

# Methane in pristine and impaired mangrove soils and its possible effect on establishment of mangrove seedlings

Antje Strangmann · Yoav Bashan · Luise Gianì

Received: 14 February 2007 / Revised: 13 July 2007 / Accepted: 27 July 2007 / Published online: 24 August 2007  
© Springer-Verlag 2007

**Abstract** Pristine and impaired mangrove soils (from road construction, aquaculture, and sewage) in Baja California Sur, Mexico were investigated for methane dynamics, related soil properties, and their impact on initial establishment of black mangrove propagules. All soils (Salic Fluvisols and Histosols) had neutral to alkaline pH, were saline, and had variable organic carbon content, and redox potentials. Most pristine mangrove soils showed low methane concentration, low methane production rates, and no methane emission. Impaired mangrove soil (from aquaculture) and mangrove soil affected by sewage water showed high methane concentration, high methane production rates, and high methane emission, thus acting as a methane source. Elevated methane concentrations, similar to levels detected in the impaired mangrove soil, reduce the growth of seedlings under closed chamber conditions. Addition of sulfate to the soil reversed the trend. These results indicate that impaired mangrove soils in dry climatic regions produce and emit methane and that elevated methane concentration in

the vicinity of propagules may affect establishment of mangrove seedlings in impaired mangrove soils.

**Keywords** Anthropogenic impact · Mangroves · Methane dynamics · Propagules · Wetland soils

## Introduction

Establishment of mangrove seedlings in arid climatic regions after natural or man-made disasters is low. The State of Baja California Sur, Mexico shows some examples of this phenomenon: Patches of clear-cut mangrove forest at Laguna Balandra (30 km north of the city of La Paz) remained almost barren from 1990 until 1995, when it was artificially reforested (Toledo et al. 2001). Hurricane winds and currents blocked the inlet channel of a mangrove community on the El Mogote barrier sand spit (1 km from the city of La Paz). Most trees died after the 2001 event. In 2004, the inlet channel was opened, and very slow recovering is underway (<http://www.bashanfoundation.org/conservation2.html>, accessed July 2007). A mangrove community at Enfermeria (20 km north of the city of La Paz) was blocked by road construction in 1995 and reopened a year later. Most of the trees died after the construction; almost no mangrove seedlings have been established to this day (A. Ortega, CIBNOR, personal communication). Explanations for this die-off were attributed to high salinity (Patterson et al. 1993) and high temperature (McMillan 1971). The edaphic parameters and gasses emitted from these lagoons, as possible inhibitors, have not been studied. At the same time, there is no difficulty of mangrove establishment in pristine mangroves (<http://www.bashanfoundation.org/balandra/balandra.html>, accessed July 2007). Tropical and subtropical mangroves (humid and arid regions) with naturally high productivity

---

This paper is in memory of the late mangrove researcher Dr. Gina Holguin of Mexico.

---

A. Strangmann · L. Gianì  
Department of Soil Science,  
Institute of Biology and Environmental Science,  
Carl von Ossietzky Universität Oldenburg,  
Postal Box 2503, 26111 Oldenburg, Germany

Y. Bashan (✉)  
Environmental Microbiology Group, Northwestern Center for  
Biological Research (CIBNOR),  
Mar Bermejo 195, Colonia Playa Palo de Santa Rita,  
La Paz 23090, Mexico  
e-mail: bashan@cibnor.mx

Y. Bashan  
Department of Soil, Water, and Environmental Science,  
University of Arizona,  
Tucson, AZ 85721, USA

of energy (Lee 1999) cover approximately 160,000 km<sup>2</sup> (Spalding et al. 1997), which is nearly 60–75% of the world's tropical and subtropical coastlines (Jennerjahn and Ittekkot 2002). Mangrove ecosystems are known to support coastal fishery resources (Warne and Laman 2007), to protect coastlines from erosion, and to contribute to the conservation of coastal resources by regulating nutrient and suspended sediment levels (Holguin et al. 2001; Spalding et al. 1997).

In general, natural wetlands are considered a natural source of atmospheric methane (CH<sub>4</sub>) (Hütsch 1998), contributing 115 Tg year<sup>-1</sup> (22%) to global annual methane production. Methanogenesis is thought to be negligible for mangrove soils, a minor contributor to methane production and emission on a global scale (Harris et al. 1988; Alongi et al. 2000, 2001). This assumption is based on the fact that sulfate reducing and methane producing bacteria compete for the same substrates (H<sub>2</sub>/CO<sub>2</sub>, acetate), but the sulfate reducers are better competitors (King 1984). Consequently, sulfate reduction prevents methanogenesis in sulfate-rich environments, such as salt marshes and mangroves. Methanogenesis within mangrove soils can occur if substrates are abundant, the supply of substrates (listed above) exceeds the sulfate supply, or large amounts of methylated amines are present (Giani et al. 1996a). Considerable methane concentrations and methane production rates were found in an anthropogenically impacted section of a pristine mangrove ecosystem in Laguna Balandra near La Paz, Baja California Sur, Mexico (Giani et al. 1996b). A mangrove forest in Puerto Rico subjected to secondary sewage sludge showed high methane fluxes. The forest emitted 40 times more methane than unimpacted pristine areas (Sotomayor et al. 1994). High methane levels were measured in an impaired Indian mangrove on the coast of the Bay of Bengal (Mukhopadhyay et al. 2001, 2002), and an intense methane flux was also found in a pristine mangroves in India (Purvaja and Ramesh 2001; Mukhopadhyay et al. 2001, 2002).

This study investigated (1) methane production and related soil properties in pristine mangroves and in mangroves that had been impaired by different anthropogenic activities and (2) the impact of methane generation on germinating propagules of black mangrove growing in these soils.

## Materials and methods

### Sample sites

Salic Fluvisols and Histosols (FAO/ISRIC/ISSS 1998) of pristine and impacted mangrove ecosystems in the area of La Paz, Baja California Sur, Mexico were sampled between January and March 1999.

The first location, the lagoon at Balandra, covering 10 ha (Perdrin-Aviles et al. 1990), is an almost undisturbed mangrove

(Holguin et al. 1992; Toledo et al. 1995a) 30 km north of La Paz. The vegetation shows a typical landward zonation, beginning at the sea with *Rhizophora mangle* L. (red mangrove), followed by *Laguncularia racemosa* Gaertn. (white mangrove), and then *Avicennia germinans* (L.) Stern (black mangrove; Toledo et al. 2001). Further inland, the mangrove is adjacent to salt marsh, which is dominated by three herbaceous species: *Salicornia bigelovii*, *Allenrolefea occidentalis*, and *Monanochloe littoralis*. Especially near the sea, a dense population of grapsid crabs (*Uca vocator*) is common (also see Holguin et al. 1992; Giani et al. 1996b). Sampling was performed along two transects from sea to land (sampling sites: Balandra 1.1–2.3). Additionally, two solitary profiles, each with different textures, were sampled (sampling sites: Balandra 3, 4). The latter locations were undisturbed mangrove.

The second location, currently a shrimp aquaculture facility that started production in 1989, is located in a former mangrove 13 km north of La Paz. The vegetation was cleared inside the mangrove pond. The feeder channel supplying the ponds with seawater is dominated by *Avicennia germinans* and *L. racemosa*. Sampling took place in the feeder channel, inside the oldest shrimp-pond, and within the outlet (sampling sites: Shrimp 1–3). Aquaculture that is generally believed to induce increased methane production comes from increased substrates, like fertilizers, decomposition products of fish and shrimp, or sewage waters.

The third location, Playa Tesoro, is a small mangrove ecosystem located near the bay at Pichilingue, 20 km north of La Paz. Except for very small channels, the former mangrove is cut off from the sea by road construction, which has changed the water regime. Cut off from the sea is supposed to lead to increased methane production because the starting substrates for methanogens is more favorable, as sulfate supply from the sea is no longer available for the sulfate reducers. The site contains some *Avicennia germinans* but is mostly bare. Samples were obtained next to the trees area (sampling site: Playa Tesoro).

The fourth location is a mangrove ecosystem along the western side of La Paz, within the Ensenada de La Paz, a large lagoon formed by the large El Mogote barrier sand spit. Until 1994, when a wastewater treatment facility was constructed, this site had been impacted by municipal sewage for a long time (A. Ortega, CIBNOR, personal communication). Sewage induces increased methane production similar to the aquaculture operation. Samples were taken seaward of a patch of *Avicennia germinans* that is topped with a layer of decomposing algae (sampling site: Bay La Paz).

### Sampling procedures for methane analysis and soil characterization

At each sample site, subsites were chosen according to their representative pedological, hydrological, and plant commu-

nity features. Within each subsite, an area (10×30 m) was selected at random as representative of the soil. Profiles were excavated to water level (0–105 cm soil depth) during low tides and described morphologically.

Triplicate soil samples were collected from each distinct horizon and placed in new polyethylene bags. The tightly sealed bags were immediately taken to the laboratory, where redox potential and pH were immediately determined by pushing the electrodes into the samples when the bags were opened. The same day, a second, fresh soil sample was prepared for soil saturation extraction. This sample was centrifuged for 10 min at 3,500×g within 24 h. The extract was used to assay sulfates, phosphates, chlorides, nitrates, and dissolved organic carbon. The rest of the fresh soil was air-dried or dried at 105°C in an oven, passed through a 2-mm screen. This soil was used for measuring total carbon, nitrogen, and carbonate. Organic carbon was calculated by subtraction of C derived from carbonates.

Triplicate field samples (1 ml from each horizon within each profile) for measurements of methane concentration were immediately put into pre-weighed serum flasks containing 1.5 N NaOH and then sealed. After partitioning of the methane gas dissolved or trapped in the interstitial waters of the soil into the gas phase, the headspace was sampled with a gas-tight syringe and analyzed by gas chromatography in the laboratory. For measurements of methane production rates, triplicate samples (1 ml) from each horizon were extruded into vacutainers and sealed under N<sub>2</sub> atmosphere immediately after sampling. The tubes were flushed with N<sub>2</sub>, after which a starting time was established for the time course of methane production. The tubes were incubated at room temperature (25–28°C). Every 24 h, the headspace of the tubes were sampled with a gas-tight syringe and measured by gas chromatography. Methane accumulation in the headspace of the tubes was determined by the change in concentration of methane per volume of soil in the tubes. In situ methane flux measurements were made at the same places and dates, using a static chamber technique, which consisted of a PVC chamber with a septum-closed serum flask at its top (12 l<sup>-1</sup> vol) and an extra ring of steel. The ring was installed 2–3 cm into the soil, filled with water, and the chamber placed in the ring. Using a gas-tight syringe, aliquots (10 ml) of the chamber air were withdrawn at the beginning and end (after 2–3 h) of the experiment. The samples were transferred into vacutainers and analyzed by gas chromatography.

#### Growth of mangrove seedlings

Propagules of black mangroves (*Avicennia germinans*) were collected from the same collection sites at the lagoon at Balandra. Propagules were selected and treated before planting, as described by Toledo et al. (1995b). Intact surface mud samples were collected from Station 1 at

Balandra and from the fourth sampling areas described above. The simple core sampler was a PVC pipe (9.5 cm inner diameter) pressed into the soil to the depth of 5 cm. Careful handling was practiced to keep the mud samples from being disturbed. The mud samples (260±10 g fresh weight) were immediately placed in small, black plastic pots without drainage (10 cm diameter, 7 cm height). The mud did not receive any additional treatment. After placing the core samples in the pots, the space between the core and the side of the pot (<5 mm) was filled with inert, fine pre-wetted perlite to prevent moving or breaking of the core sample. The pots were placed in large plastic containers (45 cm diameter, 30 cm high) containing a bottom cover of 0.5-cm water to maintain high humidity and prevent drying of the pots. The large containers were covered with transparent plastic film to prevent exchange of methane with the outside atmosphere. The surface that could emit methane from all pots within one container was 708 cm<sup>2</sup>. From each pot, three 6-mm vertical cores were taken with a small manual core sample. These small soil cores were discarded. Three 8-cm plastic straws (5 mm inner diameter), each perforated with about 50 holes smaller than 1 mm each, were carefully inserted into the holes to provide the soil samples easier access to gasses from the atmosphere to the lower parts of the soil in the pot. Three propagules were planted in each pot at 1 cm depth (Toledo et al. 1995b), leaving part of the propagule above the mud substrate. The mud was covered with a 0.5-cm layer of synthetic seawater. Propagules were allowed to germinate and to grow for 4 weeks in an uncontrolled, shaded greenhouse (average day temperature of 32±3°C) under natural light (average, 1,100 μmole m<sup>2</sup> s<sup>-1</sup> at midday). The containers were ventilated (opened), once a week and new doses of methane were applied. Commercial methane was supplied from a sealed chamber in the container at concentrations of 80 or 150 μM. The level of methane in the closed atmosphere was measured in each container every 7 days, as described below (before the weekly ventilation). Analysis of the purity of the gas showed only traces of other gases. The following treatments were carried out using pristine (undisturbed) and impaired mangrove soils: (1) seedlings growing in an atmosphere containing methane, (2) seedlings growing without methane, and (3) seedlings growing in soil amended with irrigated water containing magnesium sulfate (MgSO<sub>4</sub>•7H<sub>2</sub>O, 1.5 mM dissolved in seawater) as an inhibitor of methane. After the treatment period, plant parameters of height and dry weight were recorded (Bashan and de-Bashan 2005).

#### Analytical analyses

Standard laboratory analyses of soil properties and methane production were made according to the following proce-

dures: pH and redox potential according to Giani et al. (1996b). Soil saturation extract analyses of several inorganic chemicals were made according to the following procedures: sulfate was measured spectrometrically according to Giani et al. (1996b); chloride was measured with a potentiometer (E-536, Methrom Potentiograph, Germany); nitrate was measured spectrophotometrically according to Dewes and Schmitt (1990); phosphate was measured spectrophotometrically according to standard methods (APHA, AWWA, WPCF, 1992); and carbon was determined with a C/N analyzer (NA 2000, Fisons Instruments, Germany) after pulverizing the sample. Organic carbon was calculated as the difference between total carbon and carbonate carbon. Dissolved organic carbon was spectrophotometrically determined by absorbance at 254 nm according to Brandstetter et al. (1996). Methane was determined by withdrawing a 0.5-ml sample from the serum flasks ( $\text{CH}_4$  concentration) and vialtainers ( $\text{CH}_4$  production and flux) and direct injection into a gas chromatograph (Hewlett Packard 5890, Series II equipped with a FID and a HP capillary column with internal diameter of 0.32 mm and length of 50 m maintained at  $90^\circ\text{C}$ ). The carrier gas was nitrogen, and the flow rate was  $15 \text{ ml min}^{-1}$ . Injector and detector temperatures were set at  $250^\circ\text{C}$ , retention time was 4 min. Sample peaks were compared to commercial preparations of 10 to  $100 \mu\text{l l}^{-1}$  methane standards. Methane concentrations, methane production rates, and methane emissions were analyzed in triplicate samples.

#### Experimental design and statistical analysis

Soil samples were taken in triplicate and each soil or methane analysis were repeated three times from each sample. Ten pots served as a treatment ( $n=110$  pots, 330 seedlings) and the experiment was repeated twice. Results were analyzed by one-way analysis of variance (ANOVA) and then by Tukey's post hoc analysis at  $P \leq 0.05$ . Computations used Statistica 6.0™ software (StatSoft, Tulsa, OK, USA). Soil and methane data are accompanied by standard error.

## Results

### Soil properties

All soils had neutral to alkaline pH values and were saline (Table 1). At the soil surface, salt (chloride) content at Playa Tesoro was high ( $113\text{--}139 \text{ g l}^{-1}$ ) from restricted tidal exchange and high evaporation in this arid climate. Organic carbon varied widely ( $0.0\text{--}184 \text{ g kg}^{-1}$ ) with concentrations of  $2.9\text{--}81.2 \text{ mg l}^{-1}$ . C/N ratios were often extraordinarily

high because nitrogen content was extremely low ( $<0.1 \text{ g kg}^{-1}$ ). Playa Tesoro and most Balandra soils showed high Eh values (Table 1), indicating unfavorable conditions for methanogens. Lowest redox potential was found at the Shrimp 2 site ( $-276 \text{ mV}$ ). Subsequent Eh measurements in the same pond were in the same range (data not shown).

Marine conditions were evident in the high sulfate content of the soils (Table 1), mostly similar to its concentration in seawater ( $28\text{--}44 \text{ mM}$ ). Salinization was extraordinarily high from the sulfate contents (up to  $280.2 \text{ mM}$ ) at Playa Tesoro. Some sites (Balandra 3, Shrimp 2, Bay La Paz) showed low sulfate content ( $6.8$ ,  $6.4$ , and  $4.7 \text{ mM}$ , respectively), indicating sulfate depletion in the soil solution from intense microbial sulfate reduction. However, sulfate concentration is not only exclusively controlled by biological factors but also by rain and transpiration. Thus, it has to be related to the conservative chloride ion, expressed as a sulfate/chloride ratio (Lord and Church 1983). Using this parameter, a tendency toward depletion of sulfate in the soil solution was confirmed for Bay La Paz and Shrimp 2, with ratios of 0.2 and 0.4, respectively.

Phosphate in most soils was low, almost below detection level (Table 1). However, Shrimp soils (2 and 3) contained moderate levels of P (up to  $11.2 \text{ mg P l}^{-1}$ ) and Bay La Paz soils had up to  $8.4 \text{ mg P l}^{-1}$ , indicating eutrophic conditions.

### Methane production

Most Balandra soils contained methane at  $<10 \mu\text{M}$  and production rates generally  $<20 \text{ nmol CH}_4 \text{ ml}^{-1} \text{ d}^{-1}$  (Table 2). Distinctly higher concentrations of up to  $77.7 \mu\text{M}$  and production rates of up to  $102.0 \text{ nmol CH}_4 \text{ ml}^{-1} \text{ d}^{-1}$  were found within the subsoil of the lagoon site, Balandra 1.1, at low redox potentials and with considerable organic carbon and dissolved organic content. But methane production did not lead to release of methane. This was also true for emission experiments conducted at Balandra 2.2, where gas domes covered the black mangrove pneumatophores. The same experiment at Balandra 3 yielded detectable emission of methane ( $19.2 \mu\text{g m}^{-2} \text{ d}^{-1}$ ) along with distinct methane production; however, the large standard deviation suggests a weak result.

Methane production at Playa Tesoro, where road construction essentially devastated the mangrove, was up to  $15 \mu\text{M}$ , production rates of up to  $38.1 \text{ nmol ml}^{-1} \text{ day}^{-1}$ , and no methane emission (Table 2). This is comparable to most of the Balandra soils but not to the other sites where the soils were affected.

Some soils at the aquaculture facility showed intensified methane production (Table 2). Highest methane concentrations ( $13.5\text{--}79.1 \mu\text{M}$ ) and production rates ( $157.2\text{--}$

**Table 1** Properties of mangrove soils: pristine mangrove at Balandra (Balandra 1.1–4) and impaired mangrove from aquaculture wastes (Shrimp 1–3) from road construction (Playa Tesoro) and from sewage (Bay La Paz)

Site	Depth (cm)	Vegetation	pH (H <sub>2</sub> O)	Eh (mV)	C org (g kg <sup>-1</sup> )	C/N <sup>a</sup>	DOC (mg l <sup>-1</sup> )	Sulfate (mM)	Chloride (g l <sup>-1</sup> )	Sulfate/ chloride ratio	Nitrate (mg l <sup>-1</sup> )	Phosphate (mg l <sup>-1</sup> )
Balandra 1.1	0–27	<i>Rhizophora</i>	7.2	331.3	176.9	18.4	47.3	34.8	26.1	1.3	12.9	1.2
	27–40	<i>Avicennia</i>	7.5	-92.0	183.4	21.5	58.3	29.8	26.2	1.1	27.7	0.9
	>40		7.4	-195.3	86.8	>868	81.2	31.2	26.4	1.2	11.3	1.4
Balandra 1.2 <sup>ab</sup>	0–7	<i>Salicornia</i>	8.1	387.0	1.1	>11	25.7	61.9	33.8	1.8	3.9	n.n
	7–21		8.2	440.0	3.8	>38	3.8	20.6	13.0	1.6	1.9	n.n
	35–55		8.2	435.7	0.0	n.d.	3.2	20.7	13.1	1.6	n.n.	n.n
	55–85		8.1	475.3	1.6	>16	3.1	27.8	14.6	1.9	2.5	n.n
	>85		7.8	-114.7	0.0	n.d.	4.1	47.0	22.8	2.1	0.9	n.n
Balandra 2.1	0–10	No Vegetation	7.3	-100.0	3.3	>33	5.4	18.4	11.7	1.6	6.1	<0.35
	10–50	( <i>Rhizophora</i> )	7.3	126.0	114.7	>1147	10.1	31.5	20.9	1.5	7.2	<0.35
Balandra 2.2	0–40	<i>Avicennia</i>	8.0	441.3	1.7	>17	5.8	30.5	19.7	1.6	3.0	n.n.
	40–65		7.7	449.0	1.8	>18	5.8	37.3	20.2	1.8	0.5	<0.35
	65–105		6.9	278.7	42.7	>427	8.1	48.4	37.1	1.3	16.4	<0.35
	>105		7.6	163.0	11.8	>118	12.0	46.9	22.3	2.1	1.0	n.n.
Balandra 2.3	0–13	<i>Salicornia</i>	8.2	531.0	6.4	>64	3.5	16.9	19.5	0.9	2.6	n.n.
	13–46		8.0	530.0	1.3	>13	3.4	18.9	13.6	1.4	0.8	n.n.
	46–70		7.8	540.7	1.0	>10	4.4	23.6	17.4	1.4	2.9	n.n.
	>70		7.7	272.7	1.9	>19	n.b.	n.b.	n.b.	n.b.	n.b.	n.b.
Balandra 3	0–28	<i>Avicennia</i>	7.6	-201.7	23.4	>234	18.4	31.2	21.2	1.5	4.8	<0.35
	>28		6.8	-220.7	83.9	>839	3.5	6.8	4.6	1.5	0.7	n.n.
Balandra 4	0–15	<i>Avicennia</i>	6.6	108.0	57.6	>576	61.4	25.0	33.3	0.8	1.4	n.n.
Shrimp 1	0–10	<i>Avicennia</i>	8.2	260.0	2.3	9.76	3.9	15.2	11.2	1.4	6.0	<0.35
	>10	<i>Laguncularia</i>	8.3	-155.0	1.9	>19.4	2.9	16.7	11.6	1.4	4.7	<0.35
Shrimp 2 <sup>b</sup>	0–5	No Vegetation	7.9	-108.3	6.2	6.8	13.3	59.8	15.4	3.9	9.6	1.0
	5–20		7.9	-94.7	2.7	>26.8	9.4	50.8	12.8	4.0	10.6	0.8
	0–5		7.6	-276.3	14.9	5.6	33.7	6.4	14.7	0.4	1.6	11.2
Shrimp 3	0–20	<i>Avicennia</i>	7.6	184.4	7.5	1.5	8.1	48.6	12.6	3.9	11.4	1.1
	>20		8.3	294.0	1.9	>19.2	4.4	22.9	15.4	1.5	7.5	<0.35
Playa Tesoro	0–0.5	<i>Avicennia</i>	7.2	150.3	20.4	5.4	59.9	280.2	138.5	2.0	1.2	1.0
	0.5–5		7.8	-41.0	1.8	0.5	13.5	202.0	113.1	1.8	n.n.	n.n.
	7–25		8.3	90.7	0.0	n.d.	6.6	74.7	39.2	1.9	6.8	n.n.
	>25		8.2	358.3	0.0	n.d.	5.2	91.3	41.4	2.2	0.3	n.n.
Bay La Paz	+3	<i>Avicennia</i>	7.0	-243.0	89.6	5.9	75.8	4.7	20.1	0.2	n.n.	8.38
	0–3		7.9	-77.3	0.1	>0.7	4.8	22.5	11.2	2.0	21.0	<0,35

The data were based on two replicates, the standard deviations were always less than 10% of the mean.

nn Not detectable; nd not determined

<sup>a</sup>>Means N <0.1 g kg<sup>-1</sup>

<sup>b</sup>Sampled 1 m beside the soil profile

377.8 nmol ml<sup>-1</sup> day<sup>-1</sup>) were detected in the pond at the Shrimp 2 site. Although methane concentrations were in the same range as in the subsoil of Balandra 1.1, production rates were distinctly higher. Intensive methane production was accompanied by perceptible methane flux (911.5 µg m<sup>-2</sup> h<sup>-1</sup>). The feeder channel at Shrimp 1 and at the outlet of Shrimp 3 had methane emission rate of 2.3 and 16.9 µg m<sup>-2</sup> h<sup>-1</sup>, respectively, but concentrations (<10 µM) and rate of production was low (<10 nmol ml<sup>-1</sup> day<sup>-1</sup>).

The highest methane contents (147.5 µM) were found in the mangrove (Bay La Paz) affected by wastewater (Table 2). Simultaneously, very high methane production rates (up to 4,155.5 nmol ml<sup>-1</sup> day<sup>-1</sup>) were measured.

### Effect of application and inhibition of methane on development of black mangrove seedlings

Over 95% of all propagules germinated when sown in mangrove soils; however, seedlings in impaired mangrove soils were smaller than in pristine soils. Application of high doses of methane to pristine soil stopped development in seedlings by 56% of the germinating propagules, and the seedlings that continued to develop were significantly smaller than the control plants. Application of moderate doses of methane to both pristine and impaired soils has smaller but significant inhibition of seedling development; the effect was more pronounced in impaired soil. Applica-

**Table 2** Methane concentration, methane production rates, and methane emission from pristine mangrove soils at Balandra (Balandra 1.1–4) and from impaired mangrove soils impacted by aquaculture (Shrimp 1–3), road construction (Playa Tesoro), and sewage (Bay La Paz)

Site	Depth (cm)	CH <sub>4</sub> Concentration (μM)			CH <sub>4</sub> Production Rates (nmol CH <sub>4</sub> ·ml <sup>-1</sup> day <sup>-1</sup> )						CH <sub>4</sub> Flux (μg CH <sub>4</sub> ·m <sup>-2</sup> h <sup>-1</sup> )		
		Average	SEM	N	t1	SEM	N	t2	SEM	N	Average	SEM	N
Balandra 1.1	0–27	0.68	1.0	3	8.91	3.0	3	13.61	19.3	3	0.00	0.0	3
	27–40	36.06	21.9	3	64.06	18.3	3	0.00	0.0	3			
	>40	77.67	9.0	3	102.03	1.3	3	0.00	0.0	3			
Balandra 1.2 <sup>a</sup>	0–7	2.83	1.2	3	1.63	1.7	3	1.08	1.0	3	0.00	0.0	2
	7–21	3.02	0.8	3	3.22	3.5	3	0.18	0.3	3			
	>85	4.38	1.2	3	1.75	2.5	3	1.18	1.7	3			
Balandra 2.1	0–10	0.33	0.5	3	1.79	2.5	3	7.81	2.7	3	0.00	0.0	3
	10–50	2.63	0.4	3	3.42	2.9	3	0.92	1.3	3			
Balandra 2.2	0–40	5.71	4.3	3	0.60	0.8	3	0.42	0.3	3	0.00	0.0	2
	40–65	5.78	0.2	2	0.61	0.9	3	0.61	0.5	3			
	65–105	1.27	1.8	3	0.66	0.9	3	0.60	0.4	3			
	>105	4.43	4.2	3	2.28	1.6	3	0.00	0.0	3			
Balandra 2.3	0–13	0.00	0.0	2	0.00	0.0	3	0.32	0.5	3	0.00	0.0	2
	13–46	0.00	0.0	3	0.00	0.0	3	0.70	1.0	3			
	46–70	1.56	2.2	3	0.76	1.1	3	0.00	0.0	3			
	>70	0.00	0.0	3	0.52	0.7	3	0.00	0.0	3			
Balandra 3	0–28	5.93	1.6	3	19.18	9.4	3	39.04	36.8	3	19.15	19.1	2
	>28	15.40	3.6	3	19.69	3.9	3	0.00	0.0	3			
Balandra 4	0–15	3.25	0.0	3	14.80	7.0	3	39.56	17.9	3	0.00	n.d.	1
Shrimp 1	0–10	1.57	1.2	3	9.63	1.2	3	1.92	1.7	3	2.28	3.2	3
	>10	1.58	2.2	3	7.79	5.0	3	3.60	2.9	3			
Shrimp 2 <sup>a</sup>	0–5	28.88	4.1	3	377.81	69.3	3	41.17	43.0	3	911.47	939.5	3
	5–20	13.50	8.4	3	157.23	59.8	3	29.20	21.1	3			
	0–5	79.11	18.9	3	160.06	29.0	3	104.04	31.9	3			
Shrimp 3	0–20	9.67	3.4	3	11.79	3.7	3	0.00	0.0	3	16.89	6.6	3
	>20	9.13	1.5	3	9.90	2.7	3	1.62	2.3	3			
Playa Tesoro	0–0.5	14.90	12.2	3	22.42	24.3	3	0.48	0.5	3	0.00	0.0	3
	0.5–5	7.16	1.9	3	38.12	18.4	3	41.77	52.6	3			
	7–25	0.00	0.0	3	14.55	2.2	3	10.34	12.3	3			
	>25	0.00	0.0	3	0.64	0.9	3	2.00	2.1	3			
Bay La Paz	+3	147.52	47.0	3	4155.50	547.8	3	2117.55	879.6	3	n.d.	n.d.	n.d.
	0–3	22.27	6.4	3	126.77	58.4	3	13.04	13.0	3			

<sup>a</sup> Sampled 1 m beside the soil profile

t1 Measurement 24 h after starting; t2 measurement 24 h after t1; SEM standard error of the mean; n.d. not determined

tion of sulfate, an inhibitor of methane production, to both kinds of soil yielded seedlings comparable to those growing under pristine mangrove conditions. Complementation treatment (addition of exogenous methane to soils incapable of producing methane) led to inhibition of plant development. Seedlings grew slightly better under sealed atmosphere conditions than controls growing in the open, but the difference was not statistically significant (Table 3).

## Discussion

Worldwide, mangroves are challenged by human activities (Moreno-Casasola 2000; Holguin et al. 2006) and natural disasters, especially tropical storms. Damaged mangroves need restoration for general ecological and economic activ-

ities, including serving as nurseries for young marine animals and fish and timber production (Kakuma 2000; Holguin et al. 2001). In the wet tropics, this has been left to nature or to simple reforestation techniques (Chen et al. 2000; Ida 2000). In the arid subtropics, damaged mangroves are very slow to recover on their own (Cintron et al. 1978; Toledo et al. 2001). Two plausible reasons explain this difference: higher salinity from intense evaporation under cloudless skies and lack of a fresh water supply (Gonzalez-Acosta et al. 2006) and high temperatures that desiccate the unsettled propagules (McMillan 1971). Although mangrove plants grow in seawater, mangrove seedlings develop more slowly as salinity increases (Clarke and Hannon 1970; Downton 1982; Naidoo 1987; Patterson et al. 1993; Parida et al. 2004).

Independent of climate, natural pristine coastal wetlands, mangroves included, are regarded as minor contributors to

**Table 3** The effect on growth of seedlings of black mangroves of exogenous and indigenous methane and a methane inhibitor in a closed environment

Type of soil and methane treatment	Plant parameter		
	Height (cm)	Dry weight (g)	Methane in soil grown in sealed compartment ( $\mu\text{M}$ )
Pristine (control)	15.3 $\pm$ 0.5 d	3.5 $\pm$ 0.3 e	8 $\pm$ 3
Impaired (control)	13.5 $\pm$ 0.4 b	2.4 $\pm$ 0.2 c	60 $\pm$ 5
Pristine+high methane	10.4 $\pm$ 0.3 a <sup>a</sup>	1.4 $\pm$ 0.1 a <sup>a</sup>	145 $\pm$ 8
Pristine+moderate methane	14.6 $\pm$ 0.6 c	2.7 $\pm$ 0.2 d	75 $\pm$ 6
Impaired+ moderate methane	12.7 $\pm$ 0.4 b	1.9 $\pm$ 0.1 b	82 $\pm$ 5
Pristine+sulfate	15.8 $\pm$ 0.5 d	3.6 $\pm$ 0.3 e	Trace
Impaired+sulfate	14.7 $\pm$ 0.3 c	3.0 $\pm$ 0.2 de	Trace
Pristine+sulfate+methane	14.8 $\pm$ 0.4 c	2.8 $\pm$ 0.2 d	72 $\pm$ 4
Impaired+sulfate+methane	14.4 $\pm$ 0.4 c	2.6 $\pm$ 0.2 d	77 $\pm$ 5
Pristine, growing in open container (control)	14.9 $\pm$ 0.6 cd	3.1 $\pm$ 0.3 e	5 $\pm$ 3
Impaired, growing in open container (control)	13.1 $\pm$ 0.4 b	2.2 $\pm$ 0.2 bc	58 $\pm$ 6

Numbers denoted by a different letter in each column separately differ significantly at  $P \leq 0.05$  by ANOVA and Tukey's post-hoc analysis.

*Pristine* Soil from Laguna Balandra station # 1; *Impaired* soil from the Ensenada de La Paz, site 4; high methane concentration applied=150  $\mu\text{M}$ ; moderate methane concentration applied = 80  $\mu\text{M}$ ; sulfate= $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ; 1.5 mM;  $\pm$  standard error

<sup>a</sup> Calculated only from propagules growing into seedlings

atmospheric methane on a global scale (Bartlett et al. 1985; Giani et al. 1996a). This view is also supported by this study. For example, low methane flux was found in a Chinese mangrove (Lu et al. 1999), and methane was not detected in a Thailand mangrove forest (Alongi et al. 2001) or in a Vietnamese mangrove plantation (Alongi et al. 2000). This can be explained because methanogenesis is limited by the availability of appropriate substrates and low redox potentials, <100 mV (Heyer 1990). Low redox potentials, per se, do not automatically indicate increased methanogenesis, as obvious in the case of topsoil at Balandra 2.1. The limited substrate is the most important reason for the minor contribution of marine coastal environments to the global methane budget (Giani et al. 1996b). Sulfate reducers and methane producers compete for the same substrates, but the former have a competition advantage (Heyer 1990). At sulfate concentrations >1 mM (King 1984) or >0.5 mM (Watson and Nedwell 1998), methanogenesis is inhibited. High sulfate concentrations and high  $\text{SO}_4/\text{Cl}$  ratios in Balandra soils and high sulfate contents in soils of other mangroves (Alongi et al. 2000; Purvaja and Ramesh 2000, 2001) indicate restricted methanogenesis in pristine mangrove soils, in principle.

Despite these findings, reasonable methanogenesis was reported in a pristine mangrove in India (Purvaja and Ramesh 2000, 2001; Mukhopadhyay et al. 2001, 2002), indicating that intact mangroves may become a methane source. Soil turnover generated by a crab (*U. vocator*), often found in subtropical mangrove soils (Giani et al. 1996b), significantly enhances soil aeration (Lee 1998), which enhances the oxidation potential for methane.

Methane production in anaerobic, coastal soils is generally negligible (Giani et al. 1996a) because sulfate-reducing bacteria and methane-producing bacteria compete for the same substrates ( $\text{H}_2/\text{CO}_2$ , acetate-competitive substrates), but as a consequence of a stronger affinity to the substrates, sulfate reducers are better competitors. However, this negligible amount of methane dynamics will increase if there is abundant competitive substrates (Holmer and Kristensen 1994) or after sulfate-depletion (Tyler 1991). These conditions are common in areas disturbed by human activities. For example, organic-rich sediments from a salmon farm, amended with fish pellets, showed high methane production resulting from abundant competitive substrates (Holmer and Kristensen 1994). This probably accounts for the intense methane production in the soils at the shrimp aquaculture facility. Production of methane was greatest at the site affected by sewage.

Methane is not specifically a toxic substance for plants. The most common plant that grows under constant methane emission is rice in flooded fields (Aselmann and Crutzen 1989). Rice transports a large quantity of methane through the plant system without any negative effect on growth in the field (Cicerone and Shetter 1981). However, under closed chamber conditions, similar to those tested in this study, which is a common procedure for testing effects of gasses on plant growth, higher concentrations of methane significantly reduced the yield of rice plants (Yu et al. 2006). Finally, young rice seedlings are usually grown in nurseries, where methane is not emitted from the soils. Only later, as the young plants are transplanted to the paddies that emit methane. Although mangrove ecosystems

have a potential to produce and emit methane, undisturbed tropical and arid subtropical mangrove soils usually do not emit methane (Tyler 1991), as also confirmed in this study. Yet, mature, arid subtropical mangrove trees survive for years in impaired areas that produce methane (this study). Similarly, mature, wet tropical mangroves (Purvaja and Ramesh 2000; Verma et al. 1999; Mukhopadhyay et al. 2001) survive, and a negative effect on tree growth, if there is any, has not been reported so far. In contrast to transplanted rice, mangrove seedlings in arid subtropical areas are established from floating propagules that settle on mudflats that, if disturbed and producing methane, negatively impact very early plant growth. Our results show that more than half of the propagules did not continue development into seedlings, and those that did develop had slower growth. It is unknown if this inhibition of growth is permanent or transient or what physiological mechanism(s) are involved. A plausible explanation for the positive effect of sulfate on growth of seedlings is that adding sulfate reduces methane production in the soil, not necessarily reducing methane emission to the atmosphere, as demonstrated in this study; only traces of methane were found in the soil amended with sulfate. Because the very early development of propagules is in the soil, they perhaps benefited from the reduction of methane in their microenvironment. On a regional comparison, black mangrove communities in arid subtropical regions are far smaller in height and general development than the same species growing in wet tropical areas, such as the southern USA and Hainan Island off southern China (Spalding et al. 1997; Baowen L, Research Institute of Tropical Forestry, China, personal communication).

In summary, this study showed that pristine or undisturbed arid subtropical mangrove soils release only minor amounts of methane, similar to wet tropical mangroves, but they are very close to turning into a methane source. In many cases, human disturbances of mangrove soils, predominantly caused by enrichment of organic matter, may lead to increased methane production. Increased methane production in these soils has a negative impact on the initial development of propagules.

**Acknowledgements** We thank Alejandro Amador and Monika Noormann for helping with field sampling and Ariel Cruz and Gunda Sangerlaub for technical assistance. This study was partially supported by the Bashan Foundation.

## References

- Alongi DM, Tirendi F, Trott LA, Xuan TT (2000) Benthic decomposition rates and pathways in plantations of the mangrove *Thizophora apiculata* in the Mekong delta, Vietnam. *Mar Ecol Prog Ser* 194:87–101
- Alongi DM, Wattayakorn G, Pfitzner J, Tirendi F, Zagorskis I, Brunskill GJ, Davidson A, Clough BF (2001) Organic carbon accumulation and metabolic pathways in sediments of mangrove forests in southern Thailand. *Mar Geol* 179:85–103
- APHA, AWWA, WPCF (1992) Standard methods for the examination of water and wastewater, 18th edn. American Public Health Association, American Waterworks Association, Water Pollution Control Federation, Washington, DC
- Aselmann I, Crutzen PJ (1989) Global distribution of natural freshwater wetlands and rice paddies, their net primary productivity, seasonality and possible methane emissions. *J Atmos Chem* 8:307–358
- Bartlett KB, Harriss RC, Sebacher DI (1985) Methane flux from coastal salt marshes. *J Geophys Res* 90:5710–5720
- Bashan Y, de-Bashan LE (2005) Fresh-weight measurements of roots provide inaccurate estimates of the effects of plant growth-promoting bacteria on root growth: a critical examination. *Soil Biol Biochem* 37:1795–1804
- Brandstetter A, Sletten RS, Mentler A, Wenzel WW (1996) Estimating dissolved organic carbon in natural waters by UV absorbance (254 nm). *J Plant Nutr Soil Sci* 159:605–607 (in German)
- Chen Z, Wang R, Miao Z (2000) Introduction of *Sonneratia* species in Guangdong province, China. In: Asia-pacific cooperation on research for conservation of mangroves. Proceedings of an international workshop 26–30 March 2000, Okinawa, Japan. The United Nations University Publ. Tokyo, Japan, pp 257–263
- Cicerone RJ, Shetter JD (1981) Sources of atmospheric methane—measurements in rice paddies and a discussion. *J Geophys Res* 86:7203–7209
- Cintron G, Lugo AE, Pool DJ, Moris G (1978) Mangroves of arid environments in Puerto Rico and adjacent islands. *Biotropica* 10:110–121
- Clarke LD, Hannon NJ (1970) The mangrove swamp and salt marsh communities of the Sydney District: III. Plant growth in relation to salinity and water logging. *Ecol* 58:351–369
- Dewes T, Schmitt L (1990) Nitrat-bestimmung in mist-sickersaft mittels UV-absorption. *Z Pflanzenemahr Bodenkd* 153:365–367
- Downton WJS (1982) Growth and osmotic relations of the mangrove *Avicennia marina*, as influenced by salinity. *Aust J Plant Physiol* 9:519–528
- FAO/ISSS/ISRIC (1998) World reference base for soil resources. World soil resources reports no. 84. FAO, Rome, pp 1–101
- Giani L, Dittrich K, Martsfeld-Hartmann A, Peters G (1996a) Methanogenesis in salt marsh soils of the North Sea coast of Germany. *Eur J Soil Sci* 47:175–182
- Giani L, Bashan Y, Holguin G, Strangmann A (1996b) Characteristics and methanogenesis of the Balandra lagoon mangrove soils, Baja California Sur, Mexico. *Geoderma* 72:149–160
- Gonzalez-Acosta B, Bashan Y, Hernandez-Saavedra NY, Ascencio F, De la Cruz-Agüero G (2006) Seasonal seawater temperature as the major determinant for populations of culturable bacteria in the sediments of an intact mangrove in an arid region. *FEMS Microbiol Ecol* 55:311–321
- Harris RC, Sebacher DI, Bartlett KB, Bartlett DS, Crill PM (1988) Sources of atmospheric methane in the South Florida environment. *Global Biogeochem Cy* 2:231–243
- Heyer J (1990) Der Kreislauf des Methans. Akademie-Verlag, Berlin (in German)
- Holguin G, Guzman MA, Bashan Y (1992) Two new nitrogen-fixing bacteria from the rhizosphere of mangrove trees: their isolation, identification and in vitro interaction with rhizosphere *Staphylococcus* sp. *FEMS Microbiol Ecol* 101:207–216
- Holguin G, Vazquez P, Bashan Y (2001) The role of sediment microorganisms in the productivity, conservation, and rehabilitation of mangrove ecosystems: an overview. *Biol Fertil Soils* 33:265–278



- Holguin G, Gonzalez-Zamorano P, de-Bashan LE, Mendoza R, Amador E, Bashan Y (2006) Mangrove health in an arid environment encroached by urban development – a case study. *Sci Total Environ* 363:260–274
- Holmer M, Kristensen E (1994) Coexistence of sulfate reduction and methane production in an organic-rich sediment. *Mar Ecol Prog Ser* 107:177–184
- Hütsch BW (1998) Sources and sinks of methane in German agroecosystems in context of the global methane budget. . *Agrobiological Research–Zeitschrift für Agrarbiologie, Agrikulturchemie, Ökologie* 51:75–87
- Ida A (2000) Sustainable mangrove management in Indonesia. In: Asia-pacific cooperation on research for conservation of mangroves. Proceedings of an international workshop 26–30 March 2000, Okinawa, Japan. The United Nations University Publ. Tokyo, Japan, pp 233–239
- Jennerjahn TC, Ittekkot V (2002) Relevance of mangroves for the production and deposition of organic matter along tropical continental margins. *Naturwissenschaften* 89:23–30
- Kakuma S (2000) Co-management of coastal fisheries resources in tropic and sub-tropic regions. In: Asia-pacific cooperation on research for conservation of mangroves. Proceedings of an international workshop 26–30 March 2000, Okinawa, Japan. The United Nations University Publ. Tokyo, Japan, pp 171–182
- King GM (1984) Utilization of hydrogen, acetate and “non-competitive” substrates by methanogenic bacteria in marine sediments. *Geomicrobiol J* 3:275–306
- Lee SY (1998) Ecology role of grapsid crabs in mangrove ecosystems—a review. *Mar Freshwater Res* 49:335–343
- Lee SY (1999) Tropical mangrove ecology: physical and biotic factors influencing ecosystem structure and function. *Aust J Ecol* 24:355–366
- Lord CJ III, Church TM (1983) The geochemistry of salt marshes: sedimentary ion diffusion, sulfate reduction and pyritization. *Geochim Cosmochim Acta* 47:11–18
- Lu CY, Wong YS, Tam NFY, Ye Y, Lin P (1999) Methane flux and production from sediments of a mangrove wetland on Hainan Island, China. *Mangroves Salt Marshes* 3:41–49
- McMillan C (1971) Environmental factors affecting seedling establishment of the black mangrove on the central Texas coast. *Ecology* 52:927–930
- Moreno-Casasola P (2000) Mangroves, an area of conflict between cattle ranchers and fishermen. In: Asia-pacific cooperation on research for conservation of mangroves. Proceedings of an international workshop 26–30 March 2000, Okinawa, Japan. The United Nations University Publ. Tokyo, Japan, pp 153–167
- Mukhopadhyay SK, Biswas H, Das KL, De TK, Jana TK (2001) Diurnal variation of carbon dioxide and methane exchange above Sundarbans mangrove forest, in NW coast of India. *Indian J Mar Sci* 30:70–74
- Mukhopadhyay SK, Biswas H, De TK, Sen BK, Sen S, Jana TK (2002) Impact of Sundarban mangrove biosphere on the carbon dioxide and methane mixing ratios at the NE coast of Bay of Bengal, India. *Atmos Environ* 36:629–638
- Naidoo G (1987) Effects of salinity and nitrogen on growth and water relations in the mangrove, *Avicennia marina* (Forsk.) Vierh. *New Phytol* 107:317–325
- Parida AK, Das AB, Mitra B (2004) Effects of salt on growth, ion accumulation, photosynthesis and leaf anatomy of the mangrove, *Bruguiera parviflora*. *Trees Struct Funct* 18:167–174
- Patterson CS, Mendelssohn IA, Swenson EM (1993) Growth and survival of *Avicennia germinans* seedlings in a mangal/salt marsh community in Louisiana, U.S.A. *J Coast Res* 9:801–810
- Perdrin-Aviles S, Padilla-Arredondo G, Diaz-Rivera E, Sirkin L, Stuckenrath R (1990) Estadigrafía del pleistoceno superior-holoceno en el área de la laguna costera de Balandra, estado de Baja California Sur. *Revista de la Universidad Nacional Autónoma de México, Instituto de Geología* 9:170–176
- Purvaja R, Ramesh R (2000) Human impacts on methane emission from mangrove ecosystems in India. *Regional Environmental Change* 1:86–97
- Purvaja R, Ramesh R (2001) Natural and anthropogenic methane emission from coastal wetlands of South India. *Environ Manag* 27:547–557
- Sotomayor D, Corredor JE, Morell JM (1994) Methane flux from mangrove sediments along the southwestern coast of Puerto Rico. *Estuaries* 17:140–147
- Spalding MD, Blasco F, Field CD (eds)(1997) World mangrove atlas. The International Society for Mangrove Ecosystems, Okinawa, Japan. 178 pp
- Toledo G, Bashan Y, Soeldner A (1995a) Cyanobacteria and black mangroves in Northwestern Mexico: colonization, and diurnal and seasonal nitrogen fixation on aerial roots. *Can J Microbiol* 41:999–1011
- Toledo G, Bashan Y, Soeldner A (1995b) In vitro colonization and increase in nitrogen fixation of seedling roots of black mangrove inoculated by a filamentous cyanobacteria. *Can J Microbiol* 41:1012–1020
- Toledo G, Rojas A, Bashan Y (2001) Monitoring of black mangrove restoration with nursery-reared seedlings on an arid coastal lagoon. *Hydrobiología* 444:101–109
- Tyler SC (1991) The global methane budget. In: Rogers JE, Whitman WB (eds) Microbial production and consumption of greenhouse gases: methane, nitrogen oxides, and halomethanes. American Society for Microbiology, Washington DC, pp 7–38
- Verma A, Subramanian V, Ramesh R (1999) Day-time variation in methane emission from two tropical urban wetlands in Chennai, Tamil Nadu, India. *Curr Sci* 76:1020–1021
- Warne K, Laman T (2007) Forests of the tide. *National Geog* 211 (2):132–151
- Watson A, Nedwell DB (1998) Methane production and emission from peat—the influence of anions (sulfate, nitrate) from acid-rain. *Atmos Environ* 32:3239–3245
- Yu KW, Chen GX, Xu H (2006) Rice yield reduction by chamber enclosure: a possible effect on enhancing methane production. *Biol Fertil Soils* 43:257–261