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Acorn removal and seedling age determine oak (*Quercus ilex* L. and *Q. suber* L.) restoration outcome in ungulate-dominated Mediterranean environments

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Abstract

Key message We test the potential benefits of planting 2-year vs. 1-year-old seedlings to restore Mediterranean oak-dominated systems. Planting 2-year-old *Quercus suber* L. seedlings is recommended for improved survival and resilience against wild boar (*Sus scrofa* L.) and drought in dry sandy soils. The removal of acorns in seedlings did not apparently influence leaf biochemical traits and could reduce wild boar damage, particularly in 1-year-old seedlings.

Context In the face of anthropogenic global change, Mediterranean oak-dominated ecosystems confront increased biotic (ungulate herbivory) and abiotic (drought) stressors, compromising forest regeneration. Restoration measures are imperative to address this scenario.

Aims This study assesses the impact of different mitigation measures on the survival and biochemical traits of two oak species.

Methods We planted *Quercus ilex L.* and *Q. suber L.* seedlings in Cabañeros and Doñana National Parks (Spain), subjecting them to three treatments: cotyledon/acorn removal, seedling age (1- vs. 2-year-old), and herbivore protection (fenced vs. non-fenced).

Results Wild boar (*Sus scrofa L.*) damage peaked in winter and early spring, while drought prevailed from late spring to fall. In sandy soils, wild boar uprooted 1-year-old more often than 2-years-old seedlings (40% vs. 18%). One-year-old seedlings without acorns showed higher survival rates against wild boar only in sandy soils. The removal of acorns in seedlings did not influence plant biochemical traits.

Conclusions Planting 2-year-old seedlings in sandy soils may mitigate wild boar damage and improve drought resilience. Seedling age seems more important than acorn removal against biotic and abiotic stressors although acorn removal could reduce wild boar damage in 1-year-old seedlings. Our results underscore the importance of considering multiple stressors in oak restoration strategies.

Keywords *Sus scrofa* L., *Quercus*, Assisted regeneration, Herbivory, Seed predation, Climate change

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1 Introduction

A major challenge facing ecologists today is understanding how global change is altering the structure, diversity, and dynamics of plant communities (Franklin et al. 2016; Pugnaire et al. 2019). Anthropogenic global changes are considered important drivers of these processes, contributing, among other factors, to the decline in tree recruitment rates in many areas of the world (Kerr 2007; Wright 2010; Wu et al. 2019). Analyzing the impact of different mitigation measures aimed at reducing anthropogenic stressors on tree regeneration is imperative and deserves further attention.

In the Mediterranean basin, climate change has produced an increase in the frequency and intensity of drought episodes and heat waves (IPCC 2019) as well as a variation in precipitation patterns (Peñuelas et al. 2004; Fernández-Manjarrés et al. 2018; Noto et al. 2023), leading also to an increase in the intensity of wildfires (Ruffault et al. 2020). Thus, drought is already the main abiotic stress threatening Mediterranean ecosystems and compromising forest regeneration (Peñuelas et al. 2017). In addition, land use changes driven by socio-economic factors are favoring a rapid increase of ungulate populations across the Northern Hemisphere (Côté et al. 2004; Valente et al. 2020; Carpio et al. 2021a). Particularly striking is the dramatic increase in wild ungulate populations in Mediterranean environments in recent decades (Acevedo et al. 2008), especially in highly-diverse Mediterranean oak (genus *Quercus*) ecosystems. Ungulates exert a disproportional effect on plant communities, as ecosystem engineers, which can prevent or hinder natural regeneration of key native Mediterranean oak species (Tyler et al. 2006; Muñoz et al. 2009; San Miguel et al. 2010; Davis et al. 2011; Perea et al. 2014b; López-Sánchez et al. 2021). Ungulates can modify vegetation and steer succession through a variety of mechanisms, such as herbivory (browsing, grazing), physical disturbance (trampling, fraying, uprooting), and nutrient translocation (defecation) (Ramirez et al. 2019; Reimoser et al. 2023).

The Mediterranean basin is a highly anthropized landscape with a long history of human occupation (Blondel 2006). It contains extensive degraded areas due to the excessive exploitation of the land (i.e., intensive agriculture, overgrazing, repeated burning, and deforestation). Restoration is a necessary tool to recover functioning of degraded areas, but this task has become difficult in the new context of climate change. Therefore, many restoration efforts are failing due to the complexities of overcoming the major abiotic and biotic stressors (Castro et al. 2004; Perea and Gil 2014a; Rey-Benayas et al. 2015; López-Sánchez et al. 2019). Thus, new restoration tools are urgently needed based on a better understanding of tree regeneration under the new context and should mostly focus on making ecosystems more resistant (Castro et al. 2004; Pulido et al. 2010).

Plant recruitment is a multi-stage demographic process that can be affected by multiple abiotic and biotic stressors, limiting seed dispersal and germination or seedling and sapling survival. A bottleneck on any of these stages can arrest the entire recruitment process (Pulido and Díaz 2005; Ramirez et al. 2023). In Mediterranean systems, seedling establishment and survival are critical processes for the regeneration of *Quercus* species (Espelta et al. 1995; Retana et al. 1999; Rodríguez-Calcerrada et al. 2010; Arosa et al. 2015; Martínez-Baroja et al. 2022). Among abiotic stresses, summer drought causes most seedling mortality (Rey-Benayas 1998; Gómez-Aparicio et al. 2004, 2008; Marañón et al. 2005; Pulido and Díaz 2005; Gómez and Hódar 2008; Rodríguez-Calcerrada et al. 2010; López-Sánchez et al. 2019) as seedlings have poorly developed root systems and cannot access deep water in the dry period (Matzner et al. 2003; Tyler et al. 2008). Additionally, many biotic stressors affect oak seedlings including pathogens, herbivores, and competition with other plants (Cuesta et al. 2010). Among the damages caused by ungulates, such as deer (Cervidae) or bovids (Bovidae), browsing stands out as one of the major factors constraining oak regeneration in Mediterranean environments (Pulido and Díaz 2005; López-Sánchez et al. 2016; Leal et al. 2022), typically affecting saplings and young bushes more intensively than seedlings (López-Sánchez et al. 2014; Perea et al. 2016; Peláez et al. 2019). In addition, plant palatability plays an important role as preferred plants are more heavily browsed than non-preferred plants (Rooney and Waller 2003; Perea et al. 2014a). Other ungulates, such as wild boars (*Sus scrofa*; Suidae), kill the plants mostly by digging up the whole seedling (Focardi et al. 2000; Gómez et al. 2003; Pulido and Díaz 2005; Gómez and Hódar 2008). Moreover, unlike deer or bovids that primarily feed on fallen acorns, wild boars also consume hoarded acorns that are dispersed by scatter hoarders away from trees, demonstrating a remarkable efficiency in foraging buried acorns in both open areas and shrublands (Herrera 1995; Focardi et al. 2000; Gómez et al. 2003). Wild boars can also uproot growing seedlings to consume their acorn, or accidentally, when looking for other buried food items, leaving the roots exposed and thus causing the death of the plant (Herrera 1995; Focardi et al. 2000; Gómez et al. 2003; Gómez and Hódar 2008; Mayer et al. 2000; Perea and Gil 2014a). Although active restoration by planting is particularly useful to promote forest regeneration (Rey-Benayas 2005; Leiva et al. 2013; Andivia et al. 2021), current ungulate densities in many areas compromise planting success (Perea et al. 2014b; Fern et al. 2020; Skotál et al. 2021).

Some restoration studies have shown that considering spatio-temporal variability of different stressors may improve our understanding of the mechanism underlying seedling survival (Gómez et al. 2003; Gómez-Aparicio et al. 2005; López-Sánchez et al. 2019). Numerous studies have

already pointed out the importance of microsite location for seedling survival, for example, those created by nurse plants, which provide protection against biotic and abiotic factors (Castro et al. 2002; Rey-Benayas et al. 2002; Gómez-Aparicio et al. 2005; Cuesta et al. 2010; Garrote et al. 2019; Peláez et al. 2019). Nursing effects include hiding seedlings from predators (Callaway et al. 2000; Zamora et al. 2004). However, ungulates such as wild boars are able to detect acorns very efficiently, even after they have germinated and remain attached to the seedlings (Groot Bruinderink et al. 1994; Gómez et al. 2003). When seedlings are older and the cotyledon reserves are almost depleted, acorn predators become less pressing (Gómez et al. 2003). Therefore, removing the acorn from the seedling before planting may reduce the impact of wild boars; however, it can also weaken the seedling by arresting the supply of nutrients from the acorn to the growing plant parts. The effect of removing the acorn attached to the seedling on wild boar damage and restoration projects success remains largely unknown (but see Yi et al. 2015). The use of individual protection methods, such as wire cages, has been shown to be an effective method to reduce ungulate damage (Reque and Martin 2015) but it can increase considerably the project budget (Perea and Gil 2014a) and involves a source of waste that is rarely accounted for in restoration management projects. The use of large exclosures is also effective against wild ungulates but their economic (e.g., 2 m high fences, electrified fences, or high-tensile ground cables) and environmental costs (animal collision, habitat fragmentation, unesthetic views) may deter managers from using them, particularly in protected areas (Smith et al. 2020).

Another important consideration in assisted oak regeneration is the intraspecific differences in seedling survival according to their attributes, such as plant size or age (Andivia et al. 2021; Navarro et al. 2006). However, previous studies have reported mixed findings. Some studies have shown higher survival rates among 1-year-old compared to 2-year-old seedlings (González-Rodríguez et al. 2011; Navarro et al. 2006), while others have observed the opposite trend (Bonfil et al. 2020). Furthermore, most of these studies have focused on the effect of seedling age on abiotic stress resistance, and there is limited research on the effect of plant age to improve survival or physiological performance under biotic factors such as wild boar. Leaf biochemical traits are easy to measure in the field (e.g., using leaf-clip optical devices) and may provide useful information about the seedling status (Agati et al. 2016). The physiological responses of oak seedlings may vary depending on the different plant traits and treatments (López-Sánchez et al. 2021). For example, chlorophyll is related to photosynthetic activity and plant vigor (Percival 2005), flavonols and anthocyanins provide a defense against biotic and abiotic stressors (Chalker-Scott 1999; Hernández et al. 2004; López-Sánchez et al. 2021),

and nitrogen, a major plant food, is an essential constituent of proteins and chlorophyll and plays an important role in plant metabolism (Leghari et al. 2016). Thus, the inclusion of biochemical measures provides a more comprehensive understanding of the processes affecting seedling survival and performance and can support the assessment of plant responses to applied treatments.

In this study, we evaluate the effectiveness of two restoration practices to increase *Quercus ilex* L. and *Quercus suber* L. seedling survival in two Mediterranean National Parks in the Iberian Peninsula, where wild ungulates (red deer—*Cervus elaphus* L. and wild boar—*Sus scrofa* L.) occur in high densities. Specifically, we investigated the effect of cotyledon removal (manual acorn extirpation) and plant age (1- vs. 2-year-old seedlings) on seedling survival and leaf biochemical traits.

We specifically hypothesized that:

a) Higher mortality due to wild boars will be observed in 1-year-old vs. 2-year-old seedlings because of the higher acorn nutrient content in 1-year-olds (Gómez et al. 2003).
b) Seedlings without the acorn attached would display higher survival rates as they will not be so frequently damaged by wild boars.
c) Removing the acorn attached to the seedling will not significantly affect leaf biochemical traits especially in the case of 2-year-old seedlings.
d) Two-year-old seedlings will show higher resilience against drought than 1-year-old because of their deeper and more developed root system.

2 Materials and methods
2.1 Study area
The experiment was conducted in two Spanish National Parks (NPs): Cabañeros NP (central Spain; 39°23′ N, 4°29′ W) and Doñana NP (southwestern Spain; 37°01′ N, 6°30′ W; Fig. 1), both with Mediterranean climate characterized by dry and hot summers. Doñana NP is considered sub-humid, with an Atlantic influence that moderates temperatures in winter and summer (600 mm average annual rainfall, AAR, and 17 °C mean T; Miteco 2011). Cabañeros NP experiences colder winters (550 mm AAR, 12.4 °C mean T; García-Herrera et al. 2011), owing to its inland location and higher elevation compared to Doñana (700 m a.s.l. vs. 10 m a.s.l.). Cabañeros NP has two distinct geographical features known as the "Sierras" and "Rañas". The Sierras are mountainous terrains characterized by the presence of quartzite and siliceous slate, while the "Rañas" are expansive plains formed by the accumulation of clay and quartzite pebbles from alluvial deposits along the hillside. These Rañas have an argillic horizon that causes an extensive hydromorphy that acidifies the soil (Pardo
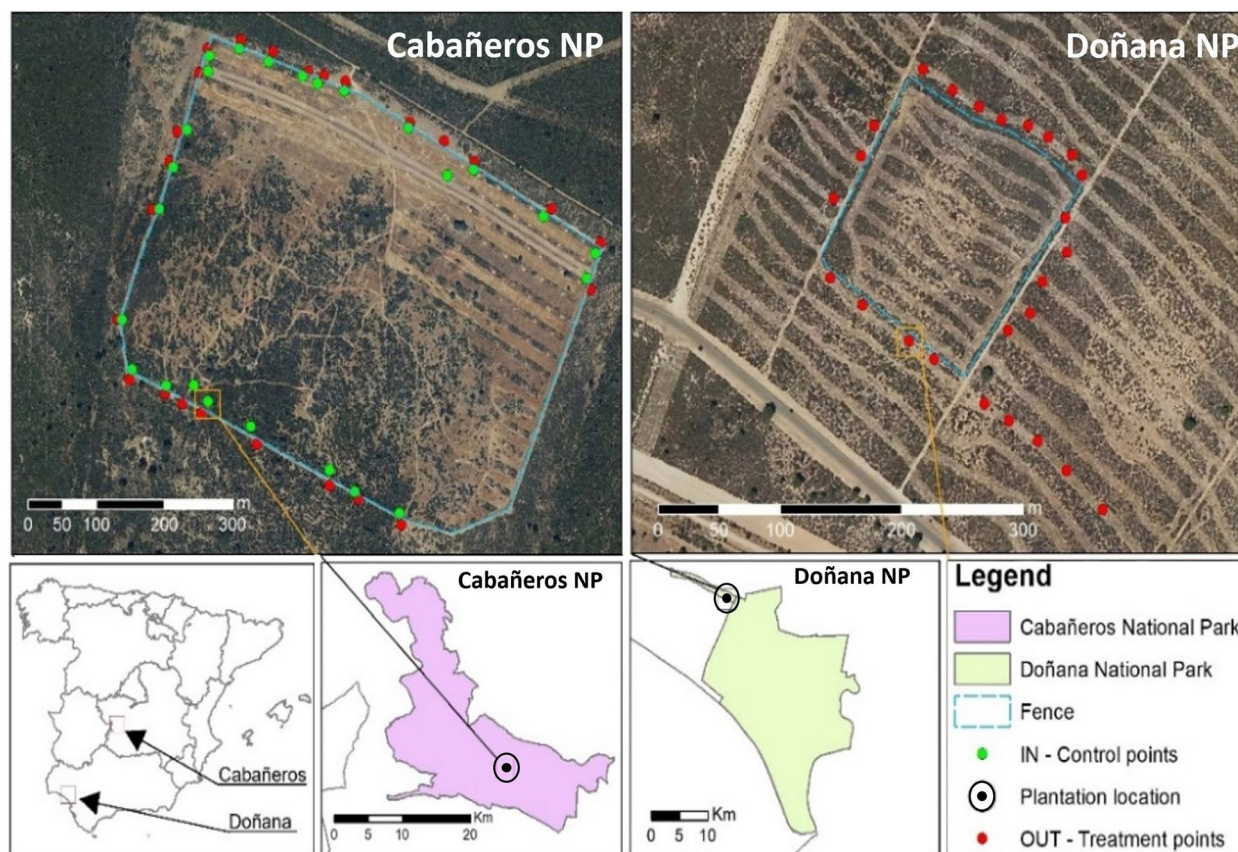
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Fig. 1 Experimental design with the location of the sites. Dots show the locations where seedlings were planted, both outside the fence (red) and inside the fence (green). Blue lines delimit the fenced exclosure perimeter

García 1995). Doñana is the result of coastal-littoral sedimentation processes that have formed a large aeolian landform. Soils are poor in organic matter, slightly acidic, and consisting mainly of sand with some silt and clay. Low water retention capacity of these soils allows rapid water circulation (Clemente et al. 1998).

The vegetation in Cabañeros is dominated by sclerophyllous and semideciduous oak forests and woodlands composed by *Quercus ilex* L., *Quercus suber* L., *Quercus faginea* Lam., and *Quercus pyrenaica* Willd., with an understory dominated mainly by evergreen shrubs such as *Cistus ladanifer* L., *Erica* spp., *Rosmarinus officinalis* L., *Lavandula pedunculata* (Mill.) Cav., *Thymus mastichina* L., *Phillyrea angustifolia* L., and *Rubus ulmifolius* Schott (Perea et al. 2015a). Doñana vegetation is dominated by both monospecific and mixed forests of *Pinus pinea* L. and *Quercus suber*, alongside extensive areas of shrubland. The composition of the shrubland vegetation varies significantly depending on the soil phreatic properties, resulting in two distinct categories referred to as "Monte Blanco" and "Monte Negro". "Monte Blanco" encompasses open shrublands found on drier soils, featuring a variety of plant species, including *Halimium halimifolium* (L.) Willk., *Rosmarinus officinalis*

L., *Ulex australis* Clemente, *Cistus libanotis* L., *Lavandula stoechas* L., and *Thymus mastichina* L. In contrast, "Monte Negro" is distinguished by its dense shrublands, thriving on humid soils and boasting a diverse flora that includes *Erica* spp., *Calluna vulgaris* L., *Phillyrea angustifolia*, *Rubus ulmifolius*, *Ulex minor* Roth, and *Ulex australis*.

Both National Parks host high density of wild ungulates, with wild boar and the red deer as the most abundant species (Perea and Gil 2014a; Gortázar et al. 2008). In Cabañeros, current densities are 31 individuals (ind.) per km^2 for red deer and 2.5 ind./km^2 for wild boar (Linares and Urivelarrea 2020), and in Doñana, 6 and 5 ind./km^2, respectively (Laguna 2015), although ungulate pressure strongly varies spatially and seasonally, depending on food resource distribution. In both parks, the regeneration processes of the main oak species are in decline partly due to high pressure from wild ungulates (Perea and Gil 2014a; Fedriani et al. 2016). Currently, populations of red deer and wild boar numbers are controlled by rangers to reduce ungulate overabundance (Linares and Urivelarrea 2020). Density of lagomorphs (rabbit—*Oryctolagus cuniculus* L. and hare—*Lepus granatensis* Rosenhauer) for Doñana are 2.5 individuals per km^2 (Beltrán et al. 2022).

In Cabañeros, lagomorph density is unknown but presumably very low (Delibes-Mateos et al. 2008).

During the study period (February to September), the accumulated precipitation in Cabañeros and Doñana was 210 mm and 174 mm, respectively, 4% and 34% lower than the average of the last 20 years (2000–2020; Figure 5 in Appendix). Moreover, in the crucial month of May (López-Sánchez et al. 2019), which marks the onset and length of summer drought in Mediterranean environments, both Cabañeros and Doñana had 11 mm and 10 mm of rain, 70% and 63% below the respective averages, resulting in an earlier onset of the summer drought.

2.2 Experimental design

The experimental sites in both NPs were situated in shrublands characterized by a low tree density that historically had higher tree density. Specifically, in Cabañeros NP, the study was conducted in a transition zone between the "Raña" and the mountain ranges "Sierras." In Doñana, the experimental site was located in an area dominated by "Monte Blanco" vegetation. In both parks, a wild ungulate fenced exclosure, proved effective for wild boar, was used as the study site (see Fig. 1).

In Cabañeros, the fence consisted of a 2-m-high wire fence. The planting was made inside and outside the fence to evaluate ungulate pressure. Photo-trapping (Browning motion detection cameras, Model BTC-5PXD) was used to confirm that no ungulates enter the exclosure and to identify the animals interacting with the planted seedlings. Thus, three cameras were located inside and three outside the exclosure from February to July. Cameras were pointing at the seedlings and were rotated among plantation sites as they were killed or died (Figure 6 in Appendix). No medium-size mammals (lagomorphs) or large ungulate were detected during photo-trapping inside the fence in Cabañeros and thus, the inside area (with none of these herbivores) was used as the control treatment (see Fig. 1). In Doñana NP, there was no control treatment as rabbits were abundant within the fenced area. Thus, only the outside area of the fence was used as a replicate of the "outside the fence" treatment in Cabañeros NP.

In each park, 25 planting sites (ca. 7 m×7 m each) around the fenced area were selected at a minimum distance of 20 m from each other to ensure independence. Each planting site had the same ecological conditions (soil, rock cover, microtopography, plant composition). Two different treatments were set-up at each point: (i) cotyledon removal: seedlings with acorn attached and seedlings without acorn and (ii) plant age: a pair of 1- and 2-year-old seedlings was planted at a distance of 50 cm from each other. In Cabañeros, this was repeated inside the fence as a control plot without mammal herbivory (fenced treatment). In February 2021, just before the onset of the growing season, we planted 200

Quercus ilex seedlings in 25 points×2 acorn treatments×2 ages×2 fenced sites; in Doñana, we planted 100 *Quercus suber* seedlings in 25 points×2 acorn treatments×2 ages. We selected pairs of seedlings of similar height, basal diameter, and number of leaves for each treatment.

Plant material employed in this experiment was sourced from the same locations as the respective park authorities' ongoing restoration projects. Seedlings for Cabañeros National Park were obtained from the park's nursery, while those for Doñana National Park were acquired from the Seville Council plant nursery (Junta de Andalucía). In both cases, the genetic material was collected within each park.

All seedlings were supplied in standard propagation trays, each containing 35 individual plants. Each individual container measured 50 mm in diameter and was 300 mm in depth and was filled with a growth medium of a mixture of peat and perlite (3:1) with mycorrhiza. Prior to planting, the plants underwent a hardening process by spending several months outdoors.

For soil preparation, we created conical holes with the aid of hoes, ensuring they had a minimum of 40 cm diameter and 40 cm depth. When positioning the plant, we ensured that the root base was appropriately covered, after which we proceeded to fill the hole and gently pressed the soil around the root system, eliminating any air pockets. At the time of planting, seedlings were labeled and their GPS coordinates recorded.

Monthly measurements were conducted from February to September. Planting and following measurements were performed 15 days earlier in Doñana (beginning of each month) than in Cabañeros (middle of each month). Each visit consisted of recording: the seedling survival status (alive vs. dead), the damage caused by biotic and abiotic stress agents, and the identification of the main stress agent, namely wild boar, deer, rabbit, fungi, insect, or drought. The damage caused by wild boar was identified by overturned soil from rooting and plant digging or uprooting (Herrera 1995); deer damage was visible by torn twigs from browsing; rabbit damage was characterized by the sharp clipping of terminal shoots at an angle; fungal damage was observed as patterns of foliar chlorosis and wilting compatible with different fungal infestations; insect damage was recognized as defoliated leaves or the presence of nests; and drought was evidenced by leaf dry-out (see López-Sánchez et al. 2019; Perea et al. 2015a, b). Seedling damage was quantified as an indicator of potential stress, using a damage score ranging from 0 to 5: 0=no evidence of damage; 1=light (<10% of the plant biomass damaged); 2=low (10–30% of damaged); 3=intense (30–60% of damaged); 4=heavy (60–90%); and 5=maximum damage (>90% of the plant biomass damaged; Fernández-Olalla et al. 2006; Perea et al. 2015a, b). Fungi, rabbits, and insects were minor stress agents in our study area compared to ungulates and drought and barely

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caused the mortality of plants. For simplicity, we considered the main stress agent the one leading to the plant death, or, in the case of surviving seedlings, the one identified with the highest intensity score.

A final follow-up assessment was conducted 6 months later (February or March 2022), to evaluate seedling long-term survival and to identify any instances of resprouting after the above-ground portions had dried out in the last visit, which were then classified as dead. To compare leaf biochemical responses to different treatments and plant ages, several non-destructive measures of chlorophyll, anthocyanin, and flavonoid concentrations and the nitrogen balance index (NBI) were taken using the leaf clamp fluorescence sensor Dualex® TMScientific+, Force One (Agati et al. 2016). The Dualex device estimates the chlorophyll content of the leaf using a transmittance ratio at two different wavelengths, and the flavonoid and anthocyanin content of the leaf epidermis using a differential chlorophyll fluorescence ratio (ForceA-Dualex n.d.; Zhang et al. 2019). NBI is a proxy of nitrogen status and nitrogen to carbon allocation in plants based on the ratio between the chlorophyll and flavonol content (Cartelat et al. 2005; Agati et al. 2016). The Dualex measures were taken on 5 leaves previously marked, from top to bottom, only in alive seedlings.

2.3 Data analysis

We used multinomial models to assess the probability of stress agent occurrence on each park. The response variable was the type of stressor (a multinomial factor) while predictors were time (month of main stress occurrence) and the treatments: acorn (with acorn vs. no acorn; hereafter Acorn), seedling age (1 vs. 2 years; hereafter Age), fencing (inside vs. outside the fence; only in Cabañeros; hereafter Fence), and their two-way interactions. Due to the differences in experimental design, different models were performed for each NP. The location point of each seedling ($n=25$ points per NP) was also included as cluster (random effect) in the model to group correlated observations. To build the models, we used the function *multinom* from the R package "nnet" (Ripley and Venables 2016). We also used the function "*mnl_pred_ova*" from the package "MNLpred" to graphically represent model predictions. We selected all variables from models with ΔAIC < 2 (top models) and calculated the importance of each variable following Burnham and Anderson (2002). For this purpose, we used the functions *dredge* and *importance* "*sw*" from the package "MuMIn" (Bartón 2023).

Similarly, to analyze differences in seedling survival according to the different treatments (Acorn, Age, and Fence), we used Cox proportional hazard models (Cox 1972), checking the assumptions through model diagnostics. Response variable was time at death (in months). Seedling survival was also included in the model codified

as 0 if the seedling was alive at the end of the study (censored data) and 1 if it was dead (event). Predictors were the treatments and their two-way interactions. We also included the location point of each seedling ($n=25$) as cluster (random effect) in the model to group correlated observations. We used the function *coxph* from the R "survival" package (Therneau 2015) to calculate the estimated time at death for each treatment and to disentangle differences in predicted time at death among treatments. We used the function *survfit* and *ggsurvplot* from the library "survminer" (Kassambara et al. 2017) to generate the figures. We reported full model results and compared differences among predictors and their significance levels.

In addition, to evaluate the effect of removing the acorn on seedling leaf biochemical variables, we computed least-squares means predictions from a linear model to compare seedlings of the same age with and without acorn for each month. Response variables were the four biochemical metrics (chlorophyll, flavonols, anthocyanins, and the nitrogen balance index). As predictors, we included the two-way interaction between month and treatments (Age and Acorn). We used the function *lm* from the package "stats" to perform the model and the function "lsmeans" to generate the contrasts. The analysis utilized the dataset available from the e-cienciaDatos—UPM repository (Pelaez Beato et al. 2024). Statistical analyses were performed using the R software, version 4.1.2 (R Core Team 2024), available at www.r-project.org.

3 Results

3.1 Stress agents and their effect on oak seedling survival

In Cabañeros NP, almost all seedlings ($N=197/200$) experienced some type of stress; 4% of seedlings experienced at least one, 33% two, 49% three, and 12% four or more stress agents. Thus, at the end of the experiment, 95.5% of the seedlings died ($N=191$). Inside the fence, drought killed 96% of the seedlings and was the only cause of mortality (100%). In the non-fenced treatment, 95% of seedlings died, with 74% affected mainly by drought, 23% by wild boar damage, 2% by transplanting shock, and 1% by lagomorphs (hare/rabbit) herbivory. When wild boar or rabbit was the main stress agent, no seedling survived and when drought was the main stress, only 4% of seedlings survived. Damage caused by deer, fungi, and insect was not strong enough to cause the death of any seedling, even though the damage caused in some seedlings was severe, with 50% of the leaves showing damage.

In Doñana (non-fenced), most seedlings ($N=99/100$) experienced at least one stressor during the study period. Many of those also suffered from two (10%), three (26%), or even four or more stress agents (35%). The damage caused by these stress agents was so strong that no seedlings survived at the end of the experiment (all were dead

by the beginning of August). Main mortality agents were drought (68%) and wild boar (31%) while 1% died for unexpected anthropogenic causes (e.g., crushed by a car).

As expected, we found a strong spatio-temporal variation in the occurrence of the main stress agents in Cabañeros. Results from the multinomial model suggested that the predominant stress type was determined by Time, Fence, and Age (model averaged results based on ΔAIC < 2; variable importance was 1 for Time and Fenced and 0.43 for Age). Acorn or any interaction among variables were not included in the top models (ΔAIC < 2).

First, at the spatial level, we found that drought was the only cause of mortality inside the fence, while biotic factors also influenced mortality outside the fence, such as wild boar and rabbit (Fig. 2a). Second, these factors also had a marked temporal pattern, with higher risk of mortality by wild boar at the beginning of the study (from February to mid-May) and higher risk of mortality by drought after the onset of the dry season (from June to September). Finally, although Age was an important factor in the model, we found no significant differences in the incidence of different stressors between 1- and 2-year-old seedlings ($P = 0.154$).

In Doñana (non-fenced), we also found a clear temporal pattern of the main stresses. Model selection indicated that only the variable Time was key to predict the main stress probability. Wild boars were particularly active from February to mid-April, reducing their activity after mid-April (Fig. 2b). In contrast, drought dramatically increased from mid-April onwards, being the main cause of mortality. Finally, the effect of drought started 1 month earlier in Doñana compared to Cabañeros (Fig. 2).

3.2 Effect of acorn removal and plant age on the survival of oak seedlings

In Cabañeros, results from the proportional hazard model revealed no significant differences in seedling survival between any of the treatments at the end of the experiment (Table 1 in Appendix). No significant differences were found between the fenced vs. non-fenced areas, as seedling survival in September was very low (4.5% of the total; $N = 9$). Furthermore, seedling survival depending on plant age or acorn treatment was similar outside and inside the fence at the end of the experiment (Table 1 in Appendix).

However, as Fig. 2a shows, some temporal variation of seedling survival were observed within the first months of the study (February to May), when wild boar was the main mortality cause (90% of mortality caused by wild boar outside the fenced exclosure in Cabañeros NP). For this reason, we repeated the analysis using only the subset of data before June (Table 1 in Appendix).

As expected, the results in Cabañeros for this time period showed a significant increase in seedling survival inside the fenced exclosure for all combinations of plant

age and acorn presence (Table 2 in Appendix). Furthermore, before June all seedlings inside the fenced area were still alive (100%) while, outside, there was a significant decrease in survival (89%). No significant differences for plant Age or Acorn treatment were found outside or inside the fence. However, as indicated by the model coefficient sign, a non-significant decrease in survival probability was found on seedlings with acorns when compared to their same-age pair without acorn (80% 1-year-Acorn vs. 84% 1-year-No Acorn; $P = 0.578$ and 76% 2-years-Acorn vs. 84% 2-years-No Acorn; $P = 0.698$; Table 3 in Appendix).

In Doñana (non-fenced), survival results mirrored those in Cabañeros, although in this case, no plant survived by the end of the experiment (0% of the total; $N = 0$). Hence, cox proportional hazard models showed no significant differences in seedling survival at the end of the study for any of the treatments (Age or Acorn; Table 1 in Appendix). However, when restricting the analysis to the period of activity of wild boar (i.e., from February until mid-April), 1-year-old seedling survival was significantly lower compared to 2-year-old seedlings ($P < 0.05$; Fig. 3; Table 4 in Appendix). Thus, wild boar killed up to 40% of all 1-year-old seedlings and 18% of 2-years-old during the first months of study. Furthermore, within the 1-year-old group, a non-significant but noticeable decrease in survival probability for the plants with acorns was observed (48% 1-year-acorn vs. 64% 1-year-No Acorns; $P = 0.2455$; Table 4 in Appendix) but not for the 2-years-old group (80% 2-year-Acorn vs. 80% 2-year-No Acorn; $P = 0.81$; Table 4 in Appendix).

Next year, in February and March 2022, we could confirm that no plant resprouted in Doñana, indicating that all plants died before September 2021. In Cabañeros, however, out of the nine seedlings that initially survived until September ($N = 9/200$), a 2-year-old without acorns outside the fence did not survive the winter, while a 1-year-old seedling with an acorn placed outside the fence, previously categorized as deceased, exhibited signs of resprouting.

3.3 Effect of seedling age and acorn treatment on leaf biochemical parameters

At the beginning of the study, there were no significant differences between seedlings of the same age assigned to each treatment (Acorn and Fence; Fig. 4). However, slightly higher values of chlorophyll and nitrogen (nitrogen balance index; NBI) could be observed for 2-year-old compared to 1-year-old seedlings, although these differences were not significant. Overall, we observed a decreasing trend in content of chlorophyll and NBI over time and an increase in anthocyanins. Flavonol content did not show a clear temporal pattern.

For each month and plant age, the removal of plant cotyledons did not significantly affect chlorophyll, NBI, flavonols, or anthocyanins (Fig. 4; Table 5 in Appendix).
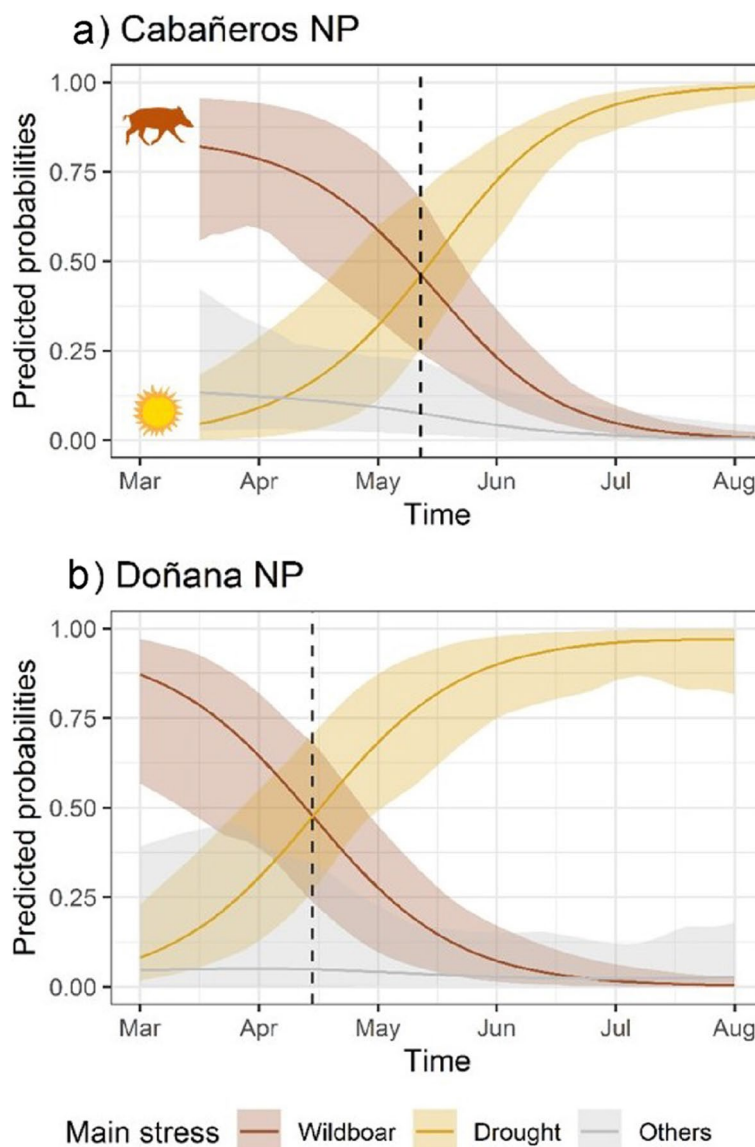
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Fig. 2 Results from the multinomial model to analyze the temporal variation of main stress occurrence outside the fence in Cabañeros (**a**) and Doñana (**b**). Note that data collection started 15 days later for Cabañeros compared to Doñana

Similarly, in the fenced treatment, with no wild boar activity, we found higher chlorophyll and NBI in April in 2-year-old seedlings than in 1-year-old seedlings. In the following months (at the onset of the drought season; June) 1-year-old seedlings showed higher mortality rates (Fig. 3a).

Sample size in Doñana was too small for statistical analysis of leaf biochemical variables due to high wild boar damage at the beginning of the study and the severity of drought early in the dry season.

4 Discussion

4.1 Spatio-temporal variation in stress agent occurrence

In this study, drought emerged as the predominant abiotic stressor contributing to seedling mortality, with wild boar

as the secondary (biotic) factor, consistent with previous research conducted in Mediterranean environments (Rey-Benayas 1998; Gómez-Aparicio et al. 2008; Perea and Gil 2014a). However, our results show a strong temporal variation in the dominance and intensity of these biotic and abiotic stressors that can influence the establishment of *Quercus* seedlings. Furthermore, this temporal dimension determined the lethality of each stressor: biotic effects on mortality occurred earlier and lasted longer than the abiotic stressors, a hierarchical effect that has also been reported in other studies (Cahill et al. 2003; Gómez-Aparicio et al. 2008; Perea et al. 2014b). Therefore, when performing forest restorations with seedlings to assist natural regeneration, we should evaluate not only the main biotic and
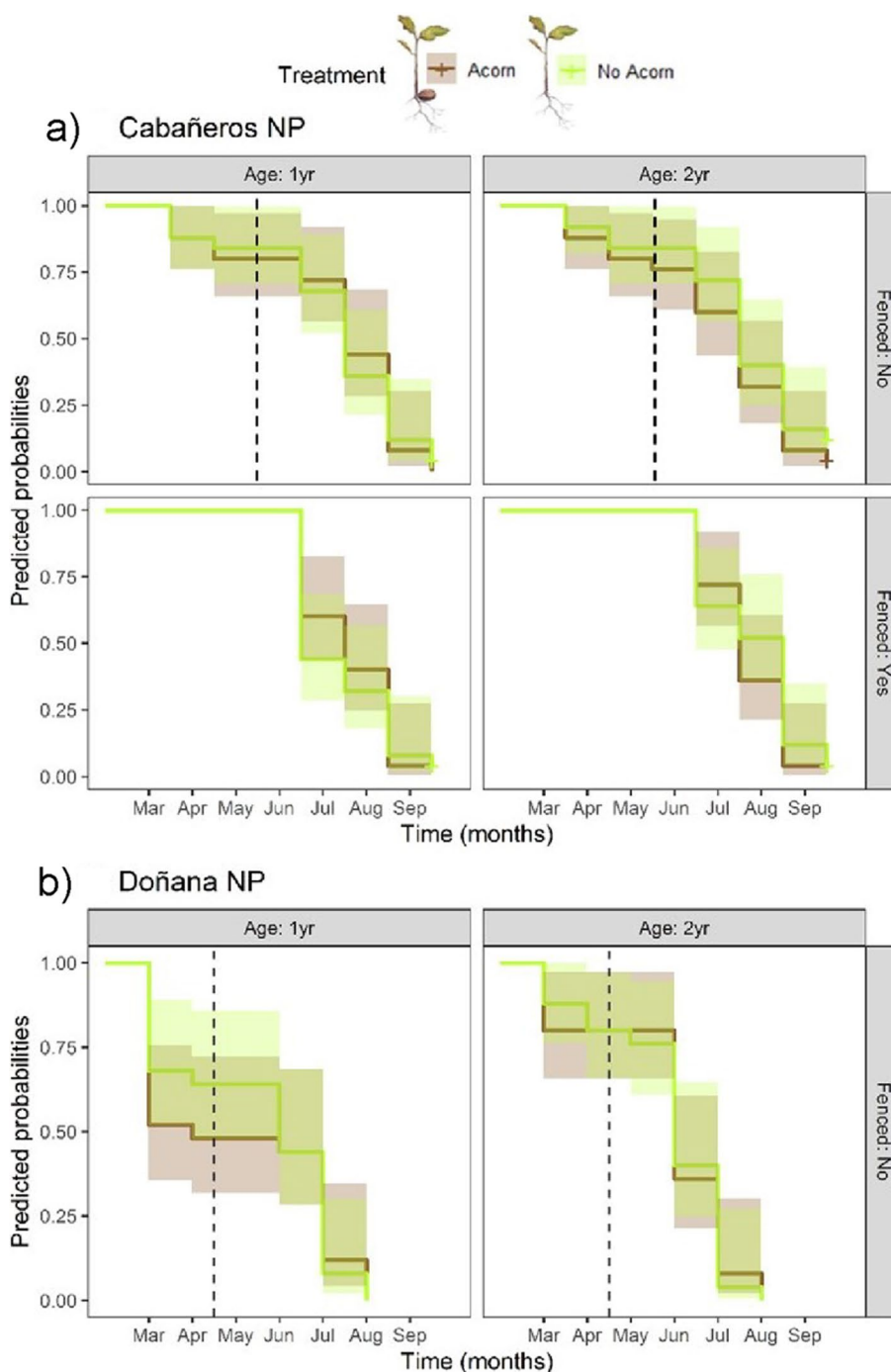
Fig. 3 Seedling survival probability over time (in months) across the different treatments: (1) acorn: with (dark-brown) and without cotyledons (light-green); plant age: 1-year vs. 2-years-old; and fenced: fenced vs. non-fenced. **a** Results observed in Cabañeros National Park and **b** in Doñana National Park. Dashed vertical lines divides the two periods of the study where importance of main stressors differed: (1) wild boar (left) and (2) drought (right)

abiotic factors occurring at a particular site but also their time-dependent effect in order to increase the success of the planting (Gómez-Aparicio et al. 2012). In the context of global change, drought is a major cause of seedling mortality in restoration projects in Mediterranean environments

(Rey-Benayas 1998; Gómez-Aparicio et al. 2008; Perea and Gil 2014a; López-Sánchez et al. 2019). A decrease in the frequency of wet years can be detrimental for recruitment of woody species in the Mediterranean (more than an increase in drought intensity), given the window of opportunity that
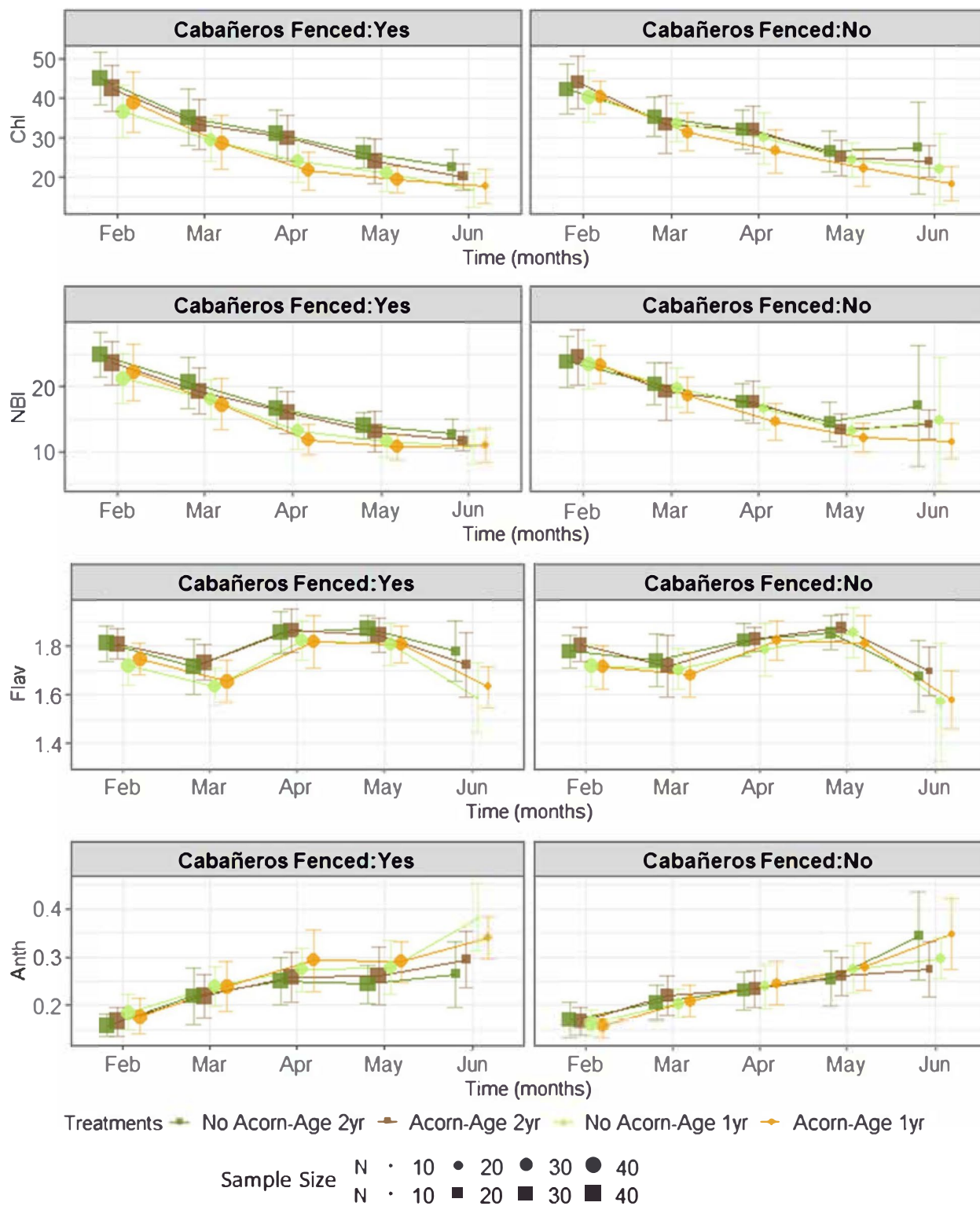
Fig. 4 Average values of leaf biochemical parameters of the seedlings of Cabañeros NP [chlorophyll content (Chl), nitrogen balance index (NBI), flavonoid content (Flav), and anthocyanin content (Anth)] for the three factors: (1) age (1- and 2-year-old seedlings; dark colors vs. light colors), (2) acorn attached or not (brown vs. green), and (3) fenced vs. non-fenced (left column vs. right). The standard deviation for the data set is shown. We displayed only data from February to June as sample size during July, August, and September was very low. Size of symbols is proportional to sample size

wet years imply for seedling survival (Matías et al. 2012). However, many oak seedlings will have no chance to survive the dry period if they are previously damaged by biotic stresses (i.e., wild boar) as very few seedlings will reach the onset of the dry season (Gómez and Hódar 2008; Perea and Gil 2014a). Reducing or controlling wild ungulate overpopulation is advisable in most ecosystems (Martínez-Jauregui et al. 2020; Carpio et al. 2021a, b) although might be very challenging, particularly in protected areas where hunting is typically forbidden or incompatible with other uses (recreation, wildlife preservation and observation, hiking, etc.). However, most studies agree that oak systems are highly resilient and provide more useful ecosystem services than other systems such as shrublands (e.g., more valuable food sources for wildlife, shading for animals and plant species, reduced risk of wildfire compared to shrublands, and increased biodiversity levels). Our results confirm that these ancient systems (oak savannas) are currently in danger because of high seed predation and seedling mortality, mostly due to ungulate management and summer drought (Pulido and Díaz 2005; Perea and Gil 2014a, b; Perea et al. 2017). Our findings suggest that alternative restoration approaches should adapt to future scenarios of increasing drought and herbivory, for instance by using 2-year-old seedlings, which are seemingly more resistant to drought and less attractive to wild boars. Importantly, the use of evergreen oaks compared to deciduous oak species seems recommended as Mediterranean evergreen oaks are typically less damaged or more resistant to both herbivory and drought (Montserrat-Martí et al. 2009; Perea et al. 2017; López-Sánchez et al. 2019). However, further studies should compare the effect of acorn removal on seedling survival of evergreen vs. deciduous oak species.

We observed that wild boar rooting activity was higher at the beginning of the study (late winter and spring) than during the summer. This agrees with most of the studies reporting wild boar rooting activity in Mediterranean environments (Focardi et al. 2000; Mayer et al. 2000; Cahill et al. 2003; Gómez et al. 2003; Pulido and Díaz 2005; Gómez and Hódar 2008; Perea and Gil 2014a). This behavior is usually attributed to the fact that late winter and early spring soils contained more water and are softer and easier to dig up by boars than in summer (Cahill et al. 2003) and that food availability in winter is typically lower. Also buried seeds might be easier to detect olfactorily at this time when they have higher water content than during the summer, when they are desiccated (Perea and Gil 2014a). Spatially, we observed that the ungulate fenced exclosure was the best method to increase survival of the seedlings during the first months after planting. However, fencing inside National Parks is a contentious topic because of the high visual impact and its associated high cost to build and maintain (Hayward and Kerley 2009). Fencing is very

costly (2 m high wire fence, ca. 15–25€/m), and particularly against wild boars because a tense thick wire at the ground level is necessary to avoid wild boar entering. In addition, having lots of wire exclosures in protected areas may cause important detrimental effects such as animal collision, habitat fragmentation, or decreased ecological connectivity (Hayward and Kerley 2009; Smith et al. 2020). Therefore, testing different restoration techniques such as the use of older seedlings and/or removing the acorns attached to the seedlings before planting them can potentially reduce the negative impacts of wild ungulates more efficiently and with less economic and environmental cost.

4.2 Effect of acorn removal and plant age on the survival of oak seedlings

Overall, our results agreed with our main hypothesis that wild boars, while actively searching for acorns during early spring, mostly focused on seedlings with a potentially nutrient-filled acorn still attached. As seedlings age (from 1 to 2 years), acorns become rotten and less nutritious (Gómez et al. 2003) and therefore, less attractive to wild boar. However, this preference was only found in Doñana (Fig. 3b) where two main factors (1) higher wild boar densities and (2) decreased soil resistance associated to sandy soils (compared to the rocky and highly clay dominant soils of Cabañeros) may explain the increased seedling damage. The combined effect of both factors may enable wild boar to overturn extensive areas in Doñana with minimum effort (see Fern et al. 2020). Thus, the higher damage inflicted in Doñana has probably facilitated the detection of a significant trend in the selection pattern of wild boar.

Furthermore, we observed a hierarchical pattern of wild boar preferring 1-year-old seedlings over 2-year-old seedlings and, within each age group, a non-significant but consistent preference toward those with the acorn still attached, especially among the 1-year-old group. Indeed, as acorns decompose, they give off an odor that can attract animals (Colville et al. 2012; Löf et al. 2019). However, our results indicate that acorn presence may not be as relevant as plant age. Fern et al. (2020) found that wild boars uproot seedlings more frequently if they recognize the species as a good food, and thus, their decisions may be influenced by their previous experiences. Similarly, we hypothesize that wild boar could be more attracted to certain plant traits based on experiences finding nutritious acorns in younger plants. Other authors have suggested that freshly overturned or disturbed soil around the seedlings is their main attractant (Mayer et al. 2000; Skoták et al. 2021). Although we believe this could be true in some cases and it is a factor to consider after digging the holes for planting, in this study, wild boar displayed a clear selective pattern toward 1-year-old seedlings in the early period after planting (Feb–May). Furthermore, Mayer et al. (2000) found that wild

boar did not randomly uproot all nine species used in their experimental restoration project. In contrast, only four of the nine species were affected by wild boar, being two of them oak species. Out of these four species, three had been uprooted and had their rootstock consumed: cherrybark oaks (*Q. pagodaefolia* Raf.), swamp chestnut oak (*Q. michauxii* Nutt.), and water hickory (*Carya aquatica* (F.Michx) Nutt.). It is noteworthy that these three species are recognized as being preyed upon by wild boars for their seeds. Thus, we strongly believe that, in their search, wild boars are showing a preference for some species that they feed on, although sometimes they may also investigate disturbed soils. In summary, a combination of ecological factors likely influence wild boar's decision to uproot specific seedlings (Mayer et al. 2000; Fern et al. 2020; Skoták et al. 2021). Unfortunately, sample size in our study did not allow for strong conclusions on the interaction between the main treatments (i.e., age and acorn). Nevertheless, our findings do reveal a noteworthy trend of preference toward plants with acorns still attached, particularly within the 1-year-old group. We are confident that future investigations would gain greater insight by increasing the sample size.

Unfortunately, water stress is a very difficult abiotic factor to overcome, and droughts are expected to increase in the Mediterranean basin due to climate change (IPCC 2021). To enable seedlings to resist water stress during the summer, some authors recommend applying irrigation during the peak of the dry season (Rey-Benayas 1998; Siles et al. 2010) or applying drought hardening in the nursery to gain drought tolerance and transplanting performance in some oak species (Villar-Salvador et al. 2004). Further experimental studies on the recognized benefits of irrigation during the first summer (Rey-Benayas 1998; Siles et al. 2010) should also quantify the possible detrimental effect of this treatment in ungulate-dominated areas as it may imply a trade-off, by re-hydrating the acorn, and thus making the acorn easier to detect olfactorily by wild boars (Perea and Gil 2014a).

4.3 Effect of acorn removal and plant age on leaf biochemical traits

Additionally, we found that removing the cotyledon reserves did not affect biochemical traits of the seedlings (i.e., content of chlorophyll, NBI, flavonols, and anthocyanin). Indeed, these results are in line with other studies, which have also demonstrated that removing the cotyledons to oak seedlings does not affect seedling growth or survival (Sonesson 1994; Yi et al. 2015), as long as acorn removal is performed when the seedling is no longer heavily dependent on these resources (i.e., 1 month after emergence; Li and Ma 2003). While more research is needed to determine the positive impact of removing the acorn in wild boar-dominated environments, the current

evidence suggests that, at least, it is not harmful to the future survival and biochemical traits of the seedling. This is important because acorn removal in 1-year-old seedlings would not apparently involve any additional cost to this restoration approach compared to fencing or waiting for another year (2-year-old seedlings) to plant. However, 2-year-old seedling performed better for some biochemical variables such as chlorophyll and nitrogen (Fig. 4), two important parameters in determining plant vigor (Capó et al. 2024). These differences seem negligible in early spring and become particularly larger during the summer drought, suggesting greater performance for 2-year-old seedling compared to 1-year-old seedling under dry conditions. This better performance is eventually transferred to the overall seedling survival, suggesting the use of 2-year-old seedlings in the future Mediterranean scenarios with longer and more severe summer droughts (IPCC 2021).

5 Conclusions

This study provides valuable insights into the role of wild boar as a limiting agent for oak recruitment and its negative impact on the management of restoration projects. A combination of factors, such as age and acorn removal, can significantly influence the level of wild boar damage on oak seedlings and their overall survival.

The main findings of this study are:

- Two-year-old seedlings were less likely to be damaged by wild boars than 1-year-old seedlings in areas with high wild boar abundance and sandy soils, such as Doñana NP but not on the clay dominant soils of Cabañeros NP.
- Despite the lack of statistical significance, a trend suggesting decreased survival probability was observed in seedlings of the same age when acorns were present compared to those without acorns.
- Since the removal of acorns did not appear to negatively influence leaf biochemical traits, our findings imply that, for restoration projects with 1-year-old seedlings, detaching the acorn could be a useful management strategy to reduce wild boar damage.
- Finally, 2-year-old seedlings seem to increase resilience against drought in both sites. Thus, planting 2-year-old seedlings instead of 1-year-old seedlings could be a good strategy to avoid damage by wild boar just after planting and improve drought resistance.

Early mortality and, thus, sample size was one of the main limitations of our study and further experimental restoration projects should focus on planting higher number of seedlings per treatment. These investigations are important due to the significant damage that both wild boars and summer drought may inflict on oak seedlings.

Appendix

Fig. 5 **a** Meteorological data from the Porzuna weather station (14.3 km away from the plantation in Cabañeros) obtained through the SIAR (Irrigation
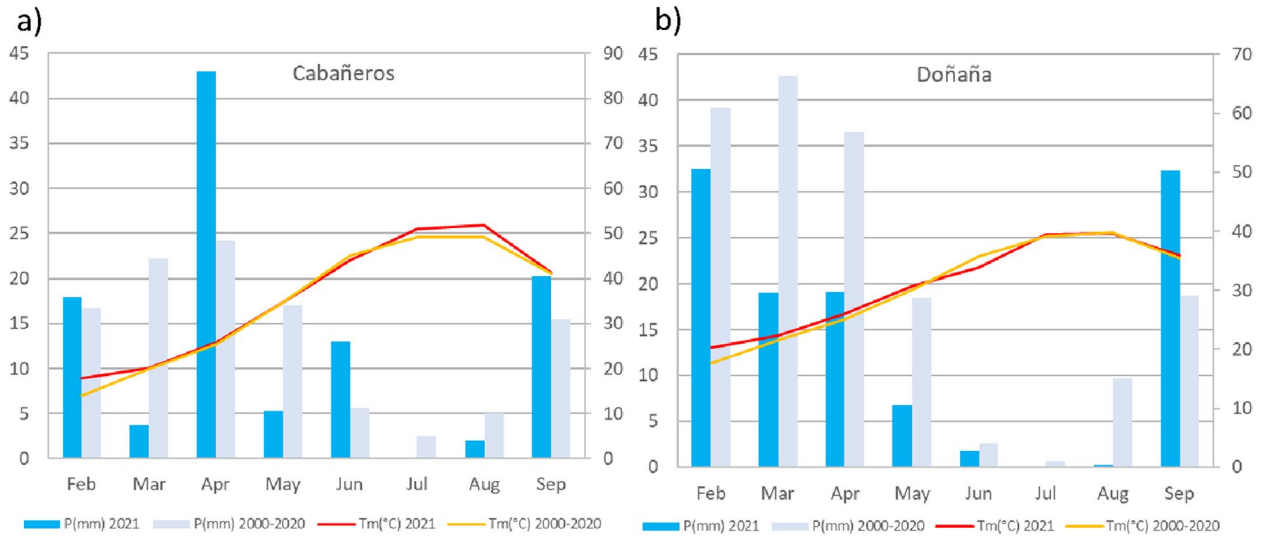
Advisory Service of Castilla-La Mancha) of the Consejería de Agricultura, Agua y Desarrollo Rural de Castilla-La Mancha (Department of Agriculture, Water and Rural Development of Castilla-La Mancha). **b** Meteorological data from the Almonte station (14.1 km away from the plantation in Doñana) obtained through the RIA (Red de Información Agroclimática de Andalucía) of the Junta de Andalucía. P (mm): precipitation in millimeters. Tm (°C): mean temperature in degrees Celsius

Fig. 6 Wild boar uprooting one of the seedlings of the experiment caught by one of the trap cameras set up for the experiment (date: 16/04/2021)

Table 1 Pair-wise comparison of survival during the whole study period (February–September 2021) based on Cox proportional hazard model significance levels. Comparisons between 1- and 2-year-old seedlings with or without acorns and between seedlings with and without acorns of a given age were analyzed separately inside and outside the fence in Cabañeros and outside the fence in Doñana. *yr* year

Site	Contrasts	coef	SE	z ratio	P value
Cabañeros NP (inside the fence)	1 yr, Acorn–1 yr, No Acorn	0.099888	0.322448	0.310	0.757
	1 yr, Acorn–2 yr, Acorn	−0.02327	0.228537	−0.102	0.919
	1 yr, Acorn–2 yr, No Acorn	−0.22452	0.215296	−1.043	0.297
	1 yr, No Acorn–2 yr, No Acorn	−0.32441	0.196004	−1.655	0.098
	1 yr, No Acorn–2 yr, Acorn	−0.12316	0.308035	−0.400	0.689
	2 yr, Acorn–2 yr, No Acorn	−0.20125	0.243527	−0.826	0.409
Cabañeros NP (outside the fence)	1 yr, Acorn–1 yr, No Acorn	−0.02538	0.217206	−0.117	0.907
	1 yr, Acorn–2 yr, Acorn	0.103435	0.250238	0.413	0.679
	1 yr, Acorn–2 yr, No Acorn	−0.23603	0.289015	−0.817	0.414
	1 yr, No Acorn–2 yr, No Acorn	−0.21065	0.288046	−0.731	0.465
	1 yr, No Acorn–2 yr, Acorn	0.128813	0.281482	0.458	0.647
	2 yr, Acorn–2 yr, No Acorn	−0.33947	0.192479	−1.764	0.078
Doñana NP (outside the fence)	1 yr, Acorn–1 yr, No Acorn	−0.03385	0.216701	−0.156	0.876
	1 yr, Acorn–2 yr, Acorn	−0.04069	0.192753	−0.211	0.833
	1 yr, Acorn–2 yr, No Acorn	−0.022	0.206007	−0.107	0.915
	1 yr, No Acorn–2 yr, No Acorn	0.040367	0.193682	0.208	0.835
	1 yr, No Acorn–2 yr, Acorn	0.002412	0.253994	0.009	0.992
	2 yr, Acorn–2 yr, No Acorn	0.037955	0.227939	0.167	0.868

Table 2 Pair-wise comparison of survival before the onset of the dry season based on Cox proportional hazard model significance levels. Comparisons are among seedlings of 1–2 years old, with or without acorns, inside or outside the fence in Cabañeros. *yr* year

Fenced-1 yr-Acorn	Fenced-1 yr-No Acorn	Fenced-2 yr-Acorn	Fenced 2 yr-No Acorn	
$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	No Fenced-Acorn-1 yr
	$P < 0.001$	$P < 0.001$	$P < 0.001$	No Fenced-No Acorn-1 yr
		$P < 0.001$	$P < 0.001$	No Fenced-Acorn-2 yr
			$P < 0.001$	No Fenced-No Acorn-2 yr

Table 3 Pair-wise comparison of survival before the onset of the dry season based on Cox proportional hazard model significance levels. Comparisons are made among all combinations of 1–2-year-old seedlings, with or without acorns outside the fence in Cabañeros. Model coefficient and *P* values are indicated. Positive coefficients indicate an increased survival of the treatment in the columns compared to the treatments in the rows. *yr*, year

1 yr-No Acorn	2 yr-Acorn	2 yr-No Acorn	
0.233 $P = 0.578$	-1.51×10^{-15} $P = 1$	0.253 $P = 0.649$	1 yr-Acorn
	−0.231 $P = 0.578$	0.022 $P = 0.974$	1 yr-No Acorn
		0.253 $P = 0.698$	2 yr-Acorn

Table 4 Pair-wise comparison of survival before the onset of the dry season (April) based on Cox proportional hazard model significance levels. Comparisons are made among all combinations of seedlings of 1–2 years old, with or without acorns outside the fence area in Doñana. Positive sign means the risk of death is higher for the treatment in the rows that the one on the columns (i.e., 1 yr-No Acorn has higher risk than 1 yr-No Acorn). *yr* year

1 yr-No Acorn	2 yr-Acorn	2 yr-No Acorn	
0. 398 *P* = 0.255	**1.008** ***P* = 0.006**	**1.023** ***P* = 0.024**	1 yr-Acorn
	0.675 *P* = 0.231	**0.722** ***P* = 0.023**	1 yr-No Acorn
		0.047 *P* = 0.940	2 yr-Acorn

Table 5 Least squares mean comparison of leaf biochemical traits between seedlings with and without acorn, at different months, and for each plant age (1- or 2-year-old; *yr*, year)

Site	Biochemical traits	Age	Month	Estimate—contrast (Acorn vs. No Acorn)	SE	df	*t* ratio	*P* value
Cabañeros (inside the fence)	Chl	1 yr	Feb	−0.253	0.392	6	−0.646	1
			Mar	−0.54	0.392	6	−1.378	0.994
			Apr	−0.64	0.392	6	−1.634	0.972
			May	−0.593	0.392	6	−1.514	0.985
			Jun	−1.211	0.392	6	−3.091	0.464
			Jul	−1.454	0.392	6	−3.713	0.277
			Aug	−0.778	0.392	6	−1.986	0.897
		2 yr	Feb	0.638	0.392	6	1.629	0.973
			Mar	0.351	0.392	6	0.897	1
			Apr	0.251	0.392	6	0.64	1
			May	0.298	0.392	6	0.761	1
			Jun	−0.32	0.392	6	−0.817	1
			Jul	−0.563	0.392	6	−1.438	0.991
			Aug	0.113	0.392	6	0.288	1
Cabañeros (inside the fence)	NBI	1 yr	Feb	−0.1	0.269	6	−0.374	1
			Mar	−0.268	0.269	6	−0.996	1
			Apr	−0.361	0.269	6	−1.342	0.995
			May	−0.299	0.269	6	−1.112	0.999
			Jun	−0.813	0.269	6	−3.026	0.489
			Jul	−0.91	0.269	6	−3.388	0.365
			Aug	−0.475	0.269	6	−1.767	0.95
		2 yr	Feb	0.44	0.269	6	1.637	0.972
			Mar	0.273	0.269	6	1.015	1
			Apr	0.18	0.269	6	0.669	1
			May	0.242	0.269	6	0.899	1
			Jun	−0.273	0.269	6	−1.015	1
			Jul	−0.37	0.269	6	−1.377	0.994
			Aug	0.066	0.269	6	0.244	1

Site	Biochemical traits	Age	Month	Estimate—contrast (Acorn vs. No Acorn)	SE	df	*t* ratio	*P* value
Cabañeros (inside the fence)	Anth	1 yr	Feb	0.003	0.008	6	0.386	1
			Mar	0.006	0.008	6	0.77	1
			Apr	0.006	0.008	6	0.715	1
			May	0.003	0.008	6	0.419	1
			Jun	0.004	0.008	6	0.556	1
			Jul	0.021	0.008	6	2.689	0.624
			Aug	0.013	0.008	6	1.573	0.98
		2 yr	Feb	−0.005	0.008	6	−0.571	1
			Mar	−0.001	0.008	6	−0.186	1
			Apr	−0.002	0.008	6	−0.241	1
			May	−0.004	0.008	6	−0.538	1
			Jun	−0.003	0.008	6	−0.4	1
			Jul	0.014	0.008	6	1.732	0.957
			Aug	0.005	0.008	6	0.617	1
Cabañeros (inside the fence)	Flav	1 yr	Feb	−0.001	0.01	6	−0.065	1
			Mar	−0.002	0.01	6	−0.189	1
			Apr	0	0.01	6	0.015	1
			May	−0.002	0.01	6	−0.162	1
			Jun	−0.002	0.01	6	−0.237	1
			Jul	0.023	0.01	6	2.339	0.771
			Aug	0	0.01	6	−0.003	1
		2 yr	Feb	−0.005	0.01	6	−0.465	1
			Mar	−0.006	0.01	6	−0.59	1
			Apr	−0.004	0.01	6	−0.385	1
			May	−0.006	0.01	6	−0.562	1
			Jun	−0.006	0.01	6	−0.637	1
			Jul	0.019	0.01	6	1.939	0.91
			Aug	−0.004	0.01	6	−0.403	1
Cabañeros (outside the fence)	Chl	1 yr	Feb	0.172	0.244	6	0.706	1
			Mar	0.102	0.244	6	0.419	1
			Apr	0.018	0.244	6	0.072	1
			May	0	0.244	6	0.002	1
			Jun	−0.132	0.244	6	−0.541	1
			Aug	−0.79	0.244	6	−3.243	0.411
		2 yr	Feb	0.257	0.244	6	1.056	1
			Mar	0.462	0.244	6	1.895	0.922
			Apr	0.392	0.244	6	1.608	0.976
			May	0.307	0.244	6	1.261	0.998
			Jun	0.29	0.244	6	1.191	0.999
			Aug	0.158	0.244	6	0.648	1

Site	Biochemical traits	Age	Month	Estimate—contrast (Acorn vs. No Acorn)	SE	df	*t* ratio	*P* value
Cabañeros (outside the fence)	NBI	1 yr	Feb	−0.501	0.244	6	−2.054	0.876
			Mar	0.547	0.244	6	2.245	0.808
			Apr	0.16	0.181	6	0.88	1
			May	0.094	0.181	6	0.52	1
			Jun	−0.027	0.181	6	−0.15	1
			Aug	−0.022	0.181	6	−0.119	1
		2 yr	Feb	−0.235	0.181	6	−1.297	0.997
			Mar	−0.524	0.181	6	−2.889	0.542
			Apr	0.194	0.181	6	1.071	1
			May	0.332	0.181	6	1.832	0.937
			Jun	0.267	0.181	6	1.472	0.989
			Aug	0.145	0.181	6	0.802	1
Cabañeros (outside the fence)	Anth	1 yr	Feb	0.151	0.181	6	0.833	1
			Mar	−0.063	0.181	6	−0.345	1
			Apr	−0.351	0.181	6	−1.937	0.911
			May	0.367	0.181	6	2.023	0.885
			Jun	0.003	0.004	6	0.579	1
			Aug	0	0.004	6	0.048	1
		2 yr	Feb	0	0.004	6	−0.002	1
			Mar	0.001	0.004	6	0.245	1
			Apr	−0.002	0.004	6	−0.49	1
			May	0.014	0.004	6	3.12	0.454
			Jun	0.012	0.004	6	2.735	0.605
			Aug	−0.005	0.004	6	−1.196	0.999
Cabañeros (outside the fence)	Flav	1 yr	Feb	−0.008	0.004	6	−1.726	0.958
			Mar	−0.008	0.004	6	−1.776	0.949
			Apr	−0.007	0.004	6	−1.529	0.984
			May	−0.01	0.004	6	−2.264	0.8
			Jun	0.006	0.004	6	1.345	0.995
			Aug	0.004	0.004	6	0.961	1
		2 yr	Feb	−0.003	0.008	6	−0.38	1
			Mar	0.001	0.008	6	0.091	1
			Apr	0.005	0.008	6	0.592	1
			May	0.002	0.008	6	0.31	1
			Jun	0.014	0.008	6	1.788	0.946
			Aug	0.02	0.008	6	2.61	0.657

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Availability of data and materials

The datasets generated during and/or analyzed during the current study are available in the e-cienciaDatos—UPM repository, https://doi.org/10.21950/VF8MWV.

Declarations

Ethics approval and consent to participate

All work conforms to the legal requirements of EU (the country in which this work was carried out), including those relating to conservation and welfare, and to the journal's policy on these matters.

Consent for publication

All authors gave their informed consent to this publication and its content.

Competing interests

I, as the corresponding author of this scientific article, declare that there are no conflicts of interest related to the content of this manuscript. This manuscript describes original work and is not under consideration by any other journal.

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