

## Denitrification activity in mangrove sediments varies with associated vegetation

Sheryl Oliveira Fernandes <sup>a,#</sup>, Pinky Dutta <sup>b</sup>, Maria-Judith Gonsalves <sup>a</sup>,  
Patricia C. Bonin <sup>c</sup>, P. A. LokaBharathi <sup>a,\*</sup>

<sup>a</sup> Biological Oceanography Division, CSIR-National Institute of Oceanography (NIO), Dona Paula, Goa – 403 004, India.

<sup>b</sup> Hislop School of Biotechnology, Civil Lines, Nagpur, Maharashtra – 440001, India.

<sup>c</sup> Aix-Marseille Université, MIO-UM 110, CNRS/INSU, IRD, 13288, Marseille, Cedex 09, France.

\*Corresponding author: Tel.: +91-832-2458108;  
E-mail: [loka@nio.org](mailto:loka@nio.org); [lokabharathiponnapakkam@gmail.com](mailto:lokabharathiponnapakkam@gmail.com)

### ABSTRACT

To test the hypothesis that mangrove vegetation associated sediment characteristics could influence an ecologically important function such as denitrification, a study was carried out at the Divar mangrove ecosystem in Goa, India. The composition of intertidal mangroves was assessed based on which the down-core (0-10 cm) variation in environmental variables and denitrification activity (DNT) associated with dominant vegetation types was examined. Our observations revealed a distinct zonation pattern of mangroves with the dominance of *Rhizophora mucronata* (RM) in the lower intertidal zone followed by *Avicennia marina* (AM) in the mid region. *Acanthus ilicifolius* (AI) was restricted to the upper intertidal zone (UIZ) which was characterized by relatively lower sediment temperatures, higher porewater salinity and intense reducing conditions. A zonation pattern in occurrence of DNT was also observed. Denitrification activity generally decreased with depth and increased in intensity from the seaward to the landward side. Maximum DNT of  $4.06 \pm 0.44$  nmol N<sub>2</sub>O g<sup>-1</sup> h<sup>-1</sup> was recorded in the surficial AI sediments followed by AM and RM sediments. Surface plant litter content also increased from the lower to UIZ. A negative relationship of NH<sub>4</sub><sup>+</sup> and total organic carbon content (n = 15, r = -0.388, p < 0.05) in AI sediments was observed. This suggested that re-mineralization of accumulated organic matter (up to  $3.07 \pm 0.58$  % at 0-2 cm) enhanced NH<sub>4</sub><sup>+</sup> availability in the porewater which in turn could support nitrification-denitrification. Thus, degradation of vegetation-derived organic matter was important in sustaining nutrients mainly towards the landward side proving our hypothesis that vegetation could have an influence on DNT, albeit indirectly.

**Keywords:** Denitrification; Intertidal; Mangroves; Nitrate; Sediments; Vegetation

## 1. Introduction

Research on biogeochemical cycles in coastal environments has intensified following evidence that wetlands have a strong impact on human activities and on water quality. One such ecologically important coastal ecosystem is mangroves which occupy ~ 14 million hectares in tropical and subtropical regions of the world (Giri et al., 2011). They provide a range of ecosystem services like soil formation, wood production, fish spawning grounds, carbon (C) storage and nutrient cycling (Murdiyarso et al., 2015). However, over the past few decades, there has been an alarming loss (1 to 2%  $y^{-1}$ ) of these valuable ecosystems globally (Duke et al., 2007). This loss has been attributed to human related activities viz., land competition for aquaculture, agriculture, infrastructure, coastal landfill, tourism (FAO, 2007), overexploitation for timber, fish, crustaceans, shellfish, alteration of hydrology, pollution and global warming (Alongi, 2002). Duke et al. (2007) predict that any further decline in mangrove area is likely to be followed by accelerated functional losses.

The occurrence of biogeochemically important microbially-mediated processes like denitrification (Chiu et al., 1996; Yu et al., 2008) and anammox in mangrove sediments contribute to eutrophication control by mitigating excess inorganic nutrients (Fernandes et al., 2012). Mangrove sediments are largely anaerobic and  $NO_3^-$  availability is a major factor controlling denitrification activity (DNT) (Fernandes and LokaBharathi, 2011). Some other environmental parameters known to influence DNT include variation in oxygen concentrations (Bonin and Raymond, 1990), quantity and quality of organic matter (Bonin et al., 1999; McCarty and Bremner, 2003; Hill and Cardaci, 2004; Qin et al., 2005; Welti et al., 2012) sediment temperature (Holtan-Hartwig et al., 2002; El-Sayed, 2003), sediment pH (Rust et al., 2000; Simek et al., 2002), bioturbation (Gilbert et al., 1995) and trace metal content (Labbé et al., 2003; Fernandes et al., 2013). So far, little information is available on the influence of vegetation-associated sediment characteristics on N removal processes like denitrification. Some studies have indicated that community type, vegetation cover, biomass, and plant invasion are significantly correlated with DNT (Bachand and Horne, 2000; Evans et al., 2001; Hernandez and Mitsch, 2007; Yang et al., 2007). Planting of high-productivity species has been suggested as an economic way to increase organic matter in sediments which in turn will enhance denitrification rates in wetlands (Liu et al., 2011).

To maintain good water quality in the estuarine system, it is important to protect and restore fringing mangrove habitats. As mangrove sediments have the potential to eliminate excess nutrients from the aquatic system (Corredor and Morell, 1994), it was hypothesized that changes in sediment chemistry associated with vegetation type could play an important role in influencing DNT. Hence, this study aimed to elucidate (i) the composition of mangroves in the intertidal region of a mangrove ecosystem

(ii) assess the down-core variation in sediment physico-chemical characteristics and DNT associated with dominant vegetation types and (iii) understand the influence of environmental parameters associated with dominant mangrove vegetation on benthic denitrification. These findings would help to generate awareness on the link between mangrove vegetation and N removal potential in estuarine sediments. It would also contribute to the efforts in planning vegetation afforestation / restoration of mangroves along intertidal zones.

## **2. Materials and methods**

### *2.1. Study area*

Major mangrove zones (> 90%) in Goa extend along swampy banks of two economically important rivers - Mandovi and Zuari (including the connecting Cumbarjua canal) (Jagtap, 1985; Jagtap et al., 1993). These sensitive and fragile biodiversity hotspots house a diverse community of mangroves and are a rich repository of other associated faunal species. In recent years, Goa has made rapid strides in the field of industrialisation and tourism. Undoubtedly, this has led to urbanisation mostly in the coastal zones. As a result, mangroves now face a number of threats, including de-forestation, elevated anthropogenic inputs, etc. Our study area was located along the mangrove-fringed island of Divar (Fig. 1a) which is separated from the mainland by the Mandovi. This mangrove ecosystem is subjected to anthropogenic influences arising from mining activities upstream of the Mandovi (De Sousa, 1999) and nutrient input from domestic sewage discharge (Ansari et al., 1986). Previous studies have shown relatively higher DNT at the anthropogenically-influenced Divar mangrove ecosystem as compared to other relatively pristine areas (Fernandes et al., 2012; Fernandes et al., 2013). The mangrove fringed intertidal region at Divar was selected to understand the influence of vegetation-associated sediment characteristics on DNT. Sampling locations (Fig. 1b) were chosen nearby to the mangrove zone that was recently cleared for ecotourism activities. The study area is influenced by mixed semi-diurnal tides and the tidal ranges are ~ 2.3 and 1.5 m during the spring and neap tides respectively (Manoj and Unnikrishnan, 2009). During high tide the intertidal region is inundated with seawater while at low tide it gets exposed.

### *2.2. Mangrove community composition*

Studies on distribution and composition of mangroves were carried out according to Jagtap (1985). Six study locations were demarcated (Table S1) which extended perpendicularly from the landward to seaward side (Fig. 1b). Quadrats (10 × 10 m) were laid out at 10 m intervals along each location. Number of quadrats at each location varied depending upon the extent of the intertidal expanse (~ 15 to 50 m). Based on the width of each location (land to estuary), they were further sub-divided as low,

mid and high tide zones to enable grouping of mangrove species as per their zonation pattern. Trees larger than 2.5 cm in diameter (at 10 cm above the root collar for genus *Rhizophora* and at breast height for species without prop roots) were recorded in each plot to assess the number of individuals of plant species by actual count. Relative abundance of mangrove trees was calculated as:

$$\text{Relative abundance} = (\text{No. of individuals of species} / \text{Total individuals of all species}) \times 100$$

### 2.3. Sampling

Based on abundance of mangrove species in various zones of the intertidal region (Table 1), three sampling sites were selected for sediment core retrieval as follows:

Site 1: located in the lower intertidal zone (LIZ) of location 3 for *Rhizophora mucronata* (RM) associated sediments.

Site 2: located in the mid intertidal zone (MIZ) of location 3 for *Avicennia marina* (AM) associated sediments.

Site 3: located in the upper intertidal zone (UIZ) of location 5 for *Acanthus ilicifolius* (AI) associated sediments.

Sediment sampling was carried out at the selected sites in close proximity to the plant species at low tide during April, 2014 (pre-monsoon season). Hand-held push cores (7.5 cm inner diameter, 20 cm length; 12 sediment cores per site) were used to retrieve sediment samples which were used as follows:

(i) Three sediment cores were collected for physical measurements which were capped at both ends after retrieval. They were transferred to an insulated box for transportation to the laboratory. Physical parameters were measured on arrival to the laboratory within 1 h of sampling.

(ii) Nine cores were collected for chemical, microbiological analyses and microbial activity measurements. These were transported to the laboratory in an ice box. On arrival, the cores were maintained at 4°C until analysis and analysed < 48 h of retrieval. Seawater from the sampling sites was collected in clean, well-rinsed carbuoys and used for media preparation during microbiological analyses.

### 2.4. Physico-chemical analyses

Sediment cores were marked at 2 cm intervals to obtain representative sample readings at 0–2, 2–4, 4–6, 6–8 and 8–10 cm. Sediment temperature was measured using a digital hand held probe (Fisher Scientific UK Ltd.). Hydrogen ion concentration (pH) and oxidation- reduction potential (Eh) was

measured using a HACH-ISFET pH meter (Hach Company, Loveland, CO, USA). The core was then sectioned and ~ 0.5 g sub-samples (triplicates) were centrifuged at 8000 rpm for 10 min at 20 °C (centrifuge model: 3-18 K; Sigma Laborzentrifugen GmbH, Osterode, Germany). Salinity of the extracted porewater was then measured using a hand-held refractometer (Model: S/Mill-E, ATAGO, Shimadzu Corporation, Japan).

For porewater nutrient analyses, sediment sub-samples were taken at every 2 cm intervals from surface to 10 cm by carefully sectioning the cores. 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 (centrifuge model: CPR-24; Remi Instrument Ltd., India). 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, NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> were measured colorimetrically (Model: UV Mini-1240 UV/VIS spectrophotometer; Shimadzu Corporation; precision: ± 0.01 µmol L<sup>-1</sup>) as described by Koroleff (1970), Bendschneider and Robinson (1952) and Wood et al. (1967) respectively. The sediment used in the extractions was filtered through pre-weighed laboratory grade filter papers and dried in a hot air oven at 60 ± 2 °C for ~ 48 h. After drying, the filter papers were weighed to assess the dry weight of the sediment used in the extraction.

For total organic carbon (TOC) analysis, triplicate sediment samples from each section of the core were dried in a hot air oven at 60 ± 2 °C for 48 h. A colorimetric method was adopted for the determination of TOC content as described by Azam and Sajjad (2005). Briefly, the method involves addition of conc. H<sub>2</sub>SO<sub>4</sub> and 2N K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> to the sediment samples, heating the treated samples in a microwave oven equipped with a rotating plate, dilution of the contents with distilled water and determination of optical density of the samples at 590 nm. Glucose solution (5 mg C mL<sup>-1</sup>) was used as standard.

Earlier studies in a mangrove system along the Mandovi-Zuari estuarine complex have shown that litter yield for four mangrove species viz., *Rhizophora apiculata*, *R. mucronata*, *Sonneratia alba* and *Avicennia officinalis* can vary from 2.79 – 4.65 g m<sup>-2</sup> d<sup>-1</sup> (Wafar et al., 1997). In this study, we focused on collection of plant litter at the sediment surface at the time of sampling as its subsequent degradation would play a crucial role in influencing the chemistry of the benthic environment and heterotrophic denitrification. Therefore, surface plant litter content was collected by demarcating three 1 m × 1 m plots at the site of core collection. The collected material from each plot was dried to constant weight at 80 °C before weighing.

## 2.5. Bacteriological studies

Sub-samples of ~ 5 g wet weight sediment from each section of the core were extruded using syringe cores. These sub-samples were transferred to 45 mL of filter sterilized seawater ( $10^{-1}$  dilution). Tween 80 (50  $\mu$ L) 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 prokaryotic cells (TPC) by epifluorescence microscopy. An aliquot of 5 mL sample from  $10^{-2}$  dilution was fixed using 250  $\mu$ L of buffered formalin (2% final concentration). Two millilitres of sub-sample was filtered over a 0.2  $\mu$ m black Isopore polycarbonate filter paper (Millipore, Darmstadt, Germany) and stained with acridine orange (final concentration 0.01% w/v). The sample was then incubated for 2 min and then filtered. Microbial cells retained on the filter paper were counted using an epifluorescence microscope (Nikon 50i microscope; Nikon Instruments Inc., Melville, NY, USA) equipped with a 100X oil immersion objective. Cells were counted from 10-100 microscopic fields and the TPC values were expressed as number of cells  $g^{-1}$  (dry sediment).

To enumerate total heterotrophic bacteria (THB), petri plates containing nutrient agar (NA) medium were prepared using seawater 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  $\mu$ L inoculum from  $10^{-2}$  dilution was used to spread on to the NA plates. The plates were then incubated at room temperature  $28 \pm 2$  °C for 7 days. Bacterial counts were taken as colony forming units (CFU)  $g^{-1}$  (dry sediment). Initial weight of the sediment used in the dilutions was determined by drying the filtered sediment at  $60 \pm 2$  °C for ~ 48 h.

Denitrifiers were enumerated by the  $N_2O$  based-most probable number (MPN) technique as described by Michotey et al. (2000) and Fernandes et al. (2013). The culture medium was prepared using substrates suggested by Michotey et al. (2000) 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). Positive tubes were scored based on an increase in  $N_2O$  content in the culture vials. The MPN was assessed from McCready's table (Rodina, 1972) and denitrifier counts have been expressed as MPN cells  $g^{-1}$  (dry sediment).

## 2.6. Microbial activity

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 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<sup>3</sup> 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 µmol NO<sub>3</sub><sup>-</sup>-N L<sup>-1</sup>) was added to the sediment. No additional carbon or NO<sub>3</sub><sup>-</sup> 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<sub>2</sub> 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<sub>2</sub>O to N<sub>2</sub>. 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, microbial activity in all incubations was terminated using HgCl<sub>2</sub> at a final concentration of 10 mmol L<sup>-1</sup>.

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<sub>2</sub>O over time and DNT has been expressed as nmol N<sub>2</sub>O g<sup>-1</sup> h<sup>-1</sup> of dry sediment.

### 2.7. Statistical analyses

The Primer (Plymouth Routines in Multivariate Ecological Research) software (version 6) was used for calculating various univariate biodiversity measures (for mangroves) such as Margalef's index (d) for species richness, Shannon-Weiner index (H'; commonly used to characterize species diversity in a community providing important information about rarity and commonness of species) and the Pielou's evenness index (J'; a measure of how evenly the individuals are distributed among the different species).

Bacterial numbers were log<sub>10</sub> transformed before analysis. One-way analysis of variance (ANOVA) test was performed in *Statistica* software (*STATISTICA* version 6, StatSoft, Inc. 2001) to find out significant difference in benthic DNT associated with dominant mangrove types. For significant ANOVA results, Tukey's *post-hoc* test was used to examine the difference in the means. Pearson's

correlation coefficients were also used to assess one-to-one relationship between microbial activity and/or environmental parameters. Only significant relationships have been discussed.

To understand the combined influence of abiotic and biotic parameters on DNT, principal component analysis (PCA) was performed on the pooled dataset using the XLSTAT version 2015.1.03.15440 (Addinsoft, France). The results of the ordination analyses were visualized as a bi-plot (Cattell, 1966; Gabriel, 1971).

### 3. Results

#### 3.1. Mangrove abundance and diversity

*R. mucronata* and *A. marina* occurred in all three zones of the inter-tidal region (Table 1). However, *R. mucronata* was most abundant in the LIZ (50%) whereas *A. marina* was maximum in the MIZ (42%) followed by *S. alba* (17%). *Acanthus ilicifolius* was mainly restricted to the UIZ (64%) and occurred along with *Avicennia spp.* Other mangrove species with sparse distribution recorded at the study locations were *Bruguiera cylindrica* and *R. apiculata*.

The highest number of mangrove species (up to 5) was recorded in the LIZ followed by UIZ and MIZ (Table 1). Species richness and evenness was maximum at location 5\_LIZ while the highest plant diversity was recorded at location 6\_LIZ (Table 2).

#### 3.2. Physico-chemical parameters

Sediment temperature generally ranged between  $28.00 \pm 0.10$  to  $29.00 \pm 0.66^\circ\text{C}$  except at 8-10 cm in AI associated sediments where  $26.77 \pm 0.40^\circ\text{C}$  was recorded at 8-10 cm (Fig. 2a; Table S2). The sediments were acidic in nature with pH values varying between  $5.94 \pm 0.05$  at 8-10 cm in AI sediments to  $6.83 \pm 0.10$  (Fig. 2b; Table S2) at 0-2 cm in AM sediments. They were also largely anaerobic with lowest Eh value of  $-1.67 \pm 4.04$  mV in the AI sediments (Fig. 2c; Table S2). In RM sediments, Eh varied between  $43.20 \pm 8.07$  to  $57.67 \pm 5.66$  mV. Salinity generally increased with depth (Fig. 2d; Table S2). In RM sediments, it varied from  $27.00 \pm 0.00$  to  $29.33 \pm 2.52$ . In AM sediments, maximum of  $33.00 \pm 1.73$  was recorded at 8-10 cm while in the AI dominant zone, the maximum was  $34.33 \pm 0.58$  at the same depth.

Ammonium concentration showed similar down-core profile for sediments dominated by RM and AM with high values at the surface followed by a sub-surface minimum at 4-6 cm (Fig. 3a). In AI sediments, high  $\text{NH}_4^+$  content of up to  $32.52 \pm 0.99 \mu\text{mol L}^{-1}$  was recorded within the first 4 cm (Fig. 3a; Table S2). Interestingly, the deeper layer (8-10 cm) also showed the high  $\text{NH}_4^+$  content of  $32.75 \pm 0.81 \mu\text{mol L}^{-1}$  in these sediments. Nitrite concentration showed high variation between the



sediments associated with the three mangrove species. The least concentration of  $1.10 \pm 2.32 \mu\text{mol NO}_2\text{-N L}^{-1}$  (Fig. 3b; Table S2) was recorded at 8-10 cm in AI sediments while the highest was  $9.30 \pm 0.08 \mu\text{mol L}^{-1}$  at 4-6 cm for RM sediments. The AI and RM associated sediments showed similar down-core trend for  $\text{NO}_3^-$  content with values varying between 9.55 and  $14.95 \pm 0.00 \mu\text{mol L}^{-1}$  (Fig. 3c; Table S2). A negative relationship between DNT and  $\text{NO}_3^-$  concentration was observed in the RM sediments ( $n = 15$ ,  $r = -0.391$ ,  $p < 0.05$ ; Table S3). In AM sediments,  $\text{NO}_3^-$  concentration was far greater reaching up to  $27.94 \pm 0.62 \mu\text{mol NO}_3\text{-N L}^{-1}$  at 2-4 cm.

TOC content differed in sediments associated with all the 3 plant species (Fig. 3d; Table S2). Surficial AI sediments had the highest TOC content of  $3.07 \pm 0.58 \%$ . A negative relationship of  $\text{NH}_4^+$  and TOC ( $r = -0.388$ ,  $p < 0.05$ ; Table S3) was also observed.

### 3.3. Microbial abundance

Total prokaryotic cell abundance in AI sediments was one order higher ( $10^9 \text{ cells g}^{-1}$ ) as compared to the RM and AM sediments (Fig. 4a; Table S4). A sub-surface (4-6 cm) maxima in cell number was also observed in AM and AI sediments. In RM sediments, maximum TPC abundance was found even deeper i.e. at 6-8 cm. Heterotrophic abundance generally decreased with depth and varied between  $10^{3-4} \text{ CFU g}^{-1}$  in the mangrove sediments (Fig. 4b; Table S4). The highest THB counts of  $5.22 \pm 2.09 \times 10^4 \text{ CFU g}^{-1}$  were recorded in the surficial AM sediments. The denitrifier abundance varied with depth and numbered between  $10^{4-8} \text{ MPN cells g}^{-1}$ . Maximum denitrifier abundance of  $3.64 \times 10^9 \text{ MPN cells g}^{-1}$  was recorded in the surficial AI sediments (Fig. 4c).

### 3.4. Microbial activity and influencing factors

At all three sampling sites, maximum DNT was recorded in the surficial sediments (Fig. 5). Denitrification rates in surficial AI sediments were about 2 times higher ( $4.06 \pm 0.44 \text{ nmol N}_2\text{O g}^{-1} \text{ h}^{-1}$ ) as compared to RM and AM sediments (Fig. 5). One-way ANOVA also showed significant differences in benthic DNT between the three plant communities ( $df = 2$ ;  $p < 0.000003$ ). The significant effect was further examined by Tukey's HSD *post hoc* test, which showed appreciably high values of denitrifying activity in AI sediments ( $P < 0.0001$ ). Denitrifiers ( $r = 0.649$ ,  $p < 0.01$ ; Table S3) strongly influenced variation of DNT in AI sediments.

Principal component analysis was applied to the matrix of correlation coefficients between biotic and abiotic parameters to understand the combined influence of environmental parameters on DNT. The first three factors (eigenvalues  $> 1$ ) corresponded to 72.8% of total data variability (Table 3). The first component (PC 1) explained 36.1% of total data variability. Sediment Eh was positively correlated to the axis while total prokaryotic cell counts, denitrifier and heterotrophic abundance,

total organic carbon content and porewater  $\text{NH}_4^+$  concentration were negatively correlated. The sampling points in the AI sediments clustered along with most of the aforementioned parameters (Fig. 6). The second component (PC 2) explained 23.6% of data variability. Nitrite, pH and  $\text{NO}_3^-$  concentrations were major factors influencing denitrification rates mainly in AM sediments. The most significant variables in the third component (PC 3) were salinity and temperature which explained 13.1% of the variation.

#### 4. Discussion

About 13 mangrove species have been recorded in the Mandovi-Zuari estuarine complex (Misra et al., 2015) of which about 7 species were recorded along the sampled area in the present study. These comprised of *R. mucronata*, *R. apiculata*, *A. officinalis*, *A. marina*, *S. alba*, *Bruguiera gymnorhiza* and *A. ilicifolius*. A distinct zonation pattern in the distribution of mangroves was also observed with the dominance of *R. mucronata* in the LIZ, *A. marina* in the MIZ and *A. ilicifolius* in the UIZ (Table 1). Tidal elevation, ambient salinity (Satyanarayana et al., 2002), sediment redox potential (Matthijs et al., 1999) and inundation periods (Lewis, 2005) are important factors affecting the zonation pattern of mangroves. *Rhizophora* spp. are common on the seaward side and have extensive prop roots to withstand wave action along the tidal channel. Their habitat is characterized by low sediment salinity, silt and pH values as compared to those of *A. marina* that grow in more saline sediments (Ahmed and Abdel-Hamid, 2007). Measurements of physico-chemical variables in the present study have also revealed low pH (Fig. 2b) and salinity (Fig. 2d) values in RM sediments. Further, we observed relatively higher sediment redox potential (Fig. 2c) and lower  $\text{NO}_3^-$  (Fig. 3c) content in these sediments as compared to other zones in the intertidal region. Generally, RM sediments are sandy in nature (Ahmed and Abdel-Hamid, 2007; Fernandes et al., 2012). These types of sediments promote permeability of ambient seawater thereby influencing nutrient availability. Our findings are in congruence with earlier studies by Middleburg et al. (1996) who have shown low  $\text{NO}_3^-$  concentrations in RM sediments. Low porewater nutrient concentrations probably reflect nutrient uptake by mangroves/microbes. At higher elevation, mangrove biomass and number of roots per unit surface decrease which results in lower nutrient uptake rates by plants consequently increasing concentration of  $\text{NO}_3^-$  (Middleburg et al., 1996). This trend has also been observed in the present study. Clay content in mangrove systems too has been found to increase with distance from the sea edge (Sukardjo, 1994). Clayey soils possess higher nutrient content than sandy ones (Prema et al., 2013). A large quantity of  $\text{NH}_4^+$  is also known to get adsorbed on to clay particles (Mackin and Aller, 1984). Transformation of the nutrient via microbially-mediated anaerobic nitrification could result in elevated  $\text{NO}_3^-$  content in AM and AI sediments. In the UIZ, salinity values were relatively

higher than in the LIZ and MIZ which favour the proliferation of *A. ilicifolius* that is known to tolerate salinity above 25 (Yong et al., 2004). *Acanthus ilicifolius* has a short rooting time and their diaspores are floating type, which are known to be more abundant in the backshore locations (Yong et al., 2004).

Sediment quality is an important factor affecting the distribution of mangroves in the intertidal region. However, microbial communities are influenced by multiple environmental factors, some of which are directly or indirectly affected by plants due to their role in changing sediment characteristics. Therefore, understanding relationship between plant communities, ecosystem functioning and other associated environmental variables is crucial for appreciating and predicting the impact of vegetation type on ecosystem processes. Locations chosen for detailed vegetation-microbe interaction studies in the present study had a fairly rich, diverse and even distribution of mangroves (Table 2; Fig. 1b). Previous studies in wetlands have shown that plant community composition rather than diversity has a significant effect in regulating denitrification (Liu et al., 2011). In this study, we examined DNT and other environmental factors likely to influence the process in sediments of three dominant mangrove species from Divar. Interestingly, our study has revealed a zonation pattern in DNT as well which is linked to changes in sediment characteristics in different zones of the intertidal region. Previous DNT values from RM sediments (Fernandes et al., 2013) are comparable to those in the present study. Highest DNT of up to  $4.06 \pm 0.44 \text{ nmol N}_2\text{O g}^{-1} \text{ h}^{-1}$  (Fig. 5) was recorded in *A. ilicifolius* sediments. The *A. ilicifolius* dominated UIZ is characterized by intense reducing conditions (Fig. 2c), higher porewater  $\text{NH}_4^+$  (Fig. 3a) and organic carbon content (Fig. 3d). Surface plant litter content recorded in the present study showed a decreasing trend from the landward to the seaward side i.e. AI > AM > RM (Table S5). Plant material like leaves/twigs are major contributors of organic matter in mangrove systems. Lower surface plant litter content in the RM sediments (Table S5) indicate flushing of organic material into the adjoining estuary. *A. ilicifolius* is widespread towards the landward side where removal of plant material due to wave action is likely to be relatively lesser. Though *A. ilicifolius* leaves have a higher protein content as compared to those of *R. apiculata* (Basak et al., 1999), studies by Herbon and Nordhaus (2013) have revealed that leaf eating crabs in mangrove forests prefer to consume leaves of *Rhizophora* sp. to meet their N demand. The consumption of *A. ilicifolius* leaves was least as compared to those of other mangrove species (Herbon and Nordhaus, 2013). Leaves of *A. ilicifolius* have about 33% more tannin content as compared to *R. apiculata* (Basak et al., 1999). Ravichandran et al. (2006) have shown that the leaf-eating mangrove crab - *Neosarmartum smithi* stores leaves in burrows indicating that tannins are left to leach before consumption. The loss of polyphenols in aged

litter (~ 6 weeks old) increases its palatability and is then consumed. Visual examination of the Divar sediments have revealed a large number of fiddler crabs belonging to the genus *Uca* surfacing out from burrows during low tide. These crabs comprise one of the dominant macro benthic communities in this ecosystem. The selective feeding habits of benthic organisms could contribute to an accumulation of plant material in the UIZ. Thus, re-mineralization of accumulated organic material in this zone could lead to generation of  $\text{NH}_4^+$ , a substrate for nitrification. A negative relationship of  $\text{NH}_4^+$  and TOC observed in the present study could imply production of the nutrient through organic matter degradation in AI sediments. Krishnan and LokaBharathi (2009) have demonstrated the occurrence of anaerobic nitrification in the Divar sediments. Further, nitrification is strongly coupled to denitrification in mangrove sediments (Fernandes et al., 2012). Re-mineralization of accumulated organic matter in AI sediments could contribute to  $\text{NH}_4^+$  generation which in turn could support anaerobically-mediated nitrification-denitrification.

Principal component analysis elucidated the collective influence of abiotic and biotic components on DNT in sediments associated with dominant mangrove types. A visual examination of PCA plots clearly showed the clustering of sampling points from AI sediments along with denitrifier abundance,  $\text{NH}_4^+$  and organic carbon content (Fig. 6). This observation further re-affirms that re-mineralization of organic matter in AI sediments results in generation of  $\text{NH}_4^+$  which in turn supports the occurrence of anaerobic nitrification-denitrification. A significant negative relationship of DNT and  $\text{NO}_3^-$  in RM sediments was also observed in the present study (Table S3) suggesting transformation of the nutrient via the reductive pathway. This relationship weakened in the AM sediments and furthermore in AI sediments. In LIZ,  $\text{NO}_3^-$  could originate either intrinsically or get replenished from the ambient seawater. Earlier observations carried out in the LIZ (RM sediments) have also revealed that DNT is mainly dependent on  $\text{NO}_3^-$  content (Fernandes et al., 2012). However, observations in the present study suggest that DNT in the UIZ could be largely dependent on intrinsically produced  $\text{NO}_3^-$ . Thus, high DNT in AI sediments could contribute in modulating the level of nutrients.

## 5. Conclusion

The present study highlights the significance of fringing mangrove vegetation and their influence in supporting an ecologically important function such as denitrification. A distinct zonation pattern of mangroves in the intertidal region was observed. Likewise, a discrete zonation pattern in the occurrence of DNT was also observed which increased in intensity from the seaward to the landward side. These changes were caused by the variation in vegetation-associated sediment characteristics. Based on our earlier studies (Fernandes et al., 2012) and current observations, DNT in LIZ

dominated RM sediments was governed by porewater  $\text{NO}_3^-$  concentration. In the UIZ dominated AI sediments, accumulation and subsequent degradation of plant-derived organic material enhances  $\text{NH}_4^+$  content. The nutrient is further transformed (via anaerobic nitrification; Krishnan and LokaBharathi, 2009; Fernandes et al., 2012) and supports denitrification under intense reducing conditions. Thus, coupled nitrification-denitrification could be stronger in the AI sediments and serves in modulating nutrient levels. However, elevated rates of organic matter decomposition and DNT are likely to contribute to a greater production and subsequent flux of radiative gases like  $\text{CO}_2$  and  $\text{N}_2\text{O}$  respectively. Conversely, chlorophyll content and photosynthetic enzyme activity of *A. ilicifolius* is relatively higher as compared to *R. mucronata* (Joshi et al., 1984) which implies that they could fix much more atmospheric  $\text{CO}_2$  via photosynthesis. Recently, Misra et al. (2015) have raised concern over slow growth of mangroves in estuarine systems attributing it to increasing anthropogenic activities. Furthermore, the effects of global climate change e.g. increased frequency and severity of pulsed heavy rains are apparent (Nandargi and Mulye, 2014). In the near future, sea level changes could inundate larger tracts of the intertidal region affecting the distribution/zonation of mangrove vegetation and potentially nitrogen chemistry. Therefore, findings from the present study would be useful for guiding vegetation afforestation / restoration and enhancing  $\text{NO}_3^-$  removal mechanisms in estuarine systems.

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## Legend to figures

**Fig. 1.** Study area located at Divar island (★) along the river Mandovi (a) and locations (L1 to L6) for study of mangrove composition in intertidal region (b). Sediment samples for physico-chemical and microbiological analyses were retrieved from location 3 (L3; *R. mucronata* and *A. marina* associated sediments) and 5 (L5; *A. ilicifolius* associated sediments).

**Fig. 2.** Down-core variation in sediment temperature (a), pH (b), Eh (c) and salinity (d). Standard deviation (SD) values have been provided in Table S2.

**Fig. 3.** Down-core variation in porewater ammonium (a), nitrite (b), nitrate (c) and total organic carbon (d). Standard deviation values have been provided in Table S2.

**Fig. 4.** Down-core variation in the abundance of total prokaryotic cells (a), heterotrophic bacteria (b) and denitrifiers (c) at Divar. Due to large variation, denitrifier abundance has been presented using a log scale. Standard deviation values have been provided in Table S4.

**Fig. 5.** Down-core variation of denitrifying activity ( $\pm$  SD) in sediments of dominant mangrove vegetation.

**Fig. 6.** Principal component analysis (PCA) plots (first and second component) of environmental variables and microbial activity (a) and sampling points (b) in the Divar mangrove sediments. Variables Temp, temperature; TOC, total organic carbon; TPC, total prokaryotic cells; THB, total heterotrophic bacteria; DNT, denitrification activity. In the first plot, eigenvectors (projected parameters) point in the direction of maximum variation and the length of each parameter is equivalent to their rate of change in the dataset. Eigenvectors in opposite directions imply negative correlations while those in the same direction are positively correlated. Oblique angles between two vectors imply in a certain degree of relatedness between vectors whereas right angles between vectors show they are independent from one another (Couto de Brito, 2009).

**Table 1.** Abundance of mangroves (10 m × 10 m quadrats) in the intertidal region at Divar

	Location 1		Location 2		Location 3		Location 4		Location 5		Location 6		Relative abundance (%)		
	LIZ	UIZ	LIZ	UIZ	MIZ	LIZ	LIZ	UIZ	LIZ	LIZ	LIZ	LIZ	MIZ	UIZ	
<b>Mangrove species</b>															
<i>Avicennia officinalis</i>	24	5	0	2	0	0	1	3	2	2	24	0	14		
<i>Avicennia marina</i>	2	1	1	3	5	1	0	4	0	1	4	42	11		
<i>Acanthus ilicifolius</i>	0	0	0	0	0	0	2	47	0	6	7	0	64		
<i>Bruguiera cylindrical</i>	0	1	0	0	0	0	2	0	1	0	3	0	1		
<i>Rhizophora apiculata</i>	0	0	0	0	0	0	0	0	0	1	1	0	0		
<i>Rhizophora mucronata</i>	12	2	30	1	5	10	0	4	0	8	50	42	10		
<i>Sonneratia alba</i>	0	0	0	0	2	2	10	0	1	0	11	17	0		

Note: Classification of various zones in the intertidal region has been carried out based on the width of each location (landward to seaward side); LIZ = Lower intertidal zone; MIZ = Mid intertidal zone; UIZ = Upper intertidal zone; Relative abundance = (No. of individuals of species / Total individuals of all species) x 100

**Table 2.** Mangrove richness and diversity in the intertidal region at Divar

<b>Location</b>	<b>S</b>	<b>N</b>	<b>d</b>	<b>H'</b>	<b>J'</b>
Location 1_LIZ	3	38	0.550	0.809	0.737
Location 2_UIZ	4	9	1.365	1.149	0.829
Location 2_LIZ	2	31	0.291	0.143	0.206
Location 3_UIZ	3	6	1.116	1.011	0.921
Location 3_MIZ	3	12	0.805	1.028	0.936
Location 3_LIZ	3	13	0.780	0.687	0.625
Location 4_LIZ	4	15	1.108	0.988	0.713
Location 5_UIZ	4	58	0.739	0.693	0.500
Location 5_LIZ	3	4	1.443	1.040	0.946
Location 6_LIZ	5	18	1.384	1.292	0.803

Note:

S = number of species in each sample. i.e. species with non zero counts

N = The number of individuals in each sample

d = Margalef's species richness for each sample. It is a measure of the number of species present

$$d = (S-1)/\text{Log}(N)$$

H' = Shannon–Wiener diversity index

$$H' = - \sum_{i=1}^s p_i \ln p_i$$

where,

p = proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N)

ln = natural log

Σ = sum of the calculations

s = number of species

J' = Pielou's evenness index is measure of equitability. When there are similar proportions of all species then evenness is one, but when the abundance are very dissimilar (some rare and some common species) then the value increases.

**Table 3.** Principal component (PC) analysis of environmental variables and denitrification activity.

Variable	PC 1	PC 2	PC 3
Ammonium	<b>-0.537</b>	-0.536	-0.221
Denitrifiers	<b>-0.828</b>	-0.123	-0.254
DNT	<b>-0.564</b>	-0.300	-0.476
Eh	<b>0.938</b>	0.024	-0.203
Nitrate	-0.341	<b>0.755</b>	0.167
Nitrite	0.314	<b>0.792</b>	-0.127
pH	-0.321	<b>0.775</b>	-0.060
Salinity	-0.400	0.014	<b>0.758</b>
TPC	<b>-0.672</b>	0.499	0.233
Temperature	0.135	0.487	<b>-0.673</b>
THB	<b>-0.783</b>	0.305	-0.245
TOC	<b>-0.775</b>	-0.245	-0.038
Eigenvalue	4.334	2.829	1.572
Variability (%)	36.1	23.6	13.1
Cumulative %	36.1	59.7	72.8

**Note:** TOC, total organic carbon; TPC, total prokaryotic cells; THB, total heterotrophic bacteria; DNT, denitrification activity. The most significant variables in each component (at the 0.05 probability level) have been highlighted bold.

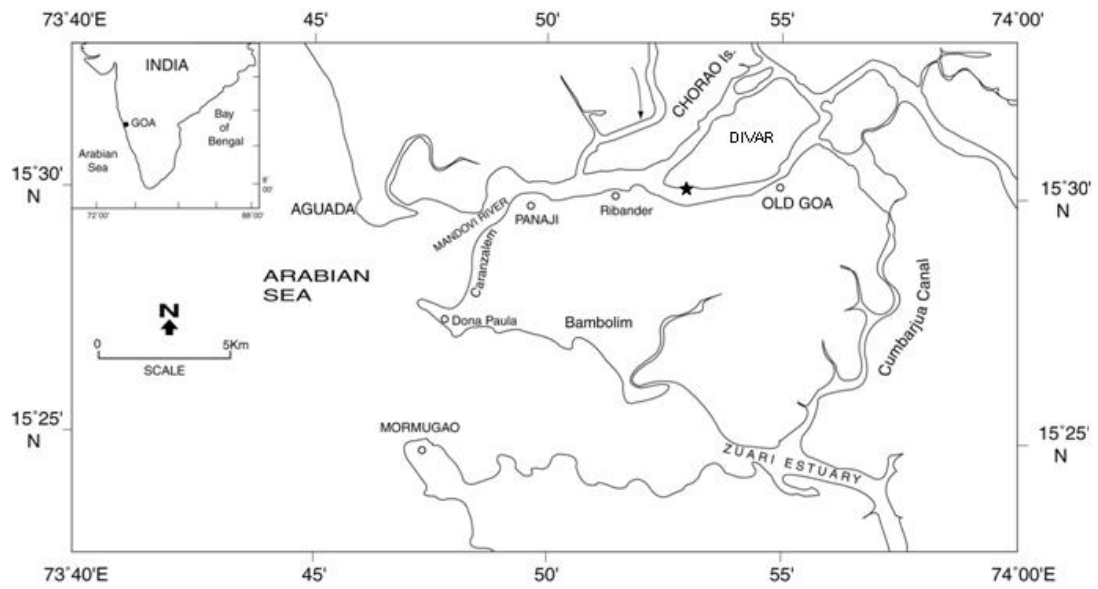


Fig. 1a



Fig. 1b

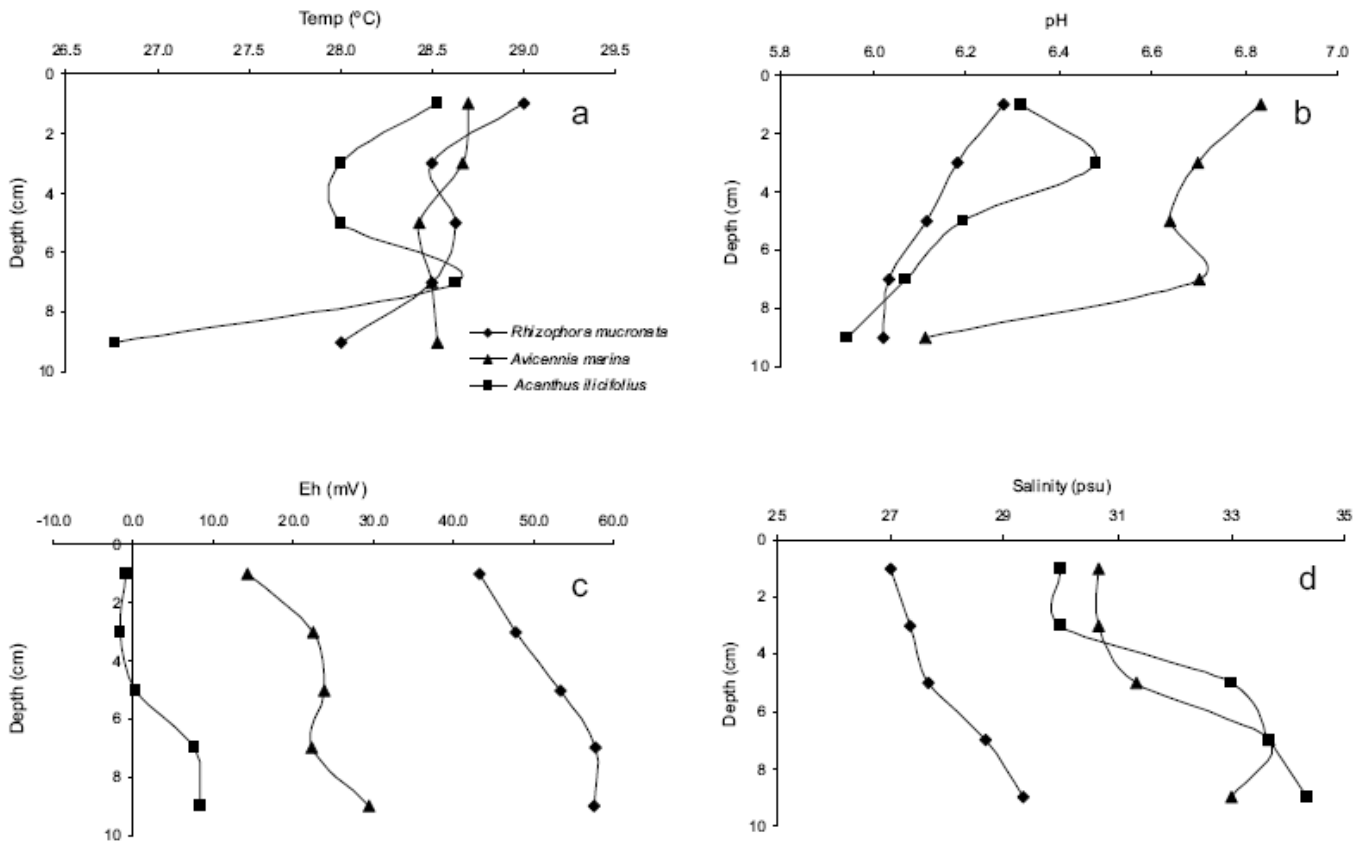


Fig. 2

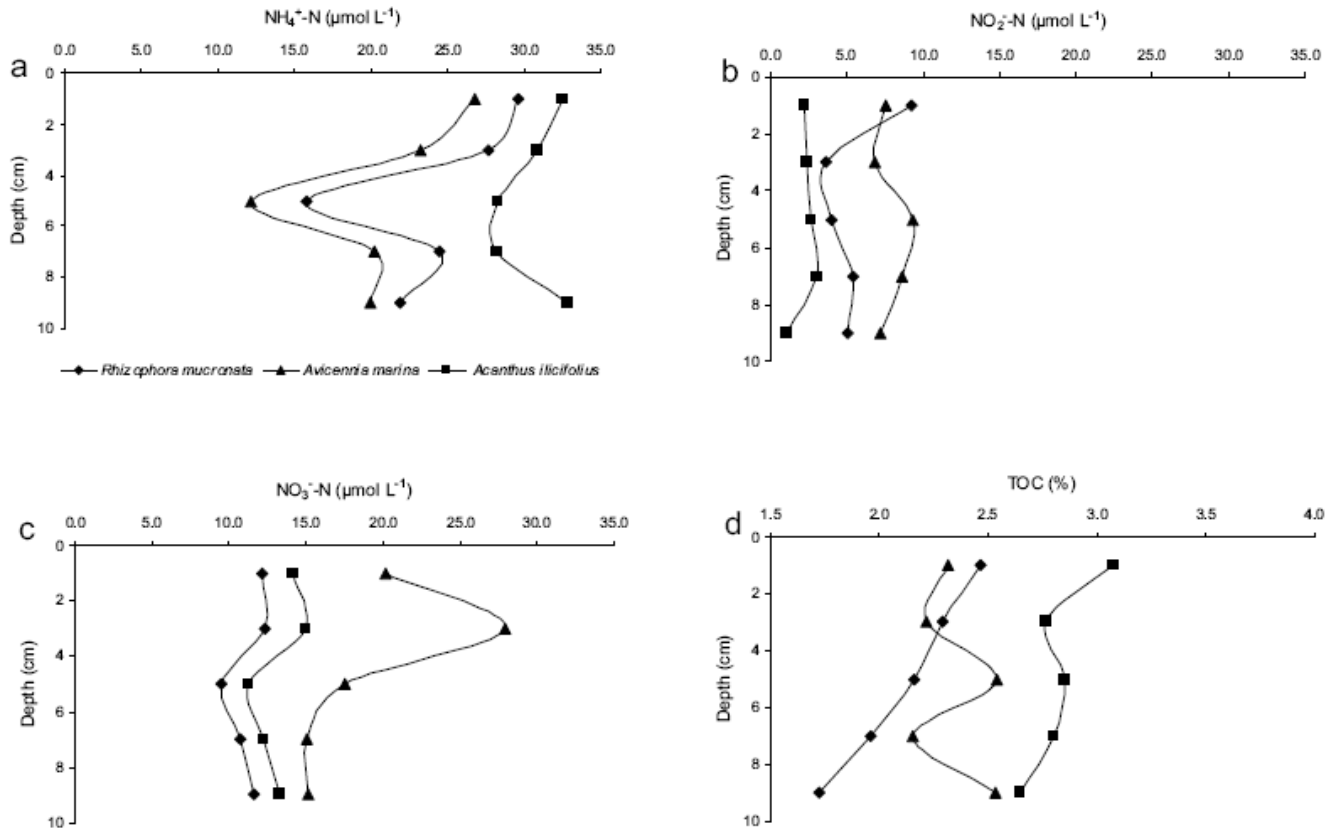


Fig. 3



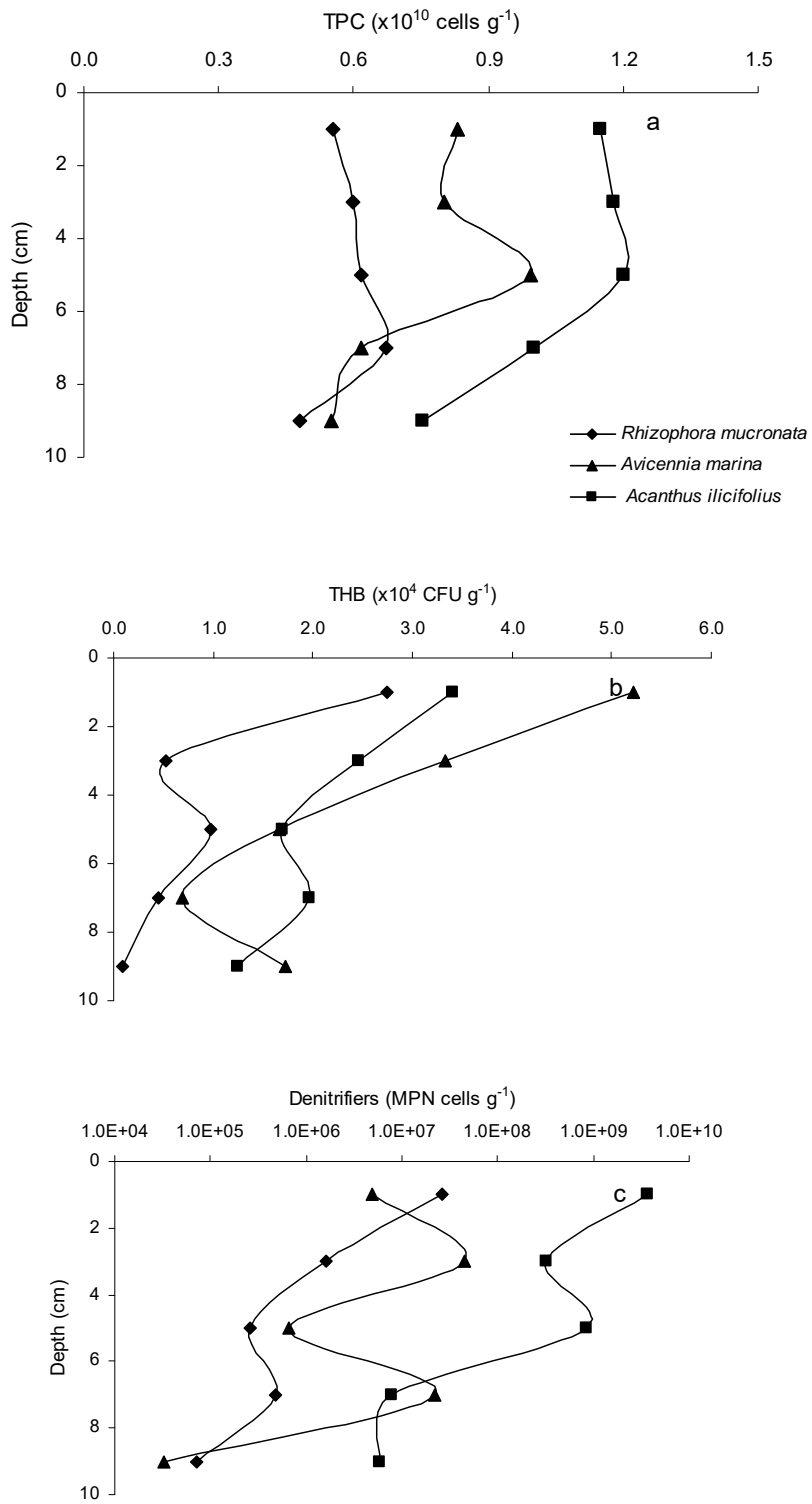


Fig. 4

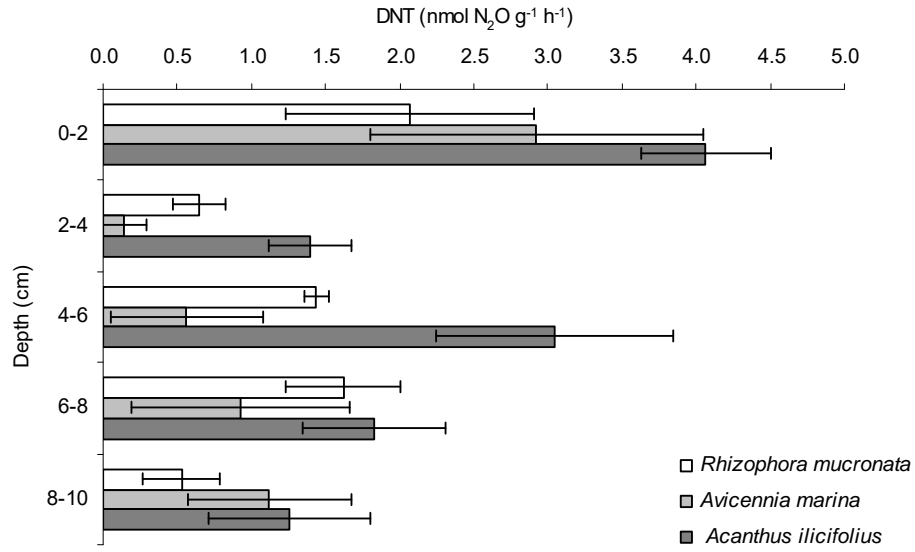


Fig. 5

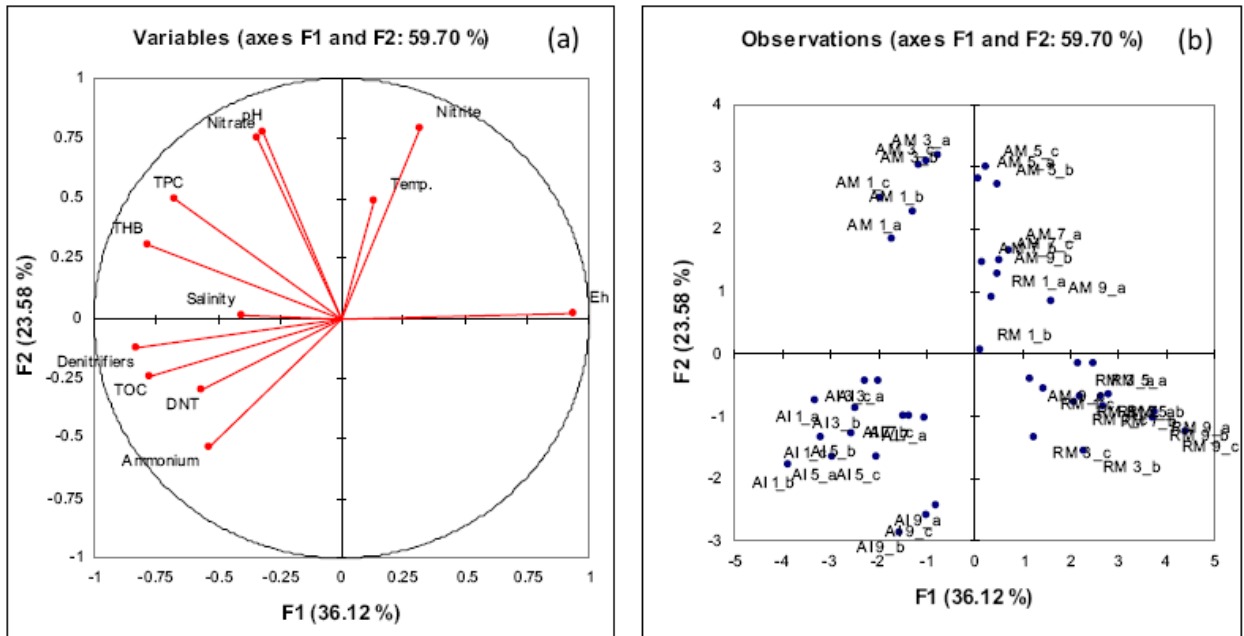


Fig. 6

## Supplementary information

**Table S1.** Study locations in the intertidal region at Divar

<b>Location (L)</b>	<b>Latitude (°N)</b>	<b>Longitude (°E)</b>
L1	15°30'19.03"N	73°52'45.91"E
L2	15°30'18.67"N	73°53'22.01"E
L3	15°30'26.75"N	73°54'26.71"E
L4	15°30'33.33"N	73°54'46.40"E
L5	15°30'35.64"N	73°54'49.29"E
L6	15°30'37.64"N	73°54'51.45"E

**Table S2.** Average and standard deviation values (n = 3) for physico-chemical parameters associated with various plant types.

Temp (°C)	pH	Eh	Salinity	NH <sub>4</sub> <sup>+</sup> (μmol L <sup>-1</sup> )	NO <sub>2</sub> <sup>-</sup> (μmol L <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> (μmol L <sup>-1</sup> )	TOC (%)
<i>Rhizophora mucronata</i>							
29.0 ± 0.66	6.28 ± 0.15	43.20 ± 8.07	27.00 ± 0.00	29.61 ± 1.40	9.26 ± 0.07	12.20 ± 0.00	2.47 ± 0.14
28.50 ± 0.85	6.18 ± 0.03	47.80 ± 1.57	27.33 ± 0.58	27.60 ± 3.23	3.66 ± 0.09	12.30 ± 0.09	2.29 ± 0.25
28.63 ± 0.50	6.12 ± 0.08	53.33 ± 4.08	27.67 ± 1.15	15.81 ± 0.52	3.99 ± 0.00	9.55 ± 0.00	2.16 ± 0.04
28.50 ± 0.53	6.03 ± 0.10	57.67 ± 5.66	28.67 ± 1.53	24.49 ± 1.27	5.41 ± 0.15	10.79 ± 0.25	1.96 ± 0.21
28.0 ± 0.10	6.02 ± 0.10	57.50 ± 5.43	29.33 ± 2.52	21.89 ± 2.98	5.03 ± 0.07	11.60 ± 0.15	1.72 ± 0.03
<i>Avicennia marina</i>							
28.70 ± 0.36	6.83 ± 0.10	14.27 ± 5.34	30.67 ± 0.58	26.79 ± 0.00	7.56 ± 0.22	20.19 ± 0.27	2.32 ± 0.05
28.67 ± 0.15	6.70 ± 0.08	22.40 ± 5.76	30.67 ± 1.15	23.20 ± 4.05	6.80 ± 0.00	27.94 ± 0.62	2.22 ± 0.06
28.43 ± 0.23	6.64 ± 0.08	23.87 ± 4.02	31.33 ± 1.15	12.11 ± 0.26	9.30 ± 0.08	17.51 ± 0.16	2.54 ± 0.22
28.50 ± 0.26	6.70 ± 0.05	22.30 ± 1.47	33.67 ± 1.15	20.23 ± 0.26	8.64 ± 0.09	15.05 ± 0.32	2.15 ± 0.06
28.53 ± 0.06	6.11 ± 0.73	29.43 ± 1.93	33.00 ± 1.73	19.90 ± 1.84	7.22 ± 0.00	15.12 ± 0.21	2.53 ± 0.19
<i>Acanthus ilicifolius</i>							
28.53 ± 0.68	6.32 ± 0.10	-1.00 ± 2.65	30.00 ± 1.00	32.49 ± 5.52	2.23 ± 0.35	14.24 ± 0.09	3.07 ± 0.58
28.0 ± 0.36	6.48 ± 0.05	-1.67 ± 4.04	30.00 ± 1.00	30.84 ± 1.74	2.39 ± 0.03	14.95 ± 0.00	2.77 ± 0.28
28.0 ± 0.10	6.19 ± 0.15	0.33 ± 4.73	33.00 ± 1.73	28.25 ± 3.05	2.67 ± 0.23	11.32 ± 0.00	2.85 ± 0.35
28.63 ± 0.50	6.07 ± 0.06	7.67 ± 0.58	33.67 ± 2.31	28.17 ± 1.95	3.01 ± 0.13	12.27 ± 0.06	2.81 ± 0.14
26.77 ± 0.40	5.94 ± 0.06	8.33 ± 0.58	34.33 ± 0.58	32.75 ± 0.81	1.10 ± 1.90	13.28 ± 0.06	2.65 ± 0.24

**Table S3.** Resulting *r* values of Pearson’s correlation analysis carried out between benthic denitrification activity (DNT) and environmental variables (n = 15) associated with 3 dominant mangrove types at Divar.

*R. mucronata*

	Depth	Temp.	pH	Eh	Salinity	NH <sub>4</sub> <sup>+</sup>	NO <sub>2</sub> <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	TOC	TPC	THB	Denitrifiers	DNT
Depth	1.000												
Temp.	-0.492	1.000											
pH	-0.751	0.507	1.000										
Eh	0.760	-0.467	-0.994	1.000									
Salinity	0.581	-0.540	-0.676	0.664	1.000								
NH <sub>4</sub> <sup>+</sup>	-0.511	0.098	0.478	-0.535	-0.294	1.000							
NO <sub>2</sub> <sup>-</sup>	-0.473	0.328	0.446	-0.436	-0.188	0.546	1.000						
NO <sub>3</sub> <sup>-</sup>	-0.371	-0.013	0.359	-0.433	-0.106	0.859	0.396	1.000					
TOC	-0.886	0.355	0.557	-0.576	-0.462	0.471	0.388	0.254	1.000				
TPC	0.033	0.041	0.003	-0.021	0.142	-0.060	-0.065	-0.198	0.099	1.000			
THB	-0.858	0.587	0.703	-0.672	-0.633	0.329	0.538	0.003	0.780	0.094	1.000		
Denitrifiers	-0.921	0.487	0.708	-0.717	-0.489	0.704	0.733	0.518	0.801	-0.028	0.817	1.000	
DNT	-0.320	0.324	0.038	0.001	-0.313	-0.093	-0.074	-0.391	0.313	0.040	0.480	0.229	1.000

*marina*

	Depth	Temp.	pH	Eh	Salinity	NH <sub>4</sub> <sup>+</sup>	NO <sub>2</sub> <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	TOC	TPC	THB	Denitrifiers	DNT
Depth	1												
Temp.	-0.32425	1											
pH	-0.55061	0.086161	1										
Eh	0.725569	-0.07747	-0.55098	1									
Salinity	0.683604	-0.24415	-0.00273	0.500068	1								
NH <sub>4</sub> <sup>+</sup>	-0.50825	0.424939	0.254485	-0.5271	-0.26439	1							
NO <sub>2</sub> <sup>-</sup>	0.163009	-0.3758	0.17241	-0.0324	0.200008	-0.71649	1						
NO <sub>3</sub> <sup>-</sup>	-0.68192	0.302957	0.304101	-0.28956	-0.60478	0.483339	-0.56632	1					
TOC	0.269531	-0.24145	-0.58292	0.371766	-0.18036	-0.51902	0.170706	-0.3202	1				
TPC	-0.74936	-0.09977	0.354617	-0.44004	-0.64795	-0.10715	0.187489	0.509359	0.185709	1			
THB	-0.72478	0.102055	0.022708	-0.49771	-0.76326	0.433344	-0.42412	0.594792	0.172961	0.637494	1		
Denitrifiers	-0.58	0.195799	0.565035	-0.49483	-0.21939	0.442089	-0.05559	0.597487	-0.74044	0.232715	0.171366	1	
DNT	-0.35831	0.273485	0.187368	-0.45947	-0.02983	0.422153	-0.09031	-0.19637	-0.07827	0.077373	0.242664	-0.09135	1

*A. ilicifolius*

	Depth	Temp.	pH	Eh	Salinity	NH <sub>4</sub> <sup>+</sup>	NO <sub>2</sub> <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	TOC	TPC	THB	Denitrifiers	DNT
Depth	1												
Temp.	-0.53984	1											
pH	-0.81457	0.344841	1										
Eh	0.789926	-0.33529	-0.72367	1									
Salinity	0.793911	-0.3499	-0.8042	0.743356	1								
NH <sub>4</sub> <sup>+</sup>	-0.09429	-0.37444	-0.00916	-0.04036	-0.38649	1							
NO <sub>2</sub> <sup>-</sup>	-0.24302	0.327495	0.305955	-0.10922	-0.13086	-0.36032	1						
NO <sub>3</sub> <sup>-</sup>	-0.49803	-0.01665	0.550107	-0.34499	-0.60728	0.428081	-0.22457	1					
TOC	-0.36404	0.39657	0.142988	-0.44102	-0.193	-0.38789	-0.06993	0.065356	1				
TPC	-0.70497	0.538494	0.650678	-0.61482	-0.45339	-0.06961	0.263615	0.04869	0.087463	1			
THB	-0.91144	0.614306	0.721271	-0.57547	-0.69774	0.057815	0.261495	0.53222	0.412257	0.611943	1		
Denitrifiers	-0.9132	0.392137	0.695061	-0.81084	-0.6579	0.054202	0.185227	0.231932	0.352312	0.702041	0.712667	1	
DNT	-0.62996	0.390004	0.323259	-0.43072	-0.41177	0.006342	0.26345	-0.10961	0.435664	0.369397	0.559122	0.736553	1

**Table S4.** Average and standard deviation values (n = 3) for total prokaryotic cells and heterotrophs associated with sediments of dominant mangrove plants.

Depth (cm)	TPC ( $\times 10^9$ cells g <sup>-1</sup> )	THB ( $\times 10^4$ CFU g <sup>-1</sup> )
<i>R. mucronata</i>		
0-2	5.54 $\pm$ 2.31	2.74 $\pm$ 0.66
2-4	5.98 $\pm$ 3.09	0.52 $\pm$ 0.14
4-6	6.17 $\pm$ 2.12	0.98 $\pm$ 0.36
6-8	6.74 $\pm$ 1.11	0.44 $\pm$ 0.09
8-10	4.79 $\pm$ 0.48	0.09 $\pm$ 0.04
<i>A. marina</i>		
0-2	8.31 $\pm$ 1.29	5.22 $\pm$ 2.09
2-4	8.02 $\pm$ 0.94	3.33 $\pm$ 0.42
4-6	9.95 $\pm$ 0.64	1.67 $\pm$ 0.60
6-8	6.18 $\pm$ 0.87	0.69 $\pm$ 0.25
8-10	5.49 $\pm$ 0.73	1.73 $\pm$ 1.35
<i>A. ilicifolius</i>		
0-2	11.5 $\pm$ 1.79	3.40 $\pm$ 0.19
2-4	11.8 $\pm$ 1.39	2.46 $\pm$ 0.25
4-6	12.0 $\pm$ 0.77	1.70 $\pm$ 0.14
6-8	10.0 $\pm$ 1.42	1.96 $\pm$ 0.27
8-10	7.52 $\pm$ 1.00	1.24 $\pm$ 0.06

**Table S5.** Average and standard deviation values (n = 3) for surface plant litter content in sediments of dominant mangrove vegetation.

Mangrove species	Surface plant litter content (g m <sup>-2</sup> )
<i>R. mucronata</i>	12.21 $\pm$ 4.39
<i>A. marina</i>	15.67 $\pm$ 5.41
<i>A. ilicifolius</i>	18.37 $\pm$ 9.17