

# Spatial and seasonal variations in the stable carbon isotopic composition of methane in stream sediments of eastern Amazonia

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## ABSTRACT

The stable carbon isotopic composition of methane ( $\delta^{13}\text{C}\text{-CH}_4$ ) gas bubbles formed in the sediments of three Amazonian streams was determined over a 5-yr period. The study sites were two 'várzea' floodplain (Açu and Maicá) and one 'terra-firme' (Jamaraquá) streams. The  $\delta^{13}\text{C}$  of sedimentary organic matter (SOM) from the surrounding vegetation and bottom sediments were also determined. The mean  $\delta^{13}\text{C}$  value of SOM was lower in the *terra-firme* ( $-29.6\text{‰}$ ) than in the *várzea* stream ( $-23.8\text{‰}$ ) as a result of less  $\text{C}_4$  plant deposition in the former. The  $\delta^{13}\text{C}\text{-CH}_4$  values varied systematically both seasonally and spatially among the sites during all 5 yr of the study, in association with changes during hydrologic cycle. Overall, the variation in values of  $\delta^{13}\text{C}\text{-CH}_4$  during the high water phase covered a narrower range of values,  $-63$  to  $-56\text{‰}$ . Contrastively, during the low water phase the  $\delta^{13}\text{C}\text{-CH}_4$  values for *várzea* and *terra-firme* streams are different and are in direct opposition. At this phase, the  $\delta^{13}\text{C}\text{-CH}_4$  at *terra-firme* stream is at least  $20\text{‰}$  depleted of  $^{13}\text{C}$  compared to *várzea* streams. Changes in organic matter sources, water levels and associated microbial degradation processes control the observed seasonal and spatial variations in net stable carbon isotopic composition of methane.

## 1. Introduction

Methane atmospheric concentration has doubled since the pre-industrial period (Chappellaz et al., 1990; Adams et al., 1996; Etheridge et al., 1998), reaching an average value of  $1.7 \text{ ppmv}^1$  in 1990 (Breas et al., 2002). Ice cores records, solar spectra, and direct atmospheric measurements document increases in methane emissions thought to result from human sources including rice cultivation, domestic ruminants and increased burning of fossil fuels (Wuebbles and Hayhoe, 2002). Changes in the concentration of atmospheric methane have important potential environmental consequences in addition to temperature alterations. These consequences include potential changes in ozone formation rates and mixing ratios of important radicals, water vapour

and chlorine (Gupta et al., 1996; Ridal and Siskind, 2002; Khalil and Rasmussen, 2004). Predictions of future changes in the atmospheric concentration of methane and their effect on the earth's atmosphere remain highly uncertain. The magnitude of methane emissions from a wide variety of sources could be affected by many factors including energy use, agricultural practices, land use change, human population distributions and climate (Wuebbles and Hayhoe, 2002).

Wetlands are the most important natural source of methane, emitting approximately  $92\text{--}237 \text{ TgCH}_4 \text{ yr}^{-1}$ , which corresponds to approximately 39–94% of total natural emissions (IPCC, 2001). Tropical wetlands are known to be the biggest natural source of methane to the atmosphere and are responsible for approximately 60% of the total flux from all wetlands on Earth (Cicerone and Oremland, 1988; Bartlett and Harriss, 1993). The Amazon basin represents a large portion of the world's humid tropics with a drainage system that exceeds six million square kilometres (Junk, 1997) and thus, assumes an important role in determining the total wetland flux of methane. Measurements of

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<sup>1</sup>ppmv = parts per million ( $10^6$ ) per volume

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methane emissions from flooded forests, lakes and macrophyte meadows spread out along the Amazon river basin indicated high fluxes of methane to the atmosphere (Bartlett et al., 1990). Recently, Melack et al. (2004) estimated the total methane flux for the Amazon River floodplain at  $1.3 \text{ TgC yr}^{-1}$  using field methane flux measurements and geoprocessing techniques. Extrapolation to the total area of the Amazon basin led them to estimate a total flux of  $22 \text{ TgC yr}^{-1}$ .

However, not only flooded areas but also upland forests in the Amazon may be a net source of methane. Carmo et al. (2006) estimated upland forest methane emissions to range from 4 to  $38 \text{ TgCH}_4 \text{ yr}^{-1}$  using data from three tower sites across the Amazon basin. Keppler et al. (2006) suggested, on the basis of laboratory plant incubation studies, that significant methane emissions from plant tissues occur even under aerobic conditions. Extrapolation of their laboratory results led them to estimate a global flux of methane up to  $120 \text{ TgCH}_4 \text{ yr}^{-1}$  for tropical forests, however, recent discussions by Kirschbaum et al. (2006) suggest an estimate at least six times lower ( $18.8 \text{ TgCH}_4 \text{ yr}^{-1}$ ) than the Keppler et al. (2006) estimate.

The stable carbon isotopic composition of atmospheric methane ( $\delta^{13}\text{C-CH}_4$ ) provides an important constraint on the global budget of  $\text{CH}_4$  (Stevens and Engelkemeir, 1988). The mean atmospheric global  $\delta^{13}\text{C-CH}_4$  value is equal to the weighted average composition of all methane sources corrected for fractionation occurring during removal by hydroxyl radical and others oxidative processes (Quay et al., 1988; Stevens, 1988; Stevens and Engelkemeir, 1988; Wahlen et al., 1989). Evaluation of the importance of individual methane sources using stable isotopes requires a capability to differentiate various biogenic (microbial) sources such as wetlands, rice paddies and ruminant animals from thermogenic sources such as natural gas venting and biomass burning. Another issue that needs to be addressed, besides the differentiation of individual sources of methane, is the detection of spatial and temporal variations in these sources in terms of both concentration and isotopic composition of the released gas. Understanding such variations in remote, annually flooded areas, such as the subbasins of the Amazon River, is especially important.

A wide range of  $\delta^{13}\text{C-CH}_4$  values has been reported from various wetlands around the world (Voytov, 1975; Chanton and Martens, 1988; Martens et al., 1992; Devol et al., 1996; Sugimoto and Fujita, 1997; Hornibrook et al., 2000b). In addition, the  $\delta^{13}\text{C}$  values of biogenic methane from temperate and sub-Arctic wetlands are known to vary seasonally as a result of changes in production mechanisms (Kelley et al., 1992; Martens et al., 1992; Chanton, 2005), oxidation during transport through overlying sediments and water (Chanton et al., 1992; Kruger et al., 2002; Marik et al., 2002), and gas transportation through aquatic plant tissues (Chanton, 2005).

Previous measurements of  $\delta^{13}\text{C-CH}_4$  from less accessible tropical *várzea*, primarily from the Amazon River basin (Tyler et al., 1987; Quay et al., 1988; Chanton et al., 1989; Devol et al.,

1996), show that there are significant differences in values even on small spatial scales. For example, Devol et al. (1996) observed that the methane in bubbles released from underlying sediments has lower values of  $\delta^{13}\text{C}$  than the methane released from floating meadows ('*capims*') overhead. Nevertheless, most methane flux and isotopic composition studies in the Amazon region have been restricted to the floodplain areas of the Amazon River's main channel because of obvious difficulties associated with collecting and analysing samples from remote areas of the basin. As a result, the potential existence of significant spatial and seasonal variations in concentrations, fluxes and isotopic composition of sedimentary methane from extensive smaller wetlands, lakes and tributaries and seasonally flooded areas has not been systematically explored.

Here we present the results of a 5-yr study of the carbon isotopic composition of methane in gas bubbles collected from sediments of two Amazon floodplain (*várzea*) streams and from an upland forest (*terra-firme*) stream in the eastern Brazilian Amazon near Santarém, Pará. Monthly collection of samples from these three stream sites allowed us to investigate both spatial and temporal variations in methane stable isotopic composition and to investigate the mechanisms controlling these variations. We also investigated the isotopic composition of vegetation surrounding the stream basins and of stream sedimentary organic matter (SOM) that allowed us to infer the influence of different sources of organic matter deposited in the stream sediments on the isotopic composition of sedimentary methane.

We hypothesize that the  $\delta^{13}\text{C}$  of methane in gas bubbles at shallow depths in the sediments of a specific stream should reflect differences in sources of organic matter and in processes acting to fractionate methane during production, oxidation and transport. We expected to find that methane released from the sediments in floodplain areas of the Amazon river (*várzea* streams) would be enriched in  $^{13}\text{C}$  compared to methane released from sediments in flooded areas in upland forests of the Amazon region (*terra-firme* stream), because of the greater abundance of  $\text{C}_4$  plants (grasses) and their relatively greater contribution to sedimentary organic matter in the floodplains.

## 2. Materials and methods

### 2.1. Study area and field sites

The study area is located in the midwest of the state of Pará in the municipalities of Santarém and Belterra. Three streambank transect stations were selected for repeated sampling: a single transect in the *terra-firme* stream Jamaraguá and two transects, one each in the *várzea* streams Açú and Maicá, as illustrated in (Fig. 1). It was necessary to move sampling operations toward and away from the stream bank along each transect in association with seasonal changes in water level. The characteristics of these streams differ in topography, water flow patterns in their channels and changes in the surrounding vegetation in their drainage

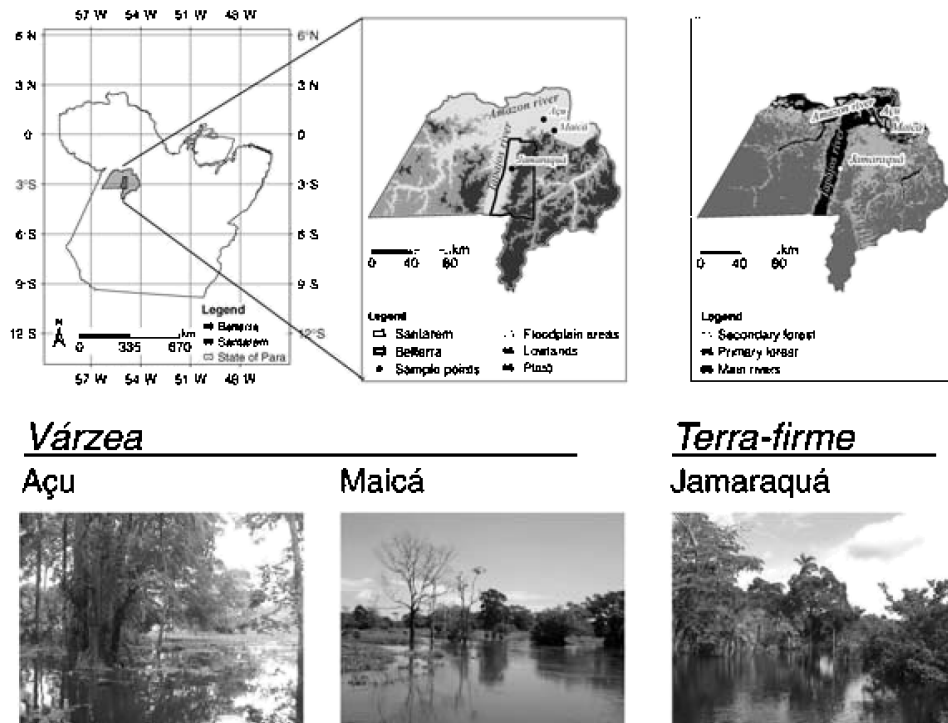


Fig. 1. Localization of the study area and illustration of studied streams.

basins. It is important to note that Maicá and Açu streams do not have well defined drainage basins and headsprings. In both cases, the formation of the streams is largely controlled by flooding of topographic depressions by the Amazon and Tapajós rivers, forming very common watercourses types in the Amazon region that connect two rivers (Açu Stream) or just pass through periodically flooded areas of the Amazon River (Maicá Stream).

The Jamaraguá Stream is located in the Tapajós National Forest and its entire basin is surrounded by primary tropical forest (predominately  $C_3$  plants). The drainage area of the basin is approximately 46 km<sup>2</sup> with 44% corresponding to areas with altitudes ranging from 5 to 131 m (lowlands), and 56% corresponding to the *platô* area with altitudes ranging from 132 to 203 m. The width of the channel is about 1.5 m near the stream's headspring. Our sampling transect is located approximately 900 m from the stream's entrance into the Tapajós River and is not covered by the forest; therefore the light penetrates the water allowing the growth of a large number of different  $C_3$  macrophytes that totally cover the bottom. During the high water phase, the stream is approximately 150 m wide. Rice et al. (2004), working in a nearby site reported a mean value of 143.7 Mg C ha<sup>-1</sup> for aboveground live biomass and an additional 48.0 Mg C ha<sup>-1</sup> of coarse woody debris.

The Maicá Stream is a white-water (sediment-laden) side channel (*paraná*) of the Amazon River covered by characteristic *várzea* vegetation. White-water streams are loaded with suspended sediments and dissolved nutrients provided by the weathering of Andean rocks and are characterized by a yellow tone and a 10–50 cm limit of visibility (Sioli, 1970). In the Ama-

zon, white-water floodplains are characterized by the presence of species well adapted to annual flood pulses that reduce oxygen levels in the rhizosphere, requiring the development of specialized survival and reproduction strategies (Junk et al., 2001; De Simone et al., 2002; De Simone et al., 2003). High growth rates of *Paspalum* sp. grass ( $C_4$  plant); locally known as '*premembeca*', occur during the high water phase. This vegetation forms extensive meadows within the white-water channels and in their floodplain areas. Junk and Piedade (1993) reported a biomass of 22 t ha<sup>-1</sup> of this grass in a lake located in the Amazon River floodplain near Manaus-AM. The grass biomass and exudates are known to be substrates for methanogenesis (Junk, 1970; Hedges et al., 1986; Devol et al., 1995).

The Açu Stream has similar vegetation to the Maicá Stream; however, there is far less growth of *premembeca* as compared to Maicá. Also, the forest surrounding Açu is a mix of *várzea* and *igapó*; which is the most common vegetation cover found in black or clear-water river floodplains in the Amazon region. Near both streams local inhabitants raise cattle using the grass to feed the animals. Although sporadically visited by the animals during the low water phase, our sampling sites were located upstream and away from these activities.

## 2.2. Sampling approaches

### 2.2.1. Methane gas bubble samples

Methane-rich gas bubble samples were taken in water depths varying from 0.5 to 3.0 m according to seasonal changes in water level and location along each transect. As the dry season advanced we moved toward

the stream channels, moving back towards the banks during the wet season. To collect the samples we followed the method described by Martens et al. (1992). The gas was collected in an inverted funnel (30 cm diameter; 4.1 L volume) mounted on the end of an extension plastic pole placed just below the waterline. The funnel allowed us to trap rising bubbles released from the sediment immediately after its disturbance with another pole, thus preventing extensive gas exchange with overlying waters. A 60 mL syringe was used to pull the gas out of the funnel through low gas permeability nylon tubing connected to the apex of the funnel. The gas samples were then transferred into pre-cleaned glass vials by water displacement and sealed with thick butyl rubber stoppers without air contact. Ten samples were taken monthly at each site, totalling over 360 samples per year. Although the sampling points were located along the same transect throughout the year, samples were periodically collected from both shores of the streams in order to help determine local spatial variability near our transects.

**2.2.2. Core sediment sampling.** Based on the methods described by Alperin et al. (1992), sediment samples were taken for analysing the C and N abundances and stable C isotopic composition of the organic matter deposited in the stream sediments. A 10 cm diameter PVC tube was inserted into the sediment until approximately 30 cm depth and then capped on the bottom by digging down before withdrawal. A pole with a rubber stopper tip was used to extrude sediment and the cores were sectioned at 5 cm intervals. The average wet weight for each sub-sample was 180 g. After oven drying (48 hr at 100 °C) the samples were processed and analysed. Three core sampling campaigns were conducted, one during the high water phase (June/2004) and two during the low water phase (November/2003 and January/2005).

**2.2.3. Vegetation and ground deposited material sampling.** Samples of the upper 5 cm of material found exposed on the stream banks during the low water phase were taken along a 30 m transect from sediments 10 m offshore to 20 m into the forest. We also collected green leaves from understory plants and grasses located on the stream shores.

### 2.3. Analytical methods

**2.3.1. CH<sub>4</sub> stable carbon isotopic composition.** The stable carbon isotopic composition of methane in all gas samples was determined by isotope ratio mass spectrometry (Thermo Electron Delta Plus IRMS coupled to a PRECON gas concentrator). This device allowed the injection of gas samples (~20 μL) into a continuous flow system (Helium as carrier), which moved the gas sample through a cryogenic trap (-196 °C) and a GC column (Poraplot Q at 30 °C) in order to separate the CH<sub>4</sub> from any other gas. The CH<sub>4</sub> was combusted over an oxidation reactor (at 1000 °C) into CO<sub>2</sub> and H<sub>2</sub>O. The water was removed downstream by a Nafion trap in the Precon/MS interface. Finally, the isolated CO<sub>2</sub> was injected into the mass spectrometer and analysed for its 13C/12C ratio. The isotopic composition of methane (δ<sup>13</sup>C-

CH<sub>4</sub>) was expressed as the deviation in per thousand (‰) relative to a primary standard according to the following equation:

$$\delta^{13}\text{C} = 1000 \times \left( \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right), \quad (1)$$

where  $R_{\text{sample}}$  is the isotope ratio <sup>13</sup>C/<sup>12</sup>C of the sample and  $R_{\text{standard}}$  is the isotope ratio <sup>13</sup>C/<sup>12</sup>C of the standard PDB ( $R_{\text{standard}} = 0.0112372$ ).

Analytical calibration was provided based on comparison with a methane standard (δ<sup>13</sup>C-CH<sub>4</sub> = -18.6‰). The standard was analysed every 10 samples to insure accuracy. The isotopic composition of this standard was first determined by vacuum line combustion at University of North Carolina at Chapel Hill and then verified by cross calibrating against various other independent laboratories, including those operated at North Carolina State University by Neal Blair, and Florida State University by Jeff Chanton.

**2.3.2. C and N elemental abundances and stable C isotopic composition of sediment and vegetation.** The collected material (sediment, leaves and soil) was dried, ground and weighed in tin capsules (Elemental Microanalysis) for combustion in a continuous flow EA-IRMS for elemental abundance (EA) and stable isotope ratio measurements using a Carlo Erba NA 1110 CHNS, coupled to the mass spectrometer through a ConFlo interface. Sample sizes ranged from 10 to 44 mg for soil and sediment samples and from 1.5 to 1.8 mg for leaf samples. The capsules were burned at 1020 °C and the CO<sub>2</sub> and N<sub>2</sub> gases from the combustion process were purified before entering the mass spectrometer. The ratio of <sup>13</sup>C/<sup>12</sup>C was measured in each sediment sample and the δ<sup>13</sup>C value was calculated according to eq. (1). Elemental abundances of C and N were simultaneously measured for each sample. Standard reference materials; soil (LECO Corporation, PN: 502-308) and atropine (Elemental Microanalysis Limited, PN: 338.24400), were used to calibrate sediment and vegetation samples analysis, respectively) and to verify the accuracy of the instrumentation. For C and N elemental concentrations the accepted errors were always less than 5% while for carbon isotope measurements the error was less than 0.3‰.

**2.3.3. Statistical analysis.** All analysis were made using SAS System for Windows, version 8.2 (SAS, 2001). We used the Kolmogorov–Smirnov tool to test data normality. The T-Turkey test was used to detect differences between groups with equal variances and the weighted ANOVA was used to compare data sets with different variances. The significance level considered for all analysis was 5%.

## 3. Results

### 3.1. Carbon isotopic composition of methane (bubbles)

The mean δ<sup>13</sup>C values of methane trapped in bubbles collected from the stream sediments are summarized in Table 1 and range from -75.1 to -52.7‰. We can divide the years into four

Table 1. Means ( $\bar{x}$ ), standard deviation ( $\sigma$ ), and number of samples ( $n$ ) for the  $\delta^{13}\text{C}\text{-CH}_4$  values during hydrograph phases

Site		Phases of hydrograph			
		High water	Falling water	Low water	Rising water
Maicá	$\bar{x}$	-58.5	-56.5	-52.7	-54.2
	$\sigma$	2.3	4.0	5.2	5.8
	$n$	122	73	100	153
Açu	$\bar{x}$	-57.8	-58.2	-55.1	-56.7
	$\sigma$	2.2	3.0	4.7	2.4
	$n$	117	107	100	132
Jamaraquá	$\bar{x}$	-63.0	-64.0	-75.1	-64.5
	$\sigma$	3.9	4.8	7.2	4.3
	$n$	117	131	108	129

Maicá: High water (May–July), Falling water (August–September), Low water (October–December) and Rising water (January–April)

Açu: High water (May–July), Falling water (August–September), Low water (October–December) and Rising water (January–April)

Jamaraquá: High water (April–June), Falling water (July–September), Low water (October–December) and Rising water (January–March)

periods on the basis of changes in water level in order to make quantitative seasonal comparisons. Systematic seasonal differences occur during all 5 yr of the study in association with changes between the two extreme water level stages. During the low water phase the  $\delta^{13}\text{C}\text{-CH}_4$  differences between the most distinct streams (Maicá and Jamaraquá) are greater than 21%. At both Açu and Maicá (*várzea* streams), the higher values of  $\delta^{13}\text{C}\text{-CH}_4$  appear during the low water phase,  $-55.1 \pm 4.7$  and  $-52.7 \pm 5.2\%$ , respectively. The lower  $\delta^{13}\text{C}\text{-CH}_4$  values for these streams were measured during the high water phase at Maicá and during the falling water phase at Açu. Methane from the Maicá Stream had a mean value of  $-58.5 \pm 2.3\%$  during the high water period, slightly more depleted of  $^{13}\text{C}$  than Açu Stream ( $-57.8 \pm 2.2\%$ ). Conversely, at the *terra-firme* stream (Jamaraquá) the low water phase is the period when the most depleted values of  $\delta^{13}\text{C}\text{-CH}_4$  are measured, with a mean value of  $-75.1 \pm 7.2\%$ . The highest  $\delta^{13}\text{C}\text{-CH}_4$  value for this stream ( $-63.0\%$ ) was measured during the high water phase. Among the three sites, carbon methane isotopic composition at the *terra-firme* stream (Jamaraquá) had the highest range between the high and low water phases (12.1%). For the *várzea* streams, the differences between the two phases were only 5.8% (Maicá) and 2.7% (Açu).

At Jamaraquá, the carbon isotopic composition of methane had a strong positive correlation ( $p = 0.0001$ ) with the variation of water level ( $Y = 3.121x - 75.87$ ;  $R^2 = 0.80$ ), and the gas tended to show higher  $\delta^{13}\text{C}$  values as the water column depth increased.

At Maicá, the mean values of  $\delta^{13}\text{C}\text{-CH}_4$  on individual sampling dates ranged from  $-64.4$  to  $-45.0\%$ . We observed less

variation in values during the high water phase than during the low water phase. At this stream, we also determined an inverse correlation ( $p = 0.002$ ) between the  $\delta^{13}\text{C}\text{-CH}_4$  and the discharge<sup>2</sup> of the river ( $Y = -0.062x - 44.62$ ;  $R^2 = 0.62$ ). The same trend was observed for the variation of dissolved oxygen against depth at this site ( $Y = -3.591x + 7.736$ ;  $R^2 = 0.93$ ).

Overall, the observed variation in mean values of  $\delta^{13}\text{C}\text{-CH}_4$  during the high water phase covered a narrower range of values,  $-63$  to  $-58\%$ , throughout the entire 5-yr period of observation (August/2000 to August/2005). However, during the low water phase, the range in  $\delta^{13}\text{C}\text{-CH}_4$  values was much greater. At low water phase the gas from the *terra-firme* stream reached extremely negative values ( $\delta^{13}\text{C}\text{-CH}_4 \sim -75\%$ ), while at the *várzea* stream (Maicá) the reverse trend is observed and the gas becomes more enriched than during the high water phase ( $\delta^{13}\text{C}\text{-CH}_4 \sim -53\%$ ). This same trend was observed at the Açu Stream, but the variation at this site differed among years during our 5-yr observation period (Fig. 2).

### 3.2. Sedimentary organic matter %C<sub>org</sub>, $\delta^{13}\text{C}$ and atomic C:N ratios

The %C<sub>org</sub>,  $\delta^{13}\text{C}$  values and atomic C:N ratios of sedimentary organic matter (SOM) varied significantly between the two stream types. Data from the sediment cores collected during the high water phase (June/2004) and low water phase (November/2003 and January/2005) are illustrated in (Table 2).

The sediment at *terra-firme* stream, Jamaraquá, had mean values of  $-29.6 \pm 0.4\%$ ,  $14.6 \pm 3.1\%$ ,  $0.9 \pm 0.2\%$  and  $17.1 \pm 1.2$  for the  $\delta^{13}\text{C}$  of SOM, %C, %N and C:N ratio, respectively. There was no statistically valid seasonal variation among these parameters in sediments from this stream. The *várzea* stream, Maicá, had mean values of  $-23.8 \pm 2.2\%$ ,  $1.1 \pm 0.8\%$ ,  $0.1 \pm 0.08\%$  and  $8.8 \pm 1.1$  for the same parameters. We observed statistically significant seasonal variations for all parameters, except for C:N ratios.

The  $\delta^{13}\text{C}$  values of SOM in Jamaraquá, ranged slightly from  $-30.4$  to  $-28.9\%$ . During the high water phase SOM  $\delta^{13}\text{C}$  values tended to be lower than during the low water period, however, the variation was not statistically significant ( $p = 0.174$ ). The values for %C, %N and atomic C:N ratios at this site showed just a small difference between high and low water phases. The carbon content ranged from 10.9 to 14.6% and tended to decrease with depth. During the low water phase the average carbon content was only 1.7% smaller than during the high water phase and was not statistically different ( $p = 0.296$ ). The N content ranged from 0.6 to 0.9% and did not show any seasonal variation. The same trend was observed for C:N ratios, which ranged from 15.1

<sup>2</sup>As we did not have a reliable data set for the discharge or variation of the water level at this site we used the long data set of the Amazon River, collected at the Municipality of Óbidos-PA by the Agência Nacional das Águas-ANA.

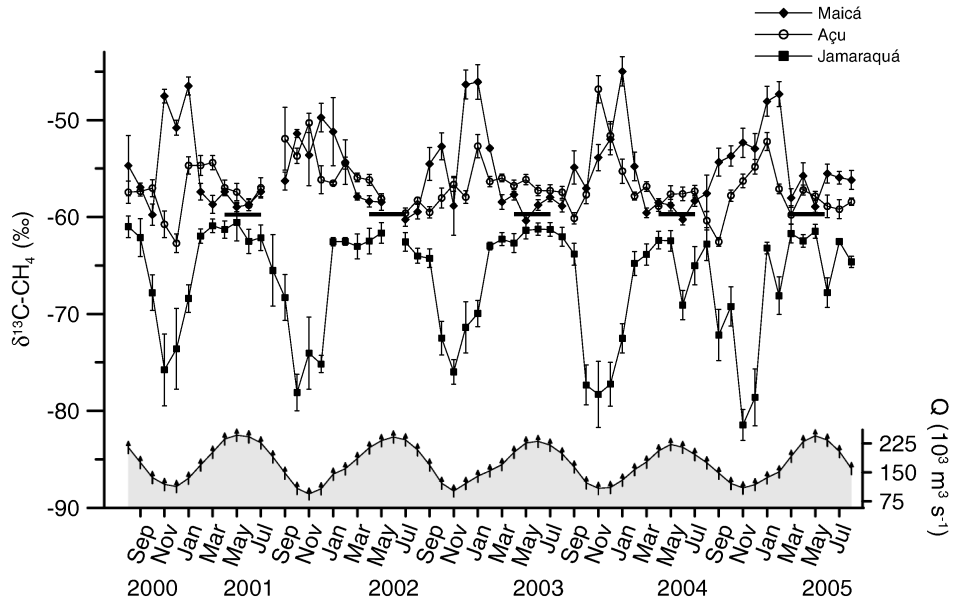


Fig. 2. Seasonal variations of  $\delta^{13}\text{C-CH}_4$  at the várzea streams (Maicá and Açú) and at the terra-firme stream (Jamaraquá) for 5 yr. The values represent the monthly average and respective standard error for each site during the period from August/2000 to August/2005. The dashed marks represent the mean value for all streams during the high water phase ( $-59.74\%$ ,  $n = 356$ ). The inferior graphic shows the discharge of the Amazon River, measured at the Municipality of Óbidos-PA by the Agência Nacional das Águas-ANA.

Table 2. Density ( $\rho$ ),  $\delta^{13}\text{C}$ , carbon content, nitrogen content and C:N ratio of sedimentary organic matter in two Amazonian streams. The data represents the average values ( $\bar{x}$ ) and the corresponding 1 standard deviation ( $\sigma$ ), in the form:  $\bar{x} \pm \sigma$

Site	Phase	Depth (cm)	$\rho$ ( $\text{g cm}^{-3}$ )	$\delta^{13}\text{C}$ (‰)	C (%)	N (%)	C:N
Jamaraquá ( <i>terra-firme</i> )	High water	0 – 15	$0.38 \pm 0.03$	$-29.7 \pm 0.2$	$15.8 \pm 5.1$	$0.9 \pm 0.2$	$18.2 \pm 1.0$
	Low water	0 – 15	$0.51 \pm 0.21$	$-29.5 \pm 0.5$	$14.1 \pm 2.0$	$0.8 \pm 0.1$	$16.5 \pm 0.9$
Maicá ( <i>várzea</i> )	High water	0 – 15	$0.49 \pm 0.19$	$-27.5 \pm 1.7$	$2.5 \pm 0.8$	$0.3 \pm 0.1$	$9.7 \pm 1.0$
	Low water	0 – 15	$0.79 \pm 0.33$	$-22.8 \pm 1.0$	$0.8 \pm 0.4$	$0.1 \pm 0.0$	$8.6 \pm 1.0$

to 17.1, being just a little higher during the high water phase ( $p = 0.110$ ). Some of these differences may result from changes in sediment composition along our stream edge transects as we followed seasonal water level change.

At Maicá, the *várzea* stream, the  $\delta^{13}\text{C}$  of SOM ranged seasonally from  $-28.6$  to  $-21.1\%$ . As opposed to the *terra-firme* stream, we detected significant differences in the isotopic composition of the sediment between the high and low water phases ( $p = 0.024$ ). The carbon content at the *várzea* stream differs greatly from the *terra-firme* stream ( $p = 0.000$ ) and was much lower at the former. We also found evidence of seasonal differences in the %C ( $C_{\text{org}}$ ) at the *várzea* stream ( $p = 0.033$ ). During high water phase the concentration of  $C_{\text{org}}$  was at least three times higher than during low water phase. The same trend was observed for total N (TN) content, which was lower during the low water phase ( $p = 0.022$ ). The atomic C:N ratio was slightly lower during the low water period, however, there was no significant seasonal variation ( $p = 2.920$ ).

### 3.3. Vegetation $\delta^{13}\text{C}$ values, water temperature and dissolved oxygen

Water temperature and dissolved oxygen profiles (1 m depth) data from the *várzea* (Maicá) and at the *terra-firme* (Jamaraquá) streams are summarized in Table 3. Stable C isotopic values of living vegetation are also included.

## 4. Discussion

### 4.1. Organic matter sources and stream sediment composition

A comparison between the  $\delta^{13}\text{C}$  values and C:N ratios of sedimentary organic matter reveal a clear distinction between the *terra-firme* and *várzea* streams. The SOM from *várzea* (Maicá) had higher values of  $\delta^{13}\text{C}$  and lower C:N ratios than the *terra-firme* stream, Jamaraquá, presumably because of differences in the source inputs of  $\text{C}_3$  versus  $\text{C}_4$  plant material. The  $\text{C}_4$  plant

Table 3. Water temperature, dissolved oxygen (DO), and stable C isotopic composition of vegetation at *terra-firme* site (Jamaraguá) and at the *várzea* site (Maicá). Means ( $\bar{x}$ ) are presented together with the corresponding 1 standard deviations ( $\sigma$ ) and number of observations ( $n$ ), in the form:  $\bar{x} \pm \sigma(n)$

Site	Temperature ( $^{\circ}\text{C}$ )	DO ( $\text{mg L}^{-1}$ )	$\delta^{13}\text{C}$ ( $\text{‰}$ )	
			$\text{C}_4$ plant (grass)	$\text{C}_3$ plant
Jamaraguá	$28 \pm 0.7$ (28)	$8.8 \pm 2.7$ (30)	–	$-31.7 \pm 2.0$ (11)
Maicá	$31 \pm 0.7$ (21)	$5.6 \pm 1.5$ (21)	$-11.5 \pm 0.5$ (4)	$-29.2 \pm 1.9$ (3)

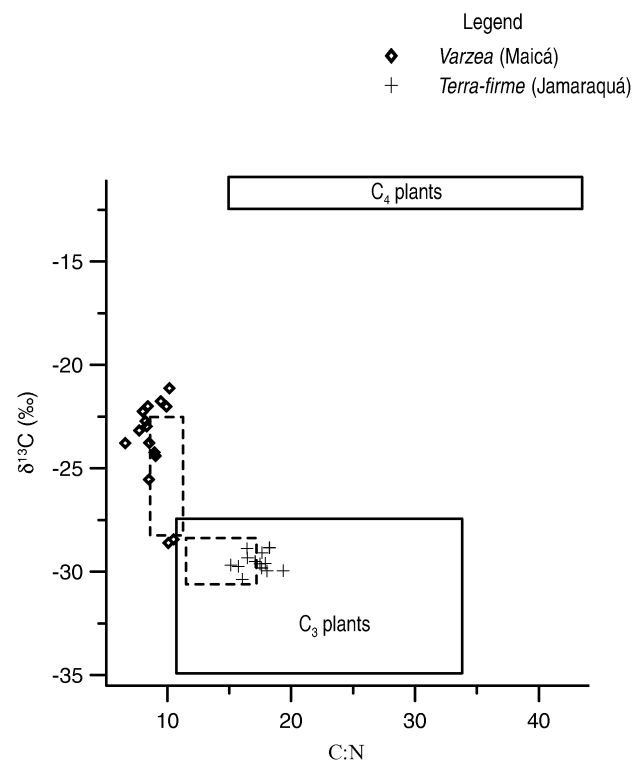


Fig. 3. Organic matter  $\delta^{13}\text{C}$  values versus C:N ratios for the sediments deposited at *várzea* (diamonds) and *terra-firme* (crossings) streams. The solid line rectangles indicate the expected distributions of values for  $\text{C}_3$  and  $\text{C}_4$  plant leaves. The dashed rectangles represent the values for SOM collected at the *várzea* transect (grey) and *terra-firme* transect (black).

tissue collected at Maicá was at least 15‰ enriched in  $^{13}\text{C}$  relative to  $\text{C}_3$  plants, and its C:N ratio tended to be higher than that of  $\text{C}_3$  plants. The  $\delta^{13}\text{C}$  values and C:N ratio for the SOM deposited at the *terra-firme* site, and along its superficial sediment transect, showed values representative of the  $\text{C}_3$  plants dominant in the stream understory, indicating that they have a common origin (Fig. 3).

#### 4.2. Calculated contributions of $\text{C}_3$ and $\text{C}_4$ plants to stream SOM

The relative contributions of  $\text{C}_3$  and  $\text{C}_4$  plants to total sediment composition were calculated using the following simple isotope

mass balance approach:

$$P_{\text{C}_3} = \left( \frac{\delta_{\text{sed}} - \delta_{\text{C}_4}}{\delta_{\text{C}_3} - \delta_{\text{C}_4}} \right) \times 10^2 \quad (2)$$

$$P_{\text{C}_4} = 100 - P_{\text{C}_3}, \quad (3)$$

where  $P$  is percentage of  $\text{C}_3$  or  $\text{C}_4$  plant;  $\delta_{\text{sed}}$  is mean value of  $\delta^{13}\text{C}$  for the sedimentary organic matter from the Maicá Stream and Jamaraguá Stream;  $\delta_{\text{C}_3}$  is mean value of  $\delta^{13}\text{C}$  for the forest understory leaf samples and  $\delta_{\text{C}_4}$  is mean value of  $\delta^{13}\text{C}$  for grass (*Paspalum*) collected at Maicá Stream. The  $\delta^{13}\text{C}$  values of the organic matter deposited in the sediment profiles (0–15 cm) showed that the contribution of  $\text{C}_3$  and  $\text{C}_4$  plants varied spatially among the streams. According to the isotope mass balance (eqs 2 and 3), at the *terra-firme* stream, approximately 92% of the matter that compound the sediment comes from  $\text{C}_3$  plants and less than 10% comes from  $\text{C}_4$  plants (Fig. 4a).

At the *várzea* stream, Maicá, the contribution of  $\text{C}_4$  plants is relatively higher than at the *terra-firme* stream and averages around 41% (Fig. 4b).

#### 4.3. Water level and SOM source influences on $\delta^{13}\text{C}\text{-CH}_4$ values

The total seasonal range in  $\delta^{13}\text{C}\text{-CH}_4$  values observed during our 5-yr study,  $-75.1$  to  $-52.8\text{‰}$ , is similar to seasonal and spatial ranges measured in other wetland environments around the world including the Amazon basin. The observed seasonal ranges at all three stream sites are similar in magnitude to those measured in marine (Martens et al., 1986) and freshwater systems (Kelley et al., 1992; Martens et al., 1992). Sugimoto and Fujita (1997) reported  $\delta^{13}\text{C}$  values ranging from  $-78.8$  to  $-52.5\text{‰}$  for  $\text{CH}_4$  from a temperate bog in Japan. Less seasonal variation was reported by Chanton and Martens (1988) in the White Oak River Estuary in North Carolina, USA, where  $\delta^{13}\text{C}\text{-CH}_4$  values ranged seasonally from  $-77.9$  to  $-62.6\text{‰}$ . Wassmann et al. (1992) reported values ranging from  $-68.7$  to  $-49.7\text{‰}$  for sediment gas bubbles from spatial survey at *várzea* sites along the Amazon River. Devol et al. (1996), also working along the Amazon River at *várzea* sites, reported values ranging from  $-63.1$  to  $-50.5\text{‰}$ .

Our detailed study of seasonal variability at the three stream sampling sites has revealed dynamic water level and SOM

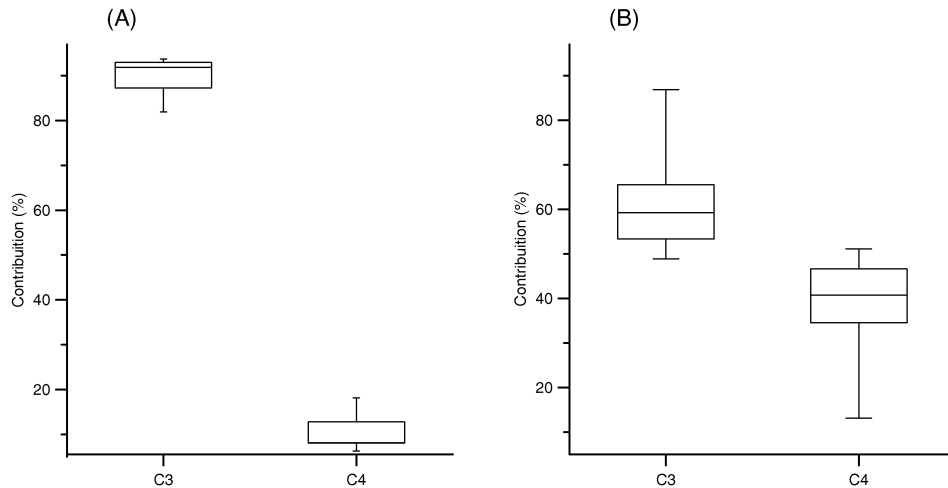


Fig. 4. Contributions of C3 and C4 plant material to organic matter present in bottom sediment of the terra-firme stream; Jamaraquá (A) and várzea stream, Maicá (B). The caps represent the minimum and maximum values and the horizontal bars indicate the median, lower and upper quartiles for the distribution.

sources influences on methane stable isotopic composition that can lead to dramatic and seasonally reproducible differences between individual sites. The most striking finding is that seasonal variations in  $\delta^{13}\text{C}\text{-CH}_4$  values at the *terra-firme* stream, Jamaraquá, are in direct opposition to and markedly different from those at Maicá and Açú. Possible factors controlling these differences include site-specific differences in sources of reactive SOM and/or variations in the mechanisms of methane production and aerobic (Rudd et al., 1974) or anaerobic (Alperin et al., 1992) methane oxidation processes. Gas bubbles collected at our stream sampling sites were released from organic-rich, anoxic sediments containing little dissolved sulphate, thus, we did not expect any methane isotopic variation to result from aerobic or anaerobic oxidation processes. Therefore, we might expect the systematic variability observed to result from either differences in organic matter sources and/or variations in methane production pathways (acetoclastic versus  $\text{CO}_2/\text{H}_2$  reduction).

Methane produced from C<sub>3</sub> plant tissue should be depleted of  $^{13}\text{C}$  compared to methane produced from C<sub>4</sub> plants (Chanton et al., 1989). In addition, lower C:N ratios and freshness of sedimentary organic matter are useful indicators of its reactivity (Martens et al., 1992). Mathuriau and Chauvet (2002) demonstrated that the decomposition of leaves in streams is correlated with their C:N ratio, observing that decomposition of N-enriched material is faster than in N-depleted materials. The lower values of the C:N ratio of SOM measured in the *várzea* stream, Maicá, as compared to the *terra-firme* stream (Fig. 3), suggest that the SOM at the *várzea* stream sites, which is degraded to produce substrates for methanogenesis, should be more reactive.

There is a clear relationship between the composition of living vegetation present at each stream site and the sediment, even after the decomposition of fresh material has reached advanced stages (Fig. 3). At Maicá, as in most other streams in the Amazon

*várzea*, a considerable amount of grass (*Paspalum* sp.) grows on the stream shores and in their channels during the high water phase of the hydrograph. These grasses remain alive, during the high and falling water phases or only partially decompose in these environments. The visible deposition of large quantities of these C<sub>4</sub> grasses (enriched in  $^{13}\text{C}$ ) undoubtedly accounts for the higher  $\delta^{13}\text{C}$  values for SOM deposited on the shores and at the bottom of the stream channels compared to material deposited on the surrounding forest floor (Table 2).

Furthermore, at the *várzea* sites the values of  $\delta^{13}\text{C}$  for sediment were 5‰ higher during the low water phase than during high water phase (Table 2). A correlation between gas bubble  $\delta^{13}\text{C}\text{-CH}_4$  values and SOM  $\delta^{13}\text{C}$  values at Maicá provides evidence that the SOM sources at that site control the observed changes in methane isotopic composition. The higher early variability at Açú probably occurs due to a combination of changes in the environmental conditions (e.g. water temperature, dissolved oxygen level, column water pressure and light penetration) and the contributions of seasonally changing in the vegetation that grows along the stream channel.

The similar values of  $\delta^{13}\text{C}\text{-CH}_4$  found at all three stream sites during the high water phase (Fig. 2, see the dashed marks) suggest that there is a common source of fresh, highly degradable organic matter produced and deposited in the sediments of Maicá, Açú and Jamaraquá during those months. If highly reactive, this organic may be entirely consumed during the dry season and not leave an isotopic signature in the remaining SOM.

Martinelli et al. (2003) analysed samples of the sediment deposited in *várzea* streams located in the western portion of the Amazon basin and reported similar values for the parameters we analysed ( $\delta^{13}\text{C} = -27.6 \pm 1.8\text{‰}$ , C =  $0.92 \pm 0.28\%$ , N =  $0.11 \pm 0.03\%$  and C:N =  $8.6 \pm 2.1$ ). The  $\delta^{13}\text{C}$  values we measured are  $^{13}\text{C}$ -enriched in comparison to those present by Martinelli et al. (2003) presumably due to the contribution of



$^{13}\text{C}$ -enriched  $\text{C}_4$  grasses, which are more abundant in the eastern portions of the Amazon basin (Victoria et al., 1992; Martinelli et al., 2003).

At the *terra-firme* stream the elemental and stable carbon isotopic compositions of the surrounding  $\text{C}_3$  plants and SOM are similar and the  $\delta^{13}\text{C}\text{-CH}_4$  values for methane seen during the low water phase are  $^{13}\text{C}$ -depleted relative to high water phase values, exactly the opposite of the seasonal trends in values from *várzea* streams (Fig. 2). A comparative analysis of the isotopic composition of SOM at the *terra-firme* streams between the high and low water phases does not show any significant difference (Table 2), suggesting that changes in organic matter sources may not be responsible for controlling the extreme seasonal variability in  $\delta^{13}\text{C}\text{-CH}_4$  values. However, a lack of seasonal changes in the isotopic composition of the SOM at Jamaraquá might be expected since the drainage basin of this stream is located in a primary forest area surrounded by  $\text{C}_3$  plants (Fig. 1) where the turnover of reactive organic matter during the high water phase is fast (months). The mean  $\delta^{13}\text{C}$  value for organic carbon in fresh leaves collected at Jamaraquá was  $-31.7\text{‰}$  (Table 3), which was similar to values reported by Ometto et al. (2006) in nearby sites at the Tapajós National Forest averaging  $-32.5\text{‰}$ .

As discussed above, the similar values of  $\delta^{13}\text{C}\text{-CH}_4$  found at all three stream sites during the high water phase (Fig. 2) may result from a common source of fresh, highly degradable organic matter at Maicá, Açu and Jamaraquá during those months. Replacement of this common source by organic matter from surrounding forest  $\text{C}_3$  plants during the dry season could then result in the observed swing towards lower  $\delta^{13}\text{C}\text{-CH}_4$  values during the low water phase.

#### 4.4. Influence of methane production pathways on $\delta^{13}\text{C}\text{-CH}_4$

The isotopic composition of methane produced in a natural sediment should reflect a combination of the isotopic composition of specific substrates (Martens et al., 1986; Gelwicks et al., 1994) and fractionations occurring during different methane production pathways (Martens et al., 1986; Alperin et al., 1992; Marik et al., 2002). For example, Sugimoto and Wada (1993) demonstrated that methane produced during acetate fermentation is relatively  $^{13}\text{C}$ -enriched as compared to methane produced from  $\text{CO}_2$  reduction, producing  $\delta^{13}\text{C}\text{-CH}_4$  values that are only 11‰ depleted relative to the  $\delta^{13}\text{C}$  of the acetate methyl group. Due to the low abundance of acetate during aceticlastic methanogenesis, a barely expression of metabolic kinetic isotope effects (KIEs) is expected, resulting in a gas production,  $\text{CH}_4$  and  $\text{CO}_2$ , with  $\delta^{13}\text{C}$  values similar to the intramolecular distribution of sedimentary acetate (Hornibrook et al., 2000a).

At the *várzea* streams, Maicá and Açu, where the values of  $\delta^{13}\text{C}\text{-CH}_4$  are higher during the low water phase (Fig. 2), the contribution of the  $^{13}\text{C}$ -enriched  $\text{C}_4$  grass (*Paspalum* sp.) appears to be the primary controlling factor of the seasonal variation in

$\delta^{13}\text{C}\text{-CH}_4$ . At the *terra-firme* stream, Jamaraquá, where we reported the most depleted values of  $\delta^{13}\text{C}\text{-CH}_4$  (Fig. 2), we cannot rule out the possibility that changes in the dominant pathway of methane formation might have important influences on the  $\delta^{13}\text{C}$  of the gas bubble methane because of the lack of direct evidence for concurrent changes in methane and SOM carbon isotopic values. During methane production higher values of  $\delta^{13}\text{C}$  are generally expected to result from acetate fermentation while lower values (less enriched in  $^{13}\text{C}$ ) occur during  $\text{CO}_2$  reduction (Alperin et al., 1992; Sugimoto and Wada, 1993; Marik et al., 2002). Whiticar et al. (1986) demonstrated that aceticlastic methane generally had  $\delta^{13}\text{C}$  values ranging from  $-70$  to  $-45\text{‰}$  while methane with lower values of  $\delta^{13}\text{C}$  ( $-110$  to  $-60\text{‰}$ ) resulted from  $\text{CO}_2$  reduction. In natural environments the  $\delta^{13}\text{C}$  value of acetate produced during organic matter decomposition is not significantly different from the  $\delta^{13}\text{C}$  of its organic matter source (Blair et al., 1987), which for  $\text{C}_3$  plants is approximately equal to  $-28\text{‰}$ .

In addition to differences in production pathways, physical factors could be important in controlling gas bubble  $\delta^{13}\text{C}\text{-CH}_4$  values in shallow Amazonian streams with luxurious plant growth along bank margins. Kelley et al. (1992), working in peat wetland areas in Minnesota, USA, reported an inverse correlation between  $\delta^{13}\text{C}\text{-CH}_4$  values and water column depth. They hypothesized that the reduction of water column pressure would favour the penetration of atmospheric oxygen via aquatic plants, which in turn, should result in an increase in oxidation rates of methane and isotopic enrichment of the remaining gas.

## 5. Conclusions

We observed large, systematic seasonal variations over a 5-yr period in the stable isotopic composition of methane from the sediments of three stream sites in the eastern Amazon basin near Santarém, Pará. We also observed significant differences between the three streams that appear to be based on a combination of organic matter source and gas production pathways. The seasonal and spatial isotopic variations in methane and in sedimentary organic matter have proven to be useful for determining differences in the mechanisms and relative importance of processes controlling methane production and fluxes. The observed variations occur as a consequence of the differences in physical environmental characteristics of each site, the contribution of different sources of organic matter, and the processes by which this organic matter is decomposed.

During the high water period, the sources of organic matter and the processes by which they are decomposed appear to be similar in all three streams since the range in the average values of  $\delta^{13}\text{C}\text{-CH}_4$  produced in this period is relatively small (from  $-63$  to  $-58\text{‰}$ ). However, during the low water period a much larger range is observed ( $-75$  to  $-53\text{‰}$ ). The relative contribution of organic matter from  $\text{C}_3$  versus  $\text{C}_4$  plants is key to explaining differences in the composition isotopic of the methane during

this phase. At the *várzea* streams (Maicá and Açú) the  $\delta^{13}\text{C}\text{-CH}_4$  is generally higher than at the *terra-firme* stream (Jamaraquá) due to the fact that in the latter the organic matter in the sediment is composed almost entirely of  $\text{C}_3$  plant matter which has lower values of  $\delta^{13}\text{C}$  than  $\text{C}_4$  plant material more commonly seen at the *várzea* sites.

Higher isotopic values measured in *várzea* stream sediments during the low water period appear to result primarily from degradation  $\text{C}_4$  grass that grows at the *varzea* at the end of high water period. Conversely, gas bubble  $\delta^{13}\text{C}\text{-CH}_4$  values in the *terra-firme* stream sediments exhibit lower values during the low water period. This behaviour could possibly result from a change in the methanogenic pathway, from acetate fermentation to  $\text{CO}_2$  reduction.

Our knowledge about the mechanisms and rates of methane generation in *várzea* and *terra-firme* wetlands in the Amazon remains limited; however, it is clear that systematic changes in organic matter sources, water levels and associated microbial degradation processes control the observed seasonal and spatial variations in net stable carbon isotopic composition of methane emitted to the atmosphere. Changes in land use, the organic matter sources, and loading of streams should have a strong impact on the isotopic composition of methane emitted to the atmosphere. In order to understand and predict the impact of land-use changes on methane fluxes from the Amazon basin to the atmosphere, it is important to quantify the effects of changes in organic matter sources and fluxes.

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