

# High resolution ancient sedimentary DNA shows that alpine plant biodiversity is a result of human land use

**Sandra Garcés-Pastor** (✉ [sandra.garces-pastor@uit.no](mailto:sandra.garces-pastor@uit.no))

The Arctic University Museum of Norway <https://orcid.org/0000-0001-5652-7264>

**Eric Coissac**

Univ. Grenoble Alpes, CNRS

**Sebastien Lavergne**

French National Centre for Scientific Research

**Christoph Schwoerer**

Oeschger Centre for Climate Change Research, University of Bern & Institute of Plant Sciences, University of Bern

**Jean-Paul Theurillat**

Université de Genève <https://orcid.org/0000-0002-1843-5809>

**Pete Heintzman**

The Arctic University of Norway

**Owen Wangensteen**

Norwegian College of Fishery Science, UiT - The Arctic University of Norway

**Willy Tinner**

Oeschger Centre for Climate Change Research, University of Bern & Institute of Plant Sciences, University of Bern

**Fabian Rey**

Department of Environmental Sciences, University of Basel

**Martina Heer**

Department of Environmental Sciences, University of Basel

**Astrid Rutzer**

Department of Environmental Sciences, University of Basel

**Kevin Walsh**

University of York <https://orcid.org/0000-0003-1621-2625>

**Youri Lammers**

The Arctic University of Norway <https://orcid.org/0000-0003-0952-2668>

**Antony Brown**

Arctic University of Norway

**Tomasz Goslar**

**PhyloAlps Consortium .**

University Grenoble-Alpes · Laboratoire d'écologie alpine (LECA) France

**Oliver Heiri**

University of Basel

**Inger Alsos**

Tromsø University Museum <https://orcid.org/0000-0002-8610-1085>

---

**Article**

**Keywords:** alpine biodiversity, sedaDNA, ecological indicators, grazing, flora, alpine farming

**Posted Date:** September 22nd, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-908129/v1>

**License:**   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

**Version of Record:** A version of this preprint was published at Nature Communications on November 4th, 2022. See the published version at <https://doi.org/10.1038/s41467-022-34010-4>.

# Abstract

Alpine areas are well known biodiversity hotspots, but their future may be threatened by expanding forest and changing human land use. Here, we reconstructed past vegetation, climate, and livestock over the past ~ 12,000 years from Lake Sulsseewli (European Alps), based on sedimentary ancient DNA, pollen, spores, chironomids, and microcharcoal. We assembled a highly-complete local DNA reference library (PhyloAlps, 3,923 plant species), and used this to obtain an exceptionally rich *sedaDNA* record of 366 plant taxa. The vegetation mainly responded to temperature during the first half of the Holocene, while human activity drove changes from 6 ka onwards. Land-use shifted from episodic grazing (Neolithic, Bronze Age) to agropastoral intensification (Medieval Age). This prompted a coexistence of species typically found at different elevational belts, thereby increasing plant richness to levels that characterise present-day alpine diversity. Our results indicate that traditional agropastoral activities should be maintained to prevent reforestation and preserve alpine plant biodiversity.

## Introduction

Changing environmental conditions are displacing organisms out of their ranges, causing severe threats to biodiversity<sup>1</sup>. In mountains, vegetation is strongly determined by temperature and forms elevational belts, in the European Alps ranging from the warm lowland Colline belt to the cold highland Nival belt<sup>2</sup>. Climate-based projections indicate an expected upward displacement of vegetation that will reduce habitat for present-day alpine species and especially cold adapted taxa that inhabit the highest elevations<sup>3</sup>. This process is already being observed on European summits, with an increase in total plant species richness due to the arrival of lowland vegetation over the last century<sup>4,5</sup>. Plant remains in lake sediments allow us to explore vegetation responses to past climate changes and human activity, particularly at the decadal and longer time scales relevant for understanding future vegetation response to global warming. Therefore, detailed palaeoecological records representing the full range of plant types and functional groups that compose alpine and subalpine vegetation are needed to understand how long-term interactions of climate and humans affect overall biodiversity and survival of high-altitude plants. However, some ecologically relevant groups such as grasses and sedges are poorly represented in conventional palaeoecological records due to limited taxonomic resolution<sup>6</sup>. Recent advances in sedimentary ancient DNA (*sedaDNA*) have greatly improved our ability to get detailed insight into past diversity processes<sup>7-9</sup>.

The European Alps are an important plant biodiversity hotspot<sup>10</sup>, with ~4,000 native plant species<sup>11</sup>. This diversity results from a complex interplay of both "natural" and "human" factors over both geological and more recent timescales. Natural climatic and environmental conditions in alpine regions as well as palaeoclimatological and palaeogeographic changes resulted in suitable conditions for plant immigration, speciation, and endemism<sup>12,13</sup>. However, traditional human activities over millennia have modified and in many cases helped to maintain a part of this diversity<sup>14</sup>. Humans have modified the alpine landscapes since the Mesolithic, ca. 10 ka (1 ka = 1,000 yr ago), by clearing small areas of forest

to attract prey for hunting<sup>15,16</sup> while the introduction of agropastoral activities during the Neolithic (ca. 7 ka) drove a downward shift of alpine treelines<sup>15–18</sup>. Human-environment interactions in forested and open vegetation types such as the Subalpine zone led to a mosaic of different habitats that include species-rich meadows<sup>2,9,16</sup>. As a result, future changes in land use might imply a reduction in biodiversity of subalpine and alpine landscapes<sup>19</sup>. For example, the abandonment of high-mountain traditional practices during the last half-century has reduced the composition of alpine pastures in many mountain ranges such as the Alps<sup>14,20</sup>, the Pyrenees<sup>21</sup>, and the Himalayas<sup>22</sup>.

Here, we reconstruct the response of the vegetation around Lake Sulsseewli, located in the northern Swiss Alps (Figure 1), to climate and human activities over the past 12,000 years. We used a multiproxy approach consisting of plant *sed*aDNA, pollen, fossil chironomids for summer temperature reconstruction, geochemical proxies, and multiple independent indicators of human activity, that included microscopic charcoal (reflecting fire activity) and grazing indicators (coprophilous fungi spores and mammalian *sed*aDNA). Also, we assembled *trnL* P6 loop locus data from a new comprehensive taxonomic DNA reference database consisting of 3,923 plant species collected in the Alps and 417 from the Carpathians (the PhyloAlps database; <http://phyloalps.org/>). We demonstrate that PhyloAlps provides superior identification accuracy for plant *sed*aDNA as compared to three other non-local databases. The exceptionally high taxonomic resolution of the plant *sed*aDNA data allowed us to reconstruct both long-term changes in plant diversity and changes in plants that are particularly temperature-sensitive (i.e. with restricted elevational distributions), or are considered pastoral and arable indicators<sup>23</sup>. Our results show that the vegetation was mainly driven by temperature during the first half of the Holocene (11 to 6 ka), while human activity drove changes from 6 ka. We find that millennia of intensive human pressure prompted the rise of diversity that characterizes the present alpine diversity by favouring the coexistence of taxa normally found in different vegetation belts. These findings suggest that in order to maintain the current high plant diversity of subalpine and alpine ecosystems in the face of ongoing climate warming, governments should aim to maintain moderate land use practices.

## Results

### Improved identification of plants and ecological indicators from *sed*aDNA

After final data filtering, we obtained sequences from 366 unique plant taxa from 73 sediment samples of Sulsseewli (see Methods, Supplementary dataset S1, Supplementary Figure SF4, Supplementary Tables ST8, ST9). We first identified plant taxa in our *sed*aDNA data set using four reference databases (PhyloAlps, PhyloNorway, ArctBorBryo, and EMBL). PhyloAlps provided the highest number of sequences assigned to vascular plants overall and increased the number of taxa assigned at genus or species level by 30% compared to EMBL at genus or lower. After consolidating the identifications from the four reference databases, PhyloAlps identified 87% of the total sequences, followed by EMBL which assigned 12% (Supplementary Tables ST3, ST4.1, ST4.2).

A total of 91 of our 366 identified plant taxa were informative of land use or vegetation belts: 87 were identified as indicators of specific elevational vegetation belts (Material and methods, Supplementary Table ST2), one indicated arable land (*Myosotis arvensis*), eight taxa were used for pastoral inference (Supplementary Table ST5, Figure 2, B) and five taxa are indicators for both vegetation belts and pastoral inference. For data analysis, all *sed*aDNA results are expressed as the relative abundance index (RAI), which integrates information from the relative proportion of reads and replicability of metabarcoding PCR (see Material and methods).

## A local Holocene temperature reconstruction

Fossil chironomids are excellent indicators of past summer temperature change, as many chironomid taxa are indicative of prevailing temperature conditions<sup>24</sup>. The remains of chironomid larvae are well preserved and remain identifiable in lake sediment records. Chironomids from Sulsseewli indicate several major community composition shifts, which are indicative of changing temperatures (Supplementary Figure SF3). The compositions of chironomid communities were translated to estimates of past summer temperature change using a Swiss-Norwegian chironomid - July air temperature transfer function<sup>24</sup> that has been extensively used and tested in the Alps<sup>25</sup>. Reconstructed mean July temperatures range between 7 and 11 °C (Figure 2, D). The earliest Holocene presents a phase of relatively cool inferred temperatures (ca. 7.9 °C, before 11 ka), which was followed by an extended phase of relatively warm temperatures in the mid-Holocene (ca. 10.8 °C, 9.2 to 5.5 ka) and again cooler temperatures in the late Holocene (ca. 9.7°C, from 4 ka). This temperature development is in agreement with other climate records from the Alps<sup>25,26</sup>.

## Determining drivers of vegetation changes

We conducted a redundancy analysis (RDA) of the plant *sed*aDNA data across all samples and added 21 explanatory variables to assess how the relative abundance index (RAI) of plants changed in relation to potential drivers, such as early human activities (represented by plant *sed*aDNA of pastoral and arable indicators, coprophilous fungal spores, *sed*aDNA of key mammals groups, and microcharcoal) and climatic changes (represented by chironomid-inferred temperatures, and organic matter content) (Figure 4). The results of this analysis show that the first axis (RDA 1, 53.35% of variance explained) is mainly related with multiple independent variables related to human influence (plant *sed*aDNA of pastoral and arable indicators, the coprophilous fungi spores), and also separated samples older or younger than ~6 ka. Axis 1 was also related with the RAI of species representing the Colline-Montane and Montane-Subalpine vegetation belts, a group that also includes some taxa used as pastoral/arable indicators. This suggests that the distribution of these taxa may be influenced by human activity rather than climate (estimated summer temperatures). *Sed*aDNA of domestic mammals such as domestic goat and sheep strongly correlate with these indicators, providing independent evidence of this ecological inference. While sheep are more related to Colline-Montane taxa, goats correlate with our arable indicator and Montane-Subalpine taxa. The second axis (RDA 2, 17.06%) is related to variables reflecting climate change, such as the temperature inferred by chironomids and organic matter content of the sediments, as

well as the relative proportion of Alpine-Subalpine communities. This second axis also broadly separates the Early and Mid Holocene samples. Given the results of the RDA analysis, we split our description of the proxy results into the two periods highlighted by RDA 1.

## Temperature-driven vegetation changes during the Early to Mid-Holocene

The oldest analysed sediments of the Sulsseewli record are challenging to date precisely based on the available chronological and sedimentological data (Supplementary Figure SF2) but likely originate from the Younger Dryas-Holocene transition at 11.7 ka or the period just preceding this transition. Highly variable and low chironomid-derived July temperatures are inferred for these lowest sediment sections, which is consistent with the transition from the Younger Dryas into the Early Holocene. Based on the plant *sed*aDNA, this period was associated with a high abundance of cold-adapted Alpine forbs such as *Achillea atrata*, *Crepis rhaetica* and *Silene acaulis* with some *Dryas octopetala* dwarf shrubs. This period had the lowest values of organic matter (LOI), suggesting limited vegetation cover and organic production for this interval (Figure 2, F, G, Zone 1, 12-11.35 ka, T range = 7-9 °C). An increase in Subalpine/Alpine plant taxa (*Anthyllis vulneraria*, *Athamanta cretensis*, *Chaerophyllum* and *Carex frigida*), suggests an upward migration of the vegetation that could be associated with an initial plateau of inferred temperature of ~8.5 °C at 11.35-10.55 ka (Figure 2, B, Zone 2). This resulted in the largest forb introduction in the catchment (~50 taxa) and the highest diversity of *sed*aDNA plant species for the Early Holocene (~120 taxa per sample)(Supplementary Figure SF7). This climate-related establishment of new species apparently triggered a series of biotic interactions that, together with further increasing temperatures, changed the community composition and displaced alpine species and caused a decrease in richness (Figure 2, B). The amount of forbs in our *sed*aDNA results are in line with the high proportions of Poaceae and *Artemisia* pollen recorded during the earliest Holocene (Supplementary Figure SF5, Zone 1). However, the lower taxonomic resolution of forb, grass and sedge pollen (96 taxa) does not reveal the exceptionally high diversity of the herb community that is recovered by *sed*aDNA (232 taxa) or the full breadth of cold-adapted species, and shows lower richness than indicated by *sed*aDNA throughout the Early to Mid Holocene (Supplementary Figure SF8, Supplementary dataset S2). During this earliest Holocene interval, we observe only sporadic mammalian *sed*aDNA sequences of two wildlife taxa, ibex (*Capra ibex*) and chamois (*Rupicapra rupicapra*), suggesting a low mammalian biomass and natural landscape at this time (Figure 3, B, Supplementary Figure SF9).

Temperatures then increased and reached a second plateau by ~9.5 ka that were as much as ca. 3 °C higher than in the inferred Younger Dryas interval. This represents the most dramatic temperature increase in our record and, based on plant *sed*aDNA, prompted the upward migration of Subalpine taxa like *Crepis bocconi* whereas Subalpine-Alpine taxa almost disappear, and richness decreases in the vicinity of the lake (Figure 2, B, Zone 3, 10.55 - 9.2 ka). The warmer and relatively stable temperatures of the Holocene Climatic Optimum (9-5.5 ka, ~11 °C) also allowed the upward expansion of Montane-Subalpine taxa (*Abies alba*, *Chaerophyllum aureum*, *Lonicera alpigena*), along with a dominance of woody taxa (Supplementary Figure SF4, SF5, Figure 2, B, C, Zone 4, 9.2 to 6 ka). The rise of shrubs (Maleae, Rosoideae), which began at ~10.5 ka, might have transformed ecosystem productivity and

produced the highest values of sedimentary organic matter (LOI) (Figure 2, A, E, F). As shrubs expanded, the relative abundance index of herbs decreased and diversity dropped to only a third of the taxa that dominated in the earliest Holocene (~50 taxa) (Figure 2, C, Supplementary Figure SF7). We recovered a single occurrence of domestic cow (*Bos taurus*) at ~9 ka, which we attribute to stochastic contamination (see Methods) as there is no archaeological evidence to support this occurrence<sup>27</sup>. A rise in coprophilous fungi spores between 7.9-7.15 ka suggests a greater abundance of mammalian herbivores and therefore higher grazing pressure in the vicinity of Sulsseewli (Figure 3, A, B). The lack of arable and pastoral indicators and domesticated livestock *seDa*DNA suggest that this may have resulted from wild animals. The decrease in trees and shrubs in the plant *seDa*DNA record beginning at 7 ka led to the introduction of new herb species and the rise of total plant diversity (~90 taxa) (Figure 2, A, D, Supplementary Figure SF8). During this interval, red deer (*Cervus elaphus*) are first detected in the record.

## Human-driven vegetation changes during the Mid-Late Holocene

The first major evidence of human disturbance through fire and grazing appears at 6.35 ka during the latter half of the Neolithic (7.5–4.2 ka) (Figure 3), as inferred from the rise of several indicators of human activity (microcharcoal, coprophilous fungi spores, plant *seDa*DNA indicating pastoral activities) (Figure 2, A, E). The first cereal pollen appeared in the sediments around this time, indicating agricultural practices in the vicinity of the Sulsseewli catchment, presumably at lower elevations in the main Lauterbrunnen valley (Figure 1, A, Supplementary Figure SF6). An increase in grazing pressure is evidenced by a rise of coprophilous fungi spores, pastoral plant *seDa*DNA and microcharcoal during the late Neolithic (Figure 3, A). This human impact signal continues in the interval between the late Neolithic and earliest Bronze Age, 5.7-4.2 ka, with multiple detections of red deer and a rise of mesophilous, Montane-Subalpine plant taxa such as *Saxifraga rotundifolia*, *Vicia sylvatica* (indicative of forests with open canopies), and *Trollius europaeus* (wet meadows) by 5 ka (Figure 2, B, Zone 5, Supplementary Figure SF5).

From the start of the Bronze Age (ca. 4.2 ka) onwards, the continuous presence of diverse indicators of human activity suggest that Sulsseewli has been exploited as a grazing area for domesticated livestock. This interpretation is supported by an increase in the pollen and the appearance of *seDa*DNA of species favoured by human disturbances such as *Alnus alnobetula* (Supplementary Figure SF5). A short period of agricultural activity at 3.9-3.8 ka during the Early Bronze Age is marked by the first appearances of the arable plant *seDa*DNA indicator and cereal pollen including subtypes Cerealina, *Hordeum* and *Triticum* (Figure 3, A, Supplementary Figure SF6). This period also includes the first occurrence of sheep *seDa*DNA, which is suggestive of pastoral farming during this time. The Late Bronze Age (3.35–2.8 ka) is characterised by intensifying human impact. The rise of indicators of human impact suggests that humans burned forests and/or grasslands (microcharcoal), cultivated crops (arable plant *seDa*DNA and pollen of Cerealina and *Hordeum*, Supplementary Figure SF6), and pastured sheep (*Ovis seDa*DNA, coprophilous fungi spores) in the Montane-Subalpine plant communities of Sulsseewli, although we note the potential disappearance of sheep in the latest Bronze Age (Figure 3, A, B). After the Bronze Age, high microcharcoal concentrations together with high RAI values of sheep *seDa*DNA point to recurrent burning

to maintain nearby pastures and crops (Figure 3, A). At this time, the proportion of Colline-Montane plant taxa increased (Figure 2, B). However, a drop in all human indicators and the reappearance of red deer around 2.2-2 ka preceding the Roman period suggests the temporary abandonment of agropastoral activities in the zone, which apparently favoured Subalpine plant taxa and conifer reforestation (*Pinus cembra*, *Picea*, Supplementary Figure SF4, SF5), leading to another decrease in meadows (Figure 2, A). By the Late Roman period (ca. 1.6 ka), a marked decrease of trees reflected by plant *sed*aDNA functional groups and increased microcharcoal concentrations suggest a high fire incidence to clear the forest. The high proportions of pastoral plant *sed*aDNA and co-occurrence of domestic goat (*Capra hircus*), sheep and cow *sed*aDNA also support that forest was cleared for pastures of diverse livestock (Figure 3, A, B). After the Roman period, human activity continued with the presence of arable *sed*aDNA, pollen from cereals (*Cerealia*, *Hordeum*, *Triticum* and *Secale*), *sed*aDNA of Colline cultivated trees (*Juglans regia*, *Castanea sativa*), and a continued decrease in tree pollen and *sed*aDNA (Supplementary Figure SF4, SF5, SF6). However, *sed*aDNA of domestic livestock taxa was not present during this brief interval prior to the Middle Ages.

The Middle Ages (1.4-0.5 ka) saw a massive increase of microcharcoal and a near loss of tree pollen and *sed*aDNA (Figure 2, A, Figure 3, A), suggesting high fire activity to clear the forest<sup>17</sup>. The rise of coprophilous fungi spores, plant *sed*aDNA pastoral indicators, as well as sheep, goat, cow, and horse (*Equus caballus*) *sed*aDNA also points to large-scale grazing (Figure SF4). Total plant diversity (~160 taxa) and the abundances of both Subalpine-Alpine and lowland plant species rose (Figure 2, A, B, C, Zone 6, Supplementary Figure SF8, SF5). The contribution from higher elevation taxa could have been favoured by a reduction of competition by shrub and tree species in open meadows together with a greater erosion due to deforestation. The rise of grazing activities might have facilitated the proliferation of lowland taxa that could have been transported or favoured by livestock herds, increasing the total floral diversity (Figure 2, A, B, D, E). The greater influence from lower elevation vegetation would have led to a contraction of space and increased competition for Alpine species (Figure 2, B, Zone 6). Finally, the recent increase in plant *sed*aDNA of Subalpine-Alpine taxa during Medieval and Modern times (1.2-0 ka) coincides with some Colline-Montane taxa (Figure 2, B, Zone 7). Increased human activity and grazing may have reduced the standing biomass of the more competitive Subalpine taxa (grasses, dwarf shrubs), allowing heliophilous, shorter Subalpine-Alpine taxa to occur at a lower elevation, resulting in an overall increase in species richness (Figure 2, C). The highest values of coprophilous fungi spores, together with plant *sed*aDNA pastoral indicators and cow-dominated *sed*aDNA suggest a return to high mountain grazing during the 18<sup>th</sup> Century CE (Figure 2, Figure 3).

## Discussion

The diverse multiproxy and high resolution approach of this study makes it the most detailed palaeoecological reconstruction of Holocene Alpine vegetation to date. With 366 identified plant taxa, Sulsseewli yields the richest single record studied to date with *sed*aDNA using an exact match. This is 2-3 times as many taxa as found in any previous study of ancient plant *sed*aDNA<sup>7,8,28,29</sup>, and highlights the

advantage of using a region-wide comprehensive, rather than global and incomplete reference databases<sup>9,30,31</sup>. Our *sedDNA* results uncover a hidden diversity of taxa that are unrepresented by pollen analysis<sup>32-34</sup>, including many insect-pollinated plants normally underrepresented by pollen, and highlights the importance of forbs in Subalpine communities<sup>35</sup>. The pollen of Sulsseewli (166 vascular taxa) present a similar diversity as nearby lakes Iffigsee (143 taxa)<sup>17</sup> and Lac de Bretaye (137 taxa)<sup>36</sup>. These results suggest that the high values of richness found in Sulsseewli are representative and not only related to good DNA preservation and recovery, thereby indicating that the study region has been a biodiversity hotspot over the entire Holocene.

The high number and resolution of detected taxa allowed us to identify those that are informative about elevational vegetation belts and therefore reconstruct how Alpine, Subalpine and Montane plant communities changed throughout the Holocene at Sulsseewli, which is coherent with the regional trends of pollen and macrofossil studies<sup>37-39</sup>. Pollen studies reconstructed the shifts of the Montane-Subalpine ecotone by grouping characteristic pollen taxa, with the limitation that pollen taxa can often include several species and autecologies<sup>17,40</sup>. Some *sedDNA* studies used single genera such as *Plantago* to infer human activities<sup>9,30</sup>. However, this genus has ~16 species that are found in the Alps of which only four are favoured by grazing (*P. alpina*, *P. lanceolata*, *P. media*, *P. major*<sup>23,41</sup>). On the other hand, Liu et al.<sup>7</sup> used single species such as *Sanguisorba officinalis* to infer human activities. However, this species can also be found in undisturbed sites such as wetlands or meadows. Our *sedDNA* approach provides a more robust inference since its ecological interpretations are based on groups of well-characterised taxa. We have also overcome the taxonomic limitation of some species sharing the same p6 loop sequence<sup>42</sup> by retaining the sequences as indicators only if their autecological category is shared among all species assigned to that sequence (see Methods). The information obtained from these stricter indicator sequences opens up a range of possibilities to robustly reconstruct unexplored parameters in palaeoecology.

The rise of DNA richness in Sulsseewli during the Early Holocene contrasts with the minor increase in palynological richness in Sulsseewli and the Alps<sup>43</sup> (Figure 2, A). In this study, the higher representation of forbs by *sedDNA* fills a gap of the herbaceous taxa that could not have been resolved by pollen or might have been masked by high pollen-producing taxa (Supplementary Figure SF7). Our *sedDNA* results paint a more detailed picture than the pollen results of how higher temperatures led to increased plant diversity during the Early Holocene and suggest that temperature was the main driver of vegetation changes during the first part of the Holocene. This finding diverges from the weak relationship between *sedDNA*-inferred plant taxa richness and independent temperature reconstructions that Liu et al.<sup>7</sup> found in Lake Naleng (4,200 m a.s.l, subalpine meadows, Tibet) during the Holocene. However, the different thresholds of the identification match for the sequences used in both studies limits the comparability of these results.

Our multiproxy palaeoecological approach also reveals that from the Neolithic onwards human activities promoted the co-occurrence of taxa typical for different elevation belts. This resulted in a rise of diversity

in the vicinity of Sulsseewli, a change also supported by a clear increase of the regional pollen diversity<sup>44,45</sup>. Considering the strong evidence of intensified grazing, it seems likely that grazing activities during the Neolithic modified the structure and composition of the vegetation by patchy disturbance, dung deposition and grazing preference. This is in line with social and human genetic changes that occurred in the Late Neolithic population<sup>46</sup> associated with a rise in transhumance activities in the region<sup>37,47–50</sup>. The input of nutrients and seed by livestock when moving across valleys and high-mountains during seasonal transhumance<sup>51</sup> could have introduced new species that might have been able to grow in the new microhabitats created around the lake (e.g. nutrient-rich depressions, open areas suitable for pioneer taxa)<sup>45,52,53</sup>. Indeed, part of the pastoral plant *seda*DNA indicators are species dispersed by animals<sup>23</sup>. The shift towards combined arable and pastoral farming during the Bronze Age (3.4 ka) prompted a further disruption of the landscape, where sheep pastoralism, fires and deforestation favoured the presence of Colline-Montane taxa. This trend agrees with a rise of pastoralism in nearby lakes Lac de Bretaye (68 km south-west of Sulsseewli<sup>36</sup>), Sägitalsee and Bachalpsee (10 and 14 km north-east respectively<sup>37</sup>). Sulsseewli offers the earliest robust evidence of domesticated livestock DNA in the Northern Swiss Alps, which occurs at least 1,000 years earlier than in the western Alps (Lake Anterne, Iron Age, 2.5 ka<sup>54</sup>) although plant grazing indicators suggest that the first significant human impact might have occurred as early as 3.5 ka in that region of the Alps<sup>30</sup>. The shift towards mainly cow dominated livestock farming by 700 years ago coincides with the establishment of an Augustinian monastery in the Interlaken valley (20 km from Sulsseewli), which might have facilitated the intensification of pastoral practices in the region<sup>9,37</sup> as part of the cheesemaking economy<sup>55,56</sup>.

Our study showcases how human activity can promote a co-occurrence of taxa typical for different elevational vegetation belts. Based on the intermediate disturbance hypothesis<sup>57,58</sup>, intermediate levels of agropastoral disturbance during the last millennia may have maximized the spatial heterogeneity and plant species diversity. This is corroborated by contemporary studies that relate land abandonment during the past decades to a significant reduction in plant biodiversity<sup>59</sup> as well as by observations of peak plant richness with moderate levels of grazing<sup>58</sup>. Therefore, our long term reconstruction of plant diversity based on *seda*DNA and other palaeoecological indicators confirms earlier studies that suggested that maintaining high plant diversity in the Alps requires conserving or promoting moderate land use practices<sup>60</sup>. This contrasts with palaeoecological studies elsewhere that suggest that grazing effects might be too weak to compensate for climate warming impacts<sup>7,61</sup>. This discrepancy might be explained by a lesser human impact in their study site, located at a much higher altitude (4,200 m a.s.l.). However, our findings stand with palaeoecological and contemporary studies that registered the potential of grazing to mitigate the elevational advance of climate-driven shifts in plant communities through increasing total stress<sup>45,62</sup>. Furthermore, recent studies suggest that shifts from intensive to extensive agricultural land use can favour native species while controlling the oversimplification of the flora due to plant invasion<sup>61</sup>.

Considering the observed general decrease of species' richness with elevation<sup>2</sup>, we conclude that a rise in diversity in alpine systems might be a sign that land-use allows the coexistence of species adapted to different habitats. This is in line with many studies that point out that regional richness may increase with warmer temperatures<sup>2</sup>, prompting an acceleration of changes in mountain summits during the past century due to the upward movement of lower vegetation belts<sup>4</sup>. In the view of our results, we question the use of total diversity as a measure of ecosystem health in alpine systems and consider the introduction of other diversity metrics. In contrast with Liu et al.<sup>7</sup>, we propose metrics that are based on particular elevational vegetation belts, as demonstrated here, instead of the within dominant alpine families since they can contain taxa belonging to different elevational habitats and communities. Therefore, if preserving the high biodiversity of alpine landscapes is a conservation goal, then policies should support the maintenance of traditional land-use practices in high-elevation areas<sup>19,20,44,59,61</sup> that will ensure the continuity of the ecological niches for alpine species and limit the spread of forest species that threaten them<sup>45,63</sup>. These measures are also important for other organisms that inhabit such habitats, such as the mountain hare or ring ouzel, which are especially vulnerable to global warming<sup>64,65</sup>.

## Methods

### Study Site

Sulsseewli is a small lake (2 ha) in the Bernese Alps (northern Swiss Alps), located in the subalpine vegetation zone below the present treeline (Figure 1, 46.617639° N, 7.864028° E; 1,921 m a.s.l.). The catchment lies on calcareous bedrock, surrounded by bare rock and scree slopes, together with meadows and a scattered spruce (*Picea abies*) forest on the eastern side. Sulsseewli has two water inlets and a maximum water depth of ca 7.5 m. The northern Alps have a cool temperate climate with mild summers (18 °C July) and cool winters (1 °C January) in the lowlands and very cold conditions above treeline<sup>66</sup>.

### Core sampling, lithology and chronology

Four parallel cores (SUL A, B, C, D) were retrieved in August 2018 with a modified Streif-Livingstone piston corer<sup>67</sup>. Sediment cores were sealed in the field to avoid contamination. Sediments were then extruded in half-pipes and immediately sealed to minimize the risk of modern DNA contamination at the Institute of Plant Sciences & Oeschger Centre for Climate Change Research (University of Bern, Switzerland). Cores A and B were stored at the University of Bern, while cores C and D were stored at the The Arctic University Museum of Norway in Tromsø (TMU, Norway). All cores were stored at 4 °C. At both facilities, core sections were opened by longitudinal splitting. One half was used for proxy subsampling, and the other half was used for photography. Lithostratigraphic markers were used to correlate cores A, B and C resulting in a continuous composite sequence of 716 cm (Supplementary Figure SF1), whereas SUL D was not used.

The organic content of the sediments was measured by mass loss-on-ignition (LOI) following the Lamb method<sup>68</sup>. Samples were taken at the same intervals as DNA, dried overnight at 105 °C and burned at 550

°C for 2 h to oxidize the organic matter. Total LOI was calculated as the percentage loss of dry weight after ignition.

Twenty-three plant macrofossil remains were sampled and radiocarbon-dated using accelerator mass spectrometry (AMS) at either the Laboratory for the Analysis of Radiocarbon at the University of Bern ('BE' accessions) or the Poznan Radiocarbon Laboratory ('Poz' accessions, Poland) (Supplementary Table ST1). AMS radiocarbon dates were calibrated using the terrestrial IntCal20 curve<sup>69</sup> within an age-depth model that was constructed using the Bayesian framework calibration software 'Bacon' v.2.3.4<sup>70</sup> in R v3.4.2 (R Core Team 2017).

## Chironomid-based temperature reconstruction

For chironomid analysis, 75 sediment subsamples of 0.125 to 9 cm<sup>3</sup> were sieved (100 µm) from a correlated composite section of cores SUL A and B without chemical pretreatment following Brooks<sup>71</sup>. Chironomid head capsules were recovered from the sieve residue with a fine forceps under a stereomicroscope at 30-50x magnification and mounted on slides in Euparal mounting medium. Identification was performed by examining the mounted specimens under a compound microscope (100-400x magnification) and using keys for Chironomidae Larvae<sup>71,72</sup>.

A 274-sample modern chironomid-temperature calibration dataset from Switzerland and Norway, describing the distribution of chironomid taxa in small lakes relative to observed mean July air temperatures, was used to develop a Weighted-Averaging-Partial Least Squares (WA-PLS) regression model that predicts mean July air temperatures based on the composition of fossil chironomid assemblages<sup>24</sup>. This model, based on two WA-PLS components and square root transformed chironomid assemblage data predicted mean July air temperatures with a root mean square error of prediction of 1.40°C and an  $r^2$  of 0.87 in the modern environment (estimated based on cross-validation (bootstrapping) with 9999 bootstrapping cycles). Before application to the downcore record several samples with low counts were amalgamated with adjacent samples to result in a higher count size for reconstruction. The final record consisted of 73 samples with a minimum count sum of 40 chironomids, except for a single sample at 389.5 cm sediment depth (36 chironomids). Sample-specific estimated standard errors of prediction were calculated using 9999 bootstrapping cycles. The transfer function and reconstruction were developed with the program C2<sup>73</sup>.

## Pollen, spores, and microcharcoal

A total of 75 samples of 1 cm<sup>3</sup> were sampled from a correlated composite section of cores SUL A and B for pollen, spores and microscopic charcoal analyses. Pollen samples were processed following standard palynological approaches<sup>74</sup> with KOH, HCl, HF, acetolysis and mounting in glycerine. *Lycopodium* tablets were added before the chemical treatment as a control and to estimate microfossil concentrations<sup>75</sup>. Pollen, spores and non-pollen palynomorphs (NPPs) were identified at 400× magnification according to Moore et al.<sup>74</sup> and Reille<sup>76</sup> and the reference collection at the Institute of Plant Sciences (University of

Bern). A minimum of 500 terrestrial pollen grains per sample was counted. Up to 166 pollen types of terrestrial and aquatic vascular plants were identified. NPP and coprophilous fungal spores were identified according to van Geel et al.<sup>77</sup>. Diagrams were plotted with the program Tilia. Statistically significant pollen zones were determined with partitioning using optimal sum of squares and the broken stick method<sup>78</sup>. To study the regional fire activity, microscopic charcoal was analysed<sup>79,80</sup>. Particles between 10-500 µm were counted on the pollen slides following Tinner et al.<sup>81</sup>. Coprophilous fungi spores were analyzed to infer grazing pressure (*Cercophora*, *Delitschia*, *Podospora*, *Sordaria*, *Sporormiella* and *Trichodelitschia*). The palynological results are presented as percentages of the terrestrial pollen sum excluding aquatic plants whereas charcoals and fungal spores were presented as influx (particles cm<sup>-2</sup> year<sup>-1</sup>, Figure SF5).

## Sedimentary ancient DNA data generation

Core SUL C was subsampled at 10-cm resolution in the ancient DNA lab at TMU. DNA extraction was conducted in a clean ancient DNA laboratory and followed the protocols of Rijal et al.<sup>8</sup>. DNA was extracted from 80 sediment samples and 13 extraction/sampling negative controls using a modified DNeasy PowerSoil kit (Qiagen, Germany) protocol in the ancient DNA laboratory at TMU. DNA extracts and negative extraction/sampling controls, along with two positive PCR controls, were amplified using a uniquely dual-tagged generic primer sets (Supplementary dataset S5) that amplify either the *trnL* P6 loop region of the chloroplast genome, a locus that has proven most successful for studies of plant *sedDNA*<sup>82,83</sup>, for plants (gh primers;<sup>84</sup> or a section of the mammalian mitochondrial 16S locus (MamP007 primers;<sup>9</sup>). Reaction and cycling conditions for all PCRs followed Voldstad et al.<sup>85</sup>, with the following exceptions for the 16S PCRs: (1) forward and reverse primer concentration was reduced to 0.1 µM each, and (2) forward and reverse blocking primers were added at 1 µM each. We used a forward blocking primer modified from Giguet-Covex et al.<sup>9</sup> with a sequence of GGAGCTTTAATTTATTAATGCAAACAGTAGG-C3 and a reverse blocking primer with a sequence of CCCAACCGAAATTTTTAATGCAGGTTTGGTGA-C3. Eight PCR replicates were carried out for each sample or control for *trnL*, whereas four replicates were performed for 16S. The PCR replicates were pooled and cleaned, and the pools were converted into 4 DNA libraries using a modified TruSeq PCR-free library kit (Illumina) and unique dual indexing (Rijal et al. 2021). The library was quantified by qPCR using the Library Quantification Kit for Illumina sequencing platforms (KAPA Biosystems, Boston, USA), using a Prism 7500 Real-Time PCR System (Life Technologies, The Norwegian College of Fishery Science, UiT). Each library was sequenced on ~10% of a flow cell on an Illumina NextSeq 500 platform (2x150 bp, mid-output mode) at the Genomics Support Centre Tromsø (UiT).

## Database construction and sedimentary ancient DNA data analysis

The OBITools software package<sup>86</sup> was used for the bioinformatics pipeline, following the protocol and criteria defined by Rijal et al.<sup>8</sup>. Briefly, paired-end reads were aligned using SeqPrep (<https://github.com/jstjohn/SeqPrep/releases>, v1.2). Merged reads were demultiplexed according to the 8

bp unique primer tags and identical sequences were collapsed. Singleton sequences and those shorter than 10 bp were removed and putative artifactual sequences were identified and removed from the dataset.<sup>29</sup>

Previous metabarcoding studies of the Alps that targeted the *trnL* P6 loop have relied on global databases with a sparse representation of the Alpine flora resulting in limited, and potentially inaccurate, identifications<sup>9,30,31</sup>. Here we generated a local DNA reference library. We used the PhyloAlps genome skim database that consist of 4604 specimens of 4437 taxa collected in the Alps (n=4280) and the Carpates (n=324) (<https://data.phyloalps.org/browse/>). A full description of this database including the standard barcodes ITS2, *matK* and *rbcl* are available in Alsos et al.<sup>83</sup>. Here, we compiled a P6loop database by running the ecoPCR software<sup>87</sup> on their corresponding raw databases with the gh primers. The same approach was also used for the PhyloNorway genome skim database<sup>29,83</sup>, and the global EMBL r1143 database.

The assignment of the reads were then done to the four different reference libraries: (1) PhyloAlps; (2) ArctBorBryo (regional arctic/boreal reference library compiled from Willerslev et al.<sup>28</sup>, Sønstebo et al.<sup>42</sup> and Soininen et al.<sup>88</sup>); (3) PhyloNorway<sup>83</sup>; and (4) the global reference library based on the EMBL r1143 database. For mammals, we generated a reference library from the EMBL r1143 database as described above except using the 16S primers. The identified sequences were filtered in R using a custom script (available at <https://github.com/Y-Lammers/MergeAndFilter>). Only sequences with a 100% match to a reference sequence, represented by three reads per replicate, and with a minimum of 10 total reads and three replicates across the entire data set were retained. Furthermore, artificial sequences that were the result of sequencing errors, such as homopolymer length variation, were merged with the source sequence (following Rijal et al.<sup>8</sup>). Additionally, sequences that displayed higher average read counts in negative extraction or PCR controls than lake sediment samples were removed, as well as common laboratory contaminants (Supplementary Table ST6). Final taxonomic assignments for plants were based on the collapsed assignments to the four reference databases, giving priority to the local PhyloAlps identifications. The identified taxa were compared with the species in Flora Alpina (Aeschimann et al 2004) and Flora indicativa (Landolt et al 2010). We removed 8 samples for plants with low metabarcoding data quality, which had technical quality (MTQ) scores <0.45 and/or analytical quality (MAQ) scores <0.175. These MTQ/MAQ score thresholds were derived from the negative controls following Rijal et al.<sup>8</sup> (Supplementary material, Table ST6, Supplementary dataset S5). In the 16S data set, we determined final taxonomic assignments based on scrutiny of NCBI BLAST<sup>89</sup> hits and comparison to known temporal biogeographic distributions (full justifications are given in Supplementary dataset S5). Data for 16S sequences assigned to the same taxon were collapsed. We discarded all non-mammalian sequences and those assigned as human (*Homo sapiens*) or wild boar (*Sus scrofa*), as the former is a common contaminant (also found in the negative controls) and the latter cannot be differentiated from sympatric domestic pig at this locus (Supplementary dataset S5). Although absent from the negative controls, we note that domesticated taxa (pig, cow, sheep, goat) can be sources of sporadic PCR contamination. We therefore interpret sporadic occurrences of these taxa, defined as single,

isolated PCR replicate detections, as likely deriving from contamination. We note that these occur in otherwise low diversity samples that are most at risk of sporadic contamination detections (e.g. sheep at 6.1 ka; cow at 7.2, 8.9, 12.5 ka).

We present a novel relative abundance index (RAI) to integrate the information of the relative abundance of reads and PCR replicability. It is a proportional index multiplying the proportion of obtained reads by the proportion of weighted PCR replicates (weighted PCR replicates are defined in Rijal et al.<sup>8</sup>). This study follows the line of a growing number of environmental DNA modern studies that suggest that the number of DNA copies contain information about the species' relative abundance<sup>90</sup>.

## **DNA sequences to identify plants associated with distinct elevational belts, arable and pastoral activities**

We used all the obtained sequences to get the maximum autecological information, even if not assigned at species level, for reconstructing distributional changes of plants typical for particular vegetation belts, arable and pastoral activities. From all the obtained DNA sequences, only taxa with narrow elevational, climatic and/or habitat requirements are good environmental indicators that can provide insights into past environmental change, while other sequences that do not originate from specific indicator taxa might smooth the results, and by consequence, our palaeoecological interpretation. First, we matched the obtained sequences from Sulsseewli against the PhyloAlps database to obtain a list of the species that share the same sequence (haplotype-sharing species from now on). Then, we checked these haplotype-sharing species with the ecological parameters of Flora Indicativa for temperature, which is the main parameter that characterizes the elevational vegetation belts. The obtained results were graded from optimal to not suitable indicators. e.g.: if one sequence has 4 haplotype-sharing species and all of them have the same ecological value, it is considered an optimal indicator for temperature, while conflicting values indicate a non-suitable indicator. Similarly as for temperature, indicators for pastoral and arable activities were obtained from the sequence data based on the review of palaeoecological literature and Flora indicativa. The regional coherence of all sequences was checked. Optimal indicator sequences for elevational vegetation belts, and arable and pastoral activities as identified based on this procedure were plotted versus other palaeoecological indicators in Figures 2 and 3. These comparisons formed the basis for interpretations regarding changes in vegetation caused by immigrations or expansions of plant species typical for various altitudinal belts or promoted by human activities. If climate was the main driver of past vegetation change, then changes in taxa representative for different vegetation belts are expected to track the chironomid temperature record. If human impact was the main driver, then these changes would be expected to follow the patterns of other palaeoecological indicators of human activities. Constrained incremental sum of squares (Coniss) zonation for all samples was used to infer statistically significant changes of plant DNA sequences along the core. Finally, we performed a Redundancy analysis (RDA) of the relative abundance index of all samples with 21 added explanatory variables to explore the influence of climate (represented by chironomid-inferred temperatures, organic matter (LOI)) and human impacts (represented by plant *seada*DNA of pastoral and arable indicators, coprophilous fungi spores, *seada*DNA from domesticated mammals, and microcharcoal) on the plant

communities. Plots were made with R v3.4.2 using the Vegan, Rioja and Ggplot2 packages, RDA and CONISS were calculated in R using the vegan and rioja packages, respectively<sup>91–93</sup>.

## Declarations

# Data and Code Accessibility

We will deposit raw DNA sequence data on the European Nucleotide Archive (ENA) with accessions xxxxxx-xxxxxx. The unfiltered Obitools output tsv files will be on figshare (doi: xxxxxxxx). All other raw and processed data and links to code will be within the manuscript or supplementary information. Chironomid count data and chironomid-inferred temperatures will be uploaded to the Dryad database (datadryad.org, doi: xxxxxxxx) and pollen data to the Neotoma database (<https://www.neotomadb.org>).

## Acknowledgements

Special thanks to César Morales-Molino and Willi Tanner who helped with fieldwork. To Dilli Prasad Rijal for his input with ecological discussions. Lucas Dane Elliott for doing part of the bioinformatic pipeline. Scarlett Zetter for preparing part of the metabarcoding libraries and Amy Mcdermott for analysing the LOI. Bioinformatic analyses were performed on resources provided by UNINETT Sigma2 - the National Infrastructure for High-Performance Computing and Data Storage in Norway. This study has been financed by the project “ECOGEN - Ecosystem change and species persistence over time: a genome-based approach”, funded by the Research Council of Norway (grant number: 250963/F20 to Alsos). The PhyloAlps reference database was built thanks to the following projects : the joint ANR-SNF project Origin-Alps (ANR-16-CE93-0004, SNF-310030L\_170059), European Research Council under the European Community’s Seventh Framework Programme FP7/2007-2013 grant agreement 281422 (TEEMBIO) and by the SNF grant 31003A\_149508. The sequencing for the PhyloAlps reference database was performed within the framework of the PhyloAlps project, funded by France Génomique (ANR-10-INBS-09-08). Some computations related to the elaboration of PhyloAlps data were carried out with the GRICAD infrastructure (<https://gricad.univ-grenoble-alpes.fr>).

## Author contributions

IGA and SL designed the research, raised the funding, and provided resources; SL, PAC and EC generated and curated the PhyloAlps database; IGA, CS, and FR did the fieldwork; SGP did the ancient DNA laboratory work with input from IGA and PDH; TG performed radiocarbon dating; CS built composite cores and SGP performed age-depth modeling with input from CS and OH; FR performed pollen, charcoal

and non-pollen palynomorphs analysis; MH and AR performed chironomid analysis supervised by OH, who also built the temperature reconstruction; SGP verified and curated the plant barcode sequence taxonomic assignments with input from IGA and JPT, who also verified their botanic origin; PDH designed the blocking primers and verified the mammal barcode sequence taxonomic assignments; OW and SGP designed the bioinformatic pipeline to obtain the indicator sequences, which were verified by JPT; SGP and OW developed the Relative Abundance Index; SGP and YL performed bioinformatics and the quality control checks; SGP did the statistical analyses with input from OW; SGP curated the data; WT, IGA, SL, CS, JPT, AGB, FB and OH contributed with the palaeoecological and botanical interpretation; PDH and KW provided interpretation of the mammalian data; KW provided archaeological interpretation of the region. SGP, IGA and OH wrote the first draft of the manuscript, which JPT, PDH, IGA, OH, WT, CS and the remaining co authors commented on; All authors have reviewed and approved the final manuscript.

## Composition of the PhyloAlps consortium

- Laboratoire d'Ecologie Alpine (LECA), Université Grenoble Alpes, CNRS, FR-38000 Grenoble, France. Sébastien Lavergne, Charles Pouchon, Eric Coissac, Cristina Roquet, Jan Smycka, Martí Boleda, Wilfried Thuiller, Ludovic Gielly, Pierre Taberlet, Delphine Rioux, Frédéric Boyer, Anthony Hombiat, Bruno Bzeznick.
- Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Univ Evry, Université Paris-Saclay, FR-91057 Evry, France. Adriana Alberti, France Denoeud, Patrick Wincker.
- Jardin Alpin du Lautaret, Université Grenoble Alpes, CNRS, FR-38000 Grenoble, France. Christophe Perrier, Rolland Douzet, Maxime Rome, Jean-Gabriel Valay, Serge Aubert.
- Swiss Federal Research Institute WSL, CH-8903 Birmensdorf, Switzerland. Niklaus Zimmermann, Rafael O. Wüest, Sonia Latzin.
- Conservatoire Botanique National Alpin, Domaine de Charance, FR-05000 Gap, France. Jérémie Van Es, Luc Garraud, Jean-Charles Villaret, Sylvain Abdulhak, Véronique Bonnet, Stéphanie Huc, Noémie Fort, Thomas Legland, Thomas Sanz, Gilles Pache, Alexis Mikolajczak.

- Conservatoire Botanique National Méditerranéen, FR-83400 Hyères, France. Virgile Noble, Henri Michaud, Benoît Offerhaus.
- Parc National des Ecrins, FR-05000 Gap, France. Cédric Dentant, Pierre Salomez, Richard Bonet.
- Parc National de la Vanoise, FR-73000 Chambéry, France. Thierry Delahaye.
- Parc National du Mercantour, FR-06006 Nice Cedex 1, France. Marie-France Leccia, Monique Perfus.
- Info-Flora – Centre national de données et d'informations sur la flore de Suisse, Genève, CH-3001 Bern, Switzerland. Stefan Eggenberg, Adrian Möhl.
- Faculty of Biology and Geology, Babeş Bolyai University, RO-400015 Cluj Napoca, Romania. Bogdan Hurdu, Mihai Puscas.
- Conservatoire d'espaces naturels de Provence-Alpes-Côte d'Azur. Héloïse Vanderpert.

## Abbreviations

SGP - Sandra Garcés-Pastor\*

SL - Sébastien Lavergne

EC - Eric Coissac

CS - Christoph Schwörer

JPT - Jean-Paul Theurillat

YL - Youri Lammers

OW - Owen S. Wangensteen

WT - Willy Tinner

FR - Fabian Rey

MH - Martina Heer

AR - Astrid Rutzer

KW - Kevin Walsh

PDH - Peter D. Heintzman

AGB - Antony G. Brown

TG - Tomasz Goslar

PAC - PhyloAlps Consortium

OH - Oliver Heiri#

IGA - Inger Greve Alsos#

## References

1. Trisos, C. H., Merow, C. & Pigot, A. L. The projected timing of abrupt ecological disruption from climate change. *Nature* **580**, 496–501 (2020).
2. Theurillat, J.-P. & Guisan, A. Potential Impact of Climate Change on Vegetation in the European Alps: A Review. *Springer Science and Business Media LLC* (2001) doi:10.1023/a:1010632015572.
3. Masson-Delmotte, V. *et al.* IPCC. in *Summary for Policymakers. In: Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* (ed. Cambridge University Press) (2021).
4. Steinbauer, M. J. *et al.* Accelerated increase in plant species richness on mountain summits is linked to warming. *Nature* **556**, 231–234 (2018).
5. Pauli, G. Climate effects on mountain plants. *Nature* (1994).
6. Bennett, K. D. & Willis, K. J. Pollen. in *Tracking environmental change using lake sediments* (eds. Smol, J. P., Birks, H. J. B., Last, W. M., Bradley, R. S. & Alverson, K.) 5–32 (Kluwer Academic Publishers, 2002). doi:10.1007/0-306-47668-1\_2.
7. Liu, S. *et al.* Sedimentary ancient DNA reveals a threat of warming-induced alpine habitat loss to Tibetan Plateau plant diversity. *Nat. Commun.* **12**, 2995 (2021).
8. Rijal, D. P. *et al.* Sedimentary ancient DNA shows terrestrial plant richness continuously increased over the Holocene in northern Fennoscandia. *Sci. Adv.* **7**, (2021).

9. Giguet-Covex, C. *et al.* Long livestock farming history and human landscape shaping revealed by lake sediment DNA. *Nat. Commun.* **5**, 3211 (2014).
10. Väre, H., Lampinen, R., Humphries, C. & Williams, P. Taxonomic diversity of vascular plants in the European alpine areas. in *Alpine biodiversity in Europe* (eds. Nagy, L., Grabherr, G., Körner, C. & Thompson, D. B. A.) vol. 167 133–148 (Springer Berlin Heidelberg, 2003).
11. Aeschimann, D., Rasolofo, N. & Theurillat, J.-P. Analyse de la flore des Alpes. 1: historique et biodiversité. *Candollea* **66**, 27–55 (2011).
12. Hewitt, G. The genetic legacy of the Quaternary ice ages. *Nature* **405**, 907–913 (2000).
13. Tribsch, A. & Schönswetter, P. Patterns of endemism and comparative phylogeography confirm palaeo-environmental evidence for Pleistocene refugia in the Eastern Alps. *Taxon* **52**, 477–497 (2003).
14. Rudmann-Maurer, K., Weyand, A., Fischer, M. & Stöcklin, J. The role of land use and natural determinants for grassland vegetation composition in the Swiss Alps. *Basic and Applied Ecology* **9**, 494–503 (2008).
15. Walsh, K. *et al.* A historical ecology of the Ecrins (Southern French Alps): Archaeology and palaeoecology of the Mesolithic to the Medieval period. *Quaternary International* **353**, 52–73 (2014).
16. Walsh, K. & Giguet-Covex, C. A history of human exploitation of alpine regions. in *Encyclopedia of the world's biomes* 555–573 (Elsevier, 2020). doi:10.1016/B978-0-12-409548-9.11908-6.
17. Schwörer, C. *et al.* Holocene climate, fire and vegetation dynamics at the treeline in the Northwestern Swiss Alps. *Veg. Hist. Archaeobot.* **23**, 479–496 (2014).
18. Schwörer, C., Henne, P. D. & Tinner, W. A model-data comparison of Holocene timberline changes in the Swiss Alps reveals past and future drivers of mountain forest dynamics. *Glob. Chang. Biol.* **20**, 1512–1526 (2014).
19. Henne, P. D. *et al.* An empirical perspective for understanding climate change impacts in Switzerland. *Reg. Environ. Change* **18**, 1–17 (2017).
20. Niedrist, G., Tasser, E., Lüth, C., Dalla Via, J. & Tappeiner, U. Plant diversity declines with recent land use changes in European Alps. *Plant Ecol.* **202**, 195–210 (2009).
21. Lasanta-Martínez, T., Vicente-Serrano, S. M. & Cuadrat-Prats, J. M. Mountain Mediterranean landscape evolution caused by the abandonment of traditional primary activities: a study of the Spanish Central Pyrenees. *Applied Geography* **25**, 47–65 (2005).
22. Nautiyal, S. & Kaechele, H. Adverse impacts of pasture abandonment in Himalayan protected areas: Testing the efficiency of a Natural Resource Management Plan (NRMP). *Environ. Impact Assess. Rev.* **27**,

109–125 (2007).

23. Landolt, E. *et al.* *Flora indicativa: Ökologische Zeigerwerte und biologische Kennzeichen zur Flora der Schweiz und der Alpen*. (Haupt, 2010).

24. Heiri, O., Brooks, S. J., Birks, H. J. B. & Lotter, A. F. A 274-lake calibration data-set and inference model for chironomid-based summer air temperature reconstruction in Europe. *Quat. Sci. Rev.* **30**, 3445–3456 (2011).

25. Heiri, O., Ilyashuk, B., Millet, L., Samartin, S. & Lotter, A. F. Stacking of discontinuous regional palaeoclimate records: Chironomid-based summer temperatures from the Alpine region. *The Holocene* **25**, 137–149 (2015).

26. Ivy-Ochs, S. *et al.* Latest Pleistocene and Holocene glacier variations in the European Alps. *Quat. Sci. Rev.* **28**, 2137–2149 (2009).

27. Schibler, J., Elsner, J. & Schlumbaum, A. Incorporation of aurochs into a cattle herd in Neolithic Europe: single event or breeding? *Sci. Rep.* **4**, 5798 (2014).

28. Willerslev, E. *et al.* Fifty thousand years of Arctic vegetation and megafaunal diet. *Nature* **506**, 47–51 (2014).

29. Alsos, I. G. *et al.* Ancient sedimentary DNA shows rapid post-glacial colonisation of Iceland followed by relatively stable vegetation until the Norse settlement (Landnám) AD 870. *Quat. Sci. Rev.* **259**, 106903 (2021).

30. Pansu, J. *et al.* Reconstructing long-term human impacts on plant communities: an ecological approach based on lake sediment DNA. *Mol. Ecol.* **24**, 1485–1498 (2015).

31. Varotto, C. *et al.* A pilot study of eDNA metabarcoding to estimate plant biodiversity by an alpine glacier core (Adamello glacier, North Italy). *Sci. Rep.* **11**, 1208 (2021).

32. Parducci, L. *et al.* Proxy comparison in ancient peat sediments: pollen, macrofossil and plant DNA. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **370**, 20130382 (2015).

33. Clarke, C. L. *et al.* A 24,000-year ancient DNA and pollen record from the Polar Urals reveals temporal dynamics of arctic and boreal plant communities. *Quat. Sci. Rev.* **247**, 106564 (2020).

34. Niemeyer, B., Epp, L. S., Stoof-Leichsenring, K. R., Pestryakova, L. A. & Herzschuh, U. A comparison of sedimentary DNA and pollen from lake sediments in recording vegetation composition at the Siberian treeline. *Mol. Ecol. Resour.* **17**, e46–e62 (2017).

35. Wilson, J. B., Peet, R. K., Dengler, J. & Pärtel, M. Plant species richness: the world records. *J. Veg. Sci.* **23**, 796–802 (2012).

36. Thöle, L. *et al.* Reconstruction of Holocene vegetation dynamics at Lac de Bretaye, a high-mountain lake in the Swiss Alps. *The Holocene* **26**, 380–396 (2016).
37. Lotter, A. F. *et al.* Holocene timber-line dynamics at Bachalpsee, a lake at 2265 m a.s.l. in the northern Swiss Alps. *Veg. Hist. Archaeobot.* **15**, 295–307 (2006).
38. Heiri, O., Lotter, A. F., Hausmann, S. & Kienast, F. A chironomid-based Holocene summer air temperature reconstruction from the Swiss Alps. *The Holocene* **13**, 477–484 (2003).
39. Finsinger, W. & Tinner, W. Pollen and plant macrofossils at Lac de Fully (2135 m a.s.l.): Holocene forest dynamics on a highland plateau in the Valais, Switzerland. *The Holocene* **17**, 1119–1127 (2007).
40. Garcés-Pastor, S., Cañellas-Boltà, N., Clavaguera, A., Calero, M. A. & Vegas-Vilarrúbia, T. Vegetation shifts, human impact and peat bog development in Bassa Nera pond (Central Pyrenees) during the last millennium. *The Holocene* **27**, 553–565 (2017).
41. Aeschimann, D., Lauber, K., Moser, D. M. & Theurillat, J. P. Flora alpina: atlas des 4500 plantes vasculaires des Alpes. (2004).
42. Sørensen, J. H. *et al.* Using next-generation sequencing for molecular reconstruction of past Arctic vegetation and climate. *Mol. Ecol. Resour.* **10**, 1009–1018 (2010).
43. Giesecke, T. *et al.* Postglacial change of the floristic diversity gradient in Europe. *Nat. Commun.* **10**, 5422 (2019).
44. Colombaroli, D. & Tinner, W. Determining the long-term changes in biodiversity and provisioning services along a transect from Central Europe to the Mediterranean. *The Holocene* **23**, 1625–1634 (2013).
45. 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).
46. Furtwängler, A. *et al.* Ancient genomes reveal social and genetic structure of Late Neolithic Switzerland. *Nat. Commun.* **11**, 1915 (2020).
47. Gilck, F. & Poschod, P. The origin of alpine farming: A review of archaeological, linguistic and archaeobotanical studies in the Alps. *The Holocene* **29**, 1503–1511 (2019).
48. Tinner, W., Nielsen, E. H. & Lotter, A. F. Mesolithic agriculture in Switzerland? A critical review of the evidence. *Quat. Sci. Rev.* **26**, 1416–1431 (2007).
49. Berthel, N., Schwörer, C. & Tinner, W. Impact of Holocene climate changes on alpine and treeline vegetation at Sanetsch Pass, Bernese Alps, Switzerland. *Rev. Palaeobot. Palynol.* **174**, 91–100 (2012).
50. Hafner, A. & Schwörer, C. Vertical mobility around the high-alpine Schnidejoch Pass. Indications of Neolithic and Bronze Age pastoralism in the Swiss Alps from paleoecological and archaeological

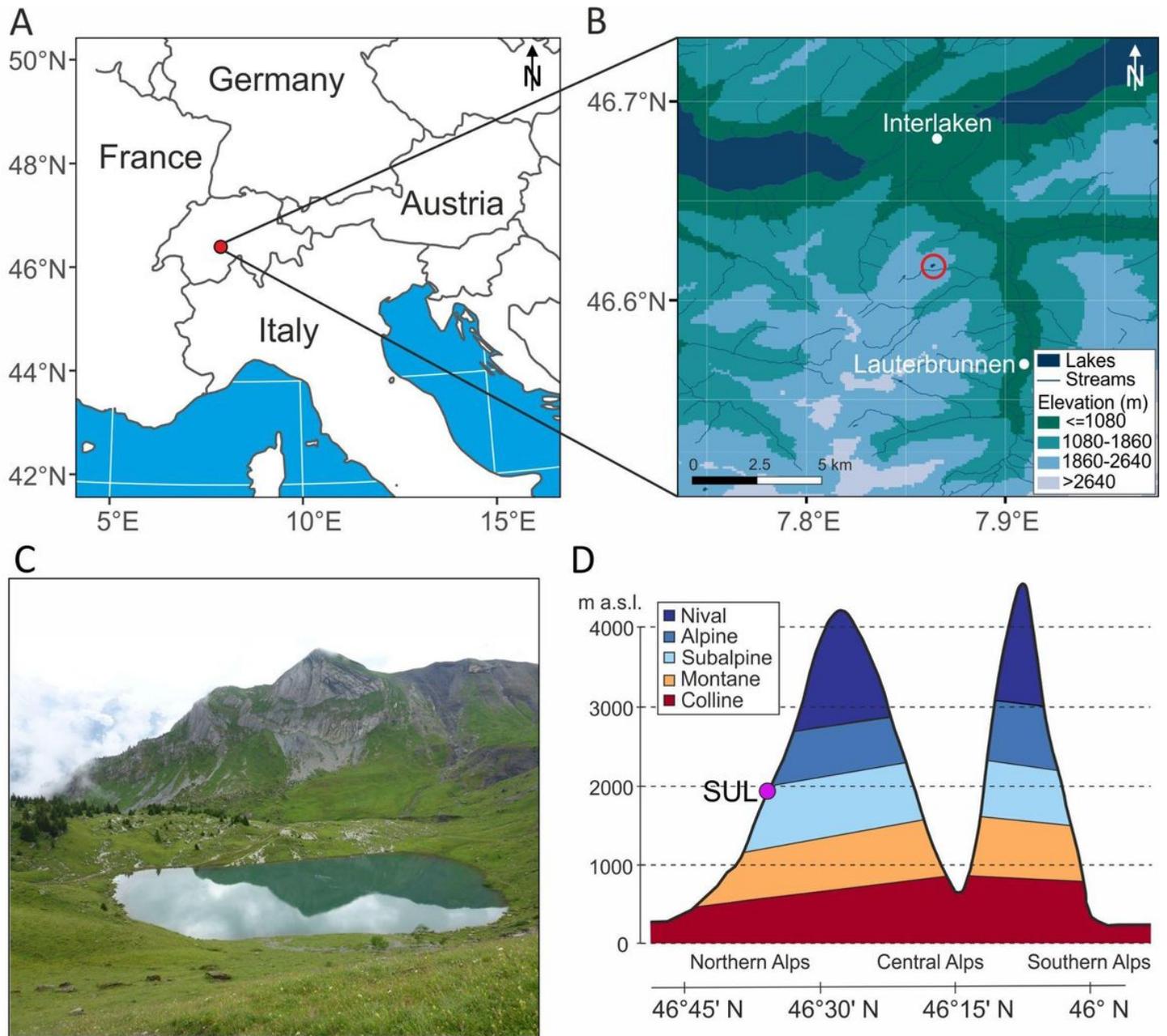
sources. *Quaternary International* (2017) doi:10.1016/j.quaint.2016.12.049.

51. Oveisi, M. *et al.* Potential for endozoochorous seed dispersal by sheep and goats: Risk of weed seed transport via animal faeces. *Weed Res.* **61**, 1–12 (2021).
52. Bardgett, R. D. & Wardle, D. A. Herbivore-mediated linkages between aboveground and belowground communities. *Ecology* **84**, 2258–2268 (2003).
53. Scherrer, D. & Körner, C. Topographically controlled thermal-habitat differentiation buffers alpine plant diversity against climate warming. *J. Biogeogr.* **38**, 406–416 (2011).
54. Giguet-Covex, C. *et al.* New insights on lake sediment DNA from the catchment: importance of taphonomic and analytical issues on the record quality. *Sci. Rep.* **9**, 14676 (2019).
55. Andres, B. Alpine settlement remains in the Bernese Alps (Switzerland) in medieval and modern times. in *Historical Archaeologies of Transhumance across Europe* (eds. Costello, E. & Svensson, E.) 155–169 (Routledge, 2018). doi:10.4324/9781351213394-11.
56. Andres, B. Alpine Summer Farms–Upland Animal Husbandry and Land Use Strategies in the Bernese Alps (Switzerland). (2012).
57. Grime, J. P. Competitive exclusion in herbaceous vegetation. *Nature* **242**, 344–347 (1973).
58. Yuan, Z. Y., Jiao, F., Li, Y. H. & Kallenbach, R. L. Anthropogenic disturbances are key to maintaining the biodiversity of grasslands. *Sci. Rep.* **6**, 22132 (2016).
59. Spiegelberger, T., Matthies, D., Müller-Schärer, H. & Schaffner, U. Scale-dependent effects of land use on plant species richness of mountain grassland in the European Alps. *Ecography* **29**, 541–548 (2006).
60. Maurer, K., Weyand, A., Fischer, M. & Stöcklin, J. Old cultural traditions, in addition to land use and topography, are shaping plant diversity of grasslands in the Alps. *Biological Conservation* **130**, 438–446 (2006).
61. Pellegrini, E., Buccheri, M., Martini, F. & Boscutti, F. Agricultural land use curbs exotic invasion but sustains native plant diversity at intermediate levels. *Sci. Rep.* **11**, 8385 (2021).
62. Speed, J. D. M., Austrheim, G., Hester, A. J. & Myserud, A. Elevational advance of alpine plant communities is buffered by herbivory. *J. Veg. Sci.* **23**, 617–625 (2012).
63. Orlandi, S. *et al.* Environmental and land use determinants of grassland patch diversity in the western and eastern Alps under agro-pastoral abandonment. *Biodivers. Conserv.* **25**, 275–293 (2016).
64. Barras, A. G., Braunisch, V. & Arlettaz, R. Predictive models of distribution and abundance of a threatened mountain species show that impacts of climate change overrule those of land use change. *Divers. Distrib.* (2021) doi:10.1111/ddi.13247.

65. Rehnus, M., Bollmann, K., Schmatz, D. R., Hackländer, K. & Braunisch, V. Alpine glacial relict species losing out to climate change: The case of the fragmented mountain hare population (*Lepus timidus*) in the Alps. *Glob. Chang. Biol.* **24**, 3236–3253 (2018).
66. Conedera, M., Colombaroli, D., Tinner, W., Krebs, P. & Whitlock, C. Insights about past forest dynamics as a tool for present and future forest management in Switzerland. *Forest Ecology and Management* **388**, 100–112 (2017).
67. Merkt, J. & Streif, H. Stechrohr-Bohrgeräte für limnische und marine Lockersedimente. in *Geologisches Jahrbuch* 137–148 (1970).
68. Lamb, A. L. Determination of organic and carbonate content in soils and sediments by loss on ignition (LOI). *197*(2004).
69. Reimer, P. J. *et al.* The IntCal20 Northern Hemisphere radiocarbon age calibration curve (0–55 cal kBP). *Radiocarbon* 1–33 (2020) doi:10.1017/RDC.2020.41.
70. Blaauw, M. & Christen, J. A. Flexible paleoclimate age-depth models using an autoregressive gamma process. *Bayesian Anal.* **6**, 457–474 (2011).
71. Brooks, S. J., Langdon, P. G. & Heiri, O. The identification and use of Palaeartic Chironomidae larvae in palaeoecology. *Quaternary Research Association ...* (2007).
72. Schulze, E. A Key to the Larval Chironomidae and their Instars from Austrian Danube Region Streams and Rivers with Particular Reference to a Numerical Taxonomic Approach. Part I. In: Wasser und Abwasser, Supplementband 3/93. Hrsg.: Bundesamt für Wassergüte, Wien-Kaisermühlen. Schriftenleitung: Werner Kohl. Selbstverlag, 1993, 514 S., öS 562,—. *Acta hydrochim. hydrobiol.* **22**, 191–191 (1994).
73. Juggins, S. C2: Software for ecological and palaeoecological data analysis and visualisation (user guide version 1.5). *Newcastle upon Tyne: Newcastle University* (2007).
74. Moore, P. D., Webb, J. A. & Collison, M. E. Pollen analysis. *Pollen analysis.* (1991).
75. Stockmarr & Ja. Tabletes with spores used in absolute pollen analysis. *Pollen Spores* (1971).
76. Reille, M. Pollen et spores d'Europe et d'Afrique du Nord. (1992).
77. van Geel, B. *et al.* Environmental reconstruction of a Roman Period settlement site in Uitgeest (The Netherlands), with special reference to coprophilous fungi. *J. Archaeol. Sci.* **30**, 873–883 (2003).
78. Bennett, K. D. Determination of the number of zones in a biostratigraphical sequence. *New Phytol.* **132**, 155–170 (1996).

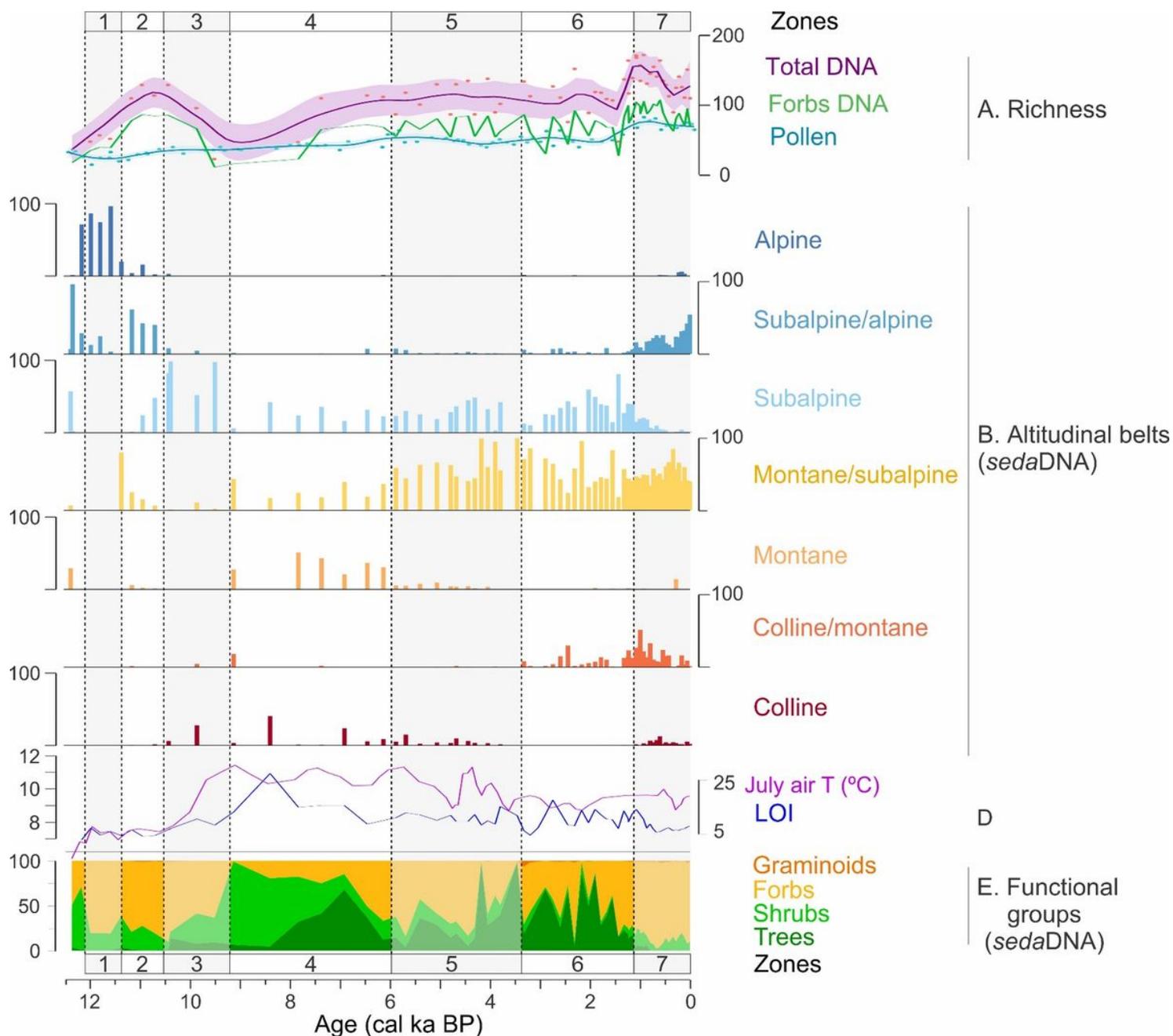
79. Tinner, W. *et al.* Pollen and charcoal in lake sediments compared with historically documented forest fires in southern Switzerland since AD 1920. *The Holocene* **8**, 31–42 (1998).
80. Adolf, C. *et al.* The sedimentary and remote-sensing reflection of biomass burning in Europe. *Global Ecol. Biogeogr.* **27**, 199–212 (2018).
81. Tinner, W. & Hu, F. S. Size parameters, size-class distribution and area-number relationship of microscopic charcoal: relevance for fire reconstruction. *The Holocene* **13**, 499–505 (2003).
82. Parducci, L. *et al.* Ancient plant DNA in lake sediments. *New Phytol.* **214**, 924–942 (2017).
83. 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**, (2020).
84. Taberlet, P. *et al.* Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. *Nucleic Acids Res.* **35**, e14 (2007).
85. 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).
86. Boyer, F. *et al.* obitools: a unix-inspired software package for DNA metabarcoding. *Mol. Ecol. Resour.* **16**, 176–182 (2016).
87. Ficetola, G. F. *et al.* An in silico approach for the evaluation of DNA barcodes. *BMC Genomics* **11**, 434 (2010).
88. Soininen, E. M. *et al.* Highly overlapping winter diet in two sympatric lemming species revealed by DNA metabarcoding. *PLoS ONE* **10**, e0115335 (2015).
89. Boratyn, G. M. *et al.* BLAST: a more efficient report with usability improvements. *Nucleic Acids Res.* **41**, W29-33 (2013).
90. Deiner, K. *et al.* Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Mol. Ecol.* **26**, 5872–5895 (2017).
91. Juggins, S. Rioja: Analysis of Quaternary Science Data. R package version 0.9-26. <https://cran.r-project.org/web/packages/rioja/index.html> (2020).
92. Legendre, P., Minchin, P. R. & O'hara, R. B. Package “vegan.” *Community ecology ...* (2013).
93. Wickham, H. ggplot2-Elegant Graphics for Data Analysis. Springer International Publishing. *Springer International Publishing* (2016).

## Figures



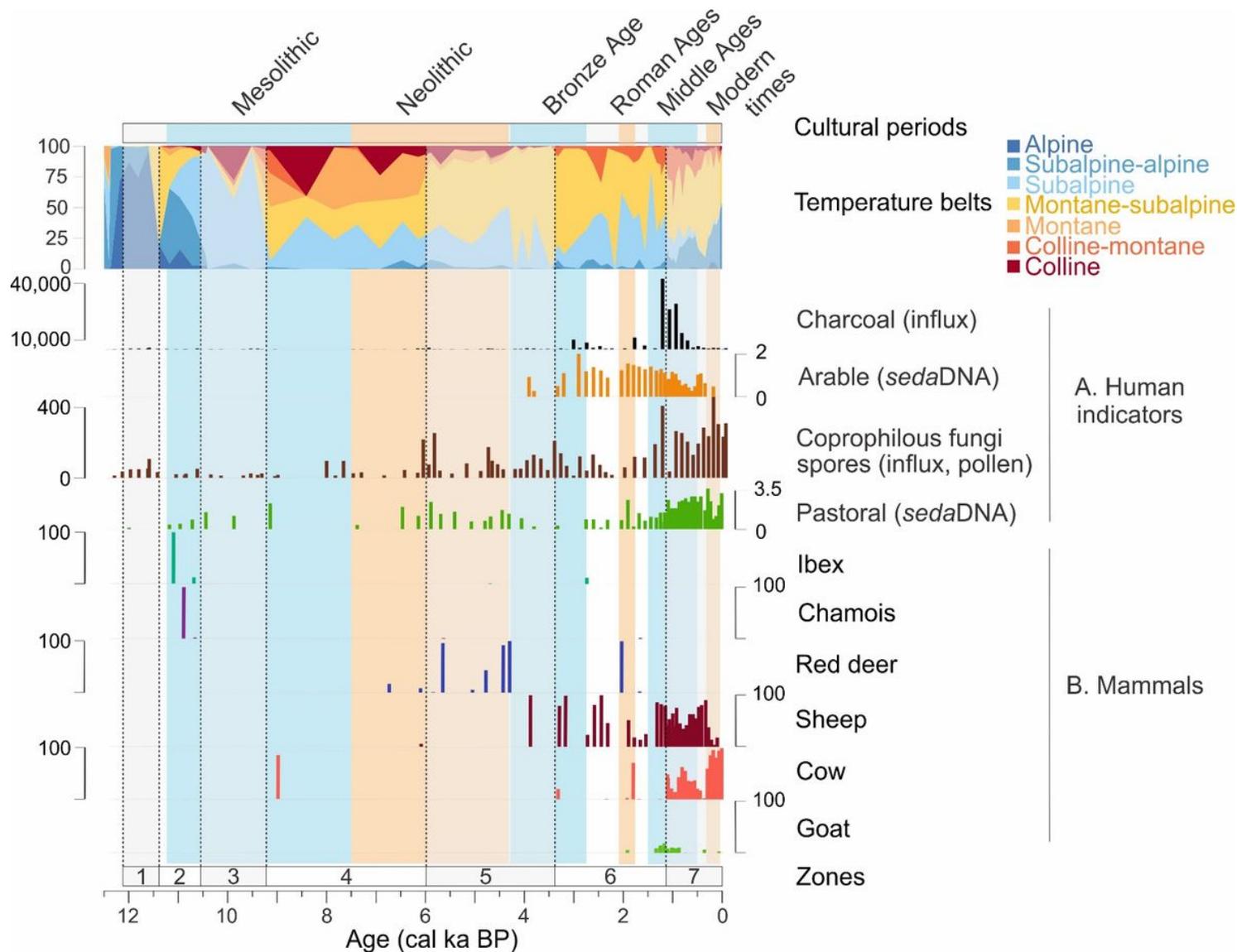
**Figure 1**

Location of lake Sulsseewli, Switzerland. (A) European map. (B) Location of Sulsseewli and the catchment, map from Federal Office of Topography swisstopo. (C) Photo of the lake taken from the side (Inger Greve Alsos). (D) Elevational vegetation belts in Switzerland (Figure of Tinner and Ammann 2005 modified and redrawn according to Theurillat and Guisan 2001).



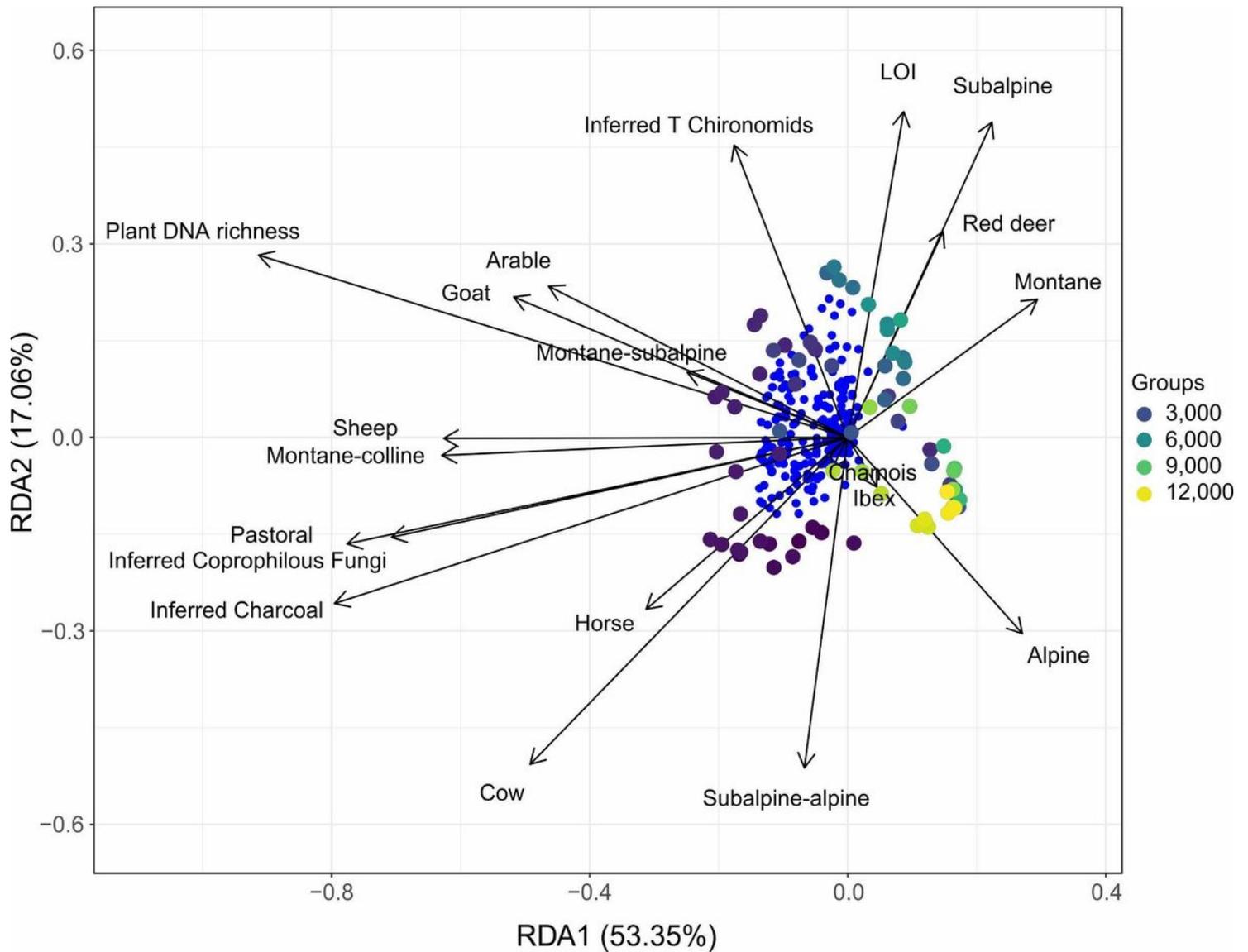
**Figure 2**

Development of the vegetation surrounding Lake Sulsseewli during the Holocene. Total plant and forb taxonomic richness based on sedaDNA, as well as total plant taxonomic richness based on pollen (A); proportion of sedaDNA plant taxa characteristic of altitudinal vegetation belts ordered from high to low elevation (B); chironomid reconstructed temperature (as 3 sample running average) and organic content of the sediments (as loss on Ignition (LOI), D); relative proportions of plant sedaDNA representing different functional plant groups (E). Vegetation zones 1-7 based on constrained cluster analysis (CONISS) of sedaDNA are indicated by vertical lines.



**Figure 3**

Expanding human impact through the Holocene at Lake Sulsseewli. Human indicators are (A): arable plant *sedaDNA*, microcharcoal, pastoral plant *sedaDNA*, coprophilous fungi spores and (B) mammal *sedaDNA*: Ibex (*Capra ibex*), Chamois (*Rupicapra rupicapra*), Red deer (*Cervus elaphus*), Sheep (*Ovis aries*), Cow (*Bos taurus*), Goat (*Capra hircus*). Vegetation zones 1-7 based on constrained cluster analysis (CONISS) of *sedaDNA* are indicated by vertical lines. Vertical colored bars correspond to the Mesolithic, Neolithic, Bronze Age, Medieval Age and Modern times.



**Figure 4**

Redundancy analysis (RDA) of the plant sedaDNA across the record with 21 added explanatory variables representing human activities (plant sedaDNA pastoral and arable indicators, coprophilous fungi spores, mammal DNA and microcharcoal) and climatic changes (chironomid-inferred temperatures and organic matter (LOI)). Small blue dots represent the plant species. Note: Colline belt is not seen.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [GarcesPastoretalSupplementarydataset.xlsx](#)
- [GarcesPastoretalSupplementarymaterial.pdf](#)