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Full Length Research Paper

Phosphorus fractions, microbial biomass and enzyme activities in some alkaline calcareous subtropical soils

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A study was conducted to estimate different phosphorus fractions in some alkaline calcareous subtropical soils and evaluate their relationship with microbial and biochemical properties of the soils. Soil samples (0 to 15 cm) belonging to 15 soil series were collected from the agricultural fields of Potohar, northern Punjab, Pakistan in September, 2008 and analysed for P fractions and microbial parameters including microbial biomass C, microbial biomass N, microbial biomass P, and activities of dehydrogenase and alkaline phosphatase enzymes. The average size of different P fractions (% of total P) in the soils varied in the order: $_d$HCl-P (63.7%) > $_c$HCl-P_i (14.8%) > residual-P (9.4%) > $_c$HCl-P_o (3.8%) > $NaHCO_3$-P_i (2.4%) > NaOH-P_i (2.0%) > $NaHCO_3$-P_o (1.4%) > resin-P (1.3%) > NaOH-P_o (1.2%). The organic P fractions collectively formed 6.4% of the total P, while the inorganic P fractions were 93.6%. The bio-available P fractions that is, resin-P, $NaHCO_3$-P_i and $NaHCO_3$-P_o showed significant positive relationship with Olsen-P, microbial biomass P, and total organic C, but also with microbial biomass C, microbial biomass N, dehydrogenase activity and alkaline phosphatase activity in the soils. Among other P fractions, NaOH-P_i had strong positive correlation with total organic C, alkaline phosphatase and dehydrogenase activities, while the NaOH-P_o had strong positive correlation with total organic C, dehydrogenase activity, microbial biomass N, alkaline phosphatase activity and microbial biomass C. The $_d$HCl-P fraction did not show any strong correlation with microbial parameters, whereas the $_c$HCl-P_o had strong positive correlation with microbial biomass N and microbial biomass C. The results demonstrate positive contribution of soil microbial biomass, especially the microbial biomass P to P availability in the soils under present study.

Key words: Phosphorus fractions, microbial biomass, enzyme activities, alkaline calcareous soils.

INTRODUCTION

Phosphorus (P) is an element essential for the growth of all living organisms including plants. Despite its wide distribution in nature, P is a limited resource for plant growth and its low availability is a major constraint to crop production in most arable soils (Ayaga et al., 2006; Redel et al., 2011). According to some estimates, P deficiency spreads over an area of 2 billion hectares worldwide and about 80 to 90% soils of the arid and semi-arid regions are deficient in plant-available P (Fairhurst et al., 1999; NFDC, 2001). In alkaline calcareous soils, low P availability was attributed to the adsorption/precipitation reactions of both the indigenous and the applied P with calcium or iron and aluminium components of the soil (Brady and Weil, 2008). Although plants absorb phosphorus from the labile P pool consisting of resin-P, $NaHCO_3$-P_i and $NaHCO_3$-P_o fractions in soil, but other P fractions like dilute HCl extractable P, and microbial biomass P also contribute variably to plant-available P (Gichangi et al., 2009; Gichangi et al., 2009). Therefore, knowledge of different P fractions in soil is important to understand P availability and its mana-gement in soil-plant system.

Microbial biomass P is regarded as an easily

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mineralisable P fraction of the soil (Redel et al., 2008; Sugito et al., 2010). Particularly in soils with low total P content, soil microorganisms are capable of storing substantial amounts of P into their biomass often above their own physiological P requirements, and thus prevent its fixation by soil components (Achat et al., 2010). According to Anderson and Domsch (1980), P up to 83 kg ha^{-1} could be present in the microflora of upper 12.5 cm soil layer. Brookes et al. (1984) found mean annual flux of 7 kg P ha^{-1} $year^{-1}$ in arable soils and 23 kg P ha^{-1} $year^{-1}$ in grassland soils. Kondo et al. (1989) observed that almost 39% of the Olsen P originates from soil microbial biomass. Achat et al. (2010) reported P storage of up to 21.6 kg ha^{-1} in the soil microbial biomass. Although the P contained in soil microbial biomass cannot be directly used by the plants, but the turnover of microbial biomass may release P slowly into the soil solution (Gichangi et al., 2009). This slowly released P, if synchronized with crop P requirements, can be utilized by crop plants more efficiently (Ayaga et al., 2006).

Soil microorganisms also play an important role in organic P transformations in soil through excretion of enzymes like phosphatase and dehydrogenase. Phosphatase catalyses the hydrolysis of esters and anhydrides of phosphoric acid and thus its activity indicates the mineralization potential of organic P in soils (Dick and Tabatabai, 1993). The dehydrogenase activity being an index of microbial redox system and oxidative activities is also considered as an indicator of biological activity in soil (Trevors, 1984).

Sequential extraction procedures like those of Hedley et al. (1982) have been widely used to characterise soil phosphorus into various inorganic and organic P fractions differing in their availability to microorganisms and plants (Redel et al., 2008). However, information regarding relationships of P fractions with microbial biomass and enzyme activities in soils is quite limited. This is particularly true for the alkaline calcareous subtropical soils of the Potohar, Pakistan where no such work has been reported so far. The present study was therefore conducted with the objectives: i) to estimate different P fractions in alkaline subtropical soils of Potohar, Pakistan under rainfed dry farming, and ii) to elucidate the relationships between P fractions, microbial biomass and enzyme activities in the soils.

MATERIALS AND METHODS

Experimental sites and soil sampling

Soil samples of 15 prominent soil series were collected from agricultural fields of the Potohar plateau in northern Punjab, Pakistan during the September, 2008. The soils belonged to five soil orders including the *Aridisols, Entisols, Inceptisols*, *Alfisols and Vertisols.* Among 15 soils, three (Guliana, Kahuta and Rawalpindi) were non-calcareous while remaining 12 soils varied between slightly and strongly calcareous. The geographic locations, classification and other information about the soils are presented in Table 1. The mean annual temperature of the experimental area is 24.5°C and the mean annual rainfall is 750 mm, of which more than two third is received in the months of July and August in the form of monsoon rains. Main crops of the area are wheat, maize, groundnut, pearl millet and oilseed rape. Tillage is carried out with cultivators up to 15 to 18 cm depth, whereas mouldboard plough down to 20 cm depth is also used occasionally. Use of inorganic fertilizers is not up to the mark because of uncertainty of precipitation, high cost, and poor economic conditions of the farmers. Addition of organic manures is also limited due to alternate use of crop residues and animal dung (Ali et al., 2002; Mufti, 2011). The soil samples of roughly 1.5 kg size were taken from four different locations of the selected fields at a depth of 0 to 15 cm with the help of soil auger. All the sites were lying fallow at the time of sampling and the moisture contents were below field capacity as sampling was carried out during dry period following the rainy season (monsoon). The field moist samples were hand-picked to remove stones, larger plant residues and soil animals (earth worms etc.). The samples were then passed through a 2-mm sieve, mixed thoroughly and stored in polyethylene bags in freezer at -15°C prior to biological analysis. A portion of each soil sample was air-dried, ground to powder form and used for the determination of different P fractions.

Soil analysis

Particle size distribution was carried out by Hydrometer method after dispersion with sodium hexametaphosphate (Gee and Bauder, 1986). Soil pH was determined in 1:2.5 (w/v) soil water suspensions using a glass electrode (Anderson and Ingram, 1993). Calcium carbonate was measured by acid neutralization (Ryan et al., 2001). Organic C was determined by dichromate digestion method (Nelson and Sommers, 1982). Olsen P was measured by extracting soil with 0.5 M $NaHCO_3$ at pH 8.5 (Olsen and Sommers, 1982). For the determination of total P, 0.2 g soil samples were digested with 4.4 ml of digestion mixture containing selenium powder, lithium sulphate, hydrogen per oxide (30%) and sulphuric acid at 360°C for at least two hours (till the contents were colorless). In the clear digest, P contents were determined using spectrophotometer at 882 nm wavelength (Anderson and Ingram, 1993).

Phosphorus fractionation

Soil samples were subjected to sequential P extraction using a modified Hedley fractionation method (Hedley et al., 1982) as described by Tiessen and Moir (1993). The air-dried samples of 0.5 g (on oven-dry basis) soil were placed in 50 ml centrifuge tubes and horizontally shaken for 16 h at 25°C at 175 rev min^{-1} with one resin strip (6×2 cm, anion-exchange resin) in 30 ml deionized H_2O. The P adsorbed by resin strip was recovered in 30 ml elution solution (0.1 M NaCl + HCl) after shaking for 2 h and inorganic P in the eluents was determined (Kouno et al., 1995). The suspension was centrifuged for 10 min at 8000 rpm and the supernatant discarded. The soil residues left in the centrifuge tubes were then sequentially extracted with 30 ml each of 0.5 M $NaHCO_3$ (pH 8.5), 0.1 M NaOH and 1.0 M HCl ($_d$HCl) after 16 h shaking (end to end) at 25°C at 175 rev min^{-1}. The suspensions were centrifuged at 8000 rpm for 15 min and filtered with Whatman no. 42. A portion of $NaHCO_3$ and NaOH extracts was acidified to precipitate extracted organic matter and the supernatant was analysed for inorganic P (P_i). Another portion of $NaHCO_3$ and NaOH extracts was digested with acidified ammonium persulphate in an autoclave at 120 kPa and 121°C (60

Table 1. Information about the soils collected.

Soil series	Classification		Parent material	Location	
	Great group	Order		N	E
Argan	Ustochrepts	Inceptisol	Alluvium	33° (32.546')	073° (09.264')
Balkassar	Haplargids	Aridisol	Sandstone	32° (55.377')	072° (45.398')
Basal	Camborthids	Aridisol	Loess	33° (30.585')	072° (14.242')
Chakwal	Haplargids	Aridisol	Loess	32° (54.245')	072° (52.518')
Dhumman	Haplargids	Aridisol	Alluvium	32° (55.828')	072° (43.799')
Domel	Ustochrepts	Inceptisol	Loess	33° (30.300')	072° (38.069')
Guliana	Haplustalf	Alfisol	Loess	33° (33.382')	072° (38.545')
Kahuta	Ustochrepts	Inceptisol	Sandstone	33° (02.066')	072° 57.428')
Missa	Ustochrepts	Inceptisol	Loess	33° (35.905')	072° (48.497')
Qutbal	Ustorthents	Entisol	Loess	33° (35.868')	072° (48.621')
Rajar	Torriorthents	Entisol	Loess	33° (17.299')	073° (10.667')
Rawalpindi	Ustochrepts	Inceptisol	Loess	33° (29.343')	073° (13.118')
Satwal	Chromusterts	Vertisol	Alluvium	32° (55.790')	072° (43.038')
Therpal	Camborthids	Aridisol	Alluvium	32° (51.145')	072° (54.977')
Tirnaul	Ustochrepts	Inceptisol	Sandstone	33° (39.327')	072° (54.218')

min for $NaHCO_3$ and 90 min for NaOH extract) and analysed for total P (P_t). For the determination of concentrated HCl-P ($_c$HCl-P), the soil residue (obtained after 1.0 M HCl extraction) was heated for 10 min in 10 ml concentrated HCl at 80°C in a water bath. Then further 5 ml concentrated HCl was added and centrifuged, and P was measured in HCl solution after adjusting pH to1.5. P_t in $_c$HCl fraction was measured by digesting 5 ml of the $_c$HCl solution in acidified ammonium per sulphate for 60 min in an autoclave at 120 kPa and 121°C. The organic P (P_o) in $NaHCO_3$, NaOH and $_c$HCl extracts was obtained as the difference between P_t and P_i of respective extracts. Finally the residual P in soil samples was determined after digestion with H_2SO_4-H_2O_2. The P concentration in all extracts and digest solutions was determined colorimetrically at 882 nm (Murphy and Riley, 1962).

Microbial parameters

The soil samples stored for microbial analysis at -15°C were taken out of the freezer, equilibrated to room temperature, adjusted to 50% water holding capacity and pre-incubated for 10 days. Microbial biomass C and biomass N contents in pre-incubated soil samples were determined by fumigation-extraction method (Vance et al., 1987). The samples were fumigated with ethanol free $CHCl_3$ and extracted with 0.5 M K_2SO_4. The organic carbon in the soil extracts was measured using an automated total organic carbon (TOC) analyzer and the microbial biomass C was calculated by a k_{EC} value of 0.45. The total N in soil extracts was measured after kjeldahl digestion, and the microbial biomass N was calculated by a k_{EN} value of 0.54.

Soil microbial biomass P was also determined by fumigation-extraction method (Brookes et al., 1982) as described by Joergensen et al. (1995). Three portions of pre-incubated moist soil (equivalent to 2.5 g oven-dry weight) were taken and each of them was extracted with 50 ml 0.5 M $NaHCO_3$ (pH 8.5) after different pre-treatment that is, the first after fumigation, the second after non-fumigation, and the third after the addition of 25 µg P g^{-1} soil as KH_2PO_4 to the extractant. P contents were analyzed and the microbial biomass P was calculated by a k_{EP} value of 0.40.

Dehydrogenase activity in the soils was measured as the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) in triphenyl formazan (TPF). Moist pre-incubated 5.0 g soil was weighed, mixed with 5 ml TTC and incubated for 24 h at 30°C. After the incubation, 40 ml acetone was added, the contents were shaken and further incubated at room temperature for 2 h. The soil suspension (15 ml) was then filtered and the optical density of the supernatant was measured at 546 nm wavelength on spectrophotometer. The dehydrogenase activity (TPF µg g^{-1} dwt soil) was calculated as TPF (µg ml^{-1}) x 45/dwt/5, where dwt is dry weight of 1 g moist soil (Alef, 1995).

Alkaline phosphatase activity was measured using *p*-nitrophenyl phosphate as the substrate. One gram moist soil was incubated with 0.2 ml toluene, 4 ml modified universal buffer (pH 11), and 1 ml *p*-nitrophenyl phosphate solution at 37°C for 1 h. Then, 1 ml 0.5 M $CaCl_2$ and 4 ml 0.5 M NaOH were added, the suspension was filtered and the *p*-nitrophenol released was measured at 400 nm wavelength using a spectrophotometer (Alef et al. 1995).

Statistical analysis

The results given are arithmetic means of 15 soil series showing variability of various experimental sites and expressed on an oven dry basis (24 h at 105°C). Arithmetic means and coefficients of variation (CV) of different soil parameters were estimated by using Microsoft Excel package (Office-2007). This package was also used for plotting graphs and drawing figures. Regression and correlation analysis were carried out using mean values to see interrelation between P fractions and soil physico-chemical and microbial properties. Confidence interval (CI) of different P fractions was measured by Statistica 9.0.

RESULTS

Physico-chemical soil properties

Texture of the soils varied from sandy loam to clay loam

Table 2. Different P fractions (µg g^{-1} soil) in the soils.

Soil	Resin P	0.5 M $NaHCO_3$-P		0.1 M NaOH-P		1.0 M HCl-P	$_c$HCl-P		Residual P
		P_i	P_o	P_i	P_o		P_i	P_o	
Argan	14.1	25.4	6.0	12.8	7.7	377.4	57.8	12.8	38.2
Balkassar	10.1	5.5	5.2	3.5	1.9	429.4	54.9	10.4	46.2
Basal	3.1	5.4	4.9	5.9	3.4	346.8	117.0	18.5	52.2
Chakwal	6.3	6.7	5.7	8.6	5.8	272.3	62.2	23.4	45.4
Dhumman	6.4	5.1	7.6	5.0	4.2	287.3	48.8	19.7	41.8
Domel	5.5	12.8	2.3	5.6	5.6	367.5	81.4	21.9	55.6
Guliana	2.5	3.6	9.3	13.7	12.6	122.2	128.9	29.4	60.5
Kahuta	2.2	5.9	9.2	8.1	3.9	74.5	37.2	7.1	29.7
Missa	3.3	12.4	5.8	7.7	6.7	409.8	86.5	21.5	63.2
Qutbal	3.2	9.2	3.8	4.7	5.4	435.5	68.6	18.1	52.2
Rajar	3.0	11.4	2.4	7.0	3.9	424.9	76.0	4.9	29.5
Rawalpindi	2.5	14.7	4.7	24.6	6.5	137.0	102.4	21.9	46.9
Satwal	17.6	29.4	17.1	16.4	8.9	437.4	61.8	28.9	35.5
Therpal	7.2	9.1	8.2	7.2	2.2	345.9	40.7	14.0	37.3
Tirnaul	8.5	21.2	10.8	15.0	9.2	265.9	77.7	31.1	61.2
Mean	6.4	11.9	6.9	9.7	5.9	315.6	73.5	18.9	46.4
CV (±%)	5.1	7.2	4.5	6.5	4.6	2.5	4.7	8.4	4.6
CI (95%)	3.8 - 8.9	7.5 - 16.2	4.8 - 8.9	6.5 - 12.9	4.3 - 7.5	249 - 382	58.7 - 88.2	14.5 - 23.3	40.3 - 52.5

CI, Confidence interval at 95% probability level.

with silt loam being the dominant textural class (data not shown). Clay contents varied from 11 to 43% with the mean of 22% and the soil pH ranged from 7.2 to 8.3 with an average of 8.0. Calcium carbonate contents ranged widely from 0.2 to 17.6% with the mean around 7.9%. The total organic C contents in the soils were 1.5 to 10.1 mg g^{-1} soil with an average value of 5.0 mg g^{-1} soil. Olsen P (0.5 M $NaHCO_3$-extractable P) ranged from 2.2 to 19.7 µg g^{-1} soil with the mean of 6.4 µg g^{-1} soil. Total P contents in the soils ranged from 178 to 653 µg g^{-1} soil with the mean of 495 µg g^{-1} soil.

Phosphorus fractions in soil

The average size of different P fractions in the soils (Table 2; Figure 1) varied in the order (% of total P in soils): NaOH-extractable P_o (1.2%) < resin P (1.3%) < $NaHCO_3$-extractable P_o (1.4%) < NaOH-extractable P_i (2.0%) < $NaHCO_3$-extractable P_i (2.4%) < $_c$HCl-extractable P_o (3.8%) < residual P (9.4%) < $_c$HCl-extractable P_i (14.8%) < $_d$HCl-extractable P (63.7%). The labile P fractions (resin-P, $NaHCO_3$-P_i and $NaHCO_3$-P_o) accounted for 5.1% whereas hydroxide fraction constituted 3.2% of the total P. The largest P fraction in the soils was Ca-associated 1.0 M HCl extractable P ($_d$HCl-P) which comprised of 63.7% of the total P. The highly stable and recalcitrant P forms that is, $_c$HCl-P_i, $_c$HCl-P_o and the residual-P constituted 28% of the total P in soils. Organic P fractions on the whole made up a small portion that is, 6.4% of the total P, while the remaining 93.6% was contributed by the inorganic P fractions.

The confidence intervals for different P fractions in soils are given in Table 2 which indicates 95% probability of mean being in between the range given against each.

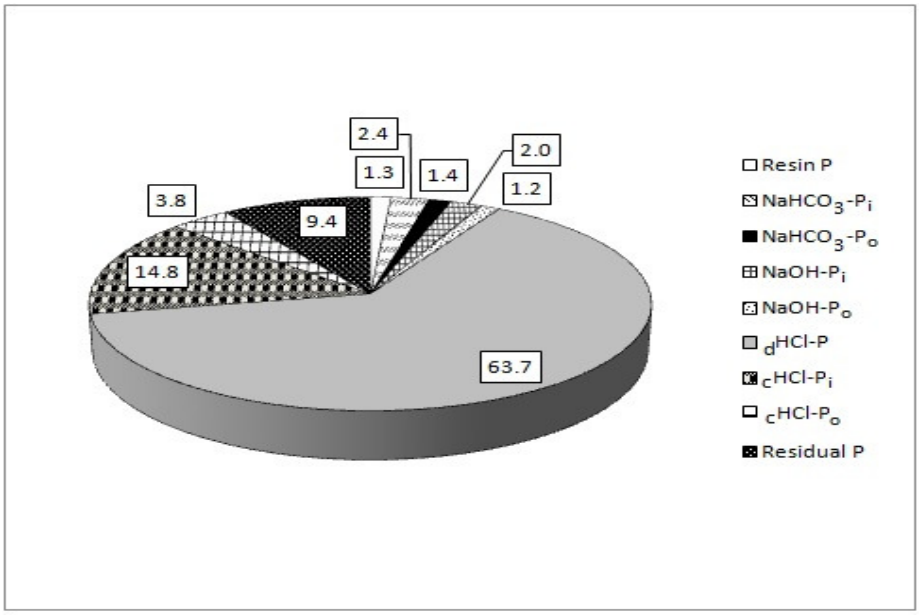

Figure 1. P fractions in semi arid alkaline soils of Potohar, Pakistan (% of total P).

Table 3. Microbial and biochemical properties of soils.

Soil series	Microbial biomass C (µg g-1 soil)	Microbial biomass N (µg g^{-1} soil)	Microbial biomass P (µg g^{-1} soil)	Dehydrogenase TPF (µg g^{-1} soil 24 h^{-1})	Alkaline Phosphatase *p*-nitrophenol (µg g^{-1} soil h^{-1})	Microbial biomass C/ P
Argan	117.9	13.1	8.2	45.5	118.2	14.4
Balkassar	58.2	6.8	4.4	15.9	59.6	13.2
Basal	94.8	11.2	4.3	13.7	27.1	22.0
Chakwal	110.6	12.8	6.1	19.2	110.3	18.1
Dhumman	73.9	8.7	6.3	29.4	90.4	11.8
Domel	61.8	8.1	2.0	27.1	67.9	31.1
Guliana	147.2	17.2	7.6	70.6	185.0	19.4
Kahuta	59.2	6.5	5.4	30.1	109.9	11.0
Missa	100.0	11.8	3.6	27.6	126.7	27.8
Qutbal	83.2	9.7	2.6	23.7	102.0	31.8
Rajar	45.3	5.4	1.6	10.8	35.8	28.4
Rawalpindi	75.3	10.2	2.3	32.3	112.5	32.2
Satwal	143.9	16.5	11.6	53.2	171.6	12.4
Therpal	101.9	10.9	5.8	20.7	103.3	17.5
Tirnaul	131.6	16.2	9.1	80.3	230.6	14.4
Mean	93.7	11.0	5.4	33.3	110.1	20.4
CV (±%)	2.8	2.8	4.8	3.8	1.6	5.1

Microbial biomass Indices

The mean contents of microbial biomass C and microbial biomass N in the soils were 93.7 and 11.0 µg g^{-1} soils with the ranges of 45.3 to 147.2 and 5.4 to 17.2 µg g^{-1} soils, respectively (Table 3). The mean contents of microbial biomass P were 5.4 µg g^{-1} soils ranging from 1.6 to 11.6 µg g^{-1} soil. The activity of alkaline phosphatase was in the range of 27.1 to 230.6 *p*-nitrophenol µg g^{-1} dwt h^{-1} with the mean of 110.1 *p*-nitrophenol µg g^{-1}

dwt h^{-1}. The activity of dehydrogenase ranged from 10.8 to 80.3 TPF µg g^{-1} dwt 24 h^{-1} with the mean of 33.3 TPF µg g^{-1} dwt 24 h^{-1}. The microbial biomass C to P ratio ranged from 11.0 to 32.2 with the mean value of 20.4.

Correlation between P fractions and microbial indices

The resin P showed significant positive relationship with Olsen P and microbial biomass P (Table 4). The $NaHCO_3$-P_i had highly significant positive relationship with Olsen P but also with total organic C and microbial biomass P. The $NaHCO_3$-P_o was most strongly positively related to microbial biomass P, but also with alkaline phosphatase activity, microbial biomass C, biomass N, dehydrogenase activity and total organic C, and negatively related to microbial biomass C to P ratio. The NaOH-P_i was positively related to total organic C, and also with alkaline phosphatase and dehydrogenase activities. The NaOH-P_o had highly strong positive relationship with total organic C and also with dehydrogenase activity, microbial biomass N, alkaline phosphatase activity, microbial biomass C, and fairly strong positive relationship with clay contents. The $_d$HCl-P fraction showed very strong positive relationship with total P, and also strong positive relationship with soil pH, and $CaCO_3$ contents. The $_c$HCl-P_o had strong positive relationship with microbial biomass N, clay contents, total organic C and microbial biomass C, but was also significantly positively related to alkaline phosphatase and dehydrogenase activities.

DISCUSSION

Among different P fractions, resin P and bicarbonate extractable P (P_i and P_o) collectively form the bio-available or labile P in the soil (Sui et al, 1999). The labile P pool was 5.1% of total P in the present set of soils. In general, the soils having high contents of microbial biomass P had more labile or bio-available P. This indicates the importance of microbial biomass P to P availability in the soils. Regression analysis (Figure 2) reveals that 52% variability in resin P fraction among the soils was due to variation in microbial biomass P contents. There was also a significant positive relationship between $NaHCO_3$-P_i and total organic C contents in the soils. Different organic components in soil, particularly those containing P, may release phosphorus to the $NaHCO_3$-P_i pool through microbial decomposition (Cassagne et al., 2000). The soils having high contents of total organic C and microbial biomass P also contained high contents of $NaHCO_3$-P_o. Actually, more availability of organic C in the soils results in higher microbial populations and more P assimilation into the microbial cells. Upon microbial turnover, a significant proportion of this organic P released becomes part of the $NaHCO_3$-P_o fraction in the soils (Wang et al., 2006). The negative relationship between microbial biomass C/P ratio and the $NaHCO_3$-P_o also strengthens the above interpretation that P contained in microbial biomass replenishes the available P pool on the lyses of microbial cells and thus contributes to bicarbonate extractable organic P fraction in the soils (Achat et al., 2010).

The NaOH-P (P_i and P_o) is considered to be the P associated with the surface of amorphous and some crystalline Fe and Al minerals (Cross and Schlesinger, 2001). This P fraction constituted 3.2% of the total P in the soils, with 2.0 and 1.2% contributions from the NaOH-P_i and NaOH-P_o pools respectively. The above valuesare within the ranges reported by Roberts et al. (1985) and Cross and Schlesinger (2001) for Mollisols and Aridisols. A significant positive correlation existed between NaOH-P_i and the soil organic C which indicates that during the microbial decomposition of organic substrates, a significant proportion of the mineralized P goes to the NaOH-P_i pool as well. There was also a strong positive correlation between NaOH-P_o, soil organic C and the microbial parameters in the soils. This confirms the conclusion drawn by Wang et al. (2006) who reported NaOH-P_o as the P strongly associated with organic soil components. According to Cross and Schlesinger (2001), soil organic C controls variability of NaOH-P_o pool in the soils. The NaOH extracts P mainly from organic components and some amorphous aluminium-containing compounds in soil (Cassagne et al., 2000) that is, the P associated with humic acids or chemisorbed to the surfaces of Fe and Al compounds (Schoenau et al., 1989; Schlesinger et al., 1998). The products of microbial decomposition of organic materials might solubilise some of the sorbed or recalcitrant P in soil causing an increase in organic P associated with the humic substances (Reddy et al., 2005).

The $_d$HCl-P dominated among all the P fractions in the soils. Although this P fraction was 63.7% of the total P and 68.1% of total inorganic P, it did not show a significant relationship with any microbial parameter in the soils. This might be due to great influence of the geochemical properties on P cycling in the soils under study dominated by high concentrations of $CaCO_3$ minerals in the soils (Cross and Schlesinger, 2001).

The concentrated HCl removes inorganic and organic P occluded with Fe and Al containing minerals and from apatite (Tiessen and Moir, 1993). This P fraction ($_c$HCl-P) accounted for 18.6% of the total P in the present set of soils, with 14.8 and 3.8% contributions as the $_c$HCl-P_i and $_c$HCl-P_o respectively, similar to those reported by Cross and Schlesinger (2001) in the Aridisols. The $_c$HCl-P_o is considered to be derived from particulate organic matter which is unavailable in its current form but may become bio-available after microbial decomposition (Tiessen and Moir, 1993). Like other organic P pools, $_c$HCl-P_o also had

Table 4. Correlation coefficients between soil microbial properties and different P pools in soils

Property	Resin-P	$NaHCO_3$-P_i	$NaHCO_3$-P_o	NaOH-P_i	NaOH-P_o	$_d$HCl-P	$_c$HCl-P_i	$_c$HCl-P_o	Residual-P
Clay			0.59*		0.62*			0.79**	
pH				-0.71**		0.72**			
$CaCO_3$						0.66**			
Total organic carbon		0.53*	0.59*	0.68**	0.89**			0.76**	
Olsen P	0.85**	0.92**	0.63*	0.56*					
Total P						0.93**			
Microbial C			0.68**		0.77**			0.75**	
Microbial N			0.65**	0.53*	0.82**			0.83**	
Microbial P	0.72**	0.52*	0.89**		0.53*				
Dehydrogenase			0.64*	0.58*	0.86**			0.69**	
Alkaline phosphatase			0.71**	0.58*	0.79**			0.71**	
Microbial C/P			-0.66**						

*, **Significant at 5% and 1% levels of probability, respectively.

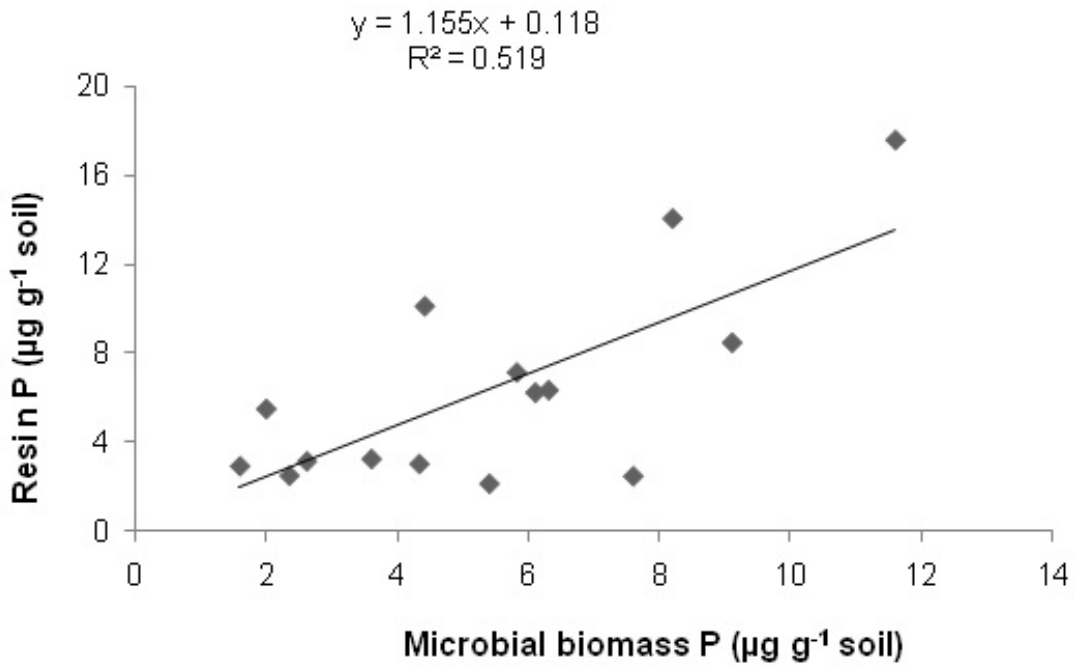

Figure 2. Correlation between resin P and microbial biomass P in soil.

a highly significant positive correlation with microbial parameters highlighting once again the significance of microbial biomass in making this organic P fraction available to plants via microbial decomposition.

The residual P is a highly stable part of the total P pool in soil which is available to plants in the long-term only (Cross and Schlesinger, 1995). The values of residual P in our soils are similar to those reported by Bowman and Cole (1978) and Cross and Schlesinger (2001) for Mollisols and Aridisols. In general, the residual P comprises of high molecular weight organic compounds (humus) and originates from Fe and Al compounds. Therefore, the binding partners for P in this form may be highly stable complexes of pedogenic oxides (Tiessen et al., 1983).

The microbial biomass P in the soils was 1.1% of the total P and 17.1% of total organic P. These values are within the range of percentages reported for red soils of China by Chen et al. (2000) where microbial biomass P accounted for 2.2 to 17.9% of the organic P and 0.7 to

8.4% of the total P in the soils. However, the percentages found in our soils were slightly lower than those reported by Tate (1985) and Wang et al. (2004) for other soil types. This variability might be explained by relatively low total organic C contents, and less P availability in the soils in our study. Differences in microbial community structure and P availability can also contribute to variation in the size of microbial biomass P in the soils (Oberson and Joner, 2005). Brookes et al. (1982) and Myers et al. (1999) found about two times higher P contents in bacteria than fungi.

The activities of alkaline phosphatase and dehydrogenase enzymes in the present study had significant positive correlation with all organic P fractions in the soils along with NaOH-P_i fraction as well. These P fractions being bio-available in the short-term are of paramount importance in the sustainability of soil P availability. Such positive relationships between enzyme activities and organic P fractions highlight the role of microbial biomass in P availability in soils. The positive relationships among soil organic C, enzymes activities and organic P fractions support the hypothesis that microbial decomposition products may cause desorption of some sorbed P and solubilise some part of the recalcitrant P in the soil to favour the accumulation of organic P in the soil (Reddy et al., 2005).

Conclusion

All the microbial parameters that is, microbial biomass C, microbial biomass N, dehydrogenase activity, and alkaline phosphatase activity in general, while the microbial biomass P in particular had a highly significant positive correlation with the bio-available P fractions and organic P fractions in the soils. This demonstrates the importance of microbial parameters, particularly the microbial biomass P for P availability in alkaline calcareous soils. The positive relationship between microbial biomass P, labile P fractions and the Olsen-P confirmed the hypothesis that soil microbial biomass plays an important role in P cycling and its availability to crop plants.

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