Effect of Cropping Systems and Seasonal Variations on Soil Microbial Biomass and Enzymatic Activities in Arid Soils

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Abstract: This study aimed to investigate the effects of different cropping systems and seasonal variations on soil microbial biomass and enzymatic activities in arid soils. For this purpose, soil samples were collected from the soils under wheat *(Triticum aestivum)* – maize *(Zea* mays. L*)* and wheat – mung bean *(Vigna radiata*) cropping systems. The data showed that the soil microbial biomass Carbon (MBC), Nitrogen (MBN), Phosphorous (MBP) and soil enzymes such as dehydrogenase (DH) and alkaline phosphatase (AP) activities varied in all seasons. Overall, summer showed more soil MBC, MBN and MBP contents and relatively more DH and AP activities as compared to the other seasons. The soil MBC contents were higher under wheat-maize cropping system, while the soil MBN and MBP contents were higher under wheat-mung bean cropping systems in Kahuta areas. But the soil AP and DH activities were more pronounced under wheat-maize and wheat-mung bean cropping systems, respectively. We suggest that the inclusion of leguminous crops in cropping system is more suitable for arid areas, which tend to sustain soil fertility and preserve soil microbial biomass.
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1. Introduction

Soil productivity primarily depends on its soil biological health, which reflects the magnitude of soil microbial biomass C (MBC), soil microbial biomass N (MBN), soil microbial biomass P (MBP) and enzymatic activities (Kawabiah *et al.,* 2003; Hussain *et al.,* 2009a). In present scenario, the exhaustive and intensive cropping systems have endangered the health of soil ecosystem and its services as well. The preservation and sustainable utilization of soil ecosystem services is one of the key burning questions confronted to soil scientists across the globe (Foley *et al.,* 2005; Hussain *et al.,* 2009a; 2009b).

Recently several researchers have reported the adverse affects of different land uses practices on tropical forest ecosystem (Islam and Weil, 2000), grass land ecosystems (Garnier *et al.,* 2007), wetlands ecosystems (Acosta-Martínez *et al.,* 2007), appalachian forests ecosystems (Fraterrigo *et al.,* 2005), streams ecosystems (Allan, 2004) and on riparian ecosystem (Wang *et al.,* 2009) etc. Little is known about the consequences of different cropping systems and seasonal variations on soil biological health in arid soils.

At present, about 60-70 percent area of Pakistan is arid to semi-arid in nature. Owing to pre-existing climatic and environmental conditions, the annual precipitation in these areas is insufficient to support crop production on large scale to feed the masses. The currently used cropping systems in Pothowar (arid zone of northern Pakistan) are exhaustive, instead of restorative. In addition, the soils of this area are less productive because of low fertility status. This study aimed to investigate the effects of different cropping systems on soil MBC, MBN and MBP contents and enzymes activities in the soil occurring in this area. On the basis of this study, we attempt to suggest suitable cropping system under pre-existing arid environmental conditions to sustain crop production and soil health as well.

2. Material and Methods

2.1 Study site and soil sampling

Kahuta is situated in Pothowar region receiving an annual rainfall from 750 to 1000 mm per annum. In this area, the wheat-maize cropping system has been adopted more than 20 years before, while the wheat-mung bean cropping system is a newly (five years old) adopted cropping system. From the selected study sites**,** eighteen soil samples were taken from the soils (0-30 cm depth) under these cropping systems. The soil samples were air dried, sieved (2 mm) and preserved into polythene bags, each having 1.5 kg soil sample and were kept frozen before physio-chemical analysis. In addition to this, moist 1 kg field soil

samples were also collected from these sites and stored in ice tubes in fields. These soil samples were brought to laboratory for analyses of soil microbial biomass C (MBC), soil microbial biomass N (MBN), soil microbial biomass P (MBP) contents and also of soil dehydrogenase (DH) and alkaline phosphatase (AP) activities. Soil phsio-chemical and soil microbial biomass and enzyme activities were replicated six times from the selected sites of both cropping systems.

2.2 Soil chemical analysis

Soil samples collected from the selected sites were also analyzed for the soil chemical analysis. The brief soil chemical analysis is shown in Table 1.The soil reaction; calcareousness and salinity were determined by the established methods (Page *et al.,* 1982; FAO, 1974). Similarly the total organic C, total N, available P, soluble K, soluble Na, Cation exchange capacity (CEC) and Ca ± Mg of the soil samples were also determined by already established methods (Richards, 1954; FAO, 1974; Buresh *et al.,*1982; Knudsen *et al.,*1982; Olsen and Sommers, 1982; Rhoades, 1982).

2.3 Soil microbial biomass C (MBC) analysis

About 50 g soil sample was taken from representative sample for the said analysis. From this, 25g was fumigated at 25°C for 24h with ethanol free chloroform ($CHCl_3$). The fumigant was removed before taking soil extract. The soil extract was obtained by mixing soil with 100 ml 0.5 M K_2SO_4 and horizontal shaking at 200 revs min^{-1} shaking for 30 minutes. Soil extract was filtered through a folded filter paper. The non-fumigated portion (25g) also followed the same procedure. The organic carbon in the extracts was measured as CO_2 emission by infrared absorption after combustion at 850 °C by using a Dimatoc 100 automatic analyzer. The microbial biomass Carbon (MBC) was calculated by using previously published method (Wu *et al.,* 1985; Joergensen and Mueller 1996).

2.4 Soil microbial biomass N (MBN) analysis

Soil MBN was measured by using method developed by Brookes and colleagues, (1985). The soil sample of 30g in a 100-ml beaker containing 50 ml chloroform was placed in the desiccator. In addition, the pumice boiling granules were also added into the chloroform containing baker to assists rapid volatilization of the chloroform. The control non-fumigated soil samples also followed the same procedure. The vacuum was applied to the fumigated treatment during the chloroform was boiling. Then, we evacuated the fumigated treatment by using a vacuum pump repeatedly (8 – 12 times). From the desiccators, the fumigated and non-fumigated soil samples were transferred to 250 ml Erlenmeyer flasks and 100 ml 0.5 M potassium sulfate solution was added into each sample. The samples were shaken on an orbital shaker for 1 hour. Then, the suspension was filtered through Whatman No. 42 paper. The filtrates were added into a 250 ml calibrated digestion tube containing 1 ml 0.2 M copper sulfate solution, 10 ml concentrated sulfuric acid and a few pumice boiling granules. Then, the tubes in racks were placed in the block-digester. The temperature was set to 150 °C to remove extra water and was increased up to 380 °C. This digestion process was sustained for 3 hours. The tubes in racks were cooled to room temperature. The total N in the extracts was measured as NO_2 after combustion at 760 ^{0}C by using a Shimadzu-N chemo luminescence detector (Shimadzu Corp. Japan). The microbial biomass N was calculated as follows:

$$\text{Microbial biomass N} = E_N / k_{EN}$$

Where E_N = (total N extracted from fumigated soils) – (total N extracted from non-fumigated soils) and $k_{EN} = 0.54$.

Table 1. Physico-chemical characteristics of soil under various cropping system in Kahuta area

Seasons	Summer		Winter		Spring		Autumn	
Cropping Pattern	Wheat-Maize	Wheat-Mung bean	Wheat-Maize	Wheat-Mung bean	Wheat-Maize	Wheat-Mung bean	Wheat-Maize	Wheat-Mung bean
Soil Parameters								
pHs	7.32 ± 0.10	6.76 ± 0.13	7.39±0.028	6.80±0.014	7.43±0.03	6.87±0.08	7.8±0.03	7.01±0.02
ECe (ds m^{-1})	0.36 ± 0.03	0.26 ± 0.02	0.33±0.021	0.29±0.03	0.325±0.02	0.29±0.06	0.35±0.01	0.38±0.007
CEC (meq $100g^{-1}$)	9.8 ± 1.55	8.8 ± 0.56	14.47±0.62	12.2±2.53	13.65±0.21	12.09±2.80	10.21±2.40	8.56±0.64
$CaCO_3$ (%)	9.4 ± 0.98	4.4 ± 1.41	8.3± 0.84	4.75±0.49	7.9±0.28	5.15±0.78	6.95±0.30	7.075±0.99
TOC (%)	1.01 ±0.12	0.26 ±0.05	0.64±0.042	0.41±0.03	0.545±0.06	0.46±0.04	0.14±0.01	0.30±0.06
Total Nitrogen (%)	0.08 ±0.01	0.02 ±0.004	0.052±0.001	0.034±0.002	0.075±0.006	0.039±0.003	0.026±0.0007	0.036±0.001
Available Phosphorous (µg g^{-1})	4.65 ±0.77	5.85 ± 0.91	5.15±0.35	5.95±0.21	4.40±0.99	5.95±0.21	2.45±0.19	3.38±0.45
Soluble Potassium (meq L^{-1})	2.53 ±0.03	3.15 ±0.39	2.67±1.19	3.15±0.36	2.85±0.23	3.17±0.03	172.1±5.52	4.61±0.32
Soluble Sodium (meq L^{-1})	3.46 ±0.04	2.2 ±0.11	3.31±1.62	2.01±1.83	3.01±0.11	1.97±0.15	66.45±1.20	2.01±0.22
Ca ± Mg (meq L^{-1})	0.37 ±0.03	0.38 ± 0.03	0.35±0.042	0.355±0.04	0.33±0.03	0.35±0.01	0.53±0.06	0.515±0.15

2.5 Soil microbial biomass P (MBP) analysis

The soil MBP was also measured by fumigation-extraction technique (Brookes *et al.,*1982). About 30 g soil was taken from the representative soil sample for analysis The soil extract from a sub-sample of 10 g was taken by mixing soil with 100 ml of 0.5 M $NaHCO_3$ (pH 8.5). The mixture was horizontally shaken at 200 rev min^{-1} for 30 min. Afterwards, the soil suspension was centrifuged for 15 min at (2000 rev min^{-1}) and the extract was filtered subsequently. Similarly, 10 g of soil sample was also used as control for estimating the recovery of 25 µg P g^{-1} soil added as KH_2PO_4. The total phosphoric content was analyzed by a modified ammonium molybdate

ascorbic acid method (Joergensen *et al.,* 1995). The soil MBP was determined by method developed by Brookes and colleagues, (1985).

2.6 Soil alkaline phosphatase (AP) analysis

For estimation of alkaline phosphatase, one gram of soil sample was mixed with 0.2 ml toluene, 4 ml of MUB (modified universal buffer having pH 11) and 1 ml of *p*-nitrophenyl phosphatase solution. The mixture in the flask was placed in an incubator at 37 ^{0}C for 24 hours. Then, 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 N NaOH were added into the mixture. Afterwards, the soil suspension was filtered through a Whatman No.2 filter paper. The yellow color intensity was measured at 400 nm wavelength by using a Pharmaspec UV-1700 spectrophotometer Shimadzu (Eivazi and Tabatabai, 1977).

2.7 Soil dehydrgenase (DH) analysis

For this, 0.2 g of $CaCO_3$, 1 ml of 3% aqueous solution of TTC (triphenyl tetrazolium chloride) and 2.5 ml of distilled water were added into 10 g soil sample. The samples were incubated into tubes at 37 ^{0}C. Then, 10 ml of methanol was added into tubes and filtered after shaking. The red color intensity was measured by using a Pharmaspec UV-1700 spectrophotometer Shimadzu at a wavelength of 485 nm (Casida *et al.,* 1964).

2.8 Statistical analyses

The average of each sample for seasonal variation and microbial biomass were calculated and the standard deviation was tested at α 5% probability by using Stat View 5.0 (SAS Inst., Inc.).

3. Results

3.1 Soil microbial biomass C (MBC)

The MBC was monitored under wheat – maize and wheat – mung bean cropping system in Kahuta area in summer, winter, spring and autumn seasons (Fig. 1). Under wheat-maize cropping system, the average MBC contents differed significantly ($P < 0.05$) in all seasons. The average MBC contents under wheat – maize cropping system were 155.8, 136.3, 130.0 and 140.4 µg g^{-1} in summer, winter, spring and autumn, respectively. The wheat–maize cropping system had significantly ($P < 0.05$) more average MBC in summer as compared to other seasons. The average MBC contents under wheat – mung bean cropping system were 132.1, 137.5, 121.0 and 145.9 µg g^{-1} in summer, winter, spring and autumn, respectively. In this case, the average MBC contents were significantly ($P \leq 0.05$) lower in spring and were non-significantly ($P > 0.05$) higher in summer, winter and autumn. However, the MBC contents were similar to that of wheat – maize cropping system. Wheat – maize cropping pattern generally showed more average soil MBC contents in summer as compared to wheat – mung bean cropping system that showed more soil MBC contents in autumn. Hence, the soil MBC contents were higher under wheat-maize showed more as compared to wheat-mung bean cropping systems in Kahuta area.

3.2 Soil microbial biomass N (MBN)

Similarly soil MBN contents were monitored in all seasons under these cropping systems (Fig 2). The average soil MBN contents under wheat – maize were 7.9, 6.15, 7.3, 7.01 µg g^{-1} in summer, winter, spring and autumn, respectively. The average soil MBN contents were significantly ($P < 0.05$) lower in winter and high in spring as compared to other. Under wheat – mung bean cropping system, the average MBN contents were significantly ($P < 0.05$) lower in spring as compared to other seasons. The average

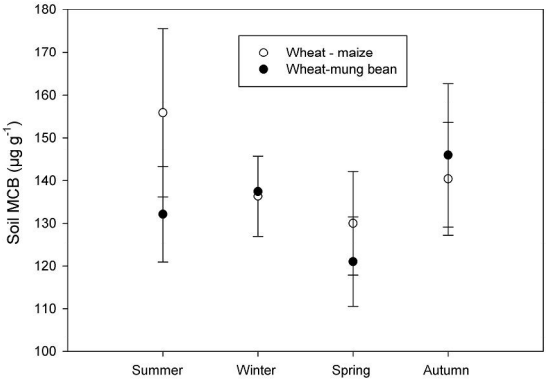

Figure.1 Effect of seasonal variations on soil MBC under different cropping systems

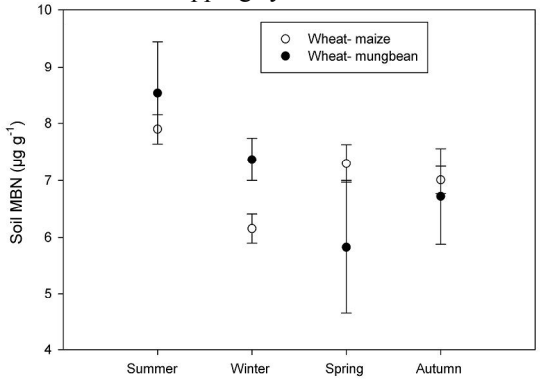

Figure. 2 Effect of seasonal variations on soil MBN under different cropping systems

MBN contents were 8.54, 7.37, 5.83 and 6.72 µg g^{-1} in summer, winter, spring and in autumn, respectively. Pertaining to seasonal impact, the soil MBN contents were found higher in spring and summer under wheat – maize and wheat – mung bean cropping system, respectively. Comparatively the wheat – mung bean cropping pattern had more

average soil MBN contents as compared to those observed under wheat – maize cropping system.

3.3 Soil microbial biomass P (MBP)

The average soil MBP contents under wheat – maize and wheat – mung bean cropping system also differed in all seasons (Fig. 3). The average soil MBP contents under wheat – maize were 5.84, 3.91, 4.42, 4.11 µg g^{-1} in summer, winter, spring and in autumn, respectively. The average MBP contents were non-significantly ($P > 0.05$) lower in winter season as compared to other seasons. Similarly the average soil MBP contents under wheat – mung bean cropping system were 6.12, 5.42, 4.38 and 3.13 µg g^{-1} in summer, winter, spring and autumn, respectively. The average soil MBP contents were significantly ($P < 0.05$) higher in summer followed by other seasons. In general, the wheat – mung bean cropping system showed more average MBP contents as compared to wheat – maize in Kahuta area.

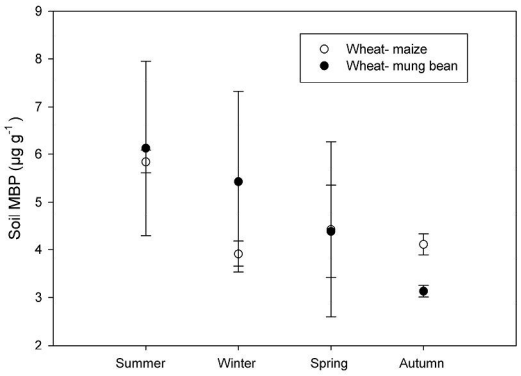

Fig. 3 Effect of seasonal variations on soil MBP under different cropping systems

3.4 Soil dehydrogenase (DH)

The soil DH activities under wheat– maize and wheat – mung bean cropping systems were also monitored in all seasons (Fig. 4). The DH activities under wheat-maize cropping pattern were 45.01, 43.3, 43.67 and 43.15 µg TPF g^{-1} soil in summer, winter, spring and in autumn, respectively. The average soil DH activity did not differ significantly ($P > 0.05$) among all seasons. Contrarily, the DH activity under wheat – mung bean was significantly ($P < 0.05$) higher in summer as compared to all other seasons and was non-significantly ($P > 0.05$) lower in winter, spring and autumn as compared to summer. Hence, the DH activities under wheat – mung bean cropping system were 45.30, 44.2, 44.04 and 43.92 µg TPF g^{-1} soil in summer, winter, spring and in autumn, respectively.

3.5Alkaline phosphatase (AP)

The AP activity was monitored under wheat – maize and wheat – mung bean cropping systems in all seasons (Fig. 5). The AP activities under wheat – maize cropping system were 21.8, 16.6, 18.9 and 17.8 µg *p*-NP g^{-1} soil 24 h^{-1} soil in summer, winter, spring and in autumn, respectively. The AP activity was non-significantly ($P > 0.05$) lower in winter compared to other seasons. The AP activities under wheat- mung bean cropping pattern were 23.9, 19.8, 20.0 and 17.4 µg *p*-NP g^{-1} soil 24 h^{-1} soil in summer, winter, spring and in autumn, respectively. The average AP activity under wheat-mung bean was significantly ($P < 0.05$) lower in winter, spring and autumn as compared to summer.

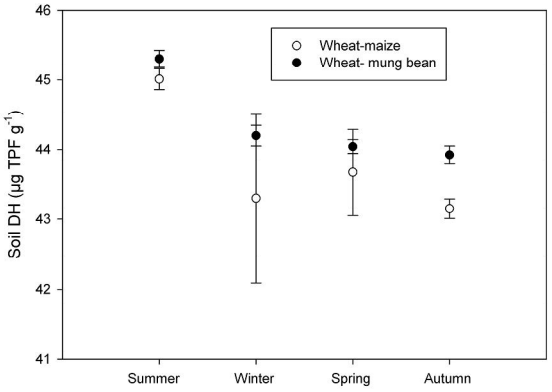

Fig. 4 Effect of seasonal variations on soil DH activities under different cropping systems

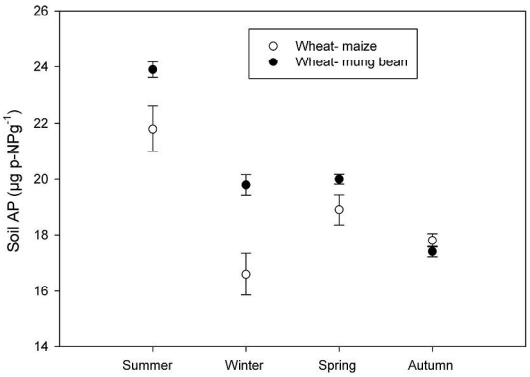

Fig. 5 Effect of seasonal variations on soil AP activities under different cropping systems

4. Discussions

Owing to a limited precipitation, an optimum soil health index is pre-request for sustainable crop production, particularly, in arid areas. That is why, it is imperative to elucidate the impact of land use including cropping system on soil health in these remote areas of the world. The magnitude of soil microbial activities/biomass, nutrients bioavailability and enzymatic activities determine the health and

productivity standards of soil environment. This study was conducted to determine the impact of most commonly use cropping system (wheat – maize and wheat – mung bean) on soil MBC, MBN and MBP contents and enzymatic activities.

Soil MBC, as an indicator soil of quality, is supposed to be influenced by different land use practices. Several researchers have investigated the relationship between soil MBC and soil prosperities like moisture (Herron *et al.,* 2009), texture (Grandy *et al.,* 2009) and temperature etc., (Fang *et al.,* 2005). Hence, MBC is also sensitive to numerous other land use practices (e.g.,) pesticides applications (Hussain *et al.,* 20009a). In our case, seasonal variations and cropping system together influence the soil MBC. The MBC contents are mostly higher under wheat – maize cropping system in summer as compared to other seasons. This could be due to more crop residues under this cropping system coupled with more microbial incorporation and/or decomposition in summer (Petersen *et al.,* 2002; Williams and Rice, 2007). Our results are similar to the finding of Gong *et al.* (2009) who reported addition in soil organic pool under long-term applications of manures and fertilizers under a wheat–maize cropping system in North China Plain under irrigated conditions. Contrarily, the wheat – mung bean cropping system show more MBC contents in autumn season. Similarly Song *et al.* (2007) described an increase in MBC contents under inter-cropping of wheat and *Vicia faba* L. Overall wheat – maize cropping system show more MBC contents, which could be due to more crop residues production by maize compared to mung bean.

Soil MBN is also a major source of N for microbial activities (mineralization and nutrient cycling) and possesses several other environmental implications (mineralization to inorganic forms and consequently environmental quality). The soil MBN contents are higher in spring and summer under wheat – maize and wheat – mung bean cropping system, respectively. In general, the MBN contents under wheat – mung bean cropping system are higher as compared to those observed under wheat – maize cropping system. Likewise Song and colleagues (2007) showed an increase in MBC, MBN and MBP contents under various inter-cropping systems (wheat/faba bean, wheat/maize, and maize/faba bean). Contrarily, Wright and colleagues, (2005) showed a decrease in MBN contents under maize cropping. Moreover, the higher contents of soil MBN under wheat – mung bean cropping system could be due to more fixation of atmospheric nitrogen by leguminous crops like mung bean (Saleem *et al.,* 2007). However, increase in soil MBN contents were not related DH activity which did not show any significant (P=0.05) change in any of both cropping system. We suppose that the soil samples were taken after crop harvesting, therefore, we do not see any dynamics in DH activities, which primarily depends upon the root associated soil micro organisms in the pre-existing crops in the field (Saleem *et al.,*2007). In broader context, in arid regions having limited water availability, the selection of nutrient preserving and N-fixing crops (like legumes) could be best strategy to achieve the goal of sustainable agriculture as compared to nutrient exhausting crops like Maize.

Similarly soil MBP is a major source of plants available phosphorus as a nutrient. Its contents are more important under arid environmental condition where soil edaphic features (pH and moisture) are not feasible for its availability to plants. The soil MBP contents are relatively more in summer under wheat – mung bean cropping system as compared to wheat – maize in Kahuta area. Our results partially differed from He *et al.* (1997) who did not see any difference in MBP contents with seasonal variations; however the MBP contents were decreased in summer the presence of pastures. In our case, more MBP could be due to more affiliation and interaction of P- phosphate solubilizing microorganisms with mung bean plants, which resulted in more soil MBP contents (Gaind and Gaur, 1991; Rodríguez and Fraga. 1999; Saleem *et al.,* 2007). In addition, soil AP activities were relatively higher wheat – mung bean cropping system in summer, which further supports our observation about soil MBP contents (Fig. 5).

In conclusions, we found relatively higher soil microbial biomass(C, N and P) contents and enzymatic activities under wheat – mung bean as compared to wheat – maize cropping system under arid environmental conditions. Our finding possesses broad implications in agricultural, ecological and soil ecosystem restoration perspectives. We suggest that leguminous crops are best option for sustainable soil productivity under arid condition.

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