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COMMUNITY LEVEL PHYSIOLOGICAL PROFILES (CLPP) IN THE RHIZOSPHERE OF CASSAVA AND FORESTED AGROECOSYSTEMS

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ABSTRACT

The functional diversity and similarity in soil microbial community in a cultivated and forested tropical agroecosystem was assessed using the Biolog ecoplate. The CLPP of the soil extract was able to detect the effect of increasing N-level of the cultivated soil with fertilizer NPK on the pattern of substrate utilization in the Biolog ecoplate. Similarity in microbial nutrition pattern as indicated by colour change in the Biolog ecoplate wells inoculated with soil samples from NPK fertilized plot was compared with that from the forest plot. Results from a multivariate analysis of variance (MANOVA) of the normalized average-well colour density (AWCD) values of ecoplate readings indicated a close relationship between CLPP in the NPK and forested plots. The functional diversity (H') of the NPK fertilized and unfertilized plots were higher when compared to the forest plot. The tetrazolium reduction, as a result of microbial activity, increased with incubation time. Sixteen (16) out of the 31 substrate wells were significantly utilized with increased incubation of extracts from both soils. The utilization of substrates (N-acetyl-D-glucosamine, L-asparagine, 2-hydroxybenzoate, γ-hydroxybutyric acid, D-malic acid and L-serine) were significantly enhanced by NPK fertilizer in the cassava plot.

Keywords: Substrate utilization, Biolog ecoplates, Functional diversity Tetrazolium reduction, Microbial activity, CLPP.

INTRODUCTION

The rhizosphere is a dynamic environment, specifically, that part of the soil (within 1 or 2mm of the root surface) whose properties are influenced by the presence of roots. Certain microorganisms (notably bacteria) naturally inhabit the rhizosphere. However, depending on the root elongation, development of root hairs and adventitious roots, different stages of maturation and senescence, the soil microorganisms that inhabit the rhizosphere meet with a lot of challenges and risks of extinction. Thus, the microbial community is composed of microorganisms with different types of metabolism and adaptive responses to the variable supply of water, oxygen, organic carbon sources and nutrients (Sorensen, 1997). The rhizosphere can be categorized into two types: the endorhizosphere and ectorhizosphere. The endorhizosphere is described as the multilayered microenvironment, which includes a mucoid layer of

plant-or microbe-derived polysaccharide, the epidermal layer including the root hairs, and the cortical layer, while the ecto -rhizosphere, usually extends a few millimeters from the root surface. For the purpose of this study, the ecto-rhizosphere is of interest.

Microbial activity is expected to be high in the rhizosphere where readily degradable substrates are exuded from the plant roots. According to Sorensen (1997), substrates from root exudates significantly supported microbial activity. Though he reported that N-fertilization does not lead always to higher microbial activity and growth in the rhizosphere, however in non -fertilized soils low in nutrient availability the utilization of root released carbon compounds may be limited. Therefore plants may have dual and counteracting effects on microbial activity. This means that even when microbial activity is stimulated as a result of organic substrates, plants may inhibit microorganisms by scavenging inorganic nutrients such as ammonium (NH_4^+), nitrate (NO_3^-) and phosphate (PO_4^{3-}) ions. This view is supported by the research work of Griffiths et al. (1997), which indicated that that the relationships between genetic diversity and functional diversity of microorganisms are complex, but show that at a critical level of species richness, function is impaired.

There is, therefore, great expectation that resilience, which defines the ability of the community to withstand or recover from perturbation, be studied to help establish critical levels of functional diversity. Therefore, in this study, community level physiological profiles (CLPP) were used to determine fertilization and cultivation effects on the functional diversity in a tropical soil; we were able to compare the functional diversity of the cultivated and forested soil and to determine if the microbial community in the cultivated soil, as indicated by the pattern of substrate utilization of sole-C source in Biolog Ecoplate returned to that in the undisturbed forest soil.

MATERIALS AND METHODS

Study site/Area

The soils used for this study were collected from the research and experimental farms of the University of Nigeria Nsukka and a nearby forest. The area, classified as Nsukka zone, is located at 06° 51'N, 07° 24'E, 400amsl. The forest soil represents the climax vegetation, not affected by annual bush burning and the cultivated soil has been cultivated annually to cassava for over five years (Spaccini et al., 2001).

Incubation process

Soil bacteria were extracted from soils by Hopkins method (Hopkins et al., 1991) and the bacterial cell inoculum's density was estimated through the acridine orange direct count, as described by Binnerup et al. (1993). An estimated bacterial cell density of 2×10^5 to 2×10^7 cells was used for the inoculation. Using approximately 12.5ml of the extracted sample for each plate of the Biolog Ecoplate (120µlper well), the inoculated plates were incubated at 21°C for approximately one week. An initial scan or absorbance reading was performed immediately after inoculation, and then readings with a micro-plate reader were taken in twelve hourly intervals. The substrates used in this study are those of Insam (1997). The Biolog Ecolpates (Insam,

1997) consist of 96-well microplates with 31 substrates plus control; each replicated three times (Table1).

Data Collection

The results from the ecoplate readings were normalized using the Garland-Mills scaling (Howard, 1997) given below;

Garland-Mills scaling = R-C/ AWCD…….(1)
Where: AWCD = average well colour density.
C = amount of colour development in the control cell.
R = the value for an experimental cell.

The normalized values were subjected to Stepwise discriminant analysis (DA). The discriminant analysis is a method to analyze differences between known groups. It searches for those variables that separate the groups at their best. The stepwise selection procedure adds variables one by one to the analysis until no further increase in the separation power is achievable (Backhaus et al. 1990).

Table 1: Substrates offered in the Biolog Ecoplate*

Amines	**Carbohydrates**
Putrescine	α-D-lactose
Phenyl ethylamine	β-methyl D-glucoside
Amino Acids	Cellobiose
Arginine	D-mannitol
L-asparagine	I-erythritol
L-phenylalanine	Glucose-1-phosphate
L-serine	Xylose
L-threonine	D-galactonic acid lactone
Glycyl-L-glutamic acid	N-acetyl-D-glucosamine
Polymers	D-L-α-glycerol phosphate
α-cyclodextrin	**Carboxylic acids**
Glycogen	α-keto glutaric acid
Tween 40	D-galacturonic acid
Tween 80	D-glucosaminic acid
Phenolic compound	D-malic acid
2-hydroxybenzoate	Itaconic acid
4-hydroxybenzoate	Methylpyruvate
	γ-hydroxybutyric acid
	Control
	H_2O

*Adapted from Insam, H. (1997)

Functional Diversity

Functional diversity defines the species richness, evenness and composition (Griffith et al. 1997), this was assessed in this study using the Shannon-Weaver diversity index according to Zak et al. (1994):

$$H' = \sum Pi \ (\log Pi)$$

where:

Pi = ratio of the absorbance value of each well to the sum of absorbance values of all wells

H' = functional diversity of microbial community of the study soil

A summary of the method used in this study is shown in figure 1 below.

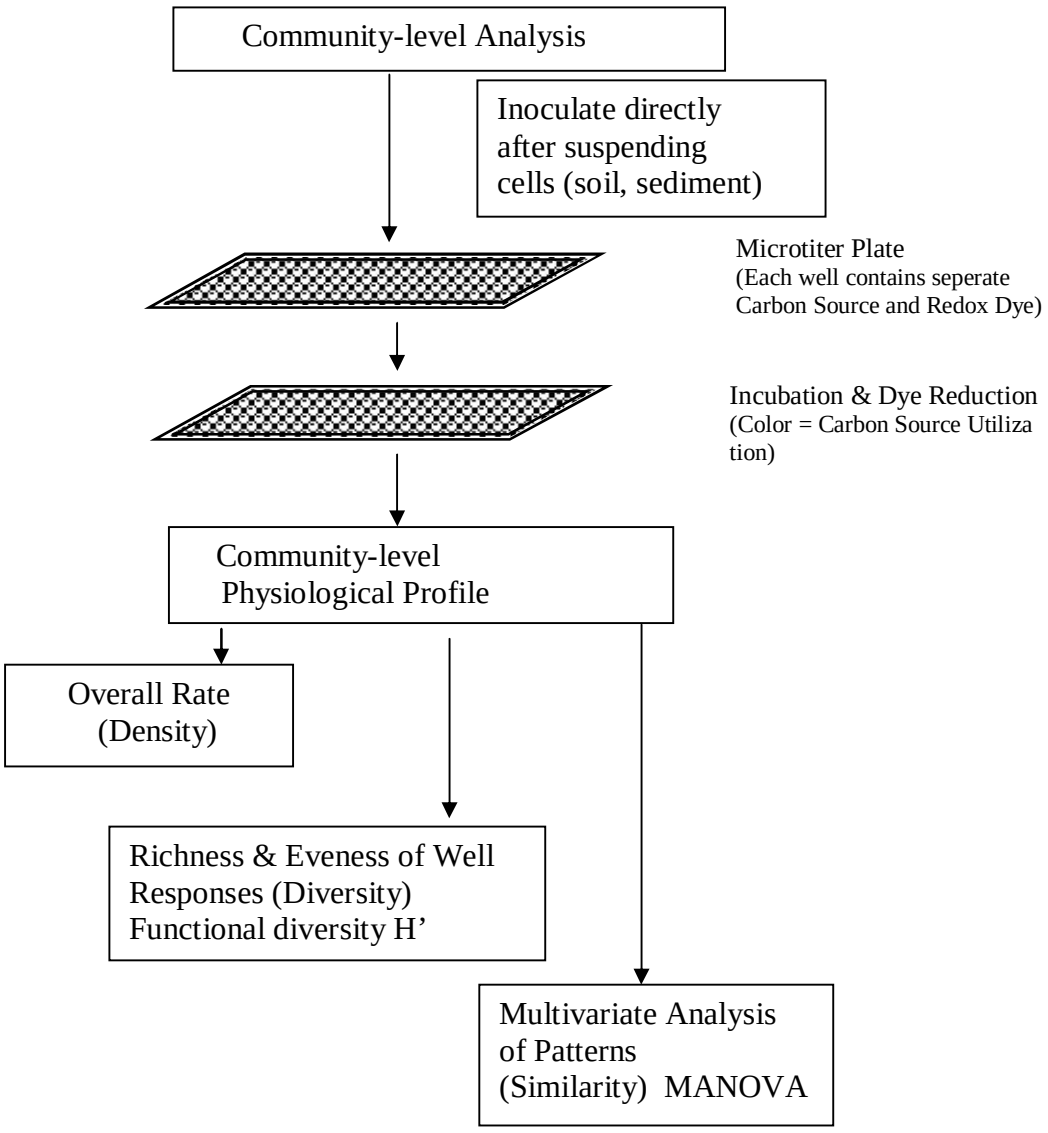

Fig. 1: A summary of the method used to profile microbial community (Adapted from Garland, 1997)

RESULTS AND DISCUSSION

The discriminant analysis clearly separated the substrate utilization pattern of the cultivated plots without NPK fertilizer, or that with NPK fertilizer and the forest plots (Figs. 2-3), suggesting that there were specific bacterial activities depending on the availability of nutrients. This observation is supported by Garland (1996), who reported that viable organisms respond to substrates they can metabolize. This can provide basis for grouping soils with respect to microbial community similarities and differences. Thus shift in substrate utilization measures of diversity suggest changes in microorganisms performing similar function. Also these changes can be viewed as differences in the availability of carbon substrates as a result of the composition of root exudates from different plant species, e.g. cassava and tree plants.

Substrate utilization is also used to monitor carbon source utilization based on the assumption that shifts in utilization are linked to availability within the environment. Thus nine months after the amendment of the cultivated soil with NPK fertilizer, there was a shift in the substrate utilization pattern (Fig. 3), suggesting a dominance of carbon mobilization and metabolization by the soil bacteria, causing an overlap in substrate utilization pattern in the plots exposed to NPK and that in the control plot. As the ecoplate incubation progressed (0hr, 12hrs, 24hrs and one week), the absorbance scan indicated nutrient mineralization (Figs. 4-7(a-d)) such that the substrate utilization pattern indicated a close relationship between bacterial nutrition in the NPK and forest plots.

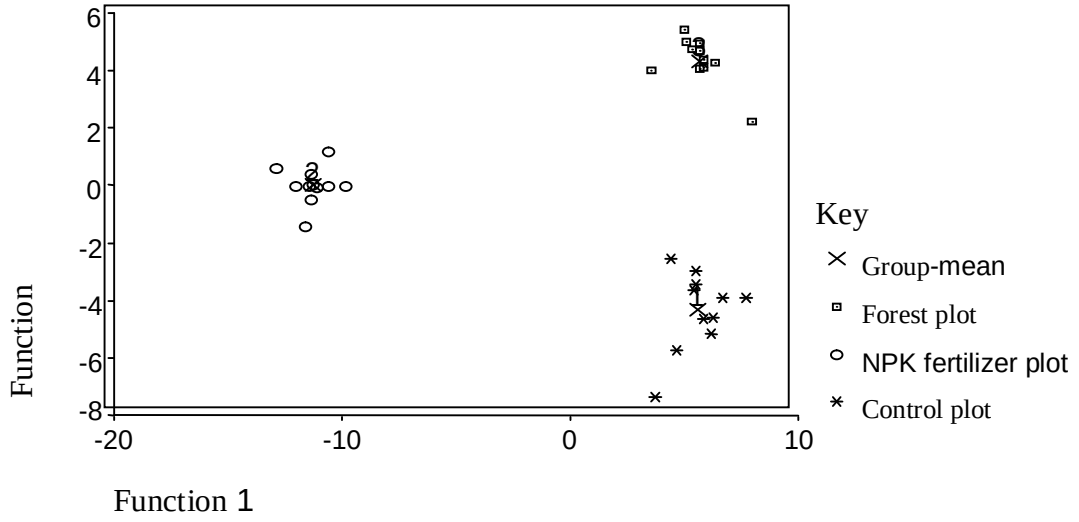

Fig.2: Group scatter plot after discriminant analysis of normalized absorbance values showing the effects of different treatments on CLPP of bacteria in study soil after three months

All the 31 substrates of the ecoplate indicated dissimilarity in CLPP of bacteria of the forest and NPK fertilized plots as shown by the initial absorbance scan of the ecoplates (4a-d). However, at one week of ecoplate incubation, the CLPP of both the forest and NPK fertilized plots became similar as each of the 31 substrates inoculated with soil extracts from the different management types were positively utilized by the soil microorganisms (7a-d).

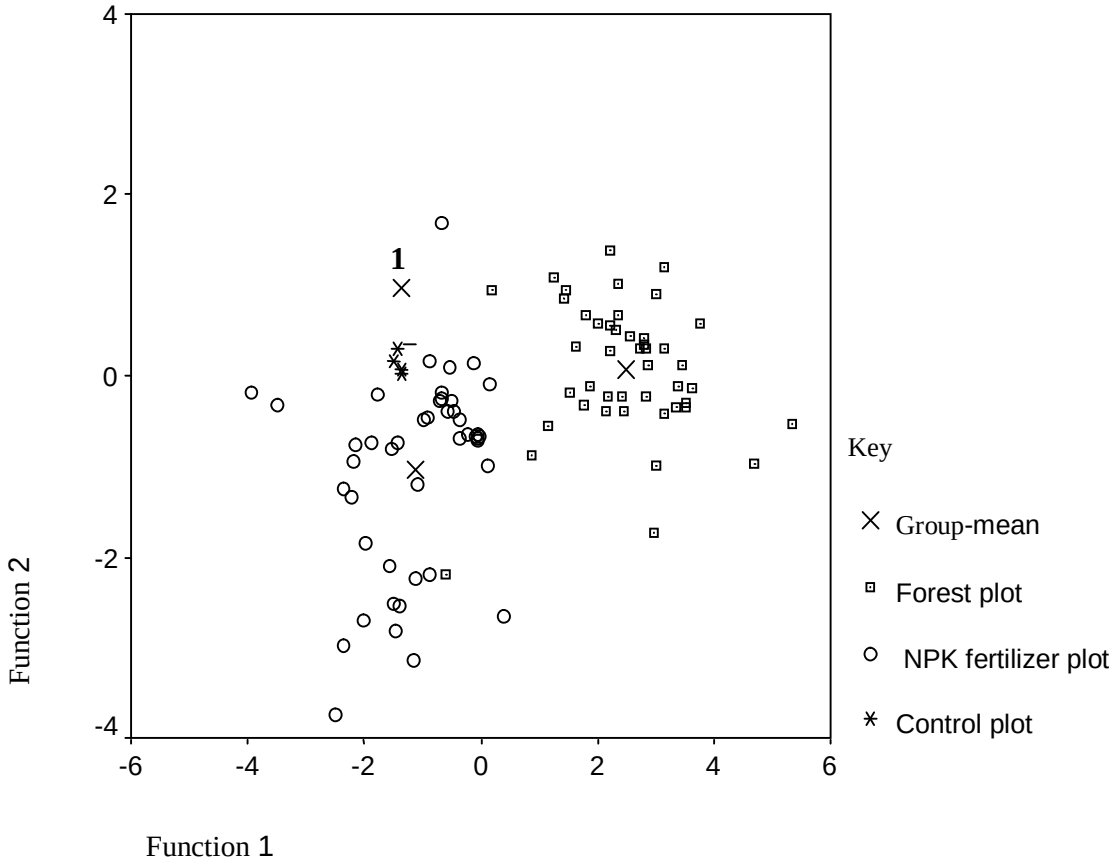

Fig. 3: Group scatter plot after discriminant analysis of normalized absorbance values showing the effects of different treatments on CLPP of bacteria in the study soil after nine months

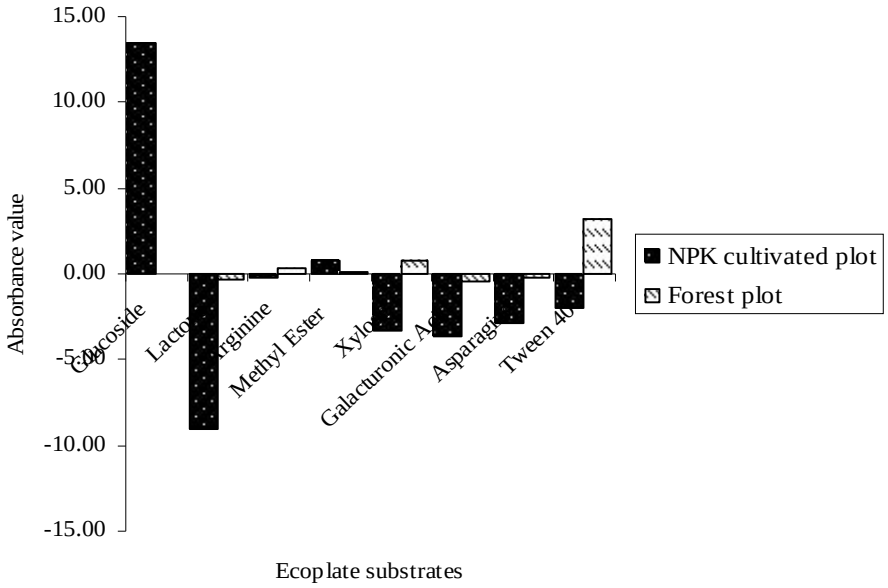

Fig4a:A comparison of CLPP of bacteria in NPK cultivated and forest plot as shown by Initial absorbance scan of biolog ecoplate

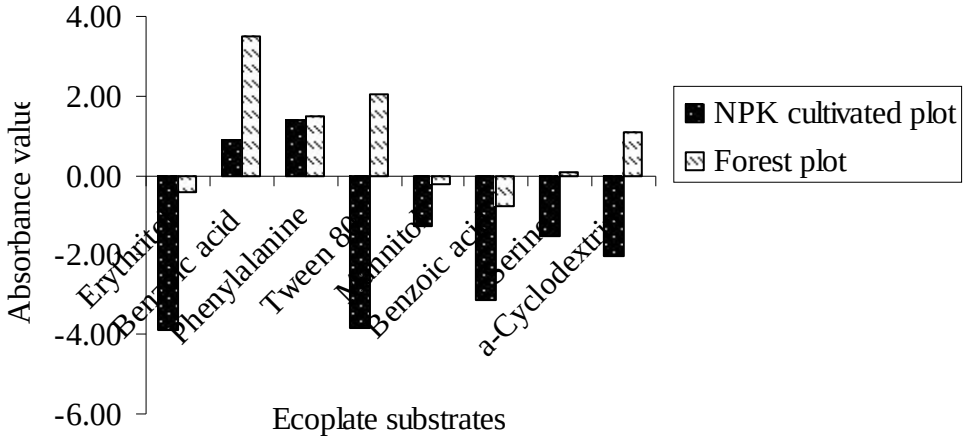

Fig. 4b: A comparison of CLPP of bacteria in the forest and cultivated plots as shown by the initial biolog ecoplate absorbance scan

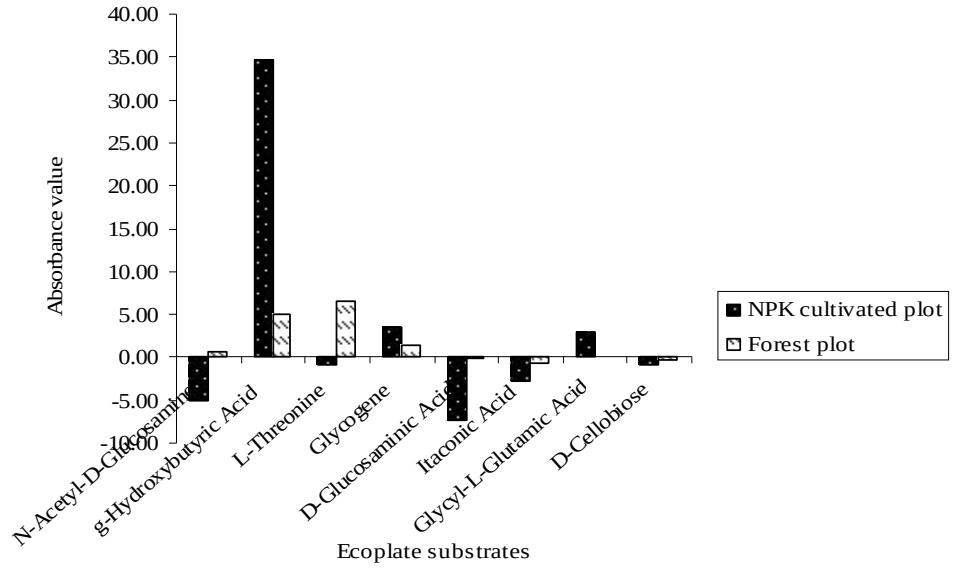

Fig. 4c: A comparison of the CLPP of bacteria in the forest and NPK cultivated plots as shown by the initial absorbance scan of biolog ecoplate

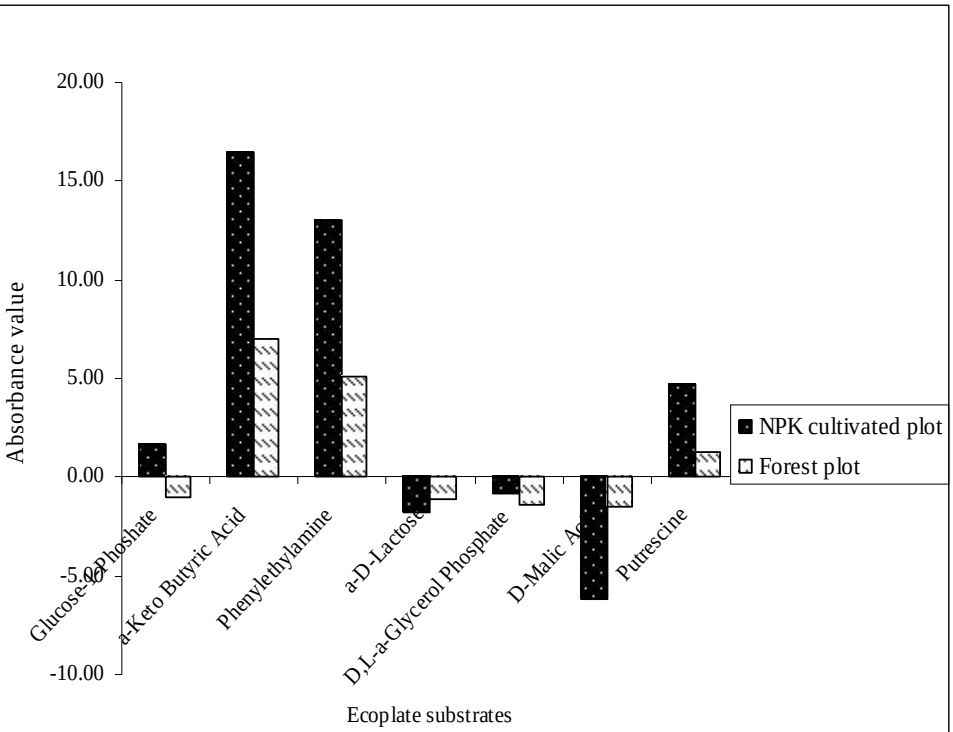

Fig. 4d: A comparison of the CLPP of bacteria in the forest and NPK cultivated plots as shown by the initial absorbance scan of biolog ecoplate

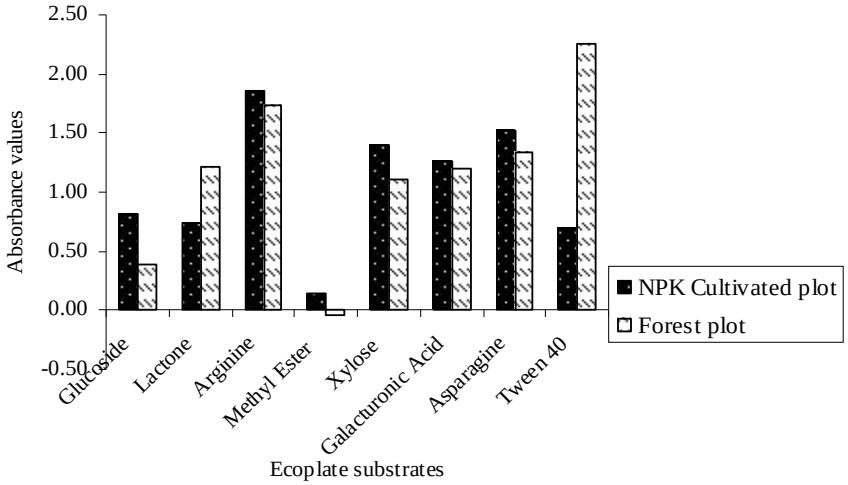

Fig 5a: A Comparison of CLPP of bacteria in forested and cultivated plots as shown by biolog ecoplate absorbance scan after twelve hours of incubation.

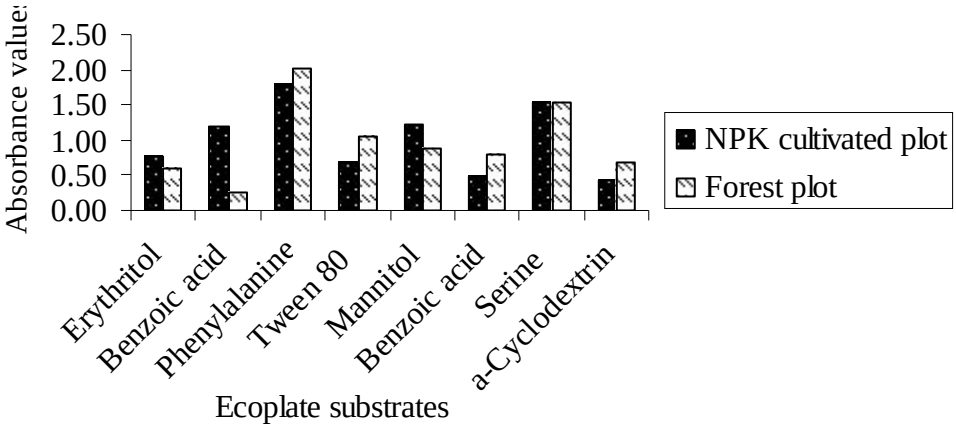

Fig. 5b: A comparison of CLPP of bacteria of the forest and NPK cultivated plots as shown by biolog ecoplate absorbance scan after twelve hours of incubation

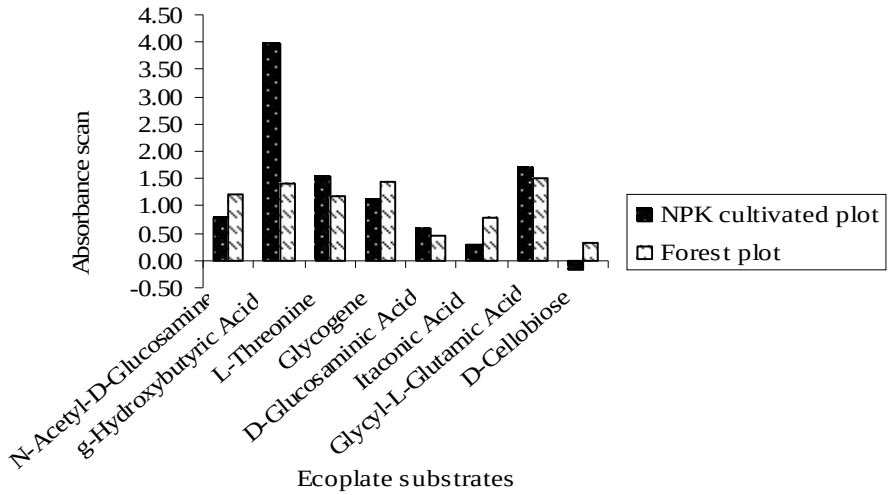

Fig. 5c: A comparison of the CLPP of bacteria in the forest and NPK cultivated plots as shown by biolog ecoplate absorbance scan after twelve hours of incubation

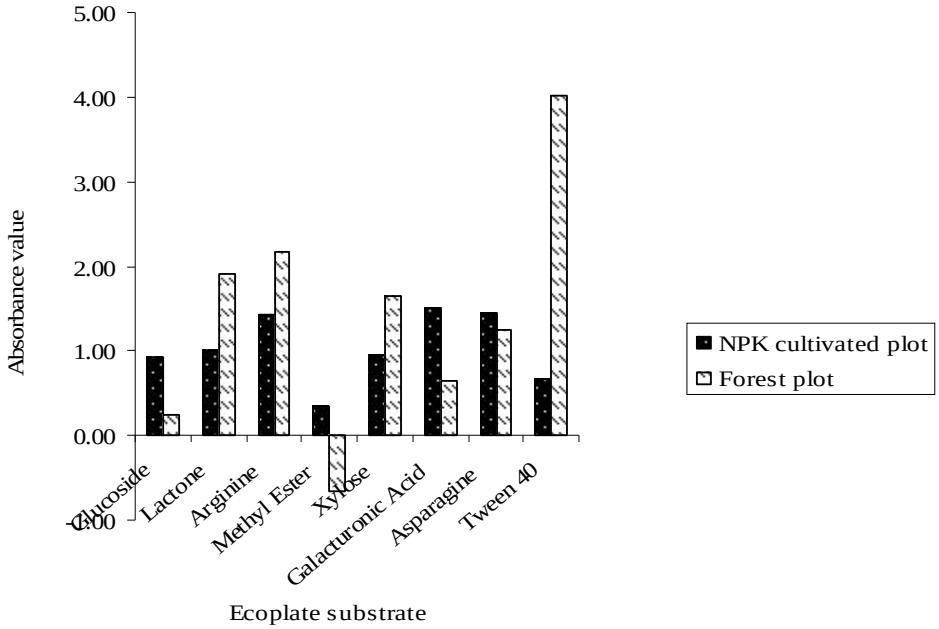

Fig. 6a: A comparison of CLPP of bacteria of the forested and NPK cultivated plots as shown by biolog ecoplate absorbance scan after twenty-four hours of incubation

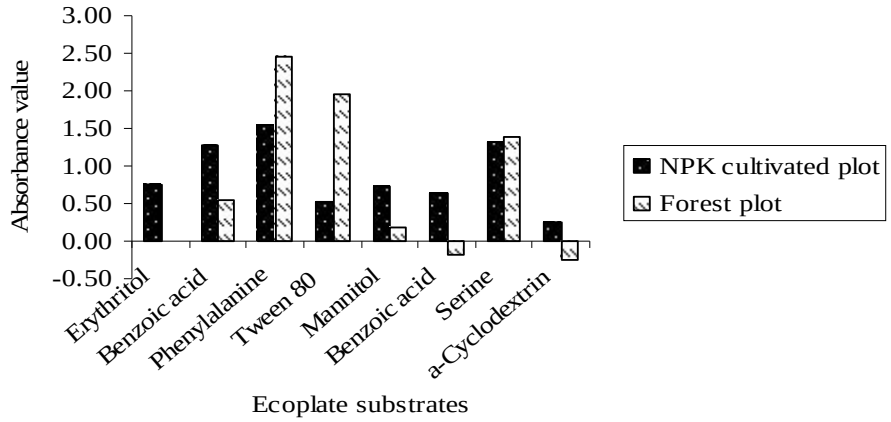

Fig. 6b:A comparison of the CLPP of bacteria of the forest and NPK cultivated plots as shown by the biolog ecoplate absorbance scan after twenty-four hours of incubation

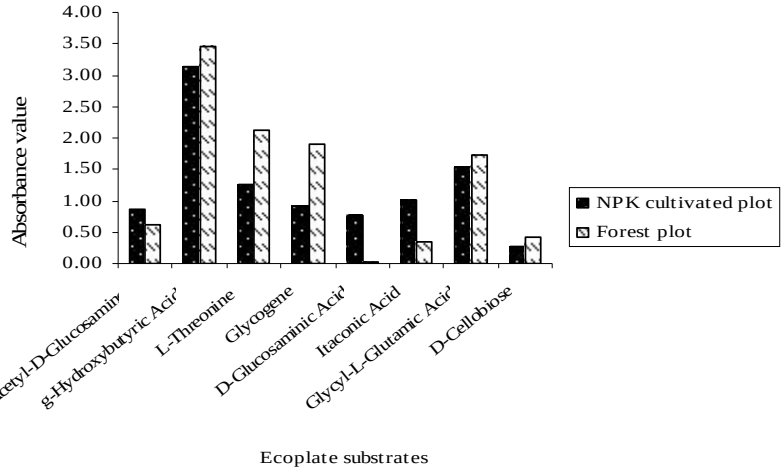

Fig. 6c: A comparison of the CLPP of bacteria in the forest and NPK cultivated plots as shown by biolog ecoplate absorbance scan after twenty-four hours

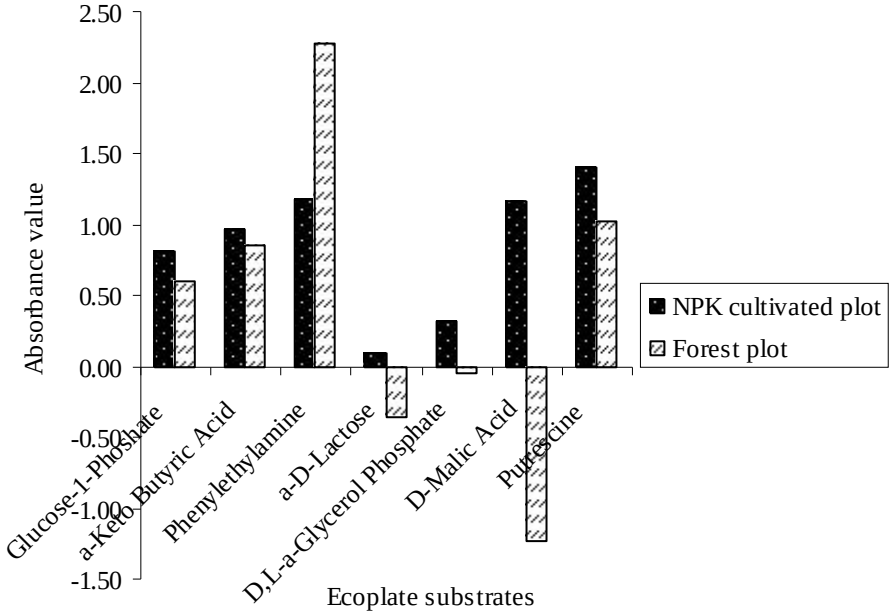

Fig. 6d: A comparison of the CLPP of bacteria in the forest and NPK cultivated plots as shown by biolog ecoplate absorbance scan after twenty-four hours of incubation

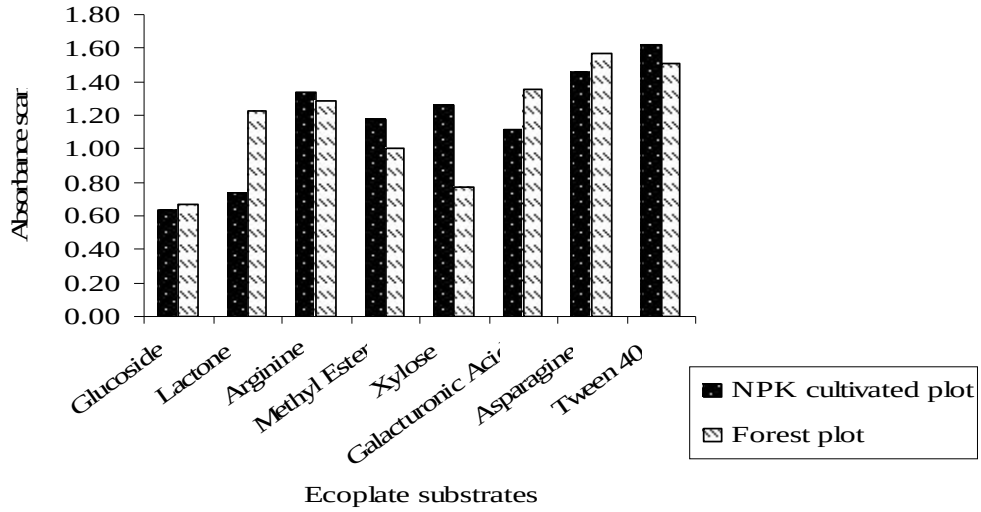

Fig. 7a: A comparison of CLPP of bacteria of the forest and NPK cultivated plots as shown by biolog ecoplate absorbance scan after one week of incubation

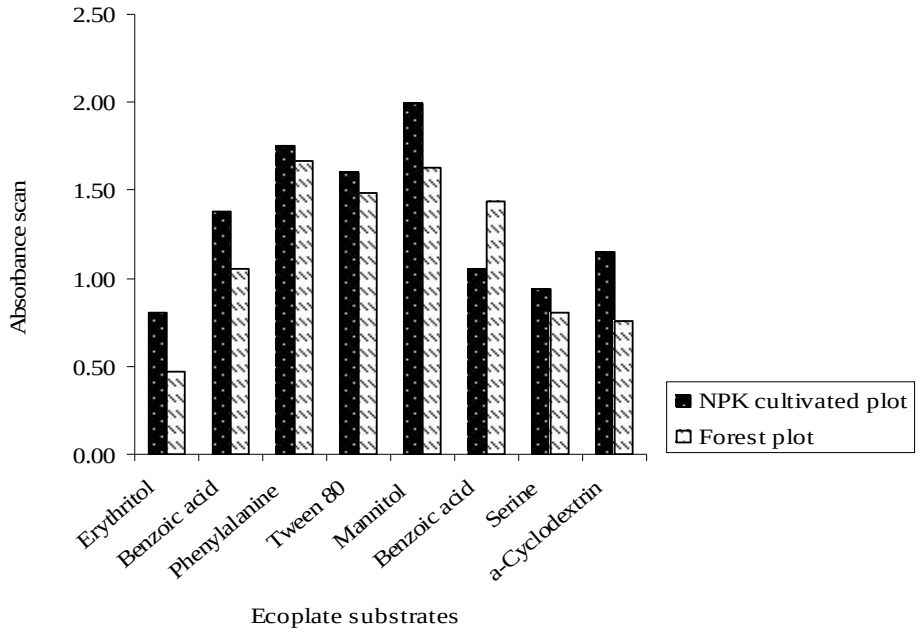

Fig. 7b: A comparison of CLPP of bacteria in the forest and NPK cultivated plots as shown by biolog ecoplate absorbance scan after one week of incubation

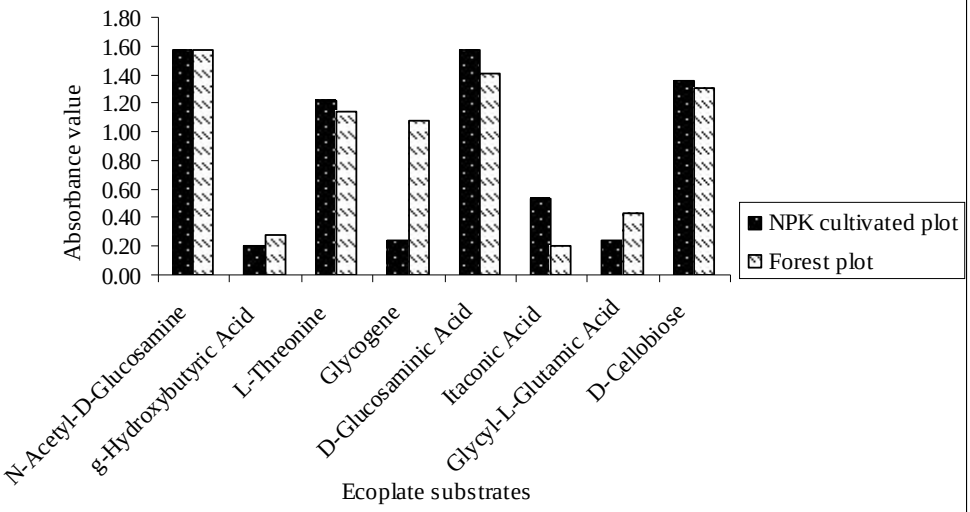

Fig. 7c: A comparison of the CLPP of bacteria of the forest and NPK cultivated plots as shown by the absorbance scan of biolog ecoplate after one week of incubation

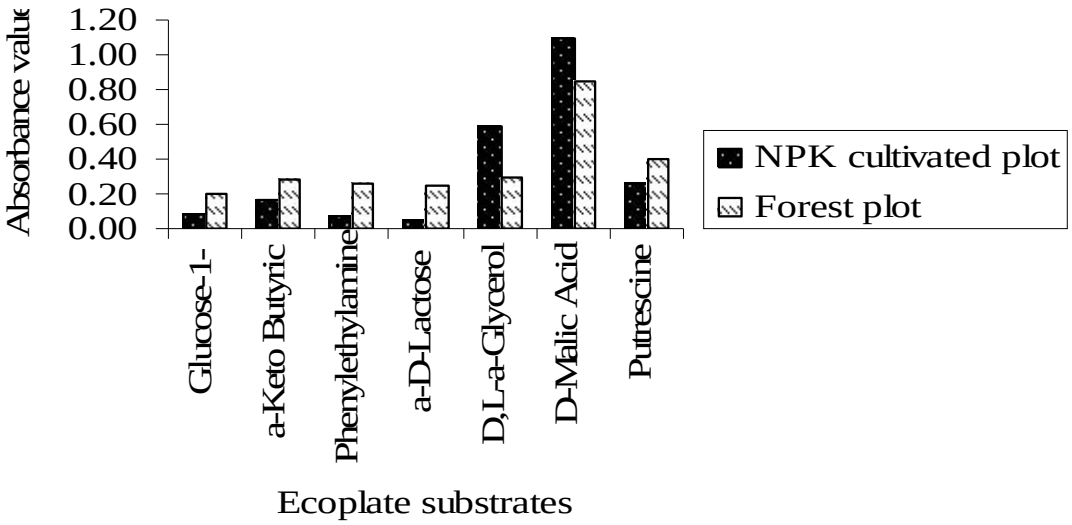

Fig. 7d: A comparison of the CLPP of bacteria in the forest and cultivated plots as shwon by biolog ecoplate absorbance scan after on week of incubation

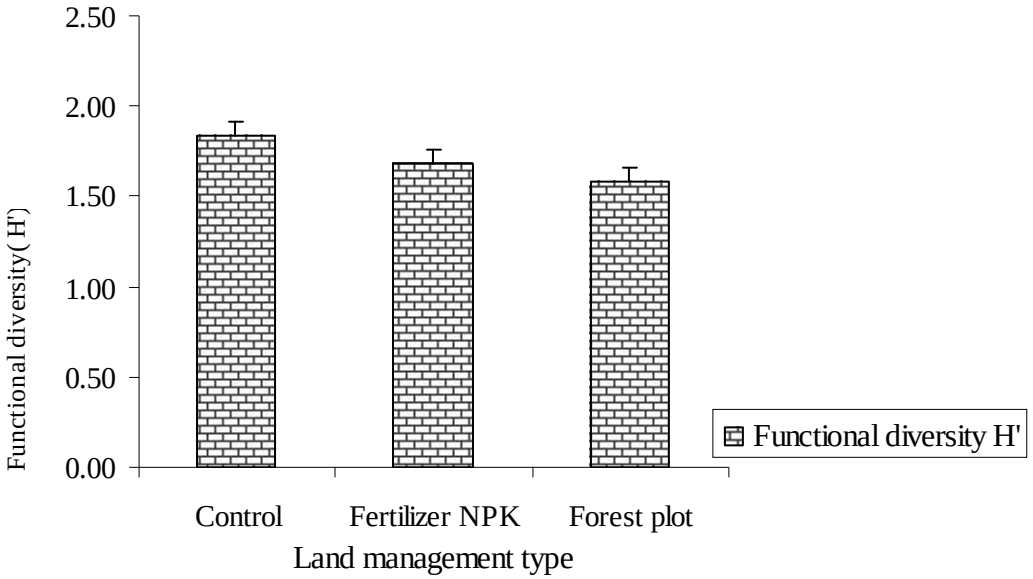

Fig. 8: A comparison of the functional diversity (H) of soil microorganism in the different land management systems.

The influence of NPK fertilizer on specific substrate, i.e. the substrates that may have spiked the level of bacterial activity in the cultivated soil as a result of N addition, assessed using MANOVA, indicated that only six out of the 31 substrate wells were significantly influenced by the addition of NPK fertilizer in the cultivated plots (Table2).

Table 2: Effects of NPK fertilizer on Biolog substrate utilization assays of cultivated soil

Substrate	Error MS	F-Cal	Sig. of F	Power at 0.05
N-acetyl-D-glucosamine	4.79	7.34	0.000***	0.937
L-asparagine	19.87	3.20	0.04*	0.610
2-hydroxybenzoate	5.63	3.10	0.05*	0.595
γ-hydroxybutyric acid	9.22	5.48	0.004**	0.818
D-malic acid	1.82	4.34	0.014**	0.751
L-serine	2.12	3.03	0.05*	0.585

The utilization of 25 other substrates in the Biolog Ecoplates responded, but not significantly, to the effect of N. According to Winding and Hendriksen (1997), the tetrazolium reduction detected in the Biolog assays is attributed to microbial activity and not to soil enzyme present at the time of incubation. They also reported increases in formazan formation with increases in incubation time as observed in this study (Table 3). Here 16 substrate wells were significantly utilized or oxidized with increase in time of incubation of the soils with NPK and maturity/ decomposition of the forest litter. Clearly more of the substrates were utilized much latter with incorporation of organic amendments suggesting that these nutrients were not accessible to the specific soil bacteria immediately after incorporation. However, two of these substrates, N-acetyl-D-glucosamine, and Serine, were rather enhanced with time, as indicated by significant effect of both NPK amendment and length of residence or incubation in the soil.

The functional diversity of soil bacteria in the cultivated and forested ecosystems were different as indicated by differences in the Shannon diversity index, H' of the bacterial communities in the different management systems (Fig.8), with the control plot giving the highest values for the twelve hourly incubation scan (Table 4). This can be explained by the observation of Kennedy and Gewin (1997) that microorganisms (e.g. actinomyces) responded better to stress agents such as streptomycin and penicillin, as well as to carbon sources like arginine, sorbitol and xylan, and the management system influence the response of these substrates. Also microbial isolates from the tilled system utilized maltose than the isolates from the praire system, equivalent to our tropical forest system, while simple sugars are more prevalent in tilled systems, complex substrates e.g. xylan, are more prevalent in undisturbed systems such as the forest. It becomes obvious that the cultivated soil (Control without fertilizer and NPK fertilized plots) which is more exposed to very simple substrates as

a result farming activities would experience greater microbial functionality compared to the forest plot.

Table 3: Effect of time of incubation on substrate utilization assay of a forest and cultivated soil

Substrate	Error MS	F-Cal	Sig. of F	Power at 0.05
N-acetyl-D-glucosamine	4.79	6.42	0.000***	0.965
Arginine	2.81	4.47	0.004**	0.878
γ-hydroxybutyric acid	3.81	3.46	0.016**	0.774
Cellobiose	7.13	7.19	0.000***	0.982
I-erythritol	1.60	2.68	0.047*	0.651
Glucose-I-phosphate	2.23	7.88	0.000***	0.990
β-methyl D-glucoside	2.38	18.79	0.000***	1.00
D- glucosaminic acid	2.03	5.49	0.001***	0.939
D-L-α-glycerol phosphate	3.46	3.58	0.014**	0.789
α-D-lactose	16.34	2.82	0.038*	0.680
Phenyl ethylamine	2.12	5.58	0.001***	0.942
L-phenylalanine	9.60	4.52	0.004**	0.882
Methyl pyruvate	3.97	2.69	0.046*	0.654
L-serine	2.12	2.96	0.032*	0.701
Tween 40	24.35	8.09	0.000***	0.991
Xylose	7.10	2.77	0.041*	0.668

CONCLUSION

It has been observed in this study that patterns of substrate utilization varied with soil management and these patterns indicate differences in type of microbe in the system. Therefore, a combined determination of the extent of microbial activity, taxonomic and functional diversity in agro-ecosystems would increase our knowledge of the function of microbes, for agro-ecosystem quality and productivity. Microbial functional diversity is a beneficial index to the agro-ecologist to enable the control and maintenance of the resilience of the community to stress. In this study, initially the three soil management conditions (forest, cultivation with NPK fertilizer, cultivation without fertilizer) showed a triaxiale substrate utilization pattern, but with time as probably more forest litter decomposed and fertilizer NPK decomposed, there was merging of the substrate utilization pattern of fertilizer NPK plot and the forest plot with a clear parallel pattern of nutrition, especially after twelve months, This suggests that the influence of soil bacteria on substrate utilization pattern of NPK fertilizer in cultivated and forest soils were clearly the same. Thus by isolating individual subsets of a community, and

monitoring nutritional pattern and performance under stress, we can establish repeatable predictions on agro-ecological responses from different management systems.

Table 4: Functional diversity of microorganisms at different incubation times under different land management in the month of August

Land management	Absorbance reading (every 12hrs)	Functional diversityH'
Control	0	1.49
Control	12	1.67
Control	24	2.08
Control	36	1.84
Control	48	1.62
Control	60	1.84
Control	72	2.17
Control	84	1.91
Control	96	1.96
Control	108	1.96
Control	120	1.71
Average		1.84
NPK fertilizer	0	1.78
NPK fertilizer	12	1.81
NPK fertilizer	24	1.70
NPK fertilizer	36	1.67
NPK fertilizer	48	1.70
NPK fertilizer	60	1.67
NPK fertilizer	72	1.60
NPK fertilizer	84	1.66
NPK fertilizer	96	1.63
NPK fertilizer	108	1.58
NPK fertilizer	120	1.63
Average		1.68
Forest	0	1.61
Forest	12	1.56
Forest	24	1.62
Forest	36	1.59
Forest	48	1.55
Forest	60	1.60
Forest	72	1.58
Forest	84	1.54
Forest	96	1.59
Forest	108	1.57
Forest	120	1.54
Average		1.58

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