# Soil Microbial Diversity in Long Term Fertilizer Experiments in different Agroecological Zones in India

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#### **Abstract**

On-going long term fertilizer experiments results illustrated that nutrient management options have influenced the biological properties of soil. Imbalanced fertilizer application adversely affected the biological state of the soil. Across the agroecological zones (AEZ), the balance nutrient application significantly improved the count of microorganisms i.e. bacteria, fungi, actinomycetes; their enzymatic activities and also active pools of nutrients. However, NPK+hand weeding treatment has edge over treatment 100% NPK with herbicide in terms of microbial count, microbial biomass carbon, dehygrogenase activities. The use of NPK along with farmyard manure has further enhanced all the stated soil biological properties of alfisols, vertisols and inceptisols. In alfisols, lime amendment has beneficial effect on biological health.

## 1. Introduction

Researchers during last few decades have identified specific pools of soil organic carbon (SOC) with functional significance in the turnover of organic matter in the soil especially under agriculture (Lal, 2009). These processes depended on the richness of soil in terms of microbial count and their enzymatic activities. Soil microbial biomass C and water soluble fractions of carbon are considered as the most active and highly labile fractions of SOC which enhances nutrient supply in response to management practices (Manna et al., 2007). Similarly, dehydrogenase activity is considered as one of the key indicator of biological status of soil. According to Oades and Ladd (1977) humic and fulvic acids are the most recalcitrant fractions of SOM but their fractional appearance seem to be of little significance in the turnover of SOC under cultivation. Indian Council of Agricultural Research (ICAR) has established AICRP on Long Term Fertilizer Experiments at different agroecological zones with different cropping

system and soil type in India during 1971-72. The information generated from these sites found to be very useful to calibrate the future strategies. Here attempt has been made to assess the biological state of the soils in terms of microbial count, their enzymatic activity and impact on organic pools and yield of crops under different nutrient management options over the years in long term experiments.

#### 2. Materials and Methods

The AICRP LTFE consists of ten treatments in each experiment at PDKV, Akola (sorghum-wheat), BAU, Ranchi (soybean-wheat), OUAT, Bhubaneshwar (rice-rice), TNAU, Coimbatore (maize-wheat) and CRIJAF, Barrackpore (rice-wheat-jute). There are 10 treatments in each experiment viz., 50% NPK, 100% NPK, 150% NPK, 100% NPK+hand weeding, 100% NPK+Zinc or lime, 100% NP, 100% N, 100% NPK+FYM, 100% NPK (sulphur free), Unmanured (control). The treatments were replicated four times in a randomized block design. The soil samples were collected from surface

layer up to 15 cm. The biological properties were estimated as per standard procedure. The recommended doses of nutrient applied are as per requirement of crops and FYM was over and above the nutrients.

#### 3. Results and Discussion

Soil organic matter acts as substrate for micro-organisms and helps in maintaining their population. Soil biota also needs nutrient for their multiplication and growth. In this context nutrient management enhances the biomass that proportionately adds crop residues in the soil influencing the biological soil properties.

# 3.1. Impact on microbial diversity

Microbial counts determined after harvest of sorghum clearly demonstrated that application of fertilizer improved their number. Irrespective of nutrient combination, application of chemical fertilizer resulted increase in population of bacteria, fungi and actnomycetes at Akola (Table 1), at Coimabatore (Table 2) and Bhubaneshwar (Table 3). Imbalance use of nutrients say N alone had negative effect on population of these organisms when compared with balanced treatment but superior to control. Incorporation of biomass in larger quantity through root and stubble added to soil as a result of higher productivity in plot receiving balanced nutrient application favored microbial population.

In Inceptisols of Coimbatore, also gradual increase in microbial population was observed with the addition of N, P and K over the years. It indicates that nutrients are sufficient to support growth of soil microorganism in these soils even with merely N and NP treatments. However, very sharp increase was recorded

Table 1: Impact of long term treatments on microbial count after harvest of sorghum at Akola

Treatment	Bacteria	Fungi	Actinomycetes
	$(\times 10^7  \mathrm{cfu})$	(×10 <sup>4</sup> cfu g <sup>-1</sup>	(×106 cfu g-1
	g-1 soil)	soil)	soil)
Control	6.25	4.50	5.00
100% N	8.75	6.25	5.75
100% NP	13.00	8.25	8.75
50% NPK	9.50	7.25	6.00
100% NPK	15.50	11.25	11.75
150% NPK	22.75	13.25	13.5
100% NPK (S free)	14.75	12.00	10.5
100% NPK+Zn @ 2.5 kg ha <sup>-1</sup>	17.75	11.50	12.00
100% NPK+ FYM @ 10 t ha <sup>-1</sup>	30.50	15.50	16.25
CD(p=0.05)	2.05	1.94	1.74

in population of organism with integrated nutrient management (NPK+FYM) treatment. It suggests that application of fertilizer has positive effect on microbial population and incorporation of FYM further favoured build up in their population. Similarly, at Bhubaneshwar also, application of fertilizer did not have adverse effect on biological health (Table 3). Amending soil with lime and FYM over and above NPK enhanced the microbial population in these soils.

# 3.2. Impact on enzymatic activities and organic pools

Nutrient management also has consequence on the enzymatic activities. Evolution of CO<sub>2</sub>-C with proportional presence of soil microbial biomass carbon (SMBC) in larger quantity supports the higher microbial population on balanced application of nutrient at Akola (Table 4). Evolution of CO<sub>2</sub>-C and SMBC are running parallel to population of these organisms. Detection of greater amount of dehydrogenase activity (DHA) further supports the findings, as DHA activity gives an overall indication about the biological health of soil.

At Barrackpore, higher microbial biomass carbon was observed in FYM amended treatment (Table 5). Basal soil respiration followed the trend of SMBC. Many workers have adopted measure of enzymatic activities of soil as an index of soil quality. Most studied enzymes include urease and phosphatase. There is evidence that some of enzymes may be confounded by soil pH and long-term application of fertilizers or liming. Hydrolysis of fluorescein diacetate (FDA) appears to be widely spread among the primary producers, bacteria and fungi. This hydrolysis is mediated by a number of enzymes like proteases, lipases and esterase, at a single time. Therefore, the assay is not specific to a particular enzyme, which in terms of soil quality assessment could be advantageous over individual enzyme assays.

Application of lime and FYM (at Bhubaneshwar) moderate

Table 2: Microbial population after finger millet at Coimbatore					
Treatments	Bacteria Fungi Ac		Actinomycetes		
	$(\times 10^7  \mathrm{cfu})$	(×10 <sup>4</sup> cfu	$(\times 10^3 \text{ cfu g}^{-1})$		
	g-1 soil)	g-1 soil)	soil)		
Control	4.7	20	7		
100% N	6.8	21	8		
100% NP	6.7	20	9		
50% NPK	7.8	22	9		
100% NPK	8.5	22	11		
150% NPK	7.7	23	10		
100% NPK+HW	8.3	24	11		
100% NPK (S free)	8.4	22	10		
100% NPK+Zn	7.9	22	10		
100% NPK+FYM	11.0	24	15		
CD ( <i>p</i> =0.05)	0.57	2.95	3.42		

Table 3: Effect of nutrient management on microorganisms count at Bhubaneshwar

Treatment	Bacteria	Fungi	Actinomy-
	(x10 <sup>7</sup> cfu	(x10 <sup>5</sup> cfu	cetes
	g-1 soil)	g <sup>-1</sup> ) soil)	(x10 <sup>5</sup> cfu g <sup>-1</sup> )
Control	5.78	4.6	0.92
50% NPK	5.13	6.6	1.77
100% NPK	6.20	7.45	1.42
100% NPK+FYM	8.70	3.03	7.23
100% NPK +FYM+Lime	12.77	5.90	0.32
100% NPK+Lime	10.70	5.32	0.47
100% NPK (S free)	14.75	12.00	10.5
100% NPK+Zn @ 2.5 kg ha <sup>-1</sup>	17.75	11.50	12.00
100% NPK+FYM @ 10 t ha <sup>-1</sup>	30.50	15.50	16.25
CD ( <i>p</i> =0.05)	2.05	1.94	1.74

Table 4: Impact of long term treatments on soil biological properties after sorghum at Akola

properties after sorghum at Akora					
Treatment	CO <sub>2</sub> evolution	SMBC	DHA (μg TPF		
	(mg 100 g <sup>-1</sup> )	(mg kg <sup>-1</sup> )	g <sup>-1</sup> 24 h <sup>-1</sup> )		
Control	22.00	137.81	32.62		
100% N	26.07	180.17	36.28		
100% NP	28.32	204.39	39.57		
50% NPK	27.35	197.49	37.60		
100% NPK	31.62	216.39	41.19		
150% NPK	40.42	239.79	46.10		
100% NPK	29.42	206.40	41.16		
(S free)					
100% NPK+Zn	32.72	218.70	42.28		
@ 2.5 kg ha <sup>-1</sup>					
100% NPK+	41.52	249.01	47.70		
FYM @ 10 t ha <sup>-1</sup>					
100% NPK+S @	35.47	221.05	44.45		
37.5 kg ha <sup>-1</sup>					
CD(p=0.05)	2.10	14.88	2.20		

the soil environment and provide more carbon to soil microbes through more root biomass due to larger yield. Increase in activity of urease and amidase (Table 6) on application of nutrients and soil amendments proved that integrated nutrient management with FYM or lime favours the growth and activities of microbes.

At Coimbatore, increase in biomass carbon was noted due to application of fertilizer with and without FYM. Microbial biomass carbon and nitrogen followed trend similar to that

Table 5: Microbial properties of soil after completion of 39 years of cropping at Barrackpore

A	В	С	D	Е	F	G	
Control	132	85	2.99	120	130	430	
100% N	104	123	0.18	142	159	422	
100% NP	108	79	0.80	161	124	322	
50% NPK	88	128	0.76	168	163	444	
100% NPK	118	101	1.81	134	167	390	
150% NPK	119	74	4.13	133	157	382	
100% NPK (-S)	134	86	2.45	150	147	299	
100% PK+HW	151	136	1.14	168	195	407	
100% NPK+ZnSO <sub>4</sub>	157	160	0.22	145	192	398	
NPK+FYM	148	221	3.45	181	228	436	
A=Treatments: B=FDA (ug g-1): C=MBC (ug g-1): D=DHA							

A=Treatments; B=FDA ( $\mu g g^{-1}$ ); C=MBC ( $\mu g g^{-1}$ ); D=DHA ( $\mu g TPF g^{-1} 24 hr^{-1}$ ); E=Urease ( $\mu g NH_4^+N g^{-1}$ ); F=ACPA ( $\mu g PNP g^{-1}$ ); G=ALPA ( $\mu g PNP g^{-1}$ )

FDA, fluorescein diacetate; DHA, dehydrogenase activity, ACPA, acid phosphatase activity; ALPA, Alkaline phosphatase activity.

Table 6: Effect of soil amendments on urease and amidase activities in soil (Bhubaneshwar)

Treatments	Enzymatic activities				
_	(mg NH <sub>4</sub> <sup>+</sup> -N kg <sup>-1</sup> soil 2 hr <sup>-1</sup> )				
	Urease Amidase				
Control	11.7	4.8			
100% NPK	13.5	8.8			
100% NPK+FYM	16.5	11.7			
100% NPK+Lime	23.3	12.7			

of microbial population which is expected as amount of SMBC and SMBN are depend on the active population of soil micro-organisms. At Ranchi also, active fraction of soil organic carbon (SMBC) and easily available N (SMBN) changed significantly and substantial decrease was recorded in these parameters under N or NP treatments as compared to balanced NPK use (Table 7). A decline in active fractions of C and N on long term cultivation of soil led to depletion of soil fertility through reduction of labile sources of nutrients, faster decomposition and lower bio-available nutrients. Furthermore, continuous cultivation and less aboveground biomass production significantly reduced total amount of nutrients as well as soil microbial biomass, which could have led to degradation of soil biological function.

The long term treatments have significant impact on carbon and its fractions under soybean-wheat system at Ranchi (Table 8). Redistribution of SOM from labile to more humified fractions was observed with cropping which points towards carbon sequestration. Thus, balanced plant nutrition (NPK+FYM or NPK+lime) was necessary to increase total amount of organic

Table 7: Microbial biomass C and N in LTFEs at Coimbatore and Ranchi

Treatment	Coim	batore	Ranchi		
	SMBC SMBN		SMBC	SMBN	
	(mg	(mg	(mg	(mg	
	kg-1)	kg-1)	kg-1)	kg-1)	
Control	219	24.6	175	16.9	
100% N	230	32.4	132	11.7	
100% NP	287	32.4	156	13.9	
100% NPK	329	54.0	186	18.3	
150% NPK	327	54.4	217	22.8	
100% NPK+HW/	220	10.5	207	23.0	
Lime	320	42.5			
100% NPK+FYM	346	67.9	237	24.9	
CD ( <i>p</i> =0.05)	11.4	3.75	8.8	1.4	

SMBC, soil microbial biomass carbon; SMBN, soil microbial biomass nitrogen.

Table 8: Long term effect of manure, fertilizer and lime on distribution of carbon in different fractions of soil humus after soybean at Ranchi

A	В	C	D	E	F
Control	0.32	0.062	0.091	0.153	0.167
100% N	0.36	0.039	0.076	0.115	0.245
100% NP	0.37	0.053	0.069	0.122	0.248
100% NPK	0.39	0.118	0.138	0.256	0.134
100% NPK+Lime	0.33	0.067	0.065	0.132	0.198
100% NPK+FYM	0.50	0.073	0.165	0.238	0.262
150% NPK	0.42	0.074	0.098	0.172	0.248
CD ( <i>p</i> =0.05)	0.08	0.01	0.02	0.025	0.04

A=Treatment; B=Total organic C(%); C=Humic C(%); D=Fulvic C(%); E=Humic+Fulvic C(%); F=Humin C(%)

fractions in Alfisol under soybean-wheat system. Similarly, carbon in humic and fulvic acid fractions was generally higher in 100% NPK+FYM treatments as compared to others except 100% NPK. The fulvic acid carbon was higher than humic acid carbon in all the treatments except in 100% NPK+lime.

#### 4. Conclusion

Long term fertilizer experiments have clearly proved that balance nutrient application is valuable with respect to growth of soil microorganism and their development. Application of recommended dose of NPK along with FYM or lime (in Alfisols) has further improved the richness of soil microbial fauna in terms of their count, enzymatic activity and active pools of nutrients. Thus, soil biological properties improved greatly with integration of nutrient and FYM or lime in each agro-ecological zone. However, microbial diversity and composition has to be adequately characterized under vivid nutrient management options and agroecological zones.

#### 5. References

Lal, R., 2009. Challenges and opportunities in soil organic matter research. European Journal of Soil Science 60, 1-12.

Manna, M.C., Swarup, A., Wanjari, R.H., Mishra, B., 2007. Long-term fertilization, manure and liming effects on soil organic matter and crop yields. Soil and Tillage Research 94, 397-409.

Oades, J.M., Ladd, J.N., 1977. Biochemical properties: Carbon and Nitrogen metabolism. In: Russell, J.S., Greacen, E.L.,(Eds.). Soil factors in crop production in a semi-arid environment. University of Queenland Press, St. Lucia, Queensland. 127-162.