New insights on lake sediment DNA from the catchment: importance of taphonomic and analytical issues on the record quality

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Abstract

Over the last decade, an increasing number of studies have used lake sediment DNA to trace past landscape changes, agricultural activities or human presence. However, the processes responsible for lake sediment formation might affect DNA archiving via taphonomic and analytical processes. It is crucial to understand these processes to ensure reliable interpretations for "palaeo" studies. Here, we combined plant and mammal DNA *metabarcoding* analyses with sedimentological and geochemical analyses from three lake-catchment systems that are characterised by different erosion dynamics. The new knowledge from this approach concern the DNA sources and transfer processes. The sources of eroded materials strongly affect the "catchment-DNA" concentration in the sediments. For instance, erosion of upper organic and organo-mineral soil horizons provides higher plant DNA quantities than deep horizons, bare soils or glacial flours. Moreover, high erosion rates along with a well-developed hydrographic network, are proposed as positive factors for the good representation of the catchment flora. The development of open and agricultural landscapes, which favour the erosion, could thus bias the reconstructed landscape trajectory. Regarding domestic animals, pastoral practices and animal behaviour might affect their DNA record because they control the type of source of DNA ("point" *vs*. "diffuse").

Key words: ancient DNA (aDNA), extracellular DNA, catchment DNA, lake sediment DNA, metabarcoding, taphonomy, plant cover, agriculture, landscape archaeology

1. Introduction

1.1. History and potential of the lake sediment DNA (sedDNA)

The earliest studies on ancient DNA (aDNA) from lake sediment archives date to the mid-1990 [1,2]. However, molecular biology techniques have been applied more extensively on lake sediments for the last eight years only (reviews from [3] and Domaizon et al., 2017; [4–8]. Lake sediments accumulate through time both autochthonous (in-lake biological production and chemical precipitation) and allochthonous (particles brought from the catchment and beyond) potentially DNA-bearing materials. Their study using molecular biology techniques, therefore, has a great potential to identify any of organisms present within the "lake's sediment source area" (i.e., the lake itself, its catchment area as well as the atmosphere). Downstream, this could help to trace changes of biodiversity over time, from the scale of the population to that of the ecosystem and to address a wide range of questions, especially in ecology [5,9,10]. Before 2008, only a few studies were performed on aDNA of terrestrial organisms from lake sediments, and all focused on pollen DNA [2,11,12]. In the meantime, most studies focused on aquatic organisms [1,13–19]. This may be due to the perception that the DNA from organisms within the lake would be preferentially archived in the sediments (or in higher quantities) compared to the DNA derived from the catchment area. However, since 2008, researchers have successfully tracked organisms derived from terrestrial environments, using bulk sediments and focusing on extracellular or total DNA [20]. These studies on bulk sediments targeted plants [21–29], mammals [25,30], humans and/or animal specific faecal bacteria [30–35] and more recently eukaryotes [6] in the aim of reconstructing past vegetation cover, landscape, climate, agro-pastoral activities, human occupation or the relationships between humans and landscapes and the wide spectrum of diversity. They demonstrated the great potential of this tool in providing new knowledge for palaeoecology and archaeology.

1.2. Issues and limits: taphonomic considerations

1.2.1. Plant DNA records

However, several studies questioned the interpretation of DNA results, suggesting concern over analytical and/or taphonomic processes, i.e. all the processes that govern the production, transfer, incorporation and preservation of the aDNA (modified from [36]). For instance, [37] did not detect a substantial proportion of DNA from the local flora which was independently identified by macrofossils.

They proposed multiple, non-exclusive explanations, such as the high abundance of some taxa that may overwhelm the rarest taxa. The taxonomic resolution and assignment rate could have been limited by the degradation of DNA sequences, the sequencing depth or the incompleteness of the reference database. Indeed, in a more recent study, also from the Arctic, the authors obtained superior taxonomic recovery between aDNA and macrofossils, probably due to the use of an almost complete reference library, as well as optimised extraction protocols (sediment quantity) and sequencing conditions [21]. Several studies also revealed discrepancies between records of plant DNA, pollen and macrofossils, which may reflect differences in the source (production, origin), transfer modalities, depositional environment as well as preservation conditions for these different vegetation-cover proxies [23,29,37]. Whereas taphonomic processes are relatively well-known for pollen and plant macroremains, their understanding for lake sediment DNA is still limited [3,9,36], especially for extracellular DNA, which by definition excludes the DNA from pollen and plant macroremains. However, a recent review [3,20] and two studies based on the comparison of modern vegetation with pollen and DNA analyses from surface sediments of a large set of lakes in different vegetation environments (tundra to forest tundra environments [38]; boreal and alpine [39]), provides some lessons and hypotheses: 1) pollen does not significantly contribute to the DNA records, 2) the DNA has a local origin and probably has a similar source as the macrofossils, 3) aquatic plants are well-represented, 4) taxa detection seems to depend on the distance to lake shore, the relief and its abundance (biomass) in the vegetation, 5) different types of sediments might have an impact on the DNA preservation. These studies targeted both, intra and extracellular DNA, of which the respective contribution to the sediments remains unclear [3] while the taphonomic processes affecting each of these DNA pools can be expected to differ.

1.2.2. Mammal DNA records

Regarding animal DNA, some studies also raised questions about taphonomic processes which might affect the DNA records. For instance, [25] did not find sheep extracellular DNA in modern sediments from a small subalpine lake (Lake Anterne, 2063 m a.s.l, Northern French Alps), while sheep flocks are present today in the catchment. Here, low stocking-rates (low biomass) and scattered distributions of domestic animals (representing a “diffuse source” of DNA) have been proposed as an explanation for the non-detection of DNA. On the contrary, high stocking-rates and/or the existence of areas used for the herding or flocking of animals (e.g. enclosures or folds, representing a “point source” of DNA because of the « concentration effect » of animals) might explain the enhanced supply of mammal DNA in the sediments during previous periods [25]. Moreover, urinary and faecal eliminations - two main sources of animal DNA [40,41] - are produced especially during the night within the enclosures or folds [42]. The presence of enclosures within a catchment is thus expected to significantly favour the detection of domestic animal DNA. Another study that aimed to identify the presence of humans in a catchment using human-specific bacteria DNA also proposed potential biases in the record due to taphonomic

issues [31]. In fact, the absence of human-specific bacteria DNA while pollen data suggests the presence of humans might be due to a DNA concentration below the limit of detection, for instance, if human camps/villages are at some distance from the lake or the inlet (thus limiting the DNA transfer to the lake), and/or as a consequence of a low population density (thus limiting the DNA production and biomass). An alternative explanation might also be that pollen reflect a more regional record.

1.2.3. "Time shifts"

Several studies raised the question of potential "time shifts" in lake sediment DNA records related to DNA leaching through the sediment layers [29] or DNA preservation and storage in soils and its release into the environment several centuries after its production [25]. The release into the environment of molecules stored in soils for decades has already been observed for pesticides, which are persistent molecules [43]. For DNA in alpine soils, it has been shown that very little DNA from crops cultivated more than 50 years ago, can be detected [44]. This study also shows a significant correlation between the proportion of DNA in soils and the proportion of above ground biomass for different functional plant groups. These two results suggest that the DNA brought by soil erosion will mainly reflect the ecosystem established at the time of the erosion event and will only weakly be influenced by long-term DNA storage in soils. This is supported by a recent study in which DNA accurately recorded the timing of changes in a vegetation cover detailed in historical evidence [29]. This good concordance with an independent approach highlights not only the absence of release of old DNA stored in soils but also suggests limited DNA leaching through the sediment layers.

1.2.4. DNA Degradation/preservation processes

DNA degradation/preservation processes have also to be considered within the lake water-column and sediments. DNA preservation/degradation is the most studied taphonomic process because it concerns several research communities and issues, including nutrient cycles, gene transfer, palaeoenvironmental reconstructions or genetic studies from archaeological remains like bones.

DNA degradation is triggered by both abiotic and biotic mechanisms. From the cell death, mechanisms of DNA repair cease and DNA starts to degrade through several chemical reactions (oxidation, hydrolysis, alkylation and Maillard reaction) acting both inside and outside the cells after their lysis, thus affecting both intracellular and extracellular DNA [45,46]. The rate of chemically-induced degradation is controlled by several environmental factors. Low temperature, high salt concentration (high ionic strength) and high pH limit the hydrolysis and thus favour the DNA preservation [47–49]. Environments protected from ultraviolet (UV) radiation also favour DNA preservation as this radiation causes DNA damage [49]. The extracellular DNA is also affected by microbial activity. In fact, the degradation by DNases produced by bacteria is considered as the primary mechanism of extracellular DNA degradation [50]. However, DNA can be protected from this process when it is adsorbed onto charged surfaces (clays

and humic substances), or absorbed into the crystal lattice of fine particles, amorphous crystals and particulate organic compounds [50,51]. This protection can also be due to the inactivation of DNases via their binding on particles [52].The binding of extracellular DNA on particles, as well as the degree of protection, are complex processes as they are dependent on the mineralogy of the sorbent, the presence of organic material, pH conditions, the ionic strength and length of the DNA molecules [52,53]. In soils, nucleic acids released from cells were found to be quickly bound to particles [50,52,54,55], which delays the DNA degradation and might explain the detection of the few DNA sequences in the alpine soils, 50 years after the stop of crops [44]. Inside the lake, bacterial activity, oxygenation, salt concentration, organic and mineral particles, UV penetration and pH conditions can vary through time and thus differentially affect the DNA preservation. When sediments are deposited in the lake bottom, they quickly become anoxic after burying, which limits microbial activity and thus favours long-term DNA preservation. However, the uppermost sediments often represent an active layer which has been shown to significantly modify the concentration and composition of microbial DNA [8]. With burial, DNA becomes also totally protected from UV radiation. In marine sediments, it has also been shown that a high proportion of extracellular DNA is bound to minerals or humic substances [56,57]. Given the mechanism of DNA protection provided by the binding, the absence of oxygen and UV, aquatic sediments are, *a priori*, good environments for DNA preservation [20]. However, the low bacterial activity and the DNA binding on particles do not prevent chemically induced DNA degradation, especially hydrolysis. DNA degradation should trigger a decrease of the DNA pool with time and decrease the size of DNA fragments still present. A time-dependent DNA decrease was reported in a study of dinoflagellate DNA from fjord sediments in Antarctica [58], and several studies reported the loss of long fragments with age [5,28,59]. Ageing also triggers cytosine to thymine substitutions at the single-stranded ends of the DNA fragments, which was used to discriminate between ancient DNA sequences and contaminations from modern DNA [28,60,61]. DNA preservation can also vary among different groups of organisms as well as among different species of the same group [58,62,63].

1.3. Challenges ahead

In the light of all the previous considerations, there is a need to investigate the potential distortions of the lake sediment DNA record due to taphonomic processes (production, transfer, preservation of DNA) and/or analytical procedures (extraction/amplification/identification) [9,10,36] (Fig. 1). Without a good understanding of these processes, the full potential of lake sediment DNA cannot be realised. Especially important is the issue as to whether the DNA archived in the sediment represents a reliable diachronic signal; i.e. are the following characteristics or processes constant over time: 1) the source of DNA, 2) processes and efficiency of DNA transfer, and 3) preservation conditions of DNA?

Our review of the literature demonstrates a good knowledge of the DNA preservation processes. However, few studies have focused on identifying terrestrial DNA sources and transfer processes from catchments to lakes.

Here, we analysed temporal DNA datasets from three mountainous lake-catchment systems characterised by various erosion dynamics due to the different geological formations, topographical characteristics and vegetation and soil covers (Figure 2A/B), in order to get information on these taphonomic (i.e. source and transfer) processes. Both plant and mammal extracellular DNA were investigated using the DNA *metabarcoding* approach, which is the amplification and sequencing of DNA molecules found in the environment using universal markers [64]. This extracellular DNA may represent the main DNA pool in sediments [56,65] and is of great interest as it may provide the most integrated view of aquatic, sedimentary and terrestrial biodiversity [57]. Here, we only focused on this particular DNA pool to avoid the extraction of DNA from plant macroremains, which might lead to an overrepresentation of these taxa and limit the detection of the other, rarer taxa. Sedimentological and geochemical data were also acquired to get information about the processes of sediment production, transfer and deposit as well as of lake water physico-chemical conditions. Pollen or coprophilous fungi data were included in the study as complementary evidence of vegetation cover changes and domestic herds presence. All these data are key to understand the processes which drive the DNA records as well as to emphasise how changes in taphonomic conditions over time can affect the quality of the DNA record and thus of the landscape and land-use reconstructions.

2. Results and interpretations

2.1. Plant and mammal DNA detected in the three lakes

After the filtering procedure, 107 and 83 MOTU of plants are detected in lakes La Thuile and Muzelle, respectively, while only 19 MOTU are found in Lake Serre de l'Homme. Lake Muzelle exclusively records terrestrial plant DNA (100% of the reads). Lake La Thuile presents a mixed recording, but most of the DNA reads are of terrestrial origin (71% of reads distributed in 96 MOTU, Table 1). Conversely, most of the DNA reads detected in Lake Serre de l'Homme are aquatic in origin (79% of reads distributed in 7 MOTU but probably only 3 different taxa, Table 1 and Supplementary figure 3).

Based on the comparison between the proportions of samples in which terrestrial plants are detected in 0, 1, 2, 3 or 4 replicates, it is clear that the low terrestrial plant richness detected in Lake Serre de l'Homme also corresponds to very low quantities of DNA extracted from the samples compared to the two other lakes. In fact, we never detected terrestrial plants in more than three replicates over eight and in 85% of the samples, either we did not detect terrestrial plants, or we only detect them in one replicate (Table 1). On the contrary, in most of the samples from lakes Muzelle and La Thuile (87% and 76%, respectively), terrestrial plant DNA is detected in the four replicates performed on these lakes (Table 1). However, in 12% of the samples from Lake Serre de l'Homme, aquatic plants are detected in more

than 4 replicates (44% of the samples detect aquatic plants in more than 1 replicate). The three lake-catchment systems are thus characterised by different plant DNA records in terms of quantity and of quality.

Mammal DNA is only detected in the sediments from Lake La Thuile (Table 2), while herds/flocks of domestic animals currently graze on all study sites, with high pastoral pressure around lakes Serre de l'Homme and Muzelle (Figure 2). In the first run of sequencing performed on four replicates, only cattle are detected (Table 2) and always in only one replicate. In the second run of sequencing performed on twelve replicates, the number of positive replicates (where mammals were detected) increases to four. Moreover, two additional taxa are found (*Ovis sp.* and *Canis sp.* in addition to *Bos sp.*) (Table 2).

2.2. Plant DNA: what can we learn from the sedimentological/geochemical records and pollen?

2.2.1. La Thuile

The record of terrestrial plant DNA content (Figure 3) can be divided into seven phases ((a) from 0 to 1000 cal. BP, (b) from 1000 to 1400 cal. BP, (c) from 1400 to 2500 cal. BP, (d) from 2500 to 3600 cal. BP, phase (e) 3600 to 4500 cal. BP, (f) from 4500 to 5200 cal. BP and (g) from 5200 to 6400 cal. BP), corresponding to changes in environmental conditions inferred from the sedimentological and geochemical proxies (Bajard et al. 2016). In most of these phases (a, b, c, e and g), the terrestrial plant DNA content is positively correlated with the organic matter content ($r=0.82$, $p<0.001$; Figure 3). This relationship probably reflects the significant role of the biomass production described in previous studies [3,39]. Phases (d) and (f) does not follow this relationship. They are, respectively, impoverished and enriched in DNA compared to the organic content. Phase (d) is also characterised by a very low carbonate content (<4%) (Figure 3), which might indicate the presence of acid conditions in the water column. Acid conditions are not favourable for DNA preservation [47–49]. Moreover, our method of DNA extraction might not be efficient enough to unbound organically (humic substances)-complexed DNA [57], which might be an important pool of extracellular DNA in this part of the sediment pile mostly made of leaves and needles [66]. Humic substances are also known to inhibit the PCR reaction [67]. The poor-DNA content in phase (d) might thus be due to unfavourable preservation conditions and/or analytical limits. Phase (f) contains as much organic matter as phase (g), but the DNA content is higher. However, phase (f) contains much more organic matter of terrestrial origin (vs aquatic; cf Figure 3) and more precisely, coming from the erosion of a forest litter and/or the direct fall of the upper parts of plants inside the lake [66]. Very high content in organic matter from the forest litter is also recorded in phase (e), but the DNA content does not significantly increase relative to the phase (f). This result is probably due

to the presence of humic substances and the acidic conditions suggested by the low carbonate content as in phase (d). Phase (b) has a slightly lower DNA content than in phase (a), while there is as much organic matter. Moreover, this phase presents a very low number of MOTU, especially compared to those detected by pollen analyses (Figure 4). However, this phase is dominated by a contribution from deep soils, i.e. mineral soil horizons, while phase (a) is dominated by a contribution of the soil surface, i.e. organo-mineral soil horizons (Figure 3, [68]). The sediments are thus enriched in terrestrial plant DNA when the erosion strongly affect the soil surface horizons, such as the litters and the organo-mineral soil horizons (except when the lake water is acidic and/or contains humic substances, which does not favour the DNA preservation/recovery). Consequently, the erosion processes (e.g. sheet erosion, gully erosion or bank undercutting), controlling the origin of the organic matter, are key processes driving the terrestrial plant DNA concentration in the sediments.

Both pollen and DNA records show an increase in floristic diversity from 2500 cal. BP, i.e. from phase (c) (Figure 4). Before this period, 31 and 11 taxa on average, are detected by pollen and DNA analyses, respectively (without taking into account phases d and e of lower DNA detection). From 2500 cal. BP, the number of taxa detected with pollen increases to 34 on average for the phase (c) and to 38 for the phase (a). With the DNA analyses, the mean number of MOTU in phases (c) and (a) are 19 and 30, respectively. The number of MOTU detected by DNA is thus always lower than that obtained from pollen analyses. However, the increases of floristic diversity in phases (c) and mostly (a) are more significant with the DNA analyses. The efficiency in detecting plant communities through DNA analyses might thus be higher after 2500 cal. BP than during the previous period. Moreover, from this moment up to 1400 cal. BP (i.e. in phase (c)), an increase of the proportion of arboreal taxa is recorded by DNA whereas pollen data suggests deforestation. The significant increase of the erosion from 2500 cal. BP (Figure 3; [66,68]), which led to a high increase of the total flux of sediments (13 to 504 mg/cm^2/yr), is in agreement with this assumption of deforestation, as this human activity decreased soil stability. Consequently, the higher detection of trees (for instance, *Quercus sp*., *Acer sp.*, Betulaceae, Ulmaceae and to a lesser extent *Viburnum opulus* and *lantana,* Figure 4 and Supplementary figure 8) and the higher increase of the richness in the DNA dataset (compared to the pollen dataset) might be due to this higher erosion. In fact, the erosion increases the degree of connectivity of the catchment area. On the contrary, before 2500 cal. BP in the forested landscape, there is a probable bias towards recording plants growing on the lakeshore and the riverside (through the proximal litter erosion or the direct fall of tree leaves) as suggested by the dominance of *Alnus sp.*, which over the three possible species, has two riparian species (*Alnus glutinosa* and *incana*) and by the presence of *Frangula sp.* (Supplementary figure 8).

Temporal inconsistencies are recorded between *Cannabis sativa*, detected via DNA analyses, and *Cannabis sativa* or *Humulus lupulus*, detected via pollen analyses (Figure 4). The percentages of pollen are quite high (around 10-15%), suggesting that it originates from retting activity. In this case, both

pollen and DNA are directly transferred to the lake. Consequently, high quantities of DNA from *Cannabis sativa* can be transferred to the sediments which might explain the high detection during the phase (b), i.e. when the erosion affects the deep soil horizons and dilutes the DNA inputs of other terrestrial plants (Figure 3 and 4). On the contrary, in phases (a) and (c), i.e. when the erosion predominantly affects soil surface horizons, the DNA from *Cannabis sativa* may be diluted by the DNA from the plants in the catchment. As the DNA from this species becomes rarer, it competes with other more abundant DNA fragments and is therefore no longer amplified. Nevertheless, we can point out that for many taxa, DNA and pollen signals are the same (excluding phases b and d). Especially, several tree taxa show the same (or very close) trends over time: *Taxus sp.*, *Tilia sp.*, *Abies sp.*, *Alnus sp.*, *Fagus sp.*, *Cupressaceae* (*Juniperus* with pollen) and *Juglandaceae* (*Juglans* with pollen). Herbaceous plants, like *Rumex sp.*, *Plantago sp.*, *Mentha sp.*/ Mentheae, *Helianthemum nummularium* (*Helianthemum* with pollen) and others (Figure 4 and Supplementary figure 8) also record the same history.

2.2.2. Serre de l'Homme

Very little land plant DNA (low DNA concentration and richness) is recorded in the Lake Serre de l'Homme (Figure 5). The sediments mostly comprised non-carbonate mineral matters (35.5-78 %) of clastic and biogenic (diatoms) origins and organic matter (20.4-62%). The C:N atomic ratio fluctuates from 9.3 to 15.4, i.e. between a pure aquatic end-member and a mixed terrestrial/aquatic end-member [69–73] (Figure 5). The sediments contain terrestrial plant macrofossils. The lake catchment is flat and the "lake surface: catchment surface" ratio is high, which explains the low terrigenous inputs reflected by the low total flux of sediments (between 1 and 20 mg/cm^2/yr). In these topographical conditions, only the most easily erodible materials are mobilised. These materials may be the plant remains fallen on the soils (constituting the source of terrestrial plant macrofossils) as well as the bare soils on sandstones (Figure 2), which contribute to the non-carbonate mineral matter. These materials are not expected to bear extracellular DNA from plants, which probably participate to the poor detection of terrestrial plant DNA. Moreover, poor-DNA preservation conditions may be triggered by the soil acidity (pH of 4.3-5.3 have been measured on soils developed on the same geological substratum and close to the catchment) and/or by the low water depth favouring 1) high temperature, 2) UV penetration and 3) oxygenation in the lake bottom. Higher detection probability of taxa was demonstrated in deeper lakes in boreal to alpine environments in Northern Norway [39]. In Lake Serre de l'Homme, better in-lake preservation conditions are assumed from 300-100 cal. BP due 1) to the higher organic matter production favouring the establishment of anoxic conditions and thus reducing the bacterial activity and 2) to the important development of aquatic plants limiting UV penetration in the lake bottom. These good preservation conditions may contribute to the detection of high quantity of aquatic plant DNA, which is otherwise in agreement with the decrease of the C:N atomic ratio (Figure 5).

The poor quality of the terrestrial flora reconstruction is characterised by a stochastic detection of only eight different taxa (Figure 6). At least four of these plants live in wet environments (*Athyrium sp.*, *Caltha sp.*, *Saliceae* and *Filipendula ulmaria*). The proximity or good connection between these wet environments and the lake might have favoured the DNA transfer of plants that grow in these environments, like the DNA from the aquatic plants [29,39], which are nearly continuously detected in Serre de l'Homme (successions of *Myriophyllum sp.*, *Sparganium sp.* and *Potamogeton sp.* as well as *Potamogetonaceae*, Figure 6). On the contrary, the very poor spatial representativeness of the catchment-scale flora at Serre de l'Homme probably reflects the low connectivity between the whole catchment and the lake due to the absence of a well-developed hydrographic network and the low erosion, both due to the flat topography. The role of catchment relief on catchment flora reconstructions has also been proposed in two recent studies, in Arctic and African environments [39,74].

2.2.3. Muzelle

The sediments from Lake Muzelle present substantial variations in terrestrial-plant DNA concentration (from 0.28 to 2.10, Figure 7A) but have nearly homogeneous concentrations all along the core in non-carbonate mineral matter (93.6% +/-0.8), total organic matter (4.2% +/-0.6) and carbonates (2.2% +/-0.4). However, the sedimentological dynamic of this lake is dominated by significant changes in grain size [75]. We observe that the quantity of terrestrial-plant DNA tends to decrease with the increase in clay content ($r=-0.72$, $p<0.0001$; Figure 7B). These inputs of clays increase substantially during two phases, i.e. 750-625 and 310-50 cal. BP (Figure 7A), which are in the Little Ice Age (LIA) [76]. In this context and given the presence of a glacier in the catchment, clays are interpreted as representing a proxy of inputs in glacial flour to the lake. In fact, glacier advances triggered by colder and/or wetter conditions produce more glacial flour, which increase the input of clays into the lake, especially during high precipitation events as shown by the increase of the flood frequency [75]. Because these clays do not come from soils covered by plants, no extracellular DNA fragments from terrestrial plants are expected to be bound to these clays. Thus, the inputs of these DNA-free clays might dilute the DNA coming from vegetated-soil erosion and thereby explain the decreases in DNA content when clays increase (Figure 7A).

The taxonomic richness strongly increases from 550 cal. BP, i.e. when the tree-shrub cover % decreases. From this period, plant communities with different ecological preferences are recorded. In fact, plants of heathland characteristic of well-developed acid soils (e.g. *Vaccinium uliginosum*) are detected together with plants of calcareous meadow (*Myosotis alpestris*), siliceous screes, snow beds or moraines (*Oxyria digyna*, *Veronica alpina*), siliceous rocks (*Eritrichium sp.*), calcareous rocks (*Saxifraga paniculata*), nutrient rich soils (*Rumex sp.*, most of *Mentheae sp.*) and wet environments (*Bartsia alpina*) (Figure 9). This record of a mosaic landscape may have been favoured by the well-developed

hydrographic network connecting different parts of the catchment to the lake (Figure 2) and the high erosion dynamic as shown by the high total sediment flux (14-77 mg/cm^2/yr) and contribution of non-carbonate mineral matter (Figure 7). This mosaic landscape is probably the result of the landscape opening for the development of pastoral activities, as suggested by the presence of plants that have preferences for nutrient-rich soils. Mammal DNA analyses can be performed to test this hypothesis.

2.3. Mammal DNA detection and indirect evidence of pastoral activities

In Lake La Thuile, more mammal DNA is detected for the last thousand years, which is in agreement with the detection of *Rumex sp.* (Figure 8A), a nitrophilous plant commonly associated with animal stalls. *Plantago sp.*, generally associated with grazing activity because it is resistant to trampling and not eaten by animals (especially *P. alpina* and *P. Lanceolata*), is also detected in previous periods (DNA and pollen, Figure 4), e.g. from the Late Iron Age to the Early Medieval Period. Its occurrence suggests that herds/flocks of domestic animals might have been present in the catchment before the last millennia, although they are not detected from the mammal DNA analyses. This possible divergence between the proxies might be due to 1) a low number of animals and/or a dominance of sheep or goats relative to cattle (i.e. a smaller biomass leading to less DNA production) before 1000 cal. BP, 2) the fact that areas of animal stalls like enclosures (representing high stock density and favouring the development of nitrophilous plants such as *Rumex sp.*) increase the detection probability of livestock farming relative to scattered distributions of animals, because they represent "point sources" vs "diffuse sources", 3) the relatively low DNA transfer due to the high deep soil horizons erosion between 1400 and 1000 yr cal. BP (Figure 3) or 4) a combination of these factors. In another alpine lake (Anterne), sheep DNA was detected in only one over eight replicates during the Late Bronze Age, whereas *Plantago sp.* DNA started to be regularly recorded [25,26]. In this case, the low DNA content may also be explained by a dilution triggered by the significant increase in deep soil horizons erosion [26,77]. Furthermore, as observed for Lake La Thuile (Figure 3), this period was also characterised by the detection of few terrestrial plant taxa [26].

The absence of mammal DNA in sediments from Lake Muzelle is quite unexpected. Indeed, DNA from *Rumex sp.* and coprophilous fungi spores (*Sporomiella sp.*) are found in the sediments dated to the last few centuries ([75], Figure 9B), which attests the presence of domestic flocks/herds at least during this period. Coprophilous fungi spores, as well as extracellular DNA from both *Rumex sp.* and domestic animals, are supposed to share the same area of production. *Sporomiella* spores mainly come from the faeces of herbivores, mammal DNA is assumed to be largely derived from dung and urine [41] and DNA from *Rumex* comes from places of high nutrient accumulation, such as domestic animal stalls where

faeces accumulate (hence the good correspondence with the mammal DNA observed for La Thuile). However, the production (and thus concentration) of each of these proxies as well as their distribution in the soil profiles may be different. Consequently, the non-detection of mammal DNA in the sediments from Lake Muzelle might be due to low production/concentration of mammal DNA compared to DNA from *Rumex sp.* and to spores of *sporomiella sp.*, and/or to differential limit of detection between the different proxies. This issue of the limit of detection of the DNA is well illustrated by the results of the two experiments performed on Lake La Thuile. In fact, a better detection (higher number of positive replicates and more taxa) of mammal DNA is recorded when increasing the number of DNA replicates (Lake La Thuile Table 2 and Figure 9A), because it increases the detection probability of "rare" taxa [78,79]. In particular, *Ovis sp.* is consistently detected in Lake La Thuile only when many PCR replicates are performed (Table 2). Even if these taxa are not "rare" in the catchment, because of contaminations by human DNA (still high even with the use of blocking primers, see supplementary figure 6) of samples, these taxa have to be considered as "rare" in the sediments. Consequently, the low number of replicates analysed in Lake Muzelle (only four), could contribute to the non-detection of the domestic animals.

The absence of mammal DNA in the sediments from Lake Serre de l'Homme, where spores of *Sporomiella sp.* are also detected, is probably due to the low detrital supplies combined to the poor-DNA preservation conditions as was hypothesised for terrestrial plants.

3. Discussion

From the lessons provided by our case studies and the review of our knowledge about the fate of the DNA in the environment, we propose a model summarising the archiving of the extracellular DNA from the catchment in a lake (Figure 10). This model can be used to guide the choice of lakes most suitable for the reconstruction of the catchment history (landscape changes, agropastoral activities, biodiversity).

It integrates three equations. The first one is a mixing equation between the different materials affected by erosion in the catchment and transferred to the lake. This equation can be written as follow, for one taxon (Eq 1) and several taxa (Eq 1'):

Eq 1)

$$\left[\mathrm{DNA}_{Taxa\ j,TERRinit}\right] = \sum_{i=0}^{x1}\left[\mathrm{DNA}_{Taxa\ j,Source\ i}\right][\mathrm{Source}\ i]$$

Eq 1')

$$[\mathrm{DNA}_{TERRinit}] = \sum_{i=0}^{x1} \left(\sum_{j=0}^{x2} [\mathrm{DNA}_{Taxa\ j,Source\ i}] \right) [\mathrm{Source}\ i]$$

, where $[\mathrm{DNA}_{Taxa\ j,TERRinit}]$ and $[\mathrm{DNA}_{TERRinit}]$ are the concentrations, respectively of the taxon j and of a group of taxa targeted by the primer (from 0 to x2), in the terrigenous materials affected by the erosion (log(N reads+1)/g of terrigenous materials) and *Source i* represents the different sources of terrigenous materials (from 0 to x1 sources). We hypothesise that these materials contain different concentrations of DNA from different taxa j ($[\mathrm{DNA}_{Taxa\ j,\ Source\ i}]$) due to variations in 1) spatial distribution of the taxa in the catchment, 2) DNA distribution in soil profiles, 3) soil type, and 4) biomass produced by each taxon. For instance, according to our interpretations from Lake La Thuile, the soil litter is the most extracellular DNA-rich source for plants (humic substances-bound DNA; Figure 10). However, we anticipate different DNA contents in different types of litter (for instance forest vs meadow), especially due to the different biomass production, litter turnover, and pH conditions, as proposed by a study in boreal environments but on total DNA [44]. Data from La Thuile also suggests that the organo-mineral soil horizons contain less extracellular plant DNA (clay-bound DNA) than the litter, but much more than the mineral (deep) soil horizons. The distribution of extracellular plant DNA in soil profiles should thus have a decreasing trend from the top to the bottom (Figure 2). A lower total extracellular DNA concentration was also observed in deeper horizons (B) than in upper horizons (A) from Inceptisols (forest soils from Mediterranean regions) [80]. In case of presence of buried palaeosoils [81] higher DNA content might be expected in the "palaeo" soil surface horizon. Acidic soils and bare soils would be very poor or free of extracellular plant DNA which probably contributes to the poor DNA record from Lake Serre de l'Homme. Moreover, glacial flour is free of extracellular plant DNA, as exemplified by the data from Muzelle.

The content of extracellular DNA from animals in soil profiles can be different from that of plants. Total DNA was shown to be strongly related to the animal biomass as well as to the soil texture, with significant leaching in sandy soils and for larger animals [40]. For the livestock, this biomass depends on the stocking rate and more precisely on the stock density, which is driven by the animal behaviour and pastoral practices (Figure 10). These factors will also produce spatial variations in mammal DNA distribution in the catchment. However, as for plants and microbes, the highest animal DNA quantities are found in top soils [40].

The concentration of the different sources of terrigenous materials ([Source *i*]) will depend on their erodibility (capacity to be mobilised), the slope and the connections between the sources and the lake (direct or via runoff waters and tributaries). A well-developed hydrographic web should provide terrigenous inputs from the different parts of the catchment and thus afford a more reliable reconstruction of the floristic diversity at the catchment scale, as exemplified by the records of a landscape mosaic in the sediments from Lake Muzelle as well as another mountain lake, Anterne [26]. Moreover, open landscapes, with a higher erosion dynamic triggered by higher soil erodibility should

yield better spatial representativeness, for example, the range of plants in the catchment. This process is well exemplified on Lake La Thuile. However, the erosion should preferentially affect the upper parts of the soils as previously written. This also means that any significant developments in agricultural activities should be well reflected in the aDNA record of this activity. On the contrary, extensive practices, such as unmanaged grazing without stockading or animal enclosures, with less impact on the erosion dynamic, might be more difficult to detect.

Previous studies proposed that the biomass, distance and relief determine the terrestrial plant DNA record in the sediments [3,39]. Here, our model goes further, integrating more explicitly the mechanisms behind the production and transfer of extracellular DNA in lake sediments. In fact, our data demonstrate that the nature of erosion processes (such as sheet erosion, gully erosion, bank undercutting or glacial erosion) is important to consider because it controls the sources and quantity of catchment derived extracellular DNA inputs to the lake. Furthermore, the concept of "catchment connectivity" combining the hydrographic web and the catchment erodibility, reflects the features and processes controlling the spatial representativeness of the DNA record, which is key for good quality reconstructions, especially when landscapes have high habitat diversity (i.e. are made of plant metacommunity).

The second equation of the model reflects the dilution by the autochthonous production (lake production):

Eq 2) $[\mathrm{DNA}_{TERRSED}] = [\mathrm{DNA}_{TERRinit}][\mathrm{TERR}_{SED}]$ or $[\mathrm{DNA}_{TERRinit}](1 - [\mathrm{AquaMat}_{SED}])$

where $[\mathrm{DNA}_{TERRSED}]$ is the concentration of terrestrial DNA in the sediments (log(N reads+1)/g dry sediments), $[\mathrm{TERR}_{SED}]$ is the concentration of terrigenous materials in the sediments (g of terrigenous materials/g of dry sediments) and $[\mathrm{AquaMat}_{SED}]$ represents the concentration of the aquatic production. The aquatic end-member of the sediments can include organic matter from microalgae, and aquatic plants as well as mineral matters produced or induced by aquatic organisms or chemical reactions. The dilution effect by the aquatic end-member is illustrated by the records from phases (a), (c) and (g) at Lake La Thuile and probably contributes to the poor terrestrial DNA record in lake Serre de L'Homme. In the dilution equation, we did not consider the materials coming from the atmosphere because they represent very low quantities beside the aquatic and terrestrial materials.

Finally, the third equation integrates the DNA degradation process in the lake water column and the sediments into the model.

Eq 3)

$$[\mathrm{DNA}_{TERRSED}] = (1-\alpha)\left(\sum_{i=0}^{x1}\left(\sum_{j=0}^{x2}\left[\mathrm{DNA}_{Taxa\ j\ Source\ i}\right]\right)[\mathrm{Source}\ i]\right)[\mathrm{TERR}_{SED}]$$

where α is a factor of degradation (if α=1 all the DNA is degraded and if α=0 all the DNA is preserved). Theoretically,

$$\alpha = f(\mathrm{pH}, \mathrm{T°}, \mathrm{UV}, \mathrm{O_2}, \text{microbial activity}, \text{salinity}, \text{sediment composition}, \text{time})$$

In case of Lake La Thuile, we were able to recognise a probable negative impact of acidic conditions in the water column on the DNA preservation (or on the capacity of our method to detect DNA due to the presence of humic substances). A hypothesis of DNA degradation in the lake Serre de l'Homme due to low water depth favouring warm conditions, UV penetration, and oxygenation is also proposed. Interestingly, our data do not provide any clear evidence for a significant effect of the DNA degradation over time. Indeed, the DNA concentration is not especially higher in the top cores, and all changes of DNA content occur abruptly and are always associated with sedimentological and/or geochemical changes.

Some of the factors influencing the quantity and the spatial representativeness of the DNA archived in the lake sediments are relatively constant over time (catchment slopes, lake surface/catchment surface ratio and the hydrographic web at the scale of the Holocene). Therefore, they can be used to initially guide the choice of lakes most suitable for the reconstruction of the catchment history (landscape and agropastoral activities). However, as the other factors could change over time (especially the soil erodibility), a DNA record of good quality cannot be guaranteed throughout the DNA record and thus required to be assessed. In fact, changes in the quality of the DNA record over time will result in the limitation of inter-period comparisons. This assessment is particularly essential because the palaeosciences are largely concerned with the identification and understanding of changes in socio-ecosystem trajectories, including tipping points and resilience. We demonstrate that the integration of data from sedimentary geology, geochemistry and soil studies is a powerful approach to assess the potential taphonomic biases in the DNA records. Similar approaches, i.e. better considering the context of sediment formation, should be more routinely adopted as interpretative tools.

The model that we propose is based on the study of only three lake-catchment systems. Therefore, a similar empirical field-study on modern sediments from a larger collection of lakes located in diverse geological and ecological environments, in order to avoid confounding variables, would be relevant. Studies on soil collections integrating the different soil horizons would also be informative and complementary. Moreover, there would be a need for experimental projects that recreate a series of different taphonomic scenarios. These projects will thus test and enhance the model proposed in the manuscript.

Lake sediment DNA is often considered as a biological/ecological proxy because it gives information about organisms. Here, we rather propose that lake sediment DNA is a bio-geological proxy because 1) the understanding of the record requires to involve earth scientists (taphonomic study) and 2) it might be used to answer questions about the evolution of geological processes of the critical zone. Indeed, we feel that there is a potential to use the terrestrial DNA composition detected in lake sediments as a

signature of the sources mobilised in a catchment to determine areas affected by erosion, today [82] and in the past.

4. Material and methods

4.1. Regional setting and site presentation

All three study sites are located in the French Alps, although in different ecological zones (Figure 2A/B). The catchment of Lake La Thuile (874 m above sea level (asl)) is located in the mountainous belt of a pre-alpine massif (the Bauges Massif, Northern French Alps). The catchment of lakes Muzelle (2105 m asl) and Serre de l'Homme (2235 m asl) are located in the Ecrins massif (central part of the French Alps), i.e. in a more internal position relative to the alpine range. These sites are at a higher altitude than Lake La Thuile. Lake Muzelle's catchment area includes several ecological zones/ecotones: the upper subalpine zone, the alpine zone, and the nival zone, with the presence of a relict glacier in the catchment (Figure 2B). Serre de l'Homme is in the subalpine zone. The subalpine belt comprises the so-called "alpages" areas (i.e. high-altitude pastoral units used in summer following the growth of grass). Given the range of altitudes covered by the sites, they cover zones that can support different types of agricultural activity. Until recently, the Lake La Thuile catchment hosted pastoral activities (including the presence of permanent farms), and multiple crops. The two other sites only support pastoral activity, nowdays (Figure 2B; [66]).

4.2. Sites topography/ geology

Each of the catchment areas studied possesses different physical characteristics (Figure 2B). The Lake Muzelle catchment area has the highest proportion of steep slopes of the three sites, a well-developed hydrographic network, highly erodible rocks, including schist, and partial meadow vegetation, with some bare soils exposed to erosion. The lake surface constitutes <2% of the catchment, which implies there is an important "concentration effect" of sediments derived from the catchment. Combined, these characteristics lead to significant terrigenous inputs to the lake. Furthermore, the catchment comprises a glacier. Thus, a part of these terrigenous inputs comes from glacial erosion. This type of erosion provides glacial clayey materials ("glacial flour") [75].

At Lake La Thuile, the lake surface to catchment surface ratio is 4.7%, i.e. 2.4 times higher than for Muzelle. This implies that in Lake La Thuile the "concentration effect" is lower than in Lake Muzelle.

The slopes are also less steep, the hydrographic network is poorly developed, and the vegetation cover greater (meadows, some agricultural and forested areas) than in the catchment of Lake Muzelle. However, the presence of agricultural activities triggers significant soil erosion and thus terrigenous inputs to the lake [66,68]. The physical characteristics of Serre de l'Homme's catchment are the opposite of those at Muzelle: high lake to catchment surface ratio (12.9%), gentle slopes, and no hydrographic network. These characteristics are not favourable for detrital supplies into the lake. However, rocks around the lake are easily erodible (sandstones), and there are some small barren/exposed areas (bare soils), which are susceptible to provide a few terrigenous (and more precisely clastic) inputs.

4.3. Vegetation cover

Around Lake La Muzelle, the vegetation cover is dominated by subalpine and alpine meadows with herbs such as grasses (*Poaceae*), wormwood (*Artemisia*), sedges (*Cyperaceae*) and creeping willows (*Salix*) [83]. Lake Serre de l'Homme is surrounded by a eutrophic subalpine meadow with goosefoot (*Chenopodium bonus henricus*), yellow gentian (*Gentiana lutea*) and docks (*Rumex sp.*) (H. Cortot, Pers. Com.). Lake La Thuile (in mountainous area) is surrounded by meadows and pastures. According to the exhaustive floristic survey undertaken around the lake (M. Pienne, T. Delahaye, S. Henriquet; Conservatoire Naturel de Savoie, 1999 and 2000), two types of meadows are present: a meadow with orchard grass (*Dactylis glomerata*) and heath false brome (*Brachypodium pinnatum*), which is sometimes grazed, and a mesophylic meadow dominated by grasses such as crested dogstail (*Cynosurus cristatus*), and ryegrass (*Lolium perenne*) used for grazing and mowing. Artificial grassland and kitchen garden are found in the northwest and southeast extremities of the lake. White willow (*Salix alba*), ashy willow (*Salix cinerea*), black poplar (*Populus nigra*), ash tree (*Fraxinus excelsior*) were also described at the edge of the lake. In the higher part of the catchment, there are coniferous forests comprised of spruce (*Picea abies*) on the north side, and of deciduous forest on the east side.

4.4. Coring and dating

All lake sediment cores were taken in the deepest part of the lakes, which are located approximately in the centre of the lakes (Figure 2). For lake La Thuile, cores were taken using a UWITEC platform and coring devices. The sediment sequence comprises two core sites. Sections from the second hole are shifted by one meter in depth in order to have overlapping sections and create a continuous sequence (THU10, N45 31.813, E6 03.394, IGSN:IEFRA00BB – IGSN codes refer to an open international database. www.geosamples.org). Cores from lake Muzelle (MUZ12, N44 57.037, E6 05.845, IGSN : IEFRA00A4) and two from lake Serre de l'Homme (SDH-09-P1 and P2, N44 77.459 , E6 23.772,

IGSN : IEFRA00AW and IEFRA00AV, respectively) were taken using a UWITEC gravity corer. Core diameters are 90 mm for La Thuile and Serre de l'Homme and 63 and 90 mm for Muzelle. Another core on Lake Serre de l'Homme (SDH-1) was also taken with a Russian corer close to the shore line. After coring, sediment cores were stored at 4°C.

The lake sediment cores used for DNA analyses as well as sedimentological/geochemical analyses measured 283.5 cm at Muzelle (core MUZ-12, 90 mm diameter from 0 to 130 cm depth and 63 mm from 130 to 183.5 cm depth), 549 cm at La Thuile (upper part of the core THU-10) and 81.5 cm (core SDH-09-P1) and 93 cm (core SDH-09-P2) at Serre de L'Homme. These cores cover different periods: 1700 years for Muzelle, 6450 years for La Thuile and 4000 years for Serre de L'Homme. Depending on the lakes, age-depth models are based on ^{14}C dates, geomagnetic field secular variations, short-lived radionuclide measurements and known lead-pollution levels. All age-depth models were generated using the *R software* and the *R-code package 'Clam' version 2.2* [84]. Details about sediment lithology and the age-depth models are provided in the "sediment lithology and dating" section of the supplementary materials. For Lake Serre de l'Homme, several cores were used. Thus, core correlations are also presented in detail in the "sediment lithology and dating" section of the supplementary materials. Age-depth models were used to estimate the sedimentation rate for each lake (cm/yr).

4.5. Sedimentological, geochemical and microfossils analyses

The cores were longitudinally cut, and a half-core was subsampled for DNA analyses (the heart of the slices, see section 2.7.) and for basic sedimentological analyses (edges of the slices). Samples reserved for DNA analyses were weighed wet. Edges of the sediment slices were weighed wet (Wet $weight_{Edge}$; g) and dry (dried at 60°C, Dry $weight_{Edge}$; g) to determine the water content (WC) and be able to calculate the total dry weight of the sediments (Dry $weight_{Total}$; g) and finally the total flux of sediments ($Flux_{Totsed}$; $g/cm^2/yr$), as follow:

1) $Flux_{Totsed}$ = (Dry $weight_{Total}$ * Sedimentation rate) / (Half core surface * Sample thickness)

Where, Dry $weight_{Total}$ = Dry $weight_{Edge}$ + Wet $weight_{Heart}$ - (WC* Wet $weight_{Heart}$);

and WC = (Wet $weight_{Edge}$ -Dry $weight_{Edge}$)/Wet $weight_{Edge}$

The edge samples were then used for Loss on Ignition (LOI) analyses, except for Lake Serre de l'Homme for which the analyses were performed on another core (SDH-09-P2). Samples were firstly ground in an agate mortar, and then the standardised procedure proposed by [85] was applied. The LOI at 550°C and then at 950°C burns the organic matter and carbonate particles, respectively. The contributions (%) of these two components can thus be estimated. The residue of these two successive ignitions provides an estimation of the content in non-carbonate mineral matter (%) and corresponds to alumina and silica-rich particles, i.e. clastic particles and/or biogenic silica.

In Lake Muzelle, where the sediments are dominated by the mineral terrigenous fraction, grain size measurements were also undertaken at the same sampling resolution as that employed for DNA analyses (on the other half of the core). Particle size analyses were carried out on bulk sediments using a Malvern Mastersizer S, which operates on the laser diffraction principle. Only the proportion of clays (< 2 μm), will be used in this study.

Complementary information about organic matter quality is used for lakes La Thuile and Serre de L'Homme (i.e. for which sediments are the richest in organic matter). In the case of Lake La Thuile, pyrolysis Rock Eval and XRF core scanner analyses from a previous study provide indices (Hydrogen Index, HI mgHC/gTOC, Oxygen Index, OI mgO_2/gTOC and Si/Ti as proxy of biogenic silica production) allowing us to distinguish the aquatic organic matter, the organic matter produced in the litter, the soil surface organo-mineral horizons, and the deep mineral soil horizons [66,68]. For Serre de l'Homme, the C/N atomic ratio was used as indicator of aquatic organic matter and organic matter derived from soils and land plant macroremains [69,86]. The carbon (C) and nitrogen (N) contents were measured with an elemental analyser (CEREGE, Aix en Provence).

Pollen analyses from Lake La Thuile and spores of coprophilous fungi from Lake Muzelle were already published in [66] and [75], respectively. For Lake La Thuile, samples do not correspond to those used for the lake sediment DNA analyses. For Lake Muzelle, samples analysed for coprophilous fungi are the same as those for DNA.

4.6. DNA metabarcoding approach

4.6.1. Lake sediment core sub-sampling

To avoid contamination, the sampling of the three half-cores was performed in a room dedicated to sedimentological analyses at the EDYTEM laboratory (University of Savoie Mont Blanc, Le Bourget du Lac-France), where no DNA analyses were previously performed. Sediment core slices were taken using sterilised metal plates. The edges of slices were removed using sterile scalpels as the surface of the half-core was in contact with the air, and the concave edge was in contact with water that circulates along the coring tubes. For each lake, samples were cut in two parts to perform two extractions by sediment slices. Fifty, 30 and 41 samples were taken from the cores corresponding to lakes - La Thuile, Muzelle and Serre de l'Homme, respectively. The thicknesses of sediment slices are 1 cm for lakes Muzelle and Serre de l'Homme but 0.5 or 1 cm for Lake La Thuile due to substantial variations in the sedimentation rate (greater than 10-fold variations) and thus to avoid high differences in time covered by the different samples. Sample wet weights were between 2.22 and 13.04 g for Lake La Thuile, between 4.08 and 15.63 g for Lake La Muzelle and 10.49 and 23.92 g for Lake Serre de l'Homme. These significant differences are due to different water content values, particle densities (organic vs mineral) and, in cases of lakes La Thuile and Muzelle, also due to the changes in sample thickness and

core diameters, respectively. In dry weights, these differences are higher because of the wide variability of the water content, especially between the top and bottom sediments (0.58 to 9.46 g for Lake La Thuile, 1.97 to 10.88 g for Lake La Muzelle and 0.76 to 14.3 g for Lake Serre de l'Homme.

4.6.2. DNA extraction

DNA extractions were performed in the Laboratoire d'Ecologie Alpine (University Grenoble-Alpes, France), in a room dedicated to ancient DNA extraction. Eleven extraction controls were performed (3 for lakes Muzelle and La Thuile and 8 for Lake Serre de L'Homme).

DNA extraction was performed by mixing the sediment with 20 mL of saturated phosphate buffer (0.12 M Na_2HPO_4; pH ≈ 8) for 15 minutes. Then, the mixture was centrifuged (10 minutes at 10000 g) to recover 400 µL of the resulting supernatant. DNA was extracted from the supernatant using the NucleoSpin® Soil commercial kit (Macherey-Nagel, Düren, Germany), following the manufacturer's instructions but omitting the lysis step. The DNA extract was eluted in 100 µL of SE buffer. This method of extraction allows the retrieval of the extracellular DNA pool that is dissolved in pore water and adsorbed onto mineral surfaces. It is unlikely that organically/inorganically complexed DNA is released by DNA-desorbing phosphate buffer [57].

4.6.3. DNA amplification and high-throughput sequencing

DNA amplification was realised in a second room of the ancient DNA laboratory using PCR. For the amplification of plants, we used the primers g-h, targeting the P6 loop region of the chloroplast trnL (UAA) intron [87]. For the amplification of mammals, we used universal primer MamP007 amplifying 60-84 bp fragment of the mitochondrial 16S gene [25]. To limit the amplification of human DNA, we used a human-specific blocking oligonucleotide (MamP007_B_Hum1, 5'-GGAGCTTTAATTTATTAATGCAAACAGTACC-C3-3'). A unique combination of 8 bp long sequence of nucleotides (tag) was added at the 5'end of each primer, in order to recognise each sample after the parallel sequencing of multiple samples [88].

To improve the reliability of the detection/ non-detection pattern, we performed multiple PCR replicates on each DNA extract [78]. For Lake Serre de l'Homme, we performed four PCR replicates on two DNA extraction replicates, yielding eight analyses replicates. For Muzelle and La Thuile samples we performed four PCR replicates on one single extraction replicate using the g-h and Mam-P007 primers. For mammals in the La Thuile samples, we performed 12 additional PCR replicates per sample (33 over 50 selected samples) on a second extract obtained from the same samples (which were divided into two parts).

All DNA amplifications were carried out at a final volume of 30 µL containing 2.5 µL of DNA template. The amplification mixture contained 1 U of AmpliTaq Gold® DNA polymerase (Applied Biosystems), 15 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.1 µM of each primer and 4.8 µg of bovine serum albumin (Roche Diagnostic). We added 2 µM of the human-specific blocking oligonucleotide to the PCR mixture in mammal analyses. For all primer pairs, the PCR mixture was denatured at 95°C for 10 minutes, followed by 45 cycles of 30 s at 95°C also for the denaturation, 30 s at 50°C for the hybridation and 1 min at 72°C for the elongation. A final elongation step was applied for 7 min at 72°C. The PCR products were then purified and mixed (equivolume mixes) before sequencing. Seventy-two PCR controls were included for each primer.

Sequencing was carried out using the Illumina Hi-seq technology (2*100 bp, paired-end reads), in three separate runs, one comprising four PCR replicates for plants and mammals from La Thuile and Muzelle samples; one for the additional 12 replicates of mammals in La Thuile samples and one for mammals and plants in Serre de l'Homme samples.

4.6.4. Data treatment and representation

The analysis of sequences and the taxonomic assignment were realised using the OBITOOLS software (http://www.grenoble.prabi.fr/trac/OBITools) [89]. The forward and reverse reads corresponding to the same DNA fragment were aligned and merged applying the *IlluminaPairEnd* function that takes into account the quality of merging. An "ngsfilter" file containing the list of samples and their associated combination of primer and tag was created and then used to assign each sequence to the relevant sample applying the *ngsfilter* function. Only sequences containing perfect tags and primers with a maximum of three errors were considered. The next step was to identify and merge the identical sequences for each sample using the *obiuniq* function. Afterwards, the *obigrep* function allowed the filtering of sequences based on two parameters, 1) the sequence length and 2) the sequence occurrence in the entire dataset. For plants, sequences shorter than 10 bp and sequences detected less than 100 times were removed. The same filters were applied for mammals, but the shortest sequences retained in the datasets had 60 bp. *Obiclean* was then used to determine the status of each sequence in each PCR product: "head", "internal" or "singleton" [89]. Only sequences that were more often "head" and "singleton" than "internal" in the global dataset were retained for the subsequent steps. Reference databases were built from the EMBL database with the *ecoPCR* program (gh-database-r113, mamP007-database-r113) and then used to assign a taxon to each unique sequence with the *ecoTag* function (the % of sequence similarity was calculated and specified in the final file).

For the subsequent analyses, only the sequences with a similarity >95% to taxa in the reference database were selected. We considered a sequence as present in a PCR replicate when at least five reads were counted [25]. In each lake dataset, we did not consider taxa that were only detected in one sample, or

stochastically in less than two replicates (i.e. taxa always detected in only one replicate but with detections in consecutive samples were kept). To remove contaminants, we excluded taxa frequently present in extraction and PCR negative controls (in more than 5 controls, where the total number of reads was greater than 10000), and taxa allochthonous in the Alps (like *Actinidia* sp.) (see Supplementary section 2.1 as well as Supplementary figures 3, 6 and table 2 for more details on contamination and on the data filtering steps). Potential impacts of the filtering procedure on the main results of the study are also presented and discussed in the supplementary material (Supplementary section 2.2. and Supplementary figures 4 and 5).

For each PCR replicate, we summed the total number of reads corresponding to terrestrial plants, aquatic plants and mammals separately. Then, we determined the mean and standard deviation of the log-transformed total number of reads across PCR replicates, as well as the number of replicates where more than 20 reads were detected. These two parameters are positively correlated (see Supplementary section 3), which supports the assumption that the number of reads is correlated to the DNA quantity available for amplification as suggested by previous studies on soils and lake sediments [29,44]. We normalised the log-transformed number of reads by the dry weight of sediments used for the extractions in order to obtain a proxy of the DNA concentration that we can compare with the concentrations of the main sediment components. The log-transformation allows correction of exponential DNA amplification during the PCR. We also determined a proxy of the richness (number of MOTUs: Molecular Operational Taxonomic Units) of mammals and plants, considering the presence of the taxa (more than 5 reads). As part of this process, for terrestrial plants, the mean value and standard deviation across replicates were calculated. We also determined a "maximum richness" from the sum of reads obtained in all the replicates for each detected taxa. For Lake La Thuile, we also calculated the pollen taxon richness to compare it with the proxy of the plant DNA richness, as that had already been carried out on another lake, but with plant macroremain data [21]. For mammals, we only determined the maximum richness from the sum of reads obtained in all the replicates for each detected taxa.

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Author contributions

C.G.-C., J. P and F.A. and K.J.W., contributed to the concept and designed the study. C.G.-C. and L.G. performed the DNA experiments, the sequence analyses and taxa assignment. M. B., L. F., A.-L.D., P.S., E.B., R.S., F.G., F.D. created the sedimentological, geochemical and pollen datasets. C.G.-C. analysed the data with the help of F.G.F. and P.S. F.A. and J. Poulenard contributed their expertise on the reconstruction of soil erosion and dynamics. E.M. provided expertise on the reconstructions of plant cover based on pollen analyses. K.J.W. provided its expertise on taphonomic processes in archaeological contexts and and skills in english. C.G.-C. wrote the manuscript with the contributions of all co-authors. C.G.-C. and K.J.W. coordinated the Marie Sklodowska Curie Individual fellowship (PALEO-AGRI project, EC grant number 655331) and the program funded by the Parc national des Ecrins. C.G.-C. and P.T. coordinated the Amaryllis program funded by the Conseil Général of Isère.

Data availability

Sequences for plant and mammal DNA (Raw and filtered data with the obitools) will be deposited in the DRYAD database under an accession number that will be provided after the acceptation of the manuscript.

The final DNA datasets and sedimentological/geochemical data will be available in the PANGAEA repository.

Competing financial interest

L.G. and P.T. are co-inventors of patents related to the gh primers and the use of the P6 loop of the chloroplast trnL (UAA) intron for plant identification using degraded template DNA. These patents only restrict commercial applications and have no impact on the use of this locus by academic researchers.

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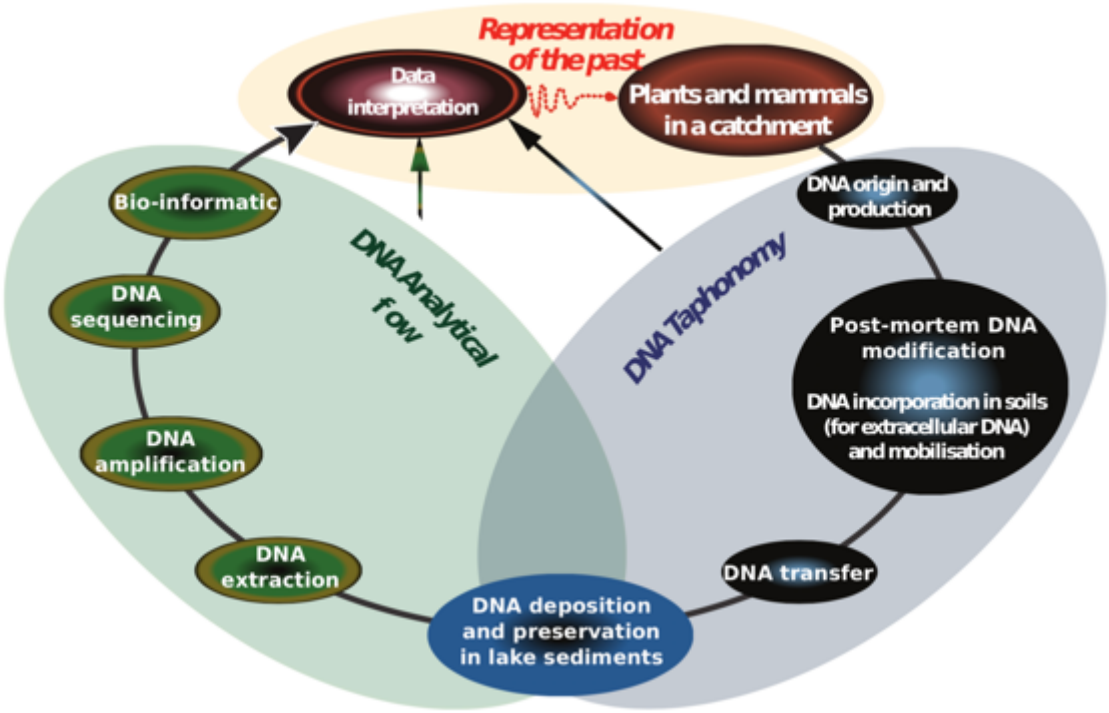

Figure 1. Flow chart of taphonomic processes and analytical process likely to affect reconstructions of the past, especially reconstructions of landscapes and agricultural activities.

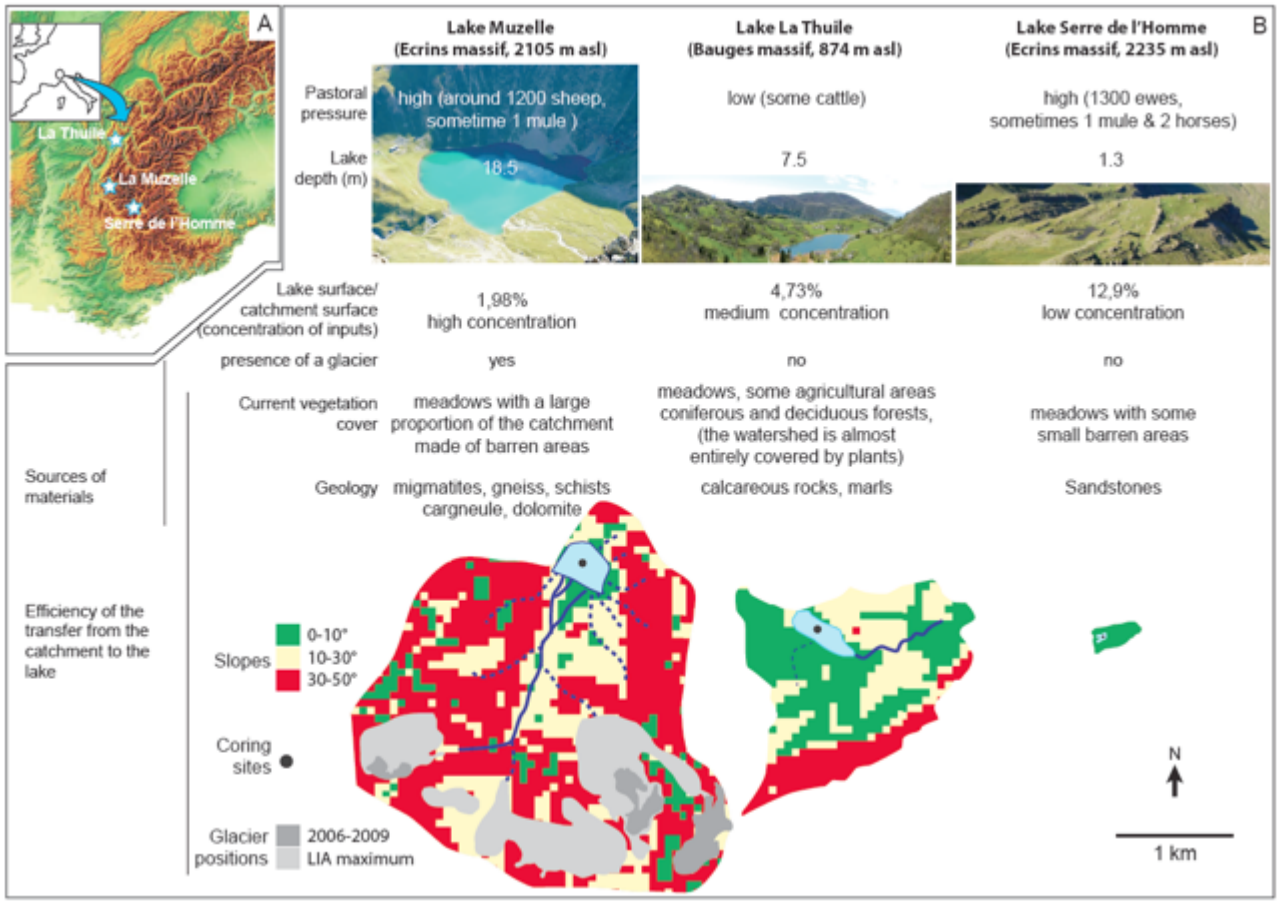

Figure 2. Presentation of the study sites. A) Location of sites. B) Presentation of the characteristics of each catchment-lake systems (pastoral pressure, physical characteristics and plant cover).

Table 1. Synthesis of plant DNA results for the three lakes. Grey shaded areas mean no analyses with these analytical conditions were realised. La Thuile and Muzelle were analysed in the same sequencing run.

	Lakes	*La Thuile*	*Muzelle*	*Serre de l'Homme*
	number of samples	50	30	41
	replicate number performed	4	4	8
	Illumina Hi-seq run numero	run 1	run 1	run 2
number of MOTU	***Terrestrial***	96	83	12
	Aquatic	11	0	7
number of reads	***Terrestrial***	796266	1836110	1205395
	Aquatic	326988	0	4517931
	Terrestrial (%)	70,90	100	21,10
	Aquatic (%)	29,10	0	78,90
% of samples with x positive replicates	***Terrestrial*** ***0***	2	0	**58,5**
	Terrestrial ***1***	4	0,3	**26,8**
	Terrestrial ***2***	6	0	4,9
	Terrestrial ***3***	**12**	**10**	9,8
	Terrestrial ***4***	**76**	**86,7**	0
	Terrestrial ***>4***			0
	Aquatic ***0***	28	0	44
	Aquatic ***1***	22	0	22
	Aquatic ***2***	2	0	22
	Aquatic ***3***	14	0	0
	Aquatic ***4***	34	0	0
	Aquatic ***>4***			**12**

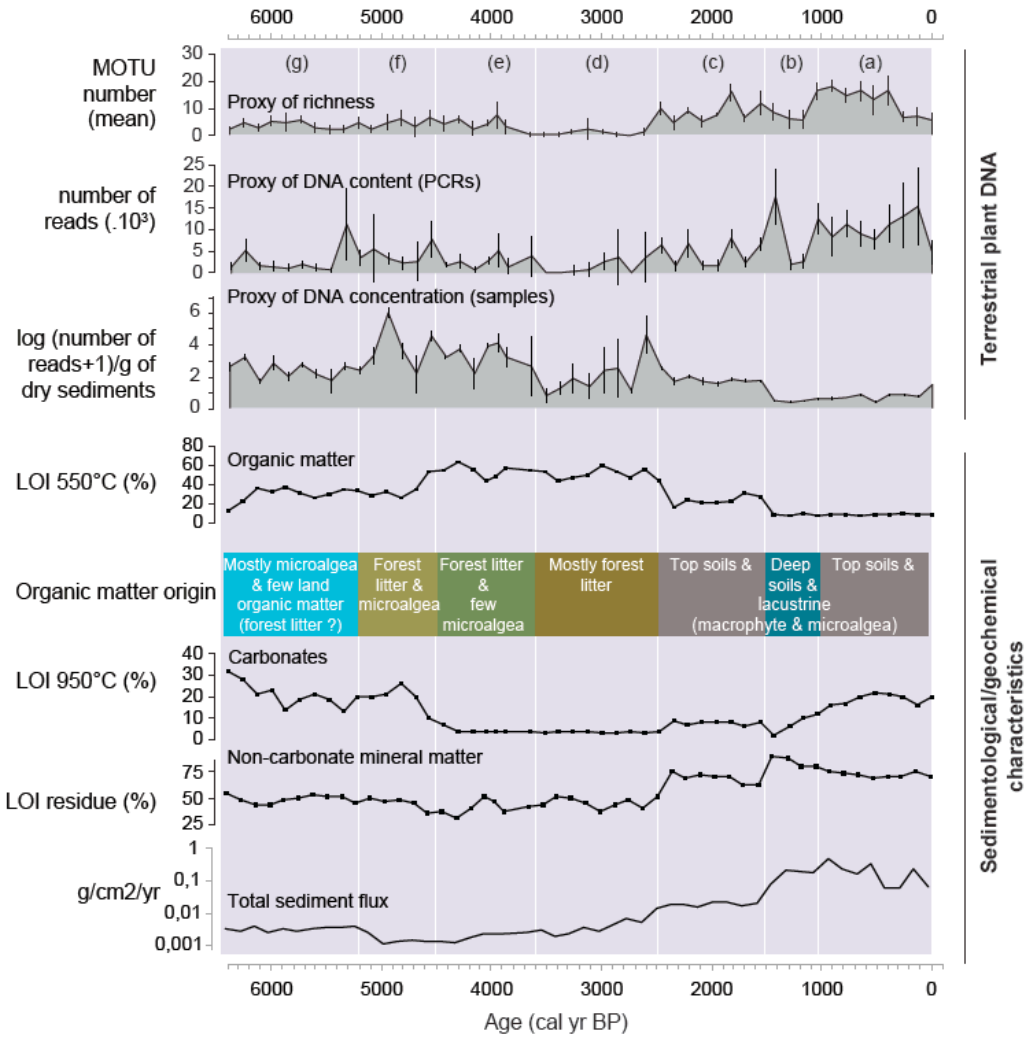

Figure 3. Comparison between global terrestrial plant DNA and the sedimentological/geochemical properties of sediments in Lake La Thuile over the last 6500 years. To study the behaviour of land plant extracellular DNA we focused on the proxies of the richness (mean and standard deviations of the number of MOTU) and the DNA contents in the extracts (number of DNA reads) and the samples (mean and standard deviations of the log(number of DNA reads+1)/dry mass of sediment). These variables were compared to several selected sedimentological and geochemical data: the organic matter content ($LOI_{550°C}$) and origin, the contents in non-carbonate mineral matter (LOI residue) and carbonates ($LOI_{950°C}$) and the total sediment flux (g/cm^2/yr). The organic matter origin is determined from the combination of data from pyrolysis Rock Eval analyses (Hydrogen Index in mg HC/ g TOC and Oxygen Index in mg O_2/g TOC, Bajard et al. 2017), X-Ray fluorescence core scanner analyses (Si/Ti as a proxy of biogenic silica, Bajard et al. 2016), the lithological description and the aquatic plant DNA analyses (Supplementary Material figures 2 and 4). Seven specific phases of changes in DNA content were defined and discussed in the text (purple shaded areas a, b, c, d, e, f and g). They correspond to different sedimentological and geochemical characteristics, which inform hypotheses explaining the behaviour of the extracellular DNA from the catchment.

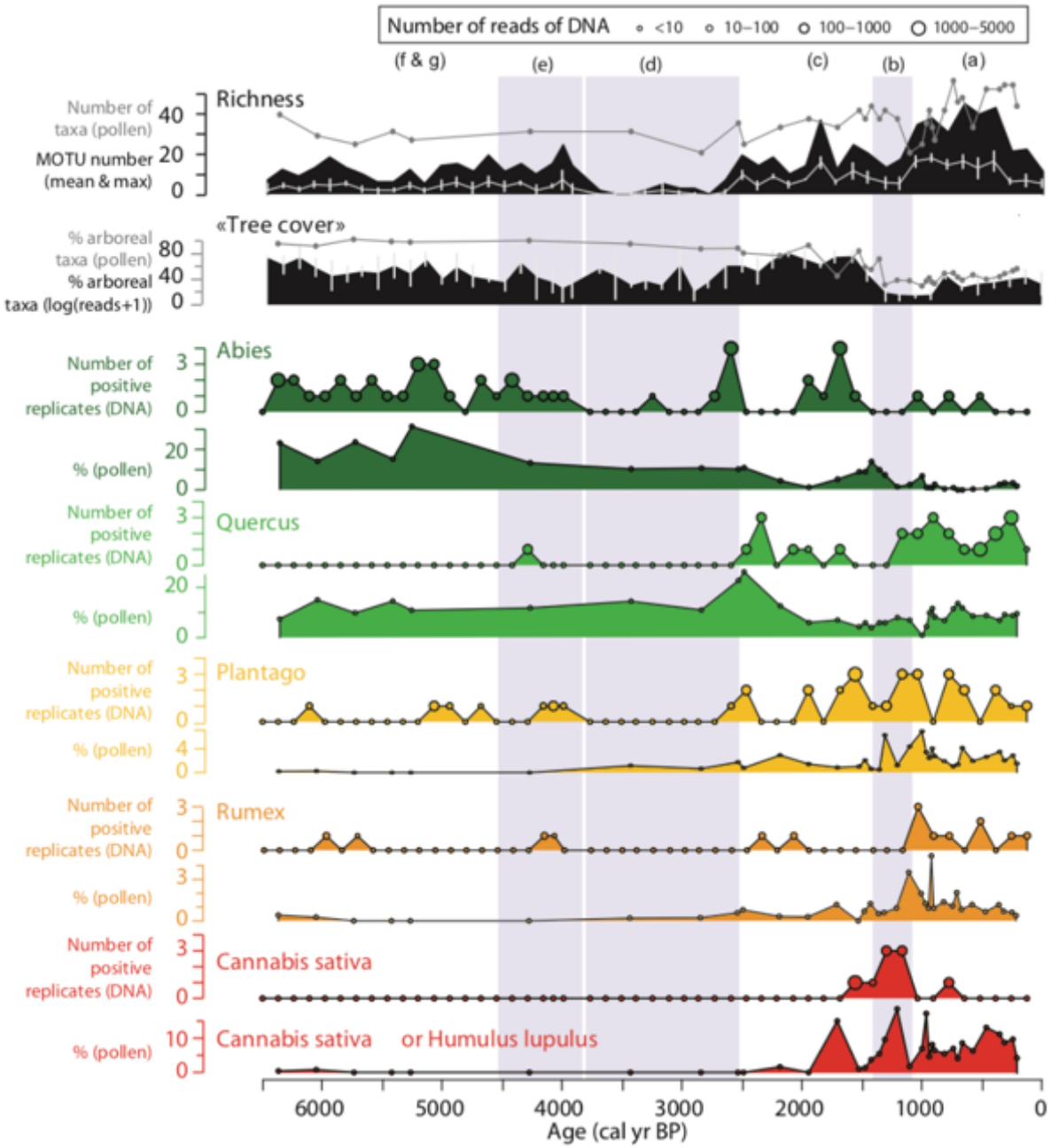

Figure 4. Comparison between lake sediment DNA and pollen data from Lake La Thuile. The temporal evolution of the richness, the percentage of arboreal taxa, and several selected taxa are presented for the both methods. For the richness and the percentage of arboreal taxa determined from the terrestrial plant DNA dataset, we present the mean values and standard deviations of the four replicates. The maximum richness, i.e. cumulating all the replicates is also presented as it provide a more pertinent absolute value to compare with that of pollen. For each taxon in DNA, the size of circles is proportional to the number of reads (see scale on the top of the figure). The purple shaded areas underline the periods (b), (d) and (e), when no or very few DNA was detected.

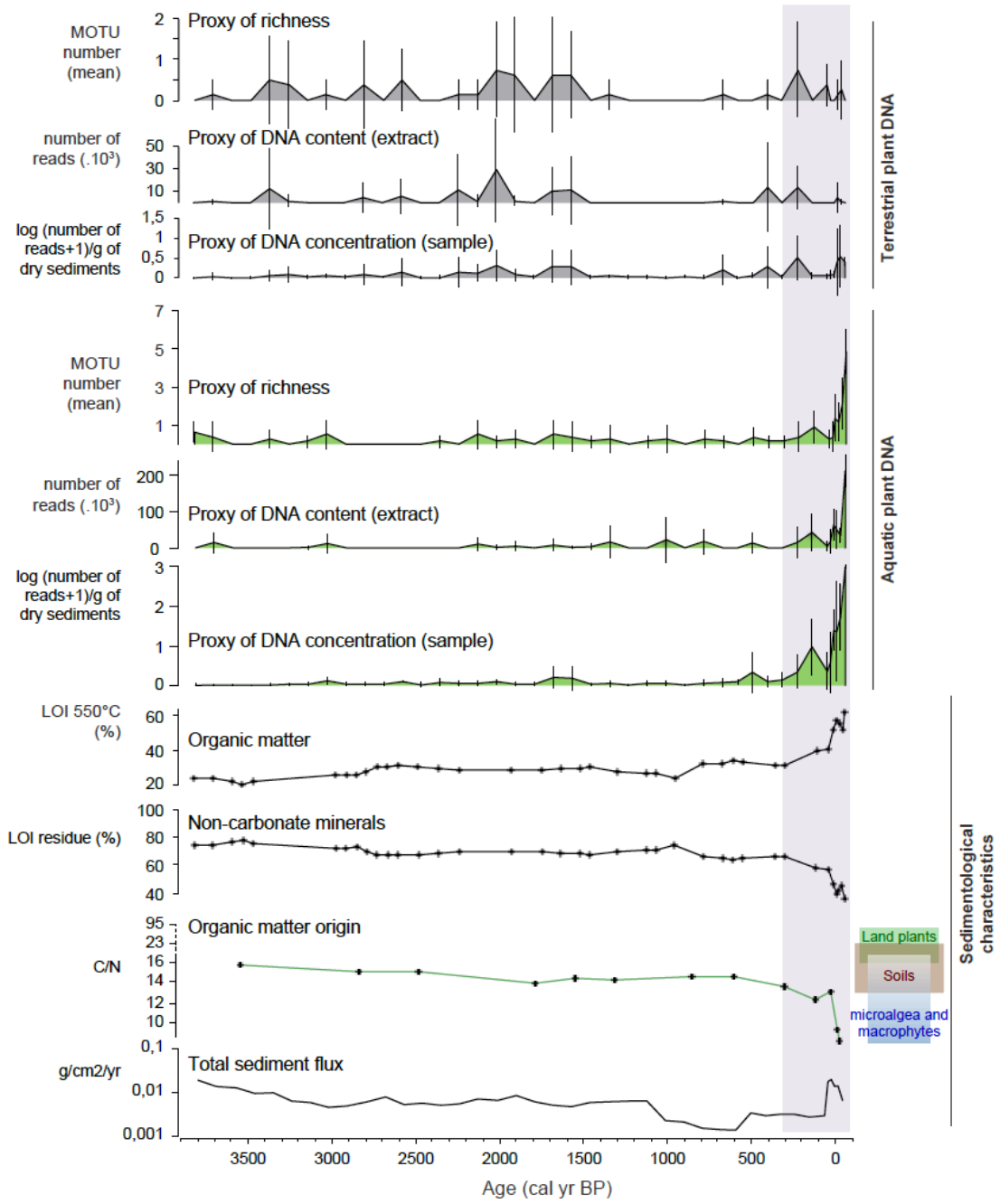

Figure 5. Comparison between plant DNA (terrestrial and aquatic) and the sedimentological/geochemical properties of sediments from Lake Serre de L'Homme over the last 3800 years. To study the behaviour of plant extracellular DNA we focused on the proxies of the richness (mean and standard deviations of the number of MOTU) and the DNA content (mean and standard deviations of the log(number of DNA reads+1)/dry mass of sediment), These variables were compared to the organic matter content ($LOI_{550°C}$) and origin (C/N atomic ratio), the content in non-carbonate mineral matter (LOI residue) and the total sediment flux (g/cm2/yr). The ranges of C/N values of land plants (green shaded area), soils (brown shaded area) and algea and aquatic plants (blue shaded area) come from the literature (Bertrand et al., 2010; Duarte, 1992; Li et al. 2013; Meyers, 1997; Thevenon et al., 2012). The main change in sediment composition is characterised by an increase in aquatic organic matter production corresponding to an increase in aquatic plant DNA.

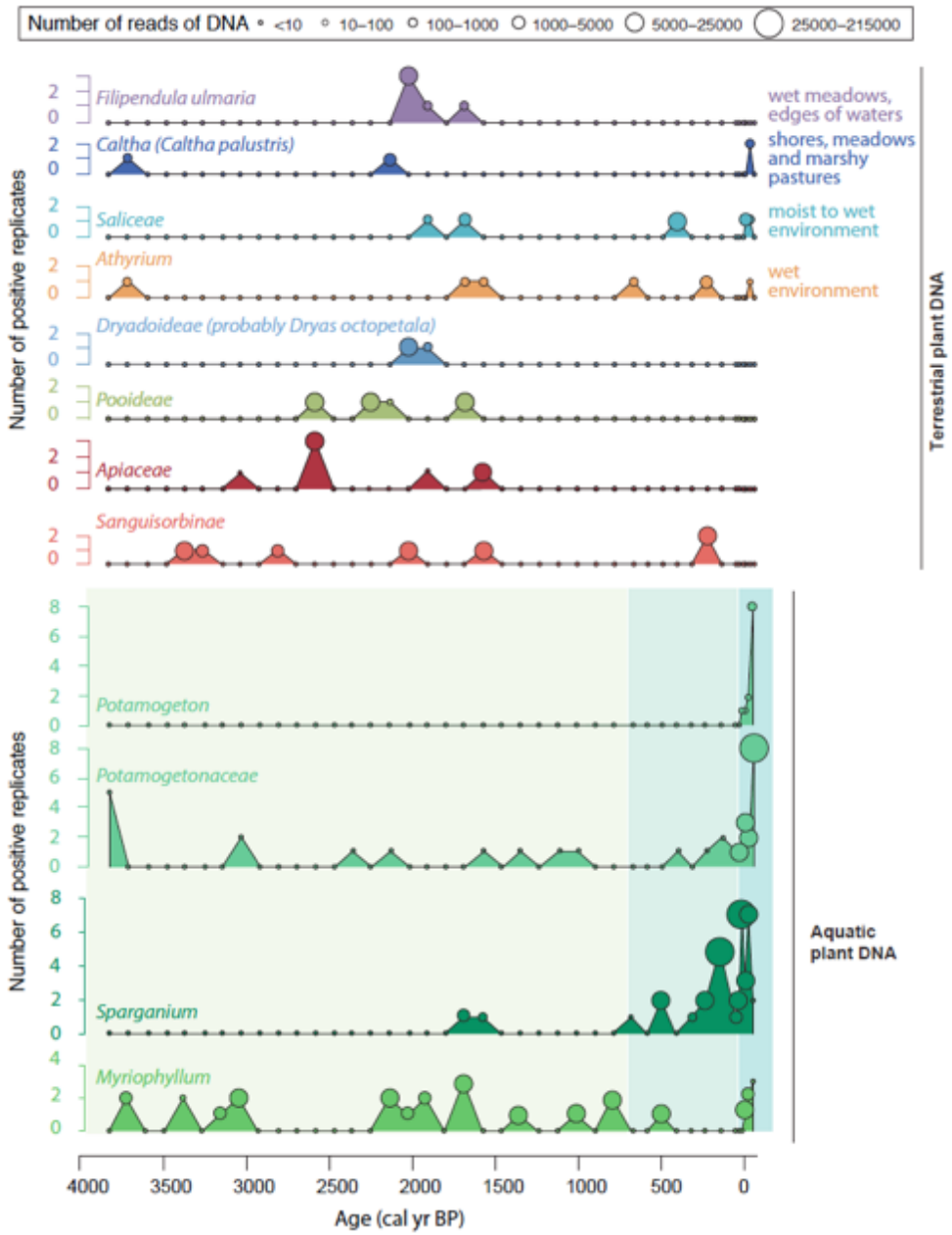

Figure 6. Community composition of terrestrial and aquatic plants provided by the DNA analyses. For each taxon, the size of circles is proportional to the number of reads (see scale on the top of the figure). Four over eight terrestrial taxa are specific of wet environments. The detection of terrestrial taxa is relatively stochastic and only three taxa are detected in more than one replicate but in one sample (*Filipendula ulmaria*, *Caltha* and *Apiaceae*). However, each aquatic taxon is more frequently detected and often in at least two replicates. Moreover, their detections are clustered in specific periods highlighted by the green areas: the periods 3800-2950 and 2250-700 cal. BP are mostly characterised by *Myriophyllum sp.*, the period 700-10 cal. BP by *Sparganium sp.* and the period from 10 to -59 cal. BP the three taxa.

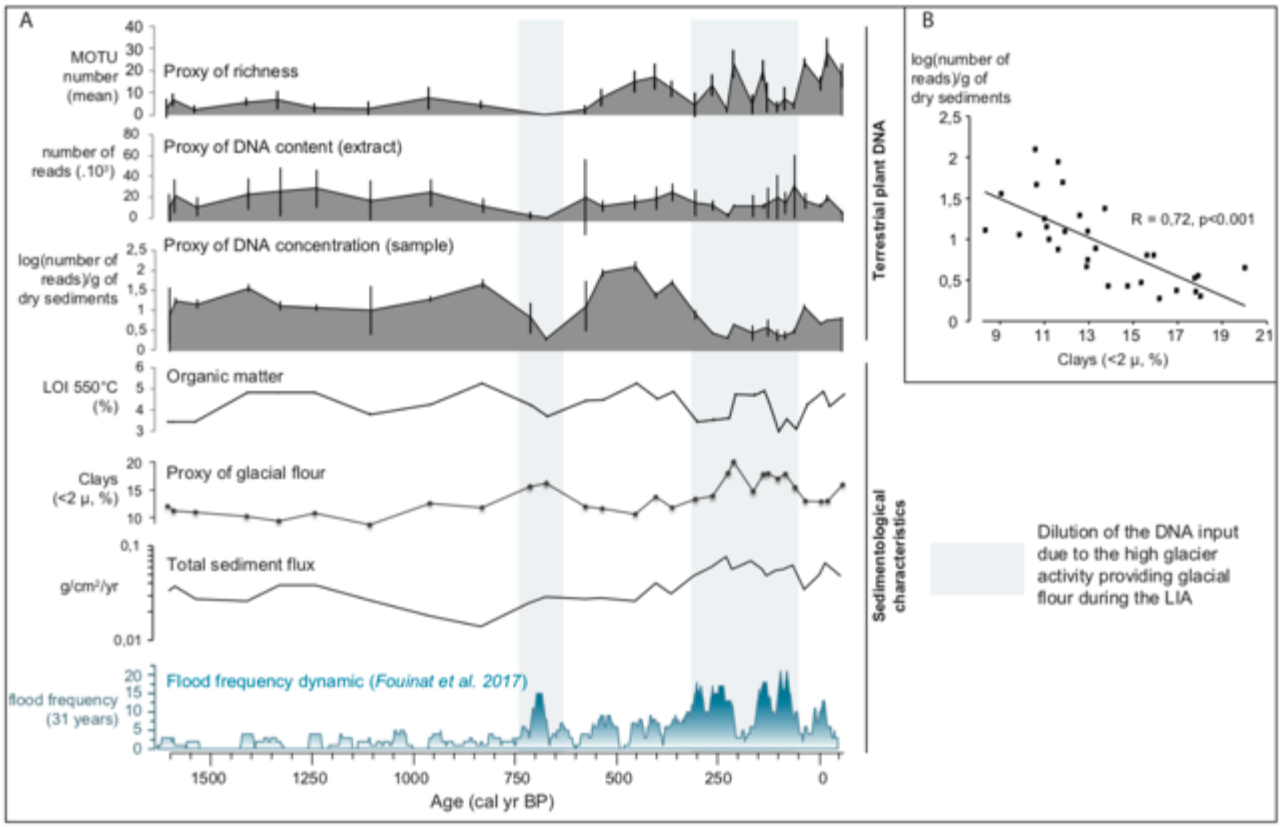

Figure 7. Comparison between terrestrial plants DNA archived in Lake Muzelle sediments and the sedimentological/geochemical properties of sediments. A) Evolutions of the richness (mean values and standard deviations of the four replicates), the contents in DNA reads in the extracts (mean number of DNA reads) and the samples (mean number of DNA reads normalised by the dry mass of sediment and standard deviations of the four replicates), the organic matter content (LOI 550°C), the clay content and the flood frequency over the last 1600 years. Blue areas highlight phases of high inputs of clays and high flood frequency, which corresponds to low DNA concentration in the sediments samples. B) Relationship between the DNA content in the samples and the clay content.

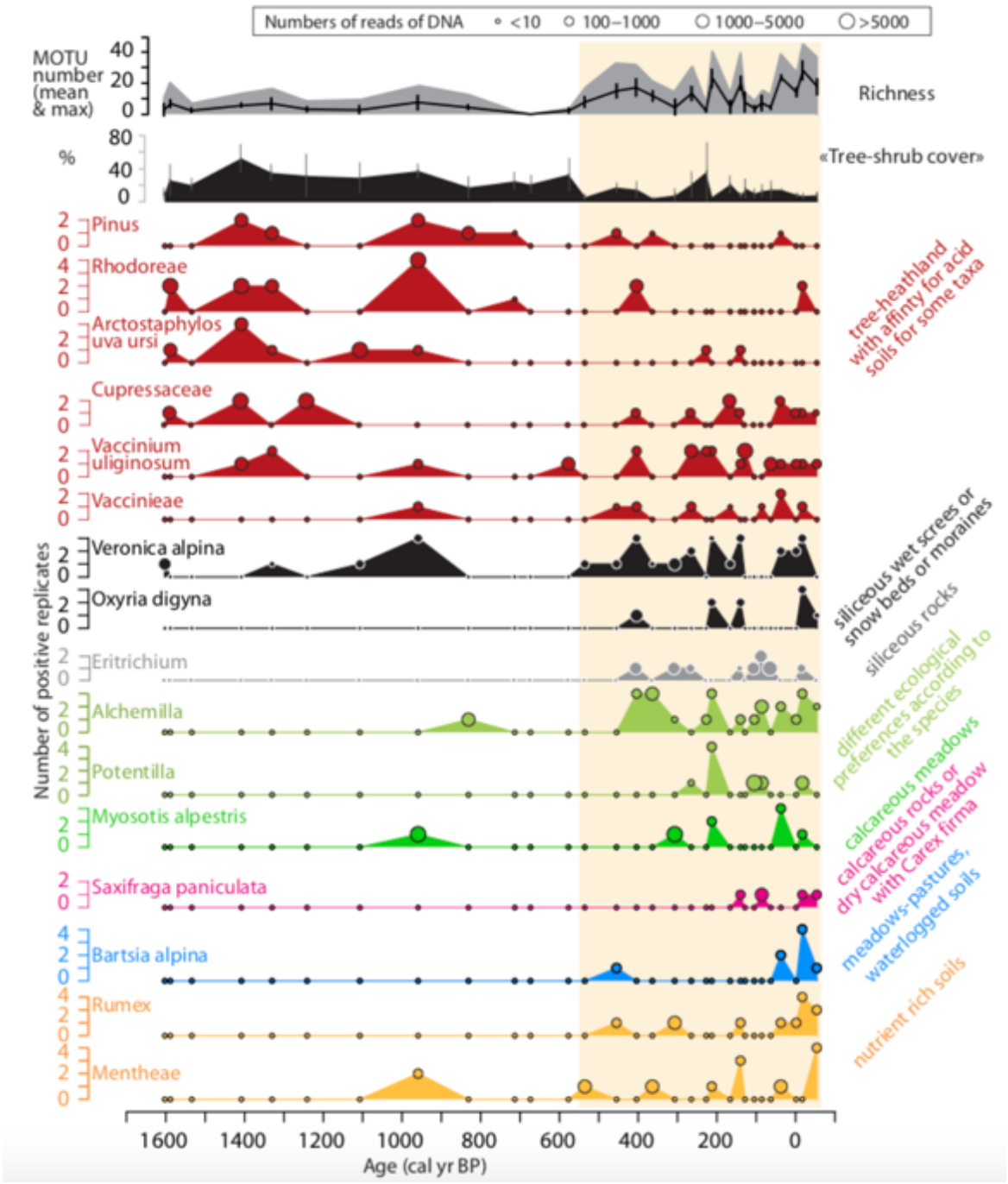

Figure 8. Plant cover evolution around Lake Muzelle from lake sediment DNA analyses. The richness (mean and maximum), the percentage of arboreal taxa and several taxa (species and genus) of different ecological preferences (mentioned on the right side of the figure) were selected to document the landscape and environmental changes. *Alchemilla* sp. and *Potentilla* sp. can have different ecological preferences according to the species. However, these pollen types were frequently observed in overgrazed and trampling sites (Court-Picon et al. 2005). A study on lake sediments DNA also observed these taxa during phases when pastoral activities with sheep and/or cow were recognised (Pansu et al. 2015). For each taxon, the size of circles is proportional to the number of reads (see scale on the top of the figure).

Table 2. Synthesis of mammal DNA results from the three lake sediment cores. Grey shaded areas mean no analyses with these analytical conditions were realised.

primer	replicate number	Illumina Hi-seq run	detected taxa		
			La Thuile	Muzelle	Serre de l'Homme
Mam-P007	4	1	*Bos sp.*	No DNA	
Mam-P007	12	2	*Bos sp., Ovis sp., (Canis sp.)*		
Mam-P007	8	3			No DNA

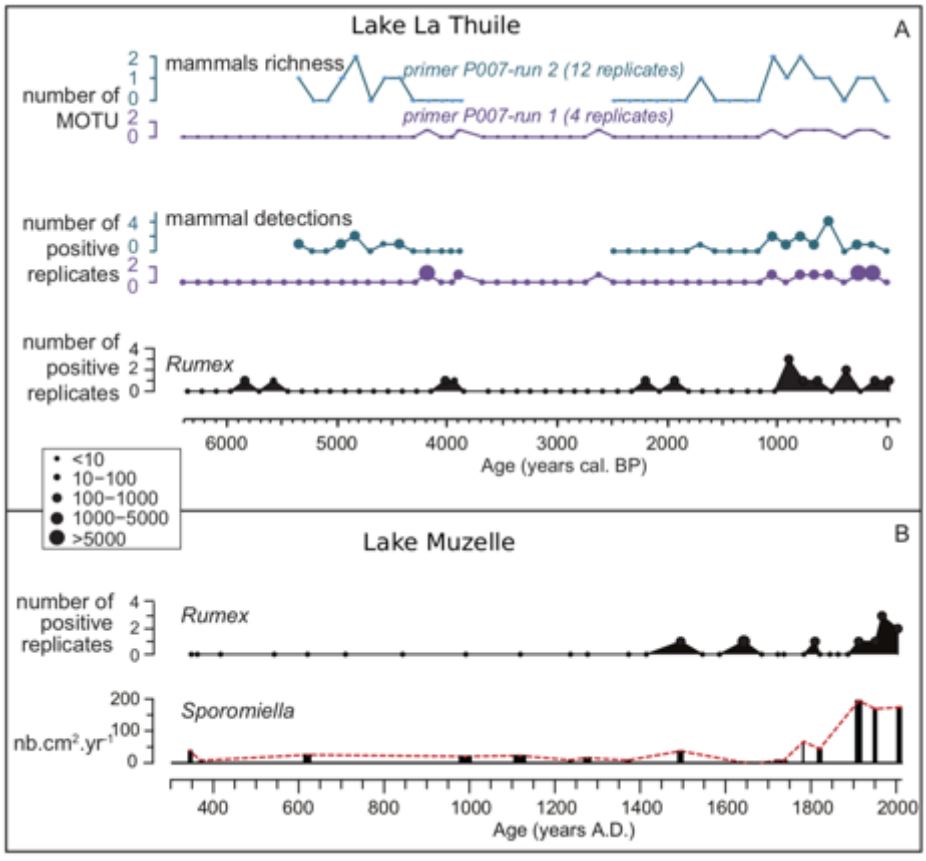

Figure 9. Comparison of proxies of the presence of domestic animals in the aim of studying the taphonomic processes and analytical biases affecting mammal DNA. A) Comparison for Lake La Thuile between the mammal DNA results obtained from the same primer "mam P007", but not with the same replicate numbers (4 vs 12). The DNA from *Rumex sp.* is also presented as a proxy of high animal stocking rate or stock density (nitrophilous plant) to compare with the mammal DNA. B) Comparison on Lake Muzelle between the DNA from *Rumex sp.* and spores of coprophilous fungi (*Sporomiella sp.*).

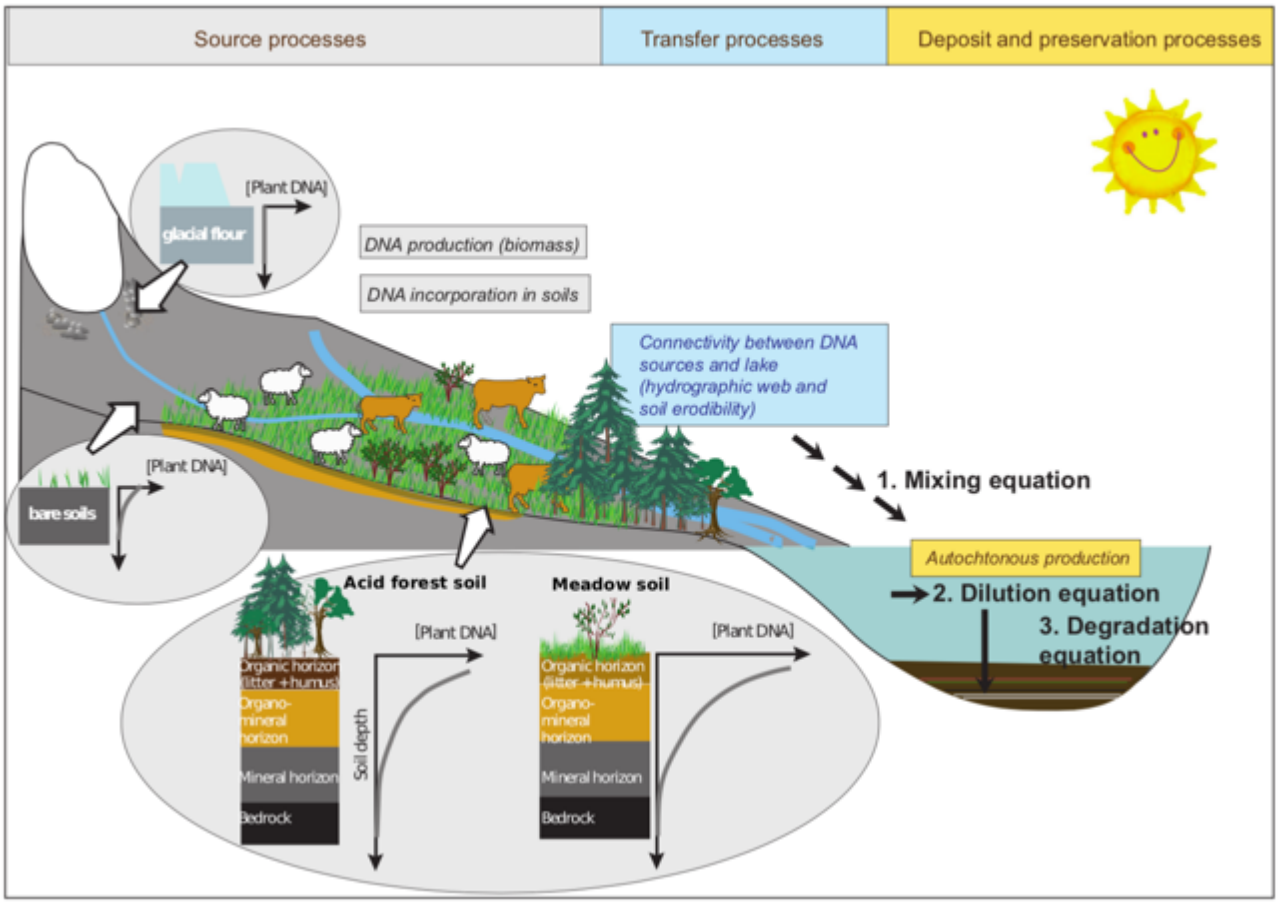

Figure 10. Proposition of a model describing the processes driving the archiving of extracellular DNA from plants and mammals in the lake sediments. Taphonomic processes acting at the source and driving the transfer, deposit and preservation of the DNA in the lake sediments are summarised.