Article:

Zetter, Scarlett, Garcés-Pastor, Sandra, Lammers, Youri et al. (9 more authors) (2024) SedaDNA shows that transhumance of domestic herbivores has enhanced plant diversity over the Holocene in the Eastern European Alps. The Holocene. ISSN 0959-6836

https://doi.org/10.1177/09596836241307304

The Holocene

SedaDNA shows that transhumance of domestic herbivores has enhanced plant diversity over the Holocene in the Eastern European Alps

Journal:	*The Holocene*
Manuscript ID	HOL-24-0103.R1
Manuscript Type:	Paper
Date Submitted by the Author:	14-Oct-2024
Complete List of Authors:	Zetter, Scarlett; UiT The Arctic University of Norway, The Arctic University Museum of Norway Garcés-Pastor, Sandra; UiT The Arctic University of Norway, The Arctic University Museum of Norway; Universitat de Barcelona Facultat de Biologia, Department of Ecology Lammers, Youri; UiT The Arctic University of Norway, The Arctic University Museum of Norway Brown, Antony; UiT The Arctic University of Norway, The Arctic University Museum of Norway Walsh, Kevin; University of York, Department of Archaeology Goslar, Tomasz; Poznań Park of Science and Technology, Poznań Radiocarbon Laboratory; Adam Mickiewicz University, Faculty of Physics Lavergne, Sebastien; Université Grenoble-Alpes, Université Savoie Mont Blanc, CNRS, LECA Coissac, Eric; Université Grenoble-Alpes, Université Savoie Mont Blanc, CNRS, LECA Consortium, PhyloAlps; Université Grenoble-Alpes, Université Savoie Mont Blanc, CNRS, LECA Tribsch, Andreas; University of Salzburg, Department of Environment and Biodiversity Heintzman, Peter; UiT The Arctic University of Norway, The Arctic University Museum of Norway; Stockholm University, Department of Geological Sciences; Centre for Palaeogenetics, Stockholm Alsos, Inger; UiT The Arctic University of Norway, The Arctic University Museum of Norway
Keywords:	European Alps, <i>sed</i>aDNA, Anthropogenic impacts, Climate change, Austria, Holocene, Metabarcoding, Alpine pasture management, Central Eastern Alps
Abstract:	The Eastern European Alps boasts highly biodiverse ecosystems and a rich archaeological history. However, there is limited research on the enduring impacts of historical climate change and human activities on plant biodiversity in this region. Using sedimentary ancient DNA, we reconstructed plant and animal dynamics from 8.5 thousand years before present (ka BP) around Großer Winterleitensee (Zirbitzkogel, Austria). Variable intensities of human activities since the Middle Bronze Age (~3.5 ka BP) facilitated the persistence of biodiverse Alpine meadow

communities and lowered timberlines below their natural limit. Since the end of the Bronze Age (~2.8 ka BP), human activities, particularly pasturing, and increasing temperatures, emerged as significant drivers of plant community dynamics. The introduction of sheep (Ovis aries) did not reduce wild mammal presence, including red deer (Cervus elaphus), hare (Lepus), European mole (Talpa europaea), bank vole (Myodes glareolus), and short-tailed field vole (Microtus agrestis). During the High & Late Mediaeval Period, (~1150 - 450 BP), cattle (Bos taurus) and horses (Equus caballus) became the dominant domesticates, while all wild mammals except the short-tailed field vole and Eurasian water shrew (Neomys fodiens) declined or even disappeared. These changes are also accompanied by a significant transformation of plant community structure. The ability to determine both plant responses and animal drivers from the same palaeolimnological sequence vastly improves our ability to partition causes of vegetation change over the Holocene. Here, we reveal that plant biodiversity is maintained or increased by moderate cattle grazing. Therefore, non-intensive domesticated stock grazing is essential for maintaining diverse Alpine meadows.

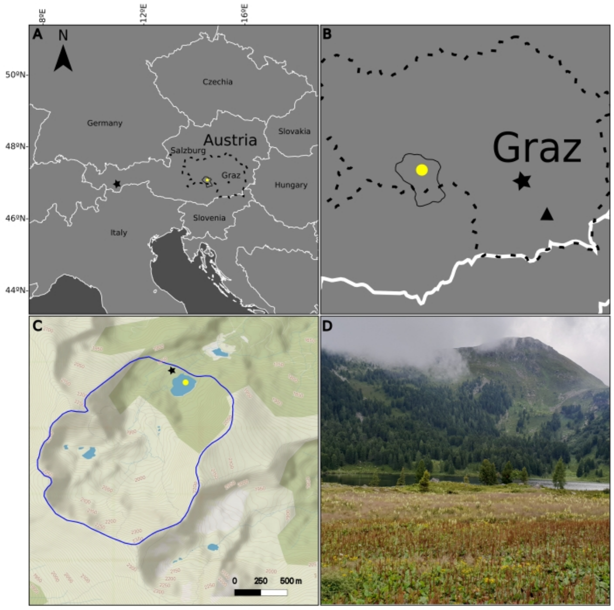


Figure 1

Figure 1. Maps and coring site: A. A map of Europe centred around Austria, indicating Styria (dashed line), the Seetaler Alps (solid line), Großer Winterleitensee (yellow circle), and Schwarzsee ob Sölden (black star). Map drawn with vemaps.com basemap. **B.** A zoomed in image of Styria, (dashed border) and the Seetaler Alps (solid border). The triangle indicates Wildon Schlossberg and the yellow circle, Großer Winterleitensee. Map drawn using vemaps.com basemap. **C.** A map of Großer Winterleitensee with the catchment area (blue border), sediment core coring site (yellow circle) and moor core coring site (black star) indicated. Map drawn in QGIS using MapTiler Topo basemap. **D.** Photo taken from downslope of the northwestern side of the lake (I. G. Alsos).

1321x915mm (118 x 118 DPI)

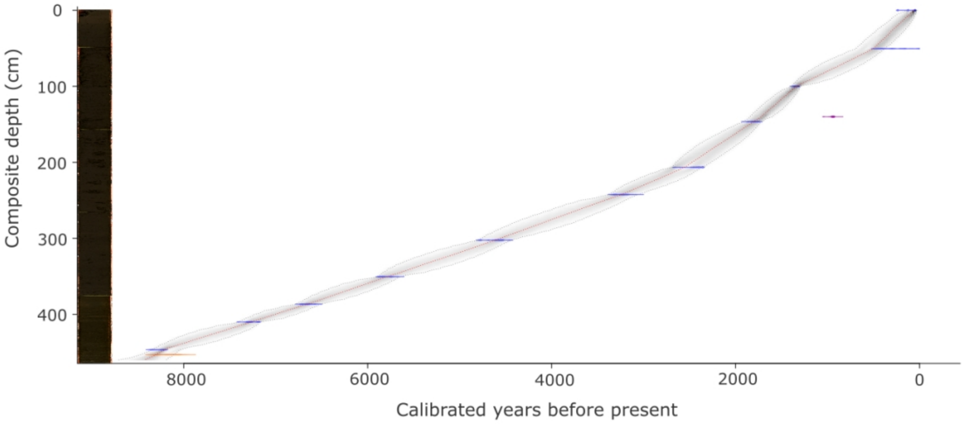


Figure 2. Age depth model, core image and lithology: The Age depth model with the calibrated radiocarbon dates (one standard error) in blue. The age-depth relationship is illustrated by a curve: the most probable calendar ages (darker grey), the 95% confidence interval (grey stippled line), and the optimal model derived from a weighted average of the mean (red line). Two dates were discarded, one with an error margin >100 years (orange) and the other lies outside of the model (purple). The core comprises dark-brown silty-gyttja and some lighter coloured bands throughout.

357x176mm (96 x 96 DPI)

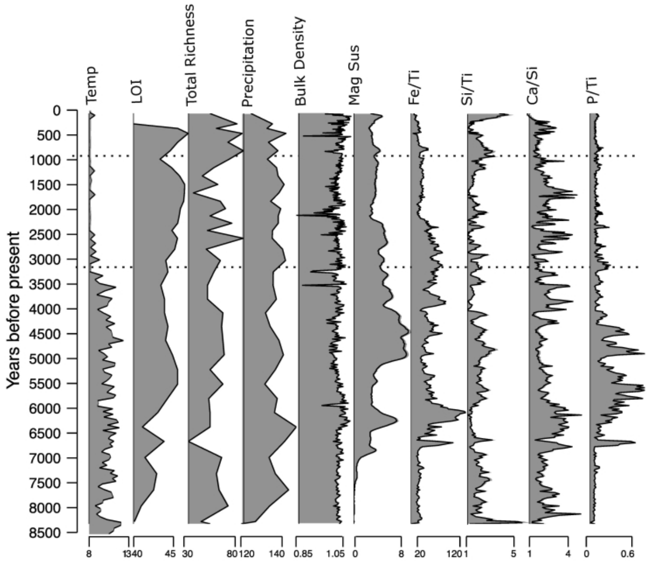


Figure 3 The XRF data with temperature and precipitation data: XRF data of the core taken from Großer Winterleitensee, Austria, alongside mean July temperature (Temp, ºC), % loss on ignition (LOI), total plant richness (Hill where q = 0), mean precipitation (kg m^{-2}) of water in all phases, bulk density, and magnetic susceptibility (mag sus). Full XRF data shown in Supplementary Figure 1.

246x144mm (600 x 600 DPI)

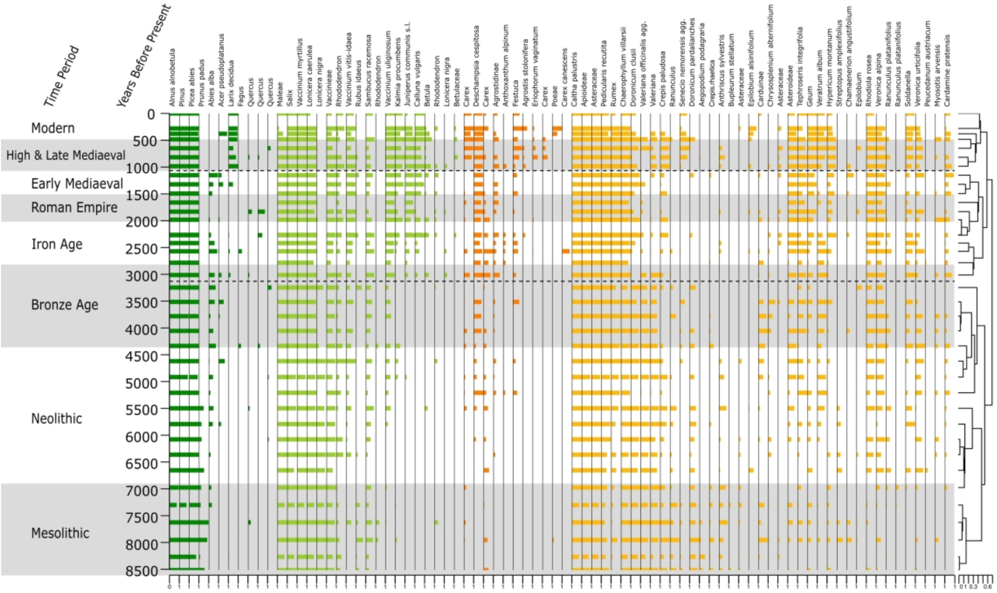
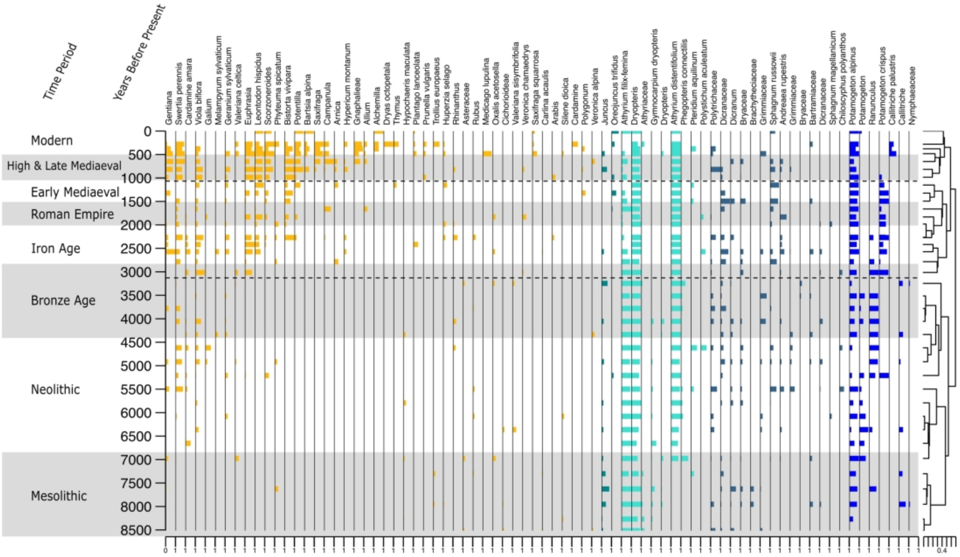


Figure 4 Plant *seda*DNA diagram, split into two: A representation of the plant taxa present through time in years before present. Each taxon is represented by its proportion of weighted PCR replicates from the *seda*DNA, where 0 represents that the taxon is present in no replicates and 1 represents that the DNA it is detected in all 8 replicates. Data is plotted with CONISS statistical zonation using the plant *seda*DNA data. Archeological time periods are alternately shaded grey and white.

302x189mm (300 x 300 DPI)



252x146mm (300 x 300 DPI)

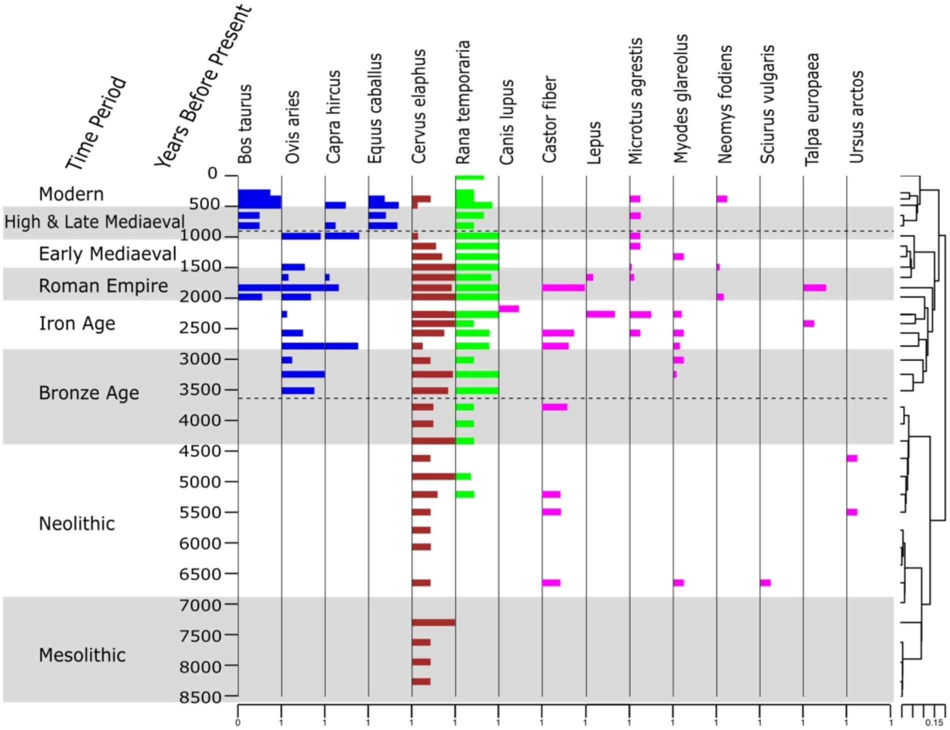


Figure 5. Vertebrate *sed*aDNA diagram The proportion of weighted PCR replicates of the vertebrate *sed*aDNA data where 0 represents *sed*aDNA present in no replicates and 1 represents all 8 replicates. Data is with CONISS statistical zonation using the vertebrate *sed*aDNA data. Archeological time periods are alternately shaded grey and white.

194x145mm (300 x 300 DPI)

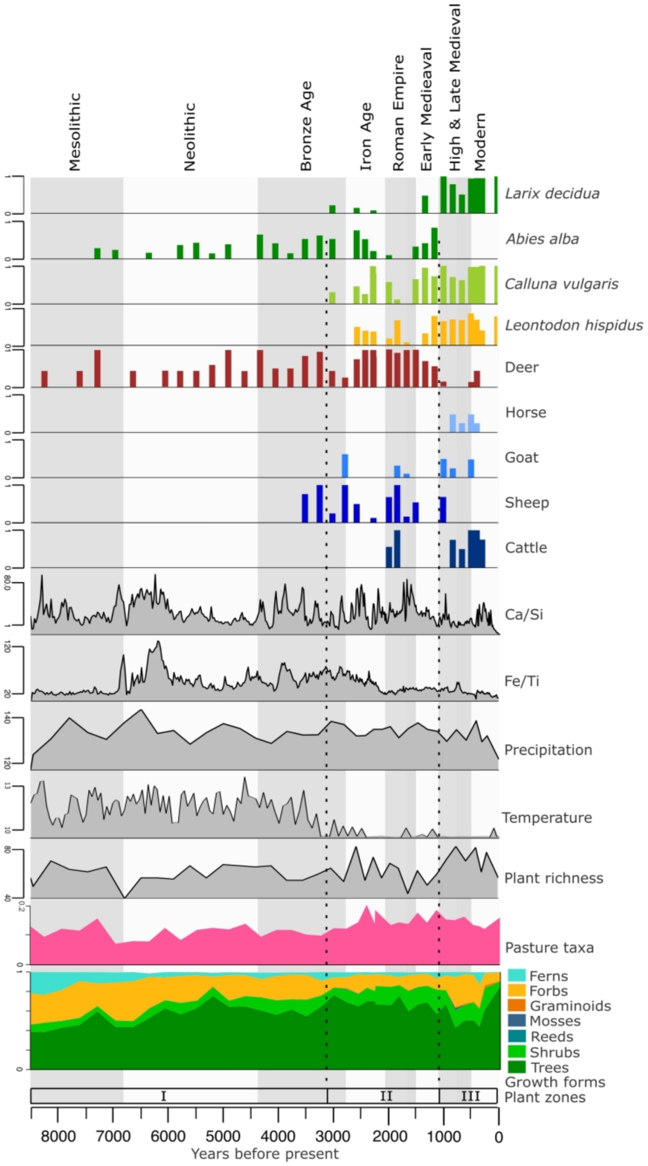


Figure 6 Overall figure: Cultural periods are indicated by the vertical grey and white boxes. Dotted lines are the zones (Plant DNA zones I - III) based on constrained cluster analysis of plant *seda*DNA data. Plant taxa that are indicative of the changing environment are plotted with red deer (*Cervus elaphus*)and four domesticated taxa from the vertebrate *seda*DNA data. Also plotted are Ca/Si and Fe/Ti, (indicators of erosion and weathering), precipitation (kg m^{-2}), mean July temperature (℃), plant richness (Hill where q = 0), relative proportion of meadow related taxa, and the relative proportions of plant*seda*DNA taxa reads separated by growth form.
insert Figure 6.

158x267mm (300 x 300 DPI)

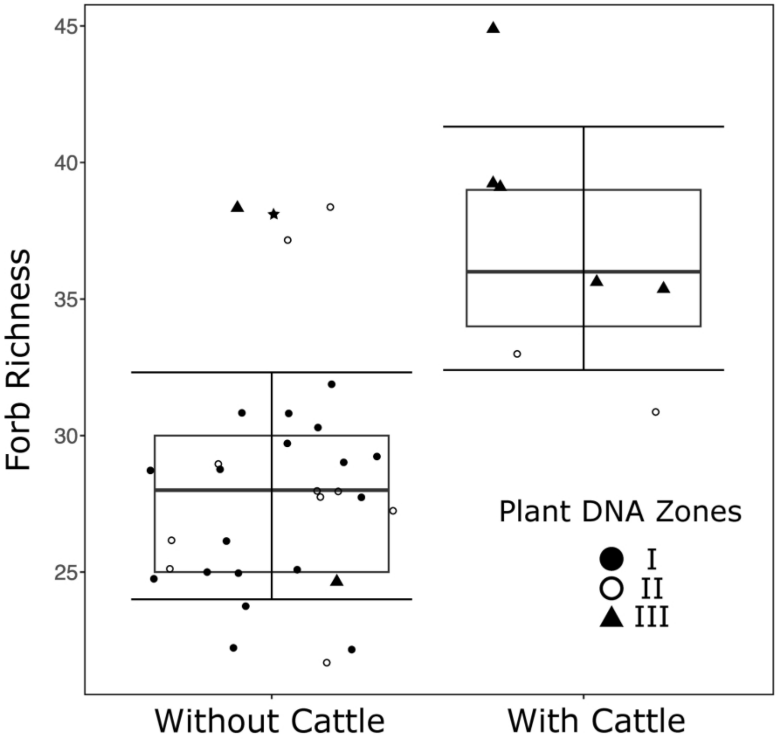


Figure 7 Boxplot: A visual representation showing the statistically significant difference in forb richness (Hill were q = 0) with and without the presence of cattle (*Bos taurus*). The Error bars indicate one standard deviation and the points represent the constrained cluster analysis Plant DNA Zones I - III. The outliers are indicated by the star.

219x189mm (300 x 300 DPI)

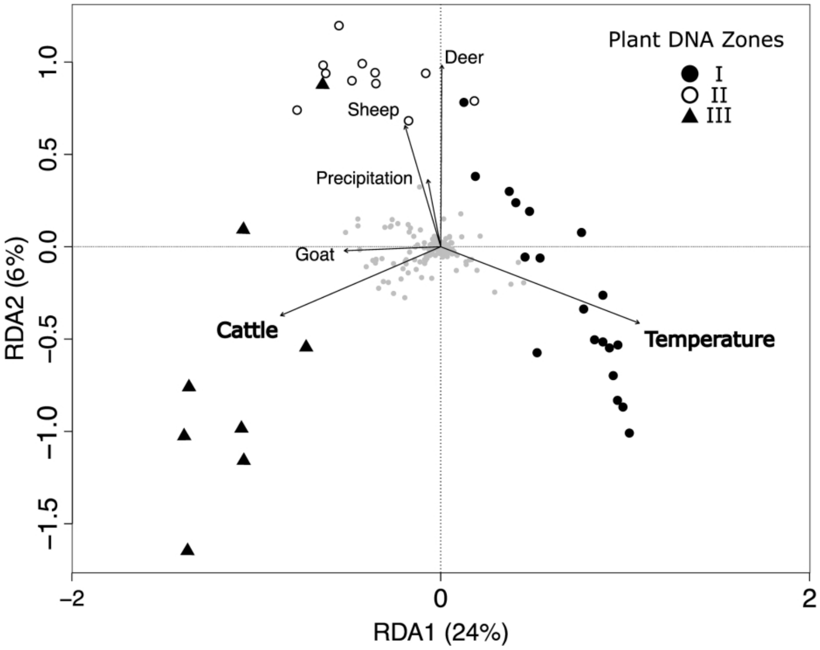
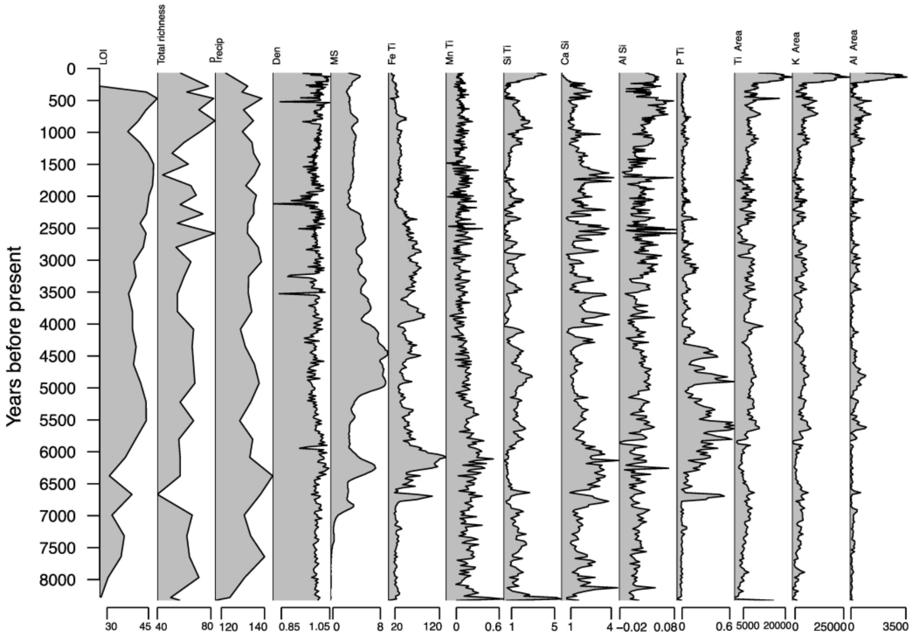


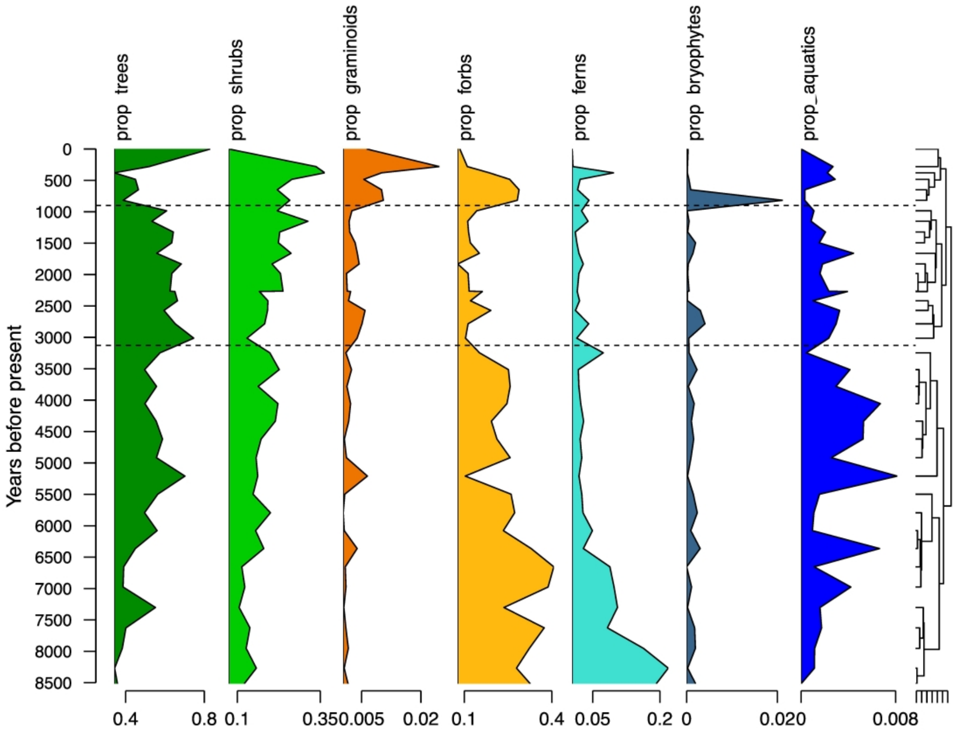
Figure 8. Redundancy Analysis (RDA): Redundancy analysis of the plant *seda*DNA taxa (quantified as 0-8 weighted PCR repeats) composition across the explanatory variables representing environmental (precipitation (kg m^{-2}), temperature (℃)) and animal factors. The sample points' represents the constrained cluster analysis Plant DNA Zones I-III, the grey dots, the plant taxa. The significant variables that explained the plant diversity pattern are shown in bold.

304x219mm (300 x 300 DPI)



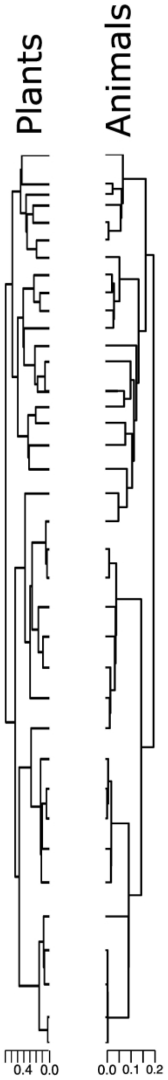
Supplementary Figure 1. Full XRF data plotted against years before present. Data is plotted with CONISS statistical zonation using the plant *sed*aDNA data

214x154mm (600 x 600 DPI)



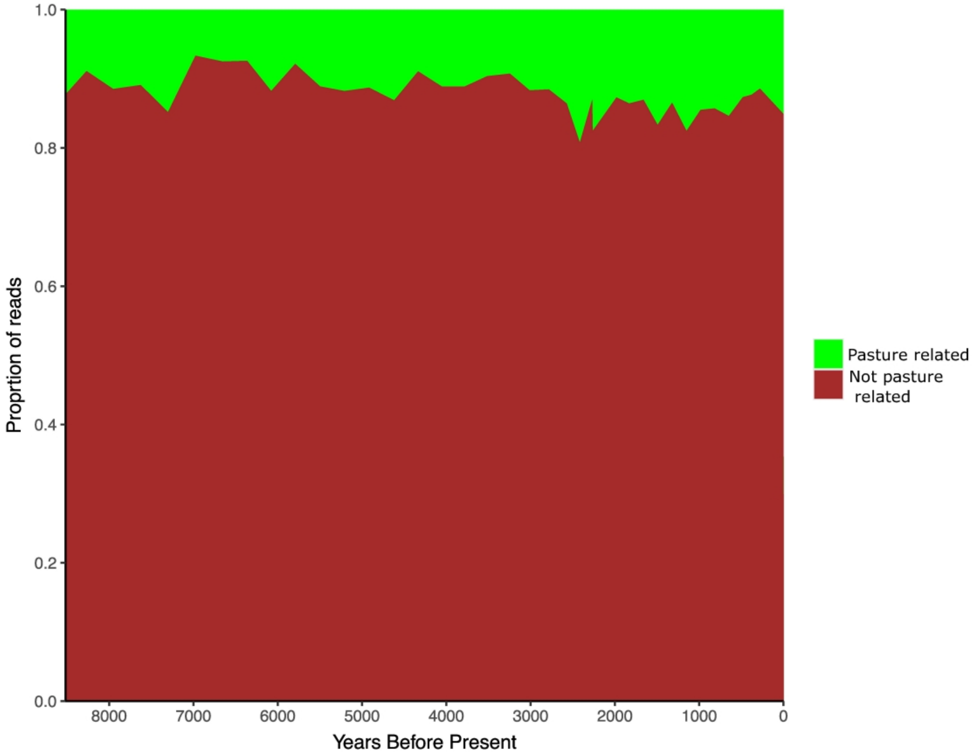
Supplementary Figure 2. Plant growth forms plotted as a proportion of total reads. Data is plotted with CONISS statistical zonation using the plant *seda*DNA data

212x155mm (600 x 600 DPI)



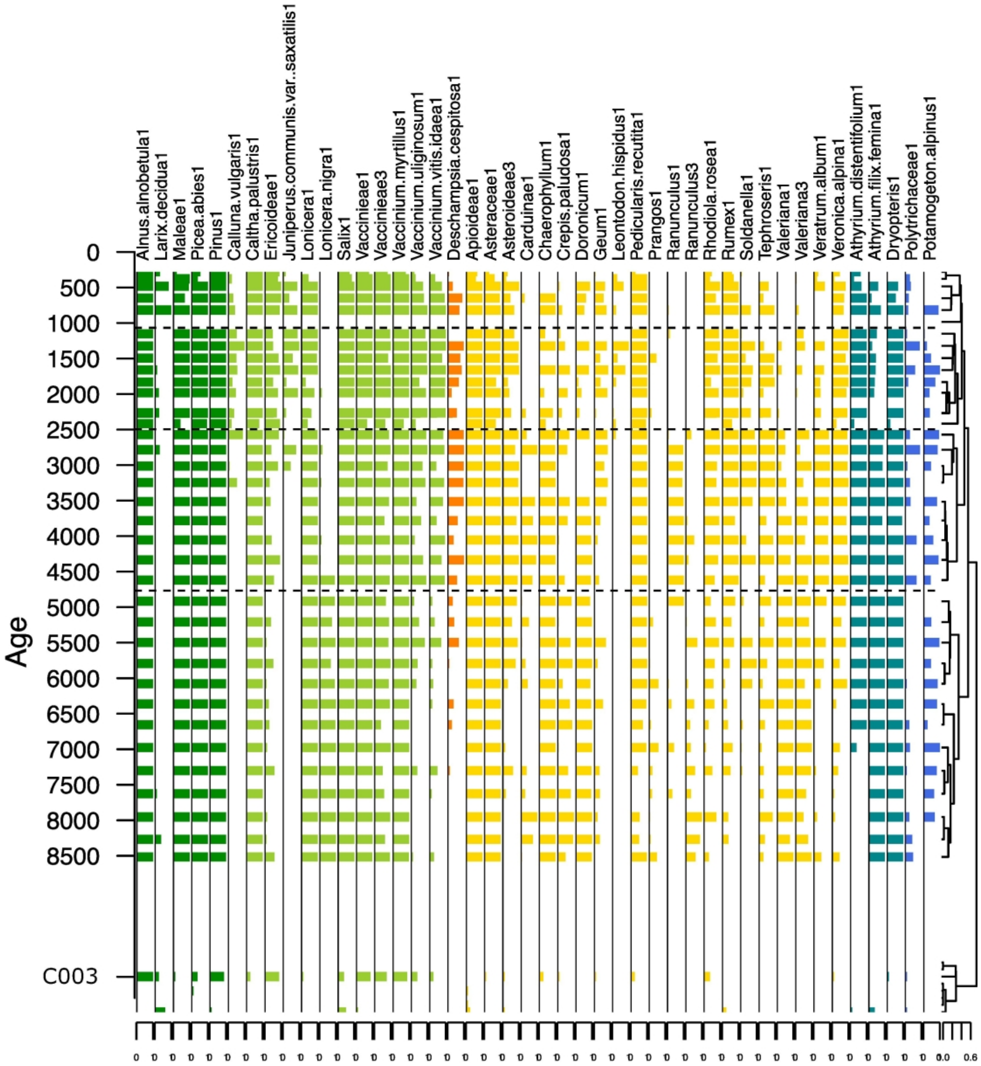
Supplementary Figure 3. Comparison of CONISS statistical zones for the plant and vertebrate *sed*aDNA.

103x250mm (600 x 600 DPI)



Supplementary Figure 4. The proportion of reads of 19 alpine pasture related taxa and the remaining taxa, not related to alpine pastures. Alpine pasture related taxa consist of shrubs, graminoids, forbs, and ferns that are commonly found within Alpine pastures. They can be indicators of disturbances from both human and non-human related events. A list of all alpine related taxa can be found in Supplementary table 4"

197x159mm (600 x 600 DPI)



Supplementary Figure 5. Rioja plot of the top 40 taxa from the TEG003 P6 loop data with the switched C003 control and sample no. EG23_087.

146x162mm (600 x 600 DPI)

Supplementary

Supplementary information

Glacial History

Großer Winterleitensee (1845 m a.s.l) is situated within Winterleitenkar, a cirque in the Seetaler Alps, Styria. The lake is near the modern municipality of Neumarkt in der Steiermark. The area is situated on what was the easternmost margin of the Mur Glacial ice sheet during the Würm high glacial period (Gasser et al., 2009). The glacier occupied the basin in and around Neumarkt in der Steiermark and Perchauer pass, up to ~1400 m a.s.l. Winterleitenkar, however, is situated above 1400 m a.s.l, and so was created, and occupied, by hanging glaciers during the last glacial maximum (Lieb, 1989).

Other studies of the lake

Two previous vegetation studies around Großer Winterleitensee have been carried out. A pollen analysis was carried out in 1975 from a core taken near the lake (Zukrigl, 1975) and a contemporary vegetation analysis of Winterleitenkar was done in 2018 (Zernig and Berg, 2018). It is not possible to fully align the core taken for the pollen analysis with ours due as it only has two published radiocarbon dates (3320 +/- 160 BC and 500 +/- 130 BC).

Supplementary Tables

Supplementary table 1. Radiocarbon dates of Großer Winterleitensee. These macrofossil remains were dated in the Poznan (Poz) laboratory. The sample indicated with a star (Figure 2, orange) was removed due to standard deviation of >100 years. The date marked with † fell outside of the age depth model (Figure 2, purple).

Lab. code	Depth [cm]	Material	14C age [BP]
Poz-123846	446 - 447	Plant remains	7490 ± 50 BP
*Poz-131032	453 - 454	* Plant remains & Daphnia eggs	*7350 ±110 BP
Poz-131034	410 - 411	Plant remains	6350 ± 40 BP
Poz-123847	386 - 387	Plant remains	5840 ± 40 BP
Poz-123848	350 - 351	Plant remains	5035 ± 35 BP
Poz-123849	302 - 303	Plant remains	4085 ± 35 BP
Poz-123850	242 - 243	Plant remains	3030 ± 50 BP
Poz-123851	206 - 207	Plant remains	2380 ± 30 BP
Poz-123852	146 - 147	Plant remains	1900 ± 35 BP
†Poz-131031	140 - 141	† Plant remains	1040 ± 30 BP
Poz-131035	100 - 101	Plant remains	1475 ± 30 BP
Poz-123853	50 - 51	Plant remains	250 ± 90 BP

Supplementary table 2. Summary of plant and vertebrate *sed*aDNA results

Primer region	Plant *sed*aDNA	Vertebrate *sed*aDNA
Raw paired-end plant DNA sequences	2 572 655	405 060
After filtering and identification	1 626 989	65 152
Total samples	38	38
Total plant sequences	137	15
Removed sequences	12	95

Supplementary table 3. Taxa with 100% match to a DNA reference library, but removed from the final dataset due to the reasons given. The number of reads of these taxa are <1% of the total number of reads with 100% match within their respective datasets.

Removed taxa	**Reason for removal**	***Sed*aDNA data set**
Capsicum	Common lab contaminant	Plant
Rutaceae	Common lab contaminant	Plant
Solanum lycopersicum	Common lab contaminant	Plant
Vicia faba	Common lab contaminant	Plant
Tritaceae	Common lab contaminant	Plant
Litorella uniflora	Only in post PCR controls, no samples	Plant
Hipperus Vulgaris	Only in post PCR controls, no samples	Plant
Quercus	Sporadic and unreliable detections	Plant
Asterales	Too high taxonomic level	Plant
Hypnales	Too high taxonomic level	Plant
Indigoferoid clade	Too high taxonomic level	Plant

PACMAD Clade	Too high taxonomic level	Plant
Sus	Only in negative controls	Vertebrate
Sus scrofa	Only in negative controls	Vertebrate
Bilateria	Misidentification of human DNA in reference database	Vertebrate
Catarrhini	Misidentification of human DNA in reference database	Vertebrate
Homininae	Human DNA	Vertebrate
Hominoidea	Human DNA	Vertebrate
Homo	Human DNA	Vertebrate
Homo sapiens	Human DNA	Vertebrate

Supplementary table 4. Plant taxa identified in Großer Winterleitensee in all 38 samples or more than 32 samples (84%).

Trees 100% samples	**Shrubs 100% sample**	**Shrubs > 84% samples**	**Forbs 100% sample**	**Forbs > 84% samples**	**Ferns 100% sample**	**Ferns > 84% samples**	**Aquatics > 84% samples**
Alnus alnobetula	*Salix*	*Rhododendron*	*Caltha palustris*	*Rumex*	*Dryopteris*	*Athyrium filix-femina*	*Potamogeton alpinus*
Pinus	*Vaccinium myrtillus*	*Vaccinium vitis-idaea*	Apioideae	*Doronicum clusii*			
Maleae	*Lonicera caerulea*	*Sambucus racemosa subsp pubens*	Asteraceae	Asteroideae			
Pica abies	Vaccinieae	*Vaccinium uliginosum*	*Pedicularis recutita*	*Tephroseris integrifolia*			
			Chaerophyllum villarsii	*Geum*			
				Veratrum album			

				Rhodiola rosea			
				Veronica alpina			

Supplementary table 5. Plant taxa typically found in pasture in the Alps today.

Taxa that are commonly found in Alpine Pastures
Plantago lanceolata
Rhinanthus sp.
Deschampsia cespitosa
Juniperus communis s.l
Valeriana officinalis agg.
Veratrum album
Leontodon hispidus
Aegopodium podagraria
Rubus idaeus
Pteridium aquilinum
Anthriscus sylvestris
Chamaenerion angustifolium
Alchemilla vulgaris agg.
Arnica montana
Anthoxanthum alpinum
Medicago lupina
Veronica chamaedrys
Carlina acaulis
Sambucus nigra

Supplementary Figures

[insert Supp figure 1.]

[insert Supp figure 2.]

[insert Supp figure 3.]

[insert Supp figure 4]

[insert Supp figure 5]

References

Gasser D, Gusterhuber J, Krische O, et al. (2009) Geology of Styria: an overview. *Mitt. Naturwiss. Ver. Steiermark* 139: 5–36.

Lieb GK (1989) Die Seetaler Alpen (Steiermark) - Länderkundliche Grundstrukturen und Pleistozäne Landschaftsgenes. *Arb. Geogr. Inst. Graz, Bd.* 29: 243–276.

Zernig K and Berg C (2018) Durch das Winterleitenkar: Floristische Kleinode der Seetaler Alpen. *Tuexenia Beiheft* 11: 171–188.

Zukrigl K (1975) Zur Geschichte der Hochlagenwälder in den Seetaler Alpen (Steiermark) Eine pollenanalytische Untersuchung des kleinen Moores im Winterleitenkessel. *Centralblatt fur das gesamte Forstwesen* 92: 175–188.

*Sed*aDNA shows that transhumance of domestic herbivores has enhanced plant diversity over the Holocene in the Eastern European Alps

Scarlett Zetter*, Sandra Garcés-Pastor, Youri Lammers, Andreas Tribsch, Antony G. Brown, Sébastien Lavergne, Eric Coissac, Kevin Walsh, Tomasz Goslar, Peter D. Heintzman, Inger Greve Alsos*

* Author of correspondence

Scarlett Zetter, The Arctic University Museum of Norway, The Arctic University of Norway, UiT - The Arctic University of Norway, NO-9037 Tromsø, Norway. Email: scarlett.zetter@uit.no. https://orcid.org/0000-0002-4712-0005.

Sandra Garcés-Pastor, The Arctic University Museum of Norway, The Arctic University of Norway, UiT - The Arctic University of Norway, NO-9037 Tromsø, Norway. https://orcid.org/0000-0001-5652-7264.

Department of Evolutionary Biology, Ecology and Environmental Sciences, University of Barcelona, Barcelona, 08007, Spain. Marine Sciences Institute, ICM-CSIC, Barcelona, 08003, Spain. sgarcespastor@gmail.com. https://orcid.org/0000-0001-5652-7264.

Youri Lammers, The Arctic University Museum of Norway, The Arctic University of Norway, UiT - The Arctic University of Norway, NO-9037 Tromsø, Norway. Email: youri.lammers@uit.no. https://orcid.org/0000-0003-0952-2668.

Antony G. Brown, The Arctic University Museum of Norway, The Arctic University of Norway, UiT - The Arctic University of Norway, NO-9037 Tromsø, Norway. Email: antony.g.brown@uit.no. https://orcid.org/0000-0002-1990-4654

Kevin Walsh, Department of Archaeology, University of York, York, UK Email: kevin.walsh@york.ac.uk. https://orcid.org/0000-0003-1621-2625

Tomasz Goslar, Faculty of Physics, Adam Mickiewicz University, Poznań, Poland. Email: tomasz.goslar@radiocarbon.pl. https://orcid.org/0000-0002-1346-3122.

Sébastien Lavergne, Université Grenoble-Alpes, Université Savoie Mont Blanc, CNRS, LECA, 38000 Grenoble, France Email: sebastien.lavergne@univ-grenoble-alpes.fr. https://orcid.org/0000-0001-8842-7495.

Eric Coissac,Université Grenoble-Alpes, Université Savoie Mont Blanc, CNRS, LECA, 38000 Grenoble, FranceEmail: eric.coissac@metabarcoding.org. https://orcid.org/0000-0001-7507-6729.

The PhyloAlps Consortium France. Email: info@phyloalps.org

Andreas Tribsch, Department of Environment and Biodiversity, University of Salzburg, Salzburg, Austria. Email: andreas.tribsch@plus.ac.at. https://orcid.org/0000-0002-5081-1675.

Peter D. Heintzman, The Arctic University Museum of Norway, The Arctic University of Norway, UiT - The Arctic University of Norway, NO-9037 Tromsø, Norway. https://orcid.org/0000-0002-6449-0219.

Department of Geological Sciences, Stockholm University, Stockholm, Sweden. Email: peter.d.heintzman@geo.su.se. https://orcid.org/0000-0002-6449-0219.

Centre for Palaeogenetics, Stockholm, Sweden. https://orcid.org/0000-0002-6449-0219.

Inger Greve Alsos, The Arctic University Museum of Norway, The Arctic University of Norway, UiT - The Arctic University of Norway, NO-9037 Tromsø, Norway. Email: inger.g.alsos@uit.no, https://orcid.org/0000-0002-8610-1085.

Abstract

The Eastern European Alps boasts highly biodiverse ecosystems and a rich archaeological history. However, there is limited research on the enduring impacts of historical climate change and human activities on plant biodiversity in this region. Using sedimentary ancient DNA, we reconstructed plant and animal dynamics from 8.5 thousand years before present (ka BP) around Großer Winterleitensee (Zirbitzkogel, Austria). Variable intensities of human activities since the Middle Bronze Age (~3.5 ka BP) facilitated the persistence of biodiverse Alpine meadow communities and lowered timberlines below their natural limit. Since the end of the Bronze Age (~2.8 ka BP), human activities, particularly pasturing, and increasing temperatures, emerged as significant drivers of plant community dynamics. The introduction of sheep (*Ovis aries*) did not reduce wild mammal presence, including red deer (*Cervus elaphus*), hare (*Lepus*), European mole (*Talpa europaea*), bank vole (*Myodes glareolus*), and short-tailed field vole (*Microtus agrestis*). During the High & Late Mediaeval Period, (~1150 - 450 BP), cattle (*Bos taurus*) and horses (*Equus caballus*) became the dominant domesticates, while all wild mammals except the short-tailed field vole and Eurasian water shrew (*Neomys fodiens*) declined or even disappeared. These changes are also accompanied by a significant transformation of plant community structure. The ability to determine both plant responses and animal drivers from the same palaeolimnological sequence vastly improves our ability to partition causes of vegetation change over the Holocene. Here, we reveal that plant biodiversity is maintained or increased by moderate cattle grazing. Therefore, non-intensive domesticated stock grazing is essential for maintaining diverse Alpine meadows.

Key words:

European Alps, anthropogenic impacts, climate change, environmental management, metabarcoding, *sed*aDNA

Introduction

The impacts of climate change on alpine plant communities are raising concern due to their potential repercussions on biodiversity, ecosystem functions and services (Liu et al., 2021; Steinbauer et al., 2018; Zu et al., 2021). The rate of climate warming in the European Alps is reaching up to three times the average observed in the northern hemisphere (Hock et al., 2019), which is predicted to result in biodiversity decline in the coming decades (Schwager and Berg, 2019). Pressures from human land use could potentially exacerbate these effects causing further losses (Wang et al., 2018). The Central Eastern Alps, recognized as a biodiversity hotspot harbouring numerous endemic species (Tribsch and Schönswetter, 2003), is now facing significant ecological threats due to both anthropogenic climate change and land use (Schwager and Berg, 2019). Long-term studies are needed to understand the drivers of such changes, but most palaeoecological studies so far do not allow for simultaneous statistical analyses of climate and land use effects.

Recent advances in *sed*aDNA (sedimentary ancient DNA) methods enable comprehensive reconstructions of the surrounding vegetation and husbandry dynamics using plant and animal DNA from lake sediment deposits, providing an opportunity for statistical testing to elucidate drivers of vegetation changes (Garcés-Pastor et al., 2022; Giguet-Covex et al., 2023; van Vugt et al., 2022). This enhanced understanding can provide pivotal insights into the preservation and management of these invaluable habitats for future generations, particularly regarding the impact of grazing in the management of Alpine ecosystems in the face of ongoing climate change.

From the Last Glacial Maximum (Local glacial history in Supplementary information) to the Holocene climatic optimum (~9.5 - 5.5 ka BP (thousand years before present)), the Alps underwent a progressive increase in temperature (Fohlmeister et al., 2013). The short-lived 8.2 ka BP climatic event was characterized by generally cooler conditions (Tinner and Lotter, 2001), along with increased humidity and precipitation (Drescher-Schneider, 2007). Vegetation reconstructions indicate that the 8.2 ka BP event caused a restructuring of terrestrial plant communities (Tinner and Lotter, 2001). Following this event, the climate remained relatively stable until the end of the Holocene climatic optimum at ~5.5 ka BP.

Climate is not the sole driver of plant community dynamics. Throughout the Holocene, Alpine plant communities have faced significant impacts from human land use practices. These include early Holocene vegetation clearance through deliberate burning (~9 ka uncalibrated BP) (Bos and Urz, 2003; Lechterbeck and Rösch, 2021), the artificial lowering of timberlines resulting from Bronze Age (~4.5 - 3 ka B) mining (~3.5 ka BP) (Knierzinger et al., 2020) and transhumance (Pini et al., 2017; Schmidl et al., 2005), to modern day recreational hiking (Aziz et al., 2023).

[insert Figure 1.]

Although the Austrian Neolithic period began ~6.9 ka BP (Löcker et al., 2009), the earliest known human settlement phase in Styria (Figure 1B) dates to ~6.3 ka BP (Wildon Schlossberg) (Brandl et al., 2015). By this time, the adjacent province to the west (Salzburg) already had evidence of Neolithic activity at over 2000 m a.s.l (Gilck and Poschlod, 2019). Austria in general remained sparsely populated until the Late Neolithic (~4.6 ka BP). After which Styria experienced a significant population increase, becoming progressively more inhabited (Brandl et al., 2015). Extensive copper ore mining emerged across Styria during the Neolithic to Bronze Age transition ~4.5 ka BP (Knierzinger et al., 2021). This widespread mining activity led to significant local deforestation (Drescher-Schneider, 2007) and an increased local demand for animal products. In the Late Bronze/Early Iron Age, sheep (*Ovis aries*) alpine transhumance, the seasonal mobility of pastoral activities across altitudes, became a common practice (Saliari et al., 2020). Following this, during the Iron Age (2.8 - 2 ka BP), there was a shift in farming practices from sheep to cattle (*Bos taurus*) alpine transhumance. However, a few strongholds of sheep alpine transhumance remained in East Austria (Schmölcke et al., 2018). The cooler temperatures toward the end of this period likely decreased the viability of cattle farming, leading to a resurgence of sheep as the primary domesticate in Austria (Schmölcke et al., 2018). Throughout the period of the Western Roman Empire (2 - 1.5 ka BP), the prosperity in Austria was mainly derived from mining (stone, salt, metal ores) and agriculture (Schmidl et al., 2005). Facilitated by a warmer, more stable climate (Marx et al., 2018), farmers of this period took to a reinvestment in cattle alpine transhumance. To support both mining processes and cattle pasturing, further tree clearing was undertaken (Drescher-Schneider, 2007). Following the collapse of the Western Roman Empire ~1.5 ka BP, the Early Mediaeval Period (~1.5 - 1 ka BP) was a time of war and mass movement across Europe, but also of an unpredictable climate (Marx et al., 2018). At the onset of the Early Mediaeval Period, the cooler, wetter climate resulted in a return to sheep alpine transhumance in some areas across Austria (Marx et al., 2018). A new intensification of cattle alpine transhumance took place at the start of the High Mediaeval Period (~1 - 0.7 ka BP), once again causing significant habitat disturbances and plant community turnover (Drescher-Schneider, 1998) that has continued to the present day. This historical reconstruction is based on pollen and archaeological records. It creates a general narrative, but cannot provide species-specific and local biodiversity data for statistically testing the drivers of change: climate and grazing.

In this paper, we analyse plant and mammal *sed*aDNA from an Alpine lake (Großer Winterleitensee, Easternmost Central Alps) to identify species-level ecological histories and test the main drivers of plant diversity trends. We further aim to identify from when the landscape around Großer Winterleitensee has been used for Alpine transhumance, which animal species were the dominant grazers, and when, in order to resolve the debate concerning human impacts on Alpine biodiversity in the Eastern European Alps. This has further implications for the management of high-altitude Alpine landscapes in general. If biodiversity has increased through the grazing of domesticates then it follows that maintaining grazing is essential to sustain high biodiversity in the face of climate change.

Materials and Methods

Großer Winterleitensee is a subalpine lake in Winterleitenkar, Seetaler Alpen, Austrian Alps (47.089653 N, 14.566058 E, 1845 m a.s.l., Figure 1), with an area of 4.5 ha, a maximum water depth of 9.6 m, and a pH of 6.98 (July, 2018). The lake catchment is 2.7 km^2 and comprises a diverse landscape including mire, Alpine meadows and dwarf shrubs. On the steep south-eastern facing slope, there is a closed Swiss pine (*Pinus cembra*) forest with scattered spruce (*Picea abies*) and larch (*Larix decidua*) (Figure 1D). The lake is situated in the Styrian crystalline region, which is dominated by metamorphic rock (garnet, mica, schist) with rarer occurrences of plagioclase, gneiss, and pegmatite, and scattered marble and amphibolites on the north-west ridge above the lake (Zernig and Berg, 2018). Trees in this area can grow at a maximum elevation of 1950 m a.s.l, allowing for an elevational difference between lake and upper tree limit of c. 105 m. A moor core was taken within Winterleitenkar (47.0916667 N, 14.5638888 E, 1850 m a.s.l, Figure 1C) for pollen analysis (Zukrigl, 1975). However, with only two absolute dates provided (500 ± 130 and

3320 ± 160 BCE or ~2.5 and ~5.3 ka BP) we are unable to fully align this core with our lake sediment core.

A 4.6 m sediment core was taken from the deepest point of the lake from a floating platform using a Nesje coring system fitted with a 5 m long, 110 mm diameter pipe. For transportation, the core was cut into one metre sections with the newly exposed ends immediately sealed. A surface sediment core was also taken with a UWITEC gravity corer using a 1 m long, 100 mm diameter pipe. All core sections were transported at 4 ˚C to the Arctic University of Norway in Tromsø (UiT). Prior to opening, the core sections were scanned for magnetic susceptibility using a GEOTEK Multi Sensor Core Logger (MSCL-S) with a point sensor at the Department of Geosciences, UiT. The sections were then cut longitudinally and opened and sampled in a dedicated ancient DNA laboratory, UiT. We took 37 subsamples at 12 cm intervals for *sed*aDNA analysis from one core half, the other half was archived. During subsampling, samples were also taken for Loss on Ignition (LOI) and plant macrofossil remains were opportunistically collected for radiocarbon dating. A subsample from the sediment/water interface was collected from the surface core in a dedicated environmental DNA laboratory, UiT.

Radiocarbon dating, age-depth model construction, and geochemistry

Sixteen terrestrial plant macrofossils were radiocarbon dated at the Poznan Radiocarbon Laboratory (Goslar et al., 2004) using accelerator mass spectrometry (AMS). The calibration of AMS dates used the terrestrial IntCal20 curve (Reimer et al., 2020). The age-depth model was constructed using the Bayesian framework calibration software ‘rbacon’ (v2.5.0) (Blaauw and Andrés Christen, 2011), executed in R version 4.3.1 (2023-06-16) (R Core Team, 2023).

The organic content of the sediment samples was measured using the LOI method by Lamb (2004). The archival core halves were scanned using an AVAATECH XRF core scanner with measurements taken at 10 mm resolution using a 1.5 mA current and 10 kV voltage for 10 seconds.

High-resolution imaging was carried out using a Jai L-107CC 3 CCD RGB Line Scan Camera mounted to the XRF scanner. The raw peak area data was normalized using either Ti and Si in order to mitigate the influence of water and matrix effects (Croudace et al., 2006). Both elements are reliable indicators of input of allochthonous material from the catchment. Iron/titanium (Fe/Ti) is used to indicate changing redox conditions, phosphorus/titanium (P/Ti) is used as a weathering indicator and calcium/silicon (Ca/Si) is used as an erosion indicator (Davies et al., 2015). Magnetic susceptibility, measured using a GEOTEK Multi Sensor Core Logger, can be used as an indicator for precipitation (Balsam et al., 2011).

*Sed*aDNA data generation and sequence assignment

All *sed*aDNA data generation steps mainly followed Rijal *et al.* (2021). Briefly, all 38 samples, four sampling controls, and four extraction negative controls were extracted using a modified DNeasy PowerSoil kit (Qiagen, Germany). Amplification of the samples used two uniquely dual-tagged generic primer sets. For plant *sed*aDNA, the trnL P6 loop region of the chloroplast genome was targeted using gh primers (Taberlet et al., 2006). For animal *sed*aDNA, we targeted the mitochondrial 16S locus using MamP007 primers (Giguet-Covex et al., 2014) including an updated human blocking primer strategy (Garcés-Pastor et al., 2022). Eight uniquely dual tagged (8 or 9-bp) amplicon replicates were created for both plant and animal *sed*aDNA. PCR reactions and cycling conditions for plants followed Voldstad *et al.* (2020), while animals followed Garcés-Pastor *et al.* (2022). We pooled and cleaned PCR products creating two pools, one for each primer set. A sequencing library was created for each pool and sequenced at Genomics Support Centre Tromsø, UiT on an Illumina NextSeq platform (2x150 bp, mid-output mode, dual indexing). Following data analysis (outlined below), plant negative control EG23_C003 was indiscernible from the plant *sed*aDNA samples, while plant *sed*aDNA sample EG23_087 contained no taxa (Supplementary Figure 4). We suspected a switch between the sample and control. We therefore re-extracted and re-amplified the batch of 20 samples (EG23_B series) in which the switch could have occurred. The resulting library was sequenced using an Illumina MiSeq platform (2x150 bp, mid-output mode, dual indexing) at the Faculty of Biosciences, Fisheries and Economics, UiT. For downstream data analyses, we combined the re-extracted samples with the remaining unaffected samples.

Following Rijal *et al.,* (2001), plant *sed*aDNA data was processed using a bioinformatic pipeline based on the OBITools software package (Boyer et al., 2016). The reference databases PhyloAlps (Garcés-Pastor et al., 2022), Arctborbryo (Soininen et al., 2015; Sønstebø et al., 2010; Willerslev et al., 2014), PhyloNorway (Alsos et al., 2020, 2022) and EMBL release 143 (Kanz et al., 2005) were used. The identified sequences were filtered using custom R scripts (available at https://github.com/Y-Lammers/MergeAndFilter). Sequences were retained if they matched at 100% to at least one reference database, were present in at least three PCR replicates and had a read count of >10. Sequences that were detected in more replicates of the negative control samples than the sediment samples and/or are known common laboratory contaminants (Supplementary Table 3) were removed. Co-occurring sequences were assigned to the same taxon (Alsos et al., 2022). For the animal *sed*aDNA data, we used the aforementioned pipeline with adjustments outlined in Garcés-Pastor *et al.* (2022). The single reference database, EMBL release 143 was used. As the concentration of animal *sed*aDNA is typically lower than terrestrial plant *sed*aDNA (Murchie et al., 2023), we used more relaxed filtering criteria to maximize detections. Sequences were retained if they were present in at least one replicate, with a read count >1, and a match of ≥95% to the reference database. Co-occurring sequences assigned to the same taxon were merged. We discarded sequences matching human (*Homo sapiens*), whereas sequences matching invertebrates were not used in downstream analyses. Sequences identified as *Sus scrofa* were discarded as we are unable to distinguish domesticated pig from wild boar at this locus. One of the three unique pig sequences was also present in the extraction control at the same frequency as the sediment samples, leading us to believe this may be contamination from the extraction reagents. Sequences that were only present with a read count of one were also removed.

Climate reconstruction and statistical analysis

We reconstructed the Holocene climate around Großer Winterleitensee using local temperature and precipitation datasets. The temperature reconstruction is based on chironomid assemblage data taken from the Austrian lake Schwarzsee ob Sölden (SoS) (Ilyashuk et al., 2011; Wick and Tinner, 1997), a lake at 2796 m a.s.l, 951 m higher elevation than Großer Winterleitensee. We adjusted the temperature reconstruction using a mean environmental lapse rate of -6.5 ℃ km^{-1} (Lute and Abatzoglou, 2021). Therefore, by adding 6 °C to each SoS temperature point an approximate local temperature reconstruction appropriate for Großer Winterleitensee was created. We reconstructed precipitation at 100-year resolution using the CHELSA-TraCE21k v1.0 model (Karger et al., 2023). Precipitation (kg m^{-2}) refers to the mean mass (kg) of water in all phases (rain, snow, etc.) per square metre per 100 years.

We plotted the proportions of weighted PCR replicates (wtRep) (Rijal et al., 2021) for plant and animal *sed*aDNA data using the Rioja package (Juggins, 2015) as implemented in R. This conservative measure of abundance avoids patterns of change being masked by read-dominant taxa and thereby highlights the diversity detected (Alsos *et al*., 2022). We conducted a constrained cluster analysis (CONISS) to identify statistically significant changes in the plant and animal community compositions using the Vegan package (Oksanen et al., 2013) in R.

Box plots were used to determine significant differences in total richness and individual plant growth form richness (Hill number for q = 0) with and without the presence of grazing animals. The data for cattle (*Bos taurus*), sheep (*Ovis aries*), horses (*Equus caballus*), goats (*Capra hircus*), and red deer (*Cervus elaphus*) were transformed into binary presence/absence data. Boxplots were plotted using the geom_boxplot() and the error bars were created and plotted using stat_summary() and stat_boxplot() from R package ggplot2 (Ginestet, 2011). The error bars indicate one standard deviation from each group mean. Plant richness is taken as the total count of taxa identified to family level and lower within each sample. Whereas the plant growth form richness is this data separated into each growth form, e.g tree, shrub etc.

To explore the effects of temperature, precipitation and animals (wtRep cow, sheep, horse, goat, red deer) on vegetation changes (plant wtRep data), a redundancy analysis (RDA) was performed using Vegan, and plotted using ggplot2. The drivers were checked for co-correlations that could impact the results using cor(). Horse was highly correlated with cattle, thus it was removed. Scaling of the RDA axes was set to two so the angle between the arrows reflects their relative relationship; an angle <90˚ representing a positive relationship, 90˚ a neutral relationship, and >90˚ a negative relationship. A transformation, such as the Hellinger transformation was not required as the data grouping had already reduced the ecological gradient. Model and constraint significance testing was achieved using anova.cca() in Vegan.

Results

[insert Figure 2.]

Age-depth model and lithology

The sixteen AMS radiocarbon dates range between 250±90 and 7490±50 years BP (Supplementary Table 1). Two dates were rejected; one had an error margin of 110 years, and the other fell outside of the model. The age-depth model (Figure 2) displays a near linear sediment accumulation rate (SAR), well captured by the Bayesian model but also well described by two linear rates with a break-point at ~2.8 ka BP. At approximately this date the SAR increases x4.5 from 0.2 mm yr^{-1} to 0.9 mm yr^{-1}.

The core consists of dark-brown silty-gyttja with organic macro-remains and lighter coloured bands throughout (Figure 2). LOI (Figure 3) indicates a core rich in organic matter. In the oldest sample (8.5 ka BP), only 20% of the core comprises organic material. Over the following three thousand years, this percentage increases, culminating in a peak at 5.5 - 5.2 ka BP (44%) before plateauing and stabilising around a mean of 43±5%. The youngest sample however is composed of only 27% organic content.

[insert Figure 3.]

XRF data (Figure 3) reveals an increase in Fe/Ti and Ca/Si input in the early Neolithic period ~6.8 - 6 ka BP, indicative of a period of increased weathering and erosion respectively. The ratio of P/Ti 6.5 - 4.5 ka BP also signals an alteration in precipitation patterns during this period. However, while a downturn in input takes place, Fe/TI and Ca/Si remain noisy throughout, suggesting that while the transport of material into the lake is still occurring, it is at a reduced rate than before, possibly due to decreased precipitation. The intervals at 2.4 - 2.2 (Iron Age) and 1.3 - 1 ka BP (Early Mediaeval Period) are intervals characterized by lower erosion as inferred from Ca/Si. Magnetic susceptibility of this core remains low until ~6.8 ka BP, subsequently peaking at ~4.5 ka BP and then gradually decreasing towards the upper section of the core, suggesting a decrease in precipitation regimes and/or a decrease in input of magnetic materials such as Fe.

Precipitation reconstruction

Modelled precipitation reconstruction data ranges from 117 (8.5 ka BP) to 144 kg m^{-2} (6.4 ka BP) (Figure 3) with the record starting with the lowest precipitation value (until 6.8 ka BP; mean = 129 kg m^{-2}). This is followed by a peak with the aforementioned highest value and then decreases through the Neolithic period (6.9 - 4.4 ka BP; mean = 136 kg m^{-2}). The mean precipitation remains relatively stable (mean = 134 kg m^{-2}) until the Modern period, where precipitation decreases once more (600 years BP - present day; mean = 131 kg m^{-2}).

*Sed*aDNA

[insert Figure 4.]

Plant data

A total of 1,642,012 reads with 100% match to at least one of the reference libraries were obtained after bioinformatic filtering (Supplementary Table 2). Following the collapse of homopolymers and post-identification filtering, we retained 1,626,989 reads, (99%) across 137 unique sequences: 11 identified to family-level, 30 to genus-level and 84 species-level (61%) and 12 to other taxonomic levels (sub-family, tribe, and sub-tribe). These taxa can be broken down into the following growth forms: 8 trees, 16 shrubs, 14 graminoids, 72 forbs, 8 ferns, 13 bryophytes and 6 aquatic plants (Figure 4). The number of taxa per sample ranged between 40 - 81 with a mean and median of 58.5 and 57 taxa respectively. Removed taxa and taxa that are consistently present throughout the core can be found in Supplementary Tables 3 and 4, respectively.

Animal data

The animal data comprises 84,062 reads identified with 95% or higher to 246 identified sequences (Supplementary Table 2). Fifteen unique sequences representing 15 taxa were retained after post-identification filtering and haplotype collapsing, comprising 65,152 reads: one taxon at genus-level and 14 at species-level. Whilst these are mammal specific primers, amphibian by-catch does occur. The 15 taxa can be broken down into the following groups: 4 domesticate mammals, 10 wild mammals, and 1 amphibian. Red deer (*Cervus elaphus*) and, from 5.2 ka BP, common frogs (*Rana temporaria*) were detected consistently throughout the core, being absent in only a few samples across the record (Figure 5).

[insert Figure 5.]

CONISS zone analysis

Constrained cluster analysis (CONISS) using a broken stick model of both the plant and mammal data identified three statistically significant zones each according to main changes (Supplementary Figure 3). The breakpoints of the CONISS zones for these two data sets are similar. We chose to use the plant CONISS zones as these zones are based on more taxa than those of the mammal data, giving us more confidence in these zones. We call these zones Plant DNA Zones (PDZ) I - III (Figure 6). For comparison, the CONISS zones for the animals are plotted in Figure 5 (See direct comparison of zones in Supplementary Figure 3).

[insert Figure 6.]

PDZ I (459 - 235 cm, 8500 - 3010 yrs BP Mesolithic, Neolithic, Bronze Age)

PDZ I is characterized by unstable environmental conditions (Figure 6). At the core's base around 8.5 ka BP, the models indicate a relatively high temperature (12.02 ˚C), the lowest precipitation value for the record (117.08 kg m^{-2}) and a median starting point for plant richness at 57 taxa. Pasture related plant taxa (Figure 6; Supplementary Table 5) are present across the entire core, however PDZ I displays the lowest proportion (mean = 10±2 %).

Over the Mesolithic period, Fe/Ti remains stable and low suggesting low weathering. Ca/Si indicates variable inputs of material from erosion processes, while becoming more stable towards the end of the Mesolithic period. At the start of this core, the LOI values are at their lowest, possibly due to few aquatic species and/or less erosion at this time. Progressing through the Mesolithic period (until ~6.8 ka BP), the temperature is fluctuating, and the precipitation is highest in the middle of the period. Total plant richness (Hill q = 0, PDZ I mean = 54.5±6.0) fluctuates in tandem with variations in precipitation. Ferns and forbs have high abundance (average 22% and 32%, respectively), whereas trees only constitute an average of 39% of the reads across these samples.

As the climate warms and dries ~7.5 ka BP, the proportion of ferns decreases, while that of trees increases. Following a peak of pasture related taxa at 7.3 ka BP, these decrease over the subsequent samples.

The Neolithic Period (6.8 - 4.6 ka BP) begins with the highest precipitation levels of the core and corresponds with a peak in mineral in-wash (Fe/Ti, Ca/Si & P/Ti; Figure 3 and 6) into the lake and low abundance of trees. The proceeding drier, more stable climate through the Holocene climatic optimum is reflected in an increase in trees, the first appearance of beaver (*Castor fiber*) and bank vole (*Myodes glareolus*), as well as the only record of red squirrel (*Sciurus vulgaris*) at 6.8 ka BP (Figure 5). We note that red deer decreases during the early Neolithic but increases again during the Late Neolithic, when also brown bear (*Ursus arctos*) appears.

The cooling throughout the Bronze Age coincided with once again an opening of the forest environment with a decrease in trees and an increase in forb and shrub growth forms. In the final 500 years of PDZ I, while detecting the domesticated sheep (*Ovis aries*), there is an increase of trees and ferns once more with a reduction in shrubs and pasture related taxa suggesting, once again, a closing forest environment. With the exception of one detection at ~6.8 k yrs BP, the bank vole is first detected in consecutive samples at ~3.25 k yrs BP, coinciding with the aforementioned increase in forest environment.

PDZ II (235 - 105 cm, 3010 - 1020 yrs BP, Iron Age, Western Roman Empire, Early Mediaeval Period)

PDZ II can be characterized as a period where human land use is having a profound effect on plant dynamics. Trees maintain dominance throughout PDZ II with the proportion of reads fluctuating around a mean of 68%. The early phase of PDZ II, is represented by the Iron age (2.8 - 2.2 ka BP) and plant richness (mean = 59.2±9.2 %) and temperature are both decreasing. An initial increase in shrub diversity includes *Juniperus communis*, *Vaccinium uliginosum*, and *Calluna vulgaris* indicating habitat disturbance and open land creation, possibly from the pasturing of domesticates.

The proportion of pasture related taxa across this zone (Figure 6; mean = 14±0.02 %) is highest at 2.5 ka BP at 19 %. Pasture related taxa including *Calluna vulgaris*, and *Leontodon hispidus* are first detected between ~3 and 2.5 ka BP. Taxa that are present only sporadically prior to PDZ II, are detected more frequently and in more subsequent samples, e.g *Deschampsia cespitosa*, *Juniperus communis s.l.* The bank vole is detected from the start of PDZ II until the end of the Iron Age at 2.3 ka BP. The detection of goats (*Capra hircus*) at the Bronze Age/Iron Age boundary indicates the presence of more domesticate varieties potentially having a more profound effect on the vegetation than sheep alone. After the introduction of goats, the short-tailed field vole is detected in two samples (2.5 and 2.2 ka BP), before being detected once again from 1.7 ka BP until the end of this zone. The presence of Eurasian water shrew (*Neomys fodiens*), European mole (*Talpa europaea*), hare (*Lepus sp.*) and one instance of wolf (*Canis lupus*) can also be detected in this zone. *Abies alba*, is a disturbance sensitive species that is not detected when cattle are detected at this lake. The aquatic plant *Potamogeton crispus* is present almost exclusively in this zone. This species can persist in nitrogen-rich waters, allowing it to thrive in heavily farmed areas where more sensitive species cannot. The onset of the Iron Age (2.8 - 2 ka BP) sees an increase and then a gradual overall decline in plant richness, although there is no sharp change in plant community structure (Figure 4 and 6).

The climate during the Roman Empire has less precipitation relative to other time periods. However, towards the start of the fall of the Western Roman Empire, a warmer temperature is recorded. Plant richness is overall high through this period, coinciding with the detection of sheep, cattle (*Bos taurus*) and goats. At 1.8 ka BP, a peak in Ca/Si indicates increased erosion around the lake. The increased erosion, coupled with the detection of multiple domesticates, points to heightened human activity in the vicinity.

The Early Mediaeval Period (1.5 - 1.1 ka BP) has relatively higher precipitation and lower temperatures. It is characterized by a lower plant richness, however *Abies alba* is detected throughout. Of the domesticates, only sheep are present at the start of this time period. After this, sheep also are no longer detected in the record. Red deer however, maintain their presence around the lake.

PDZ III (105 - 0 cm, 1020 - 0 yrs BP, High & Late Mediaeval Period, Modern Age)

PDZ III is defined by significant human disturbance and demarcates the discontinuation of *Abies alba.* Of the wild mammals, only red deer, short-tailed field vole, and the Eurasian water shrew are detected. Red deer however, are only detected in two samples in PDZ III. This could be due to an increased effect of human activity around the lake, suggesting that throughout previous domesticate grazing periods, the natural community structure has not been so greatly impacted. Pasture related taxa remain at around 14±2% throughout this zone, indicating that the grazing of the domesticated animals is maintaining the openness of the alpine pasture and halting the expansion of the forest. LOI values are low at this time, possibly due to lower in-lake organic production as the nitrogen indicator *Potamogeton crispus* is no longer detected. Plant richness remains high throughout PDZ III.

At the start of the High & Late Mediaeval Period richness is increasing alongside forbs and graminoids which increase from 15% to 30% and > 0.1% to 1%, respectively. Sheep and goat are detected during the start of the High & Late Mediaeval Period, with input of Ca/Si indicating increased erosion. while cattle and horse (*Equus caballus*) are detected later during the Modern Age. The proportion of tree reads decline, meaning that the relative biomass of trees around the lake is decreased, suggesting some tree clearing. This is in contrast to the previous periods of pasturing.

Drivers of changes

Box plots

The box plot (Figure 7) shows that forb richness is significantly higher when there are cattle present compared to when there are not. Samples are plotted with colour representing the different CONISS Zones. There are three outliers in the without cattle group, these three are all samples with sheep present.

[insert Figure 7.]

RDA

The RDA (Figure 8) environmental variables (cattle, goat, sheep, red deer, temperature, and precipitation) account for 35% of the variation in plant composition across samples. Only the variables cattle (F = 2.80, p = 0.01) and temperature (F = 9.51, p = 0.001) are shown to be significant; the other variables were not and cumulatively accounted for only 3% of the variation in the plant composition. All explanatory variables are included in this RDA plot to emphasize the patterns of the data, however significant variables are in bold. The cluster clearly separates samples belonging to the three different time periods, PDZ I - III. Both RDA axis 1 (24%) and RDA axis 2 (6%) are most affected by temperature and cattle. The non-significant grazers (sheep, horses, and red deer) also fall on RDA axis 2, except goats, which falls on RDA axis 1. RDA axis 1 separates samples by plant CONISS zones (PDZ I-III), and therefore temporally. RDA 2 separates the samples by animal type showing that sheep cause a similar non-significant effect on the vegetation to that of red deer, and an opposing effect to that of cattle. Thus, cattle cause a different effect on the vegetation from that of large native mammals.

[insert Figure 8.]

Discussion

Dynamics before the detection of domesticated animals.

Climate drove vegetation composition before the introduction of domesticated animals. Climate change is complex as there are different aspects to which plants can respond. Locally, at Großer Winterleitensee, and regionally across the wider Austrian Alps (Moser et al., 2005), precipitation does not have a significant impact on vascular plant richness. Precipitation however, does have a significant effect on plant richness at Sulsseewli (Garcés-Pastor et al., 2022). The Northern experience relatively high humidity that decreases as we move more towards the interior of the Alps, resulting in a more continental climate (Fink, 1993; Moser et al., 2005). While there is increased humidity in the Northern Alps, there is also greater variability in precipitation through the year causing pronounced drier months (Scherrer et al., 2022). Plant richness in environments with prolonged drier periods, like at Sulsseewli, is more likely to be affected by precipitation regime changes (Korell et al., 2021) than plant richness in environments with more stable regimes. Precipitation in the Seetaler Alpen is more evenly spread throughout the year (Hiebl and Frei, 2018) and therefore no relationship between precipitation and plant richness is found. We did not observe any strong increase in plant richness over time, in contrast to results from pollen records from the wider Alps (Giesecke et al., 2019) Taxa expansion occurred at different rates in the Central Eastern Alps relative to the rest of the Alps due to many factors including a more continental climate (Ilyashuk et al., 2011; Wick and Tinner, 1997), human impact (Latałowa and van der Knaap, 2006; Rey et al., 2013; Schwörer et al., 2015), and interspecific competition (Ravazzi, 2002). Pollen analyses revealed the expansion of *Picea abies* in the Eastern European Alps between 10 - 9 ka BP (Drescher-Schneider, 2007) compared to that of the expansion in the Western Alps at 6 - 5 ka BP (Latałowa and van der Knaap, 2006; Thöle et al., 2016). This is corroborated with *P. abies* detected from the start of the Großer Winterleitensee record at ~8.5 ka BP, contrasting with its late detection around ~6 - 5.5 ka BP (Garcés-Pastor et al., 2022; Thöle et al., 2016; Tinner and Theurillat, 2003) in the Swiss Alps. The mass expansion of *P. abies* into the

Swiss alps was facilitated by anthropogenic activities such as fire, grazing and logging (Schwörer et al., 2015). *Abies alba*, a taxon that is limited by late frosts and low spring precipitation, is not detected at Großer Winterleitensee until ~7.4 ka BP, some 600 years delayed from presence at Lago Sangiatto in the Italian Alps (van Vugt et al., 2022). This suggests that the change in precipitation regimes and a more oceanic climate (Heiri et al., 2003) allowed for *A. alba* to establish successfully in the catchment of Großer Winterleitensee. Multiproxy palaeoecological studies in the Central and Western Alps suggest that summer high-altitude pasturing was already taking place by the Late Neolithic (Dietre et al., 2014, 2020; Garcés-Pastor et al., 2022; Schwörer et al., 2015). However, no evidence of this is found at Großer WInterleitensee. From the Middle Neolithic the tree reads decline, we can assume that relative biomass of the trees, and thus forest cover, around the lake is decreasing. The decline is corroborated by the local pollen core with tree pollen decreasing at one of the 2 given dates, ~5.3 ka BP (Zukrigl, 1975). This can be attributed to a known wetting (Zukrigl, 1975) and cold phase ~5.5 - 5 ka BP in the Alpine region (Ilyashuk et al., 2011; Thöle et al., 2016; Wick and Tinner, 1997). Plant richness was at a low during this period in agreement with general Alps pollen analyses showing a lower richness in Early compared to Middle and Late Holocene (Giesecke *et al.,* 2019). While some pasture related taxa are present around the lake, they are at their lowest level during this period. Indicating that before domesticates were introduced to pasture, a less diverse alpine meadow was already present. The consistent detection of wild grazers such as red deer, together with a rich forb community suggests that the wild grazer population densities were high enough to maintain the openness of such a meadow.

After the introduction of domesticated animals

The introduction of sheep around the lake during the late Bronze Age aligns with the notion that vertical alpine transhumance systems began during this period in the Eastern Alps (Gilck and Poschlod, 2019; Schmidt et al., 2002). By the Bronze Age, sheep products were integral for daily life (Grömer and Saliari, 2018). Wool was a valuable resource during the Middle Bronze Age, used for textiles and tradable goods (Schmölcke et al., 2018) and sheep meat and milk products were well established dietary staples for the communities of what is now Austria (Schmölcke et al., 2018). A shift in land use around Großer Winterleitensee is reflected in the change of sediment accumulation rate (SAR) from ~2.8 ka BP, the Bronze Age/Iron Age boundary. It coincides with an increased representation of domesticates in the *sed*aDNA record and significant vegetation change. Human land use, pasturing (Giguet-Covex et al., 2014; Rapuc et al., 2024), and increased precipitation (Arnaud et al., 2016) can increase be drivers of changing SAR. The opening of the landscape and greater pasturing could in increase erosion, thereby increasing the SAR and allowing more *sed*aDNA to be transported into the sediment from the wider catchment area. The delay in SAR increase from the first introduction of sheep during the Bronze Age, and no great change in precipitation indicate that domesticate pasturing and human disturbance is intensifying. Later, during the Roman Period, the presence of cattle, sheep, and goats are detected. The wealthy Romans in Central Europe were known for their advanced culinary culture and their riches afforded them the luxury of a varied and complex diet (Bakels and Jacomet, 2003) consisting of goat and sheep meat and milk products from all three animals. Wool manufacturing also played a crucial role in the Roman economy (Schmölcke et al., 2018), with what is now Austria becoming a centre for textile manufacturing (Gostenčnik, 2013). The leather of sheep, cattle and goats were used for garments throughout the Roman Empire, especially those worn by Roman military personnel (Grömer et al., 2017). All of these factors could have led to the intensification of alpine transhumance of these animals around Großer Winterleitensee. Towards the end of the Roman Period and into the Early Mediaeval period, tree clearing may be related to the mining of Noric iron and a large human population (Drescher-Schneider, 2007). During the High & Late Mediaeval Period, the abandonment of sheep farming after 1 ka BP coincided with the intensification of cattle farming and dairying, and the introduction of horses around the lake. Horses during this period were used for, among other things, rural heavy transportation (Henning, 2014) and could have been used to cart heavy items to and from the area around Großer Winterleitense, such as logs from tree clearance processes.

While there is an overall increase in plant taxa from pre to post introduction of domesticated mammal grazing, much of the local flora already had arrived prior to human alteration of the landscape. Conversely, in the Western Alps richness is increased greatly due to the presence of domestic animals (Thöle et al., 2016), especially cattle (Garcés-Pastor et al., 2022). At Großer Winterleitensee, there is a reorganisation of the existing plant communities around the lake. The introduction of domesticated animals to an area can heavily modify vegetation and soil through several factors caused by animals at varying intensities; husbandry, grazing, excretion (fertilisation) and trampling. Cattle and sheep have largely differing grazing methods, while sheep prefer to graze on forbs and close to the ground surface, cattle prefer to eat more moderately high level forage (Cutter et al., 2022). Another way in which cattle can facilitate forb growth is impeding the growth of trees and shrubs, thus diminishing the forest expansion (Wieczorkowski and Lehmann, 2022). We observed the highest mean total richness in the periods of cattle presence. The trampling of cattle has a strong influence on ground cover by forming bare patches (Hiltbrunner et al., 2012) suitable for the germination of seeds and the establishment of less dominant taxa such as pasture related taxa *Leontodon hispidus* and *Calluna vulgaris* (Mitchell et al., 2008). Seeds could arrive via wind transportation, but also deposited through cattle faecal matter (Traba et al., 2003) A potential way cattle presence caused a significant change to forb richness may be the larger body size and differing grazing patterns compared to both sheep and goats and the usually larger population density than the horses. Conversely, at Sulsseewli both sheep and cattle that showed a significant relationship with plant richness (Garcés-Pastor et al., 2022), possibly due to a higher sheep density than at Großer Winterleitensee. While both sheep and cattle have a trampling effect (Chai et al., 2019), the cattle would have a greater impact as they are larger and heavier than sheep (Yang et al., 2019). Despite the differences, both these lakes display that light to moderate grazing of either animal increases plant biodiversity.

Abies alba was not detected around Großer Winterleitensee at multiple periods, suggesting significant human disturbance likely by tree removal, browsing from domesticates, and forest regeneration prevention. *Abies alba* germinates within thick humus layers, so regenerates best in forest environments. It is also a browsing intolerant taxon, where dense populations of even wild ungulates can significantly hinder its growth and subsequent regeneration (Frei et al., 2024;

Kupferschmid et al., 2015; Unkule et al., 2022). While *A. alba* populations demonstrate resilience mostly to the presence of sheep, there is a disappearance of *A. alba* once at 2.8 ka BP and then every time cattle are present. The *A. alba* population declines in the Bernese Alps, Switzerland (Rey et al., 2013; Schwörer et al., 2015) at 5.5 ka BP, attributed to anthropogenic disturbances such as fire, grazing and logging, and the expansion of *Picea abies,* a taxon that is ever present around Großer Winterleitensee. In the Lepontine Alps, Italy (van Vugt et al., 2022) the *A. alba* population declines and 5.1 ka BP, also attributed to intense human land use. The final collapse of *A. alba* at Großer Winterleitensee is ~1ka BP coinciding with heavy disturbance and tree removal around the lake for grazing of domesticates. *Larix decidua* is seen to establish a persistent population around Großer Winterleitensee instead, at ~1 ka BP. Normally, *L. decidua* stands would occur where soil is exposed, as the seeds of *L. decidua* are dispersed by wind and thus are very efficient at colonising bare ground and disturbed habitats (index of colonising success of 7/10) (Prach et al., 2017). While *L. decidua* is an efficient colonizer and more robust against disturbances than *A. alba*, it was also spread by humans to form larch forest-meadows for grazing and timber production (van Vugt et al., 2022). Species-rich *L. decidua* stands were already detected by the Bronze Age in the neighbouring Bernese Alps, Switzerland (Rey et al., 2013) and Lepontine Alps, Italy (van Vugt et al., 2022). While a smaller presence of *L. decidua* is detected around the lake at these times, a continued strong detection of *L. decidua* occurs much later around Großer Winterleitensee, in the High & Late Medieval Period, suggesting an extremely long delay in the creation of these stands.

Wild animals around the lake

Contrary to subalpine lake Sulsseewli record (Garcés-Pastor et al., 2022) the introduction of domesticated animals did not coincide with the disappearance of red deer (*Cervus elaphus*). This may be since the domestic grazing pressure around Großer Witnerleitensee was relatively low during this period, unlike Sulsseewli. Thus, enough tree coverage was maintained, or habitat disturbance was not intense enough, and the red deer population was able to maintain a presence at the lake. From the High & Late Mediaeval Period, ~1 ka BP, the red deer are no longer detected.

Red deer live between woodland and open grassland areas, suggesting the intensification of farming and escalation of tree removal forced the red deer to retreat from this lake, and it did not fully re-establish or the population was kept low due to hunting. Other wild animals are also affected by the plant growth forms around the lake. The bank vole's preferred habitat is forests with high canopy and shrub cover (Hille and Mortelliti, 2011) while the field vole's preferred habitat is forests with dense herbaceous cover and wet meadows (Mathias et al., 2017). We see that the bank vole disappears from the record during the Roman Period, however the field vole remains through the intensification of human land use.

Implications for conservation

The preservation of Alpine meadows has become increasingly important in recent years with respect to climate change and Alpine pasture abandonment (Piccinelli et al., 2020; Thöle et al., 2016). The conservation of Alpine pastures not only directly impacts plant biodiversity, but also other organisms dependent upon the open grassland (Cutter et al., 2022). In the past, prolonged heavy grazing has led to significant harm, causing impoverished soils and decreased biodiversity (Cislaghi et al., 2019). However, the undergrazing, or complete abandonment of these pastures leads to an expansion of trees and progressive ecological succession to closed forest (Cislaghi et al., 2019). Compounding this, increasing temperatures are further promoting the growth of trees (Snell et al., 2022) and therefore leads to a decrease in plant biodiversity. Our data shows that the light to moderate grazing of cattle increases not only forb richness, but also total plant richness. This is in agreement with the intermediate disturbance hypothesis which suggests that moderate levels of habitat disturbance allows for highest plant species diversity (Hobbs and Huenneke, 1992). Furthermore, it shows that grazing by sheep has a similar effect on the plant diversity to that of red deer. Moderate levels of cattle or sheep alpine transhumance should be continued to encourage the maintenance of these pastures and upkeep of plant biodiversity. Especially in a manner that could help mitigate the promotion of climate change on tree growth.

Conclusion

This sedaDNA-based research from Großer Witnerleitensee emphasizes the importance of interdisciplinary approaches to unravel complex ecosystem dynamics over long timescales. By using a multi-proxy approach, we are able to tease-apart and evaluate the impacts of climate change, native mammals and alpine transhumance on the vegetation at high taxonomic resolution. Our study represents the first in the Eastern European Alps identifying how and when alpine transhumance occurred using *sed*aDNA and DNA-barocoding technology. Our findings demonstrate that moderate-intensity cattle grazing significantly enriches the plant community. The insights gained from this research contributes to a deeper understanding of the historical and ongoing drivers of Alpine plant communities, thereby providing a foundation for informed conservation efforts and sustainable, evidence-lead, management practices.

Acknowledgements:

We thank Kristin Heggland (Tromsø) and Roland Kaiser (Salzburg) for invaluable assistance with fieldwork and Karina Monsen (Tromsø) for assistance with the geological measurements. Scarlett Zetter, 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). Andreas Tribsch was supported by ABOL (Austrian Barcode of Life) via an HRSM-project supported by the Bundesministerium für Bildung, Wissenschaft und Forschung (BMBWF) in Austria.

Consortia:

The PhyloAlps Consortium

Sébastien Lavergne, Eric Coissac, Charles Pouchon, Cristina Roquet, Wilfried Thuiller, Niklaus E. Zimmermann, Adriana Alberti, Patrick Wincker, Martí Boleda, Frédéric Boyer, Anthony Hombiat, Christophe Perrier, Rolland Douzet, Jean-Gabriel Valay, Serge Aubert, France Denoeud, Bruno Bzeznick, Ludovic Gielly, Pierre Taberlet, Delphine Rioux, Céline Orvain, Maxime Rome, Rafael O. Wüest, Sonia Latzin, John Spillmann, Linda Feichtinger, Jérémie Van Es, Luc Garraud, Jean-Charles Villaret, Sylvain Abdulhak, Véronique Bonnet, Stéphanie Huc, Noémie Fort, Thomas Legland, Thomas Sanz, Gilles Pache, Alexis Mikolajczak, Virgile Noble, Henri Michaud, Benoît Offerhaus, Cédric Dentant, Pierre Salomez, Richard Bonet, Thierry Delahaye, Marie-France Leccia, Monique Perfus, Stefan Eggenberg, Adrian Möhl, Bogdan-Iuliu Hurdu, Paul-Marian Szatmari, Mihai Pușcaș, Jan Smyčka, Patrik Mráz, Kristýna Šemberová, Michał Ronikier & Marek Slovák

Author Contribution:

IGA designed the research and raised the funding and provided resources. IGA, AGB, AT, SGP, KW and PDH did the fieldwork. SZ did the DNA lab work with input from SGP. TG performed radiocarbon dating. SZ performed XRF interpretation with input from AGB. AGB and SZ performed age-depth modelling with input from PDH. SZ verified and curated the plant sequences with input from SGP and AT, whereas PDH did the same for animals. YL and SZ performed the bioinformatics. SZ performed the statistical analysis with input from SGP and IGA. SZ wrote the first draft of the manuscript with input from SGP, PDH, IGA, YL, AT, AGB, and KW, the latter two also provided input on the wider archaeological/historical interpretation.

IGA - Inger Greve Alsos
AGB - Anthony Gavin Brown
AT - Andreas Tribsch
SGP - Sandra Garcés-Pastor
TG - Tomas Gozlar
KW - Kevin Walsh
SZ - Scarlett Zetter
PDH - Peter D. Heintzman
YL - Youri Lammers

References

Alsos IG, Lavergne S, Merkel MKF, et al. (2020) The Treasure Vault Can be Opened: Large-Scale Genome Skimming Works Well Using Herbarium and Silica Gel Dried Material. *Plants* 9(4).

Alsos IG, Rijal DP, Ehrich D, et al. (2022) Postglacial species arrival and diversity buildup of northern ecosystems took millennia. *Science advances* 8(39): eabo7434.

Arnaud F, Poulenard J, Giguet-Covex C, et al. (2016) Erosion under climate and human pressures: An alpine lake sediment perspective. *Quaternary science reviews* 152. Elsevier BV: 1–18.

Aziz N, Holmes MA, Bennion LD, et al. (2023) Hiking trailheads, but not trailsides, associated with higher cover of non-native plants' trail impact on non-native plant cover. *Applied Vegetation Science* 26(1). Wiley.

Bakels C and Jacomet S (2003) Access to luxury foods in Central Europe during the Roman period: the archaeobotanical evidence. *World archaeology* 34(3). Routledge: 542–557.

Balsam WL, Ellwood BB, Ji J, et al. (2011) Magnetic susceptibility as a proxy for rainfall: Worldwide data from tropical and temperate climate. *Quaternary science reviews* 30(19): 2732–2744.

Blaauw M and Andrés Christen J (2011) Flexible paleoclimate age-depth models using an autoregressive gamma process. *Bayesian Analysis* 6(3). International Society for Bayesian Analysis: 457–474.

Bos JAA and Urz R (2003) Late Glacial and early Holocene environment in the middle Lahn river valley (Hessen, central-west Germany) and the local impact of early Mesolithic people - pollen and macrofossil evidence. *Vegetation history and archaeobotany* 12(1): 19–36.

Boyer F, Mercier C, Bonin A, et al. (2016) obitools: a unix-inspired software package for DNA metabarcoding. *Molecular ecology resources* 16(1). Wiley: 176–182.

Brandl M, Martinez MM, Modl D, et al. (2015) Chert from the Rein Basin (Styria, Austria): prehistoric use and distribution. *Connecting Networks: Characterising Contact by Measuring Lithic Exchange in the European Neolithic. Oxford.* torrossa.com: 103–115.

Chai J, Yu X, Xu C, et al. (2019) Effects of yak and Tibetan sheep trampling on soil properties in the northeastern Qinghai-Tibetan Plateau. *Applied soil ecology: a section of Agriculture, Ecosystems & Environment* 144: 147–154.

Cislaghi A, Giupponi L, Tamburini A, et al. (2019) The effects of mountain grazing abandonment on plant community, forage value and soil properties: observations and field measurements in an alpine area. *Catena* 181: 104086.

Croudace IW, Rindby A and Rothwell RG (2006) ITRAX: description and evaluation of a new multi-function X-ray core scanner. *Geological Society, London, Special Publications.* Epub ahead of print 2006. DOI: 10.1144/gsl.sp.2006.267.01.04.

Cutter J, Hovick T, McGranahan D, et al. (2022) Cattle grazing results in greater floral resources and pollinators than sheep grazing in low-diversity grasslands. *Ecology and evolution* 12(1). Wiley: e8396.

Davies SJ, Lamb HF and Roberts SJ (2015) Micro-XRF Core Scanning in Palaeolimnology: Recent Developments. In: Croudace IW and Rothwell RG (eds) *Micro-XRF Studies of Sediment Cores: Applications of a Non-Destructive Tool for the Environmental Sciences*. Dordrecht: Springer Netherlands, pp. 189–226.

Dietre B, Walser C, Lambers K, et al. (2014) Palaeoecological evidence for Mesolithic to Medieval climatic change and anthropogenic impact on the Alpine flora and vegetation of the Silvretta Massif (Switzerland/Austria). *Quaternary international: the journal of the International Union for Quaternary Research* 353. Elsevier BV: 3–16.

Dietre B, Reitmaier T, Walser C, et al. (2020) Steady transformation of primeval forest into subalpine pasture during the Late Neolithic to Early Bronze Age (2300−1700 BC) in the Silvretta Alps, Switzerland. *Holocene* 30(3). SAGE Publications: 355–368.

Drescher-Schneider R (1998) Ergebnisse der pollen-und großrestanalytischen Untersuchungen im Gebiet der Plankenalm, Dachstein (Österreich). *Dachstein. Vier Jahrtausende Almen im Hochgebirge* 2: 46–61.

Drescher-Schneider R (2007) Das Kohltratten-Moor in Süden von Schloss Lind. Neue pollen- und großrestanalytische Ergebnisse zur spät- und postglazialen Vegetations- und Klimaentwucklung im Gebiet Neumarkt (Steirmark, Österreich). *Naturwissenschaftlicher Verein für Steiermark* 137: 63–84.

Fink MH (1993) *Die Pflanzengesellschaften Österreichs* (L Mucina et al.eds). Gustav Fischer Verlag, Jena.

Fohlmeister J, Vollweiler N, Spötl C, et al. (2013) COMNISPA II: Update of a mid-European isotope climate record, 11 ka to present. *Holocene* 23(5). SAGE Publications Ltd: 749–754.

Frei ER, Conedera M, Bebi P, et al. (2024) High potential but little success: ungulate browsing increasingly impairs silver fir regeneration in mountain forests in the southern Swiss Alps. *Forestry* . Oxford University Press (OUP). Epub ahead of print 15 June 2024. DOI: 10.1093/forestry/cpae028.

Garcés-Pastor S, Coissac E, Lavergne S, et al. (2022) High resolution ancient sedimentary DNA shows that alpine plant diversity is associated with human land use and climate change. *Nature communications* 13(1): 6559.

Giesecke T, Wolters S, van Leeuwen JFN, et al. (2019) Postglacial change of the floristic diversity gradient in Europe. *Nature communications* 10(1): 5422.

Giguet-Covex C, Pansu J, Arnaud F, et al. (2014) Long livestock farming history and human landscape shaping revealed by lake sediment DNA. *Nature communications* 5: 3211.

Giguet-Covex C, Bajard M, Chen W, et al. (2023) Long-term trajectories of mountain agro-ecosystems in the North-Western Alps. *Regional Environmental Change* 23(2): 58.

Gilck F and Poschlod P (2019) The origin of alpine farming: A review of archaeological, linguistic and archaeobotanical studies in the Alps. *Holocene* 29(9). SAGE Publications Ltd: 1503–1511.

Ginestet C (2011) ggplot2: Elegant Graphics for Data Analysis. *Journal of the Royal Statistical Society. Series A,* 174(1). Oxford Academic: 245–246.

Goslar T, Czernik J and Goslar E (2004) Low-energy 14C AMS in Poznań radiocarbon laboratory, Poland. *Nuclear instruments & methods in physics research. Section B, Beam interactions with materials and atoms* 223-224. Elsevier BV: 5–11.

Gostenčnik K (2013) Textile production and trade in Roman Noricum. *Making Textiles in Pre-Roman and Roman Times: People, Places, Identities. (Ancient Textiles Series, 4). --pp*: 60–86.

Grömer K and Saliari K (2018) Dressing Central European prehistory – the sheep's contribution An interdisciplinary study about archaeological textile finds and archaeozoology. *Annalen des Naturhistorischen Museums in Wien. Serie A, Fur Mineralogie und Petrographie, Geologie und Palaontologie, Anthropologie und Prahistorie* 120. Naturhistorisches Museum: 127–156.

Grömer K, Russ-Popa G and Saliari K (2017) Products of animal skin from Antiquity to the Medieval Period. *Annalen des Naturhistorischen Museums in Wien. Serie A. Mineralogie und Petrographie, Geologie und Palaeontologie, Anthropologie und Praehistorie* 119. Naturhistorisches Museum: 69–93.

Henning J (2014) Did the 'agricultural revolution' go east with Carolingian conquest? Some reflections on early medieval rural economics of the Baiuvarii and Thuringi. In: Fries-Knoblach, J., Steuer, H., Hines, J. (ed.) *Baiuvarii and Thuringi: An Ethnographic Perspective. Studies in Historical Archaeoethnology*. Woodbridge: the Boydell Press, pp. 331–360.

Hiebl J and Frei C (2018) Daily precipitation grids for Austria since 1961—development and evaluation of a spatial dataset for hydroclimatic monitoring and modelling. *Theoretical and Applied Climatology* 132(1-2). Springer Science and Business Media LLC: 327–345.

Hille SM and Mortelliti A (2011) Microhabitat partitioning of Apodemus flavicollis and Myodes glareolus in the sub-montane Alps: a preliminary assessment. *Hystrix, the Italian Journal of Mammalogy* 21(2).

Hiltbrunner D, Schulze S, Hagedorn F, et al. (2012) Cattle trampling alters soil properties and changes soil microbial communities in a Swiss sub-alpine pasture. *Geoderma* 170: 369–377.

Hobbs RJ and Huenneke LF (1992) Disturbance, diversity, and invasion: Implications for conservation. *Conservation biology: the journal of the Society for Conservation Biology* 6(3). Wiley: 324–337.

Hock R, Rasul G, Adler C, et al. (2019) High mountain areas. In: *IPCC Special Report on the Ocean and Cryosphere in a Changing Climate*. H.-O. Pörtner, DC Roberts, V. Masson-Delmotte, P. Zhai, M. Tignor, E …, pp. 131–202.

Ilyashuk EA, Koinig KA, Heiri O, et al. (2011) Holocene temperature variations at a high-altitude site in the Eastern Alps: a chironomid record from Schwarzsee ob Sölden, Austria. *Quaternary science reviews* 30(1-2). Pergamon Press: 176–191.

Juggins S (2015) rioja: Analysis of Quaternary Science Data. Newcastle University. Epub ahead of print 2015.

Kanz C, Aldebert P, Althorpe N, et al. (2005) The EMBL Nucleotide Sequence Database. *Nucleic acids research* 33(Database issue). Oxford University Press (OUP): D29–33.

Karger DN, Nobis MP, Normand S, et al. (2023) CHELSA-TraCE21k--high-resolution (1 km) downscaled transient temperature and precipitation data since the Last Glacial Maximum. *Climate of the Past* 19(2). Copernicus GmbH: 439–456.

Knierzinger W, Drescher-Schneider R, Knorr K-H, et al. (2020) Anthropogenic and climate signals in late-Holocene peat layers of an ombrotrophic bog in the Styrian Enns valley (Austrian Alps). *E& G Quaternary Science Journal* 69(2). Copernicus Publications: 121–137.

Knierzinger W, Huang J-JS, Strasser M, et al. (2021) Late Holocene periods of copper mining in the Eisenerz Alps (Austria) deduced from calcareous lake deposits. *Anthropocene* 33: 100273.

Korell L, Auge H, Chase JM, et al. (2021) Responses of plant diversity to precipitation change are strongest at local spatial scales and in drylands. *Nature communications* 12(1). Springer Science and Business Media LLC: 2489.

Kupferschmid AD, Wasem U and Bugmann H (2015) Browsing regime and growth response of Abies alba saplings planted along light gradients. *European journal of forest research* 134(1). Springer Science and Business Media LLC: 75–87.

Lamb AL (2004) Determination of organic and carbonate content in soils and sediments by loss on ignition (LOI). *NERC Isotope Geosciences Laboratory Report* 197.

Latałowa M and van der Knaap WO (2006) Late Quaternary expansion of Norway spruce Picea abies (L.) Karst. in Europe according to pollen data. *Quaternary science reviews* 25(21): 2780–2805.

Lechterbeck J and Rösch M (2021) Böhringer See, western Lake Constance (Germany): an 8500 year record of vegetation change. *Grana* 60(2). Taylor & Francis: 119–131.

Liu S, Kruse S, Scherler D, et al. (2021) Sedimentary ancient DNA reveals a threat of warming-induced alpine habitat loss to Tibetan Plateau plant diversity. *Nature communications* 12(1): 2995.

Löcker K, Nau E, Neubauer W, et al. (2009) Magnetic surveys of Early and Middle Neolithic settlements in Austria. *ArchéoSciences* (33 (suppl.)). OpenEdition: 101–104.

Lute AC and Abatzoglou JT (2021) Best practices for estimating near-surface air temperature lapse rates. *International Journal of Climatology* 41(S1). Wiley.

Marx W, Haunschild R and Bornmann L (2018) Climate and the Decline and Fall of the Western Roman

Empire: A Bibliometric View on an Interdisciplinary Approach to Answer a Most Classic Historical Question. *Climate* 6(4). Multidisciplinary Digital Publishing Institute: 90.

Mathias M da L, Hart EB, Ramalhinho M da G, et al. (2017) Microtus agrestis (Rodentia: Cricetidae). *Mammalian Species* 49(944). Oxford Academic: 23–39.

Mitchell RJ, Rose RJ and Palmer SCF (2008) Restoration of Calluna vulgaris on grass-dominated moorlands: The importance of disturbance, grazing and seeding. *Biological conservation* 141(8): 2100–2111.

Moser D, Dullinger S, Englisch T, et al. (2005) Environmental determinants of vascular plant species richness in the Austrian Alps. *Journal of Biogeography* 32(7). Wiley Online Library: 1117–1127.

Murchie TJ, Giguet-Covex C, Heintzman PD, et al. (2023) Terrestrial Fauna and Hominin DNA from Sedimentary Archives. In: Capo, E., Barouillet, C., Smol, J. P. (ed.) *Tracking Environmental Change Using Lake Sediments: Volume 6: Sedimentary DNA*. Cham: Springer International Publishing, pp. 299–379.

Oksanen J, Blanchet FG, Kindt R, et al. (2013) Community ecology package. *R package version* 2(0): 321–326.

Piccinelli S, Brusa G and Cannone N (2020) Climate warming accelerates forest encroachment triggered by land use change: A case study in the Italian Prealps (Triangolo Lariano, Italy). *Catena* 195: 104870.

Pini R, Ravazzi C, Raiteri L, et al. (2017) From pristine forests to high-altitude pastures: an ecological approach to prehistoric human impact on vegetation and landscapes in the western Italian Alps. *The Journal of ecology* 105(6). Wiley: 1580–1597.

Prach K, Tichý L, Vítovcová K, et al. (2017) Participation of the Czech flora in succession at disturbed sites: quantifying species' colonization ability. *Preslia* 89(2). Czech Botanical Society: 87–100.

Rapuc W, Giguet-Covex C, Bouchez J, et al. (2024) Human-triggered magnification of erosion rates in European Alps since the Bronze Age. *Nature communications* 15(1): 1246.

Ravazzi C (2002) Late Quaternary history of spruce in southern Europe. *Review of palaeobotany and palynology* 120(1-2). Elsevier BV: 131–177.

R Core Team, (2023) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Reimer PJ, Austin WEN, Bard E, et al. (2020) The IntCal20 Northern hemisphere radiocarbon age calibration curve (0–55 cal kBP). *Radiocarbon* 62(4). Cambridge University Press (CUP): 725–757.

Rey F, Schwörer C, Gobet E, et al. (2013) Climatic and human impacts on mountain vegetation at Lauenensee (Bernese Alps, Switzerland) during the last 14,000 years. *Holocene* 23(10). SAGE Publications Ltd: 1415–1427.

Rijal DP, Heintzman PD, Lammers Y, et al. (2021) Sedimentary ancient DNA shows terrestrial plant richness continuously increased over the Holocene in northern Fennoscandia. *Science advances* 7(31).

Saliari K, Pucher E, Staudt M, et al. (2020) Continuities and changes of animal exploitation across the Bronze Age—Iron Age boundary at mining sites in the Eastern Alps. *Archaeofauna* 29. revistas.uam.es: 77–160.

Scherrer SC, Hirschi M, Spirig C, et al. (2022) Trends and drivers of recent summer drying in Switzerland. *Environmental research communications* 4(2). IOP Publishing: 025004.

Schmidl A, Kofler W, Oeggl-wahlmüller N, et al. (2005) Land use in The Eastern Alps during the Bronze Age—an archaeobotanical case study of a hilltop settlement in the montafon (western Austria). *Archaeometry* 47(2). Wiley: 455–470.

Schmidt R, Koinig KA, Thompson R, et al. (2002) A multi proxy core study of the last 7000 years of climate and alpine land-use impacts on an Austrian mountain lake (Unterer Landschitzsee, Niedere Tauern). *Palaeogeography, palaeoclimatology, palaeoecology* 187(1-2). Elsevier BV: 101–120.

Schmölcke U, Gross D and Nikulina EA (2018) The history of sheep husbandry in Austria from the Neolithic to the Roman Period. *Annalen des Naturhistorischen Museums in Wien. Serie A. Mineralogie und Petrographie, Geologie und Palaeontologie, Anthropologie und Praehistorie* 120. Naturhistorisches Museum: 101–126.

Schwager P and Berg C (2019) Global warming threatens conservation status of alpine EU habitat types in the European Eastern Alps. *Regional Environmental Change* 19(8): 2411–2421.

Schwörer C, Colombaroli D, Kaltenrieder P, et al. (2015) Early human impact (5000–3000 BC) affects mountain forest dynamics in the Alps. *The Journal of ecology* 103(2). Wiley: 281–295.

Snell RS, Peringer A, Frank V, et al. (2022) Management-based mitigation of the impacts of climate-driven woody encroachment in high elevation pasture woodlands. *The Journal of applied ecology* 59(7). Wiley: 1925–1936.

Soininen EM, Gauthier G, Bilodeau F, et al. (2015) Highly overlapping winter diet in two sympatric lemming species revealed by DNA metabarcoding. *PloS one* 10(1): e0115335.

Sønstebø JH, Gielly L, Brysting AK, et al. (2010) Using next-generation sequencing for molecular reconstruction of past Arctic vegetation and climate. *Molecular ecology resources* 10(6). Wiley: 1009–1018.

Steinbauer MJ, Grytnes J-A, Jurasinski G, et al. (2018) Accelerated increase in plant species richness on mountain summits is linked to warming. *Nature* 556(7700): 231–234.

Taberlet P, Coissac E, Pompanon F, et al. (2006) Power and limitations of the chloroplast trn L (UAA) intron for plant DNA barcoding. *Nucleic acids research* 35(3). Oxford Academic: e14–e14.

Thöle L, Schwörer C, Colombaroli D, et al. (2016) Reconstruction of Holocene vegetation dynamics at

Lac de Bretaye, a high-mountain lake in the Swiss Alps. *Holocene* 26(3). SAGE Publications: 380–396.

Tinner W and Lotter AF (2001) Central European vegetation response to abrupt climate change at 8.2 ka. *Geology* 29(6). GeoScienceWorld: 551–554.

Tinner W and Theurillat J-P (2003) Uppermost limit, extent, and fluctuations of the timberline and treeline ecocline in the Swiss central alps during the past 11,500 years. *Arctic, antarctic, and alpine research* 35(2). Informa UK Limited: 158–169.

Traba, J, Levassor, C, Peco, B (2003) Restoration of species richness in abandoned Mediterranean grasslands: seeds in cattle dung. *Restoration Ecology* 11(3): 378–384.

Tribsch A and Schönswetter P (2003) Patterns of endemism and comparative phylogeography confirm palaeo-environmental evidence for Pleistocene refugia in the Eastern Alps. *Taxon* 52(3). Wiley: 477–497.

Unkule M, Piedallu C, Balandier P, et al. (2022) Climate and ungulate browsing impair regeneration dynamics in spruce-fir-beech forests in the French Alps. *Annals of forest science* 79(1). Springer Science and Business Media LLC.

van Vugt L, Garcés-Pastor S, Gobet E, et al. (2022) Pollen, macrofossils and sedaDNA reveal climate and land use impacts on Holocene mountain vegetation of the Lepontine Alps, Italy. *Quaternary science reviews* 296: 107749.

Voldstad LH, Alsos IG, Farnsworth WR, et al. (2020) A complete Holocene lake sediment ancient DNA record reveals long-standing high Arctic plant diversity hotspot in northern Svalbard. *Quaternary science reviews* 234: 106207.

Wang J, Wu R, He D, et al. (2018) Spatial relationship between climatic diversity and biodiversity conservation value. *Conservation biology: the journal of the Society for Conservation Biology* 32(6): 1266–1277.

Wick L and Tinner W (1997) 1997: Vegetation changes and timberline fluctuations in the Central Alps as indicators of Holocene climatic oscillations. Arctic and Alpine Research 29, 445--458. *Arctic and Alpine Research* 29(4): 445–458.

Wieczorkowski JD and Lehmann CER (2022) Encroachment diminishes herbaceous plant diversity in grassy ecosystems worldwide. *Global change biology* 28(18): 5532–5546.

Willerslev E, Davison J, Moora M, et al. (2014) Fifty thousand years of Arctic vegetation and megafaunal diet. *Nature* 506(7486): 47–51.

Yang H, Sun J, Xu C, et al. (2019) Hoof pressure and trampling intensity of yaks are higher than those of Tibetan sheep in a Tianzhu alpine meadow. *Rangeland Journal* 41(2). CSIRO PUBLISHING: 125–133.

Zernig K and Berg C (2018) Durch das Winterleitenkar: Floristische Kleinode der Seetaler Alpen.

Tuexenia Beiheft 11: 171–188.

Zu K, Wang Z, Zhu X, et al. (2021) Upward shift and elevational range contractions of subtropical mountain plants in response to climate change. *The Science of the total environment* 783: 146896.

Zukrigl K (1975) Zur Geschichte der Hochlagenwälder in den Seetaler Alpen (Steiermark) Eine pollenanalytische Untersuchung des kleinen Moores im Winterleitenkessel. *Centralblatt fur das gesamte Forstwesen* 92: 175–188.