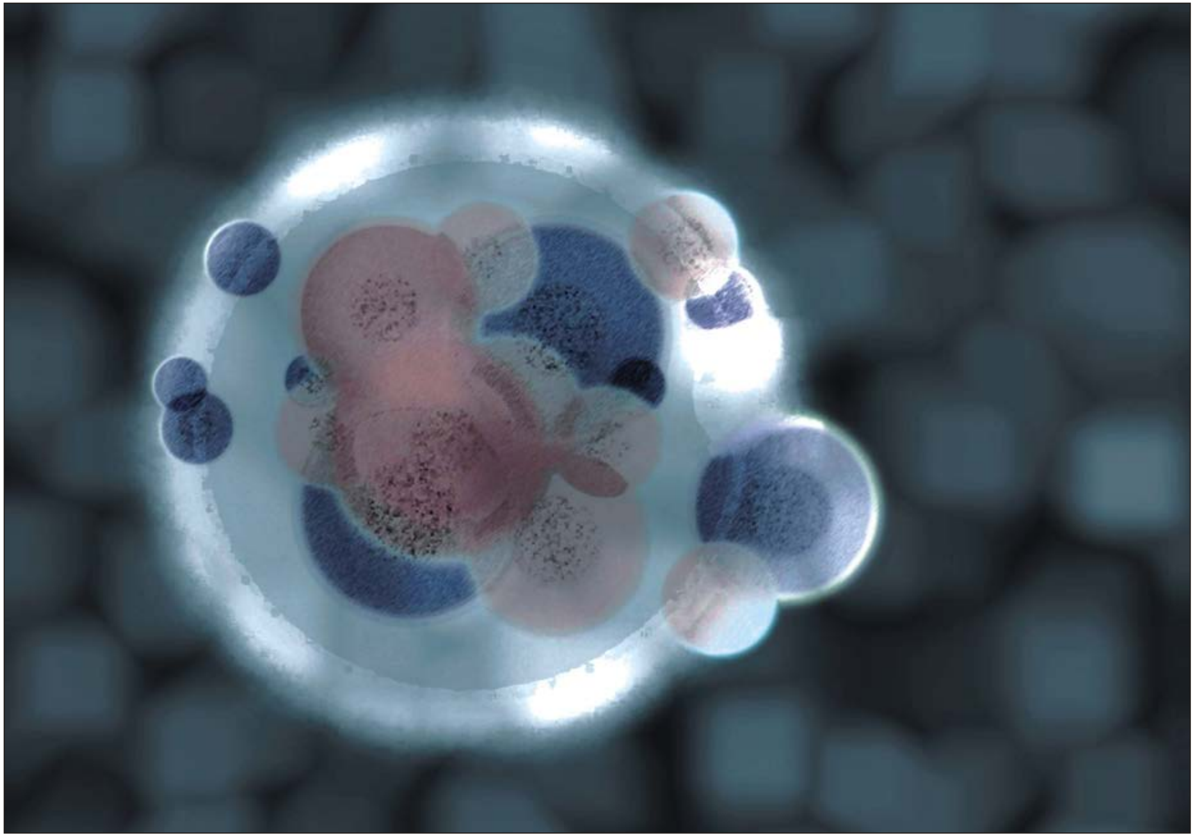

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Culturable Microbial Population Dynamics During Decomposition of *Theobroma cacao* Leaf Litters in a Tropical Soil Setting

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Abstract: The culturable aerobic heterotrophic bacterial and fungal distributions and population dynamics during decomposition of cocoa leaf litters were investigated between February 2002 and January 2003 using standard litterbag studies and microbial cultivation techniques in a confined and unconfined setting. Organic carbon, soil pH, ambient temperature and rainfall regimes were also monitored in the experimental plot. Bacterial counts were of the order of 10^5-10^7 cfu g^{-1} wet weight of litter or soil, while fungal counts were of the order of 10^3-10^4 cfu g^{-1} wet weight of litter or soil. Counts of bacteria and fungi were highest during the rainy season months and reduces on either sides of the rainy season divide. Bacteria diversity index ranged between 0.95 and 2.21, while fungal diversity index varied between 0.56 and 2.54 and a total of nineteen culturable bacterial and fourteen fungal strains were identified. The organic carbon contents of the leaf litters were consistently significantly ($p<0.05$) higher than those of the soils in the experimental plot. Rainfall and temperature ranged from 0.07-8.17 mm and 21.7-32.4°C, respectively during the period of sampling. There was no correlation between total bacterial or total fungal counts with rainfall. The pH of the soil samples was near neutral pH throughout the study period. We conclude that decomposition of cocoa leaf litters in a confined or unconfined setting does not appear to significantly affect the stability of the culturable microbial milieu.

Key words: Culturable bacteria, fungi, cocoa leaf litters, decomposition

INTRODUCTION

Plant decomposition is the physical and chemical breakdown of dead plants, animals and microbial material. It is a complex process made of a number of sub-processes and involving complete breakdown of organic matter into CO_2 and the mineral forms of nutrients like nitrogen, phosphorus etc. (Aerts, 1997) depending on such factors as temperature, moisture and chemical composition of the organic matter (Dickinson and Pugh, 1974). Hence, plant residues are a crucial source of nutrients in both natural and agricultural ecosystems, where synchronous plant growth and residue decomposition are essential for soil fertility and represents a readily available substrate for both soil fauna and soil microorganisms, with the main mineralization activity being performed by soil microbial communities (Dilly *et al*., 2004) and with the specific quality of organic residues controlling the decomposition rate and related release of nutrients (Neely *et al*., 2001).

The substrate, which the litter decomposers may influence, has a reservoir of organisms which can colonize the litter resulting in changes in the quality of the organic matter, which then induces a succession of microbial communities as shown in litter bag studies (Diil *et al*., 2001; Rosenbrock *et al*., 1995) and in vertical soil horizons (Zvyagintsev, 1994) in forest ecosystems. Based on their functions and ecological strategies, different dominant genera and species of microorganisms are present in biotopes (Zvyagintsev, 1994) with the diversity increasing during succession (Atlas and Bartha, 1998). Hence, the evolutionary forces that shape decomposition are those that maximize the maintenance, growth and reproduction of microbiota. Controls over decomposition are therefore best understood based on the controls over the activities of these organisms (Becker and Deamer, 1991). In this study, we report the effect of decomposition of *Theobroma cacao* (cocoa) leaf litters on the distribution and species variation of the culturable heterotrophic bacteria and fungi communities

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under a typical tropical setting within the confines of established physico-chemical regimes of the leaf litters and environment.

MATERIALS AND METHODS

Site location and sample collection: The study area is located in a cocoa plot at Abagboro, a village within Obafemi Awolowo University, Ile-Ife, Nigeria and occupies a dimensions of 50×50 m within latitudes N07°32.649′-N07°32.654′, longitudes E004 30.661′ - E004°30.699′ and an elevation range of 844 to 975ft. Samples were collected on a monthly basis for a period of one calendar year (February, 2002-January 2003). Two treatments were set up comprising of a confined treatment in which leaf litters were confined within a 30×30 cm litter bag the leaves were previously air-dried and 20 g was weighed in each of the bags and positioned randomly in the plot) and another treatment in which the litters were not confined. During each sampling regime the following categories of samples were collected viz., leaf litters inside litter bag (confined treatment); leaf litters not inside litter bags (unconfined treatment); soil directly under confined treatment and soil directly under the unconfined treatment. A total of four samples were collected at random for each category of treatment for laboratory analysis. Other sampling protocol are as described elsewhere (Okoh *et al*., 1999).

Microbial analysis: Nutrient agar which has been reported to support the growth of most heterotrophic bacteria (Davis *et al*., 1980), containing 0.015% (w/v) nystatin (to inhibit fungi growth) was used for bacteria isolation and incubation was at 35°C for five days. Potato dextrose agar to which 0.05% (w/v) chloramphenicol has been added (to inhibit bacteria growth) was used for fungal isolation and incubation was at ambient temperature for seven days. Microbial enumeration and isolation were carried out as described by Seeley and Vandenmark (Seeley and Vandenmmark, 1981). Representative bacteria and fungi colonies were identified as described elsewhere (Bergey, 1977; Talbot, 1978). Species diversity index where determined as previously described (Shannon and Weaver, 1963).

Soil pH and organic carbon determination: Soil pH was determined with the aid of a glass electrode pH meter. Ten grams of dry soil was weighed into a 50 mL size beaker and 20 mL of 0.01 M $CaCl_2$ was added to the soil. The preparation was allowed to stand for 30 min with occasional stirring before determination of pH. Soil organic carbon was estimated using the chromic acid digestion method as described by Black (1965).

RESULTS

The total bacterial counts ranged from 8.0×10^5-5.1×10^7 cfu g^{-1} for leaf litter inside litter bag (ILB); 2.4×10^6-9.9×10^7 cfu g^{-1} for leaf litter not inside litter bag (OLB); 8.0×10^5-8.3×10^7 cfu g^{-1} for soil directly under leaf litter in litter bag (SILB) and 1.1×10^6-6.5×10^7 cfu g^{-1} for soil under leaf litter that is not inside litter bag (SNILB) (Fig. 1). These differences were however not significant. The highest counts were observed in the months of August for ILB and September for OLB, SILB and SNILB, while the lowest counts were observed in January for ILB and December for OLB, SILB and SNILB.

The total fungal counts ranged from 3.0×10^3-7.5×10^4 cfu g^{-1} for ILB, 1.1×10^3-6.4×10^4 cfu g^{-1} for OLB, 2.0×10^3-5.1×10^4 cfu g^{-1} for SILB and 1.0×10^3-5.2×10^4 cfu g^{-1} for SNILB (Fig. 2). Highest counts were observed in the months of May for ILB, OLB and SILB and October for SNILB. Lowest counts were observed in the months of March for ILB and SILB and April for OLB and SNILB (Fig. 2).

The bacteria diversity index ranged from 0.95-2.21 for ILB, with the months of April and January having the lowest and highest values, respectively. The diversity index for OLB, SILB and SNILB ranged from 0.97-1.35, 0.69-1.36 and 0.52-1.85, respectively (Table 1). The fungi diversity index on the other hand was found to fall within

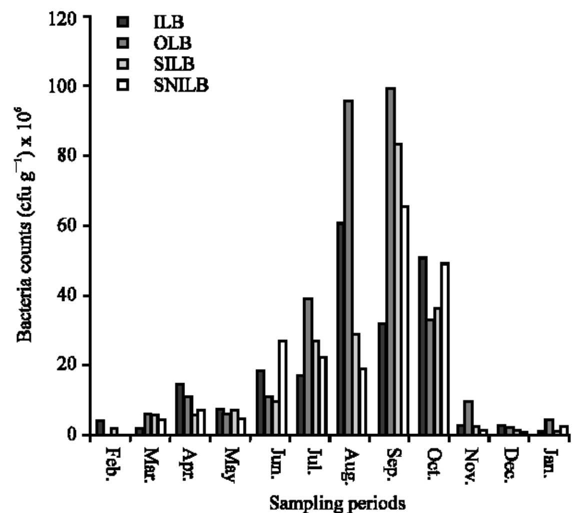

Fig. 1: Total culturable aerobic bacteria counts of the litter and soil samples represented by ILB (litter inside litterbag or confined); OLB (litter in unconfined treatment); SILB (soil under confined treatment); SNILB (soil under unconfined treatment)

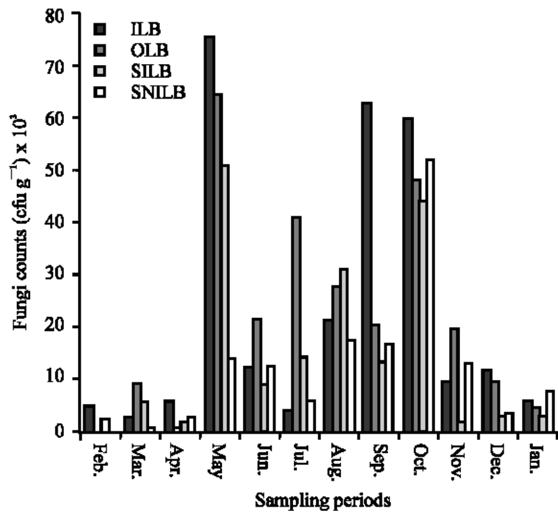

Fig. 2: Total culturable fungi counts of the litter and soil samples represented by ILB (litter inside litterbag or confined); OLB (litter in unconfined treatment); SILB (soil under confined treatment); SNILB (soil under unconfined treatment)

Table 1: Culturable earobic bacterial diversity indices of the litter and soil samples represented by ILB (litter inside litterbag or confined); OLB (litter in unconfined treatment); SILB (soil under confined treatment); SNILB (soil under unconfined treatment)

	Bacterial diversity index			
Months	ILB	OLB	SILB	SNILB
Feb.	1.28	nd	1.02	nd
Mar.	1.27	1.24	1.34	1.28
Apr.	0.95	0.97	0.97	0.52
May	1.48	1.22	1.20	1.04
Jun.	1.22	1.08	0.69	1.10
Jul.	1.01	1.10	1.36	1.04
Aug.	1.45	1.24	1.03	1.23
Sept.	1.49	1.42	1.49	1.85
Oct.	1.45	1.35	1.23	1.43
Nov.	1.33	1.41	1.28	1.42
Dec.	1.06	1.04	1.04	1.00
Jan.	2.21	1.19	1.01	1.08

Table 2: Culturable fungal diversity indices of the litter and soil samples represented by ILB (litter inside litterbag or confined); OLB (litter in unconfined treatment); SILB (soil under confined treatment); SNILB (soil under unconfined treatment)

	Fungal diversity index			
Months	ILB	OLB	SILB	SNILB
Feb.	1.31	nd	1.39	nd
Mar.	1.64	1.28	1.54	1.50
Apr.	1.44	1.72	1.09	1.36
May	1.76	1.21	1.44	1.56
Jun.	1.52	1.30	1.03	1.01
Jul.	2.04	1.56	1.19	1.01
Aug.	1.34	1.08	0.91	1.50
Sept.	1.67	1.28	1.56	1.49
Oct.	1.47	1.24	0.82	1.47
Nov.	1.03	1.51	0.69	1.30
Dec.	1.20	1.03	0.64	0.56
Jan.	0.64	1.01	1.06	1.32

Table 3: List of culturable bacteria species isolated from the litter and soil samples represented by ILB (litter inside litterbag or confined); OLB (litter in unconfined treatment); SILB (soil under confined treatment); SNILB (soil under unconfined treatment)

	Bacterial isolates			
Organisms	ILB	OLB	SILB	SNILB
Bacillus spp.	√	√	√	√
Flavobacterium spp.	√	√	√	√
Corynebacterium spp.	√	√	√	√
Actinomyces spp.	√	√	√	√
Lactobacillus spp.	√	√	√	√
Pseudomonas spp.	√	√	√	√
Pasteurella spp.	√	√	√	√
Aeromonas spp.	√	√	√	√
Chromobacterium spp.	√	√	√	
Shigella spp.	√	√	√	√
Enterobacter spp.	√		√	√
Staphylococcus spp.	√	√	√	√
Klebsiella spp.	√	√	√	√
Micrococcus spp.	√		√	
Nocardia spp.	√	√	√	√
Acinetobacter spp.	√	√	√	√
Proteus spp.	√			
Alcaligenes spp.			√	
Streptococcus spp.				√
Total	17	14	17	15

Table 4: List of fungal species isolated from the litter and soil samples represented by ILB (litter inside litterbag or confined); OLB (litter in unconfined treatment); SILB (soil under confined treatment); SNILB (soil under unconfined treatment)

	Fungal Isolates			
Organisms	ILB	OLB	SILB	SNILB
Aspergillus niger	√	√	√	√
Penicillium spp.	√	√	√	√
Alternaria spp.	√	√	√	√
Aspergillus fumigatus	√	√	√	√
Botrytis spp.	√	√	√	√
Trichoderma spp.	√		√	√
Fusarium spp.	√	√	√	√
Aspergillus flavus	√	√	√	√
Cladosporium spp.	√	√	√	√
Trichophyton spp.	√	√	√	√
Mucor spp.	√	√	√	√
Microsporum spp.	√	√	√	√
Cephalosporium spp.	√	√		√
Gliocladium spp.	√			
Total	14	12	12	13

the ranges of 0.64-2.54 (ILB); 1.01-1.72 (OLB); 0.64-1.56 (SILB) and 0.56-1.56 (SNILB) (Table 2).

A total of nineteen bacterial (Table 3) and fourteen fungal (Table 4) strains were identified, at least, to genus levels. Seventeen bacteria species were each isolated from the ILB and SILB samples while OLB and SNILB yielded fourteen and fifteen bacteria isolates each. Fourteen fungal strains were identified in the ILB samples. OLB and SILB samples respectively yielded twelve fungal strains while SNILB samples yielded thirteen strains.

Soil pH regime ranged between 6.1 and 7.9 for SILB and 6.8 to 8.0 for SNILB (Fig. 3). The pH of soil directly under leaf litter in the unconfined treatment were

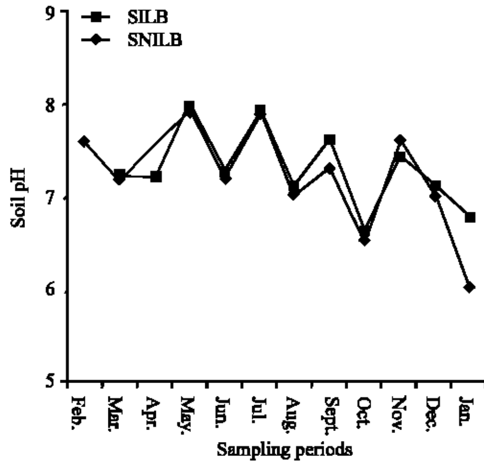

Fig. 3: pH profile of the soil samples represented by SILB (soil under confined treatment); SNILB (soil under unconfined treatment)

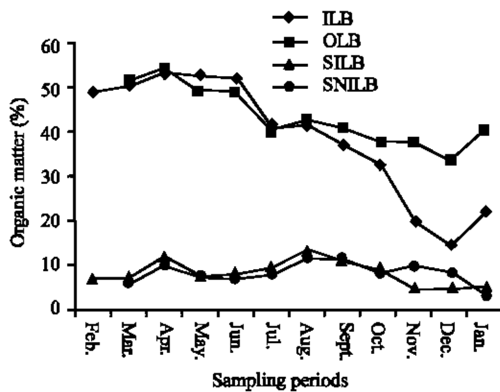

Fig. 4: Organic matter contents of the litter and soil samples represented by ILB (litter inside litterbag or confined); OLB (litter in unconfined treatment); SILB (soil under confined treatment); SNILB (soil under unconfined treatment)

consistently higher than the pH of soil under the confined system. The percentage organic carbon content of the leaf litters were significantly ($p<0.05$) higher than those of the soil throughout the sampling period and ranged from 8.39-30.84% (ILB); 19.50-31.47% (OLB); 2.69-7.80% (SILB) and 1.95-6.83% (SNILB), respectively (Fig. 4). However, the variation in organic matter content between the confined and unconfined leaf litters were not significant.

As shown in Fig. 5, the average monthly temperature values during the sampling period ranged from 27.1-32.4°C, with the highest temperature being recorded in April, while the lowest were in September. The monthly rainfall ranged from 0.07-8.17 mm, with months of January and February having no rainfall at all. The temperature was generally on the decline during the rainy season.

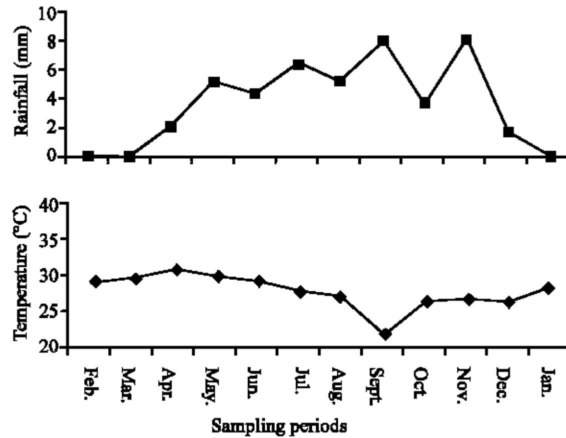

Fig. 5: Rainfall and temperature regimes during the study periods

DISCUSSION

The methods used to collect the leaf litter samples can generally be divided into two, namely, the litter bag (confining) method and the no-litter bag (non confining) method. Both methods have their advantages and disadvantages (Huang *et al*., 1998). The principle of confining known amounts of litter in order to follow its decomposition was used much earlier (Swift *et al*., 1979; Berg *et al*., 1993). However, litter bags have several limitations, despite being the most frequently used method to study litter decomposition (Gilbert and Bocok, 1962; Edwards, 1977; Tanner, 1981; Anderson and Swift, 1983; Suberkropp and Chauvet, 1995). Litter bags may also alter decomposition rates by altering litter microclimate, hindering soil contact and excluding litter macrofauna, such as arthropods, millipedes, termites and earthworms (Edwrds, 1977; Ewel, 1976; Hanlon and Anderson, 1979; St. John, 1980). The two methods (litter bag and no-litter bag) discussed above were used in this study for the purpose of comparing them and hence highlighting any possible differences. In this study not much significant variation was observed between litter that was confined inside litter bag and the ones that were not confined. This was true for almost all the parameters measured (i.e., total bacterial counts, total fungal counts, organic matter and species diversity index).

The range of bacterial densities observed in this study for the two treatments fell within the range reported by earlier workers (Okoh *et al*., 1999, 1999; Rangaswami, 1966) and were generally higher in the unconfined litter samples. The peak values were recorded in the month of September, while decline were observed on either sides of the divide and is probably due to the low moisture

content during the months of low counts, as similarly observed in previous report (Fernando *et al*., 1994). Throughout the sampling period, the total fungal counts were generally lower than those of bacteria and peaked in the month of May. A similar range of fungal density has been reported before (Amir and Pineau, 1998). However, there was no significant difference in these variations in support of the findings of some others (Fernando *et al*., 1994; Amir and Pineau, 1998). Also, the predominance of bacteria over fungi observed throughout the sampling period supports our earlier reports (Okoh *et al*., 1999, 1999).

Low microbial counts were observed in the months of December, January and February. It is reasonable to deduce that this may have resulted from, at least in part, the extremely dry conditions experienced in these months due to the low rainfall and high temperature. Aerts (1997) reported that temperature and moisture affect the population of microorganisms during decomposition. Microbial population density was generally lower during the dry season and a similar trend has been reported by Rheinheimer (1997). In this study, rainfall showed stepwise increases, attaining peak density in November, after which there was a drop, which however did not correlate generally with total microbial counts. Jordan (1989) reported that water affects decomposition directly through leaching and indirectly through its effect on microbial decomposers. It would appear that moisture and temperature conditions were most conducive during the wet season when microbial population and diversity flourished, thus suggesting that drought affects the compositions and populations of microbial communities, with fungi, being more tolerant of dry conditions than bacteria (Hendrix *et al*., 1986).

The organic carbon contents of the leaf litters and soil samples in the confined and unconfined treatments fell within the ranges that have been reported by Ekanade *et al.* (1991). While the organic matter regimes of the two treatments were not significantly different, that of litters were always significantly ($p<0.05$) higher than that of the soil, as similarly reported elsewhere (Martin *et al*., 2003).

A significant positive correlation was also observed between the organic matter and temperature ($r = 0.503$, $p<0.05$). It is conceivable that increase in temperature should promote microbial activity during decomposition, thus leading to increase in the organic carbon content released (Adejuwon and Ekanade, 1988). On the other hand, there was a significant negative correlation between rainfall and temperature ($r = -0.538$, $p<0.01$). However no significant correlation was observed between the organic carbon content of the different treatments with rainfall.

Although some studies (Areola, 1984; Ekanade, 1998) have reported that, under forest conditions, pH and organic carbon were positively correlated. This was not observed in this study. Nevertheless, all the soil pH in this study was near neutral ranges, which favour microbial growth. The composition and diversity of culturable heterotrophic bacteria and fungi observed in this study were not much different amongst the treatments, in support of earlier reports (Okoh *et al*., 1999; Qiu *et al*., 1998) and most of the organisms isolated are similar to those reported before (Amir and Pineau, 1988) and their composition does not appear to follow any defined pattern (Okoh *et al*., 1999).

This study has shown that diverse types of bacteria and fungi were involved in the decomposition of cocoa leaf litters, with their population dynamics following specific fashions during decomposition of the leaf litters. The microbial diversity does not appear to follow any defined pattern and their population dynamics does not appear to be affected by confinement in litterbag. Organic matter regimes correlated significantly with atmospheric temperature of the study area, thus supporting the fact that increases in temperature enhances microbial decomposition of litter and hence release of organic matter. An insight into the population dynamics and distribution of culturable aerobic bacterial and fungal diversity during the decomposition of cocoa leaf litters in a tropical setting has been elucidated by this study. This is without prejudice to the possible influence which a substantial proportion of bacteria and fungi that are not culturable *in vitro* could have on the overall picture of event and this is the subject of our on-going study.

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