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# Nitrogenase Activity in Intertidal Sediment Along the Tanzanian Coast, Western Indian Ocean

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***Key words:*** Nitrogen fixation, Acetylene Reduction Assay, diazotrophs, intertidal sediment, Tanzania

***Abstract***—Nitrogenase activity was determined using the acetylene reduction assay in the littoral areas near Tanga, Dar es Salaam and Mafia with the aim of investigating its spatial and temporal distribution along the Tanzanian coastal line. Ethylene ($C_2H_4$) production rates ranged from 0.07 - 13.5 nmol $C_2H_4$ $cm^{-2}$ $h^{-1}$ in Tanga area, 0.30 - 5.43 nmol $C_2H_4$ $cm^{-2}$ $h^{-1}$ in Dar es Salaam and from 0.10 - 6.25 nmol $C_2H_4$ $cm^{-2}$ $h^{-1}$ in Mafia. Significantly higher rates of nitrogenase activity were measured during night-incubated samples compared to daytime incubated samples suggesting that the sediments were mostly composed of non-heterocystous diazotrophic organisms. However, there were no significant differences in rates of nitrogenase activity between the rainy and dry season and between the sandy and muddy beaches. Also, there were no significant differences in nitrogenase activity between the upper, mid and lower littoral zones, and between the three sampling locations, i.e. Tanga, Dar es Salaam and Mafia. A nitrogen fixation rate of 38 mmol N $m^{-2}y^{-1}$ was estimated. We conclude that diazotrophs contribute a substantial amount of nitrogen input to the Tanzania coastal ecosystems.

## INTRODUCTION

Biological nitrogen fixation is an enzymatic (nitrogenase) process exclusively limited to some free-living and symbiotic prokaryotic organisms collectively known as diazotrophs. In nature the process of nitrogen fixation is of utmost importance because it counterbalances losses of combined nitrogen from the environment by denitrification and assimilation. In the marine environment nitrogen fixation process substantially contributes to 'new production' i.e., primary production associated with newly available nitrogen coming from outside the euphotic zone (Dugdale and Goering, 1967; Bergman, 2001). In the open ocean for example, Karl *et al.* (1997) estimated that *Trichodesmium* species alone, a filamentous colony forming planktonic cyanobacterium, contributes up to 50% of 'new production' in the subtropical North Pacific Ocean. The overall global oceanic nitrogen fixation is in the range of 1 - 2 g N x $10^{14}$ $yr^{-1}$, approaching modern estimates of oceanic denitrification (Karl *et al.*, 2002).

In the Tanzanian coastal waters rates of nitrogen fixation by *Trichodesmium* species in the surface waters were estimated by Lugomela *et al.* (2002) to be of the order of 42.7 mmol N $m^{-3}y^{-1}$. In a study conducted from one mangrove stand at Mazizini, Zanzibar, Lugomela and Bergman (2002) reported nitrogenase activity to range from 6.0 to 118.5 nmol $C_2H_4$ $cm^{-2}h^{-1}$ on mangrove pneumatophores and from 1.3 to 16.8 nmol $C_2H_4$

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$cm^{-2}h^{-1}$ in adjacent sediments. In addition, Kyaruzi *et al.* (2003) reported rates of nitrogen fixation to be in the order of 1.64 and 1.34 nmole N $m^{-2}$ $h^{-1}$ in sandy and muddy areas, respectively, of a mangrove ecosystem near Zanzibar town while Hamisi (2005) reported that the nitrogen fixation in bare sediments were significantly lower than in sediments under seagrasses.

However, studies on nitrogen fixation rates in open littoral areas (sand or muddy) without associated mangrove and/or seagrass vegetations along the Tanzanian coastline are missing. In addition, nitrogen budgets for most tropical coastal areas have not been established due to lack of enough qualitative and quantitative data on diazotrophy and other sources of nitrogen inputs as well as nitrogen outputs. The current study was aimed at assessing the spatial and temporal variability of nitrogenase activity in littoral sandy and muddy beaches along the Tanzanian coastline. It is expected that the data presented here will be useful when working out nitrogen budgets for marine coastal ecosystems in the area.

## MATERIAL AND METHODS

This study was conducted at three distantly located sites along the Tanzanian coast. These were Sahare, within Tanga municipality, Kunduchi, north of the central Dar es Salaam city, and Kilindoni in Mafia Island (Fig. 1). All sites visited had muddy and sandy beaches. Sandy shores were visually characterized by having large grain sizes and whitish brown in colour while muddy shores were soft with fine grain sizes and somewhat black in colour. Sahare site was fringed by a mangrove forest though samples were taken in the open areas away from the forest. At Kunduchi the mangrove

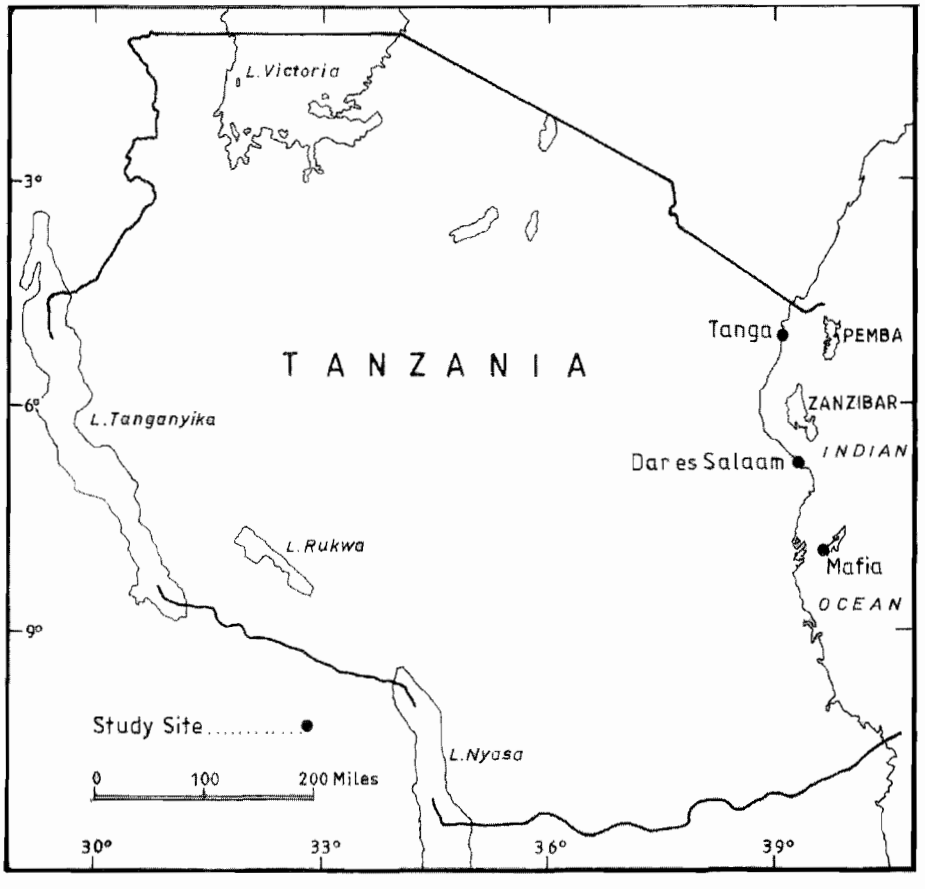



**Fig. 1. Map of Tanzania showing the study sites**

forest occurred on the side with muddy beach while sandy areas were found away from the mangroves. The Kilindoni site lacked mangroves. All sites were located close to landing sites for fishermen, as a result there were significant human activities taking place on the beaches e.g. trampling, some boat anchorage and collection of bivalves.

Samples for nutrients analyses were collected from intertidal pool waters, filtered through GF/F filters into acid-cleaned 50 ml plastic vials and immediately stored in a cool box containing ice. In the laboratory, nutrient samples were kept frozen at -20°C when immediate analyses were not possible. Analyses for $PO_4^+$, $NO_3^-$ and $NO_2^-$ were conducted according to Parsons *et al.* (1989). Quadruplicate sediment samples for Chlorophyll a (Chl. a) analysis were collected in a similar way as those intended for nitrogen fixation as described below. The samples were blotted dry using a tissue paper and kept in 10 ml serum bottles. In the laboratory 5 ml of 90% acetone was added to each serum bottle and left to extract the pigment overnight in a refrigerator (4°C). Following this, the samples were transferred into 10 ml centrifuge tubes and spinned at 10,000 rpm before decanting the pigment extracts (supernatant) into clean test tubes. Then the extracts were analyzed using a spectrophotometer as described by Parsons *et al.* (1989). Salinity and temperature were measured *in situ* using an ATAGO (Japan) refractometer and an alcohol thermometer, respectively.

Nitrogenase activity was measured using the acetylene reduction assay (Capone, 1993). At all sampling sites two transects were established, one in the sandy area and the other on the muddy area. Transects were perpendicular to the shoreline running from the upper littoral to the lower littoral zone. On every transect quadruplicate samples were collected on the upper littoral zone, mid-littoral zone and lower littoral zone during the spring low tide. Sediment samples were randomly collected using a corer made out of a 10 mm in diameter tip cut syringe. The corer was inserted to about 5 mm depth into the sediment and the samples collected into 10 ml serum bottles. The bottles were then sealed with gastight rubber stoppers and aluminum caps. On return to the shore 10% of the gas phase on the serum bottles were removed and replaced with the same volumes of acetylene gas generated from calcium carbide. Daytime incubations were done on shore receiving full sun light in a 10 litter capacity basin filled with seawater to maintain ambient temperature. All incubations were carried out for two hours between noon and 1500 hrs. All night incubations were conducted between 2200 to 2400 hrs in an empty dark box at room temperature.

After 2 hrs of incubation, 1 ml gas samples were withdrawn from the serum bottle by gas tight syringe and pierced (to seal) in a rubber block. Upon return to the laboratory, ethylene production was assayed using gas chromatography (HP 5890 series II) equipped with Flame Ionisation Detector (FID) and a column packed with porapack N, 80/100-mesh size. The carrier gas was nitrogen at 35 ml/min; oven, detection and injection port temperatures were 80, 200 and 170°C, respectively. The concentrations of ethylene were obtained from a calibration curve obtained using pure ethylene gas from a commercial cylinder (UCAR-Belgium).

## RESULTS

### Environmental variables

Results for the environmental parameters are shown on Table 1. Intertidal pool water temperatures ranged from 29.5 - 35°C in the sandy beaches and between 28.0- 36°C in the muddy beaches. Salinity ranged from 35 - 39‰ in the sandy beaches while in the muddy beaches it varied from 28 - 36‰. Nitrate concentration in the sandy beaches ranged from undetected values to 3.80 μM while nitrite concentrations in the sandy beaches ranged from undetectable values to 0.87 μM. Phosphate concentrations in the sandy beaches ranged from 0.03 - 0.17 μM. In muddy beaches the nitrate, nitrite and phosphate concentration ranges were undetectable values to 7.22 μM, undetectable values to 1.77 μM and 0.01 - 0.66 μM, respectively.

### Nitrogen fixation

The average nitrogenase activity in both rain and dry seasons at Tanga, Dar-es-Salaam and Mafia are shown in figure 2. During the rain season,

**Table 1. Physical and chemical parameters at the sampling sites during the rain season (April-May) and dry season (August-September) in 2003**

| Parameter | | Kunduchi | | | Tanga | | | Mafia | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Upper | Mid | Lower | Upper | Mid | Lower | Upper | Mid | Lower |
| Temperature | Sandy | 35.0/31.0 | 34.0/30.5 | 34.0/30.0 | 33.0/35.0 | 32.0/33.0 | 31.0/31.0 | 29.5/32.0 | 29.5/35.0 | 29.5/34.0 |
| °C | Muddy | 32.0/30 | 33.0/28.0 | 34.0/29.0 | 31.0/29.0 | 31.0/30.5 | 30.0/30.5 | 31.0/36.0 | 30.0/36.0 | 30.5/35.0 |
| Salinity | Sandy | 36.5/35.0 | 35.5/35.0 | 37.0/35.0 | 38.0/39.0 | 37.0/38.0 | 36.0/38.0 | 33.0/35.0 | 33.0/35.0 | 36.0/35.0 |
| (%) | Muddy | 36.0/36.0 | 36.0/37.0 | 38.0/37.0 | 37.0/38.0 | 36.0/38.0 | 36.0/38.0 | 38.0/36.0 | 38.0/36.0 | 36.0/36.0 |
| Nitrate | Sandy | 1.5/1.8 | 1.9/1.7 | 1.1/1.7 | Nd/1.7 | 0.5/1.6 | 0.5/1.8 | 0.8/3.8 | 0.5/1.9 | 0.4/1.7 |
| (μM) | Muddy | 1.7/1.7 | 1.7/1.9 | 1.7/7.2 | 2.0/1.9 | 0.6/1.6 | 0.4/1.7 | 0.5/3.0 | 0.6/1.6 | 0.5/1.6 |
| Nitrite | Sandy | 0.05/0.24 | 0.02/0.14 | 0.02/0.08 | Nd/0.19 | 0.02/0.19 | 0.13/0.19 | 0.08/0.87 | 0.02/0.24 | Nd/0.14 |
| (μM) | Muddy | 0.0/0.14 | 0.0/0.19 | 0.0/1.30 | 0.10/0.19 | 0.10/0.14 | 0.13/0.14 | 0.02/1.77 | 0.02/0.19 | 0.24/0.19 |
| Phosphate | Sandy | 0.03/0.09 | 0.03/0.05 | 0.05/0.74 | 0.04/0.09 | 0.05/0.17 | 0.08/0.26 | 0.16/1.67 | 0.04/1.35 | 0.04/0.17 |
| (μM) | Muddy | 0.07/0.66 | 0.07/0.13 | 0.07/0.09 | 0.57/0.34 | 0.07/0.09 | 0.08/0.01 | 0.04/0.26 | 0.04/0.09 | 0.08/0.05 |

Nd = not determined

nitrogenase activity at Tanga, Dar-es-Salaam and Mafia stations ranged from 0.07 to 13.5, 0.18 to 1.7 and from 0.36 to 5.43 nmol $C_2H_4$ $cm^{-2}$ $h^{-1}$, respectively. During the dry season the rates ranged from 0.04 to 3.14 in Tanga, while in Dar es Salaam and Mafia the ranges were from 0.10 to 6.25 and from 0.33 to 4.64 nmol $C_2H_4$ $cm^{-2}$ $h^{-1}$, respectively. By using a ratio of $C_2H_4$ produced to $N_2$ fixed of 4:1 (Capone, 1993), an estimated average nitrogen fixation rate of 38 ± 36 mmol N $m^{-2}$ $year^{-1}$ was obtained.

In most cases nitrogen fixation was high during the night-incubated samples compared to daytime-incubated sample (Fig. 2 ), confirmed by the two sample t-test showing a significant difference between the two incubation periods ($t = 3.94$, $p = 0.004$). However, there were no significant differences in the rates of acetylene reduction between the rainy and dry seasons and between the sandy and muddy beaches, $t = 0.10$, $p = 0.92$ and $t = 0.16$, $p = 0.87$, respectively. With exception of some specific sampling dates, there were no clear general trends in the rates of nitrogenase activity from the upper littoral zones to the lower littoral zones. Indeed, Kruskal Wallis test showed no significant differences between the upper, middle and lower littoral zones ($KW = 1.89$, $p = 0.39$). There were also no significant differences between the three sampling sites, i.e. Tanga, Dar es Salaam and Mafia ($KW = 8.06$, $p = 0.18$).

## Microalgal biomass

Microalgal biomass (Chl. *a*) in sediment at the study sites are given in (Fig. 3). During the rain season, Chl. *a* ranged from 3.2 - 13.4, 3.6 - 33.1 and 2.7 to 13.3 μg Chl. *a* $cm^{-2}$ in Tanga, Dar es Salaam and Mafia, respectively. During the dry season, Chl. *a* ranged from 2.2 - 10.3, 1.7 - 6.9 and 3.7 - 18.3 μg Chl. *a* $cm^{-2}$ in Tanga, Dar es Salaam and Mafia, respectively. A significantly higher microalgal biomass ($t = 2.9$, $p = 0.01$) was observed on muddy beaches compared to sandy beaches. However, there was no significant different in microalgal biomass at the sampling sites between the rain and dry seasons, ($t = 1.45$, $p = 0.16$). Also, there was no significant correlation between rates of nitrogen fixation and microalgal biomass in sediment, $r = 0.16$, $p = 0.34$.

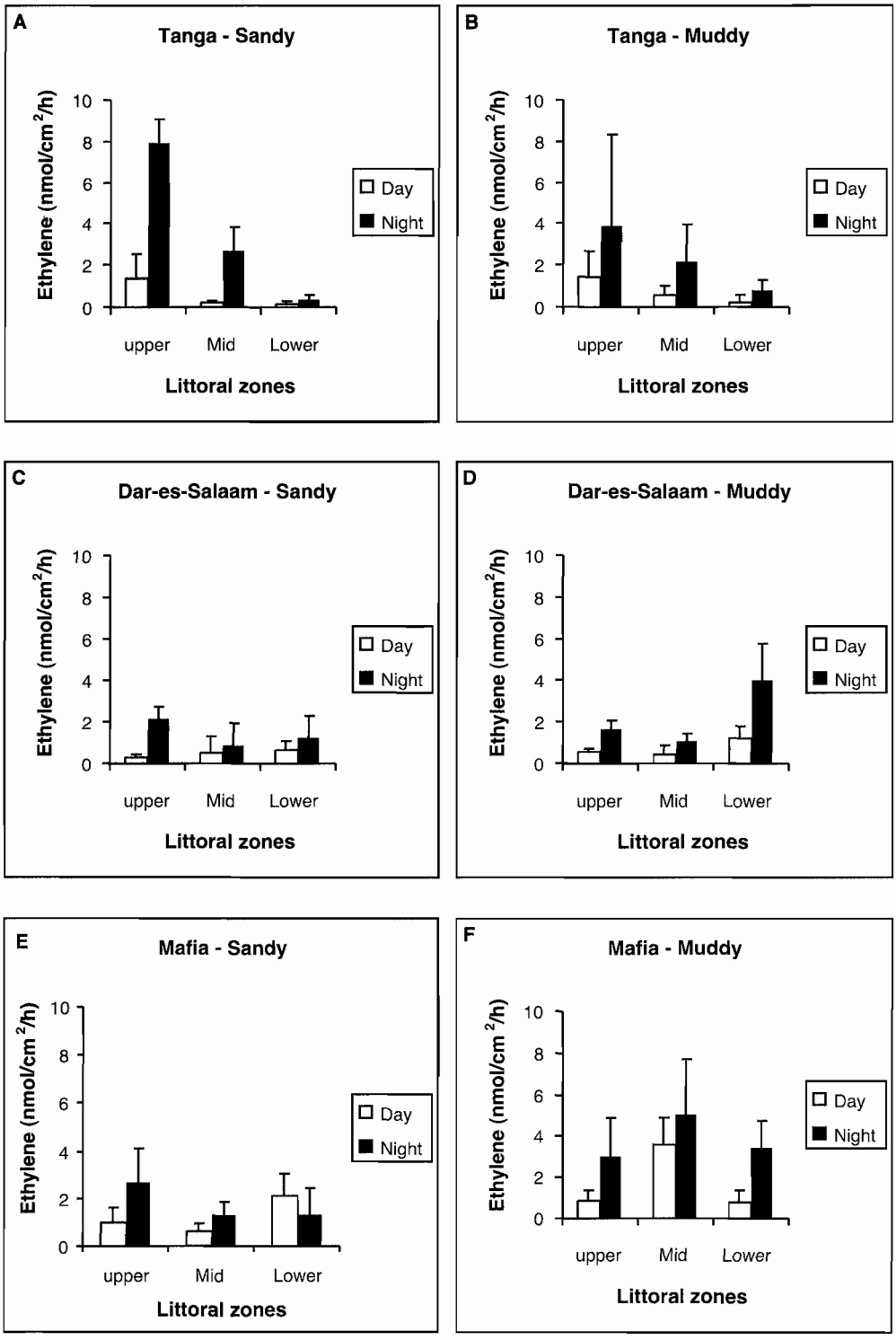



**Fig. 2. Nitrogenase activities measured as ethylene production in sandy and muddy areas at Tanga (A and B), Dar es Salaam (C and D) and Mafia (E and F) during the day (light bars) and at night (black bars); error bars = standard deviation, n = 6**

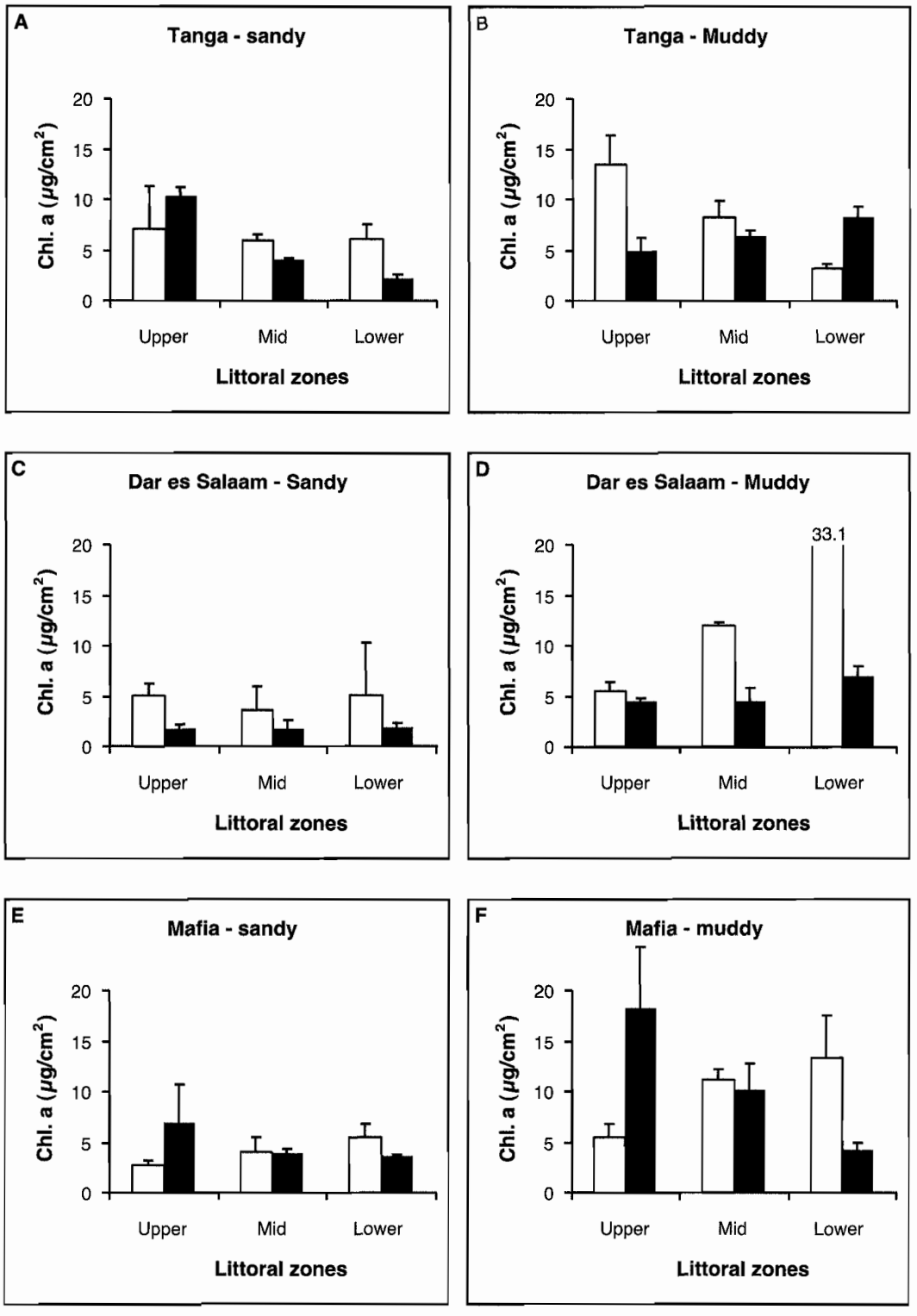



**Fig. 3. Amount of Chlorophyll a in sediment at Tanga (A and B), Dar es Salaam (C and D) and Mafia (E and F) during the rain season (light bars) and dry season (black bars); error bars = standard deviation, n = 3**

## DISCUSSION

Rates of nitrogen fixation recorded in this study fall within the range as those recorded previously in similar environment within the region and elsewhere. For example, Lugomela and Bergman (2002) reported ethylene production rates to range from 1.3 - 16.8 nmol $C_2H_4$ $cm^{-2}$ $hr^{-1}$ in mangrove sediments near Zanzibar town while Paerl *et al.* (1996) reported nitrogenase activity to range from 0.0 to 9.0 $C_2H_4$ $cm^{-2}$ $hr^{-1}$ during the winter and in the summer, respectively, in the intertidal mudflats of north Carolina. Also, Stal *et al.* (1984) reported ethylene production rates ranging from 0.6 - 87.2 $C_2H_4$ $cm^{-2}$ $hr^{-1}$ in the intertidal flats of Mellum Island, North Sea. The spatial variability of nitrogenase activity observed in this study and in relation to other studies may be due to variability of the biomass and/or composition of nitrogen fixing organisms and to variability in environmental parameters such as temperature, light and inorganic nutrient concentration in the respective environment.

The significant higher nitrogen fixation rates at night compared to daytime-measured samples in the study area suggests that non-heterocystous cyanobacteria may be the dominating diazotrophic organims in the litoral sediments along the Tanzanian costline. In most cases, non-heterocystous cyanobacteria and bacteria fix nitrogen at night in order to protect nitrogenase from the endogenously synthesized oxygen during photosynthesis (Bergman *et al.*, 1997) although some can still fix during the day utilizing other protective mechanisms (Berman-Frank *et al.*, 2001). Diurnal variations in nitrogenase activity are known in natural populations of cyanobacteria-dominated systems (Stal *et al.*, 1984; Stal, 1995; Stal, 2000). This depends on the diazotrophic cyanobacteria dominating one particular system. Nitrogen fixations in communities of heterocystous cyanobacteria are strongly light (daytime) dependent, while the daily pattern is less predictable in systems dominated by non-heterocystous cyanobacteria. The latter depends on the type of organisms present and on prevailing conditions such as oxygen tension of the system under consideration (Paerl *et al.*, 1996). In addition, these conditions may also vary from day to day as a result of other factors such as tidal movement, light regime and temperature. As a result, the daily pattern of nitrogen fixation in non-heterocystous cyanobacterial communities may also change considerably (Stal, 1995; 2000).

The high standard deviations in nitrogenase activity recorded in the current study may be attributed to high spatial and temporal fluctuations (patchiness) in the biomass and composition of the diazotrophic organisms in the different, randomly-collected corers. Patchiness distribution in microalgae biomass in intertidal sediment has been reported elsewhere (e.g. Pinckney *et al.*, 1994). The lack of a clear trend in nitrogenase activity between the upper, mid and lower littoral zone and between muddy and sandy beaches suggests that diazotrophic organisms are more or less distributed homogeneously (although with patchiness) in the intertidal areas. Also, the lack of significant difference between rainy and dry season during the current study suggests that there is little seasonal influence on diazotrophy in intertidal sediments in the area.

The insignificant correlation between nitrogenase activity and microalgal biomass suggests that the major part of microalgal biomass in sediment is not composed of diazotrophic cyanobacteria. Sediments are characterized by development of various microflora such as cyanobacteria, diatoms and dinoflagellates. Not even all cyanobacteria are capable of fixing molecular nitrogen (e.g. Bergman *et al.*, 1997). In addition, Olson *et al.* (1999) have reported a ubiquity of diazotrophic hetetrophic eubacteria in marine sediment and microbial mats, which may also have contributed to the observed nitrogenase activity.

We conclude that diazotrophs contribute a substantial amount of nitrogen input to the Tanzania coastal ecosystems. However, further studies are required to corroborate the current study. In addition, other sources of nitrogen input to the coastal ecosystems e.g. terrestrial runoff and upwelling need to be quantified in order to determine the nitrogen budget for such ecosystems.

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