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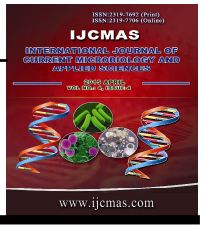

Original Research Article

Comparative Diversity Study of Nitrifying and Denitrifying bacteria from National Parks of Gujarat, India

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A B S T R A C T

Keywords

Nitrifying Bacteria, Denitrifying Bacteria, Forest soil

In this paper, a database of nitrifying and denitrifying bacterial species present in four national parks of Gujarat has been created. A quantitative and qualitative estimation of soil microorganisms is, therefore, necessary to know the role of different microbes, operating in the soil which, in turn, helps in various ways. Thus as per forest soil types, it will surely help in suggesting strategies for sustainable forest management. This kind of Identification will help evaluating the role of the same in forest soil in mitigation of greenhouse gas emissions as managing terrestrial microbial processes is a tantalizing prospect for the future.

Introduction

Soil is a dynamic habitat for an enormous variety of life-forms. It gives a mechanical support to plants from which they extract nutrients (Heritage et al. 1998). Microbes are major contributors to the functioning of the biosphere, being indispensable for the cycling of the elements essential for life. They also are a source of nutrients at the base of all ecological food chains and webs (Willey et al., 2008).

Nitrogen is often considered as one of the most important factors for primary production in terrestrial plants (Hara A, S. et al., (2010)

Nitrifying bacteria change ammonium (NH_4^+) to nitrite (NO_2^-) then to nitrate (NO_3), a preferred form of nitrogen for grasses and most row crops. Thus the nitrification process can be defined as the biological transformation of reduced forms of nitrogen to nitrate. Nitrate is leached more easily from the soil, so some farmers use nitrification inhibitors to reduce the activity of one type of nitrifying bacteria. Nitrifying bacteria are suppressed in forest soils, so that most of the nitrogen remains as ammonium (Rygiewicz, P. T., & Ingham, E. R. (1999).

Nitrification is an important soil process that can affect fertilizer use efficiency, the potential for NO^{3-} movement into potable water sources, and loss of N_2O to the atmosphere as a global warming gas (Hanson, E. J. et al, 2002)

The production of N_2O during nitrification by microorganisms in soils is also apparently an important source of atmospheric N_2O (Simpson et al., 1977). Jetten M.S.M. (2008) came up with the novel ideas regarding the microbial nitrogen cycle which also includes few species of nitrogen-fixing bacteria in special habitats.

Denitrification where the reduction of nitrate to nitrous oxide or nitrogen by denitrifying bacteria, is the major biological mechanism by which fixed nitrogen returns to the atmosphere from soil and water (Henry et al., 2004). Denitrifiers are anaerobic in saturated soils or inside soil aggregates. Globally, the production of N oxides by denitrification has been implicated as an important source of greenhouse gases (Matson & Vitousek 1990) and as one of the gaseous products of denitrification, N_2O, affects levels of stratospheric ozone and is an important absorber of infrared radiation (Rasmussen and Khalil 1986). Denitrification is also important in reducing NO_2^- and pollution of groundwater (Keeney 1987). In terrestrial habitats, the microbial fixation of atmospheric nitrogen is carried out by free living bacteria and by bacteria living in systemic association with plants like Rhizobia (Atlas R.,1984) and its relation to climate change is well understood, both in terms of physiology and community structure (Singh et al. 2010). The biological communities vary with different locations and these complex communities contribute significantly to the continuous cycling of nutrients across the globe (Heritage et al. 1998). Regional Isolation and Identification of such communities could prove of utmost significance as delivery of microbially mediated ecosystem functions, such as denitrification, is influenced by both the structure and activity of the microbial community (Peralta et al., 2010). Bhatt et al., 2015 came up with such study for national parks of Gujarat. Because N supply often limits primary production and other ecosystem processes over much of the natural world, human alteration of the N cycle has the capacity to change Earth's ecosystem substantially (Matson et al., 2002). The biogeochemical cycling of nitrogen is largely dependent on the metabolic activities of microorganisms alone (Atlas R., 1984). And hence the need for identification of microorganisms becomes inevitable. Also recently, Bardgett et al., 2008 emphasized the urgent need for greater understanding of how soil microbial ecology contributes to land–atmosphere carbon exchange in the context of climate change, and identify some challenges for the future.

Materials and Methodology

Study area

Random sampling of soil collection was done using GPS. The GPS points were decided randomly knowing the different eco-zone in the park and were decided according to the area of National Park which should be at least 25% of the area of National Park Published by FAO-Data. The soil samples were collected from on site at 3 different depths: 0-10cm, 10-20cm, and 20-30 cm in the months of December 2011, January and February and March 2012 for four National parks respectively.

1) **The Gir National Park** (also known as Sasan- Gir) is a dry deciduous forest in the semi-arid western part of India covering area of 258.71 square kilometer in Gujarat, India established in 1965. Gir has a topography made up of succession of

rugged ridges, isolated hills, plateaus and valleys.

2) Velavadar National Park: Established in 1976 in the in the Bhavnagar District of Gujarat state, India. It is spread over an area of 34.08 sq.km and typically Grassland, semi-arid bio-geographical zone about 50 kilometers west of the Gulf of Cambay.

3) **Marine National Park:** Situated on the southern shore of the Gulf of Kachchh in the Jamnagar District of Gujarat state, India. It is Mangrove forest and area 162.89 sq.km. There are 42 islands; best known ones are Pirotan, Narara, Sikka and kalubhar on the Jamnagar coast in the Marine National Park, most of them surrounded by reefs.

4) **Vansda National Park**: Representing moist deciduous forest covering area of 23.09sq.km having thick woodlands of the Dangs and southern Gujarat, in the mountains of Western Ghats or Sahyadris. It is situated in the Navsari District of Gujarat state, India. Vansda, the town is an important trading place for the surrounding area where the majority of the population is represented by adivasis.

Soil sample collection and preservation:

The Soil Samples were collected from four National parks mentioned above. The random soil sampling was done from the surface at the depth of 0-30 cm using GPS. Sampling Tool Hand auger used was also sterilized. Samples were put in sterile Plastic zip locked bags and immediately refrigerated in Laboratory.

Soil analysis (Physiochemical and Microbial)

Physicochemical analysis of soil

The physicochemical characteristics of soils were analyzed by standard methods. Soil pH, Electrical conductivity of soil, Bulk density of soil, Soil organic carbon percentage, Soil organic carbon density, these all characteristics were measured by standard methods like respectively.

1. The newer methods of measuring soil pH in a laboratory by taking 1:5 suspension of soil in 0.01 M $CaCl_2$ is being followed to measure pH of all four national park soil samples.
2. Electrical Conductivity was measured by standard Electrical Conductivity Meter.
3. The core method was used for the collected of samples for the bulk density determination. Soil samples were air dried ground and passed through a 2mm sieve before being used for analysis.

Microbial analysis of soil: Microbial analysis of soil was done by Media preparation, Autoclaving, Serial Dilution, Inoculation of the media for Isolation of organisms, Colony counting and identification of the same.

Media preparation: The media used were Nutrient Agar, Asparagines Nitrate medium and Inorganic salt medium (Frankland et al., 1995) respectively to isolate different species of nitrifying and denitrifying bacteria. Appropriate amount of media powder was suspended in respective amount of distilled water. To dissolve the medium completely, it had to be heated till boiling. The media have been autoclaved at 121°C and 15 lbs pressure.

Nutrient agar and asparagine nitrate medium: Isolation and identification: An accurately weighed sample of 1g fresh soil was mixed with 10ml of D/W to make sample suspension. This sample was diluted up to 10^{-3} dilution by serial dilution method

and the suspension was spread over media with the help of sterile glass spreader in aseptic condition (Robertson and Egger, 2010). The spread plates were permitted to absorb the inoculums for at 37°C temperature for 24 hrs. They were inverted and incubated as desired. Morphological characteristics of all different colonies were observed which included size, shape, colour, elevation and transparency. Each and every different colony was identified with Gram stain Method (Gupta P.K., 2006) then was followed by Colony Counting (Ronald, 1984). Plates with 30 to 300 CFUs/plate were used to calculate CFUs/ml. If fewer than 30, it runs into greater statistical in accuracy and if greater than 300, the colonies would be tedious to count and also would tend to run together (Davis K. E., e t al., (2005).

Inorganic salt medium: Isolation and identification:

An accurately weighed sample of 1g fresh soil was mixed with 10ml of D/W to make sample suspension. This sample was diluted up to 10^{-3} dilution by serial dilution method. Using the autoclaved test tubes filled with the medium inoculated with the soil sample, were left for 24-48 hours. Then, it was observed for turbidity and in turn the growth of rhizobia in specific.

Result and Discussion

Gir National Park

On nutrient agar, bacteria found and identified were *Nitrobacter species* and *Nitrosomonas species* are nitrifying bacteria where as on Aspargine Nitrate Medium the species found were *Micrococcus species, Bacillus species* and *Pseudomonas species* and were denitrifying bacteria.

Velavadar national park

On nutrient agar, both nitrifying and denitrifying bacteria were identified. *Nitrobacter species* is nitrifying bacteria where as on Aspargine Nitrate Medium the species found was *Bacillus species* which is denitrifying bacteria.

Vansda national park

On nutrient agar, both nitrifying and denitrifying bacteria are identified. *Nitrobacter species* is nitrifying bacteria where as on Aspargine Nitrate Medium the species found was again *Bacillus species* which is denitrifying bacteria.

Marine national park

On nutrient agar, both nitrifying and denitrifying bacteria are identified. *Nitrobacter species* and *Nitrosomonas species* are nitrifying bacteria where as on Aspargine Nitrate Medium the species found were, *Bacillus species*, Seratia species and *Pseudomonas species* which were denitrifying bacteria.

Inorganic salt medium

Inorganic salt medium showed luxuriant growth which indicates presence of rhizobia species in all four national parks. In terrestrial ecosystems, the responses of plant communities and symbiotic microorganisms, such as rhizobia and nitrogen fixing bacteria, to climate change is well understood, both in terms of physiology and community structure (Singh et al. 2010). Bacteria in the genus Rhizobia, methanotrophs and Archaea are involved in a complex interplay that affects the N cycle dynamics (Lowe et al. 2012). Rhizobium is of extreme importance for maintaining soil fertility as able to fix atmospheric nitrogen (Atlas, 1984). Marine national park showed

maximum turbidity indicating highest growth of rhizobia followed by Gir then Vansda and least growth in Velavadar National park.

When growing in association with plants, symbiotic nitrogen fixing bacteria, such as rhizobium species, generally exhibit rates of nitrogen fixation that are two to three orders of magnitude higher than those accomplished by free-living, nitrogen fixing soil bacteria (Atlas R., 1984). Also, nitrogen-fixing bacteria may play an important role in forest bed soil for nitrogen supply (Hara A et al., (2010). In forest, symbiotic nitrogen fixing bacteria, including actinomycetes, live in association with various trees and make significant contributions to the soil nitrogen needed to support the growth of forests (Atlas R., 1984). Our results were in accordance with again ecological type of the forest with Gir and Vansda showing luxuriant growth whereas Velavadar and Marine national park showing the decreasing order. Following graph shows park wise species obtained. Gir being deciduous forest can be a home to varied biodiversity as we are getting maximum nitrifying and denitrifying bacterial species present in Gir National Park.

Bacillus and Nitrobacter are common species found in all four national parks. Pseudomonas species is found in Gir and Marine National Park signifies the healthy environment in the said forest as *Pseudomonas* help to keep harmful organisms like parasitic fungi in check as well as it forms mutualistic relationships where both parties benefit and can help keep plants healthy. Bacteria of the genus *Pseudomonas,* and *Thiobacillus* species can also reduce nitrates to liberate nitrogen gas into the environment (Heritage et al. 1998).

Minimum pH of 5.82 in Dang and maximum value for pH of 10.25 is obtained in MNP. A pH range (Gir and Dang) of approx. 6-7.5 promotes the most ready availability of plant nutrients. Bhatt, M. & Sapra, N. (2015) showed the estimates of total nitrogen availability in same forest soil types of Gujarat.

Slightly acidic soils to moderately alkaline soils found in Gir and Dang shows high rate of decomposition. Highly alkaline pH values shows low decomposition rates increasing turn over times of nutrient. Though Gir and Marine both show highest amount and number of species but as the pH in Marine is alkaline in nature signifies low decomposition rates as compared to Gir and Vansda National Park.

Conclusion and future scope of the study

Following table and graph both concludes the results obtained in form of percent bacteria found in respective national parks.

Graphs and table showing comparison of both type shows the dominating presence of nitrifying bacterial community as compared with denitrifying bacterial community. Thus, the community composition is related to ecological properties such as vegetation type, soil type and forest type (Rich et al., 2003). Identifying soil factors like pH, EC, bulk density, total available N and soil organic carbon that are primary ecological drivers of soil bacterial communities, especially denitrifying populations, can potentially aid the development of predictive models for restoration of biogeochemical transformations (Peralta et al. 2008). All organisms in the biosphere depend on the microbial activity. They are vital for the continuing cycling of nutrients and for driving above ground ecosystems (Kirk et al., 2004).

	National Park	Nitrifying bacteria	Denitrifying Bacteria
1	Gir National Park	71%	29%
2	Velavadar National Park	80%	20%
3	Vansda National Park	67%	33%
4	Marine National Park	59%	41%

Figure.1 shows the site area of four national park in Gujarat state

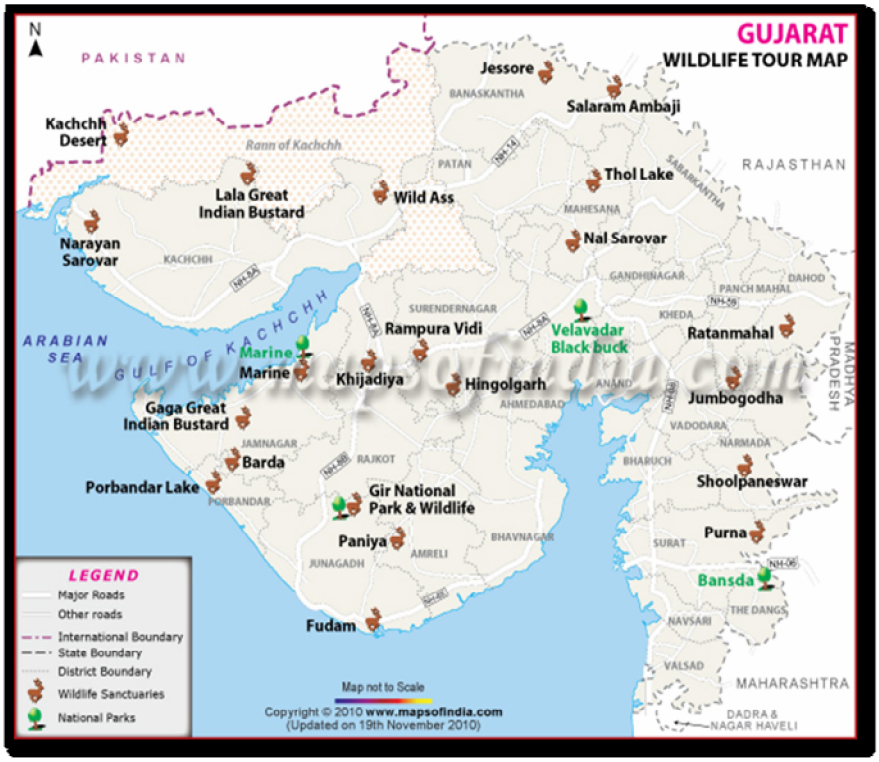
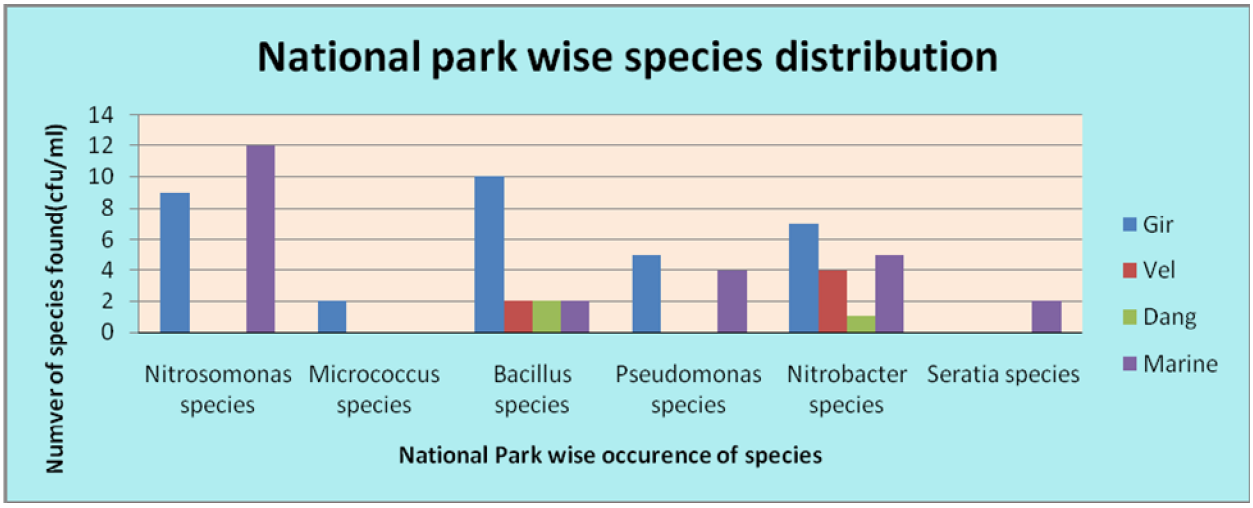
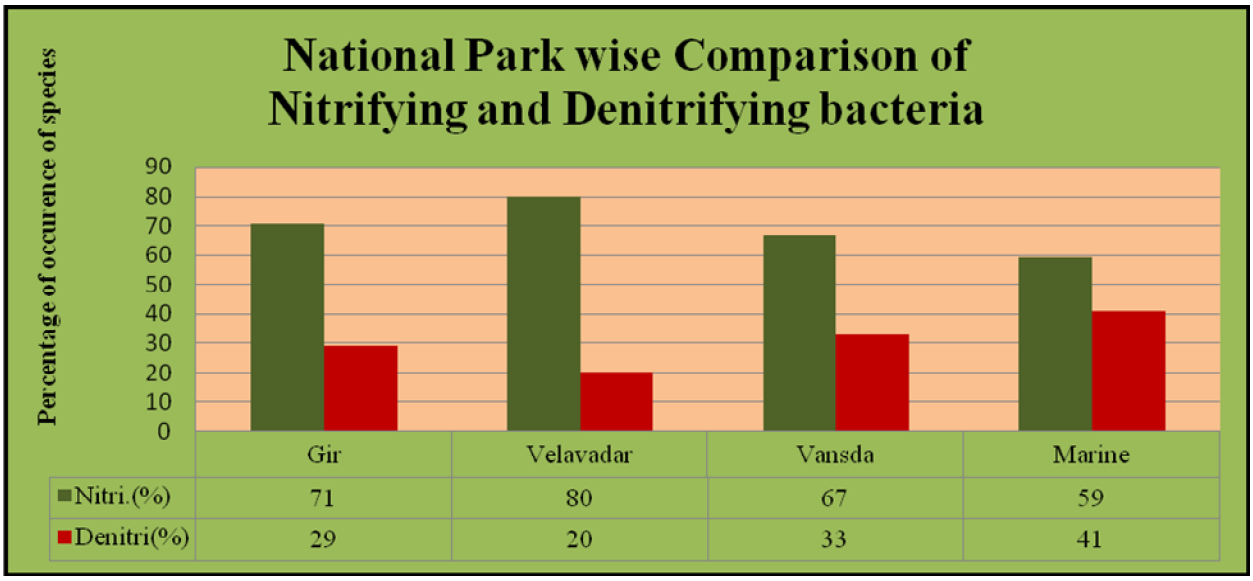


Fig.2 Luxuriant growth seen in Inorganic Salt Medium





Denitrifying community composition, as well as environmental factors, may contribute to the variability of denitrification rates in these systems (Rich et al., 2003). As per the results, the amount of denitrifying bacteria found was much less compared to nitrifying and hence we can conclude that there soil fertility is still intact as denitrification leads to the loss of nitrogen from the soil which results into the depletion of an essential nutrient N for plant growth and therefore, it is an undesirable process reducing the soil fertility. Especially Within forest ecosystems, removal of N by denitrification may be important in reducing site fertility (even small yearly rates may add up to a sizeable loss over the length of a rotation)(Matson and Vitousek 1990) thus Marine ecosystem where the denitrifiers form 40% of the total community is at risk losing N at a faster rate compared to other national parks. Denitrification has been studied in bacterial cultures and in field plots for over 100 years (Payne 1981). Such estimates are needed to evaluate the role of denitrification in atmospheric chemistry and for calculating large scale N budgets for agricultural, forestry, municipal waste treatment and coastal management practices. Bhatt M.S. (2014) studied terrestrial nitrogen cycle in India where our kind of study would contribute to latest N fluxes at

least for Gujarat region. Rhizobium species association with alfalfa, for example, can account for an input of 250 kg of nitrogen fixed per hectare per year (Atlas R., 1984). Thus, abundance of rhizobia in all four national parks can surely assure the fixation of N at decent rate.

Bardgett et al., 2008 made an attempt to understand the potential negative and positive contributions of soil microbes to land–atmosphere carbon exchange and global warming requires explicit consideration of both direct and indirect impacts of climate change on microorganisms. Also a better understanding of the functions of these microbial groups could provide some clarity on the impact of different land development practices and a changing climate on soil ecosystems (Lowe et al. 2012). Hence this study will contribute to the amount of regional community in turn deciding the intensity of the function done by the same community, will surely be instrumental indirectly to climate change studies.

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