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Biofilm biofertilizer application rapidly increases soil quality and grain yield in large scale conventional rice cultivation: a case study

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ABSTRACT
Excessive use of chemical fertilizers (CF) has led to degrade agroecosystems, and human and environmental health. In this context, maintaining soil quality has been challenging. As alternatives to CF, ecofriendly substitutes are being tested, but to a lesser extent. Amongst, Biofilm biofertilizers (BFBF) which improve soil and crop yields are being popularized among farmers. Thus far, there is no published research regarding the effect of BFBF on soil quality and crop production at large scale cultivations. Therefore, present study investigated this in lowland rice farming of Sri Lanka. The study was carried out in 54 farmer fields spreading over thousands of hectares in three districts representing major rice growing areas. In each location, two consecutive, uniform paddy fields were applied separately with farmers' CF practice and the newly introduced BFBF practice. Root zone soil samples were collected and analyzed for 16 physico-chemical and microbial parameters, and grain yield was recorded at harvest. The results showed that the application of BFBF increased soil nutrient contents and microbial communities, and finally it led to increase grain yield over the CF alone practice. A significant relationship between a developed soil quality index (SQI) and grain yield only in the BFBF practice indicated that the soil has a major role to play only in ecofriendly rice cultivation. It is concluded from this study that the CF alone application would not be sufficient to break yield barrier for which the reinstated microbial action of BFBF is a must.

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KEYWORDS
BFBF; grain yield; rice cultivation; soil quality

Introduction

Soil quality maintenance is critical for long-term agricultural productivity. However, to sustain crop productivity, excessive use of chemical fertilizers and agrochemicals has been a common practice, which has adversely affected not only on the agricultural ecosystems themselves, but also on human and environmental health (Beiying, Tianlin, and Bing 2010). This has demanded us to evaluate the physicochemical and biological properties of soil under different types of fertilizer application, in order to select ecofriendly alternatives (Filip 2002). Rice is the staple food in Asia and it covers approximately 160 Mha of croplands in the region (Ricepedia 2021). However, with the population increase in the region, the demand for rice production has increased in several folds. This has led to the indiscriminate use of CF, particularly nitrogenous (N) fertilizers, which, in turn, has increased production cost, and also has created many problems such as deterioration of soil quality and environmental pollution because of leaching, emissions and volatilization in soil, water, and air (Singh et al. 2017).

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In modern agriculture, alternative sources to CF and the use of organic inputs (e.g., Biofertilizers) are considered as sustainable options to improve soil quality (Meena et al. 2015). The favorable effects of biofertilizers on crop productivity, soil quality, disease resistance, and plant growth hormones have been widely discussed (Yang, Kloepper, and Ryu 2009). N_2 fixers and P solubilizers are mainly used in the production of biofertilizers and they enrich soil N and P, reduce the need of inorganic N and P fertilizers, which reduce environmental risks (Bhattacharjee and Dey 2014). Further, the application of biofertilizers increases soil organic matter (SOM), by inducing changes within the soil organic carbon (SOC) pools, play important roles in soil nutrient status and stabilization of SOC (Li et al. 2013; Meena et al. 2015; Wang et al. 2021) and ultimately improve the soil quality and crop productivity.

Biofilm biofertilizer (BFBF) is an effective bio-product introduced during 2000s to agriculture (Seneviratne, Kecskés, and Kennedy 2008a). Biofilm consists of a community of microbial cells and extracellular polymeric substances (EPS), which are secreted by the resident microbes to have protection to the community. The use of BFBF can reduce CF application, increase crop yield, and improve soil quality while recovering soil livability that has been damaged by conventional agriculture practices (Seneviratne, Kecskés, and Kennedy 2008a; Seneviratne et al. 2011). Currently the BFBF practice has been adopted by farmers in thousands of hectares throughout the rice growing areas in Sri Lanka. Therefore, the BFBF practice has become an acceptable remedy to reduce the amount of CF in the country (Seneviratne et al. 2011). However, there is no scientific evidence on the effect of BFBF application on soil quality and its impact on rice yield. Therefore, the present study focused on investigating this in large scale rice cultivation of Sri Lanka. Here, indices were developed to predict soil quality and sustainability of the crop yield using several soil attributes related to physicochemical and microbial properties.

Materials and methods

Experimental sites

The experiment was conducted in Ampara (7.2912°N, 81.6724° E), Kurunegala (7.4871° N, 80.3649° E) and Polonnaruwa (7.9403° N, 81.0188° E) districts in Sri Lanka. The research sites had a typical subtropical climate with a mean annual rainfall of 1000 mm, 2000 mm and 1678 mm in Ampara, and Kurunegala and Polonnaruwa districts, respectively during the study period of 2018-2020. The locations consist of variable soil types, particularly red yellow podzolic with laterite, low-humic gley, non-calcic brown, reddish brown earth, solodize solonets and regosol (Sri Lanka Ministry of Agriculture 2014). According to WRB soil classification system, all these soil types, particularly in paddy cultivation have been classified as Anthrosols (IUSS Working Group WRB 2015). Initial soil properties of the three districts were not significantly different due to high variability and therefore they were pooled and the means were calculated (Table 1).

Treatments and sample collection

In 54 locations in the three districts, farmers' maintained two consecutive, uniform paddy field plots, each ca. 0.4 ha for two major treatments viz. the farmers' CF practice [425 kg CF ha^{-1} (Urea 284, TSP 76 and MOP 66 kg ha^{-1})] alone; and the BFBF practice [2.5 L of BFBF with 225 kg CF ha^{-1} (Urea 150, TSP 40 and MOP 35 kg ha^{-1})]. Previously, Amarathunga et al. (2018) and Wickramasinghe et al. (2018) tested a range of treatments consisting of different levels of CF alone and CF + BFBF combinations {0, 65%, 80% and 100% of CF recommended by the Department of Agriculture of Sri Lanka (DOA), i.e., 340 kg CF ha^{-1} (Urea 225, TSP 55 and MOP 60 kg ha^{-1}); and BFBF + 65% CF & BFBF + 80% CF}. Those two studies showed that the optimum level of CF that should be coupled with BFBF was 225 kg ha^{-1}, as aforementioned. When it was coupled with BFBF, it gave a higher grain yield than 225 kg CF ha^{-1} alone application. Thus,

Table 1. Initial soil properties of the three districts.

Parameter	Mean (n = 54)	Range
STN (%)	0.08	0.04 – 0.1
SOC (%)	0.75	0.6 – 1.2
pH	6.48	6.0 – 7.0
STP (%)	0.2	0.1 – 0.6
SP (%)	0.3	0.2 – 0.8
SLC (%)	0.06	0.05 – 0.08
MBC ($mgkg^{-1}$)	110	100 – 130
STB (x $10^6 CFUml^{-1}$)	1.8	1.5 – 2.5
SD (x $10^4 CFUml^{-1}$)	5.2	5.0 – 6.5
SF (x 10^4 g^{-1})	2.4	2.0 – 2.5
PSB (x 10^4 g^{-1})	1.7	1.5 – 2.0
BD (gcm^{-3})	1.63	1.6 – 1.7
PD (gcm^{-3})	2.13	1.0 – 2.3
PO (%)	35.32	30 – 36
Sand (%)	52.36	47.25 – 54.13
Silt (%)	15.32	14.35 – 17.45
Clay (%)	32.32	31.04 – 33.24
SM (%)	55.7	50 – 58

Soil pH, moisture (SM), bulk density (BD), particle density (PD), porosity (PO), organic carbon (SOC), labile carbon (SLC), total phosphorus (STP), total nitrogen (STN), total potassium (SP), microbial biomass carbon (MBC), fungi (SF), diazotrophs (SD), total bacteria (STB), and phosphate solubilizing bacteria (PSB).

we used this as the recommended practice of BFBF. The BFBF is a fungal-bacterial biofilm (Seneviratne et al. 2008b), which is now a patented [Sri Lanka patent no. 15958 (2013)] commercial product, and hence we are not allowed to reveal its exact composition due to Intellectual Property Right reasons. 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. These two methods are two independent practices, which are being used extensively by farmers. The plots were treated separately without mixing the treatments and the rice crop was cultivated according to cultural practices recommended by the DOA. The study continued for four seasons, wet 2018/2019, dry 2019, wet 2019/2020 and dry 2020 seasons in the three districts. The two consecutive treatment plots were taken as a block design in each site. Fifty-four field locations acted as replicates. The rice varieties used were At 373, At 362, At 311, Bg 358, Bg 350 and Bg 300, all of which are 3.5 month old varieties. At flowering stage, five plants (hills) were carefully uprooted with root zone soil (ca. 0.2 m depth) from random positions in each plot by digging around the root zone without damaging the root system. Root zone soil was sampled because it is the main sphere in which the root system explores for resources that are important for plant growth, and also a significant portion of the water cycle. Moreover, the root zone is still understudied (McNear 2013). The collected samples were immediately brought to the laboratory of the NIFS, Kandy, Sri Lanka. At the crop maturity, five crop-cuts from each plot, each 1 m x 1 m were harvested from random positions to evaluate grain yield.

Sample preparation

Soil was carefully removed without damaging to the root system of each plant. The soil sample was mixed thoroughly and divided into three subsamples; one subsample was used for analyzing moisture (105 °C), pH and microbiological attributes. Soil bulk density was measured according to the method described in Anderson and Ingram (1993). The second subsample was air dried and sieved (< 2 mm), and used for analyzing other properties. In evaluating grain yield, the collected crop-cuts were threshed and cleaned separately, grains were dried to 14% moisture, and crop yield per plot was calculated.

Soil physicochemical analyses

Soil pH was estimated in 1:2.5 (w/v) soil: water suspension using a digital pH meter. BD, PD, PO, ST, SOC, STP, STN and SP were estimated according to the methods given in Anderson and Ingram (1993). The MBC was determined using the $CHCl_3$ fumigation–extraction method (Vance, Brookes, and Jenkinson 1987). The SLC was determined the method of oxidation described by Shang and Tiessen (1997)

Soil microbiological analyses

Soil fungi (SF), diazotrophs (SD), and soil total bacteria (STB), defined as the total number of colony-forming units, were enumerated using a classical serial dilution technique (at a dilution of 1:1000) using Czapek-Dox agar, N free Combined Carbon (CCM) and Nutrient Agar (NA) media, respectively. The serial dilution technique was also used for enumeration of P solubilizing bacteria (PSB)by the spread plate technique on Pikovskaya's solid medium.

Sustainable yield index

At the end of the four seasons, the recorded yield data were pooled and used to calculate the sustainable yield index (SYI) according to Nayak et al. (2012) as follows.

Where, Y is the average yield of rice over the four seasons, sd is the standard deviation and Y_{max} is the maximum yield observed during the four seasons.

Soil quality index

To assess the soil quality index (SQI), three steps were followed: (1) the selection of the appropriate indicators for a minimum data set (MDS) using principle component analysis (PCA), (2) scoring the MDS indicators based on their performance; and (3) incorporation of indicators in a relative index of soil quality (Buragohain et al. 2018; Li et al. 2013; Romaniuk et al. 2011; Raiesi and Kabiri 2016). The selection of indicators for the MDS was based on PCA. PCA is used to reduce data space, thus removing redundant variance (Martens and Naes 1989; Sena et al. 2002). The redundant variance shows the correlated variables in the soil data set which are not essential for the analysis. PCA helps to explain general hypotheses from the collected data. The difference between PCA and statistics used to analyze planned experiments is that the latter uses a priori hypotheses testing. The main rationale of exploratory analysis like PCA is that it is used to learn about interrelationships between variables and objects. Only the factors with eigenvalues ≥ 1 were considered in the selection of indicators in the MDS after applying varimax rotation of the factor analysis. Soil parameters which had a higher correlation ($r > 0.7$) with the PCA and the variable with the highest loading factor were considered in the MDS (Li et al. 2013). Selected parameters were transformed into numerical scores that ranged from 0 to 1 in the second step (Armenise et al. 2013). After all, indicators in the MDS were scored and weighted, and soil quality index (SQI) was calculated using the method described by Buragohain et al. (2018) and Romaniuk et al. (2011).

$$\mathrm{SQI} = \sum_{i=1}^{n} (Wi\ x\ Qi)$$

where, SQI is the soil quality index ranging from 0 to 1.0, W_i is the assigned weight of each indicator, Q_i is the indicator score, and n is the number of indicators in the final MDS. Regression analysis was done to see if there were relationships between SYI and SQI and paddy grain yield of the treatments.

Table 2. Mean physico-chemical and microbial properties of root zone soil, rice grain yield, sustainable yield index (SYI), and soil quality index (SQI) after four seasons of the two treatments in the three districts.

Parameter	BFBF practice (n = 54)	Farmers' CF practice (n = 54)
STN (%)	0.96[a]	0.44[b]
SOC (%)	2.04[a]	0.77[b]
pH	5.63[b]	6.58[a]
STP (%)	0.85[a]	0.66[b]
SP (%)	1.65[a]	1.17[b]
SLC (%)	0.13[a]	0.04[b]
MBC (mgkg^{-1})	156[a]	109[b]
STB (x 10^{6}CFUml^{-1})	10.3[a]	2.0[b]
SD (x 10^{5}CFUml^{-1})	4.9[a]	0.8[b]
SF (x10^{4} g^{-1})	6.0[a]	2.3[b]
PSB (x10^{4} g^{-1})	5.0[a]	1.8[b]
BD (gcm^{-3})	1.47[b]	1.67[a]
PD (gcm^{-3})	2.59[a]	2.42[a]
PO (%)	37.3[a]	33.5[a]
Sand (%)	40.2[b]	43.3[a]
Silt (%)	30.7[a]	28.3[a]
Clay (%)	18.3[a]	11.2[b]
SM (%)	54.9[a]	54.7[a]
Grain yield (kgha^{-1})	6440[a]	4270[b]
SYI	0.91	0.85
SQI	0.71	0.15

Within rows, values with different letters differ significantly ($P < 0.05$, Duncan's multiple-range test). Soil pH, moisture (SM), bulk density (BD), particle density (PD), porosity (PO), organic carbon (SOC), labile carbon (SLC), total phosphorus (STP), total nitrogen (STN), total potassium (SP), microbial biomass carbon (MBC), fungi (SF), diazotrophs (SD), total bacteria (STB), phosphate solubilizing bacteria (PSB), sustainable yield index (SYI), soil quality index (SQI), and paddy grain yield.

Statistical analysis

Means and correlations of all the variables of BFBF practice and farmers' CF practice 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.

Results and discussion

Soil physicochemical and biological properties

With the application of BFBF, SOC and clay contents increased significantly ($P < 0.05$) by 165% and 63%, respectively, while BD and sand content decreased significantly by 14% and 8%, respectively, compared to the farmers' CF practice (Table 2). The increased SOC content with the use of BFBF in paddy cultivation has also been observed previously by Seneviratne et al. (2009). The simultaneous increase of the SOC and clay contents in our study can be ascribed to,1) augmented root exudates C and their incorporation in to the root zone due to improved photosynthesis with the BFBF practice (Buddhika, Seneviratne, and Abayasekara 2014; Dignac et al. 2017), and 2) SOC and clay interactions via a range of physico-chemical processes (Singh et al. 2018) that traps clay particles in the SOC-rich root zone without letting them to lose through the downward transport which is generally observed in the lowland paddy field conditions, respectively (Li et al. 2005; Chen, Zhang, and Effland 2011). The decreased BD and sand content with the BFBF application are attributable to reduced soil compaction and dilution of the sand fraction, respectively with vast accumulation of SOC in the root zone. Generally, soil texture is an inherited soil property that may not change significantly within a short period of time. However, in the present study, soil texture was observed to alter because we analyzed only the root zone soil with the aforementioned changes.

There was a significantly low soil pH, but within the favorable limits with the BFBF practice, compared to the farmers' CF practice (Table 2), possibly due to enhanced production of

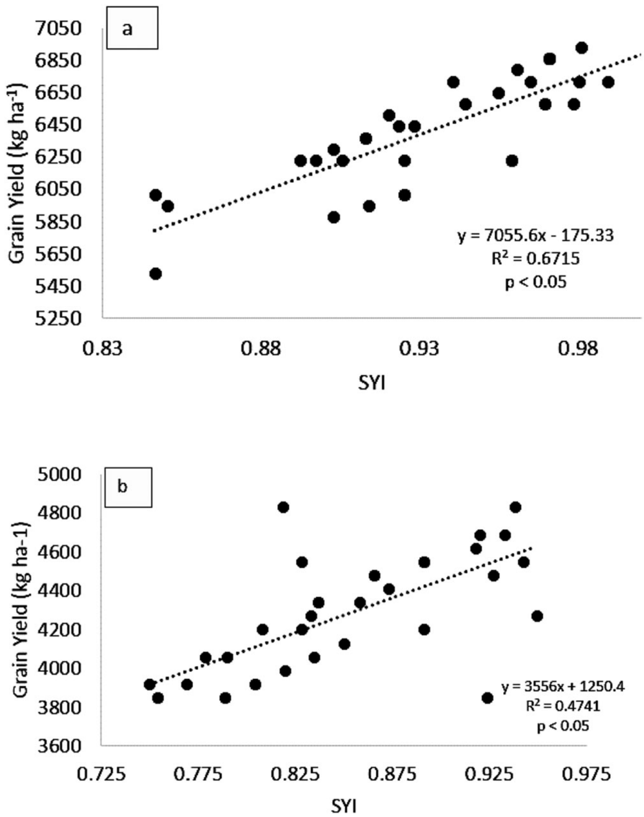

Figure 1. (a) Relationship between paddy grain yield and sustainable yield index (SYI) of BFBF practice and (b) relationship between paddy grain yield and sustainable yield index (SYI) of farmers' CF practice.

microbial acids like formic, acetic, propionic, lactic, glycolic, fumaric and succinic acids. Moreover, the increased acid production plays an integral role in improved primary metabolism in plants such as C3 photosynthesis (Rodrı́guez and Fraga 1999), which might also have contributed to the increased SOC with BFBF application, as explained above.

The STN, STP, SP, MBC and SF of the BFBF practice were significantly higher than that of the farmers' CF practice ($P < 0.05$; Tables 2).This clearly shows that excessive CF NPK that is not taken up by the plants in the BFBF practice is incorporated in to the microbial biomass, as reflected in MBC, mainly SF, which contributes immensely to microbial immobilization of the nutrients in paddy soils (Inubushi and Nagano 2017).Furthermore, the increased STN in the BFBF practice should also have been contributed from biologically fixed N by the increased SD, as evidenced by the increased soil C availability (i.e., SLC) and PSB, which are essential in supplying energy for the energy-costly biological nitrogen fixation (Stam, Stouthamer, and Verseveld 1987). Generally, SD provide N to the rest of the soil microbial community for their growth and persistence (Seneviratne et al. 2011), which was reflected by the increased STB in our study (Table 2).

Rice yield and SYI

Mean rice yield of the two treatments ranged between 4270 kg $ha^{-1}$and 6440 kg ha^{-1}over the four seasons, with the highest recorded yield in the BFBF practice (Table 2). This can be ascribed to improved nutrient availability with the mineralization of increased soil NPK pools of the BFBF

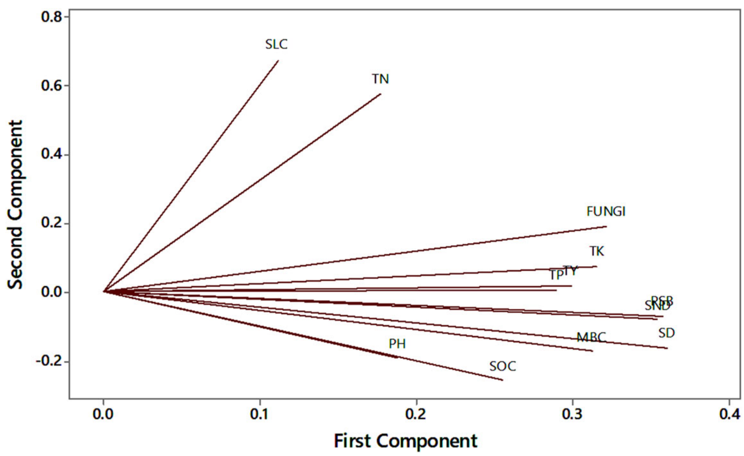

Figure 2. Principal component analysis loadings of the soil physicochemical and microbial parameters investigated in the BFBF practice. Soil pH, organic carbon (SOC), labile carbon (SLC), total phosphorus (STP), total nitrogen (STN), total potassium (SP), microbial biomass carbon (MBC), fungi (SF), diazotrophs (SD), total bacteria (STB), phosphate solubilizing bacteria (PSB).

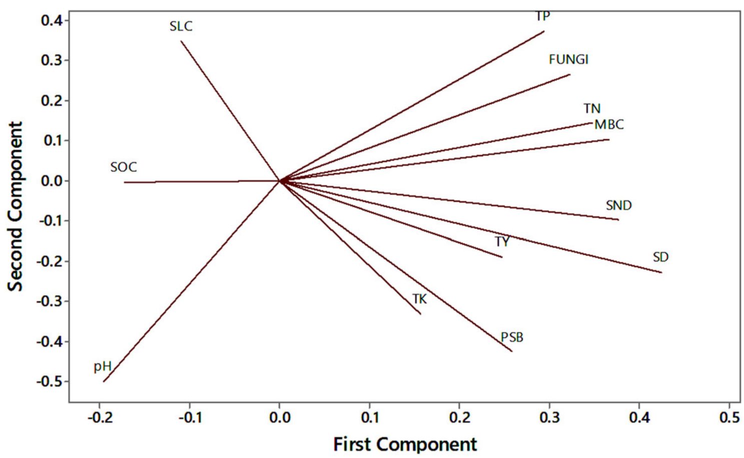

Figure 3. Principal component analysis loadings of the soil physicochemical and biological parameters investigated in the farmers' CF practice. Soil pH, organic carbon (SOC), labile carbon (SLC), total phosphorus (STP), total nitrogen (STN), total potassium (SP), microbial biomass carbon (MBC), fungi (SF), diazotrophs (SD), total bacteria (STB), phosphate solubilizing bacteria (PSB).

practice. Although there was a higher yield with the BFBF application, it is important to evaluate sustainability of crop production, which can be estimated by SYI (Figure 1). This index helps to establish the minimum guaranteed yield that can be obtained compared to the maximum observed yield. An SYI close to 1 indicates the capacity of a fertilizer to sustain high crop yields, whereas deviation from 1 indicates loss of sustainability (Bhindhu and Gaikawad 1998; Li et al. 2013). As such, the higher value of SYI (i.e., 0.91) of the BFBF practice indicates higher sustainability of the BFBF practice than that of the farmers' CF practice (Table 2).

Soil quality index

All three PCs were considered to develop the SQI (Figures 2 and 3). The variables included in the correlation analyses (i.e., STN, SOC, pH, STP, SP, SLC, MBC, STB, SD, SF and PSB) were finally selected to represent the soil quality indicators and they were given weights when calculating the SQI (Tables 3 and 4).

Table 3. Communality and weight of soil quality indicators in MDS of the BFBF practice.

Variable	Factor 1	Factor 2	Communality	Weight
STN	0.462	0.636	0.618	0.0829
SOC	0.683	0.275	0.543	0.0728
pH	0.505	0.208	0.298	0.0399
STP	0.771	0.013	0.595	0.0798
SP	0.821	0.084	0.681	0.0913
SLC	0.300	0.752	0.655	0.0878
MBC	0.835	0.184	0.731	0.0980
STB	0.910	0.090	0.835	0.1120
SD	0.932	0.181	0.901	0.1208
SF	0.827	0.207	0.727	0.0975
PSB	0.929	0.079	0.569	0.1165

Soil pH, organic carbon (SOC), labile carbon (SLC), total phosphorus (STP), total nitrogen (STN), total potassium (SP), microbial biomass carbon (MBC), fungi (SF), diazotrophs (SD), total bacteria (STB), phosphate solubilizing bacteria (PSB).

Table 4. Communality and weight of soil quality indicators in MDS of the farmers' CF practice.

Variable	Factor 1	Factor 2	Communality	Weight
STN	0.714	0.210	0.554	0.0925
SOC	0.300	0.221	0.139	0.0232
pH	0.723	0.336	0.635	0.1060
STP	0.794	0.074	0.635	0.1060
SP	0.023	0.578	0.334	0.0557
SLC	0.079	0.591	0.356	0.0594
MBC	0.716	0.348	0.634	0.1058
STB	0.574	0.545	0.626	0.1045
SD	0.561	0.755	0.886	0.1479
SF	0.764	0.082	0.591	0.0986
PSB	0.126	0.766	0.602	0.1005

Soil pH, organic carbon (SOC), labile carbon (SLC), total phosphorus (STP), total nitrogen (STN), total potassium (SP), microbial biomass carbon (MBC), fungi (SF), diazotrophs (SD), total bacteria (STB), phosphate solubilizing bacteria (PSB).

Mean SQIs of the BFBF practice and farmers' CF practice of the 54 field locations were 0.71 and 0.15, respectively (Table 2). It can be clearly observed that the highest SQI belongs to the BFBF practice over the farmers' CF practice. The SQI proved that the application of inorganic fertilizers had adverse effects on soil quality, whereas the application of biofertilizers boosted soil quality (Macci et al. 2012). Results demonstrated that STN, SOC, pH, STP, SP, SLC, MBC, STB, SD, SF and PSB were the key parameters that contributed to the improvement of soil quality and noticeably indicates that the contributors to SQI significantly affect the rice yield. Therefore, these parameters should be included to the MDS to evaluate agricultural soil quality with the biofertilizer incorporation in the present context.

Regression analysis showed that SQI was significantly related with rice yield in the BFBF practice ($p < 0.05$; Figure 4a) whereas there was no significant relationship in the farmers' CF practice ($p > 0.05$; Figure 4b).

In general, the objective of soil quality evaluation is to determine soil quality grade by analyzing soil indicators, and then to evaluate the potential in increasing rice yield. In our study, soil quality was determined using all indicators, but only key indicators were selected from the data analysis. Each indicator in the MDS was scored and weighted, and then the SQI was obtained using the Integrated Quality Index (IQI) equation. In the present study, the significant relationship between SQI and grain yield only in the BFBF practice indicated that the Integrated Quality Index equation is a good method for developing a biologically significant soil quality indicator system for ecofriendly rice cultivation in Sri Lanka in future.

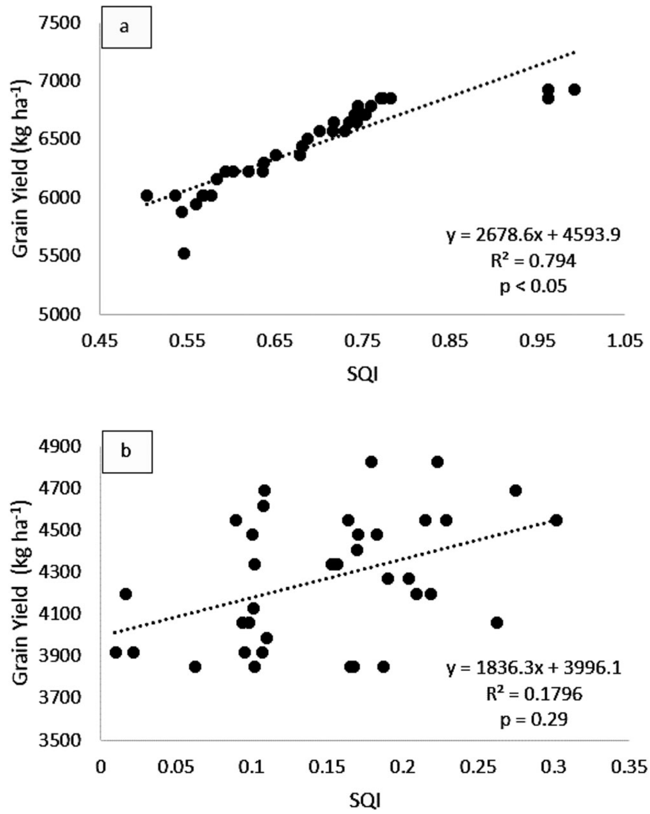

Figure 4. (a) Relationship between paddy grain yield and soil quality index (SQI) of BFBF practice and (b) relationship between paddy grain yield and soil quality index (SQI) of farmers' CF practice.

Conclusions

This study concludes that soil quality can predict paddy grain yield only in ecofriendly BFBF practice. This implies that the grain yield in the farmers' CF practice is determined by other factors than soil parameters, possibly the temporal availability of applied CF in the soil, which varies depending on spatial soil heterogeneity. The significantly increased grain yield of the BFBF practice over the farmers' CF practice indicates that CF alone would not be sufficient to break the yield barrier for which the reinstated microbial action is a must.

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