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Mildly toxic shrubs as indicators of goats herbivory give information for the management of natural landscapes on Mediterranean islands

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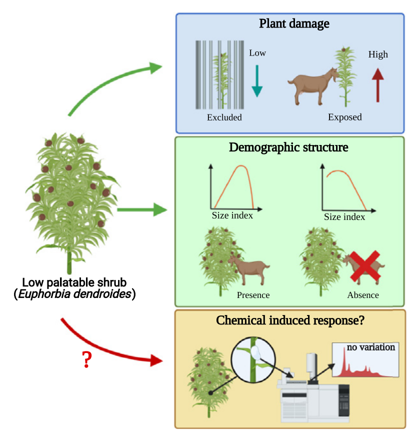
HIGHLIGHTS

- Herbivory by introduced goats has been scarcely focused on their impact on low-palatability species.
- High affection of low-palatability shrubs by ungulates can indicate height herbivory pressure.
- Juveniles of the low-palatability shrub *Euphorbia dendroides* are highly predated in some areas of Mallorca island.
- *E. dendroides* demographic structure is influenced by goats presence.
- The impact on low-palatability shrubs demography is indicator of high herbivory pressure in the mediterranean ecosystems.

GRAPHICAL ABSTRACT



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ABSTRACT

Herbivory of insular plant communities by introduced animals has been widely studied for decades. Though their diet mainly includes palatable and highly nutritive species, goats will also eat plants that are toxic to other animals. Thus, severe affection of toxic species may indicate high herbivore pressure or a low quality of vegetative food. To evaluate whether herbivory damage to low-palatability shrubs could give us information about feral goat pressure on vegetation, we assessed the predation impact of feral goats on the shrub *Euphorbia dendroides* (Euphorbiaceae) on Mallorca Island (Spain). We aimed to investigate whether goats consume juvenile *E. dendroides* and affect their population structure and determine if the plants increase the concentrations of toxic compounds as an adaptation to herbivory. Overall, two experimental plots and analysis of eleven natural populations indicated *E. dendroides* is affected by ungulates and that the population structure change with the presence of feral goats. *Euphorbia dendroides* could be used as an ecological indicator to determine the extent of ungulate damage to vegetation or indicate poor food availability, and thus inform the maintenance of optimal animal populations. Depending on the management objective for the territory, *E. dendroides* could be used as an ecological indicator to determine the extent of ungulate damage to vegetation or indicate poor food availability for animals, and thus inform the maintenance of optimal animal populations.

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1. Introduction

The impact of non-native mammal herbivores such as rats, rabbits or goats on island ecosystems has been widely studied on oceanic islands (Campbell and Donlan, 2005; Coblentz, 1978; Garzón-Machado et al., 2010; Shiels and Drake, 2015; Vitousek et al., 1997) and more recently Mediterranean island ecosystems (Gizicki et al., 2018). However, the impacts of herbivores—especially ungulates—are unpredictable, as the consequences of herbivory strongly depend on the local context and herbivory may even be necessary for conservation of plant communities in some situations (Arévalo et al., 2011; Fernández-Lugo et al., 2013, 2011). Moreover, conservation of ungulates of taxonomic or economical interest may require specific management measures (Papadopoulos, 2002; Pérez-Solano et al., 2020). As a result, an integrative approach involving the use of appropriate ecological indicators that reflect the intensity of the impact of ungulates on vegetation is required to adjust the management of ungulate populations based on their impact (Perea et al., 2015) or their needs (Papadopoulos, 2002). Some authors have proposed successful regeneration of sensitive or threatened woody taxa as adequate indicators of the ecological sustainability of ungulate populations (Perea et al., 2015; Velamazán et al., 2017). Observation of intense browsing of adult plants and a lack of regeneration of populations are considered red flags that may indicate a need to reduce the ungulate populations (Perea et al., 2015; Velamazán et al., 2017). In these cases, herbivores can be expected to induce demographic effects in plant populations; due to the low tolerance to herbivory of seedlings (Barton and Hanley, 2013), since seedlings are considered the most vulnerable stage (Barton, 2016; Latorre et al., 2013). Consequently, early plant stages would be more disproportionately and more severely damaged, whereas older plants would be overrepresented, leading to an imbalance between recruitment and mortality (Gómez-Aparicio et al., 2005; Rhodes et al., 2017). However, plants that are sufficiently preadapted to non-native herbivores may display defence strategies, such as constitutive toxic secondary compounds (Agrawal and Fishbein, 2006), and may increase the concentrations of these metabolites in response to herbivory (Agrawal, 1998).

Despite the fact that goats mostly select palatable and highly nutritious species for their diet, they will also consume other less-suitable species if resource availability is low (Baraza et al., 2010; Osuga et al., 2020). Thus, when the herbivore pressure is high or nutritious vegetation is scarce, even low-palatability and toxic species may exhibit demographic damage due to herbivores. In this context, we hypothesized that the demography of low-palatability shrubs could function as an indicator of goat herbivory pressure on vegetation or the low availability of more palatable.

In this context, using Mallorca as a case study, we analysed the demographic impact of feral goats on *Euphorbia dendroides* L., a low-palatability shrub species (Eichberger, 2003) distributed throughout the island, as well as some islets where ungulates have been eradicated. The extinction of native mammals coincided with the introduction of exotic herbivores such as goats *ca.* 4300–4050 yr BP (Seguí et al., 2005). Feral populations of goats were controlled by humans until the 1960s, when changes in land-use caused by the economic shift towards tourism implied an abandonment of the rural management (Salva Tomas, 1993) and conversion of most goat populations in the mountains from domestic to feral (Limpens et al., 2020; Vives and Baraza, 2010). At present, as their impact on endemic biodiversity and vegetation is considered an important conservation issue, the populations of feral goats are controlled by the local government in public areas and by hunters in designated hunting areas (Cursach et al., 2013; Moragues et al., 2015; Limpens et al., 2020).

Euphorbia dendroides (Euphorbiaceae) is a shrub that grows up to *ca.* 2 m tall and wide. The species is distributed in the western and central Mediterranean region (Traveset and Sáez, 1997) and flowers between mid-March and April. After producing fruit, the species exhibits summer-deciduous behaviour that coincides with the dry summer period, and loses its leaves until the autumn. Similarly to many other species from the same genus, *E. dendroides* produces a toxic, gummy latex that can lead to irritant damage when in contact with mammal tissues (Eichberger, 2003). Thus, *E. dendroides* is considered a low-palatability species and an unlikely component of the diet of many mammal herbivores. However, while this species was highly abundant in the mountain area named La Victoria in 2011, it is now very rare inside the hunting area on this mountain, but not outside the hunting area (Fig. 1). Moreover, some individuals of this species still grow in places inaccessible to goats inside the hunting area (pers. obs.), so the decrease in the *E. dendroides* population may possibly be attributed to goat herbivory pressure.

The aims of this study were (i) to assess the impact of goats on the survival of juvenile *E. dendroides* under experimental conditions, (ii) to compare the population structure of natural *E. dendroides* populations in the presence and absence of goats, and (iii) to analyse interpopulation variability in the main chemical compounds related to toxicity in relation to the presence of goats.

2. Materials and methods

2.1. Study system

Populations of *E. dendroides* are located in littoral Mediterranean shrublands with a predominance of *Olea europaea* L. (Oleaceae) and especially in xeric soils. *E. dendroides* populations were selected in eleven areas around Mallorca island (Balearic Islands archipelago); feral goats are present in eight of these areas and totally absent from three of these areas (Fig. 2, Table 1). The populations without goats are located on islets (Dragonera and Cabrera) and in the south of Mallorca, where no feral goats are reported. The populations that coexist with feral goats are located in the north of Mallorca island. Essentially, with the exception of Sa Dragonera islet, where alien mammals have been absent since a recent eradication program (Mayol et al., 2012), all of the selected *E. dendroides* populations interact with introduced rodents such as rats or rabbits.

2.2. Assessment of the effect of herbivory on the survival of juvenile *E. dendroides*

During 2017, we established an experimental plantation of juvenile *E. dendroides* individuals in the La Victoria locality (39.855 N/3.155 E) to evaluate the effect of herbivores on the survival of the plants. The following year, a new plantation was established at Mortitx (39.868 N/2.922 E), where *E. dendroides* populations coexist with other herds of goats.

E. dendroides seeds were germinated and the plants grown for one year at the Forestal Center of the Balearic Islands (Balearic Islands Government). In 2017, we planted 46 individuals protected by lattice fences (21 cm diameter, 85 cm high; 2.7 × 3-cm mesh size) and 46 individuals with no protection at La Victoria. In 2018, we planted 40 individuals protected with the same fencing and 40 individuals with no protection at Mortitx. For each population, we evaluated the status of the individuals five weeks after the plantation, which coincided with the flowering time of the species. A second evaluation was conducted in September, after the dry period. Each individual was categorized as: (i) Not eaten; plant in good condition, (ii) Eaten/Alive; signs of predation, but the individual had resprouted, (iii) Eaten/Dead; signs of predation and the individual was dead or had been ripped out of the soil, and (iv) Dry/Dead; dead due to drought without signs of predation.

Data was analysed by conducting multinomial logistic regression with the '*multinom*' function of the '*lme4*' package (Bates et al., 2015) in R software version 3.6.1 (R Core Team, 2019) considering the number of individuals included in each browsing category as the response variable, with treatment (exposed or protected) and area (Victoria or Mortitx) as fixed factors. When significant differences were reported, the Tukey *ad hoc* test was used to evaluate the significance of the differences between groups.

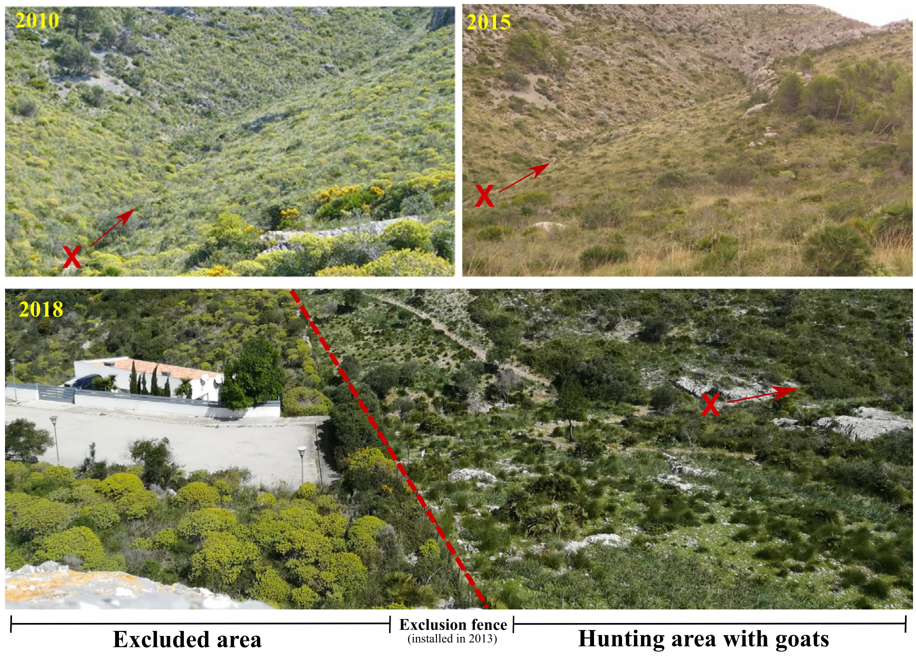

Fig. 1. Images of La Victoria mountain area, where *E. dendroides* were abundant in 2010 (top right) but had almost disappeared by 2015 (bottom right). At present, the access of goats to this area is restricted by a fence constructed in 2013 to separate a hunting area from the urban area (left), where *E. dendroides* is still abundant. Crosses (position) and arrow (orientation) are used as references to compare the pictures.

2.3. Assessment of the demographic structure of natural populations

Three plots of 10 × 10 m were established in each selected population, and the number of individuals inside the plots was counted to evaluate plant density. The maximum height and perpendicular diameter of each plant were measured, as well as the diameter of the trunk. To evaluate the population structure, we used the size index (Height + Diameter)/2 described by De Cáceres et al. (2013). Then, the individuals

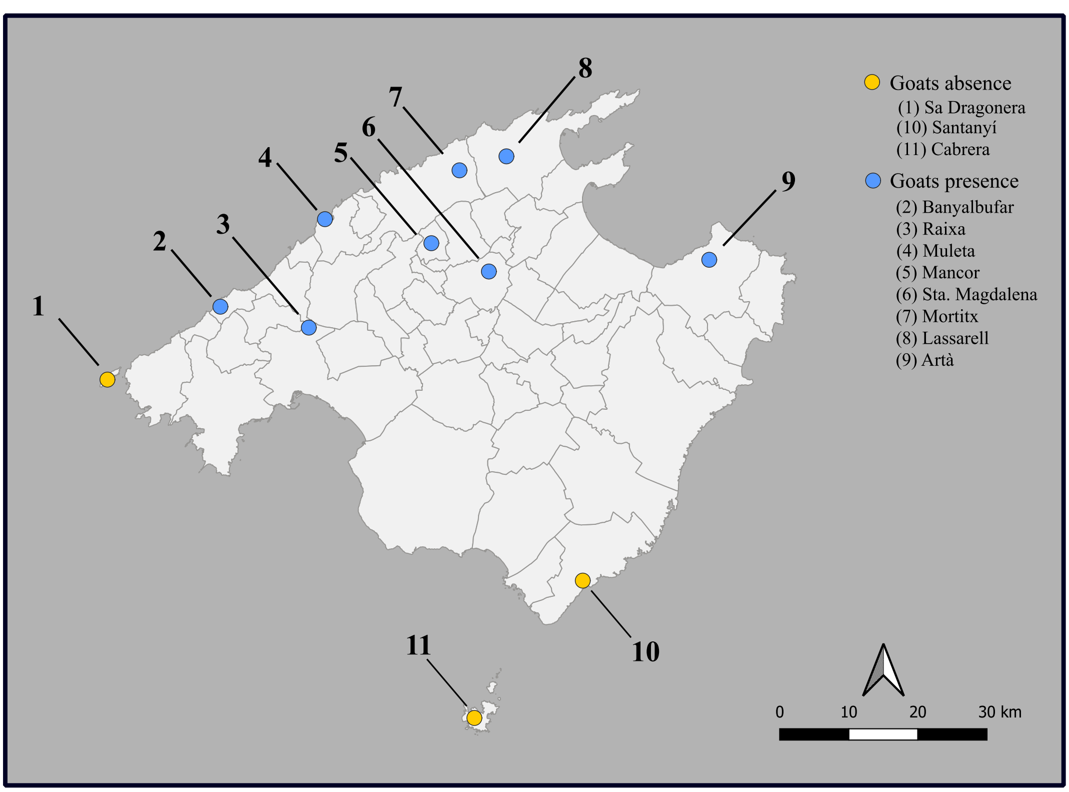

Fig. 2. Map showing the locations of the populations studied on Mallorca island (A) and La Victoria (B), where fencing against herbivores affected the *E. dendroides* population. Blue dots indicate populations where goats are present and red dots indicate areas where goats are absent.

Table 1
Populations studied in the experiments conducted during 2017 and 2018. Populations were selected based on current records of *E. dendroides* populations. Coastal habitats were defined as populations less than 100 m from the sea.

Map code	Study site	Year	Coordinates	Habitat type	Presence of goats	Human action
1	Sa Dragonera	2017	39.587 N/2.325 E	Coastal	No	No
2	Banyalbufar	2017	39.681 N/2.498 E	Coastal	Yes	Yes
3	Raixa	2017	39.681 N/2.671 E	Inland	Yes	Yes
4	Muleta	2017	39.791 N/2.685 E	Coastal	Yes	Yes
5	Mancor	2017	39.750 N/2.866 E	Inland	Yes	Yes
6	Sta. Magdalena	2018	39.725 N/2.954 E	Inland	Yes	Yes
7	Mortitx	2017	39.868 N/2.922 E	Inland	Yes	No
8	Assarell	2017	39.849 N/2.984 E	Inland	Yes	Yes
9	Artà	2018	39.733 N/3.337 E	Inland	No	No
10	Mondragó	2017	39.332 N/3.175 E	Coastal	No	No
11	Cabrera	2018	39.151 N/2.934 E	Coastal	No	No

were grouped into size classes as follows: stage 1 (size index < 8), stage 2 ($8 <$ size index < 40), stage 3 ($40 <$ size index < 120), or stage 4 (size index > 120).

Population histograms were plotted using SigmaPlot 11 (Systat Software, Inc., San Jose, CA, USA) and the frequency of each size class was numerically obtained using a new balance index, calculated using mean population size index/mid-range population size index (see Supplementary Information 1 for further information). First, for each size class studied, we evaluated plant density as a response variable, the presence of goats as a fixed factor and population as a random factor. Then, the population balance indices were evaluated as response variables; the presence of goats, the mean annual temperature between 1979 and 2013 (Karger et al., 2017), the altitude and the distance from the sea were used as fixed factors, and population was considered as a random factor. For these purposes, we generated linear mixed models accepting normality and homoscedasticity using the '*lme*' function of the '*nlme*' package (Pinheiro et al., 2019) and model selection was performed using the '*dredge*' function of the '*MuMIn*' package (Barton, 2020) in R software 3.6.1 (R Core Team, 2019). The best-fit model was selected according to the minimum $\Delta AIC < 2$, then analysis of variance was executed to evaluate the results using the '*anova*' function of '*car*' package (Fox and Weisberg, 2019).

2.4. Chemical responses to herbivory in latex majority compounds

From each population, 250 μL samples of fresh latex were collected from six juvenile plants and six adult plants ($n = 12$) during early spring, prior to flowering. Samples were stored in Eppendorf tubes at 4 °C until analysis. Aliquots of 10 μL were pre-weighed, dried at 60 °C for two days and weighed again to calculate the latex dry weight and percentage water content (*i.e.*, the difference between the fresh and dried weights per gram fresh weight). To determine the presence of low-molecular-weight metabolites, 5 μL samples of latex were extracted in 1 mL of ethyl acetate containing 0.01% (v/v) hexadecane as an internal standard. The extracts were vortexed for 10 s, incubated in an ultrasonic bath at 25 °C for 20 min, placed in an oven at 60 °C for 20 min, and the final extracts were centrifuged for 1 min at 11000 rpm. Samples were analysed using a flame ionization detector on a gas chromatograph coupled to an autosampler (GC-FID, Agilent 7820A) equipped with a capillary column (HP-1MS, 30 m, 0.25 mm). The program used for the run was: 200 °C initial temperature, ramp of 4 °C min^{-1} up to 320 °C, and 3 min at 320 °C. The concentrations of each compound were calculated from the peak areas as hexadecane equivalents. Concentrations were normalized to the total latex dry mass using the density value of each sample (mg compound/g dry latex).

To determine the identity of the compounds, one representative sample was analysed by gas chromatography coupled to a mass spectrometer (MS, Agilent 6890 with an Agilent 5973 mass spectrometer) using the same column and temperature program as for GC-FID. Compounds were tentatively identified as triterpenoids based on their mass fragmentation spectra in comparison with spectra libraries.

Principal Component Analysis (PCA) was used to evaluate the effect of the presence of goats on the general composition of triterpenoids. Each principal component was used as a response variable in a linear model to evaluate the effect of the presence of goats as a fixed factor. The total concentration of triterpenoids was assessed to evaluate the variation in this molecular family due to herbivory pressure. For this purpose, a linear mixed model was generated using the total triterpenoids concentrations as a response variable, the presence of goats and plant life stage as fixed factors, and population as a random factor. Model selection and analysis of variance were performed as previously explained.

3. Results

3.1. Juvenile E. dendroides are highly predated by goats

The exposed juvenile *E. dendroides* individuals planted without protective fencing structures at La Victoria in spring were severely affected by herbivory consuming the 100% of the branches; 76% died and 21% were eaten but survived (Fig. 3). Moreover, in the same area and season, even 23.9% of plants protected by fencing exhibited signs of herbivory if their branches grew outside the fence (considered eaten/alive in our data). The fencing protection significantly prevented herbivory damage in both spring (df = 3, $\chi^2 = 98.299$, $p < 0.001$) and autumn (df = 3, $\chi^2 = 138.162$, $p < 0.001$). However, the plants at the two test sites exhibited significantly different herbivory damage in both spring (df = 3, $\chi^2 = 77.833$, $p < 0.001$) and autumn (df = 3, $\chi^2 = 93.673$, $p < 0.001$). In spring, the proportion of exposed individuals that died due to herbivory was significantly higher at La Victoria (76% in spring and 100% in autumn) than Mortitx (2.6% in spring and 21.6% in autumn) (df = 9, $t = -10.84$, $p < 0.001$), while the proportion of protected plants with branches affected outside the fencing was significantly higher at Mortitx (df = 9, $t = 5.20$, $p = 0.002$). At the end of the experiment, nearly all exposed *E. dendroides* individuals at La Victoria had died due to predation (76.1% in spring and 63.6% in autumn), while the majority of exposed plants at Mortitx remained alive after herbivore damage (85.7% in spring and 70.2% in autumn). On the contrary, individuals with clear signs of death due to drought were more abundant at La Victoria than Mortitx (df = 9, $t = -4.42$, $p = 0.007$).

3.2. Goats negatively affect the demographic structure of natural E. dendroides populations

The abundance of *E. dendroides* was related to the presence of goats (Table 2). Areas with goats contained lower numbers of *E. dendroides* individuals and had a lower plant density. The Dragonera islet population had the highest number of individuals (410 individuals in total), and seedlings were the most common age category in this population.

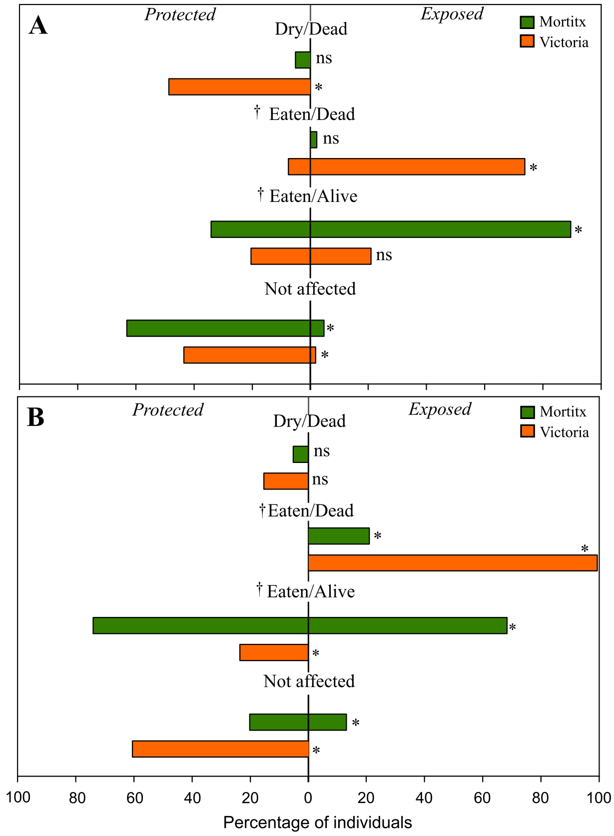

Fig. 3. Percentage of planted individuals that survived in spring (A) and in autumn (B). Asterisks indicate significant differences between both treatments for each site, season and affection level using *ad hoc* Tukey tests.

When each size class was assessed, areas where goats were present showed lower densities of plants in the middle stages of the population, but there were no significant differences in the densities of the oldest individuals (Fig. 4). Moreover, higher densities of stage 1 plants (df = 1, F = 8.40, $p < 0.017$), stage 2 plants (df = 1, F = 49.41, $p < 0.001$) and stage 3 plants (df = 1, F = 16, $p = 0.003$) were observed in areas from which goats are absent, though the density of stage 4 plants was similar between treatments (df = 1, F = 0.74, $p = 0.412$; Fig. 4).

Frequency distributions were generated for each population using the size indexes (Fig. S1) and balance indexes were calculated for each plot in each population. Model selection to explain balance index of the population considered the best-fit model as the one that included the presence of goats as the unique significant predicted variable (df = 8, F = 7.65, $p = 0.024$; Fig. 5). However, based on the AIC, the second model included both presence of goats and temperature whereas the third one only included temperature (Table 3). Therefore, even without significant effect, the temperature seems to slightly influence the balance index of the population. Areas with goats presented populations with higher balance indexes—that is, a greater representation of old individuals and a lack of juveniles—than areas without herbivores. In the same way areas with higher temperatures presented higher balance indexes, especially if goats were present.

Table 2
Comparison of population density indicators between areas with and without goats. Density was evaluated both including and excluding seedlings. Parameters were analysed using the Kruskal-Wallis chi-squared test.

		Total individuals	Total density (ind/m^2)	Adult density (ind/m^2)
Goats	Artà	91	0.3	0.3
	Assarell	89	0.3	0.3
	Mancor	71	0.24	0.24
	Mortitx	65	0.22	0.2
	Muleta	55	0.18	0.18
	Raixa	38	0.13	0.11
	Sta. Magdalena	129	0.43	0.42
	Verger	79	0.26	0.26
No goats	Cabrera	316	0.97	0.97
	Dragonera	410	1.37	0.59
	Mondragó	348	1.17	1.02
χ^2 *p*-value		>0.001***	>0.001***	>0.001***

* < 0.05, ** < 0.01, *** < 0.001.

3.3. The chemical composition of latex extracts does not differ among *E. dendroides* populations

Seven compounds were detected in the latex samples, all of which exhibited fragmentation patterns compatible with triterpene sterols, such as obtusifoliol (Table S1, Fig. S2). The concentrations of total triterpenoids ranged from 123.97 mg/g to 699.51 mg/g. PCA showed that principal component 1 (PC1) explained 79% of the variability in the data, while principal component 2 (PC2) explained 6.9% (Fig. S3). However, neither PC1 (df = 1, F = 0.24, $p = 0.625$) or PC2 (df = 1, F = 2.71, $p = 0.101$) were affected by the presence of goats. After model selection, altitude, temperature and distance to the coast were not included in the final model. The presence of goats (df = 1, F = 0.05, $p = 0.813$) and life stage (df = 1, F = 1.92, $p = 0.167$) did not significantly influence the concentration of triterpenoids.

4. Discussion

The structure of *E. dendroides* populations varied between natural areas with and without goats. Furthermore, the experimental plantations suggest a high probability of consumption of juveniles which can lead to low recruitment and overrepresentation of old individuals.

4.1. Juvenile *E. dendroides* are highly predated by goats

The exclusion experiments at La Victoria and Mortitx clearly indicate that juvenile *E. dendroides* are strongly predated by goats, although the intensity of this pressure was more severe at La Victoria, where the stocking rate is higher (between 0.2 and 0.4 goats/ha, according to the range managers). Indeed, most of the experimental *E. dendroides* individuals at La Victoria died after predation, while the experimental plants at Mortitx successfully resprouted after damage. This difference is probably caused by the different herbivory pressure and its interaction with water stress that was great at La Victoria (Huberty and Denno, 2004). Currently, populations of *E. dendroides* are only present on cliffs at La Victoria, while some populations are present in the shrublands at Mortitx. In agreement with our findings, Rivera-Sánchez et al. (2011) and Limpens et al. (2020) found that goat herbivory pressure affects the majority of plants in the community at La Victoria.

Death by drought was also observed among protected individuals at both sites. In fact, our results show that temperature also has some influence on the population structure of this species. Areas with higher temperatures have a lower probability of juvenile survival. However, the majority of deaths by herbivory are likely to be due to a combination of both events. The interaction between temperature and damage by ungulates can vary between species, so that higher temperatures decrease or increase the negative effect of herbivory (Vuorinen et al., 2020). In our case predation interferes with correct development, and then individuals are unable to resprout due to high temperatures during summer.

Protected individuals also suffered the effects of drought during the summer at both sites, but especially at La Victoria where the temperatures are higher and the rainfall is lower. This observation indicates that the growth of juvenile *E. dendroides* plants is limited during the dry season -particularly, this species is summer deciduous-, as observed for many other Mediterranean species (Llorens et al., 2004). However,

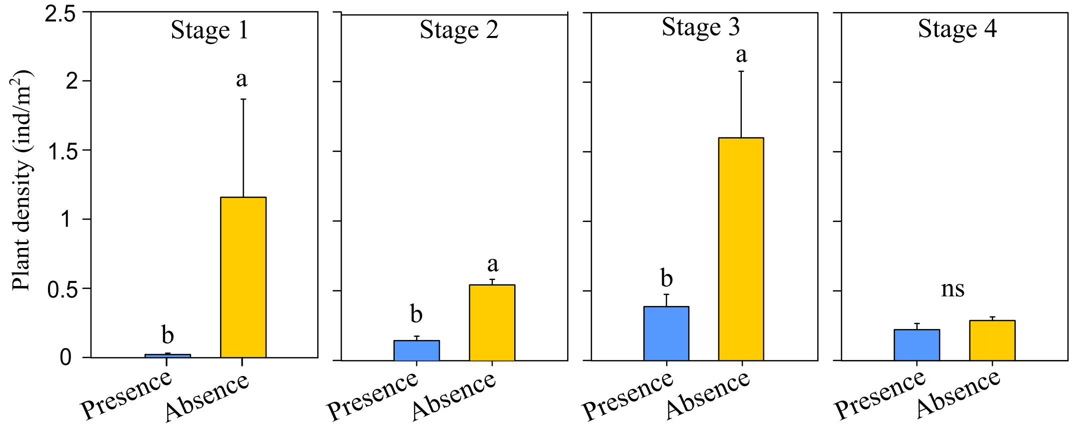

Fig. 4. Plant density for each of the four life stages in populations where goats were absent (red) and present (blue). Percentages were calculated by dividing the number of individuals in each age class by area. Significance is shown in letters; 'ns' means not significant. Error bars indicate the standard error.

herbivory has a major impact on juvenile *E. dendroides* and leads to higher death ratios than other no controlled biotic and abiotic stresses alone.

4.2. Goats negatively affect the demographic structure of E. dendroides populations

The natural populations of *E. dendroides* were strongly affected by the presence of goats, especially in terms of seedling recruitment. The effects of the herbivores on young natural individuals are concordant with the results of the experimental plantations. Interference with recruitment can severely affect the demographic dynamics of a population (Llorens et al., 2004). Moreover, three life stages were affected by the presence of goats, which indicates grazing not only limits current plant recruitment, but also increases the presence of older individuals. Specifically, the highest recruitment rate was found on the islet of Dragonera, where other mammal herbivores such as rabbits or rats are also absent. Thus, both rats and rabbits may also severely affect *E. dendroides* plant recruitment, as reported for other species such as *Medicago citrina* (Font Quer) Greuter, which is only found on islets were herbivores are absent (Latorre et al., 2013). Overall, it seems that herbivores affect both the germination and development of the early life stages of *E. dendroides*, and only older, adult individuals are able to survive in a high browsing pressure scenario. Similarly, in other Mediterranean islands such as Sardinia, the presence of feral goats negatively affected to the thorny endemic *Centaurea horrida* population structure: saplings and adults were more abundant in those areas where herbivores were absent (Pisanu et al., 2012). Even less browsing ungulates such as semi-feral cattle decrease the density of juveniles not only of deciduous woody species usually more nutritious such as *Acer* but also of perennial species such as *Pinus* (Fortuny et al., 2020).

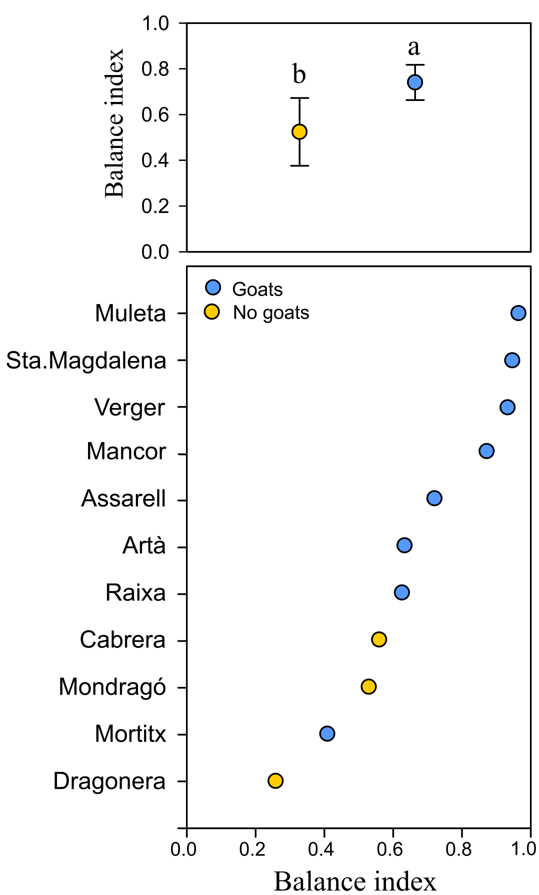

Fig. 5. Balance index values for populations where goats were absent (red) and present (blue). Error bars indicate standard the error and letters indicate significant differences among groups using *ad hoc* Tukey tests.

However, for certain woody species a strong impact of various factors such as the structure of the stand and the micro-topography or climatic conditions on regeneration has been observed, beyond the impact of browsing (Fazan et al., 2021). In our study case, the environmental conditions evaluated did not show significant effects on *E. dendroides* demography although, in areas where goat populations are actively controlled, exhibited higher representation of younger plant stages, as was the case at Mortitx This could indicate that certain level of herbivory pressure is necessary to affect *E. dendroides* populations, especially in areas where the summer climate is milder. These results prove that the demographic structure of the low-palatable *E. dendroides* is caused by herbivores despite being mildly toxic. Therefore, the demographic structure of low-palatable or toxic shrubs can help managers to assess the herbivory impact on natural landscapes and evaluate the necessity to reduce the presence of herbivores in the area.

4.3. The chemical composition of latex extracts does not differ among E. dendroides populations

The majority of compounds detected in *E. dendroides* latex in this study are major precursors of triterpenoids, which are related to plant defence against herbivores, mainly insects and fungi (Sytwala et al., 2015). After evaluating the significance of the influence of herbivores on the population structure of *E. dendroides*, we expected that

Table 3
Selection of the seven best-fit models for the analysis. Variables included in each model are marked with a cross. The AIC value and its delta (Δ) against the first model are indicated in the last two columns.

Model	Goats presence	Temperature	Altitude	Distance to the sea	AIC	ΔAIC
M01	X				−2.3	0
M02	X	X			4.5	6.8
M03		X			5.2	7.4
M04	X		X		7.4	9.6
M05			X		14.7	17.01
M06	X	X	X		15.1	17.40
M07				X	20.9	23.19

individuals severely affected by ungulate herbivory would increase the biosynthesis of triterpenoids as a response to predation, as observed in other species (Giner and Djerassi, 1995). However, the concentrations of secondary compounds in latex did not differ between the populations coexisting with goats and populations without goats. Some species exhibit induced resistance responses by increasing the total amount of latex present in their plant tissues, though the initial concentrations of triterpenoids in the latex remain the same. Additionally, some herbivores can tolerate low amounts of toxic compounds in their diet, and may prefer to graze on juvenile plants that invest more resources in growth than toxic compounds compared to adult plants (Konno, 2011). Nevertheless, the induced chemical response of the species remains unknown. The sampling methodology, the temporal effect and the resolution of chemical analysis can be optimized in the future. For instance, further studies must consider to sample the whole plant tissue or to include the interannual or seasonal variation in the concentration of latex compounds. On the other side, we did not evaluate the complete inventory of toxic compounds in the *E. dendroides* latex samples. It is possible that other molecules that play a role in plant defences, as proteases can respond to the presence of ungulates (Sytwala et al., 2015). Therefore, an exhaustive proteomic or metabolomic study might reveal other chemical responses to herbivores not assessed in our study.

5. Conclusion

This evaluation indicates goats negatively impact the demographic structure of the mildly toxic shrub *E. dendroides,* possibly as a consequence of high goat population or a scarcity of more palatable species. Therefore, management strategies must focus on highly affected *E. dendroides* populations, such as those at Muleta, es Verger and Sta. Magdalena. Also, we suggest other low-palatability species that are eventually eaten by ungulates could potentially act as indicators of high herbivory pressure in other regions of the world, such as the case of *Taxus baccata* in mountains and valleys. Therefore, it could be used as a tool for managers to evaluate the ungulates pressure on plant communities through low-palatable shrubs demography evaluations.

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Ethical approval

Not applicable.

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CRediT authorship contribution statement

MC, CC and EB designed the experiment and planted the individuals for the experimental exclusion. EB and JB obtained the financial support for the experiment. MC, CE, MR and EB collected the information of the population structure. MC and EC developed the chemical analysis of latex triterpenoids. MC, CE and EB analysed the data. MC and EB wrote the first draft of the manuscript. All authors have contributed with the definitive version of the manuscript and accepted the final version.

Declaration of competing interest

The authors declare no conflicts of interest.

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