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Department of Environment and Development
Environment and Development Engineering



Graduation Special Project
Analysis of soil carbon storage and carbon sequestration potential of
permanent pastures for beef cattle at Zamorano, Honduras

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Abstract

Agriculture is a significant contributor to greenhouse gas (GHG) emissions. Various strategies have been implemented to mitigate this, including grazing systems for beef production and soil carbon (C) sequestration. The potential of these alternatives has not been quantified in the permanent pastures of Zamorano, so the objective of this study was to analyze the soil carbon content and carbon sequestration potential through permanent pasture management for beef cattle during the dry season. Carbon in dry biomass (DM) was estimated using the removal factor of 0.47 ton C/ton DM of aboveground biomass from the Intergovernmental Panel on Climate Change (IPCC) guidelines. Soil organic carbon (SOC) content was determined using the Walkley-Black and loss weight on ignition method. Roots were analyzed using WinRHIZO® to measure length and volume. Normality tests, analysis of variance (ANOVA), and least significant difference (LSD) Fischer tests and measures of central tendency were used to analyze the data. The results indicated that beef cattle pasture in Monte Redondo, sequestered approximately 7,135 ton of carbon in biomass during the dry season. Grazed plots showed higher SOC compared to plots used to harvest hay to feed the cattle suggesting the potential for carbon incorporation through cattle manure. The study showed that SOC content was influenced by soil depth and root growth, with the top 5 cm of soil having a higher concentration of volatile matter (VM). These findings emphasize the importance of managed pastures in carbon sequestration and greenhouse gas (GHG) mitigation. The implementation of grazing systems and the consideration of soil depth and root growth in the selection of pasture species can contribute to enhanced carbon storage in agricultural systems.

Key words: Carbon sequestration, climate change, grazing systems, greenhouse gasses, soil organic carbon

Resumen

La agricultura es un importante contribuyente a las emisiones de gases de efecto invernadero (GEI). Se han implementado diversas estrategias para mitigar este impacto, incluyendo los sistemas de pastoreo en la producción de carne de vacuno y el almacenamiento de carbono (C) en el suelo. El potencial de estas alternativas no ha sido cuantificado en los pastos permanentes de Zamorano por lo que el objetivo de este estudio es analizar el contenido de carbono del suelo y el potencial de captura de carbono a través del manejo de pasturas permanentes para ganado de carne durante la época seca. La fracción de carbono en biomasa seca (BS) se estimó utilizando el factor de remoción de 0.47 t C/t BS, según las directrices del Grupo Intergubernamental de Expertos sobre el Cambio Climático (IPCC). El contenido de carbono orgánico del suelo (COS) se determinó mediante los métodos de “Walkley-Black” y pérdida de peso por ignición. Además, se analizaron las raíces utilizando el “software WinRHIZO®” para medir su longitud y volumen. Los resultados revelaron que los pastos en Monte Redondo secuestran aproximadamente 7,135 ton de carbono en biomasa durante la estación seca. Las parcelas pastoreadas mostraron un mayor contenido de COS en comparación con las parcelas utilizadas para la cosecha de heno, lo que indica un mayor potencial de incorporación de carbono a través del estiércol. El estudio también reveló que el contenido de COS estaba influenciado por la profundidad del suelo y el crecimiento de las raíces, donde los 5 cm superiores del suelo tenían una mayor concentración de materia volátil. Estos hallazgos enfatizan la importancia de las prácticas de manejo de pasturas para optimizar el secuestro de carbono y la mitigación de emisiones de gases de efecto invernadero.

Palabras clave: Cambio climático, captura de carbono, carbono orgánico del suelo, gases de efecto invernadero, sistemas de pastoreo

Introduction

Climate change is a global growing problem that has serious impacts on water resources, biodiversity, ecosystems, productive processes, and health. This phenomenon is linked with global temperature increases and changes in climate patterns as a result of the accumulation of greenhouse gases (GHG) in the atmosphere. In the past century, the concentration of greenhouse gasses has grown exponentially, with carbon dioxide, methane, and nitrous oxide increasing by 150, 40 and 20% respectively since 1950. These increases are mainly due to the burning of fossil fuels and land-use change by agricultural expansion (Intergovernmental Panel on Climate Change [IPCC], 2015).

One of the most important sectors of the economy, both regionally and globally, is agriculture. Globally, in 2020, the agricultural sector contributed an average of 4.3% of gross domestic product (GDP) in terms of value added per country, and in Latin America and the Caribbean, it had a major contribution of 6.9% of GDP (World Bank, 2022). The beef production sector has grown significantly as its major source of protein for human nutrition. In 2019, beef meat demand was 70 million ton and it is projected to increase to 74 million ton by 2023 (Greenwood, 2021).

On the other hand, the Agriculture Forestry and Other Land Use (AFOLU) sector contributes 18.4% of global GHG emissions; with livestock and manure management accounting for 5.8% of these emissions (Ritchie et al., 2020). In the same way, Costantini et al. (2018), states that the main contributors to GHG in the atmosphere are the release of nitrous oxide (N_2O) from animal liquid excreta and the release of methane (CH_4) from cattle enteric fermentation. Although the sector is an important contributor to climate change, it is a key player in reducing poverty, increasing incomes, and improving food security by 80%.

Climate change mitigation consists of a planned intervention where different strategies, policies, and actions focused on the reduction of GHG emissions are incorporated to promote the use and installation of natural sinks that reduce the impacts derived from this problematic (Guerrero Gutiérrez et al., 2014). In this context, different mitigation strategies have been designed and applied

in several agricultural practices. These practices include: Agroforestry, soil conservation for carbon (C) sequestration, manure management, correct application and reduction of agrochemicals, and high biomass production for grazing livestock as a tool for carbon sequestration (Rosenzweig & Tubiello, 2007).

The implementation of grazing systems for meat production is one of the methods used in livestock management as a way of carbon storage, due to the processes of photosynthesis, the natural cycle of carbon in the soil, and the accumulation of organic matter in the soil (Lok et al., 2013). The correct use of grazing systems in livestock production can potentially work as a tool to counteract climate change, however, there is a lack of information corresponding to its mitigation potential.

According to Follett and Reed (2010), grazing lands represent 3.6 billion ha and have a quarter of soil's carbon sequestration potential, capturing about 20% of carbon dioxide emissions for land-use change. Under grazing systems, captured carbon has a permanence from 10 to 50 days in the above layers of the soil and from 1 to 1,000 years underground, containing more than 50% of the captured carbon in the horizons below 30 cm where most root activity is found (Soussana & Lemaire, 2014). Rhizodeposition favors carbon storage due to the physical stabilization of soil organic carbon through the processes of exudation, mucilage production, and root molting (Balesdent & Balabane, 1996; Jones & Donnelly, 2004).

Carbon sinks in grazing land soils are influenced by human activities. Soil carbon can be lost by many factors such as soil disturbances, vegetation degradation, fires, erosion, and water and nutrient shortage in the soil (Soussana & Lemaire, 2014). In tropical and subtropical regions, the primary source of grazing land degradation is the systematic use of population rates that exceed pastures' capacity for recovering from grazing (Dias-Filho, 2014). Soil degradation product of systematic mismanagement causes a decrease in biomass production yields and the quality of biomass that returns to the soil, consequently reducing carbon pools in the soil (dos Santos et al., 2022).

Despite its carbon sequestration potential, there is a lack of information regarding grazing potential and practices to enhance this activity in the Central American region. Investigations conducted in Cerrado, Brazil by Sant-Anna et al. (2017) showed that the change of native forest to grazing lands not necessarily leads to an increase in soil carbon. Also, it indicated that the benefit of the implementation of intensified grazing systems using improved pastures and fertilizers and the implementation of integrated crop-livestock systems is not the sequestration of carbon dioxide from the atmosphere but the reduction of GHG emissions per kilogram of animal.

The estimation of the carbon sequestration potential in different pasture species for beef cattle production in Zamorano will serve as a tool to identify the activities that have a climate change mitigation potential. Moreover, it will serve to identify management practices that can improve this activity and counteract climate change. In addition, this quantification is relevant for the determination of the carbon footprint, which is used as an indicator of environmental performance and marks a path toward carbon neutrality in livestock production. Consequently, it will serve as a baseline to develop improvement techniques for the strengthening of pasture management. Due to the lack of information about the sequestration and storage of carbon that grazing lands can have in existent soils, this study can serve as a baseline to establish different alternatives for livestock and grazing land management in the Central American region.

The main objective of this study is to analyze the potential for carbon content in the soil and its sequestration through permanent pasture management during the dry season, for which the following specific objectives are proposed: a) To estimate the carbon sequestration potential for Circuit No. 12 used for beef cattle nutrition, b) To determine the relationship between soil organic carbon (SOC) and soil volatile matter content, c) To analyze the soil organic carbon (SOC) distribution at different depths and d) To analyze the relationship between soil carbon content and root growth of the pastures under study.

Materials and Methods

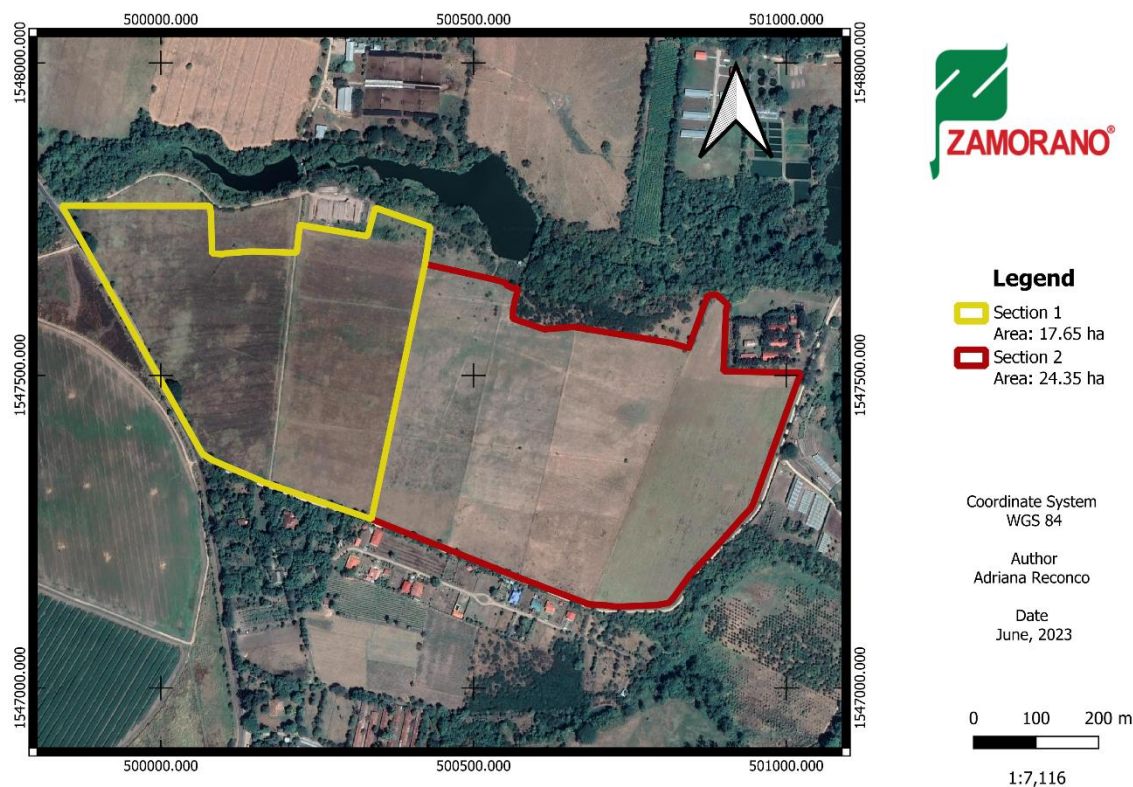
Study Site

The study was carried out in the permanent pastures of Monte Redondo's Circuit No. 12 (Figure 1), at the Pan-American Agricultural School, Zamorano, located at 800 meters above sea level in the municipality of San Antonio de Oriente, department of Francisco Morazán, Honduras (coordinates, 14°00'24"N 87°00'58"W). Circuit No. 12 covers an area of 42.16 ha divided into six sub-circuits. These pastures are used by livestock for meat production on a rotational basis and for hay harvest. The area is in a humid tropical climate, which experiences two different seasons a year: the rainy and the dry season. The rainy season lasts from May to October and the dry season from November to April. The annual rainfall in this territory averages 1,048.453 mm and the average monthly temperature is 23 °C (Hernández Castro, 2021).

For this study, Monte Redondo's Circuit. No. 12 was divided into two sections: subcircuits one and two, which are used for harvesting hay to feed the cattle during the dry season, and subcircuits three, four, five, and six which are used for grazing the cattle during the rainy season (Figure 1). Each section was then divided into three equal plots according to the section's area. The first section consisted of an estimated area of 17.65 ha with a subdivision of 5.88 ha plots. Likewise, the second section consisted of an estimated area of 24.35 ha and each plot of 8.12 ha.

Figure 1

Circuit No. 12 Monte Redondo's beef livestock unit, Zamorano, Honduras



Estimation of Carbon Sequestration per Aboveground Biomass

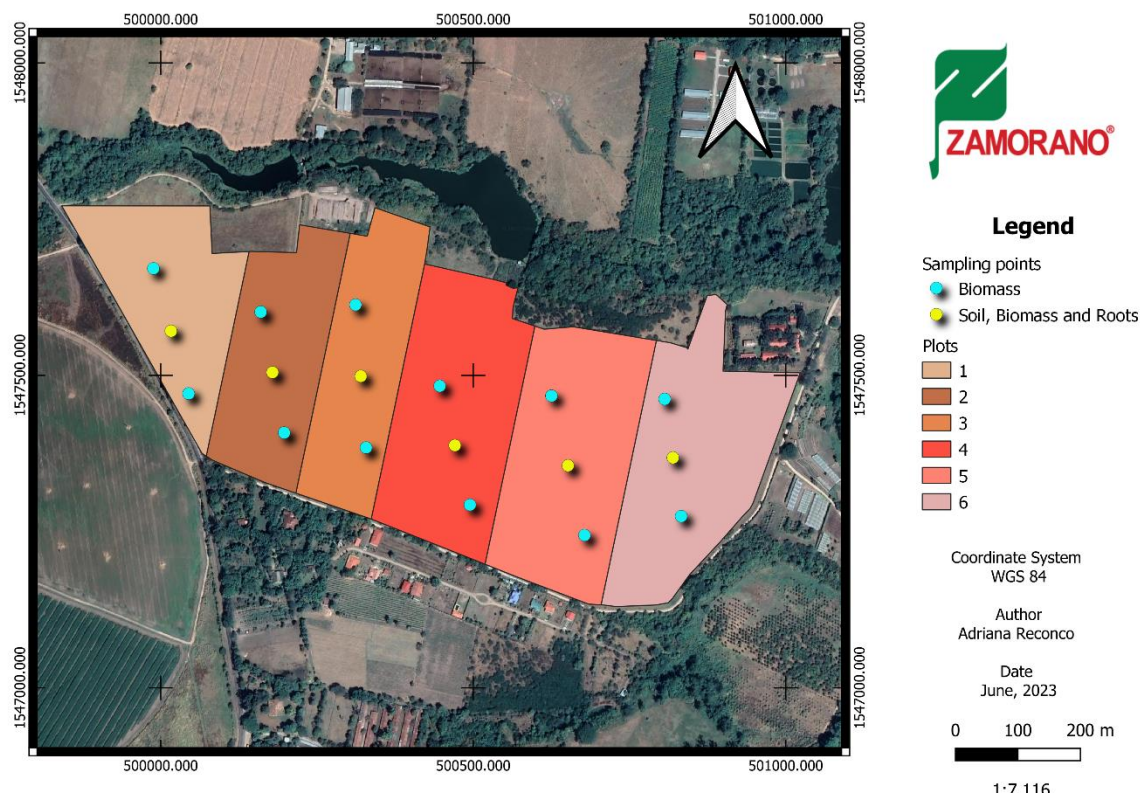
To estimate the carbon sequestration potential of the pastures in each plot, factors such as biomass production per year per cycle, which varied according to the management practices used in each plot, and the dry matter content within the biomass were considered.

Mapping and Identification of Pastures Species

The first activity carried out in this study was the mapping and identification of the existent pastures found in Monte Redondo's Circuit No.12. As a first step, a semi-structured interview (Annex A) was conducted with the person in charge of the Zamorano beef cattle unit to collect information on the existing pasture species, their management, and use during the different seasons of the year. Once the information from the interview was collected, a field visit was carried out to identify the existent pastures and their proportion in each plot. This was done with the help of an expert in the

field who identified the different pasture species in the area. The location of the pastures was then determined.

The reference coordinates were assessed through the GPS receptor Garmin eTrex 10®. Then, the coordinates X, Y, Z of each one of the pasture plot limits were collected and downloaded in comma-separated values (CSV) format. The software QGIS® version 3.24.3 (Quantum Geographic Information System [QGIS]) was used to download the coordinates in GPX format and then turned into a shapefile format. The coordinates in shapefile format were digitalized and turned into polygons in shapefile format, one per plot. Afterwards, the area of the polygons was determined using the QGIS® tool, field calculator. Using QGIS® complement, cadastral, a division of No. 12 Circuit was made into two sections, the first being made up of subcircuits one and two and the second one being made up of subcircuits three, four, five, and six (Figure 2). With the same tool, the division of the subcircuit was made for its identification. For sampling purposes, diagonal lines were traced in the plots where three strategic points were located: two at the end of each line and one point respectively in the midpoint of the lines.

Figure 2*Plots and sampling points****Biomass Sampling***

A systematic process of plots methodology was used to conduct biomass sampling to assess dry biomass content per unit area on the study site during the dry season. In plots one, two, and three, samples were taken right before hay harvesting which is done twice a year every six months. Likewise, in plots four, five and, six samples were recollected after the end of the pasture's resting period (55 days). Three samples were taken per plot, two at the extremes and one at the midpoint of each drawn diagonal line (Figure 2 and Annex B).

For sampling, a 1 m² measuring wooden square table was used to delineate the biomass sampling area. At the end of the rotational grazing period, the pastures were mown at a height of 10 cm with a large pruning shear to simulate herd grazing and collected in sacks to avoid possible damage from moisture (Villalobos & Arce, 2014). The samples were then taken to Zamorano's Stove Center

where they were weighed and registered. After weighing, the samples were transferred to the Bioenergy Laboratory of the Environmental Development Department for its analysis.

Estimation of Carbon Fraction from Biomass

Biomass is composed of total solids and moisture. The concentration of moisture in biomass was determined using the ASTM D3173-11 Standard Test Method for Moisture in the Analysis Sample of Coal and Coke (Annex C). The result of the moisture content is used to determine the dry matter content of biomass, which is used to calculate the carbon content of the biomass.

Samples were homogenized and then three 1 g subsamples were extracted. The subsamples were dried in an oven set at a temperature of 105 °C for a duration of 24 hours. After the drying process, the subsamples were weighed to determine the moisture content of each sample (Equation 1).

$$\text{Moisture \%} = \frac{(\text{initial weight}) - (\text{weight after } 105^{\circ}\text{C}) \times 100}{\text{initial weight}} \quad [1]$$

Average moisture was calculated for each plot using data from each point. Dry matter was calculated using average moisture and fresh biomass weight for each plot. This was obtained through by following the Equations 2 and 3.

$$\text{Dry matter per plot kg} = \left(\text{Dry matter } \frac{\text{kg}}{\text{m}^2} \right) \times (\text{Area m}^2) \quad [2]$$

$$\text{Total dry matter (kg)} = \sum \text{dry matter per plot kg} \quad [3]$$

The carbon content of the pastures was determined from each plot's dry matter content (kg/m²). Using the Intergovernmental Panel on Climate Change IPCC (2006) Guidelines for National Greenhouse Gas Inventories Volume 4 Agriculture, Forestry and Other Land Use (2006) Tier 1 categorization of tropical and subtropical zones for herbaceous biomass, a removal factor of 0.47 ton

of carbon per ton of aboveground biomass was applied (Equations 4, 5, and 6). The results of this analysis resulted in the amount of carbon found in the biomass at Monte Redondo 's Circuit No. 12.

$$\text{Biomass carbon content } \frac{t}{ha} = \left(\text{dry matter } \frac{kg}{m^2} \right) \times (0.47 \text{ tonne (ton d.m)}) \quad [4]$$

$$\text{Biomass C per plot } t = \left(\text{Biomass carbon content } \frac{t}{ha} \right) \times (\text{Area } ha) \quad [5]$$

$$\text{Total biomass C } t = \left(\sum \text{Biomass C per plot } t \right) \quad [6]$$

The statistical analysis was performed using Infostat® software. A normality test was carried out on the data to assess whether they were homogeneous and normal distributed. Then, an Analysis of Variance (ANOVA) was performed to determine if there were significant differences between the volatile matter (VM) and biomass observed in each plot. The ANOVA was performed at a 95% confidence level. A Fisher's LSD test was then performed to identify any statistical differences between the volatile matter and moisture content of the biomass in the different plots.

Soil Organic Carbon

Soil organic carbon is defined as the carbon found in soil that is derived from organic origins (Stockmann et al., 2013). The concentration of soil organic carbon between depths can vary by many factors like its texture, density, root activity, biomass density, and the management practices implemented in the area. A direct method using Walkley-Black analysis and an indirect method using volatile matter content were used to estimate the Soil Organic Carbon (SOC) content.

Estimation of Soil Organic Carbon (SOC) from Soil Volatile Matter

Soil Sampling

Sampling pits were excavated in the midpoints of the traced diagonal lines in each one of the plots. To determine if volatile matter is a good determination of soil organic carbon two samples were collected with a small hand shovel at depths ranging from 0 cm to 30 cm and from 30 cm to 80 cm

(Annex D). The samples were sent to Zamorano's Soil Laboratory (LSZ) and the Bioenergy Laboratory of the Environmental Development Department for its analysis.

Estimation of Soil Carbon Content from Volatile Matter

Two methods were used to estimate soil organic carbon concentration: Walkley-Black and Loss of Weight on Ignition (LOI) (Table 1). The soil organic carbon (SOC) of the twelve samples collected at the depths of 0 to 30 cm and 30 to 80 cm was estimated by the Walkley-Black method. Additionally, the same soil samples (0 to 30 cm and 30 to 80 cm) were subjected to LOI at 600 °C (Annex E) to compare both methods and to determine if volatile matter obtained by LOI is a good estimation for soil organic carbon. According to Izquierdo Bautista and Arévalo Hernández (2021) results obtained by both methods display a strong correlation making LOI a valid alternative to save time and resources.

Table 1

Methods for SOC and volatile matter analysis

Analysis	Method	Reference
Soil organic carbon	Walkley-Black	Black SSSA Book series. Methods of soil analysis Part 3 Chemical Methods
Volatile matter	Loss weight on ignition	

For the Walkley-Black analysis, the Black SSSA Book series. Methods of Soil Analysis Part 3 (Klute, 1986-), (Chemical Methods) was used to guide the procedure. In this method, organic carbon was determined by the milliequivalents (meq) of potassium dichromate ($K_2Cr_2O_7$) which reacts with the soil to oxidize organic carbon. This includes a proposed Equation 7, that is based on the difference between the total meq of potassium dichromate added and the meq of ferrous sulfate titrated. The formula assumes that the oxidation state of carbon is 0. Carbon in organic matter is oxidized to become carbon dioxide CO_2 with an oxidation state of + 4, which has a carbon equivalence of 3 g.

$$OC\% = (meq\ k_2Cr_2O_7 - meq\ FeSO_4) \times (0.003\ g\ C\ meq - 1) \times cf \div soil\ g \times 100 \quad [7]$$

With the Loss of Weight on Ignition method, the samples were subjected to a temperature of 600 °C using a muffle furnace. Initially, the humidity of the samples was determined. To begin, a 10 g soil sample was placed in a porcelain crucible that had been previously tared at 600 °C. The initial mass of the sample was recorded. Subsequently, the samples were exposed to a temperature of 105 °C for 48 hours to remove humidity from the soil. Using the same samples undergone through a drying process, 3 g subsamples were extracted in porcelain crucibles tared at 600 °C and placed in the oven at 105 °C for a further eight hours for stabilization. The samples were then subjected to two hours in the muffle at 600 °C. Each time the samples were exposed to a thermal process, their weight was recorded to calculate humidity and volatile matter. The following Equations 8 and 9 were used to determine the humidity, volatile matter present in the samples:

$$\text{Humidity \%} = \frac{(\text{initial weight}) - (\text{weight after } 105^{\circ}\text{C}) \times 100}{\text{initial weight}} \quad [8]$$

$$\text{Volatile matter \%} = \frac{(\text{weight after } 105^{\circ}\text{C}) - (\text{weight after } 600^{\circ}\text{C}) \times 100}{\text{initial weight}} \quad [9]$$

A correlation analysis was performed using the results from plots 1 - 6 and depth ranges of 0 to 30 cm and 30 to 80 cm, respectively. This correlation analysis aimed to determine if there was a relationship between the concentration of VM determined through LOI and the SOC determined through Walkley-Black method. This was done to validate the VM concentration as an indicator of soil organic carbon content. A linear regression was then applied to determine if there was a statistical relationship between the variables and obtain an equation for determining SOC from VM.

Determination of Soil Organic Carbon at Different Depths

Soil Sampling

The analysis of the relationship between soil carbon content at different depths started with the excavation of soil sampling pits. One pit per plot was made at the midpoint of the drawn diagonal lines. Samples were taken with a small hand shovel at the depths of 0 - 5, 5 - 10, 10 - 20, 20 - 30, 30 -

40, 40 - 60, and 60 - 80 cm in each pit (Oliveira et al., 2021; Sant-Anna et al., 2017) . The samples were stored in plastic Ziplock bags and labeled for their identification. Additionally, samples for texture analysis and the apparent density (PB) were taken in the same location where the pits were made, the samples for this last analysis were recollected using a 100 cm³ metal ring. Samples were taken to Zamorano's Soil Laboratory (LSZ) where apparent density (PB) and texture of the soil were evaluated. Samples were then prepared by allowing the soil to dry in plastic trays at room temperature for a few days until it was visibly dry. All soil samples except PB samples were ground in a porcelain mortar and sieved through a 2 mm sieve (Soon & Abboud, 1991).

Soil Characterization

To determine the apparent density of the soil samples, a semi-analytic scale was employed, where a plastic bag was initially weighed to establish a baseline measurement. Dried portions of 100 cm³ for each sample were placed in individual plastic bags and weighed. This process was repeated for each plot to determine the mass of the respective soil volume sample. By evaluating the weighted mass, the apparent density of each plot's soil was subsequently determined (Equation 10).

$$PB \left(\frac{g}{cm^3} \right) = \frac{\text{soil's mass } g}{100 \text{ cm}^3} \quad [10]$$

At Zamorano's Soil Laboratory, the soil texture analysis for each plot was conducted using the Hydrometer Method (Annex C). The primary objective was to investigate any potential relationship between soil texture and carbon content. To begin the procedure, 50 g of soil was carefully weighed and placed in graduated cylinders. Then, 100 mL of NaOH solution was added to each cylinder. The samples were allowed to settle undisturbed for 24 hours. After the designated time had elapsed, the contents of each graduated cylinder were stirred until there were no visible sediments in the solution. The solution was then made up with water to reach a final volume of 1,000 mL. After the adjustment, hydrometer readings were taken at 40 seconds and 2 hours. Based on the obtained results, the soil texture of each plot was subsequently determined (Table 2).

Table 2*Texture and apparent density analysis methods*

Analysis	Method	Reference
Soil texture	Texture by bouyoucos	LSZ-MT-P12
Apparent density	Cylinder method	

Importance of Soil Characterization

A comprehensive soil characterization plays a pivotal role in understanding the intricate relationship between soil properties and the content of soil organic carbon (SOC). Various soil properties can directly influence the capacity of carbon storage within the soil, as they can significantly impact the biological, chemical, and physical processes occurring within the soil matrix.

Estimation of Soil Organic Carbon

To estimate the soil organic carbon, samples collected at (0 - 5, 5 - 10, 10 - 20, 20 - 30, 30 - 40, 40 - 60, and 60 - 80 cm) were analyzed under the LOI method at 600 °C to determine its volatile matter. The SOC content was determined from the volatile matter using the regression-based model formula derived from the linear regression submitted to the Walkley-Black method and LOI. Correspondingly, the volume, the apparent density, and organic carbon concentration of the soil were used to obtain the total SOC content per plot and the total area of Monte Redondo's Circuit No. 12 (Equations 11, 12, and 13):

$$SOC \text{ kg} = (\text{soil's volume } m^3) \times \left(PB \frac{kg}{m^3} \right) \times (SOC \%) \quad [11]$$

$$Total \text{ SOC } \frac{kg}{ha} \text{ per plot} = (SOC \text{ kg}) \times (\text{area ha}) \quad [12]$$

$$Total \text{ SOC } \frac{kg}{ha} = \sum \text{ total SOC per plot} \quad [13]$$

To conduct a statistical analysis of soil several procedures were carried out. Firstly, a normality test was conducted to assess the homogeneity and normal distribution of the data from plots one to six and depth ranges of 0 - 5 cm, 5 - 10, 10 - 20, 20 - 30, 30 - 40, 40 - 60, and 60 - 80 cm. Then, an Analysis of Variance (ANOVA) was performed under a blocks experimental design to determine the presence of significant differences the concentration of volatile matter among the different plots and depth ranges. Subsequently, an LSD Fischer test was applied to identify any existing statistical differences between the volatile matter content among the various plots and depth ranges. The software Infostat® was used to do all correspondent statistical analysis.

Analysis of the Relationship Between Soil Carbon Content and Root Growth

Soils represent the largest biogeochemically active carbon reservoir on a global scale and are crucial in maintaining the stability of atmospheric CO₂ concentrations (Jackson et al., 2017). Within the carbon cycle, roots play a significant role in carbon stabilization within the soil environment. Rhizodeposition, the process by which plants sequester carbon through photosynthesis and subsequently deposit a portion of the captured carbon into the soil via their roots, is a key factor in this natural cycle. This mechanism contributes to the long-term storage of carbon in the soil, thus impacting overall carbon dynamics and influencing the global carbon balance.

Root Samples

Pasture roots are a key factor for carbon sequestration and storage both for the plant and for the soil. At the midpoint of the traced diagonal lines, the same point where the pits were made, samples of the entire root pasture were taken for characterization. Using a pickaxe, hoe, and shovel three plants with their entire root system were extracted from the soil at a depth of 30 cm, taking care not to damage the plant. The samples were stored in a plastic bag for transport to the laboratory of the Bean Research Project (PIF) where they were kept in a refrigerator until analysis. At the time of analysis, the samples were washed to remove soil adhered to the roots. The samples were cut to

separate the stem and root and then scanned with the software WinRHIZO® to determine the root's volume and length (Annexes F and G).

In this investigation, the analysis of roots was conducted from a descriptive standpoint. The collected data underwent a thorough analysis to determine measures of central tendency and dispersion. This analysis resulted in the calculation of the mean and standard deviation, providing valuable insights into the central values and variability of the obtained root data.

Results and Discussion

Mapping and Identification of Circuit No. 12, Monte Redondo

A semi structured interview was conducted to the person in charge of the beef livestock unit of the Panamerican Agricultural University. Through the interview, a detailed characterization of the original established divisions of the circuit (Figure 3) according to its usage was made, including the pasture species found, their use and management. The pastures of Monte Redondo 's Circuit No.12 were established 20 years ago and re-established 10 years after their initial establishment.

Different types of pastures were found in the study area, such as: *Cynodon nlemfluensis*, *Digitaria eriantha*, *Panicum maximum* (cv. Tanzania), and *Panicum maximum* (cv. Tobiata). The area is divided into six subcircuits in which all with the same type of pasture, but in different proportions (Error! Reference source not found.4). In subcircuits one and two most of the pastures are *Cynodon nlemfluensis*, *Digitaria eriantha* and the other species are found in smaller proportions. These are used to harvest hay to feed the livestock in the dry season two times per year, with a six-month difference. On the other hand, subcircuits three, four, five, and six contain in greater proportion *Cynodon nlemfluensis* and *Panicum maximum*, and the other pasture species a lower proportion which are used for cattle grazing.

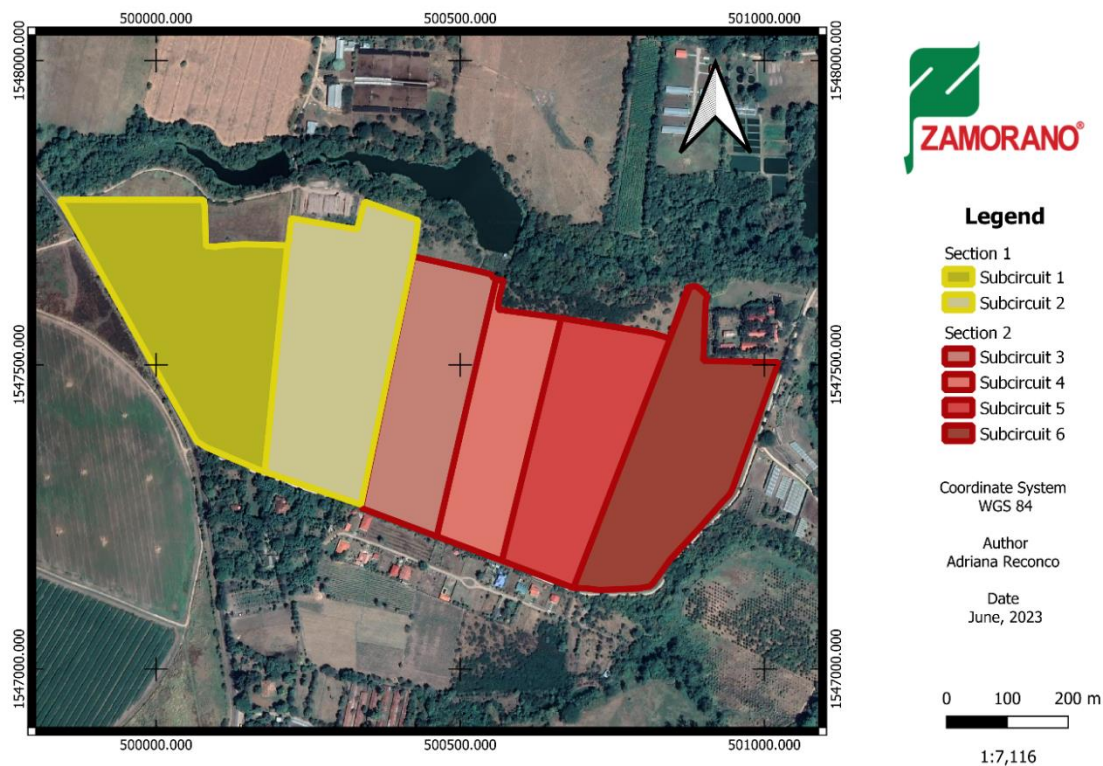
Cynodon nlemfluensis is a low-growing type of pasture that possesses a dense and expansive carpet-like appearance due to its finely textured turf. It is characterized by a prostrate growth pattern, with stems capable of rooting at the nodes, facilitating horizontal spread (Rojas-Sandoval & Acevedo-Rodríguez, 2014). Conversely, *Digitaria eriantha* exhibits an upright growth habit, with its stems reaching heights ranging from 30 to 120 cm (Tow et al., 1997). *Panicum maximum*, specifically the cultivars Tanzania and Tobiata, is a tall grass known for its tendency to form large, upright clumps or tufts. It has a clump-like growth habit, with individual plants growing densely in distinct clusters (Agyare et al., 2013).

The herd was previously grazed under an intensive rotational grazing system, but then switched to a Voisin rotational grazing system during the rainy season and an ultra-high density grazing management system during the critical dry season. The cattle herd is constituted of 600 animals conformed by pure breed of Brahman and the rest as crossbreeding between Brahman and Senepol, Beefmaster, Drahmaster, Charolais and Brown Swiss, yet not all the herd grazes simultaneously in the same circuit. The carrying capacity of the herd in the plots designated for grazing is designed to provide the animal with 10% of its live weight in fresh biomass per day considering the factor that there is 1 pound of fresh biomass per m². The herd is left no more than three days to graze to avoid plow pan and a good development of the plant for the next grazing period. Furthermore, the regrowth period consists of 50 to 55 days.

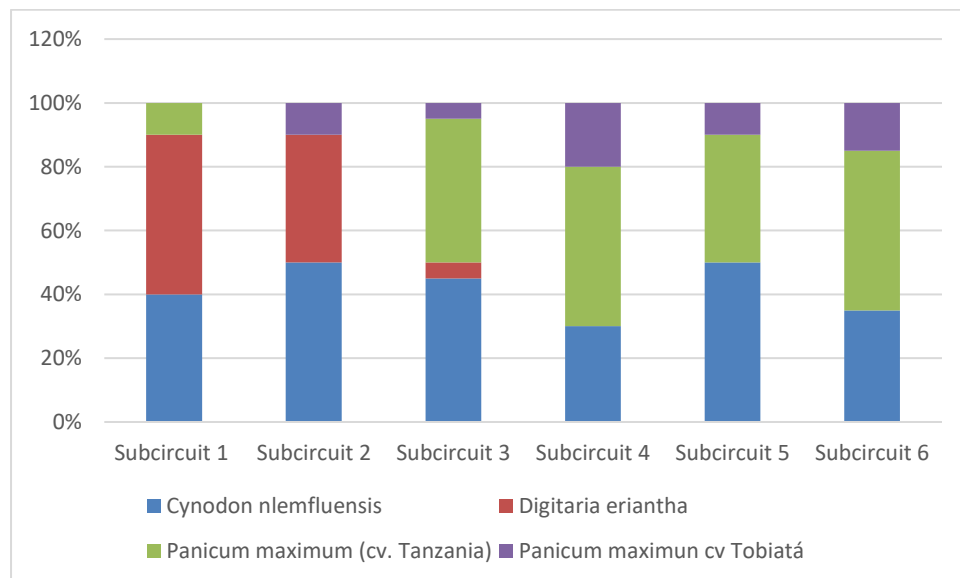
Regarding the pasture's management, fertilization of the pastures is done once or twice a year with DAP, SUL-PO-MAG, Nitro Xtend, and potassium chloride (KCL) with an application of 500 pounds per hectare of a mix of two parts of all fertilizers mentioned above and one part of SUL-PO-MAG. During the field study conducted in the company of an expert, valuable information regarding the pasture species and their specific locations within the circuit was corroborated. Furthermore, the field visit unveiled a significant prevalence of the weed *Sporobolus poiretii* throughout the circuit, particularly concentrated in plots one, two, and three.

Figure 3

Circuit No. 12, Monte Redondo original established divisions according to pasture usage

**Figure 4**

Proportions of pasture species in subcircuits



Estimation of Carbon Sequestration per Biomass

An estimation of biomass dry matter (DM) was conducted. The results from the new divided plots for the purpose of this investigation of Monte Redondo's Circuit No.12 indicate that plot one had the highest dry matter production per square meter and plot five the lowest (Table 3). Using the obtained dry matter content, the carbon fraction in biomass was estimated by applying a conversion factor of 0.47 ton of carbon per ton of aboveground biomass. The results indicated that approximately 7,135 ton of carbon were sequestered in biomass within Circuit No. 12 (Table 3). It is important to note that this estimation was done in dry season resulting in a reduced quantity of biomass observed and collected. It is also important to note that only one cycle of each plot was considered. Plots one, two, and three have higher carbon content per plot, but the cycles for these plots last 6 months, and a cycle for plots four, five, and six lasts only 55 days.

An investigation was conducted in Costa Rica where different grassland systems were evaluated to estimate their carbon sequestration potential (L. 't Mannetje et al., 2008). The results of this study show that degraded pastures had an average annual mean amount of 0.65 ± 0.07 t/ha of C in available DM. The results obtained in Circuit No. 12 (Table 3), indicate that an average of 0.292 t/ha of C were captured in biomass in section one (plots one, two, and three) and 0.082 in section two (plots four, five, and six). However, the data obtained from Monte Redondo, was considering only one cycle during the dry season. With this data an annual estimation was determine resulting that in section one a total of 0.584 t/ha of C (considering it has two cycles per year) and in section two 0.41 t/ha of C (considering it has five cycles per year).

When comparing both studies, carbon content per hectare in Circuit No. 12, Monte Redondo, has significantly less carbon sequestration. Yet, it is important to take into consideration that the data obtained in the study conducted in Costa Rica were evaluated over of two years considering both rainy and dry seasons leading to a possible increase in dry matter content in the rainy season resulting in greater carbon sequestration.

Table 3*Carbon sequestration content per plot per cycle*

Plots	Dry matter (kg/m ² /cycle)	Carbon t/ha	Carbon t/plot/cycle
P1	0.915	0.430	2.530
P2	0.506	0.238	1.399
P3	0.441	0.207	1.219
P4	0.170	0.080	0.647
P5	0.154	0.073	0.589
P6	0.197	0.092	0.750
Total			7.135

Note. P mean plots**Volatile Matter and Soil Organic Carbon**

When the SOC values obtained from the Walkley-Black method were correlated with the volatile matter content, a high positive correlation was found between these variables. The correlation coefficient ($r = 0.82$) indicated a high positive correlation between SOC and VM. This suggests that as the VM content increases, the soil organic carbon (SOC) content also increases. These findings contribute to a better understanding of the relationship between VM measured from LOI and SOC measured with the Walkley-Black method, providing valuable insights for further research and practical applications in soil management and carbon sequestration efforts.

Based on the linear regression applied to the data (Table 4), a regression-based model was obtained to estimate SOC from soil's VM (Equation 14). Additionally, the soil organic matter explains approximately 66% ($R^2 = 0.66$) of the variance in the volatile matter of the sample (Figure 5 and Annex H). This implies that a substantial proportion of the variability observed in the volatile matter can be accounted for by variations in the soil organic matter (Annex H).

Table 4*Linear regression volatile matter and soil organic carbon*

Coefficient	Estimates	P-value
Soil organic carbon	-1.02	0.0410
Volatile matter %	0.37	0.0012

$$y = -1.02 + 0.37x \quad [14]$$

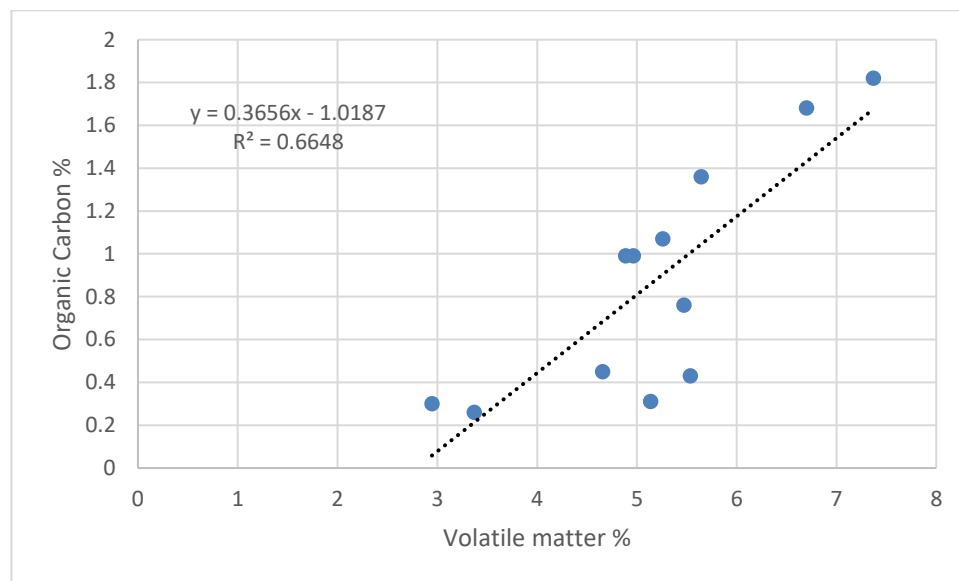
Where:

y = soil organic carbon

x = volatile matter content

Figure 5

Linear regression for volatile matter and soil organic carbon



Soil Organic Carbon (SOC) Distribution at Different Plots and Depths

The analysis of variance (ANOVA) with a block design helps to assess the influence of the depths on the VM data excluding the possible influence of the blocks, which in this case are each one of the six plots. This analysis showed evidence of significant differences ($p \leq 0.05$) between the volatile matter content of the soil across different plots and depths (Table 5 and Annex I). These findings suggest a clear relationship between the volatile matter content and both the plots and soil depths studied.

Table 5*Analysis of variance (ANOVA) of volatile matter, plots, and depth*

Variables	F-value	p-value
Model	2131.57	<0.0001
Plot	15.85	<0.0001
Depth	23.43	<0.0001

The results obtained through the LSD Fischer test showed that plot six is statistically different from the rest of the plots having a higher content of volatile matter (mean = 7.8%), indicating that it has a higher SOC (Table 6). On the contrary plots two and four showed statistical differences between other plots, yet statistical similarity among them. These two plots contain the least amount of volatile matter content in the soil compared to the rest of the plots indicating that they have the least amount of SOC content.

Table 6*LSD Fischer Test of soil volatile matter content and plots*

Plot	Mean	S.E.				
P6	7.8	0.35	A			
P5	6.24	0.28		B		
P1	5.6	0.25		B	C	
P3	5.17	0.22			C	D
P4	4.89	0.21				D
P2	4.73	0.2				D

Note. P means plots

Volatile matter concentration within circuit's plots can be due to different factors one of them being soil's texture and clay content. According to Silver et al. (2010) there was a positive correlation between soil carbon pools and clay content in the soil indicating that when clay content increased, the soil carbon pools also increased. In the context of this investigation, a texture analysis was conducted, revealing that plot five and six exhibited the highest clay content among all the analyzed plots (Table 7). This notable clay composition in plot six provides is a possible explanation for its higher carbon content when compared to the other plots.

Another factor that could potentially influence the soil organic carbon (SOC) content is soil management. Studies have suggested that well-managed soils generally exhibit higher SOC content and better carbon dynamics compared to degraded grasslands (Maia et al., 2009). During the field visit, plots one, two, three, and four were observed to have a notable presence of the weed species such as *Sporobolus poiretii*. The high prevalence of weeds could be indicative of lower grassland quality. As stated by Peprah (2015) the presence and increase of weeds serve as a biological indicator of land degradation. In contrast, plot six displayed the least presence of weeds, which may suggest a better quality and more likely to have higher content of SOC.

Table 7

Soil texture, granulometry and apparent density

Plot	Apparent density (g/cm ³)	Texture	Granulometry %		
			Sand	Silt	Clay
P1	1.232	Loam	44	40	16
P2	1.227	Loam	38	38	24
P3	1.024	Loam	34	42	24
P4	1.249	Loam	34	44	22
P5	1.302	Loam	34	40	26
P6	1.499	Clay loam	30	36	34

Note: P means plots

Several studies have shown that there is a negative strong correlation between SOC and apparent density PB (Alvarado et al., 2013; Chaudhari et al., 2013; Sakin, 2012). In this investigation, comparing the SOC in the plots and its corresponding density yielded opposite results. In this context, plot six has a greater VM content (mean = 7.8) indicating a greater SOC content, yet a higher PB than the rest of the plot. These findings can be due to the presence of cattle which can have contrasting effects on soil properties. On one hand, livestock activity contributes to soil compaction, as evidenced by previous studies (Lai & Kumar, 2020), which in turn can lead to increased apparent density (PB) (Shah et al., 2017). However, the incorporation of carbon into the soil is influenced by the grazing intensity, whereby greater grazing intensity can result in a higher carbon input by manure excretions (Cecagno et al., 2018).

When comparing volatile matter content with soil depth ranges in the plots, LSD Fischer test revealed depths from 0 to 5 cm have a greater volatile matter content (mean = 9.2) (Table 8). Depths between 30 to 40 cm and 20 to 30 cm are statistically different from the rest of the studied depth ranges holding the lowest amount of volatile matter (mean = 4.47).

Table 8

LSD Fischer Test of soil volatile matter content and soil depth

Depth (cm)	Mean	E.E.			
0 - 5	9.2	0.4	A		
5 - 10	6.61	0.24		B	
10 - 20	5.51	0.14			C
40 - 60	4.95	0.42			C
60 - 80	4.95	0.32			C
30 - 40	4.76	0.28			D
20 - 30	4.19	0.29			D

Variation in carbon content with soil depth can be due to several factors. Higher carbon concentrations in the topsoil may be due to vegetative activity such as plant litterfall and root activity which leads to an accumulation of soil organic matter and consequently an increase in soil organic carbon (Laskar et al., 2021). In the top centimeters of the soil, higher carbon concentration are observed as a result of root activity in the rhizosphere. Roots release various carbon-containing substances into the rhizosphere where these substances undergo additional transformations resulting in the formation of a wide range of carbon-containing small and large molecules that can persist in the soil and influence its structure (Kell, 2012).

A study done in Boone County, Iowa, USA revealed similar results where the majority of soil organic carbon content found in maize and prairie soils was found in soil depth below 20 cm (Dietzel et al., 2017). A study done by Tückmantel et al. (2017) revealed that root exudation diminishes substantially with increasing soil depth. This decline in exudation is primarily attributed to a significant decrease in the specific exudation activity per unit of root mass, which diminishes to approximately

one-fifth of the activity observed in the topsoil. The decrease of root exudation can lead to low levels of SOC content as fewer organic compounds are being incorporated into the soil.

Given the substantial proportion of soil organic carbon (SOC) that was found at depths ranging from 0 - 5 cm, the estimation of carbon content in Monte Redondo's Circuit No.2 was specifically focused on this depth range. Results from this calculation revealed that approximately 639,132.01 kg of SOC were found at the soil depths of 0 - 5 cm considering the total area in each plot of Monte Redondo's Circuit No.12 (Table 9 and **Error! Reference source not found.**).

Table 9

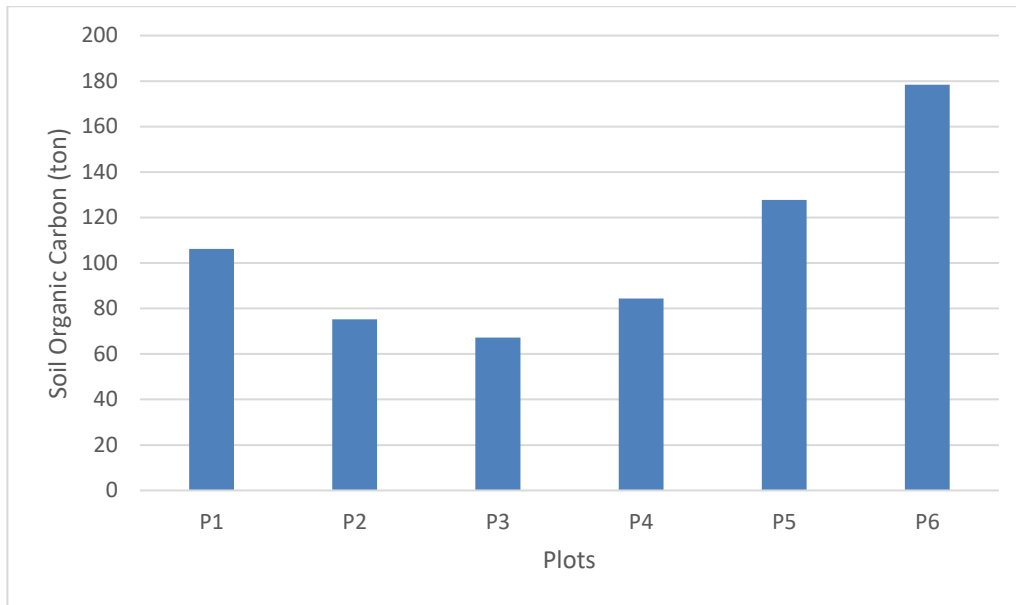
Soil organic carbon content from 0 - 5 cm

Plot	Depth cm	SOC %	SOC (kg)
P1	0-5	2.9	106,186.79
P2	0-5	2.1	75,203.83
P3	0-5	2.2	67,254.28
P4	0-5	1.7	84,315.26
P5	0-5	2.4	127,789.22
P6	0-5	2.9	178,382.63
Total			639,132.01

Note. P means plots

Figure 6

Soil organic carbon content per plot (0 - 5 cm)



Analysis of the Relationship Between Soil Carbon Content and Root Growth

Root analysis

Roots were analyzed using the software Whinrizo to determine their total length (cm) and volume (cm³). When analyzing the data, results showed, under an observational perspective, that roots in plot six have a bigger volume and length in their roots than the other plots (Table 10). This may contribute to a higher SOC content due to increased root activity. Soil's biological activity and associated processes such as nutrient cycling are mainly concentrated in the area around living plant roots and influenced by root activity (Pierret et al., 2007). Rhizodeposition is a key factor for carbon content found in soil as it consists of the release of organic compounds by exudation which are a flux for organic C pools in soil (Nguyen, 2009). As Tückmantel et al. (2017) revealed in their investigation, root morphology has a big influence on carbon exudation, the bigger the morphological parameters of the roots (volume, length, and surface area) the bigger the C flux done by exudation.

The variation in pasture species composition and their respective proportions can account for the observed differences in volume and length measurements across the plots. Plots found in section

one (plot one two and three) contain on average 45% of *Cynodon nlemfluensis* and *Digitaria decumbens* Stent, and 5% of *Panicum maximum* cv. Tanzania and *Panicum maximum* cv. Tobiata. Moreover, in section two existent pasture species and their proportion changed having on average 40% of *Cynodon nlemfluensis*, 5% of *Digitaria decumbens* Stent, 45% of *Panicum maximum* cv. Tanzania, and 10% of *Panicum maximum* cv. Tobiata. One of the dominant pasture species found in plots of the first section is *Digitaria decumbens* Stent. Unlike the other species in Circuit No.12, this one has fibrous roots which only extend in the first of the soil's horizons whereas *Panicum maximum* cv. Tanzania and cv. Tobiata and *Cynodon nlemfluensis* which have denser fibrous roots and can reach deeper into the soil profile (Table 10). These characteristics between different pasture species among plots were clearly observed.

Table 10

Root's total length and volume per plot

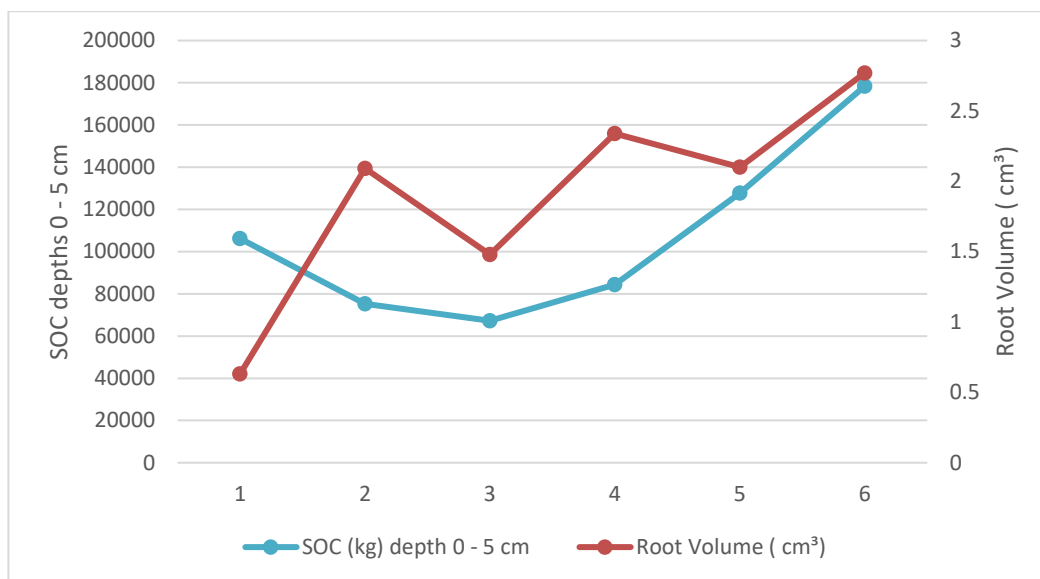
Plot	Summary	Total Length (cm)	Root Volume (cm ³)
P1	Mean	375.49	0.63
	S. D	5.96	0.4
P2	Mean	363.6	2.09
	S. D	15.4	0.21
P3	Mean	344.25	1.48
	S. D	28.54	0.55
P4	Mean	378.88	2.34
	S. D	3.4	0.44
P5	Mean	365.89	2.1
	S. D	2.6	0.21
P6	Mean	380.72	2.77
	S. D	9.69	0.48

Note. P means plots

Descriptively, a relationship between root volume and soil carbon content is observed. Roots in plot six exhibit a higher volume in comparison with the rest of the plots under investigation as well as a higher SOC (Figure 7). This indicates root volume can be a contributing factor in soil organic carbon content such as other factors as land management, presence of livestock and soil physical characteristics.

Figure 7

SOC in depth from 0 to 5 cm and average root volume (cm³) per plot



Conclusions

Pasture for beef cattle in Circuit No. 12 of Monte Redondo, Zamorano, Honduras, has an estimated carbon sequestration potential due to biomass growth for both above ground biomass and roots of 7.135 ton of carbon. Considering an area of 42.16 ha during a single cycle in the dry season which consists of a period from November to April (5 months). The highest levels of sequestration were found in the plots used for hay harvesting, as these plots have a longer cutting cycle, however, the situation may change during the rainy season as the production of biomass will be higher resulting in more dry matter content per cycle.

The statistical analysis conducted in this study supports the proposition that volatile matter concentrations in soil serve as a reliable estimation for soil organic carbon content. This finding indicates that the volatile matter concentration can be effectively utilized to estimate the soil organic carbon present in the soil.

The pastures used for cattle grazing, showed significantly higher levels of soil organic carbon (SOC) compared to the plots used for hay harvesting. This suggests that grazing activities can promote the incorporation of larger amounts of carbon into the soil due to the deposition of animal manure.

The study found variations in soil organic carbon (SOC) at different depths, likely influenced by soil compaction and management practices applied to permanent pastures. The top 5 cm of soil exhibited the highest concentration of soil organic carbon (SOC).

The results indicate that the type and growth of roots among different species, both in volume and length, has a relationship with the amount of SOC content. The study revealed a substantial amount of approximately 639,132 kg of SOC in the top 0 - 5 cm of soil.

Recommendation

Implementing practices which can further enhance carbon sequestration capabilities. These practices may include rotational grazing, proper fertilization techniques, and the implementation of woody vegetation in the area.

Conduct a twelve-month analysis to assess how the different seasons influence the pastures' ability to sequester carbon.

Undertake further investigations to assess additional pasture species, refine management techniques for a greater carbon storage in soil and sequestration in biomass, and comprehensively evaluate long-term carbon sequestration trends.

Analyze variations in SOC content over time, to evaluate the carbon sequestration potential of soils used in pastures.

Conduct an emission balance of greenhouse gasses released by the beef cattle of Zamorano and the carbon sequestration of the pastures used to feed the cattle.

Conduct an estimation of the carbon sequestration potential of the rest of the pastures used for the feeding of Zamorano's beef cattle.

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Annexes

Annex A

Interview

Entrevista Encargado de la Unidad de Ganado de Carne

1. ¿Qué pasturas se encuentran en el circuito no? 12 de la unidad de ganado de carne?
 - ☐Cynodon nlemfluensis (Pasto Estrella)
 - ☐Digitaria eriantha (Transvala)
 - ☐Panicum máximum (Tanzania)
 - ☐Otros:
2. ¿Se encuentran las mismas especies de pasturas en cada subcircuito del circuito no? 12, Monte Redondo?
3. En el caso de que no sean las mismas pasturas, ¿qué especies de pasturas se encuentran en cada subcircuito del circuito no. 12 de la unidad de ganado de carne?
 - Subcircuito 1
 - ☐Cynodon nlemfluensis (Pasto Estrella)
 - ☐Digitaria eriantha (Transvala)
 - ☐Panicum máximum (Tanzania) invasion de panicum lo mismo para el 2
 - ☐Otros: mayoría Estrella y trasvala
 - Subcircuito 2
 - ☐Cynodon nlemfluensis (Pasto Estrella)
 - ☐Digitaria eriantha (Transvala)
 - ☐Panicum máximum (Tanzania)
 - ☐Otros:
 - Subcircuito 3
 - ☐Cynodon nlemfluensis (Pasto Estrella)
 - ☐Digitaria eriantha (Transvala)
 - ☐Panicum máximum (Tanzania)
 - ☐Otros:
 - Subcircuito 4
 - ☐Cynodon nlemfluensis (Pasto Estrella)
 - ☐Digitaria eriantha (Transvala)
 - ☐Panicum máximum (Tanzania)
 - ☐Otros:
 - Subcircuito 5
 - ☐Cynodon nlemfluensis (Pasto Estrella)
 - ☐Digitaria eriantha (Transvala)
 - ☐Panicum máximum (Tanzania)
 - ☐Otros:
 - Subcircuito 6
 - ☐Cynodon nlemfluensis (Pasto Estrella)
 - ☐Digitaria eriantha (Transvala)

☐ Panicum máximo (Tanzania)

☐ Otros:

4. ¿Hace cuanto se establecieron las pasturas del circuito No. 12?
5. ¿Conocen que se encontraba en ese terreno antes del establecimiento de las pasturas?
6. ¿Qué uso se le da actualmente al circuito no. 12?

☐ Pastoreo

☐ Elaboración de heno

☐ Otras:

7. ¿Qué manejo se les da a las pasturas del circuito no. 12?
8. ¿A las pasturas se les fertiliza?
9. Si se fertilizan, ¿qué productos se le aplican y con qué frecuencia?
10. ¿Se utiliza maquinaria en este terreno?
11. Si se utiliza maquinaria, ¿Qué tipo de maquinaria se utiliza?
12. ¿Con que propósito se utiliza la maquinaria?
13. ¿Con qué frecuencia?
14. ¿Las pasturas dentro del circuito no. 12 cuentan con sistema de riego?
15. Si cuentan con sistema de riego, ¿con que sistema cuenta y con que frecuencia se riega?
16. Al momento del pastoreo del hato, ¿que sistema de pastoreo utilizan?
17. ¿Se ha utilizado siempre este sistema de pastoreo?
18. Si no se ha utilizado siempre ¿Cuál se utilizaba anteriormente y hace cuanto implementaron el nuevo sistema de pastoreo?
19. ¿En qué consiste el pastoreo que utilizan en la actualidad?
20. ¿Cuál es el periodo de descanso que se le brinda a las pasturas después del pastoreo?
21. ¿Cuánto tiempo permanece el hato por m² de pastura?
22. ¿Cuál es la densidad de hato por m² de terreno?

Annex B

Biomass sampling



Annex C

Biomass analysis



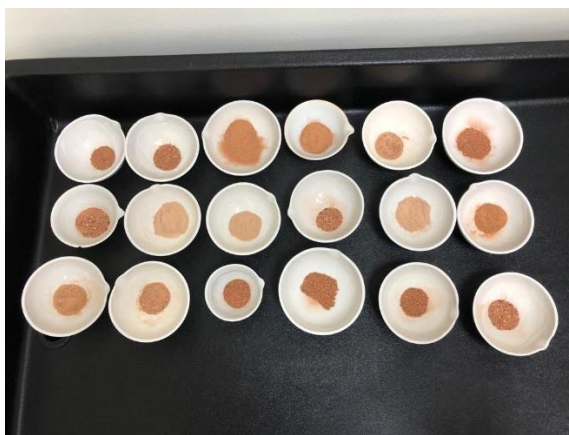
Annex D

Soil sampling



Annex E

Soil analysis



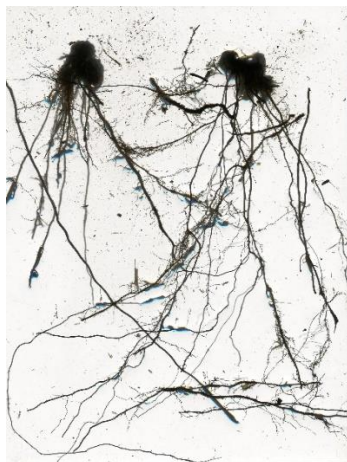
Annex F

Root sampling



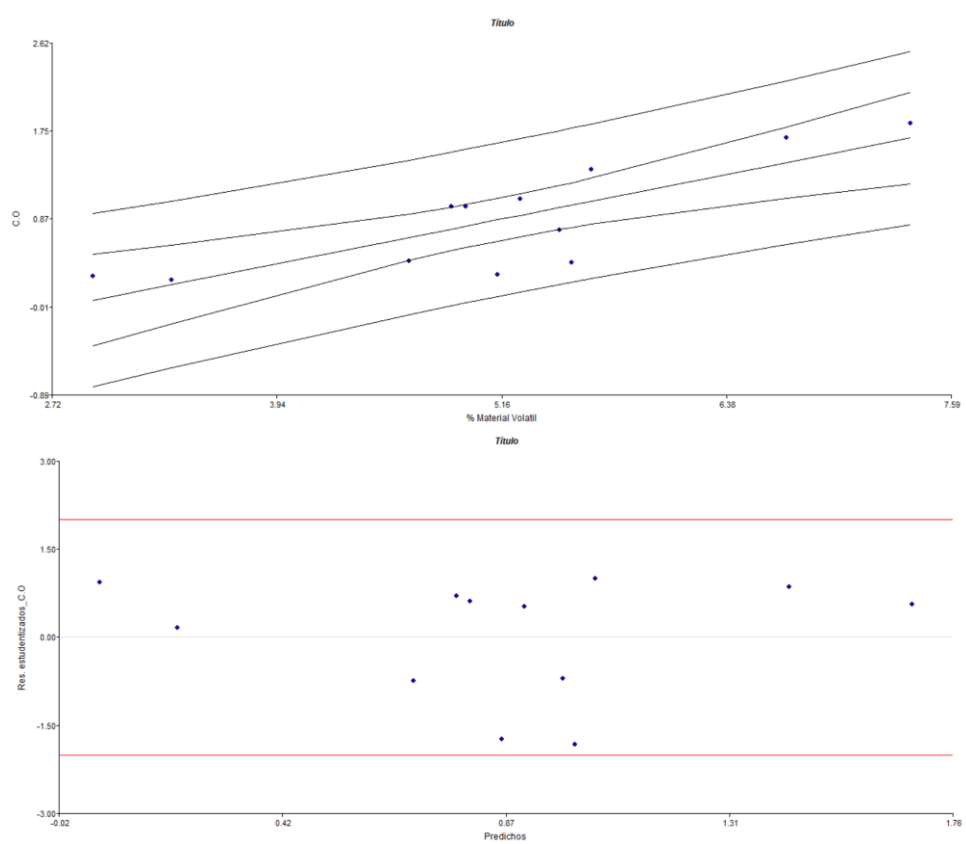
Annex G

Root analysis



Annex H

Residuals



Annex I

Soil crude statistical analysis

	numDF	F-value	p-value
(Intercept)	1	2131.57	<0.0001
Plot	5	15.85	<0.0001
Depth	6	23.43	<0.0001

Depth	Medias	E.E.	
0-5	9.20	0.40	A
5-10	6.61	0.24	B
10-20	5.51	0.14	C
40-60	4.95	0.42	C D
60-80	4.95	0.32	C D
30-40	4.76	0.28	D
20-30	4.19	0.29	D

Medias con una letra común no son significativamente diferentes ($p > 0.05$)

Plot	Medias	E.E.	
P6	7.80	0.35	A
P5	6.24	0.28	B
P1	5.60	0.25	B C
P3	5.17	0.22	C D
P4	4.89	0.21	D
P2	4.73	0.20	D

Medias con una letra común no son significativamente diferentes ($p > 0.05$)