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ANALYSIS OF SOIL MICROBIAL BIOMASS DYNAMICS IN RAINFED WHEAT FIELDS IN ARID ZONE OF PAKISTAN

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Abstract

Soil Microbial Biomass (SMB) dynamics in rainfed wheat fields was assessed. In study ½ NP @ of 40:20 kg ha^{-1} and full recommended dose of NP @ of 80:40 kg ha^{-1} was applied. A composite soil sample was taken and its electrical conductivity (EC_e) was 0.38 dS m^{-1}. SMB parameters such as C_{mic}, N_{mic}, P_{mic}, C_{mic}: N_{mic} and C_{mic}: P_{mic} were determined by fumigation extraction method. The C_{mic}, C_{mic}: N_{mic} and C_{mic}: P_{mic} values were highest at the early stage of crop then declined in the middle and lowest at the crop maturity. Whilst N_{mic} and P_{mic} were low in the initial growth period and at peak in middle and lowest at maturity. All indices of SMB were increased by utilization of a balance fertilizer as in CK, the total amount of C_{mic}, N_{mic}, P_{mic}, C_{mic}: N_{mic} and C_{mic}: P_{mic} during wheat growth was 861.08 mg kg^{-1}, 84 mg kg^{-1}, 31.91 mg kg^{-1}, 83.81 mg kg^{-1} and 224.19 mg kg^{-1}, respectively. In ½ NP dose it was1260.4 mg kg^{-1}, 104.07 mg kg^{-1}, 45.69 mg kg^{-1}, 98.35 mg kg^{-1} and 230.33 mg kg^{-1}, respectively whilst in NP dose it was 1435.42 mg kg^{-1},112.68 mg kg^{-1},59.65 mg kg^{-1},102.78 mg kg^{-1} and 198.5 mg kg^{-1}, respectively. Seasonal variation depicted prevalent effect on SMB in the study. The average values of C_{mic}, C_{mic}: N_{mic} and C_{mic}: P_{mic} during drought period were higher than in rainy season. SMB fluctuation was ambient with regard to change in air and soil temperature. The considerable seasonal changes and variation due to fertilization in SMB indicated a direct relationship with nutrients availability which shed impact on growth and yield of crop.

Introduction

Pakistan is agricultural country and agriculture is backbone of economy. Agri-products do contribute @ of 24% in gross domestic product (GDP) and 47% national employments. Most area of Pakistan is arid to semi-arid region with 68% area has of the 250 mm precipitation (FAO, 2004). The arid area under research (Pothwar plateau- Rawalpindi) is situated between 32- 34° N and 71-73° E. The annual rainfall in the study area ranges from 500 to 1000 mm (Nizami *et al*., 2004). The soil of the area is loess, alluvial, colluvial and calcareous with high pH and low organic matter (OM). The mostly cultivated crop in this area is wheat (Halvin *et al*., 2004). The fertility of soil is important for better yield of crops and fertility means prevalence and availability of different micro and macro nutrients for plants (Hamayun *et al*., 2011).

Soil microbial biomass (SMB) is the living portion of organic matter. It transforms organic matter which is already present in the soil and added for improvement of soil fertility and it is a labile reservoir of available nitrogen (N), phosphorous (P) and sulfur (S) (Jenkinson & Ladd, 1981). The soil microbial biomass plays an important role as a dynamic source and sink of nutrients and it main is the driving force behind. SOM transformations showed that soil microbial biomass can be a source of plant nutrients in nutrient-poor tropical soils (Sakamoto & Hodono, 2004; Singh *et al*., 1989; Kang *et al*., 2012). Soil microbial biomass plays a key role in controlling nutrient cycle and energy flow (Bardgett *et al.,* 1997). It also plays an important role in regulating crop yield, such as reflected by positive correlations between grain yield and microbial biomass Carbon (C_{mic}), Nitrogen (N_{mic}) and Phosphorous (P_{mic}) (Frostega & Baath, 1996). The C_{mic} has been correlated with several functional microorganisms, such as microbial diversity (Nziguheba *et al*., 2006), legume-nodulating bacterial populations (Petersen & Klug, 1994) and enzyme activities in the soil (Bossio *et al*., 1998). Microbial activity is influenced by temperature, moisture and organic matter (Beier *et al*., 2008). Microbes regulate nutrient availability, and potentially immobilize large amounts of added nutrients and increase plant growth in nutrient limited ecosystems so SMB also plays an important role in carbon and nitrogen cycle (Ruan *et al*., 2004; Khan *et al*., 2012).

SMB is being directly and indirectly influenced by various factors such as soil texture, seasonal change, rhizosphere products (exudates), organic farming, continuous tillage and vegetation cover and depth of soil (Ross, 1987; Shabeg *et al*., 2007; Ferris *et al*., 2004). The numerous studies have evaluated the seasonal dynamics of C_{mic} and N_{mic} (Hamel *et al*., 2006; Montano *et al*., 2007; Murphy *et al*., 2007). Spedding *et al*., (2004) noticed greater effect of season than tillage and residue management on SMB. Campbell *et al*., (2010) predicted change in SMB, as decline in summer and rising in winter. SMB at the surface (0-15cm) is in higher amount but decrease with depth of soil. Vegetation cover increases soil microbial biomass as compared to barren soil. The type of vegetation is also important to enhance soil microbial biomass (Dijkstra *et al*., 2006; Russell *et al*., 2007).

SMB is influenced by inputs added to soil, either through plant productivity or exogenous soil amendments (Jackson, 2003).The addition of a significant amount of organic matter in the soil will increase SMB (Papatheodorou *et al*., 2004). SMB in wheat field is affected by fertilizers provision (Xie *et al.,* 2010). There was a positive increase in microbial biomass during seasonal-wheat crop. In the spring the amount of microbial biomass is highest in wheat field. The purpose

of this research was multifarious which included to investigate the dynamics of soil microbial biomass in relation with seasonal changes; secondly to explore the correlationship between soil microbial biomass (C, N and P) and nutrients availability to the wheat crop and thirdly to find out role of SMB on growth and yield of wheat.

Materials and Methods

Experiment layout: The experiment was conducted at research farm of Pir Mehr Ali Shah (PMAS) Arid Agriculture University Rawalpindi. An area of one kanal was selected which was provided three treatments as control (CK), half recommended dose of nitrogen and phosphorous (1/2NP) @ 40:20 and full recommended dose of nitrogen and phosphorous (NP) @ 80:40 in randomized complete block design (RCBD). Each treatment was replicated for four times. Wheat (*Triticum aestivum,* Chakwal-50) was sown on 27 October and was harvested on 20th May. Urea and DAP were used for N & P, respectively. At sowing time, complete dose of DAP was added but split doses of urea were given to the field. Other cultural practices (application of weedicides, pesticides and removal of weeds manually) were done during whole growing season.

Soil sampling and analyses: A composite soil sample was taken on regular basis each month during whole period of crop growth from 0-15 cm depth. A sub-sample of soil was air dried in the laboratory, ground in mortar and pestle, sieved through 2 mm stainless steel sieve and was stored in plastic containers for further experiments. This sample was analyzed for soil texture, soil bulk, soil moisture content, density, pH, EC_e, TOC, TN and available P. The moist soil samples was passed through a 2.0 mm sieve and mixed thoroughly which was used to determine microbial biomass C, N and P.

Physico chemical analyses: Soil texture was determined with Boyoucos Hydrometer method and textural class was determined by using ISSS triangle (Gee & Bauder, 1986). Total nitrogen (TN) was determined by colorimetric analysis (CMA) of digested soil samples and absorbance of samples was measured using Spectrophotometer at 665 nm (Anderson & Ingram, 1993). Available phosphorus (P) was determined by Olsen's method and transmittance was recorded by using Spectrophotometer (Olsen & Sommers, 1982). Soil pH was determined by 1:1 (soil: water) suspension procedure by using soil pH meter (Mc-Lean, 1982) and Electrical conductivity (EC_e) was determined by extracting the soil sample in a saturated paste and was measured with the help of conductivity meter (Rhoades, 1982). Bulk density was determined by core method and calculated by using the following formula (Blake & Hartge, 1986).

$$\text{Bulk density} = \frac{\text{Weight of oven dry soil}}{\text{Volume of core sampler}}$$

Total organic carbon (TOC) was determined by acid digestion method (Heans, 1984).
Soil total organic carbon was calculated by:

$$\% \text{ C} = \frac{\text{mg C}}{\text{Oven dry soil wt}} \times 100$$

Microbial biomass analysis

Soil microbial biomass carbon (C_{mic}): Microbial biomass C was estimated by fumigation extraction method (Brooks *et al.,* 1985). 10 g (on oven dry basis) of moist soil was fumigated for 24 h at 25°C with ethanol-free Chloroform ($CHCl_3$). After fumigation the sample was extracted with 50 ml 0.5 M K_2SO_4 for 30 minutes on horizontal shaker at 200 revs min^{-1} and filtered through paper (Whatman No. 42). The non fumigated 10 g portion was extracted similarly at the time when fumigation commenced. Total Organic C in the extracts was determined by titration method (TTM). Then microbial biomass C was calculated as:

$$\text{Microbial biomass C} = (C_{fumigated} - C_{unfumigated}) \times 2.64 \text{ (Anderson \& Ingram, 1993)}$$

Soil microbial biomass nitrogen (N_{mic}): Microbial biomass N was also measured by fumigation extraction method. Total N in the K_2SO_4 extract was measured after Kajeldahl digestion. For this, 30 ml of K_2SO_4 soil extract was taken in each Kajeldahl digestion tube, 0.5 ml conc. H_2SO_4 was added and the volume of extract was reduced to 1-2 ml by heating at 70^{o}C. At cooling, 1.0 g of digestion mixture ($FeSO_4$ 10: $CuSO_4$ 1: Se 0.1) and 4.5 ml of conc. sulfuric acid (H_2SO_4) was added to every digestion tube and refluxed the mixture for 3 hours. At cooling, 20 ml of distilled water was added to the digestion tube. Then 25 ml of 10 M NaOH was added slowly to the tubes, mixing the contents thoroughly after each addition of alkali. The digested material was transferred into steam distillation chamber of the Kajeldahl distillation apparatus by using 10 M NaOH and 2% H_3BO_3. The 40 ml of distillate collected and titrated to bluish red end point with 50 mM H_2SO_4. The soil microbial biomass N was calculated as:

$$\text{Microbial biomass N} = (N_{fumigated} - N_{unfumigated}) \times 1.46 \text{ (Anderson \& Ingram, 1993)}$$

Soil microbial biomass phosphorus (P_{mic}): The soil microbial biomass P was measured by same fumigation extraction method as used in C_{mic} and N_{mic} but difference was that the extract was taken in 0.5 M $NaHCO_3$. The pH of $NaHCO_3$ solution was adjusted to 8.5. Phosphorus was measured by Osen's method as, 1ml sample and standard were taken in test tubes and 4 ml ascorbic acid was added. After mixing color developing reagent was added and the

color was fully developed after 1hr. The reading was taken on spectrophotometer at 880 nm wave length. The microbial biomass P was measured by using the following formula:

$$\text{Microbial Biomass P} = (P_{\text{fumigated}} - P_{\text{unfumigated}}) \times 2.5 \text{ (Anderson \& Ingram, 1993)}$$

Statistical analysis: The data was subjected to Analysis of Variance and the means obtained was compared by LSD at 5 % level of significance. The results were co-related by correlation (Steel & Torri, 1980).

Results

Dynamics of soil microbial biomass carbon (C_{mic}): Soil microbial biomass carbon (C_{mic}) indicated considerable temporal fluctuation during the growth period of wheat crop as indicated in Table 2. It was highest in the initial growth period of wheat, which is also an active growth period but it was reduced near the time of maturity. It was also observed that the effect of treatments on C_{mic} was also valuable. C_{mic} was different in all three treatments. It was lowest (9.3 mg Kg^{-1}) without fertilizer or in control (CK) but in half recommended dose, in ½ NP (40:20 Kg hac^{-1}) was 13.1 mg Kg^{-1} and in full recommended dose (80:40 Kg hac^{-1}) was 16.1 mg Kg^{-1}. This variation was more significantly due to seasonal dynamics. During the extreme drought period (October, November, December and January) C_{mic} was higher (14.0 mg Kg^{-1}) than in pre-spring February with value up to 12.5 mg Kg^{-1}. When the temperature was optimum (24°C) in March and April C_{mic} was increased (13.3 mg Kg^{-1}) but again suddenly declined in May when the crop was matured @ temperature 29°C (Table 1 & Fig. 1). The relationship of C_{mic} was negative with soil microbial biomass nitrogen (N_{mic}) but was positively related to soil microbial biomass phosphorous (P_{mic}) and soil pH (Tables 8 & 10). The affect of all climatic parameters (rainfall, temperature, soil moisture and temperature) was inversely proportion to the C_{mic} (Table 1).

Table 1. Effect of climate on dynamics of Soil Microbial Biomass Carbon (C_{mic}).

Average of C_{mic}	Treatments (mg/Kg)			
Months	**1CK**	**2NP**	**NP**	**Grand Total**
9-Nov	11.7	12.9	14.3	13.0
9-Dec	11.8	13.9	16.3	14.0
10-Jan	11.5	13.4	16.4	13.8
10-Feb	10.0	12.1	15.3	12.5
10-Mar	11.2	13.6	16.9	13.9
10-Apr	10.3	12.4	17.2	13.3
10-May	9.8	13.5	16.6	13.1
Grand Total	10.8	13.1	16.1	13.4

Table 2. Effect of different treatments on dynamics of Soil Microbial Biomass Carbon (C_{mic}).

Average of C_{mic}	Months					
Treatments (mg/Kg)	**Dec-09**	**Jan-10**	**Feb-10**	**Mar-10**	**Apr-10**	**May-10**
CK	11.8	11.5	10.0	11.2	10.3	9.3
NP40: 20	13.9	13.4	12.1	13.6	12.4	11.5
NP80: 40	16.3	15.8	15.3	16.9	15.9	14.6

Dynamics of soil microbial biomass nitrogen (N_{mic}): Soil microbial biomass nitrogen (N_{mic}) was fluctuated under temporal change, climatic variation as well as at different levels of treatments. In temporal change it was low during early growth period, in middle it was high and near maturity was declined. This temporal change was very small. There was a considerable difference in N_{mic} at three levels of treatments. In CK (10.4 mgKg^{-1}) it was much low as compared to ½ NP (12.8 mgKg^{-1}) and NP (16.5 mgKg^{-1}) (Table 3). At three different levels of treatment there is no significant difference in N_{mic} values during whole growth period of crop. In NP and ½ NP, the difference was not larger but in case of CK it was significantly different in all months except in October and November (Table 4). Seasonal fluctuation was more substantial than temporal changes and treatment effects. In October, November, December and January it was low. During this period temperature was much low and there was extreme drought. In January, it was low (12.2 mg Kg^{-1}) when the temperature (12.1°C) was extremely low (Table 3). In February it was in high (13.9 mg Kg^{-1}) amount and the maximum level was obtained. The temperature (13.7°C) of the February was optimum and it was frost free. In March and April N_{mic} was also high (14.8 mgKg^{-1}) but in May it was decreased (12.7 mg Kg^{-1}). During May temperature was high (29°C) and the rain fall was low (22.6 mm) and crop was fully mature and was harvested. It was negatively correlated with C_{mic}

while positively with P_{mic}, these both relations were very weak (Fig. 2). It has a little increase with decrease in soil pH, so a weak negative relationship was between N_{mic} and pH. C_{mic}:N_{mic} and C_{mic}:P_{mic} have strong negative relation with $N_{mic,}$ it means that when it was high they were both in low amount. At high rain fall and soil moisture it was higher while the affect of temperature was negative but was much small.

Dynamics of soil microbial biomass phosphorous (P_{mic}): The temporal variation in soil microbial biomass phosphorous (P_{mic}) during whole period of wheat crop was observed throughout the experiment. But this difference was small and it fluctuated at different stages. It was low in early growing period then increased gradually in middle and then at the time of maturity it was suddenly declined. This difference was not as much. The variation in P_{mic} was more considerable at different levels of treatments. In CK it was much low (3.9 mg Kg^{-1}) while in ½ NP and NP it was maximum (4.4 mg Kg^{-1} and 5.2 mg Kg^{-1}) during all months (Table 5). It fluctuated during month to month and season to season. During winter when there was drought period it was low while in spring (February, March and April) was high. But during the rainy season it was much high. In January it reached to highest value (6.0 mg Kg^{-1}), suddenly when the weather was so cold but some rain (16 mm) was occurred. During spring it was also maximum (av. 6.8 mg Kg^{-1}). In this period the rain fall was maximum (av. 82 mm) and temperature was optimum (21°C). Maximum rainfall (115 mm) was in March and P_{mic} was also high (6.5 mg Kg^{-1}) while in December rainfall was minimum (trace) and P_{mic} was also low (4.9 mg Kg^{-1}) (Table 6). P_{mic} was significantly different in all three treatments in early growth period, in January there was a little difference between P_{mic} values in CK and ½ NP .There was a considerable difference between CK and NP while between ½ NP and NP it was non significant. In February P_{mic} in NP was much different than in CK and ½ NP treatments but very small difference between CK and ½ NP. In March this trend was opposite to February. In April P_{mic} in all three treatments was substantially different from each other while during May there was no difference between P_{mic} in CK and ½ NP but both were significantly different from NP (Fig. 3). The relationship of P_{mic} with C_{mic} was negative while was positively correlated with N_{mic} and the affect of pH on P_{mic} was negative. It was decreased at high pH and vise versa. There was a weak positive relationship between P_{mic} and C_{mic}:N_{mic} while strong negative relation with C_{mic}: P_{mic}. When rainfall and soil moisture content was high P_{mic} was also greater. With increase in both soil and air temperature there was a little decline in P_{mic}. At very low it was highest. So it was mostly affected by climatic factors (Fig. 3).

Table 3. Effect of climate on dynamics of Soil Microbial Biomass Nitrogen (N_{mic}).

Average of N_{mic}	Treatments (mg/kg)			
Months	**1CK**	**2NP**	**NP**	**Grand Total**
Nov-09	10.1	12.3	14.3	12.5
Dec-09	11.0	13.9	17.6	14.2
Jan-10	9.7	11.8	15.9	12.2
Feb-10	11.1	13.1	17.5	13.9
Mar-10	10.0	12.4	16.7	13.0
Apr-10	10.3	13.2	17.2	14.8
May-10	10.3	12.8	16.6	12.7
Grand Total	10.4	12.8	16.5	13.2

Table 4. Effect of treatments on dynamics of Soil Microbial Biomass Nitrogen (N_{mic}).

Average of N_{mic}	Months					
Treatments (mg/kg)	**Dec-09**	**Jan-10**	**Feb-10**	**Mar-10**	**Apr-10**	**May-10**
CK	11.0	9.7	11.1	10.0	10.3	10.3
NP40: 20	13.9	11.8	13.1	12.4	13.2	12.8
NP80: 40	17.6	15.9	17.5	16.7	17.2	16.6

Table 5. Effect of climate on dynamics of Soil Microbial Biomass Phosphorus (P_{mic}).

Average of P_{mic}	Treatments (mg/kg)			
Months	**1CK**	**2NP**	**NP**	**Grand Total**
Nov-09	3.9	4.1	4.8	4.3
Dec-09	4.1	4.4	5.2	4.6
Jan-10	4.7	5.0	6.0	5.2
Feb-10	4.0	4.5	5.2	4.6
Mar-10	3.3	4.0	5.0	4.1
Apr-10	4.0	4.7	5.3	4.7
May-10	3.6	4.2	5.0	4.3
Grand Total	3.9	4.4	5.2	4.5

Table 6. Effect of treatments on dynamics of Soil Microbial Biomass Phosphorus (P_{mic}).

Average of P_{mic}	Months					
Treatments (mg/kg)	Dec-09	Jan-10	Feb-10	Mar-10	Apr-10	May-10
CK	4.1	4.7	4.0	3.3	4.0	3.6
NP40: 20	4.4	5.0	4.5	4.0	4.7	4.2
NP80: 40	4.9	6.0	5.2	6.5	6.8	5.0

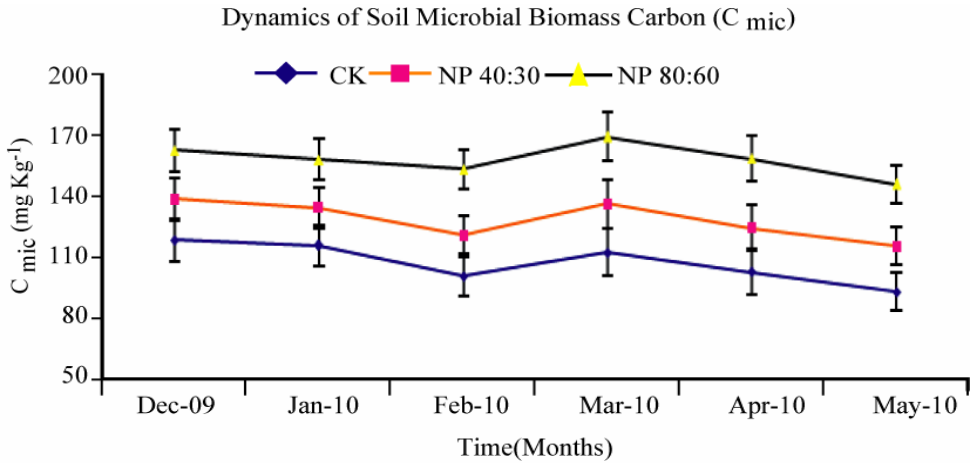

Fig. 1. Effect of Temperature (seasons) on dynamics of Soil Microbial Biomass Carbon (C_{mic}).

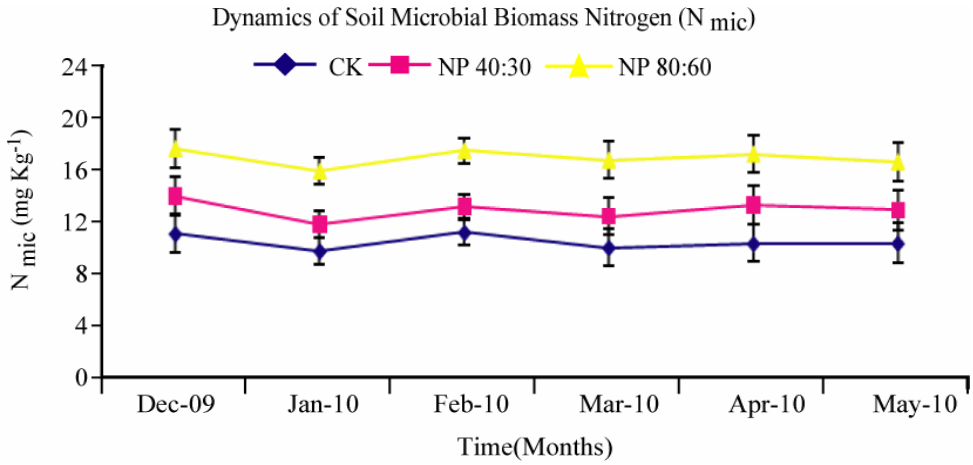

Fig. 2. Effect of Temperature (seasons) on dynamics of Soil Microbial Biomass Nitrogen (N_{mic}).

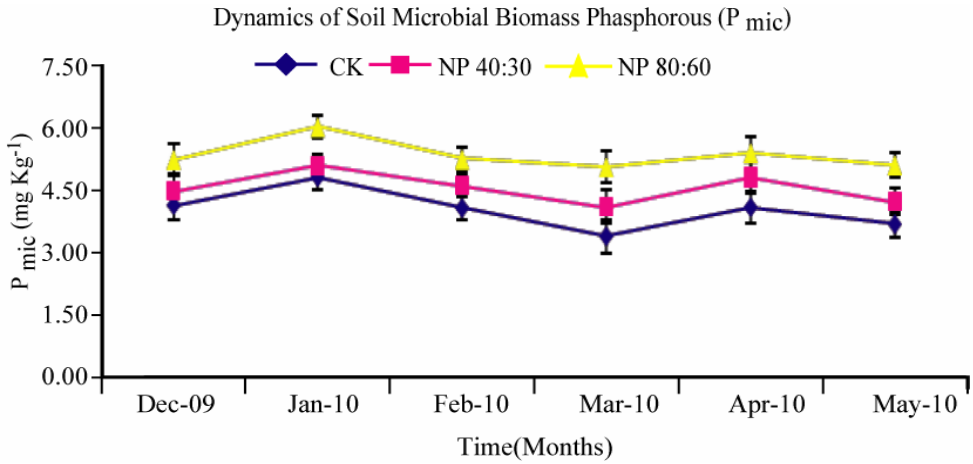

Fig. 3. Effect of Temperature (seasons) on dynamics of Soil Microbial Biomass Phosphorus (P_{mic}).

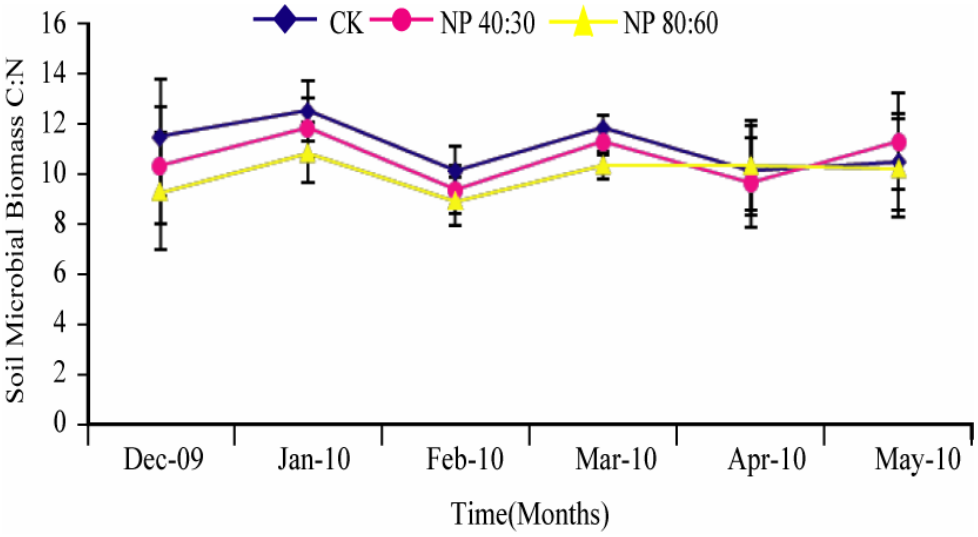

Fig. 4. Effect of Temperature (seasons) on dynamics of Soil Microbial Biomass C:N ratio.

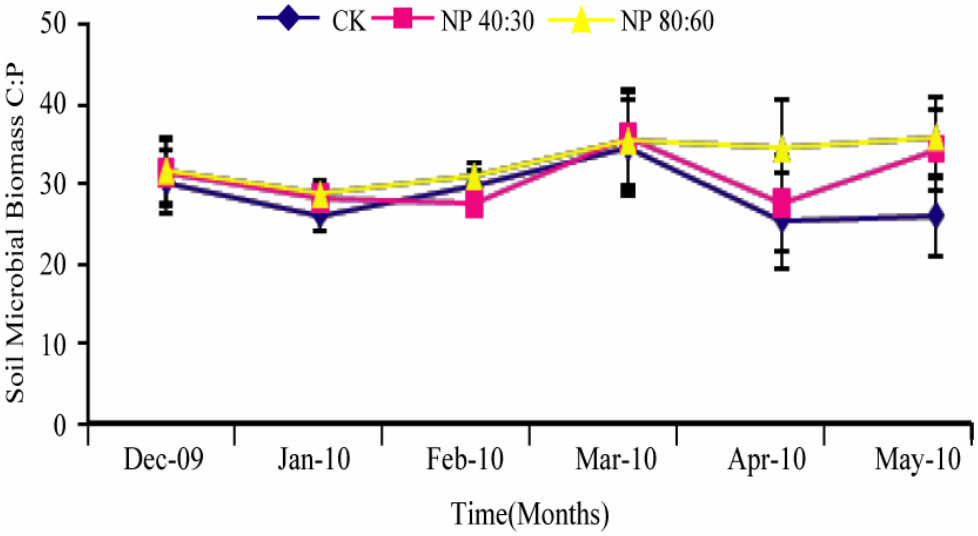

Fig. 5. Effect of Temperature (seasons) on dynamics of Soil Microbial Biomass C:P ratio

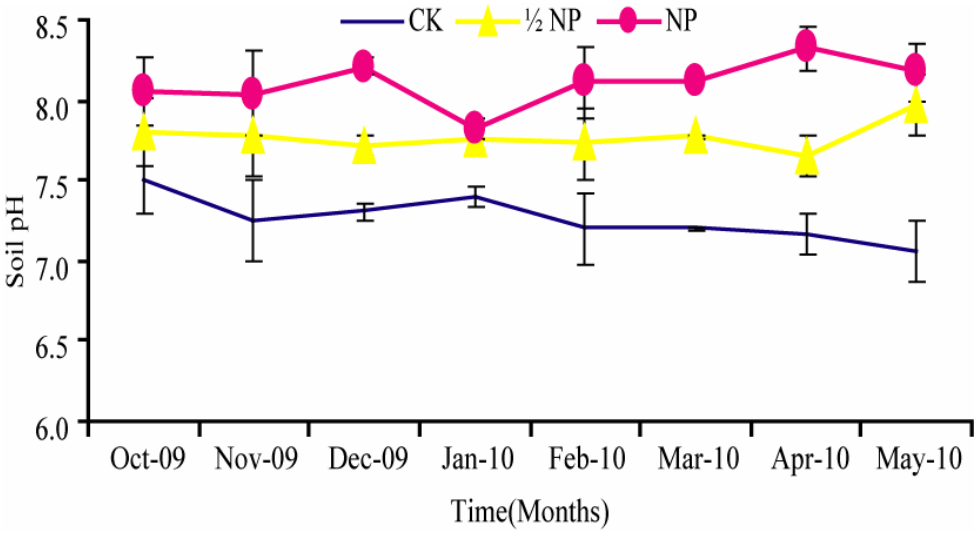

Fig. 6. Dynamics of soil pH in the rain-fed wheat field.

Dynamics of soil microbial biomass carbon and nitrogen ratio (C_{mic}: N_{mic}): The change in C_{mic}: N_{mic} ratio during growth period of wheat crop was much slight but as comparison it was greater in early stage of crop growth. It was declined at maturity and near the harvest. The fluctuation in C_{mic}:N_{mic} was not mark able in all three treatments although it was highest in NP then in ½NP while was lowest in CK (Table 7). The difference in all these value was not large in almost all months but in May CK was much different than NP and ½ NP. The average values of C_{mic} :N_{mic} in all three treatments were 12.8 mg Kg^{-1}, 12.2 mg Kg^{-1} and 10.4 mg Kg^{-1} in NP, ½ NP and CK, respectively. It has strong positive correlation with C_{mic} but weak with P_{mic} and has a much weak negative relation with soil pH (Tables 7 & 8). It means, the affect of pH on C_{mic}:N_{mic} was negligible. Its relation with C_{mic}:P_{mic} was positive but was also weak. All climatic factors (rainfall, temperature and soil moisture content) were inversely proportion to C_{mic}:N_{mic}. The affect of rainfall and soil moisture on C_{mic}:N_{mic} was more significant while the affect of temperature(air and soil temperature) was non significant (Fig. 4).

Dynamics of soil microbial biomass carbon and phosphorous ratio (C_{mic}:P_{mic}): The temporal change in C_{mic}:P_{mic} was also considerable and it was clear that in the initial growth period it was high then gradually decreased till maturity. C_{mic}:P_{mic} was positively correlated with treatments. It has a narrow range under the treatment as it was low without any treatment and also low in full recommended dose of NP than half recommended and was highest in ½ NP (28.8 mg Kg^{-1}) then in CK (28.0 mg Kg^{-1}) and was lowest in NP (24.8 mg Kg^{-1}) (Table 10). The climatic fluctuation was not considerable as compared to temporal and treatment flux. During December and January when the temperature was much low so C_{mic}:P_{mic} was 27.5 and in May it was again increased up to 31.8 (Table 9). C_{mic}:P_{mic} has a weak positive relationship with C_{mic} and C_{mic}:N_{mic} strong negative relation with $P_{mic.}$ High rainfall and soil moisture negatively affected C_{mic}:P_{mic} while it was directly proportion to temperature because at high temperature it was high while was decreased at low temperature (Fig. 5).

Table 7. Effect of climate on dynamics of Soil Microbial Biomass Carbon and Nitrogen Ratio (C:N).

Average of C:P	Treatments (mg/Kg)			
Months	1CK	2NP	NP	Grand Total
Nov-09	12.6	11.9	10.3	11.31
Dec-09	11.5	10.3	9.3	9.3
Jan-10	12.5	11.8	10.8	10.8
Feb-10	10.1	9.3	8.9	8.9
Mar-10	11.8	11.3	10.3	10.3
Apr-10	10.1	9.7	10.3	10.3
May-10	10.5	11.3	10.2	10.2
Grand Total	11.30	10.68	10.02	10.67

Table 8. Effect of treatments on dynamics of Soil Microbial Biomass Carbon and Nitrogen Ratio (C:N).

Average of C:N	Months					
Treatments(mg/Kg)	Dec-09	Jan-10	Feb-10	Mar-10	Apr-10	May-10
CK	11.5	12.5	10.1	11.8	10.1	10.5
NP40: 30	10.3	11.8	9.3	11.3	9.7	11.3
NP80: 60	9.3	10.8	8.9	10.3	10.3	10.2

Table 9. Effect of climate on dynamics of Soil Microbial Biomass Carbon and Nitrogen Ratio (C:P).

Average of C:P	Treatments (mg/Kg)			
Months	1CK	2NP	NP	Grand Total
Nov-09	30.9	32.7	29.6	31.0
Dec-09	30.0	31.2	31.5	30.9
Jan-10	25.9	28.0	28.5	27.5
Feb-10	29.7	27.4	30.7	29.3
Mar-10	34.3	35.6	35.2	35.0
Apr-10	25.2	27.4	34.2	28.9
May-10	25.8	34.1	35.6	31.8
Grand Total	28.0	28.8	32.2	30.6

Table 10. Effect of treatments on dynamics of Soil Microbial Biomass Carbon and Nitrogen Ratio (C:P).

Average of C:P	Months					
Treatments (mg/Kg)	Dec-09	Jan-10	Feb-10	Mar-10	Apr-10	May-10
CK	30.0	25.9	29.7	34.3	25.2	25.8
NP40: 20	31.2	28.0	27.4	35.6	27.4	34.1
NP80: 30	31.5	28.5	30.7	35.2	34.2	35.6

Dynamics of pH: The difference in pH values was very small. It was affected by different treatments. With increasing fertilizer it was increased to some extent. In CK it was low (7.3) but in ½ NP (7.8) and NP (8.1) it was high (Fig. 5). There was no considerable temporal and climatic fluctuation in pH. It was inversely proportion to three main parameters of soil *viz*: microbial biomass (C_{mic}, N_{mic} and P_{mic}). So, at low pH they were increased and the ratios of these parameters (C_{mic}:N_{mic} and C_{mic}:P_{mic}). It was low at high rainfall and soil moisture while was directly proportion to air and soil temperature. At high temperature it was also high and vice versa (Fig. 6).

Discussion

Soil is very essential for plants to grow and flourish. Different soils have different composition of micro and macro elements and texture which definitely influence the yield of crops and plants. Soil is also habitat of microorganisms which determine soil microbial biomass (SMB) that do have significant effect on soil and flora too. In this research it was found that soil microbial biomass (SMB) for carbon (C), nitrogen (N) and phosphorus (P) was determined at different stages of crop growth and considerable variations was found (Tables 2, 4, 6 & 8). C_{mic} was lowest (10.5 mg Kg^{-1}) in control (CK) and highest (4.0 mg Kg^{-1}) in full recommended dose, 80:40 Kg hac^{-1} NP (NP) and similar findings were observed in work of Franzluebbers *et al.,* (1995). Singh & Tripathi, (2007) reported that SMB was low in beginning but was considerable high at maturity but our results do not coincide with these. In our findings overall SMB was high at early stage till flowering but near to maturity it was substantially decreased that may due to experiment plan variations or eco-climatic difference. Patra *et al*., (1990) estimated a very small temporal difference in SMB. A considerable temporal fluctuation in soil microbial biomass carbon (C_{mic}) was proved by a continuous wheat (*Triticum aestivum* L.) cultivation as described in Table 1 and Fig. 1 (Franzluebbers *et al*., 1994). It was highest in the initial growth period of wheat which is an active growth period but it was gradually reduced at maturity (Muhammad *et al.,* 2007). The same trend was shown in another experiment according to which tillering stage of wheat crop had the maximum impact in enhancing MBC (414 mg kg^{-1}) in the soil followed by the flowering (396 mg kg^{-1}) and dough (297 mg kg^{-1}) stages (Shahandeh *et al.,* 2004). This temporal changes in C_{mic} also consistent with the work of (Singh *et al*., 2007b) and (Manjaiah *et al.,* 2000).

The considerable difference in N_{mic} at various crop growth stages was also observed by Shahandeh *et al*., (2004) but there was a minor difference in our findings, as N_{mic} was low at stage of tillering as compare to flowering stage (Table 3, Fig. 2). Murphy *et al.,* (1998) visualized a significant difference in N_{mic} during continuous wheat cultivation. According to him it remained half at maturity. From these investigations it was clear that most of the temporal changes in both C_{mic} and Nmic were related with the time of soil sampling. During early period it was high but was low near to end of experiment (Table 3) (Shahandeh *et al.,* 2004).

In the case of C_{mic}: N_{mic} temporal change was observed. It fluctuated at various stages of crop growth (Shahandeh *et al.,* 2004). Although this difference was so small and likewise C_{mic} and N_{mic}, their ratio was high at early growth period of wheat crop and declined near to maturity (Table 8, Fig. 4). There are certain possibilities of low C_{mic}: N_{mic} at the end of wheat crop growth. This decline might be due to variation in soil microbial community which could be as a result of change in N (Tables 8, 10 and Fig. 5) as added through urea or already present in the trial field and/or this decrease might be due to change in soil ability during the crop growth (Sugihara *et al*., 2010). High level of C_{mic}: N_{mic} in stages just prior to maturity or harvesting stage might be due to temporal change in two active group of microbes (Table 8): bacteria and fungi (Eberhardt *et al.,* 1996; Wardle *et al*., 2004). Lateral stages of wheat growth depicted considerable decline in C_{mic} and similar temporal trend was arrested in the case of P_{mic} which confer upon the work of Shahandeh *et al.,* (2004) but in this case change was so small and trend was similar to N_{mic}.

Overall P_{mic} was low during whole period of wheat crop growth, it might be due low availability of P in rain-fed area (Muhammad *et al*., 2006) and C_{mic} : P_{mic} was high which might be due to high C and low P availability (Table 10, Fig. 5) (Eberhardt *et al.,* 1996) at the early stage of wheat crop. Its trend of decreasing was similar results were observed by Shahandeh *et al.,* (2004). The annual changes in soil microbial biomass in C, N and P constituents under regular wheat cultivation were very minute and rare (Patra *et al*., 1990). It is paramount that there is scarce work done on temporal change in soil microbial biomass, so it is necessary to provide a serious attention to conduct comprehensive experiments and applied research for understanding this factor and its subsequent impacts on growth of wheat crop.

Soil microbial biomass was strongly affected by treatments as inorganic fertilizers. All three indices of soil microbial biomass (C,N and P) were fluctuated by the application of chemical fertilizers (Khan & Joergensen, 2009) as urea and DAP (Di-ammoniame phosphate) were used in this experiment as a source of NP (Table 10). It could be assumed that fertilization might be increased the soil fertility due to which the growth of crop was high which might be the cause of high SMB. C_{mic} was increasing with increasing fertilizers all values of soil microbial biomass were highest in plots where balanced dose of NPK was used, the same result was shown by Shahandeh *et al.,* 2004. The effect of fertilizer on C_{mic} was also investigated by Liu *et al*., (2003) and this investigation is consistent with our work by the following statement as C_{mic} was high by the application of N and P fertilizer (Figs. 3, 4). This might be a reason that microbes obtained more substrate by utilizing them they got energy which helped them for high metabolic processes culminating into more release of C_{mi}. Ayaga *et al*., (2006) reported that C_{mic} was related with inorganic P fertilizer. These our findings of increasing tendency in C_{mic} was also confirmed by Kouno *et al*., (2001) in their investigation it was observed that the value of C_{mic} was double to triple by the application of fertilizer as compared to control (Table 2, Fig. 1). The change in C_{mic} was also supported by another scientist Li *et al*., (2008).

Like C_{mic}, fluctuation in N_{mic} was very significant and its trend corroborates same as in C_{mic} even during fertilizer application and these results are also supported by the previous work of Shahandeh *et al.,* (2004). The application of DAP with urea increased N_{mic} (Khan & Joergensen, 2009) and it was also proved from the work of several scientists as Iyamuremye & Dick (1996), Hinsinger (2001) & Ayaga *et al*., (2006). From the results of this experiment it was clear that by increasing the fertilizer not only improve the availability of N for crop growth (Table 4, Fig. 2) but also a great source of N_{mic} which is another indirect source of N through microbial activity. The research described that soil microbial biomass phosphorous (P_{mic}) is also affected by type and quantity of fertilizers as urea and specially DAP indicated a considerable increase in it (Table 6, Fig. 3) and similar results were presented by Khan & Joergensen, 2009. P_{mic} was readily increased by the addition of inorganic source of P due to its excessive and easy availability and it was already demonstrated by Khan & Joergensen, 2009. It is found that high value of P_{mic} in initial growth period of crop, inorganic source of P is immediately incorporated into P_{mic} (Figs. 3,5) which corroborates past research outcomes (Muhammad *et al*., 2007).

Other parameter analyzed was ratio of (C:N) in soil microbial biomass which showed a very small variation against treatments during the whole study but their trend of improvement was similar to that of C_{mic} and N_{mic}; enhances with increasing the fertilizers application (Khan & Joergensen, 2009; Li *et al*., (2008).

The increase in C_{mic}: N_{mic} might be due enhancing soil metabolic process which might be speed up due to fertilizer used. As a result of this the lyses of microbes might be occurred due to which C was released from the body of microbes (Zahir *et al.,* 2010). The information about the effect of fertilizers on C_{mic} : N_{mic} was scarce as observed by Shahandeh *et al.,* (2004) that there was no affect of treatments on C_{mic} : N_{mic} but in this study we observed a little change in C_{mic} : N_{mic} which might be due to the more available sources of C and scarce of N source (Table 8, Fig. 4) (Khan & Joergensen, 2009).

As it was discussed previous that C_{mic} responded more actively against balanced P fertilizers (Shahandeh *et al.,* 2004) it might be a reason of low C_{mic}: P_{mic} at half dose of fertilizer in this experiment but full dose indicated the lowest value it was clear that P_{mic} could be increased by only excess of P fertilizer due to its low availability (Khan & Joergensen, 2009).

The seasonal fluctuation SMB was observed during this experiment (Tables 1,3,5,7,9) and this phenomenon has been proved by a number of studies (Wardle, 1992, 1998; Hamel *et al*., 2006; Montano *et al*., 2007 & Murphy *et al*., 2007). Both temperature and moisture are the limiting factor for the crop growth and nutrient availability to the plant, and microbes also depend on these factors for their activities to release different indices of soil microbial biomass (Fig. 5) and this study corroborates with work of Abbasi *et al.,* 2009. This discussion reveled that it was high in extreme drought period while it was low under heavy rain fall (Singh *et al.,* 1989 and Michelsen *et al.,* 2004) the main reason of low may be due lysis of microbes cell due to destructive effect of moisture (Halverson *et al.,* 2000 and Fierer & Schimel, 2003). In other research contrary findings were also reported that SMB was greater during drought period and this might be due to less competition with plants they absorbed more nutrient (Michelsen *et al*., 2004). Generally is claimed that SMB aid in prevention and/or minimizing leaching of N during heavy rains (Singh *et al.,* 2007b). The affect of temperature was also clear in this study that SMB was highest at low temperature during winter (Shahandeh *et al.,* 2004) and it was high in spring and in summer due to optimum temperature (Tables 1,3, 5, 7 and Fig. 2).

$C_{mic:}$ P_{mic} was strongly correlated with the individual values of C_{mic} and P_{mic} so seasonal changes in $C_{mic:}$ P_{mic} depends upon the changes in C_{mic} and P_{mic} values (Table 10, 8 and Fig. 5). The overall information about seasonal changes about SMB was much scarce agro ecosystem (Lulu & Insam, 2000). In this study soil pH was also monitored because it is a very important parameter which was affected by all same factors as which affected SMB but variation in pH was much small (Fig. 6). There was no visible temporal change was investigated in this study as well as no previous work showed this but a minute variation due to chemical fertilizer was observed (Li *et al*., 2008; Grayston *et al*., 2001). All parameters of SMB were directly correlated with soil pH in this study which corroborates previous findings (Anderson & Domsch, 1993). It is clear from this discussion that pH was it self affected in the same way as SMB was affected by the several factors but it also severed as a variable for SMB. Seasonal variation and treatment affect was more prominent factor to change SMB while other factors as temporal variation of wheat crop and soil pH also contributed to the SMB yield and productivity of plants.

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