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Effects of grazing on soil properties in Mediterranean forests (Central-Eastern Spain)

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Abstract

Traditional management practices, such as grazing, can have adverse impact on soils. Despite an extensive body of literature exploring the effects of grazing on soil and plants worldwide, there is a notable lack of research on its impacts in Mediterranean forests within the Iberian Peninsula Furthermore, there is a knowledge gap on the enzymatic activities and basal respiration of soil in forest after grazing. To address these gaps, this study aimed to investigate the impact of grazing on various important physicochemical and biological soil properties along with vegetation richness in a Mediterranean forest located in Castilla-La Mancha (Central Eastern Spain). Relative to undisturbed sites, grazing significantly reduced soil water content (-53%) and available water (-59%). However, soil hydraulic conductivity remained unaffected by animal trampling and the soil water repellency observed in ungrazed sites disappeared. Grazed soils experienced a slight increase in pH (+18%). Among the biochemical properties studied, only dehydrogenase showed a significant increase (+100%) while basal respiration exhibited a notable decrease (-24%). Grazing resulted in a reduction of plant species richness (-34%) indicating a loss of biodiversity in grazed areas. The observed significant alterations in key soil and plant properties due to livestock activity suggest that grazing has the potential to modify the overall soil quality of these sites. Certain variables that exhibited noteworthy differences between grazed and ungrazed sites could serve as indicators of grazing impacts in Mediterranean forests. These indicators may be considered proxies for establishing effective land management strategies to mitigate degradation in the Mediterranean forest ecosystem.

Keywords: grazing effects; semi-arid pastures; soil hydraulic characteristics soil enzymatic activities; soil basal respiration.

1. Introduction

In recent decades, there has been an increasing interest in both traditional and innovative agroforestry practices. These practices involve land use scenarios where trees, crops, pasture and livestock coexist on the same sites (Reyna-Bowen et al., 2020). They have been promoted as measures to mitigate and reverse degradation in rural ecosystems (Moreno Marcos et al., 2007). To achieve environmentally sustainable management, strategies must consider the ecological impacts of traditional agroforestry practices, such as grazing by domestic livestock. This consideration is particularly important in regions with a long history, where these practices play a crucial role in supporting several ecosystem functions and services (Oggioni et al., 2020). When focusing on the impacts on soils and plants, it is well-established that agroforestry systems exhibit a higher potential for carbon sequestration compared to pastures or intensive croplands (Uribe et al., 2015). This becomes essential in arid and semi-arid areas, where soil organic matter is typically low and the carbon sequestration potential is limited in comparison to more humid sites (Uribe et al., 2015).

Commonly, Mediterranean forests offer a diverse array of landscapes where various types of shrubs or herbaceous vegetation serve as grazing resources (Joffre et al., 1999). This agroforestry system is structurally complex, accommodating at least two layers of vegetation (trees and herbaceous plants coexist through a harmonized recycling of organic matter and nutrients, along with the mobilization of other natural resources) (Tárrega et al., 2009). Additionally, this agroforestry system is characterized by significant levels of plant and animal diversity and offers numerous ecosystem services, including carbon storage (Parras-Alcántara et al., 2014) and commercial goods (Rodríguez-Rojo et al., 2022). This multifunctionality underscores its economic and environmental importance (Oggioni et al., 2020). As a result, Mediterranean forests have been considered for decades as an exemplar of sustainable management (Tárrega et al., 2009).

Human management practices can lead to significant changes in the Mediterranean forest ecosystems (Tárrega et al., 2009). In Spain, for instance, economic development and policies have led to the gradual underutilization or abandonment of vast rural and forest areas, resulting in insufficient tree regeneration (Uribe et al., 2015) and an increase in flammable biomass load. As a consequence, there is a greater risk of wildfires, resulting in changes to species composition and soil quality (Tárrega et al., 2009). The scientific literature has focused on the Mediterranean forest due to its conservation significance as a Mediterranean landscape or agroforestry system, its support of high levels of biodiversity (Rodríguez-Rojo et al., 2022), economic importance, and susceptibility to climate change (Moreno and Pulido, 2009).

Grazing has a long history as a management practice, and its impact has influenced the ecological dynamics of Mediterranean forests in various locations, contributing to the development of more resilient grazing systems (Carreira et al., 2023). However, grazing often results in ongoing landscape changes influenced by various environmental and animal-induced factors. Soils in Mediterranean forests are characterized by low fertility, shallow depth, and limited water and nutrient availability, resulting in poor productivity and quality of natural pastures (Hernández-Esteban et al., 2019). Unsustainable grazing practices, commonly referred as "overgrazing" (Seligman and Perevolotsky, 1994), exacerbate these natural constraints, altering important soil properties (e.g., organic matter dynamic, Bartley et al., 2010), and disrupting the water cycle (Vörösmarty and Sahagian, 2000). In certain Mediterranean environments, such as Spain, overgrazing has intensified landscape degradation (Minea et al., 2022). Additionally, increased livestock densities associated with grazing have led to several environmental and socio-economic problems on a global scale, raising concerns about the sustainability of this practice (Pulido et al., 2018). Consequently, grazing has emerged as one of the most significant human disturbances affecting grasslands (Hu et al., 2016).

In many biomes, such as the Mediterranean forest, grazing has increased the risk of soil desertification and biodiversity loss (Steffens et al., 2008), resulting in severe changes in ecosystem function and giving rise to socio-economic issues (Li et al., 2021). While the scientific literature extensively addresses the effects of grazing on ecosystem structure and functions globally (Paz-Kagan et al., 2016), research specific to the Mediterranean forest is comparatively limited. Studies have explored the impacts of grazing on vegetation cover and composition (e.g., Moreno Marcos et al., 2007; Rodríguez-Rojo et al., 2022; Tárrega et al., 2009), soil properties (e.g., Parras-Alcántara et al., 2014; Reyna-Bowen et al., 2020; Uribe et al., 2015), and hydrology (e.g., Schnabel et al., 2018). However, studies focusing on the overall quality of ecosystems (both plant and soil) and enzymatic activities of soil are relatively scarce (e.g., Hidalgo-Galvez et al., 2023; Oggioni et al., 2020; Peco et al., 2017; Santos-Francés et al., 2021). For example, Oggioni et al. (2020) explored the impacts of grazing abandonment on soil microbial activity and carbon storage but did not analyze these effects on undisturbed pastures. Hidalgo-Galvez et al. (2023) studied the effects of grazing on pasture quality (chemical properties of soil, tree cover, water and temperature stress) with a specific focus on grazing histories. Moreover, Peco et al. (2017) measured the effects of grazing abandonment, calculating a soil multifunctionality index (including many physiochemical properties and soil respiration, but not the enzymatic activities). Finally, Santos-Francés et al. (2021) evaluated a set of 16 soil properties, but did not specifically investigate the impacts of grazing on the enzymatic activities. This state-of-the-art analysis reveals a gap in understanding key soil properties, such as the enzymatic activities and basal respiration, along with modifications in plant diversity due to grazing, under a comprehensive and integrated approach in the Mediterranean forest. These soil characteristics are proxies of the ecosystem functionality, in which soil and plants play a key role to maintain natural functions and to provide the ecosystem services against the possible natural and anthropogenic threats. Hence, there is a lack of knowledge about important indicators of ecosystem functionality, such as hydrological parameters, biochemical activities and biodiversity of plants in these agroforestry systems, with a specific emphasis on the water infiltration, repellency, enzymatic activities and basal respiration of the soil as well as species richness of the plants.

To address these knowledge gaps, this study investigates the impacts of grazing on a selection of important physiochemical and biological soil properties, including enzymatic activities and basal respiration, as well as vegetation richness in a Mediterranean forest located in Castilla-La Mancha (Central Eastern Spain), which is subjected to grazing. The specific objectives are as follows: (i) Assessing the sensitivity of studied soil and plant variables ofto grazing in comparison to undisturbed sites; (ii) Evaluating the extent to which grazing influences the overall quality of this agroforestry system though the calculation of the Soil Quality Index (SQI); (iii) Determining whether the overall quality of the ecosystem significantly differs between grazed and ungrazed sites using multivariate statistical analysis. We hypothesize that grazing alters key soil properties and the overall quality of the ecosystem, leading to significant environmental degradation when compared to undisturbed sites. The outcomes of this research aim to contribute valuable insights into the intricate interactions between grazing practices and the Mediterranean forest ecosystem, shedding light on potential environmental consequences and guiding sustainable land management strategies.

2. Materials and methods

2.1. Study area

The study area is located in “Los Palancares y Agregados” forest (40º01’50”N; 1º59’10”W, average elevation of 1200 m above the mean sea level), covering about 5000 ha in the Cuenca Mountain area of Castilla-La Mancha (Central-Eastern Spain, Figure 1). The experimental area belongs to the largest Natural Monument in Castilla La Mancha (Central-Eastern Spain). Moreover, this natural ecosystem is included in the endangered habitats of European Union, since it belongs to a natural habitat requiring specific conservation measures (Resolution 4/1996 by the Convention on the Conservation of European Wildlife and Natural Habitats). The climate is Mediterranean humid (Csa), according to the Köppen-Geiger classification (Kottek et al., 2006). The annual mean lowest and highest temperatures are -0.5 ºC and 30.5 ºC, respectively, while the mean annual precipitation is 595 mm (only 99 mm concentrated in summer). The soils are classified as Entisols (USDA soil taxonomy, USDA, 1999) with a clay loam or loam texture. The area is mainly covered by natural forests of Spanish black pine (*Pinus nigra* Arn. ssp *salzmannii*), forming mixed stands with Holm oak (*Quercus ilex* L.), Portuguese oak (*Quercus faginea* Lam.) and Spanish juniper (*Juniperus thurifera* L.). The herbaceous and shrub vegetation mainly consist of *Juniperus oxycedrus* L., *Rosa* sp., *Eryngium campestre* L., *Geranium selvaticum* L., *Centaurea paniculata* L., and *Plantago media* L. Sustainable forest management has been implemented in “Los Palancares y Agregados” forest, where timber exploitation is balanced with economic benefits from various uses, including livestock, hunting, mycology, beekeeping, etc. Shrubs and grasslands, often sparse between the pine and mixed forests, are closely linked to human activities, particularly grazing. Since the end of the 19th century, Spanish black pine stands in Spain have been managed under different systems. A shelterwood system with a 100-to-120-year rotation and a 20 to 30-year regeneration period is the most commonly used method. Initial seedling recruitment has always been difficult to achieve due to factors such as summer drought, soil compaction, masting conditions, seed predation, inadequate overstorey density, attacks by European pine shoot moth on seedlings, and damage from grazing animals, which is often a major problem. During the last century, forest managers tried to enhance regeneration by using soil treatment, planting, and introducing new varieties of species, but the results have been inconsistent. In this context, the management system in grazed areas has been continuous, with cows serving as the primary livestock species in the study for the past 20 years. Intensive grazing was observed in plots near water ponds, which served as the sole drinking water source for the cows. The areas have been not logged or influenced by other anthropogenic action. According to the forest service of the Castilla-La Mancha region, the background values can be considered as the same for all studied sites. In the study areas, we conducted identification and measurements of cow dung. Dung counts and pellets are widely used to estimate abundance of large herbivores (Eldridge et al., 2017; Eldridge and Delgado-Baquerizo, 2017), and have also been proposed as indicators for evaluating grazing intensity (Sheidai-Karkaj et al., 2022; Stumpp et al., 2005). According to previous studies, the grazing intensity in the monitored sites, particularly those near water ponds, can be considered high due to the dense concentration of livestock. Control sites (i.e., without grazing) were included for comparison with grazed areas (see Figure 1). The contrasting grazing regimes were characterized by a single factor: grazing (yes vs. no). The grazing regimes resulted in fence-line contrasted with marked differences in structure and composition of the vegetation, being control areas totally covered by trees, whereas in grazed sites the tree cover was low (Figure 1). For detailed information on the climate, soils, vegetation units, and livestock in the two areas, readers can refer to Lucas-Borja et al. (2012), and Zhou et al. (2022a, 2022b).

Figure 1 – Geographical location and aerial view of the study area ("Los Palancares y Agregados", Castilla-La Mancha, Spain).

2.2. Experimental design

In the study area, three sites were identified (G1 and G2, Figure 1), of which two sites were subjected to animal grazing (mainly ruminants, e.g., cows), hereafter indicated as "grazing" (G). G1 and G2 sites experienced the same grazing intensity, and were adopted in ordert o better capture the intrinsic variability of soil and vegetation characteristics. A third site without any excrements and visual tracks of animals was selected as “control” (C). In each of G and C sites, three plots 5 x 5 m^2 were randomly chosen at a minimum reciprocal distance of 25 meters (to avoid pseudo-replications) for the measurement of soil properties and plant characteristics. The grazing sites plots were always located by the water ponds (between 15 and 20 meters away of the water ponds), thus being exposed to heavy grazing. We visually assessed and counted the dung number at each plot as a proxy of grazing intensity. This experimental approach is composed of three sites and three plots by site (9 plots in total). The higher density of soil and vegetation surveys in G plots was due to the lower homogeneity of soil and plant characteristics compared to the C plots (Figure 2).

Figure 2 – Aerial photographs showing differences between sites subjected to animal grazing (G, 2a) and control (C, 2b).

2.3. Infiltration and water repellency measurement

At each plot, a measurement of infiltration and water repellency was carried out. The Mini-Disk Infiltrometer (MDI, Decagon, 2013) was used to measure the hydraulic conductivity of unsaturated soil (K), following the MDI technical manual and the procedure suggested by Robichaud et al. (2008a, 2008b). Before placing the infiltrometer on the measurement area, the litter was removed using a shovel, and the soil surface was leveled and cleaned using a brush. After starting each measurement, the volume of water infiltrated in the soil was measured each 30 s until the water volume fully infiltrated or a maximum time of 10 minutes. The equations proposed by Zhang (1997) were used to estimate K, regressing the infiltrated water (I, [m]) to time (t, [s]) at steps of 30 s:

$$I = C_1 t + C_2 t^{1/2} \tag{1}$$

C_1 are C_2 being two coefficients related to the hydraulic conductivity [m s^{-1}] and absorption capacity [m $s^{-1/2}$] of soil, respectively.

K ([mm h^{-1}]) was estimated as:

$$K = C_1/A \tag{2}$$

where A (equal to 1, (Devices, 2013) is a coefficient that corresponds to the Van Genuchten's parameters (n and α) for a given soil type at the suction rate h_0 (equal in this study to -2 cm) and for the disk radius of the infiltrometer (2.25 cm).

The Water Drop Penetration Test (WDPT) method (Letey, 1969; Woudt, 1959) was used to measure the soil water repellency (SWR) very close to the point of K measurement (distance lower than 15-20 cm). WDPT measures the time a drop of distilled water needs to infiltrate into soil. In more detail, 15 drops were left from a pipette on the soil surface, and the infiltration time was recorded by a stopwatch (Zema et al., 2021a, 2021b).

The measured values of WDPT were classified to estimate SWR according to Bisdom et al. (1993): (i) non water-repellent or wettable soil (class 0, WDPT < 5 s); (ii) slightly water-repellent soil (class 1, 5 < WDPT < 60 s); (iii) strongly water-repellent soil (class 2, 60 < WDPT < 600 s); (iv) severely water-repellent soil (class 3, 600 < WDPT < 3600 s); and (v) extremely water-repellent soil (WDPT > 3600 s) with three sub-classes (class 4, 1 < WDPT < 3 h, class 5 (3 < WDPT < 6 h) and class 6 (WDPT > 6 h).

It is well known that the soil water content (SWC, e.g., Dekker and Ritsema, 1994; Lichner et al., 2013; Vogelmann et al., 2013) plays a noticeable role on influencing SWR. For this reason, SWC was measured simultaneously with SWR, using a VG400 (Vegetronix, Utah, USA) device placed on the soil surface and connected to a data logger (Onset HOBO, Massachusetts, USA). When SWC measurements differed from each other by over 10%, the corresponding measures of SWR were discarded.

2.4. Soil sampling and analysis

At each plot, close to the point of K and SWR measurement, a sample of 600 g was collected in the layer between 0 and 5 cm in April 2023, passed in laboratory through a 2 mm sieve and then stored at 4 °C.

The following soil properties were determined on the collected samples:

- pH and Electrical Conductivity (EC, in distilled water, at a soil:solution ratio of 1:2.5 and 1:5 respectively, using a digital pHmeter, LAQUA PH1100, HORIBA, Tokio, Japan, and a conductivity meter, Crison 522, Barcelona, Spain).
- Total Organic Carbon (TOC, by Walkley and Black, 1934 method, modified by Mingorance et al. (2007), and measured in a spectrophotometer Spectronic Helios Gamma UV-Vis, Thermo Fisher Scientific, Massachusetts, USA).
- Basal Soil Respiration (BSR, expressed as mg C-CO_2 day^{-1} kg^{-1} of dry soil), using an infrared CO_2 sensor (IRGA S151, Qubit Systems Inc., Canada).
- Dehydrogenase Activity (DHA, expressed as µmol INTF $hour^{-1}$ g^{-1} of dry soil), by reduction of p-iodonitrotetrazolium chloride (INT) to p-iodonitrotetrazolium formazan (INTF) following García et al. (1997).
- Urease Activity (UA, expressed as µmol N-NH_4^+ $hour^{-1}$ g^{-1} of dry soil), using urea as a substrate and a borate buffer at pH = 10 (Kandeler and Gerber, 1988; Tabatabai and Bremner, 1969).
- Acid Phosphatase (PA) and β-glucosidase (BGA) (both expressed as µmol pNP g^{-1} of dry soil), using the methods of Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1988), respectively.
- Available Water for plants (AW), by measuring the pFs at -1500 and -33 kPa by Richard's method (Richards, 1947).

2.5. Plant survey

Simultaneously to soil sampling, a survey of vegetation species was carried out at each plot (5 m x 5 m, area of 25 m^2) to measure the plant diversity, adopting the species richness (SR, the total number of the different species detected in each plot) as indicator.

2.6. Evaluation of soil quality index

To assess the overall soil quality, the well-known Soil Quality Index (SQI) proposed by Andrews et al. (2002) was calculated for each soil condition (G and C) in three main steps: (i) choice of appropriate indicators for a minimum data set (MDS); (ii) conversion of the indicators into scores; and (iii) aggregation of the scores into the SQI. In more detail, a principal component analysis (PCA) was applied to all physical, chemical, and biological properties, and used as Minimum Data Set (MDS) method, for the selection of the most suitable soil quality indicators. Only highly-weighted variables (i.e., those loadings having absolute values within 10% of the highest factor loading or ≥ 0.40) were retained from each PC for the MDS (Wander and Bollero, 1999).

After selecting the MDS, all indicators were converted into scores for inclusion in the SQI using a linear method. Indicators for plots under each soil condition were ranked in ascending or descending order, depending on whether a higher value was considered “good” or “bad” in terms of soil functions. For “more is better” indicators, each observation was divided by the highest observed values, and therefore the highest observed value received a score of one. For “less is better” indicators (in our case EC and SWR), the lowest observed value (in the numerator) was divided by each observation (in the denominator), so that the lowest observed value received a score of one (Andrews et al., 2002). A mid-point optima method, such as Gaussian function, was used to standardize pH values (Andrews et al., 2002). Then, the scores of the plots were weighted using the PCA results. The amount (in %) of the variation in the total data set explained by each PC, standardized to unity, was considered as the weight for scores under a given PC. Finally, the weighted scores for each plot were summed, and the mean and standard errors for each soil condition were calculated.

2.7. Statistical analysis

A t-test was applied to the soil and plant properties as well as SQI (dependent or response variables), to evaluate the statistical significance of the differences between the two soil conditions (G and C, independent variables or factors). The equality of variance and normal distribution, which are assumptions of the t-test, were checked by normality tests.

Then, PCA was used to select derivative variables (Principal Components, PCs) (Rodgers and Nicewander, 1988) and simplify the analysis of the large number of soil properties, losing as little information as possible. In this study, PCA was carried out by standardizing the original variables (expressed by different measuring units) and using Pearson’s method to compute the correlation matrix. The first three PCs, explaining at least at least a percentage of 70% of the original variance, were retained.

Finally, the observations were grouped in clusters using the Agglomerative Hierarchical Cluster Analysis (AHCA) applied to the original variables. AHCA is a distribution-free ordination technique to group samples with similar characteristics by considering an original group of variables. The Euclidean distance was used as similarity-dissimilarity measure for this approach. The statistical analysis was carried out using the XLSTAT software (release 2019, Addinsoft, Paris, France).

3. Results

The dung counts in grazed plots was 5.83 ± 1.83 animal imprints per plot (5 ± 2.7, G1 site, and 6.7 ± 3.2, G2 site, without any statistical difference) compared to absence of dungs in control sites. The t-tests revealed that pH, DHA, d-BSR, AW, SWC, SWR and SR were significantly different among the two soil (i.e., C vs G) conditions (Table 1.SM). In more detail, the plots that were subjected to grazing (G) showed higher pH (7.72 ± 0.03) and DHA (4.87 ± 0.47 µmol INTF h^{-1} g^{-1} of soil) compared to the control (C) soils (6.56 ± 0.14 and 2.43 ± 0.89 µmol of INTF h^{-1} g^{-1} of soil, respectively). In contrast, daily BSR decreased in G plots (57.1 ± 4.73 against 75.3 ± 3.68 mg of C-CO_2 day^{-1} kg^{-1} of soil) (Table 1).

Table 1 – Mean ± standard error of the physiochemical and biochemical properties of soils under two soil conditions (G = grazing; C = control) in the study area ("Los Palancares y Agregados", Castilla-La Mancha, Spain)

Soil parameter	**Soil condition**	
	G	C
EC (mS cm^{-1})	0.11 ± 0.01 a	0.11 ± 0.01 a
pH	7.72 ± 0.03 a	6.56 ± 0.14 b
TOC (%)	5.04 ± 0.53 a	5.58 ± 0.20 a
AW (%)	9.45 ± 1.10 a	23.0 ± 3.41 b
SWC (%)	14.4 ± 2.63 a	30.6 ± 4.28 b
K (mm h^{-1})	18.3 ± 4.84 a	2.22 ± 0.46 a
SWR	1.00 ± 0.00 a	4.40 ± 0.17 b
DHA (µmol of INTF h^{-1} g^{-1} soil)	4.87 ± 0.47 a	2.43 ± 0.89 b
BGA (µmol of PNF h^{-1} g^{-1} soil)	3.87 ± 0.26 a	3.63 ± 0.13 a
PA (µmol of PNF $h^{-1}g^{-1}$ soil)	3.51 ± 0.23 a	4.13 ± 1.00 a
UA (µmol of N-NH_4^+ h^{-1} g^{-1} soil)	3.99 ± 0.62 a	2.03 ± 0.58 a
BSR (mg of C-CO_2 day^{-1} kg^{-1} soil)	57.1 ± 4.73 a	75.3 ± 3.68 b

Notes: EC = electrical conductivity; TOC = total organic carbon; DHA = dehydrogenase activity; BGA = β-glucosidase activity; PA = phosphatase activity; UA = urease activity; d-BSR = daily basal soil respiration; c-BSR = cumulative basal soil respiration. *Different letters indicate significant differences among soil conditions after t-test ($p < 0.05$).*

G soils showed significantly lower SWR (class 1 for all sites) compared to the C plots (2.22 ± 0.46 mm/h and class 4, respectively). Moreover, a significant decrease in both AW (9.45 ± 1.10% against 23.0 ± 3.41% of C plots) and SWC (14.4 ± 2.63% against 30.7 ± 4.28%) was measured (Table 1). Plant richness was significantly lower in G plots (6.2 ± 0.48 species) and higher (9.3 ± 0.33) in the control.

PCA provided three principal components (PCs), which together explain 82.7% of the total variance of the soil and plant parameters measured under the two soil conditions. PC1 and PC2 explained 49.5% and 17.5% of this variance, respectively, while PC3 gave another 15.7%. Specifically, pH, DHA, UA, d-BSR, and SR and all the hydrological variables (K, AW, SWC and SWR) had high loadings (absolute value > |0.584|) on the first PC. PC1 increased with pH, DHA, UA and K positive loadings, while decreasing with increasing AW, SWC, d-BSR, SR and SWR. PC2 was strongly associated to EC (with a negative loading, -0.703), and to TOC and PA (positive loadings, > 0.638), while BGA only noticeably weighed on the third PC with a positive loading (0.778) (Table 2.SM and Figure 3a). More specifically, sites subjected to grazing are associated to high values of PC1, corresponding to lower SWC, AW, d-BSR and SR (having negative loadings on this PC) compared to ungrazed plots (being instead in the negative quadrant of the first PC) (Figure 3b).

The samples of soils and plants collected under the two soil conditions were clearly discriminated by PCA in two separate clusters, of which a first group, consisting of samples of C plots, was associated to negative values of PC1, while the second cluster, which only contains variables sampled in G sites, is in the negative quadrant of PC1 (Figure 3b). Moreover, AHCA separated the G samples in two distinct clusters, each one containing three of the six plots (Figure 4).

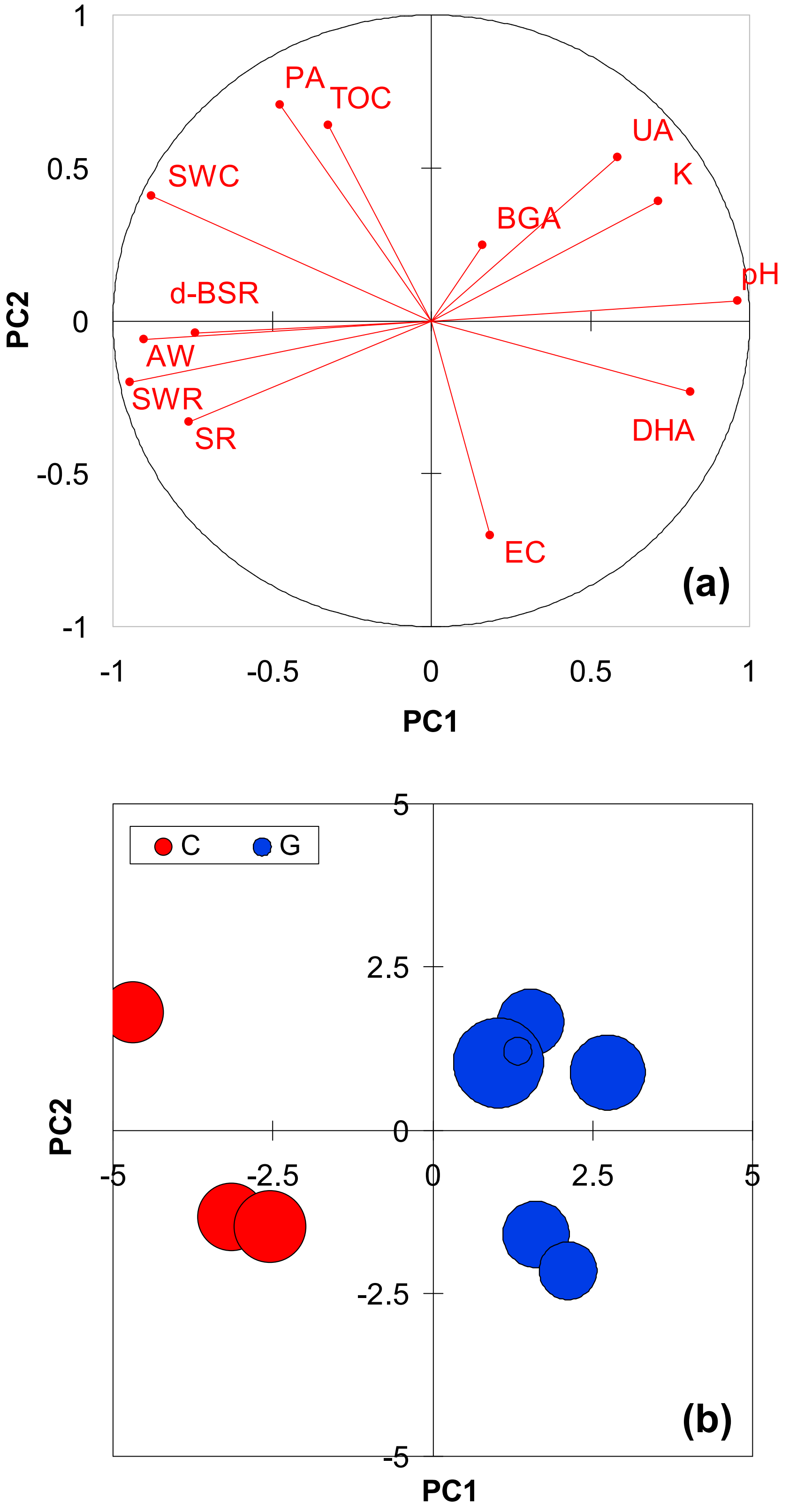

Figure 3 - Loadings of the original variables (a, soil properties), and scores (b) on the first three Principal Components (PC1, PC2 and PC3) provided by the Principal Component Analysis applied to plots under two soil conditions (G = grazing; C = control) the study area ("Los Palancares y

Agregados", Castilla-La Mancha, Spain). Notes: EC = electrical conductivity; TOC = total organic carbon; DHA = dehydrogenase activity; BGA = β-glucosidase activity; PA = phosphatase activity; UA = urease activity; d-BSR = daily basal soil respiration; c-BSR = cumulative basal soil respiration; K = unsaturated hydraulic conductivity; SWR = soil water repellency; AW = available water; SWC = soil water content; SR = plant species richness; the diameter of bubbles in Figure 6b is proportional to the value of PC3.

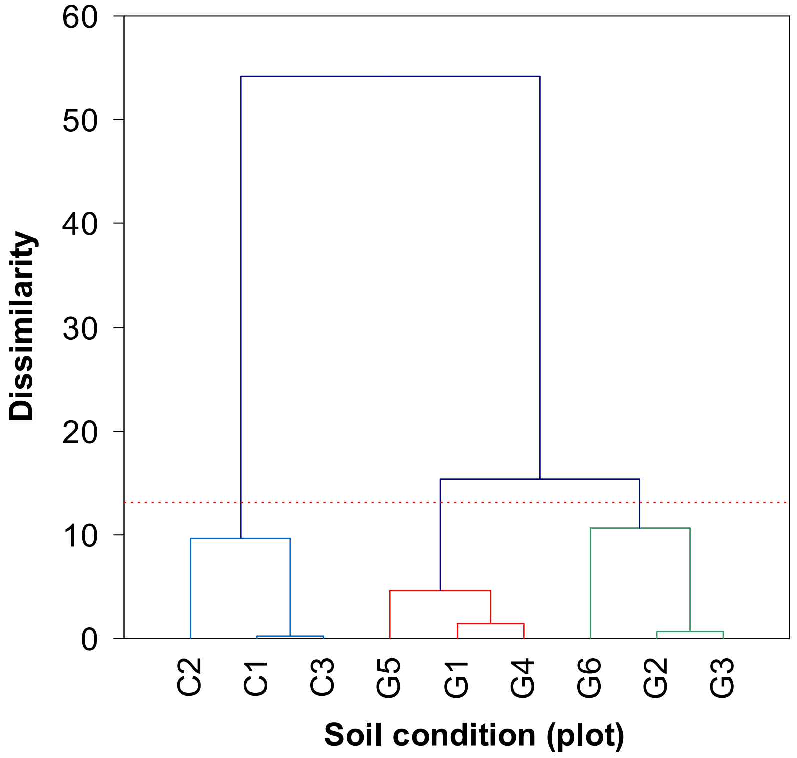

Figure 4 - Dendrogram provided by the Agglomerative Hierarchical Cluster Analysis (AHCA) applied to plots under two soil conditions (G = grazing; C = control) in the study area ("Los Palancares y Agregados", Castilla-La Mancha, Spain). *Notes: the dashed red line indicates the level of similarity, and the letter and number on the x axis refer to the sampling plot.*

The distinction between the two clusters formed in Figure 4 was mainly attributed to differences in OC (+32.6% in plots of cluster C1 compared to C2), SWC (+34%), UA (+31.3%), and K (+59.8%). Finally, the t-test showed that SQI was significantly different between G and C plots ($F = 12.210$; $p < 0.05$), and SQI of grazed sites (1.32 ± 0.06) was lower compared to ungrazed plots (1.78 ± 0.16).

4. Discussions

Mediterranean forests subjected to grazing may experience contrasting changes in physiochemical and biological soil properties as well as on vegetation richness. The non-significant impact of grazing on K, as revealed by this study, contrasts with findings by Cerda et al. (1998), who observed lower infiltration rates in a Mediterranean agroforestry system, except in sites with shrubs and areas with low grazing pressure. Contrasting results, influenced by soil characteristics of grazing systems such the hydraulic conductivity and water content, were also noted in studies by Minea et al. (2022) and Weltz et al. (1989). Animal trampling generally increases bulk density and compacts the topsoil layers, which reduces total porosity. The negligible effect of grazing on K detected in this study may have been counterbalanced by the SWR disappearance compared to the severe levels observed in the undisturbed soils. The disappearance of SWR can be attributed to trampling, primarily affecting the surface soil layer, which, in turn, facilitated water infiltration rather than resulting in increased compaction. The removal of vegetation through defoliation due to grazing, causing damage to plants through animal trampling and feeding could further contribute to enhanced infiltration in the grazed plots. Although a reduction in soil water content negatively impacts plant growth in semi-arid environments, the lack of pronounced effects on water infiltration and the disappearance of soil water repellency should not necessarily imply an exacerbation of surface runoff and soil erosion on grazed hillslopes. Specific measurements of these variables would be needed to support this general observation.

The significantly lower SWC and AW measured in the disturbed soils in this study represent some of the negative impacts of grazing in semi-arid areas, where soils are generally dry for extended periods throughout the year. This condition poses challenges for the growth of herbaceous and shrub vegetation. SWC exhibits variability in areas subject to grazing due to changes in water infiltration, pasture composition, and pasture growth. Moreno Marcos et al. (2007) noted that SWC in Mediterranean forests shows low variability with distance from trees, attributed to the extensive root system of oaks. However, this result might have been influenced by the specific focus of that study on sites covered by tree canopies.

In general, changes in hydraulic properties are often associated with other soil characteristics, particularly the organic matter (OM) content. However, the lack of significance of changes in K between grazed and control soils in this study may also be attributed to the non-significant variations in OM after grazing. It is well-established that a higher OM content supports enhanced infiltration. The impacts of grazing systems on soil OM are known to be highly variable.

Additionally, inputs of livestock excreta into ecosystems increase the rates of carbon and nitrogen cycling. The carbon dynamics in Mediterranean agroforestry systems are quite contrasting. Studies have shown increases in carbon stock in the soil under the tree canopies (Uribe et al., 2015), but also in cleared sites, while the lowest OM content was found in grazed areas by Tárrega et al. (2009). In contrast, Reyna-Bowen et al. (2020) reported higher TOC in grazed sites compared to the cultivated sites, with the influence of trees on soil OM noticed under mature trees. These contrasting results support the lack of significance in TOC between grazed and control sites detected in our study, where the difference is likely attributed to the natural soil variability. The slight but significant increase in pH in grazed sites compared to control sites contrasts with several studies reporting acidification in pasturelands due to urine and fece flows (Carreira et al., 2023; Haynes and Williams, 1993). This increase in pH may be due to the higher (although non-significant) infiltration rates, which could have leached acid compounds (such as nitrates) into the surface soil layer, consequently reducing nutrient extraction.

Grazing has the potential to impact soil microbial communities and processes, often serving as indicators of soil health in agricultural systems, although the quantification of soil quality and health indices in rangelands has been less common. The analysis of the biochemical properties of soil conducted in this study revealed significant variations only in DHA and d-BSR between grazed and ungrazed sites, with BGA, PA and UA remaining unaffected by grazing. There is limited literature specifically addressing the impacts of grazing on soil biochemistry in Mediterranean agroforestry systems. Only Oggioni et al. (2020) reported a negative effect of grazing abandonment on enzyme activities, highlighting the significant role of topography on microbial dynamics, while Uribe et al. (2015) showed increases in soil respiration under tree canopies. Wang et al. (2014), working under different environmental conditions in the rangelands of China, provided clear evidence that grazing affects the activity of micro-organisms in the soil. In this study, the effects were only observed for a few biochemical properties, and to varying extents. These changes may be attributed to changes in soil water and temperature associated with a different plant cover between grazed and control sites.

Floristic composition is a reliable indicator of pasture quality. The agro-forestry systems of the Iberian Peninsula, as noted by Peco et al. (2006), exhibited extremely high plant species richness due to the wide heterogeneity in soil and micro-climate, animal grazing, and low-impact management practices. Grazing has a direct impact on plant cover and biomass, directly altering vegetation community structure, and this influence may lead to positive or negative evolutionary trends. In this study, the impact of grazing was significant and negative, as shown by a notable reduction in plant species richness in grazed sites compared to the ungrazed soils. The effects of grazing on vegetation in Mediterranean agroforestry systems vary across studies. Some studies reported that livestock, particularly cattle, enhances plant diversity in microsites created by trees compared to herbaceous communities (López-Sánchez et al., 2016), while others observed no significant differences in vegetation richness (Tárrega et al., 2009). Also, pasture productivity and the species of grazing animals are key drivers of plant richness, with higher richness observed with large herbivores grazing in high-productivity sites, and minimal changes in low-productivity areas. The negative impacts on species richness found in this study are likely attributed to the presence of large ruminants, causing a decrease in herbaceous cover resulting in increased bare and crusted soils. This effect generally leads to a significant reduction in biomass and an increase in height heterogeneity in pasture plants. Our results align with the weak or negative effects of grazing on species richness reported by Olff and Ritchie (1998) and Peco et al. (2006).

Grazing has significantly affected the overall quality of the Mediterranean agroforestry ecosystem, demonstrated through two approaches. First, the significant differences in species richness and key soil properties resulted in a significantly lower SQI. This aligns with the findings of Paz-Kagan et al. (2016), who, though working in Mediterranean typical grasslands of Israel, observed a lower SQI, indicating clear effects of grazing activity and landscape cover degradation on the undisturbed pasture. Moreover, since six out of the evaluated soil properties (SWR, SWC, AW, pH, DHA and SBR) showed sensitivity to grazing, these parameters may serve as easy-to-measure and meaningful indicators for grazing management in Mediterranean forests. Second, the multivariate statistical approach using PCA and AHCA demonstrated clear differences in soil and plant characteristics between grazed and undisturbed soils. This was quantitatively shown by the evident gradient along the first PC, as well as by the discrimination between grazed and ungrazed soils grouped into different clusters. Despite similar animal loads, some soil properties within the grazed sites (especially TOC, K and some enzymatic activities) underwent noticeable differences. (e.g., plots of clusters C1 and C2). This highlights the large variability in important soil properties, especially those associated with the carbon and nitrogen cycles (TOC and UA).

The noticeable impact of grazing in Mediterranean agroforestry systems somewhat contrasts with the literature, which generally demostrates the positive influence of agroforestry on the supply of regulating services and their role in enhancing landscape structure. Tarrasón et al. (2014) suggested that Mediterranean agroforestry systems may be useful for recovering productive and regulatory functions of ecosystems, such as grazing potential, soil erosion control, and for reducing the risk of wildfire. However, the sustainability of the Mediterranean agroforestry systems has been questioned by some studies (e.g., Cubera and Moreno, 2007; Pulido et al., 2001) due to intensive and simplified management, leading to changes in vegetation and soil properties, as detected in our study. This does not imply that the Mediterranean forest must be abandoned or converted to other land uses since livestock exclusion may cause a decrease in pasture quality or ecosystem invasion by Mediterranean shrub species. Rather, a moderate grazing intensity is suggested, which exerts positive effects on the overall system quality. Implementing strategies to reduce livestock pressure or prevent access of animals to sensitive areas may effectively mitigate declines in soil and plant health due to overgrazing. In other words, as suggested by Peco et al. (2006), landscape managers should promote agri-environmental policies aimed at lightening and diversifying grazing regimes across the Mediterranean forest landscape. This approach may increase vegetation and animal species diversity, improve soil quality and health, and enhance other ecosystem functions, such as productivity and stability.

A possible limitation of this study stands in the fact that the results were obtained from short-term measurements after grazing and with a limited number of soil samples. Increasing the number of cores collected per site and comparing with other grazing systems in Mediterranean forests of other regions would enhance the reliability of our findings. A temporal extension of the monitoring activity over two or three years after grazing could elucidate whether the effects observed are transient or permanent. Moreover, a more in-depth investigation into nutrient cycling and the composition of microbial biomass in grazed soils is suggested (although not assessed in this study) to provide a more comprehensive overview of the grazing effects in semi-arid landscapes.

5. Conclusions

The impacts of grazing in a Mediterranean forest (Castilla-La Mancha, Central Eastern Spain) were detected in comparison to ungrazed sites: (i) a significant reduction in the soil water content and available water; (ii) no significant changes in soil hydraulic conductivity, and the disappearance of soil water repellency in grazed sites; (iii) significant increases in soil pH and dehydrogenase activity, and a decrease in basal respiration; (iv) no significant changes in other enzymatic activities; (v) a noticeable decrease in plant species richness.

These findings suggest that while grazing may not significantly alter soil hydrology (water infiltration and repellency), the reduction in soil water content could have negative implications for plant growth in this semi-arid environment. Additionally, the significant changes in other key soil properties and species richness indicate that grazing has the potential to modify the overall soil quality of Mediterranean forests. Hence, the initial hypothesis that grazing alters the overall ecosystem quality compared to the undisturbed sites appears to be well-founded. This underscores the importance of careful monitoring of certain chemical (pH), biochemical (respiration and dehydrogenase), and physical (SWR and SWC) soil properties, as well as plant diversity (species richness). These parameters can serve as indicators of grazing impacts on vegetation and soil properties, providing valuable insights for the management of the Mediterranean forests. In summary, this research sheds light into the effects of grazing systems on the soil and environmental characteristics of Mediterranean forests, offering insights into land management strategies for the mitigation of ecosystem degradation in this important agroforestry system.

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SUPPLEMENTARY MATERIAL

Table 1.SM – Results of t-tests applied to the main hydrological, chemical and biochemical properties, plant species richness and SQI under two soil conditions (G = grazing; C = control) in the study area (“Los Palancares y Agregados”, Castilla-La Mancha, Spain)

Soil/plant parameters	**Degrees of freedom**	**Sum of squares**	**Mean squares**	**F**	**Pr > F**
EC	1	0.000	0.000	0.011	0.918
pH		2.683	2.683	129.413	**< 0.0001**
TOC		0.574	0.574	0.468	0.516
DHA		11.915	11.915	7.313	**0.030**
BGA		0.109	0.109	0.355	0.570
PA		0.781	0.781	0.730	0.421
UA		7.758	7.758	4.033	0.085
d-BSR		666.179	666.179	6.202	**0.042**
c-BSR		682168	682168	6.202	0.042
K		518.665	518.665	5.160	0.057
AW		364.643	364.643	24.151	**0.002**
SWC		527.800	527.800	11.642	**0.011**
SWR		23.170	23.170	935.283	**< 0.0001**
SR		20.056	20.056	18.719	**0.003**
SQI		0.433	0.432	12.210	**0.0107**

Notes: EC = electrical conductivity; TOC = total organic carbon; DHA = dehydrogenase activity; BGA = β-glucosidase activity; PA = phosphatase activity; UA = urease activity; d-BSR = daily basal soil respiration; c-BSR = cumulative basal soil respiration; K = unsaturated hydraulic conductivity; AW = available water; SWC = soil water content; SWR = soil water repellency; SR = plant species richness; SQI = soil quality index

Table 2.SM - Factor loadings of the soil and plant parameters on the first two Principal Components provided by the Principal Component Analysis applied to plots under two soil conditions (G = grazing; C = control) in the study area (“Los Palancares y Agregados”, Castilla-La Mancha, Spain)

Soil/plant parameters	**Principal components**		
	PC1	**PC2**	**PC3**
EC	0.184	**-0.703**	0.663
pH	**0.964**	0.063	-0.073
TOC	-0.322	**0.638**	0.576
AW	**-0.901**	-0.062	0.151
SWC	**-0.880**	0.407	0.076
DHA	**0.816**	-0.233	0.306
BGA	0.160	0.247	**0.778**
PA	-0.475	**0.707**	-0.294
UA	**0.584**	0.533	0.194
d-BSR	**-0.739**	-0.040	0.587
K	**0.715**	0.390	0.245
SWR	**-0.946**	-0.201	0.039
SR	**-0.760**	-0.332	-0.005

Notes: EC = electrical conductivity; TOC = total organic carbon; AW = available water; SWC = soil water content; DHA = dehydrogenase activity; BGA = β-glucosidase activity; PA = phosphatase activity; UA = urease activity; d-BSR = daily basal soil respiration; c-BSR = cumulative basal soil respiration; K = unsaturated hydraulic conductivity; SWR = soil water repellency; SR = plant species richness