



Long-term effect of rice-based cropping systems on pools of soil organic carbon in farmer's field in hilly agroecosystem of Manipur, India

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Abstract A comprehensive study on various pools of soil organic carbon (SOC) under different rice-based cropping systems is necessary for predicting their effect on soil quality through carbon build-up in soil and their impact on global climate change. The present investigation was undertaken to study the long-term effect of six different rice-based cropping systems (continuously followed by farmers > 10 years) on various SOC pools viz., total organic carbon (TOC), oxidizable organic carbon (C_{oc}) and its different fractions [C_{frac1} (very labile), C_{frac2} (labile), C_{frac3} (less labile) and C_{frac4} (non-labile)], soil microbial biomass carbon (SMBC) and lability index (LI) and SOC stock at the farmer's field of Kakching district under hilly ecosystems of Manipur, India. In every cropping system, all the fractions of C_{oc} were significantly decreased with increasing soil depth.

Among all the fractions, C_{frac4} (non-labile) constituted the largest percentage of TOC for both surface (0–20 cm) and sub-surface (20–40 cm) soil varying from 47.95–58.45% and 55.76–64.83% with average values of 51.87 and 59.73% respectively. Results also revealed that the C_{frac1} (very labile) of C_{oc} constituted highest (42.79%) percentage of C_{oc} and that of C_{frac4} constituted highest percentage (55.80%) of TOC. In both soil depths, rice-pea cropping system recorded highest TOC, C_{oc} and SMBC followed by rice-French bean and rice-potato. In surface soil, the lowest TOC, C_{oc} and SMBC were recorded in rice-mustard which was statistically at par with rice-cabbage. The SOC stock of both soil layers was also recorded highest in rice-pea. The highest LI of surface soil was recorded in rice-potato which was statistically equal with rice-pea and rice-French bean. Significant correlations among different pools/fractions of C and with available nutrients indicate their importance in improving soil quality. Long-term combination of rice with the leguminous crops and/or potato enhanced C_{oc} , TOC, SMBC, LI and active pools ($C_{frac1} + C_{frac2}$) of rapid turnover rate that may influence the quality and productivity of soil. Long-term cultivation of rice-French bean with high passive C along with good active C and LI is proved to be a good cropping system for sustaining soil and environment by enhancing quality and C reserve of degraded soils of hilly agroecosystem.

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Introduction

Understanding the dynamics of soil organic carbon (SOC) in agriculture context is essential because of its effects on global C cycle and crop production. In the global terrestrial ecosystem, soil is an extensive C reservoir storing approximately 1505 Pg of SOC in it (Lal 2018), more or less three times that stored in vegetation which is approximately twice the contents stored in the atmosphere (Yang et al. 2016). Thus, the SOC and its fractions affect the global C cycle besides changing the atmospheric chemistry with the emission of greenhouse gases (GHGs) (Ladha et al. 2016). So, a small increase of the SOC and its fractions in the soil could impact the C concentration in atmosphere (Mi et al. 2016). Also, the sustainable productivity of agroecosystems is greatly influenced by SOC and its fractions in particular, as it plays a prime role in soil quality and influences physical, chemical and biological properties of soil (Kumar et al. 2018). Besides, SOC build-up is a means of C sequestration which increases the potential of agroecosystems to absorb atmospheric carbon dioxide (CO₂) and thereby storing it in soils. Thus, rotating different crops after rice in the same piece of land and its effect on SOC dynamics are very important to envisage the effect on soil quality and its consequences on global climate change. Different agricultural activities have significantly contributed to the increase of GHG concentrations in atmosphere (Lal 2004) and also have been known to affect soil C sequestration (Jaiarree et al. 2011). With the adoptions of different management practices, agricultural soils may become an effective C sink which can balance C inputs from plant production and outputs through decomposition and controlling the SOC storage in soil (Minasny et al. 2017). Certain land use and management practices influence C input and soil C lost (Paustian et al. 2016). Among different management practices, crop rotation, reduced tillage and fertilization practices maintained SOC at optimum level (Gong et al. 2009), increased the grain yield, maintained soil quality, developed sustainable agro-ecosystem (Zhou et al. 2015) and improved the C reserve to ameliorate the present global climate change (Paustian et al. 2016).

The hilly ecosystems of North-Eastern (NE) India are characterized by diverse agro-climatic and geographical situation. Most of the land cover is under forest (54.1% of total geographical area (TGA)) and rest is under cropped (16.6%) and non-agricultural holdings (29.3%) (Saha et al. 2012). The region has climate

ranging from tropical to alpine type, and altitude varies from 15 to > 5000 m above mean sea level. These diverse land holdings, physiography and climate in the region resulted in rich biological diversity than other parts of India. Due to the undulating physiographic features of the region (> 15% slope), agricultural land holding is low. The faulty land use practices like shifting cultivation and cultivation along the steep slope lead to the dilution of forest cover in the region resulting in degradation of soil health and environment in the hilly ecosystem.

Rice (*Oryza sativa* L.) fields are considered to have high potential in sequestering C from the atmosphere (Song et al. 2015) because of prolonged submergence, reduced decomposition of organic matter, higher productivity and its subsequent contribution to organic C in hidden half, affecting the global C cycle (Rajkishore et al. 2015). Therefore, the need to study accumulation and stabilization of C is of major importance (Weller et al. 2016). For a larger part of world agriculture, rice is giving health benefit by feeding nearly 50% of world population (Fuhrmann et al. 2019) and is the staple food for people especially in the Asia-Pacific region including India (Ladha et al. 2009). Long-term and intensive cultivation of rice with puddling affect the SOC dynamics, soil nutrient elements and their use efficiency which may lead to diminished soil quality and productivity (Bhattacharyya et al. 2012). However, higher soil organic C storage and sequestration is reported in rice fields compared with upland arable crops (Wissing et al. 2011). Due to excessive reduced conditions and shortfall of oxygen for microbial activity under submerged conditions, organic C build-up in rice field is faster and more conspicuous than other arable land, as organic matter decomposition is reduced in lowland rice fields (Yadav et al. 2017). In south Asian countries, rice-upland crop rotations are one of the most important agricultural production systems for food security (Yadav et al. 2000) and are known to increase the SOC build-up and improve soil nutrients content in juxtaposition with monocultures (Moore et al. 2000). However, rice-upland crop rotation is the conversion of soil from anaerobic to aerobic and then back to anaerobic annually which leads to more and more losses of SOC due to frequent tillage creating difficulties for soil organic matter (SOM) conservation. On the other hand, due to overall increased biomass production from the succeeding crop, enormous amount of C is added to the soil through crop residues (Hazra et al. 2018). Inclusion

of leguminous crop in the rotation can help in reducing the use of fertilizer owing to the reduction of greenhouse gases (GHGs) (Stagnari et al. 2017). There may be either increase or decrease of SOC and its pools in the soil owing to crop rotation of rice with arable crops that may also relies on the crop species which are cultivated with, since both the quality and quantity of their residue after harvesting returned to the soils may affect the turnover or residence time in soils (Mandal et al. 2007). Further, fallowing time and duration within a particular cropping system can also affect the SOC content in the soil (Halvorson et al. 2002).

To understand the SOC dynamics, SOC is separated into labile or actively cycling pool and stable, resistant or recalcitrant pools with varying residence time (Chan et al. 2001; Jat et al. 2019). Different SOC fractions or pools of varying complexity and stability provides valuable information regarding the quality of soil as well as the preservation of C in the soil and also help to monitor the changes in SOC dynamics under varied land uses and climatic condition (Venkatesh et al. 2013). Labile C pool is defined as the C with a turnover time of less than a few years (residence time 1–5 years), and is characterized by rapid turnover rates and it responds more quickly (Nandan et al. 2019), therefore with a slight disturbance in the soil can also oxidize this pool leading to the movement of the quantity of CO₂ per unit time from soils to atmosphere. Whereas, the recalcitrant C pool with a turnover time of few thousand years is very slowly changed by microbial activities (Sherrod et al. 2005). The labile C pool influences the quality and productivity of soil and has been regarded as the main source of nutrition in soil (Chan et al. 2001). Highly recalcitrant C pool which is slowly altered by microbial activities may not be a good soil quality parameter but contributes towards overall TOC stock (Mandal et al. 2008). Many researchers have studied the changes in labile and non-labile fraction of C in rice field under different soil management practices including irrigation facilities in tropical and sub-tropical regions in India. However, there is scanty of information on the changes in various SOC pools or fractions on surface and sub-surface soil in long-term rice-based cropping systems in hilly agroecosystems of Manipur, India. In the present investigation, efforts were, therefore, made to evaluate the changes in various pools/fractions of SOC along two depths in different long-term rice-based cropping systems in hilly areas of Manipur continuously followed by farmers greater than 10 years.

Materials and methods

Description of the study area

Manipur is predominantly a hill state of NE India, and its major area is under forest (92% of the TGA of the state), and rest 8% area situated in the central part of the state is plain. The study was conducted in Wabagai, located in Kakching, a valley district of Manipur, which is 39 km to the south-east from Imphal city (Fig. 1). It is located on the bank to the Sekmai River. Geographically, it lies in between 24° 32' 45.276" North latitude and 93° 56' 16.0188" East longitude. The altitude of the area is on average 778 m above mean sea level (msl). The study area is specified by humid sub-tropical climate, and the soil is moderately fertile with clay loam to silty clay loam soil with little patches of clay and loam. The temperature ranges from a minimum of 2.4 °C to a maximum of 34.1 °C, and the annual rainfall ranges from 670 to 1450 mm. Rice is the main crop grown in Manipur. Six different rice-based cropping systems were selected for the study viz., (i) rice (*Oryza sativa* L.)-fallow, (ii) rice-pea (*Pisum sativum* L.), (iii) rice-mustard (*Brassica campestris* L.), (iv) rice-potato (*Solanum tuberosum* L.), (v) rice-cabbage (*Brassica oleracea* L.) and (vi) rice-French bean (*Phaseolus vulgaris* L.) (Table 1). The studied area has maintained the similar cropping sequence for more than 10 years and were cultivated twice a year (except rice-fallow) with rice in wet season and the succeeding crops in dry season. Before transplanting of rice seedlings, the fields were puddled with power tiller and levelled the surface with laddering. Then, 30-day-old rice seedlings were transplanted maintaining the spacing of 30 cm × 30 cm in first week of July every year. Recommended doses of nitrogen (N)-phosphorus (P)-potassium (K), i.e. 60-40-30, were applied in rice in the form of urea, single super phosphate (SSP) and muriate of potash (MOP) respectively. Full doses of P and K were applied during land preparation. However, N was applied in three equal splits—first at land preparation, second at the tillering stage and third at flowering stage of rice. All other recommended package of practices for rice cultivation like irrigation, weeding, plant protection etc. were followed by the farmers as when required. After rice harvesting in second week of November, land preparations were done each year for sowing/planting of each of the succeeding crops with their appropriate spacings except rice-fallow, rice-pea and rice-mustard cropping sequences. In rice-fallow cropping system, the land was remained

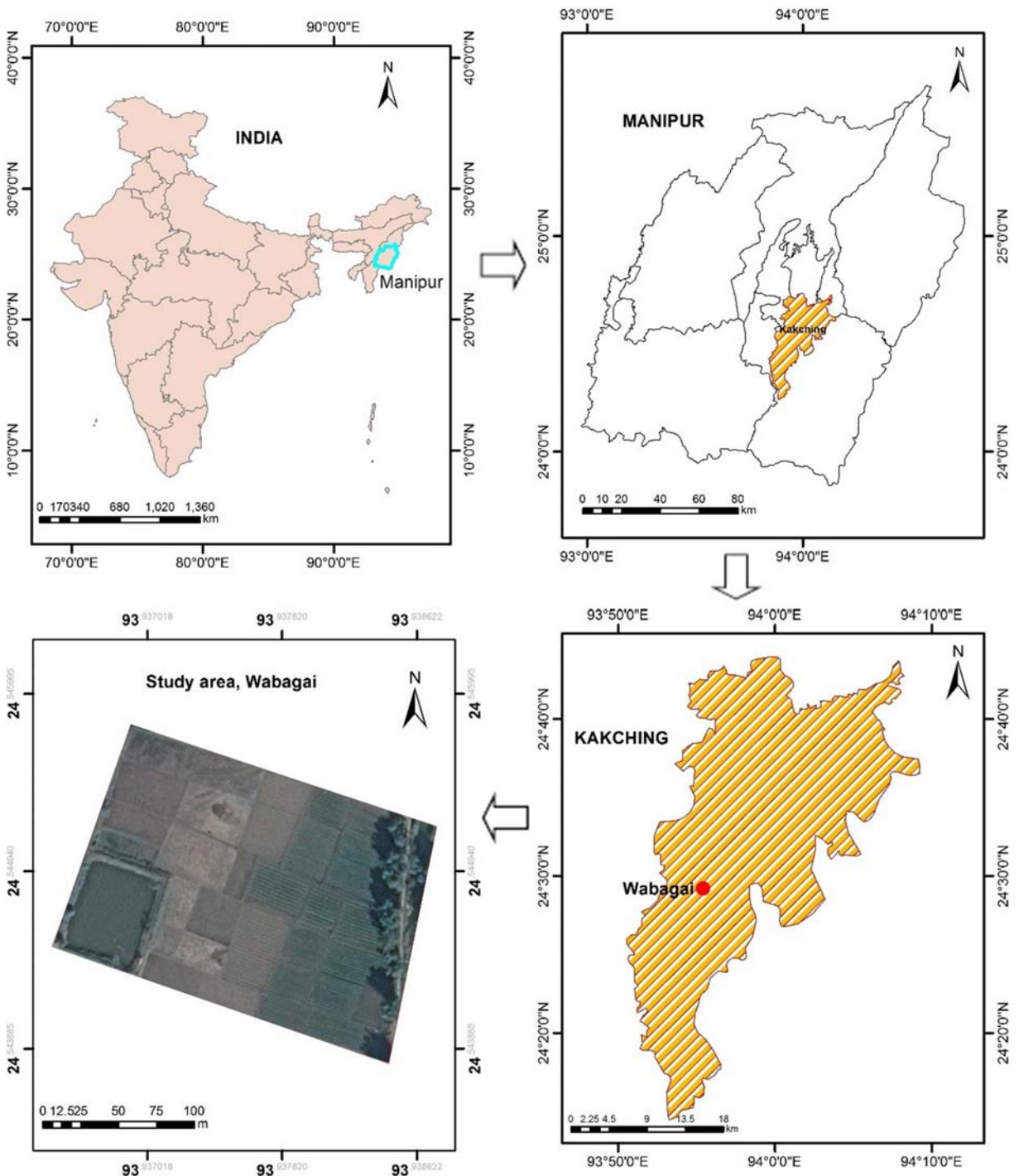


Fig. 1 Location of the study area (Manipur, India)

empty after harvest of rice till the next wet season. Again, in rice-pea and rice-mustard cropping sequences, seeds of pea and mustard were broadcasted in moist soil of standing rice crops just 1 week before harvesting of rice and

were grown as paira crops each year. For the succeeding crops (after rice) except potato, only half of the full recommended doses of N-P-K, i.e. 10-30-20, 15-30-20, 10-30-20 and 10-30-20, were applied in the form of urea,

Table 1 Variations in soil properties at different soil layers under different rice-based cropping systems

Soil properties	Soil layers (cm)	Rice-fallow	Rice-pea	Rice-mustard	Rice-French bean	Rice-cabbage	Rice-potato
Bulk density (g cm ⁻³)	0–20	1.18a	1.02c	1.09b	1.06bc	1.15a	1.05bc
	20–40	1.41a	1.19d	1.37ab	1.36ab	1.40a	1.27cd
Sand (%)	0–20	47.00a	44.16ab	43.50ab	39.28bc	42.20ab	32.83c
	20–40	43.56a	39.16b	39.16b	36.28bc	33.20c	35.83bc
Silt (%)	0–20	32.50bc	30.34c	37.00ab	35.00abc	35.00abc	39.67a
	20–40	34.00ab	30.10b	39.34a	37.28a	39.80a	39.34a
Clay (%)	0–20	20.50ns	25.50ns	19.50ns	25.72ns	22.80ns	24.50ns
	20–40	22.44b	30.83a	21.50b	26.44ab	26.90ab	30.34a
pH	0–20	5.97ab	6.07a	5.94ab	5.55abc	5.40bc	5.04c
	20–40	6.24a	6.41a	6.07a	5.85ab	6.08a	5.43b
Available N (kg ha ⁻¹)	0–20	276.41b	313.66a	251.94bc	296.65c	264.43b	285.12bc
	20–40	168.09b	183.33a	153.23d	178.78c	155.02d	147.96e
Available P (kg ha ⁻¹)	0–20	18.76b	31.02a	18.29b	16.83b	18.01b	28.19a
	20–40	12.17c	21.97a	15.45bc	14.25bc	15.55bc	17.63b
Available K (kg ha ⁻¹)	0–20	181.34a	155.69ab	113.40d	122.04cd	107.53d	143.10bc
	20–40	135.18a	127.58a	100.67bc	107.09b	70.37c	91.95c
Available S (kg ha ⁻¹)	0–20	40.14b	45.54a	36.58bc	34.36c	38.40b	47.04a
	20–40	24.41abc	26.62a	22.25c	23.06bc	22.51bc	25.73ab
Available Zn (mg kg ⁻¹)	0–20	0.637ab	0.864a	0.526b	0.628ab	0.556b	0.670ab
	20–40	0.465bc	0.534a	0.389d	0.423cd	0.326e	0.498ab

Means in each row followed by different letters refers to significant differences between the treatments at 5% level of significance according to DMRT for separation of means; *ns* non-significant

SSP and MOP in pea, mustard, cabbage and French bean respectively because of the residual effect of fertilizer from rice. However, in potato being a heavy feeding crop, full recommended doses of N-P-K, i.e. 80-60-40, were applied. However, no organic manures were added in the fields, since root and shoot biomass return from the rice (stubbles) and succeeding crops (root and leaf litter) after harvesting supplies the organic matter required for the cultivation. In addition to fertilization, all other recommended package of practices like irrigation, weeding, plant protection, earthing up (for potato only), etc. for each crop were followed by the farmers as and when required for raising these crops. The pea, mustard, potato, cabbage and French bean were harvested in the month of February/March each year.

Soil sampling, sample preparation and analysis of soil properties

For each cropping system, four composite soil samples were collected from the surface (0–20 cm) and another

four composite soil samples were collected from sub-surface (20–40 cm) soil just after harvesting of rice during 2015 to 2016. Composites were made in such a way by mixing three different soil samples collected from three locations of each surrounding areas. Approximately 1 kg of composite sample was taken from each sampling sites and put into plastic bags. The soil samples were air-dried at room temperature, crushed, homogenized and passed through a 2-mm sieve before laboratory analysis. Thus, a total of 48 composite soil samples (eight from each land use types) were collected for soil analysis. For analyses of soil microbial biomass carbon (SMBC), fresh undisturbed sub-samples were placed in sealed plastic bags, and stored at 2 °C in the refrigerator. Different soil properties of the experimental sites were determined as per the following procedures: pH in soil/water suspension with a ratio of 1:2.5 (Jackson 1973), available nitrogen (Av. N) by alkaline potassium permanganate method (Subbiah and Asija 1956), available phosphorus (Av. P) by Bray’s II method (Bray and Kurtz 1945), available potassium (Av. K) by

neutral normal ammonium acetate extractant (Jackson 1973), available sulphur (Av. S) by turbidometric method (Bardsley and Lancaster 1960), DTPA extractable zinc (Zn) by the method described by Lindsay and Norvell (1978), soil separates (sand, silt and clay) by hydrometer method (Bouyoucos 1962) and bulk density (BD) by core method.

Carbon pools

Total organic carbon (TOC) by modified method of Nelson and Sommers (1982) as described by Majumder (2007) and SMBC was determined by chloroform fumigation extraction method (Vance et al. 1987). Oxidizable organic carbon (C_{oc}) was determined by wet digestion method (Walkley and Black 1934).

Fractions of oxidizable organic carbon (C_{oc})

The different fractions of C_{oc} were estimated through a modified Walkley and Black method as described by Chan et al. (2001). In this method, different concentrations (12 N, 18 N and 24 N) of concentrated sulphuric acid (H_2SO_4) were used with 5, 10 and 20 ml respectively. The amount of SOC that was determined using 5, 10 and 20 ml of concentrated H_2SO_4 when compared with TOC gives the separation of TOC into following four different fractions of decreasing oxidizability.

C_{frac1} (very labile)	Organic C oxidizable under 12.0 N H_2SO_4
C_{frac2} (labile)	Difference in SOC oxidizable under 18.0 N and that under 12.0 N H_2SO_4
C_{frac3} (less labile)	Difference in SOC oxidizable under 24.0 N and that under 18.0 N H_2SO_4 (the 24.0 N H_2SO_4 is equivalent to the standard Walkley and Black method)
C_{frac4} (recalcitrant)	Residual organic C after reaction with 24.0 N H_2SO_4 when compared with the TOC (modified method of Nelson and Sommers 1982) (Majumder 2007)

Active and passive C pools

C_{frac1} and C_{frac2} together are designated as ‘active pool’ of SOC because of their easy oxidizability (by weak 12.0 and 18.0 N H_2SO_4). Again, summation of C_{frac3}

and C_{frac4} together constitutes the ‘passive pool’ of SOC in the experimental soils.

Lability index

The lability index (LI) for the SOC was computed using C_{frac1} , C_{frac2} and C_{frac3} . The C_{frac1} , C_{frac2} and C_{frac3} , which will be considered as very labile, labile and less labile pools, were given weightage of 3, 2 and 1 respectively. Afterwards, their actual values were transformed to a proportional amount of TOC and were weighed with the weighing factor to get a lability index for the SOC content of the studied cropping systems. The index will be computed as (Hazra et al. 2018):

$$\text{Lability index} = \left[\frac{C_{frac1}}{TOC} \times 3 + \frac{C_{frac2}}{TOC} \times 2 + \frac{C_{frac3}}{TOC} \times 1 \right]$$

Soil organic carbon stock

The SOC stocks were calculated as follows (Sharma et al. 2014):

$$\text{SOC stocks (Mg ha}^{-1}\text{)} = \text{SOC} \times \rho \times d \times 10,000$$

where SOC is the soil organic carbon measured in $g\ g^{-1}$, ρ is the soil bulk density ($g\ cm^{-3}$) and d is the depth of soil layer (m). The value of 10,000 indicates the stock for 1 ha of land.

Statistical analysis

The data obtained from the laboratory analysis were analysed statistically using Microsoft Excel 2007 and the SPSS v. 21 statistical software packages. The means and standard errors were calculated and presented for each of the measurements. One-way analysis (one-way ANOVA) and means of the treatments were compared using the Duncan’s multiple range test (DMRT) at 5% probability level.

Results and discussion

Effect on total organic carbon (TOC), oxidizable organic carbon (C_{oc}) and microbial biomass carbon (SMBC) of soil

Results showed that there were significant depth-wise variations of TOC, C_{oc} and SMBC in the studied different rice-based cropping systems (Table 2). While comparing the TOC content of surface (0–20 cm) soil, the highest TOC was recorded in rice-pea (32.61 g kg⁻¹) which was, however, statistically at par with rice-French bean (30.33 g kg⁻¹) and rice-fallow (29.13 g kg⁻¹) and the lowest TOC was recorded in rice-mustard (23.76 g kg⁻¹). In sub-surface (20–40 cm) soil also, the highest TOC was recorded in rice-pea (24.10 g kg⁻¹) but found statistically at par with rice-potato (21.85 g kg⁻¹) whereas the lowest was recorded in rice-cabbage (15.28 g kg⁻¹) which was again statistically equal with rice-mustard (15.29 g kg⁻¹). During the regular period of flooding in rice-fallow, anaerobic soil condition prevailed minimizing soil oxidation, which limited the degradation of native SOM as well as added crop residues (Nandan et al. 2019). The higher topsoil TOC content in paddy soil compared with upland arable cropped soil was mainly due to excessively reduced conditions and shortfall of oxygen for microbial activity under submerged conditions reducing the organic matter decomposition in lowland rice fields (Bhattacharyya et al. 2013). Different long-term experiments under rice

have recorded that application of balanced fertilizer with paddy straw and green manure (Majumder et al. 2008; Sathish et al. 2011) enhanced TOC in surface soil with increased in yield (Mitran and Mani 2017). Thus, in the present study, residual effect of root and shoot biomass returned in soil and regular application of recommended fertilizer for long time may be the reason for high TOC (Merante et al. 2017) in legume-included cropping systems (Table 2). In rice-mustard and rice-cabbage, most of the above-ground biomass (mustard and cabbage as vegetable) were removed and very less residue returned to the soil. The only input of organic matter was through root biomass, which may be the reason of lowest organic carbon content. Tripathi et al. (2010) mentioned that mustard requires relatively large amount of nutrients for growth and production which feeds large quantity of nutrients but return less. Further, Biswas et al. (2006) found that in Brassicaceae family (both mustard and cabbage) because of its nutrient-exhaustive nature, there was a decline in the productivity of crop as well as soil.

While comparing the oxidizable organic carbon (C_{oc}) content of surface (0–20 cm) soil, similar trend like that of TOC were observed, with rice-pea (16.98 g kg⁻¹) recorded highest followed by rice-French bean (14.95 g kg⁻¹), which was, however, statistically at par to each other. The lowest C_{oc} was recorded under rice-mustard (11.15 g kg⁻¹) which was, however, statistically at par with and rice-cabbage (12.08 g kg⁻¹). Again, rice-pea (10.66 g kg⁻¹) was observed significantly higher C_{oc} than other cropping systems in sub-surface soil (20–

Table 2 Variations in total organic C, oxidizable organic C and microbial biomass C content at different soil layers under different rice-based cropping systems

Cropping systems	Soil organic carbon (g kg ⁻¹ soil)					
	Total organic carbon (TOC)		Oxidizable organic carbon (C_{oc})		Soil microbial biomass carbon (SMBC)	
	0–20 cm	20–40 cm	0–20 cm	20–40 cm	0–20 cm	20–40 cm
Rice-fallow	29.13ab	16.25b	12.10cd	6.03d	0.140c	0.096b
Rice-pea	32.61a	24.10a	16.98a	10.66a	0.222a	0.107a
Rice-mustard	23.76d	15.29c	11.15d	6.60bc	0.126c	0.097b
Rice-French bean	30.33ab	19.55b	14.95ab	6.88bc	0.204ab	0.103ab
Rice-cabbage	25.24cd	15.28c	12.08cd	6.40bc	0.159bc	0.098b
Rice-potato	28.03bc	21.85ab	14.13bc	8.66b	0.179abc	0.102ab
Mean	27.35	18.72	13.56	7.54	0.172	0.100

Means in the column followed by different letters refers to significant differences between the treatments at 5% level of significance according to DMRT for separation of means

40 cm), and this was followed by rice-potato (8.66 g kg^{-1}) but statistically at par with rice-French bean (6.88 g kg^{-1}), rice-mustard (6.60 g kg^{-1}) and rice-cabbage (6.40 g kg^{-1}) in descending order, and the lowest was recorded under rice-fallow (6.03 g kg^{-1}) (Table 2). Bhattacharyya et al. (2012) found that with addition of rice straw and green manure at rice field, there is an increment of C_{oc} content in soil by 34%. So, the incorporation of pea and French bean biomass in soil along with rice straw for a long time may lead to higher C_{oc} than other cropping systems. In both surface and sub-surface soil, rice-potato was found to be comparable with pea and French-bean rotation, suggesting the benefits of storage and accumulation of SOC. Incorporation of potato haulm into the soil after harvest (Biswas et al. 2006), residual effect of high fertilizer doses applied (Banerjee et al. 2016) and intensive soil aeration during cropping of potato may have caused these benefit when compared with leguminous species. Increase in organic matter with potato cropping system has also been observed in the Eastern Indo-Gangetic plain of West Bengal (Biswas et al. 2006). And also, in a research conducted by Chen et al. (2012) under different rice-upland crop rotation, the rice-potato rotation was found producing highest biomass which suggested highest value of organic material returned into the soil.

The SMBC concentrations in rice-pea cropping system (0.222 g kg^{-1} and 0.107 g kg^{-1}) were recorded higher than other cropping system in both depths though found statistically similar with rice-French bean (0.204 g kg^{-1} and 0.103 g kg^{-1}) and rice-potato (0.179 g kg^{-1} and 0.102 g kg^{-1}). Lowest were observed under rice-fallow

(0.140 g kg^{-1} and 0.096 g kg^{-1}) and rice-mustard (0.126 g kg^{-1} and 0.097 g kg^{-1}) in both the surface and sub-surface soil. During the crop cycle of rice with the upland plant species for long time, root and shoot biomass returned to the soil enriched the surface soils (0–20 cm), which leads to higher SMBC (Inubushi and Nagano 2017) in surface soil, whereas with increasing depth, the optimum soil characteristics (SOC, water holding capacity, BD, etc.) that governs the microbial activities in the soil declines which resulted in drop of SMBC (Choudhary and Gill 2013). So, the rate of microbial activity and amount of microbial biomass decrease below the surface layer (Jones et al. 2018). Lamb et al. (2011) reported that the quality and the quantity of litter returned to soil are the major factors that determined the rate of carbon accumulation, microbial biomass and microbe's activity. In the present study, pea plant because of its taproots nature can absorb plant nutrients and water from different layers of soil (Duzdemir et al. 2009), and on top of that, pea is a leguminous crop that can fix nitrogen in the soil; further addition of this N-rich leaf litter and biomass to soil makes rice-pea cropping system a system of highest SOC, TOC and SMBC on both surface and sub-surface soil. Similar result of high SMBC content in soil with inclusion of legumes species in the rotation was recorded by many researchers (Das et al. 2017; Majumder et al. 2008).

Effect on fractions of oxidizable organic carbon

The amounts of different fractions of C_{oc} (C_{frac1} , C_{frac2} , C_{frac3} and C_{frac4}) varied significantly among the cropping

Fig. 2 Percent distribution of various fractions of oxidizable organic carbon (C_{oc}) of varying lability in surface soil (0–20 cm) of different rice-based cropping systems

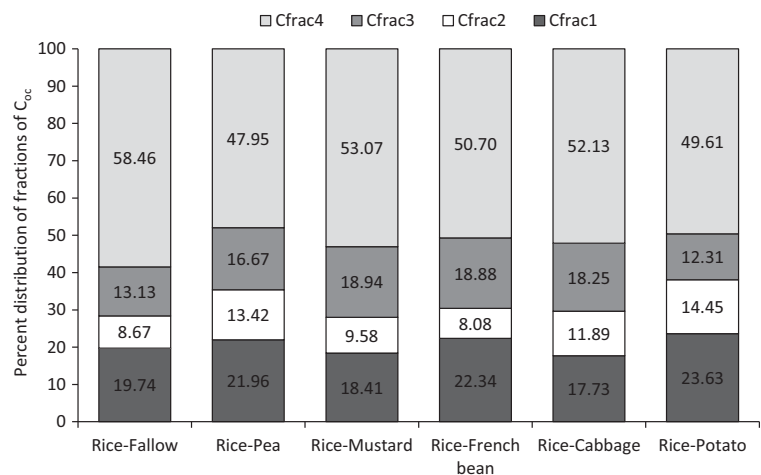
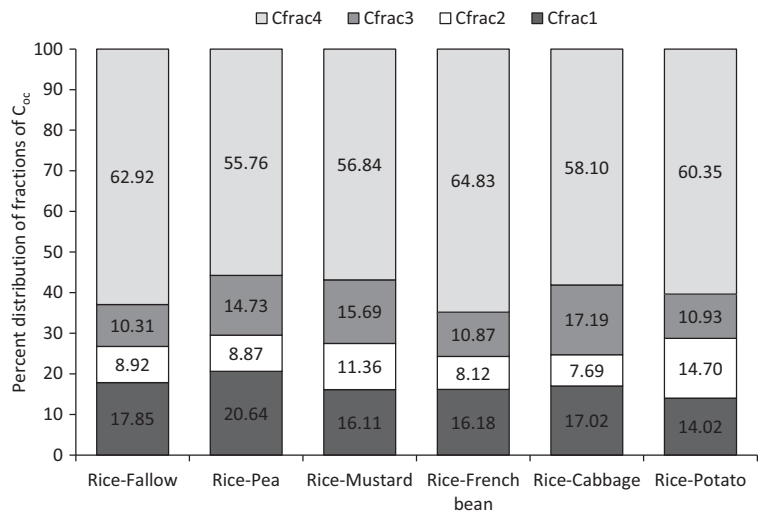


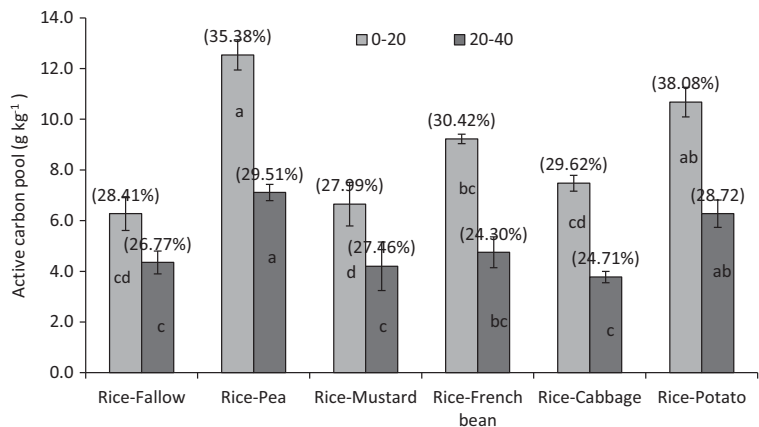
Fig. 3 Percent distribution of various fractions of oxidizable organic carbon (C_{oc}) of varying lability in sub-surface soil (20–40 cm) of different rice-based cropping systems



systems and with depth (Table 3). Irrespective of different cropping systems, the recalcitrant C pool (C_{frac4}) occupied highest proportion (47.95 to 58.46%) in the surface soil (Fig. 2) which further increased with increasing depth (55.76 to 64.83%) (Fig. 3). In the surface soil, C_{frac4} was recorded highest under rice-fallow (58.46%) and lowest was under rice-pea (47.95%) cropping system (Fig. 2). On the other hand, the very labile fraction (C_{frac1}) was observed highest under rice-potato (23.63%) and the lowest under rice-cabbage (17.73%) which was recorded lowest of all (Fig. 2). It was also found that with increasing depth, the percent (%) C_{frac1} , C_{frac2} and C_{frac3} for all the cropping systems studied decreases, except for C_{frac4} (Fig. 3). In the sub-surface soil, rice-French bean (64.83%) recorded highest C_{frac4} and the lowest under rice-pea (55.76%) (Fig. 3). The C_{frac1} was found highest under rice-pea (20.64%) followed by rice-fallow (17.85%) and lowest under rice-mustard (16.11%). In surface soil due to high

input of crop residues and fine roots, substrate availability will be more for microbial activity thereby increasing the labile carbon content (C_{frac1} and C_{frac2}) in soil (Carpenter-Boggs et al. 2003). Comparable findings of previous study by Padbhusan et al. (2016) under different rice-based cropping systems are available. Crop residue returns in the surface soil enhance the microbial activity with increasing the mineralisation processes making more labile fraction of C (Fang et al. 2018). But in the sub-surface soil due to lacked of crop residue, the microbial activities decrease which in turn may decrease the labile fraction of TOC but non-labile fraction increases. Similar result of increasing non-labile fractions of TOC with increasing depth has also been recorded by Nath et al. (2015) in lowland rain-fed paddy soil and also in different cropping systems by Ghosh et al. (2010) in the eastern cereal belt of India. Application of balanced fertilizer with biomass returned from potato, and pea may increase polysaccharide

Fig. 4 Active carbon pools ($C_{frac1} + C_{frac2}$) of surface (0–20 cm) and sub-surface soil (20–40 cm) of different rice-based cropping systems. [Parentheses show percent of TOC. Line bars indicate standard error mean. Different letters written in each column refer to significant differences between the treatments at 5% level of significance according to DMRT for separation of means]



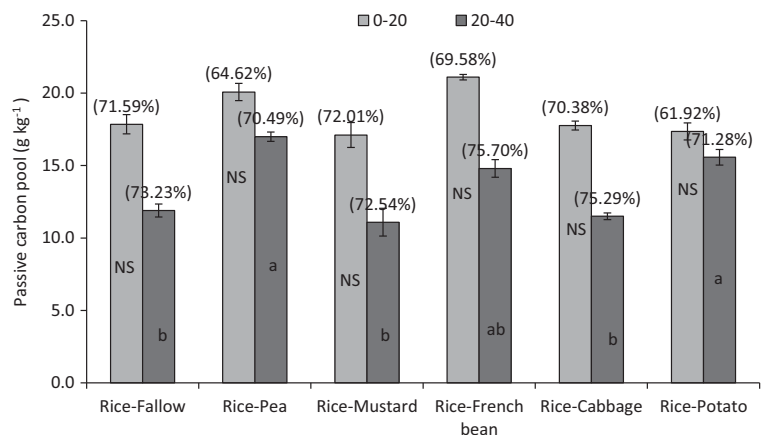
(cellulose and hemi-cellulose) content in soil that lead to production of higher amounts of very labile carbon fraction (Sathish et al. 2016) in both the cropping system. Whereas, during the regular period of flooding in the rice fallow, excessively reduced conditions and shortfall of oxygen for microbial activity prevail which limit the degradation of native soil organic matter as well as that added with crop residues (Wu 2011), organic carbon build-up is also faster and more conspicuous than other arable land, which may be the reason for high recalcitrant C fraction in both the soil depths. In the present study, overall mean evaluation of different C fractions (in percent) out of TOC (mean along depth) of different cropping systems was found to be in the trend of $C_{\text{frac4}} > C_{\text{frac1}} > C_{\text{frac3}} > C_{\text{frac2}}$, constituting about 55.80%, 18.93%, 14.72% and 10.55% respectively (Table 3). Highest C_{frac4} in overall TOC (mean along depth) of different cropping systems may be due to the presence of partially decomposed plant material in soil (Nandan et al. 2019). But the overall % of different C fractions out of C_{oc} (mean along depth), C_{frac1} recorded highest which contributes 42.79% followed by C_{frac3} (33.24%). Also, it has been found that the C_{frac1} contributes highest percentage (out of the overall C_{oc}) in the surface soil rather than sub-surface (Table 3), which indicates higher proportion of active C fraction in the surface soil of all the studied cropping systems and decrease with increasing soil depth.

Effect on active and passive pools of SOC

Irrespective of all the studied cropping systems, passive C pool dominated active C pool in both surface and sub-surface soil. In surface soil, proportion of active pool

($C_{\text{frac1}} + C_{\text{frac2}}$) from TOC in g kg^{-1} follows the order: rice-pea > rice-potato > rice-French bean > rice-cabbage > rice-mustard > rice fallow (Fig. 4). Crop residue returns in the surface soil enhance the microbial activity with increasing the mineralisation processes making more active C fraction (Fang et al. 2018). Same trend was observed in sub-surface soil with only exception of rice-cabbage cropping system being last. In surface soil, even though the active C pools of rice-pea was higher than rice-potato, the percent (%) active C pools out of the TOC was found higher in rice-potato (38.08%), which indicates the sensitivity of the soil (Moharana et al. 2012) in both rice-pea and rice-potato cropping systems. The production of higher amount of very labile carbon fraction and high microbial activity in pea included cropping system and potato haulm incorporated with high fertilizer doses may have resulted in higher active pools of carbon in the soil of these concerned cropping systems. On the other hand, the passive C pool ($C_{\text{frac2}} + C_{\text{frac3}}$) of the surface soil (g kg^{-1}) is dominated by rice-French bean (21.10 g kg^{-1}), but no significant difference was observed with other cropping systems (Fig. 5). However, the percentage of passive C pool of the individual cropping system out of their respective TOC was found highest under rice fallow and rice-mustard (~ 72%) (Fig. 5). This indicates higher proportion of recalcitrant carbon fractions of the TOC stored in rice-fallow, enhancing stable C storage. Due to low microbial activity and less disturbance of the soil (no tillage) in rice-fallow and rice-mustard (SMBC), there may be less degradation of SOC which might be the reason for higher passive C in soil. In sub-surface soil, rice-pea (16.99 g kg^{-1}) recorded highest passive C pools

Fig. 5 Passive carbon pools ($C_{\text{frac3}} + C_{\text{frac4}}$) of surface (0–20 cm) and sub-surface soil (20–40 cm) of different rice-based cropping systems. [Parentheses show percent of TOC. Line bars indicate standard error mean. Different letters written in each column refer to significant differences between the treatments at 5% level of significance according to DMRT for separation of means, NS non-significant]



but statistically at par with the rice-potato (15.58 g kg⁻¹) and rice-French bean (14.80 g kg⁻¹). But, the percentage of passive C pool of the individual cropping system out of their respective TOC was found highest under rice-French bean followed by rice-cabbage and rice fallow contributing 75.70%, 75.29% and 73.23% respectively (Fig. 5). This indicates higher proportion of recalcitrant carbon fractions of the TOC stored in the mentioned cropping systems. Rice-pea, rice-potato and rice-French bean cropping systems because of its high quantity of active and passive pools of TOC in both surface and sub-surface may be deliberated as a good cropping system for soil quality as well as for stable C storage for mitigating climate change and crop production.

Effect on lability index

The values of LI (Table 4) recorded from different cropping systems showed different trend as of SOC stated in the above section. With increase in depth layer of soil the LI decreases. The significant changes of LI from surface to sub-surface soil may be attributed due higher BD and non-availability of easily decomposable C substrate (Datta et al. 2017; Jat et al. 2018). It was also recorded that there was significant difference in the vertical distribution of LI only in rice-pea, rice-French bean and rice-potato out of the six cropping systems studied (Table 4). LI of surface soil was highest in rice-potato which was, however, statistically at par with rice-

pea and rice-French bean. Legumes because of their low C/N ratio contribute high-quality organic matter to the soil which in turn increased the microbial activity making higher LI in legume included cropping systems (Hazra et al. 2018). Whereas in rice-potato due to incorporation of potato haulm and regular higher doses of fertilizer application in soil increased the LI which showed good agreement with the findings of Sharma et al. (2017). In rice-mustard and rice-cabbage cropping systems due to harvest of whole plant as vegetable less residue returned to the soil which resulted lower LI in the those cropping systems. In surface soil (0–20 cm), the LI values for SOC were ranged from 0.90 to 1.12, and with increasing depth, there were significant decrement of LI value in rice-pea (13.65%), rice-French bean (25.91%) and rice-potato (26.60%). The higher LI value for rice-potato, rice-pea and rice-French bean among all the cropping systems tested indicated that they would maintain proportionately a higher amount of C in labile pools and thereby good for upkeeping soil health.

Effect on soil organic carbon stocks

The effects of the different crop combination with rice on SOC stock under soil depth are presented in Table 4. On an average, the SOC stock (Mg ha⁻¹) was higher in the surface soil (0–20 cm) compared with sub-surface soil (20–40 cm). The per cent decrease of SOC stock from surface to sub-surface soil were 40.19, 26.48, 25.33, 40.90,

Table 3 Variations in oxidizable organic C pools at different soil layers under different rice-based cropping systems

Cropping systems	Soil organic carbon pools (g kg ⁻¹ soil)											
	C _{frac1}			C _{frac2}			C _{frac3}			C _{frac4}		
	0–20	20–40	Mean	0–20	20–40	Mean	0–20	20–40	Mean	0–20	20–40	Mean
Rice-fallow	5.75b	2.90b	4.33bc	2.53b	1.45b	1.99b	3.82c	1.68b	2.75c	17.03a	10.23ab	13.63ab
Rice-pea	7.16a	4.98a	6.07a	4.38a	2.14ab	3.26a	5.44ab	3.55a	4.49a	15.64ab	13.44a	14.54a
Rice-mustard	4.38c	2.46b	3.42d	2.28b	1.74b	2.01b	4.50bc	2.40ab	3.45abc	12.61b	8.69b	10.65b
Rice-French bean	6.78ab	3.16b	4.97b	2.45b	1.59b	2.02b	5.73a	2.13b	3.93ab	15.38ab	12.68a	14.03ab
Rice-cabbage	4.48c	2.60b	3.54cd	3.00ab	1.17b	2.09b	4.61ab	2.63ab	3.62abc	13.16ab	8.88b	11.02ab
Rice-potato	6.63ab	3.06b	4.84b	4.05a	3.21a	3.63a	3.45c	2.39ab	2.92bc	13.91ab	13.19a	13.55ab
Mean	5.86	3.19	4.53	2.95	1.88	2.41	4.76	2.46	3.61	13.78	11.18	12.48
% of TOC	20.80	17.06	18.93*	11.04	10.06	10.55*	16.29	13.14	14.72*	51.87	59.73	55.80*
% of oxidizable SOC	43.21	42.37	42.79*	22.95	24.99	23.97*	33.84	32.64	33.24*			

Means in the column followed by different letters refer to significant differences between the treatments at 5% level of significance according to DMRT for separation of means

*Mean value

Table 4 Variations in lability index (LI) and SOC stock (Mg ha⁻¹) at different soil layers under different rice-based cropping systems

Land uses	Lability index (LI)		SOC stock (Mg ha ⁻¹)	
	0–20 cm	20–40 cm	0–20 cm	20–40 cm
Rice-fallow	0.90bNS	0.82bNS	28.49bcA	17.04cB
Rice-pea	1.09aA	0.94aB	34.52aA	25.38aB
Rice-mustard	0.93bNS	0.87bNS	24.28cA	18.13cB
Rice-French bean	1.02abA	0.76cB	31.64abA	18.70bcB
Rice-cabbage	0.95bNS	0.84bNS	27.69bcA	17.88cB
Rice-potato	1.12aA	0.82bB	29.76abA	22.05abB
Mean	1.00A	0.84B	29.40A	19.86B

Different small letters between columns and capital letters between rows indicate significant differences ($P < 0.05$) according to DMRT for separation of means; NS non-significant

35.43 and 25.91% for rice fallow, rice-pea, rice-mustard, rice-French bean, rice-cabbage and rice-potato respectively. Among the cropping systems studied, the highest SOC stock in surface soil was recorded under rice-pea (34.52 Mg ha⁻¹), which was, however, statistically at par with rice-French bean (31.64 Mg ha⁻¹) and rice-potato (29.76 Mg ha⁻¹) (Table 4). The SOC stock of surface soil of rice-fallow, rice-mustard, rice-French bean, rice-cabbage and rice-potato cropping systems were 17.47%, 29.66%, 8.34%, 19.79% and 13.79% less compared with rice-pea. In the sub-surface soil (20–40 cm), the SOC stock was lowest (17.04 Mg ha⁻¹) in rice-fallow and highest (25.38 Mg ha⁻¹) in rice-pea (Table 4). The ability of pea to fix atmospheric nitrogen reduces the nitrogen fertilizer requirement and has a beneficial effect on soil fertility (Soon and Arshad 2004) and also on biomass return to

Table 5 Relationship (Person's correlation coefficient, r) of different pools of C with soil properties of both surface and sub-surface soil of the rice-based cropping systems

	pH	Av N	Av P	Av K	Av S	Av Zn	BD	Sand%	Silt%	Clay%
Surface soil (0–20 cm)										
SMBC	-0.13	0.42*	0.72**	0.31	0.67**	0.45*	-0.67**	-0.34	-0.12	0.51*
TOC	-0.07	0.49*	0.43*	0.16	0.21	0.49*	-0.52**	-0.17	-0.18	0.33
SOC	0.06	0.50*	0.54**	0.30	0.32	0.32	-0.70**	-0.25	-0.22	0.48*
C _{frac1}	-0.05	0.43*	0.59**	0.26	0.43*	0.30	-0.60**	-0.24	-0.22	0.39
C _{frac2}	-0.11	0.40*	0.63**	0.02	0.53**	0.26	-0.53**	-0.43*	0.09	0.51
C _{frac3}	0.36	-0.18	-0.44*	0.27	-0.57**	0.08	-0.06	0.31	-0.29	-0.11
C _{frac4}	-0.16	0.01	0.15	-0.04	0.03	0.37	-0.13	-0.03	-0.07	0.05
Ac C	-0.09	0.47*	0.71**	0.18	0.55**	0.35	-0.66**	-0.38	-0.09	0.52**
Pa C	-0.02	-0.05	-0.02	0.06	-0.18	0.36	-0.14	0.09	-0.17	0.01
LI	0.04	0.52**	0.53**	0.21	0.46*	-0.02	-0.52**	-0.29	-0.10	0.42*
Sub-surface soil (20–40 cm)										
SMBC	0.26	0.47*	0.63**	0.24	0.38	0.40	-0.54**	0.09	-0.27	0.08
TOC	-0.21	0.39	0.73**	0.26	0.54**	0.62**	-0.63**	-0.01	-0.38	0.59**
SOC	0.00	0.44*	0.64**	0.24	0.43*	0.56**	-0.49*	0.01	-0.32	0.48*
C _{frac1}	0.28	0.71**	0.51*	0.46*	0.58**	0.63**	-0.49*	0.07	-0.53**	0.32
C _{frac2}	-0.30	-0.12	0.27	0.03	0.37	0.38	-0.15	0.00	0.08	0.24
C _{frac3}	0.02	0.33	0.56**	0.00	-0.08	0.14	-0.38	-0.05	-0.22	0.45*
C _{frac4}	-0.30	0.24	0.58**	0.20	0.46*	0.48*	-0.54**	-0.02	-0.31	0.50*
Ac C	-0.01	0.37	0.49*	0.31	0.60**	0.63**	-0.40*	0.05	-0.29	0.35
Pa C	-0.27	0.32	0.69**	0.18	0.39	0.48*	-0.60**	-0.03	-0.35	0.58**
LI	0.34	0.24	0.02	0.17	0.17	0.22	0.04	0.04	-0.08	-0.10

TOC total organic carbon, SOC soil organic carbon, C_{frac1} very labile carbon fraction, C_{frac2} labile carbon fraction, C_{frac3} less labile carbon fraction, C_{frac4} recalcitrant carbon fraction, Ac C active carbon fraction, Pa C passive carbon fraction, LI lability index, SMBC soil microbial biomass carbon, Av N available nitrogen, Av P available phosphorus, Av K available potassium, Av S available sulphur, BD bulk density

*The correlation is significant at 5% level of significance respectively where $n = 24$ and $df = 22$

**The correlation is significant at 1% level of significance respectively where $n = 24$ and $df = 22$

both surface and sub-surface soil which ultimately resulted higher SOC stock in rice-pea. Balance C input through litter fall and root exudates makes the SOC stock to increase (Benbi et al. 2015). On contrary to this, there is low biomass return in crops under *Brassicaceae* family (cabbage and mustard), due to the removal of above-ground biomass as vegetable and little incorporation of residues to the soil which resulted low SOC stock in soils of rice-cabbage and rice-mustard cropping systems.

Correlation study between pools of C and soil properties and among various pools of C

Simple Pearson’s correlation coefficient values (*r*) of different pools of C with soil properties of both soil depths are given in Table 5. It showed that available N

significantly and positively influenced SMBC, TOC, SOC, C_{frac1} , active C and LI of surface soil and SMBC, SOC and C_{frac1} of sub-surface soil. Again, there lies significant positive correlation of available P with SMBC, TOC, SOC, C_{frac1} , C_{frac2} , active C and LI of surface soil and with SMBC, TOC, SOC, C_{frac1} , C_{frac3} , C_{frac4} , active C and passive C of sub-surface soil. The available S showed significant positive correlation with SMBC, C_{frac2} , active C and LI of surface soil and that of TOC, SOC, C_{frac1} , C_{frac4} and active C of sub-surface soil. There was significant positive relationship of available Zn with SMBC and TOC of surface soil and with TOC, SOC, C_{frac1} , C_{frac4} , active C and passive C of sub-surface soil. Clay content significantly and positively influenced SMBC, SOC, active C, LI of surface soil and TOC, SOC, C_{frac1} , C_{frac2} , passive C of sub-surface soil.

Table 6 Inter-relationship (Person’s correlation coefficient, *r*) among the different pools of C of both surface and sub-surface soil of the rice-based cropping systems

	SMBC	TOC	SOC	C_{frac1}	C_{frac2}	C_{frac3}	C_{frac4}	Ac C	Pa C	LI
Surface soil (0–20 cm)										
SMBC	1.00									
TOC	0.66**	1.00								
SOC	0.76**	0.76**	1.00							
C_{frac1}	0.83**	0.84**	0.89**	1.00						
C_{frac2}	0.66**	0.28	0.64**	0.47*	1.00					
C_{frac3}	-0.34	0.15	0.15	-0.03	-0.47*	1.00				
C_{frac4}	0.29	0.80**	0.23	0.44*	-0.17	0.09	1.00			
Ac C	0.88**	0.68**	0.91**	0.89**	0.83**	-0.27	0.19	1.00		
Pa C	0.14	0.78**	0.26	0.38	-0.33	0.44*	0.93**	0.07	1.00	
LI	0.62**	0.18	0.74**	0.62**	0.76**	-0.19	-0.41*	0.80**	-0.44*	1.00
Sub-surface soil (20–40 cm)										
SMBC	1.00									
TOC	0.49*	1.00								
SOC	0.62**	0.75**	1.00							
C_{frac1}	0.60**	0.65**	0.79**	1.00						
C_{frac2}	0.32	0.46*	0.65**	0.28	1.00					
C_{frac3}	0.39	0.45*	0.65**	0.35	0.04	1.00				
C_{frac4}	0.25	0.88**	0.34	0.36	0.19	0.17	1.00			
Ac C	0.57**	0.69**	0.90**	0.80**	0.80**	0.25	0.34	1.00		
Pa C	0.34	0.93**	0.51**	0.43*	0.18	0.46*	0.96**	0.38	1.00	
LI	0.37	-0.06	0.56**	0.53**	0.46*	0.16	-0.50*	0.62**	-0.40*	1.00

TOC total organic carbon, SOC soil organic carbon, C_{frac1} very labile carbon fraction, C_{frac2} labile carbon fraction, C_{frac3} less labile carbon fraction, C_{frac4} recalcitrant carbon fraction, Ac C active carbon fraction, Pa C passive carbon fraction, LI liability index, SMBC soil microbial biomass carbon

*The correlation is significant at 5% level of significance respectively where *n* = 24 and *df* = 22

**The correlation is significant at 1% level of significance respectively where *n* = 24 and *df* = 22

However, BD significantly and negatively influenced SMBC, TOC, SOC, C_{frac1} , C_{frac2} , active C and LI of surface soil and SMBC, TOC, SOC, C_{frac1} , C_{frac4} , active C and passive C of sub-surface soil.

Simple Pearson's correlation coefficient (r) values among different pools of C of both the soil depths (Table 6) revealed that SMBC content was positively correlated with TOC, SOC, C_{frac1} , C_{frac2} , active C and LI of surface soil and with TOC, SOC, C_{frac1} and active C of sub-surface soil. Again, there lies significant positive correlation of TOC with all the C pools except C_{frac2} , C_{frac3} and LI of surface soil. The SOC content significantly and positively influenced all the C pools except C_{frac3} , C_{frac4} and passive C of surface soil and except C_{frac4} of sub-surface soil. The C_{frac1} was positively correlated with all the C pools except C_{frac3} and passive C of surface soil and except C_{frac2} , C_{frac3} , C_{frac4} of sub-surface soil. However, C_{frac2} was significantly and positively correlated with SMBC, SOC, C_{frac1} , active C and LI and negatively correlated with C_{frac3} of surface soil and positively correlated with TOC, SOC, active C and LI of sub-surface soil. Again, C_{frac3} was positively correlated with passive C and negatively correlated with C_{frac2} of surface soil and positively correlated with TOC, SOC, passive C of sub-surface soil. The, C_{frac4} was significantly and positively correlated with TOC, C_{frac1} and passive C and negatively correlated with LI of surface soil. However, C_{frac4} was significantly and positively correlated with TOC and passive C and negatively correlated with LI of sub-surface soil. Active C was significantly and positively correlated with all the C pools except C_{frac3} , C_{frac4} and passive C of both soil depths. However, passive C was significantly and positively correlated with TOC, C_{frac3} , C_{frac4} and negatively correlated with LI of surface soil and positively correlated with all the pools of C except SMBC, C_{frac2} , active C of sub-surface soil. LI was significantly and positively correlated with SMBC, SOC, C_{frac1} , C_{frac2} , active C and negatively correlated with C_{frac4} , passive C of surface soil and positively correlated with SOC, C_{frac1} , C_{frac2} , active C and negatively correlated with C_{frac4} , passive C of sub-surface soil.

Conclusion

Among all the different long-term rice-based cropping systems, rice in combination with leguminous crops due to their taproot systems and

nitrogen-rich biomass return to soil and/or with potato contributes high C_{oc} , TOC, SMBC, LI and active C pools ($C_{frac1} + C_{frac2}$) of rapid turnover rate that can oxidize more quickly and affect the quality and productivity of soil. The correlation between the important soil nutrients and pools of SOC indicates their importance in improving soil quality. Long-term cultivation of rice-French bean with high passive C pool along with good active C and high LI is proved to be a good cropping system for capturing C in soil and thereby maintaining the better quality of degraded soils of hilly agroecosystem. On the other hand, rice-fallow and rice-mustard although possess low soil active C pools but retain high passive C pools which may not be good with respect to soil quality but may increase carbon reserve/stock in the soil. Therefore, it can be concluded that rice-French bean, because of its ability to increase both quality and C reserve to the soil, is highly recommended for both soil and environmental sustainability of hilly agroecosystem.

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