

## Microbial activity determining soil CO<sub>2</sub> emission in the Sundarban mangrove forest, India

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**Abstract:** Temporal variation in the soil CO<sub>2</sub> emission from the worlds largest mangrove forest was studied on a monthly basis. *In situ* measurements were carried out from three different parts of the mangrove forest, i.e. (1) deep forest (with limited tidal influence), (2) rooted (with pneumatophores, medium tree density and tidally active), and (3) un-rooted region (tidal flat). Subsequently, the role of soil salinity, temperature and the enzyme activities (Urease, β-D glucosidase and Dehydrogenase activity) in regulating the microbial mineralization processes were also studied to understand their contribution to the soil CO<sub>2</sub> emission. Irrespective of the sampling locations, the highest and lowest CO<sub>2</sub> efflux was recorded during month from October to January and from June to September, respectively. Among the three different regions, the highest and the lowest CO<sub>2</sub> emission was recorded from the deep forest ( $8.34 \pm 1.04$  mmol C m<sup>-2</sup>h<sup>-1</sup>) and un-rooted soil ( $0.81 \pm 0.07$  mmol C m<sup>-2</sup> h<sup>-1</sup>), respectively. Soil CO<sub>2</sub> emission did not show any significant response to salinity. However, soil temperature and available enzyme activities showed significant control over soil CO<sub>2</sub> emission. The results indicated that the microbial community in this tidally active zone is well adapted within a large salinity range. Among the soil enzymes, β-D glucosidase activity showed the highest contribution, in regulating the soil CO<sub>2</sub> emissions, in all the studied locations.

**Key Words:** Enzyme activity, microbial mineralization, salinity, soil CO<sub>2</sub> emission, Sundarban mangroves, temperature.

**Handling Editro:** Emma J. Rochelle-Newall

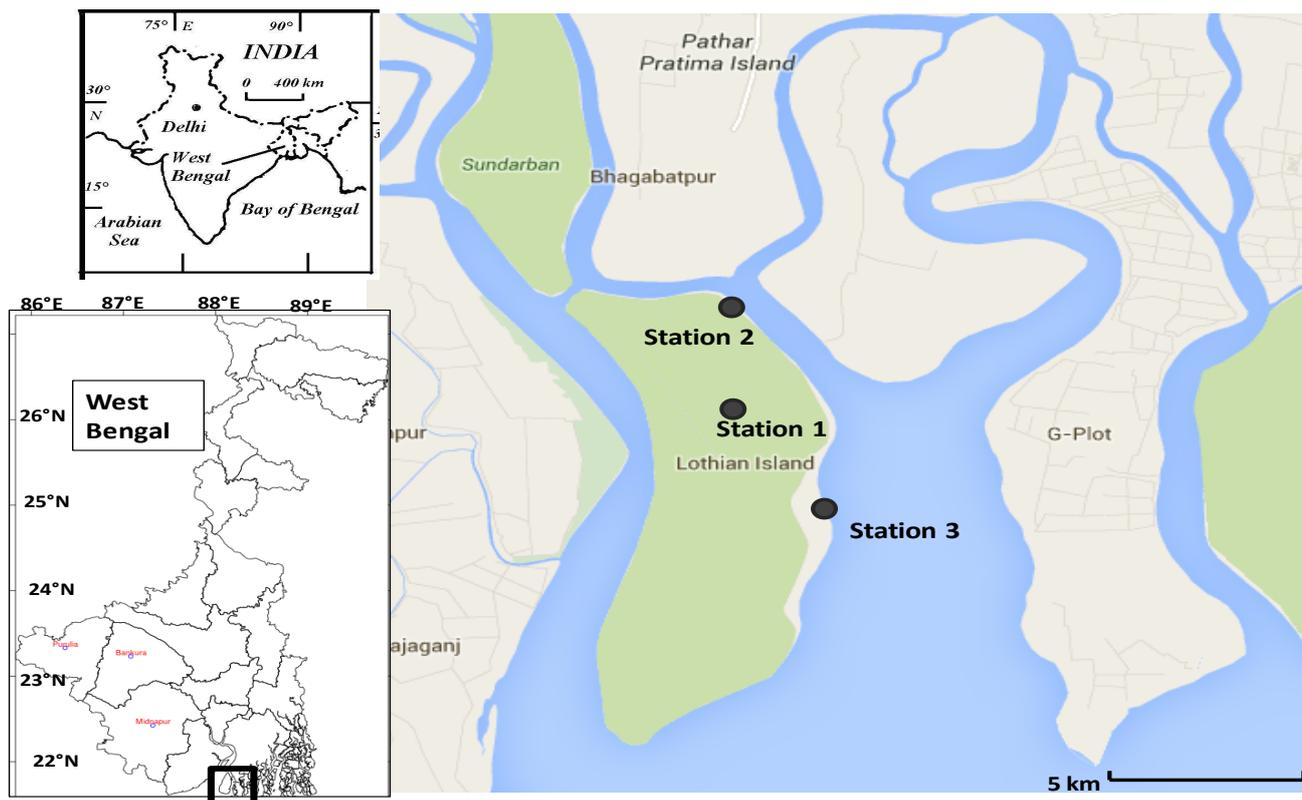
### Introduction

Soil is one of the major sources of atmospheric CO<sub>2</sub> and the global rate of soil CO<sub>2</sub> emission is 10 times higher than that from fossil fuel combustion (Raich & Potter 1995). Degradable organic carbon present in the soil is the main fuel responsible for the CO<sub>2</sub> emission during soil respiration and any alteration of that degradable organic carbon due to environmental factors may show an effect on global warming by changing overall CO<sub>2</sub> emission rates from the soil (Bohn 1982; Eswaran *et al.* 1993; Eswaran *et al.* 1995; Jenkinson *et al.* 1991;

Kirschbaum 1995; Raich & Schlesinger 1992; Schleser 1982). Soil-surface CO<sub>2</sub> efflux accounts for 67–76% of total ecosystem respiration in forests (Littton *et al.* 2003). It is estimated that a global warming of 0.03 °C per year will enhance soil respiration, producing a net release of an additional 60 Pg of carbon (equivalent to a 19% increase in fossil fuel combustion) from the soil to the atmosphere between 1990 and 2050 (Xu & Qi 2001). Soil CO<sub>2</sub> emission has been reported from various forest ecosystems all over the world with high spatial and temporal variability (Davidson *et al.* 1998; Russell & Voroney 1998). The variability has

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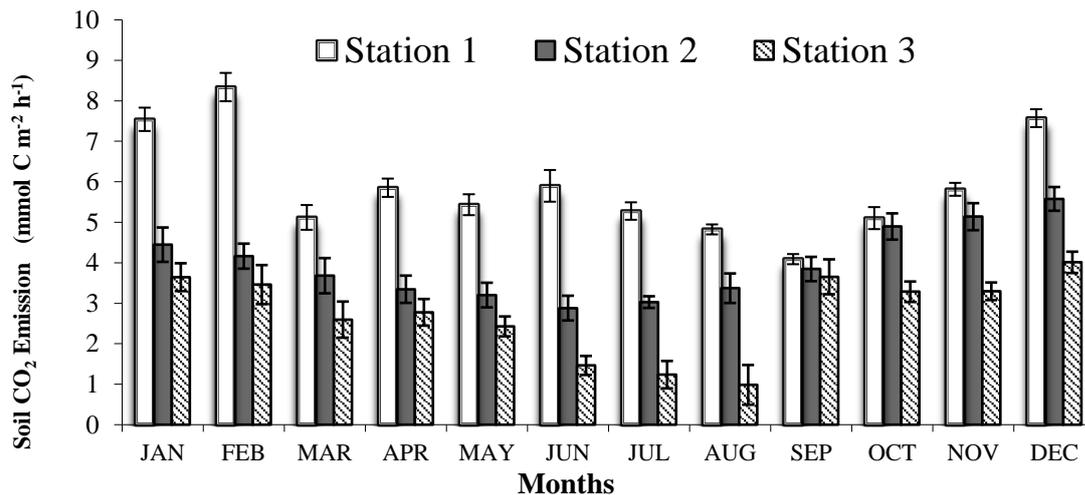


**Fig 1:** Map showing the study area. Three sampling stations represent three distinct zones, namely 1. deep forest region (with limited tidal influence; Station1), 2. rooted region (with pneumatophores, medium mangrove tree density and tidally active; Station2) and 3. un-rooted region (exposed tidal flat with no pneumatophores, Station3)

been attributed to species composition, stand age, management practices, and climatic and edaphic conditions (Edwards & Ross-Todd 1983; Ewel *et al.* 1987; Hanson *et al.* 1993; Nakane & Lee 1995; Toland & Zak 1994). Among forest ecosystems, mangroves are one of the most productive ecosystems (net primary production  $218 \pm 72$  TgC $\cdot$ yr<sup>-1</sup>; Bouillon *et al.* 2008), found in tropical and sub-tropical intertidal areas and are known for the high deposition rates of autochthonous and allochthonous organic matter, followed by low rates of organic matter oxidation (under the dominance of anaerobic decay processes) (Kristensen *et al.* 2008). Factors like degree of tidal influence, supply of oxygen to the soil, proximity to the C sources and other environmental parameters may significantly modify C biogeochemistry in the soil (Bano *et al.* 1997; Włodarczyk *et al.* 2002).

Soil enzymes play a significant role in maintaining soil ecology, physical and chemical properties, and soil fertility. Among the different

enzymes in soils, dehydrogenase,  $\beta$ -D-glucosidase, urease and phosphatases are important in the transformation of different plant nutrients. Dehydrogenase activity reflects the total oxidative activity of the microbial biomass (Nannipieri *et al.* 1990). The  $\beta$ -D-glucosidase is an important enzyme in terrestrial carbon cycle in producing glucose, which constitutes an important energy source for microbial biomass (Tabatabai 1994). Urease catalyzes the hydrolysis of urea into ammonia or ammonium ion depending on soil pH, and carbon dioxide. Understanding the responses of various enzymes present in the mangrove soil and their relative importance in regulating the CO<sub>2</sub> emission under the different environmental conditions could be useful to predict the future scenarios. However, because of the technical difficulties in setting up CO<sub>2</sub> emission experimentation in the water logged soil during high tide, exposed soil surface were preferred for the measurement of direct soil CO<sub>2</sub> emissions. Overall, this present study was aimed to



**Fig 2:** Seasonal variation of CO<sub>2</sub> emission from the soil of deep forest region (station 1), rooted region (station 2) and un-rooted region (station 3).

understand the relation between the soil CO<sub>2</sub> efflux and microbial activities, under varied environmental settings on a seasonal basis in a tropical mangrove forest. Further, it was hypothesized that organic carbon, temperature and associated soil enzyme activity may cause significant spatial variation in the soil CO<sub>2</sub> emission from this unique bioclimatic zone.

## Material and methods

### Study Site

The Sundarban Mangrove forest is located geographically in between 21°31'N 22°30'N and longitude 88°10'E to 89°51'E along the North East coast of Bay of Bengal, India. This mangrove forest is a part of the estuarine system of the River Ganges, NE coast of Bay of Bengal (Fig. 1), which covers 9630 km<sup>2</sup>. Several numbers of discrete islands constitute Sundarbans. The tide in this eastern complex is semidiurnal in nature with spring tide ranging between 4.27 m and 4.75 m and neap tide range between 1.83 m and 2.83 m. It is a unique bioclimatic zone in between the land and ocean boundaries of the Bay of Bengal and the largest delta on the globe. The deltaic terrain of Sundarban Biosphere Reserve comprises mainly saline alluvial soil consisting of clay (4.66–14.99%), silt (72–88%) and coarse sand (5.44–15.12%) particles (Dey *et al.* 2012).

### Description of the three sampling stations

Soil samples were collected on a monthly basis

from three distinct zones, namely, 1. deep forest region (with limited tidal influence; Station1), 2. rooted region (with pneumatophores, medium mangrove tree density and tidally active, Station.2), and 3. un-rooted region (exposed tidal flat with no pneumatophores, Station. 3) from the mangrove ecosystem (Fig. 1). Station 3 experienced regular tidal inundation whereas station 1 used to get inundated only during the highest high tide. Number of pneumatophores (aerial roots) was maximum in station 2 (~120 m<sup>-2</sup>) followed by station 1 (~45 m<sup>-2</sup>). *Avecennia marina* (70%) and *Avecennia officinalis* (30%) are the two major species found in these sampling locations.

### Sampling and analysis

#### Quantification of different types of bacteria and fungi of soil

For quantification of bacteria and fungi the procedure as described by Ramanathan *et al.* (2008) was followed. The soil samples were collected from the superficial sediment (2–10 cm depth; upper layer was removed) using a stainless steel sterile spatula. Immediately after collection, the samples were aseptically stored in tightly sealed sterilized plastic bags and transported to the laboratory at 4 °C. A total of five sub-samples were collected (5 different location) from each station. From three different stations, 10 grams of soil were taken and homozinised in 100 ml sterile phosphate buffer saline solution (isotonic). Serial dilutions up to 10<sup>-4</sup> were made and inoculation was performed with 0.1 ml soil suspension.

### Measurement of soil enzyme activity

Soil dehydrogenase activity was measured following standard method depicted by Mersi & Schinner (1991). In this method, 2(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride was used as the substrate. Soil dehydrogenase activity was estimated by spectrophotometry (464 nm) by measuring the reduced iodonitro-tetrazolium formazan extraction with N, N-dimethylformamide and ethanol. The urease activity was determined by measuring the amount of ammonium released from 1 g soil sample incubated for 1 h at 37 °C, using urea (79.7 mmol l<sup>-1</sup>) as substrate. The released ammonium was determined spectrophotometrically at 660 nm (Paolini & Sánchez-Arias 2008).

For estimation of soil phosphatase activity, 1 g soil sample was incubated with, *p*-nitro phenyl phosphate (*p*-NPP) for 1 h at 37 °C. After extraction with NaOH solution the activity was estimated spectrophotometrically (430 nm) (Zhou *et al.* 2005). Soil β-D- glucosidase activity was determined by the colorimetric method described by Timothy *et al.* (2004). One gram soil was incubated with β-D-glucopyranoside (substrate) at pH 6.0 and 37 °C. After 1 h of incubation, 0.5 M CaCl<sub>2</sub> and pH 12.0 modified universal buffer were added to extract *p*-nitrophenol. The amount of *p*-nitrophenol released

by glycosidases was determined colorimetrically at 410 nm.

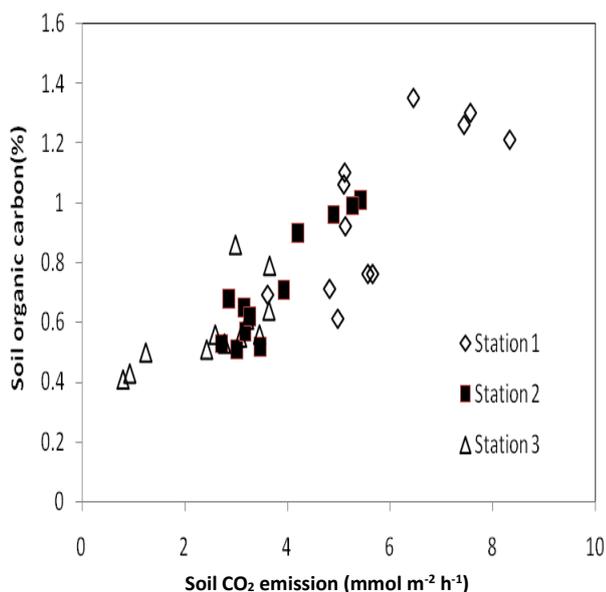
### Soil quality measurement

Concentrations of sulfate-sulfur of soil was measured after extraction of soil sample into aqueous solution, followed by turbidimetric determination (Grasshoff 1983; Mussa *et al.* 2009). Concentration of Silicate-silicon in the soil sample was determined by using acidified molybdate solution and ascorbic acid solution after extracting the soil suspension in 2M KCl solution (20 g soil in 100 ml 2M KCl solution) and the absorbance of the blue complex was measured photometrically at 810 following Grasshoff *et al.* 1983. Soil temperature was measured using thermocouple probes (± 0.1 °C accuracy). The pH value was measured in a 1:5 (w/w) soil water suspension using an electric digital pH meter (Tiwari *et al.* 1989) and salinity of a soil saturation extract (ECe) was determined by measuring the electrical conductance of soil water saturation extract with the help of a conductivity meter (Richards 1968). Soil organic carbon was measured by dichromate oxidation and subsequent titration by Mohr's salt (Walkley & Black 1934).

### Measurement of CO<sub>2</sub> emission rate from soil

Rate of emission of soil CO<sub>2</sub> was measured following enclosed static chamber technique (Bartlett *et al.* 1987; Van den Nat & Middelburg 2000). The air samples were collected (1 ml each) from the enclosed static chamber in the pre-evacuated air sampling glass bulbs under the field condition. The samples were then transported to the laboratory and injected into the injection port of the gas chromatography (Varian CP 3800) with the help of gas tight syringe within a day of collection. Carbon dioxide was reduced to methane, after passing through Methanizer (Nickel catalyst system, Model No. MTN-1) maintained at 350 °C followed by its determination with Flame Ionization Detector (FID). Temperature of chrompack capillary column (12.5 m, 0.53 mm) and FID were maintained at 50 and 150 °C, respectively. Standard carbon dioxide (320 ppm), procured from EDT Instruments Ltd. was used for the calibration.

Finally the mixing ratio of CO<sub>2</sub> in the air sample was determined by comparison of peak areas for samples and standard followed by the compensation for the ambient CH<sub>4</sub>.



**Fig 3.** Correlation graph for soil organic carbon and soil emission rate in the deep forest region (station 1), rooted region (station 2) and un-rooted region (station 3).

**Table 1.** Monthly variations of pH, temperature (°C) and salinity (psu) of soil at different stations.

Month	pH			Temp			Salinity		
	St <sub>1</sub>	St <sub>2</sub>	St <sub>3</sub>	St <sub>1</sub>	St <sub>2</sub>	St <sub>3</sub>	St <sub>1</sub>	St <sub>2</sub>	St <sub>3</sub>
Jan	8.23 ± 0.37	8.24 ± 0.12	8.28 ± 0.23	12.76 ± 2.1	12.12 ± 2.4	11.23 ± 2.7	15.28 ± 2.3	19.68 ± 1.8	18.15 ± 3.1
Feb	8.16 ± 0.29	8.15 ± 0.22	8.13 ± 0.12	17.47 ± 2.7	16.51 ± 1.3	15.68 ± 1.7	16.34 ± 2.6	16.77 ± 1.4	18.22 ± 3.5
Mar	8.45 ± 0.17	8.12 ± 0.51	8.37 ± 0.42	21.32 ± 1.5	22.40 ± 1.1	23.54 ± 1.1	17.33 ± 2.4	20.18 ± 1.7	21.15 ± 2.7
Apr	8.28 ± 0.12	8.11 ± 0.38	8.12 ± 0.33	22.25 ± 1.2	23.38 ± 1.5	24.56 ± 1.2	18.50 ± 2.1	21.60 ± 1.2	21.78 ± 1.3
May	8.02 ± 0.24	8.41 ± 0.23	7.98 ± 0.29	24.32 ± 1.5	25.31 ± 1.7	26.59 ± 1.2	20.34 ± 2.2	22.8 ± 1.1	22.85 ± 2.1
Jun	7.91 ± 0.56	8.28 ± 0.37	8.17 ± 0.35	26.32 ± 1.4	27.65 ± 1.6	28.04 ± 1.6	19.13 ± 2.5	20.50 ± 1.9	17.86 ± 2.1
Jul	8.13 ± 0.33	7.59 ± 0.41	7.43 ± 0.58	25.15 ± 1.6	26.43 ± 1.3	27.17 ± 1.1	16.55 ± 2.1	17.50 ± 1.7	16.74 ± 2.6
Aug	8.09 ± 0.27	8.21 ± 0.14	8.38 ± 0.29	23.15 ± 1.8	24.32 ± 1.8	25.56 ± 1.4	14.06 ± 1.5	16.45 ± 1.4	13.44 ± 2.8
Sep	8.21 ± 0.11	7.72 ± 0.37	7.98 ± 0.16	22.3 ± 1.6	22.98 ± 1.5	24.15 ± 1.5	15.60 ± 1.8	17.75 ± 1.5	16.85 ± 1.3
Oct	8.31 ± 0.14	8.20 ± 0.18	8.22 ± 0.33	21.16 ± 2.1	22.23 ± 1.4	22.47 ± 1.3	18.50 ± 1.5	19.10 ± 1.1	18.57 ± 1.5
Nov	8.29 ± 0.21	8.31 ± 0.26	8.28 ± 0.37	19.01 ± 1.8	19.28 ± 1.7	20.11 ± 1.7	17.10 ± 2.1	20.47 ± 1.3	18.45 ± 1.1
Dec	8.67 ± 0.16	8.35 ± 0.19	8.16 ± 0.15	15.23 ± 1.5	15.52 ± 1.8	15.93 ± 1.5	18.44 ± 1.4	21.22 ± 1.6	19.12 ± 1.8

**Table 2.** Monthly variations of organic carbon (%), sulfate (mg g<sup>-1</sup> dry wt. of soil), silicate (μg g<sup>-1</sup> dry wt. of soil), and total microbial population (CFU × 10<sup>6</sup> g<sup>-1</sup> dry soil) of soil at different stations.

Month	Org.C			S-SO <sub>4</sub> <sup>-2</sup>			Si-SiO <sub>4</sub> <sup>-2</sup>			Total Microbial population		
	St <sub>1</sub>	St <sub>2</sub>	St <sub>3</sub>	St <sub>1</sub>	St <sub>2</sub>	St <sub>3</sub>	St <sub>1</sub>	St <sub>2</sub>	St <sub>3</sub>	St <sub>1</sub>	St <sub>2</sub>	St <sub>3</sub>
Jan	1.28	0.87	0.62	1.25	1.32	0.91	7.35	8.91	11.23	18.013	12.657	8.719
Feb	1.24	0.77	0.59	1.21	1.07	0.82	8.37	10.12	12.37	15.101	9.669	9.215
Mar	0.92	0.56	0.52	0.54	0.47	0.41	8.12	17.33	11.36	13.121	11.775	8.709
Apr	0.81	0.67	0.57	0.65	0.57	0.42	7.22	9.18	10.23	11.212	10.889	8.102
May	0.91	0.54	0.55	1.70	0.73	1.03	7.65	12.08	16.71	15.878	10.114	8.218
Jun	0.78	0.56	0.46	1.57	1.61	1.51	7.79	9.7	11.13	9.986	9.958	8.498
Jul	0.63	0.67	0.45	1.41	1.14	1.21	8.25	14.96	20.86	11.899	7.779	7.114
Aug	0.74	0.59	0.55	1.33	0.93	1.61	7.36	9.12	11.03	6.124	6.921	5.972
Sep	1.25	0.66	0.58	1.31	1.12	0.81	8.41	6.12	7.35	12.102	14.738	6.442
Oct	1.30	0.97	0.91	1.23	1.18	0.88	7.43	8.28	9.47	17.982	8.981	6.991
Nov	1.37	1.11	0.67	1.21	0.86	0.80	12.07	16.48	18.93	9.112	8.882	7.487
Dec	1.31	0.97	0.74	1.30	0.82	0.75	9.12	9.31	13.45	10.941	9.893	7.908

**Table 3.** The result of the two way ANOVA (between 3 different stations and 3 different seasons) for the soil parameter including salinity, pH, organic carbon and total microbial population.

Variable	Source	Sum of squares	df	Mean Sum of squares	F	P
S	Station	54.4	2	27.2	4.89	0.015
	Season	62.61	2	31.3	5.64	0.009
pH	Station	868.8	2	434.4	96.16	<0.001
	Season	127.4	2	63.7	14.25	<0.001
Org C	Station	1.47	2	0.7	21.93	<0.001
	Season	0.58	2	0.3	8.59	0.003
Total microbial population	Station	123.1	2	61.6	8.88	0.001
	Season	25.67	2	12.8	1.85	0.136

### Statistical analyses

Two way ANOVA test was performed considering the seasons and stations as independent factors with three levels each, to determine the variation in soil salinity, pH, soil organic carbon and total microbial population with respect to space and time. For the temporal variations, the months were grouped into three different seasons namely, pre-monsoon (March-June), monsoon (July-October), and post-monsoon (November-February). Additionally, linear mixed effects models, the plot as random factor (*lmer* function in the *lme4* package version 1.1–7 in *R* software package; Bates *et al.* 2014) were used to avoid pseudo-replication in the design for both factors. Stepwise multiple regression analysis was carried out to determine the relative importance of various enzyme activities (independent variable) in regulating the soil CO<sub>2</sub> emission (dependent variable) from the mangrove forest. All mathematical and statistical computations were made using Excel 2007 and Minitab 16.

### Result and Discussion

Monthly variations of physico-chemical parameters at the three locations are depicted in Tables 1 and 2. Minimum and maximum soil temperatures were observed during post-monsoon and monsoon, respectively, at all the stations (Table 1). The mean salinity of the soil decreased from 19.8 to 16.8 from the pre-monsoon to the monsoon period. The soil pH, sulphate and organic carbon content (Table 2) were found to be the maximum during post-monsoon with lower values during the monsoonal season.

A two way ANOVA (Table 3) with the stations and the seasons as factors, showed significant spatial and seasonal variations in soil salinity, pH and organic carbon. The variation in total microbial population between the stations was significant, whereas the variation between the season (pre-monsoon, monsoon and post-monsoon) was not statistically significant.

Irrespective of the sampling locations, the highest and the lowest soil CO<sub>2</sub> emission was recorded during post-monsoon and monsoon seasons, respectively. Among the three different locations, deep forest region consistently showed the highest soil CO<sub>2</sub> emission with seasonal mean of  $5.37 \pm 0.87$ ,  $4.63 \pm 0.56$  and  $7.45 \pm 0.92$  mmol C m<sup>-2</sup>h<sup>-1</sup> respectively during the pre-monsoon, monsoon and post-monsoon periods. In contrary, the unrooted region with frequent tidal influences showed lowest soil CO<sub>2</sub> emission compared to the other regions in all the three seasons. Spatial variation in soil CO<sub>2</sub> emission between the three sampling regions was found significant ( $P < 0.05$ ). Soil organic carbon known to be the major fuel for bacterial respiration and corresponding soil CO<sub>2</sub> emission was recorded maximum during post-monsoon season (Fig. 2). Earlier studies by Ray *et al.* (2011) showed that the supply of mangrove litters, the most important autochthonous organic carbon source in these ecosystems, was the maximum during the same period of the year. Shao *et al.* (2015) showed that soil labile organic carbon and soil enzymes play important roles in the carbon cycle of coastal wetlands. Highest CO<sub>2</sub> emission from station 1, which is the least influenced by tidal transport of allochthonous carbon could also be attributed to the relatively higher labile nature of

**Table 4.** Range of CO<sub>2</sub> fluxes from mangrove sediments at different parts of the globe.

Location	CO <sub>2</sub> fluxes (mmol C m <sup>-2</sup> h <sup>-1</sup> )		References
	Low	High	
Mangroves from Semi-arid condition (New Caledonia)	-0.71	2.41	Leopold <i>et al.</i> 2015
Subtropical mangrove (China)	0.69	20.56	Chen <i>et al.</i> 2010
Subtropical mangrove (China)	0.24	31.23	Chen <i>et al.</i> 2012
Tropical mangrove (Indonesia)	-1.34	3.88	Chen <i>et al.</i> 2014
Subtropical mangrove (China)	0.35	6.05	Jin <i>et al.</i> 2013
Tropical mangrove (Tanzania)	1.17	4.67	Kristensen <i>et al.</i> 2011;
Varied mangrove (Caribbean, Australia, New-Zealand)	-0.97	10.69	Lovelock 2008; Lovelock <i>et al.</i> 2014
Tropical mangrove (Thailand)	1.67	3.21	Poungparn <i>et al.</i> 2009
Globally compilation data from mangroves	0.25	10.04	Bouillon <i>et al.</i> 2008
Tropical mangrove (India)	0.15	2.32	Chanda <i>et al.</i> 2013
Tropical mangroves (India)			
Deep forest	3.62	8.34	
Rooted	2.72	5.42	
Unrooted	0.80	3.86	Present study

mangrove organic carbon than the exported organic carbon. Previous research revealed that a major portion of the leached DOM from mangrove litters was degraded efficiently due to its labile nature under oxic and nutrient limiting conditions and 90% of conversion efficiencies into microbial biomass was found (Kristensen & Pilgaard 2001). In contrary, under partly anoxic mangrove sediments (without pneumatophores; unrooted), the microbial incorporation occurs with a lower average efficiency of roughly 35% (Kristensen *et al.* 2008). Most of the previous studies from various tropical and subtropical conditions, on CO<sub>2</sub> emissions indicated the mangrove soil as a persistent source of CO<sub>2</sub> to the atmosphere (Table 4). However, these measurements did not account for the fact that certain parts of the mangrove NPP can be mineralized and/or emitted toward the atmosphere as CO<sub>2</sub> after export to adjacent ecosystems.

The present study revealed significant spatio-temporal variation ( $P < 0.05$ ) in soil CO<sub>2</sub> emission within the mangrove ecosystem, mostly depending on the varying degree of supply and removal of organic carbon. However, the CO<sub>2</sub> emission rates (0.8–8.34 mmol m<sup>-2</sup> hr<sup>-1</sup>) were well within range reported by Chanda *et al.* (2011). Ganguly *et al.* (2008) and Chanda *et al.* (2013) advocated the role of Sundarban mangrove forest as a net sink of CO<sub>2</sub> from the atmosphere. However, the present study demonstrates that a major part of these photosynthetically fixed C gets transported to the

sediment in terms of mangrove organic matter (litter, etc.) and subsequently respired back as CO<sub>2</sub> to the atmosphere.

Strong correlation between soil organic carbon and CO<sub>2</sub> emission in all the three stations (Fig. 3) and their seasonal variations indicated the importance of autochthonous carbon sources to the bacterial mineralization in these unique ecosystems. According to Raich & Tufekcioglu (2000) under favorable conditions, enhanced supply of organic carbon may significantly increase the heterotrophic microbial activity and subsequent soil CO<sub>2</sub> emission rates. Similarly, total microbial load showed a positive correlation with the soil CO<sub>2</sub> emission rate. Carbon dioxide evolution is a useful index to assess the influence of soil conditions and management practices on microbial biomass (Brookes 1995; Nannipieri *et al.* 1990) as the metabolically active heterotrophs release CO<sub>2</sub> when they utilize organic C (metabolizable) as a substrate for their maintenance and growth. The present result signifies the role of microbial community in supporting the heterotrophic mineralization process in the carbon cycle of Sundarban mangrove ecosystems.

However, the correlations between the soil salinity and soil emission rate in all the three stations were statically insignificant ( $R^2 = 0.6\%$ ,  $P = 0.816$ ), which indicated limited dependency of microbial respiration over the salinity fluctuation (between 12 to 26) in these mangrove soils.

**Table 5.** Multiple regression analysis with a stepwise variable selection. Dependent variables: Soil CO<sub>2</sub> emission (F<sub>CO2</sub>), independent variables: Glucosidase activity, phosphatase activity, urease activity, dehydrogenase activity; where, SS = sum of squares, *df* = degree of freedom, *f* = critical value of the F-distribution, *P* = probability level, *r*<sup>2</sup> = coefficient of multiple determination (i.e. the cumulative percentage of the response variable variation that is explained by the linear model).

Station 1	<i>df</i>	SS	<i>f</i>	<i>P</i>	<i>r</i> <sup>2</sup> (stepwise)
Glucosidase activity	12	12.58	15.71	0.003	56.3
Phosphatase activity	12	15.83	14.94	0.001	76.8
Urease activity	12	15.91	9.06	0.005	82.3
Dehydrogenase activity	12	16.14	6.34	0.018	83.4
F <sub>CO2</sub> = -3.03 + 0.0345 glucosidase activity + 0.00177 Phosphatase Activity + 0.0035 Urease Activity -0.00365 dehydrogenase activity.					
<b>Station 2</b>					
Glucosidase activity	12	5.07	10.3	0.009	50.7
Phosphatase Activity	12	5.14	4.77	0.039	51.6
Urease Activity	12	8.05	11	0.003	80.5
Dehydrogenase activity	12	8.74	12.17	0.003	81.2
F <sub>CO2</sub> = -3.51 + 0.0092 glucosidase activity + 0.000254 phosphatase Activity + 0.0156 Urease Activity + 0.00642 dehydrogenase activity					
<b>Station 3</b>					
Glucosidase activity	12	3.31	3.95	0.075	28.4
Phosphatase Activity	12	3.72	2.11	0.178	32.3
Urease Activity	12	6.87	3.81	0.058	58.6
Dehydrogenase activity	12	7.82	3.54	0.043	68.9
F <sub>CO2</sub> = -3.25 + 0.0009 glucosidase activity + 0.000675 Phosphatase Activity + 0.0143 Urease Activity + 0.00750 dehydrogenase activity					

This observation may further be explained by the salt tolerance ability of the microbes present at that particular ecosystem, because change in soil salinity could not affect them too much in microbial degradation which ultimately yield less influence on CO<sub>2</sub> emission from the soil. In contrary to this, soil temperature, in these conditions, showed quite significant control over soil emission rates (F<sub>CO2</sub> = 37.1–0.60 Temp [R<sup>2</sup> = 39.9%, *P*=0.046, *df*=41]). This finding can be explained by the high temperature sensitivity of the microbial population responsible for soil CO<sub>2</sub> emission to the atmosphere. These results indicate relatively higher influence of soil temperature (Lloyd & Taylor 1994) than soil salinity on the microbial utilization of organic carbon in the soil and subsequent soil CO<sub>2</sub> emission from Sundarban mangrove forest. To evaluate the relative importance of these soil enzyme activities on CO<sub>2</sub> efflux from the mangrove soil, the stepwise multiple regression procedure was applied and explained variability of F<sub>CO2</sub> was found most significant for β-D-glucosidase activity relative to the other enzyme activities, at station 1 and 2

(Table 5). In contrary, only dehydrogenase activity showed significant control over the soil CO<sub>2</sub> emissions (*P*<0.05) from the tidally active unrooted area (station 3).

It was hypothesized that the activity of β-D glucosidase, which is involved in the breakdown of polysaccharides would increase in response to elevated CO<sub>2</sub> (Hugh *et al.* 2005). The future increase in atmospheric CO<sub>2</sub> would increase the productivity of mangrove vegetation leading to more litter fall, which could provide more polysaccharides in the mangrove soil to accelerate β-D glucosidase activity. Soil urease on the other hand, transforms urea to NH<sub>3</sub> and CO<sub>2</sub> and the ammonia released may be converted to ammonium ion and then easily be absorbed by plant roots, but released CO<sub>2</sub> cannot be used up by any organisms and may increase the soil emission rate. Dehydrogenase enzyme belongs to intracellular enzyme that are involved in catalyzing of oxido-reductive reactions (Alef 1995) and is found to be active both in aerobic and anaerobic conditions, although most dehydrogenases are

produced by anaerobic microorganisms (Subhani *et al.* 2001).

The relatively higher significance of dehydrogenase activity in controlling the soil CO<sub>2</sub> emissions was observed at station 3, where the root system was absent. Mangrove pneumatophores play a profound role in the exchange of gases in between the soil and atmosphere (Kristensen & Alongi 2006; Lyimo *et al.* 2002). Unrooted areas, without any root system often gets tidally inundated which may enhance the anoxicity of soil. This soil anoxicity may influence the dehydrogenase activity and subsequent soil CO<sub>2</sub> emission process. Włodarczyk *et al.* (2002) showed that the emissions of CO<sub>2</sub> were significantly related to dehydrogenase activity. All the observed results indicated the dependence of soil enzyme activity on temperature, which in turn regulates the soil CO<sub>2</sub> emission flux to the atmosphere.

The results indicated that bacterial population in mangrove soils could be limited by the availability of organic carbon, whereas the soil enzyme activity and CO<sub>2</sub> emission was strongly regulated by organic carbon and soil temperature. Supply of oxygen to the mangrove sediment also could be a deciding factor in the bacterial mineralization processes.

### Conclusion

This study presents the results of soil CO<sub>2</sub> efflux and its regulating factors obtained from the mangrove forest floor of Indian Sundarban. Soil enzyme activities, regulated by the bacterial population and other biogeochemical conditions, showed positive feedback towards the CO<sub>2</sub> emission rates from these mangrove soils. The results clearly indicate the importance of microbial control over the soil mineralization processes in this unique bioclimatic zone. The results further indicated the relative importance of soil temperature over the soil salinity on microbial activities which in turn is largely responsible for soil CO<sub>2</sub> emission from these mangrove forests.

The results depict that CO<sub>2</sub> emissions from mangrove soil may vary significantly within the same forest, depending on the supply and removal of organic carbon and other environmental parameters.

It can be concluded that future fluctuations in global as well as the regional temperature could affect net carbon budget by altering the turnover time, in this unique ecological system. It may further be concluded that the microbes of the

mangrove soil are well adapted to the fluctuations in the salinity conditions.

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