International Scholars Journals

Advances in Agriculture and Agricultural Sciences ISSN 2381-3911 Vol. 3 (6), pp. 001-005, June, 2017. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Studies of physiochemical and microbial properties of soils from rainforest and plantation in Ondo state, Nigeria

Oseni, O. A.*, Ekperigin, M. M., Akindahunsi, A. A. and Oboh, G.

Department Of Biochemistry, Federal University of Technology, Akure, Ondo State, Nigeria.

Accepted25 November, 2016

Microbial population, and biomass as well as hydrolytic enzymes of soil that are responsible for biodegradation of various structural complexes of plant litters and dead organic compounds from Ondo State Aforestation project at Lisagbede (Oluwa forest reserve) were studied. Soil fungi and bacteria were isolated and identified from two sources namely *Gmelina aborea* plantation (planted for over 15 years) and an adjacent natural forest at different depth levels (0 - 10, 10 - 20 and 20 - 30 cm) by weighing 1.0 g of soil into 9.0 ml of sterilized water, shaken and diluted 1 in 1000, 0.5 ml each of this was poured onto separate sterilized Petri dish and overlaid with potato dextrose agar and nutrient agar, allowed to incubate at 27 and 32^{o}C for five and one day respectively. The results of the study revealed that all the parameters showed slight increase in natural forest soil as compared to the *G. aborea* plantation soil. Substantive differences were obtained in soil microbial biomass for each sampling depth in the natural forest compared to the *G. aborea* plantation. While there were no much differences in the soil physicochemical properties of the forest and plantation. Bacteria and fungi isolates of the two locations and their enzymes activities were similar. Two fungi *Aspegillus niger* and *Penicillium italicum* isolated from the two locations produced hydrolytic enzyme activities of cellulase, -amylase, - amylase and protease which are degrading enzymes.

Keywords: Natural forest, *Gmelina aborea* plantation, physicochemical parameters, enzyme activities, soil microbial biomass.

INTRODUCTION

Forest reserves are areas where no activities are taking place. Human intervention are excluded in order to guarantee that these ecosystems develop (Bachamn et al., 2002). The main interest in creating forest reserves has always been to develop strategies of nature oriented silviculture. Moreover, the forest reserves offer the possibility of studying the structure and function of ecosystems widely unaffected by anthropogenic influences (Bachamn et al., 2002). It is essential for the understanding of both natural forest ecosystems functioning and impacts of forest conversion to plantation, to study vegetation characteristics and soil microbial processes by enzyme activities (Bachamn et al., 2002; Piriyaprin et al., 2002). The soil microflora and the vegetation of an ecosystem are closely interrelated. Plants influence soil biotic processes by delivering organic compounds, whereas soil microbes have an impact on plant growth by the decomposition and mineralization of plant material (Pietikainen, 1999; Bachamn et al., 2002). Most tropical rainforest soils are relatively poor in nutrients (MBG, 2002). Millions of years of weathering and torrential rains have washed out most of soil nutrients (MBG, 2002). More recent volcanic soils, how-ever, can be very fertile (MBG, 2002). Tropical rain forest soils contain less organic matter than temperate forests and most of the available nutrients are found in living plant and animal material (MBG, 2002). Soil microbial biomass, a living part of soil organic matter, is an agent of transformation for added and native organic matter and acts as a labile reservoir for plant-available N, P, and S (Jenkinson and Ladd, 1981). The activity of the microbial biomass is commonly used to characterize the microbio-

*Corresponding author. E-mail: ooseni2003@yahoo.com.

Table 1. Physicochemical Properties of the natural forest and plantation soils at different soil depths

Locations/	Natural Forest			Gmelina Plantation		
Depths(cm)	0 – 10	10 – 20	0 – 10	0 – 10	10 – 20	20 – 30
pH	6.89±0.01	6.72±0.02	6.67±0.01	7.02±0.01	6.99±0.02	6.69±0.01
C org. (%)	1.19±0.03	1.19±0.03	0.76±0.10	2.72±0.08	1.61±0.10	1.30±0.08
N (%) Organic	0.13±0.02	0.03±0.01	0.03±0.01	0.14±0.01	0.08±0.02	0.07±0.01
Matter (%)	3.73±0.07	2.07±0.03	1.03±0.03	4.71±0.06	2.91±0.04	2.36±0.08
Phosphorus(%)	11.41±0.06	7.81±0.10	5.11±0.05	6.00±0.10	4.13±0.08	4.18±0.23
Moisture(%)	26.27±0.25	26.11±0.25	18.01±0.10	13.81±0.15	16.51±0.21	17.97±0.05
CEC(Cmol. Kg^{-1})	11.99±0.09	5.34±0.10	5.06±0.07	10.25±0.26	10.29±0.15	5.87±0.06
Clay (%)	21.67±0.29	23.67±0.29	26.00±0.50	24.00±0.50	30.00±0.20	27.00±0.00
Sand (%)	63.50±0.50	62.50±0.50	64.17±0.80	65.00±0.00	60.00±0.00	62.00±0.00
Silt (%)	14.67±0.29	13.83±0.29	9.83±0.29	11.00±0.00	11.00±0.00	11.00±0.00

logical status of a soil (Nanniperi et al., 1990), and to determine the effects of cultivation (Beyer et al., 1991; Anderson and Domsch, 1993), field management (Perott et al., 1992), or contamination (Chander and Brookes, 1993) on soil microorganisms. The aim of this work was to assess and compare the physicochemical parameters, microbial biomass and the hydrolytic enzyme activities of both natural forest and *G. aborea* plantation of Oluwa forest reserve.

MATERIALS AND METHODS

Soil sampling

The Ondo State Aforestation project at Lisagbede (usually called Oluwa forest reserve) is located in the humid tropical zone of South-western Nigeria. Oluwa forest reserve is in Odigbo local government area and is situated between latitude 6^{o} 55^{1} and 7^{o} 20^{1} N and longitude 3^{o} 45^{1} and 4^{o} 32^{1} E. Soil samples were collected from two locations at the reserve:- the *G. aborea* plantation - the adjacent natural rainforest

Soils were sampled at three depth levels (GM 0 - 10, 10 - 20 and 20 - 30cm and NF 0 – 10, 10 - 20 and 20 – 30 cm) respectively according to standard sampling techniques (FORMECU, 1997) where a 20 x 20 m and 25 x 25 m plots were mapped out in the *G. aborea* plantation and natural forest respectively. Soils were collected in three equal distance spots of the diagonal within *G. aborea* plantation and in four different spots of the natural forest with soil auger.

Soil analysis

Soil samples were sieved (2 mm) and stored at 4°C until needed while microbial isolations were done on freshly collected samples by weighing 1.0 g of soil into 9.0 ml of sterilized water, shaken and diluted 1 in 1000, 0.5 ml each of this was poured onto separate sterilized Petri dish and overlaid with potato dextrose agar and nutrient agar, allowed to incubate at 27 and 32^{o}C for five and one day, Kader et al. (1999), Wirth and Ulrich (2002) respectively. Sub samples for the determination of physicochemical parameters were air dried before analysis. Organic C and organic matter were determined as described by Walkley and Black (1934) wet oxidation method and, total N by Regular Macro- Kjeldahl method AOAC (1990). Other physicochemical analyses (pH, cation exchange capacity (CEC) and particle size) were determined according to standard methods (AOAC, 1990; Udo and Ogunwale, 1986 and Black et al., 1965) respectively.

Microbial biomass was determined by fumigation - extraction using ninhydrin-reactive N method of Amato and Ladd (1988) as modified by Joergenson and Brookes (1990) and Ocio and Brookes (1990) Cellulase activity was determined by its effect on cellulose with respect to glucose formation (Miller et al., 1960). Protease activity was assayed by the modified method of Ladd and Butler (1972) which involved determination of aromatic amino acids released during the hydrolysis of casein. The methods of Bernfeld (1951, 1955) were used for -amylase and -amylase which hydrolyze starch and maltose respectively to give reducing groups measured by reduction of 3, 5-dinitrosalicylic acid.(DNSA).

RESULTS AND DISCUSSION

The pH of soils from the two locations (pH range of 6.89 and 6.67 in the natural forest and 7.02 and 6.69 in the *Gmelina* plantation) could be described as slightly acidic to neutral, from all indications the pH decreased with soil depths in both natural forest and plantation as acidity was observed to increase with increasingly soil depth (Table 1), implying that subsoil is more acidic than topsoil. It has been reported by Han et al., (2002) that pH of lands with high tea production normally have low pH as the natural forest is slightly acidic than the aged plantation soil. The pH of similar soil depths in the two locations were not statistically different ($p > 0.05$) as presented in Table 1. The organic matter contents of the natural forest and aged *Gmelina* plantation did not show any significant difference though the values in the plantation are relatively higher than those of natural forest with a range of 4.71 ± 0.06 and 2.36 ± 0.08% for plantation and 3.73 ± 0.07 and 1.03 ± 0.03% for natural forest showing a decrease down the soil depth in each case. A similar trend was observed in the organic carbon and nitrogen contents of the soils, though Pansombat et al. (1997a) demonstrated that long term tea cultivation resulted in accumulation of organic C and total N. Phosphorus and cation exchange capacity of the natural forest soil are higher than in the plantation with decreasing values down

Table 2a. Fungi population of the natural forest and plantation soils at different depths.

Locations	No of Fungi isolated	No. of Fungi identified
Natural Forest		
0 – 10	3	*Aspergillus niger*, *Penicillium italliccum*
Gonatobotryx spp.		
10 – 20	2	*Aspergillus niger*, *Penicillium italliccum*
20 – 30	1	*Aspergillus niger*
***Gmelina* Plantation**		
0 – 10	2	*Aspergillus niger*, *Penicillium italliccum*
10 – 20	2	*Aspergillus niger*, *Penicillium italliccum*
20 – 30	2	*Aspergillus niger*, *Peacelomyces spp.*

Table 2b. Bacteria population of both natural forest and plantation soils at different depths

Location	Gram Reaction	Number of colonies	Number of isolated (cfu/ml)x10^3 bacteria	Bacteria count/gram soil (cfu/g) x10^5
Natural Forest				
0 – 10	+ve, -ve	2	260	2.60
10 – 20	+ve	1	120	1.20
20 – 30	ND	1	70	0.70
***Gmelina* Plantation**				
0 – 10	+ve	2	123	1.23
10 – 20	+ve, -ve	2	120	1.20
20 – 30	ND	1	59	0.59

the sampling depth for both soils. The particle sizes of the soils (sand, clay and silt) were similar across sampling locations with some differences at various depths as seen in Table 1. Both the Natural forest and aged *Gmelina* plantation show similar particle size characte-ristics of sand, clay and silt as shown in Table 1. The texture of the topsoil in both natural forest and plantation is sandy loam (FAO 1996) while subsoil consists of clay, these observations were similar to that reported by Onyekwelu et al. 2006. The physical, chemical and biological characteristics of a particular soil, as well as growing plants influence the number and activities of its various microbial components (Germida 1993; Uckan and Okur, 2004).

The fungi isolated from the two study locations were similar with the exceptions of *Gonatobotryx spp.* obtained from 0-10cm depths in the forest soil and the *Peacelomyces spp*. obtained from 20 -30 cm depth in the plantation soil. (Table 2a).

In the two study locations, the numbers of bacteria colonies were between 1 and 2 colonies of both Gram-positive and Gram-negative organisms. However, the natural forest soil at 0 – 10cm depth has the greatest number of both fungi and bacteria count in both cases (Tables 2a and 2b), this is supported by the observation of Piriyaprin et al., (2002) that the number of micro-organisms and microbial activities in the upper soil depth were higher than those of the lower. The various depths in forest and plantation soils however showed similar organisms and the isolated organisms were also found to possess amylase, protease and cellulase activities as seen from Table 4. This observation is however corro-borated with the work of Kader and Omar (1998) and Kader et al. (1999).

Microbial biomass C and N

There were significant changes in the microbial biomass C and N across sampling locations and various depths (Table 3). Microbial biomass C was the greatest (836.42 ± 8.44 to 169.75 ± 3.97 µgNg^{-1} Oven dry soil) in the forest

Table 3. Microbial biomass carbon and nitrogen of natural forest and plantation soils at different depths

Microbial biomass µgNg^{-1} Oven dry soil		Microbial biomass C µgNg^{-1} Oven dry soil	Microbial biomass N µgNg^{-1} Oven dry soil
Natural Forest			
0 – 10	26.45±0.80	836.42±8.44	127.29±4.80
10 – 20	21.34±0.78	666.90±5.55	98.10±0.47
20 – 30	5.33±0.33	169.75±3.97	24.39±5.44
***Gmelina* Plantation**			
0 – 10	2.25±0.05	68.37±0.37	10.23±0.27
10 – 20	0.72±0.03	22.03±0.08	3.23±0.10
20 – 30	0.25±0.02	7.56±0.10	1.11±0.10

Table 4. Some hydrolytic activities of *Aspergillua niger* and *Penicillium italicum* isolated from natural forest and *Gmellina* plantation.

Enzyme activities				
Fungi	CMCase µmoleGlucose/min/ml	-Amylase µmoleMaltose/min/ml	-Amylase	Protease µmoleTyrosin/min/ml
Natural Forest				
Natural Forest *Aspergillus niger*	0.46	2.00	2.44	1.0x10^{-2}
Penicillium itallicum	0.61	1.89	2.26	6.0x10^{-3}
Gmelina Plantation *Aspergillus niger*	0.40	1.95	2.37	1.0x10^{-2}
Penicillium itallicum	0.30	1.89	2.27	6.0x10^{-3}

Note: CMCase is 'cellulase activities using carboxyl methylcellulose as substrates.

soil compared to the *Gmelina* plantation soils 68.37 ± 0.37 to 7.55 ± 0.10 µgNg^{-1} Oven dry soil for the soil depths (0 – 10, 10 - 20 and 20 – 30)cm respectively. This observation were similar to the work of Han et al. (2007) who reported that microbial biomass C in various tea stands declined as compared to forest. There was a significant difference in the soil microbial biomass of the two locations, (Table 3) though they show similar trends in depths. The natural forest ranged from 26.45 ± 0.80 to 5.33 ± 0.33 µgNg^{-1} oven dried soil while the plantation ranged from 2.25 ± 0.05 to 0.25 ± 0.02 µgNg^{-1} oven dried soil. Measurements of soil microbial biomass have been used in studies of the flow of carbon, cycling of nutrients and plant productivity in a variety of terrestrial ecosystems (Voroney et al., 1993)

Enzyme activities

The number of hydrolytic enzyme - producing fungi isolated and identified from the various locations are shown in Table 2a. Two of them *A. niger* and *Penicillium italicum* were investigated for their cellulolytic, amylolytic and proteolytic activities. Hydrolytic enzymes are produced by a wide range of fungi and bacteria, which are either cell-bound or extra cellular (Kader and Omar, 1998)

Table 4 shows the cellulase, alpha- and beta-amylases and the protease activities of *A. niger* and *P. italicum*. A knowledge of the spectrum of enzymatic activities of a soil is important since it will indicate the potential of the soil to permit the basic biochemical processes necessary for maintaining soil fertility. Slight differences in the enzyme activities of the two organisms were observed. There was no significant difference between the cellulase activity of the two organisms in both the natural forest and *Gmelina* plantation .This work agreed with that of Kader et al., 1999 who reported CMCase of 0.58IU/ml for *Aspergillus* species isolated from Bario Highlands soil. There is also no significant differences in the amylase activities of the two organisms from both locations. Protease activities of *A. niger* and *P. itallicum* from natural forest and plantation soils were found to be similar, though the low values might have been due to lack of nitrogen enrichment of the medium. The results of the enzyme activities in this work is consistent with the observations of Bardick and Dick (1999) that various soils contain enzyme activities but differ based on the respective crops and are related to microbial biomass, therefore changes in enzyme and microbial activities

could alter the availability of nutrients for plant uptake (Dick et al., 1988a,b) and these changes are potential sensitive indicators of soil quality (Dick, 1994).

Conclusion

Though the *G. aborea* plantation was aged, there was still significant reduction in the parameters studied relative to the forest without any human disturbance; this is in agreement with Ajwa et al. (1999) that human activities have diverse effects on organic matter turnover and therefore, affect microbial biomass and the enzyme production, because many soil enzymes and microbial biomass nitrogen (MBN) are immediately responsive to disturbance or restoration.

From the results of the work, it can be concluded that the pH of both soil samples were slightly acidic, microorganisms and microbial activities in both natural forest and plantation in the upper soil depth of 0 -10 cm were higher than those of the lower soil depths of 10 - 20 and 20 – 30 cm. The two species of fungi isolated produced hydrolytic enzyme activities.

ACKNOWLEDGEMENT

We wish to acknowledge and appreciate the African Forest Research Network (AFORNET) for the grant to execute this research work.

REFERENCE

Ajwa HA, Dell CJ, Rice CW (1999). Changes in enzyme activities and Microbial biomass of tall grass prairie soil as related to burning and nitrogen fertilization. Soil Boil. Biochem. 31: 769-777.

Amato M, Ladd JN (1988) . Assay for microbial bioman based on ninhydrin reactivenitrogen in extraction of funmigation soils. Soil Boil. Biochem. 70: 107-114.

Anderson TH, Domsch KH (1993). The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. Soil Biol. Biochem. 25: 393-395

ASA Monograph (1982). Methods of Soil analysis, Part 2 No. 9.

Bardick AK, Dick RP (1999). Field Management effects on soil enzyme activities. Soil Sci. Biochem. 31: 1471-1479

Bachman G, Zechmeister – Boltenstenm B, Pfefer M, Hack E (2002). Microbial Nutrient Turnover in soils of Natural forest communities htpp//chemisrvo.pph.univie.ac.at. leha/biogeoch.litm.

Beyer L, Blume HP, Peters M, (1991). Biological activity and organic matter transformations in typical soils of Schleswig-Holstein. Geoderma 49: 273-284.

Bernfeld P (1951). Enzymes of Starch Degradation and Synthesis. Advances in Enzymol., 12: 379.

Bernfeld P (1955). Amylases and . Methods in Enzymology, I, (Colowick SP, Kaplan NO eds.) Academic Press Inc., New York p. 149.

Chander K, Brookes PC (1993). Residual effects of zinc, copper and nickel in sewage sludge on microbial biomass in a sandy loam. Soil Biol. and Biochem. 25:1231-1239.

Dick RP, Myrold DD, Kerle EA (1988a). Microbial Biomass and soil enzyme activities in compacted and rehabilitated skin trail soils. Soil Sci. Soc. Am. J. 52: 512-516.

Dick RP, Rasmussen PE, Kerle,EA, (1988b). Influence of long term residue management on soil enzyme activity in relation to soil chemical properties of a wheat-fallow system. Biol. Fertility Soils 6: 159-164.

Dick RP (1994). Soil enzyme activeities as indicators of soil quality. In: Doran JW, Colman DC, Bexdicek DF, Stewart BA (Eds.), Defining Soil Quality for asustainable Environment. American Society of Agronomy , Madison, WI, pp. 107-124.

FAO (1996). Food and Agricultural Organization publication Soil bulletin No 63.

Germida JJ, (1993). Cultural method for soil microorganism soil sampling and methods of analysis M.R carter Ed Canadian Society of Soil Science. Lewis Publication, p. 263.

Han WY, Ruan JY, Lin Z, (2002). The major nutrient limiting factors in tea soils and development of series tea speciality fertilizers. J. Tea Sci. 22: 70-74.

Han WY, Kemmitt SJ, Brookes PC (2007). Soil microbial biomass and activity in Chinese tea gardens of varying stand age and productivity. Soil Biol. Biochem 39: 1468-1478.

Jenkinson DS, Ladd JN, (1981) Microbial biomass in soil measurement and turnover. In Soil Biochemistry (E.A. Paul and J.N. Ladd, eds.) 5: 415-471. Marcell Decker, New York.

Joergensen RG, Brokes PC (1990) Ninhydrin-reactive nitrogen measurement of microbial biomass in 0.5M K_2SO_4 soil extracts. Soil Biol. Biochem. 22: 1023-1027.

Kader AJ, Omar O. (1998) Isolation of cellulytic fungi from Sayep. Kinabalu park. Sabah. ASEAN Review of Biochemistry and Environmental Conservation (ARBEC) Article II.

Kader AJ, Omar O, Feng LS, (1999) Isolation of cellulytic fungi from Bario Hyghlands, Sarawak. ASEAN Review of Biochemistry and Environmental Conservation (ARBEC) Article.

Ladd J N, Butler, JHA, (1972) Short-term assay of soil proteolytic enzyme activities using proteins and dipeptide substrates. Soil Biol. Biochem. 4:19-39

MBG (2002) http//www.mbgnet.net.bioplant.Missouri Botanical Garden.

Miller GL, Blum R, Glennon WE, Burton AL (1960) Measurement of carboxymethyl cellulose activity. Anal. Biochem. 2: 127-132.

Nannipieri P, Grego S, Ceccanti B, (1990) Ecological significance of the biological activity in soil. Soil Biochem 6:293-355

Ocio JA, Brookes PC, (1990) An evaluation of methods for measuring the microbial biomass in soils following resent addition of wheat straw and characterization of the biomass that develops. Soil Biol. and Biochem. 22: 685-694.

Onyekwelu JC, Mosandl R, Stimm B (2006) Productivity, site evaluation and state of nutrition of *Gmelina arborea* plantation in Oluwa and Omo forest reserves, Nigeria. J. For. Ecol. Manage. 229: 214-227.

Pansombat K, Kanazawa S, Horinguchi T, (1997a) Microbial ecology in tea soils. 1. Soil properties and microbial populations. Soil Sci. Plant Nutr. 43: 431-438.

Perrott KW, Sarathchandra SU, Dow BW (1992) Seasonal and fertilizer effects on the organic cycle and microbial biomass in a hill country soil under pasture. Aust. J. Soil Res. 30:383-394.

Pietikainen J (1999) Soil microbes in boreal forest humus after five. Finish forest Research Institute. Research Paper, 750(50): 78.

Piriyaprin S, Sunanthapongsout V, Limlong P, Leavngv-utiviroj C, Pasda, N (2002) Study on soil microbial biodiversity in rhizophore of vetiver grass in degrading soil. 17th WCSS, 14 – 21 August 2002. Thailand htpp//www.sfts.org/proceeds/17wcss find/papers//1896.pdf.

Uckan HS Okur N. (2004): Seasonal changes in soil microbial biomass and Enzyme activity in Arable and Grassland soil Bildiri Ozetleri (Anas Sayfaya Donus ISD Ana Sayfasi. Finnish Environment Institute Laboratory 00250 Helsinki.

Voromey RP Winter JP, Beyast RP (1993) Cultural method for soil Microorganism soil sampling and methods of analysis M.R carter Ed Canadian Society of Soil Science. Lewis Publication, p. 263.

Walkley, A, Black, IB (1934) An examination of the Degtjareff method of determining soil organic matter a modification of the chromic acid titration method. Soil Sci. 37: 29-38.