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# Pollen, macrofossils and *seda*DNA reveal climate and land use impacts on Holocene mountain vegetation of the Lepontine Alps, Italy

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ABSTRACT

Both climate change and anthropogenic disturbance affect vegetation composition, but it is difficult to separate these drivers of vegetation change from one another. A better understanding of past vegetation dynamics is necessary to disentangle the influence of different forcing factors and assess future vegetation change. Here we present the first multi-proxy palaeoecological study combining sedimentary ancient DNA (*seda*DNA), pollen, spores, stomata, charcoal and plant macrofossils from the Alps. We reconstructed the Holocene vegetation dynamics and fire history at Lago Inferiore del Sangiatto (1980 m asl), a small lake in the subalpine belt of the Ossola region, Italian Lepontine Alps. Afforestation in response to climate warming started at 10,700 cal yr BP with *Larix decidua* and tree *Betula*, which formed open forests together with *Pinus cembra* from 10,500 cal yr BP onwards. Human impact on the regional vegetation started at 5100 cal yr BP, resulting in expansions of *Picea abies* and *Alnus viridis* and the collapse of *Abies alba*. Species response models and ordination analysis show that livestock grazing and fire were major drivers of vegetation change at Lago Inferiore del Sangiatto during the late Holocene. Finally, increasing human impact during the Bronze (ca. 4200–2900 cal yr BP) and Iron Age (ca. 2900–2000 cal yr BP) led to the formation of species-rich larch meadows and alpine pastures that are still dominant today. The palaeoecological data suggest that under projected climate change and land abandonment, the treeline ecotone will likely shift to higher altitudes, leading to important changes in species composition and increasing the risk of biodiversity loss.

## 1. Introduction

Climate change has a large impact on ecosystems; the ongoing rise in temperatures will likely lead to changing habitats, local species extinctions, shifts in species distributions and biodiversity losses (Kovats et al., 2014; European Environment Agency, 2017; IPCC, 2021). The treeline ecotone, i.e. the transition between closed forests (timberline) to open parkland (treeline; uppermost limit of trees >3 m) and open alpine meadows, is especially sensitive to climate change due to thermal limitations of tree growth (Körner, 2012). Today's treeline as well as the distribution ranges of many plants in the Alps, are already shifting upwards in response to climate warming (Leonelli et al., 2011; Gottfried et al., 2012; Steinbauer et al., 2018). These range shifts will lead to significant habitat losses and potential extinction of present-day alpine species (Dirnböck et al., 2011).

Climate, however, is not the only factor affecting ecosystem dynamics in the Alps; the vegetation composition and the altitude of the treeline ecotone are strongly influenced by human activity. There is evidence that humans have changed the landscape and vegetation for thousands of years (e.g. Rey et al., 2013; Schwörer et al., 2015; Vescovi et al., 2018). Fire and livestock grazing at the treeline ecotone lowered timberline 300 m below its natural elevation from the Bronze Age onwards (Tinner and Theurillat,

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2003), whereas treeline was less affected. Anthropogenic disturbance does not only modify the location of the treeline ecotone, but also changes the composition of the vegetation. An increase in fire frequency and grazing activities led to the decline of disturbance-sensitive species like *Abies alba*, and/or to an increase of disturbance-tolerant species like *Alnus viridis* (Conedera et al., 2017). It is therefore clear that both climate change and anthropogenic disturbance can have a large impact on the vegetation. However, it is difficult to separate these two drivers of vegetation change from one another. To predict future vegetation change, we need to better understand and quantify the relative impacts that climate change and anthropogenic disturbance have on the environment.

Here, we present the first palaeoecological reconstruction of the vegetation history in the Ossola region (Lepontine Alps), Italy. This region was an important corridor linking Last Glacial Maximum plant refugia in the Po Plain and in the south-eastern Alpine forelands with the Central Alps (Vescovi et al., 2007; Birks and Willis, 2008). The region also played an important role in human history; its many mountain passes allowed people to cross the Alps connecting southern and central Europe (Curdy et al., 2010; Rizzi, 2014). For instance, the Albrun Pass (6 km distance to Lago Sangiatto) has a long history of human activity, as shown by archaeological finds dating from the Mesolithic onwards (Curdy et al., 2010). Despite its ecological and historical importance (Rizzi, 2014; Berruti et al., 2016), little palaeoecological research has been done in the valleys of Ossola. This is in strong contrast to the neighbouring areas of Valais and Ticino, which have been extensively studied (e.g. Zoller and Kleiber, 1971; Tinner et al., 1999; Tinner and Theurillat, 2003; Vescovi et al., 2018).

The location of Lago Inferiore del Sangiatto (1980 m asl) in the subalpine belt, just below the current treeline ecotone, is especially well suited for assessing past treeline and timberline dynamics. We studied pollen, stomata, dung fungal and fern spores, sedimentary ancient DNA (*seda*DNA), plant macrofossils and charcoal preserved in the sediments of Lago Inferiore del Sangiatto. Based on the interpretation of these different proxies we aim to (1) reconstruct the vegetation and fire history of the last 12,000 years; (2) infer vegetation dynamics in response to past climate change and disturbance; and (3) assess the impact of land use on the vegetation and biodiversity in the subalpine belt of the Lepontine Alps.

## 2. Study site

Lago Inferiore del Sangiatto (Lago Sangiatto) is located in the subalpine belt of the Lepontine Alps at 1980 m asl (Italy; 46°19′09″N, 8°17′13″E; Fig. 1). The lake is small (1 ha surface area; 3 m maximum water depth), with one minor surface inflow and no surface outflow. It is of glacial origin and was likely formed by a moraine. Geologically, the area around the lake belongs to the Lebendun nappe and mainly consists of calcareous schists and mica-schists (Piana et al., 2017). The present climate is subalpine

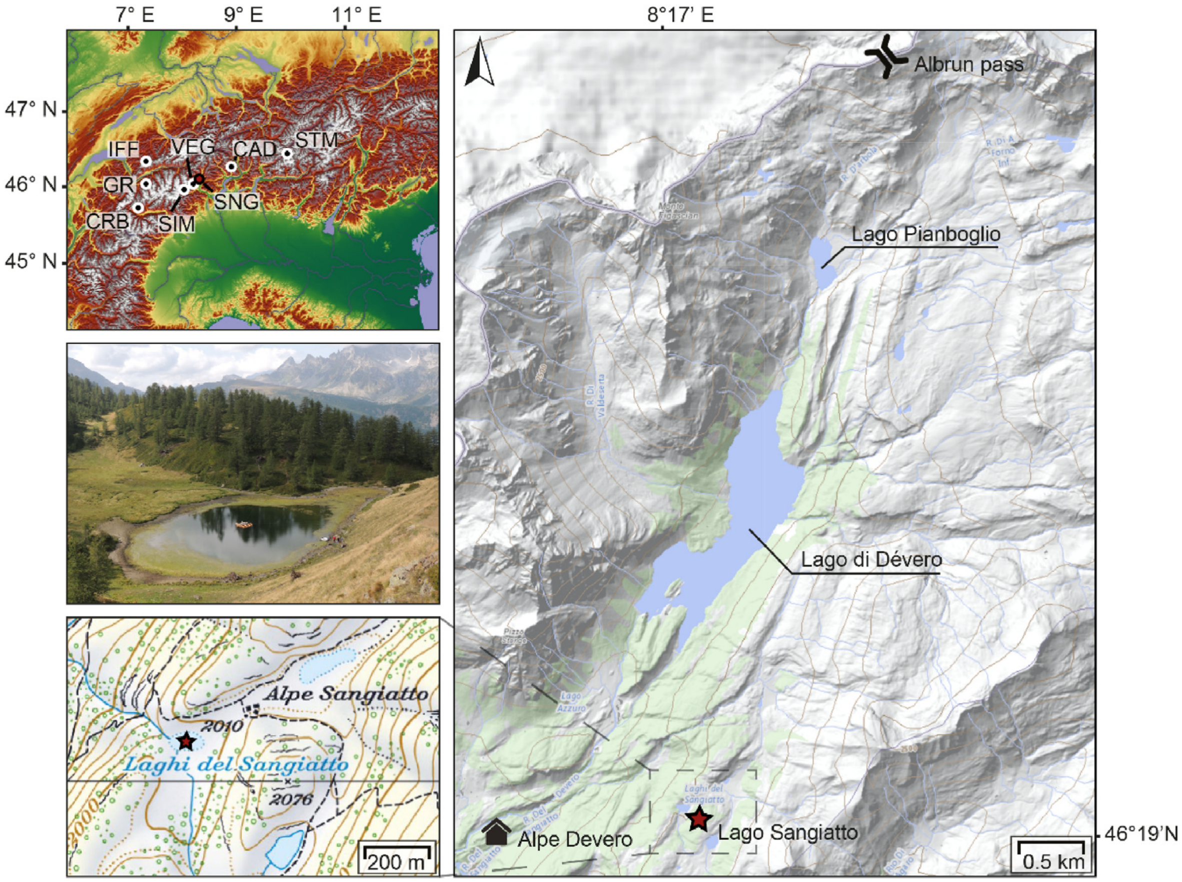


**Fig. 1.** Overview of the study site and its surroundings. Top left: Map overview with palaeoecological sites mentioned in the text (Perconte, 2006). SNG = Lago Sangiatto, VEG = Alpe Veglia, CAD = Lago Cadagno, SIM = Simplon-Hobschensee, IFF = Iffigsee, STM = St. Moritz/Upper Engadin, CRB = Crotte Basse, GR = Gouillé Rion. Middle left: Photograph of Lago Inferiore del Sangiatto (C. Morales-Molino). Bottom left: Map of the study site. Star = coring site, 46°19′09″N, 8°17′13″E (© Swisstopo). Right: Map of the study area with Lago Inferiore del Sangiatto (red star), Albrun mountain pass and the settlement of Alpe Devero (Arpa Piemonte, 2016). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Insubrian, with a mean annual temperature of ca. 2.0 °C and 1530 mm of annual precipitation. The mean temperature of the coldest (January) and warmest month (July) are ca. −6.0 °C and 11 °C respectively (adjusted to the elevation of Lago Sangiatto with a lapse rate of −6 °C/1000 m of altitude from the nearby meteorological station at Alpe Devero for 1991–2020; Arpa Piemonte, 2022). The lake is situated 200 m above the valley floor of Alpe Devero, on a southwest-exposed mountain slope. The present vegetation around Lago Sangiatto consists of open conifer forests and larch-meadows dominated by *Larix decidua*, with single *Pinus cembra* trees, dwarf shrubs (*Juniperus communis, Rhododendron ferrugineum, Vaccinium* spp.) and alpine meadows used as pastures. The treeline is located at ca. 2250 m asl and the timberline at ca. 2050 m asl, placing Lago Sangiatto just below the treeline ecotone.

## 3. Methods

### 3.1. Coring

In August 2018, we took four sediment cores (A–D) from the deepest part of Lago Sangiatto (3 m water depth) by using a modified Streif-Livingston piston corer with a diameter of 8 or 5 cm (Merkt and Streif, 1970). The distance between the cores was 0.5 m. Additionally, we took a surface core of the upper 60 cm with a UWITEC gravity corer. All cores were correlated visually using litho-stratigraphic markers and the cores A and B were combined into one master core totalling 4.23 m. The master core was studied at the Institute of Plant Sciences (IPS) of the University of Bern and used for the age-depth model, and the palynological, macrofossil and charcoal analyses. Cores C and D were studied at The Arctic University Museum of Norway (UiT) and used for the *sed*aDNA analysis. All cores were stored at 4 °C.

### 3.2. Radiocarbon dating

During the macrofossil analysis, 15 samples of terrestrial plant macrofossils (Table 1) were selected for dating with accelerator-mass spectrometry (AMS) in the Laboratory for the Analysis of Radiocarbon with AMS (LARA) at the University of Bern. We used the program Bacon (Blaauw and Christen, 2011), with the IntCal20 calibration curve (Reimer et al., 2020), to calibrate the radiocarbon dates to years before present (cal yr BP) and to construct the age-depth model (Fig. 2). An additional 8 samples from the C and D cores were radiocarbon dated at the Poznan Radiocarbon Laboratory (Table 2, Fig. 2) to confirm our litho-stratigraphic correlation with the master core but were not used in the final age-depth model.

### 3.3. Pollen, stomata and charcoal analyses

For the pollen, stomata and microscopic charcoal analyses, we took a total of 64 samples of 1 $cm^3$ volume. Generally, we sub-sampled every 8 cm, except between 0–160 cm and 368–416 cm depth where we sub-sampled every 16 cm, and between 312 and 352 cm depth where we sub-sampled every 2 cm to account for changes in sediment accumulation. We prepared the samples following standard procedure with HCl, HF, KOH and acetolysis (Moore et al., 1991). We added a known number of *Lycopodium* spores to the samples before chemical preparation to estimate concentration and influx values (Stockmarr, 1971). The samples were mounted in glycerine and analysed under a light microscope at 400 × or 1000 × magnification. Pollen, spores, stomata and other non-pollen palynomorphs (NPP) were identified using different keys (Trautmann, 1953; Moore et al., 1991; Beug, 2004) and the reference collection at the IPS. Generally, the samples have sums >500, excluding pollen of aquatic plants and spores. Pollen percentages can be used to infer forest and timberline conditions, with values of 70–80% of arboreal pollen (AP, includes tree and shrub taxa) considered typical for timberline (Tinner and Theurillat, 2003). Microscopic charcoal (>10 μm) in pollen slides was analysed by following Tinner and Hu (2003) and Finsinger and Tinner (2005) to reconstruct regional fire activity (Whitlock and Larsen, 2002; Conedera et al., 2009). We subdivided the pollen diagram into local pollen assemblage zones (LPAZ), by using the zonation method of optimal sum of squares partitioning (Birks and Gordon, 1985) and by identifying statistically significant zones with the broken-stick method (Bennett, 1996). Zone SNG 2 was subdivided into two non-significant subzones (SNG-2a/b) to highlight changes in the vegetation composition at 5100 cal yr BP.

### 3.4. Plant macrofossil and macroscopic charcoal analysis

For the macrofossil analysis, we took a total of 94 samples. Most of the samples form a continuous section from 294 to 369 cm and 411–423 cm depth, the others were taken at regular intervals through the core. The samples have a thickness of 1, 2 or 4 cm, with a volume between 4 and 25 $cm^3$. They were soaked overnight in KOH and sieved with a mesh size of 200 μm. The plant macrofossils were analysed under a stereo microscope and identified using macrofossil keys (e.g. Cappers et al., 2006) and the reference

**Table 1**
Radiocarbon dates from the master core of the Lago Inferiore del Sangiatto sediment record. N: needles, BS: bud scales, S: seeds, L: leaves.

| Depth (cm) | Lab. code | Material | $^{14}$C-Age (yr BP) | Median age (cal yr BP) | Calibrated age (cal yr BP, 2σ-range) | Modelled age (cal yr BP) in diagrams |
|---|---|---|---|---|---|---|
| 101–102 | BE-10652.1.1 | *Larix decidua* N | 670 ± 20 | 643 | 590–670 | 648 |
| 139–141 | BE-9747.1.1 | *Larix decidua* N, *Picea abies* N | 1305 ± 20 | 1221 | 1225–1290 | 1224 |
| 217–219 | BE-9748.1.1 | *Larix decidua* N, coniferous cone | 2330 ± 20 | 2346 | 2330–2360 | 2359 |
| 309–310 | BE-10653.1.1 | *Larix decidua* N, *Picea abies* N, *Pinus cembra* N | 3700 ± 20 | 4035 | 3976–4144 | 4081 |
| 317–319 | BE-9749.1.1 | *Larix decidua* N, *Picea abies* N, *Pinus cembra* N, seeds indet., BS | 4095 ± 20 | 4592 | 4455–4800 | 4583 |
| 325–326 | BE-10654.1.1 | *Larix decidua* N, *Picea abies* N, cone | 4550 ± 20 | 5168 | 5056–5318 | 5177 |
| 331–332 | BE-12194.1.1 | *Larix decidua* N, *Pinus cembra* N, *Abies alba* N | 5805 ± 25 | 6611 | 6499–6672 | 6564 |
| 335–336 | BE-12195.1.1 | *Abies alba* S, *Larix decidua* N | 6470 ± 25 | 7368 | 7324–7429 | 7340 |
| 341–343 | BE-9750.1.1 | *Larix decidua* N, *Pinus cembra* N, short shoot | 7320 ± 25 | 8102 | 8032–8180 | 7959 |
| 359–361 | BE-9751.1.1 | Bark, *Pinus* sp. S | 9240 ± 25 | 10,408 | 10,283–10506 | 10,393 |
| 365–367 | BE-10655.1.1 | Twig (deciduous) | 9710 ± 50 | 11,141 | 10,809–11240 | 10,765 |
| 385–387 | BE-12196.1.1 | *Dryas octopetala* L | 9670 ± 30 | 11,115 | 10,812–11198 | 11,139 |
| 391–393 | BE-12417.1.1 | *Salix* sp. L | 9930 ± 60 | 11,362 | 11,220–11688 | 11,295 |
| 415–417 | BE-10656.1.1 | *Draba* cf. L, *Thymus* cf. L, coniferous BS | 10,240 ± 90 | 11,969 | 11,509–12473 | 11,822 |
| 421-423[a] | BE-9752.1.1 | *Draba* cf. L, *Thymus* cf. L, BS, *Saxifraga* S, Poaceae S | 5660 ± 90 | Rejected | Rejected | Rejected |

[a] Rejected, as too young.

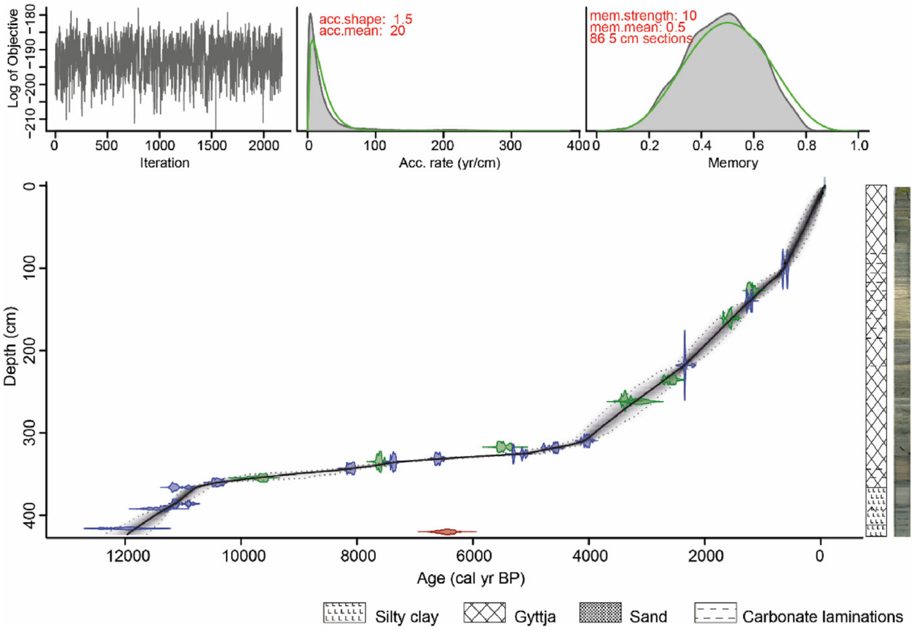


**Fig. 2.** Age-depth model and lithology of the sediment from Lago Inferiore del Sangiatto. The black line shows the single best model based on the mean age for each depth, the grey area shows the 95% confidence interval. Only dates from the master core (blue probability distributions) were used to construct the model, dates from the C/D cores (green probability distributions) were only used to check the correlation. One date was rejected for falling well outside the expected age range (red date). Top left: Number of Markov Chain Monte Carlo (MCMC) iterations used to generate the grey-scale graphs. Top middle: Prior (green) and posterior (grey) distributions of the sediment accumulation rates. Top right: Prior (green) and posterior (grey) distributions of memory (dependence of the sediment accumulation rate between neighbouring depths). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

**Table 2**
Radiocarbon dates from the C and D cores of the Lago Inferiore del Sangiatto sediment record.

| Depth (cm) | Lab. code | Material | $^{14}$C-Age (yr BP) | Median age (cal yr BP) | Calibrated age (cal yr BP, 2σ-range) |
|---|---|---|---|---|---|
| 127.5 | Poz-111,434 | Rootlet | 1260 ± 30 | 1217 | 1077–1282 |
| 161 | Poz-123,994 | Needles | 1675 ± 30 | 1566 | 1421–1695 |
| 235.5 | Poz-123,995 | Rootlet, needles, moss | 2500 ± 30 | 2585 | 2469–2727 |
| 261 | Poz-111,433 | Wood, branch, needles, moss | 3150 ± 35 | 3377 | 3257–3451 |
| 262 | Poz-123,993 | Wood, needles | 2970 ± 80 | 3137 | 2890–3362 |
| 317 | Poz-123,915 | Rootlet | 4750 ± 50 | 5494 | 5326–5587 |
| 335 | Poz-111,120 | Wood | 6750 ± 40 | 7609 | 7520–7672 |
| 354.5 | Poz-111,509 | Wood | 8690 ± 60 | 9653 | 9539–9891 |

collection at the IPS. Generally, single findings of plant macrofossils indicate the local presence of a taxon (Tinner and Theurillat, 2003). We interpret the presence of single needles as treeline conditions and the presence of multiple needles as timberline conditions (Tinner and Theurillat, 2003; Schwörer et al., 2014). Macroscopic charcoal particles (>200 μm) were counted and their area was measured in mm$^2$. These charcoal particles mostly originate from local sources and can be used to reconstruct local fire activity (Whitlock and Larsen, 2002). The macrofossil concentrations were downscaled to the lowest volume of 4 cm$^3$ for the macrofossil diagram. To compare the results, we used the pollen zonation (LPAZ) to subdivide the diagram.

### 3.5. SedaDNA analysis

We followed the protocols of Alsos et al. (2021) for DNA extraction, PCR amplification and further sequencing. We extracted DNA from 34 sediment samples taken at 11 cm intervals using a DNeasy PowerSoil kit (Qiagen, Germany) in the ancient DNA laboratory at the Arctic University of Tromsø (UiT). DNA extracts and negative extraction/sampling controls, along with one positive PCR control, were amplified using uniquely dual-tagged generic primer sets (Supplementary Table A1) that amplify the *trn*L P6 loop region of the chloroplast genome (Taberlet et al., 2007). PCR reaction and cycling conditions followed Voldstad et al. (2020). Each sample and control underwent eight PCR replicates; these were pooled, cleaned and converted into one DNA library using a modified TruSeq PCR-free library kit (Illumina) with unique dual indexing (Rijal et al., 2021). The library was quantified with qPCR using the Library Quantification Kit for Illumina sequencing platforms (KAPA Biosystems, Boston, USA), using a Prism 7500 Real-Time PCR System (Life Technologies, The Norwegian College of Fishery Science, UiT), and normalised to a working concentration of 10 nM. The library was sequenced on an Illumina NextSeq platform (2 × 250 bp, mid-output mode) at the Genomics Support Centre Tromsø (UiT). In total, 34 samples, 2 sampling negatives, 2 extraction negatives, 3 PCR negatives and 1 PCR positive were analysed.

We used OBITools (Boyer et al., 2016) for the bioinformatics pipeline following the protocol and criteria defined by Rijal et al. (2021). Paired-end reads were aligned using SeqPrep (https://github.com/jstjohn/SeqPrep/releases, v1.2). Identical sequences were collapsed with *obiuniq*, and sequences shorter than 10 base pairs and singletons were removed. To correct for PCR and sequencing errors, we used *obiclean*. Taxonomic assignment was done with *ecotag* by matching them against four different reference libraries: (1) PhyloAlps library (Garcés-Pastor et al., in press); (2) ArctBorBryo (regional arctic/boreal reference library compiled from Sønstebø et al., 2010; Willerslev et al., 2014; Soininen et al., 2015); (3) PhyloNorway (Alsos et al., 2020a); and (4) a global reference library based on the EMBL rl143 database. We only kept sequences with a 100% match to the reference library, prioritising PhyloAlps, represented by three reads per replicate and with a minimum of 10 total reads and three replicates across the entire dataset. We also removed sequences that displayed higher average read counts in the negative extraction PCR controls than in the sediment samples (e.g. *Picea abies*). Taxonomy was compared and corrected against Flora Helvetica (Lauber et al., 2018). The results from the *sed*aDNA analysis are displayed as either wtRep (defined as the number of reads detected for a taxon divided by all the reads detected in a sample; Rijal et al., 2021) or as the proportion of PCR replicates (number of replicates the taxon was detected in divided by the total number of replicates). By using wtRep, we account for differences in relative counts of retained reads across PCR replicates (Rijal et al., 2021). To compare the results, we used the pollen zonation (LPAZ) to subdivide the *sed*aDNA record.

### 3.6. Numerical methods

#### 3.6.1. Ordination analysis

To infer environmental gradients in the pollen data, we used a principal component analysis (PCA) performed in Canoco 5 (ter Braak and Šmilauer, 2012). Before the PCA we square-root transformed the percentage data to reduce the effects of dominant species. Microscopic charcoal influx (as a proxy for fire) and *Sporormiella* influx (as a proxy for grazing) were added passively to the analysis as supplementary variables. Additionally, we added the isolines of the rarefied number of pollen types to the PCA plot to study the relationship between the different environmental gradients and palynological richness. We also calculated a PCA for the *sed*aDNA wtRep of tree, shrub and herb taxa using log transformed wtRep data (Supplementary Figure A1).

### 3.7. Species response curves

To examine the response of different subalpine species to disturbance, we assessed the relationship between pollen abundance (as a proxy for plant abundance), microscopic charcoal influx (as a proxy for fire) and *Sporormiella* influx (as a proxy for grazing) by fitting pollen percentage data with multiple regression models (Colombaroli et al., 2010; Birks et al., 2012). Influxes were used for the explanatory variables to avoid proportional distortion problems and thus spurious correlations. We used Generalised Additive Models (GAM; Hastie and Tibshirani, 1990) to depict the responses of *Pinus cembra*, *Larix decidua*, *Picea abies*, *Abies alba* and *Alnus viridis* to the impacts of fire and grazing. The models were created by using Canoco 5 (ter Braak and Šmilauer, 2012), with a maximum of two degrees of freedom and a Gaussian distribution of the data. We chose the best fitting model that was alternative to the null model by using a stepwise selection based on the degrees of freedom and the AIC value (Akaike Information Criterion).

#### 3.7.1. Biodiversity analysis

Species diversity is a complex function of species richness and species evenness (Legendre and Legendre, 2012). To investigate biodiversity trends in the past, we calculated the Hill numbers $N0$ (also referred to as palynological richness index; PRI) and $N2$ (Hill, 1973) for both the pollen and *sed*aDNA records. Hill's $N0$ represents the total number of taxa in a sample and is a proxy for species richness, whereas Hill's $N2$ is an estimate of the number of dominant taxa in a sample and is related to diversity and evenness (Legendre and Legendre, 2012; Felde et al., 2016). To account for differences in sample size (i.e. differences in pollen sums or *sed*aDNA reads) we used a rarefaction analysis (Birks and Line, 1992; Clarke et al., 2019; Rijal et al., 2021). The rarefaction analysis was performed with a minimum pollen sum of 473 (excluding aquatic taxa and ferns) and a minimum *sed*aDNA read count of 12,605 (excluding aquatic taxa and ferns) by using the Vegan package in R (Version 4.1.1; Oksanen et al., 2019). In addition, we also calculated the probability of interspecific encounter (PIE; Hurlbert, 1971) for the pollen record. PIE has been used here as a proxy for evenness to estimate to what extent the total number of taxa in a sample is influenced by dominant taxa (e.g. Lepori et al., 2005; Lestienne et al., 2020; Tolmos et al., 2022). However, it is important to note that no diversity index can completely disentangle richness from evenness as the two concepts are intimately related (Gotteli and Ellison, 2013).

## 4. Results and interpretation

### 4.1. Lithology and chronology

The sediments (Fig. 2) at the bottom of the core (365–423 cm depth) consist of silty clay, with a coarse sand layer between 411 and 409 cm depth. From 365 cm depth upwards, the composition of the sediment changes to gyttja. There are three sections in the gyttja with carbonate laminations (78–185 cm, 221–246 cm and 335–356 cm depth), whereas the rest of the gyttja regularly contains silt.

We rejected one radiocarbon date from the bottom of the core (sample BE-9752.1.1), as it is clearly too young based on the other dates and the biostratigraphy. The age-depth model (Fig. 2) suggests a likely age of 12,000 cal yr BP for the oldest sediments of Lago Sangiatto. Because of a plateau in the radiocarbon calibration curve during the Younger Dryas (ca. 12,700–11,700 cal yr BP), this date has large uncertainties (Table 1) and could be younger or older than depicted in our model. The age-depth model suggests three major changes in the sedimentation rates. The oldest part of the core (ca. 12,000–10,800 cal yr BP, 423–365 cm depth) is characterised by a high sedimentation rate (0.05 cm $yr^{-1}$) and consists of silty clay (Fig. 2), suggesting bare soils, high erosion rates, or inflow of glacial meltwater. From 10,800–5100 cal yr BP (365–325 cm depth), the sedimentation rate is constant and extremely low (0.007 cm $yr^{-1}$), indicating little allochthonous input. From 5100 to 4050 cal yr BP (325–308 cm depth), the sedimentation rate (0.05–0.1 cm $yr^{-1}$) increases and remains high to the present, pointing to enhanced erosion in the catchment.

### 4.2. Pollen, macrofossil, sedaDNA and charcoal analyses

As a result of different sampling strategies and conspicuous changes in sediment accumulation rates, the pollen, plant macrofossil and *sed*aDNA records have divergent sample resolutions (Figs. 3–6). The analyses of our different proxies resulted in a total of 153 pollen taxa, 46 plant macrofossil taxa and 273 *sed*aDNA taxa; all identified to different taxonomic levels (Table 3). For the *sed*aDNA record, a total of 15,150,672 merged paired-end reads were obtained. After filtering and cross-checking, we retained 5,827,071 reads, belonging to 273 sequences of vascular plants. The pollen record (Fig. 3) is largely dominated by wind-pollinated coniferous species (e.g. *Pinus cembra*, *Abies alba*), disturbance-adapted shrubs (*Alnus viridis*) and grasses (Poaceae). The macrofossil and *sed*aDNA records (Figs. 4, 5 and 9) are dominated by *Larix decidua*, a coniferous tree that sheds needles every year. Additionally, the *sed*aDNA record shows a high variety of forbs, grasses and other herbaceous taxa.

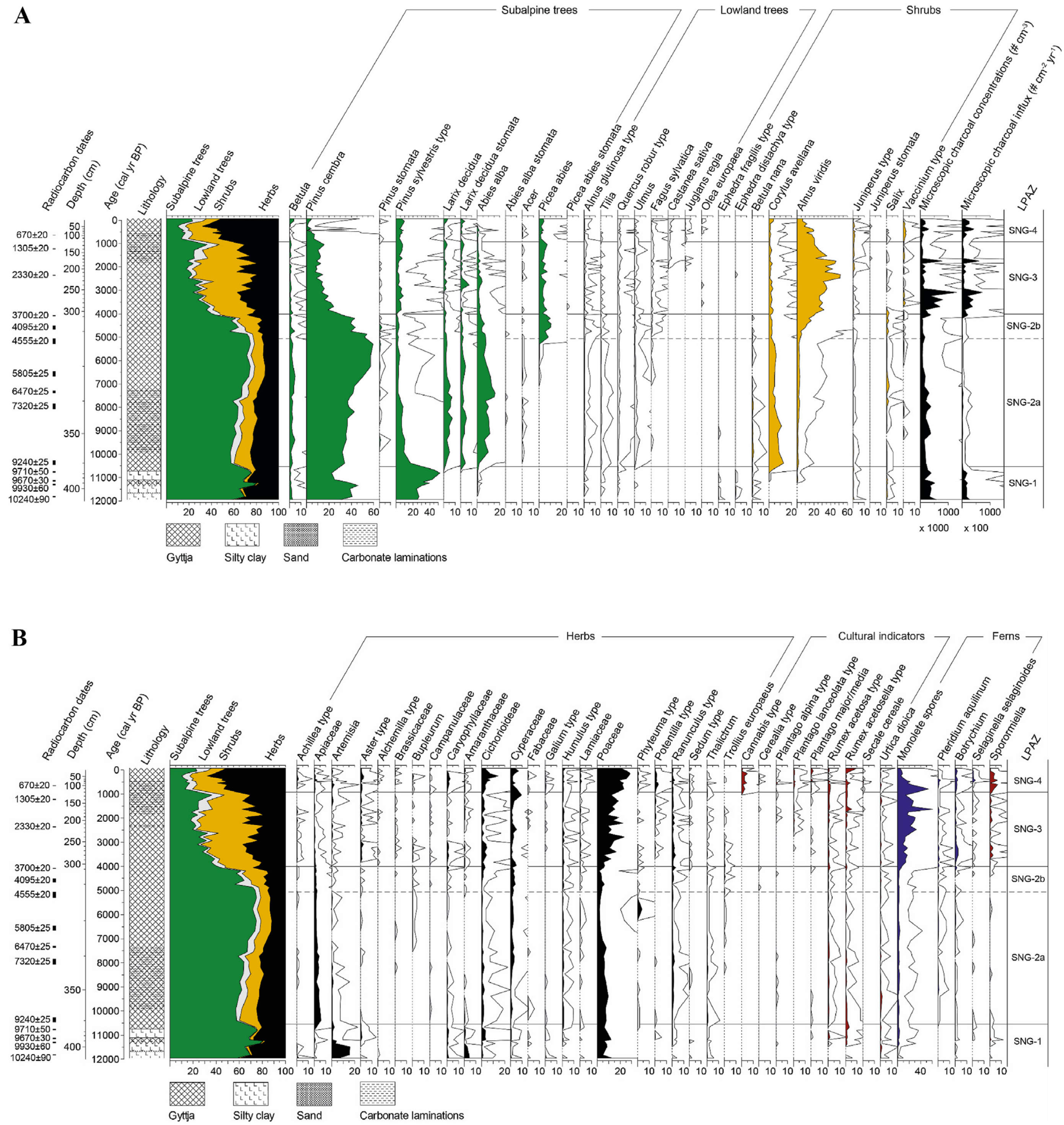


**Fig. 3.** a: Selected pollen and stomata percentages of the arboreal vegetation at Lago Inferiore del Sangiatto based on the total terrestrial pollen sum, together with $^{14}$C dates, lithology and microscopic charcoal concentrations and influx values. Empty curves show 10x exaggeration. *LPAZ* correspond with statistically significant local pollen assemblage zones 1–4. b: Selected pollen and spores of the non-arboreal vegetation.

#### *4.2.1. SNG-1 (12,000-10,550 cal yr BP)*

This zone is dominated by *Pinus* and herb pollen (Poaceae, *Artemisia* and Amaranthaceae, Fig. 3). Subalpine tree pollen is around 70%, but is likely from long-distance transport, as tree *sed*aDNA proportions are low (Fig. 5). The macrofossil and *sed*aDNA records consist of dwarf shrubs like *Dryas octopetala, Salix retusa, S. serpillifolia* and various herbs generally found in rocky habitats or in alpine meadows, such as *Astragalus australis, Bartsia alpina, Campanula thyrsoides* and *Rumex scutatus* (Figs. 4 and 5). All our proxies indicate a diverse alpine tundra and unforested conditions around Lago Sangiatto. Low concentrations of coniferous bud scales at ~12,000 cal yr BP and 11,300–11,000 cal yr BP, the first *Betula* (tree birch) fruit at ~11,300 cal yr BP and low amounts of *Pinus* and *Larix decidua sed*aDNA suggest that trees were growing close to the

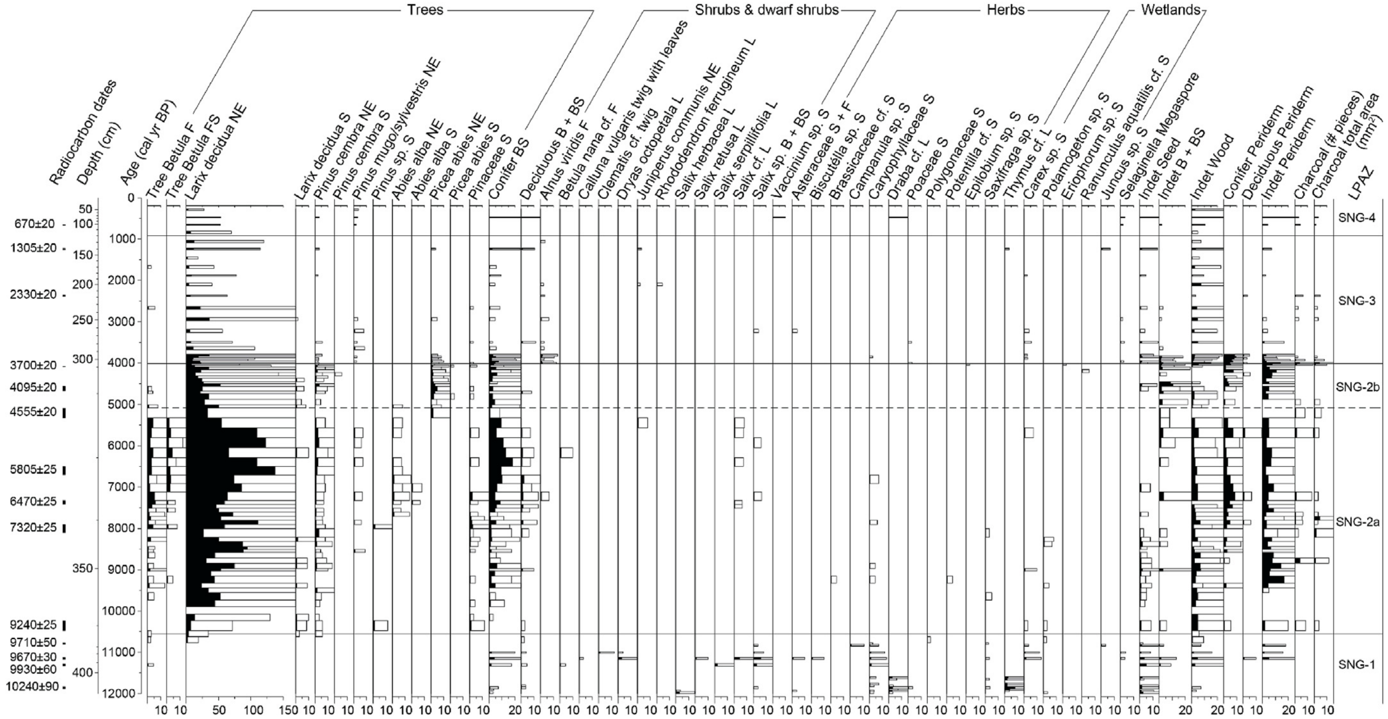


**Fig. 4.** Plant macrofossil concentrations diagram of Lago Inferiore del Sangiatto for selected taxa. All the volumes are standardised to 4 cm$^3$ of sediment. *B* buds, *BS* bud scales, *F* fruit, *FS* fruit scale, *S* seed, *NE* needle equivalent, *L* leaf, *LPAZ* correspond with local pollen assemblage zones. Empty bars show 10x exaggeration.

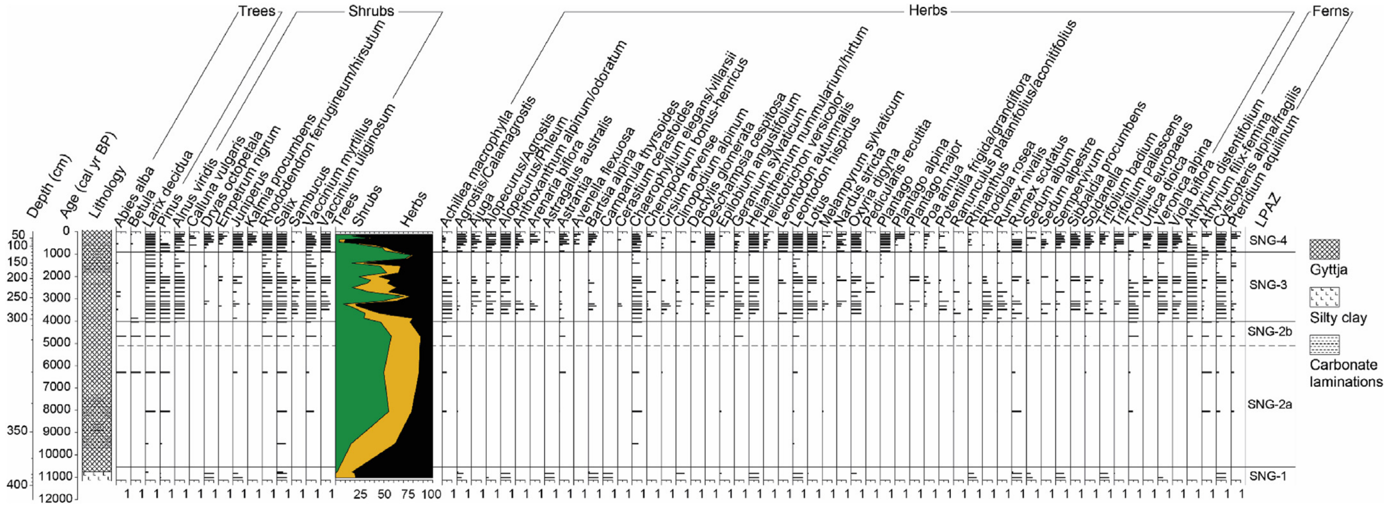


**Fig. 5.** Sedimentary ancient DNA (*sed*aDNA) diagram from Lago Inferiore del Sangiatto for selected taxa only. Individual *sed*aDNA bars are presented as a proportion out of 8 PCR repeats scaled to 1. The overview diagram presents the *sed*aDNA as a percentage of the total reads of tree, shrub and herb taxa. *LPAZ* correspond with local pollen assemblage zones.

site (Fig. 6). Microscopic charcoal influx values are relatively high in this zone, indicating rather high regional fire activity. The absence of macroscopic charcoal, however, suggests that local fires did not occur around Lago Sangiatto.

Towards the end of this zone (~10,700 cal yr BP), the first appearance of *Larix decidua* pollen, stomata and needles together with tree *Betula* fruits (likely *B. pendula*) indicate the establishment of open birch-larch stands around Lago Sangiatto. The decrease in sedimentation rate and the lithologic change from silty clay to gyttja at the same time suggests that the establishment of trees, stabilised soils and limited erosion.

### 4.2.2. SNG-2a (10,550–5100 cal yr BP)

At the start of this zone, the first presence of *Pinus cembra* needles indicates that *P. cembra* expanded locally around Lago Sangiatto. High concentrations of *Larix decidua* needles together with *P. cembra* and tree *Betula* macrofossils and *Betula sed*aDNA, suggest that the lake was surrounded by a larch-dominated forest with stone pine and birch stands. The high percentages of subalpine tree pollen (~70%) and the stable reads of tree *sed*aDNA (~50%) indicate closed forest conditions near the lake. The relatively high pollen percentages of Apiaceae (~5%) and Poaceae (~8%), combined with the presence of taxa like *Achillea macrophylla* and

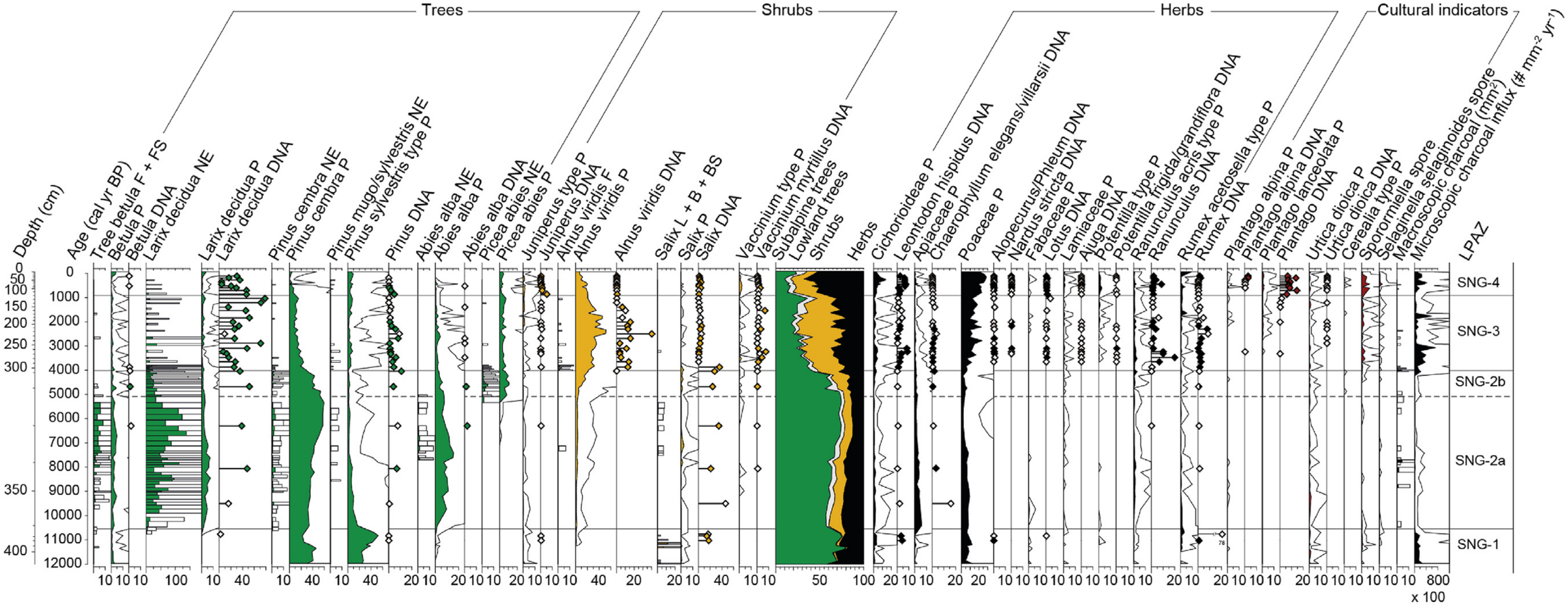


**Fig. 6.** Combination diagram of selected pollen percentages (P, exaggeration x10), *seda*DNA reads percentages and replicates (DNA, bars = percentage of reads, coloured diamonds = DNA present in all 8 replicates, open diamonds = DNA present in less than 8 replicates), microscopic charcoal influx (exaggeration x10), macrofossil- and macroscopic charcoal concentrations (NE = needle equivalent, F = fruit, FS = fruit scale, exaggeration x10). *LPAZ* correspond with statistically significant local pollen assemblage zones 1–4.

**Table 3**
Total number of pollen, *sed*aDNA and macrofossil taxa present, with their taxonomic resolution, for the Lago Inferiore del Sangiatto sediment record.

| | Pollen | | | | *sed*aDNA | | | | Macrofossils | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Family | Genus | Subgenus | Species | Family | Genus | Subgenus | Species | Family | Genus | Subgenus | Species |
| Trees | 1 | 8 | 6 | 11 | 2 | 6 | | 4 | 1 | 2 | 2 | 4 |
| Shrubs | 2 | 8 | 2 | 6 | 3 | 5 | 1 | 10 | 1 | 4 | | 9 |
| Herbs | 29 | 38 | 10 | 16 | 29 | 82 | 28 | 83 | 5 | 13 | | 2 |
| Ferns | | 5 | | 6 | | 5 | 3 | 9 | | 1 | | |
| Aquatic taxa | 1 | 4 | | | | 1 | 1 | 1 | | 1 | | 1 |
| Total | 153 | | | | 273 | | | | 46 | | | |

*Chaerophyllum elegans/villarsii* in the *sed*aDNA record suggest an undergrowth of tall forbs and grasses. The first *Abies alba* stomata at about 9000 cal yr BP and the regular presence of *A. alba* needles after 8000 cal yr BP show the expansion of *A. alba* stands around the lake. The appearance of *Picea abies* pollen around 6700 cal yr BP points to the establishment of spruce stands in the region. Low microscopic charcoal concentrations and influx values indicate reduced regional fire activity. However, the presence of macroscopic charcoal in several samples does suggest occasional local fires in the catchment of Lago Sangiatto.

#### *4.2.3. SNG 2b (5100–4000 cal yr BP)*

The sharp increase in pollen percentages of *Picea abies* and continuous presence of its needles at the start of this zone indicate that the species expanded at about 5100 cal yr BP. Decreasing subalpine tree pollen (60–70%) and a decrease in *Larix decidua* and *Abies alba* needles suggest, that concurrent with the expansion of *P. abies,* other subalpine tree species declined, the forest became more open and its composition changed. At the same time, the rise of *Alnus viridis* pollen suggests its expansion in the region.

The first Cerealia type pollen appeared at 4900 cal yr BP, indicating human activity in the area. The rise of human indicators after 4300 cal yr BP, along with the regular occurrence of *Sporormiella* dung spores (a proxy for grazing; Cugny et al., 2010; Etienne et al., 2013), point to the beginning of high-elevation farming activities at the onset of the Bronze Age (4200–2900 cal yr BP). The small increase in sedimentation rates points to erosion in the catchment of Lago Sangiatto, likely caused by the opening of the forest. For most of this zone, regional and local fire activity was low. At about 5000 cal yr BP, a small peak in the macro- and microscopic charcoal suggests an increase in local and regional fire frequency. At the end of the zone (~4000 cal yr BP), several large peaks in the micro- and macroscopic charcoal record suggest increasing fire occurrence that continued into the next zone.

#### *4.2.4. SNG 3 (4000–900 cal yr BP)*

The appearance of *Alnus viridis* in the macrofossil and the *sed*aDNA record, together with a rapid increase in its pollen percentages, indicate local expansion of green alder thickets. Subalpine tree pollen percentages continue to decline (20–40%) from the previous zone, especially *Pinus cembra* pollen, which drops below 10%. This decline, together with a decline in the proportion of tree and shrub *sed*aDNA and strongly decreasing needle concentrations, suggest disruption of the remaining stone pine, larch and spruce stands and a marked deforestation of the catchment. At the same time, the pollen and *sed*aDNA records show an increase in the number of taxa and a high abundance of forbs, herbs and grasses, documenting the establishment of diverse alpine meadows around the site. The increase in *Sporormiella* dung fungal spores as well as the presence of *sed*aDNA from the apophytes *Cirsium arvense* and *Chenopodium bonus-henricus* point to alpine farming activities. Furthermore, the sharp increase in sedimentation rates suggests increased erosion, exposed bare soils and open landscape conditions. Maximum Holocene microscopic charcoal concentrations and influx values indicate peaking regional fire activity during this zone. Macroscopic charcoal particles suggest local fires at the onset of this zone (~4000 cal yr BP).

#### *4.2.5. SNG 4 (900 cal yr BP–today)*

Subalpine tree pollen percentages show minimum values (10–20%); *P. cembra* has particularly low values and *A. alba* disappears. Similarly, the tree macrofossil concentrations are low, pointing to an open landscape. Herbs and grasses dominate the pollen (>50%) and *sed*aDNA (>80%) record. Grazing indicators are at their maximum, with peaks in e.g. *Plantago major/media* pollen, *Plantago alpina* and *Urtica dioica sed*aDNA, and *Sporormiella* spores. The grazing indicators, together with the presence of many herbaceous species and the widespread deforestation, indicate a landscape of alpine meadows with scattered tree stands surrounding the lake. Small peaks in *Secale* and Cerealia type pollen suggest nearby cultivation of cereals. Although high-altitude cereal production in the subalpine belt has been historically documented in the Alps (Mathieu, 1992), we assume that most Cerealia pollen was transported from the valley or lower slopes and cereal crops did not grow around the lake. Peaks in the microscopic charcoal concentration and influx values, together with the presence of macroscopic charcoal particles, suggest that local and regional fire activities remained rather high.

During the last 50 years, the percentage of subalpine tree pollen has increased again, suggesting that the forest, and especially *L. decidua* as a pioneer, is expanding, leading to today's young open larch forests around the lake.

### *4.3. Ordination*

The biplot of the PCA (Fig. 7) shows the pollen samples clustered by LPAZ, in which even the non-significant zones SNG-2a/b are two separate clusters. Axis 1 explains 39.9% of the variance and likely reflects anthropogenic vegetation disturbance. The gradient shows the transformation from undisturbed subalpine forests, characterised by *Pinus cembra* and *Abies alba*, to a more anthropogenic vegetation, characterised by disturbance-adapted species like *Alnus viridis* and human indicators, such as *Cannabis* type and *Plantago lanceolata* type. Axis 2 explains 22.0% of the variance and shows a gradient from open, continental and light-demanding vegetation (*Pinus sylvestris* type, *Artemisia*) to closed, shade-tolerant or oceanic vegetation (*Picea abies, Abies alba*).

The passive plotting of *Sporormiella* and charcoal influx as explanatory variables into the PCA (Fig. 7 top) shows the correlation between grazing, fire and open anthropogenic landscapes, suggesting that most fires were of human origin. The added isolines of rarefied pollen types in the PCA plot (Fig. 7 bottom) show that the number of different pollen types increases as the landscape becomes more open and disturbed, with anthropogenic subalpine meadows having the highest biodiversity.

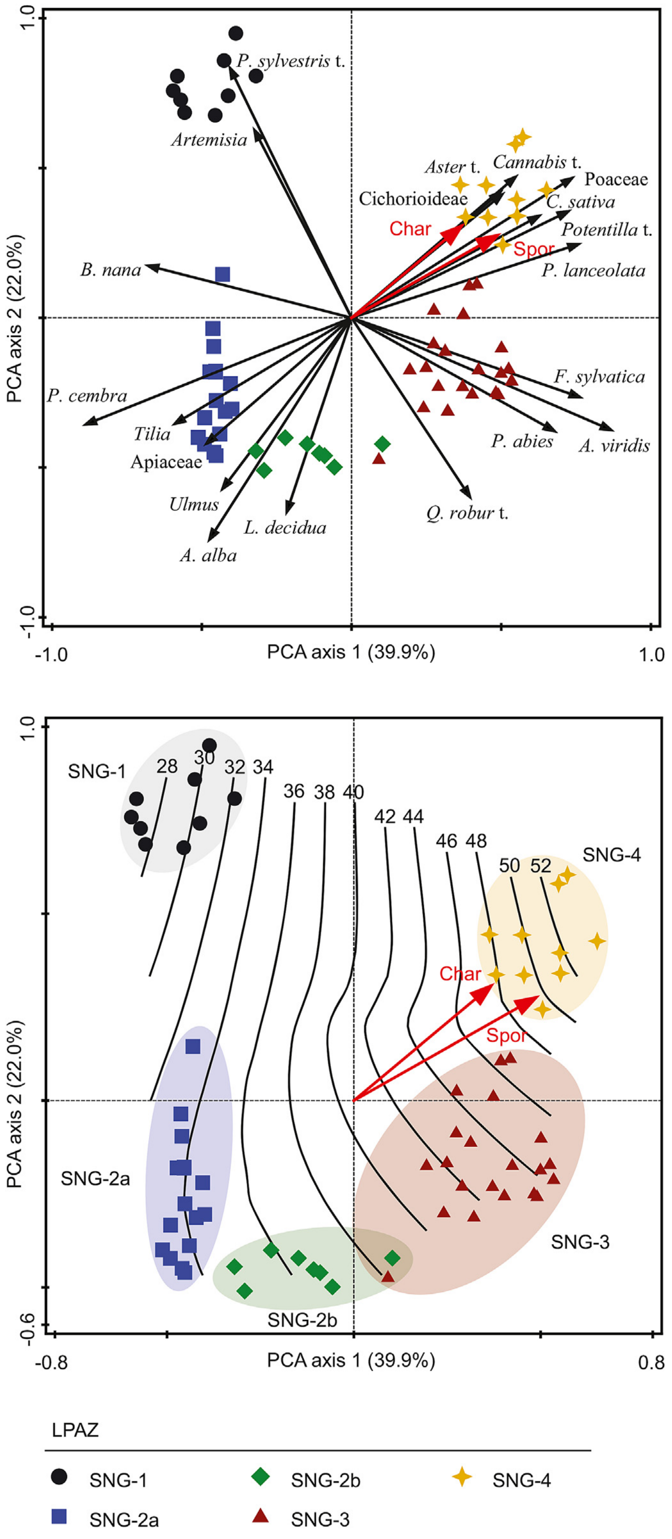


**Fig. 7.** Top: Principal component analysis (PCA) biplot showing both species (black arrows) and sample scores (symbols). Charcoal influx (Char) and *Sporormiella* influx (Spor) are supplementary explanatory variables and were projected onto the ordination (red arrows). The first axis explains 39.9% of the variance and the second axis 22.0%. Bottom: Principal component analysis (PCA) scatterplot showing sample scores with their respective local pollen assemblage zones (LPAZ) highlighted. The isolines represent the rarefied number of different pollen types found in the samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

### 4.4. Species response curves

GAM models (Fig. 8) show how different pollen taxa respond to changes in *Sporormiella* influx or microscopic charcoal influx as proxies for grazing and fire. The models for *Picea abies* are not depicted, as they are not statistically significant. *Pinus cembra, Abies alba* and *Larix decidua* show negative responses to both fire and grazing. *P. cembra* is especially sensitive and disappears with heavy grazing. The slight increases of *A. alba* and *L. decidua* at very high influx values are an artefact of the chosen model. Interestingly, only disturbance-adapted *Alnus viridis* shows a steady positive response to an increase in fire. In response to grazing, *A. viridis* shows a 'humpbacked' curve with highest abundance at intermediate disturbance levels. The results suggest that intense grazing, together with regular fire activity, leads to deforestation of the subalpine belt, including the removal of disturbance-adapted shrubs like *A. viridis* (Welten, 1982).

### 4.5. Diversity proxies

Palynological richness (PRI/*N*0) is very low at the beginning of our record (Fig. 9f), then rises quickly and stabilises at the onset of the Holocene (11,500 cal yr BP). PRI stays constant until ~5000 cal yr BP, after which the values gradually increase again. The last 800 years show highest values of PRI, pointing to highest pollen diversity. Two distinct drops in PRI, at 1050 cal yr BP and at the present day, are likely caused by forest regeneration, since they coincide with increasing percentages of subalpine tree pollen and tree *sed*aDNA. Pollen *N*2 and PIE show a similar trend (Fig. 9f) as PRI. The drop in values between 7500 and 5000 cal yr BP corresponds to the period with the highest percentage of subalpine tree pollen (mainly *Pinus cembra*), suggesting that a few dominant taxa and rather uneven conditions are lowering pollen richness estimates.

The *sed*aDNA richness estimates (*N*0; Fig. 9f) show a similar pattern as the PRI curve, with lower values during the early Holocene as compared to the late Holocene. Generally, *sed*aDNA richness gives an up to twofold higher estimation of plant richness than PRI. This difference suggests that *sed*aDNA, with its higher taxonomic resolution, is better at capturing total plant diversity than pollen. However, it is important to keep in mind that sediment composition can have an effect on DNA preservation (Giguet-Covex et al., 2019), while pollen and spores are generally more resistant to decay (Moore et al., 1991). The distinct drop in *sed*aDNA richness between 2000 and 800 cal yr BP coincides with a drop in *sed*aDNA *N*2 and a drop in total reads. This drop could be a limitation of the DNA extraction protocol when used on sediments with a high carbonate content or a result of taphonomic processes (Parducci et al., 2017; Giguet-Covex et al., 2019).

## 5. Discussion

### 5.1. Late-glacial and early Holocene vegetation dynamics driven by climate and soil development

According to the age-depth model (Fig. 2), the oldest sediments of Lago Sangiatto were deposited during the Younger Dryas (ca. 12,700–11,700 cal yr BP). The presence of coniferous bud scales in the oldest samples suggests that coniferous trees were growing near the site during this period. However, considering the large dating uncertainties, these macrofossils could also reflect trees that were present either at the end of the Bølling-Allerød interstadial, when summer temperatures were only slightly cooler than today (Samartin et al., 2012; Heiri et al., 2015), or at the beginning of the Holocene, when summer temperatures rapidly increased. Other palaeoecological records from the Southern Alps indicate that

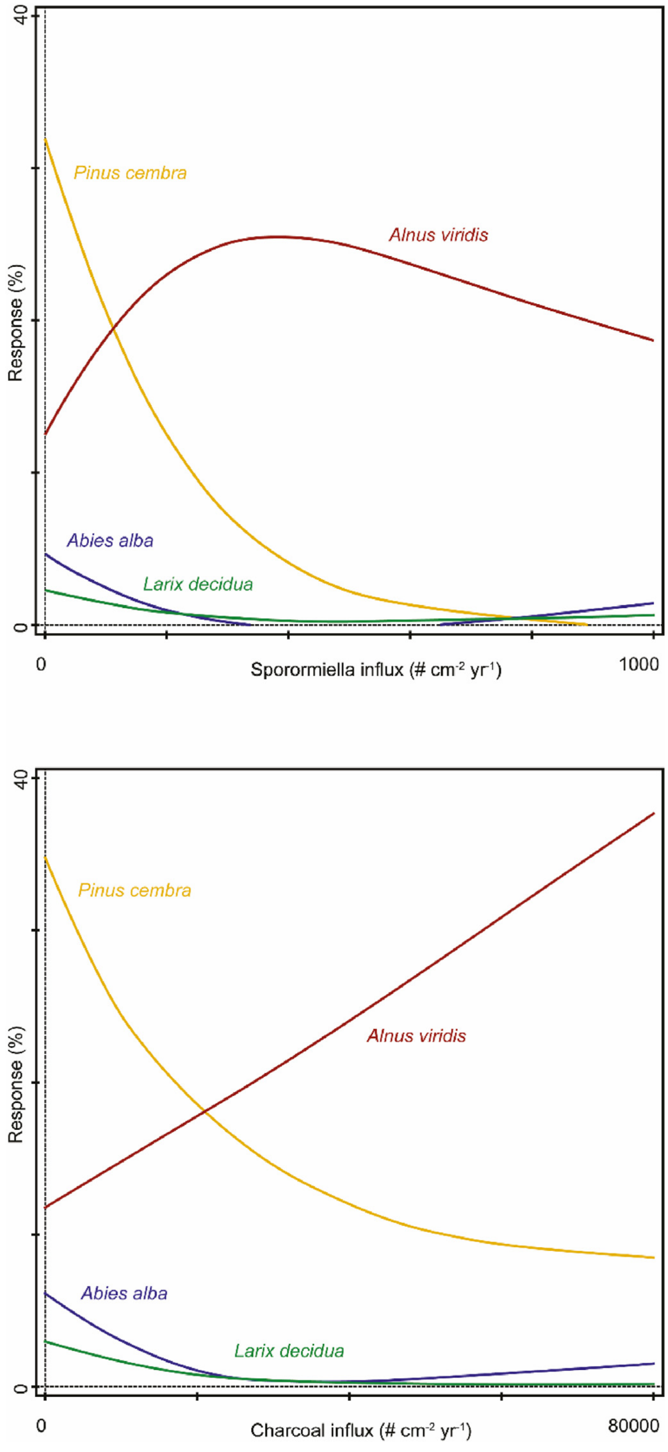
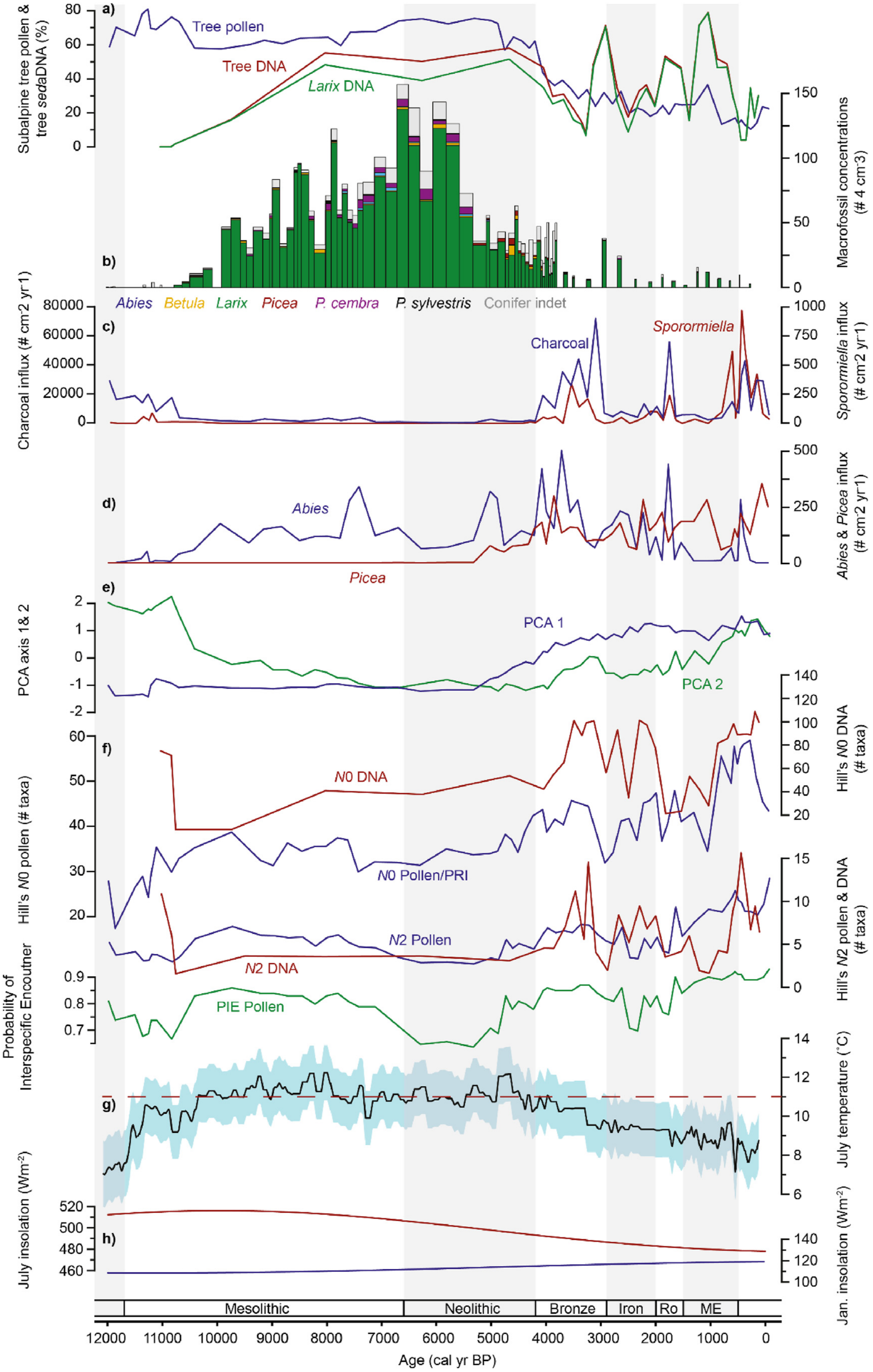


**Fig. 8.** Species response curves to *Sporormiella* influx as a proxy for grazing (top) and microscopic charcoal influx as a proxy for fire (bottom).

treeline was well above 1500 m asl during the Bølling-Allerød (ca.14,700–12,700 cal yr BP), possibly even as high as 2000 m asl (Lang and Tobolski, 1985; Vescovi et al., 2007, 2018), and at about 1300–1800 m asl during the Younger Dryas (Gobet et al., 2005; Tinner and Vescovi, 2005; Vescovi et al., 2007). Therefore, the bud scales may have originated from lower elevations and were transported by wind to our site. In agreement, chironomid temperature reconstructions from the Central and Southern Alps provide mean July temperatures of about 7.0–8.8 °C for our site during the Younger Dryas (Heiri et al., 2007; Ilyashuk et al., 2009; Heiri et al., 2015, Fig. 9g). These temperatures would be at the lowest limit of tree growth (Landolt et al., 2010) and agree well with the presence of alpine tundra inferred at Lago Sangiatto (predominance of herbaceous taxa, see Fig. 5).

During the Younger Dryas/Holocene transition (ca. 11,700 cal yr BP), temperatures abruptly increased by 2.5–4 °C (Heiri et al., 2007; Ilyashuk et al., 2009; Heiri et al., 2015, Fig. 9g), leading to an upward movement of the treeline ecotone. Although the first evidence of trees is dated to ~11,300 cal yr BP (winged fruits of tree *Betula*), the first unambiguous evidence of local tree establishment are *Larix decidua* needles dated to 10,700 cal yr BP, followed by *Pinus cembra* needles at ~10,500 cal yr BP, significantly later than the rapid warming at 11,600 cal yr BP. However, the upward shift of the treeline ecotone was possibly slowed down by the Preboreal Oscillation, a short period (150–250 yr) of cooling (1–2 °C) that started at 11,300 cal yr BP (Schwander et al., 2000; Tinner and Kaltenrieder, 2005; Höhn et al., 2022, Fig. 9g). Other palaeobotanical records in the Lepontine Alps show similar afforestation processes as at Lago Sangiatto. For example, *Larix decidua* stands established at Lago Cadagno (1921 m asl; Fig. 1) at about 10,950 cal yr BP (Vescovi et al., 2018) and *Betula*, *L. decidua* and *P. cembra* were present at Alpe Veglia (1700 m asl; Fig. 1) at the onset of the Holocene (Paganelli and Borgato, 2000; Rigamonti and Uggeri, 2016), although determining the exact timing is difficult due to the low temporal resolution of the records.

At Gouillé Rion (2343 m asl; Fig. 1), a site in the neighbouring Central Swiss Alps, establishment of *Larix decidua* stands occurred much earlier, at 11,350 cal yr BP, suggesting a rapid upward shift of the treeline ecotone in response to climate warming (ca. 800 altitudinal meters within 350 years; Tinner and Kaltenrieder, 2005). In the Central Alps however, lower air temperatures are partly compensated by higher solar radiation (i.e. mass elevation effect), allowing plant species to reach higher elevations than in the Northern and Southern Alps (Ellenberg, 2009; Landolt et al., 2010). This mass elevation effect was probably influencing vegetation dynamics during the early Holocene (Tinner and Kaltenrieder, 2005). Under current climatic conditions, the Insubrian Alps receive about 400 mm more annual precipitation than the Central Alps (1520 vs. 1100 mm) and have higher cloud cover. Furthermore, the early Holocene had a pronounced seasonality because of higher summer and lower winter insolation compared to today (Fig. 9h; Laskar et al., 2004). This seasonality, in combination with the higher cloud cover, would have resulted in a shorter growing season that might have delayed the early establishment of the treeline ecotone at its present elevation in the Lepontine Alps compared to the Central Alps by about 650 years (10,700 vs 11,350 cal yr BP). Another factor that may explain the lag in afforestation is soil development. At Hobschensee (2017 m asl; Fig. 1), at the border between Valais and the Lepontine Alps, *Larix decidua* and *Betula* appeared at about 11,700 cal yr BP (Welten, 1982; Lang and Tobolski, 1985; van der Knaap and Ammann, 1997). This site has a similar climate to Lago Sangiatto but was already deglaciated from the Bølling-Allerød onwards (Dielforder and Hetzel, 2014). We speculate that early deglaciation provided more time for soils to develop, which may have facilitated tree survival under the dry and warm summer conditions of the early Holocene. In the Northern Alps, Holocene afforestation was even more delayed compared to the Southern Alps. At Iffigsee (2065 m asl; Fig. 1), for example, treeline conditions did not establish until 10,000 cal yr BP and timberline reached the site at about 9800 cal yr BP (Schwörer et al., 2014). Palaeoclimatic studies suggest a pronounced latitudinal temperature gradient across the Alps during the Younger Dryas cooling and less warming at the beginning of the Holocene in the


a)
Subalpine tree pollen & tree *sed*aDNA (%)
80
60
40
20
0
Tree pollen
Tree DNA
*Larix* DNA
b)
Macrofossil concentrations (# 4 cm⁻3)
150
100
50
0
*Abies* *Betula* *Larix* *Picea* *P. cembra* *P. sylvestris* Conifer indet
c)
Charcoal influx (# cm⁻2 yr⁻1)
80000
60000
40000
20000
0
Charcoal
*Sporormiella*
*Sporormiella* influx (# cm⁻2 yr⁻1)
1000
750
500
250
0
d)
*Abies*
*Picea*
*Abies* & *Picea* influx (# cm⁻2 yr⁻1)
500
250
0
e)
PCA axis 1& 2
2
1
0
-1
-2
PCA 1
PCA 2
f)
Hill's *N0* pollen (# taxa)
60
50
40
30
20
*N0* DNA
*N0* Pollen/PRI
Hill's *N0* DNA (# taxa)
140
120
100
80
60
40
20
*N2* Pollen
*N2* DNA
Hill's *N2* pollen & DNA (# taxa)
15
10
5
0
Probability of Interspecific Encoutner
0.9
0.8
0.7
PIE Pollen
g)
July temperature (°C)
14
12
10
8
6
h)
July insolation (Wm⁻²)
520
500
480
460
Jan. insolation (Wm⁻²)
140
120
100
Mesolithic
Neolithic
Bronze
Iron
Ro
ME
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Age (cal yr BP)

Northern Alps compared with the Central and Southern Alps (Heiri et al., 2014; Schwörer et al., 2014). Such a palaeoclimatic pattern may indeed explain the 700–1350 year delay in forest development in the Northern Alps when compared to the Central and Southern Alps.

After the Preboreal Oscillation (ca. 11,300–11,100 cal yr BP), summer temperatures increased again and rose above present-day values (Ilyashuk et al., 2009; Heiri et al., 2015, Fig. 9g), moving the treeline ecotone farther upwards. *Pinus cembra* had a first peak in macrofossils coincident with the occurrence of *Pinus* DNA at about 9500 cal yr BP (Fig. 6). The sequence of *P. cembra* following *L. decidua* is typical for palaeorecords in the Alps and may be explained by the marked continental conditions and pronounced seasonality of the early Holocene (Fig. 9h; Laskar et al., 2004), which led to a shorter growing season and possibly more late-winter drought stress when compared to today (Tinner and Kaltenrieder, 2005; Schwörer et al., 2014). Both *P. cembra* and *L. decidua* are well adapted to cold, but as *L. decidua* loses its needles every year it is more resilient to late-winter drought stress than *P. cembra* (Ellenberg, 2009; Körner, 2012). Another explanation for the observed lag might be the more efficient wind dispersal of the winged seeds of *L. decidua* compared to the heavy seeds of *P. cembra*. The seeds of the stone pine are dispersed by the nutcracker (*Nucifraga caryocatactes*, a bird species), which prefers to cache them in deeper soils (Hättenschwiler and Körner, 1995). This explains why *L. decidua* is more abundant on bare soils, although both species can germinate well in the absence of developed soils or humus horizons (Ellenberg, 2009). At Lago Cadagno (1921 m asl; Fig. 1), the delay in the establishment of *P. cembra* was more pronounced; there *L. decidua* established at 10,950 cal yr BP followed by *P. cembra* at 9950 cal yr BP (Vescovi et al., 2018). The greater lag at Lago Cadagno is best explained by more pronounced continental and drier conditions than in other areas of the Lepontine Alps (Vescovi et al., 2018). A similar lag is also observed in the more continental and drier Central Alps at Gouillé Rion (2343 m asl; Fig. 1), where the succession from pure *L. decidua* to mixed *P. cembra* forests took about 1000 years (Tinner and Kaltenrieder, 2005).

At about 8200 cal yr BP, mean annual temperatures abruptly decreased by about 2–3 °C for several decades (Fig. 9g; Heiri et al., 2015). This event led to a reorganisation of atmospheric circulation patterns, resulting in more oceanic conditions and an increase in moisture availability in Europe (Seppä and Birks, 2001; Tinner and Lotter, 2001). In response to more humid summer conditions, *Abies alba* was able to expand at Lago Sangiatto at 8000 cal yr BP (Fig. 6). Even though *A. alba* can grow in dry locations, it is sensitive to late frost, low air humidity and low spring precipitation (Tinner et al., 2013). During the more continental and drier conditions of the early Holocene, *A. alba* was not able to co-dominate in the vegetation (Tinner and Lotter, 2006; Tinner et al., 2013). A similar pattern is recognised at Lago Cadagno (1921 m asl; Fig. 1), where *Abies* stomata indicate its presence from ~9000 cal yr BP but pollen data indicate that the species was most abundant after 8000 cal yr BP (Vescovi et al., 2018).

### 5.2. Earliest human impact on the vegetation

A Mesolithic camp at Alpe Veglia and multiple archaeological finds around the Simplon Pass suggest regular human activity in the subalpine and alpine regions of the Lepontine Alps after 10,000 BP (Gambari et al., 1989; Crotti et al., 2004). Findings from the Albrun Pass (Fig. 1) indicate its use from at least 8400 cal yr BP (Curdy et al., 2010). Despite these activities, no unambiguous signs of early human impact during the Mesolithic (ca. 11,700–6600 cal yr BP) or the Early Neolithic (ca. 6600–5600 cal yr BP) can be detected in the vegetation record of Lago Sangiatto.

The earliest evidence of agricultural activities at Lago Sangiatto dates to the Late Neolithic at 4900 cal yr BP, when the first Cerealia type pollen appears in our record. Clear indicators for grazing occur after 4300 cal yr BP with the continuous presence of *Sporormiella*, and the expansion of alpine meadows. Palaeoecological records from other alpine sites also show evidence of anthropogenic activity during the Neolithic. However, near alpine mountain passes the signs of farming and grazing appear (much) earlier than at Lago Sangiatto. For example, at Hobschensee near the Simplon Pass and at Iffigsee near Schnidejoch (Fig. 1), the first indications of agriculture appear from ~7000 to 6500 cal yr BP (Welten, 1982; van der Knaap and Ammann, 1997; Schwörer et al., 2014). At Crotte Basse (2365 m asl; Fig. 1) in the Aosta Valley, the formation of alpine pastures around 5600 cal yr BP was preceded by a ~200 year phase of sporadic grazing activity (Pini et al., 2017). At these sites, humans used a combination of fire and livestock grazing to clear subalpine forests and expand alpine meadows (Schwörer et al., 2015; Pini et al., 2017). Disturbance-sensitive tree species, such as *Abies alba* or *Pinus cembra* suffered, whereas disturbance-adapted trees and shrubs, such as *Alnus viridis* or *Picea abies* thrived.

The expansion of *Picea abies* across the Alps has been attributed to climate change, migrational lags, soil development, human impact (including fire and browsing), or a combination of those (Markgraf, 1970; Lang, 1994; Wick et al., 2003; Henne et al., 2011; Rey et al., 2013; Schwörer et al., 2015). *Picea abies* survived the Last Glacial Maximum in refugia in the south-eastern Alps and then spread west during the Holocene (Ravazzi, 2002; Latałowa and van der Knaap, 2006). The first appearance of *P. abies* pollen at Lago Sangiatto at about 6700 cal yr BP and its subsequent expansion at ~5100 cal yr BP might be related to changing climate conditions, from warm and humid conditions during the Holocene Thermal Maximum (ca. 9000–5000 cal yr BP) to colder and more humid climates with the onset of the Neoglacial (ca. 5000 cal yr BP; Wick et al., 2003; Fischer et al., 2018). For our site, it is unclear if fire altered the vegetation at this time, as there is only a minor increase in microscopic charcoal at about 5100 cal yr BP when *Picea abies* expanded. Assessing this question would require continuous high-resolution charcoal and pollen records and time-series analyses (Tinner et al., 1999; Rey et al., 2013; Schwörer et al., 2015). However, it is intriguing that the spread of the species approximately corresponds to the first occurrence of cereal pollen at Lago Sangiatto (4900 cal yr BP). Usually, cereal pollen is poorly dispersed (Behre and Kucan, 1986), but in mountain areas it can be transported to higher locations by strong anabatic winds (Vescovi et al., 2018). We assume that arable farming and pastoral activities took place on the

**Fig. 9.** Comparison of the main biotic proxies of Lago Inferiore del Sangiatto with different climate records: **a)** Percentage of subalpine tree pollen (blue), percentage of tree *sed*aDNA reads (red) and percentage of *Larix decidua* reads (green); **b)** Stacked macrofossil record of subalpine tree macrofossils. Green: *Larix decidua*, yellow: *Betula*, purple: *Pinus cembra*, blue: *Abies alba*, red: *Picea abies*, black: *Pinus mugo/sylvestris*, grey: coniferous tree remains indet; **c)** Influx values of microscopic charcoal (blue) and *Sporormiella* (red); **d)** Pollen influx values of *Abies alba* (blue) and *Picea abies* (red); **e)** PCA axis 1 (blue) and PCA axis 2 (green); **f)** Hill's numbers *N*0 and *N*2 for the *sed*aDNA record (red) and the pollen record (blue), and pollen evenness (PIE, green) as proxies for species richness and diversity; **g)** July temperature reconstruction for Lago Sangiatto calculated from a stacked chironomid-based temperature reconstruction for the Alps (Heiri et al., 2015). The dotted line is today's mean July temperature at the site; **h)** July insolation (red) and January insolation (blue; Laskar et al., 2004). Alternating white and grey shading indicates cultural time periods; *Ro* Roman period, *ME* Medieval period (Pini et al., 2017). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

valley floor at Alpe Devero (Fig. 1), facilitating regional vegetation changes. The lack of a clear signal for local human activities at 4900 cal yr BP can be explained by the small diameter (~60 m) of Lago Sangiatto, which would tend to catch a local signal (Jacobson and Bradshaw, 1981; Conedera et al., 2006; Gill et al., 2013; Alsos et al., 2018; Giguet-Covex et al., 2019).

Our numerical analyses suggest that disturbance was an important driver of vegetation change at Lago Sangiatto. Specifically, the PCA axis 1 (Figs. 7 and 9f) shows the change from undisturbed landscapes to disturbed vegetation, including the expansion of *P. abies* at 5100 cal yr BP that coincided with the most significant change in the vegetation composition at Lago Sangiatto during the Holocene. Also, the decline of *Pinus cembra* and *Abies alba*, as well as the expansion of *Alnus viridis* suggest that disturbance played a role in changing vegetation composition (Gobet et al., 2003; Rey et al., 2013; Conedera et al., 2017). The species response curves (GAM, Fig. 8) show the negative effects of grazing and fire disturbance on *P. cembra* and *A. alba* and the positive effect on *A. viridis*. This finding supports previous numerical evidence from time-series and GAM analyses, that indicate that late-successional *P. cembra* and *A. alba* were strongly reduced by prehistoric fires, while early-successional *A. viridis* was strongly promoted (e.g. Tinner et al., 1999; Gobet et al., 2003; Colombaroli et al., 2010; Rey et al., 2013; Schwörer et al., 2015). Interestingly, grazing and fire do not have a significant effect on *P. abies* according to our analyses, in contrast to other Alpine sites (e.g. Schwörer et al., 2015). However, the species response curves (Fig. 8) show that fire and grazing have very similar effects on vegetation and have a large impact on tree cover. In forests with heavy browsing, herbivores eat many of the saplings, preventing the establishment of new trees and thus control the tree cover. Fire has a similar effect, by reducing seedling establishment and destroying young saplings (Bond and Keeley, 2005). However, the main difference is that animals have strong preferences regarding palatability, while fires just consume the burnable biomass.

### 5.3. Impact of livestock grazing since the Bronze Age

During the Bronze Age (ca. 4200–2900 cal yr BP), human population increased and the exploitation of copper deposits led to stronger connections between the Ossola and the Upper Valais, increasing traffic over the Simplon and Albrun mountain passes (Curdy, 2007; Gambari, 2007). Our vegetation reconstruction and numerical analyses suggest that people started using fire at Lago Sangiatto to open the forest and gain space for grazing livestock during this period. This land use is documented by the highest charcoal concentration and influx values, as well as high *Sporormiella* percentages in our record. People created larch-meadows (open *Larix decidua* stands with an herbaceous understory), for livestock grazing, timber and fodder production (Garbarino et al., 2013). A combination of persistent disturbance and human preference for larch-meadows led to the decline of *Pinus cembra* and *Abies alba*. Specifically, *P. cembra* and *A. alba* are sensitive to fire and browsing, whereas *L. decidua* is more resistant to disturbance (Conedera et al., 2017). People also favoured *L. decidua* at the expense of other less desirable tree species such as *P. cembra* (Garbarino et al., 2013). In the past, farmers leasing pastures in some areas of the western Italian Alps had to remove any *P. cembra* seedlings to maintain the economically important meadows (Motta et al., 2006). A similar process occurred in the Upper Engadine, where persistent regional population growth during the Bronze Age led to intensified land use and the creation of larch-meadows in combination with prehistoric copper exploitation (Gobet et al., 2003).

Human disturbance continued to increase during the Iron Age (ca. 2900–2000 cal yr BP) and Roman Period (ca. 2000–1474 cal yr BP) after which it decreased during the Migration Period (ca. 1600–1400 cal yr BP). Societal changes after the fall of the Roman Empire led to land abandonment and re-expansion of forests around Lago Sangiatto. The largest impact of agricultural activities on the vegetation were recorded during the Medieval to modern period. From 750 to 650 cal yr BP (1200–1300 CE) Allemanic speaking settlers from the Upper Valais moved into the Valle Devero and surroundings to settle the higher valleys and mountain flanks (Kreisel, 1991). These 'Walser' settlers opened the forest to create space for crops and their livestock. Large-scale deforestation by the Walser left few trees standing around Lago Sangiatto, as indicated by the lowest subalpine tree pollen percentages and extensive grass and herb communities in our record (Figs. 5 and 6). Historical sources mention activities like charcoal production, pitch extraction from the bark of larch trees and rye cultivation during the 1300s CE at Alpe Devero (Garbarino et al., 2013). These activities are registered in our record (Figs. 3 and 6) as charcoal and *Secale* peaks. However, the most important activities were cattle and goat grazing and hay making on the subalpine pastures and larch-meadows (Garbarino et al., 2013), as evidenced by high *Sporormiella* values and *seda*DNA of a wide variety of meadow species (Fig. 5).

### 5.4. Impacts of fire and grazing on biodiversity

Intensive fire and grazing not only affected the tree cover, but also influenced plant biodiversity. The diversity indicators (Hill's *N*0, Fig. 9) and the PCA plot (Fig. 7) suggest that fires and livestock grazing led to higher biodiversity. Even though disturbance negatively impacted tree species diversity, it promoted species typical of open meadows and grasslands (e.g. *Leontodon hispidus, Plantago alpina, Potentilla* sp., *Trifolium badium*; Figs. 5 and 6), a feature which is common for Europe (Colombaroli et al., 2013). Land use fragmented the vegetation into mosaics of novel vegetation communities, creating high overall diversity (Thöle et al., 2016). Over the last 100 years, grazing has decreased at Alpe Devero due to land abandonment (Garbarino et al., 2013), which has allowed establishment of new seedlings and forest regeneration. *Larix decidua* now dominates the open forest, causing a decline in species richness. The same pattern can also be observed during other phases of land abandonment, such as the Migration Period (ca. 1600–1400 cal yr BP; Fig. 9). With continuing low anthropogenic disturbance, forest succession will displace the diverse alpine meadows (Colombaroli et al., 2010; Schwörer et al., 2014), but increase the arboreal diversity within a few centuries at most.

### 5.5. SedaDNA as a proxy for local vegetation dynamics

The analysis of *seda*DNA complements other established palaeoecological proxies such as pollen and macrofossil analyses (Birks and Birks, 2016; Parducci et al., 2017; Alsos et al., 2020b). To explore the potential of *seda*DNA analysis, it is essential to compare it with palynological and macrofossil analyses over the entire Holocene. Each proxy has its own strengths and weaknesses regarding catchment area, taphonomy and taxonomic resolution; by combining them a more complete picture of past vegetation dynamics can be reconstructed. Our study is the first to combine pollen, macrofossils and *seda*DNA to reconstruct vegetation dynamics in the Alps.

Generally, the different proxies at Lago Sangiatto show similar patterns (Fig. 6). For example, macrofossils, pollen and *seda*DNA of *Larix decidua* all appear at about 10,700 cal yr BP and are present throughout the Holocene (Fig. 6). Similarly, percentages of *sed*aDNA reads and pollen of *Salix* follow similar trends (Fig. 6). This observation is further corroborated by the similar patterns of the ordination analysis of both pollen and *sed*aDNA data (Fig. 7; Supplementary Figures A1, A2). Studies from the Arctic show that *sed*aDNA mainly represents local vegetation and is more sensitive to site-specific vegetation compositional change than pollen (Sjögren et al., 2017; Alsos et al., 2018; Liu et al., 2020). Our results generally confirm the local origin of *sed*aDNA; for example, *Alnus viridis sed*aDNA and macrofossils appear concurrently at 4000 cal yr BP even though pollen percentages increased earlier (Fig. 6).

Furthermore, our results suggest that the percentage of reads can be used as a qualitative measure of species abundance. This relationship is evident when comparing tree pollen and *sed*aDNA percentages, as well as macrofossil concentrations (Figs. 6 and 9). All three proxies show low tree abundance at the beginning of the record, a gradual increase during the early Holocene, highest values indicating closed forests during the Holocene Thermal Maximum and a rapid decline at the beginning of the Bronze Age with anthropogenic deforestation. The high fluctuations of *sed*aDNA tree percentages during the late Holocene, however, are not reflected in either the pollen or macrofossil record and might be explained by the sensitivity of *sed*aDNA to species-specific local biomass input into the lake (Parducci et al., 2017). It is possible that the presence of a few *Larix decidua* trees around the lake, shedding needles every year, led to a higher biomass input and thus higher reads compared to the otherwise abundant herbaceous vegetation. This 'swamping the signal' effect is very likely as the tree *sed*aDNA signal is mostly driven by *Larix decidua*.

A major advantage of *sed*aDNA analysis is the high taxonomic resolution that can be achieved (Table 3), especially for herbs and insect-pollinated species that are underrepresented in pollen records (Jørgensen et al., 2012; Clarke et al., 2019). The expansion of species-rich meadows due to anthropogenic land use from the Bronze Age onwards is captured in far more detail in the *sed*aDNA data at Lago Sangiatto than in the pollen and macrofossil records (Fig. 9f). Combining both classical palaeoecological approaches, which can quantitatively reconstruct vegetation dynamics, with *sed*aDNA analyses, which can contribute taxonomic information for underrepresented plant groups, provides novel insights. Furthermore, because *sed*aDNA is supposed to directly derive from the local vegetation and because pollen has a much wider spatial catchment, a combination of classic and genetic palaeoecological proxies may help to refine the spatial resolution and coverage of vegetation reconstructions.

## 6. Conclusions

Lago Inferiore del Sangiatto provides the first palaeoenvironmental reconstruction in the Alps that combines pollen, stomata, spores, charcoal, macrofossils and *sed*aDNA data to provide detailed insights into vegetation dynamics in response to climate and land-use change. Our study shows that a multi-proxy approach including *sed*aDNA enables higher taxonomic resolution and a better understanding of vegetation responses to climate and anthropogenic disturbance. The data suggest that the treeline reached the lake around 10,700 cal yr BP, followed by the timberline around 10,500 cal yr BP, which seems to agree with other sites in the Southern Alps. The apparent lag in afforestation in response to the rapid warming of 2–4 °C at the Younger Dryas/Holocene transition is not as pronounced as in the Northern Alps, but still larger than in the Central Alps. Afforestation may have been stalled by a short cooling phase (Preboreal Oscillation) and a less pronounced mass elevation effect. The 200-year delay in the establishment of late-successional *Pinus cembra* forests was most likely caused by dry conditions (including soil moisture) in the early Holocene. From 5100 cal yr BP, anthropogenic burning and grazing led to a decrease in subalpine tree species such as *Larix decidua*, *Abies alba* and *Pinus cembra* and to the creation of larch-meadows during the Bronze Age that are still widespread today in the Southern and Central Alps.

Vegetation at Lago Sangiatto is shaped by thousands of years of anthropogenic disturbance. The current decline in land use, caused by decreasing populations in the south-western Alps, is enabling successional change and altering the subalpine vegetation composition (Falcucci et al., 2007; Bätzing, 2015). In the absence of land use, our data suggest that the current pure *Larix decidua* forests will likely be replaced by more diverse communities including *Pinus cembra, Picea abies* and, under low disturbance, *Abies alba* within at most a few centuries. Additionally, the latest climate projections indicate that by the end of this century, temperatures could increase up to 7 °C and summer precipitation could decrease up to 30% in the Insubrian Alps, if greenhouse gas emissions are not drastically reduced (CH2018, 2018; IPCC, 2021). According to our data, such a drastic warming would lead to an unprecedented upward shift of the treeline ecotone, potentially displacing the species-rich alpine meadow communities and leading to an overall loss of biodiversity (Gottfried et al., 2012). Our data suggest that promoting traditional alpine agriculture, especially in marginal areas, may be an effective way to maintain species-rich meadows and protect alpine ecosystems and their biodiversity.

## Author contributions

**Lieveke van Vugt**: conceptualisation, palynological and macrofossil analysis, chronology, data analysis, visualisation, writing – original draft; **Sandra Garcés-Pastor**: DNA analysis, data analysis, writing – reviewing and editing; **Erika Gobet**: palynological and macrofossil analysis, supervision, writing – reviewing and editing; **Sarah Brechbühl**: macrofossil analysis; **Antonietta Knetge**: macrofossil analysis; **Youri Lammers**: DNA analysis, data analysis, writing – reviewing and editing; **Katja Stengele**: macrofossil analysis; **Inger Greve Alsos**: funding acquisition, conceptualisation, project management, writing – review and editing; **Willy Tinner**: conceptualisation, project management, writing – review and editing, **Christoph Schwörer**: supervision, conceptualisation, project management, writing – review and editing.

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## Declaration of competing interest

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

## Data availability

The pollen- and macrofossil datasets will be available from Neotoma and the Alpine Palynological Data-Base (ALPADABA); the sedaDNA dataset will be available from the ENA (European Nucleotide Archive).

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## Appendix A. Supplementary data

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