STUDIES ON BENTHIC DENITRIFICATION IN THE CHWAKA BAY MANGROVE SEDIMENTS, ZANZIBAR

SM Mohammed[1] and RW Johnstone[2]
[1]Institute of Marine Sciences, University of Dar es Salaam, P.O. Box 668, Zanzibar
[2]Formerly of Stockholm University, S-106 91 Stockholm, Sweden

ABSTRACT
Denitrification was measured at three sites in a narrow tidal creek in the Chwaka mangrove ecosystem. Denitrification arising from water column nitrate (D_w) was low (< 0.1μM $Nm^{-2}h^{-1}$) and there was little spatial and seasonal variation in D_w rates in the creek. Coupled nitrification-denitrification (Dn) was found to be low, but within the range measured in other mangrove sediments. Average values ranged from 0.01 to 0.45 μM $Nm^{-2}h^{-1}$ and showed strong spatial variations. The low denitrification rates observed in the creek were possibly as a result of the low availability of NO_3^- and high C: N values of the organic matter in the sediment. Spatial variations in denitrification rates were due to variations in concentration levels of organic matter and possibly to disproportionate competition for inorganic nitrogen between denitrifiers and benthic autotrophs among sites. There were no seasonal differences in denitrification rates. Results from the present study suggest that sediment denitrification does not play a major role as a sink for N in this ecosystem.

INTRODUCTION
The physical location of tropical mangrove ecosystems at the interface between nutrient-limited marine aquatic ecosystems, such as coral reefs and seagrasses, and often more nutrient-rich land masses, make them ideally situated to exert a strong influence on the overall nutrient dynamics of the coastal environment. Various studies have indicated nitrogen to be the most common limitation to coastal phytoplankton production (eg. Rhyther & Dunstan 1971 and references therein) and so any transfer of nutrients from mangroves may be highly significant for the production of coastal aquatic ecosystems (eg. Moran *et al.* 1991, Robertson & Alongi 1995). However, this role could be severely curtailed by processes that hinder the transfer of these nutrients from mangroves to adjacent ecosystems such as uptake by the biota and by denitrification.

Extensive mangrove forests occur along the East African coastline. Despite their significance for coastal communities and ecosystem function, there has only been limited research conducted on them (Hemminga *et al.* 1994, Cederlöf *et al.* 1995, Slim *et al.* 1996). One area that has recently received considerable attention is the Mapopwe creek mangrove forest in Zanzibar (Wolanski 1989, Cederlöf *et al.* 1995, Mohammed & Johnstone 1995, Shunula 1996). In an earlier study at this site, Mohammed and Johnstone (1995) found a low exchange of nutrients, including N, between the creek and the open Chwaka bay water. Furthermore, a study of nutrient exchanges between sediment and the water column in the creek showed generally low rates, despite strong concentration gradients (Mohammed & Johnstone, in prep). It was therefore speculated that, among other factors, sediment based processes may be responsible for the uptake or removal of nutrients. In particular, it was proposed that benthic denitrification may be important for the removal of nitrogen. As has been shown in other coastal ecosystems, denitrification can play a key role in controlling water column nitrogen levels and may therefore control ecosystem production (Seitzinger *et al.* 1984, Jensen *et al.* 1988, Nielsen *et al.* 1995). However, available information in the literature shows that denitrification rates in these ecosystems are not consistent and vary from one mangrove ecosystem to another.

In the light of these observations, an understanding of the role of denitrification in the Mapopwe creek ecosystem is vital if the other nutrient studies are to be synthesised. Moreover, given the variability of denitrification rates reported in other studies, there has been the need to investigate the process over a range of sediment types and seasons. In the present study, we measured denitrification rates at three locations in the Mapopwe creek in order to assess the significance of the process as a sink for nitrogen. Furthermore, in order to assess the seasonal and spatial variabilities of the process, measurements were carried out during the wet and dry seasons.

METHODS

Study sites and sampling

Mapopwe creek is the largest of a series of tidal creeks that drain the Chwaka mangrove forest and which opens into the Chwaka bay located on the east coast of Zanzibar (Fig. 1). This ecosystem is tidally dominated with no significant surficial freshwater input other than periodic events during monsoon periods. Three sampling sites were chosen, namely the channel site (site C), the channel bank site (site CB), and a site within the forest proper (site F). The channel site lies in the creek channel as it leaves the central channel towards the forest. This site lies at a depth of approximately 0.5 m during low tide and 2 m at high tide. The channel bank site lies on one bank of the creek while the forest site lies under mangrove canopies, approximately 15 m from the central channel. Unlike site C, site CB is inundated only during high tides (tide elevation 3 m). On the other hand, site F is only inundated during high water springs. Sampling was carried out during both dry and wet period of the year.

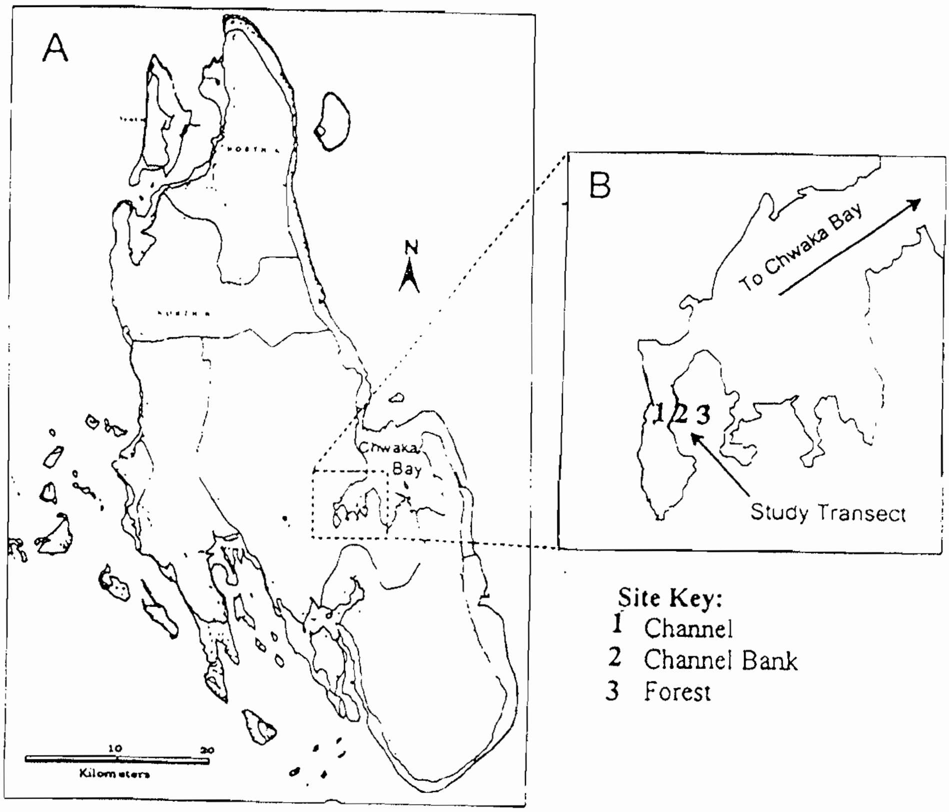

Fig. 1: Map of Zanzibar (A) and the study sites in the Mapopwe creek in Chwaka bay (B)

Sediment organic matter content

Sediment samples were collected from the three sites (six cores per site) using a modified 60 ml plastic syringe. Sub-samples from the sediment cores were ground in an agate mortar and treated with 25 % HCl to remove carbonates. They were then analysed for total organic carbon (TOC) and total nitrogen (TN) on a CHN analyser (LECO CHN-900) against EDTA. Because it was not possible to investigate seasonal variations of organic matter concentrations using elemental analysis, organic matter was determined using combustion method. In this case, six sediment cores were taken randomly from the three study sites as above and a known weight of sediment from the upper 1 cm was sub-sampled from each of them. The sub-samples were then placed in an oven and heated at 400 °C for 24 h, cooled and then reweighed. The difference in weight after ignition was taken as an estimate of organic matter content in the sediment.

Measurement of dissolved nitrate and ammonium (DIN)

Multiple cores (6-8 cores per site) were taken randomly from the three sites using Plexiglass corers (15 cm long and 8 cm internal diameter) and the top 1 cm of sediment was sectioned and collected. The samples were then centrifuged at 3000 rpm for 25 minutes. The supernatant was siphoned off and filtered through acid washed 0.45 (GF/F microfibre filters before they were analysed for dissolved $NO_3^- + NO_2^-$ (hereafter referred to as NO_X) and NH_4^+ according to the spectophotometric methods of Parsons *et al* (1984). Filtered samples of the overlying water in the sediment cores were also collected and analysed in the same manner to give an estimation of water column concentration for these nutrients.

Denitrification assays

Denitrification was measured using the nitrogen isotope pairing method of Nielsen (1992). Between 15 and 22 cores were collected as above from each of the three sites across the Mapopwe creek. The sediment height was adjusted so that each core was approximately 7.5 cm in height and with 64 ml of headwater. Incubations were started by injecting 50 µl of $^{15}NO_3^-$ from a 100 mM stock solution into the water phase. The cores were left to incubate under aerobic conditions and at laboratory temperatures, for approximately three hours. The overlying water was kept in motion by means of a small magnetic stirrer placed about 1 cm above the sediment surface. After the incubation period, 50 µl of $ZnCl_2$ (1 g/ml) was added to the overlying water to stop microbial activity. The water column and the sediment were then mixed thoroughly and samples of the resulting slurry were collected by means of a modified syringe. The samples were analysed on a mass spectrometer for the different isotopic formations of N (National Environmental Research, Silkeborg, Denmark).

Calculation of denitrification rates

Rates of denitrification were calculated using Nielsen's (1992) equations:

$$D_{15} = {}^{29}N_2 + 2 * {}^{30}N_2 \text{ and}$$

$$D_{14} = ({}^{29}N_2/2 * {}^{30}N_2) * ({}^{29}N_2 + 2 * {}^{30}N_2)$$

where D_{15} and D_{14} are denitrification rates of $^{15}NO_3^-$ and $^{14}NO_3^-$ respectively.

Ambient denitrification of $^{14}NO_3^-$ from the water column (D^W_{14}) was calculated as:

$$D^W_{14} = D_{15} * f^W_{14}/f^W_{15}$$

where f^{W}_{14} and f^{W}_{15} are ^{14}N and ^{15}N frequencies of nitrate in the overlying water. Coupled nitrification and denitrification was thus given as the difference.

$$D^{n}_{14} = D_{14} - D^{W}_{14}$$

RESULTS

Sediment TOC and TN levels

Generally, there was no significant difference in concentration of TOC at the different sites (Kruskal-Wallis, $p > 0.05$). Mean concentration of TOC in the top 1 cm of sedi.nents was 15.60 %(± 0.9) dry weight. On the other hand, depth distribution patterns of TN were slightly more variable among sites but the spatial variations were not statistically significant (Kruskal-Wallis, $p > 0.05$). Mean levels of TN in the top 0.5 cm of sediment (by dry weight of sediment) did not vary significantly among sites; the values were 0.31 % (± 0.04), 0.49 % (±0.05), 0.51 % (±0.06), dry weight at sites C, CB and F respectively.

Site F showed a much higher organic matter content compared to the other sites, which showed more or less similar concentrations. Concentrations at this site averaged 36 % (±9.0) by dry weight during the wet season and 38 % ((6.9) during the dry season. By comparison, at site C concentrations were 16.2 % (±8.1) by dry weight during wet season and 16.2 (±4.3) by dry weight during the dry season; and at site CB concentrations were 19 % (±4.3) by dry weight during the wet season and 18.9 % (±5.1) during the dry season (Table 1). There were no significant differences in sediment organic matter concentration between wet and dry seasons at all sites (Kruskal-Wallis, $p > 0.05$).

C:N values did not vary significantly with depth but there were significant spatial variations (Kruskal-Wallis, $p < 0.001$) in mean C:N values over the 10 cm depth (Fig. 2). Highest values were recorded at site C (46.88). Sites F and CB showed similar C:N ratios with average values of 32.56 and 32.57 respectively. Generally, there were no significant differences in C:N depth distribution patterns between sites F and CB where C:N was uniformly. distributed with depth. However, at site C, there was a sharp increase in C:N ratios with depth from approximately 3.5 cm, which was not observed at the other sites.

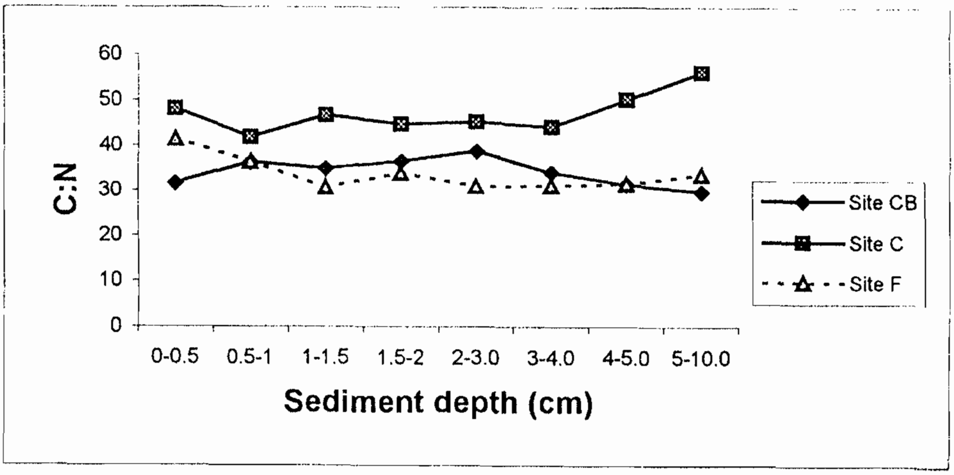

Fig. 2: Sediment C:N ratios (mean±SD) in the Mapopwe Creek, Chwaka Bay, Zanzibar

DIN Concentrations

Dissolved nitrate concentration in the water column was consistently low during both seasons, with values ≤ 0.5 µM N/l (±0.1) during both the rainy and dry seasons. Similarly, porewater concentrations of dissolved nitrate in the top cm of sediment were equally low and did not show any seasonal differences. Concentrations averaged 1.1 µM N l^{-1} (±0.3) at all sites. By contrast, pore water concentrations of dissolved ammonium were high, and with the exception of site C did not vary significantly between sites and between wet and dry periods (Kruskal-Wallis, $p > 0.05$, Table 1.)

Table 1: Concentration of ammonium and organic matter in the top cm of sediment in the Mapopwe creek, Chwaka bay, Zanzibar (mean ± SD)

	Ammonium (µM)		Organic matter (% dry weight sediment)	
Site	Wet Season	Dry season	Wet season	Dry season
Channel Bank	121.69±58.26	91.06±90.92	19.01±4.27	18.85±5.0
Channel	109.32±20.1	28.63±19.22	16.23±8.11	16.21±4.43
Forest	123.66±49.93	103.46±49.92	35.55±8.96	37.91±6.93

Denitrification rates

Generally, the sediments in the Mapopwe creek showed low denitrification activities with no significant spatial differences in non-coupled (D^W) denitrification between sites (Kruskal-Wallis, p>0.05, Table 2). By comparison, coupled denitrification (D^n) was approximately 10 times higher than D^W. There were also significant spatial variations in D^n in the Mapopwe sediments (Kruskal-Wallis, $p < 0.001$) with the Forest site demonstrating comparatively higher rates than the other sites, which showed more or less similar rates. Seasonally, there were no significant differences in the denitrification rates between wet and dry seasons at the different sites (Kruskal-Wallis, $p > 0.05$). Generally, there was a strong correlation between organic matter in sediment and total denitrification (R2 = 0.91).

Table 2: Seasonal variations of denitrification of (a) nitrate from the overlying water (D^W14) and (b) denitrification coupled to nitrification (D^n14) in the Mapopwe Creek, Chwaka Bay, Zanzibar (mean ± SD)

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DISCUSSION

As mentioned previously, earlier studies in the Mapopwe creek have suggested that sediment denitrification might be an important sink for nitrogen in this ecosystem. The present study, however, indicates that this is not the case, and highlights the probable role of benthic microphytobenthos in controlling sediment DIN in the creek. The denitrification rates obtained in this study are within the range of values recorded in other mangrove ecosystems; however, these values are at the lower end of the range. For example, Rivera-Monroy *et al* (1995) recorded denitrification rates ranging from 0.08 to 9.4 (M N $m^{-2}h^{-1}$ in a fringe mangrove stand and 1.9 to 4.5 (M $Nm^{-2}h^{-1}$ in a basin mangrove forest in Terminos Lagoon, Mexico.

Among the factors that are important in regulating denitrification rates are temperature, oxygen concentration, nitrate concentration, and a supply of organic matter. The role of temperature in tropical mangrove ecosystems is possibly insignificant given that temperatures are generally constant and vary over only a small scale on a daily and seasonal basis. On the other hand, nitrate concentration in water and sediment is generally regarded as the most important parameter in controlling denitrification rates (eg. Rysgaard *et al.* 1995, Rivera-Monroy *et al.* 1995). Denitrification is dependent on either a supply of NO_3^- from the water column or *in situ* nitrate production (nitrification). Mapopwe creek is almost entirely tidal in nature and hence receives very little terrigenous input except during the heavy rains. A previous study (Mohammed & Johnstone 1995) and this study have found low levels of NOx in the creek. This may account for the low water column NO_3^- denitrification.

Another factor that may explain the low denitrification rates observed in the creek is competition for inorganic nitrogen in the sediment. Microbial populations in sediments convert inorganic nitrogen (NH_4^+ and NO_3^-) into organic N during initial stages of leaf litter (organic matter) decomposition. This is especially the case where decomposing litter has high C: N ratios, such as mangrove litter (Twilley *et al.* 1986), as opposed to litter rich in N (low C:N) which favours mineralisation (Alongi *et al.* 1992, Kristensen *et al.* 1991). Rivera-Monroy and Twilley (1996), in their study in the Terminos Lagoon, were able to stimulate nitrification and denitrification in mangrove sediments by increasing nitrogen enrichment thus lowering the C:N ratio. In the present study, we found high sediment C:N levels in the Mapopwe creek which should favour immobilisation and hence low denitrification rates.

Bioturbating benthic invertebrates are known to enhance the denitrification activity by pumping water through the sediment (Kristensen *et al* 1991, Nielsen *et al.* 1995). Unlike the other sites, the Forest site has been observed to accommodate a large number of bioturbating organisms. Moreover, numerous crab holes are found scattered over a vast area under mangrove canopies. The sum total of these activities is to create oxygenated micro zones deep in the anoxic zone of the sediment where nitrifier activities can then take place. These might have been responsible in providing the supply of nitrate needed in the coupling of nitrification and denitrification at the Forest site resulting in the relatively higher denitrification rates observed at this site as compared to the other sites.

The supply rate of organic matter has been shown to be an important parameter in controlling the extent of denitrification. Studies have shown that there is a direct relationship between organic matter concentration in sediment and denitrification activity (Terry & Nelson 1975, Koike & Hattori 1978, Joy & Pearl 1993). The presence of high organic matter generally associated with mangrove sediments should presumably favour the denitrification process. In addition to supplying the ammonia needed for nitrification in the coupling of nitrification and denitrification (Seitzinger 1988) organic matter in sediment increases the availability of electron donors, which stimulates denitrifier growth (Twilley & Kemp 1986). The high organic carbon content at Station F may also account for the relatively high denitrification rate while the other two sites which had more or less similar concentrations of organic matter showed somewhat similar rates. The lack of seasonal variations in organic matter input into the creek may account for the observed lack of variations in the denitrification rates between wet and dry seasons.

In an earlier study (Mohammed & Johnstone 1995, Mohammed & Johnstone in prep.), it was shown that Mapopwe creek sediments retain a high concentration of N in the form of NH_4^+ but low levels of NO_3^-. It was also implied that the low level of NO_3^- within the creek was possibly due to denitrification. However results of this study show that N uptake in the creek does not necessarily represent a nitrogen sink via denitrification. It is speculated that the low NO_3^- concentrations observed in the Mapopwe creek sediments and the generally low fluxes of N, as observed in earlier studies, are possibly a result of immobilisation through microbial and benthic autotrophic activities. It is obvious that the forest conserve nitrogen through an efficient recycling mechanism, possibly micro-algal mediated, which ensures that the little nitrogen present in the system is retained. This is corroborated by the observation (Mohammed & Johnstone 1995) that little inorganic nitrogen is exported from the system.

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