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Distribution of soil carbon and nitrogen in Mediterranean oak silvopastoral systems under grazing

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Soil organic carbon (SOC) plays a key role in climate regulation and the functioning of ecosystems. However, the impact of grazing on its distribution in Mediterranean oak silvopastoral soils is not well understood. This study analysed the effects of grazing on soil carbon and nitrogen dynamics in two oak-based silvopastoral systems in northeastern Portugal, namely *Quercus pyrenaica* and *Quercus rotundifolia*. Soil samples were collected at depths of 0–10 cm and 10–30 cm before and after grazing cycles. The concentrations of carbon and nitrogen in total soil and in three particle-size fractions (sand, silt, and clay) were quantified following physical fractionation. Multivariate analysis (PCA) and generalized linear mixed models (GLMM) were used to evaluate the effects of stand-forming tree species, presence of grazing, and soil depth. Soil carbon and nitrogen were significantly influenced by stand-type and soil depth, whereas grazing had no significant effect. Higher SOC values were observed under *Q. pyrenaica*, reaching 35.00 ($\pm$2.07 SE) g kg^{-1} in the 0–10 cm layer, compared with 24.6 g kg^{-1} under *Q. rotundifolia*. Carbon stocks ranged from 30.42 $\pm$ 2.12 Mg ha^{-1} under *Q. pyrenaica* to 13.25 $\pm$ 2.15 Mg ha^{-1} under *Q. rotundifolia* across the soil profile. A similar pattern was observed for soil nitrogen, with total N values reaching up to 2.28 $\pm$ 0.17 g kg^{-1} under *Q. pyrenaica*, compared to approximately 0.52 $\pm$ 0.05 g kg^{-1} under *Q. rotundifolia*. Most of the soil carbon and nitrogen was associated with the sand fraction. These results suggest that the dynamics of soil carbon and nitrogen in Mediterranean silvopastoral systems are primarily driven by vegetation type and soil depth, whereas grazing under extensive management conditions has a limited influence on SOC stocks and their distribution among soil fractions.

KEYWORDS
bulk density, ecosystem resilience, land-use systems, soil carbon fractions, soil carbon stocks

Introduction

Soil organic carbon (SOC) represents one of the largest carbon reservoirs in the Earth system and plays a critical role in regulating the global climate, supporting nutrient cycling, and sustaining the productivity of agroecosystems (Lal et al., 2021; Kopittke et al., 2022; Da Gama, 2023). The stability and dynamics of SOC are modulated by biogeochemical processes, which are, in turn, influenced by land use and management practices as well as local environmental conditions (Zhang et al., 2024; Maqbool et al., 2025). Understanding how SOC stocks respond to different management practices is essential for evaluating ecosystem carbon-sink potential and their contribution to mitigating global climate change (Ramesh et al., 2019; Mäkipää et al., 2023; Schreiner-McGraw et al., 2024).

Studies indicate that most of the organic carbon in the soil is associated with macroaggregates, this includes carbon that is trapped within these aggregates, this carbon represents a substantial proportion of the total stock and responds more quickly to changes in management (Howlett et al., 2011; Gelaw et al., 2015). By contrast, carbon associated with finer fractions, microaggregates and silt-clay complex, tends to have lower absolute concentrations, but is more stable due to organomineral protection and a longer residence time in the soil (Mustafa et al., 2021; Delahaie et al., 2024). This structural understanding of organic matter supports the hypothesis that changes in disturbance intensity, such as grazing, primarily affect the most labile and superficially distributed compartments, while the more stable pools remain relatively resilient (Dong et al., 2020; Encarnation et al., 2025). In this context, silvopastoral systems have been described as being structurally resilient to moderate disturbances, as they maintain the integrity of soil aggregates and the biogeochemical functionality of the soil (Mosquera-Losada et al., 2018).

When managed sustainably, grazing can contribute to the maintenance or even enhancement of soil organic carbon (SOC) through several mechanisms, including the incorporation of plant residues into the soil, stimulation of root turnover, and the addition of organic inputs via animal excreta (McDonald et al., 2023; Ritchie, 2020; Gross and Glaser, 2021). However, excessive grazing pressure may lead to soil compaction, reduced water infiltration, and loss of vegetation cover, which can ultimately reduce SOC stocks and alter soil functioning (Abdalla et al., 2018; Donovan and Monaghan, 2021; Centeri, 2022; Fonseca et al., 2023). Consequently, the overall impact of grazing on soil carbon dynamics is highly context-dependent, being influenced by factors such as stocking rate, grazing season, management practices, and local environmental conditions (Eze et al., 2018; Santos et al., 2022; Stanley et al., 2024).

However, several studies have also reported limited or non-significant effects of grazing on soil carbon and nitrogen stocks, particularly in extensive or well-managed silvopastoral systems (Piñeiro et al., 2010; Ritchie, 2020; Abagandura et al., 2024). Under moderate grazing pressure and with the maintenance of vegetation cover, soil disturbance tends to be reduced, allowing soil organic matter stocks to remain relatively stable over time (Mosier et al., 2021; Zhou et al., 2022). In such situations, the balance between organic matter inputs from plant residues and animal excreta and potential losses caused by trampling or biomass removal may result in neutral net effects on soil carbon and nitrogen dynamics (Coonan et al., 2020; Zhu et al., 2021; Li et al., 2025). These findings highlight the importance of evaluating grazing impacts within specific ecological and management contexts, as soil responses may vary considerably depending on site conditions, grazing intensity, and soil characteristics (Eze et al., 2018; Liu et al., 2021).

In the face of climate change and growing pressure on agri-food systems, the integration of grazing practices that align with carbon sequestration is emerging as a promising strategy for reconciling sustainable livestock production and climate regulation (Godde et al., 2020; Rui et al., 2022; Stanley et al., 2024). However, grazing intensity plays an important role. Low stocking rates in silvopastoral systems tend to promote greater carbon sequestration in the upper soil layers compared with intensive grazing or grazing exclusion (Ferreiro-Domínguez et al., 2016). In addition, the integration of trees into pasturelands through silvopastoral management aligns livestock productivity with long-term strategies for soil carbon conservation and climate change mitigation (Torres et al., 2022; Aryal et al., 2022).

In northeastern Portugal, native oak forests such as *Quercus rotundifolia* and *Quercus pyrenaica* are renowned for their high ecological and cultural relevance (Carvalho, 2011; Pérez-Luque et al., 2020; Pousa et al., 2025). Hese woodlands support a variety of traditional practices, with extensive grazing playing a significant part in shaping the region's rural landscape (Guadilla-Sáez et al., 2019; Plieninger et al., 2021; Castillo-Garcia et al., 2022). Maintaining an appropriate balance between conservation goals and pastoral use is essential to preserving ecosystem services, including soil protection, nutrient cycling, biodiversity conservation, and the provision of silvopastoral products (Díaz-Pereira et al., 2020; Lecegui et al., 2022; Amorim et al., 2023). In addition, grazing plays an important socio-economic role in north-eastern Portugal, particularly for small and medium-sized family farms, contributing to income generation, rural population retention and the preservation of traditional knowledge (Torres-Manso et al., 2017; Castro et al., 2021; Rego et al., 2022; Sharifian et al., 2022).

Despite the ecological and climatic importance of the subject, significant gaps remain in the literature concerning the impact of grazing on soil carbon stocks in native forests, particularly in oak forests in Portugal. Most available studies focus on permanent pastures (Deosaran et al., 2024), intensive agricultural systems (Rodrigues et al., 2019), or forest ecosystems evaluated in isolation (Patrício et al., 2023). Integrated systems, such as silvopastoral systems, remain relatively understudied. This hinders our understanding of the mechanisms that regulate soil carbon dynamics and stability in environments where trees, shrubs, herbaceous plants, and pastoral management interact simultaneously.

Therefore, this study aimed to evaluate the effects of grazing on soil carbon and nitrogen dynamics and their distribution among particle-size fractions, in two Mediterranean oak silvopastoral systems in northeastern Portugal, namely *Quercus pyrenaica* and *Quercus rotundifolia*.

Materials and methods

Study area

The study was conducted in north-eastern Portugal (41°53.59' N; 6°52.8' W), within the Montesinho Natural Park (PNM; Figure 1). It targeted two forest habitats of Community interest listed under the EU Habitats Directive (92/43/EEC): Galician Portuguese oak woods dominated by *Quercus robur* and *Quercus pyrenaica* (habitat 9230), and evergreen oak forests dominated by *Quercus ilex* and *Quercus rotundifolia* (habitat 9340). Within these habitats, stands of *Q. pyrenaica* and *Q. rotundifolia were studied*. Both habitats are of

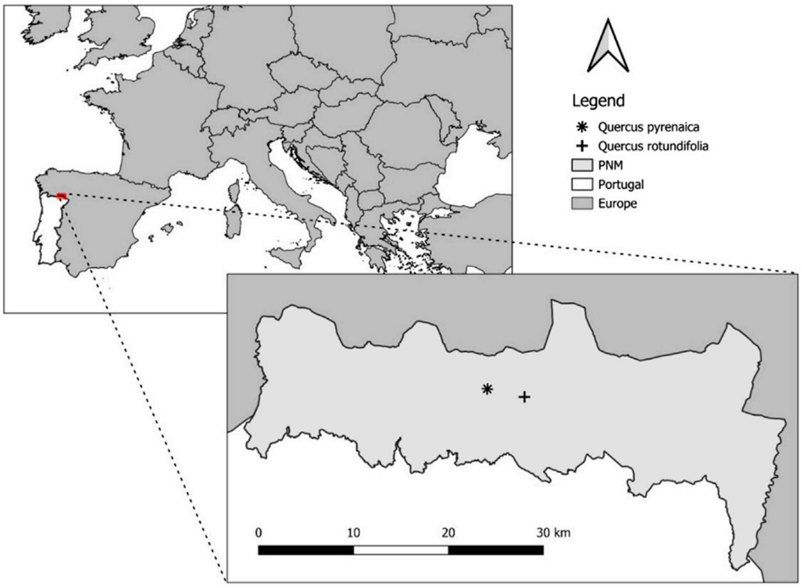

FIGURE 1
Location of the study area in Montesinho Natural Park (PNM), NE Portugal. Reproduced from "Location of the study area in Montesinho Natural Park (PNM), NE Portugal" by Júlio Henrique Germano de Souza, Maria Rosa Mosquera-Losada and Marina Castro, licensed under CC BY 4.0.

natural origin, having developed without silvicultural intervention, and are occasionally subjected to extensive grazing.

The territory of PNM is characterised by a markedly heterogeneous relief, consisting of plateaus interspersed with deep valleys and mountainous formations (Castro et al., 2021). The slopes vary from flat to very steep, with altitudes ranging from 438 to 1,486 m asl. Most of the territory falls within the supra-Mediterranean bioclimatic zone, with an average annual temperature of between 8 °C and 12 °C and, annual rainfall of between 1,000 and 1,600 mm, mostly concentrated between the months of October and March (Castro et al., 2021). Climate data for the years 2023, 2024, and 2025 are presented, as these years encompass the period during which changes in soil carbon stocks were assessed in grazed and ungrazed areas (Supplementary File).

Experimental sites

The area consists of a multifunctional natural forest which has historically been used for various purposes, such as firewood collection, grazing, and mushroom harvesting (Castro et al., 2025). Around 30 years ago, the area underwent traditional small-scale firewood and timber extraction. This mainly involved the selective removal of branches or a limited number of trees. These interventions resulted in low-intensity timber extraction, although no quantitative records are available.

One of the sites includes two fenced plots of 1.3 ha each, located within a stand dominated by *Q. rotundifolia*, with an estimated tree density of approximately 965 trees per hectare. The tree layer is accompanied by a relatively dense understory with approximately 51% shrub cover, composed of *Genista falcata* Brot., *Pterospartum tridentatum* (L.) Willk., *Cistus salviifolius* L., *Halimium lasianthum* subsp. *alyssoides*, *Cytisus multiflorus* (L'Hér.) Sweet, and *Erica australis* L. A sparse herbaceous layer is also present, consisting mainly of annual grasses.

Soil is classified as Leptosol derived from basic rocks (Agroconsultores and Coba, 1991), which are the dominant soil in the region. These soils are poorly developed and have a shallow profile with a surface A horizon varying between 10 and 30 cm (Calzolari et al., 2021). The soil has a sandy loam texture, with an average clay content of 11.15%, and is often gravelly (De Souza et al., 2026). The underlying C horizon consists mainly of fragmented parent material with a low proportion of fine earth. Coherent bedrock is typically found at depths of less than 50 cm, constraining both water retention and root development. Chemically, the soil is moderately acidic, with an average pH (H_2O) of 5.3.

In July 2024, a local flock of 25 late-gestation *Preta de Montesinho* goats, weighing between 45 and 50 kg, grazed one of the experimental plots for 15 consecutive days, while the other plot remained ungrazed as a control. The animals spent approximately 15 h per day on the plot and were gathered at night due to the presence of wolves in the area. During the grazing period 5 kg of energy-rich concentrate feed (crude protein 18.4%, crude fiber 11.4%, crude fat 3.1%, crude ash 8.5%, calcium 1.32%) was distributed in the plot to stimulate the goats' appetite. Pastoral management in this plot can be described as low-intensity and short-duration grazing.

The other experimental site consists of two fenced plots of approximately 1.3 ha each, located within a stand dominated by *Q. pyrenaica*, with an estimated tree density of approximately 1,545 trees per hectare. The understory with approximately 32% shrub cover, is mainly composed of *Cytisus striatus* (Hill) Rothm. and *Rubus ulmifolius* Schott. The soil is predominantly classified as Umbric Leptosol, characterised by a low degree of pedogenetic development (Agroconsultores and Coba, 1991). It features a dark-coloured, humic A horizon with a sandy loam texture (average clay content = 8.91%) and frequent gravel inclusions (De Souza et al., 2026). This horizon overlies a C horizon composed mainly of coarse fragments resulting from the physical weathering of bedrock, with a low proportion of fine earth. A sharp lithological discontinuity is present, with unweathered bedrock occurring abruptly at depths between 10 and 50 cm. These conditions significantly limit the effective soil depth, constraining both root development and water retention capacity. Chemically, the soil is acidic, with an average pH (H_2O) of 5.1. A local flock of 220 pregnant *Churra Galega Bragançana* ewes, each weighing between 50 and 60 kg, grazed on the *Q. pyrenaica* site. Between May and October 2024, the animals grazed on the plot for a total of 55 days, which were not consecutive. They spent around 17 h a day on the plot. In short, the *Q. pyrenaica* habitat can be characterised by high grazing intensity on non-consecutive days.

Soil sampling

Soil samples were collected twice: once before the first grazing cycle, in June 2023, and again after grazing, in June 2025. Samples were collected at depths of 0–10 cm and 10–30 cm for the analysis of bulk density (using a cylinder of known volume) and particle size distribution (texture) according to the methodology described by Howlett et al. (2011). To ensure representativeness and capture spatial variability, five samples were collected per plot, using the composite sampling method, whereby each composite sample was made up of three simple samples homogenised together. Soil samples were collected using a probe sampler, which preserves the natural soil structure, particularly important when assessing bulk density. After collection, the samples were placed in labelled containers and transported to the laboratory for drying, sieving, and subsequent physico-chemical analysis.

Soil samples were air dried at room temperature (20 ˚C–25 ˚C) to a constant weight and passed through a 2-mm sieve (#10 U.S. Standard Testing Sieve). The portion of soil that did not pass the 2-mm sieve was separated, dried overnight at 70 ˚C (with the weight noted), and discarded. The weight of the discarded fraction would be used to convert the eventual data derived from the 2-mm sieved fraction (hereafter referred to as "whole soil") back to fi eld conditions (Rodríguez-Murillo, 2001; Six et al., 2002a).

Soil fractionation and carbon determination

The whole soil was physically fractionated according to Elliott (1986) and Six et al. (2002b). A 25 g sample of 2 mm sieved air-dried soil of known moisture content was placed in a 250 mL beaker. Distilled water (150 mL), enough to completely cover the soil, was poured into the beaker to promote slaking. The slaking process breaks up water-unstable aggregates in the soil, leaving behind water-stable aggregates for further analysis. After 5 min, using 250 and 53-µm sieves (60 and 270 U.S. Standard Testing Sieves, respectively), slaked soil was poured on top of the 250-µm sieve. Soil solution was wet-sieved manually by moving the sieve up and down about 5 cm each, 50 times in 2 min. What did not pass the 250-µm sieve was backwashed, with a distilled water-filled wash bottle, into a preweighed and numbered aluminum plate. The remaining soil solution was poured over the 53-µm sieve and given the same 2-min manual sieving. What did not pass the 53-µm sieve was backwashed into a preweighed and numbered aluminum plate. The remaining soil solution that passed the 53-µm sieve was poured into a preweighed and numbered aluminum plate. The three soil fractions (250–2000, 53–250, and <53 µm) were dried at 60 ˚C overnight, weighed, ground for homogenization, and stored in individually sealed and labeled plastic bags for further C analysis. Samples were then analyzed by a LECO C.N.H.S. Elemental Analyzer for percentage C within 2 weeks after whole soil was air dry. Laboratory analysis was conducted at the University of Santiago de Compostela, Crop Production Department laboratories in Lugo, Galicia, Spain.

The bulk density of the soil, measured at each sampling depth, was used to convert the concentrations of C in the total soil and its fractions into Mg C ha−1 for each depth interval (0–10 and 10–30 cm), using the methodology of Howlett et al. (2011). The carbon stock (C) in each soil fraction (Mg C ha^{-1}) was estimated based on the C concentration in the fine soil fraction (<2 mm), multiplied by the proportion of soil < 2 mm in relation to the raw soil, the soil density (g cm^{-3}) and the proportion of the specific fraction's mass in relation to the soil < 2 mm (Equation 1). The resulting values were converted to carbon stocks and expressed as Mg C ha^{-1} using the appropriate conversion factor. The equation used therefore integrates: (i) the carbon concentration per unit of soil < 2 mm, (ii) the ratio between fine soil and raw soil, (iii) soil density, (iv) the proportion of the soil fraction of interest, and (v) the conversion factors for the sampled depth (10 cm or 20 cm) and unit area.

$$\mathrm{Mg\,C\,ha^{-1}} = \left(\frac{\mathrm{gC}}{\mathrm{g < 2\,mm\,soil}}\right) \times \left(\frac{\mathrm{g < 2\,mm\,soil\,C}}{g\,bulk\,soil}\right) \times \left(\frac{g\,bulk\,soil}{cm^3}\right) \times \left(\frac{g\,fraction\,soil}{\mathrm{g < 2\,mm\,soil}}\right) \times \left(\frac{sample\,depth}{1}\right) \times \left(\frac{100}{1}\right) \quad (1)$$

Statistical analysis

Multivariate indirect ordination analysis was applied to the set of soil variables analysed in this study. In particular, we applied Principal Component Analysis (PCA). The response of carbon stock (g. kg ha^{-1}) in the total soil fractions (sum of all soil fractions), macro (sand), micro (silt) and clay to the type of stand (levels: *Q. pyrenaica* and *Q. rotundifolia*), the treatment (levels: with grazing and without grazing), sample depth (levels: 0–10 and 10–30 cm) and the triple interaction between stand, treatment and depth was analysed using four generalised linear mixed models (GLMM), one for each fraction, using the glmmTMB package (Brooks et al., 2017). A Gaussian distribution (family = gaussian()) was used, which is appropriate when the residuals exhibit normal behaviour. Sampling point was included as a random effect to consider the dependence between samples collected at 10 and 30 cm at the same point. The effect of the explanatory variables and their interactions was evaluated using Wald tests, based on analysis of variance tables obtained with the ANOVA function of the car package (Fox and Weisberg, 2018). The adequacy of the model was assessed by analysing simulated residuals using the DHARMa

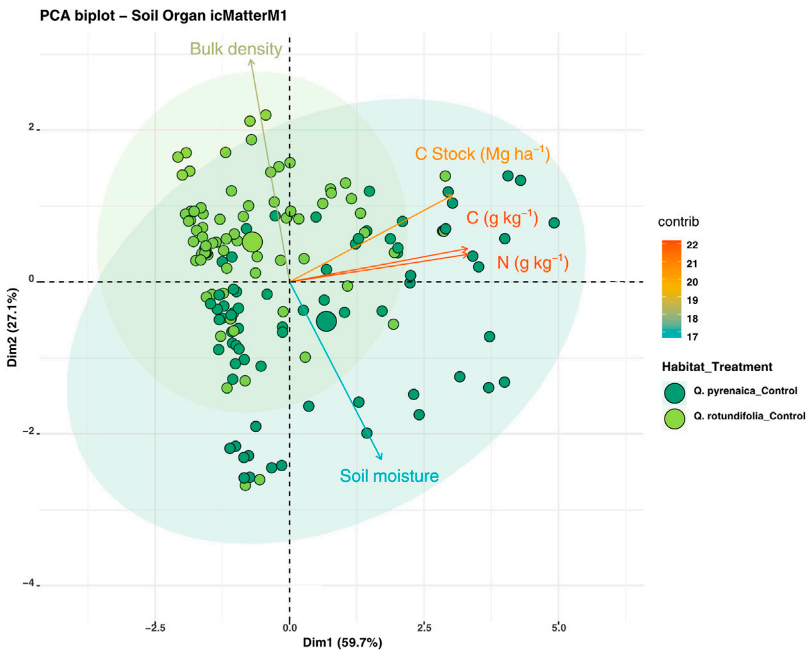

FIGURE 2
Principal component analysis (PCA) biplot of soil properties in ungrazed plots. Points represent individual samples coloured by stand type (*Q. pyrenaica* and *Q. rotundifolia*), with ellipses indicating group dispersion. Vectors represent soil variables (carbon stock, C Mg ha^{-1}; carbon content, C g kg^{-1}; nitrogen content, N g kg^{-1}; bulk density (g cm^{-3}); and soil moisture (%)), with direction and length indicating their correlation with the ordination axes. Colour gradients of vectors ("contrib") represent their relative contribution to Dim1 and Dim2.

package (Hartig, 2024), which verified normality, homogeneity of variances, the presence of outliers, and possible structural patterns in the residuals. Next, we performed Tukey's *post hoc* analysis with a significance level of $p < 0.05$. All statistical analyses were conducted in R version 4.0.2.

Results

Principal component analysis (PCA) of soil properties in ungrazed plots showed that the first two components explained 86.8% of the total variance (Dim1: 59.7%; Dim2: 27.1%) (Figure 2). Dim1 represented a clear gradient of soil organic matter accumulation, strongly driven by carbon stocks (C Mg ha^{-1}), carbon content (C g kg^{-1}) and nitrogen content (N g kg^{-1}), which exhibited the highest contributions (indicated by longer vectors and warmer colours). In contrast, bulk density was negatively associated with this axis, indicating that soils with higher compaction were characterised by lower C and N levels. Dim2 was primarily associated with soil moisture, reflecting a secondary environmental gradient independent from carbon and nitrogen dynamics. The orthogonal orientation of moisture relative to C and N variables suggests that water availability was not the main driver of nutrient distribution in these systems. The ordination revealed a clear separation between stand types. Samples under *Q. pyrenaica* were predominantly associated with the positive side of Dim1, indicating higher soil carbon and nitrogen contents and stocks. In contrast, samples under *Q. rotundifolia* were grouped towards the negative or central region of the axis, reflecting soils with lower nutrient levels and higher bulk density. This pattern indicates that vegetation type is the main factor structuring soil C and N distribution, overriding potential variability associated with soil moisture.

Following the grazing period, the PCA revealed a consistent ordination structure, with the first two principal components explaining 62.1% and 24.8% of the total variance, respectively (Figure 3). As observed prior to grazing, Dim1 represented a strong gradient of soil carbon and nitrogen status, driven primarily by carbon stock (C Mg ha^{-1}), carbon content (C g kg^{-1}), and nitrogen content (N g kg^{-1}), which exhibited the highest contributions (longer vectors with warmer colours). Bulk density remained negatively associated with this axis, reinforcing the inverse relationship between soil compaction and organic matter accumulation. Dim2 was mainly associated with soil moisture, indicating a secondary environmental gradient largely independent of the distribution of carbon and nitrogen. The near-orthogonal orientation between moisture and the C–N variables suggests that water availability did not directly control the spatial variability of soil organic matter in the studied systems. The distribution of samples after grazing closely mirrored the pattern observed in ungrazed conditions, with a clear separation between stand types along Dim1. Samples under *Q. pyrenaica* remained associated with higher carbon and nitrogen contents and stocks, whereas those under *Q. rotundifolia* were predominantly located in regions characterised by lower nutrient levels and higher bulk density. No substantial temporal shifts were detected in the ungrazed sites during the study period, with control samples maintaining a relatively stable position and dispersion in the ordination space compared with the grazed treatments.

FIGURE 3
Principal component analysis (PCA) biplot of soil properties after grazing. Points represent individual samples coloured by stand type and grazing treatment (*Quercus pyrenaica* and *Quercus rotundifolia*, grazed and control), with ellipses indicating group dispersion. Vectors represent soil variables (carbon stock, C Mg ha^{-1}; carbon content, C g kg^{-1}; nitrogen content, N g kg^{-1}; bulk density (g cm^{-3}); and soil moisture (%)), with direction and length indicating their correlation with the ordination axes. Colour gradients of vectors ("contrib") indicate the relative contribution of each variable to Dim1 and Dim2.

TABLE 1 **Final models for all C g kg^{-1} variables in the study *p*-values from the mixed model regressions on C variables, for the following tested explanatory variables: Grazing, habitats and soil depth.**

Explanatory Variables	Total Soil Carbon	Carbon in Sand	Carbon in Silt	Carbon in Clay
Grazing	0.958	0.833	0.132	0.073
Habitat	0.000 ***	0.000 ***	0.004 **	0.020*
Soil depth	0.000 ***	0.000 ***	0.001 ***	0.000***
Habitat * grazing	0.646	0.999	0.140	0.872
Grazing * soil depth	0.318	0.411	0.198	0.132
Habitat * soil depth	0.238	0.584	0.952	0.268
Habitat * grazing * soil depth	0.085	0.093	0.297	0.713

$p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***.

Overall, the stability of the ordination pattern between sampling periods indicates that grazing, under the conditions evaluated, did not substantially alter the multivariate structure of soil properties. Instead, vegetation type and inherent soil characteristics remained the dominant factors controlling the distribution of soil carbon and nitrogen.

The sandy fraction showed the highest levels of C and N in the soil. It was found that most of the variables evaluated were significantly influenced by habitat type and soil depth, while grazing had no statistically significant effect (Table 1; Tables 2 and 3).

Soil carbon (g kg^{-1}) was significantly influenced by habitat type (*Q. rotundifolia* vs. *Q. pyrenaica*) ($p < 0.001$) and soil depth ($p < 0.001$) in all fractions analysed (Table 1). Grazing had no significant effect on soil carbon fractions ($p > 0.05$). None of the interactions between the factors evaluated were significant for this variable ($p > 0.05$).

The Tukey test indicated significant differences mainly between habitats, with higher soil carbon contents (C g kg^{-1}) in areas dominated by *Q. pyrenaica* compared to *Q. rotundifolia* (Figure 4). In the surface layer (10 cm), C values ranged from approximately 35.00 g kg^{-1} (±2.07 SE) under *Q. pyrenaica* to 24.60 g kg^{-1} (±1.45 SE) under *Q. rotundifolia*. At a depth of

TABLE 2 Final models for all N g kg^{-1} variables in the study. *p*-values from the mixed model regressions on N variables, for the following tested explanatory variables: Grazing, habitats and soil depth.

Explanatory Variables	Total Soil Nitrogen	Nitrogen in Sand	Nitrogen in Silt	Nitrogen in Clay
Grazing	0.995	0.826	0.187	0.173
Habitat	0.000 ***	0.000 ***	0.000 **	0.023*
Soil depth	0.000 ***	0.000 ***	0.007 **	0.077
Habitat * grazing	0.459	0.837	0.631	0.384
Grazing * soil depth	0.987	0.728	0.396	0.273
Habitat * soil depth	0.000***	0.000***	0.974	0.211
Habitat * grazing * soil depth	0.290	0.331	0.946	0.620

p < 0.05 *; p < 0.01 **; p < 0.001 ***.

TABLE 3 Final models for all C Mg ha^{-1} variables in the study. *p*-values from the mixed model regressions on C Mg ha^{-1} variables, for the following tested explanatory variables: Grazing, habitats and soil depth.

Explanatory Variables	Total soil Carbon	Carbon in sand	Carbon in silt	Carbon in clay
Grazing	0.995	0.973	0.781	0.236
Habitat	0.000 ***	0.000 ***	0.038 **	0.172
Soil depth	0.079	0.300	0.919	0.460
Habitat * grazing	0.364	0.677	0.194	0.768
Grazing * soil depth	0.264	0.470	0.765	0.886
Habitat * soil depth	0.014*	0.007**	0.252	0.046*
Habitat * grazing * soil depth	0.629	0.599	0.850	0.766

p < 0.05 *; p < 0.01 **; p < 0.001 ***.

30 cm, the contents were lower, ranging from 25.86 g kg^{-1} (±1.45 SE) to 10.52 g kg^{-1} (±1.06 SE) respectively.

When the distribution of carbon among the soil particle size fractions was considered, it was observed that the sand fraction concentrated most of the carbon at both depths and in both stands. In the 10 cm layer, values ranged from approximately 26.39 g kg^{-1} (±1.61 SE) to 19.20 (±0.66 SE) under *Q. pyrenaica* and *Q. rotundifolia*. At a depth of 30 cm, the values were approximately 19.53 g kg^{-1} (±1.97 SE) in *Q. pyrenaica* and 8.70 g kg^{-1} (±0.25 SE) in *Q. rotundifolia*. In the silt fraction, carbon content was substantially lower, ranging in the surface layer from 3.81 g kg^{-1} (±0.25 SE) in *Q. pyrenaica* to 2.56 g kg^{-1} (±0.29 SE) in *Q. rotundifolia* in the surface layer. At a depth of 30 cm, the values ranged from approximately 2.25 g kg^{-1} (±0.24 SE) in *Q. pyrenaica* to 1.39 g kg^{-1} (±0.25 SE) in *Q. rotundifolia*. For the clay fraction, carbon content was the lowest of all the analysed fractions. These values ranged from 1.52 g kg^{-1} (±0.18 SE) to 1.2 g kg^{-1} (±0.15 SE) in *Q. pyrenaica* and from 1.54 g kg^{-1} (±0.07 SE) to 0.61 g kg^{-1} (±0.05 SE) in *Q. rotundifolia*.

Soil nitrogen (g kg^{-1}) was significantly influenced by habitat type (*Q. rotundifolia* vs. *Q. pyrenaica*) ($p < 0.001$) and soil depth ($p < 0.001$) in all fractions analysed (Table 2). Grazing had no significant effect on soil fractionated nitrogen ($p > 0.05$). None of the interactions between the factors evaluated were significant for this variable ($p > 0.05$).

The pattern observed for total soil nitrogen (N g kg^{-1}) was similar to that observed for carbon, with differences primarily due to habitat type and soil depth (Figure 4). The means ranged from 2.28 g kg^{-1} (±0.17 SE) to 0.52 g kg^{-1} (±0.05 SE) under *Q. pyrenaica* and *Q. rotundifolia*, respectively. Among the granulometric fractions, the sand fraction contained the highest concentrations of the N, with averages ranging from of 1.63 g kg^{-1} (±0.12 SE) under *Q. pyrenaica* to 0.44 g kg^{-1} (±0.02 SE) under *Q. rotundifolia*. In the silt fraction, the values ranged from 0.25 g kg^{-1} (±0.05 SE) to 0.09 g kg^{-1} (±0.01 SE), under *Q. pyrenaica* and *Q. rotundifolia*, respectively, while in the clay fraction they were lower, ranging from 0.12 g kg^{-1} (±0.01 SE) in *Q. pyrenaica* to 0.05 g kg^{-1} (±0.01 SE) in *Q. rotundifolia*. In general, N contents decreased with depth in all soil fractions.

Soil carbon stock (Mg ha^{-1}) was significantly influenced by habitat type for total carbon ($p < 0.001$) and for sand ($p < 0.001$) and silt ($p < 0.05$) fractions, while the clay fraction did not show a significant effect ($p > 0.05$) (Table 3). Grazing and soil depth, alone, had no significant effect on soil carbon stock ($p > 0.05$). The habitat × soil depth interaction was significant for total carbon stock ($p < 0.05$) and for sand ($p < 0.01$) and clay ($p < 0.05$) fractions, while the other interactions were not significant ($p > 0.05$).

Soil carbon stock (C, Mg ha^{-1}) varied according to both habitat type and soil depth (Figure 3). In the surface layer, the mean values ranged from 30.42 Mg ha^{-1} (±2.12 SE) under *Q. pyrenaica* to

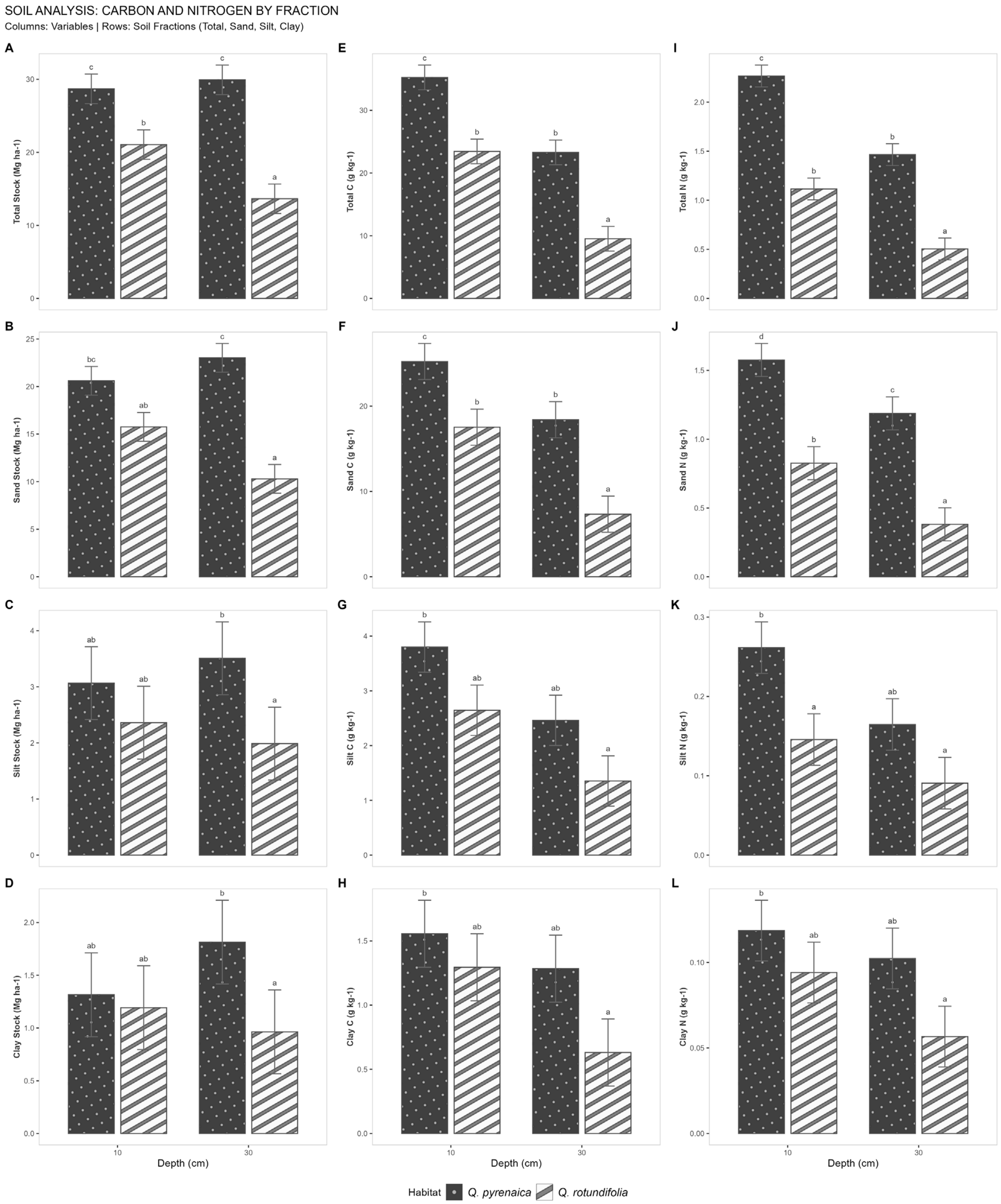

FIGURE 4
Mean ± SE total and fractional (top to bottom) soil C g kg^{-1} **(A–D)**, N **(E–H)** and C Mg ha^{-1} ratio **(I–L)** distribution in the 0–10 cm and 10–30 cm soil layers. Different letters indicate significant differences (p < 0.05) among treatments according to multiple Tukey mean comparison tests (soil layers and burning).

13.25 Mg ha^{-1} (±2.15 SE) under *Q. rotundifolia*. Of the granulometric fractions, the sand fraction contained the most carbon, with mean values of 22.51 Mg ha^{-1} (±2.52 SE) in *Q. pyrenaica* and 10.61 Mg ha^{-1} (±2.10 SE) in *Q. rotundifolia*. In the silt fraction, mean values ranged from 3.54 Mg ha^{-1} (±0.98 SE) in *Q. pyrenaica* to 2.42 Mg ha^{-1} (±0.52 SE) in *Q. rotundifolia*. The lowest values were found in the clay fraction, ranging from 1.86 Mg ha^{-1} (±0.32 SE) in *Q. pyrenaica* to 0.9 Mg ha^{-1} (±0.28 SE) in *Q. rotundifolia*. Overall, the highest carbon stocks were observed under *Q. pyrenaica*, particularly in the sand fraction and in the deeper soil layers.

Discussion

The results showed that habitat and soil depth were the main determinants of carbon fraction dynamics, whereas the effect of grazing was limited and inconsistent across fractions. *Q. pyrenaica* exhibited higher C content, particularly in the surface layer (0–10 cm), in terms of both total C and C associated with the sand, silt and clay fractions. This pattern is consistent with a greater potential for carbon accumulation and retention in the *Q. pyrenaica* stand, although site-specific environmental conditions may also have contributed to the observed differences.

These findings suggests that intrinsic attributes of the habitat, such as greaterlitterfall and litter quality (Zhang et al., 2016; Fonseca and Figueiredo, 2018), root architecture, and intensity of biogeochemical cycling, may play a central role in the formation and stabilisation of carbon in different granulometric fractions (Turrión et al., 2009; Fernández-Getino et al., 2018).

The consistent reduction in content in the 10–30 cm layer, particularly in *Q. rotundifolia*, highlights the importance of vertical stratification in controlling the distribution of carbon, particularly in terms of total carbon and for the sand and silt fractions. This pattern indicates either lower organic input at depth or greater susceptibility to mineralisation (Jobbágy and Jackson, 2000). Although the absolute values were lower in the clay fraction, the variations associated with the interaction between stand and depth suggest differences in organomineral stabilisation mechanisms between systems (Zhang et al., 2024). These results suggest that the variability in the fractional distribution of carbon is primarily influenced by ecological and structural attributes of Mediterranean oak forests, as well as the vertical organisation of the soil, rather than by pastoral management (Abdalla et al., 2018).

The observed patterns are consistent with the literature on the physical fractionation of soil organic carbon. Studies indicate that most C tends to be associated with macroaggregates, including C enclosed by macroaggregates, while finer fractions (microaggregates and silt + clay) have lower absolute concentrations but greater relative stability (Howlett et al., 2011; Gelaw et al., 2015; Ferreiro-Domínguez et al., 2016). A synthesis of 41 studies by King et al. (2019) showed that macroaggregates account for approximately 83% of the variations in total C stock, with proportionally greater increases in soils with higher carbon contents. The predominance of higher carbon content in the surface fractions associated with macroaggregates, observed in this study, is consistent with this global pattern and reinforces the role of aggregation as a fundamental mechanism for carbon protection.

The absence of a significant effect of grazing on the silt and clay fractions, particularly the latter, which offers the greatest physical-chemical protection, suggests that the management strategy did not encourage sufficient aggregate restructuring to affect the stabilisation of C in the fine fractions. This result is consistent with studies of Mediterranean silvopastoral which show that moderate or rotational grazing does not necessarily result in losses of carbon associated with stable aggregates (Wang et al., 2016; Manetti et al., 2025; Ishaq et al., 2025). Similarly, Lai and Kumar (2020) observed that controlled management systems maintained aggregate stability and carbon associated with macro and microaggregate fractions, even under continuous animal pressure. Therefore, the data suggest that grazing was not a disruptive driver of the structural dynamics of carbon in these areas under a put-and-take grazing system.

Total C content ranged from 10 to 32 g kg^{-1}, which is similar to Turrión et al. (2009) (1.3–18 g kg^{-1}) for different stands of *Q. pyrenaica* in central-western Spain. This is lower than the values reported by Castro and Fernandez-Nuñez (2014) (6–60 g kg^{-1}) for different Quercus stands in north-eastern Portugal, and substantially lower than the values described by Fonseca et al. (2019) for *Q. pyrenaica* in north-eastern Portugal (55–100 g kg^{-1} at 0–10 cm). Similarly, the estimated stocks (13–31 Mg ha^{-1}) fall within the range reported for Mediterranean silvopastoral systems, though they are often lower than the values observed in mature forests or with less disturbed systems. Cardinael et al. (2017) reported values 30–60 Mg ha^{-1} (0–30 cm) in French agroforestry systems, while Díaz-Pinés et al. (2011) found values of 40–80 Mg ha^{-1} in *Q. pyrenaica* stands in central Spain. Lara-Gómez et al. (2020) reported values of 42 Mg ha^{-1} at depths of up to 30 cm in systems with *Q. ilex* and *Q. suber* under traditional management. Conversely, Paustian et al. (2016) described lower values (15–35 Mg ha^{-1} at 30 cm) were described by Paustian et al. (2016) in temperate pastures with a history of intensive use. Thus, the stocks observed in this study are in an intermediate range, reflecting both soil characteristics and management history.

Regarding nitrogen, all soil layers and fractions had concentrations below 2.3 g kg^{-1} in ecosystems dominated by *Q. pyrenaica*, with the exception of total N in the surface layer (0–10 cm). These values are consistent with those reported for Mediterranean Quercus forests. Castro and Fernández-Núñez (2014) reported values of 1.03–8.80 g kg^{-1} for *Q. pyrenaica* and 0.61–2.56 g kg^{-1} for *Q. rotundifolia* in various locations in Trás-os-Montes (northeastern Portugal), suggesting that the values observed in this study fall within the regional variability previously reported for these ecosystems. In systems dominated by *Q. rotundifolia*, total N content remained within the range previously reported for Mediterranean Quercus forests, including the values observed for *Q. ilex* in north-eastern Spain (Canadell and Vilá, 1992) and *Q. suber* in southern Portugal (Otieno et al., 2011).

In addition to the differences observed between species, the vertical profile of N followed the typical pattern seen in terrestrial ecosystems, with higher concentrations in the surface layer and a progressive decrease down to 30 cm (Wang et al., 2022; Li et al., 2022; Chau et al., 2024). Higher concentrations under *Q. pyrenaica* (2.2–2.3 g kg^{-1}) than under *Q. rotundifolia* (1.1–1.2 g kg^{-1}) at the surface suggest a marked effect of stand type on soil N accumulation. This is probably associated with differences in litter quantity and quality, root architecture, and associated microbial activity (Lorenz et al., 2020; Sarıyıldız et al., 2022; Huang et al., 2023; Bashir et al., 2024; He et al., 2023). Overall, these results suggest that the distribution of soil N is influenced by the interaction between habitats (stand-forming tree species) characteristics, edaphic properties, and biogeochemical processes that regulate the decomposition and accumulation of organic matter.

Together, the results support the idea that silvopastoral systems can withstand moderate disturbances fairly well (Mosquera-Losada et al., 2018). The stability of the main carbon compartments in the 0–30 cm layer following grazing. Although the assessment covered a relatively short period, this response may reflect physical protection mechanisms, particularly aggregation, which can help preserve organic matter integrity under disturbance (Sharrow, 2007; Byrnes et al., 2018; Jensen et al., 2019; Meyer et al., 2025).This reinforces, the compatibility between controlled grazing and surface carbon conservation, providing further evidence that well-managed practices can maintain or even sustain the ecosystem service of carbon sequestration (Upson et al., 2016; Teague et al., 2016; Bastani et al., 2023; Guimarães et al., 2023; Lamb et al., 2024). Overall, these results highlights the importance of tree species composition, particularly the dominance of *Q. pyrenaica*, in controlling soil carbon and nitrogen dynamics in Mediterranean silvopastoral systems. However, given the lack of habitat replication, these differences should be interpreted cautiously, as site-specific factors may also have influenced the observed patterns.

Within the timeframe of this study, soil carbon pools remained relatively stable under the grazing regimes evaluated. However, long-term monitoring is needed to determine whether these systems can consistently sustain their carbon sequestration potential.

Conclusions

The results of this study show that the dynamics of soil carbon and nitrogen dynamics in Mediterranean silvopastoral systems are influenced by the stand-forming tree species and soil depth. Under the evaluated conditions, grazing had a minor effect. *Q. pyrenaica* consistently presented higher total and fractionated carbon contents and stocks, particularly in the topsoil (0–10 cm), showing a greater capacity for C accumulation and retention compared to *Q. rotundifolia*. The strong vertical stratification, with a particularly notable reduction in the 10–30 cm horizons (more pronounced in *Q. rotundifolia*), confirms that carbon dynamics primarily depend on the input of organic matter to the surface and the biological activity concentrated in the upper horizons.

Fractional analysis indicated that the greatest variations occurred in the sand-associated fractions (macroaggregates), whereas the silt and clay fractions presented lower absolute values but greater relative stability. These results reinforce the idea that the ecological and structural attributes of Mediterranean oak forests, combined with the vertical organisation of the soil, are the main determinants of carbon distribution and stabilisation in silvopastoral systems.

Finally, this study highlights the importance of considering both stand characteristics and soil stratification when evaluating soil carbon dynamics in Mediterranean silvopastoral systems. However, given the relatively short duration of the study, the observed stability of soil carbon stocks should not be interpreted as direct evidence of long-term carbon sequestration. Long-term monitoring is needed to determine the persistence of these patterns and to better assess the contribution of these systems to climate change mitigation.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

Conceptualization: MC and GD; Research: JD and MC; Data analysis: JD, MC, GD, and OF; Writing – original draft: JD and MC; Editing: JD, MC, GD and OF; Supervision: MC, GD, and MM-L. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors(s) declared that this work 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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/past.2026.16765/full#supplementary-material

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