



Effect of rate and source of phosphorus application on soil organic carbon pools under rice (*Oryza sativa*)-wheat (*Triticum aestivum*) cropping system

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ABSTRACT

A field experiment was conducted during 2006-2013 on rice (*Oryza sativa* L.) - wheat (*Triticum aestivum* L.) cropping system at research farm of Punjab Agricultural University, Ludhiana, India. The study aimed to evaluate the effect of two rates of phosphorus (P) application, viz. 0 and 30 kg P₂O₅/ha to rice in rice-wheat sequence on soil organic C pools. Phosphorus was applied through either single super phosphate (SSP) or rock phosphate (RP) with and without farmyard manure (FYM). After 7 years of cropping total organic carbon (TOC) and labile C fractions were higher under FYM and RP treated plots and the lowest in unfertilized control. Plots treated with FYM and fertilizer-P either individually or in combination significantly ($P < 0.05$) increased TOC and water extractable organic C (WEOC) in surface (0-7.5 cm) and subsurface (7.5-15 cm) soil. Beneficial effects of fertilizer-P application through RP were lower than SSP. Potassium permanganate oxidizable C (KMnO₄-C) comprised the largest labile pool of soil organic C and represented 13.6% of TOC. Carbon management index (CMI) improved with FYM and fertilizer-P applications indicating the favorable impact of these treatments on C stabilization in soil. Conjoint application of FYM and RP improved soil organic C pools to a greater extent than their individual application, suggesting the need for integrated use of FYM and RP in these alluvial soils.

Key words: Carbon management index, C sequestration, Labile C, Rice-wheat, Rock phosphate, Soil P

Rice (*Oryza sativa* L.)-wheat (*Triticum aestivum* L.) is a dominant cropping system in the Indo-Gangetic Plains (IGP) of India. This is an exhaustive cropping system and results in nutrient mining from soil. Imbalanced application of nutrients and little or no recycling of organic sources adversely affect soil organic carbon and its labile pools (Benbi *et al.* 2015a). Organic carbon is a key attribute of soil fertility, which serves as soil conditioner, nutrients source, and substrate for microbial activity, preserver of the environment and sustainer of agricultural productivity (Benbi *et al.* 2011). Fertilizer application stimulates crop biomass production, which results in an increase in the amount of residue returned and C accumulation in soil. Integrated use of FYM along with inorganic fertilizer leads to higher crop yields and greater accrual of C in soil (Benbi *et al.* 2015b). However, changes in SOC are slow to occur and because of larger background levels the short-term effect of nutrient management practices on total organic C is not discernible. Labile C pools determined by chemical extraction techniques are considered early indicators of management induced changes in quality and composition of soil organic matter

(Benbi and Senapati 2010, Benbi *et al.* 2015b). The labile pools being readily accessible to microorganisms directly impact plant nutrient supply. According to Schulz *et al.* (2011), hot water soluble C (HWSC) is an indicator of decomposable SOC pool and serves as a source of nutrient supply during organic matter decomposition. Microbial biomass C (MBC) plays an important role in nutrient cycling and was recognized as a component of active soil organic matter that helps in maintaining functions and sustainability of terrestrial ecosystems. Carbon management index (CMI) is a sensitive indicator of changes in SOC and was used to quantify the effect of management practices on C rehabilitation (Sodhi *et al.* 2009). While the effect of manure and fertilizer N application on SOC and its labile pools is fairly well-documented (Sodhi *et al.* 2009, Tong *et al.* 2014, Benbi *et al.* 2015b), the effect of phosphorus application through different sources (rock phosphate and single superphosphate) on SOC pools under rice-wheat system is not known. Combination of FYM with rock phosphate could be cost-effective and appropriate strategy for enhancing crop yield and maintaining soil health. The present study was, therefore conducted to investigate the effect of rates and sources of P application on SOC and its labile fractions.

MATERIALS AND METHODS

A field experiment was established during 2006 on

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rice-wheat cropping systems at research farm of Punjab Agricultural University, Ludhiana, India (Latitude: 30° 56'N, Longitude: 75° 52'E and mean sea level 247.5 m). The study location is at the centre of Punjab state which forms the north-western part of the country. The climate of the place is semi-arid subtropical. During 1970-2005, the experimental area received ~750 mm rainfall annually, of which ~80% was received during the *kharif* season extending from 1 May to 31 October. The mean monthly minimum and maximum air temperatures averaged 18 and 35°C during rice season (June-October) and 6.7 and 22.6°C during wheat season (November-April), respectively. The soil of the experimental field was a *Typic Ustorthent*, developed on alluvial material with a sandy loam texture. At the start of the experiment, the field soil (0–15 cm) had a pH (1:2 soil: water suspension) of 7.22, electrical conductivity (1: 2 soil: water supernatant) of 0.21 dS/m, and SOC of 5.72 g/kg. The experimental soil tested 141 kg/ha in available N, 22 kg/ha in available P and 90 kg/ha in available K.

The treatment details for rice and wheat are given in Table 1. Briefly, the treatments comprised i) application of nil and 30 kg P₂O₅/ha to rice through single super phosphate (SSP) and rock phosphate (RP) and ii) application of farmyard manure (FYM) at 10 Mg/ha with or without fertilizer application to rice. Except in control plots wheat was fertilized with 60 kg P₂O₅/ha through SSP. Each treatment was replicated three times and arranged in a randomized complete block design (RBD) on a plot size of 3.0 m × 8.0 m (24 m²). Fertilizer N to rice was applied through urea in three equal splits, viz. at the time of puddling, 3 and 6 weeks after transplanting. Whole of P as SSP or RP and K as potassium chloride was applied as a basal dose to rice and wheat. Requisite amount of FYM was spread on the soil surface and mixed in the top 5-7.5 cm soil with a cultivator. The field was submerged with 5-6 cm water. After puddling, 30-35 d old rice seedlings were transplanted in second week of June in rows 20 cm apart with plant-to-plant distance of 15 cm, ensuring 33 plants/m². Rice was harvested manually in the fourth week of October each year. After rice, wheat was sown in second week of November at a seed rate of 100 kg/ha in rows 22.5 cm apart (*rabi* season). In wheat, half dose of fertilizer N was applied at the time of sowing and the remaining half one week after first irrigation. Wheat was irrigated with canal or ground water and an irrigation of about 7.5 cm was applied as and when required

depending on the visual inspection of the field. Wheat was harvested manually in the third week of April each year.

Soil samples were collected from 0-7.5, 7.5-15, 15-30 and 30-60 cm soil depths after wheat harvest in May 2013. Soil samples were collected with a metallic core sampler (7 cm inner diameter and 7.5 cm length) from three sites for each depth within a plot and composited. Soil samples were air dried in shade, ground to pass 2 mm sieve for analysis. Total organic carbon was determined by using 1N potassium dichromate and concentrated H₂SO₄, followed by heating at 150°C for 1 hr and titration with ammonium ferrous sulphate and diphenylamine indicator (Snyder and Trofymow 1984). Water extractable organic C was determined by shaking 10 g soil with 20 ml deionized water for 1 hr (McGill *et al.* 1986). Hot water soluble C was determined by moderately boiling 20 g soil with 100 ml distilled water for 1 hr under reflux condenser. The amount of C in the extract was determined by chromosulphuric acid method (Schulz *et al.* 2003). Soil microbial biomass C was determined by chloroform fumigation extraction method (Vance *et al.* 1987) using a recovery factor (K_{EC}) of 0.41 (Voroney and Paul 1984). Potassium permanganate oxidizable C was determined by oxidation with 33 mmol KMnO₄ solution (Blair *et al.* 1995). Non-labile C was calculated as the difference between TOC and KMnO₄-C.

Carbon management index (CMI) was computed for two soil depths (0-7.5 and 7.5-15 cm). The mean value of control soil samples was used as a reference. Based on changes in TOC between the reference (control) and a treated sample, carbon management index (CMI) was calculated as per the procedure described by Blair *et al.* (1995):

$$\text{CMI} = \text{CPI} \times \text{LI} \times 100$$

where, CPI is the C pool index and LI is the lability index.

The CPI and the LI were calculated as:

$$\text{CPI} = \frac{\text{TOC treated (g/kg soil)}}{\text{TOC control (reference) (g/kg soil)}}$$

$$\text{LI} = \frac{\text{L}_{\text{treated}}}{\text{L}_{\text{control (reference)}}}$$

where, L refers to the C lability, calculated as

$$= \frac{\text{Content of labile C (KMnO}_4 - \text{C) (g/kg soil)}}{\text{Content of non-labile C (g/kg soil)}}$$

Data were subjected to analysis of variance (ANOVA) in a completely randomized block design. Mean separation

Table 1 Treatment details applied to rice and wheat crops

Treatment reference	Treatment acronym		Nutrient applied (kg/ha/yr) to	
	Rice	Wheat	Rice	Wheat
CK	P ₀	P ₀	120 N + 30 K ₂ O	120N + 30 K ₂ O
F ₀ P ₀	P ₀	P ₆₀ SSP	120 N + 30 K ₂ O	120N + 60 P ₂ O ₅ + 30 K ₂ O
F ₀ P ₃₀ RP	P ₃₀ RP	P ₆₀ SSP	120 N + 30 P ₂ O ₅ (RP) +30 K ₂ O	120N + 60 P ₂ O ₅ + 30 K ₂ O
F ₀ P ₃₀ SSP	P ₃₀ SSP	P ₆₀ SSP	120 N + 30 P ₂ O ₅ (SSP) +30 K ₂ O	120N + 60 P ₂ O ₅ + 30 K ₂ O
F ₁ P ₀	FYM	P ₆₀ SSP	120 N + 30 K ₂ O + 10 t FYM	120N + 60 P ₂ O ₅ + 30 K ₂ O
F ₁ P ₃₀ RP	FYM+P ₃₀ RP	P ₆₀ SSP	120 N + 30 P ₂ O ₅ (RP) +30 K ₂ O + 10 t FYM	120 N + 60 P ₂ O ₅ + 30 K ₂ O

CK=Control, RP=Rock phosphate, SSP=Single super phosphate, FYM=Farmyard manure.

for different treatments was evaluated at 95% confidence interval using the Duncan's multiple range test (DMRT). Statistical and correlation analysis were performed with SPSS for Windows 16.0 (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

Soil organic carbon fractions

Plots treated with FYM and fertilizer-P either alone or in combination significantly increased TOC and WEOC in

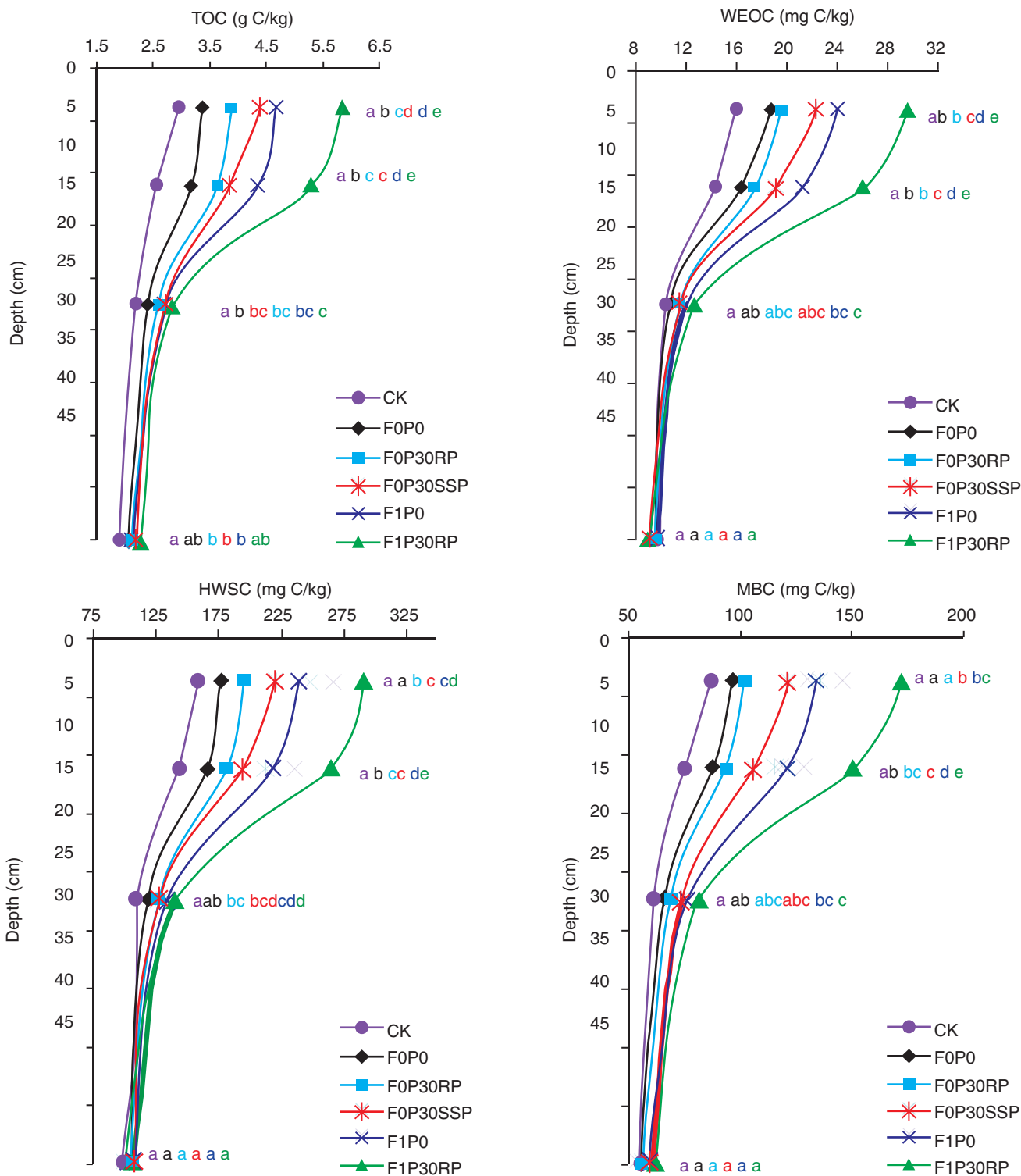


Fig 1 Effect of amount and source of P application on profile distribution of total organic carbon (TOC), water extractable organic carbon (WEOC), hot water soluble carbon (HWSC) and microbial biomass carbon (MBC) in soil after 7 cycles of rice-wheat cropping. Mean values for a soil property at a given depth followed by different letters differ significantly (P<0.05) by Duncan's multiple range test (DMRT)

surface (0-7.5 cm) and subsurface (7.5-15 cm) soil (Fig 1). Compared to CK, fertilizer-P along with FYM applications increased TOC by 97%. Fertilizer application stimulates crop biomass production and increases the amount of residue retained in the soil and this enhances C accumulation in the soil. An improvement in TOC in soil with FYM application may also be because of additional C input through manure. Adoption of integrated nutrient management and FYM application was reported to significantly increase SOC concentration in surface soil under rice-wheat system (Benbi *et al.* 2015b). Under chickpea-wheat cropping system, Deshpande *et al.* (2015) reported 41-51% increase in SOC with the application of RP along with fresh cow dung and phosphorus solubilising bacteria. Fertilizer-P application to wheat at 60 kg P₂O₅/ha (F₀P₀) resulted in 14.3% increase in TOC in surface soil (0-7.5 cm), compared to CK. Application of fertilizer-P to both the crops through SSP (F₀P_{30SSP}) significantly increased TOC in 0-7.5 cm soil compared to its application through RP (F₀P_{30RP}). Compared to RP application of fertilizer-P through SSP to both the crops in the sequence increased TOC. It may be because of higher availability of P to plant roots when P is applied through SSP than RP. This could have resulted in increased root growth and biomass leading to greater C return to the soil. Akande *et al.* (2011) reported greater P availability in SSP treated soils compared to rock phosphate treated soils, because of higher solubility of SSP. Application of FYM in combination with P fertilizer (F₁P_{30RP}) to rice significantly increased TOC concentration by 24.6% over FYM application alone (F₁P₀). Beneficial effects of fertilizers and manure application on TOC was similar in the sub-surface soil (7.5-15 cm).

Water extractable organic C (WEOC), which represents an array of molecules in a soluble phase that remain in equilibrium with solid SOC, responded to rates and sources of P application (Fig 1). Compared to application of FYM alone (F₁P₀), conjoint application of FYM and fertilizer-P (F₁P_{30RP}) significantly increased WEOC by 23.3%. Plots treated with fertilizer-P during both the crops through SSP (F₀P_{30SSP}) showed 14.3% increase in WEOC than treated with RP (F₀P_{30RP}). On an average, WEOC comprised ~0.5% of TOC concentration in the soil. In 15-30 cm soil, application

of FYM either alone or in combination with fertilizer-P significantly increased WEOC compared to unfertilized control. A significant increase (16-70%) in WEOC in the surface soil as a result of 11-years of rice-wheat cropping with NPK plus FYM and rice straw application has earlier been reported (Benbi *et al.* 2015b). In 30-60 cm soil depth, all the treatments exhibited similar WEOC concentration. A higher increase in WEOC in FYM amended plots compared to chemical fertilizers applications indicates that added organic manure contained much soluble organic matter (Chantigny *et al.* 2002).

Fertilizer-P and FYM application either alone or in combination to both the crops led to significant increase in HWSC compared to CK (Fig 1). Hot water soluble C was significantly higher in soils receiving FYM along with fertilizer-P (F₁P_{30RP}), compared to soils receiving FYM alone (F₁P₀). Application of P through SSP (F₀P_{30SSP}) significantly increased HWSC by 12.8% over F₀P_{30RP} treatment. On an average, HWSC comprised ~5.1% of TOC in soil and it was 10-times higher than the WEOC concentration (Fig 1). Considerably higher amount of HWSC was due to the fact that hot water extracted not only microbial biomass-C but also root exudates, soluble carbohydrates and amino acids (Gregorich *et al.* 2003). Carbon bound to soil enzymes may also be extracted because most of soil enzymes are denatured at 80 °C. Application of inorganic fertilizers either alone or in combination led to significant increase in HWSC probably because of greater plant mediated C input (Liang *et al.* 2012, Benbi *et al.* 2015b).

Microbial biomass C plays an important role in nutrient cycling and was recognized as a component of active soil organic matter that helps in maintaining function and sustainability of terrestrial ecosystems (He *et al.* 2003). In surface soil (0-7.5 cm), application of P through SSP significantly increased MBC by 19.2% over rock phosphate (RP) treatment (Fig 1). Plots receiving FYM along with fertilizer-P (F₁P_{30RP}) showed 28.2% higher MBC than those receiving only FYM (F₁P₀). In sub-surface soil (7.5-15 cm), the highest concentration (151 mg/kg) of soil MBC was observed in FYM and RP treated plots and the lowest (75 mg/kg) in control plots. Soil MBC comprised 2.8% of TOC concentration in the plough layer. This concentration is in

Table 2 Effect of amount and source of P application on KMnO₄-C (g/kg), non-labile C (g/kg), lability index (LI), soil carbon pool index (CPI) and carbon management index (CMI) in the surface (0-7.5 cm) and sub-surface (7.5-15 cm) soil after 7 cycles of rice-wheat cropping

Treatment	KMnO ₄ -C		Non labile-C		Lability		LI		CPI		CMI	
	Depth (cm)											
	0-7.5	7.5-15	0-7.5	7.5-15	0-7.5	7.5-15	0-7.5	7.5-15	0-7.5	7.5-15	0-7.5	7.5-15
CK	0.319a [†]	0.305a	2.62a	2.26a	0.122	0.135						
F ₀ P ₀	0.388a	0.356a	2.97a	2.80a	0.131	0.127	1.07	0.94	1.14	1.23	122.4	116.1
F ₀ P _{30RP}	0.524b	0.472b	3.36b	3.16b	0.156	0.149	1.28	1.11	1.32	1.41	169.1	156.9
F ₀ P _{30SSP}	0.568b	0.512b	3.82c	3.35b	0.149	0.153	1.22	1.14	1.49	1.50	182.3	171.0
F ₁ P ₀	0.685c	0.582bc	3.98c	3.76bc	0.172	0.155	1.41	1.15	1.59	1.69	224.3	194.5
F ₁ P _{30RP}	0.805d	0.682c	5.01d	4.61c	0.161	0.148	1.32	1.10	1.98	2.06	260.9	226.5

[†]Mean values within a column followed by different letters differ significantly (P<0.05) by Duncan's multiple range test (DMRT)

Table 3 Correlation matrix depicting relationship among different soil carbon pools and soil properties

Variable	KMnO ₄ -C	WEOC	HWSC	TOC
WEOC	0.97**			
HWSC	0.98**	0.99**		
TOC	0.98**	0.99**	0.99**	
MBC	0.95**	0.99**	0.99**	0.99**

*Correlation is significant at $P < 0.05$ level (2-tailed),
 **Correlation is significant at $P < 0.01$ level (2-tailed)

the range (1-5% of TOC) reported by several researchers. The results showed that regular application of FYM and RP to the soil improved soil biological activity and labile pools of TOC.

Beneficial effects of different treatments on TOC and its labile fractions were higher in surface (0-7.5 cm) soil and decreased with depth (Fig 1). It is because of the fact that C input through root biomass and leaf litter as well as applied FYM is mainly limited to the soil plough layer (0-15 cm). In 15-30 cm soil, compared to CK, FYM along with fertilizer-P (F_1P_{30RP}) application increased TOC, WEOC, HWSC and MBC significantly by 32.3, 23.0, 28.1 and 34.5%, respectively. However, all the treatments exhibited similar WEOC, HWSC and MBC at 30-60 cm soil depth. Farmyard manure along with RP application besides being source of organic matter, results in greater crop mediated C input to the soil through enhanced crop yield (Benbi 2015).

In 0-7.5 cm and 7.5-15 cm soil depths, fertilizer P and FYM application either alone or in combination to rice increased KMnO₄-C by 64-152% than CK (Table 2). The KMnO₄-C increased by 17.5% in soils receiving fertilizer-P and FYM (F_1P_{30RP}) over plots receiving FYM alone (F_1P_0). Moharana *et al.* (2012) reported that FYM at 20 Mg/ha/yr application either alone or in conjunction with NPK significantly increased KMnO₄-C over control. In sub-surface soil (7.5-15 cm), effect of different treatments was similar to that observed in 0-7.5 cm soil. However, the KMnO₄-C did not differ significantly between treatments F_0P_{30SSP} , F_1P_0 and F_1P_{30RP} (Table 2). The effect of different treatment decreased with depth and the treatments did not differ significantly at 30-60 cm soil depth (Data not shown).

In surface soil (0-7.5 cm), application of fertilizer P through SSP (F_0P_{30SSP}) increased non-labile C by 13.6% over RP treated (F_0P_{30RP}) plots (Table 2). Significantly higher (25.9%) non-labile C was observed in F_1P_{30RP} than F_1P_0 treatment. In surface (0-7.5 cm) and sub-surface (7.5-15 cm) soils, non-labile C was the highest in plots amended with fertilizer-P along with FYM (F_1P_{30RP}) and the lowest in control. The C lability, lability index (LI), C pool index (CPI) and C management index (CMI) also improved considerably with fertilizer-P application along with FYM at both the soil depths (Table 2). Carbon management index (CMI) followed the order $F_1P_{30RP} > F_1P_0 > F_0P_{30SSP} > F_0P_{30RP} > F_0P_0 > CK$. Higher concentration of non-labile C in treatments involving FYM application shows the stabilization of organic C in soil. This could be due to the application of already

stabilized material in soil through FYM.

Correlation among carbon pools

Correlation matrix showed positive relationship among soil C pools. Highly significant positive correlation among TOC, KMnO₄-C, WEOC, HWSC and MBC suggests a dynamic relationship among various C pools (Table 3). Benbi *et al.* (2015b) also reported significant correlation among TOC, HWSC, MBC and WEOC.

The results of the study showed that beneficial effects of RP were lower than SSP. However, integrated use of RP and FYM brought about greater increase in TOC compared to SSP application alone. Irrespective of the treatment, the effect on soil organic C pools was higher in surface (0-7.5 cm) soil, which decreased with depth. The KMnO₄-C was one of the largest pool of soil organic C (13.6% of TOC) and WEOC was the smallest one (0.51% of TOC). Carbon management index (CMI) also improved considerably with fertilizer-P and FYM applications in surface and subsurface soils, indicating greater C stabilization in soil. Thus application of RP with FYM could be a sustainable management option for crop production. Combined application of FYM and RP improved soil organic C pools to a greater extent than their individual applications, suggesting the need for their integrated use.

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