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SHORT COMMUNICATION

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Microbial biomass and its activities in salt-affected coastal soils

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Abstract Seasonal fluctuations in salinity are typical in coastal soils due to the intrusion of seawater in the groundwater. We studied the effect of salinity on the microbial and biochemical parameters of the salt-affected soils of the coastal region of Bay of Bengal, Sundarbans, India. The average pH values and average organic C (OC) contents of soils from nine different sites cultivated with rice (*Oryza sativa*) ranged from 4.8 to 7.8 and from 5.2 to 14.1 g kg^{-1}, respectively. The average electrical conductivity of the saturation extract (EC_e) during the summer season was about five times higher than that during the monsoon season. Within the nine sites, three soils (S_3, S_4, and S_5) were the most saline. The average microbial biomass C (MBC), average basal soil respiration (BSR), and average fluorescein diacetate hydrolyzing activity (FDHA) were lowest during the summer season, indicating a negative influence of soil salinity. About 59%, 50%, and 20% variation in MBC/OC, FDHA/OC, and BSR/MBC (metabolic quotient, qCO_2), respectively, which are indicators of environmental stress, could be explained by the variation in EC_e. The decrease in MBC and microbial activities with a rise in salinity is probably one of the reasons for the poor crop growth in salt-affected coastal soils.

Keywords Microbial biomass carbon · Soil respiration · Fluorescein diacetate hydrolyzing activity · Metabolic quotient · Salt-affected coastal soils

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Introduction

Coastal soils are generally salt-affected due to certain hydrologic and geographical reasons, and the salinity in coastal areas is the main factor for poor crop yields (Kaur et al. 1998). In India, 8 Mha of land is salt-affected, of which more than 2 Mha is in coastal regions. Monoculture of rice is predominant during the monsoon season in these areas with poor yields (Lenka 1996). A plethora of information is available on the effects of salinity on the physical and chemical properties of soil (Sardinha et al. 2003); however, soil microbial aspects of natural saline environments are scantily studied (Zahran 1997; Rietz and Haynes 2003). Soil microbial communities and their activity are greatly influenced by salinity (Rietz and Haynes 2003), which is a concern because microbial processes in soils control ecological function and soil fertility. In addition, abiotic and biotic environmental variables can affect microbial parameters (Alexander 1977). Microbial biomass, soil respiration, and fluorescein diacetate hydrolyzing activity (FDHA) can be measured as sensitive indicators of ecosystem development and disturbance. Brookes (1995) emphasized that in case of nonexperimental field data, microbial biomass C (MBC) as percentage of soil organic C (OC) content may itself constitute an internal control and provide information on early warning of soil disturbances. Anderson (2003) pointed out that the MBC/OC ratio and the metabolic quotient (qCO_2) could be used as more sensitive indicators of soil microbial response to land use, soil management, and environmental variables. Dick (1994)

suggested that a correct comparison between soils under different environmental conditions and management could be made based on the enzyme activities and OC content of the soil. We studied the influence of salinity in different seasons on the MBC, basal soil respiration (BSR), and FDHA of some agricultural soils. We also tried to understand the relationships between salinity as a stress factor and different soil microbial variables.

Materials and methods

Study site and soil sampling

The study site is situated at the experimental farm of Central Soil Salinity Research Institute, Regional Research Station, Canning Town (22°15′N and 88°40′E), in a coastal saline region of Bay of Bengal, Sundarbans, West Bengal, India. Soils with variable salinity having acidic, neutral, and alkaline pH were selected from different sites cultivated with rice (*Oryza sativa*), which is the major land use in the region. The region is typically monsoonal, with three distinct seasons in a year: a warm and wet rainy season (June to September), a mild winter (October to February), and a hot and relatively dry summer (March to May). The long-term (1983 to 2003) yearly average rainfall is 1,886 mm; the average maximum and minimum temperatures are 34.5°C (May) and 12.5°C (January), respectively. The average maximum relative humidity reaches 97% during September, and the minimum, 38% in March. The soils were classified as typic endoaquents, and three replicate soil samples (100 g) were taken from the A horizon (0–20 cm soil depth) of each study site during the monsoon, winter, and summer seasons of 2003 through 2004. The soils were sieved (<2 mm) after sorting out the plant debris and visible fauna. Microbiological parameters were monitored on field moist soil samples, and physicochemical properties, on air-dried soil samples.

Analytical procedure

Soil pH was measured in a 1:2.5 soil–water suspension using a glass electrode. Soil EC_e was determined by measuring the electrical conductance of soil saturation extract with a conductivity meter (United States Department of Agriculture [USDA] 1954). Soil OC was determined according to Nelson and Somers (1975). MBC was determined by the fumigation extraction method using a correction factor (K_{ec}) of 0.38 according to Vance et al. (1987). The BSR was measured according to the method of Alef (1995a), modified in the following way: 20 g of moist soil was placed in a 500-mL airtight conical flask with a vial containing 10 mL 1 M NaOH. The sample was incubated for 10 days in the dark at 25°C. The CO_2–C evolved was determined every 2 days by titration and was calculated based on cumulative CO_2 evolution over a 10-day period. FDHA of the soils was measured, as reported by Schnürer and Rosswall (1982), under shaking for 3 h at 24°C.

Statistical analyses

Regression between soil microbial parameters and EC_e was fitted to linear, power, quadratic, and polynomial functions, and the best fits were graphically represented. Correlations were determined on the basis of the Pearson product–moment correlation coefficient with the help of Microsoft Excel software. Statistical analyses using STATISTICA 6.0 (Statsoft Inc., USA) were carried out with three replicated data of the soil parameters. Season and soil were considered as two treatment factors. The factor season had three levels, i.e., monsoon, summer, and winter. The soil had nine levels depending upon soil properties, as given in Table 1. The upper series in Table 1 represents the data of each season averaged over nine soils collected from different sites. The lower series represents the data for each soil averaged over three seasons. Assigning season to the main plot and soil to the subplot, analysis of variance (ANOVA) was carried out by split-plot design. The least significant difference (LSD) test was applied to evaluate the significance of the differences between the individual treatments.

Results and discussion

The average soil pH (Table 1) did not differ significantly ($P \leq 0.05$) between the seasons. Soils collected from the different sites exhibited distinct variation in soil pH, ranging from 4.8 to 7.8. Most of the soils were acidic, except for S_1 and S_2. Bandyapadhyay et al. (2003) reported that both saline (high pH) and saline acid soils (low pH) are distinctive features of the coastal saline soils. Seasonality had an important bearing on the EC_e of soil (Table 1). In the monsoon season, the average EC_e was 2.7 dS m^{-1}, and then the average among the various soil samples increased during the winter season (8.3 dS m^{-1}) and peaked in the summer season (14.0 dS m^{-1}). Salinity is seldom constant with time or uniform in space, and fluctuating trends in salinity levels generally occur in coastal saline soils (Bandyapadhyay et al. 1982). During the season of limited rainfall, a drought-type condition prevails and water is lost from the system. Shallow groundwater in the coastal region is saline, and rapid evaporation during the summer months brings the salts on the surface through capillary action. Thus the salts get deposited on the upper surface of the soil, increasing the salinity of the A horizon (0–20 cm). There was considerable variation in the EC_e values of the soils from the different sites, ranging from 2.2 to 16.3 dS m^{-1}. The S_1 (2.2 dS m^{-1}), S_8 (3.1 dS m^{-1}), and S_2 (3.5 dS m^{-1}) soils were nonsaline soils, whereas the remaining soils were saline according to the classification by USDA (1954). The average OC content of soil varied significantly ($P \leq 0.05$) between the seasons, with the highest value in the winter season (10.1 g kg^{-1}), whereas values for the summer and monsoon seasons were statistically similar (Table 1). Since soil samples were collected from arable lands cultivated with rice during the monsoon season, the leftover stubbles and root masses might have contributed to the increase in

Table 1 Physicochemical and microbial properties of the soils under study

Treatment	pH (1:2.5 water)	EC_e (dS m^{-1})	OC (g kg^{-1})	MBC (μg g^{-1} soil)	BSR (μg CO_2–C g^{-1} soil h^{-1} at 25°C)	FDHA (μg fluorescein g^{-1} soil h^{-1} at 24°C)
Season (S) (average values of soils in different seasons)						
Monsoon	6.2	2.7	9.1	305	1.3	75
Winter	6.16	8.3	10.1	308	1.3	63
Summer	6.16	14.0	9.2	253	1.5	49
LSD ($P \leq 0.05$)	NS	0.16	0.24	8.97	0.066	3.51
Soil (L) (average of three seasons in different soils)						
S_1	7.2	2.2	14.1	446	2.2	111
S_2	7.8	3.5	8.7	274	1.4	56
S_3	4.8	14.7	10.2	268	1.2	32
S_4	6.1	16.3	6.9	199	0.9	29
S_5	6.7	15.7	5.2	125	0.7	33
S_6	5.2	6.4	8.9	284	1.0	53
S_7	5.5	7.1	10.4	312	1.7	61
S_8	7.2	3.1	10.7	344	1.3	102
S_9	5.13	5.3	10.3	346	2.0	82
LSD ($P \leq 0.05$)	0.32	0.27	0.42	15.74	0.115	6.08
S×L	0.56	0.47	0.73	27.27	0.2	10.54

NS not significant

the OC content during the winter season, when crops were harvested in December. The average OC content varied between the sites, ranging from 5.2 g kg^{-1} in S_5 to 14.1 g kg^{-1} in S_1 during different seasons. Soil pH did not seem to exert any significant effect on OC ($r = 0.08$, $P \leq 0.05$, $n = 81$), whereas the OC content of soil decreased by increasing salinity ($r = -0.39$, $P \leq 0.01$, $n = 81$). Kaur et al. (1998) also indicated a significant negative relationship between the OC content and the EC_e value of the soil.

The average MBC of soil was greatly influenced by the time of soil sampling (Table 1). The highest average value was recorded in the winter samples (308 μg g^{-1} soil), but this value was not statistically different with respect to that of the monsoon samples (305 μg g^{-1} soil). The summer samples recorded the lowest average value (253 μg g^{-1} soil) when salinity was the highest and hot dry spell prevailed. Soil desiccation due to dry weather (Van Gestel et al. 1992) coupled with increase in soil salinity (Batra and Manna 1997; Rietz and Haynes 2003) can decrease the MBC value during the summer season. Sardinha et al. (2003) suggested that salinization has stronger effects on soil microbial properties than heavy-metal pollution observed at other sites has (Chander et al. 2001), and it is probably one of the most stressing environmental conditions for microbial growth and proliferation in soil. The presence of measurable soil MBC during summer indicated that some microbial species have adapted to salinity in these soils. A wide range (125–446 μg g^{-1}) of average MBC, averaged over seasons, could be detected in the different sites under study, and the values were higher in soils with higher OC contents (Sparling 1997). The MBC contents and the corresponding EC_e values of the soil samples during all the seasons were linked by an exponential relationship (Fig. 1). About 53% variation in MBC could be explained by the variation in EC_e. Our results substantiate a pattern found in naturally occurring saline soils, where microbial biomass is usually negatively correlated with total soluble salt content (Mallouhi and Jacquin 1985; Ragab 1993; Garcia et al. 1994).

There was a statistically significant difference in the BSR values of the soils collected in different seasons (Table 1). The average BSR in the summer season (1.5 μg CO_2–C g^{-1} soil h^{-1} at 25°C) was higher than in the monsoon and winter seasons (1.3 μg CO_2–C g^{-1} soil h^{-1} at 25°C). Variability in soil respiration mainly depended on weather variables (Kucera and Kirkham 1971) and soil moisture (Gupta and Singh 1981), along with the salt content of salt-affected soils (Garcia and Hernandez 1996). The soils collected from the different sites showed wide differences in BSR values, varying from 0.7 to 2.2 μg CO_2–C g^{-1} soil h^{-1} at 25°C. The soils with higher OC respired more CO_2. The BSR is related to the amount of available C and may not be related to microbial biomass in soil (Nannipieri et al. 1990). According to what was reported by Sommers et al. (1981), the average BSR values of these soils decreased with salinity, irrespective of season. Figure 1 shows that BSR exponentially decreased by increasing the EC_e value, and the variation caused by EC_e was 33%, which could be considered low. Nonactive microorganisms, including dormant forms, can prevail in microbial communities of natural ecosystems (Heinemeyer et al. 1999). It may be possible that basal respiration can also reflect the activity of dormant populations (Ohya et al. 1988); thus, the lesser magnitude of the effect of EC_e on BSR may be due to the ability of the dormant soil microbial populations to overcome the stress caused by salinity.

Hydrolysis of fluorescein esters has been used to measure microbial activity in environmental samples because it reflects protease, lipase, and esterase activities (Schnürer and Rosswall 1982). The average among soil FDHAs showed clear seasonal variation (Table 1), with the highest value in the monsoon season (75 μg fluorescein g^{-1} soil h^{-1}

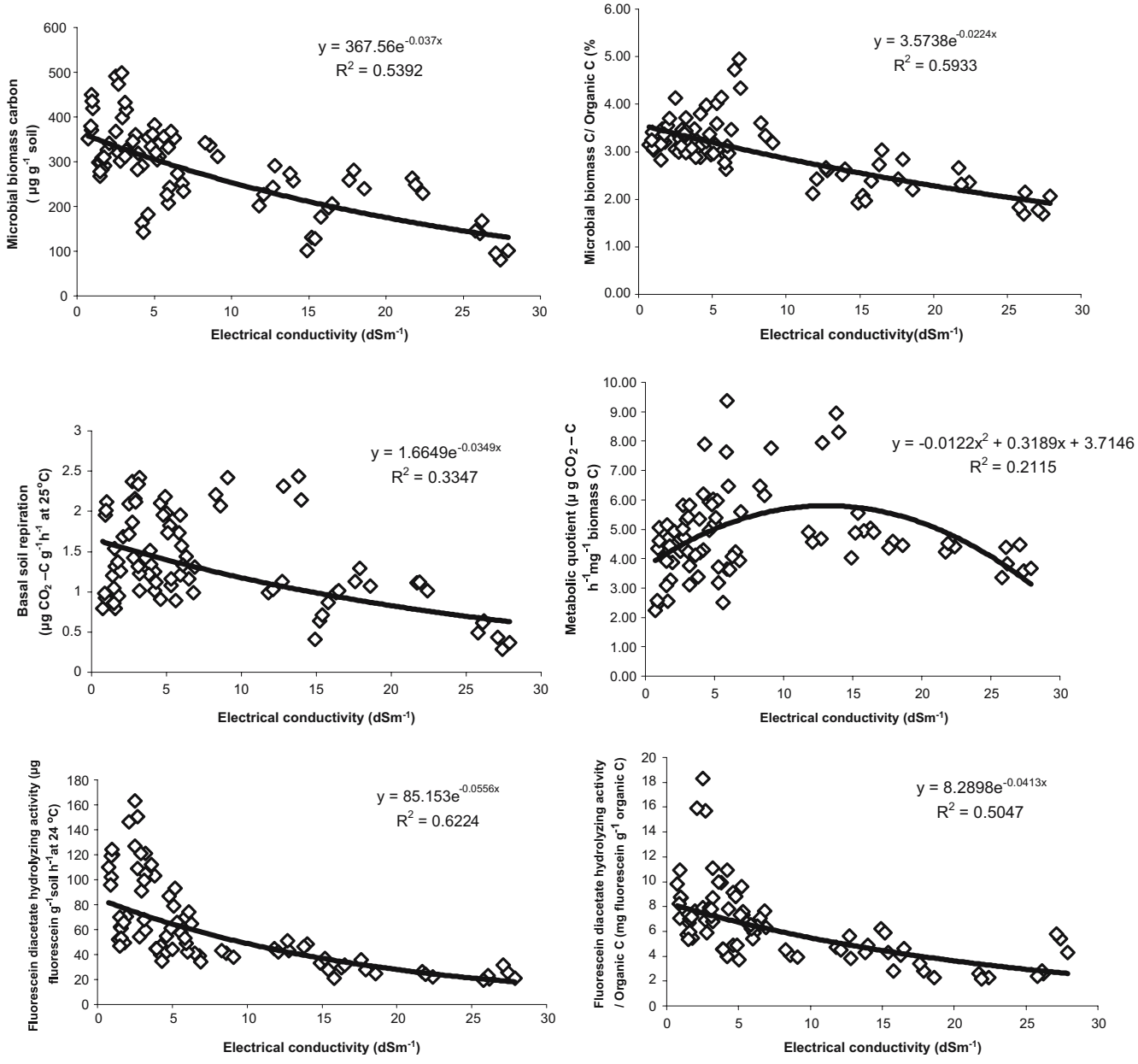

Fig. 1 Relationship of microbial parameters and electrical conductivity of the salt-affected coastal soils

at 24°C) and the average values decreasing to 63 µg fluorescein g^{-1} soil h^{-1} at 24°C in the winter and 49 µg fluorescein g^{-1} soil h^{-1} at 24°C in the summer season. Active microbial cells transport fluorescein diacetate inside the cell, where it is hydrolyzed into polar fluorescein (Alef 1995b), and then, when the storage capacity of the cell with respect to fluorescein is exceeded, it is released in the extracellular environment (Schnürer and Rosswall 1982). The remarkable reduction of FDHA during summer might be attributed to the lower uptake of fluorescein diacetate by the soil microbial cells due to the high salt stress conditions. The average FDHA among seasons greatly varied among the soils from the different sites, and the respective pattern was similar to that of MBC and OC contents. The S_1 soil, which also had the highest MBC and OC, showed the maximum average FDHA (111 µg fluorescein g^{-1} soil h^{-1} at 24°C). There were positive correlations between the FDHA and OC ($r = 0.59$, $P \leq 0.01$, $n = 81$) and between the FDHA and MBC ($r = 0.74$, $P \leq 0.01$, $n = 81$) values. Several previous studies have reported a positive correlation between enzyme activities and the OC content (Burns 1978). The FDHA decreased in an exponential pattern (Fig. 1) by increasing salinity, which conforms to Rietz and Haynes (2003). The equation suggests that EC_e caused 62% variation in FDHA.

Both MBC/OC and FDHA/OC ratios decreased from monsoon to winter, with the lowest values in the summer season. The soils from the different sites showed wide variation in the percentage of MBC/OC and FDHA/OC. The MBC/OC percentages ranged from 1% to 4%, as

observed in other soils (Sardinha et al. 2003). Generally soils with higher EC_e values had lower ratios, and the relationship between MBC/OC and FDHA/OC and the salinity was exponential (Fig. 1). The regression equations demonstrated that 59% and 50% variation in MBC/OC and FDHA/OC, respectively, could be explained by the variation in EC_e. The qCO_2 values were higher in summer, probably as a result of stress by salinity on soil microflora (Anderson and Domsch 1993). The soils from the different sites showed statistically significant variation in qCO_2, possibly because their microbial communities showed different compositions. The qCO_2 showed a quadratic relationship with EC_e (Fig. 1). Probably, under high salinity conditions, a greater proportion of substrate C is lost as CO_2 for cell maintenance; however, only 21% variation in qCO_2 could be explained by the variation in EC_e.

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