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Methane in sulfate-rich and sulfate-poor wetland sediments

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Abstract. Wetlands of northern Belize provide a unique opportunity to study methane production and emissions from marshes dominated by identical species (*Typha domingensis, Cladium jamaicense*) and genus (*Eleocharis* spp.), but differing substantially in the amount of sulfates present in the sediments. Some marshes occur on limestone marls rich in gypsum ($CaSO_4$) while others are underlain by alluvial sands poor in sulfates. Concentrations of methane and sulfates in the sediment interstitial water are one or more orders of magnitude different for these two geological substrata averaging 139.2 and 14.9 μM of CH_4, and 0.08 and 11.53 mM of SO_4^{-2} on alluvial sands and limestone respectively. The amount of methane found in the internal atmosphere of plants from alluvial sands is significantly higher (6.3 μM) than in plants from limestone (0.19 μM). The average methane emissions measured in wetlands located on alluvial sands were 25.2 mg m^{-2} h^{-1}while emissions from marshes on limestone were only 2.4 mg m^{-2} h^{-1}. These values extrapolated for the entire year and the respective wetland areas resulted in the estimate of total CH4 emissions from northern Belize of 0.066 Tg per year.

Introduction

Atmospheric concentrations of methane have been increasing at an annual rate of 0.9% over the last 200 years (Etheridge et al. 1992; Stauffer et al. 1985). Wetlands are a major source of atmospheric methane, contributing about 50% of the global flux. In the past few years the major attention of the 'methane' community has concentrated on northern wetlands, mostly boreal peatlands (Morrissey & Livingston 1992; Valentine et al. 1994; Bubier & Moore 1994; Bridgham et al. 1995). It has been predicted that northern wetlands would be most affected by global warming although recent studies indicate that the increase in methane production may not be as high as initially expected (Bubier & Moore 1994). Compared to high-latitude wetlands, tropical wetlands have received limited attention. One exception is the Amazon region where the methane fluxes from the floodplain have been studied extensively since 1985 (e.g. Bartlett et al. 1988; Richey et al. 1988; Devol et al. 1990; Devol et al. 1994). Methane emissions by bubbling from Lake Gatun in Panama were measured by Keller & Stallard (1994). Methane emissions from the Florida Everglades, considered a subtropical wetland, have been studied

in great detail (e.g. Sebacher et al. 1985; Barber et al. 1988; King et al. 1990; Bachoon & Jones 1992; Chanton et al. 1993). With these few exceptions, very little information is available on methane production and emissions in tropical and subtropical wetlands. Yet it has been suggested (Blunier et al. 1995) that low-latitude wetlands exert the main controlling influence on atmospheric methane.

Methane is generated by the microbial decomposition of organic matter in wetland sediments (Barber et al. 1988). Methane producing bacteria, methanogens, are considered terminal organisms in the anaerobic microbial foodweb. They can utilize carbon from simple compounds, such as acetate, provided by fermentation processes (Wolin & Miller 1987; Schipper & Reddy 1994; Valentine et al. 1994). Due to the low energy yield of methanogenesis, it normally occurs after all other oxidazing agents, such as oxygen, nitrate and sulfate, have been exhausted. In freshwater wetland sediments, nitrates and sulfates are often in short supply and methanogenesis is ultimately responsible for the mineralization of organic carbon. In these areas methane production can be substantial. In coastal wetlands and marine sediments, the mineralization of organic matter occurs primarily through sulfate reduction and methane emissions from these environments are generally low (Bartlett et al. 1987; Bartlett & Hariss 1993; Holmer & Kristensen 1994). Sulfate-reducing bacteria are regarded as better competitors for electron donors (e.g. acetate) than methanogens (Martens & Klump 1984). However, there is evidence that methane production rates can be sustained even with relatively high (5–60 mM) concentrations of sulfates in environments where organic-rich sediments provide enough competitive substrata (Holmer & Kristensen 1994). Most of these studies have been conducted on sediment cores in laboratory conditions, resulting in valuable information on **potential** processes for the particular environments from which cores were collected (King et al. 1990; Bachoon & Jones 1992). The information on **actual** in situ CH/H_2S concentrations in wetlands has been assessed much less frequently.

Gas flux across the water-air interface, ebullition, and transport through aerenchyma of emergent aquatic plants are the main mechanisms of transport of methane from sediments. When emergent plants are present, they often dominate methane emission relative to ebullition or water-air transport (Chanton & Whiting 1995). Different plants transport methane with varying efficiency, yet individuals of the same species/growth form (e.g. *Typha domingensis*, rhizomatous emergent macrophyte) generally exhibit similar pattern of methane transport (Sebacher et al. 1985). Methane concentrations in plant aerenchyma should therefore reflect the levels present in the sediment providing the measurements are taken from the same species using similar methodology.

Wetlands of northern Belize provide a unique opportunity to study methane production and emissions from marshes dominated by the same vegetation, growing under very similar climatic and hydrologic conditions but differing widely in the level of sulfates present in the sediments. Some marshes occur on limestone marls rich in gypsum ($CaSO_4$) while others are underlain by alluvial sands poor in sulfates. As Matson & Harriss (1995) pointed out, gas producing processes and flux measurements placed in the context of the coarser scale factors that regulate gas flux, such as soil type or parent material, are important for the extrapolation and development of regional (and global) models. Accordingly, by relating the methane fluxes to geology and distribution of herbaceous wetlands in northern Belize, we can estimate methane emission from this region. The questions that we wanted to answer by this study were: (1) Do sediment methane concentrations in sulfate-rich vs. sulfate-poor wetland sediments differ significantly? (2) Are there any differences in methane concentrations inside stems/leaves of the same plant species/genera growing in sulfate-rich vs. sulfate-poor wetlands? (3) What are the methane fluxes from these wetlands? Finally, a prediction of methane emissions from the region can be made using this information in conjunction with existing land cover maps (King et al. 1992) and geologic map (Wright et al. 1959).

Study site

Methane sampling was conducted in several marshes of northern Belize which is located in the southeastern Yucatan Peninsula (Fig. 1). Detailed description of these marshes is provided elsewhere (Rejmankova et al. 1996). The climate of Belize is characterized by a predictable temperature regime and unpredictable rain patterns. The mean annual rainfall is 1300–1500 mm, the range of mean annual minimum and maximum temperatures is 22.8 °C to 30.2 °C (Department of Meteorology, unpublished data).

Geologically, the Yucatan Peninsula is an uplifted marine platform (Lopez Ramos 1979; Weidie 1985). Soft calcareous mudstones (marls) of the Orange Walk Group, abundant in gypsum, typify the bedrock in northern Belize. A thin cover of Quaternary beach sands, or lagoon and marsh clays and marls, is found along the coast (High 1975; Ward 1985). A unique aspect of the geology of the area is the presence of remnants of extensive alluvial sands and gravels of probable Pleistocene age (Wright et al. 1959). These alluvial sands are responsible for strikingly different water and sediment chemistry of the marshes located in areas underlain by sands. Soils in marshes of northern Belize are quite diverse, ranging from peat to marl or clay, with most soil profiles being some mixture of the three.

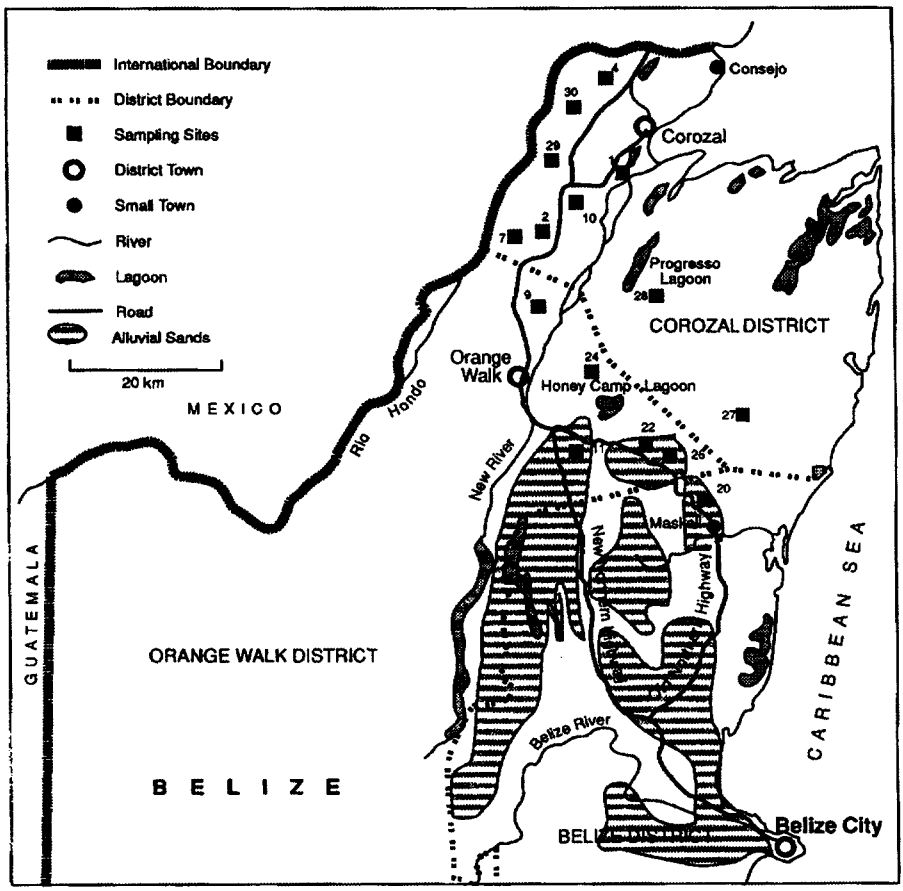

Figure 1. Map of northern Belize indicating locations of marshes from which we collected samples of interstitial water in February 1995. Methane emissions were measured in August 1994 in marshes 1, 2, 4, 11, 20 and 24. Striped pattern indicates the alluvial sand areas. (The marsh numbers are not consecutive because methane was sampled only in the subset of marshes described in Rejmankova et al. 1996)

Some of the uplands in the study area are in sugar cane cultivation, with minor amounts of pastures and other crops. Little to no attempt has been made to drain the swamps or marshes and they remain little disturbed other than by fire. Most of the marshes are dominated by emergent macrophytes, specifically rushes, *Eleocharis cellulosa*, *E. interstincta* and *E. elegans*, sawgrass, *Cladium jamaicense*, and cattail, *Typha domingensis*. Frequent codominants are submersed species of the genus *Utricularia* and *Chara*, floating-leaved *Nymphaea ampla* and algal mats consisting mostly of blue green algae often

growing periphytically on dead shoots of *Eleocharis* spp. or on *Chara* spp. Most of the wetlands in northern Belize stay flooded or at least saturated the entire year.

Methods

In situ CH_4 emissions were measured in six marshes (Fig. 1, Nos. 1, 2, 4, 11, 20 and 24) in August 1994 using a closed chamber technique (Livingston & Hutchinson 1995). All marshes were either inundated or had sediments saturated with water. In each marsh, replicate measurements were conducted in zones dominated by the different species, *Typha domingensis, Eleocharis* spp. and *Cladium jamaicense*, as well as in areas of bare ground or algal mats. The chambers were placed over several shoots of each particular species or over bare ground. Numbers and lengths of shoots/leaves enclosed in a chamber were recorded. The lower part of the chamber was made from an opaque PVC cylinder (25.4 cm diameter), the upper portion was clear plexiglass and the top with the sampling port was again opaque. Gas samples were collected in 1, 5, 10, 15 and 20 minute intervals in air-tight syringes. Samples in syringes were stored on ice until the measurements about 10 days later in the laboratory at Davis, CA. To control for potential losses of CH_4 through the long storage period, we filled several syringes with a CH_4 standard (10 ppm CH_4, Scott Specialty Gases) and exposed them to the same handling as samples. In the leakage control test syringes did not loose more than 6% over 20 days. Sampled syringes that were obviously mishandled (e.g. valve handle askew) were discarded. Results were screened for an indication of sediment disturbance and samples with high CH_4 concentration (>10 ppm) in the first subsample collected after the cylinder closure were discarded. Unfortunately, only a small portion of samples could be used for flux calculations. For the February 1995 field work we therefore decided to concentrate on the sampling of the sediment interstitial CH_4 concentrations combined with the sampling of gas from the internal plant atmosphere. This enabled us to obtain a larger data set without the technical difficulties of building boardwalks or developing other precautionary measures to ensure undisturbed sediments for chamber measurements.

Methane concentrations were measured on a Shimadzu 14A gas chromatograph with a flame ionizing detector and Haysep D column. Peak areas were integrated on an CR 501 integrator and calibrated with Scott CH_4 standard (10 ppm; 1 point calibration). The rate of CH_4 increase within chambers was calculated from the linear regression of concentration measured vs. time. From the rate of increase and the surface area and volume of the chamber, we calculated emission fluxes in mg CH_4 m^{-2} h^{-1}.

The concentration of methane within plant stems or leaves was sampled 10–20 cm above water/substrate surface with a needle and syringe. The reason for sampling at this height was that in *Typha domingensis* the leaves are so tightly packed closer to the water/substrate level that it was difficult to sample. To stay consistent, we sampled all three species at the same height. We could not be consistent and sample only leaves or only stems because *Eleocharis cellulosa*, whose aerial organs are erect spike-like stems, has leaves consisting of bladeless sheath while *Typha domingensis* and *Cladium jamaicense* are mostly present in the leafy form. Three replicates, each from a different stem/leave were taken for each species. The sampling was conducted during the midday (10 am to 4 pm).

Interstitial water from the depth of approximately −20 to −30 cm below the sediment surface was sampled using a sampler described by McKee et al. (1988). The sampler consists of a perforated plastic pipette with a sealed end connected to a 50 ml syringe. The first 5–10 ml of each sample was discarded. For methane, about 8 ml of water was collected in 10 ml S.E.S.I. nylon air-tight syringe and stored on ice until the analysis. To determine CH concentration in the interstitial water, the volume of water in syringes was adjusted to 5ml, then 5ml of ambient air was added, and the syringe was thoroughly shaken. Virtually all of the methane is stripped from the water by this process (McAullife 1971). Methane in the headspace was analyzed on the gas chromatograph (see above). For hydrogen sulfide analysis, 7 ml of water was transferred into a plastic centrifuge tube containing an equal volume of a sulfide antioxidant buffer (SAOB). The SAOB prevents the oxidation of sulfide and converts H_2S and HS-forms of sulfur to S^{-2}. Total sulfide was analyzed using an ORION silver/sulfide ion-selective electrode and ORION 530 meter. A standard curve was constructed with a series of solutions of Na_2S prepared with the antioxidant buffer in the laboratory at UC Davis and kept in air-tight vials until needed in the field lab. The concentrations of standard solutions did not change over three weeks and the standard curve was almost identical to the one measured in Davis. In addition, 30 ml of interstitial water were transferred into a Nalgene bottle for analyses of sulfates, nitrogen and phosphorus. The samples were transported in a cooler to the field lab, filtered through GF filters and transported to UC Davis. Sulfates were analyzed on a Dionex Ion chromatograph with As4A column. Ammonium nitrogen and total soluble phosphorus were analyzed colorimetrically according to Janik & Byron (1987).

Soil samples were collected from the top 20 cm sediments, dried and analyzed for the amount of organic material expressed as loss on ignition (Allen 1989).

Results

The CH_4, SO_4^{-2} and H_2S concentrations in the sediment interstitial water are one or more orders of magnitude different for the two geological substrata (Table 1). Methane is on average 10x higher in the wetlands from the alluvial sand locations (139 μM) and, correspondingly, the amount of CH_4 found in the internal plant atmosphere from these locations are significantly higher (6.29 μM). Interstitial CH_4 concentrations measured at corresponding sites in August 1994 (wet season) and February 1995 (dry season) were very close, documenting a rather stable situation (data not shown). Soils from marshes on the two geological substrata contained significantly different percentages of organic material with the mean loss on ignition for the alluvial sand sites being almost double of that from sulfate-rich limestone.

Concentration of methane decreases exponentially with an increasing sulfate concentration (Fig. 2). The amount of methane in the internal atmosphere of shoots/leaves in relation to the concentration of methane in the sediment is plotted in Fig. 3. There is a highly significant linear relationship between the amount of CH_4 in sediments and CH_4 within shoots of *Eleocharis* spp. Three species of *Eleocharis* were included, *E. interstincta* with the highest values, *E. elegans*, and *E. cellulosa* with the lowest values. *Eleocharis interstincta* is the most common species in the alluvial sand (= sulfate-poor) areas while *E. cellulosa* and *E. elegans* are mostly found on sulfate-rich limestone. Data from a related greenhouse study (Dillon, unpublished data) indicate that CH_4 concentration within shoots of *E. cellulosa* grown in a sulfate-poor sediment are quite high (13 μM). All three species have a very similar growth form and we believe that the differences in internal methane concentration reflect the availability of methane in sediments rather than species specific differences in the ability to conduct methane. *Typha domingensis* showed less significant ($p < 0.1$) relationship between sediment and within plant CH_4. Methane within *Cladium jamaicense* leaves was always low.

The CH_4 emissions from undisturbed sites are summarized in Table 2. Even with a very small sample size, we found a statistically significant difference ($p < 0.01$) between emissions from marshes located on limestone vs. alluvial sands.

Discussion

Our *in situ* data on sediment methane concentrations confirmed what has been shown from previous studies conducted in salt marshes, that methanogenesis is reduced in the presence of high sulfate concentrations (DeLaune et al. 1983;

Table 1. Mean values and ranges of variables measured in sulfate-poor (alluvial sands) and sulfate-rich (limestone/gypsum) marshes, Belize, February 1995. LOI = loss on ignition. Significance of differences between means was tested by two-tailed t-test.

			Interstitial Water				Plant	Soil
	CH_4 [μM]	H_2S [μM]	Conductivity [mS cm^{-1}]	SO_4 [mM]	NH [$\mu g\ l^{-1}$]	TSP [$\mu g\ l^{-1}$]	CH [μM]	LOI [%]
Alluvial sand ($n = 11$)	139.2	11.9	0.5	0.08	480	66.4	6.29	65
Range	9.1–409.2	1.5–58.1	0.1–1.5	0.02–0.19	80–2210	27.8–251.2	0.16–19.05	43–76
Limestone/gypsum ($n = 21$)	14.9	663.7	1.56	11.54	990	72.5	0.19	35
Range	0.2–87.8	1.9–2021.7	0.32–7.02	1.86–31.23	110–2730	37.3–101.6	0.07–1.05	13–71
$P <$	0.0001	0.001	0.0001	0.0001	ns	ns	0.0002	0.01

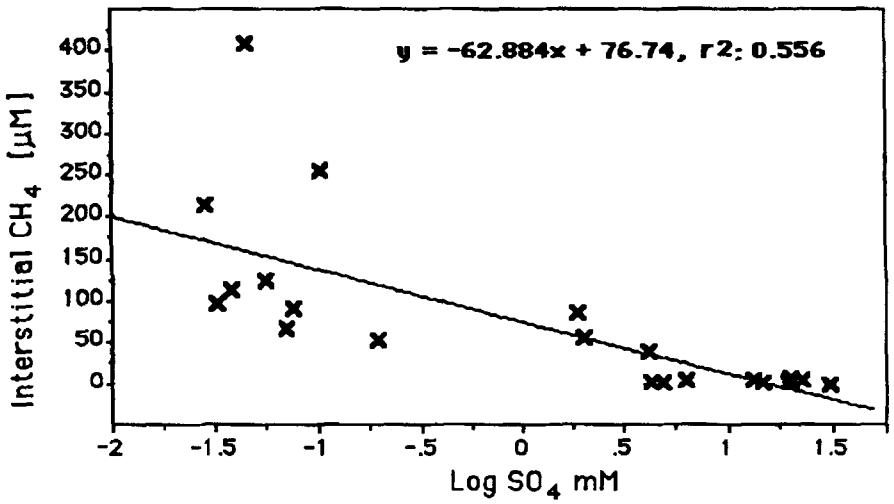

Figure 2. Relationship between sulfate concentration (x-axis, log scale) and CH_4 concentration in the sediment interstitial water.

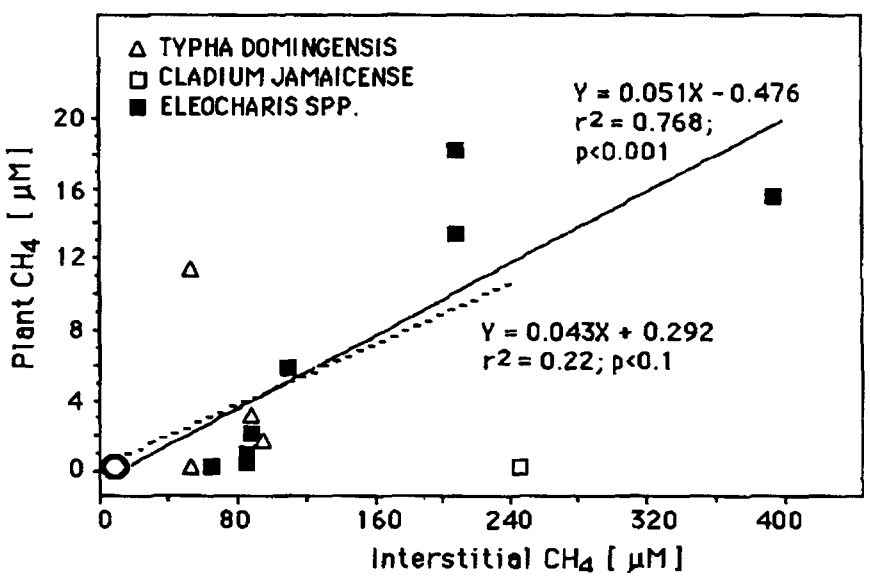

Figure 3. Relationship between CH_4 concentration in the sediment interstitial water and in the internal atmosphere in shoots of *Eleocharis* spp. and leaves of *Typha domingensis* and *Cladium jamaicense*. The large circle includes five values for *Eleocharis*, five for Cladium, and nine for *Typha* ranging from 0.08 to 0.25 μM on y-axis and 0.15 to 8 μM on x-axis.

Table 2. Methane emissions from wetlands located on alluvial sand *vs.* limestone/gypsum. Numbers of marshes correspond to Fig. 1. Numbers in parentheses show the standard deviation. The means are significantly different at $P < 0.01$ level.

Location	Plant Species	CH_4 [mg m^{-2} h^{-1}]	Interstitial Water	
			CH [μM]	SO [mM]
Alluvial sand				
Marsh 11	*Eleocharis interstincta*	12.34		
	Typha domingensis	52.16		
	Typha domingensis	37.58	25.2–91.3	0.01–0.07
	Bare ground	9.93		
Marsh 20	*Cladium jamaicense*	13.82		
	Mean	**25.17** (18.7)		
Limestone				
Marsh 4	*Eleocharis cellulosa*	1.27		
	Cladium jamaicense	4.57		
	Algal mats	0.35		
	Bare ground	0.20		
Marsh 1	*Typha domingensis*	1.33	2.33–7.24	6.50–35.10
	Typha domingensis	1.67		
Marsh 2	*Typha domingensis*	7.38		
Marsh 4	Bare ground	0.36		
	Mean	**2.40** (2.63)		

Bartlett et al. 1987). Interstitial methane concentrations in our wetlands rich in sulfates were about 10 times lower than in sulfate-poor wetlands.

In our study area, the concentration of sulfate was the best determinant of methane in the interstitial water. However, marshes on limestone had, on average, lower percentages of organic material in the soil than marshes from the alluvial sands. One possible explanation for lower amount of organic material in our sulfate-rich sediments is the faster decomposition mediated by sulfate reducing bacteria (Rejmankova et al. 1996). We have no data on the specific composition of the organic material from the Belizean soils. In the Everglades Bachoon & Jones (1992) reported higher methanogenesis from marl soils than from peaty sawgrass marshes and explained this by organic materials in the marl soils being more amenable to microbial fermentation than the sawgrass peat. They did not report data on sulfate concentrations at their sites but they were probably lower than at our sites since, according to Missimer (1984), the Everglades marles have lower gypsum concentrations

than the marles in northern Belize. More specific study is needed to elucidate the relationship between the sulfates, amount and quality of organic material and methane production in sediments of Belizean wetlands.

There were no differences in the amount of nitrogen (NH_4-N) or phosphorus (total soluble P) between marshes on the two substrata. This is in agreement with Bachoon & Jones (1992) who did not find any effect of ammonium or phosphorus on methanogenesis in the Everglades.

Our sampling method did not allow for sampling interstitial water from a precisely defined depth. The sampling device allowed us to draw samples from a layer about five cm thick centered approximately 20 cm below the sediment surface. Therefore the fact that in several cases we found sulfide and methane in the interstitial water drawn from the same sampler and the same depth range does not necessarily mean that methanogenesis was occurring at the same layer as sulfate reduction. Methane or sulfide could be diffusing through the sediment from the sites of production to the sampler. Schipper & Reddy (1994) suggested that methanogenesis may occur in microsites where sulfate concentrations were depleted.

Plant internal methane in our data set may be underestimated because we collected samples between 10 and 20 cm above water/sediment level. It has been demonstrated that most methane is released immediately above the water line (Sebacher et al. 1985; Chanton et al. 1993). The highest concentrations were found in *Eleocharis interstincta*, followed by *Typha domingensis*. This is in agreement with Sebacher et al. (1985) who placed both species into a group of plants that contributed to high methane emissions. *Typha domingensis* has been reported to possess pressurized ventilation (Chanton et al. 1993). It remains to be found whether *E. cellulosa* employs diffusive gas exchange or pressurized flow. Gas transport by pressurized convective flow was described for an Australian species of the same genus, *E. sphacelata* (Sorrell & Boon 1994). Higher internal CH concentrations in plants from sulfate-poor marshes indicate that not only is there more methane produced but also more is transported through plants to the atmosphere.

The fluxes of methane from sulfate-poor wetlands in northern Belize (604 mg m^{-2} d^{-1}) are comparable to rates reported from other warm climate regions. Schutz et al. (1989) present 580 mg m^{-2} d^{-1} as a value for rice from tropical regions. Fluxes reported by Devol et al. (1994) from macrophyte beds (mostly floating meadows) in the Amazon Basin range from 130 to 390 mg m^{-2} d^{-1} and the maximum flux reported from that region was 3,674 mg m^{-2} d^{-1}. In the Everglades, Chanton et al. (1993) found emission rates of 143 mg m^{-2} d^{-1} and 45 mg m^{-2} d^{-1} for *Typha domingensis* and *Cladium jamaicense* respectively. Their measurements were conducted in January and the temperature was probably much lower than what we recorded in Belize.

This could explain why we found higher emissions in Belizean marshes which are in many respects similar to the Everglades.

The land use map of Belize (King et al. 1992) indicates that 1,236 km^2 of northern Belize are covered by herbaceous wetlands. Of these, roughly 200 km^2 occur on alluvial sands, i.e. are potentially high methane producers. Most of the wetlands in northern Belize stay flooded or at least saturated the entire year. Seasonal wetlands are rather limited. Since the temperature fluctuates very little (T in −10 cm in sediments ranges from 25 °C to 28 °C in different marshes; Rejmankova unpublished data), we can assume that methanogenesis proceeds the entire year and is mainly influenced by the concentration of sulfates in sediments. If we use the average emission rates found for sand and limestone sites, 25.17 mg CH_4 $m^{-2}h^{-1}$ and 2.40 mg CH_4 $m^{-2}h^{-1}$ respectively (see Table 2), and extrapolate these for the entire year and respective areas, the total CH_4 emission from northern Belize would be 0.066 Tg per year. We need more emission measurements for more accurate estimates but we are confident that the relative differences between sulfate-rich and sulfate-poor wetlands will remain constant.

This region is not a major contributor to the global CH_4 budget, but does provide an opportunity for further research on interactions between methanogenic vs. sulfate reducing processes in wetland sediments.

There is an emphasis on using remote sensing to map wetland vegetation and use this for methane flux estimates (Gross et al. 1993). Our research documents that wetlands dominated by almost identical vegetation (no differences in wetland classes between alluvial sand and limestone area on a SPOT image; Rejmankova unpublished data) can differ significantly in methane emissions depending on their geological substrata. Therefore caution is necessary when using exclusively vegetation for regional gas emission estimates.

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