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SHORT COMMUNICATION

Emergence of Diverse Microbes on Application of Biofilmed Biofertilizers to a Maize Growing Soil

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ABSTRACT

Diverse microbial communities in the rhizosphere perform an amazing role in plant growth and productivity. However, conventional agricultural practices such as the use of chemical fertilizer (CF) and tillage have collapsed the diversity of the microbial communities. Direct application of developed fungal-bacterial communities known as biofilmed biofertilizers (BFBFs) to the soil has been introduced recently, and observed to be multi-functional and more effective than conventional biofertilizers. However, the effect of such biofertilizers on soil microbial diversity has not been studied sufficiently world over. Therefore, the current study was carried out to investigate the effects BFBFs on bacterial, cyanobacterial and fungal species in a maize growing soil. A pot experiment was conducted under greenhouse conditions with different fertilizer treatments; 100% chemical fertilizers (CF) recommended for maize, 50% CF, 50% CF + BFBF, and no fertilizer, as the control. Microbial species richness and abundance of bacteria were evaluated after two months of plant growth. Microbial biomass carbon (MBC) was estimated by using chloroform fumigation-extraction technique. Results showed that the species richness of bacteria, fungi and cyanobacteria, and abundance of bacteria were higher in 50% CF + BFBF, compared to 50% and 100% CF. This implies that the action of BFBFs tends to break dormancy of microbial seeds in the soil, resulting in emergence of a diverse microbial community, which may support natural biocontrol of pathogens. An interesting observation was the stimulation of an additional microflora of cyanobacteria by the application of BFBF. MBC levels did not show a significant difference between any of the treatments. We recommend further testing of the BFBFs for increasing microbial diversity and ecosystem functioning and hence the sustainability of maize cultivating agroecosystems.

Keywords: bacteria, cyanobacteria, fungi, *Zea mays*

INTRODUCTION

Agriculture in Sri Lanka is mainly focused on plantations, cereals, vegetables and field crops. Among cereals, maize is a widely utilized crop for human consumption and as animal feed. Currently in Sri Lanka, 30,000 ha, the highest extent of land next to rice, is cultivated with maize (Department of Agriculture, 2006). Modern farming methods such as the use of machineries, pesticide and chemical fertilizer (CF) *etc*. have achieved increased productivity in the sector. However, the CF application adversely affects natural microbial communities in agricultural ecosystems and thereby affect soil fertility and crop productivity in the long run (Seneviratne, 2009). Particularly, chemical N fertilizers tend to produce soil microbial communities having reduced N nutrition and hence low biomass (Priha and Smolander, 1995; Cerny *et al*., 2003; Cruz *et al*., 2009; Strickland and Rousk, 2010), possibly due to low N supply by suppressed N_2 fixers.

Diverse microflora in soil has been identified for its beneficial functions in the soil-plant system (Kennedy and Smith, 1995). They perform various catabolic activities, and are involved in primary production, nutrient recycling *etc*. In addition, their interactions with plant root system play key roles in several other ecosystem functions, such as decomposition of organic matter and nutrient balancing, rhizoremediation, pathogen suppression and, the maintenance of soil structure and water relationships (Sharma *et*

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al., 2010). Hence, the diversity of soil microorganisms is considered as one of the most important indicators in soil quality (Bastidia *et al*., 2008; Sharma *et al*., 2010). Increased microbial biomass with the enrichment of microbial diversity is also an important determinant of quality and productive capacity of soil (Fernandes *et al*., 1997), since it contributes to beneficial functions such as biosolubilization and mineralization (Brookes, 1995; Pankhurst *et al*., 1995; Yao *et al*., 2000).

Generally, research studies pertaining to the application of microbes as biofertilizers in agriculture have been focused to reduce the dependence on CF, due to economic and environmental concerns. Rhizobia and mycorrhizae are the two major candidates of biofertilizers that are used worldwide. In addition, biocontrolling and soil conditioning microbes are also used with a limited application (Milton and Joseph, 1982; Thomas *et al*., 1984; An *et al*., 2009). They are generally applied as mono or mixed cultures. Direct application of developed microbial communities known as BFBFs has been introduced recently, and observed to be multi-functional and more effective than conventional biofertilizers (Seneviratne *et al*., 2011). Application of BFBFs with low levels of CF (*e.g*. 50%) has given yield comparable to 100% CF in tea (Seneviratne *et al*., 2011), rice (Weeraratne *et al*., 2012) and in maize (Buddhika *et al*., 2012a). Further, preliminary studies based on BFBFs for maize showed their positive effects on rhizoremediation, nitrogenase activity, seed germination, seedling growth, photosynthesis and soil N accumulation (Buddhika *et al*., 2012b & c). Such beneficial biological functions have been reported to be due to diversified microbial communities in the soil (Kennedy and Smith, 1995). However, effect of such biofertilizers on soil microbial diversity has not been studied sufficiently. Therefore, in the current study, the effect of the BFBFs on diversity of microbes, particularly bacteria, cyanobacteria and fungi in a maize growing soil was investigated.

MATERIALS AND METHODS

The study was carried out in a green house at the Institute of Fundamental Studies (IFS), Kandy, Central Province, Sri Lanka. A maize-growing, clay loamy soil collected from a home garden in Kandy was used (pH 6.52, organic C 1.87 %, available NH_4^+-N and NO_3^-N 43 µg g^{-1} soil and 15 µg g^{-1} soil, respectively, and available PO_4^{3-} 2.4 µg g^{-1} soil).

Biofilmed biofertilizer inoculum and other treatments

The BFBF that has already been developed (Seneviratne *et al*., 2011) and tested for maize was used in the study. It contains one fungal species (*Aspergillus* sp.) isolated from maize rhizosphere and seven bacterial species (*Azorhizobium* sp., *Rhizobium* sp., *Acetobacter* sp., *Azotobacter* sp, *Azospirillum* sp. and two unidentified bacteria) derived from various sources including maize rhizosphere. All of them are in the culture collection of the Institute of Fundamental Studies, Kandy, Sri Lanka. These microbes were inoculated into a low cost commercial culture medium (exact composition of the medium and the biofilm development method are not revealed due to intellectual property rights) and incubated at 27-30 ºC for 7 days to form fully matured biofilm, and the cell concentration was 10^{-10} ml^{-1}. Developed BFBF was used in the study together with a reduced dose (*i.e*. 50 %) of recommended CF for maize, and compared with 50 % CF alone, 100 % CF and no fertilizers as the control. Generally, BFBF alone is not recommended, since fungal counterpart in the BFBF acquires some nutrients from the soil system, thus reducing plant growth (Seneviratne *et al*., 2011).

Seed material

Zea mays seeds, 'Pacific', a hybrid variety, which is recommended by the Department of Agriculture, Sri Lanka was used in the experiment.

Inoculation, fertilizer application and experimental design

An experiment was conducted in the green house to examine the effect of the BFBFs on microbes that are only in the rhizosphere soil, but not on the root surface, in maize crop. Twelve plastic pots (4 treatments x 3 replicates) were arranged in a completely randomized design (CRD). The pots were filled with 3 kg of a soil mixture (1:1 weight ratio of sand and maize growing soil, < 2 mm), and seeded with maize. Soil and seed inoculations of the BFBF were done after diluting it with water at 1:16. Maize seeds were soaked overnight in tap water and then one half of them were separately soaked in the BFBF, while the other half was continued to be soaked in distilled water for 2 hours, to be placed in CF treatment and no fertilizer control. Seeds were placed at 5 cm depth in all treatments. Remaining suspension of the BFBF was sprayed on to soil at the rate of 10 L ha^{-1}. In 100 % CF treatment, the soil was applied with N, P and K at rates of 200, 100 and 50 kg ha^{-1} as urea, triple super phosphate and muriate of

potash, respectively. Nitrogen at the rate of 80 kg per hectare was applied at sowing time and the rest at the start of tussling. The crop was managed according to the method recommended by the Department of Agriculture, Sri Lanka (Department of Agriculture, 2006). The pots were watered daily to maintain soil moisture at ca. 60% of water holding capacity. At two months soils from the surface and root zone of each pot were collected (using a hand-held auger) for the isolation of cyanobacteria, and bacteria and fungi.

Isolation of rhizosphere bacteria and fungi

Soil samples of all replicates of each treatment were composited. One gram of the composite soil was serially diluted and 50 µl from 10^{-3}, 10^{-4} and 10^{-6} dilutions were spread on nutrient agar (NA) and potato dextrose agar (PDA) plates. Thirty six petri plates were incubated at 25 ºC for 5 days for fungi, and at 30 ºC for 24 hrs for bacteria. After the incubation period, colony forming units (CFU) on NA plates were counted. Morphologically different bacterial colonies were picked from all plates and streaked on another set of new NA plates. Similarly different fungal colonies were also picked from the PDA plates and sub cultured on new PDA plates. This was continued until pure colonies have been achieved.

Identification of isolated bacteria and fungi

Gram's staining was carried out for all isolates of bacteria and morphological characterization was done prior to the biochemical tests. Identification was carried out using standard biochemical tests in the Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). Slide cultures were prepared to identify fungi (Riddell, 1950). Morphological features and sporulating structures of the fungi were used in the identifications.

Identification of cyanobacteria

Cyanobacteria growing on the soil crust in each treatment were carefully scraped without soil by using a sharp needle and were transferred into the BG11 medium (Rippka *et al*., 1979) with or without $NaNO_3$ as the N source, in tubes plugged with sterilized cotton plugs. The tubes were incubated at 28 ± 2 ºC under a continuous light source from fluorescent tubes at an intensity of 7.5 W m^{-2} for the growth of cyanobacteria. When the medium of the tubes turned green, slides were prepared and observed under an oil immersion lens. Species that grew in different fertilizer treatments were recorded separately.

Determination of microbial biomass carbon in soil

The total microbial C in the soil was measured by the chloroform fumigation-extraction technique described by Vance *et al*. (1987). Briefly, 10 g of fresh soil from each sample was fumigated with 30 ml alcohol free chloroform and incubated in dark for 24 hrs. It was followed by the soil extraction using 0.5M K_2SO_4. The extract was titrated using acidified ferrous ammonium sulphate after dichromate digestion. Microbial biomass C (MBC) was calculated using the equation (Vance *et al*., 1987), MBC = 2.64 x E_C where, E_C = (organic C from fumigated soil) – (organic C from non-fumigated soil), expressed as mg kg^{-1} soil.

Statistical analysis

Data were subjected to one way analysis of variance (ANOVA) and means were compared using Tukey's HSD test at 5% probability level. All statistical analyses were performed using SAS (1998) software. The CFU were expressed as $\log_{10}$ (g^{-1} soil dry weight).

RESULTS AND DISCUSSION

NA was used for the isolation of bacteria, since it is a well-known universal medium that serves the role of supporting many microbes randomly without being selective, whereas other media that are used may be of a selective nature. PDA and BG 11 were used for the isolation of fungi and cyanobacteria, respectively, media generally used for the culture of the relevant groups of microorganisms. The higher species richness of bacteria and the highest number of CFU were observed in 50% CF + BFBF application (Figure 1 and Table 1) compared to control soil. Major species observed were *Azorhizobium*, *Acetobacter*, *Bacillus* and two species of gram positive rods (Table 2). Among those species, *Acetobacter* was more frequently identified in the BFBF treated soil, compared to other treatments. Originally, the BFBF contained 7 bacterial genera, of which only 2 genera were isolated from the BFBF treated soil. This could be due to complexity of the microbial community in succession under the selection pressure which has not been understood yet in the BFBF applied soil-plant system. Other possibilities are the suppression of introduced bacteria, due to the competition of better adapted soil native microbes (van Veen *et al*., 1997), and also changes in community structure and function of microbes with biofertilizer application (Ge *et al*., 2003). Gram positive rods were frequent in 100% CF treated soil (ca. 65% of total colonies). Application of 100% CF resulted in significantly

high CFU counts, but an average species number, compared to no fertilizer (Table 1), confirming reduction of species richness and evenness with the CF (Sun *et al*., 2004). No fertilizer application showed the lowest bacterial richness and CFU count. Therefore, results indicated that the BFBF when applied together with 50% CF supported emergence of new bacteria with their increased abundance in the soil-plant system of maize.

The highest number of fungal species was observed in the 50% CF + BFBF application (Table 2), which was similar to the trend observed with bacteria. *Aspergillus* spp. are natural inhabitants in the maize rhizosphere (Gomes *et al*., 2003). There were two new *Aspergillus* spp. in addition to the introduced species from the BFBF treatment (Table 2). In 100% CF treatment, only *Fusarium* sp. was observed, which is a common plant pathogen. This may be due to the fact that CF application collapses natural balance of soil microbial communities (Seneviratne, 2009), which may lead to disease transmission (Keesing *et al*., 2010), possibly due to lack of natural control of pathogens. Interestingly, *Trichoderma* sp. was observed with *Fusarium* sp. only in the BFBF applied soil (Table 2), thus increasing the fungal species richness. Generally, majority of *Trichoderma* species isolated from tropical soils has been identified as biocontrolling agents (*e.g.* Affokpon *et al*., 2011), in addition to other eco-friendly effects (Fravel, 2005; Benitez *et al*., 2004; Zeilinger and Omann, 2007). The above facts are indications of possible effects of the BFBFs along with 50% CF in maintaining microbial balance for natural biocontrol of pathogens.

With regard to cyanobacterial diversity, the highest species number was recorded again in the BFBF application (Table 2). *Anabaena* sp., *Nostoc* sp., and *Oscillatoria* sp. were recorded as dominant species in all treatments whereas *Lyngbya* sp., *Scytonema* sp., *Calothrix* sp., *Fischerella* sp. and *Cylindrospermum* sp. (Table 2), with relatively high visual heterocyst number (U. V. A. Buddhika *et al*., unpublished data), were present in BFBF applied soils. In general, cyanobacteria are applied in agriculture to gain beneficial effects through their ability to fix atmospheric nitrogen (Nayak *et al*., 2004; Kaushik, 2004; Ahmad *et al*., 2008; Prasanna *et al*., 2010). An interesting observation of this study was that the emergence of additional diverse beneficial cyanobacteria in soil treated with 50% CF + BFBF, this is presumably the first observation of this nature.

Table 1. Number of colony forming units (CFU) of bacteria and microbial biomass carbon (MBC) in maize rhizosphere under different treatments. Values followed by different letters are significantly different at 5% probability level, according to Tukey's HSD test (CV = Coefficient of Variation; MSD = Minimum Significant Difference).

Treatment	Log_{10}(CFU g^{-1} soil)	MBC (µg g^{-1} soil)
100% CF	$7.40^{b} \pm 0.02$	$241^{a} \pm 26$
50% CF	$7.30^{bc} \pm 0.03$	$202^{ab} \pm 46$
50% CF + BFBF	$7.56^{a} \pm 0.00$	$266^{a} \pm 21$
No fertilizer	$7.26^{c} \pm 0.05$	$108^{b} \pm 13$
F- value	19.28	6.73
MSD (0.05)	0.14	131
CV (%)	0.70	24.8

Table 2. Microbes found in maize rhizosphere under different treatments

Treatment	Bacteria	Fungi	Cyanobacteria
100% CF	*Rhizobium* sp. *Bacillus* sp. 1 Gram (+) rod 1	*Fusarium* sp.	*Nostoc* sp. *Oscillatoria* sp. *Anabaena* sp.
50% CF	*Acetobacter* sp. *Azotobacter* sp. Gram (+) rod 2	*Aspergillus* sp. 1 Unidentified sp. 1	*Nostoc* sp. *Oscillatoria* sp. *Anabaena*sp. *Cylindrospermum* sp.
50% CF+ BFBF	*Azorhizobium* sp. *Acetobacter* sp *Bacillus* sp. 2 Gram (+) cocci Gram (+) rod 3	*Aspergillus* sp. 1 *Aspergillus* sp. 2 *Aspergillus* sp. 3 *Trichoderma* sp. *Fusarium* sp. Unidentified sp. 1	*Nostoc* sp. *Oscillatoria* sp. *Anabaena* sp. *Lyngbya* sp. *Scytonema* sp. *Calothrix* sp. *Fischerella* sp. *Cylindrospermum* sp.
No fertilizer	Gram (+) rod 4	Unidentified sp. 2	*Nostoc* sp. *Anabaena* sp. *Oscillatoria* sp.

Soil MBC was not significantly different among treated soils, but it was significantly low in the non-treated soil ($P<0.05$) (Table 1). Thus, it did not reflect differences of microbial species richness among the treatments. Generally, MBC depends on soil C and nutrient availability. Therefore, higher nutrient availability in the 100% CF resulted in high MBC, as also observed previously (Zhong and Cai, 2007; Lynch and Panting, 1982; Goyal *et al*., 1992), though there was relatively a low microbial richness. Apparently, higher MBC observed in 50% CF + BFBF than the 50% CF could be due to the introduced microbes and subsequent emergence of cyanobacterial and fungal communities with the application of the BFBF. Cyanobacteria and fungi have been reported to be major contributors to soil MBC (Henrot and Robertson, 1994; Lovell *et al*., 1995; Prasanna *et al*., 2009).

Unfavorable environmental conditions and nutrient depletion are some of the many reasons which make microbial cells to become dormant and to incorporate into soil microbial seed bank (Coates, 2003). Adverse conditions created by the 100% CF application collapse microbial communities (Seneviratne, 2009), leading metabolically active cells to become dormant and hence altering the composition of microbial communities. However, our results suggested that the application of the BFBFs along with 50% CF tends to break dormancy of microbial seeds in the soil, which causes emergence of diverse microbes with a possible indication of pathogen suppression through increased food web interactions.

Generally, dormancy of cysts, spores, akinetes and conidia are broken in the presence of organic compounds in various exudates (*e.g.* low molecular weight sugars and amino acids). Interactions among microbes in the BFBFs have also been observed to release diverse organic compounds including those mentioned above (Herath *et al*., 2013), as was also observed by Saini *et al*. (1986) and De Boer *et al*. (2005). When such compounds become increasingly available, it resuscitates microbial cells to grow on a broader substrate spectrum (Lennon and Jones, 2011; Teeling *et al*., 2012). In some cases, cell to cell communication via quorum sensing is reported to resuscitate cells to break dormancy (Lennon and Jones, 2011). In biofilm formation, quorum sensing is a prerequisite, which helps to establish the biofilm. Therefore, the role of BFBFs in breaking dormancy of the microbial

seed bank in this manner could be expected obviously.

It is concluded from the results of this study that the BFBFs allow a diverse community of bacteria, fungi and cyanobacteria to emerge when coupled with 50% of CF inputs. This contributes to strengthen biodiversity-ecosystem functioning relationship (Langenheder *et al*., 2010), which leads to ecosystem sustainability (Tilman *et al*., 1996). In order to have a better understanding of the effect of application of the BFBFs and CF on microbial diversity, the use of molecular assisted technologies would be the next step, because culturable microbial community represents less than 1% of the total microbes in some soils.

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REFERENCES

Affokpon, A., Coyne, D. L., Htay, C. C., Agbèdè, R. D., Lawouin, L. and Coosemans, J. (2011). Biocontrol potential of native *Trichoderma* isolates against root-knot nematodes in West African vegetable production systems. *Soil Biology and Biochemistry* **43**: 600–608.

Ahmad, F., Ahmad, I. and Khan, M. S. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological Research* **163**: 173-181.

An, S. S., Mentler, A., Acosta-Martínez, V. and Blum, W.E.H. (2009). Soil microbial parameters and stability of soil aggregate fractions under different grassland communities on the Loess Plateau. *China Biologia* **64**: 424-427.

Bastidia, F., Zsolnay, A., Hernandez, T. and García, C. (2008). Past, present and future of soil quality indices: a biological perspective. *Geoderma* **147**: 159–171.

Benitez, T., Rincon, M.A., Limon, M. C. and Codon, C.A. (2004). Biocontrol mechanisms of *Trichoderma* strains. *International Microbiology* **7**: 249-260.

Brookes, P.C. (1995). The use of microbial parameters in monitoring soil pollution by heavy metals. *Biology and Fertility of Soils* **19**: 269-279.

Buddhika, U. V. A., Kulasooriya, S. A., Seneviratne, G. and Abayasekara, C. L. (2012a). Potential of biofilmed microbial communities as biofertilizers for maize (*Zea mays* L.). *Proceedings of India-Sri Lanka Conference on Agrobiotechnology for Sustainable Development*, Colombo, Sri Lanka. P. 63.

Buddhika, U. V. A., Seneviratne, G. and Abayasekara, C. L. (2012b). Biofilmed biofertilizers for sustaining maize cultivation. Available from http://brightice.org/biotechnology 2012.

Buddhika, U. V. A., Seneviratne, G., and Abayasekara, C. L. (2012c). Biofilmed biofertilizers for maize (*Zea mays* L.): effect of plant growth under reduced doses of chemical fertilizers. In: B. Nimalathasan, A. Ramanan and K. Thabotharan (Eds), *Jaffna University International Research Conference-2012*, Jaffna, P. 8.

Coates, A. R. M. (2003). *Dormancy and Low-Growth States in Microbial Disease*. Cambridge Univ. Press, Cambridge.

Cerny, J., Balik, J., Pavlikova, D., Zitkova, M. and Sykora, K. (2003). The influence of organic and mineral nitrogen fertilizers on microbial biomass nitrogen and extractable organic nitrogen in long term experiments with maize. *Plant, Soil and Environment* **49**: 560-564.

Cruz, A. F., Hamel, C., Hanson, K., Selles, F. and Zentner, R.P. (2009). Thirty-seven years of soil nitrogen and phosphorus fertility management shapes the structure and function of the soil microbial community in a Brown Chernozem. *Plant and Soil* **315**: 173-184.

De Boer, W., Folman, L. B. and Summerbell, R. C. (2005). Living in a fungal world, impact of fungi on soil bacterial niche development. FEMS.*Microbiological Reviews* **29**: 795-811.

Department of Agriculture (2006). http://www.agridept.gov.lk/index.php/en/crop-recommendations/1026.

Fernandes, E. C. M., Motavallic, P. P., Castilla, C. and Mukurimbira, L. (1997). Management control of soil organic matter dynamics in tropical land use systems. *Geoderma* **79**: 49-67.

Fravel, R. D. (2005). Commercialization and implementation of biocontrol. *Annual Review of Phytopathology* **43**: 337-359.

Ge, J. Q., Yu, X. C. and Wang, Z. H. (2003). The function of microbial fertilizer and its application prospects. *Chinese Journal of Eco-Agriculture* **11**: 87–88.

Gomes, N. C. M., Fagbola, O., Costa, R.,

Rumjanek, N. G., Buchner, A., Mendona-Hagler, L. and Smalla, K. (2003). Dynamics of fungal communities in bulk and maize rhizosphere soil in the tropics. *Applied and Environmental Microbiology* **69**: 3758-3766.

Goyal, S., Mishra, M. M., Hooda, I.S. and Singh, R. (1992). Organic matter-microbial biomass relationships in field experiments under tropical conditions: effects of inorganic fertilization and organic amendments. *Soil Biology and Biochemistry* **24**: 1081–1084.

Henrot, J. and Robertson, G. P. (1994). Vegetation removal in two soils of humid tropics: effect on microbial biomass. *Soil Biology & Biochemistry* **26**: 111-116.

Herath, H. M. L. I., Senanayeke, D. M. N., Seneviratne, G. and Bandara, D. C. (2013).Variation of biochemical expressions of developed fungal-bacterial biofilms over their monocultures and its effect on plant growth. *Tropical Agricultural Research* **24**:186 – 192.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. and Williams, S. T. (1994). Bergey's *Manual of Determinative Bacteriology.* (9th ed.), Williams and Wilkins, Baltimore.

Kaushik, B. D. (2004). Use of blue-green algae and *Azolla* biofertilizers in rice cultivation and their influence on soil properties. In: P.C. Jain (Ed), *Microbiology and Biotechnology for Sustainable Development.* CBS Publishers & Distributors, New Delhi, Pp. 166-184.

Keesing, F., Belden, L. K., Daszak, P., Dobson, A., Harvell, C. D., Holt, R. D., Hudson, P., Jolles, A., Jones, K. E., Mitchell, C. E., Myers, S. S., Bogich, T., and Ostfeld, R. S. (2010). Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature* **468**: 647-652.

Kennedy, A. C. and Smith, K. L. (1995). Soil microbial diversity and the sustainability of agricultural soils. *Plant and Soil* **170**: 75-86.

Langenheder, S., Bulling, M. T., Solan, M., Prosser, J. I. (2010). Bacterial biodiversity ecosystem functioning relations are modified by environmental complexity. *PLoS ONE* **5**(5): e10834. doi:10.1371/journal.pone.0010834.

Lennon, J. T. and Jones, S. E. (2011). Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nature Reviews Microbiology* **9**: 119-130.

Lynch, J. M. and Panting, L. M. (1982). Effects of season, cultivation and nitrogen fertiliser on the size of the soil microbial biomass. *Journal of the Science of Food and Agriculture* **33**: 249–252.

Lovell, R. D., Jarvis, S. C. and Bardgett, R. D. (1995). Soil microbial biomass and activity in long term grass land: effects of management changes. *Soil Biology and Biochemistry* **27**: 969-975.

Milton, N. S. and Joseph, G. H. (1982). Disease-suppressive soil and root-colonizing bacteria. *Science* **216**: 1376-1381.

Nayak, S., Prasanna, R., Pabby, A., Dominic, T. K. and Singh, P. K. (2004). Effect of BGA-*Azolla* biofertilizers on nitrogen fixation and chlorophyll accumulation at different depths in soil cores. *Biology and Fertility of Soils* **40**: 67-72.

Pankhurst, C. E., Hawke, B. G., McDonald, H. J., Bukerfield, J. C., Michellsen, P., O'Brien, K. A., Gupta, V. V. S. R. and Double, B. M. (1995). Evaluation of soil biological properties as potential bioindicators of soil health. *Australian Journal of Experimental Agriculture* **35**: 1015-1028.

Prasanna, R., Joshi, M., Rana, A. and Nain, L. (2010). Modulation of IAA production in cyanobacteria by tryptophan and light. *Polish Journal of Microbiology* **59**: 99-105.

Prasanna, R., Nain, L., Ancha, R., Shrikrishna, J., Joshi, M. and Kaushik, B. D. (2009). Rhizosphere dynamics of inoculated cyanobacteria and their growth-promoting role in rice crop. *Egyptian Journal of Biology* **11**: 26–36.

Priha, O. and Smolander, A. (1995). Nitrification, denitrification and microbial biomass N in soil from two N-fertilized and limed Norway spruce forests. *Soil Biology and Biochemistry* **27**: 305-310.

Riddell, R. W. (1950). Permanent stained mycological preparations obtained by slide culture. *Mycologia* **42**: 265-270.

Rippka, R., Deruells, J., Waterbury, J. B., Herdman, M. and Stanier, R. (1979). Generic assignment, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology* **111**: 1-61.

Saini, H. S., Bassi, P. K., Consolacion, E. D. and Spencer, M. S. (1986). Interactions among plant hormones, carbon dioxide, and light in the relief of thermo inhibition of lettuce seed germination, Studies in a flow- through gaseous system. *Canadian Journal of Botany* **64**: 2322-2326.

SAS (1998). SAS/STAT *User's guide, Release 6.0.* SAS Institute Inc., Cary, NC.

Seneviratne, G. (2009). Collapse of beneficial microbial communities and deterioration of soil health: a cause for reduced crop productivity. *Current Science* **96**: 633.

Seneviratne, G., Jayasekara, A. P. D. A., De Silva, M. S .D. L. and Abeysekara, U. P. (2011). Developed microbial biofilms can restore deteriorated conventional agricultural soils.

Soil Biology and Biochemistry **43**: 1059-1062.

Sharma, S. K., Ramesh A., Sharma M. P., Joshi, O.P., Govaerts, B., Steenwerth, K.L. and Karlen, D.L. (2010). Microbial community structure and diversity as indicators for evaluating soil quality. In: E. Lichtfoust (Ed), *Biodiversity, Biofuels, Agroforestry and Conservation Agriculture*. Springer Science **5**: 317-358.

Strickland, M. S. and Rousk, J. (2010). Considering fungal:bacterial dominance in soils- Methods, controls, and ecosystem implications. *Soil Biology and Biochemistry* **42**: 1385-1395.

Sun, H. Y., Deng, S. P. and Raun, W. R. (2004). Bacterial community structure and diversity in a century-old manure-treated agroecosystem. *Applied and Environmental Microbiology* **70**: 5868–5874.

Teeling, H., Fuchs, B. M., Becher, D., Klockow, C., Gardebrecht, A., Bennke, C. M., Kassabgy, M., Huang, S., Mann, A. J., Waldmann, J., Weber, M., Klindworth, A., Otto, A., Lange, J., Bernhardt, J., Reinsch, C., Hecker, M., Peplies, J., Bockelmann, F. D., Callies, U., Gerdts, G., Wichels, A., Wiltshire, K. H., Glöckner, F. O., Schweder, T. and Amann, R. (2012). Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science* **336**: 608-611.

Thomas, J. B., Anthony, C. and Schrolh, M. N. (1984). Beneficial plant bacteria. Critical *Reviews in Plant Sciences* **2**: 1 – 20.

Tilman, D., Wedin, D., Knops, J. (1996). Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* **379**: 718-720.

Vance, E. D., Brookers, P. C. and Jenkinson, D. S. (1987). An extraction method for measuring microbial biomass C. *Soil Biology and Biochemistry* **19**: 703-707.

van Veen, J. A., Overbeek, L. S. and van Elsas, J.D. (1997). Fate and activity of microorganisms introduced into soil. *Microbiology and Molecular BiologyReviews* **61**: 121–135.

Weeraratne, N., Seneviratne, G. and Subasinghe, A. (2012). The potential of biofilmed biofertilizers to reduce chemical fertilizer use in lowland rice cultivation. Available from http://brightice.org/biotechnology 2012.

Yao, H., He, Z., Wilson, M. J. and Campbell, C. D. (2000). Microbial biomass and community structure in a sequence of soils with increasing fertility and changing land use. *Microbial Ecology* **40**: 223-237.

Zeilinger, S. and Omann, M. (2007). *Trichoderma* biocontrol: signal transduction pathways involved in host sensing and mycoparasitism. *Gene Regulation and System Biology* **1**: 227-234.

Zhong, W. H. and Cai, Z. C. (2007). Long-term effects of inorganic fertilizers on microbial biomass and community functional diversity in a paddy soil derived from quaternary red clay. *Applied Soil Ecology* **36**: 84–91.