Chapter 6

Summary & conclusion
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The study on the "Ecology of denitrifiers in mangrove sediments" elucidates the role of environmental parameters in governing the reductive phase of the N cycle in two mangrove ecosystems of Goa, India - the anthropogenically influenced Divar and the relatively pristine site Tuvem. Field and lab based experiments were carried out to meet the following objectives:

➢ To quantify the abundance and activity of denitrifying bacteria
➢ To understand the influence of environmental parameters on denitrification
➢ To identify the denitrifiers at cellular and molecular level
➢ To delineate the influence of bioturbating organisms on denitrification

Down-core investigations were carried out at 2 cm intervals within 0-10 cm depth range at both the locations. Some of the salient findings from this study are as follows:

1. Denitrifier abundance in mangrove sediments varied from $10^{5-8}$ cells g$^{-1}$ and constituted an important fraction of the total bacterial community ($10^{9-10}$ cells g$^{-1}$) in mangrove sediments. Maximum denitrifier abundance by both culturable and molecular methods showed higher abundance at deeper depths (within 4-8 cm) at both the sites.

2. Denitrification activity (DNT) was found to be maximum within 0-4 cm at both the sites suggesting that the activity depends on the prevailing environmental conditions and is not a function of bacterial biomass.

3. DNT at Divar was nearly 3 times the value at Tuvem with maximum activity of 224.51 nmol g$^{-1}$ h$^{-1}$ observed at 0-2 cm. Other co-occurring processes in the N cycle were also measured in conjunction with denitrification. Highest anammox activity of 101.15 nmol N$_2$ g$^{-1}$ h$^{-1}$ at Divar was recorded at 8-10 cm and was 5 times higher than at Tuvem. Di-nitrogen fixation was minimal in estuarine habitats prone to high nitrate inputs and denitrification rather than anammox served as an important mechanism for counteracting N loading.

4. Alternate respiratory pathways like dissimilatory nitrate reduction to ammonium (DNRA) removes up to 3 times more nitrate than DNT resulting in N retention.
5. DNT results in a significant flux of nitrous oxide (N\textsubscript{2}O), a potent greenhouse gas. Microcosm studies showed that net nitrous oxide production at Divar occurred at a maximum rate of 22 \( \mu \text{mol N}_2\text{O-N m}^{-2} \text{ h}^{-1} \) which was 3 times higher than at Tuvem and is indicative of higher emission of the radiative gas in anthropogenically influenced regions.

6. Among the environmental factors influencing DNT, nitrate had a larger influence \((n=15; p<0.001)\) than organic carbon suggesting that mangrove sediments are NO\textsubscript{3}\textsuperscript{-} limited and these regions could act as a sink for nitrate. Multiple regression analysis showed that Fe and Mn also influenced DNT which is indicative of DNT coupled to metal oxidation.

7. Bioturbating infauna were responsible for only 18% of the variation in DNT. The little influence on the process is attributed to the low and patchy distribution of macrofauna in the sediments.

8. Culturable methods have shown that up to 43% of culturable denitrifiers belonged to Gammaproteobacteria.

9. The dominant denitrifier community probed based on the functional gene (nosZ) phylogeny showed that they belonged to the sequences of uncultured organisms and were clustered within phylum Proteobacteria. However, it is possible that some of these genes belong to the culturable counterparts.

10. Analysis of bacterial diversity using the 454 pyrosequencing technology revealed a complex and rich bacterial community in mangrove sediments with \( \sim 3300 \) phylotypes recorded at Divar. The phylum Proteobacteria was the most dominant phylum at both the locations. The class Deltaproteobacteria dominated the Tuvem sediments while the Gammaproteobacteria were more dominant at Divar. Deltaproteobacteria include most of the sulfur cycle bacteria whereas the Gamma and Alphaproteobacteria are involved in N cycling. The existence of these bacteria in mangrove sediments reflects their ability to thrive on reduced substrates and could therefore play an important role in altering the chemistry of inorganic N compounds in coastal ecosystems.
11. Though benthic DNT in mangrove ecosystems serves as an important mechanism for counteracting N loading, it can be concluded that these habitats effectively conserve N through the DNRA pathway thereby minimizing nutrient loss that would otherwise occur through DNT. Most importantly DNRA contributes to minimizing the flux of green house gas N₂O to the atmosphere.
Chapter 7

Implication, application

Future scope
Chapter 7. Implication, application and future scope

Implication and application

- Mangrove systems overcome N limitation by effectively conserving N through the dissimilatory nitrate reduction to ammonium (DNRA) pathway thereby minimizing nutrient loss that would otherwise occur through denitrification (DNT). As the reduction of nitrate through the DNRA pathway in anoxic sediments could be coupled to the oxidation of reduced forms of sulfur, the process contributes to lowering levels of toxic ions in the system. Most importantly this process contributes to minimizing the emission of the potent radiative gas \( \text{N}_2\text{O} \) to the atmosphere.

- In estuarine habitats prone to high nitrate inputs, DNT overrides other co-occurring processes like anammox. Consequently, the process serves as an important mechanism for counteracting N loading.

Future scope

1. Examination of oxidizing and reducing processes of N cycle could be carried out in tandem to elucidate spatial/temporal coupling at the genetic, cellular and community level.

2. Mn/Fe coupled denitrification in mangrove sediments could be examined to gain deeper understanding of their contribution to the reductive phase of the N cycle.

3. Nitrous oxide flux across the sediment - atmosphere interface could be quantified to enhance the contribution of mangrove ecosystem to \( \text{N}_2\text{O} \) inventory from the Indian Ocean region.

4. Contrary to our understanding that pristine mangrove habitats have a higher bacterial diversity, taxonomic investigations in the present study have shown
anthropogenically influenced mangrove sediments to contain more complex and diverse bacterial communities. It would also be interesting to examine if the metabolic diversity also follows a similar trend.