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Developed microbial biofilms can restore deteriorated conventional agricultural soils

G. Seneviratne [a,*], A.P.D.A. Jayasekara [b], M.S.D.L. De Silva [c], U.P. Abeysekera [c]

[a] Microbial Biotechnology Unit, Institute of Fundamental Studies, Hantana Road, Kandy, Sri Lanka
[b] Tea Research Institute of Sri Lanka, Hantana, Sri Lanka
[c] Tea Research Institute of Sri Lanka, St. Coombs, Talawakelle, Sri Lanka

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ABSTRACT

Nitrogen fixing bacteria play a key role in the growth and persistence of effective microbial communities in the soil by supplying N through biological nitrogen fixation (BNF). In the long run, chemical inputs, particularly N fertilisers are known to adversely affect N_2 fixers and hence maintenance of soil fertility and crop productivity. This study examined the effect of developed microbial biofilms with N_2 fixers on restoration of soils deteriorated by conventional agricultural practices in tea cultivation. Just reducing recommended chemical fertiliser use by 50% significantly increased soil microbial biomass and BNF, and decreased soil NO_3^- and pest infestation. The lower chemical fertiliser addition coupled with the biofilm-based biofertilisers known as biofilmed biofertilisers (BFBFs) further increased BNF significantly. The combined application significantly increased soil organic C by ca. 20%, and reduced leaf transpiration by ca. 40%. It also supported plant growth, rhizoremediation and soil moisture conservation in comparison to the 100% chemical fertilisation. Those improved performances were observed to be proportional to the increased density of soil bacteria, and have several agronomic and environmental implications. It is apparent from this study that replenishing the depleted soil microbial communities by applying such biofertilisers is likely to be beneficial in agroecosystems with chemical N fertiliser use, if they are to be sustained for crop production.

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1. Introduction

In conventional agriculture, high cost inputs, particularly chemical fertilisers and agrochemicals have been reported to adversely affect maintenance of soil fertility and crop productivity in the long term, mainly due to their effects on soil fauna and microbes (Bedano et al., 2006; Seneviratne, 2009). This has been demonstrated in China, where farmers are now showing that reducing chemical fertiliser use can improve crop yields without contributing to environmental problems (Hvistendahl, 2010). It is speculated in this report that this may be attributed to immobilized, large nutrient reserves built up previously in the soil under high fertiliser rates that provide the balance of nutrients in synchrony with crop demand, thus improving crop uptake and yields. If that is the case, this, being a temporary effect would vanish when the nutrient reserves are depleted. However, it is well-known that the N fertilisers suppress the action of microbes (Kolb and Martin, 1988; Cruz et al., 2009), particularly N_2 fixers. This tends to produce N-poor soil microbial communities with low biomass (Priha and Smolander, 1995; Hossain et al., 1995; Černý et al., 2003; Cruz et al., 2009), due to diminished N supply by the N_2 fixers. Thus, reduced fertiliser rates should also enhance growth of the microbes and consequently their beneficial effects on the plant growth and the soil.

The N_2 fixers play a key role in the growth and persistence of effective microbial communities by supplying N through BNF. This has been demonstrated in a number of studies, as explained below. An increased microbial efficiency of P biosolubilization was illustrated when an N source was added to naturally occurring, surface-attached microbial communities or biofilms (Singh and Amberger, 1998). Further, several studies indicated the improved efficiency of beneficial microbial action in N_2 fixation, P biosolubilization, plant growth hormone production etc., when N_2 fixers were incorporated to microbial biofilms, as was reviewed by Seneviratne et al. (2008a). As a recent development in biofertiliser research, biofertilisers have been produced from developed fungal–rhizobial biofilms *in vitro* (Seneviratne et al., 2008a), which are now known as BFBFs (Seneviratne et al., 2008b). The BFBFs showed increased BNF, mineral nutrient release in the soil, organic acid and plant

* Corresponding author. Tel.: +94 81 2232002; fax: +94 81 2232131.
E-mail address: gaminis@ifs.ac.lk (G. Seneviratne).

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growth hormone production etc., compared to mono- or mixed cultures of the microbes without biofilm formation (Seneviratne et al., 2008a). The present study examined the effect of the BFBFs on restoration of soils deteriorated by conventional agricultural practices in tea cultivation of Sri Lanka as a test case.

2. Materials and methods

2.1. Experimental setup

The study was conducted in an ongoing trial of about one year old tea (*Camellia sinensis* cv. DT1) of the Tea Research Institute at Talawakelle (up country 1382 m amsl, mean annual temperature 18 °C, mean annual precipitation 2250 mm) of Sri Lanka. The site was previously under tea with conventional chemical fertilisers and agrochemical applications for 50–55 years. There were three treatments in the present study; i) 100% of recommended chemical fertilisers (CF, 113 kg N ha^{-1} y^{-1} as sulphate of ammonia, 33 kg P ha^{-1} y^{-1} as rock phosphate, 69 kg K ha^{-1} y^{-1} as muriate of potash and 20 kg Mg ha^{-1} y^{-1} as kieserite), applied in every two months, and 1.6 kg Zn ha^{-1} y^{-1} as zinc sulphate sprayed within 7–14 days after application of other fertilisers), ii) 50% of CF or iii) 50% of CF together with BFBFs (*Acetobacter* spp., *Azotobacter* spp., *Rhizobium* spp., *Bradyrhizobium* spp. and a nonpathogenic *Colletotrichum* spp. in the fungal surface-attached biofilm mode). The treatments were replicated three times and arranged in a randomized complete block design. Generally, the BFBFs alone application is not recommended, since they are fungal-bacterial biofertilisers, which may incorporate a considerable fraction of plant available soil nutrients to the fungal biomass, thus reducing plant growth. All microbes isolated from the soil–plant system of tea in Sri Lanka are stored in the culture collection of the Institute of Fundamental Studies, Sri Lanka. Bacterial cell densities of the BFBFs are in the order of 10^9 cfu mL^{-1} broth. Microbial biomass of the biofilm is ca. 0.03 g dry weight mL^{-1} broth. A low-cost liquid medium composed of locally available C and nutrient sources (exact composition is not revealed due to Intellectual Property Right reasons) is used to grow the BFBFs. It is a liquid biofertiliser diluted with water at 1:15 and sprayed on to the soil just after CF application (i.e. once in every two months). In the trial, there were 20 plants per plot sized 6 × 4 m. There was a negligible slope of the land. Soil type in the site is Red yellow podzolic with a texture of clay loam, pH 4.1, 1.45% C and 0.08% N.

In addition, a nursery experiment was established in a planthouse of the Tea Research Institute with the same treatments as above (4 replicates per treatment, arranged in a completely randomized design) for evaluating nitrogenase activity of tea roots at maturity. Tea cuttings of the same cultivar DT1 were planted in standard nursery bags with the soil collected from the above site, and the experiment with standard nursery practices was maintained for 8 months.

2.2. Sampling and analyses

Soil was collected from 0 to 10 cm depth of the plots only once on the 20 July 2010. All field measurements were also done on the same day of soil sampling. Four soil samples were collected from random position of each plot using a soil auger (cross section area 19.6 cm^2). They were mixed thoroughly to form one composite sample and transported to the laboratory. After sub sampling a portion from each sample for soil microbial biomass C (MBC), NO_3^-, pH and rhizoremediation, the rest of the sample was air-dried, sieved (<2 mm) and stored for later analysis. Soil moisture was measured *in situ* using Theta Probe capacitance soil moisture instrument type ML1 from Delta-T Devices, Inc., Cambridge, England. The MBC, organic C and NO_3^- were measured by chloroform fumigation-extraction method (Vance et al., 1987), wet oxidation method and colorimetric method using a spectrophotometer (Anderson and Ingram, 1993), respectively. Soil pH was measured in 1:2.5 soil to distilled water ratio, using a pH meter with a glass electrode. Plant height was recorded, because harvested biomass was only available after 1–1.5 years after planting of the young tea. Leaf transpiration was measured using a portable infrared gas analyser (ADC-LCA4, Analytical Development Company Ltd., Hoddeson, England). Shothole borer infestation was recorded from a visual count of the plots. Rhizoremediation was evaluated by lettuce seed germination method (Marwood et al., 1998). Total soil bacteria extracted from sterilized distilled water (1:25 soil to water, w/v) were diluted to 10^{-4} and plated on nutrient agar for visual observation of the colonies.

In the nursery experiment, plants were carefully uprooted after 8 months and nitrogenase activity of tea roots was measured by acetylene reduction assay (Zuberer and Silver, 1978).

2.3. Data analysis

For data, lettuce seed germination percentages were compared using χ^2-test. Effects of the three treatments on other parameters were analysed by ANOVA and the means were separated by LSD test at 5% probability level. Data were analysed using SAS (1998) software.

3. Results and discussion

The present study showed that just reducing recommended chemical fertiliser use by 50% improved plant and soil parameters in tea plantation (Fig. 1). Soil microbial biomass increased significantly by 65–80% ($p < 0.05$), when the chemical fertiliser rate was reduced by 50% in the presence or absence of the BFBFs. Nitrogenase activity, which reflects tea root associated with BNF also increased significantly once the fertiliser rate was decreased ($p < 0.05$). This clearly supports our argument that N_2 fixers are suppressed by chemical fertilisers. Soil nitrate levels were reduced significantly by ca. 70% ($p < 0.05$) when the chemical fertiliser use was decreased. Generally, this could lead to lower nitrate leaching to ground water and emission of N_2O, a greenhouse gas with a very high global warming potential (GWP, 310 times CO_2 eq.). The lowered chemical fertiliser use significantly decreased infestation of shothole borer, a common pest in tea by ca. 65% ($p < 0.05$). It has been shown that altered morphology and reduced availability of nutrients in plant parts under lowered chemical fertiliser rates are attributable to the diminished pest infestation (Altieri and Nicholls, 2003; Garratt et al., 2010). Further, the reduced chemical fertiliser rate increased soil bacterial density by ca. 100-fold (Fig. 2) and may have contributed to the pest control, as plant growth promoting rhizobacteria (PGPR) have been shown to induce systemic acquired resistance of some plants against pests (Ramamoorthy et al., 2001). Overall, various positive responses mentioned above could possibly be due to the increased cell density of bacteria (Fig. 2), having soil ameliorating and biocontroling abilities. The decreased rate of the chemical fertilisers may have contributed to a reduced suppression of the soil bacteria, as was also reflected in the soil microbial biomass (Fig. 1). Improved crop yields with the reduced rates of chemical fertilisers in China (Hvistendahl, 2010) could also be attributed to the reduced suppression of the soil microbes, as was argued above. When the rates of N fertilisers are compared, it is clear that relatively lower level of the fertilisers (113 kg N ha^{-1} y^{-1}; De Silva (2007) and this study) is applied in the tea cultivation of Sri Lanka against China (300–900 kg N ha^{-1} y^{-1}; Han et al., 2007), but, still plant growth and some soil properties are negatively impacted in Sri Lanka. This could be due to relatively low range of soil

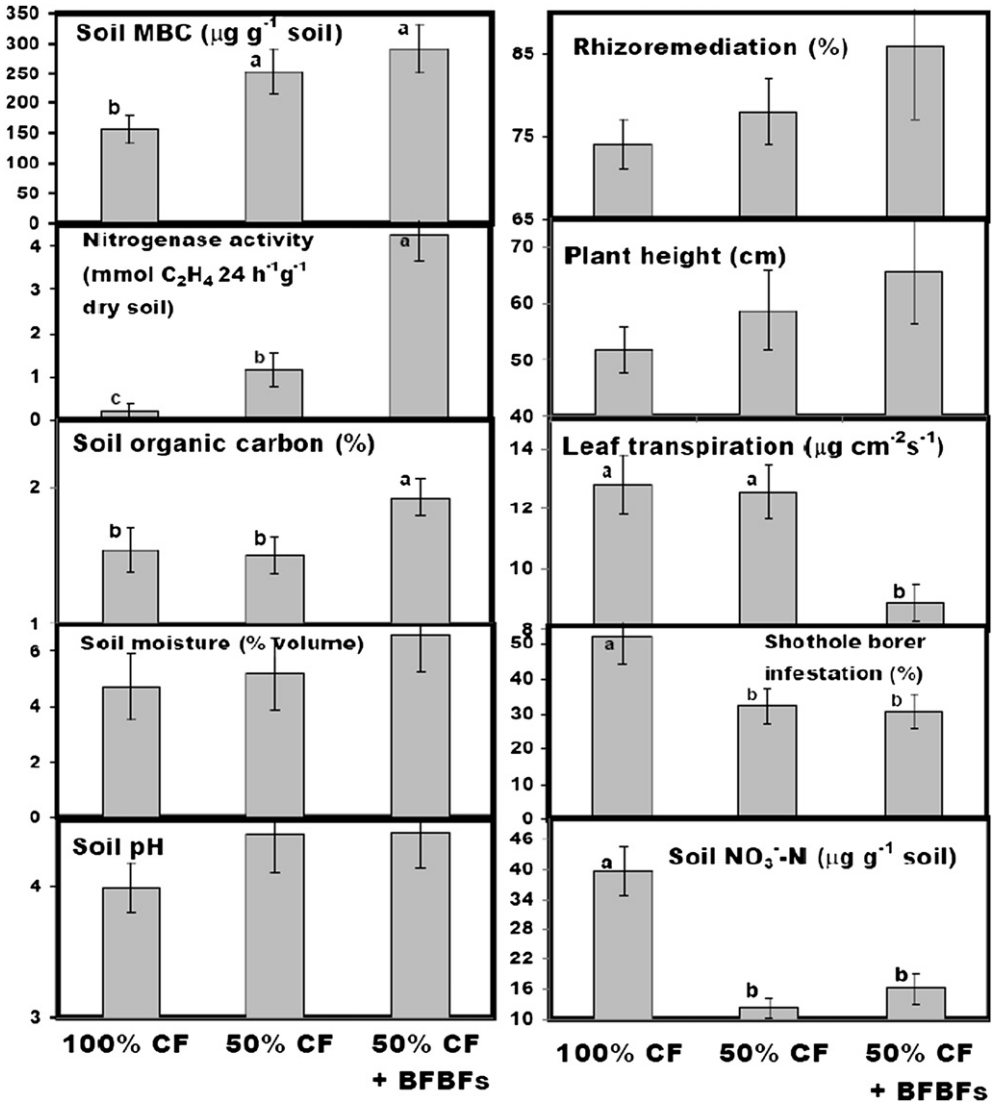

Fig. 1. Responses of soil and plant parameters, when 100% of recommended chemical fertilisers (CF), 50% of CF or 50% of CF together with biofilmed biofertilisers (BFBFs) were applied under nursery and field conditions of tea cultivation in Sri Lanka. Different letters on the columns show significant differences at 5% probability level whereas the columns without letters are not significantly different at the same probability level. Vertical bars on the columns show standard errors.

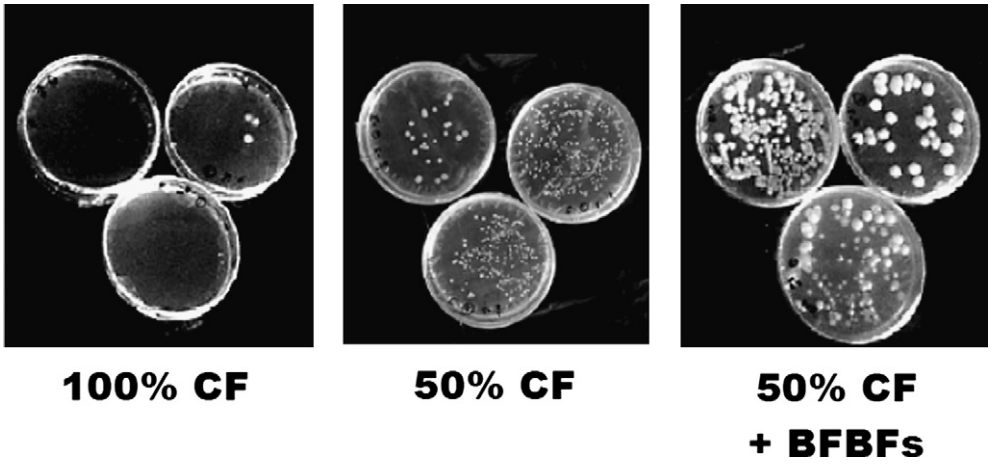

Fig. 2. Total soil bacterial colonies isolated on nutrient agar, when water extracts of soils treated with 100% of recommended chemical fertilisers (CF), 50% of CF or 50% of CF together with biofilmed biofertilisers (BFBFs) in a tea nursery were plated on Petri dishes and observed on day 7. Persistence of the applied BFBFs in the soil was evident from larger colonies developed from large colony forming units or biofilms of the 50% CF + BFBFs treatment.

microbial biomass (120–150 μg C g^{-1} soil; De Silva, 2007; this study) existing in the tea soils of Sri Lanka, compared to China (98–214 μg C g^{-1} soil; Han et al., 2007), which may have been suppressed by even the relatively lower rate of N fertilisers used in Sri Lanka. Thus, the plant and soil properties are negatively impacted by the deteriorated microbial community. This is an understudied area of research which needs further investigations.

Several studies conducted so far with the BFBFs under laboratory, nursery and field conditions of tea in Sri Lanka have shown encouraging results for soil restoration and improved crop production (Seneviratne et al., 2009). In the present study, it was revealed that the use of the BFBFs together with a 50% of the chemical fertilisers can further improve the parameters discussed above with the fertiliser reduction alone. Application of BFBFs together with reduced fertiliser inputs increased the nitrogenase activity significantly ($p < 0.05$) by ca. 4-fold compared to the 50% chemical fertilisers alone (Fig. 1). There were four N_2 fixers in the BFBFs, which could have contributed to the increased nitrogenase activity. Soil organic C increased significantly ($p < 0.05$) by ca. 20% with the use of BFBFs, which could be attributed to increased storage of root exudate C by the fungal components of the BFBFs, forming biofilms on the root surface and in the rhizosphere (Seneviratne et al., 2009). Thus, these biofilms appear to have similar effects as those seen for ectomycorrhizae. Ectomycorrhizal fungi associated with roots of a range of tree species have been observed to assimilate up to ca. 10 t C ha^{-1} y^{-1}, derived from root exudates (Fogel, 1988). This is within the order of magnitude of the observed soil C increase in the present study. The C accumulation is important for better plant growth as well as C sequestration in agroecosystems, thus the use of BFBFs can be considered as a means to enhance soil C storage, contributing to mitigate global warming. With the application of BFBFs, leaf transpiration decreased significantly ($p < 0.05$) by ca. 40%, favouring plant growth. This could be due to decreased transpiration by N_2 fixers like rhizobia, which lessen stomatal conductance, when applied to some crops (Matiru and Dakora, 2005). Plant height and rhizoremediation showed increasing trends as the fertiliser rate was reduced and the BFBFs were coupled with it, but the increases were not significant ($p > 0.05$). In general, plant growth hormonal action of biofertilisers increases plant height (Dardanelli et al., 2010), which translates into increased biomass (Niklas and Enquist, 2001). Soil pH and moisture favoured the plant growth when the chemical fertiliser use was reduced, though insignificantly ($p > 0.05$). All those enhanced performances of the BFBFs application over the reduced rate of chemical fertilisers alone may be due to persistence of biofilms (Seneviratne et al., 2008a), introduced to the soil by the applied BFBFs, as was evident from larger colonies developed from large colony forming units or biofilms of the 50% CF + BFBFs treatment (Fig. 2).

4. Conclusion

It seems that replenishing the depleted soil microbial communities by means of biofertiliser application is likely to be beneficial in agriculture with chemical N fertiliser use. In conventional biofertilisers, monocultures of microbes or mixed cultures are used, which do not render the maximal microbial action to the soil–plant system in terms of plant growth and soil quality improvement. The BFBFs have thus been shown to replenish and revive the soil microbial communities affected by the chemical agriculture. As such, it appears that the crop-soil system also needs Probiotics, possibly in the form of BFBFs for sustenance under conventional agriculture. The BFBFs can also be extended to other crops for which further research are needed.

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