Co-application of organic amendments and inorganic P increase maize growth and soil carbon, phosphorus availability in calcareous soil

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Phosphorus (P) constraint can be alleviated by increasing C inputs, which can help to improve crop production and P fertilizer use efficiency. However, the effects of different manures on soil microbial biomass P (MBP) and P fractions as well as C fractions in calcareous soils remain poorly understood. Soil MBP pool involves the P mineralization and immobilization processes, potentially changing P fractions and P availability. Therefore, the effects of different manures on soil microbial biomass (MBP, MBC) pool, P, and C fractions and crop P utilization were evaluated in greenhouse experiments with maize plantation. Treatments included no manure (control), poultry manure (PM), cow manure (CM), goat manure (GM), mixed manure (MM), and three inorganic P (Pi) rates; P0: 0 mg kg-1, P50: 50 mg kg-1, and P100: P100 mg kg-1 (P2O5). For plant growth comparison, crop physiological growth indices, shoot P contents and total P uptake were increased by PM and P100 as compared to other treatments. The PM with P100 significantly increased the plant growth by inducing P uptake of ~18% compared with control. The results exhibited that Pi (P100) combined with manure (PM) significantly ($p < 0.05$) increased the soil physicochemical properties, that is, 683.76 mg kg-1 total P, 21.5 mg kg-1 Olsen P, 4.26 g kg-1 SOC, 2.41 g kg-1 POC, as well as microbial biomass C and P increased by 152.84 mg kg-1 and 36.83 mg kg-1, respectively. Consequently, we concluded that PM with Pi (P100) application builds up soil microbial biomass, which is more beneficial for promoting P utilization for maize.

Introduction

Phosphorus deficiency is the world's second most serious soil fertility issue and a major constraint to agricultural productivity (Gichangi et al., 2011). In Pakistan, soils have low organic matter levels, ranging from 2.6 to 6.4 mg C g^{-1}, and extracted phosphate (NaHCO$_3$), about 6 µg P g^{-1} (Khan and Joergensen 2009). Low P availability in soil (pH 8.2–9.3) is mainly due to the rapid formation of non-labile P forms after inorganic P (Pi) addition (Khan and Joergensen 2012). P precipitated and adsorbed with native soil P and calcium, iron, and aluminum ions which become unavailable to plants. According to Huang et al. (2017), the Olsen P contents in soil ranging from 5 to 10 mg kg^{-1} would be optimum for crop production, while lower contents (<5 mg kg^{-1}) affect plant growth.

Many agricultural soils require a substantial amount of costly fertilizer to achieve adequate crop production (Muhammad et al., 2007). This causes serious environmental challenges such as P leaching, soil acidity, and soil hardness, which reduce soil fertility (Ahmed et al., 2019). To address these issues, Khan et al. (2019) found the impact of the combined application of organic addition with various inorganic inputs on soil fertility and rice production. Their findings show that the cumulative use of organic and inorganic fertilizer increased P availability and crop yield. The inorganic fertilizer's use efficiency could be decreased by improving soil fertility with organic manure (Ayaga et al., 2006). The combination of inorganic and organic amendments such as farmyard manure and crop residues is recommended to overcome soil fertility challenges (Biswas et al., 2017).

Organic amendments, including farmyard, poultry, and goat manure, are commonly used to mitigate soil alkalinity by improving the nutrient supply and water retention capacity of soils (Ojo et al., 2016). The P availability for plants was increased by adding organic amendments to calcareous soils (Huang et al., 2017). This was attributed to the release of organic acids contending with phosphates for adsorption sites on the soil colloid's surface or P mineralization during decomposition. Many cumulative mechanisms might be involved, such as soil pH improvement and inorganic P release from decomposing residues (Chen et al., 2016). There is considerable information that applying organic manure to soil can increase P solubility, decrease P fixation, and improve P availability to plants (Bader et al., 2020).

Soil microbial biomass is a living component of soil organic matter and an active pool for plant-available N and P. Several scientists have previously documented increases in microbial biomass carbon after adding organic amendments to the soil, such as compost, poultry manure, and compost (Marschner,

2008). Phosphorus (P) incorporation into the microbial biomass and its associated pool are reported mechanisms that substantially increase P availability in soil-plant systems (Muhammad et al., 2007). The use of organic manure and inorganic P together promotes soil microbial biomass growth and labile microbial P enriched metabolites, which serve as a labile P pool in soil (Brookes, 2001). During the microbial biomass turnover process, this phosphorus could be released slowly and more efficiently taken up by the plants (Kouno et al., 2002). Several studies indicated that when organic manure and P fertilizer were applied together, the microbial biomass phosphorus (MBP) pool was larger than when P fertilizer or organic manure were applied separately (Reddy et al., 2005; Ayaga et al., 2006; Khan and Joergensen 2009; Li and Marschner 2019; Peng et al., 2021). According to most studies, calcareous soils have a negative impact on plant development by restricting root carbon release into the soil (Zhang et al., 2016). As a result, soil alkalinity causes a decrease in soil microbial biomass (Halvorson et al., 1990).

Soil organic carbon (SOC) is the largest C pool in the terrestrial ecosystem, which directly or indirectly affects the soil's physicochemical and biological properties (Kan et al., 2020; Zhao et al., 2020). Particulate organic matter (POC) is a labile fraction of SOC that could reflect initial changes in the soil immediately as compared to other fractions. Furthermore, mineral-associated organic carbon (MAOC) is a relatively non-labile fraction associated with silt and clay-sized particles, and it is primarily composed of low molecular weight compounds derived from microbial activities (Kleber et al., 2015). Soil fertility is influenced due to variations in SOC under organic and inorganic amendments (Lal 2013).

Soil phosphorus forms and their distributions in the soil vary under different sources of manure application, including compost and animal manure (Schachtman et al., 1998). The soil's chemical characteristics are potentially affected by the application of different types of manure sources, which change the quantity and distribution of distinct soil P forms. Calcium-bounded phosphorus is the dominant fraction and is frequently present in calcareous soils (Khan and Joergensen, 2009). Calcium phosphate minerals were divided into three groups: 1) Ca$_2$-P (brushite and monetite) is water-soluble P, which is readily available to plants; and 2) Ca$_8$-P (b-tricalcium and P octacalcium P) is a moderately labile P fraction that can be used by selective plants (Jiang and Gu 1989); and 3) Ca$_{10}$-P (hydroxyapatite), is a stable form that is unavailable to plants. Al-P and Fe-P are less available to plants, and occluded P (goethite) is not available to plants (Adhikari and Pandey 2019). In maize soils, organic and Pi addition can influence the total P storage and create changes in soil P contents, which could provide useful

TABLE 1 Selected properties of the soil and different manure used in the present study.

Properties	Soil	Manures		
		PM	CM	GM
Total N%	0.056	2.04	1.34	0.21
Total P%	0.041	2.06	0.78	0.66
Total K%	0.0086	1.86	0.15	0.71
pH	7.93	#FF0000; 6.67	#FF0000; 7.23	#FF0000; 7.87
Soil texture	Sandy clay loam	—	—	—
EC (dSm^{-1})	1.89	—	—	—
Organic C (mg kg^{-1})	3.34	#FF0000; 23.90	#FF0000; 19.64	#FF0000; 18.45
Olsen P (mg kg^{-1})	2.37	—	—	—
MBC (mg kg^{-1})	36.75	—	—	—
MBP (mg kg^{-1})	3.37	—	—	—
#FF0000; NaHCO$_3$-Pi (g kg^{-1})	—	#FF0000; 1.73	#FF0000; 0.96	#FF0000; 0.76
#FF0000; NaOH-Pi (g kg^{-1})	—	#FF0000; 0.34	#FF0000; 0.20	#FF0000; 0.16
#FF0000; HCl-Pi (g kg^{-1})	—	#FF0000; 3.28	#FF0000; 2.01	#FF0000; 1.26

information on the effects of P inputs and P transformations (Linu et al., 2019). In P-limited soil conditions, the bioavailability of both the soil organic and inorganic P chemical fractions can be different (Bol et al., 2016). Consequently, exploring the behaviors of soil P at the expense of organic and inorganic inputs is critical to clarifying the P fraction distribution and maize P uptake mechanism in calcareous soil.

Recent studies have resulted that P-enriched organic treatments could improve P availability and plant growth more than the sole addition of Pi fertilizers (Aziz et al., 2010). This might be mainly because of microorganisms that act as a sink for inorganic P during the decomposition of soil organic matter or organic amendments (Rawat et al., 2020). According to Muhammad et al. (2007), the addition of P-enriched compost significantly increased P availability and maize yield, possibly due to higher microbial growth and turnover rate. Some studies have suggested that soil microbial activities are quite linked with SOC, P fractions, and utilization in different soil systems.

However, the relationship of soil microbial pools with SOC and P factions has not been fully understood. Therefore, this study was conducted to evaluate the response of SOC, P fraction, and microbial pool as well as plant growth. We hypothesized that organic amendments could induce microbial activity by providing soil C as an energy source, and in return, microbes could acquire more P from the soil Pi pool, thereby increasing plant growth. The basic objectives of this study are 1) to evaluate variation in MBP and P fractions in maize soil under inorganic and organic applications to better understand soil P behavior; and 2) to assess the relationship between SOC and P fractions and the plant's P uptake.

Materials and methods

Collection and pre-analysis sample

Organic manures used in this study were collected from a livestock farm located at the University of Agriculture in Faisalabad, Pakistan, and were sieved (2 mm) before the start of the experiment in order to ensure a homogeneous product. A subsample of manure is sieved and dried at 65°C for elemental analysis by following standard procedures (Peters et al., 2003). Soil samples were collected from the university ISES research farm (Table 1).

Growth conditions and plant material

A pot trial was set up in the greenhouse of the Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan. The study treatments were organized in a completely randomized design (CRD) with four replicates using organic C sources (goat, cow, poultry, and mixed manures) and inorganic P (P$_2$O$_5$ form) applied at three different application rates, that is, P0 = 0, P50 = 50, and P100 = 100 mg kg^{-1} by using diammonium phosphate (DAP) fertilizer source. The treatments consisted of: no manure (control treatment with P0, P50, and P100 mg P$_2$O$_5$ kg^{-1}; first, goat manure (GM) with P0, P50, and P100 mg kg^{-1} P$_2$O$_5$; second cow manure (CM) with P0, P50, and P100 mg kg^{-1} P$_2$O$_5$; third, poultry manure (PM) with P0, P50, and P100 mg kg^{-1} P$_2$O$_5$ and finally, mixed manure (MM) fertilization with P0, P50, and P100 mg kg^{-1} P$_2$O$_5$. The mixed manure (MM) was developed by the combination of all of the three aforementioned manures in an

equal ratio (1:1:1). Considering a manure input rate of 5% (w/w) (oven-dried weight) was taken. The soil was thoroughly mixed and three maize seeds (cv Nilam) were initially sown in each plastic pot (32 cm in diameter, 24 cm in depth). Water holding capacity was maintained at 50%. In addition, the recommended dose of nitrogen and potassium were applied to the pots at the rate of 120:40 kg ha^{-1} in split doses for normal plant growth, and development and to avoid nutrient deficiency (Reddy 2012). After 7 days of emergence, the plants were thinned to one plant per pot. Eight weeks of plants were harvested to measure the plant growth indexes. Plant height was measured by using a measuring tape. Leaf chlorophyll contents (SPAD) were recorded with a SPAD-502 m, while leaf area (LA) was recorded by using a measurement tape. Plant roots were removed gently from each pot and washed to remove soil particles and allowed to be stored in paper bags. Furthermore, an electric balance was used to measure the fresh root and shoot weights.

Determination of plant nutrient uptake

Shoots and roots were carefully washed using water (distilled) and were dried in an oven (105°C) for 1 h and then at 70°C to ensure final weight. Dry shoot and root samples of 200 mg were processed with sulfuric acid 8 ml in a sealed chamber (Li and Marschner 2019). Total P concentration in dried samples was measured with the vanadate molybdate method by visible spectrophotometer/UV as recommended by El-Gizawy et al. (1992). Total P uptake was calculated from the formula given below;

$$Total\ plant\ P - uptake = P - uptake\,(shoot) + P - uptake\,(root).$$

After the final harvest (56 days), the soil sample (50 g moist bases) was taken and analyzed for different P fractions, microbial biomass C, and microbial biomass P contents. Additionally, samples collected (air-dried) were analyzed for soil available P.

Determination of microbial biomass C, P, and olsen P

Microbial biomass P was estimated by the chloroform fumigation extraction method (Brookes et al., 1982) in the 50 g sample taken from each treated pot. Three parts of soil weighing 5 g (oven-dried for 24 h at 105°C), were separated into a 50 ml flask. One part was untreated, the second part was treated with P KH_2PO_4, and the third part was fumigated (24h, 25°C) with ethanol-free chloroform. These three soil parts were extracted with 0.5 M $NaHCO_3$ by shaking at 150 rev min^{-1} for 30 min and filtered by using a 0.45 μm filter paper. MBP was calculated by using a 0.4 conversion digit. The MBC was analyzed by the chloroform fumigation-extraction method and

calculated as follows: $C = E_C/K_{EC}$, where E_C is the difference between organic carbon extracted from fumigated and non-fumigated samples, and K_{EC} is 0.4 (Wu et al., 1990). The Olsen-P concentration was analyzed by extracting 0.5 M $NaHCO_3$ (8.5 pH) following Olsen (1954).

Determination of soil C fractions

For soil organic C analysis, the soil samples (oven-dried) were ground, sieved (0.25-mm), and analyzed as suggested by Walkley and Black, (1934). Particulate organic matter (POM) and mineral-associated organic carbon (MAOC) were analyzed by the method of Cambardella and Elliott, (1992). POM was determined by shaking 10 g of oven-dried soil for 18 h in 30 ml of sodium hexametaphosphate (5 g/L). The supernatant was passed through a sieve (53-μm). The retained material passed on the screen was oven-dried at 50°C for 24 h and used to measure C according to the aforementioned method. POC was determined as the carbon (C) content in the screen retained POM. Meanwhile, the screen-passed material was also measured for C and identified as mineral-associated organic carbon (MAOC).

Determination of soil pi fractions

According to Jiang and Gu (1989), soil inorganic P (Pi) was divided into distinct inorganic P fractions; this extraction method has been used for P fractionation of alkaline soils (Wang et al., 2010b). Different inorganic P fractions such as Ca_2-P, Ca_8-P, Ca_{10}-P, Al-P, Fe-P, and O-P were extracted by following the sequential extraction method (Figure 1).

Briefly, the soil sample of about 0.5 g (oven-dried bases) was added into centrifuge tubes of 50 ml. By following the sequence shown in Figure 1, the selected extractants of 25 ml (with a defined pH) were taken into tubes and shaken by using an automated reciprocating shaker machine for the required time. After shaking, the tubes were allowed to centrifuge (10 min, 4500 rpm). After, completing the centrifugation process, the supernatants were transferred carefully into a new tube to avoid any contamination. Finally, the extracted supernatants were evaluated with a UV/visible spectrophotometer at 880 nm following the molybdate blue method of Murphy and Riley (1962) to analyze the inorganic phosphorus (Pi) contents in the soil fractions.

Statistical analysis

SPSS version 25.0 was used for the statistical analysis. Two-way ANOVA (analysis of variance) was used to analyze the influence of organic manure and inorganic P (Pi) on recorded data. To compare the cumulative effects of organic C and Pi
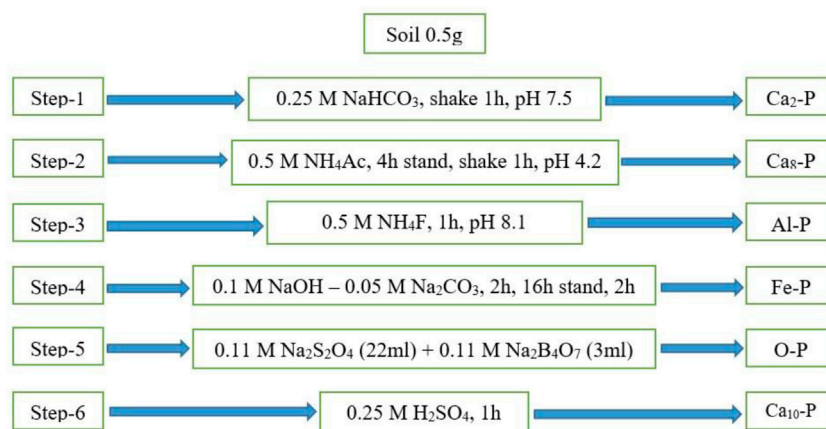
FIGURE 1
Sequential extraction steps for the inorganic P fractions by Jiang and Gu 1989.

amendments, the significance of the difference between treatments was evaluated by the HSD test (honestly significant difference test).

Results

Plant growth and P uptake

Inorganic and organic fertilization alone or in combination affect the growth indexes of maize. In this study, the combined application of PM and P100 significantly increased the maize growth attributes (Table 2). The average increase in plant height (117 cm), shoot fresh weight (101 g), shoot dry weight (13 g), root fresh weight (33.34 g), root dry weight (4.8 g), and leaf area (422 cm^2) was observed under the combined application of PM and P100 to the maize plant. The chlorophyll content of maize leaf increased under the cumulative effect of manures and Pi than that of control (Table 2). The addition of PM and P100 together increased the 180 mg P uptake by the plant compared to the control. Compared with the control, the P contents were significantly increased in the root (0.21%) and shoot (0.4%) of maize (Table 2). The P concentration in different plant parts of maize (shoot and root) were increased in the order of PM > CM > MM > GM > control, respectively (Table 2).

Variation in soil pi fractions

In the current study, the size of the individual P pool was significantly ($p < 0.05$) affected by organic and inorganic amendments (Table 3). The ANOVA demonstrated a significant effect of the treatments with inorganic P (Pi)

addition rates of P0, P50, and P100 on the relative proportion of extracted P in bulk soil fractions. The overall organic treatment effect indicated the increasing trends, that is, PM > CM > GM > MM > control. The PM showed a significant increase in the Pi fractions $NaHCO_3$-P, NH_4Ac-P, NH_4F-P, and NaOH-P occluded P (O-P), and Ca_{10}-P in calcareous soils.

In general, under no manure treatment (control) the average size of Pi fractions increased in the order of Ca_8-P > Ca_2-P (labile pool), Al-P > Fe-P (moderately labile pool), and Ca_{10}-P > O-P (non-labile pool), respectively (Table 3). In the control treatment, the Ca_2-P and C_8-P were 1.89 and 9.16% of the total P in the soil (Figure 2). The P100 showed the highest increase in Ca_2-P that was about 40.3%, whereas, the C_8-P was increased by 14.8% (was more or less constant) as compared to P0. Moreover, Fe-P and Al-P constituted 2.31 and 4.51% of the total P in the soil in control, respectively (Figure 2). Compared with the control, the average increase of Fe-P was about 32.6% of the added P50 rate and 35.22% of the added P100 rate. The P50 and P100 exhibited the highest increase in Al-P about 27.3 and 30.75% with respect to control, respectively. In addition, P100 showed the maximum concentration of occluded P approximately, 46.96%. Similarly, P100 depicted the highest Ca_{10}-P about 7.4% compared to P0. The occluded P and Ca_{10}-P were 0.68 and 81.34% of the total P in the soil in control (Figure 2).

Data in Table 3 showed that the PM ($p < 0.05$) increased the concentration of Pi fractions in the following sequence: C_8-P > Ca_2-P, Al-P > Fe-P and Ca_{10}-P > O-P. By applying PM, the Ca_2-P and C_8-P fractions were significantly increased, with a mean value of 32.30 and 76.56 mg kg^{-1}, respectively. These fractions had a higher concentration of about 5.09% (Ca_2-P) and 12.06% (C_8-P) of the total soil P under PM (Figure 2). The increase in Ca_2-P was observed in other organic manure that is 28.91 (CM), 19.33 (GM), 23.34 (MM), and 8.3 mg kg^{-1} (control). Whereas,

TABLE 2 Maize plant growth characteristics and P uptake under different manure types and inorganic P rates.

Treatment	Plant height (cm)	Leaf area (cm²)	Chlorophyll content	Shoot fresh weight (g/plant)	Shoot dry weight (g/plant)	Root fresh weight (g/plant)	Root dry weight (g/plant)	Shoot P concentration (%)	Root P concentration (%)	Plant P uptake (mg/plant)
#FF0000; Control	75.25c	230d	30.09d	57.00d	7.70d	24d	3.42e	0.47e	0.18e	57.00e
GM	86.57b	328.59c	33.68c	72.98c	8.10d	25.50cd	3.66d	0.59d	0.24d	72.98c
CM	105.92a	403.66a	40.54a	92.23 ab	10.66b	30.52 ab	4.26b	0.78b	0.35b	92.23 ab
PM	112.93a	415a	42.18a	98.00a	12.22a	32.08a	4.60a	0.86a	0.39a	98.00a
MM	95.02b	362.83b	36.13b	84.05b	9.72c	27.89bc	3.87c	0.67c	0.28c	84.05b
P0	90.08b	336.18b	34.87c	73.33a	8.36c	26.42b	3.74c	0.62c	0.26c	73.33b
P50	95.10 ab	346.13 ab	36.59b	82.64a	9.96b	27.95 ab	3.97b	0.67b	0.29b	82.64a
P100	100.23a	361.73a	38.07a	86.57a	10.72a	29.62a	4.18a	0.72a	0.31a	86.57a
P0-Control	72.34	215.16	27.90	46.50	6.40	22.22	3.23	0.43	0.15	46.50
P0-GM	80.83	313.61	32.79	64.73	7	23.91	3.54	0.55	0.22	64.73
P0-CM	100.04	398.81	39.19	84.47	9.47	28.86	3.96	0.72	0.31	84.47
P0-PM	108.38	407.94	40.45	93.00	10.34	30.17	4.45	0.83	0.37	93.00
P0-MM	88.82	345.37	34.01	77.99	8.59	26.97	3.60	0.59	0.24	77.99
P50-Control	75.47	228.04	30.23	61	7.70	23.92	3.48	0.47	0.18	61.00
P50-GM	86.34	321.09	33.04	74.20	8.37	25.21	3.64	0.59	0.24	74.20
P50-CM	105.73	402.61	41.35	93.86	10.85	30.06	4.30	0.78	0.35	93.86
P50-PM	113.12	414.90	42.02	100	13.01	32.74	4.54	0.86	0.36	100.00
P50-MM	94.81	364.04	36.31	84.19	9.87	27.84	3.90	0.66	0.28	84.19
P100-Control	77.94	246.80	31.91	63.50	9.00	25.85	3.56	0.50	0.20	63.50
P100-GM	92.54	351.07	35.20	80.01	8.94	27.39	3.81	0.63	0.25	80.01
P100-CM	111.97	409.55	41.08	98.39	11.67	32.63	4.54	0.83	0.38	98.39
P100-PM	117.29	422.16	44.09	101	13.30	33.34	4.88	0.89	0.41	101.00
P100-MM	101.43	379.07	38.08	90	10.70	28.88	4.11cd	0.74	0.33	90.00
p-Value (OT)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
p-Value (P)	<0.01	<0.01	<0.01	<0.01	<0.01	0.0130	<0.01	<0.01	<0.01	<0.01
p-Value (OT*P)	0.9959	0.9389	0.7664	0.9386	0.3816	0.9989	0.3582	0.8278	0.5663	0.0049

the Ca_8-P average increase by using other manure sources was about 70.61 (CM), 50.52 (GM), 57.08 (MM), and 40.57 mg kg^{-1} (control). The Ca_2-P was increased by 17.89, 24.32, and 25.11 mg kg^{-1}, while the Ca_8-P was increased by 53.77, 61.67, and 61.76 mg kg^{-1} in P0, P50, and P100, respectively. The current study results showed that the Ca_8-P was larger than the Ca_2-P with a mean difference value of 44.29 mg kg^{-1} under the P100 and PM (Table 3). The significantly increased in Al-P with PM, the mean value was 49.27 mg kg^{-1}, while other manures had mean values of 41.24 (CM), 29.95 (GM), 37.40 (MM), and 20 mg kg^{-1} (control) (Table 3). In addition, Al-P had almost 7.8% contribution to total P (Figure 2). Compared with P0, the maximum increase of Al-P was about 31% by P50, and similar results were observed with P100. When the PM and P100 were applied together, the average increase in Al-P contents was about 53.07 mg kg^{-1}. The PM also significantly increase the

Fe-P by about 36.92 mg kg^{-1} compared to the control. This fraction made up 6.28% of total P in soil (Figure 2). By using other manure, the mean value of Fe-P was approximately 47.51 (CM), 31.10 (GM), 33.03 (MM) and 10.57 mg kg^{-1} (control), respectively. The P0, P50, and P100 significantly increased the Fe-P concentration by approximately 26.42, 35.08, and 35.73 mg kg^{-1}, respectively. In this study, the combined application of P100 and PM significantly increased the Fe-P fraction by almost 43.29 mg kg^{-1}. In comparison to the labile and moderately labile pools, the occluded P (O-P) was slightly affected by PM addition (Table 3). The PM increased the O-P contents by 13.89 mg kg^{-1}, which accounted for 2.24% of total P in soil (Figure 2). The O-P fraction was significantly affected with other manure types approximately of 12.49 (CM), 7.89 (GM), 5.84 (MM), and 2.53 mg kg^{-1} (control). The PM addition and P100 together increased the O-P concentration by about

TABLE 3 Different phosphorus and carbon fractions distribution under different manure types and inorganic P rates.

Treatment	Olsen P (mg kg^{-1})	Ca$_2$P (mg kg^{-1})	Ca$_8$P (mg kg^{-1})	Al-P (mg kg^{-1})	Fe-P (mg kg^{-1})	Occ-P (mg kg^{-1})	Ca$_{10}$-P (mg kg^{-1})	MBP (mg kg^{-1})	MBC (mg kg^{-1})	SOC (g kg^{-1})	POC (g kg^{-1})	MAOC (g kg^{-1})
#FF0000; Control	6.05e	8.34e	40.57e	20.0e	10.58d	2.53e	358.8d	7.82e	70.60d	2.90c	1.20c	1.70a
GM	11.03d	19.33d	50.52d	29.96d	31.11c	7.89c	381.19cd	14.27d	90.25c	3.98b	1.97b	2.01a
CM	15.79b	28.91b	70.61b	41.24b	33.04c	12.49b	410.29 ab	25.87b	110.21b	4.30 ab	2.50a	1.80a
PM	18.87a	32.30a	76.56a	49.56a	39.81b	14.24a	421.05a	32.87a	140.09a	4.50a	2.71a	1.79a
MM	12.52c	23.31c	57.07c	37.40c	33.04c	5.84d	391.53bc	17.12c	105.35b	4.10b	2.10b	1.99a
P0	8.44c	17.89b	53.78b	29.85b	26.43b	6.68b	375.23b	15.63c	76.90b	4.61a	2.76a	1.84a
P50	14.49b	24.32a	61.67a	38.0a	35.05a	9.37a	399.17a	19.87b	115.06a	3.69b	1.77b	1.91a
P100	8.44c	25.10a	61.76a	39.03a	35.74a	9.73a	403.31a	23.28a	117.93a	3.57b	1.74b	1.82a
P0-Control	2.54	5.60	35.11	13.94	6.60	1.30	348.91	3.395	37.93	3.63	2.11	1.51
P0-GM	5.76	14.04	45.02	22.64	23.47	5.99	366.70	11.00	60.57	4.96	2.60	2.36
P0-CM	11.82	24.90	64.41	36.98	40.15	10.16	389.05	22.15	90.10	4.84	3.08	1.76
P0-PM	14.48	27.56	71.02	44.15	34.21	12.32	399.94	28.65	115.32	4.94	3.32	1.62
P0-MM	7.62	17.32	53.34	31.53	27.74	3.66	371.53	12.96	80.61	4.68	2.71	1.97
P50-Control	7.65	9.35	43.06	22.44	12.39	2.97	361.60	9.11	84.66	2.60	0.74	1.85
P50-GM	13.32	21.85	53.02	33.02	34.74	8.59	383.94	14.36	102.72	3.54	1.68	1.85
P50-CM	16.69	30.76	73.01	43.05	50.70	13.45	419.26	25.46	119.06	4.18	2.21	1.97
P50-PM	20.65	34.04	79.057	51.44	41.92	15.10	430.70	33.12	152.09	4.30	2.42	1.88
P50-MM	14.14	25.60	59.70	40.07	35.49	6.75	400.36	17.29	116.77	3.80	1.82	1.97
P100-Control	7.96	10.06	43.55	23.62	12.76	3.30	365.88	10.97	89.20	2.49	0.73	1.75
P100-GM	14.02	22.09	53.51	34.20	35.12	9.08	392.91	17.46	107.46	3.43	1.62	1.81
P100-CM	18.85	31.07	73.92	43.66	51.68	13.84	422.57	10.97	121.49	3.86	2.20	1.66
P100-PM	15.80	35.29	79.61	51.44	43.29	15.32	432.50	36.83	152.84	4.26	2.41	1.85
P100-MM	21.49	27.01	58.19	53.08	35.87	7.10	402.70	21.12	118.67	3.80	1.75	2.04
p-Value (OT)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.197
p-Value (P)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.767
p-Value (OT*P)	<0.01	0.085	0.773	0.327	0.096	0.160	0.996	0.152	0.490	0.284	0.342	0.348

14.02 mg kg^{-1}, as compared to the control. The O-P increased about 45.75% under P100 (same for P50) compared to P0. Moreover, the Ca$_{10}$-P was 66.46% of the total soil P (Figure 2). By PM, the highest Ca$_{10}$-P contents (17.59%) were observed with respect to control. The PM and P100 together increased the Ca$_{10}$-P concentration by about 432.50 mg kg^{-1}. Whereas, the Ca$_{10}$-P concentration increased about 410.26 (CM), 381.50 (GM), 391.53 (MM) and 358.8 mg kg^{-1} (control). In P0, P50, and P100, the Ca$_{10}$-P fraction was increased by almost 375.23, 399.17, and 403.31 mg kg^{-1}, respectively.

Variation in soil C fractions, olsen P and microbial biomass indices

The SOC, POC, and MAOC concentrations varied between the different organic amendments. The SOC fraction increased in

the following order: PM > CM > MM > GT > control (Table 3). The maximum SOC was 4.2 g kg^{-1} under the combined addition of P100 and PM. Compared to the control, the average increase in SOC was about, 1.6 (PM), 1.4 (CM), 1.08 (GM), and 1.2 (MM) g kg^{-1}. The Pi had increased the SOC, approximately 4.61, 3.69, and 3.57 g kg^{-1} under P0, P50, and P100, respectively. The different organic manure additions significantly increased POC that ranging between 1.2 and 2.7 g kg^{-1} (Table 3). The Pi significantly ($p < 0.0 5$) increased the POC that was 1.77 g kg^{-1} under P50 and 1.74 g kg^{-1} under P100. The higher POC was 2.4 g kg^{-1}observed in the combined addition of P100 and PM. The effect of treatments on MAOC fraction increased in the following order: GT > MM > CM > PM > control (Table 3). Overall, the maximum C fractions size increased in PM by the following order: SOC > POC > MAOC.

The different organic manure amendments with Pi caused variations in Olsen P, MBC, and MBP (Table 3). The maximum Olsen P 21.5 mg kg^{-1} was observed by the combined addition of

FIGURE 2
Total Pi proportion in each fraction under organic manure addition.
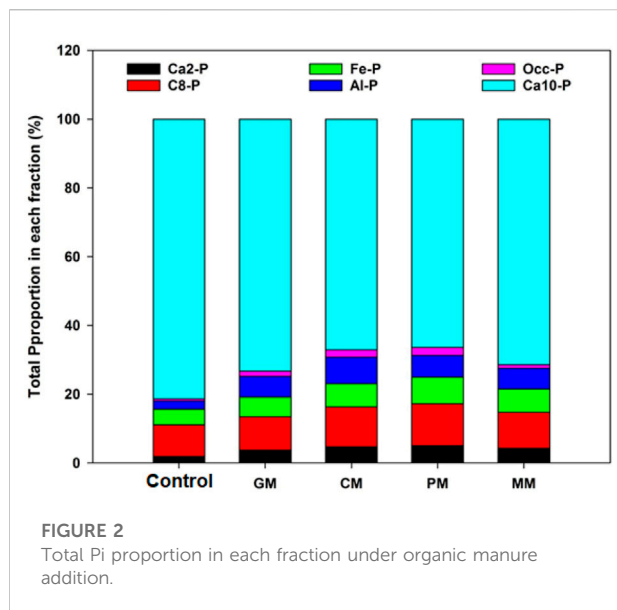
PM and P100. The Olsen P was increased by about 72 and 85% under P50 and P100. The average increase of Olsen P was 18.88 (PM), 15.79 (CM), 11.04 (GM), and 12.51 (MM) mg kg^{-1} than that of control (Table 3). Collectively, the MBC and MBP pools were higher at almost 69.4.5, 39.9, 20.25, 34.75 mg kg^{-1} and 25.07, 18.07, 6.47, 9.32 mg kg$_{-1}$ in PM, CM, GM, MM in comparison to control, respectively (Table 3).

Discussion

Changes in soil properties (SOC, POC, and olsen P)

In our study, organic and inorganic fertilization significantly ($p < 0.05$) affected the soil chemical properties, microbial biomass indices, and inorganic P (Pi) fractions (Table 3), that could be due to the manure types and P application rates. Many previous studies have presented that the Pi forms are mainly influenced by microbial activities and soil chemical properties (Spohn and Widdig, 2017). Furthermore, the soil physicochemical properties significantly affected the microbial biomass and Pi fraction in calcareous soil. Among different organic amendments, PM had the greater effect on soil chemical and biological properties, resulting in native soil P lability. This was mainly due to organic amendments chemical composition, where PM had higher organic-P contents as compared to other amendments (CM, GM). Moreover, higher microbial activities (MBC, MBP) under PM might increase the soil available and total P by inducing SOC and POC concentration in the soil (Table 3). In the present research, total soil P (683.76 mg kg^{-1}) and Olsen-P (21.5 mg kg^{-1}) contents were highest in the PM with

P100 (Figure 2; Table 3). According to Hassan et al. (2012), the critical Olsen-P level for wheat and maize growth is 14.6 and 13.2 mg kg-1, respectively. This study achieves quite a similar Olsen-P threshold hold levels with PM and P100 applications. The significant increase in the Olsen-P showed a positive correlation with Pi fractions pools, showing that the combined organic and inorganic fertilizer addition had positive effects on Olsen-P contents. This might be due to the organic manures being sufficient in P supply and organic manures with inorganic P are prevented from the adsorption and insoluble complexes in the soils (Chen et al., 2006). Moreover, organic inputs mobilized native soil P by accelerating microbial activities (Kouno et al., 2002).

Overall, organic amendments significantly stimulated the soil microbial pool by increasing the availability of C sources (POC). Among other amendments, PM had a higher moderately labile (Ca$_8$-P) fraction, possibly due to higher nutrient contents, POC availability, and water holding capacity (Hoover et al., 2019). The MAOC had no relationship with microbial activities because that was known to be a recalcitrant C and a non-usable pool (Virk et al., 2021). This indicates that the only labile pool of C in the organic manures could increase P availability by inducing microbial activity. Natural manure fertilization is the source of essential nutrients for plants. Plants require P in the vegetative, and during reproductive development (Richardson et al., 2009). The data in Table 2 shows that plant biomass is considerably affected by both manures and Pi. The higher amount of approximately 236 mg of P uptake by the maize plant was estimated as compared to CM (197.74 mg) and GM (113.54 mg) under the dose rate of P100 (Table 2). This might be because PM contains about three to four times higher P concentration than manure from other livestock, except for pigs (Kleinman et al., 2005), which was also seen in the current study. The nutrient concentration for all types of manure varied when compared to other published studies, which was probably attributed to dietary habits, animal age, and the season (Penhallegon 2003; Brown 2013). In addition, this higher plant P uptake might be due to PM-treated soil having the potential to increase the labile SOC pool mainly POC, resulting in higher microbial activities and higher P availability (Ca$_8$-P) inducing plant growth. Similarly, our results are also consistent with the previous research, which reported that applied Pi is mostly transformed into Ca$_2$-P (more available for plant growth) instead of Ca$_8$-P in calcareous soil (Marriott and Wander 2006; Liebisch et al., 2014). Furthermore, Hassan et al. (2012), found that organic input with Pi leads to a synergistic effect on plant growth compared to the application of the sole fertilizer in calcareous soils. The labile P and moderately labile P are always known as bioavailable P forms for plant uptake. Consequently, it improves the physicochemical and biological soil conditions for plant growth and development.

Changes in microbial biomass indices

Soil microbial dynamics are affected by various organic amendments. In C and P deficient soils, the microbial community acts more effectively when energy source C is added. A significantly higher MBC of 152.8 mg kg^{-1} was observed under the cumulative effect of organic and inorganic amendments (Table 3). In addition, a significant positive relationship between MBC with Pi fractions and a negative correlation with SOC and POC was observed, suggesting that an increase in MBC at the expense of SOC and POC resulted in P release from Pi fractions, in parallel to these results. Wu et al. (1990) reported that MBC pool mainly depends on the organic C availability. Moreover, the native microbial population is stimulated in response to the Pi addition (Ayaga et al., 2006). However, the relationship of MBC to organic C is inconsistent, showing either a positive or no correlation due to the microbial community structure and organic C quality (Brookes 2001).

The SOC and POC had a positive correlation with MBP, the increase in microbial biomass indicating the strong potential to accumulate radially available P into their bodies (Spohn and Widdig, 2017). The maximum increase in MBP of 36.8 mg kg^{-1} due to the cumulative effect of organic manure and Pi amendments was observed (Table 3). As suggested before, organic amendments induce MBC (in response to POC and SOC increment), but Pi with organic amendments could stimulate MBP and Olsen-P availability (Tang et al., 2014). An important finding was that the manure-treated soil (PM) had a larger size of microbial biomass C and microbial biomass P than that of other organic manures. This might be due to the difference in microbial P acquisition ability in different manure sources, or might be due to the difference in availability of C and P in manure sources (Kouno et al., 2002). In the current study, microbial biomass dynamics showed variations in different organic amendments, indicating that microbial activity depends on organic C availability (Nyawade et al., 2019).

Changes in soil P fractionation

The application of Pi and different manure together ($p < 0.05$) enhanced the P concentration in soil, this result is consistent with Zhang et al. (2021). P input increased the size of all Pi fractions such as the labile, moderately-labile, and non-labile (Table 3). Our study describes that the manure applied to soil has a direct influence on P availability. This was possibly due to different Pi amendment rates and manure having various impacts on P immobilization and its mobilization in calcareous soils (Khan and Joergensen 2009).

The Ca-P fraction was identified as the dominant P fraction in calcareous soil. Soil Pi forms Ca$_2$-P considered as labile P or bio-available P form for plant uptake (Jiang and Gu 1989). In the current study, Ca$_2$-P accounted for 5.09%, of total P as shown in
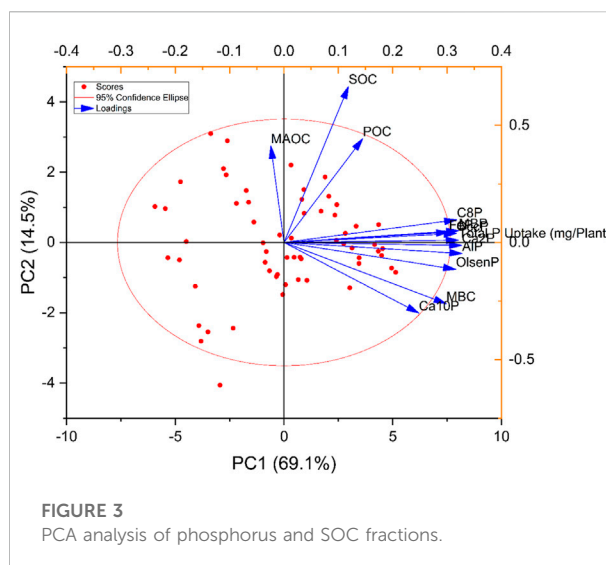

FIGURE 3
PCA analysis of phosphorus and SOC fractions.

Figure 2 by the combined addition of PM and P100. This indicated that the available P supply ability was well maintained by the Pi input combined with manure over inorganic input only (control with P100). According to Delgado et al. (2002), P fixation with Ca-P reduces the P accessibility for plant growth that may be available after the manure input into the soil, which has been proven in our PCA analysis (Figure 3). These study results are in accordance with Ahmed et al. (2019), which found that the Pi and pig manure addition increased the P labile forms by inducing microbial activities in response to C addition in soil. The average labile Pi content increases in the PM, possibly due to the higher SOC (4.5 g kg^{-1}) and POC (2.7 g kg^{-1}) concentration in the soil (Virk et al., 2021), that might move the P adsorption sites by exchanging the clay mineral surface charges, decreasing the sorption sites and improving the soil P availability. Noticeably, the association of Pi fractions and soil carbon (SOC, POC) also indicated that the input of organic manure reduced the Pi fixation and increase Ca$_2$-P availability (Delgado et al., 2002).

The inorganic fractions (Ca$_8$-P, Fe-P, and Al-P) were known as the moderately labile P pool. Ca$_8$-P, Fe-P, and Al-P fractions accounted for 79.61, 43.29, and 50.03 mg kg^{-1}, respectively, with the PM and Pi. During the manure decomposition process, organic acids are released, which lead to chemical bonding of P with minerals ions (Fe and Al), decreasing the soil P availability. As the labile P, the moderately labile P also improved by the PM, compared to the other manure source and control (Table 3). That is possibly due to the high nutrient content in PM mainly POC and SOC as compared to other manures (GM, CM), which have higher nutrients and water holding capacity. Several studies also resulted that the various source of organic amendments increased the moderately labile Pi pool as compared to inorganic fertilizer (Li et al., 2012). Malik

et al. (2012) identified that manure sources containing P content can have synergetic effects on increasing organic P content and retention in soils that could be due to the P adsorption in organic matter that is transformed into inorganic P by plant roots. In calcareous soil, the Ca_8-P fraction is mostly greater than Ca_2-P this may explain that Ca_8-P is more stable in P-limited soil and it needs higher Pi in the soil for the solution to transform into Ca_2-P, which may not be possible under low Pi addition (Lindsay et al., 1962; Ayaga et al., 2006). However, the increase in the moderately labile P pool in the combined use of PM and Pi may explain the slow P release from an organic source which can contribute to the P cycling process in soil.

The non-labile P fractions which are stable and insoluble P form, such as occluded P (O-P) and apatite P (Ca_{10}-P), are known as the unavailable P pools for plant uptake (Cao et al., 2020). The Ca_{10}-P fraction is dominated by all the P fractions in the soils. Noticeably, it had a significant correlation with microbial index, this might be due to higher organic source amendments (Yong-Fu et al., 2008). The Ca_{10}-P is the recalcitrant pool due to geochemical properties on P cycling and higher $CaCO_3$ contents in the soils, as suggested by Mehmood et al. (2015). Our results support the study of Zhang et al. (2021), who suggested that adding P fertilizer had a slight influence on the residual P forms but substantially enhanced the soluble Pi. While the stable P was supposed to be less available P pool, the variation in this P pool shows P cycling on a long-term basis in the soil system (Huang et al., 2017). It was concluded by Meason et al. (2009) that applying P adsorbed on the surface of the mineral can contribute to increasing the stable pool. Our study resulted that non-labile P concentrations were higher in Pi with manure addition, that resulted in the P sorption under excessive Pi input, that may be transformed to the moderately or labile P pools over time.

Conclusion

The Poultry manure addition increased the plant growth, P uptake, and Ca_8-P pool (after Ca_2-P is depleted), by improving the total SOC contents and labile organic C (POC) pool in P-limited soil. Present research demonstrated that SOC and microbial biomass (MBP, MBC) are the key drivers to improve the soil P availability and the cumulative effect of PM and Pi is not only a better approach for P acquisition for the maize plant but also has a positive effect on the chemical properties (SOC, POC and Olsen P) of the soil. Indeed, the increase in the soil labile and moderately labile P fractions are likely due to MBP turnover processes mediated by PM. Therefore, we concluded PM with Pi promotes soil MBP pool, which in turn improves soil P availability and utilization efficiency in maize crops. This study shed light on the Pi

addition rate with PM should be further evaluated for P balance and crop yield in field conditions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

Conceptualization, KK, and GF; methodology, KK, MN, and MR; software, KK; validation, MA and MN; data curation, MN; writing—original draft preparation, KK; writing—review and editing, KK, MA, MN, MS, NA, HA, and RG; supervision, MN and GF.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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