

ORGANIC CARBON AND IRON MODULATE NITRIFICATION RATES IN MANGROVE SWAMPS OF GOA, SOUTH WEST COAST OF INDIA

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Abstract:

Nitrification, fuelled by ammonium is the pivotal oxidative pathway to nitrogen cycling. In spite of its ecological significance, the factors regulating nitrification rates in the benthic realm remain poorly understood. The present study therefore examines some of the factors like ammonium, nitrite, nitrate, organic carbon, iron and manganese on down-core variability in benthic nitrification rates in two different mangrove ecosystems, one under the influence of ferromanganese ore mining (experiment) and the other relatively undisturbed (control). We hypothesize that besides organic carbon, iron could also influence the rate of nitrification. The study also contrasts the distributive pattern of autotrophic and heterotrophic nitrifiers in the two regions. The concentration of iron at the control site ranged from 1.1 to 15.1% while at the experimental site it ranged from 2.9-46%. The levels of organic carbon at control and experimental site ranged from 0.02-6.9 and 0.1-6.5% respectively. The nitrification rates at the control and experimental sites are comparable and ranged from 3.2 ± 1.2 to 18.4 ± 1.9 and 2.7 ± 1.5 to 18.2 ± 0.6 ng at-N g (sediment)⁻¹h⁻¹ respectively. While the abundance of heterotrophic nitrifiers at both the sites ranged from 10^{2-3} cells g⁻¹ sediment, the autotrophic nitrifiers at the experimental site was higher by an order at $\sim 10^3$ cells g⁻¹ sediment reflecting the relatively higher refractile nature of organic carbon at the experimental site (Straus and Lamberti, 2000). Though organic carbon and nitrification rates are similar in both the sites, the underlying mechanisms governing the processes could be different. Our studies suggest that at the control site, heterotrophic nitrifiers govern nitrification rates ($r=0.28$, $p<0.05$, $n=64$) using organic carbon ($r=0.32$, $p<0.01$, $n=64$). At the experimental site, nitrification was governed more by autotrophic nitrifiers ($r=0.43$, $p<0.001$, $n=64$) at the expense of iron ($r=0.47$, $p<0.001$, $n=64$). Therefore at the experimental site with higher load of iron, autotrophic nitrification could be more important. It is therefore inferred that both the quality of organic carbon and quantity of iron govern nitrification rates in these mangrove swamps.

Key words: Nitrification, Nitrifiers, Mangroves, Organic carbon, Manganese, Iron

1. Introduction:

Nitrification is a microbial process that converts ammonium (NH_4^+) to nitrate (NO_3^-) via nitrite (NO_2^-) and occupies a central position within the global nitrogen cycle. Hence, the factors regulating this process are vital to eutrophication as well as to health concerns related to enhanced nitrate levels in aquatic ecosystems. Nitrification also serves as a significant link between nitrogen mineralization and potential nitrogen loss from the benthic system through denitrification (Seitzinger, 1990 and Sloth et al., 1992). Nitrification was traditionally considered to be restricted to aerobic environments (e.g. Froelich et al., 1979), but studies (Mortimer et al., 2004) have shown that nitrification does happen in anoxic environments at the expense of elements like manganese (Mn) and/or iron (Fe). In a recent study, Krishnan et al. (2007) had suggested the possibility of anaerobic nitrification in mangroves as a mechanism to ward off the excess manganese entering the sediments as a result of pollution. Nitrification also depends on NH_4^+ regeneration rates, which in turn is positively influenced by temperature (Nixon, 1981). The presence of organic carbon (OC) compounds may diminish the rate and the yield of nitrate formed, by diverting nitrogen from the nitrifiers to the heterotrophs proliferating at the expense of easily assimilable carbon (Delwiche and Finstein, 1965). However, this depends mainly on the magnitude of C:N ratio and the quality of organic compound (Straus and Lamberti, 2000). Straus and Lamberti (2000) have hypothesized that high C:N and higher levels of labile organic carbon does inhibit nitrification, whereas low C:N and higher levels of refractory organic carbon enhance nitrification rates. Moreover, though mangroves are considered to be productive coastal marine ecosystems (Qasim and Wafar, 1990), nutrient measurements, especially those of nitrogen which is an important factor sustaining this productivity has been sparse (Dham et al., 2002). Hence, in order to understand some of the important factors regulating nitrification rates (N_{rate}) two different mangrove sites with different ecological setup were studied (south-west coast of India, Figure 1). We

hypothesize that besides organic carbon, iron could also influence the rate of nitrification.

2. Materials and methods:

2.1. Site description and sampling procedure

Sediment cores were collected from mangrove swamps along the Chapora and Mandovi estuaries. The control site (15° 38.28' N and 73° 47.71' E) which is relatively free from metal pollution is located at Tuvem in the Chapora estuary, while the experimental site which is under the influence of ferromanganese ore mining and is exposed to enrichment of metal ores of Fe and Mn is located at Diwar (15° 30.42' N and 73° 52.28' E) in the Mandovi estuary (Figure1). Monthly sampling was carried out at both the control and experimental sites from April 2005 to April 2006 using a PVC hand-held sediment corer. Sediment core was sectioned at 2 cm intervals in sterile conditions to obtain representative samples at 0–2, 2–4, 4–6, 6–8 and 8–10 cm depths.

2.2. Pore water extraction and chemical analyses

After sectioning the cores, each fraction was made into slurry with a known volume of saline and then loaded separately into centrifuge tubes. The tubes were spun at low R.P.M (5000) at 4 °C for 10 minutes. The water was then carefully siphoned out into a pre-cleaned 100ml polyethylene bottle and allowed to stand for 15 minutes in cold conditions in order to sediment out the coarse particles. Further, the diluted pore water was filtered on GF/F and then subsequently on 0.22 µ membrane filter. The filtrate was stored in cold for further analysis. By spinning at low temperature and RPM it was ensured that minimal disturbance was caused to the benthic organisms, which on lysis could change the pore water chemistry. NH_4^+ , NO_2^- and NO_3^- were measured spectrophotometrically as described by Koroleff (1969), Bendschneider & Robinson (1952); and Wood et al. (1967) respectively. OC was determined by the wet oxidation method with a precision of 0.01% (El Wakeel and Riley, 1957). Sub samples for metal analyses were dried at 60(±2)°C for 48 h and

disaggregated in an agate mortar before chemical treatment for Fe and Mn analysis. The detailed procedure of sediment digestion is given in Balaram et al. (1995). Trace metal concentrations (Fe and Mn) were measured using a flame atomic absorption spectrophotometer (AAS, Perkin Elmer Model 5000). The accuracy of the analytical procedures was assessed using the certified reference material MAG-1 (USGS) that yielded results within the reference value range (Flanagan, 1967, 1976).

2.3. Measurement of nitrification rates

N_{rate} was measured by the method of Schell (1978). Each intact section was transferred to a beaker (500 ml capacity), to which filtered estuarine water was added. Samples were then incubated with $^{15}\text{N-NH}_4\text{Cl}$ (10% of the ambient) in the dark for 24 hours. At the end of the incubation period, samples were gently mixed and pre-screened through a 200 μm mesh net. Samples were then filtered onto Whatman GF/F filter pads (pre-ignited at 400 $^{\circ}\text{C}$ for 4 hours) and 200ml of the filtrate was recovered for the extraction of nitrite. NO_2^- in the sample was extracted as a dye (1-benzene-azo-2-naphthol) by using an organic solvent. $^{15}\text{N}:^{14}\text{N}$ isotope ratios of the NO_2^- extracted from the incubation medium were measured by emission spectrometry using a Jasco N-150 Nitrogen analyzer. N_{rate} was calculated according to Lipschultz (1984). All measurements were done in triplicate.

2.4. Microbial analyses

Sub samples of approximately 5 g wet weight sediment were sampled using sterile syringe cores. The sub samples were transferred to 45 ml full strength sterile seawater (10^{-1} dilution). Tween80 (50 μL) was added and the mixture was sonicated at 40 mHz for 10 s. Serial dilutions of the sediment samples were made in autoclaved seawater to yield dilutions from 10^{-1} to $10^{-6/-7}$. Nitrifiers were enumerated by the most probable number (MPN) method of Alexander and Clark (1965) on both inorganic and organic media. Winogradsky's inorganic nitrifying media (seawater amended with NH_4Cl of 2 mM final concentration) was

distributed in 5 ml quantities in 15 ml screw capped tubes. The organic nitrifying media was prepared by adding 0.01% final concentration of glucose to Winogradsky's inorganic nitrifying media. From each dilution ranging from 10^{-1} to $10^{-6/-7}$, 500 μ l was inoculated in triplicate in the nitrifying media until the inoculation from the highest dilution into the culture tubes yielded negative results. The culture tubes were incubated in the dark for a period ~60 days at $28(\pm 1)^{\circ}\text{C}$. After incubation, the tubes were tested for the presence of NO_2^- and/or NO_3^- . The combinations of positive and negative tubes were scored and MPN was assessed from McCready's table (Rodina, 1972).

Statistical analysis was carried out using Pearson's Correlation Coefficient and Analysis of Variance in order to explore the possible associations and variances existing between/within different variables. The analysis was done using Microsoft Excel 2000. The bacterial parameters were normalized by log transformation before analyses.

3. Results:

3.1. Pore water chemistry

Monthly down-core variation in NH_4^+ , NO_2^- and NO_3^- at both the control and experimental sites have been illustrated in the figures 2 a, b and c respectively. Depth compromised pre-monsoon (February-April) average of NH_4^+ at the control site was $19.1 \pm 4 \mu\text{g}$ at NH_4^+ -NL $^{-1}$ pore water (n=25). Similar values were encountered at the experimental site with NH_4^+ averaging $19.9 \pm 2.4 \mu\text{g}$ at NH_4^+ -NL $^{-1}$ pore water (n=25). However, the monsoon season (June-September) was characterized by lower levels of NH_4^+ at both the control ($16.8 \pm 3.6 \mu\text{g}$ at NH_4^+ -NL $^{-1}$ pore water; n=20) and experimental sites ($14.1 \pm 2.1 \mu\text{g}$ at NH_4^+ -NL $^{-1}$ pore water; n=20). Interestingly, the post-monsoon (October-January) values at the control site showed an increase in NH_4^+ accumulation ($17.9 \pm 7.9 \mu\text{g}$ at NH_4^+ -NL $^{-1}$ pore water; n=20) as compared to the monsoon, but they still remained lesser than the pre-monsoon values. NH_4^+ accumulation at the experimental site was highest ($27.8 \pm 5.6 \mu\text{g}$ at NH_4^+ -NL $^{-1}$ pore water; n=20) during post-monsoon season as compared to monsoon and pre-monsoon seasons. NH_4^+ showed

significant monthly variability at the experimental site ($p < 0.001$, $df = 12$), while there was no significant variability at the control site. Similar trend was observed inter-seasonally with no variability at the control site but with a significant variation at the experimental site ($p = 1.5 \times 10^{-6}$, $df = 2$). When compared, the control and experiment site differed only in the post-monsoon season ($p < 0.001$, $df = 1$) and this could be attributed to the variability observed at 0-2 cm ($p < 0.001$, $df = 1$).

Average pre-monsoon NO_2^- concentration at control and experimental sites were 2.4 ± 0.8 and 2.1 ± 0.1 μg at NO_2^- -NL⁻¹ pore water ($n=25$) respectively. During monsoon season, NO_2^- values at control (2.3 ± 0.5 μg at NO_2^- -NL⁻¹ pore water; $n=20$) and experimental sites (2.7 ± 0.6 μg at NO_2^- -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. NO_2^- showed a significant monthly variability at the experimental site ($p < 0.1$, $df = 12$) where as the control site was marked by significant down-core variability ($p < 0.01$, $df = 12$). 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 NO_2^- both at the control and experimental sites.

Levels of NO_3^- at the control site were 9.3 ± 2.9 μg at NO_3^- -NL⁻¹ pore water ($n=25$) during the pre-monsoon season, while, the monsoon values were lower (6.1 ± 1.2 μg at NO_3^- -NL⁻¹ pore water; $n=20$). In contrast, highest NO_3^- values at the experimental site (13.2 ± 2.0 μg at NO_3^- -NL⁻¹ pore water; $n=20$) were recorded during the monsoons. The pre and post-monsoon values were 8.6 ± 1 μg at NO_3^- -NL⁻¹ pore water; $n=25$ and 8 ± 2.1 μg at NO_3^- -NL⁻¹ pore water; $n=20$ 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. NO_3^- showed significant

monthly variability at both the control ($p = 2.9 \times 10^{-5}$, $df = 12$) and the experimental site ($p = 4.4 \times 10^{-3}$, $df = 12$). Contrary to the pattern of inter-seasonal stability in NO_2^- , NO_3^- values were significantly variable at both the control ($p < 0.1$, $df = 2$) and experimental sites ($p < 0.01$, $df = 2$). There exists considerable variability at both the control and experimental sites during the monsoon and post-monsoon seasons ($p < 0.1$, $df = 1$).

3.2. Iron, manganese and organic carbon

Monthly down-core variation in Fe, Mn and OC at both the control and experimental sites have been illustrated in the figures 3 a, b and c respectively. Depth compromised average values of Fe 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 Fe ($24 \pm 3.2\%$) followed by post-monsoon ($18.7 \pm 3.6\%$) and monsoon ($10.2 \pm 1.9\%$) seasons respectively. 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 ($p < 0.001$, $df = 2$).

Even though there was significant monthly variability in the accumulation of Mn 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 Mn levels remained $\sim 0.5\%$ at the control site throughout the sampling period, whereas at the experimental site it ranged from $1.1 (\pm 0.6)\%$ in the post-monsoon to $1.7 (\pm 0.6)\%$ in the monsoon season with intermediate values of $1.4 (\pm 0.2)\%$ in the pre-monsoon season. Notable difference 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 ($p < 0.01 = 3.4 \times 10^{-2}$, $df = 1$) and further decreases in the pre-monsoon season ($p < 0.0001$, $df = 1$).

A study of the OC at the control and experimental sites, revealed lowest values of $1.2 (\pm 0.1)$ and $1.9 (\pm 0.3)$ % respectively during the monsoon season. Both the sites showed differences in pre and post-monsoon OC accumulation. Highest accumulation at the control site was in the pre-monsoon season ($3.1 \pm 0.8\%$) while at the experimental site it was in the post-monsoon season ($3.4 \pm 1\%$). In contrast, intermediate values were observed at the control site during post-monsoon ($2.9 \pm 1.3\%$) 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, $p < 0.1$, $df = 2$). The control and experimental sites showed similar values during non monsoon seasons but different levels during the monsoon ($p < 0.1$, $df = 1$). This variability could be attributed to that observed in the depth intervals 0-2 ($p < 0.1$, $df = 1$) and 2-4 cm ($p < 0.1$, $df = 1$).

3.3. Nitrification rates

N_{rate} (Figure 4 a) at the control site ranged from 3.2 ± 1.2 in the monsoon to 6 ± 1.1 and 18.4 ± 1.9 $\text{ng at-N g (sediment)}^{-1} \text{h}^{-1}$ in the post and pre-monsoon respectively. The trend was similar at the experimental site with pre-monsoon showing the highest rates (18.2 ± 0.6), followed by post-monsoon (5.8 ± 1.1) and monsoon (2.7 ± 1.5) seasons. There was significant monthly and inter seasonal variation at both the control ($p = 7.2 \times 10^{-21}$, $df = 12$ and $p = 9.3 \times 10^{-26}$, $df = 2$) and experimental sites ($p = 6.5 \times 10^{-23}$, $df = 12$ and $p = 6.3 \times 10^{-28}$, $df = 2$). There was no down-core variability in N_{rate} for both the control and experimental sites.

3.4. Autotrophic and heterotrophic nitrifiers

The abundance of autotrophic (A_{Ntb}) and heterotrophic nitrifiers (H_{Ntb}) is illustrated in the figures 4b and c. The A_{Ntb} abundance at control site for all the seasons were $\sim 10^6$ cells per g dry weight of sediment, where as at the

experimental site highest abundance was encountered during the pre-monsoon season [$2.1 (\pm 1) \times 10^7$ cells per g dry weight of sediment] and the lowest abundance was during the monsoon season [$3(\pm 7) \times 10^5$ cells per g wet weight of sediment]. The post-monsoon recorded a marginal increase [$7(\pm 2) \times 10^5$ cells per g wet weight of sediment]. The seasonal average of H_{Ntb} showed that they were one to two orders higher than the A_{Ntb} . At the control site, highest abundance of H_{Ntb} was during the pre-monsoon season [$3.0 (\pm 1.0) \times 10^8$ cells per g wet weight of sediment], while the lowest was in the post-monsoon season [$1.5 (\pm 0.4) \times 10^8$ cells per g wet weight of sediment]. The monsoon season recorded intermediate values [$2.4 (\pm 2.3) \times 10^8$ cells per g wet weight of sediment]. The abundance of H_{Ntb} at the experimental site was uniform ($2.2-2.5 \times 10^8$ cells per g wet weight of the sediment) through out the monsoon cycle with highest in the pre-monsoon and lowest in the post-monsoon. There was no inter-monthly variation in the abundance of A_{Ntb} at the control site while there was significant variability at the experimental site ($p= 1.31 \times 10^{-6}$, $df = 12$). In contrast, the H_{Ntb} abundance showed significant monthly variability at both the control ($p= 1.7 \times 10^{-4}$, $df = 12$) and experimental sites ($p= 1.5 \times 10^{-3}$, $df = 12$). Neither A_{Ntb} nor H_{Ntb} showed any significant down-core variation at the control and experimental sites. There was considerable similarity between the control and experimental sites for all the depth intervals for both the A_{Ntb} and H_{Ntb} . A comparison of inter seasonal abundance revealed variability at the experimental site for A_{Ntb} ($p= 4.2 \times 10^{-3}$, $df = 2$) where as the abundance was uniform at the control site. In contrast, the H_{Ntb} showed significant monthly variability at the control site ($p= 1.4 \times 10^{-3}$, $df = 2$), while their inter seasonal abundance at the experimental site was uniform. On comparing the control and experimental sites for the different seasons, the A_{Ntb} varied during both the pre ($p= 4.6 \times 10^{-3}$, $df = 1$) and monsoon ($p= 4.8 \times 10^{-2}$, $df = 1$) seasons while the post-monsoon season was comparable. Similar comparisons for H_{Ntb} yielded contrasting results. There was variability only in the post-monsoon season ($p= 3.6 \times 10^{-2}$, $df = 1$) whereas the abundance was uniform for both the pre and monsoon seasons.

4. Discussion:

4.1 Nitrification rates: process and controls

Lack of seasonal monitoring especially in the benthic realm in mangroves has hampered our understanding on these productive coastal marine ecosystems which are vulnerable to human impacts. Most of the studies conducted in the mangrove forests report sporadic measurements, though some studies conducted in Australia (Boto and Wellington 1988; Trott and Alongi 1999), Pakistan (Harrison et al, 1997), Mexico (Rivera-Monroy et al 1995), and India (Krishnamurthy et al., 1975 and Dham et al., 2002), discuss seasonal cycles. Further, very few studies have attempted to investigate benthic nitrification and factors governing the same. Nitrifying bacteria and N_{rate} in general, may be regulated by many factors including NH_4^+ (Triska et al., 1990; Jones et al., 1995), pH (Sarathchandra 1978), temperature (Paul and Clark 1989), oxygen concentration (Stenstrom and Poduska 1980; Triska et al., 1990), competition for NH_4^+ (Verhagen and Laanbroek 1991), and OC availability (Verhagen and Laanbroek 1991). In the present study, multiple regression analysis was employed along with simple correlation analysis to elucidate the factors governing N_{rate} . The results of the multiple regression analysis are given in Table 1. In general, N_{rate} was governed by the availability of OC ($r=0.32$, $p<0.01$, $n=64$) at the control site irrespective of season or depth of sampling. This is further supported by a positive correlation of OC with H_{Ntb} ($r=0.28$, $p<0.05$, $n=64$). Depth wise correlation analysis gives additional insights into these relationships. N_{rate} at the top layer (0-2cm) of the core from the control site was governed by the abundance of H_{Ntb} ($r=0.58$, $p<0.02$, $n=12$); whereas at the bottom of the core (8-10cm) it appears to be regulated by both the abundance of H_{Ntb} ($r=0.46$, $p<0.1$, $n=12$) as well as the OC ($r=0.45$, $p<0.1$, $n=12$). A positive correlation with NO_2^- ($r=0.48$, $p<0.1$, $n=12$), probably indicates that the NH_4^+ oxidation might be of a higher magnitude than NO_2^- oxidation. In the pre-monsoon season N_{rate} increased depth ($r=0.67$, $p<0.001$, $n=25$) and was limited by the availability of NH_4^+ ($r=0.57$, $p<0.001$, $n=25$). A scatter plot of OC:DIN vs Nitrification rate (Figure 5) represents three well defined clusters. The cluster representing the

pre-monsoon is well differentiated from the other two clusters. In the monsoon season, OC to DIN ratio is low (Figure 5). This favors enhanced N_{rate} (Straus and Lamberti, 2002), N_{rate} is negatively linked to OC ($r=-0.4$, $p<0.05$, $n=25$) suggesting that the bulk fraction of the carbon is labile. In addition, there was no single set of factors governing N_{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). Though the OC:DIN ratio is high there is no relation between OC and N_{rate} for the pre and post-monsoon season indicating that OC is either unlimiting or dominated by the recalcitrant fraction (Figure 5). In general the system evolves from a 'Low OC:DIN – Low N_{rate} ' system in the monsoon season to a 'High OC:DIN – High N_{rate} ' system in the pre-monsoon season with the post-monsoon representing a transition period where the OC:DIN ratio gradually increased. Hence, our study indicates that the quality of OC could be a more important proxy to N_{rate} rather than OC:DIN ratio. In addition multiple regression analysis (Table 1) shows that NH_4^+ is probably the only ion involved in the regulation of N_{rate} . Further, it could also be deduced that maximum fraction of recalcitrant carbon during the pre and post-monsoon season is accumulated at 4-6cm.

N_{rate} at experimental site showed a positive relation with Fe ($r=0.47$, $p<0.001$, $n=64$) and A_{Ntb} ($r=0.43$, $p<0.001$, $n=64$) which is indicative of anaerobic autotrophic nitrification. Further, a negative relation with NO_3^- ($r=0.25$, $p<0.05$, $n=64$) signifies feedback inhibition indicating that at the experimental site, N_{rate} is at the optimum. Hence it appears that the control site is dominated by heterotrophic nitrification whereas autotrophic nitrification governs the experimental site. In addition, OC ($3.4\pm 1\%$) and NH_4^+ ($27.8\pm 5.6 \mu\text{g at } NH_4^+-NL^{-1}$) peak at the experimental site during the post-monsoon season while at the control site it was during the pre-monsoon season (OC; $3.1\pm 0.8\%$ and NH_4^+ ; $27.8\pm 4 \mu\text{g at } NH_4^+-NL^{-1}$). However, the pre-monsoon of the experimental site was marked by highest levels of nitrification rates ($18.2\pm 0.6 \text{ ng at-N g (sediment)}^{-1}\text{h}^{-1}$) indicating a decoupling with OC and NH_4^+ . These parameters

remain coupled during the pre-monsoon season at the control site. Multiple regression analysis (Table 1) shows that NH_4^+ ($r=0.55$, $p<0.001$, $n=25$) plays a limiting role in the pre-monsoon season even though the OC: DIN ratio is comparatively higher (Figure 5). Further, the production of nitrite appears to govern the nitrification rates during the monsoon season. Depth wise correlation analysis shows that the abundance of A_{Ntb} is the major factor governing the N_{rate} for most of the depth intervals sampled. Positive relations with A_{Ntb} at 0-2 ($r=0.54$, $p<0.05$, $n=12$), 2-4 ($r=0.47$, $p<0.1$, $n=12$), 6-8 ($r=0.49$, $p<0.1$, $n=12$) and 8-10 ($r=0.68$, $p<0.01$, $n=12$) cm is in accordance with the earlier observation. In addition, Fe appears to be the next critical factor regulating N_{rate} at the experimental site with maximum coupling observed at 4-6 ($r=0.7$, $p<0.001$, $n=12$) and 6-8 cm ($r=0.6$, $p<0.01$, $n=12$) intervals. The feed back inhibition of NO_3^- is restricted to the bottom layer of 8-10cm depth indicating enhanced nitrification potential down the core till this depth??

4.2 Factors governing the abundance and distribution of nitrifiers

Even though there are reports on the occurrence of heterotrophic nitrification (Verstraete and Alexander, 1972; Castignetti and Holocher, 1984, Barraclough and Puri, 1995; Gupta, 1997; Lu et al., 2008; Ahmad et al., 2008), most of the studies consider nitrification as a purely chemoautotrophic process. In the present study, we were able observe the production of $\text{NO}_2^-/\text{NO}_3^-$ even when the nitrifying media was amended with 0.01% glucose favoring higher ratios of OC:DIN. Hence, heterotrophic nitrification could contribute to the total $\text{NO}_2^-/\text{NO}_3^-$ pool. Though quantification of heterotrophic nitrification is yet to be done, we tried to explore the factors governing the abundance of H_{Ntb} . The abundance of A_{Ntb} and H_{Ntb} at the control and the former at the experimental site were found to be unlimited by any of the factor measured in this study. However, the H_{Ntb} at the experimental site increased with depth ($r=0.33$, $p<0.01$, $n=64$) and were governed by concentration of NH_4^+ ($r=0.23$, $p<0.1$, $n=64$). Since the A_{Ntb} fraction is dominant at the experimental site it might out compete the H_{Ntb} for NH_4^+ . At the control site in the depth interval 0-2 cm, the NO_3^- production is principally

governed by the abundance of H_{Ntb} ($r=0.55$, $p<0.05$, $n=12$), while Mn levels in turn govern ($r=0.5$, $p<0.05$, $n=12$), the abundance of these nitrifiers. Another significant relationship at the control site was observed at 4-6cm, where H_{Ntb} and A_{Ntb} were found to be mutually exclusive ($r=-0.5$, $p<0.05$, $n=12$) with NO_2^- having a feed back inhibition on the former ($r=-0.48$, $p<0.1$, $n=12$). The abundance of A_{Ntb} in the depth intervals of 6-8 and 8-10 cm was governed by the levels of Mn ($r=0.55$, $p<0.05$, $n=12$) and Fe ($r=0.54$, $p<0.05$, $n=12$). Moreover the autotrophic nitrifiers govern the production of NO_3^- at 6-8cm ($r=0.56$, $p<0.05$, $n=12$). Similarly the abundance of H_{Ntb} in the deepest section of the core is governed by the availability of Mn ($r=0.47$, $p<0.1$, $n=12$). Similar to the control site, at the experimental site (0-2 cm) the abundance of H_{Ntb} is governed by the levels of Mn in the sediment. The H_{Ntb} at 4-6 cm were governed by the levels of NH_4^+ ($r=0.79$, $p<0.001$, $n=12$) and they could be actively linked to NO_3^- production ($r=0.46$, $p<0.1$, $n=12$). In a seasonal perspective it was found that at the control site the availability of NH_4^+ is the most important factor governing the abundance of A_{Ntb} during all the seasons, except for the post-monsoon season when there is a feed back inhibition by NO_3^- ; the abundance of H_{Ntb} is probably not limited by any factor under consideration in the present study. At the experimental site, only NH_4^+ and OC were found to regulate the abundance of H_{Ntb} during the monsoon season. However, Fe and depth were the two parameters governing the abundance of A_{Ntb} during pre-monsoon and monsoon seasons. Their abundance was not limited by any factor during the post-monsoon season.

Conclusion:

The role of iron in governing N_{rate} in mangrove sediments has been demonstrated. Our studies suggest that heterotrophic nitrification could be more ubiquitous and significant in mangrove ecosystems. In spite of having similar levels of OC and N_{rate} at both the sites, the underlying mechanisms governing the processes could be different. Higher abundance of H_{Ntb} at both the sites (10^{2-3} cells g^{-1}) and the A_{Ntb} at the experimental site ($\sim 10^3$ cells g^{-1}) reflect the relatively higher refractile nature of organic carbon at the experimental site. We conclude

that both the quality of organic carbon and quantity of iron govern nitrification rates in these mangrove swamps.

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References:

- Ahmad, N., Haiying, X.U., Liping, C., Zhipei, L., Shuangjiang, L., 2008. Enhanced biological nutrient removal by the alliance of a heterotrophic nitrifying strain with a nitrogen removing ecosystem. *Journal of Environmental Sciences* 20,216-223.
- Alexander, M., Clark, F.E., 1965. Nitrifying bacteria. *Methods of Soil Analysis, Part 2. American Society Agronomy, Madison, Wis, 1477–1483.*
- Balaram, V., Ramesh, S.L., Anjaiah, K.V., 1995. Comparative study of the sample decomposition procedures in determination of trace and rare elements in anorthosites and related rocks by ICP-MS. *Fresenius Journal Analytical Chemistry* 353,176-182.
- Barracough, D., Puri, G., 1995. The use of ^{15}N pool dilution and enrichment to separate the heterotrophic and autotrophic pathways of nitrification. *Soil Biology and Biochemistry* 27,17-22.
- Bendschneider, K., Robinson, N.J., 1952. A new spectrophotometric determination of nitrite in sea water. *Journal of Marine Research* 11,87-96.
- Boto, K.G., Wellington, J.T. 1988. Seasonal variations in concentrations and fluxes of dissolved organic and inorganic materials in a tropical, tidally dominated mangrove waterway. *Marine Ecology Progress Series* 50,151–160.

- Castignetti, D., Holocher, T.C., 1984. Heterotrophic nitrification among denitrifiers. *Applied and Environmental Microbiology* 47,620-623.
- Delwiche, C.C., Finstein, M.S., 1965. Carbon and energy sources for the nitrifying autotroph *Nitrobacter*. *Journal of Bacteriology* 90,102-107.
- Dham, V.V., Heredia, A.M., Wafar, S., Wafar, M., 2002. Seasonal variations in uptake and in situ regeneration of nitrogen in mangrove waters. *Limnology and Oceanography* 47,241–254.
- El Wakeel, S.K., Riley, J.P., 1957. Determination of organic carbon in the marine muds. *Journal Du Conseil International Pour L'exploration De La Mer* 22,180-183.
- Flanagan, F.J., 1967. U.S. Geological Survey silicate rock standards. *Geochimica et Cosmochimica Acta* 31,289-308.
- Flanagan, F.J., 1976. Descriptions and Analyses of Eight New USGS Rock Standards. U.S. Geological Survey Professional Paper 840, 192.
- Froelich, P.N., Klinkhammer, G.P., Bender, M.L., Luedtke, N.A., Heath, G.R., Cullen, D., Dauphin, P., Hammond, D., Hartman, B., Maynard, V., 1979. Early oxidation of organic matter in pelagic sediments of the Eastern Equatorial Atlantic: suboxic diagenesis. *Geochimica et Cosmochimica Acta* 43,1075–1090.
- Gupta, A.B., 1997. *Thiosphaera pantotropha*: a sulphur bacterium capable of simultaneous heterotrophic nitrification and aerobic denitrification. *Enzyme and Microbial Technology* 21,589-595.
- Harisson, P.H., Khan, N., Yin, K., Saleem, M., Bano, N., Nisa, M., Ahmed, S.I., Rizvi, N., Azam, F., 1997. Nutrient and phytoplankton dynamics in two mangrove tidal creeks of the Indus River delta, Pakistan. *Marine Ecology Progress Series* 157,13-19.
- Jones, J.B., Fisher, S.G., Grimm, N.B., 1995. Nitrification in the hyporheic zone of a desert stream ecosystem. *Journal of the North American Benthological Society* 14, 249-258.

- Koroleff, F., 1969. Direct determination of ammonia in natural waters as indophenol blue. International Conference in the Exploration of the Sea. 1969/ C. 9: 4 p
- Krishnamurthy, K., Sundararaj, V., Santhanam, R., 1975. Aspects of an Indian Mangrove, p. 88–95. In G. Walsh, S. Snedaker, and I. Teas [eds.], Proceedings of the International Symposium on Biology and Management of Mangroves.
- Krishnan, K.P., Fernandes, S.O., Chandan, G.S., Loka Bharathi, P.A., 2007. Bacterial contribution to mitigation of iron and manganese in mangrove sediments. *Marine Pollution Bulletin*. 54, 1427-1433.
- Lu, J., Jin, Q., He, Y., Wu, J., Zhao, J., 2008. Biodegradation of nonylphenol ethoxylates by *Bacillus* sp. LY capable of heterotrophic nitrification. *FEMS Microbiology Letters* 280, 28-33.
- Mortimer, R.J.G., Harris, S.J., Krom, M.D., Freitag, T.E., Prosser, J.I., Barnes, J., Anschutz, P., Hayes, P.J., Davie, I.M., 2004. Anoxic nitrification in marine sediments. *Marine Ecology Progress Series* 276, 37–51.
- Nixon, S.W., 1981. Remineralization and nutrient cycling in coastal marine ecosystems. p. 111–138. In *Estuarine and Nutrients*, ed. by B. J. Neilson and L. E. Cronin, Humand.
- Paul, E.A., Clark, E.E., 1989. *Soil microbiology and biochemistry*. Academic Press, Inc., Harcourt Brace Jovanovich, Publishers, San Diego, CA. 275p.
- Qasim, S.Z., Wafar, M.V.M., 1990. Marine resources in the tropics. *Resource Management and Optimization* 7, 141–169.
- Rivera-Monroy, V.H., Day, J.D., Twilley, R.R., Vera-Herrera, F., Coronado-Molina 1995. Flux of nitrogen and sediment in a fringe mangrove forest in Terminos Lagoon, Mexico. *Estuarine, Coastal Shelf Science* 40, 139–160.
- Rodina, A.G., 1972. *Methods in aquatic microbiology*. University Park Press, Butterworths, Baltimore London, 269–291.

- Saractchandra, A.U.,1978. Nitrification activities and the changes in the populations of nitrifying bacteria in soil perfused at two different H-ion concentrations. *Plant and Soil* 50, 99-111.
- Schell, D.M.,1978. Chemical and isotopic methods in nitrification studies. In: "Microbiology-1978". Eds. Schlessinger D. American Society for Microbiology, Washington DC.292-295.
- Seitzinger SP (1990). Denitrification in aquatic sediments. p. 301–322. In *Denitrification in Soil and Sediment*, ed. by N. P. Revsbech and J. Sørensen, Plenum.
- Sloth, N.P., Nielson, L.P., Blackburn, T.H.,1992. Nitrification in sediment cores measured with acetylene inhibition. *Limnology and Oceanography* 37, 1108–1112.
- Stenstromm, K., Podlska, R.A.,1980. The effect of dissolved oxygen concentration on nitrification. *Water Research* 14,643-649.
- Straus, E.A., Lamberti, G.A,2000. Regulation of nitrification in aquatic sediments by organic carbon. *Limnology and oceanography* 45,1854-1859.
- Straus, E.A., Lamberti, G.A.,2002. Effect of dissolved organic carbon quality on microbial decomposition and nitrification rates in stream sediments. *Freshwater Biology* 47,65-74.
- Triska, F.J., Duff, J.H., Avanzino, R.J.,1990. Influence of exchange flow between the channel and hyporheic zone on nitrate production in a small mountain stream. *Canadian Journal of Fisheries and Aquatic Science* 47,2099-2111.
- Trott, L.A., Alongi, D.M.,1999. Variability in surface water chemistry and phytoplankton biomass in two tropical, tidally dominated mangrove creeks. *Marine and Freshwater Research* 50, 451–457.
- Verhagene, J.M., Laanbroek, 1991.Competition for ammonium between nitrifying and heterotrophic bacteria in dual energy-limited chemostats. *Applied and Environmental Microbiology* 57,3255-3263.
- Verstraete, W., Alexander, M.,1972. Mechanism of nitrification by *Arthrobacter* sp. *Journal of Bacteriology* 110,962-967.

Wood, E.D., Armstrong, F.A.J., Richards, F.A., 1967. Determination of nitrate in seawater by cadmium-copper reduction to nitrite. *Journal of Marine Biological Association of United Kingdom* 47,23-31.

Legends to Table and figures:

Fig. 1. Location of sampling sites in the Chapora and Mandovi estuary.

Fig. 2. Seasonal down-core variation in pore water ammonium (a), nitrite (b) and nitrate (c) at both the control and experimental site for the Pre-monsoon ●; Monsoon ○ and Post-monsoon ▼ seasons. Only positive standard deviation is shown in the figure.

Fig. 3. Seasonal down-core variation in iron (a), manganese (b) and OC (c) at both the control and experimental site for the Pre-monsoon ●; Monsoon ○ and Post-monsoon ▼ seasons. Only positive standard deviation is shown in the figure.

Fig. 4. Seasonal down-core variation in nitrification rates (a), autotrophic nitrifiers (b) and heterotrophic nitrifiers (c) at both the control and experimental site for the Pre-monsoon ●; Monsoon ○ and Post-monsoon ▼ seasons. Only positive standard deviation is shown in the figure.

Fig. 5. OC: DIN Vs Nitrification rate for both the control and experimental sites. Pre-monsoon control (●), Monsoon Control (○), Post-monsoon control (▼), Pre-monsoon experiment (▽), Monsoon experiment (■), Post-monsoon experiment (□).

Table 1: Summary of multiple regression analysis. Only the significant factors are listed ($p < 0.05$) in the descending order of their significance. 'r' value is given in brackets.

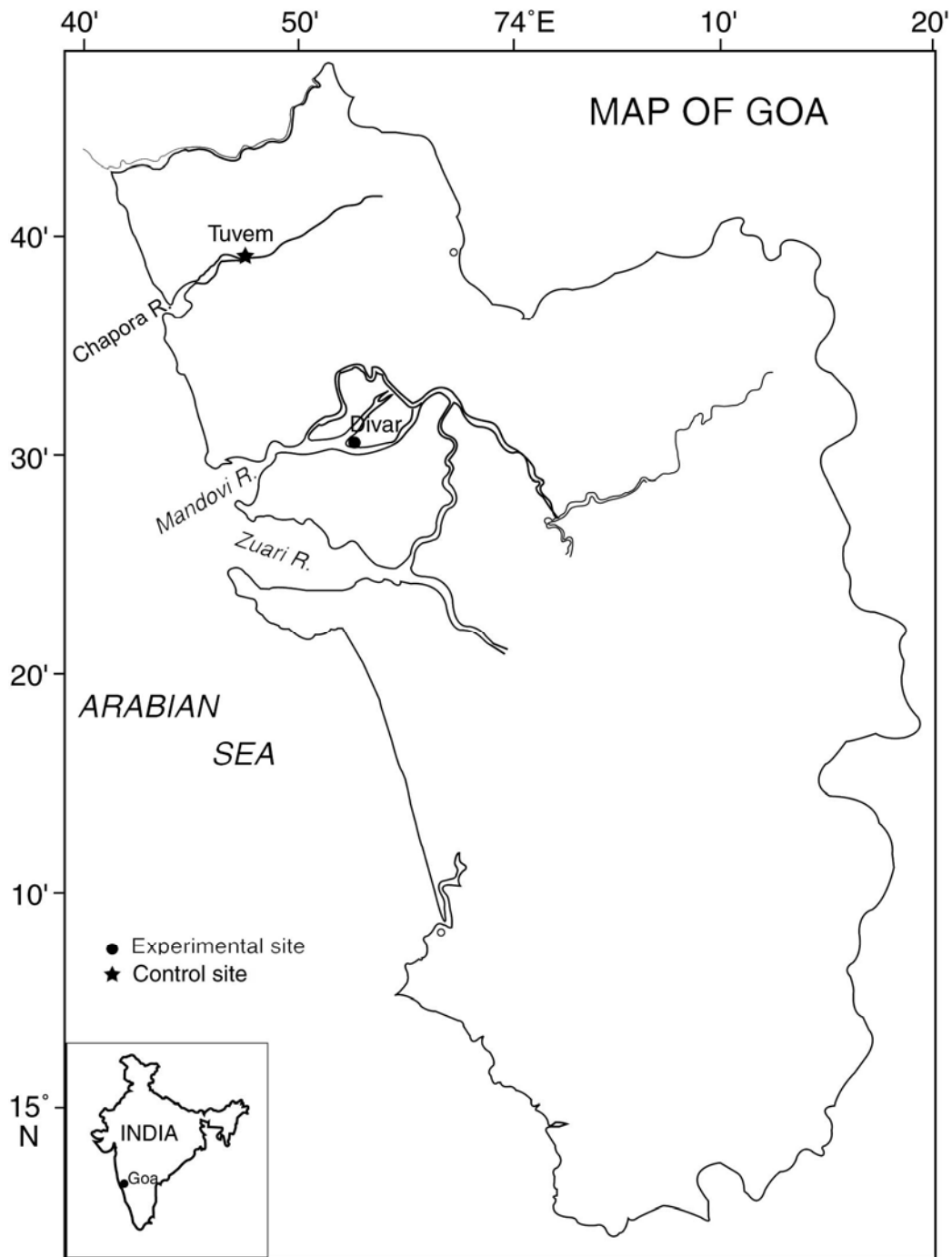


Fig. 1.

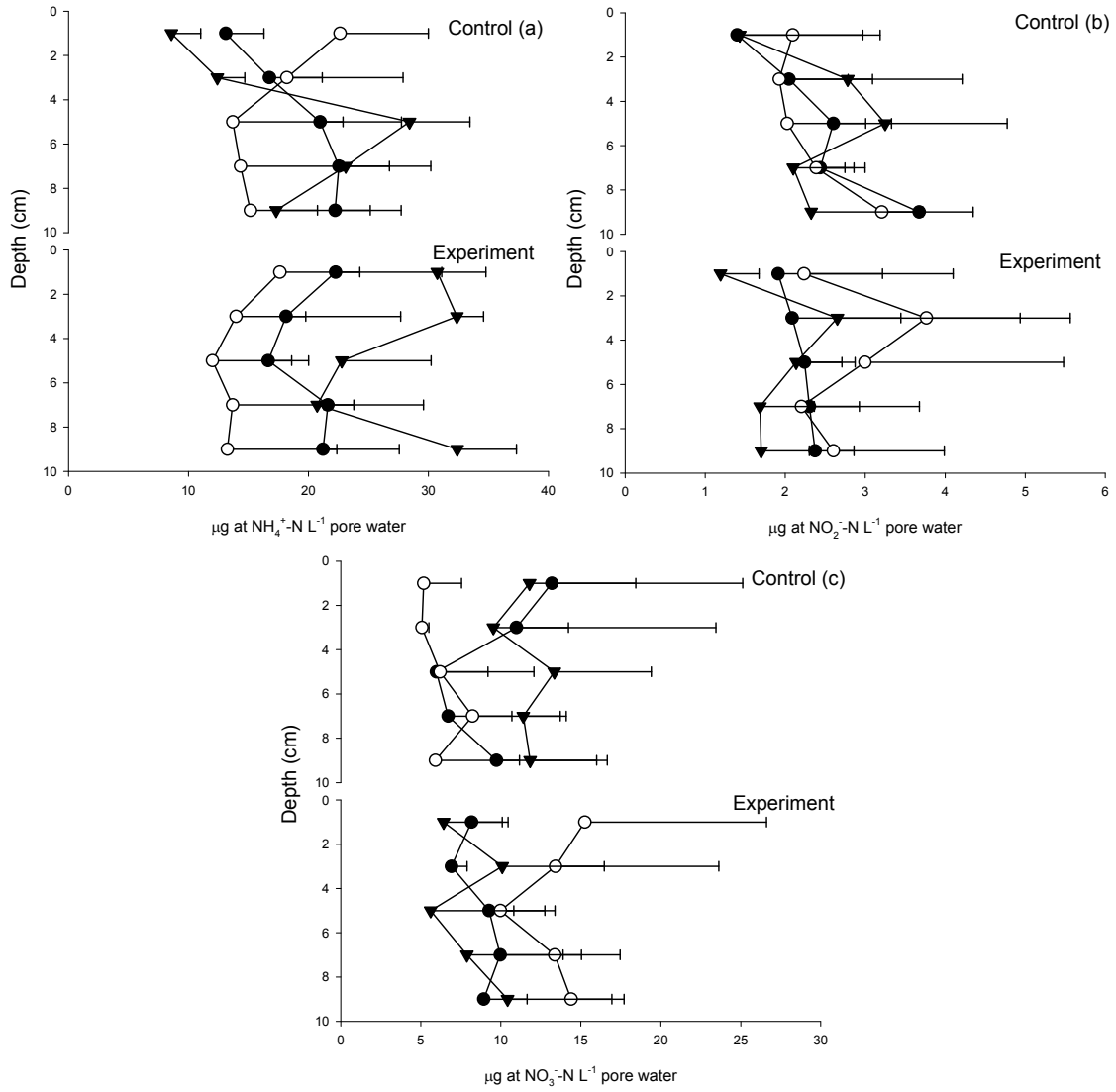


Fig. 2.

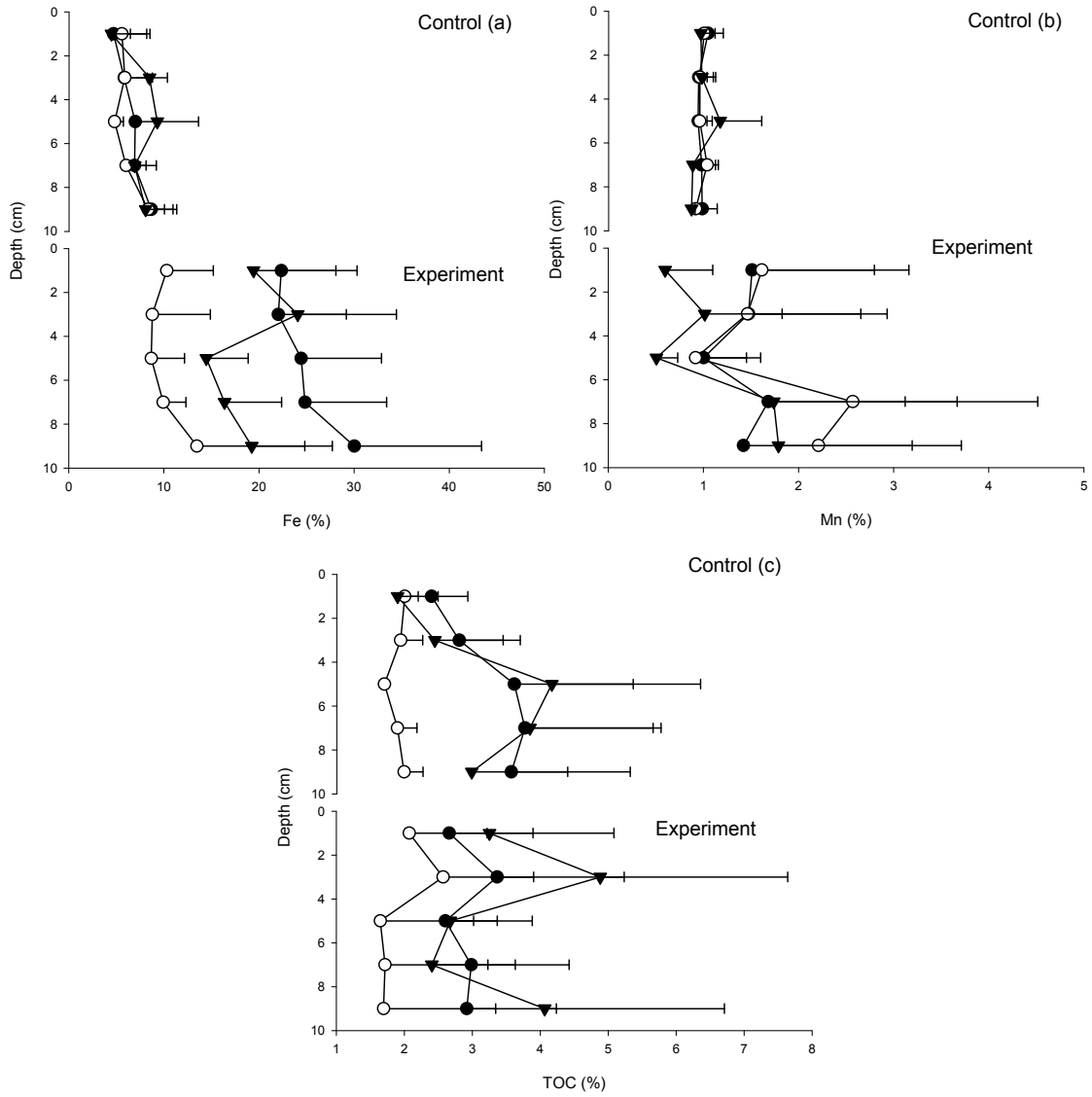


Fig. 3.

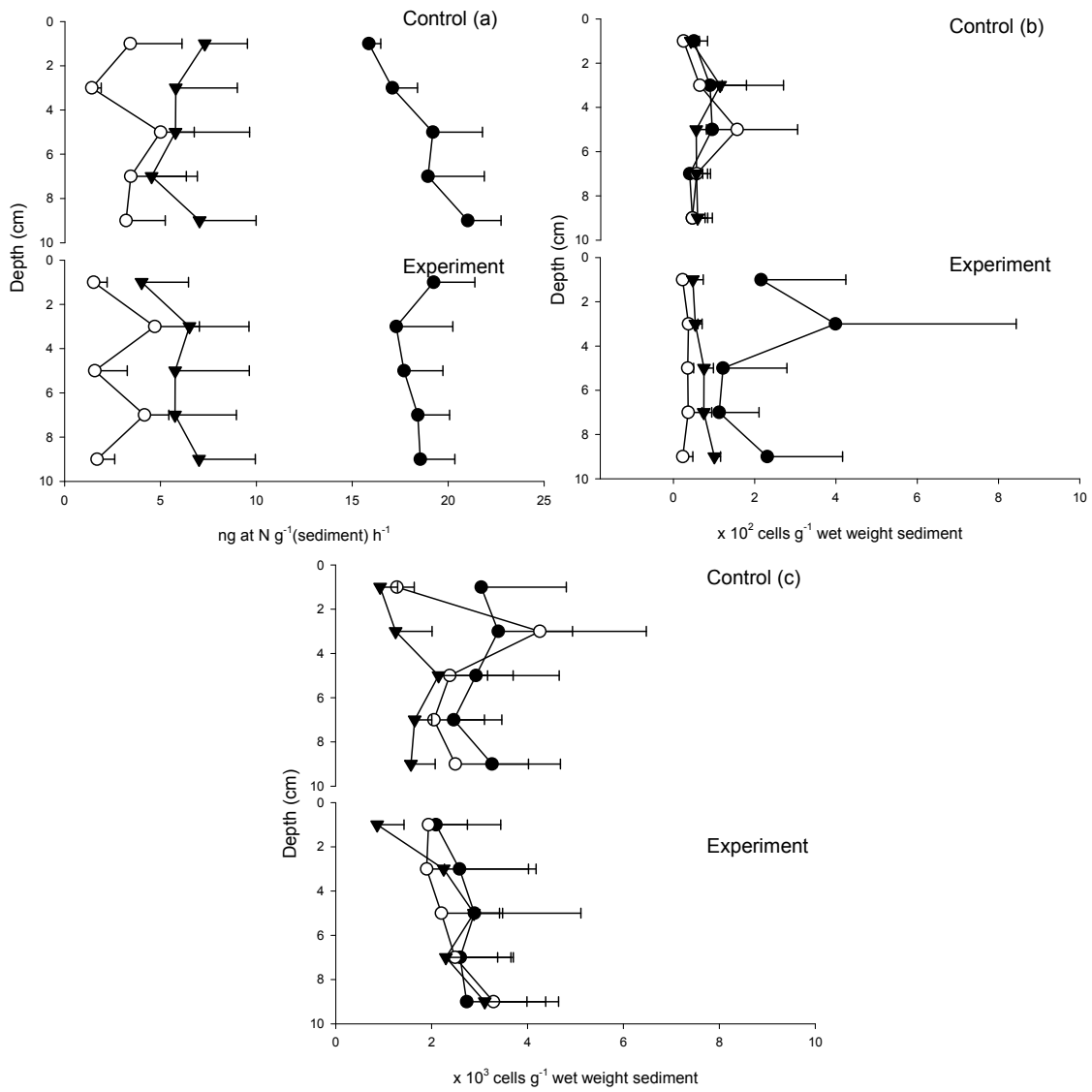


Fig. 4.

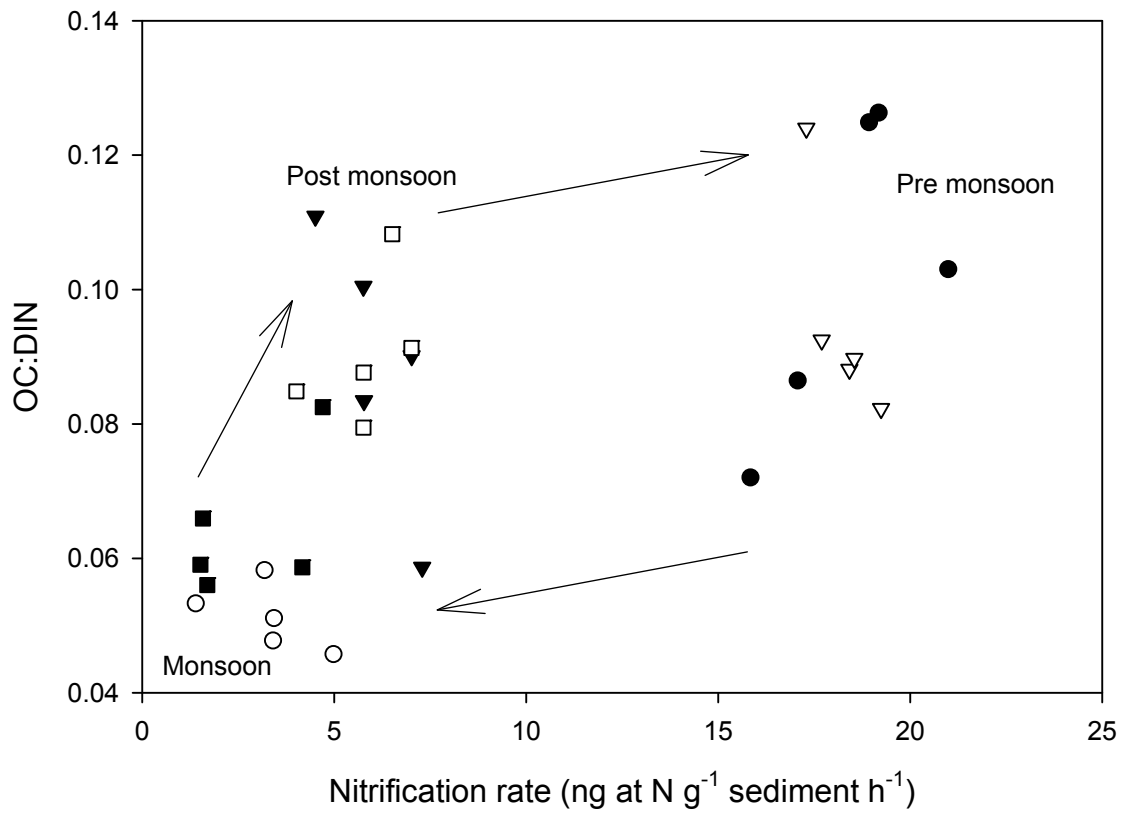


Fig. 5

Season	Factor under consideration	Control (Parameters influencing the factor under consideration)	Experiment (Parameters influencing the factor under consideration)
Pre-monsoon	Nitrification rate	Depth (0.67)	NH ₄ ⁺ (0.55)
	Autotrophic nitrifiers	OC(0.71)> NH ₄ ⁺ (0.64)> Mn(0.61)	Fe (0.34)
	Heterotrophic nitrifiers	Nil	Nil
Monsoon	Nitrification rate	Nil	NO ₂ ⁻ (0.67)
	Autotrophic nitrifiers	NH ₄ ⁺ (0.58)	Depth(0.54)> NO ₂ ⁻ (0.47)
	Heterotrophic nitrifiers	Nil	NH ₄ ⁺ (0.71)> OC(0.62)
Post-monsoon	Nitrification rate	NH ₄ ⁺ (0.69)	Nil
	Autotrophic nitrifiers	NH ₄ ⁺ (0.74)	Nil
	Heterotrophic nitrifiers	NO ₃ ⁻ (0.50)	Nil
Full monsoon cycle	Nitrification rate	OC(0.76)>Hntb(0.68)	Fe(0.82)>Antb(0.71)
	Autotrophic nitrifiers	N _{rate} (0.82)	NH ₄ ⁺ (0.73)> NO ₂ ⁻ (0.62)> Depth(0.61)> OC(0.58)> N _{rate} (0.51)
	Heterotrophic nitrifiers	NO ₂ ⁻ (0.74)	N _{rate} (0.65)

Table 1