

Seasonal Changes of Organic Carbon Content in a Mild Climate Meadow Cambios Estacionales del Contenido de Carbono Orgánico en una Pradera de Clima Templado

María Fernanda García-Domínguez¹ , Gerardo Antonio Pámanes-Carrasco² ,
Daniel Sierra-Franco³ , Rafael Jiménez-Ocampo⁴ ,
Elia Esther Araiza-Rosales³ , and Esperanza Herrera-Torres^{5†}

¹ Universidad Juárez del Estado de Durango, Programa Institucional de Doctorado en Ciencias Agropecuarias y Forestales; (M.F.G.D.). ² SECIHTI, Universidad Juárez del Estado de Durango, Instituto de Silvicultura e Industria de la Madera; (G.A.P.C.). Boulevard Guadiana No. 501, Ciudad Universitaria. 34120, Durango, Durango, México.

³ Universidad Juárez del Estado de Durango Facultad de Medicina Veterinaria y Zootecnia. Carretera Durango-Mezquital km 11.5, Col. Valle del Sur. 34162 Durango, Durango, México; (D.S.F.), (E.E.A.R.).

⁴ Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campo Experimental del Valle del Guadiana. Carretera Durango-Mezquital km 4.5, Col. El Mezquital. 34170 Durango, Durango, México; (R.J.O.).

⁵ Tecnológico Nacional de México, Instituto Tecnológico del Valle del Guadiana. Carretera Durango-México km 22.5, Col. Villa Montemorelos. 34308, Durango, Durango, México; (E.H.T.).

† Corresponding author: hetoies99@yahoo.com.mx

SUMMARY

Soils are the largest reservoir of carbon in terrestrial ecosystems. Meadows have been established to offer a different source of forage and show high potential in carbon sequestration. This study aimed to evaluate the seasonal changes in the concentrations of soil organic carbon, total nitrogen, vegetative coverage, and the physicochemical properties of the soil in a mild climate meadow in Durango. The study was carried out in a mild climate meadow during the four seasons of the year, in which the vegetative coverage, dominant species, physicochemical properties, organic carbon, and nitrogen of the soil were determined. Vegetative coverage was different between seasons ($p < 0.05$); the percentage of herb decreased in winter, whereas the percentage of nude soil was lower in summer compared to the other seasons. On the other hand, the lowest values of organic matter and organic carbon in soil were found in fall ($p < 0.05$), therefore, the C:N ratio was also lower ($p < 0.05$). Carbon accumulated in soil increased from 21 Mg C ha⁻¹ in fall to 27.5 ton C ha⁻¹ in summer ($p < 0.05$); apparent density and total nitrogen showed no changes among seasons ($p < 0.05$). The results suggest that mild meadows have a high potential for carbon sequestration to reduce GHG emissions and contribute to climate change mitigation.

Index words: carbon sequestration, climate change mitigation, grassland soils.

RESUMEN

Los suelos son el mayor reservorio de carbono en los ecosistemas terrestres. Las praderas se han establecido para ofrecer una fuente diferente de forraje y muestran un alto potencial en el secuestro de carbono. Este estudio tuvo como objetivo evaluar los cambios estacionales en las concentraciones de carbono orgánico y nitrógeno total del suelo, la cobertura vegetal y las propiedades fisicoquímicas del suelo en una pradera de clima templado en Durango. El estudio se llevó a cabo durante las cuatro estaciones del año, en las que se determinaron la cobertura vegetal, las especies dominantes, las propiedades fisicoquímicas, el carbono orgánico y el nitrógeno del suelo. La cobertura vegetal fue diferente entre estaciones ($p < 0.05$); el porcentaje de hierba disminuyó en invierno, mientras que el porcentaje de suelo desnudo fue menor en verano con respecto a las demás estaciones. Por otro lado, se encontraron los menores valores de materia orgánica y carbono orgánico del suelo en otoño



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($p < 0.05$), por lo que la relación C:N también fue más baja ($p < 0.05$). Por su parte, el carbono acumulado en el suelo aumentó de 21 Mg C ha⁻¹ en otoño a 27.5 Mg C ha⁻¹ en verano ($p < 0.05$); la densidad aparente y el nitrógeno total no mostraron cambios entre estaciones ($p < 0.05$). Los resultados sugieren que las praderas de clima templado muestran un alto potencial en el secuestro de carbono para reducir las emisiones de GEI y contribuir a la mitigación del cambio climático.

Palabras clave: secuestro de carbono, mitigación del cambio climático, suelos de pradera.

INTRODUCTION

Carbon sequestration became into a very popular term since it has been used as a mitigation strategy for climate change. The soils are the largest source of carbon, since they store more than the 69% of the total carbon in the biosphere. It is estimated that the C content in the first 3 ft depth is equal to 1500 Pg; however, there are some places that have ranged data among 500-3000 Pg (Vargas-Larreta *et al.*, 2023).

Carbon sequestration takes place when plants absorb carbon dioxide from the atmosphere and then use it through the photosynthesis pathway to fix it into plants in the form of carbohydrates. Eventually, carbon exudates lixiviate to the soil, where microorganisms use it to decompose it and then to integrate it into organic matter (Lei *et al.*, 2023).

On the other hand, soil organic carbon (SOC) is used as an indicator of soil health since it improves soil properties when the cation exchange capacity increases. Likewise, SOC contributes to soil pH neutralization, improves the water retention capacity, increases the stability of clay soils with the formation of aggregates, and enhances the nutrients release (Núñez-Ravelo, Ugas, Calderón, and Rivas, 2021).

Grasslands are systems that can sequester carbon and may contribute to climate change mitigation. Nevertheless, carbon sequestration in these systems depends on soil health. In addition, soil health depends on climatic conditions and water precipitation (Nigussie, 2024). However, meadows are irrigated systems mainly created for grazing livestock, and in contrast to grasslands, these meadows do not present water supply limitation (Zapata, Cabanillas, Burboa, and Domínguez, 2021). Additionally, these systems offer other benefits associated with carbon sequestration through the plant's diversity, biomass production, livestock excreta, and the improved management of grazing livestock (Barrezueta-Unda, Velepucha, Solano, and Hurtado, 2020; Bai and Cotrufo, 2022). Otherwise, seasonal changes can affect carbon reservoir and organic matter in soils through variations in temperature, water precipitation, and plant growth (Wuest, 2014). Based on the above, this study aimed to evaluate the changes in the concentrations of SOC, vegetative coverage, and the physicochemical properties of the soil across the seasons of the year in a mild climate meadow.

MATERIALS AND METHODS

Studio Area

The research was carried out from October 2023 to September 2024 in a mixed mild climate meadow located in the INIFAP's Valle del Gadiana Experimental Camp in Durango, Durango, México at 23° 56' 07" N and 104° 37' 45" and 1880 m of altitude. The climate in this region is classified as dry and semi-dry, with a lowest and maximum annual mean temperature of 5 °C and 39°C, respectively. Annual precipitation is reported in 550 mm, distributed mainly in July, August, September, and October (data obtained from the meteorological station of the Valle del Gadiana Experimental Camp, INIFAP, Durango). The meadow possesses 1.7 ha of surface and was established 10 years ago. The soil texture was determined as clay loam, with a 40.8% sand, 29.5% clay, and 29.7% silt. Vegetation is composed of the species *Lolium Perenne*, *L. multiflorum* L., *Festuca arundinacea* S., *Dactylis glomerata*, *Bromus wildenowii*, and *Trifolium repens*. Meadow is watered every 30 days, and the animal load is 2.5 Mg ha⁻¹, which is organized in a rotational grazing system.

Vegetation Samples and Dominant Species

Eight samples per season of the year were selected randomly using Daubenmire's quadrant method, cited by Jones and Tracy (2017). One m² quadrant was located on the soil, and then the coverage of grass, herbs, legumes, other types of cover, and bare soil were measured and registered on the most representative points of the meadows. Also, dominant species inside the quadrant were identified through visual estimation, and their distribution were estimated.

Soil Sampling

Soil sampling was carried out randomly during each season of the year. A composite sample per season of the year was elaborated with eight simple samples which were taken at depth of 30 cm with a metal auger (De la Cruz-López, Villanueva, Casanova, Martínez y Aryal, 2024). The soil samples were dried and sieved using a 2 mm mesh for subsequent physicochemical analysis.

Analysis of the Chemical Properties of the Soil

The physicochemical properties of the soil were carried out in the soil laboratory of the Valle del Guadiana Institute of Technology (24° 00' 38.4" N, 104° 26' 40.74" and 24° 00' 32.7" N, 104° 26' 40.95" W).

A saturation extract was performed. Thus, 250 g of the sample was weighed and then saturated with distilled water to obtain a saturated paste. Then, the paste was left to stand for 24 h and filtered using a vacuum pump. The pH was measured in the saturation extract with a potentiometer (model HI8314-1, Hanna, USA). Also, the extract was used to determine minerals as Calcium (Ca) and Magnesium (Mg) by EDTA titration; bicarbonate was evaluated by H₂SO₄ titration, and chlorides (Cl) by AgNO₃ titration (Bashour and Sayegh, 2007). The determination of phosphorus (P) was performed by Olsen method (FAO, 2021a).

Soil Carbon Content

The soil apparent density (AD) was determined by the cylinder method (Sandoval, Dorner, Seguel, Cuevas, and Rivera, 2012); soil samples were collected using a metal cylinder core at a depth of 30 cm, and later were dried at 105 °C until constant weight to determine AD as the proportion of the soil mass and the volume of the cylinder. Soil organic matter (SOM) was estimated using the proposed method of Walkley and Black (1934). This value is multiplied by 0.58 to obtain soil organic carbon (SOC), assuming that 58% of SOM is C. The carbon accumulated in soil (CAS) was estimated from the percentage of SOC in the soil using Equation 1.

$$CAS = SOC * AD * D * 10^{-3} \text{ Mg C ha}^{-1} * 10^4 \text{ m}^2 \text{ ha}^{-1} \quad (1)$$

Where CAS = carbon accumulated in soil (Mg C ha⁻¹), SOC = soil organic carbon (kg Mg⁻¹), AD = apparent density (Mg m⁻³), and D = depth of the soil sample (m).

Soil Nitrogen Content

Total nitrogen (TN) was determined using the Kjeldahl method (FAO, 2021b). The soil ratio C/N was obtained by dividing the SOC and TN percentages.

Statistical Analyses

The data obtained were analyzed with a completely randomized design with a level of significance of $p < 0.05$, to determine if there are significant effects on different variables.

Tukey's multiple range test was used ($p < 0.05$) to separate the means, establishing the season of the year as treatments with 8 replicas each; the statistical package SAS was used.

The multivariate principal component analysis was performed using R Studio software to identify the variables that explain the greatest variability in the dataset. Seasons of the year were included as principal components to determine the relationship between the soil variables and the season of the year.

RESULTS AND DISCUSSION

Vegetation Coverage and Dominant Species

The vegetation coverage of the mild climate meadow is presented in Table 1. The results show that grass is the pasture with the greatest coverage surface in all seasons but spring; the greatest plant coverage in spring is provided by legumes. However, some legumes in mixture with grasses in meadows tend to produce a higher quality forage and also tend to reduce the need for external N inputs (Lüscher, Mueller, Soussana, Rees, and Peyraud, 2014).

Table 1. Vegetation coverage (%) of the mild climate meadow during the four seasons of the year.

Coverage	Season				SEM
	Fall	Winter	Spring	Summer	
Grass	55.5 ± 17.647 a	48.38 ± 27.107 a	36.13 ± 20.992 a	47.5 ± 23.44 a	3.19
Herb	26 ± 11.174 a	-	13.38 ± 9.303 ab	19 ± 6.86 ab	1.54
Legume	12.63 ± 6.116 a	32.88 ± 21.10 a	42.75 ± 25.001 a	32.25 ± 22.657 a	2.91
Other coverage	2.00 ± 1.69 ab	4.88 ± 3.603 a	3.75 ± 1.77ab	0 ± 0.00 b	0.438
Nude soil	3.88 ± 3.482 a	7.13 ± 11.331 a	4.00 ± 0.41a	1.25 ± 1.16 b	0.794

NF = no found.

These mixtures also showed enhanced resilience to drought stress and weed suppression potential when compared to monocultures (Hofer *et al.*, 2016; Connolly *et al.*, 2018). The quality of the pasture in terms of vegetation shows good quality; during all the seasons of the year, the nude soil was presented in a very low percentage.

The distribution of species of the meadow is shown in Table 2. As can be observed, all the dominant species are present in summer, whereas only three are present in winter. *L. perenne* or commonly known as ray grass, is the major observed species; however, there is no presence of this grass during summer. On the contrary, *T. repens* increases throughout the seasons. Otherwise, *S. iridis*, *P. notatun*, and *B. gracilis* were observed only in summer, whereas *B. wildenowii* was observed in spring and summer.

As can be noticed, *L. perenne* is more abundant in the fall than in other seasons. This may be attributed to the fact that it is sensitive to high summer temperatures. Moreover, *L. perenne* is a species representative of mild climates, and its optimal growth temperature is 18°C (Mendoza-Pedroza, Maldonado, Rojas, Cruz, Torres, and Vaquera, 2018), whereby it is the grass with the highest percentage during the fall, as can be observed in Table 1.

Otherwise, legume species such as *T. repens* presented a higher development in spring than in the other seasons of the year. This species requires specific weather conditions, such as more sunlight for carbon fixation and photosynthesis (Ventura-Ríos *et al.*, 2020); this is the reason why its growth is more efficient in spring.

Chemical Analysis of Soil

Table 3 presents the chemical analysis of the soil in the meadow.

The SOM content in the fall was lower than that observed for the other seasons of the year. According to CONAGUA (2023), October had the most abundant rainfall (78mm) compared to the years 2022 and 2024 (41.7 and 4.9 mm, respectively). Therefore, it is viable that nutrients lixiviation in the analyzed soil samples, whereby fall had

Table 2. Distribution of dominant species (%) in the mild meadow during the four seasons of the year.

Dominant species	Season			
	Fall	Winter	Spring	Summer
<i>Lolium perenne</i>	61	54	41	NF
<i>Chloris radiata</i>	15	1	NF	5
<i>Dactylis glomerata</i>	12	NF	2	22
<i>Trifolium repens</i>	12	45	53	27
<i>Setaria viridis</i>	NF	NF	NF	28
<i>Paspalum notatun</i>	NF	NF	NF	12
<i>Bromus wildenowii</i>	NF	NF	4	4
<i>Bouteloa gracilis</i>	NF	NF	NF	2

NF = no found.

Table 3. Physicochemical properties of mild meadow soil during four seasons of the year.

Variable	Season				SEM
	Fall	Winter	Spring	Summer	
SOM	1.2 ± 0.05 b	1.4 ± 0.13 a	1.5 ± 0.04 a	1.6 ± 0.04a	0.042
AD	0.98 ± 0.24 a	1.00 ± 0.011 a	0.98 ± 0.031 a	1.02 ± 0.009 a	0.012
pH	6.72 ± 0.16 b	6.62 ± 0.05 b	6.19 ± 0.09 c	7.08 ± 0.02 a	0.055
Ca	50 ± 0.00 ab	60 ± 0.00 a	46.6 ± 5.77 b	33.3 ± 5.77 c	2.35
Mg	14 ± 3.36 a	8 ± 3.46 a	14 ± 3.46 a	12 ± 0.00 a	1.73
HCO ₃	471.1 ± 40.73 a	542.4 ± 40.85 a	206.1 ± 35.7 b	287.4 ± 38.29 b	22.51
Cl	420 ± 8.75 b	422 ± 13.36 ab	396.66 ± 5.05 b	294.58 ± 10.11c	5.64
P	2.08 ± 0.13 d	2.72 ± 0.12 c	3.32 ± 0.19 b	4.75 ± 0.20 a	0.096

Mean ± standard deviation. ^{ab} Different superscript letters between rows indicate statistical difference among means ($p \leq 0.05$). SOM = soil organic matter (%); AD = apparent density (g cm^{-3}); Ca = calcium (mg kg^{-1}); Mg = magnesium (mg kg^{-1}); HCO₃ = bicarbonates (mg kg^{-1}); Cl = chlorides (mg kg^{-1}); P = phosphorus (mg kg^{-1}); SEM= standard error between means.

the lowest SOM content. In fact, in this study, the measured SOM content was lower than that reported by Kitczak, Jänicke, Bury, and Jarnuszewski (2023), which was 3.3% in a mixed meadow located in Germany, but was similar to Aguirre *et al.* (2025), who reported a SOM of 1.5% in a cultivated soil in an arid zone of Mexico. However, the SOM values observed in this study were lower than those reported by Research Grasslands Institute of New Zealand (2010) to meet the needs of pastures (8-12%). In spite of the latter, the meadow fulfills one of its main functions, related to carbon sequestration, hydrological and erosion regulation, nitrogen removal, invasion regulation, and livestock number; the above gives a high value to grasslands (Plantureux *et al.*, 2016). Moreover, it is also known that the SOM content in natural grasslands is higher than in cultivated ones due to agronomic management.

According to Crespo-López (2018), the SOM content is higher in grasslands composed of perennial grasses compared with monocultures, which leads to more carbon accumulation in soil. In fact, Ordoñez-Flores, Huamán, and Rojas (2019) mentioned that pasture associations between grasses (as ray grass) and legumes (as alfalfa and clover) are ideal for producing more green forage, which will provide greater vegetation cover, which may contribute to an increase in the sequestration of organic carbon in the soil.

On the other hand, the AD values obtained in this study were similar to those obtained by Naeth, Dhar, and Wilkinson (2023) in a mixed grass meadow; AD may be modified because, during the season, the availability of water and nutrients promotes biomass production and decreases density. Nevertheless, this study observed no changes in AD among seasons. Additionally, AD values above 1.39 g cm^{-3} in clay soils indicate problems such as compaction, which might cause limitations in the growth and development of roots (Contreras-Santos, Martínez, Cadena, and Falla, 2020); the soil in this meadow doesn't show this problem, which may restrict the growing forage. In fact, Ma *et al.* (2020) reported that increasing AD leads to a decrease in SOM content; this may be attributed to reduced aggregates due to over-compaction. Therefore, no observed changes in AD in the present study may enhance SOM and C storage. Moreover, recorded values are suitable for better water infiltration, aeration, and root development (Bravo *et al.*, 2013).

With respect to pH, although there were differences between seasons, these values did not exceed 7, which classifies it as a neutral soil according to NOM-021-RECNAT-2000 (SEMARNAT, 2002). This value is ideal for the development of crops. Thus, pH should not limit the availability of nutrients in the soil.

On the other hand, as presented above in this study, the concentrations of Ca, Mg, and P in the soil are deficient. This effect shows the soil's low capacity to retain nutrients. For their part, López-Báez *et al.* (2019) explained that when the SOM content is limited, the nutrients are unprotected against the lixiviation process. This effect was also observed in soils cultivated in northern Mexico; in these soils, Cervantes-Vázquez *et al.* (2018) found low values of Ca and Mg (13.3 and 3.17 mg kg^{-1} , respectively) and a low SOM content (1.3%), which agrees with this study, pointing out the nutritional deficiencies in the area of northern Mexico.

However, P is one of the elements with the greatest deficiency in arid and semi-arid soils. In this study, an increase in P content was observed across the seasons (fall to summer), likely due to the constant deposition of feces by grazing animals, which can improve fertility. In addition, there is a lack of rain among these seasons,

which may suggest a reduction in the lixiviation of nutrients to the deeper layers of soil in the meadow. In a similar study to this one, Suárez-Hernández, García, Mellado, and Dueñez (2015) reported an increase in the content of SOM and P in grazing areas compared to ungrazed area throughout the seasons. Based on the latter, it can be inferred that grazing can show a beneficial effect by contributing to the deposition of this element in areas where P deficiencies are present.

Bicarbonates and carbonates are considered important forms of C storage in arid and semi-arid areas (Ayala-Niño, Maya, and Troyo, 2018). The differences in HCO_3^- between seasons could be related to climatic variations, such as the increase in humidity that can cause the lixiviation of these compounds. Regarding Cl, there is little information on concentrations in different productive soils; however, it is known that the requirement for this element is low, so it is not common to find deficiencies of it in the soil (Zárate-Martínez *et al.*, 2024). Otherwise, if the presence of this element is high, it could be related to soil salinity problems. Thus, high Cl values in the soil could be associated with the water used for irrigation; water quality is commonly poor in arid and semi-arid areas where irrigation is used due to lack of rainfall, and this effect may promote salinization problems that contribute to reducing crop productivity. Commonly, the energy that the plant uses for normal physiological processes is redirected to processes that involve water acquisition when osmotic stress occurs (Castillo-Valdéz, Etchevers, Hidalgo y Aguirre, 2021). Therefore, a water analysis is highly recommended to elucidate this point of view.

In this study, it can be observed that plant species, such as *L. perenne*, is not a metal hyperaccumulator plant; it shows a high tolerance to some metals, resistance to unfavorable conditions, and rapid establishment, which allows a rapid soil cover once planted (Casler and Undersander, 2018). Although in this study it is observed that when the concentration of chlorides and calcium increases, the percentage of *L. perenne* decreases during the winter, nevertheless, a greater adaptation of this plant to the concentrations of Cl and Ca is observed compared to the others. Otherwise, *T. repens* shows a greater tolerance to these minerals; when higher concentrations of these metals are present, the coverage of *T. repens* is greater.

Soil Carbon Stock and Soil Nitrogen Content

Table 4 shows the SOC, CAS, TN, and C:N ratio of the meadow.

Regarding SOC and CAS, an increase of 30% in summer when compared to fall, showing an effect with a trend in carbon accumulation. Jurado, Saucedo, Morales y Martínez (2013) observed similar values of CAS in grasslands in arid zones to those presented in this study, whereas Bravo *et al.* (2017) registered higher values in a mixed grass meadow. Contrarily, Ali and Shukla (2024) reported a mean value of 18 Mg ha^{-1} in rangelands of arid zones, which confirms that weather is an important factor that affects the carbon capture in arid zones due to high temperatures, low precipitation, and the prolonged droughts caused by the climatic changes.

Otherwise, plant species observed in this study, as *L. perenne*, *D. glomerata*, *T. repens* and *B. wildenowii* are plants with C3 metabolism; however, species such as *C. radiata*, *S. viridis*, *P. notatum* and *B. gracilis* are of C4 metabolism, which according to Ramos-Hernández and Martínez-Sánchez (2020), these species may enhance carbon capture in soil, because they have a greater capacity to integrate CO_2 from atmosphere into organic matter than C3 grasses, due to their higher sugar content and lower nitrogen content. Thus, the increase in

Table 4. Organic carbon accumulated and total nitrogen of mild meadow soil during four seasons of the year.

Variable	Season				SEM
	Fall	Winter	Spring	Summer	
SOC	0.71 ± 0.03 b	0.85 ± 0.07 a	0.91 ± 0.02 a	0.92 ± 0.04 a	0.026
CAS	21.0 ± 1.002 b	25.6 ± 2.19 a	26.8 ± 0.61 a	27.5 ± 1.06 a	0.780
TN	0.20 ± 0.02 a	0.23 ± 0.03 a	0.22 ± 0.01 a	0.19 ± 0.02 a	0.011
C:N	2.96 ± 0.32 b	3.71 ± 0.67 ab	4.24 ± 0.42 a	4.72 ± 0.47 a	0.283

Mean \pm standard deviation. SOC = soil organic carbon (%); CAS = carbon accumulated in soil (Mg C ha^{-1}); TN = total nitrogen (%); C:N = carbon:nitrogen ratio.
^{ab} Different superscript letters between rows indicate statistical difference among means ($p \leq 0.05$). SEM = standard error between means.

SOC in the summer season compared to fall may be explained by the presence of these species, where their growth is improved by better climatic conditions for growing of plants (Table 2). As a matter of fact, the greatest cover during spring was *T. repens* (53%); this legume has the property of leaf expansion, which allows better interception of solar radiation and therefore a greater CO₂ sequestration (Flores-Santiago, Hernández, Guerrero, Quero, and Martínez, 2015). Likewise, perennial crops, such as *L. perenne*, has shown a significantly increase in the organic carbon reserves compared to monocultures or crop rotations; this plant is known to possess a remarkable capacity to fix and retain organic carbon in the soil (Jiménez, 2018¹), therefore its high presence in all seasons, may result in the incorporation of C into the soil.

In this sense, the variations in climatic conditions may affect the carbon content by changing the ratio of grasses, herbs, and other components of the meadow throughout the seasons of the year. In fact, Dhakal and Anowarul (2018) found that legume-grass mixtures increased the C and N in the soil compared to monoculture, and the productivity of aerial biomass was better, which promotes greater root biomass that represents the main source of C in the soil (FAO, 2018). Furthermore, possible explanations for the differences that meadows present with CAS could be associated with decomposition rates and patterns of the different types of vegetation (Rojas, Brenes, and Abarca, 2022).

In addition, as previously mentioned, the soil in this study is classified as clay loam, and it has been reported that the soil textural class is closely related to carbon and nitrogen storage in soils. Previous studies have shown that soils with fine textures tend to have higher SOM and SOC contents than sandy soils (Sánchez-Hernández *et al.*, 2011), because fine particles such as silt and clay provide greater physical protection of organic matter through the adsorption of organic compounds and the formation of soil aggregates, which make their mineralization rate lower (Ordaz-Gallegos, Rodríguez, García, and Pimentel, 2020). Likewise, Zhao, Kubota, and Hernandez (2024) reported a positive correlation between fine textured soils and soil carbon concentration, suggesting that soil texture plays an important role in soil organic carbon accumulation. The latter implies that fine textured soils have a higher potential for carbon sequestration, particularly under regenerative management practices.

It should be mentioned that CAS is the main component of SOM and is an indicator of soil health, and is important for its contributions to food production, climate change mitigation and adaptation, and the achievement of the Sustainable Development Goals. In fact, this type of soil shows an effect on the rate of mineralization and nutrient retention due to its balance of sand, silt, and clay (Matus, 2018). Thus, when organic matter levels are between 2 and 4%, it can be available as a source of nitrogen when it is mineralized, meaning it is available for plant nutrition.

Total Nitrogen and Ratio C:N

As presented earlier in this study, the concentration of TN in soil showed no changes in this meadow throughout the seasons of the year (Table 4). However, the values registered in this study were higher than those reported by Álvarez-Sánchez, Améndola, Cristóbal, and Soto (2014) in a mixed meadow of mild climate. This effect can be attributed to the fact that the TN content of soil in meadows may present variations depending on the type of meadow and the land use. In the case of soils located in grasslands, Cantú and Yáñez (2018) reported a similar TN content to that obtained in this study (0.27%), while others, like Martínez-Soto, Cantú, Yáñez, González, and Béjar (2023), recorded average concentrations of TN in grassland soils of 0.25%. It is worth mentioning that N is the main element that provides organic matter for plant growth; it is considered to be a primary macronutrient because it is used by plants in large amounts, and unfortunately, it is not always available in the soil in sufficient quantities to allow plants to grow better (Gamarra-Lezcano, Díaz, Vera, Galeano, and Cabrera, 2018).

As shown previously, the C:N ratio increased 59% in summer when compared to fall (Table 3). However, these values are lower than those reported as the optimal ratio in meadows (10-12). In addition, Spohn and Stendahl (2024) reported that total nitrogen (TN) content may also be related to soil texture, since they found a lower C:N ratio in fine textured soils than in coarse textured soils; this was attributed to a greater enrichment and retention of nitrogen in fine textured soils. According to USDA (2024), if the amount of N in the added material is inadequate to support the increased growth of microbes, the microbes will absorb N from the soil and immobilize it in their tissues. This will limit growing plants from obtaining the N they need for immediate growth. In this case, as discussed earlier, there is no loss of TN among seasons. Nevertheless, a low C:N ratio may indicate a faster mineralization of SOM content and a loss of COS by CO₂ emissions through soil respiration (Skersiene, Slepeliene, Stukonis, and Norkeviciene, 2024). In order to confirm the latter, better management strategies must be applied to promote the entry of SOM into the system and continue evaluating soil carbon reserves over a longer period.

¹ Jiménez, J. P. (2018). Effect of season and regrowth days on the production and nutritive quality of grasses in Costa Rica. Master's thesis, Regional Graduate Program in Tropical Veterinary Sciences, Costa Rica

Multivariate Analysis

Eigen vectors of principal component analysis are presented in Figure 1; given values show that the three components explain 58.8, 23.22, and 17.98% of the variance, respectively. For a cumulative percentage of 100% of the variance as a linear result of the 12 variables studied. In the first component, the greatest variation is given by SOM, SOC, CAS, TN, and C:N ratio, while in the second component, it is given by AD and TN. Similarly, in the third component, the one that presents the greatest variation is due to pH and HCO_3 . As can be observed, SOC is the variable that presents the greatest contribution to the variance. In addition, TN appears in the first two components, which suggests that it possesses a strong influence on the variance. Furthermore, it can be observed that the cumulative variance is mainly given by the second component (82%), which, when added to the first component, gives 100% of the variance. Moreover, Figure 1 shows a negative correlation between the contents of P, pH, AD, SOC, CAS, SOM, and the C:N ratio.

According to the negative correlation shown in Figure 1, between the contents of P, pH, BD, and SOC, TOC, OM, and the C:N ratio, we can understand that the higher the BD, the lower the OM, TOC, and SOC contents. That is, in grasslands, there is a negative relationship between soil bulk density and organic carbon content, as well as an influence of organic matter on pH. Generally, soils with higher organic carbon content present lower bulk density, and organic matter tends to low pH. According to Murray-Núñez, Orozco, Flores, Marcelleño, and Nájera (2021), the relationship between organic matter and bulk density is reflected in soil structure, improving when organic matter increases above the soil surface and reaches the subsoil. However, the observed behavior in multivariate analysis can be explained by the season, which in this case shows that TN, OM, and SOC content are influenced by winter.

Furthermore, Rivera, Chará, and Barahona (2019) note that cover and apparent density are variables that may influence C sequestration in soil. Unlikely, He, Sun, Hu, Zhu, and Zhang (2023) reported that there are no differences in the carbon contributed by grass cover under different shrub covers with respect to the general contribution of carbon to the soil, and according to the type of cover and apparent density.

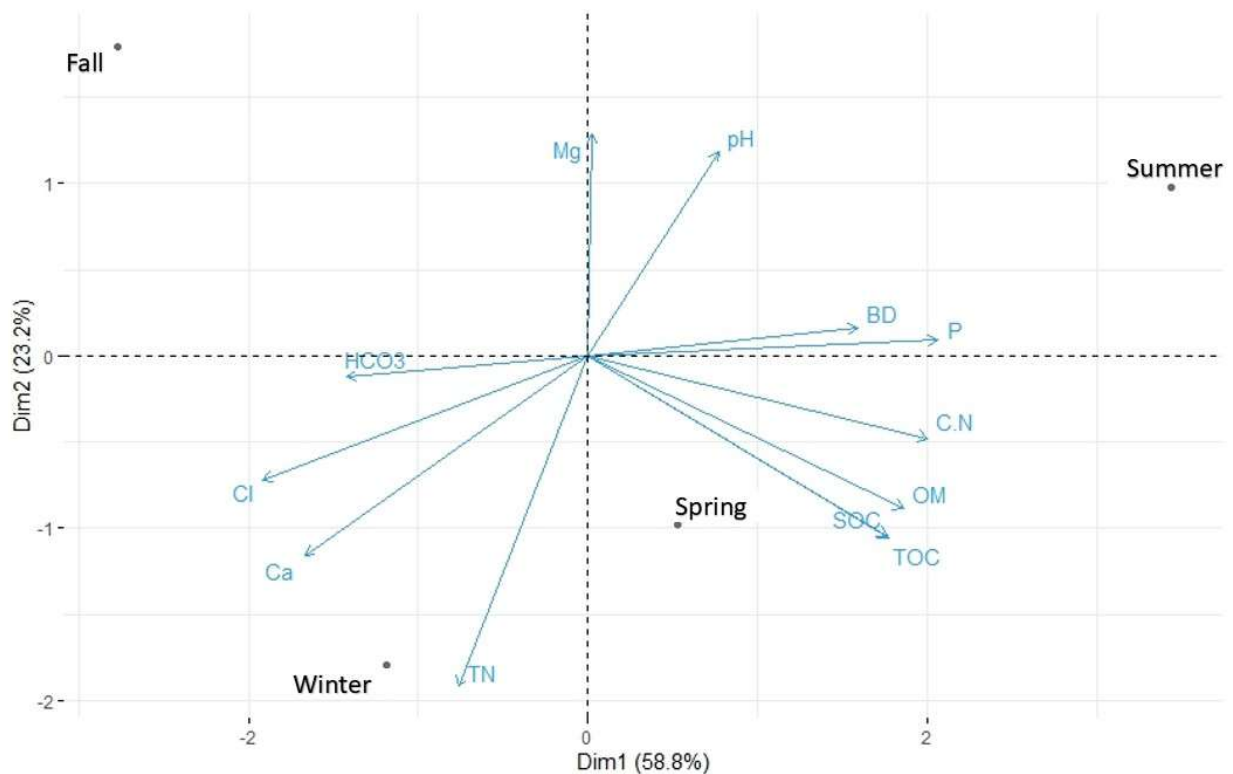


Figure 1. Principal component analysis (PCA) for pH, apparent density = BD, P, Mg, Cl, HCO_3 , Ca, total nitrogen = TN, soil organic matter = OM, soil organic carbon = SOC and the seasons of the year.

CONCLUSIONS

The studied meadow showed very low nude soil, which suggests that the involved species represent a good source of nutrients for grazing animals. On the other hand, similar values in AD among seasons indicate no compaction in soils, which may promote biomass production and enhance the organic matter content in soils, so erosion can be avoided. Additionally, similar contents of N suggest that good vegetative coverage remains among seasons, and assumably there are no losses of N through lixiviation. Otherwise, in summer, the presence of species such as *C. radiata*, *S. viridis*, *P. notatum*, and *B. gracilis*, which are identified as C4 metabolism plants, increases SOM and SOC by a potential fixation of C in soils. Therefore, the absence of these species in the other seasons reduces the C:N ratio by a potential decrease in C fixation. However, it is highly recommended to evaluate the ratio among C3:C4 plants in a meadow to assess fixation and content of C in soil. These results suggest that meadows show a high potential in carbon sequestration to reduce GHG emissions and mitigate the effects of climate change. Moreover, it is imperative to continue with this evaluation as long term research.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

All data generated or analyzed during this study are included in this published article.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHORS' CONTRIBUTIONS

Investigation: M.F.G.D. and E.H.T.; Formal analysis: G.A.P.C., D.S.F. and E.H.T.; Data curation: G.A.P.C. and D.S.F.; Methodology: R.J.O.; Resources: E.E.A.R.; Conceptualization: E.H.T.; Supervision: E.H.T.; Funding acquisition: E.H.T.; Writing - review and editing: M.F.G.D., G.A.P.C., D.S.F., R.J.O., E.E.A.R. and E.H.T.

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