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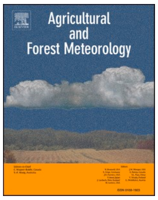

Intraspecific trait variation, growth, and altered soil conditions at tree species distribution limits: From the alpine treeline to the rear edge

Antonio Gazol [a,*], J. Julio Camarero [a], José M. Igual [b,c], Ester González de Andrés [a], Michele Colangelo [a,d], Cristina Valeriano [a]

[a] Pyrenean Institute of Ecology (IPE-CSIC), Zaragoza E-50059, Spain
[b] Institute of Natural Resources and Agrobiology of Salamanca (IRNASA-CSIC), Salamanca E-37008, Spain
[c] Unidad Asociada Grupo de Interacción Planta-Microorganismo (Universidad de Salamanca-IRNASA-CSIC), Salamanca, Spain
[d] School of Agricultural, Forest, Food and Environmental Sciences (SAFE), University of Basilicata, Potenza 85100, Italy

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ABSTRACT

Alpine treelines are expected to shift upward due to climate warming, whereas warmer conditions can have negative impacts on forests located near the xeric, equatorward limit of the distribution of tree species (rear edge). We compare tree populations forming the distribution limits of mountain pine (*Pinus uncinata*) in north-eastern Spain: two cold-limited alpine treeline populations, and a rear-edge drought-prone stand. In the treelines, trees were sampled in three altitudinal belts to evaluate within-site variations considering the forest, the transitional ecotone and the treeline.

Tree growth was markedly higher in one of the treeline sites (Tesso), particularly in the forest belt, as compared to the other treeline (Las Cutas), and to the warmest rear-edge stand. Tree growth in the rear-edge population depended on June precipitation. Trees presented also comparatively smaller leaves with higher Specific Leaf Area (SLA) there than in the two treelines. Regarding the differences between altitudinal belts within the treelines, treeline soils showed lower N concentrations and a lower content of sand than in forests. Soil microbiota was dominated by bacteria in the treeline and by fungi in the forest, although with differences between sites. Soil characteristics and microbial composition were strongly related, whereas its relationship with tree growth and functional traits was less clear.

The marked differences in leaf traits and growth response to climate found when comparing treeline and rear-edge sites highlights the biogeographical uniqueness of sites forming the equatorward distribution limit. The greater tree growth in the Tesso treeline as compared to Las Cutas indicate that interactions between climate and physical and chemical properties of the soil influence tree growth and its interactions with soil microbial communities in the treeline. A better understanding of plant-soil feedbacks may help to understand the future dynamics of tree populations forming the species' climatic and geographic limits of distribution.

1. Introduction

Mountains are present in all biomes and cover around of 12.3% of all terrestrial land area excluding the Antarctica (Körner et al., 2011). A common characteristic of mountains is the presence of steep environmental gradients over short distances which induce changes in vegetation composition and function (Körner, 2007). Since vegetation development is strongly contingent on temperature variations (cf. Körner, 2007), mountain forests are noticeably sensitive to rises in temperature because of climate change (Pepin et al., 2015; Hagedorn et al., 2020). Changes in vegetation and associated environmental variables such as forest productivity, tree growth, soil nutrient concentration and microbial composition in response to climate change are expected to be more evident in ecotones, because they represent transitional zones between different communities (Allen and Breshears, 1998).

The treeline ecotone, which is the temperature-controlled transition from the potentially forested area to the alpine or arctic tundra, represents an ideal natural setting to test ecological hypothesis (Körner, 2012). The cold-limited treeless environments located above the treeline

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* Corresponding author.
E-mail address: agazol@ipe.csic.es (A. Gazol).

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represent around 20% of the total mountain areas (Körner et al., 2011), but their extension might change due to climate change inducing upslope tree expansion and encroachment into grasslands (Harsh et al., 2009). These upward migrations can be accompanied by enhanced tree growth due to longer and warmer growing seasons (Camarero et al., 2015a, 2015b, 2021a). However, climate change might also limit treeline expansions by increasing drought sensitivity in seasonally water limited regions (González de Andrés et al., 2015; Lyu et al., 2019). Soil nutrient concentration can also limit tree growth in harsh treeline environments because low temperatures limit soil nutrient mineralization and acquisition (Ellison et al., 2019; Hagedorn et al., 2019). Nevertheless, some authors have stressed that nutrient shortages play a minor role in treeline dynamics as compared to climatic limitations (Fajardo and Piper, 2017). Treeline shifts would affect carbon storage, nutrient cycling and hydrological properties in mountains, with significant feedbacks on climate and human populations which depend on mountain water resources (Greenwood and Jump, 2014). Thus, further studies are required to understand how climate impacts tree growth in alpine treelines and how treeline shifts relate to changes in soil conditions.

Given that tree growth is limited by thermal and water constraints, the limitations posed to forest expansion by low temperatures upwards or polewards may transform into water limitations equatorwards (Babst et al., 2019). The result of the interplay between altitudinal and latitudinal gradients is that some tree species forming alpine treelines in Eurasia also find their southernmost distribution limit (rear edge) in the Iberian Peninsula (de Vries et al., 2015), where drought limits its growth (e.g., Galván et al., 2014). This is the case of mountain pine (*Pinus uncinata* Ram. ex DC.) that forms the core of its distribution area in the western Alps, Massif central and the Pyrenees, but which also present relict and isolated populations located in mountains of north-eastern Spain such as the Iberian System (Camarero et al., 2017, 2021b; González-Díaz et al., 2020). It is expected that such isolation may influence the performance of tree species in relict stands as compared to other interconnected populations as has been found for *Pinus cembra* in the Alps (Tóth et al., 2019). Being subjected to drier conditions, Spanish mountain pine rear-edge populations are susceptible to water deficit during summer (Galván et al., 2014), and so they may be more sensitive to the amplification of water deficit because of climate warming (Sánchez-Salguero et al., 2017). By contrast, mountain pine populations located in the Pyrenees, often forming the alpine treeline, may benefit from a longer growing season as a consequence of climate warming (Sánchez-Salguero et al., 2017). What remains unclear is whether trees in isolated rear-edge populations differ in functional characteristics from those trees located in interconnected, core treeline populations. It can be expected that isolation will result in a genetic differentiation potentially leading to legacies in the form of morphological traits adapted to couple with drier conditions in the case of relict, equatorward mountain stands (Tóth et al., 2019; González-Díaz et al., 2020).

While differences in tree performance and associated environmental conditions between isolated, rear-edge and interconnected core populations are conspicuous, potential variations along the treeline ecotone, i.e., from the closed forest to the alpine treeline, are less evident. Several local factors can affect the performance of trees along the treeline such as stand structure or tree age (Holtmeier and Broll, 2005; Camarero et al., 2017; Sánchez-Salguero et al., 2018) or the extent and duration of the snow cover (Sanmiguel-Vallelado et al., 2020). However, differences in tree growth do not necessarily have a direct link with differences in functional characteristics such as wood density or leaf shape and nutrient concentrations (Fajardo and Piper, 2017; Fajardo et al., 2018). Given that temperature is the most conspicuous factor limiting upward tree expansion of alpine treelines, we can expect differences in tree sensitivity to climate along the treeline ecotone also related with local changes in tree age and environmental conditions (Camarero and Gutiérrez, 2004; Batllori and Gutiérrez, 2008). In a context of treeline expansion, the higher in altitude a tree is sampled the closer it is to the treeline isotherm (Körner, 2021) and the lower is the time it has had to modify the soil conditions (Hagedorn et al., 2020).

In this context, as trees colonize areas formerly dominated by herbaceous and shrubby species, they might modify the soil conditions by altering nutrient availability and soil microbial composition (Benett et al., 2017). Plant-soil feedbacks play a fundamental role in the development of plant populations and communities (Bennett and Klironomos, 2019). This has important implications in sight of climate change as both, vegetation, and soil composition, can be influenced by climate change (Pugnaire et al., 2019). Climatic gradients are of primary importance to understand changes in plant functional traits as well as on plant-soil feedbacks (e.g., Rutten and Gómez Aparicio, 2018; Laughlin et al., 2021). In this respect, alpine treelines represent ideal settings to test how local climate change affects plant performance and associated changes in soil conditions (Mayor et al., 2017; Collins et al., 2018; Hagedorn et al., 2019).

Land-use changes and recent warming trends have enhanced the expansion of trees beyond the alpine treeline in several regions (Harsh et al., 2009), including the Spanish Pyrenees (Camarero et al., 2015a; Vitali et al., 2019). However, it is also expected that local microsite conditions can determine the performance of tree regeneration (Batllori et al., 2009), thus also affecting plant-soil feedbacks in complex ways (De Long et al., 2019). Changes in soil conditions can also be delayed with respect to treeline advances (Hagedorn et al., 2019), leading to lagged effects of vegetation on soil conditions (Heinen et al., 2018). Recently, it has been found that temperature influences plant root traits and the association between plants and fungi (Laughlin et al., 2021). Therefore, soil microbial communities may differ beneath trees located in the forest as compared to trees colonizing the alpine grasslands because of treeline expansion.

Here we study how trees grow and soil conditions shift along two *P. uncinata* alpine treelines located in the Pyrenees (north of Spain). Furthermore, we compare these treelines, located at the core of *P. uncinata* distribution range, with an isolated population forming the species rear edge in north-eastern Spain. We quantify how tree radial growth, intraspecific trait variations (wood specific gravity, leaf area, specific leaf area –SLA, leaf dry matter content), soil conditions and microbial biomass vary between sites and along the treeline ecotone. That is, we aim to understand how environmental differences determine tree performance when comparing sites subjected to contrasted climate conditions (i.e. between sites) but also when comparing the altitudinal gradient of the treeline ecotone (i.e. within sites). We expect that site differences will be more important than differences along the treeline ecotone in terms of tree performance. Thus, we expect that the rear-edge population may present functional characteristics adapted to a drier climate in comparison to the two well connected treelines (core populations) such as smaller leaves and higher Specific Leaf Area (hereafter SLA). Further, we expect temperature to limit growth in the treelines more intensely, whilst water shortage during summer to be more important in the rear-edge population. Despite such between site differences, we also expect soil conditions and tree growth to vary within each treeline site, i.e. along the altitudinal gradient of the treeline ecotone, reflecting the impact of local conditions on tree growth and its environment. Greater exposure to harsh environmental conditions may make recently recruited trees more sensitive to temperature at higher elevations than older individuals located downslope. Along this, less developed soils from higher elevations may have lower nutrient concentrations and different microbial composition than fungi-dominated soils from the low-elevation, long-established forests. Finally, we also explore the covariations between tree functional traits, growth, soil conditions and microbial biomass along the treeline ecotones.

2. Materials and methods

2.1. Study sites

We studied two treelines formed by *Pinus uncinata* Ram. ex DC. and located in the “Ordesa y Monte Perdido” and “Aigüestorters i Estany de Sant Maurici” National Parks (Las Cutas and Tesso, respectively) situated in the Central Spanish Pyrenees. These two sites are located at the core of *P. uncinata* distribution range (Camarero et al., 2021b), and in those sites treelines are shifting upwards (Camarero and Gutiérrez 2004; Camarero et al., 2017). In Las Cutas (42.63º N, 0.08º W, 2100–2110 m a.s.l.), the treeline is abrupt with strong differences in tree cover and height from the forest (ca. 5–10 m in height) to the treeline (ca. 2–3 m), located 10 m upslope (Camarero and Gutiérrez, 2004). The aspect of the site is south, and the slope is shallow (10–20º). Soils are rocky and mixed with calcareous and acid spots. In Tesso (42.58º N, 1.03º E, 2330–2360 m a.s.l.), the treeline is diffuse with a gradual decrease in tree cover and height (from ca. 15 to 2 m) as we move upwards from the forest to the treeline located 30 m upslope (Camarero and Gutiérrez, 2004). The aspect of the site is north-eastern, the slope is steep (25–30º) and soils are calcareous and developed on shales. Basal area values in the forest of Las Cutas and Tesso are 8.9 and 12.1 cm^2 ha^{-1}, respectively (Camarero and Gutiérrez, 2002).

The climate in the treeline sites is continental with mean annual temperatures ranging between 3º and 5º C (mean maximum and minimum temperatures range 18–20 ºC and −10–−7 ºC, respectively) and total annual precipitation is ca. 1200–1660 mm (Camarero and Gutiérrez, 2002, 2004). Maximum snow cover may range from 0.5 to 1.5 to 1.5–3.5 m in Las Cutas and Tesso sites, respectively. This difference is due to their contrasting aspect and to the fact that strong winds blowing from NW-W remove part of the winter snowpack in Las Cutas (Camarero and Gutiérrez, 2004). Both sites have not been disturbed by local disturbances (fire, logging, overgrazing, snow avalanches) at least since the 1960s. In both treelines, vegetation changes along the treeline ecotone depend on treeline structure and dynamics (Camarero and Gutiérrez, 2002). In Las Cutas, the forest is rapidly encroaching into the grassland forming a belt of shrubby krummholz pines with other smaller shrubs (e.g., *Juniperus communis* L., *Calluna vulgaris* L.). However, in Tesso the gradual change in tree cover and height going upslope is paralleled by a gradual change in understory with *Rodhodendron ferrugineum* L., *Vaccinium myrtillus* L. and *Dryas octopetala* L. dominating the forest and the treeline, respectively (Camarero and Gutiérrez, 2002; Pardo et al., 2013).

We also studied a rear-edge population of *P. uncinata* located in Peñarroya, Teruel, north-eastern Spain (40.39º N, 0.57º W, 2010 m a.s.l.). This population represents the southernmost distribution limit (rear edge) of the species, in contrast to Las Cutas and Tesso sites which are located at the core of the species distribution range (Camarero et al., 2021b). The Peñarroyra site is a mixed stand with Scots pine (*Pinus sylvestris* L.), where both species hybridize (Galván et al., 2014). The climate is continental with mean annual temperature of 9º C (mean maximum and minimum temperatures are 25º C and −5º C, respectively) and total annual precipitation of ca. 580 mm (Camarero and Gutiérrez, 2002). Soils are developed on limestones. Maximum snow cover ranges 0.5–1.5 m. The understory is dominated by *J. communis* and *J. sabina* L. shrubs. This site has not been locally disturbed through logging or fire since the 1960s.

2.2. *Sampling design*

In the two treelines, samples were collected along three altitudinal bands within a rectangular plot of 30 m x 140 m located with its longest side along the maximum slope (Camarero and Gutiérrez, 2004). Tree cover was measured by Camarero and Gutiérrez (2002) in 1998 and re-measured by Pardo et al. (2013) in 2009 by noting tree presence using the point-intercept method along six, 140-m line transects placed along the maximum slope in both treeline sites. Transects were separated from each other by 6 m along the 30-m axis, and their position was permanently marked. We considered the following altitudinal bands along the treeline ecotone (see also Pardo et al., 2013): the forest with abundant mature trees and tree cover > 40% (lowermost level), the transitional treeline ecotone also with mature trees but tree cover between 20% and 40% (intermediate level), and the treeline with scattered trees (cover < 20%) with height > 2 m found in a matrix of alpine grasslands and shrublands (uppermost level). These three levels will be referred as the forest, the ecotone, and the treeline, respectively. In the rear-edge population, samples were collected in the forest as *P. uncinata* is only present on a mountain top. In the treelines, we selected ten trees in each altitudinal band and measured their diameter at breast height (1.3 m; Dbh) and total height with tapes. In the rear-edge forest, 15 trees were sampled and similarly measured. To quantify radial growth, two wood cores were extracted at 1.3 m and perpendicular to the slope using 5-mm increment borers (Haglöf, Sweden) . A total of 75 trees were sampled.

2.3. *Climate data*

Since long-term records are not available for the study sites, monthly climate data (mean maximum temperature, total precipitation) for each location were obtained from the 1-km^2 gridded E-OBS v. 22.0e database (Cornes et al., 2018). We calculated the climate parameters at every site from that grid point of the corresponding 1-km^2 pixel containing the closest location to the study site. The remaining elevational distance was accounted for by assuming a common altitudinal lapse rate of air temperature of −0.0055 K m^{-1} during the growing season (Körner, 2012).

2.4. *Soil texture, nutrient content and microbial community structure*

We collected three soil samples around the trunk (less than 50 cm in distance) and below the canopy projection of each sampled tree. Samples were taken using a 5-cm diameter soil auger from the uppermost 15 cm where most fine roots are found (Janssens et al., 2002). The litter layer was removed carefully prior to the collection of the samples. Soil samples were stored in plastic bags and transported to the laboratory for their analyses. Samples for soil microbial structure analyses were frozen and stored in the freezer prior to the analyses. Soil samples for physical-chemical analyses were air dried on a glasshouse and sieved with a 2-mm mesh size. Soil texture was determined with a laser diffraction method in a particle analyzer (Coulter Mastersizer, 2000), and clay content was corrected following (Taubner et al., 2009). Soil C and N concentrations were determined with an elemental analyzer (Elementar VarioMAX N/CM, Hanau, Germany).

We used Phospholipid Fatty Acids (PLFA) to assess changes in microbial community structure as well as biomarkers for specific microbial groups. PLFAs were extracted from 2 g of sieved (2 mm) and lyophilized soil, separated and methylated following the methodology described by Buyer and Sasser (2012). The resulting fatty acids methyl esters (FAMEs) were separated by gas chromatography using an Agilent 7890A GC System equipped with a HP-ULTRA 2 column (length 25 m, ID 0.20 mm; J&W Scientific Inc.) and a flame ionization detector. The individual FAME peaks were identified and quantified by the PLFAD1 method of Sherlock® software version 6.3 from MIDI, Inc (Newark, USA). The internal standard 19:0 phosphatidylcholine (Avanti Polar Lipids, Alabaster, USA) was used for quantification of FAMEs. Total microbial biomass was estimated by summing the contents of all individual PLFAs and reported as nanomoles of PLFAs per gram of soil. Specific PLFAs were used as biomarkers to quantify biomasses of eukaryotes, Gram-positive and Gram-negative bacteria, actinobacteria, fungi, arbuscular mycorrhizal (AM), fungi and anaerobic bacteria (Frostegård and Bååth, 1996; Zelles, 1997, 1999).

2.5. *Functional traits*

Functional traits were measured following Cornelissen et al. (2003) and Pérez-Harguindeguy et al. (2013). We considered four functional traits from leaves and wood: leaf area (LA), specific leaf area (SLA), leaf dry matter content (LDMC), and wood specific gravity (WSG). For each sampled tree, we cut a fully expanded sun-exposed branch using a telescoping pole. Fully developed, sun-exposed and undamaged leaves from the last cohort were placed in sealed plastic bags and taken to the laboratory within 24 h. There, their projected areas (10 needles per tree) were scanned and measured using the ImageJ image analysis software (Schneider et al., 2012) while being still fresh. After measuring leaf areas, leaves were weighted fresh, and dried in the oven at 72 h at 70 ºC prior obtaining dry weights. SLA, which is associated to net assimilation and growth rates (Reich et al., 1997), was obtained by dividing the LA by leaf dry weight. LDMC correlates negatively with relative growth rate and positively with leaf life span and is related to nutrient retention within the plant and resistance to physical hazards (Poorter and Garnier, 1999). LDMC was calculated as the ratio of the oven-dry mass to its water-saturated fresh mass. Finally, WSG describes the carbon investment in woody tissues and has important implications in mechanical support and water transport (Chave et al., 2009). Bark was removed from one 2- to 3-year old twig segments per tree and its fresh volume (volume replacement method) was measured. Then, twigs were oven-dried 72 h at 70 ºC and dry weight was measured. WSG was estimated as the ratio between dry weight and fresh volume.

2.6. *Tree-ring width data*

Wood samples were air-dried, glued and polished using a series of sand-paper grids until tree-ring boundaries were clearly visible. Cores were visually cross-dated and tree-ring widths were measured with a 0.001 resolution using scanned images (resolution 2400 dpi) and the CDendro software (Larsson and Larsson, 2018). Cross-dating accuracy was checked by using the software COFECHA (Holmes, 1983).

Tree ring width was transformed to basal area increment (BAI) assuming a circular shape of stems and using the formula:

$$\mathrm{BAI}_t = \pi\left(\mathrm{R}_t^2 - \mathrm{R}_{t-1}^2\right) \tag{1}$$

where R_t and $\mathrm{R}_{t\text{-}1}$ are the radius of the tree in the year of growth (*t*) and the previous year (*t*-1).

To detrend each individual tree-ring width series, we applied a cubic regression spline with a frequency response of 0.5 at a wavelength of 2/3 of the series length. The resulting standardized tree-level series were pre-whitened (fitting an autoregressive model to the time series) to obtain series of pre-whitened or residual ring-width indices (RWIres). To obtain standardized and residual site chronologies, individual series were averaged year-by-year using a biweight robust mean (Fritts, 1976). All the processes of tree-ring series detrending and chronology computation were performed using the dplR package in the R environment (Bunn et al., 2020).

2.7. *Statistical analyses*

Pearson correlation analyses were used to test for the relationship between ring-width indices (RWIres) and monthly climate variables (mean maximum temperature, precipitation). Separate analyses were done for each site and also splitting in altitudinal bands (treeline, ecotone and forest) in the two treelines studied. Correlations were done between residual chronologies (RWIres) and climate data from October in the year before tree ring formation to September in the year of tree ring formation.

The Kruskal-Wallis test was used to test for the differences in tree features, intraspecific trait variation, soil parameters and soil microbial composition (PLFA) between sites (Las Cutas, Tesso and Peñarroya) and along treeline altitudinal gradients (treeline, forest and ecotone). Separate analyses were performed to test for differences among altitudinal belts in the treeline sites (i.e. one analyses for each treeline site). After that, separate analyses were performed to test for the presence of differences between treelines and ecotones in Las Cutas and Tesso, and between forests in Las Cutas, Tesso and Peñarroya. When significant differences were found the Dunn's (1964) test of multiple comparisons was performed.

We used Non-Metric Multidimensional Scaling (NMDS; Legendre and Legendre, 2012) with Euclidean dissimilarity to visualize the differences in soil microbial community structure (PLFA) across sites and altitudinal belts (forest, ecotone and treeline). Prior to the analyses, PLFAs were converted into arcsin square root transformed molar percentages. We used Permutational Multivariate Analysis of Variance (PERMANOVA; McArdle and Anderson, 2001) to test for the differences between sites and altitudinal belts. To facilitate the interpretation of the results, the first and the second ordination axes were correlated with the total microbial biomass, the arcsin square root transformed molar percentages biomasses of eukaryotes, Gram-positive and Gram-negative bacteria, actinobacteria, fungi, arbuscular mycorrhizal (AM), fungi and anaerobic bacteria and the ratios between fungi and bacteria and between Gram-positive and Gram-negative bacteria. NMDS ordinations and PERMANOVAs were performed applying the “metaMDS” and “adonis” functions from the vegan package (Oksanen et al., 2020) in the R software (R Core Team, 2020).

Pairwise Spearman rank correlations were used to test for the covariations between tree features, intraspecific trait variation, soil conditions and soil microbial biomass and community structure. Separate analyses were performed in each alpine treeline site to study the covariation between factors along the altitudinal gradient. In order to facilitate the interpretability of the analyses, the following variables were included in the analyses: percentage of sand content, soil pH, carbon and nitrogen content and C : N ratio (soil conditions), the first and second axes of the PLFAs in each treeline (representing soil microbial biomass and community structure), the average tree-ring width of the last 10 years (tree growth), and the WSG, LA, SLA and LDMC (intraspecific trait variation).

3. Results

Tree growth varied between sites and along altitudinal gradients within each treeline (Fig. 1). The BAI in Tesso was markedly higher than in Las Cutas and Peñarroya. Furthermore, in Tesso the BAI of the trees in the forest was larger than the BAI of the trees in the ecotone and treeline. Conversely, in Las Cutas the BAI of the treeline and ecotone trees surpassed the BAI of the forest trees (Fig. 1). Along the two altitudinal gradients, trees differed in diameter at breast height and height (Table 1). As expected, trees were higher and presented larger Dbh in the forest than in the ecotone and the treeline (Fig. S1). No differences in Dbh between trees from the different altitudinal belts were found between treelines, but they differed when comparing Las Cutas and Tesso ecotone trees (Tables 1 and S2). Strong differences in mean tree ring width, the ring-width of the last 10 years, autocorrelation and standard deviation were found between forests (Table 1 and S2).

In the Peñarroya rear-edge site the relationship between tree growth rates (ring-width indices) and the precipitation in June was stronger than in Las Cutas and Tesso treeline sites (Fig. 2). Besides, the correlation between average maximum temperature and growth occurred earlier in Peñarroya (April) than in the two treeline sites (May). Temperature-growth couplings were more intense in trees at the treeline (Las Cutas and Tesso sites) and the ecotone (only Tesso site) than in the forest (Fig. 2). A positive correlation between February temperature and growth was also observed in Las Cutas.

Leaf traits varied considerably between forests (Table 1; Fig. 3), whereas no differences were found along the altitudinal gradient in the treeline sites. These differences were mainly driven by the presence of leaves with lower LA and LDMS and larger SLA in the Peñarroya rear-edge site than in the two Pyrenean treeline sites (Las Cutas and Tesso). Regarding variations along the altitudinal gradient, we found differences in WSG between the treelines and ecotones in Tesso and Las Cutas and between the treeline, ecotone and forest in Tesso (Table 1; Fig. 3).

Soil conditions varied considerably between sites and along the

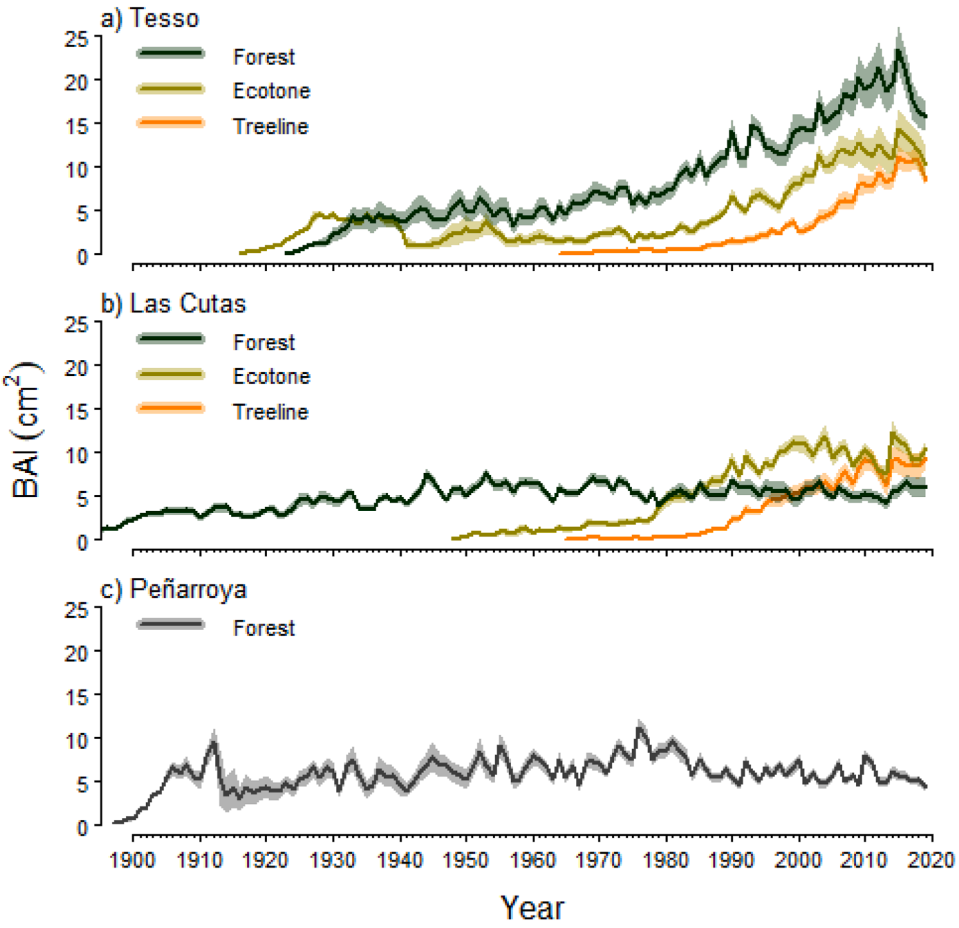

Fig. 1. Basal area increment (BAI) for the trees in the different *P. uncinata* sites studied. The mean BAI (solid line) and standard error (dashed semi-transparent area) are presented for each altitudinal belt (forest, ecotone and treeline) in the two treeline sites (Tesso (a); Las Cutas (b)) and for the rear-edge population (c) Peñarroya) are shown.

Table 1
Site differences in tree growth features and intraspecific trait variation in the studied *P. uncinata* sites. The Kruskal Wallis test was performed to test for differences along altitudinal gradients in the two treeline sites (Las Cutas and Tesso) and between comparable locations (treeline, ecotone and forest) in the two treelines and the rear-edge Peñarroya population (forest only). Variables' abbreviations: Dbh, Diameter at breast height; TRW, tree-ring width; TRW 10, mean ring width of the last 10 years; SD, standard deviation; AR1, first-order aurocorrelation; WSG, wood specific gravity; LA, leaf area; SLA, specific leaf area.

	Altitudinal gradient		Site differences		
	Las Cutas	Tesso	Treeline	Ecotone	Forest
Dbh	20.029**	19.234**	0.082	0.143	2.02
Tree age	24.292**	13.956**	1.319	0.367	20.974**
Mean TRW	20.612**	4.359	5.227*	1.286	19.721**
TRW 10	17.902**	1.946	1.707	0.006	23.08**
TRW SD	12.41**	1.457	0.027	1.651	18.948**
TRW AR1	9.572**	0.006	0.327	1.751	4.484
Height	22.683**	22.874**	2.195	7.924**	3.684
WSG	5.726	16.925**	9.135**	8.691**	6.455*
LA	3.425	4.8	3.84*	3.291	10.476**
SLA	3.724	1.158	2.667	0.051	23.4**
LDMC	3.014	1.027	0.427	1.286	22.146**
LMA	3.725	1.158	2.667	0.051	23.403**

altitudinal gradients within Las Cutas and Tesso (Table 2; Figs. 4 and S2). Differences in soil pH were found between sites with high values in Peñarroya (Fig. 4), and altitudinal belts (increasing upslope). The percentage of sand was higher in Las Cutas than in Tesso and Peñarroya, and it presented higher values in the forest than in the ecotone (Las Cutas and Tesso) and the treeline (Las Cutas). The C:N ratio was lower in Peñarroya than in Tesso and Las Cutas forests, and peaked in Las Cutas. The assimilable phosphorus presented very high values in the Las Cutas forest and it was always higher than in Tesso treeline regardless the altitudinal belt. Carbon and nitrogen concentrations changed between sites and along altitudinal gradients, but no differences between ecotones and treelines were found (Table 2).

Soil microbial composition varied considerably between sites and along altitudinal gradients (Table 2; Figs. 5, S3 and S4). The PERMANOVA analyses showed the existence of a significant site by altitudinal belt interaction (Fig. 5). These differences in the multivariate space strongly depended on the covariation with soil pH, the percentage of sand, and the C:N ratio with opposite sign. Overall, microbial biomass varied along the altitudinal gradient and changed between ecotones and forests (Fig. 5). The differences between groups were stronger when comparing forests, due to the inclusion of the rear-edge Peñarroya site, than along altitudinal gradients. Eukaryote and total biomass varied more in Las Cutas, whereas the variation in the rest of soil groups was more marked in Tesso. In Las Cutas and Tesso treeline sites, AM fungi, Gram – bacteria and Actinomycetes were relatively more abundant in the treeline whereas Eukaryotes and other Fungi were more abundant in the forest (Fig. S3 and S4). However, total biomass in Las Cutas was higher in the forest whereas in Tesso it was higher in the treeline and the ecotone. In Las Cutas, the forest and the ecotone separated from the treeline whereas in Tesso the forest separated from the ecotone and the treeline.

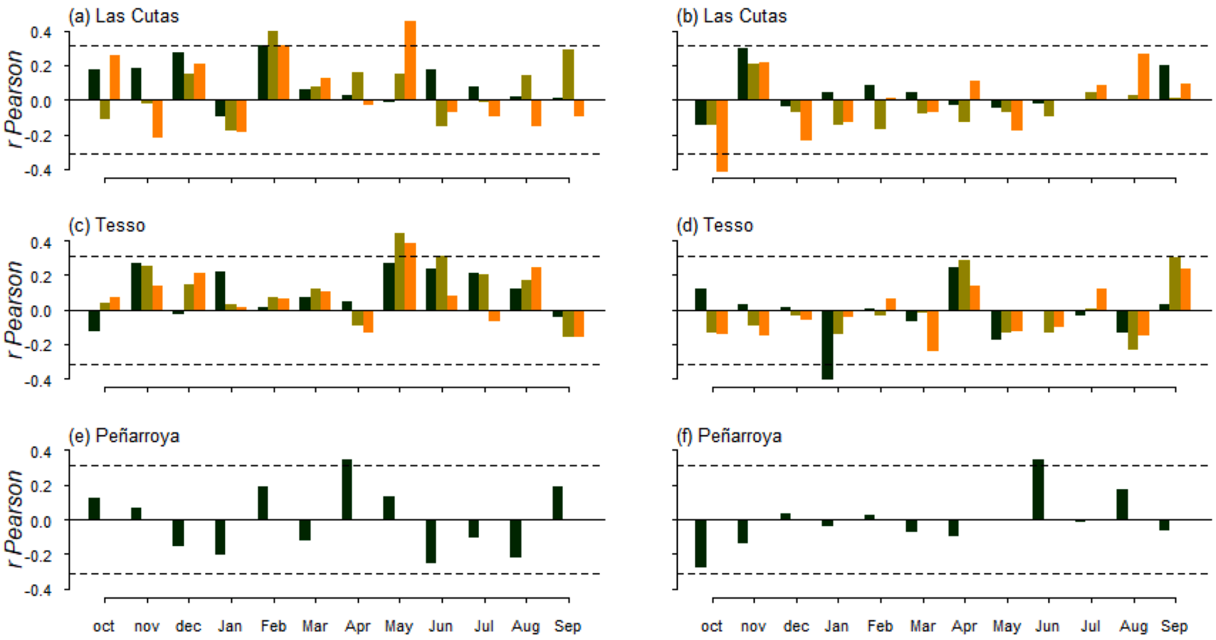

Fig. 2. Climate-growth relationships for the trees in the different *P. uncinata* sites studied. The Pearson correlation between the ring-width residual chronologies and mean monthly maximum temperature (a, c, e) and total precipitation (b, d, f) in the two treeline sites (Las Cutas (a, b); Tesso (c, d)) and for the rear-edge population (e, f) Peñarroya) are shown.

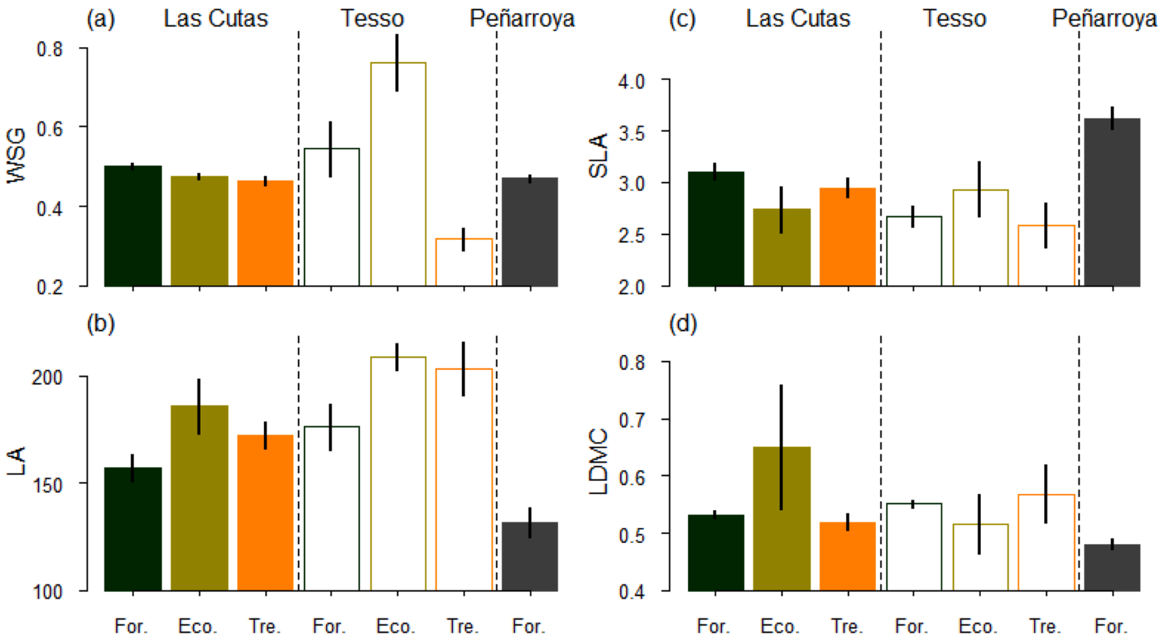

Fig. 3. Intraspecific trait variation for wood and leaf traits in the *P. uncinata* sites studied. The wood specific gravity (WSG; a in g cm^{-3}), leaf area (LA; b in mm^2), Specific leaf area (SLA; c in mm^2 mg^{-1}) and Leaf Dry Matter Content (LDMC; d in g $^{-1}$) are shown for the trees in each altitudinal belt (forest - For., ecotone - Eco., and treeline - Tre.) in the two treeline sites (Tesso, Las Cutas) and for the rear-edge population (Peñarroya).

Covariations between the soil conditions, microbial composition, tree functional traits and growth showed similarities and differences in the two treeline sites (Fig. 6). Relationships between soil conditions and tree growth, and between soil conditions and soil microbial biomass and community structure were stronger in Las Cutas than in Tesso. In general, covariations were clear between soil conditions in the two treeline sites. The percentage of sand in the soil was positively related with C, N and P concentrations and with the C:N ratio in the two sites. Conversely, it was negatively related with the soil pH and the first NMDS axis of the soil microbial composition (Fig. 6). Average growth for the last 10 years was positively related with the soil pH and the NMDS1, and negatively with soil nutrients, in Las Cutas but not in Tesso. Tree functional traits showed low covariation with soil features and tree growth in the two sites, but a positive relationship between WSG and concentration of soil N and C was found in Tesso, and with soil P in Las Cutas. Tree growth (tree-ring width of the last 10 years) was negatively related with WSG in Las Cutas.

Table 2
Site differences in soil conditions and the abundance of different soil microbial groups (according to PLFAs) in the studied *Pinus uncinata* forests. The Kruskal Wallis test was performed to test for differences along altitudinal gradients in the two treelines (Las Cutas and Tesso) and between comparable sites (treeline, ecotone and forest) in the two treelines and the rear-edge Peñarroya population (forest only). Significant differences are indicated with asterisks ($p < 0.05$ *; $p < 0.01$ **).

Variables	Altitudinal gradient			Site differences	
	Las Cutas	Tesso	Treeline	Ecotone	Forest
pH	23.532**	18.222**	6.213*	14.296**	28.052**
% sand	14.937**	18.139**	13.500**	13.720**	23.576**
C org.	19.311**	14.284**	0.96	2.174	20.966**
N	19.346	10.475**	0.96	11.063**	10.996**
C : N	24.938**	18.624**	10.685**	14.307**	21.19**
P asimmilable	20.333**	19.031**	14.574**	5.533*	19.673**
Biomass	20.201**	5.992*	2.94	11.571**	15.62**
Eukaryote	16.601**	8.646*	4.86*	14.286**	17.325**
Gram (-)	5.853	3.344	0	8.251**	22.503**
Gram (+)	7.203*	7.963*	6*	0.28	23.581**
Actinomycetes	9.043*	13.672**	12.907**	9.143**	26.236**
Fungi	4.653	7.779*	7.707*	9.606**	26.905**
AM Fungi	2.717	5.851	3.227	10.566**	15.49**
Anaerobe	0.939	11.544**	13.5**	3.291	20.819**
Fungi : Bacteria	4.653	7.372*	7.26**	8.251**	26.905**
Gram (+) : Gram (-)	6.599*	9.409**	5.607*	2.766	7.87*

4. Discussion

Mountain forests are expected to change in terms of structure and function because of climate change during the next decades, but we are still far from a fully understanding of such changes. Here we show that the functional characteristics and growth of trees markedly differed between the two treelines located in the core distribution range of *P. uncinata* and an isolated population forming the species' rear edge. Tree growth in the rear-edge population was more impacted by drought and those trees showed morphological adaptations to face drier conditions. However, it is important to note that the growth of trees in Tesso was markedly larger than the growth of trees in Las Cutas indicating that the Mediterranean influences in the climate of the later treeline site and its soil conditions makes this site less suitable for *P. uncinata* growth as previously reported (Camarero and Gutiérrez, 2004). Thus, according to our hypothesis, site differences play an important role on determining

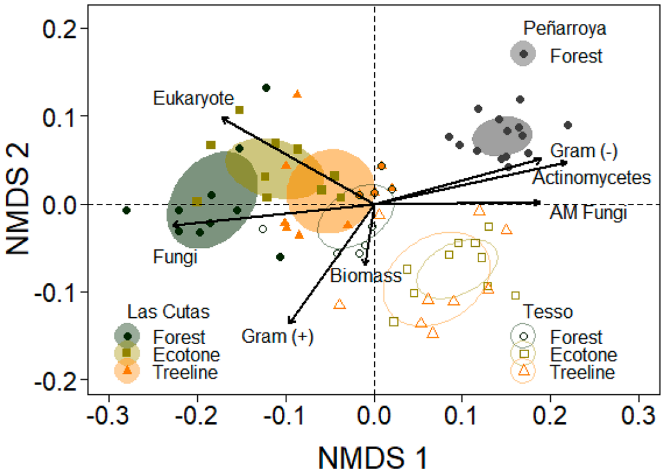

Fig. 5. Ordination diagram of the soil microbial composition in the *P. uncinata* sites studied. A Non-Metric Multidimensional Scaling (NMDS) was performed and the two first axes (NMDS1 and NMDS2) are represented to show differences between sites. Points represent different sites and altitudinal belts (shown with different colors) and formations in each site. Shaded areas represent the centroid of the values for each site and formation. The soil conditions with a stronger correlation with the axes (see Table S1) are displayed with arrows.

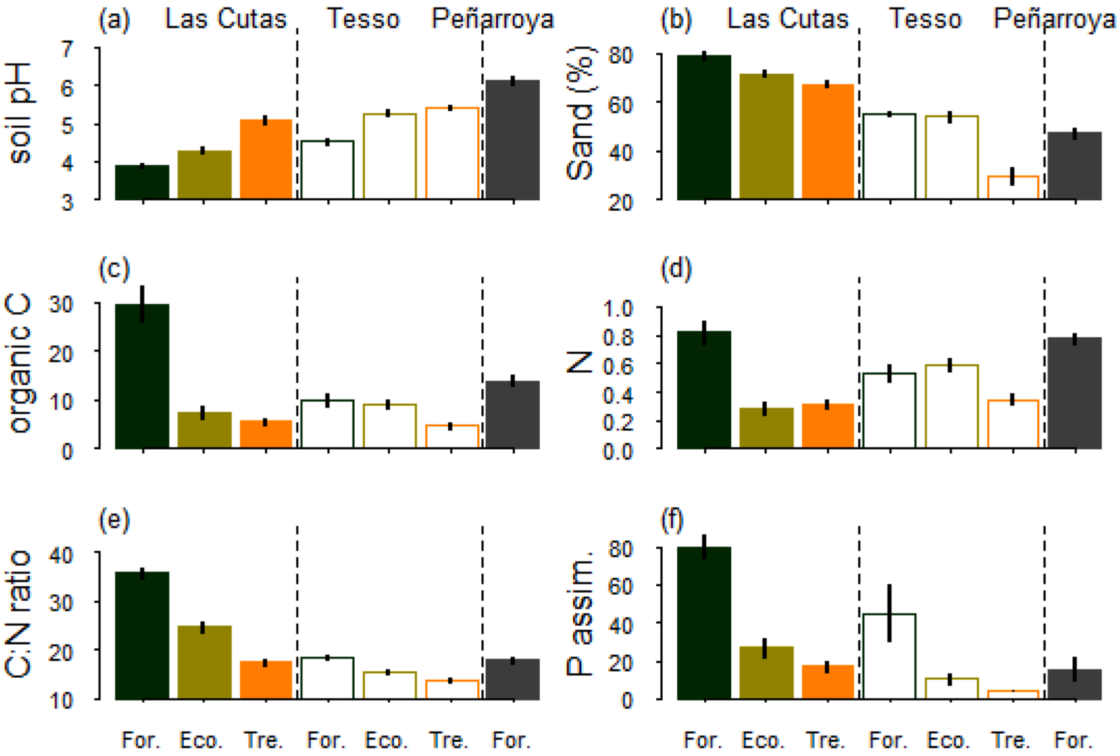

Fig. 4. Variation in soil conditions between the different *P. uncinata* populations studied. The soil pH (a), the percentage of sand in the soil (b), the organic carbon (c, in%), the nitrogen concentration (d, in%), the carbon : nitrogen ratio (C:N ratio; e), and the assimilable phosphorus content (P assim; f, in ppm) are shown for the trees in each altitudinal belt (forest, ecotone and treeline) in the two treeline sites (Tesso, Las Cutas) and also for the rear-edge population (Peñarroya).

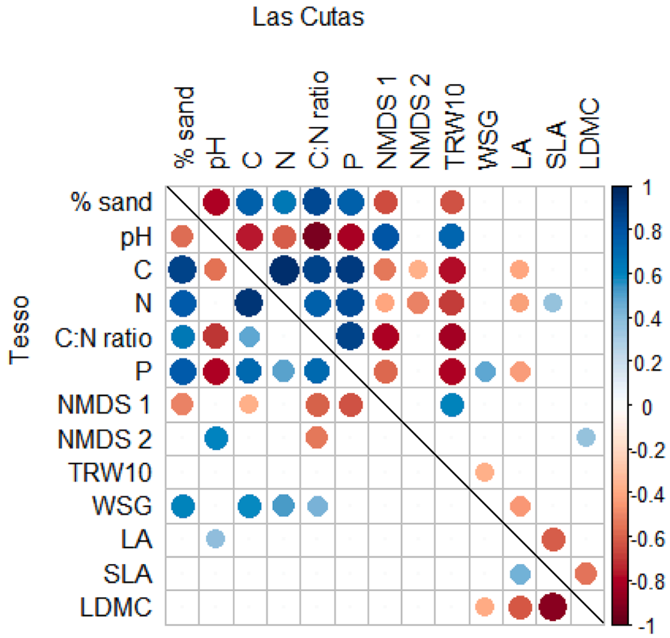

Fig. 6. Correlations between soil parameters, PLFA ordination of soil groups, tree growth and trait variation in the treeline. The Spearman rank correlation between each pair of variables is represented with symbols proportional to the correlation coefficient and colours (see color scale). The upper triangle shows the relationships in the Las Cutas treeline site and the lower triangle in the Tesso treeline site. Only significant correlations (p. < 0.05) are shown.

tree performance. However, we also showed that soil characteristics vary along the treeline ecotone as does tree growth and its relationship with climate. While we did not find clear and directional differences in functional traits along the treeline ecotone, the stronger sensitivity of trees for the upper level to temperature suggests they are more constrained by low temperatures, as can be expected as they form the current forest barrier (Körner, 2012; Camarero et al., 2021). The marked differences in soil microbial community composition between the closed forest and beneath the scattered trees from the upper treeline limit are in accordance with previous studies reporting variations in soil conditions across treeline ecotones (Djukic et al., 2010; Sun et al., 2020). Despite young trees from the upper treeline present higher growth rates than those from the forest, the upward advancement of the treeline will require time as trees will need longer time to modify soil conditions. In other words, even in the case that treeline isotherm moves upslope and trees became less temperature-limited (e.g. Camarero et al., 2021) strong differences in soil conditions along short gradients may constrain local population fluctuations (e.g. Hagedorn et al., 2019) making treeline advances slower than expected and contingent on local conditions (Batllori et al., 2009; McIntire et al., 2016).

In Peñarroya, the isolated rear-edge population, trees present smaller leaves and grow less than in one of the treeline sites (Tesso; Fig. 1). We also show that the tree growth in Peñarroya is more limited by the precipitation during the growing season (Fig. 2). All these factors point that this population has been subjected to a drier environment than those treeline populations from the Pyrenees (Galván et al., 2014). The fact that trees here present different functional characteristics suggest that its genetic isolation has leaved an imprint in the functioning of the trees. In a comparable mountain pine forest, González-Díaz et al. (2020) found genetic differences with the Las Cutas treeline. We do not have here information on genetic composition, but functional traits indicate strong variability in leaf traits which in turn can be also explained due to phenotypic plasticity (Vilá-Cabrera et al. 2015). Alternatively, the Peñarroya population may be experiencing strong hybridization with Scots pine thus favouring more drought-tolerant leaf traits. In any case, our analyses reveal a stronger dependency of growth on water availability in this relict, rear-edge population than in the two alpine treeline sites, and this has important implications in sight of climate change. Recent studies suggest that drought is becoming a more important driver of tree growth than temperature globally (Babst et al., 2019; Pompa-García et al., 2021). Along this, results based on modeling approaches suggest a growth enhancement of mountain pine in the treeline of the Pyrenees because of climate warming (Sánchez-Salguero et al., 2017). However, the growth projections of forests such as the one in Peñarroya are missing despite those will be the most vulnerable sites to the increase in drought intensity associated to climate warming.

We also found differences between trees when comparing the two treelines suggesting that local factors are important on modulating mountain pine dynamics in the Pyrenees (Batllori et al., 2009). A higher growth rate was observed in Tesso than in Las Cutas probably due to the presence of higher precipitations and less Mediterranean influence (Tardif et al., 2003; Galván et al., 2014). Larger growth in trees from Tesso can be also attributed to the local conditions given that soils are less acidic and present lower percentage of sand than those in Las Cutas (Fig. 4). Insufficient water retention and low nutrient availability can limit tree growth in alpine treelines (e.g. Hagedorn et al., 2019). Nevertheless, this is not so clear when comparing the soil nutrient content that present very high values in Las Cutas. However, we measured soil conditions in the top-soil layers and tree growth may be dependent on soil conditions at deeper layers. Differences in growth between sites can be also caused by differences in tree age, because the sampled trees are younger in Tesso. In fact, the main differences in growth between trees were found in the forest belts (Fig. 1; Table 1), with less or no differences when the ecotones and treeline belts were compared. We conclude that tree growth differences between the two treelines depended on the combination of climate and soil factors (Ellison et al., 2019), which favor *P. uncinata* growth in Tesso as compared to Las Cutas.

Differences between the two treelines were also reflected at the intraspecific trait level mainly in the case of WSG and in LA to a lesser extent (Table 1; Fig. 3). According to the wood economic spectrum, higher wood density and higher growth rates are expected in resource-acquisitive species (Chave et al., 2009). However, translating these conclusions to the intraspecific level remains challenging (Fajardo, 2016, 2018). In our study, we found the highest wood density in the Tesso site, where trees present the largest growth rates (Figs. 1 and 3). This was due to the presence of some individuals with values far beyond the limits reported commonly (above 1.0 g cm^{-3}; Chave et al., 2009). Two of these individuals were located in the ecotone and another one was in the forest. Thus, differences in intraspecific trait variations between the two alpine treelines did not follow a clear pattern.

In general, we found that wood density was lower in the treeline as compared to the ecotone and the forest, despite these differences were only significant in Tesso (Table 1). Fajardo (2018) suggested that trees in the treeline will have lower wood density because they are smaller and so they have lower structural requirements than trees in the forest or the ecotone. According to Chave et al. (2009), treeline trees will display lower wood density because they are young and thus have a fast growth, which could apply to the ecotone trees in Tesso. Unfortunately, the lack of consistency between the two treelines does not allow us to draw strong conclusions regarding this topic. Conversely, we found that the growth of treeline trees was more sensitive to temperature that the growth of trees in the ecotone and the forest (Fig. 2). Recent studies suggest that the growth of treeline trees is becoming less temperature limited because of climate change (Camarero et al., 2021). However, as Körner (2021) pointed out in a response letter the isotherm that defines the treeline moves upward making difficult to assess whether the growth of those leading trees is or not temperature limited. The results presented here suggest that the growth of the most exposed trees will be limited by temperature at the same time as those trees located a few meters lower forming the ecotone. However, it is important to note that these temperature-growth couplings will be site dependent as suggested by the differences observed between Tesso and Las Cutas, and in agreement with previous results (Galván et al., 2014). Nevertheless, tree growth is only one component driving treeline dynamics which depend on other local and regional factors (Batllori et al., 2009; Camarero et al., 2017).

In this respect, we found marked differences in soil conditions between the treeline, the ecotone and the forest in the two alpine treelines studied. It is expected that soil conditions can play a fundamental role in the process of vegetation expansion because of climate change (HilleRisLambers et al., 2013). As tree density increases, carbon accumulation shifts from the soil to the tree biomass (Hagedorn et al., 2019) because organic matter decomposition is higher under the favourable conditions of the forest than in the alpine treeline. These differences are clearer when comparing the forest with the ecotone and the treeline, and when comparing the ecotone and the treeline. Due to the harsh conditions above the treeline and the poorly developed soils, soil nutrient concentrations can increase in the forest affecting also soil microbial composition (Donhauser and Frey, 2018). These results differ from those reported by Ren et al. (2018) in China where they found no differences in nutrient concentrations along the altitudinal gradient. Similar decreases in nutrient concentration were found by Drollinger et al. (2017) in Nepal as those presented here. Liu et al. (2020) also found changes in soil organic carbon, nitrogen and phosphorus concentrations along the treeline. Interestingly, they also found that these changes were dependent on soil depth, with mean values across depths differing from the values in the top-soil layer.

Changes in soil conditions and associated vegetation are also linked to changes in the soil microbial community composition (Thébault et al., 2014; Hagedorn et al., 2019). Our results indicate that the fungi biomass is higher in the forest than in the ecotone and the treeline in contrast to the biomass of bacteria (Fig. 5). These results contrast with what Thébault et al. (2014) found in the treeline of the Andes. However, we also found a higher relative abundance of AM fungi in the treeline which is mainly dominated by herbaceous vegetation (Pardo et al., 2013) which is more likely associated to AM fungi (Laughlin et al., 2021). The results also suggest the presence of more favourable environments in the forest enhancing soil microbial development (higher biomass) and probably promoting higher diversity (Hagedorn et al., 2019). However, it is important to note that total microbial biomass did not differ along the altitudinal gradient in Tesso, probably due to the diffuse structure of this treeline. Thus, treeline encroachment enhances the development of soil microbial communities which in turn may enhance vegetation development via positive plant-soil feedbacks (Bennett et al., 2017). Our results highlight the strong linkage between soil conditions and microbial community structure (Fig. 6). At the same time, the covariation between tree growth and altitude suggests that treeline advances and soil conditions are linked to some degree. However, establishing links between intraspecific trait variations and soil variations is less straightforward pointing to several factors influencing the variation in these different ecosystem components.

5. Conclusions

Site conditions played an important role in determining tree growth, intraspecific trait variations and the relationship between trees and soil characteristics including microbial biomass. When talking about the differences between sites (i.e. comparing sites in the rear-edge and the core of the distribution range) the isolation and climatic marginality of certain tree populations which are located near the species rear edge may determine its long-term performance. This is the case of the Peñarroya relict rear-edge mountain pine population, whose growth is constrained by summer drought and where trees present leaves adapted to higher levels of water scarcity than trees from the treeline ecotones. These results suggest the existence of differences in genetic composition between the two Pyrenean treeline ecotones and the Peñarroya mountain pine population. Nevertheless, differences between sites also emerge when comparing two treeline populations located in the Pyrenees and which represent the core of the species distribution range. Here, these differences can be attributed to the more favourable conditions for tree growth in Tesso than in Las Cutas. In Tesso, the combined effect of more favourable climate conditions and less acidic soils with higher water retention capacity than in Las Cutas may enhance *P. uncinata* growth and plant-soil feedbacks. Finally, differences in growth also emerge when comparing trees within each treeline because the growth of trees in the forest is less dependent on temperature than the growth of trees in the treeline. Besides, soil nutrient content and soil microbial community biomass is higher in the forest than in the treeline ecotones.

Observed upward treeline shifts lead to changes in plant-soil feedbacks, and so we need to advance in the understanding of how trees affect and are affected by soil conditions. This gap of knowledge should be filled in tree populations forming the species' distribution limits, including alpine treelines and rear-edge stands, which are expected to be impacted by climate warming in contrasting ways. This knowledge would allow better forecasting the future persistence of forests and related plant-soil dynamics.

Declaration of Competing Interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.agrformet.2022.108811.

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