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NETWORK INTERACTIONS OF SOIL CARBON SEQUESTRATION IN PADDY GROWN SOILS AMENDED WITH BIOFILM BIOFERTILIZER VERSUS CHEMICAL FERTILIZERS

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ABSTRACT

Soil carbon (C) sequestration (SCS), the widely accepted strategy to mitigate global warming and climate change, also restores degraded soils and remediates the environment, while increasing plant growth and yields. Limitations in forest SCS and predicted reduction of grain production have prompted to plant food crops rather than planting trees. Here, lowland paddy cultivation is more effective in SCS than upland cropping. However, overuse of chemical fertilizers (CFs) has contributed to deplete soil and the environmental quality. Ecofriendly biofilm biofertilizers (BFBFs) are reported to reduce the use of CFs, while increasing grain yield and SCS. However, the main soil factors governing the SCS with BFBF have not been investigated yet. The present study investigated this in the BFBF practice in comparison to farmers' CF practice, using network analysis, by conducting a field experiment in four districts during Maha (wet) 2020/21 season. Results revealed that the BFBF application significantly increased plant rooting depth, SCS and grain yield over the farmers' CF practice ($P < 0.10$). A higher number of interactions in the soil C sequestering network of the BFBF practice implied the strengthening of the network interactions. There were strong positive and negative effects of soil total N and total P, respectively, on C accumulation in both practices, showing complexity of the network interactions. In conclusion, it is clear that nutrient management is the key for maximizing SCS, and of course yield with the BFBF application in paddy cultivation in the Sri Lankan context.

Keywords: Agroecosystems, Network interactions, Paddy, Soil carbon sequestration

INTRODUCTION

Atmospheric concentration of CO_2 has increased by 31% since 1750, mainly contributed by the land use change and fossil fuel combustion and hence identification of approaches for mitigating the accompanied global warming has been an urgent need (Lal, 2004). In this context, soil C sequestration (SCS) has been observed to be a win–win strategy, because it restores degraded soils and remediates the environment, while increasing plant growth and yields.

Although forests are traditionally regarded as strong carbon (C) sequesters or sinkers, high mortality, drought, nutrient limitations and wildfires that have been occurring frequently from the recent past have made the forests C sources, particularly in widely arid areas, which account for ca. 41% of Earth's land area (Dass *et al*., 2018). On the other hand, grasslands have been observed to be C sinks, this is because, grasslands are capable to withstand high temperatures, drought and fire, while storing C, thus helping to conserve sequential terrestrial C and preventing it from re-entering the atmosphere (Dass, 2018). In agroecosystems, it has been predicted that the total grain production might drop by about 10% due to climate change and extreme climate events during the period of 2030~2050 (Kim, 2012). As a consequence, the production of three major crops – wheat, rice and maize – might all be reduced. Under such circumstance, the priority should be given to planting food crops rather than planting trees for environmental benefits.

Among food crops, lowland paddy cultivation has been observed to be more effective in terms of SCS than upland cropping. It has been shown that changing cropping systems from lowland rice to upland annual crops reduces the soil C stocks to a significant level after 10 years of cultivation (Ratnayake *et al*., 2017). The reduction of C stocks in the upland cropping has been attributed mainly to increased SOC and crop residue decompositions under tillage. In addition, extensive utilization of CFs in crop cultivation has contributed to deplete soil and the environmental quality. This has led to collapse the sustainability of agroecosystems while increasing the cost of cultivation as well. In this context, maintaining food security and environmental quality has become a daunting challenge.

To address the above, research studies for seeking ecofriendly alternatives to CFs have been conducted worldwide. Amongst, microbial biofertilizers have gained attention, because they are less bulky and hence user-friendly, compared to other alternative fertilizer sources. However, the relatively low efficacy and inconsistent performance under field conditions have made them less attractive to farmers (Batista and Singh, 2021). As a recent development, engineered microbial biofilm-based biofertilizers known as biofilm biofertilizers (BFBFs) have proven their effectiveness over the conventional biofertilizers internationally (Hassani *et al*., 2018; Korniichuk and Zayarnyuk, 2018; Ricci *et al*., 2019; Santos *et al*., 2017; Sudadi and Triharyanto, 2018; Swarnalakshmi *et al*., 2013; Triveni *et al*., 2012; Velmourougane *et al*., 2017; Singhalage *et al*., 2021; Seneviratne, 2021), and hence they have been accepted by farmers. In paddy cultivation, the BFBFs have contributed to reduce chemical fertilizer use up to 50%, while increasing grain yield by 20-30% on average in Sri Lanka (Rathnathilaka *et al*., 2022; Premarathna *et al*., 2021).

Jayasekara *et al*. (2022) reported that the BFBF practice could increase rooting depth of paddy and microbial C assimilation in the root-zone soil, thus sequestering up to 15 t C ha^{-1} $season^{-1}$ over the farmers' CF alone practice. However, they have not investigated the main soil factors governing the C accumulation. Generally, soil processes take place via complex interaction networks of several variables (Premarathna *et al*., 2021; Meepegamage *et al*., 2021), which are of utmost importance for understanding the real actions under field conditions in order to address soil and crop issues. Therefore, the present study was designed to reveal the soil parameters regulating SCS through network interactions in BFBF practice in comparison to farmers' CF alone practice in paddy cultivation.

MATERIALS AND METHODS

A field experiment was carried out during *Maha* (wet) 2020/21 season under the long-term BFBF research project conducted by the Microbial Biotechnology Unit (MBU) of the National Institute of Fundamental Studies (NIFS), Kandy. The MBU has formulated an effective BFBF for paddy (Premarathna *et al*., 2021), which has been patented and used commercially in extensive cultivations. Generally, the BFBF contains fungal-bacterial biofilms and exuded biochemicals by the biofilm, which are important for various processes in the soil-plant system.

Field sites

Fourteen farmer fields in four districts viz. Ampara (07˚ 05′ N 81˚ 45′ E, average annual temperature (AAT) 27 ºC, elevation above sea level (EASL) 37 m, average annual rainfall (AAR) 1,858 mm), Kurunegala (07˚ 45′ N 80˚ 15′ E, AAT 26 ºC, EASL 116 m, AAR 2000 mm), Kegalle (07˚ 24′ N 80˚ 34′ E, AAT 25 ºC, EASL 1800 m, AAR 2,306 mm), and Polonnaruwa (07˚ 56′ N 81˚ 0′ E, AAT 27 ºC, EASL 60 m, AAR 1,678 mm) were selected to conduct the field experiment. The locations consisted of variable soil types, particularly red yellow podzolic, and low humic gley great soil groups (Ministry of Agriculture, 2014). Initial soil properties of the four districts were measured, and found to be not significantly different due to high variability, and they ranged; soil pH 5.9–6.4, bulk density 1.33 Mg m^{-3}, labile carbon (SLC) 12700–12900 mg kg^{-1}, total nitrogen (STN) 0.08–0.18%, total phosphorus (STP) 0.065–0.073%, and SOC 0.7–1.0%.

Experimental Design

Uniform, two consecutive paddy fields, each of ca. 0.4 ha with two different farming practices, (a) farmers' CF alone practice (340 kg NPK/ha), and (b) BFBF practice (2.5 L BFBF/ha + 225 kg NPK/ha) were arranged in a randomized block design in each of the selected 14 locations in Ampara (n = 3), Kurunegala (n = 3), Kegalle (n = 3), and Polonnaruwa (n = 5). The 14 field locations acted as replicates. Paddy (various varieties used by farmers in the four districts, viz. BG 352, BG 357, BG 360, BG 366, and AT 302) was broadcasted and irrigation water was managed separately in the fields, without mixing from surrounding fields. In the BFBF application, 500 ml of BFBF was soaked to ca. 7 kg of fine sand and this mixture was remixed with the solid CF top dressing and broadcasted at two weeks and six weeks of the crop.

Sampling and laboratory preparations

Four random plant hills with root-zone soil were uprooted carefully at 50% flowering stage from each experimental paddy plot to measure the root length. The samples were brought to the laboratory of the NIFS, and the plant root-zone soil was removed carefully. Soil moisture (SM), pH and conductivity were measured using the fresh soils. Then, the rest of the soil samples were air-dried, and the subsamples of the air-dried soils were ground and passed through 0.5 mm sieve.

Soil analysis

Soil moisture was determined by oven drying fresh soil at 105 ºC to a constant weight. Soil pH was determined using soil:water 1:2.5 ratio. Using the ground soils, SOC was determined using Walkley-Black colorimetric method (Baker, 1976), whereas STN and STP were measured using distillation and titration method (Bremner and Mulvaney, 1982) and colorimetric method (Anderson and Ingram, 1993), respectively. SLC was analyzed using permanganate oxidizable carbon method (Weil *et al*., 2003).

Soil stable C

Following formula was used to calculate soil stable C (SSC, g/100 g).

$$SSC = SOC - SLC$$

During cropping season in paddy cultivation, drying and rewetting cycles (DRC) were observed in well-drained soils. Simultaneous fluctuations of soil moisture can be seen with the DRC, thus leading to loss of SLC from the paddy soils (Dong *et al*. (2021). This is why we have to deduct SLC from SOC in calculating SSC.

Then, the SSC density or SCS (t ha^{-1}) was calculated based on the thickness of the soil layer [L (m)], bulk density [ρ (initial soil value of 1.33 Mg m^{-3} was used)], and SSC (Veldkamp, 1994).

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

Yield Analysis

Paddy grain yield (kg/ha) was evaluated by collecting five 1 m x 1 m crop cuts in each field, threshing them manually, cleaning and weighing the seeds.

Data 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 soil parameters between the two practices, pairwise Tukey's multiple range test ($p < 0.10$) was used. Probability level considered for statistical significance of the results was 0.10, because in agricultural field research, there is an allowance to consider the significance even up to 10% probability level (Mullen *et al*., 2008).

Network construction

First, densities of SOC, SLC, STN and STP were calculated based on the initial soil bulk density and plant rooting depth. Then, network analysis was performed by Gephi software based on the correlation analysis of the parameters i.e. soil pH, conductivity, SM, SOC, SLC, STN, and STP. Gephi has been used widely in visualizing soil-plant-microbial networks, especially in paddy cultivation (Bakker *et al.*, 2014; Sun *et al.*, 2018; Ji *et al.*, 2018).

Multiple nonlinear regression analysis

The complex interactions among the main variables were evaluated using multiple nonlinear regression, which is used to model complex phenomena that cannot be handled by linear regression models. The regression analysis was done using Statistics Kingdom (2023) software, whereas the 3D response surface plots were constructed using Academo (2022) software.

RESULTS AND DISCUSSION

The rice plant rooting depth was significantly higher in the BFBF practice than that of the farmers' CF practice (Table 1; P = 0.001), showing a potential to increase SOC accumulation (Jayasekara *et al*., 2022). Moreover, the SLC concentration was significantly lower in the BFBF practice than the farmers' CF practice (Table 1; P = 0.044), which may have contributed to increase SSC via low priming effect, thus leading to increased SCS (Premarathna *et al*., 2023). Further, the BFBF practice significantly increased paddy grain yield by ca. 43% over the farmers' CF practice (Table 1). Such yield increases have also been observed previously by Premarathna et al. (2021) and Rathnathilaka et al. (2022).

Network Analysis

Networks of farmers' CF and BFBF practices

Ten relationships (five positively and five negatively correlations) were observed in the network of the farmers' CF practice whereas 12 relationships (eight positively and four negatively correlations) were observed in the BFBF practice. In the farmers' CF practice, the SOC was directly related positively to SM (r = 0.856; P = 0.000), STN (r = 0.794; P = 0.000) and STP (r = 0.421; P = 0.015), and negatively related to soil pH (r = -0.501; P = 0.003) (Figure 1). In the BFBF practice, SM (r = 0.766; P = 0.000), STN (r = 0.546; P = 0.006), STP (r = 0.340; P = 0.104), and SLC (r = 0.521; P = 0.039) were positively, and soil pH (r = -0.633; P = 0.001) and conductivity (r = -0.404; P = 0.050) were negatively related to the SOC (Figure 2). Only in the BFBF practice, the SLC contributed to SOC stock because the density of SLC with the increased rooting depth was relatively higher in the BFBF practice than that of the farmers' CF practice (Figures 1 and 2). The SLC is a short-lived vital component in SOC that determines the soil biological fertility, acting as a soil microbial energy source. However, the SLC is highly sensitive to soil management (Magdoff and Weil, 2004; Duval *et al.*, 2018). Soil improvement via SLC enhances the plant-available nutrients such as N and P, and holds enough SM (Hammad *et al.*, 2018), as was observed from the nutrient densities of the present study (Figures 1 and 2).

Table 1. Soil and plant parameters of the BFBF and farmers' CF practices.

Parameter	**BFBF practice**	**Farmers' CF practice**	**Difference**
SM (%)	32.40 ± 2.18	32.45 ± 1.94	0.05 (0.985)
pH	5.85 ± 0.11	5.92 ± 0.10	0.07 (0.665)
Conductivity (µs/cm)	14.13 ± 1.81	15.53 ± 1.28	1.39 (0.541)
SOC (%)	1.79 ± 0.18	1.95 ± 0.16	0.17 (0.500)
SLC (mg/kg)	12,438 ± 109	12,805 ± 73	367 (0.044)
SCS (t/ha)	26.5 ± 2.62	20.3 ± 1.70	6.2 (0.062)
STN (%)	0.15 ± 0.019	0.16 ± 0.013	0.003 (0.901)
STP (%)	0.07 ± 0.006	0.07 ± 0.005	0.001 (0.943)
Plant rooting depth (cm)	11.2 ± 0.64	7.8 ± 0.52	3.4 (0.001)
Grain yield (kg/ha)	6350 ± 220	4425 ± 180	1925 (0.002)

Mean ± SE in each column. SE of the means was calculated using the four district means of each parameter. *Values within parentheses are probability levels at which differences are significant. Soil moisture (SM), pH, conductivity, organic C (SOC), labile C (SLC), total nitrogen (STN) and total phosphorus (STP).

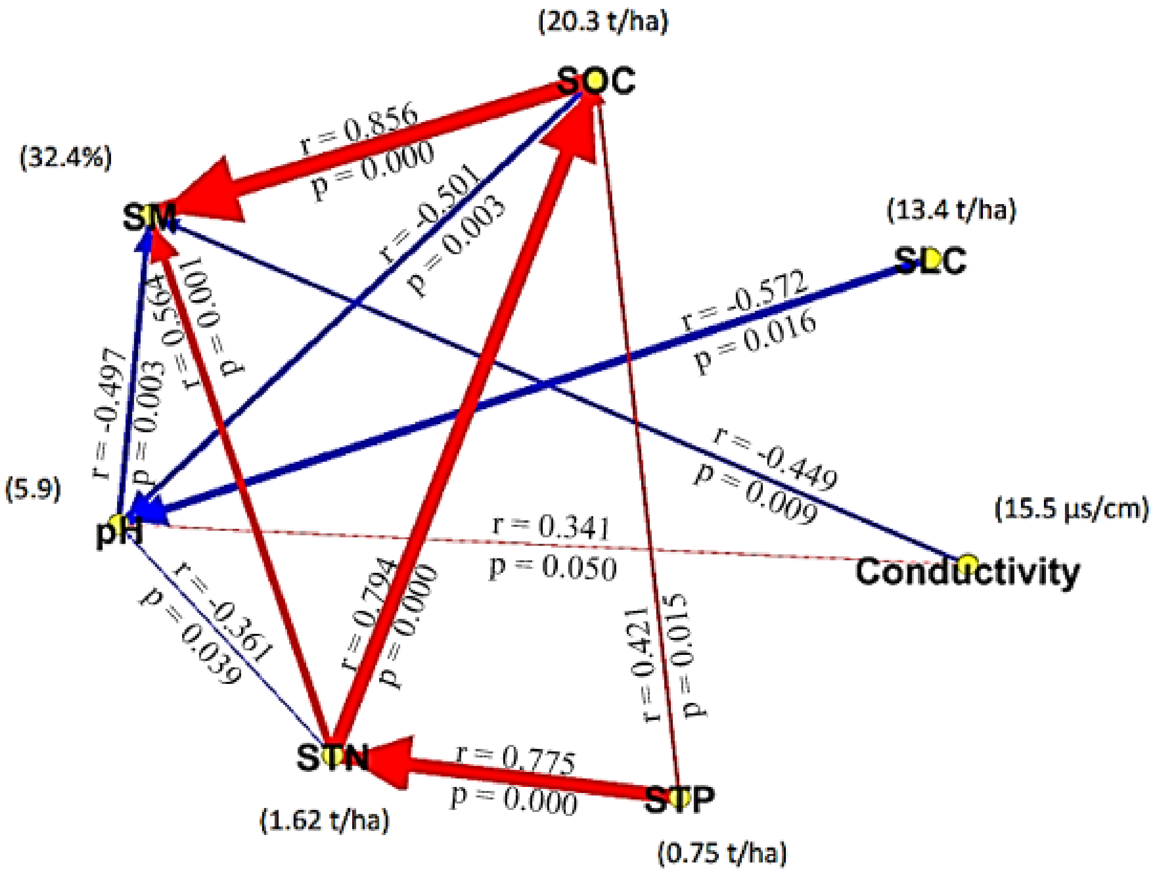

Figure 1. Network of soil physico-chemical parameters in the farmers' CF practice. Values within parenthesis are mean values of each parameter. Red and blue arrows are positive and negative interactions, respectively. Thickness of the arrows represents the strength of the interaction. r: correlation coefficient, p: probability level.

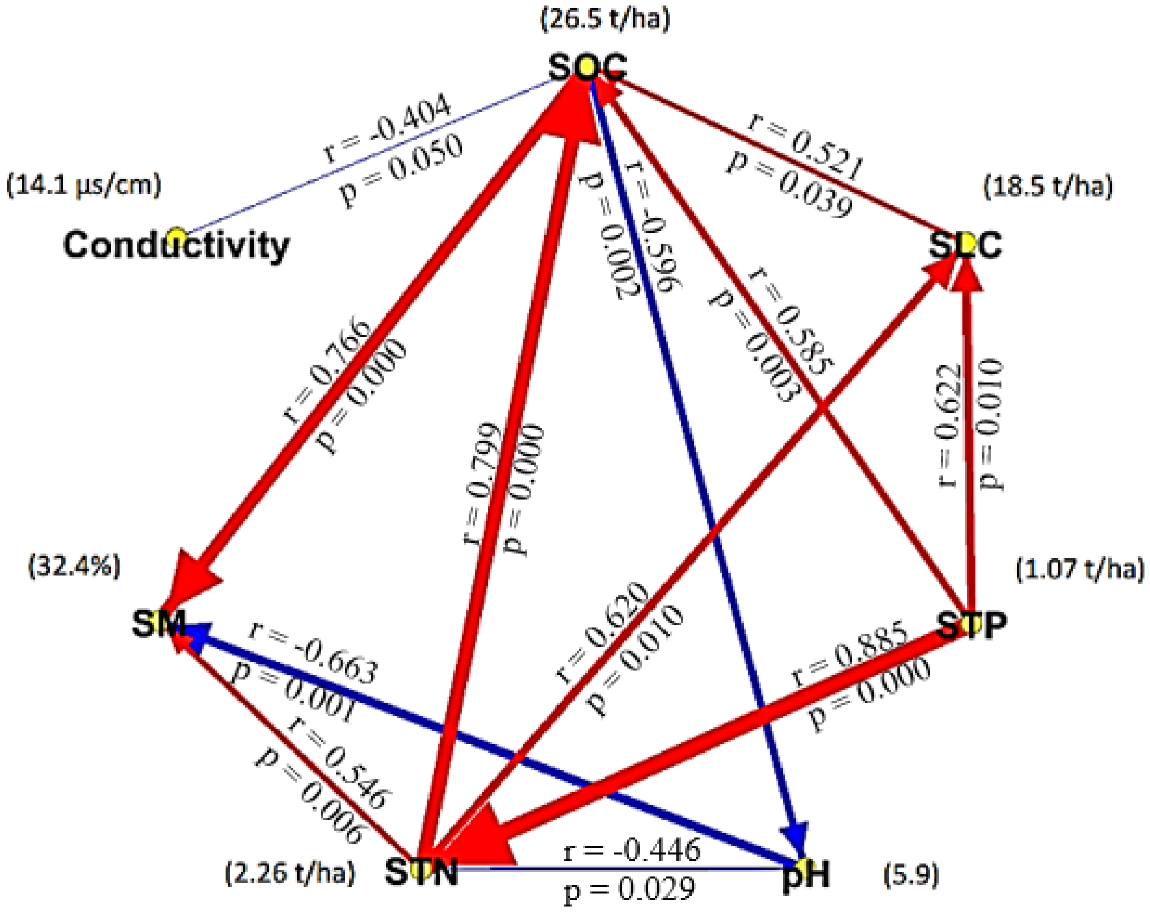

Figure 2. Network of soil physico-chemical parameters in the BFBF practice. Values within parenthesis are mean values of each parameter. Red and blue arrows are positive and negative interactions, respectively. Thickness of the arrows represents the strength of the interaction. r: correlation coefficient, p: probability level.

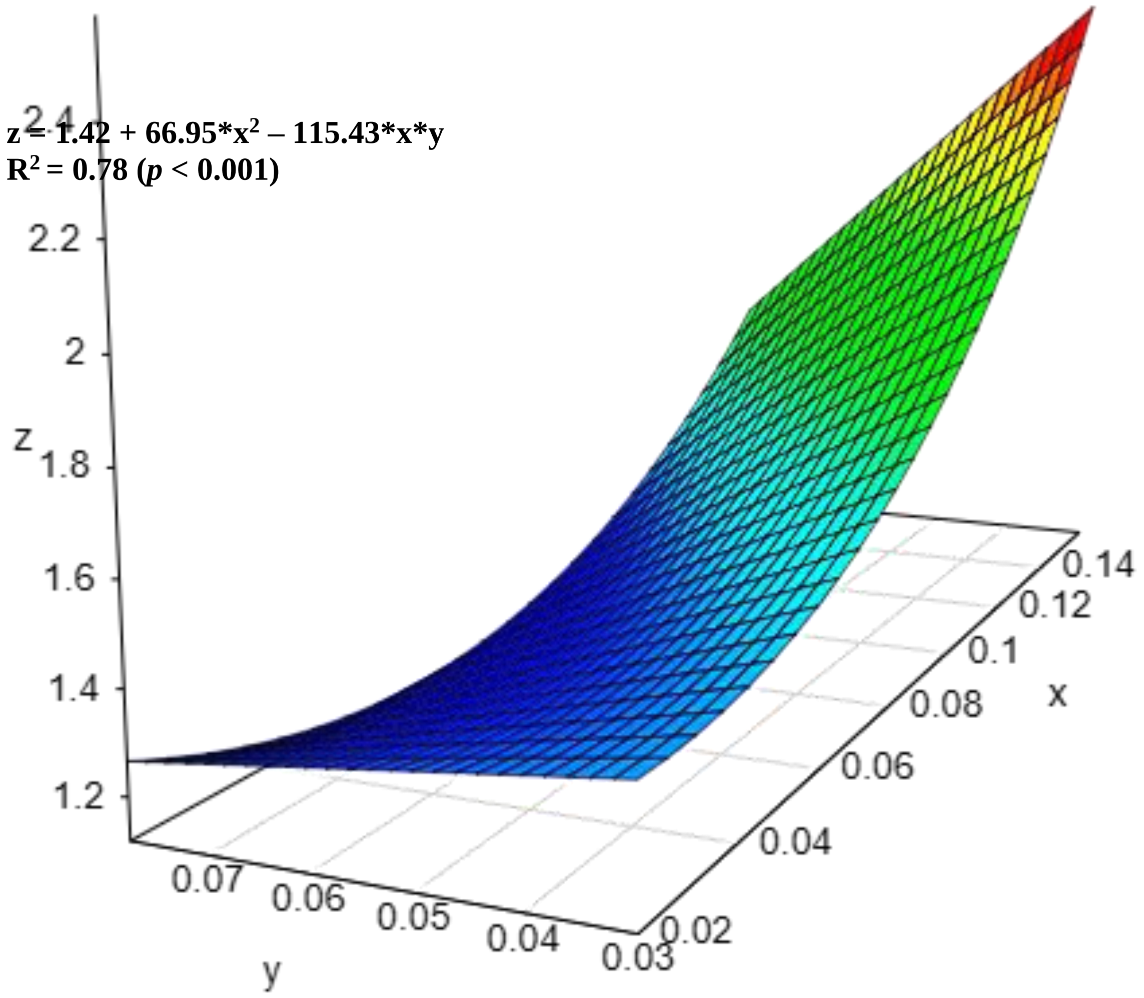

Figure 3. 3D response surface plot among SOC (z), STN (x) and STP (y) in the farmers' CF practice.

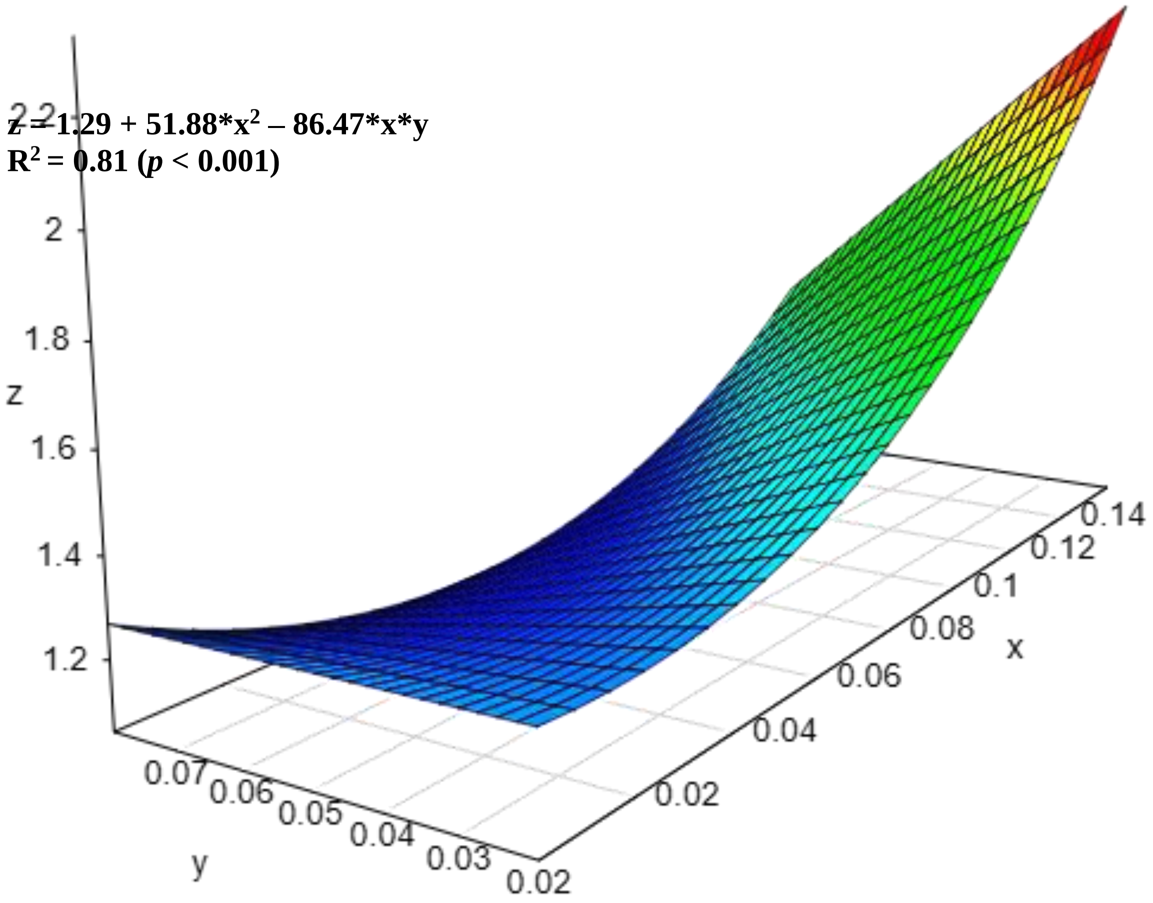

Figure 4. 3D response surface plot among SOC (z), STN (x) and STP (y) in the BFBF practice.

The results of the non-linear regression indicated that there were very strong relationships among STN, STP, and SOC [F value (2, 30) = 53.77, $P < 0.001$, $R^2 = 0.78$] in fields with farmers' CF practice (Figure 3). It was indicated that STN ($T = 6.892$, $P < 0.001$) and STP ($T = -4.298$, $P < 0.001$) were significant predictors in the model. Similarly, the non-linear regression of the BFBF practice also indicated that there were very strong relationships among STN, STP, and SOC [F value (2, 21) = 44.81, $P < 0.001$, $R^2 = 0.81$] (Figure 4), while STN ($T = 4.997$, $p < 0.001$) and STP ($T = -2.983$, $p = 0.007$) being significant predictors in the model.

The non-linear regression equations of the both practices take the form,

$$SOC = c + b.STN^{\wedge}2 - a.STN.STP \;\ldots\ldots\ldots\ldots\ldots (1)$$

where a, b and c are coefficients.

By differentiating this partially with respect to STN and STP, we get the following two equations.

$$\frac{\partial(SOC)}{\partial(STN)} = 2b.STN - a.STP \;\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

$$\frac{\partial(SOC)}{\partial(STP)} = -a.STN \;\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

This shows that with any increase in STN, the change in SOC depends on both STN and STP, whereas with any increase in STP, the change in SOC depends only on STN negatively. Further, the equation (2) shows that at constant STP, the increase of STN increases SOC accumulation, whereas at constant STN, the increase of STP decreases SOC accumulation. This is because P addition increases nutrient imbalances, limiting N and P in the rhizosphere for the decreased SOC storage (Wei et al., 2017). This decreased SOC accumulation could also be attributed to increased CH_4 emissions with the P addition to lowland paddy soils, as was reported by Shah *et al*. (2022). As such, the regression analysis revealed the complexity of the network interactions among SOC, STN and STP in the soil C sequestering network within the limitations of this study.

CONCLUSION

A higher number of interactions in the soil C sequestering network of the BFBF practice implied the strengthening of the network interactions. The complex N and P network interactions in SCS of the two practices indicated the need of careful management of the nutrients for soil C storage. Increased density of the SLC along the elongated rooting depth of the BFBF practice should have contributed to increased nutrient cycling and soil microbial C immobilization. In conclusion, it is clear that nutrient management is the key for maximizing SCS, and of course yield with the BFBF application in paddy cultivation in the Sri Lankan context.

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