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Assessment of soil organic carbon fractions under sugarcane harvest residue management

Evaluación de las fracciones de carbono orgánico del suelo en el marco de la gestión de residuos de la cosecha de caña de azúcar

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ABSTRACT

Labile carbon (LC), measured by Permanganate oxidizable Carbon (POXC) techniques, and mineralizable carbon (MC), assessed through microbial CO_2 flux, are soil organic carbon (SOC) fractions that serve as sensitive indicators of soil management practices and soil organic matter (SOM) composition. Thus, this type of study is necessary for managing soil fertility in sugarcane cultivation systems. The experiment was based on measuring SOC fractions with different percentages of crop residue removal (T1:0%, T2:20%, T3:40%, T4:60%, T5:80%, and T6:100%) in a Pachic Vertic Haplustolls soil whose plots had the crop variety CC 93 4418. The experimental design was Randomized Complete Blocks. Samples were collected at three depths during the seventh ratoon. The plant biomass variables were expressed in tons per hectare. The effects of treatments on LC and MC were compared using analysis of variance (P-value of 0.05 or less), the Tukey test, a Pearson correlation analysis among soil properties, and a conceptual analysis between LC and MC was established through least squares linear regression. The LC across the three depths recorded significant differences, with T2 exhibiting the highest value of 328.91(mg. kg^{-1}) and T6 exhibiting the lowest average of 272.75 (mg. kg^{-1}). High correlations were observed between LC and soil properties such as CEC, volumetric water content, and gravimetric water content. Treatments could be directed towards high accumulation for T1 and T2 and high mineralization for T6 based on the correlation between LC and MC. Regarding the productivity parameter, T2 showed a higher TCH (211.66) with a more significant percentage change in LC increase over time. It is concluded that a soil without cover presents high CM dynamics but with low nutrient storage reflected in the CL.

Keywords: carbon storage; coverage; decomposition; labile carbon; mineralizable carbon; soil respiration

RESUMEN

El carbono lábil (CL), medido mediante técnicas de carbono oxidable con permanganato (POXC), y el carbono mineralizable (CM), evaluado a través del flujo de CO_2 microbiano, son fracciones de carbono orgánico del suelo (COS) que sirven como indicadores sensibles de las prácticas de manejo del suelo y la composición de la materia orgánica del suelo (MOS). Por lo tanto, este tipo de estudio es necesario para el manejo de la fertilidad del suelo en sistemas de cultivo de caña de azúcar. El experimento se basó en la medición de las fracciones de COS con diferentes porcentajes de remoción de residuos de cultivo (T1:0%, T2:20%, T3:40%, T4:60%, T5:80% y T6:100%) en un suelo Pachic Vertic Haplustolls con variedad de cultivo CC 93 4418,con el diseño experimental de Bloques Completos al Azar. Se recolectaron muestras a tres profundidades durante el séptimo ciclo de soca. Las variables de biomasa vegetal se expresaron en toneladas por hectárea. Los efectos de los tratamientos sobre CL y CM se compararon mediante análisis de varianza (valor p de 0,05 o inferior), prueba de Tukey, correlación de Pearson entre propiedades del suelo y se estableció un análisis conceptual entre CL y CM mediante regresión lineal de mínimos cuadrados.Los resultados indican que El CL registró diferencias significativas en las tres profundidades, identificando a T2 con el mayor valor 328,91(mg. kg^{-1}) y a T6 con el menor 272,75 (mg. kg^{-1}). Se observaron altas correlaciones de CL con propiedades CIC, contenidos de agua volumétrico y gravimétrico. Los tratamientos podrían dirigirse hacia alta acumulación para T1 y T2 y alta mineralización para T6 realizando correlación entre CL y CM. En cuanto al parámetro productividad, T2 obtuvo mayor TCH (211,66) con cambio porcentual más significativo en el aumento de CL a lo largo del tiempo. Se concluye que un suelo sin cobertura presenta alta dinámica de CM, pero con bajo almacenamiento de nutrientes reflejado en el CL.

Palabras clave: almacenamiento de carbono; carbono lábil; carbono mineralizable; cobertura; descomposición; respiración del suelo

INTRODUCTION

Monitoring soil variables is crucial for preventing physical, chemical, or biological degradation in agroecosystems. Among these variables, specific fractions of soil organic carbon (SOC) have been identified as key indicators for monitoring and assessing soil quality and fertility. Consequently, it is crucial to establish the relationship between these fractions and fundamental natural processes such as nutrient storage and availability. Regarding the challenges facing the sugarcane agro-industrial sector, developing technological alternatives to enhance soil fertility while minimizing environmental impact on soil and water resources represents a crucial area of research (Sanclemente Reyes *et al*., 2015).

It is pertinent to align local actions with global recommendations, particularly those focused on developing policies and strategies for soil management. These policies should prioritize the protection, capture, measurement, mapping, monitoring, and reporting of soil organic carbon (SOC). Furthermore, the Food and Agriculture Organization of the United Nations (FAO) has supported this effort with its initiative, "Unlocking the Potential of Soil Organic Carbon." This program presents the initial theme of measuring, mapping, monitoring, reporting, and verifying SOC reserves and changes in these reserves. Consequently, recommendations have been derived from industrial crops, such as sugarcane, to build capacity for expanding national reference values for SOC stocks, and to develop the necessary infrastructure for effective data management (FAO, 2018).

A reduction in soil organic matter stocks directly affects the potential yield of crops in the short term (Manna *et al*., 2003). Soil organic carbon (SOC) is therefore crucial in forming and stabilizing soil structure, enhancing water infiltration, promoting root development, and increasing resistance to erosion. The degradation processes on these properties are irreversible (Stevenson & Cole, 1999). Reports indicate that increasing soil organic carbon improves soil fertility, enhances microbial activity, raises water retention, and improves soil structure, thereby protecting it from erosion.

While the surface layers or horizons of tillage soils contain relatively low percentages of organic matter (OM) (approximately 1 to 3%), their role in the soil-water-solute dynamics and overall structure is crucial. Conceptually, a distinction is made between total organic carbon (which encompasses all organic compounds) and oxidizable organic carbon (which is completely transformed and typically affects the physicochemical properties of soil). This distinction is vital as it enables the establishment of a correlation with other fundamental properties, such as adsorption-desorption and mineral transport (Prieto *et al*., 2014).

The constant quantification of sensitive soil organic carbon (SOC) fractions provides valuable information that relates to the effects of management changes in crops (Campitelli *et al*., 2010). These fractions serve as raw materials for soil microorganisms, fueling their metabolism and subsequent transformation into complex organic compounds (Ortega, 1982). Furthermore, labile organic carbon (LOC) is closely associated with short-term soil responses due to management changes (Dou *et al*., 2008). This is evidenced by its strong relationship with microbial biomass carbon, total SOC, and particulate organic matter (Weil *et al*., 2003).

In terms of soil quality, the agronomic practices employed are expected to have few correlations in organic matter (OM) dynamics in the short term (Tan *et al*., 2007). A long-term observation period is therefore necessary, as organic fractions have a characteristically slow cycling rate. According to Haynes (2000) and Six *et al*. (2002), labile fractions of soil carbon are highly sensitive to soil use, serving as key indicators of various management practices on soil quality, including crop rotation, fertilization, and tillage systems.

In agricultural production systems, the mineralization of Soil Organic Matter (SOM) is significantly influenced by clay content. Clay provides a key mechanism for the physical stabilization of SOM, protecting it from rapid transformation (Hassink *et al*., 1993). Understanding this dynamic motivates the quantification of the soil's activated carbon (labile carbon - LC) to help determine how crops will respond to the fertilization of key elements such as nitrogen (N).

The permanganate oxidizable carbon (POXC) method was proposed, as a way to measure soil organic carbon (SOC) fraction that is closely linked to several parameters of soil quality (Weil *et al*., 2003). In conjunction with N mineralization, the POXC method enables the estimation of the labile C/N ratio in soil. This approach allows for the fine-tuning of nitrogen fertilization. As reported by Delgado Restrepo *et al*. in 2016, this method produced better results for quantifying N mineralization in an organic sugarcane system using the Direct Steam Distillation (DSD) method and in a conventional system using the Illinois Soil Nitrogen Test (ISNT) method.

Soil organic matter (SOM) levels are the direct result of the balance between carbon (C) inputs from sources like crop residues and amendments, and losses from mineralization (i.e., the release of CO_2 through soil respiration). These dynamics (stabilization versus mineralization) are intervened through the soil food web, which plays a vital role in the decomposition of SOM and ultimately supports crop nutrition. Agricultural producers have vested interest in both processes: they rely on mineralization for short-term crop productivity but also strive for stabilization to build resilience, tillage, and soil quality (Culman *et al*., 2012). This study's objectives focus on the importance of organic carbon fractions, the relationship with other soil properties and their effect on sugarcane yield.

MATERIALS AND METHODS

Soil samples were collected from a sugarcane cultivation system (*Saccharum officinarum*) variety CC 93-4418 located at the Colombian Sugar Cane Research Center (CENICAÑA) specifically from experimental lot 19B, in Agro-ecological Zone 6H1. Cantarina (CN) as the predominant soil in this area, is classified as Pachic Vertic Haplustolls, fine, smectitic and isohyperthermic (Carbonell *et al.*, 2011). The experimental lot had a record of seven crops (one plant cane and six crops). The study was arranged in a randomized complete block design with six treatments and three replications, comprising 18 plots. Each plot measured 10m x 1.5m, with 10 furrows, for a total area of 150 m². The treatments correspond to the increasing removal of crop residues (leaf litter from manual harvesting) as shown in Table 1.

The treatments for each plot were established by removing biomass along 2, 4, 6, 8, and 10 linear meters, which corresponded to 20, 40, 60, 80, and 100% removal, respectively. Subsequently, the remaining material was evenly distributed across the soil surface to ensure homogeneous coverage. The soil organic carbon fractions were measured 4 years after the different crop residue covers were established, along with simultaneous measurements of electrical conductivity and soil moisture.

In the initial characterization of the soil, the following averages were obtained: pH=7.4, organic matter=2.16%, phosphorus=14.5 ppm, sulfur=10.7 ppm, calcium=15.4 meq/100g, magnesium=6.41 meq/100g, sodium=0.22 meq/100g, and potassium=0.18 meq/100g.

Table 1. *Description of Treatments Associated with the Removal Proportion of Sugarcane Harvest Residue*

Treatment.	Percentage of waste removal	Withdrawal expressed in kg/ha
T1	0.%	0.00
T2	20%	12,700.72
T3	40%	18,748.68
T4	60%	22,881.45
T5	80%	33,162.98
T6	100%	33,263.78

Sugarcane crop conditions were under conventional irrigation management with scheduling based on water balance estimation for determining the frequencies and the K factor for crop's age (1-3 months of age: 0.3; 3-4 months of age: 0.4; 4-5 months of age: 0.6). Based on the soil and crop analysis, fertilization was applied at rates of 120 $kg.ha^{-1}$ N, of 26 $kg.ha^{-1}$ P, and 56 $kg.ha^{-1}$ K.

Soil samples were collected from three depths: 0-2, 10-12, and 20-22 cm. For the 2021 analysis, labile carbon (LC) was determined. In 2022, the same depths were used to estimate both LC and microbial carbon (MC) fractions. A total of 54 samples were collected from 18 plots, with one sample taken from each of the three depths per plot and three sampling depths. All samples were obtained using steel rings with a height of 2 cm and a diameter of 7.78 cm, resulting in a volume of 48.98 cm^3. The contents were mixed to make up the sample of each depth. Laboratory sampling was performed 15 days prior harvest, a period that coincided with the lowest rainfall in the year.

The Permanganate Oxidizable Carbon (POXC) method determines labile carbon (LC) by oxidizing the most readily available organic matter in the soil with a slightly alkaline solution of potassium permanganate (KMnO4). This fraction of LC includes simple carbohydrates, amino acids, amino sugars, and other carbon compounds containing hydroxyl, ketone, carboxyl, double bonds, and aliphatic compounds (Weil *et al*., 2003).

According to the method of Culman *et al*. (2012), 20 ml of $KMNO_4$ 0.02 mol. L^{-1} and 2.5g of air-dried soil were added to screw-cap polypropylene centrifuge tubes. The tubes were agitated for 2 minutes at 240 oscillations. min^{-1}. Subsequently, a 0.5 mL aliquot of the supernatant was transferred to a second 50 mL centrifuge tube and mixed with 49.5 mL of deionized water. The absorbance of this solution was measured with a spectrophotometer at 550 nm, and the Permanganate Oxidizable Carbon (POXC) was calculated in mg. kg^{-1} of soil using the following equation 1:

$$\frac{mg\ C}{kg\ sample} = \frac{9000\ mg\ C}{mol} * \left[\frac{\left(0{,}02\ L * \frac{0{,}02\ mol}{L}\right) - \left(0.02\ L * \frac{abs - b}{m}\frac{mol}{L} * \frac{10\ mL}{0.5\ mL}\right)}{0{,}005\ kg\ sample}\right] \quad (1)$$

The calculation for POXC was based on several key factors. The value 9000 represents the milligrams of carbons oxidized by one mol of $KMnO_4$, as it is reduced from Mn^{7+} to Mn^{2+}; The initial volume and concentration of the KMnO4 added were 0.02 L and 0.02 M, respectively. The absorbance (abs) was read from a calibration curve with a slope (m) and intercept (b). A dilution factor of 20 was also applied to the samples (10 mL / 0.5 mL). The excess potassium permanganate (KMnO4) in the sample was measured by UV-visible spectroscopy at 550nm.

Mineralizable carbon was determined using the soil microorganism respirometry technique. The dried soil sample was rewetted to 50% of its initial volumetric water content (Franzluebbers *et al*., 2000). A metal cylinder containing the soil was then placed inside a hermetically sealed container with a volumetric capacity of 1920 cm^3. A beaker with a one mol NaOH solution (10 mL) was included to act as a CO_2 trap, along with a separate vial containing 10 mL of water to maintain a humid atmosphere. The incubation chamber was kept at a constant temperature of 25 °C for three days. For every 18 soil samples, a blank container with Sodium Hydroxide (NaOH) and water, but no soil, was established to serve as a control. At the end of the incubation, the NaOH vessels were removed and sealed until titration. During the titration of all samples and the blank, the following reagents were added in the same sequence:

- 2.5mL of 1.5M $BaCl_2$ solution to precipitate bicarbonate as $BaCO_3$.
- Drops of phenolphthalein color indicator.
- Magnetic stir bar.

The vial was placed on a magnetic stirrer plate, and 1 M of HCl was slowly added to the solution until the color changed from pink to colorless. The amount of CO_2 released was calculated using Anderson's equation 2, where the mineralizable carbon content was expressed in mg of C per kg of soil (Anderson, 1982).

$$\frac{mg\ CO_2 - C}{kg\ sample} = \left[\frac{mL\ (white) - mL(sample) * N * M}{S}\right] \qquad (2)$$

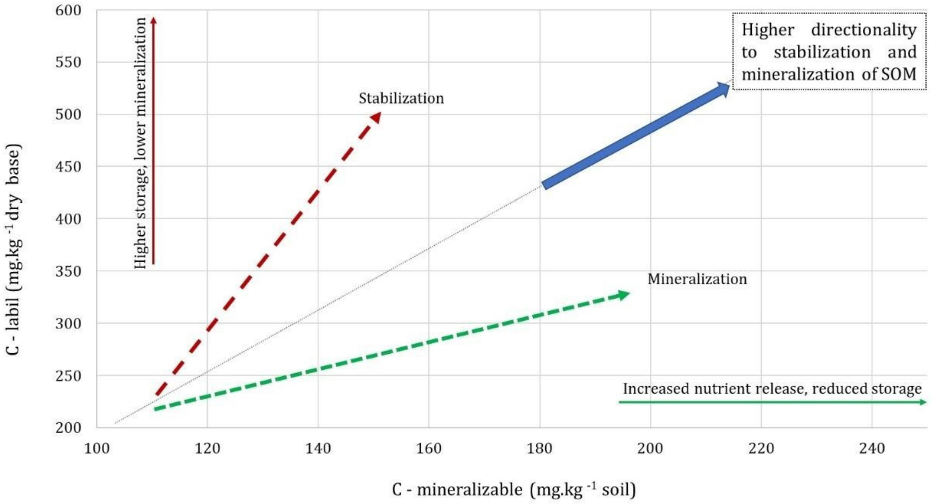
In which: N = acid normality (mol. L^{-1}; e.g., 1), M = mass conversion from C mol to C g (6000), S = soil weight (e.g., 100g).

The final value was multiplied by the factor 0.273 corresponding to the amount of C in the molecule (Franzluebbers *et al*., 2000, Haney *et al*., 2001).

Once the LC and MC were obtained, a conceptual analysis of these soil organic carbon fractions was conducted using least squares linear regression (Hurisso *et al*., 2016) to examine the treatments in relation to storage trends and/or nutrient availability.

Data were analyzed using Analysis of Variance (ANOVA), and mean comparisons were conducted with Tukey test. A Pearson's correlation was also performed to determine the coefficients among linearly related soil variables. All statistical analyses were conducted using SAS statistical software, version 9.1 for Windows (SAS Institute Inc., Cary, NC, USA), with a significance level of $p<0.05$.

To approximate the functional differences between the LC and MC fractions, a model was developed for each experimental factor using least squares linear regression, which reflected observations and forecasts with negative or positive residual values depending on the soil management (Figure 1, adapted from Hurisso *et al*., 2016).



From left to right: Labile Carbon (dry base); Higher storage, lower mineralization; Stabilization; Higher directionality to stabilization and mineralization of SOM; Mineralization; Increased nutrient release, reduced storage; Mineralizable carbon (soil)

Figure 1. *Concept Diagram with Figures of Labile Carbon (LC) and Mineralizable Carbon (MC) Fractions in Soil under a Cultivation System of Sugarcane*

A partial least squares (PLS) regression was employed to develop an explanatory model for crop productivity. The dependent variable, tons of cane per hectare (TCH), was regressed against a set of independent variables, which included foliar parameters (Chlorophyll, N, P, K, Ca, Mg, Fe, Mn, Cu, Zn, B) and soil parameters (pH, Soil Organic Matter (SOM), P, K, Ca, Mg, Na, Fe, Mn) measured at month 6 of the crop cycle, prior to flowering. This analysis allowed for the identification of significant variables based on their coefficients. The interpretation of these coefficients was based on their sign: a positive value (+) indicated a direct relationship, while a negative value (-) indicated an inverse relationship.

RESULTS

The values of LC and MC in the field reached a normal distribution, with low coefficients of variation (15.34 and 15.72, respectively). For the first sampling (2021), the overall average of LC was estimated across the three depths, revealing significant differences among treatments (Table 2). Specifically, T2 showed the highest value (328.91 mg. kg^{-1}) while T6 had the lowest (272.75 mg. kg^{-1}).

Table 2. *The Comparison of Averages in Soil Labile Carbon (LC) Contents for the Year Sampling, 2021*

Removal of residues	Labile C (mg. kg^{-1} Dry Base)
0% (T1)	312.51(ab)
20% (T2)	328.91(a)
40% (T3)	277.15(c)
60% (T4)	294.81(ab)
80% (T5)	319.27(a)
100% (T6)	272.75(c)

Values of means with different letters in each column indicated statistically significant differences, according to the Tukey test (p≤0.05). The value of each treatment corresponds to the ratio of the three depths evaluated.

Table 3, which corresponds to the second sampling (2022), shows a similar trend in LC with significant statistical differences among treatments. T2 again had the highest average of 494.38 mg. kg^{-1}, followed by T4 with 450.44 mg. kg^{-1} (also a statistically significant difference), The lowest amount was once more found in T6, which had an average of 399.78 mg. kg^{-1} (this value also showed statistical contrast with the others.).

Table 3. *Comparison of Averages in Soil Labile Carbon (LC) Contents for the Year Sampling 2022*

Removal of residues	Labile C (mg. kg $^{-1}$ Dry Base)
0% (T1)	403.63(c)
20% (T2)	494.38(a)
40% (T3)	415.82(c)
60% (T4)	450.44(ab)
80% (T5)	422.47(c)
100% (T6)	399.78(c)

The values of means with different letters in each column indicated statistically significant differences, according to the Tukey test (p≤0.05). The value of each treatment corresponds to the ratio of the three depths evaluated.

Analysis of the chemical properties from the first sampling depth in 2022 revealed, a strong correlation between CEC of soil and LC. This relationship was confirmed by a linear regression with R^2=0.60 (as shown by the shaded points in Figure 2) and a Pearson correlation coefficient of 0.77 (Table 2). These results are consistent with the findings reported by Mikutta *et al.* (2005), who attributed similar correlation to the oxidation of C organic fractions. This process creates new carboxylic groups that could increase load density on particle surfaces (clays), thereby, enhancing the soil's cation exchange capacity (CEC).

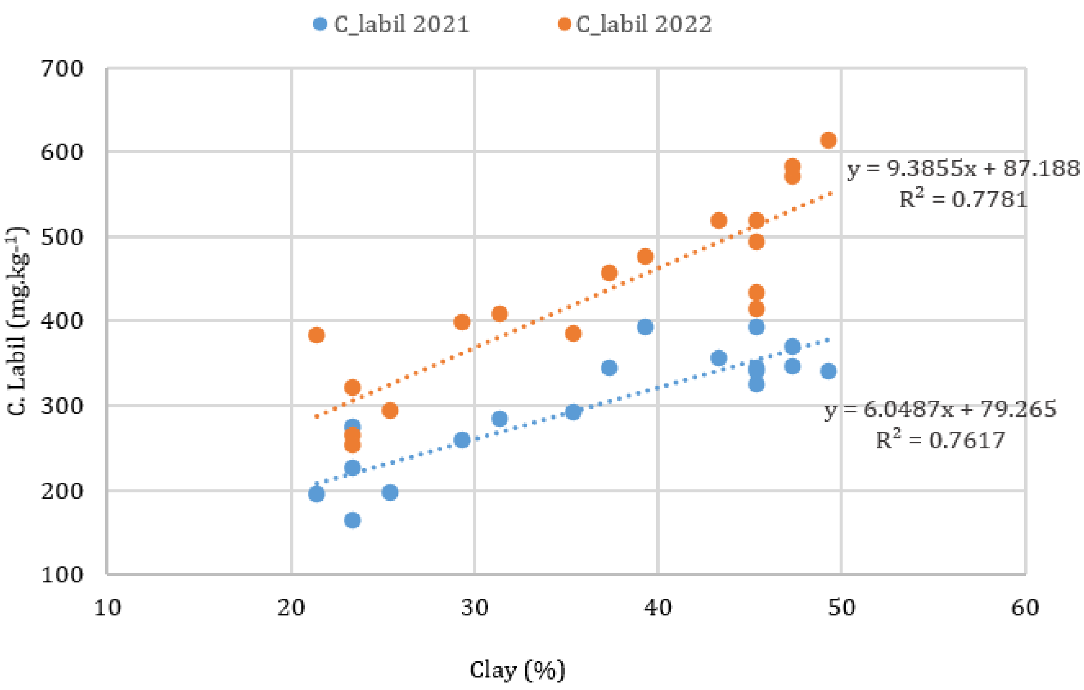


Figure 2. *The Ratio of Labile Soil Carbon to Clay Content*

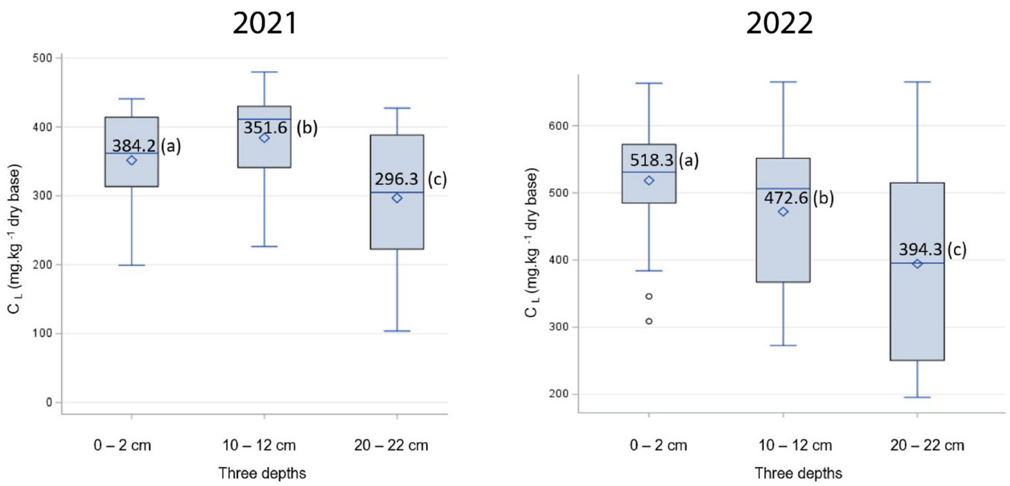
In the first sampling depth, moisture exhibited a directly proportional relationship with other soil properties, as shown by correlation coefficients of 0.48 and 0.42, respectively (Table 4). This finding highlights the crucial significance of moisture for microbial metabolism, a factor heavily influenced by environmental conditions. These results are supported by the work of Martínez *et al.* (2008), who explained how organic carbon can intervene in the distribution of soil pore space, thereby influencing key physical properties, such as available moisture, water movement and soil gas exchange.

Table 4. *Pearson Correlation Among Soil Variables*

	G. Water Content	Vol. Water Content	Labile Carbon	%SOM	CEC	Pb
G. Water Content	1.00					
Vol. Water Content	0.99	1.00				
Labile Carbon	0.48	0.42	1.00			
%SOM	0.35	0.33	0.41	1.00		
CEC	0.27	0.20	**0.78***	0.32	1.00	
pb	0.97	0.99	0.41	0.33	0.19	1.00

** Significance at 0.05 of the probability level. G. Water Content: Gravimetric water content; Vol. Water Content: Volumetric water content; Labile Carbon: Carbon Labile; %SOM: percentage of soil organic matter; CEC: cation exchange capacity; pb: apparent density.*

The mean comparison test for LC did not reflect significant statistical differences among treatments, but there was a statistical contrast (Figure 3) for the three depths in each measurement year.



From left to right: 2021 Measurement; 2022 Measurement; (Dry Base); (Dry Base) Depth; Depth

Figure 3. *Overall Average Labile Carbon (LC) of Soil in Three Depths*

These findings on the decrease of lignin content (LC) with depth align with previous research. Guo and Gifford (2002) reported a 10% reduction in LC associated with conventional agricultural soil management. Conversely, they observed an increasing trend with depth (a 53% increase) when agricultural land was converted to secondary forest management. Similarly, Collins *et al.* (2010) reported statistically significant decreases in LC with depth, showing up to a 40% reduction in soils under conventional tillage systems compared to those with minimum tillage cover.

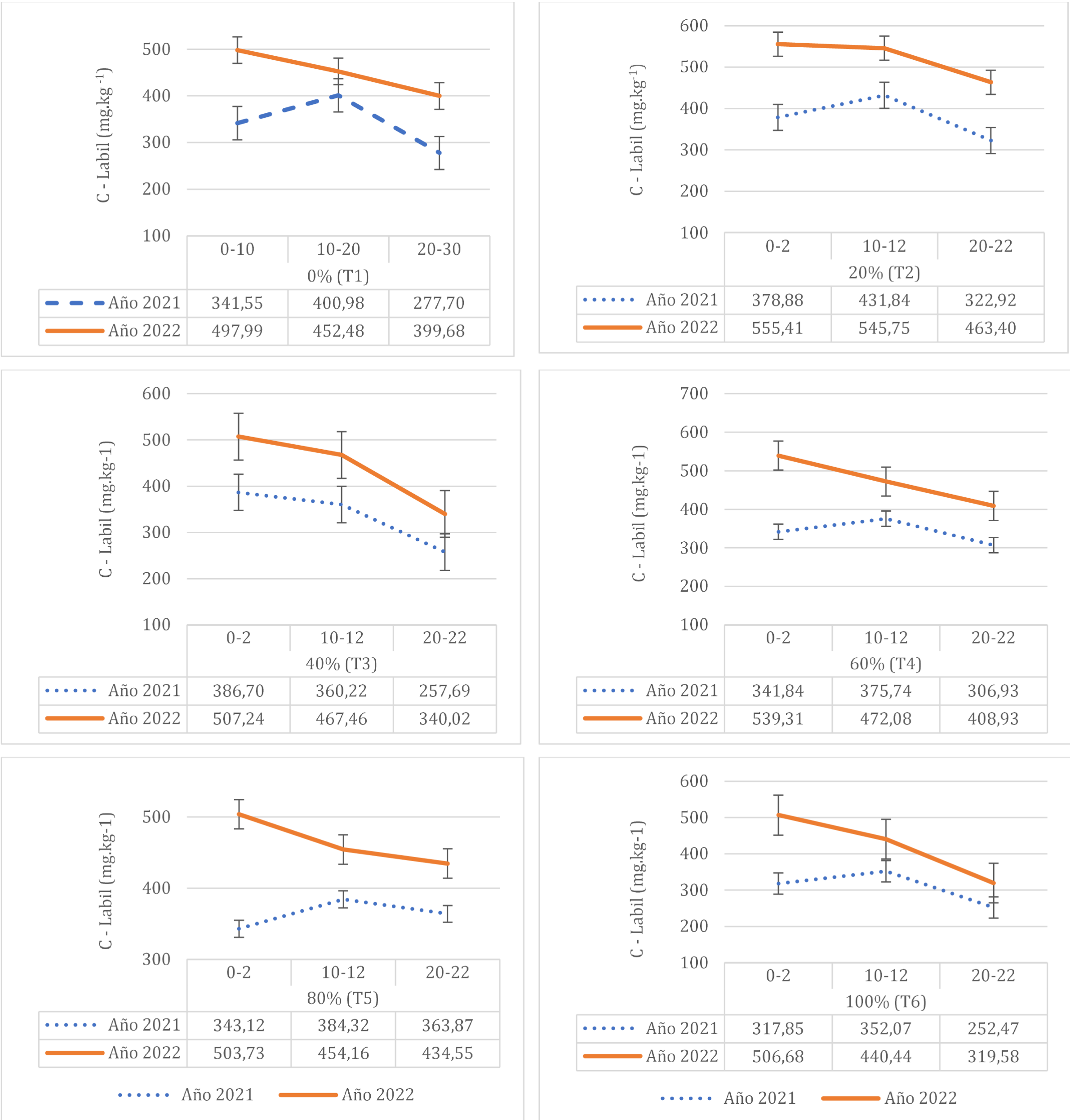
In the 2021 sampling, there was a trend of increasing labile carbon (LC) at the 10-12 cm depth (the broken line in Figure 4), with the exception of T3. An analysis of the increase in labile carbon (LC) suggests that climate and soil moisture parameters, particularly higher solar radiation and lower soil water content compared to 2022, created favorable conditions for this increase at the 10-12 cm depth (Table 5). The increased accumulation of labile carbon (LC) under low-moisture conditions may also be attributed to crop residue coverage.

Table 5. *Climate Parameters, Soil Moisture, and Labile Carbon.*

Parameter	Year 2021	Year 2022	Difference
Precipitation (mm)	1131.7	1338.6	206.90
Average temperature °C	23.37	23.11	0.26
Minimum solar radiation (cal.cm^{-2})	153.70	122.60	**31.10**
Max. solar radiation (cal.cm^{-2})	646.10	632.50	**13.60**
Excess of water in soil (mm)	12.37	16.14	3.77
LAS (mm)	9.88	15.16	5.28
Labile C (mg.kg^{-1}) 0-2cm depth	351.65	518.39	166.74
Labile C (mg.kg^{-1}) 10-12cm depth	**384.19**	472.06	87.87
Labile C (mg.kg^{-1}) 20-22cm depth	296.92	394.36	97.44

According to Moorhead and Callaghan (1994), under certain soil conditions in semi-arid environments, the degradation of organic compounds (e.g., lignins) could be profoundly affected by solar radiation, generating reactions that contribute significantly to the transformation of soil organic carbon (SOC).

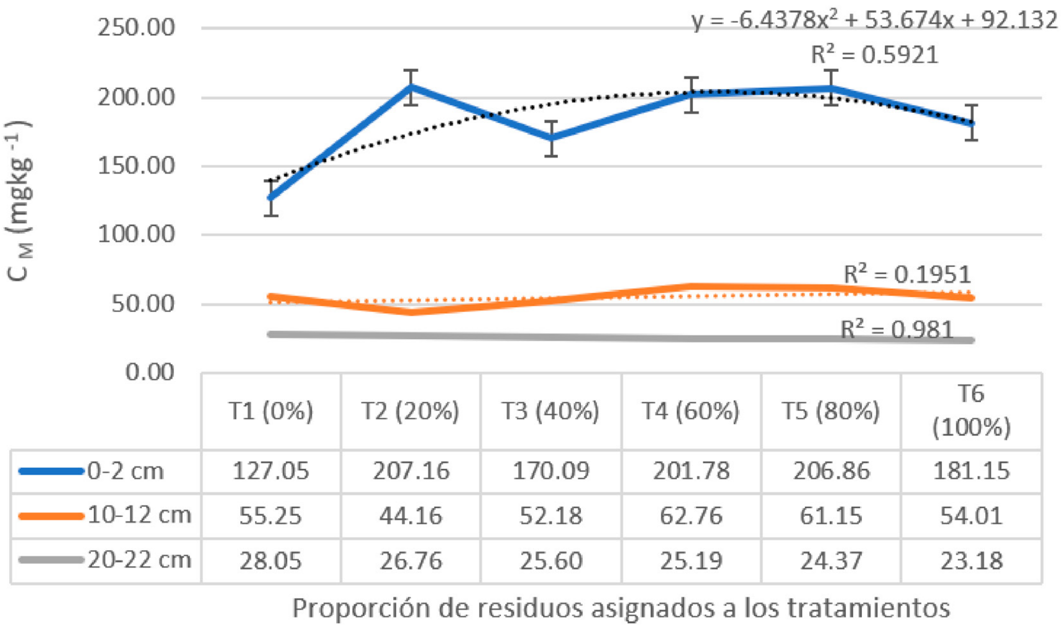
However, in 2022, the labile carbon (LC) showed a consistent decrease with depth for all treatments, and this reduction was more significant than what was observed in 2021 (Figure 4, solid lines). This finding may be linked to a rapid cycle of LC increase over a short period (ranging from months to a few years), as indicated by Gregorich *et al.* (1994).



From left to right: 0% removal; Year 2021; Year 2022

Figure 4. *Comparison of Labile Carbon (LC) of Soil for Two Years of Evaluation and at Three Depths: 0-2 cm, 10-12 cm, and 20-22 cm (Residual Removal Treatments: T1, T2, T3, T4, and T5).*

Similarly, the Tukey test revealed no significant differences in moisture content (MC) among treatments. However, a progressive trend was observed based on the proportions of harvest residues. For example, at the 0-2 cm depth, a polynomial model yielded an $R^2 = 0.59$, which likely indicates a fluctuating effect of the surface cover on microbial CO_2 emissions (Figure 5). In contrast, the deeper layers showed different relationships: the 10-12 cm depth had an $R^2=0.21$, while the 20-22 cm depth showed a high linear adjustment with an $R^2=0.98$, indicating a more stable relationship without the fluctuations seen at the surface.

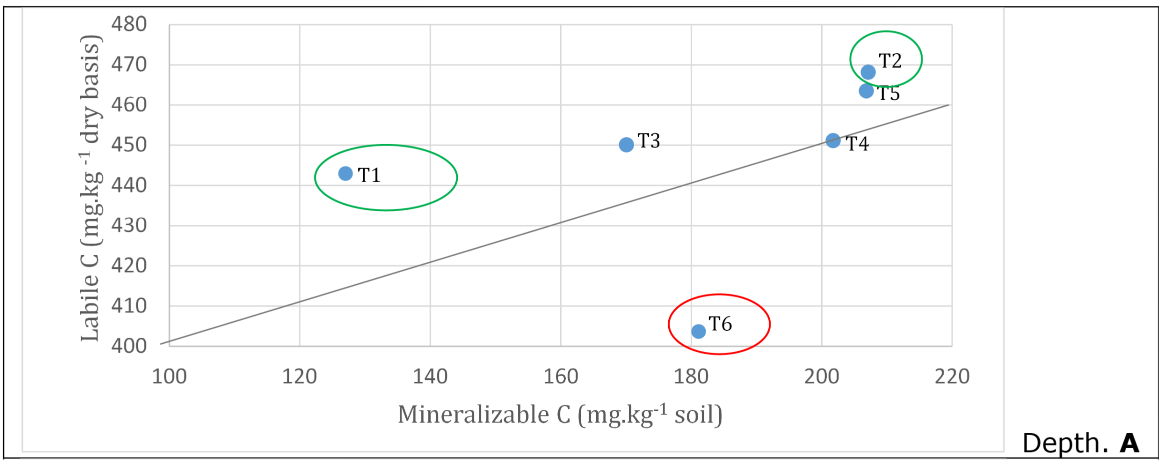


	T1 (0%)	T2 (20%)	T3 (40%)	T4 (60%)	T5 (80%)	T6 (100%)
0-2 cm	127.05	207.16	170.09	201.78	206.86	181.15
10-12 cm	55.25	44.16	52.18	62.76	61.15	54.01
20-22 cm	28.05	26.76	25.60	25.19	24.37	23.18

Figure 5. *Mineralizable Carbon (MC) based on the amount of harvest residue removal. Data for three depths.*

According to Fang and Moncrieff (2005), this vertical variation could be related to organic carbon stocks in each stratum, showing significant effects on the concentration of C fraction and the soil microbial activity.

Therefore, the treatments could be directed toward accumulation and mineralization, respectively (Figure 6, Depth A), by correlating LC and MC for the first depth. Based on this analysis, it was inferred that Treatment 1 (T1) with LC of 442.96 mg.kg^{-1} and MC of 127.05 mg.kg^{-1} (upper left quadrant) was associated with organic matter (OM) stabilization processes. In contrast, soils with the T6, corresponding to 100% residue removal, were associated with a high potential for nutrient mineralization and low organic matter (OM) stability (lower right quadrant). This scenario projects a very low C/N ratio with intense biological activity, which is characteristic of a mollic epipedon.



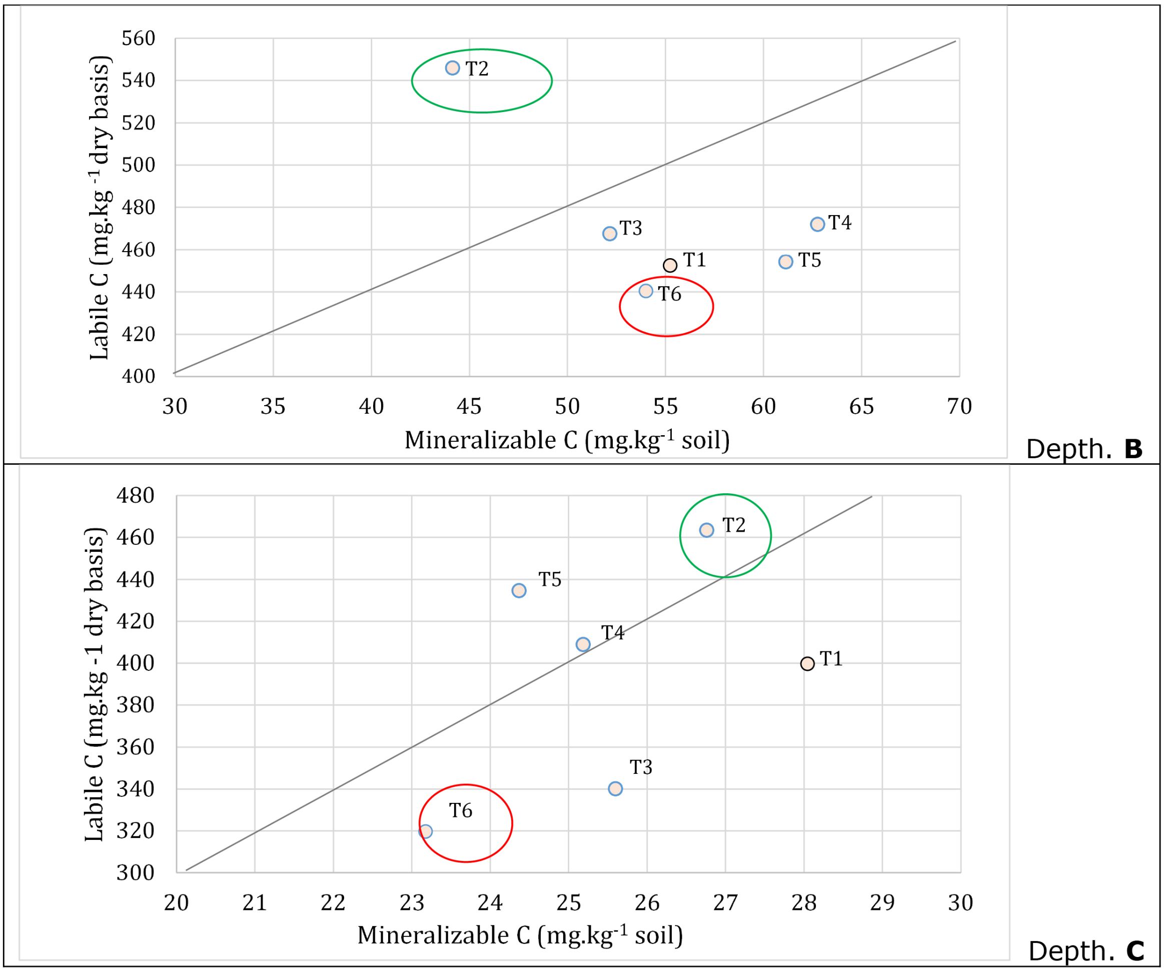


Figure 6. *Conceptual Diagram of Soil Organic Carbon Fractions (LC vs MC) Through Least Squares Linear Regression. Depths A (0-2cm), B (10-12cm) and C (20-22cm).*

The location of T6 in this correlation is consistent with the findings of Robert (2002) which indicate that that increased soil aeration and coverage disturbance were the main factors that stimulated the mineralization of organic matter by soil microorganisms. According to Hurisso *et al.* (2016), ideal soils would have high values of the two C fractions, ensuring long-term organic matter reconstruction with simultaneous short-term nutrient availability. For this purpose, the other treatments were identified with the values of LC in the intermediate line with positive averages (T2 with 468.16, T3 with 450.10, T4 with 451.08, and T5 with 463.54 mg.kg^{-1} respectively).

For the second depth (10-22cm), Treatment 2 (T2) exhibited a greater tendency toward stabilization with LC of 545.72 mg.kg^{-1}. In contrast, the other treatments were located in the lower left quadrant, indicating a tendency toward mineralization. T6 was again notable for having the lowest value of LC at this depth (440.44 mg.kg^{-1}).

Considering the morphology of sugarcane roots and the primary absorption zone (between 15-30cm) Morris, (2005); the correlation analysis identified Treatment 2 (T2) at the 20-22 cm depth with higher averages of LC values.

Position in the upper right quadrant of the plot, T2 was characterized as having a highly significant positive residual effect near the centerline, as recommended by Culman *et al*., (2012). This likely indicates that the mineralization process in T2 at this depth retained an equilibrium C/N ratio.

A partial least squares (PLS) analysis was conducted in 2022 to determine the influence of leaf and soil variables on the response variable, tons of cane per hectare (TCH). This analysis provided standardized coefficients that were used to find relationships between productivity and key variables such as lignin content (LC), as shown in Table 6.

Table 6. *Standardized Coefficients with Leaf and Soil Variables Related to crop yield (TCH).*

Parameter estimations for center and scaled data	Standardized coefficients
Chlorophyll	0.20
Leaf N	**-0.57**
Leaf P	0.24
Leaf K	***0.39***
Leaf Ca	-0.04
Leaf Mg	-0.26
Leaf Fe	0.15
Leaf Mn	**0.30**
Leaf Cu	-0.27
Leaf Zn	-0.21
Leaf B	-0.06
Soil pH	-0.18
SOM - soil	0.10
Soil - P	**0.34**
Soil - K	0.11
Soil - Ca	**0.40**
Soil - Mg	**0.33**
Soil - Na	0.18
Soil - Fe	-0.10
Soil - Mn	**0.60**

Based on the analysis of standardized coefficients, the most influential variables contributing to the crop's biological response (TCH) were identified. The key leaf parameters were N, K, and Mn, while the most significant soil variables were P, Ca, Mg, and Mn. All of these variables had a positive effect on yield, with the exception of leaf N, which had a negative influence, as indicated by a coefficient of -0.57.

The data suggested that the favorable conditions observed in Treatment 2 (T2) were due to a balanced state of soil organic carbon (SOC), where both accumulation and mineralization were occurring in both the surface and 20 cm depth. This dynamic could have contributed to the higher crop productivity in T2, as supported by the positive standardized coefficients of the key soil variables: P, Ca, Mg, and Mn.

As shown in Table 7, treatments with total crop harvest (TCH) values above 200, such as (T2) and (T3) which had residue removal fractions of 20% and 40%, respectively, were associated with high MC values (26.76 and 25.60 $mg.kg^{-1}$,) In contrast, although T6 also had a high HCT (202.46) and a large variation in LC over time, it obtained the lowest LC content (319.58mg.kg^{-1}). This indicates a net loss of carbon (C) storage for this treatment, as illustrated by the negative residual gradient shown in Figure 6, Depth C.

Table 7. *Sugarcane Crop Harvest Biomass Expressed in Tonnes per Hectare (TCH) Reported in 2022 and SOC Fractions at 20-22cm Depth.*

Treatment of residue removal	Average of TCH	LC (mg.kg^{-1})	(%) Change in LC compared to 2018	MC (mg.kg^{-1})
T2 (20%)	**211.66 (a)**	**463.40 (a)**	**43.5**	**26.76 (a)**
T3 (40%)	**210.56 (ab)**	340.02 (a)	32.0	**25.50 (a)**
T6 (100%)	202.46 (ab)	319.58 (a)	26.6	23.18 (a)
T4 (60%)	194.87 (ab)	408.93 (a)	33.2	25.19 (a)
T1 (0%)	188.11 (ab)	399.68 (a)	43.9	28.05 (a)
T5 (80%)	151.81 (b)	434.55 (a)	19.5	19.50 (a)

Means values in the TCH, LC, and MC columns with different letters indicate statistically significant differences, according to the Tukey test ($p \leq 0.05$). SOC: soil organic carbon; LC: labile carbon; MC: mineralizable carbon.

Thus, Figure 7 illustrates a generalized correspondence between the percentage change of LC soil and TCH, in which this R^2=0.76, associating nutrient availability with the standardized coefficients of Table 5.

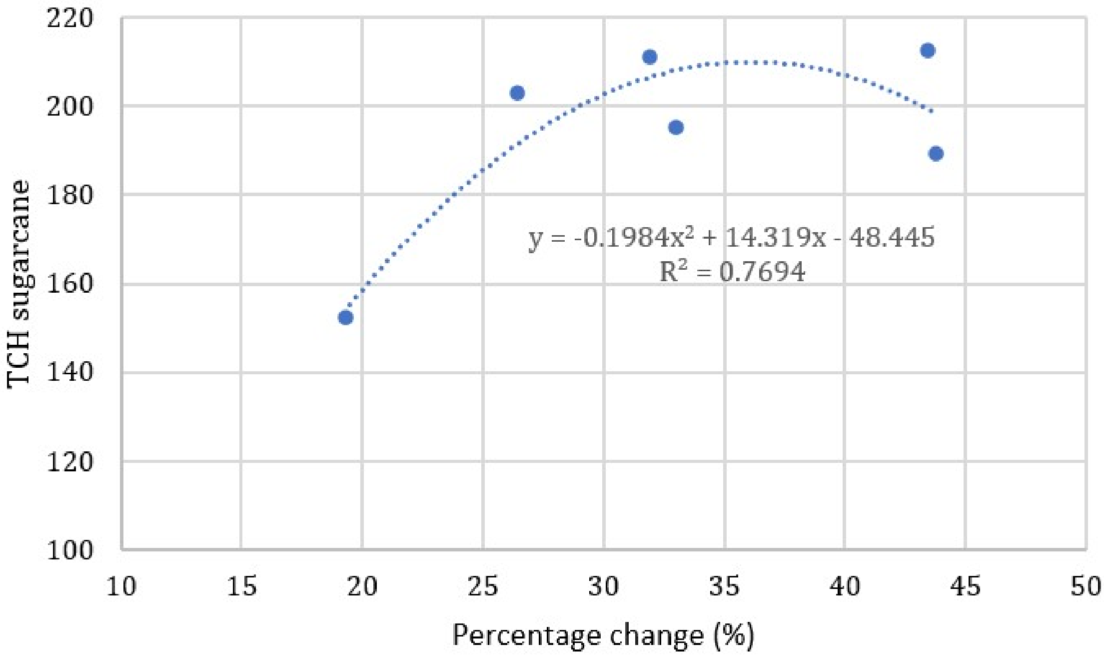


Figure 7. *Relationship between tonnes of Cane per Hectare (TCH) and Labile Carbon Variation (LC) at 20-22cm Depth for 2022.*

This result provided a time correspondence to estimate the variation of LC and the dynamics of SOM stabilization and mineralization under the environmental conditions of the experiment.

DISCUSSION

The higher LC content in T2 can be attributed to favorable environmental conditions, which resulted from a slight change in factors such as moisture, aeration, and temperature compared to T1 (0% residue removal). These conditions likely provided an optimal oxygen level for the enzymatic activity of specific microorganisms, particularly those that produce cellulases and chitinases essential for the decomposition of plant litter. This enzymatic activity promoted the greater accumulation of labile carbon compared to treatments with less soil coverage (40, 60, 80, and 100% residue removal). This finding is consistent with the research of Schnurer *et al.* (1985), who found a significant correlation between cellulase enzymatic activity and soil CO2 evolution, influenced by minor changes in plant residue amounts and soil moisture conditions.

A critical analysis of this research reveals a potential bias introduced by the quantities of crop residues, as current studies often rely on mechanized harvesting methods. This mechanized approach can generate inconsistencies in residue distribution and quantity, which could, in turn, introduce confounding variables and bias the results of future research.

All treatments showed an increase in LC since 2018. However, T6 (without coverage) presented consistently demonstrated the lowest storage values. A balanced condition of temperature, aeration and humidity of T2 (20% removal of crop residues) could have contributed to its superior crop yield.

The moisture content (MC) values were decisive in positioning T6 towards the highest mineralization and lowest storage. This is in direct contrast to T2, which was characterized by the highest positive residual effect. According to Culman *et al.*, (2012), the position T2 near the centerline of the conceptual diagram of soil organic carbon fractions, indicates its favorable conditions.

CONCLUSIONS

Significant differences were recorded when establishing the LC fractions in the three depths. T2 consistently had the highest values, while the total removal of residues resulted in the lowest values. In the second sampling (2022), the LC contents increased across all treatments, maintaining the same downward trend with increasing depth.

The favorable conditions observed in T2 are attributed to the high correlations of LC with key soil properties including clay content, volumetric water content, and gravimetric water content. This strong relationship underscores the significant role of these factors in enhancing soil organic carbon (SOC) storage.

The combined assessment of Labile Carbon (LC) and Mineralizable Carbon (MC) for each treatment provided specific information on how different percentages of sugarcane crop residues influenced the stabilization and mineralization of soil organic matter.

Through the use of standardized coefficients, several variables were identified as having a significant influence on TCH, including leaf elements (N, K, and Mn) and soil elements (P, Ca, Mg, and Mn). Treatment T2 produced the highest average yield (211.66 TCH) and resulted in the most significant percentage increase in labile carbon (LC) at the 20-22 cm depth.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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