

## CHARACTERIZATION OF ORGANIC CARBON FRACTIONS IN A SOUTH-WESTERN NIGERIAN SOIL: AN ACID HYDROLYSIS APPROACH.

S. J. Akinsete<sup>a\*</sup>, O. F. Adebayo<sup>a</sup> and J. I. Uponi<sup>b</sup>

<sup>a</sup> *Department of Soil Science and Land Management, College of Plant Science and Crop Production, Federal Univ. of Agriculture, Abeokuta. P.M.B. 2240, Abeokuta, Ogun State, Nigeria*

<sup>b</sup> *Analytical Services Laboratory, International Institute of Tropical Agriculture, P.M.B. 5320, Ibadan, Oyo State, Nigeria*

\*Corresponding author's email Address: [sjohnadisa@yahoo.com](mailto:sjohnadisa@yahoo.com); Tel: +234813453 9776;

### ABSTRACT

Soil organic carbon characterization using acid hydrolysis is commonly performed at high temperatures but rarely at room temperature. This study used acid hydrolysis at 95 °C and 25 °C to quantify recalcitrant (non-hydrolyzable carbon, NHC) and labile C fractions of three depths in a planted fallow (*Mucuna jaspidea*) soil. Across depths, SOC fractions were: total SOC (11.83 – 21.57 g kg<sup>-1</sup>) > NHC 25 °C (8.43 – 16.51 g kg<sup>-1</sup>) > NHC 95°C (5.09 – 11.41 g kg<sup>-1</sup>) ≥ labile C (5.03 – 10.16 g kg<sup>-1</sup>). Recalcitrant C fraction isolated at room temperature (NHC 25°C) was not significantly different ( $P = 0.05$ ) from total SOC concentrations across the soil depths, and contributed to a large amount of total SOC (71 – 92%), indicating incomplete hydrolysis of the labile C fraction. Conversely, recalcitrant C fraction isolated at 95 °C (NHC 95°C) contributed about 43 – 66 % to the total SOC and was significantly lower ( $P = 0.05$ ) than total SOC, suggesting a meaningful separation between the recalcitrant and labile C fractions. The labile C fractions accounted for 34 – 57 % of total SOC and these amounts were generally similar but not significantly different from those of the NHC 95°C. The recalcitrant C fractions (NHC 25°C and NHC 95°C) were significantly correlated with total SOC ( $R^2 = 0.88$  and  $0.87$  respectively) and with each other ( $R^2 = 0.97$ ) across the soil depths, but labile C was weakly correlated ( $R^2 = 0.38$ ;  $0.20$ ) with total SOC and NHC 25°C. The stratification ratio (concentration at the soil surface/concentration at a lower depth) of SOC fractions were below the threshold value of 2 and was 1.0 to 1.7 for SOC, 1.1 to 1.8 for NHC, and 0.7 to 1.7 for labile C, suggesting a disturbed or degraded condition. The information provided from this study showed that acid hydrolysis at room temperature (25°C) cannot be used as an alternative to hydrolysis performed at 95°C.

**Keywords:** labile, recalcitrant, organic carbon, stratification ratio.

### INTRODUCTION

Globally, soils remain the largest terrestrial store of organic carbon. Consequently, total soil organic carbon (SOC) pool is 3.3 times larger than the atmospheric pool and 4.5 times

the biotic pool (Lal, 2004). Soil C sequestration has been studied in several soils (Hernandez-Ramirez *et al.*, 2009), however, more research is needed to better characterize soil organic

carbon (SOC) fractions and their potential for sequestration in the South Western, Nigeria.

SOC is an important ecosystem property, controlling other soil properties which are important to long-term productivity, ecological stability, soil quality, and long-term C storage (Franzluebbers, 2002). An increase in SOC is a desirable aim as it is associated with better plant nutrition and better soil physical properties (Moreno *et al.*, 2006), however, this results mostly in the enrichment of the soil surface. The vertical distribution and accumulation of not only total SOC but its fractions within the profile is essential for long-term C storage since the soil surface is particularly prone to disturbance and eventual deterioration. To better understand C vertical distribution, C status and potential to sequester (Gerzabek *et al.*, 2002) in the soils of this study, the stratification ratio concept was employed. Franzluebbers (2002), defined stratification ratio as a soil property at the soil surface divided by the same soil property at a lower depth, such as the bottom of the tillage layer. A stratification ratio threshold value of 2 distinguishes degraded soil with improved soil conditions from degraded soils (Franzluebbers, 2002).

Soil OC contains complex and heterogeneous compounds derived from different organic materials that can be compartmentalized into a labile fraction with a small size and a recalcitrant fraction with a large size using acid hydrolysis (Belay-Tedla *et al.*, 2009). Long term carbon storage, is often determined by the long-lived recalcitrant fraction (Trumbore *et al.*, 1990), sequestered in soil for several thousands of years (Cheng *et al.*, 2007), and are the more appropriate and representative fractions of sequestered C in the soil (Silveira *et al.*, 2008). The labile fractions of SOC known to exhibit relatively fast turnover rates may not contribute significantly to C sequestration in soils (Silveira *et al.*, 2008). The chemical resistant or recalcitrant SOC can be estimated by determining non-hydrolysable carbon

(NHC) in the soil residue remaining after acid hydrolysis with 6 M HCl and this has been demonstrated to provide meaningful estimates of C fractions (Silveira *et al.*, 2008).

Acid hydrolysis is a commonly used technique to isolate and quantify labile and recalcitrant fractions of SOC. In most acid hydrolysis fractionation studies, hydrochloric acid (HCl) is the conventionally used extractant, however, hydrolysis with sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) has also been used widely (Belay-Tedla *et al.*, 2009). Acid hydrolysis has been performed most commonly by incubation at 95-116 °C for 16 or 18 hr (Paul *et al.*, 2006; Rovira and Vallejo, 2007). Although, it has been suggested that exposure to high temperatures may cause soil sugars to caramelize during acid hydrolysis, a two-step procedure with a weak acid pretreatment should be employed especially where NHC will be used for further chemical characterization (Paul *et al.*, 2006). However, to reduce possible alterations of SOC, acid hydrolysis has been performed at room temperature (Lorenz *et al.*, 2006).

Owing to increasing unsteady, surges and seizures of power supply in our nation, acid hydrolysis at room temperature (25°C) was adopted in this study to provide possible alternative to hydrolysis at higher temperatures. To this end, this study quantified non-hydrolysable carbon (NHC) concentrations in bulk soils (< 2 mm) at two temperatures, i.e., 25°C (room temperature) and 95°C. Therefore, the objectives of this study were:

1. To quantify the abundance of recalcitrant (NHC) and labile OC fractions isolated at different temperatures (25°C (room temperature) and 95°C).
2. To compare the effectiveness of acid hydrolysis (6 M HCl) for estimating SOC fractions at 25°C (room temperature) with 95°C.
3. To correlate labile C and NHC fractions with total soil organic carbon.

## MATERIALS AND METHODS

### *Site description*

The study was conducted at a field located at the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria (latitude – 7°12' N and longitude - 3°23' E). The site was previously a fallow of mixed shrubs, weeds and grasses but was planted with *Mucuna jaspidea* at three plant densities [25% (1 x 0.25 m), 50% (1 x 0.5 m), and 75% (1 x 0.75 m)] for this study in September 2011. The site is situated in the Derived savanna of Nigeria with a bimodal rainfall distribution pattern. The annual rainfall is about 1000 – 1500 mm with two distinct seasons; the wet season (March to October) and the short dry season (November to February). Mean temperature ranges from 21.9 °C (minimum) to 29.8 °C (maximum). The experimental area (40 m x 19 m) was laid out in a Randomized Complete Block Design with three replicates and divided into plot sizes of 3 m x 3 m. The soil had differing texture: loamy sand, sandy loam, and sandy clay loam, at 0 – 15 cm, 15 – 30 cm and 30 – 45 cm depths respectively.

### *Soil sampling*

Soil samples were collected from three depths (0 – 15; 15 – 30; 30 – 45 cm) in two mini-pits (60 cm x 60 cm) dug per plot in November 2011 (12 weeks after planting). Two or more soil cores (7.5 cm diameter x 20 cm deep) were taken from each depth, and samples from the same treatment were pooled together and composite samples taken and air-dried. The air-dried samples were then passed through a sieve (< 2 mm diameter), and any visible plant materials were manually removed from the sieved soil. Finely ground soil samples were used for all carbon analysis.

### *Soil organic carbon determination*

Soil organic carbon (SOC) for soil samples was determined by wet oxidation method. The SOC was measured by the colorimetric procedure using the UV/visible spectrophotometer model (Genesys™8) at the International Institute for

Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria.

### *Determination of labile and recalcitrant C fractions*

Soil C was separated into two chemical fractions, labile and recalcitrant, using acid hydrolysis modified from Plante *et al.* (2006). Soil samples (2.00 g) were digested for 16 hr in digestion tubes with 20 ml of 6 M hydrochloric acid solution at two temperatures: 25°C (room temperature) and 95°C. The residue after hydrolysis was separated from the supernatant (which was discarded) by centrifugation and washed three times with a total of 60 ml of deionized water. The non-hydrolysable residue was dried at 60 °C, and analyzed for C using the Walkley and Black, (1934) procedure. The C fraction which represents the recalcitrant fraction (NHC) was measured by the colorimetric procedure using the UV/visible spectrophotometer model (Genesys™8) at IITA, Ibadan. The hydrolysable fraction which represents the labile C in this study was obtained by subtracting the non-hydrolysable C from total SOC.

### *Calculation of stratification ratio*

Stratification ratio is defined as a soil property at the soil surface divided by the same soil property at a lower depth, such as the bottom of the tillage layer (Franzluebbers, 2002). Stratification ratios were calculated for C fractions at the 0 – 15 cm depth divided by 15 – 30 cm and/or 30 – 45 cm depths.

### *Statistical analysis*

Analysis of variance and linear regression analysis were performed using SPSS (15.0) package for Windows (SPSS Inc., Chicago, IL, USA). Treatments were considered different at  $P = 0.05$  using Duncan's multiple range test.

## RESULTS AND DISCUSSION

### *Abundance of recalcitrant and labile C fractions*

Generally, the concentrations ( $\text{g kg}^{-1}$ ) of SOC fractions significantly ( $P = 0.05$ ) decreased in the order: total SOC (11.83 – 21.57) >

NHC<sub>25°C</sub> (8.43 – 16.51) > NHC<sub>95°C</sub> (5.09 – 11.41) ≥ labile C (5.03 – 10.16) across the soil depths (Table 1). Overall, NHC<sub>25°C</sub> contributed 71 to 92% to total SOC, while NHC<sub>95°C</sub> contributed 43 to 66% to total SOC and labile C accounted for 34 to 57% of the total SOC across the treatments. Some studies have found that the recalcitrant (NHC) C fraction contributes a larger proportion to total SOC (Silveria *et al.*, 2008). On average, carbon (C) in the labile fraction measured by acid hydrolysis (hydrolysable C) represented about half (47 %) of total SOC in this study, suggesting hydrolysable C includes more C than just biologically labile C (Duo *et al.*, 2008). The size of the labile fraction measured by acid hydrolysis is regularly > 50% of total SOC in most studies (McLauchlan, and Hobbie, 2004), implying it is not a small fraction with short turnover time as with cold- and hot water extractable C (Adisa and Nortcliff, 2011).

The vertical distribution of SOC fractions revealed a decrease with depth in most treatments in this study. These results support those of Zhang *et al.* (2006), who reported higher total OC and labile C concentrations in the topsoil in their study. Increasing plant density should increase SOC because intensification results in greater amounts of biomass being returned to the soil (Sherrod *et al.*, 2003). These authors reported cropping intensification increased SOC after 12 yrs. It is therefore, not surprising to observe that plant density did not influence SOC fractions over the short period (12 weeks after planting) in the current study (statistics not shown). Other studies (Halvorson *et al.*, 2002; Ortega *et al.*, 2002), have also reported the lack of significant increases in SOC with increased biomass after 5- and 8 yr respectively.

**Table 1: Soil carbon fractions concentrations in three soil depths under different plant densities**

Plant density (%)	Soil depth (cm)	Soil organic carbon fractions (g kg <sup>-1</sup> soil)			
		SOC	NHC <sub>25°C</sub>	NHC <sub>95°C</sub>	Labile C <sub>95°C</sub>
75	0 – 15	21.57 (4.63)a	16.51 (2.62)ab	11.41 (2.84)ab	10.16 (1.85)b
	15 – 30	12.43 (1.13)a	11.04 (0.21)a	6.18 (1.41)b	6.25 (0.44)b
	30 – 45	11.87 (1.54)a	8.43 (1.19)ab	6.16 (0.84)b	5.71 (0.77)b
50	0 – 15	14.63 (0.34)a	13.52 (0.74)ab	9.60 (1.88)b	5.03 (1.56)c
	15 – 30	14.93 (1.93)a	11.60 (1.40)ab	6.86 (0.54)b	8.08 (1.44)b
	30 – 45	11.83 (1.09)a	9.71 (0.89)ab	5.09 (0.95)c	6.74 (0.92)bc
25	0 – 15	14.97 (2.10)a	13.06 (1.49)a	7.78 (1.10)b	7.18 (1.00)b
	15 – 30	13.47 (1.54)a	11.30 (1.42)a	7.23 (1.16)b	6.24 (0.57)b
	30 – 45	15.33 (3.53)a	13.50 (2.62)ab	9.52 (1.42)ab	5.82 (2.11)b

Values followed by different lowercase letters are significantly different at  $P=0.05$  along the row, using the Duncan's Multiple Range Test (DMRT); Standard error of means in parentheses: SOC – Total soil organic carbon; NHC<sub>25°C</sub> – nonhydrolysable carbon at room temperature; NHC<sub>95°C</sub> – nonhydrolysable carbon at 95°C; Labile C<sub>95°C</sub> – labile carbon at 95°C.

#### *Efficiency of acid hydrolysis to isolate recalcitrant C at different temperatures*

Acid hydrolysis at two different temperatures (25°C and 95°C) resulted in the recovery of varying amounts of NHC. Although, not statistically different, hydrolysis at room temperature (25 °C) recovered higher concentrations (~ twice) of NHC than at 95°C (Table 1). For acid hydrolysis at 25 °C (room

temperature), 8 to 29 % of the total C was hydrolyzed, while 34 to 57 % of total C was hydrolyzed at 95 °C. The greater proportion of C hydrolyzed at 95 °C suggests that more labile C were hydrolyzed at the higher temperature, thereby resulting in a complete hydrolysis. These results support those obtained in other studies (Cheng *et al.*, 2007; Silveria *et al.*, 2008), that found that hydrolysis at higher temperature effectively separated the

recalcitrant C fraction from the labile C, thus, providing meaningful estimates of C fractions.

Acid hydrolysis at room temperature (25°C) was adopted in this study to provide possible alternative to hydrolysis at higher temperatures, due to increasing unsteady power supply in our nation. From the results in this study, a lesser

amount (8 to 29 %) of SOC was hydrolyzed at 25 °C demonstrating that it is less effective in separating C fractions and as such cannot replace hydrolysis in hot HCl. Lorenz *et al.* (2006), suggested that acid hydrolysis with 6 M HCl at room temperature probably extracted mainly fulvic acids (labile C) as in hydrolysis with 1 M HCl at room temperature.

**Table 2: Correlation matrix ( $r^2$ ) for total soil organic carbon fractions for all treatments across three soil depths (n = 9)**

Soil carbon fractions	SOC	NHC25°C	NHC95°C	Labile carbon
SOC	1			
NHC <sub>25oC</sub>	0.88**	1		
NHC <sub>95oC</sub>	0.87**	0.97**	1	
Labile carbon	0.38 <sup>ns</sup>	0.20 <sup>ns</sup>	0.07 <sup>ns</sup>	1

\*\* Significant at  $P = 0.01$ ; ns – not significant

SOC – total soil organic carbon; NHC25°C - Non-hydrolyzable carbon at 25°C; NHC95°C - Non-hydrolyzable carbon at 95°C

#### *Relationships between OC fractions*

The recalcitrant C fractions (NHC25°C and NHC95°C) were significantly ( $P = 0.01$ ) correlated ( $R^2 = 0.88$  and  $0.87$  respectively) with total SOC (Table 2), suggesting that total SOC was a major determinant of the amount of recalcitrant C fraction. There was a strong relationship (Table 2) between NHC25°C and NHC95°C ( $R^2 = 0.97$ ;  $P = 0.01$ ), indicating the presence of similar organic materials within these recalcitrant C fractions. The similarities in the composition of the material in these

recalcitrant C fractions can be further supported by their very close relationships with total SOC mentioned earlier. Even though, there is a strong relationship between NHC25°C and NHC95°C, the difference among them is revealed when compared with the labile C fraction. The labile C was weakly correlated with total SOC and NHC25°C ( $R^2 = 0.38$ ;  $0.20$  respectively), but there was not relationship ( $R^2 = 0.07$ ) with NHC95°C (Table 2).

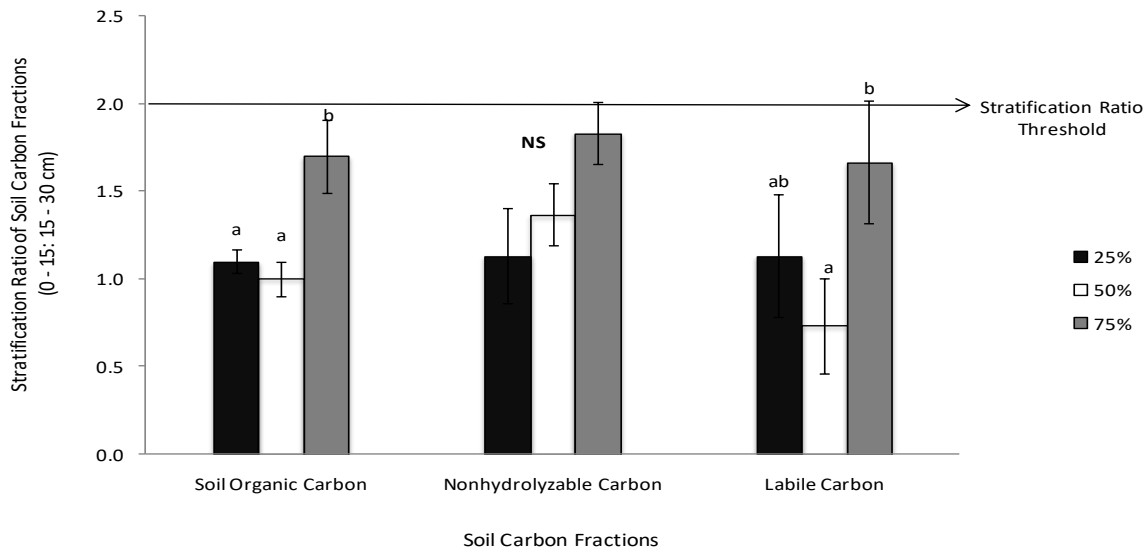


Fig. 1. Stratification ratio of soil organic carbon fractions at soil depths (cm) 0 – 15:15-30; Different lowercase letters on top of standard error bars indicates significant difference ( $P=0.05$ ); NS – Not significant; Plant densities (25, 50 and 75%)

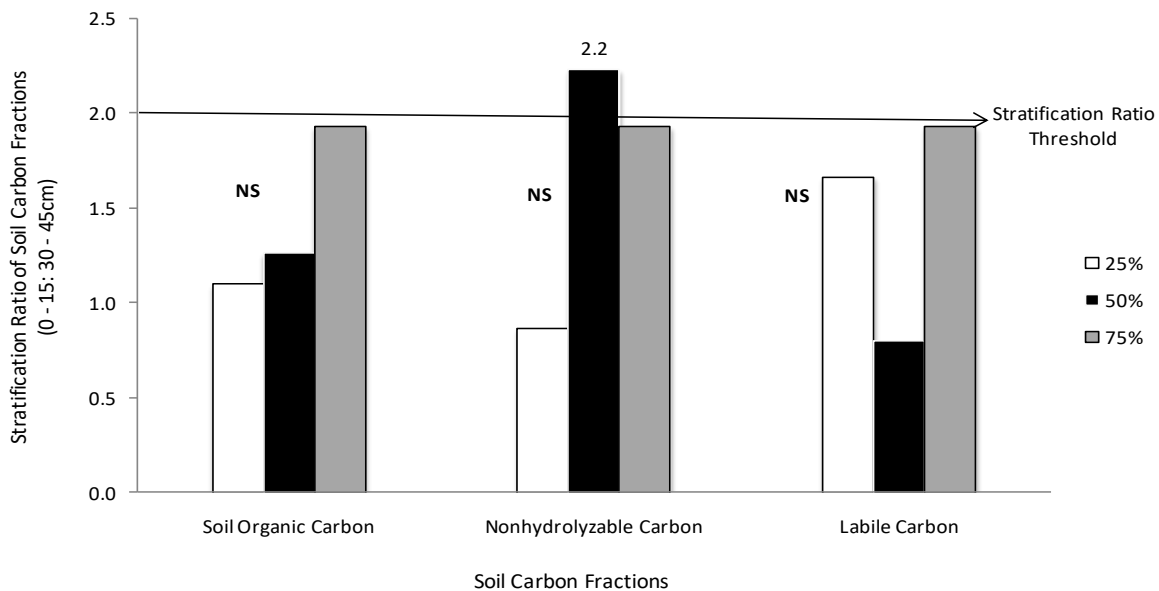


Fig. 2. Stratification ratio of soil organic carbon fractions at soil depths (cm) 0 – 15:30 - 45; NS – Not significant; Plant densities (25, 50 and 75%)

The lack of relationship of labile C with  $NHC_{95^{\circ}C}$  gives additional explanation for the complete hydrolysis achieved in hot acid ( $95^{\circ}C$ ), as a result, labile C did not contribute to this recalcitrant (NHC) fraction. However, the very weak relationship ( $R^2 = 0.20$ ) of labile

C with  $NHC_{25^{\circ}C}$  implies that some labile C fraction remained unhydrolyzed at room temperature ( $25^{\circ}C$ ), which further confirms the inadequacy of this method to characterize recalcitrant C fraction.

### *Soil organic carbon fractions stratification ratio*

The stratification ratio of SOC fractions were significantly below the threshold value of 2 (Franzluebbers, 2002) and was 1.0 to 1.7 for SOC, 1.1 to 1.8 for NHC<sub>95°C</sub>, and 0.7 to 1.7 for labile C at the 0 – 15 cm; 15 – 30 cm depth (Fig. 1). When the stratification ratio was calculated at the 0 – 15 cm; 30 – 45 cm depth, the ratios were 1.1 to 1.9 for SOC, 0.9 to 2.2 for NHC<sub>95°C</sub> and 0.8 to 1.9 for labile C (Fig. 2). However, these ratios were not significantly different. Stratification ratio < 2 as revealed in most instances in this study (Figs. 1 and 2) implies a disturbed soil condition as higher ratios (> 2) reflect relatively undisturbed soil condition or an improving quality on previously degraded soils (Causarano *et al.*, 2008) and are not frequently found in degraded soils (Franzluebbers, 2002). The stratification ratio is also an indicator of present C status and the potential to sequester (Gerzabek *et al.*, 2002). With most ratios < 2 in this study, it implies the opportunity for C sequestration exists for the soils in this current study.

### CONCLUSION

This study demonstrated the ability of 6 M HCl acid at a higher temperature (95°C) to characterize labile and recalcitrant C fractions better than at room temperature (25°C). Acid hydrolysis at room temperature (25°C) was employed in this study to provide possible alternative to hydrolysis at higher temperatures, due to increasing unsteady power supply in our nation (Nigeria). However, the results clearly show that hydrolysis at 25°C cannot replace that performed at higher temperatures, e.g. 95°C. The lack of correlation ( $R^2 = 0.07$ ) between NHC<sub>95°C</sub> and labile C provided additional explanation for the effective hydrolysis achieved at a higher temperature. The total SOC and C fractions status is revealed and there is great potential for C sequestration for the soils in this study since most C stratification ratios were < 2.

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