CULTURABLE MICROBIAL POPULATION DYNAMICS DURING DECOMPOSITION OF *COLA NITIDA* 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 kolanut 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 and soil pH were also monitored in the experimental plot. Bacterial counts ranged between 6.0×10^5 and 8.1×10^7 cfu/g wet weight of litter or soil, while fungal counts were of the order of $10^3 – 10^4$ cfu/g wet weight of litter or soil. Counts of bacteria and fungi were highest during the rainy season months and reduce on either sides of the rainy season divide. Bacteria diversity index ranged between 0.42 and 1.69, while fungal diversity index varied between 0.56 and 2.54, and a total of eighteen 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 and ranged between 13.42 and 59% for the leaf litter and 4.02 – 14.76% for the soil samples. pH of the soil samples were observed to vary between 5.3 and 7.98. We conclude that decomposition of kolanut leaf litters in a confined or unconfined setting does not appear to significantly affect the stability of the culturable microbial milieu.

Keywords: Culturable bacteria, fungi, kolanut leaf litters, decomposition

Tropik Toprak Koşullarında *Cola nitida* Yaprak Artıklarının Parçalanması Sürecinde Kültüre Alınabilir Mikrobiyal Populasyon Dinamikleri

Özet

Çürümekte olan *Cola nitida* yaprak artıklarında bulunan kültüre alınabilir aerobik, heterotrofik bakteri ve fungus dağılımı ve populasyon dinamikleri, Şubat 2002 ve Ocak 2003 tarihleri arasında çalışılmıştır. Bu amaçla, standart çöp-torba ve mikrobiyal kültivasyon teknikleri, dışa kapalı ve kapalı olmayan ortamlar düzeneğinde kullanılmıştır. Ayrıca, organik karbon ve toprak pH' sı takip edilmiştir. Bakteri sayıları 6.0×10^5 ve 8.1×10^7 cfu/g yaş artık veya toprak ağırlığı arasında değişirken, fungal sayımlar $10^3 – 10^4$ cfu/g yaş artık veya toprak ağırlığı civarında olmuştur. Bakteri ve fungus sayıları en çok yağmurlu sezon içerisindeki aylarda olmuş, bunun dışındaki aylarda düşmüştür. Bakteri çeşitlilik indeksi 0.42 ve 1.69, fungal çeşitlilik indeksi ise 0.56 ve 2.54 arasında değişmiş ve toplam onsekiz kültüre alınabilir bakteri ve ondört fungal strain bulunmuştur. Yaprak artıklarında bulunan organik karbon miktarı toprakta bulunandan devamlı olarak yüksek bulunmuş, yaprak artıkları için % 13.42 ve 59.00 arasında değişirken, toprak örnekleri için % 4.02 ve 14.76 arasında seyretmiştir. Toprak örneklerinde pH, 5.3 ve 7.98 arasında bulunmuştur. *Cola nitida* yaprak artıklarının kapalı veya kapalı olmayan ortamlarda dekompozisyonun, kültüre alınabilir mikrobiyal ortamın stabilitesini önemli ölçüde etkilemediği sonucuna varılmıştır..

Anahtar Kelimeler: Kültüre Alınabilir Bakteri, *Cola nitida*, Yaprak Artığı, Ayrışma.

1. Introduction

Plant decomposition is the physical and chemical breakdown of dead plant material and it involves a number of sub-processes resulting in complete breakdown of organic matter into CO_2 and the mineral forms of nutrients like nitrogen, phosphorus (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. 1991).

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 (Dilly et al. 2001) 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 paper, we report the effect of decomposition of *Cola nitida* (kolanut) leaf litters on the population dynamics of the culturable heterotrophic bacteria and fungi communities under a typical tropical setting and within a confined and unconfined litterbag setup.

2. Materials and Methods

The study area is located in a kolanut in Nigeria occupying a dimension within latitudes N07°32.649/ - N07°32.654/, longitudes E004°30.661/ - E004°30.699/ and an elevation range of 844ft 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 cm × 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, ILB); leaf litters not inside litter bags (unconfined treatment, OLB); soil directly under confined treatment (SILB); and soil directly under the unconfined treatment (SNILB). A total of four samples were collected at random for each category of treatment for laboratory analysis. Other sampling protocols are as described elsewhere (Okoh et al. 1999b).

Nutrient agar 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 (1981). Representative bacteria and fungi colonies were identified according to Bergey (1977) and Talbot (1978) respectively. Species diversity index where determined as previously described (Shannon and Weaver, 1963).

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 0.01M $CaCl_2$ was added to the soil. The preparation was allowed to stand for 30 minutes with occasional stirring before determination of pH. Soil organic carbon was estimated using the chromic acid digestion method as described by Black (1965).

3. Results

The total bacterial counts ranged from $1.2 \times 10^6 - 8.1 \times 10^7$ cfu/g for leaf litter inside litter bag (ILB); $1.1 \times 10^6 - 7.1 \times 10^7$ cfu/g for leaf litter not inside litter bag (OLB); $6.0 \times 10^5 - 4.4 \times 10^7$ cfu/g for soil directly under leaf litter in litter bag (SILB), and $1.0 \times 10^6 - 3.8 \times 10^7$ cfu/g for soil under leaf litter that is not inside litter bag (SNILB) (Figure 1). These differences were however not significant. The highest counts were observed in the months of August for ILB and October for OLB and September for SILB and SNILB, while the lowest counts

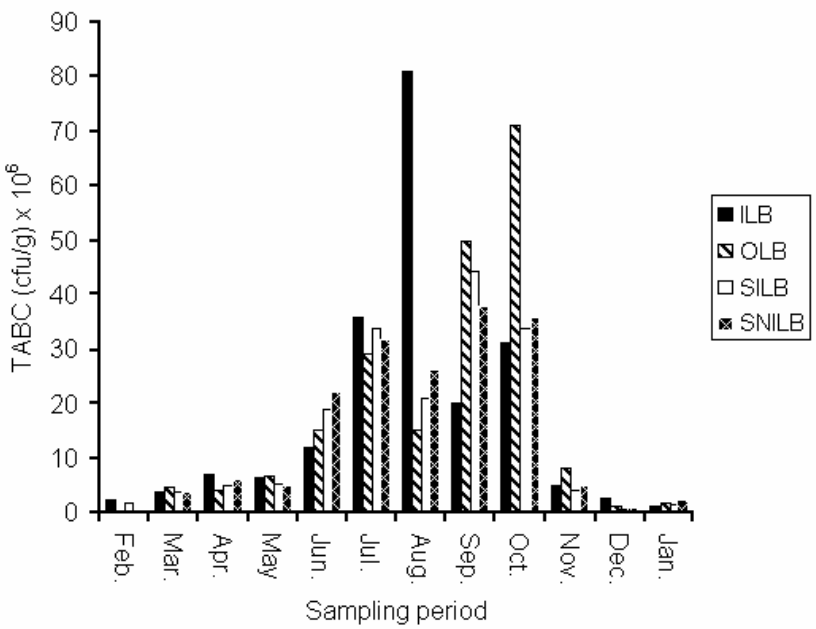

Figure 1. Total culturable aerobic bacteria counts (TABC) of the kolanut leave litter and soil samples represented by ILB (litter inside litterbag or confined); OLB (litter in unconfined treatment); SILB (soil under confined treatment); SNILB (soil under unconfined treatment).

were generally observed from the month of November through March.

The total fungal counts ranged from $2.1 \times 10^3 - 9.1 \times 10^4$ cfu/g for ILB, $2.3 \times 10^3 - 7.5 \times 10^4$ cfu/g for OLB, $2.6 \times 10^3 - 6.4 \times 10^4$ cfu/g for SILB and $2.0 \times 10^3 - 9.8 \times 10^4$ cfu/g for SNILB (Figure 2). Highest counts were observed in the months of May for ILB, OLB and SILB and September for SNILB. Lowest counts were also generally observed from the month of November through to April. (Figure 2).

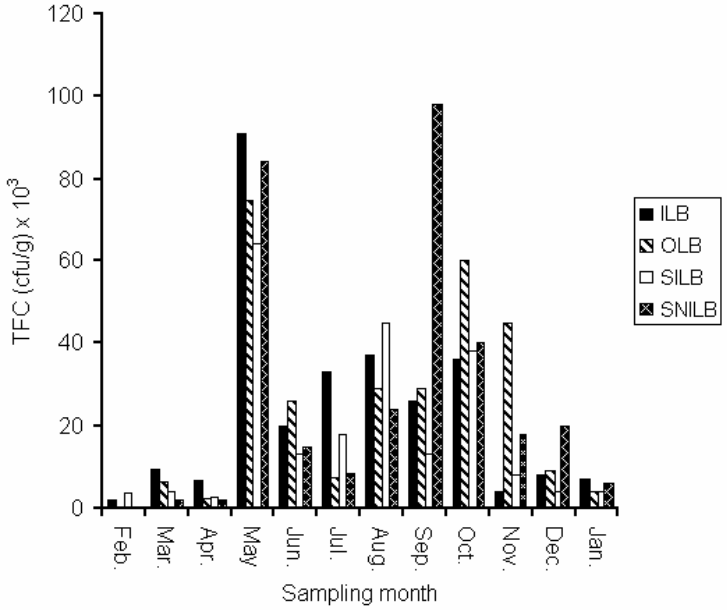

Figure 2. Total culturable fungi counts (TFC) of the kolanut leaf litter and soil samples represented by ILB (litter inside litterbag or confined); OLB (litter in unconfined treatment); SILB (soil under confined treatment); SNILB (soil under unconfined treatment).

The bacteria diversity index ranged from 1.02 – 1.57 for ILB, and 1.01 – 1.69, 0.49 - 1.63 and 1.05 - 1.57 for OLB, SILB and SNILB respectively (Table 1). The fungi diversity index on the other hand was found to range as follows: 1.04 – 2.54 (ILB); 0.56 – 1.38 (OLB); 0.56 – 1.65 (SILB); and 0.64 – 1.56 (SNILB) (Table 2).

A total of eighteen bacterial (Table 3) and fourteen fungal (Table 4) strains were identified, at least, to genus levels. Fifteen bacteria species were each isolated from the ILB and OLB samples while SILB and SNILB yielded seventeen bacteria isolates each. Thirteen fungal strains were identified in the ILB samples. OLB and SILB samples respectively yielded eleven and nine fungal strains respectively, while SNILB samples yielded twelve strains.

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).

	BACTERIA DIVERSITY INDEX			
Months	ILB	OLB	SILB	SNILB
February	1.02	-	1.07	-
March	1.35	1.32	1.63	1.55
April	1.3	1.01	0.94	1.33
May	1.28	1.06	1.51	1.27
June	1.31	1.08	0.42	1.09
July	1.57	1.05	1.34	1.05
August	1.49	1.52	1.23	1.08
September	1.26	1.47	1.02	1.57
October	1.12	1.69	1.55	1.55
November	1.31	1.53	1.58	1.33
December	1.22	1.34	0.64	1.55
January	1.08	1.34	1.06	1.19

Table 2. Culturable fungal diversity indices of the kolanut leaf 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).

	FUNGI DIVERSITY INDEX			
Months	ILB	OLB	SILB	SNILB
February	1.26	-	1.29	-
March	2.54	0.95	1.42	1.47
April	1.57	1.22	1.18	1
May	1.26	1.17	1.41	1.42
June	1.25	1.71	1.07	1.17
July	1.52	1.08	1.65	0.64
August	1.15	0.64	0.86	1.56
September	1.35	0.7	0.9	0.8
October	1.15	1.09	0.86	0.79
November	1.04	1.28	0.66	0.94
December	1.08	1.37	0.56	1.3
January	1.08	0.56	0.69	1.01

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).

	Treatment Plots			
Bacterial isolate	ILB	OLB	SILB	SNILB
Bacillus	√	√	√	√
Actinomyces	√	√	√	√
Lactobacillus	√	√	√	√
Corynebacterium	√	√	√	√
Flavobacterium	√	√	√	√
Shigella	√	√	√	√
Aeromonas	√	√	√	√
Klebsiella	√	√	√	√
Acinetobacter	√	√	√	√
Pseudomonas	√	√	√	√
Staphylococcus	√	√	√	√
Alcaligenes	√		√	√
Enterobacter	√			√
Nocardia	√	√	√	√
Pasteurella	√	√	√	√
Chromobacterium		√	√	√
Streptococcus		√	√	√
Proteus			√	
Total	15	15	17	17

The percentage organic matter content of the leaf litters were significantly ($p < 0.05$) higher than those of the soil throughout the sampling period, and ranged from 13.42 % - 53.31% (ILB); 20.12 % - 59.03% (OLB); 4.02 % - 14.76 % (SILB) and 4.5 % - 11.54 % (SNILB) respectively (Figure 3).

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).

	Treatment Plots			
Fungal isolates	ILB	OLB	SILB	SNILB
Aspergillus niger	√	√	√	√
Penicillium	√	√	√	√
Alternaria	√	√	√	√
Fusarium	√	√	√	√
Cladosporium	√	√	√	
Trichoderma	√			√
Botrytis	√			√
Aspergillus flavus	√	√	√	√
Cephalosporium	√	√		√
Mucor	√	√	√	√
Aspergillus fumigatus	√	√	√	√
Trichophyton	√	√		√
Microsporum	√		√	√
Pullularia pullulans		√		
Total	13	11	9	12

However, the variation in organic matter content between the confined and unconfined leaf litters were not significant. Soil pH regime ranged between 5.3 and 7.72 for SILB and 5.86 to 7.98 for SNILB (Figure 4). The pH of soil directly under leaf litter in the unconfined treatment was consistently higher than the pH of soil under the confined system.

4. Discussion

The range of bacterial densities observed in this study for the two treatments fell within that reported elsewhere (Okoh et al. 1999a, b), and were generally higher in the unconfined litter samples. Counts were generally high within the months of July and October while decline were observed on either sides of the divide in support of earlier 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, although these variations were not significant in consonance with the report of Amir and Pineau (1998). Also, the predominance of bacteria over fungi observed throughout the sampling period supports our earlier reports (Okoh et al. 1999a, b).

Low microbial counts were observed in the months of December through to April and this is probably due to the low rainfall and high temperature conditions that are

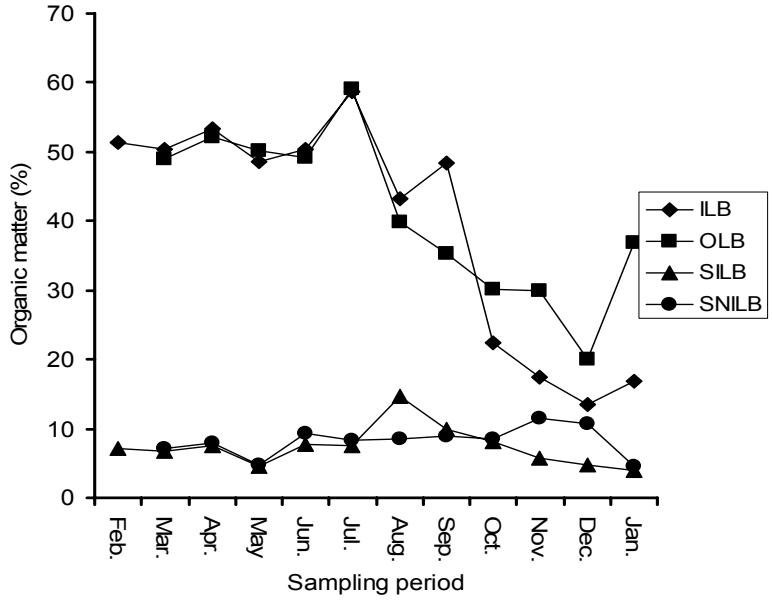

Figure 3. Organic matter contents of the kolanut leaf litter and soil samples represented by ILB (litter inside litterbag or confined); OLB (litter in unconfined treatment); SILB (soil under confined treatment); SNILB (soil under unconfined treatment).

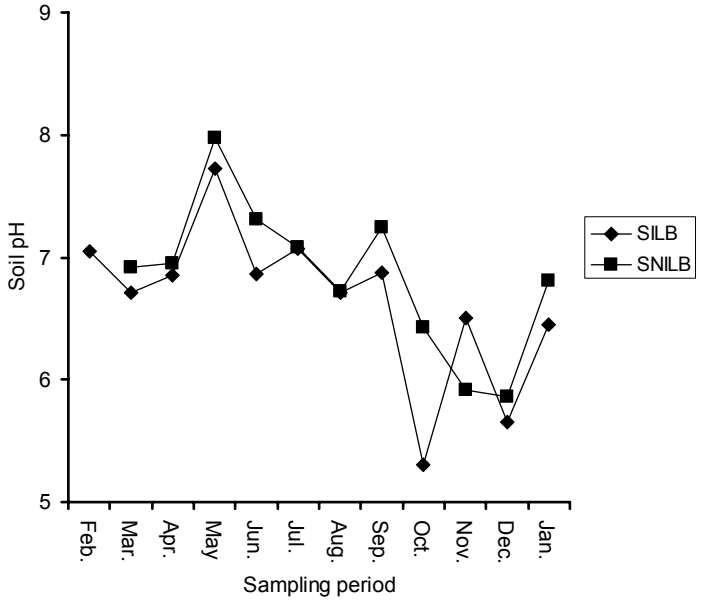

Figure 4. pH profile of the soil samples represented by SILB (soil under confined treatment); SNILB (soil under unconfined treatment).

characteristic of this period (Aerts 1997). 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 earlier workers (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).

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 within the limits that favours 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 (Qiu et al. 1998; Okoh et al. 1999b). Also, most of the organisms isolated are similar to those reported before (Amir and Pineau 1998) and their composition does not appear to follow any defined pattern (Okoh et al. 1999b).

5. Conclusion

The confined and unconfined litterbag setups no doubt have their advantages and disadvantages (Huang et al 1998) and the two methods have been used in this study for the purpose of comparing them and hence highlighting any possible effects they could have on the microbial regimes during decomposition of the leaf litters. In this study no significant variation was observed between litter that was confined inside litter bag and those 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 microbial diversity does not appear to follow any defined pattern, and their population

dynamics does not appear to be affected by confinement in litterbag. 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, which is the subject of our on-going study.

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