

Tree – Open Grassland Structure Drives Carbon And Nitrogen Cycling In Mediterranean Wood Pastures of The Iberian Peninsula

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Abstract

Purpose: Mediterranean wood pastures are the largest agroforestry system in Europe. Traditional silvo-pastoral uses shaped these systems into a mosaic of trees and open grassland. However, little is known about how this structure may influence ecosystem carbon (C) and nitrogen (N) dynamics, considering different canopy types and interactions with the herbaceous layer.

Aims: To unravel the canopy effect on C and N dynamics (1) under representative canopy types, including traditional *Quercus* stands and *Pinus pinea* L. plantations; and (2) in interaction with the herbaceous plant functional types (PFT).

Methods: We analysed C and N content and the isotopic natural abundance on soil and plant materials at the open grassland and under the different tree canopies.

Results: Under the canopy, soil C and N content, and N availability for the herbaceous layer increased. The canopy effect performed differences between *Quercus* and *P. pinea* plots, as detected on the belowground biomass C content. Also, the canopy effect was more pronounced with increasing environmental constraints, highlighting the relevance of trees in more restrictive environments. PFT ecophysiological characteristics interacted with tree canopies, as reflected the enhanced efficiency taking-up N, and/or exploiting symbiotic N of grasses, which was probably one of the causes of their dominance under the canopy, at higher N availability.

Conclusion: Changes in the tree coverage and tree species will significantly change ecosystem C and N storage and cycling. Therefore, it is highly advisable to preserve a balance between trees and open grassland, and traditional *Quercus* stands, to keep ecosystem services provisioning.

1. Introduction

Mediterranean wood pastures or oak savannahs (called dehesas in Spain and montados in Portugal) are semi-natural savannah-like systems that result from traditional silvo-pastoral uses, in which an herbaceous and an arboreal layer (mostly *Quercus* species) coexist (Ibañez et al. 2021b). They are one of the largest agroforestry systems in Europe (Eichhorn et al. 2006), covering 3.5 – 4 million ha, mostly along the South West of the Iberian Peninsula (Olea et al. 2005), and are also present in other world regions with Mediterranean climates, mainly in California (Gaman and Firman 2006; Marañón et al. 2009; Huntsinger et al. 2013).

Mediterranean wood pastures have traditionally provided a wide variety of silvo-pastoral goods and services, including production of forage, acorns, timber, and cork. Uses that shaped these systems into a mosaic of trees and open grassland. However, the tree coverage in Mediterranean wood pastures is changing. Traditional silvo-pastoral 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 (Costa Pérez et al. 2006; Costa et al. 2011). These

changes are reshaping the tree – open grassland mosaic, with the consequent implications that this may have on ecosystem functioning, and specifically on carbon (C) and nitrogen (N) cycling and storage.

To this regard, the natural abundance of C and N isotopes – here given as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values – can be used as a proxy to assess C and N acquisition and processing (Dawson et al. 2002; Ferrio et al. 2005; Kahmen and Buchmann 2007). Discrimination against ^{13}C in C_3 plants is directly linked to photosynthetic net CO_2 assimilation and stomatal conductance, increasing discrimination against ^{13}C with increasing stomatal conductance (Farquhar et al. 1989). Variations in $\delta^{13}\text{C}$ are then controlled by physiological and environmental factors (Farquhar et al. 1982, 1989), and $\delta^{13}\text{C}$ of plant organic matter has been identified as a useful tool to assess CO_2 and water exchange relationships (Ehleringer et al. 1990). Thus, tree canopies directly influence light availability, which in turn controls photosynthetic activity and C cycling (Bonafini et al. 2013). The light reduction below the canopy generally increases stomatal conductance, making possible more discrimination against ^{13}C , and generating organic matter depleted in ^{13}C (van der Merwe and Medina 1991; Buchmann et al. 1997; Della Coletta et al. 2009; Bonafini et al. 2013).

On the other hand, $\delta^{15}\text{N}$ in the above and belowground biomass will depend mostly on the amount and the type of N sources, N cycling steps, and the microbial community (Robinson 2001; Dawson et al. 2002). Particularly with regard to N availability, plants tend to fractionate against ^{15}N at high N availability, meaning that the remaining source will be more ^{15}N enriched, while the biomass exploiting the N source will be ^{15}N depleted (Yoneyama et al. 2001). On the contrary, at limiting N conditions there will be very low ^{15}N discrimination, and the exploiting biomass will have a $\delta^{15}\text{N}$ similar to the source (Evans et al. 1996). Also, at low N availability, plants will be more dependent on symbiotic N fixed from atmospheric N_2 by bacteria (Evans 2001; Santi et al. 2013).

Thus, a “canopy effect” on the C and N content and the natural isotopic composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of the different ecosystem compartments is expected. However, still little is known about how such “canopy effect” may differ among canopy types of Iberian wood pastures. Even less is known about how the different plant functional types (PFT) of the herbaceous layer may differ in their response to the presence/absence of tree canopies in terms of C and N cycling. Indeed, the herbaceous layer of Iberian wood pastures is highly diverse (Marañón 1985), and the two microhabitats (open grassland and under the canopy) are known to differ in terms of plant specific and functional diversity and composition. Grasses being dominant under the canopy, while legume forbs (hereafter “legumes”) and non-legume forbs (hereafter “forbs”) are limited in their growth under the canopy (Olsvig-Whittaker et al. 1992; Gea-Izquierdo et al. 2009; Marañón et al. 2009; Lopez-Carrasco et al. 2015; Ibañez 2019).

Also, these PFT are known to differ in their N and light (and therefore CO_2) use and acquisition strategies (Tilman et al. 1997; Symstad 2000; Lavorel et al. 2007; Sebastià 2007; Ibañez et al. 2020). Legumes being widely known for their capacity to fix symbiotic N, which may result in higher rates of net CO_2 uptake (Ibañez et al. 2020, 2021b, a); while grasses have erect and high-density leaves that

ensure good light penetration (Craine et al. 2001). Whereby a differential C and N cycling response to tree canopies among those PFT is expected.

Hence, in the present study we specifically aim at assessing such canopy effect on the different ecosystem compartments, including the soil and the above and belowground biomass. Specifically aiming at unravelling the canopy effect (1) under representative canopy types of Iberian wood pastures, considering traditional *Quercus* stands and *Pinus pinea* L. plantations, a common tree plantation replacing traditional canopies (Costa Pérez et al. 2006); and (2) in interaction with the PFT of the herbaceous layer.

2. Material And Methods

2.1 Study sites and sampling design

Study sites were the same as reported in Ibañez et al. (2021), 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 (Peel et al. 2007). However, SM is slightly cooler and wetter than DN, with a mean annual temperature in SM of 16.8 °C and in DN of 18.1 °C, and a mean annual precipitation in SM of 648 mm and in DN of 543 mm. Grasslands in both locations are dominated by herbaceous annual species, including grasses, forbs, and 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).

Study plots were selected according to their tree composition, with representative canopy types of Iberian wood pastures (Costa Pérez et al. 2006). 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 in the Iberian Peninsula; one *Q. ilex* and *Q. suber* mixed stand (DN-mixed), the next most abundant stand; and a pure *Pinus pinea* L. stand (DN-pinea), a common tree plantation replacing traditional canopies (Costa Pérez et al. 2006).

Field work was carried out in spring (2016//04/05 – 2016/04/10), coinciding with the most productive moment of the system. Study treatments were therefore established according to plot (SM-ilex, DN-mixed, DN-suber and DN-pinea), and canopy (open grassland, OG, and under the canopy, UC). At each treatment level we sampled 4 replicates, resulting in 40 sampling points, sampling aboveground biomass (Section 2.2), belowground biomass and soil (Section 2.3). In the DN-mixed plot we discriminated between both *Quercus* species (*Q. suber* and *Q. ilex*) to establish sampling points. However, we performed preliminary comparative analysis in the DN-mixed plot on environmental and vegetation characteristics under the canopy of both *Quercus* species and no relevant differences between *Quercus* species were found. DN-mixed results are then always presented combining both tree species.

2.2 Aboveground biomass sampling

At each sampling point we sampled same dominant species of each PFT including grasses (*Bromus hordeaceus* L. and *Hordeum vulgare* L.); forbs (*Calendula arvensis* L., *Chamamelum mixtum* L., *Crepis capillaris* L., *Erodium moschatum* L., and *Geranium molle* L.); and legumes (*Ornithopus sativus* Brot. and *Trifolium subterraneum* L.). Also, PFT composition from the same study plots (Ibañez et al. 2021b) was used to interpret and discuss our results.

2.3 Soil and belowground biomass sampling

Two soil cores of 9 cm² surface and 0 – 10 cm depth were extracted at each sampling point. In the laboratory, one of the cores was processed for soil analysis, and the second core washed and filtered with a 0.2 mm pore size strainer to obtain belowground biomass (BGB).

2.4 Determination of carbon and nitrogen content and isotopic natural abundance

All collected samples, including soil, BGB, and the herbaceous layer sorted by PFT – grasses, forbs, and legumes –, were oven dried at 60°C until constant weight, powdered with a ball mill (MM200, Retsch, Asturias, Spain) and tin capsuled (Courtage Analyse Service, Mont Saint-Aignan, France). For determining C content and $\delta^{13}\text{C}$ in all our samples, except soils, we used glutamic acid and acetanilide laboratory standards, both calibrated with USGS40 international reference material. For determining the percentage of N and $\delta^{15}\text{N}$ an additional standard was also used (N_1), calibrated using IAEA- N_1 . Samples were prepared in the Institut de Biologie des Plantes (<http://www.ips2.u-psud.fr>). Afterwards, samples were analysed in the Isolab of the Grassland Sciences group at ETH Zurich (<http://www.gl.ethz.ch/>), with a Flash EA 1112 Series elemental analyser (Finnigan MAT, Bremen, Germany), coupled to a Delta^{plus}XP isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany) via a 6-port valve (Brooks et al. 2003) and a ConFlo III interface (Werner et al. 1999).

For determining C content and $\delta^{13}\text{C}$ in the soil, we used acetanilide and caffeine as standards, both calibrated using glutamic acid from IAEA, USGS40, and NBS-22. In determining the percentage of N and $\delta^{15}\text{N}$ in the soil, tyrosine was also used as standard calibrated using IAEA- N_1 . Soil samples were prepared and analysed in the Isolab of the Grassland Sciences group at ETH Zurich, as described above.

Isotopic composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were both calculated as deviation of the isotope ratio ($R = {}^{13}\text{C}/{}^{12}\text{C}$ or $R = {}^{15}\text{N}/{}^{14}\text{N}$) of the samples from the ratio of the corresponding international standard ($\delta = (R_{\text{sample}} / R_{\text{standard}}) - 1$), VPDB for $\delta^{13}\text{C}$, and air- N_2 for $\delta^{15}\text{N}$ (Coplen 2011).

2.4 Data analysis

All data analyses were performed using R software (R Core Team 2019). We ran linear models on C and N content and isotopic composition ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) of each ecosystem compartment as function of plot (SM-ilex, DN-mixed, DN-suber, DN-pinea), and canopy (OG, UC). 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). Linear models were also applied to explore the relationships between C and N dynamics within or between ecosystem compartments when applicable. Finally, we assessed differences among PFT on the C and N content and isotopic composition ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) by one way ANOVAs, and tukey post-hoc tests, using the HSD.test function of the agricolae package (Mendiburu 2017). Only significant models ($p < 0.05$) are presented and discussed, selecting the most explanatory and parsimonious ones.

3. Results

Both soil C and N contents were consistently higher under the canopy than in the open grassland in all the study plots (canopy effect, Table 1 and Fig. 1a-b). Soil C and N were positively correlated (R^2 0.9, $p < 0.001$, Fig. 1c). At the same time, the environment under the canopy, with enhanced soil N content, favoured ^{15}N fractionation by the BGB (decreasing $\delta^{15}\text{N}$, Fig. 2).

Tree canopies influenced the C content in the BGB, but with differences between *Quercus* species and *P. pinea* dominated plots (Fig. 3). BGB presented higher C content under the canopy than in the open grassland in all the plots dominated by *Quercus* species (SM-ilex, DN-mixed, DN-suber, Fig. 3); while in the *P. pinea* dominated plot the C content in the BGB decreased under the canopy compared to the open grassland (DN-pinea x canopy effect, Table 2 and Fig. 3).

Among PFT of the herbaceous layer, forbs also responded to tree canopies, but with some differences between locations (DN vs. SM). Forbs presented more ^{13}C depleted tissues under the canopy than in the open grassland in all DN plots, especially in DN-mixed and DN-pinea plots (DN-mixed and DN-pinea effect, Table 3); while such effect was not noticeable in the SM-ilex plot (Table 3 and Fig. 4a). Similarly, the N content in forbs increased under the canopy in all DN plots, especially noticeable in the DN-mixed and DN-pinea plots (DN-mixed x canopy and DN-pinea x canopy effect, Table 3); while the N content in forbs in the SM-ilex plot did not show any difference between under the canopy and the open grassland (Table 3 and Fig. 4b).

In addition, legumes presented the highest N content (Fig. 5a), as well as the most ^{15}N (Fig. 5b), and ^{13}C (Fig. 5c) depleted tissues among the PFT of the herbaceous layer. Conversely, when comparing grasses and forbs, grasses had generally higher N content than forbs (Fig. 6a), and lower values of $\delta^{15}\text{N}$ (Fig. 6b).

4. Discussion

4.1 The canopy effect under representative canopy types of Iberian wood pastures

Intra-plot soil C and N dynamics were strongly driven by the presence of trees, as shows the higher soil C and N content under the canopy compared to the open grassland (Fig. 1 and Table 1). This was most likely related to a higher input of litter from the trees; which in addition was probably more recalcitrant than that from the herbaceous species, increasing the organic matter accumulation and soil C and N stocks under tree canopies (Dahlgren et al. 1997; Howlett et al. 2011; Gómez-Rey et al. 2013; Pulido-Fernández et al. 2013; Andivia et al. 2015). At the same time, the environment under the canopies favoured the ^{15}N fractionation by plants, as shown by the lower $\delta^{15}\text{N}$ in the BGB compared to the open grassland. (Fig. 2). This higher ^{15}N fractionation by plants might be related to a higher soil N availability under canopies than in the open grassland (Fig. 2), conditions that can allow a higher ^{15}N fractionation (Dawson et al. 2002; Kalcsits et al. 2014). However, the type of N sources and N cycling steps (Robinson 2001; Dawson et al. 2002), as well as the microbial community (Handley and Raven 1992; Unkovich 2013; Liu et al. 2017), can also leave an imprint the ^{15}N composition

Moreover, the canopy had a differential effect depending on the tree species that dominated the plot. This was detected on the C content in the BGB, as showed the higher C content under the canopy than in the open grassland in all the plots dominated by *Quercus* species (SM-ilex, DN-suber and DN-mixed), while the *P. pinea* dominated plot (DN-pinea) presented the opposite pattern (Fig. 3). Fact that suggests us a differential magnitude of the drivers in each case. First, the higher C content in the BGB under the canopies detected in the plots dominated by *Quercus* species may be related to the presence of the tree itself. Although BGB samples were quite superficial (0-10 cm), tree fine roots may increase the overall C content in the BGB (Kumar et al. 2010). Second, PFT composition of the herbaceous layer differed between under the canopy and the open grassland – therefore most likely there was a change in the BGB composition –, with legumes mostly appearing in the open grassland, while grasses were dominant under the canopy (Ibañez et al. 2021b). Thus, legumes have higher root respiration rates, lower C storage, and lower C/N ratio than forbs and grasses (Warembourg et al. 2003), properties that could result in a lower C content in the BGB in the open grassland compared to under the canopy (Fig. 3).

Conversely, the lower C content in the BGB detected in the DN-pinea plot under the canopy compared to the open grassland (Table 2 and Fig. 3) could be driven by the low N availability. The DN-pinea plot in the open grassland presented the lowest soil N content and the BGB tissue with the highest ^{15}N enrichment (Fig. 2), suggesting that N availability for plants could be here quite low and ^{15}N fractionation could be more restricted. Conditions that may increase root C content (van Diest 1987) to get a limiting resource (Ågren and Ingestad 1987; Gargallo-Garriga et al. 2014). On the other hand, the BGB under *P. pinea* canopy had the most ^{15}N enriched tissues, even though soil N content was not the lowest one under the canopy (Fig. 2); and $\delta^{15}\text{N}$ differences between both microenvironments (open grassland and under the tree) were much lower for *P. pinea* than for *Quercus* species (Fig. 2). Overall, these suggesting that N availability for plants in the *P. pinea* plot might be more limited than that on plots dominated by

Quercus species. The litter characteristics of *P. pinea* is known for its allelopathic properties (Valera-Burgos et al. 2012) and poor in N content, lower than litter of *Quercus* species (Fioretto et al. 2008; Sheffer et al. 2015). Facts that may be driving these differences in terms of N availability. Nevertheless, we recommend further research on the effect of tree litter on soil-belowground biomass interactions to clarify this point.

The canopy effect was also dependent on the location (DN vs. SM), as showed the almost neutral canopy effect found on the $\delta^{13}\text{C}$ and the N content of forbs, in contrast to the strong canopy effect detected in all DN plots (Fig. 4). This results suggests a coupling between C and N cycling (Aljazairi et al. 2014), and interestingly agree with the net ecosystem CO_2 exchange patterns reported by Ibañez et al. (2021) from the same study plots. The authors reported that: (a) in the SM-ilex plot CO_2 exchange was dominated by net CO_2 uptake both under the canopy and in the open grassland; while (b) in all DN plots there was net CO_2 uptake in the open grassland, but CO_2 emissions under the canopy. These results combined with our $\delta^{13}\text{C}$ values suggest that the stomatal conductance is similar between both microenvironments in SM, therefore similar ^{13}C discrimination rates are possible, in opposition to the strong differences found in DN. Similarly, the differential canopy effect found on the N content of forbs, suggests that also in terms of N availability, both microenvironments in the SM-ilex did not differ much, in contrast to the pronounced differences found in DN. Eventually, these facts highlighting the relevance of trees as drivers of ecosystem fertility and buffers of extreme conditions, especially in more constrained environments, as is DN vs. SM.

4.2 Interactions among tree canopies and plant functional types of the herbaceous layer

PFT of the herbaceous layer (forbs, grasses, and legumes) were exploiting C and N resources in particular ways under trees' influence, and this in turn driving ecosystem C and N cycling. As expected, legumes highlighted in terms of N and C acquisition and use (Fig. 5). Legumes are known for their higher leaf N content (Fig. 5a), coming mostly from symbiotically fixed sources (Fig. 5a), and generally present a low leaf mass/area ratio and thickness. These traits result in a shorter internal diffusion pathway from stomata to chloroplasts, and generate greater CO_2 conductance and consequently greater CO_2 supply for carboxylation (Li et al. 2016). The greater CO_2 supply leads to both higher root respiration (Warembourg et al. 2003) and leaf CO_2 exchange rates (Li et al. 2016), and eventually more depleted ^{13}C tissues than those of grasses and forbs (more negative $\delta^{13}\text{C}$, Fig. 5c).

However, although legumes perform higher CO_2 exchange rates than grasses and forbs (Ibañez et al. 2020), the environment under the canopy did not favour their abundance (Ibañez et al. 2021b). Legumes are limited for their high light demand (Gea-Izquierdo et al. 2009; Marañón et al. 2009; Lopez-Carrasco et al. 2015), and competition with species that are favoured under higher N availability, as is the case of grasses. Indeed, the differences in the N acquisition and use detected between forbs and grasses (Fig. 6)

is suggesting such competitive advantage for grasses. The higher N content (Fig. 6a) and the generally lower $\delta^{15}\text{N}$ (Fig. 6b) in grasses compared to forbs, suggests that grasses may be highly efficient taking-up N and/or exploiting symbiotic N sources (Yoneyama et al. 2001).

Thus, N-transfer between grasses and legumes (source of symbiotic N, Fig. 5b) has been described to be more efficient than between legumes and forbs (Pirhofer-Walzl et al. 2012). Also, grasses usually have fibrous roots (Weaver 1958; Schenk and Jackson 2002; Pirhofer-Walzl et al. 2012), trait that may facilitate higher N absorption from the most superficial soil layers, and from symbiotically fixed N; while forbs have generally taproots that are not so efficient to this effect (Pirhofer-Walzl et al. 2012). Overall, grasses being very competitive at high N availability (Song et al. 2011), via increasing their biomass at the expense of forbs and legumes, which is probably one of the causes of their dominance under the canopy, where there is higher soil N (Fig. 1b).

Conclusion

Changes in the tree coverage and tree species may cause profound changes on C and N dynamics in Mediterranean wood pastures. Overall, trees acted as islands of fertility, as reflected by the higher soil N and C content, and the higher rates of ^{15}N discrimination by the BGB under the canopy compared to the open grassland, which indicates higher N availability for plants. However, such canopy effect presented some differences related to the dominant tree species and local conditions. In terms of tree species, N availability in the *P. pinea* dominated plot seemed to be lower than that in *Quercus* species dominated plots, and this in turn changed the canopy effect over the C content of the BGB. Second, in terms of local conditions, the canopy effect on C and N cycling was generally more pronounced with increasing environmental constraints (as is DN vs. SM), highlighting the relevance of trees as drivers of ecosystem fertility and C and N relationships, especially in more constrained environments. On the other hand, each PFT drove ecosystem C and N cycling in particular ways, as for instance reflected the higher N content and the generally lower $\delta^{15}\text{N}$ of grasses compared to forbs, which suggests that grasses were highly competitive under the canopy, where there was higher soil N content. Our results indicate that it is highly advisable to preserve a balance between trees and open grassland spaces, as well as traditional *Quercus* stands; to keep ecosystem fertility and the structure and composition of the herbaceous layer, in order to guarantee ecosystem services provisioning and preservation of Mediterranean wood pastures.

Declarations

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Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Linear modelling of soil C and N content (%) as function of plot and canopy. Plot with SM-ilex 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.

	Soil C content (%)				Soil N content (%)			
	Est.	SE	t	p	Est.	SE	t	p
(Intercept)	2.1	0.4	5.73	< 0.001	0.18	0.03	6.77	< 0.001
Plot (DN-mixed)	-1.1	0.4	-2.65	0.01	-0.10	0.03	-3.31	0.002
Plot (DN-suber)	-0.2	0.5	-0.43	0.7	-0.02	0.03	-0.68	0.5
Plot (DN-pinea)	-0.8	0.5	-1.73	0.09	-0.09	0.03	-2.63	0.01
Canopy	1.8	0.3	6.20	< 0.001	0.11	0.02	5.39	< 0.001
Plot (DN-mixed) x canopy								
Plot (DN-suber) x canopy								
Plot (DN-pinea) x canopy								
R^2_{Adj}	0.53			< 0.001	0.51			< 0.001

Table 2. Linear modelling of C content (%) in belowground biomass (BGB) as function of plot and canopy. Plot with SM-ilex 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.

Table 3. Linear modelling of $\delta^{13}C$ (‰) and N content (%) in forbs as function of plot and canopy. Plot with SM-ilex 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.

Figures

	BGB C content (%)			
	Est.	SE	t	p
(Intercept)	30	1	20.12	< 0.001
Plot (DN-mixed)	2	2	1.03	0.3
Plot (DN-suber)	4	2	2.04	0.05
Plot (DN-pinea)	9	2	4.07	< 0.001
Canopy	4	3	1.37	0.2
Plot (DN-mixed) x canopy	2	3	0.55	0.6
Plot (DN-suber) x canopy	-1	3	-0.25	0.8
Plot (DN-pinea) x canopy	-8	3	-2.23	0.03
R ² _{Adj}	0.41			0.001

	Forbs $\delta^{13}\text{C}$ (‰)				Forbs N content (%)			
	Est.	SE	t	p	Est.	SE	t	p
(Intercept)	-28.2	0.2	-154.92	< 0.001	2.2	0.2	10.70	< 0.001
Plot (DN-mixed)	-0.5	0.2	-2.34	0.02	-0.8	0.2	-3.14	0.002
Plot (DN-suber)	-0.2	0.2	-0.79	0.4	-0.9	0.3	-3.43	< 0.001
Plot (DN-pinea)	-0.4	0.2	-1.69	0.09	-0.6	0.3	-2.33	0.02
Canopy	-0.5	0.1	-3.72	< 0.001	0.0	0.3	0.17	0.9
DN-mixed x canopy					0.9	0.3	2.48	0.01
DN-suber x canopy					0.4	0.4	1.07	0.3
DN-pinea x canopy					0.9	0.4	2.24	0.03
R ² _{Adj}	0.15				0.34			
				< 0.001				< 0.001

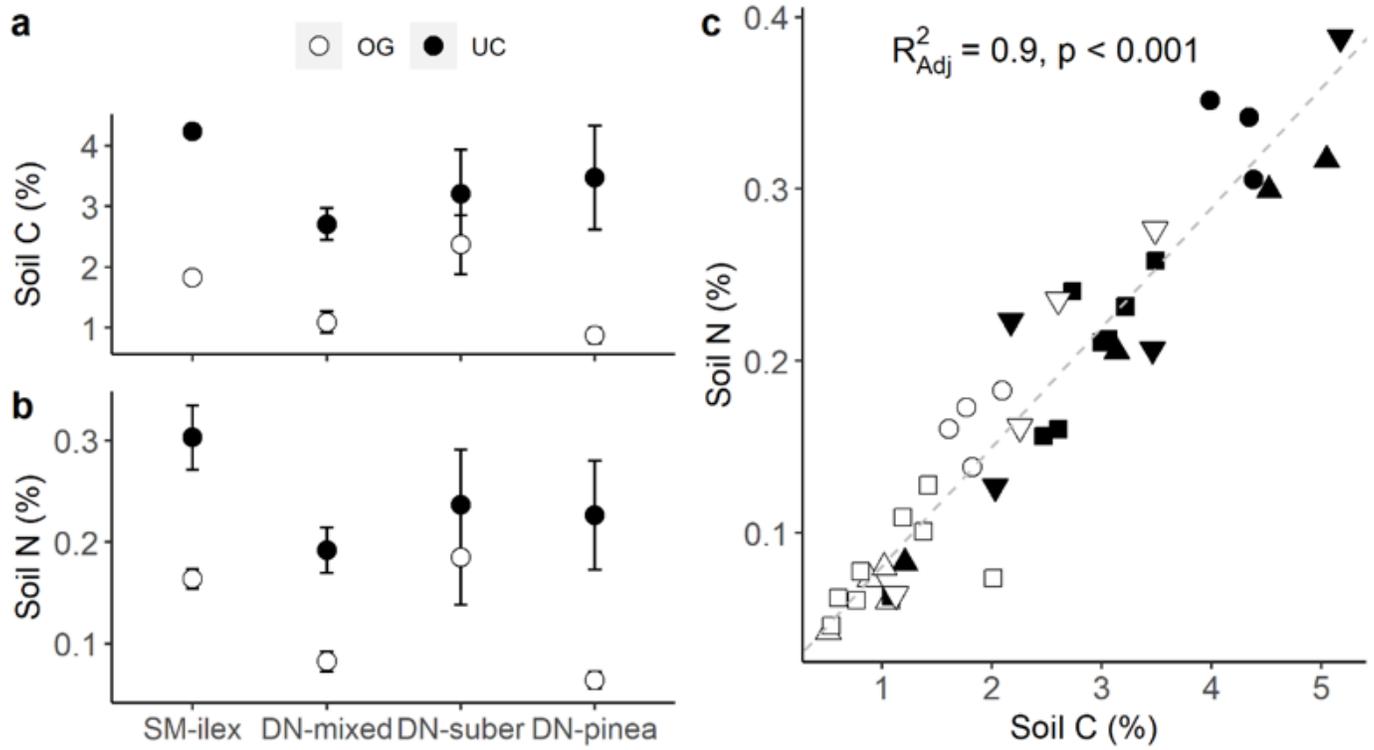


Figure 1

(a) Mean soil C \pm 1 standard error; (b) mean soil N \pm 1 standard error; and (c) soil N versus soil C per plot (shapes = ●●SM-ilex, ○■DN-mixed, ▽▼DN suber, △▲DN pinea), and canopy (open symbols indicate open grassland and solid symbols indicate under the canopy).

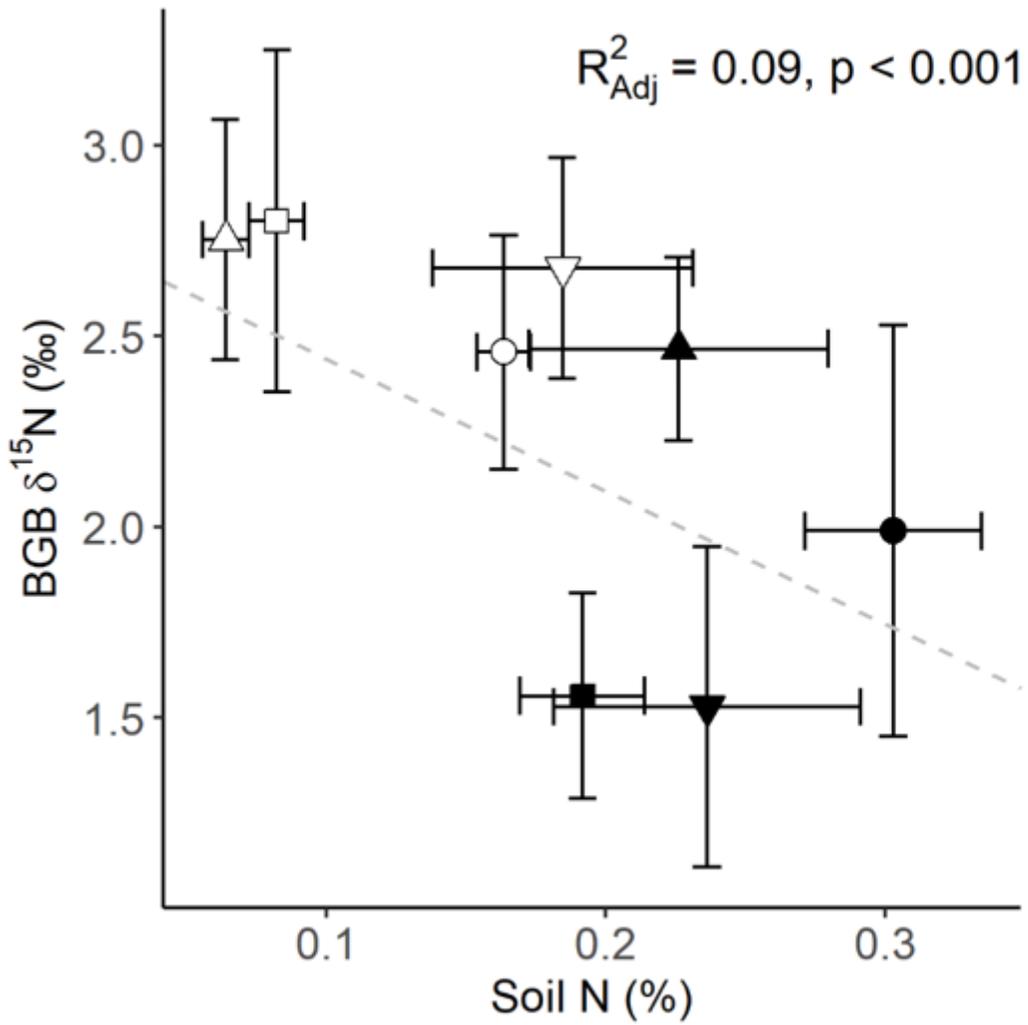


Figure 2

Relationship between belowground biomass (BGB) $\delta^{15}N$ and soil N, per plot (shapes = ●●SM-ilex, ○■DN-mixed, ▽▽DN suber, △▲DN pinea) and canopy (open symbols indicate open grassland and solid symbols indicate under the canopy). Mean \pm 1 standard error, and linear trend line.

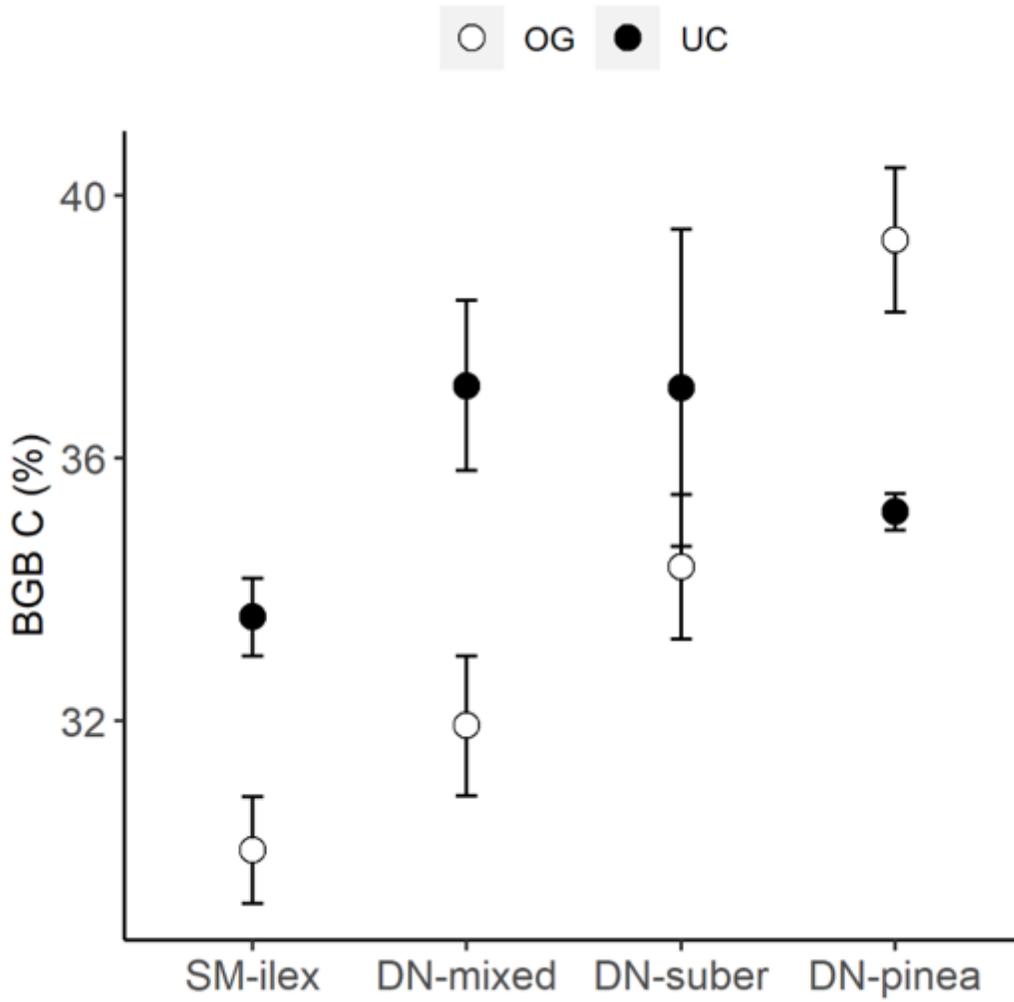


Figure 3

Mean C content in belowground biomass (BGB) \pm 1 standard error, per plot and canopy: open grassland (OG) and under the canopy (UC).

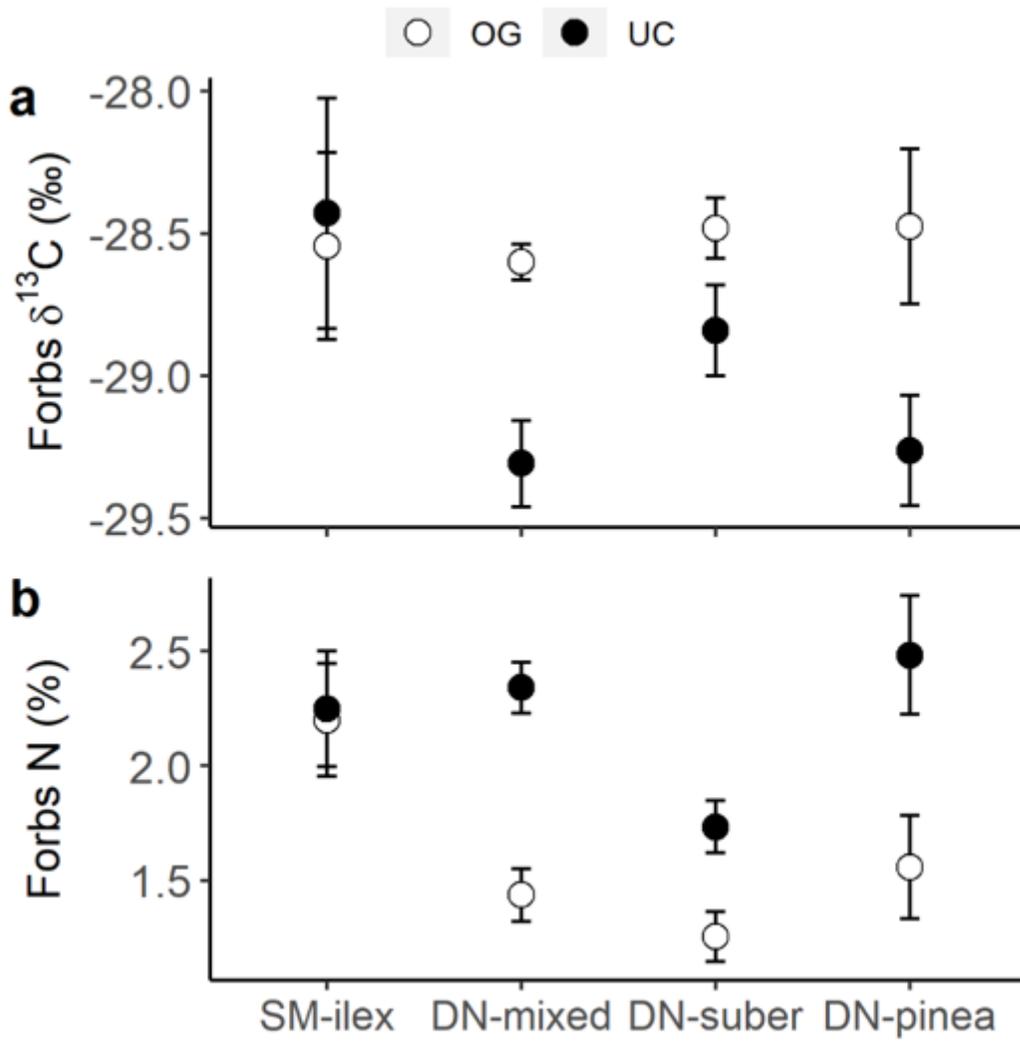


Figure 4

(a) Forbs $\delta^{13}\text{C}$; and (b) forbs N content per plot and canopy: open grassland (OG) and under the canopy (UC). Mean \pm 1 standard error.

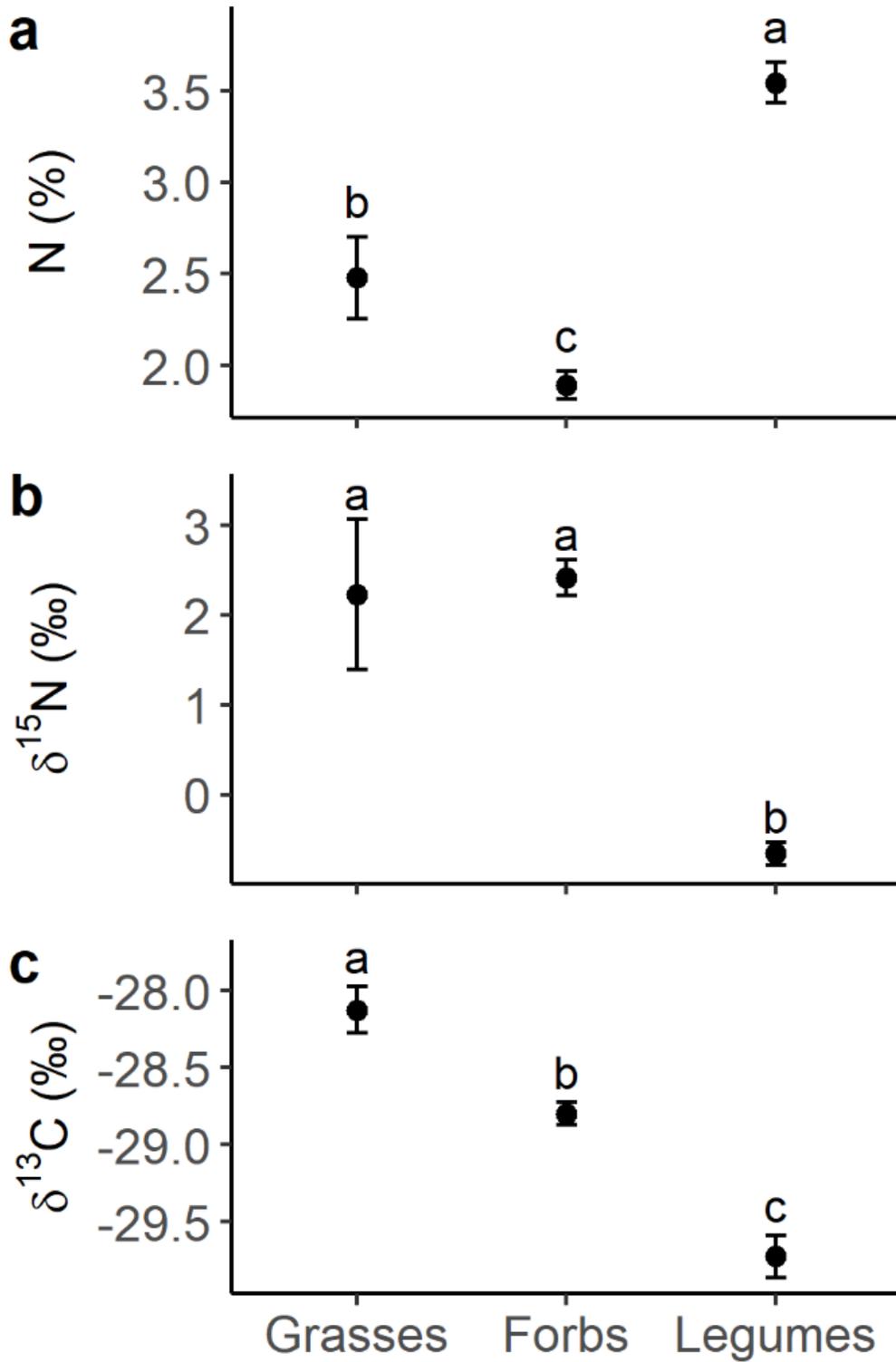


Figure 5

(a) Nitrogen content; (b) $\delta^{15}\text{N}$; and (c) $\delta^{13}\text{C}$ in grasses, forbs, and legumes. Mean \pm 1 standard error. Letters indicate significant differences among groups ($p < 0.05$) based on Tukey post hoc tests.

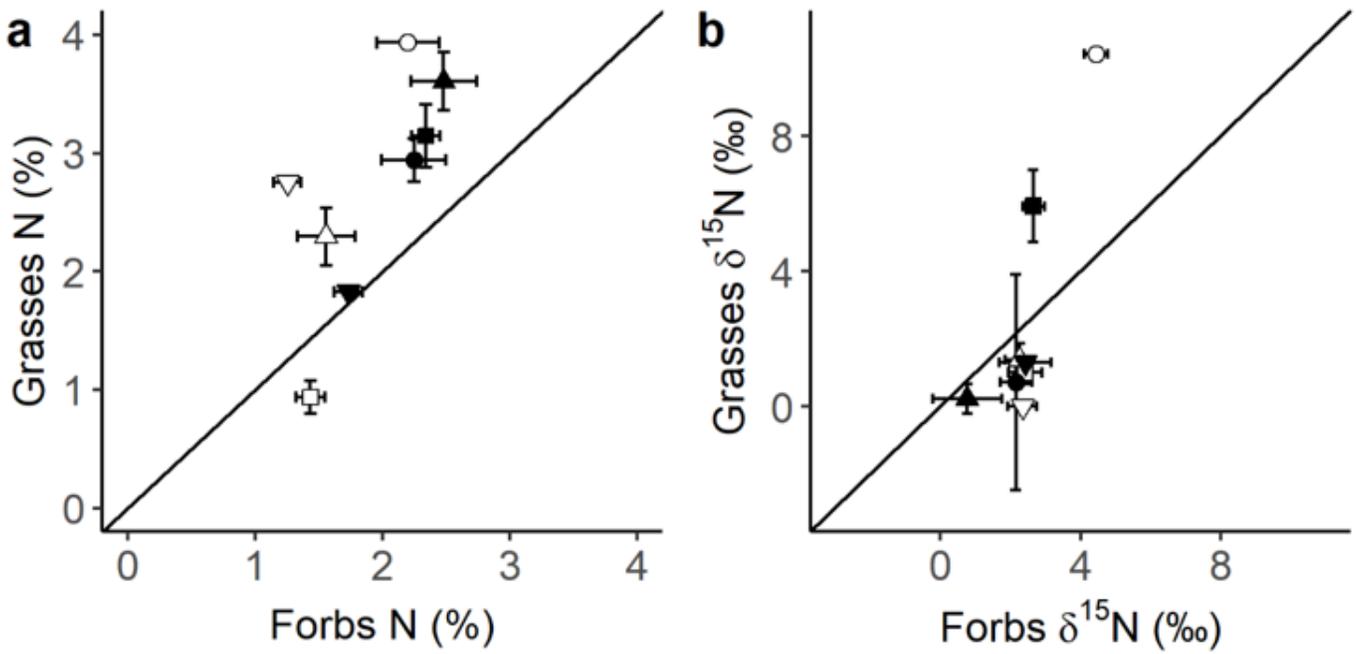


Figure 6

Relationship between (a) grasses N content and forbs N content; and (b) grasses $\delta^{15}\text{N}$ and forbs $\delta^{15}\text{N}$, per plot (shapes = ●● SM-ilex, ○■ DN-mixed, ▽▼ DN suber, △▲ DN pinea) and canopy (open symbols indicate open grassland, and solid symbols indicate under the canopy). Mean \pm 1 standard error. Diagonal line indicates the 1:1 relationship.