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SOIL MICROBIAL ACTIVITY AS BIO-INDICATORS DURING THE DECOMPOSITION OF GRASS RESIDUES IN SANDY CLAY LOAM SOIL

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Abstract- Assessing the quality of the soil is an important method in determining the sustainability and environmental impact of agricultural ecosystems. Soil microbial indices like microbial biomass and microbial activity are important criteria for the determination of soil quality. Laboratory incubation study was undertaken to examine the influence of 2 grass residues on changes in soil microbial indices {(microbial (Cmic: Corg), metabolic (qCO2), carbon mineralization (qM) and microbial biomass change rate (qC) quotients)} in a sandy clay loam soil. Both *P.maximum* and *D.horizontalis* amended soil showed maximum soil respiration rate (11.90 mgCO_2-C g^{-1} soil) while *P.maximum* had maximum cumulative respiration rate (199.90 mgCO_2-C g^{-1} soil) .Microbial biomass C was significantly improved by both *P.maximum* and *D.horizontalis* residues under study (158.08 and 162 mg/g respetively). The qCO_2 among different grass residues ranged from 1.23 to 1.24µg CO_2-C µg-biomass-C^{-1} on the 82nd day. The results showed that incorporation of both grass residues impacted a positive effect on microbial flora and their activity. The study suggests that the despite the high C:N ratio of grasses, its seems to be of advantage in the maintenance of soil quality in a sandy clay loam soil.

Keywords: grass residues, Microbial biomass, Microbial indices, Soil quality, qCO_2, eco-physiological quotients.

I. INTRODUCTION

Soil organic matter (SOM) plays a key role in determining soil health, which is defined as the soil's capacity to maintain environmental functions and biological productivity [6] with soil and crop management practices involving cropping system, residue management and fertilization exerting a considerable influence on the levelf organic matter over time [8]. In bid to reducing organic matter loss incorporation of plants residue has been employed as its decomposition leads to an increases the supply of organic compounds rich in carbon and nitrogen serving as energy sources for the living microorganisms and the dead microbial biomass providing the substrate. Thus, leading to a series of biological transformations. Singh et al 2004 explained that decomposition is a biologically driven process that is determined by nutrient availability, soil microorganisms, physical environment, crop residue quality and the combination of this factors determine the determine the rate of plant residue decomposition facilitating the release of nutrients as evidenced by increased release of CO_2 leaving the soil through respiration.

Soil microbial biomass (SMB) comprises only a small percentage of the total mass of SOM, these microorganisms play a critical role in the decomposition of plant and animal residues and the associated release of plant-available nutrients (Gonzalez-Quiñones *et al*., 2011) playing a key role in controlling the nutrient cycling and energy flow in soil ecosystems [10].Soil respiration rate, C_{mic}:C_{org} and qCO_2 quotients directly reflects soil microbial activities, and are affected by the eco-environmental changes [1]. The Cmic:Corg quotients calculated as ratio of microbial biomass carbon (MBC) to soil organic carbon (SOC) is mainly used to measure soil C lost during decomposition as well as the efficiency of organic C conversion into microbial C. It is a qualitative soil parameter that allows comparison across soils with different organic matter content [16, 23]. qCO_2, the rate of basal respiration per unit of MBC is a very valuable way to measure the relative efficiency of the soil microbial biomass in utilizing C resources and the degree of substrate limitation for soil microorganisms [23,12] while it also expresses the metabolic effectiveness of soil microbial communities [15] . The qM expresses the fraction of total organic carbon mineralized during incubation period [14] and the qC expresses the daily enrichment or loss of soil microbial C [5].

A better understanding of soil microbial indices and the mineralization of plant residues added to the soil is of high importance in addressing the soil management practices for proper soil quality maintenance. Therefore, soil microbial indices is of importance as it provides necessary, immediate and accurate information on changes occurring within soil

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during decomposition of plant residues. The objective of the present research was to evaluate the changes in soil microbial indices that have been amended with plant residues of high C/N ratio in Sandy clay soils.

II. MATERIALS AND METHOD

Experimental location and soil properties

This study was carried out in the laboratory of Crop, soil and pest management of the Federal University of Technology, Akure. The soil used in this study was collected from the Teaching and research farm of the Federal University of Technology, Akure. The initial physiochemical properties are summarized in Table 1.

Experiment treatments and Experimental design

Treatments consisted of control and 2 grass residue of *Panicum maximum* (Guinea grass) and *Digitaria horizontalis* (Crab grass) in 3 replications. The grass residues were air dried and crushed into powder. Then, 500mg of the grounded plant residues was added to 50g of plastic jars. The soils were air dried and adjusted to 60% WHC prior to the addition of the grounded plant residues. This Plastic jar was well shaken to allow proper mixture of the plant residue and the soil. The control consisted of soil without the straw powder. 20 mL solution of 0.5M NaOH was dispensed into a 50mL beaker and placed inside the glass jars containing the treated soil to trap CO_2 evolved from the soil. The Jars were arranged in a completely Randomized design.

Table 1. Initial physiochemical properties of the soil

pH (2:1 H_2O)	5.16
C (%)	1.07
N (%)	0.16
P m/kg	5.36
Ca (col/kg)	1.60
Mg (col/kg)	0.60
Na (%)	0.15
K (%)	0.11
Sand (%)	54.80
Silt (%)	20.00
Clay (%)	25.20
Soil classification	Sandy clay loam
Bulk density	1.65

Soil respiration Measurements

The amount of CO_2–C released from each jar was determined by titrating the residual NaOH solution with 0.5M HCl in the presence of excess $BaCl_2$ to a phenolphthalein end point. The titration was carried out 1st, 3rd, 5th and 7th day after the mixing the grounded soil while subsequent titrations were carried every 14 days till the 84th day. The release rate of CO_2–C for different treatments was calculated as the function of incubation duration in days. Jars without soil but with a vial of NaOH solution were also included as blanks during the incubation, period. The soil respiration rate was calculated as the difference in the amount of HCl consumed.

Determination of Physico-Chemical Properties of Soil

Soil texture, pH, Organic matter and soil nutrient status of the air dried soil sample were determined following standard methods of Association of Official Analytical Chemists [6]. The soil samples were analysed for total N using Kjeldahl digestion and distillation method. Available phosphorus was by the Bray 1 method, exchangeable K, Ca and Mg were determined by extraction with 1M ammonium acetate at pH 7.0. K, Ca and Mg contents were determined with flame photometer. Soil pH (1:2 soil-water) was determined by pH meter, while organic matter (OM) was determined by dichromate oxidation method.

Plant analysis

Prior to incorporation leaf samples of *Panicum maximum* and *Digitaria horizontalis* were collected randomly, oven-dried for 24 h at 80°C and ground in a Willey mill. The samples were analyzed for leaf N, P, K, Ca and Mg as described by Tel and Hagarty (1984). Leaf N was determined by the micro-Kjeldahl digestion method. Ground samples were digested with nitric-perchloric-sulphuric acid mixture for determination of P, K, Ca and Mg. Phosphorus was determined colorimetrically using the vanadomolybdate method, K was determined using a flame photometer and Ca and Mg were determined by the EDTA titration method. The percentage of organic carbon (OC) in the green manure leaves was determined by the Walkley and Black procedure using the dichromate wet oxidation method.

Determination of Soil Microbial Biomass Carbon (Cmic)

Soil microbial biomass C (Cmic) was determined by the fumigation and extraction method described by Vance *et al.* (1987) [22]. 10g of unfumigated soil was extracted with 50 mL of 0.5 M K_2SO_4 by shaking for 45 min with a rotary shaker at 180 rpm, and the suspension filtered using a Whatman No. 2 filter paper. A separate portion was fumigated by placing it in a 50-mL beaker inside a desiccator alongside with another beaker containing ethanol-free chloroform. The desiccator was covered and evacuated with a vacuum pump until the chloroform boiled vigorously for 5 min. The evacuation was repeated three times at intervals of 15 min, letting air pass back into the desiccator to facilitate the distribution of the chloroform throughout the soil. The desiccator was evacuated a fourth time until the chloroform

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boils vigorously for 2 min. 24 hours later, the $CHCl_3$ was removed by vacuum extraction and the fumigated sample was extracted as above. Organic carbon in the extract was determined by the wet combustion method of Walkley and Black. MBC was calculated by the differences between the fumigated and non-fumigated samples divided by the K_2SO_4 extract efficiency factor (Kc = 0.35) [19] for MBC.

Determination of Eco-physiological indices (qCO_2, qM and qD / qC, and C_{mic}:C_{org})

qCO_2 (the community respiration per biomass unit or the metabolic quotient) was measure every 14 days while the qM mineralization quotient), and C_{mic}:C_{org} were measured at the end of incubation. qCO_2 was determined as the ratio of cumulative CO_2-C (µg CO_2-C g-1 soil) to the soil microbial biomass carbon, while qM was determined as the ratio of CO_2-C (µg CO_2-C g^{-1} soil) to the soil organic carbon (mg g^{-1} soil). Estimation of Cmic:Corg was based on the ratio of soil microbial biomass carbon to the total organic carbon.

Determination of the microbial biomass change rate quotient (qC) which expresses the daily enrichment or loss of soil microbial C was calculated based on qD (death rate) as reported by Anderson and Domsch [3]. In this study, the biomass C (Cmic) measured at 14 days after treatment was used as the initial microbial biomass of the soil. The incubating soils were maintained at room temperature in the dark and Cmic was recorded at each time of sampling. The data were based on arithmetic means of four replicates soil samples. The C-loss quotient was calculated based on total microbial-C-loss after the number of days of incubation before sample collection using the following equation [3]:

$qD = [(Cmic)t1–(Cmic)t_2/(Cmic)t_1]/ t_2–t_1$.

In this study, the qC was preferred to the qD of Anderson and Domsch [3] because both C-loss and C-enrichment were encountered in the effects of the treatment applied.

Data Analysis

Data collected from the experiment were subjected to an analysis of variance while treatment means were compared using the Tukey test at 5% level of probability. Graphs were designed using Minitab 19 statistical software package.

Results

The chemical compositions of the different crop residues studied are summarized in table 2.The C content ranged from 468.4mg/kg (*D. horizontalis)* to 458.65mg/kg (*P. maximum*), and the N contents ranged from 17.73mg/kg (*D. horizontalis*) to 20.44mg/kg (*P. maximum*), resulting in C/N ratios ranging from 26.41(*D. horizontalis*) to 22.44 (*P. maximum*).

Table 2: chemical constituent of grass residue

Grass Residue	C (mg/kg)	N (mg/kg)	C:N	P (%)	K (%)	Mg (%)	Ca (%)	Na (%)
DH	468.40	17.73	26.41	0.41	2.31	0.10	0.64	1.85
PM	458.65	20.44	22.44	0.51	2.30	0.97	1.93	3.19

Results of grass residue on soil respiration (table 3) showed that residue treatments of *Panicum maximum* and *Digitaria horizontalis* significantly improved the CO_2 from the 3rd day (not displayed) till the 84th day at the termination of the experiment. Peak CO_2 efflux was recorded on the 14th day after treatments and a drop in CO_2 was recorded every time of sampling till the termination on the 84th day this could be as a result of the depletion of easily available water-soluble compounds and all that are left within soil are the not easily decomposed components. This was consistent with previous studies of Trinsoutrot *et al.* [21], Wang *et al*. [24] high respiration rates in the first few days after residue addition to easily decomposable and readily available water-soluble compounds in the residues, to water-soluble compounds in the residues, thus enhancing the microbial activity. This was further explained by studies carried out by Cogle *et al.* [11]; Wang *et al.* [24] and Stemmer *et al.* [20] explaining the two phases involved in plant residue decomposition; The first phase is characterized by a more rapid reaction, this is due to the degradation of water-soluble compounds such as amino acids, amino sugars and carbohydrates. In the second stage which tends to be slower, structural and recalcitrant components such as lignin, cellulose is decomposed. When residues are added, respiration rates are initially high due to the decomposition of easily available compounds. Thereafter,

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respiration rates decrease as easily available compounds are depleted. No significant difference was recorded in the CO_2 efflux but CO_2 efflux production was figuratively higher in *Panicum maximum* in all the days of sampling.

Cumulative respiration (table 4) was significantly improved by the residue treatments in comparison to the control. Amongst both grass residue treatments, *Panicum maximum* significantly increased total CO_2 efflux till the end of the experiment. The cumulative CO_2 production indicated an elevation in respiration across all treatments, this is most likely due to the stimulation of the soil microbial activity by the greater oxygen availability attributable to physical mixing of the soil and residue amendments facilitating the release of more C and N during decomposition serving as substrates.

Table 3. Effects of grass residues on soil respiration (mg CO_2-C g^{-1})

Treatments	Days after Treatments						
	7	14	28	42	56	70	84
CONTROL	5.90b	5.30b	3.90b	4.80b	5.10b	4.20b	3.70b
DH	29.10a	33.00a	21.60a	21.00a	19.20a	18.00a	11.90a
PM	30.00a	33.00a	21.90a	21.00a	19.50a	18.60a	11.90a

Means that do not share a letter are significantly different

PM- *Panicum maximum* DH-*Digitaria horizontalis*

Table 4. Effects of grass residues on soil cumulative respiration (mg CO_2-C g^{-1})

Treatments	Days after Treatment						
	7	14	28	42	56	70	84
CONTROL	29.60b	34.90b	38.80c	43.60c	48.70c	52.90c	56.60c
DH	71.70a	104.70a	126.30b	147.30b	166.50b	184.50b	196.40b
PM	74.00a	107.00a	128.90a	149.90a	169.40a	188.0a	199.90a

Means that do not share a letter are significantly different.

PM- *Panicum* maximum, DH-*Digitaria horizontalis*

Effects of grass residues on Microbial biomass carbon

The presence of *Panicum maximum* and *Digitaria horizontalis* residues within the soil significantly raised the soil microbial biomass C from the 14th day after application (Table 5). This trend was continuous till the end of the experiment. Although there was no significant amongst both residue treatments, but addition of *Panicum maximum residues* recorded the higher microbial biomass C from the 14th day to the termination of the experiment compared to *Digiitaria horizontalis residue* treatments. While C_{mic} declined as incubation progressed from the 28th day to the termination of the experiment in the control the same cannot be said for soils under residue treatments of *Panicum maximum* and *Digitaria horizontalis*. The significant increase in soil microbial biomass can be linked to the addition of organic matter to the soil as result of decomposition of these incorporated residues. This was corroborated by Ye *at al* [27] who stated that residue amendments can provide adequate organic nutrition and promote the large reproduction of soil microorganisms based on their findings on green manure stimulating higher Microbial biomass under tobacco cultivation. Higher microbial biomass figures recorded under residue treatments of *Panicum maximum* can be as a result of

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faster release of nutrients that guarantees a more sustainable bio-stimulation of native microbial community.

Table 5. *Effect of grass residues on microbial biomass C*

Treatment	Days after Treatment					
	14	28	42	56	70	84
Control	74.48b	83.60b	60.80a	51.68b	41.04b	41.04b
DH	141.36a	167.20a	144.40a	135.28a	162.64a	158.08a
PM	144.40a	170.24a	148.96a	139.80a	167.20a	162.64a

Means that do not share a letter are significantly different.

PM- *Panicum maximum* DH-*Digitaria horizontalis*

Decomposition of grass residues on Eco-physiological quotients

qCO_2 was significantly higher in treatments of both grass residues compared to the control, this significantly higher vales continued till the 8th week after treatments where qCO_2 experienced a significant drop compared to the control. Although, there was no significant difference in qCO_2 of soils treated with residues of *Digitaria horizontalis* and *Panicum maximum* but *Digitaria horizontalis* had higher qCO_2. The rise in the qCO_2 shows that the indigenous microbial population used up more energy in the decomposition of the grass residues. This could be due to the high C:N ratio, changes in pH and most probably the quantity of recalcitrant materials like lignin within the tissues of both residues and also the soil. According to the concluding remarks of Anderson and Domsch, [2] a rise in qCO_2 might mean more C is lost in form of CO_2 but it shows high microbial activity and can be interpreted as a positive property. However, a high qCO_2 is a clear indication of high maintenance carbon demand, and if the carbon lost through respiration cannot be replenish within the soil system microbial biomass must decline. Thus, the decline in qCO_2 in the 70th and 82nd day experienced in all the treatments compared to the control showed that a balance might have been reached and stability has being attained. This result was in correlation with Balota and Chaves [7] who suggest that the qCO_2 decreases in more stable systems when explaining the decrease of qCO_2 in the soil under *L. leucocephala* and *Arachis hypogea* treatments and linking the variations in qCO_2 to differences in the accessibility of C substrates to the microorganisms, changes in the microbial metabolic rates and changes in the microbial community composition and physiological standards. Anderson (2003) explained that pH influences rate of qCO_2, as micro-organisms undergo an initial pH stress which is characterized by a high initial qCO_2 and immediate biomass loss till the survivors of the microbial community becomes adapted to the new established soil pH, then a continuous decrease of qCO_2 will be experienced.

For the qD, there was C enrichment recorded in all treatments including the control on the 28th day (table 7). This was followed by significantly loss of carbon as denoted by negative values in the 42nd day and 52nd day after treatment and the percentage C loss relative to the non-amended soil was in the order 65% > 54% for *Panicum maximum* and *Digitaria horizontalis* , respectively . At the termination of the experiment, although no C loss or enrichment was recorded in the control it wasn't statistically different from the loss experienced in both residue treatments.

Residue decomposition of both *Panicum maximum* and *Digitaria horizontalis* stimulated a significant increase in the C_{mic}:C_{org} of the soil compared to the control. The increase in this parameter recorded in all treatments is an indication that the microbes were able to immobilize more C for biomass production under the influence of these treatments. Changes in the C_{mic}:C_{org} relationship may be related to organic matter formation and the efficiency of conversion of the recalcitrant C pools into MBC [17].

The qM, or the potential C mineralization activity that was measured under controlled conditions of temperature and humidity, didn't show any significant change amongst the treatment but compared to untreated soil caused a significant

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change. This Indicates that a larger substrate availability to the soil microorganisms and a positive trend for organic C accumulation. The decaying litter constitutes the site of an active microbial pool because of the availability of eco-physiological maintenance carbon that serves as their energy source [5].

Table 6. *Effect of grass residues on qCO_2*

TREATMENT	Days After Treatments					
	14	28	46	52	70	84
CONTROL	0.47b	0.47a	0.72b	0.95b	1.29a	1.38a
DH	0.74a	0.76a	1.02a	1.24a	1.13b	1.24b
PM	0.741a	0.76a	1.01a	1.23a	1.14b	1.23b

Means that do not share a letter are significantly different.

PM- *Panicum maximum*, DH-*Digitaria horizontalis*

Table 7. Effect of grass residues on $C_{mic:}C_{org}$, qM

Treatments	C_{mic}:C_{org}	qM
CONTROL	0.38b	0.53b
DH	1.27a	1.58a
PM	1.29a	1.59a

Means that do not share a letter are significantly different.

PM- *Panicum maximum* DH-*Digitaria horizontalis*

Table 8: Effect grass residue on qD (x 10^{-3})

Treatments	Days after Treatment				
	28	42	56	70	84
CONTROL	7.80a	-26.82b	-12.63b	-18.52b	0.00a
DH	11.03a	-11.3a	-4.84a	12.02a	-2.08a
PM	10.79a	-10.19a	-4.69a	12.37a	-1.32a

Means that do not share a letter are significantly different.

PM- *Panicum maximum* DH-*Digitaria horizontalis*

III. CONCLUSION

In conclusion, the microbial index of the soil proved to be sensitive to changes occurring in soil processes as a result of adding grass residues .On observation, an overall increase in microbial biomass and their activity was recorded in soil amended plant residues as compared to unamended control. In addition, results obtained under laboratory conditions suggest that the decomposition of plant residues can be fairly predicted. Thus, addition of plant residue as a source of organic matter to the soil can help maintain the ecological balance of soil microbes and may also help in enhancing the biological status of soil. The Microbial (Cmic: Corg), metabolic (qCO_2), carbon mineralization (qM) and microbial biomass change rate (qC) quotients, all seem to be suitable diagnostic indicator of the quality of the soil.

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