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Research article

Influence of land management on soil organic matter pools, plant traits and enzymatic activity in mountain grasslands

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A B S T R A C T

Mountain grasslands are globally widespread ecosystems which play a pivotal role in several provisioning, regulating, supporting and cultural ecosystem services. Often shaped over centuries by traditional agricultural activities, including mowing and livestock grazing, mountain grasslands are integral to both ecological function and local livelihood. This study investigated the impact of light grazing on the soil-plant system in extensively managed grasslands, with a focus on functional structure and soil-associated ecosystem functions, including soil organic carbon accrual. Five meadows and five pastures were identified in the Central Italian Alps to simulate land-use intensification along an elevational gradient. Both plant compartment and topsoil samples were collected from each site and characterized. Grazed sites showed higher organic carbon, total nitrogen, and available phosphorus contents as well as higher urease activity, resulting in a higher soil organic matter accrual compared to meadows. In contrast, meadows were characterised by higher fluorescein diacetate hydrolase and phosphomonoesterase activities as well as by greater plant biomass and specific leaf area values. The mineral-associated organic matter (MAOM) fraction was the main carbon and nitrogen pool, especially in meadows. Correlations found between MAOM features and plant traits/soil enzymatic activities suggest that MAOM, in both management systems, is not exclusively of microbial origin, but also influenced by the plant component. Finally, particulate organic matter and MAOM showed a different stability both within and between management systems. These findings underscore the importance of a sustainable grassland management in storing organic matter, thus contributing to climate change mitigation, as well as to enhance nutrient cycling and ecosystem health.

1. Introduction

Grassland ecosystems cover approximately 40 % of the Earth's land surface (Suttie et al., 2005) and provide a wide range of essential ecosystem services such as carbon (C) sequestration, primary productivity support, biodiversity conservation, soil retention, water storage, and cultural and aesthetic values (Bengtsson et al., 2019; Paudel et al., 2021). Being grasslands a large store of soil organic carbon (SOC), and considering that it is easier and faster for soils to lose C than to gain C (Johnson et al., 2009), a sustainable management of these ecosystems becomes imperative (Smith, 2014). In fact, Reed et al. (2021) showed that mountain meadow soils can be either large net C sinks (578 ± 251 g C m^{-2} y^{-1}) or sources (392 ± 154 g C m^{-2} y^{-1}) depending on their disturbance level.

Alpine seminatural grasslands are generally grouped based on the type of management in hay meadows and pastures. Alpine hay meadows, typically situated on fertile soils in valley floors with gentle slopes, are primarily managed through mowing and represent a crucial source of high-quality forage for domestic herbivores. In some cases, these meadows are also grazed at the end of the summer (Marini et al., 2007; Pittarello et al., 2020), a practice that can alter the ecosystem balance. Unlike hay meadows, alpine pastures are not mown but are maintained through continuous or rotational grazing, which influences plant community composition and ecosystem processes. Due to their ecological fragility and increasing threats, certain types of hay meadows and pastures have been recognized as priority habitats under the EU Habitat Directive (Council Directive 92/43/EEC).

Livestock grazing is the most widespread form of grassland use globally (Dong et al., 2011), and one of the most important drivers of grassland degradation and C turnover alteration due to overgrazing (Steffens et al., 2009; Ma et al., 2018a). The impact of the grazing regime (high or low intensity) on SOC can be diverse and significant. For

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example, contrary to intensively grazed grasslands, those adapted to low grazing levels are generally dominated by slow growing species resulting in a slow litter decomposition (Milchunas and Lauenroth, 1993). In this context, McSherry and Ritchie (2013) reported cumulative changes up to 16 t C ha^{-1} over 10–30 years, and annual variations reaching 1.5 t C ha^{-1} yr^{-1}. Livestock grazing encompasses three main key mechanisms: removal of aboveground plant material, dung and urine deposition, and soil trampling. These mechanisms, individually or combined, influence the biological processes involved in C, nitrogen (N) and phosphorus (P) cycling, and cause a loss of biodiversity. Liu et al. (2015) showed that most of the variation in the grazing effects on grassland was explained by defoliation, which alters belowground C allocation (Holland et al., 1996) and promotes N-mineralization (Hamilton et al., 2008). Actually, both grazing and mowing have may alter soil organic matter (SOM) accrual in soil stimulating in a different way plant physiology and rhizospheric C input (Rumpel et al., 2015b).

Grassland management impacts coupling and decoupling of C, N and P cycles through regulation of plant production and the grazing regime. In particular, grazing leads to uncoupling of C from N and P (Soussana and Lemaire, 2014), as most of N and P are returned to soil in form of urine or dung inputs, whereas the majority of the ingested C is released to the atmosphere via respiration (Parsons et al., 2013). Moreover, the intensity of microbial decomposition, and, consequently, C accrual is strongly affected by plant residue stoichiometry. In temperate grasslands, variations in soil microbial communities have been ascribed to specific plant traits, in addition to other pedoclimatic-related factors (De Vries et al., 2012). Likewise, shifts in leaf economic traits have been associated with changes in microbial community structure, particularly the relative abundance of fungi and soil bacteria, mirroring differences in biomass quality and turnover (Pakeman, 2011). This highlights the strong interdependence between above- and belowground processes, suggesting that plant traits could be valuable indicators for predicting and understanding how plants influence nutrient cycling mechanisms (De Deyn et al., 2008). Furthermore, understanding how soil microbial communities respond to disturbances is critical for developing sustainable and regenerative management practices. Thus, having soil enzyme activities a huge influence on SOM turnover and nutrient cycling, and responding to land management practices (Burns et al., 2013), they have been widely used as a soil quality indicator (Schloter et al., 2017).

Similarly, land management can strongly impact SOC and total nitrogen (TN) content and distribution into particulate (POM) and mineral-associated organic matter (MAOM) (Giannetta et al., 2018), which exhibit different formation pathways, functions, turnover rates, and sensitivities to management practices and climate change (Cotrufo and Lavallee, 2022). Generally, POM is more susceptible to disturbance and rapid decomposition, unless physically protected (occluded) within soil aggregates, while MAOM shows slower turnover rates and represents the most persistent SOM component (Cotrufo et al., 2019). Among management practices, grazing deeply affects a wide range of ecophysiological and biogeochemical processes which govern SOC formation and partitioning into SOM pools (Maestre et al., 2022; Stanley et al., 2024). In fact, differences in SOM quality and inputs across ecosystems, including grasslands, can result in different patterns of MAOM and POM accrual (Castellano et al., 2015; Galluzzi et al., 2025). Therefore, investigating the soil-plant system as a whole becomes crucial to correctly interpret its response to land management practices.

Despite livestock grazing accounts for 77 % of global agricultural land and serves as a livelihood for billions of people worldwide, its effects on ecosystem services are still a matter of ongoing debate (Maestre et al., 2022). Numerous studies have examined the impact of grazing on ecosystem functioning (McSherry and Ritchie, 2013). For example, Zhou et al. (2017) showed that low-intensity promoted C and N accumulation, whereas moderate to heavy grazing significantly increases belowground C and N losses, particularly microbial biomass C and N (21.6 and 24.4 %, respectively). Conversely, they found that belowground fluxes, such as soil respiration, net N mineralization, and nitrification, increased by 4.3, 34.7, and 25.9 %, respectively, in grazed meadows compared to ungrazed controls. These findings underscore how grazing intensity can significantly affect both the magnitude and the direction of changes in belowground C and N pools, fluxes, and C/N ratios.

Thus, grazing may be more beneficial for SOM accrual than mowing up to a certain animal density depending on soil type and pedoclimatic context (Rumpel et al., 2015b). Increased grazing intensity is generally assumed to reduce SOC, potentially reducing both carbon dioxide (CO_2) fixation, due to the loss of photosynthetic tissue, and C input to the soil, through lower root production and faster litter turnover (Klumpp et al., 2009). Thus, considering the vulnerability of grasslands and their SOC stock to climate change (Díaz-Martínez et al., 2024; Galluzzi et al., 2024), further research is needed to clarify the impacts of grazing on the soil-plant system in extensively managed mountain grasslands.

In this study, we assess the effects of light grazing – defined as low-intensity grazing where livestock density is kept minimal to avoid overgrazing - on extensively managed mountain meadows. Our aim is to evaluate the system functional resilience and SOC dynamics under such conditions. We hypothesize that even a low level of grazing intensity could influence the soil-plant system by affecting the plant compartment and the microbial activity, thereby altering SOC distribution and stability.

2. Materials and methods

2.1. Site selection and sampling

The study was carried out in South Tyrol (Central Alps, Italy). Ten extensive grasslands, showing different management regimes (i.e., 5 meadows and 5 pastures) (Fig. S1), were selected from the pool of grasslands monitored within the framework of the project “Biodiversity Monitoring South Tyrol” (Hilpold et al., 2023). Each grassland type was equally distributed across the elevational gradient, which ranged from 732 to 1548 m a.s.l. (Table 1). The mean annual temperature and precipitation were in the range 6.0–10.5 °C and 630–789 mm, respectively.

From each site, topsoil (0–15 cm) samples were collected in June 2023 in triplicate, and then mixed in a final sample. Bulk density (BD) was measured using steel soil sampling rings (Eijkelkamp, Netherlands) inserted perpendicularly into the soil using an impact-absorbing rubber hammer. Additionally, leaf samples were collected to measure leaf functional traits for the most representative species, covering 80 % of the recorded species by the “Biodiversity Monitoring South Tyrol” (BMS, 2023) (Table S1). For each species, 20 to 30 leaves were collected from healthy individuals, targeting both sun-exposed leaves from the top of the plant and leaves from the base for variability. Diseased or damaged plants were excluded. Leaves were immediately placed between moist paper sheets in sealed plastic bags. Aboveground plant biomass was also collected by clipping the vegetation above the soil surface in a sample plot of 30 × 50 cm. Once collected, all samples were kept at 4 °C until arrival in the laboratory.

2.2. Soil sample preparation and analyses

Samples for biological analyses were stored at −80 °C, whereas samples for physical and chemical analyses were air-dried, gently crushed and passed through a 2-mm sieve.

Soil reaction (pH) was measured on suspensions of 1:2.5 sample: water ratio (Thomas, 1996), whereas electrical conductivity (EC) was tested on water extracts obtained at a sample-to-water ratio of 1:5 (Rhoades, 1996). BD was determined as the ratio between the bulk soil dry mass (at 105 °C for 24 h), corrected for the skeleton, and the sampling ring volume. Texture was determined using a laser diffraction particle size analyzer (Microtrac MRB Sync) provided with 3 red lasers and a Flowsync wet dispersion system equipped with ultrasound. The semi-quantitative mineralogical composition was determined on sample powders by X-ray diffraction (XRD) in the range 3–70° 2θ using a Philips

Table 1
Sites description. Soil texture, density (corrected for the skeleton), pH and EC are also reported, while main mineralogical phases are summarized in Table S2. The site location is also summarized in Fig. S1.

Land management	Site	Coordinates (Latitude, Longitude)	Elevation (m a.s.l.)	MAT (°C)	MAP (mm)	Sand (%)	Silt (%)	Clay (%)	Density (g/cm^3)	EC (μS cm^{-1})	pH
Meadow	Eggental/Valdega I	46.462233, 11.420063	732	10.5	683	67	31	2	0.82	49	7.4
Meadow	Truden/Trodena	46.338601, 11.357089	928	8.4	726	18	71	11	0.90	47	7.3
Meadow	Klobenstein/Collalbo	46.528022, 11.442162	1173	7.7	704	44	51	5	0.66	69	6.2
Meadow	Eggental/Valdega II	46.419562, 11.501205	1326	6.8	760	63	35	2	0.66	68	6.3
Meadow	Flaas/Valas	46.603585, 11.298000	1531	6.2	702	49	49	2	0.56	193	6.5
Pasture	Vintl/Vandoies	46.815303, 11.748955	769	10.5	676	62	36	2	0.68	162	7.2
Pasture	Verdings/Verdignes	46.653465, 11.571354	925	10.0	630	51	46	3	0.54	54	6.8
Pasture	Gaid/Gaido	46.5129056, 11.2059085	1068	8.2	638	49	46	5	0.69	224	6.9
Pasture	Latzfons/Chiusa	46.67485, 11.555955	1328	7.6	714	46	52	2	0.58	98	6.9
Pasture	Aldino/Aldein	46.370142, 11.387693	1548	6.0	789	34	62	4	0.53	174	6.5

X'Pert diffractometer (Bragg-Brentano geometry) with Cu Kα (40 kV, 45 mA) radiation and a scan rate of 0.02° 2θ s^{-1}. Available phosphorus (AP) was determined according to Olsen method (Olsen and Sommers, 1982). For each sample, an extraction lasting 30 min was performed using a solution of sodium bicarbonate followed by molybdenum blue colorimetry. Absorbance was read on a UV spectrophotometer at 882 nm.

2.3. Physical fractionation of SOM

SOM pools were isolated from all bulk soil samples using the physical fraction method proposed by Cambardella and Elliott (1992). Briefly, 10 g of sieved soil (2 mm) were shaken in a solution of sodium hexametaphosphate (5 g L^{-1}) for 18 h. After dispersion, the samples were passed through a vibratory sieve shaker (AS 200, Retsch, Germany) to achieve the size separation into POM (>53 μm) and MAOM (<53 μm). Obtained fractions were then oven-dried at 60 °C and ground with a zirconium ball mill (MM 400, Retsch, Germany) for further analysis. The recovery was 98.6 ± 0.9 %.

2.4. Organic C and total N analysis

Both bulk soil samples and corresponding SOM fractions were analysed for total concentration of C and N by flash combustion using an elemental analyzer (CHNS, Vario Macro Cube, Elementar, Germany). To determine the concentration of organic C (OC), acid (HCl) fumigation was carried out on each sample before analysis to remove carbonates (Harris et al., 2001).

SOC stock in the topsoil was determined following the following formula:

$$SOC\left(\mathrm{Mg\,C\,ha^{-1}}\right) = BD \cdot OC \cdot L \cdot 0.1$$

where *BD* is the bulk density, following skeleton correction (g cm^{-3}), *OC* is the organic C concentration (mg C g^{-1}), and *L* is sample depth (15 cm). The same formula was used for TN stock.

OC concentrations in POM and MAOM fractions are reported both in mg g_{soil}^{-1} and as % of total OC (TOC) content in bulk soil. The SOC recovery, calculated as the sum of the relative OC percentage in MAOM and POM compared to the TOC in bulk soil, was 98.2 ± 3.6 %.

2.5. Thermal analysis

The stability of SOM in POM and MAOM fractions was investigated by thermal analysis using a thermogravimetric analyzer coupled with differential scanning calorimetry (TGA-DSC 3+, Mettler Toledo, Switzerland). Approximately 20–30 mg of each sample was placed in an alumina crucible and heated from 30 to 700 °C at a constant rate of 10 °C min^{-1} under a synthetic air flow of 50 mL min^{-1}. SOM loss was determined by the weight loss (WL) measured between 105 and 550 °C (i.e., loss-of-ignition; LOI). The $WL_{450\text{-}550/250\text{-}350}$ was calculated as the ratio between WL occurring within higher (450–550 °C) versus lower (250–350 °C) temperature ranges. The energy density (E_{dens}) was calculated dividing the total exothermic energy content, determined integrating the area under the DSC curve between 105 and 550 °C, by the OC content (J mg OC^{-1}).

2.6. Enzymatic assays

Fluorescein diacetate hydrolase (FDAH) is generally used as a proxy of total microbial biomass activity (Adam and Duncan, 2001), while urease (Ure) and acid phosphomonoesterase (PME) enzyme activities were chosen because they are key indicators of N and P cycling, which are impacted by grazing through dung inputs and changes in plant biomass. Enzymatic activities were measured by spectrophotometric methods using fluorescein diacetate as substrate for FDAH (Green et al., 2006), urea as substrate for Ure activity (EC 3.5.1.5) (Kandeler, 1988), and 4-nitrophenyl phosphate bis(cyclohexylammonium) salt as a substrate for PME activity (EC 3.1.3.2) (Eivazi, 1977). An aliquot of soil (from 0.2 to 1.0 g, depending on the SOM content) was put in a centrifuge tube and, according to the specific assay, a substrate (fluorescein diacetate, urea or 4-nitrophenyl phosphate, respectively) and a buffer solution were added. The sample was then incubated in a water bath at 37 °C, for 3, 2 and 1 h for the determination of FDAH, Ure and PME, respectively. Following incubation, (i) acetone was added for the FDAH assay, and the absorbance at 490 nm was read in a UV–VIS spectrophotometer; (ii) a 1:1 Na-salicylate/NaOH and sodium dichloroisocyanurate 0.1 % solutions were added for the Ure assay, and the absorbance at 690 nm was read, (iii) $CaCl_2$ and NaOH 0.5 M solutions were added for PME determination, and the absorbance at 405 nm was read. Enzyme activities were reported as μmol of product developed in 1 h per g of OC.

2.7. Plant parameters determination

In the laboratory, leaves were rehydrated for 30 min and weighed to determine fresh weight. Leaf area was measured on three subsamples of

5 leaves each using ImageJ software (http://rsb.info.nih.gov/ij/). Measurements were taken within 48 h from the field sampling. Each leaf sample was oven-dried at 72 °C for 24 h and then weighed to determine dry weight. Both specific leaf area (SLA) and leaf dry matter content (LDMC) were determined according to Cornelissen et al. (2003). SLA is defined as the one-sided surface area of a fresh leaf per unit dry mass (mm^2 mg^{-1}), while LDMC quantifies the ratio of a leaf dry mass to its water-saturated fresh mass (mg g^{-1}). Aboveground plant biomass was measured on sample plots by drying plant material at 70 °C for 72 h.

2.8. Statistics

Statistical analysis was performed using the R 4.2.1 software (R Core Team, 2022). One-way analysis of variance (ANOVA) was used to test the effects of the two land-use on the response variables, followed by Tukey's post-hoc test ($\alpha = 0.05$). The identification of outliers has been carried out by means of Z-scores. Data normality was checked by the Shapiro-Wilk test and Q-Q plots, while the homogeneity of variance was checked by Levene's test. Correlations among parameters were assessed using Pearson's correlation coefficients ($p < 0.05$).

3. Results and discussions

3.1. Influence of land management on soil features

Soil physical and chemical characteristics are reported in Table 1. Soil texture ranges from sandy loam to silt loam (Table 1), thus having a different influence on microbial habitat, with finer textures typically promoting higher microbial biomass. Soils are mainly characterized by the presence of quartz (30–48 %), followed by feldspars (24–46 %) and amorphous material (16–25 %) (Table S2). The density ranges from 0.53 to 0.90 g cm^{-3}, the EC between 47 and 224 µS cm^{-1}, and the pH between 6.2 and 7.4.

Previous studies on the influence of livestock grazing on C and N cycles in soils showed often contrasting results, with SOC sometimes declining (Liu et al., 2015), increasing (Bai et al., 2012), or even being almost unaffected (Bagchi and Ritchie, 2010). However, livestock grazing appears to consistently suppress microbial biomass C and N (Wen et al., 2013). This is because grazing intensity alters the plant community, the microenvironment within the soil, and the diversity and activity of soil microorganisms (Stavi et al., 2008). In our study, most of the investigated parameters (i.e., TOC, SOC stock and TN) show significantly higher values in pastures than in meadows (Table 2). Light or moderate grazing not necessarily causes a decrease of SOM. Actually, Franzluebbers et al. (2000) found that long-term grazing was more beneficial for SOC storage than mowing. Lin et al. (2010) and Liu et al. (2015) reported that a low level of grazing intensity could act as a catalyst for both SOC and TN sequestration, while moderate and high levels of grazing intensity promote their loss. Zhou et al. (2017) showed that light grazing increased TOC and TN by 0.78 and 3.24 %, respectively, compared to the ungrazed site, while Bi et al. (2018) found TOC contents of 67 and 55 % and TN contents of 6.8 and 6 %, respectively, in grazed and ungrazed mountain meadows. In our study, TOC, TN and AP concentrations are higher (90, 65 and 30 %, respectively) in pastures compared to meadows. Thus, extensive grazing has the potential to significantly improve the pool of these elements. Zhou et al. (2017) showed that light grazing promotes greater inputs of photosynthetically fixed carbon to belowground roots, resulting in an increase of root exudates and biomass, as well as an alteration of root C/N (Bardgett et al., 1998). In addition, the AP increment found in pastures compared to meadows, although not statistically significant (Table 2), agrees with a previous study by He et al. (2020), who reported that grazing increased the soil P pool by 4 %. Thus, the observed increase in soil AP could be due to a higher rock weathering rate promoted by a lower plant cover, and the exposure of the soil to trampling (Delgado-Baquerizo et al., 2017), which may promote the release of P from mineral surfaces. Indeed, the magnitude of grazing effects on C/N/P stoichiometry depends on grazing intensity, vegetation type and environmental components (McSherry and Ritchie, 2013). Zhou et al. (2017) found that light grazing increased both C/N and C/P ratios. In our study, although these stoichiometric ratios do not show statistically significant differences between the two management systems, they are higher in pastures than in meadows (Table 2).

3.2. Plants parameters

Herbivores, depending on grazing intensities, can substantially alter the primary productivity of an ecosystem (De Mazancourt et al., 1998). Grazing reduces aboveground biomass, which in turn leads to decreased litter mass and quality (Liu et al., 2015), root elongation and biomass (Bagchi and Ritchie, 2010), and C allocation to roots (McSherry and Ritchie, 2013), while increasing the C/N ratio and the resistance to decomposition (Bardgett et al., 1998). Pastures typically show lower plant biomass compared to meadows; however, the lack of a statistically significant difference in our findings (Fig. 1A) could reflect the similarly extensive management regimes applied to both grassland types.

In this context, plant functional traits provide valuable insights into local ecological dynamics. Individual plant species can influence soil microbial community structure and activity, through alterations in the quantity and quality of inputs to the soil, particularly via litter and root exudates (Bardgett and Wardle, 2010; Harrison and Bardgett, 2010). Traits associated with the leaf economic spectrum are particularly relevant, as they reflect plant litter quality and have been linked to broader ecosystem processes (Freschet et al., 2010). Communities dominated by exploitative species, characterized by high SLA and low LDMC, tend to support faster nutrient cycling, whereas those dominated by conservative species (with opposite traits) exhibit slower turnover rates (Lavorel and Grigulis, 2012). In line with this, SLA is positively associated with plant biomass and is indicative of faster growth rates (Pontes et al., 2007). Our data further support this relationship, showing statistically higher SLA values in meadows compared to pastures (Fig. 1C). Liu et al. (2015) attributed most of the variability observed in grazing impacts on grasslands to defoliation through mechanisms such as decreased plant productivity, reduced soil respiration, and altered plant community composition. While dung and urine depositions represent additional C and N inputs to the soil, their potential positive effects may be offset by increased microbial activity and higher soil respiration (Lovell and Jarvis, 1996). Overall, these results underscore the relevance of the leaf economics spectrum not only for predicting aboveground biomass and litter decomposition, but also for better understand key soil processes (De Deyn et al., 2008).

Table 2
Bulk soil chemical characteristics. Data are expressed as mean ± SE. Superscript letters indicate significant differences within means in the column according to one-way ANOVA at $p \leq 0.05$ (Table S3) and Tukey's test. *TOC* = total organic C; *Stock OC* = organic C stock; *TN* = total N; *AP* = available P; *C/N* = ratio between organic C and total N; *C/P* = ratio between organic C and available P; *N/P* = ratio between total N and available P.

Land management	pH	TOC (mg g^{-1})	Stock OC (Mg ha^{-1})	TN (mg g^{-1})	AP (mg kg^{-1})	C/N	C/P	N/P
Meadow	6.7 ± 0.2^a	31.5 ± 2.9^a	34.5 ± 1.8^a	3.3 ± 0.4^a	3.8 ± 0.7^a	9.8 ± 0.5^a	9.6 ± 1.7^a	1.0 ± 0.2^a
Pasture	6.9 ± 0.1^a	60.7 ± 3.6^b	52.9 ± 3.4^b	5.5 ± 0.3^b	5.0 ± 0.8^a	11.1 ± 0.5^a	15.7 ± 2.1^a	1.4 ± 0.3^a

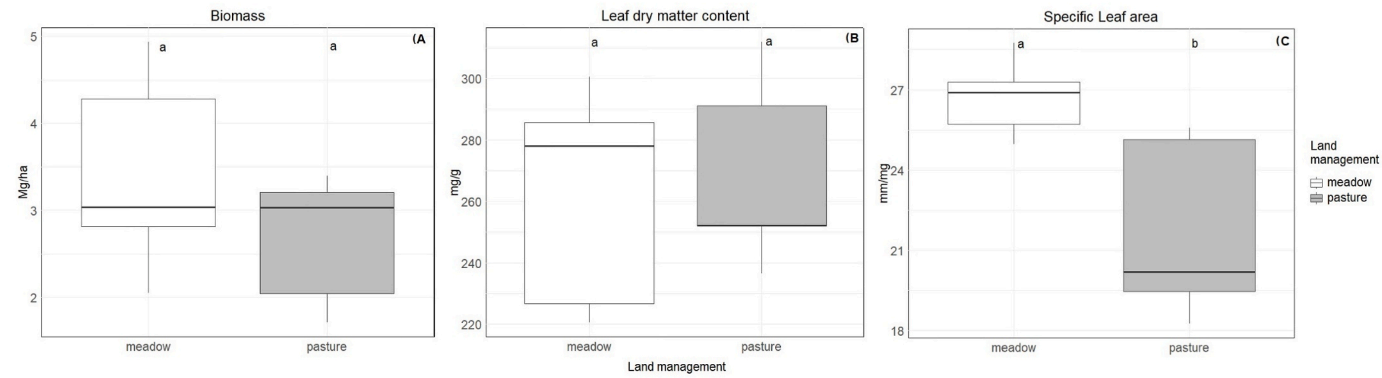

Fig. 1. Plant biomass (A), leaf dry matter content (B) and specific leaf area (C) in the two different management systems. Superscript letters indicate significant differences according to one-way ANOVA at $p \leq 0.05$ (Table S3) and Tukey's test.

3.3. Soil enzyme activity

The enzyme activity is a sensitive indicator of ecosystem functioning mirroring the ongoing changes within soil and in biogeochemical cycles. In our study, enzymatic activities show different trends depending on land management (Fig. 2) and were expected to be higher in sites with higher nutrient concentrations, i.e. pastures (Table 1). Previous studies showed that grazed soils were commonly characterized by a higher urease activity and a lower dehydrogenase (considered a total biomass activity proxy as FDAH), as well as by acid and alkaline phosphatases activity, than the ungrazed soils (Mencel et al., 2022). Consistently, in our study urease shows significantly higher activity in pastures than in meadows, whereas FDAH and PME show significantly higher values in meadows (Fig. 2). Thus, the higher urease activity in pastures reflects increased N inputs from dung and urine, which may enhance microbial N cycling, while the higher FDAH and PME activities in meadows suggest that lower grazing intensity supports more diverse microbial communities and greater nutrient cycling. Cui and Holden (2015), investigating the effect of grassland stocking rate on the soil enzymatic activity, found a positive correlation between animal density and urease activity, as larger N inputs were supplied to the soil. Chmolowska et al. (2017), comparing enzyme activity in wastelands and extensively managed meadows (mown once a year and extensively grazed in the late summer), however, revealed a pattern not in line with our results. Despite infrequent mowing in the meadows, this practice nevertheless led to an increase in the overall enzyme activities. Furthermore, specific enzymes, including acid and alkaline phosphatases, showed a positive response to both mowing and light grazing.

Changes in litter and root biomass due to grazing, linked to a decline in soil bacterial community diversity (Knops et al., 2002), have an impact on the total microbial biomass and SOC pool (Danise et al., 2022). This could explain the higher total microbial activity, as expressed by FDAH, found in meadows. Furthermore, trampling can alter soil microbial community structure due to its effects on soil physical properties, i.e., decreasing porosity (Hiltbrunner et al., 2012), and, thus, oxygen concentration and diffusion (Cid-Rodríguez et al., 2024). Trampling alone can induce a shift in plant resource allocation, favouring belowground tissues. However, when combined with defoliation, trampling can have a contrasting effect; this combined disturbance further reduces the abundance of soil microbes, potentially leading to a slower soil C cycle and a longer retention time for SOC (Liu et al., 2015).

3.4. OC and TN distribution between SOM pools

Pasture generally shows higher values of TN and OC in both SOM fractions (i.e., MAOM and POM) compared to meadow (Table 3), thus confirming the trend observed in the corresponding bulk soils (Table 2). Differences between the two management systems, despite not statistically significant, are found mainly in the POM fraction where both OC and TN are higher in pasture than in meadow. POM primarily forms from plant structural components and turns over a period of time ranging from years to decades (Lavallee et al., 2020). Despite the chemical complexity of organic inputs does not seem to directly influence their long-term persistence (Kleber and Johnson, 2010), their quality is likely to have an impact on POM-C storage (Huys et al., 2022). Some studies suggested that a temporary increase in POM accumulation at higher input rates (e.g., lignin-rich plant material) leads to a higher C/N ratio in POM compared to MAOM (Cotrufo et al., 2019). This is confirmed also in our study (Table 3), while the slight differences

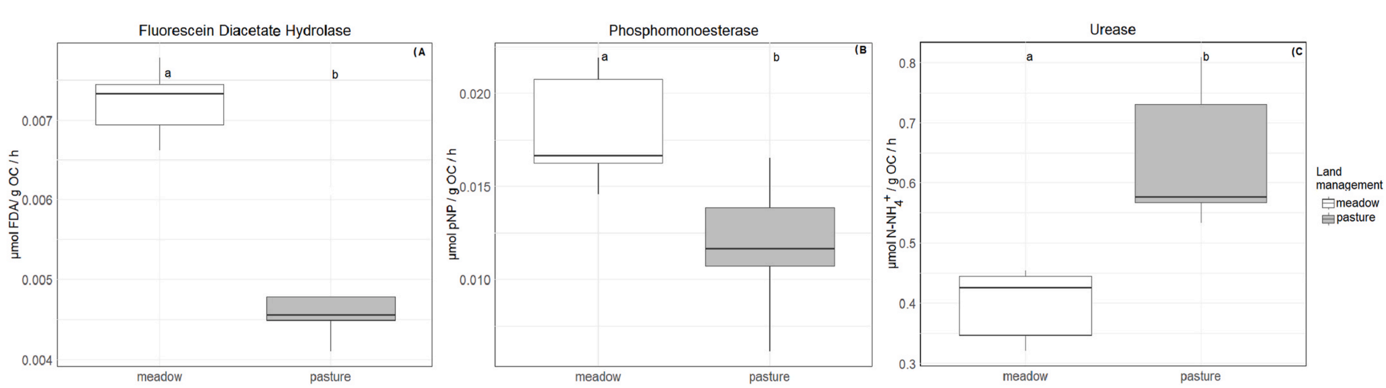

Fig. 2. Fluorescein Diacetate Hydrolase (A), Phosphomonoesterase (B) and Urease (C) enzyme activity in the two different management systems. Superscript letters indicate significant differences according to one-way ANOVA at $p \leq 0.05$ (Table S3) and Tukey's test.

Table 3
Chemical composition and thermal stability of mineral-associated (MAOM) and particulate (POM) organic matter. Data are expressed as mean ± SE. Superscript letters indicate significant differences within means in the column according to one-way ANOVA at $p \leq 0.05$ and Tukey's test. Capital letters indicate differences within land management (Table S4) while lower case letters indicate difference between land management (Table S3). *OC* = organic C; *TN* = total N; *C/N* = ratio between organic C and total N; $WL_{450\text{-}550/250\text{-}350}$ = ratio between weight losses at 450–550 °C and at 250–350 °C; E_{dens} = energy density.

	Land management	OC (mg g_{soil}^{-1})	OC (% TOC)	TN (mg g_{soil}^{-1})	C/N	$WL_{450\text{-}550/250\text{-}350}$	E_{dens} (J mg^{-1} OC)
MAOM	Meadow	21.1 ± 3.0 ^{aA}	57.8 ± 4.4 ^{aA}	2.1 ± 0.2 ^{aA}	9.7 ± 1.1 ^{aA}	0.44 ± 0.02 ^{aA}	22.8 ± 1.1 ^{aA}
	Pasture	28.2 ± 5.0 ^{aA}	53.1 ± 2.9 ^{aA}	2.8 ± 0.5 ^{aA}	10.2 ± 0.8 ^{aA}	0.31 ± 0.03 ^{bA}	25.2 ± 1.6 ^{aA}
POM	Meadow	15.4 ± 4.7 ^{aA}	38.7 ± 4.5 ^{aB}	1.1 ± 0.4 ^{aB}	13.9 ± 1.0 ^{aB}	1.51 ± 0.40 ^{aB}	27.6 ± 2.3 ^{aA}
	Pasture	24.9 ± 4.8 ^{aA}	46.2 ± 3.5 ^{aA}	2.0 ± 0.4 ^{abA}	12.7 ± 0.8 ^{aA}	2.33 ± 0.40 ^{aB}	25.3 ± 0.6 ^{aA}

observed between the two management systems probably reflect the diverse litter quality as indicated by plant traits (Fig. 1). Nevertheless, the C/N ratio of POM found in our study (Table 3) is generally lower respect to those reported by Rocci et al. (2022) (16.7 ± 0.8) for grazing lands.

In contrast, analysing data within land management, both meadow and pasture are characterized by higher values of TN and OC concentrations and OC proportion (as %TOC) in the MAOM fraction (Table 3); with the exception of OC concentration (mg g_{soil}^{-1}), these differences are statistically significant in meadows (Table 3; Table S4). Yu et al. (2022) showed that most grassland/shrubland soils stored more SOC in MAOM than in POM - although the MAOM/POM ratio changes with soil depth and age, and climatic conditions (Galluzzi et al., 2025) -, while Lugato et al. (2021) reported that SOC in grazing lands consists of 70–72 % MAOM and 28–30 % POM. The lower MAOM relative content found in our study (<60 %; Table 3) could be due to the extensive management regime; in fact, according to Six et al. (2004), in extensively managed systems, the resulting SOM is generally consisting mainly of occluded POM. In detail, in the present work, the OC in MAOM (as a %TOC) is 1.56 ± 0.33 (mean ± SE) times greater than in POM in meadow soils, and 1.18 ± 0.10 times greater in pasture soils; however, such a trend is not always statistically significant (Table S3). MAOM formation follows a distinct pathway compared to POM, being mainly composed of microbial necromass and dissolved organic matter which undergo chemical bonding with soil minerals, mainly those present in the silt and clay fractions (Kleber et al., 2015; Zaccone et al., 2018a). Thus, microbial activity is especially critical for MAOM formation in grazing lands, where microbial necromass contributes over 60 % of the SOC (Liang et al., 2019). Plant productivity and litter C/N ratio significantly impact MAOM formation (Hansen et al., 2024), as well as soil mineral properties (King et al., 2023), and microbial transformations (Kallenbach et al., 2016), especially in grazing areas (Stanley et al., 2024). Increased inputs of high-quality plant litter, characterized by a lower C/N ratio and more soluble components, can enhance MAOM accumulation. The C/N ratio is lower in MAOM than in POM in both management systems, but statistically significant differences are observed only in meadows (Table 3). On a global scale, grazing lands exhibit an average MAOM C/N ratio around 12.1 ± 0.6 (Rocci et al., 2022), which is slightly higher compared to our findings (Table 3).

Factors that increase N availability for microbes can potentially lead to greater MAOM accumulation. Dung inputs by grazing animals directly contribute to MAOM formation by promoting the adsorption of dissolved organic matter on mineral surfaces (Brewer et al., 2023). Thus, analysing the pattern of data within management systems (Table 3; Figs. 3 and 4), it is possible to hypothesize that, despite the differences found in plant traits (Fig. 1) and soil microbial activity (Fig. 2), both management systems have the same SOM distribution pattern, but probably a different synthesis pathway. In fact, the inverse correlation between MAOM TN and FDAH found in pasture (Fig. 4) suggests that, in this management system, MAOM is not exclusively of microbial origin but also influenced by the plant component (Whalen et al., 2022). Literature on MAOM formation often overlooks the direct plant contribution, although the lysis of plant cells could directly introduce proteins, peptides, and amino acids into the mineral soil (e.g., RuBisCo; Kögel-Knabner, 2002). Furthermore, Rumpel et al. (2015a) and Sanaullah et al. (2011) suggested that structural litter can also contribute to MAOM, particularly when decomposing near mineral surfaces. This is in line with the high adsorption affinity of proteins, amino acids and nucleic acids (Kleber et al., 2007; Zaccone et al., 2018a), suggesting that their release from decomposing plant litter could directly contribute to MAOM formation if their adsorption on mineral surfaces outcompetes microbial uptake (Dippold et al., 2014). On the other hand, the positive correlation found between FDAH and MAOM E_{dens} in pasture (Fig. 4) implies that a higher microbial activity promotes higher energetic MAOM compounds.

3.5. SOM pools stability

Thermal indices (E_{dens}, $WL_{450\text{-}550/250\text{-}350}$) were used as proxy of SOM stability in both pools (Table 3). The $WL_{450\text{-}550/250\text{-}350}$ values highlight that the two fractions show a statistically different SOM stability both within and between management systems. In particular, (i) within land management, POM shows a higher thermal stability (recalcitrance) than MAOM, while (ii) between management systems, meadows show a higher MAOM stability and pastures a greater POM stability. Both pasture and meadow results confirm the "decoupled" hypothesis (Yu et al., 2022) that infer that POM should present generally higher C/N ratios than MAOM, as the C/N of POM and MAOM should largely correspond to the C/N of plant litter and microbial necromass, respectively. Thus, the higher thermal stability of POM (higher $WL_{450\text{-}550/250\text{-}350}$) in both management systems (Table 3) generally reflects the occurrence of more aromatic, biologically recalcitrant compounds (e.g., lignin; Giannetta et al., 2018; Zaccone et al., 2018b); this is true especially in pastures, where ecophysiological responses (e.g., plant productivity and plant litter C/N ratio, and microbial transformations) seem to induce the formation of more stable POM fraction than in meadows (Stanley et al., 2024), which could potentially contribute to long-term C sequestration.

The E_{dens} show no significant differences either between pasture and meadow or between POM and MAOM (Table 3). At the same time, a negative correlation is observed between E_{dens} and both OC and TN in the POM fraction from meadows (Fig. 3), characterized by a higher MAOM stability (Table 3), as well as between E_{dens} and both OC and TN in the MAOM fraction from pastures (Fig. 4), where a higher POM stability is observed (Table 3).

Moreover, in meadows a positive correlation between SLA and MAOM E_{dens} is found (Fig. 3). This may suggest that, also in meadows, MAOM could have a significant contribution of plant material; in fact, a litter consisting of higher SLA inputs may originate more bioavailable and, therefore, more energy-rich compounds which in turn result in a greater microbial activity and thus MAOM formation.

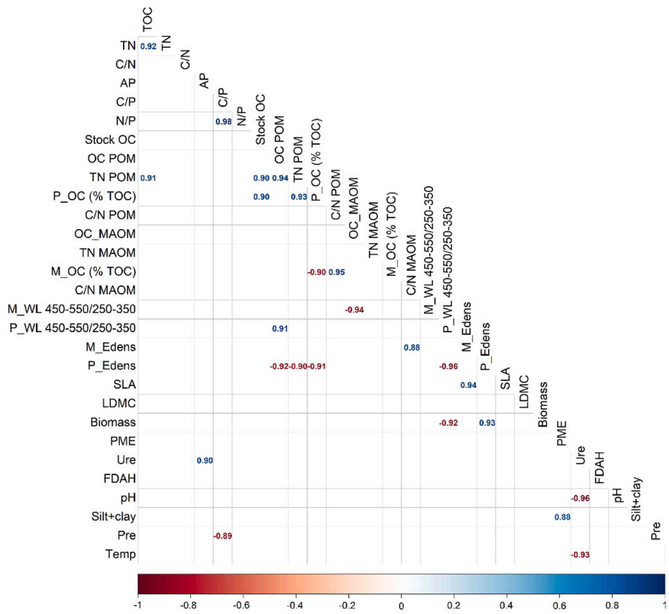

Fig. 3. Pearson's correlations in meadow (only significant correlations are reported; $p \leq 0.05$). *TOC* = total organic C; *TN* = total N; *C/N* = ratio between organic C and total N; *AP* = available P; *C/P* = ratio between organic C and available P; *N/P* = ratio between total N and available P; *Stock OC* = organic C stock; *OC POM* = organic C in POM; *TN POM* = total N in POM; *P_OC_POM* = organic C in POM (as %TOC); *C/N POM* = ratio between organic C and total N in POM; *OC MAOM* = organic C in MAOM; *TN MAOM* = total N in MAOM; *M_OC_MAOM* = organic C in MAOM (as %TOC); *C/N MAOM* = ratio between organic C and total N in MAOM; *M_WL450-550/250–350* = ratio between weight losses at 450–550 °C and at 250–350 °C in MAOM; *P_WL450-550/250–350* = ratio between weight losses at 450–550 °C and at 250–350 °C in POM; *M_Edens* = energy density in MAOM; *P_Edens* = energy density in POM; *SLA* = specific leaf area; *LDMC* = leaf dry matter content; *Biomass* = aboveground plant biomass; *PME* = acid phosphomonoesterase; *Ure* = urease; *FDAH* = fluorescein diacetate hydrolase; *pH* = soil reaction; *Silt + clay* = fine texture; *Pre* = mean annual precipitations; *Temp* = mean annual temperatures.

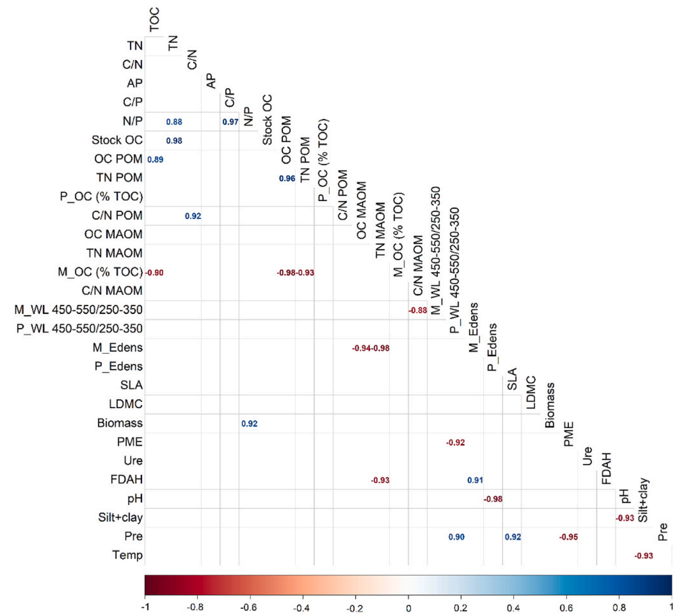

Fig. 4. Pearson's correlations in pasture (only significant correlations are reported; $p \leq 0.05$). *TOC* = total organic C; *TN* = total N; *C/N* = ratio between organic C and total N; *AP* = available P; *C/P* = ratio between organic C and available P; *N/P* = ratio between total N and available P; *Stock OC* = organic C stock; *OC POM* = organic C in POM; *TN POM* = total N in POM; *P_OC_POM* = organic C in POM (as %TOC); *C/N POM* = ratio between organic C and total N in POM; *OC MAOM* = organic C in MAOM; *TN MAOM* = total N in MAOM; *M_OC_MAOM* = organic C in MAOM (as %TOC); *C/N MAOM* = ratio between organic C and total N in MAOM; *M_WL450-550/250–350* = ratio between weight losses at 450–550 °C and at 250–350 °C in MAOM; *P_WL450-550/250–350* = ratio between weight losses at 450–550 °C and at 250–350 °C in POM; *M_Edens* = energy density in MAOM; *P_Edens* = energy density in POM; *SLA* = specific leaf area; *LDMC* = leaf dry matter content; *Biomass* = aboveground plant biomass; *PME* = acid phosphomonoesterase; *Ure* = urease; *FDAH* = fluorescein diacetate hydrolase; *pH* = soil reaction; *Silt + clay* = fine texture; *Pre* = mean annual precipitations; *Temp* = mean annual temperatures.

3.6. *Grazing-induced plant-soil interaction*

Plant and soil compartments are tightly linked in grassland ecosystems (McSherry and Ritchie, 2013). Among the various influencing factors, grazing intensity is likely the most critical one affecting this ecosystem, as it causes alterations in plant community structure, soil microenvironment, and soil microbial diversity (Dainese et al., 2015; Sebastià et al., 2008). Grazing practices affect several key processes within the root-microorganism-soil system, including plant C sequestration, root exudation, and microbial activity, as well as several biogeochemical cycles (C, N, P) (Steffens et al., 2009; Teague et al., 2011; Zhou et al., 2007). Our results indicate that pastures show slightly higher AP content and lower aboveground plant biomass values than meadows. Craine and Jackson (2010), investigating 98 North American grassland soils, showed that plants growing in environments with a higher P than N availability were primarily N-limited. Because N is required to produce phosphatases, key enzymes to increase P uptake, plant responses to P availability are often indirectly driven by N availability. This mechanism may explain why N inputs can enhance P acquisition, suggesting that grasslands may respond to increased availability in the soil of both elements (He et al., 2020). However, the long-term ecological implications of increased P availability on mountain grassland productivity and community composition remain an open question.

Understanding how grazing intensity influences soil C/N/P stoichiometry is therefore crucial for developing sustainable grassland management practices. De Deyn et al. (2009) demonstrated that changes in plant species diversity and functional group richness influence C and N storage (or loss). He et al. (2011) found positive correlations between grazing intensity and root C/P and soil C/P under low and moderate grazing intensity. An increase in the root C/N ratio, possibly due to greater C translocation to roots than N induced by grazing (Ritchie et al., 1998), may represent a defence mechanism, whereby plants allocate more non-structural carbohydrates to belowground organs to compensate for aboveground biomass loss (Whigham and Simpson, 1978). A positive correlation between soil N/P ratio and plant biomass is observed in pasture (Fig. 4). This agrees with findings reported by Bai et al. (2012) and suggests that an increase in biomass, which implies a greater P demand, could lead to a greater P uptake resulting in a reduction of AP content in soil (higher N/P ratio).

A negative relationship between POM stability ($WL_{450\text{-}550/250\text{-}350}$) and plant biomass is observed in meadows (Fig. 3), suggesting a greater nutrient bioavailability for plant growth due to a more labile litter. The higher SLA values found in meadows (Fig. 1) reflect the dominance of nutrient-demanding, fast-growing plant species, which promote faster nutrient cycling (and SOM turnover) and higher microbial activity (higher FDAH), and result in a litter characterised by more readily available material for decomposition (POM showing lower $WL_{450\text{-}550/250\text{-}350}$ values). In pasture, an opposite trend is observed: plant species with higher LDMC values (Fig. 1), indicative of slower growth rates and greater structural investment, are associated with lower microbial activity (FDAH) (Fig. 2), thus suggesting a SOM accumulation (Table 1) mainly in the form of more stable POM (higher $WL_{450\text{-}550/250\text{-}350}$ values). The negative relationship between this thermal proxy and phosphatase

(Fig. 4) further supports the hypothesis that pasture-derived POM is more recalcitrant to decomposition, contributing to the observed lower microbial activity.

3.7. Future perspective

Although climate change is expected to affect several ecosystem functions, including C accrual and distribution in soil as well as plant and microbial biodiversity (Dainese et al., 2024; Díaz-Martínez et al., 2024; Galluzzi et al., 2024; Xu et al., 2013), our results did not reveal statistically significant differences along the elevational/temperature gradient. This finding contrasts with previous studies reporting that the warming treatment significantly affected soil bacterial community structure and chemical characteristics (e.g., Ma et al., 2018b), while other studies showed no significantly changes under similar experimental conditions (e.g., Zi et al., 2018). We hypothesize that, in our study, the effects of land management may have masked potential climate-related impacts. Therefore, further research is needed to disentangle these interacting drivers and assess the resilience of mountain grasslands to climate change more explicitly.

4. Conclusions

This study demonstrates that even low-intensity grazing can affect the soil-plant system in extensively managed mountain grasslands. TOC, TN and AP concentrations were consistently higher in pastures compared to meadows, while soil enzymatic activities showed divergent responses based on land management. In fact, urease activity was higher in pastures than in meadows, likely due to dung inputs, whereas FDAH and PME, both indicator of microbial activity, were higher in meadows, which also exhibited greater SLA values. Total N and OC was higher in MAOM and (particularly) POM from pasture soils. Regardless of land management, the MAOM fraction consistently showed higher TN and OC concentrations and OC proportion (as %TOC) than POM, with these differences particularly significant in meadows. Correlations found between MAOM characteristics and plant traits/soil enzymatic activities suggest that MAOM, in both pasture and meadow, is not exclusively of microbial origin but also influenced by the plant component. Furthermore, our findings underscore different patterns of SOM stability both within and between management systems. Within land management, POM showed a higher thermal stability (recalcitrance) than MAOM, while between management systems, meadows showed a higher MAOM stability and pastures showed a greater POM stability, likely associated with the prevalence of conservative plant species characterized by high LDMC. These results underscore the pivotal role of grassland management in enhancing SOC sequestration to mitigate climate change. Moreover, given the different susceptibility of MAOM and POM to decomposition, resulting in distinct mean residence times, targeted grazing practices could be instrumental to enhance SOM stability and nutrient cycling. Ultimately, this highlights the need for management strategies that balance productivity with long-term soil health and climate mitigation goals.

CRediT authorship contribution statement

Tiziana Danise: Writing – original draft, Investigation, Formal analysis, Data curation. **Sara E. Goldoni:** Writing – review & editing, Formal analysis, Data curation. **Matteo Dainese:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Claudio Zaccone:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2025.125846.

Data availability

Data will be made available on request.

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