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Rhizosphere Microbial Populations and Physico Chemical Properties as Affected by Organic and Inorganic Farming Practices

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Abstract: The present study was undertaken May-September (2007) to investigate the effect of organic viz., plant compost (PC), vermicompost (VC), integrated plant compost (IPC) and farmyard manure (FYM) and inorganic (NPK) fertilisers on the rhizosphere microbial population and soil physico chemical properties of soybean variety JS80-21. Control (CON) plot was also maintained without any fertilisers. Results showed that application of organic fertilisers had enhanced the microbial population compared to NPK and CON. The highest fungal and bacterial population was recorded in VC and the least in CON plot. Application of organic fertilisers also showed increase in rhizosphere soil physicochemical properties compared to NPK and CON plots. The above finding revealed that organic fertilisers would be able to sustain the soil fertility for a longer period by meeting the demand of present and future generation.

Key words: Microbial population · Organic and inorganic farming · Rhizosphere

INTRODUCTION

The rhizosphere is that region of the soil and the root influence by enhanced microbial activity [1]. A plant is a partner in the biocenotic system and all the physiological changes it undergoes during vegetation are reflected in the feature of coexisting microorganisms. The inseparable plant microorganism system is set up which undergoes short and long term fluctuation depending on plant development stage as well as agro-ecological condition [2]. Rhizosphere region provides better sites for the isolation of beneficial microorganisms than the bulk soil [3]. The physico-chemical properties of the region create different growing condition for microorganisms in comparison to root free soil. Land used activities particularly related to agricultural practices can have considerable impact on the size and activity of soil microbial community and biological health of soil.

Farming practices have undergone various changed from time to time with new technologies. For higher productivity, heavy doses of fertilizers and other agrochemicals are applied. These practices even though increase yield, make the microbial and plant system more vulnerable to various stresses beside deleterious effect on the environment. Because of the effect of synthetic chemicals and the associated quality, some ecologist and environmentalists have promoted the other extreme, i.e., to demand agricultural commodities produced in accordance with specialized system that is totally independent of any form of synthetic chemical influence. This has led to the emergence of a movement toward specified farming concept based on the traditional farming philosophy, which is popularly known as 'organic farming'. Organic farming has increased in recent years in many parts of the world. Organic systems do not use synthetic chemicals and in the long run may be more sustainable than inorganic or conventional farming.

In a long-term field trial in which organic and conventional agricultural systems were compared, microbial biomass was higher in soils from organic plots [4-7]. A 10-26% increase in microbial biomass under organic management was reported [8]. The addition of animal or green manures on organic farms provided a significantly greater input of organic carbon, which increased bacterial population. A 21-year study of agronomic and ecological performance of biodynamic, bioorganic and conventional farming systems in central Europe reported enhanced soil fertility and higher biodiversity in organic than conventional plots and concluded organic systems less dependent on external

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inputs[9]. Moreover, other researchers have shown that incorporation of organic amendments increased soil microbial activity [10], microbial diversity [11, 12], densities of bacteria [13], fluorescent Pseudomonas spp., fungi and nematodes [14]. Although the majority of research has shown increased microbial diversity in soils from organic farming systems compared to conventional farming systems [15] studied microbial communities in soils managed under organic and conventional regimes and found conflicting evidence that the size, composition and activity of the soil microbial biomass were attributed to management practice. They found that differences in microbial communities in soils under different management practices were subtle rather than dramatic. Conventional farming systems have been associated with loss of soil fertility, soil erosion and ground water pollution [16]. In addition, some conventional agricultural practices inhibit the activity and function of soil microbes. For instance, insecticide applications may promote changes in population biodiversity and dynamics by inhibiting or killing components of the soil microbial community. Fungicide application can cause significant changes to the relative sizes of the bacterial and fungal communities in soil [17, 18].

The purpose of this study aims to investigate the potential of organic fertilizers to improve rhizosphere microbial population and soil characteristics to obtain higher crop production without the need of tremendous inorganic fertilizers.

MATERIALS AND METHODS

Experimental Design: High yielding variety of soybean (JS80-21) was sown for investigation. The experimental field was divided into six plots with each having three replicates, among six plots; four were treated with organic amendments, one with inorganic fertilizer and one plot without any treatment and was considered as control plot. The inorganic plot was prepared about 100m away from the organic and control plot so as to avoid any mixing of inorganic fertilizers in the organic and control plots. The optimum dose of fertilizer applied in the field (Table 1). According to the type of treatment each of the plots were organic (PC, VER, IPC, FYM), control and inorganic (NPK). Each triplicate plot had a size of 6.5 x 6.5 m^2 with spacing of 30 x 15cm RxP (row by row and plot by plot).

Soil Sampling and Processing: Soybean roots and rhizosphere soil were collected directly beneath plant crown from experimental plots under different organic, inorganic and control plots. At each plot, three plants located in the middle were randomly selected for sampling. The soil containing soybean crown and root material, as collected in the field, were stored in a refrigerator at 4°C until they were processed. Inoculum from each collection was prepared by compositing subsamples from the three plants sampled at each plots and sieving the rhizosphere soil through a 2-mm sieve to remove rocks and large litter fragments. All adhering root material was removed and the sieves were cleaned in distilled water and allowed to dry between samples to avoid cross-contamination. Additional root material was clipped free from the rhizomes and crown, cut into 2-cm pieces and combined with the soil sieving for isolation of microorganisms and about 300g of each rhizosphere soil samples were air dried and stored at 4°C for chemical analysis. The mean data was used to interpret as the effect of organic amendments and inorganic fertilizer on microbial and chemical characteristics.

Isolation and Counting of Microorganisms: The serial dilution plate technique [19] was employed to enumerate the rhizosphere soil fungi and bacteria. Martin's rose Bengal agar medium [20] and nutrient agar medium was used for isolation of fungi and bacteria respectively. The inoculated Petri plates were incubated at 25±1°C for 5 days and 30±1for 24hr for fungi and bacteria respectively. After the incubation period, the colony forming units were counted and expressed as CFU g^{-1} of soil on a moisture free basis.

Table1: Type of fertilizer treatments and doses

Treatments	Source	Doses
1. Farmyard manure (FYM)	Cow dung	15t/hec
2. Vermicompost (Vermi)	Earthworm	7.5t/hec
3. Integrated plant compost(IPC)	1/3rd FYM:Vermi:PC	5t/hec
4. Plant compost(PC)	Plant debris	15t/hec
5. NPK	N	10kg/hec
	P	30kg/hec
	K	20kg/hec
6. Control (Con)	-	-

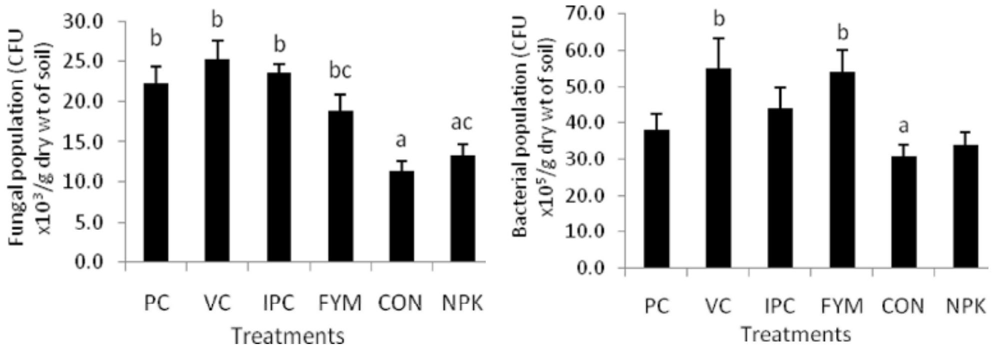

Fig. 1: Effect on rhizosphere microbial community (fungi and bacteria) as influenced by organic and inorganic fertiliser treatments. Mean±SE with the same letter on top does not differ significantly whereas different letters differs significantly according to Tukey test (ANOVA) (P=0.05)

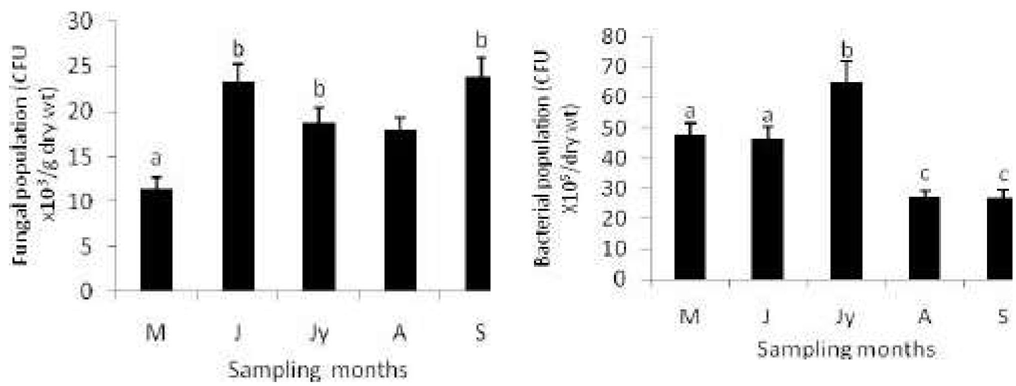

Fig. 2: Monthly variations in rhizosphere microbial community (fungi and bacteria) as influenced by organic and inorganic fertiliser treatment. Mean±SE with the same letter on top does not differ significantly whereas different letters on top differs significantly according to Tukey test (ANOVA) (P=0.05).
(Note: M=May, J=June, Jy=July, A=August, S=September)

Table2: Correlation between microbial population and physicochemical properties in organic and inorganic fertiliser treatments

Treatment		pH	MC	SOC	N	K	P
PC	FP	-	-	-	-	-	-
	BP	-	-	-	-	-	-0.62*
VC	FP	-	-	-	-	-0.52*	-
	BP	-0.62*	-	0.53*	-	-	-0.62*
IPC	FP	-	-	-	-0.60*	-0.55*	-
	BP	-	-	0.62*	-0.91***	-	-
FYM	FP	-	-	-	-	-	-
	BP	0.60*	-	-	-	-	-
CON	FP	-	-	-	-	-	-
	BP	-	-	-0.56*	-	-	-0.56*
NPK	FP	-0.85***	-	-	-	-0.53*	-
	BP	-0.76***	-	-	-	-	-

(Note: BP=Bacterial population, FP= Fungal population, MC = moisture content, SOC = soil organic carbon, N = nitrogen, K = potassium, P = Phosphorus, = not significant. *,**,*** represents significance at p=0.05, 0.01, 0.001 respectively and-represents not significant.

Soil Physico Chemical Properties: Soil temperature was noted using soil thermometer at the time of sample collection. pH of the samples was read using an electronic digital pH meter. The moisture content was determined by drying the samples in hot air oven at 105°C for 24h. Organic carbon was measured by the method given by Anderson and Ingram [21]. Total N, available P and K was determined by kjeldahl method [22] molybdenum blue method [23] and flame photometer method [22], respectively.

Soil respiration was determined by the absorption and titration method [24]. Soil microbial biomass carbon (MBC) was determined using the chloroform-fumigation-extraction method given by Anderson and Ingram [21].
Results and Discussions:

Microbial Populations: The fungal populations showed significant difference among treatments according to Tukey's test at p=0.05 (Fig 1). The greatest number of fungi was significantly greater in VC (25.23 x10^3 $CFUg^{-1}$ dry soil) followed by integrated PC (23.54 x10^3 $CFUg^{-1}$ dry soil) and the least was observed in CON (11.37 x10^3 $CFUg^{-1}$ dry soil). In this study the greater fungal population in vermicompost was further evidenced from the correlation between fungal population and available phosphorus. Fungal population in vermicompost was positively correlated to available phosphorus (Table 2). Monthly variation in fungal population also showed significant statistical difference among month according to Tukey's test at p=0.05 with the maximum in September (23.85 x10^3 $CFUg^{-1}$) and the least observed in the month of May (11.46 x10^3 $CFUg^{-1}$ dry soil) (Fig2). The application of fertiliser increased the soil fertility level, the number of microorganisms and also agricultural practices particularly input of manure and cover crop has large impact on the size and activity of soil microbial population [25, 26]. The enrichment of soil nitrogen through biological fixation of nitrogen by the host legume plant could have also affected the microbial diversity [27].

In the rhizosphere soil the highest bacterial population was exhibited by Vermicompost (55.19 x10^5 $CFUg^{-1}$ dry soil) followed by farmyard manure (54.26 x10^5 $CFUg^{-1}$ dry soil) and the least was observed in CON (30.89 x10^5 $CFUg^{-1}$ dry soil)(Fig.1). There was a significant variation in bacterial population between control, vermicompost and farmyard manure according to Tukey test at P=0.05 (Fig 1). Also a positive correlation was observed between bacterial population and available phosphorus in VC treatments (Table 2). Monthly variations of bacterial population exhibited highest bacterial population in the month of July (64.94 x10^5 $CFUg^{-1}$ dry soil) followed by May (47.86 x10^5 $CFUg^{-1}$ dry soil) and the least was observed in the month of August (27.30 x10^5 $CFUg^{-1}$ dry soil). Also a significant monthly variation was observed in the bacterial population according to Tukey test at P=0.05 (Fig 2). Organically managed soils usually exhibit higher microbial biomass and activity [28, 9]. The highest bacterial population in Vermicompost treatment may be due to the ratio of Gram +ve to gram-ve bacteria and of bacteria to fungi as determined signature phospholipids fatty acids, were higher in organic treatment than in inorganic treatments [29]. They also concluded that organic amendment increased the C_{org} of the soil whereas C_{org} and C/N ratio significantly affect bacteria and eukaryotic community structure. The manure soils also supplied large amounts of readily available C, resulting in a more diverse and dynamic microbial system than in inorganically fertilized soil [30]. The soil microbial activity was always higher in organic plots than conventional plots [31].

pH and Moisture Content: All the treated plots with organic and inorganic treatment showed higher pH values in the rhizosphere soil except for NPK and CON. The highest pH value was observed in integrated plant compost (5.68) and the lowest was observed on CON (4.68). Significant difference in pH was observed between organic, NPK and CON according to Tukey's test at p=0.05 (Fig 3). Monthly variation in pH value was found to be highest in June and July and the lowest was observed in September (Fig 4). Changes in soil pH over time occur by the displacement of cations or by additions of sources of acidity like hydrogen and aluminum ions [32]. This increment in pH in the organic treatments could be ascribed that the decomposition of organic products released Ca and Mg nutrients which could slightly increase the soil pH [33]. Organic manures can increase the buffering capacity of soils, preventing swings in pH, because of the additional organic matter [34]. Comparison studies of conventional and organic agroecosystems revealed that organic systems sometimes have higher pH levels in mildly acidic soils than their conventional counterparts [35, 36, 16, 37, 38]. This illustrates the important role organic manures and other organic matter inputs can have in buffering the soil [34, 39]. A comparison on organic pineapple production system was done, with compost as its only fertility input, with a conventional system using chemical nitrogen, phosphorus and potassium fertilizer. It was found that pH and available Ca and Mg were higher with the compost application [35]. Moreover N-containing inorganic fertilizers unless specially treated tends to acidify soil. This is due to the fact that most fertiliser supplies NH^{4+} or results in its production. Upon oxidation NH^{4+} can release H-ions which are potential source of soil acidity [40].

Moisture content was recorded to be highest in PC (24.91%) and the least was observed in NPK (Fig 3), no significant difference in moisture content was found between treatments. A significant monthly variation was found in moisture content with the highest in June and

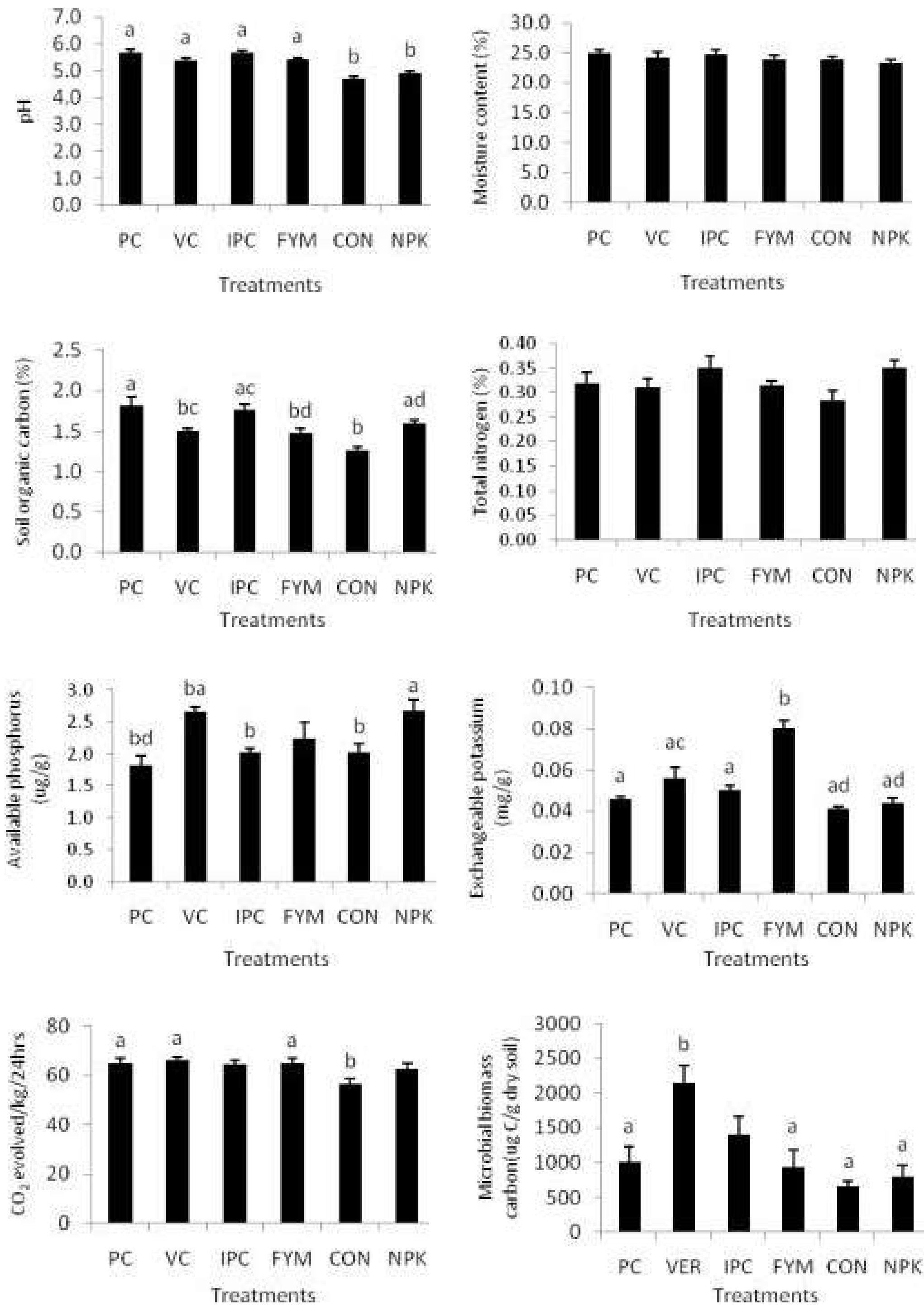

Fig. 3: Effect on rhizosphere physico chemical properties as influenced by organic and inorganic fertiliser treatments. Mean±SE with the same letter on top does not differ significantly whereas different letters on top differs significantly according to Tukey test (ANOVA) (P=0.05).

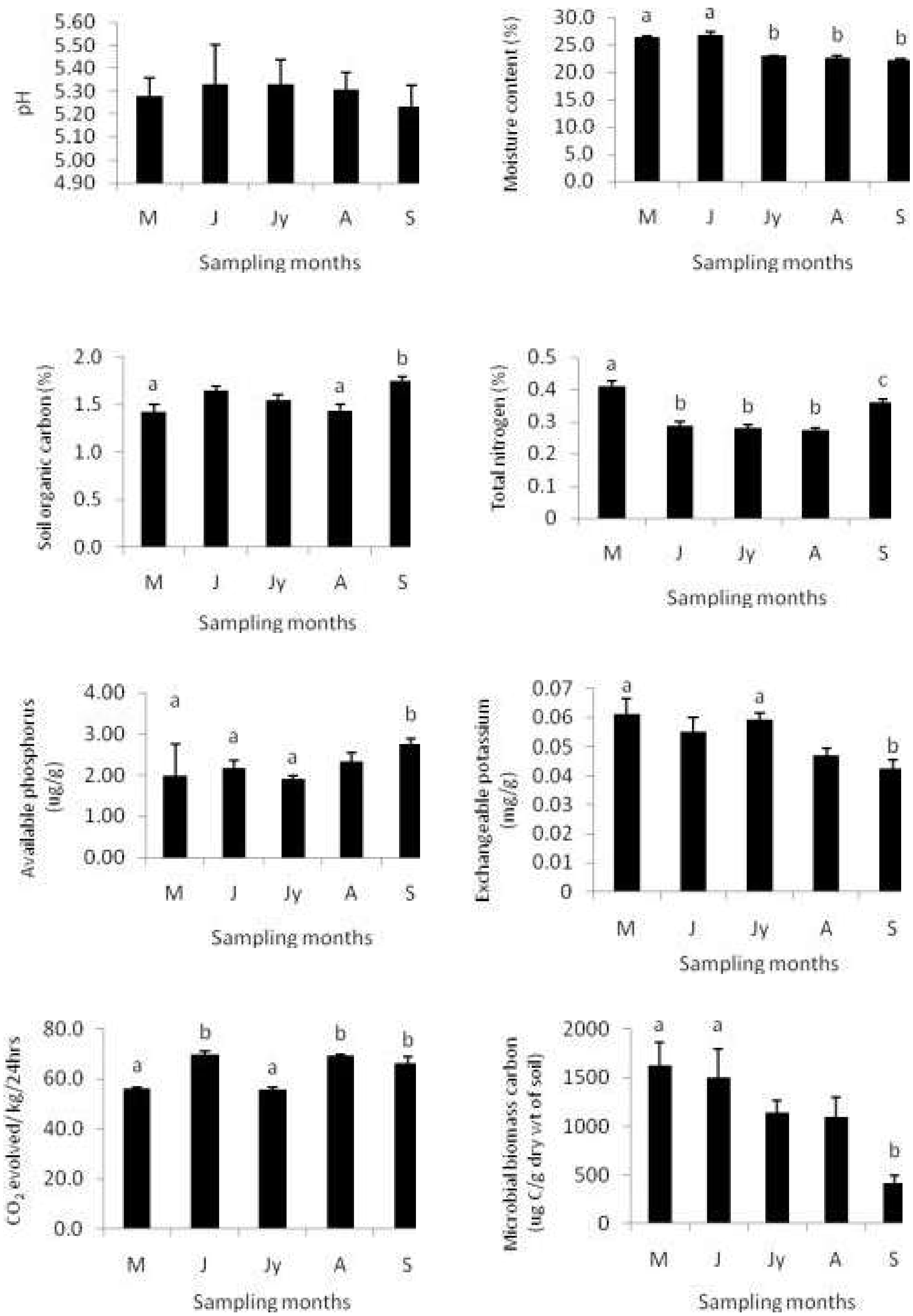

Fig. 4: Monthly variations in rhizosphere physico chemical properties as influenced by organic and inorganic fertiliser treatment. Mean±SE with the same letter on top does not differ significantly whereas different letters on top differs significantly according to Tukey test (ANOVA) (P=0.05).
(Note: M=May, J=June, Jy=July, A=August, S=September)

Table 3: Physico chemical properties of rhizosphere soil influenced by organic and in organic fertilizers

Treatment	pH	MC%	SOC%	TN%	µ /g AP	mg/gK	mg/gSR	µ /g MBC
PC	(5.2-6.9)	(21.8-28.1)	(0.43-2.16)	(0.16-0.46)	(1.04-3.12)	(0.03-0.05)	(48.8-73.0)	(130.5-2610.6)
	5.6±0.01	24.9±0.54	1.80±0.11	0.32±0.02	1.18±0.15	0.04±0.001	65.1±2.18	1015±207.3
VC	(5.1-5.8)	(19.4-29)	(1.4-1.65)	(0.24-0.47)	(2.32-3.2)	(0.035-0.089)	(56.98-71.94)	(984.9-3803.5)
	5.4±0.05	24.24±0.9	1.50±0.02	0.31±0.01	2.66±0.07	0.05±00005	66.11±1.54	2145.7±248.9
IPC	(5.3-6.2)	(21.4-33.9)	(1.02-2.13)	(0.20-0.53)	(1.57-2.52)	(0.029-0.06)	(52.14-72.38)	(131.5-3308.9)
	5.6±0.05	24.68±0.8	1.75±0.07	0.35±0.02	2.01±0.08	0.04±0.002	64.56±1.95	1385.1±264.4
FYM	(5.0-5.7)	(20.1-27.4)	(1.12-2.04)	(0.25-0.37)	(1.21-4.40)	(0.063-0.102)	(52.14-72.6)	(14.1-2453.09)
	4.6±0.08	23.82±0.6	1.27±0.03	0.31±0.01	2.24±0.25	0.08±0.003	56.5±1.93	940.9±240.3
CON	(4.1-5.1)	(20.4-27.4)	(1.05-1.46)	(0.19-0.44)	(1.20-3.16)	(0.041-0.063)	(34-34-71.5)	(173.0-1210.5)
	4.9±0.06	23.39±0.44	1.60±0.03	0.28±0.01	2.01±0.15	0.05±0.001	56.56±2.39	656.5±83.08
NPK	(4.5-5.3)	(20.7-26.8)	(1.43-1.86)	(0.22-0.46)	(1.84-3.72)	(0.035-0.046)	(48.84-72.38)	(86.7-1756.4)
	4.9±0.06	23.39±0.44	1.60±0.03	0.35±0.01	2.68±0.16	0.04±0.001	62.89±2.23	798.9±167.7

Note: Min and max range followed by mean ± Standard error. PC= Plant compost, VC= Vermicompost, IPC= Integrated plant compost, FYM= Farm yard manure, CON= Control, MC= moisture content, SR= Soil respiration, SOC= Soil organic carbon, TN= Total nitrogen, AP= Available phosphorous, K= Potassium, MBC= Microbial biomass carbon.

the least in September according Tukey's test at p=0.05 (Fig 4). Improved soil moisture associated with organic manure is attributable to mulching effect of organic matter and improved moisture retention and water acceptance as a result of improved soil structure and macro porosity [41].

Soil Organic Carbon: Soil organic carbon was recorded to be highest in PC (1.81%) and the least was observed in CON (1.27%). Significant difference was found in soil organic carbon among different treatments according to Tukey's test at p=0.05 (Fig 3). Monthly variation in soil organic carbon was found to be highest in the month of September (1.75%) and the least was found in the month of May (1.42%) (Fig4). The higher value of soil organic carbon in PC, IPC and NPK might be due to higher organic matter content in organic amendments. This is in accordance to the finding that soil organic matter levels and soil microbial activities vital for nutrient turnover and long term productivity of soil were enhanced by use of organic amendments along with inorganic fertilizers [42].

Total Nitrogen: In the rhizosphere soil was found to be highest in both IPC and NPK (0.35%) treated plots and the lowest was observed in CON (0.28%) (Fig3). No significant difference was found statistically among treatments. Monthly variation in total nitrogen was exhibited to be highest in the month of May (0.41%) and the least was observed in the month of August (0.27%) (Fig4). Significant difference in total nitrogen among months was observed according to Tukey's test at p=0.05. The insignificant variation within treatment could be due to fixation of atmospheric nitrogen by soybean through it root nodules present in the root system. The higher soil N content in the treated plot was greater in all fertilized treatment as compared to CON [43, 44].

Available Phosphorus: In the rhizosphere soil available phosphorus was found to be highest in NPK (2.68µg/g) and the least was observed in PC (1.81µg/g). Significant variation was found among all the treatments according to Tukey's test at p=0.05 (Fig 3). Monthly variation in available phosphorus was recorded to be highest in the month of September (2.77µg/g) and the least was recorded in July (1.9µg/g). Significant variation in available phosphorus was observed between months according to Tukey's test at p=0.05 (Fig 4). The highest value in NPK plot indicates that P fertilizer application significantly increased soil P concentration [45, 46]. Low value of P in PC may be attributed to more P uptake by crop in plant compost treatment, so less P was left for raising its status in soil.

Exchangeable Potassium: In the rhizosphere soil exchangeable potassium was found to be high in all the organically treated plots with the highest value observed in FYM (0.08mg/g) and the least was recorded in CON (0.04mg/g). FYM showed a significant difference with all the treatments, NPK and CON also exhibited significant difference in potassium level with VC according to Tukey's test at p=0.05 (Fig 3). Monthly variation in exchangeable potassium was found to be highest in the month of May (0.06mg/g) and the least was observed in the month of September (0.04mg/g). May and July exhibited significant difference according to Tukey's test at p=0.05 (Fig 4). The higher amount of fixed K in FYM treatment may be due to the fact that FYM increases CEC, which holds more exchangeable K by mass action. Similar results were reported by Dhanorkar *et al*. [47] in a study of forms of soil potassium as influenced by long-term application of FYM and NPK in vertisol and they further observed that distinct built up in total K was observed where K was applied and FYM alone increased total K by 40 per cent. The built up in total K may be attributed to fixation of added soluble K by clay mineral. On the other hand, Singh *et al*. [48] observed that there had been a net negative balance of total K in soil the decline in total K was larger in plots that received chemical fertilizer along with organic manure which was due to greater release of K from non exchangeable K pool and its subsequent uptake by crops.

Soil Respiration: Soil respiration among all the treatment in the rhizosphere ranged from 56.56 CO_2 kg^{-1} $24hrs^{-1}$ in CON to 66.11CO_2 kg^{-1} $24hrs^{-1}$ in VC treated plot. Soil respiration in VC, FYM and PC showed higher value and are significantly different then CON plots according to Tukey's test at p=0.05 (Fig 3). Monthly variation in soil respiration was recorded to be highest in June and the least was found in July. May and July exhibited significant difference in soil respiration according to Tukey's test at p=0.05 (Fig 4). The maximum soil respiration values in VC and FYM might be due to enrichment of soil nutrient through the addition of high organic carbon content or the availability of active organic carbon of VC and FYM. The higher value of soil respiration during month of June, August and September rather than May and July, may be due to the lower amount of organic carbon where VC and FYM were suppose to provide higher amount of organic carbon. Further it was reported that the mean soil CO_2 evolution was greater during the growing season than during fallow in all crops [49] and legumes could enrich their immediate soil environment with rhizobia through rhizosphere effect [50].

Microbial Biomass Carbon: In the rhizosphere soil microbial biomass carbon was found to be highest in VC (2145.77 µg C/g dry soil) and the least recorded in CON (656.52 µg C/g dry soil). Significant variation in microbial biomass carbon was found between VC and rest of the treatments except for IPC according to Tukey's test at p=0.05 (Fig 3). Monthly microbial biomass carbon was highest in May (1625.6 µg C/g dry soil) and least in Sep (82.27 µg C/g dry soil). May and June exhibited significant difference in biomass carbon with Sep according to Tukey's test at p=0.05 (Fig 4). Determination of soil microbial biomass carbon is an indicator of the response of microbial biomass to changes in soil management that affects the turnover of organic matter [51]. Result of this study indicates that the soil amended with PC and IPC increased MBC significantly more than that observed in control and inorganic fertilisers. Increasing MBC after application of organic amendments at a higher rate of application suggested that this soil is C-limited and that the labile carbon provided by PC and IPC may have been used as a source of energy by the microorganisms [52] indicated that soils treated with FYM and composts showed a significant increase in total organic C and biomass C in response to the increasing amounts of organic C added. It has also been reported that the soil receiving manure has larger MBC pool then in the same soil receiving only chemical fertilizers [53]. The addition of organic amendments increased the soil MBC compared to the fertilizer and control. Similar observations were observed in organic recycling experiments [54] Soil systems receiving more organic matter tends to harbour higher levels of MBC with higher microbial activity [55]. The higher MBC in PC treated soils is related to the quality of organic matter with respect to decomposition in these materials which is important for proliferation of microorganisms in soil. [56].

CONCLUSION

The application of organic and inorganic treatments differently affected the rhizosphere microbial population and physicochemical properties. The application of vermicompost resulted in most pronounced growth of microbial population compared to inorganic treatment. Also application of organic treatments showed increased rhizosphere soil physicochemical properties which in return lead to the increased microbial population which is of great importance in nutrient availability of the studied soil.

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