

Nitrate levels modulate denitrification activity in tropical mangrove sediments (Goa, India)

Sheryl Oliveira Fernandes, P. A. Loka Bharathi*

Abstract

A study to examine the short-term effect of nitrate and organic carbon addition on denitrification activity was carried out on sediments from a mangrove ecosystem prone to anthropogenic activities (Divar, Goa-India). Laboratory microcosms were prepared using sediment sectioned at every 2 cm depth interval from the surface to 10 cm. The incubations were subjected to varying nitrate amendments at concentrations ranging from 0, 5, 10, 20, 40 and 60 $\mu\text{mol l}^{-1}$ (three times more than measured in field). Nitrous oxide production rates increased significantly ($n=15$; $p<0.001$) on addition of the nutrient at all depths investigated indicating that denitrification in mangrove sediments was NO_3^- limited. Incubations amended with organic carbon were prepared using glucose as a substrate with concentrations ranging from 0, 0.1, 0.3, 0.5, 0.75 and 1%. No significant increase in N_2O production was observed on organic C addition. When both the substrates were in excess (1mmol KNO_3 + 1mmol glucose), potential denitrification rates decreased with depth and were up to 38 times higher than the *in situ* denitrification activity varying from 81.26-304.09 $\mu\text{mol N}_2\text{O-N m}^{-2} \text{ h}^{-1}$. These results reveal that mangrove sediments could act as a sink for nitrate and microbially-mediated denitrification could effectively reduce N load controlling any adverse environmental impact in the adjoining estuarine system.

Keywords Denitrification · Organic carbon · Nitrate · Mangroves · Sediments

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Introduction

In ecosystems with high inputs of nitrogen such as estuaries, denitrification mediates reduction of nitrogen load and therefore contributes to eutrophication control (Nogales et al. 2002). One such ecosystem is the mangroves which constitute nearly 75% of tidal vegetation in tropical regions (Alongi et al. 1989). These habitats lie in close proximity to areas prone to anthropogenic activity (Muñoz-Hincapié et al. 2002). Mangroves play an important role in the biogeochemical cycles of coastal and marine ecosystems (Thorsten and José 2001). They function as efficient buffer zones mitigating large amounts of excess nutrients in the estuarine system reducing water pollution.

The nitrogen cycle within mangrove forests is mediated pre-dominantly by microbial rather than chemical processes (Alongi et al. 1992). Inorganic N (nitrogen) and other parameters important for nitrogen turnover can fluctuate widely due to the position of mangroves in the intertidal zone (Meyer et al. 2008). Chiu et al. (2004) state that as much as 55% of the N loss in mangrove sediments occurs through denitrification. Mangrove sediments are largely anaerobic and rich in organic matter providing favourable conditions for denitrification. The process is mediated mainly by facultatively anaerobic heterotrophic bacteria which utilize oxidized N compounds for respiration under oxygen deficient conditions (Zumft 1997). Earlier studies have highlighted nitrate (Morell and Corredor 1993; Corredor and Morell 1994; Rivera-Monroy and Twilley 1996; Kana et al. 1998) and organic carbon (McCarty and Bremner 1992; Pfenning and McMahon 1997; Hill and Cardaci 2004) as important factors affecting denitrification. In sediment, denitrifying activity is highly correlated with water-extractable organic carbon and is frequently stimulated by the addition of exogenous carbon (Knowles 1982; Hahndel and Isermann 1993). Sufficient organic carbon is required for denitrification to occur as it is a source of energy for the conversion of nitrate to nitrogen gas. In the marine environment, high denitrifying activity has been reported to occur within the oxygen minimum zones (OMZs) contributing to 30-50% of the total nitrogen loss and has been commonly attributed to heterotrophic denitrification (Gruber and Sarmiento 1997; Codispoti et al. 2001). In the OMZs of the Eastern Tropical North and South Pacific, denitrification appeared to be limited by organic carbon (Ward et al. 2008). In the Indian Ocean region, denitrification in the Arabian Sea has also been shown to be dependant on the organic carbon regime (Anderson et al. 2007). So far, there have been no reports on the factors limiting denitrification from nearby estuarine zones.

In the present study, we examined the short-term effect of nitrate and organic carbon addition on denitrification activity in mangrove sediments prone to nutrient input from anthropogenic activities. We

hypothesize that in organically rich mangrove sediments, denitrification could be more dependent on nitrate availability. The significant increase in denitrification activity on nitrate amendments observed in the present study further corroborates our hypothesis suggesting that nitrate is the main limiting factor for denitrification in mangrove sediments.

Materials and methods

Study area and sampling

Investigations were carried out at fringing mangrove forest located at Divar in Goa, west coast of India (Fig. 1) during January 2008 (post-monsoon season). The Divar mangrove ecosystem (15°30'35" N and 73°52'63" E) is separated from the mainland by the river Mandovi and is accessible by ferry. A significant number of inhabitants colonize along the banks. The Mandovi 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 on the riverbank, discharge effluents directly into the estuary. This discharge contains high quantities of NH_4NO_3 used as explosive in ferromanganese mining operations (De Sousa 1999).

Sediment cores were collected at low tide and transported in an ice box. Cores for activity measurements were maintained at 4°C. For measurement of labile organic matter (LOM), cores were immediately sectioned at 0-2, 2-4, 4-6, 6-8 and 8-10 cm interval. The sections were dried at 60°C \pm 2, powdered and sieved through a 200 μm sieve and stored in clean PVC vials until analysis.

Pore water nitrate

For the estimation of pore water nitrate concentration, 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 homogenized using a glass rod. The slurry was centrifuged at 5000 rpm for 10 mins and 4°C. Low spin was maintained during centrifugation to ensure minimal change in nutrient concentration due to lysis of benthic infauna. The supernatant was filtered through a 0.2 μm filter and stored at -20°C until analysis. Nitrate was measured colorimetrically (SHIMADZU UV/VIS spectrophotometer; precision: \pm 0.01 $\mu\text{mol l}^{-1}$) as described by Wood et al. (1967). Weight of the sediment used in the extraction was estimated by drying in a hot air oven at 60°C for 48 hours.

Total organic carbon

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

Labile organic matter

The labile organic matter (LOM) was measured as a sum of proteins, carbohydrate and lipid content in the sediments. Proteins were estimated as described by Lowry et al. (1951). Carbohydrate was estimated by phenol-sulphuric acid method (Dubois et al. 1956) using glucose as standard. Lipid content in the sediment was estimated by using the acid dichromate method outlined by Parson et al. (1984).

Effect of NO_3^- addition on denitrification

Sediment cores were demarcated into five sections (0-2, 2-4, 4-6, 6-8 and 8-10 cm). About 1 cm³ 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 seawater from the sampling site was added to the sediment. This seawater used for slurry preparation was spiked with a KNO₃ solution to give final concentrations of 0 (unamended to reflect *in situ* denitrification activity), 5, 10, 20, 40 and 60 μmol NO₃-N l⁻¹. The seawater was also amended with chloramphenicol at a final concentration of 1 g l⁻¹ (Bonin et al. 2002) to inhibit *de novo* synthesis of denitrifying enzymes thus reflecting *in situ* activity at the time of sampling (Brooks et al. 1992). No additional carbon substrates were added. The vials were capped with butyl stoppers and were briefly vortexed to form slurry. The vials were purged with high purity N₂ for 10 minutes to induce anaerobic conditions. Acetylene gas at 20 kPa (Bonin et al. 2002) was injected into the headspace to inhibit N₂O production by nitrification and its reduction by denitrification (Castro-González and Farias 2004). Triplicates were maintained at each depth and the vials were incubated in the dark for 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 hours. At the end of the incubation period, bacterial activity was terminated using 0.1 ml of 1M HgCl₂.

Nitrous oxide in the headspace was analyzed using a Shimadzu 2010 gas chromatograph fitted with a electron capture detector and Porapak Q column (1/8" SS column, 3.05 m length, 80/100 mesh). The oven and detector temperatures were 40°C 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 ±0.38 nmol N₂O in nitrogen (National Physical Laboratory (NPL), New Delhi). Denitrification activity was calculated based on the linear accumulation of N₂O over time. The solubility

coefficients of N_2O were used to correct for dissolved N_2O in the microcosms (Weiss and Price 1980). Sediment used in each microcosm was filtered through a laboratory grade filter paper, dried at 60°C for approximately 48 hours and weighed. Denitrification activity was calculated as $\text{nmol N}_2\text{O-N g}^{-1} \text{ h}^{-1}$ of dry sediment, extrapolated to a unit area basis (m^{-2}) and expressed as $\mu\text{mol N}_2\text{O-N m}^{-2} \text{ h}^{-1}$.

Effect of organic C addition

Sterilized seawater used for slurry preparation was amended with 1 g l^{-1} chloramphenicol and glucose solution at a final concentration of 0, 0.1, 0.3, 0.5, 0.75 and 1%. Samples were prepared and analyzed as described above. No additional nitrate was added.

Potential denitrification rates

Samples were prepared and analyzed as described above except that the seawater used for slurry preparation was amended with KNO_3 and glucose at a final concentration of 1 mmol l^{-1} in addition to chloramphenicol.

Statistical analyses

Statistical analyses have been carried out using analysis tool pack in *Microsoft Excel*. Significant differences in denitrification rates have been determined using analysis of variance (ANOVA).

Results and discussion

Estuarine sediments are known to have considerable nutrient loading mostly derived from extraneous inputs like sewage outfall (King and Nedwell 1987). The present study showed that the Divar sediments harbored measurable pore water nitrate content which increased with depth (Fig. 2) ranging from $4.15 (\pm 0.21)$ to $18.71 (\pm 0.28) \mu\text{mol l}^{-1}$. Down-core profiling of denitrification showed a sub-surface maxima at 2-4 cm with a rate of $20.08 \mu\text{mol N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ ($1.43 \pm 0.66 \text{ nmol g}^{-1} \text{ h}^{-1}$). Isotopic measurements by Chiu et al. (2004) have shown much higher denitrification activity in surficial mangrove sediments as compared to the deeper non-rhizosphere soil with rates of up to $120 \text{ nmol g}^{-1} \text{ h}^{-1}$. Thomas and Lloyd (1994) have also reported maximum denitrification to occur at the surface in estuarine sediment. The process is known to be dependent on nitrate availability (Seitzinger 1990). Low nitrate values encountered at the surface could thus be attributed to higher nitrate removal in the upper few centimeters of the sediment.

High denitrification activity at 2-4 cm coincided with increased organic carbon availability at this depth which varied from 2.88% at 4-6 cm to 4.95% at 2-4 cm (Fig. 3). Naturally occurring organic carbon in sediments is critical because it can influence nutrient availability (Moore 1989), enhance biological activity and can increase acidity of ambient waters through organic acids (Eshleman and Hemond 1985; Kerekes et al. 1986). The primary source of TOC in mangrove swamps is plant material which is decomposed by sediment organisms and converted to organic compounds viz., carbohydrates, proteins and lipids. A sizeable fraction of organic matter could be transported to deeper depths by bioturbating infauna and retained within the system. Natural processes and human activities can also result in elevated content of TOC in the area. Sardessai (1993) have shown that decomposition of mangrove litter and influx of fresh water during monsoons contributes to organic matter derived from humic acids. In the present investigation, labile organic matter showed a similar depth-wise distribution like TOC with a maximum concentration of 0.68% LOM at 2-4 cm (Fig. 4). Labile organic matter is known to limit denitrification (McCutchan and Lewis 2008). In the reductive phase of the N cycle, NO_3^- ions are reduced to N_2O or N_2 whereas organic C gets oxidized to CO_2 and H_2O . Our findings suggest that about 11-19% of the TOC is present in readily utilizable form (LOM) in mangrove sediments and could be important for heterotrophic metabolism.

Denitrification activity in mangrove sediments was monitored when subjected to a wide range of nitrate amendments (three times higher than the ambient concentration). Denitrification activity was stimulated in microcosms containing seawater with all combinations of nitrate amendments i.e. 5, 10, 20, 40 and 60 $\mu\text{mol NO}_3\text{-N l}^{-1}$. Higher rates were observed especially at depth ≤ 4 cm. Highest activity of 129.22 (± 31.94) $\mu\text{mol N}_2\text{O-N m}^{-2} \text{h}^{-1}$ at 0-2 cm was observed at nitrate amendment of 40 $\mu\text{mol NO}_3\text{-N l}^{-1}$ (Fig. 5). At depths > 4 cm, the activity was less pronounced. In estuarine sediments prone to high nitrate inputs, the bacterial communities adapt to changes in the concentration exhibiting higher rates of nitrate reduction and also increasing the proportion of nitrate reduced to gaseous products (King and Nedwell 1987). Laverman et al. (2007) have shown that denitrification in estuarine sediments is nitrate limited and the resident denitrifying community rapidly adjusts its level of activity to increased nitrate availability. Denitrification accounts for 27 to 57% of the nitrate consumption in estuarine and coastal sediments (Nishio et al. 1982). The Divar mangrove ecosystem is prone to high nutrient input from mining rejects, land runoff and domestic sewage discharge. The increase in denitrification activity especially in the first few centimeters is indicative of the high nitrate removal capacity of these sediments. Corredor and Morell (1994) have confirmed that mangrove sediment-microbial communities

are capable of depleting up to 10 to 15 times the nitrate added. Recently, Krishnan and Loka Bharathi (2009) have shown that nitrification rates in the Divar sediments vary between 2.7 to 18.2 $\mu\text{mol N}_2\text{O-N m}^{-2} \text{ h}^{-1}$. A strong coupling between redox processes of the N cycle could exist in these sediments wherein nitrate supplied continuously through the nitrification process could be fuelling denitrification especially in the upper few centimeters.

Organic carbon addition stimulated denitrification activity mostly at depths ≥ 4 cm (Fig. 6). However, maximum activity of only 35.24 (± 9.93) $\mu\text{mol N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ was recorded at 4-6 cm depth. A 0.5% amendment of labile organic carbon (glucose) was found to effectively stimulate denitrification activity at all depths suggesting that the process is optimal at this concentration. Statistical analyses did not show significant increase in denitrification activity on organic carbon addition as compared to amendments with nitrate (one way ANOVA; $n=15$; $p<0.001$) at all depths indicating that organic carbon was not a limiting factor for denitrification in mangrove sediments. Denitrification in oxygen minimum zones is known to be fuelled almost entirely by organic matter supplied by particles sinking vertically from the euphotic zone (Anderson et al. 2007; Ward et al. 2008). In these environs, organic carbon is the main limiting factor controlling denitrification. In contrast, estuarine systems have considerable organic C loading and labile organic matter is readily available for metabolic activity. In such circumstances, denitrification is more dependent on nitrate availability. Similar observations have been made by Davidsson and Leonardson (1996) using peaty and sandy soil in which NO_3^- has been shown to be a stronger regulator of denitrification than organic carbon.

Potential denitrification rates decreased with depth and were 15-38 times higher (within 0-10 cm core) than *in situ* denitrification activity when both nitrate and organic carbon were in excess. Highest activity of up to 304.09 (± 47.6) $\mu\text{mol N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ (Fig. 7) was recorded at 2-4 cm. At 8-10 cm the activity decreased to 81.25 (± 22.58) $\mu\text{mol N}_2\text{O-N m}^{-2} \text{ h}^{-1}$. Flemer et al. (1998) have reported relatively high potential denitrification rates in estuarine sediments ranging between 500 to 1000 $\mu\text{mol N m}^{-2} \text{ h}^{-1}$ which were also limited by nitrate availability. Laverman et al. (2007) have encountered maximum *in situ* rates two fold lower than the maximum potential rate in surficial sediment indicating that *in situ* denitrification was nitrate limited. Our observations indicate that the surficial sediments have a comparatively higher denitrifying potential. This could be possible since the denitrifying communities at depths < 4 cm get a continuous availability of electron acceptors through lateral supply from the ambient seawater in addition to relatively intense nitrification in the surficial sediments. Thus, these microbes are able to eliminate elevated nitrate concentration through the denitrification pathway.

Conclusion

The Divar mangrove sediments harboured sufficient amount of labile organic matter to support denitrification activity. Microcosm experiments have indicated that nitrate addition enhanced denitrification at *in situ* organic carbon concentration. The benthic denitrifying community in these habitats respond rapidly to episodic events of elevated nutrient supply by increasing the rate of nitrate removal through the denitrification pathway. Thus, these sediments could act as a sink for nitrate and this nutrient is more crucial in controlling denitrification activity. Potential denitrification rates were up to 38 times higher than the *in situ* denitrification activity indicating that the process was important in maintaining low concentration of nitrate and helps to maintain the water quality of the adjoining aquatic system.

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Legend to figures:

Fig. 1: Sampling location at Divar Island, Goa

Fig. 2: Down-core variation in pore water nitrate (\pm SD) at Divar

Fig. 3: Down-core variation in total organic carbon (TOC) (\pm SD) at Divar

Fig. 4: Down-core variation in labile organic matter (LOM) (\pm SD) at Divar

Fig. 5: Effect of nitrate addition on denitrification activity (\pm SD)

Fig. 6: Effect of varying organic carbon amendments on denitrification (\pm SD)

Fig. 7: Down-core variation in potential denitrification activity (\pm SD)

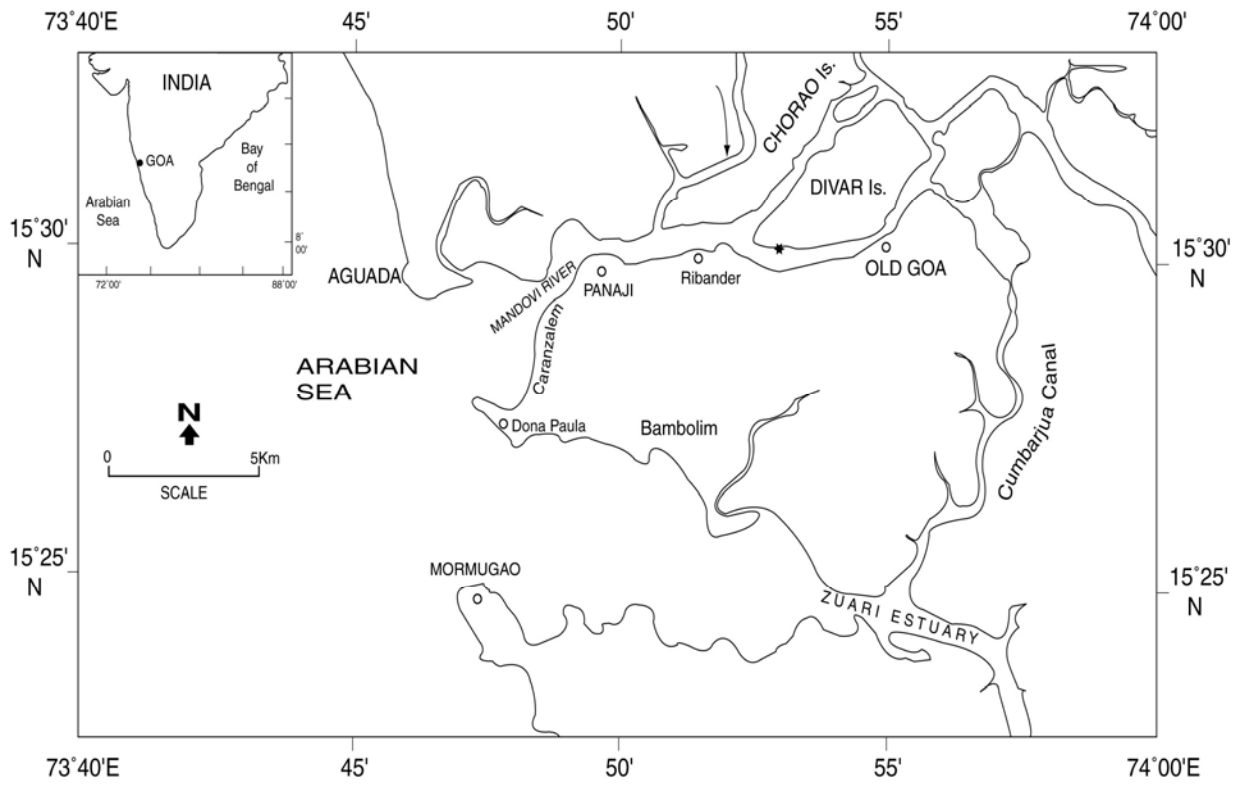


Fig. 1

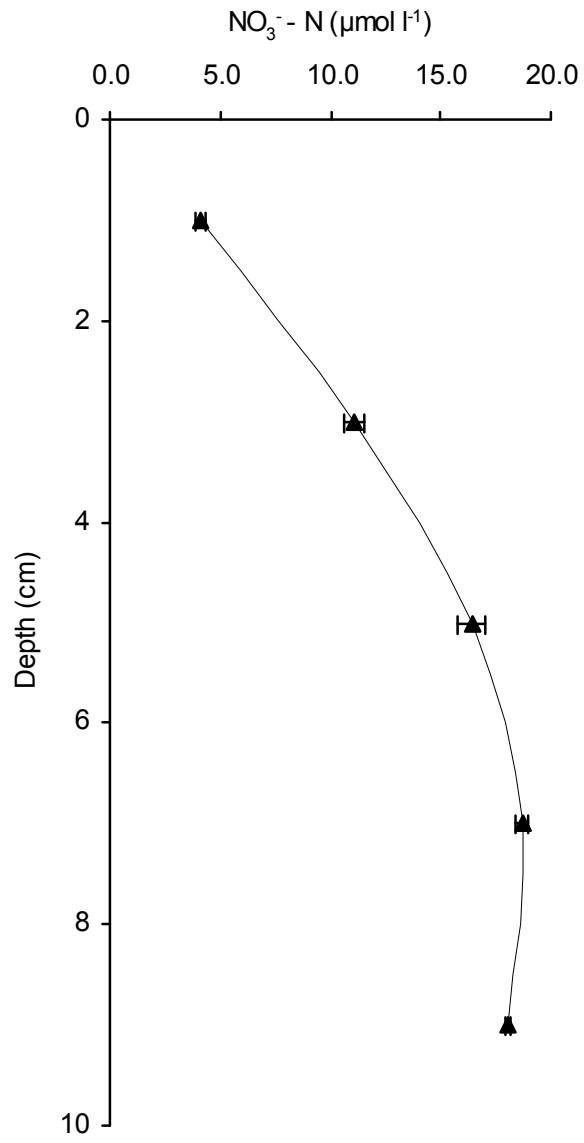


Fig. 2

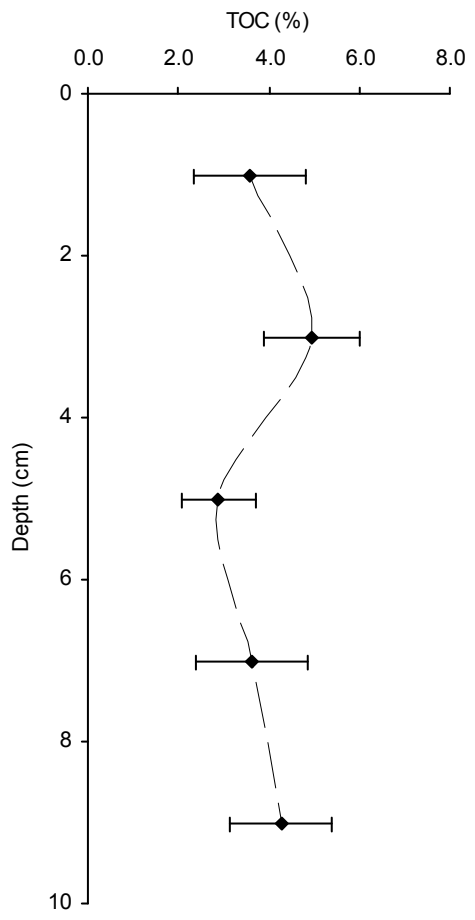


Fig. 3

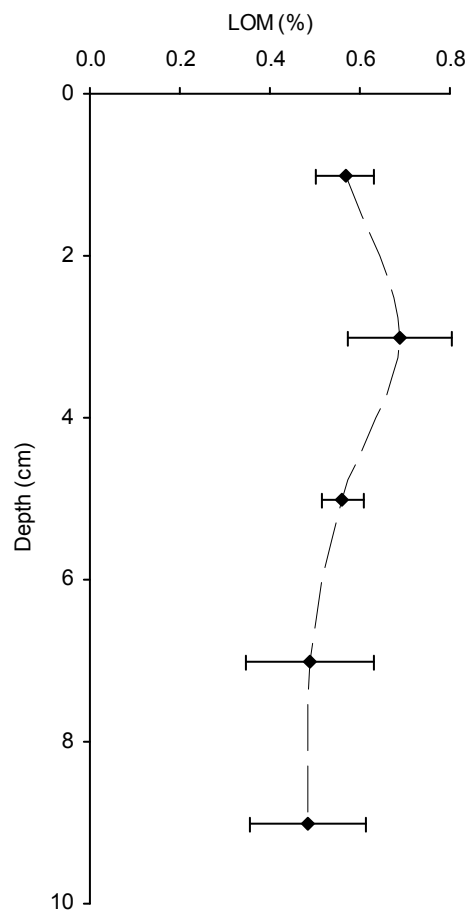


Fig. 4

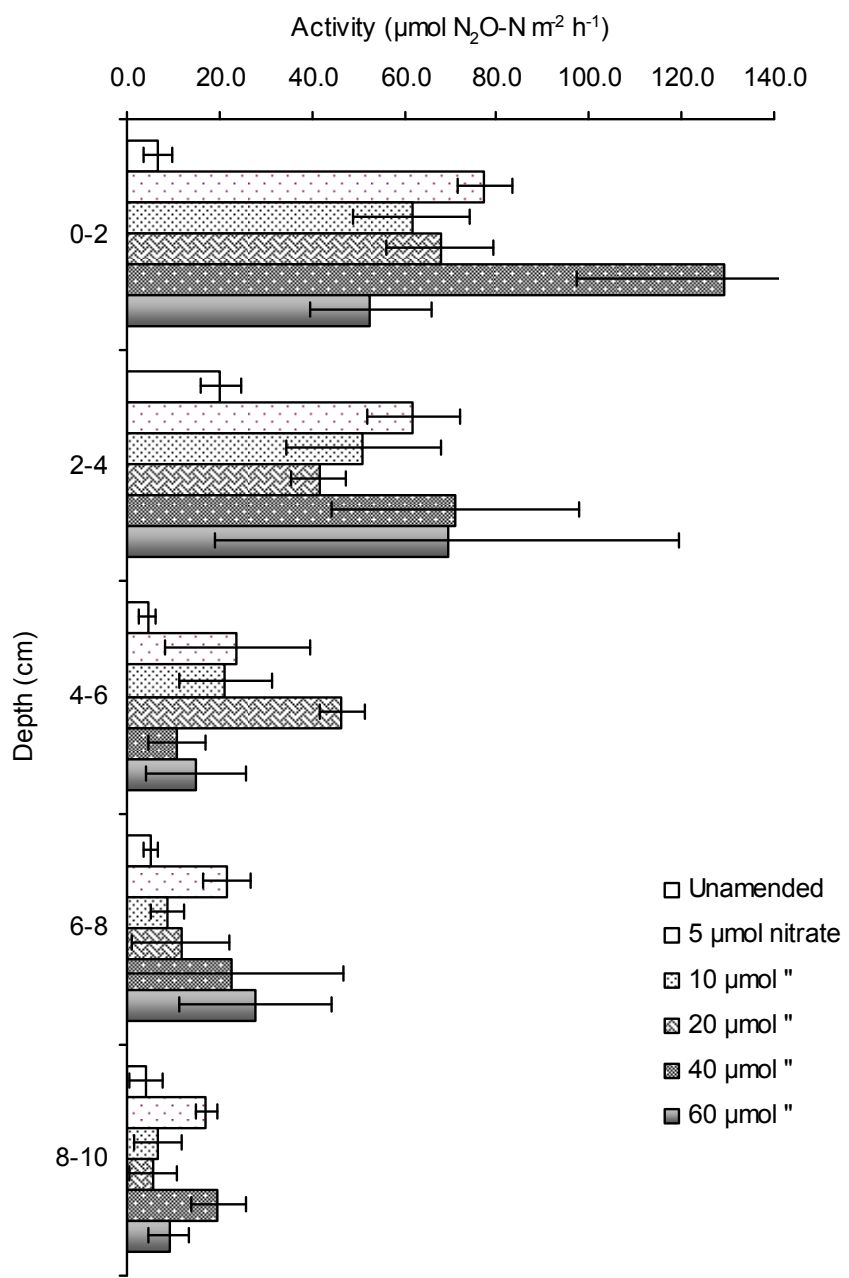


Fig. 5

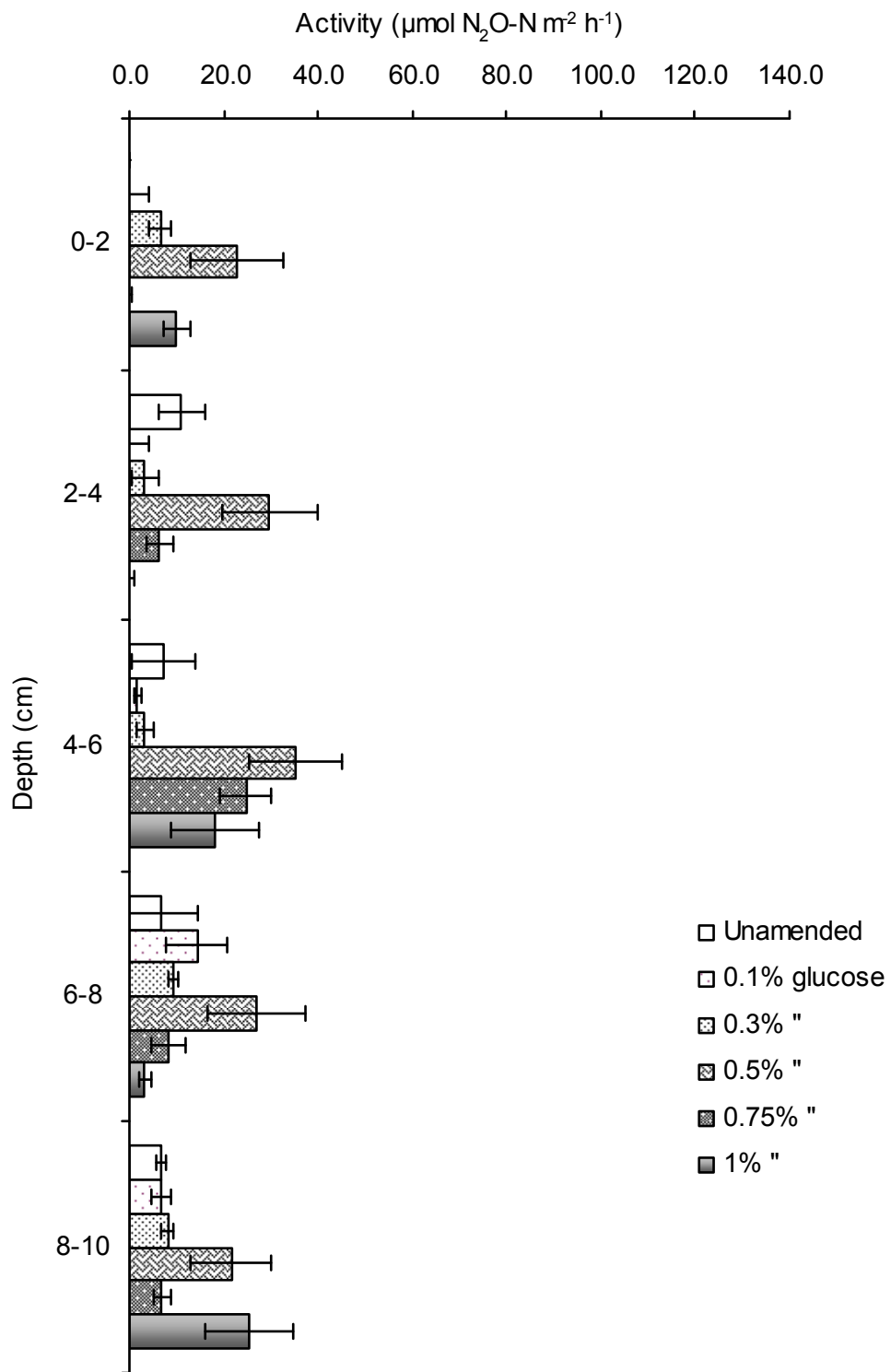


Fig. 6

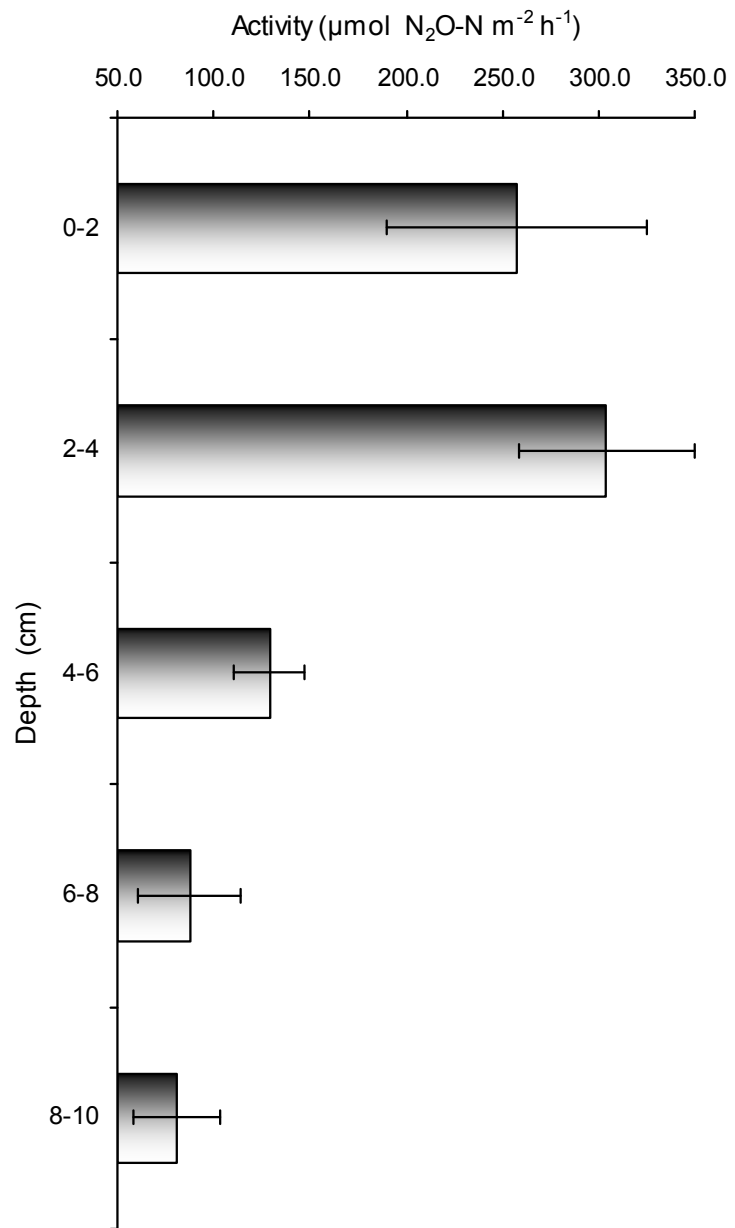


Fig. 7