Chapter 5.
Discussion
This section begins with a brief introduction on the role of bacteria on the biogeochemical cycling of nitrogen in mangrove environments. Further, the discussions have been done in three sections. The first section (5.1) discusses the ambient nitrogen, organic carbon and metals in the mangrove sediments. The second section (5.2) dwells into the abundance and identity of nitrifiers with specific reference to their trophic status in mangrove sediments. The final section (5.3) discusses both field and laboratory results and aims to understand the factors regulating nitrification in mangrove sediments.

Microbial activity controls the mineralization of organic matter in intertidal soils and sediments thereby influencing pore water nutrient availability and the speciation of redox sensitive ions [Paerl and Pinckney 1996]. Organic matter oxidation is coupled to the reduction of an oxidant by terminal metabolism and produces a variety of products, including nitrogen and nitrous oxide gases, reduced iron and manganese, sulfide and methane which are indicative of denitrification, metal reduction, sulfate reduction and methanogenesis, respectively. This process recycles complex organic matter back to inorganic forms such as bicarbonate, ammonium, and phosphate, which are critical for primary production in oligotrophic mode.

Environmental pollution by inorganic nitrogen is the result of disequilibrium between input of fixed nitrogen (biotic and abiotic), usually in the form of ammonia, and its output, usually in the form of dinitrogen, the only form of nitrogen which can be considered as environmentally safe. All other forms of nitrogen, which can accumulate due to the establishment of unbalanced fluxes,
may create problems ranging in their severity from mild nuisances to serious ecological hazards. Only by manipulating and maintaining environmental factors to allow microorganisms to transform fixed nitrogen to dinitrogen gas, can we avoid the consequences of the accumulation of biologically active inorganic nitrogen species. Success depends on an in depth understanding of the processes involved, at all levels, from biochemistry to ecology, as with any other biological activity. Pollution of the environment with nitrogen differs from pollution by other biogenic pollutants such as carbon and phosphorous, because not only can it trigger eutrophication of water bodies or affect soils and atmosphere, but also cause toxicity to various life forms, depending on the organisms exposed and upon environmental factors such as pH and temperature. When compared to other environmentally important biogenic inorganic ions, the major nitrogenous end products of degradation of organic except for dinitrogen are very soluble, and can therefore reach high concentrations. Of the various forms of nitrogen, ammonia and nitrite are the most toxic to aquatic wildlife (fish, crustaceans, mollusks). It is therefore obvious that for all practical reasons nitrogen assimilation and dissimilatory denitrification must be optimally balanced. If not, either excess assimilation will cause accumulation of toxic inorganic nitrogen, or, on the other hand, denitrification might reduce primary productivity, affecting agriculture and the capacity of any ecosystem to sustain life. Main inputs of ammonia and nitrite into the environment are through microbial nitrogen fixation and anthropogenic sources such as wastewater discharge and the widespread
use of industrial fertilizers. Ammonification resulting from the degradation of organic matter could also contribute substantially.

Mangrove soils are usually nutrient deficient [Alongi 1996; Alongi and Sasekumar, 1992; Boto and Wellington, 1984] though rich in organic matter, suggesting highly throughput recycling of the inorganic nutrients [Holguin et al 2001]. Nitrogen fixation is an important source of new nitrogen but this process is influenced by spatial and temporal variability [Lee and Joye, 2006]. The high productivity of mangroves is thus sustained by internal nutrient recycling, which could be coupled to organic matter mineralization. Mangrove soils can be both hypersaline and at the same time biochemically reducing [Kathiresan and Bingham, 2001]. Increased rainfall during the wet season can affect pore water salinity, redox potential, and pH and other soil biogeochemical processes [Alongi et al, 1999;2004]. Earlier studies of benthic metabolism and nutrient transformations in mangrove soils fringing oceans or rivers have a marked relationship between organic matter availability, elemental cycling and mangrove density [Nedwell et al, 1994, Sherman et al, 1998]. This study focuses on the nitrification pathway, understanding the factors regulating and modulating this process in the mangrove sediments.

5.1. Ambient nitrogen, organic carbon and metals

Ammonium: The significance of ammonium in the marine environment is recognized from the following facts. These include 1) the most of preferred form of dissolved inorganic nitrogen (DIN) by the phytoplankton [McCarthy and
Goldman, 1979; Billen, 1984] as well as heterotrophs 2) major form of biogenic input to the ocean [Sharp, 1983] and 3) central component in the regeneration pathway [Boucher et al, 1994]. Ammonium is produced in sediments through different processes: (i) decomposition of organic matter by various heterotrophic organisms [Billen, 1978; Boynton et al, 1980], (ii) primary excretory product of microheterotrophs [Blackburn and Henriksen, 1983; Svensson, 1997], (iii) nitrogen fixation by prokaryotic organisms [Zuberer and Silver, 1979; Potts, 1979; Boto and Robertson, 1990], (iv) turnover of urea [Lomstein and Blackburn, 1992] and (v) dissimilatory reduction of nitrate to ammonium [Cole, 1988; Koike and Hattori, 1978; Sorensen, 1978]. Ammonium produced by these processes can be reoxidized, reincorporated into the organisms, adsorbed onto the particles, and diffused along concentration gradients to other regions of the sediment or to the overlying water. However, high concentrations of ammonium could also reprecipitate as a constituent of authigenic minerals [Martens et al, 1978]. Almost all the measurements of pore water ammonium in mangrove sediments show that the concentrations are higher than in sediments of other ecosystems [Alongi, 1996]. Generally, mangrove sediments are considered as anaerobic and ammonium is the major form of inorganic nitrogen [Alongi et al, 1992; Kristensen et al, 1988]. Hulth et al [1999] suggested that, due to lack of oxygen, the transformation of ammonium into oxidized or gaseous forms by the activity of microorganisms becomes slower and therefore the ammonium gets accumulated in the sediments. However, studies conducted by Lizumi [1986] on the nutrient pools in mangrove soils showed that ammonium concentrations are low in the
mangrove zone compared with the non-mangrove zone and suggested that this low level of ambient ammonium could be due to uptake of mangrove plants. Boto and Wellington [1984] also reported variations in ammonium concentrations with depth as well as season in mangrove sediments, and opined that the variation in ammonium uptake by mangrove plants could be a major controlling factor. In a Bermuda mangrove system, ammonium concentrations ranged from 31 to 114 μM [Hines and Lyons, 1982]. Carlson et al [1983] noted that the ammonium concentrations were reported to range from 5-11 μM in an Avicennia germinans dominated sediment. Rosenfeld [1979] reported that pore water ammonium in eutrophicated Florida mangroves ranged from 390 to 760 μM, which was much higher than those recorded from other mangroves areas. In the present study, depth compromised ammonium concentrations in pore water during the pre-monsoon season at the control site was 19.1±4 μg at NH₄⁺-NL⁻¹. Similar values were encountered in the pore water at the experimental site with ammonium averaging 19.9 ±2.4 μg at NH₄⁺-NL⁻¹. However, the monsoon season was characterized by lower levels of ammonium in the pore water at both the control (16.8 ±3.6 μg at NH₄⁺-NL⁻¹) and experimental sites (14.1±2.1 μg at NH₄⁺-NL⁻¹ pore water). Physical processes also significantly influence the nutrient pool. In a study carried out in the Ems estuary, de Jonge and Colijn [1994] observed that high water movement and wave action resulted in higher efflux of pore water nutrients. Similar conditions were also observed in the present study. This resulted in the loss of nutrients from the sediment during the monsoon season. Also, the Mandovi is characterized by a large seasonal influx of freshwater into
the estuary, with unchanged tidal amplitude over large distances [Unnikrishnan et al, 1997]. During the monsoon season the concentrations of ammonium decreased, supporting this observation. This fall could be explained by the advection of fresh water during these months. In this season, due to high speed of freshwater, the erosion of the surface sediments and the efflux of pore water ammonium to the overlying waters led to the fall. In this study, the post-monsoon values at the control site showed an increase in ammonium accumulation (17.9±7.9 µg at NH₄⁺-NL⁻¹ pore water) as compared to the monsoon, but they were still lower than the pre-monsoon values. Ammonium accumulation at the experimental site was the highest (27.8±5.6 µg at NH₄⁺-NL⁻¹ pore water) during post-monsoon season as compared to monsoon and pre-monsoon seasons. This probably due to the effect of higher organic load coming into the estuary due to a wider catchments area or due to higher anthropogenic impact in the Mandovi estuary. Most of these values, except for the lower range (monsoon value) fall within the range recorded at Phuket, Thailand [Kristensen et al, 1988].

During the non-monsoon seasons, the physical conditions favor physiological and regenerative process of ammonium production in the sediment and, as water movement is low, there is accumulation of ammonium in the sediment. Throughout the sampling period there was a significant variation in ammonium concentration between the control and experimental sites only at the depth interval 0-2 cm (F = 7.9, df = 1, p = 0.009). This could be attributed to sediment composition (percentage of sand, silt and clay) and adsorption, terrigenous input and biological processes. Since it is dominated by clay, the
fluxes could be more restricted to the sediment water interface. Also, Adsorption experiments on mangrove sediments described by Rosenfeld [1979] indicate that ammonium adsorption was low compared with other marine sediments. The low absorption capacity of ammonium by mangrove sediments may be attributed to the low sediment porosity, density and cation exchange capacity [Boto, 1982]. The differences in the particle size and the porosity of the sediment are also important factors which can influence ammonium concentrations significantly [Rocha, 1998]. At the mangrove zones, the sediment is composed mostly of clay and silt. As a result the sediments are compact and less porous and hence the diffusion of ammonium ion from sediments is less. Terrigenous input also plays an important role in influencing ammonium concentrations. In shallow mangrove-lined estuaries, litter forms a significant fraction of particulate organic matter [Wafar et al, 1997].

**Nitrite:** Nitrite is a well studied transient compound in the nitrogen cycle. It is an intermediate product in many nitrogen transformation processes such as: nitrification, denitrification, dissimilatory nitrate reduction and assimilatory nitrate reduction. The first three processes are mediated by bacteria and the fourth one by autotrophic organisms and also a number of aerobic bacteria and fungi [Hattori, 1983]. In assimilatory nitrate reduction nitrite is released when light is not adequate for photosynthesis and when nitrate is abundant. However, the extent of nitrite production by this process is generally not significant enough to affect regenerated production [Bronk et al, 1994; Collos et al, 1996]. Nitrite produced
during any of these processes would get released into the pore water. The rates at which these processes proceed vary in the sediment, depending on the amount of substrate available and its supply (either by biogenic or external sources). The rate of these processes also depends on the geomorphology as well as the biochemical composition of the sediments. Availability of oxygen in the sediments and the quantity of organic load also influence the nitrite concentrations in the sediment. The benthic microorganisms oxidize organic nitrogen, using at first, the oxygen dissolved in pore water when oxygen is insufficient, nitrite and nitrate are used as electron acceptors, as in the first three process mentioned above [Cartaxana and Lloyd, 1999]. In mangroves the distribution of nitrite has not been studied extensively. In most of the studies, the estimation of nitrite has been made in connection with nitrification or denitrification processes. Morell and Corredor [1993] estimated the pore water nitrite at various depths (1-9cm) in different localities. They reported concentrations ranging from 0.02 to 0.35 µM in the mangrove lagoon. Alongi [1996] compared the nitrite concentrations in different locations (mangroves and mudflats) and found that the concentrations were significantly lower (0.02-0.05µM) in sediments in which mangrove roots were found.

In the present study, the average pre-monsoon NO₂⁻ concentration at control and experimental sites were 2.4±0.8 and 2.1±0.1 µg at NO₂⁻-NL⁻¹ pore water respectively. During monsoon season, NO₂⁻ values at control (2.3±0.5 µg at NO₂⁻-NL⁻¹ pore water; n=20) and experimental sites (2.7±0.6 µg at NO₂⁻-NL⁻¹ pore water; n=20) were comparable to their pre-monsoon counterparts. The post-
monsoon nitrite levels at the control site (2.3±0.6 μg at NO$_2$-NL$^{-1}$ pore water; n=20) showed a similar trend as that of the other seasons, but experimental site recorded the lowest values (1.8±0.5 μg at NO$_2$-NL$^{-1}$ pore water; n=20) during the post-monsoon season. As described in the previous section, the ammonium levels were higher at the experiment site during the post-monsoon season which in conjunction with low nitrite could mean that the conditions prevailing are not very conducive for nitrification.

In addition, the present study, there is no significant correlation between nitrite and nitrate, suggesting that these two species of nitrogen had different controlling mechanisms. Nitrite showed a significant monthly variability at the experimental site (p <0.1, df = 11) where as the control site was marked by significant down-core variability (p <0.01, df = 11). The monthly variability in pore water nitrite at the experimental site is probably due to the following reasons. The physical process that is fresh water advection and diffusion of nitrite from the overlying waters are the major sources of nitrite and the variations in the supply leads to the monthly changes. The down core variability observed at the control site could be due to the variations in sediment composition and utilization of nitrite by the autotrophic biomass and mangrove vegetation. Maximum down-core variation was observed during the pre-monsoon season (p <0.01, df = 4). Even though the inter seasonal variability occurred neither at the experimental nor at the control site, the bottom most layer of the core (8-10cm) showed significant variation in nitrite both at the control and experimental sites. This could be due to the differential impact of denitrification. Denitrification being a
respiratory pathway promotes quick fluxes there by bringing in profound variation in various niches.

This study shows that the supply of oxidized forms of nitrogen is from different sources. Nitrite may also have been produced through denitrification as the mangrove sediment is generally with low oxygen concentrations and this may facilitate the nitrite production [Cartaxana and Lloyd, 1999; Alongi et al, 1999]. The utilization of nitrite by the autotrophs is also an important factor that could influence the nitrite pool significantly. Nitrite uptake rates of phytobenthos were reported to be low and insignificant considering the ambient concentrations in mangrove sediments by Dham [2000]. However, Boto and Wellington [1983] observed that mangrove roots can take up nitrite efficiently and may influence the nutrient pool significantly. This phenomenon is further discussed with respect to spatial variation in nitrite concentrations.

Spatial variations in pore water nitrite concentrations could be controlled by factors such as: 1) sediment composition and oxygen levels 2) topography of the area and 3) flux processes or diffusion.

**Nitrate:** Nitrate is thermodynamically stable and the most oxidized form of inorganic nitrogen. The availability of nitrate has been attributed a special status in marine primary production [Eppley et al, 1979]. Its importance lies in its abundance as a species of nitrogen (more than any inorganic dissolved form), next to N\(_2\) and secondly its abundance as a biologically assimilable form in the dissolved nitrogen pool in the seas. Nitrate is also involved in reduction pathways
and serves as the terminal electron acceptor in microbially-mediated processes such as denitrification and dissimilatory nitrate reduction. Nitrate production and its utilization in the benthic component are much less understood. The production of nitrate in the benthic pool could be linked with physical as well as biological processes. Among the physical processes, nitrate could be supplied by external sources mainly through overlying waters via percolation [Henriksen et al, 1984] and advection of fresh water [Smith et al, 1985]. The biological processes involved in nitrate production include bioturbation and other microbial processes [Nixon et al, 1976]. The in situ production of nitrate in the sediment via nitrification [Kristensen et al, 1988] is considered as a major biological process that adds nitrate into the dissolved pool. Equally important as the supply and production of nitrate in the sediment is its loss or removal by biological means that determines the net ambient nitrate levels in the dissolved pool [Alongi et al, 1999; Cartaxana and Lloyd, 1999]. Nitrate uptake [Boto et al, 1985; Alongi, 1996], denitrification [Sorensen, 1978; Cartaxana and Lloyd, 1999] and dissimilatory nitrate reduction [MacFarlane and Herbert, 1982; 1984] are the major processes through which nitrate can be lost significantly from the sediments or transformed into another compound. In denitrification and dissimilatory nitrate reduction, nitrate replaces oxygen as the terminal electron acceptor when oxygen diffusion rates in the soil or sediment are insufficient to fulfill the demand for microbial respiration [Jensen et al, 1994]. Measurement of nitrate from mangrove sediments by several workers have shown that nitrate concentrations are generally lower than those of ammonium [Boto and
Wellington, 1984; Carlson et al, 1984], in comparison with coast, estuarine and sea grass ecosystems.

In this study, the concentration of nitrate at the control site were 9.3±2.9 μg at NO$_3^-$-NL$^{-1}$ pore water during the pre-monsoon season, while, the monsoon values were lower (6.1±1.2 μg at NO$_3^-$-NL$^{-1}$ pore water). In contrast, highest nitrate values at the experimental site (13.2±2.0 μg at NO$_3^-$-NL$^{-1}$ pore water) were recorded during the monsoons. This could be due to enhanced river runoff in monsoon seasons with more nitrates flushed down from the catchment areas. The pre and post-monsoon values were 8.6±1 μg at NO$_3^-$-NL$^{-1}$ pore water and 8±2.1 μg at NO$_3^-$-NL$^{-1}$ pore water respectively. However highest levels were recorded at the control site (11.5±1.3 μg at NO$_3^-$-NL$^{-1}$ pore water; n=20) during the post-monsoon season. A similar range was reported by [Kristensen et al, 1988] from the mangrove sediments of Phuket, Thailand.

Nitrate showed significant monthly variability at both the control (p=2.9x10$^{-5}$, df = 11) and the experimental site (p= 4.4x10$^{-3}$, df = 11). The monthly variability in the present study can be explained in the context of the physical and biological processes. In the monsoon season, the high nitrate concentrations were noticed at the experimental site. This sharp increase in the nitrate concentrations can be mainly attributed to transport with fresh water as a result of heavy river discharge into the estuary caused by southwest monsoon rainfall. Nitrate concentrations in the overlying waters at this time are ten times higher than in the non monsoon months [Heredia, 2000] and this could have influenced
the pore water concentrations. Alongi [1988] made a similar observation during the rainy season in a mangrove forest of Australia.

There was no down core variation neither at the control nor at the experimental site. In spite of having a well packed nature, the sediment pore water dissolved nitrogen variability is less probably indicating that the microbes have a profound role which is similar or even more than physical processes. There exists considerable variability in nitrate at both the control and experimental sites during the monsoon and post-monsoon seasons ($p < 0.1$, $df = 1$). The nitrate concentrations reported in this study is similar to the range reported by [Kristensen et al, 1988] from the mangrove sediments of Phuket, Thailand.

In the present study there was no significant correlation between nitrification rates and nitrate concentrations at the control site, while there was a significant negative correlation ($r = -0.25$, $p < 0.05$, $n = 65$) ($p<0.05$ at the experimental site probably indicating a mechanism of feedback inhibition at the experimental site. In the control system the nitrate pool could be either non limited by nitrification or there are alternative local sources for nitrate input. The nitrification rates are higher in the non monsoon seasons at both the sites. It can, therefore, be suggested that in the non-monsoon months, the pore water nitrate concentrations are controlled by microbial activity (nitrification) whereas, in the monsoon season it may have been influenced by overlying waters. The rate at which the nitrate is utilized could also be a factor influencing the monthly variations of nitrate concentrations. Uptake studies revealed that the preference
of the autotrophs to utilize a particular nutrient leads to the variations in the
dissolve pool [Stanley et al., 1987; Alongi, 1988; 1996; Boto and Wellington, 1988; Revera-Monroy et al., 1995]. Boto et al. [1985] reported significant uptake of nitrate by mangrove roots and suggested that pore water nitrate could be depleted due to high uptake. Gilbert and McCarthy [1984] showed that in shallow ecosystems, variation in the uptake rates and nitrogen demand of the autotrophs are the major causes for the seasonality in nitrate concentrations.

**Organic carbon:** Mangroves are known to be highly productive ecosystems (global litter fall of 100 Tg C y⁻¹ [Jennerjahn and Ittekot, 2002]). Recent estimates show that as much as 11% of the total organic carbon inputs across the coastal zone (i.e. through riverine transport) is of mangrove origin. show that the carbon fixed by mangroves is could be significant in the carbon budget of the coastal zone [Jennerjahn and Ittekot, 2002]. It has become clear that a strong interaction exists between the intertidal zone and the adjacent aquatic environment. Both import (of terrestrial material, phytoplankton, sea grasses, etc.) and export (of mangrove-derived organic matter) are expected to have major consequences for the carbon dynamics in both compartments [Hemminga et al, 1994; Bouillon et al, 2003; Marchan et al, 2003]. The imported carbon sources have an important trophic role in sustaining macro-invertebrate communities of the estuarine zones [Bouillon et al, 2002]. Mineralization, however, could represent a major fate for exported organic matter [Bouillon et al, 2002; Borges et al, 2003]. Similarly, estimates of iron available [Alongi, 1988;
Alongi et al, 1993; Middelburg et al, 1996; Alongi et al, 2000] indicate that intense mineralization takes place in intertidal region of mangrove sediments. A continuous range of mangrove ecosystem types exists, from ‘retention’ systems where sediments are rich in organic carbon which is almost entirely of local origin, to ‘flow-through’ systems with mineral sediments (i.e. relatively low in organic carbon) where the organic matter can be dominated by imported sources [Bouillon et al, 2003].

In this study, organic carbon revealed lowest values of 1.2 (±0.1) and 1.9 (±0.3) %, at the control and experimental sites respectively during the monsoon season. This could again be attributed the higher flow rates and lower residence times associated with the southwest monsoon. Both the sites showed difference in pre and post-monsoon organic carbon accumulation. Highest accumulation at the control site was in the pre-monsoon season (3.1±0.8%) while at the experimental site it was in the post-monsoon season (3.4±1%). This compliments the earlier observation made on the levels of ammonium which was higher at the experimental site during the post-monsoon season. In contrast, intermediate values were observed at the control site during post-monsoon (2.9±1.3%) and experimental site during pre-monsoon (2.9±0.3). Though there was no monthly variability at the control and experimental sites there was considerable inter seasonal variability at both the sites (control, p<0.01, df = 2; experiment, p<0.1, df = 2).

Organic carbon related positively to iron (r = 0.67, p < 0.01) and manganese (r = 0.92, p < 0.001, n = 15) at the experimental site, whereas the
relationships were negative at the control site \((r = -0.72, p < 0.001)\) for iron and \((r = -0.51, p < 0.05)\) for manganese. These relationships could imply that at the experimental site there was considerable extraneous input of organic carbon favoring the accumulation of iron and manganese. Wangersky [1986] has reported that coatings of organic matter prevalent in fine grained sediments bind a variety of trace elements.

**Iron and Manganese:** Depth compromised average values of iron at the control site ranged from 6-7.4% for the entire sampling period. There was no inter-seasonal variability at the control site but there was considerable down-core variation \((p < 0.1, df = 4)\) irrespective of the month. In contrast, high inter-seasonal variability was observed at the experimental site \((p<0.001, df = 2)\), with pre-monsoon showing the highest accumulation of Iron \((24\pm3.2\%)\) followed by post-monsoon \((18.7\pm3.6\%)\) and monsoon \((10.2\pm1.9\%)\) seasons respectively. This is because of the fact that the experimental site is under the influence if the movement of iron ore bearing barges which traverse the estuary in large numbers especially in the pre and post-monsoon season. Maximum disparity between the control and experimental sites was observed during the pre-monsoon season \((p<0.001, df = 2)\) while the lowest was during the monsoon \((p<0.01, df = 2)\). The post-monsoon values depicted a transitional stage with considerable variability \((p<0.001, df = 2)\). The values reported in the present study are comparatively higher to those reported by Ray et al [2006] from the Godavari estuarine mangrove ecosystem on the eastern coastline of India.
These authors reported that the average sedimentary iron and manganese values were 0.004% and 0.001%, respectively. Studies by Alagarsamy [2006] showed that the concentrations of iron varied from 2.2% to 49.7% on the surface sediments of the Mandovi estuary, while the concentration of manganese ranged below detection limit to 1.61%. Though reports from the Mandovi estuary [Alagarsamy, 2006] showed that metal concentrations were generally low during monsoon, compared to the pre and post-monsoon seasons, at the adjoining mangrove sediments, they were found to vary. In the present study, the highest accumulation of iron was observed during pre-monsoon, while the values in the monsoon and post-monsoon seasons were lower and comparable. The variation of Manganese values was similar to observations made by Alagarsamy [2006] in the surface sediments of the adjoining Mandovi estuary.

Even though there was significant monthly variability in the accumulation of manganese at both the control (p<0.01, df = 12) and experimental site (p<0.01, df = 12), there was no significant inter seasonal variability at both the sites. In general the Manganese levels remained ~0.5% at the control site throughout the sampling period, whereas at the experimental site it ranged from 1.1(±0.6)% in the post-monsoon to 1.7(±0.6)% in the monsoon season with intermediate values of 1.4 (±0.2%) in the pre-monsoon season. Notable variation between the control and experimental site was observed in the monsoon season (p<0.001, df = 1). The degree of similarity appears to increase as it progresses to post-monsoon season (p<0.01, df = 1) and further decreases in the pre-monsoon season (p<0.0001, df = 1).
In general, the enrichment of iron increases with depth to reach values >165% at an 8–10 cm interval during pre-monsoon. During monsoon, the general enrichment pattern is reversed with the highest enrichment (85.6%) at 0–2 cm. There was no significant correlation between iron and manganese in the monsoon months, neither at the control nor at the experimental site. However, a direction is suggested in the relationship. It is negative at the experimental site and positive at the control site, perhaps suggesting that under a lower concentration of iron, manganese concentration tends to increase. With a higher concentration of iron, this trend changes, suggesting that the increase of both of the elements do not get coupled after a threshold. These observations are contrasted by very high enrichment (393–773%) of manganese at the experimental site during the pre-monsoon season, especially in the depth range of 4–6 cm. A positive relation between iron and manganese during the non-monsoon months at the control site and the absence of such a relation at the experimental site showed that, though the chemistry of iron and manganese are closely related, they could be differentially preferred by organisms, which in turn is influenced by the prevailing environment.

The geoaccumulation index ($I_{geo}$) was originally defined by Müller [1979] for metal concentrations in the <2 μ fraction and developed for global standard shale values. The choice of the background value plays an important role in the interpretation of geological data. $I_{geo}$ has been widely utilized as a measure of pollution in freshwater [e.g. Müller, 1980; Singh et al, 1997; Kralik, 1999] and marine sediments [e.g. Stoffers et al, 1986; Bryan and Langston, 1992; Dickinson
et al, 1996]. Igeo of iron and manganese in the experimental site with control as reference showed that the sediments in the depth range 0–10 cm fall in the 'uncontaminated to moderately contaminated by iron' category during the pre-monsoon and monsoon season. While, in the post-monsoon season, though the 0–4 cm still remains 'uncontaminated to moderately contaminated by iron', the 4–10 cm layer has recovered from Iron contamination and could be termed as 'Uncontaminated'.

The contamination due to manganese is more acute than iron during the pre-monsoon season. During the pre-monsoon, the 0–8 cm section falls under the 'Moderately to strongly contaminated' category, while the 8–10 cm section falls under the 'Moderately contaminated category'. All the depths fall in the 'Uncontaminated' group during the monsoon and post-monsoon and hence could be assessed as free from the manganese pollution. These observations could again suggest that, though the elements are closely related, the biogeochemical cycling of manganese could be more efficient and rapid when compared to iron in the mangrove sediments. Moreover, the mangrove ecosystems play a buffering role by reducing the enrichment levels of iron and manganese in the sediments. The overall assessment could be that, though the Mandovi estuary is under the influence of ferromanganese ore mining with significant impact on the estuarine sediments, the sediments of the adjoining mangroves are comparatively less contaminated, but when strongly contaminated, could be self-regulatory and recover in the time scale tested.
5.2. Nitrifiers –Insights into their trophic structure, abundance and diversity

Occurrence of heterotrophic nitrification have been widely reported [Verstraete and Alexander, 1972; Castignetti and Holocher, 1984; Barraclough and Puri, 1995; Gupta, 1997; Lu et al, 2008; Ahmad et al, 2008], though most of them categorize nitrification as an exclusively chemoautotrophic process. Specifically, the potential of heterotrophic nitrification in sediments has been previously reported by Schimel et al [1984]. In this study, it was observed that nitrite/nitrate was produced by nitrifiers even when the media was supplemented with 0.01% glucose favoring higher organic carbon: dissolved inorganic nitrogen ratio. Hence, the total dissolved inorganic nitrogen pool could be significantly governed by heterotrophic nitrification. The abundance of heterotrophic nitrifiers increased with depth at the experimental site and was governed by concentration of ammonium in the pore water ($r=0.23$, $p < 0.1$). Since the autotrophic nitrifiers were dominant at the experimental site it might pose as a strict competitor for their heterotrophic counterpart for ammonium. Retrievable nitrifier counts showed more significant monthly variation at the control site than the experimental site. In general, the retrievable nitrifier counts at the control site were higher than the experimental site for all the seasons.

At the control site, in the top 2 cm, nitrification could be primarily governed by the abundance of heterotrophic nitrifiers ($r=0.55$, $p <0.05$), while manganese levels in turn govern ($r =0.5$, $p <0.05$) the abundance of these nitrifiers. Another significant relationship at the control site was observed at 4–6 cm, where both the groups of nitrifiers were found to be mutually exclusive ($r=0.5$, $p <0.05$) with
nitrite having a feedback inhibition on the former ($r=0.48$, $p<0.1$). Iron and manganese governed the abundance of autotrophic nitrifiers in the depth intervals of 6–8 ($r=0.55$, $p<0.05$) and 8–10 cm ($r = 0.54$, $p <0.05$). The autotrophic nitrifiers governed the production of nitrate at 6–8 cm ($r=0.56$, $p <0.05$). The concentration of manganese had significant impact on the abundance of heterotrophic nitrifiers ($r=0.47$, $p< 0.1$). As was the case at the control site, the abundance of heterotrophic nitrifiers at the experimental site (0–2 cm) was controlled by the levels of manganese in the sediment. The heterotrophic nitrifiers at 4–6 cm were governed by the levels of ammonium ($r=0.79$, $p< 0.001$) which could be linked to nitrification ($r=0.46$, $p < 0.1$).

Lack of seasonal variability in manganese indicate that manganese turnover time could be much less, as it is actively removed both by physical processes and biogeochemical sequestration. The positive correlations observed between the nitrifiers and manganese could be due to the fact that manganese could be actively used as a co-factor for the ammonia monoxygenase enzyme. More importantly, it could also serve as an alternate terminal electron acceptor in anaerobic respiration [Hulth et al, 1999]. Absence of a significant relationship between iron with the heterotrophic and autotrophic nitrifiers at the both the control and experimental sites suggested that iron was present in excess and perhaps non-limiting. Moreover, manganese (IV) reduction precedes that of Iron (III) because of reduction energetics of the solid phases [Burdige et al, 1992].

The relationship between manganese and general heterotrophs indicate that the latter has a considerable influence in regulating the levels of Manganese.
During post-monsoon, the variation in heterotrophs affected the variation in manganese concentration up to 90% \((r = 0.949, p < 0.001)\). These relationships demonstrate that the nitrifiers and general heterotrophs could be actively involved in maintaining the level of manganese on par with the reference levels at the control site.

In a seasonal perspective, at the control site the availability of ammonium is the most important factor governing the abundance of autotrophic nitrifiers during all the seasons, except for the post-monsoon season when there is a feedback inhibition by nitrate. At the experimental site, only ammonium and organic carbon were found to regulate the abundance of heterotrophic nitrifiers during the monsoon season. However, iron and depth were the two parameters governing the abundance of autotrophic nitrifiers during pre-monsoon and monsoon seasons.

The above observed function diversity is not well supported by the taxonomic diversity of nitrifiers hitherto recorded. After the first reports on successful isolation of chemolithoautotrophic ammonia oxidizers at the end of the 19th century [Frankland et al, 1890; Winogradsky, 1890], researchers continued to investigate the diversity of ammonium oxidizers in natural and engineered environments by applying enrichment and isolation techniques. These efforts resulted in the description of sixteen ammonium oxidizing bacterial species [Watson, 1965; Jones et al, 1988; Koops et al, 1976; 1990; 1991]. Furthermore, DNA-DNA hybridization studies provided evidence for the existence of at least fifteen additional species [Koops and Harms, 1985; Koops et al, 1991; Stehr et al,
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1995]. However, low maximum growth rates and growth yields of ammonium oxidizers render cultivation-based analysis of their environmental diversity extremely time-consuming and tedious. Furthermore, all culture techniques are potentially selective and thus bear the risk of incomplete coverage of the actually existing bacterial diversity [Wagner et al, 1993; Amann et al, 1995; Juretschko et al, 1998]. Comparative 16S rRNA sequence analyses of cultured ammonium oxidizing bacteria revealed that members of this physiological group are confined to two monophyletic lineages within the Proteobacteria. *Nitrosococcus oceani* [Watson, 1965; Trüper and de Clari, 1997] is affiliated with the gamma-subclass of the class Proteobacteria, while members of the genera *Nitrosomonas* (including *Nitrosococcus mobilis*), *Nitrosospira*, *Nitrosolobus*, and *Nitrosovibrio* form a closely related grouping within the beta-subclass of Proteobacteria [Woese et al, 1984; Head et al, 1993; Teske et al, 1994; Stehr et al, 1995; Utåker et al, 1995; Princic et al, 1998]. It has been suggested [Head et al, 1993] and subsequently questioned [Teske et al, 1994] that the latter three genera should be reclassified in the single genus *Nitrosospira*. The availability of 16S rRNA sequences also provided a basis for the development of cultivation-independent methods to investigate the diversity and community composition of these microorganisms in complex environments. PCR-mediated preferential amplification of ammonium oxidizing bacterial 16S rDNA and subsequent cloning and sequencing have been extensively applied to create phylogenetic inventories of various environments [Kowalchuk et al, 1997; 1998; 2000; Pommerening-Röser et al, 1996; McCaig et al, 1999; Mendum et al, 1999; Phillips et al, 1999;
Whitby et al., 1999; Bano and Hollibaugh, 2000], which led to the recognition of seven 16S rRNA beta-subclass ammonium oxidizing bacterial sequence clusters. Recently, the battery of molecular tools to infer the presence of ammonium oxidizing bacteria in the environment has been supplemented by PCR primers for specific amplification of the ammonia monooxygenase structural gene amoA [Holmes et al., 1995; Mendum et al., 1999; Rotthauwe et al., 1997; Sinigalliano et al., 1995]. While environmental 16S rDNA and amoA libraries significantly extended our knowledge on the natural diversity of ammonium oxidizing bacteria, biases introduced by DNA extraction, PCR amplification, and cloning methods [Reysenbach et al., 1992; Farrelly et al., 1995; Suzuki and Giovannoni, 1996; Chandler et al., 1997; Wintzigerode and Goebel, 1997; Polz and Cavanaugh, 1998; Suzuki et al., 1998] have impacts on quantitative information on the community composition. In contrast to PCR-based methods, quantitative information on ammonium oxidizing bacterial population structure and dynamics in the environment is obtainable via membrane or in situ hybridization techniques in combination with ammonium oxidizing bacteria specific oligonucleotide probes [Wagner et al., 1995; Mobarry et al., 1996; Schramm et al., 1996; Juretschko et al., 1998; Logemann et al., 1998]. The latter approach also allows one to directly relate community structure with the morphology and spatial distribution of the detected organisms.

Enumeration of total eubacterial counts by fluorescence in situ hybridization showed that the probe used had detected 34-43% of acridine orange counts. This probably has some implications. One being that the range of
the probe, though considered universal is limited when used on mangrove sediments. The other reason for the underestimate could be due the detrital load in the sediments which could give false signals. The probe Nso1225 is known to cover most of the β proteobacterial ammonium oxidizers, however the detection in the range 52-62% probably signal the existence of novel groups of nitrifiers including the heterotrophs. Incomplete coverage of cultured ammonium oxidizing bacteria in the current 16S rRNA and amoA gene data sets hampered the design and evaluation of specific primers. It was also interesting to note that the sequence match for many of the isolates was less than 98% (16SrDNA) probably indicating its genetic novelty. In addition, this study shows that well known heterotrophs like Pseudomonas, Janthinobacterium, Alcaligenes etc have potential to convert ammonium to its oxidized forms, a process which was thought to be exclusively due to autotrophic nitrifiers. Heterotrophic nitrification, not coupled to any energy yielding metabolic pathways, has been demonstrated in many organisms, including bacteria, such as Arthrobacter sp. [Witzel and Overback 1979], Alcaligenes faecalis [Papen et al, 1989], Thiosphaera pantotropha [Robertson and Kuenen 1988], and fungi [Killham, 1986]. A possible mechanism for the accumulation of nitrite by heterotrophic organisms is the cooxidation of ammonia to nitrite, coupled to the xanthine oxidase reaction [Nagano and Fridovich, 1985]. The most active heterotrophic nitrifier as yet reported belongs to the genus Alcaligenes isolated from soil [Castignetti and Gunner, 1980; 1981; Castignetti and Hollocher, 1982; Castignetti et al, 1983]. It is also capable of the assimilation of nitrate [Castignetti and Gunner, 1980] and
denitrification [Castignetti and Hollocher, 1982]. The ability to carry out both heterotrophic nitrification and denitrification has been established with certainty among bacteria only with the genus Alcaligenes, but this dual ability can be presumed to apply also to a strain of *Pseudomonas aeruginosa* which can oxidize acetaldoxime nitrogen to nitrite [Amarger and Alexander, 1968; Obaton *et al*, 1968]. While Poth and Focht [1985] have stated that *Nitrosomonas* releases nitrous oxide only by denitrifying reaction, Hooper *et al* [1990] also showed that some of the nitrous oxide may be produced through oxidation of ammonium. Also, the data presented by Kim and Craig [1990] suggested that nitrification is the origin of some nitrous oxide in deep ocean water. A possible mechanism of accumulation of nitric and nitrous oxides during nitrification by *Nitrosomonas* is that under certain conditions (e.g. the presence of organic reductants) excess hydroxylamine is released by the cells and reacts chemically with nitrite to form nitric and nitrous oxides. The data presented by Klemendtsson *et al* [1988] and Downs [1988] suggested that nitrifiers play a major role in nitrous oxide production in soils and freshwater lakes, according to Yoshida [1988] the role of nitrifiers in the production of nitrous oxide in the oceans has been overestimated.

The present study gives provides additional proof to this process and thereby strengthens the argument on the existence of heterotrophic nitrification and also the possibility of denitrification to coexist with nitrification in the same organism. The functional diversity of the existing as well as the new genera of nitrifiers could also be very high and this is probably reflected in the metabolic finger prints [details given is section 4.5]. Most of the isolates (22 of the 30
isolates) had signature metabolic profiles which are tuned to a particular carbon substrate. For eg. In the present study, *Nitrobacter winogradskyi* was the only nitrite oxidizer that was capable of using formic acid, while the use of Turanose, Uridine and Acetic acid were restricted to the genus Pseudomonas among the ammonium oxidizers. Incidence of signature substrates was comparatively less among the coupled ammonium-nitrite oxidizers that were identified in this study, however α-Ketobutyric acid and D-Gluconic acid was signature to Janthinobacterium and Alcaligenes respectively.

This work also throws light on the occurrence of wide variety of microbes catalyzing critical reactions which were hitherto considered as a strictly autotrophic process. Also, many isolates generally known to be heterotrophs were able to oxidize ammonium up to its most oxidized product nitrate, a feature that could be subject to further studies in the future. Also, most of the exclusive nitrite oxidizers encountered in this study except for *Nitrobacter winogradskyi* showed higher activity in the presence of organic carbon.

5.3. Regeneration of nitrogen by nitrification: Process and Controls

5.3.1. Inter parameter relationships

Benthic nitrogen regeneration may supply an important, but variable fraction of the nitrogen requirements for plankton production in marine coastal and shelf regions. The amount of nitrogen recycled by the benthos depends on the pelagic-benthic coupling, i.e. the quantity and quality of the organic matter sinking to the sediments, but also on the nitrogen transformations taking place in
the sediments. In the sediments nitrification plays a key role. Its coupling with N-mineralization and denitrification, together with nitrate, determines the concentrations in the overlaying water and the species of inorganic nitrogen effluxing from the sediment and the amount of nitrogen shunted into the denitrification sink. Numerous investigations have described benthic nitrogen exchange in estuarine and coastal sediments [Klump and Martens, 1983; Seitzinger, 1988]. The variability in temporal and seasonal patterns indicates a complex relationship between physical-chemical and biological control factors, but there are some general trends: higher inputs of organic nitrogen to the sediments generally increase both nitrification and denitrification. However, at high organic inputs and/or increasing temperatures, nitrification becomes low or zero due to limited oxygen penetration and sulfide inhibition [Hansen et al, 1981; Kemp et al, 1982; 1990; Jensen et al, 1988; Billen, 1988; Seitzinger, 1988]. The increase in nitrification rate at higher ammonium availability is usually small due to increased oxygen uptake by other heterotrophic processes. A higher organic input to the benthos is often associated with increased macrofaunal abundance [Nixon 1981; Kemp et al, 1982; Grebmeier et al, 1988]. The macrofauna may further contribute to increased rates of nitrification and denitrification and to a stronger coupling between the two processes due to the effect of ventilated macrofaunal burrows [Kristensen et al, 1988; Aller, 1988]. Macrofaunal activity also tends to increase the total rate of nitrogen mineralization [Kristensen and Blackburn, 1987; Lomstein et al, 1989] and the flux of ammonium from the sediments.
The benthic chamber in mangroves has been least studied in terms of nitrogen cycling. This has hampered our understanding on these productive coastal marine ecosystems which are vulnerable to human impacts. Most of the studies reported from mangrove forests are sporadic measurements, though some studies conducted in Australia [Boto and Wellington, 1988; Trott and Alongi, 1999], Pakistan [Harisson et al, 1997], Mexico [Rivera-Monroy et al, 1995], and India [Krishnamurthy et al, 1975; Dham et al, 2002] discuss seasonal cycles. Nitrifying bacteria and nitrification rates in general, may be regulated by many factors including ammonium [Triska et al, 1990; Jones et al, 1995], pH [Saractchandra, 1978], temperature [Paul and Clark, 1989], oxygen concentration [Stenstromm and Podlska, 1980; Triska et al, 1990], competition for ammonium [Verhagene and Laanbroek, 1991], and organic carbon availability [Verhagene and Laanbroek, 1991]. Irrespective of season or depth of sampling, nitrification rates were predominantly governed by the availability of organic carbon ($r=0.32, p<0.01$) at the control site. This is supported by a positive correlation of organic carbon with heterotrophic nitrifiers ($r=0.28, p<0.05$). Nitrification rates at the top layer (0–2 cm) of the core from the control site was governed by the abundance of heterotrophic nitrifiers ($r=0.58, p<0.02$); whereas at the bottom (8–10 cm) it appears to be regulated by both the abundance of heterotrophic nitrifiers ($r=0.46, p<0.1$) as well as organic carbon ($r=0.45, p<0.1$). A positive correlation with nitrite ($r=0.48, p<0.1$) probably indicates that the ammonium oxidation might be of a higher magnitude than nitrite oxidation. In the pre-monsoon season nitrification rates increased with depth ($r=0.67, p<$
and were limited by the availability of ammonium ($r = 0.57$, $p < 0.001$). This favors enhanced nitrification rate [Straus and Lamberti, 2000]. Bulk of the carbon could be labile as the nitrification rate is negatively linked to organic carbon ($r=0.4$, $p < 0.05$). In addition, there was no single set of factors governing nitrification rate during the monsoon season. However, the recalcitrant fraction of organic carbon is considered to favor enhanced nitrification rates by reducing the utilization pressure on ammonium between heterotrophs and nitrifiers [Straus and Lamberti, 2000]. Even though the organic carbon: dissolved inorganic nitrogen ratio is high there was no relation between organic carbon and nitrification rates for the pre and post-monsoon season indicating that organic carbon is either unlimiting or dominated by the recalcitrant fraction. In general, the system evolved from a ‘low organic carbon: dissolved inorganic nitrogen – low nitrification rate’ system in the monsoon season to a ‘high organic carbon: dissolved inorganic nitrogen – high nitrification rate’ system in the pre-monsoon season with the post-monsoon representing a transition period where the organic carbon: dissolved inorganic nitrogen ratio gradually increased. This study indicates that the quality of organic carbon could be a more important proxy to nitrification rate rather than organic carbon: dissolved inorganic nitrogen ratio. Further, it could also be inferred that maximum fraction of recalcitrant carbon during the pre and post-monsoon seasons accumulated at 4–6 cm.

Nitrification rates at experimental site showed a positive relation with iron ($r = 0.47$, $p < 0.001$) and autotrophic nitrifiers ($r=0.43$, $p < 0.001$) which is indicative of anaerobic autotrophic nitrification. An inverse relation with nitrate ($r$
=-0.25, p <0.05) signifies feedback inhibition indicating that at the experimental site, nitrification rate at the optimum. Hence it appears that the control site is dominated by heterotrophic nitrification whereas autotrophic nitrification governs the experimental site. In addition, organic carbon (3.4±1%) and ammonium (27.8±5.6 µg at NH₄⁺-NL⁻¹) peaked at the experimental site during the post-monsoon season while at the control site it was during the pre-monsoon season (organic carbon 3.1±0.8% and ammonium 27.8±4 µg at NH₄⁺-NL⁻¹). However, the pre-monsoon of the experimental site was marked by highest levels of nitrification rates (18.2±0.6 ng at-N g (sediment)⁻¹ h⁻¹) indicating a decoupling with organic carbon and ammonium. These parameters remain coupled during the pre-monsoon season at the control site. Ammonium plays (r= 0.55, p< 0.001) plays a limiting role in the pre-monsoon season even though the organic carbon: dissolved inorganic nitrogen ratio is comparatively higher. Further, the production of nitrite appears to govern the nitrification rates during the monsoon season. The abundance of autotrophic nitrifiers is the major factor governing the nitrification rate for most of the depth intervals sampled. Positive relations with autotrophic nitrifiers at 0–2 cm (r =0.54, p< 0.05), 2– 4 cm(r=0.47, p <0.1), 6–8 cm (r=0.49, p< 0.1) and 8–10 cm (r =0.68, p <0.01) is in accordance with the earlier observation. In addition, iron appears to be the next critical factor regulating nitrification at the experimental site with maximum coupling observed at 4–6 cm (r =0.7, p < 0.001) and 6–8 cm (r =0.6, p <0.01) intervals. The feedback inhibition of nitrate is restricted to the bottom layer of 8–10 cm depth indicating enhanced nitrification potential down the core till this depth.
5.3.2. Experimental studies

The mangrove environments are subject to several influences, the most profound could be those with an anthropogenic origin. The impact on nitrification can be significant even when pollution sources are remote and contact indirect. For instance, although there is very little human activity on the shores of Lake Superior, its levels of nitrates increased 4-fold during the past century, primarily due to loading of nitrogenous compounds from the atmosphere [Bennet, 1986]. When the ecosystem is disturbed certain key reactions like nitrification is disturbed resulting in ecosystem malfunction. When nitrite accumulates, due to incomplete nitrification or denitrification, it reacts readily with chlorine, decreasing effective residual chlorine concentrations. It also interferes with chloramination [Wolfe et al, 1988]. Heavy agricultural fertilization frequently results in massive nitrification and leaching of nitrates to ground water, increasing concentrations of nitrates to levels beyond permitted standards [Soares et al, 1991].

The experimental studies [details given in section 4.6] reported in the present study have been conducted in dark, as light has a detrimental effect on nitrification. Hooper and Terry [1973, 1974] have shown that visible light (420 nm) inhibits oxidation of ammonia but not of hydroxylamine, at a rate constant proportional to its intensity. Inhibition by light also abolishes the ability of the putative ammonia monooxygenase peptide to bind acetylene [Hyman and Arp, 1992].

Although nitrification is considered to be sensitive to excess ammonia and nitrite, Blouin et al [1989] have reported a complete oxidation of very high
concentrations of ammonia nitrogen in farm wastes in 5 days to nitrite, and another 10 days were needed for oxidation of all nitrite to nitrate. In the present study, the addition of ammonium was found to have a positive influence on nitrification rates with maximum stimulation occurring at 100 μM ammonium (159±21 nM NO₃⁻·h⁻¹). Though denitrification rates retarded in 50μM ammonium amendment, higher amendments stimulated the reduction of nitrate. Unlike that of ammonium, addition of nitrite had a very strong negative impact on nitrification rates. Even additions of lower concentrations brought down the nitrification rates by an order of ten. Higher additions of nitrite had a stimulatory effect on nitrite reduction. The addition of 50μM nitrate triggered the increase of nitrification rates by almost double to reach ~65 nM NO₃⁻·h⁻¹, however on prolonged incubation the rates came down to ~43 nM NO₃⁻·h⁻¹. All the nitrate enrichments facilitated the proportional increase of denitrification rates in the system, the increase as compared to the controls were from ~18 to 60 nM NO₃⁻·h⁻¹. At a concentration of 2-3 mM, ammonia will cause 50-90% inhibition in the rate of oxygen generation by any algal population. Un-ionized ammonia penetrates freely through the cell membrane and, unless kept below ~2 mM, reaches intracellular concentrations which strongly inhibit photosynthesis and oxygenation. This can be interrupted only by enhancing nitrification [Abeliovich, 1983].

The majority of nitrogen fertilizer added in agricultural practice is in the form of ammonia, which is adsorbed by the soil particles and then slowly released. However, in the presence of nitrifiers, ammonium is also rapidly oxidized to nitrate which is then lost either through leaching to groundwater
(polluting aquifers) or to surface waters (polluting lakes and rivers) or it is oxidised to nitrous and nitric oxides. Under anaerobic conditions, if electron donors are available in sufficient concentrations, nitrates may be lost by their reduction to dinitrogen. Therefore, direct measurements of nitrification potential, number of nitrifiers, and actual nitrification rates, in coastal ecosystems are needed for accurate estimates of nitrogen fluxes through these habitats. In the present study, in contrast to expectations, the addition of fertilizers only marginally increased the nitrification rates in the system (\(-25 \text{ nM NO}_3^- \text{ h}^{-1}\)). Addition of 1% w/w had a profound influence on triggering denitrification rates in the system to reach values close to 80 nM NO$_3^-$ h$^{-1}$.

The addition of dissolved organic carbon (glucose) had a negative impact on nitrification rates while it supported enhanced denitrification rates. Nitrification rates were found to decrease with increasing dissolved organic carbon to reach very low values of \(-3 \text{ nM NO}_3^- \text{ h}^{-1}\) at 50 mgC/l amendment. Probably, the addition of dissolved organic carbon into the system resulted in enhanced heterotrophic activity which resulted in enhanced competition for ammonium between the nitrifiers and other heterotrophs. Similar observations have been made by Strauss and Lamberti [2000] in stream sediments.

Contamination of mangrove soils with liquid hydrocarbons is yet another aspect of anthropogenic activity. Addition of liquid hydrocarbon had tremendous negative impact on nitrification. An addition of 10 and 50 mg/g retarded nitrification rates to as low as \(-1 \text{ nM NO}_3^- \text{ h}^{-1}\) while 100mg/g was found to block nitrification completely. The same was also true for denitrification rates but the
extent of inhibition was much lower. Unlike the present report, Drozd [1976] had demonstrated that hydrocarbons are metabolized by a wide range of chemolithotrophic ammonia oxidizers [Hyman and Wood 1983; Jones and Morita 1983; Voysey and Wood 1987], apparently due to the non-specificity of ammonia monoxygenase. Other hydrocarbons were also co-metabolized by ammonia oxidizers. Ethylene is oxidized by *N. europaea* to ethylene oxide, a reaction sensitive to inhibitors of ammonia oxidation. Ethylene oxide is also further metabolized [Hyman and Wood 1984]. Hyman *et al.* [1988] also demonstrated that n-alkanes (C$_x$-C$_8$) were oxidized to their respective alcohols, with increasing rates from C$_1$ to C$_4$. Halogenated hydrocarbons were also degraded by ammonia oxidizers Vannelli *et al.*[1990].

Much is known about the possible toxicity of pesticides to microorganisms growing in laboratory media and to microbial populations and communities in soils. For example, the effects and fate of propoxur [Kuseske *et al.*, 1974; Gupta *et al.*, 1975], dichlorvos [Ballington *et al.*, 1978], chlorpyrifos [Miles *et al.*, 1979] and carbaryl [Rodriguez and Dorough, 1977] in soil have been investigated. Butcher *et al.* [1977] suggested that chlorpyrifos enhanced algal blooms in pond water. Studies of the effects of a number of pesticides on nitrification in soil have shown some inhibition at high concentrations (usually greater than 50 ppm) of the test compounds [Kuseske *et al.*,1974; Gupta *et al.*, 1975]. In the present study, the pesticide chlorpyrifos was found to have a marginal stimulatory effect on nitrification in both amendments of 10 and 50ppm. As was the case in earlier reports concentrations above 50ppm retarded nitrification rates to almost 13 nM
NO₃⁻ h⁻¹. The case was almost different for the denitrification mechanism, addition of chloropyrifos at concentrations of 50 and 100ppm retarded denitrification rates in the system to ~ 12 and 8 nM NO₃⁻ h⁻¹.

Schoberl and Engel [1964] evaluated the effect of dissolved oxygen concentration by observing dissolved oxygen uptake rates as a function of its concentration. They found that the growth rate for Nitrosomonas was independent of the dissolved oxygen concentration above 1.0 mg/l, and that for Nitrobacter, growth rate was independent above 2.0 mg/l. Limiting amounts of dissolved oxygen (concentrations below 2 mg/l) inhibit nitrification and cause nitrite accumulation or nitrous and nitric oxide production [Goreau et al, 1980; Painter, 1986]. Ammonia oxidizing bacteria are the key functional group in removing ammonium from wastewaters. Knowledge of the effect of oxygen on nitrification and nitrifying populations has economic importance since aeration of activated sludge is one of the most costly items in the operation of a wastewater treatment plant [Painter, 1986]. In environments with high inputs of ammonium, such as wastewaters, bio-oxidation of this substrate increases the oxygen uptake and lowers the pH. Such modifications of the environment not only affect the production of nitrite and nitrate but can also select a different nitrifying community that is perhaps specialized for these new conditions. Nitrification does occur in extreme environments that pure cultures of nitrifiers cannot tolerate [Bock et al, 1986]. The effects of extremely high dissolved oxygen concentration have received less attention than low dissolved oxygen concentrations. Okun [1949] and Haug and McCarty [1972] investigated high dissolved oxygen concentration
and report no adverse effect for dissolved oxygen concentrations of 33 and 60 mg/l respectively. Since there was a lacuna on the understanding of the impact of elevated oxygen levels in the system on nitrification, differential aeration experiments were conducted which yielded results in favor for nitrification. Though both the aeration strategies could lower the ammonium levels by nitrification, the pulse mode incubations were found to have a better effect compared to the continuous mode. This could mean that in continuous mode there could be a possibility of supersaturation of dissolved oxygen which could be limiting nitrification.