Effect of Biofilm Biofertilizer on Agro-ecosystems: A Network Science Approach

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ABSTRACT

Biofilms are complex communities of multiple microbial species which are attached to surfaces or with interfaces. Such beneficial biofilms can be developed *in vitro* and be used as Biofilm biofertilizer (BFBF). Agro-ecosystems consisting of soil, plant and microbial communities are affected by farmers' practices (e.g. excessive use of chemical fertilizers, CF), disrupting its natural cycles through depleting beneficial microorganisms. BFBF application to a crop soil increases N_2 fixing bacteria, P-solubilizing fungi and many other useful microorganisms which help reinstate the degraded networks in the agro-ecosystem by replacing lost organisms and also producing various biochemicals such as phytohormones, signaling molecules etc. Thus, the present study was designed for analyzing soil, plant and microbial parameters with the application of farmers' CF practice and BFBF practice in 25 different locations using rice (*Oryza sativa* L.) as the test crop, and to reveal the effect of BFBF in re-establishing networks in the agro-ecosystem. Results indicated that parameters were variable among different locations; however there was an increasing trend of rice grain yield in the BFBF practice. BFBF application has improved rhizosphere soil organic matter through microbial action, which has in turn increased rice grain yield over CF alone application in the farmers' practice. It helped in cutting down chemical fertilizers use up to 50% of the farmers' practice without hampering yields. This has been attributed to the positive effects of the biofilms in the BFBF practice towards soil and microbial properties. Therefore, it is concluded from this study that the BFBF practice can be considered as an eco-friendly and economically viable method to replace current farmers' practice of CF alone application.

KEYWORDS: Agro-ecosystem, Biofilm biofertilizers, Microbial communities, Networks.

INTRODUCTION

Functional stability of ecosystems is strongly influenced by diverse microbial communities in the soil (Wittebolle *et al*., 2009). There are signal-mediated interactions between microorganisms which are parasitic/symbiotic with plants, animals, fungi, dry/wet land decomposers, aerobes, anaerobes, N_2 fixers, disease suppressors, actinobacteria, sulfur oxidizers, phages, plasmids, genetic parasites and viral colonies. Plant-microbe interactions in the soil mediate key processes that control ecosystems, and they potentially represent a mechanistic link between plant diversity and ecosystem function (Zak *et al.,* 2003).

Extreme uses of the soil and hazardous practices in the cropping systems lead the ecosystems to degrade. Modern agriculture is one of the greatest extinction threats to biodiversity in most of the agro-ecosystems (Jackson *et al.,* 2005). Use of chemical inputs in agro-ecosystem results in collapse of microbial communities, particularly N_2 fixers, which leads to a reduction in microbial diversity (Van der Heijden *et al.,* 2006; Hadgu *et al.,* 2009). Regaining the degraded microbial community structure and complex networks in agro-ecosystems is important for sustainability of agriculture.

Microbes are found in the agro-ecosystems as planktonic (freely living) or as biofilm (complex communities) forms. Biofilms are assembling of microorganisms adherent to each other and/or biotic or abiotic surfaces and embedded in a matrix of polymers. Such biofilms consist of microbial cells (algal, fungal, bacterial and/or other microbial) and extracellular polymeric substances (EPS), which are secreted by themselves to have structural protection from environmental stresses such as UV radiation, extreme pH, osmotic shock, desiccation.

Beneficial biofilms in rhizosphere enhance cycling of nutrients and their availability for crop growth as well as biocontrol of pests and diseases, thus improving crop productivity and soil fertility than their monoculture forms (Seneviratne and Jayasinghearachchi, 2005). Although these biofilms occur naturally in the soil, there density is not enough to have a significant effect. Therefore, *in vitro* development and application of biofilms as biofertilizers is essential for augmenting agricultural productivity in eco-friendly manner (Bandara *et al*., 2007).

As an agricultural country, majority of people in Sri Lanka is engaged in agriculture, and the highest land area is occupied by rice cultivation. Rice (*Oryza sativa)* is the staple food in Sri Lanka and it is cultivated as a wetland crop in all the districts. About 34% of total cultivated area (0.88 million ha) is occupied by paddy lands. About 1.8 million farm families are engaged in paddy cultivation island-wide producing 4,819 Mt rice annually (Census and Statistics, 2016). It is mainly cultivated using chemical inputs. Therefore, it is very important to develop methods to cultivate rice using eco-friendly biological methods, which should directly contribute to enhance its productivity and quality in a sustainable manner.

Generally, BFBFs influence on microbial activities in soil and rice plants (Feng *et al.*, 2006). Among plant microbes of exophytes and endophytes, endophytes play a major role in crop production, promoting plant growth and resistivity to pathogens (Feng *et al*., 2006). Endophytic bacteria are defined as nonpathogenic bacteria that can be isolated from surface disinfected plant tissues or extracted within the plants. BFBFs act as not only a biofertilizer, but also as one of very effective novel treatment applications which can be used to reduce chemical fertilizer application, improve the crop growth, while recovering soil livability that was damaged by conventional agriculture practices (Seneviratne *et al.*, 2011; Seneviratne *et al.*, 2007). This is a product which contains beneficial fungal-bacterial communities (Seneviratne *et al.*, 2008) in a biofilm mode. Studies that have been carried out with BFBF application have shown that it facilitates biological N_2 fixation etc. in non-legumes (e.g. rice, tea, wheat and vegetables), while solubilizing phosphorous and other nutrients required for crop growth via beneficial interactions between microbes and the soil (Seneviratne and Indrasena, 2006; Seneviratne *et al*., 2008).

Inoculation of the developed microbial biofilms influences microbial and plant diversity and soil quality parameters positively. Soils treated with a developed biofilm and evaluated after three months for plant and culturable microbial species richness, microfaunal count, nitrogenase activity and selected soil parameters showed that the biofilm application resulted in significantly higher plant species richness than respective monocultures of the biofilm. Further, culturable bacterial and fungal species richness, soil nitrogenase activity, total organic carbon and available ammonium and nitrate also increased significantly with the biofilm application. Thus, the present study was designed for analyzing soil, plant and microbial parameters with the application of farmers' CF alone practice and BFBF practice using rice (*Oryza sativa* L.) as the test crop, and to reveal the effect of BFBF in re-establishing networks in the rice agro-ecosystem.

MATERIALS AND METHODS

Field Sites

Twenty five farmer fields in Ampara (n=3), Nikaweratiya (n=14), Ambalanthota (n=8) districts with variable soil types were selected to conduct the field experiments. Two consecutive, uniformly managed paddy fields were used to apply treatments separately. Two treatments applied were (a) BFBF practice (1000 ml of Biofilm-R with 90 kg NPK per acre). (b) Farmers' practices (180 kg NPK per acre). The two consecutive treatment plots were taken in a block design in each site. Twenty five field locations acted as replicates.

Sample Collection

Two plant samples (hills) with their rhizosphere soils around the root system were collected from each treatment plot. They were collected at flowering and harvesting stages.

Determination of Characteristics

Soil pH was determined by using fresh soil: water ratio 1:2.5(Rhoades, 1982). Soil moisture content was measured by oven drying fresh soil at 105 ^oC to a constant weight. To determine Total Organic Carbon (TOC) content the rhizosphere soil was removed from plant roots and air dried. Sub samples were ground and passed through two step sieving with a 2mm and 0.5mm sieves. TOC was measured using Walkley-Black colorimetric method (Baker, 1976).

Root and Shoot Dry Mass

Roots and shoots were separated and oven-dried at 65 ^oC to measure root and shoot dry mass (g).

Total Endophytic Bacterial Count

Total endophytes in plant leaves were enumerated by culturing them at 10^{-5} dilution in water agar + mineral salts. Total number of colonies was taken after 48 hours. Nitrogen fixing bacterial count (diazotroph count) was taken in water agar + 5 carbon sources(Glucose, Manitol, Sucrose, Malic Acid, Lactic Acid)of combined carbon medium (CCM).

Data Analysis

Data were analyzed statistically using Statistical analysis system software (Minitab 17 and Stata, R). Network analysis was performed by Gephi software.

RESULTS AND DISCUSSION

Soil, plant and microbial parameters of the two treatments in different locations are summarized in Table 1 and depicted in Figures 1and2. They indicate that although the parameters are variable among different locations, there is an increasing trend of rice grain yield in the BFBF practice.

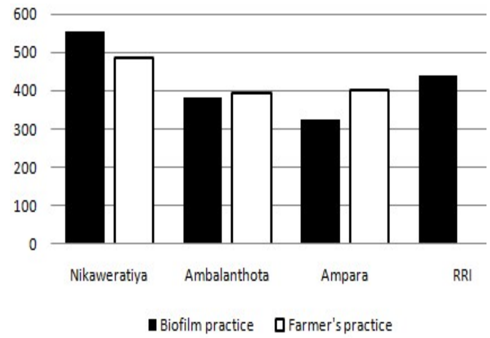

Figure 1. Total endophytes per plate of the two treatments in different locations.

Table 1: Soil pH, moisture, TOC, total endophytes, endophytic diazotrophs, shoot dry weight, root dry weight and yield of the two treatments (1- BFBF practice, 2- farmers' practice) in different locations.

Location	Treatment	Total endophytes	Endophytic diazotrophs	pH	Moistu-re	Total C	Root DW	Shoot DW	Yield
Nikaweratiya (n=14)	BFBF	553.0[a] ± 264.5	2741.3[a] ±3214.6	6.5[a] ±0.6	23.9[a] ± 8.5	11.0[a] ± 4.4	4.7[a] ±2.0	13.7[a] ± 4.6	2200[a] ±336.3
	CF	486.2[b] ± 326.6	1727.5[b] ± 2538.5	6.3[b] ±0.5	32.2[b] ± 5.8	11.3[b] ± 2.3	3.6[b] ±4.2	8.3[b] ± 2.7	2113.8[b] ±328.1
Ambalanthota (n=5)	BFBF	382.6[a] ±153.0	366.0[a] ± 246.0	nd	nd	16.1[a] ±3.5	3.7[a] ±2.6	14.1[a] ± 9.4	3434.8[a] ±259.9
	CF	395.0[b] ± 176.7	566.0[b]	nd	nd	13.1[b] ±5.2	2.7[b] ±2.0	10.6[b] ± 4.8	nd
Ampara (n=3)	BFBF	324.5[a] ±150.9	177.6[a] ±191.8	5.7[a] ±0.5	43.5[a] ± 6.1	17.4[a] ± 6.6	2.0[a] ±1.0	7.3[a] ±0.2	2246.6[a] ± 251.6
	CF	400.8[b] ±227.0	162.5[b] ±165.6	5.9[b] ±0.1	37.4[b] ± 3.0	16.5[b] ± 4.2	3.2[b] ±1.7	9.2[b] ± 1.0	2015.6[b] ± 72.2
RRI (n=3)	BFBF	441.3[a] ± 366.8	3136.0[a] ± 2596.9	7.2[a] ±0.1	nd	16.2[a] ±0.8	8.0[a] ±1.1	21.1[a] ±6.5	nd

(Total endophytes - per plate, Endophytic diazotrophs – per plate, Moisture - %, Root DW – g, Shoot DW – g, Yield – kg/acre, RRI – Rice Research Institute, Ambalanthota, nd – not determined)

Significant differences were not observed for soil pH, moisture and TOC between two treatments. However, yield and shoot dry weight was significantly higher in BFBF compared with farmer's practice.

Total Endophytes and Endophytic Diazotrophs

Total endophytes and Endophytic Diazotrophs were higher with BFBF practice compared to farmers' practice in Nikaweratiya district (Figure 1 and 2). In Ambalanthota and Ampara these values were higher with farmer's practice except Endophytic Diazotrophs in Ampara. Only BFBF fields were available in RRI, where highest endophytic diazotrophs were found.

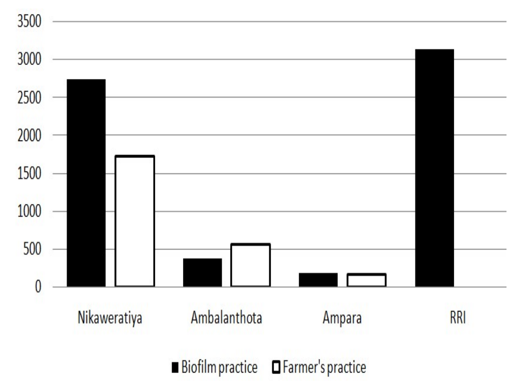

Figure 2. Endophytic diazotrophs per plate of the two treatments in different locations.

Correlations among different parameters

In the BFBF practice, soil pH and moisture positively and negatively correlated with total endophytes, endophytic diazotrophs, respectively (Figure 3, $P < 0.05$). In the farmers' practice, only total endophytes negatively correlated to soil moisture (Figure 4, $P < 0.05$). Further, in the BFBF practice, root dry weight was positively correlated with shoot dry weight and moisture was negatively correlated with shoot dry weight (Figure 3, $P < 0.05$).

	WA+MS	MS+CCM	pH	Moisture	Total C	Root DW	Shoot DW
MS+CCM	0.401						
	0.089						
pH	0.182	0.494					
	0.485	0.032					
Moisture	-0.918	-0.737	-0.537				
	0.000	0.023	0.109				
Total C	0.013	-0.100	0.194	0.527			
	0.956	0.675	0.441	0.144			
Root DW	0.224	0.132	0.311	-0.459	-0.144		
	0.342	0.568	0.182	0.182	0.513		
Shoot DW	0.274	0.103	0.395	-0.841	0.105	0.722	
	0.242	0.656	0.085	0.002	0.634	0.000	
Yield	-0.350	-0.391	-0.201	0.256	0.357	-0.082	-0.051
	0.184	0.121	0.455	0.476	0.122	0.725	0.827

Cell Contents: Pearson correlation
P-Value

(WA + MS = Total Endophytes, MS + CCM = Endophytic Diazotrophs)

Figure3. Correlations among different soil, plant and microbial parameters in biofilm practice.

	WA+MS	MS+CCM	pH	Moisture	Total C	Root DW	Shoot DW
MS+CCM	0.468						
	0.125						
pH	0.310	0.483					
	0.327	0.095					
Moisture	-0.845	-0.632	-0.285				
	0.008	0.128	0.494				
Total C	-0.188	-0.457	-0.060	0.634			
	0.603	0.184	0.868	0.091			
Root DW	0.248	0.102	0.013	-0.495	-0.209		
	0.392	0.729	0.965	0.213	0.493		
Shoot DW	0.266	0.531	0.120	0.519	0.009	0.312	
	0.358	0.051	0.682	0.188	0.977	0.207	
Yield	0.053	-0.099	-0.377	-0.414	-0.179	0.360	0.055
	0.891	0.785	0.253	0.308	0.644	0.276	0.871

Cell Contents: Pearson correlation
P-Value

(WA + MS = Total Endophytes, MS + CCM = Endophytic Diazotrophs)

Figure 4. Correlations among different soil, plant and microbial parameters in farmers' practice.

Network analysis

Network analysis for farmer's practice revealed that rice grain yield is more correlated with plant biomass and endophytes (according to the width of the arrows), which are also strongly correlated with each other (Figure 5). Here, plant biomass and endophytes are weakly related to rhizosphere soil C. However, in the BF treatments, rice yield has been governed mainly by rhizosphere soil C (Figure 6). With the BFBF application, endophytes are more related to rhizosphere soil C (width of the edge is high). This clearly shows that BFBF application has improved rhizosphere soil organic matter through microbial action, which has in turn increased rice grain yield over CF alone application in the farmers' practice.

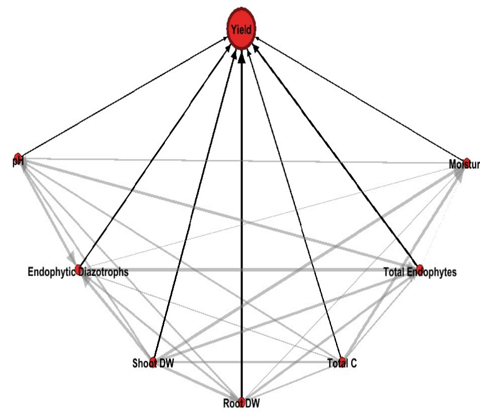

Figure 5. Network analysis of farmer'sCF alone practice.

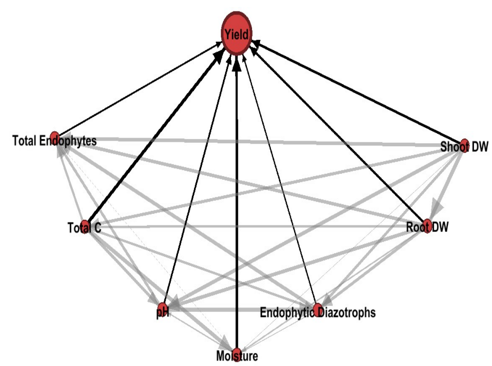

Figure 6. Network analysis of biofilm practice.

CONCLUSIONS

It is clear from this study that biofilm practice helps increase rice grain yield, while cutting down chemical fertilizers up to 50% of farmers' practice without hampering yields.
Significant differences were not observed for soil pH, moisture and TOC between two treatments. Soil TOC was higher in Biofilm practice than Farmer's CF alone practice. However, yield, root dry weight and shoot dry weight were significantly higher in BFBF compared with farmer's practice.

In the CF alone treatments, rice grain yield is more correlated to plant biomass and endophytes. Plant biomass and endophytes are weakly related to rhizosphere soil C. In the BFBF treatments, rice yield has been governed mainly by rhizosphere soil C. Endophytes are more related to rhizosphere soil C. BF application has improved rhizosphere soil organic matter through microbial action, which has in turn increased rice grain yield over CF alone application in the farmers' practice. Therefore, the biofilm practice can be considered as an eco-friendly and economically viable method to replace current farmers' practice of CF alone application.

ACKNOWLEDGEMENTS

All the members of Microbial Biotechnology Unit of the NIFS and Department of Biotechnology WUSL 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.

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