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Methane Production by Seagrass Ecosystems in the Red Sea

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Atmospheric methane (CH_4) is the second strongest greenhouse gas and it is emitted to the atmosphere naturally by different sources. It is crucial to define the dimension of these natural emissions in order to forecast changes in atmospheric CH_4 mixing ratio in future scenarios. However, CH_4 emissions by seagrass ecosystems in shallow marine coastal systems have been neglected although their global extension. Here we quantify the CH_4 production rates of seagrass ecosystems in the Red Sea. We measured changes in CH_4 concentration and its isotopic signature by cavity ring-down spectroscopy on chambers containing sediment and plants. We detected CH_4 production in all the seagrass stations with an average rate of 85.09 ± 27.80 μmol CH_4 m^{-2} d^{-1}. Our results show that there is no seasonal or daily pattern in the CH_4 production rates by seagrass ecosystems in the Red Sea. Taking in account the range of global estimates for seagrass coverage and the average seagrass CH_4 production, the global CH_4 production and emission by seagrass ecosystems could range from 0.09 to 2.7 Tg yr^{-1}. Because CH_4 emission by seagrass ecosystems had not been included in previous global CH_4 budgets, our estimate would increase the contribution of marine global emissions, hitherto estimated at 9.1 Tg yr^{-1}, by about 30%. Thus, the potential contribution of seagrass ecosystems to marine CH_4 emissions provides sufficient evidence of the relevance of these fluxes as to include seagrass ecosystems in future assessments of the global CH_4 budgets.

Keywords: methane, greenhouse gas, seagrass ecosystems, cavity ring-down spectroscopy, Red Sea

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INTRODUCTION

Atmospheric methane (CH_4) is a strong greenhouse gas (Lashof and Ahuja, 1990), with a 34-fold higher global warming potential relative to the CO_2 for a time horizon of 100 years, taking in account the climate-carbon feedback (IPCC, 2013). Atmospheric CH_4 mixing ratio has more than doubled from 722 ppb in pre-industrial times to 1,803 ppb in 2011 (IPCC, 2013) with high inter-annual variability. The highest rates of CH_4 growth have been reported for the 1980's (12 ppb yr^{-1}) followed by a decrease in the 1990's (6 ppb yr^{-1}) and the lowest growth rate (2 ppb yr^{-1}) or even stabilization of CH_4 atmospheric levels in the 2000's (Dlugokencky et al., 2003; Kirschke et al., 2013). Since 2007, atmospheric CH_4 mixing ratio is increasing at high rates again (10 ppb yr^{-1}; Rigby et al., 2008). The causes of this inter-annual variability in the growth rate of CH_4 remain unclear, with difficulties in attributing this trend to specific contributions of different sources (biogenic, thermogenic, and pyrogenic emissions) and sinks (mostly, photo-oxidation by hydroxyl free radicals in the troposphere) (Bousquet et al., 2006; Kirschke et al., 2013; McNorton et al., 2016; Turner et al., 2017). The biogenic sources of methane include natural and anthropogenic emissions from wetlands, agriculture, fresh water reservoirs and oceans, ruminants, termites and organic waste.

Bottom-up estimates identify biogenic emissions from natural freshwater wetlands as the main sources of CH_4 since 1980 (Kirschke et al., 2013). In the last decade, the average global CH_4 emissions by wetlands was estimated at 190 Tg CH_4 yr^{-1}, ranging from 177 to 284 Tg CH_4 yr^{-1} due to uncertainties mainly in coverage estimates (Kirschke et al., 2013; Melton et al., 2013). Oceanic and marine systems are also net sources of CH_4 although with lower global emissions than other sources. Yet, CH_4 emissions reported for the coastal ocean (5.5 and 1.9 Tg CH_4 yr^{-1} for the continental shelf and estuaries, respectively; Rhee et al., 2009; EPA, 2010) exceed those reported from the open ocean (1.8 Tg CH_4 yr^{-1}; EPA, 2010), despite the coastal ocean represents <15% of the global ocean area. The sources of CH_4 in the oceans are both biogenic, mediated by microbial processes, and thermogenic, through marine seeps. In open waters, biogenic CH_4 can be produced anaerobically by methanogens associated to particulate organic matter (Karl and Tilbrook, 1994; EPA, 2010) and aerobically as by-products during phosphonate uptake by heterotrophic bacteria under phosphate-limiting conditions (Karl et al., 2008). In coastal waters, biogenic CH_4 is mainly produced in sediments by anaerobic methanogenesis decomposing organic matter. Anaerobic methanogenesis requires anoxic conditions and redox potentials lower than −100 mV (Conrad, 2007), typically found in marine sediments (Gray, 1981). The contribution of seep and groundwater sources to CH_4 emissions in coastal waters remains unclear. Few data are available for oceanic CH_4 fluxes compared to other natural and anthropogenic sources. Oceanic CH_4 emissions were omitted in earlier assessments and have only been included in the last, the fifth, IPCC Assessment Report (IPCC, 2013), although its contribution accounts for 1–4% of the global CH_4 emissions. The paucity of data is especially important for tropical latitudes and for estuaries and shallow coastal areas, where the role of sediments colonized by seagrasses has been largely neglected. Indeed, CH_4 production rates, ranging from 5.8 to 307.2 μmol CH_4 m^{-2} d^{-1} on average, have been only reported for 4 different seagrass species in 5 locations (**Table 1**).

Seagrass ecosystems cover shallow coastal areas from all continents, except Antarctica, with an estimated global coverage ranging from 0.15×10^6 to 4.32×10^6 Km^2 (Duarte, 2017). Thus, due to their global coverage, high productivity and high organic matter content in their sediments supporting high microbial activity compared to adjacent bare sediments, seagrass ecosystems could represent a potential important source to be included in global CH_4 budgets. Here, we report CH_4 production rates by seagrass ecosystems along the eastern Red Sea coast, encompassing 7 different species distributed across 9 locations spanning 10 degrees latitude. Moreover, in one of the seagrass meadow we measured CH_4 production rates periodically during 1 year in order to examine the existence of seasonal patterns.

MATERIALS AND METHODS

We sampled 9 seagrass stations (S1–S9) along the Saudi coast of the Red Sea, onboard the R/V Thuwal on March 2017 as part of a scientific cruise covering more than 1,300 km of the Saudi Red Sea coast (**Figure 1**). The Red Sea is the northernmost tropical sea, reaching the 28° N parallel, with an extension of about 450,000 km^2 and an average depth of 490 m (Bruckner et al., 2012). Approximately 25% of its extension is occupied by shallow shelves (less than 50 m deep) holding rich and diverse marine ecosystems (Rasul et al., 2015). We sampled 7 different seagrass species: 7 stations out of the total were monospecific meadows and the other 2 were mixed meadows. Moreover, in one of the stations, an *Enhalus acoroides* meadow adjacent to our laboratory (S5), we also sampled periodically during 1 year, starting on June 2016 until April 2017, in order to detect seasonal patterns in CH_4 emissions.

At each station and sampling event, we collected 3 or 4 replicated cylindrical plastics cores (inner diameter = 9.5 cm, height = 30 cm) containing ∼10 cm of sediment (without disturbing its structure) and seagrass shots, in order to measure CH_4 production rates. We used a rubber hammer to push the plastic cores down into the sediment. The lower edges of the plastic cores were sharpened in order to facilitate the penetration of the cores into the seagrass meadow sediments. Once the core was at the desired depth into the sediment (∼10 cm), we closed the upper end with a rubber cap and pulled the core up carefully, ensuring that the sediment structure was not disturbed and finally we closed immediately the lower end of the core with another rubber cap. For those stations sampled during the scientific cruise, the cores were transported immediately onboard and were secured on the upper deck in incubators equipped with a running seawater system, maintaining the temperature close to *in situ* temperature. For the periodical sampling events at station S5, the cores were transported immediately to the laboratory and placed in an incubator (Percival chambers) set at *in situ* temperature and simulating the natural photoperiod (12 h light: 12 h dark). At the end of each incubation, we collected the aboveground seagrass tissues present in each core, in order to calculate the aboveground seagrass biomass. We dried the plant material at 60°C and recorded the dry weight. Additionally, we collected 3 cores to analyze the organic matter and nutrient (C and N) content of the sediment from each station. The organic matter content was estimated by loss on ignition method, while the C and N concentration were analyzed on an CHN Elemental Analyzer (Flash 2000) after acidification of the sediment samples, in order to remove carbonates.

CH_4 Concentrations in Equilibrated Air

The concentration of CH_4 in equilibrated air along with its isotopic composition ($\delta^{13}C$-CH_4) was measured by cavity ring-down spectroscopy (Picarro G2201-*i*). The precision and accuracy of the CH_4 concentration analysis was ±0.01 and ±0.05 pmm, respectively. The precision and accuracy of the $\delta^{13}C$-CH_4 analysis was ±0.02 and ±1.01‰, respectively. We used an air mixture (CH_4 concentration = 9.7 ppm and CO_2 concentration = 750 ppm, Abdullah Hashim Industrial Gases & Equipment Co. Ltd., Jeddah, Saudi Arabia) as a standard for the gas concentrations and Stable Isotope Calibration Standard UN1956 ($\delta^{13}C$-CH_4 = −45‰, Air Liquide America Specialty Gases, Plumsteadville, PA, USA), as standard for the isotopic signature. We analyzed the concentration and isotopic composition of

TABLE 1 | CH_4 production rates by seagrasses reported in the literature and in this study.

Seagrass species	Location	CH_4 production rates (μmol CH_4 m^{-2} d^{-1})		References
		Range (min–max)	Average	
Syringodium sp.	Bimini, Bahamas	3.6–7.9	5.8	Oremland, 1975
Thalassia testudinum	Florida, USA	43.4–44.6	44.0	Oremland, 1975
Thalassia testudinum	Florida, USA	25.8–341	183.4	Barber and Carlson, 1993
Enhalus acoroides	Sulawesi, Indonesia	4.5–233.1	118.8	Alongi et al., 2008
Zostera noltii	Arcachon Bay, France	4.8–868.8	98.4	Deborde et al., 2010
Zostera noltii	Ria Formosa, Portugal	105.6–1,704	307.2	Bahlmann et al., 2015
Halophila stipulacea and *Halodule uninervis*	Red Sea, Saudi Arabia	24.5–111.1	61.0	This study
Thalassodendron ciliatum	Red Sea, Saudi Arabia	0.1–6.9	3.2	This study
Thalassia hemprichii	Red Sea, Saudi Arabia	0.3–16	6.5	This study
Halophila decipiens	Red Sea, Saudi Arabia	0.7–1.9	1.4	This study
Enhalus acoroides	Red Sea, Saudi Arabia	−11.9–270.1	96.2	This study
Cymodocea serrulata and *Halodule uninervis*	Red Sea, Saudi Arabia	135.5–565.3	401.3	This study
Halodule uninervis	Red Sea, Saudi Arabia	25.4–59.9	48.1	This study

The CH_4 production rates from this study are averaged rates per each seagrass species, pooling data from different stations or sampling events.

CH_4 in equilibrated air in two different setups. First, for the stations sampled during the scientific cruise and analyzed on board we used the headspace technique (section Headspace Technique: Discrete Gas Sample Analysis) and, second, for the cores collected at station S5 along 1 year we used the closed recirculating circuits technique with the cores placed in the laboratory incubation system (section Closed Recirculating Circuits: In Continuum Gas Analysis).

Headspace Technique: Discrete Gas Sample Analysis

Once on board the research vessel, the upper cap of the core was removed and seawater from the core was replaced by fresh seawater from the running surface seawater system without disturbing the sediment. Seawater temperature and salinity was recorded for further calculations. The cores were closed again with a stopper fitted with a gas-tight valve, leaving a headspace of about 250 ml. The specific volume of the sediment, seawater and headspace of each core was calculated. We left the cores to stabilize for 1 h until the first headspace sample was taken. The air sample (15 ml) from the headspace of each replicate core was withdrawn with a syringe through the gas-tight valve and the CH_4 concentration along with its isotopic composition ($\delta^{13}C$-CH_4) were analyzed immediately on board using a cavity ring-down spectrometer (Picarro G2201-*i*), equipped with a module for discrete small volume gas samples (Picarro Small Sample Isotope Module, SSIM A0314). Several headspace samples were taken for each core at 3–7 time intervals along the incubation and analyzed in the cavity ring-down spectrometer. The incubations lasted about 24 h for all the stations, thereby encompassing day and night periods, except for station S3 for which the incubation was extended up to 35.43 h.

Closed Recirculating Circuits: In Continuum Gas Analysis

Once the cores were settled in the incubator chamber in the laboratory, the seawater from the core was replaced by fresh seawater collected from the same location without disturbing the sediments. The cores were closed again with a stopper with two holes fitted with silicone tubbing connected to an air-water exchange module to equilibrate the gas concentration in the closed water circuit with that in the closed air circuit. The closed seawater circuit was filled with seawater,

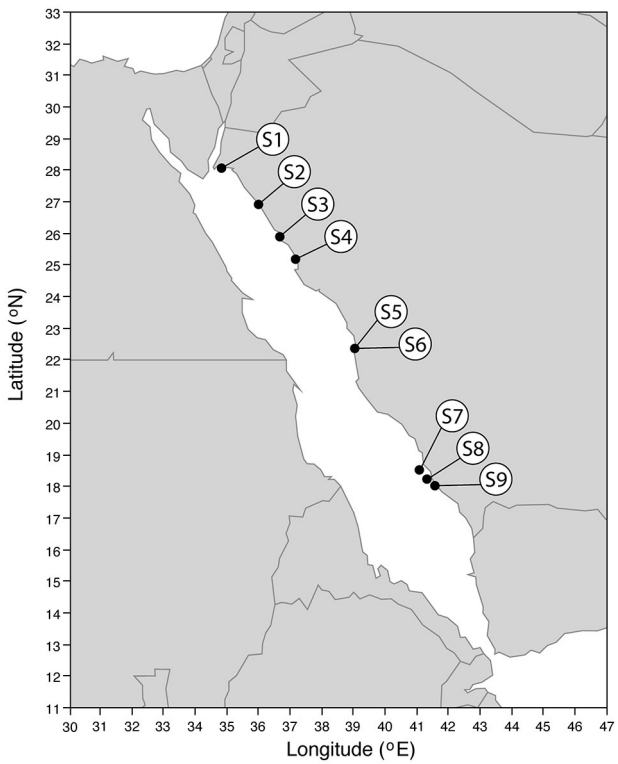

FIGURE 1 | Location of the seagrass stations sampled along the Saudi coast of the Red Sea.

carefully removing any gas bubble. The seawater was pumped through the air-water exchange module with a peristaltic pump, recirculating the seawater from the core. The closed air circuit was connected to the cavity ring-down spectrometer (Picarro G2201-*i*), thereby continuously recording the CH_4 concentration in the air, equilibrated with the water circuit, along with its isotopic composition (δ^{13}C- CH_4). These incubations lasted for at least 1 h, allowing net CH_4 production rates to be established, and were performed under light (200 μmol photons m^{-2} s^{-1}) and dark conditions for each of the samplings.

CH_4 Concentration in Seawater

The concentration of dissolved CH_4 in seawater (in nmol CH_4 L^{-1}) was calculated from the concentration of CH_4 (in ppm) measured in air samples after equilibration from both approaches (discrete headspace samples and closed circuit in continuum) as described previously for other gases (Wilson et al., 2012). Briefly, we calculate the dissolved CH_4 remaining in seawater after equilibration with the air phase ($[CH_4]_{SW-eq}$) by,

$$[CH_4]_{SW-eq} = 10^{-6}\ \beta\ [CH_4]_{Air}\ \mathcal{P}$$

where β is the Bunsen solubility coefficient of CH_4, calculated according Wiesenburg and Guinasso (1979), as a function of seawater temperature and salinity; $[CH_4]_{Air}$ is the CH_4 concentration measured in the air from headspace samples or closed air circuit (in ppm) and $\mathcal{P}$ is the atmospheric pressure (in atm) of dry air that was corrected by the effect of multiple sampling applying Boyle's Law. Then, the initial CH_4 concentration in seawater before the equilibrium ($[CH_4]_{SW-before\ eq}$) was calculated (in ml CH_4/ml H_2O) by,

$$[CH_4]_{SW-before\ eq} = ([CH_4]_{SW-eq}\ V_{Sw} + 10^{-6}\ ([CH_4]_{Air} - [CH_4]_{Air\ background})\ V_{Air})/V_{SW}$$

where V_{Sw} is the volume of seawater in the core or in the seawater closed circuit, $[CH_4]_{Air\ background}$ is the atmospheric CH_4 background level and V_{Air} is the volume of the headspace or the closed air circuit. Finally, the initial CH_4 concentration was transformed to nmol CH_4 L^{-1} by applying the ideal gas law.

CH_4 Production Rates by Seagrass Meadows

CH_4 production rates (in nmol CH_4 L^{-1} h^{-1}) were calculated based on the change in the concentration of CH_4 during the incubation time for each replicate core for each station and each sampling event. Then, we converted the rates to a daily and aerial (taking in account the core surface) base (in μmol CH_4 m^{-2} d^{-1}).

Isotopic Composition (δ^{13}C) of the Source CH_4

The isotopic composition (δ^{13}C) of the source CH_4, i.e., CH_4 produced by seagrass ecosystems, in the Red Sea was analyzed by conducting keeling plots (Thom et al., 1993) for each of the seagrass stations sampled in this study. The δ^{13}C of the source CH_4 was inferred from the Y axis intercept of the linear regression between the inverse of the CH_4 concentration measured in the equilibrated air (in ppm^{-1}) and its δ^{13}C-CH_4 during our incubations.

RESULTS

Methane production was detected at all seagrass meadows sampled, although the CH_4 emission rate varied greatly among replicates within stations and among stations (**Figure 2**). Some incubations reached a CH_4 concentration as high as 8,521.56 nM, (**Figure 2**, S6 replicate 1), whereas in others the CH_4 concentration remained very low throughout the incubation time (**Figure 2**, S4 replicate 2). Some incubations showed a linear increase in CH_4 concentration (**Figure 2**, S2 and S6), but in other incubations CH_4 concentration experienced fluctuations (**Figure 2**, S1 and S3), indicative of methanogenesis and methanotrophy processes. Moreover, the isotopic δ^{13}C signature of CH_4 decreased when CH_4 concentration increased, confirming its biogenic origin amidst the largely thermogenic background of Red Sea waters. Conversely, the isotopic δ^{13}C signature of CH_4 increased when CH_4 was oxidized by methanotrophs. The biogenic origin of the CH_4 produced in our seagrass incubations was confirmed by the δ^{13}C of source CH_4, inferred from keeling plots (**Figure 3**). The mean δ^{13}C of the CH_4 produced by seagrasses in the Red Sea was −59.36‰, ranging from −73.81 to −46.65‰ (**Table 2**).

The CH_4 production rates varied among stations, ranging from 0.09 to 565.27 μmol CH_4 m^{-2} d^{-1} (**Table 2**) with an overall mean of 85.09 ± 27.8 μmol CH_4 m^{-2} d^{-1} for the Red Sea seagrass meadows studied here. The lowest rates were detected in a *Thalassodendron ciliatum* meadow, followed by *Thalassia hemprichii* and *Halophila decipiens* meadows and the highest rates were detected in a *Cymodocea serrulata* and *Halodule uninervis* mixed meadow. The CH_4 production rates increased with sediment organic matter content (**Figure 4A**, $R^2 = 0.54$, $p < 0.0001$), and less strongly with increasing salinity (**Figure 4B**, $R^2 = 0.39$, $p = 0.0003$) and temperature (**Figure 4C**, $R^2 = 0.23$, $p = 0.0092$). The highest CH_4 production rates were observed in seagrass growing in enclosed marine coastal lagoons and the lowest rates were observed in pristine areas in shallow lagoons offshore from the mainland (**Figure 4D**, non-linear log fit $R^2 = 0.54$). The CH_4 production rates were independent of sediment nutrient (C and N) concentration ($p = 0.77$ and $p = 0.25$, respectively) or aboveground seagrass biomass ($p = 0.11$).

There was no evident seasonal pattern in CH_4 production rates at the *Enhalus acoroides* seagrass meadow (Station S5) sampled along the year, with an annually averaged daily CH_4 production rate of 92.1 ± 21.2 μmol CH_4 m^{-2} d^{-1}. The CH_4 production rates measured under light and dark conditions (**Figure 5A**) and the daily rates (**Figure 5B**) did not differ among the five sampling events (ANOVA test, $p = 0.43$, $p = 0.79$, and $p = 0.74$, respectively). Similarly, there was no clear daily pattern in the CH_4 production rates with no tendency for rates under the light to be consistently higher or lower than those measured under dark conditions (**Figure 6**). The annual mean of the CH_4

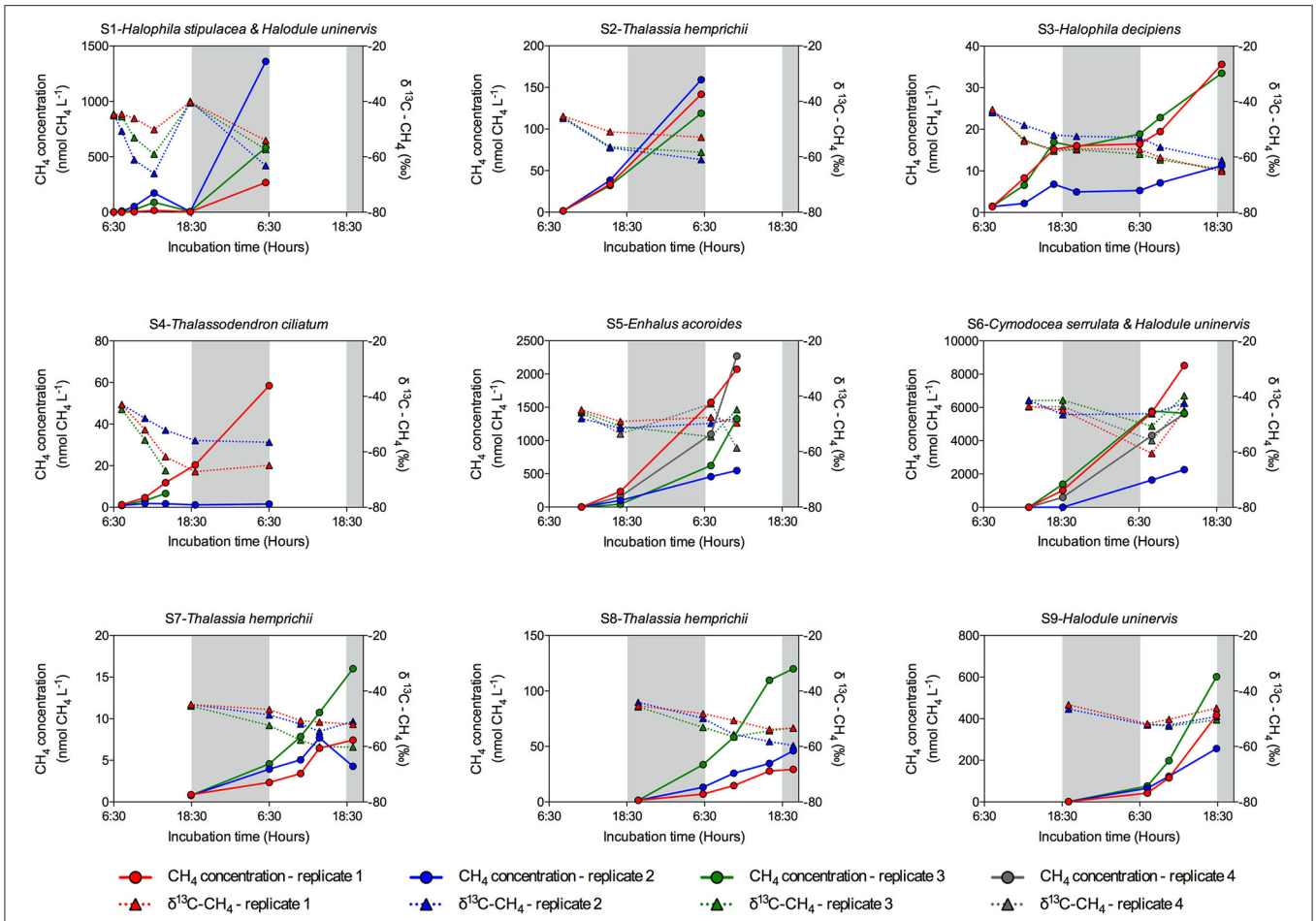

FIGURE 2 | Evolution of CH_4 concentration (circles and solid lines) and its isotopic ($\delta^{13}C$–CH_4) signature (triangles and dashed lines) in the sediment and seagrass incubations for each seagrass station. Data from each replicate (in color code) are shown. Gray shaded area corresponds to night time. Note the different scale in Y left axis.

production rates measured under light conditions (3.30 ± 0.92 μmol CH_4 m^{-2} h^{-1}) did not differ from the annual mean of the rates measured under dark conditions (4.22 ± 0.92 μmol CH_4 m^{-2} h^{-1}).

Finally, the two different technical approaches used to measure CH_4 production rate on *E. acoroides* seagrass meadow (Station S5) gave similar results. The CH_4 production rate for *E. acoroides* using the headspace technique resulted in 115.62 ± 23.7 μmol CH_4 m^{-2} d^{-1} and the CH_4 production rate using the technique of the closed recirculating circuits for the same date resulted in 114.5 ± 59.4 μmol CH_4 m^{-2} d^{-1}.

DISCUSSION

Here, we present, for the first time, rates of CH_4 production by seagrass ecosystems in the Red Sea. The mean CH_4 production rate for the seagrass meadows analyzed in this study was 85.09 ± 27.8 μmol CH_4 m^{-2} d^{-1}, ranging from 0.81 ± 0.34 to 401.32 ± 95.59 μmol CH_4 m^{-2} d^{-1} on average for a *Thalassia hemprichii* meadow and a *Cymodocea serrulata* and *Halodule uninervis* mixed meadow, respectively. These rates measured here are consistent with previous estimates in seagrass meadows elsewhere, with mean rates ranging from 5.8 to 307.2 μmol CH_4 m^{-2} d^{-1} for *Syringodium* sp. and *Zostera noltii*, respectively (**Table 1**). From all the seagrass species analyzed here, *Enhalus acoroides* is the only one for which CH_4 production rates were previously reported (Alongi et al., 2008), with very similar values. The CH_4 production rate reported for this seagrass in Indonesia was on average 118.8 μmol CH_4 m^{-2} d^{-1} (ranging from 4.5 to 233.1 μmol CH_4 m^{-2} d^{-1}) and the rate reported here for the Red Sea was 115.6 ± 23.7 μmol CH_4 m^{-2} d^{-1} (**Table 2**) for the single sampling on April and 96.2 ± 17.9 μmol CH_4 m^{-2} d^{-1} when averaging the rates measured along a year (**Figure 5**). The high variability that we observe in our measurements has been described as well for other systems, such as freshwater wetlands (EPA, 2010).

Methane production in Red Sea seagrass meadows increased with increasing organic matter content in the sediment (**Figure 4A**), in agreement with previous findings of higher CH_4

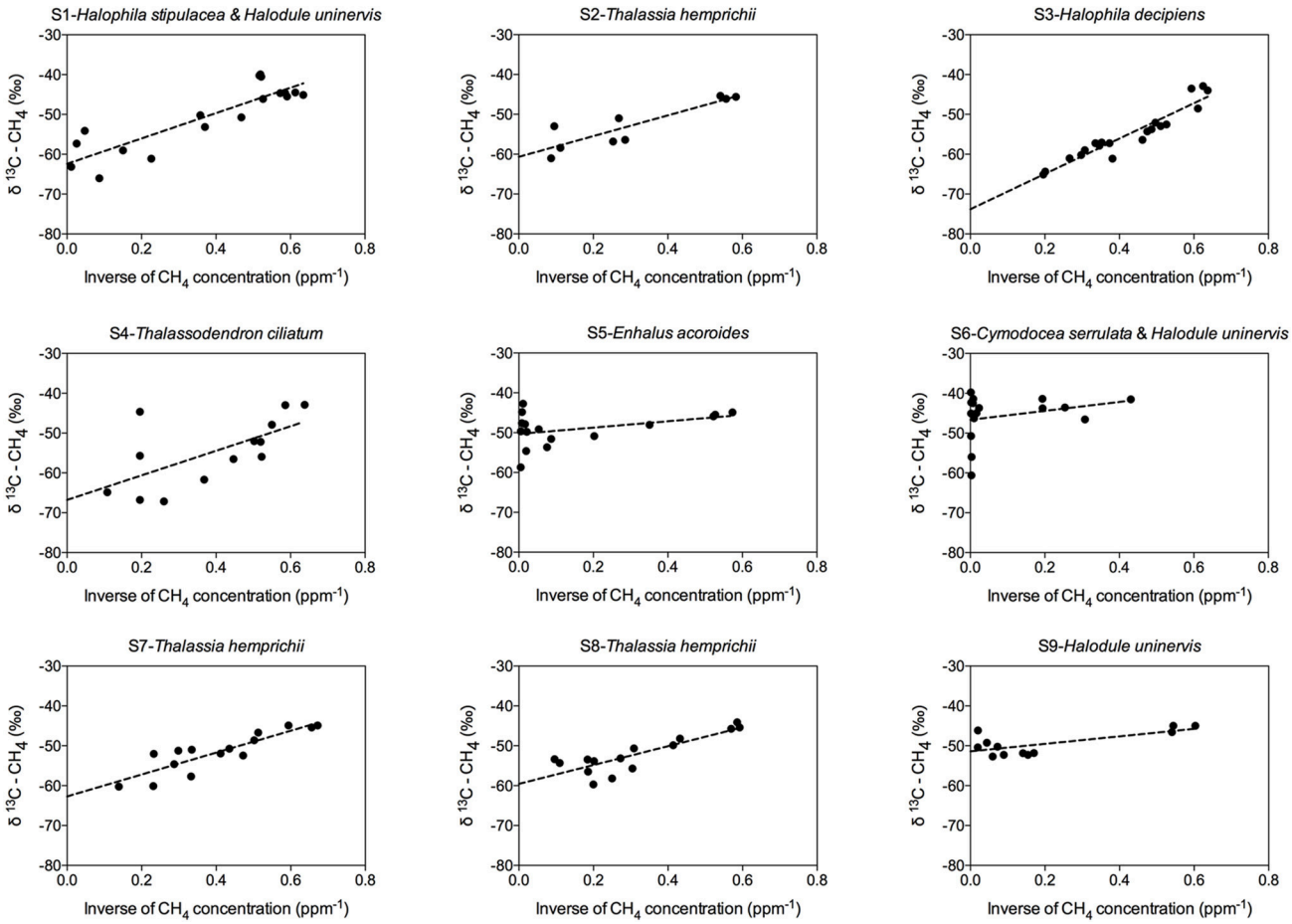

FIGURE 3 | Keeling plots for each seagrass station, showing the linear regression fitting (dashed line) between the inverse of the CH_4 concentration and the $\delta^{13}C$–CH_4, used to calculate the isotopic signature of the source CH_4 as the intercept of Y-axis.

concentration in porewater of seagrass sediments compared to adjacent bare sediments (Barber and Carlson, 1993). Similarly, CH_4 fluxes were more than 4-fold higher in *Zostera noltii* sediments compared to bare sediments in a temperate intertidal system (12.8 and 3 μmol CH_4 m^{-2} h^{-1}, respectively) (Bahlmann et al., 2015), and Alongi et al. (2008) reported an increase in CH_4 release associated to an increase in seagrass productivity. Altogether, our findings and consistent literature reports suggest that organic exudates from seagrasses are fueling a complex microbial community including methanogenic Archea. Moreover, a trend toward increasing CH_4 production with increasing salinity (**Figure 4B**) suggests that salinity slightly enhances CH_4 production in the Red Sea, contrary to what has been previously described for estuaries and intertidal systems with freshwater inputs (Bartlett et al., 1987; Middelburg et al., 2002; Deborde et al., 2010). However, this relationship may be spurious, rather than causal, as salinity, increasing from south to north along the Red Sea, is also related to gradients in climate and productivity (Raitsos et al., 2013; Wafar et al., 2016), which may be driving the apparent relationship with salinity. Moreover, the highest salinity was observed in enclosed coastal lagoons, suggesting high rates of CH_4 production to be reached in these environments. Similarly, CH_4 production increased with seawater temperature, accounting for 25% of the variability in seagrass meadows along the Red Sea (**Figure 4C**). Barber and Carlson (1993) also reported the lowest values of CH_4 concentration in porewater of *Thalassia testudinum* sediments in Florida at 22 °C during winter. However, we did not detect any clear seasonal pattern in the CH_4 production for the *E. acoroides* meadow (**Figure 5**), even though the seawater temperature ranged from 22 °C in February to 34°C in August, during our study. This again suggest that, as for the positive relationship between salinity and CH_4 production, temperature may reflect north-south gradients in the Red Sea rather than a functional response. Similarly, we did not detect any clear diurnal patter in CH_4 production rates (**Figures 2**, **6**). For some of our incubations, the CH_4 concentration remains stable during night time (**Figure 2** S3, S7, and S9) but for others, the CH_4 concentration seems to increase faster during night time (**Figure 2** S1). Our findings are in contrast with previous studies where CH_4 concentration in *Thalassia testudinum* rhizosphere followed diurnal cycles,

TABLE 2 | Summary of daily CH_4 production rates (range, mean ± SEM and *n*) and the isotopic signature ($\delta^{13}C$) of the source CH_4 for each seagrass station, along with their location and other environmental variables.

						Organic matter (%)	Aboveground biomass (gDW m^{-2})	CH_4 production rates (μmol CH_4 m^{-2} d^{-1})		$\delta^{13}C$ of the source CH4 (‰)
Seagrass species (Station number)	**Coordinates**	**Distance to mainland (m)**	**Depth (m)**	**Salinity (‰)**	**Temp (°C)**	**Mean ± SEM (*n*)**	**Mean ± SEM (*n*)**	**Range (min-max)**	**Mean ± SEM (*n*)**	**Keeling plot intercept ± SE**
Halophila stipulacea and *Halodule uninervis* (1)	28°4′17.9574″N 34°50′44.5668″E	97.7	1	40.19	23.33	3.51 ± 0.17 (3)	209.07 ± 23.69 (3)	24.47–111.09	61.01 ± 25.90 (3)	−62.39 ± 1.94
Thalassia hemprichii (2)	26°55′2.9388″N 36°0′27.4782″E	664.8	1	40.18	23.4	3.65 ± 0.08 (3)	151.13 ± 37.51 (3)	11.89–15.96	14.01 ± 1.18 (3)	−60.65 ± 1.86
Halophila decipiens (3)	25°59′21.7212″N 36°41′57.5988″E	1175	2	40.12	23.6	3.97 ± 0.08 (3)	21.77 ± 7.19 (3)	0.67–1.85	1.42 ± 0.38 (3)	−73.81 ± 1.49
Thalassodendron ciliatum (4)	25°9′28.371″N 37°9′45.8526″E	7220	3.5	39.76	24.01	3.65 ± 0.23 (3)	347.17 ± 125.12 (3)	0.09–6.95	3.23 ± 2.00 (3)	−66.78 ± 4.78
Enhalus acoroides (5)	22°23′35.0016″N 39°8′5.1324″E	*	2	42.29	30	12.42 ± 0.23 (4)	463.3 ± 41.99 (4)	54.68–165.62	115.62 ± 23.68 (4)	−50.31 ± 1.23
Cymodocea serrulata and *Halodule uninervis* (6)	22°22′50.0622“N 39°7′54.825″E	*	0.5	42.29	30	nd	239.81 ± 35.29 (4)	135.51–565.27	401.32 ± 95.59 (4)	−46.65 ± 1.70
Thalassia hemprichii (7)	18°31′11.9994″N 41°5′2.8206″E	21010	1	38.07	28.4	2.95 ± 0.16 (3)	94.9 ± 26.51 (3)	0.34–1.46	0.81 ± 0.34 (3)	−62.69 ± 1.83
Thalassia hemprichii (8)	18°13′44.1654″N 41°19′23.88″E	17625	1	38.13	28.61	2.58 ± 0.36 (3)	40.45 ± 3.15 (3)	2.91–7.73	4.67 ± 1.54 (3)	−59.55 ± 1.48
Halodule uninervis (9)	18°7′6.7182″N 41°33′55.4394″E	1616	0.5	37.98	29.03	2.33 ± 0.07 (3)	105.76 ± 30.09 (3)	25.43–59.95	48.18 ± 11.34 (3)	−51.39 ± 0.90
						Overall CH_4 production rates for the Red Sea		0.09–565.27	85.09 ± 27.80 (29)	

*Enclosed marine coastal lagoons.

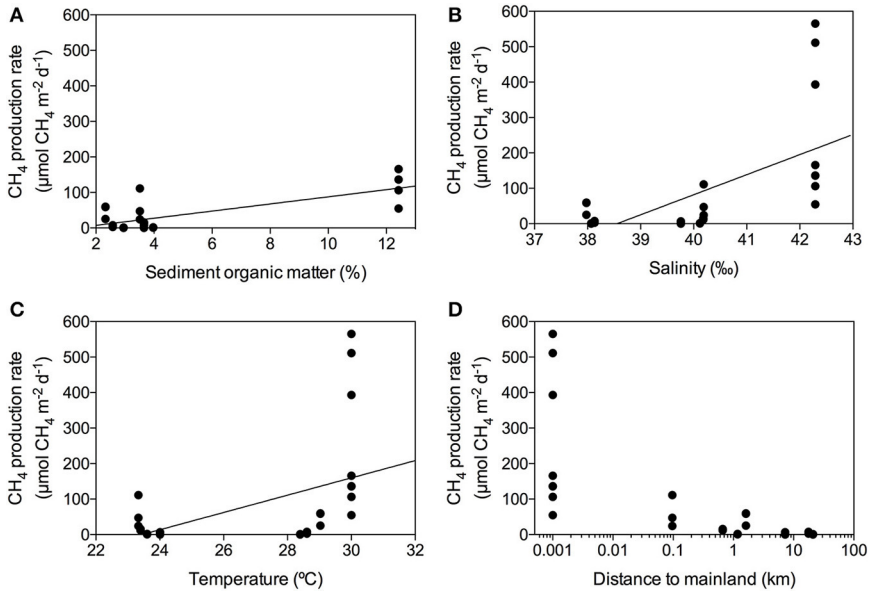

FIGURE 4 | Linear response of CH_4 production rates to changes in sediment organic matter content **(A),** salinity **(B)** and temperature **(C),** showing the fitted linear model (solid line). Decline of CH_4 production rates with increasing distance from the seagrass meadow to mainland **(D).**

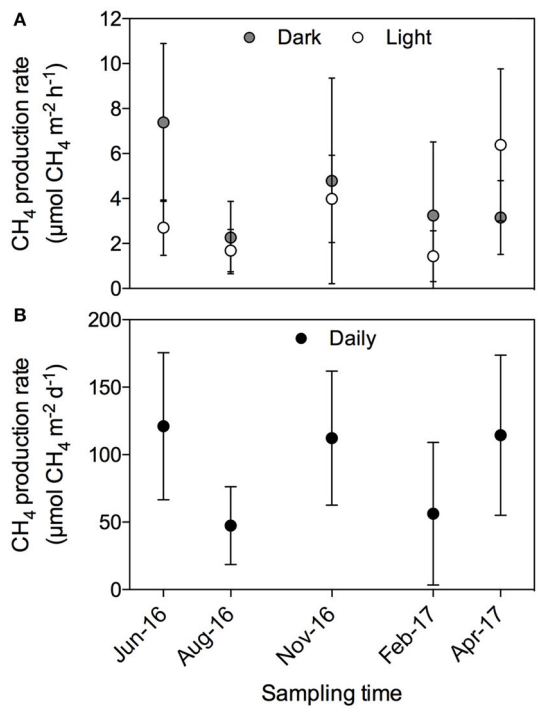

FIGURE 5 | CH_4 production rates measured periodically during 1 year on *Enhalus acoroides* seagrass meadow (Station S5). **(A)** CH_4 production rates measured during light (open circles) and dark (gray circles) conditions. **(B)** Daily CH_4 production rates.

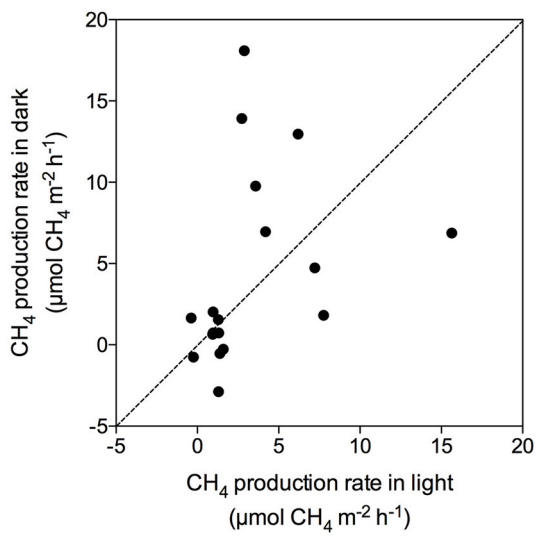

FIGURE 6 | Relationship of CH_4 production rates measured under light and dark conditions, showing line 1:1 (dashed line).

with minimum values coinciding with maximum oxygen levels (Oremland and Taylor, 1977) and higher CH_4 fluxes during night in an intertidal *Zostera noltii* meadow (Bahlmann et al., 2015), suggesting CH_4 oxidation by photosynthetic oxygen. Part of the CH_4 produced in seagrass meadows can be oxidized before reaching the atmosphere by methanotrophic bacteria inhabiting seagrass sediments (Jones et al., 2003) consisting of an anaerobic consortium between Archea and sulfate-reducing bacteria (Boetius et al., 2000). Similarly, Oremland and Taylor (1977) found that CH_4 concentration within aerenchymatic seagrass tissues was much lower than the concentration recorded in the rhizosphere, suggesting the presence of methanotrophic

bacteria inhabiting the rhizome surface. The fluctuations in CH_4 concentration and in its isotopic composition during our incubations (**Figure 2**), points out that both methanogenesis and methanotrophy are happening concurrently in Red Sea seagrass meadows. Bacterial methanogenesis depletes total CH_4 in ^{13}C, shifting to lighter isotopic signature with respect to the starting CH_4 isotopic values, whereas CH_4 oxidation by bacteria enriches total CH_4 in ^{13}C, shifting to heavier isotopic signature (Whiticar, 1990). The mean $\delta^{13}C$ of the CH_4 produced by seagrasses in the Red Sea (−59.36‰) confirmed its biogenic origin. The isotopic signature of biogenic CH_4 ranges from −40 to −80‰, depending on the isotopic signature of the methanogenic substrates (Reeburgh, 2014).

Interestingly, we detected a sharp decrease in seagrass CH_4 production rates with increasing distance to the mainland, with the lowest rates detected in pristine and remote seagrass meadows (**Figure 4D**). Eutrophication has been related to an increase in N_2O and CH_4 production in shallow coastal areas (Bange, 2006), suggesting that further deterioration and anthropogenic disturbance on seagrass ecosystems may lead to an increase in CH_4 production and therefore CH_4 emissions. However, the Saudi Red Sea coast is relatively pristine, except for high discharges associated with main urban areas, so the reasons for the decrease in seagrass CH_4 production rates with increasing distance to the mainland remain unclear. CH_4 production rates were independent of the aboveground seagrass biomass and the C and N sediment content. The aboveground seagrass biomass varies greatly among species, depending on their size, tissue turnover and growth rate (Duarte and Chiscano, 1999). Thus, the difference in CH_4 production rates might be due to different sediment microbial communities, specific of each seagrass species.

Net fluxes or emissions to the atmosphere of the CH_4 produced by these ecosystems will depend on the solubility of CH_4 in seawater as a function of salinity and temperature, the saturation ratio (observed/equilibrium concentrations), the air-water concentration gradient and the exchange coefficient as a function of wind or turbulence (Middelburg et al., 2002). Here, we report CH_4 production rates by seagrasses, but due to the high salinity and temperatures found in the Red Sea waters that reduce the solubility of CH_4 leading to low equilibrium levels and high saturation ratios, combined with low atmospheric concentrations, we believe that the air-water concentration gradient and the prevalent winds are enough to force a significant flux of CH_4 from seawater to the atmosphere.

Assuming that the net CH_4 produced by seagrass ecosystems is emitted to the atmosphere, the CH_4 emissions of Red Sea seagrass meadows would be 19.3–70.9-fold higher, on average, than the emissions reported for the open ocean, with emissions ranging from 1.2 to 4.4 $\mu mol\ m^{-2}\ d^{-1}$ (Holmes et al., 2000; Oudot et al., 2002; EPA, 2010). Moreover, taking in account the range of global estimates for seagrass coverage, from 0.15×10^6 to $4.32 \times 10^6\ Km^2$ (Duarte, 2017) and using the mean seagrass CH_4 production rate calculated by compounding our estimates with those available in the literature ($105.8 \pm 34.4\ \mu mol\ CH_4\ m^{-2}\ d^{-1}$), the global CH_4 production and emission by seagrass ecosystems could range from 0.09 to 2.7 Tg yr^{-1}. Because CH_4 emission by seagrass ecosystems had not been included in previous global estimates, the estimate provided here would increase the contribution of marine global emissions, hitherto estimated at 9.1 Tg yr^{-1} (EPA, 2010), by about 30%. In terms of CO_2-equivalents, calculated with the global warming potential for a time horizon of 100 years taking in account the climate-carbon feedback (IPCC, 2013), the CH_4 emissions by seagrass ecosystems would represent 3.2–90.9 Tg CO_{2-eq} yr^{-1}. This would imply a modest, 4.8%, reduction in the carbon sink capacity of seagrass ecosystems, estimated between 65.5 and 1,887.5 Tg $CO_2\ yr^{-1}$ for the lower and upper limit of the seagrass coverage range (Duarte et al., 2010). However, these estimates are tentative for both the regional variation in CH_4 emissions by seagrass ecosystems and the poorly constrained global seagrass area. In any case, the first order estimate of potential contribution of seagrass ecosystems to marine CH_4 emissions provides sufficient evidence of the relevance of these fluxes as to include seagrass ecosystems in future assessments of the global CH_4 budgets.

AUTHOR CONTRIBUTIONS

NG-B and CD designed the study and collected samples. NG-B acquired and analyzed the data. NG-B and CD interpreted data, wrote and reviewed the manuscript.

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