

Denitrification: an important pathway for nitrous oxide production in tropical mangrove sediments (Goa, India)

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ABSTRACT

Net nitrous oxide production and denitrification activity were measured in two mangrove ecosystems of Goa, India. The relatively pristine site Tuvem was compared to Divar which is prone to high nutrient input. Stratified sampling at 2 cm intervals within the 0-10 cm depth range showed that N₂O production at both the locations decreased with depth. Elevated denitrification activity at Divar resulted in maximum production of up to 1.95 nmol N₂O-N g⁻¹ h⁻¹ at 2-4 cm which was 3 times higher than at Tuvem. Detailed investigations to understand the major pathway contributing to nitrous oxide production carried out at Tuvem showed that incomplete denitrification was responsible for up to 43-93% of N₂O production. N₂O production rates closely correlated to nitrite concentration (n=15; r=-0.47; p<0.05) and denitrifier abundance (r=0.55; p<0.05) suggesting that nitrite utilisation by microbial activity leads to N₂O production. Nitrous oxide production through nitrification was below detection affirming that denitrification is the major pathway responsible for production of the greenhouse gas. Net N₂O production in these mangrove systems are comparatively higher than those reported from other natural estuarine sediments and therefore warrant mitigation measures.

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Abbreviations: DNT, denitrification activity; ^DN₂O, incomplete denitrification; ^IN₂O, percentage of N₂O through incomplete denitrification; MPN, most probable number; ^NN₂O, nitrous oxide produced through nitrification; NPL, National Physical Laboratory; PCA, Principal component analysis; TOC, total organic carbon.

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Introduction

The marine environment is recognized as a net source of nitrous oxide (N₂O) to the atmosphere (Corredor et al., 1999). Estuaries and coastal regions account for approximately 60% of the total oceanic N₂O flux (Bange et al., 1996). The N₂O molecule is a precursor to compounds involved in the destruction of the stratospheric ozone layer (Yamagishi et al., 2007) which protects Earth from harmful ultraviolet radiation. Since ~1750 onwards, industrialisation has increased the global atmospheric N₂O concentration from ~270 to 319 μL L⁻¹ (IPCC, 2007). Though N₂O is responsible for 5–6% of the greenhouse effect (Houghton et al., 1996), its lifetime of about 114 years makes the global warming potential of this biogenic gas 298 times greater than that of CO₂ over a 100-year time frame (Forster et al., 2007). Nitrous oxide is produced as a by-product during several microbiological processes including nitrification, denitrification and dissimilatory nitrate reduction to ammonium (De Wilde and De Bie, 2000). However, denitrification and chemolithotrophic nitrification appear to be the main biological sources of N₂O emission in natural systems (Bremner and Blackmer, 1978; Firestone and Davidson, 1989; Bonin et al., 2002).

In ecosystems with high inputs of nitrogen (N) such as estuaries, denitrification mediates reduction of N loadings and therefore contributes to control of eutrophication (Nogales et al., 2002). Mangroves ecosystems constitute nearly 75% of tidal vegetation in tropical regions (Alongi et al., 1989) and they play an important role in the biogeochemical cycles of coastal and marine ecosystems (Thorsten and José, 2001). The N cycle within mangrove forests is mediated predominantly by microbial rather than chemical processes (Alongi et al., 1992). Inorganic N and other parameters important for N turnover can fluctuate widely due to the position of mangroves in the intertidal zone (Meyer et al., 2008). Anthropogenic inputs such as like effluents from sewage treatment plants (Corredor and Morell, 1994) increase the rate of N loading to mangroves (Muñoz-Hincapié et al., 2002). They function as efficient buffer zones mitigating large amounts of nutrients (Corredor and Morell, 1994) in the estuarine system and reduce water pollution. Chiu et al. (2004) state that as much as 55% of the N loss in mangrove sediments occurs through the denitrification pathway. Earlier studies have recorded a substantial benthic flux of N₂O in mangroves (Corredor et al., 1999; Muñoz-Hincapié et al. 2002; Kreuzwieser et al., 2003). Mangrove sediments are largely anaerobic and rich in organic matter providing favourable conditions for denitrification. However, redox processes in the N cycle operate in tandem stressing the importance of considering total N₂O production rates from nitrification and

denitrification (Meyer et al., 2008). Further, N₂O production is dependant on a number of environmental factors such as inorganic N concentrations (Dong et al., 2002), sediment redox potential (Van Cleemput and Samater, 1996) and organic carbon availability (Rosswell et al., 1989). Therefore, assessment of environmental parameters and their inter-relationships with net N₂O production in mangrove sediments is crucial in determining the key parameters governing its formation.

In the Indian Ocean region, high N₂O emission has so far been reported within the oxygen minimum zones in the Arabian Sea (Naqvi et al., 2000; Bange et al., 2001). Recently, investigations by Krithika et al. (2008) in a South Indian mangrove system have shown that the benthic N₂O flux varies between 0.41 and 0.77 $\mu\text{mol m}^{-2} \text{h}^{-1}$ indicating that these wetlands are significant contributors of the radiative gas to the atmosphere. However, little is known about the net production or origin of N₂O in these environments. In the present study, we quantified down-core variation in denitrification activity and net N₂O production in two mangrove ecosystems of Goa, India - one relatively pristine and the other influenced by extraneous nutrient input. Detailed studies to assess the major pathway for N₂O production and environmental factors responsible for its generation have been restricted to the relatively undisturbed site. Since there is growing concern over the role of mangrove ecosystems with respect to increased N₂O fluxes to the atmosphere, the study would enhance our knowledge on the contribution of the N cycle processes in N₂O production. In addition, our study would help initiate the formulation of mitigatory measures to minimize N₂O production and its emission from estuarine zones of the Indian Ocean region.

Materials and methods

Study area and sampling

Investigations were carried out at mangrove forests located at Tuvem and Divar along the rivers Chapora and Mandovi respectively, in Goa on the west coast of India (Fig. 1). The site at Tuvem (15°39'94" N and 73°47'65" E) is set amidst coconut, cashew and banana plantations and is comparatively less influenced by anthropogenic activities. The Divar mangrove ecosystem (15°30'35" N and 73°52'63" E) is separated from the mainland by the river Mandovi. The Mandovi is prone to high nutrient concentrations especially during the monsoon season which has been attributed to riverine and land runoff (Divya et al., 2009) and anthropogenic sources (De Souza, 1983). Approximately 10 Mg/month of NH₄NO₃ is used as explosive in ferromanganese mining operations upstream of the

Mandovi (De Sousa, 1999). Iron ore beneficiation plants situated on the riverbanks carry out treatment and up-gradation of low grade ore fines. These plants use river water to wash the iron ore and discharge effluents directly into the aquatic system. This wastewater contains $\sim 80 \mu\text{mol NO}_3\text{-N L}^{-1}$ forming a major source of the nutrient to the estuary (De Sousa, 1999). Annual variation of $\text{NO}_3\text{-N}$ concentrations along the Mandovi estuary has been reported by Divya et al., (2009). Further, Krishnan et al. (2007) have shown that the Divar mangrove sediments are also enriched with metals (primarily Fe and Mn) as a result of ferromanganese mining upstream of the Mandovi. These metals mainly Fe have been shown to influence N transformations (Krishnan and Loka Bharathi, 2009).

Sediment samples were collected at low tide during January 2008 (post-monsoon season) using hand-held PVC push cores (inner diameter 7.5 cm, 15 cm length). The cores were immediately capped at both ends and transported to the laboratory in an ice box. Cores for pore water nutrient and denitrification rate measurements were maintained at 4°C until analysis. Water from the sampling sites was collected in carbuoys for media preparation.

Physico-chemical parameters

Hydrogen ion concentration (pH) was measured upon sample arrival at the laboratory using an *Orion 4-Star Plus* benchtop pH/ISE meter (Thermo Fisher Scientific Inc., USA). Sediment oxidation-reduction potential (Eh) was measured using an *Orion* platinum redox in combination with a Ag/AgCl_2 reference electrode (Thermo Fisher Scientific Inc., USA).

For nutrient analyses, triplicate sub-samples from sediment cores were taken at 2 cm increments from 0 to 10 cm depth by careful sectioning of sediment. Each section (7.5 cm diameter and 2 cm thick) was transferred to 100 mL of sterile saline (0.85% NaCl) and gently homogenized using a glass rod. The slurry was centrifuged at 4°C for 10 minutes at 5000 rpm (x 1803 g) with a high speed cooling centrifuge (Model CPR-24; Remi Instrument Ltd., India). A low spin speed was maintained during centrifugation to ensure minimal change in nutrient concentrations due to lysis of benthic infauna. The supernatant was filtered through a $0.2 \mu\text{m}$ filter and stored at -20°C until analysis. Ammonium, $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ were measured colorimetrically (UV mini 1240 spectrophotometer; Shimadzu Corp., Japan) as described by Koroleff (1969), Bendschneider and Robinson (1952) and Wood et al. (1967). The weight of the sediment used in the extraction was estimated by drying the wet samples at 60°C for 48

hours. Total organic carbon (TOC) was determined by wet oxidation method with a precision of 0.01% (El Wakeel and Riley, 1957).

Denitrification rate, net nitrous oxide production and its origin

Sediment cores were demarcated into five sections (0-2, 2-4, 4-6, 6-8 and 8-10 cm). Then 1 mL of sediment was extruded from each section using a syringe core and transferred aseptically to sterile 20 mL headspace vials. Three mL of sterilized ambient sea water from the sampling site (containing $4.5 \mu\text{mol NO}_3^- \text{-N L}^{-1}$) was added. Further, sample preparations were amended with chloramphenicol (1 g L^{-1}) to prevent de novo enzyme synthesis during the incubations (Bonin et al., 2002). No additional C or $\text{NO}_3^- \text{-N}$ was added as substrate. The vials were capped with butyl stoppers, sealed with Al crimps and then briefly vortexed to form a slurry.

Denitrification activity (DNT) 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). Thus to measure DNT, some of the vials were made anaerobic by flushing with N_2 for 15 min. The headspace over these slurries was amended with acetylene at 20 kPa (Bonin et al., 2002) and the tubes were briefly vortexed.

To determine net N_2O production, aerobic conditions were maintained in the vials and no acetylene was added to the headspace. However, to measure N_2O produced by denitrifiers ($^{15}\text{N}_2\text{O}$), aerobic conditions were maintained and the headspace was adjusted to an acetylene concentration of 10 Pa to inhibit nitrification (Berg et al., 1982; Bonin et al., 2002). Triplicate measurements were performed at each depth and the vials were incubated in the dark for 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 hours. Following incubation, each vial was treated with 0.1 mL of 1 molar HgCl_2 solution and vigorously shaken for 2 min to stop the reaction.

Nitrous oxide in the headspace was analyzed using a gas chromatograph (Model 2010; Shimadzu Corp., Japan) fitted with an electron capture detector and Poropak Q column (1/8" SS column, 3.05 m length, 80/100 mesh; Chromatopak Analytical Instrumentation, India). The oven and detector temperatures were 40 and 300°C respectively. High purity nitrogen at a flow rate of 35 mL min^{-1} was used as a carrier gas. The gas chromatograph was calibrated using a secondary standard $44 \pm 0.38 \text{ nmol}$

N₂O in N₂ (National Physical Laboratory (NPL), New Delhi). The rate of N₂O production was determined based on its linear accumulation over time (Tiedje, 1982). The Bunsen solubility coefficient for the measured salinity and temperature in the microcosms was used to correct for dissolved N₂O (Weiss and Price, 1980). 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 hours. Average N₂O production and DNT were calculated as nmol N₂O-N g⁻¹ h⁻¹ of dry sediment.

Nitrous oxide produced through nitrification (^NN₂O) was calculated as:

$${}^N N_2O = \text{Net } N_2O \text{ production} - {}^D N_2O$$

where,

Net N₂O production = N₂O production from denitrification plus nitrification (without nitrification inhibitor)

^DN₂O = N₂O produced by denitrifiers only (nitrification inhibited in presence of 10 Pa C₂H₂)

Percentage of N₂O through incomplete denitrification (^IN₂O) was calculated as:

$$\% {}^I N_2O = \frac{{}^D N_2O}{DNT} \times \frac{100}{1}$$

where,

DNT = Denitrification activity (in presence of 20 kPa C₂H₂)

Quantification of denitrifiers

Denitrifiers were enumerated by the N₂O-most probable number (MPN) technique. The sediment core was thawed and sectioned at 2 cm intervals using a sterile core cutter to obtain representative samples at 0-2, 2-4, 4-6, 6-8 and 8-10 cm. Sediment from each depth was homogenised by mixing. Approximately 1 g of wet sediment was sub-sampled from each depth using sterile syringes. The sub-samples were transferred to 9 mL of sterile culture medium to give a 10⁻¹ dilution. The culture medium was prepared as described by Michotey et al. (2000). Tween 80 (20 µL) was added and the mixture was sonicated at 40 MHz for 15 seconds. Serial dilutions for each section of the core were prepared in triplicates. The vials were purged with high purity N₂ for 10 minutes to induce anaerobic conditions and supplemented with 20 kPa acetylene (Bonin et al., 1994). The vials were incubated at room temperature in the dark for 10 days and the positive tubes were scored based on the accumulation of N₂O. Subsequent quantification

was made using standard McCready's table (Rodina, 1972). Denitrifier abundance has been expressed as MPN cells g⁻¹ of dry sediment.

Statistical analyses

All analyses were performed using *Statistica* version 6. Bacterial numbers were log₁₀ transformed before analysis. Nitrous oxide production rates were checked for normal distribution using the Kolmogorov-Smirnov test. As the data was normally distributed ($p > 0.2$), t-Test was used to check for statistically significant differences in mean value of N₂O production between the two sites. Pearson's correlation coefficients were used to assess relationships between biotic and abiotic parameters. The correlation values were plotted using *Cytoscape 2.6.3* software which enabled an open-source network visualization. Principal component analysis (PCA) was used to examine the combined influence of environmental parameters on N₂O production.

Results

Physico-chemical characteristics

The mangrove sediments were acidic in nature with pH ranging from 5.8-6.1 (Table 1). Sediment redox potentials were consistently low, in the range of -27.8 (±0.9) to -6 (±16.45) mV at Tuvem while the Divar sediments exhibited the lowest redox potential of -5.7 (±25.10) at a depth of 8-10 cm.

Pore water nutrient content varied widely with depth with 15.1 (±3.4) μmol NH₄⁺-N L⁻¹ recorded at 8-10 cm depth at Tuvem, while 31.34 (±1.83) was recorded at Divar at a depth of 6-8 cm. Nitrite and nitrate concentration generally increased with depth with up to 11.7 (±0.8) μmol NO₂⁻-N L⁻¹ and 14.2 (±0.4) μmol NO₃⁻-N L⁻¹ respectively at Tuvem while at Divar the maximum levels were 14.2 (±0.0) μmol NO₂⁻-N L⁻¹ 10.1 (±0.2) μmol NO₃⁻-N L⁻¹ at 6-8 cm depth respectively. Total organic carbon at Tuvem varied from 2.1-4.5% while at Divar it ranged between 2.5-4.0%.

Denitrification & net nitrous oxide production

The profile of DNT at Tuvem, showed a maxima at 2-4 cm (2.23 (±0.34) nmol N₂O-N g⁻¹ h⁻¹) which decreased with depth (Fig. 2). Similarly at Divar, the highest DNT activity was observed within 0-4 cm and this was ~5 times higher than at Tuvem.

Nitrous oxide production was significantly different (two-tailed t-test, $P = 0.003$, $n = 15$) at both the locations and varied with depth. At Tuvem, a steady decrease in N_2O production with depth was observed. The highest production rate of $0.71 (\pm 0.11) \text{ nmol } N_2O\text{-N } g^{-1} \text{ h}^{-1}$ was recorded at 0-2 cm (Fig. 3). At the deepest layer investigated (8-10 cm), production of N_2O had decreased to a minimum of $0.12 (\pm 0.02) \text{ nmol } N_2O\text{-N } g^{-1} \text{ h}^{-1}$. At Divar, the 2-4 cm layer had the maximum N_2O production rate of $1.95 (\pm 1.20) \text{ nmol } N_2O\text{-N } g^{-1} \text{ h}^{-1}$. Depth integrated values for net N_2O production and denitrification activity at both the locations are shown in Table 2.

N_2O production by denitrifiers

Detailed investigations to elucidate the major pathway for N_2O production at the relatively pristine site Tuvem showed that these sediments harbored up to 10^7 denitrifiers g^{-1} sediment. Their abundance was maximum at 4-6 cm (7.14×10^7 cells g^{-1}) as compared to the other sections of the core where their number was relatively stable. In the Tuvem sediments, the denitrifiers significantly influenced N_2O production ($n=15$; $r=0.55$; $p<0.05$) as visualized in Fig. 4. They also showed an inverse relationship with pore water $NH_4^+\text{-N}$ ($r=-0.57$; $p<0.05$) and $NO_2^-\text{-N}$ ($r=-0.55$; $p<0.05$) concentrations.

In presence of a nitrification inhibitor, denitrifiers produced a maximum of $1.26 (\pm 0.74) \text{ nmol } N_2O\text{-N } g^{-1} \text{ h}^{-1}$ at 0-2 cm (Fig. 5). Incomplete denitrification ($^{15}N_2O$) was responsible for 43-93% (Fig. 6) of the N_2O production which accounts for about 13-52% ($\text{net } N_2O/DNT \times 100$) of the N_2O produced through denitrification. N_2O production through nitrification ($^{14}N_2O$) was below detection.

Environmental factors influencing N_2O production

Relationships of N_2O production rates with physico-chemical parameters at Tuvem showed an inverse relationship between N_2O production and pore water $NO_2^-\text{-N}$ concentration ($n=15$; $r=-0.47$; $p<0.05$). Up to 32% of the variation in N_2O production was negatively influenced by sediment pH ($r=-0.57$; $p<0.05$). Even though the relationship between N_2O concentration and total organic carbon was not statistically significant, a positive relationship existed between these parameters.

Principal component analysis (PCA) on sediment variables resulted in four main components explaining nearly 80% of the total variance (Table 3) in N_2O production. The first component in the correlation plot explained 29% of the observed variance. A forward regression (Fig. 7) of the reductive processes in the N cycle (DNT and N_2O production) was observed in the first component. Sediment pH,

denitrifier abundance and NO_2^- -N significantly influenced this component. The second component explained 22% of the variance with pore water NO_3^- -N and organic carbon concentrations correlating strongly with this component. The third component correlated with pore water NH_4^+ -N and denitrification activity and explained 16% of the variation. The only and most significant variable in the fourth component was sediment redox potential which explained 12% of the variation.

Discussion

The Mandovi estuary receives a considerable nutrient input from various sources such as mining wastes (De Sousa, 1983; De Sousa, 1999), land runoff during the summer monsoon (Sardesai and Sundar, 2007; Divya et al., 2009), remineralization of organic matter (Pratihary et al., 2009), sewage effluents (Ansari et al., 1986), etc. Hence we expected the adjoining Divar mangrove ecosystem fringing the estuary to also contain elevated inorganic N levels favouring denitrification and consequently N_2O production. As hypothesized, down-core DNT at Divar was found to be comparatively higher than at the relatively pristine site Tuvem (Fig. 2). Low redox potentials (<115 mV) at the sampling sites (Table 1) are indicative of anaerobic conditions in the sediment which are conducive for alternate respiratory pathways like denitrification and sulfate reduction. Pore water profiles in the present study revealed that low NO_3^- -N concentrations within 0-4 cm layer in the Divar sediments coincided with elevated DNT. Denitrification is dependent on NO_3^- -N supply either from nitrification (Klingensmith and Alexander, 1983) or availability of the nutrient from the ambient seawater. The surficial sediments especially, are continuously replenished with nutrients from the estuarine water. These sediments (≤ 4 cm) act as efficient traps to immobilize nutrients (Tam and Wong, 1993) which consequently enhances N metabolism within this depth range. Our observation is consistent with earlier studies by Jørgensen (1989) who has shown that the denitrification capacity of estuarine sediments was always highest at the surface and declined with depth. To compare DNT measured using the acetylene block technique in the present study with other similar measurements in mangrove ecosystems, values were integrated to 10 cm depth. Denitrification occurred at a rate of up to $0.67 \text{ mmol N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ in the sediments examined (Table 2). These values are similar in range to those reported from a mangrove system prone to secondary sewage effluents (Corredor and Morell, 1994) and in other estuaries (Barnes and Owens, 1998; Bernot et al., 2003). Though over-enrichment of nutrients in coastal waters has ecological implications (Howarth et al., 2000), very little is known about the fate of terrestrially-derived nutrients in the Mandovi estuarine system. Pratihary et al., (2009) state that benthic denitrification is responsible

for 22% removal of riverine dissolved inorganic N in the Mandovi estuary. The high denitrifying capacity of the Divar sediments suggests that this ecosystem also acts as a buffer zone by reducing nutrient levels through the denitrification process and helps to maintain the water quality of the adjoining estuary.

High DNT at Divar was accompanied by elevated N_2O production (Fig. 3) as compared to Tuvem. Net N_2O production at Divar occurred at a rate of up to $1.95 \text{ nmol g}^{-1} \text{ h}^{-1}$ which is almost three times higher than the relatively pristine site Tuvem. Natural N_2O production rates in estuarine sediments range from 0.1 to $8.5 \text{ } \mu\text{mol m}^{-2} \text{ h}^{-1}$ (Wang et al., 2007). At NO_3^- -N concentrations of ~ 10 - $15 \text{ } \mu\text{mol}$ (*in situ* + ambient seawater used in medium), depth integrated N_2O production values of up to $0.1 \text{ mmol N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ (Table 2) recorded in our study are far greater than those reported by Dong et al. (2002) from the anthropogenically influenced Colne estuary at similar NO_3^- concentration. A microsensor approach by Meyer et al. (2008) has shown that in sub-tropical mangrove sediments, anaerobic N_2O production (through denitrification) under eutrophicated conditions occurs at a rate of $0.1 \text{ mmol m}^{-2} \text{ h}^{-1}$. These values are comparable to those recorded in the current study. Our findings emphasize that ecosystems prone to higher N loading can have a detrimental effect on the environment through increased N_2O production. Elevated levels of greenhouse gas emissions from these sediments would thus pose a major environmental issue.

Experiments to examine the major pathway for N_2O production in the present study indicates that up to 93% incomplete denitrification at the surface of mangrove sediments (Fig. 6) could contribute substantially to an increase in atmospheric N_2O . Studies by Robinson et al. (1998) in hypernutrified estuarine sediments have also shown higher N_2O concentrations in the surface layer ($\leq 2 \text{ cm}$) attributing it to denitrification fuelled by NO_3^- -N availability. Similarly, Koike and Terauchi (1996) also reported their highest concentration of N_2O in the top 1 cm of the marine sediments studied. Stratified sampling in our study showed that benthic N_2O production was found to generally decrease with depth. The flux of the radiative gas to the atmosphere would however be dependent on the diffusion coefficient and N_2O consumption rates in the sediment layers it passes through.

A number of factors are known to influence the production of N_2O in marine sediments. Physical, chemical, biological and environmental factors like temperature, pH, sediment redox potential (Van Cleemput and Samater, 1996), organic C availability (Rosswell et al., 1989), NO_2^- -N concentration

(Dong et al., 2002) and denitrifying communities play an important role in N₂O production. Statistical analysis showed that pore water nutrient concentrations, organic carbon availability and denitrifiers were some of the important factors influencing the production of N₂O in the current study. Degradation of sediment organic matter results in acidic conditions. Although the relationship between N₂O production and total organic carbon content in these sediments was not significant, a positive relationship existed and is thus indicative of its influence on the gas. Though the water soluble fraction of organic C was not estimated during the study, it is possible that it stimulated denitrifier activity and consequently N₂O production. Both denitrification rate and N₂O production followed a decreasing trend with depth, however no significant relationship was observed between the two parameters. The production of N₂O could be thus be governed by the availability of electron donors and acceptors like NH₄⁺, NO₃⁻, organic carbon rather than the rate of denitrification (Usui et al., 2001; Mathieu et al., 2006).

The denitrifier community was also found to influence N₂O production in mangrove sediments (Figs. 4, 5). They numbered up to 10⁷ cells g⁻¹ and their abundance could be regulated by the amount of organic matter available for their growth (Fig. 7). This can be easily explained by the fact that the denitrifiers are facultative aerobic microorganisms and their activity is limited by the amount of NO₃⁻-N available. Estuaries are generally heterotrophic systems, with bacterial respiration exceeding primary production (Heip et al., 1995, Gattuso et al., 1998). Consequently, removal of dissolved inorganic N from estuaries occurs through sedimentary denitrification and/or burial in the sediment (Middelburg and Nieuwenhuize, 2000). An inverse relationship observed between denitrifiers and pore water nutrient concentrations in the present study suggests that denitrification could play an important role in mitigating excess nutrients within the aquatic system preventing eutrophication.

In sulfidic sediments, the denitrification end product is known to shift from N₂ to partially reduced inorganic N forms such as NO₂⁻ and N₂O (Ebrahimipour et al., 2000). This could explain the high NO₂⁻-N pool in the largely anaerobic mangrove sediments studied. About 22% variation in N₂O production was caused by the variation in NO₂⁻-N concentrations suggesting that it was one of the important and statistically significant parameters regulating the production of N₂O in mangrove sediments. Many other studies have shown a correlation to exist between N₂O production and NO₂⁻-N concentration (He et al., 2001; Dong et al., 2004; Alinsafi et al., 2008). Denitrification activity in estuarine sediments is dependent on NO₃⁻-N availability (Kana et al., 1998) consequently leading to NO₂⁻ and N₂O production. Dong et al. (2002) state that formation of N₂O from NO₂⁻-N is

thermodynamically favourable compared to nitrate suggesting that it may be a critical factor regulating N₂O formation. Bauza et al. (2002) have reported N₂O production mainly through nitrification in red mangrove forests which are characterized by oxic conditions (redox potentials: 159–377 mV) and NH₄⁺-N concentrations varying from 0.188–0.273 mmol L⁻¹. On the contrary, (Meyer et al., 2008) have shown that nitrification and denitrification contributed almost equally to N₂O production under NH₄⁺-amended conditions. In the present study, though ammonium concentrations at Tuvem were <15 μmol L⁻¹, it did not appear to assert a strong influence on N₂O production. Nitrification could be a significant source of N₂O. In organically rich mangrove sediments, NH₄⁺-N released through re-mineralization of organic matter could easily get bound to clay particles making it unavailable for biological uptake. Thus, when NO₃⁻-N and NO₂⁻-N pre-dominate, N₂O arises from microbial denitrification (Corredor et al., 1999). Nitrate respiration is kinetically and thermodynamically favorable (Aivasidis et al., 2005) in oxygen depleted environments and is preferred over other electron acceptors (Canfield et al., 2005). Denitrification activity also enhances NO₂⁻ uptake preventing its accumulation to toxic levels. Experimental results reveal that denitrification was the major pathway for N₂O production in the mangrove ecosystems of Goa, India. Close grid measurements at sub-millimeter intervals by Meyer et al., (2008) have shown that in sub-tropical mangrove sediments, N₂O production through nitrification occurs very close to the surface while denitrification is responsible for its production in the deeper anaerobic layers. The production of N₂O through nitrification could be more prominent when the oxidative process is more pronounced. However, N₂O production through nitrification was not detected indicating that the reductive phase of the N cycle was pre-dominant at the time of sampling (post-monsoon). Despite denitrification showing highest activity during the pre-monsoon season (data not shown), measurements have shown that the mechanism of N₂O production in mangrove sediments of Goa during all the three seasons is essentially the same.

Conclusion

Denitrification and N₂O producing capacity of anthropogenically influenced mangrove ecosystems was clearly higher when compared to relatively pristine locations. N₂O production in the mangrove sediments of Goa was associated mainly with denitrification whereas its production through nitrification was non-detectable. The highest percentage of N₂O production through incomplete denitrification occurred within the first two centimetres of the sediment, a fraction of which could be lost to the atmosphere. Nitrite concentration and denitrifier abundance were the two most important environmental

parameters governing the production of N₂O in these sediments indicative of active nutrient uptake by the autochthonous denitrifier community. Though mangroves have the ability to efficiently moderate elevated nutrient concentrations in the estuarine system through the denitrification pathway, they also pose a threat by increasing greenhouse gas production. Our study shows that in mangroves prone to elevated nutrient levels, benthic N₂O production was three orders higher than natural production rates in estuarine sediments elsewhere. Thus, adequate measures like lowering use of NH₄NO₃ in mining activities and building predictive models (Valiela et al., 2000, 2004; Bowen et al., 2007) for tracking the fate of N inputs could be initiated to minimize N loading in adjoining estuarine systems. These strategies would not only help to lower N pollution but also simultaneously result in decreased N₂O emission to the atmosphere.

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Legend to figures:

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

Fig. 2: Denitrification profile (\pm SD) over depth at Tuvem and Divar

Fig. 3: Variation in net N₂O production (\pm SD) with depth from the Tuvem and Divar mangrove sediments

Fig. 4: A network visualization of factors influencing nitrous oxide production arranged based on the hierarchy of r value denoted at each node. Significant correlations at 0.05 probability level (n=15) have been highlighted in grey

Fig. 5: Nitrous oxide production by denitrifiers under nitrification inhibited conditions at Tuvem

Fig. 6: Percentage of N₂O produced through incomplete denitrification at Tuvem

Fig. 7: Correlation plot from standardized principal component analysis (PCA) on sediment variables. Variables DNT= denitrification rate, TOC=total organic carbon

Legend to tables:

Table 1: Variation in the average values of physico-chemical parameters (\pm SD; n=15) in the Tuvem and Divar mangrove sediments

Table 2: Depth integrated (0-10 cm) net N₂O production and denitrification activity in the Tuvem and Divar mangrove sediments.

Table 3: Results of principal component analysis of sediment variables and N₂O production

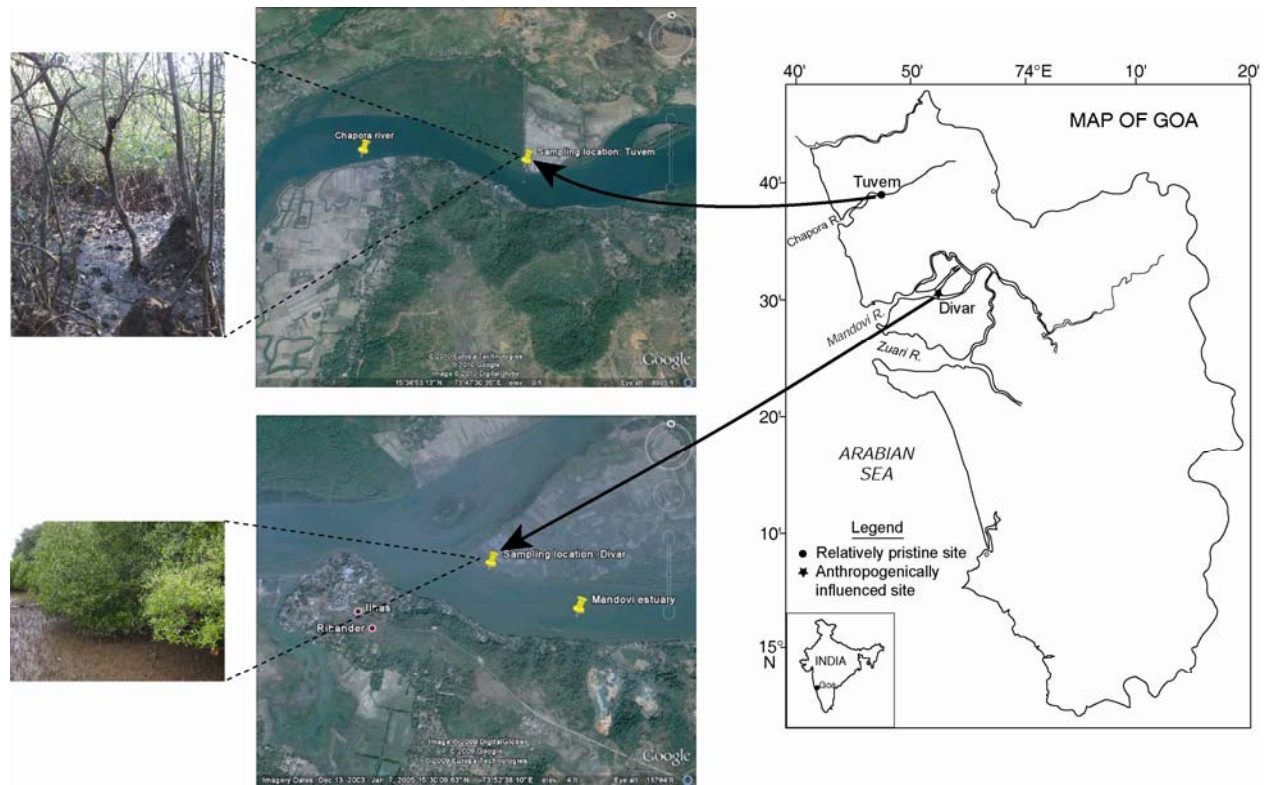


Fig. 1

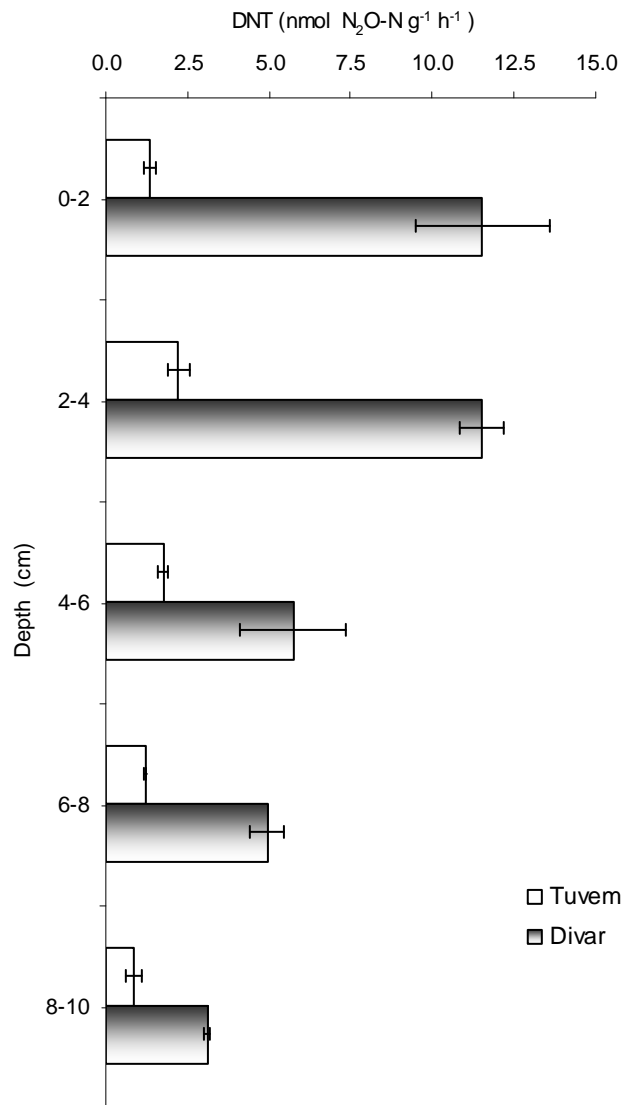


Fig. 2

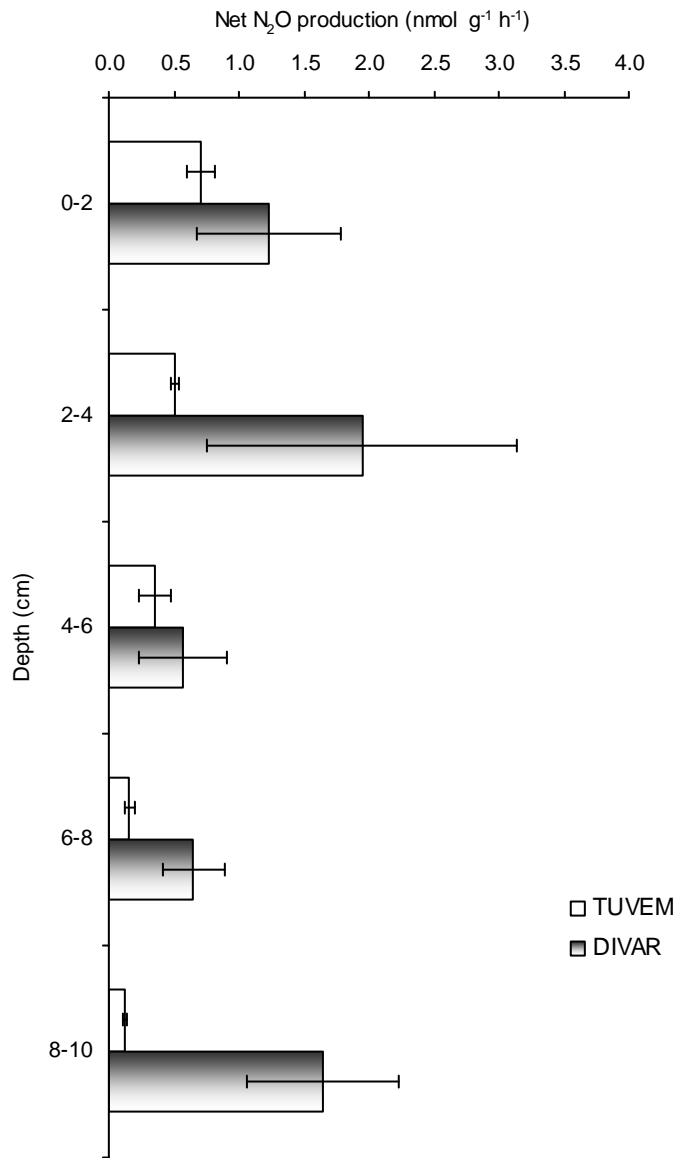


Fig. 3

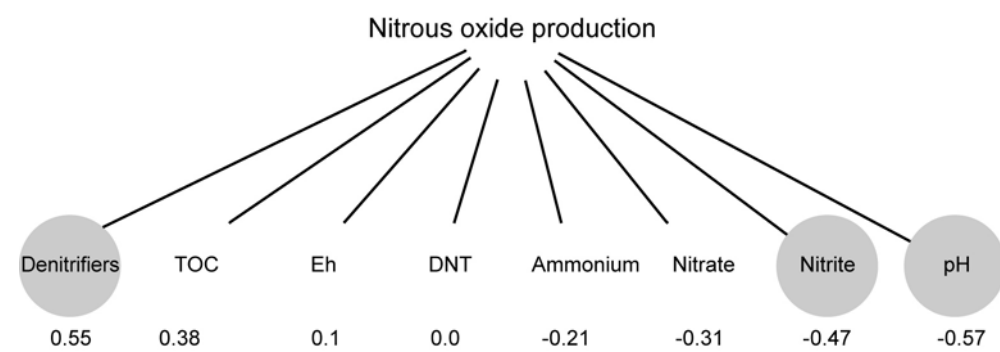


Fig. 4

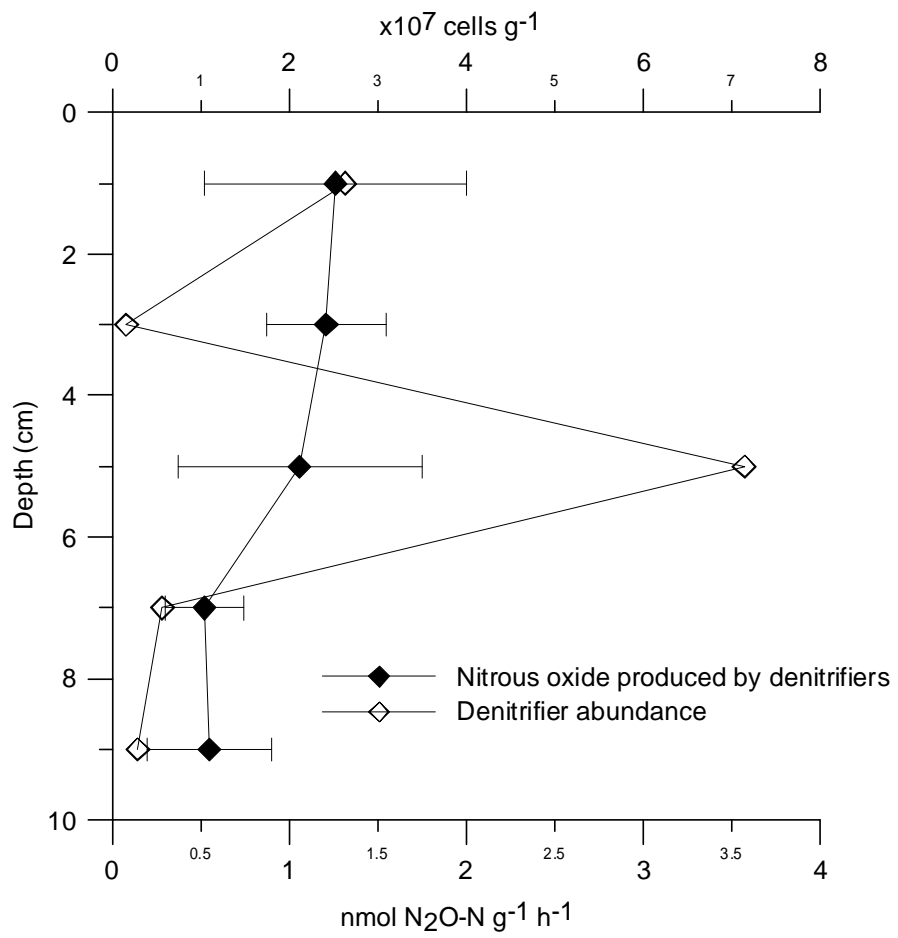


Fig.5

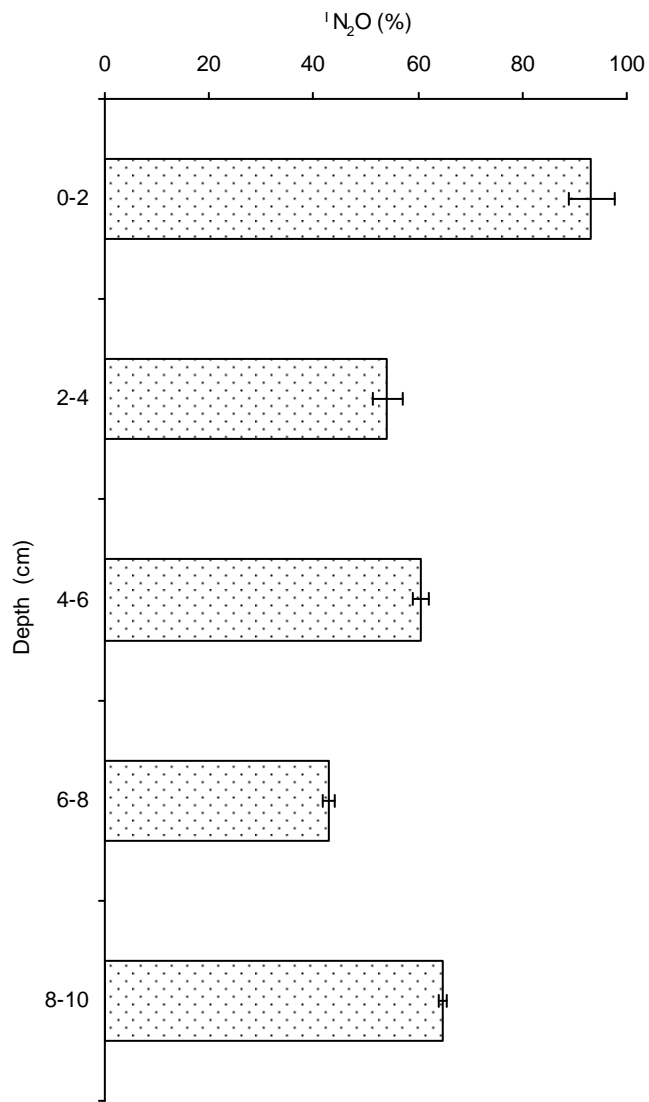


Fig.6

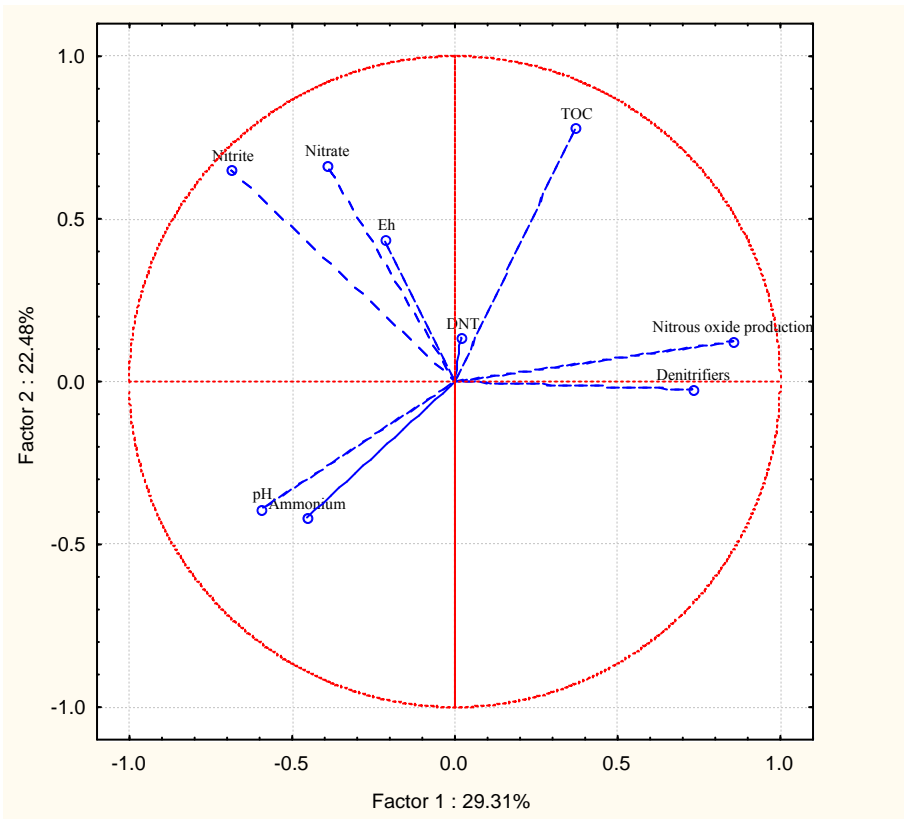


Fig. 7

Table 1:

Location: Tuvem						
Depth (cm)	pH	Eh (mV)	NH ₄ ⁺ -N (μmol l ⁻¹)	NO ₂ ⁻ -N (μmol l ⁻¹)	NO ₃ ⁻ -N (μmol l ⁻¹)	TOC (%)
0-2	5.8 (±0.2)	-27.8 (±0.9)	14.7 (±4.4)	4.5 (±0.13)	10.4 (±0.4)	2.1 (±0.7)
2-4	5.9 (±0.2)	-23.2 (±7.39)	15.0 (±1.4)	8.6 (±0.33)	9.9 (±0.4)	2.9 (±1.4)
4-6	6.0 (±0.6)	-9.5 (±15.06)	7.8 (±1.4)	8.1 (±0.1)	10.9 (±0.6)	4.5 (±2.9)
6-8	6.0 (±0.6)	-6 (±16.45)	12.0 (±0.8)	10.9 (±0.6)	14.2 (±0.4)	4.4 (±2.8)
8-10	6.1 (±0.5)	-6.3 (±14.54)	15.1 (±3.4)	11.7 (±0.8)	11.4 (±0.0)	4.0 (±2.7)
Location: Divar						
0-2	5.9 (±0.3)	115 (±64.7)	15.7 (±3.4)	7.2 (±0.0)	4.6 (±0.7)	2.9 (±1.6)
2-4	5.8 (±0.1)	94.3 (±62.6)	15.1 (±2.0)	4.1 (±0.2)	4.2 (±0.2)	4.0 (±2.1)
4-6	5.1 (±0.1)	93.5 (±45.18)	23.8 (±2.3)	6.6 (±0.6)	9.3 (±0.2)	2.5 (±1.0)
6-8	6.0 (±0.1)	50.8 (±61.38)	31.3 (±1.8)	14.2 (±0.1)	10.1 (±0.2)	3.3 (±1.7)
8-10	6.0 (±0.0)	-5.7 (±25.10)	22.1 (±3.1)	8.1 (±0.3)	10.0 (±0.2)	3.1 (±1.8)

Table 2:

Depth integrated activity	Tuvem	Divar
Denitrification (mmol N ₂ O-N m ⁻² h ⁻¹)	0.14	0.67
Net N ₂ O production (mmol N ₂ O-N m ⁻² h ⁻¹)	0.03	0.10

Table 3:

Variable	Component 1	Component 2	Component 3	Component 4
N ₂ O production	*0.856	0.124	0.206	0.266
pH	*-0.597	-0.391	-0.541	0.134
Eh	-0.215	0.438	0.106	*0.828
Ammonium	-0.453	-0.420	*0.640	0.164
Nitrite	*-0.687	0.649	0.093	-0.004
Nitrate	-0.395	*0.665	-0.367	0.027
Denitrifiers	*0.730	-0.023	-0.496	0.326
TOC	0.369	*0.779	0.286	-0.330
DNT	0.021	0.133	*-0.487	-0.189
Total variance (%)	29.30	22.47	16.26	11.71

*Correlation significant at 0.05 probability level; TOC= Total organic carbon, DNT=Denitrification rate