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Two Millennia of Complexity and Variability in a Perialpine Socioecological System (Savoie, France): The Contribution of Palynology and *sedaDNA* Analysis

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Over the last two millennia, European Alpine ecosystems have experienced major changes in response to the important, yet fluctuating, impact of human activities. This study aims to reconstruct the environmental history of the last 1800 years on the western edge of the Alps by analyzing sediments from Lake Aiguebelette, a large lake located in the perialpine area. We have combined analyses of pollen and other palynomorphs, such as coprophilous fungal spores, together with sedimentary DNA (from plants and mammals) in order to reconstruct both vegetation and land-use histories. A sedimentological and geochemical analysis was also conducted in order to gain an understanding of changes in erosion dynamics in response to landscape modifications that were influenced by climate and human activities. This work highlights alternating phases of anthropization and agricultural abandonment allowing forest recovery. While pollen reflects the major phases of regional deforestation and afforestation related to the dynamic of farming activities, plant DNA provides precise information on the plants cultivated in fields, orchards and vegetable gardens over the past centuries. The combination of mammal DNA and coprophilous fungal spores completes this work by documenting the history of pastoral practices.

Keywords: land use history, vegetation history, Alps, last two millennia, pollen, erosion processes, *sedaDNA*

INTRODUCTION

Current landscapes result from millennial-long evolutions of environments under the joint influence of human activities, climate changes, and ecosystem responses (Kaplan et al., 2009; Ellis et al., 2013; Mottl et al., 2021). In the European Alps (Mottl et al., 2021), this evolution of ecosystems during the Holocene has greatly expanded and accelerated in the last few millennia, as shown by

numerous paleoecological and paleoenvironmental records (Tinner et al., 1996; Schmidt et al., 2002; Giguët-Covex et al., 2011, 2014; Doyen et al., 2013, 2016; Walsh et al., 2014; Bajard et al., 2016, 2017; Andrič et al., 2020; Dietre et al., 2020; etc.). These records highlight the growing impact of agricultural intensification both at low altitude (Doyen et al., 2013, 2016; Simonneau et al., 2013; Rapuc et al., 2021) and in Alpine pastures (Giguët-Covex et al., 2014; Rey et al., 2017) in a period when populations were growing and agricultural techniques were being improved. The last two millennia were also marked by major shifts in climatic conditions, such as the Medieval Climate Anomaly (MCA) and the Little Ice Age (LIA) (Büntgen et al., 2011; Le Roy et al., 2015). At the same time, important socio-economic/cultural shifts occurred, among which the fall of the Roman Empire, the agricultural and economic revolution of the Middle Ages (e.g., invention of the plow) or the current agricultural decline (Mazoyer and Roudart, 1997; Ferault and Le Chatelier, 2012) and introduction of “exotic plants” are likely to have a significant impact on landscape evolutions. How have human impacts and climatic variations modified and driven the trajectories of ecosystems over the last two millennia?

Although the identification of cultivated plants is a direct indicator of past agricultural strategies, the whole vegetation history can also be a reliable indicator of such strategies and associated agrarian practices (e.g., expansion of ruderal, nitrophilous plants, weeds, changes in forest composition due to clearance, etc.) (Behre, 1981; Brun, 2007). Moreover, analysis of non-pollen palynomorphs (spores of coprophilous fungi such as *Sporormiella* sp.) provides indirect and robust information about past pastoral pressure (Ejarque et al., 2011; Etienne et al., 2013; Doyen and Etienne, 2017). Therefore, palynological analyses are traditionally used as a fundamental tool to reconstruct agropastoral activities and paleoenvironmental dynamics. For the Alps, pollen analyses are commonly used to reconstruct the vegetation and land-use history (de Beaulieu et al., 1992; Tinner et al., 1996, 2003; Haas et al., 1998; Guiter et al., 2005; Finsinger and Tinner, 2007; David, 2010; Schwörer et al., 2015; Thöle et al., 2016; Pini et al., 2017).

In perialpine areas (the regions located immediately around the Alpine range), past vegetation cover and land-use have already been documented through pollen from lake sediments (Clerc, 1988; Lotter, 1999; Tinner et al., 1999; Doyen et al., 2013, 2016; Rey et al., 2017; Andrič et al., 2020). In these regions, farming activities are more developed and diversified than in the inner Alpine areas which are not always suitable for growing cultivated plants. Indeed, previous studies have described the development of mixed agropastoral activities comprised of cereal growing, fruit tree growing and grazing, that have shaped the regional vegetation and driven soil erosion dynamics (Jacob et al., 2009; Doyen et al., 2013, 2016; Simonneau et al., 2013). Nonetheless, pollen analysis presents some limitations in reconstructing plant cover, especially in foothill areas (Muller et al., 2006). Pollen assemblages deposited in lake sediment include pollen grains from both local and regional sources, a fact which limits the reconstruction of local vegetation and land-use. The low taxonomic resolution of most of the herbaceous families and the low dispersal capacity of insect-pollinated plants,

represent important limitations to the precise identification of past plant communities and their intrinsic diversity. Spores of coprophilous fungi are robust proxies of past livestock farming but they cannot indicate the composition of past domestic herds. Consequently, the reconstructions of paleoecological trajectories and of the nature of past agropastoral activities are open to improvement.

In order to enhance our knowledge of past human-influenced environmental trajectories, it is possible to combine palynological data with plant and mammal DNA analyses. Lake sediment DNA (*sedaDNA*) analyses from plants was recently made possible thanks to the emergence of the DNA metabarcoding approach (Parducci et al., 2017), which takes advantage of the development of high-throughput sequencing techniques (Taberlet et al., 2012). This emerging indicator of past biodiversity has been successfully applied in various ecosystems ranging from tropical to boreal environments (Boessenkool et al., 2014; Epp et al., 2015; Alsos et al., 2016; Bremond et al., 2017; Sjögren et al., 2017; Ficetola et al., 2018; Tabares et al., 2020), including the Alps (Pansu et al., 2015; Bajard et al., 2017; Giguët-Covex et al., 2019). Lake *sedaDNA* analyses have also been successfully applied in different contexts to identify mammals (Giguët-Covex et al., 2014; Etienne et al., 2015; Graham et al., 2016; Pedersen et al., 2016; Sabatier et al., 2017; Ficetola et al., 2018; Brown et al., 2021). The combination of these micro-fossil and DNA-based approaches offers a new and more detailed image of past vegetation and of agricultural and pastoral activities (Giguët-Covex et al., 2014) at the local scale. Indeed, because plant DNA in lake sediments comes from the watershed (or parts of the watershed; Parducci et al., 2017; Sjögren et al., 2017; Alsos et al., 2018; Giguët-Covex et al., 2019), it potentially represents a key indicator allowing us to decipher the different pollen origins (regional/local) and to better reconstruct the spatial distribution and modification of vegetation at the catchment scale. Pollen and *sedaDNA* have already been used in combination in several studies (Parducci et al., 2005, 2013; Giguët-Covex, 2010; Jørgensen et al., 2012; Pedersen et al., 2013; Niemeyer et al., 2017; Sjögren et al., 2017). These studies have revealed the benefits of applying both methods since the results of DNA analysis provide invaluable information about past vegetation and complement the results of pollen analysis.

Therefore, in order to investigate the question of the influence of climate variations and/or human activities over the last two millennia in the perialpine area, we propose to study the Lake Aiguebelette sediment archive sequence, by combining different approaches in a multiproxy analysis: (i) sedimentological and geochemical analyses to trace the evolution of soil erosion and (ii) palynological (pollen grains and spores of *Sporormiella* sp.) and lake *sedaDNA* analyses targeting plants and mammals with a view to documenting past changes in both vegetation communities and agricultural/livestock farming activities/practices. Taken together, these proxies will provide a full picture of agricultural activities, their effects on the landscape (vegetation cover and soil erosion) and, ultimately, will give us a better understanding of the history of local livelihood strategies and land use over the past 1800 years, a period that witnessed deep social-economical mutations and significant climate changes.

STUDY SITE

Physical Setting

Lake Aiguebelette (374 m a.s.l., 5.5 km²) and its watershed (58.9 km²) are located in Savoie (France), in the northern perialpine area. The lake is bordered by the southern fringe of the Jura mountain range. It is of glacial origin and was formed after the retreat of the glacier at the end of the Würm period (Coutterand, 2010). Its catchment area ranges in altitude from 374 to 1441 m a.s.l. The geological substratum is mainly composed of Jurassic to lower Cretaceous limestone and marls, Miocene marine sandstone and Quaternary deposits composed of moraine and limestone screes in the southern part of the lake and along the limestone cliff (Eastern part) (Figure 1). The lake, which is the 7th largest natural lake in France, is composed of three main basins, respectively 30, 46, and 71 m of water depth. The main tributaries are the Gua, the Leysse de Novalaise and the Tuilerie rivers. The outlet of the lake, the Tiers, flows into the Guiers River, which itself is a tributary of the Rhône.

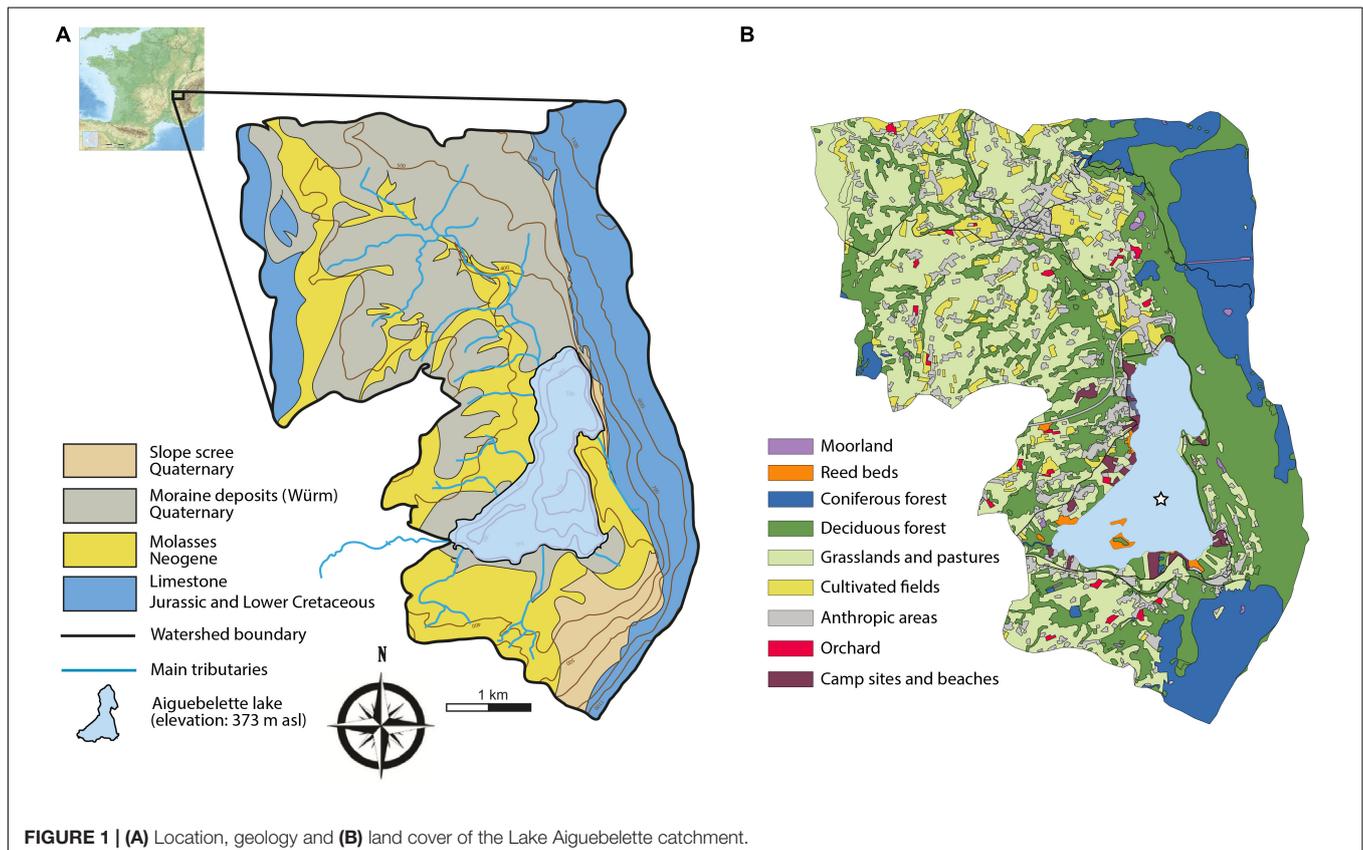
The lake water hosts a rich sub-aquatic macroflora including taxa such as *Nymphaea alba*, *Nuphar lutea*, *Najas* sp., *Potamogeton* sp., and *Myriophyllum spicatum*. The lake shores are occupied by riparian forests dominated by alder (*Alnus glutinosa*), swamps and reed beds. The eastern side of the catchment (the slopes of the Epine range) is covered by forests dominated by fir (*Abies alba*) and beech (*Fagus sylvatica*). A thin belt of white oak (*Quercus*

pubescens) and box tree (*Buxus sempervirens*) can be found on the well-exposed outcrops of the limestone massif. On this side, just a few small patches of pasture occur at the present time. The western part is mainly occupied by grasslands, cereal fields and pastures. While the farming activities are not as intense as in past centuries, this region is still an agricultural region. Forests composed of oak, hornbeam (*Carpinus betulus*), maple (*Acer* spp.) and hazel (*Corylus avellana*), are also well represented in this part of the watershed (Figure 1). Chestnut trees (*Castanea sativa*) are widespread in the forests growing on the molasses. This region was formerly known for its fruit production and while orchards were numerous in the last centuries, only a few are still in existence today (Figure 1).

MATERIALS AND METHODS

Coring

Several sediment cores were retrieved from the deepest part of the lake (71 m depth, 45,5506N; 5,80128E). In 2008, a 170-cm long sediment core (AIG08-04) was collected using an UWITEC gravity corer with hammer. The upper part of this core, dated with short-lived radionuclides was then correlated with new cores. In 2016, AIG16-08 (N° IGSN TOAE0000000168) and AIG16-06 (N° IGSN TOAE0000000166), measuring 256.5 and 125 cm respectively, were sampled using an UWITEC gravity corer with hammer.



Sedimentological and Geochemical Analysis

In the laboratory, the core was split, photographed, and logged in detail, and all physical sedimentary structures were noted as was the vertical succession of facies. From these two cores and the correlation of their sedimentary structures we were able to create a master core (AIG16-MC) measuring 261.6 cm in length (0–45.5 cm from AIG16-06, and 40.2–256.3 cm from AIG16-08). Following the standard procedure (Heiri et al., 2001), loss on ignition (LOI) analysis was performed on 179 samples taken along the AIG16-MC core in order to estimate organic matter (OM) and carbonate contents in the sediments. The non-carbonate ignition residue (NCIR) was obtained by subtracting the LOI at 550 and 950°C from the initial dry weight. X-ray fluorescence (XRF) analysis was performed on the surfaces of the split sediment cores at 1-mm intervals, using a non-destructive Avaatech core scanner (Richter et al., 2006). The relative geochemical compositions (intensities), expressed in counts per second, were obtained at various tube settings: 10 kV at 1.5 mA for Al, Si, P, S, K, Ca, Ti, Mn, and Fe; 30 kV at 1 mA for Cu, Ni, Zn, Br, Sr, Rb, Zr, and Pb with each run lasting 30 s. Principal component analysis (PCA) was performed on the geochemical results using R software (R Core Team, 2018) to identify principal sediment end-members, which were then used to better constrain each sedimentological facies and to select the best elements for tracing the erosion dynamic (e.g., Sabatier et al., 2010). Relative abundances of elements are expressed as centered log-ratio (CLR) to avoid dilution effects due to water (e.g., Weltje et al., 2015). The following elements ($n = 15$) were used to calculate the geometric mean: Si, P, S, K, Ca, Ti, Mn, Fe, Zn, Br, Rb, Sr, Zr, and Pb. For the same potential matrix effects, element ratios are expressed as logarithms (ln) of XRF count ratios that are linearly related to the log ratios of corresponding absolute concentrations (Weltje and Tjallingii, 2008).

Chronology

The core chronology is based on four AMS ^{14}C dates, which were obtained on selected terrestrial plant macroremains (Table 1), and short-lived radionuclide measurements. *Clam* (Blaauw, 2010), written for the open-source statistical software 'R', was used to calibrate the ^{14}C ages with the Intcal20 calibration curve (Reimer et al., 2020) in order to construct an age-depth model. For short-lived radionuclides, a continuous sampling step of 10 mm was applied over the first 22 cm of AIG08-04 to determine ^{210}Pb , ^{226}Ra , and ^{137}Cs activities. The analysis was carried out on well-type, germanium detectors located at the Laboratoire Souterrain de Modane following the procedure described by

Reyss et al. (1995). For each sample, the ^{210}Pb excess activities were calculated by subtracting the ^{226}Ra -supported activity from the total ^{210}Pb activity. The age model was then computed using a Constant Flux Constant Sedimentation model on the R package *serac* (Bruehl and Sabatier, 2020).

Pollen and *Sporormiella* sp. Spore Analysis

Forty-eight sediment subsamples of 1 cm^3 were taken at a mean temporal interval of ca. 40 years for pollen and Non-Pollen Palynomorph (NPPs) analyses. As far as possible, event layers were avoided during the sampling in order to avoid the taphonomic problems caused by the different origins of such sediment deposits. Samples were prepared using the standard procedure described by Faegri and Iversen (1989). *Lycopodium clavatum* tablets were added to each subsample (Stockmaar, 1971) in order to calculate *Sporormiella* sp. (HdV-113) spore concentrations. Pollen grains were identified using atlases of European and Mediterranean pollen grains (Reille, 1992; Beug, 2004). The identification of *Sporormiella* sp. is based on several published photographs (van Geel and Aptroot, 2006; Cugny et al., 2010) and follows the nomenclature established by Miola (2012). *Sporormiella* spores were counted following the procedure described by Etienne and Jouffroy-Bapicot (2014) and expressed in accumulation rates ($\text{no.cm}^{-2}\text{.yr}^{-1}$). At least 500 pollen grains of terrestrial plants (TLP: Total Land Pollen) were identified. Pollen counts were expressed as percentages of TLP, excluding pteridophytes, aquatic plants and *Cannabis/Humulus* pollen-type from the total pollen sum. Pollen data have been divided into six Local Pollen Assemblage Zones (LPAZ) by constrained cluster analysis (CONISS) using Tilia software (Grimm, 1987). In order to identify the main changes in plant community composition according to the cultural periods, a non-metric multidimensional scaling (NMDS) was performed using the Bray-Curtis dissimilarity indices with the *metaMDS* function from the VEGAN package v2.5.6 in R (Kruskal, 1964; Legendre and Legendre, 2012; Oksanen et al., 2013). This analysis was performed including three dimensions and a color code was used to distinguish the main cultural periods.

DNA Analysis: From the Sampling to the Data Treatment

Twenty-four sediment slices, each 1 cm-thick, were taken with a temporal interval fluctuating between 15 and 120 years (mean 75 years). As in the case of pollen, event layers were avoided during sampling. The edges were removed to avoid contaminations and were used to determine the sediment water

TABLE 1 | ^{14}C dates for Lake Aiguebelette related to AIG16 master core depth.

Lab. code	Samples	Material Dated	Composite MCD depth (mm)	MCD Depth without ID (mm)	Uncalibrated Age (BP)	Uncertainty	Calibrated age cal. BP (2 σ)
Poz-91100	AIG16-08a 68-70	Plant remain	742	709	80	30	25–260
Poz-91101	AIG16-08a 115	plant remain	1202	1162	470	30	495–540
Poz-91102	AIG16-08b 25-27	Plant remain	1838	1762	1115	30	938–1171
Poz-91103	AIG16-08b 66-68	Plant remain	2248	2158	1640	35	1415–1615

content. This measurement allowed the estimation of the dry sediment quantity used for the DNA extraction. This quantity varies between 1 and 4.4 g of dry sediments. The protocols applied for the sampling and the DNA extractions are described in detail on Protocols.io¹. To summarize, we mixed the wet sediments with a phosphate buffer (for 1 g of dry sediment we added 2 ml of phosphate buffer) for 15 min in a falcon tube. After a first centrifugation, supernatant was transferred to Amicon® ultra-15 10k centrifugal filters (Millipore) and centrifuged again to concentrate DNA and increase the detectability of DNA fragments of interest (Bremond et al., 2017; Capo et al., 2021). Between 500 and 700 µl of DNA extract were recovered and 400 µl was used as starting material for the DNA extraction, using a NucleoSpin® Soil kit and starting at binding step (Macherey-Nagel, Düren, Germany). Then, all of the DNA extracts were quantified by using the Quantifluor® ONE dsDNA system from Promega. Four extraction controls were performed.

The extracted DNA was then amplified using primers *g* and *h* for plants (Taberlet et al., 2007) and MamP007 (in combination with a human-specific blocking primer) for mammals (Giguet-Covex et al., 2014). For each sample and marker, nine PCR replicates were performed in order to improve the detection of rare taxa and the robustness of the data (Ficetola et al., 2015). Two negative PCR and two positive PCR controls were included. For the plants, the positive PCR controls contained 0.18 ng of DNA from a subtropical/tropical plant from the Melastomataceae family. For the mammals, the positive PCR controls contained 0.18 ng of DNA extracted from a marsupial (*Didelphis marsupialis*) that does not exist in Europe. The sequencing runs were performed by 2 × 125 bp pair-ends sequencing on an Illumina HiSeq 2500 platform. The sequences were filtered using the OBITOOLS software² (Boyer et al., 2016) and following the procedure described in Giguet-Covex et al. (2019). Reference databases used for the assignments were built from the EMBL database with the *ecoPCR* program (gh database created from release r126, mamP007 database from release r133). For the plant DNA, only DNA sequences with a > 95% similarity with a sequence in the reference database were kept. For mammals, after having removed all sequences assigned to Hominidae, only DNA sequences with a > 97% similarity with a sequence in the reference database were kept. Additional filtering steps, relating to low or stochastic detections (minimum of five reads per PCR and in two sample replicates or one replicate but in two consecutive samples) and potential contaminations (based on the extraction and PCR controls and exotic assignments), were performed (Giguet-Covex et al., 2019). For the mammals, only one taxon was frequently detected in the negative controls (in 31 over 53) and excluded from the dataset. It is also assigned to an exotic taxon, the *Meleagris gallopavo*. Among the six remaining taxa, two were assigned to water birds (*Fulica atra* and *Anas platyrhynchos*), two to fish (*Salvelinus* sp. and *Rutilus* sp.), one to an exotic rodent (*Thryonomys swinderianus*) and one to a marine worm (*Phascolosoma esculenta*). These last two taxa are the most detected in the sediment record and because

they were not frequently detected in the controls (in less than 5 over 53), we checked the suspicious assignments with Blast. The results proposed a 100% of identity with *Sus scrofa* for the rodent and with *Bos taurus* for the marine worm, which is highly plausible in our context. In this manuscript, we use only these mammals to document the past farming history. However, we note that the detection of taxa other than mammals with the primer pair MamP007 has already been demonstrated (Annelida, Lammers et al., 2018). Moreover, when we blast the primers, bony fish can also clearly be amplified. For the plants, no taxa were frequently detected in the controls (no more than four positive controls over 53). However, over the five sequences detected in four controls, two correspond also to exotic taxa (*Cedrus* sp. and Caricaceae) and were excluded from the dataset. The other excluded exotic taxa are *Grubbia rosmarinifolia*, *Passiflora* sp., Araceae, Millettieae, Musaceae, and *Persicaria* sp.

The results for each taxon are presented in numbers of positive PCR replicates, and for plants, we also integrated the sum of DNA reads. To highlight changes in groups of plants associated (or potentially associated) with human activities, we also calculated the proportion of DNA reads [log-transformed reads due to the exponential multiplication of the DNA sequences during the PCR; $\text{Log}(N \text{ reads} + 1)$] represented by each of these groups, i.e., (1) trees and shrubs, (2) ruderal and nitrophilous plants related to pastoral activities and/or hay meadows (*Urtica* sp., *Plantago* sp., *Rumex* sp., *Rhinanthus alectorolophus*, *Dactylis glomerata*) and (3) cultivated taxa (fruit trees, cereals, hemp, hops, several garden plants, and associated weeds). Based on the sum of the DNA reads from the sample replicates, we determined the number of detected taxa. A taxon was considered in the calculation when the number of reads was higher than 45 (i.e., 5 reads × 9 replicates). As in the case of pollen data, a non-metric multidimensional scaling (NMDS) was performed using the Bray-Curtis dissimilarity indices with the *metaMDS* function from the VEGAN package v2.5.6 in R. For this analysis, we summed the number of reads of three PCR replicates to finally have three replicas per sample and so decrease the stochasticity of the PCR success. This analysis was performed including three dimensions and a color code was used to distinguish the main cultural periods.

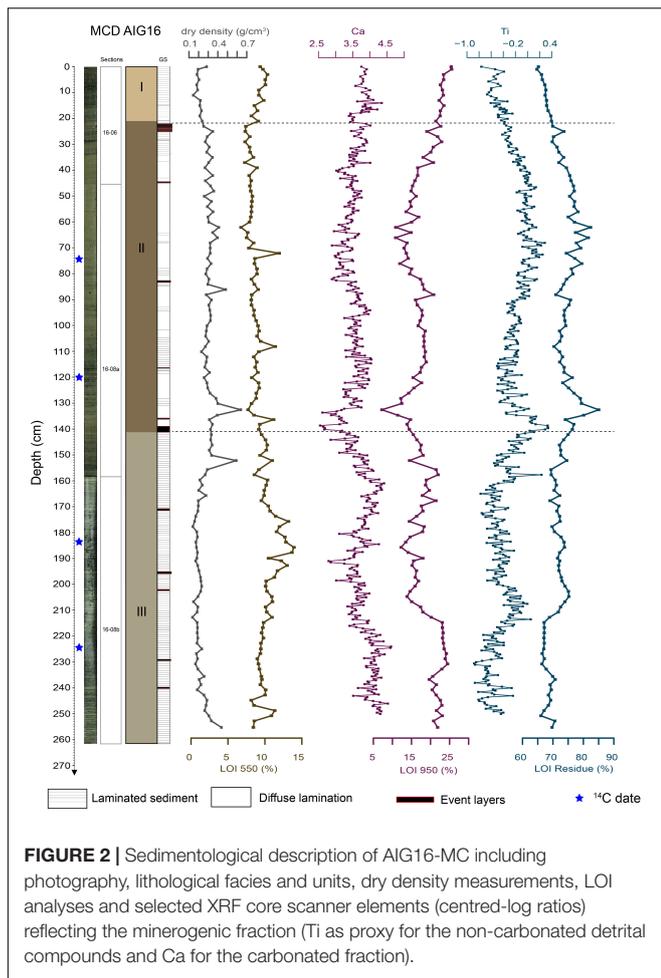
RESULTS AND INTERPRETATIONS

Sedimentary Units

The description of AIG16-MC led to the identification of different units characterized by thin laminae and interrupted by event deposits. The regular millimetric laminae are made up of an alternation of beige, grayish and green laminae. At certain depths, we observed homogenous olive-gray deposits topped by light gray mm-thick clay caps. The base of these deposits is sharp and presents high organic debris content but no visual grain size variation. Along the entire core, we observed 14 of these event deposits, with homogeneous facies ranging between 0.3 and 2.3 cm in thickness. These events are considered as instantaneous deposits (Sabatier et al., 2017) and were removed from the age-depth modeling. The origin of these deposits is currently being

¹ dx.doi.org/10.17504/protocols.io.bdws7ee

² https://git.metabarcoding.org/obitools/obitools/wiki/home



investigated (flood vs. earthquake induced deposit), but their nature (sudden erosive event) and composition mean that they fail to combine all data types for paleoecological studies. They were therefore not sampled and were omitted from the discussion regarding environmental variations.

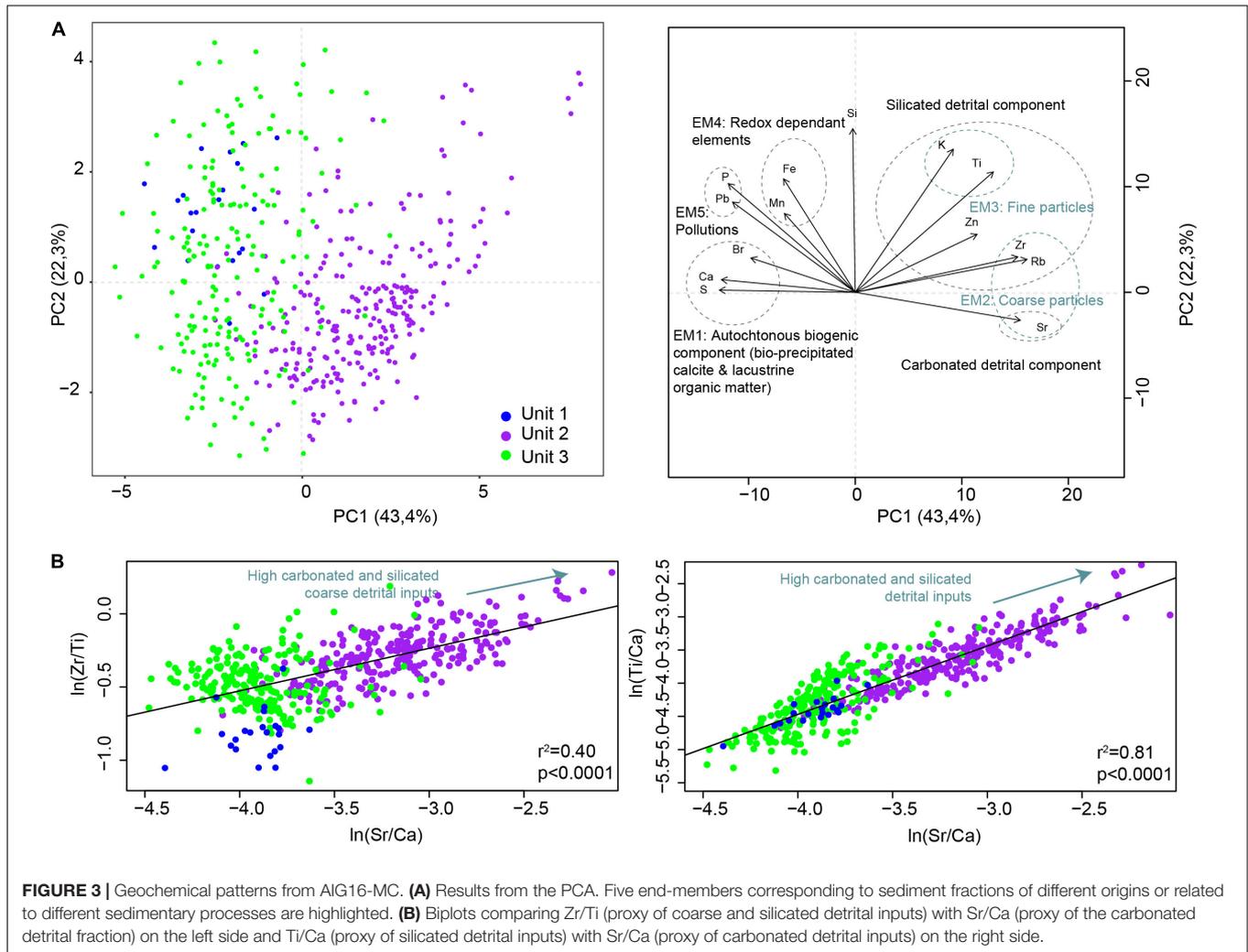
The identification of each sedimentary unit (U1, U2, and U3) is based on sedimentary characteristics such as color, grain size and sediment structures with lamination frequency and LOI contents (Figure 2). Unit 1 (U1: 0 to 21 cm) is characterized by a silty to clayey sediment with regular alternation of millimetric laminae. Mean organic content (LOI550) and carbonate content (LOI950) are relatively high with 9.3 and 23.3%, respectively. Two dark laminae appear in this unit. Unit 2 (U2: 21 to 141.2 cm) is composed of brown silty-clay sediment and presents an irregular and low presence of rhythmic lamination. Mean organic (8.6%) and carbonate (16.3%) contents are relatively low. This unit comprises 9 event deposits. It evolves progressively downwards into a more laminated and darker sediment. Unit 3 (U3: 141.2–261.6 cm) is composed of an alternation of light olive gray millimetric to sub-millimetric silty/clayey laminae. Mean organic (10.4%) and carbonate contents (19%) are slightly higher than those of U2. Five instantaneous deposits were identified in U3.

Geochemistry

A PCA analysis was performed on the following raw elements, measured with the Avaatech XRF core scanner: P, K, Ti, S, Ca, Fe, Mn, Br, Zr, Rb, Zn, Sr, and Pb. The first axis explains 43.4% of the total variance and the second, 22.3% (Figure 3A). Five different end-members can be identified. With negative loading on the first PCA axis, Ca, S, and Br form an end-member (EM1) reflecting the autochthonous biogenic production made up of bio-precipitated calcite and lacustrine organic matter, as Br content is related to lake organic matter (Lefebvre et al., 2021). With positive loading on PC1, there are the carbonated and silicated coarse detrital components characterized by Sr and Zr, respectively (EM2). With positive loading on both the first and second axes, we find the siliclastic fine detrital component made up of K and Ti (EM3). With negative loading of the first axis and positive of the second axis, the redox-dependent elements Fe and Mn are grouped together (EM4). Between this end-member and that of biogenic production, there is a last end-member (EM5) corresponding to elements typical of lead (Pb) and nutrient (P) pollution. The silicium (Si) has a positive loading on the second PCA axis. Its position between the detrital and biogenic components suggests a mixed origin, from both clastic inputs and diatoms. The first unit (U1) is mostly characterized by the pollution end-member, i.e., EM5. In the second unit (U2), the geochemical signature is dominated by the inputs of coarse carbonated and silicated detrital particles, as indicated by the PCA analysis and the bi-plots (Figure 3B). In unit 3 (U3), some depths are characterized by very low detrital inputs and are dominated by autochthonous production (especially bio-induced calcite), while others are slightly enriched in fine and silicated detrital inputs and redox-dependent elements as indicated by the PCA analysis and the bi-plots (Figure 3). Based on the PCA results and in order to better qualify the erosion dynamic, we selected three ratios, (i) $\ln(\text{Ti}/\text{Ca})$ reflecting the silicated relative to the carbonated detrital inputs, (ii) $\ln(\text{Sr}/\text{Ca})$ reflecting the carbonated detrital inputs relative to the bio-precipitated calcite, and (iii) $\ln(\text{Zr}/\text{Ti})$ reflecting the coarse relative to the fine silicated detrital inputs, and we compared them with each other (Figure 3B). The significant and high correlation ($r^2 = 0.81, p < 0.0001$) between $\ln(\text{Ti}/\text{Ca})$ and $\ln(\text{Sr}/\text{Ca})$ suggests a common origin for the silicated and carbonated detrital materials. $\ln(\text{Zr}/\text{Ti})$ and $\ln(\text{Sr}/\text{Ca})$ ($r^2 = 0.81, p < 0.0001$) are also correlated, but the lower correlation suggests the contribution of a distinct source of coarse silicated materials.

Chronology

The excess ^{210}Pb downcore profile plotted on a logarithmic scale revealed well constrained linear trends providing a mean sedimentation rate of $2.44 \pm 0.1 \text{ mm.yr}^{-1}$ for the upper 22 cm (Figure 4). The ^{137}Cs profile presented two peaks, at 4.5 and 9.5 cm, which correspond respectively to the Chernobyl accident in 1986 AD and the maximum of Nuclear Weapon Tests (NWT) in the Northern Hemisphere in 1963 AD in this region (Sabatier et al., 2014). This interpretation is confirmed by the ^{241}Am peak



attributed to the NWT (Appleby, 1991; **Figure 4**). The first ¹³⁷Cs fallout is observed at 13.5 cm and corresponds to 1955 AD (Robbins and Edgington, 1975). Ages were then calculated using the CFCS model thanks to *serac* R package (Bruehl and Sabatier, 2020) to provide a continuous age-depth relationship (**Figure 4**).

A total of four terrestrial organic macroremains were dated by ¹⁴C dating (**Table 1**). The 14 deposits interpreted as event deposits (ED), representing a total of 9.9 cm, were removed. The remaining 251.7 cm were used to build an event-free sedimentary record (Goldberg, 1963; Wilhelm et al., 2012). We then calculated an age depth relationship by a smooth spline interpolation using the R code package “*Clam*” (Blaauw, 2010). The vertical bars represent the age of event deposits (**Figure 4**). This sediment sequence from Lake Aiguebelette covers the last 1816 ± 65 yr cal BP. The mean sedimentation rates estimated from the event-free sediment record vary between 3.2 and 0.9 mm.yr⁻¹ with a higher sedimentation rate over the upper 60 cm (last 230 years).

Pollen and *Sporormiella* sp.

The NMDS analysis applied to pollen data (**Figure 5A**) highlights the difference (along the first axis) between samples

corresponding to Roman and Early Middle Ages periods, and samples corresponding to the subsequent periods. In fact, samples dated to the Roman period and the Early Middle Ages are characterized by an abundance of trees, such as *Fagus* and *Ulmus* (**Figure 5B**), which drastically declined after 1050 cal CE (**Figure 6**). The samples dated to the High Middle Ages seem to represent a transition period (middle of the first Axis, **Figure 5A**), while samples dated to subsequent periods are marked by the amount of herbs (**Figure 5C**) and cultivated taxa they contain, apart that is from the very last samples (Contemporary), which are marked by the expansion of *Populus*, *Fraxinus* and the introduced taxon *Ambrosia* (**Figure 5C**).

Based on the pollen diagram (**Figure 6**), it is possible to describe 6 main palynozones, defined according to the CONISS analysis. As the age model is defined, all pollen data are presented relative to the chronology.

LPAZ 1 (120–550 cal CE)

The vegetation was dominated by arboreal pollen (**Figure 6**), AP/TLP percentages fluctuated between 80 and 95%. Four tree taxa recorded percentages above 10%: *Alnus* increased from 10 to

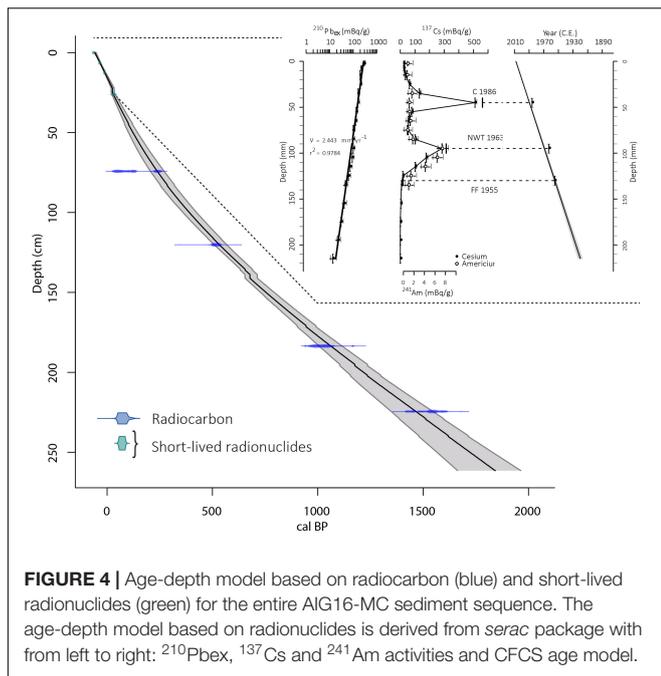


FIGURE 4 | Age-depth model based on radiocarbon (blue) and short-lived radionuclides (green) for the entire AIG16-MC sediment sequence. The age-depth model based on radionuclides is derived from *serac* package with from left to right: ²¹⁰Pb_{ex}, ¹³⁷Cs and ²⁴¹Am activities and CFCS age model.

35%, *Corylus* and *Quercus* decreased from 20 to 10% and *Fagus* oscillated between 12 and 20%. Percentages of *Betula*, *Pinus*, *Ulmus*, *Fraxinus*, and *Carpinus* were all within a range of 1–10%. Other trees and shrubs remained below 1%.

Among herb taxa pollen, Poaceae dominated and decreased from 10% to 2% before slightly increasing to 10%. Several ruderal weeds were recorded (*Plantago lanceolata*, *Rumex*-type, Urticaceae, *Artemisia*, Chenopodiaceae, *Plantago major/media*) and all percentages decreased from 250 cal CE before finally increasing at the end of the zone. In each subsample, *Cereal*-type and *Secale*-type were identified with percentages under 1%, while hemp pollen was recorded in some samples. The accumulation rate as well as the concentration of *Sporormiella* sp. stayed at low values (Figure 6).

LPAZ 2 (550–900 cal CE)

Alnus, the dominant taxon, decreased from 35 to 20% and reached its highest percentages of around 45% from 740 AD and then decreased once again from 800 AD (Figure 6). Percentages decreased and increased once again at the middle of the zone for *Betula*, *Corylus*, and *Carpinus*; and at the end of the zone for *Ulmus* and *Tilia*. Percentages of *Quercus* and *Fagus* remained at the same order of magnitude as in the previous zone. *Juglans* percentages exceeded 1% and rose to 4% around 650 cal CE. At the same period, peaks of *Cereal*-type, *Secale*-type, Poaceae, ruderal plants (*Rumex* sp. and *Urtica* sp.) and *Cannabis/Humulus* were also recorded. The concentration and the accumulation rate of *Sporormiella* sp. also showed a small peak at 50 no.cm⁻².yr⁻¹ around 700 cal CE.

LPAZ 3 (900–1300 cal CE)

Alnus decreased to 14%, increased to around 25–35% and then decreased again to 9% at the end of the zone (Figure 6).

Quercus remained stable at around 15%, except for a peak of 34% at 1270 cal CE. From 1050 cal CE, *Fagus* decreased from 16 to 7%, as did *Ulmus* and *Tilia* although to a lesser extent; at the same time, we observe an increase in *Juglans* and *Castanea* percentages.

In this zone, open areas were expanded (AP/TLP decreases from 90 to 75%), with an increase of Poaceae (to around 10%), ruderal plants and *Cannabis/Humulus*. A diversification of prairie and ruderal plants was also recorded. An important increase in *Sporormiella* sp. (accumulation rate and concentration) was recorded from 1200 cal CE (Figure 6).

LPAZ 4 (1300–1760 cal CE)

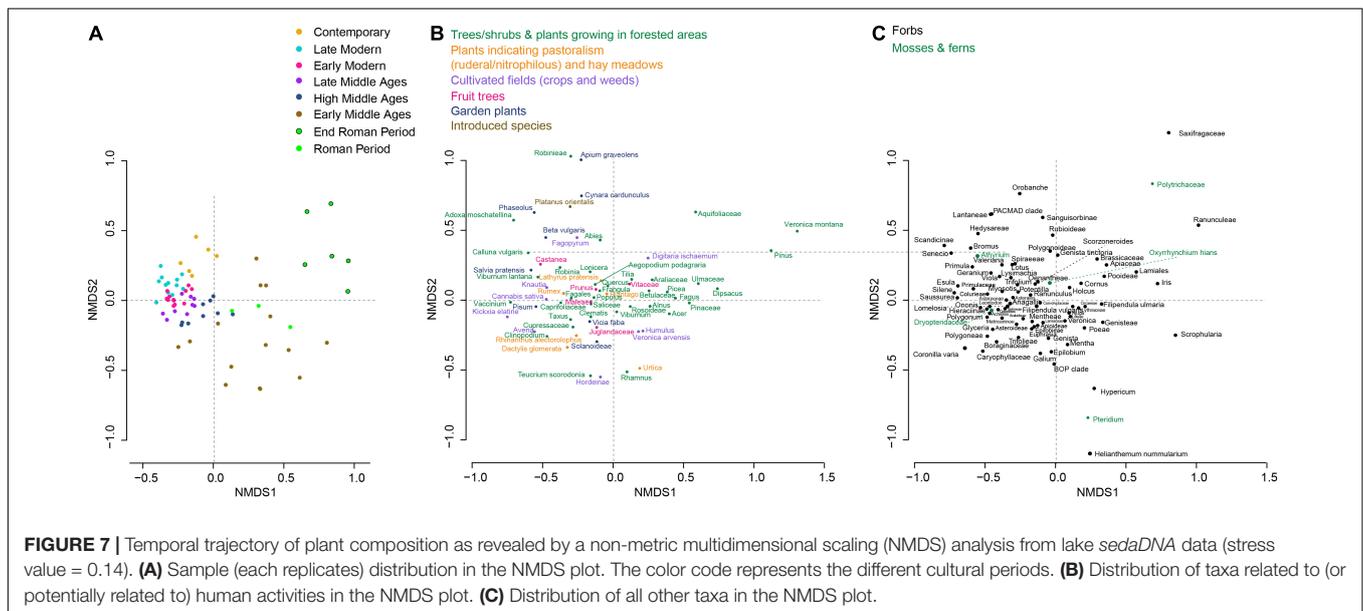
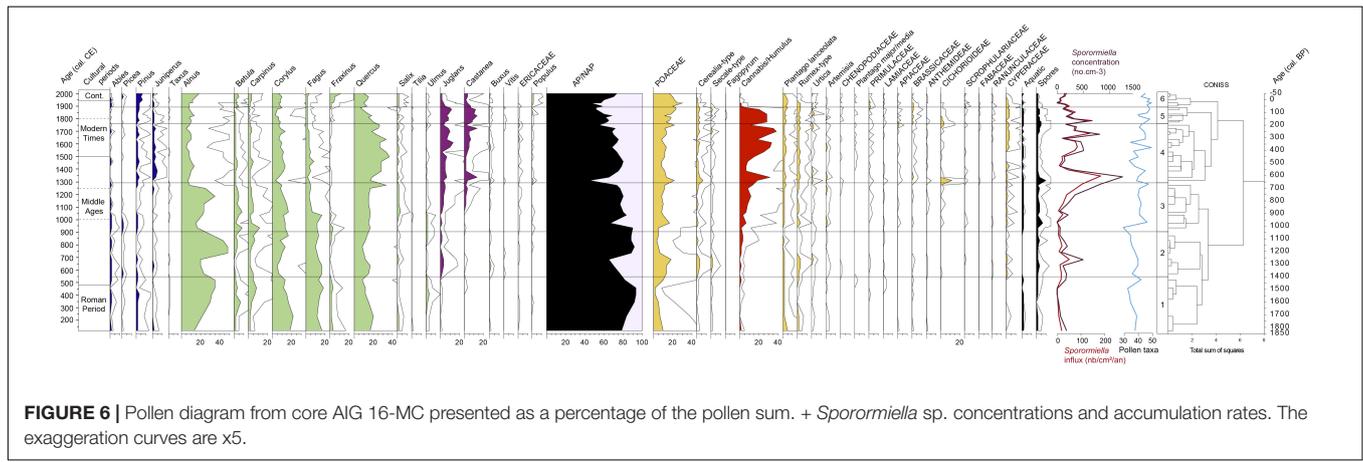
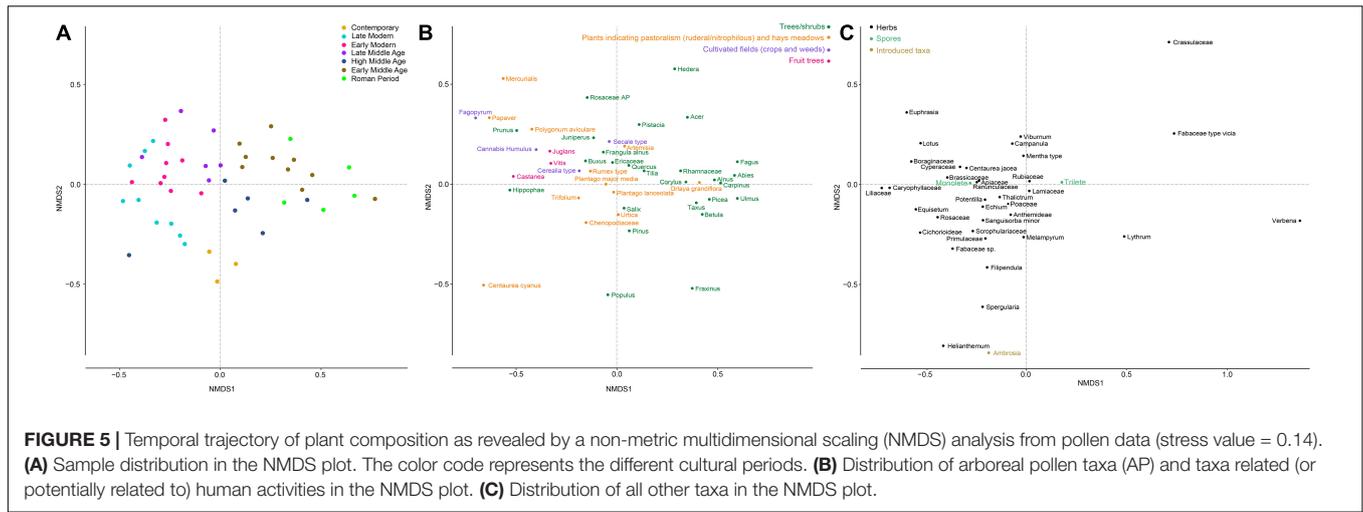
Quercus increased and became the dominant taxon with percentage values of around 20% and 35% (Figure 6). *Juglans* and *Castanea* percentages reached their highest values (13%). The percentages of *Juniperus* also increased. Most herb taxa remained at the same order of magnitude. Poaceae pollen grains were slightly more abundant than in the previous zone. It should be noted that at 1300 cal CE, a significant decrease in AP/TLP (to 45%) and peaks of *Cereal*-type, Poaceae and Cichorioideae percentages probably correspond to an assemblage of spores and pollen grains brought into the lake by a flood. This sample is an outlier in the NMDS analysis due to the high values for Cichorioideae (Figure 5A). It will not be included in the environmental interpretation. Excluding this particular sample, in general *Cereal*-type and *Secale*-type present values that are somewhat higher than those observed in LPAZ 3. Percentages of *Cannabis/Humulus* reached their highest values. Around 1350 cal CE, the accumulation rate and concentration of *Sporormiella* sp. reached its highest values (180 no.cm⁻².yr⁻¹) before decreasing to low values and then increasing again from 1550 cal CE (Figure 6).

LPAZ 5 (1760–1900 cal CE)

Quercus progressively decreased from 20 to 7% (Figure 6). The proportion of herbs increased (mean of AP/TLP decreased from 65 to 60%) mainly with the increase of Poaceae from 15% to 24%. The concentration and the accumulation rate of *Sporormiella* sp. remained relatively high (Figure 6).

LPAZ 6 (1900 cal CE to the Present Day)

Many tree taxa recorded an increase in their percentages, like *Salix*, *Alnus* (from 8 to 17%), *Betula*, *Pinus*, *Fraxinus* (from 1 to 12%), *Fagus*, *Carpinus* and *Populus* (Figure 6). On the other hand, *Juglans* and *Castanea* decreased from 10 to 1% and from 7 to 4% respectively. Among the herbs, Poaceae decreased from 30 to 15%. Cumulative percentages of ruderal plants decreased from 10 to 5% at the end of the zone, mainly due to decreases in *Plantago lanceolata* and *Rumex*-type. *Cereal*-type and *Secale*-type decreased also, from 4 to 1%, while *Cannabis/Humulus* percentages decreased sharply. The accumulation rate of *Sporormiella* sp. decreased before increasing again in the recent period (Figure 6).



Plant DNA

The filtering procedure yielded 2,305,749 DNA reads and 235 unique sequences. We grouped all unique sequences with identical assignments and finally obtained 174 plant taxa including 23 aquatic taxa. Within the 151 remaining terrestrial taxa, five were assigned to moss or ferns, six to fruit trees and shrubs, 17 to cultivated fields (cultivated plants and weeds) and vegetable gardens, five to plants associated with pastoral activity and/or hay meadows, 93 to herbaceous plants and 25 to trees and shrubs.

In order to highlight the main changes in plant community composition, an NMDS analysis was performed, and samples were grouped by different cultural periods (**Figure 7**). The most important shift appeared along the first axis and separated the Roman period and the Early Middle Ages from the following periods (**Figure 7**). It should be noted however, that the end of the Roman period can be distinguished (**Figure 7**). Unlike the pollen data, the samples related to the High Middle Ages are clearly closer to those related to the following periods. Hence, we can summarize the NMDS analysis into two main phases. Before 1050 cal CE (Roman period and Early Middle Ages), the plant community composition was characterized by the presence of many trees, shrubs, (e.g., *Pinus* sp., Aquifoliaceae, Ulmaceae, *Fagus* sp., *Picea* sp., *Alnus* sp., Betulaceae, *Hedera helix*, and *Tilia* sp.) and a number of herbaceous taxa, some of which were growing in forest or forest edge environments (e.g., *Veronica montana* and *Dipsacus* sp.). From 1050 cal CE (High Middle Ages to nowadays), the plant community composition shifted to cultivated plants and plants associated with pastoral activities as well as different herbaceous taxa. This tipping point also corresponded to the period after which the proportion of tree and shrub DNA reads decreased (**Figure 8**). A new increase only occurred in the last 50 years. In contrast to the pollen data, the proportion of DNA reads was always dominated by herbaceous taxa. The number of terrestrial taxa also strongly increased since 1050 cal CE, from 43 taxa on average (min = 20; max = 60) to 78 (min = 60; max = 90).

Before 1050 cal CE, two phases characterized by a small increase in the number of taxa and a decrease in the proportion of DNA reads from trees and shrubs were recorded, around 250 cal CE and between 500 and 800 cal CE (**Figure 8**). During these phases of the Roman period and Early Middle Ages, we see the development of plants associated with pastoral activity (*Plantago* sp., *Urtica* sp. and a few *Rumex* sp.). Fruit trees (Vitaceae, Juglandaceae, and Maleae) were recorded (**Figure 8**). In the first phase, *Vicia faba* and *Humulus* sp. were also detected. However, this phase was only represented by one sample.

From 1050 cal CE, a general trend toward a progressive intensification and diversification of agropastoral activities can be observed. In detail, from 1050 cal CE, *Castanea* sp., *Cannabis sativa* and *Vicia faba* appeared, while Vitaceae, *Prunus* sp. and *Humulus* sp. increased considerably (high N replicates). Then at 1300 cal CE, *Pisum* sp. and *Avena* sp. were introduced. Moreover, some plants associated with pastoral activities (*Lathyrus pratensis*, *Dactylis glomerata*, *Rhinanthus alectorolophus*; **Figure 7**) appeared or began to be detected

in large quantities (*Rumex* sp.). At that time, *Cannabis sativa* and *Humulus* sp. decreased. *Cannabis sativa*, *Prunus* sp. and *Castanea* sp. showed a new increase from 1500 to 1900 cal CE. During this period, *Avena* sp. and *Pisum* sp. decreased while *Fagopyrum* sp. started to be detected and *Vicia faba* appeared again. Finally, from 1850 cal CE, new taxa, i.e., *Phaseolus*, *Beta vulgaris*, *Cynara cardunculus*, and *Apium graveolens*, which we can see today in vegetable gardens were recorded. In this phase, most of the fruit tree taxa (Vitaceae, Juglandaceae, *Prunus* sp.) and *Humulus* sp. decreased and other crops (*Fagopyrum* sp., *Cannabis sativa*, and *Vicia faba*) disappeared.

Mammal DNA

Two taxa could be related to livestock farming activity, *Bos taurus* and *Sus scrofa*. They were detected almost throughout the record, but increased considerably from 1250 cal CE (**Figure 8**) as did the accumulation rate of *Sporormiella* sp. (**Figure 6**). These increases occurred when plants associated with pastoral activities (i.e., hay meadows or pastures; *Dactylis glomerata*, *Rhinanthus alectorolophus*) appeared or were detected in more PCR replicates (*Rumex* sp., *Plantago* sp.). However, the proportion of DNA from plants associated with pastoral activities remained relatively stable throughout the record. From this date, the DNA detection of *Bos taurus* presented an increasing trend until the present day, while *Sus scrofa* showed an important peak around 1350 cal CE and a long-lasting decrease between 1650 and 1950 cal CE.

DISCUSSION

Interpretative Framework of DNA and Pollen Records: Taphonomic Benefits and Drawbacks of Each Method

Pollen and DNA analyses have been combined in numerous paleoecological studies and for a variety of biomes (e.g., boreal, arctic, alpine, tropical, semi-arid). Both convergent and divergent results have been recorded (e.g., Parducci et al., 2013, 2017; Sjögren et al., 2017; Clarke et al., 2018; Giguet-Covex et al., 2019, in revision; Liu et al., 2020; Tabares et al., 2020). For instance, in boreal and arctic environments, it has been shown that pollen and DNA records were more similar in lakes with large catchment areas compared to those with smaller catchments, as these catchments cover a more regional scale pollen source (Alsos et al., 2016; Clarke et al., 2018). In our context, plant DNA and pollen records provided very similar stories, but essentially for taxa associated with agricultural and pastoral activities, i.e., for taxa growing in open areas and where soils are worked, trampled and therefore are more exposed to erosion processes. In contrast, for trees and shrubs, i.e., in areas where erosion is more reduced, we observed almost no concordance (**Supplementary Figure 4**). It has been demonstrated that extracellular DNA (i.e., the DNA fraction targeted in the study) is quickly bound to particles such as clays, which protects the molecules from microbial degradation (Blum et al., 1997; Kanbar et al., 2020). This mechanism means that we can expect to find good

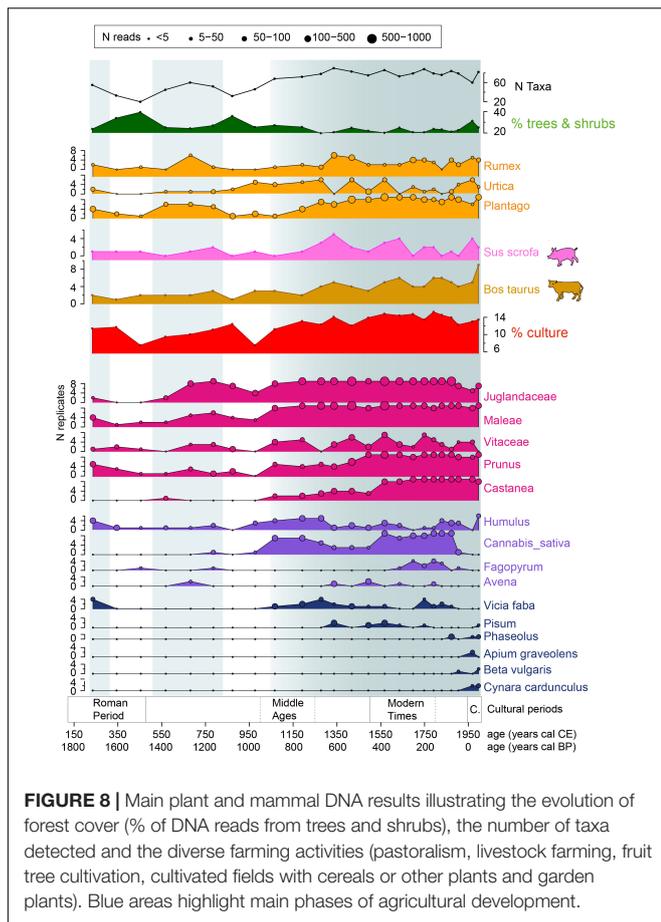


FIGURE 8 | Main plant and mammal DNA results illustrating the evolution of forest cover (% of DNA reads from trees and shrubs), the number of taxa detected and the diverse farming activities (pastoralism, livestock farming, fruit tree cultivation, cultivated fields with cereals or other plants and garden plants). Blue areas highlight main phases of agricultural development.

DNA records, in terms of spatial representativeness, in erosive contexts and periods (Giguët-Covex et al., 2019). In contrast, in forested areas (and during forested periods) where erosion is more limited, a poor spatial representativeness of taxa is to be expected which explains the poor similarities between the DNA and pollen records for several forest taxa. Moreover, the proportion of forested lands may be underestimated by the proportion of DNA reads from trees and shrubs. This assumption is verified at least for the most recent sample (25% in the 1990s compared to the 55% shown by the Corine land cover data, see **Figure 1**), where pollen analysis revealed 70% arboreal pollen (a bit overestimated).

Given the taphonomic processes outlined above and because the landscape and erosion dynamic can change markedly, especially in man-made contexts, changes in tree/shrub taxa compositions must be interpreted with caution, while the evolution of anthropogenic taxa can be considered as robust. However, while it is difficult to use DNA as a proxy of tree cover, it can be very useful for confirming the presence of a specific tree/shrub taxon in a watershed. Indeed, taxa detected by DNA are necessarily present in the catchment area and probably close to the lake border and main tributaries (especially riparian species) (Sjögren et al., 2017; Alsos et al., 2018) and at the edges of open areas.

In contrast to *sedaDNA* data and as shown for the most recent sample, percentages of tree and shrub pollen grains are often overestimated relative to the real forest cover. This phenomenon is accentuated when the landscape becomes more open because of the increase in long-distance pollen inputs (Crump et al., 2019). Indeed, because most tree pollen grains are transported by winds, the eternal question is: is the pollen the echo of a regional vegetation pattern or of local changes? This obstacle can be overcome with different approaches. When they are preserved in lake sediment, the macrofossil remains, combined with pollen can help to disentangle local and regional vegetation. Another solution would be to use current modeling approaches to modulate the overrepresentation of tree pollen, based on pollen productivity estimates (Sugita, 2007a,b). The overestimation of the forest cover in pollen assemblages is accentuated by the poor representation (or the absence) of low pollen-producing entomophilous taxa such as forbes (Clarke et al., 2018). However, these entomophilous taxa are better represented in lake *sedaDNA* datasets (Parducci et al., 2017; Clarke et al., 2018, etc.). They are also often key ecological taxa or informative taxa relating to agricultural or pastoral activities and practices.

New Insights Into Paleoecological Interpretations and Human History Based on the Combination of Pollen and DNA Analyses

The different abilities of DNA, coprophilous fungal spores and pollen grains to mirror the development of trees, crops, vegetable cultivation and pastoral activities, makes their combination particularly effective for the reconstruction of past human activities and impacts.

The ability of DNA analysis to detect entomophilous taxa is a major bonus in paleoecological studies. It offers the opportunity to identify cultivated fruit trees, such as *Prunus* sp. (potentially plums and or cherries) and Maleae (potentially apple and/or pear). For instance, in the Aiguebelette record, the development of orchards is highlighted during medieval times and in the modern period (**Figure 8**). While *Vitis* was not identified through pollen analysis, numerous DNA sequences of Vitaceae are recorded for the Middle Ages (**Figure 8**).

DNA analysis also offers a new perspective for the identification of vegetable crops, such as beans. At Aiguebelette, this allows us to identify the local cultivation of *Vicia faba* from 1050 cal CE and *Pisum* sp. from 1350 cal CE (**Figure 8**). The present study brings to light this cultivation for the first time in a natural archive. It is worth noting that this interpretation of our dataset is consistent with documented medieval practices (Colardelle and Verdel, 1993; Ruas, 1997; Ferault and Le Chatelier, 2012). DNA approaches also allow the detection of new species, such as *Apium graveolens*, *Beta vulgaris*, *Cynara cardunculus* or *Phaseolus* sp, from 1900 cal CE in local cultivation.

In many pollen records from Western Europe, the *Cannabis/Humulus* pollen type is widely recorded from the last two millennia (Doyen, 2012). Most of the time, it is interpreted as the signal of hemp cultivation, but actually it is not

easy to determine whether it reflects the growing of hemp and/or hops. This question can be resolved using DNA analysis because the technique allows us to distinguish between *Humulus* sp. and *Cannabis* sp. In the Aiguebelette sequence, the DNA analysis confirms that the high percentages of *Cannabis/Humulus* pollen type from 1050 cal CE reflect hemp cultivation (and certainly hemp retting in lake water). At the same time, the DNA analyses also demonstrate an important increase in *Humulus* (Figure 8), revealing that hops were grown too, probably for brewing. An identical decline in *Cannabis/Humulus* pollen percentages and *Cannabis* sp. DNA is concomitantly recorded from approximately 1850 cal CE and reflects the cessation of hemp cultivation in the watershed.

In paleoecological studies, cereal growing is usually recorded by two pollen types, namely “Cerealia-type” and “Secale-type.” Unfortunately, the plant DNA primers used for amplifications are not sufficiently resolutive for the Poaceae family (Yoccoz et al., 2012; Parducci et al., 2017), which means that the information provided by DNA about cereal cultivation is very limited. That represents a real weakness of *sedDNA* analysis based on the *gh* primer, but this could be overcome by using a Poaceae-specific primer targeting the internal transcribed spacer region 1 (ITS1) of nuclear ribosomal DNA (Ait Baamrane et al., 2012; De Barba et al., 2014). Nevertheless, DNA approaches highlight the cultivation of more sporadically cultivated plants such as *Avena* sp. (oat) and *Fagopyrum* sp. (buckwheat), which display their highest values between 1650 and 1850 cal CE at Aiguebelette.

The evolution of pastoral pressure is generally described in pollen diagrams using the evolution of several herbaceous taxa, combining apophytes directly favored by pastoralism (*Plantago lanceolata*, *Rumex acetosella*, etc.) and other taxa which contribute to both pasture and hay meadows (Poaceae, Anthemideae, Cichorioideae, etc.). The analysis of strict coprophilous fungal spores (i.e., *Sporormiella* sp., *Podospora* sp.) archived in lacustrine sediments provides a more specific and very useful indicator of the abundance of herds at the watershed scale (Etienne et al., 2013). The DNA analysis is fully complementary as it provides information about the livestock composition (Giguët-Covex et al., 2014). In the Aiguebelette sequence, the pollen curves of *Plantago* and *Rumex* reveal that grazing occurred throughout the record but they do not allow us to identify specific periods of increasing pastoralism, except for the last century (Figure 6). However, the curve of *Sporormiella* concentrations and accumulation rates, and mammal DNA from cattle and pigs match well and highlight phases of intensified livestock farming which are discussed below (Figure 9).

Multi-Proxy and Multi-Record Approaches for a More Comprehensive History of Land-Use in Perialpine Areas

Pollen studies have been performed on two lake systems located only a few tens of kilometers away from Lake Aiguebelette within the same geographical and geomorphological context (Supplementary Figure 5): Lake Moras (43 km, Doyen et al., 2013) and Lake Paladru (20 km, Doyen et al., 2016). These three

records (including Aiguebelette) display similar dynamics for the main tree taxa, as well as for the main agricultural and pastoral pollen indicators (Figure 10). These similarities can be explained by two main hypotheses: (i) the three lakes record the same regional pollen deposition due to overlapping RSAP (Relevant Source Area of Pollen) and their fairly large lake surface areas; (ii) the local environmental dynamics, agro-pastoral activities and socio-cultural histories were very similar within this small geographical region (i.e., they had similar trajectories).

The aim of the following sections is to put into perspective the history of the vegetation and anthropogenic activities in this area based on the pollen records from lakes Paladru, Moras and Aiguebelette, and to potentially identify the local specificities of the Aiguebelette socio-ecosystem trajectory. In that context, analyzing DNA and coprophilous fungal spores, as well as sedimentary processes ongoing in the Aiguebelette watershed, is particularly relevant for deciphering the local history of the Aiguebelette basin. This combination of data will also help us to discuss the role of cultural and climatic forcing on the erosion dynamic in the watershed of Lake Aiguebelette.

The End of the Roman Period, Late Antiquity (150–500 cal CE)

The three pollen sequences record a decline in agriculture and an increase in tree cover (Figure 10). The DNA record from Aiguebelette reflects the same trend at the local scale and provides new data on what crops were declining in the area. Indeed, the DNA reveals the existence of orchards (apples or pears and plum or cherry trees) and the cultivation of broad beans (*Vicia faba*) and probably hops, during Antiquity, but all declined from the IInd century (Figure 9). Like the pollen records, the DNA data from Aiguebelette records a recovery of forests, probably due to a decrease in human impact. The rise in pioneer trees, such as *Betula* and *Alnus*, recorded by pollen, probably indicates the recolonization of abandoned pastures, attested to by the decline in Poaceae and taxa associated with pastures (*Plantago*, *Rumex* and *Urtica* in pollen and DNA records, Supplementary Figure 3). This phase of low human activity and forest recovery is accompanied by low detrital inputs as shown by low $\ln(\text{Ti}/\text{Ca})$ and $\ln(\text{Sr}/\text{Ca})$ and low silicated detrital flux (Figure 9). However, a small increase in $\ln(\text{Zr}/\text{Ti})$, which represents a proxy of the silicated coarse detrital inputs, is recorded and may have been caused by the increase in precipitation recorded in Europe (Figure 9; Büntgen et al., 2011).

In the western Alps, the period of prosperity, characteristic of classical Antiquity (e.g., several sites have been identified to the south of Lake Aiguebelette, Nieloud-Muller, 2019), is followed by a phase of declining human impact and reforestation (Giguët-Covex et al., 2011, 2014; Doyen et al., 2013; Bajard et al., 2016). It is also well-recognized in other pollen records from the perialpine region (Clerc, 1988), as well as from other regions of the Alps. On the Swiss Plateau, pollen studies document a reduction in agricultural activities around the VIth century, accompanied by a short period of renaturation (Tinner et al., 1996, 2003). In Western Europe, this phase is a period of political turmoil, corresponding to the demise of the Roman Empire and the “Barbarian invasions,” also called the “Migration Period.” It is

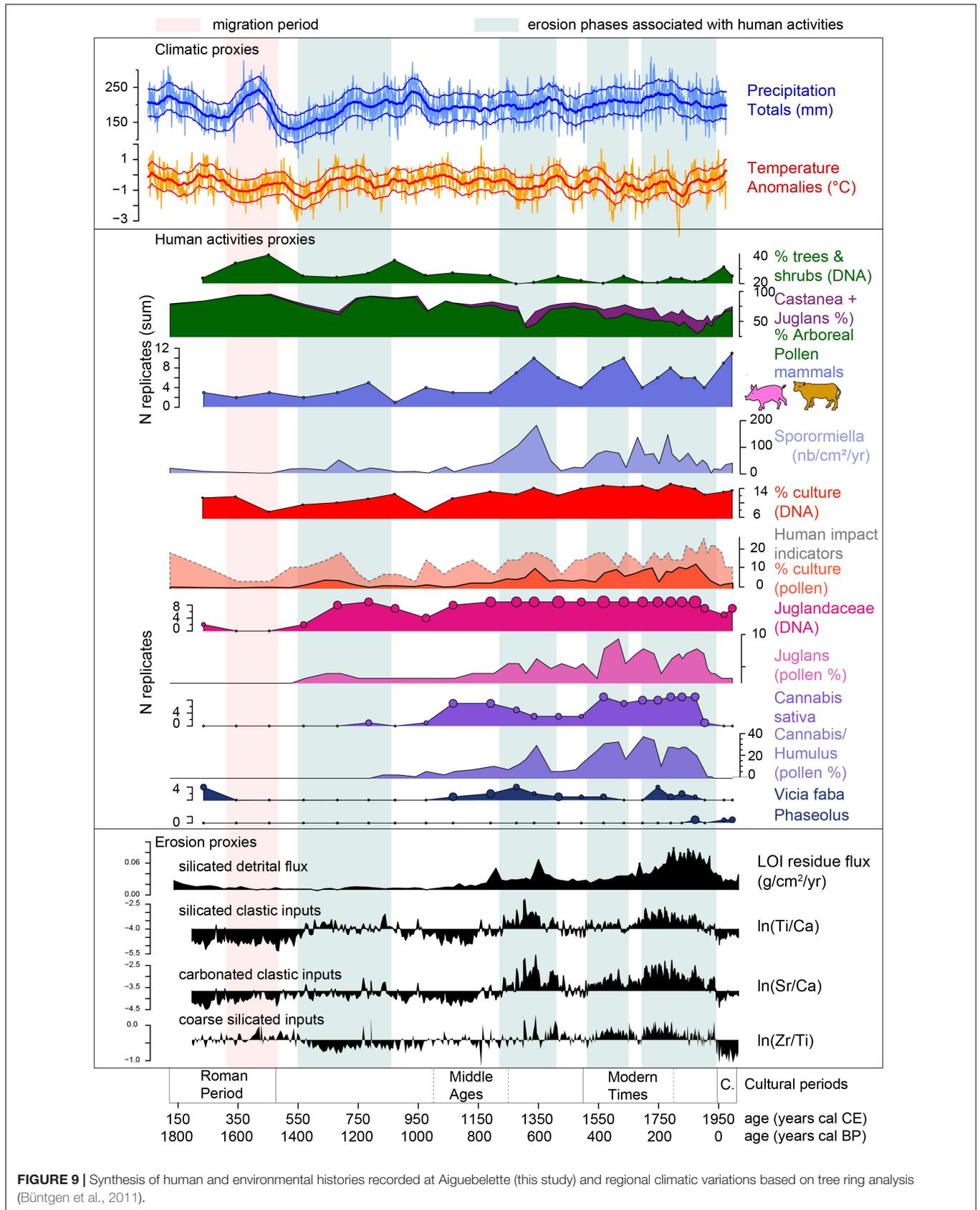
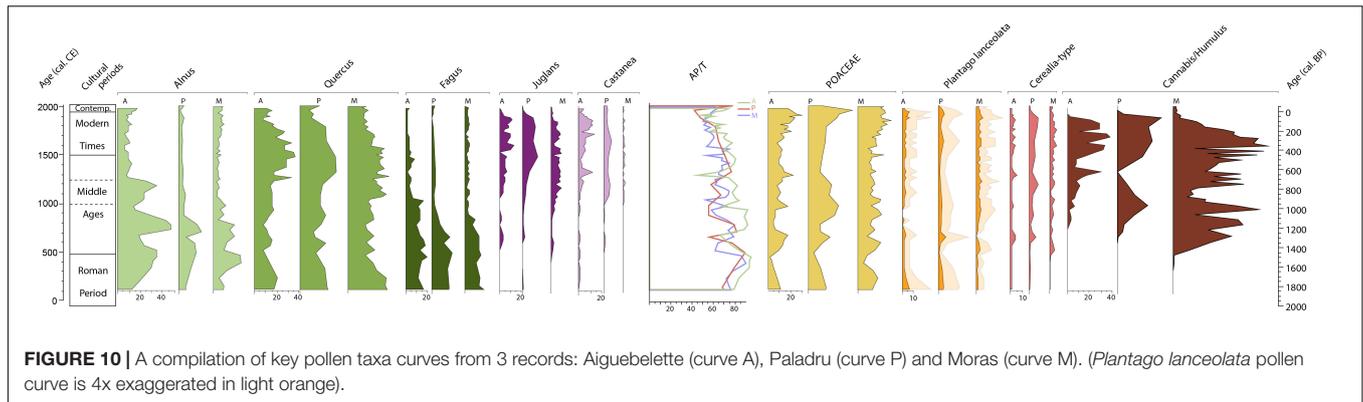


FIGURE 9 | Synthesis of human and environmental histories recorded at Aiguebelette (this study) and regional climatic variations based on tree ring analysis (Büntgen et al., 2011).



sometimes considered to be the greatest historical crisis to have occurred in central Europe. This socio-economic destabilization implied a reduced anthropogenic impact on the vegetation due to less farming activity and reduced tree harvesting (Rösch, 1992; McCormick, 2001). Additional hypotheses (other than socio-economic) have already been proposed to explain the decline in farming. Among these, the decrease in temperature and the rise of precipitation, from the mid-IIIrd century to the VIth century (Büntgen et al., 2011), could have significantly impacted agricultural production (Figure 10). An additional cause of the general degradation of production conditions might be the over-exploitation of farmland from the late Iron Age to the end of the Roman empire, which in turn would have generated the first major destabilization of the arable lands by erosion (Giguet-Covex et al., 2011, 2014; Doyen et al., 2013; Arnaud et al., 2016; Bajard et al., 2016).

Beginning of the Middle Ages (500–1000 cal CE)

In the three regional pollen records (Figure 10), the pollen values for grasses (and to a lesser extent for *Plantago* and *Rumex*) reflect an intensification of grazing. The local pastoral activity is confirmed at Aiguebelette by the DNA values of *Plantago* and *Rumex*, by the reappearance of coprophilous fungus spores (*Sporormiella*, Figure 9), and by a small increase in the detection of *Bos taurus*, all highlighting greater pastoral pressure in the watershed. This phase is also characterized by a similar decreasing pattern for *Alnus* pollen curves for the three regional records (a decrease from 500 to 650 cal CE, an increase from 650 to 800 cal CE and a main decrease from 800 to 950 cal CE) that could be interpreted as the result of human impact on wetlands and lake edges occupied by alder. It should be noted that the variations in *Alnus* are not easy to interpret because this tree grows in specific areas (swamps on waterlogged soils) directly connected to the lake. The very high quantity of *Alnus* DNA reads all along the sequence (higher values among all taxa, Supplementary Figure 2) reflects this specific situation. However, the DNA data records a slight decrease in *Alnus* reads from 800 to 1000 cal CE (Figure 8), probably confirming the decline of *Alnus* in the Aiguebelette watershed. In the pollen records, other trees such as *Fagus* and *Quercus* (Figure 6) are not affected by deforestation at this time, showing that it is possible that the humid lowlands previously occupied by *Alnus* may have been subjected to human

pressure. The decline of swamps due to human impact has already been observed in the region (Clerc, 1988; Bernigaud, 2012). These areas could have been used for cultivation and pastures and probably to manage hay meadows, because of their waterlogged soils (Mazoyer and Roudart, 1997).

At the same time, the rise of both *Cerealia* and *Secale* reflects the development of cereal cultivation. A similar increase in cultivation has been observed in the Early Middle Ages on the Swiss Plateau (Tinner et al., 2003; Soepboer et al., 2010). The diversification of farming activities is attested by the DNA record which reveals the growing of fruit trees, such as apple and/or pear, plum and/or cherry and walnut trees, in the Aiguebelette area. Viticulture also developed during this phase. According to pollen records (Figure 6), it seems that walnut trees were cultivated in the region. The DNA data from Aiguebelette display significant values for Juglandaceae confirming local cultivation of walnut. The end of this phase (700–1000 cal CE) is characterized in all three pollen diagrams by an important re-extension of alder groves. This marked increase in *Alnus* could reflect the decline in human activities in the lowlands (Berger, 2010; Bernigaud, 2012), allowing the rise in *Alnus*.

Between 550 and 800 cal CE, i.e., during the maxima of landscape opening and farming activities, a first and low increase in erosion is evidenced principally by ln(Ti/Ca) (Figure 9). This phenomenon is also recorded at lakes Paladru (Simonneau et al., 2013; Doyen et al., 2016) and Annecy (Jones et al., 2013) and may have been slightly accentuated by a trend of increasing summer precipitation as reconstructed from tree rings in central Europe (see Figure 9, Büntgen et al., 2011).

From 1000 to 1350 cal CE, the End of the Middle Ages

From 1000 cal CE, the three regional pollen sequences show a diversification of farming practices, with the development of cereal and hemp growing (Figure 10). The DNA analysis at Aiguebelette also shows an important increase in *Cannabis* and *Humulus*, revealing that hemp was widely cultivated for textiles and was processed (retting) at Aiguebelette. DNA data show that hops were also abundant. The DNA from *Humulus* could come from natural hops growing in the surrounding wetlands, but its significant presence in the record could also reflect its cultivation for brewing. The production of beer during

the Middle Age is already well known from historical archives (Poelmans and Swinnen, 2011).

The rise of *Plantago*, *Rumex* and Poaceae in pollen diagrams can be interpreted as the result of the expansion of pastoral activities. Both *Plantago* and *Rumex*, but mostly *Urtica*, are well-recorded by DNA analysis (**Supplementary Figure 3**). The *Sporormiella* accumulation rates (**Figure 9**) and the records of cow and pig DNA, which increase progressively up to a maximum value recorded at 1350 cal CE, confirm the importance of livestock farming activities at the end of this period in the Aiguebelette watershed.

The DNA record attests to the cultivation of various fruit trees, including walnuts, apples and/or pears, plums and/or cherries, grapes and chestnuts (**Figure 8**). Today grapes are not grown in the Aiguebelette area, but the historical existence of grapevines in Savoie is well-documented in written records relating to monastic and castle lands. These sources suggest an expansion of this activity before the XIIth century in some areas like the Combe de Savoie and the upper Arve Valley (Passy) (Soudan, 1978; Mouthon and Carrier, 2010). Grapevines were often planted alongside fruit trees, especially chestnuts, which acted as trellises or were planted with other trees to form hedges. Vitaceae DNA has also been detected in another nearby catchment, but at a higher altitude, between the XIth and XVIIth centuries (La Thuile in Bauges Massif; Bajard et al., 2017). The DNA record in Aiguebelette also shows the cultivation of fava beans (**Figure 8**). This result agrees with the medieval practice of legume growing (Colardelle and Verdel, 1993; Ruas, 1997; Ferault and Le Chatelier, 2012). The records for grape, hemp, cereals, hops, legumes and fruit trees reveal a transition toward a polyculture mode of farming in the Aiguebelette basin from the High Middle Ages onward.

At that time, all of these activities had an impact not only at the local scale (decrease in the DNA from trees and shrubs, **Figure 9**) but also affected regional forests. Indeed, the sum of tree pollen recorded in the three pollen sequences reflects an overall decrease in tree cover. At the regional scale, beech seems to have been considerably impacted by deforestation as early as 1050 cal CE (**Figure 10**). The abrupt decrease in the *Alnus* pollen curve, recorded between 1200 and 1300 cal CE, probably reflects other important forest clearances, but occurring in the lowlands and on the river banks.

From the Xth to the XIth century, the European population grew rapidly (Fossier, 1984), and this demographic explosion induced both an expansion and an intensification of farming activities (Mazoyer and Roudart, 1997; Ferault and Le Chatelier, 2012). The technical advances (plow, three-field rotation system, etc.) introduced to improve production (Leturcq, 2004; Ferault and Le Chatelier, 2012) caused growing disturbance to the environment (Rasmussen, 2005; Enters et al., 2008; Doyen et al., 2013, 2016). These farming activities had an impact on the forests around Aiguebelette, but the exploitation of trees for charcoal production (Crook et al., 2002; Py et al., 2012) and for the construction timber should also be considered. Another impact of the rising human activity is the increase in erosion, as evidenced first by the increase in silicated

detrital flux (**Figure 9**), and then from 1200 cal CE, by the increase in fine silicated particles [$\ln(\text{Ti}/\text{Ca})$] as well as coarse particles [carbonated with $\ln(\text{Sr}/\text{Ca})$ and sometimes silicate with $\ln(\text{Zr}/\text{Ti})$]. The contribution of coarse carbonated particles increases markedly for the first time in the record, showing new source(s) of eroded material and suggesting the exploitation of new areas (possibly on sandstone or molasses deposits **Figure 1**). The marked increase in erosion and the changes in erosion signatures occurred at a time when farming activity was highly developed, as shown by the mammal DNA and *Sporormiella* sp. records as well as the DNA and pollen indicators for cultivation. In the watershed of lakes Paladru and Annecy, new phases of soil erosion are also recorded (Jones et al., 2013; Simonneau et al., 2013; Doyen et al., 2016). Combined human activities (i.e., opening up of the landscape and agricultural developments) and climatic degradation, which included greater precipitation, undoubtedly contributed to this increase in erosion (Büntgen et al., 2011).

From 1350 to 1900 cal CE

In the regional pollen records, a short agro-pastoral decline occurs from the XIVth century (**Figure 10**). According to the pollen and DNA data, at Aiguebelette several cultivated plants (cereal, hemp, etc.) were less abundant from 1350 to 1500 cal CE. This period coincides with the recolonization of trees such as *Corylus* and *Alnus* and the main rising phase of oak, and also with a phase of decreased erosion as revealed by all geochemical indicators (**Figure 10**). The decrease in human impact at that time can be attributed to at least three main factors: (1) the effect of the second plague pandemic (Black Death); (2) the political instabilities affecting western Europe in the XIV-XVth centuries; (3) wetter and colder summers during the XIVth century, as attested by tree ring-based climate reconstructions for central Europe (Büntgen et al., 2011).

The next phase, spanning from 1500 to 1900 cal CE, is marked by renewed deforestation and a re-expansion of agropastoral activities in pollen diagrams (**Figure 6**), although it also corresponds to the Little Ice Age (Büntgen et al., 2011). The accumulation rates as well as the concentration of *Sporormiella* (**Figure 9**) indicate a constant high level of grazing throughout this period in the Aiguebelette watershed. As regards crop husbandry, cereals were still being grown in the watershed and Pollen and DNA attest to the introduction of buckwheat from around 1650 cal CE (**Figures 6, 8**). DNA results indicate an increase in fava bean and hemp growing. Both markers show that arboriculture reached its peak (**Figures 8, 9**). During the XIXth-XXth centuries, the Aiguebelette region was a region of apple production dedicated to fruit exportation (Dagenais, 1939). From 1500 cal CE, the arboreal pollen curves decrease continuously. The lowest values are recorded between the XVIIIth and XIXth centuries. Tree harvesting increased to meet the growing fuel needs of emerging industries.

In this phase, two periods of intense erosion are recorded by the geochemical tracers: from 1500 to 1650 cal CE and from 1700 to 1950 cal CE (**Figure 9**). These two phases may

reflect a response to increased livestock farming, cultivation and summer precipitation (reconstructed from tree-rings in central Europe), especially between 1700 and 1800 cal CE (Büntgen et al., 2011).

From 1900 cal CE to the Present Day

From cal CE 1900 onward, all of the regional pollen records indicate a significant environmental change with an increase in total tree pollen, except for walnut which declines in regional pollen records (Figure 10). The proportion of tree taxa in the DNA records also increases (Figure 9). The number of positive DNA replicates of *Juglans* is also in decline (Figure 8), confirming the reduction of walnut cultivation in the Aiguebelette watershed. While nut production was progressively disappearing from the Aiguebelette watershed, DNA data show that orchards, composed of cherry or plum and apple trees, were still in activity at the beginning of the XXth century.

The drastic decline in *Cannabis* in both pollen and DNA records reflects the abandonment of hemp production in the area, a pattern that is also observed in the other pollen records (a bit earlier at Moras). The cultivation of cereals decreases slightly in the pollen records but to a lesser extent around Lake Aiguebelette than around Lake Paladru. Nowadays, the watershed of Aiguebelette is a rural *terroir*, still exploited, but cereal growing represents only a small portion of the agricultural activity (only 5.65% of the watershed area). The XXth century saw the appearance of new crops in the Aiguebelette area with DNA records revealing the development of vegetable gardens where *Phaseolus*, *Cynara cardunculus*, *Apium graveolens*, *Pisum*, and *Beta vulgaris* were grown.

The general decline in cultivation and reforestation observed at Aiguebelette are also recorded in many Alpine and perialpine lakes (Doyen et al., 2013, 2016; Bajard et al., 2016) through pollen and historical documents (Crook et al., 2002; Andrič et al., 2010). The decrease in erosion recorded at Aiguebelette (lowering of all erosion proxies from 1950, Figure 9) is a probable consequence of the lower human impact and of soil reforestation.

While cultivated plants seem to be less represented in regional pollen records, pastoralism indicators such as *Rumex* and *Plantago* are still well represented. At Aiguebelette, the mammal DNA (especially cattle) and *Sporormiella* spores demonstrate the continuation of grazing in the watershed after cal CE 1950. This persistence of grazing agrees with the high values in Poaceae recorded by pollen. This result probably illustrates the cattle rearing that is still a feature of the Aiguebelette area today, with most of the farms in the area being devoted to dairy farming for the production of milk for cheese.

CONCLUSION

This work highlights the excellent complementarity between the analysis of pollen grains, coprophilous fungal spores and environmental DNA and their value for reconstructing the history of vegetation and agropastoral practices.

By combining these markers, it has been possible to reconstruct the pace, nature and sometimes the intensity of anthropization in the Lake Aiguebelette watershed over the last 1800 years.

The preliminary geochemical analysis carried out here to better understand the environmental impact of the agro-pastoral activities and climate reveals a pattern of erosion that fits very well with the dynamic of anthropization reconstructed using biotic proxies. A finer analysis of the potential sources of sediments (geochemical characterization of the watershed) would probably help to identify the different areas under human pressure throughout the record.

While the pollen seems to provide a vegetation history very similar to that recorded in two other regional lakes (Moras and Paladru), the results of the DNA analysis provide information on the agricultural activities carried out within the watershed. These results add greatly to our knowledge of the history of this *terroir* and encourage us to carry out the same type of analysis on the two other sites, to see how the exploitation of their *terroirs* differed (or not) from that of Aiguebelette.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found at: <https://doi.org/10.6084/m9.figshare.19780297.v1>. Any further queries should be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

ED, DE, and EM performed the spore and pollen analysis. CG-C and LG performed the DNA analysis. JD prepared the pollen samples. AJ managed the modern vegetation mapping. CG-C, A-LD, JP, PS, and FA performed the sedimentological and geochemical analysis. MB A-LD, CG-C, and PS managed the chronology. A-LD, CG-C, FA, and EM did the coring. All authors contributed to the article.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2022.866781/full#supplementary-material>

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