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Semi-wild horse grazing as a rewilding strategy: assessing effects on vegetation structure and composition in the Côa Valley, Portugal

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Mediterranean landscapes are characterized by fine-grained land-cover mosaics of interspersed vegetation types and high wildfire vulnerability, where grazing plays a key role in regulating vegetation structure and composition. This study explores the early effects, over a three-year period, of a transition from extensive commercial cattle grazing to semi-wild horse grazing in two rewilding areas in the Côa Valley region, Portugal. Using grazing exclusion areas as control, we test whether the less intensive regime of semi-wild horse grazing can be used to manage vegetation structure and composition, to mitigate local fire hazard and promote biodiversity. The monitoring scheme followed a paired design, where each survey site of 40 m \times 40 m comprises four sampling plots of 10 m \times 10 m, including two fenced plots (grazing exclusion) and two plots open to grazing. Effects on vegetation structure were assessed considering grass height, shrub height, shrub cover and aboveground biomass, as well as effects on plant species richness, turnover, and forbs-to-grasses ratio (F:G ratio) and the community-level importance of grasses and forbs. Results showed that grass height had a greater increase in ungrazed plots, suggesting that semi-wild horse grazing helps limit grass height. There were no significant differences in shrub metrics between treatments (i.e. horse grazing vs. no grazing), indicating that horse grazing did not effectively control woody vegetation. While species richness remained stable, species temporal turnover was higher in ungrazed plots. Additionally, the F:G ratio and the importance value of forbs were higher under horse grazing, suggesting potential benefits for anthophilous insects. These findings indicate that semi-wild horse grazing contributes to maintaining open habitats by controlling grass dominance, thereby reducing local fire hazard and potentially fostering habitat and food resources for insects. While this demonstrates the potential of using semi-wild horse grazing in rewilding, the results also suggest that horses alone, particularly at low densities, have limited impact on woody vegetation structure.

KEYWORDS

ecosystem management, rewilding, natural grazing, semi-wild horses, vegetation structure, plant community

1 Introduction

Large herbivores contribute to key ecological functions, such as nutrient cycling, seed dispersal, and the regulation of vegetation structure and composition (Mouissie, 2004; Pringle et al., 2023). Such functions result from their use of space and their feeding behavior, which includes both consumption and trampling (hereafter collectively referred to as grazing). These effects are essential for maintaining habitat and species diversity and hold significant potential for application in ecosystem management and restoration (Osem et al., 2007; Silva et al., 2019; Török et al., 2024).

In Mediterranean landscapes, characterized by fine-grained land-cover mosaics of interspersed vegetation types and high wildfire vulnerability, grazing plays an important role in regulating vegetation quantity, spatial distribution, and successional dynamics (Casasús et al., 2007; Riedel et al., 2013), ultimately influencing the landscape's fire resilience (Kirkland et al., 2024; Lecomte et al., 2024; Lovreglio et al., 2024). For these reasons, grazing has been recognized as a valuable management tool for maintaining open habitats, preventing shrub encroachment, and controlling the spread of invasive plants (Souther et al., 2019; Oikonomou et al., 2023). However, the use of grazing for the purpose of ecosystem management may also involve trade-offs (Ribeiro et al., 2023). Specifically, while higher grazing intensities may be required to effectively regulate vegetation quantity and its vertical and horizontal distribution (Ribeiro et al., 2023, 2024), they can also lead to soil degradation, loss of vegetation cover (Schoenbaum et al., 2018; Lai and Kumar, 2020), and affect the natural regeneration of sensitive plant species, leading to reduced plant diversity (Jáuregui et al., 2009; Saiz and Alados, 2012; Calleja et al., 2019). Furthermore, the risk of trade-offs can be exacerbated in areas of low fertility and degraded soils, where overgrazing, even under extensive grazing regimes, can pose significant risks if stocking densities exceed the system's carrying capacity. This is particularly concerning in Mediterranean ecosystems, which are characterized by shallow, nutrient-poor soils and high erosion potential (Van-camp et al., 2004; Teixeira et al., 2015). In such systems, these processes can limit vegetation productivity, triggering cascading effects on ecosystem functioning and the composition of ecological communities (Rogers et al., 2021; Psyllos et al., 2022). Consequently, unsustainable grazing practices risk pushing already degraded ecosystems beyond their resilience thresholds (van den Elsen et al., 2020; Zeng et al., 2021). This underscores the need for balanced grazing regimes that ensure the delivery of key ecosystem services, such as regulating biomass distribution and load to mitigate fire hazard, while also accounting for and minimizing the impacts on biodiversity (Maes et al., 2012; Torralba et al., 2016; Teague and Kreuter, 2020).

From a biodiversity and ecosystem management perspective, rewilding can be a viable strategy to address ecosystem degradation. Defined as an ecological restoration approach, rewilding aims to reestablish natural processes and dynamics in degraded landscapes, to ensure the maintenance of self-regulating and resilient ecosystems (Apollonio et al., 2017; Shackelford and McDougall, 2023; Mutillod et al., 2024). A key aspect of rewilding is the enhancement of ecological processes, such as trophic interactions, natural disturbance regimes, and species dispersal (Pereira and Navarro, 2015; Perino et al., 2019). In Mediterranean landscapes, the use of large herbivores in rewilding strategies aims to restore stochastic disturbance regimes associated with herbivory processes (Pereira and Navarro, 2015; Johnson et al., 2018; Van Meerbeek et al., 2019). These processes include regulating vegetation structure and species composition, which help maintain open habitat patches and prevent the dominance of woody plants in grassland and savanna ecosystems (Honrado et al., 2017; Silva et al., 2019). These roles and functions are particularly relevant in contexts where wild herbivores are absent or at densities too low to sustain ecological functions, or where ecological constraints cannot support economically viable livestock farming. Thus, by implementing more natural grazing regimes, rewilding approaches aim to reconcile landscapes' resilience to disturbances with the restoration of ecological processes and biodiversity conservation (Carver et al., 2021; Massenberg et al., 2023).

Here, we evaluate the early effects of a rewilding approach on vegetation structure and composition in two rewilding areas, Vale Carapito (Site 1) and Ermo das Águias (Site 2) in the Côa Valley, Portugal (Figure 1). This region, characterized by low population density and high biodiversity, has been selected by Rewilding Portugal (a conservation initiative focused on ecological restoration and biodiversity conservation in Portugal) to establish core rewilding areas, as part of a vision to create a 120,000 hectare wildlife corridor. The main management goal for these rewilding sites is to restore herbivory related ecological processes by reintroducing semi-wild herbivore populations, which will enhance habitat heterogeneity and biodiversity, as well as mitigate fire hazard by managing vegetation. The ultimate goal of Rewilding Portugal is to promote the development of self-sustaining ecosystems. Specifically, this study evaluates the effects of semiwild horse grazing (hereafter referred to as horse grazing) on vegetation, under a low-intensity "naturalistic" grazing regime that minimizes human intervention (Seddon et al., 2014), following three years after the transition from extensive commercial cattle grazing, as a first step of a long-term rewilding strategy. Additionally, no grazing areas (i.e., fenced areas) were established as control plots to assess the effect of grazing exclusion on vegetation. We aim to compare how vegetation structure and composition metrics evolve in both treatments (horse grazing vs. no grazing) and assess the potential of horse grazing to regulate vegetation, for mitigation of local fire hazard, while considering its impact on biodiversity. While cattle grazing may contribute to functions such as biomass regulation and reduction of fire hazard, overgrazing and negative impacts on biodiversity and soil can occur if stocking densities exceed the system's carrying capacity (Ribeiro et al., 2023). Particularly, the feeding of purchased crop forages to cattle in the two study sites may reflect the need to meet livestock's nutritional demands, indicating a mismatch between the available natural resources and livestock requirements.

We hypothesize that, over time, semi-wild horse grazing will exert an observable effect on vegetation structure, contributing to its regulation compared to grazing exclusion (H1: Hypothesis 1). This



FIGURE 1

Location of (a) case study region in Portugal, (b) study sites in the Côa Valley region. Grazing treatments and spatial arrangement of sampling plots are shown for (c) Site 1 and (d) Site 2. Each yellow circle represents a 10×10 m sampling plot: full circles represent ungrazed (fenced) plots and open circles represent horse grazed (open) plots.

effect is expected as horse grazing maintains some level of disturbance and influences vegetation through selective foraging and trampling (de Villalobos and Zalba, 2010; Rosa García et al., 2013; Dvorský et al., 2022; Molle et al., 2022). Regarding vegetation composition, we expect a decline in species richness, in the absence of grazing disturbance, in ungrazed areas (H2: Hypothesis 2) (Henning et al., 2017a; Dvorský et al., 2022; Bonavent et al., 2023). We also expect the composition of the plant communities to shift under the two grazing treatments, with increased temporal turnover in ungrazed areas, due to the suppression of grazing disturbance, when compared to horse grazing areas (H3: Hypothesis 3). Finally, we expect a shift in the relative representation of forb and grasses at the community level, driven by differences in grazing pressure (H4: Hypothesis 4). In ungrazed areas, the absence of herbivory is expected to promote grass dominance and impact the conditions for forb germination and growth (Bonavent et al., 2023). In contrast, horse grazing is expected to support a higher relative representation of forbs due to the maintenance of grazing but in a regime of lower grazing intensity (Garrido et al., 2019; Schmitz and Isselstein, 2020; Rakosy et al., 2022).

2 Materials and methods

2.1 Study area

Field experiments were conducted at two study sites - Vale Carapito (Site 1) and Ermo das Águias (Site 2) - in the Greater Côa Valley region in Portugal (Figure 1). This region has a Mediterranean climate, characterized by hot and dry summers, and cold winters. Both sites are characterized by woodlandshrubland mosaic habitats, with dense shrub patches dominated by Spanish broom (Cytisus multiflorus) and woodlands dominated by holm oak (Quercus rotundifolia) and Pyrenean oak (Quercus pyrenaica). Site 1 and Site 2 were grazed by small ruminants (sheep and goats) for the 40-60 years prior to being grazed by cattle in 2010 and 2005, respectively (Supplementary Table S1). The sites were then acquisitioned by Rewilding Portugal in 2020 and 2021, respectively. Site 1 experiences a wetter climate, with an average annual temperature of 12.7°C and precipitation of 795 mm (Supplementary Figure S1), and encompasses 65 hectares of grazing area. The grazing regime at Site 1 changed in 2021 from a baseline regime of 25 cattle under rotational grazing (2-3 months,

two times per year) to low intensity continuous grazing by Sorraia horses (10 horses introduced in April 2021 and later reduced to 5 to 6 horses in 2022 and onwards) (Supplementary Table S1). Site 2 has a milder and drier climate, with an average annual temperature of 13.3°C and precipitation of 544 mm (Supplementary Figure S1) and includes 330 hectares of grazing area, where the grazing regime transitioned from a baseline of 20 to 40 cattle (continuous grazing) to low intensity continuous grazing by 16 Sorraia horses in 2022 (Supplementary Table S1). The Sorraia horse is a native Portuguese breed adapted to living in wild or semi-wild conditions, making it well-suited for rewilding projects in landscapes that require high resilience. Its robustness allows it to endure harsh environmental conditions and survive on low-quality forage (Pinheiro et al., 2013).

2.2 Experimental design and survey scheme

Within each study area, six survey sites were selected prior to field surveys to cover representative habitats and dominant vegetation types. A paired design was implemented, where each survey site (approximately 40 m \times 40 m) contained four sampling plots, each measuring 100 m² (10 m \times 10 m) (Figure 1). Of these, two plots were fenced as control plots to exclude grazing, allowing vegetation to naturally regenerate, and two plots were open to grazing by semi-wild horses.

Baseline data, corresponding to the existing cattle grazing regime (Supplementary Table S1), were collected for all monitored variables (see next section) at Site 1 and Site 2 in 2021, before cattle removal and the introduction of horses. Subsequent monitoring of vegetation structure and aboveground biomass was conducted in 2023 and 2024, while plant composition was only reassessed in 2024.

Within each sampling plot (10 m \times 10 m), vegetation structure was assessed using four perpendicular 5-meter transects originating from the plot center, spaced at 90° angles or the maximum possible angles between them. Vegetation type (grasses, forbs, and shrubs) and height class (0-0.25 m; 0.25-0.50 m; 0.5-1.3 m; 1.3-2 m; 2-4 m; >4 m) was recorded at every meter along these transects. Plant community composition was surveyed in four 1 m \times 1 m quadrats, located at the end of each transect. Within each 1²m quadrat, the presence and percentage cover category of understory plant species were recorded using Braun-Blanquet's cover scale (Kent and Coker, 1994): + (<1%); 1 (1-10%); 2 (11-25%); 3 (26-50%); 4 (51-75%), 5 (76-100%). Plants were identified to the species level whenever possible, however, some taxa were only identified at the genus level for the purpose of data analysis, to harmonize species lists and enable comparison between years (Supplementary Tables S2, S3). Aboveground biomass for forbs and grasses was determined by harvesting all plant material within a 0.4 m × 0.4 m quadrat randomly placed within the sampling plot. Biomass sampling was conducted in one fenced (ungrazed) and one non-fenced (open to grazing) plot within each survey site (40 m × 40 m). Collected plant material was sorted into two functional groups (grasses and forbs), oven-dried at 60°C for 72 hours, and weighted to determine dry biomass.

Due to unforeseen land management changes in Site 1 during this study, only four of the initial six survey sites (40 m \times 40 m) remained under the planned treatments. Data analysis at Site 1 was therefore based on the remaining four survey sites.

2.3 Data processing and statistical analysis

Data collected at the survey sites (Figure 1) were used to characterize vegetation structure and composition, with data aggregated at the sampling plot level (10 m \times 10 m). Four vegetation structure metrics were evaluated: mean grass height (cm), mean shrub height (cm), shrub cover (%) and aboveground shrub biomass (t/ha), the last metric being computed using field data on shrub cover and height (Table 1). These metrics allowed the assessment of semi-wild horse grazing effects, or lack thereof, on vegetation structure in the two main vegetation layers affected by grazing, i.e., the herbaceous layer and the shrub layer, and can be used as an indicator of wildfire hazard (Table 1). Vegetation structure metrics were analyzed using linear mixed models (LMMs) with separate models fitted for each site. Grazing treatment (semi-wild horse grazing vs. no grazing), year, and their interaction were set as fixed factors, and the survey site (40 m \times 40 m) was specified as a random factor to account for the repeated measures over time. Assumptions of residuals' normality and homoscedasticity were graphically checked (Zuur et al., 2009). All variables satisfied the normality and homoscedasticity assumptions. LMMs were run with the *lme4* package (Bates et al., 2015).

Regarding plant community composition, three metrics were evaluated: species richness, the ratio of forbs to grasses based on biomass values, and the cumulative importance value (IV) of forbs and grasses based on species relative frequency and dominance in sampling plots (Table 1). Importance values were calculated at the species level (Supplementary Tables S2, S3) following Kent and Coker (1994), and then summed for forbs and grasses. Importance values were also used to identify the species with the highest IV in the baseline year and for each treatment in 2024. Forbs were defined as all species, observed at each site, excluding those from the families Poaceae, Juncaceae, Fagaceae, Dennstaedtiaceae, Aspleniaceae, and the genera Cytisus and Genista (Fabaceae). Additionally, species temporal turnover (baseline (2021) vs. last monitoring year (2024)) was assessed for each sampling plot using the codyn package (Hallett et al., 2016) to evaluate the overall compositional change in plant communities over time. Finally, the presence frequency of Stipa gigantea in 1m² quadrats was analyzed as an indicator of its dominance under each grazing treatment. This perennial grass is particularly relevant due to its fire-related traits, which contribute to fine fuel loads and fire hazard, including tall flower spikes, a dense and persistent tussock structure, and rapid post-fire regeneration (Prober et al., 2007; Cruz et al., 2017).

TABLE 1 Description of vegetation structure and composition metrics (adapted from Ribeiro et al., 2024).

Vegetation structure metric	Description
Mean grass height (cm)	Corresponds to the mean height of the highest grass in each of the point counts in the 10 m \times 10 m sampling- plots. Taller grasses are associated with higher wildfire hazard by acting as ladder fuel and facilitating contact between ground cover and the canopy (Menning and Stephens, 2007; Cardoso et al., 2022)
Mean shrub height (cm)	Corresponds to the mean height of the highest shrub in each of the point counts in the 10 m \times 10 m sampling- plots. Taller shrubs are associated with higher wildfire hazard by acting as ladder fuels and facilitating fire progression (Fernandes, 2009; Lovreglio et al., 2014)
Fractional shrub cover	Corresponds to the fraction of shrub cover in the 10 m \times 10 m sampling-plots. Represents the proportion of the ground covered by shrubs. Higher values of shrub cover are associated with higher levels of shrub encroachment and higher proneness to intense wildfires (Santana et al., 2018)
Aboveground shrub biomass (t/ha)	Corresponds to the amount of aboveground shrub biomass in the 10 m × 10 m sampling-plots. Used as an indicator of the understory structure, provides information about the available fuel load. Higher values of shrub biomass are associated with higher wildfire hazard (Kazanis et al., 2012). This metric was estimated using an allometric model (Enes et al., 2020) based on mean shrub height and fractional shrub cover (aboveground biomass = 0.0258 (%Shrub cover × Mean shrub height (cm)) ^{0.754})
Vegetation composition metric	
Species richness	Corresponds to the cumulative number of plant species observed in the 10 m \times 10 m sampling-plots. It is used as a measure of local plant richness (alfa diversity) (Herrero- Juregui and Oesterheld, 2018)
Forb to grasses ratio	Measured as an indicator of the food resources available for anthophilous (i.e. flower visiting) insects (Bonavent et al., 2023). Estimated from forb and grass biomass (F:G ratio = Forb biomass (g/m ²)/Grass biomass(g/m ²)). Higher values of the F:B ratio are associated with higher availability of flowering plants and food for insects (Norton et al., 2019; Cutter et al., 2022; Goosey et al., 2024)
Cumulative Importance Value (IV) of Grasses (or Forbs)	Corresponds to ecological importance of all grass (or forb) species within a plant community, based on species frequency (presence) and average cover in 10 m × 10 m sampling plots. The IV of each species is calculated as IV = Relative Frequency (Fr) + Relative Dominance (Dr) (Kent and Coker, 1994). Where Fr = [frequency of a species/total frequency of all species] × 100 and Dr = [dominance of a species/total dominance of all species] × 100. The cumulative IV is obtained by summing the individual IVs of all grass (or forb) species present in baseline or grazing treatment plot groups (see Supplementary Tables S2, S3 for more details).

Differences in species richness and temporal turnover were assessed through ANOVA and paired t-tests using the *stats* package (R Core Team, 2022). All analyses were conducted in R software (R Core Team, 2022).

3 Results

3.1 Vegetation structure

In Site 1, the values of shrub cover, shrub height and shrub biomass do not suggest any differences between the effects of the grazing treatment (i.e., horse grazing or no-grazing), or survey year (Figures 2a–c; p > 0.05). However, mean grass height showed a significant response to the interaction between grazing treatment and survey year (Figure 2d). More specifically, in 2023, mean grass height increased in the ungrazed plots compared to horse grazed plots (p = 0.047), with this difference becoming more pronounced in 2024 (p < 0.001).

In Site 2, shrub cover was significantly different between the plots allocated to the different treatments at the baseline year (Figure 3a; p = 0.014). While not ideal, this difference was likely caused by operational constraints during the fence installation in a challenging terrain. Shrub cover showed a marginal increase between 2024 and the baseline (p = 0.051), while significant year effects were observed for shrub height and biomass, with increases in 2023 (Figure 3b; p = 0.003 and Figure 3c; p = 0.024) and 2024 (Figures 3b, c; p < 0.001) relative to baseline. No significant year-bytreatment interactions or effects of grazing treatment alone were detected for shrub metrics. Mean grass height values (Figure 3d) showed significant changes related to the survey year, with an increase in 2024 (p = 0.002) relative to the baseline. The interaction between year and treatment was also significant in 2024 (p = 0.016), suggesting a more substantial increase in grass height in ungrazed plots during this year compared to horse grazed plots.

3.2 Vegetation composition

A total of 86 (2021) and 81 (2024) plant taxa were recorded across the four survey sites at Site 1 (Supplementary Table S2), and 130 (2021) and 129 (2024) across the six survey sites at Site 2 (Supplementary Table S3). Average richness values at the sampling plot scale were not statistically different across treatments at both Site 1 and Site 2 (Figure 4; p > 0.05).

In Site 1, the forb-to-grass (F:G) ratio increased under horse grazing by 2024 (Figure 5a; p = 0.009; Supplementary Figure S2). This ratio was also marginally influenced by the year × treatment interaction in 2024 (Figure 5a; p = 0.056). In contrast, the F:G ratio was not statistically different across years in ungrazed plots (Figure 5a; p > 0.05).

In Site 2 (Figure 5b), the F:G ratio showed a consistent increase over time, with an overall significant year effect observed in 2024 (p = 0.015). No significant year-by-treatment interactions or effects of grazing treatment were detected (Figure 5b; p > 0.05; Supplementary Figure S3).

The analysis of the cumulative importance values (IV) of grasses and forbs (Table 2) indicates similar patterns of community change at both Site 1 and Site 2. Namely, a more pronounced decline in the cumulative IV of grasses and a greater increase in that of forbs from



FIGURE 2

Shrub cover (a) Mean shrub height (b) Shrub biomass (c) and Mean grass height (d) at Site 1 under baseline conditions (i.e., rotational cattle grazing) in 2021 and in the following monitoring years, 2023 and 2024, under continuous horse grazing and no grazing. Solid line and circles represent horse grazed (natural grazing) plots, dashed line and triangles represent ungrazed plots. Solid bars represent means and standard errors. Significant *p*-values of the main effects in the linear mixed models are reported.

baseline to horse grazing conditions. In terms of dominant species (i.e., those with the highest IV), composition remained relatively stable across years and treatments at both sites (Table 2), although some notable shifts were observed. At Site 1, *Cytisus multiflorus* and *Stipa gigantea* increased their IVs, and *Avena barbata* (a tall grass species) emerged as a new dominant in ungrazed plots. At Site 2, *C. multiflorus* not only consistently exhibited the highest IV but also increased in dominance over time, with treatment differences appearing less influential.

Species turnover in Site 2 was significantly higher in the fenced plots, i.e., that shifted from the baseline to an ungrazed regime (Figure 6b; p = 0.045), whereas differences were not statistically significant at Site 1 (Figure 6a; p > 0.05).

The presence frequency of *S. gigantea* in 1 m^2 plots increased in the ungrazed areas at Site 1 (20 out of 32 plots in 2024 *vs.* 15 out of

32 in 2021) but remained stable in the horse grazed areas (13 out of 32 in 2024 *vs.* 12 out of 32 in 2021). In Site 2, the species was not recorded in any plot in 2021 but was observed in one fenced 10×10 m sampling plot in 2024.

4 Discussion

This study examined how vegetation structure and composition respond to changes in grazing management under a rewilding approach, focusing on the effects of semi-wild horse grazing and using grazing exclusion as a control treatment. Specifically, we aimed to evaluate the effectiveness of horse grazing in reducing local fire hazard through its effects on vegetation structure while promoting positive impacts on



Shrub cover (a) Mean shrub height (b) Shrub biomass (c) and Mean grass height (d) at Site 2 under baseline conditions (i.e., continuous cattle grazing) in 2021 and in the following monitoring years, 2023 and 2024, under continuous horse grazing and no grazing. Solid line and circles represent horse grazed (natural grazing) plots, dashed line and triangles represent ungrazed plots. Solid bars represent means and standard errors. *p*-values of the main effects in the linear mixed models are reported.

biodiversity, particularly with regard to the composition and richness of the plant community. Finally, we aimed to provide evidence to support adaptive management in a rewilding initiative in the Côa Valley region, Portugal. The introduction of semi-wild horses, evaluated in this study, marks the first step in a long-term strategy to restore meso-herbivore diversity in the landscape.

4.1 Vegetation structural changes

The findings for vegetation structure partially align with our first hypothesis (H1), with horse grazing showing a clear effect on herbaceous vegetation but not on shrubs. The lack of significant

treatment effects (i.e., grazed *vs.* ungrazed plots) on shrub cover and height at both sites (Figures 2, 3) suggests that horse grazing may not exert sufficient pressure to regulate woody vegetation. This is consistent with previous studies indicating that low density grazing by domestic herbivores, particularly horses, may not suppress shrub expansion in abandoned areas of the Mediterranean region (Moinardeau et al., 2016; Fagúndez et al., 2022; Ribeiro et al., 2023, 2024). Moreover, the steeper increase in shrub metrics observed over the years at Site 2 may be partially driven by postfire successional processes, as this site recently experienced a severe wildfire in 2017. Fire can reduce competing vegetation, create open space, and release nutrients, thereby promoting rapid regrowth of fire-adapted shrub species (Cruz et al., 2020; Magaña Ugarte et al., 2021; Alegria, 2022). These favorable post-disturbance conditions



FIGURE 4

Species richness at Site 1 (a) and Site 2 (b) under baseline conditions in 2021, prior to the grazing regime shift, and in 2024, the final monitoring year, under horse grazing and no grazing control plots. Baseline (group 1) includes the sampling plots that transitioned to No grazing, and Baseline (group 2) includes the sampling plots that transitioned to horse grazing. Boxplots represent species richness measured in 10 m \times 10 m plots. (mean: asterisk, median: line). No significant effects were found between treatments.

may enable shrub expansion under low grazing pressure (Bates and Davies, 2014; Smit and Coetsee, 2019; Siegel et al., 2022).

The similar trends observed for grass height at both sites, with the significant interaction between year and treatment, indicate that even in the short term, horse grazing can effectively limit grass height, compared to grazing exclusion. These findings reinforce the role of horses as herbaceous grazers, preferentially consuming grasses over woody species (Vulink et al., 2001), whereas cattle tend to include a higher proportion of woody plants in their diet (Cosyns et al., 2001; Menard et al., 2002; Lamoot et al., 2005). The increased presence of the fire-prone *Stipa gigantea* in ungrazed (control) plots in Site 1 suggests that the lack of grazing pressure may favor its establishment and aligns with previous research indicating that grazing exclusion can promote grass dominance (de Villalobos and Zalba, 2010; Schneider and Hering, 2024), potentially elevating fire hazard in these areas (Cardoso et al., 2022; Davies et al., 2022; Orr et al., 2023). Conversely, the stability of *Stipa gigantea's* occurrence in open areas indicates that horse grazing may help contain its spread.

4.2 Vegetation compositional changes

In terms of vegetation composition, although we expected a decline in species richness in ungrazed (control) plots (H2) (Papanikolaou et al., 2011; Henning et al., 2017a; Dvorský et al., 2022; Bonavent et al., 2023), no significant differences were observed in species richness between grazing treatments (horses



visualization of differences in smaller values. *p*-values of the main effects in the linear mixed models are reported.

vs. no grazing) at either site. Despite the lack of significant effects, Figure 4 shows a trend towards lower species richness in ungrazed plots, with decreasing median and mean, while plots that remained grazed maintained similar richness levels. One potential explanation for the absence of statistically significant effects may be the early monitoring stage, as the short duration of grazing exclusion in fenced plots may not yet be sufficient to produce detectable effects (Song et al., 2020). Additionally, at Site 2, species temporal turnover was significantly higher in ungrazed (control) plots when compared to horse-grazed (open) plots, while no significant differences were detected at Site 1. These results provide partial support for our third hypothesis (H3), indicating a greater shift in species composition over time in the absence of grazing. It also suggests that the removal of grazing may lead to shifts in the recruitment success among plant species, such as grazing adapted species that may decline in response to exclusion (Song et al., 2020) or competitively dominant species that were previously suppressed by grazing (Zhang et al., 2023). This result aligns with other studies showing that even when species richness remains unchanged, the cessation of grazing can lead to significant changes in plant community composition over time, as certain species may outcompete others (Schultz et al., 2011; Bar-Massada and Hadar, 2017; Kaufmann et al., 2021).

Furthermore, as expected in our fourth hypothesis (H4), which anticipated a shift in the relative representation of forbs and grasses under different grazing pressure, there was an observed increase in forb-to-grass (F:G) ratio under horse grazing at Site 1, providing additional insights into how this grazing regime can interact with plant functional groups. In contrast, ungrazed plots showed no

significant changes in the F:G ratio (Figure 5), suggesting that forb recruitment may be limited without grazing disturbances and may benefit from reduced competition for space with grasses (Fleurance et al., 2012; Tuomi et al., 2019). These results might also help explain the results observed in species temporal turnover, as changes in turnover can be influenced by changes in abundance (Peper et al., 2011; Hillebrand et al., 2018) and grasses are outcompeting forbs in ungrazed plots, potentially leading to lower structural and functional diversity (Yan and Liu, 2021; Cardoso et al., 2022). Additionally, these functional responses align with the results obtained by the analysis of species importance values (IV), which revealed parallel trends in community structure. At both sites, the sum of IV for grasses declined more sharply in horsegrazed plots compared to ungrazed areas, while forbs increased their cumulative IV under grazing. Both metrics suggest that natural horse grazing promotes a shift toward more forb dominated communities (with flowering plants), which enhance food and shelter resources for anthophilous insects (Lázaro et al., 2016; Henning et al., 2017b; Shapira et al., 2020; Dvorský et al., 2022; Garrido et al., 2022; Bonavent et al., 2023), while grazing exclusion favors grass dominance (Supplementary Figures S2, S3). Notably, Stipa gigantea increased its importance in ungrazed plots, reinforcing the previously discussed concerns about fine fuel accumulation (Menning and Stephens, 2007; Cardoso et al., 2022; Davies et al., 2022; Orr et al., 2023). Grass dominance in the absence of grazing could have adverse implications for wildfire hazard and biodiversity conservation, particularly in fire-prone Mediterranean landscapes (Kirkland et al., 2024; Lovreglio et al., 2024), such as the Côa Valley region.

	2021				2024			
	Baseline (group 1)		Baseline (group 2)		No grazing		Horse grazing	
Site 1	Species	IV	Species	IV	Species	IV	Species	IV
Species with highest IV	Cytisus multiflorus	12.63	Cytisus multiflorus	18.38	Stipa gigantea	22.85	Cytisus multiflorus	18.52
	Anthoxanthum aristatum	12.44	Stipa gigantea	13.15	Cytisus multiflorus	20.19	Stipa gigantea	15.36
	Stipa gigantea	12.39	Anthoxanthum aristatum	12.49	Avena barbata	12.53	Hypochaeris glabra	14.02
	Rumex acetosella	9.57	Tuberaria gutata	11.26	Hypochaeris glabra	9.55	Rumex acetosella	10.77
	Tuberaria gutata	8.71	Rumex acetosella	8.82	Rumex acetosella	9.10	Anthoxanthum aristatum	8.82
Cumulative IV - Poaceae (Grasses)		53.55		48.89		51.19		36.02
Cumulative IV - Forbs		131.74		132.06		125.96		144.75
Site 2								
Species with highest IV	Cytisus multiflorus	16.28	Cytisus multiflorus	18.46	Cytisus multiflorus	23.02	Cytisus multiflorus	27.50
	Erodium sp.	7.39	Quercus pyrenaica	8.06	Quercus pyrenaica	8.67	Erodium sp.	8.20
	<i>Vulpia</i> sp.	6.60	Vulpia sp.	5.95	Erodium sp.	7.79	Quercus pyrenaica	8.14
	Tuberaria gutata	6.41	Tuberaria gutata	5.35	Crepis capillaris	7.47	Crepis capillaris	5.44
	Anthemis arvensis	6.19	Anthemis arvensis	5.31	Ranunculus sp.	4.84	Ornithopus pinnatus	4.77
Cumulative IV - Poaceae (Grasses)		34.26		31.69		25.80		18.36
Cumulative IV - Forbs		136.27		134.83		134.80		141.98

TABLE 2 Summary of species importance values (IV) at Site 1 and Site 2.

For each year and grazing regime, the five species with the highest IV are shown. Additionally, the table presents the total cumulative IV of grasses (Poaceae) and forbs, with forbs defined as all observed species excluding those from the families Poaceae, Juncaceae, Fagaceae, Dennstaedtiaceae, Aspleniaceae, and the genera *Cytisus* and *Genista* (Fabaceae). Baseline (group 1) includes the sampling plots that transitioned to No grazing, and Baseline (group 2) includes the sampling plots that transitioned to horse grazing. Some plants were identified at the genus level.

4.3 Management insights

This study provides relevant evidence on the use of semi-wild horse grazing as a tool for vegetation management. On the one hand, horse grazing demonstrated an ability to reduce local fire hazard by effectively controlling the height of grasses (Figures 2, 3), while also promoting a higher representation of forbs in the plant community, as reflected by increases in the forb-to-grass ratio and cumulative importance values (Figure 5 and Table 2). On the other hand, the limited effects on shrub metrics at both sites, with increased shrub cover, height and biomass (Figures 2, 3), suggest that horse grazing alone may be insufficient to regulate woody vegetation. Furthermore, preliminary results obtained from soil and tree recruitment analysis developed in a management action for the study sites showed that shifting the grazing regimes improved soil organic matter (SOM) and oak recruitment (Supplementary Table S4 and Supplementary Figure S4), with greater increases in SOM and higher number of oak seedlings in horse grazing plots, compared to ungrazed plots. Overall, these findings suggest that while semi-wild horse grazing can contribute to multiple management goals, its effectiveness varies across vegetation and habitat types and would benefit from complementary interventions tailored to local vegetation dynamics and disturbance history.

In particular, semi-wild horse grazing alone may not be sufficient to achieve the desired management outcomes for shrub control in the Côa Valley region, which has been affected by recurrent and severe wildfires over the last two decades (Kirkland et al., 2024). The unchecked proliferation of tall grasses can also significantly heighten fire hazard (Menning and Stephens, 2007; Cardoso et al., 2022), which is particularly concerning under the hot and dry conditions typical of Mediterranean summers. However, in grassland-like habitats, where the accumulation of fine fuels can enable rapid fire spread (Turco et al., 2017; El Garroussi et al., 2024), low-intensity semi-wild horse grazing can be effective in preventing grass dominance and reducing wildfire risk, without imposing the grazing pressure that is needed to suppress shrub expansion. Notably, this finding signals the important ecological role of diverse and complementary herbivore communities (Orr et al., 2022; Pringle et al., 2023; Ribeiro et al., 2023). Management strategies should incorporate a diverse assemblage of herbivores, as this is likely to create a mosaic of grazing effects, fostering ecosystem function and resilience in Mediterranean landscapes (Liu et al., 2015; Orr et al., 2022). For example, while horses may be well-suited to certain



grazing and in ungrazed control plots, following the shift from the baseline regime in 2021. Boxplots show the distribution of values in 10 m \times 10 m plots (mean: asterisk, median: line). Significant differences between the two treatments (Paired t-Test; p < 0.05) are marked in bold.

contexts due to their lighter impact and broad grazing patterns, locally adapted cattle breeds and other rustic breeds, such as Tauros, can complement these effects by targeting different vegetation types or structures (Fleurance et al., 2012; Moinardeau et al., 2016; Schmitz and Isselstein, 2020). This diversified approach is particularly important in habitats prone to shrub encroachment, where it can help break fuel continuity and maintain landscape heterogeneity (Pausas, 2004; Nunes, 2023). Additionally, rewilding initiatives, where herds are semi-wild, social, and not reliant on supplementary feeding (except under extreme conditions), grazing patterns tend to be more heterogeneous than those in livestock production systems (Menard et al., 2002; López et al., 2019). This heterogeneity can promote a more dynamic and resilient vegetation structure by preventing the dominance of single plant functional groups (Adler et al., 2001; Pringle et al., 2023). An important consideration in implementing such management strategies is the definition of appropriate stocking densities for different herbivore assemblages. However, determining adequate densities is challenging, as grazing pressure is not defined by stocking density alone, and its ecological effects are highly contextdependent (Ribeiro et al., 2023). Moreover, in addition to promoting a diverse assemblage of herbivore, complementary management actions, such as initial shrub clearing and prescribed burning, might be needed to support vegetation regulation efforts. These actions can be particularly relevant in shrub encroached areas (Castro et al., 2022; Oikonomou et al., 2023) or where prescribed burning can reduce accumulated litter and biomass and foster herbaceous growth (Barbaro et al., 2001; O'Connor et al., 2020).

In the specific context of the study sites, and of ongoing rewilding efforts, Rewilding Portugal plans to introduce Taurus cattle (Goderie et al., 2013; Stokstad, 2015) at lower densities than those of the pre-existing commercial livestock production, aiming to complement the effects of horse grazing. As this rewilding plan is implemented, ongoing monitoring will be necessary to assess its impacts and provide evidence to evaluate this approach. Moreover, the significant effects of the year factor across sites highlight the need to account for temporal variability in future grazing management plans, as vegetation responses may lag behind grazing interventions (Song et al., 2020; Kaufmann et al., 2021; Ribeiro et al., 2023). Additionally, the interannual variation in weather conditions may have influenced plant growth and community dynamics, potentially interacting with the grazing treatments. Disentangling the effects of grazing and climatic variability also requires a longer monitoring interval to better understand the relative contribution of these factors to the observed changes (Kutiel et al., 2000; Kaufmann et al., 2021). Despite these limitations, early-stage assessments remain essential to detect initial vegetation responses (e.g. Balata et al., 2022; Li and Zhan, 2023), which can guide the adaptive management of rewilding initiatives, especially in fire-prone landscapes where early signs of structural change can inform hazard mitigation.

5 Conclusions

This study highlights the effects of semi-wild horse grazing, under a low-intensity regime, on vegetation structure and composition, after the transition from a more intensive commercial cattle grazing regime, and in comparison to ungrazed conditions. The findings indicate that semi-wild horse grazing can regulate grass height, reduce grass dominance and facilitate the persistence and relative prominence of forbs in the community. This is particularly relevant in fire-prone Mediterranean landscapes, where the unregulated growth

of grasses in abandoned ungrazed areas may heighten wildfire hazard. However, the limited effect found on shrub structure suggests that it may not provide sufficient pressure to prevent shrub expansion. From a shrub management perspective, the results suggest the need for a multi-herbivore approach. Complementary species, such as browsers like the red deer, or cattle and other large grazers, can target and exert higher pressure on woody vegetation (Venter et al., 2019), contributing to the creation of a structurally diverse mosaic of habitats. This aligns with rewilding and natural grazing frameworks, where maintaining a diverse assemblage of wild and semi-wild herbivores fosters both temporal and spatial heterogeneity. The year effects observed in this study also emphasize that vegetation responses may exhibit a time lag, requiring long-term monitoring and adaptive management to account for temporal variability in growth cycles and weather conditions. Ultimately, this study contributes to the growing body of evidence on nature-based solutions for Mediterranean ecosystem management and provides an example of integrating scientific research with practical applications to inform management strategies.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

IR: Validation, Conceptualization, Writing – review & editing, Methodology, Investigation, Writing – original draft, Visualization, Formal analysis, Software. SA: Project administration, Conceptualization, Writing – review & editing, Investigation, Supervision. TD: Writing – review & editing, Supervision. DM: Supervision, Writing – review & editing. VP: Conceptualization, Methodology, Supervision, Writing – review & editing, Investigation, Validation, Writing – original draft, Formal analysis.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2025.1596560/ full#supplementary-material

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