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Silvicultural management and altitude prevail on soil properties and fungal community in shaping understorey plant communities in a Mediterranean pine forest

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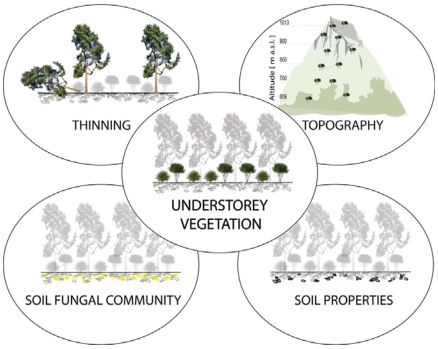
HIGHLIGHTS

- We unraveled the main drivers of understorey dynamics in a Mediterranean forest.
- We analyzed the understorey woody plant composition and diversity.
- Thinning and topography prevail on soil properties and fungal community.
- The effect of thinning on the shrub community depends on the altitude of the site.
- Changes in understorey composition were linked to microclimate conditions.

GRAPHICAL ABSTRACT



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ABSTRACT

Understorey vegetation plays a key role in Mediterranean forest ecosystem functioning. However, we still lack a thorough understanding of the patterns and drivers of understorey composition and diversity. As a result, understoreys are often ignored during assessments of forest functioning under climate change. Here we studied the effect of silvicultural management, topography, soil fungal community composition and soil physical and chemical properties on understorey community composition and diversity. The plant cover and number of individuals of understorey perennial plants, shrubs and non-dominant trees was recorded on 24 plots (paired: control-thinned) in a Mediterranean pine-dominated mountainous area in Northeast Spain. The study area represented a broad thinning intensity gradient (from 0 to 70 % in removed stand basal area) along a 400-m altitudinal range (from 609 m to 1013 m). Our results showed that thinning intensity and topography explained the greatest proportion of the total variance in the understorey species composition, i.e., 18 % and 16 %, respectively. Interestingly, the effects of the silvicultural treatments were significant only when considering the altitudinal effect, so that, the main impacts of thinning on the understorey community composition occurred at low altitudes (between 609 m and 870 m). Moreover, we found a significant decrease in both richness and abundance of understorey species in both the control and thinned plots with increasing altitude, with thinned plots being significantly richer in species compared to the control plots. The difference in the understorey community sensitivity to forest thinning along the altitudinal gradient suggests changes in factors that limit plant growth. Low elevation plots were restrained by light availability while high altitudes plots limited by winter freezing temperature.

Abbreviations: PNIN, Paraje Natural de Interés Nacional (Natural site of national interest); DCA, Detrended Correspondence Analysis; RDA, Redundancy Analysis.

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1. Introduction

The understorey vegetation in Mediterranean forests is an important biodiversity reservoir that can influence ecosystem processes and functions in multiple ways (Landuyt et al., 2019). In particular, the understorey actively influences soil nutrient cycling (Knops et al., 1996; Nilsson and Wardle, 2005), wildlife dynamics (Gunnarsson et al., 2004) and forest succession, driving forest regeneration dynamics, tree seedling establishment and growth (Messier et al., 1998; Alday et al., 2016). On the other hand, the spatial and temporal heterogeneity of the understorey vegetation can be affected by a variety of factors, like changes in the overstorey dominant layer, climate, soil nutrient availability (Qian et al., 2003; Chen et al., 2004), soil moisture (Sterkenburg et al., 2015; Urbanová et al., 2015) and distribution of soil microbiome (Fu et al., 2015; Baldrian, 2017). However, the majority of studies have focused solely on the effects of silvicultural treatments on the understorey (Thomas et al., 1999; Willms et al., 2017), neglecting the importance of many other drivers, and their potential interaction in shaping the understorey forest layer.

While light is generally not a limiting resource for dominant overstorey trees, it is considered the main limiting factor for understorey growth (Axmanová et al., 2012; Landuyt et al., 2019). In principle, a reduction of forest overstorey tree cover, for instance via selective thinning (Willms et al., 2017), will increase the light that reaches understorey plants, with positive effects on the understorey canopy height, stem density and basal area (Valladares and Guzmán, 2006). However, the increased light availability is likely to encourage some species while discouraging others, eventually affecting the richness, abundance, and composition of the understorey vegetation (Zangy et al., 2021). In parallel, silvicultural treatments may result in higher availability of water for the remaining plants (Aussenac and Granier, 1987; Sohn et al., 2016); indeed, various studies have revealed that forest stands with less dense canopies following thinning are often characterized by higher soil nutrients (Chase et al., 2016) and water availability for the residual trees (Coll et al., 2003; Kohler et al., 2010) and shrubs (Lookingbill et al., 2004; Wang, 2000).

Temperature and precipitation are other two key factors affecting the understorey species dynamics in forest ecosystems, especially in Mediterranean areas, where the vegetation copes with a generally high solar radiation and low water availability. High solar radiation implies high evapotranspiration, which leads to reduced water availability to plants (Pausas, 1994). On the other hand, low water availability (due to a combination of high temperature and low precipitation) hampers plant growth (Sardans and Peñuelas, 2013). A wide range of gradients of water availability and temperature can be found in Mediterranean montane areas, frequently over short distances, and are related to altitude. Altitudinal gradients have been successfully used as proxy of temperature (Krömer et al., 2013) and precipitation in Mediterranean forests (Coll et al., 2003). For instance, Coll et al. (2011) used site elevation as a proxy for site environmental conditions such as temperature and precipitation and found that it was negatively correlated with understorey shrub cover. Although studies focused on changes in the understorey shrubby vegetation cover (Coll et al., 2011) or diversity (Gracia et al., 2007; Salehi et al., 2013; Krömer et al., 2013) along an altitudinal gradient are still scarce, the ecological potential of altitudinal gradient has to be emphasized, in particular considering the unprecedented rise in temperatures we are experiencing nowadays (IPCC, 2021). Collecting information regarding vegetation composition along an altitudinal gradient might help in forecasting future change in the vegetation dynamics.

Soil nutrients also impact plant growth and are responsible for changes in understorey vegetation composition (Hutchinson et al., 1999), abundance and diversity (Hart and Chen, 2006). For instance, Nie et al. (2019) found that the soil properties (including available and total N, P, K, soil organic carbon, cation exchange capacity, soil texture), were more important than topography or landform in influencing understorey vegetation biomass in a subtropical hilly region. The assimilation of soil nutrients can be facilitated by the presence of belowground fungal communities, in particular mycorrhizal fungi (Amaranthus and Perry, 1987). Several studies in Mediterranean plant communities have observed that mycorrhizal fungi play an important role in the capacity of plants to take up nutrients (Martínez-García et al., 2011), like for example, P (Bell and Adams, 2004; Adamo et al., 2021). Although various studies have demonstrated that the role of mycorrhizal fungi in dry and nutrient-poor Mediterranean ecosystems is critical to improve the drought resistance capacity of plants (Sardans and Peñuelas, 2013), we still lack information related to the importance of soil fungal community regarding understorey vegetation composition.

In view of these premises, a proper understanding of understorey community dynamics is vital to address concerns for biodiversity conservation and forest management within the context of climate and land-use change. In this study, we tried to unravel the main above and belowground drivers shaping understorey species community composition and diversity (including shrubs and not dominant tree species, i.e., understorey woody species) in a Mediterranean *Pinus pinaster* forest. Maritime Pine (*Pinus pinaster* Ait.) is one of the most important and widespread forest species in Southwestern Europe (Alía et al., 1996). In Spain, *P. pinaster* constitutes pure and mixed woodlands that are both natural and planted (Bogino and Bravo, 2008). Traditionally, *P. pinaster* in Spain has played an important role in resin production, restoration programs and protection from erosion, with wood production as secondary objective (Bravo-Oviedo et al., 2007).

Our experiment was composed of 24 plots (paired: 12 control – 12 thinned) along an altitudinal range of 400 m and along a thinning intensity gradient from light (20–30 %) to medium (35–50 %) to heavy (55–70 %) in stand basal area removal. In each plot we also measured soil physical and chemical properties as well as gathered information on the soil fungal community. We hypothesized a gradual increase in the understorey species cover along the thinning gradient (from light to heavy thinning) (Thomas et al., 1999) due to the increased light and nutrient availability. Also, considering that this is a Mediterranean forest, subjected to summer drought, we expected temperature and precipitation to be key factors in explaining a shift in the understorey composition and diversity (Scarascia-Mugnozza et al., 2000; Rodriguez-Garcia et al., 2011). Finally, we thought that the soil properties might also play an important role; thus, nutrient reduction might have consequences on the survival of plants (Bedford et al., 1999). Our aims were to (a) identify the main drivers shaping understorey shrubs and non-dominant tree community structure among silvicultural treatments, topography, soil fungal community and soil properties and describe their relative importance; (b) describe how the main drivers influence understorey composition and diversity and (c) identify the specific response of understorey plant species to the altitudinal gradient. Our overarching goal was to provide insights into short to medium-term changes in the understorey community in relation to silvicultural treatment, topography, soil fungal community and soil properties, enabling better prediction of future ecosystem responses in Mediterranean pine forests.

2. Materials and methods

2.1. Study site

The study was carried out at a long-term experimental setup located at the natural site of national interest of Poblet (PNIN, Northeast Spain, 41°21′6.4728″ latitude and 1°2′25.7496″ longitude), where 12 pairs of unthinned and thinned plots (24 plots in total) were established in 2008 and 2009, respectively, to test forest thinning effects on different ecosystem features (Fig. 1) (Bonet et al., 2012). The plots consist of 65-year-old even-aged, planted *P. pinaster* stands, with isolated *Q. ilex* trees. These trees do not have a commercial value for wood production, but the area is used for recreation and mushroom collection. Various ungulates are present, in particular roe deer (*Cervus elaphus hispanicus*) and wild boar (*Sus scrofa*). However, all plots (thinned and un-thinned) are fenced and thus, there was minimal influence of domestic and wildlife grazing activity. The distribution of the plots was designed to avoid spatially driven differences so that the geographical distance between the location of the plots at lower and higher altitudes was similar (mean geographical distance and standard deviation between plots at low altitude: 1133 m ± 661 and at high altitude: 1305 m ± 928).

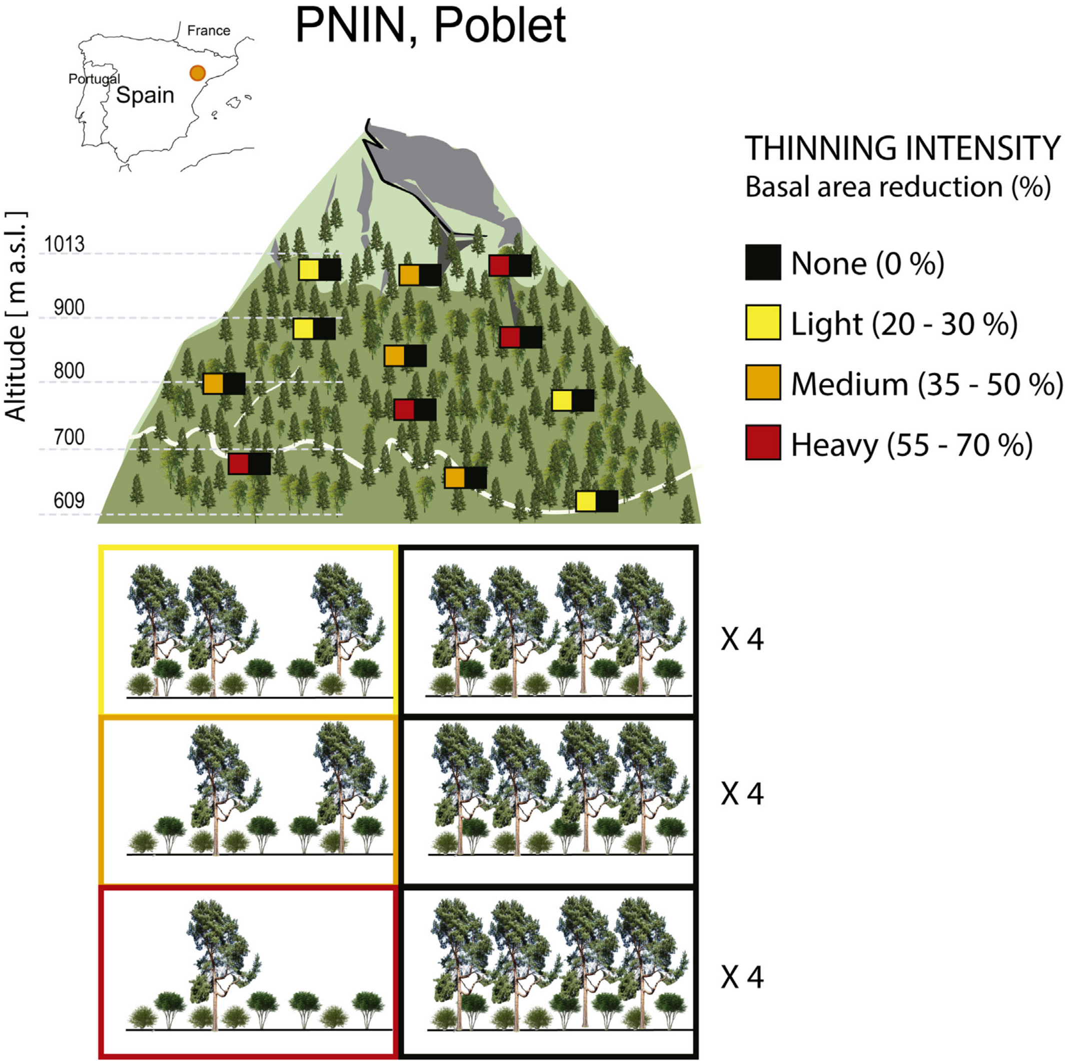

Fig. 1. Top-left corner Map of Spain showing the location of the natural site of national interest (PNIN) in Poblet (Catalunya, Spain). In the center Distribution of the 24 plots (paired: 12 control – 12 thinned) along the altitudinal gradient. The plots are highlighted according to the thinning intensity showed on the bottom: light thinning (yellow squares), medium thinning (orange squares), heavy thinning (red squares) or no thinning (black squares).

In the study site, the lowest temperatures are recorded in winter, and particularly in Jan. (monthly averaged temperature: 5.1 °C) while the highest during summer, with a peak in Jul. (monthly averaged temperature: 23 °C). Precipitation is scarce in winter (monthly averaged precipitation from Dec. till Mar.: 38 mm) and almost absent in summer (monthly averaged precipitation from Jun. till Sept.: 28 mm). In autumn (monthly averaged precipitation of Oct. and Nov.: 78 mm) and spring (monthly averaged precipitation of Apr. and May: 67 mm), the precipitation slightly increases (Fig. A.1). The lack of precipitation and the high temperatures experienced in summertime result in a pronounced summer drought that usually lasts for three months (Jun. to Aug.). Mean annual precipitation slightly increases from the bottom (530 mm year^{-1}) to the top (630 mm year^{-1}) of the experimental area, with a particularly pronounced shift in Nov., when the plots located around 900–1000 m present a mean rainfall of 90 mm and the ones located around 600–700 m reach 75 mm. Yearly mean temperature across the plots tends to decrease with altitude with a difference of 2 °C between the lowest and the highest plot (Fig. A.2).

2.2. Thinning experiment

Initially, 12 un-thinned control plots of 100 m^2 (10 m × 10 m) were established in 2008 in a forest area of approximately 300 ha (Fig. 1). For each of them, a paired plot was established in 2009 and thinned on the same year. The average distance between paired plots was of 50 m. Each thinned plot was 1600 m^2 in area (40 m × 40 m) with a central 100 m^2 (10 m × 10 m) sampling area, to reduce edge effects. In these thinned plots, three different overstorey thinning intensities in stand basal area were applied (light: 20–30 % thinned, medium: 35–50 % thinned, and heavy: 55–70 % thinned). Both thinned and un-thinned plots had a homogeneous stand structure, with even tree heights and diameters within plots. In thinned plots, trees were cut with a chainsaw and removed from the plot without using heavy machinery to avoid confounding effects caused by soil disturbance. In the control plots (and in the plots prior to thinning) the basal area ranged between 31.1 and 87.7 m^2 ha^{-1} with an average of 46 m^2 ha^{-1}, while the stand density ranged between 688 and 2657 trees ha^{-1}, with an average of 1257 trees ha^{-1}. The most intense thinning resulted in a remaining stand basal area of 16.5 m^2 ha^{-1} and a stand density of 350 trees ha^{-1}, whereas the greatest remaining stand basal area in the control plots was 81.7 m^2 ha^{-1} (2552 trees ha^{-1}). Further information about the thinning treatments and the stand variables before and after the treatments is available in Bonet et al. (2012). In 2009, at the same time of the overstorey thinning, the understorey vegetation was removed from the 12 thinned plots. The understorey clearing was repeated in 2016, this time without thinning the overstorey. The understorey clearings were part of another experiment that was partially overlapping the sampling area of this experiment (see Bonet et al., 2012). Previous research showed that the understorey clearing did not affect significantly the understorey community (i.e. herbaceous and shrub species) considering that it neither kills belowground portions of shrubs, that are able to resprout, or damages the bud bank (e.g. Fernandez et al., 2015). For these reasons, we considered that the understorey vegetation growing on the cleared plots was representative of the one growing on the uncleared plots. However, we are aware that the removal of understorey vegetation on the thinned plots might have had an impact on the understorey species abundance, richness and composition, in particular for the presence of non-dominant trees with a diameter lower than 20 cm. Thus, to clearly disentangle the overstorey thinning effects, avoiding potential overlapping effects between thinned (and cleared) plots and control (unthinned and uncleared) plots, we analyzed the changes in understorey cover using the categorical variable “intensity of thinning” (control, light, medium, heavy thinning) and the continuous variable “Basal area removed (%)”. When referring to thinned vs. control plots, the overlapping effect of overstorey thinning and understorey clearing has been highlighted in the discussion. A diagram of the experimental design is provided in Fig. 1.

2.3. Understorey data

The inventory was restricted to herbaceous perennial plants, woody shrubs, and trees with diameter at breast height lower than 20 cm, that did not belong to the dominant overstorey layer (i.e. 65 years old *P. pinaster* trees with isolated *Q. ilex*). The inventory did not include annual herbaceous plants. The understorey was monitored during October 2018, at the end of the plant growing season. In each plot, the number of individuals and the cover of all understorey species was recorded in a sampling area of 100 m^2 (10 m × 10 m). The percentage cover of all understorey plants was visually estimated always by the same person. A total of 33 understorey species was identified (Table A.2).

2.4. Topography

The 24 plots (paired: control – thinned) were distributed along an altitudinal range of 400 m, the lower plot being located at 609 m and the higher at 1013 m. The aspect was measured in degrees ranging between 0° and 360°. The degrees were converted to radians dividing the degrees by 180 and then multiplying the radians by π. Finally, the cosine function was applied to obtain the “northness” variable (from now on Aspect) which ranges between 1 (due north) and −1 (due south) with zero being neither north nor south in aspect (east or west).

2.5. Soil physical parameters

At the time of monitoring, four soil cores (12-cm deep and 5-cm in diameter) were randomly sampled with a corer in each plot to determine soil physical and chemical parameters. The soil samples were analyzed in the laboratory using the methodology described by Alday et al. (2012). Each sample was air-dried and then sieved (≤2-mm mesh). Soil texture was analyzed (i.e., clay, sand and lime proportions) using the Bouyoucos method (Day, 1965). We determined soil characteristics using the following techniques: soil pH using a pH meter in a 1:2.5 soil: deionized water slurry (Allen, 1989), total nitrogen (N) content using the Kjeldahl method (Bremner and Mulvaney, 1982), available phosphorus (P) content using the Olsen method (Olsen and Sommers, 1982); and, finally, total soil organic matter (OM) and total carbon content using loss-on-ignition (LOI) method (Nelson and Sommers, 1996). In general, soils were siliceous with sandy loam texture and a neutral pH (6.7 ± 0.6). More information about the soil physico-chemical properties together with the silvicultural and topographic characteristics of the study area can be found in Table 1.

2.6. Fungal community

Soil fungal community data was obtained from a previous study (Castaño et al., 2018) in which fungal communities were sampled in November 2009, 2012, 2013 and 2014. Each year, eight soil cores (12 cm deep and 5 cm in diameter) were systematically sampled with a corer in each plot. In these samplings, needles and partially decomposed needles were excluded to reduce the sampling of needle-associated saprotrophs (Clemmensen et al., 2013; Vořiškova et al., 2013), whereas humus and mineral soil were sampled together. The eight soil subsamples per plot were pooled in the field and the mixed sample was stored at 4 °C for <24 h before being stored at −20 °C. Each sample was lyophilized, sieved through a 1-mm mesh sieve, and then homogenized using a pestle and mortar to form a fine powder that was stored in a dark and dry place before DNA extraction. The soil samples were profiled by using high-throughput sequencing of fungal-specific internal transcribed spacer region 2 (ITS2) markers. The ITS2 sequences were quality filtered and clustered using UNITE (Abarenkov et al., 2022), to obtain approximate species-level OTUs, referred to as species hypotheses. A fungal community matrix with relative abundances of each fungal species hypothesis (i.e. OTU) in each sample was obtained (See Castaño et al. (2018) for detailed information). Since the fungal community data comprised various years and the last sampling was conducted 4 years prior to the understorey vegetation sampling, we used in this study the fungal core community for each plot; defined as the more stable group of fungi that keeps similar values across time. The soil fungal core community was obtained by fitting a redundancy analysis (RDA, see statistical analyses section for references) over the fungi data matrix, restricting the fungi data matrix across time. Afterwards, we extracted the residuals of the RDA ordination to get the variance not constrained by time, and applied a new RDA over the residual variance matrix to define the most constant and common fungal species per pairs of plots.

2.7. Statistical analysis

Statistical analyses were implemented in the R software environment (4.0.5, R Development Core Team 2021) with the following packages: VEGAN v.2.5-6 (Oksanen, 2015) for multivariate analyses, CORRPLOT v.0.84 (Wei and Simko, 2016) to compute correlation values, TIDYVERSE

Table 1
Silvicultural, topographic and soil characteristics in the natural site of national interest (PNIN) of Poblet, Northern Spain.

Driver	Variable	Units	Min	Max	Mean + SD
Silvicultural treatment (thinning of the overstorey tree cover)	Reduction of the stand basal area	%	0	68.87	21.5 ± 25
Topographic variables	Slope	%	3	23	17.1 ± 6.8
	Altitude	m	609	1013	797 ± 142
	Aspect		−0.7	1	0.5 ± 0.6
Soil physical properties	Clay content	%	9.1	19	14 ± 2.3
	Silt content	%	16.8	32.2	26 ± 3.7
	Sand content	%	37.6	60.7	45 ± 5.7
Soil chemical properties	pH		6.12	7.3	6.7 ± 0.3
	P	mg kg^{-1}	2	21	7.25 ± 4.7
	N	%	0.11	0.31	0.2 ± 0.06
	C:N ratio		8.79	19.7	14.8 ± 2.9
	SOM	%	2.95	10.53	5.6 ± 2.0

Abbreviations: SD, Standard Deviation; P, Phosphorus; N, Nitrogen; C:N ratio, Carbon: Nitrogen ratio, SOM, Soil Organic Matter.

v.1.3.0 (Wickham et al., 2019) for plot editing and GEOSPHERE v. 1.5-14 (Hijmans Robert, 2021) to compute distances for angular (longitude/latitude) locations. In all compositional analyses, the cover matrix was log transformed, while the fungal matrix was Hellinger transformed.

To test the relative importance of silvicultural treatment, topography, soil fungal community and soil properties in shaping understorey community structure in the 24 plots we used the variation partitioning approach ("varpart" function). In particular, the topography of the area was represented by altitude, slope and aspect, the silvicultural treatment by the basal area reduction after the thinning made in 2009, the soil physico-chemical characteristics by pH, sand content, P, N, SOM, and soil fungal community by the first 2 axes of the RDA obtained considering the abundance matrix of fungal species. To avoid spurious estimates of model coefficients caused by collinearity among predictors, we tested the correlation coefficients and *p*-values between the predictor variables within each group (silvicultural treatment, topography, soil fungal community and soil properties) and we excluded the variables that were significantly correlated. In the variation partitioning, we used adjusted R^2 to evaluate the contribution of each fraction (Peres-Neto et al., 2006). Through partial RDAs, we used isolated independent fractions of the variation partitioning analysis and assessed their significance with permutation-based ANOVAs ("anova.cca" function).

To describe the understorey compositional differences between plots we used a Detrended Correspondence Analysis (DCA) over the understorey cover dataset. Since there were two main drivers shaping the understorey compositional response (i.e., altitude and silvicultural treatment) we used two approaches: we fitted passively over the DCA ordination an altitudinal surface model using Generalized Additive Models (GAMs; 'ordisurf' function) and we overlaid the grouping data according to the presence and absence of thinning, and according to the thinning intensity ("ordiellipse" function). Based on the altitudinal surface model, we grouped the 4 paired plots that were located above 870 m (plot 301, 302, 303 and 304 and their control plot) and defined them as "high-altitude understorey community", while all the other plots were grouped together and defined as "low-altitude understorey community". Furthermore, to double-check the significance of the altitude × silviculture interaction we used a multivariate permutation procedure (function adonis2) considering the understorey vegetation cover in relation with a 4 levels interaction variable which represented low-high community and presence-absence of thinning. Afterwards, new ordination analyses were done separately for each community (high- and low-altitude community), grouping the plots according to presence and absence of thinning and according to the thinning intensity. Keeping the data separated in the two communities (high- and low-altitude community), we checked using linear regressions if the cover of any of the 33 understorey species was significantly affected by the thinning intensity (using the continuous variable "Basal area removed", in %). In these linear regressions, to disentangle thinning effects from understorey removal effects we analyzed only the subset of thinned plots, excluding the control plots. Considering the small sample size, the results obtained using the parametric ANOVA have been considered with caution.

Finally, considering the low-altitude and the high-altitude communities together we estimated species richness (S), evenness (J) and species abundance (N) of the understorey plant community and we tested if these indexes were affected: i) by the presence and absence of thinning running a paired *t*-tests (paring the control and the corresponding thinned plot), ii) by the thinning intensity running an ANOVA (considering S, J and N as dependent variables and the thinning intensity as an explanatory variable and running an ANOVA for each diversity index) and iii) by the altitudinal gradient performing linear regressions. To check if the altitudinal gradient affected the thinned and the control plots differently, separate linear regressions were fit to control and thinned plots, for each of the diversity indexes.

3. Results

In total, 33 understorey plant, shrub and not-dominant tree species were found in the 24 sampled plots: 29 species in the thinned plots (21 species in the light thinned, 17 species in the medium thinned, 23 species in the high thinned plots) and 23 species in the control plots. The five most frequent species were *Quercus ilex* (22/24 plots), *Lonicera implexa* (16/24 plots), *Arbutus unedo* (15/24 plots), *Ligustrum vulgare* (15/24 plots) and *Crataegus monogyna* (13/24 plots). *Quercus ilex* and *Arbutus unedo* were also the most abundant species within the plots. Conversely, *Viburnum tinus* and *Cistus salviifolius* were present just in 7/24 plots, each, but within these plots they were quite abundant, covering on average the 10 % of each plot (Fig. 2).

3.1. Main drivers of understorey plant communities

Silviculture (i.e., overstorey thinning intensity) explained the highest amount of variance of the understorey species cover (ca. 18 %), followed by topography (ca. 16 %; Fig. 3a). Conversely, soil physico-chemical properties, soil fungal community and the shared variations between pairs of variables explained a residual non-significant amount of variance (see Table A.1 for *p*-values). By removing the non-significant fungal community from the variation partitioning (Fig. 3b) the proportion of variance explained by silvicultural variables slightly decreased, while the variance explained by the topography slightly increased, overcoming the effect of silviculture. The soil variables and the shared variation between the drivers were not significantly contributing to explain understorey community variance. When excluding topography and considering just the other three drivers, the only significant variable was silviculture, explaining 10 % of the variance (not shown in Fig. 3). Finally, removing fungal community and the non-significant soil physico-chemical variables (Fig. 3c), we noticed that the topographical variables explained the highest amount of variance in the understorey community (ca. 21 %, Fig. 3c), while the silvicultural variables explained just ca. 8 % of understorey variance.

Interestingly altitude was positively related with OM ($F_{1,22} = 0.008, p = 0.003$), N ($F_{1,22} = 0.349, p < 0.001$) and P ($F_{1,22} = 0.908, p = 0.011$) (Fig. A.7 – graphs on the right), conversely the soil chemical properties did not present a significant trend along the thinning gradient (Fig. A.7 – graphs on the left).

3.2. Understorey plant species composition: thinning effects and altitude

The vegetation ordination using DCA produced eigenvalues (λ) of 0.41, 0.27, 0.23 and 0.19 and gradient lengths (GL) of 3.27, 2.6, 1.91 and 1.60 for the first four axes. The surface model of altitude fitted significantly over this DCA ordination result, showing that altitude increased from left to right of the first axis, going from 600 m to 1000 m. (Fig. 4 a, b). Most of the sites were located on the left part of axis 1 (DCA1) and were related with the majority of the species such as *Arbutus unedo*, *Rosa canina*, *Cistus salviifolius* and *Cistus albidus*. The remaining sites were located at higher altitudes (above 870 m), corresponding to the right part of axis 1 (DCA1). Here we found species such as *Quercus* spp., *Acer monspessulanum* and *Clematis vitalba*. Interestingly, the overall distribution of species along the altitudinal gradient showed that species like *Ruscus aculeatus* and *Juglans nigra* were mainly present at lower altitudes (600–700 m left-end DCA 1), species like *Pistacia terebinthus* or *Crataegus monogyna* at mid altitude (750–800 m center) and species like *Quercus faginea* and *Lonicera implexa* at higher altitudes (900–1000 m right-end DCA1). These patterns were not driven by differences in the geographical distance of the plots, as the distance between the high-altitude plots and the low altitude plots was not significantly different.

By comparing control versus thinned plots in the ordination space (Fig. A.3 a, b) we could identify how they were separated, with thinned plots located mostly on the top-left and control plots mainly on the bottom-right. The silvicultural treatment (presence - absence of thinning) was found to significantly affect the understorey composition, explaining 9 % of the variance (Fig. A.3 a, b; $R^2 = 0.09$, $p = 0.04$). Conversely, thinning intensity did not significantly impact the understorey vegetation community (Fig. A.3 c); $R^2 = 0.15$, $p = 0.288$). The high β-diversity of the plots within different treatments limited the possibility to find

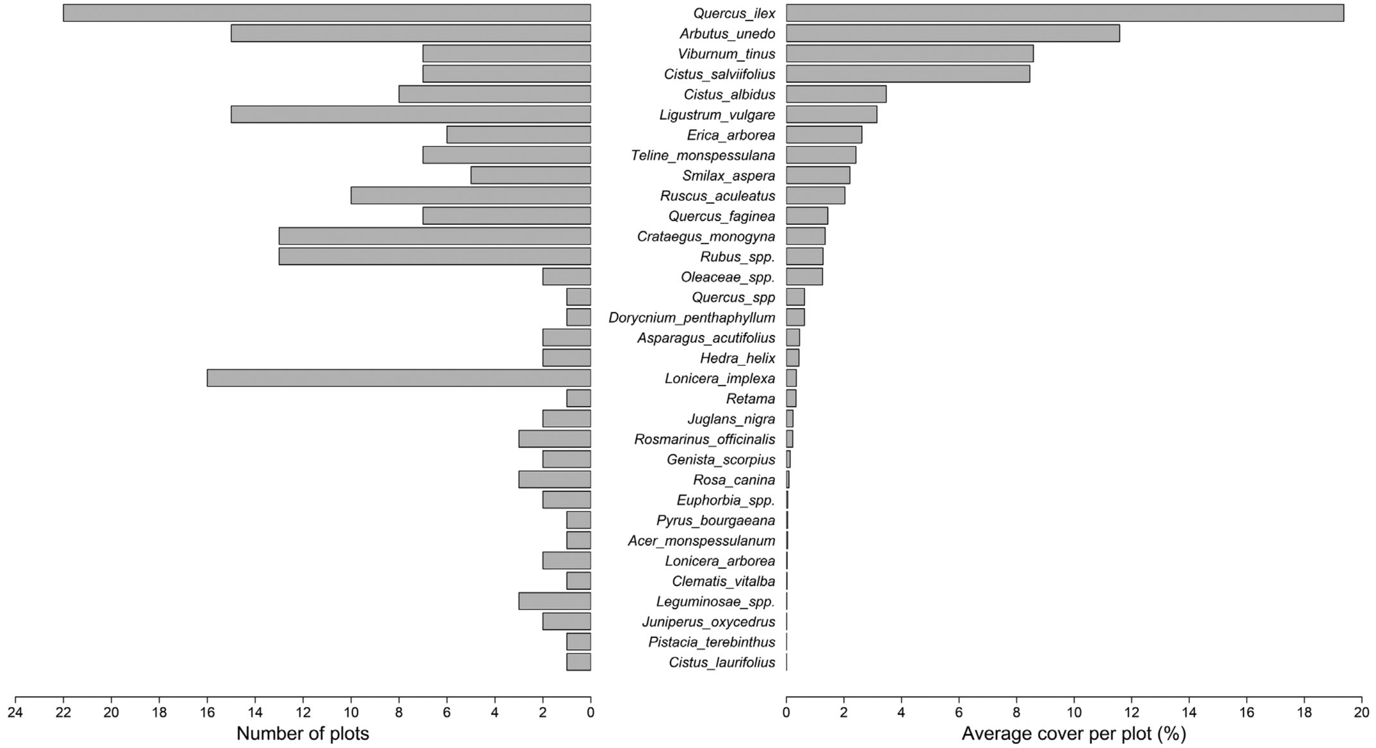

Fig. 2. Abundance (bar chart on the left) and average cover (bar chart on the right) of the 33 understorey shrub and not-dominant trees of the study area in the natural PNIN in Poblet (Catalunya, Spain).

significant differences between different treatments. Moreover, when splitting the cover data in light, medium, and high thinning intensity, and analyzing the matrices separately, no significant thinning effect was found ($p > 0.05$). Interestingly, most of the thinned plots grouped together in the middle of Fig. A.3 a, however, 3 of them were located on the bottom-right part of the figure. The 3 thinned plots on the bottom right were located at an altitude of 1013 m, 903 m and 879 m, representing 3 of the plots located at higher altitudes.

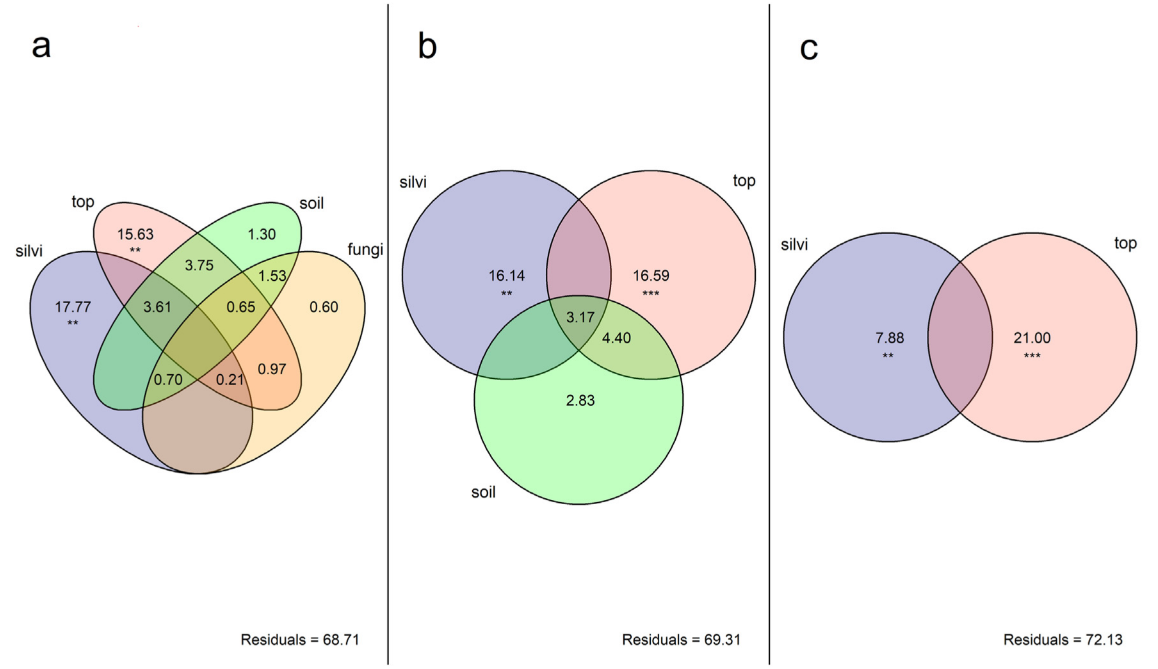

Fig. 3. Variance partitioning analysis of understorey cover data matrix in the 24 plots, explained by 4 groups of explanatory variables: silvicultural variables (silvi), topography related variables (top), soil physico- chemical variables (soil) and soil fungal community variables (fungi). a) All groups of explanatory variables included, b) three groups except fungi and c) only silvicultural treatment (silvi) and topography (top) included. All values represent the fraction of variance explained, however, the variation is partitioned into pure variation explained by each group of parameters, shared variation with the other groups, and unexplained variation (residuals). Asterisks indicate that a significant amount of variation is explained by the given fraction (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$). Values <0 not shown.

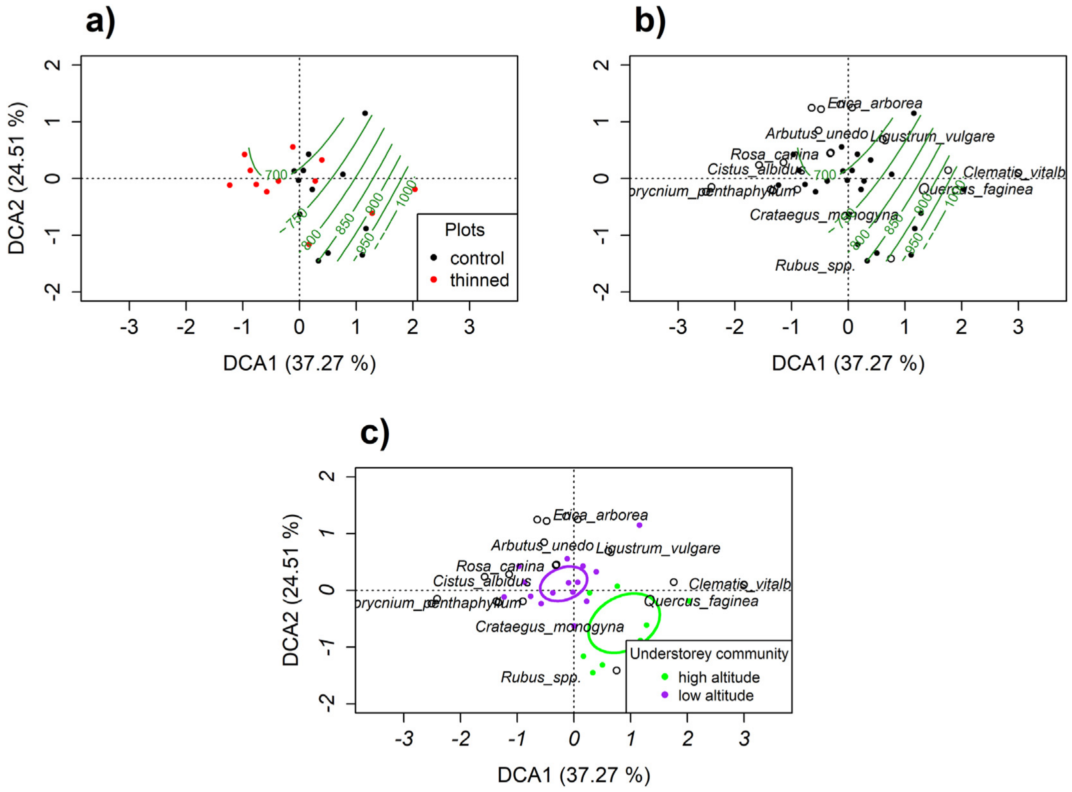

Fig. 4. DCA biplots for the first two axes of understorey shrubs compositional data in the 24 plots in the natural area of PNIN-Poblet (Northeast Spain). DCA axes 1 and 2 explained 37.27 % and 24.51 %, respectively, of the total variance. a) Site biplots in black (control) and red (thinned) overlain by a surface model of altitude, b) the most abundant understorey species biplots overlain by a surface model of altitude, c) site biplots showing high-altitude (green dots and SE ellipse) and low-altitude understorey community (purple dots and SE ellipse). The white dots with black circumference correspond to the location of the understorey species. Complete names of understorey species are listed in Table A.2.

3.3. Thinning effect on understorey plant species composition by altitude

The DCA ordination overlapping two different high- and low-altitude understorey communities showed that they are significantly different in term of composition (Fig. 4c; $R^2 = 0.19, p = 0.001$) with low-altitude communities located on the left of axis 1 (toward the negative end) with, among the others, *Rosa canina*, *Cistus albidus* and *Arbutus unedo* as associated species, while high-altitude communities located on the right of the first axis with *Quercus faginea*, *Quercus ilex* and *Clematis vitalba* as associated species. Since we found a clear silvicultural × altitude interaction ($R^2 = 0.32, p = 0.001$), with thinning effect being significant on only the low altitude community, but not on the high altitude one, hereafter, we decided to split the data in “high-altitude” and “low-altitude” understorey communities.

Considering the “low-altitude” and “high-altitude” understorey communities separately, we found that the low-altitude understorey community presented significant differences not just between thinned and control plots ($R^2 = 0.18, p = 0.01$; Fig. 5a and b) but also among thinning intensities ($R^2 = 0.33, p = 0.016$; Fig. 5c and d), although only the understorey composition of heavy thinned plots was significantly different from controls. Conversely, the high-altitude community was neither affected by the thinning presence or absence ($R^2 = 0.08, p = 0.648$) (Fig. A.4a) or by thinning intensities ($R^2 = 0.46, p = 0.343$) (Fig. A.4b).

Furthermore, we checked for possible species changes along the thinning gradient (light, medium, and heavy) over the two understorey communities. In the low-altitude understorey community, thinning favored *Cistus albidus* [$F_{1,6} = 5.55, p = 0.05$], with a significantly higher cover value in thinned plots (Fig. A.5b). Conversely, the cover of *Arbutus unedo* significantly decreased [$F_{1,6} = 12.85, p = 0.015$] with increasing % of trees basal area removed (Fig. A.5b). All the other species presented no significant cover change (Table A.2). In the high-altitude understorey community, no species presented a significant cover change along the thinning gradient (Table A.2).

3.4. Understorey species diversity responses to altitude and thinning

Overall, we found a greater number of species at lower altitudes, with on average >15 species in the plots around 600 m and <10 in the plots around 1000 m. Along the altitudinal gradient, a significant decrease in richness both in the control plots ($F_{1,10} = 17.51, p = 0.001$) and thinned plots ($F_{1,10} = 19.87, p = 0.001$) was found (Fig. A.6a). Similarly, we detected a decrease in the total number of individuals along the altitudinal gradient both in control ($F_{1,10} = 15.04, p = 0.003$) and thinned plots ($F_{1,10} = 29.74, p < 0.001$), with the plots around 1000 m having a third of the individuals compared to the plots around 600 m (Fig. A.6b). Evenness presented values that ranged between 0.5 and 0.9 and was not significantly affected by the altitude neither in the control plots ($F_{1,10} = 0.95$, $p = 0.352$) or in the thinned plots ($F_{1,10} = 0.19, p = 0.671$).

Thinning (presence - absence) significantly increased the understorey species richness ($df = 11, p = 0.003$) and total number of individuals ($df = 11, p = 0.005$) with, on average, three species and 32 individuals more in thinned plots than control plots. Conversely, the thinning did not affect the evenness ($df = 11, p = 0.6$). Moreover, the intensity of thinning (reduction in stand basal area %) did not affect understorey richness ($F_{1,22} = 2.32, p = 0.142$), total number of individuals ($F_{1,22} = 3.76, p = 0.06$) or evenness ($F_{1,22} = 0.68, p = 0.419$).

4. Discussion

Our results show that the understorey composition seems to be primarily affected by topography and silvicultural treatment. The altitudinal gradient reflected a strong decrease in the abundance and richness of the understorey species with the low-altitude understorey community including >30 species, while the high-altitude community including just a small set of 13 species. The decrease in both species richness and abundance of

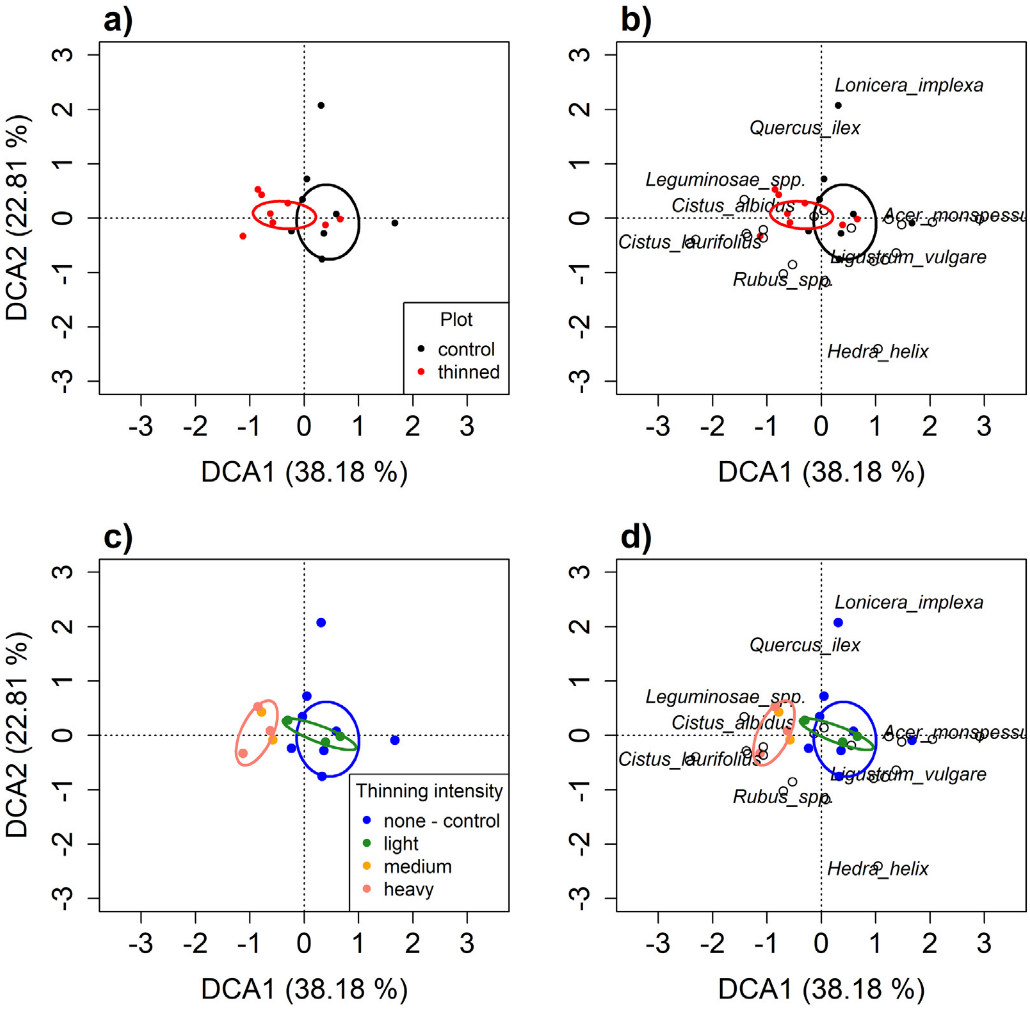

Fig. 5. DCA biplots for the first two axes of understorey shrubs compositional data in the 8 paired plots at low-altitude (below 870 m) in the natural area of PNIN-Poblet (Northeast Spain). DCA axes 1 and 2 explained 38.18 % and 22.81 %, respectively, of the total variance. a) Sites biplots with control (black) and thinned (red) factors overlapped by the SE deviational ellipses. b) Species biplots showing the most abundant understorey species and site SE ellipses. c) Sites biplots with thinning intensities, light (green), medium (yellow), heavy (salmon) and control plots (blue) overlapped by the SE deviational ellipses. d) Species biplots showing the most abundant understorey species and 3 SE ellipses. In panels c and d the ellipse of the medium intensity plots is not reported due to the presence of just 2 plots subjected to that treatment. The white dots with black circumference correspond to the location of the understorey species. Complete names of understorey species are listed in Table A.2.

the understorey community along the altitudinal gradient can probably be explained by the change in the microclimate (Sporn et al., 2009). At higher altitudes the amount of precipitation slightly increases, suggesting a higher availability of water for plants that would be also beneficial to cope with the evapotranspiration. However, here, the minimum winter temperature drops under 0 °C during January (between −1.2 and −0.3 °C) and February (between −1.7 and − 0.7 °C) while at lower altitudes the minimum temperature in the same period is ca. 1 °C higher, oscillating between −0.1 and 1.32 °C in January and between −0.2 and 1.05 °C in February. Thus, the niche at high altitude might be limited by temperature rather than by light (and resources), compared to the niche at low altitude, and therefore be less affected by the thinning treatment. This is in line with other studies that have found that freezing temperatures are an important limiting factor for plant presence and performance (e.g. Sakai and Larcher, 2012). Thus, the variation in the microclimatic conditions along the altitudinal gradient might have induced filtering of species and the creation of ecological niches with consequences for the composition and diversity of understorey species communities (Polechová and Storch, 2018).

In parallel, we noticed that, in both high and low altitude plots, the richness and the total number of individuals were generally higher in the thinned plots compared to the control plots highlighting that thinning created the conditions for the coexistence of more individuals and more species. Several studies have reported similar results where thinning altered understorey vegetation and increased plant species richness (Parker et al., 2001; Thysell and Carey, 2001; Chan et al., 2006; Metlen and Fiedler, 2006; Dodson et al., 2008; Ares et al., 2010). However, we could not differentiate the effect of overstorey thinning and understorey clearings when comparing control vs. thinned plots (e.g., Fig. 5 a and b). In these cases, we need to address the thinned plots as plots that underwent a combination of overstorey thinning and understorey (repeated) removal. Conversely, we could isolate the effect of the overstorey thinning when focusing on changes in understorey composition due to the thinning intensity (e.g., Fig. 5 c and d) or on the changes in the cover of a specific understorey species due to the % of basal area removed (Fig. A.5). In these cases, was clear that overstorey thinning played a role on the understorey vegetation, independently from the possible interference with the understorey clearings. The regression lines in Fig. A.5 show that although the understorey vegetation was cleared from all the plots with % of basal area removed major than 0, there were differences in the reaction of the understorey according to the different thinning intensities. Consequently, the differences found for *A. unedo* and *C. albidus* in the low altitude thinned plots could confidently be attributed to the overstorey thinning. Without performing the understorey clearings, the cover, and the abundance values of the understorey plants in the thinned plots would have been probably slightly higher, in particular for the small trees belonging to the understorey layer.

Interestingly, the effect of thinning on the understorey community composition depended on the altitude of the site (thinning × altitude interaction). In fact, only after sub-grouping the plots according to altitude, the silvicultural treatment was found to be significant for the understorey community composition of the plots located between 609 m and 870 m, but not for the plots at higher altitude. In the thinned plots at low altitudes, the woody community significantly changed to adapt to the increased light availability. Thinned plots favored the growth of early-serial species like *C. albidus*, that represented <5 % (each) of the cover in control plots, while gradually increasing its presence along the thinning gradient until reaching ca. 10 % of the cover in heavily thinned plots. Interestingly, the cover of *A. unedo*, a Mediterranean shrub adapted to open conditions (Helluy et al., 2021), significantly decreased along the thinning gradient. This might be due to the competition with other early-serial species, that might have grown faster. Similarly, Davis and Puettmann (2009) found that a release of early-serial species was evident in thinned stands by 5–7 years post treatment and Légaré et al. (2002) said that changes in the tree layer are likely to lead to changes in the ground vegetation (Brosofske et al., 2001; Légaré et al., 2001). Conversely to this statement, our results suggest that at higher altitude, there was no significant change in the understorey plant composition along the thinning gradient. This finding has to be interpreted with caution due to the small sampling size of the high-altitude community, however, this pattern could be explained considering a change in the understorey limiting factors along the altitudinal gradient. In fact, the main limiting factor at high altitudes does not seem to be light, nutrients, or water availability, but temperature instead. Exposure to sub-freezing winter temperatures might have hampered the growth of plants that at lower altitudes were able to flourish. Previous studies have shown that thinning might have increased not just light but also nutrients (Chase et al., 2016) and water availability (Osem et al., 2012). In our plots, soil nutrients were not affected by thinning intensity, suggesting that soil nutrients were not a limiting factor for understorey plant grow. Although in this study water availability was not measured directly, we can notice that the thinning encouraged the growth of *C. albidus* which is a semi-arid shrub species that can thrive in perturbed sunny areas (Allen, 2009) where competition by established plants is reduced (Pons, 2000). This suggest that light played a central role in the establishment of this *Cistus* species, while water availability probably was of secondary importance. Similarly, Zangy et al. (2021) found that in an East-Mediterranean conifer forests the change in understorey richness after overstorey thinning was strongly dependent on light availability as compared to a less definite dependency on water availability. These results have important implications for the conservation of the understorey communities, suggesting that, although there was a core community present along the altitudinal gradient, the understorey composition at low and high altitude presented significant differences. Thus, to preserve the ecosystem diversity the management plans should be tailored to account for the understorey differences.

Another notable result is that soil physico-chemical properties and soil fungal communities did not have a significant effect on understorey plant cover and explained a non-significant part of variance. Although the soil properties were very variable in space and found to be correlated with altitude, they were weakly correlated with the understorey structure, this being independent by the presence or absence of the topographical variables in the variation partitioning. Conversely, drivers like topography and silviculture had a stronger correlation with the vegetation, being able to explain together >30 % of the variance of the understorey composition. Our results are in line with those found by Mishra et al. (2013) that concluded that microclimate is more important than soil properties, when analyzing the same area. Furthermore, the fungal component has been repeatedly found to have no or small effects on understorey growth (West and Jones, 2000; Szefer et al., 2020). The increase of SOM, N and P with altitude might be explained considering that SOM is often positively related with mean annual precipitation (Hontoria et al., 1999) and in our plots, the mean annual precipitation is slightly higher at higher elevation (Fig. A.2). Thus, this might have positively affected the content of OM, as well as N and P.

In conclusion, in this study area soil properties and soil fungal communities were not determinant in shaping the understorey composition or diversity. Thus, to preserve the local understorey communities found in the PNIN of Poblet, as in similar Mediterranean forests, the silvicultural management should focus on the possible impact of the thinning according to the altitude or to the microclimate. At low altitude, absence of thinning or low thinning would allow the community to be preserved while medium and heavy thinning would increment the diversity of the understorey community inducing early successional species to grow with consequent compositional changes. This could reduce the conservation value of the understorey community at low altitude (Alday et al., 2021). Conversely, at higher altitude the actual understorey community would not be considerably affected by medium or heavy thinnings, allowing more flexibility in the choice of the more adequate management alternative to preserve the community structure. Furthermore, within a global warming scenario, our study could help to better understand the capacity of a Mediterranean forest to react to a potential increase in temperature and drought. In our study area, an increase in temperature (+ 0.5–1.5 °C) would probably affect the high-altitude community, characterized by its resistance to lower winter temperatures (Jan. and Feb. below 0°). The warmer microclimate would facilitate the colonization of the high-altitude area by the species located at lower altitudes, with a consequent homogenization of the local community along the altitudinal gradient and an increased, generalized response of the understorey community to silvicultural management. In a global warming scenario, we emphasize the role of forest management, and we suggest low-medium thinning as an approach to decrease water stress and to facilitate climate adaptation, at least in the short-term.

5. Conclusion

In this study, the understorey species composition dynamics have been found to be critically affected by topography and silvicultural treatment. Interestingly, the effect of thinning on the understorey community composition depended on the altitude of the site (thinning × altitude interaction). We suggest that along the altitudinal gradient there was a change in limiting factors, from light at low altitudes to winter freezing temperature at higher altitudes. These limiting factors have filtered the community creating different ecological understorey niches with a consequent different reaction to the silvicultural treatment (thinning intensity). Due to the high interconnection between the different environmental drivers and the understorey community we highlight the necessity of studying understorey diversity and composition considering a wide set of above- and below-ground drivers. This is crucial to have a deeper understanding on how the different factors interact and participate in changing the understorey community. From a management perspective, it seems clear that new/adaptative management approaches should consider topography and silvicultural treatment in the decisions plans, as well as consider the effect of warming global temperature on the vegetation. In any case, further research should be done regarding adaptative management approaches to preserve the structure of these ecosystems.

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CRediT authorship contribution statement

Giada Centenaro: Data curation, Formal analysis, Writing – original draft preparation. **Sergio de Miguel:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Writing – review and editing. **Laleh Amouzgar:** Data sampling, Data Curation. **Yasmine Piñuela:** Data sampling, Data Curation, Writing – review and editing. **Deokjoo Son:** Data sampling, Data Curation. **José Antonio Bonet:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Writing – review and editing. **Juan Martínez de Aragón:** Data sampling, Data Curation, Investigation, Writing – review and editing. **Svetlana Dashevskaya:** Writing – review and editing. **Carles Castaño:** Writing – review and editing. **Josu G. Alday:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Validation, Writing – review and editing.

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Data availability

Data will be made available on request.

Declaration of competing interest

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