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Tree – open grassland structure and composition drive greenhouse gas exchange in holm oak meadows of the Iberian Peninsula

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Abstract: Iberian holm oak meadows are savannah-like ecosystems that result from traditional silvo-pastoral practices. However, such traditional uses are declining, driving changes in the typical tree – open grassland structure of these systems. Yet, there are no studies integrating the whole ecosystem — including the arboreal and the herbaceous layer — as drivers of greenhouse gas (GHG: CO_2, CH_4 and N_2O) dynamics. Here we aim at integrating the influence of tree canopies and interactions among plant functional types (PFT: grasses, forbs, and legumes) of the herbaceous layer as GHG exchange drivers. For that purpose, we performed chamber-based GHG surveys in plots dominated by representative canopy types of Iberian holm oak meadows, including *Quercus* species and *Pinus pinea* stands, the last a common tree plantation replacing traditional stands; and unravelled GHG drivers through a diversity-interaction model approach. Our results show that the tree – open grassland structure especially drove CO_2 and N_2O fluxes, with higher emissions under the canopy than in the open grassland. Emissions under *P. pinea* canopies being higher than those under *Quercus* species. In addition, the inclusion of diversity and compositional terms of the herbaceous layer improve the explained variability, legumes enhancing CO_2 uptake and N_2O emissions. Changes in the tree cover and tree species composition, in combination with changes in the structure and composition of the herbaceous layer, will imply deep changes in the GHG exchange of Iberian holm oak meadows. These results may provide some guidelines to perform better management strategies of this vast but vulnerable ecosystem.

Keywords: Canopy, CH_4, CO_2, dehesas, diversity-interaction model, N_2O, plant functional types.

1. Introduction

Holm oak meadows, also called dehesas in Spain and montados in Portugal, are semi-natural savannah-like agroecosystems that result from the thinning of the Mediterranean forest, in which an herbaceous and an arboreal (mostly *Quercus* species) layer coexist. They are one of the largest agroforestry systems in Europe [1], covering 3.5 – 4 million ha, mostly along the South-West of the Iberian Peninsula [2], and are also present in other world regions with Mediterranean climate, mainly in California [3–5]. Also, Mediterranean savannahs, with trees belonging to different taxonomic groups, can be found in South Africa, south-western Australia and central Chile [4].

Holm oak meadows have traditionally provided a wide variety of goods and services, including pasture for livestock, acorns, timber, and cork; being ecosystems of high cultural and economic value. Such traditional uses have shaped holm oak meadows into a matrix of trees and open grassland, driving ecosystem properties. However, the tree cover is changing with the consequent implications that this may have on ecosystem functioning, since traditional uses are declining towards intensive farming; plantations of fast-growing trees, mostly *Eucalyptus* and *Pinus* species; shrub encroachment due to land abandonment; and there is a worrying lack of tree regeneration [6,7].

Hence, although the canopy influence has been described to some extent on soil [8–11], vegetation structure [12–14] and composition [4,15,16], few studies have assessed the tree – open grassland structure influence on greenhouse gas (GHG: CO_2, CH_4 and N_2O) exchange. Indeed, the only studies about GHG exchange conducted in Iberian holm oak meadows (dehesas) have mainly focused on the canopy effect on CO_2 fluxes [14,17–21], although with divergent results. Some authors have described an enhancement of soil respiration rates under the canopy compared to the open grassland [17,21], related to a higher soil C and N content, despite lower soil temperature. While on the other hand, increased CO_2 exchange rates in the open grassland have also been reported, due to higher herbaceous biomass and light availability, as main drivers of CO_2 uptake; and due to higher soil temperature, as main driver of CO_2 release [14]. On the other hand, the only previous studies addressing CH_4 and N_2O exchange in Iberian holm oak meadows, were conducted by Shvaleva et al., (2015, 2014); which related CH_4 and N_2O emissions to soil water content, but the effect of soil water content was dependent on the canopy [22].

Furthermore, there is a lack of studies integrating the whole ecosystem structure and composition, combining the arboreal and the herbaceous layer, and assessing how both interact to drive GHG exchange. To this regard, sorting the wide variety of species of the herbaceous layer into plant functional types (PFT) provides a mechanistic link between diversity and ecosystem functioning [24]. However, most diversity-GHG studies have focussed on the effect of specific or functional diversity on GHG dynamics [25,26], but many fewer have disentangled identity and interaction effects among PFT [27].

Accordingly, in the present study we aim at integrating the influence of trees and the herbaceous layer structure and composition as GHG exchange drivers. In particular, aiming at (1) assessing the canopy effect under representative canopy types of Iberian holm oak meadows, including traditional *Quercus* species and *Pinus pinea* L. stands, the last a common tree plantation replacing traditional canopies; and (2) unravelling the influence of the main PFT of the herbaceous layer, using a diversity-interaction modelling approach.

2. Materials and Methods

2.1 Study sites and sampling design

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 seasonal variability of the studied variables and effects that may be season dependent. Study plots were distributed in two locations in the South-West of the Iberian

Peninsula: Doñana Natural Park (DN, 37° 15' 34'' N, 6° 19' 55'' W, 30 m a. s. l.) and Sierra Morena mountains (SM, 37° 39' 50'' N, 5° 56' 20'' W, 296 m a. s. l.). Both locations have Mediterranean climate regime with warm, dry summers, and mild winters [28]. Mean annual temperature in DN is 18.1 ºC and in SM is 16.8 ºC, and mean annual precipitation in DN is 543 mm and in SM is 648 mm. Grassland in both locations is dominated by herbaceous annual species, including grasses, non-legume forbs (hereafter "forbs") and legume forbs (hereafter "legumes"). Both locations are extensively grazed at similar stocking rates: DN grazed by goat and cattle (0.40 livestock units (LSU) ha^{-1}), and SM by cattle and Iberian pigs (0.36 LSU ha^{-1}).

To characterize soil properties of the study plots, soil samples were extracted and analysed according to standard methods (Table 1). Texture in the SM-ilex plot varied from sandy clay loam (0 – 40 cm depth) to clay (40 – 80 cm depth). All DN soils had a sandy clay loam texture, except the deeper layer of the DN-suber plot, which was sandy loam. The SM-ilex plot had a slightly acid pH and DN plots had a neutral-basic pH. Organic C was very low in all the plots (Table 1), although the value in the first 30 cm of the DN-pinea plot was markedly above the average. Total N was in general also quite low (Table 1).

Table 1. Soil characteristics per plot and depth. Soil analysis performed according to standard methods: pH [29], organic C [30], total N (Elemental analyser CNS-Trumac, LECO Corporation, MI, USA) and texture [31].

Plot	SM-ilex		DN-mixed		DN-suber		DN-pinea	
Depth (cm)	0 – 40	40 – 80	0 – 30	30 – 60	0 – 30	30 – 60	0 – 30	30 – 60
pH	6.7	7.2	7.9	7.8	7.5	7.8	7.0	7.4
Organic C (%)	0.80	0.30	0.32	0.34	0.60	0.02	1.52	0.51
Total N (%)	0.85	0.60	0.15	0.04	0.06	0.05	0.20	0.11
Clay (%)	18	30	10	11	13	4	16	16
Silt (%)	29	28	25	15	18	9	22	21
Sand (%)	54	42	65	74	69	87	62	63

Study plots were selected according to their tree composition, with representative canopy types of Iberian holm oak meadows. One pure *Q. ilex* stand, in the SM location (SM-ilex), and one pure *Q. suber* stand in the DN location (DN-suber), both the most abundant stands; one *Q. ilex* and *Q. suber* mixed stand (DN-mixed), the next most abundant; and a pure *Pinus pinea* L. stand (DN-pinea), a common tree plantation replacing traditional canopies [6].

Study treatments were therefore established according to: plot (SM-ilex, DN-mixed, DN-suber and DN-pinea), season (spring and autumn), and canopy (open grassland, OG, and under the canopy, UC). Tree individuals of the UC treatment of each species were selected with similar cross section (*Q. ilex* 0.15 ± 0.02, *Q. suber* 0.31 ± 0.03 and *P. pinea* 0.28 ± 0.06 m^{-2}). Also, 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 influence. Sampling points were systematically placed following the north orientation respect to the tree trunks.

For each treatment level we selected 3-4 replicates, totalling 73 sampling points. In the DN-mixed plot we discriminated between both *Quercus* species (*Q. suber* and *Q. ilex*) to establish sampling points. However, preliminary comparative analysis in the DN-mixed plot on environmental and vegetation characteristics under the canopy of both *Quercus* species indicated no relevant differences. DN-mixed plot results are then always presented combining both tree species.

At each sampling point we hammered a metal collar (h = 8 cm, diameter = 25 cm), which was necessary for measuring GHG exchange (Section 2.2), and that defined the area in which vegetation (Section 2.3) and soil (Section 2.4) were sampled.

2.2 Greenhouse gas exchange (GHG) measurements

To measure GHG exchange, including CO_2, CH_4 and N_2O, we used a portable gas-exchange system, consisting of a cylindrical chamber (volume = 0.019 m^3), connected to photoacoustic spectroscopy gas analyser (PAS, INNOVA 1412, LumaSense Technologies, Denmark). Flux measurements were done closing the chamber over intact vegetation, at light and dark (covering the chamber) conditions. Afterwards, we harvested the vegetation inside the metal collar and performed bare soil measurements at dark conditions. As a result, 219 flux measurements (73 sampling points x 3 different conditions) were recorded. In the case of CO_2, resulting fluxes measured over vegetation at light conditions can be approximated as net ecosystem CO_2 exchange (NEE); over vegetation at dark conditions can be approximated as ecosystem respiration (R_{eco}); and on bare soil at dark conditions can be approximated as soil respiration (R_{soil}). In the case of CH_4 and N_2O we did not detect significant differences among measuring conditions. Therefore, we present the average value considering all three measuring conditions [32].

Flux measurements, flux calculation and data quality checks were done according to Debouk et al. (2018), including a fitting goodness assessment based on the R_{Adj}^2 value, and filtering fluxes below detection limit, calculated as the standard deviation of the ambient concentration over the measuring time. Negative values refer to the flux from the atmosphere to the biosphere and positive values correspond to the flux from the biosphere to the atmosphere, according to the micrometeorological sign convention [33].

Ancillary meteorological variables were recorded to calculate and model GHG fluxes, as well as to characterize microclimatic sampling conditions (summarized in Figure S1): photosynthetically active radiation (PAR) outside the chamber (AccuPAR model LP-80 PAR/LAI ceptometer, Decagon Devices, Inc. Washington, USA); air temperature (T_a) inside and outside the chamber (multi-logger thermometer, TMD-56, Amprove, Washington, USA); soil temperature (T_s) 1-10 cm; and soil water content (SWC, gravimetric method, Section 2.4).

2.3 Vegetation sampling

After GHG measurements were done, we harvested the vegetation at ground level at each sampling point. Thereafter, in the laboratory, we separated the vegetation into aboveground biomass (AGB) and litter (dead plant material detached from the herbaceous vegetation and tree leaves on soil surface). All fractions are summarized in Figure S2. In addition, we separated the AGB into PFT (forbs, grasses and legumes, proportions summarized in Figure S3). Vegetation was oven dried at 60 ºC until constant weight.

2.4 Belowground biomass sampling and soil water content determination

Two soil cores of 9 cm^2 surface and 0-10 cm depth were extracted at each sampling point. In the laboratory, one of the cores was washed and filtered with a 0.2 mm pore size strainer to obtain belowground biomass (BGB). The second core was used for SWC determination by gravimetric method, as the difference between fresh and dry soil weight. Both, BGB and soil samples were oven dried at 60ºC until constant weight.

2.5 Data analysis: greenhouse gas exchange modelling

To assess main GHG (CO_2, CH_4 and N_2O) drivers, especially focussing on the influence of trees and the herbaceous layer structure and composition, we run a diversity-interaction modelling [34,35]. The modelling compares a null model, in which the response variable is not affected by plant diversity and/or composition, to models that address diversity and composition at different levels. In our study, we compared the null model (Equation 1), in which the corresponding GHG depended only on treatment variables, including plot, season and canopy; environmental variables, including PAR (μmol photons m^{-2} s^{-1}), temperature (T, ºC) and SWC (fraction); and structural components of the herbaceous layer, including AGB, litter and BGB (g DW m^{-2}):

Equation 1. Null model

$$GHG = \beta_{Plot}Plot + \beta_{Season}Season * \beta_{Canopy}Canopy + \beta_{T}T + \beta_{SWC}SWC + \beta_{PAR}PAR + \beta_{AGB}AGB + \beta_{Litter}Litter + \beta_{BGB}BGB + \varepsilon$$

to models that included PFT composition and diversity of the herbaceous layer in different ways:

(a) Identity model, which includes PFT identity effects, meaning the biomass proportion of each PFT (Equation 2):

Equation 2. Identity model

$$GHG = Null\ model + \beta_F P_F + \beta_G P_G + \beta_L P_L + \varepsilon$$

(b) Average interaction model, which includes PFT identity effects, plus evenness — calculated according to Kirwan et al. (2007) —, and which acts as an average interaction term (Equation 3):

Equation 3. Average interaction model

$$GHG = Null\ model + \beta_F P_F + \beta_G P_G + \beta_L P_L + evenness + \varepsilon$$

And, (c) specific interaction model, which includes specific interactions between PFT in addition to the identity effects (Equation 4):

Equation 4. Specific interaction model

$$GHG = Null\ model + \beta_F P_F + \beta_G P_G + \beta_L P_L + \beta_{FG} P_F P_G + \beta_{FL} P_F P_L + \beta_{GL} P_G P_L + \beta_{FGL} P_F P_G P_L + \varepsilon$$

Where *P* indicates the proportion of the given PFT and the sub-index *F* indicates forbs, *G* grasses and *L* legumes respectively. The models were run without intercept to test the effect of all three PFT at the same time [34]. Models were compared at each step of modelling by analysis of variance (ANOVA) to test significant differences between models. The most explicative and parsimonious model of each GHG is shown and discussed.

3. Results

3.1 CO_2 exchange

CO_2 net uptake was similar in spring and autumn (NEE, Figure 1.A), while CO_2 emissions (R_{eco} and R_{soil}, Figure 1.A) were lower in autumn than in spring (season effect, Table 2). The SM-ilex plot showed the highest net CO_2 uptake rates (NEE, Figure 1.A) and the highest R_{eco} rates in the open grassland (Figure 1.A); while DN-pinea was the plot with the highest CO_2 emissions under the canopy (R_{eco} and R_{soil}, Figure 1.A).

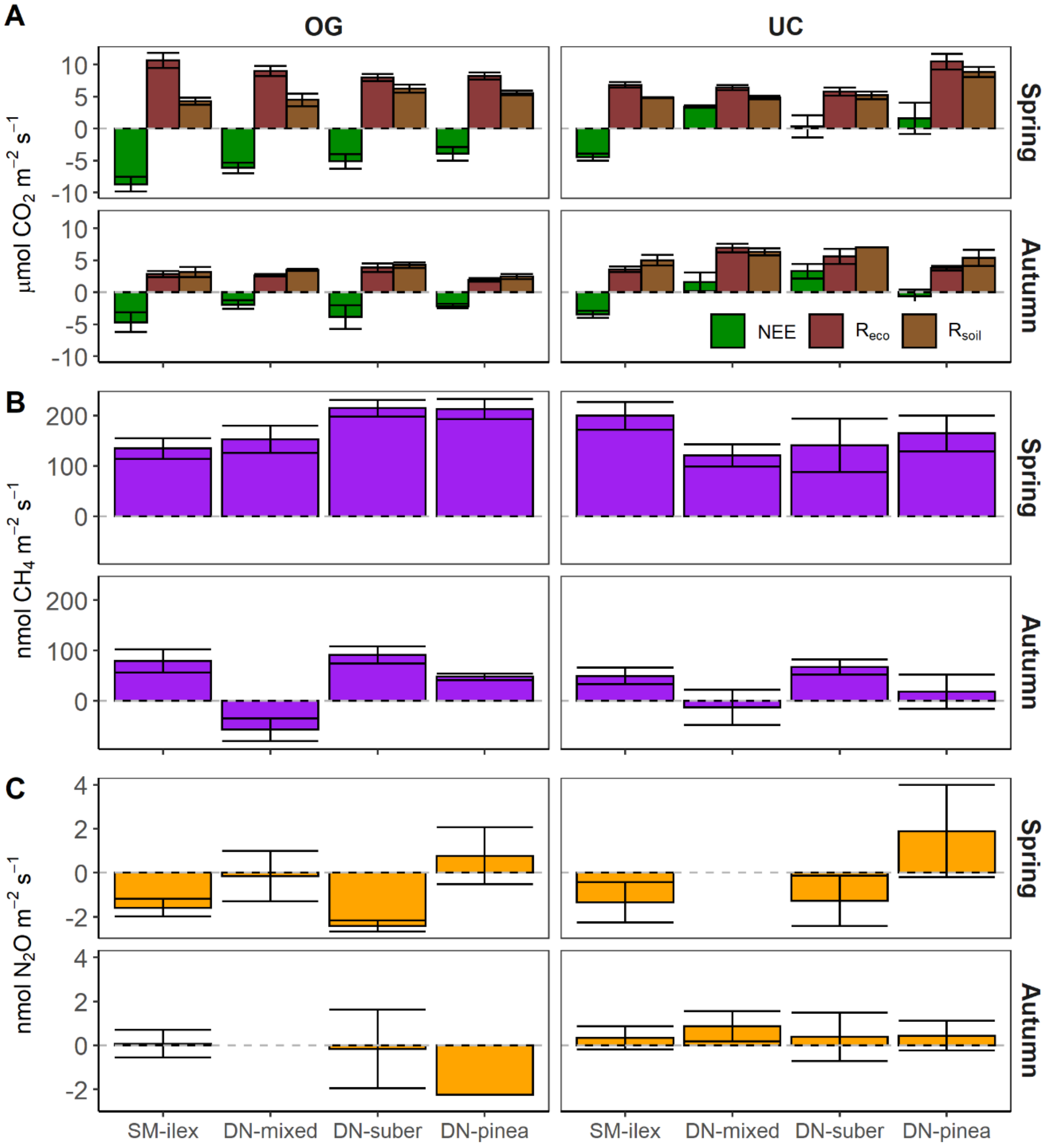

Figure 1. Greenhouse gas exchange per plot, season, and canopy: open grassland (OG) and under the canopy (UC). (A) Net ecosystem CO_2 exchange (NEE), ecosystem respiration (R_{eco}) and soil respiration (R_{soil}); (B) CH_4 and (C) N_2O exchange. Mean ± standard error.

Thus, the canopy itself had a strong influence over CO_2 fluxes. Generally, NEE under the canopy was dominated by CO_2 emissions (canopy effect, $t = 5.58$, $p < 0.001$, Table 2), NEE being strongly driven by PAR (PAR effect, $t = -4.58$, $p < 0.001$, Table 2). The exception was the SM-ilex plot, where there was net CO_2 uptake under the canopy, instead of emissions (NEE, Figure 1.A).

On the other hand, R_{eco} decreased under the canopy compared to the open grassland in spring but increased under the canopy in autumn (season x canopy effect, $t = 4.73$, $p < 0.001$, Table 2). R_{soil} increased under the canopy, especially in autumn (season x canopy effect, $t = 2.40$, $p = 0.02$, Table 2), R_{soil} being enhanced by T_a (T_a effect, $t = 3.06$, $p = 0.003$, Table 2) and BGB (BGB effect, $t = 2.21$, $p = 0.03$, Table 2).

Table 2. CO_2 exchange diversity-interaction model results. Net ecosystem exchange (NEE), ecosystem respiration (R_{eco}) and soil respiration (R_{soil}) as function of plot, season, canopy, photosynthetically active radiation (PAR), air temperature (T_a), aboveground biomass (AGB), belowground biomass (BGB) and plant functional type (forbs, grasses and legumes) proportions. Season with spring as reference level, and canopy with open grassland (OG) as reference level. Estimates of the explanatory variables (Est.), standard error (SE), t and p-value.

	CO_2 flux (µmol m^{-2} s^{-1})											
	NEE				R_{eco}				R_{soil}			
	Specific interaction model				Null model				Null model			
	Est.	SE	t	p	Est.	SE	t	p	Est.	SE	t	p
Plot SM-ilex	−6	3	−1.81	0.07	8.9	0.6	14.60	< 0.001	0	1	0.35	0.7
Plot DN-mixed	−2	3	−0.54	0.6	9.0	0.5	18.82	< 0.001	1	1	0.55	0.6
Plot DN-suber	−2	3	−0.57	0.6	8.6	0.6	13.74	< 0.001	2	1	1.33	0.2
Plot DN-pinea	−1	3	−0.38	0.7	9.2	0.6	14.69	< 0.001	1	1	0.65	0.5
Season					−6.2	0.6	−9.88	< 0.001	−1.3	0.5	−2.56	0.01
Canopy	4	0.7	5.58	< 0.001	−1.8	0.6	−2.98	0.004	1.1	0.5	2.10	0.04
Season x canopy					4.3	0.9	4.73	< 0.001	1.8	0.7	2.40	0.02
PAR (µmol photons m^{-2} s^{-1})	−0.003	0.001	−4.58	< 0.001								
T_a (ºC)									0.15	0.05	3.06	0.003
BGB (g DW m^{-2})									0.0015	0.0007	2.21	0.03
Forbs (fraction)	0	3	0.15	0.9								
Grasses (fraction)	−1	3	−0.22	< 0.001								
Legumes (fraction)	−67	17	−3.97	< 0.001								
Forbs x legumes (fraction)	97	25	3.84	< 0.001								
Grasses x legumes (fraction)	96	24	4.07	< 0.001								

Moreover, PFT composition drove significantly NEE. The most parsimonious and explanatory model was a specific interaction model (Equation 4). Legumes enhanced net CO_2 uptake (NEE, legumes effect, $t = -3.97$, $p < 0.001$, Table 2 and Figure 2), which slightly decreased with the addition of forbs (forbs x legumes effect, $t = 3.84$, $p < 0.001$, Table 2) and grasses (grasses x legumes effect, $t = 4.07$, $p < 0.001$, Table 2) in the mixture.

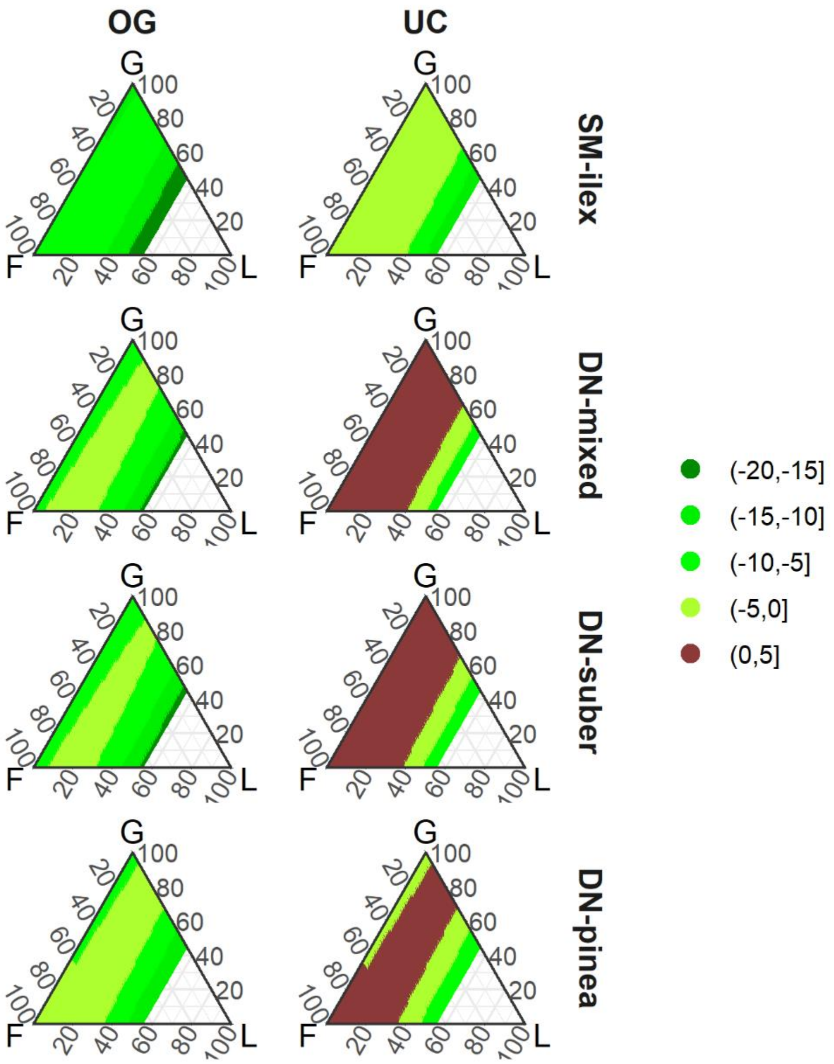

Figure 2. Predicted net ecosystem exchange (NEE, μmol CO_2 m^{-2} s^{-1}) according to the specific interaction model (Table 2). F indicates forbs, G grasses and L legumes. Predicted NEE modelled with a maximum proportion of legumes of 50%, according to the legumes proportion observed in the field (Figure S3). Negative NEE values indicate net CO_2 uptake, and positive values indicate CO_2 emissions. Environmental conditions set as the mean of the given treatment level.

3.2 *CH_4 and N_2O exchange*

CH_4 emissions were lower in autumn than in spring (season effect, $t = -9.68$, $p < 0.001$, Table 3), and were enhanced by T_s (T_s effect, $t = 2.72$, $p = 0.008$, Table 3) and SWC (marginally significant SWC effect, $t = 1.83$, $p = 0.07$, Table 3).

N_2O fluxes were uptake dominated in spring, except in the DN-pinea plot where there were emissions both under the canopy and in the open grassland (Figure 3). Under the canopy N_2O fluxes tend to increase (canopy effect $t = 2.68$, $p = 0.01$, Table 3), especially in autumn (Figure 3). N_2O emissions also increased with litter (litter effect, $t = 2.84$, $p = 0.006$, Table 3). Moreover, PFT composition of the herbaceous layer drove significantly N_2O fluxes, being the most parsimonious and explanatory model an identity model (Equation 2), with the presence of legumes significantly enhancing N_2O emissions (legumes effect, t = 2.49, p = 0.02, Table 3 and Figure 3).

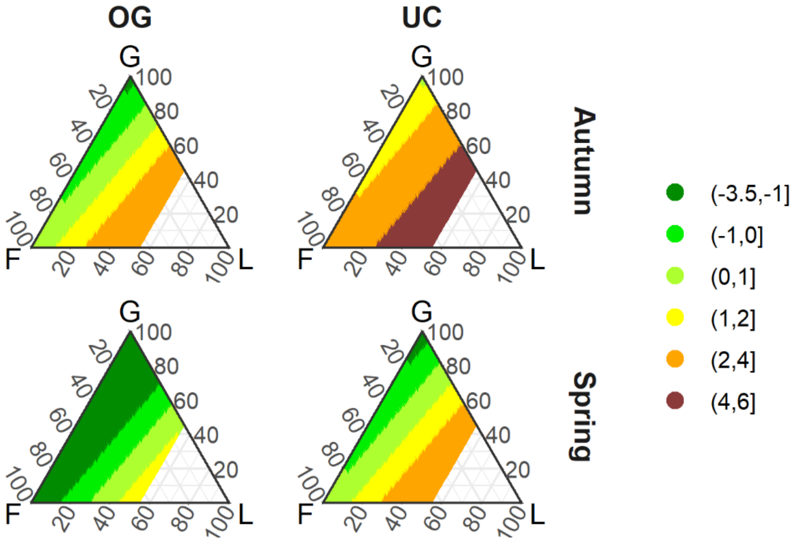

Figure 3. Predicted N_2O exchange (nmol m^{-2} s^{-1}) according to the identity model (Table 3). F indicates forbs, G grasses and L legumes. Predicted N_2O modelled with a maximum proportion of legumes of 50 %, according to the legumes proportion observed in the field (Figure S3). Negative N_2O values indicate net uptake, and positive values indicate N_2O emissions. Environmental conditions set as the mean of the given treatment level.

Table 3. CH_4 and N_2O exchange diversity-interaction model. CH_4 and N_2O exchange as function of plot, season, canopy, soil temperature (T_s), soil water content (SWC), litter, belowground biomass (BGB) and plant functional type (forbs, grasses, and legumes) proportions. Season with spring as reference level and canopy with open grassland (OG) as reference level. Estimates of the explanatory variables (Est.), standard error (SE), t and p-value.

	CH_4 (nmol m^{-2} s^{-1})				N_2O (nmol m^{-2} s^{-1})			
	Null model				Identity model			
	Est.	SE	t	p	Est.	SE	t	p
Plot SM-ilex	−24	81	−0.30	0.8				
Plot DN-mixed	−83	81	−1.03	0.3				
Plot DN-suber	−22	85	−0.26	0.8				
Plot DN-pinea	−28	85	−0.33	0.7				
Season spring					−13	5	−2.73	0.008
Season autumn	−150	15	−9.68	< 0.001	−11	5	−2.30	0.03
Canopy					2.1	0.8	2.68	0.010
T_s (ºC)	13	5	2.72	0.008	0.4	0.2	2.44	0.02
SWC (fraction)	1.4	0.8	1.83	0.07	−0.05	0.03	−2.00	0.05
Litter (g DW m^{-2})	−0.1	0.1	−1.89	0.06	0.007	0.003	2.84	0.006
BGB (g DW m^{-2})					0.002	0.001	1.75	0.09
Forbs (fraction)					3	3	1.02	0.3
Grasses (fraction)					2	3	0.47	0.6
Legumes (fraction)					10	4	2.49	0.02

4. Discussion

4.1 CO_2 exchange drivers

CO_2 exchange was closely driven by the tree – open grassland structure, with tree canopies driving microclimatic conditions (Figure S1), as well as the structure (Figure S2) and composition (Figure S3) of the herbaceous layer, factors that interacted among them to drive CO_2 fluxes. Thus, NEE was uptake dominated in the open grassland in all the study plots, while under the canopy there were emissions in all DN plots, but net CO_2 uptake in the SM-ilex plot (Figure 1.A). These results support the well-known effect of light as driver of CO_2 uptake [14,36], but also suggest interactions between the environment created by the canopy and local conditions (DN *vs.* SM). Accordingly, although light reduction under the canopy in the SM-ilex plot was in the same range of magnitude than in all DN plots (Figure S1), the vegetation in the SM-ilex plot kept taking-up CO_2 at similar rates both under the canopy and in the open grassland. Indeed, the aboveground biomass in the SM-ilex was similar under the canopy and in the open grassland, while there was a certain reduction in the aboveground biomass under the canopy compared to the open grassland in all DN plots (Figure S2). In line with these results, Ibañez (2019) reported that forbs from the same study plots presented more ^{13}C-depleted tissues under the canopy than in the open grassland in all DN plots, while this depleatment was not noticeable in the SM-ilex plot. These results indicate that the vegetation was photosynthesizing at similar rates under the canopy and in the open grassland in the SM-ilex plot, while the photosynthetic rate differed in the DN plots. Facts that suggest that the SM-ilex plot might be less environmentally constrained than DN, and the environment created under the canopy in the SM-ilex plot did not differ so much to the open grassland, in opposition to the strong differences found in DN.

Moreover, the inclusion of PFT diversity and composition terms improved NEE's explained variability, and highlighted the relevance of specific PFT identity and interaction effects (Table 2 and Figure 2), which were even more explicative than the AGB. Legumes were key NEE drivers, enhancing net CO_2 uptake (Table 2 and Figure 2), in agreement with previous studies that reported higher photosynthetic capacity of legumes compared to grasses and forbs [37–40]. Also, legumes transfer symbiotic N to other species, increasing photosynthetic rates of the overall community [41,42], and at the same time the acquisition of symbiotic N by legumes can be stimulated by the presence of grasses [43]. Accordingly, symbiotic N fixed by legumes could provide an important advantage for photosynthesis, especially in N limited systems, as might be the case of our plots, with very low soil N content (Table 1).

Ecosystem respiration (R_{eco}, Figure 1.A), was influenced by interactions between season and canopy, since R_{eco} decreased under the canopy compared to the open grassland in spring, but increased in autumn (season x canopy effect, Table 2). This suggests a differential magnitude of R_{eco} components ($R_{eco} = R_{autotrophic} + R_{heterotrophic}$) and its biotic and abiotic drivers depending on the season. In spring, R_{eco} was enhanced in the open grassland by higher temperatures (T_s effect, Table 2), which are known to be an important R_{eco} driver [14], especially enhancing the heterotrophic component of R_{eco} [44]. Also, enhanced R_{eco} rates could also be indirectly related to increased gross CO_2 uptake rates. Respiration is one of the most important C release pathways of recently fixed C photo-assimilated by plants [45], and autotrophic respiration is directly linked to photosynthetic activity [46]. Accordingly, the magnitude of the autotrophic component of R_{eco} in spring was revealed when the AGB was removed, and the remaining respiratory flux (R_{soil}) clearly decreased compared to R_{eco} (Figure S4). While on the contrary, R_{eco} and R_{soil}, equalized in autumn (Figure S4) suggesting that respiration derived from photosynthetic activity was really very low in autumn.

Furthermore, our data suggested that respiration rates (R_{eco} and R_{soil}) under *P. pinea* canopies were higher than under *Quercus* species (Figure 1.A, spring). This might be because the canopy of *P. pinea* is more open than that of *Quercus* species, with lower leaf area index [47], resulting in a lower buffering of PAR (Figure S1.A) and T_s (Figure S1.B) under the canopy, conditions that could be enhancing respiration rates. Also, litter of *P. pinea* has been reported to be more recalcitrant than litter of *Quercus* species [48,49], which could be increasing soil organic C at the plot level (Table 1), and the

C/N ratio in the soil under the canopy [50], leading to enhanced $R_{heterotrophic}$ rates, since soil microorganisms respire more C when decomposing C-rich and/or N-poor substrates [51].

In autumn conversely, the increased respiration rates (R_{eco} and R_{soil}) under the canopy compared to the open grassland (canopy x season, Table 2), were probably related to litter decomposition processes. The big amount of litter that was present under the canopy in spring was no longer present in autumn (Figure S2), suggesting that litter was already incorporated into the soil, and that was probably on mineralization process, increasing the heterotrophic component of R_{eco}. Respiratory fluxes, R_{eco} and R_{soil}, were also both influenced by the structure of the herbaceous layer, enhanced by the belowground biomass (BGB effect, Table 2), which has been directly linked to auto and heterotrophic respiration [52–54].

4.2 CH_4 and N_2O exchange drivers

CH_4 and N_2O assessments from Mediterranean grasslands or meadows are very scarce [55], and our study provides one of the first GHG datasets of a Mediterranean holm oak meadow in the Iberian Peninsula, unravelling the mechanisms behind these fluxes. Thus, CH_4 emissions were driven by seasonality rather than by the holm oak meadow structure and composition. CH_4 shifting from high emissions in spring, to low emissions or uptake in autumn (Figure 1.B), probably as result of several factors occurring simultaneously. First, higher CH_4 emissions in spring were enhanced by higher temperatures (T_s effect, Table 3), which could be favouring methanogenic activity and methane diffusion [55]. And second, SWC enhanced CH_4 emissions (although marginally significant, Table 2), which in combination with drying-rewetting cycles, typical in spring, could also be enhancing CH_4 soil emissions [56].

Regarding N_2O exchange, the DN-pinea plot had again a differential behaviour than plots dominated by *Quercus* species, with higher N_2O emissions (Figure 1.C, spring), which were probably related to the litter characteristics of *P. pinea*, also influencing N dynamics. Moreover, N_2O exchange was influenced by the structure and composition of the herbaceous layer, increasing N_2O emissions with litter and the presence of legumes in the community (Table 3). The influence of litter on N_2O exchange at field conditions is not well understood, but there are some experiments assessing the effect of cover crops on N_2O exchange that may provide some understanding [57–59]. For instance, an experimental study by Shaaban et al., (2016) reported increasing N_2O emissions with the addition of litter onto soil surface. The authors related the input of organic matter to stimulated microbial activity and denitrification, resulting in N_2O production [59]. Also, N_2O emissions have been negatively correlated to the C/N ratio of the substrate [59]. Findings that agree with our results of legumes enhancing N_2O emissions (Table 3 and Figure 3).

4.3 Management implications

Holm oak meadows are productive ecosystems, and any management strategy must take that into account. To this regard, our results suggest that an increase in the tree cover may reduce the net CO_2 sink capacity of the system, while also reduce forage production and quality. Thus, we have reported that under the canopy there is a reduction on net CO_2 uptake rates compared to the open grassland, via (a) a direct canopy effect (especially in DN, Table 2), with the corresponding implications that this may have on forage production; and (b) an indirect effect through changes in the herbaceous layer composition, since legumes, which were enhancing net CO_2 uptake rates (NEE, Table 2 and Figure 2), could mostly grow in the open grassland (Figure S3), with the implications that this may have on forage quality. Therefore, it is highly advisable to preserve open grassland spaces to maximize net CO_2 uptake and preserve forage provision.

On the other hand, a change in the tree species composition, shifting from *Quercus* species stands to *Pinus* species plantations may increase CO_2 and N_2O emissions (Figure 1.A). Therefore, it is highly advisable to preserve traditional *Quercus* stands to minimize CO_2 and N_2O emissions.

5. Conclusions

Our study provides insight into Iberian holm oak meadows functioning, integrating the arboreal and the herbaceous layer structure and composition as GHG drivers. Tree canopies especially drove CO_2 and N_2O fluxes, increasing emissions under *P. pinea* canopy compared to that of *Quercus* species. The inclusion of the herbaceous layer composition and diversity terms improved explained variability, legumes enhancing net CO_2 uptake and N_2O emissions. Thus, PFT identity and interaction effects were even more explanatory than some structural components (i.e. aboveground biomass). Changes in the tree cover and species will imply deep changes in the GHG exchange of Iberian holm oak meadows mediated by changes in the structure and composition of the herbaceous layer. This may provide some keys to improve ecosystem services provision and guarantee the preservation of this vast but vulnerable ecosystem.

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Supplementary Materials

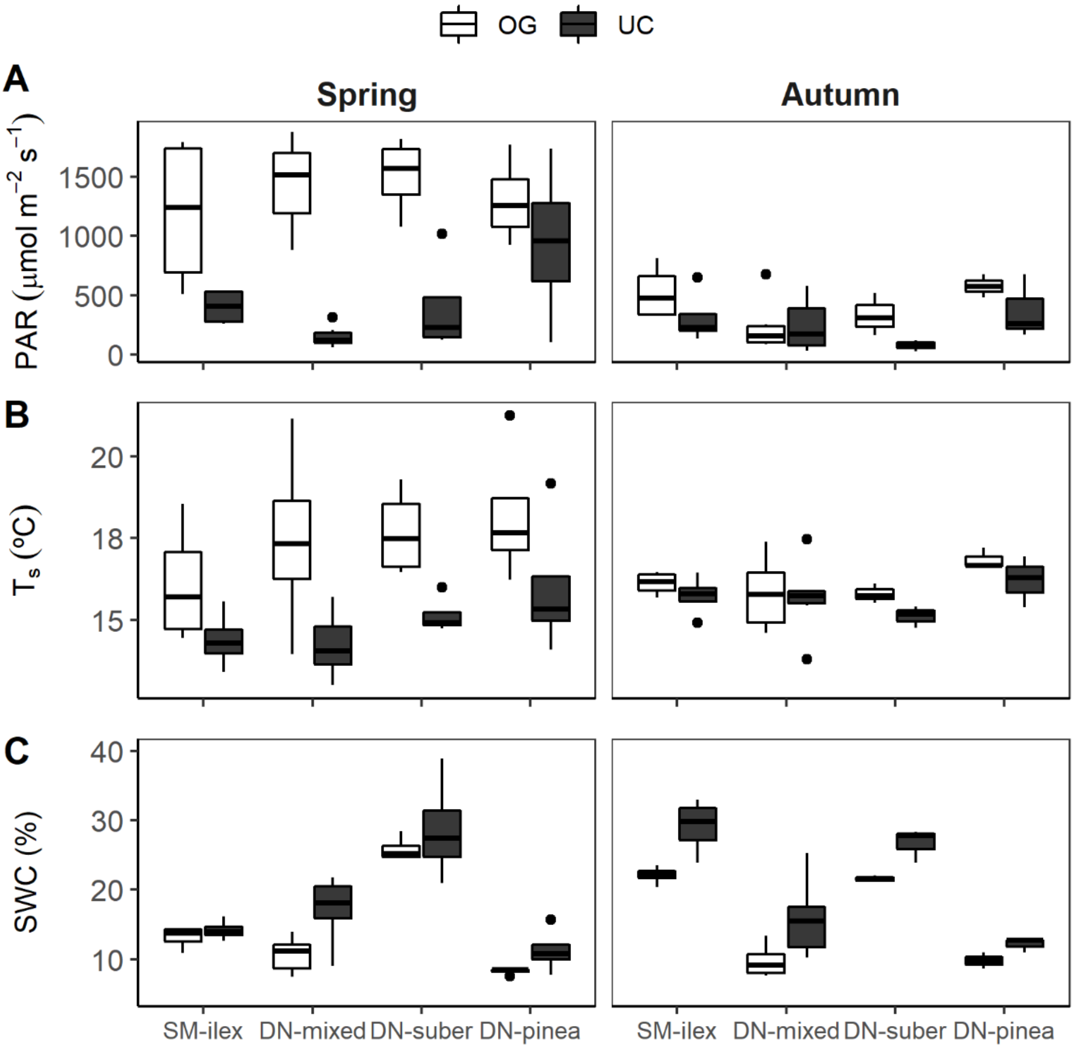

Figure S1. Microclimatic sampling conditions per plot, season, and canopy: open grassland (OG) and under the canopy (UC). (A) Photosynthetically active radiation (PAR); (B) soil temperature (T_s) and; (C) soil water content (SWC). Boxplot's midline indicates the median; upper and lower limits of the 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 points represent data beyond the whiskers.

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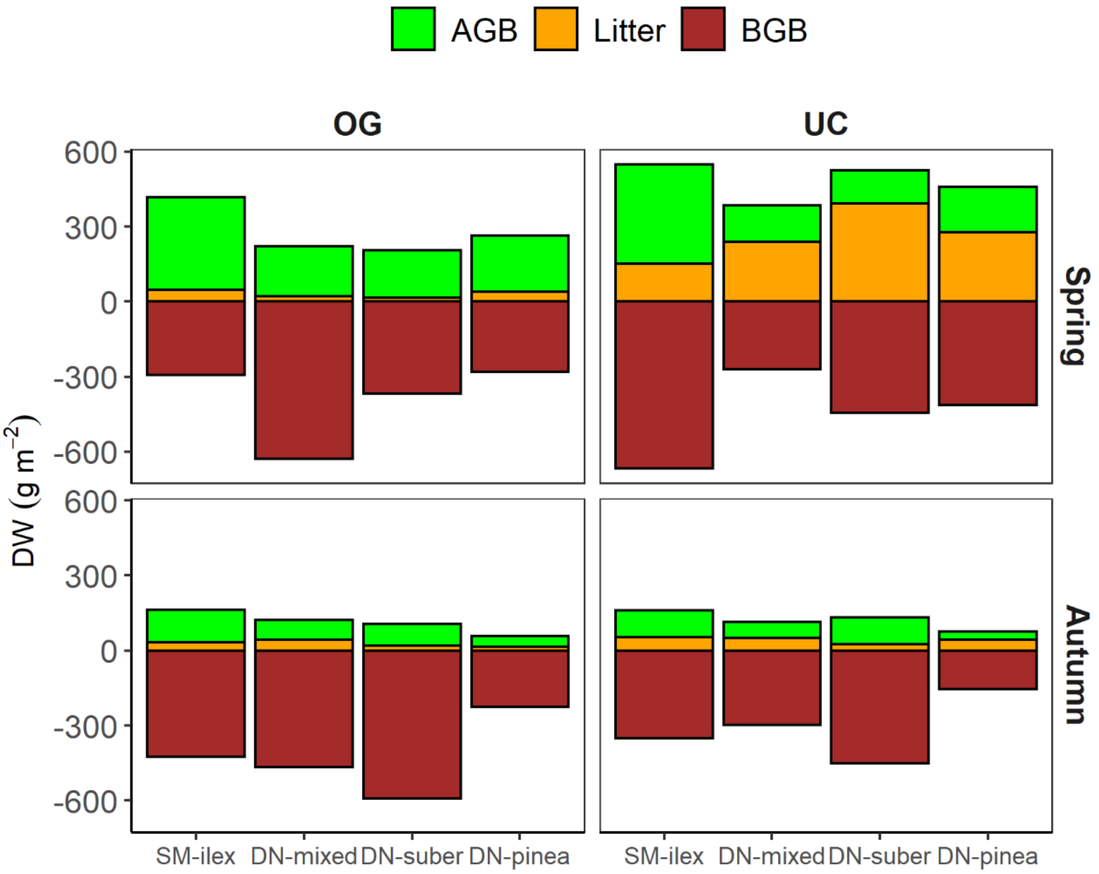

Figure S2. Mean aboveground biomass (AGB), litter and belowground biomass (BGB) per plot, season, and canopy: open grassland (OG) and under the canopy (UC).

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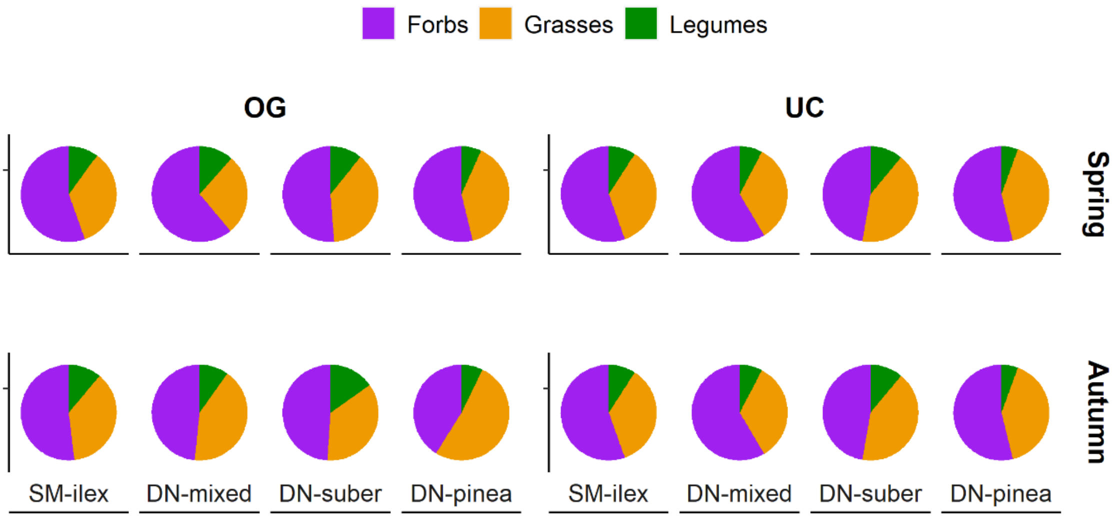

Figure S3. Plant functional types (PFT: forbs, grasses, and legumes) proportions per plot, season, and canopy: open grassland (OG) and under the canopy (UC).

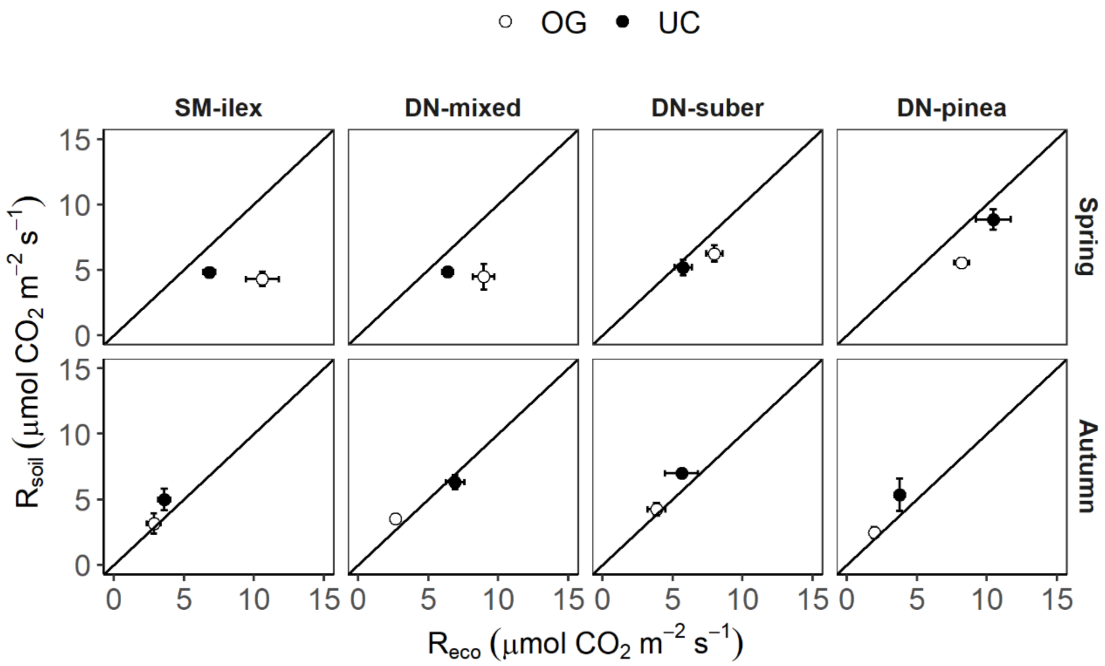

Figure S4. Soil respiration (R_{soil}) ~ ecosystem respiration (R_{eco}) relationship, mean ± standard error per plot, season, and canopy. Solid diagonal line indicates 1:1 relationship.

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References

1. Eichhorn, M.P.; Paris, P.; Herzog, F.; Incoll, L.D.; Liagre, F.; Mantzanas, K.; Mayus, M.; Moreno, G.; Papanastasis, V.P.; Pilbeam, D.J.; et al. Silvoarable systems in Europe – past , present and future prospects. *Agrofor. Syst.* **2006**, *67*, 29–50, doi:10.1007/s10457-005-1111-7.

2. Olea, L.; López-Bellido, R.J.; Poblaciones, M. Europe types of silvopastoral systems in the Mediterranean area: Dehesa. In *Silvopastoralism and sustainable land management. Proceedings of an international congress on silvopastoralism and sustainable management held in Lugo, Spain, April 2004.*; Mosquera-Losada, M.R., Rigueiro-Rodríguez, A., McAdam, J., Eds.; 2005; pp. 30–35.

3. Huntsinger, L.; Campos, P.; Starrs, P.F.; Oviedo, J.L.; Díaz, M.; Standiford, R.B.; Gregorio, M. Working Landscapes of the Spanish Dehesa and California Oak Woodlands: An Introduction. In *Mediterranean Oak Woodland Working Landscapes. Dehesas of Spain and Ranchlands of California*; Campos, P., Huntsinger, L., Oviedo, J.L., Starrs, P.F., Diaz, M., Standiford, R.B., Montero, G., Eds.; Springer New York, 2013; pp. 3–23 ISBN 978-94-007-6706-5.

4. Marañón, T.; Pugnaire, F.I.; Callaway, R.M. Mediterranean-climate oak savannas: The interplay between abiotic environment and species interactions. *Web Ecol.* **2009**, *9*, 30–43, doi:10.5194/we-9-30-2009.

5. Gaman, T.; Firman, J. *Oaks 2040. The Status and Future of Oaks in California*; Oakland, 2006;

6. Costa Pérez, J.C.; Martín Vicente, Á.; Fernández Alés, R.; Estirado Oliet, M.; Ingeniera de Montes; Consejería de Medio Ambiente *Dehesas de Andalucía. Caracterización ambiental*; Consejería de Medio Ambiente, Junta de Andalucía, Eds.; Sevilla, 2006; ISBN 849632981X.

7. Costa, A.; Madeira, M.; Lima Santos, J.; Oliveira, Â. Change and dynamics in Mediterranean evergreen oak woodlands landscapes of Southwestern Iberian Peninsula. *Landsc. Urban Plan.* **2011**, *102*, 164–176, doi:10.1016/j.landurbplan.2011.04.002.

8. Howlett, D.S.; Moreno, G.; Mosquera Losada, M.R.; Nair, P.K.R.; Nair, V.D. Soil carbon storage as influenced by tree cover in the Dehesa cork oak silvopasture of central-western Spain. *J. Environ. Monit.* **2011**, *13*, 1897–1904, doi:10.1039/c1em10059a.

9. Gómez-Rey, M.X.; Madeira, M.; Gonzalez-Prieto, S.J.; Coutinho, J. Soil C and N dynamics in a Mediterranean oak woodland with shrub encroachment. *Plant Soil* **2013**, *371*, 339–354, doi:10.1007/s11104-013-1695-z.

10. Pulido-Fernández, M.; Schnabel, S.; Lavado-Contador, J.F.; Miralles Mellado, I.; Ortega Pérez, R. Soil organic matter of Iberian open woodland rangelands as influenced by vegetation cover and land management. *Catena* **2013**, *109*, 13–24, doi:10.1016/j.catena.2013.05.002.

11. Andivia, E.; Fernández, M.; Alejano, R.; Vázquez-piqué, J. Tree patch distribution drives spatial heterogeneity of soil traits in cork oak woodlands. *Ann. For. Sci.* **2015**, 549–559, doi:10.1007/s13595-015-0475-8.

12. Gea-Izquierdo, G.; Montero, G.; Cañellas, I. Changes in limiting resources determine spatio-temporal

variability in tree-grass interactions. *Agrofor. Syst.* **2009**, *76*, 375–387, doi:10.1007/s10457-009-9211-4.

13. Moreno, G.; Obrador, J.J.; García, A. Impact of evergreen oaks on soil fertility and crop production in intercropped dehesas. *Agric. Ecosyst. Environ.* **2007**, *119*, 270–280, doi:10.1016/j.agee.2006.07.013.

14. Hussain, M.Z.; Otieno, D.O.; Mirzae, H.; Li, Y.L.; Schmidt, M.W.T.; Siebke, L.; Foken, T.; Ribeiro, N.A.; Pereira, J.S.; Tenhunen, J.D. CO_2 exchange and biomass development of the herbaceous vegetation in the Portuguese montado ecosystem during spring. *Agric. Ecosyst. Environ.* **2009**, *132*, 143–152, doi:10.1016/j.agee.2009.03.008.

15. Rossetti, I.; Bagella, S.; Cappai, C.; Caria, M.C.; Lai, R.; Roggero, P.P.; Martins, P.; Sousa, J.P.; Querner, P.; Seddaiu, G. Isolated cork oak trees affect soil properties and biodiversity in a Mediterranean wooded grassland. *Agric. Ecosyst. Environ.* **2015**, *202*, 203–216, doi:10.1016/j.agee.2015.01.008.

16. Lopez-Carrasco, C.; Lopez-Sanchez, A.; San Miguel, A.; Roig, S. The effect of tree cover on the biomass and diversity of the herbaceous layer in a Mediterranean dehesa. *Grass Forage Sci.* **2015**, *70*, 639–650, doi:10.1111/gfs.12161.

17. Uribe, C.; Inclán, R.; Hernando, L.; Román, M.; Clavero, M.A.; Roig, S.; Van Miegroet, H. Grazing, tilling and canopy effects on carbon dioxide fluxes in a Spanish dehesa. *Agroforest Syst.* **2015**, *89*, 305–318, doi:10.1007/s10457-014-9767-5.

18. Casals, P.; Gimeno, C.; Carrara, A.; Lopez-sangil, L. Soil CO_2 efflux and extractable organic carbon fractions under simulated precipitation events in a Mediterranean Dehesa. *Soil Biol. Biochem.* **2009**, *41*, 1915–1922, doi:10.1016/j.soilbio.2009.06.015.

19. Casals, P.; Sangil, L.L.; Carrara, A.; Gimeno, C.; Nogués, S. Autotrophic and heterotrophic contributions to short-term soil CO_2 efflux following simulated summer precipitation pulses in a Mediterranean dehesa. *Global Biogeochem. Cycles* **2011**, *25*, 1–12, doi:10.1029/2010GB003973.

20. Ma, S.; Baldocchi, D.D.; Xu, L.; Hehn, T. Inter-annual variability in carbon dioxide exchange of an oak/grass savanna and open grassland in California. *Agric. For. Meteorol.* **2007**, *147*, 157–171, doi:10.1016/j.agrformet.2007.07.008.

21. Tang, J.; Baldocchi, D.D. Spatial-temporal variation in soil respiration in an oak-grass savanna ecosystem in California and its partitioning into autotrophic and heterotrophic components. *Biogeochemistry* **2005**, *73*, 183–207, doi:10.1007/s10533-004-5889-6.

22. Shvaleva, A.; Costa, F.; Costa, J.M.; Correia, A.; Anderson, M.; Lobo-do-vale, R.; Fangueiro, D.; Bicho, C. Comparison of methane, nitrous oxide fluxes and CO_2 respiration rates from a Mediterranean cork oak ecosystem and improved pasture. *Plant Soil* **2014**, *374*, 883–898, doi:10.1007/s11104-013-1923-6.

23. Shvaleva, A.; Siljanen, H.M.P.; Correia, A.; Silva, F.C.; Lamprecht, R.E.; Lobo-do-Vale, R.; Bicho, C.; Fangueiro, D.; Anderson, M.; Pereira, J.S.; et al. Environmental and microbial factors influencing methane and nitrous oxide fluxes in Mediterranean cork oak woodlands: Trees make a difference. *Front. Microbiol.* **2015**, *6*, 1–11, doi:10.3389/fmicb.2015.01104.

24. Petchey, O.L.; Gaston, K.J. Functional diversity: Back to basics and looking forward. *Ecol. Lett.* **2006**, *9*, 741–758, doi:10.1111/j.1461-0248.2006.00924.x.

25. De Deyn, G.B..; Quirk, H.; Yi, Z.; Oakley, S.; Ostle, N.J.; Bardgett, R.D. Vegetation composition promotes carbon and nitrogen storage in model grassland communities of contrasting soil fertility. *J. Ecol.* **2009**, *97*, 864–875, doi:10.1111/j.1365-2745.2009.01536.x.

26. Niklaus, P.A.; Wardle, D.A.; Tate, K.R. Effects of plant species diversity and composition on nitrogen cycling and the trace gas balance of soils. **2006**, 83–98, doi:10.1007/s11104-005-5230-8.

27. Ribas, A.; Llurba, R.; Gouriveau, F.; Altimir, N.; Connolly, J.; Sebastià, M.T. Plant identity and evenness affect yield and trace gas exchanges in forage mixtures. *Plant Soil* **2015**, *391*, 93–108, doi:10.1007/s11104-015-2407-7.

28. Peel, M.C.; Finlayson, B.L.; McMahon, T.A. Updated world map of the Köppen-Geiger climate classification. *Hydrol. Earth Syst. Sci.* **2007**, *11*, 1633–1644, doi:10.5194/hess-11-1633-2007.

29. Porta Casanellas, J.; López-Acevedo Reguerín, M.; Rodríguez Ochoa, R. Determinación de pH y conductividad eléctrica en extractos acuosos 1/5 (p/v). In *Técnicas y experimentos en edafología*; Col·legi Oficial d'Enginyers Agrònoms de Catalunya, Ed.; Col·legi d'Enginyers Agrònoms de Catalunya, Universidad Politécnica de Cataluña, Escola Tècnica Superior d'Enginyers Agrònoms de Lleida, 1986.

30. Walkley, A.; Black, C.A. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* **1934**, *37*, 29–38.

31. Gee, G.W.; Bauder, J.W. Particle size analysis. In *Methods of Soil Analysis. Part 1.*; Klute, A., Ed.; Madison, 1986; pp. 383–409.

32. Debouk, H.; Altimir, N.; Sebastià, M.T. Maximizing the information obtained from chamber-based greenhouse gas exchange measurements in remote areas. *MethodsX* **2018**, *5*, 973–983, doi:10.1016/j.mex.2018.07.021.

33. *Eddy Covariance. A Practical Guide to Measurement and Data Analysis*; Aubinet, M., Vesala, T., Papale, D., Eds.; Springer Netherlands: London and New York, 2012; ISBN 9789400723504.

34. Kirwan, L.; Lüscher, A.; Sebastià, M.T.; Finn, J.A.; Collins, R.P.; Porqueddu, C.; Helgadottir, A.; Baadshaug, O.H.; Brophy, C.; Coran, C.; et al. Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. *J. Ecol.* **2007**, *95*, 530–539, doi:10.1111/j.1365-2745.2007.01225.x.

35. Kirwan, L.; Connolly, J.; Finn, J.A.; Brophy, C.; Lüscher, A.; Nyfeler, D.; Sebastia, M.-T. Diversity–interaction modeling: estimating contributions of species identities and interactions to ecosystem function. *Ecology* **2009**, *90*, 2032–2038, doi:10.1890/08-1684.1.

36. Wohlfahrt, G.; Hammerle, A.; Haslwanter, A.; Bahn, M.; Tappeiner, U.; Cernusca, A. Disentangling leaf area and environmental effects on the response of the net ecosystem CO_2 exchange to diffuse radiation. *Geophys. Res. Lett.* **2008**, *35*, 1–5, doi:10.1029/2008GL035090.

doi:10.20944/preprints202011.0220.v1

37. Busch, F.A.; Sage, R.F.; Farquhar, G.D. Plants increase CO_2 uptake by assimilating nitrogen via the photorespiratory pathway. *Nat. Plants* **2018**, *4*, 46–54, doi:10.1038/s41477-017-0065-x.

38. Lee, T.D.; Reich, P.B.; Tjoelker, M.G. Legume presence increases photosynthesis and N concentrations of co-occurring non-fixers but does not modulate their responsiveness to carbon dioxide enrichment. *Oecologia* **2003**, *137*, 22–31, doi:10.1007/s00442-003-1309-1.

39. Reich, P.B.; Walters, M.B.; Ellsworth, D.S. From tropics to tundra: Global convergence in plant functioning. *Ecology* **1997**, *94*, 13730–13734, doi:10.1073/pnas.94.25.13730.

40. Reich, P.B.; Ellsworth, D.S.; Walters, M.B. Leaf Structure (Specific Leaf Area) Modulates Photosynthesis-Nitrogen relations: Evidence from within Across Species and Functional Groups. *Funct. Ecol.* **1998**, *12*, 948–958, doi:10.1046/j.1365-2435.1998.00274.x.

41. Mulder, C.P.H.; Jumpponen, A.; Högberg, P. How plant diversity and legumes affect nitrogen dynamics in experimental grassland communities. *Community Ecol.* **2002**, *133*, 412–421, doi:10.1007/s00442-002-1043-0.

42. Pirhofer-Walzl, K.; Rasmussen, J.; Høgh-Jensen, H.; Eriksen, J.; Søegaard, K.; Rasmussen, J. Nitrogen transfer from forage legumes to nine neighbouring plants in a multi-species grassland. *Plant Soil* **2012**, *350*, 71–84, doi:10.1007/s11104-011-0882-z.

43. Nyfeler, D.; Huguenin-Elie, O.; Suter, M.; Frossard, E.; Lüscher, A. Grass-legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agric. Ecosyst. Environ.* **2011**, *140*, 155–163, doi:10.1016/j.agee.2010.11.022.

44. Davidson, E.A.; Janssens, I.A. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* **2006**, *440*, 165–173, doi:10.1038/nature04514.

45. Aljazairi, S.; Arias, C.; Nogués, S. Carbon and nitrogen allocation and partitioning in traditional and modern wheat genotypes under pre-industrial and future CO_2 conditions. *Plant Biol.* **2015**, *17*, 647–659, doi:10.1111/plb.12280.

46. Larsen, K.S.; Ibrom, A.; Beier, C.; Jonasson, S.; Michelsen, A. Ecosystem respiration depends strongly on photosynthesis in a temperate heath. *Biogeochemistry* **2007**, *85*, 201–213, doi:10.1007/s10533-007-9129-8.

47. Gratani, L.; Crescente, M. Map-Making of Plant Biomass and Leaf Area Index for Management of Protected Areas. *Aliso A J. Syst. Evol. Bot.* **2000**, *19*, 1–12, doi:10.5642/aliso.20001901.02.

48. Sheffer, E.; Canham, C.D.; Kigel, J.; Perevolotsky, A. Countervailing effects on pine and oak leaf litter decomposition in human-altered Mediterranean ecosystems. *Oecologia* **2015**, *177*, 1039–1051, doi:10.1007/s00442-015-3228-3.

49. Fioretto, A.; Papa, S.; Pellegrino, A.; Fuggi, A. Leaf litter decomposition dynamics in Mediteranean area. In *Soil Ecology Research Developements*; Tian-Xiao, L., Ed.; Nova Science Publishers, Inc.: New York, 2008.

50. Ibañez, M. Vegetation drives greenhouse gas exchange, and carbon and nitrogen cycling in grassland

ecosystems, Doctoral thesis. University of Lleida, 2019.

51. Spohn, M. Microbial respiration per unit microbial biomass depends on litter layer carbon-to-nitrogen ratio. *Biogeosciences* **2015**, *12*, 817–823, doi:10.5194/bg-12-817-2015.

52. Wang, B.; Wu, Y.; Chen, D. Stable isotopes of carbon and nitrogen help to predict the belowground communities at a regional scale. *Sci. Rep.* **2017**, *7*, 1–10, doi:10.1038/s41598-017-07517-w.

53. Zhu, C.; Ma, Y.; Wu, H.; Sun, T.; La Pierre, K.J.; Sun, Z.; Yu, Q. Divergent effects of nitrogen addition on soil respiration in a semiarid grassland. *Sci. Rep.* **2016**, 1–8, doi:10.1038/srep33541.

54. Corrêa Dias, A.T.; van Ruijven, J.; Berendse, F. Plant species richness regulates soil respiration through changes in productivity. *Oecologia* **2010**, *163*, 805–813, doi:10.1007/s00442-010-1569-5.

55. Oertel, C.; Matschullat, J.; Zurba, K.; Zimmermann, F.; Erasmi, S. Greenhouse gas emissions from soils — A review. *Chemie der Erde* **2016**, *76*, 327–352, doi:10.1016/j.chemer.2016.04.002.

56. Jugold, A.; Althoff, F.; Hurkuck, M.; Greule, M.; Lenhart, K.; Lelieveld, J.; Keppler, F. Non-microbial methane formation in oxic soils. *Biogeosciences* **2012**, *9*, 5291–5301, doi:10.5194/bg-9-5291-2012.

57. Baggs, E.M.; Rees, R.M.; Smith, K.A.; Vinten, A.J.A. Nitrous oxide emission from soils after incorporating crop crop residues. *Soil Use Manag.* **2000**, *16*, 82–87, doi:10.1002/9781118676332.ch8.

58. Lin, S.; Iqbal, J.; Hu, R.; Cai, J.; Shaaban, M.; Chen, X. Nitrous Oxide Emissions from Yellow Brown Soil as Affected by Incorporation of Crop Residues With Different Carbon-to-Nitrogen Ratios: A Case Study in Central China. *Arch. Environ. Contam. Toxicol.* **2013**, *65*, 183–192, doi:10.1007/s00244-013-9903-7.

59. Shaaban, M.; Peng, Q.; Hu, R.; Lin, S.; Zhao, J. Soil Nitrous oxide and Carbon dioxide emissions following incorporation of above- and below-ground biomass of green bean. *Int. J. Environ. Sci. Technol.* **2016**, *13*, 179–186, doi:10.1007/s13762-015-0843-9.