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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21 Supplementary materials

22 **1. Sediment lithology and dating**

23 **1.1.** Lake Serre de L'Homme

24 Three cores from Lake Serre de l'Homme (SDH-1, SDH-09-P1, SDH-09-P2) were included in our 25 study in order to provide an age-depth model and determine the sedimentation rate on the core we 26 used for DNA analyses (SDH-09-P1). These sediment cores are characterised by four different 27 units (Supplementary figure 1). Unit 1 (U1) is made of brown fibrous gyttja sediments with an 28 organic matter content around 50-60% according to the loss on ignition at 550°C (LOI 550°C). 29 Unit 2 (U2) is made of a brown non-fibrous gyttja with an organic matter content varying between 30 30 and 50%. The organic matter content varies around 30% in unit 3 (U3). The gyttja sediments 31 from this unit are grey-brown. In unit 4 (U4), sediments have lower content in organic matter and

are mostly made of grey clays. Two ¹⁴C dates were available on cores SDH-1 and five on core 32 33 SDH-09-P2 (see Supplementary table 1). Arboreal pollen/non-arboreal pollen ratio, Abies signal 34 and the ruderal-anthropic herbs signal were used to project the position of the two ¹⁴C dates from the core SDH-1 on the core SDH-09-P2 (Supplementary figure 1 and table 1). Pollen on core SDH-35 36 1 (taken in 2006 with a Russian corer close to the shoreline) was analysed by S. Richer (IMBE, Université Aix-Marseille) and published in (Walsh et al., 2014). Pollen on core SDH-09-P2 (taken 37 38 in the centre of the lake in 2009 with a UWITEC coring device) was analysed by R. Sinet (IMBE, 39 Université Aix-Marseille). Positions of dates were also projected on core SDH-09-P1 based on the 40 lithological descriptions and geochemical signals (titanium and lead/rubidium ratio) from the XRF core scanner analyses (Supplementary figure 1). SDH-09-P1 was analysed at EDYTEM 41 42 (Université Savoie-Mont Blanc) using an XRF core scanner Avaatech (X-Ray beam generated 43 with a rhodium anode and a 125 µm Beryllium window) with the following settings: run 1 at 10 44 kV, 1.2 mA, a counting time of 20 s and a resolution of 2 mm; run 2 at 30 kV, 0.75 mA, a counting 45 time of 60 s and a resolution of 2 mm. SDH-09-P2 was analysed at CEREGE (Université Aix-Marseille) with a Cox Analytics Itrax core scanner¹. The X-Ray beam was generated by a 3kW 46 47 molybdenum tube, and the following settings were applied: 30kV, 30mA, a counting time of 20 s 48 and a resolution of 2 mm.



50

51 Supplementary figure 1. Lithological descriptions and correlations for cores taken from Lake Serre de

52 l'Homme. Core correlations are based on titanium and lead/rubidium ratio measured by XRF core scanner for cores

SDH-09-P1 and P2, while SDH-09-P2 and SDH-1 were correlated using pollen analyses (arboreal pollen/non-arboreal
 pollen ratio, *Abies* and anthropogenic indicators).

55

56 Two ¹⁴C dates from the core SDH-09-P2 were too old to respect the stratigraphic principle of 57 superposition (Supplementary figure 2A). They were thus removed to generate the age-depth 58 model on core SDH-09-P2. Moreover, based on the presence of these two dates from reworked 59 materials and the enrichment in titanium only recorded in SDH-09-P2, we propose the presence of 60 a reworked sediment deposit (or of an erosive event which mobilised old plant remains buried in 61 soils) in the core SDH-09-P2 (Supplementary figures 1 and 2A). However, whether this deposit 62 corresponds to reworked materials or not has no consequences for our study. For the recent period, 63 two supplementary chronological points corresponding to recent atmospheric lead pollutions and highlighted by high Pb/Rb ratio were included to generate the age-depth model. The lead/rubidium 64 65 ratio was used to highlight these lead pollution phases because it permits a correction from the 66 enrichment effect triggered by bedrock erosion. In fact, rubidium represents a purely lithogenic 67 element and is not affected by weathering or diagenesis processes. The upper lead peak, only 68 recorded at 2.5 cm in the core SDH-09-P1 because of a gap in measurements on core SDH-09-P2, 69 is attributed to the effects of the oil crisis in 1973-74 and the introduction of unleaded gasoline in 70 1985^{2,3}. We thus proposed 1979^{+/}-6 AD as a date for this stratigraphic marker. Bellow this peak, the strong increase of Pb/Rb (at 12,5 cm for SDH-09-P2 and at 10 cm for SDH-09-P1) is attributed 71 72 to the pollution triggered by the Second Industrial Revolution at the beginning of the 20th century and the introduction of leaded gasoline in the 1920s^{2,3}. An age of 1920⁺/₋20 AD was proposed for 73 74 this increase in Pb/Rb. The age-depth models of the two cores were generated using the *R software* 75 and the *R-code package 'Clam' version 2.2*⁴. Models with linear interpolations were selected.

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79

77 Supplementary table 1. List of ¹⁴C dates for Lake Serre de l'Homme. Depth in grey and italic correspond to depth

78 determined by correlation. Samples in bold correspond to rejected dates.

		SDH 1	SI	DH-09-P2	SE	DH-09-P1			
		bottom		bottom	top	bottom		uncalibrated	Calibrated age ranges
	top depth	depth	top depth	depth	depth	depth		ages (BP) &	at 95% confidence
	(cm)	(cm)	(cm)	(cm)	(cm)	(cm)	material dated	uncertainty	intervals (cal. BP)
Poz-46475			23	24	24	25	Juniperus twig	740 +/-30	725-660
Poz-18880	33	34	31	32	32	33	bulk sediment	1385 +/-30	1345-1275
							wood pieces (including a		
Poz-46476			65	66			Brachyblast of P. cembra	3565 +/-35	3725-3970
							P.cembra and organic		
Poz-46477			75	76			matter	3375 +/-35	3510-3700
Poz-18881	73	74	78	79	65	66	bulk sediment	2930 +/-35	2965-3175
Poz-35074			86		76		Conifer (Larix/Picea)	3320 +/-35	3465-3635
							pieces of wood and organic		
Poz-46478			91	92			matter	3785 +/-35	4005-4290

The age-depth models built using the ¹⁴C dates and recent lead pollution markers are supported by 81 82 older lead pollution phases detected by the XRF core scanner (Pb/Rb ratio). The most recent one starts around 900-950 cal. BP (11th century) and fits with the exploitation of two nearby mines 83 (Faravel and L'Argentière-La Bessée) between the 10th and 13-14th centuries ⁵. The other one 84 corresponds to the Antique period (peak around 2100-2150 cal. BP, i.e. 150-200 BC). This 85 pollution was also detected in a peat core (Fangeas) located in the neighbouring valley ⁵. The 86 origin, local vs regional/global, of this pollution could not be determined by the study by Py et al 87 ⁵. However, Roman-period pollution was also detected in several lakes and peats in French Alps 88 ^{6–8} and Swiss Alps ^{9–11}, between 2500 and 1650 cal. BP, according to the sites. 89



Supplementary figure 2. Age-depth models of the three lake sediment cores. A) Age-depth model on cores SDH 09-P1 and P2. B) Age-depth model on core THU-10. C) Age-depth model considering instantaneous deposits on core
 MUZ-12. The lithological descriptions of each core are also presented.

90

95 **1.2.** Lake La Thuile

The lithology of the upper 549 cm of the core from Lake La Thuile is divided into five units, which are described in detail in Bajard et al.¹² and summarized, here (Supplementary figure 2B). The two upper units represent most of the sedimentation (0-190 cm and 190-450 cm). They are mainly made of clays (10 to 40 %) and silts (40 to 80%). The lower unit is lighter and presents fine laminations, while the upper one is darker and homogeneous. The organic matter content assessed by the LOI at 550°C is less than 10% in these two units. Between 450 and 500 cm, sediments are very dark due to higher organic-matter content (20 to 30%). White to grey laminations are visible. From 500 to 530 cm, sediments are highly enriched in organic matter (around 50 to 60%) and contain well-preserved leaves. The lower 19 cm of the core was sampled for DNA analyses and is mostly characterised by very fine rhythmic laminations alternating between bio-precipitated carbonates (mostly rhombohedral calcite crystals) and diatoms.

107 The age-depth model is based on nine ¹⁴C dates and five chronological markers provided by the 108 geomagnetic field secular variations (declination measured with three-axis 2-G enterprise 109 cryogenic magnetometer of the CEREGE laboratory at Aix-Marseille University). The last 110 hundred years are also constrained by short-lived radionuclide measurements (²¹⁰Pb, ¹³⁷Cs, ²⁴¹Am). 111 As for Lake Serre de l'Homme, the age-depth model was generated using the *R software* and the

112 *R-code package 'Clam' version 2.2*⁴. The linear interpolation was selected (Supplementary figure

- 113 2). More details about the sediment lithology and the age-depth model are available in Bajard et 114 $al.^{12}$.
- 115

116 **1.3.** Lake Muzelle

117 Sediments from Lake Muzelle are characterized by the presence of turbidite deposits all along the sediment core (Supplementary figure 2C). They are interpreted as flood deposits ¹³. In the 118 119 continuous sedimentation, three different facies were recognised: a light grey clay-rich facies, a 120 dark grey silty clay facies and a facies enriched in organic matter. The two first facies alternate 121 along the core to define seven units. The organic-rich facies mostly occurs bellow 140 cm depth 122 and in the units made of the dark grey silty clay facies (Supplementary figure 2C). The darker 123 facies is a little enriched in organic matter relative to the lighter one (4,4% vs 3,6% according to 124 the LOI 550°C).

125 As turbidite deposits represent instantaneous deposits, they were not considered (time of 126 deposition equal to zero) in the generation of the age-depth model (Supplementary figure 2C). Ten ¹⁴C dates were performed, but four were rejected as too old probably due to reworked materials. 127 128 One chronological marker provided by the geomagnetic field secular variations (also analysed at 129 CEREGE laboratory) was also included in the age-depth modelling using the *R* software and the *R-code package 'Clam' version 2.2*⁴. The smooth spline interpolation was preferred for this lake 130 (Supplementary figure 2C). More information about dating and sediment lithology are provided 131 in Fouinat et al.¹³. 132

134 **2.** Filtering steps

135 **2.1.** Dealing with true and false presences

136 The summary of the numbers of reads from the high-throughput sequencing, and after the different 137 filtering steps, is presented for plants in the supplementary figure 3. Several of these steps were 138 used to remove potential false positives. Steps 1 to 8 (Supplementary figure 3), were realised using 139 the OBITOOLS software (http://www.grenoble.prabi.fr/trac/OBITools)¹⁴, which allows, in 140 particular, the removal of potential false positives due to PCR or sequencing errors. After these 141 bioinformatic treatments, additional filtering steps (9 to 14) were applied to remove potential 142 sporadic contaminations. In step 9, negative controls were used to detect false positives. Unique 143 sequences (or Molecular Operational Taxonomic Units, MOTU) detected in at least one control 144 (>5reads) only represent 15% of the total number of MOTU assigned to 95% of similarity with 145 sequences in the reference database but represent 65 to 80% of the read numbers (whose 10 to 146 16% are detected in the negative controls). On average, they mainly occur in the extraction controls 147 for lakes Muzelle and La Thuile, and in PCR controls for Lake Serre de l'Homme (Supplementary 148 table 2). In the filtering process, we only excluded the MOTU detected in high quantity in negative 149 controls (in more than 5 controls with more than 10000 reads). These MOTU correspond to 150 Salicaceae sp., Plantago sp., Myriophyllum sp., Asteraceae sp. and Lamiale sp. for lakes Muzelle 151 and La Thuile and Betulaceae sp. for Lake Serre de l'Homme (Supplementary table 2). Except for 152 Betulaceae sp., they also occur in a high number of samples with high read number and for some 153 of them in several replicates. However, as they are detected in large quantities in the negative 154 controls, we cannot be sure that their occurrences are not affected by contaminations. Moreover, 155 the percentage of reads lost by the removal of these MOTU is higher in phases of lower total 156 numbers of reads (Supplementary figure 4 and 5), which might be due to the preferential 157 amplification of contaminants in samples containing less DNA. In Lake La Thuile, these MOTU 158 are especially detected in very high quantity (representing up to 80% of the total read number) 159 between 2600 and 3800 cal. BP (mostly Lamiale sp. and Myriophyllum sp.), which is a phase with 160 a very high total number of reads but a low number of MOTU within the context of the whole 161 dataset, i.e. without filtering (see blue area in Supplementary figure 4). Standard deviations of the 162 total number of reads (between PCR from a common sample) are also very high, which shows a 163 very stochastic amplification of the small number of MOTU detected in these samples and might 164 indicate a contamination effect. The detection of a high number of DNA reads in this period is also 165 suspicious because one extract dated to 3100 cal. BP (performed for another study) was quantified and gave a concentration lower than 0.05 ng/uL (i.e. under the detection limit of the Qubit 166

167 analysis). As presented and discussed in the manuscript, this phase is characterised by an 168 accumulation of terrestrial plant macroremains (leaves, needles), which suggests a phase of litter 169 erosion or direct fall of the macroremains in the lake. Humic substances present in the litter should 170 have led to an acidification of the lake water. In addition, this hypothesis is supported by the 171 absence of carbonate in the sediments, which only occurs during this period. Acidic conditions are 172 not favourable to DNA preservation, which might explain the poor recording of DNA during this 173 period. Moreover, DNA that might still eventually be present in this type of sediment is expected 174 to be complexed with humic substances, which are not extracted by the method we used.

175

176 The next filtering step applied to limit potential false presences (step 11), was to remove MOTU detected in only one sample in each lake dataset (51 to 89 % are also detected in only one PCR). 177 Then, MOTU only detected in one replicate per sample were discarded, unless they are detected 178 179 in contiguous samples to consider the temporal autocorrelation which often affects ecological variables¹⁵ (step 12). These two filtering steps allow the removal of MOTU considered doubtful 180 181 (possibly rare taxa but also false presences). Again, the highest quantity of these MOTU is found 182 between 2600 and 3800 cal. BP at Lake La Thuile (blue area in Supplementary figure 4). Many of 183 the MOTU detected in low quantities in negative controls (less than 5 controls) were filtered out 184 during these two filtering steps (21 and 26 over 49 for lakes Muzelle and La Thuile, respectively 185 and 47 over 60 for Lake Serre de l'Homme).

186 The final step (13) of the filtering process common to all lakes was to remove taxa allochthonous 187 in the Alps. In Lake Muzelle, six MOTU were assigned to Grubbia Rosmarinifolia, Glycine max, 188 Gaylussacia sp., Styrax sp., Cucumis sp. and Lactuca sativa. The Grubbiaceae is a family of plants 189 endemic to the Cape floristic region of South Africa. Glycine max (soy) comes from East Asia, 190 Gaylussacia sp. (Ericaceae family) from America and the genus Styrax sp. from the Far East or 191 the Mediterranean region. Cucumis sp. and Lactuca sativa are cultivated plants not supposed to 192 grow at the altitude of the lake. In addition to some of these taxa, three other exotic taxa were 193 removed from the Lake La Thuile dataset: Actinidia, Axonopus and Musaceae. Axonopus is a Poaceae from tropical and sub-tropical regions; Actinidia is the genus of kiwifruit and Musaceae 194 195 the family of bananas.

196

In case of Lake La Thuile, a fourteenth step was applied to discard the last taxa that were detected in very high quantity only in the period when we think no or nearly no DNA was extracted (between 2600 and 3800 cal. BP). These taxa are *Pinus sp., Populus sp., Frangula sp.* and 200 *Pooideae sp.*, which are not detected in the controls, and *Asteraceae sp.*, which is also detected in
201 three extraction controls.

202

203 Even if they were sometimes detected in negative controls, we think that MOTU retained for the 204 final datasets represent true signals because they possess temporal coherences, i.e. the occurrences 205 of each plant are clustered in well-defined periods. Moreover, the temporal evolution of some of 206 these MOTU is attested by independent methods. Specifically, pollen data (for La Thuile) and 207 coprophilous fungi (for Muzelle) corroborated the detection of DNA from *Rumex sp.*, which was 208 detected in 2 PCR controls (Figure 4 & 9). Likewise, for La Thuile, the DNA record of 209 Helianthemum nummularium (detected in four different extraction controls with more than 5 210 reads) is in agreement with the pollen record (Supplementary figure 8). For Lake Serre de 211 l'Homme, the true presence of *Potamogeton sp.* (detected in three controls) and *Sparganium sp.* 212 (detected in four controls) is supported by the increase of the organic matter content (LOI 550°C) 213 and the decrease in the C/N ratio (i.e. increase of aquatic organic matter relative to terrestrial 214 organic matter) (Figure 5). Moreover, the Potamogeton sp. mostly occurs at the same time as the 215 Potamogetonaceae family, which is not detected in the controls (Figure 6). However, the record of 216 the third aquatic plant detected in Lake Serre de l'Homme (Myriophyllum sp.) has to be considered 217 with caution because it is mostly detected in only one or two replicates over eight (Figure 6) and 218 is also detected in high quantities in negative controls (Supplementary table 2). Plant community 219 composition and ecological preferences can also be used to support the occurrence of taxa. For 220 instance, Pinus sp. (detected in 2 extraction controls) can be considered as accurate detections in 221 the case of Lake Muzelle because they are contemporaneous with Arctostaphylos uva-ursi, a 222 species associated with pinewood environments (Figure 8). It is also found in association with 223 Rhodoreae sp. and Vaccinium uliginosum (Figure 8); two species characteristic of acid 224 environments such as *Pinus sp.* likely to be found in this region. Moreover, pollen from *Pinus sp.* 225 (Pinus mugo or sylvestris) occur during the Subatlantic period (covering the last 2500 years) in 226 pollen diagrams from a peat area just below the lake¹⁶. By looking in detail at our datasets, we 227 emphasise that distinguishing between true and false presences is not straightforward.

	lake names	Muzelle (MUZ)	La Thuile Muzelle La Thuile (THU) (MUZ) (THU)		La Thuile (THU)	Serre de l'Homme (SDH)		
	number of samples	30	50	30	50		41	
	replicate number		4				8	
	number of controls		64				56	
				No of u	nique		No of unique	
	Data filtering steps	Noo	f reads	seque	nces	No of reads	sequences	
_1	Merging of paired-end reads			-		-	-	
_2	Primer and tag identification	12 4	01 846	-		-	-	
_ 3	Dereplication	12 4	01 846	193 0)87	-	-	
4	Filtering based on the sequence length	12 3	55 683	191 5	587	-	-	
5	Filtering of sequences without N	10 708	161 7	73	-	-		
6	Filtering based on the sequence occurrence (removal of sequences detected less than 1	11 4 00 times	78 425	3 22	24	-	-	
7	Filtering on the status of each sequence in e product (Removal of sequences more often than «head» or «singleton»	23 404	978	3	9 471 448	2 927		
8	Assignation (percent identity $\ge 95\%$ to database)	7 40)5 225	396		8 700 614	438	
9	Removal of MOTU highly detected in negative controls	4 91	0 010	391	1	8 421793	437	
10	Removal of «clean controls» and split of datasets for MUZ and THU	2 445 261	1 714 500	241	296	7 781 916	232	
11	Removal of MOTU detected in only 1 sample in each lake dataset	2 197 260	1 617 191	140	180	6 813 710	49	
12	Removal of "non-continuous detections" in less than 2 replicates	1 850 955	1 544 184	89	124	5 916 655	19	
13	Removal of exotic MOTU	1 836 110	1 475 651	83	112	(7 aqua MOTU bu	atic and 12 terrestrial t 3 different aquatic taxa	
14	Removal of other suspicious MOTU	1 124 296	107 and 8 different terrestrial taxa (10 aquatic MOTU probably corresponding to 10 different taxa)					

8. aDNA MOTU assignement (≥ 95% of similarity with taxa in the refetrence database)

Г 9. aDNA MOTU absent in controls

83 Veronica

158657

54

List of aDNA MOTU highly detected in controls (batch Muzelle & La Thuile)

	NA MO	TU in Lak	e Muzel	le		«clean co	ntrols» a	nd MOTU		scientific names	number n of reads	umber of PCR (>5 reads)
Г						only det	ected in L	a inulie	1	Lamiales	49969	5
	Uniqu	ie seauend	es dete	cted in 1 samp	е	Scientific	number	number of PCR	2	Asteraceae	32238	8
		scientific	number	number of PCR		names	of reads	(>5 reads)	3	Myriophyllum	102399	9
		names	of reads	(>5 reads)	1	Polygonoideae	e 1	0	4	Plantago	156669	12
	1	Avena	11	1	2	Medicago sativ	va 14	0	5	Saliceae	120190	30
	2	Tilia	12	1	3	3 Triticeae	14	0				
	3	campanulid	14	1								
					147	Taxus	3877	19				
	98	Trifolium	2045	2	148	Ulmaceae	16414	23				
	99	Solanaceae	15455	2	149	Quercus	18994	25				
	100	Euphorbia	2593	3	150	Nymphaeacea	ie 122372	57				
	101	Acer	7998	3								
12. aDNA N	лоти										_	
		in Lake Mu	izelle de	tected at least	in 2	consecutiv	e sample	s		«non contin in less tha	uous det an 2 repli	ection» cates
		in Lake Mu	izelle de	tected at least	in 2	consecutiv	e sample	s	-	«non contin in less tha	uous det an 2 repli	ection» cates umber of PCR
13. Final lis	st of	scientific nu	mber of	number of PCR	in 2 E)	consecutiv	e sample	S		«non contin in less tha Scientific names	uous det an 2 repli number n of reads	ection» cates umber of PCR (>5 reads)
13. Final lis	st of U in	scientific nur names	mber of read	number of PCR s (>5 reads)	in 2 E)	consecutiv		s	1	«non contin in less tha Scientific names Rosularia alpestris	uous det an 2 repli number n of reads s 53	tection» cates umber of PCR (>5 reads) 2
13. Final lis DNA MOT Lake Muz	st of U in celle 1	scientific nur names	mber of read	number of PCR s (>5 reads) 84 2	in 2 E)	consecutiv	number num preads	nber of PCR (>5 reads)	11	«non contin in less tha Scientific names Rosularia alpestris	uous det an 2 repli number n of reads s 53 ons 82	ection» cates umber of PCR (>5 reads) 2
13. Final lis aDNA MOT Lake Muz	st of U in celle 1	scientific nur names PACMAD cla Potentilleae	mber of read ade 78	number of PCR s (>5 reads) 84 2 08 2	in 2 E) 1	consecutiv cotic MOTU scientific r names c Glycine max Grubbia	number num freads	s mber of PCR (>5 reads) 4	11	«non contin in less tha Scientific names Rosularia alpestris Core eudicotyledo Repuperulus 1	uous det an 2 repli number n of reads s 53 ons 82 290	ection» cates umber of PCR (>5 reads) 2 2 2
13. Final lis aDNA MOT Lake Muz	st of U in celle 1 2 3	scientific nur names PACMAD cla Potentilleae Pamphalea	mber of read ade 78 1250	number of PCR s (>5 reads) 84 2 08 2 48 3	in 2 E) 1 2	consecutiv cotic MOTU scientific r names c Glycine max Grubbia rosmarinifolia	ve sample	S nber of PCR (>5 reads) 4 4	11	«non contin in less tha Scientific names Rosularia alpestria Core eudicotyledo Ranunculus-1	uous det an 2 repli number n of reads s 53 ons 82 290	tection» cates umber of PCR (>5 reads) 2 2 2 2
13. Final lis DNA MOT Lake Muz	st of U in celle 1 2 3	in Lake Mu scientific nui names PACMAD cla Potentilleae Pamphalea	mber of read ade 78 1250	number of PCR (>5 reads) 84 2 08 2 48 3	in 2 E) 1 2 3	consecutiv cotic MOTU scientific r <u>names</u> Glycine max Grubbia rosmarinifolia Lactuca sativa	ve sample number num of reads 1113 1942 3737	s nber of PCR (>5 reads) 4 4 5	11	«non contin in less tha Scientific names Rosularia alpestris Core eudicotyledo Ranunculus-1	uous det an 2 repli number n of reads s 53 ons 82 290	tection» cates umber of PCR (>5 reads) 2 2 2 2
13. Final lis aDNA MOT Lake Muz	st of U in celle 1 2 3 80	in Lake Mu scientific num names PACMAD da Potentilleae Pamphalea	mber of read ade 78 125 1 1	etected at least number of PCR s (>5 reads) 84 2 08 2 48 3 89 38	in 2 E) 1 2 3 4	consecutiv cotic MOTU scientific r names c Glycine max Grubbia rosmarinifolia Lactuca sativa Styrax	re sample number num of reads 1113 1942 3737 124	s (>5 reads) 4 4 5 5 5	1 2 3 49	«non contin in less tha Scientific names Rosularia alpestria Core eudicotyledo Ranunculus-1	an 2 repli number n of reads s 53 ons 82 290 a 7111	tection» cates umber of PCR (>5 reads) 2 2 2 2 5
13. Final lis 1DNA MOT Lake Muz	st of U in celle 1 2 3 80 81	in Lake Mu scientific num names PACMAD da Potentilleae Pamphalea Potentilleae Asteraceae	mber of read ade 78 1256 1 1195 1114	number of PCR (>5 reads) 84 2 08 2 48 3 89 38 45 49	in 2 E) 1 2 3 4 5	consecutiv cotic MOTU scientific r names C Glycine max Grubbia rosmarinifolia Lactuca sativa Styrax Gaylussacia	re sample number num of reads 1113 1942 3737 124 4190	S nber of PCR (>5 reads) 4 4 5 5 8	11 20 31 491 501	«non contin in less tha Scientific names Rosularia alpestria Core eudicotyledo Ranunculus-1 Filipendula ulmaria Ranunculus-2	number n of reads s 53 ons 82 290 a 7111 16723	ection» cates umber of PCR (>5 reads) 2 2 2 2 5 5

228

229 Supplementary figure 3. Information on aDNA plant datasets and flow chart of the filtering procedures. Steps 230 1 to 8, were realised using the OBITOOLS software (http://www.grenoble.prabi.fr/trac/OBITools) (Boyer et al.,

231 2016). The evolution of the number of reads and unique sequences at each step is presented, when it was calculated.

232 Supplementary tables 2. List of contaminants in the sequencing run for plant DNA in lakes La Thuile and

233 Muzelle and then in Lake Serre de l'Homme.

number of reads number of number of number of negative reads number of negative reads number of reads numbe			Lak	es MUZELLE a	nd La THUILE				
number of number of negative reads extraction negative reads extraction controls FCR negative reads rank scientific name sample PCR sciention controls Feads feads controls feads f					number of reads in	number of negative	number of reads in	number of negative	number of
rank scientific name sample PCR extraction controls (reads) controls (reads) reads) reads in the interm in			number of	number of	negative	extraction	negative	PCR	negative
rank scientific name samples >5reads controls reads) reads) reads tribe Poece 4181 7 0 0 12 1 tribe Poece 4181 7 0 0 12 1 family Salkacece 126 8 23 1 0 0 family Salkacece 126 8 23 1 0 0 subclass osterids 394 18 31 1 0 0 0 species Pertonerra camponlids 270 2 59 1 0			reads in	sample PCR	extraction	controls (>5	PCR	controls (>5	controls (>5
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family Lamiaceae 7374 8 3908 1 4 0 genus Geranium 10258 14 5582 1 8 0 family Apiaceae 110986 47 6300 1 58 0 family Ranunculaceae 23947 7 8060 1 10 0 family Caprifoliaccee 1603 3 10457 1 2 0 genus Cattha 17527 16 14973 1 8 0 1 genus Cattha 159619 66 22709 1 0 0 2 genus Veronica 159619 66 22709 1 0 0 2 genus Styrax 130 5 23 2 1 0 1 genus Styrax 130 5 23 2 1 0 1 finity Ap	2 species	Pedicularis attollens	197	1	3195	1	0	0	1
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family Apiaceae 110986 47 6300 1 58 0 family Caprifolicaceae 1603 3 10457 1 2 0 genus Caltha 17527 16 14973 1 8 0 1 genus Caltha 17527 16 14973 1 8 0 1 genus Caltha 17527 16 14973 1 8 0 1 species Medicago sativa 27 0 1 0 20024 1 1 genus Veronica 159619 66 22709 1 40 0 1 0 1 0 10 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0	species	Lomelosia brachiata	188	3	6317	1	0	0	1
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Artibe Triticeae 40 0 3 0 29205 1 genus Styrax 130 5 23 2 1 0 family Apiaceae 186 8 76 2 0 0 family Betulaceae 1162 5 9 1 67 1 2 family Betulaceae 92096 11 11 0 5754 2 2 genus Rumex 11857 26 3 0 13126 2 2 family Betulaceae 97 0 21635 1 12 1 1 subgenus Pinus 184831 19 12 0 71048 2 2 49 1 3 genus Saxifraga 47338 53 669 3 23 0 3 3 0 3 5 5 5 5 5 5	2 genus	Veronica	159619	66	22709	1	40	0	1
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B genus Rumex 11857 26 3 0 13126 2 family Betulaceae 97 0 21635 1 12 1 1 subgenus Pinus 184831 19 12 0 71048 2 16 genus Alnus 247474 114 42 2 49 1 3 genus Saxifraga 47338 53 669 3 23 0 3 genus Saxifraga oppositifolia 24340 58 2875 3 13 0 3 genus Saxifraga oppositifolia 20986 61 10168 3 13 0 3 species Saxifraga oppositifolia 20986 61 10168 3 13 0 3 family Betulaceae 90 2 23218 2 55 1 3 genus Cucumis 4569 14 8196 4 1 0 4 genus Cucumis 17393 14	tribe	Роеае	92096	11	11	0	5754	2	2
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2 genus Ainus 24/4/4 114 42 2 49 1 5 genus Saxifraga 47338 53 669 3 23 0 3 species Saxifraga oppositifolia 24340 58 2875 3 13 0 3 genus Saxifraga oppositifolia 20986 61 10168 3 13 0 3 family Asteraceae 196424 99 20762 3 54 0 3 species Athyrium vidalii 62780 37 2525 1 28189 2 3 genus Cucumis 4569 14 8196 4 1 0 4 species Helianthemum nummularium 113917 56 33361 3 65 1 4 species Helianthemum nummularium 113917 56 33361 3 65 1 4 species Helianthemum nummularium 113917 56 33361 3 65 1 4 4	species		51820	16	112146	2	16	0	4
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genus Saxifraga 47162 55 8620 3 20 0 3 species Saxifraga oppositifolia 20986 61 10168 3 13 0 3 family Asteraceae 196424 99 20762 3 54 0 3 a family Betulaceae 90 2 23218 2 55 1 3 species Athyrium vidalii 62780 37 2525 1 28189 2 3 genus Cucumis 4569 14 8196 4 1 0 4 family Betulaceae 17393 14 5728 3 27542 1 4 species Helianthemum numularium 113917 56 33361 3 65 1 4 genus Picea 31139 19 13 0 193530 4 4 order Lamiales 180033 102 24757 3 25212 2 5 genus Myriophy	species	Saxifraga oppositifolia	24340	58	2875	3	13	0	
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tatal number of DCD 230 24 110013 3 10173 21 30	trihe	Saliceae	67/820	2/7	110015	<u>4</u> ۵	10175	0 21	212
	une	total number of DCD	024030	247	110012	9	101/2	21	30

				Lake SERK	L UE L HOIVIIV	IL			
					number of	number of	number of	number of	
					reads in	negative	reads in	negative	number of
			number of	number of	negative	extraction	negative	PCR	negative
			reads in	sample PCR	extraction	controls (>5	PCR	controls (>5	controls (>5
	rank	scientific name	samples	>5reads	controls	reads)	controls	reads)	reads)
4	4	0		0		1	0		
T	tribe	Poeae	0	0	6	1	0	0	1
2	tamily	Aplaceae	0	0	6	1	0	0	1
3	genus	Solanum	0	0	6	1	0	0	1
4	tribe	Maleae	1390	1	0	0	6	1	1
5	subfamily	Vaccinioideae	0	0	0	0	8	1	1
6	species ,	Grubbia rosmarinifolia	78	1	8	1	0	0	1
7	family	Salicacaga		-	0	1	0	0	1
,		Suilcucede	0	0	9	1	0	0	1
8	family	Solanaceae	0	0	10	1	0	0	1
9	family	Apiaceae	0	0	3	0	7	1	1
10	tribe	Poeae	0	0	11	1	0	0	1
11	no rank	campanulids	0	0	11	1	0	0	1
12	subclass	asterids	41	1	11	1	0	0	1
13	genus	Fmnetrum	0	0	0	0	12	1	1
11	ordor	Empetrum	45	2	0	1	12		1
14	order	Fugules	45	2	8	1	4	0	1
15	subfamily	Solanoideae	0	0	13	1	0	0	1
16	subfamily	Asteroideae	1	0	17	1	0	0	1
17	no rank	BEP clade	20729	4	0	0	28	1	1
18	genus	Caltha	22228	4	25	1	4	0	1
19	no rank	Sanauisorhinae	398296	7	30	1	3	0	1
20	genus	Hunerzia	000100	0	0	0	36	1	1
20	genus		0	0	0	0	30	1	1
21	genus	Huperzia	0	0	0	0	44	1	1
22	genus	Huperzia	0	0	0	0	44	1	1
23	genus	Rumex	1488	2	0	0	50	1	1
24	genus	Gaylussacia	35630	3	0	0	57	1	1
25	order	Asterales	0	0	58	1	0	0	1
26	order	Fricales	0	0	58	1	0	0	1
20	gopus	Huporzia	0	0	0		70	1	1
27	genus		0	0	0	0	70	1	1
28	species	vaccinium ovalifolium	40659	3	0	0	123	1	1
29	tribe	Vaccinieae	6368	2	0	0	159	1	1
30	genus	Isoetes	24692	2	0	0	285	1	1
31	genus	Limonium	0	0	454	1	0	0	1
32	genus	Solanum	3	0	2280	1	0	0	1
22	trihe	Maleae	3155	1	0		2363	1	1
24		Charaanium	1611202	25	20	0	2303	1	1
24	genus	Spurgumum	1011592	55	50	0	2709	1	1
35	genus	Рісеа	152851	3	4647	1	1	0	1
36	family	Asteraceae	23608	1	4861	1	2	0	1
37	order	Asterales	5	0	6283	1	1	0	1
38	species	Vaccinium uliginosum	13	0	1	0	8332	1	1
39	genus	Theobroma	5767	1	0	0	11300	1	1
40	genus	Actinidia	74572	2	11895	1	2		1
11	gonus	Avena	2		0		1/2/2	1	1
41	Benus	Chucino	7110	0	40350	0	14242	1	1
42	genus	Giycine	/119	1	40250	1	2	0	1
43	genus	Bromus	9	0	45687	1	1	0	1
44	no rank	Sanguisorbinae	770	6	68980	1	1	0	1
45	subfamily	Pooideae	191706	4	0	0	77720	1	1
46	genus	Caltha	54	3	85525	1	0	0	1
47	order	Fagales	0	0	8	1	18	1	2
48	tribe	Poeae	2/12		12	1	16	1	
10	tribo	Doggo	17640	2	13	1	100	1	2
49		Filianad	12040	- 2	6/	1	109	1	2
50	species	Filipendula ulmaria	/436/	3	16	1	829	1	2
51	genus	Empetrum	9361	3	2	0	3153	2	2
52	species	Athyrium vidalii	7543	4	1739	1	1658	1	2
53	species	Filipendula ulmaria	141	3	2888	1	1090	1	2
54	order	Lamiales	32992	3	4424	2	1	0	2
55	tribe	Saliceae	17/1350	5	6865	1	 	1	2
50	tribe	Doogo	1/4333		2020	1	2727	1	2
50		Potence	29414	2	29201	1	23034	-	2
5/	genus	rotamogeton	1942111	34	2647	1	34	2	3
58	species	Athyrium vidalii	58730	7	7460	1	8215	2	3
59	family	Apiaceae	209904	7	11363	3	11013	1	4
60	genus	Myriophyllum	961603	26	20851	1	111898	3	4
61	family	Betulaceae	38738	7	7591	1	232492	4	5
	, total	number of PCR	3	20	3	-		4	56
	loidi		, s		. 3	-	. 4		50

Lake SERKE de L'HUIVIIVIE	Lake	SERRE	de	L'HOMME
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238 Supplementary figure 4. Impact of the filtering process on the number of reads and of MOTU for Lake La 239 Thuile. Curves a, b and c represent, respectively, the number of reads, the number of MOTU and the log-transformed 240 number of reads normalised by the dry weight of sediment (means and standard deviations) when no filtering is applied 241 on taxa assigned at 95 % of similarity. The curves d, e and f represent the loss percentages of DNA reads at three 242 different filtering steps: d) removal of MOTU detected in high quantity in controls, e) removal of MOTU detected in 243 only one sample or stochastically, f) removal of exotic MOTU. The curves g and h represent the distribution over time 244 of aquatic plants, in term of mean and standard deviation of the number of reads and of MOTU, respectively. The 245 curve i represent the stacking of five taxa remaining after the application of the thirteenth steps of filtering and 246 specifically detected during the period between 2600 and 3800 cal. BP (light blue area). They are removed in the 247 fourteenth step to obtain the final curves j, k and l (mean and standard deviation of the number of reads (j) and MOTU 248 (k) and of the log-transformed number of reads normalised by the dry weight of sediment (l)). 249





251 Supplementary figure 5. Impact of the filtering process on the number of reads and of MOTU for Lake Muzelle. 252 Curves a, b and c represent, respectively, the number of reads, the number of MOTU and the log-transformed number 253 of reads normalised by the dry weight of sediment (means and standard deviations) when no filtering is applied on 254 taxa assigned at 95 % of similarity. The curves d, e and f represent the loss percentages of DNA reads at three different 255 filtering steps: d) removal of MOTU detected in high quantity in controls, e) removal of MOTU detected in only one 256 sample or stochastically, f) removal of exotic MOTU. After the removal of these MOTU, the final curves g, h and i 257 are obtained (mean and standard deviation of the number of reads (g) and MOTU (h) and of the log-transformed 258 number of reads normalised by the dry weight of sediment (i)).

259

For mammals, the post-obitools filtering procedure starts, as for plants, with the removal of sequences with a similarity <95% to taxa in the reference database (step 8, Supplementary figure 6). However, the next step (9) consisted in the removal of human DNA sequences because they mostly represent contaminations during sample processing. Whereas a human-specific blocking oligonucleotide was applied, sequences from *Homo sapiens* (or assigned to lower taxonomic 265 levels, i.e. to Homo, Homininae and Hominoidea) are detected in high quantity (75 to 98% of read 266 numbers after the assignment to 95% of similarity). Then, the filtering process follows the same procedure as for plants (Supplementary figure 6). However, we removed all taxa detected in 267 268 negative controls. These taxa are Felis catus, Ovis aries, Sus sp. and Sus scrofa. Sus and Sus 269 scrofa are common contaminants. They are mostly detected in samples from Lake La Thuile, and 270 detections in more than one replicate are clustered between 1000 and 800 cal. BP, i.e. when other 271 mammals (Bos sp. and Ovis sp.) are also detected (Figure 9). However, the second run performed 272 with a higher number of replicate to increase the detection probability (12 replicates) was not able 273 to confirm the presence of Sus scrofa, supporting the assumption of false presence. Felis catus was 274 never detected in samples and Ovis aries was detected in only two PCRs in Lake Muzelle and one 275 in Lake La Thuile.

276

		Muzelle	La Thuile	Muzelle	La Thuile				
	lake names	(MUZ)	(THU)	(MUZ)	(THU)	Serre de l'	Homme (SDH)		
	number of samples	30	50	30	50	40			
	replicate number		4				8		
	number of controls		64 but 56 ide	entified		64 but	39 identified		
				No o	f unique		No of unique		
	Data filtering steps	/	lo of reads	seq	uences	No of reads	sequences		
1	Merging of paired-end reads				-	8 815 609	-		
2	Primer and tag identification		3 927 977		-	8 178 352	-		
3	Dereplication		3 927 977	18	8 256	8 178 352	86 500		
4	Filtering based on the sequence length	;	3 124 084	14	5 782	7 987 849	75 522		
5	Filtering of sequences without N		2 687 190	55	004	7 985 409	74 574		
	Filtering based on the sequence								
	occurrence (removal of sequences								
6	detected less than 100 times)		2 511 013	8	353	7 710 767	1 300		
	Filtering on the status of each								
7	sequence in each PCR product		1 940 128		319	6 917 247	279		
	Assignation (percent identity \geq 95% to	Г							
8	database)		1 322 537		34	5 518 002	40		
-	Removal of Homo sapiens, Homo,				-		_		
9	Homininae, Hominoidea		327 649		9	131 507	2		
	Removal of MOTU detected in negative					101 507			
10	controis		62506		4	131 507	2		
	Removal of negative controls and split		00400			424 502	2		
	or dataset for MO2 and THO	U	02403	U	4	131 302	2		
	Removal of unique sequences								
12	detected in only i sample in each lake		53060		1	131 3/15	1		
12	Removal of stochastic detections in	-	33000	-		101 040	1		
13	Removal of stochastic detections in		53060		1	0	0		
- 15	icas tran 2 repretes	-	00000			0	U		

8. aDNA taxa assignement (≥ 95% of similarity with taxa in the refetrence database)

9. al	DNA tax	a other th	an bel	onging to hon	ninid	s]		ba	List of tch Muzelle &	Hominids La Thuile	for plants
10. aDNA 1	10. aDNA taxa in Lake La Thuile					List of taxa in controls			scientific names		reads	PCR >5	
(11)	ouning i						scientific	number of	number of PCR >5	1	Homo sapiens-1	1212	1
							names	reaus	reads	2	Homo sapiens-2	1216	1
г						1	Ovis aries	9971	1	3	Homo sapiens-3	2965	2
11 aDNA 1	11 aDNA taxa in Lake La Thuile			ols»	2	Felis catus	10099	1					
			Tanc	(43 reads))	3	Sus	16642	2	23	Homo sapiens-23	40351	11
						4	Sus scrofa	21847	2	24	Homo sapiens-24	58319	12
Г]		5	Sus scrofa	39721	2	25	Homo sapiens-25	720492	201
12. Fina taxa in L scientific n names	al list of Lake La umber of reads	f aDNA Thuile number of PCR >5 reads	Uniq	ue sequences scientific nur names	dete mber o read:	of r	ed in 1 sa number of PCR >5 reads	mple					

Bos

53060

8

Capra hircus

3 Cervus elaphus

2

2677

Supplementary figure 6. Information on aDNA mammal datasets and flow chart of the filtering steps. Steps 1 to 8, were realised using the OBITOOLS software (http://www.grenoble.prabi.fr/trac/OBITools) (Boyer et al., 2016). The evolution of the number of reads and unique sequences at each step is presented, when it was calculated. For Lake La Thuile, we only present the results of the filtering process from the first sequencing run. Another run of sequencing was performed with a higher number of PCR replicates (12) in order to increase the detection probability of mammals and attest the presence of cattle (only detected in one over four replicates in the first run). The second run improved the detection of cattle and also allowed to detect sheep (see manuscript).

285

286 **2.2.** Impact of the filtering procedure on the main results of the study

287 In order to assess the impact of the filtering steps (post-obitools treatment, from step 9 in 288 supplementary figures 3) on the results of the study for terrestrial plants, the numbers of reads, of 289 MOTU, and the log-transformed number of reads normalised by the dry weight of sediments 290 (mean and standard deviation), are presented before and after the application of the filtering 291 procedure for both lakes, La Thuile and Muzelle (Supplementary figures 4 and 5, respectively). The loss in percentages of DNA reads during the steps of the filtering process are also presented. 292 293 The percentage of reads lost by the removal of MOTU considered as contaminants tends to be 294 higher in phases in which lower total number of reads were detected (before the filtering step 9), 295 except between 2600 and 3800 cal. BP at Lake La Thuile (blue area in the supplementary figure 296 4). As previously stated, this trend probably reflects the preferential amplification of contaminants 297 in poor DNA samples. This hypothesis is also valid for the phase between 2600 and 3800 cal. BP 298 at Lake La Thuile, but as probably almost no aDNA fragments were extracted, the contaminants 299 were "over amplified" during the PCR, which leads to the very high number of reads obtained before the application of the filtering procedure. The patterns of the number of reads in the final 300 301 datasets are thus only accentuated relative to the patterns before the filtering, except between 2600 and 3800 cal. BP at Lake La Thuile, when the highest numbers of DNA reads reach values close 302 303 to zero after the filtering steps. Regarding the number of MOTU, the temporal patterns before and 304 after the filtering are the same. The patterns are also retained (or slightly accentuated between 305 2600 and 3800 cal. BP at Lake La Thuile) for the log-transformed number of reads normalised by 306 the dry weight of sediments, i.e. for the proxy of the DNA concentration in the samples 307 (Supplementary figures 4 and 5). Consequently, whether a filtering procedure is applied or not, it 308 has no impact on the conclusions of our manuscript. If we apply a more stringent filtering, i.e. 309 removing all the taxa detected in the negative controls, it does not change the patterns either. 310

- 510
- 311

312 **3.** Metabarcoding data and DNA quantity

313 The goal of DNA metabarcoding is to identify taxa present in environmental samples¹⁷. DNA 314 metabarcoding analyses provide numbers of reads of the different taxa detected in a set of 315 environmental samples. Usually, this number of reads is not considered as representative of a DNA 316 quantity but only used to determine the presence/absence of taxa. To increase the detection probability and help detect the presence of false positives, multiple replicates can be used^{18,19}. The 317 318 number of positive replicates has also been proposed elsewhere as a proxy for the DNA quantity in a sample (e.g. in studies of lake sediment DNA^{20,21} and environmental DNA¹⁹). This proxy was 319 considered as a "more conservative interpretation more appropriate for aDNA" than the number 320 321 of reads²⁰. However, in a previous study on environmental DNA (plant DNA) from old terraces 322 representing a chronosequence of crop abandonments (French Alps), the number of reads was also used as a quantitative variable²². In fact, they showed a negative relationship between the number 323 324 of reads (log(x+1)) of a specific crop plant and the number of years since the abandonment of this 325 crop. This result was interpreted as reflecting the DNA degradation in soils, and thus implicitly 326 means the number of reads was considered as representing a DNA quantity present in the soil. 327 They also showed correlations between the aboveground plant biomass and the abundance in DNA 328 reads detected in the soil. Another study on environmental DNA from lake water also showed 329 some correlations between fish biomass and the number of fish DNA reads²³. Regarding the lake 330 sediment DNA, a study comparing the number of positive replicates, the number of reads, pollen 331 data and historical maps also highlighted the potential of the number of DNA reads as a "semi-332 quantitative" proxy, i.e. giving a magnitude of the DNA quantity in a sample compared to another one²⁴. In the present study, we also propose that the number of reads can be used as a proxy for 333 334 DNA quantity, to compare between samples (analysed in the same run of sequencing). Indeed, for 335 plants at lakes La Thuile, Muzelle and Serre de l'Homme, we found a relationship between the 336 log-transformed mean number of reads (log(N reads+1)) and the number of replicates (r = 0.78, p < 0.0001 for Muzelle; r = 0.81, p < 0.0001 for La Thuile, r = 0.74, p < 0.0001 for Serre de l'Homme; 337 338 Supplementary figure 7). A significant positive correlation is also observed for mammals detected 339 in Lake La Thuile (r = 0.83, p<0.0001; Supplementary figure 7). However, for mammals at Lake 340 La Thuile and plants at Lake Serre de l'Homme, we note a wide range of read numbers when DNA is detected in only one or two replicates. This might reflect the stochasticity of DNA amplification 341 342 when the amount of DNA is very low. Given the high correlation coefficient between the number 343 of positive replicates and the mean number of reads, we propose to use the mean number of reads

- between a reasonable number of replicates, even if it is probably less robust than the number of replicates and means cautions should be taken for the interpretations.
- 346
- 347



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Supplementary figure 7. Comparison between the means numbers of reads and the number of positive replicates (>20 reads). On the left, bi-plot for plants in lakes La Thuile (including aquatic and terrestrial plants, 50 samples) and La Muzelle (30 samples). In the middle, bi-plot for mammals in Lake La Thuile (33 samples). On the right, bi-plot for plants in Lake Serre de l'Homme (including terrestrial and aquatic plants, 40 samples).

4. Complementary analysis and data on Lake La Thuile

355 4.1. PCA analysis of terrestrial plant DNA, sedimentological and geochemical data

356 In order to synthesise the results from the comparison of the terrestrial plant DNA concentration and richness with the sedimentological and geochemical data, we perform a PCA analysis 357 358 (Supplementary Figure 8). For this purpose, we have to resample the XRF core scanner data (Si/Ti 359 and Ca/Ti used as proxies of the aquatic production) and interpolate the data from the Rock-Eval 360 Pyrolysis (Hydrogen and Oxygen Indexes used as proxies of a mixture between lacustrine and 361 litter organic matters and of organic matter from deep soil horizons, respectively). This is because 362 they were not measured at the same depths as the DNA. Despite the interpolation of some data, 363 the PCA analysis confirms our interpretations presented in the main text. A first end-member is 364 strongly correlated with the positive side of the first component. It includes the organic matter 365 (LOI 550°C) mostly derived from the litter (Hydrogen Index, HI and presence of leaves in the sediments) and the DNA concentration. This end-member confirms the role of the biomass 366 367 production and of the source of eroded materials in the DNA concentration. The second end-368 member is strongly correlated with the negative side of the first component. It represents the

369 detrital inputs (LOI residue and total sediment flux), i.e. the erosion and is also correlated with 370 terrestrial plant DNA richness. This correlation can be interpreted as follows; 1) as representing 371 the more efficient terrestrial plant DNA transfer triggered by, the higher erosion (increasing the "catchment connectivity") and/or, 2) as the result of the landscape opening which increases plant 372 373 diversity. We propose that these two processes occur because the richness in the pollen data also 374 increases; however, this increase is lower than in the DNA data. The last end-member is correlated 375 with the positive side of the second component. This end-member represents the aquatic 376 production (Si/Ti and Ca/Ti) and basic/neutral conditions (LOI 950°). The seven phases discussed in the main text are well discriminated by the PCA analysis. Phase (b), characterised by a high OI 377 378 (Oxygen Index), provides evidence that deep soil horizons have poor DNA concentration, as the 379 DNA concentration and OI are negatively correlated. Phase (d), for which most samples are 380 characterised by a low DNA concentration, is negatively correlated with the second component 381 and positively correlated with the organic matter content. This pattern probably reflects the role of 382 acid conditions and/or of humic substances on the DNA preservation and/or our analytical 383 capacity.

384



Supplementary figure 8. PCA analysis of the terrestrial plant DNA concentration and richness and the sedimentological and geochemical data from Lake La Thuile sediments. The first and second components represent 50.9 % and 25.7 % of the variance. HI is the Hydrogen Index (mg HC/g TOC) and OI, the Oxygen Index

- 389 (mg O₂/g TOC) of the organic matter. High HI values corresponds to a mixture between lacustrine and litter organic
- 390 matters and high OI values show the presence of organic matter from deep soil horizons. Si/Ti and Ca/Ti are used as
- 391 proxies of biogenic silica and bio-induced calcite, respectively. LOI residue and the total sediment flux are proxies of
- 392 the erosion. LOI 550°C and LOI 950°C represent the contents in organic matter and carbonates, respectively. The
- 393 samples from the different phases (a, b, c, d, e, f, g) used in the study (determined from the changes in the terrestrial
- 394 plant DNA concentration) are presented in different colours.
- 395
- **4.2.** Comparisons between DNA and pollen
- 397
- 398





400 Supplementary figures 9. Comparison between shared pollen (grey curves) and plant DNA taxa (black curves)

401 from Lake La Thuile sediments. Taxa possibly shared, i.e. with different taxonomic resolution like *Juglans* (pollen)
 402 and *Juglandaceae* (aDNA), are also presented.



404 Supplementary figures 9 (next).



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