Chapter 2.
Review of Literature
2.1. Definition and distribution of mangroves

Mangroves are the tidal forests of coastal wetlands, existing in the intertidal zones of sheltered shores, estuaries, tidal creeks, backwaters, lagoons, marshes and mud-flats of the tropical and sub-tropical regions of the world. They form an important ecological asset and economic resource of the coastal environment. They are the most productive ecosystems, which fertilize the sea, and also protect the coastal zone. The word "Mangroves" refer to the plants and also the forest community. According to Macnae [1968] "Mangal" refers to the habitat or the forest community and "Mangroves" to the plant species. Duke [1992] has also reported the use of "Mangrove" as an adjective for "mangrove tree" or "mangrove fauna". The origin of the word "mangrove" could be traced to the Portuguese word ‘mangue’ (= a type of trees) and the English word ‘groves’ (= a group of trees). In French, the word ‘manglier’ is similar to ‘mangue’. Probably all these words originated from the Malay word, ‘Manggi-manggi’ [Macnae, 1968]. The mangrove forests are also referred to as "tidal forests", "oceanic rain forests" and "coastal woodlands". Changing wind velocity and patterns, tides, fluctuating temperature and salinity have resulted in the evolution of varied adaptive strategies in the natural mangrove flora and fauna. So far there are no reports on any terrestrial plant that can survive these adverse conditions [Kathiresan, 1991; Kathiresan and Bingham, 2001]. The 'Mangal' are constantly exposed to water-logged and anaerobic saline soils. Occurrence of Support roots, viviparous germination, salt-excreting leaves, breathing roots, knee roots, etc makes them well-adapted to coastal environment. Other aspects
like change in climate (precipitation and temperature), sea levels rise and incidence of solar ultraviolet–B radiation also pose ecological challenges to the coastal flora and fauna [Rahaman, 1990; Swaminathan, 1991; Moorthy, 1995; Moorthy and Kathiresan, 1996].

Alluvial soil is an excellent substrate facilitating successful growth of mangroves. These substrates are fine textured, loose mud or silt, rich in humus and sulphides [Rao, 1987]. They develop in low lying and broad coastal plains where the topographic gradients are very small and the tidal amplitude is large. Their distribution is governed by temperature [Duke, 1992] and they prefer moist atmosphere and freshwater inflow, which brings in abundant nutrients and silt from terrestrial sources. Unsheltered shores pose a potential threat to mangrove seedlings from waves and currents. Periodically flooded but well-drained soils support good growth of mangroves. Improper drainage is detrimental for mangrove vegetation [Gopal and Krishnamurthy, 1993]. Indian mangroves are distributed in about 6,740 sq.km [Krishnamurthy et al, 1987] which constitute 7% of the total Indian coastline [Untawale, 1987]. In general, there are three different types of mangroves in India viz., deltaic, backwater-estuarine and insular. The backwater-estuarine type is characterized by funnel-shaped estuaries of major rivers (Indus, Narmada and Tapti) or backwaters, creeks, and neritic inlets. The insular mangroves can be found in Andaman and Nicobar islands where many tidal estuaries, small rivers, neritic islets, and lagoons support rich mangrove flora [Gopal and Krishnamurthy, 1993]. Majority (70%) of the mangrove vegetation is encountered in the east coast while the west coast accounts for
only 12%. The rest is found along the bay islands of Andaman and Nicobar [Krishnamurthy et al, 1987; Kathiresan, 1995].

Natural regeneration of mangroves in Goa have been studied [Kumar, 2000] and various methods of regeneration of mangroves were described [Kumar, 1999]. In Maharashtra and Goa, mangroves exist mainly as large patches along the Mandovi estuary, the Vasishta estuary, the Savithri estuary, the Kundalika estuary, the Dharamtar creek, the Panvel creek, the Vasai creek, the Thane creek and the Vaitarana creek [RSAM, 1992]. The mangroves occur over an area of 5 sq.km in Goa. Mangroves in the Mandovi estuary of Goa spread to an area of 2,000 ha with distinct zones, which differ in environment, species composition and growth [FSI, 1997]. Mascarenhas and Chauhan [1998] have reported that Goa once had a luxuriant mangrove swamp of around 20 km inland from the open sea coast during the recent geological past, when the sea level was 1 to 3 m lower than present.

2.2. Ecology of mangroves
2.2.1. Microbial aspects in Indian mangroves

ecological niche to a variety of microorganisms [Agate, 1991] and about 125 species of microorganisms (bacteria, fungi, algae) have been identified [Kathirvel, 1996]. The photosynthetic microorganisms behave like heterotrophs in the mangrove environment. The cyanobacteria and photosynthetic bacteria survive in low light or partially dark conditions by utilizing the suspended organic matter, which are available abundantly in the mangrove waters [Rao and Krishnamurthy, 1994]. This unique heterotrophic adaptation of photoautotrophs, is a mechanism of survival in hostile coastal anaerobic and anoxic conditions of mangrove habitat [Rao and Krishnamurthy, 1994]. Hydrocarbonoclastic bacterial isolates have been reported from mangals of Andaman [Shome et al, 1996]. The sulphate reducing bacteria have been isolated from the mangrove swamps of Goa [Saxena et al, 1988; Lokabharathi et al, 1991]. Purple photosynthetic bacteria are reportedly isolated from Pichavaram mangrove sediments: two major groups viz., purple sulphur bacteria (family- Chromatiaceae, strains belonging to Chromatium sp.) and purple non-sulphur bacteria (family-Rhodospirillaceae, strains belonging to Rhodopseudomonas spp.) [Vethanayagam, 1991]. Besides sulphur bacteria, the iron oxidizing and iron reducing bacteria do exist in mangrove habitat. This type of bacteria is higher in mining areas of Goa than in non-mining mangroves areas of Konkan [Panchanadikar, 1993]. The methanogenic bacteria have been studied for the first time for their distribution and ecology in mangrove sediments of Pichavaram [Ramamurthy et al, 1990]. In general, the bacterial counts are maximum during
the post-monsoon months and the counts of fungi and actinomycetes are maximum during the monsoon months [Mini Raman and Chandrika, 1993].

2.2.2. Value of mangroves

The value of mangrove is a measure of its importance to society. Value of mangroves can be considered of three hierarchical levels: population, ecosystem and global. At the population level, mangrove-dependent fish, shellfish, animals and timber provide important and valuable harvests and recreational fishing and hunting. At the level of the whole ecosystem, mangroves have value to the public for flood mitigation, storm abatement, aquifer recharge, water quality improvement, aesthetic and general subsistence. At the regional and global level, mangroves contribute to the stability of available nitrogen, atmospheric sulfur, carbon dioxide and methane [Mitsch and Gosselink, 2000]

Mangroves may be important in returning the ‘excess nitrogen’ to the atmosphere through denitrification. Denitrification requires the proximity of an aerobic and a reducing environment as well as a source of organic carbon, something abundant in most mangroves. As most tropical mangroves are the receivers of fertilizer-enriched agricultural runoff and are an ideal environment for denitrification, they are likely to be important to the world’s available nitrogen balance. Also, ammonia for fertilizer production is manufactured from nitrogen gas at more than double the rate of all natural fixation. Wetlands in general have been recommended as a key ecosystem in providing a solution to this eutrophication [Mitsch and Gosselink, 2000]
2.3. Dynamics of nitrogen

Nitrogen cycle is of great concern because, together with carbon, hydrogen and oxygen, it is intimately associated with reactions carried out by living organisms. The cycling of other essential nutrients, especially phosphorous and sulphur is closely linked with biochemical nitrogen transformations. Nitrogen is considered as one of the major limiting factors in coastal waters, making the nitrogen dynamics in mangroves particularly significant. Nutrient flux measurements in mangroves have been widely reported [Boto and Wellington, 1988; Trott and Alongi, 1999; Harrison et al, 1983; Rivera-Monroy et al, 1995; Krishnamurthy et al, 1975]. Since most of the studies have been restricted to certain periods of the year, there exists a dearth in understanding these processes on an intra-seasonal/annual scale. New and regenerated production in ecological systems trigger a gamut of elemental flux studies, principally that deals with the key element, nitrogen [Dugdale and Goering, 1967]. The major nitrogen pools in mangroves are total sediment nitrogen (mostly organic nitrogen), total plant nitrogen, and available inorganic nitrogen in sediments. Organic nitrogen consists of compounds from amino acids, amines, proteins and humic compounds with low nitrogen content. Inorganic nitrogen consists of ammonium nitrogen, nitrate and nitrite nitrogen. In sediments, nitrites occur in trace quantities; whereas ammonium and nitrate nitrogen is the predominant form of inorganic nitrogen and is mainly derived through mineralization of organic nitrogen and further oxidation. The gaseous form of nitrogen includes ammonia, dinitrogen and nitrous oxide [Vymazal,1995]. The total sediment pool is the
largest, ranging from 100 to 1000\text{g N/m}\textsuperscript{2}. The total plant nitrogen pool is less than total sediment nitrogen while inorganic sediment nitrogen is the lowest [Faulkner and Richardson, 1989].

The original source of soil nitrogen is atmospheric nitrogen which occupies as much as 79% of the air composition and is believed to have originated from fundamental rocks of the earth's crust and mantle [Miller, 1999]. Gains in mangrove nitrogen occur by fixation of N\textsubscript{2} into organic nitrogen and by addition of ammonia, nitrate and nitrite in rainwater. Losses occur through plant removal, leaching and volatilization in terms of organic nitrogen, nitrate and ammonia, respectively. Organic nitrogen is converted to ammonium and nitrate ions by mineralization and to ammonia gas by ammonification. Ammonium ions are oxidized to nitrate ions by nitrification while nitrate ions are reduced to nitrogen by denitrification, thus completing the cycle.

2.3.1. States of nitrogen

Nitrogen appears in both oxidized and reduced states. A single nitrogen atom can serve as a terminal electron acceptor for eight electrons, from N (+5) of nitrate ions to N (-3) of ammonium ions. In most compounds nitrogen is either bonded to carbon and hydrogen, where the oxidation state of the nitrogen is negative (such as amines, amides, proteins and urea), or bonded to oxygen (such as nitrate, nitrite and nitrous oxide), where the oxidation state is positive.

The mechanisms involved in nitrogen cycling in mangroves include N\textsubscript{2} fixation, ammonia volatilization, ammonification, nitrification, denitrification and nitrous oxide production [Wen et al, 1997].
2.3.2. Nitrogen transformations

Nitrogen fixation is a process where atmospheric nitrogen is converted into organic nitrogen, either chemically by lightning or biologically by microorganisms. The covalent triple bond of the N$_2$ molecules is highly stable and can only be broken artificially at elevated temperature and pressure. Ammonia volatilization is a process where ammonium-nitrogen is in equilibrium between gaseous and hydroxyl form. Ammonia loss to the atmosphere is related to both pH and ammonium ion concentration. About 0.036, 0.36, 3.6, and 36% of the total reduced nitrogen in the soil solution is present as ammonia at pH values of 6, 7, 8, and 9 respectively [Stevenson and Cole, 1999]. Reddy and Patrick [1984] pointed out that losses of ammonia through volatilization from flooded soils and sediments are insignificant when the pH value is above 9.3. Losses also increase when the temperature and wind speed over the soil surface increase. Ammonification is the biological transformation of organic nitrogen to ammonium or ammonium ions. The majority of the reduced nitrogen produced in this way stay within the sediment despite a small portion being volatilized. The optimal pH range for ammonification process is between 6.5 and 8.5 [Patrick and Wyatt, 1964]. The large fraction of the organic nitrogen in many wastewaters is readily converted to ammonia [Kadlec and Knight, 1996]. The rate of aerobic ammonification doubles with a temperature increase of $10^\circ$C [Reddy et al, 1979]. Nitrification is the biological oxidation of ammonium to nitrate with nitrite as an intermediate in the reaction. Firstly, ammonium is oxidized to nitrite. This step is executed by strictly aerobic bacteria which are entirely dependent on the
oxidation of ammonia for the generation of energy for growth. The second step is the oxidation of nitrite to nitrate that is performed by facultative chemolithotrophic bacteria which at the same time can utilize organic compounds for energy generation. Optimal temperature range for nitrification in soils is from 30 to 40°C whereas the optimal pH value is from 7.5 to 8.6. Denitrification is the reduction of nitrate to molecular nitrogen under anoxic conditions, where nitrogen is used as an electron acceptor. The end product of denitrification is N₂ but nitrogen oxides can be produced if electron donors are insufficient. Presence of suitable electron donors, such as organic carbon compounds, reduced sulphur compounds and molecular hydrogen, are major limits for denitrification. Denitrification is also sensitive to pH because denitrifying enzyme reductase breaks down at low pH. The optimal pH range for denitrification lies between 7 and 8. Denitrification increases at temperatures of 25°C and above, proceeding at a progressively slower rate at lower temperatures, and finally ceases at 2°C.

Almost all process in the conventional nitrogen cycle can occur in close proximity, either spatially or temporally, in the ecosystem structure of mangroves. Seasonal drying and wetting cycles allow efficient ammonification and nitrification [McLachlan, 1970]. At the same time, ideal conditions for denitrification are provided by the slow oxygen diffusion rates in hydric soils combined with an oxygen demand generated from the high primary production in mangroves. Not all transformations of soil nitrogen are mediated by microorganisms but some are chemical in nature. Ammonia can be fixed by the soil organic fraction which is not readily available to plants or microorganisms. Nitrite can react with organic
constituents, including humic and fulvic acids, with part of them being converted to organic forms and part of them being lost as gases. Ammonium ions can be fixed on interlamellar surfaces of clay minerals and the hydrated micas. The magnitude of these non-biological processes varies from one soil to another and influences the fate of inorganic forms of nitrogen in soils [Stevenson and Cole, 1999].

2.4. Factors governing nitrification rates

The benthic compartment together with the overlying pelagic system forms an important avenue for a plethora of reactions in the nitrogen cycle. It has been recognized that nitrogen plays an important role in fertility of the benthic system. A major fraction of the nitrogen in sediment is bound to organic matter and very little mineral nitrogen is present at any given time [Chu et al, 1998]. Inputs of nitrogen to terrestrial and aquatic ecosystems have increased several-fold over the last one hundred and fifty years, with a very large increase during the last forty years [Holland et al, 1999]. Higher magnitude of fertilizer production and its indiscriminate use together with increased fossil fuel combustion and widespread cultivation of nitrogen fixing crops have contributed to the striking increase in nitrogen inputs [Smil, 1990; Galloway et al, 1995 and Vitousek et al, 1997].

Triska et al [1990] and Jones et al [1995] have reported that in streams, nitrification rates were primarily governed by the supply of ammonium and oxygen. In sediments these processes occur close to the sediment-water interface and in the oxidized lining of animal burrows [Boto, 1982] and is mainly
controlled by the availability of ammonium, dissolved oxygen as well as the population dynamics of nitrifying bacteria [Hansen et al, 1981]. It has been reported that bioturbation enhances the nitrifying activity down the sediment [Aller, 1988] while benthic micro algae can become strict competitors with nitrifiers for the nitrogen source [Nielsen et al, 1990]. Trace metals like iron has a well-established role in the process of nitrification both in the aerobic and anaerobic regions. While laboratory studies have shown that anoxic nitrification to be thermodynamically possible [Anschutz et al, 2000; Hulth et al, 1999; Luther et al, 1997], Mortimer et al [2002] found significant evidence for such a reaction during high-resolution analysis of sediments. Apart from its role in respiration, iron also serves as the integral part of the enzymatic system involved in nitrification. Studies suggest that iron is capable of forming a catalytic component of ammonium monoxygenase (associated with the cell membrane) of Nitrosomonas europaea and possibly a part of the oxygen-activating center [Zahn et al, 1996]. Nitrification was traditionally considered to be restricted to aerobic environments [e.g. Froelich et al, 1979], but recent studies [Mortimer et al, 2004] have shown that nitrification does happen in anoxic environments at the expense of elements like manganese and/or iron. In general, benthic nitrification rate is regulated by the availability of dissolved oxygen [Caffrey et al, 2003] and ammonium [Henriksen and Kemp, 1988]. It also depends on ammonium regeneration rates, which in turn is positively influenced by temperature [Nixon, 1981].
2.5. Benthic nitrogen cycling in mangroves – National and International scenario

The Indian mangrove ecosystems like its other tropical counterparts form an integral part of the coastal buffer zone. In spite of its ecological significance, due to several logistic constraints, studies, especially that on nutrient chemistry and associated biological processes have been confined to a few months in an annual cycle and very few studies have traced key nitrogen conversions with respect to the various phases of the monsoon cycle. Dham (2000), Heredia (2000) and Dham et al [2002] had studied the seasonal variation in nitrogen fluxes in mangrove sediments and waters along the west coast of India. But these studies have been limited to the involvement of various fractions of algae on several aspects of the nitrogen cycle. The nitrogen fixation by microorganisms has been investigated in mangroves. Nitrogen-fixing bacteria, *Azotobacter* species have been isolated from sediments of Pichavaram mangroves and their counts were more in the mangrove habitat than in marine backwaters and estuarine systems [Lakshmanaperumalsamy, 1987]. Nitrogen fixing bacteria in the rhizosphere of mangrove plant community have been quantified in the Ganges river estuary and the bacterial counts were reported to be high in inundated swamps and low in occasionally inundated ridges and degraded areas of mangroves [Sengupta and Chaudhuri, 1990].

The international scenario is not much different from the national contributions. In fact most of the aspects we know in general come from work published on Indian mangroves. In other tropical mangroves, most of the nutrient
flux studies have been confined to a limited period of the annual cycle [Boto and Wellington, 1988; Trott and Alongi, 1999; Harrison et al, 1983; Rivera-Monroy et al, 1995].

2.6. Biodiversity of nitrifiers

Based on comparative 16S rRNA gene (rDNA) sequence analysis, cultured ammonia-oxidizing bacteria comprise two monophyletic groups within the Proteobacteria. *Nitrosoccus oceanus* and *N. halophilus* belong to the gamma subclass of the class Proteobacteria [Woese et al, 1985], while the members of the genera *Nitrosomonas* and *Nitrosospira, Nitrosovibrio, and Nitrosolobus* (the latter three being closely related to each other [Head et al, 1993]), as well as *Nitrosococcus mobilis* (actually a member of the genus *Nitrosomonas*) constitute a closely related assemblage within the beta subclass of Proteobacteria [Head et al, 1993; Pommerening-Röser et al, 1996; Stehr et al, 1995; Teske et al, 1994; Utaker et al, 1995; Woese et al, 1984] Based on ultrastructural properties, cultivable nitrite-oxidizing bacteria have been assigned to the four recognized genera, viz *Nitrobacter, Nitrospina, Nitrococcus, and Nitrospira*. Comparative 16S rRNA sequence analyses revealed that one of these genera, *Nitrobacter* [Winogradsky, 1892], with its four species namely *N. vulgaris, N. alcalicus, N. hamburgensis* and *N. winogradskyi* [Bock et al, 1983 and 1990], is a member of the alpha subclass of Proteobacteria [Orso et al, 1994; Teske et al, 1994]. The genera *Nitrospina (N.gracilis)* and *Nitrococcus (N.mobilis)* [Watson and Waterbury, 1971], with one species each, belong to the delta and gamma subclass of Proteobacteria, respectively [Teske et al, 1994].
The remaining genus, *Nitrospira* [Watson et al, 1981], encompassing the species *Nitrospira moscoviensis* [Ehrlich et al, 1995] and *N. marina* [Watson et al, 1986], is a member of the *Nitrospira* phylum of the domain *Bacteria* Ehrlich et al, 1995].

16S rRNA based tree showing the phylogenetic affiliation of ammonia and nitrite oxidizing bacteria. The scale bar indicates 0.1 estimated change per nucleotide. (Image Courtesy: http://www.microbial-ecology.net/nitrifiers.asp)

2.7. **$^{15}$N isotope as a tracer for nitrogen distribution**

2.7.1. **Natural abundance of $^{15}$N and its uses**

There are six known isotopes of N, but only $^{14}$N and $^{15}$N are stable. Most of the Earth's N occurs as the stable isotope $^{14}$N (99.634% of atmospheric N) whereas the natural abundance of $^{15}$N at atmosphere is only 0.366%. The advantage of using $^{15}$N as a tracer is that it is non radioactive. Therefore
experiments can be carried out over long period of time. Unlike those radioactive isotopes, $^{15}\text{N}$ has no health hazards and so permission is not necessary for experiments carried out in fields and research labs.

$^{15}\text{N}$ is being used in the studies related to 1) nitrogen balance in sediments and waters, 2) stabilization of N through immobilization, 3) uptake of soil and fertilizer N by plants and fate of residual fertilizer N in soil, 4) losses of soil and fertilizer N through leaching and denitrification, 5) biological $\text{N}_2$ fixation, 6) fixation of ammonium ions by clay and ammonia by organic matter and the availability of the fixed N to plants and microorganisms, and 7) relative use of ammonium and nitrate ions by microorganisms and higher plants.

2.7.2. Assumptions and possible inaccuracies

There are some key assumptions for the usage of $^{15}\text{N}$ in soil N studies. First, the isotope composition of N in the natural soil is expected to remain constant over time. Second, living organisms are believed to use $^{14}\text{N}$ and $^{15}\text{N}$ isotopes in an indiscriminate manner. Third, the chemical reactivity and response to physical factors of the two isotopes are assumed as identical and remain constant over time.

However, slight variations occur in the N isotope composition of soil. In general, $^{15}\text{N}$ values increase with depth in soil profiles while tree tissues and fresh litter are slightly depleted in $^{15}\text{N}$ relative to soils. $^{15}\text{N}$ values also increase with soil age, organic matter age and extent of decomposition. The divergence is mainly due to small difference in mass of $^{14}\text{N}$ and $^{15}\text{N}$ isotopes. For instance, the oxygen-nitrogen bond of $^{14}\text{N}-\text{NO}_3$ is weaker than that of $^{15}\text{N}-\text{NO}_3$, so
denitrification of $^{14}$N-NO$_3$ occurs more readily than of $^{15}$N-NO$_3$. Preferential sorption of $^{15}$NH$_4^+$ on clays and other cation-exchange surfaces than $^{14}$NH$_4^+$ leads to comparatively high $^{15}$N depletions in soil solution. Consequently, less $^{15}$N is available for plant uptake and in turns, results in $^{15}$N depletion in plants than in soil. $^{15}$N content of mineralized N derived from humus is not constant. Feigin et al [1974] showed that the $^{15}$N content of soil-derived nitrate increased with incubation time. Nitrogen inputs that are depleted in $^{15}$N is possible for the crucial cause of lower $^{15}$N values in vegetation and litter at the soil surface, while the discrimination against $^{15}$N during mineralization and the relative isolation of soil N from atmospheric input may result in higher $^{15}$N values at deeper soils [Lajtha and Michener, 1994]. Consistent differences in $^{15}$N between plant species are also demonstrated, through the mechanism behind is not yet known [Robinson, 2001].

2.7.3. Nitrogen balance studies with $^{15}$N isotope

As N cycle is a very complex system, it is very different to prepare reliable balance sheets where all gains and losses are accounted for. It is complicated to estimate whether the losses are through leaching, incomplete nitrification, complete or incomplete denitrification or ammonia volatilization. Also, it is intricate to determine whether the N increment in soil is owing to N$_2$ fixation, N deposition or addition of N fertilizer. The $^{15}$N-enrichment approach takes an advantage over these problems as it has the ability to quantitatively trace a given N input through the various pools.
Natural abundance studies of $^{15}\text{N}$ can provide information on overall patterns of N cycling, sources of N inputs and even perturbations in nutrient cycles from decades or centuries. A novel and rather new approach is to use $^{15}\text{N}$ labels in large scales. The use of $^{15}\text{N}$ tracers in an adequate scale is to cancel out small but detectable shifts in $^{15}\text{N}$ natural abundances in nitrogen pools. In other words, it can counteract that previously mentioned inaccuracies when quantifying the $^{15}\text{N}$ balance in ecosystems.

Several conditions must be met to obtain trustable tracing sensitivity in N addition balance work. First, reliable estimates should be obtained for the size of the various N pools. Second, representative samples have to be collected for analysis. Third, the experiment must be performed with an adequate replication and proper local controls.