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Microbial diversity in rice rhizosphere as influenced by nutrient management: A review

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Abstract

Diversity of microbial populations, various diversity indices (PLFA, Shannan index, etc) and microbial biomass carbon (MBC), are necessary to understand the effects of different nutrient management practices under rice ecosystem. Rice fields differ in many ways from other agricultural lands and natural terrestrial ecosystems. Rice fields are waterlogged for most of the time, which enhances algal photosynthesis and thus the fertility of the soil. Moreover, water-logged soils are typically anoxic, which limits the growth and activity of aerobic microorganisms, including bacteria, fungi protists etc, but favors anaerobic protists able to tolerate the temporary oxic soil conditions. The prolonged hydration also influences the ability of microeukaryotes to decompose plant residues in rice field soils.

In spite of several studies for estimating the fluxes and appraising microbial pools, the composition and functions of soil microbiota are still mostly undiscovered. Most of the soil microorganisms are still unknown, only a few have been isolated, cultured and identified, and directly related to their function in agroecosystems. Currently, several techniques, viz., analysis of DNA and phospholipid fatty acids (PLFAs) as well as cultivation on Biolog plates are available to facilitate the community-level characterization of soil microorganisms (Widmer *et al.*, 2001; Wang *et al.*, 2011).

Diversity richness, Shannon index and Evenness index indicated that bacterial functional diversity increases with increase in the application of inorganic Nitrogen (N) fertilizer, but decreased again with excessive N application. The application of N fertilizer significantly increased MBC and the main microbial groups, including bacteria, fungi, actinomycetes, Gram-positive bacteria, and Gram-negative bacteria but varied in different growth stages. Application of organic manures, especially rice straw, resulted in larger overall bacterial populations than in control or chemical fertilizer treatments (Zhang *et al.*, 2012). Organic manures significantly increased soil biological activity and microbial population by providing C source, high content of organic C due to application of rice straw along with chemical fertilizers, can provide a longer lasting C source for soil microorganisms. They provide required carbon, nitrogen and energy for microbial growth and reproduction. Organic amendments along with low amount of chemical fertilizer have a great potential to enhance paddy soil microbial biomass and microbial activity (Zhang *et al.*, 2012). Over the period of the experiment of two years, organic amendments along with low quantity of mineral fertilizer appreciably increased *Azospirillum* and PSB population as compared to the mineral fertilizers alone.

Keywords: Microbial diversity, nutrient management, organic amendments

Introduction**The soil microbial diversity concept**

Soil microbial diversity is composed of species diversity and genetic diversity, as well as ecosystem biodiversity. Species diversity consists of species richness, the total of species present, species evenness, and the distribution of species. However, the traditional species definition was based on higher plants and animals and is not readily applied to prokaryotes or asexual organisms. Microbial diversity has usually been taken as the number of individuals assigned to different taxa and their distribution among taxa. Consequently, microorganisms have been referred to collectively as functional groups or guilds. Typically, microbial diversity studies have included the relative diversities of communities across a gradient of stress, disturbance, or other biotic or abiotic differences. In spite of several studies for estimating the fluxes and appraising microbial pools, the composition and functions of soil microbiota are still mostly undiscovered.

Most of the soil microorganisms are still unknown, only a few have been isolated, cultured and identified, and directly related to their function in agroecosystems. Currently, several techniques, viz., analysis of DNA and phospholipid fatty acids (PLFAs) as well as cultivation on Bio log plates are available to facilitate the community-level characterization of soil microorganisms (Widmer *et al.*, 2001; Wang *et al.*, 2011) [8,9].

Microbial diversity in rice

Irrigated rice fields differ in many ways from other agricultural lands and natural terrestrial ecosystems, including wetlands (Kogel-Knabner *et al.*, 2010). Perhaps the most important difference is that the soil in irrigated rice fields is waterlogged for most of the time, which enhances algal photosynthesis and thus the fertility of the soil is enhanced. The prolonged hydration also influences the ability of microeukaryotes to decompose plant residues in rice field soils. Moreover, water-logged soils are typically anoxic, which limits the growth and activity of aerobic microorganisms, including bacteria, fungi and protists. Zhang Jian-fu *et al.*, in a study in 2012 [12] found Gram-positive bacteria, Gram-negative bacteria, methane-oxidizing bacteria, sulfate-reducing bacteria, fungi, actinomycete, arthropoda and protozoa were detected in anaerobic environment of paddy soil found that 99% of bacteria seen under microscope are not culturable by common lab techniques. To overcome problems associated with non-culturable bacteria and fungus various methods have been developed to identify and study these microorganisms including various traditional, biochemical and molecular procedures.

Major factors affecting rhizosphere microbial diversity:

Pertinent literature has suggested that different factors like soil P^H , salinity, Texture, organic matter content, concentration of nutrient elements, seasonal effects as well as the management practices (irrigation, tillage, cropping, fertilizer and pesticide application, residue incorporation etc.) highly influences the microbial diversity in soil. From the middle of 1990s, much more attention has been paid on the sensitive responds of soil microbial characteristics and molecular ecology footprint to the evolution of ecology system.

Microbial diversity as influenced by Nutrient Management

In recent studies on the effect of long term fertilization on soil physical and chemical properties much more efforts were focused on the impact of long-term fertilization on soil microbial characteristics. Since the soil microbial characteristic is closely related to soil quality, it is very important to understand the soil microbial diversity. Their results show that soil microbial properties respond to the different agricultural managements sensitively, and can reflect the change of soil quality. Here we shall emphasise mainly on the different management practices adopted in rice field which in turn influence the rice rhizosphere community.

Techniques used for measurement of microbial diversity

The most commonly followed methods are as follows:

1. Community Level Physiological Profiling (CLPP): It is a metabolic profiling of bacterial or fungal

community's ability to utilize specific carbon sources; and was given by Garland and Mills (1991).

2. Denaturing Gradient Gel Electrophoresis (DGGE): It is a technique used for separating DNA fragments according to their mobilities under increasingly denaturing conditions (usually increasing formamide/urea concentrations).
3. Polymerase chain reaction (PCR): It is used to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.
4. Fatty acid methyl ester (FAME) analysis provides information on the microbial community composition based on grouping of fatty acids.

However, the volume of literature on the diversity studies of fungal communities found in rhizosphere is much less than that for bacterial diversity because similar molecular tools for isolation and characterization of fungi have been developed much later. The different approaches for studying the microbial diversity under nutrient management in paddy soils are briefly discussed below

1. Effect of Intensive Inorganic Fertilizer Application on Microbial Properties in a Paddy Soil

A study was carried out by Ming, *et al.*, (2011) [4] for rice-rice (May-July and July-October) cropping system for a period of 5 years where urea was applied as N source with the following treatment levels, L1: 0, L2:207, L3:414, L4:621, L5:828 kg/h/yr. In addition, Ca (H_2PO_4)₂ (397 kg P ha/ yr) and KCl (316 kg K ha/yr) were also applied to each plot as basal fertilizers. It was found that Diversity Shannon index and Evenness index indicated that bacterial functional diversity increased with increasing of urea input, but decreased again with excessive urea application. Table 1(a)

Table 1(a): Microbial functional diversity in treatments with different levels of urea application

Treatment (Kg N /ha /year)	AWCD	Shannon index	Evenness index
L ₁ (0)	0.008±0.001 a	1.68±0.04 c	1.91±0.22 d
L ₂ (207)	0.010±0.002 a	1.93±0.06 b	2.08±0.03 b
L ₃ (414)	0.013±0.004 a	2.14±0.03 a	2.24±0.03 a
L ₄ (621)	0.016±0.005 a	2.01±0.01 ab	2.10±0.00 b
L ₅ (828)	0.016±0.004 a	1.40±0.17 d	2.02±0.03 c

Estimation of AWCD (CLPP) showed no significant difference in substrate utilization rates of microorganism. Diversity, Shannon index and Evenness index indicated that bacterial functional diversity increased with increasing of urea input, but decreased again with excessive urea application. Moisture content plays an important role in shifting of soil microbial activity and diversity because soil water status could affect microorganism directly by limiting their decomposition ability to certain compounds or by regulating substrate availability in soil. Therefore, we supposed here that soil water stress caused by intensive N fertilization might be the direct factor leading to the depression of microbial diversity after rice yellow ripening stages. This may be due to nutrient arousing or depleting, osmotic changes, and toxicity might have effect on microorganism. Low level of fertilizer input could benefit the increase of diversity.

2. Effects of N Fertilization on Microbial Community Structure at different stages of growth in Paddy Soil

A study carried out by Ya-Juan, *et. al.*, 2012 in which soil Samples were drawn at three stages: Tillering, Grain Filling and at Maturity to study the effects of N Fertilization on Microbial Community Structure at different stages of growth in Paddy Soil. Here urea was applied as N source

with the following treatments: N1: 0, N2: 126.00, N3: 157.50 kg/ha. N fertilizer was applied with 70% as basal fertilizer and 30% as topdressing at the tillering stage. Both SSP (75.0 Kg/ha) and MOP (120 Kg/ha) were applied as basal fertilizer. It was found that Total PLFA highest in grain filling stage and bacterial PLFA was highest among all the other microorganisms.

Table 2(a): Comparison of total phospholipid fatty acids (PLFAs) and main microbial groups among the three growth stages

Growth stage	Bacterial PLFA	Fungal PLFA	Acti no.	G +ve	G -ve	Total PLFAs
	(nmol g ⁻¹)					
Tillering	30.9±3.6,b	2.2±0.5b	8.2±1.0a	10.7±1.4b	19.1±2.1b	60.7±7.1b
Grain filling	33.0±3.7a	2.9±0.5a	7.3±1.1b	12.0±1.4a	19.9±2.2a	67.3±7.5a
Maturity	31.8±4.5b	2.2±0.4b	7.3±1.2b	10.6±1.4b	20.0±3.0a	62.0±7.9b

Values followed by the same letter within a column are not significantly different at $p < 0.05$ according to Duncan's multiple range test. Micro-organisms we found in the sequence of Bacteria > Actinomycetes > Fungi

Total PLFA highest in grain filling stage due to highest rate of photosynthesis leading to maximum rhizodeposition in grain filling stage. Table 2(a)

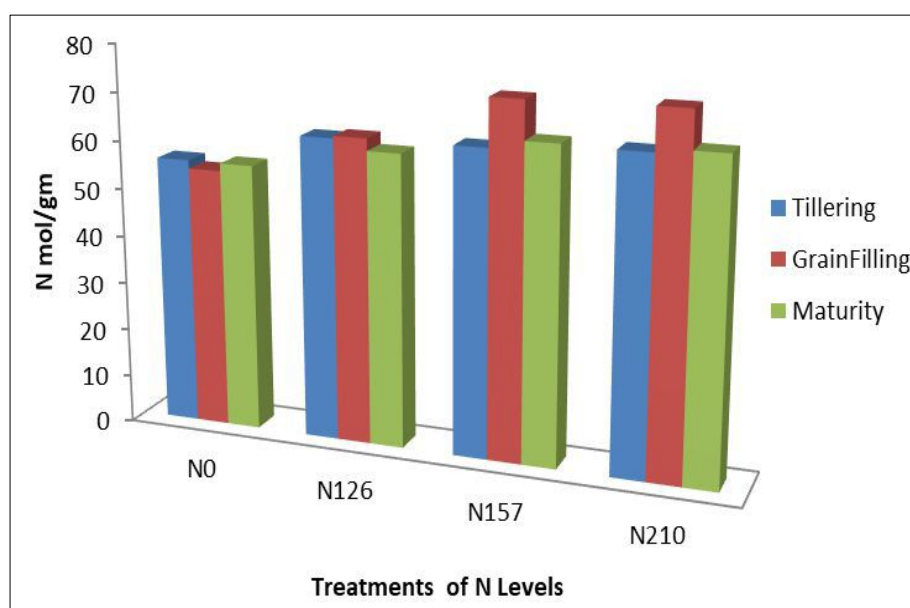


Fig 2(a): Effect of Different N levels on total PLFAs during three growth stages

With increasing levels of N, PLFA increased but up to a certain point (N157). But no significant difference between N157 and N210 levels was found. Among the growth

stages, Grain filling stages exhibited more PLFA value due to highest rhizodeposition. Fig. 2(a)

Table 2(b): Effects of different N levels on phospholipid fatty acids (PLFAs) of Diverse Microbes during the three growth stages

N (kg /ha)	Bacterial PLFAs			Fungal PLFAs		
	Tillering	Grain-filling	Maturity	Tillering	Grain-filling	Maturity
N0	27.2bc	28.8c	28.3e	1.6c	1.7d	1.6c
N1	30.9ab	32.4bc	31.2d	2.1b	2.7c	2.1b
N2	32.8a	34.6ab	34.9ab	2.2b	2.9bc	2.3ab
N3	32.3a	37.1a	34.9ab	2.1b	3.4a	2.3ab

Values followed by the same letter(s) within the same column are not significantly different at $P < 0.05$ according to Duncan's multiple range test; Not significant

The significant effects of N application on microbial communities may be due to the promotion of rice growth by N application, and the growth of rice crops, in turn, stimulates soil biological activity (Zhong and Cai, 2007). Optimum dose of nitrogen favours the proliferation of micro-organisms. Table 2(b)

3. Integrated Nutrient Management on Microbial Properties in a Paddy Soil

A Long-Term Paddy experiment (1982-2005) was carried out by Yun-fu *et.al.*, (2008) ^[10] with the following soil characteristics:

Soil type	pH	OM (g/kg)	Total NPK (G/kg)			Av.NPK(mg/kg)		
			N	P	K	N	P	K
Calcarious	8.6	15.90	1.09	1.35	26.89	66.30	3.9	130.60

The experiment had a completely randomized block design with eight treatments and four blocks. The plot size was 3.5 m × 3 m. The eight treatments include three mineral fertilizers treatments (N, NP, NPK), farmyard manure alone (M, the main component was swine manure, the content of organic matter was 1.5 g kg⁻¹), three mineral plus farmyard manure treatments (NM, NPM, NPKM), and no fertilizer control (CK). All manure, P (as single superphosphate), and K (as KCl) were applied as basal fertilizers, while N (as

urea) was split into 70 and 30% as basal application and topdressing for rice at tillering, and 60 and 40% as basal application and 40% as topdressing for wheat at tillering, respectively. The rates of fertilizers used were: manure at 3 × 10⁴ kg ha⁻¹; N at 55.2 kg ha⁻¹; P₂O₅ at 13.2 kg ha⁻¹; and KCl, at 31.5 kg ha⁻¹.

It was found that in the rice soil, the numbers of bacteria, cellulose decomposing bacteria and nitrifiers were higher.

Table 3(a): Microbial Population in Long Term Integrated Nutrient Management under Rice

Treatments	Bacteria (×10 ⁶)	Actinomycetes (×10 ⁴)	Fungi (×10 ³)	Azo to bacteria (×10 ⁴)	CDB (×10 ⁵)	Nitrifiers (×10 ⁵)	Ammonifiers (×10 ⁵)
M	1.96 c	2.94 ab	0.91 b	0.92 ab	0.94 ab	1.03 c	4.71 b
NM	2.12 c	3.48 a	1.07 ab	0.92 ab	0.97 ab	1.31 b	6.86 a
NPM	2.78 b	3.78 a	1.33 a	0.97 ab	1.07 ab	1.57 a	6.85 a
NPKM	3.99 a	3.82 a	1.51 a	1.13 a	1.23 a	1.70 a	6.27 a
CK	1.45 d	1.23 c	0.92 b	0.48 c	0.58 b	0.86 c	1.16 d
N	1.68 c	1.88 bc	0.65 b	0.61 bc	0.67 b	0.54 d	3.06 c
NP	1.75 c	2.05 bc	1.04 ab	0.67 bc	0.73 b	0.87 c	3.05 c
NPK	2.45 bc	2.35 b	1.29 a	0.78 bc	0.85 b	0.94 c	4.01 b

In the rice soil, the numbers of bacteria, cellulose degrading bacteria and nitrifiers were higher. Numbers of microbes were in the order of: bacteria > actinomycetes > fungi. Among the specific physiology kind group, Azotobacter number was the smallest and ammonifiers was the highest in rice soil. Growth of Ammonium Oxidising Bacteria (AOB) by FYM is promoted as it provides a better soil micro-habitat condition to AOB by improving the soil nutrition & aggregation structure. Table 3(a)

4. Eight Years- Long Term INM on Microbial community structure in Paddy

Zhang *et al.*, (2012) [12] conducted a long term paddy in rice-rice ecosystem after 8 years and analyzed for: 1) Bacteria, Fungi, Actinomycetes population 2) Community richness (S), Shannon (H) and McIntosh (U) indices with the following initial soil properties: Sand: 278 g/kg, Silt: 562 g/kg, Clay: 160 g/kg, total N: 2.7 g/kg, available N: 123.4 mg/kg, Olsen-P: 16.5 mg/kg, pH : 4.8, available K: 54.6 mg/kg.

The treatments taken into consideration were: T1: NF(No fertilizer) T2:CF (Chemical Fertilizer @200:50:150 NPK/h) T4:CFM (Chemical Fertilizer @200:50:150 NPK/h)+ Pig Manure@10t/ha T5:CFM (Chemical Fertilizer @200:50:150

NPK/h)+ Pig Manure@10t/ha T₆:CFS (Chemical Fertilizer @200:50:150 NPK/h)+ Rice Straw @20t/ha

Table 4(a): Number of different soil microorganisms under INM after 8 years of paddy cultivation (×10⁴ /g soil).

Treatments	Bacteria	Fungi	Actinomycetes	Total
NF	16.0 b	0.24 b	2.80 a	19.0 b
CF	25.0 b	0.48 b	2.90 a	28.4 b
CFM	131.0 a	4.50 a	2.40 a	137.9 a
CFS	154.0 a	3.80 a	1.90 b	159.7 a

It has been observed that application of organic manures, especially rice straw, resulted in larger overall bacterial populations than in control or chemical fertilizer Treatments. This was primarily due to higher bacterial counts in the CFS-treated soil. Compared to control, chemical fertilizer application also improved bacterial population. Therefore, both organic amendments and chemical fertilizers could be advantageous in exploiting all types beneficial microflora in soil and increasing the crop production. Use of organic manures that enhance the microbial diversity, biomass and activity along with chemical fertilizer could greatly benefit in the sustainable management of paddy ecosystem.

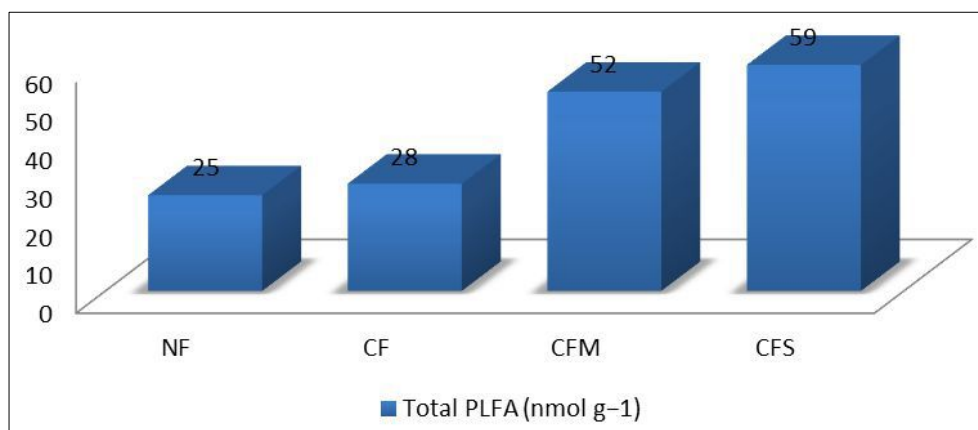


Fig 4(a): PLFA profiles as affected by different INM treatments

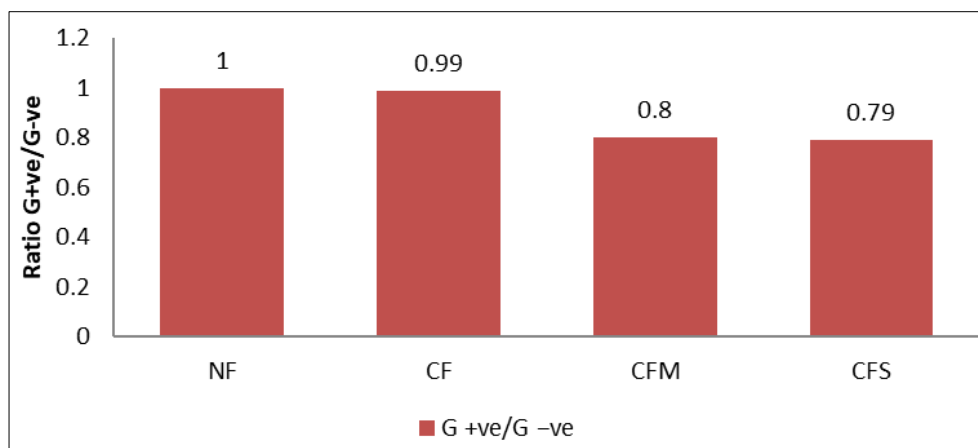


Fig 4(b): Ratio of G+ve and G-ve Bacteria as affected by different INM treatments

Table 4(b): Changes in soil microbial community richness (S), Shannon (H) and McIntosh (U) indices influenced from various INM treatments

Treatments	Total PLFA (nmol g ⁻¹)	S	H	U
T1:NF	25b	20.0 a	2.46 c	3.29 b
T2:CF	28b	14.0 b	2.69 b	3.48 ab
T3:CFM	52a	19.0 a	2.82 ab	3.98 a
T4:CFS	59a	18.5 a	2.90 a	4.00 a

Different letters within a column indicate that difference among the treatments is significant at 5% probability level. Here, it was seen that, H and U values followed the order: CFS > CFM > CF > NF. This was due to application of organic manures, especially rice straw, resulted in larger overall bacterial populations than in control or chemical fertilizer Treatments. Table 4(b)

5. Comparative study of the microbial diversity of bulk paddy soil of two rice fields subjected to organic and conventional farming

Lopes *et.al.*, 2010 ^[3] conducted a study on the of the microbial diversity of bulk paddy soil of two rice fields subjected to organic and conventional farming. Field management of paddy ORG was basal fertilization with mineral gafsa (Fertigafsa, 300 kg P ha⁻¹) and organic amendment with a plant residue commercial compost (Fertiormont, 2 t ha⁻¹) fertilization (102 kg N, 63 kg P₂O₅ and 35 kg K₂O per hectare, as ammonium sulphate, ammonium di-hydrogen phosphate and di-ammonium mono hydrogen phosphate, and potassium chloride, respectively. Soil Sampling was done three times as follows: Initial (Immediately after seeding in April), Second (Tillering in June) and Third (After Harvest in October).

Table 5(a): Enumeration of heterotrophic bacteria, actinomycetes and fungi in soil of paddies ORG and CONV over the rice cycle.

Growth Stages	Total cells		Heterotrophs		Actinomycetes		Fungi	
	log Cells /g		log CFU /g		log CFU /g		log CFU /g	
	ORG	CONV	ORG	CONV	ORG	CONV	ORG	CONV
Initial	8.0 ^A	7.9 ^A	5.0 ^A	5.6 ^A	5.2 ^A	5.5 ^A	3.4 ^A	3.7 ^A
Tillering	7.7 ^{A*}	8.1 ^{B*}	6.4 ^B	6.4 ^B	6.3 ^B	6.1 ^A	4.6 ^B	4.2 ^A
Harvest	8.1 ^A	8.2 ^B	5.8 ^{A,B}	5.9 ^{A,B}	5.6 ^{A,B}	5.6 ^A	4.3 ^B	4.3 ^A

A-B, Homogeneous subsets within each paddy (ORG or CONV), as determined by the Tukey test at $p < 0.05$. *Significant differences between paddies ORG and CONV on basis of the two-sample t-test at $P < 0.05$. It was seen that, among the stages the populations were higher in tillering

due to higher root exudations which enhances microbial proliferation. In between the practices, Conventional management exhibited more population, because of judicious use of fertilizers for six years. Table 5(a)

Table 5(b): Diversity indices of microbial communities in soil of paddies ORG and CONV over the rice cycle.

Stages	Bacterial diversity				Functional diversity			
	Shannon index(H)		Evenness (E)		Shannon index (H)		Evenness (E)	
	ORG	CONV	ORG	CONV	ORG	CONV	ORG	CONV
Initial	1.26±0.02	1.24±0.03	0.93±0.02	0.91±0.02	1.44	1.44	1.00	0.99
Tillering	1.27±0.03	1.25±0.05	0.93±0.02	0.93±0.03	1.45	1.44	1.00	0.99
Harvest	1.29±0.03	1.27±0.06	0.95±0.01	0.91±0.03	1.44	1.44	1.00	1.00

No significant differences were observed over the sampling period ($P < 0.05$).

This may be due to of judicious use of fertilizers for six years in conventional treatment which shows similar results for conventional like organic treatment. Table 5(b)

Table 5(c): Enzymatic activity of soil of paddies ORG and CONV over the rice cycle

Stages	Dehydrogenase (mmol INTF /g/h)		Urease (mmol NH ₃ /g/h)		Arylsulphatase (mmol PNP /g/h)	
	ORG	CONV	ORG	CONV	ORG	CONV
Initial	0.28 ^A	0.21 ^A	0.87 ^{A*}	1.14 ^{A*}	0.09 ^A	0.11 ^A
Tillering	0.20 ^A	0.57 ^B	1.33 ^{A,B}	1.55 ^{A,B}	0.11 ^A	0.15 ^A
Harvest	0.46 ^A	0.18 ^A	1.61 ^{B,C}	1.62 ^{A,B}	0.10 ^{A*}	0.14 ^{A*}

Increase in Dehydrogenase activity might be due the application of organic matter. Higher urease and arylsulphatase activity might be due to application of ammonium sulphate as source of N.

6. Integrated Nutrient Management on Microbial Population in Transplanted Rice in Tamil Nadu

Jeyajothi *et al.*, (2014) conducted a study on the effect of INM on Microbial Population in Transplanted Rice with the

following treatments: T₁: Control, T₂: Recommended dose of fertilizer (150/50/50 kg NPK/ha), T₃: T₂ +12.5t FYM/ha and T₄: T₃+ Azophosmet (seed + soil application) + Pink Pigmented Facultative Methyloph (foliar spray). 0.1% (PPFM) was sprayed at active tillering, panicle initiation and at 50 per cent ear head emergence stages. Analysis was done for Total bacteria, Fungi, Actinomycetes, Azo spirillum, PSB, Pseudomonas and PPFM. Results indicated the INM and application of PPFM exhibited higher total microbial populations.

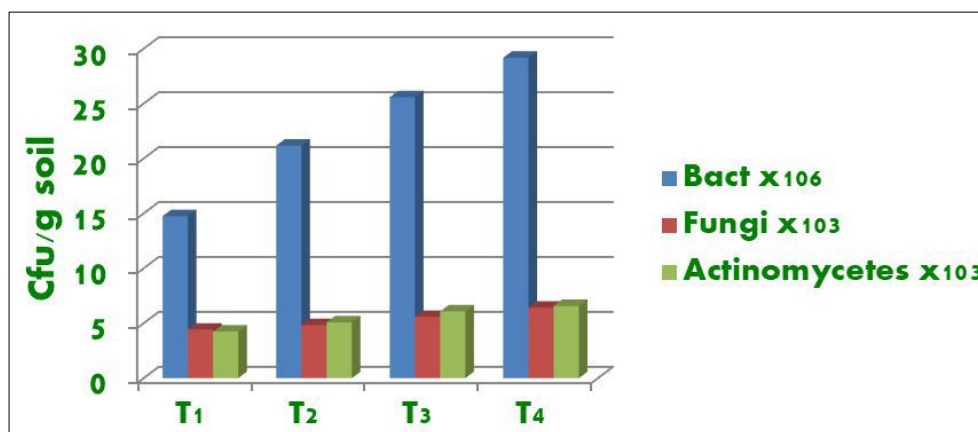


Fig 6(a): Effect of INM on microbial population in transplanted Rice eco-system

7. INM on soil microbial biomass carbon and bacterial populations under rice system

Nath *et al.*, (2011) [6] studied the effect of INM on soil microbial biomass carbon and bacterial populations under rice system with the objective of investigating how INM have affected microbial biomass and the specific bacterial

population under rice ecosystem. The RD of fertilizers were 40:20:20 (N:P₂O₅:K₂O kg/ha) for rice. The N, P and K were supplied through urea, single superphosphate and muriate of potash, respectively. Full doses of P and K were applied as basal, while N applied in three splits (50% as basal, 25% at tillering and 25% at panicle initiation) in rice.

Table 7(a): Soil microbial biomass carbon, and Azo spirillum and PSB population under INM in rice system

Treatment	MBC (µg /g)	Azospirillum	PSB
		(log cfu /g)	
T1	43.23	4.90	4.09
T2	49.60	5.22	4.31
T3	73.34	5.32	4.58
T4	76.11	5.43	4.69
T5	167.66	5.79	4.78
T6	112.80	5.39	4.72
T7	183.66	5.68	5.10
T8	61.03	5.26	4.23
CD (P=0.05)	9.29	0.29	0.46

of the organic inputs, either integration of compost (2 tonnes/ha) with bio fertilizers or application of enriched compost (2 tonnes/ha) amid 25%NP fertilizer in treatment T₅ and T₇ resulted higher MBC at 167.66 and 183.66 µg/g respectively, whereas the lowest (43.23 µg/g) was obtained from the control plots. Build-up of microbial biomass is mainly due to the microbial biomass contained in the organic residues & addition of substrate C, stimulating the indigenous soil micro-biota. Favourable effects stabilized specific populations of Azo spirillum and PSB were related to the application of compost and enriched compost & added micro organisms. Application of biofertilizers, besides showing their primary effect are also known to produce diverse growth promoting substances that might contribute to intense proliferation of microbial growth and augmented

MBC. Table 7(a)

8. Biochar amendment on soil bacterial abundance

Biochar is the product of the thermal degradation of organic materials in the absence of air (Pyrolysis) and is used as a soil amendment. (Lehmann *et al.*, 2011) selected three Sites for studying the effect of Biochar amendment on soil bacterial abundance as: Site A: Added N @ 300 kg/ha; Site B and Site C : added N @ 240kg/ha. On average, the biochar material contained on dry base organic carbon of 467 g kg⁻¹, total N of 5.9 g kg⁻¹, ash of 20.8% with a bulk density of 0.59 g cm³ and p^H (H₂O) of 10.42 as well as a CEC of 21.7 cmol kg⁻¹. The treatments were selected as: C0: 0 (Control), C1: 20 t/ha and C2:40 t/ha of biochar.

Table 8(a): MBC, MBN and Bacterial gene copy no under biochar treatment in 3 sites of Paddies

Sites and Treatments		MBC (mg/kg)	MBN (mg/kg)	Gene copy (10 ¹⁰ /g dry soil)
A	C0	338.86±18.82	20.27±7.59	1.86 0.09
	C1	336.77±3.75	42.63±5.37	2.08 0.17
	C2	321.703±6.85	32.52±3.15	2.70 0.33
B	C0	397.86±7.37	26.98±6.61	2.60 0.23
	C1	481.40±37.69	41.17±2.91	3.32 0.24
	C2	483.25±31.04	53.06±11.01	3.56 0.14
C	C0	602.67±52.21	50.47±3.00	1.31 0.06
	C1	750.76±78.85	62.35±7.15	1.69 0.20
	C2	750.22±46.07	60.41±9.72	2.10 1.22
Biochar		**	***	***
Site		***	***	***
BxS		*	NS	NS

NS, not significant ($p > 0.05$). a Significance levels for two-way ANOVA with biochar amendment and site as main effects. * $p < 0.05$ significant levels. ** $p < 0.01$ significant levels. *** $p < 0.001$ significant levels.

High dose of Nitrogen in Site A results in decrease in MBC with increasing level of biochar which causes detrimental

affect on micro-organisms. Bacterial gene copy number increases with increasing biochar level may be due to porous nature of biochar and increase in carbon source which enhances microbial proliferation. Table 8(a)

Table 8(b): Phylogenetic Diversity & Shannon index of bacteria of topsoil under biochar treatment in 3 rice paddies

Sites and Treatments		Phylogenetic diversity (PD)	Shannon index
A	C0	162	9.51
	C1	185	9.94
	C2	187	10.04
B	C0	147	9.94
	C1	155	10.12
	C2	166	10.28
C	C0	136	8.98
	C1	153	9.99
	C2	166	10.23
Biochar		***	***
Site		***	*
BxS		NS	NS

NS, not significant ($p > 0.05$). a Significance levels for two-way ANOVA with biochar amendment and site as main effects. * $p < 0.05$ significant levels. ** $p < 0.01$ significant levels. *** $p < 0.001$ significant levels.

PD higher in A even after more N dose shows presence of unculturable organism. Table 8(b)

Table 8(c): No. of sequences known of functional bacterial genera indicated in % of total of topsoil under biochar treatment

Site	T	Nitrifying bacteria			Denitrification bacteria	N fixation bacteria	
		Nitroso coccus	Nitroso Spira	Nitro Spira	Geobacter	Rhizomicrobium	Brady rhizobium
A	C0	0.67	0.33	2.56	2.00	2.78	0.78
	C1	2.44	1.56	6.44	17.00	6.56	1.44
	C2	2.33	1.00	4.22	15.11	4.22	2.56
B	C0	0.11	0.89	7.56	19.22	0.11	0.89
	C1	0.33	1.33	9.56	17.11	0.56	0.89
	C2	0.44	1.56	8.22	15.44	0.89	2.11
C	C0		1.56	14.00	8.44	1.56	0.45
	C1		3.78	12.33	16.89	0.78	0.44
	C2		3.11	9.33	10.33	0.33	0.56

NS, not significant ($p > 0.05$); NA, not applicable. Significance levels for two-way ANOVA with biochar amendment and site as main effects.

Nitrifying and denitrifying bacteria increases with medium level of biochar and decreases with higher dose of biochar. Brady rhizobium increases with increasing level of biochar. In paddy ecosystems, when organic C and N were limiting, strong competition for them in the rhizosphere soil would decrease microbial activity, thus having a negative effect on ammonium oxidization, denitrification, methanogenesis, and methane oxidation. Nitrification could also be an important

N₂O source, especially under organic C and N limitation, for rhizosphere microbial activity. Table 8(c)

Conclusion:

Soil biological properties viz. microbial biomass, soil respiration, population etc. increases with increasing of urea input on the whole. However, soil microbial functional diversity was correlated to soil moisture content. Excessive urea application caused significant water stress and microbial diversity depression after rice yellow ripening stages.

Application of chemical fertilizers alone could not be enough to support the microbial diversity and beneficial activities in paddy soil. Therefore, use of organic manures that enhance the microbial diversity, biomass and activity along with chemical fertilizer could greatly benefit in the sustainable management of agro-ecosystems. Organic amendments along with low amount of chemical fertilizer have a great potential to enhance paddy soil microbial biomass and microbial activity.

Different long term fertilization would change the microbes 'numbers and AOB communities in paddy soil. Farmyard manure plus mineral fertilizer was most effective for protecting the soil microorganism and maintaining the soil bio fertility.

In paddy ecosystems, when organic C and N were limiting, strong competition for them in the rhizosphere soil would decrease microbial activity, thus having a negative role in denitrification and other processes. Nitrification could also be an important N₂O source, especially under organic C and N limitation, for rhizosphere microbial activity.

Regular use of organic inputs (enriched compost) coupled with reduced chemical fertilizer can improve biological properties of soil under rice ecosystem such as microbial biomass carbon, microbial populations well as organic carbon concentration in soil under the sequence. Study suggests functional redundancy of the microbial communities of organically and conventionally farmed rice field soil.

Biochar plays a potential role in enhancing bacterial abundance, community diversity as well as modifying more or less the community compositions, particularly the bacteria involved in N cycling. Moreover, application of biochar has positive impact on the soil by increasing its microbial biomass carbon content. It did not affect microbial biomass nitrogen content of the soil. Further, the methanogenic archaeal diversity remains unchanged in one cropping season but its activity at varying biochar concentration may be one of the possible reasons to facilitate methane flux differentially.

Future perspectives

Understanding of microbial diversity and function in soils is limited because of the taxonomic and methodological limitations associated with studying these organisms. The level of microbial diversity and function in the soil, the link between structural diversity and function may be studied in order to understand the complexity of interacting biological, chemical, and physical factors.

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