Plant-mediated transport and isotopic composition of methane from shallow tropical wetlands

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Abstract

The methane (CH4) content of gas bubbles in sediments and CH4 emission from plants in the Pantanal wetland of Brazil was examined across a depth gradient to ascertain the relative importance of plant-mediated CH4 emission versus ebullition. Sediment bubbles contained >50% CH4 at sites with water depths >1 m but often contained <50% CH4 in shallower waters. Previous work has shown that bubble % CH4 is related to ebullition rates, and therefore these patterns are suggestive of greater CH4 emission by ebullition from deeper waters compared to waters <1 m in depth. Stable isotope ratios of carbon and hydrogen in the CH4 showed evidence for significant CH4 oxidation but no apparent relation with water depth. Shallower waters typically contain rooted emergent plants with erect, vertical stems or petioles, whereas in waters deeper than ~1 m, emergent aquatic plants are limited to rooted forms with long stems that float at the water surface. Rooted emergent plants typical of deeper waters had low rates of CH4 emission (0.0–0.2 mg d−1 CH4 for individual plants) compared to species typical of inundation depths <1 m (1.6–7.5 mg d−1 CH4). These observations suggest that tropical savanna floodplains like the Pantanal with inundation depths >1 m have low rates of CH4 emission via rooted emergent and floating-leaf plants, in contrast to results from shallower rice fields and temperate wetlands in which plant-mediated transport dominates CH4 emission from vegetated waters.

Key words: bubbles, ebullition, emission, floodplains, macrophytes, methane, tropical, wetlands

Introduction

Freshwater wetlands and rice fields are estimated to be responsible for approximately one-third of the global emission of methane (CH4), the second most important greenhouse gas responsible for climate change (Forster et al. 2007). Methane emission from wetlands has been extensively studied, yet to build process-based biogeochemical models we need a better understanding of wetland CH4 fluxes, their biophysical controls, and potential feedbacks in a changing climate (Bridgham et al. 2013). There are 3 major routes for the emission of CH4 from wetlands: diffusive evasion, ebullition, and plant-mediated transport (Chanton and Dacey 1991). Diffusive evasion is generally the least effective route in wetlands with high rates of CH4 production (Chanton and Dacey 1991). Plant-mediated transport tends to predominate in shallow waters containing rooted emergent plants (Laanbroek 2010, Bridgham et al. 2013) and has been estimated to be globally significant (Bastviken et al. 2011). Plants emit CH4 in the process of aerating their underwater tissues, which require oxygen (O2) to grow in anoxic waters or sediments. In both diffusive evasion and plant-mediated transport, the CH4 often contacts O2 belowground in the rhizosphere or in the water column before reaching the atmosphere, and large losses of CH4 can then occur through oxidation by methanotrophic bacteria (Frenzel et al. 1992, Megonigal et al. 2004,
Laanbroek 2010). In contrast, ebullition bypasses oxidizing environments (Chanton et al. 1989). Thus the relative importance of ebullition and plant-mediated transport can affect the total CH$_4$ emission rate.

Studies of CH$_4$ emission from the floodplains of the Amazon and Orinoco rivers have indicated that plant-mediated transport is insignificant, and ebullition and diffusive evasion are the predominant routes of emission (Crill et al. 1988, Devol et al. 1988, Wassmann et al. 1992, Smith et al. 2000). The Amazon and Orinoco floodplains are different from North American wetlands that have been studied because of their deep inundation (up to 10 m), and the emergent plant stands that have been investigated were composed mostly of floating grasses (e.g., *Paspalum repens*) whose roots are suspended in the water column and have little or no contact with the sediments.

Atmospheric inversion studies that combine remote sensing of atmospheric CH$_4$ with ground-based measurements and modeling indicate that tropical wetlands are responsible for 47 to 89% (median 73%) of the global CH$_4$ emission from wetlands, with a total emission rate estimated between 111 and 190 Tg y$^{-1}$ CH$_4$ (Bloom et al. 2012, Bridgham et al. 2013, Melton et al. 2013). Many tropical wetlands of the world are savanna floodplains that flood seasonally and have maximum inundation depths of at least 0.5 m, but considerably less than the 5–10 m depths common in the floodplains that fringe the Amazon and Orinoco rivers. During the inundation phase, savanna floodplains support luxuriant growths of aquatic and semi-aquatic plants with floating stems and emergent leaves (Junk 1993). Based on previous studies, it is difficult to predict the relative importance of plant-mediated transport or ebullition of CH$_4$ in these wetlands.

The Pantanal is a 140 000 km$^2$ savanna floodplain in the upper Paraguay River basin, mainly in Brazil, that is typical of savanna floodplains in South America (Hamilton et al. 1996, 2002). Seasonal inundation in the Pantanal occurs by riverine flooding and by slow drainage of local rainfall and lasts for several months in many areas. The variable depth and duration of flooding result in a wide variety of plants (Junk 1993). Shallower waters typically contain rooted emergent plants with erect, vertical stems or petioles, such as *Pontederia cordata*, *Cyperus giganteus*, *Thalia geniculata*, and *Typha domingensis*. In waters deeper than ~1 m, emergent aquatic plants are limited to rooted forms with long stems that float at the water surface, such as *Eichhormea azurea*, *Oxyccaryum cubense*, *Oryza subulata*, *Panicum elephantipes*, *Paspalum* spp., and *Polygonon* spp., and completely free-floating forms whose roots may not reach the sediments, such as *Eichhormea crassipes*. All these species can also be found in the shallower waters. The vegetated waters of the region are too shallow to have persistent thermal stratification, but despite their contact with the atmosphere, they typically have low concentrations of dissolved O$_3$ (median for 278 samples: 35 μM, or 12–15% of atmospheric equilibrium; Hamilton et al. 1995).

Recent estimates of CH$_4$ emission from the Pantanal have varied from 1.9 to 3.3 Tg y$^{-1}$ CH$_4$ (Melack et al. 2004, Marani and Alvalá 2007, Bastviken et al. 2010). Marani and Alvalá (2007) and Bastviken et al. (2010) reported static chamber measurements of CH$_4$ emission from Pantanal waters; both studies were conducted only during low water seasons. Measured rates of CH$_4$ emission were similar to those reported for the Amazon floodplain (Melack et al. 2004), and both studies inferred that ebullition accounted for ~90% of the total emission, with diffusive evasion accounting for the balance. Marani and Alvalá (2007) reported no effect of floating macrophytes on CH$_4$ emission, noting that the plants in their chambers were floating in the deeper open water of lakes, and that if they were rooted in the sediments those roots were far shoreward from the point of measurement. Bastviken et al. (2010) did find that CH$_4$ emission rates were greater near the shore and near aquatic macrophytes. Despite their high biomass and density, the role of rooted emergent plants in CH$_4$ emission from shallow waters in tropical floodplains, such as the Pantanal, has yet to be investigated.

The CH$_4$ content of gas bubbles in sediments is an indicator of gas fluxes and transport mechanisms (Chanton and Dacey 1991). Studies in North American wetlands suggest that CH$_4$ ebullition rates are directly related to the partial pressure of CH$_4$ in sediment gas bubbles because CH$_4$ ebullition strips dinitrogen (N$_2$), the other major dissolved gas, from the porewaters, and the N$_2$ content in bubbles reflects a dynamic balance between removal by bubble stripping and resupply by molecular diffusion (Chanton et al. 1989). These studies have also shown that bubbles in the sediments of unvegetated waters tend to contain ~45% CH$_4$ whereas those in waters containing rooted emergent plants tend to contain lower % CH$_4$. This difference is thought to reflect the ventilation of excess gas pressure in sediments by the root systems of emergent plants, together with enhanced aeration of sediments and the introduction of N$_2$ in addition to the O$_3$ that supports CH$_4$ oxidation in the rhizosphere. Without rooted emergent plants, an increase in the dissolved gas pressure in the sediments results in bubble formation, and ebullition occurs more frequently. Sediment gas bubbles may form not only because of high rates of CH$_4$ production, but also from a seasonal temperature increase or a decrease in hydrostatic pressure during falling water levels.

The present study analyzed measurements of the gas composition of bubbles collected from the sediments in the Pantanal, including partial pressures of CH$_4$ and N$_2$ as

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well as stable isotope ratios of carbon and hydrogen in the CH\textsubscript{4}. We also present measurements of CH\textsubscript{4} emission from several common species of emergent plants from representative environments. Our data support the hypothesis that plant-mediated transport is potentially important relative to ebullition in waters up to ~1 m depth. In deeper waters, ebullition was inferred to become the predominant route based on higher CH\textsubscript{4} content in sediment gas bubbles, despite the abundance of emergent vegetation with stems that extend from the sediments to the overlying air. In addition, we examined CH\textsubscript{4} stable isotope abundance to assess the importance of CH\textsubscript{4} oxidation. Methane oxidation preferentially consumes the lighter isotopes of methane (\textsuperscript{12}CH\textsubscript{4}, hydrogen-1 CH\textsubscript{4}), leaving residual unoxidized methane enriched in the heavy isotopes (\textsuperscript{13}CH\textsubscript{4} and deuterium DCH\textsubscript{3}).

**Methods**

We collected 64 samples of gas bubbles from a wide variety of aquatic environments in the southern Pantanal (Fig. 1). All our samples were collected during the inundation phase (mostly between Sep 1992 and Aug 1993) from sites containing herbaceous plants with emergent leaves, except for 5 samples collected from the litter layer of shallow (<0.8 m depth) flooded forests. The mean ± SD of water depth at the 64 sampling sites was 1.2 ± 0.9 m (range 0.2–4.0 m). All the sampling sites are seasonally inundated, although the deeper sites close to the Paraguay River can retain water all year in wetter years. The study area includes much of the diversity of floodplain environments in the overall Pantanal because it spans the confluence of alluvial fans of varying hydrology and geomorphology (Hamilton et al. 1996, Evans et al. 2014).

We collected bubble samples by disturbing the sediment surface (Martens and Chanton 1989), which was usually overlain by 5–20 cm of leaf litter, and trapping the rising bubbles in the water column using an inverted plastic hemisphere fitted with a septum. In most cases, gas volumes of 0.5–1.5 L were easily obtained from areas of ~0.25 m\textsuperscript{2}. A subsample of the trapped gas was quickly transferred by syringe to a gas-tight 200 cm\textsuperscript{3} multilayer gas sampling bag stored in water and analyzed at a field laboratory within 24 h by gas chromatography (thermal conductivity detector). Storage tests confirmed that the bags maintained sample integrity. Samples were dried upon injection into the gas chromatograph by passage through a column of phosphorus pentoxide (P\textsubscript{2}O\textsubscript{5}).

At 31 of the gas bubble sampling sites, a subsample was taken for stable isotopic analysis, transferred to a glass serum vial together with a small volume of sample water, and crimp-sealed with a butyl plug-type septum.
Samples were stored frozen in an upside-down position with the frozen water over the septum until they were analyzed for carbon (δ¹³C) and hydrogen (δD) isotope ratios at Florida State University using methods detailed in Chanton et al. (2006). Precision was 0.3‰ for δ¹³C and 10‰ for δD.

We measured CH₄ emission from emergent plants by fitting a custom-made polyethylene bag over the plant, closing it off around the stem or petiole just below the water surface (Dacey and Klug 1979, Chanton et al. 1992). The air inside the bag was sampled before and after a period of ~20 minutes, and a tracer gas (10% CH₄ in helium) was added after the last sampling to determine the volume inside the bag. Samples were analyzed by gas chromatography (flame ionization detector) at a field laboratory. Emission rates per plant were calculated from the increase in CH₄ inside the bag and the bag volume. We defined an individual plant for the present purposes as the above-water portion arising from a single emergent stem or petiole.

Dissolved O₂ and CH₄ concentrations in overlying water were measured following methods in Hamilton et al. (1995). Water samples were taken from mid-depth using a vertical Van Dorn sampler (none of the sampling sites showed evidence of persistent thermal stratification). Stable isotope ratios in surface waters (δ¹⁸O and δD) and in representative plant tissues (δ¹³C) were analyzed by a commercial laboratory (Geochron) using standard techniques of sample preparation and isotope ratio mass spectrometry, with a precision of about 1‰ (water) and 0.2‰ (organic carbon in plant tissues).

Results

The gas bubbles in sediments were composed mainly of CH₄ (median 58%) and N₂ (median 37%), with smaller but variable amounts of CO₂ (median 2.9%) and traces of O₂ and argon (Ar). A scatterplot of % N₂ against % CH₄ illustrates the wide variation that was encountered (Fig. 2). The CH₄ concentration in gas bubbles was related to the depth of the overlying water column (Fig. 3). In waters of <1 m depth, % CH₄ was variable with a wide range of concentrations (12–80%). In contrast, % CH₄ was always >50% in bubbles from waters of >1 m depth.

There was no relationship between the % CH₄ in sediment gas bubbles and either the dissolved CH₄ or O₂ concentration in overlying water, nor was there a systematic difference in dissolved O₂ or CH₄ concentrations (data not shown; Hamilton et al. 1995). There was no apparent difference in biomass of vascular plants or the amount or nature of sediment organic matter between the shallower and deeper waters. Both C₃ and C₄ plants occurred across the depth range, and their stable carbon isotope ratios (δ¹³C) were typical for plants obtaining their carbon dioxide from air (Table 1).

The mean ± SD of δ¹³C in methane was −55.4 ± 5.9‰ (n = 31), and the mean ± SD of stable hydrogen isotope ratios (δD) in methane was −286.7 ± 13.6‰ (n = 24). There was no correlation between δ¹³C and δD (Fig. 4A). Patterns of interest in these data (Fig. 4B–D) are considered further in the Discussion.

Methane emissions from individual plants showed a clear relation to underwater stem or petiole length, with higher emissions from plants with shorter stems or petioles (<1 m) and lower emissions from plants with longer stems (Table 2; Fig. 5). Note that stem length is not necessarily related to the depth of the water column. In general, those species with long underwater stems tended to have adventitious roots suspended in the water column, yet they were still rooted in sediments.
Discussion

This study found that rooted emergent plants growing in waters deeper than 1 m had low rates of CH₄ emission (0.0–0.2 mg d⁻¹ CH₄ for individual plants) compared to species in depths <1 m (1.6–7.5 mg d⁻¹ CH₄). Many of these vegetated waters had bubbles in sediments containing >50% CH₄, suggesting that the plants at those sites were not effective in ventilating excess gas pressure from the sediments and that high rates of CH₄ production and ebullition stripped N₂ from the porewaters. The CH₄ content of bubbles and vegetation-specific methane emission rates suggest that ebullition is the primary route of emission in deeper vegetated waters of the Pantanal. In shallower waters, the variable CH₄ concentrations in sediment gas bubbles suggest that the emergent plants are not always effective in lowering CH₄ in the sediments.

The tendency for higher CH₄ concentrations in sediment gas bubbles from deeper waters in the Pantanal can be explained if ventilation of the sediments and/or oxygenation of the rhizosphere by emergent plants becomes less efficient at water depths greater than ~1 m. Longer stem lengths between the sediments and the water surface would provide greater resistance to gas transport and more chance for equilibration with the water outside the stem. Rooted plants were present in the deeper waters that we sampled, but their stems were often much longer than the depth of the water column because of their tendency to grow horizontally.

Stable carbon and hydrogen isotope ratios in CH₄ can reveal information on organic carbon sources, methanogenic pathways, and relative losses to bacterial oxidation (methanotrophy; Whiticar 1999). A conceptual model (Fig. 6) shows the directions in which these factors are expected to change CH₄ stable isotope ratios from an arbitrary reference point. Organic carbon sources may be derived from plants of either the C₃ or C₄ photosynthetic pathway. As has been found elsewhere (Chanton and Smith 1993), C₄ vascular plants in the Pantanal are relatively ¹³C-enriched compared to C₃ vascular plants (Table 1). The isotopic composition of the flood waters also influences the isotopic composition of methane produced in aquatic sediments (Whiticar 1999, Chanton et al. 2006). The mean

<table>
<thead>
<tr>
<th>Plant</th>
<th>Photosynthetic pathway</th>
<th>δ¹³C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free floating:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eichornia crassipes</td>
<td>C3</td>
<td>−28.2</td>
</tr>
<tr>
<td>Submersed stems &lt;1 m long:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyperus giganteus</td>
<td>C4</td>
<td>−12.5</td>
</tr>
<tr>
<td>Pontederia cordata</td>
<td>C3</td>
<td>−27.0</td>
</tr>
<tr>
<td>Thalia geniculata</td>
<td>C3</td>
<td>−24.6</td>
</tr>
<tr>
<td>Submersed stems &gt;1 m long:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eichhornea azurea</td>
<td>C3</td>
<td>−29.0</td>
</tr>
<tr>
<td>Oxycaryum cubense</td>
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<tr>
<td>Paspalum pontanalis</td>
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</tr>
<tr>
<td>Paspalum wrightii</td>
<td>C4</td>
<td>−12.9</td>
</tr>
</tbody>
</table>

Fig. 4. Stable carbon (δ¹³C) and hydrogen (δD) isotope ratios in methane from sediment gas bubbles, showing (A) all data, and (B) the same data coded by depth of overlying water, by (C) dissolved O₂ concentrations in overlying water, and by (D) the photosynthetic pathway of the dominant vascular plants. These charts can be compared with the conceptual model (Fig. 6).

Fig. 5. Relation between the length of underwater stems and the methane emission rate measured on individual plants (see Table 2 for more information).
± SD of stable isotope ratios of oxygen (δ¹⁸O) and hydrogen (δD) in surface water samples collected in diverse environments throughout the Pantanal from 1992–1997 was −4.09 ± 2.04 for δ¹⁸O (n = 164) and −29.46 ± 12.55 for δD (n = 142; S. Hamilton, unpublished data).

The overall lack of correlation between δ¹³C and δD contrasts with the negative correlation for the Orinoco River floodplain reported by Smith et al. (2000); the reason for this difference is unknown. The stable isotope measurements did not provide evidence for differences in relative losses of CH₄ to bacterial oxidation between sediments of shallower and deeper waters (Fig. 4B), in contrast to what might have been expected if higher ebullition rates in deeper waters bypassed CH₄ oxidation. Gas bubbles collected from waters deeper than 1 m tended to contain CH₄ depleted in ¹³C, reflecting a greater contribution of the CO₂ reduction pathway and/or a greater relative importance of C₃ vascular plants (Fig. 4B and D; compare Fig. 6).

Gas bubbles collected beneath overlying water that was more depleted in dissolved O₂ (concentrations <50 µM) may have been more enriched in both ¹³C and D due to CH₄ oxidation losses compared to those collected from beneath waters of >50 µM O₂ (Fig. 4C; compare Fig. 6). This evidence that an isotopic fractionation effect of CH₄ oxidation in gas bubbles was greater beneath more O₂-depleted waters was unexpected; more oxic overlying waters might be expected to support more CH₄ oxidation in the surficial sediments. Anaerobic CH₄ oxidation coupled to sulfate reduction is not expected to be significant in these waters because of their low sulfate concentrations (median concentration for 540 samples = 0.5 µM: Hamilton et al. 1995; see Beal et al. 2011), although iron or humic substances are believed to also serve as potential oxidizing agents for CH₄ (Bridgham et al. 2013).

Our measurements of CH₄ emission from emergent...
plants support the hypothesis that plants with erect, vertical stems or petioles of <1 m length are more effective in the transport of gases between the sediments and the overlying air than those with longer stems or petioles (Table 2; Fig. 5). Previous studies that have identified plants as major routes of CH4 emission have examined wetlands dominated by species with erect stems or petioles of <1 m length, such as rice fields (Schütz et al. 1989), subarctic wetlands (Whiting and Chanton 1992), and the Florida Everglades (Happell et al. 1993). Wetland plants with pressurized ventilation might be able to transport CH4 over greater stem or petiole lengths, as Dacey and Klug (1979) found for Nuphar growing in depths up to 1.2 m. Studies suggest that pressurized ventilation may be widespread among certain aquatic plants with flat, floating leaves (e.g., water lilies) or with cylindrical culms and linear leaves (Grosse et al. 1991, Brix et al. 1992, Laanbroek 2010). The occurrence of pressurized ventilation in the species that predominate in deeper waters of the Pantanal, many of which have floating stems and abundant roots in the water column as well as in the sediments, has not yet been investigated, but it would seem from the the low plant-specific CH4 emission rates reported here that pressurized ventilation either does not exist or is not effective in CH4 transport in the species we investigated.

Few measurements have been reported of CH4 emission from the species of plants that are common in the Pantanal and other shallow tropical floodplains, other than the aforementioned studies in the central Amazon floodplain and in the Pantanal that found no difference in CH4 emission rates with and without floating plants in chambers. In a study of CH4 emission from shallow, interfluvial wetlands in the upper Negro River basin dominated by emergent grasses and sedges, Belger et al. (2011) measured emissions in chambers with and without emergent plants and found higher rates with plants at one site but no difference at another. Water depths at their 2 sites were no more than 1.3 m and 0.6 m.

We conclude that plant-mediated transport of CH4 to the atmosphere is likely to be relatively more important in shallow wetlands where the distance for gas transport within underwater stems or petioles is <1 m. In deeper water columns or in wetlands dominated by floating-stem growth forms, plant-mediated emission of CH4 is evidently less effective, and ebullition is thus likely to be the predominant route of CH4 emission. Many tropical wetlands, including some floodplains used for rice cultivation, have seasonally fluctuating water levels that reach depths >1 m, which encourages the growth of floating-stem forms with stems that can be much longer than the depth of the water in which they grow. Total CH4 emission may be higher in such wetlands because ebullition is thought to bypass oxidizing environments.

These observations pertain to CH4 emission from a globally significant fraction of wetlands and floodplains. Future research should further examine our hypothesis that plant-mediated CH4 emission is not necessarily the most important route of gas transport in vegetated waters and seek to understand which plant growth forms are most effective as conduits for CH4 from the sediments to the atmosphere. A better understanding of the roles of different kinds of wetland plants in the vast tropical wetlands and floodplains of the world would help focus measurement programs that strive to include all major CH4 emission pathways. Also important to understand is how the relative importance of plant-mediated versus ebullitive CH4 emission affects the fraction of CH4 that is lost to oxidation before it escapes to the atmosphere. Such research would inform biogeochemical models that seek to predict how CH4 emission rates from wetlands and floodplains may change in the future under the influence of drivers such as climate change, hydrological alterations, and invasive species.

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