

Impacts of Extensive Sheep Grazing on Soil Physical and Chemical Quality in Open Mountain Forests, NE Portugal

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Grazing and mechanical clearing are common techniques for vegetation management in open Mediterranean forests. Despite its recognized benefits in the prevention of highintensity and severity forest fires, it is essential to consider its impacts on the physical and chemical soil properties. In an open mountain forest located in the NE of Portugal, soil samples were analyzed at depths 0-5, 5-10, and 10-20 cm collected at two moments: before mechanical clearing, (Control) and after 18 months of extensive sheep grazing, in areas without grazing, only mechanical clearing (MC) and in areas with both mechanical clearing and grazing (MCG). The results indicate that vegetation cutting has induced a significant decrease in extractable potassium, and an increase in the soil organic matter and total nitrogen. The exchangeable bases and the exchangeable acidity did not undergo expressive changes, as indicated by the pH values and the cation exchangeable capacity. After grazing, extractable phosphorus and potassium, organic matter, total nitrogen, exchangeable bases, and cation exchangeable capacity have increased significantly in the topsoil (0-5 cm), reducing soil acidity. Regarding physical properties, only soil permeability has been negatively affected by grazing. Mediterranean mountain open forests management with the combination of vegetation clearing and extensive sheep grazing proved to be effective in reducing vegetable fuel availability and improving soil quality.

OPEN ACCESS

Edited by: Marco Race, University of Cassino, Italy

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Received: 31 May 2023 **Accepted:** 27 July 2023 **Published:** 10 August 2023

Citation:

Fonseca F, Castro M, Alves L, Castro J and de Figueiredo T (2023) Impacts of Extensive Sheep Grazing on Soil Physical and Chemical Quality in Open Mountain Forests, NE Portugal. Span. J. Soil Sci. 13:11632. doi: 10.3389/sjss.2023.11632 Keywords: forest, vegetation management, soil chemical properties, soil physical properties, Mediterranean region

INTRODUCTION

Grazing effects on soil quality and ecosystems services across the world are often controversial (Lai and Kumar, 2020; Fielding, 2022). However, extensive pastoral systems are an effective tool for vegetation management in open mountain forests in the Mediterranean region. It contributes to vegetation maintenance (herbaceous and shrubby) and reduces hazardous fuels, rising the prevention of wildfire occurrence (Pardini et al., 2007; Fonseca et al., 2021; Castro et al., 2022). Pastoralism should be used as a complementary technique to the prescribed fire or mechanical clearing (Torres-Manso, 2011; Castro et al., 2022), which can reduce the costs associated with these traditional practices by around 75%, following the increase in intervals of time between interventions (12-year intervals with grazing versus 3-year intervals without grazing) (Varela et al., 2007). In

addition, environmental gains must be added, as the recurrent use of prescribed fire causes substantial damage to ecosystems, such as the degradation of soil and water quality and greenhouse gases emission (Fonseca et al., 2017). Grazing by herbivores can be a climate mitigation strategy as it influences the quantity and stability of the soil carbon pool (Naidu et al., 2022).

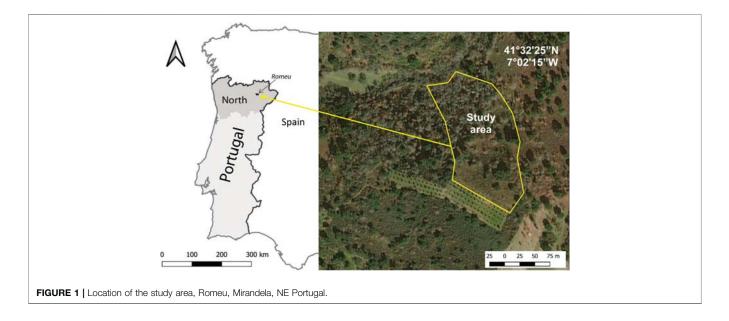
In Portugal, herding activity has been practiced mainly in mountain areas, where soil resource is scarce (Figueiredo, 2013) and dominant land use is composed of forests, bushes, and sparse pastures. In this sense, extensive grazing with species and breeds highly adapted to these constraints is, in many rural zones, one of the few economic activities capable of guaranteeing the local inhabitants' subsistence (Pinto et al., 2023). The Portuguese historical past, where extensive livestock played a crucial role in the economy of rural areas, is changing due to the scarcity and demanding workforce (shepherds and breeders), rural exodus, population aging, and lack of rural spaces organization (Fonseca et al., 2021). Effectively, in the last 100 years, there has been a sharp reduction in herding activity and in the number of shepherds and raisers (INE, 2021). The National Strategy for the Portuguese Forest indicates extensive grazing as a fuel management technique, but its use for this purpose is still very limited (Castro et al., 2018), since the most important areas for the practice of livestock are located in places with low population density.

Adequate animal load and a short residence time in a given space are essential factors to avoid excessive vegetation disappearance and consequent exposure of the soil to erosion processes and deterioration of the quality of habitats for wildlife (Díaz-Solís et al., 2016; Fielding, 2022). A well-managed pasture in space and time can provide wider biodiversity, nutrients distribution in the soil, and soil quality improvement. Soil quality is a concept centered on the dynamics of soil properties and processes (Karlen et al., 1994; Fonseca and Figueiredo, 2016), constituting an essential factor in the global sustainability of the biosphere and, in particular, in the sustainability of forest systems (Fonseca et al., 2019). In this sense, despite the recognized benefits of extensive grazing in reducing fuel and preventing forest fires, it is important to know the impacts caused on physical and chemical soil properties. Soil is a strategic resource for the sustainable development of humanity, and its preservation is of unquestionable importance. The present work aims to study the effects of mechanical clearing and extensive sheep grazing in an open Mediterranean mountain forest, on soil physical and chemical quality. Soils were sampled before the application of mechanical clearing (Control, C) and after 18 months the effects of grazing were studied. Grazed areas (mechanical clearing plus grazing, MCG) were compared with non-grazing areas (mechanical clearing, MC) and original soil (C).

MATERIALS AND METHODS

The study area, with forestry aptitude, was located in Romeu, Mirandela municipality, NE Portugal ($41^{\circ}32'25''N$ 7°02'15''W), in the range between 474 and 534 m altitude (**Figure 1**). The annual average temperature is 14° C and annual average precipitation is 508 mm, concentrated from October to April and with a marked dry season among June and August (Agroconsultores and Coba, 1991). Soils are classified as Distric Leptosols derived from schist (FAO, 2015). It is an open mountain forest where the arboreal vegetation is essentially composed of *Quercus rotundifolia*, *Quercus faginea* and *Quercus suber*. Associated with these species there is a relatively dense understory of *Cytisus multiflorus*, *Cytisus scoparius*, *Cystus ladanifer* and *Lavandula stoechas*, and some herbaceous vegetation species.

The experimental area occupies $4,300 \text{ m}^2$ and comprises three treatments: 1) plot without mechanical clearing with extensive grazing, which represents the traditional treatment, with an area of 906 m² (Control, C); 2) plot of 400 m² with mechanical clearing without grazing (MC); 3) plot of 41,694 m² with

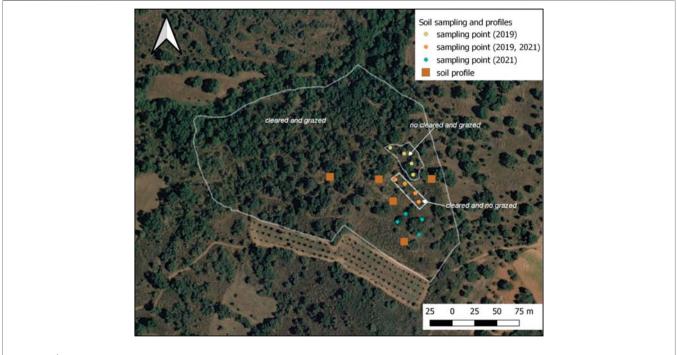


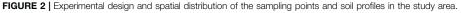
mechanical clearing plus grazing (MCG) (**Figure 2**). Extensive grazing by a flock of 150 sheep took place over an 18-month period, from December 2019 to May 2021.

In 2019, the soil was characterized before the application of mechanical clearing (C). Disturbed soil samples were collected from four randomly distributed sites at depths 0-5, 5-10, and 10-20 cm. Also, in the surface soil layer (0-5 cm) undisturbed soil samples with 100 cm³ of volume were taken to determine physical soil properties. In 2021, in the area subjected to mechanical clearing, the effects of grazing on soil physical and chemical properties were evaluated. Disturbed soil samples were collected

at depths 0–5, 5–10, and 10–20 cm in four sites randomly distributed in the area preferentially grazed by sheep (MCG) (**Figure 3**), and in the non-pastured area (MC). Permeability was measured at the same points in undisturbed soil samples taken in 100 cm³ cylinders from the topsoil (0–5 cm). In the traditional treatment (C) no evaluations were carried out in 2021, as the flock of sheep remained in this area for a very short time, and significant changes in soil properties were not foreseeable in the period considered (18 months).

Disturbed soil samples were air-dried and sieved with a 2 mm mesh sieve, in order to assess the organic matter, nutrients





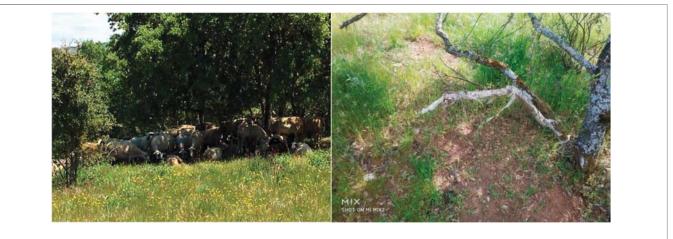


FIGURE 3 | Aspect of the most frequented places by the sheep.

Extensive Grazing and Soil Quality

concentration, soil pH, exchangeable acidity, cation exchangeable capacity, and soil particle size (soil texture). Methods applied in laboratory analysis comprised the Tinsley method for soil organic matter (Tinsley, 1950), Kjeldahl method for Total N (Bremner, 1996), the Egner-Riehm method for extractable phosphorus and potassium (Almeida and Balbino, 1960), and hydrometer method for soil texture determination. The pH was determined in a soilwater suspension (1: 2.5 soil water ratio). Exchangeable bases were evaluated by atomic adsorption (Ca^{2+} and Mg^{2+}) and by flame emission spectrophotometry (K⁺ and Na⁺). Cation exchangeable capacity was calculated as the sum of exchangeable bases and exchangeable acidity. Soil permeability was measured, in undisturbed soil samples, in a constant head closed circuit laboratory permeameter. In the same undisturbed soil samples, other physical properties were also evaluated, which are related to permeability, such as bulk density, total porosity, microporosity, macroporosity, field capacity, and water saturation capacity.

The soil classification in the study area was carried out based on the description and observation of five soil profiles and the analysis of samples collected in the horizons identified in the profiles. A representative soil profile was selected, based on the greater differentiation of horizons and their clearest delimitation.

The statistical analysis of the information collected included descriptive statistics, analysis of variance (one-way), and tests of means comparison (Tukey, p < 0.05).

RESULTS AND DISCUSSION

Soil Description and Classification

The morphological properties (Figure 4) and the physical and chemical soil properties evaluated (Table 1) show that the soils

are very shallow (<30 cm), with high stoniness, sandy loam texture, low pH (<5.5), medium to low in organic matter content, low total N, low exchangeable bases and cation exchangeable capacity, low P and moderate to high K contents. There will be no problems associated with aluminum toxicity, as the acid saturation is less than 20% (Porta et al., 2003). However, along the slope in very restricted areas, the soil thickness may slightly exceed 30 cm in depth. According to FAO (2015) and soil properties evaluated, the soils are classified as Distric Leptosols derived from schist.

Leptosols comprise very shallow soils over continuous rock to a depth of \leq 30 cm or with a high proportion of unconsolidated stony material with <20% (by volume) fine earth up to 75 cm in depth. Thus, Leptosols can be either very shallow soils directly lying on bedrock, or soils with excess rock fragments in a profile. No calcic, chernic, duric, gypsic, petrocalcic, petrogypsic, petroplinthic or spodic horizon (Agroconsultores and Coba, 1991; FAO, 2015). Leptosols are found in all climate zones (many of them in hot or cold dry regions), in particular in strongly eroding areas. Leptosols have a resource potential for wet-season grazing and as forest land. In NE Portugal, Leptosols constitute the dominant soil unit, occupying about 70% of the territory (Agroconsultores and Coba, 1991; Figueiredo, 2013).

Soil Chemical Properties Soil Organic Matter and Nitrogen

Extensive grazing (MCG) contributed to a significant increase in soil organic matter (SOM) concentration in the soil surface layer (0–5 cm). The organic matter content almost doubled in relation to the traditional treatment (C) and registered an increase of about 30% compared to the treatment with mechanical clearing (MC), presenting in the remaining layers (5–10 and 10–20 cm),

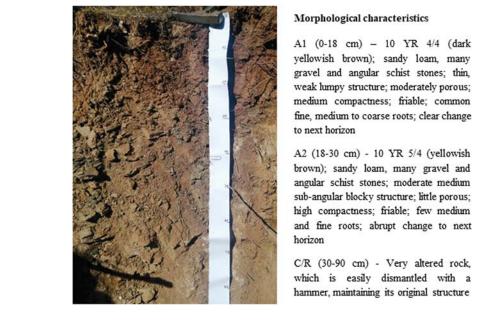


FIGURE 4 | Representative profile of the original soil in the study area.

TABLE 1 Physical and chemical	soil properties of the original soil in the study	/
area.		

Soil parameter	Depth (cm)			
	0-15/20	15/20–30		
Sand (0.02–2 mm) (%)	75 ± 1.1	65 ± 0.2		
Silt (0.002–0.02 mm) (%)	12 ± 0.5	20 ± 0.2		
Clay (<0.002 mm) (%)	13 ± 0.8	15 ± 0.5		
pH (H ₂ O)	5.5 ± 0.01	5.4 ± 0.05		
Organic matter (g kg ⁻¹)	18.2 ± 0.11	10.0 ± 0.10		
Total N (g kg ⁻¹)	0.9 ± 0.00	0.4 ± 0.00		
Extractable phosphorus (mg kg ⁻¹)	47 ± 5.1	46 ± 4.8		
Extractable potassium (mg kg ⁻¹)	52 ± 4.3	89 ± 7.9		
Exchangeable Ca (cmol kg ⁻¹)	2.02 ± 0.18	2.47 ± 0.22		
Exchangeable Mg (cmol kg ⁻¹)	0.91 ± 0.05	1.44 ± 0.27		
Exchangeable K (cmol kg ⁻¹)	0.25 ± 0.02	0.24 ± 0.00		
Exchangeable Na (cmol kg ⁻¹)	0.28 ± 0.02	0.24 ± 0.02		
SEB (cmol kg ⁻¹)	3.46 ± 0.23	4.38 ± 0.49		
BS (%)	82 ± 5.2	66 ± 4.1		
CEC (cmol kg ⁻¹)	4.20 ± 0.22	6.67 ± 0.98		
EA (cmol kg ⁻¹)	0.73 ± 0.02	1.29 ± 0.09		

SEB, sum of exchangeable bases; CEC, cation exchangeable capacity; BS, base saturation; EA, exchangeable acidity.

Mean ± standard error of the data obtained in the five soil profiles observed.

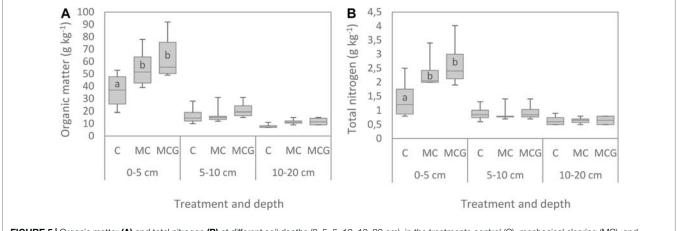
values quite similar among treatments (**Figure 5A**). As the soil samples were collected in the preferred grazing sites by the sheep, the increase in organic matter in the topsoil may be due to animal manure (feces and urine) and also to the vegetal components humification that was partially incorporated into the surface layer during mechanical clearing (Herrero et al., 2016; Fonseca and Figueiredo, 2018; Fielding, 2022). Similarly, Conant et al. (2003) and Wang et al. (2015) found only SOM increases in the topsoil layer. This increase in carbon also corroborates Abdalla et al. (2018) in a study where different grazing intensities were compared, showing increments of soil carbon under low-intensity grazing. The translocation of organic components in the soil may explain the slight increase in organic matter content in the 5–10 cm layer (Patrício et al., 2018; Fonseca et al., 2022).

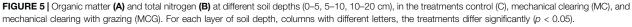
The non-significant changes observed in SOM between the areas with mechanical vegetation cutting with grazing (MCG) and without grazing (MC) agree with the low soil disturbance by the mechanized operation to cut the vegetation and its actual low impact. However, even slight soil disturbances might increase the SOM mineralization, which explains the negligible decrease in the SOM content in the surface soil layer (0–5 cm) (Ma et al., 2015; Fonseca and Figueiredo, 2018; Fonseca et al., 2019).

SOM plays a fundamental role in sustaining soil quality (Percival et al., 2000), and is often referred to as one of the main indicators of soil quality (Karlen et al., 1994; Sharma et al., 2005; Lal, 2015; Fonseca and Figueiredo, 2016). The increase in SOM due to grazing may represent an important contribution to soil fertility, water retention, aggregation stability, and carbon sequestration (C = 58% SOM), contributing to soil quality improvement and climate change mitigation (Piñeiro et al., 2010; Lal, 2015; Fonseca et al., 2019). Data obtained in this work are in line with the climate-positive strategy, as this grazing model increases the stocks of the soil organic carbon pool (Lai and Kumar, 2020). In the extensive grazing, the animals spend a short time in the same area, moving and leaving behind trampled vegetation covered by manure and urine, which turn out to be incorporated into the soil, improving physical and chemical soil properties (Abdalla et al., 2018; Fielding, 2022).

Nitrogen is found in the soil essentially in organic form (about 98%). In this sense, it presents a strict relationship with the organic matter content (N = 5% SOM), tending the carbon and nitrogen fluxes to be connected within the ecosystems (Piñeiro et al., 2010). Total nitrogen values are very low and present statistical differences (p < 0.05) only in the surface layer of the MC and MCG treatments in relation to the traditional treatment (C) (**Figure 5B**). Compared to mechanical clearing (MC), nitrogen concentration was not significantly affected by grazing (MCG), and the highest values were recorded on topsoil (0–5 cm). As expected, variations across the soil profile closely follow variations in organic matter.

Likewise, Lai and Kumar (2020), based on a meta-analysis of livestock grazing impacts on soil properties, resultant from the





analysis of 287 papers published globally from 2007 to 2019, referred that light grazing significantly increased SOM and $\rm NH_4^+$ soil concentration. Nitrogen is a limiting factor in most forest ecosystems, particularly in temperate zones (Ellsworth, 2004).

Extractable Phosphorus and Potassium

Extractable phosphorus and potassium showed different behavior by grazing effect. In grazing treatment (MCG), phosphorus increased significantly in the topsoil (0–5 cm), maintaining similar values in the deeper layers (5–10, 10–20 cm), but with a tendency to decrease in relation to mechanical clearing (MC) (**Figure 6A**). The effects of mechanical clearing without grazing (MC) are not very expressive compared to the other treatments (C and MCG). Values are considered low (26–50 mg kg⁻¹) to medium (51–100 mg kg⁻¹) (Santos, 2015).

With regard to extractable potassium, grazing (MCG) contributed to significant increases in all soil layers (0–5, 5–10, and 10–20 cm). The remaining treatments (MC and C) maintained similar values, with the exception of the topsoil layer (0–5 cm), where the vegetation cutting (MC) showed significantly lower values. Extensive grazing contributed to the values go by from medium (51–100 mg kg⁻¹) to very high (>200 mg kg⁻¹) (**Figure 6B**).

Phosphorus low values may be related to retention and fixation phenomena. Portuguese soils, in general, are very poor in this nutrient (Arrobas and Coutinho, 2002). Potassium has high mobility in the soil, undergoing redistribution along the soil profile (Fonseca et al., 2017). These parameter variations in the soil seem to be directly dependent on the effect of the manure (feces and urine) produced by the animals.

Exchangeable Bases, Exchangeable Acidity, Cation Exchangeable Capacity and pH

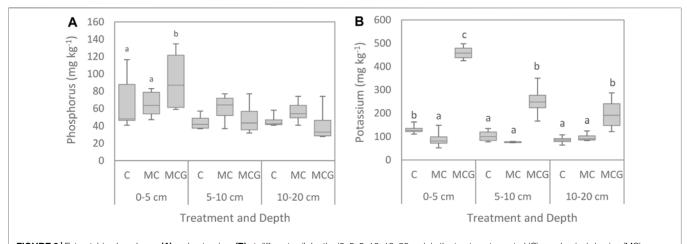
Table 2 presents the data concerning soil exchangeable complex (exchangeable bases, sum exchangeable bases, exchangeable acidity, cation exchangeable capacity) and pH values. As a result of grazing (MCG), the sum of exchangeable bases (SEB) almost doubled in the surface layer (0–5 cm),

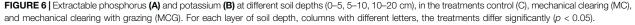
recording much lower values in the deeper layers (5-10 and 10-20 cm), but always higher in the treatment with grazing (MCG) when compared to the treatment without grazing (MC). Mechanical clearing (MC) contributed to the loss of exchangeable bases at all soil depths. This is a result of the basic cations having high mobility in the soil and the losses are associated with leaching and erosion processes (Fonseca et al., 2017), promoted by the greater exposure of the soil as a consequence of the vegetation removal. On the opposite, the exchangeable acidity (EA) decreased in the three soil layers owing to the grazing effect (MCG), leading to a reduction of the soil acidity. In general, at all soil layers and treatments, the values of base cations follow the sequence $Ca^{2+} > Mg^{2+} > K^+ >$ Na⁺, a common pattern observed in the soils. Calcium is the most important base cation, contributing more than 50% of the sum of exchangeable bases in the upper soil layers (0-5 and 5-10 cm).

Grazing produced important changes in the sum of exchangeable bases (SEB) and in the exchangeable acidity (EA), which are reflected in the cation exchangeable capacity (CEC) and in the soil pH values. The great increase in CEC in the surface layer (0–5 cm) is related to the corresponding increase in organic matter. Soil pH values were affected by the increase in base cations mainly in the most surface layer, and by the decrease in exchangeable acidity (EA). Due to the effect of grazing, in the topsoil (0–5 cm), soil changed from acid (5.3) to sub-acid (5.8) (Costa, 2011).

Soil Physical Properties

As expected, the soil permeability coefficient (k, cm/h) was significantly reduced in grazed areas (7.8 cm/h, MCG) when compared with no grazed areas (39.1 cm/h, MC) and the original soil (47.9, C) (**Figure 7**). Permeability changed from "very rapid" (25.4–100 cm/h, MC) to "moderately rapid" (6.35–12.7 cm/h, MCG) (USDA-SCS, 1985). Nonetheless, soil physical properties such as bulk density (BD), total porosity, microporosity (MicroP), macroporosity (MacroP), field capacity (FC), and water saturation capacity (WSC), showed no significant differences between

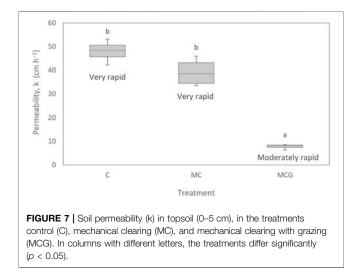




Depth	Treatment	Ca ²⁺	Mg ²⁺	K+	Na⁺	SEB	EA	CEC	pН
(cm)		(cmol kg ⁻¹)							(H ₂ O)
0–5	С	4.10 ^a	1.49 ^a	0.33 ^a	0.20 ^a	6.12 ^a	0.49 ^a	6.6 ^a	5.6 ^{ab}
	MC	3.99 ^a	1.25 ^a	0.23 ^a	0.31 ^a	5.78 ^a	0.66 ^a	6.4 ^a	5.3 ^a
	MCG	5.10 ^b	1.81 ^b	3.47 ^b	0.51 ^a	10.89 ^b	0.48 ^a	11.4 ^b	5.8 ^b
5–10	С	1.90 ^b	1.13 ^b	0.27 ^a	0.22 ^a	3.52 ^b	1.17 ^a	4.7 ^a	5.4 ^a
	MC	1.04 ^a	0.60 ^a	0.13 ^a	0.24 ^a	2.01 ^a	1.10 ^a	3.1 ^a	5.3 ^a
	MCG	0.88 ^a	0.74 ^a	0.60 ^b	0.37 ^a	2.59 ^b	1.01 ^a	3.6 ^a	5.3 ^a
10–20	С	1.34 ^b	1.08 ^b	0.26 ^a	0.22 ^a	2.90 ^c	2.04 ^b	4.9 ^b	5.2 ^a
	MC	0.39 ^a	0.40 ^a	0.12 ^a	0.34 ^{ab}	1.25 ^a	1.79 ^{ab}	3.0 ^a	5.3 ^a
	MCG	0.53 ^a	0.65 ^{ab}	0.54 ^b	0.40 ^b	2.12 ^b	1.09 ^a	3.2 ^a	5.5 ^a

TABLE 2 | Exchangeable cations (Ca, Mg, K, Na), sum exchangeable bases (SEB), exchangeable acidity (EA), cation exchangeable capacity (CEC), and pH values at different soil depths (0–5, 5–10, 10–20 cm), in the treatments control (C), mechanical clearing (MC), and mechanical clearing with grazing (MCG).

Average values of each depth with different letter are significantly different (p < 0.05).



be related to the production of hydrophobic substances on the soil surface (Keizer et al., 2008; Loss et al., 2015). On the other hand, since organic matter represents the key factor in soil quality, its significant increase in the surface layer (**Figure 5A**) may have contributed to nullifying the effect of trampling on physical soil properties. Also, Shrestha et al. (2020) did not find significant differences in soil bulk density, soil moisture, and soil compaction in extensive grazing systems of small ruminants.

In general, based on the soil physical and chemical properties evaluated, the presence of sheep flocks in the field contributed to increasing soil fertility and carbon sequestration. Identical results were obtained by Silva et al. (2020), in extensive grazing with cattle. Over a grazing period, the degree of soil physical degradation changes with factors such as animal load, hoof pressure, grazing duration, and the previous grazing history (Donovan and Monaghan, 2021). However, the existing literature and examples of light grazing effects on soil properties are limited and not conclusive.

TABLE 3 | Bulk density (BD), total porosity (P), microporosity (MicroP), macroporosity (MacroP), field capacity (FC) and water saturation capacity (WSC) in the treatments control (C), mechanical clearing (MC), and mechanical clearing with grazing (MCG).

Treatment	BD (g cm ^{−3})	Р	MicroP	MacroP	FC	WSC	
С	1.31 ± 0.05	54.2 ± 1.3	45.1 ± 0.8	9.1 ± 0.4	35.3 ± 1.1	40.8 ± 2.3	
MC	1.37 ± 0.07	51.9 ± 2.1	44.7 ± 1.9	7.1 ± 0.2	33.6 ± 3.3	38.9 ± 3.7	
MCG	1.34 ± 0.01	51.8 ± 0.7	42.6 ± 0.9	9.3 ± 0.4	31.7 ± 0.7	38.6 ± 0.3	

treatments (**Table 3**). Drewry et al. (2000) compared the effects produced on soil physical properties (macroporosity, bulk density, hydraulic conductivity), in sheep and dairy farms, and verified that the sheep affected the soil's physical attributes in a very slight way. Grazing influences the factors that control soil properties in a complex mode (Piñeiro et al., 2010; Abdalla et al., 2018).

Among other factors, soil permeability depends on soil porosity and bulk density (Leite et al., 2013; Fonseca et al., 2017). However, **Table 3** shows that grazing did not significantly affect the soil physical properties evaluated in the topsoil (0-5 cm). In this sense, the reduction in permeability may

CONCLUSION

The main effects of mechanical clearing without grazing (MC) and mechanical clearing with grazing (MCG) were recorded in the physical and chemical properties of the topsoil layer (0–5 cm), keeping the deeper layers (5–10 and 10–20 cm), in most cases, identical values to the original soil. Mechanical clearing has contributed to a significant increase in soil organic matter and total nitrogen. On the opposite, extractable potassium decreased significantly. Exchangeable bases and the exchangeable acidity did not record expressive changes, as indicated by the pH values and the cation exchangeable capacity. The soil physical properties did not change due to the cutting vegetation effect. Extensive sheep grazing contributed overall to improved soil fertility. This improvement in fertility was mainly visible in the surface layer (0-5 cm) and resulted in increases in organic matter, total nitrogen, extractable phosphorus, and potassium, the sum of exchangeable bases, cation exchangeable capacity, and soil pH values. In general, grazing did not significantly affect the physical soil properties, only a significant reduction in soil permeability was observed. Based on the results obtained, in this kind of mountain environment, grazing systems for small ruminants, in conjunction with vegetation clearing, can play an important role in reducing fuel availability (prevention of large forest fires) and in improving productive (increased fertility) and environmental functions of the soil (increased carbon sequestration).

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

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AUTHOR CONTRIBUTIONS

Conceptualization: FF, TF, and MC; methodology: FF, TF, and MC; investigation: FF, TF, MC, JC, and LA; Figures and tables: FF and JC; original draft preparation: FF; review and editing: FF and TF; project administration: MC; funding acquisition: MC; All authors contributed to the article and approved the submitted version.

FUNDING

The present work was supported by EU SUDOE, grant number SOE2/P5E0804, project entitled "OPEN2PRESERVE (SOE 2/P5/E0804)—Modelo de gestión sostenible para la preservación de espácios abiertos de montaña de alto valor ambiental."

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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