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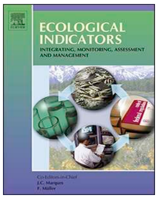

Original Articles

Soil carbon dynamics in Indian Himalayan intensified organic rice-based cropping sequences

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ABSTRACT

The contribution of soil to supporting, regulating, provisioning and cultural functions as well as its role in the ecosystem services is well-known in the international literature. However, in the domain of organic agriculture, the impact of cropping systems shifts from cereal-cereal to high-frequency diversified cropping sequences with legume as a component crop on soil carbon dynamics is not widely known. In order to identify an alternative cropping system to widely prevalent rice-fallow production system in Himalayan region of India, seven cropping sequences *viz.*, rice -fenugreek (green vegetable) - maize (R-F-M); rice -vegetable pea - maize (R-Vp-M); rice-coriander (leaves)-cowpea (R-C-Cp); rice - fenugreek (green vegetable) - baby corn (R-F-Bc); rice - broccoli - *Sesbania* (green manuring) (R-B-S); rice - buckwheat (R-Bw) and rice - maize (R-M) were assessed for five consecutive years from 2013 to 2018 for their productivity and resource conservation values. Results revealed that the inclusion of legumes in rice-based sequences increased the rice grain yield by 13.4 to 24.6% over R-M (3.13 Mg ha^{-1}) sequence. The R-B-S sequence had the highest very labile carbon (VLC) (4.6 g kg^{-1} soil) followed by the R-Vp-M. Relative proportion of various organic carbon fractions in the top 10 cm soil followed the order of VLC (30.2%) > non labile carbon (NLC, 27.6%) > labile carbon (LC, 23.4%) > less labile carbon (LLC, 18.9%). The carbon management index (CMI) was the highest (100.9%) in the R-B-S sequence followed by R-C-Cp (98.0%). The addition of a third crop in the sequence increased the active carbon (AC) pool by 1.1 to 5.8%. The passive carbon (PC) pool was highest in soil under the R-C-Cp sequence (9.15 Mg ha^{-1}) at 0–10 cm soil depth. The carbon retention efficiency under the R-C-Cp cropping sequence was the highest (15.1%) followed by the R-B-S (14.9%). R-B-S and R-C-Cp sequences had 12.5% and 10.6% higher soil microbial biomass carbon (SMBC) over the R-M sequence, respectively. Similarly, R-B-S and R-C-Cp increased the FDA by 49.6 and 41.8%, and DHA by 135.0% and 103.9%, respectively over R-M sequence. In conclusion, the management of crops from organic agriculture aimed at improving soil ecosystem services, in contrasting degradation of soil health and the decline of SOC, can also have positive effects on crop productivity in the eastern Himalayan region of India as well as all over the world.

1. Introduction

Soil system is a complex and dynamic ecosystem sustaining physical processes and chemical nutrient cycling that are vital to terrestrial life, as well as its biodiversity is vitally important to humans (Jonsson and Daviosdottir, 2016). Soils play an important role in climate regulation, particularly through the sequestration of atmospheric carbon dioxide (CO_2) and its storage within major carbon sinks (Jiang et al., 2019; Turbe et al., 2010; Haygarth and Ritz, 2009). Jonsson and Daviosdottir (2016) have provided a comprehensive list of soil ecosystem services, intended as the benefits humans derive from natural and semi-natural agro-ecosystems (Costanza et al., 1997). More specifically, soil

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contributes to supporting functions (biodiversity pool, nutrient cycling, and water cycling) (Sandhu et al., 2008; Dominati et al., 2010), regulating functions such as biological control of pests and diseases, and climate and gas regulation (Dominati et al., 2010; Turbe et al., 2010), provisioning functions as biomass production, clean water, raw materials and physical environment (Porter et al., 2009; Abrahams, 2012; Dominati et al., 2014), and cultural functions (TEEB, 2010).

In this context, the capacity of the terrestrial C pool to act as a net sink can affect the increase of atmospheric C that is estimated to increment in the order of 4 Pg per year (Stockmann et al., 2013). In the terrestrial pool, soil organic carbon (SOC) is possibly a bigger sink for atmospheric carbon dioxide (CO_2) (Abdullahi et al., 2018), and it gives indications on fertility and productivity of soil (Sahoo et al., 2019). It interacts in a complex way with the properties and functions of soil with effects on the provision of ecosystem services (Glenk et al., 2017). Taking into account the C present in the top three-meter soil, C stored in soil is second to the amount of C present in the ocean (38400 ± 2.3 Pg), and about 1500 Pg SOC is stored in the top 1-meter soil, out of which 41% (615 Pg) SOC is trapped in the top 20 cm and ~70% (1050 Pg) SOC in the top 40 cm (Lal, 2018; Yadav et al., 2019), and it is about 1.3 times higher than that present in the atmosphere (800 Pg). Thus, terrestrial carbon present in the top 40 cm soil has great significance in the global C cycle, given that a minor change in SOC has a significant impact on the atmospheric CO_2. In this perspective, the initiative "4 per 1000" promoted by COP21 Paris climate summit in 2015 aims at helping governments to implement sustainable intensification of food production, highlighting the importance of soil C sequestration to keep global warming below 1.5 °C (Chabbi et al., 2017; Minasny et al., 2017), as well as other European strategies aimed at saving resources in agricultural sector (Toma et al., 2017). Evidences indicate that the conversion of non-organic soil to organic system contributes toward SOC sequestration (Gattinger et al., 2012) and improvement in soil quality (Das et al., 2013b). Modern agriculture, involving clean cultivation with exhaustive high yielding crops led to extensive nutrient mining from the soil without natural replenishment, deteriorating the soil quality in many parts of the world (Porpavai et al., 2011). Management of SOC in agricultural systems can affect soil productive capacity by supporting the growth conditions for crops and increase the efficiency of nutrient use, reducing the amount of fertilizers required for optimal plant growth (Luxhoi et al., 2007; Pan et al., 2010; Glenk et al., 2017). In this milieu, organic farming practices have been reported to provide more soil ecosystem services than chemical farming (Williams and Hedlund, 2013; Reganold and Wachter, 2016). They can sequester SOC to the tune of 0.45 ± 0.21 Mg ha^{-1} yr^{-1} in the topsoil (Gattinger et al., 2012) and have vast potential for storing and improving SOC compared to that of conventional production systems (Gattinger et al., 2012; Cavigelli et al., 2013). But organic farming per se may not be enough to improve SOC and soil quality. von Lutzow and Ottow (1994) and Petersen et al. (1999) have reported lower SOC in organic than in conventional farms, while Burkitt et al. (2007) and Leifeld and Fuhrer (2010) have demonstrated no difference in SOC between organically and conventionally managed farms. On the other side, there have been evidences of accelerated organic carbon stock depletion and land degradation under organic farming also when nutrients scavenging cropping sequences along with improper soil and crop management practices have been carried out (Salahin et al., 2013; Yadav et al., 2018a,b). Thus, improved agronomic practices that should lead to reduced C losses or an increase in soil C storage are highly warranted. This includes improved crop varieties, diverse crop rotations notably those with a legume, deep-rooted and green manuring crops that allocate more carbon below- ground, avoiding or reducing the unplanted fallow, and application of organic amendments such as compost or waste products. Thus, redesigning and intensification of exiting cropping systems with the inclusion of legumes and other more biomass generating crops might help in increasing the SOC pool and improving soil quality (e.g., carbon management index-CMI and soil biological properties) (Yadav et al., 2019). The role of cropping sequences in reducing the adverse environmental effects has also been recognized in the current common agricultural policy (CAP) reform declared by the European Commission (Nemecek et al., 2015). The SOC (Duan et al., 2016), CMI and soil enzymatic activities (DeFelice et al., 2006) are the key indicators of soil quality due to their implications both on the environment and crop production. Diversified cropping sequences promote soil environmental sustainability by changing the quantity and quality of residues as compared to monoculture (Wright et al., 2007; Cheng et al., 2019). But, these studies are mostly confined to conventional farming (non-organic). The impact of the level and degree of intensification and diversification of traditional cropping system through the inclusion of legumes, green manures and high-value crops on soil carbon sequestration, C dynamic and CMI have not been extensively studied under organic farming. In this context, this research aims at investigating how the long-term cultivation of intensified cropping sequences under organic management influences the accumulation and distribution of total soil organic C (TOC), soil biological parameters and CMI. To this aim, a field experiment (2013–2018) was conducted for five consecutive years to assess the long term effects of different rice-based cropping sequences on soil C dynamics, soil biological health and agronomic productivity of organic farming.

1.1. Soil quality and sustainable management practices

Maintenance of soil microbial biomass and its activity is a fundamental base for any sustainable production system (Gonzalez-Quinones et al., 2011). Short and medium-term changes in total soil organic C (TOC) are difficult to detect due to the high background C and variability in non-labile C (Blair et al., 1995). Labile fraction of organic carbon (VLC – very labile carbon, and LC – labile carbon) represents the carbon active pool, comprised of physical, chemical ($KMnO_4$ C) and biological fractions, is highly responsive to changes in C input and soil management practices (land-use change) (Yadav et al., 2018a,b; Meena et al., 2018; Nandan et al., 2019). It degrades in a short span of time, and thus, is suggested as an initial and sensitive indicator of SOC changes (Ghani et al., 2003; Haynes, 2000). However, the recalcitrant pool (NLC – no labile pool, and LLC – low labile pool) is a much more stable component that can remain in the soil for a longer duration (Mandal et al., 2013) and represents the passive carbon pool (Sahoo et al., 2019). Numerous studies have reported that the VLC is a more sensitive part of SOC than the other fractions/pool and serves as an initial indicator as compared with total SOC (Moharana et al., 2012; Liu et al., 2014). The relative proportion of VLC, LC, LLC and NLC fractions determines soil quality and therefore is a crucial factor in soil C dynamics. Oxidizable soil organic C mostly represents the entire LC pool and some portion of long-lived C pools that takes a long time to change due to land and crop management effects (Benbi et al., 2015). However, the chemical nature of organic C may not always match with their degradability in soil. Hence, evaluating labile SOC fractions may be a more accurate tactic for evaluating soil changes in response to legume crops inclusion under organic farming. Based on changes in different fractions of carbon, the C pool index (CPI) has been developed to assess the capacity of management practices to improve soil quality (Blair et al., 1995; Diekow et al., 2005). Thus, the CMI developed from lability concepts are considered the most effective tool for quantitative estimation of soil quality (Blair et al., 1995). It is reported that the CMI as an early indicator of soil quality changes due to management practices and thus, able to assess best management practices that arrest the soil degradation under different management scenarios (Ghosh et al., 2016; Moharana et al., 2012; Mandal et al., 2013; Six et al., 2000). A number of available studies provide valuable information pertaining to several soil management practices, but none of them have ever integrated C pools and carbon lability into CMI for assessing capacity of the intensified cropping sequences to promote C restoration under organic management scenarios towards a holistic approach to soil management

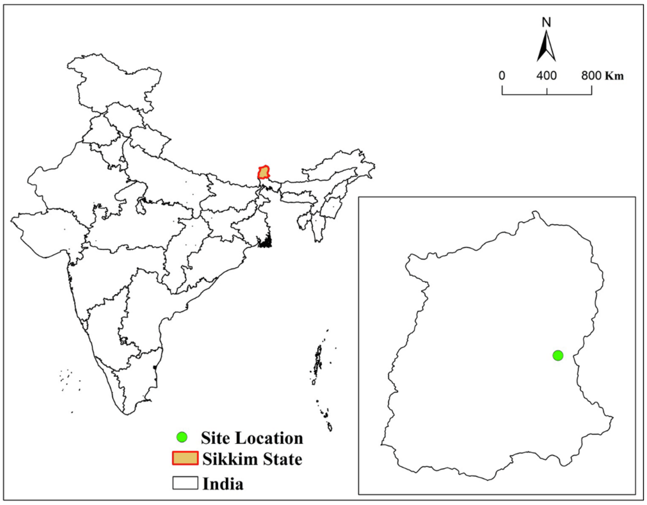

Fig. 1. Study area map.

(soil ecosystem services vs management decisions) (Jonsson and Daviosdottir, 2016).

2. Materials and methods

2.1. Study area

The Eastern Himalayan ranges (EHR) comprising of parts of Bangladesh, Bhutan, China, Nepal, Myanmar and the Indian states of Sikkim, northern part West Bengal, Arunachal Pradesh, southern Assam, Nagaland, Manipur, Meghalaya, and Mizoram are spread over 22.0 million hectares (Mha), and farming in this EHR region is either organic or with minimal use of synthetic inputs (Fig. 1).

The Indian state of Sikkim has been declared as an organic state by the Govt. of India in 2016. Few other states like Meghalaya, Arunachal Pradesh, and Mizoram are contemplating to become completely organic states in the next few years. Agriculture in the entire North-Eastern Himalayan regions (NEHR) is characterized as complex, diverse and risk-prone (Das et al., 2017). Soils of the region are highly fragile and prone to degradation. Despite this, most of the farming communities in the region follow resource exhaustive agricultural production practices like adoption of cereal-based system [mostly rice (*Oryza sativa*) and maize (*Zea mays*)], clean cultivation with minimum return of organic inputs into the soil, *etc*., which cannot support the food requirement of its growing populace and at the same time contributing to environmental unsustainability. However, increasing productivity and production while maintaining the quality of the product as well as the environment pose the greatest challenges to the researchers and policy makers to secure the immediate future of agriculture in the region (Yadav et al., 2013a). Hence, there is an urgent need to develop appropriate soil and crop management practices by involving efficient cropping sequences for sustainable hill agriculture. It has been reported that the diversified/intensified crop sequences may enhance the grain crop yields > 10% relative to simple rotations/ monocropping (Bennett et al., 2012) but their benefits on soil carbon sequestration and the CMI under organic conditions in the region are rarely known. Demand for organic produce is progressively increasing both in the international and domestic markets (Singh et al., 2016). Vegetables are the indispensable constituents in the diet of the hilly populace and also contribute to enhancing the farmer's income in organic farming. From the profitability point of view, embedding vegetables like fenugreek (*Trigonella foenumgraecum*), vegetable pea (*Pisum sativum*), and cowpea (*Vigna unguiculata*) in rice-based systems may enhance the income of the small and marginal growers in the fragile Himalayan region (Das et al., 2013a). But, their effect on SOC storage and changes in the SOC pool is not known and needs systematic study and in-depth understanding. Considering the potential and demand, vegetables/premium price fetching crops are advocated to be included in the diverse cropping sequences to identify component crops with high SOC sequestration potential and good CMI (an indicator of soil quality).

2.2. Treatment details, experimental design, and crop management

The experiment was conducted in fixed plots for five consecutive years (2013–18) at the Research Farm, Indian Council of Agricultural Research- Research Complex for North Eastern Hill Region, Sikkim Centre, Gangtok, Sikkim, India. The experimental site was situated at 27°32′ N latitude and 88°60′ E longitude and altitude of 1350 m above mean sea level (MSL). The experimental site has a mid-hill temperate climate. The mean annual maximum and minimum temperatures are 22 °C and 4 °C, respectively. Rainfall starts from pre-monsoon in February-March and reaches its peak during the monsoon in July recording the maximum monthly average of 650 mm. Annual precipitation is about 3065 mm of which 75–80% is received from June to September. The Haplumbrept soil of the experimental site is sandy loam in texture and free from hardpan. A composite soil sample from 0 to 0.4 m was collected for initial soil analysis before laying the experiment. The pre-experiment values of different soil parameters (0–0.4 m) are presented in Appendix 1.

Seven cropping sequences *viz.,* rice–fenugreek (*Trigonella foenumgraecum*, green vegetable)–maize (R-F-M); rice–vegetable pea-maize (R-Vp-M); rice–coriander (*Coriandrum sativum*, leaves)–cowpea (R-C-Cp); rice–fenugreek (green vegetable)–baby corn (*Zea mays*) (R-F-Bc); rice–broccoli (*Brassica oleracea* var. italica)–Sesbania (*Sesbania aculeate*, green manuring) (R-B-S); rice–buckwheat (*Fagopyrum esculentum*) (R-Bw) and rice–maize (R-M) were tested in a Completely Randomized Block Design. The treatments were replicated thrice in a plot size of 4 m × 3 m. To avoid intermixing of manure/compost between the plots individual plots were manually prepared with the help of a spade in each cropping season. The amount and types of organic manure/compost applied in the experiment are described in Appendix 2. Nutrient content in farmyard manure (FYM) and vermicompost (VC) was 0.50 ± 0.02 and 1.95% ± 0.04 N, 0.22 ± 0.06 and 0.88% ± 0.04 P and 0.54 ± 0.05 and 1.25% ± 0.03 K, respectively. Recommended organic cultivation practices were followed to raise the crops. Well decomposed FYM and VC were applied and incorporated into the soil at the time of last tilling before the sowing/planting of each crop. The details of important agronomic activities and inputs used under various cropping sequences are presented in Appendix 2. The nursery of the rice crop was raised in the first fortnight of June and transplanted in the first week of July in the main field during all the years. Irrespective of the treatments, rice was harvested in the first fortnight of November every year. The summer/winter-season crops were sown/planted and harvested at different times during the investigation and the sowing/ planting and harvesting schedule is presented in Fig. 2.

2.3. Harvesting and biomass measurement

All the crops were harvested manually. Grain yield of rice, buckwheat, and maize was reported at 14% moisture content. Green pods of vegetable pea and cowpea were harvested and all the above-ground biomass was incorporated into their respective plots. Fenugreek and coriander were harvested with the help of an iron sickle and the entire biomass was removed from the field as the entire above-ground biomass of these crops was economically important. Broccoli curd was harvested and leftover plant material was incorporated in the respective plots. Being a green manure crop, the total biomass of *Sesbania* was incorporated manually between 50 and 55 days after sowing (DAS) in the respective plots. Baby corn fresh ear was harvested at the first appearance of silk. After five to six plucking of fresh baby corn cobs, the entire biomass was removed from the field for fodder purposes. The

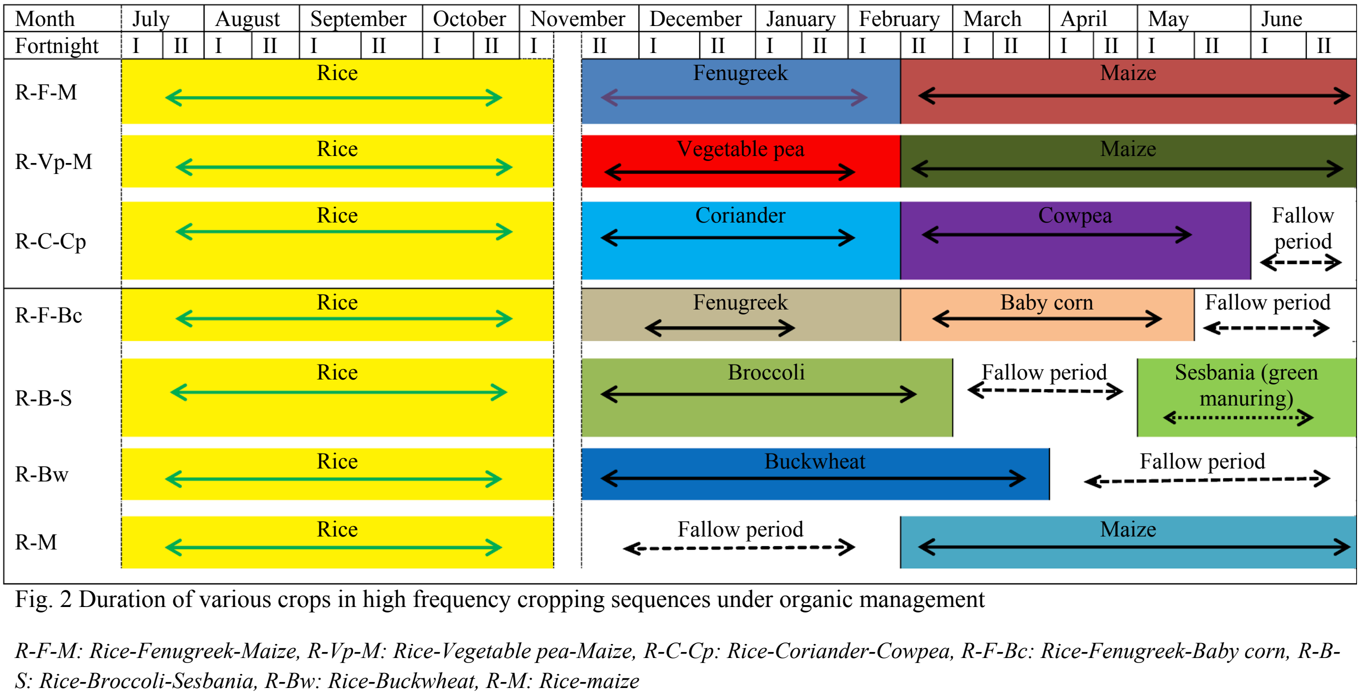

Fig. 2. Duration of various crops in high frequency cropping sequences under organic management. *R-F-M: Rice-Fenugreek-Maize, R-Vp-M: Rice-Vegetable pea-Maize, R-C-Cp: Rice-Coriander-Cowpea, R-F-Bc: Rice-Fenugreek-Baby corn, R-B-S: Rice-Broccoli-Sesbania, R-Bw: Rice-Buckwheat, R-M: Rice-maize.*

straw/stover weight of all the crops in the sequences was measured from one square meter area in each plot after oven drying at 60 ± 1 °C temperature. Root samples were obtained at harvest of rice, maize, buckwheat, fenugreek, coriander, baby corn, cowpea and before the incorporation of *Sesbania*. Root samples were obtained from 40 cm depth in each season for each crop using a core sampler (5.8 cm height and 5.4 cm inner diameter). The roots were cleaned off the soil, and the dead organic debris and the fresh roots were oven-dried at 70 ± 1 °C till constant weight. The dry biomass was determined and converted into Mg ha^{-1}. Biomass input was multiplied with a factor 0.40 assuming that the C concentration is 40% C in residues of the crops (Bolinder et al., 2007). The estimated amount of biomass and C inputs applied under different sequences from various sources is presented in Appendix 3.

2.4. Soil sampling and processing

After five cropping cycles, soil samples were collected using 10 cm scaled soil cores with 5.4 cm inner diameter at 0–10, 10–20 and 20–40 cm soil depth from each plot. Sampling was done randomly at four places from each plot and then blended to constitute a representative soil sample from each depth. The container and soil samples were weighed on an electronic balance in the laboratory before and after drying. The gravimetric moisture content in the soil was calculated by oven-drying the soil at 105 ± 1 °C for 24 h. Soil bulk density (ρ_b) was determined by the core method (Blake and Hartge, 1986) for each depth (0–10, 10–20 and 20–40 cm depths) after oven drying at 105 ± 1 °C. The collected bulk soil samples were air-dried at room temperature (25 °C), clods were broken by giving gentle strokes with a hammer, and the foreign material was removed, and sieved with 2 mm sieve. The processed soil samples were stored in airtight plastic bags for analysis of soil organic carbon and chemical properties. One part from each representative fresh soil samples from each plot (0–10 cm depth) was stored at freezing temperatures for analyzing soil microbial biomass carbon (SMBC), dehydrogenase activity (DHA), fluorescein diacetate (FDA) and acid phosphatase activities.

2.5. Analysis of carbon fractions and computation of C indices

Soil organic C content of samples was determined by the wet oxidation method (Walkley and Black, 1934). Carbon fractions at varying degrees of oxidation were determined according to the modified Walkley and Black method (Chan et al., 2001). Soil sample weighing 0.5 g was placed in each of a set of 4 oven-dried Erlenmeyer flasks of 250 ml capacity. Then 10 ml of 1 N $K_2Cr_2O_7$ followed by 2, 5, 10 and 20 ml of concentrated H_2SO_4 (98%, sp. Gr. 1.84) was added to each corresponding flasks resulting in 6 N, 12 N, 18 N and 24 N H_2SO_4 (*i.e.* 3, 6, 9 and 12 mol/L of H_2SO_4), respectively in the final oxidizing solution. After 30 min of oxidation, 200 ml of distilled water was added to each flask and the content was titrated with 0.5 N Fe $(NH_4)_2$ $(SO_4)_2$, $6H_2O$ using Phenanthroline as an indicator.

The four soil carbon fractions with decreasing degrees of oxidation were calculated as follows:

- Very labile C fraction (VLC): C oxidized by $K_2Cr_2O_7$ under 6 N H_2SO_4;
- Labile C fraction (LC): C oxidized under 12 N H_2SO_4 – oxidizable C under 6 N H_2SO_4;
- Less-labile C fraction (LLC): C oxidized under 18 N H_2SO_4 – oxidizable C under 12 NH_2SO_4;
- Non-labile C fraction (NLC): C oxidized under 24 N H_2SO_4 – oxidizable C under 18 NH_2SO_4.

Different carbon pools were estimated according to the procedure described in Chan et al., 2001:

- Active carbon pool (AC) = VLC + LC
- Passive carbon pool (PC) = LLC + NLC

Lability index (LI) was derived using very–labile, labile, and less–labile fractions of the total SOC by giving a weightage of 3, 2 and 1 to VLC, LC, and LLC, respectively (Blair et al., 1995; Hazra et al., 2018) (Eq. (1)).

$$\mathrm{LI} = [(\text{very labile C/TOC}) \times 3 + (\text{labile C/TOC}) \times 2 + (\text{less labile C/TOC})1] \times 1 \tag{1}$$

Carbon pool index (CPI) was derived as:

$$\mathrm{CPI} = \text{Sample TOC}(\mathrm{g\,kg^{-1}})/\text{Reference TOC}(\mathrm{g\,kg^{-1}}) \tag{2}$$

where, conventional cropping sequence (rice-maize) was taken for reference TOC value.

Finally, the carbon management index (CMI) was estimated using the following formula:

$$\mathrm{CMI} = \mathrm{CPI} \times \mathrm{LI} \times 100 \tag{3}$$

2.6. Soil chemical analysis

The soils were analyzed for available-N (Alkaline $KMnO_4$ method), available-P (Bray's P_1, 0.03 N NH_4F in 0.025 N HCl, pH 4.65), available-K (1 N NH_4OAc extractable K, pH 7.0), pH and EC described in Prasad et al. (2006).

2.7. Calculation of carbon pools and sequestration

Total C content was determined by the dry combustion method (Nelson and Sommers, 2005) using a TOC analyzer (Model ElementarVario Select, Germany). The SOC pools (Mg ha^{-1}) at 0–10, 10–20, 20–40 and 0–40 cm depths were calculated using the fix depth method (Lee et al., 2009) by using the following equation:

$$\begin{aligned}&\text{SOC or C fraction pool}(\mathrm{Mgha}-1)\\&= \frac{SOC\ concentration\%}{100} x \frac{Depth(cm)}{100} + \frac{\mathrm{pbMg}}{m3} x \frac{\mathrm{Area(1000m2)}}{ha}\end{aligned} \tag{4}$$

Accumulation of SOC was computed using the following equation:

$$\begin{aligned}&SOC\ accumulation(Mgha-1)\\&= FinalSOC\ pool\ (Mgha-1){-}Initial\ SOC\ pool(Mgha-1\end{aligned} \tag{5}$$

$$\begin{aligned}&\mathrm{SOCsequestration(Mgha-1yr-1)} =\\&= \frac{Final\ SOC\ pool(Mgha-1) - Initial\ SOC\ pool\ (Mgha-1)}{year}\end{aligned} \tag{6}$$

The carbon retention efficiency (CRE) is the gain in soil C in relation to carbon input applied was calculated by the following equation:

$$\mathrm{CRI}(\%) = \frac{Final\ SOC\ pool(Mgha-1) - Initial\ SOC\ pool\ (Mgha-1)}{Cumulative\ Cinput\ (Mgha-1)} \tag{7}$$

2.8. Analysis of soil biological properties

The fresh soil samples stored at freezing temperatures were used for analyzing the SMBC, DHA, FDA, and acid phosphatase activities. The SMBC was determined by the chloroform fumigation-extraction (Vance et al., 1987). SMBC was calculated as the difference in C content in the fumigated and non-fumigated sample. It was expressed in µg MBC g^{-1} soil. The DHA was estimated by reducing 2, 3, 5-triphenyl tetrazolium chloride (TTC) (Casida et al., 1964) and its activity was expressed in milligram formazan per gram dry soil per hour. The FDA was estimated as per the method outlined by Green et al. (2006) and its activity was estimated through the production of fluorescein from fluorescein diacetate by the action of hydrolytic enzymes in the soil. The Acid phosphatase activity was measured by the method given by Tabatabai and Bremner (1969). The phosphatase activity in terms of concentration of p-nitrophenol in each sample was calculated by a standard curve of p-nitrophenol in water and was expressed as a mole of p-nitrophenol released per gram of dry soil per hour.

2.9. Statistical analysis

The statistical analysis of all data was performed using the GLM procedure of the SAS 9.4 (SAS Institute, 2003) to analyze the variance and to determine the statistical significance of the treatment effects. The least significant difference (LSD) at $p = 0.05$ was used to compare the cropping sequence means.

3. Results and discussion

3.1. Agronomic productivity and biomass-carbon recycling

Agronomic yield (average of five years) of various crops varied significantly under different cropping sequences. Rice grain yield (3.91 Mg ha^{-1}) was significantly higher under the R-C-Cp sequence than those under R-Bw (3.49 Mg ha^{-1}) and R-M (3.14 Mg ha^{-1}) sequences (Appendix 3). The inclusion of legumes increased the grain yield of rice by 13.4 to 24.6% under different sequences over the R-M sequence (3.14 Mg ha^{-1}). Positive effects of legumes in cropping sequences on the yield of succeeding crops and soil productivity were ascribed to mobilization of soil phosphorus (Nuruzzaman et al., 2005), atmospheric nitrogen fixation (Yadav et al., 2013b) and improvement of the soil health to support crop growth (Kopke and Nemecek, 2010). Jensen et al. (2004) reported 17 to 21% yield increment of cereals under legume-based sequences over mono-cropping of cereals. The agronomic yield of the 2nd crop after rice varied significantly under different sequences (Appendix 3). Broccoli produced the highest marketable agronomic yield (8.32 Mg ha^{-1}) followed by fenugreek (7.62 to 7.70 Mg ha^{-1}), vegetable pea (6.88 Mg ha^{-1}) and coriander (6.5 Mg ha^{-1}) while the lowest was produced by buckwheat (0.83 Mg ha^{-1}) and maize (3.14 Mg ha^{-1}) grain yield. Among the 3rd crops, cowpea grown in the R-C-Cp sequence produced greater agronomic yield (6.62 Mg ha^{-1}) followed by baby corn (6.58 Mg ha^{-1}) under R-F-Bc than those of maize grain yield (3.48 to 3.58 Mg ha^{-1}) grown under R-F-M and R-Vp-M (Appendix 3).

System productivity in terms of rice equivalent yield (REY; as rice was the base crop) was maximum under R-F-Bc (21.52 Mg ha^{-1}) followed by R-C-Cp (17.65 Mg ha^{-1}) and R-F-M (17.44 Mg ha^{-1}) while the lowest was produced by R-Bw (4.474 Mg ha^{-1}) and R-M (7.06 Mg ha^{-1}). The higher system productivity of R-Vp-M sequences could be due to their higher agronomic yields coupled with the higher market price of the crops in this particular sequence (Babu et al., 2016). Here, the study could draw an inference that R-F-Bc and R-C-Cp (17.65 Mg ha^{-1}) cropping sequences had more system productivity and profitability under organic conditions.

Average plant biomass (sum of above and below ground) added into the soils was higher under R-Vp-M followed by R-B-S and R-C-Cp sequences than those added by other cropping sequences (Appendix 3). Many studies have established that production of high above and below-ground biomass and its retention play a substantial role in SOC restoration (Deng et al., 2017; Yadav et al., 2019) and improvement of the soil health (Das et al., 2013b). The R-Vp-M sequence in our study added the highest total biomass (plant biomass above and below ground + manure mass) and carbon inputs (sum of five years) as compared to other cropping sequences. R-Vp-M sequence added 119.5% and 67.9% higher C to the soil than those added by the R-Bw and R-M sequences. Recycling of biomass into the soil is indispensable for restoring the SOC pool and maintaining the equilibrium in the soil-crop-atmosphere band (Schulze, 2006). The variation in the amount of biomass and the resultant C in the present study was mainly due to the variation in the biomass productivity of a particular crop under different cropping sequences. We observed a significant positive correlation between the SOC content and the addition of C input in soil. Such a correlation was also reported by Liao et al. (2014).

3.2. Soil organic carbon fractions

The SOC fractions varied significantly among different cropping sequences (Appendix 4). In general, values of carbon fractions e.g., VLC, LC, LLC, and NLC were more in 0–10 cm depth than in 10–20 and 20–40 cm depth. The range of SOC fractions varied under different sequences- for example, VLC from 4 to 4.6 g kg soil^{-1}, LC 3.1 to 3.6 g kg soil^{-1}, LLC 2.5 to 2.8 g kg soil^{-1} and NLC 3.7 to 4.1 g kg soil^{-1} in 0–10 cm depth across all the cropping sequences.

The higher proportion of different carbon fractions in the top 10 cm might be due to higher microbial activity arising from the addition of mineralizable organic matter in the form of crop residues and root exudates (Naik et al., 2017). The soil under the R-B-S cropping sequence had the highest VLC (4.6 g kg soil^{-1}) followed by R-Vp-M while the lowest was observed in the soil under the R-Bw sequence. However, LC was the highest in soil under the R-C-Cp sequence (3.6 g kg soil^{-1}). However, the LLC indicated slightly different trends; the soil under R-Vp-M, R-C-Cp and R-Bw cropping sequences had more LLC (2.8 g kg soil^{-1}) than in the soils under other cropping sequences. Inclusion of legumes in the cropping sequence might be responsible for increasing the amount of various C fractions in the soil possibly due to the emanation of fathomable molecules by legumes and by the supply of fresh protein-rich biomass to microbes (Campbell et al., 1999). On the other hand, NLC had shown almost a similar trend as demonstrated by VLC among the various cropping sequences. Relative proportion of various carbon fractions followed the order of VLC (30.2%) > NLC (27.6%) > LC (23.4%) > LLC (18.9%) in the top 10 cm depth irrespective of the cropping sequences. In 10–20 cm depth, various fractions of C had shown a trend similar to those under 0–10 cm depth. VLC ranged from 3.2 to 3.9 g kg^{-1}soil, LC ranged from 2.8 to 3.1 g kg^{-1} soil, LLC ranged from 2.4 to 2.9 g kg soil^{-1}, and NLC ranged from 3.1 to 3.7 g kg^{-1} soil across the cropping sequences in 10–20 cm depth. In the lower soil layer (20–40 cm depth), VLC and LC significantly differed under different cropping sequences; conversely, LLC and NLC did not vary significantly among the sequences. The main cause of higher deposition of various carbon fractions in the top 10 cm soil attributed to the addition of higher C inputs in the upper layer in comparison to the lower depths. Furthermore, low nutrients availability and microbial activity could have decelerated the root growth resulting in the addition of a lower amount of C inputs at lower depths (Ingram and Fernandes, 2001). The variation in SOC between the soil depths in different cropping sequences could be attributed mainly due to variation in addition of root biomass (Ganeshamurthy, 2009). In these above depths, soils under triple cropping sequences (R-B-S and R-C-Cp) had higher VLC and LC than those in soils under double cropping sequences (Appendix 4). Our results corroborated the findings of other studies where SOC increased with the increasing number of crops in a sequence (Hutchinson et al., 2007) over single or double cropping on the same piece of land.

3.3. Soil organic carbon management indices

The SOC indices namely LI, CPI, and CMI which are good indicators of soil productivity and biological health, changed significantly with the inclusion of more crops and replacement of maize with other crops like legumes and high-value vegetables in the present study (Appendix 4). The LI and CPI did not vary significantly in 0–10 and 20–40 cm soil depths among the cropping sequences. However, in 10–20 cm depth, LI and CPI were significantly higher in sequences where the second crop was not maize. The soil under R-M had the lowest LI (1.41). The trend of CPI was not consistent among the cropping sequences but had shown a similar trend in all the depths of sampling. The CPI was the highest under R-C-Cp (0.64) and R-B-S (0.64), while it was the lowest under R-F-Bc (0.59) in 0–10 cm depth. The CPI ranged from 0.58 to 0.63 in 10–20 cm depth with its maximum value recorded under the R-C-Cp (0.63) and R-B-S (0.63). Effect of cropping sequences on CPI in 20–40 cm depth was not significant (Appendix 4). Although statistically, a significant difference was lacking, the R-B-S sequence had numerically the highest CPI (0.58) than those under other cropping sequences. The CMI of soil was significantly higher under R-B-S (100.9%) than those under R-B (90.9%), R-F-Bc (92.1%) and R-M (92.8%) and remained at par with the rest of the cropping sequences (Appendix 4) in top 10 cm soil. In 10–20 cm depth, the CMI was significantly higher under the R-B-S (97.2%) followed by R-C-Cp (96.2%) and R-Vp-M (95.3%). However, in 20–40 cm depth, the CMI was significantly greater under R-C-Cp (89.6%) followed by the R-B-S sequence (88.6%). CMI is an indicator of soil C restoration and soil health; the larger values show soil C rehabilitation and improvement in soil health, whereas the smaller values indicate degradation of the soil C and soil health (Blair et al., 1995). It was observed that R-Bw and R-M sequences had significantly lower rates of soil C rehabilitation than other high-frequency cropping sequences, suggesting that inclusion of legumes/green manure crops in the sequence led to better C sequestration in soils than the other cropping sequences under organic farming. In general, CMI was greater in the top 10 cm soil than that of 10–20 and 20–40 cm depths across the cropping sequences which might be due to the higher accumulation of soil carbon in the topsoil. Overall results suggested that crop intensification by including legumes (cowpea, sesbania, fenugreek, and vegetable pea) in rice-based cropping sequences improved the CMI under organic farming. Improvements in the SOC and CMI with higher cropping intensity and optimized cropping sequences had been reported in other parts of India in inorganic production systems (Ghosh et al., 2012; Ghosh et al., 2019; Nandan et al., 2019).

3.4. Soil organic carbon fraction pools

Cropping sequence engineering had a significant effect on the size of various labile and non-labile pools of SOC *viz.*, VLC, LC, LLC and NLC (Appendix 5). The highest amount of VLC pool (6.02 Mg ha^{-1}) was reported under the R-B-S in 0–10 cm soil depth while, in 10–20 and 20–40 cm soil depth, the VLC pool was the highest under R-C-Cp sequence (5.19 and 8.78 Mg ha^{-1}, respectively).

Differences in carbon pools in soils under various cropping sequences was attributed to dissimilar root exudation patterns, amount and type of C inputs added by the crops in soil (Campbell et al., 1999). The soil under the R-B sequence had the lowest VLC pool (5.4 Mg ha^{-1}) in 0–10 cm depth. However, the VLC pool was the lowest under the R-M (4.35 Mg ha^{-1}) sequence in 10–20 cm depth and under R-F-M (8.23 Mg ha^{-1}) in 20–40 cm. This kind of variation could be attributed to the rooting behavior of crops understudy because legumes generally had deeper and more vigorous root systems than other annual crops. The labile C pools ranged from 4.11 to 4.70 Mg ha^{-1} in 0–10 cm, 3.81 to 4.09 Mg ha^{-1} in 10–20 cm and 7.40 to 7.96 Mg ha^{-1} in 20–40 cm across the cropping sequences. However, different cropping sequences had different trends of labile C pools at various soil depths. R-C-Cp sequence had a significantly higher labile C pool in 0–10 cm depth than in soils under R-M (4.11 Mg ha^{-1}), R-F-Bc (4.21 Mg ha^{-1}) and R-Bw (3.32 Mg ha^{-1}). R-Vp-M sequence (4.09 Mg ha^{-1}) had a significantly higher labile C pool than the soils under R-M (3.81 Mg ha^{-1}), R-F-Bc (3.91 Mg ha^{-1}) and R-Bw (3.95 Mg ha^{-1}). However, the effects of the R-C-Cp sequence were much more visible in 20–40 cm soil depth which had significantly higher labile C pool (7.96 Mg ha^{-1}) than the other cropping sequences. As legumes have a tap root system and secrete more exudates as compared to other crops (Chen et al., 2018), they might have contributed to the higher labile pool. Comparatively higher NLC pool than VLC pool at lower depths was possibly due to the rapid conversion of crop residues and root biomass and labile C fractions to recalcitrant form (Sreekanth et al., 2013). Furthermore, at lower depths, there could be a possibility of labile C fractions chemically stabilized with silt and clay fractions of soil in the form of more stable C (Lutzow et al., 2006) thereby resulting in higher NLC pool at the lower depths. LLC pool had the highest value under the R-Bw sequence

(3.78 Mg ha^{-1}) in 0–10 cm depth. The lowest values of less labile C pool followed the trend similar to the very labile C pool in 10–20 and 20–40 cm depth and could be related to higher exudation of C substances into the soils by the legumes as compared to other crops (Ganeshamurthy, 2009). Blanco-Canqui et al. (2017) reported that the cultivation of various cropping sequences under organic farming had limited potential to accumulate SOC in greater depths of the soil profile as compared to conventional production systems. On the contrary, we observed a high amount of LLC pool under the R-B-S sequence (3.86 Mg ha^{-1}) at 10–20 cm, and under R-F-M (7.13 Mg ha^{-1}) and R-C-Cp (7.13 Mg ha^{-1}) at 20–40 cm soil depth. Generally, the amount of NLC pool present in the soils, irrespective of cropping sequences was slightly lower than the VLC pool but higher than LC and LLC pool in all the depths. Among the cropping sequences, R-C-Cp (5.46 Mg ha^{-1}) had the highest NLC pool in 0–10 cm, R-Vp-M had the highest values in 10–20 cm and R-B-S had greater values in 20–40 cm depth than the soils in other cropping sequences (Appendix 5). In the total profile depth of 0–40 cm (taking the cumulative values), the highest proportions was of VLC pools (28.4–28.8%) followed by NLC pools (26.3–26.9%), LC pool (23.4–24.4%), and LLC pool (20.6–21.3%) across all the cropping sequences after completion of five cropping cycles. The R-B-S sequence (28.8%) had the highest proportion of the VLC pool while the lowest was under R-F-M (28.4%) sequence. The share of the LC pool was the highest in soil under the R-Vp-M cropping sequence. However, R-Bw (21.3%) and R-M (26.9%) had the highest proportion of LLC and NLC pools, respectively (Fig. 3).

3.5. Active and passive soil organic carbon pools

The AC pool of soil was significantly affected by various cropping sequences in all the depths of measurement. In general, the AC pool was higher in 20–40 cm depth than those in 0–10 and 10–20 cm depths. In particular, the soil under the R-B-S sequence had a higher AC pool (10.61 Mg ha^{-1}) followed by R-C-C (10.38 Mg ha^{-1}), R-F-M (10.18 Mg ha^{-1}) and R-V-M (10.18 ha^{-1}) (Appendix 6). Higher SOC pools with intensified cropping systems were also noted by Blanco-Canqui et al. (2017). The AC pool in 10–20 and 20–40 cm depths had shown a trend similar to that of 0–10 cm depth (Appendix 6) across the cropping sequences. Replacement of maize (2nd crop) with other crops and the introduction of 3rd crop in the cropping sequence increased the AC pool by 1.1 to 5.8% over the R-M sequence in 0–40 cm depth. The PC pool was the highest in soil under the R-C-Cp sequence (9.15 Mg ha^{-1}) in 0–10 cm depth. However, in 10–20 and 20–40 cm depth, the soil under the R-B-S sequence had the highest PC pool (8.66 and 15.4 Mg ha^{-1}, respectively). The soil under the R-M cropping sequence had the lowest PC pool in all the depths (Appendix 6). Similar to the AC pool, the PC pool was increased by 0.9 to 5.9% with the replacement of maize (2nd crop) with other legumes and vegetable crops like fenugreek, vegetable pea, coriander, etc. Furthermore, cropping sequences where the third crops (e.g., cowpea, baby corn, *sesbania* etc.) were added, with a consequent crop intensification, showed a positive effect on AC than on PC and substantially improved the proportion of AP in the soil (0–40 cm depths) (Fig. 4).

This could be strong evidence that legumes had conserved soil C and arrested the losses of easily oxidizable carbon from the soil. Growing of leguminous cover crops for two years had previously reported an increase in the SOC content of degraded soil under the subtropical climate (Yadav et al., 2019). Among the cropping sequences, the R-C-Cp had the highest proportion of AC pool (52.8%) than the other cropping sequences while the PC pool was higher under R-F-B (47.8%) as compared to other sequences at 0–40 cm depth (Fig. 4) suggesting that the intensified cropping sequences promoted more SOC in AP and less SOC

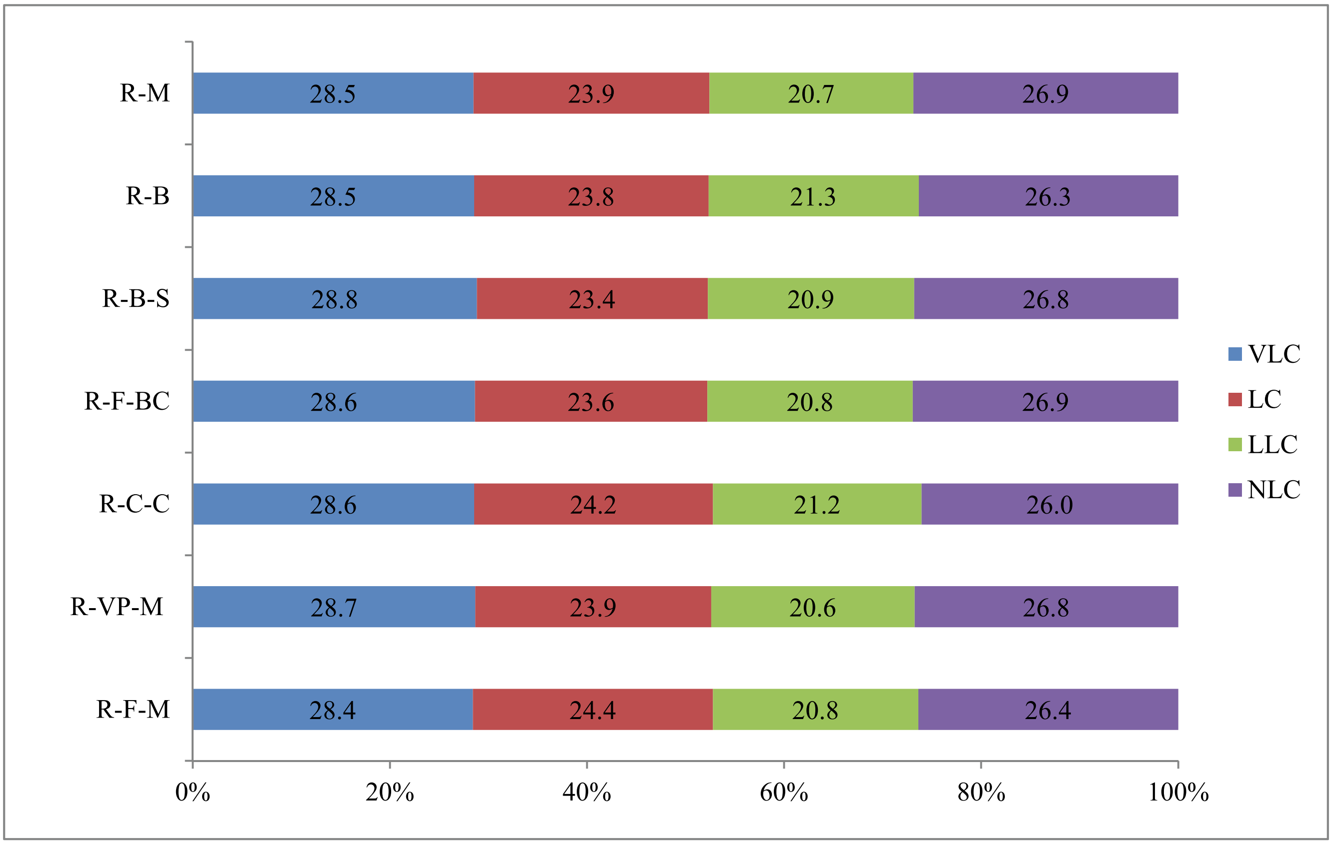

Fig. 3. Effect of cropping sequences on different carbon fractions under organic managementafter five cropping cycles in the total profile depth (0–40 cm). *R-F-M: Rice-Fenugreek-Maize, R-Vp-M: Rice-Vegetable pea-Maize, R-C-Cp: Rice-Coriander-Cowpea, R-F-Bc: Rice-Fenugreek-Baby corn, R-B-S: Rice-Broccoli-Sesbania, R-Bw: Rice-Buckwheat, R-M: Rice-maize.*

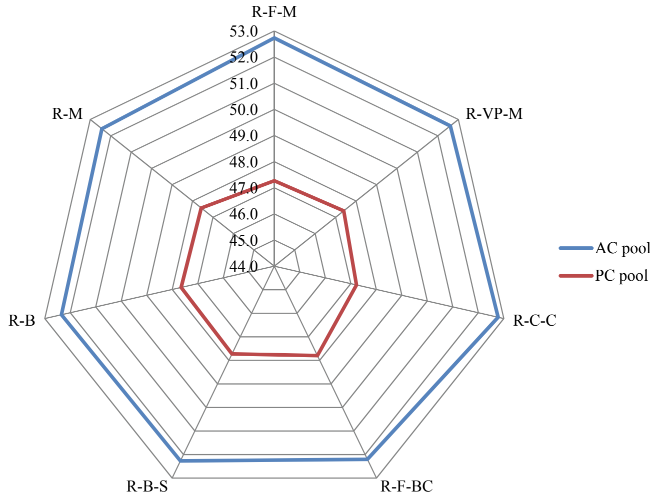

Fig. 4. Percent contribution of various cropping sequences in active and passive carbon pools under organic management. *R-F-M: Rice-Fenugreek-Maize, R-Vp-M: Rice-Vegetable pea-Maize, R-C-Cp: Rice-Coriander-Cowpea, R-F-Bc: Rice-Fenugreek-Baby corn, R-B-S: Rice-Broccoli-Sesbania, R-Bw: Rice-Buckwheat, R-M: Rice-maize.*

in PC after five years of crop cycle. Inclusion of coriander and cowpea in the rice system increased the allocation of AP over the other sequences, while the inclusion of fenugreek and baby corn increased the allocation of PC over the other cropping sequences. Thus, it could be suggested that R-C-Cp and R-F-M were the best cropping sequences in enhancing both labile and non-labile carbon in the soil under organic farming. Plant roots capture and transfer the atmospheric C into the soil in the form of C containing compounds and subsequently store in the soil for longer periods in the form of AC and PC. Root exudates and lysates contribute significant quantities of C deposition in various soil layers but the amount of C deposited in the soil layers varies with the type of crop and pedo-climatic conditions. We believe these factors might be the major regulating factors resulting in wide variability in AC and PC pools as well as their proportion in different cropping sequences in our study also.

3.6. *Total soil organic carbon pool, carbon accumulation, sequestration and retention efficiency*

The SOC storage varied significantly in different depths (0–10, 10–20, 20–40 and 0–40 cm) under diverse cropping sequences (Table1). The TOC is the balance between C inputs and C losses (Benbi et al., 2015). In the present study, the SOC pool varied from 18.4 to 19.5 Mg ha^{-1} in 0–10 cm, 16.9 to 17.8 Mg ha^{-1} in 10–20 cm, 30.7 to 31.8 Mg ha^{-1} in 20–40 cm and 66.2 to 69.2 Mg ha^{-1} in 0–40 cm depth, across the cropping sequences. Soils under the R-B-S and R-C-Cp sequences had the highest organic carbon pool in all the depths e.g., 19.5 Mg ha^{-1} in 0–10 cm, 17.8 Mg ha^{-1} in 10–20 cm, 31.5 to 31.8 Mg ha^{-1} in 20–40 cm and 68.9 to 69.2 Mg ha^{-1} in 0–40 cm depth. The total SOC in the 0–40 cm profile was enhanced by 4.2 to 5.0% in R-B-S over the R-F-Bc, R-F-M and R-Bw cropping sequences. The cropping sequences with the inclusion of *sesbania* and cowpea were most effective in enhancing the amount of total carbon after completion of five cropping cycles. These findings suggested that the crop sequence with green manure was superior in accumulating SOC relative to conventional cropping sequences (R-M) without green manure crops. The R-B-S sequence accumulated 2.8 times (5.1 Mg ha^{-1}) more total SOC in comparison to that of R-F-B (1.8 Mg ha^{-1}) which had the lowest SOC pool accumulation (Table1).

Under diverse management conditions, crop diversification/intensification usually leads to more C pool than monotonous sequences (Blanco-Canqui et al., 2010). Cropping sequences engineering promoted SOC translocation because the greater depths of the soil profile had greater potential to sequester SOC (Lorenz and Lal, 2005). That might be the reason for which R-B-S sequestrated 2.5 times (1.0 Mg ha^{-1} yr^{-1}) more C. The legumes having low C: N ratio might be additional positive factors that had contributed significantly to C stabilization (Yadav et al., 2019) as compared to the other crops under R-F-Bc (0.4 Mg ha^{-1} yr^{-1}) sequence. The value of C accumulation and sequestration of R-B-S was at par with R-C-Cp and had values significantly higher than the rest of cropping sequences (Table 1). In many instances, the cultivation of high biomass producing crops in intensified cropping sequences resulted in greater soil C sequestration (Nieder and Benbi, 2008). The CRE was significantly higher under R-C-Cp (15.1%) and R-B-S (14.9%) sequences than those of other cropping sequences (Table 1) as both the cropping sequences had legume component which had low C:N ratio that contributed effectively towards greater retention of carbon in the soil. Retention efficiency was the lowest under the R-F-Bc cropping sequence which might be due to clean cultivation practices (removal of entire biomass of fenugreek and baby corn from the field). The results of the study would be useful to researchers to understand the differential influence of cropping sequences on SOM quality and soil C sequestration in an organic production system. Soil C pools are a consequence of the balance between C inputs and C losses. Intensified cropping sequences in the present study might have influenced the SOC storage through many processes like its effect on SOM decomposition kinetics, as intensified cropping provided soil cover almost throughout the year leading to an increase the net annual C input.

3.7. *Biochemical properties of soil*

Soil pH and electrical conductivity (EC) did not change significantly after completion of five years of various cropping sequences (Table 2). Despite the lack of statistical difference, the soil under the R-B-S had the

Table 1
Effect of organically managed cropping sequences on soil carbon pool, accumulation, sequestration and retention efficiency (CRE) after five cropping cycles.

CS	Soil organic carbon pool (Mg ha^{-1})				Total soil organic carbon accumulation (Mg ha^{-1})	Soil organic carbon sequestration (Mg ha^{-1})	CRE (%)
	0–10 cm	10–20 cm	20–40 cm	0–40 cm			
R-F-M	18.6	17.1	30.7	66.4	2.3	0.5	9.4
R-Vp-M	19.1	17.4	30.7	67.3	3.2	0.6	8.7
R-C-Cp	19.5	17.8	31.5	68.9	4.8	1.0	15.1
R-F-Bc	18.4	16.9	30.7	65.9	1.8	0.4	7.5
R-B-S	19.5	17.8	31.8	69.2	5.1	1.0	14.9
R-Bw	18.5	16.9	30.9	66.2	2.1	0.4	12.7
R-M	18.8	17.0	31.0	66.7	2.6	0.5	12.3
SEm ±	0.3	0.3	1.1	1.0	0.2	0.1	0.7
LSD (P = 0.05)	0.7	0.8	2.8	2.4	0.4	0.1	1.8

CS: Cropping sequences, R-F-M: Rice-Fenugreek-Maize, R-Vp-M: Rice-Vegetable pea-Maize, R-C-Cp: Rice-Coriander-Cowpea, R-F-Bc: Rice-Fenugreek-Baby corn, R-B-S: Rice-Broccoli-Sesbania, R-Bw: Rice-Buckwheat, R-M: Rice-maize

Table 2
Effect of organically managed cropping sequences on soil biochemical properties after five cropping cycles.

CS	pH	EC (dSm^{-1})	Microbial biomass carbon (μg MBC g^{-1} soil)	Fluorescin di acetate (μg FDA g^{-1} soil h^{-1})	Dehydrogenase activity (μg TPFg^{-1} soil h^{-1})	Acid phosphatase (μg p-nitrophenol g^{-1} soil h^{-1})
R-F-M	5.80	2.40	333.2	43.2	13.1	2.41
R-Vp-M	5.70	2.30	341.3	48.0	18.1	2.35
R-C-Cp	5.90	2.10	355.1	51.2	21.0	2.45
R-F-Bc	5.80	2.50	335.1	44.1	14.2	2.43
R-B-S	6.10	1.80	361.2	54.0	24.2	2.52
R-Bw	5.50	2.20	332.1	41.2	11.3	2.21
R-M	5.53	2.60	321.0	36.1	10.3	2.11
SEm ±	0.27	0.13	4.2	2.4	1.3	0.05
LSD (P = 0.05)	0.67	0.34	10.6	6.0	3.4	0.11

CS: Cropping sequences, R-F-M: Rice-Fenugreek-Maize, R-Vp-M: Rice-Vegetable pea-Maize, R-C-Cp: Rice-Coriander-Cowpea, R-F-Bc: Rice-Fenugreek-Baby corn, R-B-S: Rice-Broccoli-Sesbania, R-Bw: Rice-Buckwheat, R-M: Rice-maize.

maximum pH (6.10) and minimum EC (1.8 dS m^{-1}) than the soils of the rest of the cropping sequences. The SMBC was significantly higher under R-B-S (361.2 μg SMBC g^{-1} soil) and R-C-Cp sequence (355.1 μg SMBC g^{-1} soil) (Table2). It reinforced the inference that the legumes could be a more sustainable alternative than non-legumes for inclusion in intensified cropping sequences. Inclusion of legumes in cropping sequences increased the inputs of biologically fixed N in the soil leading to an increase in available N, occluded more P and enhanced microbial activities (Kirkegaard et al., 2008; Monti et al., 2019). Furthermore, legume exudates contain N-enriched compounds that enter the soil that accelerates mineralization and stimulates microbial activity (Latati et al., 2017; Monti et al., 2019). The FDA, DHA and acid phosphatase (AP) had similar trends as that of SMBC across the cropping sequences (Table 2). Changes in quantity and quality of crop residue under different cropping sequences might have influenced microbial population and activity through changes in substrate supply, aeration status, and associated soil physical properties (Choudhary et al., 2018). This was demonstrated by changes in soil DHA activity which reflected the total oxidative activities of soil microorganisms and the intensity of microbial metabolism in soil (Alef and Nannipieri, 1995). The R-B-S and R-C-Cp sequences increased the FDA by 49.6 and 41.8%, respectively over the R-M cropping sequence. Similarly, DHA increased by 135.0 and 103.9% in soils under R-B-S and R-C-Cp sequences, respectively over the R-M cropping sequence. Continuous mono-cropping on the same piece of land degraded soil biological health (Zuber et al., 2015; Nunes et al., 2018) whereas, inclusion of legumes in cropping sequences increased the OM content, microbial biomass, and enzymatic activities (Sharma et al., 2013; Kinoshita et al., 2017). However, the magnitude of increase in AP was less as compared to FDA and DHA, although it increased by 19.4 and 16.1% in soil under R-B-S and R-C-Cp cropping sequences, respectively over the R-M cropping sequence.

4. Conclusions

The capacity of agro-ecosystems to supply soil ecosystem services is mainly influenced by type's crop diversity and nutrients management practices, and it is well known the highest delivery of environmental benefits from organic in comparison to conventional farm management (Boone et al., 2019). The five-year investigation proved the hypothesis that increasing the frequency of legumes and high-value vegetable crops in rice-based cropping sequences under organic farming could increase the biomass input, soil carbon storage, and the CMI. Induction of more number of crops, mainly legumes in R-M sequences, was effective in enhancing several ecosystem services provided by soil such as C recycling, SOC content, C pool, and sequestration rate, CMI, CPI and CRE in soils under organic farming. The addition of *Sesbania* (green manure) as a third crop in R-C-S sequence, cowpea in R-C-Cp, vegetable pea in R-Vp-M and fenugreek in R-F-M was effective in doubling the total C accumulation and C sequestration rate and provided three times higher system productivity over conventional R-M sequence in organic farming. Therefore, the present study inferred that sustainable intensification of rice-based systems through *Sesbania* (green manure), cowpea, vegetable pea and fenugreek under organic farming can improve system productivity and enhance the soil organic carbon pool. In this context, the "4 per mille Soils for Food Security and Climate" launched at the COP21 has the aim to offset about 30% of the global emissions of greenhouse gases related to human activities by increasing the global soil organic matter stocks. This target needs a collaborative approach among farmers, through sustainable management practices (organic farming), scientists, by the results of innovative researches, and policymakers for their contribution in terms of policies and market regulations (Minasny et al., 2017). Therefore, management of crops from organic agriculture aimed at improving soil ecosystem services, in contrasting degradation of soil health and the decline of SOC, can also have positive effects on crop productivity in the eastern Himalayan region of India as well as all over the world.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.106292.

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