Science Advances

Supplementary Materials for

Tibetan terrestrial and aquatic ecosystems collapsed with cryosphere loss inferred from sedimentary ancient metagenomics

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The PDF file includes:

Supplementary Text Figs. S1 to S16 Tables S1 to S6 Legends for data S1 to S3 References

Other Supplementary Material for this manuscript includes the following:

Data S1 to S3

Supplementary Text

Modern and late Pleistocene/Holocene site setting

The modern climate conditions are indicated by mean annual temperature (MAT) of 6.6°C, mean July temperature (MJT) of 14.3°C, mean January temperature of -3.9°C, and over 90% of annual rainfall (about 560 mm) occurring in May–October according to the closest meteorological station at Garze (31.62° N, 100.00° E, at 3,522 m a.s.l.) that is located ~ 80 km northeast of Lake Naleng. According to the observed temperature lapse rate (0.55°C 100 m⁻¹) in the Hengduan Mountains (*127*), the MAT and MJT are estimated to be 2.9°C and 10.6°C at the lake, respectively.

We simulated permafrost extent over the past 22,000 years at a resolution of 500 years (Fig. S1) with integration of our published glacier dynamics (45). The modeled glaciers dominated the lake catchment before 15 ka (median 86.4%) and rapidly disappeared by 14 ka with slight advances in the eastern margin at 12.5 ka (0.8% and 0.02 Gt, Fig. S12A, B), which agrees with the clay content (mean grain size $< 2 \mu$ m) of lake sediments (96). Before 14 ka, the permafrost extent accounted for a median value of 10.0% over the lake catchment; that is, permafrost was well developed in the glacier-free areas (median 67.9%, Fig. S12C) and lowland (4100–4500, median 62.6%, Fig. S12D) in particular. In contrast, the simulated permafrost within the lowland extensively shrank at 14 ka (7.5% remaining) and finally disappeared after 12.5 ka. The highland (4500–4900 m a.s.l.) hosted permafrost until 10 ka (Fig. S12E). Despite permafrost being restored, it was simulated in the highland only during the late Holocene (median 9.3%).

Library preparation

All libraries were quantified using quantitative real-time Polymerase chain reaction (qPCR) with 1 μ L diluted library (1:20) (*103*). In total, 24 μ L library and PCR no template controls were processed by PCR amplification and indexing as described in Gansauge and Meyer (*103*) with 11–14 cycles (Table S4) using the index primer sequences P5 (01-06, 11, 15-18, 46, 51, 64, 67, 72) and P7 (20, 21, 26, 27, 29, 40, 57, 60, 65, 82, 88, 91-96). After purification with the MinElute PCR Purification Kit (Qiagen, Germany), the initial quality control of amplified libraries was performed using the 4200 TapeStation system (Agilent, G2991AA). A total of four amplified libraries (ESL028, ESL034, ESL061, and ESL079) having high peak of adapter dimers with ~150 bp were selected to be cut out of an agarose gel (2%) after separation by electrophoresis, and then purified with E.Z.N.A.[®] MicroElute Gel Extraction Kit (Omega Bio-tek[®]). Afterwards, qPCR, indexing PCR, PCR purification, and quality control were performed for these libraries. No unexpected adapter peak was found. Two library blanks, JK125_LB and JK126_LB, without gene bands in TapeStation quality checking were not considered for shotgun sequencing.

Biota of the Tibetan Plateau: a compilation

For seed-bearing plants (phylum: Streptophyta), we downloaded species occurrence data from (i) the Global Biodiversity Information Facility (GBIF, accessed 24.10.2022, source data: (*128*)) with the following filters used: 'Geometry POLYGON ((73.499 24.814,104.672 24.814,104.672 39.826,73.499 39.826,73.499 24.814))', 'HasCoordinate is true', 'HasGeospatialIssue is false', 'TaxonKey is Tracheophyta' (Selecting Tracheophyta to ensure that taxa are not overlooked); (ii) Biodiversity the Hengduan Mountains and adjacent areas of south-central China (accessed

24.10.2022, http://hengduan.huh.harvard.edu/fieldnotes) with information on coordination and elevation; (iii) Flora of China (accessed 24.10.2022, ref. (129)) with a range of elevation; and (iv) publications with information on elevation (130, 131). The species occurrences obtained from GBIF were cropped using a merged polygon of the Tibetan Plateau (132) and the Hengduan Mountains (133) using 'over()' in the 'sp' package (v. 1.5.1, refs. (134, 135)). Elevation information was extracted from the SRTM 30m Digital Elevation Database based on the coordinates of the occurrences using the 'extract()' function in the 'raster' package (v. 3.6.11, ref. (136)). The SRTM 30m data was downloaded using the 'SRTM-Downloader' plugin in QGIS (v. 3.24.1-Tisler, https://www.qgis.org/en/site/, ref. (137)). For species lacking a family name, the family name was obtained using tax_name(db = "ncbi", get = c("family")) in the 'taxize' package (v. 0.9.98, ref. (138)). A family list, including occurrences found at elevations above 3000 m a.s.l. (referred to as high elevation region), was compiled based on these four databases. This list was then passed to the 'get_uid()' in the 'taxize' package (v. 0.9.98, ref. (138) to retrieve the taxonomy ID. As a result, the plant taxa list contains 1,615 genera and 259 families.

For mammals, we compiled the taxa list from the mammals of China (139) and GBIF (accessed 24.10.2022, source data: (140)), using the same filters as those used for vascular plants, except for the filter 'TaxonKey is Mammalia'. After retrieving the taxonomy ID and elevation information, 105 genera and 32 families were obtained. We further excluded bats (Hipposideridae, Vespertilionidae, and Rhinolophidae) and Hominidae and Camelidae.

For freshwater fish, we collected the occurrences for 15 orders of China's freshwater fish (141) from Fishbase hosted by GBIF (accessed 22.02.2023, source data: (142)) with 'TaxonKey is one Clupeiformes, of (Cypriniformes, Salmoniformes, Anguilliformes, Siluriformes, Cyprinodontiformes, Beloniformes, Gadiformes, Gasterosteiformes, Mugiliformes, Synbranchiformes, Perciformes, Scorpaeniformes, Pleuronectiformes, Tetraodontiformes). The inventory of freshwater fish was compiled following the same approach used for plants and mammals, resulting in a total of 134 genera and 62 families.

Permafrost simulation

A number of researches has demonstrated a temperature-driven permafrost distribution in the Tibetan Plateau, reviewed by Yang et al. (143). Although the soil-surface temperature (-20 cm) has been recognized to be more closely related to permafrost than air temperature (144), both variables have a strong linear relationship in the Tibetan permafrost regions (145). Due to a lack of paleo and present-day soil-surface temperature records, we used air temperature to simplify the generalized linear model (GLM). Modern estimates of the mean annual air temperature (MAAT) were derived from WorldClim (version 2, data for 1970-2000 C.E., ref. (146)) with 30 seconds (~1 km² spatial resolution). The SRTM 30-m digital elevation data was downloaded from NASA's server using QGIS (v. 3.24.1-Tisler, https://www.qgis.org/en/site/, ref. (137)) with the inherent plugin of SRTM-Downloader (v 3.1.17). To match the present-day permafrost distribution, we used a wider region of our study area with an extent of $3\overline{1.11} \pm 0.5^{\circ}$ N and $99.75 \pm 0.5^{\circ}$ E to crop both spatial datasets using the 'crop' function. Then, they were projected to coordinate reference systems (crs) of Asia North Albers Equal Area Conic (crs = 102025) to minimize the geographic distortions using the 'projectRaster' function. The present-day permafrost distribution originated from the Map of Permafrost Distribution on the Tibetan Plateau v. 2017 (crs = 102025), which is simulated based on the temperature at the top of permafrost and validated by ground-based observations (6). Subsequently, spatial datasets were downscaled to a fine resolution of 30 m using the 'resample' function with bilinear interpolation that weights the four nearest pixels to the original spatial pixel. The present-day relationship between permafrost distribution and MAAT was built using the 'glm' function with a Gaussian family. Such a relationship is statistically significant with a P value (t-test, 999 permutations) < 2e-16, suggesting that it is suitable for interpolation in a paleo temperature setting.

The past spatial temperature was simulated based on multi-proxy reconstructed paleo-temperature (113, 114) and the 30 m SRTM digital elevation data (45). The permafrost distributions for 22–0 ka at 500-year intervals were predicted using the 'predict.glm' function with parameters of type = "response" and se.fit = TRUE. We extracted the threshold (0.22052) of permafrost presence by comparing the permafrost distribution at 0 ka to present-day. Hence, any pixel with a value \geq 0.22052 was considered to contain permafrost. Then, we converted the simulated permafrost to binary spatial data and calculated the number of permafrost pixels per 100-m elevation bin under the effect of modeled glacier extent within lake catchment (45). The results are shown in Fig. S12C. We further calculated the number of permafrost pixels within 4100–4500 m a.s.l. and 4500–4900 m a.s.l to present the permafrost extent within lowland (Fig. S12D) and highland (Fig. S12E), respectively. The boundary of 4500 m a.s.l. was defined based on the reachable slope degree (38°) of yak-grazing (147). Most catchment areas above 4500 m are either far from inflows or with steep slopes (> 38°, Fig. S1). The functions for spatial data processing and simulation come from the R package 'raster' (version 3.5-15) (136) and 'stats' (126).

The composition of Proteobacteria has been reported to be distinct in soil sections in the Tibetan permafrost regions, of which Alphaproteobacteria dominate in the active layer and Gammaproteobacteria dominate in the permafrost layer (*148*). Thus, we calculated the compositional changes of Proteobacteria. Consequently, our simulated results align with the high percentages of Gammaproteobacteria before 14 ka and the pronounced decline afterward (Fig. S9A, B). Also, the percentages of Alphaproteobacteria imply that the active layers were possibly thinner in the pre-14 ka period and increased in thickness afterwards (Fig. S9C).

SedaDNA preservation and provenance assessment

The differences in sediment types and mineralogies are reported to affect DNA sources, absorption, and recovery, thereby weakening the comparison of DNA-inferred community changes across time (48, 149, 150). However, we assert that our inferences from the Lake Naleng sedaDNA record across time are both comparable and robust. The presence or absence of taxa DNA is most likely attributed to ecological changes, with limited influence from the depositional environment. The DNA preserved across sediment layers most likely tracks the biological information originating from diverse habitats within the catchment, transported via fluvial processes through time, including the influence of glacial melting before 14 ka. This reasoning is explained as follows.

First, we did not observe noticeable biases induced by lithology and sediment accumulation (Fig. S5), as neither the samples nor the taxa cluster along sediment types, and there is no skew along sedimentation rates in the PCA plots (Fig. S6).

Second, the lake-sediment source remained consistent and primarily originates from catchment area, supported by the observed uniformity in grain-size fractions and elemental components (Fig. S5). More specifically, the input of fine-grained detrital materials (clay to silt-sized particles) in the lake aligns with characteristics typical of unaltered sediments in a partly glaciated catchment

in the Tibetan alpine regions (151). Furthermore, the major element concentrations (SiO₂ and Al₂O₃) reflect the chemical composition of the granitic and granodiorite parent rock in the lake catchment. A previous study (96) discussed the deposition of lake sediments, emphasizing the direct erosion of soils derived from local sources (e.g., slopes, terraces, and topographic depressions near rivers and lakes) over time. This process was coupled with the input of local unaltered detrital sediments via glacial dynamics before 14.5 ka (96). We, therefore, assume that lake sediments continuously accumulated detrital materials eroded from the local environment.

Third, the capacity and recovery of DNA-mineral binding across time are comparable. The lake catchment area is characterized by Miocene granite and granodiorite, primarily made up of quartz, mica, and feldspar. This composition aligns with the mineral composition of a modern sediment sample from Lake Naleng, as determined by X-ray diffraction (XRD), which includes mica (48%), quartz (33%), chlorite (12%), and feldspar (7%) (152). By analogy, we deduce that a similar composition of minerals characterizes the core sediments over time, as there are no notable shifts in grain-size fractions and element concentrations throughout the core's entirety. Despite non-clay minerals (e.g., mica, quartz, chlorite, and feldspar) having relatively lower DNA adsorption capacities compared to clay minerals (e.g., smectite and illite), a high amount of DNA (40%) adsorbed to non-clay minerals is recoverable, whereas clay minerals yield up to 10% extraction of adsorbed DNA (48, 150).

Fourth, read fragmentation induced by carbonates as seen for calcite (149) and clay minerals (if applicable) is rather unlikely. The catchment area lacks calcareous rocks (e.g., calcite) and the lake-sediment core contains very low carbonate content (< 1%, Fig. S5). So far, it is unclear if clay minerals would induce fragmentation as well. Nevertheless, we observed an insignificant relationship (Spearman's rho = -0.19, Bonferroni adjusted *P*-value = 0.246) between weighted average read length (as a proxy of fragmentation) and clay-size fraction (as a proxy of clay minerals). A low standard deviation (6 bp) of weighted average read length further implies less variability in preservation conditions of DNA over time. On the other hand, weighted average read length does not significantly explain the composition turnovers of terrestrial vegetation (adjusted $R^2 = 1.9\%$, *P*-value = 0.113), terrestrial mammalian (adjusted $R^2 = -0.18\%$), suggesting that observed DNA degradation with time (Fig. S4) has contributed minimally to ecological interpretation.

Fifth, redeposition is considered rather unlikely due to the bowl-shaped structure of the lake basin, as well as relatively rapid sedimentation rates and prevailing stable chemical burial conditions.

Co-existence of alternative states in lake system

The distribution of macrophyte and phytoplankton can be horizontal, such as in Taihu (in eastern China, ref. (153)), or vertical, such as in Son Kol (in the central Tien Shan, ref. (154)). Both lakes are mesotrophic with either a large surface area (Taihu) or deep-water body (Son Kol). Similar to Lake Naleng, palynological and non-pollen palynomorph (69) as well as biogeochemical parameters (TOC, TP, and C/N ration, ref. (96)) indicate moderate nutrient levels and high lake level during 14.5–3.6 ka. These characteristics could provide suitable habitats for both macrophytes and phytoplankton. Submerged plants require nutrients and light to grow and can usually be found in the littoral zones where light can penetrate the water column, while cyanobacteria can use atmospheric nitrogen and other nutrients such as phosphorus, and can be found in deeper waters where they can access these nutrients.

Figs S1 – S16



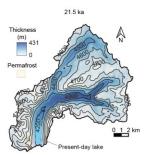
20 ka

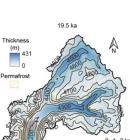
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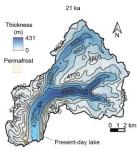
(m) 431

Permafrost





Present-day lake



19 ka

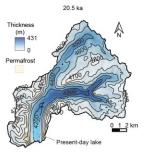
Present-day lake

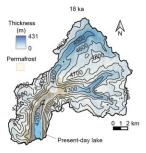
 $\bigwedge_{\mathbf{z}}$

0 1 2 km

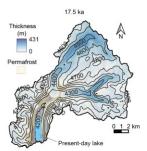
Thickness (m) 431 0

Permafros

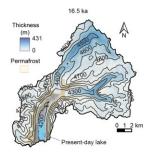


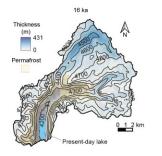


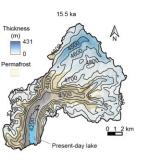
Present-day lake

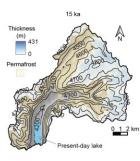


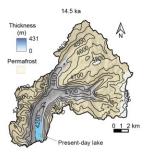
17 ka (m) 431 Permafrost (m) 421 (m) 431 (m

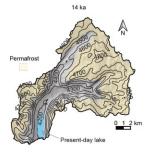




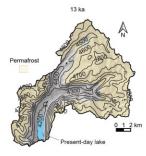


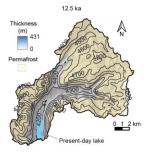


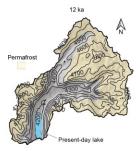


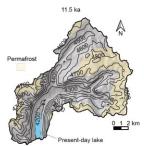




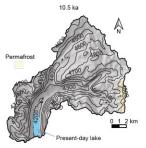


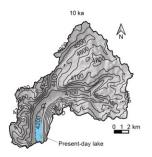


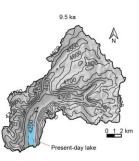




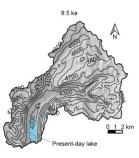
11 ka

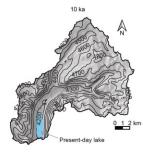


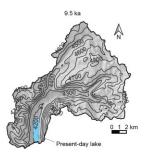




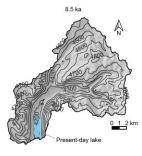


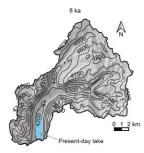




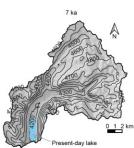














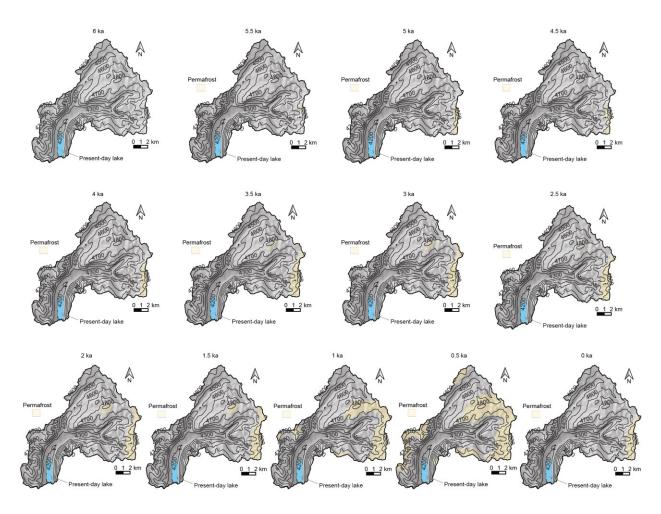


Fig. S1. Simulated permafrost extent (by taking glacier dynamics into account, ref. (45)) within Lake Naleng's catchment during 22–0 ka at a 500-year resolution, indicating substantial loss of permafrost and glaciers since 14 ka and only a small portion of permafrost restoration in the highlands during the late Holocene.

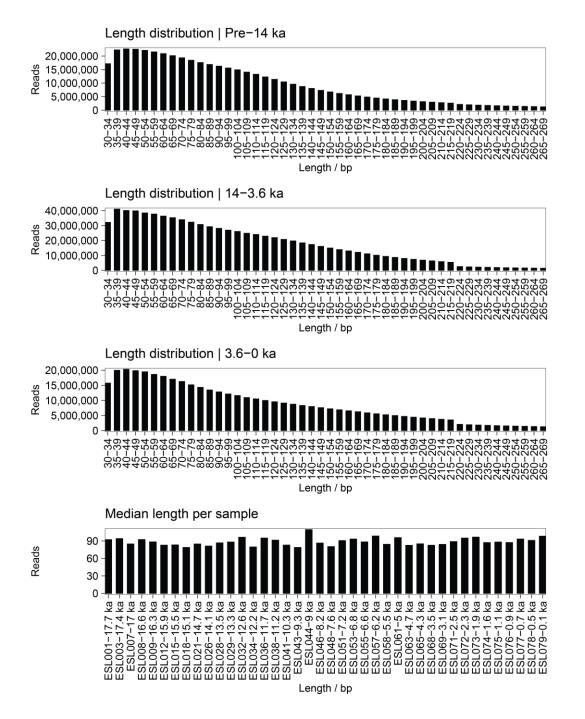
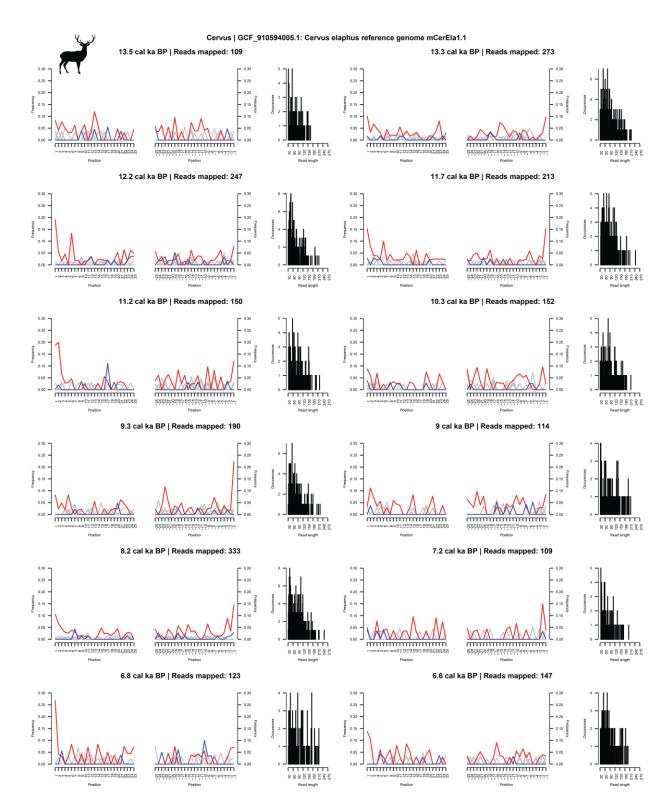
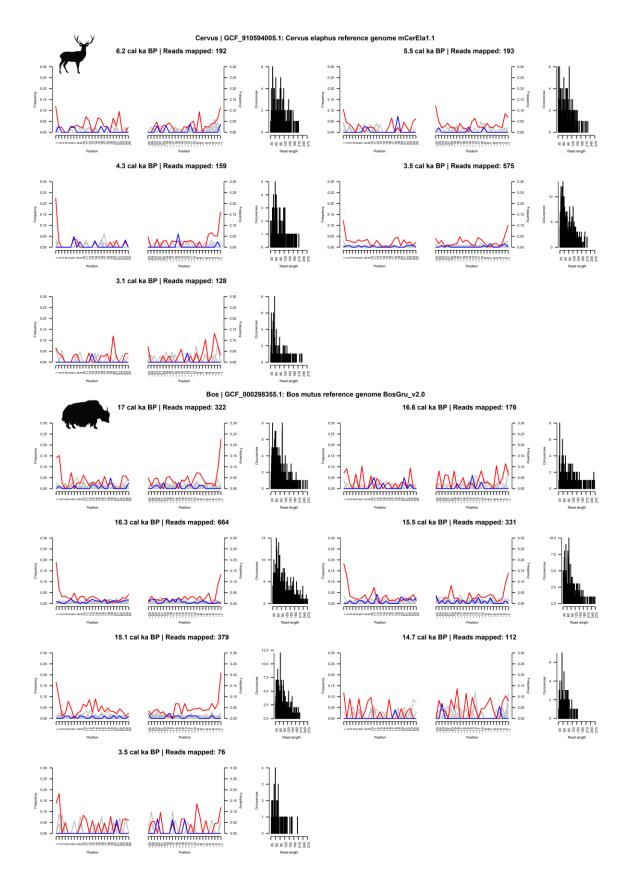


Fig. S2. Length distribution of reads of three time-intervals and median read length across time. The DNA fragments exhibit highly degraded profiles with left-skewed distributions, and there are no discernible differences in read length observed across time.

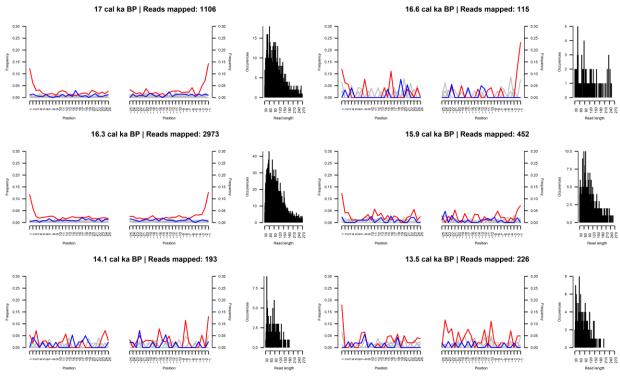


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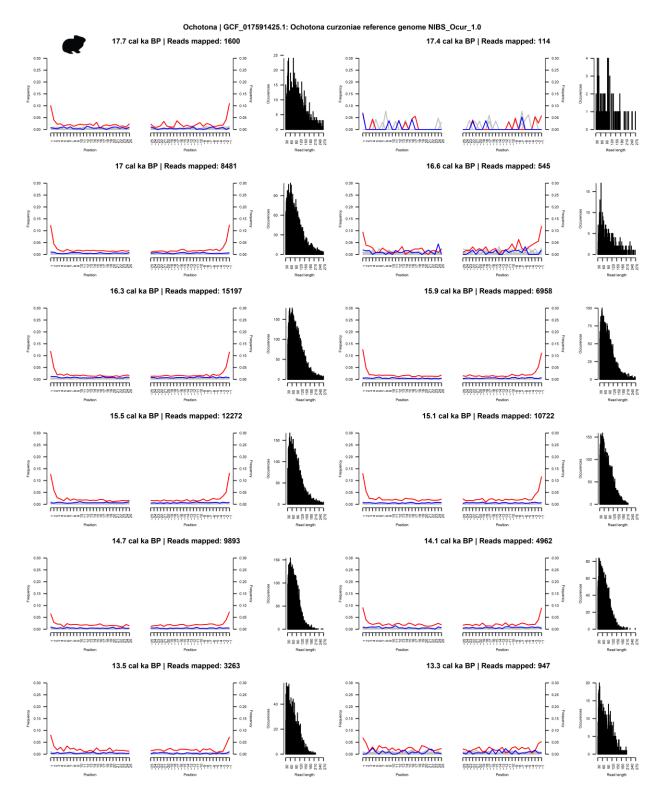


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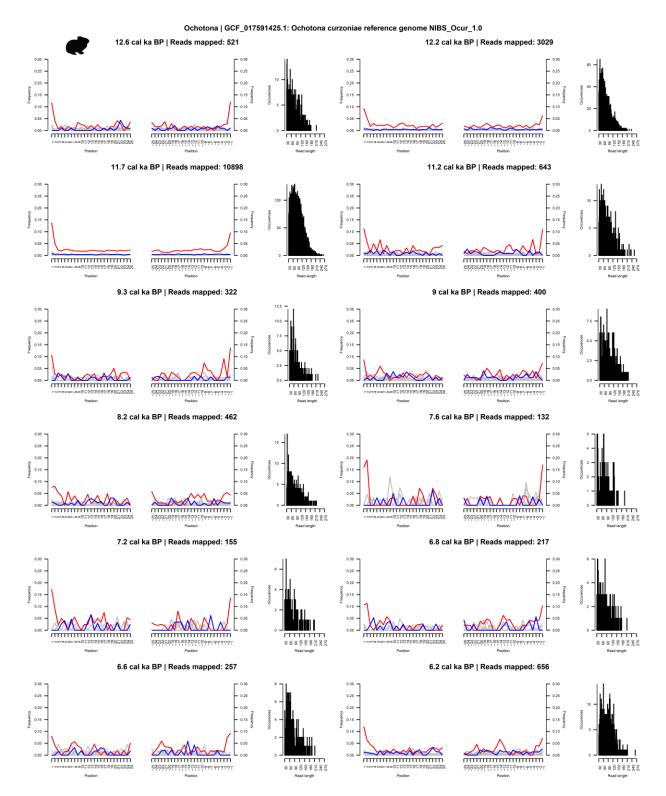
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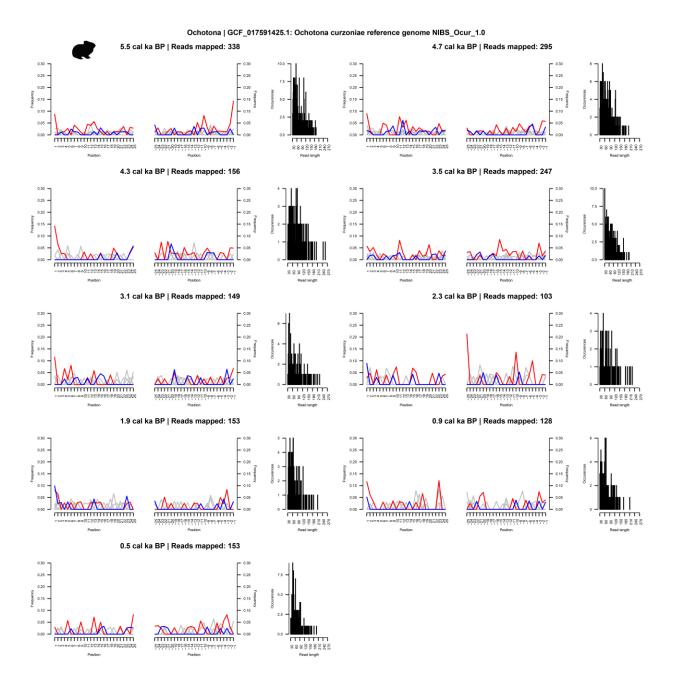
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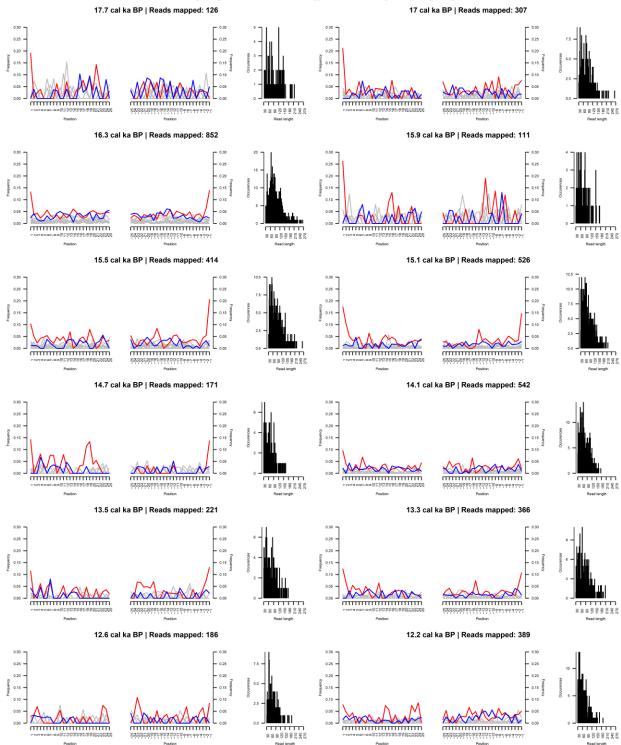


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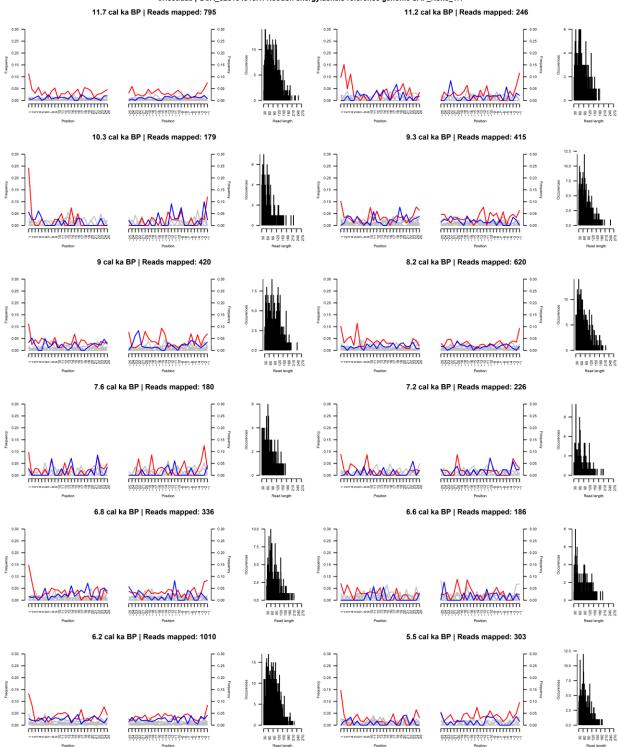
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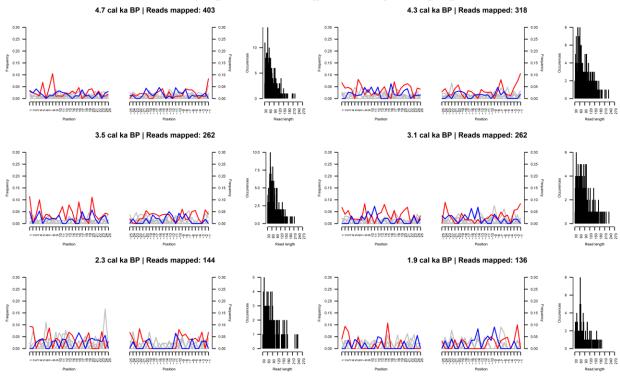


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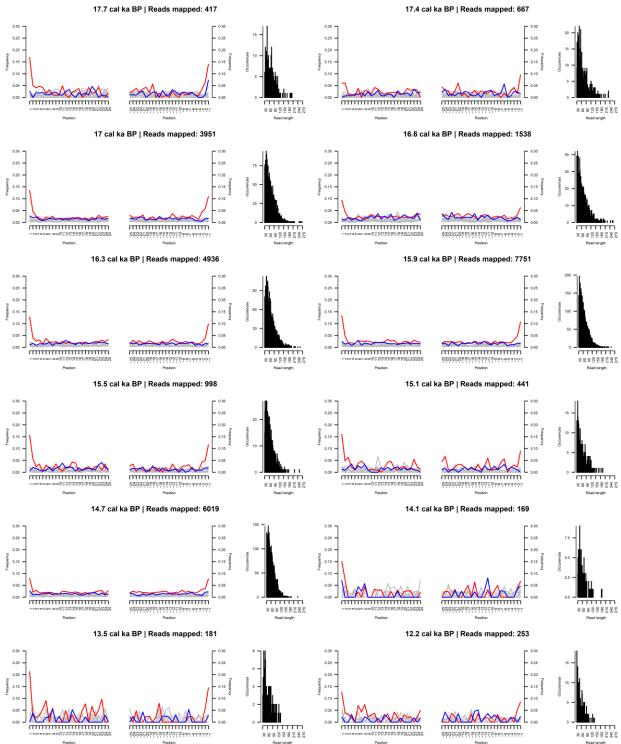
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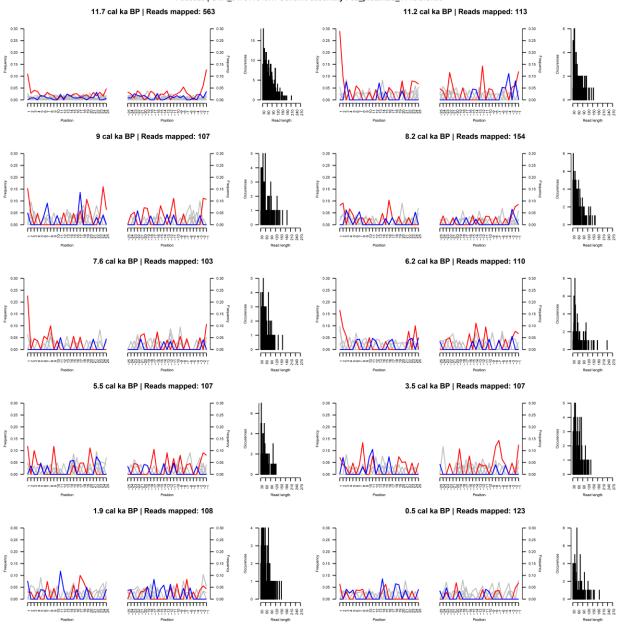
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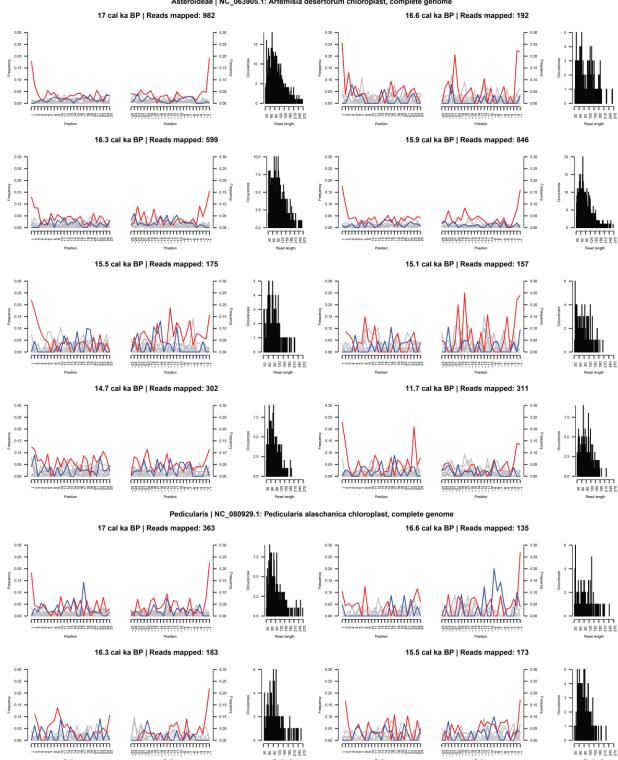


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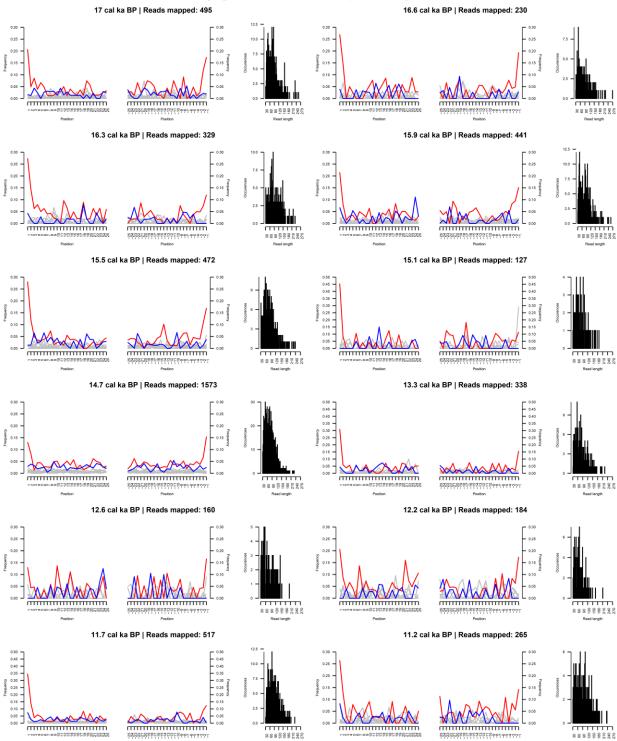


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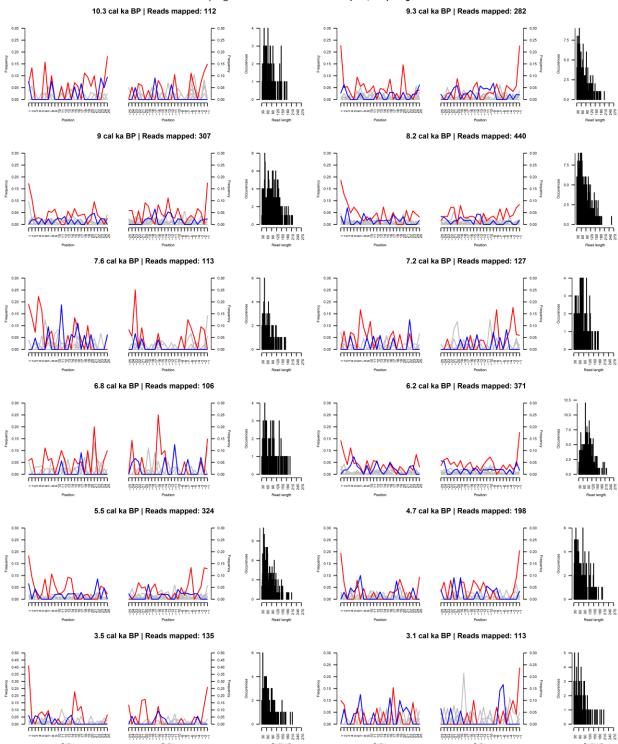
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Read length



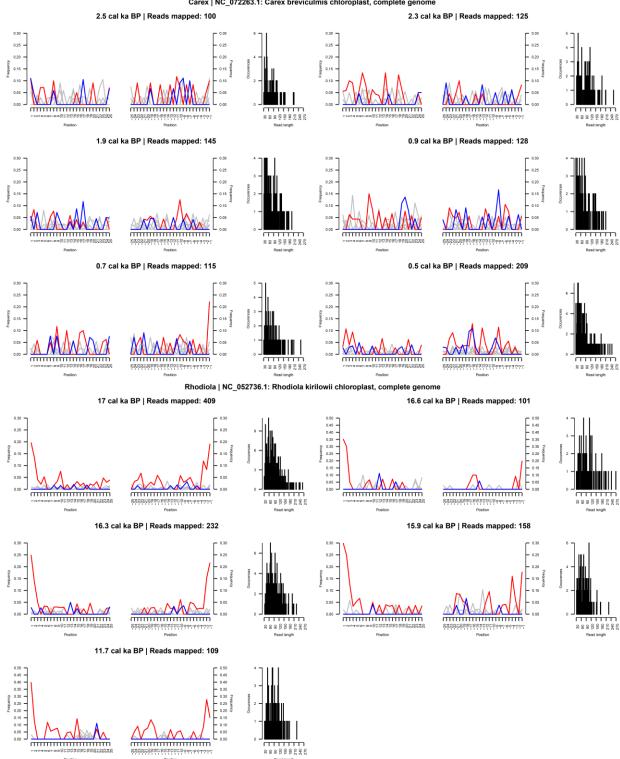
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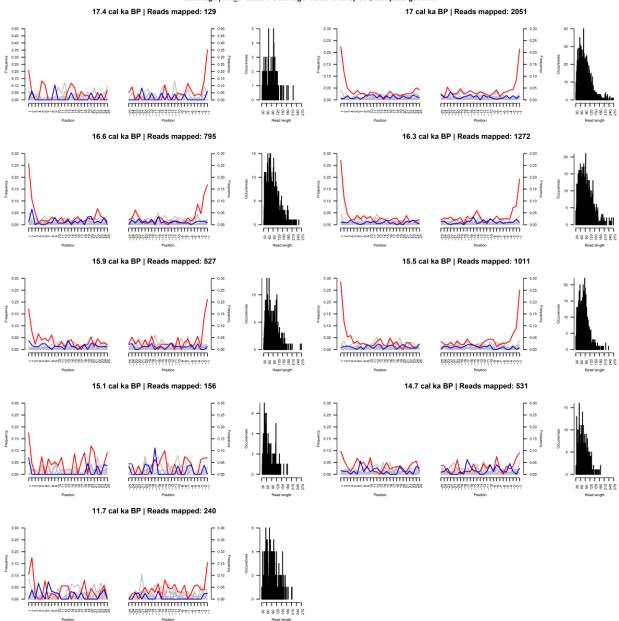
Read lengt

Read length

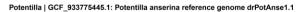
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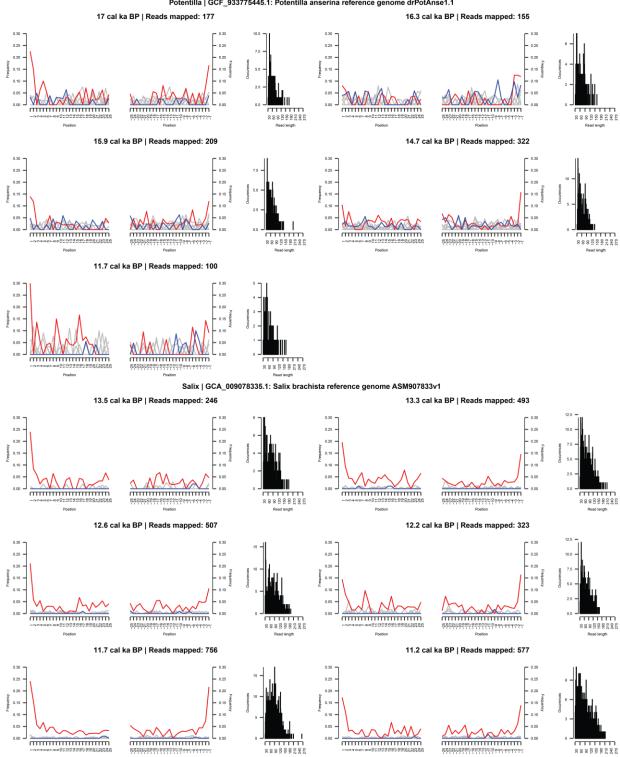


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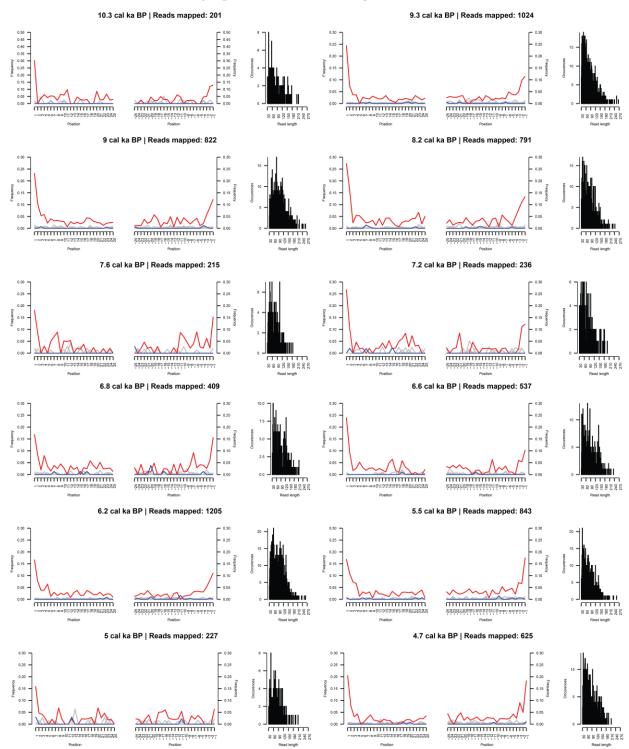


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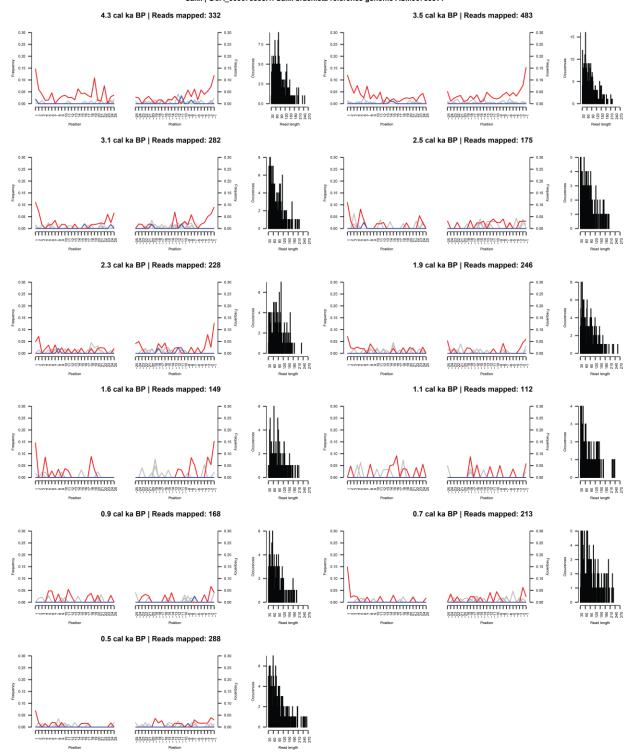




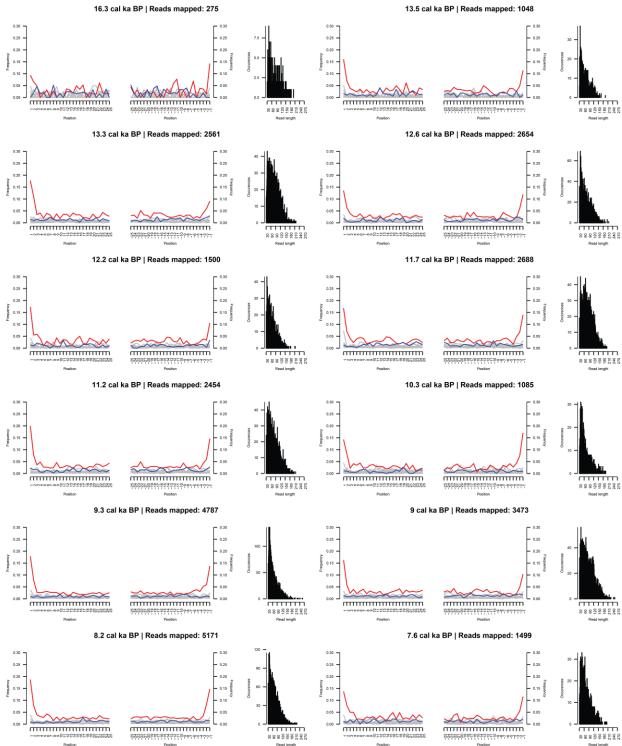
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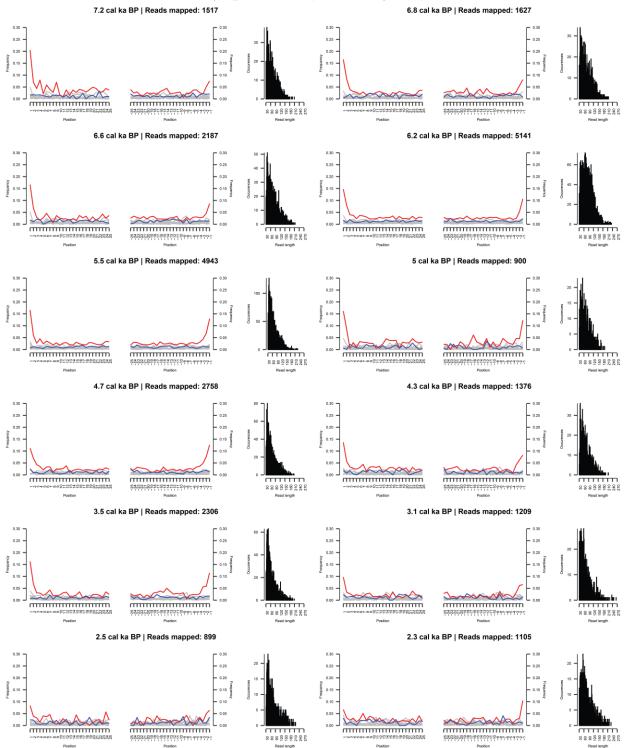




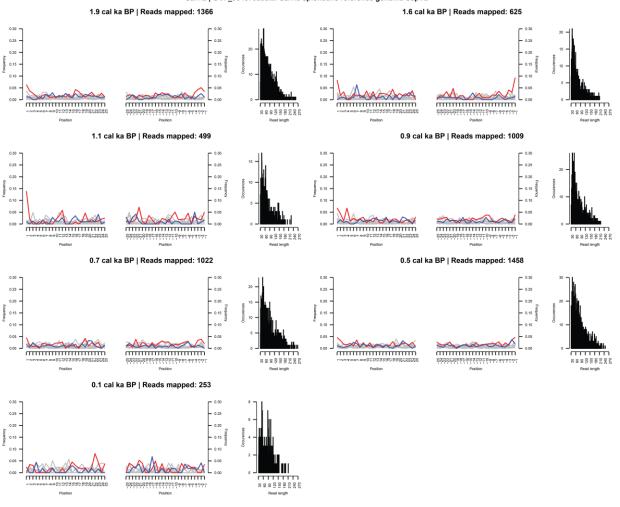
Salvia | GCF_004379255.2: Salvia splendens reference genome SspV2



Salvia | GCF_004379255.2: Salvia splendens reference genome SspV2

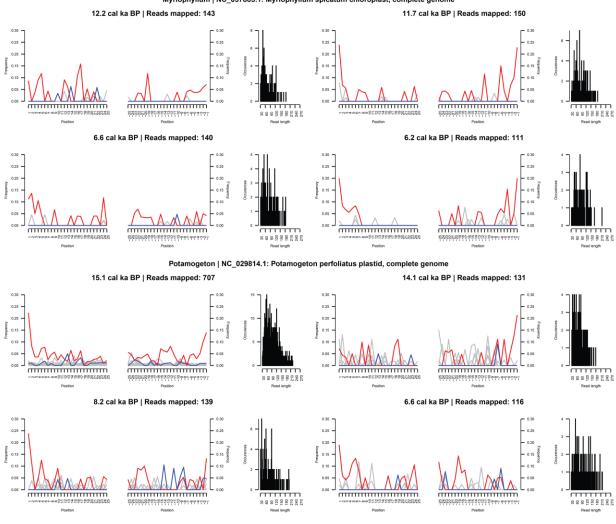


Salvia | GCF_004379255.2: Salvia splendens reference genome SspV2

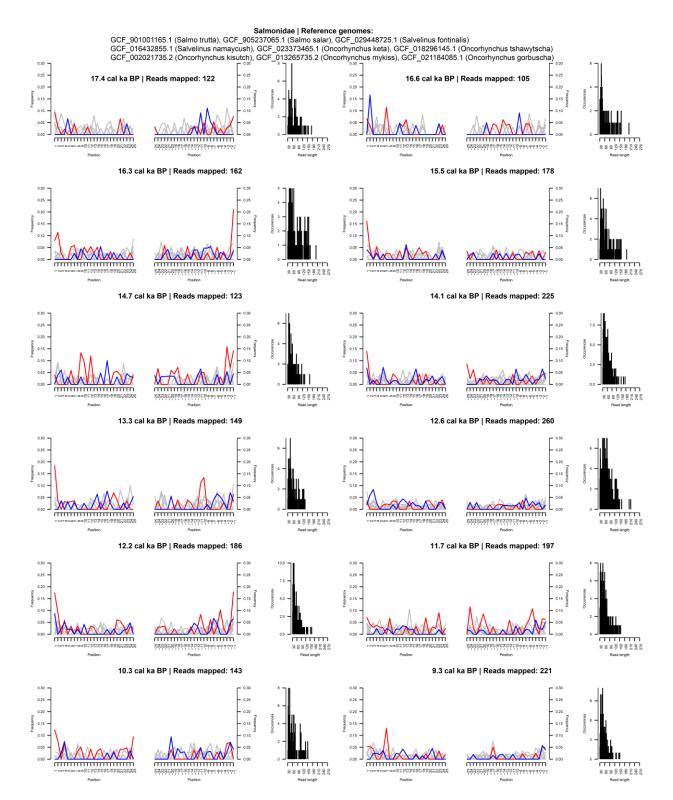


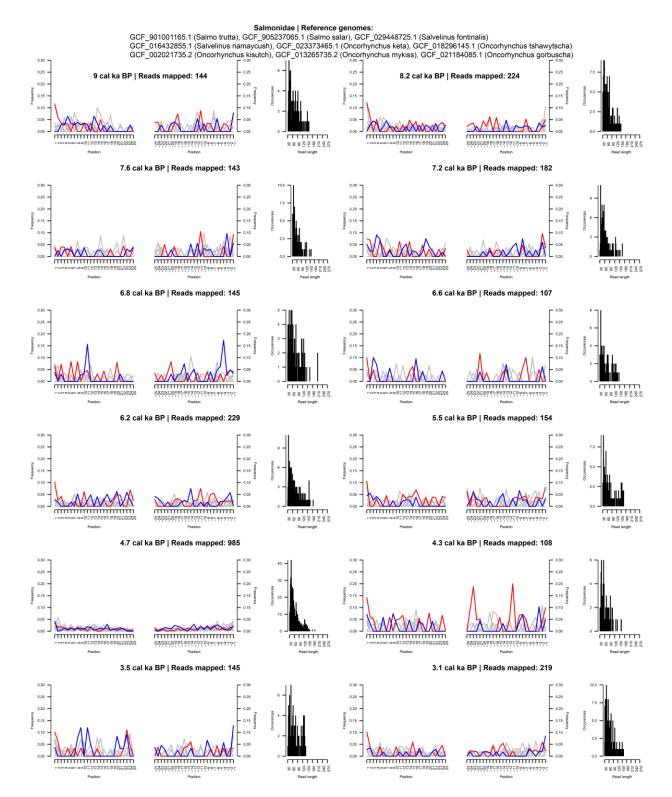
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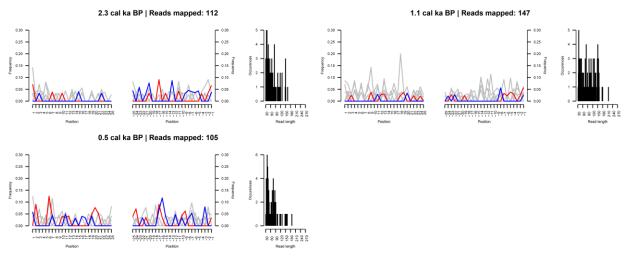




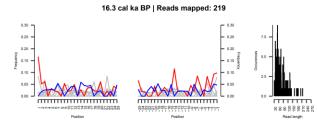
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Salmonidae | Referene genomes:

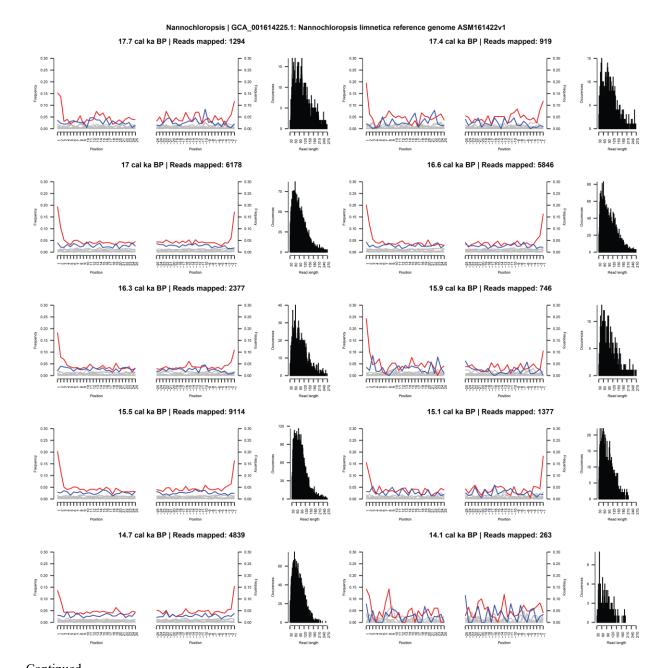
GCF_901001165.1 (Salmo trutta), GCF_905237065.1 (Salmo salar), GCF_029448725.1 (Salvelinus fontinalis) GCF_016432855.1 (Salvelinus namaycush), GCF_023373465.1 (Oncorhynchus keta), GCF_018296145.1 (Oncorhynchus tshawytscha) GCF_002021735.2 (Oncorhynchus kisutch), GCF_013265735.2 (Oncorhynchus mykiss), GCF_021184085.1 (Oncorhynchus gorbuscha)



Cyprinidae | GCF_001515625.1: Sinocyclocheilus rhinocerous Reference genome SAMN03320098_v1.1

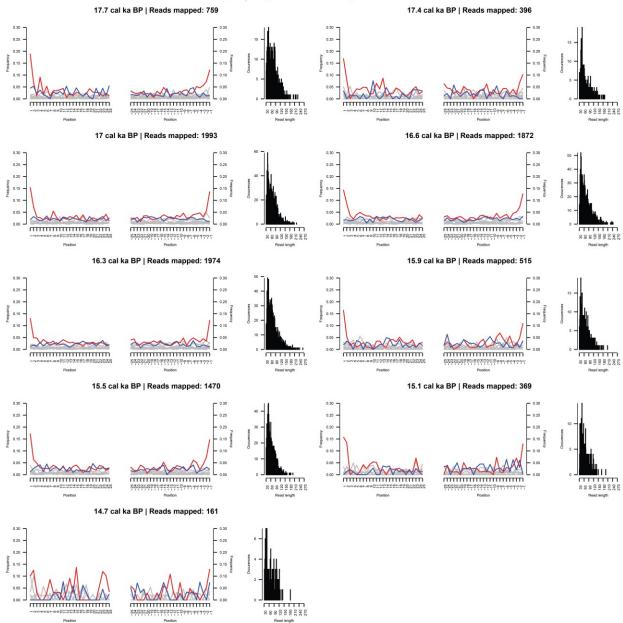


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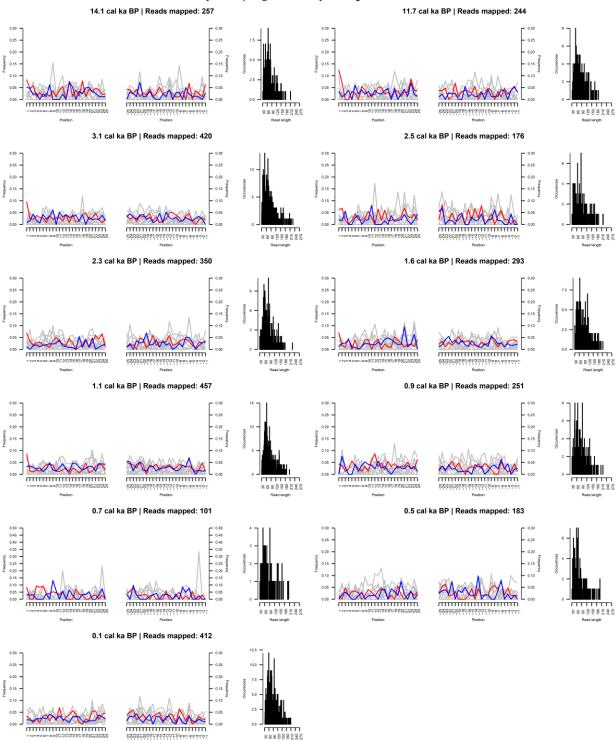


Chamaesiphon | GCF_000317145.1: Chamaesiphon minutus PCC 6605



Continued

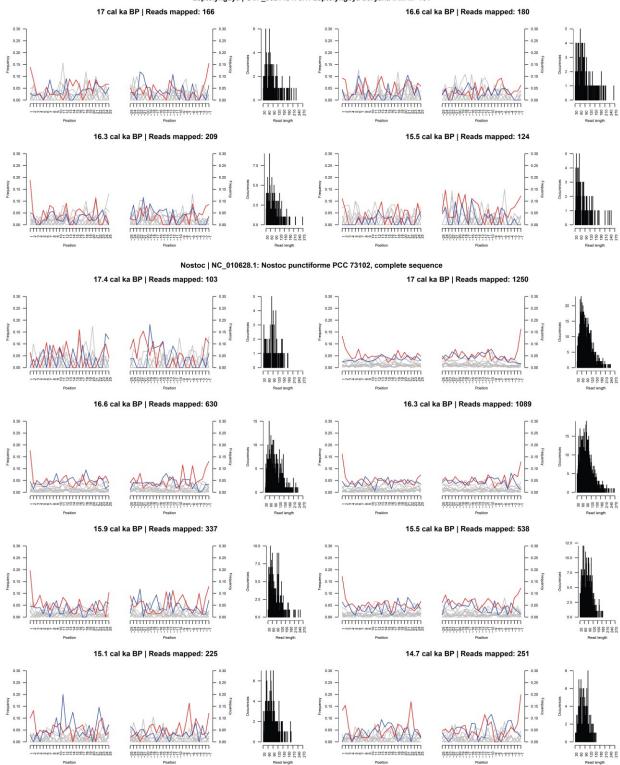
Cyanobium | GCF_000316515.1: Cyanobium gracile PCC 6307



Read length

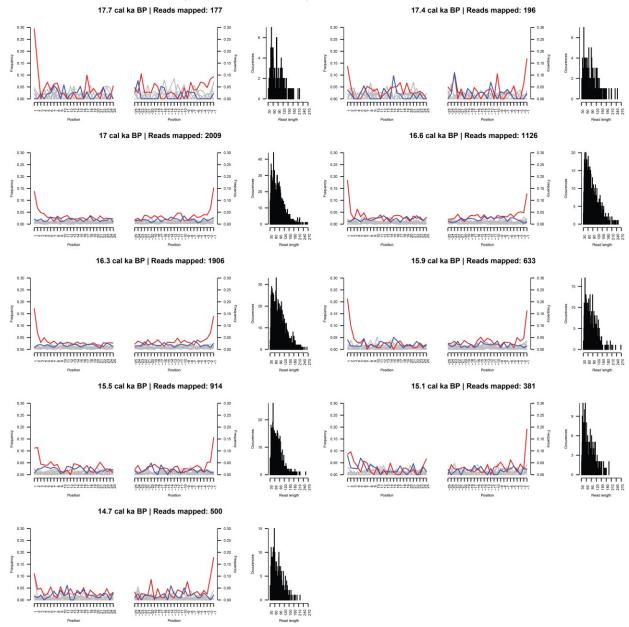


Leptolyngbya | GCF_002142475.1: Leptolyngbya boryana IAM M-101



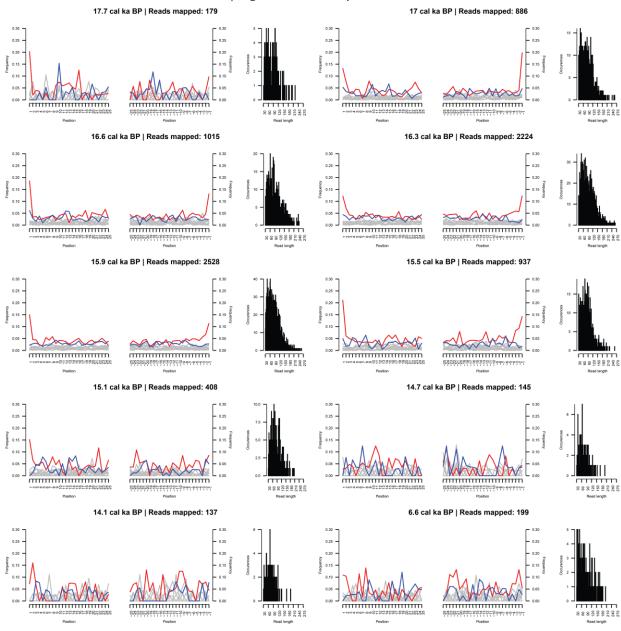
Continued

Oscillatoria | GCF_000317475.1: Oscillatoria nigro-viridis PCC 7112 reference genome ASM31747v1



Continued

Planktothrix | GCF_900009265.2: Planktothrix paucivesiculata PCC 9631



Read length

Continued

Pseudanabaena | GCF_029910235.1: Pseudanabaena galeata CCNP1313

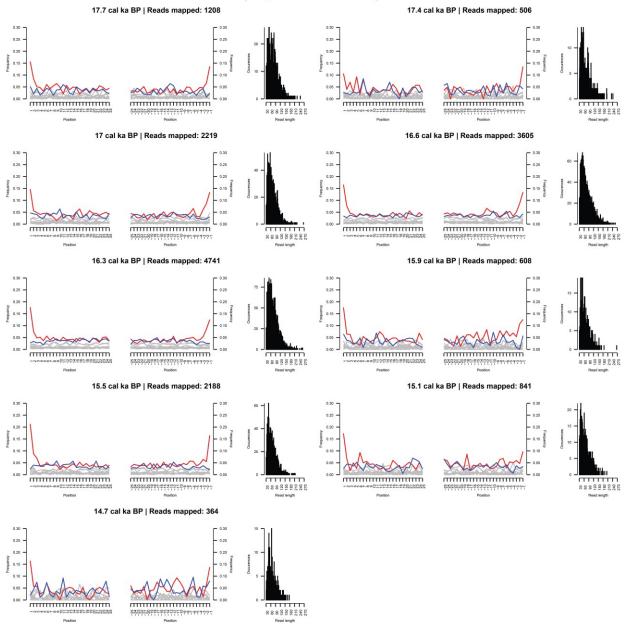


Fig. S3. Ancient damage patterns for 26 common taxa in terrestrial and aquatic ecosystems through time. Authentication of taxa at each time point was performed using mapDamage v.2.2.1 (*108*) with the settings '--rescale - single-stranded,' employing corresponding classified reads. The ancient origin of reads was evaluated using the miscoding lesion pattern (left plots, red: C to T substitutions, blue: G to A substitutions, gray: all other substitutions, y-axis is frequency on a scale of 0-0.3 and x-axis is position) and length distribution (right plot, y-axis is occurrence on a varying scale and x-axis is read length from 30 to 270 bp). The highest deamination rates, observed at the 5' and 3' end, confirm that the identified terrestrial and aquatic taxa DNA is of ancient origin.

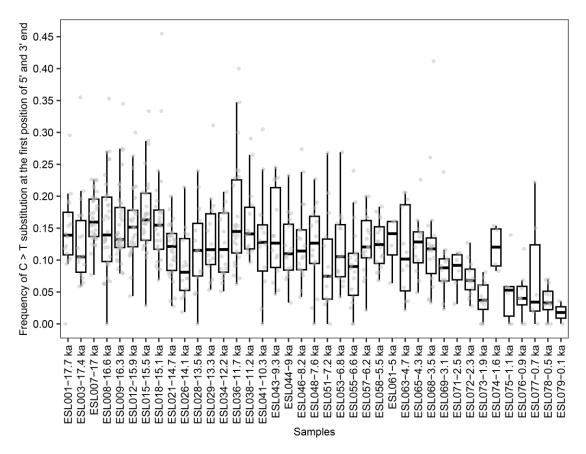


Fig. S4. Ancient damage patterns across time intervals show a clear increase in the substitution rate with age, suggesting that metagenomic DNA is ancient in origin and well-preserved in Lake Naleng. The observation of clay mineral-rich fine fractions in sediment over time (96) provides further evidence of good preservation conditions (155).

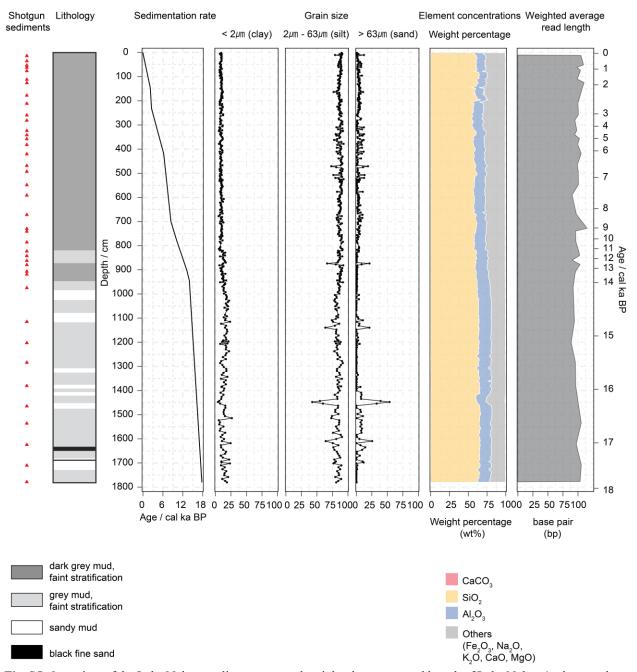


Fig. S5. Overview of the Lake Naleng sediment core and weighted average read length of Lake Naleng's shotgun data. Sediments designated for shotgun sequencing are highlighted in red. The majority of these sediments are collected from lake mud, with two additional samples obtained from sandy mud to enhance temporal resolution. The basic lithostratigraphy is established through visual inspection. The sedimentation rate in the lower section of the core (between 1780 and 950 cm) is relatively higher than in the upper part, primarily due to the enhanced accumulation of sediments derived from glacial erosion. Grain-size fractions and concentrations of major elements exhibit consistent patterns throughout the core, with higher variations observed in grain-size fractions below 950 cm. The presence of CaO is assumed to be attributed to silicates, as Lake Naleng lacks calcareous rocks in its catchment area, and the lake sediments have a low carbonate content (< 1%). The source data are derived from (96). The merged reads are applied to calculate the average read length, which is weighted by read count, considering it as a proxy for read fragmentation.

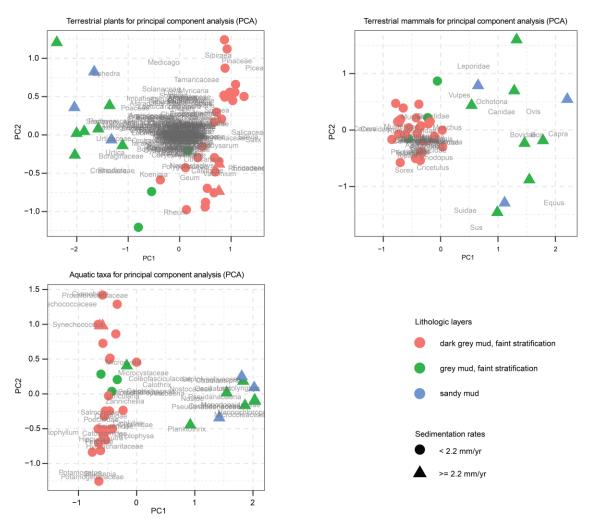
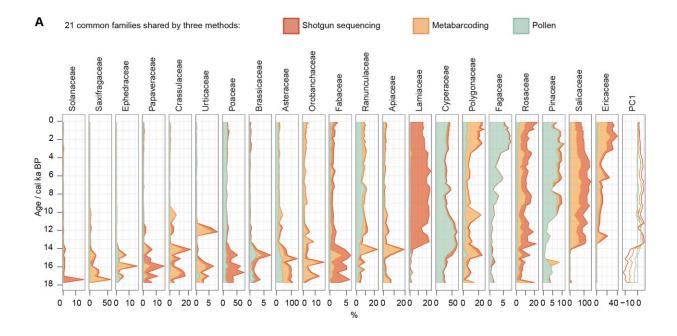


Fig. S6. Principal component analysis (PCA) of terrestrial plants, terrestrial mammals, and aquatic communities. Samples are not clustered by sediment type. No specific samples or taxa exhibit extremely large values that notably skew the PCA plots. Instead, the covariance structure of the three communities directly corresponds to past environmental changes.



Vegetation types revealed by regional pollen records and Lake Naleng

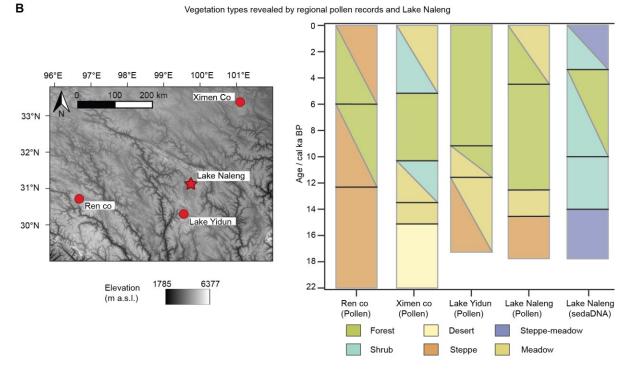


Fig. S7. Proxy records of vegetation changes archived in Lake Naleng sediments and of comparisons with surrounding lake sediment cores. (A) After comparing the relative abundance and PC1 of metagenomics/shotgun sequencing, metabarcoding, and pollen analysis, we focused on 21 common families retrieved by all three methods. (B) We compared these proxy-based vegetation types to pollen-based ones from the closest lake cores that were dated to at least the late glacial period. The synthesis of multiple proxies confirms a cold-adapted vegetation in the late glacial which turns to warm-adapted woodland in the Holocene. Plot B is modified from (15).

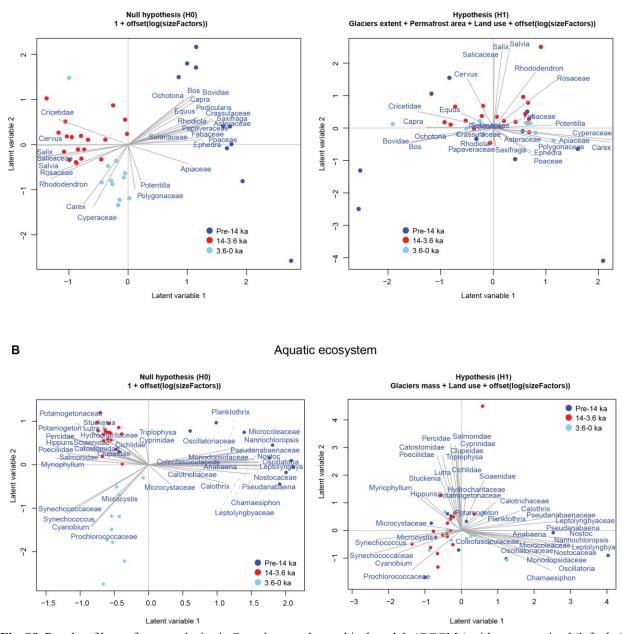


Fig. S8. Results of latent factor analysis via Gaussian copula graphical models (GCGMs) with unconstrained (left plot) and constrained latent variables (right plot) for terrestrial (A) and aquatic ecosystems (B). Three clusters of samples in terms of these covariates are in the null model (H0), while these patterns are absent after controlling for co-variance of environmental factors (H1). These shifts indicate that the co-occurrence patterns revealed by H1 are mainly generated by biotic interactions rather than shared responses to environmental covariates. The effect of sequencing depth (sampling effort) is excluded from models by taking the log of size factor.

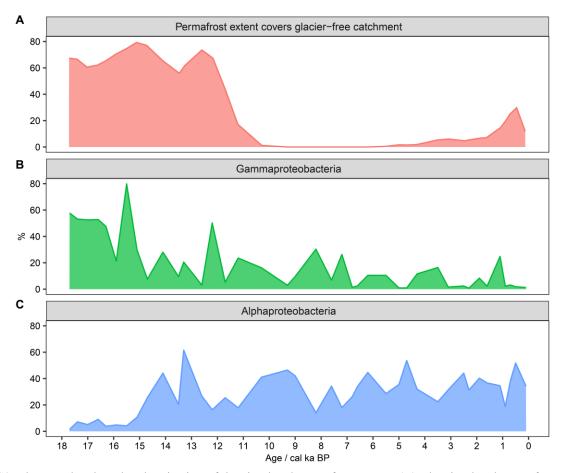


Fig. S9. Shotgun-data-based authentication of the simulated permafrost extent. (A) The simulated permafrost extent accounted for the glacier-free catchment. (B) The relative abundance of Gammaproteobacteria against Bacteria. (C) The relative abundance of Alphaproteobacteria against Bacteria. Both Proteobacteria dominate in the permafrost soils and active layers on the Tibetan Plateau, respectively (*148*). The simulated percentages of permafrost extent with 500-year intervals are linearly interpolated to the same temporal resolution as lake sediments.

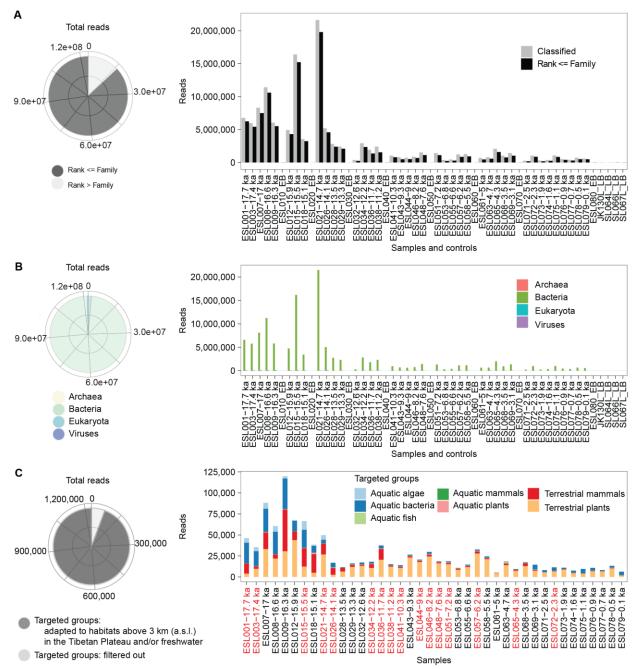
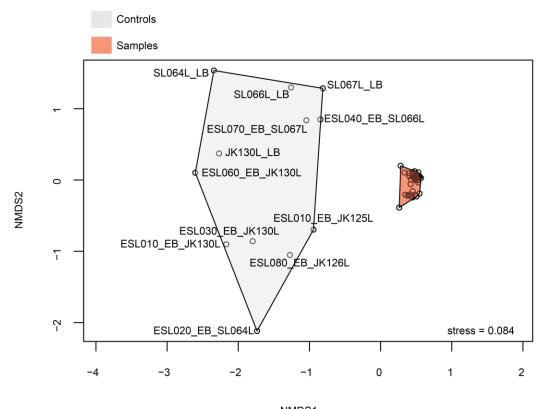


Fig. S10. Data overview. (A) Total reads of classified cellular organisms and taxa with taxonomic rank \leq Family (left plot) as well as their distribution across samples and controls (right plot). (B) The total reads of superkingdoms (left plot) and their distribution across samples and controls (right plot). (C) The total reads of targeted groups that can adapt to habitats above 3000 m a.s.l. on the Tibetan Plateau and/or in freshwater (left plot), along with their distribution across samples (right plot). Those sediments having a library with deep sequencing are marked in red once to avoid excessive coloration.



NMDS1

Fig. S11. Non-metric multidimensional scaling (NMDS) results indicate the notable dissimilarity of community composition between controls and samples.

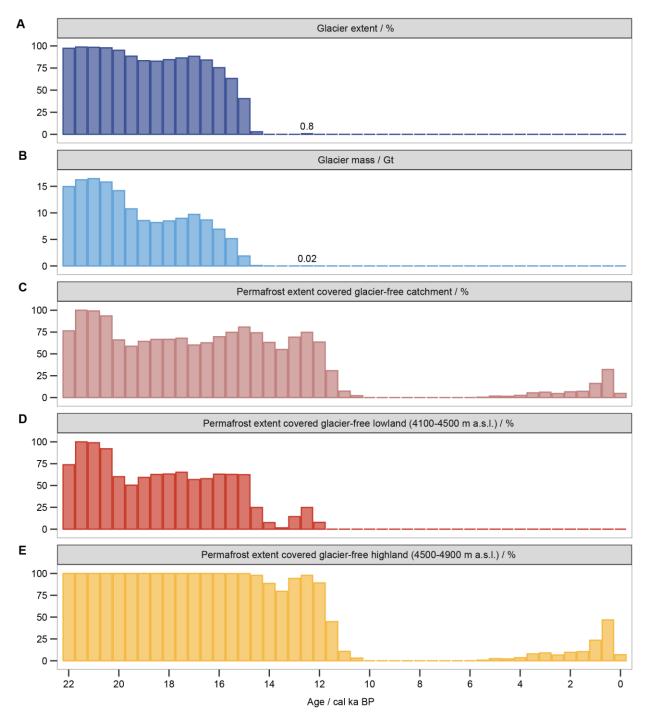


Fig. S12. Simulated past cryosphere changes are represented by the percentage of simulated glacier extent (A), glacier mass (B), and permafrost extent (C-E) within Lake Naleng's catchment from 22 to 0 ka. The simulated glacier extent was extracted from ref. ().

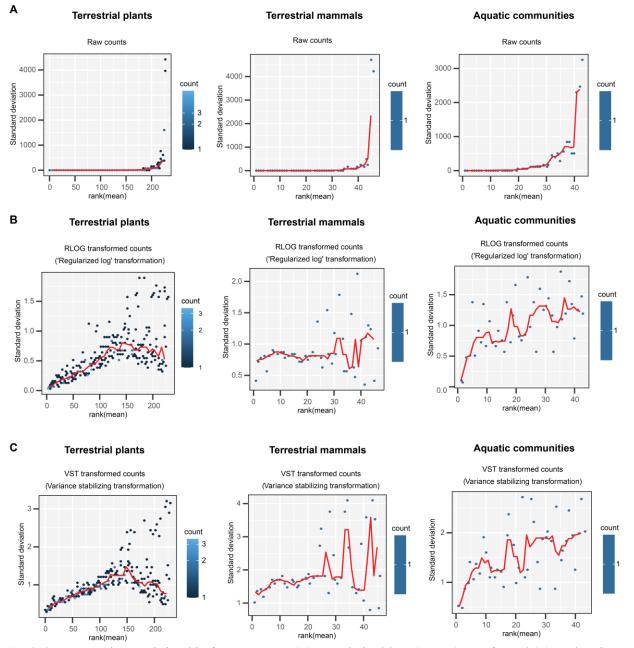


Fig. S13. Mean-variance relationship for raw counts (A), 'regularized log' (RLOG) transformed (B), and variance stabilizing transformed (VST) counts (C) of terrestrial plants, terrestrial mammals, and aquatic communities. Heteroscedasticity is clearly observed in the mean-variance relationship based on raw counts as standard deviation varies over several orders of magnitude. Both transformation approaches significantly stabilize variance across samples. RLOG transformed counts are used for ordination analysis due to their lower standard deviation. The transformation ensures that the variance and co-variance of taxa composition turnover over time are primarily influenced by predictor variables (e.g., environmental factors) rather than sampling effort or sequencing depth.

Α

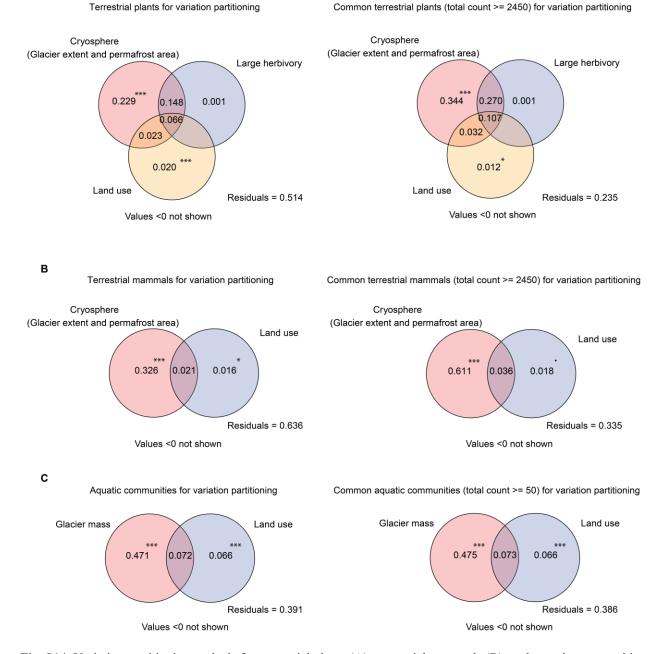


Fig. S14. Variation partitioning analysis for terrestrial plants (A), terrestrial mammals (B), and aquatic communities (C). The results based on composition of common taxa align coherently with the results for the whole composition. Significance codes of F-test with 999 permutations: '***' 0.001, '**' 0.01, '*' 0.05, '.' 0.1

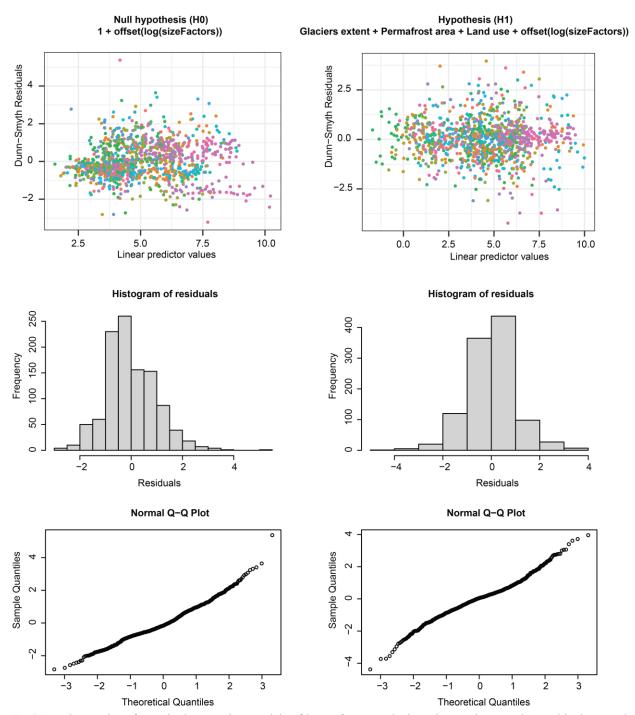


Fig. S15. Diagnostics of marginal regression models of latent factor analysis and Gaussian copula graphical network for the terrestrial ecosystem. Both the null model (left panel) and the hypothesis model (right panel) exhibit no discernible patterns in the plot of residuals against linear predictor values (top row), show no significant outliers in the histogram (middle row), and strong skewness in the Q-Q plot (bottom row). These characteristics suggest that the marginal modes adhere to the basic assumptions of the Gaussian copula graphical model.

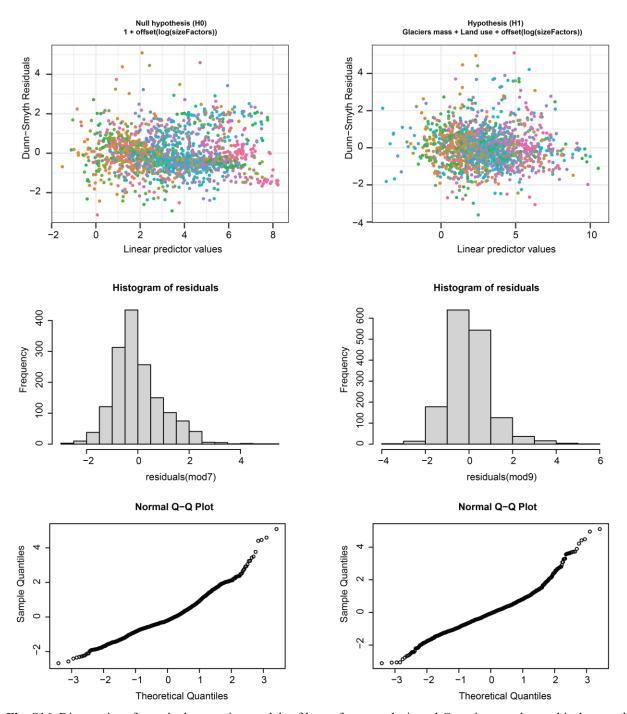


Fig. S16. Diagnostics of marginal regression models of latent factor analysis and Gaussian copula graphical network for the aquatic ecosystem. Both the null model (left panel) and the hypothesis model (right panel) display no evident patterns in the plot of residuals against linear predictor values (top row), show no significant outliers in the histogram (middle row), and strong skewness in the Q-Q plot (bottom row). These characteristics suggest that the marginal modes adhere to the basic assumptions of the Gaussian copula graphical model.

Tables S1 – S6

Table S1. Lake Naleng's shotgun sequencing, metabarcoding, and pollen analysis record similar dominant families and genera, which are among the top 20 species-rich families and genera in the Hengduan Mountains.

	Top 20 species-rich families in Hengduan Mountains	Recovered by Methods	Top 20 species-rich genera in Hengduan Mountains	Recovered by Methods
1	Asteraceae	Shotgun, Metabarcoding, Pollen	Pedicularis (Orobanchaceae)	Shotgun, Metabarcoding, Pollen
2	Rosaceae	Shotgun, Metabarcoding, Pollen	Rhododendron (Ericaceae)	Shotgun, Metabarcoding, Pollen
3	Poaceae	Shotgun, Metabarcoding, Pollen	Saxifraga (Saxifragaceae)	Shotgun, Metabarcoding, Pollen
4	Ranunculaceae	Shotgun, Metabarcoding, Pollen	Gentiana (Gentianaceae)	Shotgun, Metabarcoding, Pollen
5	Ericaceae	Shotgun, Metabarcoding, Pollen	Aconitum (Ranunculaceae)	Shotgun, Metabarcoding, Pollen
6	Fabaceae	Shotgun, Metabarcoding, Pollen	Primula (Primulaceae)	Shotgun, Metabarcoding, Pollen
7	Lamiaceae	Shotgun, Metabarcoding, Pollen	Salix (Salicaceae)	Shotgun, /, Pollen
8	Orchidaceae	Shotgun, / , /	Carex (Cyperaceae)	Shotgun, Metabarcoding
9	Orobanchaceae	Shotgun, Metabarcoding, Pollen	Corydalis (Papaveraceae)	Shotgun, Metabarcoding, Pollen
10	Apiaceae	Shotgun, Metabarcoding, Pollen	Saussurea (Asteraceae)	Shotgun, Metabarcoding, Pollen
11	Gentianaceae	Shotgun, Metabarcoding, Pollen	Delphinium (Ranunculaceae)	Shotgun, Metabarcoding
12	Primulaceae	Shotgun, Metabarcoding, Pollen	Rubus (Rosaceae)	Shotgun, /, Pollen
13	Cyperaceae	Shotgun, Metabarcoding, Pollen	Astragalus (Fabaceae)	Shotgun, Metabarcoding, Pollen
14	Saxifragaceae	Shotgun, Metabarcoding, Pollen	Berberis (Berberidaceae)	/, /, Pollen
15	Caryophyllaceae	Shotgun, Metabarcoding, Pollen	Sedum (Crassulaceae)	Shotgun, Pollen
16	Salicaceae	Shotgun, Metabarcoding, Pollen	Arenaria (Caryophyllaceae)	/
17	Papaveraceae	Shotgun, Metabarcoding, Pollen	Ligularia (Asteraceae)	/
18	Brassicaceae	Shotgun, Metabarcoding, Pollen	Poa (Poaceae)	/
19	Crassulaceae	Shotgun, Metabarcoding, Pollen	Potentilla (Rosaceae)	Shotgun, Metabarcoding, Pollen
20	Liliaceae	, / , /, Pollen	Aster (Asteraceae)	Shotgun, Pollen

 corresponding data
 93.5, 87.6, 75.8

 Source for list of top 20 species-rich families and genera in the Hengduan Mountains from ref (131).

Module	Taxa	Kleinberg's hub centrality scores	Community
Pre-14 ka (high stress)	Asteraceae	1	Terrestrial plants
Pre-14 ka (high stress)	Polygonaceae	0.909	Terrestrial plants
Pre-14 ka (high stress)	Saxifraga	0.884	Terrestrial plants
Pre-14 ka (high stress)	Pedicularis	0.801	Terrestrial plants
Pre-14 ka (high stress)	Crassulaceae	0.78	Terrestrial plants
Pre-14 ka (high stress)	Bovidae	0.76	Terrestrial mammals
Pre-14 ka (high stress)	Ochotona	0.605	Terrestrial mammals
Pre-14 ka (high stress)	Rhodiola	0.495	Terrestrial plants
Pre-14 ka (high stress)	Potentilla	0.455	Terrestrial plants
Post-14 ka (low stress)	Rosaceae	1	Terrestrial plants
Post-14 ka (low stress)	Salvia	0.993	Terrestrial plants
Post-14 ka (low stress)	Equus	0.933	Terrestrial mammals
Post-14 ka (low stress)	Capra	0.928	Terrestrial mammals
Post-14 ka (low stress)	Cervus	0.921	Terrestrial mammals
Post-14 ka (low stress)	Fabaceae	0.915	Terrestrial plants
Post-14 ka (low stress)	Poaceae	0.909	Terrestrial plants
Post-14 ka (low stress)	Papaveraceae	0.867	Terrestrial plants
Post-14 ka (low stress)	Salix	0.774	Terrestrial plants
Post-14 ka (low stress)	Salicaceae	0.732	Terrestrial plants
Post-14 ka (low stress)	Ephedra	0.713	Terrestrial plants
Post-14 ka (low stress)	Rhododendron	0.707	Terrestrial plants
Post-14 ka (low stress)	Carex	0.648	Terrestrial plants
Post-14 ka (low stress)	Bos	0.632	Terrestrial mammals
Post-14 ka (low stress)	Cricetidae	0.612	Terrestrial mammals
Post-14 ka (low stress)	Apiaceae	0.582	Terrestrial plants
Post-14 ka (low stress)	Cyperaceae	0.531	Terrestrial plants
Post-14 ka (low stress)	Solanaceae	0.359	Terrestrial plants

Table S2. Identification of influential nodes (taxa) of the weighted network for the terrestrial ecosystem.

Keystone taxa (Kleinberg's hub centrality scores ≥ 0.8) are marked in bold.

Module	Taxa	Kleinberg's hub centrality scores	Community	
Pre-14 ka (high stress)	Nannochloropsis	1	Aquatic algae	
Pre-14 ka (high stress)	Pseudanabaena	0.912	Aquatic cyanobacteria	
Pre-14 ka (high stress)	Anabaena	0.817	Aquatic cyanobacteria	
Pre-14 ka (high stress)	Leptolyngbya	0.792	Aquatic cyanobacteria	
Pre-14 ka (high stress)	Oscillatoriaceae	0.773	Aquatic cyanobacteria	
Pre-14 ka (high stress)	Leptolyngbyaceae	0.762	Aquatic cyanobacteria	
Pre-14 ka (high stress)	Nostocaceae	0.745	Aquatic cyanobacteria	
Pre-14 ka (high stress)	Chamaesiphon	0.716	Aquatic cyanobacteria	
Pre-14 ka (high stress)	Nostoc	0.683	Aquatic cyanobacteria	
Pre-14 ka (high stress)	Monodopsidaceae	0.655	Aquatic cyanobacteria	
Pre-14 ka (high stress)	Oscillatoria	0.63	Aquatic cyanobacteria	
Pre-14 ka (high stress)	Calothrix	0.598	Aquatic cyanobacteria	
Pre-14 ka (high stress)	Microcoleaceae	0.549	Aquatic cyanobacteria	
Pre-14 ka (high stress)	Coleofasciculaceae	0.521	Aquatic cyanobacteria	
Pre-14 ka (high stress)	Pseudanabaenaceae	0.39	Aquatic cyanobacteria	
Post-14 ka (low stress)	Poeciliidae	1	Aquatic fish	
Post-14 ka (low stress)	Percidae	0.9	Aquatic fish	
Post-14 ka (low stress)	Myriophyllum	0.85	Aquatic plants	
Post-14 ka (low stress)	Triplophysa	0.826	Aquatic fish	
Post-14 ka (low stress)	Salmonidae	0.823	Aquatic fish	
Post-14 ka (low stress)	Planktothrix	0.819	Aquatic cyanobacteria	
Post-14 ka (low stress)	Sciaenidae	0.776	Aquatic fish	
Post-14 ka (low stress)	Cyprinidae	0.751	Aquatic fish	
Post-14 ka (low stress)	Cichlidae	0.731	Aquatic fish	
Post-14 ka (low stress)	Hydrocharitaceae	0.713	Aquatic plants	
Post-14 ka (low stress)	Catostomidae	0.688	Aquatic fish	
Post-14 ka (low stress)	Cyanobium	0.607	Aquatic cyanobacteria	
Post-14 ka (low stress)	Lutra	0.581	Aquatic mammals	
Post-14 ka (low stress)	Hippuris	0.58	Aquatic plants	
Post-14 ka (low stress)	Stuckenia	0.578	Aquatic plants	
Post-14 ka (low stress)	Calotrichaceae	0.566	Aquatic cyanobacteria	
Post-14 ka (low stress)	Microcystaceae	0.566	Aquatic cyanobacteria	
Post-14 ka (low stress)	Synechococcus	0.537	Aquatic cyanobacteria	
Post-14 ka (low stress)	Potamogetonaceae	0.511	Aquatic plants	
Post-14 ka (low stress)	Clupeidae	0.46	Aquatic fish	
Post-14 ka (low stress)	Synechococcaceae	0.365	Aquatic cyanobacteria	
Post-14 ka (low stress)	Microcystis	0.348	Aquatic cyanobacteria	
Post-14 ka (low stress)	Potamogeton	0.268	Aquatic plants	
Post-14 ka (low stress)	Prochlorococcaceae	0.237	Aquatic cyanobacteria	

Table S3. Identification of influential nodes (taxa) of the weighted network for the aquatic ecosystem.

Keystone taxa (Kleinberg's hub centrality scores ≥ 0.8) are marked in bold

		Read count	s		Percent / %)
Library batches	Sediments	Extraction blanks	Library blanks	Sediments	Extraction blanks	Library blanks
SL064L	24,953,946	268	170	99.998	0.001	0.001
SL066L	21,054,767	2,537	1486	99.981	0.012	0.007
SL067L	2,624,963	5,207	3,991	99.651	0.198	0.152
JK125L	3,678,612	7,535	/	99.8	0.2	/
JK126L	39,550,879	2135	/	99.995	0.005	/
JK130L	15,759,638	1,806	225	99.987	0.011	0.001

Table S4. The reads in sediments and blanks per sequencing pool.

	RDA with single environmental variable			Multicollinearity evaluation						
Categories	Variables	Df	Explained (adj.r.squared) / %	P-value	Variables_01	VIF_01	Variables_02	VIF_02	Variables_03	VIF_03
	Temperature	38	44.9	0.001	Temperature	35.6	/	/	/	/
	Glacier extent	38	37.3	0.001	Glacier	9.3	Glacier	2.9	/	/
Vegetation	Permafrost	38	40.1	0.001	Permafrost	18.6	Permafrost	1.9	/	/
	Herbivory	38	21.4	0.001	Herbivory	2.4	Herbivory	2.3	/	/
	Land use	38	10.8	0.003	Land use	2.1	Land use	1.3	/	/
	Temperature	38	33.3	0.001	Temperature	35	/	/	/	/
	Glacier extent	38	33.9	0.001	Glacier	11	Glacier	5	Glacier	2
Mammalian	Permafrost	38	24.2	0.001	Permafrost	20	Permafrost	6	Permafrost	2
	PC1	38	35.2	0.001	PC1	13	PC1	C1 13 /	/	/
	Land use	38	3.8	0.001	Land use	2	Land use	1	Land use	1
	Temperature	38	71.9	0.001	Temperature	35.6	/	/	/	/
Vegetation	Glacier extent	38	62.6	0.001	Glacier	9.3	Glacier	2.9	/	/
(taxa with abundance	Permafrost	38	61.8	0.001	Permafrost	18.6	Permafrost	1.9	/	/
>= 2,450)	Herbivory	38	37.7	0.001	Herbivory	2.4	Herbivory	2.3	/	/
	Land use	38	15.1	0.008	Land use	2.1	Land use	1.3	/	/
	Temperature	38	61.6	0.001	Temperature	35	/	/	/	/
Mammalian	Glacier extent	38	64.5	0.001	Glacier	11	Glacier	5	Glacier	2
(taxa with abundance	Permafrost	38	63.4	0.001	Permafrost	20	Permafrost	6	Permafrost	2
>= 2,450)	PC1	38	64.3	0.001	PC1	13	PC1	13	/	/
	Land use	38	5.4	0.069	Land use	2	Land use	1	Land use	1

Table S5. Multicollinearity evaluation and redundancy analysis (RDA) for terrestrial vegetation and mammal data.

Vegetation and mammalian: 'regularized log' transformed shotgun DNA data (in this study).

Glacier extent: simulated glacier extent covering the lake catchment / km³ was extracted from source data (45).

Permafrost: simulated permafrost extent covering the glacier-free lake catchment / % (in this study). Large herbivory: percentage of *Bos* shotgun DNA against terrestrial mammalian shotgun DNA (in this study).

PC1: values of first axis of sample scores extracted from principal component analysis of vegetation compositional changes (in this study). Land use: percentages of Rumex and Sanguisorba relative to the pollen grains of terrestrial seed-bearing plants) recorded in Lake Naleng (25, 50).

All environmental factors are standardized using 'decostand(method = "standardize")' in the 'vegan' package (121).

adj.r.squared: adjusted R2.

P-value: F-test with 999 permutations.

Values are rounded to one decimal place except for P-values.

Independent and significant variables are marked in bold for variation partitioning analysis.

	RDA	with single environmental variable	Multicollinearity evaluation			
Categories	Variables	Explained (adj.r.squared) / %	<i>P</i> -value	Variables_01	VIF_0	
	Temperature	49.6	0.001	/	/	
	Glacier mass	54.3	0.001	Glacier mass	1.1	
Aquatic ecosystem	Land use	13.8	0.004	Land use	1.1	
A 4:	Temperature	49.8	0.001	/	/	
Aquatic ecosystem (taxa with	Glacier mass	54.7	0.001	Glacier mass	1.1	
abundance ≥ 50)	Land use	13.9	0.003	Land use	1.1	

Table S6. Multicollinearity evaluation and redundancy analysis (RDA) for the aquatic ecosystem.

Glacier mass: simulated glacier volume (km³) x density of ice (Gt/km³) were calculated from source data (45).

PC1: values of PC1 extracted from principal component analysis of vegetation (in this study). Land use: percentages of *Rumex* and *Sanguisorba* relative to the pollen grains of terrestrial seed-bearing plants recorded in Lake Naleng (25, 50). All environmental factors are standardized using 'decostand(method = "standardize")' in the 'vegan' package (121).

P-value: F-test with 999 permutations

Values are rounded one decimal place except for *P*-values. Independent and significant variables are marked in bold for variation partitioning analysis.

Legends for data S1 – S3

Data S1. The final sedaDNA shotgun count data used for taxonomic composition, ordination, and network analyses, along with related environmental variables, for the terrestrial ecosystem.

Data S2. The final sedaDNA shotgun count data used for taxonomic composition, ordination, and network analyses, along with related environmental variables, for the aquatic ecosystem.

Data S3. The sedaDNA shotgun sequencing information for 40 lake sediments and 12 controls.

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