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Labile carbon fractions build-up and dynamics under vertical stratification of *Populus deltoides* and *Eucalyptus tereticornis* based agroforestry systems in Trans-Gangetic Plains of India

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Abstract

Populus deltoides and *Eucalyptus tereticorn*is based agroforestry systems are credited for stocking significant amounts of soil carbon and hence have the potential to mitigate climate change. In this study, four different land uses were compared in terms of labile carbon fractions build up in varying soil depths. Soil organic carbon under 4-year *Populus* and *Eucalyptus* based agroforestry system was 22.9 and 6.30% higher, respectively than sole wheat crop. Microbial biomass carbon under 4-year *Populus* and *Eucalyptus* based agroforestry system was 10.6 and 12.3 % higher than sole wheat crop. Permanganate oxidizable carbon under 4-year *Populus* and *Eucalyptus* based agroforestry system was 4.4 and 23.1% higher than sole wheat crop. In *Populus* and *Eucalyptus* based agroforestry systems, significant positive correlations were recorded between POXC and MB-C (r=0.809** and 0.889**), POXC and SOC (r=0.903** and 0.793**) and SOC and MB-C (r=0.765** and 0.852**), respectively. The relationship between POXC and SOC under *Populus* (4-year) + wheat (R^2 = 0.845) had stronger relationship than *Eucalyptus* (4-year) + wheat (R^2 = 0.712). This study demonstrated the usefulness of POXC in assessing changes in the labile soil C pools quickly and inexpensively under *Populus* and *Eucalyptus* based agroforestry systems in Trans-Gangetic plains of India.

Key words: Permanganate oxidizable carbon, microbial biomass carbon, soil organic carbon, soil carbon stock, *Populus deltoides, Eucalyptus tereticornis.*

In past three decades, *Populus* and *Eucalyptus* based agroforestry systems have been exploited as a viable option for mitigating climate change, improving soil health and increasing farm productivity. *Populus* and *Eucalyptus* are widely planted tree species for short-rotation plantations due to their fast growth and high yield throughout the world especially in the tropics and subtropics including India. Trans-Gangetic plains (TGP) of India (Punjab, Haryana and parts of Uttar Pradesh) offer favourable growing conditions, good rainfall and water resources and ample sunshine for faster growth of the trees. About 0.36 and 7.5 million hectare of land is currently under *Populus* and *Eucalyptus* plantation in India, respectively. The incorporation of trees in agricultural systems has earned the name "Islands of fertility", due to the ability of trees to improve soil fertility and yield in agricultural systems (Pinho *et al.*, 2012). Increased soil fertility serves as an indicator of soil carbon build-up in agroforestry systems. In the past decades, most studies focused on the effects of forest conversion to agricultural land on soil properties, such as total SOC and other physicochemical properties (Schroth *et al.*, 2002). Total SOC is heterogeneous, and consists of different functional and biological pools with different turnover rates; labile carbon fractions are more sensitive to short-term vegetation or land-use change (von Lützow *et al.*, 2007). Estimating labile SOC is more laborious and costly than measuring total SOC. Each C component has a different residence time in soil and performs different functions. Reactive carbon (RC), also known as permanganate oxidizable carbon (POXC), is a fraction of the SOM pool that is oxidizable in the presence of potassium permanganate in solution. Carbon oxidized by this compound includes the C most readily degradable by microorganisms as well as that bound to soil minerals, making RC interpretation somewhat difficult. The microbial biomass accounts for only 1-3 % of soil organic C but it is the eye of the needle through which all organic material that enters the soil must pass (Jenkinson and Ayanaba, 1977). In the C-limited soil system, available C inorganic materials entering the soil is the driving force behind these processes but other essential nutrient elements (particularly N, P, K) are also involved. Soil being a major contributor to the carbon mitigation technology had various constrains, such as soil salinity and sodicity (Lal 2009, Singh Dagar, 2009, Felker *et al.*, 2009).

Despite reports on positive correlations between POXC and other soil biologically mediated C fractions, there remains a lack of understanding regarding which C fractions closely reflects POXC. Moreover, little is known about how these relationships might change due to land use and soil depth. Finally, there is a lack of understanding regarding the sensitivity of POXC in reflecting changes in management relative to other SOC measures. Hence this study aimed to address all three of these knowledge gaps by examining the relationships between POXC and various soil C fractions (MBC and SOC) over a wide diversity of soil types, land uses and geographic areas across the TGP. It was hypothesized that POXC would be closely associated with the other measured soil C fractions and that POXC would be a sensitive and consistent indicator of changes due to management or environmental variation. Despite limited reports of positive correlations with MBC, SOC and other soil C fractions, little is known about what soil fractions POXC most closely reflects. With these facts, the objective of this paper was to (i) quantify MBC, POXC and SOC under different land use and soil depth, and (ii) determining the relationship between MBC and SOC, POXC and MBC and POXC and SOC fractions under *Populus* and *Eucalyptus* based agroforestry systems.

Materials and Methods

The study was conducted for estimation of soil labile carbon fraction in 4-year *Populus* deltoides and *Eucalyptus* tereticornis agroforestry systems at Hara Farm, Yamunanagar (30°7'55"N, 77°22'34"E, 255 m above sea level) and Raina Farm, Kurukshetra (29° 57' 30" N, 76° 59' 40"E, 257 m above sea level), respectively, districts of Haryana state of India. Yamunanagar and Kurukshetra have sub-tropical continental monsoon climate and experience extreme conditions. May and June are the hottest months, while December and January are the coldest. The average annual rainfall of Yamunanagar and Kurukshetra districts is 1107 and 800 mm, respectively. Soil texture of the Hara Farm was sandy loam, whereas at Raina Farm it was old alluvium (Banger series) which is mainly sandy loam, loam and light loam and slightly calcareous in nature is present. Initial physicochemical properties of the soil (0-15 cm) at Hara Farm and Raina Farm showed neutral pH (7.73 and 8.31), low organic C content (0.35 and 0.28%) and low alkaline $KMnO_4$-N (204.4 and 180.3 kg/ha), high/medium Olsen's (0.5 M $NaHCO_3$ extractable) P (57.3 and 21.6 kg/ha) and medium/high 1N ammonium acetate extractable K (234.3 and 338.1 kg/ha), medium/low DTPA extractable Zn (1.21 and 0.58 ppm), high DTPA extractable Mn (110.5 and 82.2 ppm) and Cu (1.27 and 0.71 ppm) with bulk density of 1.38 and 1.47 g/cm^3, respectively.

Soil samples were randomly collected from 3 different places at 7 depths (0-15, 15-30, 30-45, 45-60, 60-75, 75-90 and 90-105 cm) in the each land use (agroforestry system and sole wheat) after wheat harvest. At each sampling point, samples were collected below the tree canopy and outside the tree canopy in 4 cardinal directions around the tree. The samples were mixed to obtain a composite sample for each depth. In total, 84 samples (4 land use × 7 depths × 3 replicates) were collected. Soil samples were analyzed for SOC by the Walkley and Black (1947) method. Bulk density was determined using metal core samplers of 4.0 cm in height and 5.0 cm in internal diameter at all the depths studied. Samples were then oven-dried separately at 105 °C for 48 h. Bulk density of soil was calculated by dividing oven-dried weight of the sample by the volume of core sampler. The amount of carbon stored per hectare was obtained by multiplying the values of soil depth (cm), bulk density (g/cm^3), and the percentage of SOC content.

Soil microbial biomass carbon was determined using the fumigation extraction methods (Vance *et al.*, 1987). The sieved, field moist soil sub-samples (equivalent to 50 g oven dry soil) were fumigated with alcohol free chloroform in vacuum desiccators and stored in the dark for 24 h. After removing the fumigant (by repeated de-evacuation of chloroform from the soils), the samples were extracted with 200 ml 0.5M K_2SO_4 for 30 minute on a shaking machine. The unfumigated soil samples were extracted similarly at the start of experiment. The filtered soil extracts of both fumigated and unfumigated samples were analyzed for organic C using the acid dichromate method. The microbial biomass carbon was estimated by multiplying by 2.64 with EC (extractable carbon), where EC is the difference between carbon extracted from fumigated and unfumigated samples both expressed in the same measurement unit (Vance *et al.*, 1987).

Permanganate oxidizable carbon analyses were based on method developed by Weil *et al.* (2003). Briefly, 2.5 g of air-dried soil was weighed into polypropylene 50-mL screw-top centrifuge tubes. To each tube, 18 mL of deionized water and 2 mL of 0.2 M $KMnO_4$ stock solution were added and tubes were shaken for exactly 2 min at 240 oscillations per minute on an oscillating shaker. Tubes were removed from the shaker and allowed to settle for exactly 10 min. After 10 min, 0.5 mL of the supernatant were transferred into a second 50-mL centrifuge tube and mixed with 49.5 mL of deionized water. An aliquot (200 µ L) of each sample was loaded into a 96-well plate containing a set of internal standards, including a blank of deionized water, four standard stock solutions (0.00005, 0.0001, 0.00015,and 0.0002 mol/l $KMnO_4$), a soil standard and a solution standard. All internal standards were analytically replicated on each plate. Sample absorbance was read with Spectra Max M5 using Softmax Pro software (Molecular Devices, Sunnyvale, CA) at 550 nm. POXC was determined using following equation:

$$\text{POXC (mg/kg soil)} = [0.02 \text{ mol/l} - (a+b\times \text{Abs})] \times (9000 \text{ mg C/mol}) (0.02 \text{ L solution})$$

where, 0.02 mol/l is the concentration of the initial $KMnO_4$ solution, 'a' is the intercept and 'b' is the slope of the standard curve, Abs is the absorbance of the unknown soil sample, 9000 mg is the amount of C oxidized by 1 mol of MnO_4 changing from Mn^{7+} to Mn^{4+}, 0.02 L is the volume of $KMnO_4$ solution reacted, and Wt is the mass of soil (kg) used in the reaction.

All the data were subjected to variance analysis using statistical software (SAS Version 9.3, SAS Institute Inc., Cary, NC, USA). The mean pair wise comparisons were based on the Duncan multiple range test (DMRT). Correlation analysis was performed to determine the relationship between the traits using the Pearson coefficient procedure.

Results and Discussion

Labile carbon fractions under different land use systems

Labile soil C fractions, such as MB-C, POXC and SOC are important indicators of changes in soil ecosystems brought about by management practices (land use change). Soil microbial biomass acts as both a pool of labile nutrients and the agent of decomposition of organic materials in soil, and can regulate flow of nutrients and energy in soils. It is also typically sensitive to small changes in soil conditions and provides early information on changes in soil organic matter. Across 105 cm soil depth MB-C was higher by 10.6 and 12.3 % under *Populus* and *Eucalyptus* based agroforestry systems than sole wheat crop (Table 1). Increased soil MB-C following a land-use change from croplands to agroforestry systems may be caused by enhanced plant residue inputs and improved understory

Table 1. Comparative study of labile carbon fractions under different land use systems across 105 cm soil depth

Source of variance	Degree of freedom	Analysis of variance (MSS)		
		MB-C (μg g^{-1})	POXC (mg kg^{-1})	SOC (%)
Replication	2	271	0.018	0.005
LUS	1	6512**	6708**	0.009*
Depth (D)	6	18931**	356804**	0.047*
LUS × D	6	389	34129**	0.003*
Error	26	235	20.9	0.005
	Comparison of Sole wheat with *Populus* (4 year) + wheat in terms of labile carbon			
Land use system		MB-C (μg g^{-1})	POXC (mg kg^{-1})	SOC (%)
Sole wheat		305.4b	547.6b	0.35b
Populus (4 year) + wheat		337.7a	674.3a	0.43a
CD (0.05%)		5.4	3.5	0.03
	Comparison of sole wheat with *Eucalyptus* (4 year) + wheat in for of labile carbon			
Sole wheat		202.3b	574.8b	0.32a
Eucalyptus (4 year) + wheat		227.2a	600.1a	0.34a
CD (0.05%)		9.7	2.9	0.04

Means with at least one letter common are not statistically significant ($p <0.05$) using DMRT LUS : Land use system

microclimate (Lee and Jose, 2003). Previous studies conducted in temperate agroforestry systems in North America (Kremer and Kussman, 2011) and under tropical conditions (Singh and Singh, 1995) reported the similar results. Among labile soil C fractions, MB-C under *Populus* and *Eucalyptus* based agroforestry system showed significant positive correlation with SOC (r=0.765 and 0.852) and POXC (r=0.889 and 0.809), respectively (Table 2). POXC under *Eucalyptus* based agroforestry system showed significantly higher positive correlation with SOC (r=0.903**).

Here, we presume if POXC can serve as an indicator of changes in management or other experimental factors, in a similar manner as SOC and MBC. POXC under 4-year *Populus* and *Eucalyptus* based agroforestry system was statistically higher (by 23.1 and 4.40 %) than sole wheat crop (Table 1). Blair *et al.* (1997) also reported that the labile carbon estimated by the $KMnO_4$ oxidation technique was extremely sensitive to soil management.

The carbon fixed by plant is the primary source of organic matter inputs in the soil both

Table 2. Pearson's correlation coefficients for association in *Populus* and *Eucalyptus* based agroforestry systems upto 105 cm soil depth

	Populus (4 year) + wheat based agroforestry system		
Soil parameters	MB-C (μg g^{-1})	POXC (mg kg^{-1})	SOC (%)
MB-C (μg g^{-1})	1	0.889**	0.765**
POXC (mg kg^{-1})		1	0.793**
SOC (%)			1
	Eucalyptus (4 year) + wheat based agroforestry system		
Soil parameters	MB-C (μg g^{-1})	POXC (mg kg^{-1})	SOC (%)
MB-C (μg g^{-1})	1	0.809**	0.852**
POXC (mg kg^{-1})		1	0.903**
SOC (%)			1

POXC= Permanganate oxidizable carbon; MB-C= Microbial biomass carbon; SOC= Soil organic carbon from above and below ground components of plants. A large fraction of the terrestrial above ground biomass finds its way to the soil surface in the form of litter fall. The organic carbon through litter, pruning material and roots of crop and trees increased the organic build-up in the surface layer. SOC under 4-year *Populus* and *Eucalyptus* based agroforestry system was higher by 22.9 and 6.30 % than sole wheat crop but the increment was statistically at par in case of *Eucalyptus* based agroforestry system. Similar observations have been reported earlier (Singh and Sharma, 2007). This may be due to sodic nature of the soil under *Eucalyptus* based agroforestry. Trees in association with agricultural crops increase the soil carbon status and the change depends upon the quality and quantity of litter input, decomposition, carbon release, *etc.* (Gera *et al.*, 2006).

The changes in micro-climate under tree canopy (soil moisture, temperature, light, humidity, *etc.*), proliferation of root system and enhanced biological activity also favour the carbon stock in the soil. However, the continuity in the system is essential; otherwise the build-up in organic carbon may revert back to its original position under commercial agriculture.

Effect of different land uses under varying soil depth on Soil C stock, POXC and MBC

In general soil carbon stock, MB-C and POXC decreased with soil depth. *Populus* (4 year) + wheat had 30.8 % higher soil carbon stock as compared to sole wheat crop (Fig. 1). Whereas, *Eucalyptus* (4 year) + wheat had 29.3 % higher soil carbon stock as compared to sole wheat crop (Fig. 2). The increased soil carbon at subsoil layer could be due to addition of organic matter through fine and coarse roots and their fast turn over. The increase in soil carbon at the surface soil layer is attributed to greater carbon input from litter fall, dead roots, and root exudates (Kaushal Belt, 2012). Soil organic C stock in agroforestry system was 65-88% higher than rice-wheat system (Benbi *et al.*, 2012), even MB-C have also been reported higher in poplar based agroforestry system than rice-wheat rotation. When *Populus* and *Eucalyptus* based agroforestry system was compared with sole wheat, 8.5 and

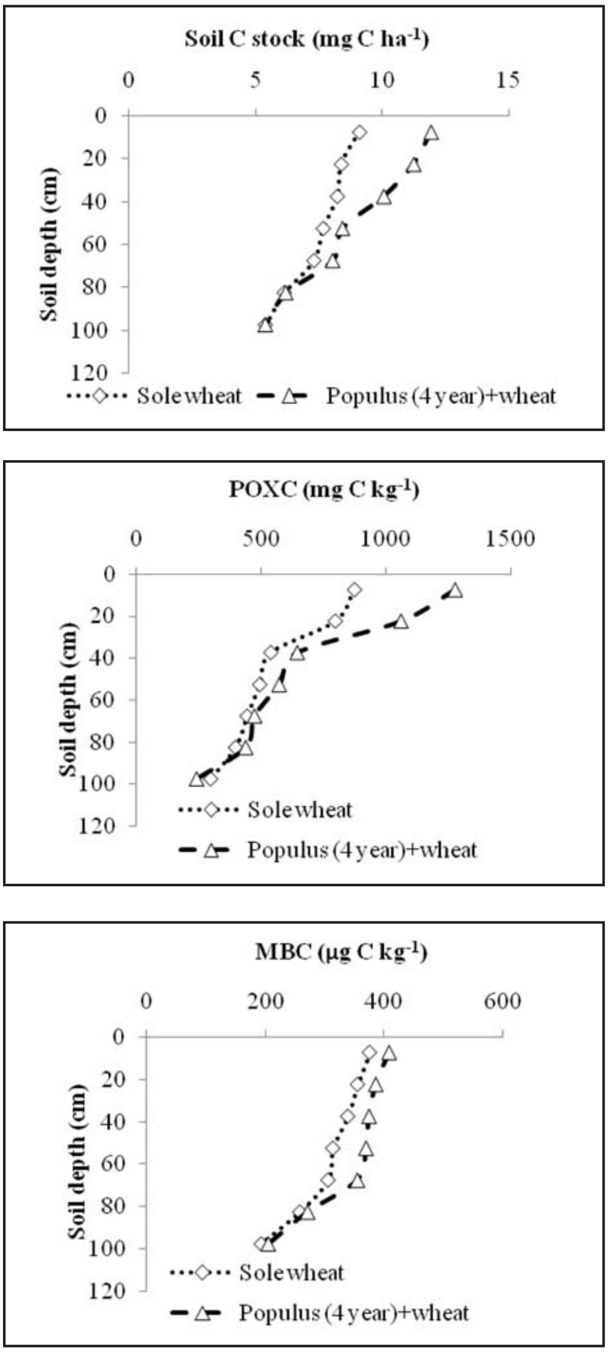

Fig. 1. Effect of *Populus* based agroforestry system on soil carbon stock, permanganate oxidizable carbon and microbial biomass carbon in different soil depths.

36.6 % increment, respectively, in MB-C was recorded (Fig. 1 and 2). The increased MB-C observed may be explained by the increase in carbon available for microorganisms derived

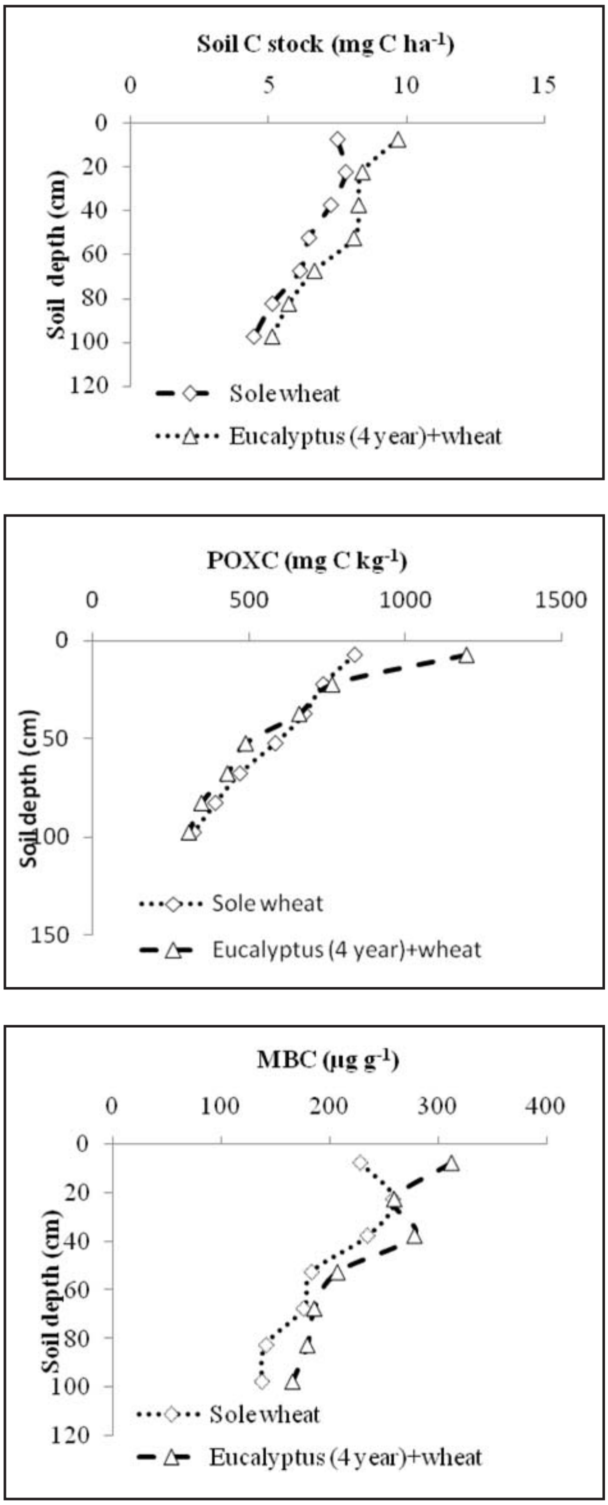

Fig. 2. Effect of *Eucalyptus* based agroforestry system on soil carbon stock, permanganate oxidizable carbon and microbial biomass carbon in different soil depths.

from rhizodeposition and from the high quality litter. These results are in agreement with other studies evaluating the effects of agricultural land conversion to *Populus*, Quercus and Salix plantations on MB-C (Kahle *et al.*, 2010). When *Populus* and *Eucalyptus* based agroforestry systems were compared with sole wheat, 46.5 and 43% increment in POXC was observed (Fig. 1 and 2). POXC may be more useful where, soil C changes markedly (Blair *et al.*, 1995), but in this study it had the same efficacy as total soil C concentration for detecting differences induced by land use change and soil depth. Mendham *et al.* (2002) also found that permanganate oxidizable C was closely related to total C.

Correlation studies

Microbial biomass carbon and soil organic carbon

MB-C and SOC were positively corelated in both *Populus* and *Eucalyptus* based agroforestry systems (Fig. 3 and 4), however, the relationship was comparatively stronger for *Populus* (4-year) + wheat (R^2 = 0.718) than *Eucalyptus* (4-year) + wheat (R^2 = 0.592). These results conform to the findings of Anderson *et al.* (2008).

Permanganate oxidizable carbon and microbial biomass carbon

Permanganate oxidizable carbon was positively correlated with MBC, although the strength of the relationship varied with the land use (Fig. 3 and 4). Eucalyptus (4-year) + wheat (R^2 = 0.796) had stronger relationship than Populus (4-year) + wheat (R^2 = 0.606). Since both MBC and POXC are based on chemical extractions of labile soil C, the agreement between these two methods is logical. Other study has also reported positive correlations between POXC and MBC (Culman *et al.*, 2010).

Permanganate oxidizable carbon and soil organic carbon

Permanganate oxidizable carbon and SOC were positively correlated with each other in both agroforestry systems studied (Fig. 3 and 4). The relationship between POXC and SOC was comparatively stronger than POXC with MBC. *Populus* (4-year) + wheat (R^2 = 0.845) had stronger

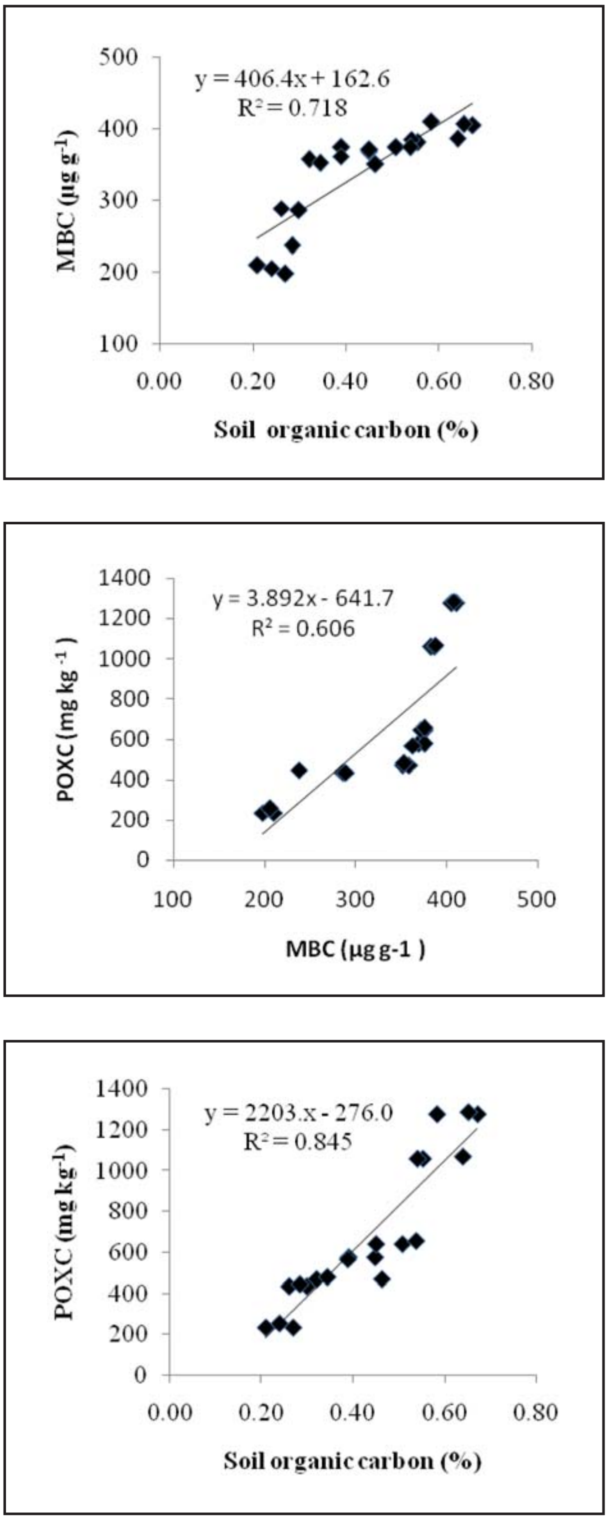

Fig. 3. Relationship between different labile forms of carbon under *Populus deltoides* based agroforestry systems

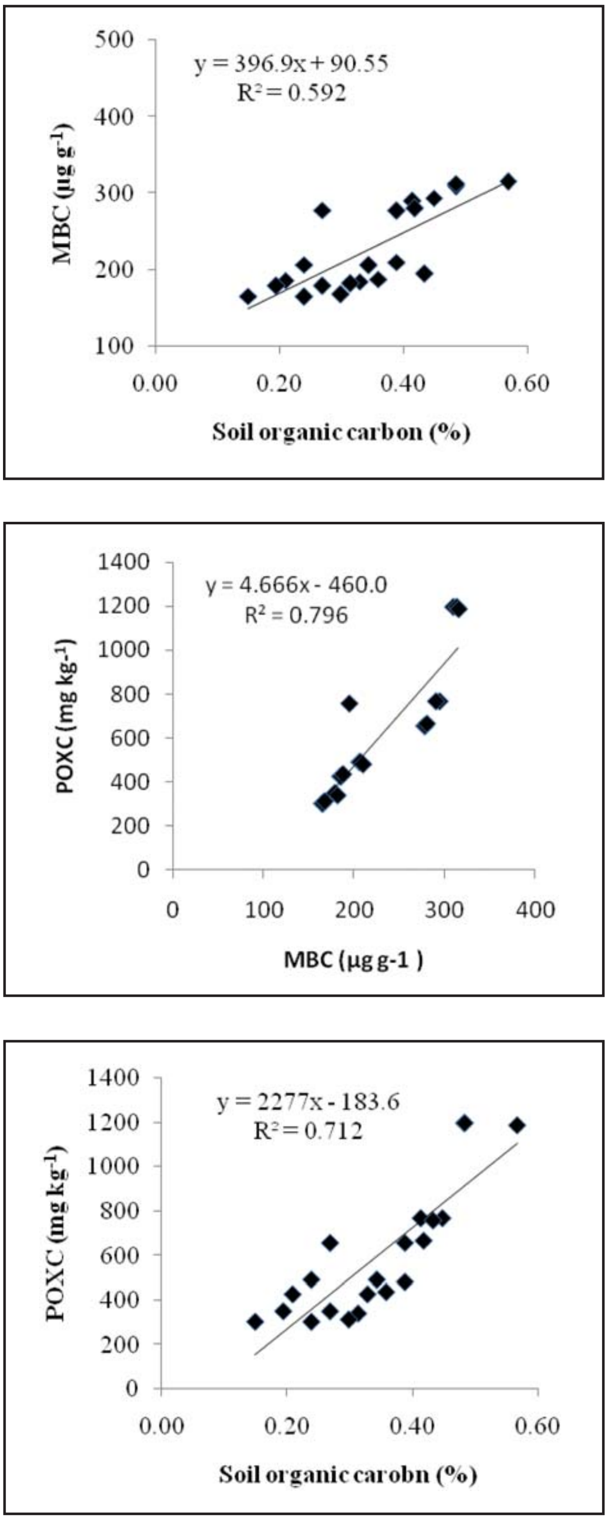

Fig. 4. Relationship between different labile forms of carbon under *Eucalyptus tereticornis* based agroforestry systems

relationship than *Eucalyptus* (4-year) + wheat (R^2 = 0.712). A strong positive relationship between POXC and SOC has previously been reported (Jokela Keltikangas, 2009). The relationship may be stronger due to similar methodologies of POXC and SOC, as SOC relies on complete oxidation of all soil C, while POXC relies on partial oxidation of the C pool.

Conclusion

Populus (4 year) + wheat and *Eucalyptus* (4 year) + wheat had 30.8 % and 29.3 % higher soil carbon stock, respectively, than sole wheat crop. The study concludes that *Populus*-wheat and *Eucalyptus*-wheat agroforestry is a better option than the sole cropping, not only for carbon mitigation but also for sustainable productivity and higher profits to the growers. However, it is essential to continue with the system otherwise the benefits gained in-terms of carbon sequestration in the system would revert back to the original state.

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