

## **Manganese cycling and its implication on methane related processes in the Andaman continental slope sediments**

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### **ABSTRACT**

In the deep subsurface sediments of the Andaman continental slope, in situ methane generation/oxidation could be coupled to the cycling of Mn, as the fluid flow characterized by high methane and Mn could occur in accretionary wedge sediments by diagenetic and tectonic processes. Laboratory studies on Mn cycling by subsurface sediment microbial communities were therefore undertaken 1) to study, further, possible in situ mechanisms of Mn cycling and 2) to examine how Mn redox reactions might be coupled to methane generation/oxidation. Biotic experiments were conducted with uniformly mixed subsamples amended with 100  $\mu\text{M}$   $\text{Mn}^{2+}$  in the presence ( $G^+$ ) and absence ( $G^-$ ) of added glucose (55.5  $\mu\text{M}$ ). The corresponding abiotic controls included set-ups poisoned with 15 mM sodium azide. Further, to relate the results of in vitro experiments on Mn cycling, to the methane related processes occurring in the subsurface sediments, pore water concentration of Mn, total cell numbers and the abundance of methanogens, methanotrophs and fermenters were determined. Results of the experiment on Mn cycling showed the immobilization of Mn occurred under oxic conditions and mobilization under suboxic conditions in the absence of added glucose at  $P \leq 0.001$  with abiotic > biotic. Whereas, in the presence of added glucose, immobilization occurred under both oxic and suboxic conditions at  $P \leq 0.001$  with biotic > abiotic, oxic > suboxic. The biotic cycling of Mn at 360 mbsf coincided with the total cell numbers ( $1.53 \times 10^8$  cells  $\text{g}^{-1}$ ), increased methane levels (89100 ppm-v) and the abundance of methanogens ( $1.0 \times 10^3$  MPN  $\text{g}^{-1}$ ). Besides, the distribution of aerobic methanotrophs decreased in abundance with depth. Also, the abundance of fermenters ( $3.5 \times 10^3$  cells  $\text{g}^{-1}$ ) at 626.7mbsf coincided with the relatively high concentration of Mn (319.1  $\mu\text{M}$ ) in sediment pore water. The results indicate that subsurface sediments harbor microorganisms that partake significantly in the cycling of Mn wherein, the availability of organic carbon dictates the direction in which the reactions occur. Besides, aerobic oxidation of methane and Mn has been reported to occur under reducing conditions. The present findings suggest that Mn redox changes affect the methane oxidation/production rates by serving either as an electron donor and/or an electron acceptor.

**Key words:** Subsurface; Sunda; Accretionary wedge; Gas Hydrate; Methanogens; Methanotrophs

## 1. Introduction

Continental margins are important for intense biogeochemical processes as they are regions for high biological production and sedimentation. During the degradation of buried organic matter in sediments, the microorganisms inhabiting the sub-surface derive energy by transferring electrons to a wide variety of electron acceptors such as nitrate, nitrite, Mn(IV), Fe(III), sulfate and CO<sub>2</sub> (DeLong, 2004). However, the preference for potential terminal electron acceptors for anaerobic respiration depends mainly on their availability, energy yield and the accessibility of a suitable carbon substrate. In the continental margins, the availability of carbon required for methanogenesis is met from the high burial of organic matter which in general consists of fine-grained terrigenous matter, biogenic opal and particulate organic matter (Rixen et al., 2005). During the organic carbon degradation great amounts of methane are formed in situ from acetate fermentation and CO<sub>2</sub> reduction. Besides, methane may also form in subsurface sediments from migration from a shorter distance, recycling during hydrate formation and dissociation following sedimentation (Kvenvolden and Lorenson, 2001).

The methane that formed from organic carbon degradation in the sub-surface, depending on its concentration, solubility in pore water and relatively high pressure and low temperature may lead to its occurrence in a dissolved state, stable form as hydrate, or a free gas (Riedel et al., 2006). Under ideal conditions, the hydrates may form typically at depths >1000m in the gas hydrate stability zone (GHSZ) (Kvenvolden and Lorenson, 2001). The sediments of the present study constrained to depths >1000m (134m and 638m) within the GHSZ is characterized by nanofossil ooze, presence of ash and methane hydrates (Collett et al., 2008) and, hence is idealistic for the understanding of sub-surface microbial processes associated with the hydrates. The global estimates show methane carbon in the hydrates to range from 0.5 to 24 teratonnes with methane representing more than 99% of the gas mixture present in the hydrate (Kvenvolden and Lorenson, 2001). The emission of methane from such sediments has increasing concerns due to its significant effect on the global climate and the carbon cycle (Jiang et al., 2013). On the other hand, the oxidation of methane by aerobic methanotrophs and anaerobic oxidation of methane (AOM) by anaerobic methanotrophic archaea (ANME) (Hanson and Hanson, 1996), controls the escape of methane to the atmosphere. Besides, AOM mediated by ANME counterbalances the net production to the oxidation of methane (Joye, 2012; Haroon et al., 2013) via “the back reaction” catalyzed by the key enzyme of methanogenesis, the methyl coenzyme M reductase (Scheller et al., 2010).

As several trace metals are important in enzymatic pathways of methanogenesis (Zerle *et al.*, 2005), the production of methane in the subsurface could be affected by the presence/absence of metals. Of the different elements, iron, nickel, cobalt, zinc, copper, molybdenum and tungsten are considered to be crucial in methanogenesis (Unal *et al.*, 2012). Nonetheless, small amounts of Mn are also important in methanogens for the stability of the enzyme methyltransferase (Scherer *et al.*, 1983; Takashima *et al.*, 1990; Glass and Orphan, 2012). Besides, Mn is a part of four metalloenzymes, manganese superoxide dismutase, mangani-catalase, arginase and O-phosphatases (Christianson, 1997; Shi, 2004). Other than functioning as a stabilizing agent and cofactor of enzymes, Mn is also required for a large number of cellular functions that includes protection from toxic metals, predation or viruses, breakdown of natural organic matter into metabolizable substrates, maintenance of an electron-acceptor reservoir for use in anaerobic respiration, oxygen production and protection against oxidative stress (Christianson, 1997; Crowley *et al.*, 2000; Spiro *et al.*, 2010). It also contributes to the stabilization of bacterial cell walls (Doyle, 1989) and plays an important role in bacterial signal transduction (Jakubovics and Jenkinson, 2001).

The oxides of Mn act as storage of an electron acceptor in sediment systems until carbon and energy become available. Through scavenging reactions, it controls the distribution and fate of trace elements that are important in different biogeochemical reactions (Tebo *et al.*, 2005). The impact of Mn on redox and cation-exchange reactions affects the chemistries of sediments and associated fluids. Also, its complexation with iron in sediments controls the availability of nutrients (Kirchner and Grabowski, 1972) and at times provides a means to derive energy from refractory organic matter through oxidative degradation of humic substances (Post 1999; Tebo *et al.*, 2005; Sujith and Loka Bharathi, 2011). The close coupling of AOM with Fe oxide, Mn oxide, sulfate, nitrate and nitrite reduction counterbalances the net production to the oxidation of methane (Beal *et al.*, 2009; Joye, 2012). Thus the cycling of Mn has considerable impact on the biogeochemical cycling of carbon in the subsurface sediments. However, we are unaware of the importance of Mn in the cycling of methane associated with the subsurface gas hydrates. In the Andaman continental slope sediments, in situ methane generation/oxidation could be coupled to the cycling of Mn as the fluid flow characterized by high methane and Mn could occur in accretionary wedge sediments, by diagenetic and tectonic processes. This Mn might be distributed in different patterns among the various phases of the metal such as water soluble, exchangeable, reducible and residual/crystalline depending on the pH and Eh (Reddy and DeLaune, 2008). Therefore, to cover the various phases of Mn formed through different mechanisms of activity, the two broader terms namely immobilization and mobilization has been used. Immobilization represents the conversion of ions present in the soluble

to the insoluble or bound state by mechanisms such as biosorption, bioaccumulation, redox reaction and complex formation. Whereas, mobilization represents the conversion of ions present in the insoluble state to the soluble state by redox, acidic and complexation reactions (Brandl and Faramarzi, 2006).

In the subsurface sediments of the Andaman continental slope in situ methane generation/oxidation could be coupled to the cycling of Mn. To further understand the implications of Mn cycling on methane related processes, laboratory studies were conducted with the following objectives 1) to study, further, possible in situ mechanisms of Mn cycling and 2) to examine how Mn redox reactions might be coupled to methane generation/oxidation.

## **2. Methods**

### *2.1. Study area and Sample collection*

The Andaman Basin has an area of 800000 km<sup>2</sup> separated from the Bay of Bengal by the Andaman-Nicobar Ridge (Rodolfo, 1969). The water depth of the site is ~1344 m and the sediments have <1% by weight total organic carbon (Ramaswamy et al., 2008; Johnson et al., 2009). The study site NGHP-01-17A is located at 10°45.1912'N (lat), 93° 6.7365'E (long) in the eastern coast of the Andaman Islands. Pressure cores were collected from this location during the Leg 4 cruise of Expedition 01 (2006). The sediments of the core are characterized by nanofossil ooze, presence of ash and methane hydrates (Collett et al., 2008). The cores identified for microbiological sampling were cored with drilling fluid spiked with contamination tracers according to ODP/IODP standard core handling procedures (Smith et al., 2000; Riedel et al., 2006). On board, the appropriate sections were taken on the catwalk and then transferred immediately to the microbiology laboratory on the forecastle deck. The core was sub-sectioned onboard for 25 depths between 134 to 637.5 mbsf (meters below sea floor) and was transported to the laboratory at 4 °C. The samples were then used for the microbiological and geochemical studies of subsurface gas hydrate sediments. It was also used as the natural medium for conducting laboratory studies on Mn cycling.

The site NGHP-01-17A was chosen for this study mainly because 1) it is located at the accretionary wedge of the Sunda subduction zone on the Andaman continental slope where fluid flow characterized by high methane and Mn could occur by diagenetic and tectonic processes and 2) to explore the biogeochemical reactions of Mn associated with subsurface gas hydrates. Besides, the deeper depths were chosen as they fall in the GHSZ and these samples were available for the study.

## *2.2. Extraction of pore water by centrifugation and analysis of geochemical parameters*

The analysis of geochemical parameters like methane and Mn in pore water was studied as it throws light on the subsurface processes with reference to organic carbon mineralization. For the extraction of pore water, all containers and tubes were cleaned carefully with acid, rinsed at least three times with double distilled water and dried before use. Sediments in its native form were purged with nitrogen while introducing into the centrifuge tubes which were capped tightly. The centrifugation of the samples was carried out at 268 g for 15 min at 4°C (Sigma 3-18K). The supernatant was collected and passed through a 0.22 µm cellulose acetate filter. The determination of Mn in the filtrate was made by adsorptive stripping voltammetry in interface with 797VA computrace in the differential pulse mode (Metrohm, Switzerland) according to [Colombini and Fuoco \(1983\)](#). Mn was quantified with two standard additions of appropriately diluted 1000-ppm concentrations of the metal standards prepared in seawater. Metal standards (1000 ppm) obtained from Merck-GMBH, were used for determining the analytical accuracy. Methane concentrations are taken from NGHP-01-17A, Expedition 01 cruise report ([Collett et al., 2007](#)).

## *2.3. Total direct counts*

To understand the contribution of microbes to the geochemical cycling and to complement the geochemical data, the abundance of microorganisms was determined in the subsurface sediments. The sediment dilution for total direct counts (TDC) was prepared by suspending ~1 g of uniformly mixed subsample in 9 ml of autoclaved and 0.22µm filtered seawater. After thorough mixing of the dilution, 2ml of the sediment slurry was withdrawn and fixed with hexamine buffered formalin to a final concentration of 2%. TDC was determined by the epifluorescent technique according to [Kogure et al. \(1979\)](#).

## *2.4. Enumeration of microorganisms associated with the subsurface sediments*

Cultivation and isolation remain the only means of identifying organisms that are physiologically adapted to the low-energy conditions prevailing in the subsurface. The enumeration of aerobic methanotrophs, methanogens and fermenters were thus undertaken which further helps relate the results of in vitro experiments on Mn cycling to the methane related processes.

#### 2.4.1. *Fermenting bacteria*

The composition of media and the substrates used for enumeration were as described by [Pfenning et al., \(1981\)](#). Colonies that formed in anaerobic agar shake tubes of SRB medium and which failed to reduce sulphate were counted as fermenters ([Gonsalves et al., 2011](#)).

#### 2.4.2. *Methanotrophs*

The medium used for isolation of methanotrophs was the nitrate-mineral-salts (NMS) medium of [Whittenbury et al. \(1970\)](#) which was solidified by the addition of 1.5% (w/v) agar. The medium was prepared in distilled water, autoclaved at 121°C for 20 min and then dispensed into plates. An appropriate dilution of sediments was prepared by suspending ~ 1g of uniformly mixed subsample in 9ml of sterile seawater. A 0.1ml aliquot of the same was spread on NMS agar plates. The plates were introduced into anaero bags, sparged to give about air: methane in the ratio of 50:50 and clamped to avoid gas leakage. Media plates without methane injection served as controls. The plates were incubated at 28±2 °C and observed at 3-day to 1-week intervals over 3–4 weeks. The colonies formed were then enumerated and expressed as colony forming units (CFU) per gram of sediment.

#### 2.4.3. *Methanogens*

The population density of methanogens was estimated by the three-tube most probable number (MPN) method. The composition of the medium and the anaerobic technique followed for their enumeration was according to [Macy et al. \(1972\)](#) and [Parkes et al. \(2010\)](#). The basal medium was prepared in distilled water that was boiled and degassed with N<sub>2</sub>. The medium was sterilized by autoclaving and on cooling 30ml of 1M NaHCO<sub>3</sub>, 1.2ml of 1M Na<sub>2</sub>S, 0.5ml of 1M FeCl<sub>2</sub> and 2ml of vitamin solution as sterile stock solutions were added. This was followed by addition of sodium acetate as substrate for methanogens and allylthiourea an inhibitor of methane oxidation, to the basal medium in final concentrations of 16.58 mM and 0.1mM respectively. The medium was purged with N<sub>2</sub>/CO<sub>2</sub> (80/20 v/v) and dispensed into 20 mL GC vials with each containing 9mL of the medium leaving sufficient head space for gas sampling. The medium in each vial was inoculated with 0.1mL of inoculum from a 10-fold serial dilution of sediments from 10<sup>-1</sup> to 10<sup>-3</sup>. They were sealed with butyl-rubber stoppers and aluminium crimp-caps. To make the conditions completely anaerobic, the vials were flushed with 5 mL of N<sub>2</sub> and 2 mL of CO<sub>2</sub> which were incubated at 28±2 °C for 60 d. Methanogenic growths was accessed by detecting methane in the head space using gas chromatograph. Populations were expressed as MPN per gram dry weight of the sediment.

## 2.5. In-vitro experiment on the cycling of manganese

It is very much unclear which type of metabolism is of key importance in methane generation/oxidation. Thus it is mandatory, to study, further possible in situ mechanisms of microbial Mn cycling and examine how Mn redox reactions might be coupled to methane generation/oxidation. In this regard a laboratory experiment was conducted with methane bearing subsurface sediments following the procedure described by [Sujith et al. \(2011\)](#). The various sterile media used for the experimental incubations were prepared in filtered and autoclaved sea water ([Table 1](#)). The experiment was conducted in 15ml screw capped tubes containing ~0.1 g of uniformly mixed subsample and 10ml of medium for oxic incubation and 15ml of medium for suboxic incubation. Each media received 100  $\mu\text{M}$   $\text{Mn}^{2+}$  as the metal of interest and 55.5 $\mu\text{M}$  glucose as a model compound for organic carbon. Media amended with 15mM sodium azide were used as abiotic controls and uninoculated media as sterile controls. The experimental setup was incubated in triplicate at  $4\pm 1$  °C in dark for 1, 20, 32, 39, 47 and 50 d. After every time interval, tubes from experiment and the respective controls were used for the determination of Mn ([Chin et al., 1992](#)), pH and Eh as described below.

### 2.5.1. Calculation of change in metal concentration

The initial measured values for Mn in solution were used as a reference to monitor the changes in metal concentrations through the incubation period. Any decrease in the level of dissolved Mn that occurred during the experiment was assumed to be caused by its immobilization and any increase in concentration to be caused by its mobilization. The concentration of Mn was determined from the curve of best fit derived from different concentrations of Mn ( $\mu\text{M}$ ) run in triplicates. The gram dry weight concentration of Mn was calculated after correcting for the respective blanks as follows:-

$$C = v(C_i - C_e)/w$$

Where:

C = concentration of Mn immobilized/mobilized,  $\text{mg g}^{-1}$

v = volume of media used, ml

$C_i$  = initial concentration of Mn in solution,  $\text{mg l}^{-1}$

$C_e$  = equilibrium/final concentration of Mn in solution,  $\text{mg l}^{-1}$

w = dry weight of sediment, mg

### 2.5.2. Eh and pH measurements

The increase in H<sup>+</sup> activity (drop in pH) leads to Eh decrease and consequently to the increased mobilization of metallic elements and vice versa when there is a decrease in H<sup>+</sup>, there is an increase in Eh (Pareuil et al., 2008). Also the growth and metabolic activity of bacteria either increases or decreases the pH and consequently the Eh of the surrounding making it vital to study the variation in pH and Eh. The pH and Eh (mV) measurements were made after calibration of the pH meter using standard reference solutions as per the protocol given in user's guide of Thermo Electron Corporation (2005). The readings for Eh and pH in experimental tubes were recorded as described by Sujith et al. (2011; 2014).

### 2.6. Statistical analysis

Two-way ANOVA and Students Newman-Keuls post hoc (SNK test) analysis were performed to find out the significant differences in the in vitro cycling of Mn between depths, incubation time and biotic and abiotic sets of incubations. The analysis was carried out using Statistica 6 software package.

## 3. Results

### 3.1. Manganese in pore water

The minimum concentration of Mn (33.97 μM) in pore water was recorded at 134 mbsf. The concentration of Mn increased at deeper depths with an initial peak of 172.65 μM at 380.5 mbsf. Beyond 498.4 mbsf depth, the Mn concentrations oscillated between high and low values. The maximum concentration of 428.99 μM Mn was at the deepest depth of 637.5 mbsf (Fig. 1).

### 3.2. Total cell abundance and culturability of fermenters, methanotrophs and methanogens

The total cell numbers in the subsurface sediments varied within the 25 depths, in the range of  $1.49 \times 10^7$  to  $1.53 \times 10^8$  cells g<sup>-1</sup> (Fig. 2). Their maximum abundance of  $1.53 \times 10^8$  cells g<sup>-1</sup> was at 360.5 mbsf. The abundance of fermenting bacteria varied from non detectable levels to  $3.5 \times 10^3$  CFU g<sup>-1</sup> with maximum counts at 626.7 mbsf. On the other hand, the methane oxidizing bacteria (methanotrophs) ranged from  $3.0 \times 10^0$  to  $4.82 \times 10^2$  CFU g<sup>-1</sup>. Their numbers were maximum at 134 mbsf and decreased gradually with depth (Fig. 2). The colonies were mostly circular, convex and opaque with smooth edges. The counts of methanogens varied from  $4.0 \times 10^1$  to  $1.0 \times 10^3$  MPN g<sup>-1</sup> of



dry sediment. Their numbers were mostly in the order of  $10^2$  MPN  $g^{-1}$  with a maximum count of  $1.0 \times 10^3$  MPN  $g^{-1}$  at 360.5 mbsf (Fig. 2).

### 3.3. *In-vitro* experimental results on manganese cycling

#### 3.3.1. *Oxic incubation with added glucose*

Significant variation by two way ANOVA ( $F=2574.1$ ;  $P \leq 0.001$ ) in Mn immobilization between the biotic and abiotic sets of incubation was observed under oxic condition with added glucose ( $G^+$ ) (Table 2). Here the immobilization of Mn was biotic  $>$  abiotic (Fig. 3a). Analyses along the core showed that Mn immobilization tend to vary depth wise from 1.07 to 4.66  $\mu M g^{-1}$  in the biotic media (Supplementary Fig. S1a). The immobilization of Mn at 360 mbsf was significantly low of 1.14  $\mu M g^{-1}$  on the 20<sup>th</sup> day of incubation. Such a trend was not apparent in the corresponding abiotic controls. There the immobilization of Mn was evident at most of the depths and mobilization of Mn ( $-0.31 \mu M g^{-1}$ ) at 360 mbsf (Supplementary Fig. S1b). The immobilization of Mn in the abiotic experiment which is used as the control was comparatively less than that of the biotic experiment, with maximum values of 1.65  $\mu M g^{-1}$  on the first day of incubation. In the biotic incubations, Eh showed a shift from lower redox potential of 181.9 mV to higher redox potential of 236.6 mV along the studied sections of the core. Interestingly, a well defined peak of 232.9 mV for Eh was also observed on the 32 d of incubation at 517.8 mbsf (Fig. 4a). Whereas, in the corresponding abiotic control the overall trend in Eh was the opposite of the biotic incubations. It shifted more towards the lower potential of 151.5 mV at 517.8 mbsf on the 32d of incubation (Fig. 4b). Variation in pH in the biotic and abiotic incubations was marginal varying between 8.22 to 8.51 in the biotic (Fig. 5a) and 8.19 to 8.55 in the abiotic controls (Fig. 5b) through the incubation period.

#### 3.3.2. *Oxic incubation without added glucose*

Unlike the former, oxic incubation without added glucose ( $G^-$ ) showed significant variation ( $F=785.2$ ;  $P \leq 0.001$ ) in Mn immobilization between the biotic and abiotic sets on the first day of incubation (Table 2). Here the immobilization of Mn was abiotic  $>$  biotic (Fig. 3b). The lower immobilization of Mn with the biotic in the absence of added glucose was not because of lesser bacterial activity but rather because of their switch over from immobilization to mobilization. Low Mn value of  $-0.75 \mu M g^{-1}$  at 360 mbsf and a high value of  $-2.8 \mu M g^{-1}$  at 343 mbsf was recorded on the first day of incubation. Overall, the mobilization of Mn varied between  $-0.01$  to  $-2.8 \mu M g^{-1}$  under biotic conditions (Supplementary Fig. S1c). The abiotic immobilization of Mn under similar conditions showed major variation in concentrations between depths on the initial day of incubation.

The immobilization of Mn in the abiotic was significant at two depths one at 343 mbsf and the other at 360 mbsf. Comparison of depths showed significantly low Mn value of  $0.09 \mu\text{M g}^{-1}$  at 360 mbsf and high value of  $1.23 \mu\text{M g}^{-1}$  at 343 mbsf on the first day of incubation. Overall, the immobilization of Mn varied between  $0.07$  to  $1.92 \mu\text{M g}^{-1}$  (Supplementary Fig. S1d) under abiotic conditions, with alternating peaks at different depths during the course of incubation. The variation of Eh in the  $G^-$  biotic experimental set up was marginal (Fig. 4c). Subtle difference in Eh was observed on the 20 d of incubation with a maximum of 185.90 mV at 517.8 mbsf. Such variations were not evident in the corresponding  $G^-$  abiotic control (Fig. 4d). On the other hand, the pH varied between 8.20 to 8.48 in the biotic (Fig. 5c) and 8.21 to 8.56 in the abiotic (Fig. 5d).

### 3.3.3. Suboxic incubation with added glucose

Under suboxic incubation with added glucose, the overall process was immobilization = mobilization with significant variation ( $F=48.26$ ;  $P \leq 0.001$ ) between the biotic and abiotic sets on the first day of incubation (Table 2). The overall process was biotic > abiotic (Fig. 3c). The immobilization of Mn was high at 441.3 mbsf ( $2.95 \mu\text{M g}^{-1}$ ) and mobilization at 531.1 mbsf ( $-2.98 \mu\text{M g}^{-1}$ ). The maximum values of immobilization and mobilization were recorded on the 20 d of incubation (Supplementary Fig. S2a). This trend was not observed in the corresponding abiotic controls (Supplementary Fig. S2b), where the immobilization-mobilization of Mn was lower than that of the biotic incubations and varied from  $1.37$  to  $-1.55 \mu\text{M g}^{-1}$ . Beyond the initial day of incubation, mobilization was prominent in the abiotic with minor difference in mobilization between the depths. Eh showed a shift from lower (177.6 mV) to higher redox potential of 235.5 mV in the biotic incubations. A well defined peak for Eh of 232.9 mV was observed on the 32 d of incubation at 475.9 mbsf (Fig. 4e). Whereas, in the corresponding abiotic control, the overall trend in Eh was the opposite of the biotic incubations. It shifted more towards the lower potential of 152.63 mV at 475.9 mbsf on the 32 d of incubation (Fig. 4f). The pH change in the biotic and abiotic incubations was marginal varying between 8.21 to 8.54 in the biotic (Fig. 5e) and 8.19 to 8.56 in the abiotic (Fig. 5f).

### 3.3.4. Suboxic incubation without added glucose

Under suboxic incubation without added glucose, the overall process was mobilization, with significant variation ( $F=110.3$ ;  $P \leq 0.001$ ) between the biotic and abiotic sets on the 20 d of incubation (Table 2). The mobilization of Mn was abiotic > biotic (Fig. 3d). Overall the concentration of Mn in the biotic experiment varied between  $0.93$  to  $-3.93 \mu\text{M g}^{-1}$  (Supplementary

Fig. S2c). On the other hand, the abiotic mobilization of Mn varied between 1.64 to  $-4.27 \mu\text{M g}^{-1}$ , with maximum immobilization on the 39 d and mobilization on the 20 d at 290.6 mbsf and 475.9 mbsf respectively. The mobilization of Mn in the abiotic was significant at two depths one at 134 mbsf and the other at 360 mbsf. Depth wise comparison showed significantly high Mn value of  $0.82 \mu\text{M g}^{-1}$  at 134 mbsf and low value of  $-2.10 \mu\text{M g}^{-1}$  at 360 mbsf (Supplementary Fig. S2d). In the biotic incubations, Eh showed a shift from lower (184.5 mV) to higher redox potential of 212.9 mV (Fig. 4g). While in the corresponding abiotic control subtle difference in Eh was observed with a minimum of 176 mV at 517.8 mbsf on the 20 d of incubation (Fig. 4h). The pH varied between 8.19 to 8.41 in the biotic (Fig. 5g) and 8.18 to 8.58 in the abiotic (Fig. 5h) incubations.

Results of the present study with subsurface sediments show microbial participation in Mn cycling. Under oxic and suboxic incubation conditions biotic immobilization of Mn dominated over abiotic in the presence of added glucose. This has been evinced by the significant variation between the biotic and abiotic incubations by two way ANOVA ( $P \leq 0.001$ ). In the absence of added glucose, immobilization was the dominant process with abiotic greater than biotic only under oxic conditions. Whereas, under suboxic conditions, the abiotic mobilization of Mn was significantly greater ( $P \leq 0.001$ ) than that of the biotic (Fig. 3). Nevertheless, the low biotic immobilization of Mn in the absence of added glucose could be because of the change in metabolism from immobilization to mobilization. This has been evidenced by the high level of soluble Mn in the biotic incubation without added glucose (Supplementary table S1). The results show that immobilization of Mn occurs under oxic conditions as opposed to its release under suboxic conditions with respect to the availability of organic carbon glucose (Fig. 3). Eh, a measure of the equilibrium potential, remained within the range of 151 to 236 mV (Fig. 4). Major variation in Eh was observed only in the presence of added glucose on the 32 d of incubation under both oxic and suboxic incubations. When Eh had a propensity to increase in the biotic it tends to decrease in the abiotic incubations. On the other hand, definite trend in pH was not apparent in the biotic or in the abiotic medium (Fig. 5) varying from 8.18 to 8.58 between depths.

#### 4. Discussion

The in situ production/oxidation of methane in the Andaman continental slope has been shown to be coupled to the cycling of Mn. This could be mainly because the region lies in the accretionary wedge of the Sunda subduction zone where there is intense recycling of sediments (Garzanti et al., 2013) and fluid flow characterized by high methane and Mn occurs by diagenetic and tectonic processes (Blanc et al., 1991). However, due to spatial heterogeneity of dynamic sediments

(Davison et al., 1997; Harper et al., 1999) and depending on the accessibility of inorganic electron acceptors like oxygen, nitrate, Mn(IV), Fe(III) and sulfate (Beal et al., 2009) the vertical separation is not very stringent and several processes may occur at discrete depths in different patches.

Under reducing conditions of the slope sediments fermenting bacteria promote methanogenesis and sulfate reduction through supply of substrates like acetate, butyrate, propionate, lactate, alcohols and H<sub>2</sub> (Fenchel et al., 2012). In the present study also, the presence of methanogens, methanotrophs and fermenters were encountered in high numbers and are in agreement with those reported by Blanc et al. (1991); Gonsalves et al. (2011) and Kallmeyer et al. (2012). The sediments also showed high levels of Mn in pore water. This probably suggests the participation of subsurface microorganisms in the geochemical cycling of diverse substrates and their active involvement in the release of Mn. Such a release of Mn<sup>2+</sup>, following the dissimilatory reduction of Mn oxides by fermenting bacteria has been reported by Lovley (1991). The resultant release of Mn could also be attributed to the slower rate of oxidation compared to that of reduction in anoxic sediments (Yadav, 1996).

Besides, the microbial mobilization of Mn from sediments at 360.5 mbsf, was also prominent under artificially altered conditions of the laboratory. The mobilization of Mn appropriately coincided with the total cell numbers, peak for CH<sub>4</sub> and maximum abundance of methanogens. Interestingly, this was the only depth that showed anomalies for calcium carbonate (Collett et al., 2007). These distinctive carbonates could be methane derived by subduction-induced dewatering, a common feature that occurs in the accretionary prism. Besides, the occurrence of common methanogenic communities, unique methanogens that subsist in these calcareous sediments could have attributed to the in-situ production of methane apart from those derived from advective fluxes from stratigraphic conduits or fractures in the shallower stratigraphy (Ritger et al., 1987). On the other hand, the solubilization of MnO<sub>2</sub> oxides could have resulted from the activity of bacteria that couples the oxidation of organic carbon to metal reduction. The present results agree with Thamdrup (2000) that when Mn or Fe reduction is a major process in the oxidation of organic carbon, respiring organisms must dominate fermenting bacteria in catalyzing the reduction of metal oxides. The fermentation of glucose to acetate under anaerobic conditions not only serves as a suitable substrate for methanogens and SRB, but also for acetate-utilizing manganese reducers (Vandieken et al. 2012). One such example could be the reduction of MnO<sub>2</sub> by acetate-utilizing manganese reducers. Here, the mobilization of Mn could be related to methanogenesis because the fermentation of glucose to acetate under anaerobic conditions could also support the growth of methanogens and acetate-utilizing manganese reducers. The acetate on oxidation by methanogens helps in the transfer of

electrons between the archaeal member and the SRB (Thauer and Shima, 2008). Besides, acetate is known to serve as a suitable substrate for methanogenesis accounting for ~70 % of the biogenic methane (Mah et al. 1977; Winter, 1980). Therefore, acetate utilization could be coupled not only to methane generation but also to Mn and sulfate reduction thus affecting the rate of methane production.

On the other hand, addition of glucose as a model compound for organic carbon stimulated the immobilization of Mn suggesting, that metabolic functions of subsurface microbial communities are regulated by organic carbon. The organic carbon required for Mn immobilization could be formed in the subsurface, by the hydrolysis reactions involving the production of extracellular enzymes. One among the different enzymes produced by bacteria is the Mn<sup>2+</sup> containing O-phosphatases that help bacteria in adapting to stress conditions, carbon and nitrogen assimilation, vegetative growth and cell segregation (Shi, 2004). Since, the remineralization of phosphorous (P) could also occur in tandem with the redox coupling of iron, which is also an important electron acceptor for anaerobic oxidation of methane (Beal et al., 2009), the cycling of Mn, Fe and P could be linked to the methane cycle in the subsurface sediments. Besides, the need for carbon by the microorganisms dwelling in the subsurface sediments may be met through methanogenesis as it contributes in the final reaction steps of the organic matter mineralization process (Megonigal et al., 2004). The present results agree with the study of Hallberg and Martinell, (1976) that more of Mn oxidation by bacteria occurs with respect to the availability of organic carbon that supports their growth and reproduction. The useful energy derived from Mn oxidation may be used by the microorganisms in the deep subsurface for organic carbon assimilation. Such an acceleration of Mn oxidation in the presence of organic carbon has been found to give the mixotrophs a selective advantage over other organisms that cannot oxidize Mn (Ehrlich, 1975). During a similar process, Mn may be utilized concurrently with glucose, or else upon the utilization of glucose. Such a related effect was also reported to occur with Fe oxidation in the presence of glucose and acetate (Ehrenreich and Widdel, 1994).

Further it was noticed that the immobilization of Mn was more under oxic than under suboxic condition in the presence of added glucose. This suggests that Mn oxidation reactions require molecular oxygen and so any reactions that produce oxygen might conceivably enhance the speed of Mn oxidation. The AOM mediated by denitrifying bacteria in the absence of an archaeal consortium leads to the reduction of NO<sub>2</sub><sup>-</sup> and the production of oxygen (Ettwig et al., 2010). The oxygen so produced from the reduction of nitrite could serve as an electron acceptor for the aerobic metabolism of methane (Smemo and Yavitt, 2011) as well as for the oxidation of reduced Mn under anaerobic

conditions. Thus, the reduced pool of Mn in the subsurface sediments could be in part derived from the reduction of Mn-oxides coupled to the oxidation of methane under anaerobic conditions (Beal et al., 2009). Besides, the production of methane leading to the formation of gas hydrate could be accelerated by Mn and at the same time the gas available to form methane hydrates could be decelerated by the aerobic and anaerobic oxidation of methane in the presence/absence of Mn.

Also, the oxidation of Mn(II) by Mn oxidizing bacteria creates conducive environment for methanogenesis by consuming oxygen and making the condition more anaerobic. The oxidation of Mn under suboxic conditions could be either because of the higher availability of soluble Mn under sub-oxic conditions which are prevalent in hemipelagic sediments on the shelf, slope and rise of continental margins (Froelich et al., 1979; Emerson et al., 1980) or else owing to the fewer requirements for oxygen in certain group of organisms which couples the reduction of nitrate to the oxidation of Mn (Tebo, 1991; Konovalov et al., 2004; Pyzola, 2013). It has been speculated that elevated CH<sub>4</sub> and ammonium in plumes stimulate Mn(II) oxidation rates (Ishibashi et al., 1997). The present results corroborate with the studies of Schippers et al. (2005) and Clement et al. (2009) which shows that microbial Mn(II) oxidation is oxygen-dependent at sub-micromolar levels in the sub-oxic zones of the Black Sea. Further, Tebo and Emerson (1985) stated that bacterial Mn oxidation under similar conditions could also be related to relatively high concentrations of Mn<sup>2+</sup>.

Immobilization of Mn also occurred in the abiotic controls by passive process. The passive immobilization of Mn in the presence of inhibitor sodium azide has been previously found to occur with deep-sea sediments (Sujith et al., 2011). The immobilization of Mn in sediments could also be followed by its release in the presence of suitable reducing agents like hydrogen sulphide, Fe<sup>2+</sup>, and humic substances which are common in the reducing slope and shelf sediments (Thamdrup, 2000). Moreover, bacterial Mn oxide reduction thought to occur mostly by enzymatic process may in some situations be triumph over by non-enzymatic reduction of MnO<sub>2</sub> in reducing environments. During CH<sub>4</sub> oxidation, sulphate is reduced to H<sub>2</sub>S by the sulphate reducing bacteria wherein the H<sub>2</sub>S, being a potent reductant of MnO<sub>2</sub> contribute to the solubilization of MnO<sub>2</sub> to Mn(II) (Burdige and Nealson, 1986). Indirectly, the microbial participation in such processes could also modify the microenvironments suitable for abiotic adsorption/dissolution reactions. This has been observed by Gachter and Meyer (1993) wherein microbial mobilization and fixation of phosphorous was accelerated by the transfer of electron from electron donors to electron acceptors, thus providing the necessary conditions for redox and pH dependant abiotic cycling of phosphorous in sediments. Therefore, Mn that undergoes oxidation under oxic conditions may get mobilized through microbial

respiration and abiotic reduction in the subsurface sediments under anoxic conditions. The reduced Mn that is been mobilized could be required by methanogens for the expression of methyl coenzyme M reductase gene, the key enzyme of methanogenesis. Such a requirement for Mn in enrichment cultures has been reported where Mn addition correlated with the increasing level of *mcrA* gene that code for methyl coenzyme M-reductase and elevated methane production (Unal et al., 2012). Also, Unal et al. (2012) reported a typical shift in the metabolically active methanogenic community from a *M. formicicum*-like group to a *M. subterraneum*-like group in the presence of Mn amendment.

The cycling of Mn could also occur by the oxidation of CH<sub>4</sub> by the methanotrophs during which MnO<sub>2</sub> could serve as the terminal electron acceptor. Such an oxidation of CH<sub>4</sub> coupled to Mn and Fe reduction has been reported (Beal et al., 2009). Compared to iron, Mn is faster to reduce, harder to oxidize and occurs at a higher level in the oxygen gradient. Because of these reasons Mn also undergoes complete reduction within suboxic regions. This was very well evident in the abiotic control where the adsorption of Mn was followed by a drop in Eh (150mV) and it occurred on the same time period when the increase in Eh occurred with biotic incubations. This could additionally be stimulated by AOM as it contributes to the slow reductive dissolution of buried Fe/Mn layers in the sediments (Och et al., 2012). Such an effect was observed in the present study wherein under suboxic conditions the mobilization of Mn was greater than the immobilization of Mn. Besides, the soluble Mn concentration in the pore water showed large variation with alternate high and low values between depths. The present results agree with the reported values for Mn<sup>2+</sup> in pore water from the Pacific Ocean (D'Hondt et al., 2003) and the Barbados accretionary complex (Blanc et al., 1991). The high soluble Mn<sup>2+</sup> concentration in the deeper layers of the sediment in the present study may be attributed to the carbonate mineral phases in the sediment. Authigenic carbonate mineral phases such as calcite and Mn-carbonate have been shown to act as sink for soluble Mn<sup>2+</sup> in the subsurface sediments (Yadav, 1996).

Moderately reduced sediments exhibit Eh variation between +100 and +400 mV (Jugsujinda et al. 1996). Comparable variation in Eh between +151 to +236 mV was also observed in the present experimental study following the biotic-abiotic cycling of Mn by the subsurface sediment microbial communities. The immobilization of Mn in the presence of added glucose was accompanied by a discernable change in Eh and it increased on the 32 d of incubation. Since, increase in redox potentials could be associated with specific oxidation-reduction process (Black et al., 2008) the shift in Eh value close to +220 mV could suggest the absolute utilization of nitrate ions in the oxidation of Mn by the sediment microbial communities. Our results agree with the typical Eh value reported

(+220 mV) at which nitrate gets completely utilized. It corroborates with the results of [Vandenabeele et al. \(1995\)](#) on the higher rate of Mn removal by the microbial consortia in the presence of nitrate and [Shaw et al. \(1990\)](#) on the oxidation of Mn by nitrate in the southern California Borderland sediments. However, beyond the fifth week of incubation the Eh values decreased to +200mV which are suggestive of the appearance of manganous ions or that the oxides of Mn in sediments are moderately reduced. The present result corroborates with the report of [DeLaune and Reddy \(2005\)](#) that redox potential (Eh) increases with increasing activity of the oxidized component and decreases with increasing activity of the reduced component. This could most probably occur where biological process is very active., as the microbial uptake of an element is always compensated by its release.

In summary, under altered conditions of the native sediments we observed a statistically significant difference ( $P \leq 0.001$ ) in the change of the microbial response to the cycling of Mn (mobilization and immobilization) in the presence of added glucose. The immobilization of Mn could be attributed to the direct requirement of the metal by methanogens for the stability of methyl transferase which catalyzes the transfer of the methyl group from a donor to an acceptor. Mn could also be immobilized as a replacement for Mg in methanogens ([Nelson and Cox, 2004](#)) which is required or stimulatory for the activity of enzymes linked to the different pathways of the methane cycle like methyl coenzyme M reductase, methyl transferase, ATPase and serine transhydromethylase ([Takashima et al., 1990](#)). Besides, mobilization of Mn could be associated with the reducing conditions of the slope sediments and the activity of methanotrophs coupled to the anaerobic oxidation of methane. The results therefore suggest that Mn redox changes affect the methane oxidation/production rates by serving either as an electron donor and/or an electron acceptor.

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## Figure captions

**Figure 1.** Pore water profile of manganese ( $Mn^{2+}$ ) for core 17A. The error bars represents the deviation of triplicate readings.

**Figure 2.** Concentration of methane, abundance of total cell numbers, methanogens, methanotrophs and fermenters in subsurface sediments of core 17A (MPN = most probable number and CFU = colony forming units).

**Figure 3.** Plots showing the significant difference ( $P < 0.001$ ) in the cycling of Mn between biotic experiments and abiotic controls under oxic versus suboxic incubation conditions in the presence ( $G^+$ ) and absence ( $G^-$ ) of added glucose ( $55.5\mu M$ ). The corresponding symbol with vertical bar under each category indicates the least square means and the standard error at 0.95 confidence intervals.

**Figure 4.** Box-whisker plots showing the variation in Eh (mV) with time in the in-vitro experiment on Mn cycling with subsurface sediments. a)  $G^+$  biotic oxic, b)  $G^+$  abiotic oxic, c)  $G^-$  biotic oxic, d)  $G^-$  abiotic oxic, e)  $G^+$  biotic suboxic, f)  $G^+$  abiotic suboxic, g)  $G^-$  biotic suboxic and h)  $G^-$  abiotic suboxic. The mean represents the average values for 25 depths in triplicates. Boxes represent the upper and lower quartiles with median (centre line) indicated in the box. Whiskers indicate the range of data and outliers are points whose value is greater than upper quartile +1.5 (interquartile distance) or less than lower quartile -1.5 (interquartile distance).

**Figure 5.** Box-whisker plots showing the variation in pH with time in the in-vitro experiment on Mn cycling with subsurface sediments. a)  $G^+$  biotic oxic, b)  $G^+$  abiotic oxic, c)  $G^-$  biotic oxic, d)  $G^-$  abiotic oxic, e)  $G^+$  biotic suboxic, f)  $G^+$  abiotic suboxic, g)  $G^-$  biotic suboxic and h)  $G^-$  abiotic suboxic. The mean represents the average values for 25 depths in triplicates. Boxes represent the upper and lower quartiles with median (centre line) indicated in the box. Whiskers indicate the range of data and outliers are points whose value is greater than upper quartile +1.5 (interquartile distance) or less than lower quartile -1.5 (interquartile distance).

**Supplementary figure S1.** The 3D plots show the variation in immobilization/mobilization of Mn with time under oxic conditions with subsurface sediments a)  $G^+$  biotic oxic, b)  $G^+$  abiotic oxic, c)  $G^-$  biotic oxic and d)  $G^-$  abiotic oxic. The yellow spheres represent the actual data points in  $\mu M g^{-1}$  where the negative values indicate mobilization and positive values indicate immobilization. The contoured surfaces are inferred from the above data points and the conjecture observed is mainly because of the gap between depths from 134 to 290.6mbsf. Abbreviations mbsf= meters below seafloor,  $G^+$  = media with added glucose and  $G^-$  = media without added glucose.

**Supplementary figure S2.** The 3D plots show the variation in immobilization/mobilization of Mn with time under suboxic conditions with subsurface sediments a)  $G^+$  biotic suboxic, b)  $G^+$  abiotic suboxic, c)  $G^-$  biotic suboxic and d)  $G^-$  abiotic suboxic. The yellow spheres represent the actual data points in  $\mu M g^{-1}$  where the negative values indicate mobilization and positive values indicate immobilization. The contoured surfaces are inferred from the above data points and the conjecture observed is mainly because of the gap between depths from 134 to 290.6mbsf. Abbreviations mbsf= meters below seafloor,  $G^+$  = media with added glucose and  $G^-$  = media without added glucose.

Figure 1.

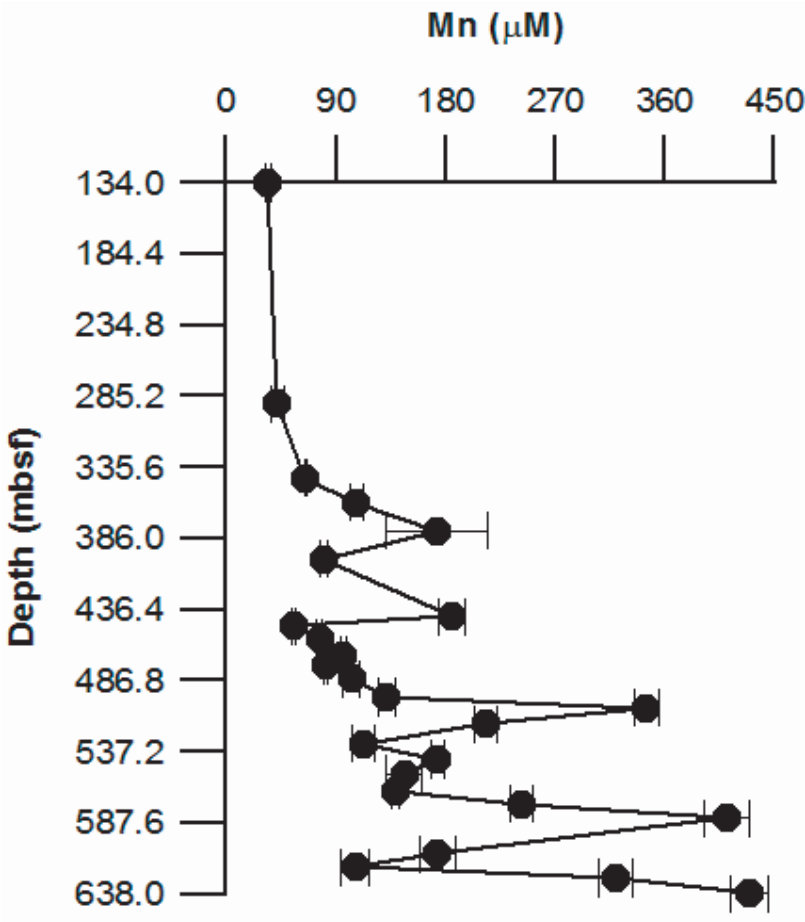




Figure 2.

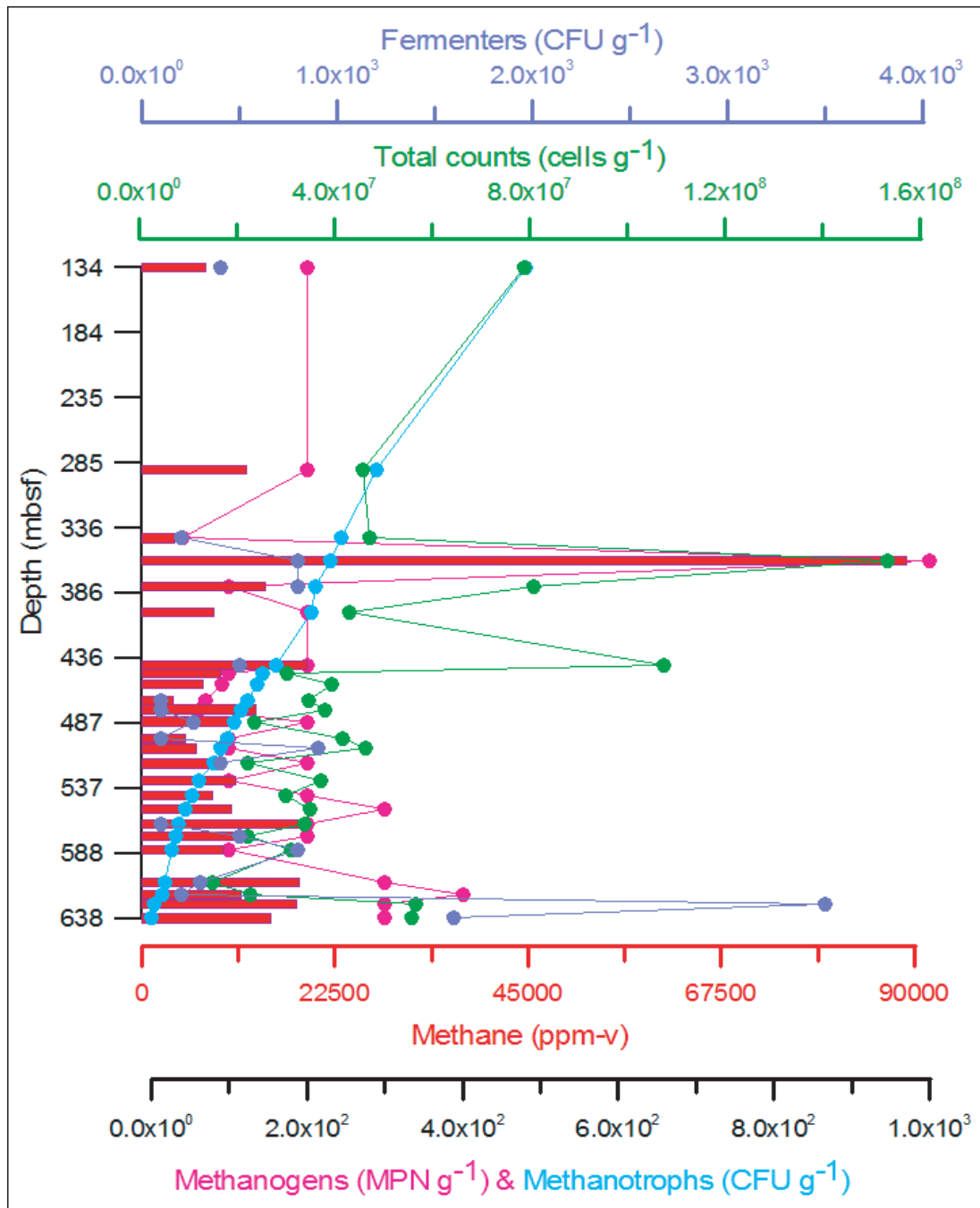


Figure 3

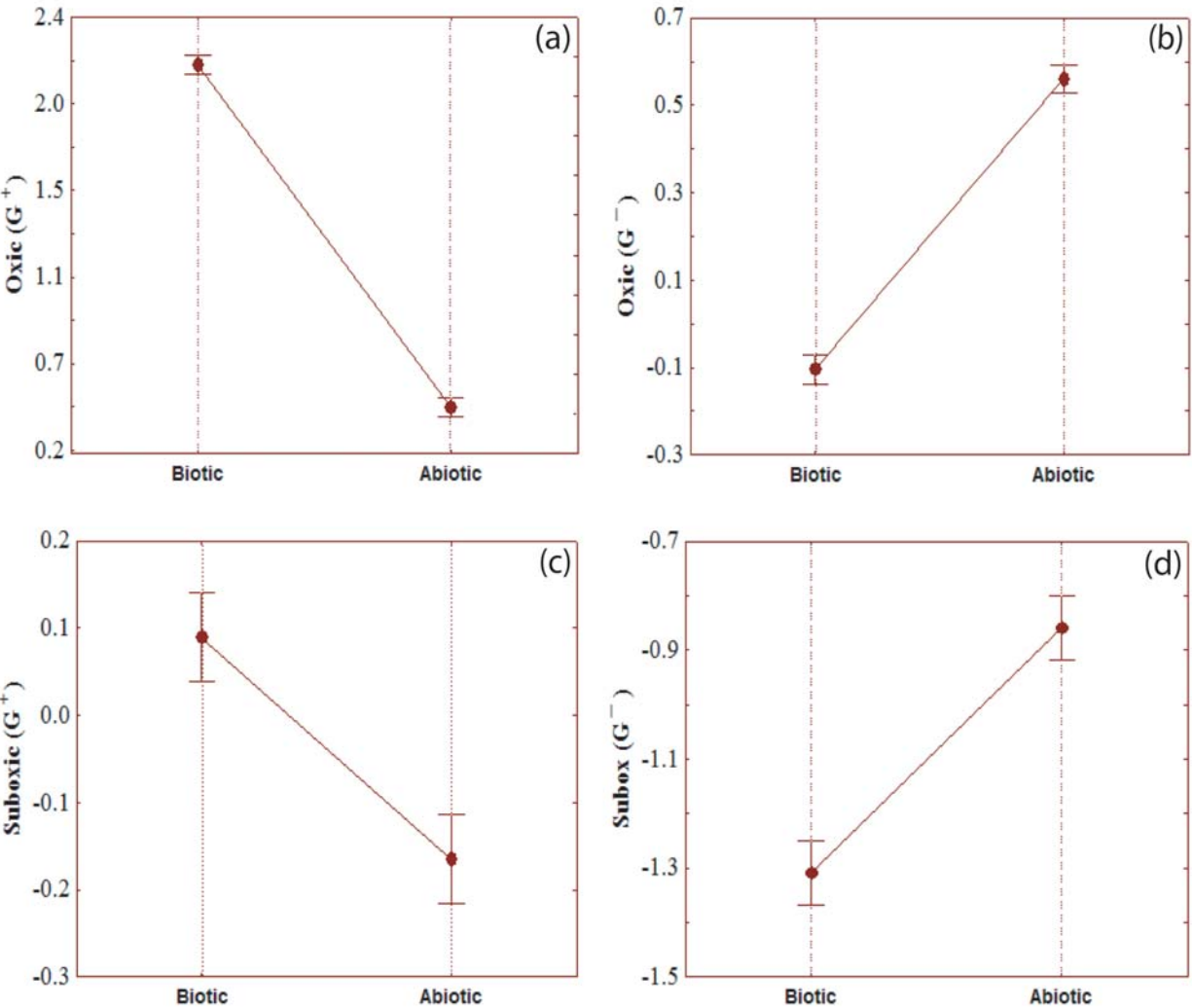


Figure 4

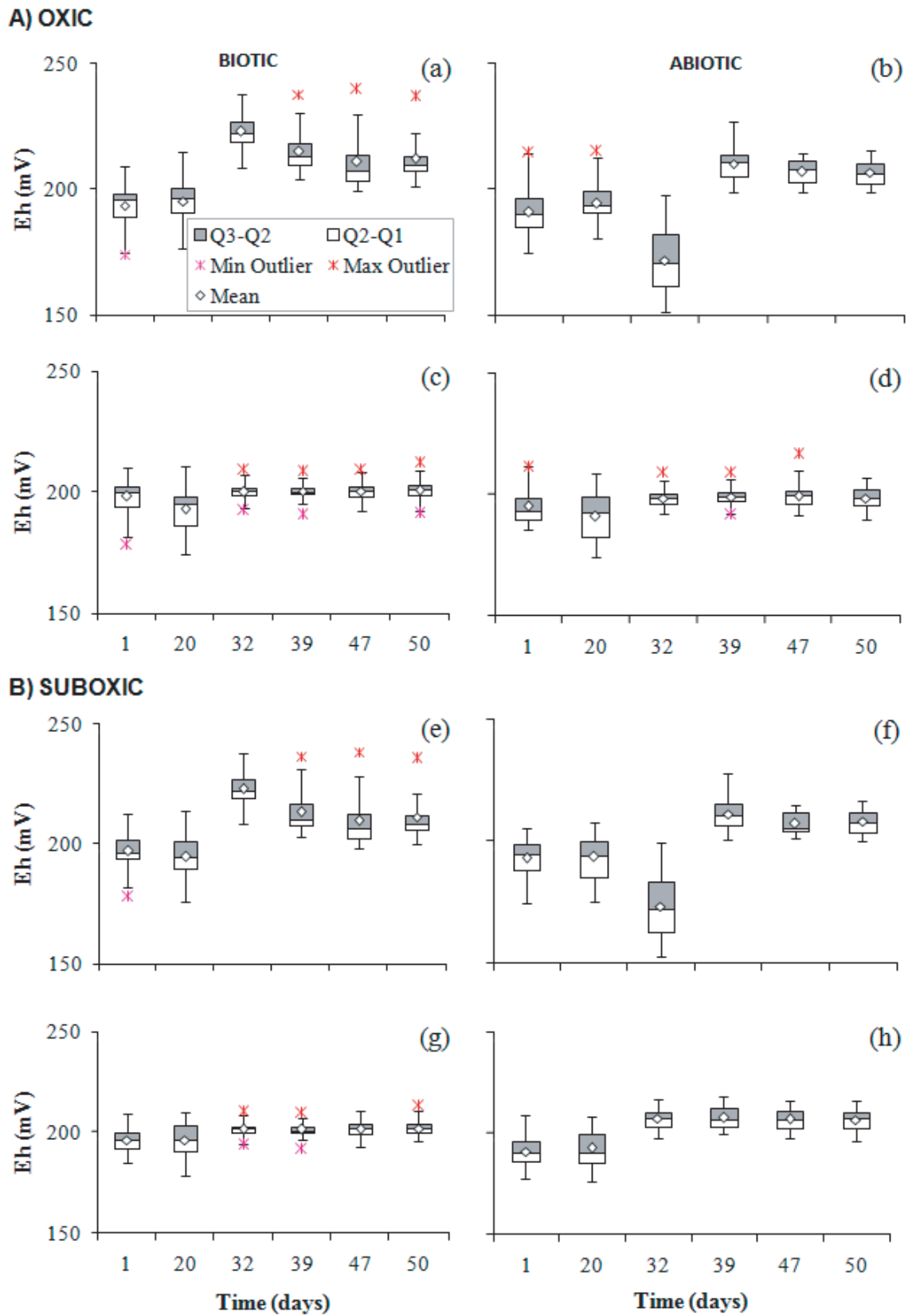
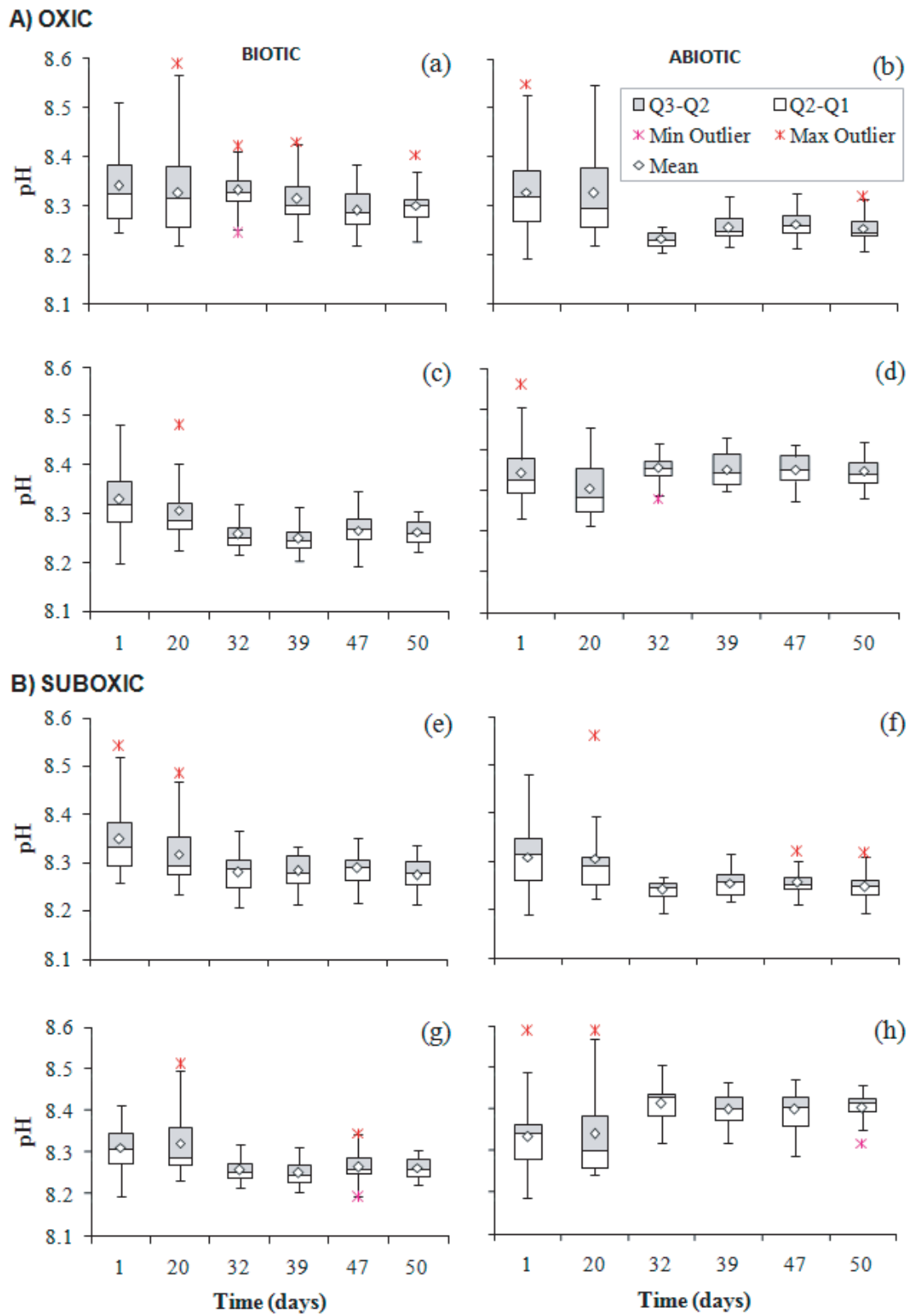
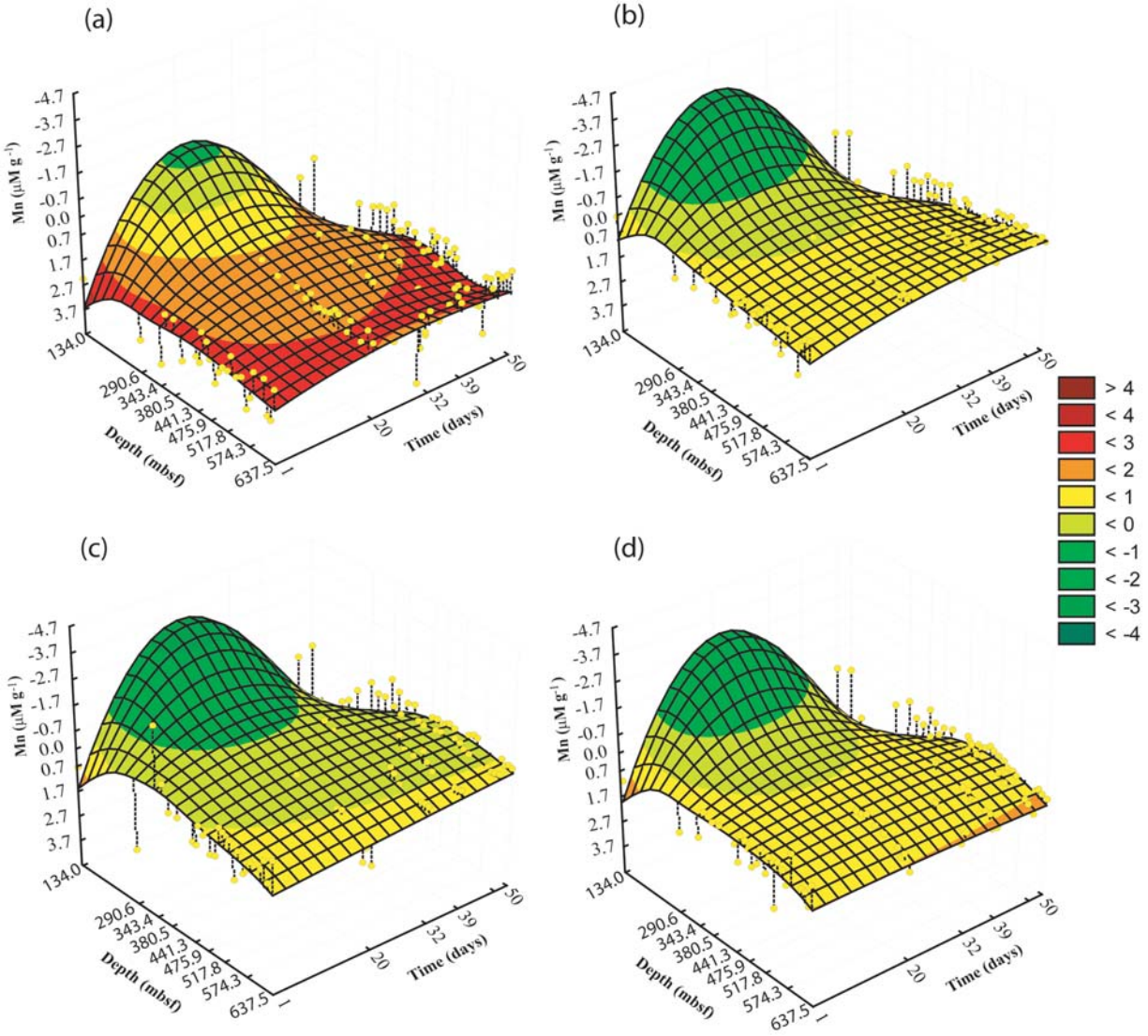


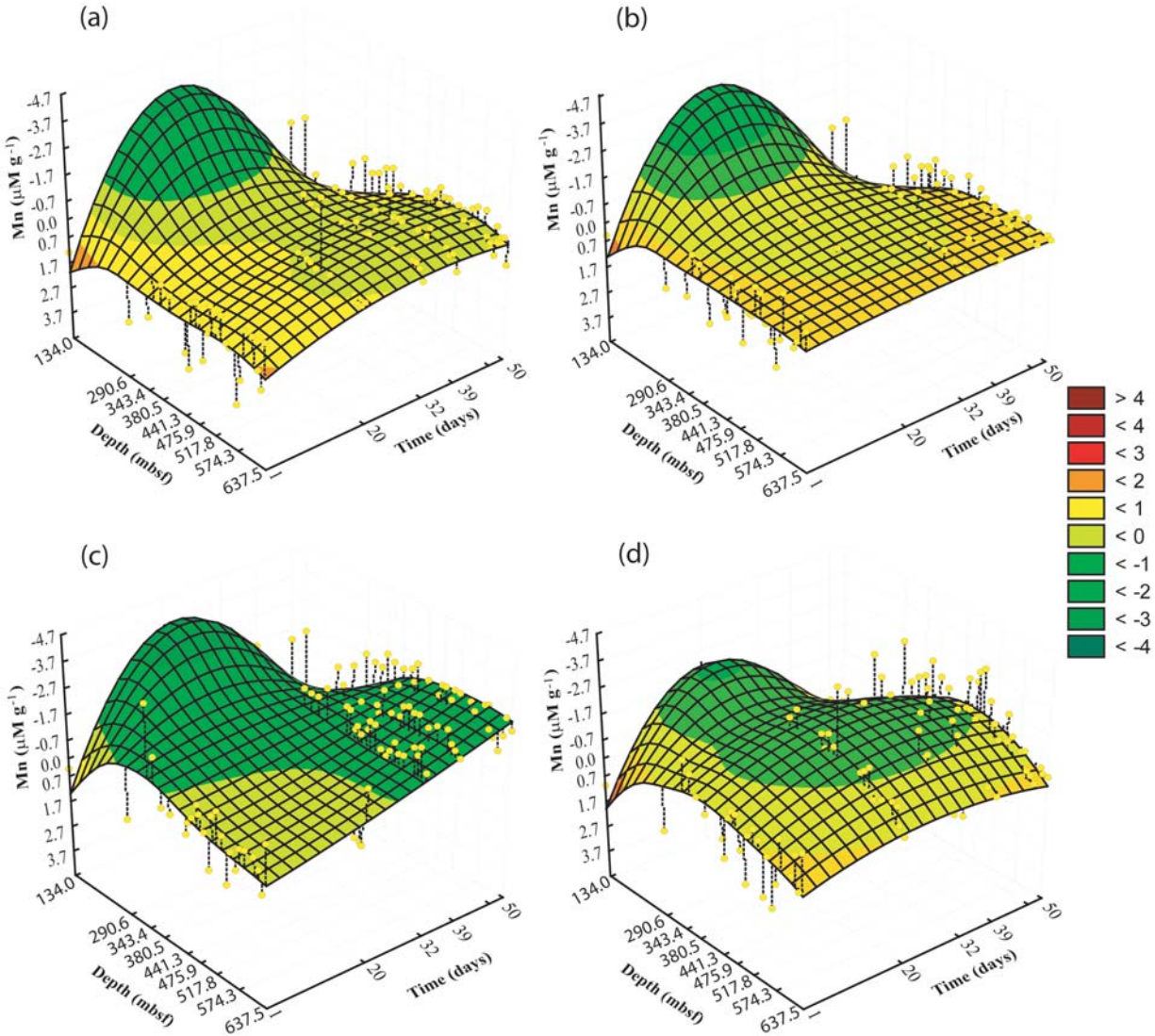
Figure 5.



Supplementary Figure S1



Supplementary Figure S2



**Table 1.** In vitro experimental set-up on the biotic/abiotic cycling of manganese by deep subsurface sediment microbial communities in the presence and absence of added glucose.

	Blanks				Chemical controls*								Azide treated controls* (Abiotic)				Experiments* (Biotic)			
	B1	B2	B3	B4	Oxic				Suboxic				Oxic		Suboxic		Oxic		Suboxic	
					C1	C2	C3	C4	C5	C6	C7	C8	Az1	Az2	Az3	Az4	E1	E2	E3	E4
55.5mM Glucose (ml) (final 55.5 $\mu$ M)	-	0.01	-	0.01	-	0.01	-	0.01	-	0.015	-	0.015	-	0.01	-	0.015	-	0.01	-	0.015
1M Azide (ml) (final 15 mM)	-	-	0.15	0.15	-	-	0.15	0.15	-	-	0.225	0.225	0.15	0.15	0.225	0.225	-	-	-	-
100mM Mn <sup>2+</sup> (ml) (final 100 $\mu$ M)	-	-	-	-	0.01	0.01	0.01	0.01	0.015	0.015	0.015	0.015	0.01	0.01	0.015	0.015	0.01	0.01	0.015	0.015
Sediment weight (g)	-	-	-	-	-	-	-	-	-	-	-	-	~1	~1	~1	~1	~1	~1	~1	~1
Sterile seawater (ml)	10	9.99	9.85	9.84	9.99	9.98	9.84	9.83	14.985	14.97	14.76	14.745	9.84	9.83	14.76	14.745	9.99	9.98	14.985	14.97
Total volume without sediment (ml)	10	10	10	10	10	10	10	10	15	15	15	15	10	10	15	15	10	10	15	15

\*Tubes incubated in triplicates at 4 $\pm$ 1°C under oxic and suboxic incubation conditions.

**Table 2.** Results of two-way analysis of variance (ANOVA) for the in-vitro experiment on the cycling of manganese in the continental slope sediments of the Andaman Sea.

<b>Variables</b>	<b>Source of variation</b>	<b>df</b>	<b>MS</b>	<b>F</b>	<b>P(≤)</b>	<b>SNK test</b>
G <sup>+</sup> oxic	Biotic vs Abiotic	1	669.9	2574.1	0.001	B > A
	Depth	24	1.8	7	0.001	360 mbsf
	Days	5	4.4	17	0.001	20 d
G <sup>-</sup> oxic	Biotic vs Abiotic	1	99.63	785.2	0.001	A > B
	Depth	24	1.97	15.5	0.001	343 & 360 mbsf
	Days	5	0.99	7.8	0.001	01 d
G <sup>+</sup> suboxic	Biotic vs Abiotic	1	14.56	48.26	0.001	B > A
	Depth	24	1.76	5.84	0.001	447 mbsf
	Days	5	10.18	33.73	0.001	01 d
G <sup>-</sup> suboxic	Biotic vs Abiotic	1	45.58	110.3	0.001	A > B
	Depth	24	7.87	19	0.001	343 & 360 mbsf
	Days	5	37.54	90.8	0.001	20 d

Note: only the significant variables are represented in the table. SNK test refers to Student-Newman-Keuls test, mbsf to meters below seafloor, G<sup>+</sup> to media with added glucose and G<sup>-</sup> to media without added glucose.



**Supplementary table S1.** Results of the in-vitro experiment on manganese cycling in the continental slope sediments of the Andaman Sea. The data in bold represents the maximum and others the minimum values for Mn, Eh and pH with negative values for Mn mobilization and positive values for Mn immobilization.

Parameters	Media	Oxic						Suboxic					
		Biotic			Abiotic			Biotic			Abiotic		
		Depth (mbsf)	Day	Value	Depth (mbsf)	Day	Value	Depth (mbsf)	Day	Value	Depth (mbsf)	Day	Value
Mn ( $\mu\text{M g}^{-1}$ )	G <sup>+</sup>	<b>609.4</b>	<b>32</b>	<b>4.66 ±0.94</b>	<b>609.4</b>	<b>1</b>	<b>1.65 ±0.52</b>	<b>441.3</b>	<b>20</b>	<b>2.95 ±0.18</b>	<b>400.8</b>	<b>1</b>	<b>1.37 ±0.70</b>
		360.5	32	1.07 ±0.58	360.5	1	-0.31 ±0.04	531.1	20	-2.98 ±0.86	584.4	1	-1.55 ±0.08
Mn ( $\mu\text{M g}^{-1}$ )	G <sup>-</sup>	<b>290.6</b>	<b>1</b>	<b>2.49 ±0.65</b>	<b>542.6</b>	<b>1</b>	<b>1.92 ±0.08</b>	<b>542.6</b>	<b>1</b>	<b>0.93 ±0.52</b>	<b>290.6</b>	<b>39</b>	<b>1.64 ±0.46</b>
		343.4	1	-2.80 ±0.39	584.4	1	-0.50 ±0.07	343.4	1	-3.93 ±1.73	475.9	20	-4.27 ±1.19
Eh (mV)	G <sup>+</sup>	<b>360.5</b>	<b>50</b>	<b>236 ±1</b>	<b>134.0</b>	<b>39</b>	<b>226 ±17</b>	<b>609.4</b>	<b>50</b>	<b>235 ±1</b>	<b>637.5</b>	<b>39</b>	<b>227 ±16</b>
		609.4	1	181 ±6	517.8	32	151 ±4.5	574.3	1	177 ±5	475.9	32	152 ±4
Eh (mV)	G <sup>-</sup>	<b>475.9</b>	<b>50</b>	<b>211 ±1</b>	<b>619.0</b>	<b>1</b>	<b>211 ±10</b>	<b>517.8</b>	<b>50</b>	<b>212 ±1</b>	<b>637.5</b>	<b>39</b>	<b>217 ±1</b>
		531.1	1	177 ±0.9	134.0	20	173 ±0.6	134.0	1	184 ±9	517.8	20	176 ±5
pH	G <sup>+</sup>	<b>290.6</b>	<b>1</b>	<b>8.51 ±0.5</b>	<b>134.0</b>	<b>1</b>	<b>8.55 ±0.1</b>	<b>290.6</b>	<b>1</b>	<b>8.54 ±0.1</b>	<b>360.5</b>	<b>20</b>	<b>8.56 ±0.1</b>
		343.4	50	8.22 ±0.4	506.2	1	8.19 ±0.5	380.5	50	8.21 ±0.2	619.0	1	8.19 ±0.5
pH	G <sup>-</sup>	<b>343.4</b>	<b>1</b>	<b>8.48 ±0.8</b>	<b>134.0</b>	<b>1</b>	<b>8.56 ±0.1</b>	<b>441.3</b>	<b>1</b>	<b>8.41 ±0.5</b>	<b>134.0</b>	<b>1</b>	<b>8.58 ±0.2</b>
		517.8	1	8.20 ±0.5	456.4	20	8.21 ±0.1	626.7	1	8.19 ±0.8	485.4	1	8.18 ±0.3

mbsf = meters below seafloor, G<sup>+</sup> = media with added glucose and G<sup>-</sup> = media without added glucose.