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FUNGAL INOCULATION WITH CLAY IMPROVES CARBON STABILIZATION OF TROPICAL FOREST FLOOR LITTER

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ABSTRACT

Carbon stabilization of tropical forest floor litter helps mitigate global warming, because it reduces an important source of CO_2 of the forest ecosystems. However, there is a lack of the C stabilization due to biological and physical effects such as low faunal activities, and segregation of floor litter layer and soil minerals by amid organic horizon, hindering organo-mineral complexation and humification. Thus, this chapter evaluates the C stabilization of tropical leaf litter, when microorganisms having different compositions and clay minerals were applied as inocula onto the litter. Litter associated fungi and bacteria separately, and their fungal-bacterial biofilms were applied with clay onto autoclaved litter, and incubated under laboratory conditions. Then, weight loss of the litter was measured, and they were analysed for organic matter fractions and organo-mineral complexation. The biofilms increased the weight loss, compared to the fungi or bacteria alone. Highest humification was observed in fungal inoculation with clay. This suggests that the humification process can be improved by altering the microbial composition on the litter. It was found that the application of clay with any microbes is essential for the organo-mineral complexation. Here, we have manipulated microbial community to enhance the forest floor litter C storage in the soils. Therefore, this method can be proposed as a Clean Development Mechanism (CDM), which can be employed for carbon trading in tropics. Further studies are however needed to quantify the effect of this biotechnology under field conditions, and then to develop an inoculation technique to forest floor litter.

Keywords: carbon sequestration, humification, litter, organo-mineral complexes, tropical forests.

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INTRODUCTION

Forest ecosystems play a significant role in regulating the CO_2 concentration in the atmosphere. They account for 3.6 billion hectares or 28% of the land area. It is estimated that as much as 90% of world's terrestrial C is stored in forests [1]. Carbon sequestration is the only method which can remove C from the atmosphere. It is also important to emphasize that increasing photosynthetic C fixation alone is not enough to reduce the atmospheric CO_2 level [2], because 70-90% of C is released back to the atmosphere during microbial decomposition of forest floor litter [3]. Based on the data set of Tropical Forest Consistent Worldwide Site Estimates, 1967-1999 [4], and the rate of microbial decomposition of forest floor litter [3], it was found that the quantity of the forest floor litter decomposed within ca. 50 years is equivalent to above and belowground total biomass of global tropical forests. This implies that the forest floor litter decomposition passively deforests and decomposes a biomass equivalent to global tropical forests once in every 50 years, emitting a huge amount of CO_2 to the atmosphere. Further, the soil organic matter (SOM) tends to decompose greatly on and in the soils of warm tropics compared to temperate regions [3]. Therefore, it is important to find out ways to fix terrestrial C in long lived soil pools. Stabilization of forest floor litter C is an important process in mitigating global warming. Organo-mineral complexation and humification are the major processes of the C stabilization. Organo-mineral complexes are the major products resulting from interactions between soil minerals and organic components in the presence of organisms [5]. Main sequestered soil C storage is persistent in the forms of organo-minerals, such as humus. Turnover time of humus is 2000-5000 years in the soils having high amounts of clay [3]. Capability of soils to accumulate and store C is greatly influenced by its ability to stabilize and protect organic matter against microbial decomposition. There is a lack of stabilization of plant litter C in the tropical ecosystems, which is mainly attributed to low faunal activities in the soils [6].

Other main reason for the low transformation of the litter into stable soil C pools in the forests is the segregation of floor litter layer and soil due to presence of amid organic horizon. It reduces the interaction between decomposing litter and soil minerals, which is essential for humification. This tends to emit a major fraction of litter C to the atmosphere through microbial respiration. We hypothesis that, if it is possible to make a contact between the decomposing litter and soil minerals in order to increase the organo-mineral complexation, the amount of litter C returned to atmosphere through microbial respiration can be reduced. Thus, objective of this study was to evaluate the C stabilization of tropical leaf litter, when microorganisms having different compositions and clay were applied as inocula onto the litter.

MATERIALS AND METHODS

A laboratory incubation study was conducted with litter, microbes and clay. *Phanerochaete* spp., *Trichoderma* spp., *Cellulomonas* spp. and *Pseudoxanthomonas* spp. isolated on Sabouraud Dextrose Agar (SDA) and Nutrient Agar (NA) previously from decomposing leaf litter of Udawattakale forest in Kandy, Sri Lanka were used in the present study. The litter collected from the same forest was cut into small pieces (0.5 cm x 0.5 cm)

and autoclaved at 121^{o}C for 20 minutes to exclude microbes. Three grams of the litter pieces were spread separately in sterilized pertidishes. Each of them was applied with 0.003 g of pre-separated and sterilized clay (*Kaolinite*) suspended in 20 ml of microbial culture broths under aseptic conditions. The cultures used were the two fungi and the two bacteria separately, and their fungal-bacterial biofilms formed by bacterial colonization on fungal mycelia [7], grown in concentrated Yeast Manitol Broth (2xYMB). This YMB is used to maintain a minimal growth of the microbes in the culturing stage. Litter alone (control), clay + litter + bacteria, clay + litter + fungi and clay + litter + biofilms were used as treatments. Clay + litter treatment was not applied, since there would not be any interactions in the absence of microbes of the sterilized clay and litter. The petridishes were incubated at 28 ^{o}C for 2 months. Sterilized distilled water was applied to the pertidishes once in four days. All the treatments received the same amount of water. After the incubation period, residual litter in the petridishes was oven dried at 65 ^{o}C to a constant weight and dry weights were recorded. Then, they were ground and used to measure lignocellulose, fulvic and humic fractions by the modified loss on ignition method [8].

The residual litter was analysed using Fourier Transformed Infrared (FTIR) spectrophotometer for detecting organo-mineral complexation. A sub sample (0.008 g) of the ground litter was mixed with 0.16 g of KBr and palettes were made. They were analysed to detect a peak at wavenumber 1643 cm^{-1}, which represents metal-coordinated carboxylates of humic fraction [9].

RESULTS AND DISCUSSION

Weight loss percentages of SOM fractions under different treatments are given in Table 1. Lowest weight loss was observed in the control. That reflects lowest decomposition rate, possibly with microbial contaminants on litter. Presence of biofilms increased the weight loss, because they are more efficient in microbial action than when the resident microbes of them are alone [10]. Lignocellulose fraction was highest in the control (Table 1). It was followed by biofilm + clay, fungi + clay and bacteria + clay treatments. This order could be attributed to transfer of litter lignin to fungal lignin production in treatments with fungi, because lignin decomposition is primarily mediated by fungi [11]. There was no significant difference in fulvic fraction. It is an intermediate product in the humification process.

Highest humic fraction was observed in fungi + clay (Table 1 and Figure 1), because fungi are effectively involved in litter decomposition and humification process [12, 13]. Lignin-degrading enzymes of fungi polymerize phenolic compounds [14]. Clay usually acts as a binding agent of minerals to organic compounds. Thus, fungal interaction with clay forms organo-mineral complexes, leading to a more stable humic fraction [15]. Humic fraction was lowest in bacteria + clay (Table 1 and Figure 1). This suggests that the humification process can be improved by altering the microbial composition on litter.

FTIR spectroscopic data showed that the application of clay with any microbes is essential for organo-mineral complexation, as reflected by a small peak at 1643 cm^{-1}, representing the presence of the complexes (Figure 2). In the absence of clay, formation of organo-minerals does not occur in the decomposing litter. This clearly indicates the reason for the lack of the litter C stabilization in long-lived soil pools, supporting our hypothesis.

Table 1. Weight losses and magnitudes of lignicellulose, fulvic and humic fractions of the residual litter when tropical forest floor litter was applied with fungi and bacteria isolated from the decomposing litter, or their developed biofilms, together with clay, and incubated for 2 months

Treatment	Weight loss (%)	Difference*	Lignocellulose (%)	Difference*	Fulvic fraction (%)	Difference*	Humic fraction (%)	Difference*
Fungi + clay	25.80 ± 0.75	4.6	8.28 ± 1.90	10.42	59.10 ± 1.72	3.2	9.26 ± 0.82	4.05
		(0.012)		(0.012)		(0.730)		(0.004)
Bacteria + clay	25.00 ± 1.46	3.9	4.21 ± 1.06	14.49	63.90 ± 2.94	8	4.90 ± 0.79	0.31
		(0.059)		(0.007)		(0.422)		(0.422)
Biofilms + clay	27.30 ± 0.56	6.1	16.67 ± 3.30	2.03	50.70 ± 3.29	5.2	6.17 ± 0.31	0.96
		(0.005)		(0.609)		(0.585)		(0.585)
Control (litter alone)	21.20 ± 0.90	0	18.70 ± 2.03	0	55.90 ± 7.86	0	5.21 ± 0.53	0

Mean ± SE. *Difference from the control. Probability levels at which the differences are significant, are within parentheses.

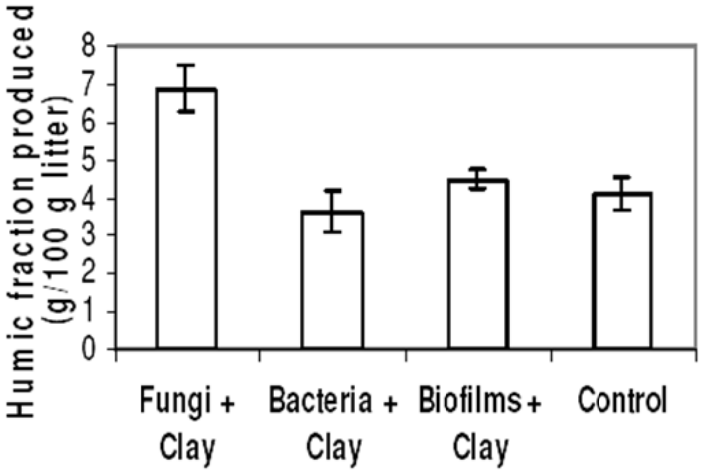

Figure 1. Humic fraction produced in the residual litter when tropical forest floor litter was applied with fungi and bacteria isolated from the decomposing litter, or their developed biofilms, together with clay, and incubated for 2 months. Vertical bars show standard error.

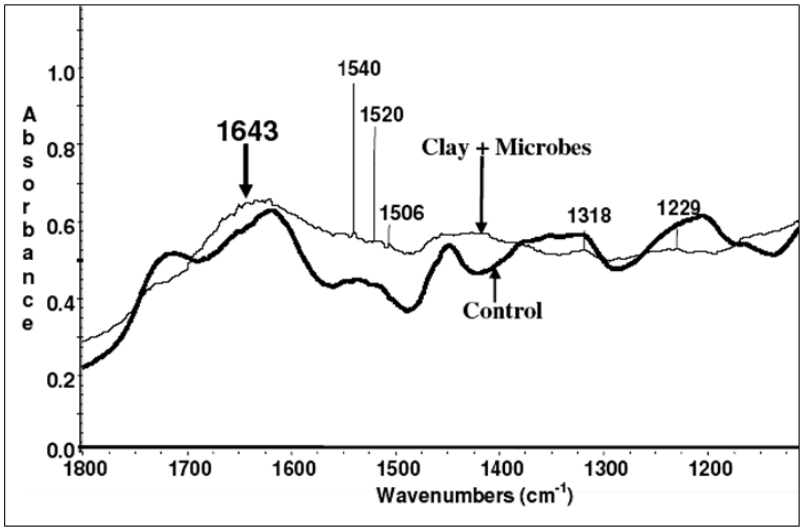

Figure 2. Fourier Transformed Infrared (FTIR) spectra of the residual litter when tropical forest floor litter was applied with fungi and bacteria isolated from the decomposing litter, or their developed biofilms, together with clay, and incubated for 2 months. Line for Clay + Microbes represents the typical spectra obtained for clay with any microbial treatments. Control was not applied clay or microbes.

Our gross calculations based on the present study show that there is a potential to increase humification process of tropical forest floor litter by ca. 60% with the fungi and clay application. Here, we have manipulated microbial community to enhance the forest floor litter C storage in the soils. Therefore, this method can be proposed as a CDM, which can be

employed for carbon trading in tropics. Further studies are however needed to quantify the effect of this biotechnology under field conditions, particularly in the presence of naturally occurring microbes on the litter. Then, it is important to develop an inoculation technique of this treatment to forest floor litter.

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