

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

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22 **Abstract**

23 Over the last decade, an increasing number of studies have used lake sediment DNA to trace past
24 landscape changes, agricultural activities or human presence. However, the processes responsible for
25 lake sediment formation might affect DNA archiving via taphonomic and analytical processes. It is
26 crucial to understand these processes to ensure reliable interpretations for “palaeo” studies. Here, we
27 combined plant and mammal DNA *metabarcoding* analyses with sedimentological and geochemical
28 analyses from three lake-catchment systems that are characterised by different erosion dynamics. The
29 new knowledge from this approach concern the DNA sources and transfer processes. The sources of
30 eroded materials strongly affect the “catchment-DNA” concentration in the sediments. For instance,
31 erosion of upper organic and organo-mineral soil horizons provides higher amount of plant DNA in
32 lake sediments than deep horizons, bare soils or glacial flours. Moreover, high erosion rates along with
33 a well-developed hydrographic network, are proposed as factors positively affecting the representation
34 of the catchment flora. The development of open and agricultural landscapes, which favour the erosion,
35 could thus bias the reconstructed landscape trajectory. Regarding domestic animals, pastoral practices

36 and animal behaviour might affect their DNA record because they control the type of source of DNA
37 (“point” vs. “diffuse”).

38

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

41

42 **1. Introduction**

43 **1.1. History and potential of the lake sediment DNA** 44 **(sedDNA)**

45 The earliest studies on ancient DNA (aDNA) from lake sediment archives date to the mid-1990s^{1,2}.
46 However, molecular biology techniques have been applied more extensively on lake sediments for the
47 last eight years only³⁻⁸. Lake sediments accumulate through time both autochthonous (in-lake biological
48 production and chemical precipitation) and allochthonous (particles brought from the catchment and
49 beyond) materials that can bear DNA. Their study using molecular biology techniques, therefore, has a
50 great potential to identify any of organisms present within the “lake’s sediment source area” (i.e., the
51 lake itself, its catchment area as well as the atmosphere). Downstream, this could help to trace changes
52 of biodiversity over time, from the scale of the population to that of the ecosystem and to address a wide
53 range of questions, especially in ecology^{5,9,10}. Before 2008, only a few studies were performed on
54 aDNA of terrestrial organisms from lake sediments, and all focused on pollen DNA^{2,11,12}. In the
55 meantime, most studies focused on aquatic organisms^{1,13-19}. This may be due to the perception that the
56 DNA from organisms within the lake would be preferentially archived in the sediments (or in higher
57 quantities) compared to the DNA derived from the catchment area. However, since 2008, researchers
58 have successfully tracked organisms derived from terrestrial environments, using bulk sediments and
59 focusing on extracellular or total DNA²⁰. These studies on bulk sediments targeted plants²¹⁻³⁰,
60 mammals^{25,31}, humans and/or animal specific faecal bacteria³¹⁻³⁶ and more recently eukaryotes⁶ in the
61 aim of reconstructing past vegetation cover, landscape, climate, agro-pastoral activities, human
62 occupation or the relationships between humans and landscapes and the wide spectrum of diversity.
63 They demonstrated the great potential of this tool in providing new knowledge for palaeoecology and
64 archaeology.

65 **1.2. Issues and limits: taphonomic considerations**

66 1.2.1. Plant DNA records

67 Despite several positive results, several studies questioned the interpretation of lake sedDNA results,
68 suggesting concern over analytical and/or taphonomic processes, i.e. all the processes that govern the
69 production, transfer, incorporation and preservation of the lake sedDNA (modified from ³⁷). For
70 instance, Pedersen et al. ³⁸ did not detect a substantial proportion of DNA from the local flora which
71 was independently identified by macrofossils. They proposed multiple, non-exclusive explanations,
72 such as the high abundance of some taxa that may overwhelm the rarest taxa. The taxonomic resolution
73 and assignment rate could have been limited by the degradation of DNA sequences, the sequencing
74 depth or the incompleteness of the reference database. Indeed, in a more recent study, also from the
75 Arctic, the authors obtained superior taxonomic recovery between aDNA and macrofossils, probably
76 due to the use of an almost complete reference library, as well as optimised extraction protocols
77 (sediment quantity) and sequencing conditions ²¹. Several studies also revealed discrepancies between
78 records of plant DNA, pollen and macrofossils, which may reflect differences in the source (production,
79 origin), transfer modalities, depositional environment as well as preservation conditions for these
80 different vegetation-cover proxies ^{23,29,38}. Whereas taphonomic processes are relatively well-known for
81 pollen and plant macroremains, their understanding for lake sediment DNA is still limited ^{3,9,37},
82 especially for extracellular DNA, which by definition excludes the DNA from pollen and plant
83 macroremains. However, a recent review ^{3,20} and two studies based on the comparison of modern
84 vegetation with pollen and DNA analyses from surface sediments of a large set of lakes in different
85 vegetation environments (tundra to forest tundra environments ³⁹; boreal and alpine ⁴⁰) suggested that
86 1) pollen does not significantly contribute to the DNA records, 2) the DNA has a local origin and
87 probably has a similar source as the macrofossils, 3) aquatic plants are well-represented, 4) taxa
88 detection seems to depend on the distance to lake shore, the relief and its abundance (biomass) in the
89 vegetation, 5) different types of sediments might have an impact on the DNA preservation. These
90 studies targeted both intra and extracellular DNA, of which the respective contribution to the sediments
91 remains unclear ³ while the taphonomic processes affecting each of these DNA pools can be expected
92 to differ.

93

94 1.2.2. Mammal DNA records

95

96 Regarding the DNA of mammals, some studies also raised questions about taphonomic processes which
97 might affect the DNA records. For instance, ²⁵ did not find sheep extracellular DNA in modern
98 sediments from a small subalpine lake (Lake Anterne, 2063 m a.s.l, Northern French Alps), while sheep
99 flocks are present today in the catchment. Here, low stocking-rates (low biomass) and scattered
100 distributions of domestic animals (representing a “diffuse source” of DNA) have been proposed as an

101 explanation for the non-detection of DNA. On the contrary, high stocking-rates and/or the existence of
102 areas used for the herding or flocking of animals (e.g. enclosures or folds, representing a “point source”
103 of DNA because of the « concentration effect » of animals) might explain the enhanced supply of
104 mammal DNA in the sediments during previous periods ²⁵. Moreover, urine and faeces - two main
105 sources of animal DNA ^{41,42} - are produced especially during the night within the enclosures or folds ⁴³.
106 The presence of enclosures within a catchment is thus expected to significantly favour the detection of
107 domestic animal DNA. Another study that aimed to identify the presence of humans in a catchment
108 using human-specific bacteria DNA also proposed potential biases in the record due to taphonomic
109 issues ³². In fact, the absence of human-specific bacteria DNA while pollen data suggests the presence
110 of humans might be due to DNA concentrations below the limit of detection, for instance, if human
111 camps/villages are at far from the lake or the inlet (thus limiting the DNA transfer to the lake), and/or
112 as a consequence of a low population density (thus limiting the DNA production and biomass). An
113 alternative explanation might also be that pollen reflect a more regional record.

114 1.2.3. “Time shifts”

115 Several studies raised the question of potential “time shifts” in lake sediment DNA records related to
116 DNA leaching through the sediment layers ²⁹ or DNA preservation and storage in soils and its release
117 into the environment several centuries after its production ²⁵. The release into the environment of
118 molecules stored in soils for decades has already been observed for pesticides, which are persistent
119 molecules ⁴⁴. Nevertheless, for DNA in alpine soils, it has been shown that very little DNA from crops
120 cultivated more than 50 years ago, can be detected ⁴⁵. This study also shows a significant correlation
121 between the proportion of DNA in soils and the proportion of above ground biomass for different
122 functional plant groups, suggesting that the DNA brought by soil erosion will mainly reflect the
123 ecosystem established at the time of the erosion event and will only weakly be influenced by long-term
124 DNA storage in soils. This is supported by recent studies in which DNA accurately recorded the timing
125 of changes in a vegetation cover and mammal distribution, in accordance with detailed evidence from
126 historical and other sedimentological sources ²⁹⁻³⁰. This good concordance with an independent
127 approach highlights not only the absence of release of old DNA stored in soils but also suggests limited
128 DNA leaching through the sediment layers.

129

130 1.2.4. DNA Degradation/preservation processes

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

135

136 DNA degradation is triggered by both abiotic and biotic mechanisms. From the cell death, mechanisms
137 of DNA repair cease and DNA starts to degrade through several chemical reactions (oxidation,
138 hydrolysis, alkylation and Maillard reaction) acting both inside and outside the cells after their lysis,
139 thus affecting both intracellular and extracellular DNA^{46,47}. The rate of chemically-induced degradation
140 is controlled by several environmental factors. Low temperature, high salt concentration (high ionic
141 strength) and high pH limit the hydrolysis and thus favour the DNA preservation⁴⁸⁻⁵⁰. Environments
142 protected from ultraviolet (UV) radiation also favour DNA preservation as this radiation causes DNA
143 damage⁵⁰. The extracellular DNA is also affected by microbial activity. In fact, the degradation by
144 DNases produced by bacteria is considered as the primary mechanism of extracellular DNA degradation
145⁵¹. However, DNA can be protected from this process when it is adsorbed onto charged surfaces (clays
146 and humic substances), or absorbed into the crystal lattice of fine particles, amorphous crystals and
147 particulate organic compounds^{51,52}. This protection can also be due to the inactivation of DNases via
148 their binding on particles⁵³. The binding of extracellular DNA on particles, as well as the degree of
149 protection, are complex processes as they are dependent on the mineralogy of the sorbent, the presence
150 of organic material, pH conditions, the ionic strength and length of the DNA molecules^{54,55}. In soils,
151 nucleic acids released from cells were found to be quickly bound to particles^{51,53,55,56}, which delays the
152 DNA degradation and might explain the detection of a few sequences of crop DNA in the alpine soils,
153 50 years after the stop of crops⁴⁵. Inside the lake, bacterial activity, oxygenation, salt concentration,
154 organic and mineral particles, UV penetration and pH conditions can vary through time and thus
155 differentially affect the DNA preservation. When sediments are deposited in the lake bottom, they
156 quickly become anoxic after burying, which limits microbial activity and thus favours long-term DNA
157 preservation. However, the uppermost sediments often represent an active layer that can significantly
158 modify the concentration and composition of microbial DNA⁸. With burial, DNA becomes also totally
159 protected from UV radiation. In marine sediments, it has also been shown that a high proportion of
160 extracellular DNA is bound to minerals or humic substances^{56,58}. Given the mechanism of DNA
161 protection provided by the binding, the absence of oxygen and UV, aquatic sediments are, *a priori*,
162 good environments for DNA preservation²⁰. However, the low bacterial activity and the DNA binding
163 on particles do not prevent chemically induced DNA degradation, especially hydrolysis. DNA
164 degradation should trigger a decrease of the DNA pool with time and decrease the size of DNA
165 fragments still present. A time-dependent DNA decrease was reported in a study of dinoflagellate DNA
166 from fjord sediments in Antarctica⁵⁹, and several studies reported the loss of long fragments with age
167^{5,28,60}. Ageing also triggers cytosine to thymine substitutions at the single-stranded ends of the DNA
168 fragments, which can be used to discriminate between ancient DNA sequences and contaminations from
169 modern DNA^{28,61,62}. DNA preservation can also vary among different groups of organisms as well as
170 among different species of the same group^{59,63,64}.

171 **1.3. Challenges ahead**

172 In the light of all the previous considerations, there is a need to investigate the potential distortions of
173 the lake sediment DNA record due to taphonomic processes (production, transfer, preservation of DNA)
174 and/or analytical procedures (extraction/amplification/identification)^{9,10,37} (Fig. 1). Without a good
175 understanding of these processes, the full potential of lake sediment DNA cannot be realised. Especially
176 important is the issue as to whether the DNA archived in the sediment represents a reliable diachronic
177 signal; i.e. are the following characteristics or processes constant over time: 1) the source of DNA, 2)
178 processes and efficiency of DNA transfer, and 3) preservation conditions of DNA?

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

182 We therefore present the empirical analysis of temporal lake sedDNA datasets from three mountainous
183 lake-catchment systems characterised by various erosion dynamics due to the different geological
184 formations, topographical characteristics and vegetation and soil covers (Figure 2A/B), in order to get
185 information on these taphonomic (i.e. source and transfer) processes. Both plant and mammal
186 extracellular DNA were investigated using the DNA *metabarcoding* approach, which is the
187 amplification and sequencing of DNA molecules found in the environment using universal markers⁶⁵.
188 This extracellular DNA may represent the main DNA pool in sediments^{57,66} and is of great interest as
189 it may provide the most integrated view of aquatic, sedimentary and terrestrial biodiversity⁵⁸. Here, we
190 only focused on this particular DNA pool to avoid the extraction of DNA from plant macroremains,
191 which might lead to an overrepresentation of these taxa and limit the detection of the other, rarer taxa.
192 Sedimentological and geochemical data were also acquired to get information about the processes of
193 sediment production, transfer and deposit as well as of lake water physico-chemical conditions. Pollen
194 or coprophilous fungi data were included in the study as complementary evidence of vegetation cover
195 changes and domestic herds presence. All these data are key to understand the processes which drive
196 the DNA records as well as to emphasise how changes in taphonomic conditions over time can affect
197 the quality of the DNA record and thus of the landscape and land-use reconstructions.

198

199 **2. Results and interpretations**

200 **2.1. Plant and mammal DNA detected in the three lakes**

201

202 After the filtering procedure, 107 and 83 MOTUs of plants are detected in lakes La Thuile and Muzelle,
203 respectively, while only 19 MOTU are found in Lake Serre de l'Homme. In Lake Muzelle, we
204 exclusively detected DNA from terrestrial plants (100% of the reads). Lake La Thuile presents a mixed

205 recording, but most of the DNA reads are of terrestrial origin (71% of reads distributed in 96 MOTU,
206 Table 1). Conversely, most of the DNA reads detected in Lake Serre de l'Homme are aquatic in origin
207 (79% of reads distributed in 7 MOTUs but probably only representing 3 different taxa, Table 1 and
208 Supplementary figure 3).

209

210 Based on the comparison between the proportions of samples in which terrestrial plants are detected in
211 0, 1, 2, 3 or 4 replicates, it is clear that the low terrestrial plant richness detected in Lake Serre de
212 l'Homme also corresponds to very low quantities of DNA extracted from the samples compared to the
213 two other lakes. In fact, we never detected terrestrial plants in more than three replicates over eight and,
214 in 85% of the samples, either we did not detect terrestrial plants, or we detect them in just one replicate
215 (Table 1). On the contrary, in most of the samples from lakes Muzelle and La Thuile (87% and 76%,
216 respectively), terrestrial plant DNA is detected in the four replicates performed on these lakes (Table
217 1). However, in 12% of the samples from Lake Serre de l'Homme, aquatic plants are detected in more
218 than 4 replicates (44% of the samples detect aquatic plants in more than 1 replicate). The three lake-
219 catchment systems are thus characterised by different plant DNA records in terms of quantity and of
220 quality.

221

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

229

230 **2.2. Plant DNA: what can we learn from the** 231 **sedimentological/geochemical records and pollen?**

232

233 **2.2.1. La Thuile**

234 The record of terrestrial plant DNA content (Figure 3) can be divided into seven phases ((a) from 0 to
235 1000 cal. BP, (b) from 1000 to 1400 cal. BP, (c) from 1400 to 2500 cal. BP, (d) from 2500 to 3600 cal.
236 BP, phase (e) 3600 to 4500 cal. BP, (f) from 4500 to 5200 cal. BP and (g) from 5200 to 6400 cal. BP).
237 These phases correspond to changes in environmental conditions inferred from the sedimentological
238 and geochemical proxies (Bajard et al. 2016). In most of these phases (a, b, c, e and g), the terrestrial
239 plant DNA content is positively correlated with the organic matter content ($r=0.82$, $p<0.001$ excluding

240 phases d and f; Figure 3). This relationship probably reflects the significant role of the biomass
241 production described in previous studies^{3,40}. However, this relationship is lacking during phases (d) and
242 (f). They are, respectively, impoverished and enriched in DNA, compared to the organic content. Phase
243 (d) is also characterised by a very low carbonate content (<4%) (Figure 3), which might indicate the
244 presence of acid conditions in the water column. Acid conditions are not favourable for DNA
245 preservation⁴⁸⁻⁵⁰. Moreover, our method of DNA extraction might not be efficient enough to unbound
246 organically (humic substances)-complexed DNA⁵⁸, which might be an important pool of extracellular
247 DNA in this part of the sediment pile mostly made of leaves and needles⁶⁷. Humic substances are also
248 known to inhibit the PCR reaction⁶⁸. The poor-DNA content in phase (d) might thus be due to
249 unfavourable preservation conditions and/or analytical limits. Phase (f) contains as much organic matter
250 as phase (g), but the DNA content is higher. However, phase (f) contains much more organic matter of
251 terrestrial origin (vs aquatic; cf Figure 3), and coming from the erosion of forest litter and/or the direct
252 fall of the upper parts of plants inside the lake⁶⁷. Very high content in organic matter from the forest
253 litter is also recorded in phase (e), but the DNA content does not significantly increase relative to the
254 phase (f). This result is probably due to the presence of humic substances and the acidic conditions
255 suggested by the low carbonate content as in phase (d). Phase (b) has a slightly lower DNA content than
256 in phase (a), while there is as much organic matter. Moreover, this phase presents a very low number
257 of MOTU, especially compared to those detected by pollen analyses (Figure 4). However, this phase is
258 dominated by a contribution from deep soils, i.e. mineral soil horizons, while phase (a) is dominated by
259 a contribution of the soil surface, i.e. organo-mineral soil horizons (Figure 3,⁶⁹). The sediments are thus
260 enriched in terrestrial plant DNA when the erosion strongly affects the soil surface horizons, such as
261 the litters and the organo-mineral soil horizons (except when the lake water is acidic and/or contains
262 humic substances, which does not favour the DNA preservation/recovery). Consequently, the erosion
263 processes (e.g. sheet erosion, gully erosion or bank undercutting), controlling the origin of the organic
264 matter, are key processes driving the terrestrial plant DNA concentration in the sediments.

265

266 Both pollen and DNA records show an increase in floristic diversity from 2500 cal. BP, i.e. from phase
267 (c) (Figure 4). Before this period, 31 and 11 taxa on average are detected by pollen and DNA analyses,
268 respectively (without taking into account phases d and e of lower DNA detection). From 2500 cal. BP,
269 the number of taxa detected with pollen increases to 34 on average for the phase (c) and to 38 for the
270 phase (a). With the DNA analyses, the mean number of MOTU in phases (c) and (a) are 19 and 30,
271 respectively. The number of MOTU detected by DNA is thus always lower than that obtained from
272 pollen analyses. However, the increases of floristic diversity in phases (c) and mostly (a) are more
273 important with the DNA analyses. The efficiency in detecting plant communities through DNA analyses
274 might thus be higher after 2500 cal. BP than during the previous period. Moreover, from this moment
275 up to 1400 cal. BP (i.e. in phase (c)), an increase of the proportion of arboreal taxa is recorded by DNA
276 whereas pollen data suggests deforestation. The significant increase of the erosion from 2500 cal. BP
277 (Figure 3; ^{67,69}), which led to a high increase of the total flux of sediments (13 to 504 mg/cm²/yr), is in

278 agreement with this assumption of deforestation, as this human activity decreased soil stability.
279 Consequently, the higher detection of trees (for instance, *Quercus sp.*, *Acer sp.*, Betulaceae, Ulmaceae
280 and to a lesser extent *Viburnum opulus* and *lantana*, Figure 4 and Supplementary figure 8) and the
281 higher increase of the richness in the DNA dataset (compared to the pollen dataset) might be due to
282 higher erosion rate. In fact, the erosion increases the degree of connectivity in the catchment area (i.e.
283 creates new connexions between patches of the catchment and the hydrographic web, including the
284 lake). On the contrary, before 2500 cal. BP, in the forested landscape there is a probable bias towards
285 recording plants growing on the lakeshore and the riverside (through the proximal litter erosion or the
286 direct fall of tree leaves) as suggested by the dominance of *Alnus sp.*, which includes two riparian
287 species (*Alnus glutinosa* and *incana*), and by the presence of *Frangula sp.* (Supplementary figure 8).

288

289 Temporal inconsistencies are recorded between *Cannabis sativa*, detected via DNA analyses, and
290 *Cannabis sativa* or *Humulus lupulus* (from the Cannabaceae family), detected via pollen analyses
291 (Figure 4). These pollens are present at rather high abundances (around 10-15%), suggesting that they
292 originate from retting activity. In this case, both pollen and DNA are directly transferred to the lake.
293 Consequently, high quantities of DNA from *Cannabis sativa* can be transferred to the sediments which
294 might explain the high detection during the phase (b), i.e. when the erosion affects the deep soil horizons
295 and dilutes the DNA inputs of other terrestrial plants (Figure 3 and 4). On the contrary, in phases (a)
296 and (c), i.e. when the erosion predominantly affects soil surface horizons, the DNA from *Cannabis*
297 *sativa* may be diluted by the DNA from other plants in the catchment. As the DNA from this species
298 becomes rarer, it competes with other more abundant DNA fragments and is therefore no longer
299 amplified. Nevertheless, we can point out that for many taxa DNA and pollen signals are the same
300 (excluding phases b and d). Trends are particularly coherent for tree taxa such as *Taxus sp.*, *Tilia sp.*,
301 *Abies sp.*, *Alnus sp.*, *Fagus sp.*, *Cupressaceae* (*Juniperus* with pollen) and *Juglandaceae* (*Juglans* with
302 pollen). Herbaceous plants, like *Rumex sp.*, *Plantago sp.*, *Mentha sp.*/ Mentheae, *Helianthemum*
303 *nummularium* (*Helianthemum* with pollen) and others (Figure 4 and Supplementary figure 8) also
304 record the same history.

305

306 2.2.2. Serre de l'Homme

307 Very little land plant DNA (low DNA concentration and richness) is recorded in the Lake Serre de
308 l'Homme (Figure 5). The sediments mostly comprised non-carbonate mineral matters (35.5-78 %) of
309 clastic and biogenic (diatoms) origins and organic matter (20.4-62%). The C:N atomic ratio fluctuates
310 from 9.3 to 15.4, i.e. between a pure aquatic end-member and a mixed terrestrial/aquatic end-member
311 ⁷⁰⁻⁷⁴ (Figure 5). The sediments contain terrestrial plant macrofossils. The lake catchment is flat and the
312 "lake surface: catchment surface" ratio is high, which explains the low terrigenous inputs reflected by
313 the low total flux of sediments (between 1 and 20 mg/cm²/yr). In these topographical conditions, only
314 the most easily erodible materials are mobilised. These materials may be the plant remains fallen on the

315 soils (constituting the source of terrestrial plant macrofossils) as well as the bare soils on sandstones
316 (Figure 2), which contribute to the non-carbonate mineral matter. These materials are not expected to
317 bear extracellular DNA from plants, which probably participate to the poor detection of terrestrial plant
318 DNA. Moreover, poor-DNA preservation conditions may be triggered by the soil acidity (pH of 4.3-5.3
319 have been measured on soils developed on the same geological substratum and close to the catchment)
320 and/or by the low water depth favouring high temperature and oxygenation in the lake bottom. Higher
321 detection probability of taxa was demonstrated in deeper lakes in boreal to alpine environments in
322 Northern Norway ⁴⁰. In Lake Serre de l'Homme, better in-lake preservation conditions are assumed
323 from 300-100 cal. BP due to the higher organic matter production favouring the establishment of anoxic
324 conditions and thus reducing the bacterial activity. These good preservation conditions may contribute
325 to the detection of high quantity of aquatic plant DNA, which is otherwise in agreement with the
326 decrease of the C:N atomic ratio (Figure 5).

327

328 The poor quality of the terrestrial flora reconstruction is characterised by a stochastic detection of only
329 eight different taxa (Figure 6). At least four of these plants live in wet environments (*Athyrium sp.*,
330 *Caltha sp.*, *Saliceae* and *Filipendula ulmaria*). The proximity or good connection between these wet
331 environments and the lake might have favoured the DNA transfer of plants that grow in these
332 environments, like the DNA from the aquatic plants ^{29,40}, which are nearly continuously detected in
333 Serre de l'Homme (successions of *Myriophyllum sp.*, *Sparganium sp.* and *Potamogeton sp.* as well as
334 *Potamogetonaceae*, Figure 6). On the contrary, the very poor spatial representativeness of the
335 catchment-scale flora at Serre de l'Homme probably reflects the low connectivity between the whole
336 catchment and the lake due to the absence of a well-developed hydrographic network and the low
337 erosion, both due to the flat topography. The role of catchment relief on catchment flora reconstructions
338 has also been proposed in two recent studies, in Arctic and African environments ^{40,75}.

339

340 2.2.3. Muzelle

341

342 The sediments from Lake Muzelle present substantial variations in terrestrial-plant DNA concentration
343 (from 0.28 to 2.10, Figure 7A) but have nearly homogeneous concentrations all along the core in non-
344 carbonate mineral matter (93.6% +/-0.8), total organic matter (4.2% +/-0.6) and carbonates (2.2% +/-
345 0.4). The sedimentological dynamic of this lake is dominated by significant changes in grain size ⁷⁶.
346 The quantity of terrestrial-plant DNA tends to decrease with the increase in clay content ($r=-0.72$,
347 $p<0.0001$; Figure 7B). These inputs of clays increase substantially during two phases, i.e. 750-625 and
348 310-50 cal. BP (Figure 7A), which are in the Little Ice Age (LIA) ⁷⁷. In this context, and given the
349 presence of a glacier in the catchment, clays are interpreted as representing a proxy of inputs in glacier
350 sediments (glacial flour) to the lake. In fact, glacier advances triggered by colder and/or wetter
351 conditions produce more glacial flour, which increase the input of clays into the lake, especially during

352 high precipitation events as shown by the increase of the flood frequency ⁷⁶. Because these clays do not
353 come from soils covered by plants, no extracellular DNA fragments from terrestrial plants are expected
354 to be bound to these clays. Thus, the inputs of these DNA-free clays might dilute the DNA coming from
355 vegetated-soil erosion and thereby explain the decreases in DNA content when clays increase (Figure
356 7A).

357

358 The taxonomic richness strongly increases from 550 cal. BP, i.e. when the tree-shrub cover % decreases.
359 From this period, plant communities with different ecological preferences are recorded. In fact,
360 heathland plants, characteristic of well-developed acid soils (e.g. *Vaccinium uliginosum*) are detected
361 together with plants of calcareous meadow (*Myosotis alpestris*), siliceous screes, snow beds or moraines
362 (*Oxyria digyna*, *Veronica alpina*), siliceous rocks (*Eritrichium sp.*), calcareous rocks (*Saxifraga*
363 *paniculata*), nutrient rich soils (*Rumex sp.*, most of *Mentheae sp.*) and wet environments (*Bartsia*
364 *alpina*) (Figure 9). This record of a mosaic landscape may have been favoured by the well-developed
365 hydrographic network connecting different parts of the catchment to the lake (Figure 2), by the high
366 erosion dynamic as shown by the high total sediment flux (14-77 mg/cm²/yr) and by the contribution
367 of non-carbonate mineral matter (Figure 7). This mosaic landscape is probably the result of the
368 landscape opening caused by the development of pastoral activities, as suggested by the presence of
369 plants that have preferences for nutrient-rich soils. Mammal DNA analyses can be performed to test
370 this hypothesis.

371

372 **2.3. Mammal DNA detection and indirect evidence of** 373 **pastoral activities**

374

375 In Lake La Thuile, more mammal DNA is detected in the last thousand years, which is in agreement
376 with the detection of *Rumex sp.* (Figure 8A), a nitrophilous plant commonly associated with animal
377 stalls. *Plantago sp.*, generally associated with grazing activity because it is resistant to trampling and
378 not eaten by animals (especially *P. alpina* and *P. Lanceolata*), is also detected in previous periods (DNA
379 and pollen, Figure 4), e.g. from the Late Iron Age to the Early Medieval Period. Its occurrence suggests
380 that herds/flocks of domestic animals might have been present in the catchment before the last
381 millennia, although they are not detected from the mammal DNA analyses. This possible divergence
382 between the proxies might be due to 1) a low number of animals and/or a dominance of sheep or goats
383 relative to cattle (the smaller biomass of these animals can lead to less DNA production) before 1000
384 cal. BP, 2) the fact that areas of animal stalls (representing high stock density and favouring the
385 development of nitrophilous plants such as *Rumex sp.*), depending on their position relative to the
386 lake/hydrographic web, can increase the detection probability of livestock farming relative to scattered
387 distributions of animals, because they represent “point sources” vs “diffuse sources”, 3) the relatively

388 low DNA transfer due to the high erosion of deep soil horizons between 1400 and 1000 yr cal. BP
389 (Figure 3) or 4) a combination of these factors. In another alpine lake (Anterne), sheep DNA was
390 detected in only one over eight replicates during the Late Bronze Age, whereas *Plantago sp.* DNA
391 started to be regularly recorded from this period ^{25,26}. In this case, the low DNA content may also be
392 explained by a dilution triggered by the significant increase in deep soil horizons erosion ^{26,78}.
393 Furthermore, as observed for Lake La Thuile (Figure 3), this period was also characterised by the
394 detection of few terrestrial plant taxa ²⁶.

395

396 The absence of mammal DNA in sediments from Lake Muzelle is quite unexpected. Indeed, DNA from
397 *Rumex sp.* and spore of coprophilous fungi (*Sporomiella sp.*) are found in the sediments dated to the
398 last few centuries (⁷⁶, Figure 9B), which strongly suggests the presence of domestic flocks/herds at least
399 during this period. Coprophilous fungi spores, as well as extracellular DNA from both *Rumex sp.* and
400 domestic animals, are supposed to share the same area of production. *Sporomiella* spores mainly come
401 from the faeces of herbivores, mammal DNA is assumed to be largely derived from dung and urine ⁴¹
402 and DNA from *Rumex* comes from places of high nutrient accumulation, such as domestic animal stalls
403 where faeces accumulate (hence the good correspondence with the mammal DNA observed for La
404 Thuile). However, the production (and thus concentration) of each of these proxies as well as their
405 distribution in the soil profiles may be different. Consequently, the non-detection of mammal DNA in
406 the sediments from Lake Muzelle might be due to low production/concentration of mammal DNA
407 compared to DNA from *Rumex sp.* and to spores of *sporomiella sp.*, and/or to differential limit of
408 detection between the different proxies. The difficulty of detecting mammal DNA is well illustrated by
409 the repeated amplification of DNA from sediments of Lake La Thuile. In fact, a better detection (higher
410 number of positive replicates and more taxa) of mammal DNA is recorded when increasing the number
411 of DNA replicates (Lake La Thuile Table 2 and Figure 9A), because this increases the detection
412 probability of “rare” taxa ^{79,80}. In particular, *Ovis sp.* is consistently detected in Lake La Thuile only
413 when many PCR replicates are performed (Table 2). Even if these taxa are not “rare” in the catchment,
414 because of contaminations by human DNA (still high even with the use of blocking primers, see
415 supplementary figure 6) of samples, these taxa have to be considered as “rare” in the sediments.
416 Consequently, the low number of replicates analysed in Lake Muzelle (only four), could contribute to
417 the non-detection of the domestic animals.

418

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

422

423

424 3. Discussion

425

426 Our case studies and the review of the literature allows to, we propose a model summarising the
427 archiving of the extracellular DNA from the catchment in a lake (Figure 10). This model can be used
428 to guide the choice of lakes most suitable for the reconstruction of the catchment history (landscape
429 changes, agropastoral activities, biodiversity).

430 It integrates three equations. The first one is a mixing equation between the different materials affected
431 by erosion in the catchment and transferred to the lake. This equation can be written as follow, for one
432 taxon (Eq 1) and several taxa (Eq 2):

433 Eq 1)

434

$$435 \quad [DNA_{Taxa\ j, TERRinit}] = \sum_{i=0}^{x1} [DNA_{Taxa\ j, Source\ i}] [Source\ i]$$

436

437 Eq 2)

438

$$439 \quad [DNA_{TERRinit}] = \sum_{i=0}^{x1} \left(\sum_{j=0}^{x2} [DNA_{Taxa\ j, Source\ i}] \right) [Source\ i]$$

440 , where $[DNA_{Taxa\ j, TERRinit}]$ and $[DNA_{TERRinit}]$ are the concentrations, respectively of the taxon j and of a
441 group of taxa targeted by the primer (from 0 to $x2$), in the terrigenous materials affected by the erosion
442 ($\log(N\ reads+1)/g$ of terrigenous materials) and $Source\ i$ represents the different sources of terrigenous
443 materials (from 0 to $x1$ sources). We hypothesise that these materials contain different concentrations
444 of DNA from different taxa j ($[DNA_{Taxa\ j, Source\ i}]$) due to variations in 1) spatial distribution of the taxa
445 in the catchment, 2) DNA distribution in soil profiles, 3) soil type, and 4) biomass produced by each
446 taxon. For instance, according to our interpretations from Lake La Thuile, the soil litter is the most
447 extracellular DNA-rich source for plants (humic substances-bound DNA; Figure 10). However, we
448 anticipate different DNA contents in different types of litter (for instance forest vs meadow), especially
449 due to the different biomass production, litter turnover, and pH conditions, as proposed by a study in
450 boreal environments but on total DNA ⁴⁵. Data from La Thuile also suggests that the organo-mineral
451 soil horizons contain less extracellular plant DNA (clay-bound DNA) than the litter, but much more
452 than the mineral (deep) soil horizons. The distribution of extracellular plant DNA in soil profiles should
453 thus have a decreasing trend from the top to the bottom (Figure 2). A lower total extracellular DNA
454 concentration was also observed in deeper horizons (B) than in upper horizons (A) from Inceptisols
455 (forest soils from Mediterranean regions) ⁸¹. In case of presence of buried palaeosoils ⁸² higher DNA
456 content might be expected in the “palaeo” soil surface horizon. Acidic soils and bare soils would be
457 very poor or free of extracellular plant DNA which probably contributes to the poor DNA record from

458 Lake Serre de l'Homme. Moreover, glacial flour is free of extracellular plant DNA, as exemplified by
459 the data from Muzelle.

460 The content of extracellular DNA from animals in soil profiles can be different from that of plants.
461 Total DNA was shown to be strongly related to the animal biomass (which is much lower than the
462 biomass of plants) as well as to the soil texture, with significant leaching in sandy soils and for larger
463 animals⁴¹. For the livestock, this biomass depends on the stocking rate and more precisely on the stock
464 density, which is driven by the animal behaviour and pastoral practices (Figure 10). These factors will
465 also produce spatial variations in mammal DNA distribution in the catchment. However, as for plants
466 and microbes, the highest animal DNA quantities are found in top soils⁴¹.

467 The concentration of the different sources of terrigenous materials ([Source *i*]) will depend on their
468 erodibility (capacity to be mobilised), the slope and the connections between the sources and the lake
469 (direct or via runoff waters and tributaries). A well-developed hydrographic web should provide
470 terrigenous inputs from the different parts of the catchment and thus afford a more reliable
471 reconstruction of the floristic diversity at the catchment scale, as exemplified by the records of a
472 landscape mosaic in the sediments from Lake Muzelle as well as another mountain lake, Anterne²⁶.
473 Moreover, open landscapes, with a higher erosion dynamic triggered by higher soil erodibility should
474 yield better spatial representativeness, for example, the range of plants in the catchment. This process
475 is well exemplified on Lake La Thuile. However, the erosion should preferentially affect the upper parts
476 of the soils as previously written. This also means that significant developments in agricultural activities
477 should be well reflected in the aDNA record of this activity. On the contrary, extensive practices, such
478 as unmanaged grazing without stockading or animal enclosures, with less impact on the erosion
479 dynamic, might be more difficult to detect.

480 Previous studies proposed that the biomass, distance and relief determine the terrestrial plant DNA
481 record in the sediments^{3,40}. Here, our model goes further, integrating more explicitly the mechanisms
482 behind the production and transfer of extracellular DNA in lake sediments. In fact, our data demonstrate
483 that the nature of erosion processes (such as sheet erosion, gully erosion, bank undercutting or glacial
484 erosion) is important to consider because it controls the sources and quantity of catchment derived
485 extracellular DNA inputs to the lake. Furthermore, the concept of "catchment connectivity" combining
486 the hydrographic web and the catchment erodibility, reflects the features and processes controlling the
487 spatial representativeness of the DNA record, which is key for good quality reconstructions, especially
488 when landscapes have high habitat diversity (i.e. are made of plant metacommunity).

489
490 The second equation of the model reflects the dilution by the autochthonous production (lake
491 production):

492 Eq 3) $[DNA_{TERRSED}] = [DNA_{TERRinit}][TERR_{SED}]$ or $[DNA_{TERRinit}](1 - [AquaMat_{SED}])$

493 where $[DNA_{TERRSED}]$ is the concentration of terrestrial DNA in the sediments (log(N reads+1)/g dry
494 sediments), $[TERR_{SED}]$ is the concentration of terrigenous materials in the sediments (g of terrigenous
495 materials/g of dry sediments) and $[AquaMat_{SED}]$ represents the concentration of the aquatic production.

496 The aquatic end-member of the sediments can include organic matter from microalgae, and aquatic
497 plants as well as mineral matters produced or induced by aquatic organisms or chemical reactions. The
498 dilution effect by the aquatic end-member is illustrated by the records from phases (a), (c) and (g) at
499 Lake La Thuile and probably contributes to the poor terrestrial DNA record in lake Serre de L'Homme.
500 In the dilution equation, we did not consider the materials coming from the atmosphere because they
501 represent very low quantities beside the aquatic and terrestrial materials.

502

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

505 Eq 4)

$$506 \quad [DNA_{TERRSED}] = (1 - \alpha) \left(\sum_{i=0}^{x1} \left(\sum_{j=0}^{x2} [DNA_{Taxa\ j\ Source\ i}] [Source\ i] \right) [TERR_{SED}] \right)$$

507

508 where α is a factor of degradation (if $\alpha=1$ all the DNA is degraded and if $\alpha=0$ all the DNA is preserved).

509 Theoretically,

$$510 \quad \alpha = f(\text{pH}, T^{\circ}, \text{UV}, O_2, \text{microbial activity}, \text{salinity}, \text{sediment composition}, \text{time})$$

511

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

519

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

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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 across 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. However, lake sediment DNA should also be considered as 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, today⁸³ and in the past.

551 **4. Material and methods**

552 **4.1. Regional setting and site presentation**

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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, nowadays (Figure 2B; ⁶⁷).

568 4.2. Sites topography/ geology

569

570 Each of the catchment areas studied possesses different physical characteristics (Figure 2B). The Lake
571 Muzelle catchment area has the highest proportion of steep slopes of the three sites, a well-developed
572 hydrographic network, highly erodible rocks, including schist, and partial meadow vegetation, with
573 some bare soils exposed to erosion. The lake surface constitutes <2% of the catchment, which implies
574 there is an important “concentration effect” of sediments derived from the catchment. Combined, these
575 characteristics lead to significant terrigenous inputs to the lake. Furthermore, the catchment comprises
576 a glacier. Thus, a part of these terrigenous inputs comes from glacial erosion. This type of erosion
577 provides glacial clayey materials (“glacial flour”) ⁷⁶.

578 At Lake La Thuile, the lake surface to catchment surface ratio is 4.7%, i.e. 2.4 times higher than for
579 Muzelle. This implies that in Lake La Thuile the “concentration effect” is lower than in Lake Muzelle.
580 The slopes are also less steep, the hydrographic network is poorly developed, and the vegetation cover
581 greater (meadows, some agricultural and forested areas) than in the catchment of Lake Muzelle.
582 However, the presence of agricultural activities triggers significant soil erosion and thus terrigenous
583 inputs to the lake ^{67,69}. The physical characteristics of Serre de l’Homme’s catchment are the opposite
584 of those at Muzelle: high lake to catchment surface ratio (12.9%), gentle slopes, and no hydrographic
585 network. These characteristics are not favourable for detrital supplies into the lake. However, rocks
586 around the lake are easily erodible (sandstones), and there are some small barren/exposed areas (bare
587 soils), which are susceptible to provide a few terrigenous (and more precisely clastic) inputs.

588

589 4.3. Vegetation cover

590

591 Around Lake La Muzelle, the vegetation cover is dominated by subalpine and alpine meadows with
592 herbs such as grasses (*Poaceae*), wormwood (*Artemisia*), sedges (*Cyperaceae*) and creeping willows
593 (*Salix*) ⁸⁴. Lake Serre de l’Homme is surrounded by a eutrophic subalpine meadow with goosefoot
594 (*Chenopodium bonus henricus*), yellow gentian (*Gentiana lutea*) and docks (*Rumex sp.*) (H. Cortot,
595 Pers. Com.). Lake La Thuile (in mountainous area) is surrounded by meadows and pastures. According
596 to the exhaustive floristic survey undertaken around the lake (M. Pienne, T. Delahaye, S. Henriquet;
597 Conservatoire Naturel de Savoie, 1999 and 2000), two types of meadows are present: a meadow with
598 orchard grass (*Dactylis glomerata*) and heath false brome (*Brachypodium pinnatum*), which is
599 sometimes grazed, and a mesophytic meadow dominated by grasses such as crested dogstail (*Cynosurus*
600 *cristatus*), and ryegrass (*Lolium perenne*) used for grazing and mowing. Artificial grassland and kitchen
601 garden are found in the northwest and southeast extremities of the lake. White willow (*Salix alba*), ashy
602 willow (*Salix cinerea*), black poplar (*Populus nigra*), ash tree (*Fraxinus excelsior*) were also described

603 at the edge of the lake. In the higher part of the catchment, there are coniferous forests comprised of
604 spruce (*Picea abies*) on the north side, and of deciduous forest on the east side.
605

606 **4.4. Coring and dating**

607
608 All lake sediment cores were taken in the deepest part of the lakes, which are located approximately in
609 the centre of the lakes (Figure 2). For lake La Thuile, cores were taken using a UWITEC platform and
610 coring devices. The sediment sequence comprises two core sites. Sections from the second hole are
611 shifted by one meter in depth in order to have overlapping sections and create a continuous sequence
612 (THU10, N45 31.813, E6 03.394, IGSN:IEFRA00BB – IGSN codes refer to an open international
613 database. www.geosamples.org). Cores from lake Muzelle (MUZ12, N44 57.037, E6 05.845, IGSN :
614 IEFRA00A4) and two from lake Serre de l’Homme (SDH-09-P1 and P2, N44 77.459 , E6 23.772,
615 IGSN : IEFRA00AW and IEFRA00AV, respectively) were taken using a UWITEC gravity corer. Core
616 diameters are 90 mm for La Thuile and Serre de l’Homme and 63 and 90 mm for Muzelle. Another core
617 on Lake Serre de l’Homme (SDH-1) was also taken with a Russian corer close to the shore line. After
618 coring, sediment cores were stored at 4°C.

619 The lake sediment cores used for DNA analyses as well as sedimentological/geochemical analyses
620 measured 283.5 cm at Muzelle (core MUZ-12, 90 mm diameter from 0 to 130 cm depth and 63 mm
621 from 130 to 183.5 cm depth), 549 cm at La Thuile (upper part of the core THU-10) and 81.5 cm (core
622 SDH-09-P1) and 93 cm (core SDH-09-P2) at Serre de L’Homme. These cores cover different periods:
623 1700 years for Muzelle, 6450 years for La Thuile and 4000 years for Serre de L’Homme. Depending
624 on the lakes, age-depth models are based on ¹⁴C dates, geomagnetic field secular variations, short-lived
625 radionuclide measurements and known lead-pollution levels. All age-depth models were generated
626 using the *R software* and the *R-code package ‘Clam’ version 2.2* ⁸⁵. Details about sediment lithology
627 and the age-depth models are provided in the “sediment lithology and dating” section of the
628 supplementary materials. For Lake Serre de l’Homme, several cores were used. Thus, core correlations
629 are also presented in detail in the “sediment lithology and dating” section of the supplementary
630 materials. Age-depth models were used to estimate the sedimentation rate for each lake (cm/yr).
631

632 **4.5. Sedimentological, geochemical and microfossils** 633 **analyses**

634
635 The cores were longitudinally cut, and a half-core was subsampled for DNA analyses (the heart of the
636 slices, see section 2.7.) and for basic sedimentological analyses (edges of the slices). Samples reserved
637 for DNA analyses were weighed wet. Edges of the sediment slices were weighed wet (Wet weight_{Edge};

638 g) and dry (dried at 60°C, Dry weight_{Edge}; g) to determine the water content (WC) and be able to
639 calculate the total dry weight of the sediments (Dry weight_{Total}; g) and finally the total flux of sediments
640 (Flux_{Totised}; g/cm²/yr), as follow:

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

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

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

644 The edge samples were then used for Loss on Ignition (LOI) analyses, except for Lake Serre de
645 l'Homme for which the analyses were performed on another core (SDH-09-P2). Samples were firstly
646 ground in an agate mortar, and then the standardised procedure proposed by ⁸⁶ was applied. The LOI at
647 550°C and then at 950°C burns the organic matter and carbonate particles, respectively. The
648 contributions (%) of these two components can thus be estimated. The residue of these two successive
649 ignitions provides an estimation of the content in non-carbonate mineral matter (%) and corresponds to
650 alumina and silica-rich particles, i.e. clastic particles and/or biogenic silica.

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

656 Complementary information about organic matter quality is used for lakes La Thuile and Serre de
657 L'Homme (i.e. for which sediments are the richest in organic matter). In the case of Lake La Thuile,
658 pyrolysis Rock Eval and XRF core scanner analyses from a previous study provide indices (Hydrogen
659 Index, HI mgHC/gTOC, Oxygen Index, OI mgO₂/gTOC and Si/Ti as proxy of biogenic silica
660 production) allowing us to distinguish the aquatic organic matter, the organic matter produced in the
661 litter, the soil surface organo-mineral horizons, and the deep mineral soil horizons ^{67,69}. For Serre de
662 l'Homme, the C/N atomic ratio was used as indicator of aquatic organic matter and organic matter
663 derived from soils and land plant macroremains ^{70,87}. The carbon (C) and nitrogen (N) contents were
664 measured with an elemental analyser (CEREGE, Aix en Provence).

665 Pollen analyses from Lake La Thuile and spores of coprophilous fungi from Lake Muzelle were already
666 published in ⁶⁷ and ⁷⁶, respectively. For Lake La Thuile, samples do not correspond to those used for
667 the lake sediment DNA analyses. For Lake Muzelle, samples analysed for coprophilous fungi are the
668 same as those for DNA.

669

670 **4.6. DNA metabarcoding approach**

671 4.6.1. Lake sediment core sub-sampling

672

673 To avoid contamination, the sampling of the three half-cores was performed in a room dedicated to
674 sedimentological analyses at the EDYTEM laboratory (University of Savoie Mont Blanc, Le Bourget
675 du Lac-France), where no DNA analyses were previously performed. Sediment core slices were taken
676 using sterilised metal plates. The edges of slices were removed using sterile scalpels as the surface of
677 the half-core was in contact with the air, and the concave edge was in contact with water that circulates
678 along the coring tubes. For each lake, samples were cut in two parts to perform two extractions by
679 sediment slices. Fifty, 30 and 41 samples were taken from the cores corresponding to lakes - La Thuile,
680 Muzelle and Serre de l'Homme, respectively. The thicknesses of sediment slices are 1 cm for lakes
681 Muzelle and Serre de l'Homme but 0.5 or 1 cm for Lake La Thuile due to substantial variations in the
682 sedimentation rate (greater than 10-fold variations) and thus to avoid high differences in time covered
683 by the different samples. Sample wet weights were between 2.22 and 13.04 g for Lake La Thuile,
684 between 4.08 and 15.63 g for Lake La Muzelle and 10.49 and 23.92 g for Lake Serre de l'Homme.
685 These significant differences are due to different water content values, particle densities (organic vs
686 mineral) and, in cases of lakes La Thuile and Muzelle, also due to the changes in sample thickness and
687 core diameters, respectively. In dry weights, these differences are higher because of the wide variability
688 of the water content, especially between the top and bottom sediments (0.58 to 9.46 g for Lake La
689 Thuile, 1.97 to 10.88 g for Lake La Muzelle and 0.76 to 14.3 g for Lake Serre de l'Homme.
690

691 4.6.2. DNA extraction

692
693 To limit artefacts and biases that can occur in metabarcoding studies, we followed strict laboratory
694 conditions, we performed multiple controls at the different steps of laboratory work (extraction, PCR
695 and blanks), we analysed samples in several replicates⁸⁸. DNA extractions were performed in the
696 Laboratoire d'Ecologie Alpine (University Grenoble-Alpes, France), in a room dedicated to ancient
697 DNA extraction. Eleven extraction controls were performed (3 for lakes Muzelle and La Thuile and 8
698 for Lake Serre de L'Homme).

699 DNA extraction was performed by mixing the sediment with 20 mL of saturated phosphate buffer (0.12
700 M Na₂HPO₄; pH ≈ 8) for 15 minutes. Then, the mixture was centrifuged (10 minutes at 10000 g) to
701 recover 400 µL of the resulting supernatant. DNA was extracted from the supernatant using the
702 NucleoSpin® Soil commercial kit (Macherey-Nagel, Düren, Germany), following the manufacturer's
703 instructions but omitting the lysis step. The DNA extract was eluted in 100 µL of SE buffer. This method
704 of extraction allows the retrieval of the extracellular DNA pool that is dissolved in pore water and
705 adsorbed onto mineral surfaces. It is unlikely that organically/inorganically complexed DNA is released
706 by DNA-desorbing phosphate buffer⁵⁸.
707

708 4.6.3. DNA amplification and high-throughput sequencing

709

710 DNA amplification was realised in a second room of the ancient DNA laboratory using PCR. For the
711 amplification of plants, we used the primers g-h, targeting the P6 loop region of the chloroplast trnL
712 (UAA) intron ⁸⁹. For the amplification of mammals, we used universal primer MamP007 amplifying
713 60-84 bp fragment of the mitochondrial 16S gene ²⁵. To limit the amplification of human DNA, we used
714 a human-specific blocking oligonucleotide (MamP007_B_Hum1, 5'-
715 GGAGCTTTAATTTATTAATGCAAACAGTACC-C3-3'). A unique combination of 8 bp long
716 sequence of nucleotides (tag) was added at the 5' end of each primer, in order to recognise each sample
717 after the parallel sequencing of multiple samples ⁹⁰.

718 To improve the reliability of the detection/ non-detection pattern, we performed multiple PCR replicates
719 on each DNA extract ⁷⁹. For Lake Serre de l'Homme, we performed four PCR replicates on two DNA
720 extraction replicates, yielding eight analyses replicates. For Muzelle and La Thuile samples we
721 performed four PCR replicates on one single extraction replicate using the g-h and Mam-P007 primers.
722 For mammals in the La Thuile samples, we performed 12 additional PCR replicates per sample (33 over
723 50 selected samples) on a second extract obtained from the same samples (which were divided into two
724 parts).

725 All DNA amplifications were carried out at a final volume of 30 µL containing 2.5 µL of DNA template.
726 The amplification mixture contained 1 U of AmpliTaq Gold[®] DNA polymerase (Applied Biosystems),
727 15 mM Tris-HCl, 50 mM KCl, 2.5 mM MgCl₂, 0.2 mM of each dNTP, 0.1 µM of each primer and 4.8
728 µg of bovine serum albumin (Roche Diagnostic). We added 2 µM of the human-specific blocking
729 oligonucleotide to the PCR mixture in mammal analyses. For all primer pairs, the PCR mixture was
730 denatured at 95°C for 10 minutes, followed by 45 cycles of 30 s at 95°C also for the denaturation, 30 s
731 at 50°C for the hybridation and 1 min at 72°C for the elongation. A final elongation step was applied
732 for 7 min at 72°C. The PCR products were then purified and mixed (equivolume mixes) before
733 sequencing. Seventy-two PCR controls were included for each primer.

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

738

739 4.6.4. Data treatment and representation

740

741 The analysis of sequences and the taxonomic assignment were realised using the OBITOOLS software
742 (<http://www.grenoble.prabi.fr/trac/OBITools>) ⁹¹. The forward and reverse reads corresponding to the
743 same DNA fragment were aligned and merged applying the *IlluminaPairEnd* function that takes into

744 account the quality of merging. An “ngsfilter” file containing the list of samples and their associated
745 combination of primer and tag was created and then used to assign each sequence to the relevant sample
746 applying the *ngsfilter* function. Only sequences containing perfect tags and primers with a maximum of
747 three errors were considered. The next step was to identify and merge the identical sequences for each
748 sample using the *obiuniq* function. Afterwards, the *obigrep* function allowed the filtering of sequences
749 based on two parameters, 1) the sequence length and 2) the sequence occurrence in the entire dataset.
750 For plants, sequences shorter than 10 bp and sequences detected less than 100 times were removed. The
751 same filters were applied for mammals, but we only retained sequences longer than 60 bp. *Obiclean*
752 was then used to determine the status of each sequence in each PCR product: “head”, “internal” or
753 “singleton”⁹¹. Only sequences that were more often “head” and “singleton” than “internal” in the global
754 dataset were retained for the subsequent steps. Reference databases were built from the EMBL database
755 with the *ecoPCR* program (gh-database-r113, mamP007-database-r113) and then used to assign a taxon
756 to each unique sequence with the *ecoTag* function (the % of sequence similarity was calculated and
757 specified in the final file).

758 For the subsequent analyses, only the sequences with a similarity >95% to taxa in the reference database
759 were selected. We considered a sequence as present in a PCR replicate when at least five reads were
760 counted²⁵. In each lake dataset, we did not consider taxa that were only detected in one sample, or
761 stochastically in less than two replicates (i.e. taxa always detected in only one replicate but with
762 detections in consecutive samples were kept). To remove contaminants, we excluded taxa frequently
763 present in extraction and PCR negative controls (in more than 5 controls, where the total number of
764 reads was greater than 10000), and taxa allochthonous in the Alps (like *Actinidia* sp.) (see
765 Supplementary section 2.1 as well as Supplementary figures 3, 6 and table 2 for more details on
766 contamination and on the data filtering steps). Potential impacts of the filtering procedure on the main
767 results of the study are also presented and discussed in the supplementary material (Supplementary
768 section 2.2. and Supplementary figures 4 and 5).

769 For each PCR replicate, we summed the total number of reads corresponding to terrestrial plants, aquatic
770 plants and mammals separately. Then, we determined the mean and standard deviation of the log-
771 transformed total number of reads across PCR replicates, as well as the number of replicates where
772 more than 20 reads were detected. These two parameters are positively correlated (see Supplementary
773 section 3), which supports the assumption that the number of reads is correlated to the DNA quantity
774 available for amplification as suggested by previous studies on soils and lake sediments^{29,45}. We
775 normalised the log-transformed number of reads by the dry weight of sediments used for the extractions
776 in order to obtain a proxy of the DNA concentration that we can compare with the concentrations of the
777 main sediment components. The log-transformation helps to correct the exponential DNA amplification
778 during the PCR. We also determined a proxy of the richness (number of MOTUs: Molecular
779 Operational Taxonomic Units) of mammals and plants, considering the presence of the taxa (more than
780 5 reads). As part of this process, for terrestrial plants, the mean value and standard deviation across
781 replicates were calculated. We also determined a “maximum richness” from the sum of reads obtained

782 in all the replicates for each detected taxa. For Lake La Thuile, we also calculated the pollen taxon
783 richness to compare it with the proxy of the plant DNA richness, as that had already been carried out
784 on another lake, but with plant macroremain data ²¹. For mammals, we only determined the maximum
785 richness from the sum of reads obtained in all the replicates for each detected taxa.
786

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798

799 **Author contributions**

800 C.G.-C., J. P and F.A. and K.J.W., contributed to the concept and designed the study. C.G.-C. and
801 L.G. performed the DNA experiments, the sequence analyses and taxa assignment. M. B., L. F., A.-
802 L.D., P.S., E.B., R.S., F.G., F.D. created the sedimentological, geochemical and pollen datasets. C.G.-
803 C. analysed the data with the help of F.G.F. and P.S. F.A. and J. Poulenard contributed their expertise
804 on the reconstruction of soil erosion and dynamics. E.M. provided expertise on the reconstructions of
805 plant cover based on pollen analyses. K.J.W. provided its expertise on taphonomic processes in
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810

811 **Data availability**

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

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

817

818 **Competing financial interest**

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

823

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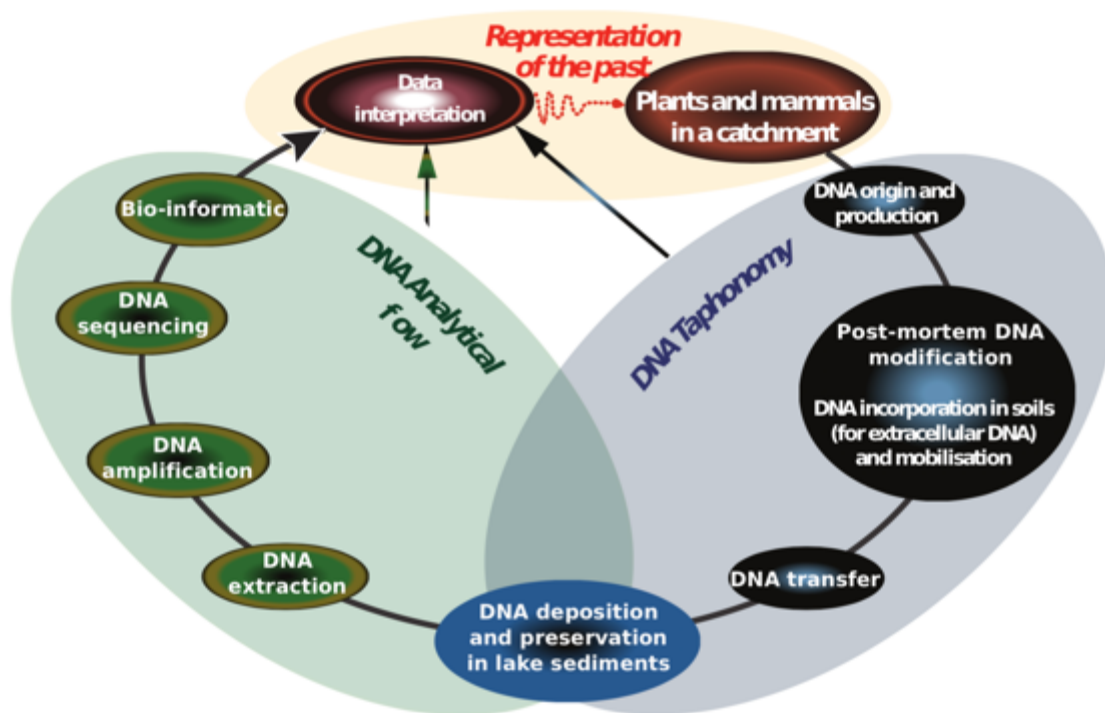
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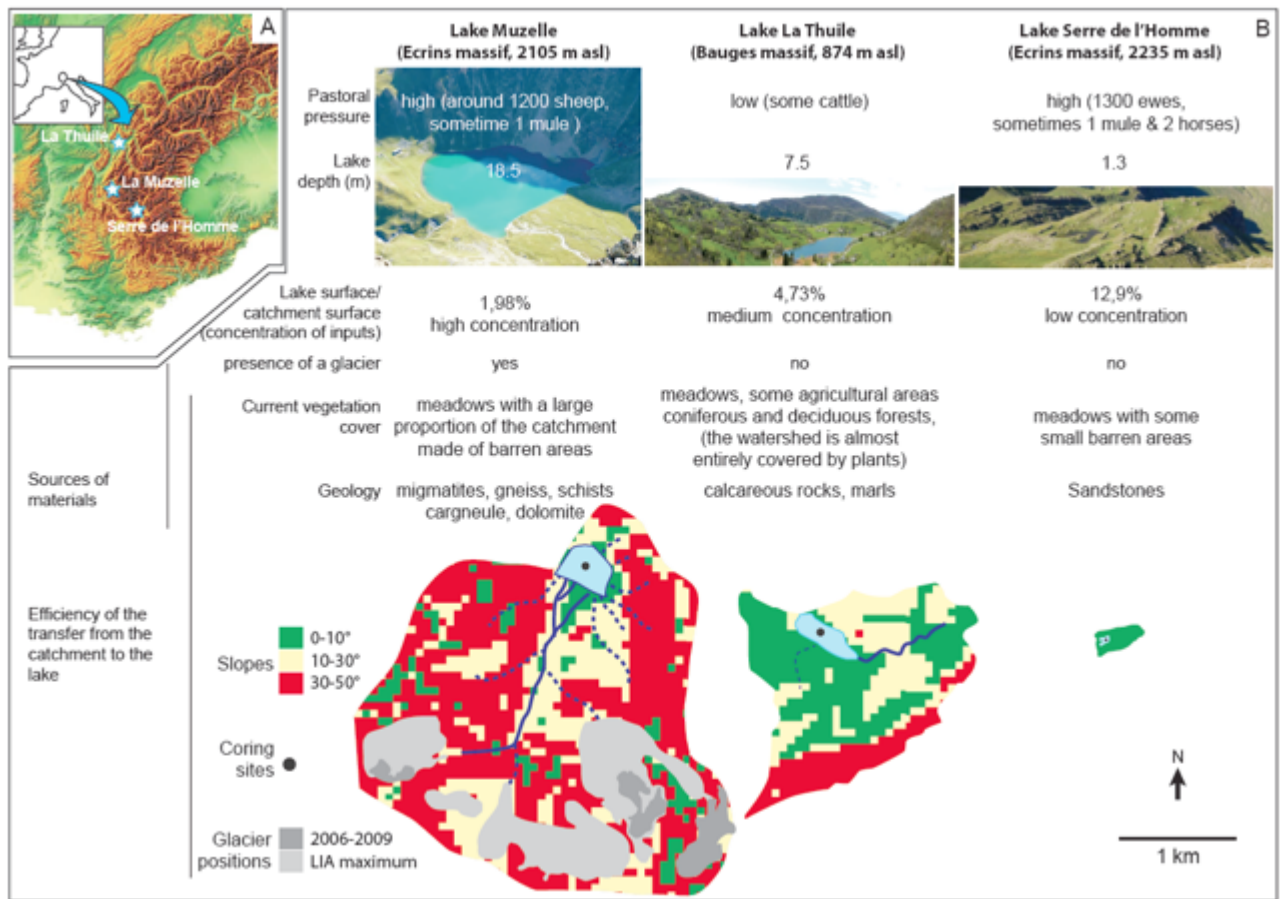
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Figure 1. Flow chart of taphonomic processes and analytical process likely to affect reconstructions of the past, especially reconstructions of landscapes and agricultural activities.

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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).

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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.

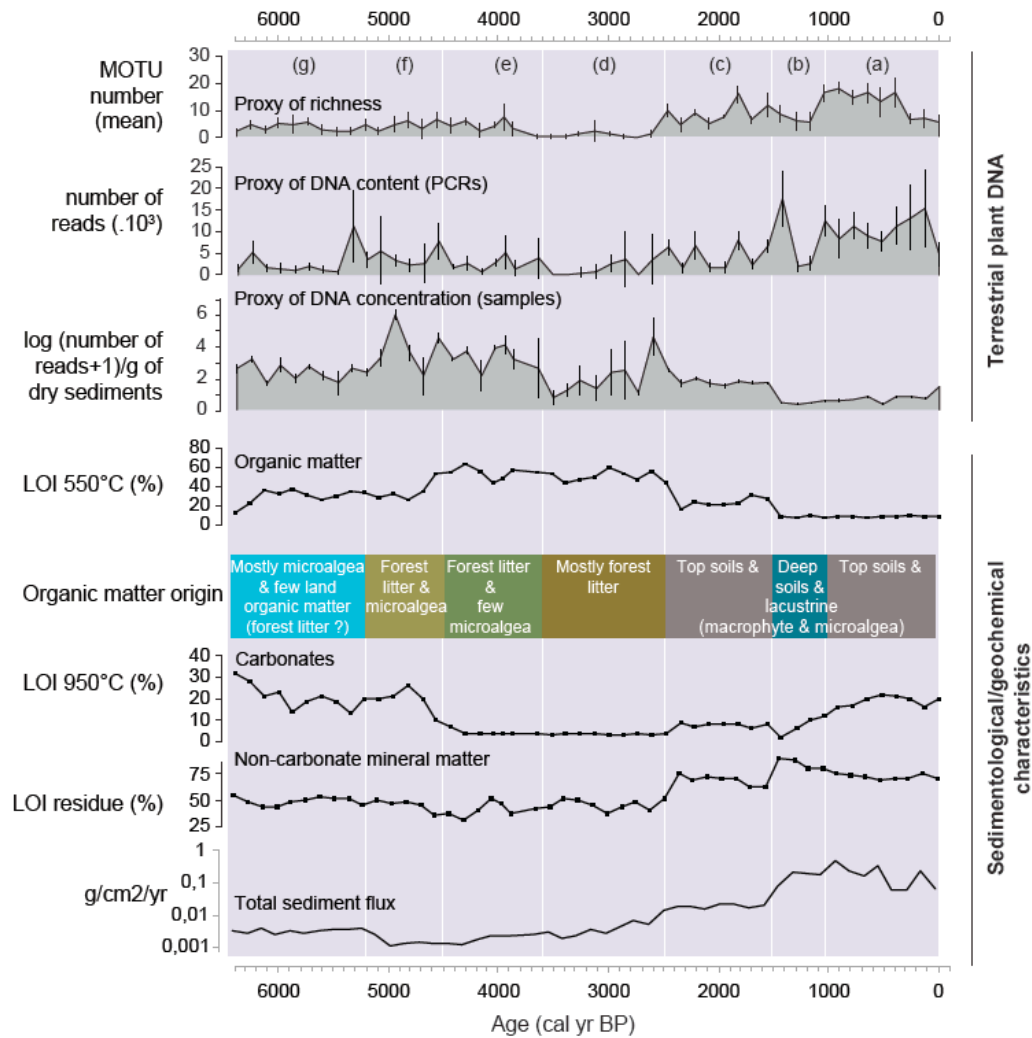
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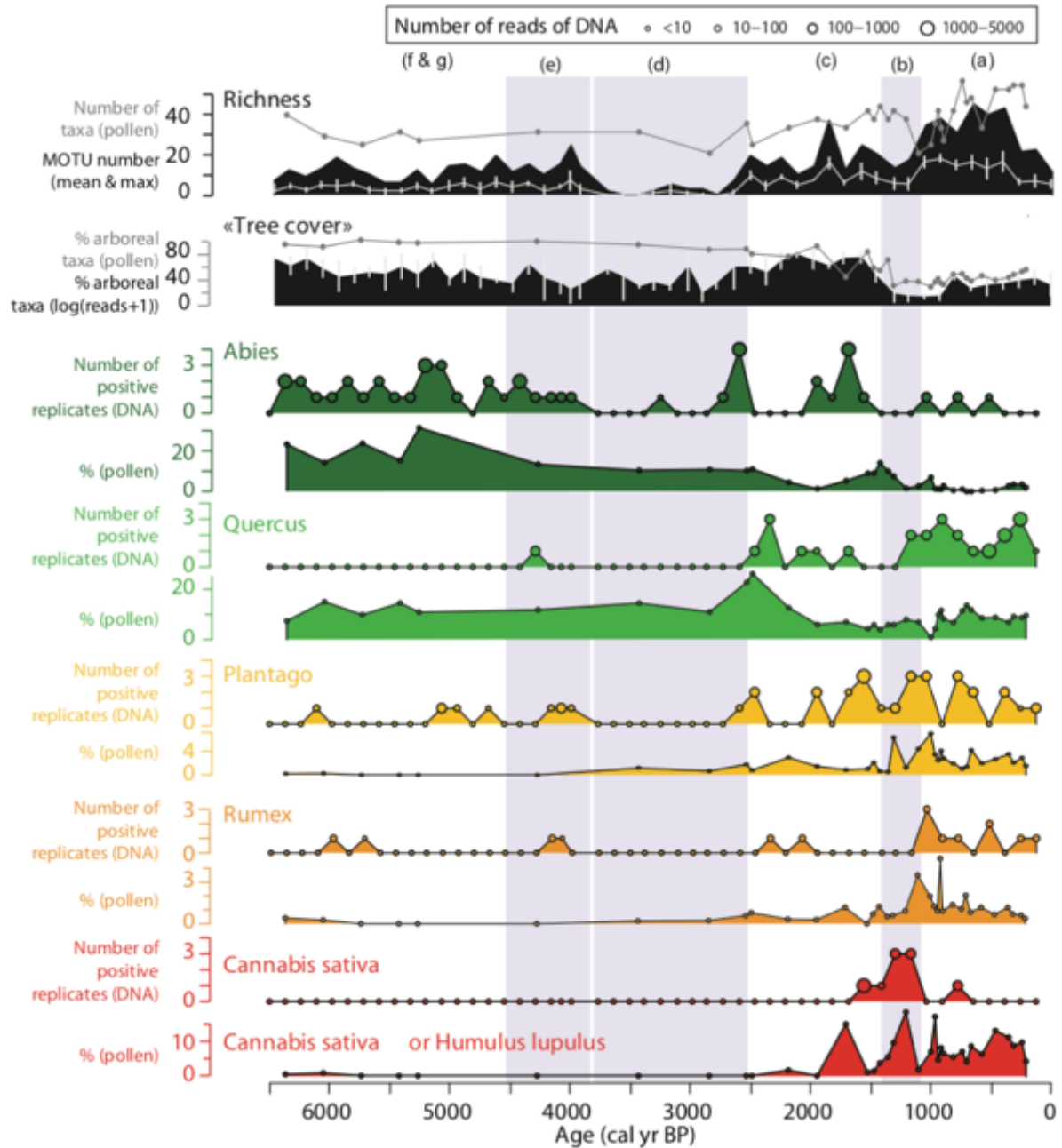
	Lakes	La Thuile	Muzelle	Serre de l'Homme
<i>number of samples</i>		50	30	41
<i>replicate number performed</i>		4	4	8
<i>Illumina Hi-seq run numero</i>		run 1	run 1	run 2
number of MOTU	Terrestrial	96	83	12
	Aquatic	11	0	7
number of reads	Terrestrial	796266	1836110	1205395
	Aquatic	326988	0	4517931
	Terrestrial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

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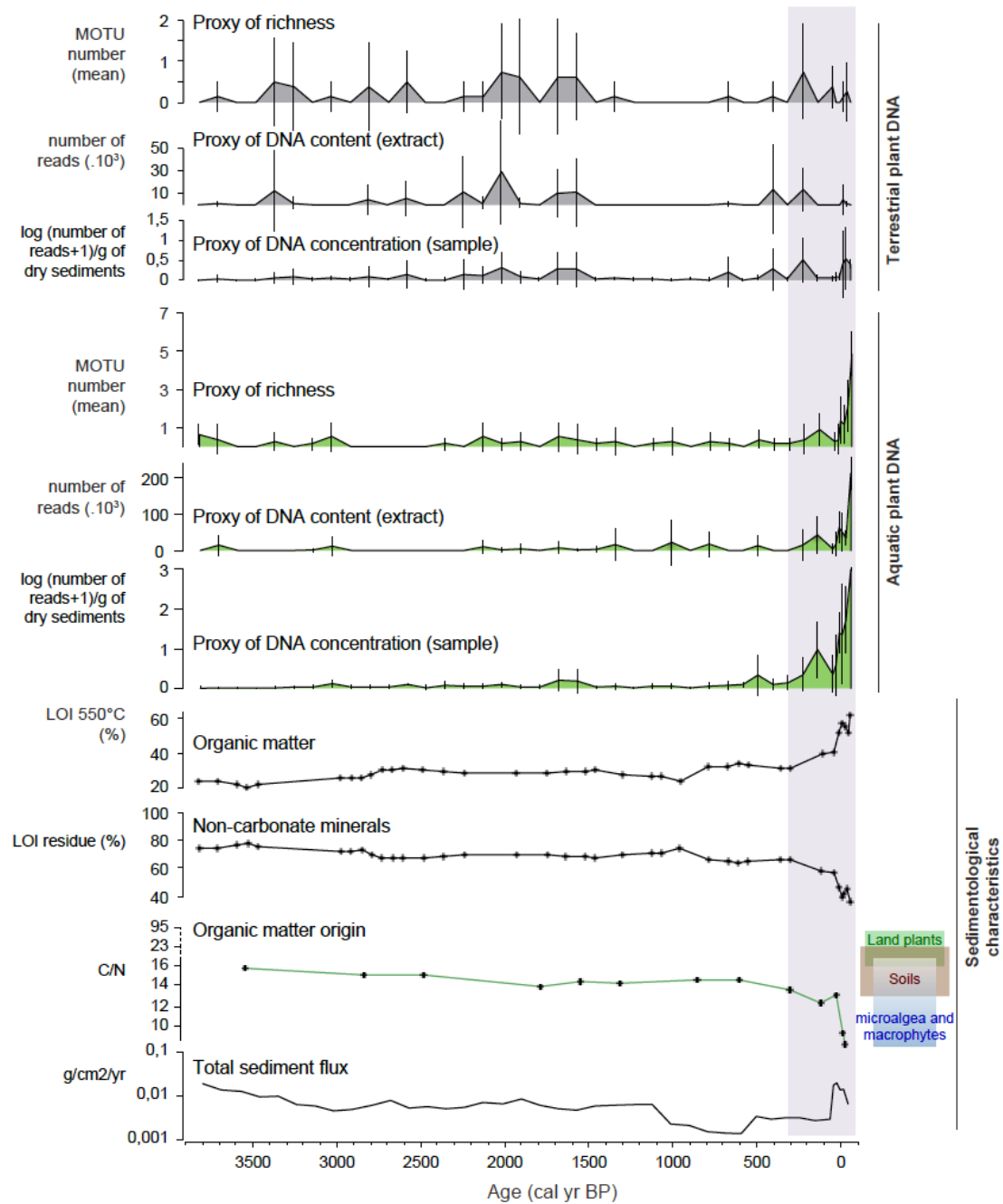
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Figure 3. Comparison between global terrestrial plant DNA and the sedimentological/geochemical properties of sediments in Lake La Thuile over the last 6500 years. To study the behaviour of land plant extracellular DNA we focused on the proxies of the 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/cm²/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 O₂/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.



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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.



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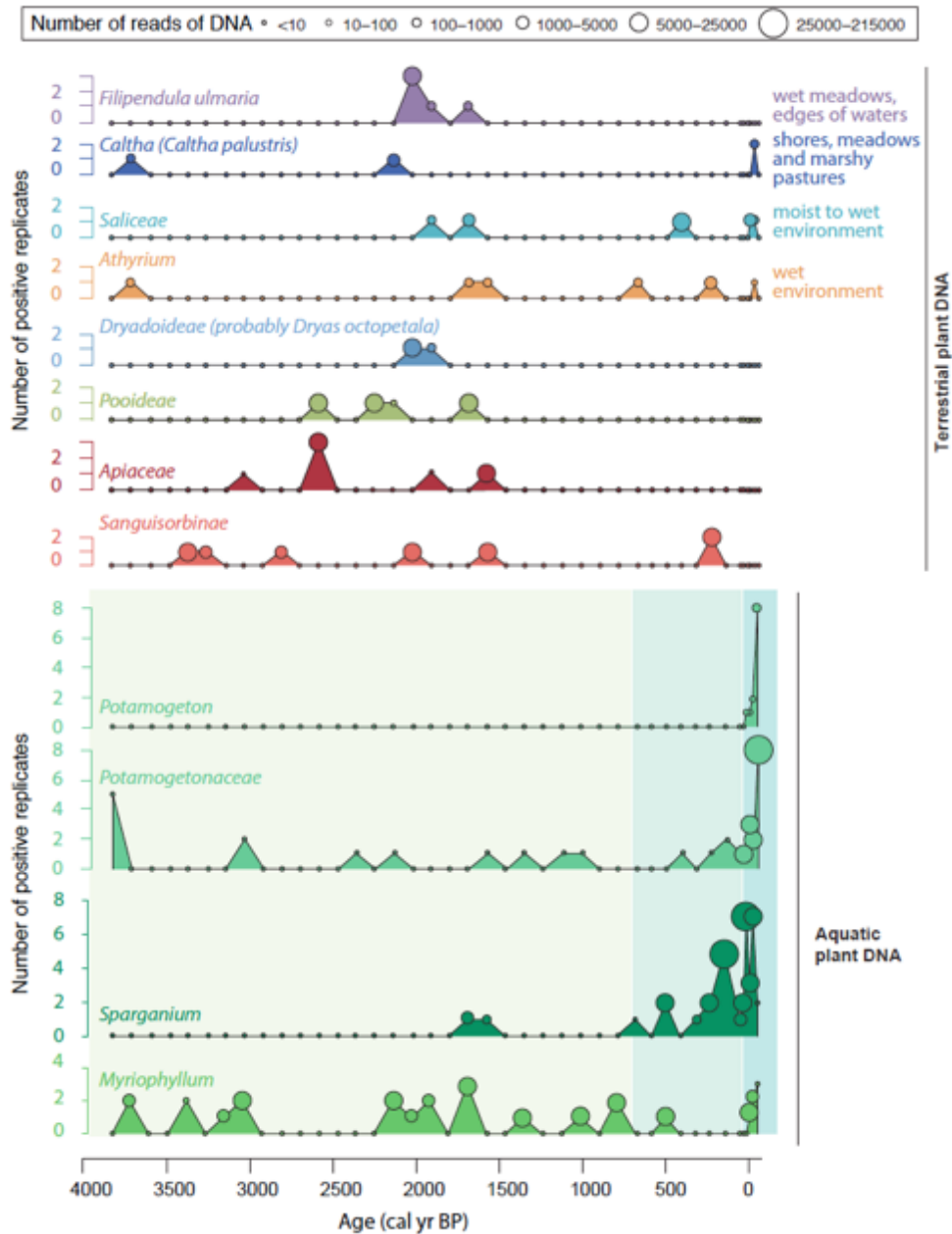
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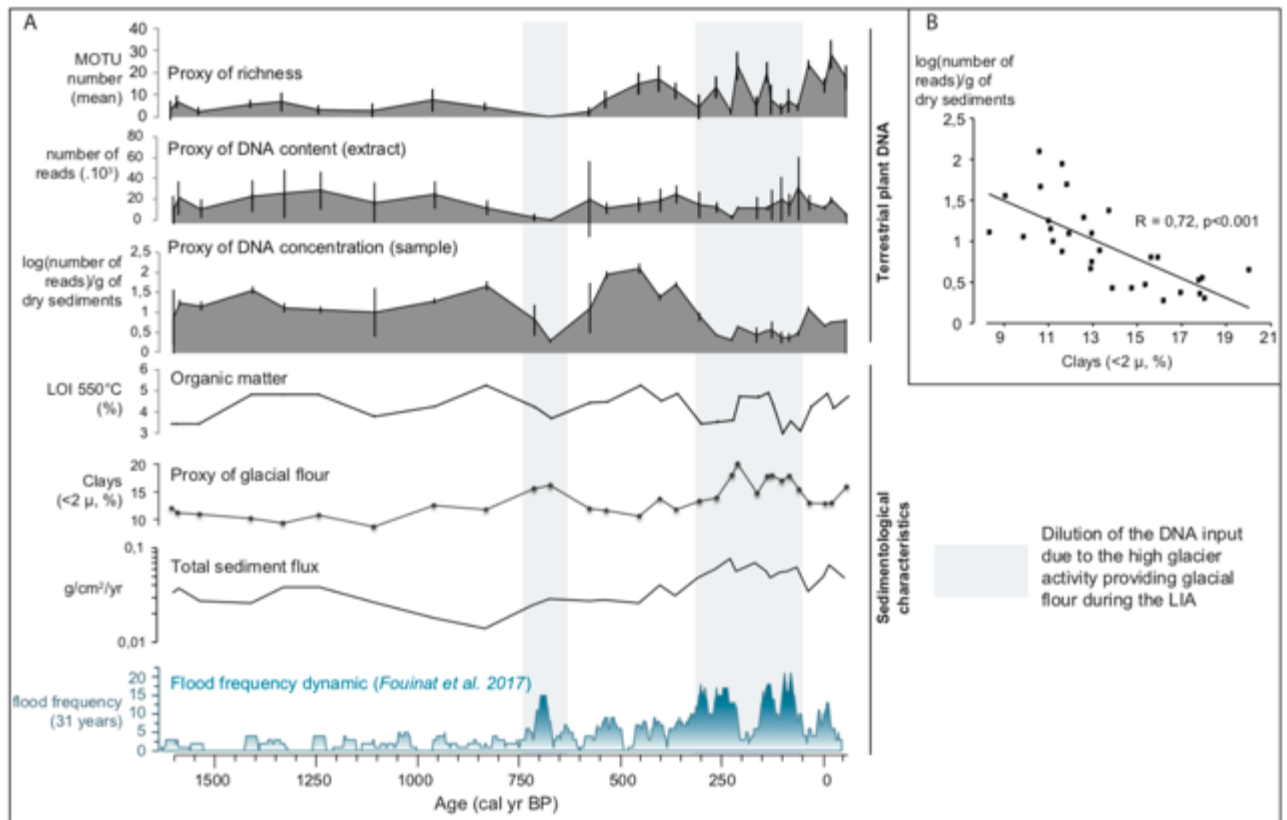
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Figure 5. Comparison between plant DNA (terrestrial and aquatic) and the sedimentological/geochemical properties of sediments from Lake Serre de L'Homme over the last 3800 years. To study the behaviour of plant extracellular DNA we focused on the proxies of the richness (mean and standard deviations of the number of MOTU) and the DNA content (mean and standard deviations of the $\log(\text{number of DNA reads}+1)/\text{dry mass of sediment}$), These variables were compared to the organic matter content ($\text{LOI}_{550^\circ\text{C}}$) and origin (C/N atomic ratio), the content in non-carbonate mineral matter (LOI residue) and the total sediment flux ($\text{g}/\text{cm}^2/\text{yr}$). The ranges of C/N values of land plants (green shaded area), soils (brown shaded area) and algae 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.



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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.



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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

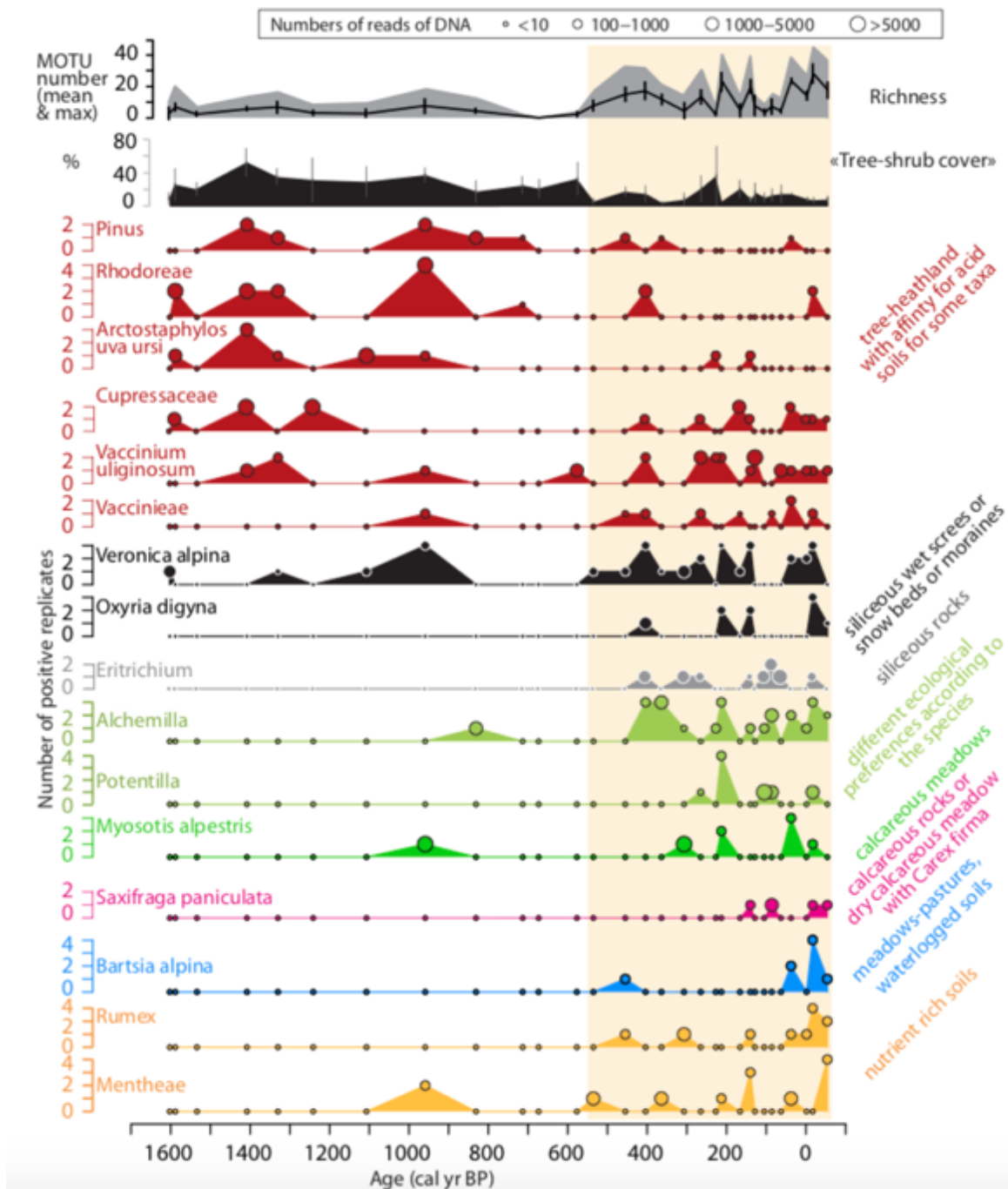
1600 years. Blue areas highlight phases of high inputs of clays and high flood frequency, which corresponds to

low DNA concentration in the sediments samples. B) Relationship between the DNA content in the samples and

the clay content.

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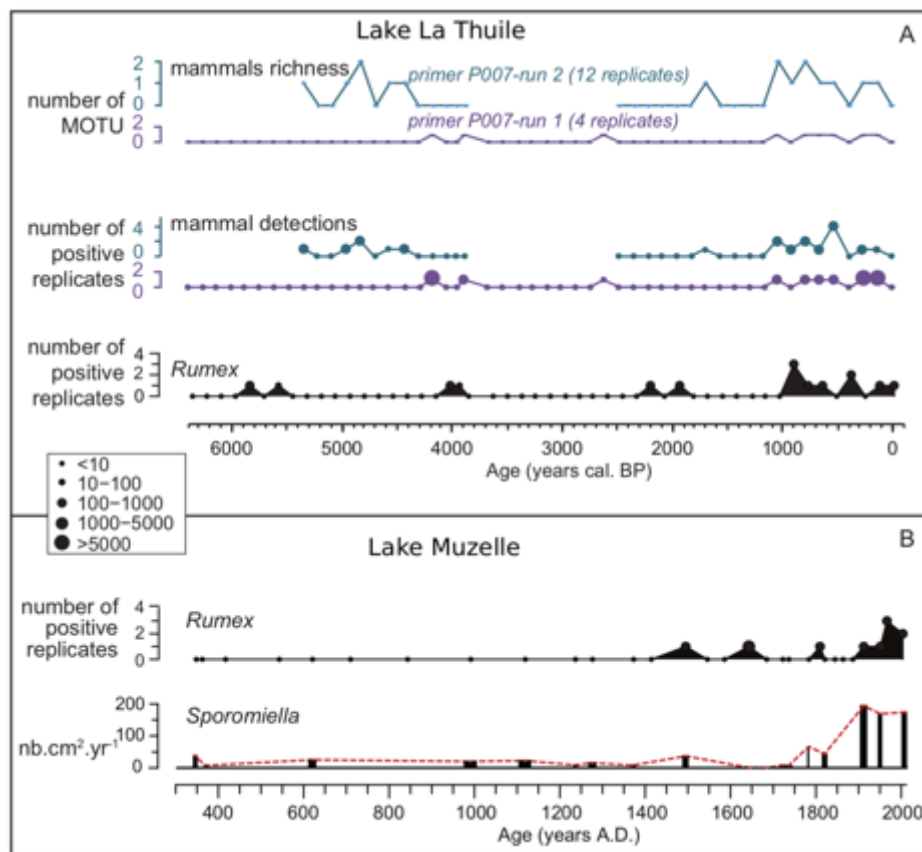
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Figure 8. Plant cover evolution around Lake Muzelle from lake sediment DNA analyses. The richness (mean and maximum), the percentage of arboreal taxa and several taxa (species and genus) of different ecological preferences (mentioned on the right side 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).

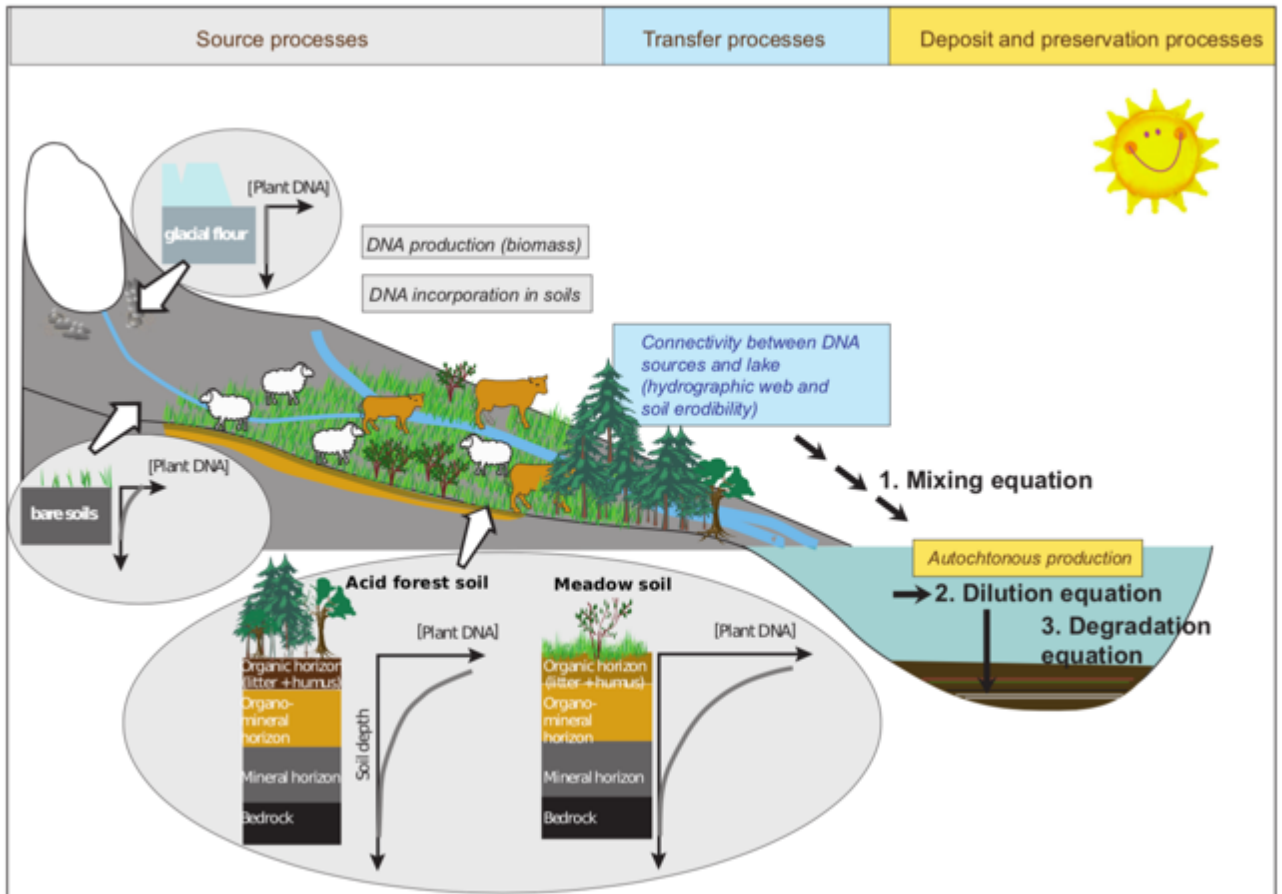
1127 **Table 2. Synthesis of mammal DNA results from the three lake sediment cores.** Grey shaded areas mean no
 1128 analyses with these analytical conditions were realised.

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

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 1133 **Figure 9. Comparison of proxies of the presence of domestic animals in the aim of studying the taphonomic**
 1134 **processes and analytical biases affecting mammal DNA.** A) Comparison for Lake La Thuile between the
 1135 mammal DNA results obtained from the same primer “mam P007”, but not with the same replicate numbers (4 vs
 1136 12). The DNA from *Rumex sp.* is also presented as a proxy of high animal stocking rate or stock density
 1137 (nitrophilous plant) to compare with the mammal DNA. B) Comparison on Lake Muzelle between the DNA from
 1138 *Rumex sp.* and spores of coprophilous fungi (*Sporomiella sp.*).



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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.