

<https://doi.org/10.1038/s43247-025-02023-8>

Neolithic pastoralism and plant community interactions at high altitudes of the Pyrenees, southern Europe

Check for updates

Irene Julián-Posada ^{1,10} ✉, Graciela Gil-Romera ^{1,10} ✉, Sandra Garcés-Pastor ^{2,3,4}, Peter D. Heintzman ^{2,5,6}, Daniel Gómez¹, Federico Fillat¹, Ana Moreno ¹, Javier Lara-Recuero¹, Pere Bover ⁷, Lourdes Montes ⁸, Alejandro Sierra ⁹, Blas Valero-Garcés ¹, Inger G. Alsos ² & Penélope González-Sampériz ¹ ✉

The Neolithization process introduced remarkable ecological impacts, especially in Mediterranean mountain areas. We generated a comprehensive sedimentary ancient DNA record from the central Pyrenees, spanning 12,200 to 1300 years before present, revealing the earliest continuous presence of sheep (6500 years before present) and cattle (5900 years before present) in alpine southern Europe. This evidence suggests pastoralism nearly concurred with the Neolithic in the Iberian lowlands, challenging prior assumptions of only sporadic occurrence and confirming Neolithic pastoralist use of mountain ecosystems. A notable plant community shift arose at 6000 years before present, with deciduous forests transitioning into diverse open grasslands. This change became pronounced at 4200 years before present, aligning with continuous presence of domesticates and a regional cooling climate, suggesting a synergistic relationship between past climate change and human-induced plant community alteration. These findings highlight complex interactions between climate, human activities, and landscape dynamics during the Neolithic in Mediterranean mountains.

The timings of the transition from foraging to farming economies is a pivotal focus of current transdisciplinary research. This transition, known as the Neolithization process, marked a considerable turning point in both human and landscape evolution¹. It started in the Fertile Crescent ca. 11.5 thousand calibrated years ago (ka BP) and reached Europe ca. 8.5 ka BP in Greece, via the introduction of domesticated plants and animals. These early introductions have spurred extensive research into the mechanisms of expansion, their cultural significance, and, more recently, their environmental impacts and long-term ecosystem effects^{2–4}. In the western Mediterranean basin, the advent of the Neolithic period instigated notable cultural, symbolic, economic, genetic, and environmental transformations^{4–6}. Nevertheless, there is a pronounced knowledge gap regarding the repercussions of early pastoralism on ecosystem dynamics in the Mediterranean region, where some of the most ancient complex societies originated, making the enduring impacts of agriculture more discernible⁷. Numerous

hypotheses have been proposed regarding alterations of the landscape due to intensified land use, particularly in central Europe^{8–10}. However, a lingering uncertainty persists concerning the potential long-term ecological impact of domesticated animals and even more so in mountain areas^{11–13}. Mountains play a crucial role in our comprehension of early animal husbandry, as they likely enabled the continuous presence of domesticated animals for a substantial part of the Holocene period¹⁴. The presence of productive pastures in summer likely facilitated the early adoption of seasonal resource utilisation strategies such as the pastoral mobility in altitude, known as transterminance^{15,16}. It has been generally accepted that montane Mediterranean environments have long been transformed by human activities^{2–4,17–22}. However, identifying clear unequivocal evidence of long-term human impact on vegetation in the high-altitude regions of the central Pyrenees has often been difficult and a true challenge, only becoming noticeable from the Mediaeval period onwards^{23–25}. This study addresses the

¹Instituto Pirenaico de Ecología (IPE-CSIC), Zaragoza, Spain. ²The Arctic University Museum of Norway, UiT - The Arctic University of Norway, Tromsø, Norway. ³Department of Evolutionary Biology, Ecology and Environmental Sciences, University of Barcelona, Barcelona, Spain. ⁴Institute of Marine Sciences (ICM-CSIC), Barcelona, Spain. ⁵Centre for Palaeogenetics, Stockholm, Sweden. ⁶Department of Geological Sciences, Stockholm University, Stockholm, Sweden. ⁷ARAID Foundation – IUCA, Universidad de Zaragoza, Zaragoza, Spain. ⁸Área de Prehistoria, P3A- IUCA, Universidad de Zaragoza, Zaragoza, Spain. ⁹Laboratori d'Arqueozoologia, Departament de Prehistòria, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain. ¹⁰These authors contributed equally: Irene Julián-Posada, Graciela Gil-Romera. ✉e-mail: irenejp@ipe.csic.es; graciela.gil@ipe.csic.es; pgonzal@ipe.csic.es

knowledge gap on the interaction between past climate changes and human landscape management in the Pyrenees, being one of the very few available studies in southern European mountains, by showcasing the early occurrence of domesticated animals and their impact on plant communities^{26–30}.

Archaeological data from the Iberian Peninsula lowlands show a rapid integration of domesticated animals at 7.5 ka BP³¹ and the subsequent occupation of mountain areas such as the Pyrenees from around 7.3 ka BP^{32,33}. However, while archaeological research provides a sound perspective on the importance of human presence in high altitude areas of the Pyrenees, it does not definitively establish clear connections between ecosystem responses and human influence there. The presence of a cattle bone at the Coro Trasito archaeological site (1600 m a.s.l., Fig. 1), dating ca. 7 ka BP, represents the earliest known instance of cattle at such high altitudes in the Iberian Peninsula³⁴. Nevertheless, a generally scattered archaeological record of cattle bones in NE Iberia, including the Pyrenees, compared to the very abundant presence of sheep bones, has prevented us from knowing the complete scenario of domesticated animal management in the mountains, thereby generalising the idea of a sparse, discontinuous presence of cattle in these mountains³⁵. Despite the wealth of archaeological data on the Neolithization process in the Pyrenees^{32,34,36,37}, there is an absence of spatially-coincident direct evidence of both ecosystem change and the presence of different animal herds. This lack of evidence under a robust quantitative framework hinders testing hypotheses regarding the main types of herds associated with Pyrenean landscape management.

The motivation for this study is thus to establish the early presence of domesticated animals in the southern Pyrenean region and their impacts on the landscape. Our objectives are twofold: (i) to test the archaeological timing of arrival for the first domesticated animals at high altitudes in the central Pyrenees, with a particular focus on distinguishing between various herbivore groups; and (ii) to assess the potential impact of these domesticated animals on the plant communities of the current subalpine area of the Pyrenees. To achieve these aims, we present the first record of both animal and plant sedimentary ancient DNA (*sedaDNA*) metabarcoding

from a lacustrine sequence in the Pyrenees, Tramacastilla Lake (TRAM21), located at 1682 m a.s.l. (Fig. 1).

Results

We developed an independent age-depth model for the Tramacastilla sediment cores acquired in 2020 and 2021, from which *sedaDNA* was extracted (Methods, Supplementary Note 1, Supplementary Figs. 1, 2 and Supplementary Table 1). The sedimentary archive from Tramacastilla spans from 25 to 1.3 ka BP, but we present here results for the period between 12.2 and 1.3 ka BP, because the sediments older than 12.2 ka BP contain limited organic matter. Besides the sedimentological constraint, the 12.2–1.3 ka BP period offers the optimal time resolution of the record, with a mean sedimentation rate of 30 yrs/cm (Standard Deviation (SD) 19 yrs/cm, see also Supplementary Table 1). The *sedaDNA* found in our core is believed to come from the lake basin as the dispersal ability and taphonomic dynamics of *sedaDNA* seem to indicate that they could be equivalent to macroremains³⁸. Future micromineralogy analyses may facilitate trace preservation rather than source³⁹.

Molecular evidence of plant and animal community changes

We present the *sedaDNA* findings using a Relative Abundance Index (RAI), which is a ratio that represents the relative abundance of each taxa, obtained by multiplying the relative proportion of total reads and the proportion of weighted PCR (Polymerase Chain Reaction) replicates in which the sequence was identified²⁶. A total of 9.8 million reads of 529 plant sequences were initially acquired in all 46 samples analysed (Supplementary Data 2), with 423 sequences meeting post-filtering criteria (Supplementary Data 3). Within this set, 192 taxa were identified at the species level, 171 at genus level and 57 at family level. Regarding animal sequences, a total of 2.9 million reads of 62 sequences were obtained in 42 samples, with no animal *sedaDNA* detected in four samples (Supplementary Data 8). Within this group, 32 taxa were excluded from analysis (31 worms and one bryozoan), retaining then 30 taxa that comprises 11 wild mammals, 10 other wild

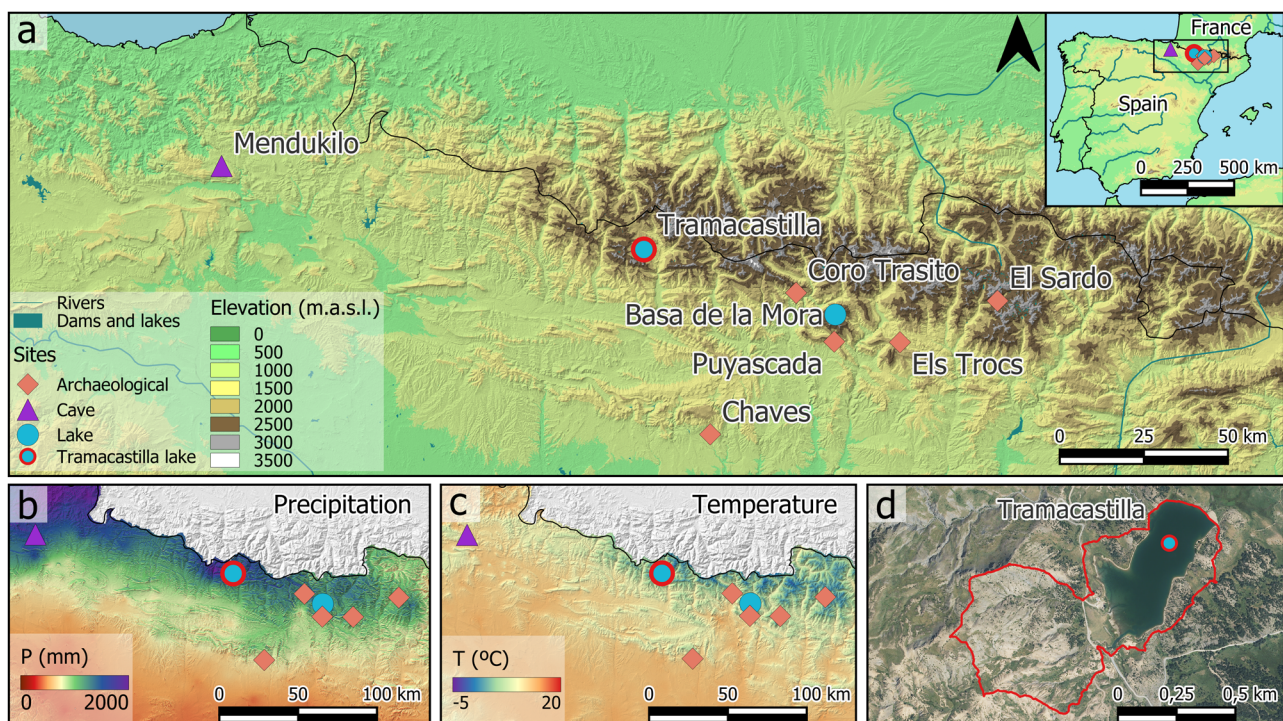


Fig. 1 | Location map of Tramacastilla Lake and other records cited in this article (cave, lake, and archaeological sites). a Altitude map; b mean annual precipitation; c mean annual temperature; d orthophoto of Tramacastilla lake with its catchment area delimited. Map sources: elevation data from ASTER GDEM v2 (METI/NASA, 2011), hydrology from IGN-CNIG 1:200k base map (BCN200), climate data from

Digital Climatic Atlas of the Iberian Peninsula⁸⁸, and orthophoto from IGN-CNIG (PNOA). Catchment area was obtained using ArcGeek calculator complement (QGIS 3.34) from Digital Elevation Model MDT02 (IGN). Projection UTM 30 Datum ETRS89 (EPSG: 25830). All coordinates in Supplementary Data 12.

animals, 3 domesticated mammals, and 6 categorised as ‘others’ that could be either domesticated or wild mammals. Notably, 27 animal taxa were identified at the species or subspecies level, demonstrating a high degree of taxonomic precision, with the remaining three classified at the genus level. We highlight the precise identification of domesticated mammals, all of which belonged to the ruminant category, including cattle, sheep and goats. The proportion of weighted PCR replicates where plant and animal sequences were found are represented in Supplementary Figs. 3–12.

The analysis of *sedaDNA* from Tramacastilla Lake reveals the prevalence of deciduous forest communities (Fig. 2a) since the onset of the Holocene, with RAI values fluctuating between 40 and 75% (Supplementary Data 5, total mean and SD $28.1 \pm 26.4\%$, Supplementary Table 2). Then, at 6 ka BP, these communities started to decrease, until the Northgrippian to Meghalayan transition (4.2 ka BP), at which point the deciduous forest practically disappears. During the first interval of the studied period (12.2–9.7 ka BP), *Pinus* sp. is consistently present and reaches up to 40%, but thereafter declines to almost negligible RAI values, aside from some expansions ca. 3.6 and 1.6 ka BP (Fig. 2b and Supplementary Data 5, total mean $4.3\% \pm 9.3\%$, Supplementary Table 2). Throughout the transition period between 6 and 4.2 ka BP, open landscape RAI increased (Fig. 2a and Supplementary Data 5), with values from 30 to 50% until the end of the record, at ~ 1.3 ka BP. Parallel to the progressive expansion of open communities, silver fir (*Abies alba* Mill.) is first detected at ca. 6 ka BP with relatively low RAI abundances until 3.2 ka BP (0.1–22%). Between 3.2 and 2.4 ka BP there is an increase in *Abies* RAI values, reaching 75% at 2.4 ka BP, and then maintains its percentages stable at around 30% until the end of the record (Fig. 2b and Supplementary Data 5).

The landscape around Tramacastilla Lake would have remained open for most of the Meghalayan period (4.2 ka BP–present) (Supplementary Data 5) (Fig. 2a). Amongst the *sedaDNA* of herbaceous taxa, there is a group whose onset happens at ca. 6.5 ka BP and becomes prevalent at 5 ka BP, that were never found before in our time series; consequently, this plant group has been designated as ‘Novel herbaceous taxa’ (Fig. 2a) indicating its unprecedented presence, but not linked to human introduction, nor an invasive character. Wild mammal and wild animal RAI values (Fig. 2c) are found scattered across most of the Holocene (particularly between 10.5–9.2 and 7–6.4 ka BP). These mainly correspond to red deer (*Cervus elaphus* L.) at ca. 10.5, 9.7 and 7.3 ka BP (RAI 100%, Supplementary Data 10) and the sporadic presence of other large mammals such as brown bear (*Ursus arctos* L.), which was observed at ca. 9.2 ka BP (RAI 100%) and 3.3 ka BP (RAI 2%). Amphibians, such as the common toad (*Bufo bufo* L.), are notably prevalent around 6.6 and 6.3 ka BP, while the common frog (*Rana temporaria* L.) becomes the dominant species in the wild animal assemblage at ~ 1.5 ka BP (RAI 100%). We found the first *sedaDNA* detections of sheep at 6.5 ka BP with varying RAI values since then, interspersed with *sedaDNA* from goats at lower values (Fig. 2d). The evidence for domesticated animals is further supported by the consistent detection of cattle *sedaDNA* in this lacustrine record for nearly the entire period spanning 6 to 1.3 ka BP (Fig. 2e), exhibiting elevated RAI values approaching 100% (mean RAI of $39.8 \pm 41.2\%$, Supplementary Table 3 and Supplementary Data 10).

In general terms, we identified a vegetation composition transition between 6 and 4 ka BP, marked by a gradual shift from a diminishing deciduous tree vegetation type to an expanding grassland community (Fig. 2a), that occurs coeval with the arrival of domesticated animals in the lake basin from 6 ka BP. Notably, an unprecedented shift in the floral composition of pastures takes place since 6.5 ka BP and steadily increased from 5 ka BP, marked by the appearance of novel, not human-introduced, nor invasive herbaceous taxa (Fig. 2a and Supplementary Data 5).

Taxonomic richness, rate of change and plant community trajectories

Excluding sample TRAM_1b_3U_136.137 (5.1 ka BP), with only 18 taxa identified, the taxonomic richness of both animals and plants, varied between 29 and 214 taxa (mean 118 ± 43 , Supplementary Table 4) reaching its highest values at 3.6 ka BP for both plants and animals (Fig. 2f). The

analysis of the rate of change (RoC) of RAI values of Tramacastilla plants and animals indicates significant changes between 4 and 3.5 ka BP (Fig. 2g).

A transformation-based canonical Redundancy Analysis (tb-RDA) of plants, and animals as explanatory variables, (Fig. 3a) (Supplementary Tables 5–10 and Supplementary Fig. 13) yielded a globally significant analysis (p -value < 0.01), where the first axis (RDA1) explains 39% of the observed variance. Cattle RAI is the only explanatory variable that is significant (p -value < 0.01), although it presents a variance inflation factor (VIF) of 1.63, indicating a slight multicollinearity. In a broader context, the tb-RDA illustrates an ecosystem trajectory that aligns with the temporal correlation between the declining deciduous forests and the emergence of open landscapes around 4–4.5 ka BP. This trend is summarised in RDA1, plotted in Fig. 2h and can be used as an open landscape index. Notably, the expansion of open landscapes and the appearance of novel herbaceous taxa (Fig. 2a) occurred mostly under the presence of cattle (5–4 ka BP, Figs. 2e, and 3a), while the proliferation of silver fir at 5 ka BP (Fig. 2b) aligned with the presence of sheep (4–1 ka BP, Figs. 2b, d, and 3a).

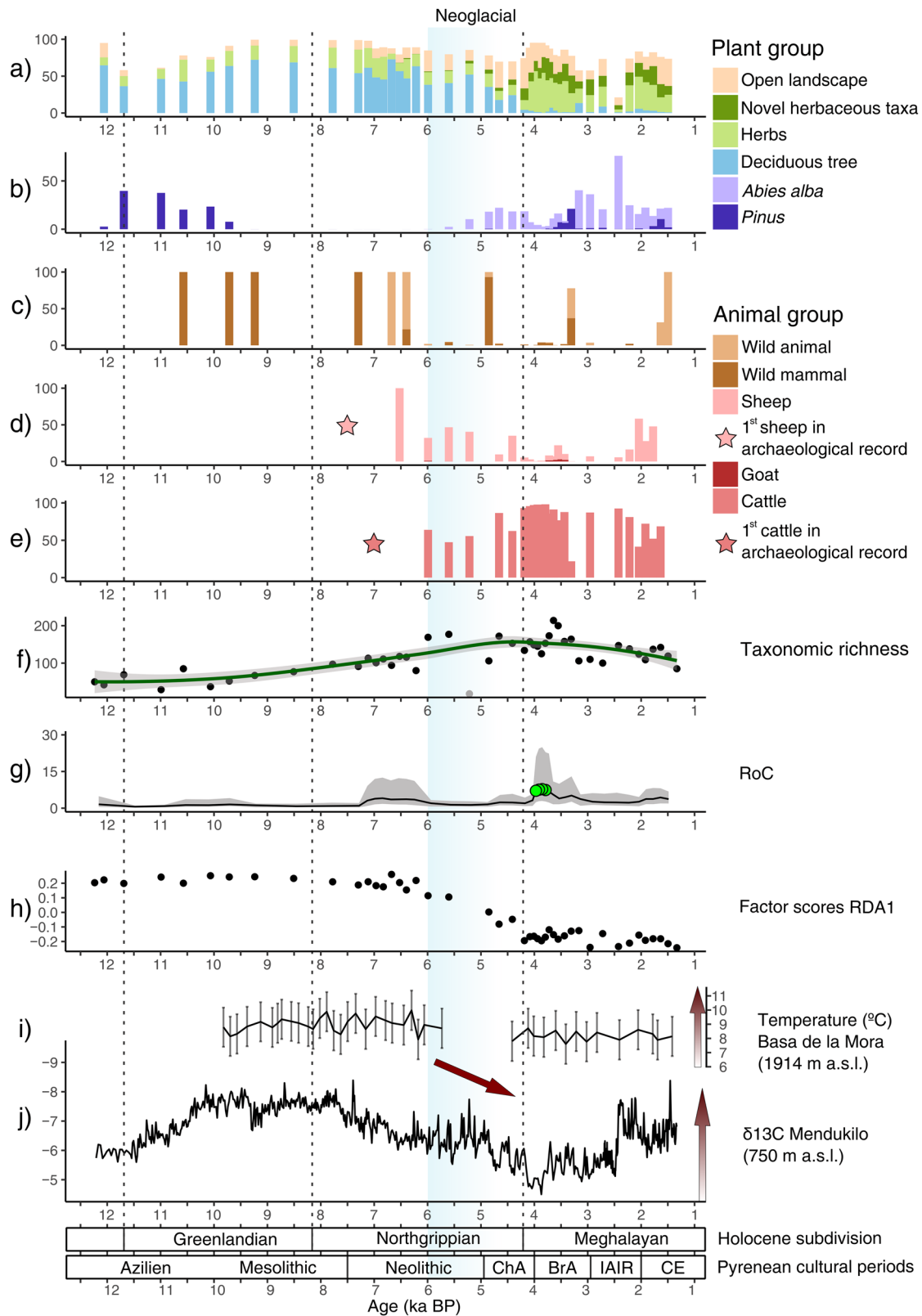
We targeted a tb-RDA on just open landscape plant groups, namely ‘Open landscape’, ‘Herbs’ and ‘Novel herbaceous taxa’ previously grouped under three functional variables (favoured by fertilisation, grazing or trampling and grazing - see Methods) (Fig. 3b) (Supplementary Tables 11–16 and Supplementary Fig. 14). This is also significant (p -value < 0.01) with RDA1 explaining 30% of the total variance. Cattle is the only significant variable among animals (p -value < 0.01), and similar to the global tb-RDA, exhibits subtle multicollinearity (VIF = 1.6). The ecosystem trajectory of the open landscape community in the tb-RDA exhibits minimal variability along both axes 1 and 2 but suggests an association of herbs favoured by fertilisation and the presence of red deer at 12 to 9 ka BP (Fig. 3b). The community trajectory between 9 to 6 and 3 to 1 ka BP was led by plants promoted by trampling and grazing. Additionally, these plants align with the presence of sheep, goats, and cattle, although not in a distinctly separated manner. The community between 6 and 3 ka BP tend to exhibit plants favoured by grazers and coexisting with other animals.

Discussion

We present, to our knowledge, the earliest molecular evidence of domesticated animal presence and grazing in European mountains based on *sedaDNA* (Fig. 2). Our results depict plant community dynamics from a mid-altitude natural archive (Tramacastilla Lake, 1682 m a.s.l.) in the Central Pyrenees, offering a continuous record of vegetation and faunal changes from 12.2 to 1.3 ka BP.

In many mid-latitude European regions, pastoral timing is frequently proposed to have been instrumental in clearing dense forest cover and shaping landscapes^{31,40–45} since the Northgrippian period (8.2–4.2 ka BP following¹⁴). However, most of the studies are based on indirect palaeoecological indicators, such as pollen assemblages^{46–48}, coprophilous fungal spores⁴⁹, or archaeological sites, including both anthracological data and the presence of bones of domestic fauna⁵⁰. They record episodic presence of domestics during the Neolithic (at 6 ka BP) and the Bronze Age (since 4 ka BP) before agro-pastoralism in the Middle Ages (more information about archaeological context in Supplementary Note 3). The first direct evidence of domesticated animals in European mountains has been recorded in Lake Anterne (northern French Alps), with cattle *sedaDNA* at ca. 5 ka BP, although sporadic until 2.4 ka BP²⁷. Complementarily, Lake Sulsseewli (central Alps) presents a more continuous record of direct evidence of domestic animals (cattle and sheep) since 4 ka BP²⁶.

Among early agro-pastoralist communities inhabiting the Mediterranean basin mountains, sheep (*Ovis aries* L.) and goat (*Capra hircus* L.) held prominence as one of the earliest domesticated animals, primarily due to their remarkable versatility and adaptability to various environments³⁶. It is hypothesised that they became the foremost species for economic exploitation, also on both slopes of the Pyrenees since 7.5–7.3 ka BP^{34,36,51–54}, with sheep forming an essential component of domestic herds throughout pre-historic times³¹. In fact, most of the zooarchaeological domestic animal remnants found in the Pyrenees correspond to sheep and goats (in Chaves, Coro



Trasito, Els Trocs, Puyascada and El Sardo caves: Fig. 1), while cattle (*Bos taurus* L.) herds have been found at low abundance albeit sparsely present during the early Neolithic^{35,36,54,55}. Traditionally, archaeological findings of domestic animals at Pyrenean high-altitude caves have suggested that these were seasonally occupied during spring and summer, likely indicating a transterminant landscape management¹⁶, thereby implying periodic

movements from lowland areas⁵⁴. This does not generally happen in lowland shelters as it is the case of Chaves Cave, at 640 m a.s.l and in the outer Pyrenean range (Fig. 1). This is a pivotal site for understanding the arrival of Neolithic and management of early domesticates in northeastern Iberia since 7.6 ka BP^{36,37,56}, and has been proved to be a permanently occupied habitat. Regarding fauna remnants, cattle is infrequent as well in lowland

Fig. 2 | Palaecological synthesis for Tramacastilla Lake throughout the Holocene. Indicated in light blue is the beginning of the period known as Neoglacial⁵⁸, and vertical dashed lines represent 11.7, 8.2 and 4.2 ka BP Holocene subdivisions. RAI percentages for: **a**, **b** selected plant groups; **c** wild mammals and wild animals; **d** sheep and goats and **e** cattle, with the star symbols indicating the chronology of the first sheep or cattle occurrence in the Pyrenean archaeological record (sheep: Chaves cave, 7.5 ka BP⁵⁶; cattle: Coro Trasito cave, 7 ka BP³⁴); **f** taxonomic richness of plants and animals with loess smooth area, with grey point representing low-DNA-quality sample TRAM_1b_4U_34.35 (6.5 ka BP); **g** rate of change (RoC) analysis results,

with uncertainty range in grey, and green circles representing statistically significant points when community composition changes; **h** RDA scores for first axis, seen as an open landscape index; **i** July temperature reconstruction for Basa de la Mora lake based on chironomid analysis, with sample-specific standard error bars⁵⁹; **j** Mendukilo cave isotope composites for $\delta^{13}\text{C}$, that represents a temperature variability reconstruction⁶⁰. The accepted subdivisions of the Holocene¹⁴ and cultural periods for the Pyrenees are shown at the base of the figure: Chalcolithic Age (ChA), Bronze Age (BrA), Iron Age and Ibero-Roman (IAIR) and Current Era (CE). All colours are suitable for colour blindness.

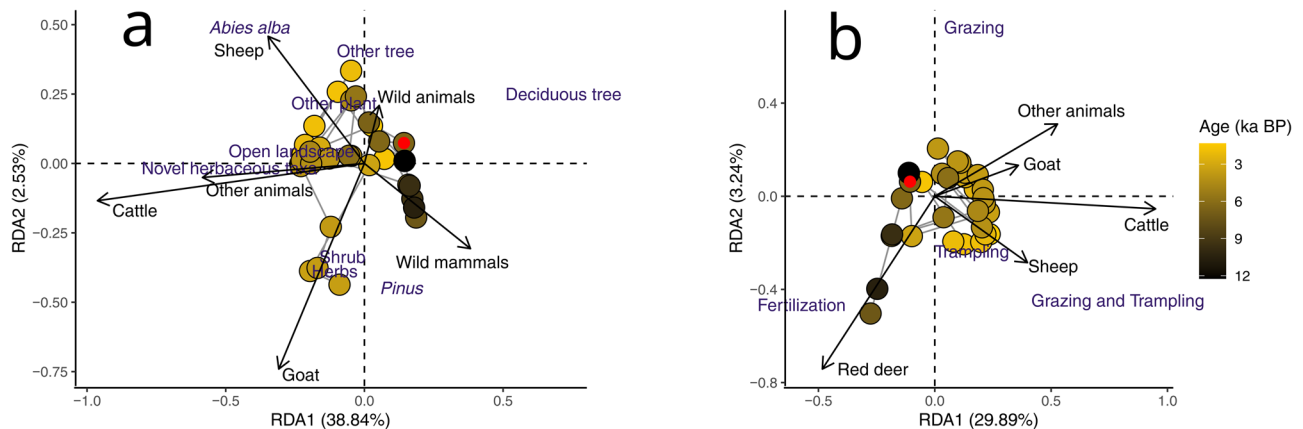


Fig. 3 | Ordination analyses with Hellinger transformation (tb-RDA). **a** for all plant taxa with all animals as explanatory variables; and **b** for all open landscape plants (i.e. ‘Open landscape’, ‘Herbs’ and ‘Novel herbaceous taxa’) classified according to whether they are favoured by grazing, trampling or fertilisation

practices, with selected animals as explanatory variables. Red circles represent the sample where the first appearance of domesticated animals is detected (6.5 ka BP, sample TRAM_1b_4U_34.35).

locations as found in Chaves, where there are mainly sheep (more than 50% including both *Ovis aries*, *Capra hircus* and *Ovis/Capra* categories) and only ca. 2% of cattle⁵³.

Our study reveals the consistent presence of *sed*aDNA from sheep (since 6.5 ± 0.3 ka BP) and cattle (since 5.9 ± 0.3 ka BP) throughout the Northgrippian and Meghalayan Holocene sub-periods in the Tramacastilla Lake record (Fig. 2d, e). This finding constitutes the earliest direct molecular and uninterrupted evidence of cattle in European mountainous regions compared to other records, like Lake Anterne or Lake Sulsseewli in the Alps, that constitute other available records of domesticates in high altitudes but nearly two thousand years later (ca. 4 ka BP)^{26,27}. Our findings reinforce the historical management of cattle, sheep and goats at high altitudes over the past seven millennia. The limited regional archaeological records of early cattle husbandry in the high altitudes of the Central Pyrenees (Coro Trasito, Els Trocs, Puyascada, Fig. 1) have provided a synchronous spatio-temporal scenario of cattle presence to that of Tramacastilla (Fig. 2e and Supplementary Data 10), confirming its presence at ca. 7 ka BP in subalpine environments. Indeed, cattle could have co-existed with native wild aurochs that were present in the Iberian Peninsula from the Middle Pleistocene to the Roman Period⁵⁷. In any case, Coro Trasito in particular has yielded the oldest dated fragment of cattle in the Pyrenees, with an age of 7.1–6.9 ka BP³⁴, about a millennium before our molecular evidence in Tramacastilla.

Both the archaeological context and our molecular evidence from Tramacastilla Lake, supports that: (i) cattle husbandry onset in the high altitudes of the central Pyrenees probably appeared at ca. 7 ka BP, although this age could be reduced by a thousand years if ages based solely on molecular evidence are considered; and (ii) there was a continuous presence of cattle since then, defying the idea of a punctuated presence given the sparse archaeological record (Fig. 2e). In this way, both the new molecular data from Tramacastilla Lake and the entire regional archaeological scenario confirm that human use of the high altitude areas of the Pyrenees only occurred three centuries after the first Neolithic human settlements in the region (Fig. 1 and Supplementary Data 7).

In the Tramacastilla record, the floristic and functional change from woodland to open landscape initiated before 6 ka BP, seems to become critical at ca. 4 ka BP (Fig. 2a and Supplementary Data 5), when significant RoC values appear in our sequence (Fig. 2g). Until then, the plant communities around Tramacastilla may have resiliently responded to environmental change, undergoing a critical transition when deforestation and grassland compositional change occurred. Considering that, in our record the primary transformation in the plant community (first appearance of ‘Novel herbaceous taxa’ category: Fig. 2a), co-occurs with the emergence of domesticated animals at 6.5 ka BP (first sheep appearance: Fig. 2b), being then tempting to propose the logical causation that Neolithic people intentionally cleared woodlands to facilitate the expansion of pastures and, perhaps, key tree taxa.

However, there is sound, quantitative, independent regional climate evidence supporting a notable temperature reduction between 6 and 4 ka BP in numerous sites in the Pyrenees (glacier advances during the beginning of the Neoglacial⁵⁸), and particularly in records relatively near to Tramacastilla (Basa de la Mora, 1903 m a.s.l., Figs. 1, and 2i⁵⁹) and in lower, more western areas of the Pyrenees (Mendukilo, 750 m a.s.l., Figs. 1 and 2j⁶⁰). Such a cooling phase could have acted either as a primary cause of the landscape opening at ca. 6 to 5 ka BP or as a reinforcing factor of the herding activity in Tramacastilla, which would have started ca. 6.5 ka BP, before the onset of the declining temperature (Fig. 2d, e, i and j). The decrease in local to regional temperatures contrasts with other paleoclimate reconstructions at the Iberian scale, where winter temperatures may have shown a consistent increase throughout the Holocene period⁶¹. However, these climate reconstructions suggest that both summer temperatures and plant moisture availability from 6 to 4 ka BP lack a discernible positive trend⁶¹, aligning with broader continental models and simulations⁶².

We hypothesise that a likely climate-driven landscape opening may have been sustained, initially by small herds of sheep and goats, and later by cattle. The progressive woodland clearing would have facilitated the expansion of herbs that are often frequent in forest margins, all those under

'Novel herbaceous taxa' (Supplementary Data 4). These plants were native and not from an exotic origin, and occupied a newly created niche, expanding into the open areas near the lake. The major floristic change at 4 ka BP, as indicated by increased richness (Fig. 2f) and the sole significant RoC values (Fig. 2g), reflects such forest opening. Our hypothesis is additionally supported by the clear presence of human activity plant indicators, like *Urtica dioica*, that only appeared in our *sedDNA* record after domestic animals arrived. The tb-RDA results further underscore the influence of domesticated animals on herbaceous plant communities. Notably, the sustained presence of cattle is associated with plants indicative of open landscapes ('Open landscape' and 'Novel herbaceous taxa' categories), contributing to the remarkable emergence of new herbs on the vegetation landscape (Fig. 3a).

Moreover, the trampling of livestock could have facilitated the proliferation of plant species, even trees, that thrive under such disturbance, a phenomenon commonly observed in mountainous grassland ecosystems (Fig. 3b). This is probably the case of Tramacastilla deciduous forest's regression at 6 ka BP, a trampling livestock scenario sharply contrasting with the spread of silver fir (*Abies alba*) at 5 ka BP. These shifts in the Tramacastilla forest community initially reflect broader dynamics observed in the Pyrenees and across Europe. In the Pyrenees, the initial colonisation of silver fir, documented between 9.5 and 8 ka BP, was primarily concentrated in the eastern part of the northern slope^{63,64}. Several millennia later, ca. 6.5 ka BP, the earliest evidence of *Abies* pollen appeared in the central Pyrenees region, reaching a peak at 4 ka BP⁶⁵. Research carried out in Central Europe has linked the decline of silver fir at 3 ka BP to climatic changes and human influences, particularly the increasing human impact during the Bronze and Iron Ages⁶⁶. While silver fir was extensively used in Central Europe for construction during these periods, leading to its gradual decline due to exploitation and agricultural expansion⁶⁶, there is evidence that low-impact pastoralism may have facilitated fir growth during certain periods, such as 5.5 to 4 ka BP in what is now the Czech Republic⁶⁷. In fact, the presence of *Abies* at Tramacastilla coincides with sustained cattle presence in the area (Fig. 2b, e), suggesting that pastoral activities did not substantially affect silver fir expansion. Although our findings diverge from broader trends observed in the Alps region^{29,68}, they align with those of the Czech Republic, indicating that grazing may have facilitated *Abies* regeneration. However, this contradicts modern ecological studies suggesting negative effects of grazing on silver fir dynamics⁶⁹. Our study emphasises the complex interplay between climate change, grazing practices, and forest dynamics since 6 ka BP, highlighting the need for further research to understand this variability. More precisely, future research should consider the study of the fire regime through the analysis of the charcoal fraction to better comprehend the interactions between climate, vegetation and human activity over time, as fires have historically played a crucial role in opening dense forests and shaping landscapes³.

In summary, our findings present the earliest direct evidence of sustained cattle presence in European mountainous regions, aligning with the concept of continuous cattle occupancy since ~6 ka BP. Furthermore, our analysis illuminates the Meghalayan transformation from dense forest to open grasslands, coinciding with the introduction of domesticated animals around 6 ka BP and a pivotal transition at 4 ka BP. Our study underscores the considerable influence of climate change, notably a discernible temperature decrease during the beginning of the Neoglacial period, on landscape dynamics. The confluence of cooler climates concurred with the expansion of grazing practices by Neolithic communities, potentially following a transterminant herding model. The synergic effect of climate and grazing likely contributed to the gradual clearing of woodlands and the emergence of open landscapes and novel pasture land communities since 6 ka BP, and a critical plant community change since 4 ka BP in the surroundings of Tramacastilla. This newly established ecosystem probably enabled the presence of mosaic-like landscapes that are currently at risk due to the abandonment of traditional land uses⁷⁰.

Methods

Geographical setting

Tramacastilla Lake (42°43'31.57"N, 0°22'03.73"W) is located at 1682 m a.s.l. in the subalpine belt of the Upper Gállego Valley of the southern Central Pyrenees (Huesca, NE Spain) (Fig. 1), in a glacial valley⁷¹. Its catchment area occupies about 50 Ha, mostly on the western side of the lake (Fig. 1d). Before the construction of a dam in 1956, the lake had a maximum depth of 4.5 m. Following the damming, the depth underwent a substantial increase, reaching 13.5 m. The lake's surroundings are deforested (Fig. 1d) due to human exploitation. Consequently, the vegetation predominantly comprises grasslands with scattered shrubs and trees such as *Pinus uncinata* Ramond ex DC., *Pinus sylvestris* L., *Rosa* sp., and *Juniperus communis* L.

Core sampling and age depth model

We retrieved four sediment cores at 13.5 m water depth, using a UWITEC platform in two fieldwork campaigns in October 2020 and October 2021, from which the two most continuous ones were selected (TRAM20-1B and TRAM21-1B; each core ~10 m long divided in five sections, 1U to 5U, Supplementary Note 1 and Supplementary Fig. 1). We excluded the other two cores because they lacked certain sections of sediment, making them discontinuous and therefore unsuitable for building a chronological model. All cores were lengthwise split, photographed, and stored at 4 °C at the Pyrenean Institute of Ecology (IPE-CSIC, Zaragoza). All analyses in the present study were performed on Sections 3 and 4 (total length of 360 cm) from core TRAM21-1B, as these reflect the most stable sedimentary dynamics of the sequence (Supplementary Fig. 1). Section 5U consisted of sediments of an age beyond the scope of the present study, while Sections 1U and 2U contain sedimentary features that render them unsuitable for establishing a reliable chronology. These reflect high-energy depositional events that can disturb the usual sequence of lacustrine sediment deposition, disrupting the chronological sequence and therefore leading to inaccuracies in age-depth models if included.

The age-depth model was obtained by combining dates from cores TRAM20-1B and TRAM21-1B, that were sedimentologically correlated in order to develop a more complete and representative composite sequence (see details in Supplementary Note 1 and Supplementary Table 1). We generated an age-depth model based on Bayesian inference using the R package rbacon v3.2.0⁷² in R (R Core Team, 2022) (Supplementary Fig. 2), which includes the IntCal curve (IntCal20⁷³) that automatically calibrates the original dates and calculates modelled ages.

sedDNA analysis

We sampled TRAM21-1B for *sedDNA* analysis under clean conditions at the Paleogenomics Laboratory of the IUCA-University of Zaragoza. We took between 2 and 3 g of 95 samples at around 5-cm resolution from the whole core, excluding the first upper section, as it was not dated.

We carried out *sedDNA* analyses at the Ancient DNA Laboratory of the Arctic University Museum of Norway in Tromsø, following a meta-barcoding approach. Data were generated in two batches (Supplementary Data 1). Altogether, we analysed 46 of the sediment samples from Sections 3U and 4U of TRAM21-1B core, which corresponds to the Holocene (Supplementary Data 11). We included 15 controls (six in batch 1, nine in batch 2) to detect potential contamination, as detailed in Supplementary Note 2. In the case of plant *sedDNA*, one control in each batch was positive and included a synthetic extract to confirm successful *sedDNA* amplification⁷⁴. We followed the protocols of ref. 74 for DNA extractions, using a modified DNeasy PowerSoil kit protocol (Qiagen, Germany). We used the 16S P007 primer set to amplify a region of the animal mitochondrial genome²⁷ and a set that amplified a chloroplast locus (*trnL* p6-loop) for plants⁷⁵. Each primer set was uniquely-dual tagged to allow for downstream demultiplexing (Supplementary Data 1). We carried out eight PCR replicates each for both primer sets, following the conditions defined by ref. 76, except for 16S PCRs, in which forward and reverse blocking primers were added, following⁷⁶. PCR products were pooled and cleaned, and library preparation was performed using a modified TruSeq PCR-free library kit (Illumina) and

unique dual indexing⁷⁴. Libraries were quantified by qPCR using the Library Quantification Kit for Illumina sequencing platforms (KAPA Biosystems, Boston, USA), and sequenced on the Illumina NextSeq platform using paired-end chemistry for 2 × 150 cycles at the Genomics Support Center Tromsø (GSCT) at The Arctic University of Norway in Tromsø. For the bioinformatics pipeline, we used the OBITools software package⁷⁷ following²⁶.

We performed a plant taxonomic assignments of *sed*aDNA metabarcodes using the following stepwise filtering process:

1. Identifying the sequences in relevant databases. We used four reference databases for plant sequences: PhyloAlps²⁶, PhyloNorway⁷⁸, ArctBorBryo^{79–81}, and EMBL r1143⁸². Note that the first two only contain vascular plants whereas the latter two also include bryophytes. Algae are generally poorly represented in reference libraries, but some are present in EMBL. Those with a 100% match to the reference sequence, and that appeared in more than one PCR replicate, were kept while the rest were discarded. We prioritised the PhyloAlps database as a confident source in case of 100% match to several databases, as it is a specific database for the flora of the Alps and Pyrenees.
2. Identifying and discarding PCR/sequencing errors. For this task we ran the LULU software⁸³, which identified erroneous molecular operational taxonomic units (OTUs), so we grouped the information of these assignment errors into the original sequence they derive from.
3. Comparison to expert knowledge. Final taxonomic assignments for plant sequences were contrasted to the Pyrenees Flora Atlas (FLOR-APYR, <http://www.atlasflorapyreneae.eu/src/home/index.php?idma=1>), plant records from the Herbarium of Jaca (<http://floragon.ipe.csic.es/>), and personal communication by botanical experts Daniel Gómez and Federico Fillat⁷⁰.
4. We finally retained 423 taxa (Supplementary Data 3) from the 529 originally identified sequences (Supplementary Data 2). From the former pool, 2 were recognized as non-native, and 10 were ‘Positive control’ identified, as expected, in positive controls from the post-PCR lab (Supplementary Data 3).
5. To facilitate data visualisation and interpretation, we organised plant taxa into 13 distinct groups, encompassing both individual taxa and functional categories. Thus, we obtained the following categories: *Abies alba*, Algae, Bryophytes, Hydrophytes, Hygrophytes, Deciduous trees, Open landscape, Novel herbaceous taxa, Herbs, *Pinus*, Shrubs, Other trees and Other plants (i.e. nemoral plants and pteridophyta) (Supplementary Data 3). Among these, we have classified all plants associated with open landscapes in three different categories: (1) ‘Novel herbaceous taxa’, that correspond to herbs that appeared for the first time only after domestic animal *sed*aDNA sequences were present in the record (6.5 ka BP onwards), despite these taxa are not introduced or invasive; (2) ‘Herbs’, that are herbaceous taxa associated with current Pyrenean pastures⁷⁰; and (3) ‘Open landscape’, that are not necessarily herbaceous taxa typical from Pyrenean pastures, but generally associated with open landscapes (e.g. *Veronica serpyllifolia*, *Pedicularis sylvatica*). We discarded Algae, Bryophytes, Hydrophytes, and Hygrophytes to retain only terrestrial vascular plants for analysis.

Animal sequences were taxonomically assigned using the EMBL r1143 database, retaining those with a 95% match or higher, detected in at least one PCR replicate, and with at least 10 reads in the entire dataset. We further removed detections in the PCR replicates if they were represented by two or fewer reads. We discarded sequences that were identified as human (*Homo sapiens*) using a blacklist (v2.1, see <https://github.com/pheintzman/metabarcoding>), as it is a common contaminant. This resulted in a dataset of 543 taxonomically assigned sequences that were collapsed into 110 taxa based on identical taxonomic information. We then manually curated these assignments and removed five Nuclear-Mitochondrial DNA Segment (NUMTs) sequences and four contaminant taxa that were found in the negative controls and were non-native to Europe (Supplementary Data 7). We then recollapsed our curated taxonomic assignments, resulting in a final dataset of 62 animal taxa (Supplementary Data 8).

We classified animal taxa according to two categories: wild or domesticated animals, obtaining the following classifications: ‘Wild mammal’, ‘Wild animal’ (including birds, amphibians and insects), cattle (*Bos taurus*), sheep (*Ovis aries*), goat (*Capra hircus*), ‘Other animals’, ‘Worms’ and ‘Bryozoan’ (Supplementary Data 8). Assignments of *Bos* sp., *Sus scrofa*, *Canis lupus* and *Equus caballus*/sp., could not be differentiated between wild and domestic animals (aurochs/cattle, wild boar/pig, wolf/dog and wild/domestic horse, respectively). Consequently, these taxa were not considered as domesticates in our analyses, but ‘Others’, as well as donkey (*Equus cf. asinus*), due to its scarce presence. We removed other invertebrates (worms and bryozoans) from our analysis, as they are regarded as by-catch when using a primer targeting mammal DNA. After filtering, we retained 30 taxa.

We used the RAI defined by ref. 26 to represent the relative proportion of plant and animal taxa (Supplementary Data 4, 5, 9, 10), calculated as the product of the proportion of weighted PCR replicates, as defined by ref. 74, and the relative proportion of reads for each taxon.

Ordination

We explored changes in plant communities related to the presence of animals by performing a transformation-based canonical Redundancy Analysis (tb-RDA) using the vegan R package⁸⁴, since our data do not have a normal distribution. We included plant RAI data classified in categories for every sample, and animal RAI data as explanatory variables. Plant data were transformed using Hellinger standardisation, in order to remove differences in absolute abundances between samples⁸⁵. We excluded sample TRAM_1b_3U_136.137, which corresponds to 5.1 ka BP, from the ordination analysis because of its limited DNA content (only 18 taxa were identified in this sample, while the mean taxonomic richness excluding this sample was 117.7, see Supplementary Table 4 and Supplementary Fig. 13).

Characterising different grassland communities in the Tramacastilla record, we expanded the whole group of plants associated with open landscapes (i.e. ‘Open landscape’, ‘Herbs’ and ‘Novel herbaceous taxa’) by accounting for every possible regional species within each taxon above the species level, by considering that there is an equal chance of each species being present (i.e. a probability factor of one divided by the total possible species within a taxon) (Supplementary Data 6). We added one variable to each of these species, following botanical expertise criteria and ref. 70, based on whether they are favoured by domesticates as grazing, trampling, fertilisation, or both grazing and trampling (Supplementary Data 6).

Using the RAI data of all plant taxa associated with open landscapes (i.e. ‘Open landscape’, ‘Herbs’ and ‘Novel herbaceous taxa’), and classified according to the way they are favoured by domesticates, we performed another ordination analysis (tb-RDA) to see how related they are with the presence of animals. We included animal RAI data as explanatory variables, although we only kept red deer (*Cervus elaphus*) from the ‘Wild mammal’ group for this dataset (Supplementary Data 8) so as to be able to see the specific signal of the most common herbivore in the record on grasslands. As with the previous tb-RDA, plant RAI data were transformed using Hellinger standardisation and sample TRAM_1b_3U_136.137 was excluded.

Rate of change

In order to see how the composition of ecological communities has changed over time we performed a Rate-of-Change (RoC) analysis with R-Ratepol R package⁸⁶ using the RAI data of plant and animal taxa. So as to have parsimonious yet consistent results and ensure the uniqueness of all taxa included in RoC analysis, we grouped together those plants that could be nested in other groups that had lower taxonomic resolution. For instance, all sequences identified as species from the Asteraceae family were grouped together under ‘Asteraceae’, as some sequences could not be identified below the family category, so those plants identified with coarser resolution could be included. This software allows for the detection of points where community composition significantly changes. We performed 10,000 randomisations, using ‘chisq’ dissimilarity coefficient, and ‘levels’ as working units. Because the median value of our data was 0, we used the mean value of all RAI data (5) as the number of individuals from each working unit to

standardise the variables in the assemblage dataset. In addition, and following the RoC workflow, we included age uncertainties from an age-depth model from Bchron R package (v4.7.6⁸⁷).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Raw *sed*aDNA sequence data have been deposited at the European Nucleotide Archive (ENA) under BioProject (accession number PRJEB83695). Blacklists and data filtering R scripts are on Github (https://github.com/irenejulianposada/tramacastilla_lake_sedaDNA.git). All processed data and the numerical workflow of this article are stored on Github and Zenodo (<https://doi.org/10.5281/zenodo.14530281>), for later integration in Neotoma (<https://www.neotomadb.org/>). All other data and information needed to replicate the findings is in Supplementary Information and Supplementary Data.

Received: 15 July 2024; Accepted: 10 January 2025;
Published online: 24 January 2025

References

- Blondel, J. The ‘Design’ of Mediterranean Landscapes: a millennial story of humans and ecological systems during the historic period. *Hum. Ecol.* **34**, 713–729 (2006).
- Revelles, J. et al. Human–environment interaction during the Mesolithic–Neolithic transition in the NE Iberian Peninsula. Vegetation history, climate change and human impact during the Early–Middle Holocene in the Eastern Pre-Pyrenees. *Quat. Sci. Rev.* **184**, 183–200 (2018).
- Orengo, H. A., Palet, J. M., Ejarque, A., Miras, Y. & Riera, S. Shifting occupation dynamics in the Madriu–Perafita–Claror valleys (Andorra) from the early Neolithic to the Chalcolithic: the onset of high mountain cultural landscapes. *Quat. Int.* **353**, 140–152 (2014).
- Branch, N. P., Black, S., Maggi, R. & Marini, N. A. F. The Neolithisation of Liguria (NW Italy): an environmental archaeological and palaeoenvironmental perspective. *Environ. Archaeol.* **19**, 196–213 (2014).
- Allentoft, M. E. et al. Population genomics of post-glacial western Eurasia. *Nature* **625**, 301–311 (2024).
- Manen, C. Dynamiques spatio-temporelles et culturelles de la néolithisation ouest-méditerranéenne. in *La transition néolithique en Méditerranée* (eds. Manen, C., Perrin, T. & Guilaine, J.), 405–418 (Toulouse, 2014).
- Zeder, M. A. Domestication and early agriculture in the Mediterranean Basin: origins, diffusion, and impact. *Proc. Natl Acad. Sci. USA* **105**, 11597–11604 (2008).
- Marquer, L. et al. Quantifying the effects of land use and climate on Holocene vegetation in Europe. *Quat. Sci. Rev.* **171**, 20–37 (2017).
- McClure, S. B. Domesticated animals and biodiversity: early agriculture at the gates of Europe and long-term ecological consequences. *Anthropocene* **4**, 57–68 (2013).
- Mottl, O. et al. Global acceleration in rates of vegetation change over the past 18,000 years. *Science* **372**, 860–864 (2021).
- Ameztegui, A., Coll, L., Brotons, L. & Ninot, J. M. Land-use legacies rather than climate change are driving the recent upward shift of the mountain tree line in the Pyrenees. *Glob. Ecol. Biogeogr.* **25**, 263–273 (2016).
- Galop, D. & Jalut, G. Differential human impact and vegetation history in two adjacent Pyrenean valleys in the Ariège basin, southern France, from 3000 B.P. to the present. *Veget. Hist. Archaeobot.* **3**, 225–244 (1994).
- Muñoz-Ulecia, E. et al. Drivers of change in mountain agriculture: A thirty-year analysis of trajectories of evolution of cattle farming systems in the Spanish Pyrenees. *Agric. Syst.* **186**, 102983 (2021).
- Walker, M. et al. Formal ratification of the subdivision of the Holocene Series/Epoch (Quaternary System/Period): two new Global Boundary Stratotype Sections and Points (GSSPs) and three new stages/subseries. *Episodes* **41**, 213–223 (2018).
- Carrer, F. & Migliavacca, M. Prehistoric Transhumance in the Northern Mediterranean. In *The Textile Revolution in Bronze Age Europe* (eds. Sabatini, S. & Bergerbrant, S.) 217–238 (Cambridge University Press, 2019) <https://doi.org/10.1017/9781108656405.009>.
- García-Ruiz, J. M. et al. Transhumance and long-term deforestation in the subalpine belt of the central Spanish Pyrenees: an interdisciplinary approach. *CATENA* **195**, 104744 (2020).
- Carrión, J. S. et al. Expected trends and surprises in the Lateglacial and Holocene vegetation history of the Iberian Peninsula and Balearic Islands. *Rev. Palaeobot. Palynol.* **162**, 458–475 (2010).
- Galop, D., Rius, D., Cugny, C. & Mazier, F. A. History of Long-Term Human–Environment Interactions in the French Pyrenees Inferred from the Pollen Data. In *Continuity and Change in Cultural Adaptation to Mountain Environments: From Prehistory to Contemporary Threats* (ed. Lozny, L. R.) **7**, 19–30 (Springer New York, New York, 2013).
- Garcés-Pastor, S. et al. Environmental history and vegetation dynamics in response to climate variations and human pressure during the Holocene in Bassa Nera, Central Pyrenees. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* **479**, 48–60 (2017).
- Morales-Molino, C. et al. Millennial land use explains modern high-elevation vegetation in the submediterranean mountains of Southern Europe. *J. Biogeogr.* **49**, 1779–1792 (2022).
- Rius, D., Vannié, B. & Galop, D. Holocene history of fire, vegetation and land use from the central Pyrenees (France). *Quat. Res.* **77**, 54–64 (2012).
- Rull, V., González-Sampériz, P., Corella, J. P., Morellón, M. & Giralt, S. Vegetation changes in the southern Pyrenean flank during the last millennium in relation to climate and human activities: the Montcortès lacustrine record. *J. Paleolimnol.* **46**, 387–404 (2011).
- González-Sampériz, P. et al. Environmental and climate change in the southern Central Pyrenees since the Last Glacial Maximum: a view from the lake records. *CATENA* **149**, 668–688 (2017).
- Leunda, M. et al. 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. *Glob. Planet. Change* **157**, 214–231 (2017).
- Pérez-Sanz, A. et al. Holocene climate variability, vegetation dynamics and fire regime in the central Pyrenees: the Basa de la Mora sequence (NE Spain). *Quat. Sci. Rev.* **73**, 149–169 (2013).
- Garcés-Pastor, S. et al. High resolution ancient sedimentary DNA shows that alpine plant diversity is associated with human land use and climate change. *Nat. Commun.* **13**, 6559 (2022).
- Giguet-Covex, C. et al. Long livestock farming history and human landscape shaping revealed by lake sediment DNA. *Nat. Commun.* **5**, 3211 (2014).
- Giguet-Covex, C. et al. Long-term trajectories of mountain agro-ecosystems in the North-Western Alps. *Reg. Environ. Change* **23**, 58 (2023).
- van Vugt, L. et al. Pollen, macrofossils and *sed*aDNA reveal climate and land use impacts on Holocene mountain vegetation of the Lepontine Alps, Italy. *Quat. Sci. Rev.* **296**, 107749 (2022).
- Morlock, M. A., Rodríguez-Martínez, S., Huang, D. Y. & Klaminder, J. Erosion regime controls sediment environmental DNA -based community reconstruction. *Environ. DNA* **5**, 1393–1404 (2023).
- Saña, M., Antolín, F., Alcántara, R., Sierra, A. & Tornero, C. Integrating domesticates: earliest farming experiences in the Iberian Peninsula. In *Farmers at the Frontier: A Pan European Perspective on Neolithisation* (eds. Gron, K. J., Sorensen, L. & Rowley-Conwy, P.) 161–175 (Oxbow Books, Oxford, 2020).
- Gassiot-Ballbè, E. et al. The Beginning of High Mountain Occupations in the Pyrenees. Human Settlements and Mobility from

- 18,000 cal BC to 2000 cal BC. In *High Mountain Conservation in a Changing World* (eds. Catalan, J., Ninot, J. M. & Aniz, M. M.) vol. 62 75–106 (Springer Nature, Cham, 2017).
33. Laborda, R. *El Neolítico antiguo en el Valle Medio del Ebro. Una visión desde la cerámica y las dataciones radiocarbónicas*. (Prensas de la Universidad de Zaragoza, 2019).
 34. Navarrete, V. et al. Early husbandry practices in highland areas during the Neolithic: the case of Coro Trasito cave (Huesca, Spain). *Front. Environ. Archaeol.* **2**, 1309907 (2023).
 35. Antolín, F., Navarrete, V., Saña, M., Viñerta, Á. & Gassiot, E. Herders in the mountains and farmers in the plains? A comparative evaluation of the archaeobiological record from Neolithic sites in the eastern Iberian Pyrenees and the southern lower lands. *Quat. Int.* **484**, 75–93 (2018).
 36. Sierra, A., Bréhard, S., Montes, L., Utrilla, P. & Saña, M. Sheep exploitation and husbandry in first farming societies: from production to consumption in Central Pyrenees in the Early Neolithic. *Archaeol. Anthropol. Sci.* **11**, 5813–5829 (2019).
 37. Utrilla, P. & Domingo, R. La transition Mésolithique-Néolithique dans la Vallée de l'Èbre. The mesolithic-neolithic transition in the Ebro Valley. In *La transition néolithique en Méditerranée. The Neolithic transition in the Mediterranean* (eds. Manen, C., Perrin, T. & Guilaine, J.) 327–358 (Errance, 2014).
 38. Edwards, M. E. The maturing relationship between Quaternary paleoecology and ancient sedimentary DNA. *Quat. Res.* **96**, 39–47 (2020).
 39. Sand, K. K., Jelavić, S., Kjær, K. H. & Prohaska, A. Importance of eDNA taphonomy and sediment provenance for robust ecological inference: Insights from interfacial geochemistry. *Environ. DNA* **6**, e519 (2024).
 40. Aurelle, D. et al. Biodiversity, climate change, and adaptation in the Mediterranean. *Ecosphere* **13**, e3915 (2022).
 41. Barker, G. Agriculture, pastoralism, and Mediterranean landscapes in prehistory. In *The Archaeology of Mediterranean Prehistory* (eds. Blake, E. & Knapp, A. B.) 46–76 (Wiley, 2005) <https://doi.org/10.1002/9780470773536.ch3>.
 42. García-Ruiz, J. M. et al. Los efectos geoecológicos del cambio global en el Pirineo Central español: una revisión a distintas escalas espaciales y temporales. *Pirineos* **170**, e012 (2015).
 43. Pini, R. et al. From pristine forests to high-altitude pastures: an ecological approach to prehistoric human impact on vegetation and landscapes in the western Italian Alps. *J. Ecol.* **105**, 1580–1597 (2017).
 44. Schwörer, C., Colombaroli, D., Kaltenrieder, P., Rey, F. & Tinner, W. Early human impact (5000–3000 BC) affects mountain forest dynamics in the Alps. *J. Ecol.* **103**, 281–295 (2015).
 45. Walsh, K. et al. A historical ecology of the Ecrins (Southern French Alps): Archaeology and palaeoecology of the Mesolithic to the Medieval period. *Quat. Int.* **353**, 52–73 (2014).
 46. Pini, R. et al. Life on a hilltop: vegetation history, plant husbandry and pastoralism at the dawn of Bergamo-Bergomum (northern Italy, 15th to 7th century bc). *Veget. Hist. Archaeobot.* **30**, 525–553 (2021).
 47. Segnana, M. et al. Holocene vegetation history and human impact in the eastern Italian Alps: a multi-proxy study on the Coltrondo peat bog, Comelico Superiore, Italy. *Veget. Hist. Archaeobot.* **29**, 407–426 (2020).
 48. Turner, J. The anthropogenic factor in vegetation history. *New Phytologist* **63**, 73–90 (1964).
 49. Cugny, C., Mazier, F. & Galop, D. Modern and fossil non-pollen palynomorphs from the Basque mountains (western Pyrenees, France): the use of coprophilous fungi to reconstruct pastoral activity. *Veget. Hist. Archaeobot.* **19**, 391–408 (2010).
 50. Alcolea, M., Utrilla, P., Piqué, R., Laborda, R. & Mazo, C. Fuel and acorns: early Neolithic plant use from Cueva de Chaves (NE Spain). *Quat. Int.* **457**, 228–239 (2017).
 51. Dumontier, P., Courtaud, P., Armand, D., Convertini, F. & Ferrier, C. Entre montagne et piémont, témoignages agropastoraux du Néolithique à l'âge du Fer. In *Estives d'Ossau: 7000 ans de pastoralisme dans les Pyrénées* (ed. Rendu, C.) 175–203 (Le Pas d'oiseau, Toulouse, 2016).
 52. Galop, D. *Estives d'Ossau: 7000 ans de pastoralisme dans les Pyrénées*. (le Pas d'oiseau FRAMESPA Parc national des Pyrénées, Toulouse Tarbes, 2016).
 53. Sierra, A. et al. Sheep husbandry in the early Neolithic of the Pyrenees: new data on feeding and reproduction in the cave of Chaves. *J. Archaeol. Sci. Rep.* **37**, 102935 (2021).
 54. Tejedor-Rodríguez, C. et al. Investigating Neolithic caprine husbandry in the Central Pyrenees: insights from a multi-proxy study at Els Trocs cave (Bisaurri, Spain). *PLoS ONE* **16**, e0244139 (2021).
 55. Sierra, A., Laborda, R., Saña, M. & Montes, L. Pastos en altura: el Neolítico antiguo de La Espluga de la Puyascada (La Fueva, Huesca). *TArqueo* **26**, 253–275 (2023).
 56. Utrilla, P. & Laborda, R. La cueva de Chaves (Bastarás, Huesca): 15 000 años de ocupación prehistórica. *Trab. Prehist.* **75**, 248 (2018).
 57. Galindo-Pellicena, M. A. et al. Metrical analysis of bovine bone remains from the Neolithic to the Bronze Age at the El Portalón site (Atapuerca, Burgos) in the Iberian context. *Quat. Int.* **566–567**, 211–223 (2020).
 58. García-Ruiz, J. M., Palacios, D., Andrés, N. & López-Moreno, J. I. Neoglaciation in the Spanish Pyrenees: a multiproxy challenge. *Med. Geosci. Rev.* **2**, 21–36 (2020).
 59. Tarrats, P. et al. Chironomid-inferred Holocene temperature reconstruction in Basa de la Mora Lake (Central Pyrenees). *Holocene* **28**, 1685–1696 (2018).
 60. Bernal-Wormull, J. L. et al. New insights into the climate of northern Iberia during the Younger Dryas and Holocene: The Mendukilo multi-speleothem record. *Quat. Sci. Rev.* **305**, 108006 (2023).
 61. Liu, M. et al. Holocene climates of the Iberian Peninsula: pollen-based reconstructions of changes in the west–east gradient of temperature and moisture. *Clim* **19**, 803–834 (2023).
 62. Kaufman, D. et al. Holocene global mean surface temperature, a multi-method reconstruction approach. *Sci. Data* **7**, 201 (2020).
 63. Jalut, G. Evolution de la végétation et variations climatiques durant les quinze derniers millénaires dans l'extrémité orientale des Pyrénées. (Toulouse III, 1974).
 64. Reille, M. & Lowe, J. J. A re-evaluation of the vegetation history of the eastern Pyrenees (France) from the end of the last glacial to the present. *Quat. Sci. Rev.* **12**, 47–77 (1993).
 65. Jalut, G., Andrieu, V., Delibrias, G., Fontugne, M. & Pages, P. Palaeoenvironment of the Valley of Ossau (western French Pyrénées) during the last 27000 years. *Pollen Spores* **30**, 357–393 (1988).
 66. Büntgen, U. et al. Placing unprecedented recent fir growth in a European-wide and Holocene-long context. *Front. Ecol. Environ.* **12**, 100–106 (2014).
 67. Kozáková, R. et al. Contrasting local and regional Holocene histories of *Abies alba* in the Czech Republic in relation to human impact: Evidence from forestry, pollen and anthracological data. *Holocene* **21**, 431–444 (2011).
 68. Tinner, W. et al. The past ecology of *Abies alba* provides new perspectives on future responses of silver fir forests to global warming. *Ecol. Monogr.* **83**, 419–439 (2013).
 69. Vandenberghe, C., Freléchoux, F., Moravie, M.-A., Gadallah, F. & Buttler, A. Short-term effects of cattle browsing on tree sapling growth in mountain wooded pastures. *Plant Ecol.* **188**, 253–264 (2007).
 70. Fillat, F., González, R. G., García, D. G., Gómez, D. & Reiné, R. *Pastos del Pirineo* (Editorial CSIC - CSIC Press, 2008).
 71. García-Ruiz, J. M., Valero-Garcés, B. L., Martí-Bono, C. & González-Sampériz, P. Asynchronicity of maximum glacier advances in the central Spanish Pyrenees: glacial advances in the Spanish Pyrenees. *J. Quat. Sci.* **18**, 61–72 (2003).
 72. Blaauw, M. & Christen, J. A. Flexible paleoclimate age-depth models using an autoregressive gamma process. *Bayesian Anal.* **6**, 457–474 (2011).

73. Reimer, P. J. et al. The IntCal20 Northern hemisphere radiocarbon age calibration curve (0–55 cal kBP). *Radiocarbon* **62**, 725–757 (2020).
 74. Rijal, D. P. et al. Sedimentary ancient DNA shows terrestrial plant richness continuously increased over the Holocene in northern Fennoscandia. *Sci. Adv.* **17**, eabf9557 (2021).
 75. Taberlet, P. et al. Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. *Nucleic Acids Res.* **35**, e14–e14 (2007).
 76. Voldstad, L. H. et al. A complete Holocene lake sediment ancient DNA record reveals long-standing high Arctic plant diversity hotspot in northern Svalbard. *Quat. Sci. Rev.* **234**, 106207 (2020).
 77. Boyer, F. et al. OBITOOLS: a UNIX-inspired software package for DNA metabarcoding. *Mol. Ecol. Resour.* **16**, 176–182 (2016).
 78. Alsos, I. G. et al. The treasure vault can be opened: large-scale genome skimming works well using herbarium and silica gel dried material. *Plants* **9**, 432 (2020).
 79. Soininen, E. M. et al. Highly overlapping winter diet in two sympatric lemming species revealed by DNA metabarcoding. *PLoS ONE* **10**, e0115335 (2015).
 80. Sønstebo, J. H. et al. Using next-generation sequencing for molecular reconstruction of past Arctic vegetation and climate. *Mol. Ecol. Resour.* **10**, 1009–1018 (2010).
 81. Willerslev, E. et al. Fifty thousand years of Arctic vegetation and megafaunal diet. *Nature* **506**, 47–51 (2014).
 82. Kanz, C. The EMBL nucleotide sequence database. *Nucleic Acids Res.* **33**, D29–D33 (2004).
 83. Frøslev, T. G. et al. Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. *Nat. Commun.* **8**, 1188 (2017).
 84. Oksanen, J. et al. *Vegan: Community Ecology Package* (2022).
 85. Legendre, P. & Gallagher, E. D. Ecologically meaningful transformations for ordination of species data. *Oecologia* **129**, 271–280 (2001).
 86. Mottl, O. et al. Rate-of-change analysis in paleoecology revisited: a new approach. *Rev. Palaeobot. Palynol.* **293**, 104483 (2021).
 87. Parnell, A. C., Haslett, J., Allen, J. R. M., Buck, C. E. & Huntley, B. A flexible approach to assessing synchronicity of past events using Bayesian reconstructions of sedimentation history. *Quat. Sci. Rev.* **27**, 1872–1885 (2008).
 88. Ninyerola, M., Pons, X. & Roure, J. M. *Atlas Climático Digital de La Península Ibérica. Metodología y Aplicaciones En Bioclimatología y Geobotánica* (Universidad Autónoma de Barcelona, Bellaterra Data, 2005).
- Sánchez for their help in the field campaigns, and Elena Royo and Inés de la Parra for the laboratory work. Bioinformatic analyses were performed on resources provided by UNINETT Sigma2—the National Infrastructure for High Performance Computing and Data Storage in Norway.

Author contributions

Penélope González-Sampérez and Graciela Gil-Romera conceived the study; Penélope González-Sampérez and Ana Moreno acquired the funding; Graciela Gil-Romera coordinated the field expedition; Graciela Gil-Romera, Irene Julián-Posada, Ana Moreno, Blas Valero-Garcés, Javier Lara-Recuero and Penélope González-Sampérez performed the fieldwork campaign; Irene Julián-Posada, Sandra Garcés-Pastor and Pere Bover sampled the sedimentary archive; Irene Julián-Posada, Sandra Garcés-Pastor and Peter D. Heintzman did the *sed*aDNA sequencing and built the molecular libraries; Sandra Garcés-Pastor, Peter D. Heintzman and Inger G. Alsos did the *sed*aDNA bioinformatics; Graciela Gil-Romera, Ana Moreno and Blas Valero-Garcés performed the depth-age model and the sedimentological analyses; Irene Julián-Posada and Graciela Gil-Romera did all numerical analyses and integrated them in an open workflow; Irene Julián-Posada, Graciela Gil-Romera and Penélope González-Sampérez led the writing; Lourdes Montes, Alejandro Sierra and Javier Lara-Recuero wrote the archaeological synthesis; Daniel Gómez and Federico Fillat contributed data and knowledge on current day grass community ecology. Each author contributed to the writing process and provided significant intellectual input.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s43247-025-02023-8>.

Correspondence and requests for materials should be addressed to Irene Julián-Posada, Graciela Gil-Romera or Penélope González-Sampérez.

Peer review information *Communications Earth & Environment* thanks Laurent Bremond and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Primary Handling Editor: Carolina Ortiz Guerrero. A peer review file is available.

Reprints and permissions information is available at <http://www.nature.com/reprints>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2025

Acknowledgements

Funding for this research was provided by the Spanish Ministry of Science and Innovation through the PYCACHU project (PID2019-106050RB-I00), GAPS AND DATES project (PID2020-116598GB-I00), CORREDORAS project (PID2022-141558NB-I00) and PASTORA project (OAPN, ref. 2852/2022), as well as the E02_23 R and H14-23R research groups of Gobierno de Aragón. Irene Julián Posada was supported by a PhD fellowship provided by the Spanish Ministry of Science, Innovation and Universities (FPI PRE2020-094749). We thank the Genomic Support Centre Tromsø (GSCT) at The Arctic University of Norway for amplicon sequencing. Sandra Garcés Pastor, Peter D. Heintzman and Inger G. Alsos were supported by a Research Council of Norway grant 250963/F20 for the ECOGEN project. Inger G. Alsos was also supported by The European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme grant agreement No 819192 for the IceAGenT project. Sandra Garcés Pastor was also supported by the Beatriu de Pinós Programme (BP-2021-00131). Peter D. Heintzman acknowledges support from the Knut and Alice Wallenberg Foundation (KAW 2021.0048 and KAW 2022.0033). Alejandro Sierra is supported by the Juan de la Cierva Formación (FJC2020-043414-I). We are grateful to the Gobierno de Aragón and the Tramacastilla de Tena town council for granting the permissions needed for the sampling process. We thank Miguel Bartolomé, Marcel Galofré, Kilian Jungkeit-Milla, Reyes Giménez, Benito Pérez, Jorge Pey, Pedro