



Testate amoebae as proxies of anthropogenic and natural impacts recorded in the sediments of the Upper Balma Lake (Piedmont, Italy)

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ABSTRACT

Testate amoebae are known among the proxies for paleolimnological investigations but, despite their recognized importance and recent findings, they have not been sufficiently studied in alpine lakes, particularly in Europe. The present study analyzes fossil testate amoeba assemblages from the Upper Balma Lake (2216 m a.s.l., Western Alps, Italy) to reconstruct ecological changes over the past 1200 years. 21 species belonging to 5 genera were identified, with *Centropyxis* and *Diffugia* being the most abundant. Multivariate statistical analyses, combined with sedimentological and geochemical data, revealed five paleoenvironmental phases corresponding to known climatic events, including the Medieval Climate Anomaly, the Little Ice Age, and the Recent Warming period. Assemblage composition and diversity were related to grain size variations, nutrient input, and trace element concentrations (e.g., As, Mn, Mo) and periods of climatic stability were associated with higher biodiversity, while cold or disturbed intervals, marked by floods or sediment reworking, resulted in reduced abundances and diversity. In recent decades, a decline was observed for the genus *Centropyxis*, possibly indicating reduced oxygenation and changes in contaminant levels. Comparative analysis with the Lower Balma Lake, located downstream in the same area, highlighted the influence of morphological/hydrological differences between lakes on species distributions.

The findings underscore the value of testate amoebae as sensitive bioindicators and their applicability in paleoecological reconstructions of alpine lakes. This study contributes to understanding long-term ecological responses to climate change and provides insights into flood regime variability in high-mountain environments.

1. Introduction

Mountain lakes are worldwide recognized as key sentinels for global environmental changes, integrating physical, chemical, and biological processes within highly sensitive alpine systems. These environments are characterized by cold temperatures, strong UV radiation, and long periods of ice cover, which significantly influence ecosystem productivity and biodiversity (Wolfe et al., 2003; Hobbs et al., 2010; Moser et al., 2019). Due to their isolation, steep climatic gradients, and oligotrophic conditions, these lakes generally host few and well-adapted communities with low biodiversity and simplified trophic webs (Füterer et al., 2006; Rahbek et al., 2019; Salvi et al., 2022). All these aspects

make mountain lakes very sensitive to little changes, due to regional and global environmental transformations, and these changes can be recorded in their sediments (Catalan et al., 2009; Williamson et al., 2009; Litaor, 2022). Such environments are ideal for paleolimnological research, as their sediments archive both natural and anthropogenic changes on decadal to millennial timescales (Catalan et al., 2013; Steffen et al., 2015). In this context, paleoecological reconstructions based on data from subfossil species associations have become essential tools for reconstructing environmental changes over long timescales (ranging from hundreds of years to millennia) (Willis and MacDonald, 2011). This approach provides valuable insights into variations in abiotic parameters such as temperature, pH, nutrient availability, and trace element

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contamination, although it offers limited information regarding changes in functional traits (Macumber et al., 2020; Marcisz et al., 2020).

Among the organisms suitable for such investigations, testate amoebae, a polyphyletic group of protists, are particularly well-suited for functional trait-based approaches. Testate amoebae are single-celled, free-living eukaryotes (Kingdom Protista; Phylum Protozoa), characterized by the presence of a shell (test) that protects the cytoplasm from negative environmental influences such as predation and dehydration (Ogden and Hedley, 1980; Adl et al., 2018; Todorov and Bankov, 2019). The shell is not only protective, as it is also involved in predation, facilitating the amoebae's attack of large prey (Dumack et al., 2024). These organisms are abundant in freshwater lakes and rivers worldwide (Asioli et al., 1996; Roe and Patterson, 2006; Yang et al., 2010; Burdřková et al., 2012; Davidova and Vasilev, 2013; Ju et al., 2014) and play an important role in lake ecosystems, contributing to material cycling, energy flow, and water purification (Han et al., 2011a; Ogden and Hedley, 1980). Testate amoebae from lake sediments have been documented at the species level and used to reconstruct past climates and environments (Asioli et al., 1996; Wall et al., 2010; Marcisz et al., 2016; Van Bellen et al., 2017; Tsyganov et al., 2019; Ndayishimiye et al., 2020a, 2021; Ndayishimiye et al., 2020a; Ndayishimiye et al., 2021; Rodas-Morán et al., 2022; Qin et al., 2024; Sysoev et al., 2024).

Besides, testate amoebae are particularly suited as bioindicators due to their sensitivity to environmental changes. In fact, the populations of testate amoebae can vary in response to: 1) natural climate variability (Han et al., 2011b; Burdřková et al., 2012; Ren et al., 2018), 2) latitude and temperature (species being abundant in subtropical floodplains than in alpine lakes) (Yang et al., 2010; Ju et al., 2014). Finally, it has been shown that the shape of the test is related to certain environmental conditions in lakes: in particular, pyriform-shaped shells are associated with oligotrophic environments with high dissolved oxygen levels, whereas more ovoid shells are found in eutrophic environments with greater macrophyte abundance, or even cap-shaped shells are observed under stressful conditions (Marcisz et al., 2020). A recent study conducted in the Western Alps revealed significant changes in testate amoeba assemblages along a 2000-year sedimentary profile of an alpine lake (Salvi et al., 2022). These changes were associated with variations in trace elements, nutrients, and grain size, and can be linked to pluvial and drought events that occurred over the past 2000 years. However, despite their recognized importance and recent findings, these organisms have not been sufficiently investigated in alpine lakes, as this is the only study in Alpine mountains. Therefore, further investigations into paleoenvironmental reconstructions using testate amoebae are of great interest. To this aim, we extended our analyses to the Western Alps, focusing specifically on Upper Balma Lake (Piedmont, Italy), a site where testate amoebae had not previously been studied.

This study examines the record of testate amoeba communities, along with trace elements (Pb, Mn, Zn, As, Cd), sediment characteristics (percentages of sand, silt, and clay), and nutrient information (percent N, total organic carbon, and C/N ratio) from a sediment core of Upper Balma Lake. The main objectives were: (i) to characterize the fossil testate amoeba communities within the lake's sedimentary sequence; (ii) to investigate the response of testate amoeba communities to major climatic phases and environmental changes that occurred during the late Holocene (approximately the last 1200 years); and (iii) to assess the influence of environmental and ecological processes, as well as potential anthropogenic impacts, on the testate amoeba community throughout different stages of the lake's history.

2. Methods

2.1. Study area

The study site is represented by the Upper Balma Lake, a typical alpine lentic environment (*sensu* Füreder et al., 2006; Fjellheim et al., 2009), located in the Cottian Alps (Municipality of Coazze, Piedmont,

Northwest Italy) at 2216 m above the sea level (a.s.l.) within the alpine belt, above the tree line (Fig. 1a and b). The Upper Balma Lake is a glacial origin basin, S-shaped, with two sub-basins separated by a shallow mid-section (Fig. 1b). The perimeter is 774 m, whereas the surface area is equal to 1.82 ha and maximum depth is 2.77 m.

Ice cover generally lasts from late October to late May/early June. The main catchment core is composed of ophiolite metamorphic bedrock, and the landscape is dominated by rocky outcrops, ridges, and mountain walls. A little meadow area is situated at the southern side of the lake, where a small inlet flows. The outlet originates from the north-eastern corner of the lake, flowing downstream where it becomes the inlet of the Lower Lake. The Upper and the Lower Lake represent a two alpine lake system, previously described by Bertoli et al. (2023). Both lakes are included in the Special Area of Conservation (SAC) and Special Protection Area (SPA) IT1110006 “Orsiera Rocciavre” and in the Orsiera Rocciavre Natural Park.

Most consistent impacts for the area over the last four decades of the 20th century are represented by the long-distance airborne transport of contaminants from the urban areas in the plain, grazing activities, and fishing (Pastorino et al., 2020a, 2020b; Perilli et al., 2020; Cantonati et al., 2021; Bertoli et al., 2023). Although the Balma Lakes were originally fishless, the brook trout *Salvelinus fontinalis* Mitchell, 1814 was introduced for recreational fishing in the 1970s (Balma et al., 1992; Pastorino et al., 2020b; Perilli et al., 2020; Cantonati et al., 2021; Salvi et al., 2022; Bertoli et al., 2023). Mean values of the main chemico-physical characteristics (such as temperature, dissolved oxygen, pH, electrical conductivity) were measured *in situ* using field probes and recorded during 2021 are reported in Table 1. Annual total rainfall in the period 2021–2024 ranged between 719.8 mm year⁻¹ (2022) and 2024 mm year⁻¹ (2024) (data obtained from the Regional Agency for Environmental Protection of the Piedmont Region, for the municipality of Coazze <https://www.arpa.piemonte.it>).

2.2. Core sampling and sediment characterization

A coring cruise activity was conducted in July 2021, using a 50 mm gravity Kajak-type sediment corer (Kajak et al., 1965; Renberg, 1991; Salvi et al., 2022). One 38 cm long core was extracted from a single sampling site in the deep area of the lake (Fig. 1b), sealed in a sampling tube, brought to the laboratory, and stored at −20 °C. Subsamples were obtained by cutting the core in 37 transverse sections of approximately 1 cm each, as described in the previous study carried out in the same site by Bertoli et al. (2024). Each section was named using the letter “L” followed by a number from “0.5” to “36.5”, indicating the mid-depth of each section (L0.5 = top section; L36.5 = bottom section) and then were frozen. The age-depth model used for the present study (Fig. 2) was developed for the Upper Balma Lake by Bertoli et al. (2024) using the RStudio package *rbacon*, updated to version 3.10 (Blaauw, 2010; Blaauw et al., 2025), which uses Bayesian statistics to rebuild accumulation histories. To achieve this aim, radiocarbon dating (¹⁴C AMS) and total lead (Pb) concentrations, determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), were analyzed. The radiocarbon ages were measured at the Poznan Radiocarbon Laboratory (Poznan, Poland), using the dataset reported by Heaton et al. (2020) for calibration. Lead pollution was integrated to build age–depth model (Renberg et al., 2001; Arnaud et al., 2003, 2004; Spadini et al., 2003; Camarero et al., 2009; Giguet-Covex et al., 2012; Perilli et al., 2020; Nedjai et al., 2021).

The sedimentation rate calculated from the model was equal to 0.029 cm year⁻¹ (Bertoli et al., 2024).

Sedimentological analyses were formerly partially reported in Bertoli et al. (2024); the grain size analysis is deepened in the present study. For the analysis, each section was filtered with a 1000 µm. The residues on 1000 µm were put aside whereas the remaining sediment was subsequently processed using a Malvern Mastersizer 3000 laser diffraction particle size analyzer, based on the technique of laser diffraction to measure the particle size by measuring the angular change

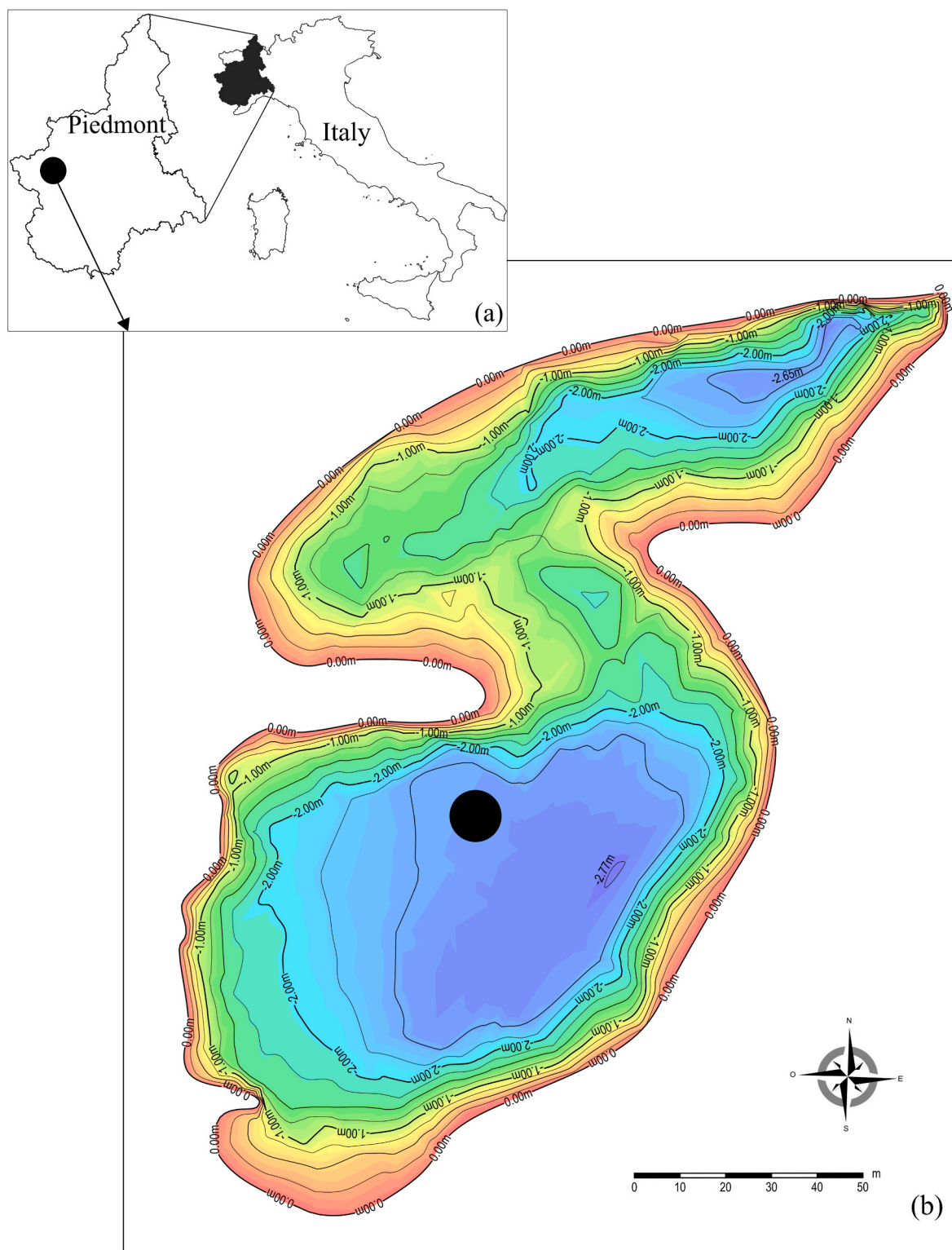


Fig. 1. Study area (a) and location of the sampling site in the Upper Balma Lake (b).

in the intensity of scattered light as a laser beam passes through a dispersed particulate sample, for both red and blue light wavelengths. Large particles scatter light at a small angle to the laser beam, and small particles scatter light at a large angle. The intensity of light is analyzed to calculate the size of the particles that created the scattering pattern. One percentile $C_{\mu m}$ (coarser granule of the sediment) and median diameter $Md_{\mu m}$ were considered as indices of high transport capacity.

2.3. *Testate amoeba* associations

Samples equal to 1 cm^3 of freeze-dried samples were cleansed with distilled water and then gently mixed for 5 min. Subsequently, samples were wet sieved, first using a $300\text{ }\mu\text{m}$ sieve and then a $25\text{ }\mu\text{m}$ sieve, in order to remove large/fine organic particles and mineral detritus, but also to recover the small specimens. The $25\text{--}300\text{ }\mu\text{m}$ sediment fractions were washed into beakers and diluted to 50 ml distilled water and later

Table 1

Mean values and standard deviations of chemico-physical parameters measured at the Balma Lake during the project including the present study. Data recorded in 2021.

Parameter	Mean	±	S.D.
Temperature (°C)	8.61	±	2.94
Dissolved oxygen concentration (mg L ⁻¹)	6.50	±	0.33
pH	7.40	±	0.19
Conductivity (µS cm ⁻¹)	12.81	±	1.35
Alkalinity (mg/L)	47.31	±	16.60
Secchi Disk (m)	1.1	±	0.1
NH ₄ ⁺ (mg L ⁻¹)	0.04	±	0.02
NO ₃ ⁻ (mg L ⁻¹)	2.75	±	1.47
P (mg L ⁻¹)	0.05	±	0.02

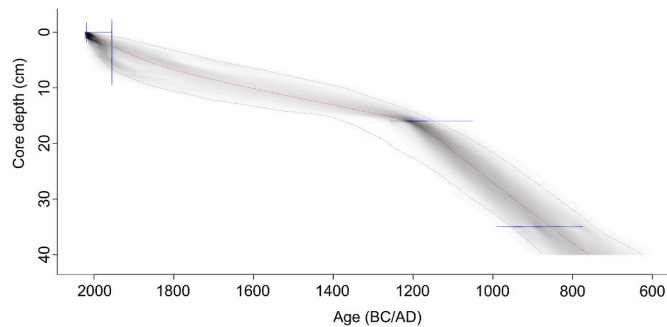


Fig. 2. Bayesian age-depth model calculated for the Upper Balma Lake (from Bertoli et al., 2024); the model is based on 4000 interactions Markov Chain Monte Carlo. The dark gray areas are the more precise dates, those in light gray the less precise; the best age estimate for each level is represented by the red line, while the black dashed lines represent the 95% confidence intervals. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

dried (Ndayishimiye et al., 2019). Testate amoebae were then extracted and mounted on slides. Their identification was carried out using a light stereomicroscope at 200–400× magnification (enlargements from 2.5× to 10.0×) LEICAMZ12. Identification and classification were achieved through the following papers considering the morphology and the materials of the shell (Mazei and Tsyganov, 2006; Mazei and Warren, 2012, 2014, 2015; Todorov and Bankov, 2019; Siemensma, 2021). For each subsample, at least 260 individuals were counted and identified; generally, an amount of 150 individuals is considered adequate to obtain information about paleolimnology, composition of testate amoeba community, the correct estimation of the rare species abundances and statistical analysis (Patterson and Fishbein, 1989; Payne and Mitchell, 2008; Wall et al., 2010; Ndayishimiye et al., 2020a). For each subsample testate amoeba densities were calculated (ind g⁻¹).

2.4. Statistical analysis

Collected data were used to investigate differences among testate amoeba assemblages along the core profile and the relationship between biotic and abiotic characteristics. All species were considered, and a dissimilarity matrix of Bray-Curtis was produced after data transformation ($\sqrt{[x+1]}$). A Q-mode hierarchical cluster analysis with the Ward's method of agglomeration was carried out and Principal Components Analysis (PCA) was performed using the Q-mode and the VARIMAX rotation (Malmgren and Haq, 1982; Palazzesi and Barreda, 2012; Auer et al., 2014; Majewski et al., 2018; Salvi et al., 2022). The cluster analysis and PCA were produced using statistical package Xlstat software Addinsoft, 2020 (XLSTAT statistical and data analysis solution, New York, USA). Non-metric Multidimensional Scaling (NMDS) was performed to summarize variations among cluster-grouped sections. For this purpose, testate amoeba densities in each sample (ind g⁻¹) were

considered, data were transformed prior to analyses ($\log(x+1)$) in order to reduce the influence of very abundant taxa (Clarke and Gorley, 2006), and a resemblance matrix was subsequently obtained using the Bray-Curtis measure. The NMDS was considered good if stress <0.2 (McCune et al., 2002; Fetzner and Taylor, 2018). For this analysis, only groups where species were present have been considered. Then, for each core section, three sub-samples were considered. To investigate differences among the communities observed in the section groups identified through cluster analysis, the homogeneity of group dispersion was tested using the PERMDISP method (Anderson, 2006). As the test was significant, the one-way ANOSIM was chosen (factor “Group”) (Clarke, 1993) to check for significant differences among identified groups. These analyses were performed using 9999 permutations. Contribution of the species to the observed variability was tested with the SIMPER test (Clarke, 1993). The contribution to testate amoeba community variation due to abiotic data was estimated using variation partitioning analysis (VPA) (Borcard et al., 1992), based on a Redundancy Analysis (RDA) (Ter Braak and Smilauer, 1998; Legendre and Legendre, 1998). In this context, abiotic features are considered as affecting the biotic compartment. For this analysis, abiotic data reported by Bertoli et al. (2024) (Fig. 3), obtained from another core sampled in the same lake and in the same sampling campaign, were used. Abiotic data regard concentrations of trace elements (Pb, Mn, Zn, As, Cd) (µg g⁻¹) along the core profile, sediment characteristics (% of sand silt and clay) and information about nutrients (% N, total organic carbon and C/N ratio). For the purposes of the present study, the testate amoebae dataset was transformed using the Hellinger transformation (Legendre and Legendre, 1998), whereas the abiotic data matrix was standardized. Before using the RDA, the axis length was tested using the Detrended Correspondence Analysis (DCA) (Hill and Gauch, 1980). To avoid multicollinearity, correlation analysis was performed using the Pearson correlation coefficient, choosing a threshold values $r > |0.7|$ and $p < 0.001$ as criteria to exclude variables from the analysis (Dormann et al., 2013). The total explained variance within the testate amoebae dataset was partitioned among three groups of variables associated with sediment characteristics (C_μ, M_μ and %sand), trace element concentrations along the core profile (As, Mn and Mo) and nutrient information (C_{tot}, and C/N). The contribution of each variable group was then plotted as a Venn diagram. The significance of each variable group and relative interactions were checked using the Monte Carlo permutation test with 9999 permutations.

All analyses were performed using RStudio, version 2025.5.0.496 (Posit Team, 2025; R Core Team, 2025), with a 0.05 *p*-level for significance. Figures are produced with RStudio and processed with software Inkscape version 0.92.

3. Results

3.1. Grain size analyses

The Upper Balma Lake core showed two main depositional stages: from the top of the core to section.

L12.5 the phase is characterized by a massive sediment without evident lamination and structures instead, from section L13.5 to the core bottom a lamination phase is evident with the alteration of coarse material and fine sediments (Fig. 4). The grain size analysis recorded the presence of two different kinds of sediment according to the Nota classification (1964), identifying them as very sandy pelite and pelitic sand. Inside the different core levels, it was possible to highlight three different sediment deposits: matrix-supported sediment, grain-supported sediment, and laminated deposits. Matrix-supported sediment deposits (level L21.5) (Fig. 4) are characterized by high sorting values and the absence of a clayey layer at the top. Grain-supported sediment deposits (upper core sediments, L17.5–L19.5, L29.5–L30.5 intervals) are characterized by a well-sorted base, while laminated deposits are those at bottom core levels and L28.5–L22.5, L16.5–L11.5

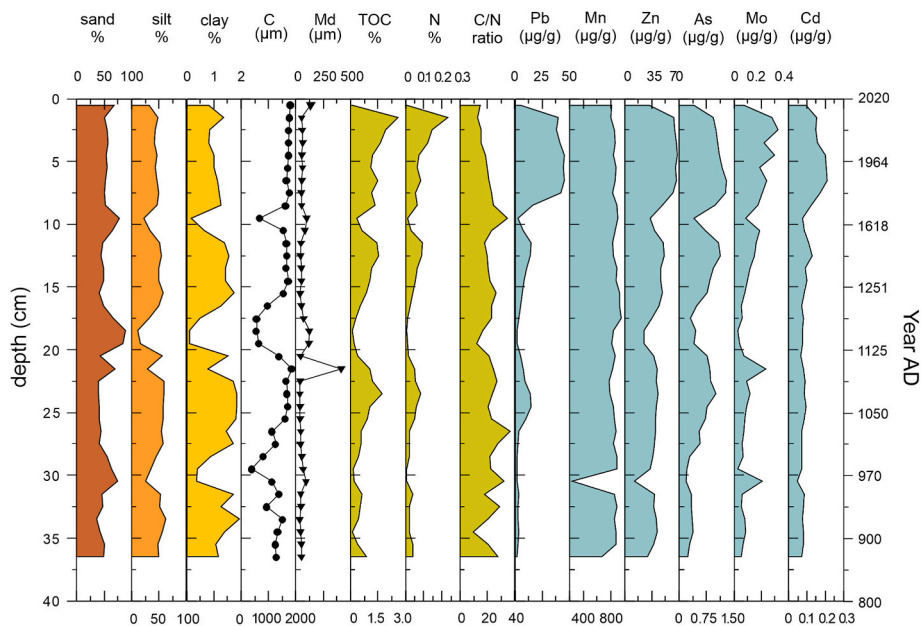


Fig. 3. Stratigraphic diagram of the sedimentological and geochemical parameters measured in core sections sampled in the Upper Balma Lake, presented by Bertoli et al. (2024) and included in the RDA analysis (orange-yellow scale indicates grain size composition, gold indicates the nutrients; light navy indicates the trace elements). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

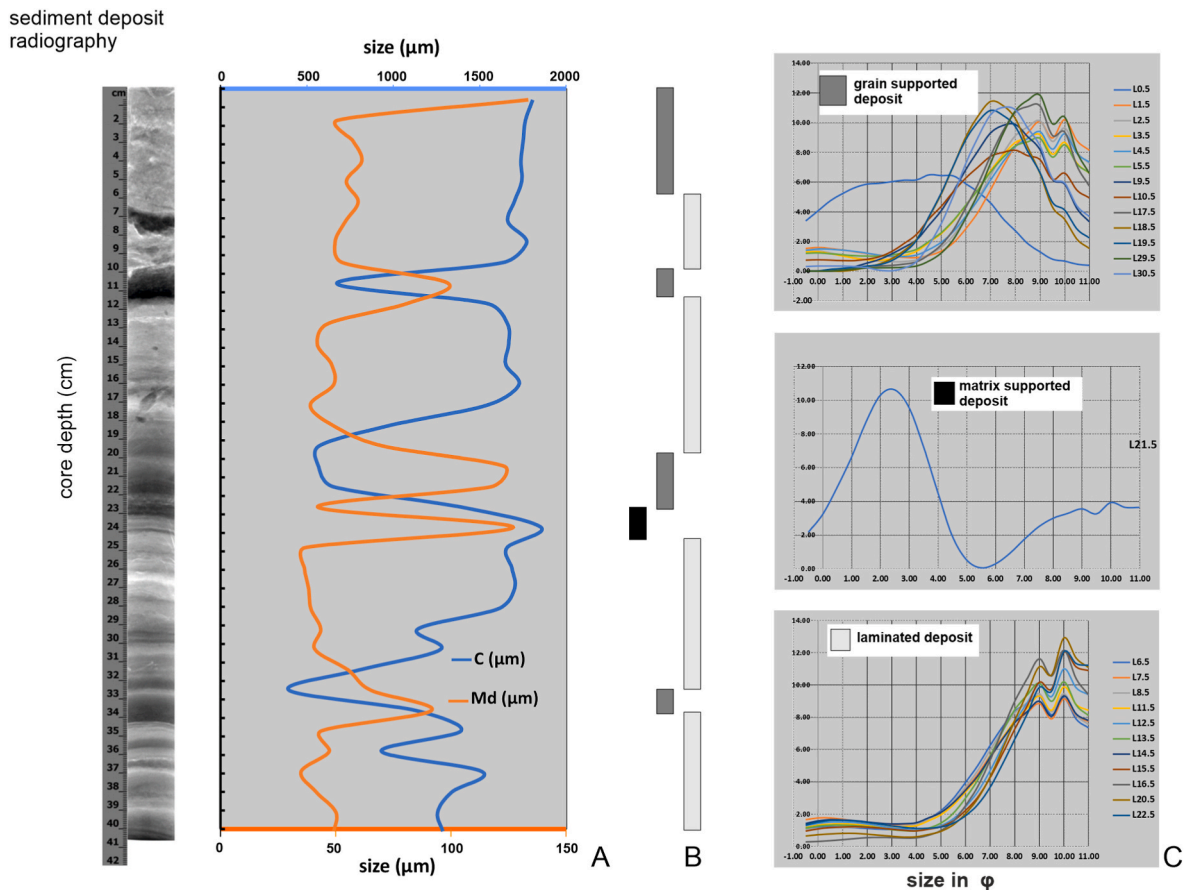


Fig. 4. Lithological description measured by XRF, and profiles versus depth of the C (coarsest grain) and the Median (Q50-median grain size) are presented in A. The different deposit thicknesses of lake sediment-core lithological units representing grain-supported sediments (flood deposits), matrix-supported sediments (gravity reworked sediment deposits) and laminated deposits are introduced in B. Grain-size distributions (phi φ - scale) of laminated sediments, grain-supported and matrix-supported sequences are shown in C. from Giguët-Covex et al. (2012) modified.

intervals.

3.2. Testate amoeba community

Analyses identified 8964 testate amoebae individuals, belonging to 5 genera (*Arcella*, *Centropyxis*, *Diffflugia*, *Lesquereusia*, and *Pontigulasia*) and 21 species of the order *Arcellinida*.

The average density along the core was 240 ± 1156.65 ind g^{-1} , ranging from 146.15 ind g^{-1} (L0.5) to 2727.27 ind g^{-1} (L20.5). No specimens were recorded in L18.5, L19.5, L29.5, and L30.5. Taxa richness ranged from 5 species (L12.5, L16.5) to 17 species (L1.5) and was generally higher in the upper core levels (L0.5–L6.5) (Fig. 5a). The Shannon–Wiener index ranged from 0.94 to 2.31, with moderate values in upper and lower sections and lower diversity in several intermediate levels (Fig. 5b). Dominance ranged between 0.127 and 0.491 while Evenness showed a similar trend (Fig. 5c and d). Two genera, *Centropyxis* and *Diffflugia*, occurred throughout the core. *Centropyxis* was the most abundant (6820 individuals; mean density 880 ind g^{-1}), mainly represented by *C. cassis* and *C. aerophila*, with other species occurring sporadically. *Diffflugia* was the second most abundant (2053 individuals; mean density 264.9 ind g^{-1}), dominated by *D. penardi*, *D. rubescens*, *D. viscidula*, *D. pulex*, and *D. globulosa*, while several other species were rare or discontinuous (Fig. S1).

Additional taxa included *Arcella hemispherica*, which was most frequent in the lower and central sections of the core, while *Lesquereusia spiralis* and *Pontigulasia bigibbosa* occurred sporadically and mostly in upper levels. Q-mode PCA identified four main components explaining 54.5% of the variance, highlighting species such as *D. penardi*, *D.*

rubescens, *D. globulosa*, *D. viscidula*, *D. pulex*, *C. aerophila*, *C. cassis*, *A. hemispherica*, *C. aculeata*, *C. elongata*, *D. viscidula*, and *D. globulosa* as the most abundant and common along the core.

3.3. Cluster analysis

The cluster analysis allowed the identification of 3 clusters based on the different testate amoebae associations (Fig. 6). Cluster 1 represents the top of the core (L0.5–L3.5, between AD, 2020 and 1983), including the levels with the lowest mean density, which ranged between 146.15 ind g^{-1} (L0.5) and 953.33 ind g^{-1} (L2.5), but they presented more diversity in species than the other groups (range of the Shannon Wiener Index 1.90–2.34; Fig. 5). Cluster 2 included most layers between L9.5 and L17.5 (aged AD 1656–1175), and the bottom of the core (L35.5–L36.5, aged AD 888–868). Additional layers enclosed in this cluster are L4.5 (AD, 1971), L28.5 (AD 995) and L32.5 (AD 933). Values of mean density ranged between 981.45 ind g^{-1} (L10.5) and 1712.50 ind g^{-1} (L4.5), and the densities of *Centropyxis* were higher than those in cluster 1 but lower than those in cluster 3 (Fig. S1, Fig. 6). Cluster 3 showed the highest mean densities and was divided into two sub-clusters: 3A and 3B (Fig. 6). Sub-cluster 3A includes layers from L5.5 to L8.5 (from AD, 1958 to AD 1732), layers L15.5 (AD 1215), L25.5 (AD 1043), L27.5 (AD 1011), L31.5 (AD 948) and the interval L33.5–L34.5 (AD 918–903) where mean density ranged between 1622.22 ind g^{-1} (L7.5) and 3000 ind g^{-1} (L25.5), and several species of the genus *Diffflugia* were observed. Sub-cluster 3B included the layers between L20.5 and L24.5 (AD 1115–

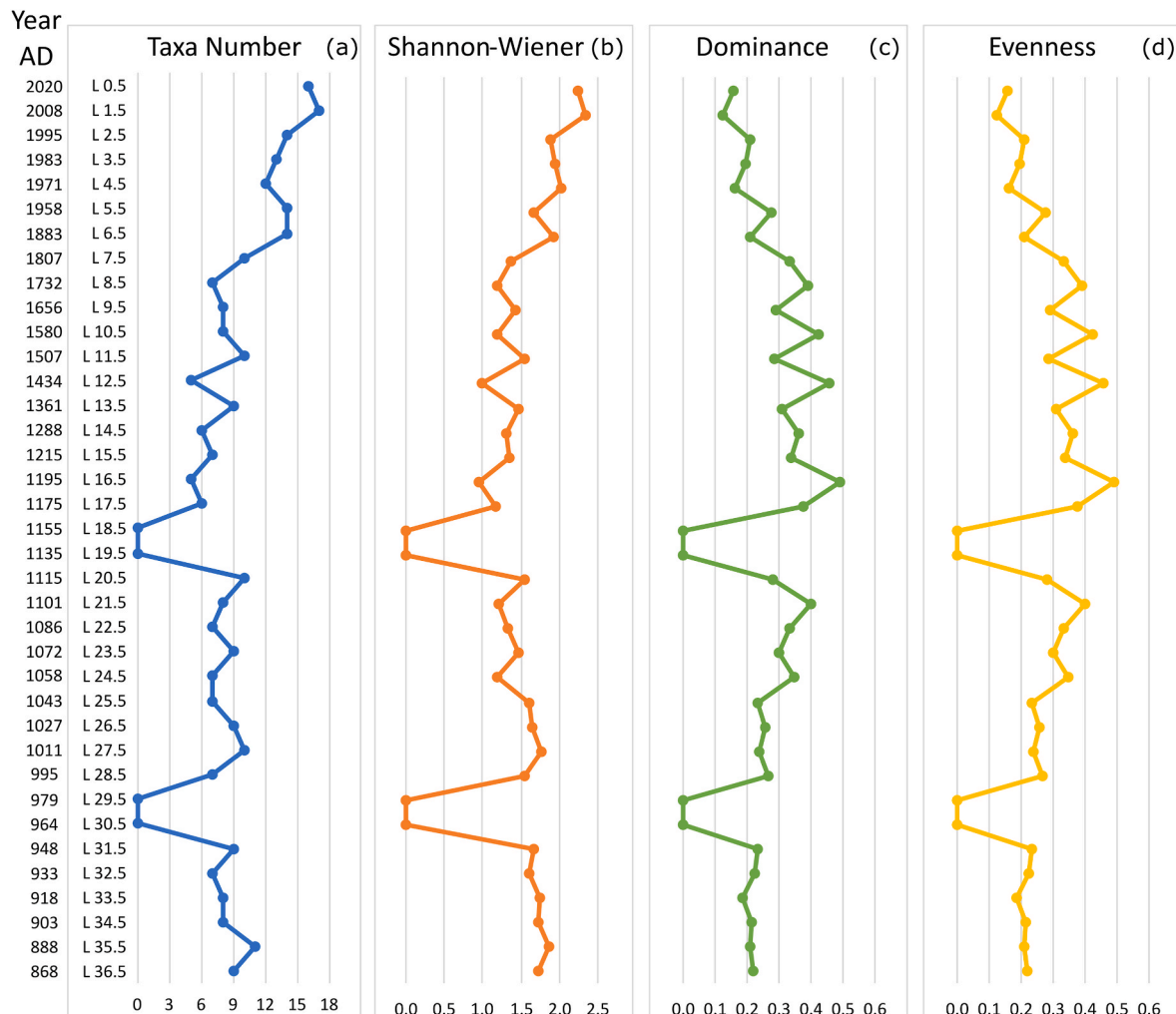


Fig. 5. Trends of the main community indices calculated for testate amoebae assemblages along the Upper Balma Lake core.

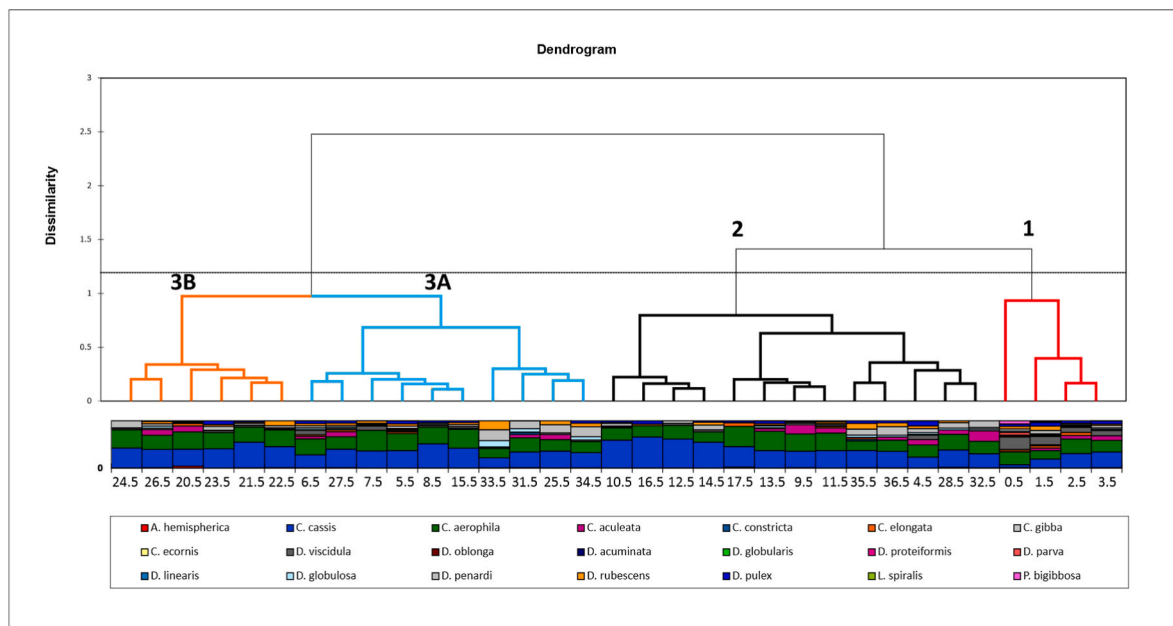


Fig. 6. Cluster analysis related to the testate amoebae associations that define the groups along the core. The different colors of the dendrogram correspond to the different groups: cluster 1 in green, cluster 2 in red and cluster 3A and 3B in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

1058) and the section L26.5 (AD 1027). Mean density ranged between 2545.45 ind g^{-1} (L23.5) and 4516.67 ind g^{-1} (L26.5), and also the presence of the genus *Centropyxis* was greater than in other clusters (Fig. S1, Fig. 6).

Groups highlighted by the cluster analysis were investigated via NMDS ordination (Fig. 7). The NMDS converged on a two-dimensional solution with an acceptable stress level (non-metric fit $r^2 = 0.98$; linear fit $r^2 = 0.95$; stress = 0.144). Groups represented by specimens observed in empty sections were excluded. Samples related to the upper core sections (L0.5–L3.5) associated with the cluster 1 take place in the left portion of the plot, close to species such as *D. oblonga*, *D. viscidula*,

P. bigibbosa and *D. parva*. Layers associated with cluster 2, 3A and 3B partially overlapped, with some species such as *C. cassis*, *C. aerophila*, *A. hemispherica*, *D. viscidula*, *C. aculeata*, *D. penardi*, *D. globulosa* and *D. rubescens* placed more centrally within the plot.

The application of the ANOSIM allowed to highlight significant differences among all the observed clusters (ANOSIM $R = 0.626$, $p < 0.001$). The SIMPER test highlighted that the species with the highest contribution to the observed variability were *C. cassis*, *C. aerophila*, *D. penardi*, *C. aculeata* and *D. rubescens*. All these species together contributed to 82.5% of the observed dissimilarity. The contribution due to other taxa was less than 5.0%, although significant (Table 2).

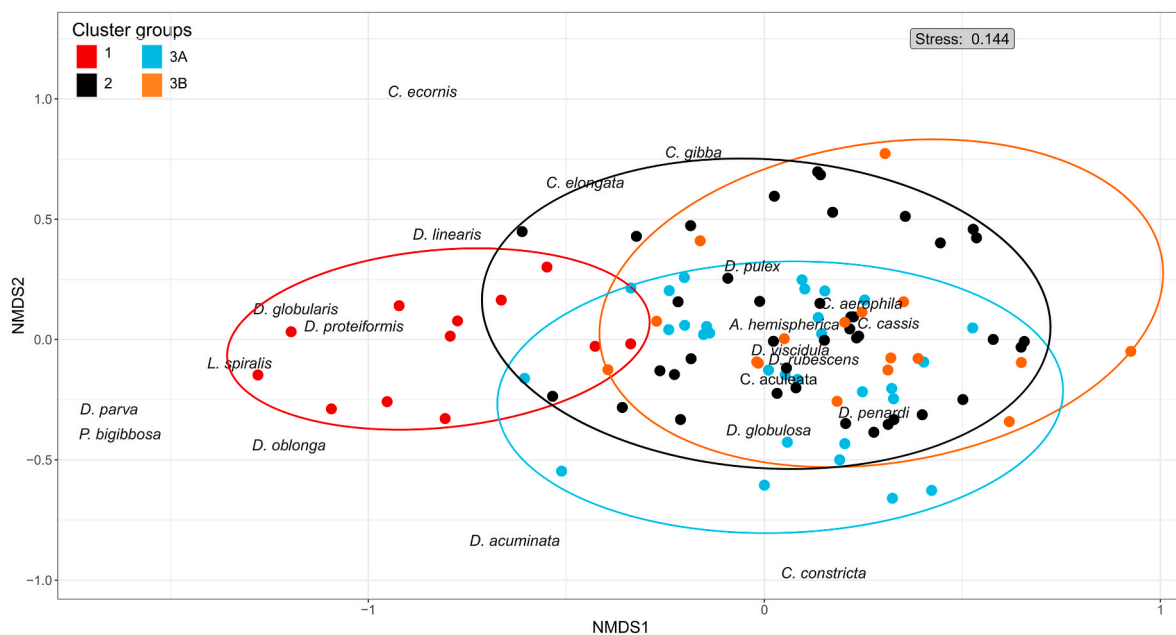


Fig. 7. Non-Metric Multidimensional Scaling (NMDS) performed on the testate amoeba densities obtained from the sections of the Balma Lake core. Colors representing the groups are the same used for the cluster in Fig. 6; The main testate amoeba taxa are indicated close to each sample (a) and subsamples for each section are also reported (b). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2

Results of the SIMPER test regarding the differences found between the groups identified in the core. The taxa whose presence significantly contributes to the dissimilarity in percentage >1% are reported.

Taxon	Av. dissim	Contrib. %	Cumulative %
<i>C. cassis</i>	15.26	31.97	31.97
<i>C. aerophila</i>	12.68	26.56	58.53
<i>D. penardi</i>	4.97	10.42	68.94
<i>C. aculeata</i>	3.53	7.40	76.35
<i>D. rubescens</i>	2.96	6.20	82.55
<i>D. viscidula</i>	1.94	4.06	86.60
<i>D. globulosa</i>	1.84	3.86	90.46
<i>D. pulex</i>	1.73	3.61	94.08
<i>C. elongata</i>	0.92	1.93	96.01
<i>A. hemispherica</i>	0.62	1.30	97.30

Regarding the relationships between testate amoebae and abiotic features, Pb is strongly correlated with other trace element ($r > 0.90$, $p < 0.001$), whereas Cd and Zn showed values of the Pearson coefficient higher than 0.75 ($p < 0.001$); therefore Mo, Mn and As were considered, because the correlation values were acceptable for the analysis ($r < |0.61|$, $p < 0.05$). The percentages of sand, silt and clay were strongly correlated ($r > 0.90$, $p < 0.001$), therefore, only sand percentage was considered to represent the sediment characteristics, together with C_p and M_d, as they showed values of the Pearson coefficient lower than the threshold value ($r < |0.55|$, $p < 0.05$). Nutrient parameters, TOC (total organic carbon) and TN (total nitrogen) were strongly correlated ($r = 0.89$, $p < 0.001$), so TOC and C/N were considered. Therefore, the variables used for the Variance Partitioning analysis were: Mo, Mn, As among the trace elements; C_p, M_d and % sand for the sediment characteristics; TOC and C/N for the nutrients.

The results of the analysis of the partitions of the variance are shown in Table 3 and in Fig. 8. The three groups of variables considered explain overall the 51.4% of the variability observed. Among individual fractions, concentrations of trace elements along the core profile explained 13.0% of the variation, followed by nutrients 7.0%, while sediment characteristics explained 21.0%. The combination between sediment characteristics and nutrients explained 3.0% of the variation, while the combination between the three groups was equal to 6.0% (Fig. 9).

4. Discussion

4.1. Grain size analysis

The grain size analysis of the Upper Balma Lake core showed depositional characteristics related to precise environmental and climatic events, previously analyzed in the northwestern French Alps (Lake Anterne, Lake Allos) (Giguet-Covex et al., 2012; Wilhelm et al., 2012; Arnaud et al., 2016). Three main deposit types were identified: matrix-supported, grain-supported, and laminated.

Matrix-supported sediments (e.g., level L21.5) (Fig. 4) are highly

Table 3

Outcomes of Variance Partitioning Analysis (VPA) conducted on testate amoebae assemblages found in sediment core samples from the Upper Balma Lake. VPA variable groups included nutrients, trace elements and sediment properties.

Groups of variables	r^2	Adjusted r^2	d.f.	F	p-level
Complete model	0.554	0.514	Model Residuals	9 101	10.514 0.001
Nutrients (TOC + C/N + N)	0.386	0.363	Model Residuals	4 106	13.544 0.001
Trace elements (As + Mo + Mn)	0.206	0.184	Model Residuals	3 107	8.217 0.001
Sediment (C _p + M _d)	0.347	0.327	Model Residuals	3 107	13.857 0.001

sorted and lack a clay cap. These deposits, associated with slope failure, are interpreted as resulting from gravity-driven, fluidized flows (Arnaud et al., 2002). A possible trigger is a seismic event recorded in 1155 AD near Lons-le-Saunier (Doubre et al., 2021), which coincided with a notably cold decade (Büntgen et al., 2006; Esper et al., 2024). Similar lacustrine seismo-turbidite deposits related to seismic events have been recorded in the Lake Anterne, NW Alps (Arnaud et al., 2002) and in the Lake of Cavazzo NE Italy (Polonia et al., 2021). This conclusion agreed with paleolimnological analyses, which highlighted the absence of testate amoebae at the sections just above (L18.5, L19.5), corresponding to the period 1155 AD. Same results were observed also in the subfossil chironomid assemblages of the Upper Balma Lake at the same core level and same age (Bertoli et al., 2024).

Grain-supported sediments (e.g., upper core levels, L17.5-L19.5, L29.5-L30.5) (Fig. 4) display well-sorted bases, indicating transport by water currents (Mulder and Alexander, 2001). These deposits are interpreted as the result of hyperpycnal turbidity currents triggered by floods in the catchment area (Arnaud et al., 2002). The size of the sediment transported is directly related to the velocity of the flow (Pidwirny and Sidney, 2008). Historical and ongoing monitoring of sediment transfer processes in the catchment confirms that sediment transport to the lake occurs predominantly during rainfall events rather than during seasonal snowmelt (Enters et al., 2009). In the Upper Balma Lake sediments, these rainfall events are associated with cold climatic phases, especially during the Little Ice Age (Büntgen et al., 2006, 2020, 2021a). Laminated deposits, found in lower core levels and in intervals L28.5-L22.5, L16.5-L11.5 (Fig. 4), also result from precipitation events (Enters et al., 2009). These monitoring efforts have not found any significant contribution from snowmelt, as its rate is likely insufficient to generate the necessary stream power for particle erosion and transport. The laminations at the Upper Balma Lake do not follow a seasonal sedimentation pattern but instead consist of individual flood deposits triggered by precipitation events, similar to grain-supported sediments. The darker gray laminae, made of coarser particles, represent the maximum stream power during the event (Cockburn and Lamoureux, 2008). These laminae are deposited at the lake bottom through hyperpycnal turbidity currents (Mulder and Alexander, 2001). The lighter-colored laminae, consisting of fine silt, reflects the settling of fine sediments when flow velocity decreases (Fig. 4). These fine-textured laminae are not necessarily associated with sedimentation beneath ice cover in winter. The Upper Balma Lake laminated deposits could be mainly associated with the climatic phase known as the Medieval Climate Anomaly (MCA).

4.2. Paleolimnological analysis

A high rate of testate amoebae individuals was identified in the Upper Balma Lake core: the dominant taxon is *Centropyxis*, characterized by a discoid, flattened, beret-shaped shell, and with mineral particles or diatoms cemented together by an organic material. The second most abundant taxon is *Diffugia*, a genus characterized by a test formed mainly by exogenous mineral particles that influence the shape and the dimension of the shell between the different species. The variations recorded in testate amoebae associations at the different sediment core levels determined through cluster analysis and principal component analysis allowed us to suppose the following sequence of paleoenvironmental and paleoclimate events (Fig. 9).

- Interval 1 (L36.5-35.5/868-888 AD): the bottom core section is characterized by growing values of the species associated with cluster 2: *D. rubescens*, *D. penardi*, *D. globulosa* and *D. acuminata*. Other species, pertaining to the cluster 3B, such as *C. cassis*, *C. aerophila*, *C. aculeata* and *A. hemispherica* are present. C/N value is equal to 27.41, indicating sediments with abundant terrestrial input of organic matter. High values of Shannon-Wiener index together

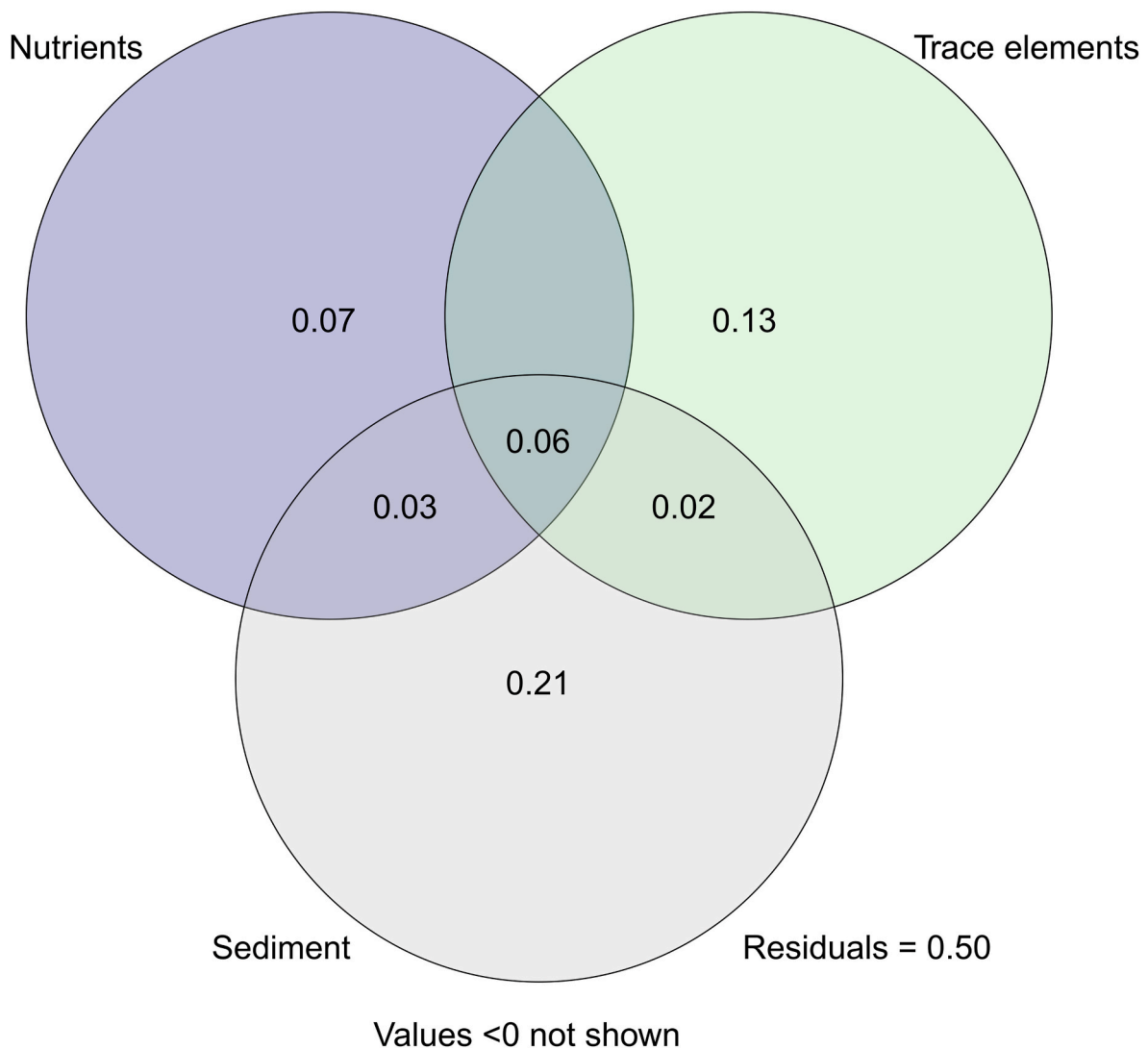


Fig. 8. Venn diagrams showing the variance partitioning (VPA) results for the variable groups associated with sediment characteristics, nutrients and trace elements along the core profile. Unexplained variance and explanations <1% are not shown.

with laminated deposition in the lake basin record a stable climate environment.

- Interval 2 (L34.5-L16.5/903-1195 AD): the interval covers the period named Medieval Climate Anomaly (MCA, around 900-1200 AD) (Büntgen et al., 2016, 2021a; Esper et al., 2024). The climatic stability, which affected European areas for about two centuries, is evidenced by higher qualitative/quantitative values of testate amoeba assemblage. The MCA period is characterized by the two warmest decades: the first, known as the Medieval Drought, around the year 1000 AD, while the second warm decade occurs just before the year 1200 AD (Büntgen et al., 2006, 2016; Esper et al., 2024). The present species are mainly associated with cluster 3B, *C. cassis*, *C. aerophila*, *C. aculeata* and *A. hemispherica* (Fig. 9).

D. viscidula and *D. oblonga* (cluster 3A), are recorded in this interval with lower density than in the others, most likely due to their association with cold precise climate phases during MCA. *C. aculeata* is considered a good indicator of oligotrophic conditions (Lorencová, 2009). Previous studies (Wall et al., 2010; Ndayishimiye et al., 2020a) suggest that warmer climatic periods could be dominated by small species such as *C. cassis*, *D. rubescens*, *D. penardi* and *D. globulosa*. Within this interval, the testate amoebae assemblages recorded two clear events presumably related to climatic events. The interval between L18.5-L19.5 (AD

1155-1135) following the presence of matrix-supported sediment deposits could highlight gravity-reworked sediments caused by slope failure due to seismic activity (Arnaud et al., 2002; Beck, 2009; Giguet-Covex et al., 2012; Polonia et al., 2021). The contemporary decrease of TOC and TN could be related both to the possible dilution of the external vegetative component and cold weather events. The effect of a possible seismic event, in fact, in conjunction with the coldest decade, named Late Medieval Pluvial, could explain the absence of testate amoebae in the interval L18.5-L19.5. Equally, the absence of testate amoebae fauna in the interval L29.5-L30.5 could record a climatically cold period prior to the MCA (Fig. 9). The decrease in TN and TOC as well as the presence of grain-supported sediment deposits could confirm the effect of climatic phenomena as a main contributor to the testate amoebae disappearance (Tsyganov et al., 2019).

- Interval 3 (L15.5-L11.5/1215-1507 AD): from a textural point of view there is an increase in the values of one percentile and no signs of important external contributions to the lake basin. As regards the predominant species of testate amoebae, the same species observed in the previous interval (*D. globulosa*, *D. penardi*, *D. rubescens*, *A. hemispherica*, *C. aculeata*, *C. aerophila*, *C. cassis*, *D. oblonga*, *D. viscidula*) are present, but with an evident decrease in density. *D. proteiformis* and *D. acuminata* disappear, while *D. parva* appears.

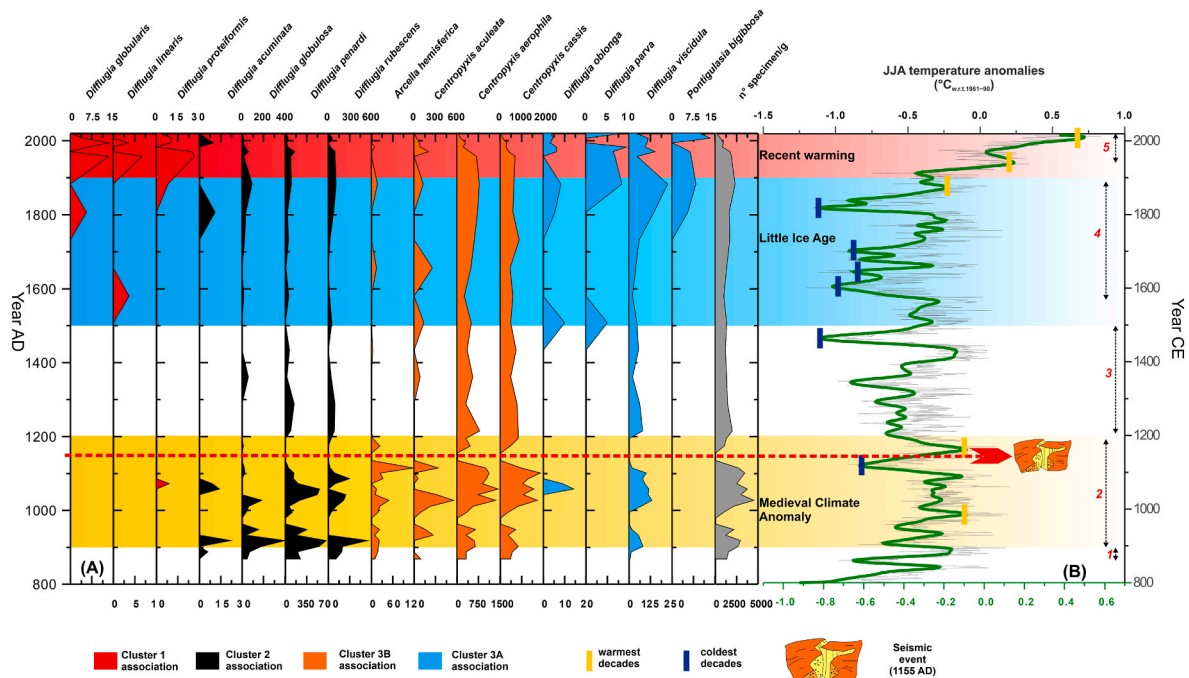


Fig. 9. Distribution of testate amoebae species (ind g^{-1}) along the sediment core of the Upper Balma Lake (A). Groups of the sediment core samples as highlighted by the Cluster associations are reported below the figure. Reconstructed temperatures from the European Alps (summer warmth between June and August JJA), with the orange and blue boxes denoting the 10 warmest and coldest decades respectively, and the smoothed green line being a 15-yr low-pass filter are reported in (B) (from Büntgen et al., 2006, 2016; Esper et al., 2024), modified. The red arrow indicates the possible seismic event recorded in 1155 AD near Lons-le-Saunier (Doubre et al., 2021). The numbers colored red on the right-hand side of the figure indicate the five intervals revealed by the testate amoebae. The colored bands (yellow, blue and red) mark the main climate phases in the last 1220 years. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

This period seems to be a transitional phase preceding the Little Ice Age. Moreover, the coldest decade around the year 1500 AD is recorded by the increasing values of *D. oblonga* and *D. parva*, which belong to the Cluster 3A association. The rising values of these species may be partly related to high concentrations of small particles in the lake sediments, linked to intense rainfall events during the Little Ice Age (Büntgen et al., 2021b). These particles can affect sediment grain sizes and mineralogy, and testate amoebae (e.g., *Diffugia* and *Centropyxis*) can use these particles or grains to construct their shells (Armynot du Châtelet et al., 2010; Ndayishimiye et al., 2020b) (Fig. 9).

- Interval 4 (L10.5-L6.5/1580-1883 AD): this interval covers a period named as Little Ice Age (LIA) (around 1600-1900 AD) characterized by four coldest decades with the peak recorded in the 1816 AD (Büntgen et al., 2006, 2016). Right before 1900 AD, a warm decade is present (Fig. 9). Two grain supported deposits at L10.5 and L9.5 evidence an increase in rainfall and flash-flood phenomena as reported by Salvi et al. (2022) in the Lower Balma Lake. As regards the trace elements, growing values of As, Pb, Zn and Cd are recorded at the end of this period and used only as supporting data. Trace element concentrations rose steadily from the late 19th century, possibly linked to increased industrial development, which has greatly contributed to the multiplication of heavy metal sources, thereby increasing their levels in the lake environment (Nedjai et al., 2021). The investigated trace elements are strongly correlated, both naturally co-occurring and associated with organic matter. Their highest levels appeared after peak contamination, suggesting accumulation with organic matter. Among them, Mn remained stable, reflecting bedrock weathering. Conversely, Zn, Pb, Mo and As may derive from both natural and anthropogenic sources (Håkanson and Jansson, 2002; Meriläinen et al., 2003); Cd, rare in nature, increased notably most likely due to human impact (Wathne et al., 1997). Similar metal trends in remote lakes suggest regional industrial

emissions as main sources (Hamerlík et al., 2016). Recent declines reflect stricter environmental policies and improved technology.

The dominant testate amoeba communities pertaining mainly to the Cluster 3. In this interval, in addition to the species observed in the previous interval, *D. acuminata* and *P. bigibbosa* appear, with the latter species being significant for cluster 3A. Besides, *D. globularis*, *D. linearis* and *D. proteiformis* were observed, despite being in low abundance (Figs. 6 and 10). Testate amoebae populations evidence lower values at the beginning of this period with an increasing trend towards the end.

- Interval 5 (L5.5-L0.5/1958-2020 AD): this interval covers the climatic period reported as Recent Warming (RW) (Büntgen et al., 2006; Büntgen et al., 2016; Büntgen et al., 2021a,b; Esper et al., 2024) (Fig. 9). This interval is characterized by high nutrient levels, including nitrogen and organic carbon, with the only exception of the top core where nutrients decrease; a gradual decrease in trace element concentrations is reported probably related to environmental policies and improved technology. The grain-supported deposits, characterizing the whole interval, highlight the ongoing warm climate evolution with prominent rainfall and flooding events. The dominant testate amoeba associations referred to Cluster 1 are *D. globularis*, *D. linearis* and *D. proteiformis* that are associated with higher values of TOC and TN and relatively low oxygen concentration (Asioli et al., 1996; Caffau et al., 2015). The same correlation is found for *D. oblonga*, *D. viscidula* (Collins et al., 1990; Asioli et al., 1996; Patterson et al., 1996) and *P. bigibbosa*. *D. oblonga* is also associated with an alkaline pH (Qin et al., 2013). In the years following the peak of trace elements such as As, Pb, Zn, Cd (1971 AD), there was a decrease in testate amoebae density mainly due to the decrease in the abundance of the genus *Centropyxis*. The taxon *Centropyxis* is highly tolerant of extreme and stressful environmental conditions, being found in both warm and cold climates (Ndayishimiye et al., 2021).

Hence, a rapid decline in *Centropyxis* in more eutrophic and warmer environments could likely be linked to changes in primary production and the availability of suitable food sources (Ndayishimiye et al., 2019, 2020a, 2021), as evidenced by the increasing values of organic carbon and nitrogen in the upper core levels. It may also have a connection to high concentrations of trace elements, including As (Patterson and Kumar, 2002; Charqueño et al., 2021). The decline in the abundance of this genus between 1983 and 2020 AD could be related to more eutrophic conditions, potentially due to expanded sedimentation rates, deforestation and agriculture (Prentice et al., 2018; Ndayishimiye et al., 2020a). Testate amoebae shifted toward a *Diffugia* spp. assemblage that is tolerant of high concentrations of terrigenous material, nutrients, and organic matter (Caballero et al., 2025 and references therein), indicating a change in sediment conditions within upper core levels.

By the analysis of testate amoeba communities over the last 1200 years, it is possible to highlight that the Upper Balma Lake experiences alternating conditions of mesotrophy and oligotrophy, due to variations in organic carbon, organic nitrogen and the C/N ratio which reveal greater or lesser exogenous contributions.

Our record shows coherent changes between environmental variables and testate amoeba abundance over the past 1200 years, despite a progressive decline in *Centropyxis* genus (Fig. 9). A marked shift in dominant taxa (from *Centropyxis* to *Diffugia*) occurred mainly during recent warming (interval, 5), coincident with increased input of organic material and a rapid increase in taxa adapted to warmer conditions. *Diffugia* is known to tolerate relatively high temperatures and more eutrophic conditions (Ndayishimiye et al., 2019; Ndayishimiye et al., 2020a and the references therein). We recorded during the intervals 1, 2, 3 (Fig. 9), species richness and abundance were largely controlled by *Centropyxis*, consistent with a dominant influence of natural climatic variability. In the upper interval, the decline of *Centropyxis* did not result in reduced total richness or abundance, as this was compensated by increased occurrence of *Diffugia* (Fig. 9). In fact, from ~1900 AD onwards, *Diffugia* became the dominant genus, primarily in response to changing environmental conditions, leading to higher overall species richness and abundance. This shift likely reflects the capacity of *Diffugia* to persist under increasingly eutrophic conditions linked to intensified climate warming and human activity (Charqueño et al., 2020; Ndayishimiye et al., 2020 and the references therein). The Shannon-Wiener index appears to confirm these findings, with the highest values (>1.5) observed in intervals 1 and 2 and at the upper level 5.

Paleolimnological analyses carried out on fossil testate amoeba associations in the Lower Balma Lake (Salvi et al., 2022), located in the same area at an altitude of 100 m lower and communicating with the Upper Balma Lake via a small stream, revealed some differences between the two lakes. The Upper Balma Lake is characterized by a sedimentation rate almost double compared to the Lower one, resulting in a greater contribution of organic material from the surrounding area. This difference is also evident by the comparison between the C/N values, which are markedly higher in the Upper Balma Lake (9.91–36.35) than in the Lower Balma Lake (9.71–11.15). Moreover, organic material input increases during the rainiest periods, as indicated by the textural data, probably influencing the testate amoebae communities. Besides, it is worth noting at the Upper Balma Lake (2.77 m maximum depth) is shallower than the Lower Lake (6.42m maximum depth).

These differences can be linked to the greater contribution by the grain-supported sediments which could have contributed to the recent burial of the Upper Balma Lake. The Upper Balma Lake is characterized by a greater number of species (21 species) compared to the Lower (11 species) (Salvi et al., 2022). Regarding species present in both lakes, *C. cassis* and *D. penardi* are more abundant in the Upper Balma Lake while *D. acuminata*, *D. oblonga*, *D. viscidula* and *P. bigibbosa* are more abundant in the Lower Balma Lake, where they are evidently favored by

more oligotrophic conditions.

The levels of water oxygenation, pH and conductivity recorded in both lakes agree with typical values reported for the alpine lakes (Füreder et al., 2006; Tiberti et al., 2010). The high P concentrations recorded in the Upper Lake are more typical for limestone basins and are not expected in granitic bedrocks.

5. Conclusions

This study presents the results of a paleolimnological investigation based on testate amoeba assemblages from a sediment core collected in the Upper Balma Lake. The analysis revealed response of testate amoeba communities to the major climatic phases and environmental changes that occurred during the late Holocene (approximately the last 1200 years). In particular, the Recent Warming phase appears to be linked to a shift in dominant testate amoebae taxa, notably a reduction in *Centropyxis* spp. Additionally, increased sediment input (964–979 AD and 1135–1155 AD), likely due to more frequent flood events, suggests a potential infilling of the lake basin. Differences in testate amoeba assemblages between the Upper and the Lower Balma Lakes may be attributed to contrasting morphologies and sediment/organic matter fluxes. This study highlights the potential of testate amoebae as sensitive indicators of past climate variability in high-altitude lakes. Notable in this regard is the disappearance of testate amoeba fauna probably in relation to the seismic event recorded in 1155 AD. Future studies should expand to other alpine sites to confirm regional patterns, integrate multi-proxy approaches (e.g., geochemical, molecular), and assess the resilience of microeukaryotic communities to ongoing climate change.

Author contributions

Marco Bertoli: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review and editing; **Gianguido Salvi** Data curation, Investigation, Methodology, Writing – review and editing; **Maira Sitta** Data curation, Investigation, **Elena Pavoni** Data curation, Methodology; **Giuseppe Esposito** Data curation, Methodology; **Fiorenza Torricella** Data curation, Methodology, Writing – review and editing; **Paolo Pastorino** Conceptualization, Funding acquisition, Writing – review and editing; **Marino Prearo** Supervision, Writing – review and editing; **Elisabetta Pizzul** Conceptualization, Supervision, Writing – review and editing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.quascirev.2026.110162>.

Data availability

All data and/or code is contained within the submission.

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