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Effect of Integrated Use of Organic and Fertilizer N on Soil Microbial Biomass Dynamics, Turnover and Activity of Enzymes under Legume-cereal System in a Swell-shrink (Typic Haplustert) Soil.

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ABSTRACT : Quantifying the changes of soil microbial biomass and activity of enzymes are important to understand the dynamics of active soil C and N pools. The dynamics of soil microbial biomass C and N and the activity of enzymes over entire growth period of soybean-(*Glycine max* (L) Merr.)-wheat (*Triticum aestivum* L.) sequence on a Typic Haplustert as influenced by organic manure and inorganic fertilizer N were investigated in a field experiment. The application of farmyard manure at 4 to 16 $Mg \cdot ha^{-1}\ y^{-1}r^{-1}$ along with fertilizer nitrogen at 50 or 180 $kg \cdot ha^{-1}$ increased the mean soil microbial biomass from 1.12 to 2.05 fold over unmanured soils under soybean-wheat system. Irrespective of organic and chemical fertilizer N application, the soil microbial biomass was maximum during the first two months at active growing stage of the crops and subsequently declined with crop maturity. The mean annual microbial activity was significantly increased when manure and chemical fertilizer at 8 $Mg \cdot ha^{-1}$ and 50/180 N $kg \cdot ha^{-1}$, respectively were applied. The C turnover rate decreased by 47 to 72 % when the level of farmyard manure was increased from 4 to 8 and 16 Mg $\cdot ha^{-1}$. There were significant correlations between biomass C, available N, dehydrogenase, phosphatase and yield of the crops.

Key words: Soil microbial biomass C and N, Dynamics, Activity of enzymes, Turnover rate, Farmyard manure, Chemical fertilizer, Legume-cereal system.

INTRODUCTION

Soil microbial biomass constitutes a transformation matrix for organic matter in soil and acts as an active reservoir for plant available nutrients (Jenkinson and Ladd, 1981). Recently, it has been established that more dynamic characteristics such as soil microbial biomass carbon (SMBC) and soil enzymes change more quickly than total soil organic matter (SOM) (Brookes, 1995). Recognition of the importance of SMBC has led to an increased interest in measuring the nutrients held in their biomass (Jenkinson and Polwson, 1976). Cultivation leads to a considerable loss of soil organic carbon (SOC) and microbial nutrients (Srivastava and Singh, 1991). Thus, the soil microbial biomass is an essential component of nutrient cycling in the agricultural ecosystem (Doran et al., 1987). However, seasonal changes in soil moisture, temperature, crop rooting and plant residues may have a great impact on soil microbial biomass (SMB), and nutrient availability status to plant through soil organic matter turnover. The turnover rate of microbial biomass is important if it is to be used as plant nutrient sources, since SOM turnover is higher in the tropical region compared with the temperate climate. Thus, crop management practices such as cultivation, crop rotation and chemical fertilizer exert a considerable influence on the level of organic matter retention in the soil over a period of time (Campbell et al., 1992), which regulates the soil microbial activity, nutrient recycling and soil organic carbon turnover (McGill et. al. 1986, Rao et al., 1990).

Continuous application of fertilizer N alone under intensive cropping system led to a substantial decrease in the SOM in tropical regions which has often been accompained by a decline in soil productivity (Swarup, 1998). Hence, low input sustainable agriculture and the reduced chemical input concept focus on the reconsideration of agricultural practices in order to maintain SOM level and health of the soil.

To understand sustainable soil fertility, therefore, the integrated nutrient supply systems under different management practices are needed to be investigated under semiarid and subtropical regions. The major objective of our present study was to assess the effect of FYM and fertilizer N on the dynamics of soil microbial biomass C and N, their turnover, and activity of enzymes in low organic matter swell-shrink soil (Typic Haplustert) under soybean-wheat-fallow system. Moreover, the contribution of microbial biomass C and N by the application of FYM and fertilizer N, is of great practical importance from several view points including their possible value as an indicator of soil quality and their relationship with yield of the crops.

MATERIALS AND METHODS

Experimental soils

The field experiment was carried out at research farm of Indian Institute of Soil Science, Bhopal, India, located in the semi-arid, sub-tropical monsoon type climate with an annual mean rainfall of 1208 mm of which 80-85 per cent falls during June-September. In January the temperature falls to a minimum of 10℃ (coldest) and the maximum of 42℃ in June (hottest). The soil was medium black clayey in texture and belongs to the Islamnagar series (Typic Haplustert). Characteristics of the soils used are 0.48% organic C; 60 % clay; pH 7.9 (1:2 :: soil : 0.01 M $CaCl_2$), 175±10 mg·kg^{-1} soil microbial biomass C and 46±15 mg·kg^{-1} day^{-1} basal soil respiration. Meteorological information during this study was also recorded (Table 1).

Field experiment

The experiment was initiated in the rainy season (June 1992), however, for the present study soil samples were collected periodically from autumn-spring-summer seasons of 1994-96. The treatments comprised of 4 levels of farmyard manure (0, 4, 8 and 16 Mg·ha^{-1}) either alone or in combination with nitrogen fertilizer (0, 50 and 180 kg·ha^{-1}) in randomized block design with four replications, having plot size of 10 m x 5 m. The treatment details are explained in Table 2. FYM was applied once in a year before sowing of soybean. Phosphorus and potassium were applied at the rate of 50 P_2O_5 kg·ha^{-1} and 40 K_2O kg·ha^{-1} through single super phosphate and muriate of potash, respectively, as a basal dose before sowing of both soybean and wheat. In soybean, the whole quantity of N was applied at sowing, while it was applied in two equal splits at sowing and 35 days after sowing of wheat every year.

Soil chemical and biochemical analysis

From each plot, moist surface soil samples (0-15cm) were

Table1. Soil physicochemical, microbiological and meteorological* properties of study site.

Soil Characteristics		Sampling time(1994-96)	Precipitation[a] (mm)	Temp.(℃)[a]	Pan evaporation
Taxomonic unit	Typic Haplustert	Janurary	--	27.5	3.6
Sand (%)	6	Feburary	0.5	27.5	5.7
Silt (%)	34	March	--	31.6	7.3
Clay(%)	60	April	3.8	37.9	183
pH(1:2)	7.9	May	1.5	37.9	16.5
CEC[cmol p(+) kg^{-1}	23	June	12.5	42.3	173
Available N (mg·kg^{-1})	159	July	355	31.1	189
Available P (mg·kg^{-1})	2.54	August	520	28.5	228
Organic C (%)	0.48	September	211	30.5	5.2
Microbial properties					
		October	4.5	32.8	152
		November	5.0	29.3	4.2
		December	5.0	24.5	225
SMBC (mg·kg^{-1}) C	175±43	January	--	27.5	3.7
SMBN (mg·kg^{-1}) biomass N	11±4	Feburary	--	28.0	215
Basal respiration (mg·Kg^{-1} day^{-1})	76±11	March	0.5	31.3	8.4

* Meteorological data collected during 1994-1996.SMBC: Soil microbial biomass carbon, SMBN: Soil microbial biomass nitrogen,

a : Values are mean of three replicates.

Table 2. Effect of organic manure and fertilizer N applicationon soil microbial biomass C and N dynamic under soybean-wheat-fallow in Typic Haplustert*

Treatment	July	Aug.	Oct.	Dec.	Feb.	April	June
	Soil microbial biomass C (mg·kg^{-1})						
T1	189	228	152	225	215	183	173
T2	201	240	163	230	220	198	185
T3	230	260	173	271	232	222	205
T4	307	338	258	282	255	234	212
T5	184	252	166	241	200	198	160
T6	239	270	179	265	240	218	205
T7	469	449	362	387	378	358	301
T8	446	468	381	425	392	363	333
LSD at 5%	33.9	26.8	29.1	23.3	19.8	17.6	23.7
	Soil microbial biomass N (mg·kg^{-1})						
T1	20	25	19	26	22	20	16
T2	23	25	20	26	22	21	18
T3	26	32	23	31	27	24	20
T4	34	40	28	30	31	32	20
T5	21	29	21	23	22	21	16
T6	28	33	25	29	24	23	17
T7	41	47	31	33	32	30	20
T8	50	52	41	46	35	31	22
LSD at 5%	6.3	5.5	4.7	4.2	5.7	3.9	2.8

T1 = Control, T2= Farmyard manure Mg·ha^{-1},
T3=FYM 8 Mg·ha^{-1}, T4=FYM 16 Mg·ha^{-1},
T5= N50/180 kg·ha^{-1}, T6=FYM 4 Mg +N 50/180 kg·ha^{-1},
T7=FYM 8 Mg +N 50/180 Mg·ha^{-1},
T8= FYM 16 Mg·ha^{-1} + N50/180 Mg·ha^{-1}
* Values are mean of two years (1994-1996) .

collected from 4-5 random points selected by leaving the border of 1 m from each side throughout the growing season of both crops as well as during the fallow period (June-July) from 1994 to 1996. The composite soil samples were brought to the laboratory, sieved through 2 mm and analysed for soil chemical and biochemical parameters. Organic C was determined by the chromic acid digestion method of Walkley and Black (1934), total N by Kjeltech Auto Analyser (Tecator Sweden model 1033) and mineral N (NH_4^+-N and NO_3^--N) was determined by steam distillation method (Bremner, 1965). Microbial biomass C was determined by the fumigated incubation method of Jenkinson and Powlson (1976). The composite soil samples from three out of four replicates fumigated with ethanol-free chloroform for 24 hr and non-fumigation soils (control) were incubated for 10 d at 25℃ in air-tight jars along with a beaker containing a vial of 10 ml 0.5 M NaOH. The evolved CO_2 was measured by titration of excess NaOH with 0.25 N HCl after addition of $BaCl_2$ to precipitate CO_3^{-2} ions. The soil microbial biomass C (SMBC), and soil microbial biomass N (SMBN) were calculated by using the following equations:

SMBC= [(CO_2-C mg kg^{-1} soil 10 d^{-1}) fumigated - (CO_2-C mg soil 10 kg^{-1} d^{-1}) nonfumigated] /k_C, where k_C = 0.45 (Jenkinson and Ladd, 1981).

SMBN = [mg NH_4^+-N + NO_3^--N kg^{-1} soil 10 d^{-1}) fumigated - (mg NH_4^+-N + NO_3^--N·kg^{-1} soil 10 d^{-1}) non fumigated] / k_N, where k_N = 0.54 (Jenkinson and Ladd, 1981).

Dehydrogenase, urease and phosphatase

The moist soil samples were analyzed with three replicates within 7 days of each sampling in triplicates. The activities of dehydrogenase, urease and phosphatase were determined by the procedure outlined by Casida et al., (1964); Tabatabai and Bremner (1969) and Tabatabai and Bremner (1972), respectively.

Statistical analyses

The significance of differences among treatments and sampling times were tested by analysis of variance and Fisher's test. Linear equations were fitted to establish the relationship of biological, climatic components and chemical parameters with soil properties.

RESULTS AND DISCUSSION

Dynamics of Microbial Biomass C and N

The soil microbial biomass C and N (Table 2) increased with increasing levels of manure (4 to 16 Mg·ha^{-1}yr^{-1}) application. Further, increase in biomass C and N was due to FYM in combination with fertilizer N throughout the growing period of crops. When FYM was applied either alone, or in combination with fertilizer N the increase in SMBC was comparatively larger in the early autumn (August) and was by 5 to 105.3% in case of SMBC and 16 to 108 % in case of SMBN in comparison with the control. The larger amount of SMBC in all treatments at the end of August was probably due to rhizodeposition which led to the significant development of microbial biomass, as was evident from the values in the active growing stage of the crops. Lynch and Panting (1980) also observed that the soil microbial activity increased with root growth and rooting intensity of the crops. As the soybean crop proceeded towards the maturity, a reduction in the microbial biomass C was recorded by about 18.6 to 34.1%, irrespective of treatment in the month of October, probably due to moisture stress and lowering in root activity at crop maturity. After sowing of the wheat crop in the first two weeks of November, the SMBC started to increase and attained maximum value in early spring (December) which again comensurate with the growth period of wheat crop. This indicated that addition of organic matter as a form of FYM supplied energy for proliferation of organisms and organic exudates released from growing roots played an important role in increasing SMBC in the succeeding crop (wheat). During the periods of both early autumn (August) and winter (December), the content of SMBN was 52 and 46 mg·kg^{-1}soil, respectively, in the plots which received 16 Mg FYM+50/180 N kg·ha^{-1}. However, the application of chemical fertilizer alone did not influence in SMBC (Table 2). There was a significant increase in SMB

Flux of microbial biomass C, N and turnover rate

The measurement of the SMBC does not reveal the dynamics of the microbial biomass population. The build up of soil microbial biomass nutrients after organic matter addition and the biomass turnover varies in different soils and with different sources of organic matter (Brookes et al., 1990). The measurement of biomass turnover can predict the flux of plant nutrients and the size of the plant nutrients pools through SMBC turnover in the soil. Jenkinson and Ladd (1981) proposed a turnover time of 1.25 year for biomass carbon by assuming that decomposition was 2 fold higher in warmer environment like Australia compared with cold environment. During the present study the highest mean annual SMBC recorded was 401 mg·kg^{-1} soil in the plot which received both FYM and nitrogen (16 Mg FYM + 50/180 N kg·ha^{-1}) (Table 2). In the present study, the flux of C and N was calculated by assuming a turnover time of 1.2 5 years for microbial nutrient (Table 3). The annual flux of C and N through SMBC in the treatment receiving both organic matter (FYM) and inorganic N fertilizer was nearly twice as large as the C and N flux in the unmanured plots. McGill et al., (1986) reported that about 32 to 72 mg 100·g^{-1} yr^{-1} carbon flux occured through microbial biomass in Canadian arable soils under different crop rotation. However, this turnover does not necessarily mean that the entire flux of microbial nutrients are available for plant growth. Brookes et al., (1984) suggested that some of microbial nutrient is transferred to the new microbial growth and some released to the soil solution which can be taken up by the plants or fixed by soil colloids. Our

Table 3. Effect of manure and fertilizer N on mean annual soil biomass C and N and the mean flux of C and N through the microbial biomass under soybean-wheat fallow system in Typic Haplustert

Treatment	Nutrient in the biomass (mg·kg^{-1})		Annual flux (mg yr^{-1})	
	C	N	C	N
T1	195 ± 28	21 ± 3	155	16.8
T2	205 ± 26	22 ± 3	164	17.6
T3	227 ± 33	26 ± 4	182	20.8
T4	269 ± 43	31 ± 6	216	24.8
T5	200 ± 35	22 ± 4	160	17.6
T6	231 ± 30	26 ± 5	185	20.8
T7	386 ± 30	33 ± 8	309	26.4
T8	401 ± 40	40 ± 4	321	32.0

Assuming a biomass turnover time 1.25 yr^{-1} as suggested by Jenkinson and Ladd (1981), Standard deviation

observation showed that the repeated application of FYM exhibited greater storage of soil C and further synergistic effect of the organic and the inorganic improved nutrients in the biomass (Table 3).

A perusal of the data in Table 4 shows that the carbon turnover rate decreased from 7.8 to 2.2 yr^{-1} by increasing level of C inputs which indicated the higher ratio of C inputs to SMBC. This suggested that combined application of both organic and chemical fertilizer N resulted in less microbial death or maximum build up of SMBC which, in turn, caused reduction in the turnover rate .

Dehydrogenase (DHA), urease and phosphatase activity

There was no definite pattern in urease (Fig.1,a & b) and DHA (Fig.1, a & b) activity at different growth stages. However, the activities were higher during autumn (August) and winter (December) which are commensurate with the

Table 4. Effect of manures and fertilizer N on C pools and carbon turnover rate under soybean-wheat system in Typic Haplustert

Treatments	Soil organic C (kg·m^{-2})	C inputs (kg·m^{-2} yr^{-1})	Carbon turnover (Yr^{-1})	C inputs/SMBC ratio
FYM(Mg·ha^{-1})				
4	1	0.128	7.8	3.12
8	1.04	0.256	4.1	5.9
16	1.12	0.512	2.2	9.7
FYM (Mg·ha^{-1}) + N (kg·ha^{-1})				
4+50 N kg·ha^{-1}	1	0.128	7.8	2.8
8+50 N kg·ha^{-1}	1.08	0.256	4.2	3.4
16+50 N kg·ha^{-1}	1.18	0.512	2.3	6.4

SMBC: Soil microbial biomass carbon

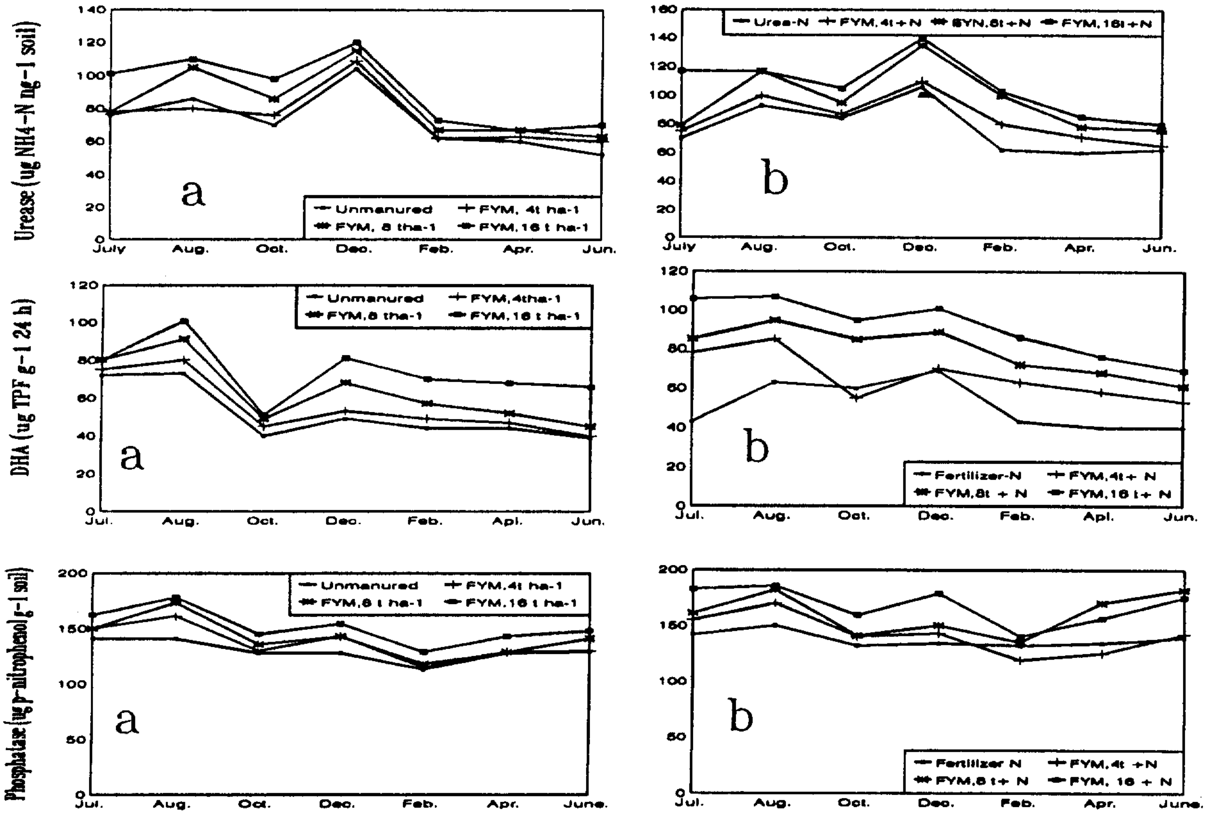

Fig. 1. Effect of organic manure (a) and organic manure + N (b) on urease, dehydrogenase and phosphatase activity under soybean-wheat system in Typic Haplustert

active growth period of soybean and wheat, respectively. In general, there is increase in microbial growth and enzyme activities with the addition of carbon and decrease as the available C is exhausted. The activities were consistently lower in the un-fertilized plots, however, the values were sustained at a higher level under integrated use of manure and chemical fertilizer application. These observations are in conformity with Nannipieri et al., (1995). Phosphatase activity showed significant changes in various treatments during growing period of soybean as well as wheat (Fig.1, a & b). During autumn (August), the maximum value of 200 mg p-nitrophenol g^{-1} soil was recorded in the both organic and fertilizer N the treated soybean growing soil, and in winter (December), a slight increase was recorded in wheat growing soil. During summer (fallow), the temporal changes of SMBC declined gradually but phosphatase activity remained higher during the period which was in contrary to the microbial biomass growth (Fig.1, a & b) possibly due to rapid cellysis during summer. Perucci (1990) reported that phosphatase activity increased with the increase in soil microbial biomass and further increase was due to cellysis of soil microbial biomass at later stage.

Soybean and wheat yield

Application of organic manure at 4 ,8 and 16 $Mg \cdot ha^{-1}$ either alone or in combination with fertilizer N increased the soybean and wheat yield over control (Table 5). There was significant positive relationship between mean annual soil microbial biomass and the grain yield of crops (r = 0.74 ; $P<0.05$) which suggests for improvement in the yield of the crops that have the synergestic effect of organic and chemical fertilizer. This equation explained about 54 percent variability ($P<0.05$) in the yield of crops due to variability of the SMBC (Fig.2).

Relationship between mean annual microbial biomass C and organic carbon, activitis of enzymes, and available N.

There were significant linear coefficient correlation between microbial biomass C and DHA (r= 0.77, $p<0.05$), urease (r=0.77, <0.05), and phosphatase (r= 0.73, $p<0.05$). There was significant correlation between soil organic carbon with mean annual biomass build up (r=0.77. $p<0.05$) .

Soil biomass is a repository of considerable quantities of N in forms which are much more labile than the N in humic

Table 5. Effect of farmyard manure and urea nitrogen on yield of soybean and wheat *

Treatment	---------------Grain yield ($kg \cdot ha^{-1}$)---------------	
	Soybean	Wheat
T1	704	2082
T2	907	2404
T3	1235	2806
T4	1465	3100
T5	1786	3451
T6	1976	3759
T7	2020	4025
T8	2135	4131
LS D at 5 %	233.4	306.1

* Values are mean of four years (1992-1996)

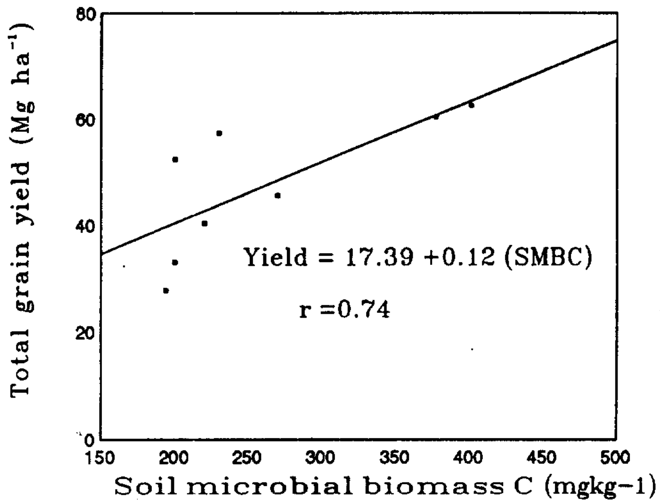

Fig. 2. Relation between grain yield and soil microbial biomass C

compounds that make of most of the soil organic matter. It contributes a significant part of the potentially mineralizable N (Bonde et al., 1988). Mineral N increased significantly following fertilizer application with no significant impact of SMBC and SMBN. However, the integrated use of FYM and chemical N increased substaintial amount of mineral N. Microbial N was also positively correlated to the mineral N ($r = 0.63$, $P<0.05$).

The amount of SMBC appeared to be controlled by fluctuating environmental condition. Considerably, more rain about 80-85 % was received from June to September. The temporal changes on soil microbial biomass was due to soil moisture changes. Further, regrowth of soil microbial biomass appeared to be related to temperature that affected by hot summer reaching about 42℃ in June (Fig 3).

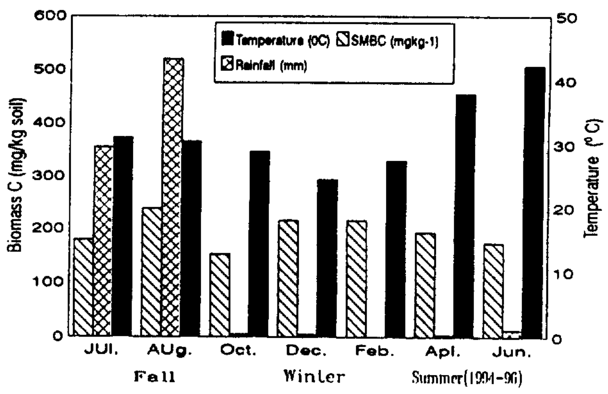

Fig. 3. Temporal changes of mean annual microbial biomass C, temperature and precipitation (1994-1996)

CONCLUSION

From these observations it may be concluded that the increased levels of manure application increase soil microbial activity and further, improve it when both organic and chemical fertilizer are used . There was no significant changes in the build up of soil microbial biomass by chemical fertilizer application alone. Microbial proliferation was maximum during active growth stage of the crops due to root exudates, irrespective of organic and chemical fertilizer application and decreased with the crop maturity. The most striking observation was the faster turnover rate, which was observed at lower level of C inputs. At higher levels of C inputs the turnover rate decreased which was probably due to less microbial biomass death or on the other hand, build up of more biomass that can be inferred from the ratio of C inputs to SMBC. The enzymes activity smaintained higher with higher level of organic inputs under integrated nutrient management system in Typic Haplustert. In addition, the management of organic manure incombination with chemical ferlizer can be optimized to achieve sustainable farming practices. As a consequence build up and turnover of native microbial biomass may contribute to an increased plant available nutrient status into soil and eventually to higher crop yields.

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