Deo Kumar
Department of Soil Science and
Agricultural Chemistry, Uttar
Banga Krishi Viswavidyalaya,
Cooch Behar, West Bengal, India

Munmun Majhi
Department of Soil Science and
Agricultural Chemistry, Uttar
Banga Krishi Viswavidyalaya,
Cooch Behar, West Bengal, India

Quantitative assessment of water soluble fractions of organic carbon in deep soils under rice and non-rice ecology

Deo Kumar and Munmun Majhi

Abstract
Soil is the largest terrestrial sink of Carbon (C). Carbon sequestration in soil is important for soil quality as well as to mitigate CO_2 loading in atmosphere. Study on C of surface soil layer is going on for long time. Only in the recent past, scientists have noticed the importance of subsoil as a store house of stable C. On the contrary, study of C dynamics in tropical rice soil is important in countries like India where rice is the predominant crop and soil C sequestration is at risk due to high temperature. In this context, this study tried to understand the dynamics of soil C in deep soil under rice and non-rice ecology. Three distinct long term experimental sites were selected for soil sampling from three eastern states of India. Results indicated high total C and total organic C in surface soil in comparison to subsoil. On the other hand, rice soils had higher C than non-rice soil. The soil C was further divided into labile and recalcitrant pools using water extraction. As per water solubility, water soluble (room temperature) and hot water soluble C was highest in surface soil. This is natural as these pools represent labile C and surface soil receives maximum fresh C input in terms of deposited leaf and litter. The remaining C, not dissolved in water, was considered as recalcitrant C. While considering the water soluble pools as well as recalcitrant C as % of total organic C, trend indicated more labile C (water soluble) in surface soil while subsoil has more recalcitrant C. Therefore, this study conclusively indicated the potential of subsoil layer to act as a C sink in comparison to surface soil. The rice soil also has been identified as a niche for soil C sequestration.

Keywords: Carbon sequestration, deep soil, labile C, recalcitrant C, rice soil

1. Introduction
Soil organic matter (SOM) is considered to be the key factor of soil quality (Friedal, 2000) [11] and an important indicator of soil productivity (Frageria, 2012) [10]. Plant litter is the primary source of SOM while microbial residues are secondary sources (Kögel-Knabner, 2002; Krull *et al*., 2003) [21, 22]. Soil organic carbon (SOC) is the chief constituent of SOM and plays a pivotal role in the global carbon (C) cycle and climate change (Lal and Kimble, 2000) [23]. Organic C is considered as basis of life in the soil as it acts as the primary source of energy for the soil microbes (Steenwerth *et al*., 2002) [44]. Thus it has a direct influence on the microbial as well as enzymatic activities occurring in the soil (Srivastava and Singh, 1991) [43]. The SOC controls many important soil physical properties like porosity, aeration, bulk density, aggregate stability (i.e. soil structure), water holding capacity (Chenu *et al*., 2000; Watts *et al*., 2006; Wendling *et al*., 2010; Powlson *et al*., 2011) [5, 49, 50, 33]. The chemical properties of soil are also very much related to the quality and quantity of organic C in soil (McCarthy, 2001) [29]. Organic C takes a crucial role in the soil ion exchange phenomena, retention and exchange of ions, specially the micronutrients (Lal *et al*., 2015) [24]. Release of various types of chemicals during the decomposition of SOC also controls the chemical properties of the soil (Rowsell *et al*., 2004; Singh *et al*., 2010). Thus, it can be said that the presence of organic C in soil is a key determinant for soil quality and productivity (Deb *et al*., 2015) [8], maintaining soil tilth, fertility as well as sustainability of the soil (Singh *et al*., 2010).
Soil C sequestration implies, "process of transferring CO_2 from the atmosphere into the soil of a land unit through its plants" (Lal *et al*., 2015) [24]. Soils are the largest global sink of C (Montagnini and Nair, 2004). Almost two-third C of all the ecosystems is retained by it (Schimel *et al*., 1994) [40].

Corresponding Author:
Deo Kumar
Department of Soil Science and
Agricultural Chemistry, Uttar
Banga Krishi Viswavidyalaya,
Cooch Behar, West Bengal, India

Thus emphasis is being laid on the various agricultural practices which can help the soil to stabilize and increase its SOC level (Wang *et al*., 2014) [48]. Although surface soil stores higher amount of organic C, the "deep" subsoil also stores a significant amount of it (Richter and Markewitz, 1995; Rumpel and Kögel-Knabner, 2011) [37, 38]. A recent study conducted in the northern circumpolar permafrost region suggests that the subsurface soil layer below 30cm depth accounts for the storage of more than 61% of the total soil C (Tarnocai *et al*., 2009) [46]. The radiocarbon study of the subsoil clearly suggests that this C is stable at longer time span (Marin-Spiotta *et al*., 2014) [28]. Further, the subsoil C is not influenced by tillage operations (Blanco-Canqui and Lal, 2008) [1]. The relatively less C saturation status of subsurface soil may result in a greater potential of these soils to serve as a niche for SOC sequestration (Lorenz and Lal, 2005) [26].

The present study has been undertaken with the objective of extraction and quantification of the most labile pools of C (water soluble) in surface as well as in deep soils under rice and non-rice ecology. To study the C dynamics in subsoil in comparison to surface soil, it is important to consider pools of SOC. Dissolved organic carbon (DOC) represents one of the most labile pools of SOC (Hedges, 2002; Callesen *et al*., 2003) [15, 3]. The DOC pools may be defined as the amount of SOC, which get dissolved in water at room temperature as well as C fraction that dissolves in hot water (Ghani *et al*., 2003) [13] and its availability in soil solution depends on soil mineral matrix (Fröberg *et al*., 2006) [12]. The dynamics of DOC in soil depends on the litter and humus decomposition, as well as root exudates (Kalbitz *et al*., 2000) [19]. As per some researches, the top litter horizon has been considered to be the most important source of DOC as it receives the fresh plant residues directly (Michalzik and Matzner, 1999; Chen *et al*., 2017) [30, 4]. During litter decomposition, the SOC gets fragmented into smaller pieces so, the specific surface area and the permeability of the litter gets increased, which leads to increased rate of DOC leaching (Kalbitz *et al*., 2000) [19]. And the leached DOC reaches the subsoil mineral horizon afterwards (Qualls and Haines, 1991; Scott and Rothstein, 2017) [34, 41]. The retention of DOC in soil depends mainly on sorption-desorption and precipitation (Qualls and Haines, 1992; Kalbitz *et al*., 2000) [19]. In soil, DOC plays an important role in transport, mineralisation and stabilization of C (Kalbitz *et al*., 2000) [19]. The DOC also regulates C flux in ecosystems and affect transfer and storage of several nutrients like N, P, S and metals (Clarke *et al*., 2007) [7]. The process of DOC production in soil is affected by soil temperature and moisture (Christ and David, 1996; Zhou *et al*., 2015) [6, 53] and a direct relation is seen between the temperature and DOC production i.e. as the temperature increases, the DOC production in the soil also consistently increases (Kaiser *et al*., 2001; Zhou *et al*., 2015) [18, 53]. The alternate drying and wetting has also been seen to influence the DOC production (Zhang *et al*., 2004) [52].

As this study considered the dynamics of subsoil C under submerged rice and upland non-rice ecology, it is important to consider the potential of these soils to capture and store C. Among the various terrestrial ecosystems, rice soils are believed to be one of the most important sites of global C cycling (Rajkishore *et al*., 2015) [35]. The area under rice cultivation is nearly 44 million hectares in India which dominantly comes under submerged system of cultivation. Though this facilitates methane emission (IPCC, 2013) [16], rice soils are known to retain higher amounts of resilient C among all terrestrial ecosystems (Liu *et al*., 2006; Stern *et al*.,

2007; Xie *et al*., 2007) [25, 45, 51]. In comparison to all the terrestrial ecosystems, rice soils have the highest C density (Stern *et al*., 2007) [45] and therefore they act as an important niche for C sequestration. Evidence showed that C density in paddy soils was higher than that in upland soils (Xie *et al*., 2007) [51].

2. Materials and Methods
In order to achieve the objective of the present study, the materials for research have been chosen purposely. The methods were selected scientifically to determine the dynamics of C pools in deep as well as in surface soils under rice and non-rice soils.

2.1 Study area
Soil sampling has been done from 3 distinct locations of eastern India, *viz*.Gayeshpur farm (22° 57' N, 88° 29' E) in West Bengal, managed by Bidhan Chandra Krishi Viswavidyalaya (BCKV), Agricultural experimental farm (AE farm) (24° 11' N, 86° 18' E) in Giridih, Jharkhand, managed by Indian Statistical Institute (ISI), and Central farm (20° 15' N, 85° 48' E) in Bhubaneswar, Odisha, managed by Orissa University of Agriculture and Technology (OUAT) (Fig. 1). All the sites were part of long term field experiment. Within each location, sampling sites were selected under rice (Rice-Rice) as well as non-rice (vegetable-vegetable, wheat-fallow, plantation crops) based cropping systems. Soil samples were collected only from those sites having at least 15-20 years continuity of same cropping system to get the signature trend of that cropping system and management practices on soil C (Carillo *et al*., 2012) [2].

2.2 Soil Sampling
Entire soil sampling was conducted in the fallow seasons of 2014-2015 winter (November, 2014 to February, 2015) to avoid impact of tillage as well as rain interruption. Within each location, soil samples were collected from rice and non-rice based cropping systems. Again, under each cropping system, soils were collected from two sites. To compare qualitative and quantitative C dynamics of surface and below-ground deep soils, samples were collected from 0-20 cm as well as from 100-120 and 120-140 cm of each field replication. Therefore, total number of soil samples for this study was 36 (3 locations x 2 cropping systems x 2 sites x 3 depth). Further, composite soil sampling was done for each depth of each sites. Spade was used for soil sampling. To exactly determine the sampling location, hand-held GPS receiver (Garmin, Olathe, KS, USA) has been used.

2.3 Soil analysis
Soil samples were air-dried, sieved with 2mm sieves and visible plant residues and stones were removed. The <2mm soil sub-samples were then finely ground to powder for different physical, chemical and biological analysis. Core sampler had been used to collect soil sub-samples for determination of bulk density.

2.4 Determination of soil properties
2.4.1 Soil total C
To estimate the total C content, the soil samples were prepared following the method of Nelson and Sommers (1982) [32]. The total C content of the soil samples was determined with the help of an elemental analyzer (Vario El III, Elementar-Hanau, Hanau, Germany). A 50 mg, 100-mesh sieved soil were placed into small tin (Sn) foil, wrapped by

pressing through a metal stick and put into the furnace of CHN analyser. Before starting the operation, the machine was allowed to warm up for about one hour and standardised through a reference soil sample.

2.4.2 Determination of inorganic C and total organic C
Soil inorganic C i.e. total carbonates content in soil were determined by rapid titration method using dilute HCl and bromothymol blue as indicator (Jackson, 1973) [17]. The total organic C was obtained by subtracting the inorganic C from total C.

2.4.3 Extraction of water soluble C (WSC)
The water soluble C fraction at normal room temperature has been named as WSC. It is mainly comprised of soluble plant residues, undecomposed animal excreta etc. (Ghani *et al*., 2003) [13]. It was estimated by mixing soil and distilled water in a 50 ml centrifuge tube at a ratio of 1:10 (in this study, 3 g of soil in 30 ml distilled water) followed by 30 minutes extraction at 20 ºC and centrifugation at 3000 rpm for 20 minutes (Ghani *et al*., 2003) [13]. After centrifugation, the supernatant was filtered through 0.45 μm cellulose nitrate membrane filter paper to get the WSC. It was named as labile pool 1 (L1).

2.4.4 Extraction of hot water extractable C (HWC)
After removing the WSC, second labile pool of C (L2) was extracted from the remaining soil samples using hot water treatment (Haynes and Francis, 1993) [14]. For this, 30 ml of distilled water was added in soil, the mixture was shook on a vortex shaker for 10 seconds and was kept in a water-bath at 80ºC for at least 16 hours. Subsequently, the solution was centrifuged for 20 minutes at 3000 rpm and finally filtered through a 0.45 μm cellulose nitrate membrane filter paper (Ghani *et al*., 2003) [13].

2.4.5 Estimation of water soluble C pools
The estimation of DOC was done following the method of Nelson and Sommers (1982) [32]. This method uses $0.0667M$ $K_2Cr_2O_7$ (prepared by dissolving 19.622 g $K_2Cr_2O_7$ in 1 litre of distilled water) as extractant (oxidizing agent) and o-phenanthroline solution (prepared by dissolving 1.485 g o-phenanthroline monohydrate and 0.695 g ferrous ammonium

sulphate (FAS) hexahydrate in 100 ml of distilled water) as an indicator. The unreacted $K_2Cr_2O_7$ is determined by titrating it against 0.033 M acidified FAS (prepared by dissolving 12.94 g FAS hexahydrate in 900 ml of distilled water, adding 50 ml concentrated H_2SO_4 and finally making the volume up to 1000 ml). Ultimately, C content is calculated on the basis of FAS consumed for titration. The organic C percentage was calculated using the formula:

$$OC\% = \left(A \times M \times \frac{0.003}{g} \right) \times \left(\frac{E}{S} \right) \times 100$$

where,
M = molarity of FAS used
A = $(ml_{HB} - ml_{sample}) \times [(ml_{UB} - ml_{HB})/ ml_{UB} + (ml_{HB} - ml_{sample})$
g = dry soil mass (g)
E = extraction volume (ml)
S = volume of sample extract used (ml)

2.4.6 Estimation of recalcitrant C pool
The recalcitrant C was detremined by subtracting the total sum of labile pools from the TOC.
RECALCITRANT POOL (RL) = TOC − (L1 + L2)

2.5 Statistical analysis
Statistical analysis has been conducted using Mirosoft Excel with Xlstat extension.
For further analysis, R statistial tool (R Core Team, 2016) has been used.

3. Results and Discussion
Analysis of water soluble C pools indicated noteworthy higher presence in surface soil (Fig 1). This was true for both water soluble C pool (L1) as well as hot water soluble pool (L2). This is quite expected as these pools represent very labile fraction of soil C (Khanna *et al*., 2001; Uchida *et al*., 2012) [20, 47] and surface soil, being the recipient of regular C input through leaf and litter fall has a large pool of labile C (Chen *et al*., 2017) [4]. Fig 2 clearly indicated the larger size of L2 in comparison to L1 pool irrespective of soil depth and crop ecology.
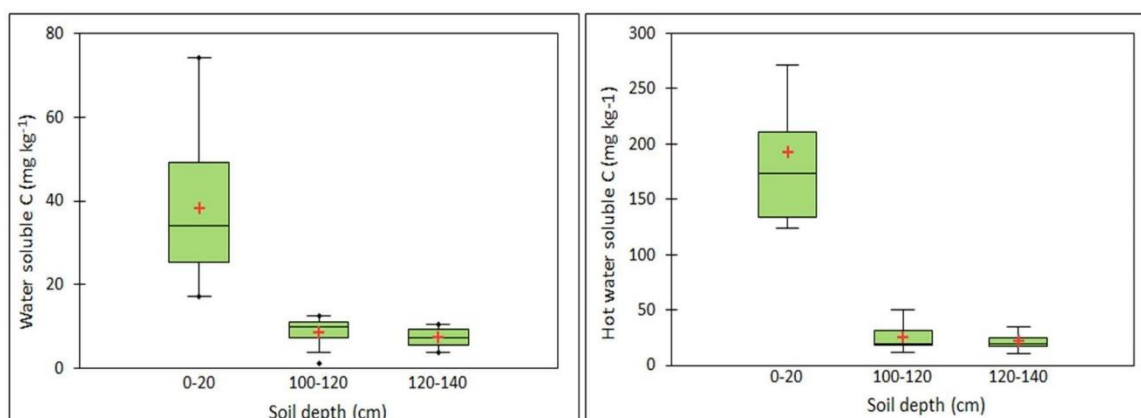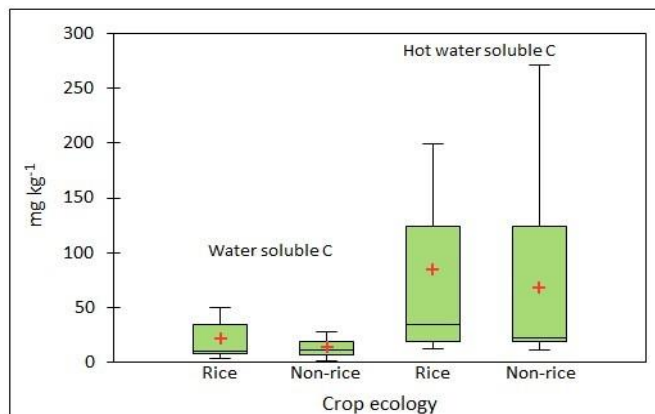

Fig 1: Distribution of water soluble (room temperature) and hot water soluble C as per soil depths

For detailed understanding of the C lability and stability, the results in this study have been examined through factorial analysis (Table 2). Apart from water and hot water soluble C, recalcitrant pool has also been derived by subtracting the earlier two pools from TOC. However, the variation of water,

hot water soluble C as well as the recalcitrant pool cannot be a representative of the true soil C dynamics, as the soil total organic C also varied a lot with depth and ecology. To resolve this issue, this study represented the sum of water soluble pools (considered as labile pool in this study, L1+ L2) as well

as recalcitrant pools as a percent of TOC. Table 2 and Fig 4 depicted higher presence of water soluble C pools (as % of TOC) in surface soil in all the locations. On the contrary, recalcitrant C as a % of TOC got increased in the subsoil layers. It indicated a higher residence time of C in subsoil, as found in earlier researches also (Deb *et al*., 2016a) [9].

Fig 2: Comparison of water soluble (room temperature) and hot water soluble

Table 1 indicated the details about the impact of soil depth and crop ecology onto soil C pools. Surface soils exhibited higher TC as well as TOC status of the surface soils, irrespective of the selected sites (Fig 3). This is quite natural as the surface soil receives the maximum C input in terms of discarded plant biomass. However, outcomes revealed a significant presence of TC and TOC in subsoil layers. As per Table 1, amount of TC and TOC was higher in soils under rice ecology in comparison to upland soils of non-rice ecology. This was possibly due to capacity of rice soil to store high amount of C (Tate, 1979). The absence of O2 as terminal electron acceptor in submerged rice soil resulted slow oxidation of C and higher turnover time (Sahrawat, 2004; Mandal *et al*., 2007) [4, 27]. All the soils stored a small amount of inorganic C. However, as evident from results, this C pool was also slightly higher in surface soils in comparison to subsoil layers.
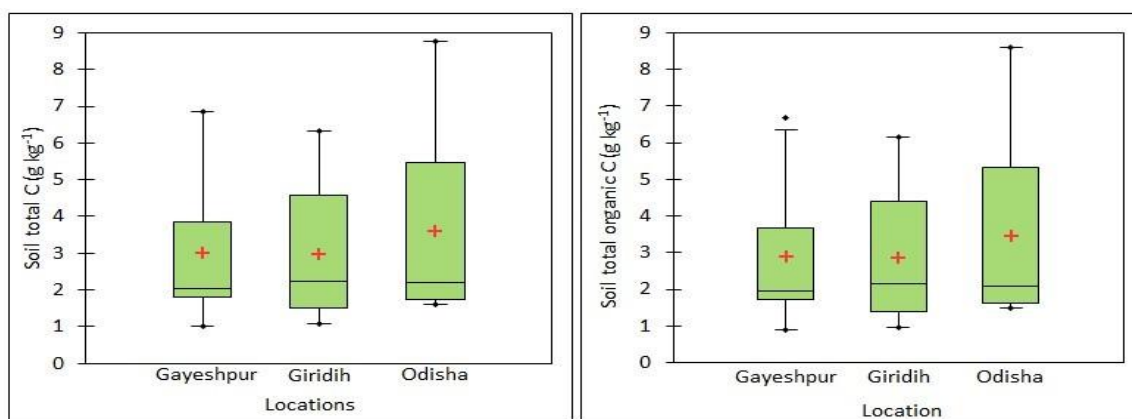
Fig 3: Distribution of total C and total organic C in soils of three study locations

Table 1: Status of soil total C, inorganic C and total organic C

Location	Soil depth (cm)/ Cropping system	Soil C status (g kg-1)		
		Total C	Total organic C	Inorganic C
Gayeshpur farm, BCKV	0-20	5.38a	5.21a	0.16a
	100-120	2.06b	1.97b	0.09b
	120-140	1.57c	1.47c	0.10b
		**	**	*
	Rice	3.40	3.28	0.11
	Non-rice	2.61	2.48	0.12
		**	**	ns
	Depth × cropping system **	**	*	
Agricultural Experimental farm, ISI, Giridih	0-20	5.36a	5.19a	0.17a
	100-120	2.06b	1.96b	0.10b
	120-140	1.56c	1.46b	0.10b
		**	**	**
	Rice	3.43	3.30	0.13
	Non-rice	2.56	2.43	0.12
		**	**	ns
	Depth × cropping system ns	*	ns	

	0-20	6.92a	6.77a	0.15a
	100-120	2.11b	1.75b	0.12b
	120-140	1.74c	1.37b	0.11b
Central farm,		**	*	*
OUAT,	Rice	4.06	3.92	0.13
Bhubaneswar	Non-rice	3.12	3.01	0.12
		Ns	**	*

Depth × cropping system ns * *

** ≤ 0.01, * ≤ 0.05, ns: not significant according to F-value of ANOVA. Different lower- case letters indicate significantly different values along soil depth at P≤0.05 according to Duncan's test for separation of means.

Table 2: Status of water soluble C (WSC), hot water soluble C (HWC), recalcitrant C in soils and their proportions in total organic C

Location	Soil depth (cm)/ Cropping system	WSC (mg kg-1)	HWC (mg kg-1)	Recalcitrant C (g kg-1)	(WSC + HWC)/ total organic C × 100	Recalcitrant C/ total organic C × 100
	0-20	40.67a	184.69a	4.99a	4.85a	95.15a
Gayeshpur farm,	100-120	10.50b	27.41b	1.93b	2.03b	97.97a
BCKV	120-140	8.00c	22.33b	1.44c	2.19b	97.81a
		**	**	*	*	Ns
	Rice	22.44	67.07	3.19	2.39	97.61
	Non-rice	17.02	89.22	2.37	3.65	96.35
		**	**	*	**	Ns
Depth × cropping system		*	**	*	*	Ns
Agricultural	0-20 cm	22.89a	160.67a	5.01a	3.47a	96.53b
Experimental farm,	100-120 cm	8.29b	16.40b	1.93b	1.37b	98.63a
ISI, Giridih	120-140 cm	4.93c	15.74b	1.44b	1.55b	98.45a
		**	**	*	*	Ns
	Rice	17.70	60.67	3.22	1.85	98.15
	Non-rice	6.37	67.87	2.36	2.41	97.59
		**	*	*	*	Ns
Depth × cropping system		*	*	**	*	Ns
Central farm, OUAT,	0-20 cm	32.17a	184.45a	6.56a	2.98a	97.02b
Bhubaneswar	100-120 cm	5.24b	31.93a	1.96b	1.86b	98.14a
	120-140 cm	7.24b	26.66a	1.59b	2.09ab	97.91ab
		**	**	**	*	*
	Rice	16.63	124.98	3.78	3.04	96.96
	Non-rice	13.33	37.04	2.96	1.59	98.41
		Ns	**	*	*	*
Depth × cropping system		*	**	*	*	*

** ≤ 0.01, * ≤ 0.05, ns: not significant according to F-value of ANOVA. Different lower-case letters indicate significantly different values along soil depth at P≤0.05 according to Duncan's test for separation of means

A comparison of rice and non-rice ecology (irrespective of soil depth) showed higher presence of water soluble C (labile pool of C) as % of TOC in soils under non-rice ecology (Table 2). The percentage of recalcitrant C pool was also higher in soils under rice ecology. It means, a greater C stability in rice soils which possibly was due to the water logging and subsequent anaerobic conditions of rice soils (Tate, 1979). The absence of O_2 possibly resulted slower microbial decomposition and low C mineralization (Witt *et al*., 2000; Guo and Lin, 2001).
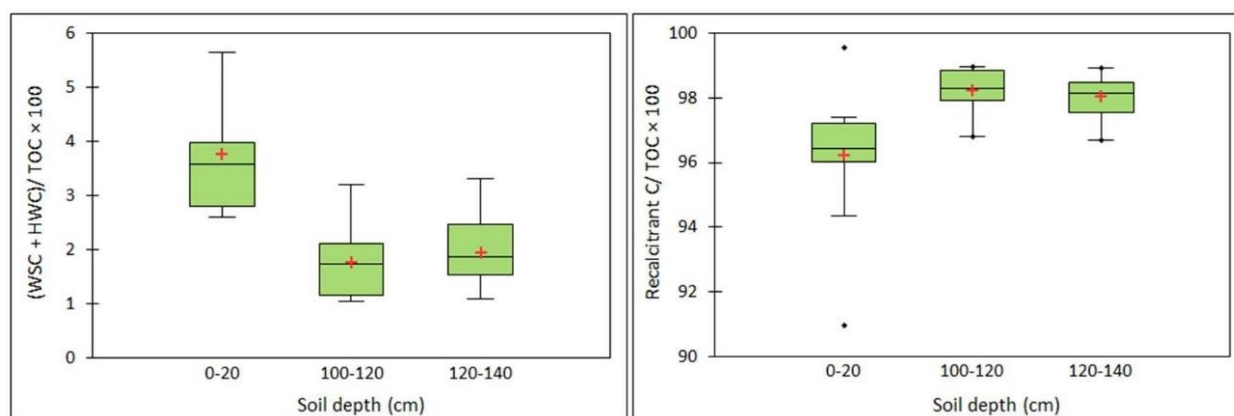
Fig 4: Relative dominance of water soluble C pools (WSC + HSC) as well as recalcitrant C pool as a fraction of soil total organic C

4. Conclusion
This study tried to understand the dynamics of C in subsoil in comparison to surface soil. Besides, the C dynamics of soils collected under rice and non-rice ecologies were also studied.

Here, attempt was done to separate soil C pools as they actually exist in soil. Thus, the method used in this study was water soluble C pools. Very labile fractions of C was mined in this study using water (at room temperature) and by hot water

extraction technique. The remaining soil C pool is considered as recalcitrant C.

Outcomes revealed higher total C and total organic C in surface soil in comparison to subsoil. The rice soil showed higher total and organic C in comparison to non-rice soil. Analysis of water soluble C pools indicated higher water soluble as well as hot water soluble C in surface soils. These two pools represent very labile C thus, their higher presence in surface soil is quite natural as this layer receives major amount of C input as leaf and litter. However, irrespective of depth and crop ecology, the hot water soluble C pool was quantitatively much higher than the water soluble pool at room temperature. The absolute quantity of this water soluble pool as well as recalcitrant C did not represent the true C dynamics of the soil as total organic C quantity also varied with soil depth and crop ecology. Therefore the summation of these two water soluble pools as well as recalcitrant pool was expressed as a ratio of total organic C. It clearly showed higher % of recalcitrant C in subsoil layer while labile pool of C (water soluble C) was higher in surface layer. This trend was same for all the three study sites. Recalcitrant C was also found higher in rice soil.

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