Society for Advancement of Wheat and Barley Research
ICAR-Indian Institute of Wheat & Barley Research
Karnal - 132 001, India

Journal of Cereal Research
11(3):286-292
Homepage: http://epubs.icar.org.in/ejournal/index.php/JWR

Research Article

Variation in soil bacterial communities by long-term application of organic and inorganic fertilizers in rice

Ashwini Shanthpure[1], Devaki Girija[2]*, Panchami Pottekkat Sidharthan[2], Moossa Puthen Peedikakkal[3] and Sneha Sasidharan Nair[2]

[1]*Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Kerala Agricultural University, Vellanikkara -680656*
[2]*Department of Agricultural Microbiology, College of Horticulture, Kerala Agricultural University, Vellanikkara -680656*
[3]*Regional Agricultural Research Station, Kerala Agricultural University, Pattambi - 679306*

Article history

Received: 29 Oct., 2019
Revised: 05 Dec., 2019
Accepted: 19 Dec., 2019

Citation

Shanthpure A, D Girija, PP Sidharthan, MP Peedikakkal and SS Nair. 2019. Variation in soil bacterial communities by long-term application of organic and inorganic fertilizers in rice. *Journal of Cereal Research* **11**(3): 286-292. http://doi.org/10.25174/2249-4065/2019/94878

***Corresponding author**
Email: devakigirija@gmail.com

© *Society for Advancement of Wheat and Barley Research*

Abstract

Fertilization plays important role in increasing the quality of soil and crop productivity. Soil bacterial communities are sensitive indicators of soil quality and their response to long-term fertilization in paddy soils of Kerala remains unexplored. In the present study, we analysed the long term effect of fertilizer regimes *viz.*, organic inputs (ONM), integrated inputs (INM) and inorganic (INF) inputs on bacterial community in paddy soils using NGS sequencing of 16S rRNA genes. The results showed that relative abundance of bacterial communities varied across organic and inorganic fertilizer treatments. The dominant phyla across all the treatments were Actinobacteria followed by Acidobacteria, Proteobacteria and Firmicutes. The long term application of organic and integrated fertilizers clearly increased the abundance of beneficial microbes like *Bacillus*, *Arthrobacter*, *Bradyrhizobium*, *Frankia* etc in soil. In contrast, continuous use of chemical fertilizers, suppressed the growth of *Rhizobium*, *Azospirillum*, *Pseudomonas* and *Burkholderia* in rhizosphere soils. Overall, the present study enhanced our understanding of relationship between soil microbiota and functioning of agro ecosystem and support the use of integrated fertilization strategy in rice.

Keywords: Bacterial community, diversity, fertilization, paddy soils

1. Introduction

Fertilization is an imperative agronomic practice for improving the soil nutrient status as well as crop productivity. Although large scale use of synthetic fertilizers contributes to impressive increase in crop production and yield, it induces soil degradation, nutrient run off and loss in biodiversity (Sun *et al.,* 2014). This has led to tremendous enthusiasm in adopting organic fertilizers, which can improve the quality of soil there inturn the nutrient status (Li *et al.,* 2010).

Soil is considered as a prime factor which affects the growth of plants and a hot spot of microorganisms. Soil microbes, plays an important role in nutrient cycling, decomposition of above and below ground plant residues, soil structure and fertility (Biswas *et al.,* 2017). Alteration or shift in the soil microbial community structure and composition may directly or indirectly affect the soil biological activity and crop productivity (Edmeades *et al.,* 2003). Therefore maintaining the diversity of microbes in soil is essential for sustainable agricultural production and for long term fertility of soils (Fierer and Jackson, 2006). Amending the soil with organic inputs, such as compost or manure resulted in higher microbial diversity which in turn may positively affect the soil health (Schmid *et al.,* 2017).

Rice (*Oryza sativa* L.) is the most important staple food for a large part of the world's human population especially in east and south Asia. Microorganisms and their activities in rice rhizosphere soil play an important role in its production and also soil fertility (Ishikawa *et al.,* 1995). Various soil and crop management practices have altered the community structure and activity of these microbial consortia (Mendum and Hirsch, 2002). There is still lack of

information about the specific changes in soil bacterial diversity and community structure as affected by long-term fertilizer application.

Understanding the shifts in soil microbial community structure following the implementation of various agronomic practices is important to improve the fertility and function of soil. The continuous monitoring of soils under organic and inorganic nutrient management practices is essential for developing strategies to sustain the agricultural production. As 99 per cent of the microbes in the environment cannot be cultured readily, culture dependent methods only offer an incomplete picture of the complex microbial communities present in soil.The recent development of high throughput next-generation sequencing with the primer spanning hyper variable regions (V1–V9) of 16S rRNA gene circumvent the limitations of earlier used methods and enlighten the way to identify total bacterial community and their subsequent classification. However, only a limited number of studies have employed Illumina-Miseq technology to investigate the microbial community structure in rice soils (de Souza *et al.,* 2016, Desantis *et al.,* 2006). With this background, the present study was initiated to assess the abundance and composition of bacterial communities based on 16S rRNA amplicon sequencing through Illumina MiSeq and their diversity in paddy soils of Kerala as influenced by the long term application of chemical and organic fertilizers.

2. Materials and methods

2.1. Experimental site

The soil samples were collected from long term field experimental rice plots receiving continuous fertilizer application since 1973 at the Regional Agricultural Research Station (RARS), Pattambi, Kerala (10° 48" N latitude, 76° 12" E longitude and 25 m altitude). The long-term experiment rice plot (variety Jaya) was laid out in a randomized complete block design with plot size 7.8 x 5.8 m. Soil samples were collected from three different fertilization treatments: 1) ONM, Organic nutrient management soil composed of cattle manure + green manure @ 9 t ha^{-1} on the nutrient equivalent basis 2) INF, inorganic fertilizer N, P_2O_5 and K_2O at the application rate of 90;45;45 Kg ha^{-1} and 3) INM, integrated treatments received cattle manure + green manure @ 4.5 t ha^{-1} along with NPK (45;45;45;Kg ha^{-1}).The manure was incorporated into the soil during last ploughing before sowing of every crop.

2.2. Soil sampling and analyses

Rice rhizosphere soil samples were collected randomly from three places from the upper surface (0-20 cm) of each plot at panicle initiation stage during the month of August. A composite sample representative of each treatment was prepared by mixing the sub-samples. The soil samples were removed from stones and stubbles, powdered and passed through a 2.0 mm sieve. Further one part of soil sample was stored at -80°C for DNA extraction and the other was packed in air tight plastic bags for analysis of soil chemical properties. The soil properties were measured following standard protocols. The soil pH and electrical conductivity was measured at a soil to water ratio of 1:25 (w/v). Soil organic carbon and total nitrogen were determined by $K_2Cr_2O_7$ oxidation–reduction titration and Kjeldahl digestion respectively. Atomic absorption spectrometer was used to measure available calcium, magnesium and Fe, Mn, Zn, Cu, respectively. Available potassium was measured using flame photometer. Available phosphorus, available sulphur and available B were determined by colorimetric methods, turbidometry methods and spectrophotometer, respectively. The microbial biomass carbon was estimated by chloroform fumigation and extraction method (Jenkinson and Powlson, 1976).

2.3. Isolation of rhizosphere micro flora

Isolation of rhizosphere microflora from three rice plots were carried out by serial dilution and plating technique (Johnson and Curl, 1972). Bacteria were isolated on nutrient agar media, fungi on Martin's rose bengal agar, actinomycetes on Kenknight's agar media, phosphate solubilizers on Pikovskaya's agar media, nitrogen fixers on Jenson's agar media and *Trichoderma* on *Trichoderma* selective media. The plates were incubated at 28 ± 2°C till the appearance of colonies. The number of colonies on each media was counted and expressed in cfu g^{-1}.

2.4. Metagenomic library preparation and sequencing

The total genomic DNA was extracted from 0.5 g of soil by lysis method (Siddhapura *et al*., 2010). The quality of extracted DNA were checked on 0.8% agarose gel, and 2µl of the DNA was loaded in a NanoDrop® ND-1000spectrophotometer (NanoDrop Technologies Inc., USA) for calculating the A260/ A280 ratios (Sambrook *et al.,* 1989).

The extracted soil metagenomic DNA was pooled and normalized to 5 ng $µl^{-1}$ (purified DNA, 10 mM Tris pH-8.5) and the amplicon libraries for the 16S rRNA sequencing were prepared. First PCR was done to amplify the V3-V4 region of 16S rRNA genes using 16S f/r (341F 5'-CCTACGGGAGGCAGCAG-3', 518R 5'-ATTACCGCGGCTGCTGG 3') primers. For the first PCR, 25 µl of the PCR master mix was prepared by adding, 5 ng of soil DNA, 2 µl each 10 pmol $µl^{-1}$ forward and reverse primers, 0.5 µl of 40 mM dNTP, 5 µl of 5X Phusion HF reaction buffer, 0.2 µl of 2U $µl^{-1}$

F-540 Special Phusion HS DNA polymerase and water. The amplification conditions were as follows: initial denaturation at 98°C for 30 sec, followed by 30 cycles of 98°C for 10 sec for denaturation, 55°C annealing for 30 sec, 72°C extension of for 30 sec and a final extension at 72°C for 5 min followed by 4°C hold. The PCR product was quantified using the fluorescence quantitative (Qubit 2.0®) fluorometer with the Qubitds DNA HS assay kit (Invitrozen, USA). This was followed by PCR clean up. Finally, the amplicon libraries were purified by AMP Pure XP beads and quantified using Qubit3.0 Fluorometer. The libraries were loaded onto was then loaded onto Illumina MiSeq TM sequencer for cluster generation.

2.5. Bioinformatic and statistical analysis

After trimming the unwanted sequences from originally paired-end data, a consensus V3 region sequence was constructed using Clustal Omega program (http://www.ebi.ac.uk/Tools/msa/clustalo/). Then multiple filters were applied *viz*, conserved region filter, spacer filter and mismatch filter and the highest quality V3 region sequences were taken for various downstream analyses. As a part of pre-processing of sequence reads, singletons that were likely due to the sequencing errors and could result in spurious operational taxonomic units (OTUs) were removed. This step was achieved by removing the reads that did not cluster with other sequences (abundances >2). Chimeras were also removed using the denovo chimera removal method UCHIME implemented in the tool USEARCH. Pre-processed reads from all samples were pooled and clustered into OTUs based on their sequence similarity using Uclust programme (similarity cutoff = 0.97). Quantitative insights in to microbial ecology (QIIME) were used for downstream analysis (Caporaso *et al.,* 2010). Representative sequences were identified for each OTU and aligned against Greengenes core set of sequences using PyNAST programme (De Santis *et al.,* 2006). Further, representative sequences were aligned against reference chimeric datasets. Taxonomy classification was performed using RDP classifier and Greengenes OTUs database and the sequence data were uploaded to MG-RAST (http://metagenomics.anl.gov/) (Meyer *et al.,* 2008)

Rarefaction was performed to compare the relative abundance of OTU richness across all the three soil samples at an OTU cut-off of 0.03. The OTU table was transformed into a suitable input file for further alpha analyses using MGRAST programme. The microbial population across three different fertilization regimes were statistically analysed using Web Agri Stat Package 2.0 (WASP 2.0) developed by ICAR-Central Coastal Agricultural Research Institute, Goa, India.

2.6. Raw sequence data submission

The sequence data were deposited in the national centre for biotechnology information (NCBI), as sequence read archive (SRA) with accession number SRP077896 (http://www.ncbi.nlm.nih.gov/).

3. Results and discussion

3.1. Effect of different fertilization regimes on soil chemical and biological properties

Long term application of inorganic and organic fertilizers in paddy soil recorded changes in soil chemical and biological properties. Under all the

Table 1: Chemical properties of soil under different fertilization regimes.

Parameters	ONM		INM		INF	
	Quantity	Remarks	Quantity	Remarks	Quantity	Remarks
pH	4.7	Very Strongly Acidic	4.6	Very Strongly Acidic	4.7	Very Strongly Acidic
Electrical Conductivity (dS m^{-1})	0.10	Normal	0.15	Normal	0.10	Normal
Total Nitrogen (%)	0.19	Normal	0.18	Normal	0.16	Normal
Available Phosphorus (Kg ha^{-1})	68.76	High	74.43	High	48.53	High
Available Potassium (Kg ha^{-1})	34.72	Low	35.84	Low	47.04	Low
Available Calcium (mg Kg^{-1})	466.25	Sufficient	277.75	Deficient	342.25	Sufficient
Available Magnesium (mg Kg^{-1})	79.75	Deficient	73.00	Deficient	79.50	Deficient
Available Sulphur (mg Kg^{-1})	13.54	Sufficient	11.20	Sufficient	6.77	Sufficient
Micronutrients						
Available Copper (mg Kg^{-1})	10.52	Sufficient	9.12	Sufficient	8.50	Sufficient
Available Iron (mg Kg^{-1})	214.80	Sufficient	196.30	Sufficient	315.40	Sufficient
Available Zinc (mg Kg^{-1})	7.05	Sufficient	5.56	Sufficient	4.79	Sufficient
Available Manganese (mg Kg^{-1})	14.42	Sufficient	8.47	Sufficient	10.73	Sufficient
Available Boron (mg Kg^{-1})	0.42	Deficient	0.35	Deficient	0.23	Deficient

treatments soil pH was recorded in the range of 4.6 to 4.7. Normally laterite soils of Kerala are acidic in nature (Chandran *et al.,* 2005). The soil salinity in terms of electrical conductivity was higher in inorganic fertilized soil compared to organic fertilized soil. The total nitrogen content and soil organic carbon were lower in inorganic fertilizer treated plots compared to organic and integrated fertilizer treated plots. Furthermore, long term application of organic fertilizers showed increased phosphorus content of 68.76 in ONM and 74.43 Kg ha^{-1} in INM respectively compared to chemical fertilization (48.53 Kg ha^{-1}). Available potassium was generally low in all the samples with highest in inorganic treated plots. The low potassium content in organic treated plots might be due to lack of external application of potassium fertilizer. The magnesium was deficient in all the three plots. The availability of micronutrients was higher in ONM compared to INM and INF. In addition microbial biomass was observed higher in INM (266 µg C g^{-1}) followed by ONM (244 µg C g^{-1}) and INF (177µg C g^{-1}) (Table 1). Most of the soil chemical and biological properties were altered by long term application of fertilization. In general, The positive effect of organic inputs on soil bacteria and actinomycetes might be due to the abundance nutrients provided during decomposition of organic nutrients (Table 2).

3.2. Isolation and quality checking of Metagenomic DNA

The metagenomic DNA isolated from the different soil samples were analyzed in terms of quality and yield. The genomic DNA was of good quality with intact bands was visualized on agarose gel. The metagenomic DNA concentration was high in ONM (80.4 ng $µl^{-1}$) followed by INM (65.11 ng $µl^{-1}$) and INF (36.01 ng $µl^{-1}$) .

3.3 Analysis of Illumina sequence data

Total raw sequencing reads (paired-end) of ONM, INM and INF DNA samples were recorded as 374632, 335666 and 381295, respectively with average sequence length of 150 bp each obtained from Illumina MiSeqTM sequencer. Nearly 90 % of the total reads had phred score greater than 30 (NQ30; sequence, the passed reads were aligned to each other with zero mismatches with an average contig length of 130–160 bp (error-probability ≥ 0.001). The average distribution of GC content for the samples ranged

Table 2: Population of rhizospheremicroflora under different fertilization.

Sample	Population of microorganisms (cfu per gram of soil)				
	Bacteria x 10^6	Fungi x 10^2	Actinomycetes x 10^4	Fluorescent Pseudomonads x 10^5	Nitrogen fixers x 10^3
ONM	29.13 (1.46)	38.50 (1.58)	50.85 (1.70)	36.00 (1.75)	22.25 (1.24)
INM	33.22 (1.52)	54.00 (1.73)	57.50 (1.75)	10.00 (0.96)	9.25 (0.54)
INF	13.00 (1.10)	93.00 (1.96)	23.00 (1.35)	05.00 (0.5)	7.00 (0.45)
CD (0.05)	NS	0.07	NS	NS	NS

Figure in parenthesis indicate log transformed values , NS – Non significant

organic fertilizers have strong positive effect on soil total nitrogen, microbial biomass carbon and most of the micro and macronutrients. This might be due to the abundance of nutrients released by organic inputs for proliferation and growth of beneficial microbes in the rhizosphere soils (Gong *et al.,* 2009).

Agricultural activities such as tillage, intercropping, fertilization and crop rotation had significant effect on soil microbial population (Hengeveld, 1996). It was observed that the integrated fertilizer treated plot (INM) recorded higher bacterial (33.22 x 10^6 cfu g^{-1}) and actinomycetes (57.50 x 10^4 cfu g^{-1}) population followed by organic (ONM) and inorganic fertilizer treated plots (INF). The fungal population was significant higher in INF (93x 10^2 cfu g^{-1}) followed by INM (54 x 10^2 cfu g^{-1}). Phosphate solubilizing bacteria and *Trichoderma* were absent in all the plots. Population of nitrogen fixing bacteria (22.25 x 10^3cfu g^{-1}) and fluorescent pseudomonads (36x10^5cfu g^{-1}) were found to be highest in ONM and lowest in INF. from 55.18 to 54.71 per cent. Application of multiple filters such as conserved region filter, spacer filter, and mismatch filter resulted in good pair end reads. From the consensus reads, singletons and chimeric sequences were removed and obtained high-quality pre-processed reads. The pre-processed reads from all samples were pooled and clustered into OTUs based on their sequence similarity (similarity cutoff = 0.97) and a total of 12860 OTUs were identified from 297,859 reads. We carried out rarefaction analysis to verify the amount of sequencing reflected in the diversity of original microbial community and the analysis revealed that the species count increased sharply before attaining a plateau. The alpha diversity obtained from rarefaction analysis indicated the extent of bacteria species diversity was highest in ONM (54.52) followed by INM (44.13) and INF (38.86). (Fig. 1). The results clearly supported the fact that addition of organic matter favours the population of bacteria.

3.4. Effects of different fertilization regimes on bacterial

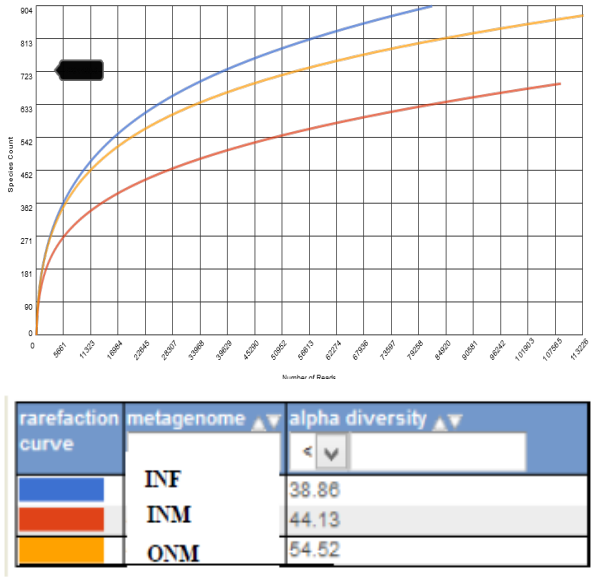

Fig. 1: Rarefaction analyses of ONM, INM and INF by MG-RAST analysis platform

community in the rhizosphere of paddy

Relative abundance of bacterial OTUs at the phylum level was variable in plots receiving organic and inorganic inputs. The most dominant phyla across all the samples were Actinobacteria, Acidobacteria, Proteobacteria, Firmicutes and Bacteroidetes. In addition Verrucomicrobia, Chlorobi, Cyanobacteria, Chloroflexia and Spirochaetes were detected in all the samples with low abundance of OTUs. Among these dominant phyla, proportion of Actinobacteria (57.95%) was more abundant in plots receiving integrated regimes (INM) and low (25.80%) in plots receiving inorganic inputs alone (INF). Conversely, Acidobacteria and Proteobacteria were more abundant in soils receiving inorganic inputs (INF) (Acidobacteria 14.31% and Proteobacteria 14.55%).

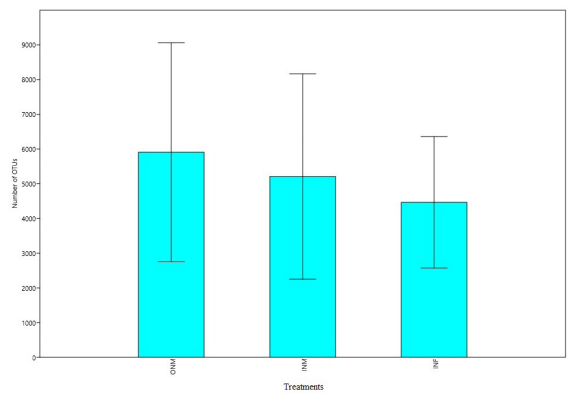

Fig. 2: Relative abundance of bacterial communities over different fertilization regimes

Firmicutes sustained more abundant in ONM (6.76%) and unclassified (derived from bacteria) were dominating in INF soils (33.57%) followed by ONM (17.78 %) and INM (16.42%) (Fig. 2 - 3). The abundance of Actinobacteria in organic and integrated treatment might be due to the incorporation of farmyard manure as organic input in those plots. Actinobacteria plays an important role in the cycling of organic compounds and have been associated with soil organic decomposition in the oxic zones of paddy soils (Ahn *et al.,* 2013). The prevalence of acidobacteria is positively related to the acidic pH prevailing in the experimental plots. Though the members of Acidobacteria are significant contributors to soil ecosystem, majority are unculturable and hence the ecology and metabolism are least understood (Eichorst, 2011). Proteobacteria was observed to be the dominant phylum present in the rice rhizosphere soil (Arjun and Harikrishnan, 2011). The phylum Proteobacteria is mainly involved in bio-recycling of essential mineral nutrients present in the soil (Lesaulnier *et al.,* 2008). Proteobacteria in the rhizosphere may contribute to improvement of soil fertility and thereby plant growth (Chaudhry *et al.,* 2012). The abundance of Proteobacteria in rhizosphere soil receiving inorganic fertilizers were reported earlier (Pisa *et al.,* 2011). Classes under phylum Proteobacteria were α, β, γ, δ, and ε Proteobacteria, amongwhich δ-Proteobacteria was dominant in all the samples analyzed.

The relative abundance of bacterial genera was represented in Fig. 4. Regarding the effect of long term application of organic and inorganic fertilization, some prevalent differences were noted at genera level. In general, application of organic and integrated fertilizers resulted in an increase in number of beneficial genera belonging to *Frankia*, *Arthrobacter*, *Bacillus*, *Nocardia*, *Saccharopolyspora*, *Bradyrhizobium*, *Pseudomonas* and *Rhodospseudomonas* compared to inorganic fertilizer treated plots. Some genera *viz.*, *Rhizobium*, *Azospirillum*, *Pseudomonas* and *Burkholderia* were completely absent in soils treated with inorganic fertilizers (Table 3). Several species of the Genus *Bacillus* are used as plant growth promoting rhizobacteria (PGPR). Several species of this genus are capable of producing antibiotics, and *B. thuringiensis* produces a toxin called 'crystal protein', which kills insects. *Clostridium* was reported highest in ONM (1.03%) and lowest in INF (0.70%). Researchers have reported an increase in Firmicutes with addition of organic matter in soil (David *et al.,* 2011). The abundance of beneficial microbes in ONM and INM might be due to the application of organic inputs which favours the microbial growth (Anisa *et al.,* 2016). Decrease in microbial population due to repeated application of inorganic fertilizers

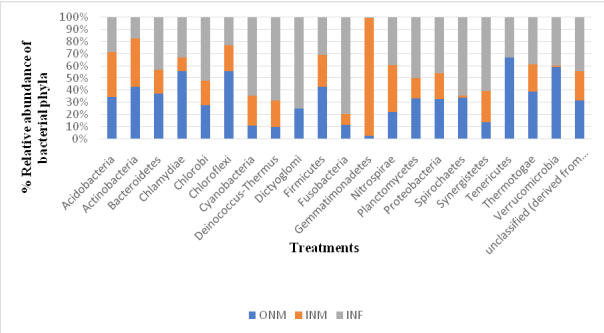

Fig. 3: Relative abundance (%) of bacterial phyla present in the 3 treatments.

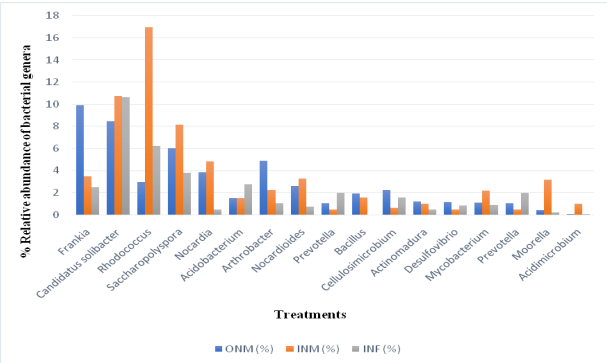

Fig. 4: The relative abundance (%) of bacterial genera under different fertilization regimes

Table 3: Abundance of beneficial microbes under different fertilization.

Sr. No	Beneficial microbes	ONM (%)	INM (%)	INF (%)
1	*Frankia*	9.89	3.50	2.49
2	*Bacillus*	1.91	1.57	0.01
3	*Rhizobium*	0.009	0.004	-
4	*Azospirillum*	0.011	0.02	-
5	*Bradyrhizobium*	0.33	0.52	0.02
6	*Pseudomonas*	0.002	0.003	-
7	*Burkholderia*	0.26	0.27	-

were already reported (Kamaa *et al.,* 2011)

The outcome of the present study also found that continuous usage of chemical fertilizers in the long run, may considerably suppress the beneficial microflora present in soil .In this regard, a balanced used of organic and inorganic fertilizers are encouraged for a sustainable agricultural management and for improving soil health.

4. Acknowledgement

The author would like to thank Kerala Agricultural University for financial support for the successful completion of the work.

5. References

1. Anisa NA, BL Markose and GK Surendra. 2016. Effect of integrated nutrient management on population of biofertilizers in rhizosphere of okra (*Abelmoschus esculentus* (L.) Moench). *International Journal of Innovative Research in Science Engineering and Technology* **5(4):** 5629-5632.
2. Arjun JK and K Harikrishnan. 2011. Metagenomicanalysisof bacterial diversity in the rice rhizosphere soil microbiome. *Biotechnology Bioinformatics and Bioengineering* **1:** 361-367.
3. Biswas T and SC Kole. 2017. Soil organic matter and microbial role in plant productivity and soil fertility. In: Adhya T, B Mishra, K Annapurna, D Verma and U Kumar. Advances in soil microbiology: Recent trends and future prospects, *Microorganisms for sustainability*. Springer, Singapore, pp219–238.
4. Caporaso JG, K Bittinger, FD Bushman, TZ DeSantis, GL Andersen and R Knight. 2010. PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics* **26:** 266–267.
5. Chandran P, SK Ray, T Bhattacharyya, P Krishnan and DK Pal. 2005. Clay minerals in two ferruginous soils of southern India. *Clay Research* **19:** 77–85.
6. Chaudhry V, A Rehman, A Mishra, PS Chauhan and CS Nautiyal. 2012. Changes in bacterial community structure of agricultural land due to long-term organic and chemical amendments. *Microbial Ecology* **64:** 450–460.
7. De Souza RSC, VK Okura, JSL Armanhi, B Jorrin, N Lozano, M Jda Silva. and HC Bagheri. 2016. Unlocking the bacterial and fungal communities assemblages of sugarcane microbiome. *Scientific Reports* **6:** 287-274.
8. De Santis TZ. P Hugenholtz , N Larsen, M Rojas, EL Brodie, K Keller, T Huber, D Dalevi, and GL Andersen. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied Environmental Microbiology* **72**(7): 5069-5072.
9. David JVH, GO Jordan, HN Buelow, MN Gooseff and JE Barrett. 2011 Soil microbial responses to increased moisture and organic resources along a salinity gradient in a polar desert. *Applied Environmental Microbiology* **80**(1): 3034-3043.
10. Edmeades DC. 2003. The long-term effects of manures and fertilisers on soil productivity and quality: a review. *Nutrient Cycle in Agroecosystem* **66**(2): 165-180.

11. Eichorst SA, CR Kuske and TM Schmidt. 2011. Influence of plant polymers on the distribution and cultivation of bacteria in the phylum acidobacteria. *Applied Environmental Microbiol*ogy **77**(3): 586–596.

12. Fierer N and B Jackson. 2006. The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences, USA* **103**: 626- 631.

13. Gong W, XY Yan, JY Wang, TX Huand YB Gong. 2009. Long term manure and fertilizer effects on soil organic matter fractions and microbes under a wheat-maize cropping system in northern China. *Geoderma* **149**: 318–324.

14. Hengeveld R. 1996. Measuring ecological biodiversity. *Biodiversity Lett*ers **3**: 58-65.

15. Ishikawa SI, A Furukawa and T Oikawa. 1995. Zonal plant distribution and edaphic and micro-meteorological conditions on a coastal sand dune. *Ecological Research* **10**(2): 259-266.

16. Jenkinson DS and DS Powlson. 1976. The effect of biocidal treatments on metabolism in soil. *Soil Biology and Biochemistry* **8:** 209-213.

17. Johnson LF and EA Curl. 1972. Methods for arch on the Ecology of Soil Born Plant Pathogens, Bargess, Minneapolis, pp247.

18. Kamaa M, H Mbruru, L Chibole, J Chotte, C Kibunja and D Lesueur. 2011. Effects of organic and inorganic fertilization on soil bacterial and fungal microbial diversity in the Kabete long-term trial, Kenya. *Biology and Fertility of Soils* **47**: 315-321.

19. Lesaulnier C, D Papamichail and S Mc Corkle. 2008. Elevated atmospheric CO_2 affects soil microbial diversity associated with trembling aspen. *Environmental Microbiology* **10**: 926–941.

20. Li BY, SM Huang, MB Wei, HL Zhang and AL Shen. 2010. Dynamics of soil and grain micronutrients as affected by long-term fertilization in an quic Inceptisol. *Pedosphere*, **20**(5): 725-735.

21. Mendum TA and PR Hirsch. 2002. Changes in the population structure of β-group autotrophic ammonia oxidising bacteria in arable soils in response to agricultural practice. *Soil Biology and Biochemistry* **34**(10): 1479-1485.

22. Meyer F, D Paarmann, MD Souza, R Olson, EM Glass and M Kubal. 2008. The metagenomics MGRAST server – a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics* **9**: 386-387.

23. Pisa G, GS Magnani, H Weber, EM Souza, H Faoro, RA Monteiro. 2011. Diversity of 16S rRNA genes from bacteria of sugarcane rhizosphere soil. *Brazilian Journal of Medical Biological Research* **44**: 1215-1221.

24. Sambrook J, EF Fritschi and T Maniatis. 1989. Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory Press, New York. pp745.

25. Schmid CAO, P Schroder, M Armbruster and M Schloter. 2017. Organic amendments in a long- term field trial consequences for the bulk soil bacterial community as revealed by network analysis. *Microbial Ecology* **29**(1): 1-4.

26. Siddhapura PK, S Vanparia, MK Purohit and SP Singh. 2010. Comparative studies on the extraction of metagenomic DNA from the saline habitats of Coastal Gujarat and Sambhar Lake, Rajasthan (India) in prospect of molecular diversity and search for novel biocatalysts. *International Journal of Biology and Macromol*ecules **47**(4): 375-379.

27. Sun W, Q Shao, J Liu and J Zhai. 2014. Assessing the effects of land use and topography on soil erosion on the loess plateau in China. *Catena* **121**(1): 151-163.