Microbial diversity of soil is an indicator of soil health as strong relationships exists between the soil microbial diversity and ecosystem sustainability. Bacteria are key players that perform unique reactions, responsible for nutrient cycling in various ecosystems, like nitrogen carbon and sulfur cycle, biodegradation of organic and inorganic compounds etc. However existing knowledge about bacterial diversity in natural environments is limited, as bacterial population less than 1% have been cultivated through traditional lab cultivation methods and characterised, indicating that much genetic and metabolic information is yet to be retrieved (Amann et al., 2001). The ‘great plate count anomaly’ states that 95–99% of the microbial community present in the environment is not accessible by traditional cultivation techniques (Nichols, 2007), and this fraction of the microbial community is referred to as uncultured micro biota or unculturables.

A plethora of molecular and biochemical methods have been applied to reveal the microbial community composition over time in response to environmental changes. Due to the limitation of cultivation based methods, recent year’s culture independent analysis has been widely used for community analysis (White et al., 2010; Jackson et al., 2013; Bevivino et al., 2014). There are mainly two approaches based on molecular analyses for studying microbial communities using DNA extracted from the soil. (i). Partial community DNA analysis investigates only a part of the genome sequence targeted and amplified by PCR. (ii). Whole community DNA analyses focuses on whole genetic information contained in the extracted DNA (Ranjard et al., 2000). Culture independent methods are based on the principle of resolving the diversity of the amplified sequences by differential electrophoretic migration on agarose or polyacrylamide
gels, depending on either size (ARDRA, RISA/RFLP) or sequence (DGGE, TGGE), the fingerprints provides the genetic structure of the community.

Mangroves ecosystems are distributed along the tropical and subtropical coastline and located at the interface between terrestrial and marine environments and frequently inundated by flooding and high tides (Holguin et al., 2001; Giri et al., 2011). Throughout the world the mangroves are the most productive ecosystem because of their exceptional floral and faunal diversity (Giri et al., 2014; Goessens et al., 2014). These forested wetlands are socio economically and ecologically important for local communities which serve as source of wood and non wood forest products (Kristensen et al., 2008; Walters et al., 2008). In addition, the mangrove ecosystems serve as the breeding grounds for fish, shellfish, spawn etc. They also act as living barrier by protecting coastal communities’ against the effect of wind, waves and water current. Mangrove are partially anaerobic, with high salinity and oxido-reductive potential, which makes it difficult for organic matter to degrade, thus limiting the nutrient availability to other living forms (Holguin et al., 2001; Ferreira et al., 2010). The bacterial communities associated with the mangroves play important ecological and biogeochemical roles in mangrove ecosystems (Xu et al., 2006; Cao et al., 2011), such as carbon, nitrogen, phosphorus, iron, and sulphur cycle (Nealson, 1997). Environmental factors like salinity (Silveira et al., 2011), organic carbon matter (Dunaj et al., 2012; He et al., 2012a), nitrogen content (Carreiro-Silva et al., 2012) and plant type (Yergeau et al., 2010) play a major role in determining the microbial community and their functions. Even though a number of environmental factors influence the microbial population of this ecosystem either directly or indirectly, the root exudates determine the microbial community and their spatial distributions (Dennis et al., 2010).
The rhizosphere associated bacteria mediate different ecological functions such as nitrogen-fixation, phosphate solubilisation and provide nutrient to plant and thus increase plant growth (Canbolat et al., 2006; Hameeda et al., 2008). It is known that mangrove microbiota is a combination of terrestrial soil, freshwater and marine microorganisms (Ananda and Sridhar, 2002). Only few studies regarding the diversity and function of bacterial has been conducted and hence little is known about their distribution among different mangrove ecosystems (Hewson and Fuhrman, 2004; Zhang et al., 2008; Zhou et al., 2009; Gomes et al., 2010; Santos et al., 2011; Silveira et al., 2011). Novel genus Swaminathania salitolerans (Loganathan, 2004) and Mangrovibacter plantisponsor from the rhizosphere of Portersia caroctata of Pichavaram mangroves have been reported (Rameshkumar et al., 2010). Along with bacteria that inhabit mangroves, the domain Archaea is also of great importance, as the anoxic conditions and high salinity in these habitats support the growth of archaea (Buckley et al., 1998; Cavicchioli, 2011; Dias et al., 2011; Yan et al., 2006). Recent developments in molecular methods have shown that Archaea are more diverse and widespread and plays a major role in nitrogen cycling and other biogeochemical cycling in an ecosystem (Chaban et al., 2006). Though culture dependant diversity analysis has been carried out, a lot need to be explored regarding the bacterial diversity of the mangrove ecosystem, which can be achieved by the culture independent methods. Though many studies have focused on phylogenetic analysis of microbial communities from mangroves based on 16S rRNA-approaches, such as PCR cloning (Li et al., 2011a), DGGE (Muyzer 1999; Tian et al., 2008), T-RFLP (Flores-Mireles et al., 2007) and Pyrosequencing (Roh et al., 2010; dos Santos et al., 2011) to understand the diversity of microbial community without the need of isolating them by traditional cultivation methods.
Nitrogen and phosphorus are the major nutrients that limit the growth of mangroves (Bashan and Holguin, 2002; Reef et al., 2010). Nitrogen cycle within mangroves is mediated mainly by microbial activity rather than other chemical process. Nitrogen cycling involves four processes: viz., nitrogen fixation, nitrification, denitrification and ammonification (Alongi et al., 1992; Purvaja et al., 2008) carried out by microbes. But N₂-fixation by diazotrophs was found to be a major input of nitrogen to the ecosystem (Sengupta and Chaudhuri, 1991; Woitchik et al., 1997; Reef et al., 2010). It has been estimated that nearly 40-60% of total nitrogen that is required by this ecosystem is contributed by microbial activity (Holguin et al., 2001). Novel nitrogen fixing Vibrio spp. and Mangrovibacter plantisponspor (Rameshkumar et al., 2010), were reported from the rhizosphere of Porteresia coarctata of Pichavaram mangroves. The nifH gene, which encodes the Fe protein component of nitrogenase enzyme complex, has been used as a functional gene to characterize diazotrophic communities in many different habitats such as marine sediments, microbial mats, terrestrial soils, sea bed, lakes, rivers, estuaries, the rhizosphere of different plants, and bioreactors (Zehr et al., 2003). Although the description and isolation of microorganisms that provides nitrogen content to this nitrogen deficit ecosystem has been done extensively, very little advancement has been made in understanding their role and function in this ecosystem.

Major factors that contribute to nitrogen loss in these ecosystems are tidal inundation, denitrification, and soil nature (Boto and Robertson, 1990; Reef et al., 2010). The occurrence of denitrifying bacteria in the mangrove rhizosphere is of interest because it implies loss of fixed nitrogen via denitrification, and it is known that nitrogen is a limiting nutrient in this ecosystem (Holguin et al., 1992, 2001). Denitrification is a dissimilatory
process in nitrogen cycle in which oxidized nitrogen is used as an alternative electron acceptor for energy production where there is a limited oxygen supply and consists of four steps in which nitrate is reduced to nitrite and then to dinitrogen gas (Zumft et al., 1997). Oxygen fluctuation that occur in the rhizosphere due to tidal inundation may support the growth and distribution of denitrifying bacteria capable of using alternate electron acceptors and thus obtaining energy without the need of oxygen.

The genetic diversity of denitrifiers in a different natural habitats and laboratory conditions have been studied based on nitrite reductase genes nirK, nirS and nitrous oxide reductase gene nosZ, the key enzymes in the denitrification process that code for copper containing and cytochrome cd1-containing nitrite reductase, and nitrous oxide reductase respectively.

Apart from nitrogen fixation and denitrification process in mangrove ecosystem, other microbial group that involved in the transformation of nitrogen is ammonia oxidizers which comprises of two major groups, ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA). Very few studies are available on this microbial distribution in mangrove ecosystem. Aerobic ammonia-oxidizing archaea and bacteria plays a major role in nitrogen cycle by converting ammonia to nitrite (Kowalchuk and Stephen, 2001; Schleper and Nicol, 2010). The converted nitrite is further oxidized to nitrate by nitrite-oxidizing bacteria or is used as an electron acceptor by the nitrate reducers and denitrifiers. Based on 16S rRNA gene and amoA gene that codes for the α-subunit of the enzyme ammonia monooxygenase in aerobic ammonia-oxidizing archaea and bacteria showed that Thaumarchaea in archaean group and betaproteobacteria (β-AOB) of the bacterial group are widely distributed (Kowalchuk and Stephen, 2001; Schleper and Nicol, 2010) and occur in
many habitats, including soils of mangrove forests (Wickramasinghe et al., 2009).

Bacterial communities in well-preserved mangroves have been studied by Dias et al. (2010), man-made mangroves (Gomes et al., 2008), and hydrocarbon contaminated mangroves (Taketani et al., 2010a). But phylogenetic and functional description of microbial diversity in the mangrove ecosystem has received less attention compared to other environments (Zhou et al., 2006). It has been proposed that studying diversity within certain functional guilds will be more useful than studying the total diversity of microbes in an ecosystem (Cavigelli and Robertson, 2001). Hence a complete description of the bacterial diversity, their distribution and in-depth exploration of their function in the mangrove would improve our understanding of their role and mode of interactions in that ecosystem.

The existing reports on the bacterial diversity of the mangrove ecosystem of South India is very limited and does not give a clear picture on the role of these microbes in this ecosystem. Previous reports supported the fact that this ecosystem harbors unreported unique bacterial populations and thus the scope of identifying novel groups of bacteria with novel functions is high. The unculturable diversity of this ecosystem has not been attempted so far and exploring the unculturable diversity may throw more light on the microbial community of this ecosystem. Further the role of the bacterial diversity in nutrient cycling is unknown.

Therefore, it is necessary to explore the diversity, composition, and structure of mangrove microbial communities and their link with environmental factors for improving our knowledge on mangrove ecosystem functioning. This work is an attempt to access the distribution of total microbial community, analysing the unculturable and culturable
diversity of nitrogen fixing bacteria and denitrifying bacteria, analysis the unculturable diversity of ammonia oxidizing bacteria and archaea from rhizosphere of four different mangrove plants through traditional cultivation methods in combination with molecular tools such as Denaturing Gradient Gel Electrophoresis (DGGE). To accomplish this, the 16S r DNA of the total community, the diversity of nirS, nosZ and nifH genes in culturable and unculturable nitrogen fixers and denitrifiers, CTO gene for AOB and AamoA for archaea diversity analysis were investigated.

**Objectives of the present study:**

1. Community profiling of Mangrove associated total bacteria and archaea by DGGE analysis


4. DGGE analysis of ammonia oxidizing bacteria (AOB) and archaea (AOA) from the total soil DNA.