ISSN 1064-2293, Eurasian Soil Science, 2023. 

AGRICULTURAL CHEMISTRY AND SOIL FERTILITY

# Biofilm Biofertilizer Stabilizes Sequestered Paddy Soil Carbon While Cutting Down Chemical Fertilizers: Answers for Climate and Fertilizer Issues

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Received January 17, 2023; revised February 9, 2023; accepted April 5, 2023

**Abstract**—Degradation of natural ecosystems demands the utilization of croplands with enhanced soil carbon sequestration. To do this, microbial biotechnologies like biofilm biofertilizers can be used because it enhances soil carbon sequestration while increasing crop yields with reduced chemical fertilizers use. However, the stabilizing sequestered soil carbon with the biofilm biofertilizer practice is yet to be revealed. Thus, present study evaluates the effect of biofilm biofertilizer on soil physicochemical and biological properties including soil respiration and organomineral complexation in paddy cultivation. The biofilm biofertilizer practice was compared with the farmers' current practice of chemical fertilizer alone application in four districts in Sri Lanka. Attenuated total reflectance Fourier-transform infrared spectroscopy was used to evaluate the chemical forms of stabilizing sequestered soil carbon. Results showed that soils with stronger organomineral complexation and reduced soil respiration down to ca. 40% were formed in the biofilm biofertilizer practice over the farmers' chemical fertilizer practice, possibly due to enhanced mineral surface-reactive metabolites, and low priming effect, respectively thus resulting to mitigate global warming. Conclusively, the biofilm biofertilizer practice is an effective method to replace farmers' chemical fertilizer practice. Reduced chemical fertilizer use down to ca. 50% with the biofilm biofertilizer application addresses the current global issue of escalating chemical fertilizer prices. Rapid implementation of such biotechnologies is important to save the globe from predicted climatic catastrophes.

**Keywords:** carbon sequestration, fertilizer prices, global warming, organomineral complexation

**DOI:** 10.1134/S1064229323600203

## INTRODUCTION

After the industrial revolution, release of more than 1 billion tons of carbon (C, as $CO_2$) into the atmosphere has been a key driver for global warming by now [18]. In this context, we should act quickly to safeguard our climate and our way of life [55]. Thus, removing $CO_2$ from the atmosphere is an urgent need. Here, sequestering C in the soil for prolonged periods is the most promising way of doing that [40]. Generally, photosynthesis is the most beneficial and least hazardous way of sequestering C in the soil [10]. The photosynthesis causes "liquid C pathway (LCP)" (rhizodeposition of C [38]) which transfers ca. 40% of the sugar products to the soil through the root system and finally feeds rhizosphere microorganisms [28]. The microorganisms have a symbiotic relationship with the plant root system throughout the lifespan of a plant. As a result, plants add more C to the soil continuously than that of decomposing plant litter [54]. Further, it helps to plant health and productivity by augmenting soil microbiota [3]. In return, the soil microbiota, predominantly fungal symbiosis [23] contributes to enriching complex soil-plant-microbial interactions.

Traditionally, forests are regarded as strong C sinkers. However, drought and wildfires have increased tree mortality, particularly in widely arid areas, which account for 41% of Earth's land area. As this is the case, grasslands have now been considered as better sinks due to their ability to withstand high temperatures, drought and fire. Moreover, grasslands conserve terrestrial C and prevent it from re-entering to the atmosphere [13].

It has been reported that global warming would reduce grain production in agriculture [58]. Under such a circumstance, planting food crops is more important than planting trees [52]. As a staple food crop in the world, rice (*Oriza sativa* L.) occupies ca. 160.5 Mha of land globally [41]. Excessive use of chemical fertilizers (CF) in food production has disabled the root-associated microbial activity by making adverse environmental conditions [66]. However, microbial biotechnological interventions like biofilm biofertilizers (BFBF) have been reported to reinstate the lost biodiversity and sustainability in agroecosystems that lead to increase soil quality and grain yield in rice cultivation [42, 46]. Further, it has also been reported that the soil carbon sequestration (SCS) is also increased with the use of BFBF in lowland paddy cultivations [27]. In fact, the BFBF practice has been reported to sequester up to 15 t stable C $ha^{-1}$ $season^{-1}$ over the farmers' current practice of CF alone application. The increased SCS has been reported to have been achieved as a result of increased rooting depth and microbial C assimilation in the root-zone soil. The BFBF practice is extensively being applied in rice cultivation in Sri Lanka, and it requires only 2.5 liters of BFBF $ha^{-1}$, whereas conventional practices need bulky quantities of organic matter inputs to sequester a comparable amount of C. Similar results were observed in the tea cultivation as well as forest plantation [7, 43]. Only disadvantage of the BFBF is that it being a liquid, its packaging and application are more difficult than the solid forms, because it requires a carrier like sand to be applied directly onto the soil.

Most of the sequestered C in any soil has a tendency to get lost due to the decomposition by microorganisms, erosion of surface soil, off-take in plant and animal production, and land preparation in agriculture [25]. In this context, stabilization of sequestered soil carbon (SSC) is vital to enhance SCS benefits in the environment.

In the soil C stabilization, added organic C is bound onto mineral surfaces through various organomineral bonding reactions such as ligand exchange, cation bridging, H-bonding, and van der Waal forces, depending on the composition of the organic inputs, soil mineralogy, and environmental factors [2, 21]. Organic C stabilization on soil minerals accounts for the majority of total soil organic C (SOC) [11, 29], and mineral-bound C has longer turnover times than other C fractions [31]. Changes in the amount or stability of organic C inputs stabilized on soil minerals therefore greatly affect bulk soil C storage or C sequestration in the long term. Moreover, organomineral complexation (OMC) is vital for the conservation of a micronutrient reservoir for crops. Therefore, these soil mineral systems are essential for agriculture [37]. Thus, in the present study, the OMC of SSC with the application of BFBF was evaluated, and it was compared with the farmers' conventional practice of CF alone application.

## EXPERIMENTAL

**Field sites.** The field experiments were carried out during 2019–2020 wet season in four locations of Sri Lanka namely viz. Kurunegala (7°45' N, 80°15' E, elevation above sea level (EASL) 116 m, average annual temperature (AAT) 26°C, average annual rainfall (AARF) 2000 mm), Ampara (07°05′ N, 81°45′ E, EASL 37 m, AAT 27°C, AARF 1858 mm), Polonnaruwa (7°56′ N, 81°0′ E, EASL 60 m, AAT 27°C, AARF 1678 mm), and Kegalle (7°18′ N, 80°24′ E, EASL 248 m, AAT 26°C, AARF 2493 mm) districts consisting of variable soil types, particularly low humic gley, red yellow podzolic with laterite, non-calcic brown, reddish brown earth, regosol and solodize solonets [15]. According to WRB soil classification system, all these soil types, particularly in paddy cultivation have been classified as Anthrosols [57]. Initial soil properties of the four districts were not significantly different due to high variability, and they ranged pH 5.9–6.2, SOC 0.7–1.6%, soil labile carbon (SLC) 0.35–1.4%, soil total nitrogen (STN) 0.10–0.17%, soil total phosphorus (STP) 0.28–0.36%, and soil potassium (SP) 0.18–0.28 $cmolkg^{-1}$.

**Experimental design.** The field experiment was done in 13 paddy field locations spreading over 0.5 Mha in Kurunegala ($n$ = 3), Ampara ($n$ = 3), Polonnaruwa ($n$ = 4), and Kegalle ($n$ = 3) districts. Two consecutive, uniformly managed paddy fields (each ca. 0.4 ha) with similar soil characteristics were used to apply the two main practices, which are extensively used by the farmers. Earlier, [62] and [1] tested a range of treatments consisting of different levels of CF [Urea, triple super phosphate (TSP) and muriate of potash (MOP) as N, P, and K fertilizers, respectively]alone and CF + BFBF combinations (0, 65, 80 and 100% of CF [14], and BFBF + 65% CF & BFBF + 80% CF). In addition, the Department of Agriculture (DOA), Sri Lanka, conducted a similar study in 2019–2020. According to their results, the optimum level of CF that should be coupled with BFBF was 225 kg NPK $ha^{-1}$. Once it was combined with BFBF, it gave an enhanced yield than 225 kg CF $ha^{-1}$ alone application. Thus, we used this rate as the recommended practice of BFBF. The two practices of the present study were (a) BFBF practice {BFBF is a fungal-bacterial biofilm [53] of *Rhizobium* sp., *Azospirillum* sp. and *Penicillium* sp., (2.5 L of BFBF with 225 kg CF $ha^{-1}$ (Urea 150, TSP 40 and MOP 35 kg $ha^{-1}$))}, and (b) Farmers' practice [425 kg NPK $ha^{-1}$ (Urea 284, TSP 76 and MOP 66 kg $ha^{-1}$)]. We used farmers' CF rate, because in an initial survey, we found that >90% of the farmers do not use the CF recommendation of the DOA. Thus, to be realistic, we used the farmers' fertilizer rate.

In the two fields, paddy was broadcasted and irrigation water was managed separately without mixing from surrounding fields. At 2 weeks and 6 weeks after broadcasting, the BFBF was applied to the paddy fields by mixing 500 mL of BFBF with 4 L of fine sand. Fine sand with CF does not show significant difference in plant growth from the CF alone application according to our preliminary studies (data not shown).

**Table 1.** Characteristic FT-IR transmittance bands in the frequency range of 500–1300 cm[1] with prominent diagnostic features

| Wavenumber, $cm^{-1}$ | Band assignment |
|---|---|
| 762–780 | Aromatic C–H bending |
| 910 | Al–OH |
| 1004 | Si–O |
| 1030 | C–O stretching of the carbohydrate and polysaccharides-like substances |
| 1124–1205 | Aliphatic C–O stretching |

Therefore, the CF was not mixed with the sand when applied. The two consecutive plots for the two practices were taken as a randomized block design in each site. Thirteen field locations acted as replicates.

**Sample collection.** Three composite soil samples were collected at the flowering stage (after 8–9 weeks from broadcasting) using a soil core (5.7 cm in diameter) from each experimental paddy plot. The sampling depth was 25 cm. The number of samples for each practice and the total number of samples were 39 and 78, respectively.

**Soil analyses.** In this study, soil pH, soil moisture (SM), SOC, SLC and soil respiration (SR) were selected as the soil parameters that reported to involved in SCS [4, 49]. The SM was determined by oven drying fresh soil at 105°C until a constant weight. Soil pH was determined using soil : water 1 : 2.5 ratio. The soil samples were then air-dried for analyzing other parameters. The air-dried soil was grinded using mortar and pestle and passed through 0.5 mm sieve. SOC and SLC were determined using Walkley-Black colorimetric method [5] and permanganate oxidizable C method [61], respectively. The SR was analyzed using MicroResp$^{TM}$ microplate-based respiration system at 25–27°C [12].

**Microbial analyses.** The cultivable soil bacterial abundance (SBA) and soil fungal abundance (SFA) were analyzed by culturing them at $10^{-3}$ dilution in nutrient agar + potato dextrose agar (NA + PDA) modified medium. The media composition (g/L) was beef extract—3, peptone—5, sliced potato—200, and Agar—20. Colony forming units (CFU) of bacteria and fungi were taken after 24 and 48 hours from the inoculation, respectively.

**ATR-FTIR spectroscopy.** Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopic study was performed to analyze OMC in the soils by using Nicolet iS 50 FT-IR spectrometer (Thermo Fisher Scientific). Based on the literature, wave number positions 762–780, 915, 1000, 1030 and 1124–1205 $cm^{-1}$ (Table 1) were selected for the analysis because they are extensively used in measuring OMC [32, 35].

**Statistical analysis.** Means and correlations of all the variables of BFBF and farmers' CF practices were calculated. T-test was performed for mean comparison after confirmation of normal distribution of data using normality test. All data were analyzed statistically using Minitab 17 version. A schematic diagram is provided to make it easier for readers to understand the methodology (Fig. 1).

## RESULTS AND DISCUSSION

**Soil parameters.** An increase though it was not significant at $P < 0.05$, was observed in SOC between consecutive seasons with the BFBF application over the farmer's CF practice (Table 2, $P = 0.154$). It is a well-known fact that rhizodeposition contributes to the interaction among soil, plant and microbes. During rice plant growth, rhizodeposits are the primary source of soil C [20, 33] which provides energy and raw materials (especially C) for the biological activity of soil microbes. It is reported that the rhizodeposition is accelerated by enhanced photosynthesis that in turn leads to incorporate C into growing microbial biomass in the root zone with the application of BFBF [6, 16]. The SBA and SFA, which reflect mainly soil microbial biomass were significantly higher in the BFBF practice than those of the farmers' CF practice ($P < 0.001$ and $<0.001$ respectively). This could be attributed to favorable microenvironment that was provided to plant growth promoting microorganisms (PGPM) by increased mycorrhizal fungi due to the accumulation of microbial biomass C along with the root development [24, 63]. The increased soil fungi can form stabilized soil organic matter (SOM) by synthesizing mineral surface-reactive metabolites (MSRMs [60]). The MSRMs affect the reactive properties of clays that directly and/or indirectly relate to soil fungal and bacterial biomass and necromass [54].

A significantly lower (up to ca. 40%) SR was observed in the BFBF practice than the farmers' CF practice (Fig. 2, $P < 0.001$). This could be ascribed to incorporating rhizodeposits into increased soil microbial biomass, i.e. increased SFA and SBA in the BFBF practice ($P < 0.001$), thus temporarily immobilizing fresh C in the biomass, and causing it to low priming effect [8, 17]. This led to reduce destabilization of SSC and the $CO_2$ release. Even a small increase in SR in agroecosystems could have a large effect on atmospheric $CO_2$ concentrations, with consequences for

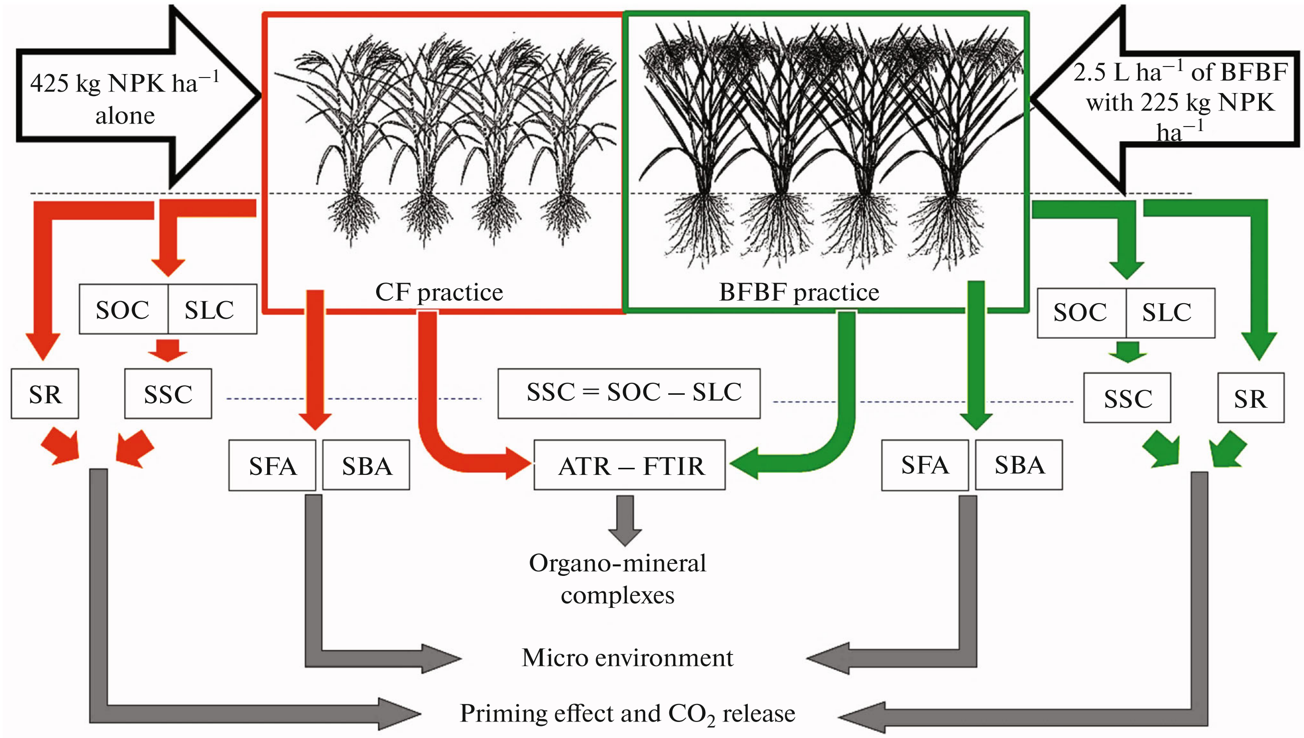


**Fig. 1.** A schematic diagram showing the process of methodology.

global warming, because ca. 10% of greenhouse gas emissions was reported from agriculture [56]. As this is the case, if we could extend the BFBF practice to the global rice cultivations, it would possibly contribute immensely to mitigate global warming and climate change.

**FTIR spectroscopic analysis.** The FTIR spectrographs of each practice showed characteristic transmittance banding patterns in the frequency range of 500–1300 cm$^{-1}$ with prominent diagnostic features (Fig. 3).

The band at 762–780 cm$^{-1}$ which corresponds to aromatic C–H bending [36] was more intense in the BFBF practice than in the farmers' CF practice. This may be due to increased aromatic C in soils treated with a combination of CF and bio/organic amendments when compared to the CF alone application [61]. The band at 2925 cm$^{-1}$ corresponds to the aliphatic C–H stretching [26]. In addition, appreciable bands at 1004 and 910 cm$^{-1}$ which resulted in polysaccharides and Si–O, and Al–OH bending vibrations of Kaolinite, respectively [47, 48, 50] were most intense in the

**Table 2.** Soil parameters of the BFBF and farmers' CF practices of paddy cultivations

| Parameter | Practice | | Difference* |
|---|---|---|---|
| | BFBF ($n$ = 13) | Farmers' CF ($n$ = 13) | |
| pH | 6.0 ± 0.14 | 6.0 ± 0.16 | 0.01 (0.933) |
| SM, % | 53.8 ± 4.9 | 54.5 ± 4.8 | 0.68 (0.922) |
| SOC, % | 1.85 ± 0.1 | 1.55 ± 0.1 | 0.30 (0.154) |
| SLC, % | 1.28 ± 134 | 1.32 ± 210 | 0.03 (0.186) |
| SR, % | 4.6 ± 0.26 | 7.7 ± 0.28 | 3.13 (0.000) |
| SBA × $10^5$ CFU mL$^{-1}$ | 97 ± 7 | 23 ± 2 | 74.18 (0.000) |
| SFA × $10^4$ CFU mL$^{-1}$ | 7.0 ± 1.0 | 1.9 ± 0.6 | 5.27 (0.000) |
| F : B ratio | 0.073 ± 0.007 | 0.104 ± 0.037 | 0.031 (0.426) |

Mean ± SE in each column. SE was calculated using means of the location means.

* Values within parentheses are probability levels at which differences are significant. Soil pH, moisture (SM), organic carbon (SOC), labile carbon (SLC), respiration (SR), bacterial abundance (SBA), fungal abundance (SFA), and fungal-bacterial (F : B) ratio.

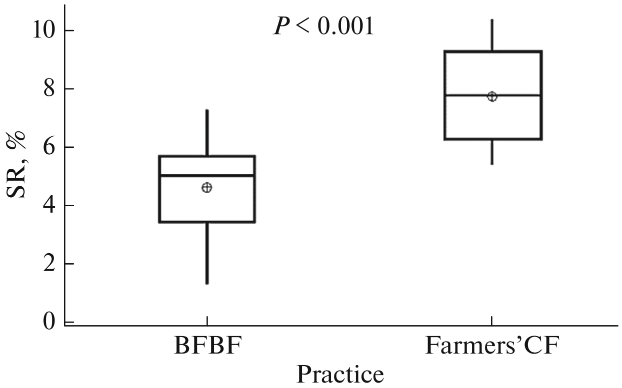


**Fig. 2.** Soil respiration (SR) of the BFBF and farmers' CF practices.

BFBF practice. The higher intensity of these bands in the BFBF practice could be ascribed to the enhanced aggregation and consolidation of soil particles due to biofertilizer application [64]. Moreover, these minerals facilitate the polymerization of organic residual substrates, leading to enhanced humification [34]. Peak at 1030 $cm^{-1}$ that corresponds to C–O stretching of carbohydrate and polysaccharides-like substances [19, 35] and high proportions of mineral-associated OM fraction [30, 35, 39, 65] was more intense in the BFBF practice. Generally, polysaccharides of microbial origin mainly bind to clay particles by promoting the formation of microaggregates <50 μm [35, 44], thus contributing to stabilization of soil C.

**Mechanism of soil C stabilization and subsequent soil respiration.** In the BFBF practice, a higher amount of fresh C is incorporated into soil through rhizodeposition [27], because of higher plant growth [42] and hence higher photosynthetic rate [6], compared to those of the farmers' CF practice (Fig. 4). It creates higher soil microbial biomass, MSRM, OMC and availability of aromatic C, which together lead to formation of stabilized soil C, lower priming effect and hence lower SR.

The OMC occurs by combining SOM with minerals in the soil, which can be highly preserved and persistent for a long time by limiting microbial effects [51] as explained above. Moreover, the OMC and its interactions control the stabilization of the SOM. The FTIR data provide evidence for interactions of abundant functional groups, such as ligand exchange, H-bonding, and π–π-bonding with aromatics of organomineral complexes qualitatively with regard to their fluctuations [45]. Moreover, the FTIR data qualitatively describe OMC and its changes with regard to its relative peak intensities and their characteristics in the application of soil amendments [9].

It is reported that the accumulation of rhizodeposits improves soil OMC via naturally existing microbial effects [22]. The BFBF application in the present

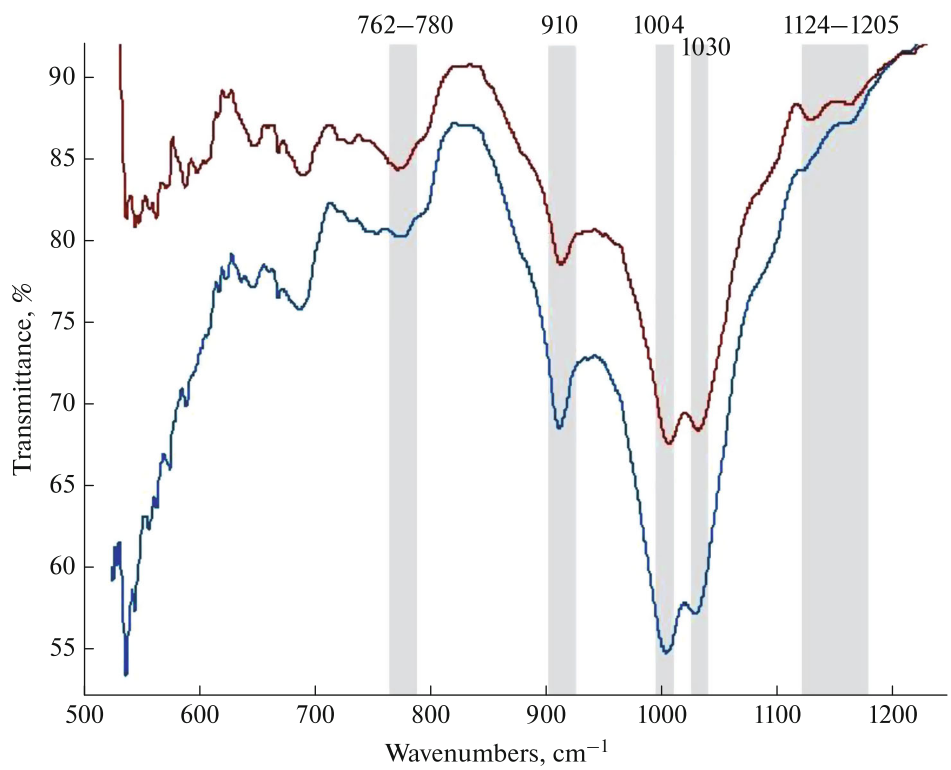


**Fig. 3.** ATR-FTIR spectra of the soils of the BFBF and farmers' CF practices, indicated by blue and red colors, respectively.

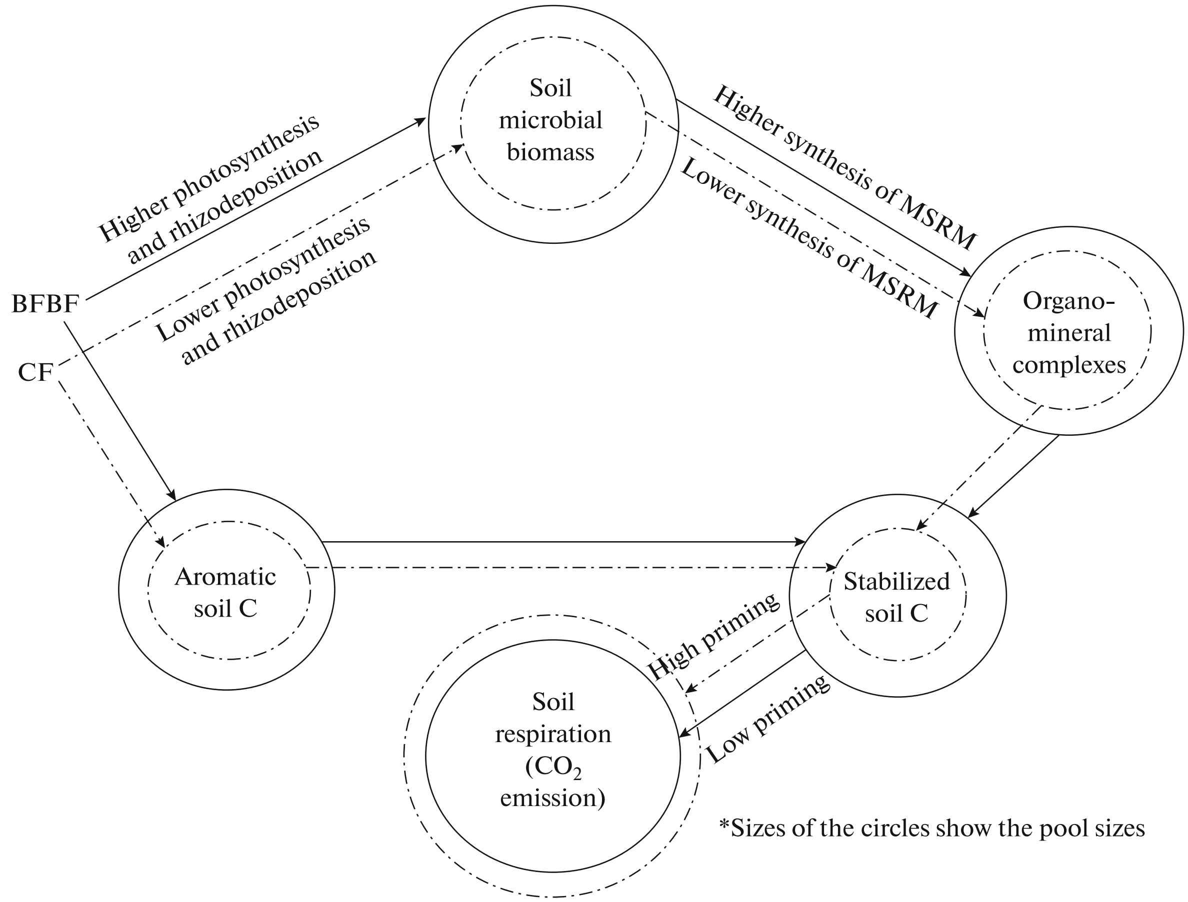


**Fig. 4.** A schematic diagram showing the stabilization of soil C and soil respiration of the BFBF and farmers' CF practices. MSRM is mineral surface-reactive metabolites.

study further increased the natural microbial actions for enhanced OMC that led to negative priming of SOC with reduced SR.

## CONCLUSIONS

The BFBF can be used to potentially increase soil stable C stock and to reduce emission of SSC back to the atmosphere while increasing grain yield. If expanded globally, this will lead to mitigate global warming and hence climate change in eco-friendly manner. Therefore, the BFBF practice can be considered as a sustainable alternative to replace farmers' current practice of CF alone application. However, this needs to be researched further under different soil and climatic conditions and for different crops, if this novel practice is to be applied extensively. The ability to reduce CF use with the BFBF application will provide an avenue to address the current issue of the escalation of CF prices. Overall, the most important thing at present is to implement such technologies after rapid evaluations in order to mitigate the climatic issues that have been predicted from recent research.

## ACKNOWLEDGMENTS

All the members of Microbial Biotechnology Unit of the NIFS are acknowledged for their support during this study. We would also like to show our gratitude to all farmers who collaborated by providing data, insights and expertise that greatly assisted the research.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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