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Effects of composition of labile organic matter on biogenic production of methane in the coastal sediments of the Arabian Sea

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Abstract Coastal regions are potential zones for production of methane which could be governed by ecological/ environmental differences or even sediment properties of a niche. In order to test the hypothesis that methanogenesis in most marine sediments could be driven more by proteins than by carbohydrates and lipid content of LOM (labile organic matter), incubation experiments were carried out with sediments from different environmental niches to measure methane production. The methane production rates were examined in relationship to the sediment biochemistry i.e. carbohydrates, proteins and lipids. The gas production measured by head space method ranged from 216 $ng.g^{-1}d^{-1}$ in the mangrove sediments to 3.1 $\mu g.g^{-1}d^{-1}$ in the shallow Arabian Sea. Labile organic matter (LOM) ranged from 1.56 to 2.85 $mg.l^{-1}$ in the shallow Arabian Sea, from 3.35 to 5.43 $mg.l^{-1}$ in the mangrove estuary and from 0.66-0.70 $mg.l^{-1}$ in the sandy sediments with proteins contributing maximum to the LOM pool. Proteins influenced methane production in the clayey sediments of shallow depths of the Arabian Sea ($r=0.933$, $p<0.001$) and mangrove estuary ($r=0.981$, $p<0.001$) but in the sandy beach sediments, carbohydrates ($r=0.924$, $p<0.001$) governed the net methane production. The gas production was more pronounced in shallow and surface sediments and it decreased with depth apparently governed by the decrease in lability index. Thus, the lability index and protein content are important factors that determine methane production rates in these coastal ecosystems.

Keywords Methane · Sediments · Organic matter · Labile · Proteins · Carbohydrates · Lipids

Introduction

Methane, a common constituent in the deep sub-surface sediments, plays an important role in many geochemical processes and thus has many environmental implications. It is important because of its high global warming potential of 26.9 for a 10 year integration period (Lelieveld et al. 1993) and as the main component of hydrocarbon deposits like gas hydrates (Hester et al. 2007). The atmospheric concentrations of this second most important greenhouse gas is determined by a balance between natural and anthropogenic methane sources and sinks (Fischer et al. 2008). Methane is 25 times more efficient in absorbing infrared radiation and its relative increase since pre-industrial times is about 150% compared to 35% for CO_2 (IPCC 2007). Global methane emissions from natural sources are around 190 Tg per year. The relative contribution of different natural sources to global atmospheric methane emissions is 76% from wetlands, 11% from termites, 8% from oceans and 5% from hydrates (IPCC 2001). Most of the methane from natural sources in Earth's atmosphere is thought to originate from biological processes in anoxic environments (Houghton et al. 2001). As much as 50% of the world's natural gas resources including gas hydrates are major sources of ''biogenic'' methane generated by microbes (Claypool 2004). One of an important sink for methane is the uptake by methanotrophic bacteria in aerated soils (Khalil et al. 1993, Quay et al. 1999). These microbes in sediments and water columns (Reeburgh 1996, 2003) regulate the methane flux to the atmosphere which is influenced by gradients in methane concentration (Sotomayor et al. 1994), the quality of sedimentary organic matter (Lojen et al. 1999) and the oxic/anoxic boundary (Ogrinc, 1997).

Methane accumulations occur in a variety of marine and non-marine depositional settings generally characterized by rapid rates of deposition (Rice 1993). In the Arabian Sea the total flux of particulate and lithogenic matter is 23.6 and 5.4 $g.m^{-2}.y^{-1}$ respectively. The vertical transport of particles is mainly associated with biogenic aggregates. The sedimentation rates of total and lithogenic material are 15.0 and 4.7 mm 1000 y^{-1} respectively (Ramaswamy et al. 1991). Three major rivers namely Indus, Narmada and Tapi, empty mainly fluvial material into the Arabian Sea. Thus the organic matter accumulated is of both terrestrial and marine origin. The other site is the mangrove estuary station which is influenced by freshwater inflow from the Mandovi River. The estuarine sediments accumulate leaf litter over time. The shallow Arabian Sea and the mangrove estuary is also a sink for the anthropogenic coastal inputs of organic and inorganic matter. The other sampling site is a sandy beach at Ratnagiri, with dune vegetation (Annual Report 2000-2004). The sediment here is considerably reworked by erosion and re-deposition. These processes could enhance microbial degradation rates (Kvenvolden & Lorenson 2001).

Microbial decomposition of organic matter leads to methane accumulation in the coastal sediments. The flux of labile organic carbon (LOC) is one factor that dictates the amount of methane that is produced in the marine sediments (Blair 1998). In the light of previous studies (Table 1) on methane production, it was hypothesized that in most marine sediments proteins more than carbohydrates and lipids could contribute to methanogenesis in the sediment. Thus, it was proposed that methane production rates are higher in sedimentary environments having more protein content in their labile organic matter (LOM).

Materials and methods

Study sites and sampling

Sediment samples of varying organic matter contents were collected from different geographical sites (Fig. 1) in the western region of India using cores (Table 2). These included shallow Arabian Sea sediments off Goa (Station 1), the fringing mangrove estuarine sediments in the Mandovi-Zuari system (Station 2) and the sandy beach placer rich regions of Kalbadevi in Ratnagiri (Station 3). The sampling depth was 125 cm and 12 cm below sea floor at the shallow Arabian Sea and the mangrove estuary respectively. Sediment cores were taken from the upper 25 cm of sandy beach sediments using piston acrylic core cylinders (6.4 cm i.d). Cores were restricted to much shorter depths in the mangrove and sandy beach station due to logistics and technical difficulties. The sediment cores were sliced at 4 cm interval and sub samples were used for laboratory experiments. In this work the sediment depths from 0 - 12 cm were used for depth-wise comparison. Salinity of the pore water obtained by centrifugation of aliquots of sediment was measured using a hand refractometer (S/Mill-E, ATAGO, Japan). The grain size of the sediments was done by the standard pipette analysis (Folk, 1968).

Methane production rates

The sediments were sliced into 4 cm thick sections from shallow Arabian Sea, the mangrove estuary and the sandy beach sediments. Slurries were made from wet sediment samples in triplicate by diluting the sediment with sea water of sample source in a ratio of 1:1. These were then incubated at near ambient temperatures in gas tight headspace vials for a week after initial readings. Methane was quantified using a head space method on a Shimadzu 2010 Gas Chromatography (GC) equipped with a 30 m long mega bore (0.53 mm) GS-Q- coated quartz capillary column and flame ionization detector (FID). Injector temperature was 100 ^{0}C and detector temperature was at 150 ^{0}C. Nitrogen was used as a carrier gas at a flow rate of 9 ml min^{-1} and the oven temperate was run isothermally at 60 ^{0}C. The GC was calibrated using reference mixture of methane standards (concentration range of 1-5 g/L) from M/S Alchemie Gases Ltd.

Biochemical parameters

Protein analyses were carried out following extraction of 3 replicates of sediment sample with NaOH (0.5 M, 4 h) according to Hartree (1972), modified by Fabiano et al. (1995) and expressed as albumin equivalents. Carbohydrate was estimated by phenol-sulphuric acid method after extraction of an aliquot of sediment sample in 5% trichloroacetic acid (Kochert 1978). The concentration was measured at 480 nm and expressed as glucose equivalents. Lipids from marine sediment samples were extracted by direct elution with chloroform and methanol (Danovaro and Fabiano, 1990). Sediment analyses were conducted using the acid dichromate method, as outlined by Bligh & Dyer (1959) and Parsons et al. (1984). Absorbance was measured at 440 nm. Carbohydrates (CHO), proteins (PRT) and lipids (LIP) were converted into carbon equivalents using conversion factors of 0.40, 0.49 and 0.70 for CHO, PRT and LIP, respectively (Fichez 1991, Fabiano & Danovaro1994).

Total bacterial counts

Total bacterial counts (TBC) was estimated by the acridine orange direct count (AODC) method (Hobbie et al. 1977) and were expressed as numbers per litre.

Total organic carbon

Organic carbon was determined by the wet oxidation method with a precision of 0.01% (El Wakeel & Riley 1957).

Stepwise analysis of variance (ANOVA) was performed to determine the depth wise differences in methane production rates. Spearman's correlation analysis was performed on the normalized data to find out the relationship between methane production rates and the different components of LOM.

Results

Salinity measured in the interstitial water varied from 1 psu in the mangrove sediments to 33 psu in the beach sediments and 35 psu in the shallow Arabian Sea sediments. The total bacterial counts (TBC) in the Arabian Sea sediments was 10^9 g^{-1}, while in the mangrove sediments it was an order higher and in the sandy sediments it was 2 orders lower at 10^7 g^{-1}. Methane production rates ranged from 0.2 to 3.1 $\mu g.g^{-1}d^{-1}$. The highest methane production was generally in the surface sediments, with the shallow

Arabian Sea and the mangrove estuarine sediments being 4-fold and 3-fold higher than the beach sediments (Fig. 2).

The average methane production rates of the sediment cores decreased in the order of shallow Arabian Sea> Mangrove estuary> Sandy beach (Fig. 2). Surface sediments of the Arabian Sea and mangrove estuary had comparatively higher methane production rates than their deeper sediments. The sandy beach sediments showed an order lower production rate than the rest of the sites. The methane production decreased with depth and was negligible in the deeper layers of mangrove and sandy sediments (Fig. 2). There was a significant difference ($p<0.01$, df=2) in the mean production of methane in the depth range from 0 to 12 cm between all the 3 stations. The average total organic carbon was 9.7 mg g^{-1} in the Arabian Sea, 19.4 mg g^{-1} in the mangrove estuary and 0.87 mg g^{-1} in the sandy beach sediments. The gradation in the sedimentary labile organic matter was mangrove estuary (4.54 mg g^{-1}) > Arabian Sea (2.77 mg g^{-1}) > sandy beach (0.68 mg g^{-1}) (Table 3). A strong relationship (Fig. 3) was observed between methane production rates and the labile organic matter in the mangroves sediments ($r=0.995$, $p<0.001$, $n=9$) and in the Arabian Sea sediments ($r= 0.948$, $p<0.001$, $n=9$). However, such a relationship was absent in the sandy beach sediments ($r=0.03$, $p>0.05$, $n=9$). Among the different macromolecules of the LOM, variations in proteins contributed positively (76%) and lipids negatively (45%) to the methane production rates in Arabian Sea sediments. In the mangrove sediments, concentrations of proteins and carbohydrates accounted for 93% and 84% of the variation in methane production rates respectively. In the sandy beach sediments carbohydrates alone explained about 78% of the variation in the methane production rates (Fig. 4).

Discussion

Methane production is an important environmental issue in the context of concerns about global climate change. Climate change and its influence on sea-level rise, present significant challenges for coastal populations. Hence, this study assesses and compares the methane production rates in the shallow sediments off Goa in the Arabian Sea with other coastal environments like the mangrove estuary and sandy beach sediments with respect to proteins, carbohydrates and lipids. Influx of a lot of sedimentary organic matter in the coastal region as reported by Ivanova et al. (2003) and high rates of decomposition of sedimentary organic carbon in the upper layers of the sediment contribute to the availability of easily degradable fraction (Lojen et al. 1999). These processes contribute to methane production and were encountered in the present study areas. This effect is reflected in the comparatively higher methane oxidation rates in the surface sediments of the Arabian Sea and mangrove estuary than in deeper sediments. Such differences have also been reported by Mitsch & Gosselink (2000) who measured generally higher methane concentrations in shallow productive aquatic environments like natural wetlands, mangrove estuary, rice fields than in deeper less productive environments e.g., open ocean (Bange et al. 1994). In this study the mangrove region had comparatively less methane production rates than the coastal Arabian Sea. Besides, though the mangrove sediments harboured higher bacterial population of 10^{10} g^{-1} as compared to the Arabian Sea sediments similar trend was not reflected in the methane production rates. One explanation could be as that reported by Sotomayor et al. (1994) where, due to the presence of freshwater and nutrient supply, there is an increased loss of methane to the atmosphere. Nevertheless, there may be many other factors that affect the methane production rates like the high sedimentation rates in the Arabian Sea (Ramaswamy et al. 1991). These rates could also be enhanced by the delivery of fine-grained riverine particles which in turn contribute to enhancing methane production (Kvenvolden & Lorenson 2001). But among these factors, the most important one is the composition and the concentration of organic matter. Soils rich in organic matter have high microbial activity and high methane formation rates (Krupadam et al. 2007). In the present study organic matter strongly influenced methane production rates (R^2 =0.899; $p<0.001$). Delwiche & Cicerone (1993) also found that without organic matter, methane flux was low. However, addition of organic matter increased the methane emission by 3-12 times. Similar observations were also found in Puerto Rico wetlands where the low availability of substrates inhibited methane emissions (Sotomayor et al. 1994). The present study is supported by a data set that includes measurements of methane production rates and components of LOM at different sediment depths from shallow Arabian Sea, estuarine mangrove, and sandy beach sediments. The sandy sediments represent a case of low LOM (Table 3). Within a habitat like sandy sediments, LOM as a whole doesn't explain much, as indicated from the absence of relationship between LOM and methane production rates. The characteristic of LOM is an important factor for methane production as the different fractions control its production rates to different extents at different sites. Thus, the components of LOM wield more influence. The methane production in the sandy beach region with sandune vegetation was influenced by the amount of carbohydrates which is responsible for generating methane in widespread pockets. Lojen et al. (1999) attributed variations in the sources of methane to differences in the quality of sedimentary organic matter. In this study, the carbohydrate fraction of sedimentary organic matter also includes the structural components. Consequently, it is possible that the dominance of carbohydrate may be because of their more refractory composition compared to lipids and proteins which are rapidly utilized (Donavaro et al. 2000, Grémare et al. 2003). Resolving the composition of the organic matter into main components would throw light on the relative contribution of each fraction. Hence, we examined the interrelationships of different fractions of LOM with methane production rates (Blair 1998). Correlation matrices give an idea of the net and dominant effect of the LOM fractions on methanogenesis. The degree of the relationships between these parameters gives an estimate of the extent of influence on each other. Although many factors affect natural methane production in sediments, it was mainly the protein fraction of sediment that was the most important in the Arabian Sea and in the mangrove sediments. Earlier observations by Yang (1998) where he showed how high protein and organic matter in lake sediments enhanced methane production rates support our present findings. Besides proteins, the availability of lipids appears to limit methane production in the shallow Arabian Sea sediments. The variation in the lipid fraction brought about 66% variations in the methane production rates negatively. In general proteins were the major component of the labile organic matter with an average of 71% followed by carbohydrates 20% and lipids 9%. The ratio of the sum of protein, carbohydrate and lipid carbon which is called labile organic matter carbon (LOM-C) to total organic carbon (TOC) is an index of lability. This index also has its influence on the methane production. Average LOM-C/TOC ratio was between 8 and 16% in the shallow Arabian Sea, from 9 to 12% for the mangrove estuary and surprisingly higher at 24 to 47.5% in the sandy beach sediments (Table 3). This suggests that the methane production in the sandy beach region can also be due to the comparatively higher lability of the organic matter. Other factors like the presence of electron acceptors - O_2, NO_3^-, SO_4^{2-} can inhibit methanogenesis in organically poor sediments. However, in this study we do not expect the shallow Arabian Sea and mangrove estuary sediments which are comparatively richer in organic matter than the sandy sediments to be influenced by the presence of energetically favorable electron acceptors. It was mainly the protein content, the index of lability and quantity of settling organic material that played a role on methane production rates. This in turn could affect the distribution and metabolic activity of the benthic assemblages in the marine and estuarine environment (Tenore 1976, Graf 1989).

Conclusions

This study has attempted to quantify methane production in sediments of three different ecosystems. The process was more rapid in upper sediments where the availability of components of LOM consisting of proteins, carbohydrates and lipids is in continuous supply, than in the deeper layers. The methane production rates varied more significantly with the changes in each labile component of organic matter than with the total LOM pool. In the present study, protein was one of the main variables that governed methane production in clayey sediments of the coastal Arabian Sea and mangrove estuary, while in the sandy beach sediments, the role of carbohydrates dominated. Environmental forcing like nature, quantity of labile organic matter, lability index and sediment depth are some of the other factors which govern biogenic methane production. Although TOC and bacterial load in the mangrove sediments were comparatively higher than those of the other sites, the lability index of mangrove sediments was comparatively lower. Thus the lability index along with protein content of the sediment could be the decisive factors for the methane production rates. Though a lot of investigations indicate the role of organic matter in methane production, there has been a paucity of information on the roles macromolecular components of LOM play in biogenic methane production. Anthropogenic disturbance of the ecosystems might bring about a change in levels of the macromolecular components in the organic matter that can contribute significantly to the variations in methane production rates and thus effect the global atmospheric environment.

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Table 1 Methane production rates at different sites

Sites	mg.m^{-2}.h^{-1}	Reference
Temperate		
Dry lands	-5 to 1	Etiope, 2005
Mud volcanoes micro seeps	103 to 105	
Organic sediment	3.4-19.0	Kankaala & Bergstrom, 2004
Sand	0.2-1.8	
Fresh water lakes	86.4	Kelly & Chynoweth, 1981
Tropical		
Kerala-rice fields	5.14	Yan et al., 2003
Gujarat-rice fields	6.17	
Paddy fields India	-0.64-84.1	Parashar et al., 1997
Mangroves		
Sundarban mangroves	-4.53-8.88	Mukhopadhyay et al., 2002
Mangrove Pichavaram undisturbed	7.38	Purvaja & Ramesh, 2001
Mangrove Pichavaram disturbed	21.56	
Mangrove swamp, China	0.02-0.20	Lu et al., 1999
SW coast Puerto Rico mangroves	0.17-3.42	Sotomayor et al., 1994
Estuaries		
Adyar estuary	15.41	Purvaja & Ramesh, 2001
Confluence of Adyar Estuary with the Bay of Bengal	3.27	
Adyar river	21.56	

Table 2 Description of sediment sampling stations

Station No.	Sites	Latitude	Longitude	Water Depth (m)	Sampling	Sediment type
1	Coastal Arabian Sea (Off Goa)	$15^0$03’300N	73 ^{0}45’000E	44	Gravity corer	Clayey
2	Mangrove (Mandovi-Zuari Estuary)	$15^0$30’357N	73 ^{0}52’634E	Intertidal zone	Push corer	Clayey
3	Placer rich Beach (Kalbadevi)	$17^0$02’409N	73 ^{0}16’561E	Intertidal zone	Push corer	Sandy

Table 3 Variations with depth of LOM, LOM-C ($mg.g^{-1}$), LOM-C/TOC (%) and total bacterial counts at the different stations

Depth (cm)	Arabian Sea				Mangrove Estuary				Sandy Beach			
	LOM	LOM-C	LOM-C /TOC	TBC x $10^9 g^{-1}$	LOM	LOM-C	LOM-C /TOC	TBC x$10^{10} g^{-1}$	LOM	LOM-C	LOM-C /TOC	TBC x $10^7 g^{-1}$
0-4	2.71	1.32	12.25	1.22	5.43	2.54	12.24	1.23	0.70	0.39	36.22	1.22
4-8	2.79	1.36	12.77	1.16	4.84	2.34	9.09	0.67	0.68	0.37	23.66	0.82
8-12	2.81	1.37	13.65	1.04	3.35	1.61	9.79	1.01	0.67	0.36	39.40	1.26
12-25	2.85	1.39	15.72	0.92	ns*	ns	ns	ns	0.66	0.37	47.48	0.69
25-50	2.12	1.05	14.04	0.84	ns	ns	ns	ns	ns	ns	ns	ns
50-75	1.61	0.83	7.91	0.79	ns	ns	ns	ns	ns	ns	ns	ns
75-110	1.75	0.89	8.61	0.80	ns	ns	ns	ns	ns	ns	ns	ns
110-125	1.56	0.87	9.68	0.70	ns	ns	ns	ns	ns	ns	ns	ns

*ns- not sampled

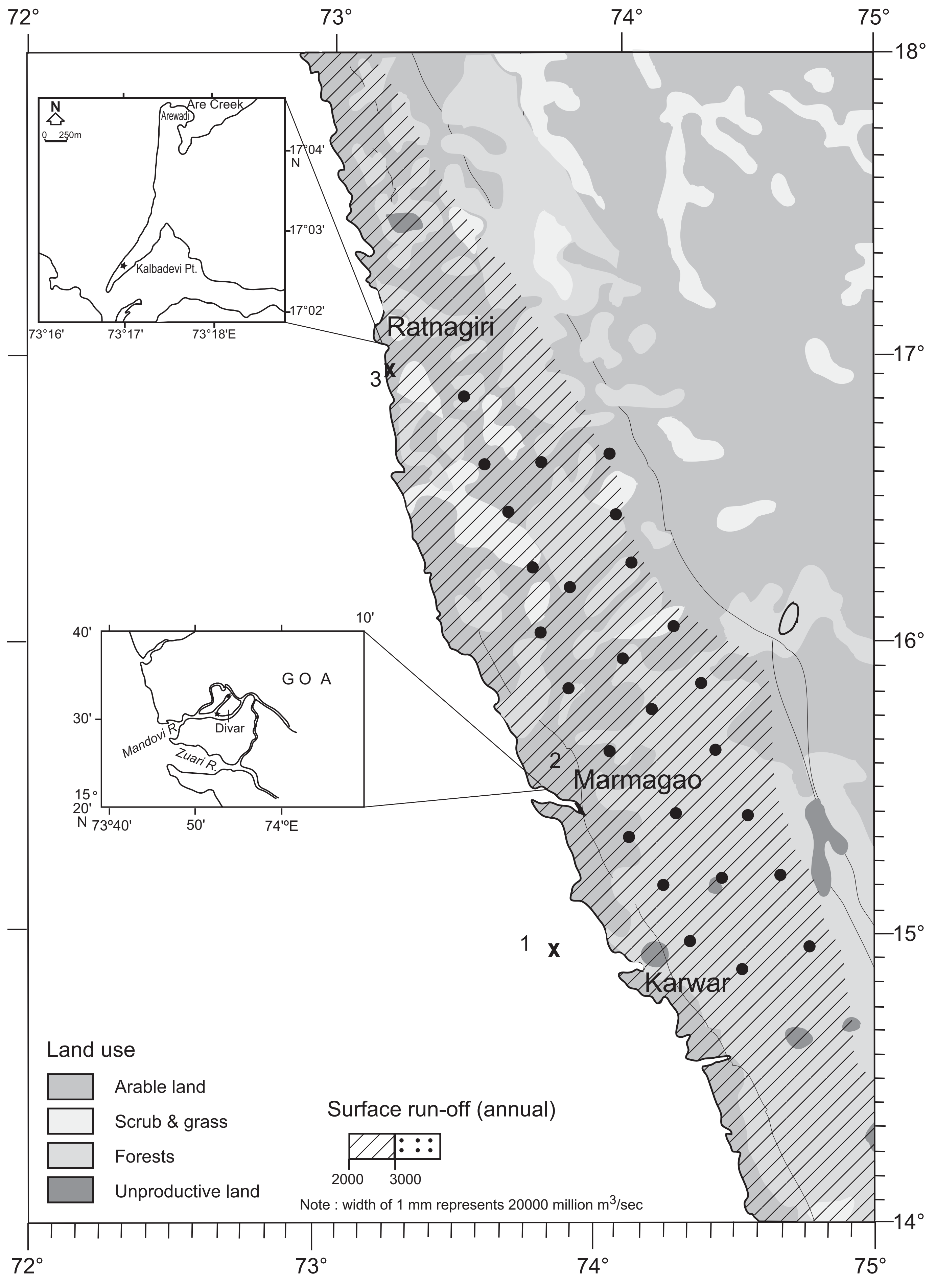
Legends to figures:

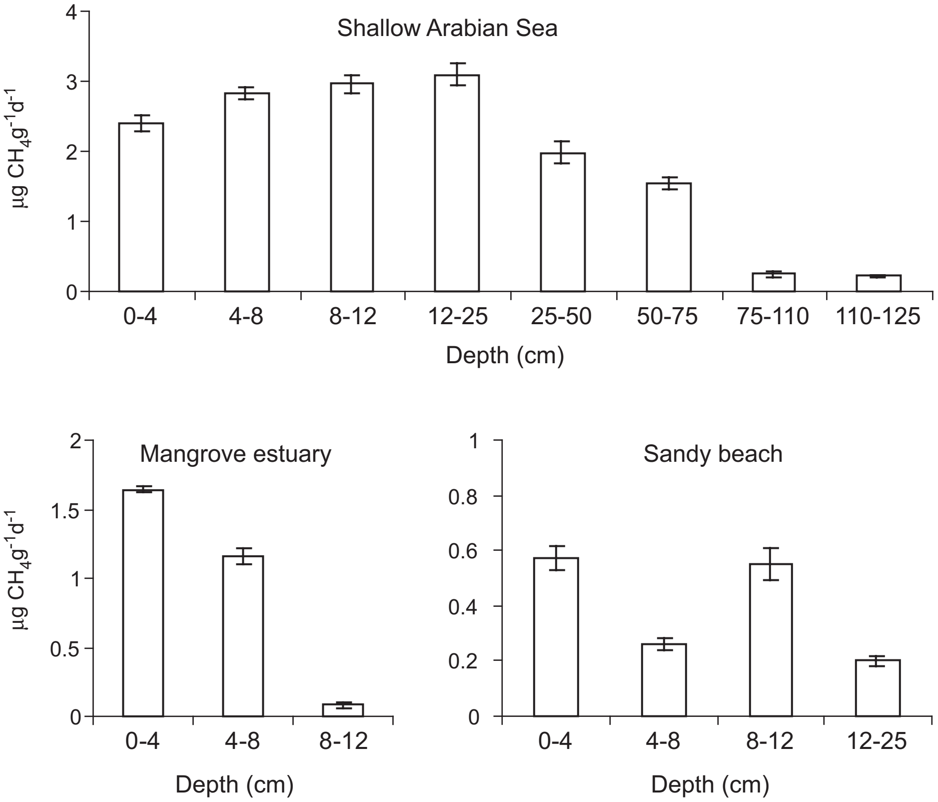
Fig. 1 Map of the sampling sites.

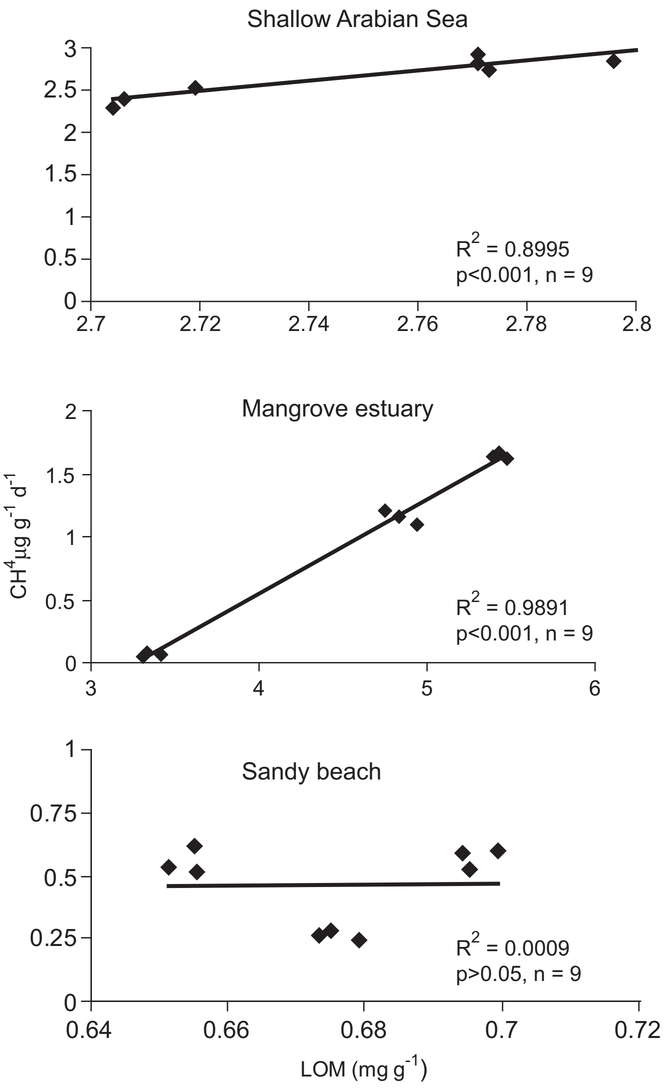
Fig. 2 Distributive pattern of methane production rates at the study sites.

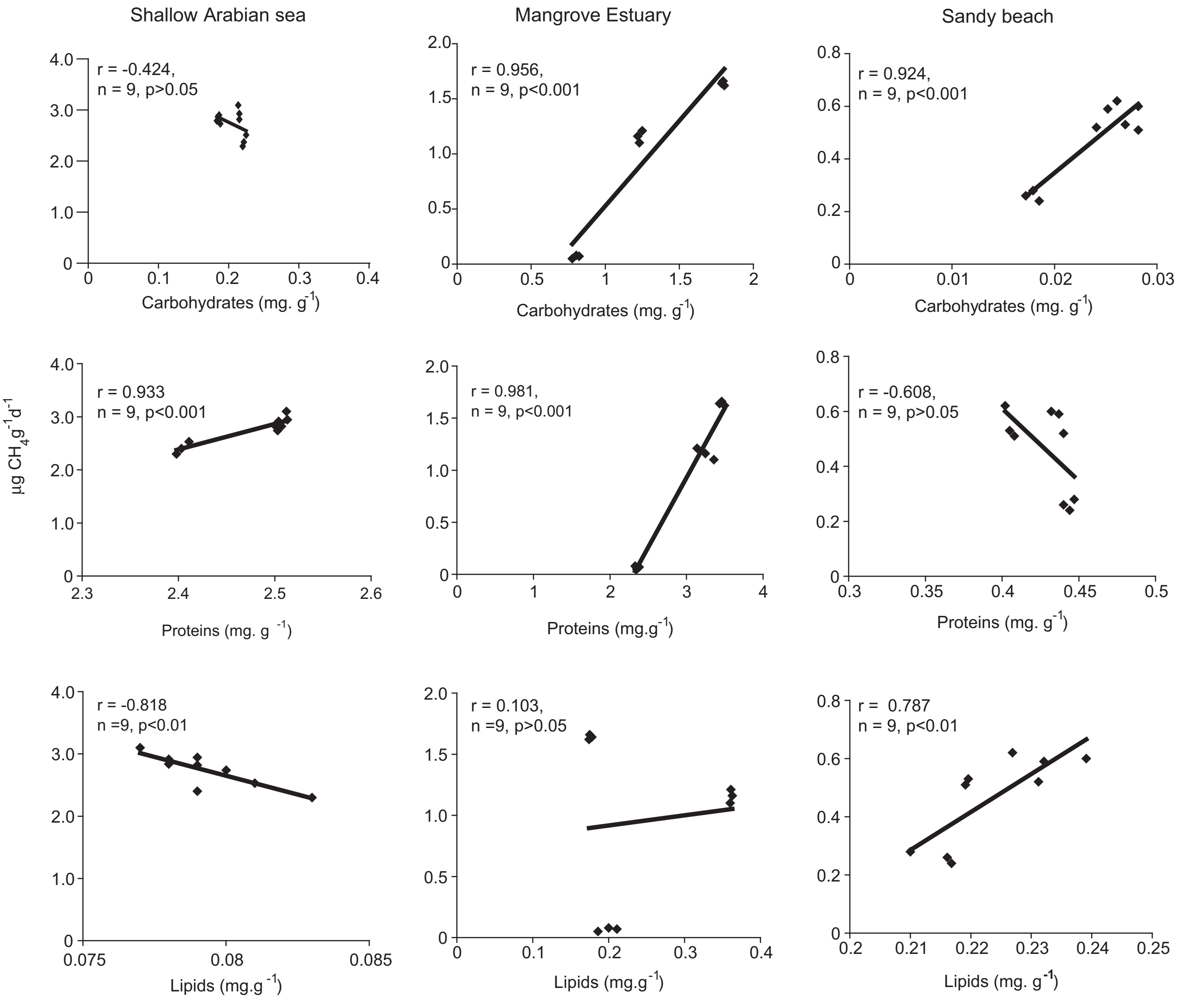
Fig. 3 Scatter diagram of methane production rates and labile organic matter.

Fig. 4 Scatter diagram showing the relationship between the methane production rate and the concentrations of carbohydrates, proteins and lipids.


72°
73°
74°
75°
18°
17°
16°
15°
14°
N
0 250m
Are Creek
Arewadi
17°04' N
17°03'
Kalbadevi Pt.
17°02'
73°16'
73°17'
73°18'E
Ratnagiri
3 x
40'
10'
G O A
30'
Mandovi R.
Divar
Zuari R.
15° 20' N
73°40'
50'
74°E
2
Marmagao
1 x
Karwar
Land use
Arable land
Scrub & grass
Forests
Unproductive land
Surface run-off (annual)
2000
3000
Note : width of 1 mm represents 20000 million m3/sec


Shallow Arabian Sea
µg $CH_4g^{-1}d^{-1}$
0
1
2
3
4
0-4
4-8
8-12
12-25
25-50
50-75
75-110
110-125
Depth (cm)
Mangrove estuary
µg $CH_4g^{-1}d^{-1}$
0
0.5
1
1.5
2
0-4
4-8
8-12
Depth (cm)
Sandy beach
0
0.2
0.4
0.6
0.8
1
0-4
4-8
8-12
12-25
Depth (cm)


Shallow Arabian Sea
3
2.5
2
1.5
1
0.5
0
2.7
2.72
2.74
2.76
2.78
2.8
R^2 = 0.8995
p<0.001, n = 9
Mangrove estuary
2
1.5
1
0.5
0
3
4
5
6
CH^{4}µg g^{-1} d^{-1}
R^2 = 0.9891
p<0.001, n = 9
Sandy beach
1
0.75
0.5
0.25
0
0.64
0.66
0.68
0.7
0.72
R^2 = 0.0009
p>0.05, n = 9
LOM (mg g^{-1})


Shallow Arabian sea
Mangrove Estuary
Sandy beach
r = -0.424,
n = 9, p>0.05
r = 0.956,
n = 9, p<0.001
r = 0.924,
n = 9, p<0.001
Carbohydrates (mg. g-1)
μg CH4g-1d-1
r = 0.933
n = 9, p<0.001
r = 0.981,
n = 9, p<0.001
r = -0.608,
n = 9, p>0.05
Proteins (mg. g -1)
Proteins (mg.g-1)
r = -0.818
n =9, p<0.01
r = 0.103,
n =9, p>0.05
r = 0.787
n = 9, p<0.01
Lipids (mg.g -1)
Lipids (mg.g-1)
Lipids (mg. g-1)