



Ecosystem shift of a mountain lake under climate and human pressure: A move out from the safe operating space

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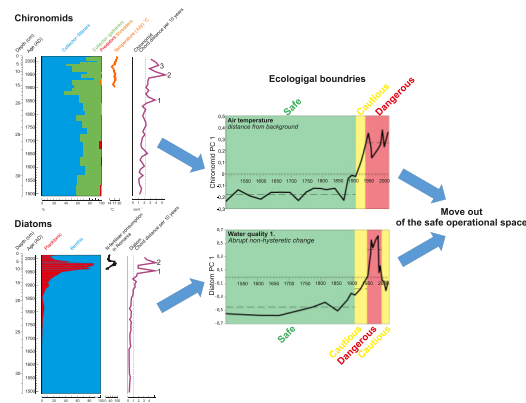
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HIGHLIGHTS

- The chironomid fauna responded primarily to temperature changes after 1926.
- Planktonic diatoms have responded to atmospheric nitrogen fertilization since 1950.
- Ecosystem reorganization showed an unprecedented increase over the last 100 years.
- Our data suggest that Lake Bâlea is outside of its safe operating space today.

GRAPHICAL ABSTRACT



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ABSTRACT

A multiproxy approach including chironomid, diatom, pollen and geochemical analyses was applied on short gravitational cores retrieved from an alpine lake (Lacul Bâlea) in the Southern Carpathians (Romania) to unveil how this lake responded to natural and anthropogenic forcing over the past 500 years. On the basis of chironomid and diatom assemblage changes, and supported by sediment chemical data and historical information, we distinguished two main phases in lake evolution. Before 1926 the lake was dominated by chironomids belonging to *Micropsectra insignilobus*-type and benthic diatoms suggesting well-oxygenated oligotrophic environment with only small-scale disturbance. We considered this state as the lake's safe operational space. After 1926 significant changes occurred: *Tanytarsus lugens*-type and *T. mendax*-type chironomids took over dominance and collector filterers increased until 1970 pointing to an increase in available nutrients. The diatom community showed the

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most pronounced change between 1950 and 1992 when planktonic diatoms increased. The highest trophic level was reconstructed between 1970 and 1992, while the indicator species of increasing nutrient availability, *Asterionella formosa* spread from 1982 and decreased rapidly at 1992. Statistical analyses evidenced that the main driver of the diatom community change was atmospheric reactive nitrogen (Nr) fertilization that drastically moved the community towards planktonic diatom dominance from 1950. The transformation of the chironomid community was primarily driven by summer mean temperature increase that also changed the dominant feeding guild from collector gatherers to collector filterers. Our results overall suggest that the speed of ecosystem reorganisation showed an unprecedented increase over the last 100 years; biological systems in many cases underwent threshold type changes, while several system components displayed non-hysteretic change between alternating community composition. We conclude that Lake Bălea is outside of its safe operating space today. The main trigger of changes since 1926 was climate change and human impact acting synergically.

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1. Introduction

Alpine lakes are regarded as sensitive indicators of both local and global environmental changes as they react rapidly to natural and anthropogenic forcing affecting their catchments (Williamson et al., 2008; Adrian et al., 2009; Catalan et al., 2013; Moser et al., 2019). Over the recent decades, with humans making a major geological imprint on the globe, mountain lake catchments and their ecosystems have faced unprecedented and possibly irreversible modifications (Lami, 2000; Messerli et al., 2000; Lotter and Birks, 2003; Steffen et al., 2015). Abrupt, non-linear changes to multiple drivers (mainly pollution, land use and climate change) have been identified worldwide using empirical time series data (Dearing et al., 2012; Battarbee et al., 2014; Wang et al., 2012; Moser et al., 2019). As a result of such studies there are increasing efforts to identify early warning signals of critical transitions and define regional safe and just operating space (RSJOS) to protect lake ecosystems and ecosystem services provided by them (Catalan et al., 2013).

The Romanian Carpathians are part of the largest continuous forested landscapes in Europe with unique ecosystems that are known for their biodiversity, impressive cultural heritage and inestimable ecological and socio-economic value (Kozak et al., 2013). These mountains hold over 200 glacial lakes that are attractive for low intensity touristic activities (Mîndrescu et al., 2017). In general, these lakes are located in remote places, away from agriculture, inhabitation and forestry, and were often regarded as pristine archives. However, more detailed investigations not only in the Carpathians (Rose et al., 2009; Hutchinson et al., 2015; Haliuc et al., 2018; Longman et al., 2018), but also in the Alps and other locations on the globe (Guilizzoni et al., 2002; Dubois et al., 2017; Andrič et al., 2020) showed that pristine/natural conditions have been disturbed since millennia; humans altered not only the lowlands, but also the upland environments since the Neolithic-Bronze Age Epoch (Feurdean et al., 2017; Vincze et al., 2017; Hubay et al., 2018). However, with the relatively low number of settlements and limited technological innovations, these earlier anthropogenic activities might have been significant only on local scales (Dubois et al., 2017).

Paleolimnological studies mainly conducted in NW Europe have shown that there are time intervals like the Pre-Industrial Revolution period between 1800 and 1850 that might be considered still minimally impacted by human activities and might be regarded as reference condition (Bennion et al., 2010, 2012, 2015; Rose et al., 2011; Battarbee et al., 2012). However, the date of first significant human impact is spatially time-transgressive and depends on the intensity of the pressure and environmental proxy responses (Bennion et al., 2010).

Recently the Carpathian Region was affected by the loss of grasslands and croplands, and also by an increase in occasional forest clear-cutting (Griffiths et al., 2012) due to post-socialist societal development, and in certain areas by unstoppable increase in tourism with the built up of large, often five-star hotels (Pehoiu, 2010). This recent intensification of human activities is translated in lacustrine records as higher sediment accumulation rates (SAR). A European survey (Rose et al., 2011) showed

that after the 1950's land-use and climate warming have been the main drivers of SAR increases. A similar study from the Romanian Carpathians showed a north-south division for SAR trends (Hutchinson et al., 2015). In the high altitude lakes of the Southern Carpathians seasonal pasturing and transhumance in the traditional period was the main driver leading to increased SARs already at the beginning of the 19th century, while in the northern Romanian Carpathians high mountain grazing became more intensive only during the socialist and post-socialist periods leading to increased SARs only in the last 70 years. Over the entire mountain range, these recent changes are overprinted by touristic activities (Hutchinson et al., 2015).

With the recent, but also with the projected trajectories of environmental change, the alpine records are of paramount importance to better understand how human activities and climatic changes impact the functioning of ecosystems; such information might further support our attempts to design restoration and management targets (Dubois et al., 2017; Larocque-Tobler, 2017). These recent human-induced environmental transformations seem unprecedented in extent and thus, developing effective management and restoration strategies are at the core point of the future health of the environment (Larocque-Tobler, 2017; Battarbee et al., 2005; Bennion et al., 2012, 2015; Dong et al., 2012; Willis et al., 2010; Willis and Birks, 2006). As long-term monitoring information to support such actions/attempts is missing, we rely on paleorecords from lake sediments as they offer the possibility to evaluate the degree of environmental degradation from a longer perspective; they can also provide reference conditions and cause-effect linkages (Larocque-Tobler, 2017; Colombaroli et al., 2017). Major gaps in our knowledge are related with how Carpathian lakes have responded to different drivers of change, what are lake system resilience times, whether the most recent human disturbances have imprinted irreversible changes in their environments, and what are the best ways to protect them. An irreversible change in a lake's environment theoretically does not exclude a return to the original state (Carpenter et al., 1999). However, in such cases no feasible reduction in pollutant inputs can move the lake back to the original steady-state. In principle, such lakes may be restorable by other intervention; regional or global intervention may help restoring the system, but its occurrence is very unlikely (Wang et al., 2012; Dearing et al., 2014). Therefore, we define these ecosystem changes irreversible. The use of paleolimnological studies in management strategies is still restricted in Romania due to data inaccessibility, lack of knowledge and communication between decision makers and scientists. With this study our aim is to contribute towards filling this gap.

The main proxy used to detect the impact of accelerated human activity and also climate change in recent decades, and on a longer perspective over the last five centuries, is fossil chironomid analysis. Chironomids (Diptera: Chironomidae) are abundant in lakes; they are widely distributed with a short life cycle, which make them a powerful tool to reconstruct environmental change (Walker, 1987, 2001; Brooks et al., 2007). Qualitative descriptions and quantitative reconstructions based on chironomids are widely employed worldwide and include

the reconstruction of temperature (Luoto, 2009; Heiri et al., 2011; Eggermont and Heiri, 2012; Tóth et al., 2015), water level, continentality (Self et al., 2011) and water quality changes including eutrophication, saprobity, dissolved oxygen and pH (Holmes, 2014; Luoto et al., 2014a).

Diatoms (Bacillariophyta) are unicellular aquatic photoautotrophs. Diatom valves, due to the siliceous cell walls, are usually well preserved in lacustrine sediments; they are extensively used in paleolimnological studies as an abundant, ecologically diverse, sensitive biological group (Smol and Stoermer, 2010). Most of the diatoms have specific ecological preferences, exhibit a variety of life strategies, and their short life spans enable them to fast respond to environmental changes (Lange-Bertalot et al., 2017; Rimet and Bouchez, 2012).

These two proxies can provide a very strong inference on lake ecological status. Studies using these proxies have demonstrated that climate change and human impact acted in tandem in most places and transformed the lake ecosystems abruptly to completely new assemblages since the 19th century, but with accelerated rate of change in most places in the second half of the 20th century. These changes were often associated with increased water productivity (Luoto et al., 2014a; Luoto et al., 2014b; Jensen et al., 2019; Smol, 2008; Millet et al., 2009; Nevalainen and Luoto, 2012).

In this study we focus on the Făgăraș Mts. of the Southern Carpathians, a formerly glaciated high-mountain area that have experienced significant socioeconomic change in the last decades due to

increased visitor numbers around its precious lakes, the best known of which is Lake Bălea (Fig. 1). The shores of this lake host three relatively recently built hotels that became popular tourist destinations both in the summer and winter due to easy access on asphalt road and by cable cars. Here we study two short sediment cores from the lake that archive almost 500 years' history of environmental change. Our working hypothesis is that the chironomid community of the lake must have gone through substantial reorganisation in response to the warming summer temperatures over the last 30 years, as these macroinvertebrate taxa are sensitive to water temperature in oligotrophic alpine lakes (Luoto, 2009; Heiri et al., 2011; Eggermont and Heiri, 2012). Similar changes were recently demonstrated in high mountain lakes in the Alps (Ilyashuk et al., 2019). An alternative hypothesis is that the effect of summer warming temperatures is less significant than the effect of accelerated human impact, and therefore our chironomid community changes are mainly explainable by these events. In case of the diatom communities we were particularly interested in whether global warming, direct or indirect human impacts are responsible for the observed amplification in diatom community change over the last 500 years. In particular, alpine lakes received much attention in the USA, where atmospheric excess nitrogen input has been demonstrated to cause planktonic eutrophication (Wolfe et al., 2001; Hobbs et al., 2010). Even though the communist era in many Eastern European countries brought about an increase in chemical fertilizer production

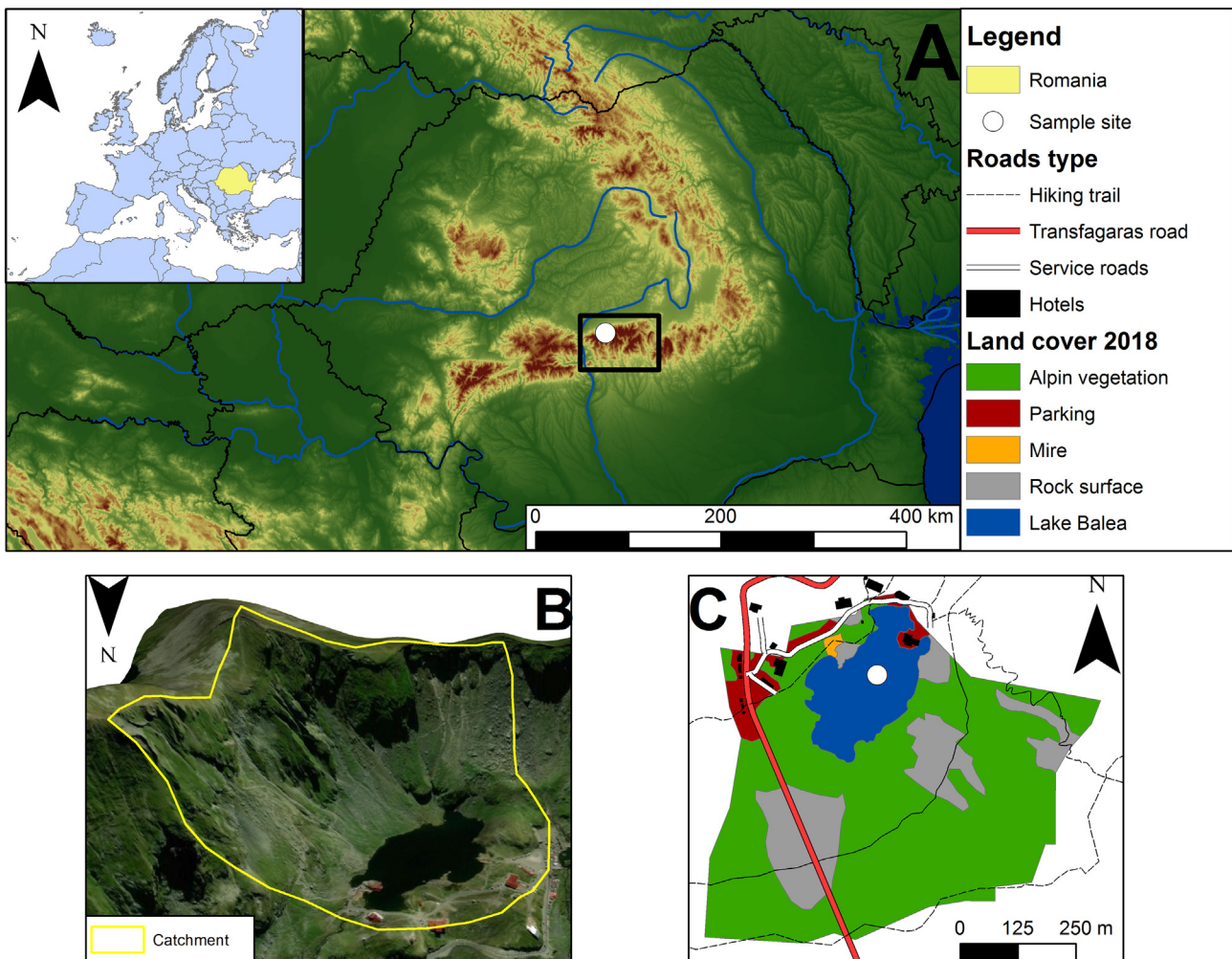


Fig. 1. The location of the study area within Europe and Romania (A) with a digital elevation model of the Lake Bălea catchment combined with satellite image (B); we also show a satellite image (2018) based land cover classification of the Lake Bălea catchment with the location of the short gravity cores (C); image source: Esri, i-cubed, USDA, USGS, AEX, GeoEye, Getmapping, AeroGRID, IGN, IGP, UPR-EGP, and the GIS User Community, ESRI.

and use (Eriksson et al., 2007; Grzebisz et al., 2012), the ecosystem effect in remote alpine environments have not yet been accessed. Our aim is to examine in the South Carpathians whether nitrogen fertilization (EEA report, 2004; Grzebisz et al., 2012) had any significant effect on the alpine lake's planktonic diatom assemblages. Even though Lake Bâlea and its surroundings are a natural reserve since 1932, protection guidelines have failed to be implemented so far, and the site is now exposed to high anthropogenic disturbance, which renders it at high risk (Pop et al., 2013).

2. Regional setting & climate history

2.1. The Făgăraș Mountains and Lacul Bâlea

The Făgăraș Mountains are located in the Southern Carpathians (Fig. 1) that is the largest crystalline range (with mica schists, gneiss, amphibolite and pure marbles) in the Carpathian Mountains. In the Lake Bâlea catchment the dominant rock type is amphibolite, while limestone outcrops occupy only a small area near the Vaiuga peak. The mountain relief is characterised by extended glacial and periglacial geomorphology (Fig. A.1). The highest peaks are over 2000 m. There are over 30 lakes with glacier origin.

The present climate is temperate continental; milder conditions prevail up to 1200 m with an average annual temperature 4–7 °C and average precipitation 700–1000 mm (Urdea, 2000; Voiculescu et al., 2016).

Lake Bâlea is the largest glacial lake situated at 2034 m a.s.l. (near Pisota peak; Fig. 1). At the altitude of the lake the mean annual temperature is ~1.7 °C, while the mean air temperature of the winter season is –6.5 °C. The coldest month is February (~–8.4 °C) and the warmest month is August (~9.9 °C). Annual precipitation is ~1706 mm, while the average annual snowfall reaches 225 cm. The highest amount of precipitation is in the summer (~619 mm). Snow cover persists for 8–9 months, average snow depth is 110 cm (Voiculescu et al., 2016, based on meteorological data from Lake Bâlea meteorological station 2010–2019).

The surface area of the lake is 4.7 ha and its maximum depth is 11.4 m. It is situated above the treeline.

Seasonal lake level fluctuation was assessed by Pișota (1971) between January 1961 and December 1963. His data suggest that the maximum water depth occurs in the spring and the minimum is in the autumn, while the seasonal fluctuation is ~1 m. Historical water-depth changes have not been recorded.

3. Materials and methods

3.1. Sediment sampling and laboratory analyses

A 32-cm sediment core (Bâlea2018-01, N 45° 36' 11", E 24°36' 55") was retrieved from the central part of Lake Bâlea in July 2018 using an Uwitec Gravity corer with a 50-cm long chamber and a diameter of 7 cm. At the core location the water depth was 8 m. The cores were sliced into 1 cm sections in the field and stored at 4 °C until further examination. On this core ²¹⁰Pb dating, chironomid, pollen and diatom analyses were done.

In 2017 another short core was taken from the lake (Bâlea2017-01, N 45°36'13", E 24°36'55") near the 2018 core. Water depth at the core location was 10 m. This core was sliced at 2 cm interval and used for loss-on-ignition and geochemical measurements. It does not have an independent ²¹⁰Pb chronology; dates were transferred from the Bâlea2018-01 core.

In 2018 and 2017 water quality measurements were done at Lake Bâlea with an HQ40d Lange multimeter for water temperature, conductivity, pH and dissolved oxygen. The water depth was measured with an RBR XRX-420 multi-channel logger.

On-site measured water temperature, conductivity and chemistry data along with land use history for the last 500 years are summarized

in Appendix Data 1. Chironomid and diatom samples were analysed down to 32 cm in core Bâlea2018-01 (1491), while pollen analysis stopped at 31 cm (1530). The lowest depth in core Bâlea2017-01 is 31 cm (1530).

3.2. Historical climate in the Făgăraș Mountains

The historical meteorological data used in the present study (temperature and precipitation) was retrieved from CRU (Climate Research Unit, version 4.03), a gridded dataset with 0.5°x0.5° spatial resolution (Harris et al., 2014). The dataset covers the interval 1901 to present. From the original temperature and precipitation data monthly means, annual means and seasonal means were calculated (Fig. A.3).

Using the meteorological data, we identified four major intervals with slightly different climatic trends between 1901 and 2018. A clear warming tendency in both the summer and annual mean temperatures is apparent in the dataset from 1988.

3.3. ²¹⁰Pb and ¹³⁷Cs dating

For aquatic sediments the use of ²¹⁰Pb isotope with a half-life of 22.2 yr (Duenas et al., 2003) is a well-established method to estimate sediment ages and sedimentation rates on a time scale up to 100 years. Samples were put in sealed 1-cm diameter, 5-cm height plastic tubes and stored for at least 28 days prior to measurement. The activity concentration of ²²⁶Ra was measured after a month of storage using the gamma lines of the short-lived radionuclide daughters of ²²²Rn (²¹⁴Pb at 295 keV and 351 keV and ²¹⁴Bi at 609 keV). Samples were analysed with a high-resolution gamma spectrometer equipped with a HPGe well-type ORTEC GEM detector, having a FWHM of 1.92 keV at 1.33 MeV permitting the detection of low gamma energies. The activity concentration was calculated using the relative method with IAEA 385,327,447 standards. Activity concentration of the ²¹⁰Pb_{tot} content from the sediment was measured by its daughter radionuclide ²¹⁰Po, the two elements reaching the equilibrium after 2 years. The ²¹⁰Po content of each sediment sample was determined using an aliquot of 0.5 g dry sample. An amount of 0.3 mL of 100 mBq mL⁻¹ ¹²⁰⁹Po tracer was added (having an alpha energy of 4.9 MeV) for determination of chemical efficiency. For age-depth model calculation the CRS model was used (Appleby, 2001). This covers the upper 21 cm of the sediment. Ages below 21 cm were calculated using extrapolation for which a polynomial fitting was used in R using third-degree polynomial.

3.4. LOI and sediment chemistry

For loss-on-ignition (LOI) analysis 1 cm³ samples taken at 1-cm interval were combusted at 550 °C for 4 h for determining the organic matter content of the sediment (Heiri et al., 2001). For the major and trace element analyses 0.25 g samples were diluted in 3 mL 65% HNO₃ (Merck, Suprapur) and 9 mL 36% HCl (Merck, Suprapur). Microwave digestion was applied (Speedwave Entry, Berghof, Germany). The solution was diluted to 50 mL with ultrapure water. Elemental analyses were performed with a microwave plasma atomic emission spectrometer (MP-AES Model 4210, Agilent Technologies, USA). Nitrogen plasma temperature in the analytical zone was 4500 K. The concentration of the following 21 elements was determined: Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb, Si, Sr, V, Zn.

3.5. Chironomid analysis

For fossil chironomid analysis (Brooks et al., 2007) 3–15.5 cm³ sediment samples were taken at 1 cm interval. Subsamples were deflocculated in 10% KOH and heated at 60 °C for 20 min, then sieved through a 100 μm mesh. Chironomid larval head capsules were picked out from the sieved sub-samples in a Bogorov-counting chamber (Gannon, 1971) under the stereomicroscope at 40–50× magnification.

Larval head capsules were mounted on microscope slides in Euparal mounting medium for microscopic identification. Chironomid head capsule concentration was estimated by counting all head capsules in the subsamples. At least 45 head capsules were identified in each sample (Quinlan and Smol, 2001; Heiri and Lotter, 2010), except for 1, 5, 6, 7, 8, 9, 10, 11, 15, 18, 19, 21, 22 cm that contained only 4–37.5 head capsules. In order to reach ~45 head capsules in a sample we merged neighbouring centimetres (0–2, 5–9, 17–19 cm). The minimum count was this way increased to 73 at 0–2 cm, 30.5 at 5–9 cm and 34.5 at 17–19 cm.

Chironomid species types were classified into feeding guilds including collector-gatherers (feed on fine particulate detritus on lake bottom), collector-filterers (filter suspended material from water column including small organism), shredders (feed on living or decomposing vascular plant tissue, coarse particulate organic matter) and predators (attack other animals and engulf whole prey or suck body fluids) (Luoto and Nevalainen, 2015; Heino, 2008). There are other point-scoring systems for defining the feeding groups (Moog, 2017) that take into account the flexibility of feeding in case of the chironomid species (e.g. they can change their feeding habit depending on external factors). However, these finely defined systems are based on recent chironomid species, while the identification of the subfossil chironomids is possible only at genus or species group level.

Chironomid identifications were made using an Olympus CX41 microscope at 100–400× magnification. Taxa were identified following Brooks et al. (2007) and Wiederholm (1983) and the taxonomy mostly follows that of Brooks et al. (2007).

3.6. Diatom analysis

Twenty-four samples were analysed for diatoms from core Bâlea2018-01. Every centimetre was analysed between 1 and 17 cm, while every second cm between 18 and 32 cm. Wet sediment was digested in hot 30% H₂O₂, then washed with deionised water several times. The cleaned valves were dried on coverslip and embedded in Naphrax resin (Battarbee, 1986). Diatom counting was performed with a Leica DM LB light microscope at 1000× magnification under oil immersion and phase contrast. A minimum of 300 valves were counted per slide. Diatom identification was based on Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b), Lange-Bertalot and Metzeltin (1996) and Lange-Bertalot et al. (2017). The planktonic:benthic (P:B) diatom ratios were calculated based on the life form grouping of Rimet and Bouchez (2012).

3.7. Pollen analysis

Samples for pollen analysis were taken at 2-cm intervals from core Bâlea2018-01 in order to examine vegetation changes during the last ~500 years. 2 cm³ subsamples were prepared for pollen using standard methods but excluding acetolysis (Bennett and Willis, 2001). Pollen, spores and microcharcoal particles were counted and identified under a Nikon Eclipse E 600 light microscope at 400× and 1000× magnification. At least 500 terrestrial pollen grains were counted on each slide. For pollen identification, the pollen atlases of Reille (1992, 1995, 1998) and the pollen identification key of Moore et al. (1991) were used. Local pollen assemblage zones were determined on the terrestrial pollen taxa. The statistical significance of the pollen assemblage zone boundaries was tested by comparison with the broken stick model (Bennett, 1996).

3.8. Statistical methods

We used rate-of-change analysis (RoC, Grimm and Jacobson, 1992) to estimate compositional change per unit time in the chironomid, pollen and diatom records. To estimate RoC, datasets were first interpolated to a constant time interval of 10 years. Subsequently the chord

distance dissimilarity (= Hellinger distance in Legendre and Birks, 2012) was calculated between adjacent interpolated samples (chord distance cent.⁻¹) following Birks (2012). The chord distance functions produce distances in the range [0, $\sqrt{2}$], with maximum values when two samples have no species in common. Periods denoted by above-than average chord-distance values are interpreted as periods of higher-than-average rates-of-change in the proxy records.

For zonation hierarchical cluster analysis (CONISS) was used in case of the pollen, chironomid and diatom datasets. The statistical significance of zone boundaries was tested by the broken stick model (Bennett, 1996).

We used the R software environment for our multivariate statistical analyses (Dalggaard, 2010). Due to the assumed linear relationship between our variables, we chose principal component analysis (PCA) for the geochemical, chironomid and diatom data. Prior to PCA, chemical data was normalized to zero mean and unit variance, while chironomid counts were transformed using Hellinger transformation (Legendre and Gallagher, 2001).

Redundancy data analysis (RDA) was used to explore the relationship between changes in the chironomid assemblages and other environmental variables. This analysis was chosen because the gradient length of the first axes was less than four SD unit (Legendre and Legendre, 2012). RDA_{Env} used Pb, LOI, planktonic diatoms, separately *Asterionella formosa* as explanatory variables, while RDA_{PCA} used the first two PC axes of the geochemical and diatom records. To examine whether chironomid community changes show any correlation with July and summer mean temperature changes in the last 120 years, we used general additive models (GAM) with Gaussian distribution, T_Jul and T_summer as predictors and a fixed variance structure in the model.

All statistical analyses were performed using the vegan (Oksanen et al., 2019) and rioja (Juggins, 2017) packages.

4. Results and interpretation

4.1. Age-depth model and sedimentation rate changes

According to our results, the upper 21 cm of the sediment covers the interval from 1814 to 2018 (approximately 200 years). Polynomial regression was used to extrapolate the age-depth model for the 21–32 cm interval, and this suggests an age of ~1491 at 32 cm. The average sediment accumulation rate is 0.14 mm year⁻¹ for the last 200 years (Fig. A.6). Rapid increases in accumulation rates were noticed at 1, 5, 7 and 10.5 cm (2013, 1989, 1982, 1959) that are likely connectable to erosion events. The ¹³⁷Cs dates fit nicely with the ²¹⁰Pb dates (Fig. A.6). Increased ¹³⁷Cs concentration points to the Chernobyl fallout at 6–8 cm (1986). Furthermore, a second ¹³⁷Cs peak can be connected to the nuclear weapon tests at 10–11 cm (1963).

4.2. Organic content, sediment chemistry and inferred environmental changes

The Bâlea sediment record is characterised by relatively low organic content (6%) in the interval between 31 and 17 cm (1530–1885) followed by a steep increase in the following interval spanning 17 to 9 cm (1885–1966) (Fig. 2). After a rapid drop in organic content in 1982, the upper 5 cm (from 1989) of the sediment is characterised by gradual increase reaching a maximum in the last 2 cm (after 2010).

From the total 21 elements measured by MP-AES we selected and discuss the elements that show high abundance, such as Fe, Al, Mg, Ca, while from the elements that show average abundance we selected only those that are usually employed as proxies in environmental reconstructions, e.g. Si, Zn, Pb and Cu. The trends in sediment chemistry are displayed in Fig. 2 that shows high concentration for Fe, Al, Mg, Ca, K (1200 to 78,000 mg/kg) and average concentration for Si, Zn, Pb and Cu (80–670 mg/kg). Two significant zones were identified based on CONISS clustering: BGC-1 (31–17 cm; 1530–1885) and BGC-2 (17–1

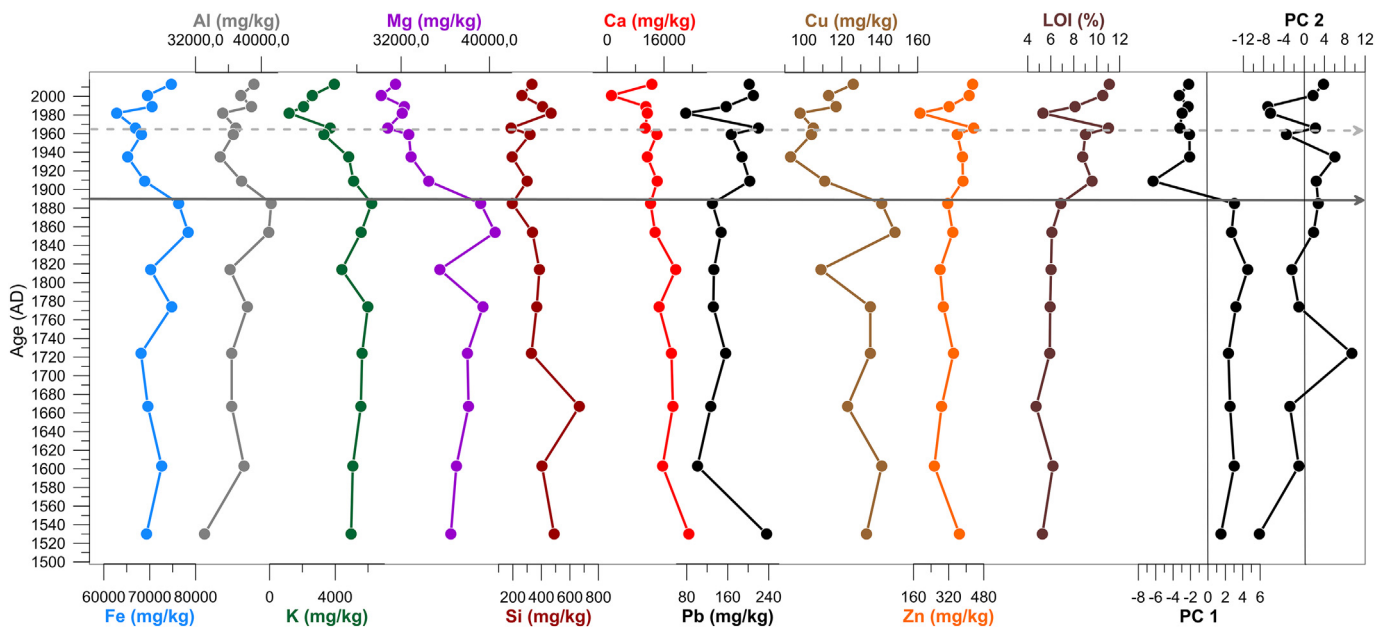


Fig. 2. Selected geochemical variables, organic content (LOI) and stratigraphic plot of the first two principal component axes (PC 1 and PC 2) for Bâlea2017-01. The lines show the concentrations of iron (Fe), aluminium (Al), potassium (K), magnesium (Mg), silica (Si), calcium (Ca), lead (Pb), copper (Cu) and zinc (Zn).

cm; 1885–2013); the latter was subdivided in two sub-zones BGC-2a, 17–9 cm (1885–1966) and BGC-2b, 9–1 cm (1966–2013).

In BGC-1 (1530–1885), Fe, Al, K, Si, Mg have similar increasing trend from the bottom to the top of the interval showing high minerogenic input likely from intense erosion activity (Cohen, 2003; Kylander et al., 2011). Considering the steep topography with poorly vegetated inclined slopes, it is expected that the Lake Bâlea catchment is highly susceptible to erosion activity. A first small decrease is visible in Fe and Al at 25 cm (1724) followed by a second one in Fe, Al, K, and Mg at 21 cm (1814) indicating a slight decrease in minerogenic input or possible change in the source of sediments. Silica (Si) displays a significant peak at 27 cm (1667) and two other small peaks are at 15 and 11 cm (1909 and 1959).

Contrary to other elements, Fe and Mg can play different roles in lake sediments, and in some cases their interpretation needs to be done in tandem. In lake sediments, changes in Fe are indicative of shifts in redox state and/or changes in the minerogenic input from the catchment usually associated with the clayey-silty fraction (Kylander et al., 2011; Naeher et al., 2013). Here, Fe shows a strong correlation with Al ($r^2 = 0.63$) supporting its interpretation as a detrital proxy. Mg can originate from bedrock, but it is also a highly mobile element and its occurrence in lake sediments can be connected to intensified weathering and in-lake precipitation through biological activity. In the Lake Bâlea record Mg has a detrital origin in the bottom part of the sequence, in BGC-1 (31–17 cm; 1530–1885) where it shows a high correlation with Al (Mg — Al $r^2 = 0.57$) and might originate through weathering of catchment's amphibolite, while in the upper part, in BGC-2 (1885–2013) it seems to be mediated by other processes, most likely by lake internal processes (Mg — Al $r^2 = 0.02$).

In the upper zone, BGC-2a (17–9 cm, 1885–1966), a decreasing trend is present in most of the minerogenic proxies like Fe, Al, Mg, K suggesting decreasing erosional activity and/or increased internal productivity. The same elements show gradual increase in the upper 6 cm, BGC-2b (1966–2013) suggesting a possible reactivation of the erosional activity and thus, delivery of minerogenic material into the lake.

Calcium has a more monotonous trend throughout the profile interrupted by a slight decrease at 29 cm (1603), and an isolated maximum at 21 cm (1814) and a minimum at 3 cm (2001). Although, the bedrock of the Bâlea catchment holds only a small pool of carbonates

(near Pisota peak) that can feed into the lake, the contrasting behaviour of Ca with weathering-resistant elements like Al, suggest that in Lake Bâlea Ca might be associated with internal biological productivity and/or deposition of organic matter with high Ca concentration.

The heavy metal, Pb, shows high values in the bottom of the core, at 31 cm (1530), followed by a decreasing trend up to 1885. This is followed by an increasing trend in the upper zone (BGC-2) that was interrupted by a decline at 7 cm (1982) Above this, exceptionally high values characterise the upper part of the core. A similar pattern is depicted in Zn. Contrary to Pb and Zn, Cu shows a different pattern in the bottom part of the core (BGC-1) with a pronounced minimum at 21 cm (1814), and with an increase thereafter (1854–1885). In BGC-2 Cu mainly follows the same pattern as Pb and Zn. In the recent sediments, Pb, Cu and Zn are mainly associated with increasing atmospheric pollution, e.g. fossil fuels combustion and vehicle emissions. Therefore, the behaviour of these elements in Lake Bâlea shows an increase after 1900 with a peak after 1990 likely reflecting regional atmospheric pollution, similar with the results of Akinyemi et al. (2013).

PCA of the chemical element data (Figs. 2 and A.7) explain 72.61% of the total variance (PC 1: 53.18%; PC 2: 19.43%). Positive values along PC axis 1 are associated with Al, Fe, K, Mg and Si. These elements were also associated with the bottom part of the core (BGC-1) and reflect a siliciclastic-dominated deposition, high terrestrial input from the catchment. The negative direction along PC 1 is associated with organic matter content (LOI), Pb, Zn and Cd. Samples from the upper geochemical zone (BGC-2) occupy this space in the biplot suggesting that organic matter deposition and heavy metals are the major components of the recent part of the record. In the upper section, 16 to 0 cm, the significant correlation between organic content and heavy metals (Pb, Zn and Cu) (Fig. 2) suggests that the absorption of these metals by organic compounds likely forms organic-metal complexes.

4.3. Chironomid stratigraphy and inferred environmental changes

Altogether 33 taxa were identified from the sediment and 23 taxa had abundances >2%. Two statistically significant chironomid zones were identified with a zone boundary at 13.5 cm (1926). These were divided further to several subzones based on smaller chironomid assemblage changes (Fig. 3).

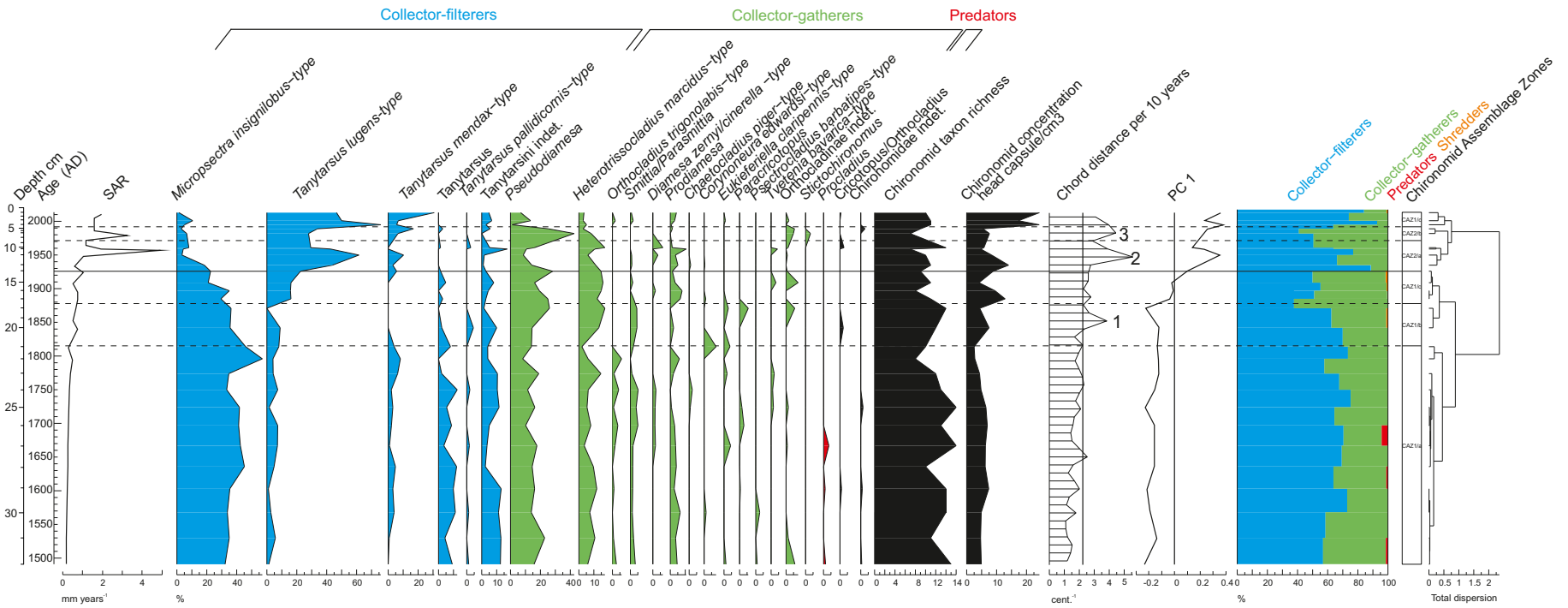


Fig. 3. Relative abundances of the most common chironomid taxa (>2%), feeding guilds, values of the taxon richness, rate-of-change (RoC), stratigraphic plot of the first principal component axis (PC 1) and sediment accumulation rate (SAR) changes for Bălea2018-01 gravity core; above average Roc values were numbered. The taxa were colored according to feeding guilds (blue: collector-filterer, green: collector-gatherer, red: predator, orange: shredders); significant chironomid assemblage zones (CAZ-) were defined by CONISS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The two significant zones are separated well along the first PC axis (Fig. A.8) explained by the very different taxon loadings for *Tanytarsus lugens*-type and *Micropsectra insignilobus*-type. Percentage changes of these two taxa are responsible for this zone boundary.

The first significant zone CAZ-1 from 32 to 13.5 cm (1491–1926) was divided into 3 subzones. The chironomid record shows high abundance of *Micropsectra insignilobus*-type. Species of this taxon have wide thermal tolerance (Brooks et al., 2007) and mostly occur in the profundal of well-oxygenated oligotrophic lakes (e.g. Brodersen and Quinlan, 2006; Heiri et al., 2011). They are sensitive to acidification (Bitušik and Kubovčík, 1999).

Subzone CAZ-1/a (32–20.5 cm; 1491–1814) was dominated by *Micropsectra insignilobus*-type (32–52%), *Pseudodiamesa* (2–22%) and *Heterotrissocladius marcidus*-type (4–14%) indicating relatively cool and deep oligotrophic lake environment (Brooks et al., 2007; Heiri et al., 2011). In addition to the dominant taxa, *Tanytarsus lugens*- and *Tanytarsus mendax*-type occurred in small but stable relative abundance (Fig. 3). Additionally, subfossil remains of several Orthoclaadiinae taxa possibly occupying to the lake littoral (like *Orthoclaadius trigonolabis*- and *Eukiefferiella claripennis*-type; Brooks et al., 2007) or even to semi-terrestrial environment (like *Smittia/Parasmittia*; Brooks et al., 2007) were also present sporadically (1–5%). Chironomid concentration was generally low in this subzone (2.5–7.5 head capsule cm^{-3} ; Fig. 3).

Subzone CAZ-1/b (20.5–17.5 cm; 1814–1871) was characterised by a gradual decrease in *M. insignilobus*-type (from 46% to 36%) (Fig. 3). At the same time, *Pseudodiamesa* and *H. marcidus*-type occurred continuously and even in larger number than in the previous sub-zone. The composition of the chironomid assemblage might still suggest relatively cold, deep and oligotrophic lake environment (Brooks et al., 2007; Heiri et al., 2011). The first peak value of the rate of change (RoC) curve indicated rapid reorganisation of the chironomid fauna at c. 1880 (Fig. 3).

Subzone CAZ-1/c (17.5–13.5 cm; 1871–1926) was characterised by the sudden increase of *Tanytarsus lugens*-type (from 0 to 22%) suggesting a possible increase in trophic level. The decrease of *Micropsectra insignilobus*-type (from 36 to 22%) continued. At the beginning of this subzone chironomid concentrations increased rapidly (4–13 head capsule cm^{-3}).

The second significant chironomid assemblage zone (CAZ-2; 13.5–0 cm; 1926–2018) was characterised by high abundance of *T. lugens*-type, which replaced *M. insignilobus*-type. Both taxa are cold stenotherm and live in oxygen rich waters, but if we compare their ecological requirements (Velle et al., 2005; Brooks et al., 2007; Heiri et al., 2011), we see that *T. lugens*-type lives in waters with higher trophic level, and its expansion indicates nutrient enrichment in cold environment (Bitušik et al., 2009; Potito et al., 2014; Taylor et al., 2017). *Pseudodiamesa* and *H. marcidus*-type occurred in relatively high and stable abundance similarly to CAZ-1 zone (Fig. 3). Moreover, *T. mendax*-type with wide thermal tolerance (Heiri et al., 2011) also increased at the beginning of this zone, but only obtained higher abundances in subzones CAZ-2/bc. This species group live in the littoral region of lakes and its presence can indicate growing trophic level (Taylor et al., 2013; Potito et al., 2014). CAZ-2/a (13.5–8.5 cm; 1926–1970) was dominated by cold stenotherm and deep water taxa (*T. lugens*-type, *Pseudodiamesa*, *Prodiamesa*, *H. marcidus*-type), and it was also characterised by the maximum abundance of *H. marcidus*-type (17%). Chironomid concentration was very high (14 head capsule cm^{-3}) at the beginning of this subzone.

Subzone CAZ-2/b (8.5–4.5 cm; 1970–1992) was characterised by the appearance and high relative abundance of *Pseudodiamesa* (25–42%). The species of this genus prefer inorganic substrate (Eggermont and Heiri, 2012; Lencioni et al., 2012) Therefore, their increase likely suggest erosion in the catchment in the case of Lake Bâlea. Subzone CAZ-2/c (4.5–0 cm; 1992–present) was dominated by *T. lugens*-type (46–50%) and *Pseudodiamesa* (6–13%). These taxa live in the profundal zone of deeper waters, require high oxygen level and are cold-tolerant. Around 1995 we noticed the second maximum abundance of *T. lugens*-type with 76%. The end of this zone was characterised by the dominance of

T. mendax-type (30%) pointing to a water body with the highest trophic level in the entire sequence (Taylor et al., 2013; Potito et al., 2014; Luoto et al., 2014b).

In order to examine when rapid reorganisation of the chironomid fauna occurred, we looked at the peak values in the rate of change (RoC) curve (Fig. 3), where the first increase appears at c. 1880. The timing of this rapid faunal reorganisation agrees well with the onset of the *Pseudodiamesa*, *H. marcidus*-type increase (Fig. 3). Much smaller RoC values characterised the bottom of the sequence, between 1491 and 1820 suggesting ecological stability. The highest chord distance value was found at c. 1950 pointing to a strong response of the fauna to an external forcing at this time, when *T. lugens*-type abundance increased greatly, while the amount of *M. insignilobus*-type suddenly decreased. A third higher increase in RoC values was at c. 1980 that also coincides with a decrease in total chironomid concentrations (Fig. 3). It is also coincident with the appearance of *T. mendax*-type.

Following Luoto and Nevalainen (2015) we grouped our fossil chironomid taxa into feeding guilds: collector-filterers, collector-gatherers, predators and shredders. The relative abundance changes of these feeding types likely indicate changes in the amount of coarse and fine (suspended) organic matter in the lake (Luoto and Nevalainen, 2015).

In the vast majority of the sequence, up until c. 1926, the relative abundance of collector-gatherers and shredders was generally higher than collector-filterers indicating that the amount of coarse-grained organic matter entering the profundal zone of the lake was high (Fig. 3). This period is indicated in blue in the PC biplot that was run on the feeding guild dataset (Fig. A.8). Between 1926 and 1970 and from 1992 to present the relative abundance of collector-filterers increased overall indicating an increase in fine-grained organic matter (suspended matter) at the expense of coarse-grained organic matter. This change also shows up in the PC biplot, where red colouring separates samples that fall in this collector-filterer dominated period. It is also notable that a return to collector gatherer dominance occurred between 1970 and 1992 suggesting a recurring increase in coarse organic input, but this change likely correlates with increased erosion that is also indicated by the LOI values (53–81 mg/kg) and the high relative abundance of *Pseudodiamesa* and *H. marcidus* (Figs. 2 and 3). The disturbance registered by the lake was likely initiated by the construction works on the Transfăgăraşan Road at this time.

4.4. Diatom inferred environmental changes

Four statistically significant diatom assemblage zones were identified using CONISS (Fig. 4). In this study we focus on the description and interpretation of selected diatom groups, particularly planktonic fragilaroid taxa (*Fragilaria tenera*, *F. nanana*, *F. nanoides*, *F. saxoplanktonica*) and centric diatoms (mainly *Discostella pseudostelligera*). They are used to infer changes in the trophic status of the pelagic zone that will be compared with the chironomid inferred trophic changes. In addition, we describe changes separately in the planktonic species *Asterionella formosa*. There is a wide literature on the ecological indicator value of planktonic diatoms (Hobbs et al., 2010; Saros and Anderson, 2015), but for ease of interpretation we use Pappas (2010). Accordingly, *A. formosa* is an indicator of mesotrophy, while the centric *D. pseudostelligera* is an indicator of mesotrophy to eutrophy. The benthic *Sellaphora seminulum* was regarded as an indicator of higher saprobic status following Lange-Bertalot et al. (2017).

The first diatom assemblage zone (DAZ-1) covers the interval from 32 to 18 cm (1491–1871). It was dominated by small-celled benthic fragilaroid taxa, but several motile biraphid species belonging to the genus *Navicula* sensu stricto and *Genkalia*, *Diploneis*, *Stauroneis*, *Placoneis*, *Geissleria* (grouped as *Navicula* sensu lato on Fig. 4) were also frequent, as well as members of the genus *Sellaphora*. The average number of taxa was high, 44 ± 7 . The diverse benthic diatom

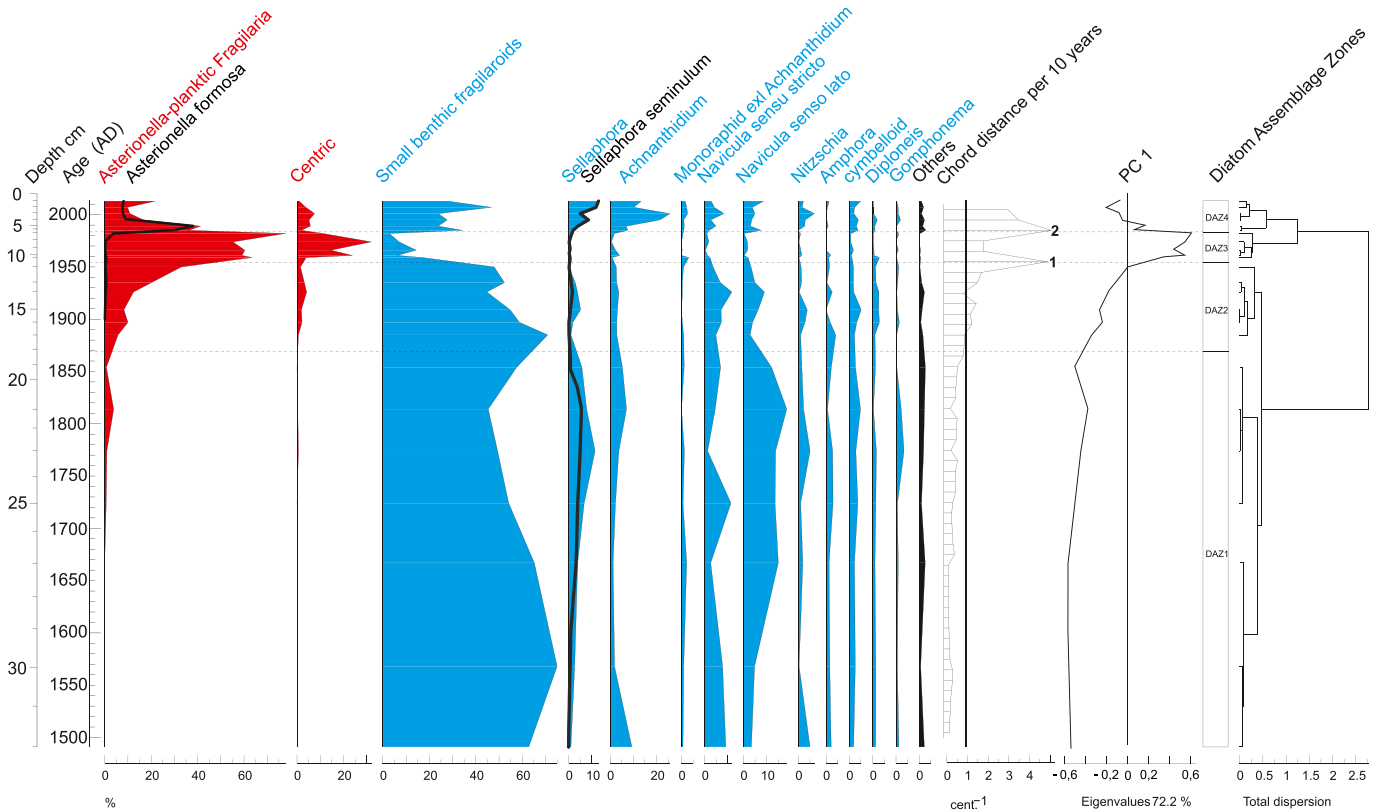


Fig. 4. Changes in the relative frequencies of main diatom taxa and life forms (red: planktonic, blue: benthic, black: others), values of rate-of-change (RoC) and the first principal component axis (PC 1) from Bălea2018-01 gravity core; above average RoC values were numbered; significant diatom assemblage zones (DAZ-) were defined by CONISS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

assemblage and the lack of planktonic diatoms suggest clear, oligotrophic lake water in this interval. The species present suggest slightly alkalic conditions, and there is no indication of peat bog in the lake margin. In the middle of the zone (between 1637 and 1871) relative frequencies of *Navicula* s.l. increase suggesting some sort of disturbance in the lake; a change from typical oligotrophic conditions to more species rich diatom assemblages.

In zone 2 (DAZ-2; 18–11.5 cm; 1871–1950) the same species were present as in DAZ-1 with the exception of *Navicula* s.l. that decreased. The most prominent change in this zone is the gradual increase in planktonic fragilaroids and *A. formosa*. The centric species, *D. pseudostelligera*, was first recorded at the onset of this zone. The number of taxa slightly decreased (37 ± 4). Two contrasting trends were depicted: the high relative abundance of benthic fragilaroids suggests a returning to the first status of the lake (oligotrophic conditions), while the continuous increase of planktonic diatoms points to increasing planktonic eutrophication.

In zone 3 (DAZ-3; 11.5–6.5 cm; 1950–1983 CE) the diatom communities changed to the dominance of *A. formosa*, *F. tenera*, *F. nanana*, *F. nanoides* and *D. pseudostelligera*. The dominance of these planktonic species suggests strong planktonic eutrophication and eutrophic conditions in the lake water that is also corroborated by a drastic decrease in the average number of taxa to 25 ± 6 .

Zone 4 (DAZ-4) covers the upper 6.5 cm (1983–2013 CE) and was characterised by a decrease in planktonic diatoms; the dominant species changed to small, benthic fragilaroids, different kind of periphytic *Achnanthydium* taxa (*A. lineata*, *A. pseudolineatum*, *A. pyrenaicum*, *A. straubianum*, but mostly *A. minutissimum*) and we see an increase in *Sellaphora seminulum*. The appearance of *Navicula reinhardtiana*, *Adlafia minuscula* var. *muralis* suggest eutrophic, meso-saprobic waters (presence of decomposing organic matter), while the increase of periphytic diatoms indicates increasing marginal vegetation cover that is visible

at the site today. The significant decrease in planktonic taxa implies lower trophic level, but the increase in benthic saprophytic taxa is a sign of high sewage water supply to the lake. In this zone one sample (5 cm; 1989 CE) had only a few, badly preserved diatoms. The top samples are quite different and suggest that the diatom assemblages of Lake Bălea are under dynamic reorganisation. This is also confirmed by the RoC curve that displays rapidly increasing and constantly high values since 1950 signalling rapid and sequential reorganisation of the diatom communities.

PC biplot of the diatom records is displayed in Fig. A.9, while the stratigraphic plot of PC 1 appears in Fig. 7. Both figures demonstrate that high positive values along PC 1 are associated with planktonic diatom abundance and hence trophic level increase in the lake, while negative values are associated with benthic diatoms and thus lower trophic state of the lake system. The highest positive loadings are attained by centric diatoms, *Asterionella* and planktonic *Fragilaria* species, while the largest negative PC loadings are ascribed to *Sellaphora*, small benthic fragilaroids, *Navicula* s.l. and cymbelloid diatom taxa.

4.5. Pollen inferred forest compositional change at lower altitudes

In the bottom part of the sequence (PAZ-1; 31–22 cm, 1530–1794) the terrestrial pollen assemblages were dominated by beech (*Fagus*), oak (*Quercus*), hazel (*Corylus*), Norway spruce (*Picea abies*) and Silver fir (*Abies alba*). Dwarf pine (*Pinus Diploxylon*-type) pollen frequencies were very low suggesting that the catchment of the lake was woodless in the 16–17th centuries. This inference is also corroborated by the absence of conifer stomata (Fig. A.10). Tree pollen in the sequence reflects the forest composition of the lower vegetation zones, and it points to still relatively extensive spruce-fir and beech-fir forests. Arboreal pollen percentages decreased at 1667 (27 cm) suggesting the downhill expansion of alpine meadows. Microcharcoal accumulation rates were

relatively low in this zone, but increased slightly together with the increase in grasses (Poaceae) around 1667 suggesting that burning was likely used to enlarge grazing meadows. Cereal pollen was present pointing to arable farming at lower altitudes (Fig. A.10).

The most conspicuous change in PAZ-2 (22–14 cm; 1794–1926) was the massive decrease in Norway spruce (*P. abies*) and Silver fir (*A. alba*) pollen frequencies and a smaller decrease in beech (*F. sylvatica*) suggesting further forest clearance mainly in the subalpine zone. At the same time grazing indicator pollen frequencies increased suggesting intensifying pastoral farming. These changes were followed by a small-scale forest recovery between 1861 and 1891 (18–16 cm), and the largest scale forest clearance afterwards, when mainly the beech (*F. sylvatica*) forests were cleared. Microcharcoal accumulation rates increased from 1930 that leads us to PAZ-3 (14–0 cm; 1926–2018). Intensifying trampling can be inferred since 1982 (7 cm), while invasion of the introduced ragweed pollen (*Ambrosia elatior*) is detectable since 1995 (4 cm). Microcharcoal accumulation rates reached very high values by 1959 (11 cm) and following a temporal decline, constantly high values were detected after 1995 (5 cm). This timing agrees well with the opening of hotels on the lakeshore of Bălea that are heated with wood-fired boilers (Fig. A.10).

5. Discussion

5.1. Is there a correlation between the instrumental summer (T_{JJA}) and July (T_{July}) mean temperature records for the last 120 yrs. and changes in the chironomid assemblages?

One key question of this research is to examine whether the observed in-lake biotic changes that accelerated during the last five decades according to our data (Fig. 5), responded to climate change or intensifying human disturbance (via both atmospheric and direct lake-shore impacts). Chironomid communities in oligotrophic high-alpine lakes are particularly sensitive to even small changes in summer, and particularly July mean temperatures (Pepin et al., 2015; Ilyashuk et al., 2019), and factors other than temperature often are much less important in affecting species composition (Lotter and Psenner, 2004) if the lake is remote. As we demonstrated in the land use history section (Appendix Data 1), the catchment of Lake Bălea has been affected by sheep grazing since at least the 16th century, but local disturbance intensified remarkably since 1970, when Cabana Paltinu and the Transfăgărașan Road were built (Fig. 5). Under such strong human disturbance, it might be unrealistic to expect that unidirectional summer warming trend since 1988 (Figs. 5 & A.3) will have a significant driving force on the chironomid community changes. In order to check and exclude this possibility, we used general additive modelling (GAM; Hastie and Tibshirani, 1990) to access the non-linear relationship between smoothed July and summer mean temperature data and the 1st principal component scores of the chironomid assemblages between 1920 and 2018 (Table 1).

The analysis results (Fig. 6cd) show significant correlation between both July mean and summer mean temperatures and PC axis 1. Increasing temperatures are clearly associated with higher PC 1 scores that are in turn correlated with *T. lugens*-type and to a smaller extent *T. mendax*-type (Figs. 6cd; A.12). We also examined the non-linear relationship between chironomid guilds and temperature changes in the last 120 years (Fig. 6d), and found again correlation at lower than 17 °C (T_{JJA}) with 1st principal component, where negative loadings are connected to collector gatherers. The number of guilds also decreased in samples characterised by >17 °C suggesting that increasing temperature may act as an environmental filter for chironomid feeding guild (functional) diversity (filtering out shredders and predators). Generally, we infer that negative PC 1 scores associated with collector filterers (grazing on suspended organic matter and smaller planktonic algae) are associated with higher temperatures with a larger uncertainty.

Overall, from these analyses we may conclude that *Tanytarsus* taxa (*T. lugens*- and *T. mendax*-type) respond in this system to increased summer temperatures after 1926. Similar results were obtained in the Tatra Mountains (Northern Carpathians), where the increase of *T. lugens*-type has been linked to warmer and more productive waters (Bitušik et al., 2009).

Even though the correlation of the chironomid community changes with the measured July and summer mean temperatures was unexpected given the relatively strong human pressure in these decades, several studies have demonstrated the direct impact of warming on lake ecosystems. For example, Porinchu et al. (2007) found significant changes in the composition of the chironomid fauna in California (Sierra Nevada, 3100 m a.s.l.) from 1970 onwards and demonstrated its close correlation with the warming of the lake water. In Spain, at Lake Río de Sec (3020 m a.s.l.) Jiménez et al. (2019) showed a major change in the chironomid community since 1950. This change was primarily attributable to temperature increase of more than 2 °C. In the Alps, in Lake Mutterbergersee (2483 m a.s.l.) Ilyashuk et al. (2019) showed that from 1920 a continuous increase in the measured air temperature correlates very well with the chironomid-inferred July mean temperature increase, and the absolute increase is about 0.5 °C in ~100 years. These changes have been found primarily in lake ecosystems that are located at high altitudes and free from direct human disturbance. The Carpathians, on the other hand, are lower, and the lakes are more easily accessible. Therefore, even in the most remote locations grazing has been present as an environmental stressor over the past 150 years (Hutchinson et al., 2015), and with lesser intensity since the Late Bronze Age (Vincze et al., 2017; Schumacher et al., 2018).

5.2. What environmental stressors drive community changes in the chironomid and diatom records?

In Fig. 5 we summarized our proxy data and land use history information in order to aid the identification of drivers. In addition, using RDA we looked at what external forcing factors may affect the community changes of the chironomid assemblages (Fig. 6ab). We used geochemical variables (LOI, Pb, Al) and diatoms (*A. formosa*, *S. seminulum*, planktonic:benthic ratio) as external variables and found that organic content (LOI, RDA 1: 15.06%) and geoPC axis 1 (RDA 1: 22.55%) have significant influence on the chironomid assemblage changes; these two variables remained as the major explanatory variables after stepwise selection that correlate with the chironomid assemblage changes (Fig. 6ab).

The negative direction of Axis 1 on RDA_{env} is associated with the organic matter content (LOI), and it suggests that changes in the chironomid assemblages are best explained by increased organic matter content since 1903. This coincides with a sudden increase in *T. lugens*-type. The positive direction of Axis 1 on RDA_{PCA} is associated with geoPC axis 1 suggesting that terrigenous elements (Rb, K, Ba, Mg, Ni, Co, Li, Cr) that increase with depth show a positive correlation with the lower chironomid assemblages dominated by *M. insignilobus*-type until 1903. Overall, this suggests that the turn of the 20th century is a major dividing line in this record, and higher organic content characterise the 20th and 21st centuries when the rate of change values, the Pb concentrations and many more paleoproxies (e.g. saprobic indicator diatom) increase suggesting an acceleration of human disturbance. Furthermore, we also see on RDA_{PCA} a negative correlation with explanatory variables (Cd, LOI, CO₃, Pb) that are associated with anthropogenic activities. These analyses overall suggest that changes in the chironomid assemblages can be partially accounted for productivity increase mainly since 1903.

If we compare our biotic and chemical proxy records with the historical information (Fig. 5; Appendix Data 1), we see that extensive grazing since 1850 correlates well with the first accelerated compositional change of the chironomid fauna. The increase in collector-gatherers around 1870 suggested coarse-grained organic material input into the

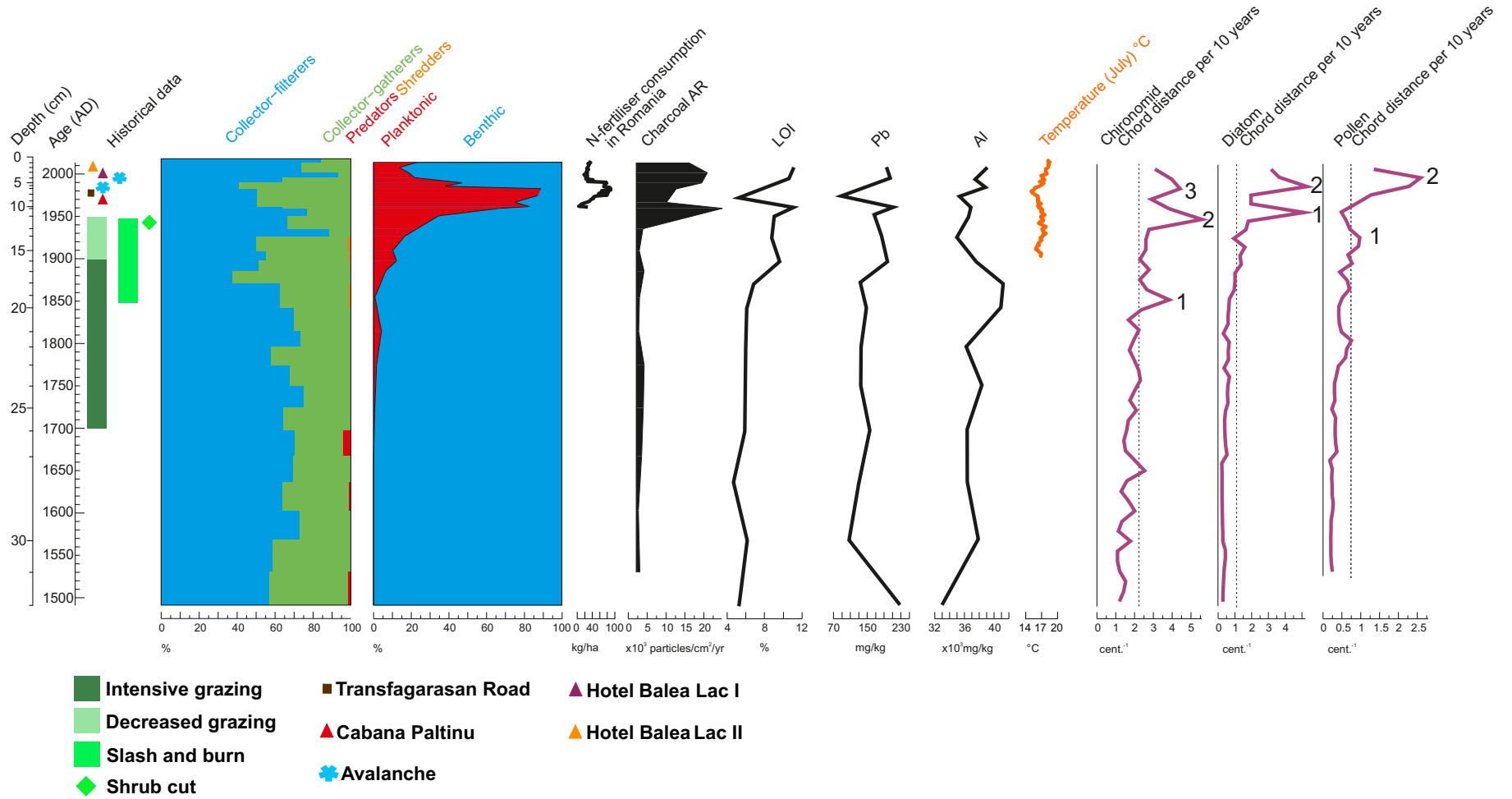


Fig. 5. Summary of biological and chemical proxy data for the last 500 years from Lake Bălea and information on land use history.

Table 1
Summary of GAMs to examine the relationship between principal component 1 scores (chironomid taxa and traits) and temperatures (T_{July} and T_{summer}).

Model	Estimated df	Reference df	F	p-Value	adj R ²	Deviance explained (%)
PC 1 of taxa ~ T_{July}	1	1	4.542	0.0585	0.244	31.2
PC 1 of taxa ~ T_{summer}	1	1	6.635	0.0272	0.339	39.9
PC 1 of traits ~ T_{July}	1.671	1.891	11.58	0.00498	0.627	68.4
PC 1 of traits ~ T_{summer}	1.714	1.918	9.192	0.0105	0.571	63.7

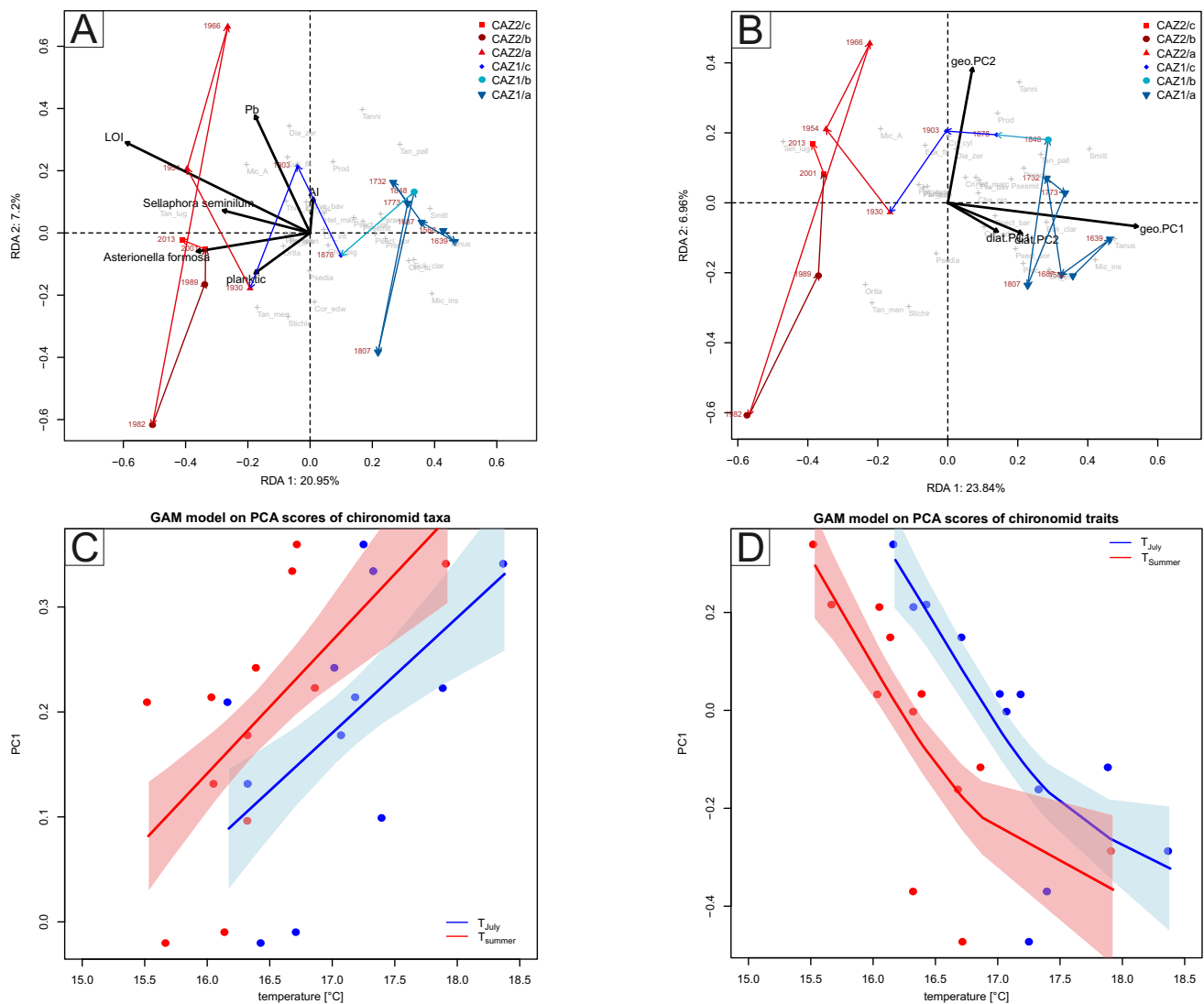


Fig. 6. Results of the redundancy analysis (RDA); (A) geochemical variables (LOI, Pb, Al) and diatoms (*Asterionella formosa*, *Sellaphora seminulum*, planktonic:benthic ratio) were used as external variables; (B) the first two principal components of diatoms and geochemical PCAs were used as external variables; (C–D) general additive models (GAMs) to examine the relationship between PC 1 scores (chironomid taxa (C) and traits (D)) and temperatures (T_{July} and T_{summer}); abbreviations include: *Dia_zer-Diamesa zernyi/cinerella*-type, *Prod-Prodiamesa*, *Psedia-Pseudodiamesa*, *Proc-Procladius*, *Cha_pig-Chaetocladus piger*-type, *Cor_edw-Corynoneura edwardsi*-type, *Thie_E-Thienemanniella type E*, *Cri_cyl-Cricotopus cylindraceus*-type, *Cri_int-Cricotopus intersextus*-type, *Ort_tri-Orthocladus trignonolabis*-type, *Euk_clar-Eukiefferiella claripennis*-type, *Euk_fit-Eukiefferiella fittkaui*-type, *Het_marc-Heterotrissocladus marcidus*-type, *Paracr-Paracricotopus*, *Psect_bar-Psectrocladius barbatipes*-type, *Psect_sor-Psectrocladius sordidellus*-type, *Psesmit-Pseudosmittia*, *Smitt-Smittia*, *Tve_bav-Tvetenia bavarica*-type, *Ortla-Ortochladinae*, *Stichir-Stitochironomus*, *Mic_ins-Micropsectra insignilobus*-type, *Mic_A-Micropsectra-type A*, *Partan-Paratanytarsus*, *Par_aus-Paratanytarsus austriacus*-type, *Par_pen-Paratanytarsus penicillatus*-type, *Tanus-Tanytarsus*, *Tan_lug-Tanytarsus lugens*-type, *Tan_men-Tanytarsus mendax*-type, *Tan_pall-Tanytarsus pallidicornis*-type, *Tanni-Tanitarsini*).

lake (Luoto and Nevalainen, 2015) (Fig. 5) that correlates well with slash and burn activity in the subalpine zone at the same time. Two chironomid groups were particularly favoured by this change: *H. marcidus*-type and *Pseudodiamesa*. These changes again show a similar trend than the North Carpathian Tatra lake study by Hamerlík et al. (2016), which demonstrated an increase in *H. marcidus*-type associated with

increasing organic matter content around 1970. During this period, planktonic algae started to increase in the diatom community (Fig. 5) signalling that intensifying grazing in the mid-19th century led to small-scale nutrient input and hence trophic level increase. Another chironomid taxon showing an increase at this time is the genus *Pseudodiamesa*, which usually live in cold, deep oligotrophic lakes

(Sæther, 1969; Tóth et al., 2017), but some species (e.g. *P. branickii*) tolerate more intense mixing and movement within the water body, supporting in this case the intensification of coarse organic debris input due to slash and burn activity (Lencioni et al., 2012). The pollen record also confirms deforestation in this period in the spruce and beech-fir forest zone, but places the onset of this regional deforestation earlier, to 1800 (Fig. A.10).

From 1926 to 1970 collector-filterers increased in relative abundance (Fig. 5) suggesting an increase in suspended material (Luoto and Nevalainen, 2015). This is the time period when grazing became less intense in the catchment, but the trophic level of the open water likely still increased progressively as indicated by the dynamic planktonic diatom increase. Both the chironomid fauna and diatom flora experienced accelerated rate of change at this time, while the terrestrial vegetation did not change too much after the initial beech (*Fagus sylvatica*) forest clearance, which took place at lower altitudes in the 1920's (Fig. 5). From 1970 the building of the Transfăgărașan Road and the construction/reconstruction of shelters on the lakeside took place (Gratton et al., 2015). The episodic increase in SAR and decrease in organic content correlates well with the timing of road works, and this disturbance (input of siliciclastic material) likely explains the increased proportion of collector-gatherers during this period accompanied by a decrease in *T. lugens*-type and chironomid taxon richness, as well as an increase in *H. marcidus*-type, *Pseudodiamesa* and collector-gatherers numbers (Fig. 3). An interesting feature of the biotic proxy records is the absence of diatom response at this time. Planktonic diatoms (DAZ-3 zone) attained their highest abundance between 1950 and 1990, when locally grazing and associated nutrient input decreased, and when road and hotel building works resulted in abrupt shifts in siliciclastic input into the lake episodically. The diatom flora shows intensive community changes in this period as depicted by the RoC curve (Fig. 5), but there is also strong indication that the increasing nutrient enrichment does not come from local sources. If we look at the curve of nitrogen fertilizer consumption in Romania since 1960 (Fig. 5), and written records from earlier periods (EEA report, 2004; Grzebisz et al., 2012), we see that artificial fertilization was the main way to boost crop production and achieve higher yields during the socialist regime. The use of artificial fertilizers started in 1900 and was on maximum between 1975 and 1990. This period corresponds tightly with the peak abundance of planktonic diatoms in Lake Bâlea, and within it the centric diatom peak what we interpreted as a clear sign of planktonic eutrophication in the lake (Figs. 4 & 5). Political changes in 1989 led to the collapse of the socialist economy, which also resulted in cutbacks in subsidies on agricultural commodities. As a consequence, fertilizer use had fallen drastically and the soil nitrogen input halved within one year (Eriksson et al., 2007; Grzebisz et al., 2012; Schmidt et al., 2017). As shown in Fig. 5, the timing of this decrease coincides within ± 5 -yr uncertainty with the dramatic decrease in planktonic diatoms suggesting a cause and effect relationship (Fig. 5). Atmospheric deposition of anthropogenic reactive nitrogen (N) and connected planktonic eutrophication has been demonstrated widely in the northern hemisphere, particularly in alpine low trophy lakes (Wolfe et al., 2001; Hobbs et al., 2010; Sheibley et al., 2014; Rhodes et al., 2017). These North American studies found that mid-latitude alpine lakes were the most impacted by high N deposition rates, and our diatom record suggests the same for the Southern Carpathian Mountains.

The diatom record has another feature that surprisingly mimics similar species reorganisations in North American alpine lakes (Wolfe et al., 2001). The nitrophilous diatom, *A. formosa* (Hobbs et al., 2010) became dominant after 1980, replacing centric diatoms and other fragilaroids within the planktonic diatom community. The magnitude of this change produced the highest RoC value in the entire record (Fig. 5). Particularly in alpine lakes of the American Cordillera (Wolfe et al., 2003; Saros et al., 2005), the increase of *A. formosa* took place in the 1950's (30 years earlier than in the Carpathians), where it has been linked causally to increase in Nr availability from atmospheric deposition. Since alpine

lakes are naturally N-limited or co-limited by N and P, they are the most susceptible (Baron, 2006). The earlier proliferation of *A. formosa* in North America is likely connectable to an earlier onset of massive artificial fertilization production and use, but if we look at the local N-fertilizer consumption in Romania (Fig. 5), it is apparent that *A. formosa* did not respond to a secondary increase in fertilizer consumption if the estimates are correct. It remains an open question what triggered its relatively late spread, but it is likely connectable to a further increase in the lake's reactive nitrogen input in the 1980's. It is also apparent that unlike in North America, the economic collapse at the transition from socialism to a market economy led into abrupt decrease in Nr availability and consequently to the halt of planktonic eutrophication in this alpine lake. Even though other anthropogenic disturbance connected to tourism and recreation brought in new environmental stressors since 1992 (more hotels were built on the lakeside with parking lots added and the number of tourists multiplied), it did not favour the sustained abundance of planktonic diatoms. These new stressors however induced further changes in the biotic assemblages, and looking at the rate of change (RoC) curves, we can conclude that both the aquatic and terrestrial environment experienced continuous high rate of change in the last nearly 30 years, which is unprecedented in the last 500 years. Saprobic indicator diatoms and the increasing abundance of *T. mendax*-type indicate warmer water and increasing nutrient loading from sewage water (Taylor et al., 2013; Luoto et al., 2014b; Tóth et al., 2017).

Similar results were published from Lake Bled in Slovenia that also highlighted the severe influence of construction activities over the sediment chemistry profile associated with productivity and nutrient growth during the last 100 years (Ogorelec et al., 2006).

5.3. What drives chironomid community changes in Lake Bâlea? Temperature, trophic state or both?

If we look at the oligotrophic lakes in Europe that have been heavily influenced by climate change in addition to humans, we see similar changes. Warming can cause significant changes within the water body, enhancing biogeochemical processes and modifying habitats, thus altering the aquatic ecosystems (Adrian et al., 2009; Schindler, 2009). Current warming is known to increase pond nutrient intake, reduce snow and ice cover, causing disturbance to a stable ecosystem (Jeppesen et al., 2009).

Overall, it is very difficult to decouple the effects of warming from the human use of landscapes in oligotrophic lake systems. There are many examples of increased lake productivity as a result of warming (Eggermont and Heiri, 2012; Luoto, 2009), while there are cases that do not show correlation between the two (Kirilova et al., 2009; Luoto et al., 2009; Tóth et al., 2017). In Lake Bâlea both factors likely played a role; in this systems diatoms are sensitive indicators and thus respond quickly to changes in limiting nutrients that had both atmospheric and land source in the last 200 years with a much stronger impact delivered by the atmospheric source, while chironomids were more sensitive to summer temperature change and were only secondarily influenced by local anthropogenic nutrient enrichment.

5.4. Ecological boundaries and restoration

Since biological systems are regulated by the tolerance ranges of their components, such systems are often characterised by threshold type responses (Langdon et al., 2016). In a rapidly changing environment biotic communities within lakes for example often show hysteresis and flickering between alternate states before a critical transition takes place (Wang et al., 2012). Small effects usually return the system to its original state, but large effects create a whole new system that is significantly different from the original. This flickering behaviour of biotic communities have recently been used to predict catastrophic ecosystem shifts that are expected to occur in the near future, and this

behaviour has been used recently to develop early warning signals to forecast undesirable change in lake and terrestrial ecosystem health (Wang et al., 2012; Dearing et al., 2014; Cooper et al., 2020).

For Lake Bălea a relatively high resolution paleoecological study was performed. Using the concept of Dearing et al. (2014), the results of the ecological regulation analysis show (Fig. 7) that even in the safe (green) part of the lake's operational space, the system shows small fluctuation around the baseline condition, and depending on the studied system component the response is either abrupt (e.g. in case of the water quality) or show a distinct one-way movement from the background level, in some cases with non-hysteretic threshold type change. Except for the terrestrial vegetation, sediment regulation and water quality, we find that our system was transformed and entered the cautious (yellow)

status in the late 19th and early 20th century (Fig. 7). Out of the examined seven system components, three measures (water quality 1 & 2 and air temperature) can be assessed as showing dangerous change (red), so the environmental ceiling is exceeded for degraded water quality, and we may conclude that the lake ecosystem has moved out of its safe operational state. This analysis warns us of expectable further catastrophic changes that require intervention (Fig. 7). Non-linear trends with unacceptable acceleration are also present in the rate-of change curves since ~1950 (Fig. 5). This multiproxy assessment suggests that protection measures have to be employed to integrate social wellbeing, (in this case sustainable tourism and herding) with sustainable lake ecosystem health. In this system increasing soil stability and decreasing organic nutrient input are likely sufficient measures to flick

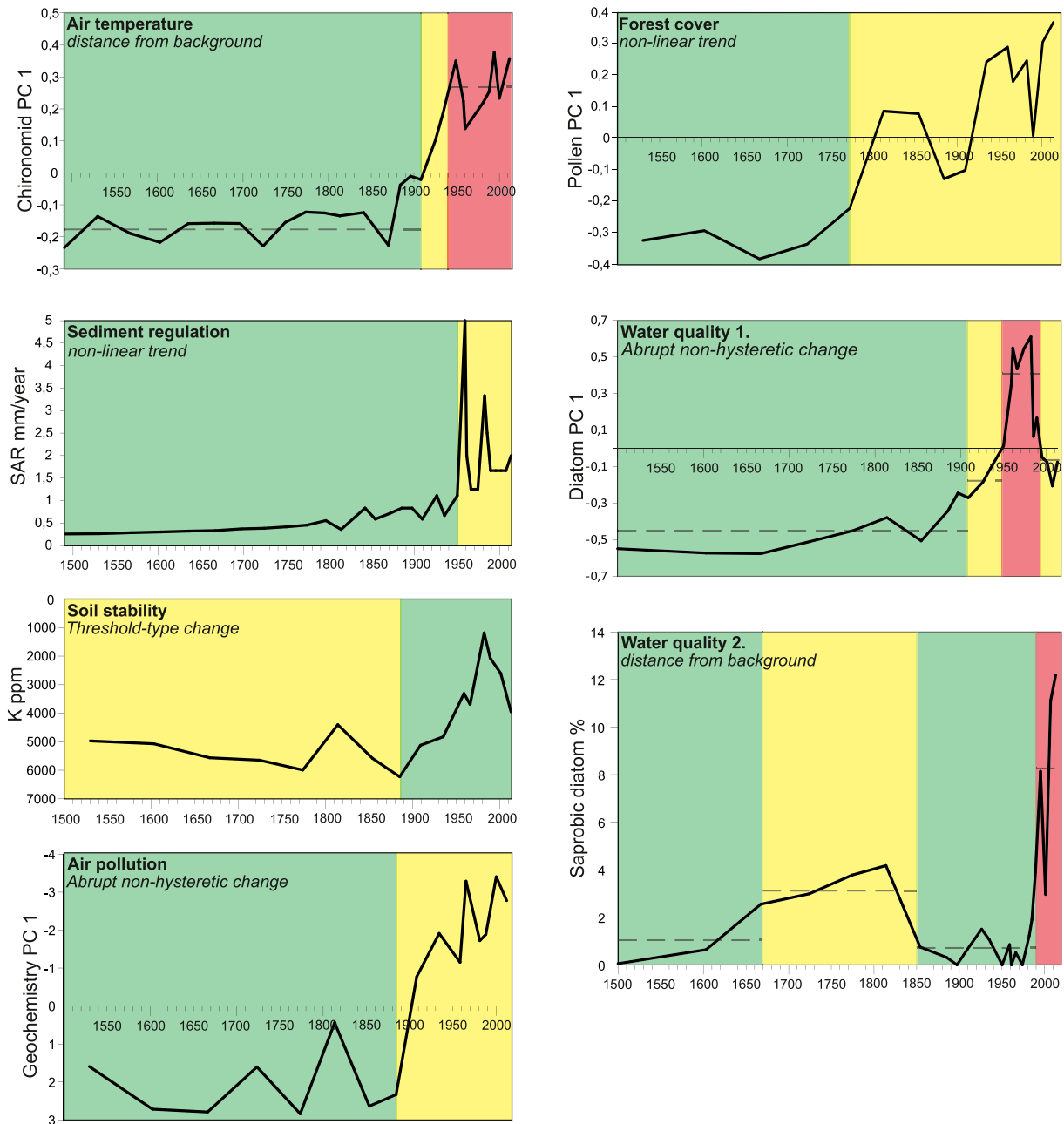


Fig. 7. Ecological boundaries from Lake Bălea (Făgăraș Mts, S Carpathians) for air temperature (chiro PC 1), sediment regulation (SAR), soil stability (K), air pollution (geochemistry PC 1), forest cover (pollen PC 1), water quality 1 (diatom PC 1) and water quality 2 (saprobic diatom %) based on a time series extending back to 1490–1530 using the concept of Dearing et al. (2014); colour coded segments show historical changes in the safe (green), cautious (yellow) and dangerous (red) status of each ecological process. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

the system back to a cautious or safe state since atmospheric Nr pollution halted; however, the ongoing climate warming may amplify ecosystem change in the future despite protection measures.

6. Conclusions

This study demonstrated the climatic and human disturbances of Lake Bâlea over the last 500 years using biological and geochemical proxies. Our results suggest that in this alpine lake chironomids responded mainly to increasing summer temperature, and higher temperatures were also associated with higher abundance of the collector filterer guild. In addition to temperature, the growing organic matter content has also influenced the change of the chironomid fauna since 1903, when the rate of change values, the Pb concentrations and several other proxies (e.g. saprobic indicator diatom) increased suggesting an acceleration of human disturbance.

The main driver of the diatom community change was atmospheric reactive N (Nr) fertilization that drastically moved the community towards planktonic diatom dominance (*A. formosa*) after 1950. We demonstrated that in East-Central Europe the trend of Nr fertilization was different than in North America, with an abrupt halt at the transition from the socialist to the market economy, unlike in North America, where the use of artificial fertilizers did not decrease. We also demonstrated that alpine lake responses were similar. Other anthropogenic disturbances connected to tourism and recreation brought in new environmental stressors since 1992. These new stressors induced further changes in the biotic assemblages, and looking at the rate of change (RoC) curves, we conclude that both the aquatic and terrestrial environment experienced continuous high rate of change in the last nearly 50 years that is unprecedented in the last 500 years. Saprobic indicator diatoms and the increasing abundance of *T. mendax*-type indicate warmer water and increasing nutrient loading from sewage water.

Except for the soil stability, we find that the system was transformed and entered the cautious status in the late 19th - early 20th century (Fig. 7). Out of the examined seven system components, three measures (water quality 1 & 2 and air temperature) showed dangerous change, so the environmental ceiling was exceeded for degraded water quality, and we conclude that the lake ecosystem has moved out of its safe operational state.

CRedit authorship contribution statement

Zoltán Szabó: Investigation, Writing - original draft, Visualization. **Krisztina Buczkó:** Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Aritina Haliuc:** Visualization, Investigation, Writing - original draft. **Ilona Pál:** Investigation, Visualization. **János Korponai:** Data curation, Visualization, Investigation, Writing - review & editing. **Róbert-Csaba Begy:** Investigation, Visualization. **Daniel Veres:** Writing - review & editing. **Tomí P. Luoto:** Writing - review & editing. **Andreea R. Zsigmond:** Investigation, Writing - review & editing. **Enikő K. Magyar:** Conceptualization, Methodology, Supervision, Project administration, Funding acquisition, Writing - original draft, Writing - review & editing.

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

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