

Denitrification activity is closely linked to the total ambient Fe concentration in mangrove sediments of Goa, India

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ABSTRACT

Denitrification activity (DNT) and associated environmental parameters were examined in two mangrove ecosystems of Goa, India- the relatively pristine Tuvem and the anthropogenically influenced Divar. Sampling was carried out at every 2 cm interval within the 0-10 cm depth range to determine (i) seasonal (pre-monsoon, monsoon and post-monsoon) down-core variation in DNT (ii) assess the environmental factors influencing the DNT and (iii) to build predictive models for benthic DNT. Denitrification generally decreased with depth and showed marked seasonal variation at both the locations ($p < 0.001$, $df = 2$). Denitrification peaked during the pre-monsoon occurring at a rate of up to $21.00 \pm 12.84 \text{ nmol N}_2\text{O g}^{-1} \text{ h}^{-1}$ within 0-4 cm at both the locations. Further, DNT at pre-monsoon was significantly influenced by Fe content at Tuvem ($n = 15$, $r = -0.660$, $p < 0.01$) and Divar ($r = -0.631$, $p < 0.01$) suggesting Fe-mediated nitrate respiration. The influence of other limiting substrates like NO_3^- and NO_2^- was most important during the monsoon and post-monsoon especially at Divar. The multiple regression models developed could predict 67-98% of the observed variability in DNT through the seasons. About 6-9 environmental variables were required to fairly predict DNT in these sediments with the complexity governing DNT decreasing from pre-monsoon to post-monsoon. Our results reveal that seasonal dynamics of DNT in tropical mangrove sediments are closely linked to the total Fe at the prevailing ambient concentration in both the systems.

Keywords: Denitrification; denitrifiers; iron; mangroves; nitrogen; sediments

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1. Introduction

Estuarine sediments provide conducive conditions for bacterially-mediated removal of anthropogenically derived inorganic nitrogen (N) from the aquatic system (Dong et al., 2009). Nitrate in particular, serves as an electron acceptor for anaerobic oxidation of organic matter and is reduced to gaseous compounds like N_2O or N_2 through the denitrification pathway. Denitrification is favourable under oxygen deficient conditions and is mediated by facultatively anaerobic bacteria. However, denitrification activity (DNT) varies in different ecosystems and could be affected by the prevailing environmental conditions. There is a general lack of information on the environmental parameters affecting benthic denitrification in near-shore coastal ecosystems such as mangroves. Most of the studies in mangroves have focused on quantifying benthic DNT (Rivera-Monroy et al., 1995; Meyer et al., 2005) to understand the potential use of mangroves in nitrate depuration (Corredor and Morell, 1994) or wastewater treatment (Nedwell, 1975). Chiu et al. (2004) have quantified denitrification and assessed the factors affecting the process in temperate mangrove sediment. Some of the factors influencing the activity of denitrifiers are oxygen concentration (Bonin and Raymond, 1990), sediment pH (Rust et al., 2000; Simek et al., 2002), redox conditions (Lee and Joye, 2006), sediment nitrification (Stockenberg and Johnstone, 1997), nitrate availability (Seitzinger, 1990; Corredor and Morell, 1994; Kana et al., 1998) and organic carbon (Mc Carty and Bremner, 1993; Pfenning and Mc Mahon 1997; Ward et al., 2008).

Earlier studies in the mangrove swamps of Goa have shown trace metal contamination derived mainly from ferro-manganese mining activities (Krishnan et al., 2007). Denitrification in the mangrove sediments of Goa has shown to be mainly limited by nitrate availability rather than organic carbon (Fernandes and LokaBharathi, 2011). The counter process of nitrification in these systems has been suggested to occur at the expense of metals (Krishnan et al., 2007; Krishnan and LokaBharathi, 2009). Therefore, these metals could also be important in influencing DNT. Though heterotrophic denitrification could be more prevalent in organic-rich mangrove sediments, we hypothesize that DNT in mangrove sediments of Goa is primarily influenced by ambient Fe concentration. The other associated abiotic variables play a secondary role. To test our hypothesis, the seasonal down-core variation in denitrification activity (DNT) and associated environmental factors likely to influence the process were measured in two tropical mangrove ecosystems of Goa- the relatively pristine site Tuvem and Divar which is influenced by extraneous nutrient input. Our results indicate marked seasonal variability in DNT with highest rates during the pre-monsoon at both the locations. This variation is

governed by the changes in sediment chemistry with metals like Fe playing an important role in influencing the process especially during the pre-monsoon period.

2. Methods

2.1. Study area and sampling

Investigations were carried out at mangrove swamps located along the Mandovi and Chapora rivers in Goa, west coast of India (Fig. 1). The relatively pristine site was located at Tuvem (15°39'94"N and 73°47'65" E) which lies along the river Chapora. This serene ecosystem is set amidst lush green hills is comparatively less influenced by anthropogenic activities (Krishnan et al., 2007). The dominant species of mangroves found at Tuvem are mainly represented by *Acanthus illicifolius*, *Excoecaria agallocha*, *Caesalpinia* spp., *Avicennia officinalis* and *Clerodendrum inerme*.

The anthropogenically-influenced site was located at Divar island (15°30'35"N and 73°52'63"E) which lies along the Mandovi estuary. The mangroves at this location consist mainly of species like *Acanthus illicifolius*, *Pongamia pinnata*, *Cyperus* spp., *Bruguiera gymnorrhiza*, *Avicennia officinalis*, *Caesalpinia* spp., *Sonneratia caseolaris*, and *Rhizophora mucronata*. The Mandovi estuary is important for the economy of the territory as it is heavily used for transportation of iron ore from mines located upstream. These iron ore beneficiation plants situated upstream, discharge effluents directly into the estuary. This discharge contains high quantities of sediment rich in iron and NH_4NO_3 used as explosive in mining operations (De Sousa, 1999). Mining activities also result in Fe-Mn enrichment in these sediments (Krishnan et al., 2007). These metals mainly Fe, has been shown to influence N transformations in mangrove sediments (Krishnan and LokaBharathi, 2009).

Sediment samples were collected at low tide during September, 2007 (representing the monsoon season), January, 2008 (post-monsoon) and April, 2008 (pre-monsoon). A total of 12 PVC push cores of 7.5 cm inner diameter and ~20 cm length were collected at each sampling and used as follows:

(i) Three cores were collected for physical measurements which were capped at both ends after sediment retrieval. They were transferred to a plastic tray for transportation to the laboratory under ambient temperature. The variation in physical parameters was measured on arrival to the laboratory within 1 h of sampling.

(ii) Nine cores were collected for chemical, microbiological analyses and DNT measurements. These were transported to the laboratory in an ice box. On arrival, the cores were maintained at 4°C until analysis and analysed within 24 h of retrieval.

Seawater from the sampling sites was collected in clean, well-rinsed carbuoys and used for media preparation during microbiological analyses.

2.2. Physico-chemical analyses

Sediment cores were sectioned at 2 cm intervals to obtain representative samples at 0–2, 2–4, 4–6, 6–8 and 8–10 cm depths. An *Orion* 4-Star Plus Benchtop pH/ISE Meter after calibration with standard buffers (pH 4, 6.9 and 9; Merck) was used to measure pH at each section of the core. Sediment oxidation-reduction potential (Eh) was measured with the help of an *Orion* platinum redox in combination with a silver/silver chloride reference electrode.

For nutrient analyses (water-extractable concentrations), sediment sub-samples were taken at every 2 cm intervals from surface to 10 cm by carefully sectioning the core. Each section (7.5 cm diameter and 2 cm thick) was transferred to 100 mL of sterile saline and homogenised using a glass rod. The slurry was centrifuged at 4°C for 10 min at 5000 rpm with a REMI CPR-24 centrifuge. Low spin was maintained during centrifugation to ensure minimal change in nutrient concentrations due to lysis of benthic infauna. The supernatant was filtered through a 0.2 µm filter and stored at -20°C until analysis. Ammonium, nitrite and nitrate were measured colorimetrically (*Shimadzu* UV/VIS spectrophotometer; precision: $\pm 0.01 \mu\text{mol l}^{-1}$) as described by Koroleff (1969), Bendschneider and Robinson (1952) and Wood et al. (1967), respectively.

Triplicate sediment samples from each section of the core were dried in a hot air oven at 60 ± 2 °C for 48 h. Total organic carbon (TOC) was then determined by wet oxidation method (El Wakeel and Riley, 1957) with a precision of 0.01%.

Sub-samples for metal analyses were also dried at 60 ± 2 °C for 48 h and disaggregated in an agate mortar, before chemical treatment for Fe and Mn analysis. Sediment digestion was carried out as described by 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).

Denitrification activity was measured by the acetylene inhibition technique based on the inhibition of the conversion of N_2O to N_2 (Sørensen, 1978). Over short incubation intervals, it is a cost-effective method for estimating denitrification, and rate estimates are comparable to those obtained by the membrane inlet mass spectrometry (Bernot et al., 2003). The slurry method was opted over the intact core method (Lowrance and Hubbard, 2001; Bernot et al., 2003) due to low permeability of mangrove sediments (Marchand et al., 2004; Schwendenmann et al., 2006) as uneven penetration of acetylene in compact sediments would lead to an underestimation of DNT. The cores were sectioned in sterile conditions. About 1 cm^3 of sediment was extruded from each section using a syringe core and transferred aseptically to sterile 20 mL headspace vials. Three millilitres of sterilized ambient sea water from the sampling site (containing $4.0 \mu\text{mol NO}_3\text{-N L}^{-1}$) was added to the sediment. No additional carbon or nitrate was amended to the seawater. The vials were capped with butyl stoppers and Al crimps. They were then vortexed for 5 s to form slurry. The vials were purged with high purity N_2 for 10 min to induce anaerobic conditions. Acetylene at 20 kPa (Bonin et al., 2002) was injected into the headspace to avoid reduction of N_2O to N_2 . Triplicates were maintained and the vials were incubated in the dark for 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 h. At the end of the incubation period, bacterial activity in all incubations was terminated using HgCl_2 at a final concentration of 10 mmol L^{-1} .

Nitrous oxide in the headspace was analyzed as described by Fernandes et al. (2010). As the experiment was carried out in microcosms, it was necessary to minimize error likely to be caused by variability during sediment transfer. Hence, sediment used in each microcosm was filtered through a laboratory grade filter paper and dried at 60°C for approximately 48 h. Activity was considered as the linear accumulation of N_2O over time and DNT has been expressed as $\text{nmol N}_2\text{O g}^{-1} \text{ h}^{-1}$ of dry sediment.

2.3. Bacteriological studies

Sub-samples of $\approx 5 \text{ g}$ wet weight sediment from each section of the core were extruded using syringe cores. The sub-samples were transferred to 45 mL of filter sterilized full strength seawater (10^{-1} dilution). Tween 80 (50 μL) was added and the mixture was sonicated at 40 MHz for 15 s. The next set of dilution (10^{-2}) for each section of the core was prepared..

The method of Hobbie et al. (1977) was used for the enumeration of total bacterial counts by epifluorescence microscopy. An aliquot of 5 mL sample from 10^{-2} dilution was fixed using 250 μL of buffered formalin (2% final concentration). Two millilitres of sub-sample was filtered over a 0.2 μm black Isopore polycarbonate filter paper (*Millipore*) and stained with acridine orange (final concentration 0.01% w/v). The sample was then incubated for 2 min and then filtered. Bacterial cells retained on the filter paper were counted using *Nikon 50i* epifluorescence microscope equipped with a 100X oil immersion objective. Cells were counted from 10-100 microscopic fields and the total counts (TC) were expressed as number of cells g^{-1} (dry sediment).

Total heterotrophic bacteria (THB) were enumerated using medium prepared in aged seawater. The seawater was amended with 0.01% nutrient broth (*HiMedia laboratories Ltd.*, India). The pH of the medium was adjusted to 8.2 before adding agar at a final concentration of 1.5%. A 50 μL inoculum from 10^{-2} dilution was used to spread on to the prepared nutrient agar plates. The plates were incubated at room temperature $28 \pm 2^\circ\text{C}$ for 7 days. Bacterial counts were taken as colony forming units (CFU) g^{-1} . Initial weight of the sediment used in the dilutions was determined by drying the filtered sediment at 60°C .

Denitrifiers were enumerated by the N_2O based-most probable number (MPN) technique as described by Fernandes et al. (2010). The culture medium was prepared using substrates suggested by Michotey et al. (2000) and prepared as follows: filtered seawater was supplemented with ammonium chloride (0.05 g L^{-1}), potassium nitrate (0.101 g L^{-1}) sodium acetate (0.2 g L^{-1}), sodium succinate (0.2 g L^{-1}), Biotrypticase (1 g L^{-1}) and 20 kPa acetylene (Bonin et al., 2002).

2.4. Statistical analyses and influence of Fe on DNT

Significant seasonal differences in DNT were analyzed using two-way Analysis of Variance (ANOVA) in Analysis tool pack of *Microsoft Excel*. At each season, environmental variables within 0-10 cm depth range were correlated (Pearson's correlation coefficient) with DNT to assess significant associations. Bacterial numbers were \log_{10} transformed before analyses. Predictive models for benthic DNT were developed using Multiple Regression Analysis (MRA) on a seasonal basis for both the study sites. The analysis was performed using *STATISTICA* software (version 6) and indicated relationship between DNT (dependent variable) and other abiotic/biotic factors (independent variables). A step-wise variable

deletion method (data not included) was used to assess the variables required to yield the best regression models on a seasonal basis within 0-10 cm at both the study sites.

To understand the influence of total Fe on DNT in mangrove sediments, data from both locations was pooled. DNT was plotted against Fe concentration in three categories i.e. Fe content 0-10, 10-20 and 20-30%.

3. Results

3.1. Physico-chemical parameters

The Tuvem sediments exhibited a marginal decrease in pH with depth during the pre-monsoon and monsoon season with lowest pH of 6.52 ± 0.65 at 4-6 cm (Fig. 2a; Table S1). At post-monsoon, lowest hydrogen ion concentration in the Tuvem sediments was also recorded at 4-6 cm (5.83 ± 0.17). In the Divar sediments, the seasonal fluctuation in pH was relatively less varying between 6.39 ± 0.19 at 6-8 cm in the post-monsoon to 7.13 ± 0.39 at 8-10 cm in the monsoon (Fig. 2b; Table S2).

The redox potential differed with seasons. The values were lowest in monsoon at Tuvem with comparatively less reducing conditions prevalent during pre and post monsoon (Fig. 2c). The Divar sediments were relatively less reduced in nature prior to the commencement of rainfall in the region. Reducing conditions were most intense in the Divar sediments during the monsoon (Fig. 2d).

Pore water ammonium at both the study sites generally showed higher values at depths ≥ 4 cm. At Tuvem, the highest average of $28.43 \pm 5.03 \mu\text{mol NH}_4^+\text{-N L}^{-1}$ was recorded at 4-6 cm depth during the post-monsoon (Fig. 2e; Table S1). Similarly at Divar, higher concentrations were recorded during the post-monsoon with average values ranging between $20.56 \pm 3.07 \mu\text{mol NH}_4^+\text{-N L}^{-1}$ at 6-8 cm to $32.05 \pm 4.63 \mu\text{mol NH}_4^+\text{-N L}^{-1}$ at 8-10 cm (Fig. 2f; Table S2).

Nitrite concentrations in the surficial sediments at Tuvem and Divar were relatively lower through the seasons as compared to the deeper layers. At Tuvem, highest concentration of $3.74 \pm 0.76 \mu\text{mol NO}_2^-\text{-N L}^{-1}$ was recorded at 8-10 cm during the pre-monsoon (Fig. 2g; Table S1). At Divar, high nitrite concentration was recorded during monsoon with up to $3.77 \pm 1.80 \mu\text{mol NO}_2^-\text{-N L}^{-1}$ at 2-4 cm (Fig. 2h; Table S1).

Nitrate concentration in mangrove sediments also varied with depth. At Tuvem, higher values were recorded during the pre-monsoon decreasing from $15.32 \pm 12.65 \mu\text{mol NO}_3^- \text{-N L}^{-1}$ at 0-2 cm to $7.66 \pm 4.85 \mu\text{mol NO}_3^- \text{-N L}^{-1}$ at 8-10 cm (Fig. 2i; Table S1). At Divar, NO_3^- content was significantly higher during the monsoon (ANOVA, $p < 0.002$, $df = 2$) as compared to the non-monsoon seasons (Fig. 2j; Table S2).

Total organic carbon content at Tuvem varied from a lowest of $1 \pm 0.05 \%$ at 4-6 cm during the monsoon to $4.52 \pm 3.13 \%$ at the same depth during post-monsoon (Fig. 3a; Table S1). Similar seasonal trend was observed at Divar where TOC varied from $1.65 \pm 1.37 \%$ at 4-6 cm during monsoon to $4.88 \pm 2.76 \%$ at 2-4 cm during the post-monsoon (Fig. 3b; Table S2).

Sediment Fe values at Tuvem ranged from $4.45 \pm 3.70 \%$ at 0-2 cm to $9.28 \pm 4.33 \%$ at 4-6 cm during the post-monsoon (Fig. 3c; Table S1). In Divar, highest Fe content of up to $30.02 \pm 13.37 \%$ was recorded at 8-10 cm during the pre-monsoon (Fig. 3d; Table S2). The maximum concentration of Mn at Tuvem was 10x lower than that of Fe (Fig. 3e; Table S1) while at Divar, Mn content was 12x lower (Fig. 3f; Table S2).

3.2. Bacteriological parameters

Total bacterial cells (TC) in the Tuvem sediments ranged from $0.67 \pm 0.28 \times 10^{10}$ cells g^{-1} at 2-4 cm during the post-monsoon to $2.10 \pm 0.77 \times 10^{10}$ cells g^{-1} at 4-6 cm during the monsoon (Fig. 4a; Table S3). Interestingly, at Divar, similar minima and maxima values for TC abundance were observed at the same depth and season (Fig. 4b) as recorded at Tuvem.

The abundance of total heterotrophic bacteria (THB) in mangrove sediments varied from 10^{2-5} CFU g^{-1} . Though THB abundance at Tuvem was higher during the post-monsoon (Fig. 4c), no inter-seasonal variability in their abundance was observed at either location. At Divar, heterotrophic abundance averaged between 10^{3-5} CFU g^{-1} decreasing steadily with depth (Fig. 4d). Lower abundance was observed during the monsoon as compared to the non-monsoon period.

The denitrifiers were most abundant at 4-6 cm at all seasons at both the sites investigated. Their abundance at Tuvem varied from $6.17 \pm 0.25 \times 10^4$ MPN cells g^{-1} at 2-4 cm to $1.87 \pm 1.20 \times 10^8$ MPN cells g^{-1} at 4-6 cm during the monsoon (Fig. 4e; Table S3). Their abundance during the pre and post-monsoon varied from 10^{6-7} MPN cells g^{-1} . At Divar, their abundance varied from $2.34 \pm 0.00 \times 10^5$ MPN

cells g^{-1} at 6-8 cm during the monsoon to $8.96 \pm 1.21 \times 10^8$ MPN cells g^{-1} at 4-6 cm during the post-monsoon (Fig. 4f; Table S3).

3.3. Seasonal variation in denitrification activity

Denitrification activity was higher at ≤ 4 cm and at pre-monsoon at both the study sites (Figs. 5a and b). Between seasons, DNT was significantly different at Tuvem (ANOVA, $p < 0.001$, $df = 2$) and Divar ($p < 0.001$, $df = 2$). During the pre-monsoon season, maximum denitrification rate of 21.00 ± 12.84 nmol N_2O $g^{-1} h^{-1}$ was recorded at the surface in Tuvem (Fig. 5a) while at Divar, a maximum of 20.81 ± 7.75 nmol N_2O $g^{-1} h^{-1}$ was recorded at the sub-surface (2-4 cm) (Fig. 5b). With the onset of the monsoon, DNT in the surficial sediments at Tuvem and Divar decreased to 1.95 ± 0.85 nmol N_2O $g^{-1} h^{-1}$ and 2.06 ± 0.99 nmol N_2O $g^{-1} h^{-1}$ respectively. At post-monsoon, DNT at Divar increased to a maximum of 11.52 ± 2.05 nmol N_2O $g^{-1} h^{-1}$ at 0-2 cm compared to Tuvem where a maximum of 2.23 ± 0.34 nmol N_2O $g^{-1} h^{-1}$ was recorded at 2-4 cm. Depth integrated (0-10 cm) DNT has been tabulated in table 1.

3.4. Factors controlling denitrification

The Pearson correlation coefficient was used to measure the strength of the linear relationship between DNT and associated environmental variables (Table 2). During the pre-monsoon, the variation in Fe concentration was seen to negatively influence DNT at Tuvem ($r = -0.66$, $p < 0.01$, $n = 15$) and Divar ($r = -0.63$, $p < 0.01$, $n = 15$). In the monsoon, nitrite showed an inverse relationship ($r = -0.62$, $p < 0.01$) at Tuvem whereas at Divar DNT was dependent on NO_3^- availability ($r = 0.56$, $p < 0.05$). During the post-monsoon, DNT at Tuvem was seen to inversely correlate to NO_3^- concentration ($r = -0.58$, $p < 0.05$). Denitrification activity at Divar during this season was influenced by a number of variables like Mn ($r = -0.64$, $p < 0.01$), Fe ($r = 0.59$, $p < 0.01$), THB ($r = 0.60$, $p < 0.01$) and pH ($r = 0.546$, $p < 0.05$) (Table 2).

MRA was used to predict the combination of factors controlling denitrification. The regression equations have been derived on the assumption that the observed values would repeat for two annual cycles without variation. The models used to predict ambient DNT in mangrove sediments explained 67-98% (model $R^2 = 0.67$ to 0.98) of the observed variability in DNT (Table 3). Based on the multiple regression models, observed DNT in the mangrove ecosystems fitted well with predicted rates (Fig. S1). Unlike highly eutrophic systems where NO_3^- concentration is the main factor controlling benthic DNT, our study revealed that a larger combination of variables (up to 9) influenced the process on a seasonal

scale at both the locations (Tables S4 & S5). As observed by correlation analysis, MRA also indicated that Fe was an important factor explaining about 16% variation in DNT at Tuvem during the pre-monsoon season (Table S4). Inorganic N compound like NO_2^- and NO_3^- had a stronger influence during the monsoon and post-monsoon respectively. At Divar, of the 7 variables in the model, the highest influence on DNT was due to NO_3^- availability (36%) during the monsoon whereas the influence of Fe was seen during the post-monsoon (Table S5).

To examine the influence of Fe on DNT, metal content at both locations was pooled and plotted against DNT in three categories in three categories (0-10, 10-20 and 20-30%). Our results showed almost no effect of Fe on DNT at lower concentrations (<10%) and an exponential increase in DNT between 10-20% of Fe (Fig. 6). At Fe concentration above 20%, DNT was likely to be inhibited.

4. Discussion

In the mangrove sediments of Tuvem and Divar, denitrifiers were maximum within 4-8 cm. However, DNT was predominant within 0-4 cm at all seasons and decreased further with depth suggesting that the activity depends mainly on the prevailing environmental conditions. The downward decreasing trend in DNT is in accordance to observations made by Chiu et al. (2004) who state that N loss occurs primarily in the surface rather than the rhizosphere sediment. Denitrification in the mangrove sediments of Goa is mainly limited by nitrate availability (Fernandes and LokaBharathi, 2011). The surficial sediments are replenished with nutrients from the ambient seawater whereas the occurrence of DNT at the deeper depths could be explained by the presence of nitrate (present study; Fernandes et al., 2012). Substrate availability at deeper depths could be attributed to the co-occurrence of anaerobic nitrification (Krishnan and LokaBharathi, 2009), sandy nature of the sediments facilitating seepage of the nutrient or percolation of the overlying ambient seawater due to bioturbation activity (Fernandes et al., 2012).

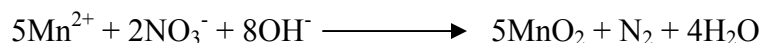
As seen in earlier studies in aquatic sediments (Cabrita and Brotas, 2000; Dong et al., 2000; Hietanen and Kuparinen, 2008), we observed distinct seasonal variability in DNT. Low DNT was recorded during the monsoon and post-monsoon period while the highest activity was recorded during the pre-monsoon season ($\sim 21 \text{ nmol N}_2\text{O g}^{-1} \text{ h}^{-1}$) at both the study sites. To compare our values with studies elsewhere, DNT within 0-10 cm was integrated (Table 1). Corredor and Morell (1994) assayed benthic DNT by the acetylene block technique in mangroves prone to excess N input through secondary sewage effluents and recorded an average DNT of $\sim 0.04 \text{ mmol N}_2\text{O m}^{-2} \text{ h}^{-1}$. In our study, DNT occurred

in a similar range ($0.08 \text{ mmol N}_2\text{O m}^{-2} \text{ h}^{-1}$) only during the monsoon season at Tuvem. Denitrification rates in the mangrove sediments of Goa were far greater than reported from estuarine sediments elsewhere (Kim et al., 1997; Barnes and Owens, 1998; Bernot et al., 2003) and indicate high N-removal capacity of these sediments. This may have implications for modulating the levels of excess nutrients in the aquatic system thereby preventing eutrophication events.

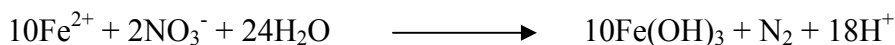
A progressive increase in DNT from monsoon to pre-monsoon was observed and could be caused due to the variation in associated environmental parameters. In this study, statistical tools were used to examine the biotic and abiotic parameters influencing DNT on a seasonal basis at both the sites. A larger number of variables (~8) affected DNT at Tuvem during the monsoon (Table S4) and the multiple regression model obtained was capable of describing up to 98% (model $R^2 = 0.98$) of the overall variation in the process at this location. The advent of the south-west monsoon results in a large input of fresh water into the adjoining estuary causing considerable changes in the water column/sediment chemistry. Runoff also results in addition of land-derived nutrients into the estuarine system as evident from the elevated pore water ammonium concentration in the surficial (0-4 cm) sediments at Tuvem. Among the variables positively influencing DNT during the monsoon, MRA showed that ambient NH_4^+ concentration accounted for about 19% variation in DNT at Tuvem (Table S4). Unlike Divar, NO_3^- availability at Tuvem is relatively low during the monsoon. DNT could be more closely linked to nitrification which oxidizes NH_4^+ and thus serves as a major source of NO_3^- (Wang et al., 2003). At Divar, up to 36% variation in DNT during the monsoon was influenced by the concentration of pore water NO_3^- (Table 2). In anaerobic environments, availability of electron acceptor often limits DNT (Seitzinger, 1990). Recently, Divya et al. (2009) have shown nitrate concentrations entering the adjoining Mandovi estuary to be particularly high during the monsoon. In the present study, the heterotrophs also contributed in influencing DNT (Table S5) during the monsoon suggesting that they could be important in reducing elevated nutrient levels in the system thereby counteracting eutrophication.

The post-monsoon marks a transition phase from highly dynamic conditions towards a period of stability. At Tuvem, an increase in pore water NO_3^- concentration was observed during this season (Fig. 2i). This brought about a shift in DNT regulation from $\text{NH}_4^+ / \text{NO}_2^-$ modulated to NO_3^- dependent (~34% variation in DNT was accounted by the variation in concentration of NO_3^- as seen in table S4). At Divar, DNT activity increased approximately 5 times during the post-monsoon as compared to the monsoon

season. Sediment pH and THB were some of the prominent variables which positively influenced DNT at Divar (Table 2). During the post-monsoon, organic carbon content decreased between 4-8 cm (Fig. 3b). Its degradation could result in shift towards acidic pH at these depths. Generally, the optimum pH for DNT to occur is 7.0–7.5 (Thomas et al., 1994). However, pH values in mangrove sediments are marginally lower than 7. The relationship of pH with DNT could be explained by the slightly acidic state of the mangrove sediments since maximum DNT occurs towards pH closer to neutral. At Divar, heterotrophs were seen to influence DNT during the post-monsoon, suggesting contribution of these bacteria to denitrification. Though MRA showed a combined influence of TC, denitrifiers, THB and NO₂ on DNT (Table S5), metals mainly Mn and Fe also contributed to controlling the process (Tables 2, S5). Nitrate or nitrite can couple their reduction to oxidants like Mn (II) (Oguz et al., 2001) as described in the equation given by Luther et al. (1997):



Experiments using denitrifying cultures by Vandenabeele et al. (1995) have shown that Mn removal rate was 38% higher in the presence of nitrate and was accompanied by nitrite accumulation. The TC also showed a positive influence on DNT indicating that a large fraction of the bacterial population could be involved in mediating the reductive phase of the N cycle and consequently play an important role in altering the sediment chemistry. The Divar ecosystem lies along the Mandovi estuary which has ferromanganese mines located upstream. Here, up to 24% Fe has been recorded at 2-4 cm during the post-monsoon season. Runoff during the monsoon and subsequent mining activities (input of mining rejects, movement of Fe ore transporting barges, etc.) during the post-monsoon could be responsible for high Fe content in the sediments. Earlier studies by Krishnan et al. (2007) at the same location have shown that the 0-4 cm remains moderately contaminated by iron during the post-monsoon while it falls in the 'uncontaminated to moderately contaminated by iron' category during the pre-monsoon and monsoon season. In anoxic sediments, Fe oxide formation could be a result of microbially-mediated oxidation of ferrous iron using nitrate as an electron acceptor following the stoichiometric equation (Straub et al., 1996):



The solubility of Fe is known to be higher in seawater due to the formation of Fe³⁺ complexes with natural organic ligands (Liu and Millero, 2002). The average pre-monsoon salinity at Divar reaches a

maximum of 28.75 ± 2.2 . Humic and fulvic acids in mangrove sediments are known to chelate Fe (III) (Ghatak et al., 2004). With high saline conditions during the pre-monsoon, enhanced bioavailability of Fe^{2+} through ligand formation could be expected. Thus, nitrate respiration by denitrifiers using various potential electron donors like metal ions could be important in reducing environmental pollution as the mangrove ecosystem shifts from heterotrophic (during post-monsoon) to autotrophic (during pre-monsoon) based respiration. The stronger influence of Mn over Fe on DNT activity in the Divar sediments during the post-monsoon further re-affirms our earlier finding that though these two elements share a similar chemistry in the environment, microbes involved in biogeochemical processes might prefer them differentially (Krishnan et al., 2007).

The influence of metals on DNT is persistent during the pre-monsoon at both the study sites albeit negatively. Metal concentration was found to increase with depth while DNT was maximum within 0-4 cm during this season. Bioturbating organisms viz., *Uca* crabs, polychaetes, etc. are abundant in the Divar region. Their burrowing action could affect the downward advection of metal oxides. In the mangrove sediments of Goa, the reductive phase of the N cycle proceeds at a much faster pace (Fernandes et al., 2012) as compared to the oxidative pathway (Krishnan and LokaBharathi, 2009). Autotrophic nitrification could be important in mangrove sediments with higher metal content as it results in metal reduction (Hulth et al., 1999). Krishnan and LokaBharathi (2009) have reported highest rates of benthic nitrification to occur during the pre-monsoon season at Tuvem and Divar. Fe^{3+} and Mn^{4+} occur as oxides and hydroxides in sediments. They play a role in serving as electron acceptors during anaerobic nitrification (Luther et al., 1997; Anschutz et al., 2000) resulting in an increase in reduced metal ions and nitrate. The lower metal content in the surficial sediments at Divar during the pre-monsoon could be attributed to higher reduction of Fe and Mn oxides and oxidation of NH_4^+ . Conversely, nitrate-dependent anaerobic ferrous iron oxidation has been reported to occur in sediments (Straub et al., 1996, 1998; Melton et al., 2012). Ferrous iron-oxidizing, denitrifying bacteria are known to form a major fraction of the total denitrifiers in sediments (Hauck et al., 2001) indicating a substantial biological potential for microbial nitrate-dependent ferrous iron oxidation. As denitrification and nitrification operate in tandem (Rysgaard et al., 1993; Nielsen et al., 1996), metal immobilization could occur through the reductive phase of the N cycle. Thus, a close coupling between metals and nitrogen redox processes in mangrove sediments could be prevalent.

Though our earlier work suggests that Divar is more influenced by anthropogenic forcing than Tuvem which is relatively pristine, there is a finer insight emerging from the MRA analysis carried out in the present study. The DNT at both sites was influenced by 9 different factors during pre-monsoon most of which are common between the two locations. However, their intensity and gradation differs. Though Divar remains moderately contaminated by Fe (Krishnan et al., 2007) and other mining associated pollutants during the pre-monsoon, the present study re-iterates the buffering capacity of the system to match that of Tuvem. Data analysis in the present study also revealed that the 20-30% range in Fe content recorded during the pre-monsoon is responsible for a negative trend in DNT (Fig. 6) for the whole year suggesting that if this tendency persists in subsequent years, it could prove to be detrimental to the mangroves and adjoining estuarine systems.

5. Conclusion

The present study reveals distinct seasonal DNT variability in the mangrove sediments of Goa with highest activity during the pre-monsoon. This variation is brought about by changes in the sediment chemistry and physiological groups of bacteria capable of denitrification. As observed by statistical analysis, 6-9 environmental variables were required to fairly predict DNT in these sediments with the complexity decreasing progressively from the pre-monsoon towards the post-monsoon. The influence of metals mainly Fe on DNT was most pronounced during the pre-monsoon at both the locations suggesting that an autotrophic mode of nitrate respiration could possibly exceed heterotrophy. Inorganic N substrates like $\text{NO}_3^-/\text{NO}_2^-$ were seen to influence DNT in these sediments after pre-monsoon. Our findings have underlined the complexity of the redox phase of the N cycle in mangrove sediments. Metal-mediated processes in the N cycle have been poorly understood in mangrove sediments and could lead to several secondary reactions of diagenesis creating new pathways of oxidation/reduction. These pathways could contribute to the production and subsequent emission of the greenhouse gas- nitrous oxide (N_2O). Thus, lowering anthropogenic inputs (N compounds, metals, sewage discharge) into estuarine systems would help minimize the flux of the radiative gas to the atmosphere.

The findings in this study could have wide ranging implications on the turnover of carbon as well as nitrogen. One immediate effect could be reduced flux of N_2O to the atmosphere during the pre-monsoon, thus prolonging the burial of organic matter in the sediments and reducing the greenhouse effect when the weather is warm. The process however could be reversed during the other seasons. On

the whole, total load of Fe in the sediments tend to decrease anaerobic respiration in these sediments. This decreased activity could also contribute to increasing the prevailing reducing conditions that could spell detrimental for the surface-dwelling fauna in these water logged systems. If the ambient concentration of Fe keeps accumulating over the years with continued mining activity, it could prove deleterious to the sensitive mangrove ecosystems in the long run. Future studies would be directed towards examining the influence of DNT on the redox chemistry of metals like Fe and Mn.

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Legend to figures

Fig. 1. Location of sampling sites at Tuvem and Divar.

Fig. 2. Down-core profile of pH, Eh, ammonium, nitrite and nitrate at Tuvem (a, c, e, g, i) and Divar (b, d, f, h, j). Standard deviation values have been shown separately along with the data in tables S1 and S2.

Fig. 3. Down-core variation in TOC, Fe and Mn at Tuvem (a, c, e) and Divar (b, d, f) during pre-monsoon (◆) monsoon (▲) and post-monsoon (■). Standard deviation values are tabulated along with the data in tables S1 and S2.

Fig. 4. Down-core variation in TC, THB and denitrifier abundance at Tuvem (a, c, e) and Divar (b, d, f). Data with standard deviation values have been provided in table S3.

Fig. 5. Seasonal down-core variation in denitrification activity (\pm SD) at Tuvem (a) and Divar (b).

Fig. 6. Trend in the variation of DNT with increase in total Fe content in mangrove sediments.

Supplementary information

Table S1. Seasonal variation of physico-chemical parameters (\pm SD) at Tuvem.

Table S2. Seasonal variation of physico-chemical parameters (\pm SD) at Divar.

Table S3. Seasonal variation of bacteriological parameters (\pm SD) at Tuvem and Divar.

Table S4. Results of multiple regression analysis of selected parameters which influence denitrification activity at Tuvem.

Table S5. Results of multiple regression analysis of selected parameters which influence denitrification activity at Divar mangrove ecosystem.

Fig. S1. Predicted versus observed denitrification activity (DNT; expressed as $\text{nmol g}^{-1} \text{h}^{-1}$) at Tuvem measured during pre-monsoon (a), monsoon (b) and post-monsoon (c). Predicted versus observed DNT at Divar during pre-monsoon, monsoon and post-monsoon have been shown in figures d, e and f respectively.

Table 1. Depth integrated (0–10 cm) denitrification activity ($\text{mmol N}_2\text{O m}^{-2} \text{h}^{-1}$) in the Tuvem and Divar mangrove sediments.

| | Tuvem | Divar |
|---------------|--------------|--------------|
| Season | | |
| Pre-monsoon | 0.72 | 1.09 |
| Monsoon | 0.08 | 0.15 |
| Post-monsoon | 0.14 | 0.66 |

Table 2. Resulting r and p-values of Pearson’s correlation analysis carried out between denitrification activity (DNT) and environmental variables (n = 15) at Tuvem and Divar.

| Location | Season | Variable | r | p-value |
|-----------------|---------------|-----------------|----------|----------------|
| Tuvem | | | | |
| | Pre-monsoon | Depth | -0.664 | 0.01 |
| | | Fe | -0.660 | 0.01 |
| | Monsoon | Depth | -0.863 | 0.001 |
| | | Denitrifiers | -0.778 | 0.001 |
| | | NO_2^- | -0.694 | 0.01 |
| | Post-monsoon | NO_3^- | -0.583 | 0.05 |
| Divar | | | | |
| | Pre-monsoon | Depth | -0.643 | 0.01 |
| | | Fe | -0.631 | 0.01 |
| | Monsoon | NO_3^- | 0.559 | 0.05 |
| | Post-monsoon | Depth | -0.092 | 0.001 |
| | | Mn | -0.643 | 0.01 |
| | | THB | 0.601 | 0.01 |
| | | pH | 0.546 | 0.05 |
| | | Fe | 0.590 | 0.05 |

Table 3. Multiple regression models obtained to predict ambient denitrification activity in mangrove sediments.

| Location | Season | Model R ² | p-value | Multiple regression model |
|---------------|--------------|----------------------|----------|---|
| Tuvenm | | | | |
| | Pre-monsoon | 0.94 | < 0.0001 | DNT = 229.90 + 2.20 * Depth + 6.65 * NO ₂ ⁻ - 2.58 * THB - 17.16 * Fe - 0.13 * Eh + 1.93 * NO ₃ ⁻ + 1.14 * NH ₄ ⁺ - 54.27 * Mn - 12.31 * TC |
| | Monsoon | 0.98 | < 0.0001 | DNT = -3.30 - 0.10 * Depth + 0.07 * NH ₄ ⁺ - 0.54 * NO ₂ ⁻ - 1.04 * THB + 0.07 * NO ₃ ⁻ + 0.89 * TOC - 1.74 * Mn + 0.87 * TC |
| | Post-monsoon | 0.67 | < 0.0001 | DNT = 15.79 - 0.13 * NO ₃ ⁻ - 2.19 * pH - 0.17 * Depth + 0.05 * NH ₄ ⁺ - 1.03 * Mn + 0.15 * NO ₂ ⁻ |
| Divar | | | | |
| | Pre-monsoon | 0.77 | < 0.0001 | DNT = -1056.72 + 6.52 * Depth + 3.94 * TOC - 0.12 * NO ₃ ⁻ + 1.92 * THB + 75.86 * Denitrifiers + 263.14 * Mn + 0.80 * Fe + 6.66 * TC + 0.87 * NO ₂ ⁻ |
| | Monsoon | 0.85 | < 0.0001 | DNT = 6.17 + 0.14 * NO ₃ ⁻ - 0.01 * Eh - 0.84 * Mn - 1.18 * THB + 0.26 * TOC + 0.08 * Depth - 0.28 * NO ₂ ⁻ |
| | Post-monsoon | 0.95 | < 0.0001 | DNT = -24.92 - 1.18 * Depth + 0.42 * Fe - 0.01 * Eh + 0.29 * NO ₂ ⁻ + 2.47 * TC + 0.93 * Denitrifiers - 0.52 * THB |

Note: DNT = denitrification rate (nmol N₂O g⁻¹ h⁻¹); concentration of NH₄⁺, NO₂⁻ & NO₃⁻ = μmol L⁻¹; Fe & Mn content expressed as %; TOC = total organic carbon expressed as %; TC = Total bacterial counts (expressed as cells g⁻¹); THB = total heterotrophic bacteria (expressed as CFU g⁻¹). Bacterial parameters have been log₁₀ transformed before statistical analysis.

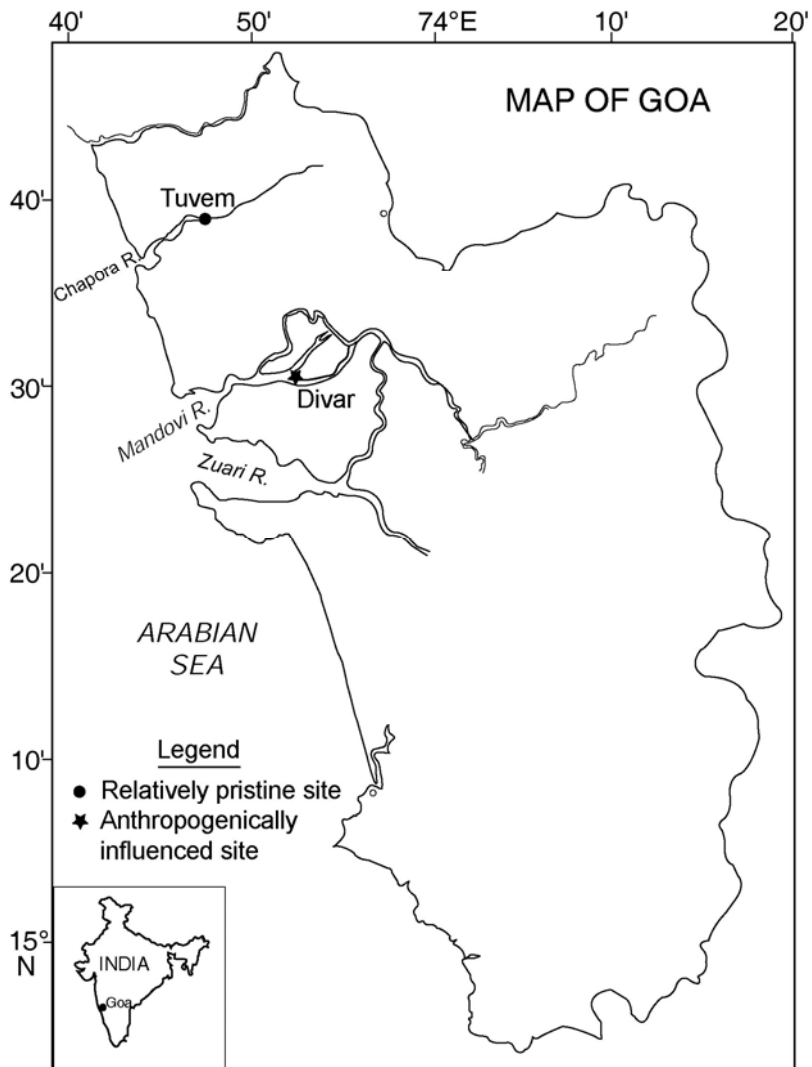


Fig. 1

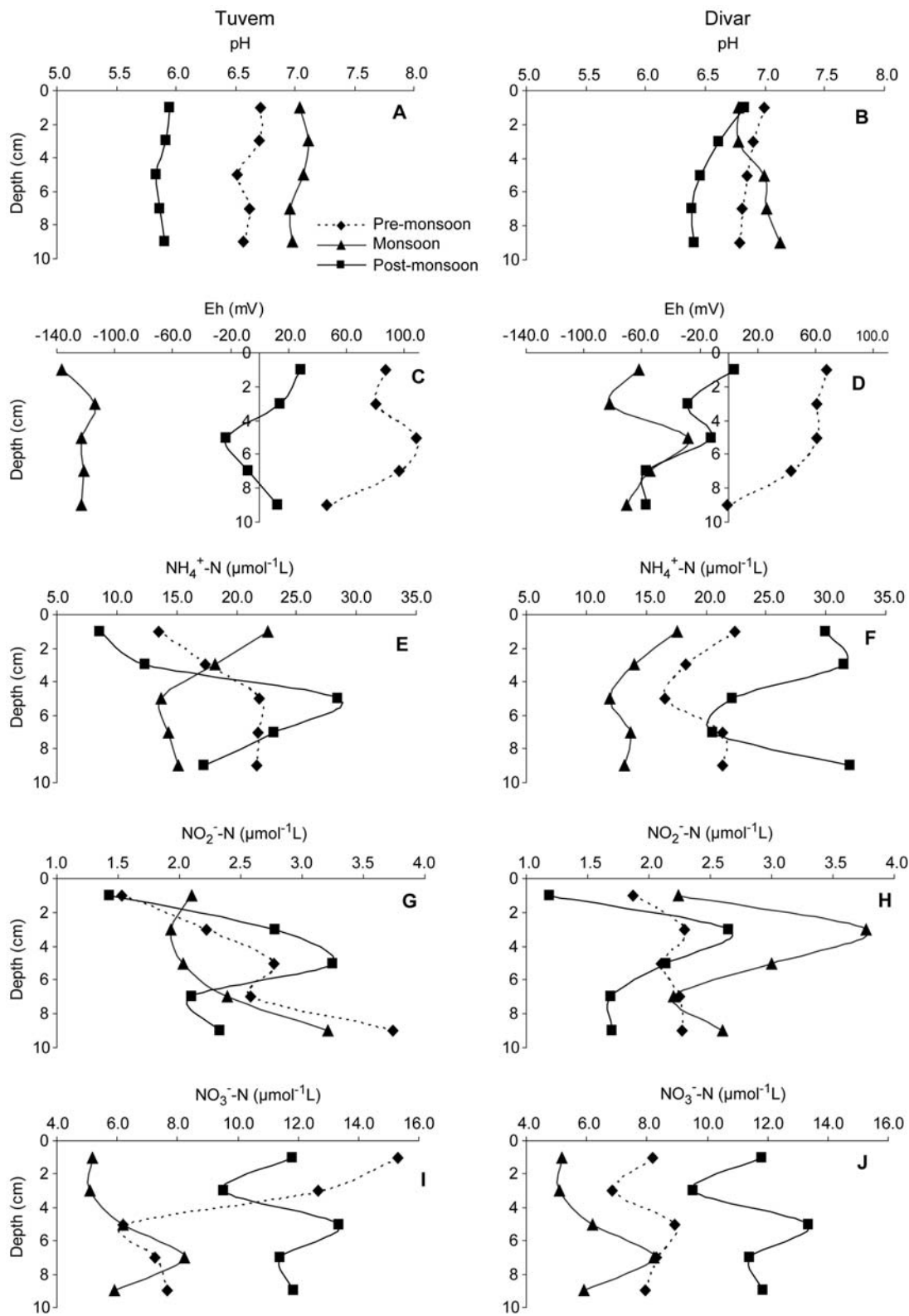


Fig. 2

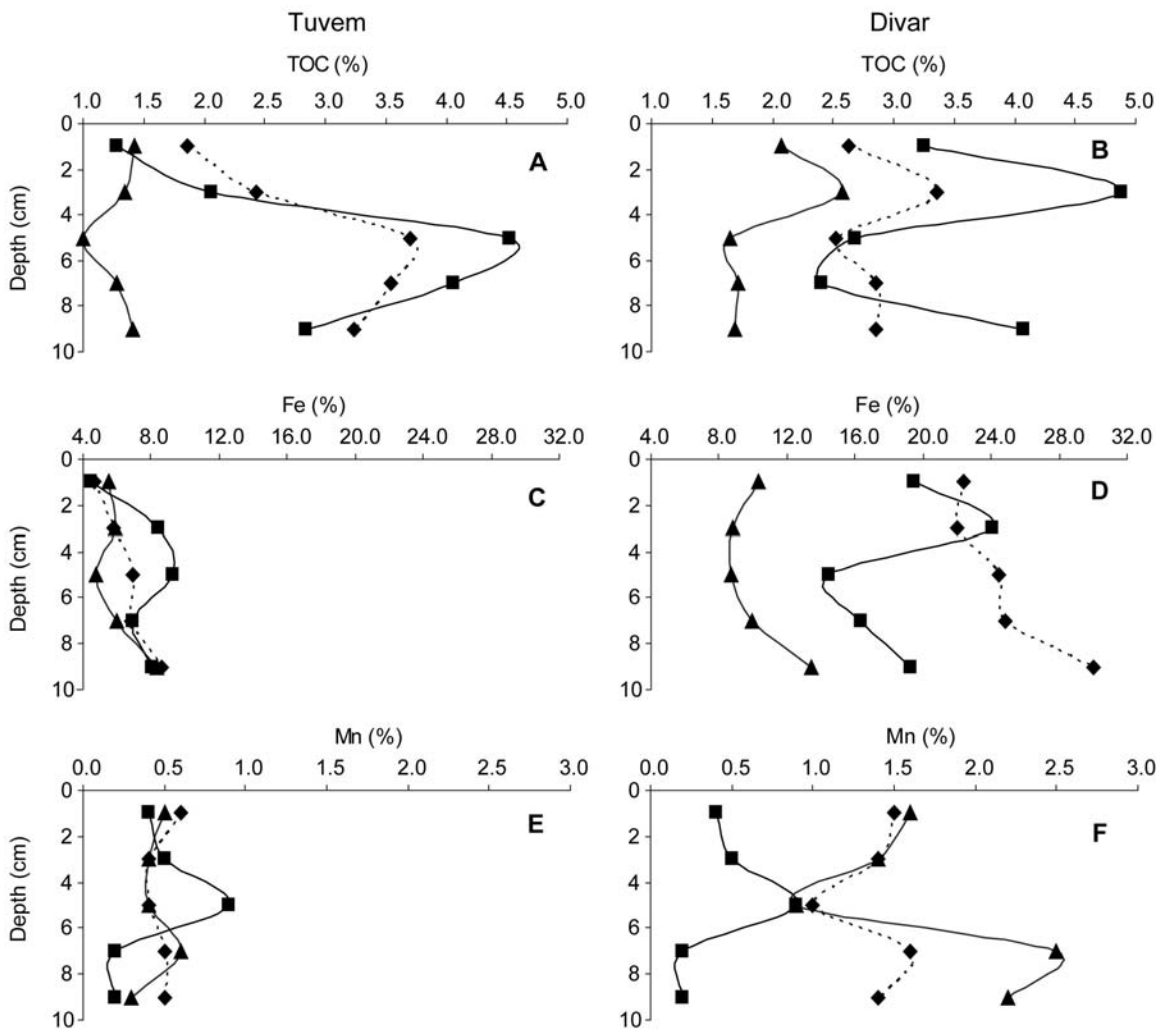


Fig. 3

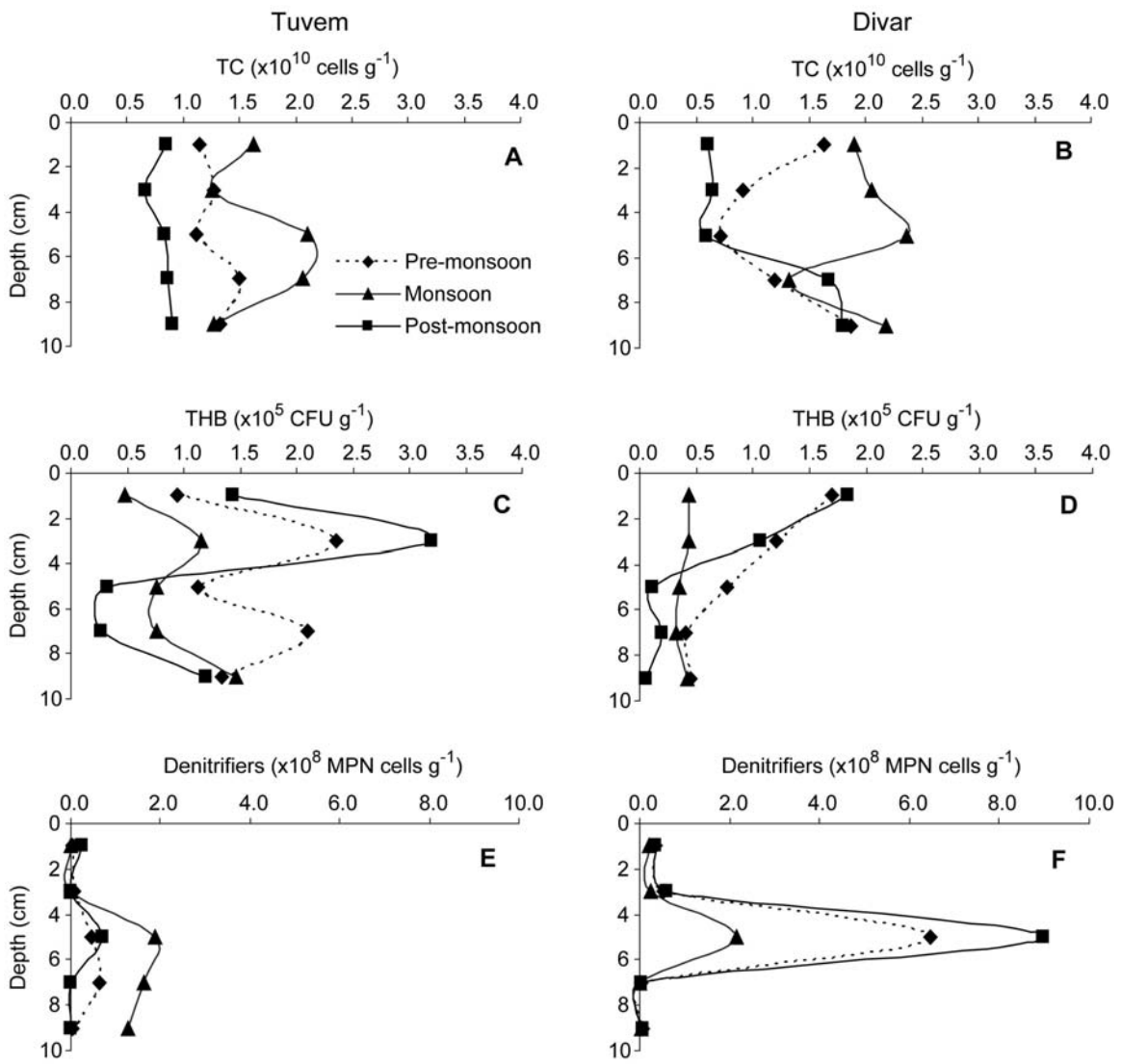


Fig. 4

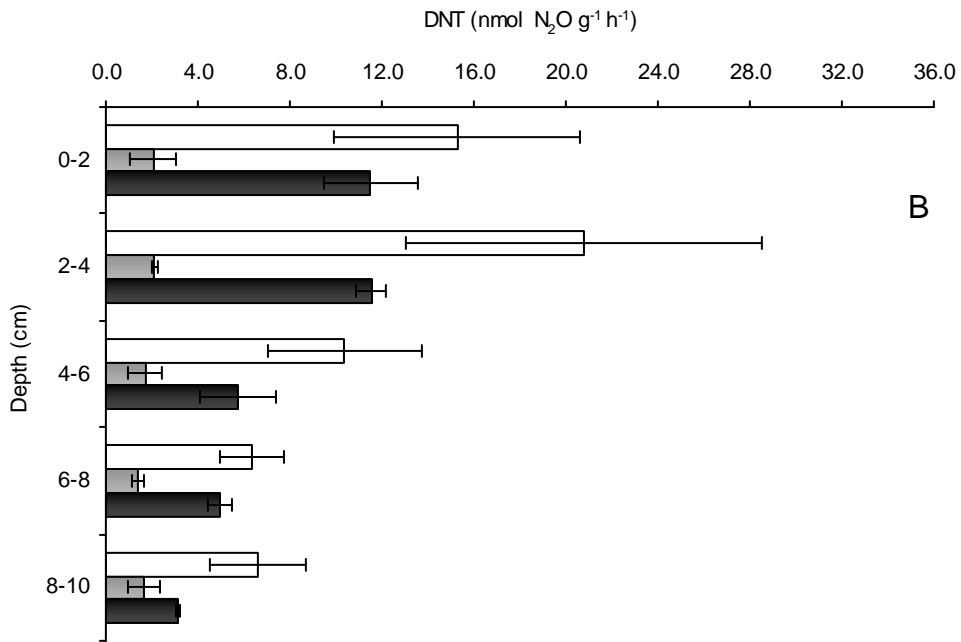
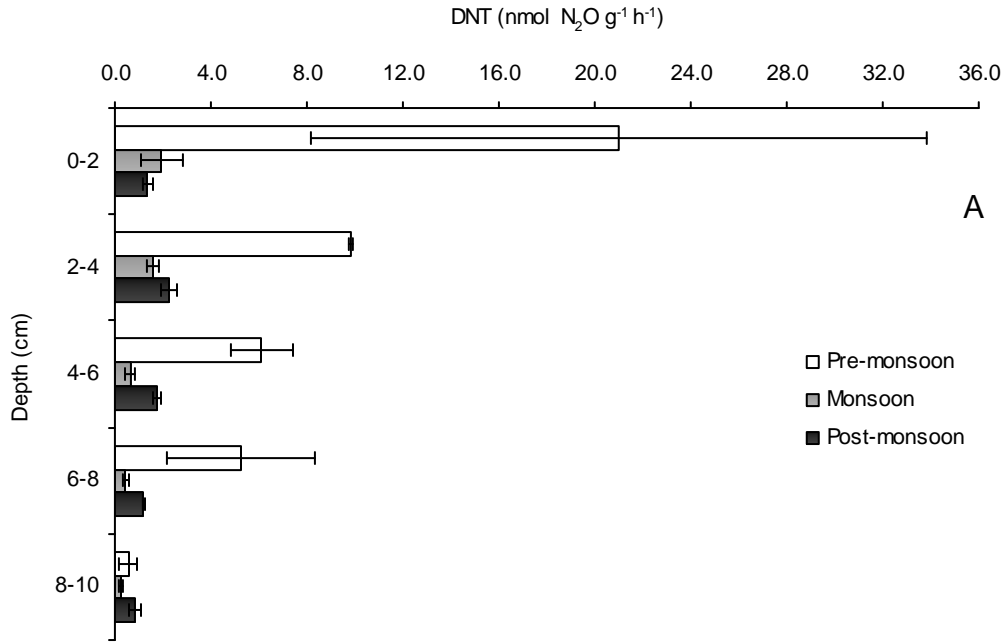
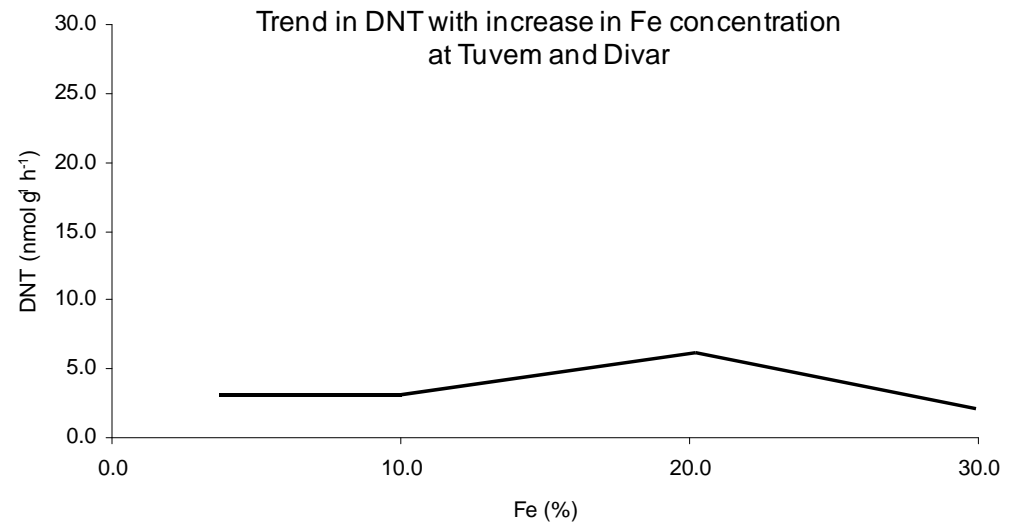
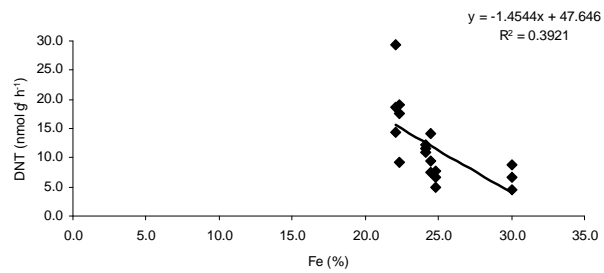
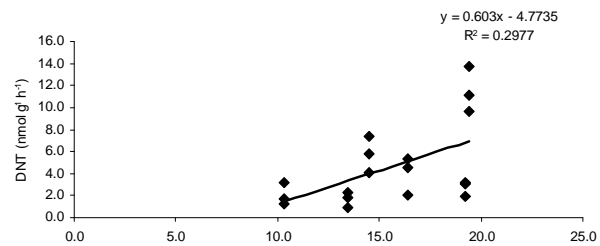
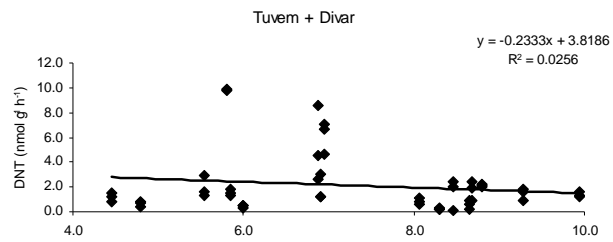


Fig. 5



Note: Figure on RHS not to scale.

Fig. 6

Table S1. Seasonal variation of physico-chemical parameters (\pm SD) at Tuvem.

| Season | Depth (cm) | pH | Eh (mV) | NH ₄ ⁺ -N (μ mol L ⁻¹) | NO ₂ ⁻ -N (μ mol L ⁻¹) | NO ₃ ⁻ -N (μ mol L ⁻¹) | TOC (%) | Fe (%) | Mn (%) |
|--------------|------------|--------------------|-------------------------|---|---|---|--------------------|--------------------|--------------------|
| Pre-monsoon | 0-2 | 6.71 (\pm 0.35) | 87.13 (\pm 137.46) | 13.52 (\pm 3.51) | 1.53 (\pm 0.72) | 15.32 (\pm 12.65) | 1.86 (\pm 0.80) | 4.68 (\pm 1.75) | 0.60 (\pm 0.30) |
| | 2-4 | 6.70 (\pm 0.51) | 80.50 (\pm 133.58) | 17.41 (\pm 4.78) | 2.22 (\pm 1.12) | 12.67 (\pm 13.73) | 2.43 (\pm 1.43) | 5.80 (\pm 2.97) | 0.40 (\pm 0.28) |
| | 4-6 | 6.52 (\pm 0.65) | 108.35 (\pm 154.17) | 21.85 (\pm 7.51) | 2.77 (\pm 0.72) | 6.21 (\pm 7.00) | 3.70 (\pm 2.88) | 6.95 (\pm 2.23) | 0.40 (\pm 0.30) |
| | 6-8 | 6.62 (\pm 0.44) | 96.08 (\pm 134.93) | 21.84 (\pm 8.65) | 2.58 (\pm 0.31) | 7.26 (\pm 4.38) | 3.54 (\pm 2.91) | 6.87 (\pm 1.23) | 0.50 (\pm 0.21) |
| | 8-10 | 6.57 (\pm 0.49) | 46.40 (\pm 102.17) | 21.71 (\pm 6.22) | 3.74 (\pm 0.76) | 7.66 (\pm 4.85) | 3.24 (\pm 2.66) | 8.64 (\pm 2.27) | 0.50 (\pm 0.30) |
| Monsoon | 0-2 | 7.04 (\pm 0.29) | -136.38 (\pm 112.58) | 22.64 (\pm 7.36) | 2.10 (\pm 1.09) | 5.18 (\pm 2.37) | 1.43 (\pm 0.28) | 5.55 (\pm 2.96) | 0.50 (\pm 0.20) |
| | 2-4 | 7.11 (\pm 0.17) | -113.43 (\pm 112.25) | 18.20 (\pm 9.67) | 1.93 (\pm 0.86) | 5.08 (\pm 0.43) | 1.34 (\pm 0.46) | 5.85 (\pm 2.36) | 0.40 (\pm 0.10) |
| | 4-6 | 7.07 (\pm 0.11) | -122.75 (\pm 123.79) | 13.69 (\pm 9.18) | 2.03 (\pm 0.98) | 6.21 (\pm 2.99) | 1.00 (\pm 0.05) | 4.79 (\pm 0.92) | 0.40 (\pm 0.10) |
| | 6-8 | 6.96 (\pm 0.18) | -121.55 (\pm 123.17) | 14.34 (\pm 8.58) | 2.39 (\pm 0.61) | 8.24 (\pm 5.48) | 1.28 (\pm 0.41) | 5.99 (\pm 1.03) | 0.60 (\pm 0.20) |
| | 8-10 | 6.98 (\pm 0.12) | -123.08 (\pm 117.64) | 15.16 (\pm 9.99) | 3.21 (\pm 0.52) | 5.92 (\pm 5.25) | 1.41 (\pm 0.40) | 8.30 (\pm 1.72) | 0.30 (\pm 0.10) |
| Post-monsoon | 0-2 | 5.95 (\pm 0.13) | 28.60 (\pm 131.78) | 8.58 (\pm 2.45) | 1.43 (\pm 1.54) | 11.80 (\pm 6.64) | 1.28 (\pm 0.85) | 4.45 (\pm 3.70) | 0.40 (\pm 0.20) |
| | 2-4 | 5.92 (\pm 0.24) | 14.15 (\pm 136.39) | 12.41 (\pm 2.28) | 2.78 (\pm 1.43) | 9.54 (\pm 4.69) | 2.06 (\pm 1.43) | 8.45 (\pm 1.87) | 0.50 (\pm 0.30) |
| | 4-6 | 5.83 (\pm 0.17) | -23.15 (\pm 117.57) | 28.43 (\pm 5.03) | 3.25 (\pm 1.53) | 13.35 (\pm 6.07) | 4.52 (\pm 3.13) | 9.28 (\pm 4.33) | 0.90 (\pm 1.00) |
| | 6-8 | 5.87 (\pm 0.28) | -7.60 (\pm 112.66) | 23.10 (\pm 3.64) | 2.10 (\pm 0.65) | 11.42 (\pm 2.68) | 4.06 (\pm 2.75) | 6.91 (\pm 2.26) | 0.20 (\pm 0.10) |
| | 8-10 | 5.91 (\pm 0.33) | 12.23 (\pm 142.12) | 17.31 (\pm 3.45) | 2.33 (\pm 1.38) | 11.84 (\pm 4.82) | 2.84 (\pm 2.02) | 8.06 (\pm 3.25) | 0.20 (\pm 0.00) |

Table S2. Seasonal variation of physico-chemical parameters (\pm SD) at Divar.

| Season | Depth (cm) | pH | Eh (mV) | NH ₄ ⁺ -N (μ mol L ⁻¹) | NO ₂ ⁻ -N (μ mol L ⁻¹) | NO ₃ ⁻ -N (μ mol L ⁻¹) | TOC (%) | Fe (%) | Mn (%) |
|--------------|------------|--------------------|------------------------|---|---|---|--------------------|----------------------|--------------------|
| Pre-monsoon | 0-2 | 6.99 (\pm 0.27) | 67.48 (\pm 114.10) | 22.41 (\pm 10.21) | 1.87 (\pm 1.50) | 8.17 (\pm 2.21) | 2.63 (\pm 1.42) | 22.36 (\pm 7.96) | 1.50 (\pm 1.20) |
| | 2-4 | 6.90 (\pm 0.10) | 60.55 (\pm 97.11) | 18.29 (\pm 11.01) | 2.29 (\pm 1.48) | 6.83 (\pm 1.10) | 3.36 (\pm 2.16) | 22.04 (\pm 7.14) | 1.40 (\pm 1.10) |
| | 4-6 | 6.85 (\pm 0.14) | 60.48 (\pm 79.93) | 16.53 (\pm 3.88) | 2.10 (\pm 0.63) | 8.94 (\pm 3.94) | 2.52 (\pm 0.86) | 24.44 (\pm 8.43) | 1.00 (\pm 0.40) |
| | 6-8 | 6.81 (\pm 0.13) | 42.68 (\pm 69.83) | 21.39 (\pm 9.18) | 2.25 (\pm 0.71) | 8.33 (\pm 1.66) | 2.86 (\pm 1.63) | 24.84 (\pm 8.57) | 1.60 (\pm 1.40) |
| | 8-10 | 6.79 (\pm 0.21) | -0.90 (\pm 24.43) | 21.35 (\pm 7.33) | 2.27 (\pm 0.48) | 7.96 (\pm 1.87) | 2.85 (\pm 1.51) | 30.02 (\pm 13.37) | 1.40 (\pm 0.70) |
| Monsoon | 0-2 | 6.78 (\pm 0.55) | -62.35 (\pm 140.37) | 17.62 (\pm 6.65) | 2.24 (\pm 1.86) | 15.26 (\pm 11.36) | 2.07 (\pm 1.14) | 10.32 (\pm 4.87) | 1.60 (\pm 1.50) |
| | 2-4 | 6.78 (\pm 0.43) | -82.50 (\pm 145.18) | 13.97 (\pm 5.81) | 3.77 (\pm 1.80) | 13.43 (\pm 10.21) | 2.57 (\pm 1.50) | 8.80 (\pm 6.10) | 1.40 (\pm 1.40) |
| | 4-6 | 6.99 (\pm 0.25) | -28.50 (\pm 100.22) | 12.00 (\pm 6.59) | 3.00 (\pm 2.48) | 9.98 (\pm 3.42) | 1.65 (\pm 1.50) | 8.68 (\pm 3.51) | 0.90 (\pm 0.60) |
| | 6-8 | 7.01 (\pm 0.31) | -54.83 (\pm 110.41) | 13.68 (\pm 7.61) | 2.20 (\pm 1.48) | 13.38 (\pm 4.09) | 1.72 (\pm 1.50) | 9.94 (\pm 2.38) | 2.50 (\pm 1.90) |
| | 8-10 | 7.13 (\pm 0.39) | -70.23 (\pm 115.40) | 13.24 (\pm 9.13) | 2.60 (\pm 1.39) | 14.40 (\pm 3.30) | 1.69 (\pm 1.50) | 13.47 (\pm 11.35) | 2.20 (\pm 1.50) |
| Post-monsoon | 0-2 | 6.83 (\pm 0.32) | 4.18 (\pm 129.16) | 29.99 (\pm 3.47) | 1.19 (\pm 0.48) | 6.43 (\pm 4.03) | 3.25 (\pm 1.50) | 19.44 (\pm 8.66) | 0.50 (\pm 0.40) |
| | 2-4 | 6.61 (\pm 0.20) | -27.88 (\pm 96.80) | 31.50 (\pm 2.04) | 2.65 (\pm 2.28) | 10.09 (\pm 6.37) | 4.88 (\pm 1.50) | 24.09 (\pm 10.36) | 1.00 (\pm 0.80) |
| | 4-6 | 6.46 (\pm 0.25) | -11.93 (\pm 115.19) | 22.21 (\pm 7.80) | 2.14 (\pm 0.57) | 5.62 (\pm 5.20) | 2.68 (\pm 1.50) | 14.47 (\pm 4.40) | 0.50 (\pm 0.20) |
| | 6-8 | 6.39 (\pm 0.19) | -56.98 (\pm 135.20) | 20.56 (\pm 3.09) | 1.69 (\pm 0.68) | 7.89 (\pm 7.16) | 2.41 (\pm 1.50) | 16.39 (\pm 6.00) | 1.70 (\pm 1.90) |
| | 8-10 | 6.41 (\pm 0.20) | -57.20 (\pm 81.34) | 32.05 (\pm 4.63) | 1.70 (\pm 0.60) | 10.43 (\pm 6.52) | 4.07 (\pm 1.50) | 19.25 (\pm 8.47) | 1.70 (\pm 1.40) |

Table S3. Seasonal variation of bacteriological parameters (\pm SD) at Tuvem and Divar.

| Season | Depth (cm) | TC $\times 10^{10}$ cells g^{-1} | THB $\times 10^5$ CFU g^{-1} | Denitrifiers $\times 10^8$ MPN cells g^{-1} |
|--------------|---------------|---------------------------------------|-----------------------------------|--|
| TUVEM | | | | |
| Pre-monsoon | 0-2 | 1.15 (± 0.65) | 0.94 (± 0.94) | 0.0369 (± 0.01) |
| | 2-4 | 1.27 (± 0.92) | 2.35 (± 4.09) | 0.0592 (± 0.02) |
| | 4-6 | 1.12 (± 0.77) | 1.12 (± 1.04) | 0.45 (± 0.15) |
| | 6-8 | 1.50 (± 1.27) | 2.10 (± 2.25) | 0.654 (± 0.32) |
| | 8-10 | 1.33 (± 0.51) | 1.34 (± 2.33) | 0.0217 (± 0.00) |
| Monsoon | 0-2 | 1.63 (± 0.76) | 0.48 (± 0.34) | 0.00962 (± 0.00) |
| | 2-4 | 1.26 (± 0.57) | 1.15 (± 0.58) | 0.000617 (± 0.00) |
| | 4-6 | 2.10 (± 0.77) | 0.76 (± 0.72) | 1.87 (± 1.20) |
| | 6-8 | 2.07 (± 0.96) | 0.76 (± 0.57) | 1.64 (± 0.21) |
| | 8-10 | 1.27 (± 0.40) | 1.46 (± 1.07) | 1.28 (± 0.44) |
| Post-monsoon | 0-2 | 0.85 (± 0.40) | 1.43 (± 0.89) | 0.263 (± 0.17) |
| | 2-4 | 0.67 (± 0.28) | 3.20 (± 2.99) | 0.0164 (± 0.00) |
| | 4-6 | 0.83 (± 0.46) | 0.32 (± 0.30) | 0.714 (± 0.33) |
| | 6-8 | 0.86 (± 0.69) | 0.27 (± 0.34) | 0.00569 (± 0.00) |
| | 8-10 | 0.90 (± 0.59) | 1.20 (± 2.18) | 0.00298 (± 0.00) |
| DIVAR | | | | |
| Pre-monsoon | 0-2 | 1.63 (± 1.41) | 1.70 (± 0.66) | 0.36 (± 0.11) |
| | 2-4 | 0.91 (± 0.43) | 1.20 (± 0.92) | 0.538 (± 0.10) |
| | 4-6 | 0.72 (± 0.34) | 0.77 (± 0.96) | 6.46 (± 0.95) |
| | 6-8 | 1.20 (± 0.50) | 0.40 (± 0.15) | 0.029 (± 0.77) |
| | 8-10 | 1.88 (± 1.58) | 0.45 (± 0.54) | 0.0848 (± 0.81) |
| Monsoon | 0-2 | 1.90 (± 1.16) | 0.43 (± 0.30) | 0.208 (± 0.28) |
| | 2-4 | 2.06 (± 0.65) | 0.44 (± 0.35) | 0.243 (± 0.09) |
| | 4-6 | 2.37 (± 2.33) | 0.35 (± 0.39) | 2.16 (± 0.23) |
| | 6-8 | 1.32 (± 1.23) | 0.32 (± 0.23) | 0.00234 (± 0.03) |
| | 8-10 | 2.19 (± 1.51) | 0.42 (± 0.38) | 0.035 (± 0.32) |
| Post-monsoon | 0-2 | 0.61 (± 0.22) | 1.84 (± 1.20) | 0.349 (± 0.34) |
| | 2-4 | 0.65 (± 0.30) | 1.06 (± 1.85) | 0.609 (± 0.14) |
| | 4-6 | 0.59 (± 0.19) | 0.11 (± 0.13) | 8.96 (± 0.10) |
| | 6-8 | 1.67 (± 2.00) | 0.19 (± 0.31) | 0.038 (± 0.05) |
| | 8-10 | 1.80 (± 2.32) | 0.05 (± 0.03) | 0.078 (± 0.10) |

Note: TC= total bacterial cells expressed as cells g^{-1} ; THB = total heterotrophic bacteria and expressed as colony forming units (CFU) g^{-1} . Denitrifiers have been expressed as most probable number (MPN) cells g^{-1} .

Table S4. Results of multiple regression analysis of selected parameters which influence denitrification activity at Tuvem.

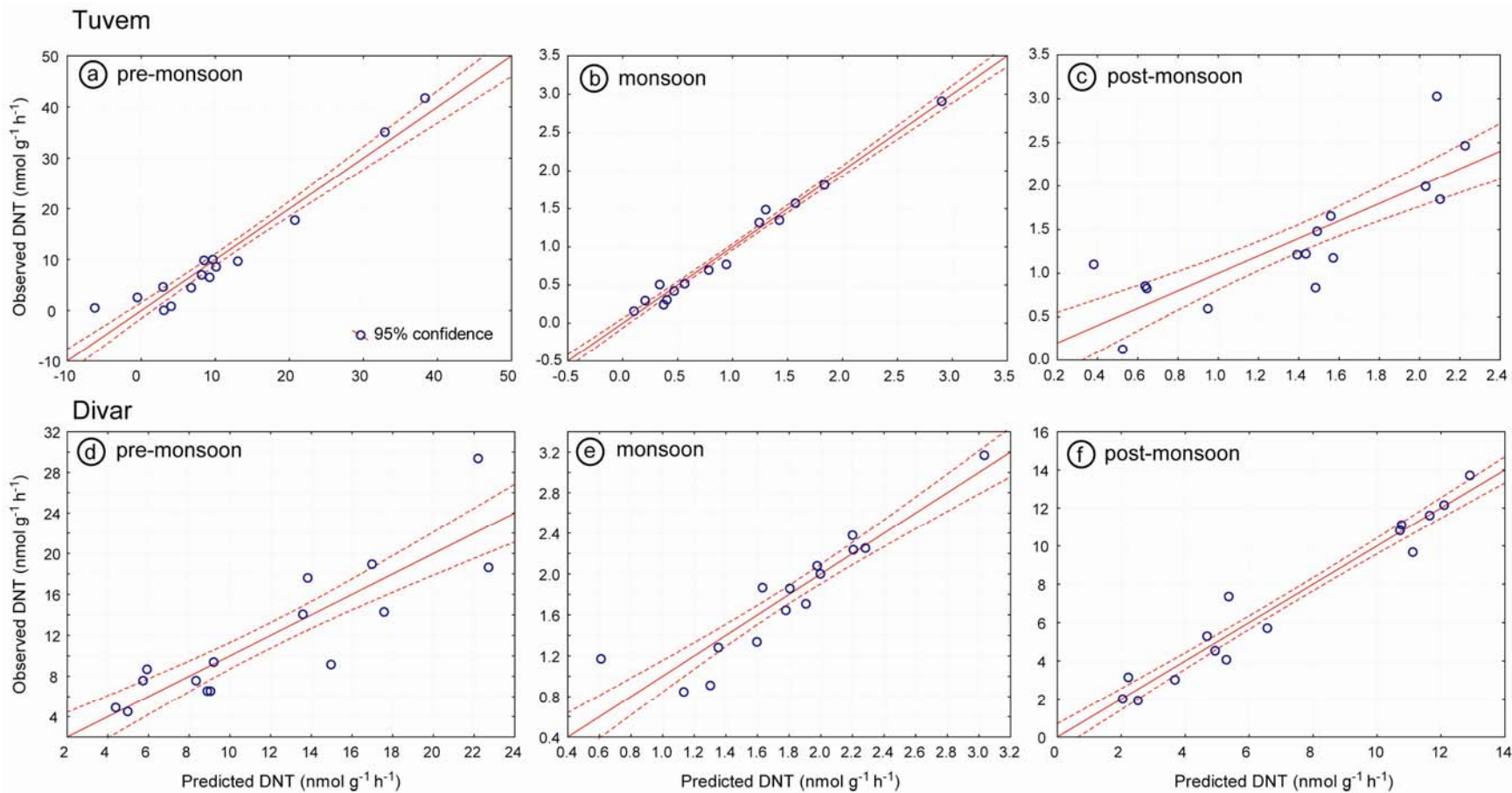
| Pre-monsoon | | | | | |
|---|---------------|----------------|---------|-----------------|--|
| R = 0.97, R ² = 0.94, Adjusted R ² = 0.91, F(9,20) = 33.14, p < 0.0001, Standard error of estimate: 3.63, n=30 | | | | | |
| | Coefficient B | Std. err. of B | p-level | Semi part. Cor. | Order of influence |
| Intercept | 229.90 | 60.58 | 0.0011 | | |
| Depth | 2.20 | 0.93 | 0.0290 | 0.13 | |
| NO ₂ ⁻ | 6.65 | 1.44 | 0.0002 | 0.26 | |
| THB | -2.58 | 1.65 | 0.1340 | -0.09 | |
| Fe | -17.16 | 2.44 | 0.0000 | -0.40 | Eh > Fe > NH ₄ ⁺ > NO ₃ ⁻ > NO ₂ ⁻ > |
| Eh | -0.13 | 0.02 | 0.0000 | -0.48 | Mn > TC > Depth > THB |
| NO ₃ ⁻ | 1.93 | 0.32 | 0.0000 | 0.34 | |
| NH ₄ ⁺ | 1.14 | 0.17 | 0.0000 | 0.38 | |
| Mn | -54.27 | 12.59 | 0.0003 | -0.24 | |
| TC | -12.31 | 4.90 | 0.0206 | -0.14 | |
| Monsoon | | | | | |
| R = 0.99, R ² = 0.98, Adjusted R ² = 0.97, F(8,21) = 139.61, p < 0.0001, Standard error of estimate: 0.12, n=30 | | | | | |
| | Coefficient B | Std. err. of B | p-level | Semi part. Cor. | Order of influence |
| Intercept | -3.30 | 1.88 | 0.0942 | | |
| Depth | -0.10 | 0.01 | 0.0000 | -0.22 | |
| NH ₄ ⁺ | 0.07 | 0.00 | 0.0000 | 0.44 | |
| NO ₂ ⁻ | -0.54 | 0.05 | 0.0000 | -0.34 | Depth > NH ₄ ⁺ > NO ₂ ⁻ > THB > |
| THB | -1.04 | 0.11 | 0.0000 | -0.28 | NO ₃ ⁻ > TOC > Mn > TC |
| NO ₃ ⁻ | 0.07 | 0.01 | 0.0000 | 0.24 | |
| TOC | 0.89 | 0.10 | 0.0000 | 0.27 | |
| Mn | -1.74 | 0.27 | 0.0000 | -0.19 | |
| TC | 0.87 | 0.18 | 0.0001 | 0.15 | |
| Post-monsoon | | | | | |
| R = 0.82, R ² = 0.67, Adjusted R ² = 0.59, F(6,23) = 7.89, p < 0.0001, Standard error of estimate: 0.47, n=30 | | | | | |
| | Coefficient B | Std. err. of B | p-level | Semi part. Cor. | Order of influence |
| Intercept | 15.79 | 3.73 | 0.0003 | | |
| NO ₃ ⁻ | -0.13 | 0.03 | 0.0001 | -0.58 | |
| pH | -2.19 | 0.62 | 0.0019 | -0.42 | NO ₃ ⁻ > pH > Depth > NH ₄ ⁺ > Mn |
| Depth | -0.17 | 0.05 | 0.0036 | -0.39 | > NO ₂ ⁻ |
| NH ₄ ⁺ | 0.05 | 0.02 | 0.0244 | 0.29 | |
| Mn | -1.03 | 0.53 | 0.0665 | -0.23 | |
| NO ₂ ⁻ | 0.15 | 0.10 | 0.1345 | 0.18 | |

Note: TC= total bacterial cells expressed as cells g⁻¹; THB = total heterotrophic bacteria and expressed as colony forming units (CFU) g⁻¹, TOC= total organic carbon.

Table S5. Results of multiple regression analysis of selected parameters which influence denitrification activity at Divar mangrove ecosystem.

| Pre-monsoon | | | | | | |
|--|----------|----------------|---------|----------------|---|--|
| R = 0.88, R ² = 0.77, Adjusted R ² = 0.66, F(9,20) = 7.35, p < 0.0001, Standard error of estimate: 3.94, n=30 | | | | | | |
| | B | Std. err. of B | p-level | Semi part Cor. | Order of influence | |
| Intercept | -1056.72 | 318.21 | 0.0034 | | | |
| Depth | 6.52 | 2.11 | 0.0058 | 0.33 | | |
| TOC | 3.94 | 1.69 | 0.0307 | 0.25 | | |
| NO ₃ ⁻ | -0.12 | 0.55 | 0.8283 | -0.02 | Denitrifiers > Mn > Depth > TOC > TC > Fe > NO ₂ ⁻ > THB | |
| THB | 1.92 | 2.84 | 0.5071 | 0.07 | | |
| Denitrifiers | 75.86 | 22.70 | 0.0032 | 0.36 | | |
| Mn | 263.14 | 80.50 | 0.0038 | 0.35 | | |
| Fe | 0.80 | 0.73 | 0.2917 | 0.12 | | |
| TC | 6.66 | 4.74 | 0.1756 | 0.15 | | |
| NO ₂ ⁻ | 0.87 | 0.85 | 0.3170 | 0.11 | | |
| | | | | | | |
| Monsoon | | | | | | |
| R = 0.92, R ² = 0.85, Adjusted R ² = 0.80, F(7,22) = 18.11, p < 0.0001, Standard error of estimate: 0.27, n=30 | | | | | | |
| | B | Std. err. of B | p-level | Semi part Cor. | Order of influence | |
| Intercept | 6.17 | 0.84 | 0.0000 | | | |
| NO ₃ ⁻ | 0.14 | 0.02 | 0.0000 | 0.60 | | |
| Eh | -0.01 | 0.00 | 0.0000 | -0.51 | NO ₃ ⁻ > Eh > THB > Mn > TOC > Depth > NO ₂ ⁻ | |
| Mn | -0.84 | 0.14 | 0.0000 | -0.48 | | |
| THB | -1.18 | 0.19 | 0.0000 | -0.50 | | |
| TOC | 0.26 | 0.06 | 0.0004 | 0.35 | | |
| Depth | 0.08 | 0.03 | 0.0080 | 0.24 | | |
| NO ₂ ⁻ | -0.28 | 0.10 | 0.0097 | -0.23 | | |
| | | | | | | |
| | | | | | | |
| Post-monsoon | | | | | | |
| R = 0.97, R ² = 0.95, Adjusted R ² = 0.94, F(7,22) = 62.01, p < 0.0001, Standard error of estimate: 1.01, n=30 | | | | | | |
| | B | Std. err. of B | p-level | Semi part Cor. | Order of influence | |
| Intercept | -24.92 | 8.63 | 0.0086 | | | |
| Depth | -1.18 | 0.10 | 0.0000 | -0.57 | Depth > Fe > Eh > TC > Denitrifiers > THB > NO ₂ ⁻ | |
| Fe | 0.42 | 0.09 | 0.0001 | 0.21 | | |
| Eh | -0.01 | 0.00 | 0.0007 | -0.18 | | |
| NO ₂ ⁻ | 0.29 | 0.24 | 0.2565 | 0.05 | | |
| TC | 2.47 | 0.73 | 0.0027 | 0.16 | | |
| Denitrifiers | 0.93 | 0.32 | 0.0079 | 0.14 | | |
| THB | -0.52 | 0.41 | 0.2141 | -0.06 | | |
| | | | | | | |

Note: Denitrifiers have been expressed as most probable number (MPN) cells g⁻¹.



Note: The diagonal reference line in each plot indicates a 1:1 relationship where observed rates would equal predicted rates.

Fig. S1