Tropical Ecology 58(3): 525–537, 2017 © International Society for Tropical Ecology www.tropecol.com

Microbial activity determining soil CO₂ emission in the Sundarban mangrove forest, India

SUBHAJIT DAS 1* , DIPNARAYAN GANGULY 2 , RAGHAB RAY 1 , TAPAN KUMAR JANA 1 & TARUN KUMAR DE 1

¹Department of Marine Science, University of Calcutta, 35, B.C. Road, Calcutta-700019 India ²Futuristic Research Division (FTR) National Centre for Sustainable Coastal Management Ministry of Environment and Forests, Govt. of India. Koodal Building Anna University Campus Chennai 600025 India

Abstract: Temporal variation in the soil CO₂ 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, 8-D glucosidase and Dehydrogenase activity) in regulating the microbial mineralization processes were also studied to understand their contribution to the soil CO₂ emission. Irrespective of the sampling locations, the highest and lowest CO₂ 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 CO₂ emission was recorded from the deep forest (8.34 ± 1.04 mmol C $m^{-2}h^{-1}$) and un-rooted soil (0.81 ± 0.07 mmol C $m^{-2}h^{-1}$), respectively. Soil CO₂ emission did not show any significant response to salinity. However, soil temperature and available enzyme activities showed significant control over soil CO2 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, 6-D glucosidase activity showed the highest contribution, in regulating the soil CO₂ emissions, in all the studied locations.

Key Words: Enzyme activity, microbial mineralization, salinity, soil CO₂ emission, Sundarban mangroves, temperature.

Handling Editro: Emma J. Rochelle-Newall

Introduction

Soil is one of the major sources of atmospheric CO₂ and the global rate of soil CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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

^{*}Corresponding Author; e-mail: Subhajit_310@yahoo.com

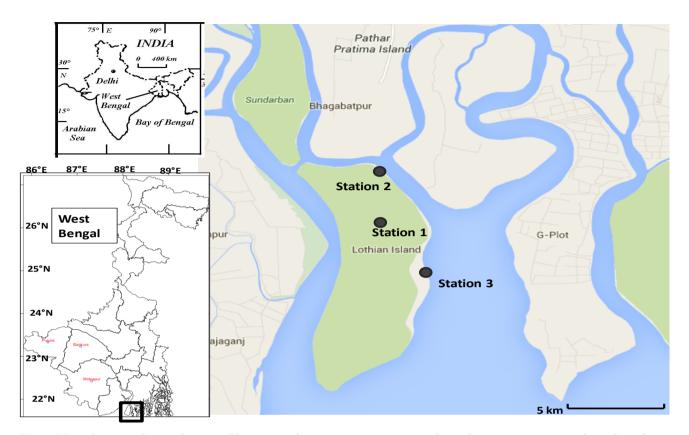


Fig 1: Map showing the study area. Three sampling stations represent three distinct zones, namely 1. deep forest region (with limited tidal influence; Station1), 2. rooted region (with pneumatophores, medium mangrove tree density and tidally active; Station2) and 3. un-rooted region (exposed tidal flat 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 ± 72 TgC yr⁻¹; 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, β-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 β-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 CO₂ emission under the different environmental conditions could be useful to predict the future scenarios. However, because of the technical difficulties in setting up CO₂ emission experimentation in the water logged soil during high tide, exposed soil surface were preferred for the measurement of direct soil CO₂ emissions. Overall, this present study was aimed to

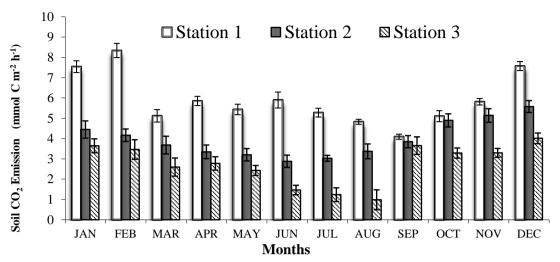


Fig 2: Seasonal variation of CO₂ emission from the soil of deep forest region (station 1), rooted region (station 2) and un-rooted region (station 3).

understand the relation between the soil CO₂ 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 CO₂ 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 km2. 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⁻²) followed by station 1 (~45 m⁻²). 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^{-4} 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⁻¹) 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 \(\textit{B-D-glucosidase}\) activity was determined by the colorimetric method described by Timothy et al. (2004). One gram soil was incubated with \(\textit{B-D-glucopyranoside}\) (substrate) at pH 6.0 and 37 °C. After 1 h of incubation, 0.5 M CaCl2 and pH 12.0 modified universal buffer were added to extract \(p\)-nitrophenol. The amount of \(p\)-nitrophenol released

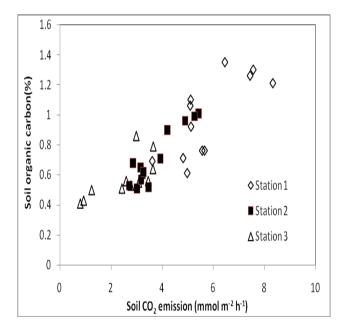


Fig 3. Correlation graph for soil organic carbon and soil emission rate in the deep forest region (station 1), rooted region (station 2) and un-rooted region (station 3).

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 CO₂ emission rate from soil

Rate of emission of soil CO2 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 preevacuated 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 CO₂ in the air sample was determined by comparison of peak areas for samples and standard followed by the compensation for the ambient CH₄.

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

Month	рН				Temp		Salinity			
	St_1	St_2	St_3	St_1	St_2	St_3	St_1	St_2	St_3	
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⁻¹ dry wt. of soil), silicate (μ g g⁻¹ dry wt. of soil), and total microbial population (CFU \times 10⁶ g⁻¹ dry soil) of soil at different stations.

Month	Org.C		$\mathrm{S} ext{-}\mathrm{SO}_4^{-2}$		$\mathrm{Si} ext{-}\mathrm{SiO_4^{-2}}$			Total Microbial population				
	St_1	St_2	St_3	St_1	St_2	St_3	St_1	St_2	St_3	St_1	St_2	St_3
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.

		Sum of		Mean Sum of		
Variable	Source	squares	df	squares	F	P
S	Station	54.4	2	27.2	4.89	0.015
	Season	62.61	2	31.3	5.64	0.009
pН	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	Station	123.1	2	61.6	8.88	0.001
population	Season	25.67	2	12.8	1.85	0.136

Statistical analyses

way ANOVA test was performed considering the seasons and stations 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 CO₂ emission (dependent from $_{
m the}$ mangrove 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 (premonsoon, monsoon and post-monsoon) was not statistically significant.

Irrespective of the sampling locations, the highest and the lowest soil CO2 emission was recorded during post-monsoon and monsoon seasons, respectively. Among the three different locations, deep forest region consistently showed the highest soil CO₂ emission with seasonal mean of 5.37 ± 0.87 , 4.63 ± 0.56 and 7.45 ± 0.92 mmol C m⁻²h⁻¹ respectively during the pre-monsoon, monsoon and post-monsoon periods. In contrary, the unrooted region with frequent tidal influences showed lowest soil CO2 emission compared to the other regions in all the three seasons. Spatial variation in soil CO2 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 CO₂ emission was recorded maximum during postmonsoon 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 CO2 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 CO₂ fluxes from mangrove sediments at different parts of the globe.

	CO ₂ fluxes (mm	ol C m ⁻² h ⁻¹)	
Location	Low	High	References
Mangroves from Semi-arid condition (New Caledonia)	-0.71	2.41	Leopold et al. 2015
Subtropical mangrove (China)	0.69	20.56	Chen et al. 2010
Subtropical mangrove (China)	0.24	31.23	Chen <i>et al.</i> 2012
Tropical mangrove (Indonesia)	-1.34	3.88	Chen et al. 2014
Subtropical mangrove (China)	0.35	6.05	Jin et al. 2013
Tropical mangrove (Tanzania) Varied mangrove (Caribbean, Australia, New-Zealand)	1.17 -0.97	4.67 10.69	Kristensen <i>et al.</i> 2011; Lovelock 2008; Lovelock <i>et al.</i> 2014
Tropical mangrove (Thailand)	1.67	3.21	Poungparn et al. 2009
Globally compilation data from mangroves	0.25	10.04	Bouillon et al. 2008
Tropical mangrove (India) Tropical mangroves (India)	0.15	2.32	Chanda et al. 2013
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 CO2 emissions indicated the mangrove soil as a persistent source of CO2 to the atmosphere (Table 4). However, measurements did not account for the fact that certain parts of the mangrove NPP can be mineralized and/or emitted toward the atmosphere as CO₂ after export to adjacent ecosystems.

The present study revealed significant spatiotemporal variation (P < 0.05) in soil CO₂ emission within the mangrove ecosystem, mostly depending on the varying degree of supply and removal of organic carbon. However, the CO₂ emission rates (0.8–8.34 mmol m⁻² hr⁻¹)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 CO₂ 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 CO₂ to the atmosphere.

Strong correlation between soil organic carbon and CO₂ emission in all the three stations (Fig. 3) and their seasonal variations indicated the importance of autochthonous carbon sources to the bacterial mineralization in theses ecosystems. According to Raich & Tufekcioglu (2000) under favorable conditions, enhanced supply of organic carbon may significantly increase the heterotrophic microbial activity and subsequent soil CO₂ emission rates. Similarly, total microbial load showed a positive correlation with the soil CO₂ 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 CO₂ when they utilize organic C (metabolizable) as a substrate for their maintenance and growth. The present result signifies the role of microbial community in the heterotrophic supporting 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_2 emission (F_{CO2}), 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, $r^2 = coefficient$ of multiple determination (i.e. the cumulative percentage of the response variable variation that is explained by the linear model).

Station 1	df	SS	f	P	r ² (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 $F_{CO2} = -3.03 + 0.0345$ glucosid dehydrogenase activity		16.14 - 0.00177 Phos	6.34 sphatase Activity	0.018 y + 0.0035	83.4 Urease Activity -0.00365
Station 2					
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 $F_{CO2} = -3.51 + 0.0092$ glucoside dehydrogenase activity		8.74 - 0.000254 pho	12.17 osphatase Activit	0.003 ty + 0.0156	81.2 Urease Activity + 0.00642
Station 3					
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 $F_{CO2} = -3.25 + 0.0009$ glucoside dehydrogenase activity	-	7.82 - 0.000675 Pho	3.54 osphatase Activit	0.043 ty + 0.0143	68.9 Urease Activity + 0.00750

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 CO₂ emission from the soil. In contrary to this, soil temperature, in these conditions, showed guite significant control over soil emission rates (F_{CO2} = $37.1-0.60 \text{ Temp } [R^2 = 39.9\%, P=0.046, df=41]). \text{ This}$ finding can be explained by the high temperature sensitivity of the microbial population responsible for soil CO₂ 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 CO2 emission from Sundarban mangrove forest. To evaluate the relative importance of these soil enzyme activities on CO₂ efflux from the mangrove soil, the stepwise multiple regression procedure was applied and explained variability of Fco2 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_2 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 CO₂ (Hugh et al. 2005). The future increase in atmospheric CO₂ 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 NH₃ and CO₂ and the ammonia released may be converted to ammonium ion and then easily be absorbed by plant roots, but released CO2 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 CO₂ emissions was observed at station 3, where the root system was absent. Mangrove pneumato phores 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 dehydrogenase activity and subsequent soil CO2 emission process. Włodarczyk et al. (2002) showed that the emissions of CO₂ 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 CO₂ 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 CO₂ 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_2 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_2 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_2 emission from these mangrove forests.

The results depict that CO₂ 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.

References

- Alef, K. 1995. Dehydrogenase activity. pp. 228–231. In: K. Alef & P. Nannipieri (eds.) Methods in Applied Soil Microbiology and Biochemistry. Academic Press, San Diego, California.
- Bano, N., M-U. Nisa, N. Khan, M. Saleem, P. J. Harrison, S. I. Ahmed & F. Azam. 1997. Significance of bacteria in the flux of organic matter in the tidal creeks of the mangrove ecosystem of the Indus river delta, Pakistan. *Marine Ecological Progress Sereis* 157: 1–12.
- Bartlett, K. B., D. S. Bartlett, R. C. Harris & D. I. Sebacher. 1987. Methane emission along a salt marsh salinity gradient. *Biogeochemistry* 4: 183–202.
- Bates, D., M. Maechler, B. Bolker, S. Walker. 2014. lme 4: Linear mixed-effects models using Eigen and S4. R package version 1:1–7.
- Bohn, H. L. 1982. Estimate of organic carbon in world soils. Soil Science Society of America Journal 40: 468–470.
- Bouillon, S., A. V. Borges, E. Castañeda-Moya, K. Diele, T. Dittmar, N. C. Duke, E. Kristensen, S. Y. Lee, C. Marchand, J. J. Middelburg, V. H. Rivera-Monroy, J. Thomas, T. J. Smith & R. R. Twilley. 2008. Mangrove production and carbon sinks: a revision of global budget estimates. Global Biogeochemical Cycles 22: (GB2013) 1–12.
- Brookes, P. C. 1995. The use of microbial parameters in monitoring soil pollution by heavy metals. *Biology and Fertility Soils* 19: 269–279.
- Chanda, A., A. Akhand, S. Manna, S. Dutta & S. Hazra. 2011. Summer fluxes of CO₂ from soil, in the coastal margin of world's largest mangrove patch of Sundarbans First report. *Journal of Basic and Applied Scientific Research* 1: 2137–2141
- Chanda, A., A. Akhand, S. Manna, S. Dutta, I. Das & S. Hazra. 2013. Spatial variability of atmosphere-biosphere CO₂ and H₂O exchange in selected sites of Indian Sundarbans during summer. *Tropical Ecology* **54**: 167–178.
- Chen, G. C., N. F. Y. Tam & Y. Ye. 2010. Summer fluxes of atmospheric greenhouse gases N₂O, CH₄ and CO₂ from mangrove soil in South China. Science of the Total Environment 408: 2761–2767.
- Chen, G. C., N. F. Y. Tam & Y. Ye. 2012. Spatial and seasonal variations of atmospheric N₂O and CO₂ fluxes from a subtropical mangrove swamp and

- their relationships with soil characteristics. Soil Biology and Biochemistry 48: 175–181.
- Chen, G. C., Y. I. Ulumuddin, S. Pramudji, Y. C. Chen, B. Chen. B, Y. Ye, D. Y. Ou, Z. Y. Ma, H. Huang & J. K. Wang. 2014. Rich soil carbon and nitrogen but low atmospheric greenhouse gas fluxes from North Sulawesi mangrove swamps in Indonesia. Science of the Total Environment 487: 91–96.
- Davidson, E. A., E. Belk & R. D. Boone. 1998. Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hard wood forest. Global Change Biology 4: 217–227.
- Dey, M., D. Ganguly, C. Chowdhury, N. Majumder & T. K. Jana. 2012. Intra-Annual Variation of Modern Foraminiferal Assemblage in a Tropical Mangrove Ecosystem in India. Wetlands 32: 813–826.
- Edwards, N. T. & B. M. Ross-Todd. 1983. Soil carbon dynamics in a mixed deciduous forest following clear-cutting with and without residue removal. Soil Science Society of America Journal 47: 1014–1021.
- Eswaran, H., E. Van den Berg & P. Reich. 1993. Organic carbon in soils of the world. *Soil Science Society of American Journal* **57**: 192–194.
- Eswaran, H., E. Van den Berg, P. Reich. & J. Kimble J. 1995. Global soil carbon resources. pp. 27–43. *In*: R. Lal, J. Kimble, E. Levine & B. A. Stewart. (eds.) *Soil and Global Change*. CRC Press, Boca Raton, FL, U.S.A.
- Ewel, K. C., W. P. Cropper, H. L. Gholz. 1987. Soil CO₂ evolution in Florida slash pine plantations. I. Changing through time. Canadian Journal of Forest Research 17: 325–329.
- Ganguly, D., M. Dey, S. K. Mandal, T. K. De & T. K. Jana. 2008. Energy dynamics and its implication to biosphere–atmosphere exchange of CO₂, H₂O and CH₄ in a tropical mangrove forest canopy. *Atmospheric Environment* 42: 4172–4184.
- Grasshoff, K., M. Ehrhardt & K. Kremling. 1983. *Methods of Sea Water Analysis*. 2nd edn. Verlag Chemie, Weinheim.
- Hanson, P. J., S. D. Wullschleger, S. A. Bohlman & D. E. Todd. 1993. Seasonal and topographic patterns of forest floor CO₂ efflux from an upland oak forest. *Tree Physiology* 13: 1–15.
- Hugh, A. L. H., D. J. John, B. F. Christopher & M. V. Peter. 2005. Interactive effects of elevated CO₂, N deposition and climate change on extracellular enzyme activity and soil density fractionation in California annual grassland. Global Change Biology 11: 1808–1815.

Jenkinson, D. S., D. E. Adams & A. Wild. 1991. Model estimates of CO₂ emissions from soil in response to global warming. *Nature* **351**: 304–306.

- Jin, L., C. Y. Lu, Y. Ye & G. F. Ye. 2013. Soil respiration in a subtropical mangrove wetland in the Jiulong river estuary, China. *Pedosphere* 23: 678–685.
- Kirschbaum, M. U. F. 1995. The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. Soil Biology and Biochemistry 27: 753–760.
- Kristensen, E. & D. M. Alongi. 2006. Control by fiddler crabs (*Uca vocans*) and plant roots (*Avicennia marina*) on carbon, iron, and sulfur biogeochemistry in mangrove sediment. *Limnology and Oceanography* **51**: 1557–1571.
- Kristensen, E., S. Bouillon, T. Dittmar & C. Marchand. 2008. Organic carbon dynamics in mangrove ecosystems: A review. Aquatic Botany 89: 201–219.
- Kristensen, E., P. Mangion, M. Tang, M. R. Flindt, M. Holmer & S. Ulomi. 2011. Microbial carbon oxidation rates and pathways in sediments of two Tanzanian mangrove forests. Biogeochemistry 103: 143–158.
- Kristensen. E. R. & Pilgaard. 2001. The role of fecal pellet deposition by leaf eating sesarmid crabs on litter decomposition in a mangrove sediment (Phuket, Thailand). pp. 369–384. *In*: J. Y. Aller, S. A. Woodin & R. C. Aller. (eds.) *Organism-Sediment Interactions*. University of South Carolina Press, Columbia.
- Leopold, A., C. Marchand, J. Deborde & M. Allenbach. 2015. Temporal variability of CO₂ fluxes at the sediment-air interface in mangroves (New Caledonia). Science of the Total Environment **502**: 617–626.
- Litton, C. M., M. G. Rayan, D. H. Knight & P. D. Stahl. 2003. Soil-surface carbon dioxide efflux and microbial biomass in relation to tree density 13 years after a stand replacing fire in a lodgepole pine ecosystem. *Global Change Biology* 9: 680–696.
- Lloyd, J. & J. A. Taylor. 1994. On the temperature dependence of soil respiration. *Functional Ecology* 8: 315–323.
- Lovelock, C. E. 2008. Soil respiration and belowground carbon allocation in mangrove forests. *Ecosystems* 11: 342–354.
- Lovelock, C. E., M. F. Adame, V. Bennion, M. Hayes, J. O'Mara, R. Reef & N. S. Santini. 2014. Contemporary rates of carbon sequestration through vertical accretion of sediments in

- mangrove forests and saltmarshes of south east queensland, Australia. *Estuaries and Coasts* **37:** 763–771.
- Lyimo, T. J., A. Pol & H. J. M. Op den Camp. 2002. Methane emission, sulphide concentration and redox potential profiles in mtoni mangrove sediment, Tanzania Western Indian Ocean Journal of Marine Science 1: 71–80.
- Mersi, W. & F. Schinner. 1991. An improved and accurate method for determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride. Biology and Fertility of Soils 11: 216–220.
- Mussa, S. A. B., H. S. Elferjani, F. A. Haroun & F. F. Abdelnabi. 2009. Determination of available nitrate, phosphate and sulfate in soil samples. *International Journal of PharmTech Research* 1: 598–604.
- Nakane, K. & N. J. Lee. 1995. Simulation of soil carbon cycling and carbon balance following clear-cutting in a mid-temperate forest and contribution to the sink of atmosphere. *Vegetatio* **121**: 147–156.
- Nannipieri, P., S. Gregos & B. Ceccanti. 1990. Ecological significance of the biological activity in soil. pp. 293–355. In: J. M. Bollag & G. Stotzy (eds.) Soil Biochemistry. Vol. 6. Marcel Dekker, New York, USA.
- Paolini, J. E & L. E. Sánchez-Arias. 2008. Comparative biochemical study of the rhizosphere of *Rhizophora mangle* and its associated species *Cyperus* sp. in the Ciénaga de Soledad (Colombia). *pp:* 79–84. In: H. Lieth, M. G. Sucre & B. Herzog (eds.) *Mangroves and Halophytes: Restoration and Utilisation*. Springer, Dordrecht.
- Poungparn, S., A. Komiyama, A. Tanaka, T. Sangtiean, C. Maknual, S. Kato, P. Tanapeampool & P. Patanaponpaiboon. 2009. Carbon di oxide emission through soil respiration in a secondary mangrove forest of eastern Thailand. *Journal of Tropical Ecology* 25: 393–400.
- Raich, J. W. & A. Tufekcioglu. 2000. Vegetation and soil respiration: correlations and controls. *Biogeochemistry* **48:** 71–90.
- Raich, J. W. & C. S. Potter. 1995. Global patterns of carbon dioxide emissions from soils. *Global Biogeochemical Cycles* 9: 23–36.
- Raich, J. W. & W. H. Schlesinger. 1992. The global carbon dioxide flux in soil respiration and its relationship to climate. *Tellus* 44B: 81–99.
- Ramanathan, A. L., G. Singh, J. Majumder, A. C. Samal, R. Chowhan, R. K. Rayan, K. Roykumar & S. C. Santra. 2008. A study of microbial diversity and its interaction with nutrients in the sediments

- of Sundarban mangroves. *Indian Journal of Marine Science* **37**: 159–165.
- Ray, R., D. Ganguly, C. Chowdhury, M. Dey, S. Das, M. K. Dutta, S. K. Mondal, N. Majumder, T. K. De, S. K. Mukhopadhyay & T. K. Jana. 2011. Carbon sequestration and annual increase of carbon stock in a mangrove forest. *Atmospheric Environment* 45: 5016–5024.
- Richards, L. A. (ed). 1968. *Diagnosis and Improvement* of Saline and Alkali Soils. USDA Agriculture Hand Book No. 60. Oxford and IBH Publishing Co., New Delhi.
- Russell, C. A. & R. P. Voroney. 1998. Carbon dioxide efflux from the floor of a boreal aspen forest. I. Relationship to environmental variables and estimates of C respired. *Canadian Journal of Soil Science* 78: 301–310.
- Schleser, G. H. 1982. The response of CO₂ evolution from soils to global temperature changes. Zeitschrift für Naturforsch 37: 287–291.
- Shao, X., W. Yang & M. Wu 2015. Seasonal dynamics of soil labile organic carbon and enzyme activities in relation to vegetation types in hangzhou bay tidal flat wetland. *PLoS ONE* **10**: e0142677.
- Subhani, A., H. Changyong, X. Zhengmiao, L. Min & A. M. El-ghamry. 2001. Impact of Soil environment and agronomic practices on microbial/dehydrogenase enzyme activity in soil. A Review. *Pakistan Journal of Biological Sciences* 4: 333–338.
- Tabatabai, M. A. 1994. Soil enzymes. pp: 775–833. In:
 S H. Mickelson (ed.) Methods of Soil Analysis, Part
 2. Microbiological and Biochemical Properties.
 Soil Science Society of America, Madison, WI.
- Timothy, R., K. Richard & P. Dick. 2004. Differentiating microbial and stabilized β-glucosidase activity relative to soil quality. *Soil Biology and Biochemistry* **36**: 2089–2096.
- Tiwari, S. C., B. K. Tiwari & R. R. Mishra. 1989. Microbial community, enzyme activity and CO₂ evolution in pineapple orchard soil. *Journal of Tropical Ecology* **30**: 265–273.
- Toland, D. E. & D. R. Zak. 1994. Seasonal patterns of soil respiration in intact and clear-cut northern hardwood forests. Canadian Journal of Forest Research 24: 1711–1716.
- Van der Nat, F. J. W. A. & J. J. Middelburg. 2000. Methane emission from tidal freshwater marsh. *Biogeochemistry* 49: 103–121.
- Walkley, A. & I. A. Black. 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science 37: 29–37.

- Wlodarczyk, T., W. Stepniewski & M. Brzezinska. 2002. Dehydrogenase activity, redox potential, and emissions of carbon dioxide and nitrous oxide from Cambisols under flooding conditions. *Biology and Fertility of Soils* 36: 200–206.
- Xu, M. & I. Qi. 2001. Soil-surface CO₂ efflux and its spatial and temporal variations in a young
- ponderosa pine plantation in northern California. Global Change Biology 7: 667– 677.
- Zhou, Q. H., Z. B. Wu, S. P. Cheng, F. He & G. P. Fu. 2005. Enzymatic activities in constructed wetlands and di-n-butyl phthalate (DBP) biodegradation. Soil Biology and Biochemistry 37: 1454–1459.

(Received on 07.01.2016 and accepted after revisions, on 02.01.2017)