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Ice cave reveals environmental forcing of long-term Pyrenean tree line dynamics

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Abstract

1. Tree lines are supposed to react sensitively to the current global change. However, the lack of a long-term (millennial) perspective on tree line shifts in the Pyrenees prevents understanding the underlying ecosystem dynamics and processes.
2. We combine multiproxy palaeoecological analyses (fossil pollen, spores, conifer stomata, plant macrofossils, and ordination) from an outstanding ice cave deposit located in the alpine belt c. 200 m above current tree line (Armeña-A294 Ice Cave, 2,238 m a.s.l.), to assess for the first time in the Pyrenees, tree line dynamics, and ecosystem resilience to climate changes 5,700–2,200 (cal.) years ago.
3. The tree line ecotone was located at the cave altitude from 5,700 to 4,650 cal year BP, when vegetation consisted of open *Pinus uncinata* Ramond ex DC and *Betula* spp. Woodlands and timberline were very close to the site. Subsequently, tree line slightly raised and timberline reached the ice cave altitude, exceeding its today's uppermost limit by c. 300–400 m during more than four centuries (4,650 and 4,200 cal year BP) at the end of the Holocene Thermal Maximum. After 4,200 cal year BP, alpine tundra communities dominated by *Dryas octopetala* L. expanded while tree line descended, most likely as a consequence of the Neoglacial cooling. Prehistoric livestock raising likely reinforced climate cooling impacts at 3,450–3,250 cal year BP. Finally, a tree line ecotone developed around the cave that was on its turn replaced by alpine communities during the past 2,000 years.
4. *Synthesis.* The long-term Pyrenean tree line ecotone sensitivity suggests that rising temperatures will trigger future *P. uncinata* and *Betula* expansions to higher elevations, replacing arctic-alpine plant species. Climate change is causing the

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rapid melting of the cave ice; rescue investigations would be urgently needed to exploit its unique ecological information.

KEYWORDS

climate impact, *Dryas octopetala*, ice deposit, *Pinus uncinata*, plant macrofossils, pollen, timberline, tree line

1 | INTRODUCTION

Mountain species and habitats are expected to be particularly sensitive to ongoing global change (IPPC, 2013; Krajick, 2004; Steinbauer et al., 2018). Tree line ecotones, that are, the transition zones between the upper limit of closed forest (timberline) and the treeless alpine grasslands (Körner, 2012), are particularly diverse and dynamic habitats (e.g., Camarero et al., 2015). Although other ecological factors such as topography, soil development, and human activities may affect the tree line ecotone position, it is mostly controlled by climate (Holtmeier & Broll, 2005; Körner, 2012). Hence, tree line ecotones are considered to be sensitive sentinels of recent climate warming (Camarero & Gutiérrez, 2004). Palaeoecological time series may contribute to a better understanding of long-term community dynamics under global change conditions in order to develop sustainable plans for maintaining diversity and ecosystem services of alpine landscapes, by, for example, providing guidelines for appropriate forest, alpine herb communities, livestock grazing, and environmental management (Kulakowski et al., 2017).

In contrast to other mountain ranges of Europe (Eide, Birks, Bigelow, Peglar, & Birks, 2006; Feurdean et al., 2016; Tinner & Theurillat, 2003), little is known about the long-term tree line and timberline shifts in the Pyrenees (Cunill, Soriano, Bal, Pèlachs, & Pérez-Obiol, 2012), a prominent Southern European mountain range. Specifically, mountain vegetation responses to major climatic shifts such as the mid-Holocene Thermal Maximum (HTM, c. 11,000–5,000 cal year BP, (Fischer et al., 2018) and the subsequent Neoglacial cooling (NGC, c. 5,000 cal year BP –1850 ad; Kumar, 2011) remain unexplored. This scientific gap is crucial, given that periods such as the HTM can be used to assess how future ecosystems may respond to ongoing global warming. Most Pyrenean Holocene palaeoecological studies have so far focused on pollen analysis, thus providing relevant information on past forest range fluctuations through time (e.g., Garcés-Pastor et al., 2017; Leunda et al., 2017; Pérez-Obiol, Bal, Pèlachs, Cunill, & Soriano, 2012; Pérez-Sanz et al., 2013) but not on processes where spatially precise information is needed such as timberline/tree line dynamics. Plant macrofossils are usually regarded as the most suitable proxy for reconstructing past tree range shifts (e.g., the altitudinal position of the tree line ecotone), as they can often be identified with greater taxonomic precision than pollen and have a mostly local source area (Birks & Birks, 2000; Tinner & Theurillat, 2003). Combined pollen and macrofossil evidence from other European mountain ranges have shown that the main changes in Holocene tree line and timberline

positions followed major climatic trends. However, land-use intensification has been invoked as the primary cause of late Holocene downward shifts of timberline, with climate playing a primary role for tree line position (Feurdean et al., 2016; Pini et al., 2017; Schwörer, Colombaroli, Kaltenrieder, Rey, & Tinner, 2015; Tinner & Theurillat, 2003). In any case, it remains unclear if this also applies to the Pyrenees, because disentangling the role of climate and human impact is challenging, sometimes provoking controversies, specifically in the Mediterranean realm (e.g., Carrión et al., 2010; González-Sampériz et al., 2017; Samartin et al., 2017).

Lakes and mires are by far the most widely used natural archives for palaeoecological research (e.g., González-Sampériz et al., 2017; Heiri et al., 2014). However, other continental palaeoenvironmental archives such as ice caves also contain information relevant to alpine environment reconstruction. In fact, ice deposits can provide outstanding palaeoenvironmental and palaeobotanical results (e.g., Eichler et al., 2011). Ice caves are rock cavities that host perennial ice resulting from the diagenesis of snow and/or the freezing of infiltrating water reaching the cave (Perşoiu & Lauritzen, 2018). Particular cave morphology and climatic conditions allowing ice formation and persistence are needed for these unique archives to develop (Luetscher & Jeannin, 2004). As a consequence, their location at mid-latitudes is generally restricted to high mountain environments, often at or above the tree line ecotone, thus representing an ideal location to study the past altitudinal tree range shifts. Although the occurrence of ice caves in the Iberian Peninsula is known since long (Casteret, 1946) growing evidence contributes to improve our knowledge about these unique natural archives (Serrano et al., 2018 and references therein). In general, little attention has so far been paid to the palaeoecological information preserved in ice caves worldwide and, to our knowledge, only one palaeobotanical study is available from this kind of natural archive (Scărișoara cave, Carpathian Mountains in Romania; Feurdean, Perşoiu, Pazdur, & Onac, 2011).

Here, we present novel palaeoecological data from the Armeña-A294 Ice Cave, a unique site located in the Central Pyrenees, well above the current tree line ecotone (Sancho et al., 2018). We use exceptionally well-preserved pollen and plant macrofossil assemblages as well as a robust chronology to address the following goals: (a) reconstructing mid-to-late Holocene altitudinal tree range shifts for the first time in the Pyrenees, (b) identifying if and how climate dynamics, including the NGC, affected Pyrenean forest and alpine vegetation, and (c) assessing if prehistoric human activities decisively contributed to the long-term Pyrenean mountain vegetation changes.

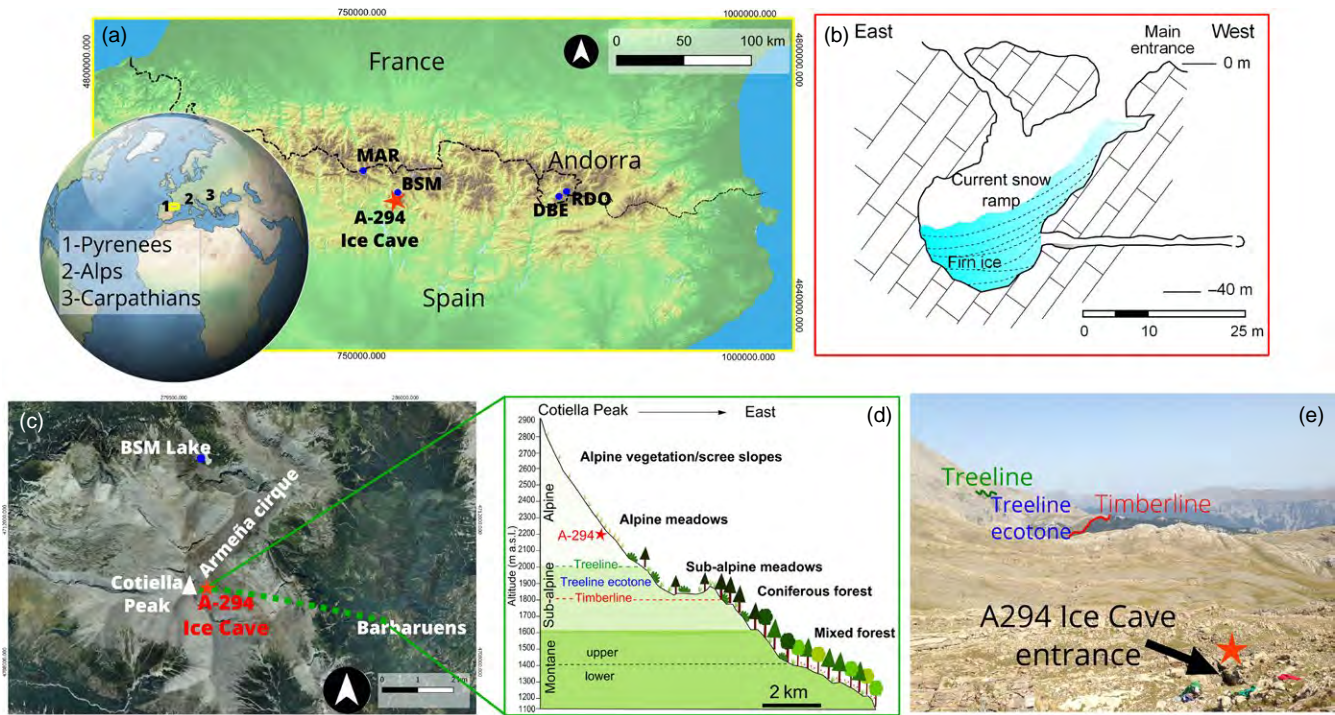


FIGURE 1 (a) Location of the main mountain ranges referred in the text (one Pyrenees, two Alps, three Carpathians) with a zoom into the Pyrenees, where the Armeña-A294 Ice Cave is located. Other Pyrenean records discussed in the text are also shown. (b) Schematic vertical cross section of the Armeña-A294 Ice Cave showing the position of the ice deposit (modified from (Belmonte-Ribas et al., 2014)). (c) Orthophotograph of the Armeña-A294 Ice Cave location and the nearby area. (d) Simplified vegetation transect from Barbaruens village (1,100 m a.s.l.) to the Cotiella Peak (2,912 m a.s.l.). (e) Photograph showing the Armeña-A294 Ice Cave entrance and the surrounding landscape. Current position of the tree line, the tree line ecotone, and the timberline is also indicated

2 | STUDY AREA

2.1 | Cave setting

The Armeña-A294 Ice Cave (42°30'52"N; 0°20'10"E, 2,238 m a.s.l.) is located in the southern central Pyrenees, Spain, on the eastern slope of the Cotiella Peak (2,912 m a.s.l.), the highest summit of the Cotiella Massif, within the Armeña glacial cirque (Figure 1a,c). The massif is mainly formed by highly folded and thrust Upper Cretaceous and Eocene carbonate rocks.

Armeña-A294 is a small sag-type cave with a main circular entrance 6-m wide where a shaft with a snow ramp about 10-m deep continues to a cavity of 40-m wide and 22-m high, where the ice deposit is hosted (Figure 1b). This particular morphology allows snow, as well as plant remains, to enter the cave and be incorporated into firn ice (Belmonte-Ribas, Sancho, Moreno, López-Martínez, & Bartolomé, 2014; Sancho et al., 2018).

2.2 | Climate and vegetation

The study site experiences a typical subalpine to alpine mountain climate, with overall cold temperatures (mean annual temperature: 1.5°C), distinct seasonality (mean winter temperature: -5°C; mean summer temperature: 9.5°C), abundant precipitation (mean annual precipitation: 1,700 mm) evenly distributed throughout the year,

and a long snow season spanning from October to May (Belmonte-Ribas et al., 2014).

Nowadays, the altitudinal limits of the different vegetation belts in the Cotiella Massif depend on slope, aspect, orography, soil development, microclimate, and past human activities (Montserrat-Martí, 1989). An altitudinal vegetation transect from the foothills of the massif (Barbaruens, 1,100 m a.s.l.) to the Cotiella Peak (2,912 m a.s.l.) including the Armeña cirque shows the typical zonation of the Central Pyrenees (Figure 1c,d). The montane belt (below 1,600 m a.s.l.) is characterized by mixed forests with *Pinus sylvestris* L., *Quercus cerrioides* Willk. & Costa, *Fraxinus excelsior* L., *Corylus avellana* L., *Sorbus aria* (L.) Crantz, *Sorbus aucuparia* L., *Fagus sylvatica* L., and *Buxus sempervirens* L. The subalpine belt (up to 2,000 m a.s.l.) comprises closed *Pinus uncinata* Ramond ex DC forests (below timberline) and open *P. uncinata* communities where the shrub *Juniperus communis* L. ssp. *alpina* (Suter) Celak is particularly relevant (tree line ecotone). Today's timberline lies at an elevation of c. 1,800 m a.s.l., with the tree line ecotone reaching up to c. 2,000 m a.s.l., that is, roughly 400 and 200 m below the altitude of the Armeña-A294 Ice Cave, respectively (Figure 1e). Finally, the alpine belt extends from c. 2,000 m a.s.l. upwards and is characterized by treeless landscapes where a diverse array of alpine herbs and dwarf shrubs thrive (e.g., *Dryas octopetala* L., *Silene acaulis* (L.) Jacq, and several grasses, sedges, and legumes among more than 300 species; Figure 1d,e). The

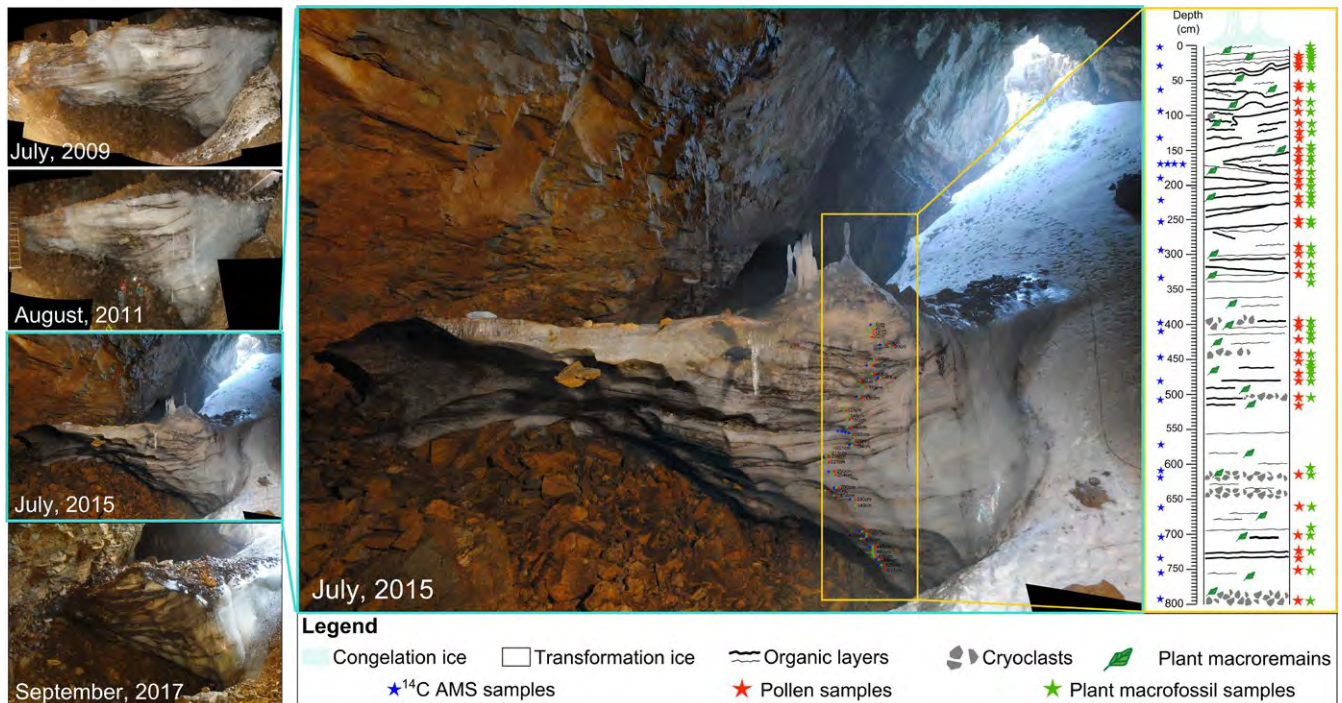


FIGURE 2 Left. Evolution of the ice deposit inside the Armeña-A294 Ice Cave (2,238 m a.s.l.) from July 2009 to September 2017. Centre. Detailed picture of the ice deposit in July 2015 when samples were taken for this study. Right. Stratigraphy of the ice deposit. The position of the samples for ^{14}C and for pollen and macrofossil analyses is also indicated

past intense grazing activities are thought to have profoundly modified the natural boundaries between the forested environments of the subalpine belt, that is, timberline and tree line ecotone, and the alpine meadows in this area (Montserrat-Martí, 1989).

3 | MATERIALS AND METHODS

3.1 | Sampling and radiocarbon dating

Samples for radiocarbon dating, pollen, and macrofossil analyses were collected in July 2015 from the exposed wall outcrop according to stratigraphic criteria along the 790 cm thickness of the ice deposit (Figure 2). We selected 27 plant macrofossil samples for accelerator mass spectroscopy (AMS) ^{14}C dating (Table 1); four of them from a single layer (165 cm depth) to test reproducibility and approximate time period embraced by these layers. Radiocarbon dates were calibrated using CALIB Rev 7.0.4 (Stuiver & Reimer, 1993) and the INTCAL 13 calibration curve (Reimer et al., 2013). To model the depth-age relationship, we used a linear interpolation between the dated levels with the Clam 2.2 package (Blaauw, 2010) running in R (R Development Core Team, 2016).

3.2 | Pollen and macrofossil analyses

The 42 samples for pollen analysis were recovered from the richer organic layers in the ice deposit at varying intervals (Figure 2). Ice was collected in plastic bottles and, when melted, the resulting water (750 cm³) was repeatedly centrifuged and decanted until getting the solid fraction.

The remaining material was used for pollen extraction following the standard chemical procedure described in Moore, Webb, and Collison (1991) including HCl, HF, and KOH digestions and dense-media separation with Thoulet solution (2.0 g/cm³). *Lycopodium* spores in a known number were added in order to calculate pollen concentrations in the firn ice samples and test laboratory procedures (Stockmarr, 1971). Pollen was identified under a light microscope using the reference collection at the Pyrenean Institute of Ecology, determination keys, and photographic atlases (Moore et al., 1991; Reille, 1992). The pollen sum, which excludes fern spores and nonpollen palynomorphs (NPPs), was usually over 300 ($M \pm SD = 334.9 \pm 36.6$ pollen grains) per sample. Local pollen assemblage zones (LPAZs) were delimited using optimal partitioning by sums-of-squares (Birks & Gordon, 1985) with the statistically significant zones obtained by comparison with a broken stick model (Bennett, 1996), using PSIMPOLL (Bennett, 2009). Conifer stomata and dung fungal spores were also identified following Trautmann (1953) and van Geel and Aptroot (2006), respectively. Conifer stomata are a good proxy for the local presence of conifer trees (Ammann et al., 2014) while coprophilous fungal spores are related to local grazing activities (Gill et al., 2013).

Samples for the identification of plant macrofossils were retrieved in 50 different levels. The samples were sieved with a mesh size of 200 μm and the plant macrofossils were identified under a binocular with the reference material at the Pyrenean Institute of Ecology and the Institute of Plant Sciences of the University of Bern. Woody remains were identified using incident light and compared with wood reference collections and wood anatomy atlases (e.g., Schweingruber, 1990). The macrofossil diagrams show the number of macrofossils (e.g., needles, seeds, fruits) and pine periderm areas per 750 cm³ of

TABLE 1 AMS radiocarbon dates from terrestrial plant macrofossils of the Armeña-A294 Ice Cave profile sampled in July 2015. Note that the rejected dates for the depth-age model are indicated in *italics*

Laboratory code	Sample	Depth (cm)	Material	Radiocarbon age (¹⁴ C year BP)	Calibrated age (2σ) (cal year BP)	Age shown in the diagram(cal year BP)
D-AMS 013241	A294-790	0	Terrestrial plant macrofossil	2,229 ± 27	2,153–2,276	2,226
D-AMS 013240	A294-765	25	<i>Terrestrial plant macrofossil</i>	2,193 ± 30	2,130–2,311	2,236
D-AMS 013239	A294-730	60	Terrestrial plant macrofossil	2,471 ± 28	2,426–2,717	2,582
D-AMS 013238	A294-700	90	Terrestrial plant macrofossil	2,529 ± 24	2,613–2,636	2,623
D-AMS 013237	A294-660	130	Terrestrial plant macrofossil	2,999 ± 23	2,957–3,080	3,032
D-AMS 013236	A294-625D	165	Terrestrial plant macrofossil	2,922 ± 24	2,985–3,159	3,067
D-AMS 013235	A294-625C	165	Terrestrial plant macrofossil	2,945 ± 24	3,004–3,171	3,106
D-AMS 013234	A294-625B	165	Terrestrial plant macrofossil	2,946 ± 28	3,000–3,180	3,106
D-AMS 013233	A294-625A	165	Terrestrial plant macrofossil	2,987 ± 23	3,076–3,228	3,167
D-AMS 013232	A294-596	194	<i>Terrestrial plant macrofossil</i>	3,331 ± 28	3,541–3,636	3,568
D-AMS 013231	A294-571	219	Terrestrial plant macrofossil	3,241 ± 23	3,395–3,510	3,459
D-AMS 013230	A294-540	250	Terrestrial plant macrofossil	3,286 ± 24	3,456–3,567	3,515
D-AMS 013229	A294-500	290	Terrestrial plant macrofossil	3,458 ± 23	3,680–3,735	3,721
D-AMS 013228	A294-460	330	Terrestrial plant macrofossil	3,878 ± 29	4,234–4,415	4,323
D-AMS 013227	A294-392	398	Terrestrial plant macrofossil	3,956 ± 28	4,377–4,449	4,425
D-AMS 013226	A294-374	416	<i>Terrestrial plant macrofossil</i>	4,150 ± 28	4,579–4,771	4,695
D-AMS 013225	A294-345	445	Terrestrial plant macrofossil	4,097 ± 28	4,520–4,652	4,605
D-AMS 013224	A294-310	480	Terrestrial plant macrofossil	4,120 ± 25	4,529–4,709	4,647
D-AMS 013223	A294-285	505	Terrestrial plant macrofossil	4,222 ± 26	4,807–4,851	4,810
D-AMS 013222	A294-220	570	<i>Terrestrial plant macrofossil</i>	4,310 ± 34	4,835–4,962	4,867
D-AMS 013221	A294-180	610	Terrestrial plant macrofossil	4,228 ± 27	4,808–4,853	4,823
D-AMS 013220	A294-175	615	Terrestrial plant macrofossil	4,318 ± 25	4,839–4,892	4,866
D-AMS 013219	A294-130	687	Terrestrial plant macrofossil	4,691 ± 30	5,320–5,423	5,396
D-AMS 013218	A294-88	702	Terrestrial plant macrofossil	4,810 ± 29	5,474–5,548	5,516
D-AMS 013217	A294-60	730	<i>Terrestrial plant macrofossil</i>	4,670 ± 32	5,316–5,470	5,400
D-AMS 013216	A294-38	752	<i>Terrestrial plant macrofossil</i>	4,777 ± 30	5,467–5,590	5,519
D-AMS 013215	A294-0	790	Terrestrial plant macrofossil	4,959 ± 28	5,610–5,738	5,684

ice. The percentage macrofossil diagram was subdivided into seven c. 500 year-bins, by pooling several adjacent samples to reach a minimum of 50 macrofossils per bin and therefore reliable percentages (Heiri & Lotter, 2001; Tinner & Kaltenrieder, 2005). Macrofossils (e.g., needles, leaves, fruits) of trees, shrubs, or herbs were considered for the percentage calculation, but fragments of wood and periderm were not included in the sum (following Tinner, 2007).

3.3 | Numerical analysis

We identified the underlying gradients in regional vegetation composition over time applying ordination analyses to the pollen percentage dataset. First, a detrended correspondence analysis (DCA; Birks and Gordon (1985) by segments without downweighting of

rare species was applied to determine the appropriate response model (unimodal vs. linear). Given the rather short length of the DCA first axis gradient (1.479 SD), we eventually used ordination techniques that assume a linear response model, in particular principal component analysis (PCA). Multivariate analyses were carried out with CANOCO 4.5 (Ter Braak & Šmilauer, 2002).

4 | RESULTS

4.1 | Ice deposit chronostratigraphy and depth-age model

The ice sequence was 9.25 m thick in July 2015 and the stratigraphy is characterized by cross-stratified ice beds resulting from

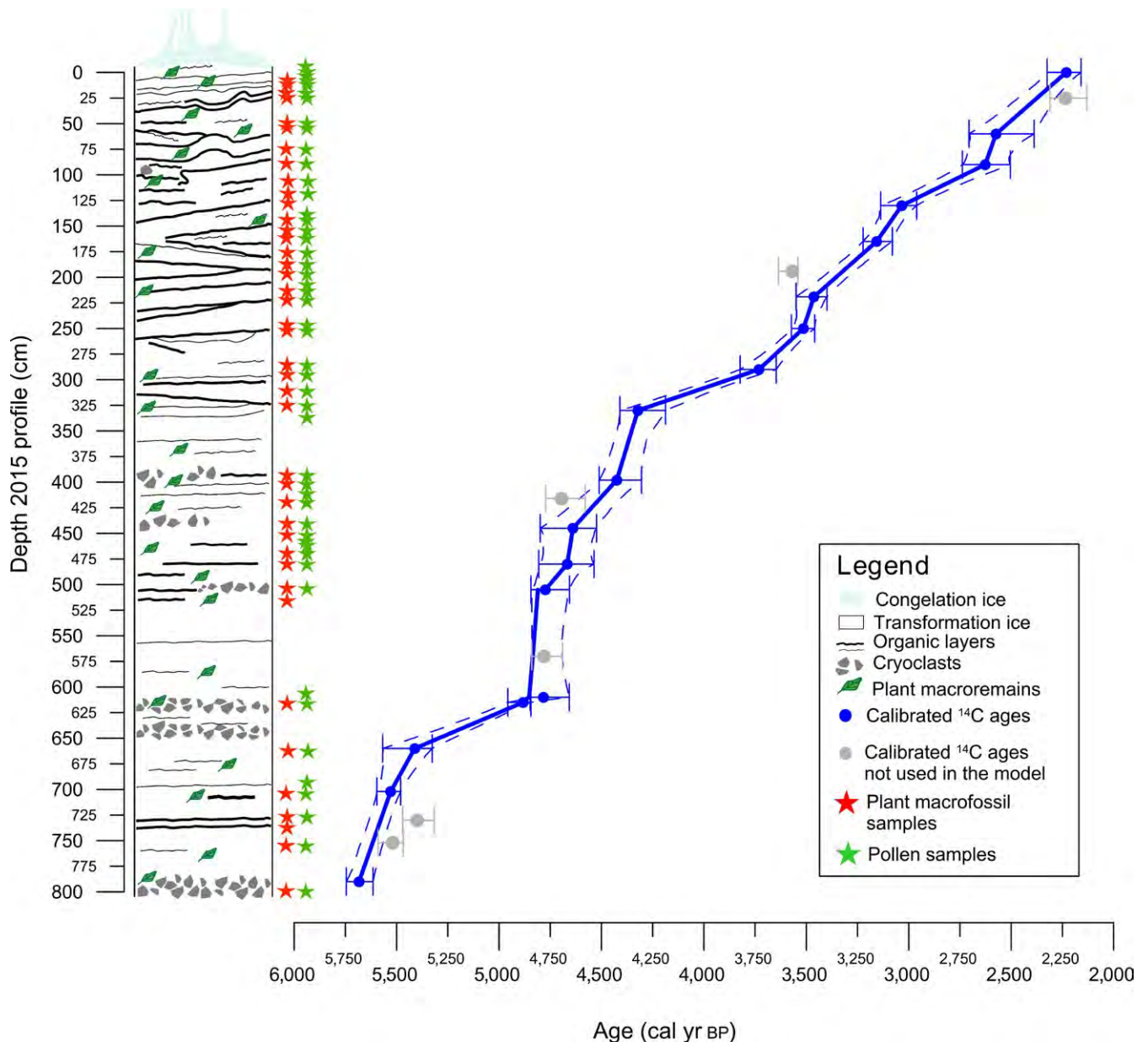


FIGURE 3 Depth-age model of the ice deposit in the Armeña-A294 Ice Cave in July 2015 based on 21 AMS ^{14}C dates

accumulation of snow entering the cave and its subsequent transformation (Figure 2). The ice profile includes detrital and organic-rich layers comprising cryoclastic rock fragments and fine detrital sediments. Organic layers rich in plant macrofossils (e.g., leaves, needles, seeds, fruits) are also common. The volume of the ice deposit is being reduced substantially with time (Figure 2, for details see Sancho et al., 2018).

The chronology of the Armeña-A294 ice deposit ranges from 5,680 to 2,230 cal year BP. Among the 27 radiocarbon dates, six of them were not considered for the depth–age model as their mean calibrated ages were not in stratigraphic order, although four of these dates (at 25, 416, 570, and 752 cm depth) are within the error of the depth–age model (Figure 3). The four samples dated from the same layer at 165 cm show very similar ages, ~3,100 cal year BP (Table 1), suggesting that the sampled detrital layers represent very short ablation periods (decadal time-scale at a maximum). We eventually built a depth–age model based on lineal interpolation using 21 dates (Figure 3).

4.2 | Pollen and plant macrofossils

Pollen and plant macrofossils are sourced from different spatial scales at the study site: while pollen may mostly reflect regional vegetation (up to 20–50 km) (Canellas-Boltà, Rull, Vigo, & Mercade, 2009) at exposed high elevation sites like the Armeña-A294 Ice Cave, plant macrofossils primarily represent local vegetation composition (c. 1–100 m around the site; Birks, 2003; Tinner, 2007). The Armeña-A294 plant macrofossil record is remarkably diverse because of the extraordinary preservation of the plant remains that, in turn, allowed the precise identification of a number of subalpine and alpine taxa (Figure 4). The record of *P. uncinata* remains is particularly diverse, including branches, entire dwarf shoots (brachyblasts), needles, fragments of male cones (with pollen inside), seeds, seed-wings and periderm. Some of these macrofossils are relatively heavy (e.g., branches) and therefore not easily transported far from their source, thus reinforcing their value as indicators of the local presence of *P. uncinata*. Additionally, *P. uncinata* macrofossils are almost continuously present, in varying amounts, throughout the sequence, indicating that mountain pines were locally abundant at ~2,250 m a.s.l. between c. 5,700 and 2,200 cal year BP. The interpretation of *Betula* macrofossils, the other relevant tree species at the Armeña-A294 sequence, is not so straightforward. In contrast to most macrofossils, where single findings indicate local presence, the winged seeds of *Betula* may be easily transported by wind. The common occurrence of fruit scales might be a better indicator of local presence (Tinner & Theurillat, 2003).

The pollen record consists of six statistically significant LPAZs (named, from base to top, A294-P6 to A294-P1), which were used to describe the pollen- and macrofossil-inferred vegetation history around the study site as both proxies present very similar trends and representing macrofossil zones may not reflect a real ecological meaning (Figure 5).



FIGURE 4 Pictures of different plant macrofossils found in the ice deposit of the Armeña-A294 Ice Cave (2,238 m a.s.l.). (a) *Pinus uncinata* needle fascicle. (b) *P. uncinata* seed-wing. (c) *P. uncinata* branch. (d) *Abies alba* seed. (e) *Betula* seeds. (f) *Arctostaphylos uva-ursi* leaf. (g) *Vaccinium myrtillus* branch. (h) *Salix herbacea* leaf. (i) *D. octopetala* leaf

4.2.1 | A294-P6; 790–462.5 cm depth; 5,700–4,650 cal year BP

The base of the pollen sequence is dominated by temperate trees and shrubs such as *Corylus*, *Betula*, *Alnus*, and *Quercus* (Figure 5). Both *Betula* seeds and seed scales are present during this period, although their number is not very high (Figure 5). *Pinus* pollen is not particularly abundant (25%–30%), but several *P. uncinata* needles occur. *Abies* pollen percentages gradually increase towards the top of the zone (up to 5%), with a single *Abies alba* Mill seed also present (Figure 5). *Juniperus* pollen values reach 20% at the base of the zone and then decrease to 5%. Herb pollen represents c. 15% of the total pollen sum mainly dominated by Poaceae, Fabaceae, and Rosaceae (Figure 5). Dwarf shrub and herb macrofossils such as Poaceae stems, *D. octopetala* leaves, Asteraceae inflorescences, and Caryophyllaceae seeds occur only sporadically in this zone (Figure 5).

4.2.2 | A294-P5; 462.5–322 cm depth; 4,650–4,200 cal year BP

Pinus dominates during this period as shown by the pollen (c. 45%), stomata, and macrofossil records (Figure 5). *Corylus*, *Betula*, and *Alnus* pollen abundances decline while *Abies* pollen percentage increases to a maximum (15%; Figure 5). Other remarkable features of the macrofossil

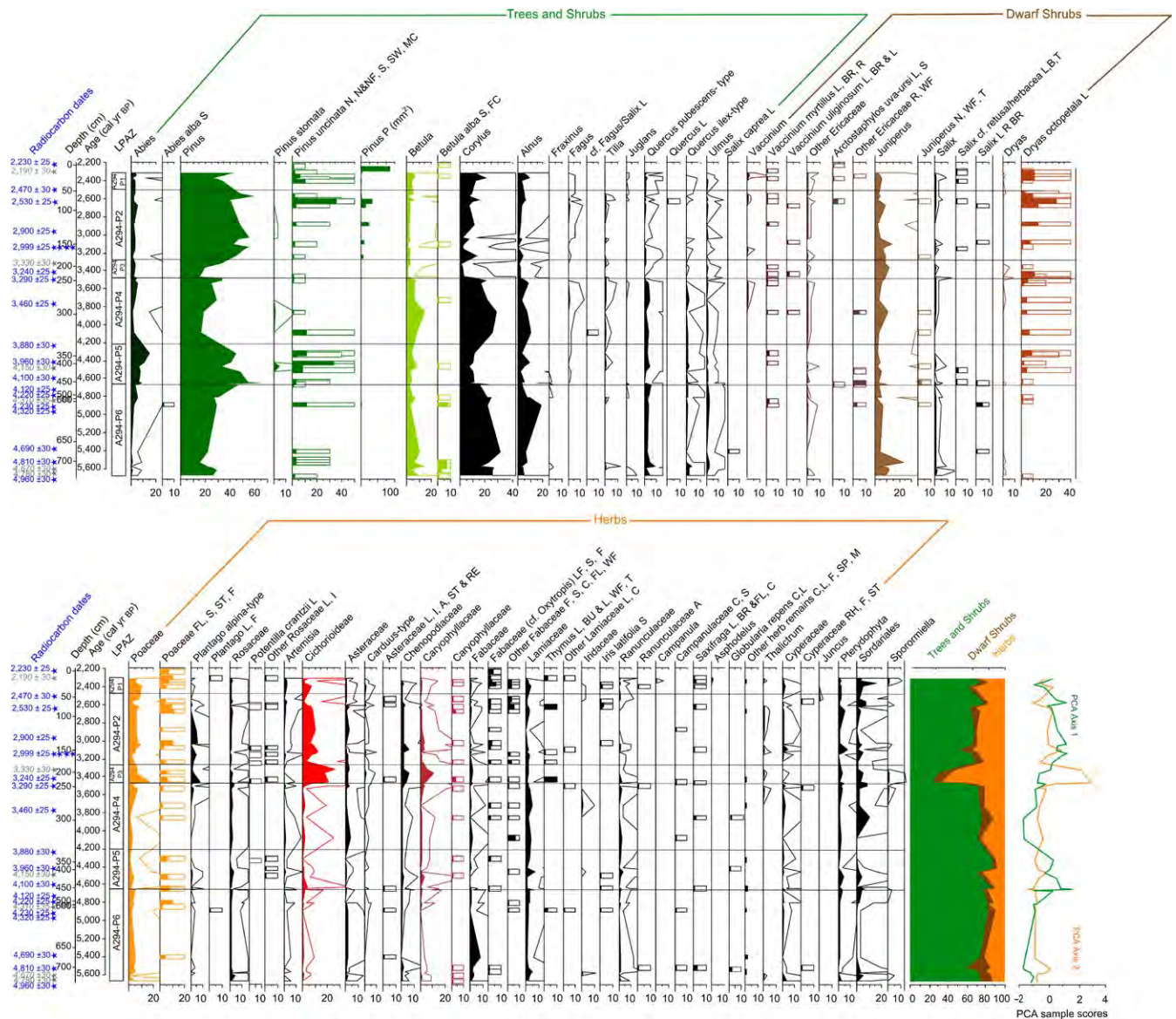


FIGURE 5 Combined diagram of pollen percentage (curves) (>1%), stomata (curve), and plant macrofossil concentration (bars) showing main taxa of the Armeña-A294 Ice Cave (2,238 m a.s.l.). PCA axis 1 and 2 sample scores of the pollen record are also shown. Plant macrofossil concentrations are expressed as total number per 750 cm³ sample. Several taxa have been included within plant macrofossil groups/families: other Ericaceae: cf. *Calluna vulgaris* (L.) Hull; Rosaceae: *Alchemilla*, *Potentilla crantzii* (Crantz) G. Beck ex Fritsch; Other Fabaceae: *Vicia*, *Medicago suffruticosa* Ramond ex DC; Caryophyllaceae: cf. *Sagina saginoides* (L.) Karsten/Minuartia, *Scleranthus*, *Silene*; Cyperaceae: *Kobresia myosuroides* (Vill.) Fiori; Other herb remains: *Androsace ciliata* DC., *Pilosella*, *Corydalis*, *Chaerophyllum*, *Hellianthemum*, *Sideritis hyssopifolia* L., *Meum athamanticum* Jacq. Empty bars represent 10× exaggerations. F = fruit, N = needle, NF = needle fascicle, S = seed, SW = seed-wing, P = periderm (expressed as mm²), MC = male cone, BR = branch, L = leaf, B = bud, FL = flower, ST = stem, I = inflorescence, LF = leaflet, C = calyx, SP = seed pod, A = achene, RE = receptacle, BU = burl, M = mericarp, RH = rhizome, FC = fruit scale, R = root, WF = wood fragment; T = twig

record are the presence of *Vaccinium* and other Ericaceae remains and an increase in *D. octopetala* leaves and Poaceae fragments (Figure 5).

4.2.3 | A294-P4; 322–223 cm depth; 4,200–3,450 cal year BP

Pinus pollen percentages decrease again (to c. 20%) (Figure 5) as well as *Pinus* macrofossil concentrations (Figure 5), whereas an increase in *Betula*, *Corylus*, and *Alnus* pollen percentages is observed. *Abies* pollen

curve experiences an important decrease (c. 3%). Herb pollen abundances slightly increase reaching 20%–25% (Figure 5). *D. octopetala* leaves maintain relatively abundant throughout this zone (Figure 5).

4.2.4 | A294-P3; 223–187 cm depth; 3,450–3,250 cal year BP

A sharp increase in herb pollen percentages (c. 65%) led by Cichorioideae, Poaceae, *Plantago*, Chenopodiaceae, and

Caryophyllaceae occurs, and consequently, the tree pollen significantly decreases (Figure 5). We observe a slight increase in the fungal spores *Sporormiella* and *Sordaria*. The macrofossil record shows a conspicuous absence of any arboreal remains (Figure 5).

4.2.5 | A294-P2; 187–42.5 cm depth; 3,250–2,450 cal year BP

Tree pollen recovers, especially *Pinus* (c. 50%), while herbs decrease although are still abundant (25%–30%) (Figure 5). Increases in *Pinus*, *Vaccinium*, *Arctostaphylos uva-ursi* (L.) Spreng., *Salix* (cf. *retusa* L./*herbacea* L.), and particularly in *D. octopetala* leaves are the major features of the macrofossil record in this zone (Figure 5). Other herbs such as Poaceae, *Medicago*, Fabaceae, *Oxytropis*, Caryophyllaceae, *Saxifraga*, and *Iris* are also represented in the macrofossil record for this period (Figure 5).

4.2.6 | A294-P1; 42.5–14 cm depth; 2,450–2,300 cal year BP

Corylus, *Betula*, and *Alnus* pollen slightly increase, while *Pinus* pollen and macrofossils slightly decrease except for *Pinus* periderm (Figure 5). *D. octopetala* as well as herbaceous macrofossils generally increase, with Fabaceae, Poaceae, and *Saxifraga* as the main taxa.

4.3 | Ordination analysis

The first and the second axes of the PCA explain 58.02% and 21.63% of the total variation in the pollen dataset respectively (Figure 6). The first axis is mainly driven by *Pinus* abundance, as evidenced by the PCA biplot (Figure 6) and the sample's factor scores (Figure 8). The underlying ecological gradient associated to PCA axis 1 reflects forest composition, with light-demanding montane taxa (*Corylus*, *Betula*, *Alnus*, *Quercus*) having low PCA axis 1 scores and shade tolerant and/or subalpine taxa *Abies* and *Pinus* reaching high scores (Figure 6). PCA axis 2 accounts for vegetation openness, with herbs showing positive values and forest taxa, negative scores (Figures 5 and 6).

5 | DISCUSSION

5.1 | Altitudinal tree range fluctuations at the end of the HTM

Our Armeña-A294 Ice Cave record suggests that the tree line ecotone with sparse *P. uncinata* trees (relatively low *Pinus* pollen percentages and limited amounts of *P. uncinata* macrofossils, Figures 5–8) was present at ~2,250 m a.s.l. during the interval 5,700–4,650 cal year BP, at the end of the HTM. We cannot assess the maximum altitude reached by the tree line in the region, as the current study is the only macrofossil sequence available so far, but we can confirm that *P. uncinata* was located at least 200 m above its present range

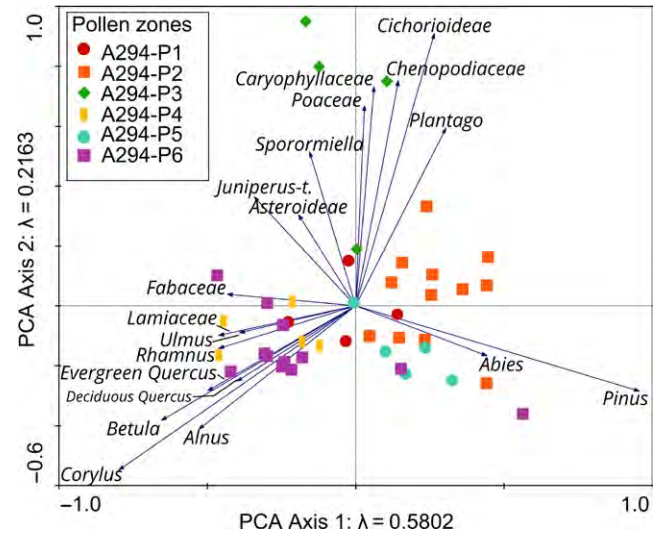


FIGURE 6 Principal component analysis (PCA) scatterplot of pollen taxa. The first axis explains 58.02% of the variance of the dataset and the second axis 21.63%

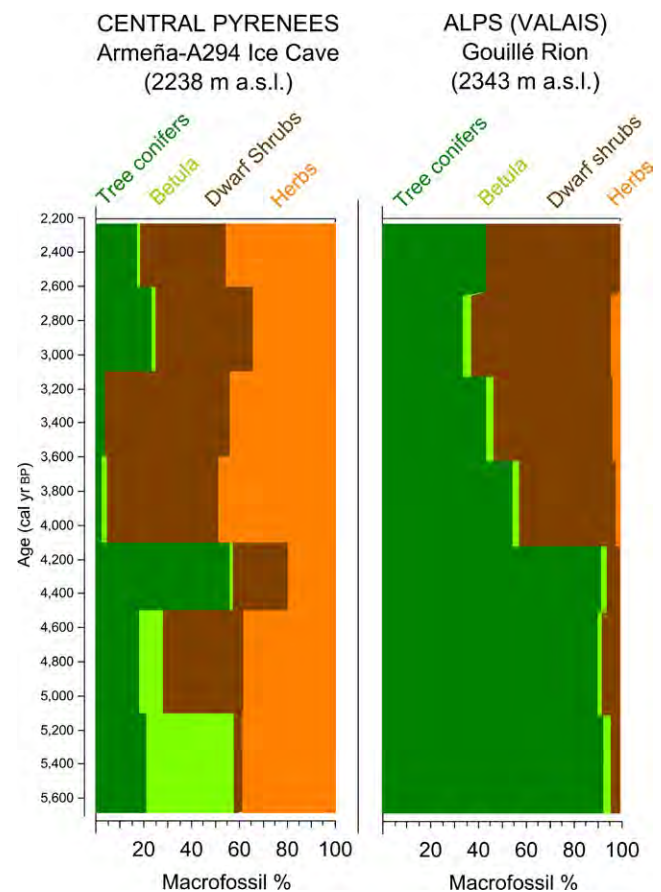


FIGURE 7 Macrofossil percentage diagrams of the Armeña-A294 Ice Cave (2,238 m a.s.l.) compared to the Gouillé Rion Lake record in the Swiss Alps (Tinner & Kaltenrieder, 2005; Tinner & Theurillat, 2003; Tinner, Ammann, & Germann, 1996). The diagrams were subdivided into ca. 500-year-bins. Note the decreasing trend in tree macrofossils from c. 4,200 cal year BP towards present in both records

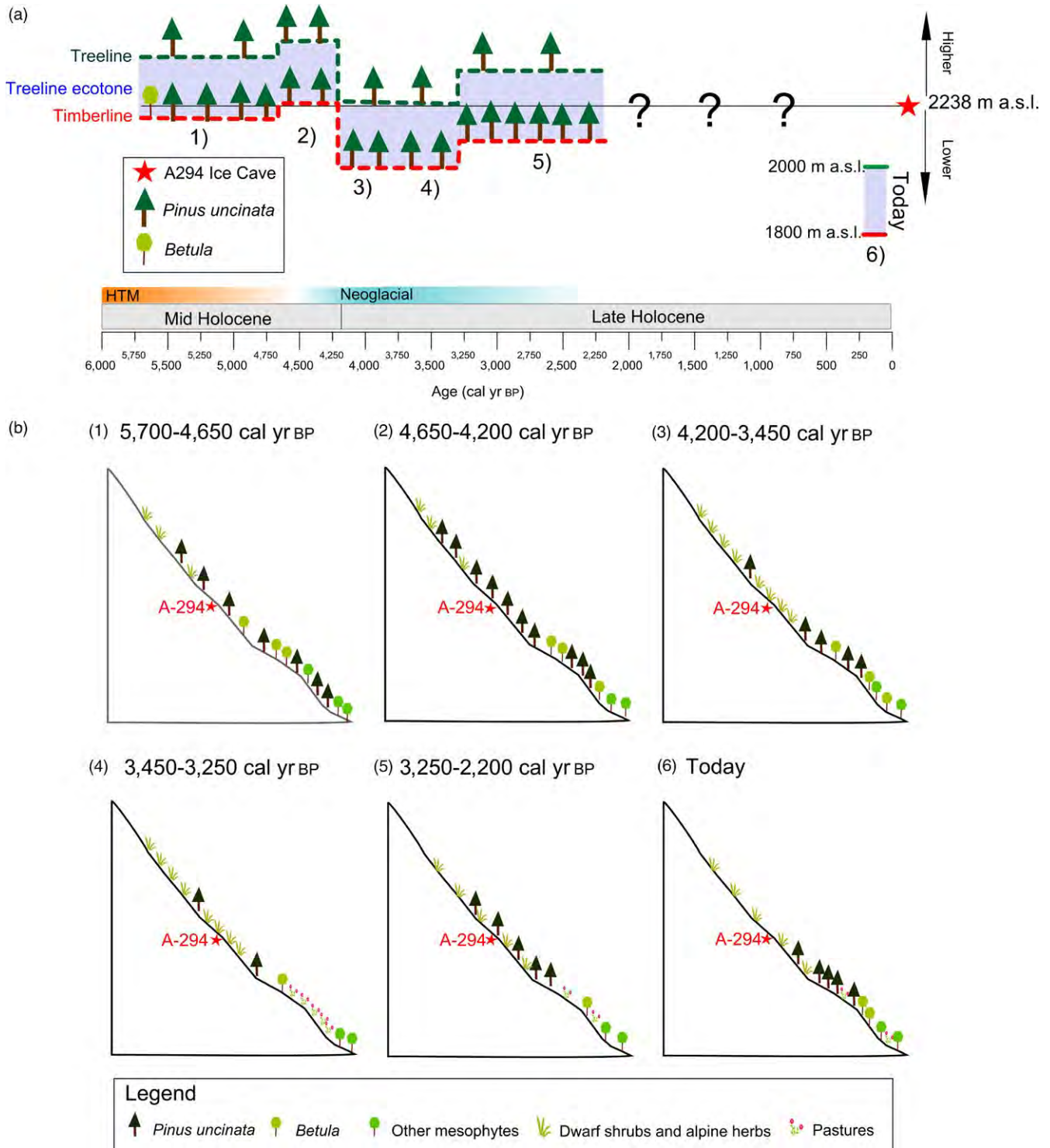


FIGURE 8 (a) Potential high-altitude tree range shifts in the Armeña cirque from 5,700 cal year BP to present based on the palaeobotanical data from the Armeña-A294 Ice Cave. Note that the height difference between the tree line and the timberline from 5,700 to 2,200 cal year BP does not reflect the real distance between both limits. To the right, current altitudes of the tree line and timberline are represented (2,000 and 1,800 m a.s.l. respectively). (b) Tentative reconstruction of the vegetation changes between 5,700 and 2,200 cal year BP in the Armeña Valley for different time windows, based on the palaeobotanical data from the Armeña-A294 Ice Cave

(Figure 8). Cunill et al. (2012) also inferred a higher than today tree line using soil charcoal analysis in the Eastern Pyrenees. The finding of seeds, fruit scales, and moderate pollen percentages suggest that

Betula might also have been growing not far from the ice cave and therefore within the tree line ecotone from 5,700 to 4,900 cal year BP (Figures 5, 7 and 8).

In the nearby Basa de la Mora Lake (BSM) sequence (1,914 m a.s.l.; Figure 1a,c), no *Betula* macrofossils were found, but the maximum pollen values of *Betula* (up to 26%) occurred at 8,100–5,700 cal year BP (Pérez-Sanz et al., 2013), suggesting that *Betula* could have reached the subalpine belt during this period. The Marboré Lake (MAR) sequence (2,612 m a.s.l.; Figure 1a) also records high deciduous forest pollen percentages until 5,200 cal year BP (Leunda et al., 2017), coinciding with HTM (Renssen et al., 2009). The chironomid-based summer temperature reconstruction from BSM shows consistently high July air temperatures in the Cotiella Massif from c. 9,000 to 6,200 cal year BP (Tarrats et al., 2018), concurring with the maximum expansion of pioneer, moisture-demanding trees, particularly *Betula* (Pérez-Sanz et al., 2013).

Betula is a heliophilous pioneer tree that was likely colonizing subalpine habitats such as open slopes, avalanche chutes, and cliffs (Costa, Morla, & Sainz, 1997) during the HTM. *Betula* macrofossils found in the ice cave (Figures 5 and 7) might represent the final stage of the trees' highest altitudinal position. It is thus likely that under warmer-than-present HTM conditions (Fischer et al., 2018), *P. uncinata* and *Betula* coexisted in the tree line ecotone until c. 4,900 cal year BP at ~2,250 m a.s.l. (Figure 8). This contrasts with the current tree line composition in the south central Pyrenees, always formed by *P. uncinata* communities irrespective of the soil conditions (Ninot et al., 2007) generally at c. 2,000–2,200 m a.s.l., while *Betula pendula* Roth grows up to 1,850 m a.s.l., only occasionally reaching 2,200 m a.s.l. (Villar, Sesé, & Ferrández, 1997). Currently, conifers are the most successful taxa forming the tree line in temperate mountain ranges (Körner, 2012; Schwörer et al., 2015), including the Pyrenees, and *Betula* only co-occurs. At higher latitudes, though, *Betula* is the main taxon in boreal tree line communities, increasingly admixed with *P. sylvestris* at lower latitudes (Bjune, 2005; Körner, 2012; Lang, 1994).

After c. 4,900 cal year BP, the range of *Betula* shifted downwards (declining pollen and macrofossil values, Figures 5, 7, and 8). Similarly, other deciduous taxa such as *Corylus* and *Alnus* (Figure 5) declined. Meanwhile, *P. uncinata* persisted locally and alpine dwarf shrubs like *S. retusa*, *S. herbacea*, *J. communis* ssp. *alpina*, *Vaccinium*, and *Calluna* established near the Armeña-A294 Ice Cave (Figure 5). *Betula* might have moved downwards in response to changed environmental conditions (e.g., less summer insolation, cooler summers at the end of the HTM) that increased competition with more shade tolerant *P. uncinata* (Beck, Caudullo, de Rigo, & Tinner, 2016; Costa et al., 1997).

Higher abundances of *P. uncinata* macrofossils and *Pinus* pollen at c. 4,650–4,200 cal year BP indicate that mountain pine expanded locally, forming relatively closed stands, locating timberline at ~2250 m a.s.l., c. 400 m higher than nowadays (Figure 8). However, while a stronger pollen and macrofossil in-wash related to a wetter climate would have also fostered larger remains abundance, in our study case, this increase is not general for all taxa and mainly happens to pines and *D. octopetala*. We, thus, infer these taxa locally spread. Scattered *A. alba* were likely growing together with mountain pines, as suggested by high *Abies* pollen percentages (Figures 5 and 6). However, given the lack of significant amounts of macrofossils (a single *A. alba*

seed found; Figure 5), we hypothesize that silver fir was not abundant at this altitude. In the Eastern Pyrenees of Andorra, the occurrence of pine stomata in both Riu del Orris (RDO; 2,390 m a.s.l.) and Bosc dels Estanyons (BDE; 2,180 m a.s.l.; Figure 1a) also points to the existence of local forested landscapes at high altitudes from c. 6,000 to 4,300 cal year BP (Ejarque, Miras, Riera, Palet, & Orengo, 2010). Wetter winters have been inferred from ice accumulation rates in the Armeña-A294 Ice Cave during 4,900–4,200 cal year BP (Sancho et al., 2018), which implies increased snowfall at the onset of the NGC, while the low chironomid content in BSM for the period c. 5,700–4,200 cal year BP prevented quantitative summer temperature reconstructions (Tarrats et al., 2018). Wetter winters likely caused increased meltwater during late-spring/early summer, which in turn might have favoured pine forest expansion as summer drought may have also been a limiting factor for *P. uncinata* (Galván et al., 2015) during the HTM, when *Betula* was dominant.

Higher-than-present tree lines and timberlines have also been recorded in most Eurasian and North American mountain ranges during the HTM (Fischer et al., 2018; Tinner, 2007). Regarding site-specific variations, both tree line and timberline in the Alps show highest positions at c. 10,000–4,500 cal year BP (Heiri et al., 2014; Tinner & Theurillat, 2003). For instance, at Gouillé Rion (2,343 m a.s.l.), located in the Upper Rhone Valley (Swiss Alps), timberline persisted until the onset of the NGC at c. 4,500 cal year BP (Figure 7). Both timberline and tree line were also higher in the Carpathians and the Scandes during the HTM until c. 5,000–4,500 cal year BP (e.g., Badino et al., 2018; Barnekow, 1999; Bergman, Hammarlund, Hannon, Barnekow, & Wohlfarth, 2005; Eide et al., 2006; Feurdean et al., 2016; Orbán et al., 2017).

5.2 | Vegetation responses to the NGC

The marked decreases in macrofossil concentrations and pollen percentages of *P. uncinata* and *Abies* indicate that their abundance significantly decreased in the Armeña cirque after c. 4,200 cal year BP, most probably associated to a marked downward shift of forest communities (Figures 5–8). *D. octopetala*-dominated communities were likely located above ~2,250 m a.s.l. until c. 4,200 cal year BP, when they expanded downwards to establish in the vicinity of the ice cave (Figures 5, 7 and 8). *D. octopetala* is a pioneer arctic-alpine species that usually colonizes open, dry, and sunny calcareous sites (Elkington, 1971). Other alpine grassland and scree species like *Potentilla crantzii*, *Globularia repens* Lam., or *Saxifraga aizoides* L. also established around the cave during this period; hence, we conclude that alpine tundra grasslands expanded to lower elevations in the Cotiella Massif from c. 4,200 cal year BP onwards.

After the HTM northern hemisphere summer temperatures declined during the NGC (Davis, Menounos, & Osborn, 2009; Kumar, 2011). In the Central Pyrenees, glaciers began to expand at c. 5,100 ± 100 cal year BP, in both the southern (García-Ruiz et al., 2014) and the northern (Gellatly, Grove, & Switsur, 1992) slopes. This is quite consistent with the chironomid-based temperature reconstruction from BSM that shows lowest summer temperatures of the Holocene at 4,200–2,000 cal year BP (Tarrats et al., 2018).

The increase in *Corylus*, *Betula*, *Alnus*, *Fagus*, *Tilia*, and *Quercus ilex* L. at c. 4,200–3,450 cal year BP (Figure 5) may reflect pollen catchment changes that may have controlled the pollen signal. Specifically, the opening of the forest and therefore of the canopy may have permitted long-distance pollen to become relatively more abundant (less pollen produced locally by trees) or alternatively to be deposited at the study site (lost pollen-filtering function of forests).

Our ice cave record thus evidences a strong sensitivity of Pyrenean ecosystems and vegetation to climatic changes such as the HTM and NGC. Although tree lowering in the Cotiella Massif during c. 4,200–3,450 was mostly controlled by climate as discussed above, the increase in herbs together with a slight increase in coprophilous *Sporormiella* at 3,450 cal year BP suggests pastoral activities during this period that may have affected the range, structure, and composition of forests. Other coprophilous fungi as *Sordaria*, do not show marked changes (Figure 5) though, indicating that some fungal spores might also have been contributed by wild ungulates (Baker, Cornelissen, Bhagwat, Vera, & Willis, 2016).

The pollen record including PCA axis 2 (Figure 6) also evidences a marked forest opening at 3,450–3,250 cal year BP. However, macrofossils do not show any abrupt change during this period but confirm the lack of forest around the study site, with *D. octopetala* present in high numbers (Figures 5 and 7). Studies on the effect of grazing activities on *D. octopetala* populations show that *Dryas* heaths might be quickly eliminated by intense grazing, giving rise to herb-rich grasslands (McVean & Ratcliffe, 1962). As *D. octopetala* does not show any response to this perturbation on the local scale, we assume that the forest clearing and subsequent pastoral activities occurred at lower altitudes. However, regional, large-scale forest clearings would have been also recorded in the nearby BSM lake sequence (Pérez-Sanz et al., 2013), which is not the case. Likewise, no abrupt vegetation changes are recorded at the nearby Marboré sequence (2,612 m a.s.l.) or in other lacustrine sequences of Central Pyrenees (González-Sampérez et al., 2017; Leunda et al., 2017). This suggests that human activities were confined to the eastern slope of the Cotiella Massif during this time period, indicating a rather localized use of prehistoric forest resources.

Human impact on the vegetation has an uneven spatial distribution in mountain environments. In the Pyrenees, for instance, some sites record a rather early human impact, since the onset of the Neolithic (c. 7,000 cal year BP) or even earlier (Ejarque et al., 2010; Pérez-Obiol et al., 2012), while at other sites, human activities were persistent and intense only since the Bronze Age (c. 4,000 cal year BP; Bal, Rendu, Ruas, & Campmajo, 2010; Galop & Jalut, 1994) or were even not noticeable at high altitudes until the last c. 1,000 years (González-Sampérez et al., 2017; Leunda et al., 2017; Pérez-Sanz et al., 2013).

At the European scale, notable depressions of timberline (more than tree line) occurred after c. 5,000–4,000 cal year BP. In the Alps, the forest downward shift (Figure 7, c. –300 to 400 m) has been primarily attributed to anthropogenic impact, with climate playing a subordinate role, while the tree line position (c. –100 to 200 m) was mainly controlled by climate (e.g., Heiri et al., 2014; Tinner &

Theurillat, 2003). However, climate has been interpreted as the main driver modifying vegetation dynamics in the La Thuile Valley (Northern Italian Alps; Badino et al., 2018). Likewise, human impact already amplified the effects of the regional cooling since c. 4,200 cal year BP in the Retezat Mountains (Southern Carpathians; Orbán et al., 2017) whereas in the Rodna Mountains, the lowering of the timberline and tree line ecotone was not strongly impacted by humans until c. 3,500 cal year BP (Feurdean et al., 2016).

In the Armeña cirque surrounding the ice cave, the tree line ecotone reestablished after c. 3,250 cal year BP at ~2,250 m a.s.l. (Figures 5, 7 and 8). Locally, scattered *P. uncinata* together with well-developed alpine grasslands and scrub tundra composed of mainly *D. octopetala*, *Salix herbacea*, *S. retusa*, *Juniperus*, and *Vaccinium* occurred (Figures 5, 7 and 8). Both *J. communis* ssp. *alpina* and *D. octopetala* are shade-intolerant species (Ellenberg et al., 1992), so it is highly unlikely that the shrub communities were growing in a closed forest, but in open communities with a wide variety of microhabitats coexisting, that is, sparse pines, dwarf shrubs, and herbaceous species (Figure 8). A similar situation was also found far below tree line Tramacastilla Lake (1,380 m a.s.l.), where forest quickly recovered after the first human-induced deforestation dated at c. 4,000 cal year BP (Montserrat-Martí, 1992).

The tree line ecotone persisted until c. 2,200 cal year BP, when the ice sequence preserved in the Armeña-A294 cave ends (Figure 3). As mentioned above, trees are absent nowadays at the study site (Figures 1 and 8), which indicates that after 2,200 cal year BP large disturbances, either climatic or human, might have led to a depression of both tree line and timberline until their current position, at c. 2,000 and 1,800 m a.s.l., respectively. Other Central Pyrenean sequences such as Basa de la Mora and Marboré (Figure 1a) also show landscape opening during the last millennia under increased human pressure (Leunda et al., 2017; Pérez-Sanz et al., 2013). As a future forecasting, we expect that both tree line and timberline will react to the projected climate warming and land-use abandonment, by shifting upwards to higher elevations, as recorded during the late HTM.

6 | CONCLUSIONS

Here, we produced the first long-term reconstruction of tree line dynamics in the Pyrenees proving that subalpine forests dominated by *Betula* and by *P. uncinata* were growing at altitudes 200–400 m higher than today during the HTM, when summer conditions were warmer. After the onset of pastoral activities c. 3,600 years ago, it is difficult to disentangle climatic and human impacts. However, it is clear that the NGC promoted the expansion of arctic-alpine species such as *D. octopetala* and that this process was likely exacerbated by prehistoric land use. Given the decline in grazing activities during the past decades in the Pyrenean alpine meadows and the rise in average temperature, both as a consequence of global change, trees may rapidly move upslope during the next decades to reach HTM positions. Even if other important controls such as poor soil development might markedly reduce the spread of tree species towards

higher altitudes (Henne, Elkin, Reineking, Bugmann, & Tinner, 2011), exceeding HTM temperature conditions may result in an unprecedented pressure on arctic alpine species, potentially affecting biodiversity. Our study emphasizes the high palaeoecological, ecological, and environmental significance of perennial ice deposits within caves. These archives, however, are severely endangered by rising temperatures. More scientific attention is needed in order to rescue their untapped scientific information that may contribute to better assessments of future ecosystem dynamics under global change conditions.

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AUTHORS' CONTRIBUTIONS

M.L., C.Sa., P.G.-S., G.G.-R., C.M.-M., C.Sc., and W.T. conceived the ideas and designed the research; M.L., C.Sa., M.B., and Á.B. collected the material; M.L., D.G.-G., C.M.-M., C.Sc., P.K., and J.M.R. identified the material and analysed data; M.L., C.M.-M., C.Sc., W.T., P.G.-S., and G.G.-R. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

All data are deposited in the European Pollen Database (<http://www.europeanpollendatabase.net/index.php>).

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REFERENCES

- Ammann, B., van der Knaap, W. O., Lang, G., Gaillard, M.-J., Kaltenrieder, P., Rösch, M., Tinner, W. (2014). The potential of stomata analysis in conifers to estimate presence of conifer trees: Examples from the Alps. *Vegetation History and Archaeobotany*, 23(3), 249–264. <https://doi.org/10.1007/s00334-014-0431-9>
- Badino, F., Ravazzi, C., Vallè, F., Pini, R., Aceti, A., Brunetti, M., Orombelli, G. (2018). 8800 years of high-altitude vegetation and climate history at the Rutor Glacier forefield, Italian Alps. Evidence of middle Holocene timberline rise and glacier contraction. *Quaternary Science Reviews*, 185, 41–68. <https://doi.org/10.1016/j.quascirev.2018.01.022>
- Baker, A. G., Cornelissen, P., Bhagwat, S. A., Vera, F. W. M., & Willis, K. J. (2016). Quantification of population sizes of large herbivores and their long-term functional role in ecosystems using dung fungal spores. *Methods in Ecology and Evolution*, 7(11), 1273–1281. <https://doi.org/10.1111/2041-210X.12580>
- Bal, M., Rendu, C., Ruas, M.-P., & Campmajo, P. (2010). Paleosol charcoal: Reconstructing vegetation history in relation to agro-pastoral activities since the Neolithic. A case study in the Eastern French Pyrenees. *Journal of Archaeological Science*, 37, 1785–1797. <https://doi.org/10.1016/j.jas.2010.01.035>
- Barnekow, L. (1999). Holocene tree-line dynamics and inferred climatic changes in the Abisko area, northern Sweden, based on macrofossil and pollen records. *The Holocene*, 9(3), 253–265. <https://doi.org/10.1191/095968399676322637>
- Beck, P., Caudullo, G., de Rigo, D., & Tinner, W. (2016). *Betula pendula*, *Betula pubescens* and other birches in Europe: Distribution, habitat, usage and threats. In J. San-Miguel-Ayanz, D. de Rigo, G. Caudullo, T. Houston Durrant, & A. Mauri (Eds.), *European atlas of forest tree species* (pp. 70–73). Luxembourg, Europe: Publication Office of the European Union. <https://doi.org/10.2788/038466>
- Belmonte-Ribas, Á., Sancho, C., Moreno, A., López-Martínez, J., & Bartolomé, M. (2014). Present-day environmental dynamics in ice cave A294, central Pyrenees, Spain. *Geografía Física E Dinámica Cuaternaria*, 37, 131–140.
- Bennett, K. D. (1996). Determination of the number of zones in a biostratigraphical sequence. *New Phytologist*, 132, 155–170. <https://doi.org/10.1111/j.1469-8137.1996.tb04521.x>
- Bennett, K. (2009). *Documentation for psimpoll 4.27 and pscomb 1.03: C programs for plotting pollen diagrams and analysing pollen data*. Belfast, UK: Queen's University of Belfast, Department of Archaeology and Palaeoecology.
- Bergman, J., Hammarlund, D., Hannon, G., Barnekow, L., & Wohlfarth, B. (2005). Deglacial vegetation succession and Holocene tree-limit dynamics in the Scandes Mountains, west-central Sweden: Stratigraphic data compared to megafossil evidence. *Review of Palaeobotany and Palynology*, 134(3–4), 129–151. <https://doi.org/10.1016/j.revpalbo.2004.12.005>
- Birks, H. H. (2003). The importance of plant macrofossils in the reconstruction of Lateglacial vegetation and climate: Examples from Scotland, western Norway and Minnesota, USA. *Quaternary Science Reviews*, 22, 453–473. [https://doi.org/10.1016/S0277-3791\(02\)00248-2](https://doi.org/10.1016/S0277-3791(02)00248-2)
- Birks, H. H., & Birks, H. J. B. (2000). Future uses of pollen analysis must include plant macrofossils. *Journal of Biogeography*, 27(1), 31–35. <https://doi.org/10.1046/j.1365-2699.2000.00375.x>
- Birks, H. J. B., & Gordon, A. D. (1985). *Numerical methods in quaternary pollen analysis*. San Diego, CA: Academic Press.
- Bjune, A. E. (2005). Holocene vegetation history and tree-line changes on a north–south transect crossing major climate gradients in southern Norway—Evidence from pollen and plant macrofossils in lake sediments. *Review of Palaeobotany and Palynology*, 133(3–4), 249–275. <https://doi.org/10.1016/j.revpalbo.2004.10.005>
- Blaauw, M. (2010). Methods and code for 'classical' age-modelling of radiocarbon sequences. *Quaternary Geochronology*, 5(5), 512–518. <https://doi.org/10.1016/j.quageo.2010.01.002>
- Camarero, J., García-Ruiz, J. M., Sangüesa-Barreda, G., Galván, J. D., Alla, A. Q., Sanjuán, Y., ... Gutiérrez, E. (2015). Recent and intense dynamics in a formerly static Pyrenean treeline. *Arctic, Antarctic, and Alpine Research*, 47, 773–783. <https://doi.org/10.1657/AAAR0015-001>

- Camarero, J., & Gutiérrez, E. (2004). Pace and pattern of recent treeline dynamics: Response of ecotones to climatic variability in the Spanish Pyrenees. *Climatic Change*, 63(1–2), 181–200. <https://doi.org/10.1023/B:CLIM.0000018507.71343.46>
- Canellas-Boltà, N., Rull, V., Vigo, J., & Mercader, A. (2009). Modern pollen-vegetation relationships along an altitudinal transect in the central Pyrenees (southwestern Europe). *The Holocene*, 19(8), 1185–1200. <https://doi.org/10.1177/0959683609345082>
- Carrión, J. S., Fernández, S., González-Sampériz, P., Gil-Romera, G., Badal, E., Carrión-Marco, Y., Burjachs, F. (2010). Expected trends and surprises in the Lateglacial and Holocene vegetation history of the Iberian Peninsula and Balearic Islands. *Review of Palaeobotany and Palynology*, 162(3), 458–475. <https://doi.org/10.1016/j.revpalbo.2009.12.007>
- Casteret, N. (1946). *Histoires au-dessous de tout*. Paris-Toulouse: Librairie Didier.
- Costa, M., Morla, C., & Sainz, H. (1997). *Los bosques ibéricos. Una interpretación geobotánica*. Barcelona, Spain: Planeta.
- Cunill, R., Soriano, J.-M., Bal, M.-C., Pèlach, A., & Pérez-Obiol, R. (2012). Holocene treeline changes on the south slope of the Pyrenees: A pedoanthracological analysis. *Vegetation History and Archaeobotany*, 21(4–5), 373–384. <https://doi.org/10.1007/s00334-011-0342-y>
- Davis, P. T., Menounos, B., & Osborn, G. (2009). Holocene and latest Pleistocene alpine glacier fluctuations: A global perspective. *Quaternary Science Reviews*, 28(21–22), 2021–2033. <https://doi.org/10.1016/j.quascirev.2009.05.020>
- Eichler, A., Tinner, W., Brüttsch, S., Olivier, S., Papina, T., & Schwikowski, M. (2011). An ice-core based history of Siberian forest fires since AD 1250. *Quaternary Science Reviews*, 30(9), 1027–1034. <https://doi.org/10.1016/j.quascirev.2011.02.007>
- Eide, W., Birks, H. H., Bigelow, N. H., Peglar, S. M., & Birks, H. J. B. (2006). Holocene forest development along the Setesdal valley, southern Norway, reconstructed from macrofossil and pollen evidence. *Vegetation History and Archaeobotany*, 15(2), 65–85. <https://doi.org/10.1007/s00334-005-0025-7>
- Ejarque, A., Miras, Y., Riera, S., Palet, J. M., & Orenco, H. A. (2010). Testing micro-regional variability in the Holocene shaping of high mountain cultural landscapes: A palaeoenvironmental case-study in the eastern Pyrenees. *Journal of Archaeological Science*, 37(7), 1468–1479. <https://doi.org/10.1016/j.jas.2010.01.007>
- Elkington, T. T. (1971). Biological flora of the British Isles. *Journal of Ecology*, 59(3), 887–905.
- Ellenberg, H., Weber, H. E., Düll, R., Wirth, V., Werner, W., & Paulissen, D. (1992). *Zeigerwerte Von Pflanzen in Mitteleuropa*. Göttingen, Germany: Scripta Geobotanica.
- Feurdean, A., Gařka, M., Tanřau, I., Geantă, A., Hutchinson, S. M., & Hickler, T. (2016). Tree and timberline shifts in the northern Romanian Carpathians during the Holocene and the responses to environmental changes. *Quaternary Science Reviews*, 134, 100–113. <https://doi.org/10.1016/j.quascirev.2015.12.020>
- Feurdean, A., Perșoiu, A., Pazdur, A., & Onac, B. P. (2011). Evaluating the palaeoecological potential of pollen recovered from ice in caves: A case study from Scărișoara Ice Cave, Romania. *Review of Palaeobotany and Palynology*, 165(1–2), 1–10. <https://doi.org/10.1016/j.revpalbo.2011.01.007>
- Fischer, H., Meissner, K. J., Mix, A. C., Abram, N. J., Austermann, J., Brovkin, V., Zhou, L. (2018). Palaeoclimate constraints on the impact of 2°C anthropogenic warming and beyond. *Nature Geoscience*, 11, 474–485. <https://doi.org/10.1038/s41561-018-0146-0>
- Galop, D., & Jalut, G. (1994). Differential human impact and vegetation history in two adjacent Pyrenean valleys in the Ariège basin, southern France, from 3000 BP to the present. *Vegetation History and Archaeobotany*, 3(4), 225–244. <https://doi.org/10.1007/BF00195199>
- Galván, J. D., Büntgen, U., Ginzler, C., Grudd, H., Gutiérrez, E., Labuhn, I., & Julio Camarero, J. (2015). Drought-induced weakening of growth-temperature associations in high-elevation Iberian pines. *Global and Planetary Change*, 124, 95–106. <https://doi.org/10.1016/j.gloplacha.2014.11.011>
- Garcés-Pastor, S., Cañellas-Boltà, N., Pèlach, A., Soriano, J.-M., Pérez-Obiol, R., Pérez-Haase, A., Vegas-Vilarrubia, T. (2017). Environmental history and vegetation dynamics in response to climate variations and human pressure during the Holocene in Bassa Nera, Central Pyrenees. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 479, 48–60. <https://doi.org/10.1016/j.palaeo.2017.04.016>
- García-Ruiz, J. M., Palacios, D., de Andres, N., Valero-Garcés, B. L., Lopez-Moreno, J. I., & Sanjuan, Y. (2014). Holocene and “little ice age” glacial activity in the Marboré cirque, Monte Perdido Massif, Central Spanish Pyrenees. *The Holocene*, 24(11), 1439–1452. <https://doi.org/10.1177/0959683614544053>
- Gellatly, A. F., Grove, J. M., & Switsur, V. R. (1992). Mid-Holocene glacial activity in the Pyrenees. *The Holocene*, 2(3), 266–270. <https://doi.org/10.1177/095968369200200309>
- Gill, J. L., McLauchlan, K. K., Skibbe, A. M., Goring, S., Zirbel, C. R., & Williams, J. W. (2013). Linking abundances of the dung fungus *Sporormiella* to the density of bison: Implications for assessing grazing by megaherbivores in palaeorecords. *Journal of Ecology*, 101(5), 1125–1136. <https://doi.org/10.1111/1365-2745.12130>
- González-Sampériz, P., Aranbarri, J., Pérez-Sanz, A., Gil-Romera, G., Moreno, A., Leunda, M., Valero-Garcés, B. (2017). Environmental and climate change in the southern Central Pyrenees since the Last Glacial Maximum: A view from the lake records. *CATENA*, 149, 668–688. <https://doi.org/10.1016/j.catena.2016.07.041>
- Heiri, O., Koinig, K. A., Spötl, C., Barrett, S., Brauer, A., Drescher-Schneider, R., Tinner, W. (2014). Palaeoclimate records 60–8 ka in the Austrian and Swiss Alps and their forelands. *Quaternary Science Reviews*, 106, 186–205. <https://doi.org/10.1016/j.quascirev.2014.05.021>
- Heiri, O., & Lotter, A. F. (2001). Effect of low count sums on quantitative environmental reconstructions: An example using subfossil chironomids. *Journal of Paleolimnology*, 26, 343–350.
- Henne, P. D., Elkin, C. M., Reineking, B., Bugmann, H., & Tinner, W. (2011). Did soil development limit spruce (*Picea abies*) expansion in the Central Alps during the Holocene? Testing a palaeobotanical hypothesis with a dynamic landscape model. *Journal of Biogeography*, 38, 933–949. <https://doi.org/10.1111/j.1365-2699.2010.02460.x>
- Holtmeier, F.-K., & Broll, G. (2005). Sensitivity and response of northern hemisphere altitudinal and polar treelines to environmental change at landscape and local scales: Treeline and environmental change. *Global Ecology and Biogeography*, 14(5), 395–410. <https://doi.org/10.1111/j.1466-822X.2005.00168.x>
- IPPC. (2013). *Climate change 2013: The physical science basis*. Cambridge, UK: Cambridge University Press.
- Körner, C. (2012). *Alpine treelines. Functional ecology of the global high elevation tree limits*. Basel, Switzerland: Springer.
- Krajick, K. (2004). All downhill from here? *Science*, 303(5664), 1600–1602. <https://doi.org/10.1126/science.303.5664.1600>
- Kulakowski, D., Seidl, R., Holeksa, J., Kuuluvainen, T., Nagel, T. A., Panayotov, M., Bebi, P. (2017). A walk on the wild side: Disturbance dynamics and the conservation and management of European mountain forest ecosystems. *Forest Ecology and Management*, 388, 120–131. <https://doi.org/10.1016/j.foreco.2016.07.037>
- Kumar, R. (2011). Neoglaciation. In V. P. Singh, P. Singh, & U. K. Haritashya (Eds.), *Encyclopedia of snow, ice and glaciers* (pp. 775). New York, NY: Springer.
- Lang, G. (1994). *Quartäre Vegetationsgeschichte Europas Methoden und Ergebnisse*. Jena, Germany: G. Fischer.
- Leunda, M., González-Sampériz, P., Gil-Romera, G., Aranbarri, J., Moreno, A., Oliva-Urcia, B., Valero-Garcés, B. (2017). The Late-Glacial and Holocene Marboré Lake sequence (2612 m a.s.l., Central Pyrenees, Spain): Testing high altitude sites sensitivity to millennial scale vegetation and climate variability. *Global and Planetary Change*, 157, 214–231. <https://doi.org/10.1016/j.gloplacha.2017.08.008>

- Luetscher, M., & Jeannin, P.-Y. (2004). A process-based classification of alpine ice caves. *Theoretical and Applied Karstology*, 17, 5–10.
- McVean, D. N., & Ratcliffe, D. A. (1962). *Plant communities of the Scottish Highlands. Monographs of the nature Conservancy, No. 1*. London, UK: H.M.S.O.
- Montserrat-Martí, G. (1989). Paisaje vegetal y síntesis de la vegetación del macizo de Cotiella y la sierra de Chía (Prepirineo central de Huesca). *Lucas Mallada, Instituto De Estudios Altoaragoneses*, 1, 119–132.
- Montserrat-Martí, J. (1992). Evolución Glaciar y Postglaciar del clima y la vegetación en la vertiente sur del Pirineo. Estudio Palinológico. Zaragoza, Spain: Monografías del Instituto Pirenaico de Ecología-CSIC.
- Moore, P. D., Webb, J. A., & Collison, M. E. (1991). *Pollen analysis* (2nd ed., pp. 1–216). Oxford, UK: Blackwell Scientific Publications.
- Ninot, J. M., Carrillo, E., Font, X., Carreras, J., Ferré, A., Masalles, R. M., Vigo, J. (2007). Altitude zonation in the Pyrenees. A geobotanic interpretation. *Phytocoenologia*, 37(3), 371–398. <https://doi.org/10.1127/0340-269X/2007/0037-0371>
- Orbán, I., Birks, H. H., Vincze, I., Finsinger, W., Pál, I., Marinova, E., Magyari, E. K. (2017). Treeline and timberline dynamics on the northern and southern slopes of the Retezat Mountains (Romania) during the late glacial and the Holocene. *Quaternary International*, 477, 59–78. <https://doi.org/10.1016/j.quaint.2017.03.012>
- Pérez-Obiol, R., Bal, M.-C., Pélachs, A., Cunill, R., & Soriano, J. M. (2012). Vegetation dynamics and anthropogenically forced changes in the Estanilles peat bog (southern Pyrenees) during the last seven millennia. *Vegetation History and Archaeobotany*, 21(4–5), 385–396. <https://doi.org/10.1007/s00334-012-0351-5>
- Pérez-Sanz, A., González-Sampériz, P., Moreno, A., Valero-Garcés, B., Gil-Romera, G., Rieradevall, M., Navas, A. (2013). Holocene climate variability, vegetation dynamics and fire regime in the central Pyrenees: The Basa de la Mora sequence (NE Spain). *Quaternary Science Reviews*, 73, 149–169. <https://doi.org/10.1016/j.quascirev.2013.05.010>
- Perşoiu, A., & Lauritzen, S.-E. (2018). *Ice caves*. Amsterdam, the Netherlands: Elsevier.
- Pini, R., Ravazzi, C., Raiteri, L., Guerreschi, A., Castellano, L., & Comolli, R. (2017). From pristine forests to high-altitude pastures: An ecological approach to prehistoric human impact on vegetation and landscapes in the western Italian Alps. *Journal of Ecology*, 105(6), 1580–1597. <https://doi.org/10.1111/1365-2745.12767>
- R Development Core Team. (2016). *R: Language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Reille, M. (1992). *Pollen et spores d'Europe et d'Afrique du Nord*. Marseille, France: Laboratoire de Botanique Historique et Palynologie.
- Reimer, P. J., Bard, E., Bayliss, A., Beck, J. W., Blackwell, P. G., Ramsey, C. B., van der Plicht, J. (2013). IntCal13 and Marine13 radiocarbon age calibration curves 0–50,000 cal years BP. *Radiocarbon*, 55(4), 1869–1887. https://doi.org/10.2458/azu_js_rc.55.16947
- Renssen, H., Seppä, H., Heiri, O., Roche, D. M., Goosse, H., & Fichefet, T. (2009). The spatial and temporal complexity of the Holocene thermal maximum. *Nature Geoscience*, 2(6), 411–414. <https://doi.org/10.1038/ngeo513>
- Samartin, S., Heiri, O., Joos, F., Renssen, H., Franke, J., Brönnimann, S., & Tinner, W. (2017). Warm Mediterranean mid-Holocene summers inferred from fossil midge assemblages. *Nature Geoscience*, 10(3), 207–212. <https://doi.org/10.1038/ngeo2891>
- Sancho, C., Belmonte, Á., Bartolomé, M., Moreno, A., Leunda, M., & López-Martínez, J. (2018). Middle-to-late Holocene palaeoenvironmental reconstruction from the A294 ice-cave record (Central Pyrenees, northern Spain). *Earth and Planetary Science Letters*, 484, 135–144. <https://doi.org/10.1016/j.epsl.2017.12.027>
- Schweingruber, F. H. (1990). *Anatomy of European woods*. Bern Germany: Verlag Paul Haupt.
- Schwörer, C., Colombaroli, D., Kaltenrieder, P., Rey, F., & Tinner, W. (2015). Early human impact (5000–3000 BC) affects mountain forest dynamics in the Alps. *Journal of Ecology*, 103(2), 281–295. <https://doi.org/10.1111/1365-2745.12354>
- Serrano, E., Gómez-Lende, M., Belmonte, Á., Sancho, C., Sánchez-Benitez, J., Bartolomé, M., Hivert, B. (2018). Ice caves in Spain. In A. Persoiu, & S.-E. Lauritzen (Eds.), *Ice caves* (p. 754). Amsterdam, the Netherlands: Elsevier.
- Steinbauer, M. J., Grytnes, J.-A., Jurasinski, G., Kulonen, A., Lenoir, J., Pauli, H., Wipf, S. (2018). Accelerated increase in plant species richness on mountain summits is linked to warming. *Nature*, 556(7700), 231–234. <https://doi.org/10.1038/s41586-018-0005-6>
- Stockmarr, J. (1971). Tablets with spores used in absolute pollen analysis. *Pollen Et Spores*, 13, 614–621.
- Stuiver, M., & Reimer, P. J. (1993). Extended (super 14) C data base and revised CALIB 3.0 (super 14) C age calibration program. *Radiocarbon*, 35, 215–230.
- Tarrats, P., Heiri, O., Valero-Garcés, B., Cañedo-Argüelles, M., Prat, N., Rieradevall, M., & González-Sampériz, P. (2018). Chironomid-inferred Holocene temperature reconstruction in Basa de la Mora Lake (Central Pyrenees). *The Holocene*, 1–12. <https://doi.org/10.1177/0959683618788662>
- Ter Braak, C. J. F., & Šmilauer, P. (2002). *CANOCO. Software for Canonical Community Ordination*. Itacha, NY: Microcomputer Power.
- Tinner, W. (2007). Plant macrofossil methods and studies: Treeline studies. In S. Elias (Ed.), *Encyclopedia of quaternary science* (2nd ed., pp. 2374–2384). Amsterdam, the Netherlands: Elsevier.
- Tinner, W., Ammann, B., & Germann, P. (1996). Treeline fluctuations recorded for 12,500 years by soil profiles, pollen, and plant macrofossils in the Central Swiss Alps. *Arctic and Alpine Research*, 28(2), 131–147. <https://doi.org/10.2307/1551753>
- Tinner, W., & Kaltenrieder, P. (2005). Rapid responses of high-mountain vegetation to early Holocene environmental changes in the Swiss Alps. *Journal of Ecology*, 93(5), 936–947. <https://doi.org/10.1111/j.1365-2745.2005.01023.x>
- Tinner, W., & Theurillat, J.-P. (2003). Uppermost limit, extent, and fluctuations of the timberline and treeline ecocline in the Swiss Central Alps during the past 11,500 years. *Arctic, Antarctic, and Alpine Research*, 35(2), 158–169. <https://www.jstor.org/stable/1552367>
- Trautmann, W. (1953). Zur unterscheidung fossiler spaltöffnungen der mitteleuropäischen coniferen. *Flora*, 140, 523–533. [https://doi.org/10.1016/s0367-1615\(17\)31952-3](https://doi.org/10.1016/s0367-1615(17)31952-3)
- van Geel, B., & Aptroot, A. (2006). Fossil ascomycetes in Quaternary deposits. *Nova Hedwigia*, 82(3), 313–329. <https://doi.org/10.1127/0029-5035/2006/0082-0313>
- Villar, L., Sesé, J. A., & Ferrández, J. V. (1997). *Atlas flora del Pirineo Aragonés* (Vol. I). Huesca, Spain: Consejo de Protección de la Naturaleza de Aragón. Instituto de Estudios Altoaragoneses.

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