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Assessing soil health under contrasting livestock grazing management systems and environmental conditions

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ABSTRACT

Soil health is essential for ecosystem health and agricultural productivity, particularly in the context of a growing global population. Grazing systems play a significant role in soil health through complex interactions influenced by soil type, climate, vegetation, and management practices. This study examines the relationships among ecoregion, grazing management, and season to soil health and productivity. A multifactorial analysis was conducted to evaluate soil health parameters, including total carbon, organic carbon, nitrogen, soil respiration, microbial biomass carbon, organic matter, electrical conductivity, pH, temperature, moisture, bulk density, and grass productivity metrics (manure biomass, forage biomass, dry matter percentage). These variables were evaluated in three ecoregions (dry-semidry, temperate, and warm), two seasons (dry and wet) and two contrasting grazing systems (continuous and regenerative): Continuous grazing is characterized by allowing livestock to freely roam over a pasture without regular rotation, a practice often associated with overgrazing and soil degradation. In contrast, regenerative grazing is characterized by a high stocking rate, short paddock occupancy times, rapid rotations between paddocks, extended rest periods for pasture recovery, and high grazing intensity during occupancy. This approach promotes pasture recovery, improves soil health, and enhances overall ecosystem resilience. The findings revealed that seasonality along with grazing management emerges as the most important factors influencing soil health parameters. The temperate and warm regions exhibited higher carbon values compared to the dry-semi-dry region, while total organic carbon levels were highest in the warm region, surpassing both the temperate and dry-semi-dry ecoregions. Furthermore, regenerative grazing management drives higher soil biological activity than continuous grazing. Also, regenerative grazing was critical in enhancing forage productivity and increasing soil organic matter and microbial activity, thereby improving overall soil health. Among the biological parameters, soil respiration emerged as a key determinant of soil health across the study sites, underscoring its importance in assessing ecosystem functionality and sustainability. This research provides valuable insights for promoting responsible grazing practices, enhancing grassland productivity, and fostering socio-ecosystem sustainability.

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1. Introduction

Soil health is defined as the capacity to function as a vital living ecosystem that sustains plants, animals, and humans. It involves maintaining soil's biological productivity, environmental quality, and promoting plant and animal health (Cherubin et al., 2021; Lehmann et al., 2020; Lima et al., 2024; Romero et al., 2024). These approaches focus on evaluating various soil chemical, physical, and biological indicators that are directly associated with essential soil ecological functions. Key indicators include organic matter content, microbial activity, nutrient availability, and soil structure (Bünemann et al., 2018; Karlen et al., 2019; Lehmann et al., 2020; Rojas et al., 2016; Zornoza et al., 2015). As a dynamic living system, healthy soil provides a variety of ecosystem services, including maintaining plant production and water quality, managing the breakdown and recycling of soil nutrients, and eliminating greenhouse emissions from the environment (Baldock et al., 2013; Tahat et al., 2020; Poppiel et al., 2025).

Ecoregions, which are defined by distinct ecological characteristics such as climate, vegetation, and geology, play a significant role in shaping soil properties and health (Pero et al., 2020). Ecoregions provide a framework for understanding spatial variability in soil health. Soil properties such as texture, organic matter content, nutrient availability, and microbial activity are strongly influenced by the ecological characteristics of a region (Dumanski and Pieri, 2000; Li et al., 2022). Understanding the interplay between ecoregions and soil health is necessary, mostly emphasizing how regional climatic, biotic, and edaphic factors influence soil properties and functions. Bünemann et al. (2018) highlight the importance of soil quality indicators in assessing ecosystem sustainability, while Ballut-Dajud et al. (2024) suggest that ecoregions influence soil organic matter, microbial diversity, and nutrient cycling in Latin America. Guevara et al. (2020) and González-Esquivel et al., (2015) link soil fertility in temperate and subhumid regions in Mexico to sustainable management practices, underscoring the need for ecoregion-specific strategies. Additionally, Zhou et al., (2023) evidence that soil organic carbon (SOC) levels vary significantly across ecoregions due to differences in vegetation type and climatic conditions. Fonteyne et al. (2021) evaluate the effects of conservation agriculture practices on soil health in different ecoregions of Mexico, finding that soil health parameters vary widely across sites and soil types. Finally, knowing how land management practices within specific ecoregions alter soil structure and fertility is needed for maintaining soil resilience (Beaumont et al., 2011; Dinerstein et al., 2017; Zhang et al., 2019).

On the other hand, season impacts soil health through fluctuations in soil moisture and temperature, which regulates microbial activity, nutrient availability, and organic matter decomposition. Studies by Schimel, (2018) highlight how moisture variability drives microbial resilience, while Sippel et al. (2018) stress temperature's role in carbon cycling. Baldock et al. (2013) link seasonal moisture changes to soil structure stability, and Islam et al. (2020) underscore the interplay of temperature and moisture in shaping soil fertility, emphasizing their importance for sustainable land management.

As a component of livestock management, grazing systems play a significant role in shaping soil health and fertility (Pereira et al., 2022). As the world seeks to balance the needs of food production and environmental sustainability, understanding how different grazing systems affect soil health is required (Bastani et al., 2023; Sarkar et al., 2020). The relationship between grazing systems and soil health is complex and influenced by different factors, including soil type, climate, vegetation type, and management practices (Debouk et al., 2020; Sato et al., 2019).

Grazing has evolved over time, with varying systems employed, such as continuous, rotational, and regenerative, which differ in intensity, frequency, and duration of livestock grazing (Bartley et al., 2023; Fan et al., 2019). Regarding this systems, continuous grazing is characterized by allowing livestock to freely roam over a pasture without regular rotation and has been associated with overgrazing, which can lead to the

depletion of plant cover, soil compaction, and soil erosion (Bartley et al., 2023; Behnke, 2021; Teague, 2018). Additionally, the continuous grazing reduces soil organic matter, nutrient content, and microbial activity (Wróbel et al., 2023). In contrast, regenerative grazing is a more recent innovation in grazing systems involving the periodic movement of livestock between different paddocks or sections of a pasture (Teague and Barnes, 2017). It is designed to mimic natural grazing patterns of large herbivores by frequently moving livestock to small, intensively grazed paddocks. This management aims to maximize the benefits of grazing, such as nutrient distribution, seed dispersal, and trampling, while minimizing the negative impacts associated with continuous managements (Johnson et al., 2022; Mosier et al., 2022). These more sustainable management practices allow for pasture recovery periods, reducing the pressure on vegetation and soil. Regenerative grazing improves soil fertility by promoting plant regrowth, nutrient cycling, and maintaining soil structure (Behnke, 2021; Döbert et al., 2021; Pereira et al., 2022; Teague, 2018).

In recent years, there has been a notable increase in scientific studies and modeling efforts aimed at unraveling the mechanisms through which grazing systems influence soil health. For example, Dastgheyb Shirazi et al. (2021), reveals that long-term grazing exclusion increased from 0.87 % to 1.36 % the soil organic matter compared to open grazing areas. Other research shows that regenerative grazing obtains 8 % more particulate organic matter than continuous grazing; additionally, it can increase the harvested forage by 10% (Mandaluniz et al., 2019). López-Santiago et al. (2019), evaluates the amount of soil organic carbon (SOC) components of a silvopasture system compared with a pasture monoculture system finding that the silvopasture shows higher values of SOC (3.1–3.7%), than the pasture monoculture (1.8–2.2%). Park et al. (2017), proves that water-holding capacity benefits from regenerative grazing, since this type of management increases water infiltration, reduces surface sliding and increases water flow by 5, 47 and $29.5\,\%$ respectively, compared to intense continuous grazing. Alfaro-Arguello et al., (2010); Ferguson et al., (2013)); Trilleras et al. (2015) conduct comprehensive comparative studies on various livestock management systems in Mexico, examining conventional, sustainable, holistic, and regenerative practices. Their studies show that productivity can not only be maintained but also enhanced as the sustainability of ranching operations improves. Notably, they highlight that holistic management strategies are especially effective in fostering both ecological resilience and economic sustainability, offering a promising pathway for the future of sustainable livestock farming. These research have yielded valuable insights, but there is still much to explore. Understanding the influence of ecoregion, climatic conditions, and grazing management on soil health indicators—and the interplay between these factors—is necessary for advancing sustainable soil management practices and enhancing agricultural productivity. This knowledge can develop strategies that ensure long-term ecological resilience and agricultural systems sustainability. The objectives of this study are: i) To assess the influence of ecoregion, grazing management, and seasonality, as well as their interactions, on key soil health parameters; and ii) To determine the relative contribution of specific variables to soil health across contrasting ecoregions, livestock management systems, and seasonal conditions. Based on the outlined objectives, the following hypotheses are proposed: i) Seasonality will exert the strongest influence on soil health parameters, though its effects will vary significantly when interacting with different ecoregions. In contrast, grazing management is expected to have the least impact on soil health outcomes. ii) Biological indicators will play a predominant role in defining soil health; however, their contributions will be modulated by the physical and chemical properties of the soil, which vary across the study sites.

2. Material and methods

2.1. Study area

The study was conducted in Jalisco, which is a state located in Northwestern Mexico, and it's considered the second most important livestock producer of the country (Rojas, 2016). Ecoregions, defined as relatively uniform areas that constitute geographical clusters or associations of ecosystems with similar functions, typically display similarities in the mosaic of environmental resources, organisms, ecosystems, and anthropogenic influences. Thus, ecoregions define extensive regions where one can anticipate encountering similar types of vegetation and soil associations in equivalent locations (Bailey, 1983; Omernik, 1995; Wright et al., 1998). Therefore, we identified three ecoregions (Table 1) based on their soil, climate, geographical characteristics, and type of vegetation. We selected the ecoregions based on different climatic conditions, defined by Köppen's climate classification system and modified by Kottek et al. (2006) (Table 1).

Each ecoregion corresponded to a cattle ranch belonging to the municipalities of Tepatitlán de Morelos, Tlajomulco de Zúñiga, and El Limón and were named Dry-Semidry, Temperate, and Warm, respectively. The physiographic provinces of the Trans-Mexican Volcanic Belt house the first two ecoregions, while the Southern Sierra Madre hosts the third one (Fig. 1; Table 1) (INEGI, 2022).

2.2. Description of grazing management

Two different types of grazing management with cattle breeding production system were considered in this study (Table 2). The continuous grazing management (CGM) was implemented in the three eco regions: the cattle remain constantly in the pasture, without rotations or rest periods. Regenerative grazing management (RGM) differs between each eco region. In the dry-semidry region, a combination of grazing systems was used, which included non-selective intensive grazing and Voisin rational grazing (PRV); three rotations per day and 8-hour permanence periods for each division were established. The temperate region established an ultra-high density grazing system (PUAD), which consists of establishing a high animal load, on a small area, with short occupation periods and rapid rotations. Finally, the warm region adopted intensive non-selective grazing alongside a silvopastoral system. This system integrates the use of all available forage resources,

Table 1Description of the geographical, ecological, and climatic characteristics of the study ecoregions.

| Eco region | Dry-semidry | Temperate | Warm |
|---|--------------------------|-------------------------|----------------|
| Municipality | Tepatitlán de Morelos | Tlajomulco de Zúñiga | El Limón |
| Latitude | 20°92'63.59"N | 20°45'35.25"N | 19°83'99.77"N |
| Longitude | 102°86'57.25"W | 103°43'61.39"W | 104°14'96.74"W |
| Height (AMSL) | 1806 | 1579 | 880 |
| Slope (%) | < 5° | < 5° | $>15^{\circ}$ |
| Köppen-Geiger | Subtropical | Subtropical | Subtropical |
| Classification* | Dry | Subhumid (Cwa) | Humid |
| | (BShw) | | (Cfa) |
| Soil type | Phaeozem | Vertisol | Regosol |
| Type of vegetation | Xerophilous | Low Deciduous | Subtropical |
| | Scrub | Forest | Dry Forest |
| Average annual temperature (°C) | 18.7 | 19.8 | 23.2 |
| Average Annual Precipitation (mm) | 669 | 765 | 1049 |
| Months with | June to | June to September | June to |
| precipitation greater than 100 mm | August | | October |

Note. Data from (IIEG, 2023). *(Kottek et al., 2006)

including trees, shrubs, and grass. Similar to the approach taken in the temperate region, a rotational grazing strategy was implemented, with two rotations per day and a 12-hour occupancy period per division.

2.3. Study paddocks and sampling

Two paddocks were selected in each ranch using the Normalized Difference Vegetation Index (NDVI) as a proxy for grass productivity (Fig. 2). This index provides a measure of the health of vegetation, based on the way plants reflect light of certain wavelengths (Huang et al., 2021). The NDVI was obtained through the application of EO Browser through the images of the Sentinel-2 satellite with a spatial resolution of 10 m, with data from level 2 A atmosphere corrector (Fedoniuk et al., 2021), and with an interval time from January to May 2022. Additionally, the information provided by the NDVI was corroborated in the field.

Sampling was conducted across the three ranches during two seasons in 2023. The first sampling, considered as the dry season, was in May, due to the warmest temperature and the lowest relative humidity. The second sampling, considered as the wet season, was in October, due to the highest accumulated precipitation record and the highest relative humidity.

Two 1-hectare paddocks were selected, corresponding to each type of livestock management in each ranch. Within each paddock, five georeferenced sampling points were identified. At each point, a 1 m² quadrat was marked for the collection of plant biomass, soil respiration measurement (Rs), and soil sampling. Within the quadrant, the manure biomass (Mb) and the grass (forage) was cut, extracted, and weighed in its wet state to calculate the production of wet forage per m². Subsequently, for the calculation of production per m², a 200 g sub-sample of green matter was dried at 65°C for 72 h in a forced ventilation oven, after which the percentage of partial dry matter (PDM) was estimated by the difference in weight before and after placing it in the oven. The partially dry samples were processed through a stationary Thomas-Wiley mill, using a 1 mm sieve. For the determination of the total dry matter percentage (TDM), 1 g of ground sample was left in the oven for an additional 3 h at 105 $^{\circ}$ C. The percentage of total dry matter (TDM) was obtained using the formula: TDM (%) = PDM*(100-Residual moisture)/100 (De La Roza-Delgado et al., 2002). Soil samples were collected within each paddock and region at the dry and wet seasons from a depth of 0–15 cm, air-dried and sieved (< 2 mm). Prior to drying, a 10 g soil sample was extracted for microbial biomass determination and transported to the laboratory at $6 \pm 2^{\circ}$ C in a cooler. Additionally, a 2.5 m radius circle (covering 19.6 m²) was delineated from the center of the quadrant for feces collection. Environmental temperature (T°) and relative humidity (RH) were recorded at each sampling site (quadrat) using the Barometric Pressure/Humidity/Temperature Datalogger Extech SD700.

2.4. Soil health parameters

Soil health parameters were determined by the guidelines provided by the Soil Quality Institute USDA (1999), Food and Agriculture Organization of the United Nations (FAO) (FAO-ITPS, 2020) and Lal (1998). Total soil carbon (%C), total organic carbon (%TOC), and total nitrogen (%N) contents in the soil were analyzed at the chemical laboratory of Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV) unidad Saltillo, using the dry combustion method of Dumas. This was performed with a Thermo Scientific™ FlashSmart™ 2000 elemental analyzer in CHNS/O analytical configuration. Soil respiration (g C m² h⁻¹) was determined in-situ with an LI-8100 Automated Soil CO2 Flux System (LI-COR Inc., Lincoln, Nebraska, USA) over a 20.3-cm diameter collar installed on the soil surface at each survey site. A 20-cm diameter (LI-8100−103) respiration survey chamber was used, in which the increase in CO₂ concentration was recorded by the LI-8100's infrared gas analyzer unit (Liu et al.,

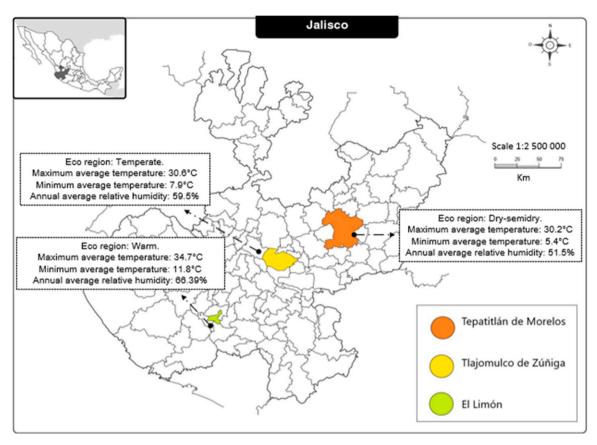


Fig. 1. Location of the study areas in Jalisco State, Mexico. The locations correspond to the ecoregions Dry-semidry, Temperate and Warm (orange, yellow, and green, respectively). See Table 1.

Table 2Description of grazing management systems across the three different ecoregions of Mexico, evaluated during two seasonal periods.

| | Dry-sen (Tepatit | n idry lán de Morel | os) | | Tempe i (Tlajom | rate ulco de Zúñia | ga) | | Warm (El Lim | ón) | | |
|------------------------------------|----------------------------|-------------------------------|---------|------|---------------------------|-----------------------|---------|------|-----------------|-------|---------|-------|
| Grazing management | Regenero | ıtive | Continu | ous | Regener | ative | Continu | ous | Regener | ative | Continu | ous |
| Rest period (days) | 90 | | N/A* | | 390 | | N/A* | | 120 | | N/A* | |
| Rotational period (hours) | 8 | | N/A* | | 12 | | N/A* | | 12 | | N/A* | |
| Season | Dry | Wet | Dry | Wet | Dry | Wet | Dry | Wet | Dry | Wet | Dry | Wet |
| Paddock productivity (t/ha) | 6.30 | 22.68 | 4.08 | 4.97 | 3.40 | 53.30 | 3.50 | 5.73 | 7.21 | 8.77 | 5.42 | 8.52 |
| Effective stocking rate (AU/ha) | 140 | 504 | 90 | 110 | 75 | 1184 | 77 | 127 | 160 | 195 | 120 | 189.3 |
| Annual stocking rate (AU /ha/year) | 1.76 | | 0.55 | | 3.45 | | 0.56 | | 0.97 | | 0.85 | |

N/A= No applicable



Fig. 2. Paddocks selection using Normalized Difference Vegetation Index (NDVI). Satellite images of the NDVI - with a spatial resolution of 10 m obtained of EO Browser through the images of the Sentinel-2 satellite. a) Dry-semidry region; b) Temperate region; c) Warm region. Capital letters in bold represent the two types of grazing managements: C= Continuous; R= Regenerative. The black line divides the paddocks for each type of grazing management.

2010; Madsen et al., 2009). Soil temperature (°CTs), and soil moisture (%Hs) were recorded using the Hanna HI98331 Soil Test™ and the Extech MO750: Soil Moisture Meter respectively. Soil organic matter content (%SOM) was determined using the gravimetric method, calcining the sample at 400 °C for 4 h (Salehi et al., 2011). Bulk and real density (g cm⁻³) were analyzed using the known volume method (Al-Shammary et al., 2018), and pycnometric method (NOM-021-SE-MARNAT-2000) respectively. Soil pH and electrical conductivity (EC) (dS m⁻¹) were measured in a soil-water suspension (1:2, w/v) using a digital pH meter (HANNA HI2211 pH/ORP Meter) and a conductometer (YSI MODEL 35), respectively. Soil texture was determined by the Bouyoucos method (NOM-021-SEMARNAT-2000). Soil microbial biomass carbon (MBC) (mg C glucose kg⁻¹) was determined by the fumigation-extraction method (Gregorich et al., 1991). The directionality of the effects of the evaluated parameters on soil health is presented in Table 3.

2.5. Experimental design

A multifactorial analysis was proposed in which soil health parameters were analyzed through several factors and their interactions. The effects of the ecoregion, grazing management type, and season on soil health were evaluated and a $3 \times 2 \times 2$ factorial design was employed. This resulted in a total of 12 treatments, each with 5 replicates.

2.6. Data analysis

All variables were tested for homogeneity of variance and data normality using Levene's test (p < 0.05) and the Shapiro-Wilk test (p < 0.05), respectively. Normal distribution was not met by 10 of the 15 variables (Hs, EC, N, SOM, C, TOC, MBC, Rs, Fb, Mb), so it was decided to use non-parametric statistics. Correlations between all measured variables were tested using Sperman's Rho with p < 0.05significance threshold with the intention of detecting and eliminating spurious correlations (Bocianowski et al., 2023).

To evaluate the main effects of each individual factor upon the fifteen soil health parameters, simple comparisons were conducted. For the 5 parameters (Ts, BD, porosity, pH, C:N ratio) that met the assumptions, a one-way ANOVA was performed with the Tukey test as a post hoc test. On the other hand, when the assumptions were not met and two groups were compared (factors GM and S), the Wilcoxon test was applied, and for more than two groups (factor R), Kruskal-Wallis with the Nemenyi test as a post hoc was conducted. A 95 % confidence interval was employed for all comparisons (p < 0.05). Subsequently, the effects of the factors interactions on individual soil physical, chemical, and biological variables were investigated using a three-way Permutational Multivariate Analysis of Variance (PERMANOVA) performed with Bray-Curtis distances as a measure of dissimilarity among the treatments (Anderson and Santana-Garcon, 2015). Significance was obtained from 9999 permutations of the data with a logarithmic transformation and an F-test based on 1000 sums of squares. Lastly, a three-way PERMANOVA was employed to investigate the relationships between the factors and its interaction with the entire set of soil health variables, adhering to the same criteria as previously considered. All analysis were performed using R software and Rstudio (R version 4.3.3 for Windows, using vegan package).

On the other hand, a Non-metric Multidimensional Scaling (NMDS) approach was implemented with the objective of visualizing and exploring patterns of similarity or dissimilarity between the study sites. Likewise, it allowed us to identify the groupings of the study sites based on soil health variables and evaluate the contribution of these variables on the composition of the groups. The "Bray-Curtis distance" matrix was created from data on soil health parameters taken at each location. The base 10 logarithmic transformation was used as a scaling method. This strategy sorted the locations into a low-dimensional space based on their ecological and management similarities. Groupings were included into

Table 3 Soil parameters and the directionality of their effect on soil health.

| Soil parameters | Directionality of effect on soil health | Description of the effect on soil health | References |
|--------------------|---|--|---------------------------------------|
| Гѕ | Non-linear | Extreme soil | (Sharma and |
| | | temperatures harm soil health: < 5°C reduces | Kumar, 2023; Z. Wang et al., 2022; |
| | | microbial activity, slows | Zhou et al., 2023, |
| | | SOM decomposition; | 2024) |
| | | > 35°C depletes SOM, | 2024) |
| | | stresses microbes, and | |
| | | worsens drought. | |
| -Is | Non-linear | Extreme moisture levels | (Allison, 2023; |
| | | affect soil health: | Pärn et al., 2025; |
| | | drought causes | Sandor et al., 2021) |
| | | microbial dormancy and | |
| | | hydrophobicity, limiting | |
| | | plant growth; | |
| | | waterlogging creates | |
| | | anaerobiosis, root | |
| | | hypoxia, and nutrient | |
| | | loss. | |
| BD | Negative | Higher bulk density | (Frene et al., 2024; |
| | | (compaction) often | Shaheb et al., |
| | | reduces soil health. | 2021) |
| | | Limits root growth, | |
| | | water infiltration, and gas exchange while also | |
| | | limiting habitat and | |
| | | oxygen for soil | |
| | | organisms. | |
| Poro | Positive | Higher porosity | (Du et al., 2022; |
| | | improved aeration & gas | Sadiq et al., 2021) |
| | | exchange, enhanced | |
| | | water infiltration & | |
| | | retention, and promotes | |
| | | better root penetration. | |
| Н | Non-linear | Soil health is optimized | (Naz et al., 2022; |
| | | at near-neutral pH | Pahalvi et al., |
| | | (6.0–7.5), while both | 2021) |
| | | acidic (<5.5) and | |
| | | alkaline (>8.5) extremes | |
| EC | Nogotiero | degrade key functions. | (Michael et al |
| 2C | Negative | Excessive EC (salinity) triggers osmotic stress, | (Mishra et al., 2023; Singh, 2022) |
| | | ion toxicity, microbial | 2023, 311igii, 2022) |
| | | suppression, and | |
| | | structural soil | |
| | | degradation. | |
| N | Non-linear | Moderate N levels | (Luo et al., 2022; |
| | | (0.1-0.5 %) enhance | Yang et al., 2023) |
| | | fertility, but excess N | = ' ' |
| | | degrades soil health, | |
| | | while deficiency limits | |
| | | productivity. | |
| C:N | Non-linear | Low C:N ratios (<20:1) | (Amorim et al., |
| | | favor nutrient release, | 2022; Gilmullina |
| | | and high ratios (>30:1) | et al., 2023; lyu |
| | | promote N | et al., 2023) |
| | | immobilization and slower decomposition. | |
| C | Positive | Higher soil organic | (Fahad et al., 2022; |
| | 1 OSILIVE | carbon (SOC) levels | Liptzin et al., 2022 |
| | | improve soil health, but | ыркын сt ан, 2022) |
| | | benefits plateau at high | |
| | | concentrations (3–6 % | |
| | | SOC, depending on soil | |
| | | type). | |
| ГОС | Positive | Higher TOC levels | (Babur and |
| - | | improve soil health, with | Dindaroglu, 2020; |
| | | benefits typically | Lal, 2016; |
| | | plateauing at soil- | Lehmann et al., |
| | | specific thresholds | 2020) |
| | | (2–4 %). Promotes | |
| | | aggregate stability, | |
| | | aggregate stability, | |

Table 3 (continued)

| Soil parameters | Directionality of effect on soil health | Description of the effect on soil health | References |
|--------------------|---|---|---|
| SOM | Positive | nutrient cycling and microbial biomass. Higher SOM levels enhance soil health, with benefits typically plateauing at soil- specific thresholds | (H. Li et al., 2020; Maestre et al., 2022) |
| MBC | Positive | (3–8 %). Higher MBC levels consistently correlate with improved soil health, serving as a | (Liu et al., 2012; KH. Wang et al., 2006) |
| Rs | Non-linear | sensitive indicator of soil biological activity and organic matter quality. Moderate soil respiration rates indicate active microbial metabolism and nutrient cycling, while both | (S. Li et al., 2024; Morris et al., 2022) |
| Fb | Positive | extremely low and high rates signal degraded soil health. Increased forage biomass generally improves soil health through organic matter inputs and root activity, | (Amelung et al., 2020; Machmuller and Dillon, 2021) |
| Mb | Negative | but benefits plateau at high productivity levels due to nutrient limitations or grazing pressure Higher amount of manure in grasslands can be related to a low degradation rate, delaying its integration into the soil, affecting soil health | (Hoffmann et al., 2001; Kao et al., 2024) |

Positive = higher value improves soil health; Negative = higher value harms soil health; Non-linear = non-uniform relationship. Ts= soil temperature (°C); Hs= soil moisture (%); BD= bulk density (g cm $^{-3}$); Poro= soil porosity (%); EC= electric conductivity (dS m $^{-1}$); N = total soil nitrogen(%); C:N = carbonnitrogen ratio; C=total soil carbon (%); TOC= total soil organic carbon (%); SOM = soil organic matter (%); MBC= microbial biomass carbon (mg C glucose kg $^{-1}$); Rs= soil respiration (g C m 2 h $^{-1}$); Fb= forage biomass (kg m $^{-2}$); Mb= manure biomass (kg m $^{-2}$).

the NMDS representation by incorporating centroids for each group, which were derived as the weighted average of the NMDS coordinates of the sites within each cluster. The centroids were utilized to interpret site groupings based on shared attributes. To determine whether the groupings seen in the NMDS are statistically significant and to assess the similarities and differences between the sites, an analysis of similarities (ANOSIM) was performed using the study sites' centroids. Finally, a similarity percentage analysis (SIMPER) was employed to ascertain the distinct contributions of each soil health variable to the observed differences or similarities among the study site groups in the NMDS. The Past 4.16c statistical software was used to perform all the above tests (Hammer, Harper, 2001).

3. Results

3.1. Effects of evaluated factors on soil health parameters

Seasonality along with grazing management emerged as the most important factors influencing soil health parameters. While grazing management significantly affected 11 out of the 15 soil health parameters analyzed (Table 4), the results highlight that seasonal variations and their interactions play a critical role in regulating soil biological processes and overall soil health. In contrast, the ecoregion had the least influence on soil health parameters. These findings reject the proposed hypothesis, underscoring the importance of considering seasonal variations and management practices over ecoregional differences in soil health assessments.

In general, the ecoregion has a significant impact on 10 out of the 15 parameters evaluated The warm ecoregion, characterized by a sandy-clay-loam soil texture, demonstrated better physical properties (BD: $0.96\pm0.15~g$ cm $^{-3}$; porosity: 54.74 ± 6.48 %; Ts: $33.46\pm3.72~^{\circ}\text{C}$) compared to the dry-semidry and temperate ecoregions, both of which have a clay-loam texture. These values indicate higher soil porosity, lower compaction, and improved aeration and water infiltration compared to the other ecoregions.

In terms of chemical parameters, the dry-semidry and warm regions exhibit similar values except for pH and nitrogen (N). The warm region demonstrates slightly acid values for pH (6.44 \pm 0.41) compared to the dry-semidry region (6.60 \pm 0.36), as well as higher N (0.27 \pm 0.09) versus the dry-semidry region (0.20 \pm 0.08). In contrast, the temperate region shows higher levels of EC (0.18 \pm 0.04) and C:N (16.10 \pm 1.55) in comparison with dry-semidry and warm regions. Regarding the parameters related to carbon dynamic, ecoregion primarily influences carbon (C) and total organic carbon (TOC). The temperate and warm regions show no significant differences in their carbon levels, both of which are higher than those in the dry-semidry region. However, for TOC, the warm region stands out with the highest values among the three regions. Regarding biological parameters, the ecoregion only influenced Mb, showing higher values for the dry-semidry (4.04 \pm 2.07) region compared to the temperate (1.07 \pm 0.76) and warm (1.94 \pm 1.35) regions.

On the other hand, grazing management had a significant impact on physical, chemical and biological parameters. Regenerative management showed better values for BD (0.95 \pm 0.08 g cm $^{-3}$), porosity (55.36 \pm 4.21 %) and N (0.29 \pm 0.08 %) compared to continuous management (1.09 \pm 0.10 g cm $^{-3}$; 50.01 \pm 4.81 %). Similarly, soil organic matter (4.37 \pm 0.36 %), organic carbon (4.19 \pm 1.19 %), total organic carbon (2.57 \pm 1.20 %), microbial biomass carbon (139.93 \pm 102.29 mg C glucose kg $^{-1}$), soil respiration (0.53 \pm 0.36 g C m 2 h $^{-1}$), and forage biomass (1.33 \pm 1.16 kg m $^{-2}$)were higher under regenerative management, indicating greater biological activity and carbon dynamics compared to continuous management.

Finally, as expected, seasonality had significant effects on soil temperature (Ts), soil moisture (Hs), and biological parameters, but its influence on chemical parameters was minimal, except for pH and TOC %. As expected, the wet season showed lower Ts (28.21 \pm 2.79 $^{\circ}$ C) and higher Hs (14.47 \pm 5.36 %) than the dry season. Additionally, biological parameters were significantly higher during the wet season (MBC: 169.12 ± 81.19 mg C glucose $kg^{-1};~Rs:~0.67\pm0.25$ g C m $^2~h^{-1};~Fb: 1.37\pm1.14~16~kg~m^{-2}).$ Finally, the chemical parameters of TOC and pH follow the same trend, which shows that the wet season obtains higher values, suggesting that temperature and moisture conditions regulate soil biological activity.

The PERMANOVA analysis revealed that the interaction between ecoregion and grazing management significantly affected EC only in the warm ecoregion, with higher values under regenerative management (0.19 \pm 0.08 dS \mbox{m}^{-1}) compared to continuous management (0.10 \pm 0.05 dS \mbox{m}^{-1}). In contrast, BD and manure biomass Mb showed similar trends across all three ecoregions, with higher values under continuous grazing than regenerative grazing. The dry-semidry ecoregion under continuous management exhibited the highest BD and Mb, while regenerative management in the warm and temperate ecoregions resulted in the lowest BD and Mb values, respectively. Conversely, Rs was consistently higher under regenerative management across all ecoregions, with the warm ecoregion under regenerative management showing the highest Rs values and the temperate ecoregion under

Table 4
Soil health parameters from three climatically different regions, two grazing management and two season of cattle ranches in Mexico.

| Type of indicator | | | Eco region | | | Grazing manage | Season | | | | |
|-------------------|------|-------------------|-------------------|-----------------------------------|-----|------------------|-----------------------------------|-----|--|------------------|-----|
| | | Dry-semidry | Temperate | Warm | P | Continuous | Regenerative | P | Dry | Wet | P |
| Phy | Ts | 29.84 + 2.79a | $30.27 \pm 4.89a$ | 33.46 + 3.72b | *** | 29.57 ± 3.84 | 32.82 ± 3.87 | *** | 34.18 ± 3.00 | 28.21 ± 2.79 | *** |
| | Hs | 6.01 ± 2.99 | 11.24 ± 8.66 | 9.33 ± 6.62 | ns | 8.25 ± 6.37 | 9.47 ± 7.21 | ns | 3.31 + 1.00 | 14.47 + 5.36 | *** |
| | BD | $1.05 \pm 0.09a$ | $1.05 \pm 0.09a$ | $0.96 \pm 0.15b$ | ** | 1.09 ± 0.10 | 0.95 ± 0.08 | *** | $\begin{matrix} -\\ 1.04 \pm 0.11\end{matrix}$ | 1.00 ± 0.12 | ns |
| | Poro | 50.86 | 52.45 ± 4.86b | 54.74 | ** | 50.01 ± 4.81 | 55.36 ± 4.21 | *** | 51.96 ± 5.20 | 53.41 ± 5.26 | ns |
| | | ± 3.40a | | ± 6.48c | | | | | | | |
| Che | pH | $6.60 \pm 0.36a$ | $6.89 \pm 0.42b$ | $6.44 \pm 0.41c$ | *** | 6.55 ± 0.45 | $\textbf{6.74} \pm \textbf{0.41}$ | ns | 6.44 ± 0.33 | 6.84 ± 0.44 | *** |
| | EC | $0.11 \pm 0.03a$ | $0.18 \pm 0.04b$ | $0.15 \pm 0.08a$ | *** | 0.13 ± 0.05 | 0.16 ± 0.06 | ns | 0.16 ± 0.06 | 0.13 ± 0.06 | ns |
| | N | $0.20 \pm 0.08a$ | $0.23 \pm 0.09b$ | $0.27 \pm 0.09c$ | ** | 0.17 ± 0.06 | 0.29 ± 0.08 | *** | 0.21 ± 0.07 | 0.25 ± 0.10 | ns |
| | C:N | 13.33 | $16.10 \pm 1.55b$ | 14.20 | *** | 14.80 ± 2.25 | 14.29 ± 2.80 | ns | 14.79 ± 1.67 | 14.30 ± 3.18 | ns |
| | | ± 2.64a | | ± 2.51a | | | | | | | |
| | C | $2.59 \pm 0.86a$ | $3.67 \pm 1.45b$ | $3.84 \pm 1.21b$ | *** | 2.55 ± 0.82 | 4.19 ± 1.19 | *** | 3.18 ± 1.19 | 3.56 ± 1.41 | ns |
| | TOC | 1.41 ± 0.62 a | $1.98 \pm 0.96a$ | $2.64 \pm 1.30b$ | *** | 1.45 ± 0.63 | 2.57 ± 1.20 | *** | 1.67 ± 0.90 | 2.34 ± 1.20 | *** |
| Bio | SOM | 3.7 ± 0.76 | 3.79 ± 0.87 | 3.84 ± 0.73 | ns | 3.18 ± 0.60 | 4.37 ± 0.36 | *** | 3.63 ± 0.77 | 3.92 ± 0.78 | ns |
| | MBC | 100.56 | 107.24 | 109.64 | ns | 71.70 | 139.93 | *** | 42.50 | 169.12 | *** |
| | | \pm 78.50 | $\pm~103.64$ | \pm 80.01 | | ± 49.28 | ± 102.29 | | ± 20.40 | ± 81.19 | |
| | Rs | 0.41 ± 0.34 | 0.37 ± 0.37 | $\textbf{0.44} \pm \textbf{0.30}$ | ns | 0.28 ± 0.27 | 0.53 ± 0.36 | ** | 0.13 ± 0.12 | 0.67 ± 0.25 | *** |
| | Fb | 1.10 ± 1.29 | 0.95 ± 0.94 | 0.75 ± 0.26 | ns | 0.54 ± 0.27 | 1.33 ± 1.16 | *** | 0.50 ± 0.23 | 1.37 ± 1.14 | *** |
| | Mb | $4.04 \pm 2.07a$ | $1.07 \pm 0.76b$ | $1.94 \pm 1.35b$ | *** | 2.90 ± 2.09 | 1.81 ± 1.61 | ** | 2.91 ± 1.99 | 1.79 ± 1.73 | ** |

Data = mean \pm SE (n = 60). The effect of factors is summarized on the left of the table. Phy= physical; Che= chemical; Bio= biological; Ts= soil temperature (°C); Hs= soil moisture (%); BD= bulk density (g cm⁻³); Poro= soil porosity (%); EC= electric conductivity (dS m⁻¹); N = total soil nitrogen(%); C:N = carbon-nitrogen ratio; C=total soil carbon (%); TOC= total soil organic carbon (%); SOM = soil organic matter (%); MBC= microbial biomass carbon (mg C glucose kg⁻¹); Rs= soil respiration (g C m² h⁻¹); Fb= forage biomass (kg m⁻²); Mb= manure biomass (kg m⁻²). Significant differences (P < 0.05) between main effects are indicated with lower-case letters (among each factor).

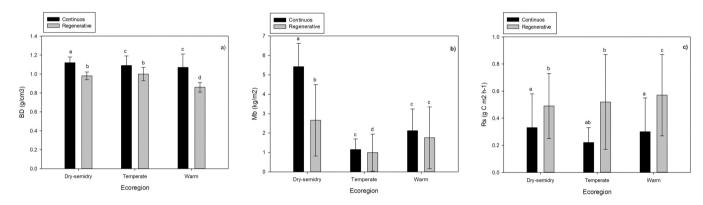


Fig. 3. Influence of the ecoregion and grazing management on bulk density (BD), manure biomass (Mb) and soil respiration (Rs). Black filled bars represent continuous grazing, grey filled bars regenerative grazing. Values are means \pm standard error. Lowercase letters represent differences among BD, Mb and Rs parameters within each grazing management and its interaction with the ecoregions (p < 0.05, n = 60), according to Pairwise post-hoc comparison.

continuous management showing the lowest (Fig. 3).

The interaction between ecoregion and season significantly influenced Mb, soil moisture (Hs), and Rs. In the dry-semidry and warm ecoregions, Mb was higher during the dry season (5.00 \pm 1.46 kg m $^{-2}$ and 2.93 \pm 0.90 kg m $^{-2}$, respectively) than in the wet season (3.09 \pm 2.21 kg m $^{-2}$ and 0.95 \pm 0.93 kg m $^{-2}$). In contrast, in the temperate ecoregion, Mb was lower during the dry season (0.81 \pm 0.34 kg m $^{-2}$) than in the wet season (1.33 \pm 0.99 kg m $^{-2}$). Meanwhile, Hs and Rs were consistently higher during the wet season across all ecoregions, with the highest Hs observed in the temperate ecoregion and the lowest in the warm ecoregion (Fig. 4).

The interaction between grazing management and season revealed that Mb differed significantly only during the wet season, with lower values under regenerative management (0.84 \pm 0.81 kg m $^{-2}$) compared to continuous management (2.77 \pm 1.85 kg m $^{-2}$). Microbial biomass carbon (MBC) and Rs varied between grazing managements in both seasons, with the highest values under regenerative management during the wet season and the lowest under continuous management during the dry season (Fig. 5).

The triple interaction (ecoregion \times management \times season) had

significant effects on pH and forage biomass (Fb). pH remained within an optimal range for plant growth across all sites, with the lowest value observed in the warm ecoregion under continuous management during the dry season (6.19 \pm 0.32) and the highest in the temperate ecoregion under regenerative management during the wet season (7.22 \pm 0.43). Fb was consistently higher under regenerative management in both seasons and across all ecoregions. The dry-semidry ecoregion under regenerative management during the wet season showed the highest forage productivity, followed by the temperate ecoregion under the same conditions. Conversely, the lowest productivity was observed in the dry-semidry and temperate ecoregions under continuous management during the dry season (Fig. 6).

Finally, The PERMANOVA analysis (Table 5) reveals global effects for ecoregion (F=18.37; p=0.0001), grazing management (F=47.89; p=0.0001), and season (F=103.30; p=0.0001), as well as significance of the interactions of ecoregion-grazing management (F=3.06; p=0.0134) and ecoregion-season (F=4.11; p=0.0029). The results reveal that all three factors had a highly significant effect, with the season being the most representative, followed by management, and lastly the ecoregion. This indicates that seasonality exerts the greatest

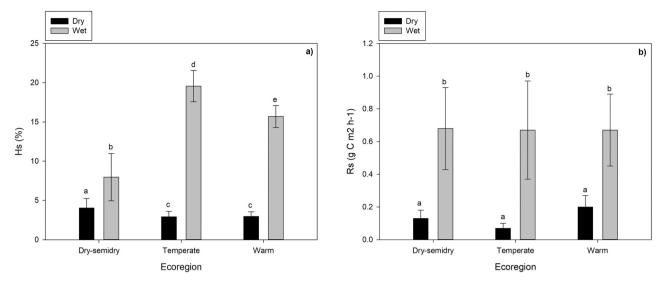


Fig. 4. Interaction of season and ecoregions on soil moister (Hs) and soil respiration (Rs). Black filled bars represent dry season, grey filled bars wet season. Values are means \pm standard error. Lowercase letters represent differences among the soil parameters within each season (p < 0.05, n = 60), according to Pairwise post-hoc comparison.

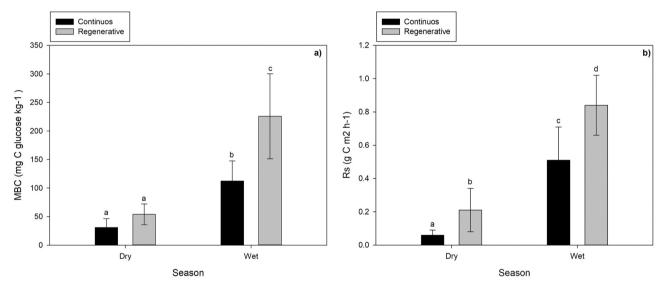


Fig. 5. Interaction of season and grazing management on microbial biomass carbon (MBC) and soil respiration (Rs). Black filled bars represent continuous grazing, grey filled bars regenerative grazing. Values are means \pm standard error. Lowercase letters represent differences among the soil parameters within each grazing management (p < 0.05, n = 60), according to Pairwise post-hoc comparison.

influence on soil health variables when they are analyzed together. On the other hand, management also significantly influences soil health. The interactions of ecoregion-management and region-season also show a significant effect, although with lesser influence.

3.2. Sites ordination and their relationship with soil health indicators

The NMDS analysis based on the Bray-Curtis dissimilarity matrix shows a clear ordination of the evaluated sites (Fig. 6). The final solution reached convergence after 9999 permutations, with a stress of 0.0455. The results revealed four clear groups along axis 1, which explains 98.4 % of the total variation (R2 = 0.9845). Group 1 (WRW, DRW, TRW) is composed of sites under regenerative management during the wet season; Group 2 (WCW, TCW, DCW) consists of sites with continuous management during the same season. In the opposite direction of axis 1, group 3 (WRD, DRD) associates the sites that implement regenerative management during the dry season, except for the one

corresponding to the temperate ecoregion; finally, group 4 (TCD, DCD, WCD, TRD) includes the sites that perform continuous management during the dry season along with the site corresponding to the temperate ecoregion with regenerative management. To verify the grouping of the sites, a similarity analysis (ANOSIM) was used along with the post hoc pairwise test with p-values, sequential Bonferroni significance, which statistically confirms ($R=0.645,\,p=0.0001$) that there are significant differences between the centroids of the study sites formed in the ordination. (Table S2). Additionally, to determine the different contributions of each soil health variable to the observed differences or similarities between the research site groups, the similarity percentage analysis (SIMPER) was conducted, showing an overall dissimilarity average of 34.98 % and finding that Rs was the soil health parameter that most influenced the site ordination, with a contribution of 17.9 %, followed by Mb, MBC, Hs, and Fb, which together achieve a cumulative contribution of 67.01 % (Table S3). These findings confirm the hypothesis that soil biological indicators, in conjunction with soil

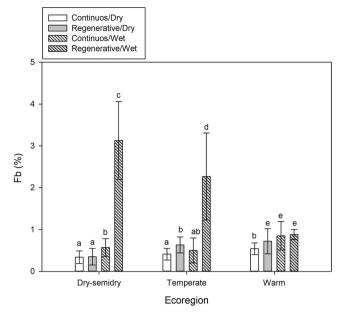


Fig. 6. Effect of the interaction of the three factors on forage biomass (Fb). The white filled bars represent continuous management in the dry season, the grey filled bars represent continuous management in the dry season, the white textured bars represent regenerative management in the dry season and the grey textured bars represent regenerative management in the wet season. Values are means \pm standard error. Lowercase letters represent differences among the Fb parameter within seasons and grazing management, according to pairwise post-hoc comparison (p < 0.05, n = 60).

moisture, are the primary factors contributing to soil health.

4. Discussions

4.1. Effects of evaluated factors on soil health parameters

The ecoregion significantly influences soil physical and chemical properties, primarily due to variations in soil type, texture, and edaphoclimatic conditions (Hidalgo et al., 2019; Mesfin et al., 2021). For example, bulk density and porosity were strongly linked to ecoregion-specific factors such as climate, topography, and vegetation (Luo et al., 2021; Zhang et al., 2019). In humid and tropical ecoregions, soils typically exhibit higher clay content and higher nutrient leaching due to increased precipitation and organic decomposition rates (Sanchez, 2019). In contrast, dry-semidry ecoregions are characterized by sandier soils with lower BD, higher porosity, and reduced organic

Table 5 Permutational Multivariate Analysis of Variance using a soil health parameters database (n=60) of three ecoregions, two grazing managements and two seasons of cattle ranches in Mexico.

| | D.f. | Sum of Sqs. | R^2 | F | Pr(>F) | |
|----------|------|-------------|-------|--------|--------|-----|
| R | 2 | 0.0035 | 0.14 | 18.37 | 0.0001 | *** |
| GM | 1 | 0.0046 | 0.18 | 47.89 | 0.0001 | *** |
| S | 1 | 0.010 | 0.40 | 103.30 | 0.0001 | *** |
| R:GM | 2 | 0.0005 | 0.02 | 3.06 | 0.0134 | * |
| R:S | 2 | 0.0008 | 0.03 | 4.11 | 0.0029 | ** |
| GM:S | 1 | 0.0001 | 0.006 | 1.56 | 0.1864 | ns |
| R:GM:S | 2 | 0.00006 | 0.002 | 0.30 | 0.9413 | ns |
| RESIDUAL | 48 | 0.004 | 0.19 | | | |
| TOTAL | 59 | 0.024 | 1 | | | |

Non-significant effects (ns>0.05); Significant effects are noted in bold (*<0.05; **< 0.01; ***<0.001); R = Ecoregion (Dry-semidry, temperate or warm); R = Ecoregion (Dry-semidry); R = Ecoregion (Dry or Wet).

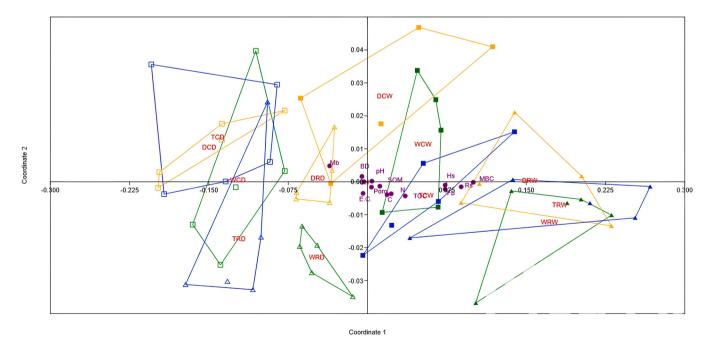


Fig. 7. Grouping of Mexican cattle ranches, plotted by Non-metric multidimensional scaling (NMDS) using soil health parameters from three ecoregions, two grazing managements and two seasons. Vector length and direction respectively represent the relative magnitude of explained variation and that of a positive increase. Study sites are represented by: Type of grazing management is represented with figures: Triangle = Regenerative; Square = Continuous; Season is represented with Fill = Wet; No filler = Dry; Ecoregion is shown with color: Blue = Temperate; Green = Warm; Orange= Dry-semi-dry: WRD (Warm regenerative dry); WCD (Warm continuous dry); DCD (Dry-semidry continuous dry); DCD (Dry-semidry continuous dry); TCD (Temperate regenerative dry); TCD (Temperate continuous dry); WCW (Warm continuous wet); WRW (Warm regenerative wet); DCW (Dry-semidry continuous wet); DRW (Dry-semidry regenerative wet); TRW (Temperate regenerative wet); TCW (Temperate continuous wet). Similarity index: Bray-Curtis; Stress: 0.0455; R2: Axis 1: 0.9846, Axis 2: 0.0129.

matter, which negatively impact fertility (Lal, 2016). According to our findings, the dry-semidry region with a clayey loam texture had the lowest porosity and carbon content among the three ecoregions studied. While the levels of BD and TOC are not significantly different from the temperate region, they are with the warm region, with the latter having the highest values. Finally, the dry-semidry zone has far higher Mb content than the temperate and warm regions, which do not differ significantly among them. This demonstrates that the edaphoclimatic conditions of the ecoregions influence the physical and chemical qualities of the soil, as well as how feces decompose and mix into it.

This study further highlights that ecoregional variations significantly affect electrical conductivity (EC), pH, nitrogen (N) content, and the C:N ratio. For instance, Rivero-Villar et al. (2022) reported notable differences in soil fertility across ecoregions, including variations in organic reserves and levels of carbon (C), nitrogen (N), and phosphorus (P). Similarly, Fonteyne et al. (2021) found that sandy soils exhibit 12 % higher EC than clay soils, while Sentis (2014) and Feng et al. (2023) demonstrated that EC is generally higher in dry-semidry due to salt accumulation, whereas humid regions tend to have more acidic pH and greater N availability. These findings align with studies emphasizing the direct influence of ecological gradients on soil parameters (Huerta Martínez et al., 2014; Murga-Orrillo et al., 2021).

Regenerative grazing management was the factor that shows the better physical and biological soil health parameters. Furthermore, regenerative management shows better values than continuous in those parameters that directly interact with the biological fraction of the soil (BD, Poro, SOM, C, TOC, MBC, Rs, Fb). This can be explained by the fact that regenerative management promotes the accumulation of particulate organic carbon (POC), humified organic matter (HOM), and mineral-associated organic carbon (MAOC), while under continuous management, the soluble fraction of SOM is easily degradable without stabilizing in the other fractions. Some studies found that regenerative grazing improves soil health by increasing the accumulation of SOC, both in its POC and MAOC components (Bansal et al., 2024; Mosier et al., 2021; Santos et al., 2024). Prairie et al. (2023) demonstrated that regenerative management practices increase SOC (12.4 %), MAOC (8.5 %), and POC (19.7 %) in the topsoil layer (0 a 20 cm). Other studies found that regenerative livestock management improve SOM by an average of 0.5-1 %, also increase microbial activity by 10-20 %, and develop soil structure, promoting an increment in soil aggregation (Gebremedhn et al., 2022; Maestre et al., 2022; Mosier et al., 2022). Additionally, the rise in porosity and the reduction of the BD observed in regenerative management may be related to a higher SOM content due to the suspension of plant biomass removal (i.e., by harvesting and/or overgrazing) and the transition to perennial grasses, which produce a substantial amount of root biomass (Deregibus et al., 2018). On the other hand, Döbert et al. (2021), showed higher values of porosity for regenerative grazing (55.09 %) in ranches that were allocated among several temperate ecoregions. This indicates that this type of livestock practice can help improve the physical properties of soils because a higher level of porosity often implies well-structured soil with sufficient pore space. These circumstances often promote microbial activity and the nutrient cycle, which contributes to improving soil health (Bork et al., 2021; McDonald et al., 2020). A study by Abdalla et al. (2018) reported a 34 % reduction in CO2 efflux rates in eroded or severely degraded plots, highlighting the critical role of soil organic carbon (SOC) in regulating soil respiration. SOC is a key driver of microbial activity, with microbial biomass typically increasing alongside organic matter content. However, this relationship can be influenced by macroclimatic conditions, fluctuations in soil moisture and temperature, grazing intensity, and grassland rest periods (Dong et al., 2015; Lima et al., 2024; Teague, 2018). These factors underscore the complex interplay between soil health, carbon dynamics, and land management practices.

Seasonal variations in soil temperature and precipitation significantly influence the composition and turnover of the labile fraction of SOM, which is directly linked to TOC levels (Chen et al., 2020; Tang et al., 2022; Zhang et al., 2019). These findings align with studies demonstrating that soil organic carbon dynamics are strongly affected by climatic fluctuations, particularly changes in moisture and temperature (Babur and Dindaroglu, 2020; Singh et al., 2021; Wang et al., 2022); During warmer and wetter seasons, microbial biomass and enzyme activity typically increase due to enhanced metabolic rates and nutrient cycling (Dietterich et al., 2022). In contrast, colder and drier periods often suppress microbial activity, slowing decomposition and carbon mineralization (Schimel, 2018). Additionally, seasonal shifts in plant growth alter root exudates and litter inputs, further regulating soil biological processes (Zhu et al., 2023).

On the other hand, the relationship between the eco-region and livestock management factors influences the parameters of EC and BD However, none of the sites were levels of EC found that could affect soil health. These findings demonstrate that maintaining an appropriate animal load, managing shorter grazing periods, and allowing long rest periods for pastures prevent soil compaction and promote the generation of important porous spaces for root development and better soil permeability, thus avoiding waterlogging (Lai and Kumar, 2020; Shawver et al., 2020).

The Rs and the amount of biomass from the feces (Mb), were the only parameters that showed a significant effect under the three types of multiple interactions (RxGM, RxS, GMxS), although the interplay between grazing management and season turned out to have a highly significant effect. Part of the CO₂ production in the soil is related to the metabolic activity of plant roots and associated microorganisms (autotrophic respiration), while another significant fraction is associated with the heterotrophic respiration of microbial communities (Huang et al., 2020; Morris et al., 2022). Soil microbial activity directly affects the stability and fertility of ecosystems, and it is widely accepted that a good level of microbial activity is essential for maintaining soil health (Kooch et al., 2022). These findings align with those reported by Li et al. (2024); Xu et al. (2023), which show that regenerative livestock management has a significant positive effect on organic matter, microbial activity, and soil structure, compared to conventional management. These positive effects were higher in studies conducted in temperate climate regions and during seasons with greater water availability. It was found that regenerative livestock management increases microbial activity by 15 % (Byrnes et al., 2018).

The levels of MBC were influenced by the type of grazing management and season. Wang et al. (2006) found that Microbial biomass C were almost two times greater in heavily grazed than in ungrazed pastures in both improved and semi-native pastures areas, also, Liu et al. (2012), demonstrated that lightly grazed management in typical and desert steppe ecosystems showed significantly higher MBC levels when compared with ungrazed systems. These findings contrast with those of Khatri-Chhetri et al. (2022) who showed that multi-paddock and conventional grazing did not differ in microbial abundance or microbial carbon biomass in prairie and boreal forest ecoregions.

The temperate region showed the maximum and minimum percentage of soil moisture, between seasons, while the dry-semidry region had the least variation in soil moisture throughout the seasons. This can be explained by the percentages of sand, silt, and clay in each type of soil were determined by the physiographic region of each site, as well as climatic conditions such as precipitation, temperature, and humidity, which lead to differences by eco-region and by season of the year (Li et al., 2020, 2022; Li et al., 2024).

The interaction of the three factors affected the parameters pH and the generation of forage biomass (Fb). The dry-semidry region showed the higher forage productivity, beneath regenerative management during the wet season; followed by the temperate region with identical management and season. Conversely, during the dry season the dry-semidry area showed reduced forage productivity for both continuous and regenerative management. The last suggests that environmental moisture controls the grass development, under very hostile

circumstances of humidity and temperature, such as during the dry season in dry-semidry regions, regardless of the grazing management, as shown by the forage biomass productivity. On the other hand, the presence of environmental and soil moisture improves the productivity of the pasture under regenerative management. Multiple researchers have linked the increase in plant biomass to soil organic matter content and moisture availability, since it promotes a beneficial interaction between fine soil particles and soil mineral complexes (Liebig et al., 2004; Machmuller and Dillon, 2021; Salcedo-Pérez et al., 2007). Simultaneously, the increase in microbial activity, the capacity of the soil to retain moisture, and the increase in grass productivity create a positive feedback loop that helps to reverse soil deterioration and restore soil health (Amelung et al., 2020).

Soil pH levels remained within the optimal range for plant development in all ecoregions, under the two grazing managements and the two seasons. Although the highest value was found in the soil from the temperate region under regenerative grazing during the wet season. This could be due to the slightly higher percentage of SOM presented on that site (Neina, 2019).

Finally, numerous studies have shown that under different climatic conditions, regenerative cattle management can reduce the negative effects of climate change and increase soil health by improving structure, fertility, and microbial activity. Maintaining soil health in different climatic zones depends on the suitable grazing practices as well as the deposition, degradation, and manure inclusion into the soil (Bastani et al., 2023; Eldridge et al., 2017; Rayne and Aula, 2020; Rottler et al., 2019; Xu et al., 2018). Trimarco et al. (2023), described that continuous grazing had lower soil microbial activity efficiency than rotational grazing, suggesting that regenerative management approaches enhance soil health.

4.2. Sites ordination and their relationship with soil health indicators

Multivariate analyzes showed that the sites were grouped by grazing management and season, suggesting that parameters related to soil biological processes were the most important for the grouping. Although the site with regenerative management in the temperate region during the dry season grouped in the NMDS along with sites with continuous management, probably due to the recent implementation of regenerative management (2 years), which makes it more similar to sites with continuous management. According to various studies, the time required for changes in management practices to influence soil health is between 3 and 5 years (Bartley et al., 2023; Lekberg et al., 2024; Teague and Kreuter, 2020).

Previous researches have also found a close relationship between a high content of microbial biomass carbon and regenerative grazing management (Bartley et al., 2023; Gulaiya et al., 2024; Jordon et al., 2022; Prescott et al., 2021).

Additionally, the overlap of the most representative soil health parameters highlighted that MBC, Rs, Hs, and Fb were more abundant in the sites with regenerative management during the wet season, showing their predominant influence on the composition of this group, whereas in the sites with continuous grazing during the same season, the variables TOC, N, SOM, C, Poro, and pH were determinants for the formation of this grouping. On the other hand, the sites with regenerative grazing during the dry season were influenced by BD and CE, and finally, the Mb was higher on those sites with continuous management during the dry season, except for the site corresponding to the temperate region with regenerative grazing in this same season. It is worth mentioning that the variables influencing the formation of the first group have an opposite effect on the last one and vice versa.

These findings demonstrate that, independently of ecoregion, soil biological parameters and forage biomass have a significant impact on cattle ranches using regenerative management. Other authors have found that high levels of biological parameters, carbon content, and plant biomass were closely related to regenerative grazing and the

relative humidity (de Otálora et al., 2021; Hao and He, 2019; Koncz et al., 2015).

Finally, it was discovered that the amount of biomass from feces (Mb) in cattle pastures under continuous management was the most important factor during the dry season and continuous management. The higher amount of feces in the sites with continuous management during the dry season can be related to a low degradation rate, delaying its integration into the soil, and is explained by low biological activity and moisture in the soil. Which may result in lower SOM, carbon, and total organic carbon content in the soil; this effect was demonstrated in the position of soil health parameters with respect to the arrangement of the sites; these findings are consistent with other studies (Hoffmann et al., 2001; Kao et al., 2024).

5. Conclusions

Our research demonstrates that implementing regenerative grazing practices significantly enhances soil health by positively influencing its physical, chemical, and biological parameters, including porosity, organic matter, nitrogen, carbon, and total organic carbon. This represents a recovery of the soil's physical, chemical, and biological properties, along with increased forage productivity. On the other hand, the ecoregion only influenced the physical and chemical properties, while the season had greater effects on the physical and biological soil parameters. Notably, the interaction between seasonality and grazing management emerged as the most critical factor in enhancing soil biological activity and carbon levels. By imitating the grazing patterns of large herbivore herds —managing high animal densities on small land areas, rotating animals rapidly, and allowing extended rest periods for pastures— regenerative grazing increases organic matter, total organic carbon, microbial biomass carbon, and soil respiration. Additionally, it improves soil properties such as porosity and bulk density while boosting forage production. These benefits surpass the effects of ecoregion, soil type, and climatic conditions on soil health. Seasonal variations and ecoregions influenced environmental parameters by altering microclimatic conditions, such as soil moisture and temperature. When combined with effective grazing management, these factors play a crucial role in improving soil fertility and regeneration while reducing greenhouse gas emissions and mitigating global warming. We anticipate that extending the duration of the study would have resulted in more pronounced differences between the treatments. Over time, the cumulative effects of regenerative and continuous grazing practices on soil health would likely have become more distinct, offering deeper insights into their long-term ecological impacts. Such extended temporal scales are critical for capturing the progressive changes and feedback mechanisms inherent in these systems, which may not be evident in shorterterm studies. This underscores the importance of long-term research in comprehensively evaluating the outcomes of different land management practices and their implications for sustainable ecosystem management. Future studies should prioritize extended timelines to better elucidate the temporal dynamics and resilience of ecosystems under varying grazing regimes. Ultimately, this research offers valuable insights to guide management decisions and promote responsible grazing practices, benefiting both grasslands productivity and ecosystem sustainability.

CRediT authorship contribution statement

Francisco Javier Padilla Ramírez: Writing – review & editing, Validation, Supervision, Methodology, Investigation. Dulce Flores-Rentería: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. Eduardo Salcedo-Pérez: Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. Francisco Martín Huerta-Martínez: Writing – review & editing, Validation,

Supervision, Methodology, Investigation, Formal analysis. **Manuel Alejandro Meléndez-Aldana:** Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Manuel Alejandro Melendez Aldana reports financial support was provided by El Consejo Nacional de Humanidades, Ciencias y Tecnologías (Conahcyt) Scholarship [grant number CVU: 970091]. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109862.

Data availability

Data will be made available on request.

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