2.25</td>
<td>± 1.81</td>
<td>± 0.16</td>
</tr>
<tr>
<td>PR-HB</td>
<td>1.70</td>
<td>± 0.1</td>
<td>± 0.27</td>
</tr>
<tr>
<td>PR-LB:SA</td>
<td>0.82</td>
<td>± 0.2</td>
<td>± 0.02</td>
</tr>
<tr>
<td>PR-LB:SA</td>
<td>0.27</td>
<td>± 0.1</td>
<td>± 0.03</td>
</tr>
<tr>
<td>PR-LB:SA</td>
<td>0.07</td>
<td>± 0.04</td>
<td>± 0.06</td>
</tr>
<tr>
<td>PR-LB:SA</td>
<td>0.11</td>
<td>± 0.05</td>
<td>± 0.05</td>
</tr>
<tr>
<td>PR-LB:SA</td>
<td>0.04</td>
<td>± 0.05</td>
<td>± 0.15</td>
</tr>
<tr>
<td>PR-LB:SA</td>
<td>0.04</td>
<td>± 0.05</td>
<td>± 0.15</td>
</tr>
<tr>
<td>PR-LB:SA</td>
<td>0.15</td>
<td>± 0.01</td>
<td>± 0.01</td>
</tr>
<tr>
<td>%SFA</td>
<td>1.76</td>
<td>± 3.77</td>
<td>± 4.67</td>
</tr>
<tr>
<td>%BFA</td>
<td>10.43</td>
<td>± 3.23</td>
<td>± 3.98</td>
</tr>
<tr>
<td>%MUFA</td>
<td>4.79</td>
<td>± 4.79</td>
<td>± 4.79</td>
</tr>
<tr>
<td>%LCFA</td>
<td>3.5</td>
<td>± 4.79</td>
<td>± 4.79</td>
</tr>
<tr>
<td>$\delta^{13}$C (%$\delta$)</td>
<td>-30.15</td>
<td>± 4.3</td>
<td>± 8.6</td>
</tr>
<tr>
<td>$\delta^{15}$N (%)</td>
<td>2.43</td>
<td>± 0.71</td>
<td>± 1.32</td>
</tr>
</tbody>
</table>

n.d. = not detected.

Table 2

Summary of ANOSIM pairwise tests for FA composition of POM between treatments. Values in italics (R < 0.3) are for high intragroup variability.

<table>
<thead>
<tr>
<th>Groups</th>
<th>R</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR-HB: Ctrl</td>
<td>0.80</td>
<td>&lt;10$^{-3}$</td>
</tr>
<tr>
<td>PR-HB: PR-LB</td>
<td>0.73</td>
<td>&lt;10$^{-3}$</td>
</tr>
<tr>
<td>PR-HB: SA-HB</td>
<td>0.75</td>
<td>&lt;10$^{-3}$</td>
</tr>
<tr>
<td>PR-LB: SA-LB</td>
<td>0.82</td>
<td>&lt;10$^{-3}$</td>
</tr>
<tr>
<td>PR-LB: Ctrl</td>
<td>0.27</td>
<td>&lt;10$^{-3}$</td>
</tr>
<tr>
<td>PR-LB: SA-HB</td>
<td>0.07</td>
<td>0.006NS</td>
</tr>
<tr>
<td>PR-LB: SA-LB</td>
<td>0.11</td>
<td>&lt;10$^{-3}$</td>
</tr>
<tr>
<td>SA-HB: Ctrl</td>
<td>0.04</td>
<td>0.15NS</td>
</tr>
<tr>
<td>SA-HB: SA-LB</td>
<td>0.04</td>
<td>0.11NS</td>
</tr>
<tr>
<td>SA-LB: Ctrl</td>
<td>0.15</td>
<td>0.01</td>
</tr>
</tbody>
</table>

NS = non-significant.

POC, DOC, DIN, PO4$^{3-}$ as well as $\delta^{13}$C-POC, $\delta^{13}$C-DIC, $\delta^{13}$C-DOC and bacterial abundance displayed significant differences between treatments (KW, p < 0.001, Table 4, Figs. 3–5), whereas $\delta^{15}$N-PON was similar between all treatments (KW, p = 0.73). The highest concentrations of POC, DOC, DIN, PO4$^{3-}$ and highest bacterial abundance, were observed in PR-HB (Figs. 3 & 5, Table 4). However, a higher POC concentration was recorded in SA-HB compared to PR-LB and SA-LB (Table 4, Fig. 3). No differences in DOC concentration were observed between SA-HB, PR-LB and Ctrl, whereas SA-LB displayed the lowest DOC concentration. In contrast, the $\delta^{13}$C-POC was significantly higher in PR-HB ($+14.3 \pm 1.0\%$) and in PR-LB ($+21.0 \pm 3.1\%$) compared to other treatments (Fig. 4, Table 4). However, no difference was found between the isotopic compositions of SA and Ctrl treatments (MWU, p > 0.05, Table 4). The contribution of P. repens to the $^{13}$C enrichment recorded in POC from PR-HB and PR-LB reached 94.2 and 63.2%, respectively (Fig. 4, Eq. (1)). Similarly, the highest $\delta^{13}$C-DOC was recorded in
PR-HB ($-17.8 \pm 1.2\%$) with a contribution of *P. repens* to the DOC of 70.7% (Fig. 4, Eq. (1)). A higher $\delta^{13}$C-DOC was also observed in PR-LB ($-24.9 \pm 1.0\%$, Fig. 4, Table 4) compared to SA-HB and SA-LB, with a contribution of *P. repens* to the DOC reaching 27.8%. A significant increase in $\delta^{13}$C-DIC was only recorded for PR-HB ($-5.9 \pm 2.9\%$, Fig. 4, Table 4), concomitantly to a decrease in $\delta^{13}$C-DIC for SA-HB ($-17.8 \pm 3.7\%$, Fig. 4, Table 4). In contrast, no differences were found between the Ctrl with both SA and PR low biomass treatments (MWW, $p > 0.05$, Table 4). $\delta^{13}$C of organic and inorganic
Table 3
Intragroup similarity of FA compositions in different treatments, and percentages of FA explaining most of this similarity (SIMPER procedure).

<table>
<thead>
<tr>
<th>Treatment (intragroup similarity)</th>
<th>14:0</th>
<th>15:0iso</th>
<th>15:0anteiso</th>
<th>15:0</th>
<th>16:0</th>
<th>18:0</th>
<th>22:5ω6</th>
<th>Σ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR-HB (77.3%)</td>
<td>6.5</td>
<td>11.2</td>
<td>6.3</td>
<td>3.9</td>
<td>4.4</td>
<td>4.9</td>
<td>5.1</td>
<td>77.1</td>
</tr>
<tr>
<td>PR-LB (85.1%)</td>
<td>8.7</td>
<td>6.8</td>
<td>1.6</td>
<td>3.7</td>
<td>33.7</td>
<td>12.7</td>
<td>4.5</td>
<td>73.7</td>
</tr>
<tr>
<td>SA-HB (82.6%)</td>
<td>8.8</td>
<td>3.9</td>
<td>15.5</td>
<td>3.4</td>
<td>37.1</td>
<td>13.8</td>
<td>5.0</td>
<td>75.4</td>
</tr>
<tr>
<td>SA-LB (81.7%)</td>
<td>9.4</td>
<td>3.4</td>
<td>1.4</td>
<td>3.5</td>
<td>33.0</td>
<td>13.2</td>
<td>4.0</td>
<td>71.7</td>
</tr>
<tr>
<td>Ctrl (83.7%)</td>
<td>8.5</td>
<td>3.8</td>
<td>1.4</td>
<td>3.3</td>
<td>39.4</td>
<td>14.3</td>
<td>4.9</td>
<td>77.7</td>
</tr>
</tbody>
</table>

Table 4
Summary of KW and MWW pairwise tests for δ13C-POC, δ13C-DOC, δ13C-DIC; POC, DOC, DIN, PO4−; concentrations and bacterial abundances of water samples between treatments.

<table>
<thead>
<tr>
<th></th>
<th>δ13C-POC</th>
<th>δ13C-DOC</th>
<th>δ13C-DIC</th>
<th>POC</th>
<th>DOC</th>
<th>DIN</th>
<th>PO4−</th>
<th>Bact</th>
</tr>
</thead>
<tbody>
<tr>
<td>KW Global test</td>
<td>5.10−4</td>
<td>3.10−4</td>
<td>6.10−4</td>
<td>5.10−4</td>
<td>4.10−4</td>
<td>1.10−3</td>
<td>1.10−3</td>
<td>10−9</td>
</tr>
<tr>
<td>PR-HB: Ctrl</td>
<td>7.10−4</td>
<td>3.10−4</td>
<td>8.10−4</td>
<td>1.10−4</td>
<td>1.10−4</td>
<td>1.10−3</td>
<td>1.10−3</td>
<td>0.05</td>
</tr>
<tr>
<td>PR-HB: SA-HB</td>
<td>7.10−4</td>
<td>3.10−4</td>
<td>8.10−4</td>
<td>1.10−4</td>
<td>1.10−4</td>
<td>1.10−3</td>
<td>1.10−3</td>
<td>&lt;10−4</td>
</tr>
<tr>
<td>PR-HB: SA-LB</td>
<td>7.10−4</td>
<td>3.10−4</td>
<td>8.10−4</td>
<td>1.10−4</td>
<td>1.10−4</td>
<td>1.10−3</td>
<td>1.10−3</td>
<td>&lt;10−4</td>
</tr>
<tr>
<td>PR-LB: Ctrl</td>
<td>9.10−4</td>
<td>3.10−4</td>
<td>8.10−4</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>&lt;10−4</td>
</tr>
<tr>
<td>PR-LB: SA-HB</td>
<td>7.10−4</td>
<td>3.10−4</td>
<td>8.10−4</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>&lt;10−4</td>
</tr>
<tr>
<td>SA-HB: Ctrl</td>
<td>0.50NS</td>
<td>0.23NS</td>
<td>0.59NS</td>
<td>0.59NS</td>
<td>0.59NS</td>
<td>0.59NS</td>
<td>0.59NS</td>
<td>0.04</td>
</tr>
<tr>
<td>SA-HB: SA-LB</td>
<td>0.11NS</td>
<td>0.54NS</td>
<td>0.11NS</td>
<td>0.11NS</td>
<td>0.11NS</td>
<td>0.11NS</td>
<td>0.11NS</td>
<td>0.04</td>
</tr>
<tr>
<td>Ctrl: Ctrl</td>
<td>0.08NS</td>
<td>0.25NS</td>
<td>0.38NS</td>
<td>0.38NS</td>
<td>0.38NS</td>
<td>0.38NS</td>
<td>0.38NS</td>
<td>0.38</td>
</tr>
</tbody>
</table>

NS = non-significant.

4. Discussion

The present microcosm experiment highlights significantly different degradation patterns of C3 and C4 Amazon macrophytes. Over a 23 day experiment, our results revealed a major impact of *P. repens* degradation, at high biomass, on OM composition. This impact was apparently related to the biomass of macrophytes used in the experiment as well as to the inherent biodegradability of C4 compared to C3 macrophytes. Indeed, even though the fast degradation of the C4 macrophyte was most evident in PR-HB, as revealed by all measured parameters, it was also observed in the PR-LB. For instance, PR-LB treatments (25 g of fresh macrophytes in a 60 L tank) showed stronger 13C enrichment in POC and DOC than SA-HB (100 g of macrophytes in a 60 L tank). There was however a slight increase in POC concentrations as well as a decrease in δ13C-DIC in the *S. auriculata* high biomass treatments that reveals on-going degradation.

In this experiment, *P. repens* displayed large proportions of 18:2ω6 and 18:3ω3 polyunsaturated FA (up to 44% of total FA), which is consistent with previous characterization of this FA as markers of macrophytes in this environment (Mortillaro et al., 2011). Similarly, carbon stable isotope compositions of both *P. repens* and *S. auriculata* were consistent with those expected from plants with C4 and C3 photosynthetic pathways (−13.0 ± 0.8 and −30.5 ± 0.5‰, respectively, Smith and Epstein, 1971).

4.1. Contribution of *P. repens* (C3) and *S. auriculata* (C4) to POM

In PR treatments, POC was significantly enriched in 13C, which indicates a contribution of *P. repens*, after its hydrolysis into the POM pool. This contribution was estimated to reach 94.2 and 63.2% of total POM composition for PR-HB and PR-LB, respectively, using a two-end-member mixing model. This was surprising as previous studies, characterizing POM in the Amazon Basin, suggested a low contribution of C4 macrophytes (Hedges et al., 1986; Mortillaro et al., 2011; Moreira-Turcq et al., 2013). The contribution of *P. repens* to POM was confirmed by the increased proportion of branched FA in PR treatments (15:0iso and 15:0anteiso). These FA are regularly described as biomarkers of bacteria (Volkman et al., 1980; Kaneda, 1991; Mfilinge et al., 2003) and suggest here that in addition to hydrolysis, *P. repens* leaves were decomposed by heterotrophic
microbial communities. Indeed, the transfer of FA to POM, including branched FA, was previously recorded from decomposing mangroves (Mfilinge et al., 2003). Similarly, the transfer of FA and the $^{13}$C enrichment of POM and sediments were evidenced in salt-marsh from the decomposition of the $C_4$ Spartina spp. (Boschker et al., 1999). In salt-marsh ecosystems, the composition of POM affected by $S$. $\text{auriculata}$ decomposition changed from predominantly unsaturated to branched and saturated FA typical of bacteria (Johnson and Calder, 1973; Schulz and Quinn, 1973). Similar findings were reported in PR-HB and differences in FA compositions and $^{13}$C enrichments reported for OM between PR and Ctrl treatments occurred in the first 3–6 days of the experiment. These changes in OM composition, as well as high bacterial abundance recorded in PR-HB, suggest a fast decomposition of this macrophyte, which may have exceeded hydrolysis (in agreement with previous studies; Fellerhoff et al., 2003).

Contrastingly to $P$. repens, no differences were reported between SA and Ctrl treatments for FA and $\delta^{13}$C-POC. Yet, decomposition of $S$. $\text{auriculata}$ could not be excluded using $\delta^{13}$C-POC analyses. Indeed, fresh leaves of $S$. $\text{auriculata}$ ($\sim 30.5 \pm 0.5\%$) had a similar composition to POM ($\sim 30.1 \pm 0.4\%$) at the beginning of the experiment. However, the higher POC concentrations measured in SA-HB compared to PR-LB and SA-LB suggest an effective hydrolysis of this macrophyte. The lack of differences between SA and Ctrl treatments for FA compositions and $\delta^{13}$C-POC suggest however a slower hydrolysis of $S$. $\text{auriculata}$ compared to $P$. repens. Indeed, Howard-Williams and Junk (1976) recovered 50% of $S$. $\text{auriculata}$ initial dry weight at the end of a 186 days decomposition experiment. Similarly, Fellerhoff et al. (2003) recovered 80% of $S$. $\text{auriculata}$ initial dry weight after 21 days of incubation. During our experiment, large macrophyte debris were observed for a much longer time in the $S$. $\text{auriculata}$ treatments than in the $P$. repens treatments. This higher resistance to fractionation of the $C_3$ macrophyte was consistent with the differences in FA, POC and $\delta^{13}$C-POC of the fine POM fraction ($<200 \mu m$).

4.2. Impact of macrophyte degradation on dissolved compounds

Decomposition of $P$. repens tissues led to a $^{13}$C enrichment of DOC and DIC. Such increase of $\delta^{13}$C-DIC resulted from bacterial mineralization of macrophyte organic carbon and CO$_2$ equilibration at the air/water interface (Quay et al., 1992; Hedges et al., 1994; Mayorga et al., 2005). Because the experiment was performed in contact with air in order to maintain aerobic conditions, the $\delta^{13}$C-DIC signature was affected by isotopic equilibration with the atmosphere. This process tends to slowly increase the $\delta^{13}$C-DIC to a value close to the isotopic equilibrium with the atmosphere at around 0% (Polsenaere and Abel, 2012). Consequently, the observed $\delta^{13}$C-DIC values are the result of a balance between the DIC production from the $C_3$ or $C_4$ plants decomposition and equilibration with the atmosphere. In SA-HB, the rapid $\delta^{13}$C-DIC decrease from $\sim 11.9 \pm 0.2\%$ at the beginning of the experiment to $\sim 20.3 \pm 7.0\%$ after 3 days of incubation reveals that the $C_3$ macrophytes were undergoing mineralization processes. Indeed, hydrolysis tends to leach out compounds relatively enriched in $^{13}$C with respect to more recalcitrant compounds (e.g. lignin) depleted in $^{13}$C (Costantini et al., 2014).

Previous works in the Amazon have attributed $^{13}$C enrichment of DIC to the preferential oxidation of organic carbon derived from $C_4$ macrophytes (Rai and Hill, 1984; Chanton et al., 1989; Quay et al., 1992; Waichman, 1996). Several other studies based on solute distribution suggested that $C_4$ grasses are more biodegradable than the bulk OM (Hedges et al., 1986; Quay et al., 1992; Mayorga et al., 2005). Ellis et al. (2012) have measured the $\delta^{13}$C of respired CO$_2$ in closed incubations at different stages of the hydrological cycle in the Solimões River and concluded that $C_3$ plants, $C_4$ plants and phytoplankton, all contributed to respiration in the Amazon River. The

---

Fig. 5. Boxplot of bacteria abundances (a), DIN (b), and PO$_4$ (c) concentrations in each treatment: PR-HB (dark green), PR-LB (light green), SA-HB (red), SA-LB (orange) and Ctrl (blue) (for interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
results of our incubations suggest however that high macrophyte biomasses are necessary in order to significantly alter the δ13C-DIC signature locally.

Besides carbon, the nitrogen (N) and phosphorus (P) concentrations in floodplain waters were also affected by the decomposition and mineralization of macrophytes. A large release of POC, DIN and potassium (K) was previously evidenced during the decomposition of P. fasciculatum (Furch and Junk, 1992). The decomposition of P. fasciculatum had the potential to supply floodplains with 242 kg ha\(^{-1}\) of N and 66 kg ha\(^{-1}\) of P in Furch and Junk (1992) nutrients budget. Following these authors’ calculations (i.e. maximum amounts of bio-elements released to water reported to maximum macrophyte biomasses), P. repens showed the potential, in our degradation experiment, to supply floodplains with 176.4 kg ha\(^{-1}\) of N and 48.2 kg ha\(^{-1}\) of P. On the other hand, S. auriculata contribution to floodplains is estimated to reach 0.13 kg ha\(^{-1}\) of N and 2.44 kg ha\(^{-1}\) of P only. Therefore, our study demonstrates that P. repens represents a predominant source of N and P.

Fast nutrient recycling from decomposing macrophytes may fertilize Amazon floodplains, where N and P are growth-limiting factors (Devol et al., 1984; Forsberg, 1984; Setaro and Melack, 1984). Within the Amazon Basin, aquatic grasses such as P. repens have been suggested to be able of atmospheric N\(_2\) fixation (Martineili et al., 1992), so that atmospheric N\(_2\) may contribute up to 90% of plant N for stands of P. repens (Kern and Darwich, 2003). Therefore, the fast decomposition of N\(_2\) fixing macrophytes may play a dominant role as a natural fertilizer for floodplains (Piedade et al., 1991; Kern and Darwich, 2003), stimulating phytoplankton production during the falling water period, when macrophytes start to decompose (Rai and Hill, 1984).

In Amazon floodplains, P. repens maximum biomasses were observed during the wet season (Junk and Piedade, 1993b; Silva et al., 2009), where C\(_4\) macrophyte contribution to the primary production in várzea was estimated to reach 65% (Melack and Forsberg, 2001). During this season, POC and DOC mainly originate from depleted carbon sources similar to C\(_3\) primary producers (Hedges et al., 1994; Mortillaro et al., 2011). However during the dry season, macrophytes are subject to intensive degradation as water level decreases (Engle et al., 2008). An increasing contribution of macrophytes to OM composition, due to the accumulation of plant detritus, was suggested in the Amazon várzea (Mortillaro et al., 2011). However, although macrophytes have been demonstrated experimentally to affect the δ13C-POC and δ13C-DOC, fast microbial mineralization of organic carbon suggests that only large macrophyte biomasses, produced during the flood season, have the potential to affect δ13C-POC and δ13C-DOC within floodplains. Indeed, C\(_4\) material may contribute to sediments OM composition (Sobrinho et al., 2016) according to spatial variability in C\(_4\) macrophytes (Hess et al., 2003), despite a low burial of organic carbon in floodplain sediments (Moreira-Turcq et al., 2004). Therefore, most C\(_4\) macrophytes are mineralized (Piedade et al., 1991; Junk and Piedade, 1993a) and thus contribute significantly to CO\(_2\) outgassing, as previously suggested (Quay et al., 1992). Moreover, bacterial growth has been shown, within Amazon floodplains, to display a low efficiency (Vidal et al., 2015). This low efficiency implies, besides high respiration rates, a low transfer of C\(_4\) carbon to higher trophic levels. The production of C\(_3\) macrophytes within the central Amazon Basin is much more limited than C\(_4\) macrophytes (Furch and Junk, 1992). However, the lower lability of these macrophyte debris compared to C\(_4\) macrophyte debris makes them available for being channeled through aquatic food webs. These findings can explain why Amazon food webs are mainly centered on a C\(_3\) carbon source (Araujo-Lima et al., 1986; Hamilton et al., 1992; Forsberg et al., 1993), although C\(_4\) macrophytes display a greater food quality and biomass for specialized herbivore fish species (Mortillaro et al., 2015).

5. Conclusion

Within the present experiment, the higher lability of C\(_4\) compared to C\(_3\) macrophytes was demonstrated. The contribution of P. repens to POC and DOC isotopes compositions reached a maximum after 3–6 days, indicating a fast decomposition rate of this macrophyte. Moreover, P. repens biomasses had a noticeable impact on OM composition. The decomposition of C\(_4\) macrophytes was followed by the mineralization into DIC, as suggested by δ13C-DIC, as well as by the release of DIN and P. Therefore, the fast mineralization of C\(_4\) macrophytes, as well as the natural mixing of POM with 13C-depleted primary producers (e.g. phytoplankton, C\(_3\) macrophytes, periphyton, and trees), should account for the overall low contribution of C\(_4\) carbon sources to the central Amazon aquatic food webs.

Acknowledgments

This research is a contribution to the CARRBAMA project, supported by the ANR (French National Agency for Research, grant number 08-BLAN-0221), and the CNPq (National Council for Scientific and Technological Development—Brazil, Universal Program grant number 477655/2010-6); it was conducted within an international cooperation agreement between the CNPq (Brazil) and the IRD (Institute for Research and Development—France), and under the auspices of the Environmental Research Observatory Hydrology and Geochemistry of the Amazon Basin (HYBAM), supported by the INSU and the IRD. We are grateful to Jessica Chichepor-tiche (LOG laboratory) for bacterial abundance estimations. We also want to thanks two anonymous reviewers whose comments helped improve this manuscript.

References


