**Supplementary Material for the article:** *Manuscript Changes in ciliate communities reveal modification of lake functioning over the last century*

**Methods Sections**

At every steps of the analysis of paleoenvironmental DNA, strict laboratory protocols and various methodological precautions were taken to achieve robust results and ensure the validity of our molecular data:

* Sterile disposable materials (labware, gloves, etc.) were used for all lab procedures; during sediment core subsampling, sediment slices were taken using sterilised metal plates.
* Separate stations were organized for subsampling, DNA extractions and PCR amplifications. To prevent contamination with modern DNA, the extraction of DNA from sediment is carried out in specific rooms dedicated to rare DNA, and PCR are prepared under designated working stations. These laboratories are physically separated from other molecular biology laboratories.
* Negative controls are included at 3 steps of the procedure: during subsampling (open tubes containing pure water), extraction (treatment of pure water) and PCR preparation (blank PCR tube). All blanks were found negative for the amplification of 18S rRNA markers; consequently, the blanks were not included in the sequencing library.
* We selected short barcodes adapted to the work on sedimentary DNA. The choice of the barcode region was previously explained in Capo et al1 based on verifications of the coverage of primers for micro-eukaryotic diversity, the ability to amplify the DNA with these primers, and the quality of taxonomic assignment obtained, the probes 960F and NSR1438 were selected.
* To evaluate the potential influence of co-extracted inhibitors present in sedimentary DNA extracts (that may reduce the efficiency of downstream PCR), we assessed the inhibition level using quantitative PCR assays. The approach applied is based on the assumption that inhibitors are diluted out when a log-linear relationship is achieved between Cq and the dilution factor2. No inhibition effect was found.
* We performed a duplicate extraction for each strata and verified the similarity of DNA results obtained for the replicates of a given strata (see Keck et al. Supplemental Material).
* In compacted lake sediments, vertical advection of pore water is minimal, and multivalent metals and organic compounds (pigments, organic molecules with more than 15 carbon atoms) are immobilized in the sediment matrix3. Large organic molecules such as DNA are likely to adhere to solid-phase sediments (particles, particulate organic matter) or are locked in dead cells or ancient dormant resting cells. Therefore, leaching of DNA is unlikely to occur in lake sediment and lake sedimentary DNA is assumed to give an accurate temporal reconstruction of the biological community succession3,4. The level of DNA preservation in the sediment (from one lake to another or when aging in sediment) is a sensitive point to be taken into account for paleo-reconstruction studies. Given the mechanisms of DNA protection by binding to mineral and organic particles and due to the absence of oxygen and UV radiation, aquatic sediments are, a priori, suitable environments for DNA preservation5,6. However, several processes can alter DNA sequences in marine7 and freshwater sediments8. It was thus important to consider whether the differences observed between top and bottom strata could be induced by diagenetic processes responsible for the modification of DNA signal over time. Though shotgun sequencing allows to differentiate ancient DNA that has been damaged (typical damage patterns of ancient DNA marked by increase in T and A at the ends of DNA fragments), the limited number of samples that can be treated in parallel and the associated cost per sample still limit routine application when a large number of samples are to be treated (as here with 96 × 2 samples). The potential distortions to lake sediment DNA records due to taphonomic processes (production, transfer, preservation of DNA) that affect DNA in sediments are not fully known; we know however that:
	+ At sites with favourable DNA preservation conditions like in lake sediments, the DNA signal is proven to be reliable for several centuries. The signal can be preserved for several millennia if the preservation conditions are very good9.
	+ The first few years after deposition are critical for DNA preservation due to the biological activity at the sediment interface and the physical and chemical changes that occur in the uppermost sediment layers1. Consequently, we chose to avoid the very recent deposits (for the sampling of modern periods) to overcome this issue; the top samples were sampled a few centimeters below the sediment surface (~year 2000).
* Different levels of taxonomy were considered throughout the data analysis in order to circumvent the potential risk associated with the use of OTUs (artifact increase of OTUs number, or loss of OTUs due to degradation/fragmentation). The choice of thresholds for the delineation of OTUs is critical, with potential risk of inflation of rare OTUs or, inversely, of lumping together OTUs with different distribution patterns. Universal thresholds also do not consider differences in substitution rates among lineages and may therefore not capture equivalent units of diversity.
* In the present study, the top strata were selected to be a few centimeters down the surface of the core in order to minimize bias associated with early diagenesis processes and living taxa. However, the presence of 59 OTUs specific to the top strata could either be related to the changes in environmental conditions in recent time or be an artifact associated with taxa living in the subsurface layers of the sediment. Importantly, the number of OTUs specific to the top strata remains low (~2% of the total OTUs) and the increase of *Metopus* genera, a ciliate associated with anoxic conditions, in the top strata supports that the observed changes likely track changes in the community composition over time. Nonetheless, a better understanding of post-depositional survival and activity of microorganisms is still required, and becomes even more important when using the top-bottom paleolimnological technique in order to insure the authenticity of the sed-DNA molecular signal as an archive of past environmental conditions10.
* It is important to note that although the set of ciliate-specific primers used (CS322F and 1147R) are known to be highly specific and resolutive, the amplification of a long DNA fragment (i.e. 800 bp) is not optimal when working on ancient DNA. As such, future studies might consider a different set of ciliate-specific primers which would target a shorter region more suitable for sed-DNA studies11.
* Molecular studies of protist communities present some challenges related to the lack of a robust reference database for ciliates12. More robust reference database would improve functional traits affiliation which would allow for a better understanding of the functional ecology of ciliates and associated studied of lake ecosystem functioning.
* The high variability in the number of copies of a gene can also present another challenge as it can result in the overrepresentation of some species that contains numerous copies of the gene13. However, for the few ciliates for which we have information about their number of copies per cells there was no clear relationship between the number of reads and the number of copies of gene per cells (Supplemental Fig. S4). Moreover, analytical methods such as the DeSeq2 analysis applied on our comparative top-bottom approach reduce interpretation errors related to these potential biases14.

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**Table S1a:** Summary of the effect of the bioinformatics steps on the DNA reads per sample. Filtering code (1) raw data obtained from the sequencing plateform, (2) conserve DNA sequences of 350±50 bp in length, with no ambiguities (N=0), 10 or less homopolymer (max homopolymer=10), (3) conserve DNA sequences with primers (no mismatch was allowed in the primer sequence), (4) ISUs aligned using an aligned version of the Silva 18S database restrained to the V7 region, removal of ISUs that were not fully aligned to the Silva 18S barcode, (5) Removal of Chimera, (6) taxonomic assignment of the ISU, and (7) removal ISU represented with only one read or that were identified as “unknown” or “Eukaryota\_unclassified”. The Final column corresponds to the final number of reads obtained after OTU clustering using the furthest neighbor approach with a similarity threshold of 97%.

| **Filtering code** | **1** | **2** | **3** | **4** | **5** | **6** | **7** |   |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample\_ID** | **Contigs** | **Trim****length\_homop\_N** | **Trim****primer** | **Trim****Alignment** | **Trim****Chimera** | **Trim****tax** | **Trim****ISU>1** | **Final**  |
| **ABB\_B** | 32791 | 32768 | 29622 | 29555 | 29388 | 29381 | 20827 | 20827 |
| **ABB\_T** | 31861 | 31835 | 29198 | 29016 | 27994 | 27991 | 20817 | 20817 |
| **AIG\_B** | 23743 | 23716 | 21009 | 20872 | 20858 | 20840 | 16319 | 16315 |
| **AIG\_T** | 25576 | 25550 | 22907 | 22785 | 22322 | 22308 | 16349 | 16349 |
| **ALA\_B** | 18905 | 18894 | 16552 | 16500 | 16472 | 16471 | 11087 | 11087 |
| **ALA\_T** | 25684 | 25662 | 23206 | 23149 | 23075 | 23075 | 15149 | 15149 |
| **ARA\_B** | 26113 | 26071 | 22740 | 22636 | 22096 | 22095 | 16729 | 16729 |
| **ARA\_T** | 30682 | 30647 | 27609 | 27515 | 26455 | 26455 | 18960 | 18960 |
| **AYD\_B** | 37525 | 37490 | 34131 | 34048 | 33669 | 33665 | 25618 | 25618 |
| **AYD\_T** | 41700 | 41655 | 37913 | 37146 | 34746 | 34731 | 25419 | 25419 |
| **AYE\_B** | 24459 | 24447 | 21933 | 21900 | 21406 | 21406 | 14921 | 14921 |
| **AYE\_T** | 25970 | 25952 | 23678 | 23619 | 22775 | 22775 | 16077 | 16077 |
| **BAR\_B** | 32700 | 32604 | 26234 | 25989 | 25955 | 25911 | 20372 | 20372 |
| **BAR\_T** | 20525 | 20515 | 18587 | 18386 | 17796 | 17791 | 12142 | 12142 |
| **BARR\_B** | 27241 | 27222 | 24693 | 24628 | 24570 | 24226 | 16646 | 16646 |
| **BARR\_T** | 17787 | 17780 | 16188 | 16159 | 16131 | 16131 | 11947 | 11945 |
| **BES\_B** | 27130 | 27117 | 24869 | 24831 | 24350 | 24349 | 16453 | 16453 |
| **BES\_T** | 17272 | 17254 | 15599 | 15188 | 14646 | 14644 | 9510 | 9510 |
| **BLAC\_B** | 44998 | 44964 | 40729 | 40633 | 39364 | 39364 | 28729 | 28729 |
| **BLAC\_T** | 30069 | 30030 | 27020 | 26577 | 24945 | 24942 | 17960 | 17960 |
| **BON\_B** | 19598 | 19573 | 17741 | 17527 | 17462 | 17412 | 12900 | 12900 |
| **BON\_T** | 38813 | 38791 | 35711 | 35593 | 34818 | 34818 | 25822 | 25822 |
| **BOR\_B** | 23568 | 23555 | 21570 | 21542 | 21486 | 21485 | 17195 | 17195 |
| **BOR\_T** | 20319 | 20301 | 18324 | 18271 | 17992 | 17992 | 14058 | 14058 |
| **BOUR\_B** | 26926 | 26912 | 24603 | 24492 | 24486 | 24472 | 20502 | 20502 |
| **BOUR\_T** | 44554 | 44529 | 40417 | 39039 | 37663 | 37658 | 28434 | 28434 |
| **CHA\_B** | 29127 | 29104 | 26549 | 26498 | 26475 | 26475 | 21528 | 21528 |
| **CHA\_T** | 37443 | 37411 | 33999 | 33864 | 32964 | 32962 | 24208 | 24208 |
| **CHE\_B** | 33782 | 33756 | 30189 | 30129 | 28904 | 28848 | 19326 | 19324 |
| **CHE\_T** | 25315 | 25295 | 22671 | 22617 | 22219 | 22183 | 16748 | 16748 |
| **COR\_B** | 38132 | 38111 | 34800 | 34748 | 32022 | 32011 | 23333 | 23333 |
| **COR\_T** | 32550 | 32536 | 29913 | 29804 | 28480 | 28480 | 20190 | 20190 |
| **CRE\_B** | 24472 | 24462 | 22352 | 22313 | 20099 | 20097 | 13925 | 13925 |
| **CRE\_T** | 29874 | 29837 | 27087 | 26171 | 23394 | 23389 | 16842 | 16842 |
| **ESP\_B** | 31863 | 31831 | 28945 | 28842 | 28064 | 28064 | 19627 | 19624 |
| **ESP\_T** | 32238 | 32219 | 29378 | 29265 | 28458 | 28453 | 19155 | 19155 |
| **ETI\_B** | 13157 | 13138 | 11657 | 11173 | 11168 | 10661 | 8785 | 8785 |
| **ETI\_T** | 29965 | 29941 | 27232 | 27153 | 26713 | 26712 | 19473 | 19473 |
| **GEN\_B** | 35088 | 35062 | 32136 | 31998 | 30073 | 30073 | 22675 | 22673 |
| **GEN\_T** | 35280 | 35252 | 31584 | 31353 | 28337 | 28337 | 21815 | 21815 |
| **GER\_B** | 36483 | 36440 | 33084 | 33018 | 31926 | 31924 | 24362 | 24362 |
| **GER\_T** | 24057 | 24040 | 22016 | 20119 | 18631 | 18631 | 13532 | 13532 |
| **GOD\_B** | 28919 | 28812 | 23738 | 23697 | 22845 | 22845 | 16949 | 16949 |
| **GOD\_T** | 25504 | 25476 | 22170 | 22148 | 22135 | 22135 | 17107 | 17107 |
| **GOU\_B** | 24522 | 24501 | 21746 | 21695 | 21617 | 21599 | 15550 | 15550 |
| **GOU\_T** | 28835 | 28822 | 26479 | 26380 | 25551 | 25547 | 18715 | 18715 |
| **GUE\_B** | 39150 | 39104 | 32940 | 32805 | 32393 | 32391 | 24929 | 24929 |
| **GUE\_T** | 15706 | 15681 | 13783 | 13679 | 12626 | 12626 | 9037 | 9037 |
| **ILA\_B** | 21376 | 21356 | 18935 | 18919 | 18785 | 18785 | 13824 | 13824 |
| **ILA\_T** | 20210 | 20198 | 18588 | 18506 | 17282 | 17279 | 12525 | 12525 |
| **ISA\_B** | 40458 | 40387 | 35698 | 35555 | 35031 | 35030 | 27756 | 27756 |
| **ISA\_T** | 23029 | 23013 | 20800 | 20682 | 19834 | 19834 | 14277 | 14277 |
| **LAG\_B** | 22377 | 22360 | 20331 | 20289 | 20203 | 20202 | 15328 | 15328 |
| **LAG\_T** | 42294 | 42269 | 38125 | 37982 | 35856 | 35856 | 26161 | 26161 |
| **LAM\_B** | 42929 | 42879 | 38936 | 38863 | 37615 | 37615 | 26347 | 26347 |
| **LAM\_T** | 21474 | 21446 | 19155 | 18925 | 18083 | 18078 | 12484 | 12484 |
| **LAN\_B** | 34345 | 34325 | 30921 | 30848 | 29449 | 29449 | 20328 | 20328 |
| **LAN\_T** | 24805 | 24783 | 22539 | 22350 | 20576 | 20573 | 15320 | 15320 |
| **LEM\_B** | 22926 | 22906 | 20640 | 20613 | 20609 | 20609 | 17155 | 17155 |
| **LEM\_T** | 33926 | 33873 | 29554 | 29309 | 28095 | 28093 | 21740 | 21740 |
| **LON\_B** | 25589 | 25578 | 23143 | 23009 | 22764 | 22762 | 15399 | 15399 |
| **LON\_T** | 26738 | 26724 | 24594 | 24432 | 23227 | 23210 | 16800 | 16800 |
| **MAI\_B** | 24442 | 24414 | 21442 | 21389 | 21293 | 21293 | 14537 | 14537 |
| **MAI\_T** | 17318 | 17309 | 15519 | 15467 | 15041 | 15038 | 9664 | 9664 |
| **MAR\_B** | 48321 | 48278 | 43800 | 43623 | 41680 | 41620 | 32126 | 32126 |
| **MAR\_D** | 30125 | 30112 | 27600 | 27543 | 27059 | 27059 | 18813 | 18813 |
| **MAR\_T** | 27336 | 27282 | 23737 | 23622 | 22603 | 22601 | 16795 | 16795 |
| **MOU\_B** | 38355 | 38305 | 34143 | 34077 | 33928 | 33869 | 25620 | 25620 |
| **MOU\_T** | 17129 | 17094 | 15269 | 15104 | 14076 | 14076 | 9999 | 9999 |
| **MTC\_B** | 24874 | 24857 | 21735 | 21665 | 21366 | 21364 | 14993 | 14993 |
| **MTC\_T** | 26554 | 26543 | 24301 | 24199 | 23072 | 23070 | 17210 | 17210 |
| **NAN\_B** | 51480 | 51449 | 47115 | 46975 | 46863 | 46839 | 38312 | 38312 |
| **NAN\_T** | 16929 | 16911 | 15223 | 14332 | 13593 | 13593 | 9842 | 9842 |
| **PAR\_B** | 48770 | 48706 | 43414 | 43372 | 43128 | 43126 | 33337 | 33337 |
| **PAR\_T** | 27870 | 27853 | 25451 | 24999 | 24109 | 24109 | 15961 | 15961 |
| **PEY\_B** | 42136 | 42104 | 37801 | 37557 | 36065 | 36064 | 29130 | 29130 |
| **PEY\_T** | 37270 | 37129 | 29381 | 29215 | 27188 | 27188 | 20764 | 20764 |
| **POR\_B** | 42691 | 42619 | 39115 | 39005 | 38468 | 38468 | 30898 | 30898 |
| **POR\_T** | 35385 | 35356 | 32382 | 32315 | 31804 | 31801 | 24469 | 24467 |
| **ROU\_B** | 30732 | 30710 | 28002 | 27964 | 26631 | 26631 | 18243 | 18243 |
| **ROU\_T** | 26952 | 26916 | 24473 | 23545 | 22833 | 22832 | 16646 | 16646 |
| **ROUM\_B** | 32782 | 32754 | 29649 | 29588 | 28963 | 28957 | 21399 | 21399 |
| **ROUM\_T** | 14109 | 14006 | 9203 | 9178 | 8693 | 8692 | 5821 | 5821 |
| **SAI\_B** | 27519 | 27503 | 25062 | 24605 | 24490 | 24490 | 18906 | 18906 |
| **SAI\_T** | 13604 | 13585 | 12266 | 11739 | 11021 | 11021 | 8213 | 8213 |
| **SEV\_B** | 40381 | 40339 | 35517 | 35426 | 33335 | 33325 | 23383 | 23383 |
| **SEV\_T** | 10530 | 10501 | 9030 | 8993 | 8977 | 8976 | 5790 | 5790 |
| **SOUC\_B** | 24161 | 24147 | 22114 | 22056 | 21265 | 21265 | 15823 | 15823 |
| **SOUC\_T** | 19759 | 19717 | 17194 | 17157 | 16709 | 16708 | 12377 | 12377 |
| **VAL\_B** | 14168 | 14145 | 12307 | 12212 | 12203 | 12168 | 9665 | 9665 |
| **VAL\_T** | 18145 | 18129 | 16368 | 16252 | 15268 | 15266 | 10653 | 10653 |
| **VALL\_B** | 32212 | 32199 | 29646 | 29548 | 29190 | 29188 | 23101 | 23101 |
| **VALL\_T** | 13506 | 13484 | 12200 | 12148 | 11813 | 11813 | 9252 | 9252 |
| **VER\_B** | 34985 | 34958 | 31315 | 31242 | 30443 | 30443 | 22147 | 22147 |
| **VER\_D** | 31295 | 31258 | 27911 | 27867 | 26439 | 26439 | 18844 | 18844 |
| **VER\_T** | 29516 | 29470 | 25749 | 25666 | 23814 | 23814 | 17350 | 17350 |
| **VERT\_B** | 23939 | 23923 | 21897 | 21657 | 21465 | 21395 | 15673 | 15671 |
| **VERT\_T** | 22012 | 21985 | 19848 | 19814 | 19744 | 19742 | 13253 | 13253 |

**Table S2:** Summary of the total number of OTUs and Reads taxonomically assigned or assigned to a functional trait.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **OTU** | **Equivalent Percentage OTU**  | **Number of Reads** | **Equivalent Percentage****Number of Reads** |
| **Taxonomic Rank** |  |  |  |  |
| **Kingdom** | 2446 | 100 | 1,745,549 | 100 |
| **Class** | 2410 | 99 | 1,741,121 | 99.7 |
| **Subclass** | 1622 | 66 | 1,525,556 | 87 |
| **Order** | 1256 | 51 | 1,282,246 | 73 |
| **Family** | 1126 | 46 | 943,172 | 54 |
| **Genus** | 660 | 27 | 722,244 | 41 |
| **Species** | 523 | 21 | 392,650 | 22 |
|  |  |  |  |  |
| **Functional Traits** |
| **Foraging Traits** | 1135 | 46 | 1,105,563 | 63 |
| **Limnetic Habitat** | 1234 | 50 | 792,622 | 45 |

**Table S3:** Summary of known physical characteristics and trophic status of the 48 studied lakes (Zmax=Maximum Depth, SA= Surface Area).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Lake Name** | **Lake Code** | **Elevation****(m)** | **Zmax****(m)** | **SA****(m2)** | **Watershed Area (m2)** | **Trophic Status** |
| Abbaye  | ABB | 910  | 19.5  | 82  | 245.8 | MESOTROPHIC  |
| Aiguebelette  | AIG | 374  | 71  | 545  | 5306.2  | OLIGO-MESOTROPHIC  |
| Alate  | ALA | 1868  | 10  | 2  | UNKNOWN  | ULTRA-OLIGOTROPHIC |
| Arratille  | ARA | 2247  | 12  | 16  | 329.9 | OLIGOTROPHIC  |
| Aydat  | AYD | 825  | 15  | 60.3  | 2551.1 | EUTROPHIC  |
| Ayes  | AYE | 1694  | 10  | 1.7  | UNKNOWN  | OLIGROTROPHIC  |
| Balcere  | BAL | 1765  | 14  | 4.5  | UNKNOWN  | MESOTROPHIC |
| Barroude  | BARR | 2355  | 9  | 9.4  | 484 | UNKNOWN |
| Barterand  | BAR | 295  | 15  | 21  | 793.1 | MESOTROPHIC  |
| Besse  | BESS | 245  | 10  | 2  | 163.3 | UNKNOWN  |
| Blanchemer  | BLA | 984  | 15  | 9  | 208.9 | UNKNOWN |
| Borderes  | BOR | 1765  | 18  | 6.5  | UNKNOWN  | OLIGOTROPHIC  |
| Bourget  | BOUR | 231  | 147  | 4396  | 57408 | OLIGO-MESOTROPHIC  |
| Chalain  | CHA | 490  | 32  | 232  | 3468.4  | MESOTROPHIC  |
| Cheserys  | CHE | 2135  | 6  | 0.4  | 35.6  | OLIGOTROPHIC  |
| Corbeaux  | COR | 887  | 27  | 10  | 91.8 | UNKNOWN  |
| Cregut  | CRE | 900  | 26  | 35.5  | 8999.7  | EUTROPHIC  |
| Espingo  | ESP | 1882  | 8  | 7.6  | UNKNOWN  | OLIGOTROPHIC |
| Etival  | ETI | 795  | 10  | 15  | 391.5  | MESOTROPHIC  |
| Gentau  | GEN | 1950  | 20  | 9.3  | 205 | MESOTROPHIC |
| Gerardmer  | GER | 660  | 38  | 116  | 1365  | MESOTROPHIC  |
| Godivelle  | GOD | 1239  | 44  | 13.8  | 12.3  | OLIGOTROPHIC  |
| Gour de Tazenat  | GOU | 630  | 66  | 32.9  | 102.8  | OLIGO-MESOTROPHIC |
| Guery  | GUE | 1246  | 20  | 26.8  | 790.1  | MESO-EUTROPHIC |
| Ilay  | ILA | 778  | 32  | 72  | 165.7  | MESO-EUTROPHIC  |
| Isaby  | ISA | 1562  | 6  | 6.3  | 755.8  | OLIGOTROPHIC  |
| Lagardelle  | LAG | 2387  | 27  | 5.8  | UNKNOWN  | OLIGOTROPHIC |
| Lamoura  | LAM | 1156  | 9  | 3.5  | 1064.8  | UNKNOWN  |
| Landie  | LAN | 1000  | 21  | 23.9  | 298.4  | MESO-EUTROPHIC  |
| Leman  | LEM | 372  | 309  | 58100  | 739500  | MESOTROPHIC  |
| Longemer  | LON | 736  | 34  | 76  | 665.2  | MESOTROPHIC  |
| Maix  | MAI | 678  | 15  | 1.5  | 17.2  | UNKNOWN  |
| Marion  | MAR | 50  | 22.8  | 3.8  | 46.3  | UNKNOWN  |
| Mont Coua  | MTC | 2797  | 10  | 2.43  | 73.5  | OLIGOTROPHIC  |
| Mouriscot  | MOU | 21  | 10  | 23  | 119  | EUTROPHIC  |
| Nantua  | NAN | 475  | 43  | 141  | 1639.8  | MESOTROPHIC  |
| Parentis  | PAR | 19  | 20  | 3502  | 53739.1  | EUTROPHIC  |
| Peyrelade  | PEY | 1919  | 28  | 9.7  | UNKNOWN  | MESOTROPHIC  |
| Port Bielh | POR | 2313 | 19 | 16.4 | 234.5 | OLIGOTROPHIC |
| Remoray | REM | 850 | 27 | 85 | 2486.4 | UNKNOWN |
| Roumazet | ROUM | 2163 | 10 | 1.8 | 22.4 | OLIGOTROPHIC |
| Rousses | ROS | 1059 | 18 | 90 | 2143.7 | OLIGO-MESOTROPHIC |
| Saint-Point | SAI | 850 | 42 | 398 | 21598.2 | MESO-EUTROPHIC |
| Serviere | SER | 1200 | 29 | 16.2 | 49.43 | MESOTROPHIC |
| Soucarrane | SOU | 2291 | 10 | 4.4 | 100.57 | OLIGOTROPHIC |
| Val | VAL | 520 | 25 | 64 | 2038.6 | MESOTROPHIC |
| Verdet | VER | 2736 | 12 | 1.9 | 56.2 | OLIGOTROPHIC |
| Vert | VERT | 1266 | 9 | 1.4 | 176.3 | NA |



**Figure S1:** NMDS of community compositions of the recent (black dots) and past (purple dots) samples with 95% confidence ellipses represented for each group based on the Bray-Curtis distances of the OTU table. Only the past samples are labelled with their corresponding lake code (cf. Table S1), the gray lines connect recent and past samples from the same lake. Note: some labels are missing to avoid overlapping labels.



**Figure S2:** Amplitude of change of ciliates community applied at the Genus level between the past and recent strata. Magnitude of change is expressed in log2 fold change, as estimated by the DESeq2 analysis (n= 48 lakes). Only the Genus for which the amplitude of change was significant are presented (two-sided Wald test corrected with the Benjamini and Hochberg method p-value < 0.05). Horizontal lines show the standard error. Any Genus terminating with one “\_NA”, two “\_NA\_NA”, or three “\_NA\_NA\_NA”, corresponds to an assignment at the Familly, Order or Class, respectively (i.e. OTUs for which the assignment stopped at the Class level the pattern “\_NA” is repeated three times corresponding to an unknown Order, an unknown Family, and an unknown Genus).



**Figure S3:** Distribution of the elevation gradient among the lake Trophic Status. The dotted line indicates the elevation of 1400 m corresponding to the split identified by the univariate regression tree analysis applied on the Bray-Curtis dissimilarity matrix. The elevation was significantly higher for the oligotrophic lakes than the other trophic status categories (Kruskal-Wallis test: χ2=27, df=5, p < 0.05; Post Hoc Wilcoxon-rank test *pajusted* <0.05 for Oligotrophic Lake against all other Trophic Status categories; for the pairwise comparison the *p* values were adjusted using the False Discovery Rate approach by Benjamini and Hochberg1).

(1) Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society: Series B (Methodological) 57:289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x



**Figure S4:** Relationship between the number of 18S gene copies as estimated by Gong et al.1 and Gong and Marchetti2 and the total number of reads all samples included for the Hypotrichia, Scuticociliatia, Oligohymenomorphorea, Prorodontida and Strombidida.

(1) Gong J, Dong J, Liu X, Massana R (2013) Extremely High Copy Numbers and Polymorphisms of the rDNA Operon Estimated from Single Cell Analysis of Oligotrich and Peritrich Ciliates. Protist 164:369–379. https://doi.org/10.1016/j.protis.2012.11.006

(2) Gong W, Marchetti A (2019) Estimation of 18S Gene Copy Number in Marine Eukaryotic Plankton Using a Next-Generation Sequencing Approach. Front Mar Sci 6:219. https://doi.org/10.3389/fmars.2019.00219