24.

SOIL BIODIVERSITY LOSS AND ITS REVERSAL BY USING DEVELOPED MICROBIAL BIOFILMS IN AGROECOSYSTEMS

U. V. A. Buddhika[1]*, G. Senevirtne[1], C. L. Abayasekara[2]

[1] Institute of Fundamental Studies, Hantana road, Kandy.
[2] Department of Botany, Faculty of Science, University of Peradeniya.

ABSTRACT

Soil biodiversity includes living organisms existing in the soil and can be categorized as micro, meso and macro biota interacting with each other forming biological web. Generally, soil biodiversity can be deteriorated and lost due to several biotic and abiotic factors. However, recovering of depleted biodiversity is a serious challenge. In agricultural ecosystems, improved biodiversity has been recorded in the application of developed microbial biofilms. N_2 fixing bacteria and rhizosphere fungal species have been used to develop fungal-bacterial biofilms *in vitro*. When such biofilms are coupled with 50% of recommended chemical fertilizers, they tend to increase soil biodiversity especially bacteria, fungi and cyanobacteria with pest and weed control. Therefore, this review is focused on the biofilms developed *in vitro* and their impact on the enrichment of soil biodiversity in agriculture. Dormant seeds of plants and microbes, which create voluminous seed bank in the soil, tend to be resuscitated with the induction of beneficial organic secretions of the applied biofilms. Consequently, the dormant seeds which are physiologically inactive are transformed into physiologically active forms. Thus, the soils applied with the *in vitro* developed biofilms can enrich the soil biodiversity, addressing many issues related to loss of biodiversity in agroecosystems for a sustainable agriculture.

INTRODUCTION

Soils are the habitat for over one fourth of all living species on earth, and one teaspoon of garden soil may contain thousands of species, millions of individuals, and hundred metres of fungal networks. They come from both functionally and taxonomically diverse groups which range from invisible microbes, bacteria and fungi to the more familiar macro-fauna such as earthworms, insects and termites. They interact in complex belowground food webs and, thus provide numerous and essential services needed for ecosystem productivity. Their functions comprise of supporting services, nutrient cycling, soil formation and primary production. Major part of the soil biodiversity is led by diverse soil microbiota which includes bacteria, fungi, cyanobacteria, protozoa and actinobacteria *etc.* Due to their biological functions including metabolism and nourishments, they decompose organic materials and also create a soil rich with nutrients through actions such as biological nitrogen fixation (Tiwary *et al.*, 1998; Cocking, 2005), mineralization (Molla *et al.*, 1984) and produce diverse organic acids (Tien *et al.*, 1979; Barea *et al.*, 2008). These biological activities interacting with soil make available and recycle nutrients back to plants. Thus, soil microbes influence above ground plant communities for improved productivity. However, the situation is completely inverted in the agroecosystems, since they are continuously exposed to different agronomic practices.

Generally, microbial diversity is reduced in agroecosystems as a result of the intensive agricultural practices (Peixoto *et al.*, 2006; Ndour, 2008). Soil tillage has been reported to have negative impacts on the soil biodiversity, particularly N_2 fixing bacterial communities (Seneviratne *et al.*, 2011). Tillage practices also alter soil physical properties and affect the habitats of soil microbiota, which lead to loss of soil microbial diversity and community structure (Hungria *et al.*, 2009). Chemical fertilizers and other agrochemicals tend to collapse soil microbial communities resulting in declining yields in the long run

* Corresponding author: : *aruniruh@gmail.com*

(Seneviratne *et al.*, 2009). Application of N fertilizers tends to produce N poor soil microbial communities with low biomass (Cerny *et al.*, 2003; Strickland and Rousk, 2010). Addition of mineral fertilizers is a selective force for the structural and functional shifts of soil microbial community since they modify available N and P fertility, thus resulting negative impacts on soil quality and nutrient fluxes (Cruz *et al.*, 2009). In this manner, conventional agricultural practices cause loss of many microbial species and their diverse functions. Some species become less abundant and others become more common due to collapse of their natural balance. This could be a real risk, especially when the abundance of unwanted species, for instances plant or human pathogens, increase. How can we rectify this imbalance of microbes? There are several options. For example, management of soil organic matter will promote soil biodiversity, so that unwanted microbes may not be able to develop in high numbers. Other possibility is to reduce soil disturbance to a minimum when managing soils.

Soil microbial diversity and its loss in agroecosystems

Diverse microflora in the soil has been identified for its beneficial functions in soil-plant system (Kennedy and Smith, 1995). Their significant functions have been identified and tested concerning plant growth benefits in many ways. They perform different catabolic activities, and hence are important in decomposition of organic matter and nutrient balancing, rhizoremediation, pathogen suppression and maintenance of soil structure and water relationships (Sharma *et al.*, 2010). In addition, their interactions with plant root system play key roles in primary production through improved photosynthesis (Ren *et al.*, 2006). Hence, the diversity of soil microorganisms is considered as one of the most important indicators of 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 soils (Fernandes *et al.*, 1997).

Unfortunately, in agroecosystems, keeping microbes in their natural balance and the diversity is difficult due to continuous alteration of soil physicochemical parameters. Jones and Lennon (2010) from their theoretical model have demonstrated that the structure of microbial communities is shaped by different biotic and abiotic factors in the environments. They have pointed out that microbes become dormant to evade such unfavourable environmental conditions. Thus, being members of a voluminous microbial seed bank, dormancy results in loss of diversity. Dormant forms sustain a minimal metabolic activity and revive if future conditions would be favourable. Thus, dormancy can be viewed as the metabolic flexibility to changing conditions and physiological tolerance of an organism, which makes it resistant to environmental disturbances. Consequently, microbial individuals in dormant forms determine the dynamics of the future microbial communities. Therefore, awakening of a previously unknown or a rare organism from the microbial seed bank can change the composition of microbial community and thereby potentially affecting ecosystem processes (Seneviratne *et al.*, 2013).

Reversal of soil microbial diversity loss by biofilm action

Since dormancy is driven by environmental factors, resuscitation of dormant microorganisms is also influenced by different biotic and abiotic factors that are present in the environment. Generally, microbial diversity and their community structure vary widely depending on environmental factors. Increasing availability of resources, typically low molecular mass compounds, such as amino acids and sugars results in the resuscitation of dormant forms. In addition, reversal of microorganisms from dormancy involves quorum sensing (QS), which leads to spontaneous resuscitation (Lennon and Jones, 2011). Further, different growth factors like cytokine secreted by metabolically active microbial cells can also do this revival (Mukamolova *et al.*, 1998).

Importance of biofilms as effective microbial communities has been described in terms of plant growth and development and other beneficial biological functions in the micro-environment of plants (Ramey *et al.*, 2004; Seneviratne *et al.*, 2011). Biofilms can be developed *in vitro* by using nitrogen fixing bacteria and rhizosphere fungal species, which can then be formulated as biofilmed biofertilizers (BFBFs) for agriculture. They can reduce chemical fertilizer use by 50% in tea (Seneviratne *et al.*, 2011) and many other crops with increased soil microbial biomass and soil functioning. Interestingly, the developed biofilms have been observed to produce a higher number of diverse organic compounds that enhance the availability of organic substrates to dormant microbial cells for resuscitation (Seneviratne and Kulasooriya 2013, Herath *et al.*, 2013). West *et al.* (2007) showed that biofilms generate public good which neighbouring microbes can utilize in their communication including exopolysaccharide, QS molecules, different growth factors and resuscitation promotion factors (RpF). Thus, soil application of BFBFs showed the resuscitation of soil microbial seed bank when it is applied with 50% of chemical fertilizers. Buddhika *et al.* (2012) clearly indicated that the soil application of the developed biofilms increased species richness and hence diversity of bacteria, fungi and cyanobacteria in comparison to soil with chemical fertilizers alone application (Buddhika *et al.*, 2013). Role of the developed biofilms in controlling the switch between active and dormant microbial cells is summarized and depicted in Fig. 1. It illustrates the relation among microbial diversity, ecosystem functioning and sustainability.

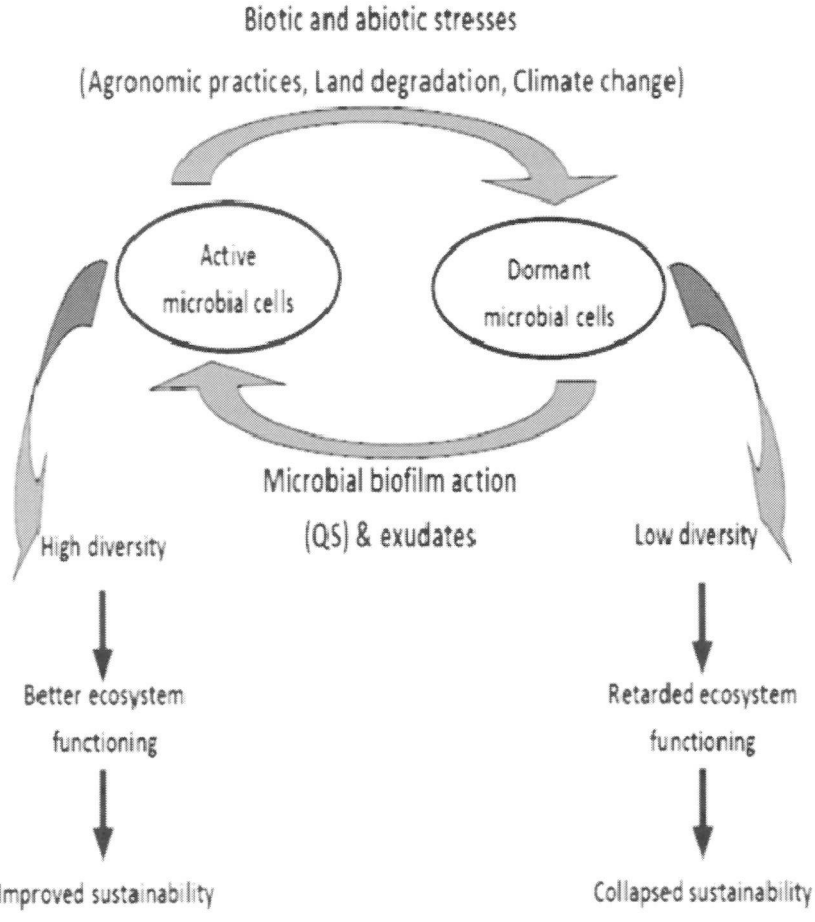


Figure 1.The interchange of dormant and active stages of microbes in the soil seed bank. Transition of metabolically active cells into dormant cells is a response to biotic and abiotic stresses induced by the human and the environment. Developed biofilms switch dormant cells to become metabolically active cells, thus improving sustainability in agroecosystems through increased microbial diversity and ecosystem functioning (Seneviratne *et al.*, 2013).

In addition, pest control has been observed in tea cultivation with the application of the developed biofilms (Seneviratne *et al.*, 2011), indicating the maintenance of natural balance of soil biodiversity. Microbes being the focal point of the ecosystem can regulate all other biotic and abiotic components (Seneviratne *et al.*, 2013), which lead to a balanced biodiversity in agroecosystems.

CONCLUSIONS AND RECOMMENDATIONS

Depleted microbiota in the agroecosystems due to conventional agronomic practices can be revived by applying developed biofilms when coupled with a reduced dose of CF. Increased microbial diversity as a response to the applied biofilms developed *in vitro* leads to a healthy soil through improved microbial diversity in the soil-plant system. Reduction of soil sickness in this manner can address soil sterility issues in the agroecosystems. Accordingly, biofilms developed *in vitro* can be a remedy for the revival of soil microbiota, the focal point of below ground food webs. Natural ecosystems affected by abiotic and biotic stress factors may also be restored from the biofilm application. Thus, the *in vitro* developed biofilms as microbial ameliorators would address issues of depleted soil biodiversity in both natural and agricultural ecosystems.

REFERENCES

Barea J.M., Navarro E., Montoya E. (1976). Production of plant growth regulators by rhizosphere phosphate-solubilizing bacteria. *Journal of Applied Microbiology,* 40, 129-134.

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

Buddhika U.V.A., Athauda A.R.W.P.K., Seneviratne G., Abayasekara C.L. (2013). Emergence of diversemicrobes on application of biofilmed biofertilizers to a maize Soil. *Ceylon Journal of Science (Biological Sciences). Accepted.*

Cerny J. Balik J., Pavlikova D., Zitkova M., 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.

Cocking E.C., Stone P.J., Davey M.R. (2005). Symbiosome-like intracellular colonization of cereals and other crop plants by nitrogen-fixing bacteria for reduced inputs of synthetic nitrogen fertilizers. *Chinese Academy of Science*, 48, 888-96.

Cruz A.F., Hamel C., Hanson K., Selles F.,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.

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

Herath H.M.L.I., Senanayeke D.M.N., Seneviratne G., 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.

Hungria M., Franchini J.C., Brandão-Junior O., *et al.* (2009). Soil microbial activity and crop sustainability in a long-term experiment with three soil-tillage and two crop-rotation systems. *Applied Soil Ecology*, 42, 288-96.

Jones S.E., Lennon J.T. (2010). Dormancy contributes to the maintenance of microbial diversity. *Proceedings of the National Academy of Sciences, USA*, 107, 5881–5886.

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

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

Molla M.A.Z., Chowdhury A.A., Islam A., Hoque S. (1984). Microbial mineralization of organic phosphate in soil. *Plant and Soil*, 78, 393-399

Mukamolova G.V., Kaprelyants A.S., Young D.I., Young M., Kell D.B. (1998). A bacterial cytokine. *Proceedings of the National Academy of Sciences, USA* 95, 8916–8921.

Ndour N.Y.B., Achouak W., Christen R. *et al.* (2008). Characteristics of microbial habitats in a tropical soil subject to different fallow management. *Applied and Environmental Microbiology,* 38, 51-61.

Peixoto R.S., Coutinho H.L.C., Madari B., *et al.* (2006). Soil aggregation and bacterial community structure as affected by tillage and cover cropping in the Brazilian Cerrados. *Soil and Tillage Research*, 90, 16-28.

Ramey B.E., Maria K., von Bodman S.B., Clay F. (2004). Biofilm formation in plant-microbe associations. Plant Science Articles. Paper 18. http://digitalcommons.uconn.edu/plsc_articles/18.

Ren A.Gao Y., Wang W., Wang J. (2006). Photosynthetic pigments and photosynthetic products of endophyte-infected and endophyte-free *Lolium perenne* L. under drought stress conditions. *Frontiers of Biology in China,*1, 168-173.

Seneviratne G., Kulasooriya S.A. (2013). Reinstating soil microbial diversity in agroecosystems: theneed of the hour for sustainability and health. *Agricultural Ecosystems and Environment*, 164,181–182.

Seneviratne G., Jayasekara A.P.D.A., De Silva M.S.D.L., Abeysekara U.P. (2011). Developed microbial biofilms can restore deteriorated conventional agricultural soils. *Soil Biology and Biochemistry*, 43, 1059-1062.

Seneviratne G., Weeraratne N., Buddhika U.V.A. (2013). Diversity of Plant Root Associated Microbes: It's Regulation by Introduced Biofilms. In: *Plant Microbe Symbiosis - Fundamentals and Advances,* (Ed). N. K. Arora, Springer, India. DOI 10.1007/978-81-322-1287-4-13.

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

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

Tien T.M., Gaskin M.H., Hubbel D.H. (1979). Plant growth substances produced by *A. brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). *Applied and Environmental Microbiology*, 37,1016–1024.

Tiwary D.K., Hasan M. A., Chattopadhyay P.K. (1998). Studies on the effect of inoculation with Azotobactor & Azospirillum on growth, yield & quality of banana. *Indian Journal of Agriculture* 42, 235- 240.

West S.A., Diggle S.P., Buckling A., Gardner A., Griffin A.S. (2007). The social lives of microbes. *Annual Review of Ecology and Systematics,* 38, 53–77