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# Organic material inputs are not essential for paddy soil carbon sequestration

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## ABSTRACT

Carbon (C) emission as CO<sub>2</sub> to the atmosphere at higher rates leads to global warming and climate change. Storing atmospheric C in the soil is achieved by soil C sequestration (SCS). In the phase of degradation of natural ecosystems, agroecosystems might play a crucial role in SCS. However, conventional methods like bulky organic material inputs in agricultural SCS will not be sufficient in the future scenarios of rapid CO<sub>2</sub> emissions to the atmosphere. Also, preserving soil C stocks for prolonged periods has been one of the biggest challenges in agricultural SCS. In recent studies, the microbial interventions like biofilm biofertilizers (BFBF) have shown its potential in SCS. However, the effect of BFBF in maintaining soil C pools for prolonged periods has not been fully elucidated thus far. Therefore, for the first time, the present study evaluated the potential of BFBF in SCS and maintaining C pools in lowland paddy cultivation in three consecutive cropping seasons. Here, the BFBF practice was compared with the farmers' current practice of chemical fertilizer (CF) alone application in 25 representative sites in four districts having ca. 0.5 Mha of paddy cultivation. In each site, two consecutive, uniformly managed paddy fields (each ca. 0.4 ha) with similar soil characteristics and grain yields were used to evaluate BFBF practice in comparison with farmers' CF practice. The two consecutive field plots in each site were taken as a randomized block design. Four random rice hills and 12 random soil samples (0 to 20 cm depth) were collected at 50% flowering stage from each experimental paddy field to measure the root length and soil parameters, respectively. Soil organic and labile C contents were analyzed to calculate SCS. The results showed that gross C pool (GCP), a portion of which is emitted as CO<sub>2</sub> during tillage, seems to play an important role in increasing the preserved soil C stock with time only in the BFBF practice, whereas it started to decline from the second season in the farmers' CF practice. Moreover, the BFBF practice sequestered up to 15 t stable C ha<sup>-1</sup> season<sup>-1</sup> over the farmers' CF practice showing the potential to mitigate global warming and to gain income through C trading. The increased SCS was due to increased rooting depth and microbial C assimilation in the root-zone soil. The BFBF practice requires only 2.5 l of BFBF ha<sup>-1</sup>, whereas conventional practices need bulky quantities of organic matter inputs to sequester a comparable amount of C. In addition, an increased grain yields up to ca. 25% was observed in the BFBF practice. Therefore, the BFBF practice can be considered as an eco-friendly and economically viable method to replace the farmers' current practice of CF alone application.

## 1. Introduction

Carbon (C) is a vital component that governs the entire biological system of the earth. The C cycle consists of a series of events that are important for sustainable life support. It is directly related to the processes of birth, growth, reproduction, death, and decay, and describes the movement of C as well as its sequestration and release from the sinks (Sedjo and Sohngen, 2012). Atmospheric carbon dioxide (CO<sub>2</sub>) is incorporated into plants through photosynthesis and is distributed among every living being. After death and decay of organisms, C leaves as CO<sub>2</sub>

back to the atmosphere. This has happened for more than half a billion years (Lal et al., 2018). The ocean near the Earth's surface contains the largest active C reservoir (Falkowski et al., 2000). Carbon exchanges between the Earth's biosphere, pedosphere, geosphere, hydrosphere, and atmosphere via various chemical, physical, geological, and biological processes (Avis et al., 2008). Jackson et al. (2018) reported that the global CO<sub>2</sub> emission was 37 Gt C in 2018, and the C store in the biosphere before agriculture began was 3,000 Gt, but now it is only ca. 2,000 Gt, of which ca. 1,580 Gt C is in its top meter. Thus, agriculture has been one of the major contributors to the global CO<sub>2</sub> emission. Its'

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impact is expected to increase in the future due to the rise of the human population, food demand, and per capita consumption (Ramankutty et al., 2018). Carbon enters into long-term soil pools through the decomposition of photosynthetically produced organic matter (OM) and the production of soil organic carbon (Lehmann and Kleber, 2015). Thus, putting so much litter on the soil does not mean that it adds a significant amount of SOC (Berg, 2000). Photosynthesis is the most effective way of withdrawing CO<sub>2</sub> from the atmosphere (Chenu et al., 2019). It gives birth to an important natural phenomenon called "liquid carbon pathway" (Jones, 2008). When the plant produces sugar by photosynthesis, around 40% of the sugar product is transferred into the soil via the root system. It feeds the rhizosphere microorganisms, creating a symbiotic relationship between the plant root system and rhizosphere microbes like bacteria and fungi over the lifespan of a plant. In this manner, the plant is continuously adding sugars to the soil. It means that the amount of C added into the soil by the plant in this way is greater than that from litter decomposition (Plante et al., 2006). The LCP enriches the diversity and abundance of soil microbiota which is important for plant health and productivity (Avis et al., 2008). Here, the soil microbiota gives back their contribution to the ecosystem processes by communicating with each other for maintaining complex soil-plant-animal-microbial network interactions (Seneviratne, 2015). However, excessive chemical fertilizer application by farmers hinders the root-associated microbial diversity and activity (Zhang et al., 2012). Interestingly, application of microbial biotechnological formulations like Biofilm microbial ameliorators [BMAs, e.g. biofilm biofertilizers (BFBFs)] to the soil has been reported to be capable of reinstating the lost biodiversity and sustainability in agroecosystems (Meepegamage et al., 2021). For example, Premarathna et al. (2021) and Rathnathilaka et al. (2022) showed that the BFBF significantly increased rice yield in thousands of hectares, and also augmented soil C possibly via the LCP in the paddy cultivation, suggesting enormous potential of the BFBF in short term SCS. Similar results were observed in the tea cultivations and forest plantations (Premetlake et al., 2011; Chandralal et al., 2019). In agricultural SCS, one of the biggest challenges is to find ways to maintain C pool for prolonged periods (Seneviratne, 2002; Powelson et al., 2016). In this context, investigating the long-term effect of BFBF application on agricultural soil C pools is the need of the hour, which has not been reported thus far. At present, only organic material inputs and conservation agriculture are considered as the major contributors to agricultural SCS (Leifeld and Fuhrer, 2010; Ghimire et al., 2017), which may not be sufficient for the current and possible future scenarios of rapid CO<sub>2</sub> emissions to the atmosphere (Hijbeek et al., 2017; Kirchmann et al., 2009). In the present study, the effect of BFBF on SOC, SLC, SCS, net C pool and gross C pool were evaluated in three consecutive farming seasons, taking paddy as the test crop.

## 2. Materials and methods

The study was carried out by the BFBF Project of the National Institute of Fundamental Studies (NIFS), Sri Lanka. The NIFS has formulated an effective BFBF for lowland paddy cultivation (Premarathna et al., 2021). Previously, a similar BFBF was tested in tea cultivation of Sri Lanka with promising results (Seneviratne et al., 2011). In the current study, the effect of the BFBF on the SCS in lowland paddy soils was studied.

### 2.1. Field sites

The field experiment was carried out in four districts in Sri Lanka, viz. Polonnaruwa (7°56'22.74" N 81°00'9.86" E, average annual temperature 27.3 °C, elevation above sea level 26 m, average annual rainfall 1678 mm), Ampara (7°17'51.14" N 81°40'55.27" E, average annual temperature 27.2 °C, elevation above sea level 37 m, average annual rainfall 1858 mm), Kurunegala (7°44'51" N 80°6'56.2" E, average an-

nual temperature 26 °C, elevation above sea level 116 m, average annual rainfall 2000 mm) and Hambanthota (6°15'N 81°10'E, average annual temperature 28 °C, elevation above sea level 1 m, average annual rainfall 1045 mm). Major soil types are reddish-brown earth (Rhodustalfs) and low humic gley (Tropaqualfs) at Polonnaruwa representing low country dry zone (DL1c), reddish-brown earth, red yellow podzolic, low humic gley and non-calcic-brown in Ampara representing low country dry zone (DL2a), red-yellow podzolic with laterite, low humic gley and non-calcic brown in Kurunegala representing low country intermediate zone (IL3), and reddish-brown earth, red-yellow podzolic, solodize solonetz and regosol in Hambanthota representing low country dry zone (DL5) (Ministry of Mahaweli Development and Environment, 2016). Initial soil properties of the four districts were not significantly different due to high variability, and they ranged pH 5.8–6.4, clay 11.2–13.1%, silt 28.3–31.5%, SOC 0.6–1.1%, soil labile carbon (SLC) 886–1100 mg kg<sup>-1</sup>, soil bulk density 133 Mg m<sup>-3</sup>, soil total nitrogen 0.08–0.15%, soil total phosphorus 0.26–0.35%, and soil potassium 0.21–0.32 cmolkg<sup>-1</sup>.

### 2.2. Experimental design

In all, 25 farmer fields spreading over thousands of hectares in Polonnaruwa (*n* = 9), Ampara (*n* = 8), Kurunegala (*n* = 5) and Hambanthota (*n* = 3) districts with variable soil types were selected to conduct the field experiments. Three composite soil samples were collected from each field for initial soil analyses.

In each site, two consecutive, uniformly managed paddy fields (each ca. 0.4 ha) with similar soil characteristics and grain yields were used to evaluate: (a) BFBF practice [2.5 L ha<sup>-1</sup> of BFBF with 225 kg NPK ha<sup>-1</sup> (Urea 150, TSP 40 and MOP 35 kg ha<sup>-1</sup>)], in comparison with, (b) farmers' chemical fertilizer (CF) practice [425 kg NPK ha<sup>-1</sup> alone (Urea 284, TSP 76 and MOP 66 kg ha<sup>-1</sup>)]. Thus, this study does not compare the two practices considering them as treatments with a control since this is merely a comparison between two existing cultivation practices being used by the farmers. Generally, in the four districts, paddy grain yields of the BFBF practice and the farmers' CF practice averaged to 5860 kg ha<sup>-1</sup> and 4733 kg ha<sup>-1</sup>, respectively (Premarathna et al., 2021). Irrigation water was managed separately in the two fields, without mixing from surrounding fields. The two consecutive field plots in each site were taken as a block in a randomized block design in order to tackle the effect of pseudoreplication (Hurlbert, 1984). The study was done in three consecutive cropping seasons; dry season 2018, wet season 2018/2019, and dry season 2019.

### 2.3. Soil sampling and preparation

Four random rice hills with root zone soil were uprooted carefully at 50% flowering stage from each experimental paddy plot to measure the root length. In addition, 12 random soil samples were collected using soil auger from 0 to 20 cm soil depth and pooled to form 3 composite samples for the soil analysis. Sampling was done in the three cropping seasons. The sample number for each practice and the total number of samples were 100 and 300, respectively. The plant samples were brought to the laboratory of the NIFS, and the root zone soil was removed carefully from the plant roots. The composite soil samples were air-dried, and the subsamples of the air-dried soils were ground and passed through 0.5 mm sieve.

### 2.4. Soil analyses

#### 2.4.1. Soil organic carbon

Briefly, 1 g (± 0.001 g) of the sieved soil was weighed into a labeled 100 ml conical flask. Ten milliliters of 5% K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> (AR, purity ≥ 99.9%) solution was added to it and allowed them to completely absorb by the soil. While gently stirring, 20 ml of concentrated H<sub>2</sub>SO<sub>4</sub>

(AR, purity 98.0%) acid was added to each mixture. Mixtures were allowed to cool. Then, 50 ml of 0.4% BaCl<sub>2</sub> (AR, purity ≥ 99%) was added and swirled to mix thoroughly and kept the samples overnight to obtain a clear supernatant solution. Finally, the supernatants were transferred into 15 ml centrifuge tubes and each sample was measured for absorbance at 600 nm using a UV-vis spectrophotometer (Baker, 1978).

The following formula was used to calculate SOC.

$$SOC(\%) = (K \times 0.1) / (W \times 0.74)$$

(K = concentration (corrected using the concentration of the blank), W = sample weight)

#### 2.4.2. . Soil labile carbon

Permanganate oxidizable C fraction is the largest labile C fraction in the soil (Bongiorno et al., 2019). Generally, labile soil organic C fractions have a maximum retention time of about 5 years (Duxbury, 1989), and hence they do not contribute to soil stable C (SSC) for long term SCS. Thus, the SLC fraction was evaluated using the permanganate oxidizable C fraction (Weil et al., 2003) to rectify the estimation of SCS. Briefly, 0.15 g ( $\pm 0.0001$ ) of the sieved soil was added to the 50 ml polycarbonate centrifuge tube. Each sample was labeled and 20 ml of 0.02 M KMnO<sub>4</sub> (AR, purity ≥ 99.0%) solution was added to each tube. Then, the tubes were shaken at 200 rpm for 20 min at room temperature followed by centrifugation at 3000 rpm for 5 min. After that, 0.2 ml of the solution was transferred to a glass tube, and it was diluted by adding 9.8 ml of distilled water. Then, the solution was vortexed to assure complete mixing, and it was measured at 550 nm wavelength using a UV-vis spectrophotometer.

The following formula was used to calculate SLC.

$$SLC = [0.02 \text{ mol/l} - (a + (b \times \text{absorbance}))] \times (9000 \text{ mgCmol}^{-1}) \times ((0.02 \text{ l}) / 0.00015 \text{ kg})$$

(0.02 mol/l = concentration of the initial solution, standard graph was constructed by assigning absorbance to X axis and concentration to Y axis, a = intercept, and b = slope of the graph).

#### 2.4.3. . Bulk density

About 2 cm of surface soil was removed from the spot where samples were taken, and leveled the spot. A 5 cm diameter thin-sheet metal tube of known weight (W<sub>1</sub>) and volume (V) was driven 5 cm into the soil surface. Excavated the soil from around the tube and cut the soil beneath the tube bottom. Trimmed the excess soil from the tube ends, and dried the soils at 105 °C for 2 days, and weighed (W<sub>2</sub>) (Blake and Hartge, 1986).

The following formula was used to calculate the bulk density ( $\rho$ , gcm<sup>-3</sup>).

$$\rho = (W_2 - W_1) / V$$

#### 2.4.5. Soil carbon sequestration

The following formula was used to calculate the SSC (g/100g).

$$SSC = SOC - SLC$$

Then, the SSC density or SCS (t ha<sup>-1</sup>) was calculated, based on the sampling depth "L", and the bulk density " $\rho$ " (Mg m<sup>-3</sup>) using the equation given below (Veldkamp, 1994).

$$SCS = SSC \times L \times \rho \times 10^4$$

Fig. 1 illustrates the flow chart of method used for analyzing and calculating SCS.

#### 2.5. Statistical analysis

Present study used a random effects model as a remedial measure for pseudoreplication (Davies and Gray, 2015; Millar and Anderson, 2004). In this context, generalized linear mixed model ANOVA in Minitab 17 package was used. For comparing SCS between the two practices, pairwise Tukey's multiple range test ( $p < 0.05$ ) was used.

### 3. Results and discussion

#### 3.1. . Soil organic and labile carbon

With the application of the BFBF, SOC content significantly increased along the three seasons as compared to the farmers' CF practice

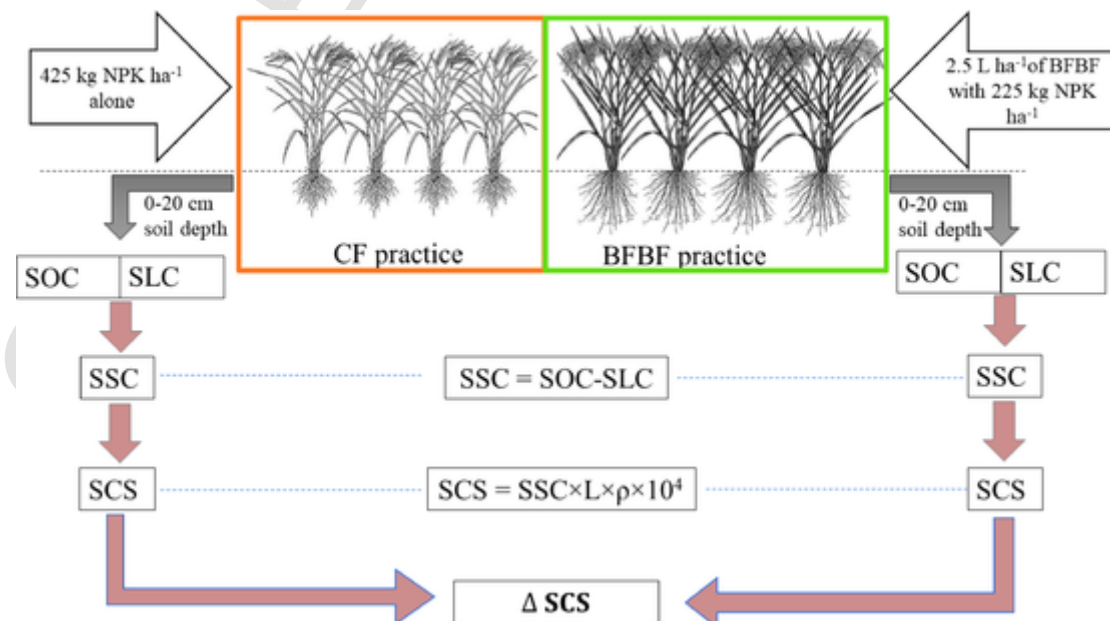


Fig. 1. A flow chart of the methodology used for quantification of SCS. SOC - soil organic carbon, SLC - soil labile carbon, SSC - sequestered soil carbon, SCS - soil carbon sequestration, L - sampling depth,  $\Delta$  - difference,  $\rho$  - bulk density, NPK - nitrogen phosphorus potassium.

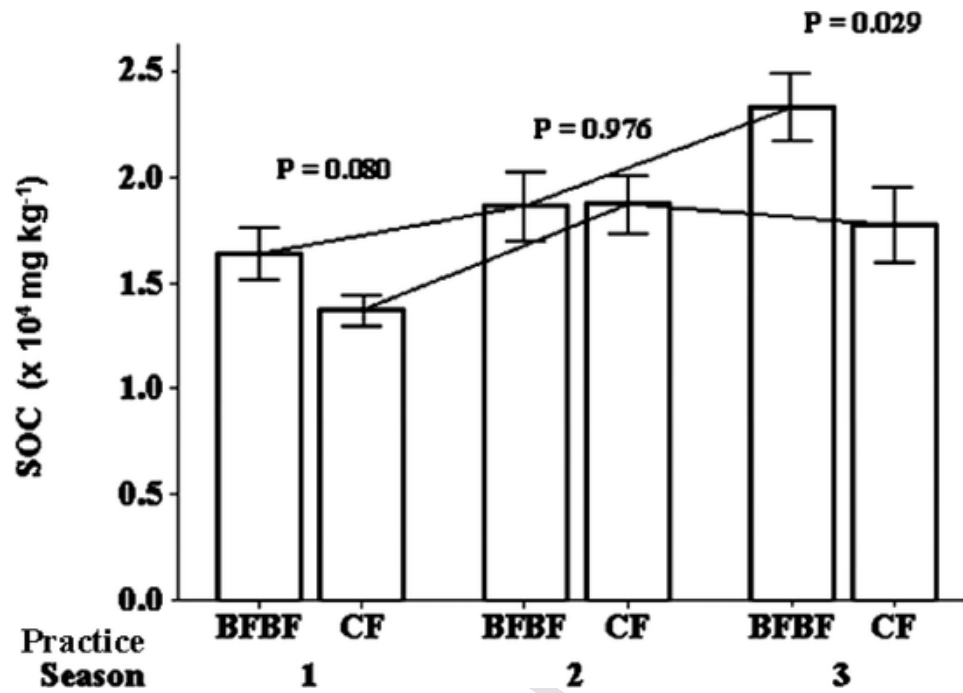


Fig. 2. Soil organic carbon (SOC) of the BFBF and farmers' CF practices in the three consecutive seasons (1 – Dry 2018, 2 – Wet 2018/19, 3 – Dry 2019).

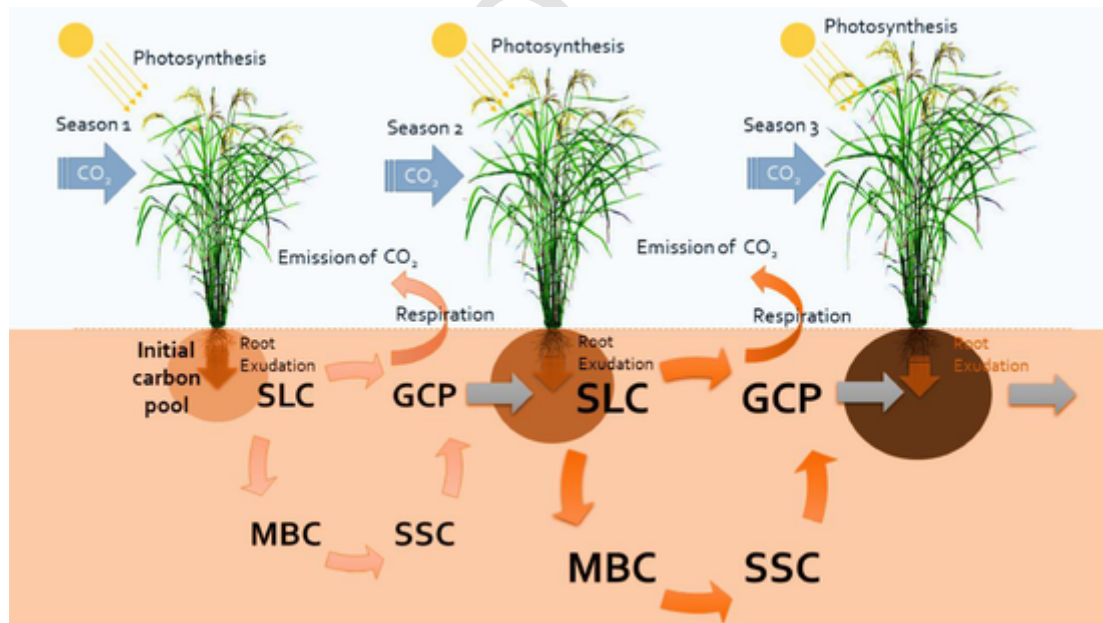


Fig. 3. A schematic diagram of the carbon sequestration pathway. SLC - soil labile carbon, MBC - microbial biomass carbon, GCP - gross carbon pool, SSC - sequestered soil carbon.

(Fig. 2). This can be attributed to increased root exudates due to enhanced photosynthesis with the application of BFBF (Buddhika et al., 2014; Dignac et al., 2017), and the exudates' incorporation in to the increased microbial biomass in the root zone, which is known as LCP (Fig. 3). Moreover, increased root growth and turnover should also have contributed to the increased SOC with the BFBF practice (Fig. 4). It has been reported that the BFBFs can optimize the production of hormones such as indoleacetic acid by having regulated metabolism, which promotes the root growth (Summuna et al., 2019). Increasing the rooting depth by applying BFBF can also result in increased plant fitness, water use efficiency and soil nutrient uptake. Thereby, root growth, which translates into increased shoot biomass production, in turn increases plant growth and ultimate yield (Akladiou and Abbas, 2012). Further,

the improved root growth of the BFBF practice has been ascribed mainly to factors beyond plant hormone production (Buddhika et al., 2014), which have not been fully understood yet.

The SLC did not differ between the two practices along the three seasons (Fig. 5), because it is a transient pool (de Souza et al., 2016), which is rapidly utilized by microbes or incorporated in to SSC pool or even lost. A sustained, increasing trend of the SLC was observed along the seasons in both practices. This clearly justifies the need of deducting SLC from SOC, if we are to quantify SCS more accurately. Also, Zou et al. (2005) attested that the SLC is the fraction of SOC with rapid turnover times. In our study, if SOC were used directly for calculating SCS, it would have overestimated SCS by 10-15%, because SOC contained that much of SLC in the soils studied (Table 1).



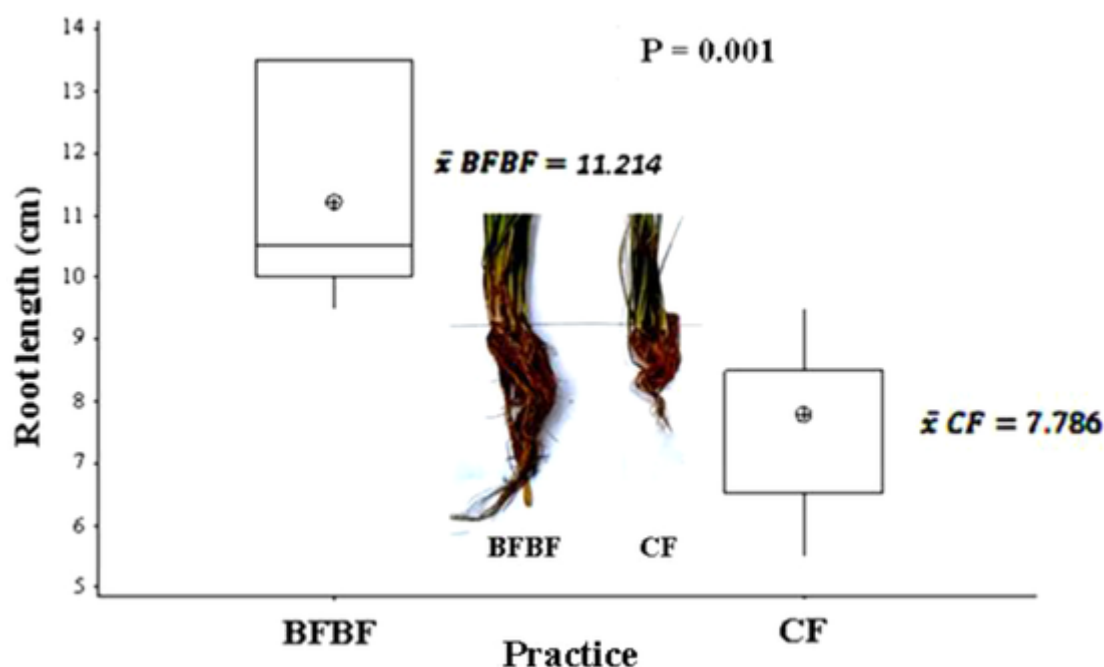


Fig. 4. Plant root lengths of the BFBF and farmers' CF practices. Inset photo shows the root growth difference of the two practices.

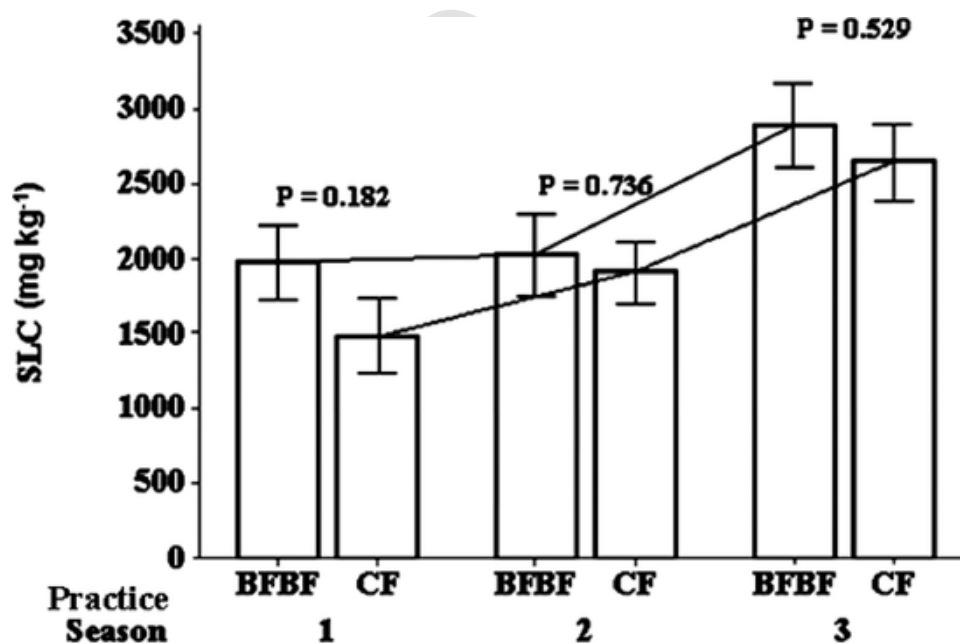


Fig. 5. Soil labile carbon (SLC) of BFBF and farmers' CF practices in the three consecutive seasons (1 – Dry 2018, 2 – Wet 2018/19, 3 – Dry 2019).

Table 1

Soil labile carbon (SLC) as a percentage of soil organic carbon (SOC) in the two practices during the three seasons.

Season	Practice	SOC ( $\times 10^4$ mg $\text{kg}^{-1}$ )	SLC ( $\times 10^3$ mg $\text{kg}^{-1}$ )	SLC as a percentage of SOC
Dry 2018	BFBF	1.60 <sup>a</sup>	1.97 <sup>a</sup>	12.3
	CF	1.35 <sup>a</sup>	1.48 <sup>a</sup>	11.0
Wet 2018/19	BFBF	1.93 <sup>a</sup>	2.03 <sup>a</sup>	10.5
	CF	1.91 <sup>a</sup>	1.91 <sup>a</sup>	10.0
Dry 2019	BFBF	2.31 <sup>a</sup>	2.89 <sup>a</sup>	12.5
	CF	1.77 <sup>b</sup>	2.64 <sup>a</sup>	15.0

### 3.2. . Soil carbon sequestration

The amount of sequestered C in the BFBF practice was continuously increasing with time, suggesting a higher potential of sequestering stable C up to a certain level at which the soil should have been saturated with C (Fig. 6). However, this trend was not seen in the farmers' CF practice. Carbon contributions from BFBF and CF were negligible compared to other C inputs because their application rates were 2.5 t BFBF  $\text{ha}^{-1}$  with 0.1% C and 284 kg Urea  $\text{ha}^{-1}$  with 20% C. Thus, the main contributor for the increased SCS was the significantly increased rooting depth (Fig. 4), as explained above. In the literatures, reports on microbial interventions that increase the sequestration of stable C in lowland paddy soils in particular, are scarce, although it has been reported that up to ca. 65 t C

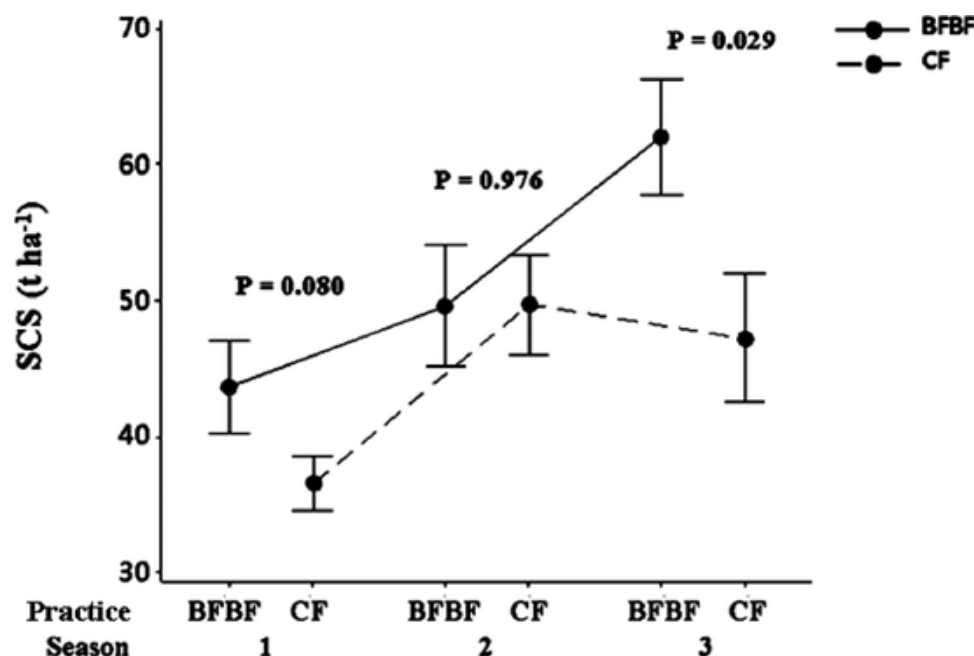


Fig. 6. Soil carbon sequestration (SCS) of the BFBF and the farmers' CF practices in the three consecutive seasons (1 – Dry 2018, 2 – Wet 2018/19, 3 –Dry 2019).

ha<sup>-1</sup> is sequestered in rice-based cropping systems in the Sri Lankan context (Ratnayake et al., 2017). Generally, paddy soils are reported to be more efficient in SCS, but the stabilization of stored C is less due to increased microbial decomposition under warmer tropical climates (Chen et al., 2021). This scenario is clearly depicted in the CF practice of our study, where there was an initial increase and then a decline of sequestered soil carbon from the first to the third season (Fig. 6). Adoption of Integrated Nutrient Management (INM) using organic and inorganic fertilizers has been shown to increase the soil C stabilization in conventional, lowland paddy cultivation (Rajkishore et al., 2015). However, bulky quantities of organic matter inputs are essential in the conventional practices to sequester even relatively low amounts of C (Sahrawat et al., 2005; Yadav et al., 2019).

When long-term SCS is considered, gross C pool (GCP), a portion of which is emitted as CO<sub>2</sub> during tillage, seems to play an important role in lowland paddy cultivation (Fig. 7), because it helps to preserve a soil C stock during the cropping seasons. In the present study, there were two cropping seasons (dry and wet) which existed ca. 7–8 months during the year. This time duration is considerable, since different rice growing countries with different cropping seasons spanning during dif-

ferent periods may contribute to a continued sequestration of more or less certain level of soil C in the GCP during the entire year. As far as the two practices are concerned, the GCP was observed to increase with time only in the BFBF practice whereas it started to decline from the second season in the farmers' CF practice. In the future studies, it is important to examine the potential of BFBF practice in sequestering C beyond the observed level.

The present study showed that the BFBF practice sequestered up to 15 t stable C ha<sup>-1</sup> season<sup>-1</sup> over the farmers' CF practice during the study period (Figs. 6 and 7), thus showing an enormous potential to mitigate global warming and to gain income through C trading (Deng et al., 2017). In the long term studies of tropical and subtropical climates, a wide range of SCS rates in paddy soils has been observed. For example, it is reported that the combined treatments of CF NPK with up to 32 t ha<sup>-1</sup> of organic amendments viz. pig manure, green manure, *Astragalus sinicus* L., and rice straw sequestered soil C at a rate from 0.20 to 0.48 t ha<sup>-1</sup> year<sup>-1</sup> in rice cultivation (Zhang et al., 2012). Moreover, the application of CF NPK with 5 t ha<sup>-1</sup> of farm yard manure sequestered 1.77 t C ha<sup>-1</sup> in paddy soils (Mandal et al., 2020). Also, the application of cow dung, poultry manure and rice straw separately up to 17 t ha<sup>-1</sup> without

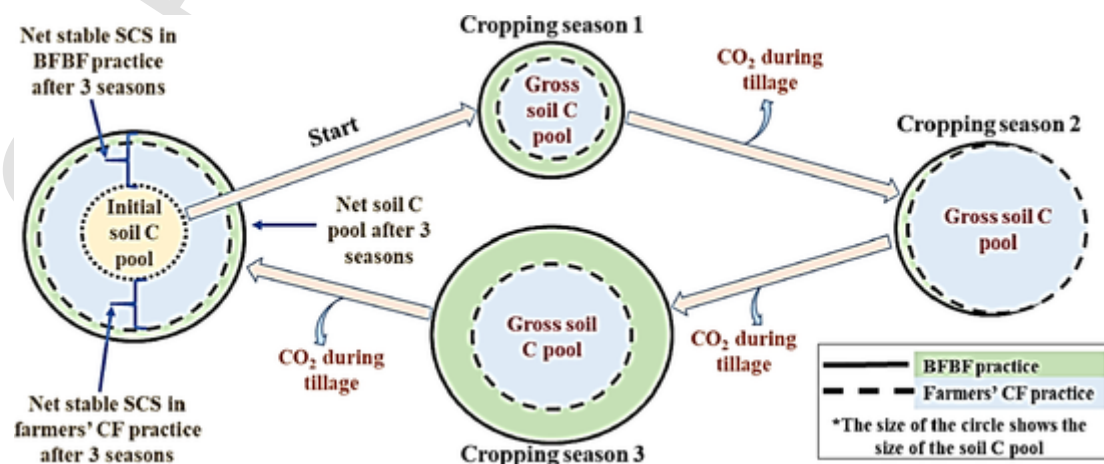


Fig. 7. A schematic diagram showing soil C pool dynamics and stable soil C sequestration at the end of three seasons

CF NPK sequestered 1–5 t C ha<sup>-1</sup> in paddy soils (Rahman et al., 2016). Those studies show that the SCS potential with the application of organic amendments even up to 32 t ha<sup>-1</sup> with or without CF NPK is only up to 5 t C ha<sup>-1</sup>. When compared with those results, an exorbitant amount of sequestered soil C over farmers' CF alone practice, i.e. 15 t stable C ha<sup>-1</sup> season<sup>-1</sup> with BFBF, but without organic amendments in the present study clearly shows that the organic material inputs are not essential for paddy SCS. Moreover, an increase of paddy grain yields up to ca. 25 % was also observed under the same practice (Premarathna et al., 2021).

#### 4. Conclusion

The BFBF practice can be considered as a novel method of sequestering soil C for prolonged periods, and it can be recommended as a means to mitigate global warming, while securing food for the future world. However, the mechanism of stabilization of sequestered soil carbon in this practice is not clear yet. Therefore, further investigations are needed to explore this. At present, the BFBF application in agriculture is practiced extensively only in Sri Lanka, though it is now being still researched in several countries with promising results. Therefore, steps should be taken to rapidly expand this practice to other parts of the world, while examining its effects and potentials in the global context. The way to reduce the vulnerability of climate change is by encouraging climate resilient-environmentally friendly and appropriate innovative technologies like BFBF. Also, recognizing and adopting appropriate traditional knowledge and practices that improve crop productivity and SCS in paddy cultivation need to be promoted.

#### Uncited references

Sahrawat et al. (2005) and Yadav et al. (2019).

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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