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Research article

Effects of livestock on arthropod biodiversity in Iberian holm oak savannas revealed by metabarcoding

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A R T I C L E I N F O

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A B S T R A C T

Increasing food production while avoiding negative impacts on biodiversity constitutes one of the main challenges of our time. Traditional silvopastoral systems like Iberian oak savannas ("dehesas") set an example, where free-range livestock has been reared for centuries while preserving a high natural value. Nevertheless, factors decreasing productivity need to be addressed, one being acorn losses provoked by pest insects. An increased and focalized grazing by livestock on infested acorns would kill the larvae inside and decrease pest numbers, but increased livestock densities could have undesired side effects on ground arthropod communities as a whole. We designed an experimental setup including areas under trees with livestock exclosures of different ages (short-term: 1-year exclusion, long-term: 10-year exclusion), along with controls (continuous grazing), using DNA metabarcoding (mitochondrial markers COI and 16S) to rapidly assess arthropod communities' composition. Livestock removal quickly increased grass cover and arthropod taxonomic richness and diversity, which was already higher in short-term (1-year exclosures) than beneath the canopies of control trees. Interestingly, arthropod diversity was not highest at long-term exclosures ($\geq$10 years), although their community composition was the most distinct. Also, regardless of treatment, we found that functional diversity strongly correlated with the vegetation structure, being higher at trees beneath which there was higher grass cover and taller herbs. Overall, the taxonomic diversity peak at short term exclosures would support the intermediate disturbance hypothesis, which relates it with the higher microhabitat heterogeneity at moderately disturbed areas. Thus, we propose a rotatory livestock management in dehesas: plots with increased grazing should co-exist with temporal short-term exclosures. Ideally, a few long-term excluded areas should be also kept for the singularity of their arthropod communities. This strategy would make possible the combination of biological pest control and arthropod conservation in Iberian dehesas.

1. Introduction

One of the great human challenges for the 21st century is to produce enough food for an increasing population, but to do so in an environmentally-friendly way (FAO and UNICEF, 2022; IPBES, 2019). In this context, traditional agroecosystems play a very important role

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combining the preservation of natural values with economic activities. Iberian silvopastoral oak savannas (so called "dehesas" in Spain and "montados" in Portugal) are a good example (Sucena-Paiva et al., 2022). Century-long shrub and tree clearing of Mediterranean forests has produced landscapes with oaks interspersed within a grassland matrix, in which livestock rearing is the main use (Bugalho et al., 2011; Moreno and Pulido, 2009; Sá-Sousa, 2014). Dehesas are also included in the Habitats Directive of the European Union, as they host a wide range of rare, threatened, or endemic animal and plant species (EC, 2013).

As in any other agricultural system, pest insects reduce the productivity in oak dehesas (Canelo et al., 2021a), but the preservation of their high natural value preclude the use of any chemical treatment. Current approaches follow the logic of ecological intensification, based on managing service-providing organisms that make a quantifiable direct or indirect contribution to agricultural and forest productivity (Bommarco et al., 2013; Montesinos, 2019). Parasitoids (frequently species of the order Hymenoptera), or vertebrates (e.g. birds), are among them (Diaz-Siefer et al., 2021; García et al., 2021; Redlich et al., 2018; Zhu et al., 2020). However, for oak dehesas, we previously proposed a novel way of biological control based on livestock management (Canelo et al., 2021b).

In dehesas, acorns are a key food resource for livestock, but their availability is limited by insect pre-dispersal seed predators, mainly *Curculio* weevils (Coleoptera: Curculionidae), which can infest half of the year's acorn production (Bogdziewicz et al., 2019; Bonal et al., 2007). The weevil larvae, however, are vulnerable to predation by large vertebrates for a short time, namely the period of 20 days that they spend inside the acorns on the ground after the premature abscission of the seed (Bonal and Muñoz, 2007). Thus, we proposed an increased livestock grazing on infested acorns during that period that proved successful to reduce pest numbers (Canelo et al., 2021b). But it is unclear whether this increased grazing could have undesired negative side effects on arthropod communities coexisting with the pest and the livestock.

Livestock plays a major role in ecosystem functioning (Eldridge et al., 2016, 2017; Eskelinen et al., 2022), but grazing consequences on biodiversity remain not fully understood (Filazzola et al., 2020; Yuan et al., 2016). Some effects of large herbivores are direct, like unintentional predation on insects while feeding (Bonal and Muñoz, 2007; Gómez and González-Megías, 2002, 2007; Retamosa et al., 2004). By contrast, others are indirect and mediated by biotic and abiotic variables (Maestre et al., 2022). For example, grazing can alter the richness, abundance, and species composition of different animal communities through changes in plant cover and/or diversity (Adler et al., 2001; Brambila et al., 2020; Kirk et al., 2019; Magnano et al., 2019; Sims et al., 2019; Song et al., 2020). Livestock also affects the functional diversity of arthropods (Torma et al., 2023), and trait-based approaches constitute an additional tool for better understanding the effects of management on vegetation and, consequently, on arthropods (Oksuz et al., 2020; Chozas et al., 2022). Additionally, grazing increases soil hardness and changes its physicochemical properties (Armas-Herrera et al., 2020; Zhang et al., 2020), as well as water composition and quality (Kilgarriff et al., 2020; O'Callaghan et al., 2019; O'Sullivan et al., 2019). Grazing effects are not always easy to predict because they result from complex cascade effects (Abdala-Roberts et al., 2019; Eldridge et al., 2016, 2017; Evans et al., 2015; Sankaran and Augustine, 2004; Vandegehuchte et al., 2017), which transcend ecosystem boundaries (Knight et al., 2005). Moreover, the variability of direct and indirect effects, along with their interactions with environmental factors (Vojta et al., 2020), may make the consequences of grazing seem counterintuitive, as different organisms may respond differently to the same disturbance events (Didham et al., 2009; Gossner et al., 2016; Jackson et al., 2015). For oak dehesas, we proposed a temporary localized increased grazing to reduce acorn pests, rotated among different farm areas every 2–3 years (Canelo et al., 2021b). In principle, grazing would be expected to reduce richness and both taxonomical and functional diversity of arthropods compared to areas with no livestock. Nonetheless, it could depend on grazing intensity, as generally predicted by the intermediate disturbance hypothesis (IDH).

The intermediate disturbance hypothesis (IDH) states that species diversity peaks at intermediate levels of environmental stress (Connell, 1978; Gao and Carmel, 2020; Roxburgh et al., 2004; Svensson et al., 2007; Yan et al., 2015). On the one hand, high disturbances result in constant selection pressure allowing only a few species to survive; on the other hand, the lack of disturbance benefits just a few strong competitors. However, in between, the competition between existing species decreases and new species can rapidly colonize. Consequently, species richness rises under intermediate disturbance regimes. This hypothesis has been proposed to explain the high arthropod diversity recorded in areas with moderate levels of grazing (Joubert et al., 2016; Kaltsas et al., 2013; Kati et al., 2012; Lázaro et al., 2016a, 2016b; Qin et al., 2017; Winck et al., 2019).

Livestock exclusion is seen as a good strategy to restore degraded ecosystems (Kröpfl et al., 2011; Prober et al., 2011; Su et al., 2015). At the same time, it is a very efficient way to evaluate the effects of grazing on other organisms (Adams, 1975; Trigo et al., 2020; Wassie et al., 2009). Such effects usually appear over time, and not always in a linear fashion (Filazzola et al., 2020), hence, the use of exclosures of different age is recommendable to study them. We did so in the present study, building exclosures and, after one year, comparing them with adjacent grazed areas and places in which livestock had been absent for a longer period of time (10-year exclusion). We performed extensive and detailed analyses on the effects on arthropod communities considering all trophic and taxonomic levels. To achieve this ambitious goal, we used DNA metabarcoding approach.

In arthropod biodiversity studies, species identification is always the main challenge (Moretti et al., 2004). Gaps in taxonomic identification are usually related to the nature of taxonomic knowledge: due to the outstanding arthropod diversity, researchers need to focus on specific taxonomic groups (Didham et al., 2009; Jackson et al., 2015), and taxonomic expertise is not always available for every study. Metabarcoding overcomes this taxonomic impediment, allowing species identification at a community level by delimiting molecular operational taxonomic units (MOTUs), which correspond to putative species (Gaytán et al., 2020). These MOTUs can be determined to the order, family, genus or species level depending on the availability of identified reference barcodes. In this sense, using more than one marker for species identification enhances detection likelihood (Kaunisto et al., 2017; Wangensteen et al., 2018). For terrestrial arthropods, the two mitochondrial genes cytochrome oxidase I (COI) and the 16S rRNA gene (16S) have shown to complement each other very well (Marquina et al., 2019a; Roger et al., 2022).

The general objective of this study is to assess, for the first time, the effects of grazing on arthropod communities of Iberian oak dehesas using DNA metabarcoding, along with the environmental factors that may drive them. Our specific goals were: i) to compare the success of two genetic markers (mitochondrial genes COI and 16S) used for arthropod species detection by DNA metabarcoding; ii) evaluate changes in vegetation structure after livestock exclusion and its effects on arthropod diversity; and iii) assess species richness, community composition and functional responses of arthropods across trophic levels throughout a gradient of grazing exclusion.

2. Material and methods

2.1. Study area and sampling design

Sampling was conducted at six holm oak (*Quercus ilex* L.) dehesa farms located in the province of Cáceres, western Spain (Fig. 1). Three of them were used for free-range livestock rearing (farms 1, 2 and 3); in the other three, livestock had been absent long-term, for at least ten years before the beginning of the study (farms 4, 5 and 6). At farms 1–3, we randomly chose eight pairs of oaks per farm in February 2016. Within

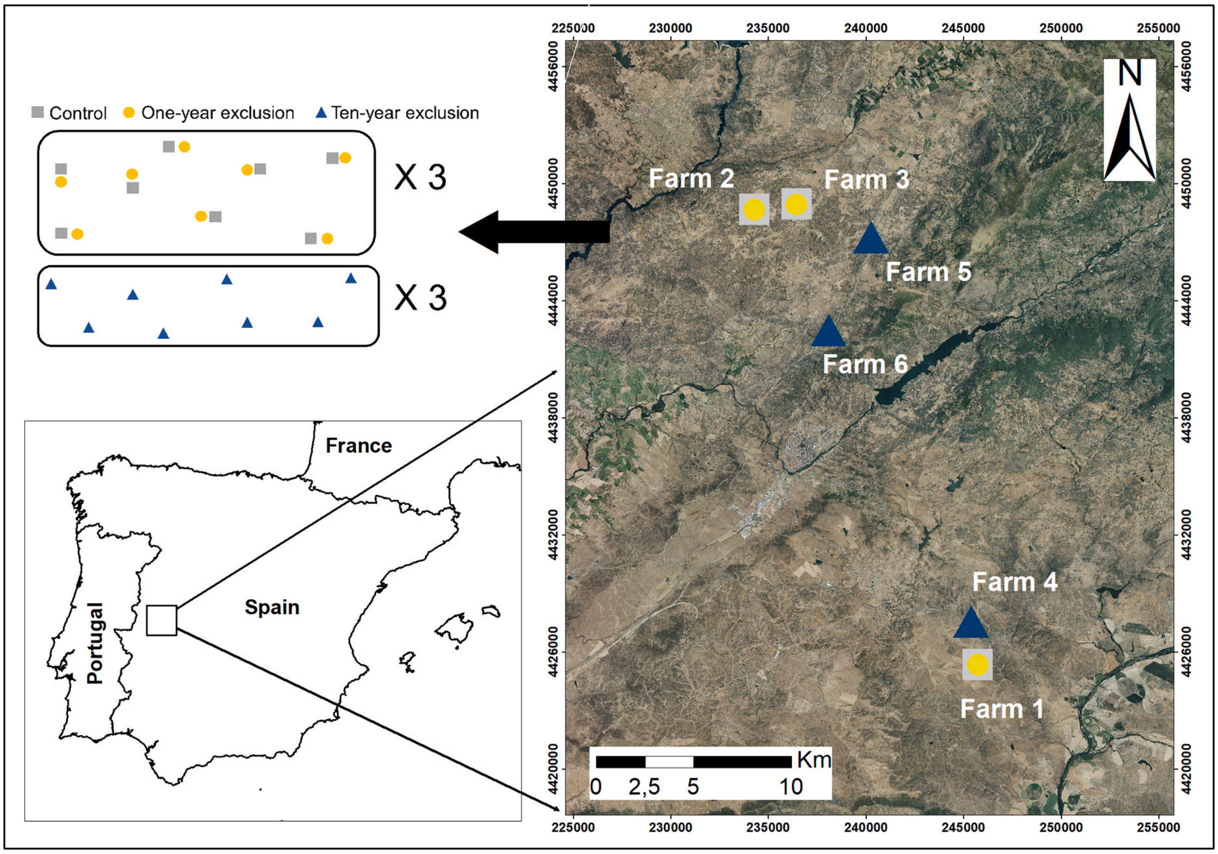

Fig. 1. Location of the study area and experimental design.

each pair, one tree was randomly chosen for experimental short-term livestock exclusion and the other one was left as control. To sum up, there were eight trees x three farms without livestock for ten years or more, and eight trees x two treatments x three farms with livestock. Therefore, 72 *Q. ilex* trees were monitored (N *(control)* = 24; N *(one-year exclusion)* = 24; and N *(ten-year exclusion)* = 24) (specific information of each farm in Table S1).

In March 2016 (early spring), experimental trees of farms 1–3 were fully enclosed by a fence to avoid livestock grazing beneath their canopies. In April 2017 (spring), we sampled all study trees following the same methodology. We did the fieldwork in spring because this is the period of the year in which vegetation productivity is highest in Iberian Mediterranean ecosystems, and so arthropod numbers and diversity peak too. Our goal was knowing how livestock affected both vegetation and arthropods at this time of the year, in which any effect could be more dramatic. We vacuumed 2 m^2 of the floor (2900 W vacuum machine) beneath the canopy of each tree over morning and early afternoon hours (from 10:00 h 16:00 h). At that time interval the temperature is high enough to allow full insect activity. Moreover, vacuum power allows collection of any insect on the grasses or on the ground, irrespective of their activity status. It took five days to complete the sampling, and the order in which study trees were sampled changed from day to day, so that the trees of a given treatment or controls were not always the first or the last. Hence, any unlikely effect of the time of the day was avoided. Beneath each canopy we set a 1 m^2 plot at the northern half of the canopy and 1 m^2 at the southern half. Each plot was vacuumed for 30 s to sample all the arthropods on the soil surface and on the vegetation. We pooled both samples of each tree and stored them in cardboard boxes that were taken to the laboratory and placed in the fridge at 4 °C until sorting. By doing this, we preserved the specimens and kept them inactive, thus preventing them flying off during sample cleaning and classification. The content of each box was emptied on a tray and carefully inspected. Plant debris and soil were discarded, and all the arthropods taken and placed in a bottle filled with 96 % ethanol. After 5 min without finding any new specimen, we considered sample revision as completed.

As livestock effects on arthropods can be mediated by changes in vegetation structure, we measured vegetation cover and height beneath the tree canopy while sampling arthropods. At each tree, we annotated vegetation height at 8 random points with a calibrated rod that we placed vertically on the ground. At each point we registered whether vegetation touched the rod at: ground level, 0–10 cm, 11–25 cm, 26–50 cm, and taller than 50 cm; we also measured the maximum vegetation height (see Muñoz et al., 2009 for a similar sampling procedure).

2.2. DNA amplification and library preparation

At the molecular laboratory we first discarded the alcohol from the tubes and let the samples dry on large petri dishes with a sterile filter paper inside a fume hood for 3 h. Once dry, we incubated the samples in a lysis buffer (2 mL buffer ASL (Qiagen) + 250 μL proteinase K) for 14 h at 56 °C with a gentle rotation. Subsequently, we recovered the lysate and rinsed the arthropods with distilled water and 90 % ethanol, and we stored them in 80 % ethanol. We used 225 μL of the lysate to perform DNA extraction on a KingFisher Duo robot (Thermo Fisher Scientific) with the Cell and Tissue kit (Thermo Fisher Scientific), with an elution volume of 100 μL. We included lysis (empty vial with just lysis buffer incubated alongside the samples) and extraction (just for the robot part) blanks.

As metabarcoding markers, we amplified two fragments of the mitochondrial cytochrome oxidase I (COI) and the 16S rRNA genes with the primers BF2-BR1 (Elbrecht and Leese, 2017) and Chiar16SF-Chiar16SR (Marquina et al., 2019b) respectively. Forward and reverse primers for both markers had attached an 8 bp tag at the 5’ end for sample multiplexing (Binladen et al., 2007). The PCR reactions consisted of one Illustra Hot Start Taq Mix RTG bead (GE Healthcare Life Sciences), 1 μL of each primer (10 nM), 2 μL DNA template and 21 μL of biology-grade water, for a final volume of 25 μL; the temperature protocol was as follows: initial DNA denaturation and Taq activation at 95 °C for 5 min, 40 cycles of denaturation at 95 °C for 30 s, annealing at 48/50 °C (COI/16S) for 45 s and extension at 68 °C for 45 s, and a final phase of extension at 72 °C for 10 min. We ran all PCRs in duplicated, and a blank was included in the batches of both markers. We confirmed PCR amplification in an agarose gel, pooled the replicates together, and measured DNA concentration using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific) with the broad-range reagents. We discarded lysis, extraction and PCR blanks after not producing a visible band in the gels and returning undetectable values of DNA concentration. We then pooled the tagged PCR products equimolarly, and cleaned-up primers and primer dimers using MinElute columns (Qiagen). We prepared libraries with the TrueSeq PCR-free kit (Illumina). We visualized ligation products on a 2 % agarose gel, cut the fragments of the desired length out of the gel, and purified them using the QIAquick gel extraction kit (Qiagen). We pooled the libraries to equimolar concentrations and sequenced them on a single Illumina MiSeq lane using 2 x 300 bp paired-end v3 chemistry run at SciLifeLab (National Genomics Infrastructure, Stockholm).

2.3. Bioinformatic processing

Raw sequencing data was processed using a pipeline based on OBITools (Boyer et al., 2016) in combination with other software (described below). A FastQC analysis (Andrews, 2010) was conducted to check sequencing quality, and sequences were trimmed at the position where the average Phred score was lower than 28. Then, they were paired-end merged and alignments with a score lower than 30 were discarded. Sequences that passed the filtering were demultiplexed based on the 8 bp sample tag combinations and the primer sequences were trimmed away. Demultiplexed sequences were then filtered based on their length, selecting only those in the range of 310–330 bp long for COI and 260–375 bp long for 16S (this range was selected based on the abundance distribution of the read lengths, which for rRNA genes is much more variable across taxonomic groups than protein-coding genes, in this case showing two peaks around 270 and 350 bp). Dereplicated reads were searched for chimeras using VSEARCH v2.7.1 (Rognes et al., 2016). Subsequently, the sequences were clustered into MOTUs (i.e. putative species) using SWARM v2.1.13 (Mahé et al., 2015), with maximum distance d = 9 and d = 5, for COI and 16S respectively, and combined with abundance information to generate MOTU occurrence tables. These were curated with LULU v0.1.0 (Frøslev et al., 2017) to reassign false MOTUs to their parent MOTU. The representative or centroid sequences of each MOTU were assigned taxonomic identity with ecotag (OBITools) using a local reference database built with a combination of i) all the barcodes from all orders of Arthropoda with terrestrial representatives from BOLD (Ratnasingham and Hebert, 2007) accessed in June 2018, and ii) the invertebrate and fungi sequences from EMBL's release r137 (Kulikova et al., 2004) (COI, ~4.1 M sequences), or just the invertebrate sequences from EMBL's release r137 (16S, ~60 k sequences). The final dataset was refined by collapsing all MOTUs with the same species identification, as well as removing those occurrences that represented less than 0.04 % of the total reads of the sample, and those MOTUs with less than 10 reads in total across all samples. The minimum abundance per sample threshold was defined after running an additional library with controlled empty sample tag combinations and calculating the proportion of reads recovered from those combinations from the total generated in that library.

2.4. Statistical analysis

All analyses were conducted in Rstudio, with different R versions (R Core Team, 2021).

2.4.1. Vegetation structure

In order to assess the effects of livestock on vegetation structure we performed two Principal Component Analyses (PCA). Both PCAs were based on standardized correlation matrix with the function *PCA* (package 'FactoMineR' (Lê et al., 2008)). The first PCA was performed using the data from all study trees (N = 72) to assess the differences among the three categories of trees (control, one-year exclusion and ten-year exclusion) (Table S2). In the second PCA, we only included control oaks (N = 24) to analyse the differences among the three oak savannas with free-range livestock excluding the effect of the experimental treatment (Table S3).

2.4.2. Richness and diversity

We draw rarefaction curves (function *rarecurve*, package 'vegan' (Oksanen et al., 2013)) to assess the sequencing depth of each sample for both COI and 16S datasets. We removed samples with a number of reads too low for downstream analyses (Fig. S1). To assess the differences in MOTU richness we performed Generalized Linear Mixed Models (GLMMs) and Generalized Linear Models (GLMs), separately for the COI and 16S datasets. We could not pool all MOTUs in one single dataset since some of them could not be identified to the species level. All analyses were done with the datasets rarefied to the lowest number of reads per sample (function *rrarefy*, package 'vegan').

First, we fit Generalized Linear Mixed Models (function *glmmTMB*, package 'glmmTMB' (Brooks et al., 2019)) with Farm as random factor to estimate the effect of Treatment on the MOTU richness in all farms (N = 72) and we ran ANOVA tests with those models (function *Anova.glmmTMB*, package 'glmmTMB'). Then, we performed Generalized Linear Models (function *glm*) with Farm and Treatment as independent factors, and richness as dependent variable, including only the experimental and control trees of Farms 1–3 (N = 48), followed by ANOVA tests of the model (function *Anova*). We did so because it was in those farms in which there were both control trees and trees subjected to short-term livestock exclosure, so that a full model including both factors (Farm and Treatment) could be performed. All models were fit with binomial negative links. This distribution was chosen after comparing it with Poisson, Generalized Poisson and Conway-Maxwell Poisson distributions. Pairwise post-hoc analyses were made with Tukey tests. We repeated the same analyses at the family and order level, merging the COI and 16S datasets. In these cases, models were fit with Gaussian distribution.

We also analysed the alpha diversity of each plot by calculating the Shannon index, H' (function *diversity*, package 'vegan'). We fit new GLMM and GLM models following the previous scheme, with Gaussian distribution, and with diversity instead of richness as dependent variables.

2.4.3. Community composition changes

To analyse the differences in sample composition at MOTU level among the treatments, we performed Non-metric Multidimensional Scaling (NMDS) ordinations of the trees (N = 72) based on the arthropod datasets of each marker. We used Bray–Curtis clustering to measure the dissimilarity between the trees (function *metaMDS*, package 'vegan'). Posteriorly, we ran a Permutation Analysis of Variance (PERMANOVA) (functions *adonis* at package 'vegan') to analyse the compositional differences between samples from different treatments. The effects of Treatment and Vegetation structure on sample composition were explored by correlating them with the NMDS ordination (function *envfit* (McCune and Grace, 2002), package 'vegan'), evaluating the strength of those relationships through the squared correlation coefficient (r^2).

We calculated the zeta diversity of each group of samples, that is, the number of species shared by a given number of trees (Hui and McGeoch, 2014), and also how the values of zeta diversity changed with increasing number of trees (McGeoch et al., 2019). We used the ratio of the zeta diversity decline to visualize the species retention rate: the probability of finding species shared by all trees as the number of trees increases. We first grouped all trees of the same treatment together, and later we separated them by Treatment and Farm. In both cases we followed the ALL model (the addition of trees does not follow any directionality, nor is dependent on proximity to one another), performing a Monte-Carlo chain sampling with 5000 samples (function *Zeta.decline.mc*, package 'zetadiv' (Latombe et al., 2020)). The better fit to a power-law or exponential regression of the zeta diversity decline was decided on the basis of the Akaike information criterion.

We also visualized the most abundant MOTUs (those whose total abundance across all samples was above the third quartile of the abundance distribution) using a semiquantitative scale in which MOTUs with an abundance an order of magnitude greater appeared as double the size in the graph (Turon et al., 2022) (see Fig. S2). To identify the MOTUs most responsible for the differences between treatments we calculated the Dufrene-Legendre indicator value *d* (Dufrene and Legendre, 1997) of the MOTUs for each treatment (function *indval*, package 'labdsv' (Roberts, 2016). We visualized a subset of the datasets containing only these MOTUs using the semiquantitative scale mentioned above.

2.4.4. Functional responses

We assigned all identified species to one of the following guilds: herbivore, parasitoid, predator, or saprophagous (Table S4). In the case of arthropods that change their trophic source (something that happens not only between genera or families, but also between life stages within the same species), we prioritized the type of food consumed during the larval stage to assign the foraging guild. We did so because arthropods are usually more voracious during their larval development which, in many cases, is also longer than any other.

We characterized guild patterns using two functional indices, namely Functional Richness and Rao's quadratic entropy, and the community-weighted mean of each gild (CWM) (Botta-Dukát, 2005; Lavorel et al., 2007) (function *dbFD*, package 'FD' (Laliberté et al., 2014)). We assessed guilds' responses to the community dynamics throughout the treatments, described by the NMDS ordination (function *envit*, package 'vegan'), evaluating the strength of these relationships through the squared correlation coefficient (r2).

3. Results

3.1. MOTU detection and taxonomic identification

The number of MOTUs detected differed between molecular markers: 398 using COI and 351 using 16S. After comparing the sequences against the reference databases, we could assign Linnean species names to 40 % of the MOTUs in the COI dataset and 37 % in the 16S dataset. Identification rates increased at higher taxonomic levels: more than 97 % of the MOTUs could be assigned to an order with either marker. The proportion was also very high at the family level, but here the success rate was considerably higher using COI: 89 % with this marker versus 76 % using 16S (Table 1).

The use of two markers increased detection success, but only at lower taxonomic levels. There were 22 different orders in total, all of them detected by COI, but only 14 picked up by 16S. The orders missed by the 16S marker were: Mantodea, Opiliones, Plecoptera, Poduromorpha, Sarcoptiformes, Symphypleona, Thysanoptera and Trombidiformes. At lower taxonomic levels, many families were detected by the two markers (N = 81), but some of them were only found by one: 36 for COI and 8 for 16S (Table 1). Table S5 shows all families recovered and marker identification, as well as the number of MOTUs classified into each family. Regarding determinations to the genus level, roughly 50 % of the MOTUs were identified per marker and differed between markers: from a total of 266 genera, 117 were exclusively identified by COI and 91 by 16S (Table 1). That is, only 58 genera were identified by both markers.

Table 1
Taxonomic identifications summary.

	COI	16S
ARTHROPODA	398 MOTUs 392 MOTUs identified	351 MOTUs 343 MOTUs identified
ORDER **22** total orders detected	98.49 % MOTUs classified into **22** orders **8** orders were **detected exclusively** 355 MOTUs identified	97.72 % MOTUs classified into **14** orders **No** order was **detected exclusively** 269 MOTUs identified
FAMILY **125** total families detected	89.19 % MOTUs classified into **117** families **36** families were **detected exclusively** 204 MOTUs identified	76.63 % MOTUs classified into **89** families **8** families were **detected exclusively** 180 MOTUs identified
GENUS **266** total genera detected	51.26 % MOTUs classified into **175** genera **117** genera were **detected exclusively**	51.28 % MOTUs classified into **149** genera **91** genera were **detected exclusively**

3.2. Vegetation structure

The results of the first Principal Component Analysis (PCA) including all trees (N = 72, Table S2) showed that vegetation structure starts to change soon after livestock is excluded. In just one year grass cover equalled that of long term exclosures, but its height was still shorter. The analyses retrieved two dimensions, which together explained 78 % of the variance. PC1 (59 % of the variance explained) was significantly correlated with all variables and defined a gradient between trees in farms with high vegetation cover and height (mostly grass) versus those with a higher percentage of short grass (i.e. ground). It showed that vegetation cover was higher beneath the canopies of oaks from which livestock had been excluded for one or ten years than in control trees. PC2 (variance explained of 16.7 %) was correlated with four variables and separated areas with high cover of short and high grass (which corresponded to long-term exclosures) from those with a prevalence of medium-sized grass (Fig. 2A).

The second PCA, performed including only the control oaks (N = 24, Table S3) from Farms 1–3 (livestock present), showed that vegetation cover differed among them (Fig. 2B). PC1 (variance explained of 59.6 %) was highly correlated with all variables, and defined a gradient between areas with short grass and those in which overall grass cover and height were higher. PC2 explained less variance (15.8 %) and was correlated with only two variables that differentiated areas with high versus medium-sized grass. Summarizing, in Farm 1 the grass beneath the canopies was scarce and shorter than in the rest, and in Farm 3 there was a high cover of grass which, on average, was also taller.

3.3. Arthropod richness and diversity

3.3.1. Richness

Neither short- nor long-term exclusion had an effect on the number of arthropod MOTUs detected using COI. The GLMM including all study farms and oaks (N = 72) showed no differences between treatments ($\chi^2 = 4.675$; $p = 0.097$) (Fig. 3, top left panel A), although the number of MOTUs found in the one-year exclosures was slightly higher than under the other trees. Analysing only the 3 farms with control and experimental one-year exclosures (N = 48), there were differences by Treatment ($\chi^2 = 5.218$; $p = 0.022$), but not by Farm ($\chi^2 = 3.959$; $p = 0.138$). Post hoc analysis, however, showed no significant differences between control and short-term exclosure trees within the same farm (Fig. 3, top left panel B).

Contrary to COI, 16S detected significant differences in arthropod richness at the MOTU level. The GLMM including all trees (N = 72) showed that there were differences between treatments ($\chi^2 = 15.302$; $p < 0.001$), and post hoc analysis showed that the number of MOTUs was significantly higher under one-year-exclusion trees than under control

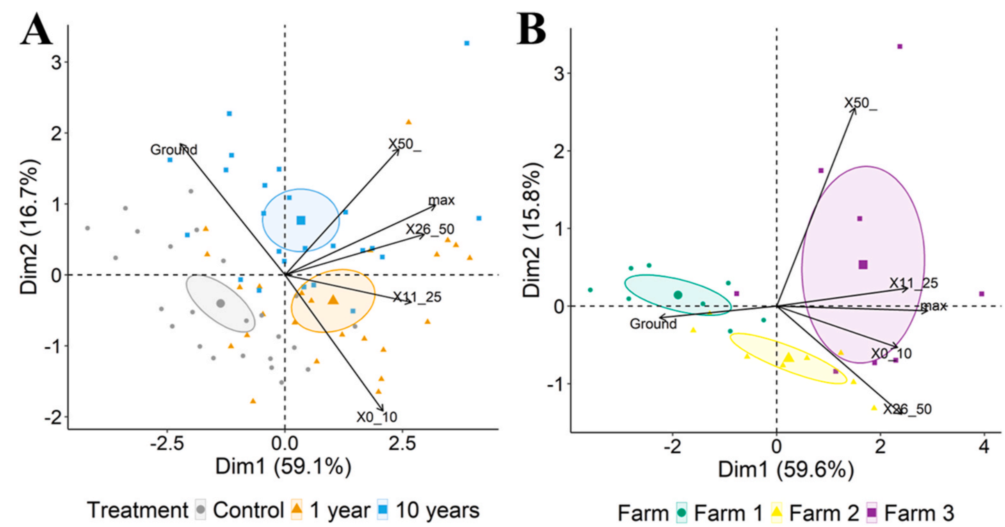

Fig. 2. Vegetation PCA biplots. (**A**) PCA biplot of all study trees (N = 72) classified by Treatment. (**B**) PCA biplot of control trees (N = 24) classified by Farm. The ellipses show the 95 % confidence variance for the mean value. The first PCA separates trees the from different treatments: low cover of short grass (Control), high cover of short/medium height grass (one-year exclusion), and high cover of high grass (ten-years exclusion). The second PCA (only control trees) segregates the three farms on basis of their vegetation at their standard livestock densities. Variable arrows are displayed to show differences in grass cover height between treatments and farms.

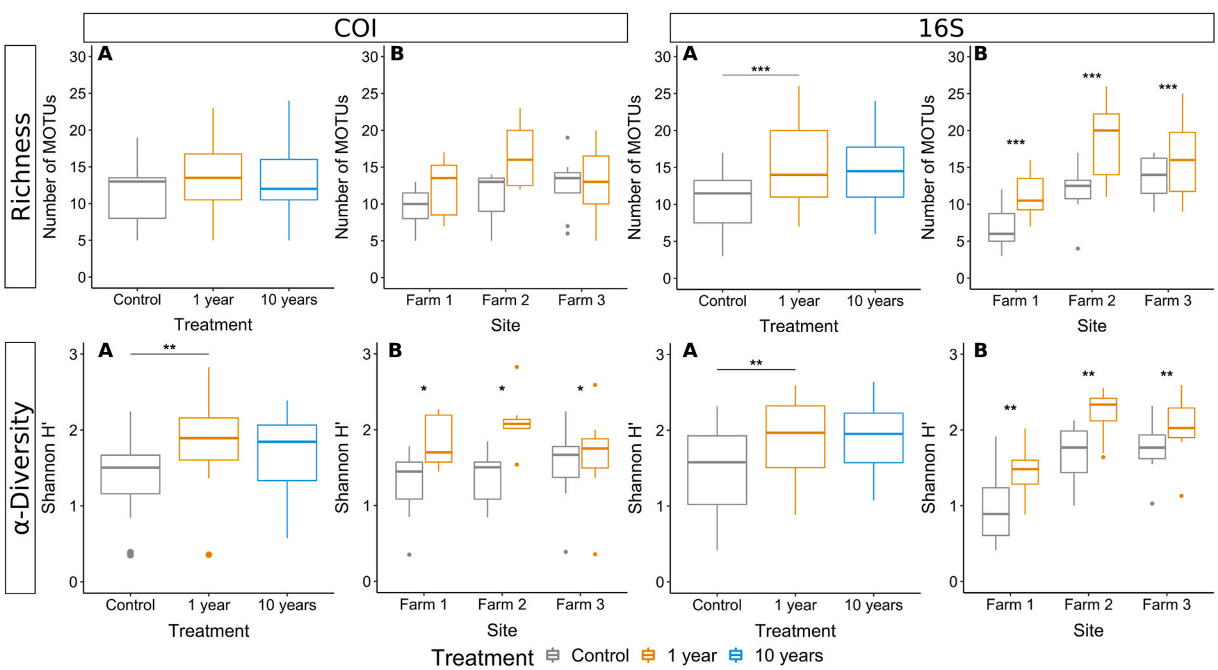

Fig. 3. Richness (top panel) and diversity (bottom panel) of the arthropod communities at MOTU level, detected with the COI (left panels) or 16S (right panels) marker. Box symbolize first to the third quartile (Q1 to Q3) with the middle line that represents the median. Whiskers lines indicate variability outside Q1 and Q3. Outliers are displayed by points. **A** graphs represent analyses performed for the trees from all treatments (N = 72), while **B** graphs are restricted to farms with paired control and one-year exclusion trees (N = 48).

trees (estimate = −0.364; T = −3.868; $p < 0.001$). However, there was no difference in richness between the arthropod communities found at long-term exclosures and control trees (Fig. 3, top right panel A), nor between short- and long-term exclosures. When looking at just the 3 farms with control and experimental one-year exclosures (N = 48), there were differences both between treatments ($\chi 2 = 16.116$; $p < 0.001$) and between farms ($\chi 2 = 26.603$; $p < 0.001$). Post hoc analysis showed that the number of 16S MOTUs was greater in one-year exclusion trees than in controls in all three farms (Fig. 3, top right panel B).

At family level, we found significant differences between treatments considering all study trees ($\chi^2 = 7.425$; p = 0.024; N = 72). Post hoc analysis showed that arthropod family richness was higher beneath one-year-exclusion trees than beneath the controls (estimate = −2.700; T = −2.542; p = 0.035), but not at ten-year-exclusion farms (estimate = −2.596; T = −1.853; p = 0.161) (Fig. 4A). Considering only the one-year exclosures and the control trees, both Treatment ($\chi^2 = 7.844$; p = 0.005) and Farm ($\chi^2 = 13.380$; p = 0.001) had significant effects on family richness. However, post hoc analysis showed only marginal significance differences within farms (Fig. 4B).

At the order level, we found no differences between treatments when

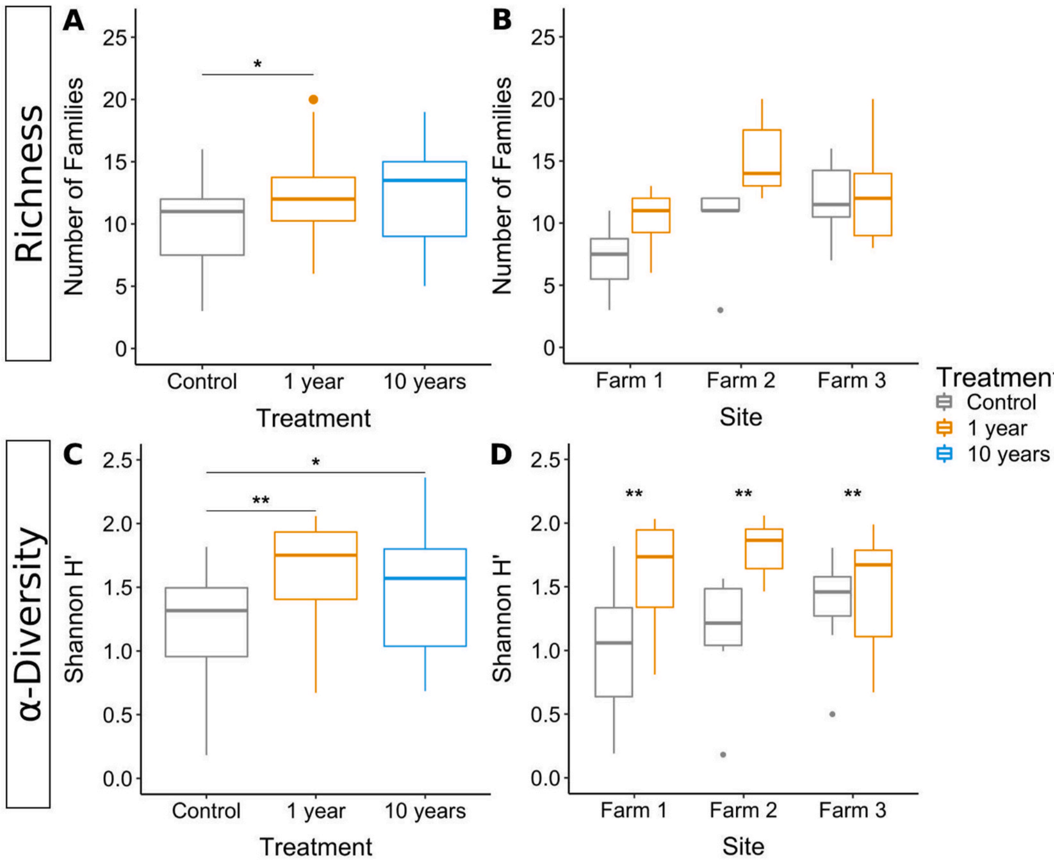

Fig. 4. Richness and diversity of arthropod families. Box symbolize first to the third quartile (Q1 to Q3) with the middle line that represents the median. Whiskers lines indicate variability outside Q1 and Q3. Outliers are displayed by points **A** when trees from all three treatments are included (N = 72), **B** only trees from farms with control and one-year exclusion are considered (N = 48). Similarly, **C** shows diversity for trees from all three treatments, and **D** only for trees from the farms with pairs of control and one-year exclusore trees.

taking all trees into consideration ($\chi^2 = 1.363$; p = 0.506). At the farms with only control and one-year-exclusion trees, significant differences were found among Farms ($\chi^2 = 11.132$; p = 0.003), but not among Treatments ($\chi^2 = 1.105$; p = 0.305).

3.3.2. Diversity

In general, considering not only richness, but also evenness and dominance, resulted in more significant differences between treatments at all taxonomic levels, except for order.

Using COI, we found significant differences in MOTU diversity among treatments when considering all 72 trees ($\chi^2 = 10.493$; p = 0.005). Pairwise analysis showed that the diversity under one-year-exclusion trees was marginally significantly higher than under control trees (estimate = −0.467; T = −3.183; p = 0.063), while no differences were found between control trees and those with long-term livestock exclusion (estimate = −0.303; T = −2.111; p = 0.095), although it was slightly higher in the latter (Fig. 3, bottom left panel A). Restricting the analysis to just the three farms with control and one-year exclosures, Treatment had significant effects on the MOTU diversity ($\chi^2 = 10.188$; p = 0.001), but not Farm ($\chi^2 = 1.104$; p = 0.576). Post hoc tests indicated that it was significantly higher at short-term livestock exclosures at the three farms (Fig. 3, bottom left panel B).

Using 16S, the results were very similar to COI. We found significant differences due to Treatment in the analysis involving the three of them ($\chi^2 = 14.417$; $p < 0.001$), although Tukey tests indicated that only in one-year exclosures MOTU diversity was higher than at the controls (estimate = −0.439; T = 3.684; p = 0.001) (Fig. 3, bottom right panel A). Considering only the farms with control and one-year exclosure trees, both Farm ($\chi^2 = 30.058$; $p < 0.001$) and Treatment ($\chi^2 = 13.677$; $p < 0.001$) had significant effects on the diversity of the arthropod community. Post hoc analysis showed that the diversity beneath the canopies of one-year-exclusion trees was higher in all three farms in comparison with their control pairs (Fig. 3, bottom right panel B).

At family level we found significant differences due to Treatment ($\chi^2 = 11.099$; p = 0.004) in the analysis that included the three categories. Interestingly, in this case the post hoc analysis showed that not only the diversity under the one-year exclusion trees was significantly higher than under the control trees (estimate = −0.442; T = −3.183; p = 0.006), the same happened when control trees were compared with those at abandoned farms (estimate = −0.336; T = −2.414; p = 0.048) (Fig. 4C). Removing the long-term-exclusion trees, we found differences due to Treatment ($\chi^2 = 10.687$; p = 0.001) but not Farm ($\chi^2 = 1.127$; p = 0.568). Arthropod diversity at family level was higher at short-term exclosures compared to controls in all the three farms (Fig. 4D).

At order level, similarly to the results obtained looking only at richness, we found no significant differences in any case.

3.4. Community composition and indicator species

MOTU community composition differed significantly between treatments. The NMDS analyses retrieved final stress values of 0.21 and 0.23 for the ordinations according to COI and 16S MOTUs, respectively (Fig. 5). For both markers, the first axis of the NMDS ordination (Axis 1) identified a gradient between control trees and one- and ten-years-excluded farms, with a clear segregation of the latter (long-term exclosures) with respect to the other two. Accordingly, correlation analyses showed that the main factor conditioning the community differences for COI and 16S markers was Treatment ($r^2 = 0.36$; p = 0.001 and $r^2 = 0.32$;

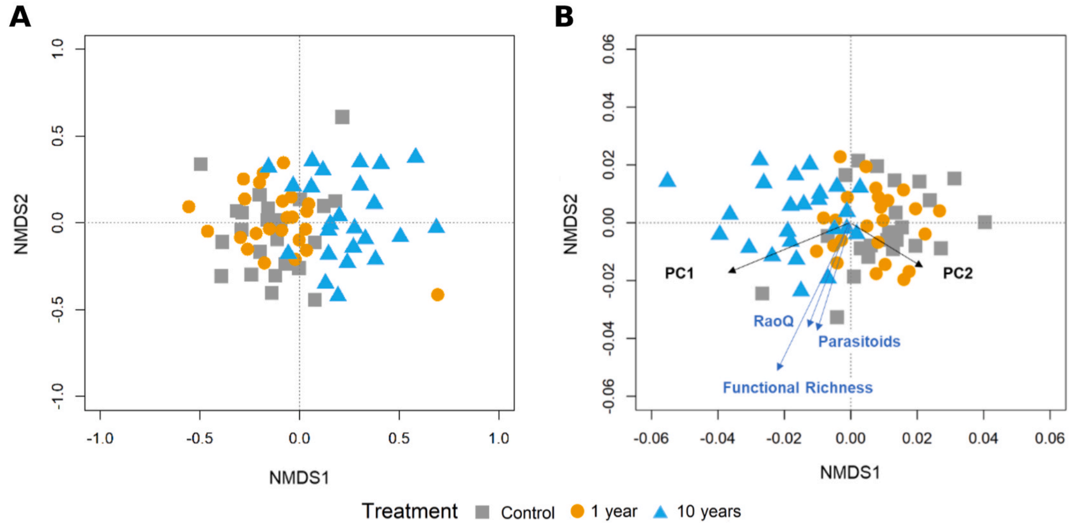

Fig. 5. Nonmetric multidimensional scaling ordination (NMDS) of study plots based on (**A**) COI or (**B**) 16S MOTU-level datasets. Vectors indicate significant correlations between MOTUs composition and i) vegetation structure (black arrows) ii) functional diversity (blue arrows). PC1 and PC2 correspond to the first and second axes of the first Principal Component Analyses (PCA); RaoQ: Rao's quadratic entropy index; and Parasitoids: Community Weighted Mean of parasitoid guild.

$p = 0.001$ respectively) and, only for the 16S marker, the vegetation structure. In the latter, both PCA factors were significantly correlated with species composition, being the first one related with long-term exclosures, which also have a high cover of tall grass (PC1: $r^2 = 0.16$; $p = 0.002$), and the second with control and one-year excluded trees in which shorter, medium-sized grass is more prevalent ($r^2 = 0.09$; $p = 0.039$) (Fig. 5B).

The species retention rate curves (Fig. 6) showed that the long-term-exclusion trees were not only the most different compared with the other treatments, but also the most different among themselves, followed by the controls and the one-year-exclusion trees. This pattern was consistent with both datasets, albeit COI (Fig. 6A) detected less species in common between samples than 16S (Fig. 6B). As an example, out of the 24 trees in each of the three treatments, with COI, there was not a single MOTU that was present in more than 5 trees in the long-term exclosures (Fig. 6A). The trend in both markers indicates that the trees under the one-year-exclusion regime were colonized by mostly the same arthropod species in many cases, while the communities in the long-term-exclusion trees had diverged towards different species assemblages. In all cases, the models better fit to an exponential than to a power-law regression, indicating that community assembly within the treatments did not respond to niche differentiation, but rather to stochastic processes (McGeoch et al., 2019).

With regards to the indicator species analysis (Table 2, Fig. 7), we identified 15 indicator MOTUs with COI and 17 with 16S. From the 15 MOTUs identified as indicators with COI, one corresponded to the control group, eight to one-year exclusion, and six to the long-term exclosures (Table 2, Fig. 7A). With 16S, on the other hand, we found no indicator MOTU for the control group, while eight were found for the one-year-exclusion group, and nine for the trees in which livestock had been excluded in the long-term (Table 2, Fig. 7B). Most MOTUs identified as indicators for the abandoned farms were herbivores and some predators, while the ones from the one-year excluded trees included a mix of herbivores (mostly), saprophagous, predators and parasitoids. In general, indicator species for one-year exclusion communities can be found abundantly in communities from other treatments, while indicator species for ten-year exclusion communities are almost exclusively found in these communities (Fig. 7). Interestingly, with 16S we

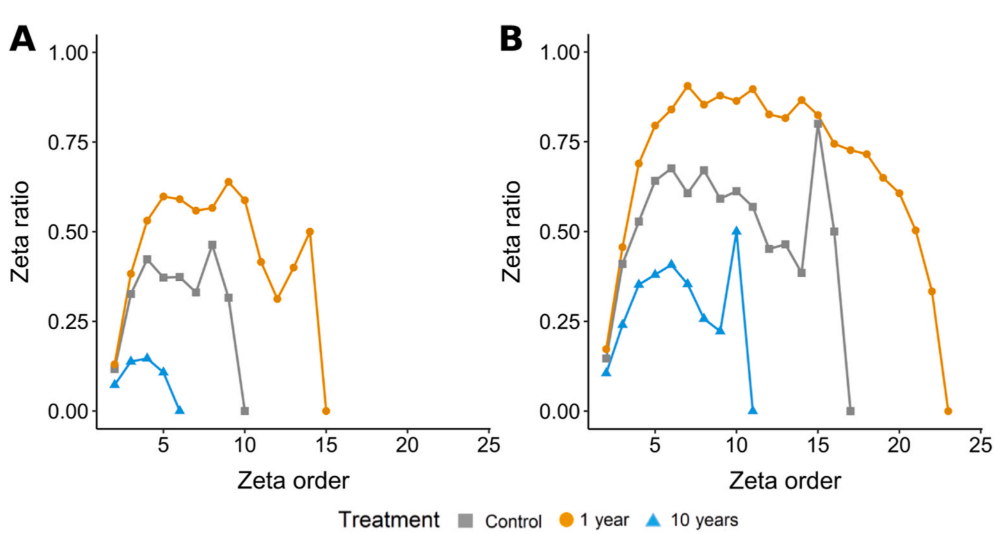

Fig. 6. Arthropod species retention rates (Zeta ratio) calculated from MOTU databases retrieved using the COI (**A**) and 16S (**B**) markers. The Y-axis (Z-ratio) shows the percentage of species shared by all trees as the number of trees included in the analyses increases (Zeta order). Control trees and those at short-term (1 year) and long-term (10 years) exclosures are depicted by grey squares, orange circles and blue triangles, respectively.

Table 2
Indicator species. This table shows COI and 16S indicator species with their sequence identification MOTU number, the treatment which represent and their taxonomical identification. Additionally, the guild associated to their larvae life stage is added on those MOTUs identified to genus name.

MOTU	Cluster	Indicator Value	Probability	Taxonomy	Guild
COI_350	Control	0.4148	0.0051	Insecta	–
COI_192	1 year	0.3343	0.0355	Hemiptera, Miridae	–
COI_213	1 year	0.3152	0.0097	Hemiptera, Miridae	–
COI_105641	1 year	0.1962	0.0354	Coleoptera, Coccinellidae, *Rhyzobius litura*	Predator
COI_103926	1 year	0.1818	0.0100	Hemiptera, Miridae, *Pachytomella parallela*	Herbivore
COI_6939	1 year	0.1771	0.0143	Collembola	–
COI_440497	1 year	0.1625	0.0192	Hemiptera, Berytidae, *Neides tipularius*	Herbivore
COI_121382	1 year	0.1364	0.0268	Araneae, Linyphiidae, *Frontinellina frutetorum*	Predator
COI_101213	1 year	0.1339	0.0436	Hemiptera, Berytidae, *Berytinus montivagus*	Herbivore
COI_273467	10 years	0.2727	0.0004	Hemiptera, Lygaeidae, *Horvathiolus superbus*	Hervibore
COI_109735	10 years	0.2241	0.0438	Hemiptera, Cicadellidae, *Macustus grisescens*	Herbivore
COI_31760	10 years	0.2083	0.0091	Araneae, Philodromidae, *Philodromus bistigma*	Predator
COI_244694	10 years	0.1818	0.0092	Hemiptera, Miridae, *Deraeocoris morio*	Predator
COI_155485	10 years	0.1732	0.0199	Hemiptera, Issidae, Mulsantereum maculifrons	Herbivore
COI_128844	10 years	0.1364	0.0301	Lepidoptera, Nymphalidae, *Pararge aegeria*	Herbivore
16S_100172	1 year	0.4580	0.0164	Hemiptera, Miridae, *Leptoterna dolabrata*	Herbivore
16S_439	1 year	0.2825	0.0062	Hemiptera, Aphididae, *Sitobion avenae*	Herbivore
16S_107957	1 year	0.2301	0.0154	Entomobryomorpha, Entomobryidae, *Willowsia nigromaculata*	Saprophagous
16S_413	1 year	0.1977	0.0223	Diptera, Phoridae, *Megaselia* sp.	Parasitoid
16S_2218	1 year	0.1807	0.0301	Diptera, Cecidomyiidae	–
16S_1025	1 year	0.1754	0.0321	Hemiptera, Miridae, *Orthocephalus* sp.	Herbivore
16S_113984	1 year	0.1667	0.0265	Entomobryomorpha, Entomobryidae, *Entomobrya proxima*	Saprophagous
16S_124014	1 year	0.1420	0.0496	Hemiptera, Berytidae, *Neoneides muticus*	Herbivore
16S_304	10 years	0.4262	0.0002	Hemiptera, Miridae	–
16S_5680	10 years	0.2456	0.0025	Araneae, Philidromidae	–
16S_160619	10 years	0.2273	0.0028	Hemiptera	–
16S_228	10 years	0.2273	0.0030	Hemiptera	–
16S_1498	10 years	0.2168	0.0043	Hymenoptera, Formicidae, *Camponotus* sp.	Saprophagous
16S_104872	10 years	0.1818	0.0096	Coleoptera, Cantharidae, *Malthodes* sp.	Predator
16S_4750	10 years	0.1818	0.0084	Hemiptera, Miridae, *Deraeocoris* sp.*1*	Predator
16S_111244	10 years	0.1364	0.0268	Hemiptera, Miridae, *Deraeocoris* sp.*2*	Predator
16S_2594	10 years	0.1364	0.0278	Hymenoptera, Formicidae	–

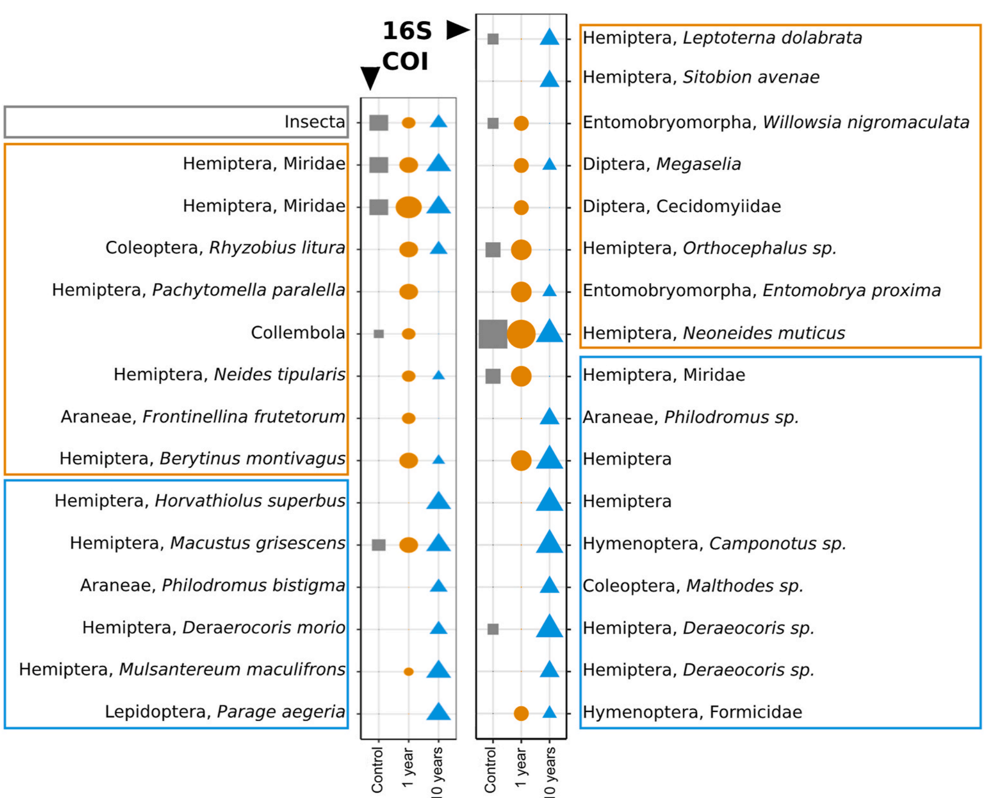

Fig. 7. Indicator species according to the datasets retrieved using the COI (left) and 16S (right) markers. Semiquantitative abundance (a relative abundance one order of magnitude larger is indicated by double the size of the bubble) under the trees of each treatment is depicted. Species are grouped in rectangles according to the group they represent: control (grey), one-year livestock exclusion (orange), or ten-years livestock exclusion (blue).

identified the aphid *Sitobion avenae* as an indicator for the one-year-exclusion communities, and with COI we identified its predator, the ladybug *Ryzhobius* sp., as an indicator for the same communities. In most cases (13 out of 15 for COI, and 11 out of 17 for 16S), the MOTUs identified as indicators for any treatment are also among the most abundant MOTUs in their respective datasets.

3.5. Functional responses

Finally, we found that Functional Richness (Fric), Rao's quadratic entropy (RaoQ), and the percentage of parasitoids species significantly covaried with the vegetation structure, being higher in trees with higher grass cover and taller herbs, independently of the treatment (Parasitoids $r^2 = 0.10$, $p = 0.045$; RaoQ: $r^2 = 0.10$, $p = 0.030$ and Fric: 0.15 $p = 0.006$; Fig. 5B).

4. Discussion

Thanks to DNA metabarcoding, we could perform the most thorough analyses up to date on the effects of livestock on arthropod communities in Iberian dehesas, the most widespread silvopastoral system in Europe (Moreno and Pulido, 2009). In control trees (livestock present) vegetation cover and height were lower, and so were the richness and diversity of arthropods at MOTU and family level. Grass cover started to increase just one year after livestock removal. However, arthropod diversity and taxonomic richness did not peak at long-term exclosures but at short-term ones. In long-term exclosures arthropod community composition showed a higher differentiation, both among them and compared to control and short-term exclosure ones.

4.1. Biodiversity detection

DNA metabarcoding has been proven to be a very effective and powerful tool to study arthropod communities as a whole by overcoming the taxonomic impediment, allowing detection of a large variety of taxa (see Liu et al., 2020 and references therein). To the best of our knowledge, this is the first time that such a wide biodiversity analysis has been carried out at the species level in Mediterranean evergreen oak woodlands ("dehesas" or "montados"). Previous studies on soil arthropod communities from this habitat had been performed by grouping at higher taxonomic levels: class, order and, in rarer cases, family (Andrés et al., 1999; Rota et al., 2015; Sadaka and Ponge, 2003). At a lower taxonomic level, only checklists of species from certain families have been reported (e.g. Zaballos, 1983), but in no case related to the effects of livestock on arthropods. In the present study we could analyse it at the MOTU level, despite not all of them could be assigned to Linnean species.

Identification success differed among taxonomic levels depending on the availability of reference sequences. Almost all arthropod MOTUs were identified to order level, and more than 80 % to family level. We could thus identify most of the arthropod families coexisting in these Iberian holm oak savannas. However, identification success at the species level was lower: we could assign Linnean species names to only 40 % of the MOTUs. This highlights the importance of promoting classical taxonomy research as the solid base to create comprehensive and reliable reference databases of DNA barcodes, indispensable for the use of molecular identification methods. Moreover, these databases should be geographically unbiased, since in the Mediterranean region, where our study took place, reference barcodes are scarcer than, for example, in Central Europe, which decreases identification likelihood (Gaytán et al., 2020).

The use of two different genetic markers increased biodiversity detection success. The complementary use of 16S in addition to COI did not contribute detection at the order level, but it did at the family level. Although more families were exclusively detected by COI, others were only retrieved by 16S. Moreover, these families may have had an important role in the biodiversity analysis, as differences between treatments in the richness at MOTU level were always more pronounced using the 16S dataset. These results agree with a previous study which concluded that the best markers to detect Hexapoda subphylum barcodes are found in the mitochondrial ribosomal RNA (rRNA) genes, such as 12S and 16S (Marquina et al., 2019b). However, our results suggest that their conclusions may be applicable not just on Hexapoda, but also to all Arthropoda phylum.

For this work, we chose a non-destructive approach for the DNA extraction from the bulk samples, as we were interested in keeping the individual arthropods for further morphological analysis. A series of recent studies have tested the suitability of these non-destructive DNA extraction protocols, finding them as a promising alternative to sample homogenization in terms of biodiversity detection (Batovska et al., 2021; Iwaszkiewicz-Eggebrecht et al., 2023; Marquina et al., 2022; Nielsen et al., 2019). However, there may be limitations relevant for the analysis of our samples: for instance, both Iwaszkiewicz-Eggebrecht et al. (2023) and Marquina et al. (2022) found that ants (Hymenoptera: Formicidae) were detected in their mock communities in much lower proportion and occasions than other taxa. This could have affected our results, as we worked with ground-dwelling arthropod communities, in which ants are prominent members. Nonetheless, we did find a great diversity of MOTUs belonging to Formicidae (18 with COI and 12 with 16S), which we consider acceptable (Arcos and García, 2023).

4.2. Effects of livestock on vegetation and arthropod community

Livestock grazing reduced vegetation cover and height, prompting trophic cascade effects on arthropod community. Grass cover differed among control trees of different farms due to differences in grazing intensity. Moreover, it was higher beneath the canopies of trees subjected to one-year and long-term livestock exclosures. Those places with lower grass height and cover (control trees in all farms) exhibited lower species richness and diversity. Grass height is a fine predictor of plant and insect diversity under contrasting grazing intensities (Kruess and Tscharntke, 2002).

Livestock and phytophagous arthropods compete for the same food resource: vegetation. Competition takes place through direct and indirect interactions; both affect each other directly by decreasing food resources (density-mediated interaction (Dennis et al., 2008; Evans et al., 2015; Feeley and Terborgh, 2006; Werner and Peacor, 2003)). The interaction is, however, frequently asymmetrical due to differences in body size that also favour other antagonistic interactions, such as incidental intraguild predation of phytophagous arthropods by livestock (Canelo et al., 2021b; Gómez and González-Megías, 2002; Zamora and Gómez, 1993). Moreover, the negative effects of livestock go beyond phytophagous arthropods and extend to predators and parasitoid species, which may also be incidentally predated by livestock albeit at lower rates than herbivores (Berman and Inbar, 2022). Predatory arthropods also suffer the scarcity of prey (phytophagous invertebrates) due to livestock grazing (King et al., 2014; Prieto-Benítez and Méndez, 2011).

The richness and diversity of arthropods increased after livestock exclusion, but, surprisingly, it did not increase with the length of the exclusion period: it peaked at short-term (one year) exclosures; only family level diversity was significantly higher at long-term exclosures compared to control trees. After one-year of livestock exclusion, vegetation cover was higher and medium-sized grass prevailed. This meant more food for herbivore arthropods –owing to the lack of competition with livestock–, a greater availability of refuge, and reduced intra-guild predation risk. Local herbivore arthropods at the excluded trees may increase their population size and new species may arrive from neighbouring areas (Catford et al., 2012; Harvey et al., 2016; Townsend et al., 1997). Higher grass cover in one year-exclosures was also related with an increase of arthropod functional diversity, including an increase in parasitic species. Therefore, the increase in primary production triggers a bottom-up trophic cascade at this initial period. This quick peak supports the predictions of the intermediate disturbance hypothesis (IDH).

The intermediate disturbance hypothesis predicts a peak of species richness at intermediate levels of disturbance (Connell, 1978; Gao and Carmel, 2020; Roxburgh et al., 2004; Svensson et al., 2007; Yan et al., 2015; Yuan et al., 2016). Within the context of our study, this would be the situation after one year of livestock exclusion. The sequence would be: high disturbance (livestock present), time-spaced disturbance (one-year exclusion, the disturbance starts to disappear) and no disturbance (ten-years exclusion). Shortly after exclusion, grass cover increases and vegetation composition changes (Sims et al., 2019). In parallel, microhabitat heterogeneity increases (Song et al., 2020), what favours species with different habitat and food requirements (Losapio et al., 2024; Stephan et al., 2017) (for example, open-habitat omnivore generalists, herbivore specialists, or parasitoids). For instance, it has been shown that, in Mediterranean regions, bee and ant richness increases after a wildfire due to the new niches created (Vidal-Cordero et al., 2023; see van Kilnk et al., 2015 for a thorough review regarding large herbivore effects on arthropod diversity).

Certain species find a suitable habitat at the recently-undisturbed areas (one-year exclosures), where they may arrive from the surroundings, and establish in the short-term. This could explain the similarity between arthropod communities under the one-year-exclusion trees seen in the Z-diversity graphs. Nevertheless, after 10 years of livestock exclusion, the competition between arthropods increases. Grass cover and height increase, and the habitat becomes more homogeneous. According to the competitive exclusion principle (Hardin, 1960; McPeek, 2014), species which compete strongly for the same resource cannot coexist; one of them will overtake the other. This is what may happen in long-term exclosures, the lack of grazing homogenizes the habitat and promotes competition among herbivores. However, the homogenization of the habitat may favour certain specialists: as times go by the overall number of species will be slightly reduced, some will disappear, but others may increase their abundance.

The slight decrease in taxonomic richness and diversity does not mean that arthropod communities become homogeneous at long-term exclosures, rather, they differed a lot among them and compared to the trees of the other categories. Species composition differed with respect to short-term exclosures probably because certain some taxa (like Colembolla) disappear as grass height increases. On the other side, the rather large variability within long-term exclosure trees shown in the Z-diversity analysis indicate that communities diverged with time towards different and unique species compositions. Further studies considering additional functional traits will help to understand the process underlying such temporal changes in arthropod communities.

5. Conclusions

In summary, our results support the intermediate-disturbance hypothesis (Connell, 1978; Gao and Carmel, 2020; Roxburgh et al., 2004; Svensson et al., 2007; Yan et al., 2015). Arthropod diversity peaks after short-time livestock exclusion, where vegetation cover has increased compared to control trees but habitat heterogeneity is still higher than at long-term exclosures dominated by tall grass. Regarding the livestock-driven biological control of acorn pests (Canelo et al., 2021b), the present study shows that intensive grazing does reduce arthropod taxonomic richness and diversity. Our results also put forward that the recovery after livestock exclusion is fast. Thus, the proposed rotative management combining, within the same dehesa farm, plots with temporary increased grazing and short-term livestock exclosures, would be appropriate. This innovative livestock management would increase the productivity of Iberian oak savannas by reducing acorn pests, while also preserving its unique and rich arthropod biodiversity.

CRediT authorship contribution statement

Tara Canelo: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Daniel Marquina:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Sergio Chozas:** Writing – review & editing, Formal analysis. **Johannes Bergsten:** Writing – review & editing, Conceptualization. **Álvaro Gaytán:** Methodology. **Carlos Pérez-Izquierdo:** Methodology. **Raúl Bonal:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2024.121619.

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