

Tree - Open Grassland Mosaics Drive the Herbaceous Structure and Diversity in Mediterranean Holm Oak Meadows

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Abstract

Background: Mediterranean holm oak meadows are semi-natural savannah-like agroecosystems that result from traditional silvo-pastoral practices, which shaped these systems into a mosaic of trees and open grassland. However, traditional silvo-pastoral uses are declining with the implications that this may have on the herbaceous layer, a very biodiverse and valuable resource of these systems. Here, we aim at assessing the influence of the tree – open grassland mosaic on the structure, diversity, and composition of the herbaceous layer. Specifically, assessing the canopy effect (a) under representative Iberian canopy types, considering traditional *Quercus* species stands and *Pinus pinea* plantations at different locations; and (b) along seasonality.

Results: The different components of the herbaceous layer performed differential responses to the presence/absence of tree canopies, as for instance shows the dominance of grasses under the canopy, while legumes and forbs were favoured in the open grassland. Also, there was a certain a reduction in the species richness in *P. pinea* dominated plots compared to plots dominated by *Quercus* species. There was a reduction of the aboveground biomass under the canopy at the more environmentally constrained location. Such canopy effects were generally more pronounced in spring than in autumn.

Conclusion: It is highly advisable preserve the tree – open grassland mosaic and traditional *Quercus* species stands to maximize and preserve plant specific and functional diversity. The optimum tree coverage might be dependent, not only on the primary ecosystem service (i. e. forage provision), but also on local conditions.

1. Background

Mediterranean holm oak meadows, also called dehesas in Spain and montados in Portugal, are seminatural savannahlike agroecosystems that result from silvopastoral practices, in which an herbaceous and an arboreal layer – mostly *Quercus* species –coexist (Ibañez et al. 2021). They are one of the largest agroforestry systems in Europe (Eichhorn et al. 2006), and are particularly abundant in the SouthWest of the Iberian Peninsula (Olea et al. 2005).

Mediterranean holm oak meadows have traditionally provided pasture and acorns for livestock, timber, and cork, and those traditional silvo-pastoral uses shaped holm oak meadows into a mosaic of trees and open grassland creating diverse ecological niches (Sankaran et al. 2004). In particular, holm oak meadows are extraordinarily rich in plant diversity (Marañón 1985; Moreno et al. 2016) and have been typified as habitat of community interest by the EU Habitats directive (6310 Dehesas with evergreen *Quercus* spp) for their singularity and potential to preserve biodiversity. However, those traditional silvo-pastoral uses are nowadays changing. Mediterranean holm oak meadows are suffering simultaneously processes of intensification and abandonment (Peco et al. 2000). Traditional grazing practices are replaced by intensive farming and plantations of fastgrowing trees, mostly *Eucalyptus* and *Pinus* species (Costa Pérez et al. 2006; Costa et al. 2011); while less productive meadows are abandoned, which results in shrub encroachment and loss of diversity (Peco et al. 2000).

Thus, the tree – open grassland mosaic itself is as a key element for the preservation and functioning of holm oak meadows. The mosaic drives microclimatic conditions (Ibañez et al. 2021), soil organic matter accumulation (Howlett et al. 2011; Gómez-Rey et al. 2013; Pulido-Fernández et al. 2013) and soil fertility

(Andivia et al. 2015), which in turn modify the productivity (Moreno et al. 2007; Gea-Izquierdo et al. 2009; Hussain et al. 2009) and diversity (Peco et al. 2006; Marañón et al. 2009; Lopez-Carrasco et al. 2015; Rossetti et al. 2015; López-Sánchez et al. 2016a, b) of the herbaceous layer.

However, although some research has been done on the influence of tree canopies on the herbaceous layer at the ecosystem scale, in this study our objective is to deepen such canopy effect under representative Iberian canopy types, comparing traditional *Quercus* species stands and *Pinus pinea* plantations; at different locations; and along seasonality. For that purpose, we considered insightfully all the herbaceous layer compartments (above and belowground biomass, and litter); specific and functional diversity and composition; and we asked the following questions: (i) are the different components of the herbaceous layer structure, composition and diversity affected in the same way by the tree - open grassland mosaic? (ii) is the canopy effect similar between *Quercus* species and *Pinus pinea* plantations? and (iii) how does the canopy effect evolve along seasonality?

2. Methodology

2.1 Study sites and sampling design

Study sites were the same as described in Ibañez et al. (2021), distributed in two locations in the SouthWest of the Iberian Peninsula (Fig. 1): Sierra Morena mountains (SM, 37° 39' 50" N, 5° 56' 20" W, 296 m a. s. l.), and Doñana Natural Park (DN, 37° 15' 34" N, 6° 19' 55" W, 30 m a. s. l.). Both locations have Mediterranean climate regime (Peel et al. 2007) with warm, dry summers, and mild winters. However, SM is slightly cooler and wetter than DN, with mean annual temperature in SM of 16.8 °C and in DN of 18.1 °C, and mean annual precipitation in SM of 648 mm and in DN of 543 mm.

SM soils have a texture between sandy clay loam and clay. DN soils are sandier than SM, with a sandy loam texture. Total soil nitrogen (N, 0–30 cm depth) is quite low in both locations, but lower in DN (0.06–0.20% N) than in SM (0.85% N Ibañez et al., 2021). Grasslands in both locations are dominated by herbaceous annual species, including grasses (i. e. *Bromus hordeaceus*), non-legume forbs (i. e. *Erodium moschatum*), and legume forbs (i. e. *Trifolium subterraneum*). Both locations are extensively grazed at similar stocking rates: SM grazed by cattle and Iberian pigs (0.36 LSU ha⁻¹), and DN grazed by cattle and goat (0.40 livestock units (LSU) ha⁻¹), both typical stocking rates in Mediterranean grazing systems.

Study plots were selected according to their tree composition, representing typical canopy types of Iberian holm oak meadows. One pure *Quercus ilex* stand, in the SM location (SM-ilex), and one pure *Quercus suber* stand in the DN location (DNsuber), both the most abundant stands in the Iberian context (Costa Pérez et al. 2006); one *Q. ilex* and *Q. suber* mixed stand (DNmixed), the next most abundant; and a pure *P. pinea* stand (DN-pinea. Figure 1.c), a common tree plantation replacing traditional *Quercus* canopies (Costa Pérez et al. 2006). Plot tree densities (trees ha⁻¹) are on the average for the region (Costa Pérez et al. 2006): 34 ± 1 in SM-ilex, 26 ± 1 in DNmixed, 26 ± 4 in DN-suber, and 48 ± 6 in DN-pinea. In the DNmixed plot, we discriminated between both *Quercus* species (*Q. suber* and *Q. ilex*) to establish sampling points. However, preliminary comparative analysis in the DNmixed plot on microenvironmental conditions and vegetation characteristics under the canopy of both *Quercus* species indicated no relevant differences. DNmixed plot results are then always presented, combining both tree species.

Field work was carried out in spring (05/04/2016 – 10/04/2016) and autumn (13/12/2016–17/12/2016), coinciding with the most productive moments of the system, to capture vegetation seasonal variability and canopy effects that may be season dependent. Study treatments were, therefore, established according to plot (SMilex, DN-mixed, DN-suber, and DNpinea), season (spring and autumn), and canopy (open grassland, OG, and under the canopy, UC). Sampling points of the UC treatment were always placed at 1 m distance from the selected tree trunk, and sampling points of the OG treatment were placed at a minimal distance of 3 m from the selected tree, clearly outside the canopy. Sampling points were systematically placed following the north orientation with respect to the tree trunks (Ibañez et al. 2021). For each treatment level, we selected 3–4 samples, totalling 73 sampling points.

2.2 Vegetation sampling

At each sampling point, we hammered a metal collar (diameter = 25 cm) that defined the sampling area, and we harvested the herbaceous vegetation at ground level rooted within each metal collar. Thereafter in the laboratory, we separated aboveground biomass (AGB) from litter (dead plant material detached from the herbaceous vegetation and tree leaves on soil surface). Also, we separated the AGB into plant species. Species were then attributed to the given plant functional type (PFT): grasses, non-legume forbs (hereafter “forbs”), and legume forbs (hereafter “legumes”). Both levels of analysis (species and PFT) provided complementary information (Zhou et al. 2017), providing the first information about the species distribution *per se*, while the latter provided a mechanistic link between vegetation and a given ecosystem function (Petchey and Gaston 2006; Debouk et al. 2015, 2020).

Finally, we determined the belowground biomass (BGB) by extracting a soil core of 9 cm² surface and 0–10 cm depth at each sampling point. Soil cores were afterwards washed and filtered with a 0.2 mm pore size in the laboratory. All vegetation samples were oven dried at 60 °C until constant weight.

2.3 Data analysis: structure, composition, and diversity of the herbaceous layer

We performed linear models on the absolute abundances of herbaceous structural components (AGB, litter and BGB) and PFT (forbs, grasses, and legumes), as function of plot, season, and canopy, and the corresponding interactions. Also, we calculated diversity indexes, including species richness (SR), defined as the number of species per sample; and species evenness according to Kirwan et al. (2007, Eq. 1). The evenness index has been defined as a measure of the distribution of the relative abundance of species, and lies between 0 for monospecific plots to 1 for a plot in which all species are equally represented (Kirwan et al., 2007):

$$\text{Evenness} = \frac{2S}{S-1} \sum_{i < j} P_i P_j \quad (\text{Equation 1})$$

Where S is the number of species in the community matrix, and $P_i P_j$ the pairwise interactions between species, meaning the product of the relative abundance of the i th and j th species (Eq. 1). SR and evenness were also modelled as function of plot, season, and canopy, and the corresponding interactions using linear models. Final models were selected by a stepwise procedure based on the Akaike information criterion (AIC) using the stepAIC function, MASS package (Venables and Ripley 2002).

Also, we described the influence of plot, season, and canopy on species composition by canonical correspondence analysis (CCA) to represent the community on a given number of dimensions (Sandau et al. 2014). We performed the CCA on species absolute abundance using the `cca` function of the `vegan` package (Oksanen et al. 2018). Significance of the CCA terms (plot, season, and canopy) and significance of the CCA axes were both tested using the `anova.cca` function, also of the `vegan` package. Afterwards, means and standard errors of the first three significant ($p < 0.001$) axes were calculated and plotted for each level of the terms included as predictors (plot, season, and canopy). Also, we selected the species with the highest CCA scores within each axis (CCA1CCA3, top five negative and top five positive), and the four species closest to the axes (0, 0), for representation purposes, and to unravel specific similarities and dissimilarities in the species composition between treatments.

3. Results

3.1 General structure of the herbaceous layer

The structure of the herbaceous layer was dependent on the particular plot, season, and the presence/absence of the tree canopy (Fig. 2). The AGB was lower in all DN plots compared to the SM-ilex plot (plot effects, Table 1); AGB decreased in autumn compared to spring (season effect, Table 1); and AGB was lower under the canopy than in the open grassland in all DN plots (canopy effect, Table 1), but not in the SM-ilex plot (Fig. 2). Litter was markedly higher under the canopy than in the open grassland in spring, but this difference between the canopy and the open grassland almost disappeared in autumn (season x canopy effect, Table 1). BGB was quite variable among treatments and neither season, nor plot or canopy explained its variability. Linear modelling on BGB is not shown.

Table 1

Modelling of the herbaceous layer structure in dry weight (DW), including aboveground (AGB) and litter biomass, as function of plot, season, and canopy. Season with spring as reference level, plot with SM-ilex as reference level, and canopy with open grassland (OG) as reference level. Parameter estimates (Par.), standard error (SE), t and p -value.

Vegetation structure (g DW m ⁻²)								
	AGB				Litter			
	Par.	SE	t	p -value	Par.	SE	t	p -value
(Intercept)	336	17	19.84	< 0.001	28	20	1.41	0.2
Plot DNmixed	-133	17	-7.62	< 0.001				
Plot DNsuber	-129	21	-6.20	< 0.001				
Plot DNpinea	-130	21	-6.28	< 0.001				
Season	-142	13	-10.76	< 0.001	3	30	0.09	0.1
Canopy	-25	13	-1.90	0.06	232	29	7.98	< 0.001
Season x canopy					-218	44	-4.99	< 0.001
R ² _{Adj}	0.70			< 0.001	0.53			< 0.001

3.2 Herbaceous plant functional types

PFT composition also changed among plots, season, and canopy (Fig. 3). Forb biomass was lower in all DN plots than in the SMilex plot (DN plots effects, Table 2). Forb biomass decreased in autumn compared to spring (season effect, Table 2); and forb biomass decreased under the canopy compared to the open grassland (canopy effect, Table 2). Grass biomass was also lower in all DN plots compared to the SMilex plot (significant DN plots effects, Table 2). Unlike forbs, grasses increased under the canopy compared to the open grassland, but such difference between the under the canopy and the open grassland was lower in autumn than in spring (season x canopy effect, Table 2), when the biomass of grasses equalized between both microenvironments (Fig. 3). Finally, legumes appeared mostly in spring and in the open grassland (season x canopy effect, Table 2).

Table 2

Modelling of plant functional type (PFT) composition in dry weight (DW), including forbs, grasses, and legumes, as function of plot, season, and canopy. Plot with SM-ilex as reference level, season with spring as reference level, and canopy with open grassland (OG) as reference level. Parameter estimates (Par.), standard error (SE), t and p-value.

	PFT composition (g DW m ⁻²)											
	Forbs				Grasses				Legumes			
	Par.	SE	t	p-value	Par.	SE	t	p-value	Par.	SE	t	p-value
(Intercept)	224	15	15.36	< 0.001	64	11	5.70	< 0.001	55	7	8.11	< 0.001
Plot DNmixed	-68	14	-4.79	< 0.001	-51	11	-4.64	< 0.001	-14	7	-2.06	0.04
Plot DNsuber	-87	17	-5.12	< 0.001	-36	13	-2.73	0.008	-11	8	-1.36	0.200
Plot DNpinea	-66	17	-3.92	< 0.001	-48	13	-3.65	0.0005	-19	8	-2.36	0.02
Season	-131	15	-8.73	< 0.001	11	12	0.93	0.4	-36	7	-5.20	< 0.001
Canopy	-46	14	-3.19	0.002	46	11	4.16	< 0.001	-40	7	-5.99	< 0.001
Season x canopy	34	22	1.59	0.1	-39	17	-2.33	0.02	35	10	3.45	< 0.001
R²_{Adj}	0.66				0.33				0.41			
	< 0.001				< 0.001				< 0.001			

3.3 Herbaceous species diversity and composition

Species evenness (Fig. 4.a) was only dependent on season, being lower in autumn than in spring (season effect, Table 3). Species richness (SR) decreased in the DN-pinea plot compared to the other plots (plot DNpinea effect), specially in autumn (Fig. 4.b). SR was also lower in autumn than in spring (season effect, Table 3); and lower under the canopy than in the open grassland (canopy effect, Table 3), being this difference between under the canopy and the open grassland specially marked in spring in the DN-suber plot (Fig. 4.b).

Table 3

Modelling of species evenness and species richness (SR), as function of plot, season, and canopy. Season with spring as reference level, plot with SMilex as reference level, and canopy with open grassland (OG) as reference level. Parameter estimates (Par.), standard error (SE), t and p-value.

	Species evenness (dimensionless)				Species richness (SR, nº species sample ⁻¹)			
	Par.	SE	t	p	Par.	SE	t	p
Intercept	0.74	0.02	30.59	< 0.001	10.6	0.6	17.12	< 0.001
Plot DNmixed					-1.3	0.6	-1.97	0.05
Plot DNsuber					-0.6	0.8	-0.79	0.4
Plot DNpinea					-1.7	0.8	-2.29	0.02
Season	-0.12	0.04	-3.21	0.002	-1.6	0.5	-3.28	0.002
Canopy					-1.6	0.5	-3.26	0.002
R²_{Adj}	0.11			0.002	0.23			< 0.001

CCA on species absolute abundance had a constrained inertia of 1.87 over a total of 10.50, representing a 17.8% of explained variance, with plot, season, canopy, and season x canopy as explanatory terms. The anova.cca by terms showed that all the terms included in the CCA were significant ($p < 0.001$), and the anova.cca by axes showed that the first four axes out of six were also significant (CCA1 – CCA3, $p < 0.001$, and CCA4, $p = 0.04$).

The first CCA axis (CCA1, 30% of total explained variance) captured mainly differences in species composition between spring and autumn (CCA1, Fig. 5). In spring, species as *Hordeum leporinum*, *Trifolium cherleri*, *Trifolium glomeratum*, and *Medicago doliata* (positive side of the CCA1, Fig. 6.a) were much more abundant than in autumn. On the contrary, species as *Myosotis ramosissima*, *Genista hirsuta*, *Hypochaeris glabra*, *Cerastium glomeratum*, and *Silene gallica* (negative side of the CCA1, Fig. 6.a) were only found in autumn. Species as *Vulpia membranacea*, *Carex divulsa*, *Ornithopus pinnatus*, *Lathyrus cicera*, and *Galium aparine* (close to the axis CCA1, Fig. 6.a) were not especially abundant, but could be found in both seasons.

The second axis (CCA2, 24% of total explained variance) explained mainly differences in species composition between plots: on one side SM-ilex and DNsuber, and on the other side DN-mixed and DNpinea plots (Fig. 5.a). SM-ilex and DN-suber being especially abundant in *Lolium perenne* (negative side of the CCA3), and DN-pinea being especially abundant in *Stachys arvensis* (positive side of the CCA2. Figure 6.b).

Finally, the third axis (CCA3, 15% of total explained variance), captured differences in species composition between under the canopy and the open grassland dependent on season (CCA3, Fig. 5.b). In the open grassland legumes as *M. doliata* and *T. glomeratum* (Fig. 6.c) were highly abundant in spring (negative side of the CCA3 and positive side of the CCA1, Fig. 6.c); while in autumn there was presence of a group of forbs, including *Taraxacum officinale*, *Leontodon longirrostris*, and *Crepis capillaris* (negative side of the CCA3 and negative side of the CCA1, Fig. 6.c). Conversely, under the canopy and in spring *Carex divulsa* was specially abundant, and some other species as *Urtica urens*, *L. cicera*, *Brassica barrelieri*, *G. aparine*, were also present, but in low proportion (positive side of the CCA3, Fig. 6.c). Under the canopy and in autumn, *Geranium molle* was specially abundant, together with many other species in lower proportion.

4. Discussion

4.1 The tree – open grassland mosaic as driver of the herbaceous layer composition

Tree canopies were important spatial drivers of the herbaceous layer, both in terms of structure (specially increasing the litter under the canopy, Fig. 2) and composition (Figs. 3–5). However, in line with our first question, the different herbaceous components presented some differences in their response to tree canopies, which was especially notable on the PFT (Fig. 3) and species distribution (Figs. 5–6). Thus, the microenvironment created under the canopy favoured the dominance of some species, mainly grasses, while the open grassland favoured the presence of legume and nonlegume forbs (Fig. 3). Previous studies have also described dominance of grasses under the canopy (Olsvig-Whittaker et al. 1992; Gea-Izquierdo et al. 2009), while the presence of forbs and legumes is limited under the canopy (Gea-Izquierdo et al. 2009; Marañón et al. 2009; Lopez-Carrasco et al. 2015). Species of high light demand – as is the case of legumes, with enhanced photosynthesis (Ibañez et al. 2020) – are limited in their growth under the canopy, affected by light constraints, litter accumulation, and competition with species tolerant to these conditions (Marañón 1986; Marañón et al. 2009). Hence, the accumulated litter may influence in different ways the growth of the different life forms, negatively affecting forbs and legumes – dicots – while not having a negative impact on the growth of grasses – monocots – with dense erect leaves (Barrantes Díaz 1986; Roldán Ruiz 1993; Sebastià 2007).

On the other hand, the higher litter input under the canopy (Fig. 2) increased soil N content (Ibañez 2019), which could favour the growth of grasses, at the expense of forbs and legumes. Song et al. (2011) found a similar result, with grasses increasing their biomass at high N availability at the expense of forbs (Song et al. 2011). Moreover, results reported by Ibañez (2019) from the same study plots, suggested that grasses could be more efficient than forbs in terms of N acquisition and use. Grasses usually have fibrous roots (Weaver 1958; Schenk and Jackson 2002; Pirhofer-Walzl et al. 2012), trait that may be facilitating N absorption from the most superficial soil layers and from symbiotically fixed N sources (Pirhofer-Walzl et al. 2012). Therefore, these differences in the N uptake could represent an important competitive advantage for grasses, displacing other species under the canopy at higher soil N availability. Fact that agrees with the lower species richness (SR), and the tendency to a lower evenness observed under the canopy in comparison to the open grassland (Fig. 4).

4.2 The canopy effect under representative canopy types of Iberian holm oak meadows

In line with our second question, the canopy effect differed between *Quercus* species and *P. pinea* plantations. This was shown by the SR decrease in the plot dominated by *P. pinea* (DNpinea) in comparison to plots dominated by *Quercus* species (specially in autumn, Table 3 and Fig. 4.b). Fact that may be related with the litter characteristics of *P. pinea*, which may be driving soil properties, and this in turn SR. The litter of *P. pinea* is known for its mulching capacity, and allelopathic properties, both factors lowering the understory growth (Valera-Burgos et al. 2012). Also, the litter of *P. pinea* has been reported to be poorer in N content than litter of *Quercus* species (Fioretto et al. 2008; Sheffer et al. 2015), which could be lowering soil N content and N availability. Indeed, N availability for plants in the DNpinea plot was reported the lowest among the study plots (Ibañez 2019). Factors that combined could be lowering SR in the DN-pinea, driving this difference in the SR between *P. pinea* and *Quercus* species dominated plots.

The different tree canopies also drove species composition, in addition to the variability associated to seasonality (Fig. 5 and Fig. 6). The mosaic of trees drove a heterogeneous distribution of the herbaceous species, with some species being dependent on the specific tree species and microclimatic conditions (Fig. 5 and Fig. 6). This combined with the PFT distribution mediated by the presence/absence of tree canopies (discussed in Sect. 4.1), interestingly, indicates that although SR decreased under the canopy (Fig. 4.b), the tree – open grassland mosaic allowed the growing of an increased variety of species, increasing plant specific and functional diversity at the ecosystem scale. Hence, it is worth mentioning that Mediterranean holm oak meadows are ecosystems of high conservation value, whose diversity and preservation is dependent on such tree - open grassland mosaic, which in turn is directly linked to the presence of grazer animals and traditional silvo-pastoral activities (López-Sánchez et al. 2016a).

On the other hand, the canopy effect differed between the two locations (DN vs. SM), as showed the neutral canopy effect on the AGB in the SM-ilex plot in contrast with the AGB decrease observed under the canopy in all DN plots (Fig. 2). This suggests that the mechanisms unravelling the canopy effect on the AGB production might differ depending on local conditions, including environmental conditions and competition/facilitation processes.

A main driver for the AGB reduction observed in all DN plots under the canopy (especially in spring, Fig. 2) could be the lower light availability compared to the open grassland (Ibañez et al. 2021), in agreement with similar light constraints on biomass production reported under the canopy in Iberian holm oak meadows (Hussain et al. 2009; Seddaiu et al. 2018). This light reduction occurs in combination with higher litter accumulation (Fig. 2), which may be mulching the soil and/or having an allelopathic effect on some species growth (Marañón et al. 2009), also possibly driving this AGB decrease under the canopy.

Nevertheless, several studies have described that although under the canopy there is less light available, the canopy in holm oak meadows can create a favourable environment, with increased soil moisture (Holmgren et al. 1997), higher nutrient availability (Gallardo et al. 2000; Gallardo 2003) and amelioration of extreme summer temperatures (Marañón et al. 2009), which may result in an enhanced productivity of the herbal layer (Moreno et al. 2007; Gea-Izquierdo et al. 2009).

In our study, both locations presented higher soil water content, lower soil temperature (Ibañez et al. 2021), and higher soil carbon and nitrogen content (Ibañez 2019) under the canopy than in the open grassland. Yet, this increase in the productivity of the herbaceous layer was not observed in our locations, which even decreased under the tree canopy in the case of DN. Accordingly, some authors have reported that competition for water resources between trees and the herbaceous layer could be limiting the productivity, especially in the most arid holm oak meadows, or when soil texture does not promote much water retention (Moreno 2008; Gea-Izquierdo et al. 2009). Plants could only profit from the increase in soil fertility mediated by the canopy without water stress, the canopy effect being dependent on water availability (Gea-Izquierdo et al. 2009; López-Sánchez et al. 2016a), which might be the case in our locations (DN vs. SM).

The DN location is drier than SM, with higher temperatures, lower precipitation, and sandier soils (Sect. 2.1); and competition for water resources could be here a limiting factor (combined with light availability and litter mulching the soil). Thus, water is especially scarce in DN during dry periods within the growing season, which are frequent under the irregular Mediterranean climate. Conversely, in the SM location, the reduced

environmental constraints (slightly cooler and wetter than DN) may allow a certain compensation between the drivers that might be reducing the biomass production under the canopy (i. e. reduced light availability), and the drivers favouring productivity (i. e. increased soil fertility), which results on a neutral canopy effect on the AGB (Fig. 2).

Also, the presence of grazer animals may be driving the general structure of the herbaceous layer, and some of those differences in the canopy effect between locations (DN vs. SM). The stocking rate was very similar in both locations (Sect. 2.1), but the productivity in the DN location was lower than in SM (Fig. 2), and the livestock impact on the AGB might be higher in DN, wherein livestock visiting the under the canopy microenvironment, looking for shadow, acorns, and fresh herb, could be more frequent.

These differential canopy effect on the AGB production between locations (DN vs. SM), interestingly links with the results reported on greenhouse gas exchange (Ibañez et al. 2021), and carbon and nitrogen dynamics (Ibañez 2019) from the same study plots. The authors reported that CO₂ exchange (Ibañez et al. 2021), and nitrogen uptake rates by plants (Ibañez 2019) under the canopy did not differ so much from the open grassland in SM, in contrast to the strong differences found in DN. Overall suggesting that SM seemed to be less environmentally constrained than DN, where the canopy effect was more pronounced. Ultimately, this differential canopy effect between locations (DN vs. SM) becomes relevant in terms of management to estimate the optimum tree coverage, since it will be dependent not only on the primary ecosystem service (i. e. forage provision) but also on local conditions.

4.3 The canopy effect along seasonality

In line with our third question, seasonality interacted with the tree – open grassland mosaic to drive the herbaceous layer structure and composition. First, this was shown by the big amount of litter that was present under the canopy in spring, but no longer present in autumn (season x canopy effect, Table 1). Fact that shows how the canopy effect on the organic matter input evolves along seasonality, suggesting a rapid incorporation of the litter into the soil along the year, in addition to the relevance of trees as sources of soil fertility in holm oak meadows (Howlett et al. 2011; Gómez-Rey et al. 2013; Pulido-Fernández et al. 2013; Andivia et al. 2015).

Second, the canopy also interacted with seasonality and drove the distribution of species, mainly of grasses and legumes, decreasing the magnitude of the difference between under the canopy and the open grassland in autumn compared to spring (season x canopy effect, Table 2). These results underscore the dynamisms of the herbaceous layer composition along seasonality, and the relevance of recording seasonal dynamics to capture canopy effects that are season dependent.

Conclusion

Our study shows how the typical tree – open grassland mosaic of holm oak meadows modifies the structure and composition of the herbaceous layer. The different components of the herbaceous layer performed differential responses to the given microenvironment (open grassland and under the canopy), as for instance is shown by the distribution of some species – with grasses being dominant under the canopy, while legumes and forbs were favoured in the open grassland. Also, the dominant tree species drove species richness and composition, with a certain a reduction in the species richness in the plot dominated by *P. pinea* compared to plots dominated by *Quercus* species. The canopy effect was also dependent on local environmental conditions, as suggested the reduction on the aboveground biomass under the canopy compared to the open grassland at

the more environmentally constrained location of DN; while such canopy effect was neutral at the cooler and fresher location of SM. Generally, such canopy effects were more pronounced in spring than in autumn. Overall, our results suggest that it is highly advisable to preserve the tree – open grassland mosaic and traditional *Quercus* species stands to maximize and preserve plant specific and functional diversity; and that the optimum tree coverage might be dependent, not only on the primary ecosystem service (i. e. forage provision), but also on local conditions. Factors that may be considered to manage and guarantee ecosystem services provision and conservation in these systems.

Abbreviations

Sierra Morena mountains (SM); Doñana Natural Park (DN); *Quercus ilex* stand in the SM location (SM-ilex); pure *Quercus suber* stand in the DN location (DNsuber); *Q. ilex* and *Q. suber* mixed stand in the DN location (DNmixed); pure *Pinus pinea* stand in the DN location (DN-pinea); open grassland (OG); under the canopy (UC); aboveground biomass (AGB); plant functional types (PFT); belowground biomass (BGB); species richness (SR); canonical correspondence analysis (CCA).

Declarations

Ethics approval

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

MI conceived the ideas, designed methodology, collected, and analysed the data, and wrote the manuscript. CC conceived the ideas, designed methodology, collected the data, and reviewed the manuscript. MJL designed methodology, executed project administration and funding acquisition, and reviewed the manuscript. M-TS conceived the ideas, designed methodology, executed project administration and funding acquisition, and reviewed the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Figures

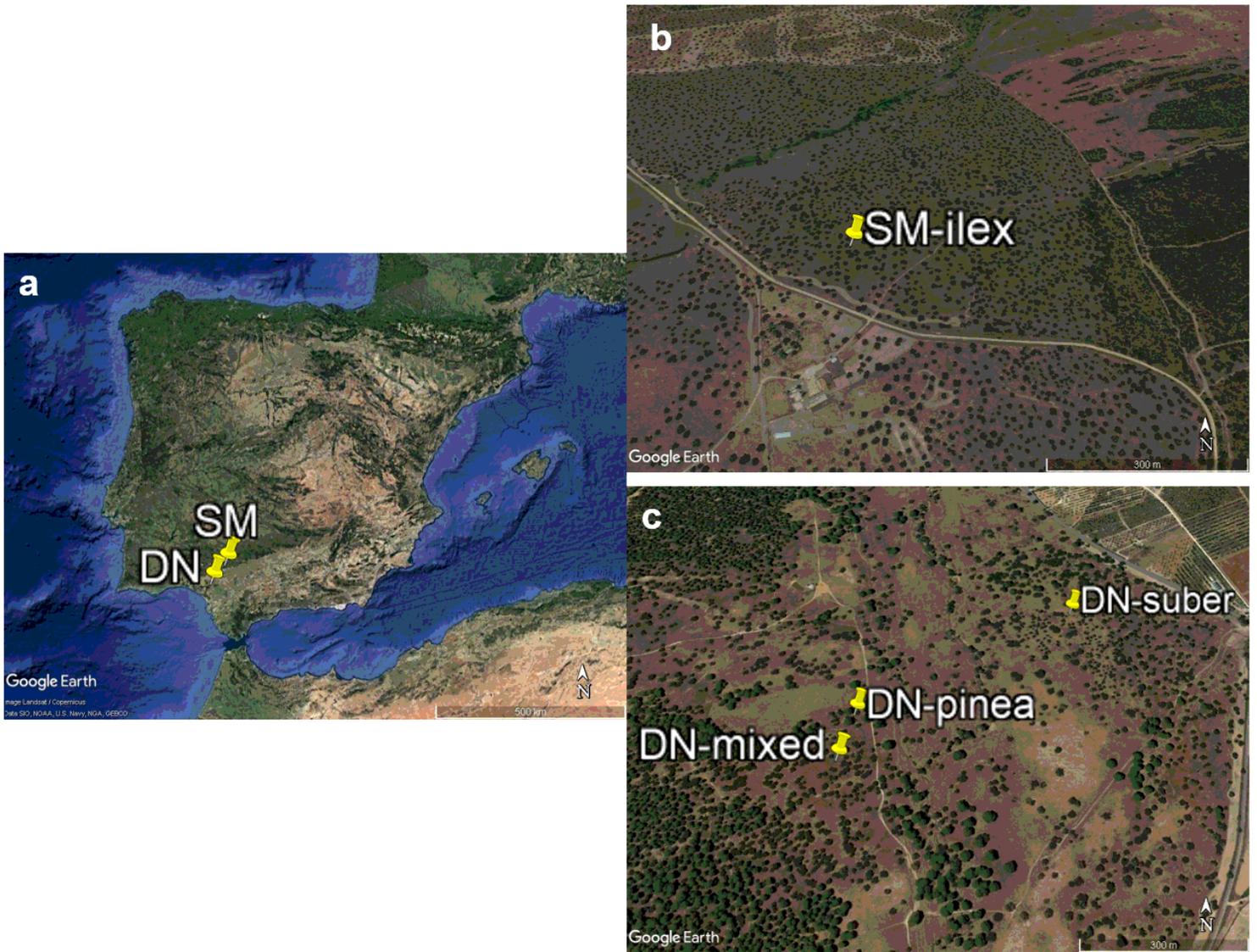


Figure 1

(a) Study sites geographical location in the Iberian Peninsula; and study plots aerial view: (b) SM-ilex; and (c) DN-mixed, DN-suber, and DN-pinea. Source: Google Earth, earth.google.com/web/

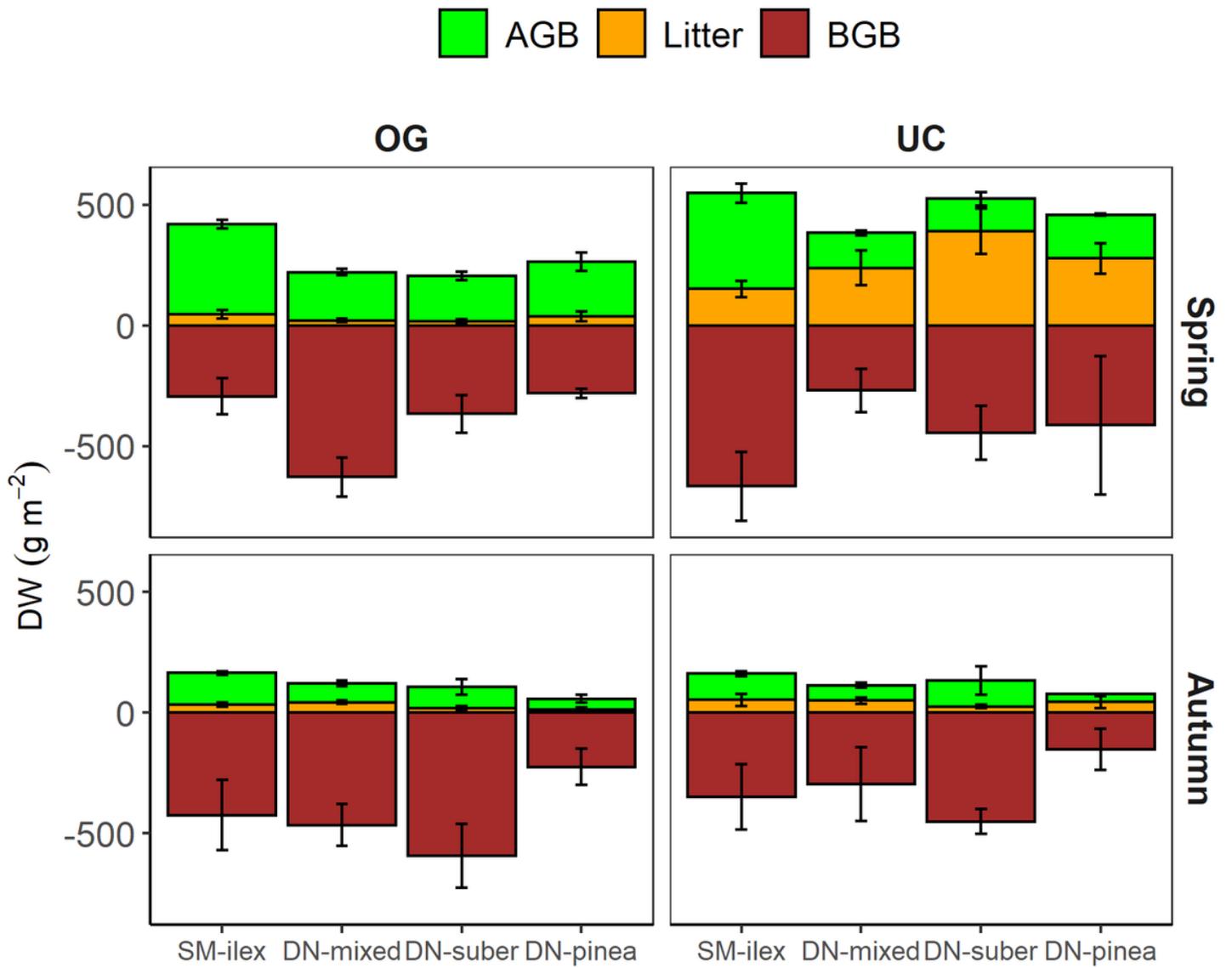


Figure 2

Dry weight (DW) mean \pm 1 standard error (SE), of aboveground biomass (AGB), litter and belowground biomass (BGB) per plot, season, and canopy: open grassland (OG) and under the canopy (UC).

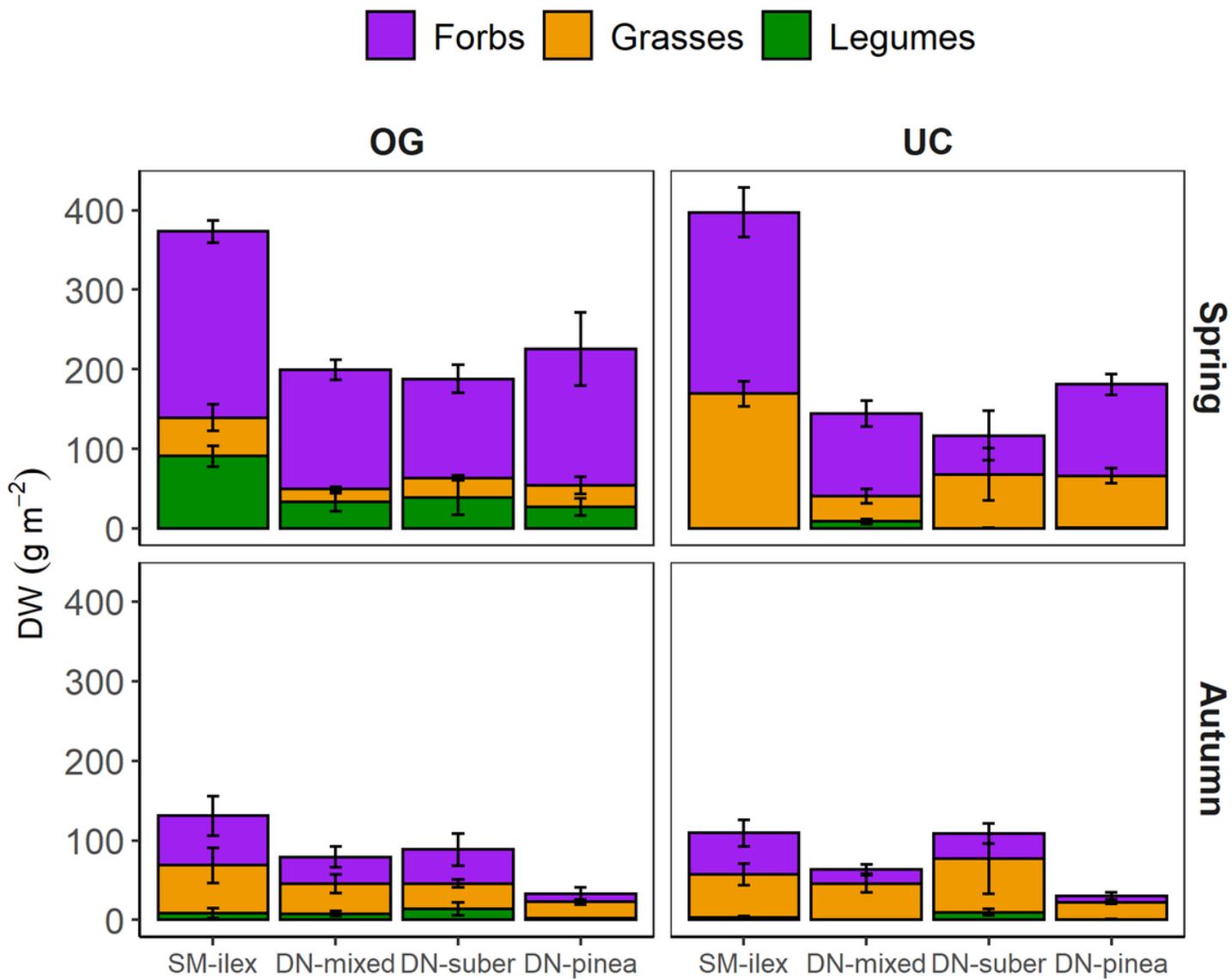


Figure 3

Dry weight (DW) mean \pm 1 SE of plant functional types (PFT: forbs, grasses, and legumes) per plot, season, and canopy: open grassland (OG) and under the canopy (UC).

OG UC

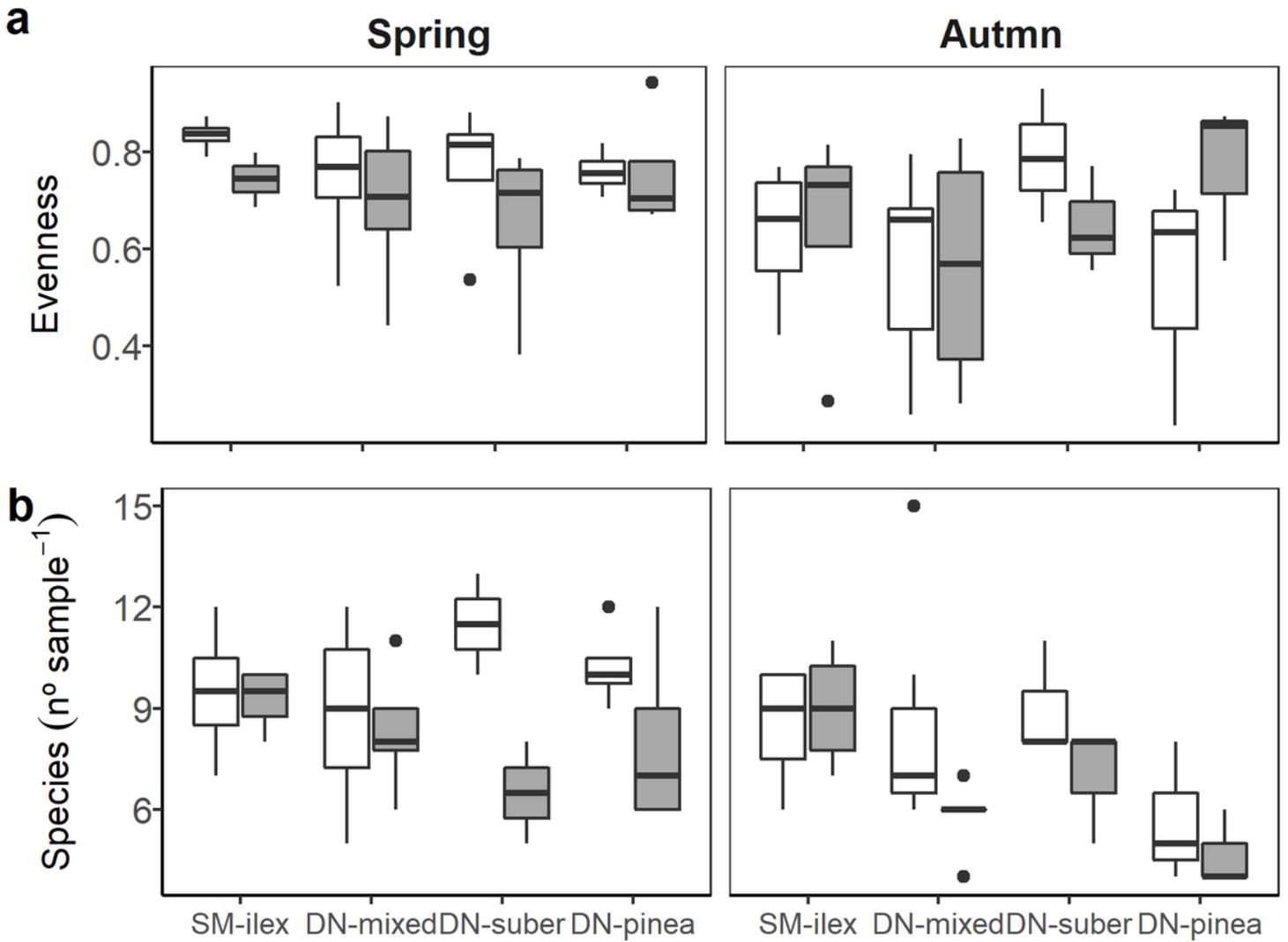


Figure 4

(a) Species evenness; and (b) species richness (SR) per plot, season, and canopy: open grassland (OG) and under the canopy (UC). Boxplot's midline indicates the median; upper and lower limits of the box indicate the third and first quartile; whiskers extend up to 1.5 times the interquartile range from the top/bottom of the respective box, and dots represent data beyond the whiskers.

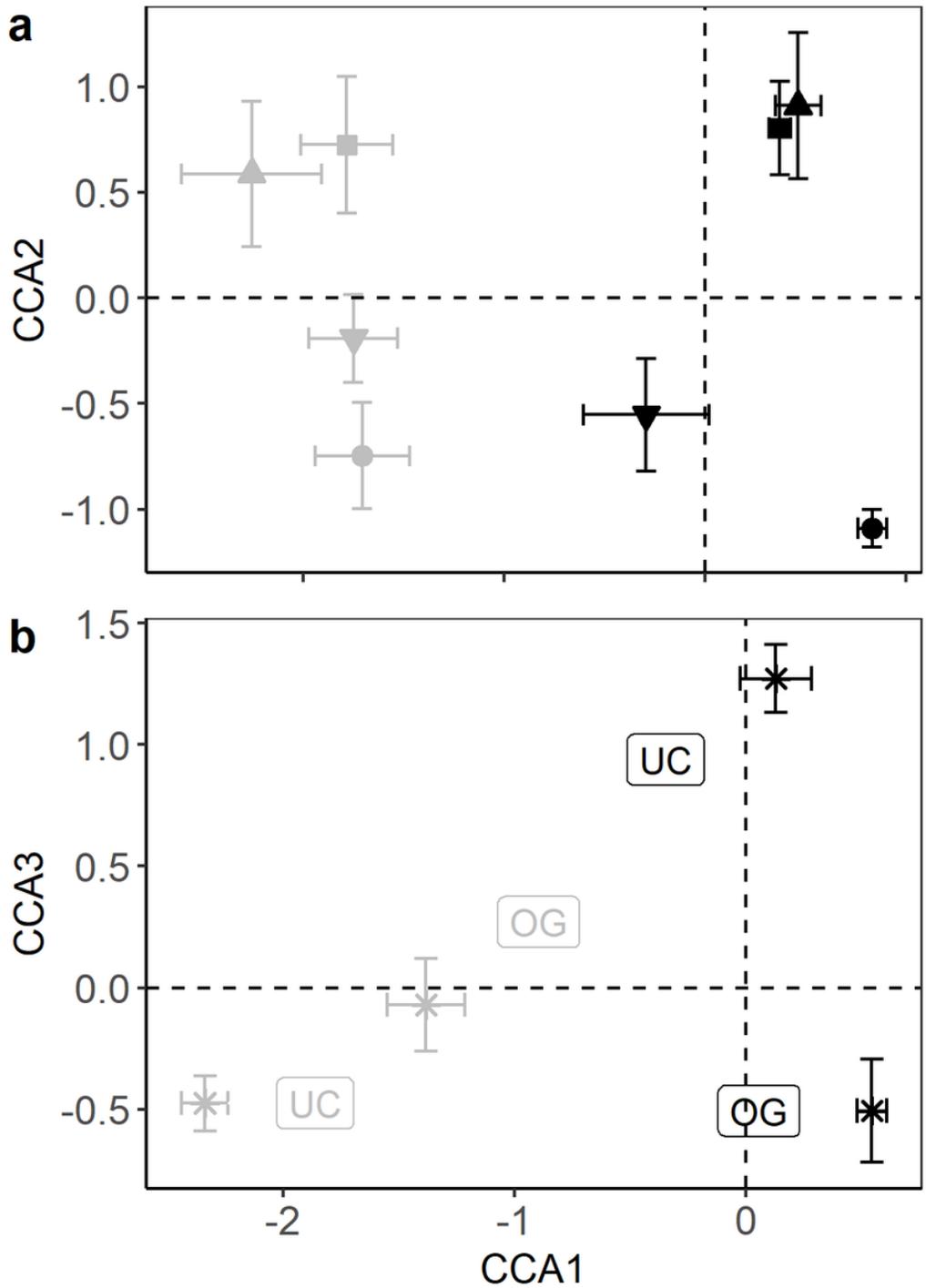


Figure 5

Canonical correspondence analysis (CCA): (a) axis 1 (CCA1) vs. axis 2 (CCA2) per season (black: spring, grey: autumn) and plot (●● SM-ilex, ■■ DN-mixed, ▼▼ DN suber, ▲▲ DN pinea); and (b) CCA1 vs. axis 3 (CCA3) per season and canopy (OG: open grassland, UC: under the canopy). Sites mean scores ± 1 SE.

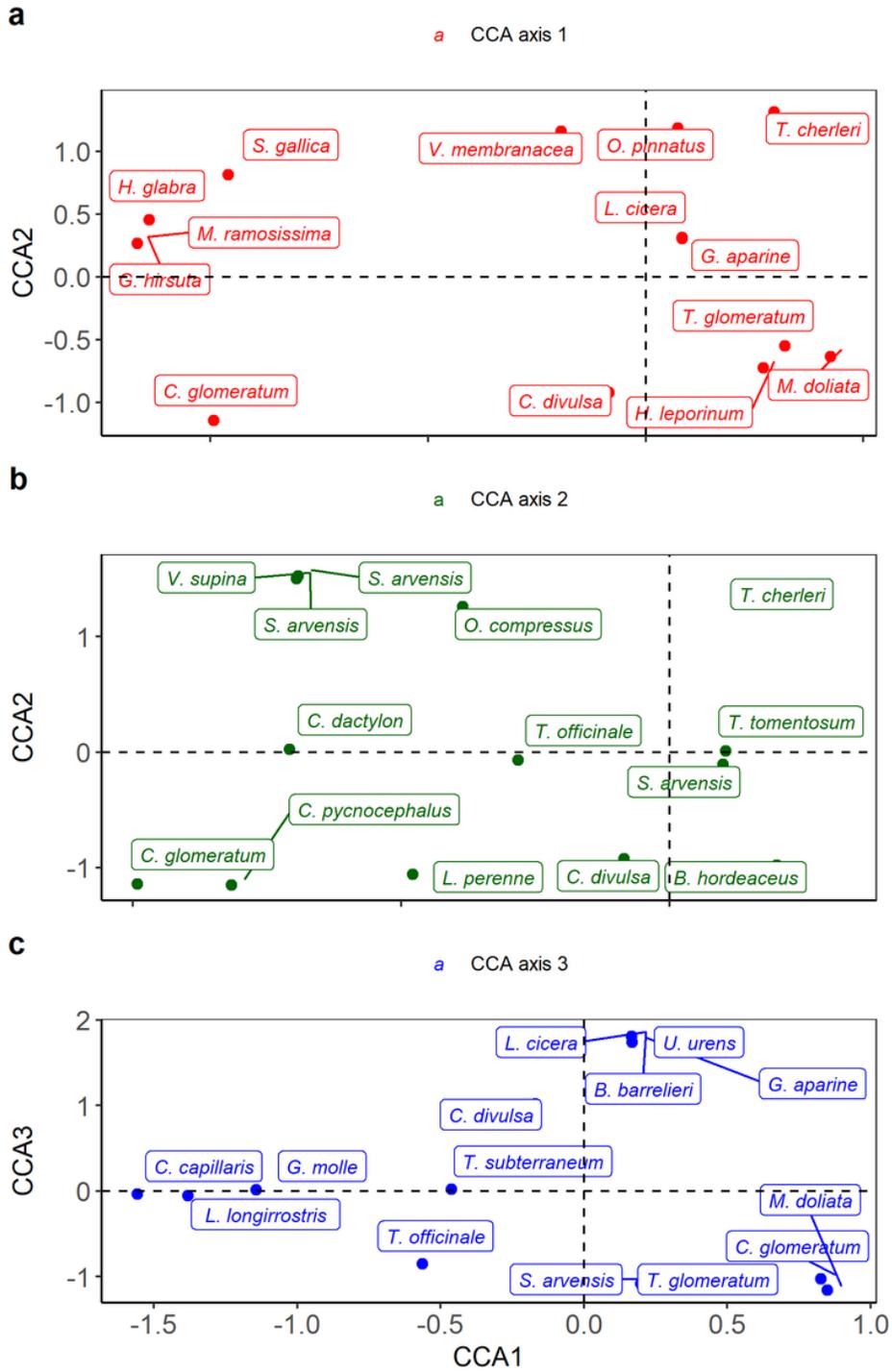


Figure 6

Species CCA scores. Species selected according to (a) axis 1 (CCA1); (b) axis 2 (CCA2); and (c) axis 3 (CCA3).