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Changes in nutrient availability imprinted in long-term diatom succession in lakes

Změny dostupnosti živin zachycené
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Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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List of original publications

This thesis is based on the following papers. The contributions of the author are as follows.

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Abstrakt

Ve vývoji jezerní primární produkce během pozdního glaciálu a holocénu lze vysledovat ustálené trendy: postupný nárůst produkce – „eutrofizaci“ a naopak pokles produkce – „oligotrofizaci“, často spojenou s přísunem organické hmoty z povodí – „dystrofizací“. Kromě nejčastějšího ovlivnění produkce dostupností živin, vstupuje do komplexního jezerního vývoje řada abiotických a biotických faktorů jako dostupnost světla, režim míchání, pH, mechanické disturbance, predace nebo kompetice. Vztah dostupnosti živin a primární produkce v minulosti v sobě tedy skrývá důležitou informaci o všech těchto faktorech ve fungování jezerního ekosystému.

Rekonstrukce minulých procesů vychází z citlivé reakce vodních organismů na změny prostředí. Jejich subfosilními zbytky se do usazenin ukládá záznam o dynamických procesech, které odhalují historii konkrétních jezer ale i události celosvětového rozsahu. Mezi organismy, z nichž lze rekonstruovat minulé fyzikálně chemické podmínky, patří významné postavení rozsivkám. Zatímco planktonní rozsivky přináší spíše informaci o celkovém stavu jezera, změny v druhově pestrém společenstvu perifytických rozsivek umožňují vhled do jemnějších změn ekosystému z pohledu litorálu.

Předkládaný výzkum pátrá po komplexních procesech v jezerním vývoji propojením kvalitativní a kvantitativní rozsivkové analýzy s dalšími ukazateli jezerních podmínek (např. pakomáry, pyly, rostlinnými makrozbytky, geochemií sedimentů). Konkrétně sleduje historii trofického stavu dvou středoevropských jezer, které byly svou morfologií a pozicí v krajině předurčeny k opačným vývojům. Zatímco rozlehlé mělké nížinné jezero prošlo časnou eutrofizací s převládajícím vlivem autochtonních faktorů, malé lesní horské jezero prodělalo dystrofizaci alochtonním přísunem rozpuštěné organické hmoty. V tomto směru potvrdila obě jezera předpoklady o svém směřování v rámci obecné představy o postglaciálním vývoji trofického stavu v jezerech. Nížinné jezero navíc ukázalo jedinečný příklad reakce ekosystému kontrolovaného převážně vnitrojezerními procesy v období prudké klimatické změny na hranici pozdního glaciálu a holocénu. A lesní horské jezero prokázalo, že velmi podobný průběh dlouhodobé dystrofizace jako v tradičně zkoumaných boreálních oblastech může být zaznamenán na špatně pufrovaných podložích ve střední Evropě.

Abstract

An examination of primary production in lakes shows regular trends during the Late Glacial and the Holocene periods: an increase (‘eutrophication’) and a decrease (‘oligotrophication’) usually connected with the input of terrestrial organic matter (‘dystrophication’). Although nutrient availability usually influences production, other abiotic and biotic factors enter lakes' complex ontogeny, such as light availability, mixing regime, pH level, mechanical disturbance, predation or competition. Consequently, the past relationship between nutrient availability and primary production contains important information about the drivers of ecosystem functioning in the past.

The reconstruction of past processes relies on the sensitive response of aquatic organisms to environmental changes. Their subfossil remains record the dynamic processes in the sediments and reveal both the local lacustrine history and more global events. Among other organisms, diatoms fulfil their function as a valuable proxy of past physical and chemical properties of water bodies. Whereas the record of planktonic diatom species dynamics represents the general ecosystem condition, variations in species-rich littoral periphytic diatoms add necessary insight into finer environmental fluctuations.

The presented research combines qualitative and quantitative diatom analysis with other proxies (e. g., chironomids, pollen, plant macrofossils, sediment geochemistry) to investigate a complex process of in-lake succession. We specifically tracked the past trophic development of two Central-European lakes predetermined towards opposite trophic trajectories by their morphology and landscape position. While the large lowland shallow lake experienced early eutrophication primarily driven by autochthonic factors, the small mountain forest lake underwent dystrophication by allochthonous input of dissolved organic matter. Both lakes confirmed their assumed tendencies in the scope of general post-glacial lake trophic trajectories. However, our record from the lowland lake provides a unique insight into the response of a highly self-controlled ecosystem to the sharp climatic change at the Late-Glacial/Holocene transition. The mountain forest lake demonstrates that long-lasting dystrophication can show a very similar pattern on poorly-buffered bedrocks in Central Europe as in the well-studied boreal regions.

Chapter 1:
Introduction

1.1 Essential role of long-term studies in research of nutrient dynamics

Nutrients are unevenly distributed in space and time. Although recent human-enhanced atmospheric nutrient deposition substantially supplies specific ecosystems (e. g. Elser et al., 2009; Camarero & Catalan, 2012), spatial transport of nutrients in terrestrial ecosystems is mediated mainly by ground and surface water. Therefore, natural water bodies such as lakes reflect nutrient availability in a surrounding landscape by collecting nutrient-bearing water from their catchment. Furthermore, lakes also archive evidence about nutrient status over time, accumulating this evidence in their sediments. As such, lakes significantly contribute to our understanding of global nutrient distribution and long-term dynamics.

Long-term records are essential in studies of nutrient cycling. However, discernible mechanisms vary with the time scale investigated; the shorter the time span, the shorter the effect of mechanisms are revealed. Decadal- and centurial-scaled studies have often failed to confirm relationships observed over broad spatial scales. Decadal-scaled monitoring suffers from a mixture of intensive anthropogenic and more extensive natural driving factors (e. g. Gavin et al., 2018), complicating the comparison between short-term observations and spatial datasets (Stetler et al., 2021). The same problem also applies to centurial-scaled studies, which can capture the direct influence of multi-seasonal climates (e. g., prolonged growing season) but cannot accommodate long-lasting indirect climate effects (e. g., vegetational succession) (e. g. Shinneman et al., 2016). In contrast, millennial-scaled studies can reveal climatically-induced mechanisms in nutrient cycles invisible during the last centuries (e. g. Dreßler et al., 2011; McLauchlan et al., 2013), although some localities were affected by profound human-induced alterations of biogeochemical processes for millennia (Anderson, 1995; Kalis et al., 2003). Palaeolimnological research enables the so-called ‘multi-proxy’ reconstruction of a broad spectrum of ecosystem responses and their driving factors using parallel records of multiple fossil remains underpinned by geochemical analyses (Birks & Birks, 2006; Bennion et al., 2015). The challenge of past nutrient trends and their driving factors was identified as one of the most important questions for future palaeoecological research (Seddon et al., 2014).

1.2 Use of the trophic state concept in palaeolimnology

Palaeolimnological research is confronted with the problem of degradation of the trophic state concept. The trophic state was originally defined by Naumann (1929) as a degree of algal production in lakes and a key role in its limitation was attributed to the concentration of nitrogen and phosphorus (Lampert & Sommer, 2007). Unfortunately, as the role of phosphorus concentration was strengthened by a discovery of a significant chlorophyll-phosphorus relationship (National Academy of Sciences, 1969; Likens, 1972), the phosphorus concentration mostly substituted the production in defining the trophic state. This shift eroded the original concept confusing the effect (production) with the cause (phosphorus concentration) and overlooking the role of other abiotic factors limiting production (e.g., light availability (Karlsson et al., 2009), pH level (Leavitt et al., 1999), mixing regime (Havens & Nürnberg, 2004), mechanical disturbance (Stevenson et al., 1996)).

If palaeolimnological studies intend to address factors of complex past processes such as eutrophication or dystrophication, they need to establish a hierarchy of factors and effects clearly. In this view, the terms ‘eutrophication’ and ‘oligotrophication’ should be assigned consistently to trends on the gradient of primary production as well as suggested for needs of recent monitoring (Carlson & Simpson, 1996). Although other factors, such as lake morphometry, oxygen budget and composition of communities (Van Dam et al., 1994 and references therein) were involved in defining the trophic state of lakes, the primary production and, particularly, algal production, remains the most comparable and easily measurable variable.

Incorporating an axis for dissolved organic carbon (DOC) concentrations into the trophic lake classification was justified by the recent browning of surface waters (Williamson et al., 1999) and can similarly serve to track millennial-scaled dystrophication in palaeolimnological studies. Advanced methods can overcome difficulties in estimating past lake production or phosphorus levels, but a lack of a unified approach to the description of fundamental processes hinders potential broader conclusions.

1.3 Phosphorus and light limitation of primary production

The paired effect of limitation by nutrients and light forms primary production in lakes. Concentrations of phosphorus and chlorophyll *a* show a significant positive relationship (Likens, 1972; Prairie et al., 1989; Phillips et al., 2008), thereby establishing phosphorus as a master variable in the limitation of aquatic primary production (Schindler, 1977). This ‘phosphorus paradigm’ has shifted recently from the singularity of phosphorus toward probable co-limitation by phosphorus and nitrogen (Lewis & Wurtsbaugh, 2008; Bratt et al., 2020). However, phosphorus's prominent impact and the necessity of its regulation in lake management are intensively advocated (Schindler, 2012; Schindler et al., 2016), and phosphorus retains its key role at least in less-productive lakes or over a longer-time perspective (Sterner, 2008; Bratt et al., 2020).

Increased phosphorus concentrations can result in the self-shading of growing primary producers in productive lakes (Sand-Jensen & Borum, 1991; Scheffer & Van Nes, 2007). A more complex coupling of light and nutrient limitation has been shown during research on surface water browning by highly pigmented organic compounds in organic matter (OM; measured as DOC). Stoichiometry of DOC and nutrients in dissolved organic matter determines the balance of its fertilizing and shading effects (Kelly et al., 2018). The nutrient content of organic matter can vary by its origin (nutrient-poor allochthonous OM, nutrient-rich autochthonous OM) (Tipping et al., 2016). The crucial question, but one that is not provable by short-term monitoring (Stetler et al., 2021), is whether the stoichiometry of DOC and nutrients changes over time. As suggested by empirical modelling (Kelly et al., 2018), a unimodal relationship applies to DOC and primary production in the case of constant stoichiometry. However, a wedge-shaped relationship appears with unstable stoichiometry, allowing both a decrease or an increase of primary production along the DOC gradient corresponding to field observations of unimodal (Jones, 1992; Seekell et al., 2015), negative (Karlsson et al., 2009) or positive responses (Nürnberg & Shaw, 1999; Zwart et al., 2016) of primary production to ongoing browning.

The distinct research bias towards pelagic primary production is clear from the overwhelming majority of studies that have focused on phytoplankton. The neglect of littoral primary production weakens whole-lake primary and secondary production estimations, especially in clear-water shallow lakes, where littoral primary producers are the dominant contributors to whole-lake primary production (Vadeboncoeur et al., 2003). Furthermore, fish populations depended strongly on littoral algal biomass, even in lakes with a relatively low contribution of littoral to total primary production (Vander Zanden et al., 2011; Finstad et al., 2014). A focus on phytoplankton might result in a serious misunderstanding of ecosystem dynamics, as nutrient and light availability disproportionally affect pelagic and littoral habitats. Littoral photosynthesis is shaded directly by thriving phytoplankton blooms during nutrient loading into clear-water shallow lakes (Scheffer & Van Nes, 2007; Vadeboncoeur et al., 2008). Humic-rich lakes experience light attenuation by organic compounds critical for primary production in deeper bottom habitats, but then pelagic growth is fertilized by nutrients mobilized to lakes within the organic matter (Karlsson et al., 2009; Brothers et al., 2014; Vasconcelos et al., 2016). The shift of primary production from bottom habitats to the water column arises from nutrient and DOC inputs. Exceptions can appear when shading by DOC crosses the threshold and suppresses pelagic primary production (Jones, 1992; Carpenter et al., 1998), or when phytoplanktonic bodies or DOC shield littoral primary producers from harmful UV radiation (Laurion et al., 2000; Pienitz & Vincent, 2000). Numerous examples of work have advocated for the essential role of littoral primary producers in estimating whole-lake primary production. Restricting the view to phototrophic primary producers can also seriously underestimate in-lake trophic pathways, since heterotrophic bacterioplankton have commonly substituted the phototrophs in primary production and have sustained the secondary production of light-limited humic-rich lakes (Jones, 1992; Nürnberg & Shaw, 1999; Drakare et al., 2002). This can complicate the response of whole-lake primary production to anthropogenic acidification and subsequent recovery (Vrba et al., 2006).

1.4 Reconstruction of past lake ontogeny

1.4.1 Motivations for reconstruction

Estimation of nutrient effect on the dynamics of past primary production is always challenging (Waters et al., 2012; Ammann et al., 2013). Parallel reconstruction of past primary productivity and phosphorus levels aims to distinguish the periods of nutrient limitation and driving by other factors, e.g., light attenuation (Waters et al., 2012; **Chapter 4**), favourable light and mixing conditions (Itkonen et al., 1999) or higher nutrient-competition (**Chapter 2**). Despite the complicated separation of drivers of past primary production changes, the coupling of nutrients and primary production reveals important aspects of past lake ecosystem functioning (e. g. Klamt et al., 2021; Tu et al., 2021). Furthermore, reconstruction of in-lake total phosphorous (TP) concentrations alone tracks broad-scaled processes, as phosphorus enters many terrestrial, freshwater and marine biogeochemical pathways (e. g. Filippelli, 2008; Lambers et al., 2008; Kuneš et al., 2011; Kopáček et al., 2015).

1.4.2 Past primary production in lakes

Estimations of past in-lake primary production proceed from sedimented biomass, although its potential reduction by mineralization must be considered. Since the biomass/OM came from both terrestrial and aquatic organisms into the sediment, the source of OM should be differentiated. A proportion of OM in the sediment can be determined by (i) loss-on-ignition (LOI), when the sedimentary sample is heated to 550°C for four hours and the burned mass represents the weight of the OM (Boyle, 2001); or (ii) concentration of total organic carbon in the sediment (Meyers & Lallier-Vergès, 1999). This general quantitative piece of information can be further qualitatively specified by the ratio of carbon and nitrogen (C/N) in the OM using different stoichiometry of C and N in terrestrial plants, with a higher proportion of C being due to developed supporting tissues compared to floated aquatic plants (Meyers & Ishiwatari, 1993). Analysis of the carbon isotopic ratio ($\delta^{13}C$) supplements the divergence between aquatic algae with C3 terrestrial plants preferring lighter isotope ^{12}C and C4 terrestrial plants discriminating less (Meyers & Lallier-Vergès, 1999; Thevenon et al., 2012). A ratio of nitrogen isotopes ($\delta^{15}N$) further specifies the fate of organic matter participating in multiple biogeochemical pathways. The $\delta^{15}N$ analysis can make some differentiation, e. g., between N_2-fixing and assimilation of nitrates, but straightforward interpretation is limited by a great complexity of nitrogen cycling (Talbot, 2001).

Production of specific groups of organisms can be estimated by counting their remains with reference particles, e. g., *Lycopodium* spores or divinylbenzene microparticles (Stockmarr, 1971; Battarbee et al., 2001). Additionally, various biomarkers are progressively employed in palaeostudies, such as pigments or lignin (Leavitt et al., 1999; Fuhrmann et al., 2004; Waters et al., 2012; Sanchini et al., 2020). To determine the production of silicified organisms (diatoms, chrysophytes, etc.), biogenic silica concentration can be analysed directly (Conley & Schelske, 2001) or indirectly estimated by the Si/Ti ratio. A correction of potentially biogenic elements (Si, Ca, Sr) by typically minerogenic Ti allows for use of the ratio as a proxy for in-lake biological production (Kylander et al., 2013).

1.4.3 Past phosphorus availability

1.4.3.1 Direct and indirect records

Sedimentary phosphorus can show the past availability of phosphorus in the water column. However, phosphorus is efficiently released from sediment, especially in anoxic conditions (Petticrew & Arocena, 2001; Søndergaard et al., 2003). Although extraction of specific fractions of sedimentary phosphorus (Paludan & Jensen, 1995) improves reconstructions of past phosphorus dynamics (e.g., Klamt et al., 2021; Tu et al., 2021), the problem of diagenetic loss of phosphorus is successfully bypassed by indirect estimations based on the phosphorus demands of aquatic organisms.

The above-mentioned close relationship between aquatic organisms and phosphorus enables reconstruction of past concentrations of epilimnetic phosphorus (i.e., TP) from species composition of subfossil assemblages. In particular, diatoms show a strong potential for reconstructions of TP, more so than other indicator groups (e.g., cladocerans, chironomids, chrysophytes) (Lotter et al., 1998; Kelly et al., 2008) and the record of sedimentary phosphorus (Anderson et al., 1993). Various possibilities of diatom-based inferring of past phosphorus levels are discussed below.

1.4.3.2 Diatom-based transfer functions

A robust tool for estimating long-term phosphorus dynamics has been required because anthropogenic eutrophication started to devastate lake ecosystems. Diatoms, abundant and diverse algae in a wide range of aquatic habitats, provided an excellent base for quantitative reconstructions and gave rise to diatom-based transfer functions for TP (Bennion et al., 1996; Hall & Smol, 2010).

The most frequently used transfer function in palaeolimnology is weighted averaging (ter Braak & Barendregt, 1986; ter Braak & Looman, 1986), which is an inverse method. It works on the assumption that particular species have a unimodal relationship with the environmental variable, i.e., an optimal variable level exists for each species. The reconstruction of the environmental variable was carried out in two steps: (i) the 'regression step', in which the species optima were determined from a calibration dataset; (ii) the 'calibration step', in which the past value of the environmental variable was calculated by weighted averaging of species optima by their abundance in a fossil sample (Juggins & Birks, 2012). The species optima were estimated from their relative abundances at localities with diverse measured concentrations of TP. If the TP gradient is long and evenly sampled, the species optima for TP concentration can be found, reasoning that a species is more abundant in more favourable conditions. The same logic is used in the second calibration step, when the most abundant species in a fossil sample were deemed to have lived in conditions closest to their optima. Possible weighting by species tolerances proved to be biased by insufficient definition of rare taxa in the calibration datasets, therefore weighting is recommended only by abundances (Birks & Simpson, 2013).

Modelling TP brings specific limitations (Juggins, 2013), resulting mainly from the fact that TP behaves more like a resource than a condition (as pH or conductivity). Therefore, the cost of simplification to the unimodal relationships is likely to be higher than in the case of pH or conductivity/salinity (Potapova et al., 2004). Potential competition for phosphorus also contributes to the variability of TP concentrations over time, making sampling more difficult (Hakanson & Boulion, 2001; Bradshaw et al., 2002; Potapova et al., 2004). These challenges of measuring direct TP concentrations led to their substitution by more accurate diatom-inferred TP in recent routine monitoring (Stevenson, 2014). Additionally, TP concentrations may correlate with other important variables, chiefly alkalinity (Cardoso et al., 2007; Kelly et al., 2020); thereby, the reconstruction of past TP can be biased, especially in periods of sharp environmental changes (Leavitt et al., 1999). The influence of secondary variables is partly reduced by an advanced approach of weighted averaging partial least squares (WA-PLS) (ter Braak & Juggins, 1993), however, a critical exploration with additional independent proxies is highly recommended (Birks et al., 2010; Rivera-Rondón & Catalan, 2020).

The use of diatom-based transfer functions for TP is especially limited in shallow lakes, where intensive mixing often favours poor nutrient-indicator diatom taxa from the *Fragilaria* species complex. A substantial proportion of the relationship between diatom species and TP is also mediated by indirect factors such as light availability or macrophyte cover (Bennion, 1995; Sayer, 2001; Sayer et al., 2010). Consequently, a ‘step back’ towards a more qualitative and functional evaluation of diatom assemblage is worth considering during the reconstruction of shallow basins (**Chapter 2**), e.g., a grouping of diatoms by phosphorus demands (Van Dam et al., 1994) and minimizing the influence of poor but abundant fragilarioid indicators (e.g. Battarbee, 1986; Heinsalu et al., 2008; Demiddele et al., 2016). Similarly, a combination of qualitative and quantitative approaches benefits the diatom-based reconstructions in the regions outside extensive lake districts which have a limited relationship to calibration datasets (**Chapter 3, 4**).

1.4.3.3 Highlighting the role of diatom periphyton

Periphytic diatoms, i.e., those living on (or associated with) substrata (Stevenson et al., 1996), respond to nutrient levels more indirectly than pelagic species, since light availability, substrate type, mechanical disturbances or grazing usually override the direct influence of water-column concentrations of nutrients in bottom habitats (Sand-Jensen & Borum, 1991; Stevenson et al., 1996; Vadeboncoeur et al., 2008; Karlsson et al., 2009). Whereas a simple reconstruction of TP from changes in littoral primary productivity or biomass is complicated, species composition of periphytic diatom communities can reflect the TP concentrations in the water column (DeNicola & Kelly, 2014). The immense diversity of diatom periphyton strengthens TP models from surface sediment samples (Denys, 2007; Bennion et al., 2010).

Although nutrient-sensitive planktonic diatoms are individually superior TP indicators (Rivera-Rondón & Catalan, 2020), the richer and more diverse periphytic community produces better models for pelagic concentrations of TP (Bradshaw et al., 2002; Philibert & Prairie, 2002). Despite this effect of periphytic richness, littoral diatoms remain problematic TP indicators.

Nevertheless, the factors complicating the response of periphyton to nutrient concentrations can help us to better understand lake development. Periphytic light or substrate demands form habitat-specific diatom communities along the depth gradient (Laird et al., 2010; Hofmann et al., 2020), which offers a different set of mechanical and chemical limitations (Cantonati et al., 2009; Pla-Rabés & Catalan, 2018). The affinity of some diatom species to macrophytes enabled the reconstruction of macrophyte cover based on diatom species composition (Vermaire et al., 2011, 2013). A useful supplement to species composition is to group diatom periphyton into guilds by their growth form. Passy (2007) defined 'low-profile', 'high-profile' and 'motile' guilds, and their responses, along gradients of nutrients, light and disturbance in running waters. Adapted to lakes, this approach provides valuable data about lakes' ecosystem functioning (Leira et al., 2015; Pla-Rabés & Catalan, 2018), although it also struggles with the limits of the periphytic complex response during the reconstruction of TP concentrations (Gottschalk & Kahlert, 2012).

A thorough analysis of periphytic assemblage can improve quantitative models of past TP concentrations and qualitative assemblage descriptions respecting the source habitat. Underpinned by other proxies, fossil periphytic diatoms can mediate past littoral heterogeneity and revise the one-sided view of plankton analysis. The approach to reducing the influence of abundant but species-poor planktonic diatoms during fossil-diatom analysis is well-established in palaeolimnology (Battarbee, 1986; **Chapter 2, 4**).

1.5 Post-glacial lake trophic trajectories

A natural development of the lake trophic state, i.e., the lake ontogeny (Deevey, 1984 in Battarbee et al., 2005), reflects a set of climatic and biogeochemical factors. Therefore, tracking millennial-scaled trends in the lake ontogeny requires understanding driving factors operating on the Holocene scale. Climatically-driven soil development coupled with vegetational succession appears to induce most of the long-lasting in-lake trends (Engstrom et al., 2000; Hu et al., 2001). However, in the initial ontogenic phase, the effect of retreating glaciers and subsequent soil base depletion most likely prevailed over climatic forcing (Birks et al., 2000; Boyle et al., 2013). Weathering then gradually differentiated source and sedimentary areas, resulting in diverse ontogenies of lakes based on their position in a landscape (lowland vs. upland) (e.g., Battarbee & Bennion, 2011). The morphology of the lake basin distinctly influences its predisposition to eutrophy or oligotrophy (i.e., an epilimnion-to-hypolimnion volume ratio<1 or >1, respectively), as described by August Thienemann (Lampert & Sommer, 2007).

Regular primary production trends were recognized along a succession of lakes during the Late Glacial and the Holocene periods. Rodhe (1969) specified two main directions in lacustrine succession (Williamson et al., 1999): (i) an autotrophic pathway from oligotrophy to eutrophy; (ii) an allotrophic pathway from oligotrophy to dystrophy. Despite the presence of a third, ‘mixotrophic’ pathway, with a combination of eutrophication and dystrophication (Poniewozik et al., 2011; Drzymulska et al., 2015), the two basic trends of eutrophication and oligotrophication/dystrophication are elaborated below.

1.5.1 Eutrophication

The first palaeolimnological investigations were conducted at lowland localities and established the basic lake trajectory of increasing production during time (Battarbee & Bennion, 2011 and references therein). The terminal hydrologic position determines lowland lakes to be naturally eutrophic together with their large ratio between the catchment and lake area (Cremer et al., 2004), and potentially enhanced weathering, nutrient cycling and terrestrial primary production at warmer low altitudes (Cardoso et al., 2007). This predisposition results in the common post-glacial naturally eutrophic character of lowland lakes. In-lake recycling of phosphorus functioned as one of the most important mechanisms of lowland lake enrichment. Shallow lakes were considered to typically suffer from nutrient resuspension because of their relatively small hypolimnion and large water-sediment surface area (Scheffer, 2004; Lampert & Sommer, 2007). However, deeper lowland lakes are also naturally eutrophized by releasing sedimented phosphorus during periods of reduced mixing depth and bottom anoxia (e.g., Sanchini et al., 2020; Tu et al., 2021).

Glacial lakes commonly increased their primary production during an initial phase of ontogeny when base-rich substrates were eroded into the lake after a glacier retreat in the Late Glacial period (Ford, 1990; Engstrom et al., 2000; Boyle et al., 2013). The cold climatic oscillation of the Younger Dryas also induced erosion from unstable catchments and in-lake nutrient recycling by anoxic conditions or sediment reworking, which supported primary production in lakes (Fuhrmann et al., 2004; Kirilova et al., 2009; Bonk et al., 2021). Moreover, the early enriched conditions were also known from non-glaciated catchments because of unleached catchment soils (Kubovčík et al., 2021) or sustained by in-lake processes (Wolfe & Härtling, 1996; **Chapter 2**). The increase in primary production was also connected to rapid warming at the onset of the Holocene (Ammann et al., 2000; Ralska-Jasiewiczowa et al., 2003; Fuhrmann et al., 2004). Several records suggest that, in lowland lakes, the rise of primary production had already begun with the second warmer half of the Younger Dryas (Goslar et al., 1993; Zawiska et al., 2015; **Chapter 2**). As well as in the case of recent abrupt warming (Smol et al., 2005; Moss et al., 2011), it is difficult to disentangle the contributions of rising temperature or nutrient input during past dynamic climatic changes (Ammann et al., 2013). A water-level rise after a humidifying climate at the Late-Glacial/Holocene boundary (Magny, 2004; Bos et al., 2007) could have particularly altered shallow-lake ecosystems with flat basins and further complicated their response to climatic change. Additionally, competition became a more important factor in community structure and production than in previous periods of severe environmental limitation (Turner et al., 2014; **Chapter 2**). Gradual natural eutrophication was supplemented by cultural eutrophication, which occurred primarily in the lowlands quite early after ~7.5 cal. ky BP (Bradshaw et al., 2005; Dreßler et al., 2006; Makri et al., 2020). The following industrial eutrophication thus often affected lake ecosystems that had already been extensively anthropogenically eutrophied for a long time (Anderson, 1995). In conclusion, this long-lasting anthropogenic impact and increasing effect of recent climate change (Moss et al., 2011) complicate the setting of target conditions for potential recovery of lake ecosystems and, consequently, enhance the need for long-term studies.

1.5.2 Oligotrophication, dystrophication

Oligotrophication events occurred even during progressive eutrophication. The most typical is a decrease in primary production after the stabilizing of catchment soils and an altered mixing regime at the Younger Dryas/Holocene transition (Kirilova et al., 2009; Apolinarska et al., 2012; Turner et al., 2014). However, the decreased nutrient availability at the onset of the Holocene did not necessarily lead to the decrease of primary production, as other factors, such as higher availability of insolated habitats, rise in water levels or decrease of mechanical disturbance, could also have been influential (**Chapter 2**).

A long-lasting oligotrophication occurred in lakes with base-poor catchments, usually after the initial nutrient-rich phase (Ford, 1990; Bradshaw et al., 2000; Björk, 2010). Primarily distinct abiotic effect of base loss (Boyle, 2007; Boyle et al., 2013) was soon supplemented and potentially substituted by climatic and biotic factors, particularly vegetation succession paired with soil development (Korsman et al., 1994; Huvane & Whitehead, 1996; Pienitz et al., 1999; Engstrom et al., 2000). Climatically-driven vegetational succession resulted in heightened input of organic matter including DOC into lakes, since the further decrease of in-lake primary production was connected to water browning in the process of dystrophication. Paedogenesis, influenced by the vegetation and the Holocene's humid climate, formed hardened horizons, which supported surface runoff and facilitated the transport of DOC into lakes (Steinberg, 1991; Engstrom et al., 2000).

Gradual but stepwise dystrophication is thought to have coincided with changes in climate humidity during the Holocene, accelerating in humid periods (Brodin, 1986; Pienitz et al., 1999; Solovieva & Jones, 2002; Myrstener et al., 2021) and ceasing during dry periods (Huvane & Whitehead, 1996; Itkonen et al., 1999; Norton et al., 2011; Ampel et al., 2015). The critical threshold of dystrophication appeared at ~6 cal. ky BP in catchments where peatlands were established (Pienitz et al., 1999; Solovieva & Jones, 2002; Myrstener et al., 2021; **Chapter 3, 4**) after the climate humidification in the northern hemisphere, during the so-called Mid-Holocene Climate Transition (Magny et al., 2006; Wanner et al., 2008). A positive correlation with rainfall (Brothers et al., 2014; Ejarque et al., 2018) or with a proportion of wetlands in a catchment (Kortelainen, 1993; Laudon et al., 2011; Rantala et al., 2016) was also observed on the spatial scale for recent concentrations of DOC in lakes.

The long-lasting naturally humic character of unproductive lakes was disrupted by anthropogenic atmospheric acid deposition, which resulted in DOC precipitation and consequent clearing of lake water (Steinberg, 1991; Anderson & Renberg, 1992; Leavitt et al., 1999; Vrba et al., 2000). Whether dystrophication was substituted by oligotrophication or eutrophication during the anthropogenic acidification remains under study (Olsson & Pettersson, 1993; Kopáček et al., 2015); it also likely depends on the source of monitored primary production (Vrba et al., 2006). The recent re-browning of lakes exceeds the assumed range of recovery from anthropogenic acidification (Clark et al., 2010; Kritzberg, 2017) and, driven by climate change or altered land use, has probably accelerated brownification in the last 30 years (Fee et al., 1996; Mattsson et al., 2009; Meyer-Jacob et al., 2015; Creed et al., 2018; Sankar et al., 2020).

1.6 Summary: research questions

This thesis aims to define the role of understudied lake types in past trophic lake trajectories, with a special focus on the potential contribution of diatom analysis. The distinct research bias towards boreal or high-alpine regions means that there is currently an incomplete range of lake ontogenies. The present work uses records from a non-glacial lowland lake and a small glacial mountain lake in the Czech Republic to challenge common understandings of long-term lake succession. The thesis consists of a general introduction to the past lake ecosystem dynamics and three chapters that take the structure of scientific articles. Two chapters have been already published, whereas the third chapter is in the form of a manuscript. All chapters are formatted according to the requirements of the particular target journals. The main research outcomes are summarized in the general conclusions. The presented research aimed to:

- reconstruct possible trophic state changes during the abrupt climate change at the Late-Glacial/Holocene transition in the very shallow large lowland lake
- identify past environmental conditions in the small mountain forest lake and its catchment, and assess their impact on *Isoëtes* dynamics
- track the onset and extent of dystrophication in the small mountain forest lake and uncover its driving factors

Chapter 2:
Nutrient availability affected shallow-lake ecosystem response along the Late Glacial/Holocene transition

Abstract

Shallow lowland lakes undergo long-lasting natural eutrophication processes, which can be studied through the development of communities of aquatic organisms. However, records showing millennial-scale trophic status variability in these water bodies are rare. Two radiocarbon-dated sedimentary profiles from former (now destroyed by brown coal mining) Lake Komořany (Central Europe, Czech Republic) served for a multi-proxy study of biological remains (diatoms, chironomids, pollen) supplemented by X-ray fluorescence (XRF) and loss-on-ignition (LOI). The age-depth model and palynostratigraphy confirm a continuous Late-Glacial to Early-Holocene record. The results suggest consistent in-lake conditions with high nutrient availability since the lake origin in the Late-Glacial period. A distinct shift at the Late Glacial/Holocene boundary evidenced by an enhancement in diatom valve concentration and a lithological interface was foregone by a qualitative change in diatom and chironomid assemblages along with rise in LOI. It suggests that a major transformation occurred before the onset of the Holocene. As this qualitative change was characterised by a decrease in relative abundance of nutrient-demanding species, we propose an indirect climatic control by means of nutrient availability as the main driver of the aquatic species composition.

2 Introduction

Abrupt climatic changes connected with the Late Glacial/Holocene (LG/H) transition distinctly altered the species composition of terrestrial (e. g., Lotter, 1999; Hošek et al., 2014; Orbán et al., 2018) and freshwater communities (e. g., Goslar et al., 1993; Birks & Ammann, 2000; Heiri et al., 2014) in Europe. Lacustrine sediments serve as sensitive natural archives of these environmental changes (Cohen, 2003). Temperate shallow lakes provide a separate and highly complex category of sedimentary records due to their polymictic regime and distinct influence of macrophyte vegetation to ecosystem dynamics (Scheffer, 2004; Bennion et al., 2010). They can remain in a stable state supported by various mechanisms until crossing the critical nutrient threshold, leading to a change in the lake's trophic state (Scheffer 1990; Jeppesen et al. 1998; Scheffer & Van Nes 2007).

The trophic state development of shallow lowland lakes is driven by natural and anthropogenic factors (Cohen, 2003; Nõges et al., 2003). Although great attention is paid to human induced eutrophication, shallow lowland lakes naturally tend towards a higher trophic state. Limited stratification in shallow basins prevents phosphorus losses to the hypolimnion and furthermore sustains phosphorus recycling at the water-sediment interface (Scheffer, 2004) which is favoured by a large bottom surface to water volume area ratio (Wetzel, 2001). Higher mean temperatures in low altitudes may further naturally enhance the input of nutrients due to faster weathering (Cardoso et al., 2007) but simply the large ratio between the catchment and lacustrine area and the terminal position in the hydrological cycle cause natural eutrophy (Cremer et al., 2004). Fortunately, the past trophic state can be estimated from the sedimentary record without directly measuring it. Such reconstructed past environmental conditions are based on multi-proxy studies combining sedimentary characteristics and biotic variables (Birks & Birks, 2006). For trophic state reconstruction, diatom and chironomid indicators are often used for their sensitivity to diverse aspects of trophic conditions (Brodersen & Lindegaard, 1999; Hall & Smol, 2010). Diatom and chironomid species composition is also affected by other factors including the abundance and diversity of macrophytes (Sayer et al., 1999; Brodersen et al., 2001; Sayer et al., 2010; Vermaire et al., 2013; Tarrats et al., 2018), water depth (Bennion, 1995; Barley et al., 2006) and chironomids additionally to the oxygen level (Brodersen & Quinlan, 2006). Despite this, and because of that their paleorecords represent an imprint of various environmental driving factors, diatoms and chironomids have a great potential to elucidate complex shallow-water ecosystem changes.

Although shallow lakes frequently occur in Central Europe, few studies have investigated the response of the lacustrine ecosystem to the LG/H transition in lowland shallow lake basins in this region. Shallow thermokarstic lakes in Poland (Apolinarska et al., 2012; Drzymulska et al., 2015; Gałka et al., 2015; Kołaczek et al., 2015; Pedziszewska et al., 2015; Mendyk et al., 2016; Zawiska et al., 2019) demonstrate lacustrine development along the gradient of glacier influence depending on the position towards the line of glaciation (Mirosław-Grabowska & Zawisza 2018). German lowlands provide records of Holocene onset from the deeper Sacrower See (Kirilova et al., 2009; Enters et al., 2010) and shallow floodplain lakes of the Jeetzel Valley (Turner et al., 2013, 2014). Late-Glacial sediments were studied in the Panonian Basin in paleolake Šúr (Petr et al., 2013) and in the largest lake of Central Europe – Balaton (Sümegi et al. 2008). In the Czech Republic, paleolimnological studies of the LG/H transition in shallow lowland lakes are lacking. From Czech non-montane localities at middle altitudes, a detailed record of the LG was found in the former Lake Švarcenberk (Pokorný & Jankovská, 2000; Hošek et al., 2014) and the LG/H transition was documented in the shallow lake Velanská Cesta (Bešta et al., 2009). Our target locality, Lake

Komořany, reached the largest surface area (max ~25 km^2) compared to other shallow lakes in the region of the Czech Republic.

A relatively high trophic state and ingrowth by aquatic plants were recorded in the Early Preboreal in Lake Komořany (Jankovská, 1983; Řeháková, 1983, 1986; Jankovská & Pokorný, 2013) but the initial trophic state of this lake before the climate change at the LG/H transition remains unexplored. As European shallow lowland lakes have undergone long-lasting cultural eutrophication (Anderson, 1995) since the Neolithic Age (Dreßler et al., 2011), scarce opportunities exist to study their dynamics and climatic relations without anthropogenic impact. Therefore, the reconstruction of past climatically-induced trophic state changes at the LG/H transition may reveal important mechanisms hardly accessible by the research of more recent records.

The aim of this study is to investigate the response of a very shallow large lowland lake ecosystem to climatic changes at the LG/H transition. By analysing sedimentary diatoms, chironomids, pollen and geochemistry, we specifically aim to (i) detect effects of the climate on the lacustrine biota and (ii) reconstruct possible trophic state changes during the investigated period.

2.1 Study site

The former Lake Komořany was situated in north-western Bohemia, the Czech Republic, at the foothills of the Krušné Mountains at 230 m a. s. l. (50°32'N, 13°32'E) (Fig. 1). Its origin is likely linked to a Late-Glacial landslide event and the subsequent damming of the Bílina River (Hurník, 1969). This lowland, large (max. surface area ~25 km^2) and very shallow lake (max. depth ~10 m) represented a unique water body type among other localities available in the region (Vondrák et al., 2015). The lake was gradually filled in with sediment until the artificial draining of its last remains (~2 km^3) and surrounding mires in 1834 (Pokorný, 1963). Unfortunately, the last remnants of the unique lake sediment archive were destroyed in the 1980s by surface coal mining, because of an overlying Tertiary coalfield located in the Most Basin. However, several profiles were taken just before the complete removal of sediments and have been stored for further research (Jankovská, 1983, 1984, 1988; Jankovská & Pokorný, 2013).

Although its sediments have been investigated since the 19th century (Wettstein, 1896; Lühne, 1897), most studies have dealt with vegetation succession during the Holocene while the Late-Glacial (LG) record was only considered marginally (Losert, 1940; Neustupný, 1985; Rudolph, 1926). Similarly, the research of diatom succession was systematically conducted only in the Holocene sedimentary sequence (Řeháková, 1983, 1986; Bešta et al., 2015; Houfková et al., 2017). Our study presents the first multi-proxy research of the Lake Komořany record representing the LG/H transition based on the basal part of the profiles PK-1-L and PK-1-M.

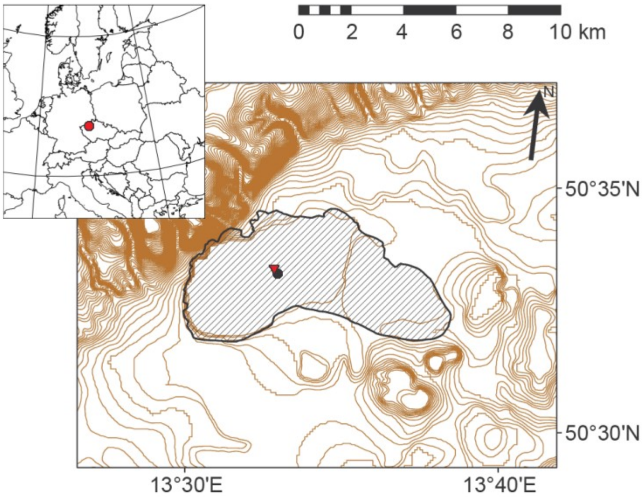

Fig. 1: Map of the study site with locations of the cores. The location of the former Lake Komořany (Czech Republic, Central Europe) indicated by a red dot. The location of the profiles PK-1-L (black dot) and PK-1-M (red triangle) indicated inside the estimated maximal area of the lake (hatched area) using a 230 m a. s. l. threshold, taken after Bešta et al. (2015).

3 Materials and methods

3.1 Sampling and lithology

Studied profiles PK-1-L and PK-1-M were sampled from the bottom of the former Dřínov reservoir by V. Jankovská, J. Kyncl, J. Klápště, and J. Beneš in 1987 using Kubiena tins (50×10×8 cm metal boxes with one 50×10 cm side open). The profiles were stored at 4°C until 2008 when they were lithologically described and subsampled.

The basal parts of the profiles PK-1-L (128–143.6 cm) and PK-1-M (134–148 cm) were chosen for further analyses due to their supposed LG age. PK-1-L was subsampled at regular intervals of 2 mm (in two sections in depth of 139.4–140 cm and 142–143.6 cm at intervals of 4 mm) and processed for diatom, pollen and X-ray fluorescence (XRF) analyses. PK-1-M was subsampled at regular intervals of 1 cm and provided sufficient material for chironomid analysis. This was done due to low chironomid head capsule concentration in the sediment (see also Houfková et al., 2017) and the resulting need for relatively large samples

(>8 g of dry sediment). Additionally, XRF analysis was also processed on samples from PK-1-M.

The frequency and size of silica grains were subjectively monitored using a light microscope in parallel to diatom analysis in the profile PK-1-L. Four categories of clastic silica quantity were determined using a nominal scale: 0 – grains absent or negligible, 1 – grains rare, 2 – grains common, 3 – grains abundant and larger pieces (>100 μm) present.

3.2 Chronology

Five bulk samples from the PK-1-L profile were dated by ^{14}C AMS at the Center for Applied Isotope Studies (CAIS), University of Georgia, USA (Table 1). A Poisson-process deposition model (Bronk Ramsey, 2008) with k=2.5 (Bronk Ramsey & Lee, 2013) based on the IntCal 13 calibration curve (Reimer et al., 2013) was constructed using the OxCal 4.2.4 application (Bronk Ramsey, 2013) with a boundary at the depth of 134 cm where sedimentation rate changed considerably. The basal bulk sample of the PK-1-M profile was dated using the same methods to date the start of sedimentation. The profiles were correlated by the fitting of Rb/Sr ratio curves acquired using XRF (Fig. 2).

Table 1: List of radiocarbon dates from the PK-1-L and PK-1-M profiles, Lake Komořany. The uncalibrated dates (^{14}C age) and their intervals (±) are quoted in radiocarbon years before 1950 AD (yr BP). Intervals of calibrated dates (cal yr BP) are counted using Poisson-process deposition model in OxCal 4.2.4 (Bronk Ramsey, 2013) using IntCal13 calibration curve (Reimer et al., 2013).

Sample ID (UGAMS#)	Profile	Depth (cm)	^{14}C age (yr BP)	±	^{14}C age (cal yr BP; interval of 95.4 probability)
10365	PK-1-L	114.2	8,420	30	9,555–9,140
9461	PK-1-L	134	9,960	30	12,020–11,195
10366	PK-1-L	136.5	10,940	30	13,100–12,670
10367	PK-1-L	139.5	12,640	35	15,465–14,230
9462	PK-1-L	144.5	13,130	40	16,265–15,230
9463	PK-1-M	147	13,810	40	15,449–15,109

3.3 Diatom analysis

The diatom record was analysed from 2 mm-thick layers in 0.4–0.6 mm intervals from the core PK-1-L. In total, 32 samples were processed from the depth interval of 128–143.6 cm. Laboratory processing and the subsequent quantitative analysis of diatom content followed standard procedures (Battarbee et al., 2001). After boiling in 30% hydrogen peroxide, the subsamples were carefully rinsed with distilled water. Purified solutions were thinned and an accurate volume gauged onto cover slides. Permanent mounts were created using Naphrax® mounting resin. The concentration of valves per dry weight (DW) of sediment was estimated using divinylbenzene microspheres as an internal standard.

At least 500 diatom valves were counted in each sample excluding dominant fragilarioid taxa (i.e., >30%: *Staurosira construens* f. *construens* Ehrenberg, *Staurosira construens* f. *venter* (Ehrenberg) Bukhtiyarova). The dominants were enumerated separately in the resolution of 100 valves per sample. A similar approach has been already adopted in several studies (e.g., Battarbee, 1986; Heinsalu et al., 2008; Demiddele et al., 2016) in the case of a high abundance of fragilarioid taxa. Diatom slides were observed under 1,000× magnification using the light microscope Nikon Eclipse E400 with a Canon EOS650D camera. Determination followed Krammer (2000, 2002, 2003), Krammer & Lange-Bertalot (1986, 1988, 1991a, 1991b) and Lange-Bertalot (2001). Nomenclature was updated according to AlgaeBase (Guiry & Guiry, 2019) and unified with the approach of last diatom analyses from Lake Komořany (Bešta et al., 2015; Houfková et al., 2017). Autecology of constituent taxa was specified using mainly Denys (1991) and Van Dam et al. (1994) and complemented with information from determination literature where necessary.

3.4 Chironomid and other Diptera analyses

For chironomid analysis, 14 1-cm thick layers from the core PK-1-M were used (depth interval of 130–144 cm according to PK-1-L profile's scale). A known weight of dry sediment (8.6–22.3 g) was deflocculated in 1% potassium hydroxide at 70°C for 20 mins and washed with distilled water onto a sieve with 100 μm mesh-size. The remaining material was transferred from the sieve into a modified Sedgewick-Rafter counting chamber and all head capsules were picked with a needle using a stereomicroscope (40–50× magnification). After dehydration in 80% ethyl alcohol, head capsules were mounted on slides in Euparal® mounting medium and identified using a light microscope (125–250× magnification). Taxonomical and autecological determination mainly follows Wiederholm (1983) and Brooks et al. (2007). When mentum was damaged or worn and mandibles or premandibles were not available, a higher taxonomic group was identified. Chironomid head capsule concentration was estimated by counting all head capsules in the samples.

3.5 Pollen analysis

Pollen analysis was conducted on the same samples as diatom analysis, i. e., from 2 mm-thick layers in 0.4–0.6 mm intervals from the core PK-1-L (128–143.6 cm depth).

Sediment samples were boiled in potassium hydroxide, sieved, acetolysed and incubated in fluoric acid according to Faegri & Iversen (1989). A known quantity of *Lycopodium* spores (three tablets) was added to each sample prior to the treatment to determine the absolute pollen concentration (Stockmarr, 1971). Pollen was counted under a Nikon Eclipse 80i optical microscope at magnifications of 400–1000x. At least 500 pollen determinations were conducted per sample. Taxonomic identifications followed Punt (1976), Eide (1981), Punt & Clarke (1980, 1981, 1984), Punt et al. (1988, 1995, 2003), Punt & Blackmore (1991), Beug (2004) and Punt & Hoen (2009). Reworked pollen grains of presumably the Tertiary period were identified according to Stuchlík (2001).

3.6 Numerical analysis

Significant diatom (DAZ), pollen (PAZ) and chironomid (CAZ) assemblage zones were based on CONISS (Constrained Incremental Sums of Squares) (Grimm, 1987). From chironomid and diatom dataset, uncertain gathering categories were excluded for further clustering using R 3.2.4 (R Core Team, 2016). From the pollen dataset, unidentified objects and Tertiary and aquatic plants' pollen were removed. Relative species abundances were processed by Hellinger transformation prior to clustering by CONISS and testing by the Broken-Stick model (MacArthur, 1957; Legendre & Legendre, 1998). Diatom assemblage zones (DAZ) were calculated both from the entire assemblage and from dominant-free species data (without taxa >30%: *Staurosira construens* f. *construens*, *Staurosira construens* f. *venter*).

3.7 Geochemistry

Dry weights of sediment samples were determined by drying them at 105°C for six hours during diatom analysis and at 50°C for 24 hours during chironomid analysis (Boyle, 2001) in order to obtain standardized sediment weights for quantitative analysis of the biological proxies.

Loss-on-ignition (LOI) analysis was conducted at 550°C for four hours according to Heiri et al. (2001) to quantify the relative proportion of organic matter in the sediment.

For the X-ray fluorescence measurements (XRF), a Delta Professional Handheld XRF analyser with 9 mm (uncollimated) spot size was used. Every 1-cm-thick layer was exposed three times for three minutes with two beams in "Geochem mode" – the first beam of up to 11 keV for lighter elements for 90 seconds and a second beam of up to 50 keV for heavier elements for 90 seconds. Presented data (%) are arithmetic averages from the three measurements.

4 Results

4.1 Lithology

A sharp lithological interface between clayey and layered gyttja was observed at the depth of 135 cm in the PK-1-L profile (Fig. 2). The PK-1-M profile was created by clayey gyttja without remarkable lithological change. The concentration of clastic silica decreased around the lithological boundary towards the Holocene and larger grains disappeared (Fig. 2). The Rb/Sr ratio increased 1.5 times at the lithological boundary in the PK-1-L profile (Fig. 2).

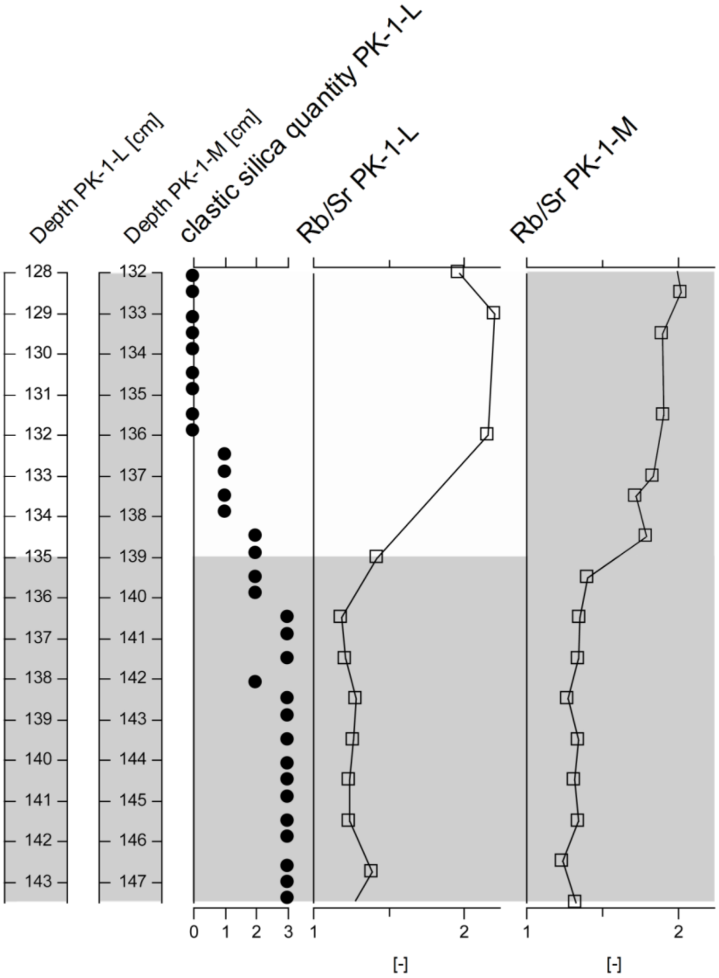

Fig. 2: Lithology and correlation of the profiles PK-1-L and PK-1-M. Clastic silica quantity from profile PK-1-L quoted in four categories: 0 – grains absent or negligible, 1 – grains rare, 2 – grains common, 3 – grains abundant and larger pieces (>100 μm) present. Rb/Sr ratios plotted for both profiles. Clayey gyttja represented by a grey background, layered gyttja represented by a white background.

4.2 Chronology

A Poisson-process deposition model (Fig. 3) based on five radiocarbon data (Tab. 1) demonstrated a change of sedimentation rate at the lithological interface in the profile PK-1-L. The studied depth interval covered period ~15,600–10,800 cal yr BP. The sedimentation rate of the clayey gyttja (depth 143.6–135 cm) was 430 yr/cm on average, while the average sedimentation rate of layered gyttja (depth 135–128 cm) was 160 yr/cm.

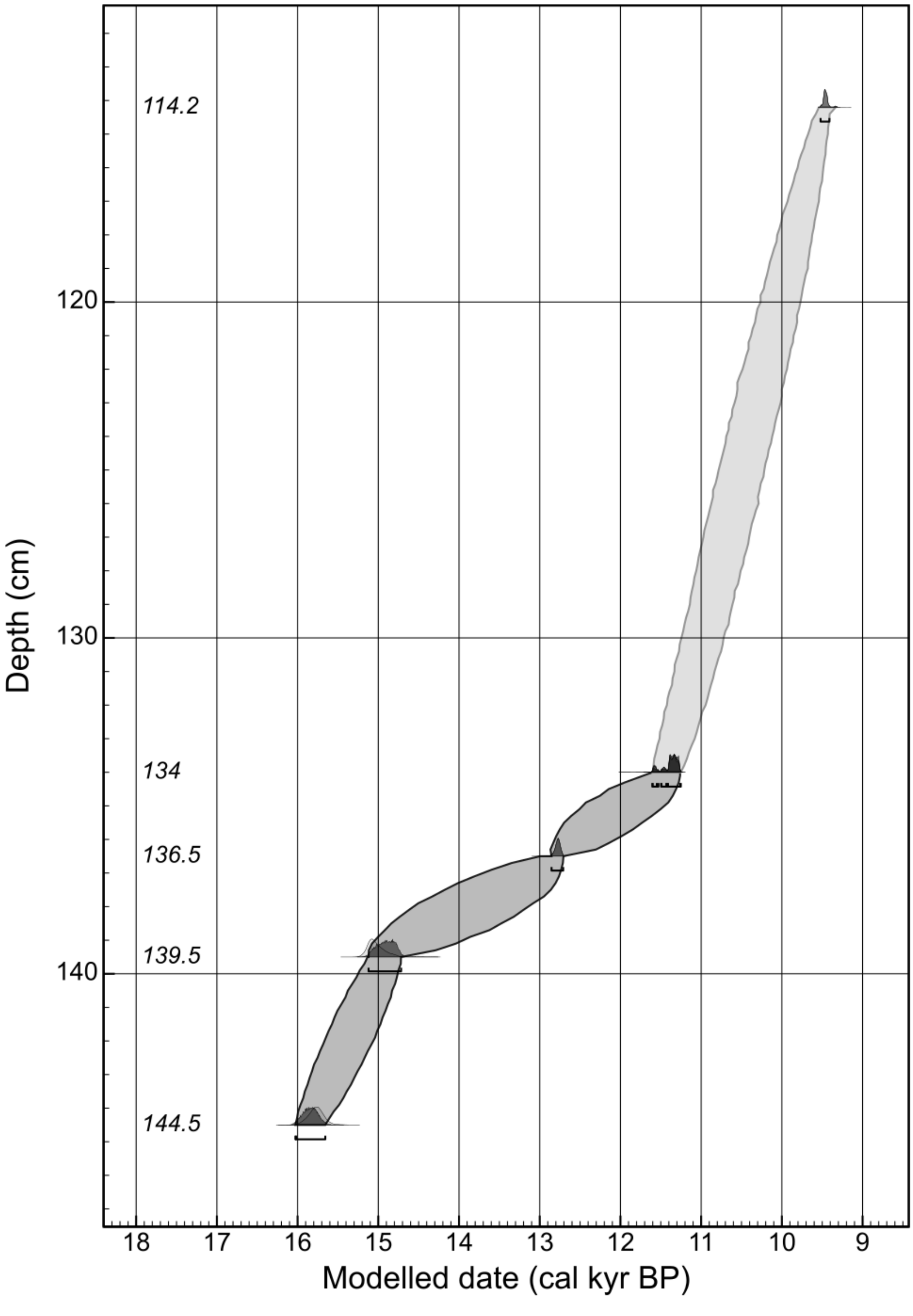

Fig. 3: Age-depth model for the profile PK-1-L. Poisson-process deposition model (Bronk Ramsey, 2008) with k=2.5 (Bronk Ramsey & Lee, 2013) based on the IntCal 13 calibration curve (Reimer et al., 2013) constructed using the OxCal 4.2.4 application (Bronk Ramsey, 2013).

4.3 Diatom record

Altogether, 105 diatom taxa were observed in the 32 studied samples. The diatom valve concentration allowed to achieve the intended total counts in all samples with the exception of the most basal one (143.2–143.6 cm). The layered gyttja provided generally higher concentrations of diatom valves than the clayey gyttja from the basal part of the profile (Fig. 4).

Tiny fragilarioid taxa (*Staurosira* Ehrenberg, *Staurosirella* Williams & Round, *Pseudostaurosira* Williams & Round) predominated along the whole studied interval. The diatom relative abundance record was divided into two significant diatom assemblage zones, (DAZ 1, DAZ 2) and the second zone was divided into two non-significant subzones (Fig. 4).

DAZ 1 (143.6–136.2 cm, 15,600–12,600 cal yr BP) was characterized by the dominance of *Staurosira construens* f. *venter*, accompanied by subdominant *Staurosira construens* var. *binodis* (Ehrenberg) Hamilton and *Fragilaria heidenii* Østrup. *Pseudostaurosira polonica* (Witak & Lange-Bertalot) Morales & Edlund, *Gyrosigma acuminatum* (Kützing) Rabenhorst and *Planothidium joursacense* (Héribaud-Joseph) Lange-Bertalot reached a considerable abundance (>0.5%) within this zone only. *Aulacoseira ambigua* (Grunow) Simonsen was the only noticeable representative of euplanktonic taxa in DAZ 1.

DAZ 2a (136.2–131.2 cm, 12,600–11,100 cal yr BP) started with a gradual increase in the total concentration of diatom valves. *S. construens* f. *venter* declined, and consequently *Staurosira construens* f. *construens* became dominant after the distinct increase in its relative abundance. *Staurosirella pinnata* (Ehrenberg) Williams & Round rose slightly at the beginning of the zone, whereas *S. construens* var. *binodis* decreased in abundance at the end of the zone. Two other euplanktonic taxa *Lindavia* cf. *balatonis* (Pantocsek) Nakov et al. and *Staurosirella berolinensis* (Lemmermann) Bukhtiyarova reached noticeable relative abundance beside *A. ambigua* being continuously present. The upper subzone boundary was defined using CONISS with excluded dominant taxa only.

DAZ 2b (131.2–128 cm, 11,100–10,800 cal yr BP) reflected growth of less abundant motile benthic taxa *Geissleria schoenfeldii* (Hustedt) Lange-Bertalot & Metzeltin, *Navicula laterostrata* Hustedt, *Navicula radiosa* Kützing and *Sellaphora vitabunda* (Hustedt) Mann, accompanied by *Fragilaria capucina* s. l. Desmazières. Prevailing *S. construens* f. *construens* remained without any significant change. *S. construens* f. *venter* rose slightly, whereas relative abundance of *S. construens* var. *binodis* and all euplanktonic species decreased.

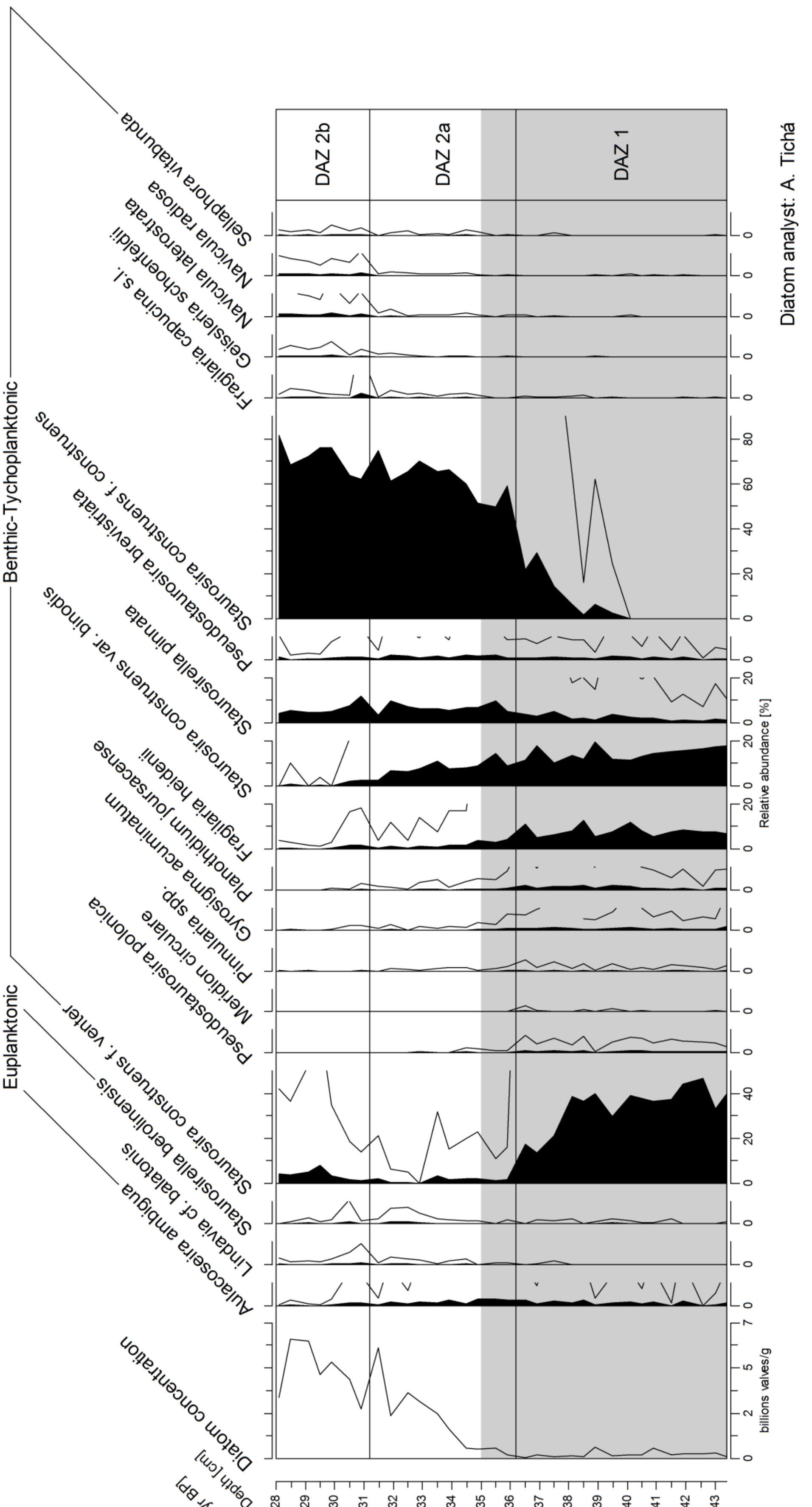
Age [cal yr BP]
Depth [cm]
Diatom concentration
Aulacoseira ambigua
Lindavia cf. balatonis
Staurosirella berolinensis
Staurosira construens f. venter
Pseudostaurosira polonica
Meridion circulare
Pinnularia spp.
Gyrosigma acuminatum
Planothidium joursacense
Fragilaria heidenii
Staurosira construens var. binodis
Staurosirella pinnata
Pseudostaurosira brevistriata
Staurosira construens f. construens
Fragilaria capucina s.l.
Geissleria schoenfeldii
Navicula laterostrata
Navicula radiosa
Sellaphora vitabunda
Euplanktonic
Benthic-Tychoplanktonic
DAZ 2b
DAZ 2a
DAZ 1
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
11000
12000
13000
14000
15000
0
2
5
7
billions valves/g
20
40
60
80
Relative abundance [%]
Diatom analyst: A. Tichá

Fig. 4: Stratigraphical diagram of diatom relative abundances from the profile PK-1-L. Diatom concentration curve expressed as number of valves per 1 g of dry sediment. Relative abundances represented by a black silhouette, lines corresponding to10-times exaggeration. Significant diatom assemblage zones (DAZ 1, DAZ 2) and non-significant subzones (DAZ 2a, DAZ 2b) defined by CONISS (stratigraphically constrained cluster analysis) and Broken-Stick model. Clayey gyttja indicated by a grey background, layered gyttja indicated by a white background.

4.4 Chironomid and other Diptera records

A total number of 35 chironomid and two other dipteran morphotaxa were identified at the studied depth interval separated into 14 1-cm-thick layers (Fig. 5, Tab. 2). The concentration of head capsules was extremely low and oscillated between two and six individuals per gram of dry sediment. To produce statistically robust reconstructions and associated interpretations, large samples of dry weight from 9 to 23 g were analysed. The subsequent picking of head capsules yielded 29–110 individuals per sample. Chironomid abundance decreased temporarily at the depth of 140.5 cm and from 135.5 cm started to decrease continually towards the onset of the Holocene. Due to poor preservation, some of the findings were identified only to genus or subfamily level. *Chironomus plumosus*-type, *C. anthracinus*-type, and *Procladius* were the most abundant taxa along the studied profile. Two significant chironomid assemblage zones were defined (CAZ 1 and 2).

CAZ 1 (143.5–136 cm, 15,600–12,500 cal yr BP) was characterized by the dominance of *C. plumosus*-type, particularly in the basal part (> 80%) and decreasing towards 24% at the depth of 140.5 cm. This temporary drop in *C. plumosus*-type relative abundance coincided with the decrease in total abundance of chironomid head capsules (number of individuals/head capsules per 1 g of dry sediment), *C. anthracinus*-type establishment, and an increase in *Procladius*. Then the relative abundance of dominant *C. plumosus*-type recovered to ~30–40% and the total chironomid abundance also increased. The dominant taxa (>15% at least in one layer) were accompanied by several sub-dominant taxa (4–15% at least in one layer) – the *Cladotanytarsus mancus*-type, *Einfeldia dissidens*/*natchitocheae*-type, *Microchironomus*, *Stictochironomus rosenschoeldi*-type, and *Tanytarsus lugens*-type. Several head capsules of the *T. lugens*-type had typical mandibles with three inner and two dorsal teeth but their surficial tooth was strongly reduced. These findings are presented as the *T.* cf. *lugens*-type.

CAZ 2 (136–130.5 cm, 12,500–11,000 cal yr BP) showed a gradual decline of *C. plumosus*-type as well as a decline in the total abundance of head capsules in the sediment. *Procladius* increased distinctly in reverse and became the most abundant taxon. Besides these two taxa, only *Einfeldia natchitocheae*-type exceeded the relative abundance of 15% in the uppermost layer. *C. anthracinus*-type and *T.* (cf.) *lugens*-type progressively disappeared from the record. *Stictochironomus rosenschoeldi*-type was completely missing, *Microchironomus* remained present, *Cladotanytarsus mancus*-type reappeared in the lower half of the CAZ 2 and disappeared again at 133 cm and the *Einfeldia dissidens*/*natchitocheae*-type increased. In addition, several new subdominants (relative abundance of 4–15%) appeared – the *Einfeldia dissidens*-type, *Polypedilum nubeculosum*-type, and *Glyptotendipes pallens*-type.

Besides the chironomid remains, the presence of *Chaoborus flavicans* (Meigen, 1830) mandibles in both CAZ 1 and CAZ 2, and one head capsule of family Simuliidae in CAZ 2 was documented.

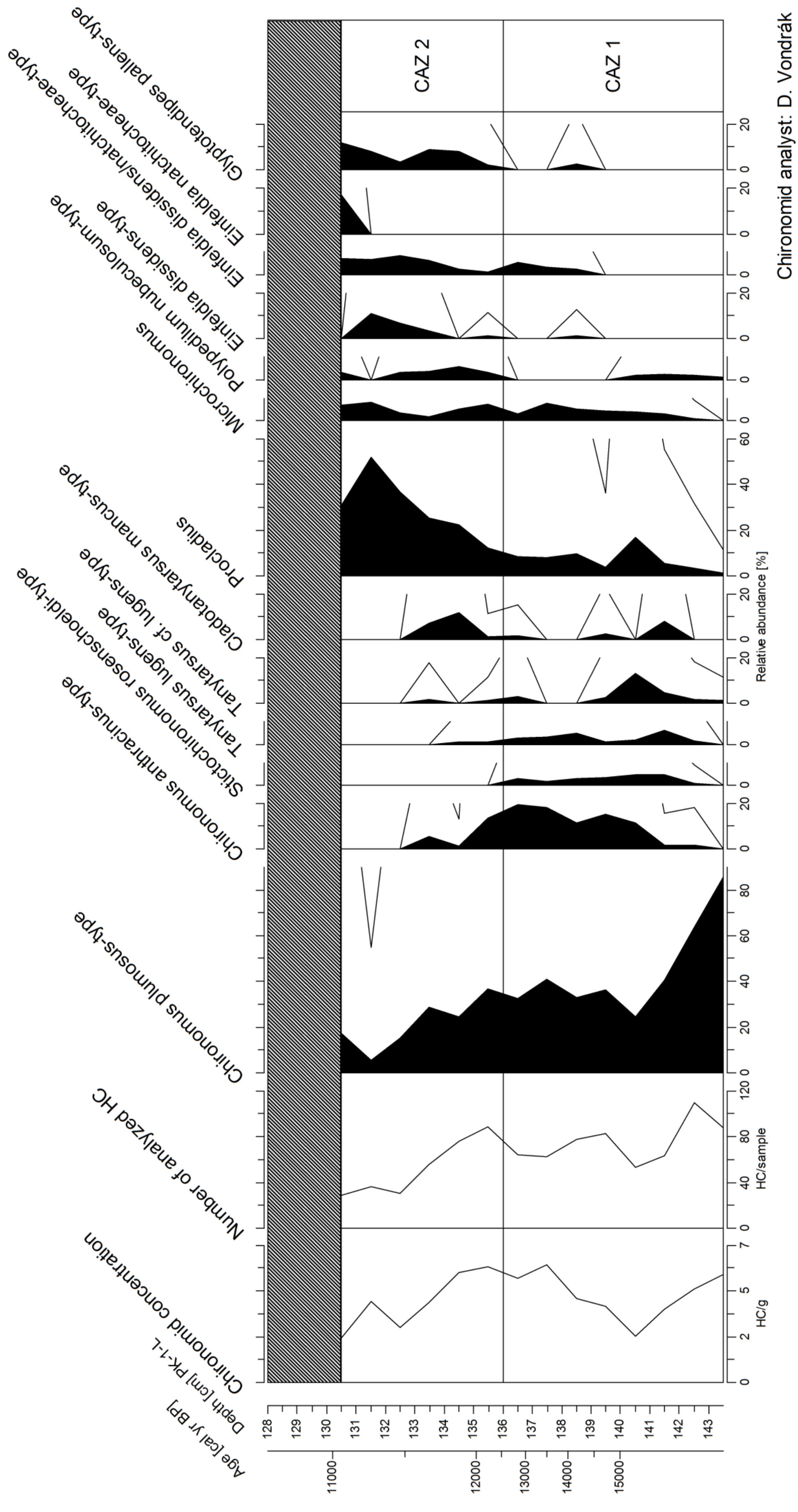
Chironomid analyst: D. Vondrák
CAZ 2
CAZ 1
Age [cal yr BP]
Depth [cm] PK-1-L
Chironomid concentration
Number of analyzed HC
Chironomus plumosus-type
Chironomus anthracinus-type
Stictochironomus rosenschoeldi-type
Tanytarsus lugens-type
Tanytarsus cf. lugens-type
Cladotanytarsus mancus-type
Procladius
Microchironomus
Polypedilum nubeculosum-type
Einfeldia dissidens-type
Einfeldia dissidens/natchitocheae-type
Einfeldia natchitocheae-type
Glyptotendipes pallens-type
HC/g
HC/sample
Relative abundance [%]
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
11000
12000
13000
14000
15000
0
2
5
7
40
80
120
20
60

Fig. 5: Stratigraphical diagram of chironomid relative abundances from the profile PK-1-M. Depth expressed in PK-1-L depth scale. Chironomid concentration curve expressed as number of HC (head capsules) per 1 g of dry sediment. Relative abundances represented by a black silhouette, lines corresponding to10-times exaggeration. Significant chironomid assemblage zones (CAZ 1, CAZ 2) defined by CONISS (stratigraphically constrained cluster analysis) and Broken-Stick model. Not-studied depth interval indicated by hatched area.

4.5 Pollen record

Three significant pollen assemblage zones (PAZ) were determined (Fig. 6) with a total of 107 pollen and spore types recorded. Pollen types of Tertiary origin numbering 15 were recorded mainly in PAZ 1. The recovered pollen grains were well preserved. Total pollen concentration reached ca 1.7–5*10^5 grains*g^{-1} in PAZ 1 and amplified to ca 1–2.5*10^6 grains*g^{-1} in PAZ 2 and 3. *Pinus sylvestris*-type and *Betula pubescens*-type pollen was the most abundant (~70–80%) in PAZ 1 (143.3–135.7 cm, 15,600–12,300 cal yr BP). Pollen of shrubs (*Juniperus*, *Salix* and *Alnus alnobetula*) reached ~5% abundance and non-arboreal pollen (NAP) ~20–30%. Aquatic species were above all represented by pollen of the *Myriophyllum spicatum*-type, *Alisma plantago-aquatica*-type, and *Oenanthe fistulosa*-type. Some rare and rather thermophilous species such as *Butomus umbellatus* were recorded. The pollen of light demanding species (the *Corylus avellana*-type), the deciduous species of the Quercetum mixtum (the *Quercus robur*-group, *Tilia cordata*-type, *Ulmus*-type, and *Fraxinus excelsior*-type), and *Picea*-type were of extremely low abundance. By the end of the zone, spores of *Sphagnum* and pollen of the *Elatine*-type were recorded. An increase in the abundance of the pollen of trees and shrubs of up to 90 % proceeded in PAZ 2 (135.7–130.2 cm, 12,300–11,000 cal yr BP). In PAZ 3 (130.2–128 cm, 11,000–10,800 cal yr BP), pollen of the *Corylus avellana* increased in abundance above 20% and was succeeded by pollen of deciduous species of the Quercetum mixtum. Hygrophilous and water species were, above all represented by pollen of the *Typha angustifolia* and the *Sparganium emersum*-type.

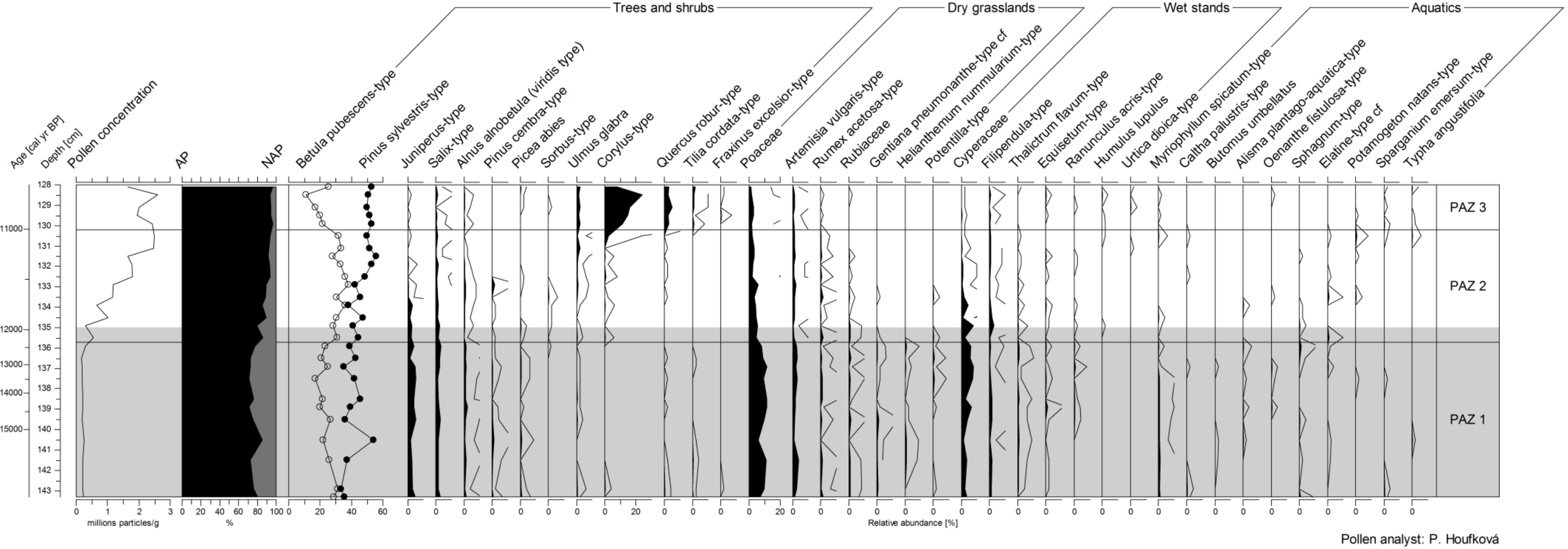
Trees and shrubs
Dry grasslands
Wet stands
Aquatics
Age [cal yr BP]
Depth [cm]
Pollen concentration
AP
NAP
Betula pubescens-type
Pinus sylvestris-type
Juniperus-type
Salix-type
Alnus alnobetula (viridis type)
Pinus cembra-type
Picea abies
Sorbus-type
Ulmus glabra
Corylus-type
Quercus robur-type
Tilia cordata-type
Fraxinus excelsior-type
Poaceae
Artemisia vulgaris-type
Rumex acetosa-type
Rubiaceae
Gentiana pneumonanthe-type cf
Helianthemum nummularium-type
Potentilla-type
Cyperaceae
Filipendula-type
Thalictrum flavum-type
Equisetum-type
Ranunculus acris-type
Humulus lupulus
Urtica dioica-type
Myriophyllum spicatum-type
Caltha palustris-type
Butomus umbellatus
Alisma plantago-aquatica-type
Oenanthe fistulosa-type
Sphagnum-type
Elatine-type cf
Potamogeton natans-type
Sparganium emersum-type
Typha angustifolia
PAZ 3
PAZ 2
PAZ 1
11000
12000
13000
14000
15000
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
millions particles/g
%
Relative abundance [%]
Pollen analyst: P. Houfková

Fig. 6: Stratigraphical diagram of pollen relative abundances from the profile PK-1-L. Pollen concentration curve expressed as number of particles per 1 g of dry sediment. Relative abundances of AP (arboreal pollen) represented by a black silhouette, relative abundances of NAP (non-arboreal pollen) represented by a dark grey silhouette. Relative species abundances represented by black silhouettes, lines corresponding to 10-times exaggeration. Significant pollen assemblage zones (PAZ 1, PAZ 2, PAZ 3) defined by CONISS (stratigraphically constrained cluster analysis) and Broken-Stick model. Clayey gyttja indicated by a grey background, layered gyttja indicated by a white background.

4.6 Trophic state indication

The succession of diatom and chironomid ecological groups suggested a decreasing trend of nutrient availability during the LG/H transition (Fig. 7). The most abundant taxa of each ecological group are listed in Table 2. Diatom ecological groups are presented without dominant fragilarioid taxa, however, they remained significant owing to the exclusion of dominants since the phase of diatom valves' counting. Eutraphentic diatom species abundance decreased at the DAZ 1/DAZ 2 boundary. Similarly, eutraphentic chironomids became less abundant after the CAZ 1/CAZ 2 boundary. While meso-eutraphentic diatoms decreased later at the DAZ 2a/DAZ 2b boundary inversely to the increase of mesotraphentic and oligotraphentic diatom species, meso-eutraphentic chironomids increased at the CAZ 1/CAZ 2 boundary. Higher relative abundances of oligotraphentic chironomids in CAZ 1 were driven by *Tanytarsus lugens*-type presence (Tab. 2).

Diatom valve concentration increased at the LG/H boundary. Similarly, the pollen concentration of aquatic macrophytes increased slightly with the onset of the Holocene during PAZ 2 and later distinctly at PAZ 3 (Fig. 8). Both variables are in more or less direct proportion to the primary productivity of diatoms and aquatic macrophytes respectively.
Loss-on-ignition (LOI) had a distinct rising trend ranging between 1.5–10.4% in the LG and between 13–16.5% in the Holocene but it remained at a relatively low level along the whole studied interval of the PK-1-L profile (max. 16.5% at 129–129.8 cm) (Fig. 8). Fe/Mn ratio decreased twice at the lithological boundary, when average values for the LG (91.1) and for the Holocene were compared (41.2) (Fig. 8).

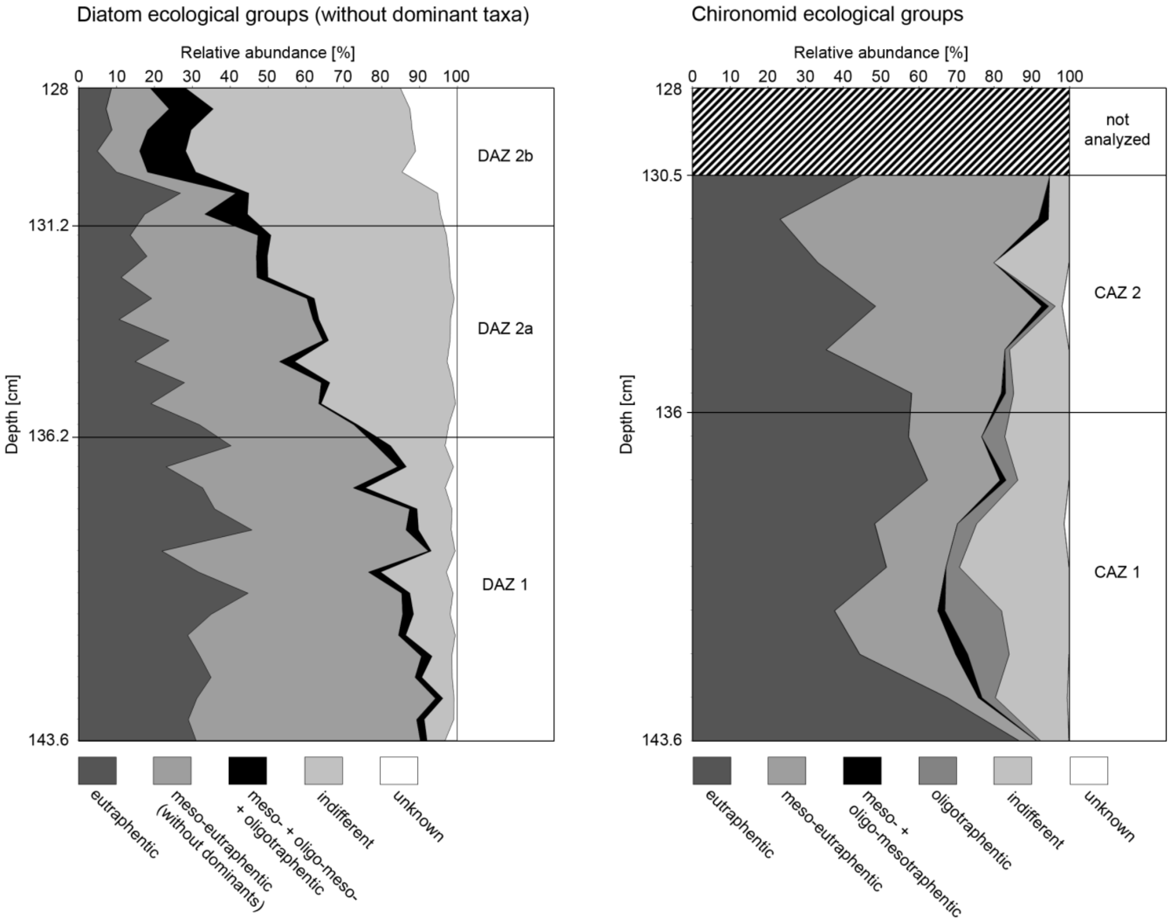

Fig. 7: Stratigraphical diagram of diatom and chironomid ecological groups. Chironomid relative abundances shown for whole assemblage, diatom relative abundances shown for assemblage counted without two dominant fragilarioid taxa. Significant diatom assemblage zones (DAZ) and chironomid assemblage zones (CAZ) defined by CONISS (stratigraphically constrained cluster analysis) and Broken-Stick model. Not-studied depth interval indicated by a hatched area.

Table 2: List of common diatom (>0.5%) and chironomid (>4%) taxa found in the studied record with affiliation according to lake trophic state preferences. Species ecology is based on observations summarised by Wiederholm (1983), Van Dam et al. (1994) and Brooks et al. (2007). *S. construens* f. *construens* and *S. construens* f. *venter* not included in the Fig. 7.

Trophic state preference	**Diatom taxa**	**Chironomid taxa**
Eutraphentic	*Aulacoseira ambigua* *Fragilaria heidenii* *Gyrosigma acuminatum* *Lindavia* cf. *balatonis* *Staurosirella berolinensis*	*Chironomus anthracinus*-type *Chironomus plumosus*-type *Einfeldia dissidens*-type *Einfeldia dissidens/natchitocheae*-type *Einfeldia natchitocheae*-type *Polypedilum nubeculosum*-type
Meso-eutraphentic	*Navicula radiosa* *Planothidium joursacense* *Staurosira construens* f. *construens* *Staurosira construens* f. *venter* *Staurosira construens* var. *binodis*	*Cladotanytarsus mancus*-type *Glyptotendipes pallens*-type *Microchironomus* *Procladius* *Stictochironomus rosenschoeldi*-type
Mesotraphentic	*Fragilaria capucina* s. l. *Geissleria schoenfeldii*	
Oligo-mesotraphentic	*Pseudostaurosira polonica* *Sellaphora vitabunda*	
Oligotraphentic		*Tanytarsus lugens*-type *? Tanytarsus* cf. *lugens*-type
Indifferent	*Pseudostaurosira brevistriata* *Staurosirella pinnata*	

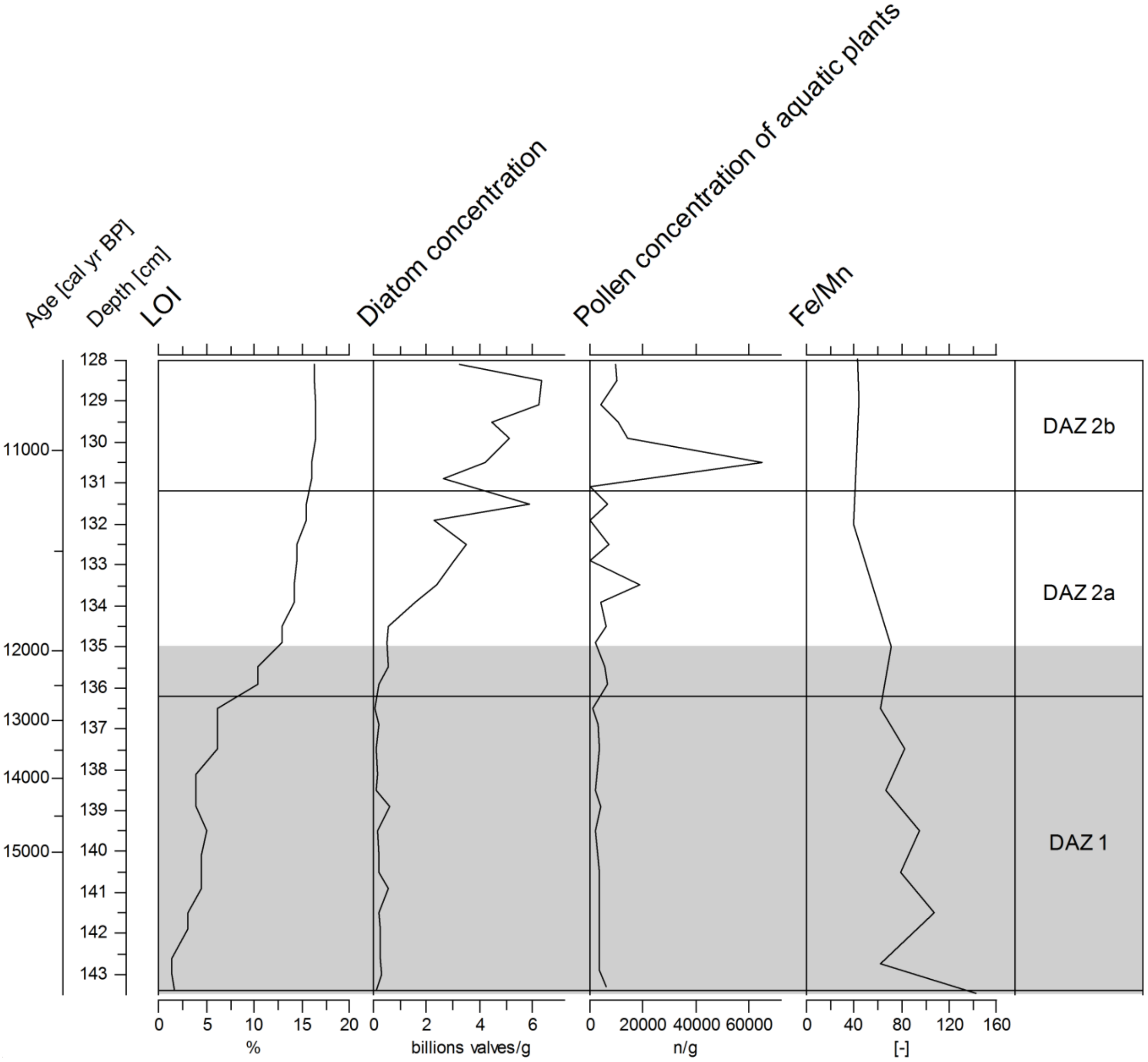

Fig. 8: Stratigraphical diagram of variables representing lacustrine trophic state from the profile PK-1-L. LOI (loss-on-ignition) represents percentages of organic matter. Diatom and pollen concentration curves expressed as number of particles per 1 g of dry sediment. Fe/Mn ratio based on XRF analysis. Significant diatom assemblage zones (DAZ 1, DAZ 2) and non-significant subzones (DAZ 2a, DAZ 2b) defined by CONISS (stratigraphically constrained cluster analysis) and Broken-Stick model. Clayey gyttja indicated by a grey background, layered gyttja indicated by a white background.

5 Discussion

5.1 The effect of climate on lacustrine biota

At the Late Glacial/Holocene transition (LG/H), a gradual succession of aquatic and terrestrial biota without any sharp interface was recorded in Lake Komořany. The remarkable feature of this transition is the asynchronous change in diatom species composition and their productivity indicated by diatom valve concentration (Fig. 4). While diatom productivity abruptly increased and changed the character of sediment at the supposed LG/H boundary (135–134.5 cm, 12,000–11,700 cal yr BP) (Fig. 2, 4), the main shift in both dominant and rarer diatom taxa along with change in chironomid assemblage and rise in LOI had taken place slightly before (136.5–136 cm, 12,800–12,500 cal yr BP) this lithological interface (Fig. 4). Therefore, there must have been two, qualitatively different, breakthroughs in ecosystem response. The chronologically first change could have resulted from ecosystem response to the warmer second half of the Younger Dryas period (Stuiver et al., 1995) which is of considerable influence on the lowland lakes (Goslar et al., 1993; Zawiska et al., 2015) and was detected also in a diatom record from a shallow lake situated in the southern part of the Czech Republic (Bešta et al., 2009). Although the climatic forcing of the first discussed event is far behind the resolution of our sedimentary record, we did not find another possible trigger of such changes. Therefore, we put it here as a provisional explanation, since a rather gradual process without the detection of generally accepted LG periods (e. g., Allerød) was observed in the profile. This is consonant with the view that neither a particular event nor even a biostratigraphic episode (cf. Rasmussen et al., 2014) can be distinguished for the LG terrestrial record from Lake Komořany. The second event, characterized by the abrupt rise in diatom productivity, is synchronous with the LG/H boundary (Fig. 2). It offers a bold conclusion that the first recorded change preceding the LG/H boundary was more prominent, since there was a shift in both chironomid and diatom species composition (quality), whereas only diatom valve concentration (quantity) changed during the second shift. The second quantitative shift in diatom assemblage, i.e. the increase in productivity of dominant fragilarioids, could be connected with spread of suitable littoral habitats with emergent aquatic macrophytes as the fragilarioids possibly prefer emergent substrates to colonize (Sayer et al., 1999). Whatever the case, the shift in productivity could not be biased by enhanced sedimentation rate (allochthonous material input), since than the valve concentration had to drop.

Terrestrial vegetation showed gradual change with the continual disappearance of steppe and tundra indicators (e. g., *Artemisia*, *Helianthemum*, *Potentilla*, *Juniperus*, *Salix*, *Betula nana*, *Alnus viridis*-type) and a successive increase in wood's proportion. *Pinus* and *Betula* trees were present in the region perhaps in the form of a patchy woodland during the LG (16,300/15,200–11,700 cal yr BP). Such scattered patches of hemiboreal forest, further indicated by the presence (<1.5%) of *Pinus cembra*-type pollen (Fig. 6), were reconstructed at low altitudes in the Bohemian Massif (Kuneš et al., 2008). The spread of mixed deciduous forest starts very early with the scattered occurrence of pollen of some thermophilous trees

(*Quercus robur*-type, *Tilia cordata*-type) (Fig. 6). Such early findings of these taxa are also usually thought to originate in a long-distance transport or reworked material (Pokorný, 2002; Ralska-Jasiewiczowa et al., 2004). Even though the steppe vegetation probably persisted during the Holocene in the adjacent thermophilous hilly region of České Středohoří (Pokorný et al., 2015), the pollen of the steppe elements almost disappeared from the Lake Komořany record until 5,500 cal yr BP (Houfková et al., 2017).

The recorded gradual development of terrestrial vegetation documents a very limited climatic forcing of the studied lowland environment at the LG/H transition transferring to a less pronounced response of the lacustrine ecosystem. We found a distinct shift in the relative abundances of diatoms and chironomids but only minor changes in species compositions (Fig. 4, 5). An addition of several new diatom and chironomid taxa to the persisting Late-Glacial species composition rather than their complete substitution generally characterizes the changes in Lake Komořany aquatic assemblages. A similar pattern was also recorded in other lowland eutrophic lakes in central Europe at the Holocene onset (Kirilova et al., 2009; Turner et al., 2014).

Low water depth is considered as one of the driving factors causing relatively stable aquatic assemblage composition in Lake Komořany because it determines the frequency of mixing in the whole water column (Scheffer, 2004). The long-lasting shallow character of Lake Komořany could explain the predominance of diatom fragilarioid taxa known to thrive in polymictic shallow waters (Bennion, 1995). Observed diatom assemblages represent a typical pioneer species composition well-known from other Late-Glacial lakes (Haworth 1976; Denys et al. 1990) and large shallow lakes in general (Heinsalu et al., 2008; Buczkó et al., 2019). This is consistent with the dominance of fragilarioid tychoplanktonic taxa throughout the existence of Lake Komořany (Bešta et al., 2015; Houfková et al., 2017). Observed invariable diatom composition indicates a shallow lacustrine character without extensive phases of deep water during the LG and weak climate forcing of the lake ecosystem. Nevertheless, subtle water-level fluctuations can be inferred from the pollen of macrophytes typical of disturbed wet stands (among others *Sphagnum* or *Elatine,* Fig. 6). The water-level rise caused by humification at the onset of the Holocene was proven by the start of sedimentation in the more elevated parts of the basin (Houfková et al., 2017). This is further supported by the outset of diatom euplanktonics *Staurosirella berolinensis* and *Lindavia* cf. *balatonis* (Fig. 4). An analogous water level rise was also described in other European records (Magny, 2004; Bos et al., 2007). The shift to the lake with more stable littoral parts is further indicated by a presence of pollen of *Typha angustifolia* or *Humulus lupulus* ~11,200 cal yr BP (PAZ 3) (Fig. 6).

Although the water level strongly influenced the general character of observed aquatic assemblages, it fails to explain species composition changes during the LG in Lake Komořany. Therefore, the change in trophic state was tracked as a potential prominent device in how climate indirectly influenced the lacustrine ecosystem.

5.2 Trophic state reconstruction

Our results document mesotrophic to eutrophic conditions already since the lake formation in the LG (Fig. 7, 8). Various aspects of the lacustrine trophic state were reconstructed, through aquatic macrophytes, primary productivity and nutrient availability.

Aquatic macrophytes shape the regime of shallow lakes (Jeppesen et al., 1998) and affect species composition of chironomid and diatom assemblages (Sayer et al., 1999; Brodersen et al., 2001; Vermaire et al., 2013; Tarrats et al., 2018). Even in the LG, *Myriophyllum spicatum* was a dominant component of aquatic macrophyte assemblage (Fig. 6) but absolute concentrations of aquatic macrophytes' pollen were distinctly lower than later on in the Early Holocene (Fig. 8). Nevertheless, the lower volume of water in the LG and submergent character of *M. spicatum* could have made its vegetation an important habitat for small fragilarioids and *Gyrosigma acuminatum* (Fig. 4), a diatom frequently observed on aquatic macrophytes (Dong et al., 2008). The first evidence of heightened macrophyte growth at the onset of the Holocene is documented by an increase in *Einfeldia dissidens*-type and *Glyptotendipes pallens*-type (Fig. 5), two chironomid taxa associated with aquatic macrophytes (Brodersen et al., 2001). The subsequent increase in the pollen concentration of aquatic macrophytes and the spread of broad-leaved species (*Potamogeton natans*-type, *Sparganium emersum*-type, *Typha angustifolia*) is linked with the onset of the Holocene (Fig. 6). This littoral vegetation provided more stable bottom habitats enabling the establishment of an epipelic diatom community including *Navicula radiosa* and other motile benthic diatoms (*Geissleria schoenfeldii*, *Navicula laterostrata*, *Sellaphora vitabunda*) (Fig. 4). Moreover, we must consider not only the substantial influence of aquatic macrophytes on nutrient availability by bottom stabilization but also the direct consumption of phosphorus during enhanced growth under the Holocene favourable climate. Aquatic macrophytes can affect the lacustrine trophic state as they serve as effective phosphorus sink and mitigate sediment resuspension (Canfield & Jones, 1984; Jeppesen et al., 1998). Furthermore, the lower abundance of macrophytes during the LG could strengthen the sensitivity of benthic diatoms including small fragilarioid taxa to the concentration of epilimnetic phosphorus (Werner & Smol, 2005).

Diatom and chironomid ecological groups in our record show a clear trend from more towards less nutrient-demanding taxa (Fig. 7). We attempted to reduce the influence of poor fragilarioid indicators (Bennion, 1995; Sayer, 2001; Bennion et al., 2010) by excluding the dominant *Staurosira* spp. from our assessment. Moreover, the nutrient concentration signal of remaining fragilarioid taxa (*F. heidenii*, *Pseudostaurosira brevistriata* (Grunow) Williams & Round, *P. polonica*, *S. berolinensis*, *S. pinnata*, *S. construens* var. *binodis*) was considered with caution. Such an approach provides a clear signal of decreasing nutrient availability towards the Holocene through decreasing eutraphentic and increasing meso- and oligotraphentic diatom species (Fig. 7). Additionally, the substitution of two diatom dominants, *S. construens* f. *venter* and *S. construens* f. *construens* (Fig. 4), could be explained by nutrient availability since *S. construens* f. *venter* tolerates higher concentrations of organically bound nitrogen compared to *S. construens* f. *construens* (Van Dam et al. 1994). Although the coenococcal algae *Pediastrum kawraiskyi* Schmidle, used typically as an indicator of cold and oligotrophic conditions, was found in Late-Glacial sediments of Lake Komořany (Komárek & Jankovská, 2001; Jankovská & Pokorný, 2013), its presence during cold periods in a naturally eutrophic lake documented by Turner et al. (2014) questioned the indicative value for nutrient levels. A more probable connection of *P. kawraiskyi* to low temperatures rather than low nutrient level supports our interpretation of there being relatively high nutrient levels since the LG.

Chironomid ecological preferences follow a similar trend showing a substitution of eutraphentic dominants for meso-eutraphentic taxa (Fig. 7). The most abundant chironomids of our record, namely *Chironomus anthracinus*-type, *C. plumosus*-type and *Procladius* (Fig. 5, Tab. 2), often produce very abundant populations in highly productive lakes with low clarity and oxygen depletion or anoxia near the bottom (Nagell & Landahl, 1978; Matěna, 1989; Hamburger et al., 1994). Characteristic cold stenothermic taxa inhabiting oligotrophic and well-oxygenated waters (e. g., the *Heterotrissocladius grimshawi*-type or *Micropsectra* spp.) are missing, although reported in many European records, including LG sediments of lowland shallow lakes (Brooks et al., 1997; Brodersen & Lindegaard, 1999; Płóciennik et al., 2011; Bos et al., 2017) or Czech LG lakes situated in elevations of above 900 m a.s.l (Kletetschka et al., 2018). Furthermore, mesotraphentic species (e. g., the *Derotanypus* or *Corynocera ambigua*-type) present in regional mid-altitude lakes (400–900 m a. s. l.) (Hošek et al., 2014; Hájková et al., 2016) are also absent from Lake Komořany records. The only exception are rare findings of *Tanytarsus lugens*-type, a morpho-taxon typical of the profundal in oligotrophic lakes (Brooks et al., 2007).

5.3 Driving mechanisms of nutrient availability

We make the case that nutrient availability was a probable factor in the Lake Komořany ecosystem change at the LG/H transition. Similar decreases in the nutrient level at the time of the climate amelioration are known from Scandinavian (Björk, 2010), Polish (Apolinarska et al., 2012) and German lowland lakes (Kirilova et al., 2009; Turner et al., 2014). In case of Lake Komořany record, this reduction in nutrient availability was likely accompanied by changes in the catchment erosion intensity, duration of seasonal anoxia and shift in primary productivity.

1. More intensive catchment erosion during the colder and drier period could have enhanced nutrient availability in the LG. A higher input of inorganic material into Lake Komořany is clearly indicated by an increased proportion of clastic particles in the LG parts of the profiles (Fig. 2). Although a decrease of Rb at the LG/H transition correspond with more intensive erosion from catchment during the LG (cf. Hošek et al., 2014), our lower values of Rb/Sr ratio in LG (Fig. 2) contradict trends usually observed for colder periods (Jin et al., 2006). This is caused by a distinct decrease of Sr at the LG/H transition, most likely linked to the affinity of Sr to Ca (Jin et al., 2006) and a decrease of alkalinity towards the Holocene.

2. Seasonal anoxia under prolonged winter stratification causes an internal recycling of sedimentary phosphorus (Wetzel, 2001). The presence of *Chironomus anthracinus*-type, *C. plumosus*-type and *Stictochironomus rosenschoeldi*-type during the LG (Fig. 5) suggests at least seasonally anoxic conditions near the lake bottom (Nagell & Landahl 1978; Int Panis et al. 1995), whereas *Tanytarsus lugens*-type presence indicates well-oxygenated conditions (Brooks et al., 2007). This discrepancy could be explained by the presence of a mosaic of different habitats in the lake and a possible restriction of *T. lugens*-type (Fig. 5) to a better oxygenated environment near the inflow of the Bílina River. The above-mentioned taxa are characteristic of our LG record and disappeared or declined with the onset of the Holocene. Higher Fe/Mn ratios (Fig. 8) also suggest at least seasonal anoxic conditions leading to higher nutrient concentrations during the LG. On the other hand, we cannot rule out the possibility that anoxic conditions affected Early Holocene chironomid assemblages as well. Chironomid concentration became very low (Fig. 5) documenting unfavourable conditions. Profundal taxa decreased and were partially replaced by littoral taxa. Therefore, the effect of oxygen

depletion caused by the enhanced decomposition of biomass produced after climate amelioration cannot be fully excluded.

3. Higher primary productivity in the Early Holocene was able to enhance competition for available nutrients and made them a limiting factor in contrast to the LG. Both growing terrestrial and aquatic vegetation serve as a phosphorus sink and can make nutrients unavailable for algal primary producers (Canfield & Jones, 1984; Tabacchi et al., 2000). While primary productivity rises, a phosphorus turnover in the lake increases but its instantaneous concentrations often decrease or fluctuate (Wetzel, 2001). In Lake Komořany, a higher biomass production of terrestrial and/or aquatic vegetation was suggested by an increasing proportion of organic matter in sediment (Fig. 8) and higher pollen concentration (Fig. 6). An increase of algal biomass in the Early Holocene is documented by higher diatom valve concentration (Fig. 4). Additionally, the limitation of biomass production by harsh conditions during the LG possibly allowed a higher availability of nutrients for surviving organisms. Light availability and mechanical disturbances are supposed key limiting factors for primary productivity during the LG in Lake Komořany: (a) Severe light attenuation by turbid inorganic particles is expected during the LG as a consequence of the potentially stronger input of minerogenic material by the tributary (the Bílina River). As we found only indications of a slightly higher input of particles in the LG (Fig. 2) and the sedimentation rate remained low (Fig. 3), the turbidity of particles by water column mixing seems to be a more important factor than the absolute amount of washed-in material. The potential input of particles could also fertilize benthic algae surviving in shallower parts where physical conditions were more stable and light more available (Stevenson et al., 1996). This could thus amplify the discrepancy between the low primary productivity and the presence of the nutrient-demanding taxa. Restricted favourable habitats did not allow heightened biomass production. (b) Mechanical disturbances by turbulent water conditions or prolonged ice-cover could impede the biomass growth of algal mats and aquatic macrophytes as frequent disturbance reduces benthic biomass (Stevenson et al., 1996).

These processes could cause either lower absolute nutrient level towards the Holocene or at least raise relative nutrient availability in the LG. Although absolute nutrient concentration in the lacustrine ecosystem could be higher in the Holocene, the instantaneous nutrient availability was lower due to the fast nutrient turnover. In Lake Komořany, the presence of nutrient-demanding diatoms and chironomids in the Late-Glacial period and their subsequent decrease towards the Holocene agrees with this hypothesis. Furthermore, the same timing of main species compositional change in diatom and chironomid assemblages with the increase in organic matter proportion supports the influence of changes in catchment and/or in-lake primary productivity to ecosystem structuring. Other enriching mechanisms such as erosion from the catchment and anoxia seem to be of less importance. Primary productivity is considered the main driving factor of change in lacustrine nutrient availability in Lake Komořany at the LG/H transition.

5.4 Limits of the sedimentary record

Interpretation of the large shallow lowland lakes' records brings various constrictions particularly due to the specific features of in-lake sedimentation such as the low inflow of allochthonous material, the possible resuspension of sediments by waves in polymictic regime or the effective oxidation of deposited organic matter (Blais & Kalff, 1995; Scheffer, 2004). The aforementioned factors result in a low sedimentation rate, which reduces stratigraphic resolution of the natural archive. Our study faced an absence of terrestrial macrofossils for radiocarbon dating and very low time-resolution of the record. Bulk sediment was used for AMS ^{14}C dating with reference to previous successful application to Holocene material from Lake Komořany (Bešta et al., 2015; Houfková et al., 2017). Although negligible bias was supposed, possible errors obliged us to take the modelled chronology with reserve.

Our results provided a clear evidence of LG record from both studied profiles. The continuous development of assemblages and geochemical variables suggested undisturbed sedimentation without more extensive reworking. The presence of minor hiatuses cannot be excluded but they do not threaten the aims of this study. The depth-age model was supported by palynostratigraphy. In particular, the observed peak of *Corylus avellana* (24%, ~10,800 cal yr BP) was seen to be identical to the peak recorded in the PK-1-C profile from Lake Komořany (26%, ~11,000 cal yr BP) (Houfková et al., 2017) which is in accordance with data from other sites in Central Europe (Theuerkauf et al., 2014).

6 Conclusion

A relatively stable ecosystem with gradual changes in aquatic and terrestrial communities was recorded in Lake Komořany at the Late Glacial/Holocene transition. Two major, almost certainly climatically driven, shifts were recorded in the studied profiles: i) the qualitative one with changes in species composition of the diatom and chironomid communities foregoing the LG/H boundary and ii) the quantitative one at the LG/H boundary distinguishable by the enhanced diatom valve concentrations and the lithological change. These shifts were linked with two opposite trends: decreasing inferred trophic status and increasing primary productivity towards the Holocene. This discrepancy between nutrient availability and primary productivity can be best explained by a sink of nutrients in biomass produced after the climatic amelioration. In other words, nutrients became a limiting factor only after the onset of Holocene favourable climate. The species composition and primary productivity were therefore shaped by the climate change indirectly by means of nutrient availability.

7 Acknowledgements

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Chapter 3:

Mountain aquatic *Isoëtes* populations reflect millennial-scale environmental changes in the Bohemian Forest Ecosystem, Central Europe

Abstract

In this study we aim to investigate millennial-scale dynamics of *Isoëtes*, a type of macrophyte well adapted to oligotrophic and clear-water lakes. Despite its wide distribution during the Early Holocene, nowadays *Isoëtes* is considered as vulnerable or critically endangered in many Central European countries. Using a multi-proxy palaeoecological reconstruction involving *Isoëtes* micro- and megaspores, pollen, plant macrofossils, macro-charcoal, diatoms and chironomids from four lakes (Prášilské jezero, Plešné jezero, Černé jezero, Rachelsee) located in the Bohemian Forest Ecosystem mountain region in Central Europe, we reconstruct *Isoëtes* dynamics and discuss how local environmental factors impacted its distribution and abundance during the Holocene. Our results show regionally concurrent patterns of *Isoëtes* colonization across all lakes beginning 10,300–9300 cal yr BP, and substantially declining around 6400 cal yr BP. Results from Prášilské jezero imply that *Isoëtes* decline and collapse in this lake reflect gradual dystrophication that led to the browning of lake water. This is evidenced by a shift in diatom assemblages towards more acidophilous taxa dominated by *Asterionella ralfsii* and by a decrease in total chironomid abundance and taxa sensitive to low oxygen levels. Dystrophication of Prášilské jezero was linked with the immigration of the late-successional tree taxa (*Picea abies* and later *Fagus sylvatica* and *Abies alba*), peatland expansion, and decreasing fire activity. Multi-site comparison of pollen records suggest that these vegetation-related environmental changes were common for the whole region. Our study demonstrates the sensitivity of *Isoëtes* to millennial-scale natural environmental changes within the surrounding lake catchment.

8 Introduction

Aquatic *Isoëtes*, commonly known as quillworts, are archaic lycopods well adapted to cold, clear-water and oligotrophic lakes. Thus, they serve as an important indicator of environmental health (Vöge, 2003). Their specific morphological and physiological adaptations to cold and oligotrophic conditions facilitated *Isoëtes* expansion during the Early Holocene, allowing them to become one of the primary colonizers of sub-arctic and mountain lakes across Europe (Birks, 2000; Pini, 2002; Guiter et al., 2005; Fajer et al., 2012). However, lake ecosystems went through substantial transitions in the Holocene as a result of climate-induced changes in vegetation composition, soil development, fire dynamics and human impacts (i.e. Bradshaw et al., 2005; Meyer-Jacob et al., 2015; Leys et al., 2016). All these factors affect both the chemical and physical properties of lake waters, and thus drove *Isoëtes* dynamics during the Holocene.

The modern distribution of two sympatric congeneric species *I. echinospora* D. and *I. lacustris* L. in Europe is situated in the northern and western part of the mainland, i.e. Scandinavia, the Netherlands and Poland. These species are also found in Southern and Central Europe, however, only in mountain lakes e.g. in the Vosges Mountains (France), the Central Alps (Switzerland), the Black Forest (Germany), the Bohemian Forest (Czechia) and the Karkonosze Mountains (Poland). As such, these mountain populations have persisted as glacial relicts. According to Rørslett and Brettum (1989), *I. lacustris* is sensitive to mechanical stress from ice scour or wave action and typically resides in deep littoral zones down to a depth of 7 m, while *I. echinospora* inhabits the shallow littoral zone and resides at depths of 2–3 m maximum in Scandinavian lakes. Similar habitats have been found among populations living in two separate Bohemian Forest lakes; *I. lacustris* inhabits depths of 1–4 m in Černé jezero, while *I. echinospora* grows in very shallow depths of 0.3–0.5 m in Plešné jezero (Čtvrtlíková et al., 2014; 2016). Recently, both *I. echinospora* and *I. lacustris* have declined across Europe as a result of increasing human impact connected with habitat degradation. Namely, lake acidification, eutrophication and humification altering lake water chemistry are the reasons for the recent *Isoëtes* population reduction and/or extinction (Arts, 2002; Grzybowski et al., 2008; Vöge, 2015; Chmara et al., 2015; Čtvrtlíková et al., 2014; 2016).

The genus *Isoëtes* belongs to the isoetid plant functional group, which are characteristically a small, slow-growing species (Hutchinson, 1975). Based on their life strategy, they are classified as S-strategists which are plants that are resistant to stress (Farmer and Spence, 1986). Extraordinary morphological traits (basal rosette growth, high root biomass, highly porous tissue) and physiological strategies (low inherent growth rate and crassulacean acid metabolism (CAM)) allow *Isoëtes* to live in carbon- and nutrient-poor environments (Rørslett and Brettum, 1989; Arts, 2002). They are also known for their high radial oxygen loss from their roots, which alters the availability of nutrients in sediments for other aquatic plants. This mechanism is crucial as *Isoëtes* are weak competitors, allowing them to overcome the potential shading effect induced by fast-growing competitors (Smolders et al., 2002). *Isoëtes* produce two types of spores: male microspores and female megaspores. In the case of *I. echinospora* and *I. lacustris*, temperature limits on spore germination are rather low, ranging between 8–12 °C (Čtvrtlíková et al. 2014; 2016). Both spores are well preserved in lake sediments which allows for reconstructing *Isoëtes* dynamics on millennial times scales.

In this study, we aim to reconstruct millennial-scale *Isoëtes* dynamics from the Bohemian Forest Ecosystem mountain region situated in Central Europe. This region is of particular interest as modern *Isoëtes* populations occupy only two of the existing eight glacial lakes (Černé jezero and Plešné jezero) and are considered to be extinct in the other six glacial lakes (Procházka, 2000). Previous palaeoecological studies showed that *Isoëtes* also historically inhabited Prášilské jezero, Čertovo jezero and the palaeolake, Stará jímka (Břízová, 2012; Carter et al., 2018). Nevertheless, the entire sedimentary sequences from these lakes were not examined, and so it remains unclear when precisely *Isoëtes* populations established and disappeared in these water bodies. Moreover, a multi-proxy palaeoecological approach investigating potential drivers behind *Isoëtes* dynamics has yet to be employed.

To fill this gap, we used a multi-proxy approach encompassing plant macrofossils, pollen, macro-charcoal, diatoms, chironomids and other aquatic proxies (chrysophytes, cladocerans and bryozoans) obtained from lake sediments from Prášilské jezero to reconstruct past environmental conditions in the lake and its catchment and assess how these affected *Isoëtes* dynamics through time. Using these proxies, we determined if changes in vegetation cover and fire dynamics affected lake water properties and influenced *Isoëtes* populations in Prášilské jezero. Finally, we compared our record to available palynological records from other lakes in the region (Černé jezero, Plešné jezero and Rachelsee) to assess spatio-temporal patterns in *Isoëtes* dynamics across the Bohemian Forest Ecosystem mountain region.

9 Study site

The Bohemian Forest Ecosystem is a low mountain region located in Central Europe along the Czech–German border, known as the Bohemian Forest (Šumava) on the Czech side, and the Bavarian Forest (Bayerischer Wald) on the German side (Fig. 1). This mountain range was subject to glaciation (Mentlík et al., 2010), and at the end of the Last Glacial Period, retreating mountain glaciers left behind eight natural lakes; five of them are situated in the Bohemian Forest, three in the Bavarian Forest.

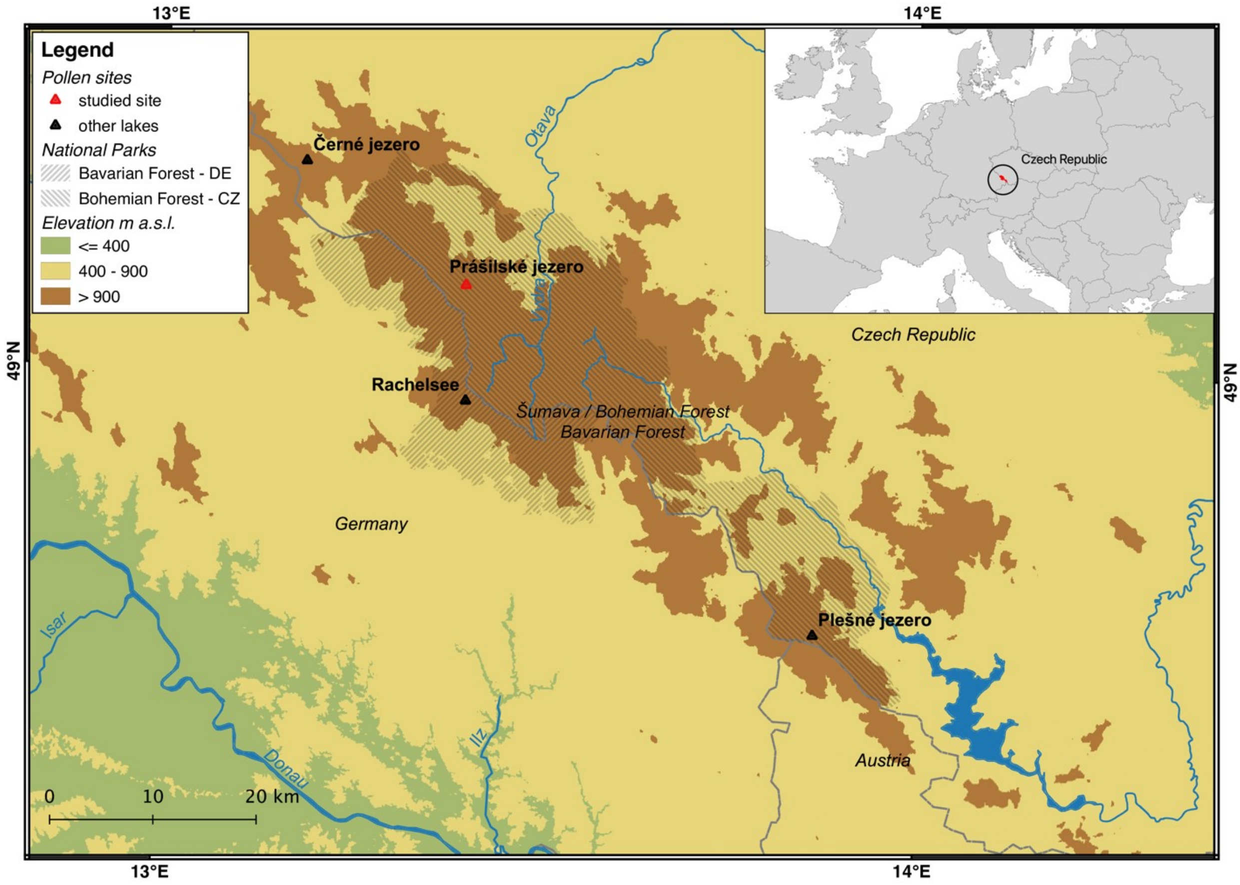

Fig. 1: Map of the Bohemian Forest Ecosystem. Black triangles indicate previously published sedimentary records used in this study (Jankovská, 2006; Carter et al., 2018a, b; van der Knaap et al., 2019). Prášilské jezero (red triangle), is the main study site used in this study, where we present for the first time *Isoëtes* megaspores, diatom and chironomid data.

Preserved microspores from Plešné jezero, Prášilské jezero, Černé jezero and Rachelsee (Fig. 1), illustrate that *Isoëtes* lived in these lakes in the past (Jankovská, 2006; Carter et al., 2018a, b; van der Knaap et al., 2019). These four lakes are situated at an altitude above 1000 m a.s.l. and are located on acidic bedrock formed from metamorphic crystalline rocks with gneiss, mica-schist and granite (Tab. 1). The acidic bedrock induces a low neutralizing capacity of the soils making lake pH-sensitive to atmospheric acid deposition (Kopáček et al., 2002), and to natural acidification triggered by changes in vegetation in the catchment (Pražáková et al., 2006; Kopáček et al., 2009). The local climate is characterized by wet and cold winters and wet and mild summers. Meteorological data from the nearest meteorological station Churáňov (Czech Hydrometeorological Institute), located ~20 km from Prášilské jezero, suggest an interpolated mean annual temperature of 4.5 °C and precipitation of 941 mm year^{-1}). The Bohemian Forest lake catchments are small and steep, covered by thin podzols and predominantly forested by Norway spruce (*Picea abies* L.), with an admixture of European beech (*Fagus sylvatica* L.), silver fir (*Abies alba* Mill.) and rowan (*Sorbus aucuparia* L.) (Table 1).

Lake Name	**Altitude (m)**	**Maximum depth (m)**	**Lake area (ha)**	**Catchment area (ha)**	**Bedrock**	**Dominant Forest Cover**
Plešné jezero	1090	18	7.5	67	granite	Norway spruce
Prášilské jezero	1079	16	4.2	65	mica schist, granite	Norway spruce
Černé jezero	1008	40	18.4	129	mica schist, quartzite	Norway spruce
Rachelsee	1071	13	5.7	58	gneiss	Norway spruce, European beech and silver fir

Table 1: Basic characteristics of the four studied Bohemian Forest lakes and their catchments (taken from Vrba et al., 2000).

10 Materials and methods

10.1 Micro-/megaspores and living macrophytes in situ, pollen, and macro-charcoal

To reconstruct local *Isoëtes* dynamics from Prášilské jezero, we analysed both micro- and megaspores from a sediment core (Pra-15) extracted from the central part of the lake. Microspores (average length for *I. lacustris*, 43µm, *I. echinospora*, 27µm) were recorded by pollen analysis but were not distinguished to the species level due to overlapping size fractions (Moore et al., 1991). The larger megaspores (average length for *I. lacustris*, 600–700 µm, *I. echinospora*, 400–500 µm) were recorded by macrofossil analysis and were distinguished to the species level, as the megaspores differ in surface structure (rugulate vs. echinate). See Carter et al. (2018 a; b) for further details regarding palynological and macrofossil methods performed on the Pra-15 core.

An accurate reconstruction of local *Isoëtes* presence/absence based on micro- and megaspores preserved in lake sediments may be limited by a "false absence" (Birks, 2014). Thus, to verify the current 'extinct' status of *Isoëtes* at Prášilské jezero previously documented by Procházka (2000), we organized a scuba diving expedition in September 2019 by M. Čtvrtlíková to inspect the current macro-vegetation. The littoral range (i.e. the zone of liveable *Isoëtes* populations) was determined visually by identifying the euphotic zone of the lake bottom. Once identified (at depths ranging between 0–2.5 m), the littoral zone was thoroughly inspected for macrophyte presence.

To discuss the effects of terrestrial vegetation composition and local fire activity on *Isoëtes* dynamics and lake water properties of Prášilské jezero, we used pollen, plant macrofossils and macro-charcoal area accumulation rates ($\mathrm{CHAR_A}$) from previously published studies (Carter et al., 2018a, b). Additionally, we compiled microspore and dominant tree pollen data from Černé jezero (Carter et al., 2018a), Rachelsee (van der Knaap et al., 2019) and Plešné jezero (Jankovská, 2006) to examine regional *Isoëtes* dynamics across the Bohemian Forest mountain region. For further details regarding palynological methods and results, see Carter et al. (2018a), van der Knaap et al. (2019), and Jankovská (2006). In this study, we used both microspore percentages and influx, as influx better reflects biomass and population size (Sëppa et al., 2009).

10.2 Diatoms, chironomids and other aquatic proxies

Diatom analysis was conducted at 2–10 cm resolution throughout the sediment core (Pra-15). Diatom cell walls were isolated using standard procedures (Battarbee et al., 2001), by boiling ~0.1 g of dry sediment in 30% hydrogen peroxide. Once cooled, a single drop of hydrochloric acid was added to each sample which was then repeatedly rinsed with distilled water. Purified solutions were diluted and 50μl of the sample (20μl for the most basal samples) was permanently mounted onto cover slides using Naphrax® (Brunel Microscopes Ltd, UK) mounting resin, together with a stable concentration ($30.65\mathrm{x}10^4$ particles/ml) of divinylbenzen microspheres used as reference particles in order to estimate diatom accumulation rates. A minimum of 400 valves were counted from each sample under 1000× magnification using a light microscope and identified using Krammer and Lange-Bertalot (1986, 1988, 1991a, b), Lange-Bertalot and Metzeltin (1996), Lange-Bertalot (2001), Krammer (2000, 2002, 2003), Houk (2003), Houk and Klee (2007), Lange-Bertalot et al. (2011) and Cantonati et al. (2017). Diatom nomenclature was updated and unified according to AlgaeBase (Guiry and Guiry, 2019). Diatom pH preferences were assessed using Van Dam et al. (1994) and complemented with information from other literature sources (see Supporting information Table S1). Chrysophycean stomatocyst total abundance was also calculated during diatom analysis. The ratio of chrysophycean stomatocyst total abundance and diatom total abundance (C:D; chrysophycean:diatom ratio) was used as a proxy for water mixing (Werner and Smol, 2005). Additionally, we counted coenobial algae, bryozoan statoblasts and *Daphnia* ephippia as these proxies provide information on past aquatic environments such as water transparency and nutrient and oxygen availability (Jankovská and Komárek, 2000; Francis, 2001; Szeroczyńska and Sarmaja-Korjonen, 2007).

Chironomid analysis was conducted at 0.5–23 cm resolution using 1–13 cm^3 of wet sediment. Samples were washed with distilled water through 100 μm sieves and transferred into a modified plastic Sedgewick-Rafter counting cell. Head capsules were picked with either fine forceps or a steel needle using a dissecting microscope. Samples were then dehydrated in ethyl alcohol and mounted on glass microscope slides in Euparal® mounting medium for microscopic identification. For taxa identification, we followed Wiederholm (1983), Rieradevall and Brooks (2001) and Brooks et al. (2007). Due to low chironomid abundances in most of the samples, we show only chironomid concentrations (head capsules per 1 cm^3), and taxa indicating a well-oxygenated profundal zone (for a detailed species list, see Supporting information Fig. S3).

10.3 Statistical analysis

To identify synchronous environmental patterns in *Isoëtes* abundance and aquatic and terrestrial ecosystem dynamics over time, we performed change point analysis (CPA). CPA was employed on both microspore and megaspore influx to identify abrupt shifts in *Isoëtes* populations using the 'change point' v2.2.2 package (Killick and Eckley, 2014) in the R studio statistical program (R Core Team, 2019). We selected a penalty of $\beta = 7\log(n)$ in order to avoid over-fitting (Killick et al., 2012). The zonation of aquatic and terrestrial proxies then follows identified shifts in *Isoëtes* abundance.

11 Results

11.1 Local *Isoëtes* history in Prášilské jezero

CPA detected 10 shifts in both the *Isoëtes* microspore and megaspore records (Fig. 2a, b, c). However, 5 of these shifts were determined by variations within a few samples or were influenced by variations in sediment accumulation rates (Fig. 2d), and therefore were not included in this analysis (following Finsinger et al., 2018). Based on the remaining 5 shifts, we grouped the *Isoëtes* records into 5 phases; P 0, P 1, P 2, P 3, and Population collapse (P C).

During P 0 (11,400–10,300 cal yr BP), *Isoëtes* megaspores were not recorded, while microspores were recorded in only three samples, which average a low influx of ~65 cm^{-2} yr^{-1} (Fig. 2c). During P 1 (10,300–9300 cal yr BP), *Isoëtes* established, averaging ~940 microspores cm^{-2} yr^{-1}. Only *I. echinospora* megaspores were identified in one sample in this zone with a concentration of 7.5 per 10 cm^3 (Fig. 2a; P 1). At the onset of P 2 (~9300 cal yr BP), *Isoëtes* microspores declined until ~6400 cal yr BP, averaging ~500 cm^{-2} yr^{-1} (Fig. 2c; P 2). Both *I. echinospora* and *I. lacustris* megaspores were identified in P 2 with an average concentration of ~0.5 and ~2 per 10 cm^3. During P 3 (~6400–4300 cal yr BP), microspore influx abruptly decreased to ~120 cm^{-2} yr^{-1} (Fig. 2c; P 3). Megaspores of *I. lacustris* were recorded in one sample in zone P 3 with a concentration of 3 per 10 cm^3. After 4300 cal yr BP, *Isoëtes* populations collapsed as demonstrated by sporadic recordings of both microspores and megaspores. In general, microspore influx did not exceed ~100 cm^{-2} yr^{-1}, while megaspore concentrations did not exceed ~3 per 10 cm^3 (Fig. 2a, c; Population collapse). Between ~2200–1750 and ~1600–950 cal yr BP, neither microspores nor megaspores were recorded. The last occurrence of either micro- or megaspores was dated to ~1947 AD; however, values were relatively low (68 microspores cm^{-2} yr^{-1}, and 1 megaspore per 10 cm^3). Moreover, the surface structuring of the megaspores was degraded and thus could not be determined to species level (see *Isoëtes* undiff. megaspores in Fig. 2a).

The 2019 scuba diving expedition showed that the current littoral zone of Prášilské jezero is neither inhabited by aquatic *Isoëtes* nor by angiosperms, but is densely grown by macroscopic algae, *Batrachospermum* sp. (determined by P. Znachor, Biology Centre CAS, České Budějovice), of 2–5 cm height and at depths 0.5–2.1 m below the water surface. The lake water was of humic brown colour with a Secchi depth estimated water transparency of 3 m.

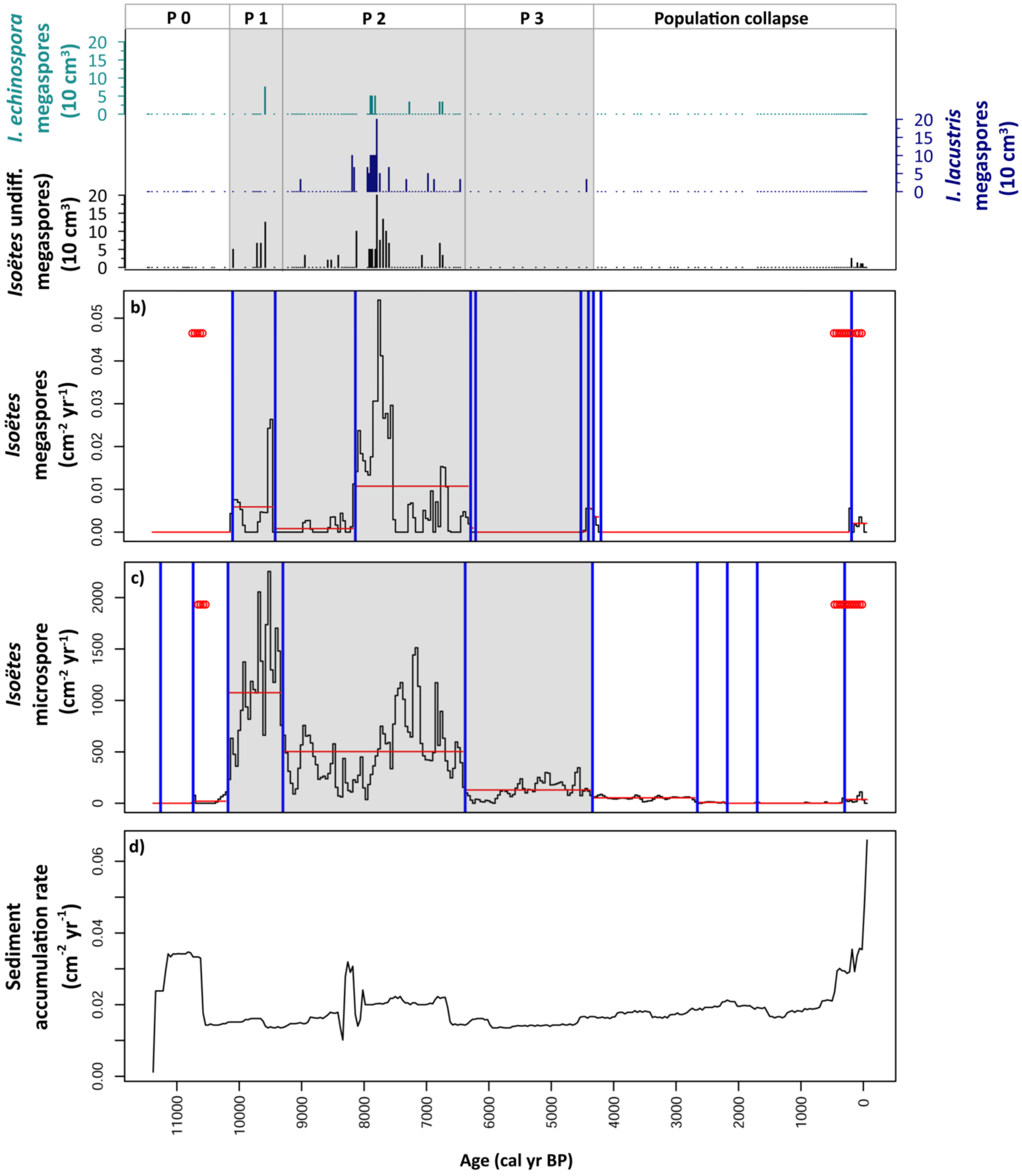

Fig. 2: Reconstructed *Isoëtes* population dynamics from Prášilské jezero based on megaspores and microspores: a) coloured bars show *I. echinospora* (light blue bars), *I. lacustris* (dark blue bars) and *Isoëtes* undiff. (black bars) megaspore concentration per 10 cm^3; b) and c): change point analysis results based on the total sum of megaspores (b) and microspore influx (c; cm^{-2} yr^{-1}); d): sediment accumulation rate (cm^{-2} yr^{-1}). Vertical blue lines in b) and c) indicate detected change points in both *Isoëtes* influx records using a penalty of $\beta = 7\log(n)$, while empty red circles indicate randomly generated change points in the *Isoëtes* influx data. Red horizontal lines in b) and c) represent the mean values of *Isoëtes* influx, while the grey shaded area highlights periods of increased *Isoëtes* influx.

11.2 Local aquatic environment history in Prášilské jezero

During P 0 (11,400–10,300 cal yr BP), tychoplanktonic diatoms (*Aulacoseira pfaffiana* and *A. alpigena* average ~50% and ~30%, respectively) dominated and benthic diatoms preferring circum-neutral or weakly acidic conditions were abundant, averaging ~20% (Fig. 3) (for details about pH preferences of diatom species, see Supporting Information Table S1). The C:D ratio was low, while the influx of *Pediastrum* (averaging ~300 cm^{-2} yr^{-1}) and concentrations of chironomids (averaging ~60 HC per 1 cm^3) peaked, reaching the highest values of the whole record in P 0. Chironomids occurring in well-oxygenated profundal environments, namely *Heterotrissocladius grimshawi*-type, *Micropsectra insignilobus/contracta*-type and *Tanytarsus lugens*-type, average ~40% (Fig. 3; P 0). The cladoceran *Daphnia* (cf.) *longispina* was common (averaging ~55 per 1 cm^3), and the bryozoan *Plumatella* was also abundant (averaging ~5 per 10 cm^3). P 1 (10,300–9300 cal yr BP) is characterised by a slight decrease in tychoplanktonic diatom species. Chironomids also decreased (averaging ~3 HC per 1 cm^3), and taxa indicating a well-oxygenated profundal environment disappeared (Fig. 3; P 1). Remaining chironomids consisted mostly of littoral and sublittoral taxa (*Ablabesmyia*, *Corynoneura*, *Heterotrissocladius marcidus*-type, *Tanytarsus pallidicornis*-type 2, *Zavrelimyia* type A; Fig. S3). Concentrations of *Daphnia longispina* increased (averaging ~80 per 1 cm^3) in P 1. During P 2 (9300–6400 cal yr BP), circum-neutral diatoms (*Stauroforma exiguiformis*, *Staurosira venter*; Fig. S2), together with *Plumatella* and *Daphnia longispina* declined, while *Botryococcus* reached its highest influx of the record (averaging ~2200 cm^{-2} yr^{-1}). Chironomid abundance was extremely low, though this trend was interrupted between 8100–7700 cal yr BP when total abundance increased (averaging ~22 HC per 1 cm^3), and *Heterotrissocladius grimshawi*-type temporally reappeared (Fig. 3; P 2). P 3 (6400–4300 cal yr BP) is characterised by substantial changes in diatom composition. Specifically, *Aulacoseira alpigena* dominated (peaking at 45%) at the beginning of the P 3, while other *Aulacoseira* taxa are less abundant (Fig. S2). Proportions of benthic diatoms prevailed over planktonic diatoms in the end of the P 3, especially indicators of moderate-to-strong acidification increased including *Brachysira brebisonnii*, which reached its highest percentages (averaging ~18%). The C:D ratio increased to its highest values during zone P 3, while *Daphnia longispina* concentrations sharply decreased (averaging ~40 per 1 cm^3) at the end of this zone. Similarly, concentrations of *Plumatella* decreased, and their findings are somewhat sporadic from the end of zone P 3 onward. Total chironomid abundance was still very low, and taxa characteristic of a well-oxygenated profundal were extremely rare (Fig. 3, P 3). At the onset of the Population collapse phase (4300 cal yr BP to present), the C:D ratio reached its highest value, then rapidly decreased. Additionally, there was a distinct shift from predominantly benthic diatoms to an overwhelming dominance of the euplanktonic taxa, *Asterionella ralfsii*. Between ~4300 and ~2200 cal yr BP, chironomid abundance was very low (averaging ~3 HC per 1 cm^3). Even though chironomid abundance slightly increased (averaging ~13 HC per 1 cm^3) after ~2200 cal yr BP, indicators of a well-oxygenated profundal environment were missing (Fig. 3; Population collapse).

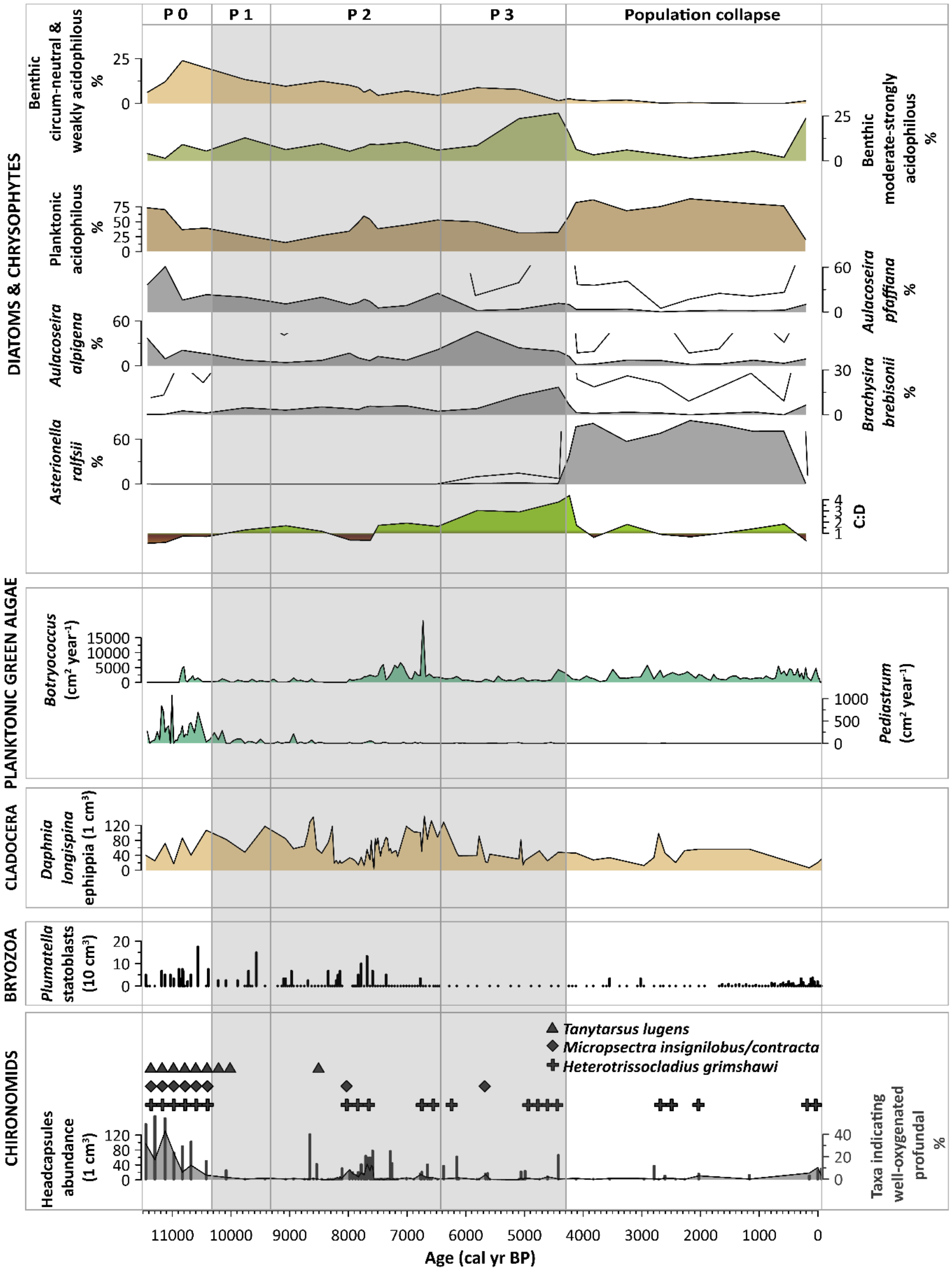

Fig. 3 Reconstructed aquatic dynamics in Prášilské jezero based on percentages of diatom ecological groups (first box titled 'Diatoms and Chrysophytes' listed from top to bottom; benthic circum-neutral and weakly acidophilous (tan silhouette), benthic moderately-to-strongly acidophilous (green silhouette), acidophilous planktonic (dark brown silhouette), and selected diatom taxa (grey silhouettes; white represents 10 x exaggeration), C:D - chrysophycean stomatocyst to diatom influx rates ratio (filled brown-green transition line), planktonic green algae influx rates (second box titled 'Planktonic green algae'; green silhouettes), *Daphnia*

longispina ephippia concentrations (third box titled ‘Cladocera’; orange silhouette), *Plumatella* statoblasts concentrations (fourth box titled ‘Bryozoa’; filled black bars), and chironomid head capsule concentrations (bottom box titled ‘Chironomids’; grey silhouette), and percentages of chironomid profundal taxa (filled grey bars)). Grey shading highlights periods of increased *Isoëtes* influx based on change point analysis.

11.3 Fire regime and terrestrial vegetation history of the Prášilské jezero catchment

During P 0 (11,400–10,300 cal yr BP), the vegetation structure is characterised by a relatively open forest canopy, shown as a high ratio of NAP:AP (non-arboreal:arboreal pollen). The ratio is consistent with high pollen percentages belonging to light-demanding pioneer trees, *Pinus* (average ~40 %), *Betula* (average ~30 %) and *Corylus* (average ~4 %), and high concentrations of their macrofossils (for *Pinus* average 1.5 needle/brachyblast/twig per 10 cm^3; for *Betula* 5.5 seed/bud scale per 10 cm^3; for *Corylus* we did not record any macrofossils). Fires were frequent as suggested by high CHAR$_A$ (Fig. 4; P 0). In P 1 (10,300–9300 cal yr BP), NAP:AP decreased coinciding with a substantial increase in *Corylus* pollen (averaging ~25 %), and an increase in *Picea abies* pollen (averaging ~5 %),and *Picea abies* needle concentrations. Pollen percentages of *Pinus* (average ~30 %) and *Betula* (average ~10 %), as well as *Betula* macrofossil concentrations (1.5 seed per 10 cm^3) all gradually decreased, despite *Pinus* macrofossil concentrations remaining high. Fires were frequent as indicated by CHAR$_A$ (Fig. 4; P 1). Zone P 2 (9300–6400 cal yr BP) is characterised by a decrease in *Corylus* (averaging ~18 %), and the expansion of *Alnus* (averaging ~5 %) and rise in *Picea abies* (averaging ~15 %) in the second half of this zone. Percentages of *Sphagnum* spores (average ~1.5 %) and leaf concentrations substantially increase (400 leaves per 10 cm^3). We inspected several samples containing *Sphagnum* stem leaves (necessary for species identification) and determined that the leaves belong to terrestrial bog and forest species *S. angustifolium* and *S. girgensohnii*. Thus, we further interpret the concentration of *Sphagnum* leaves as a proxy for peatland expansion. At the end of P 2, *Fagus sylvatica* pollen increased (averaging ~5 %), but macrofossils were not found. According to CHAR$_A$, fire activity was frequent (Fig. 4; P 2). During P 3 (6400–4300 cal yr BP), NAP:AP reaches its lowest values of the entire record simultaneous with a decrease in *Corylus* (average ~11%) and an increase in *Fagus sylvatica* pollen (average ~15%). Fire activity decreased as shown by a decrease in CHAR$_A$. The latter half of P 3 captures the increase in *Abies alba* pollen (average ~5 %) and in one sample *A. alba* needle and seed (~3 macrofossils per 10 cm^3). The Population collapse phase (4300 cal yr BP up to present) resembles P 3 in that fires were infrequent, and by a similar vegetation composition, but the vegetation structure gradually opened as shown by increasing NAP:AP (Fig. 4; P 3 and Population collapse).

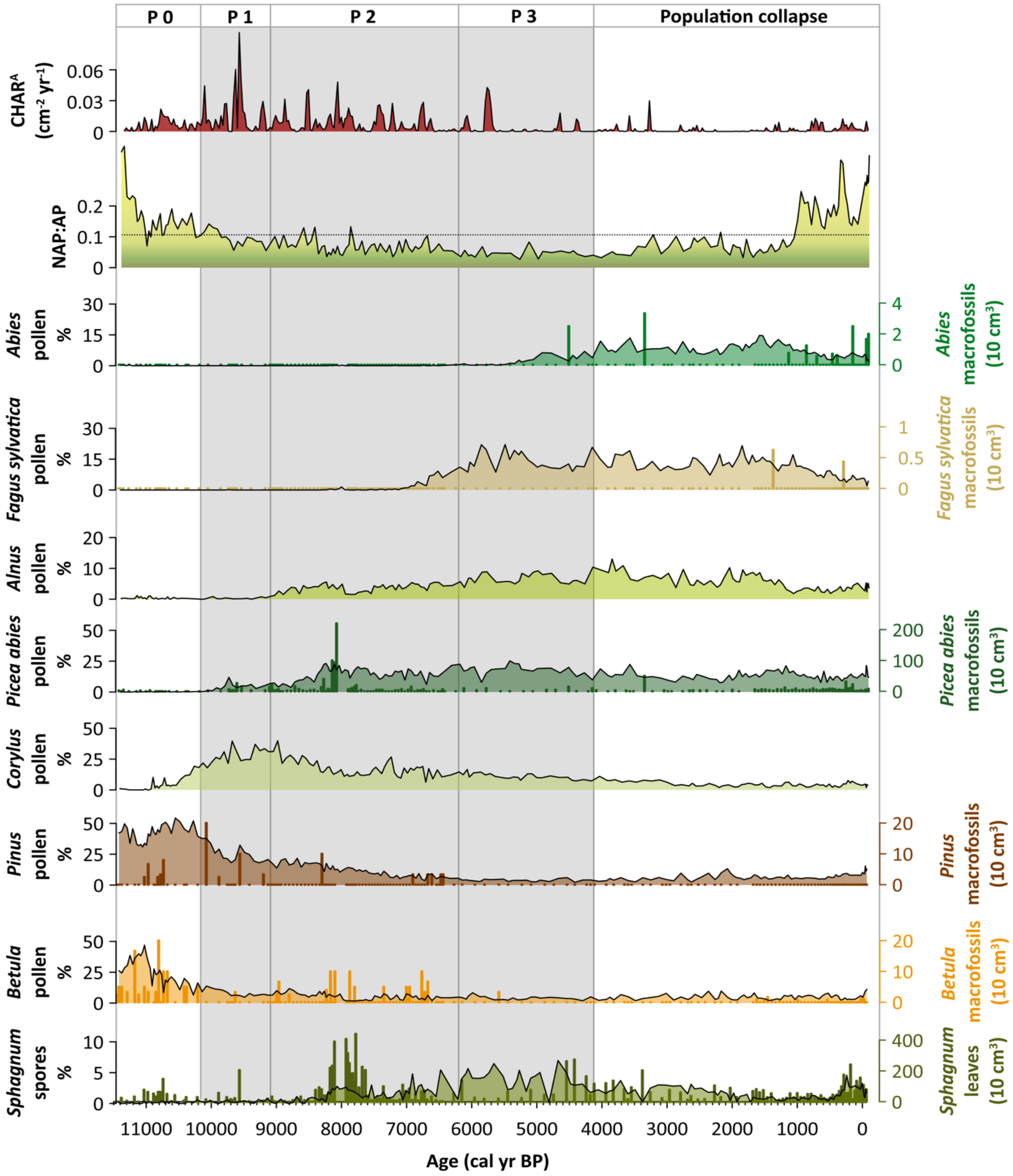

Fig. 4 Reconstructed fire history and terrestrial vegetation at Prášilské jezero using CHAR$_A$ (macro-charcoal area accumulation rate; filled red silhouette), NAP:AP (non-arboreal:arboreal pollen ratio; yellow-green silhouette; dotted line represents the long-term average), pollen/spore percentages (coloured silhouettes) and concentrations of plant macrofossil per 10 cm^3 (filled coloured vertical bars). Grey shading highlights periods of increased *Isoëtes* influx based on change point analysis. Pollen data presented come from Carter et al., 2018a, b).

11.4 Regional *Isoëtes* history in the Bohemian Forest Ecosystem lakes

The *Isoëtes* microspore records from all four study sites show a similar pattern of *Isoëtes* population dynamics across the entire Bohemian Forest Ecosystem (Fig. 5). *Isoëtes* microspores first appeared at Plešné jezero ~10,300 cal yr BP, reaching a maximum between ~10,300–7000 cal yr BP, similar to the timing of *Isoëtes* establishment and expansion at Prášilské jezero. Colonization first began ~9300 cal yr BP at Černé jezero and ~9100 cal yr BP at Rachelsee. After 6500 cal yr BP, populations decreased and either disappeared or remained extremely low at all four lakes until the present, except at Černé jezero when *Isoëtes* began to increase ~2500 cal yr BP. This concurrent population decrease after 6500 cal yr BP coincided with the regional increase in *Fagus sylvatica* and *Abies alba* pollen (Fig. 5).

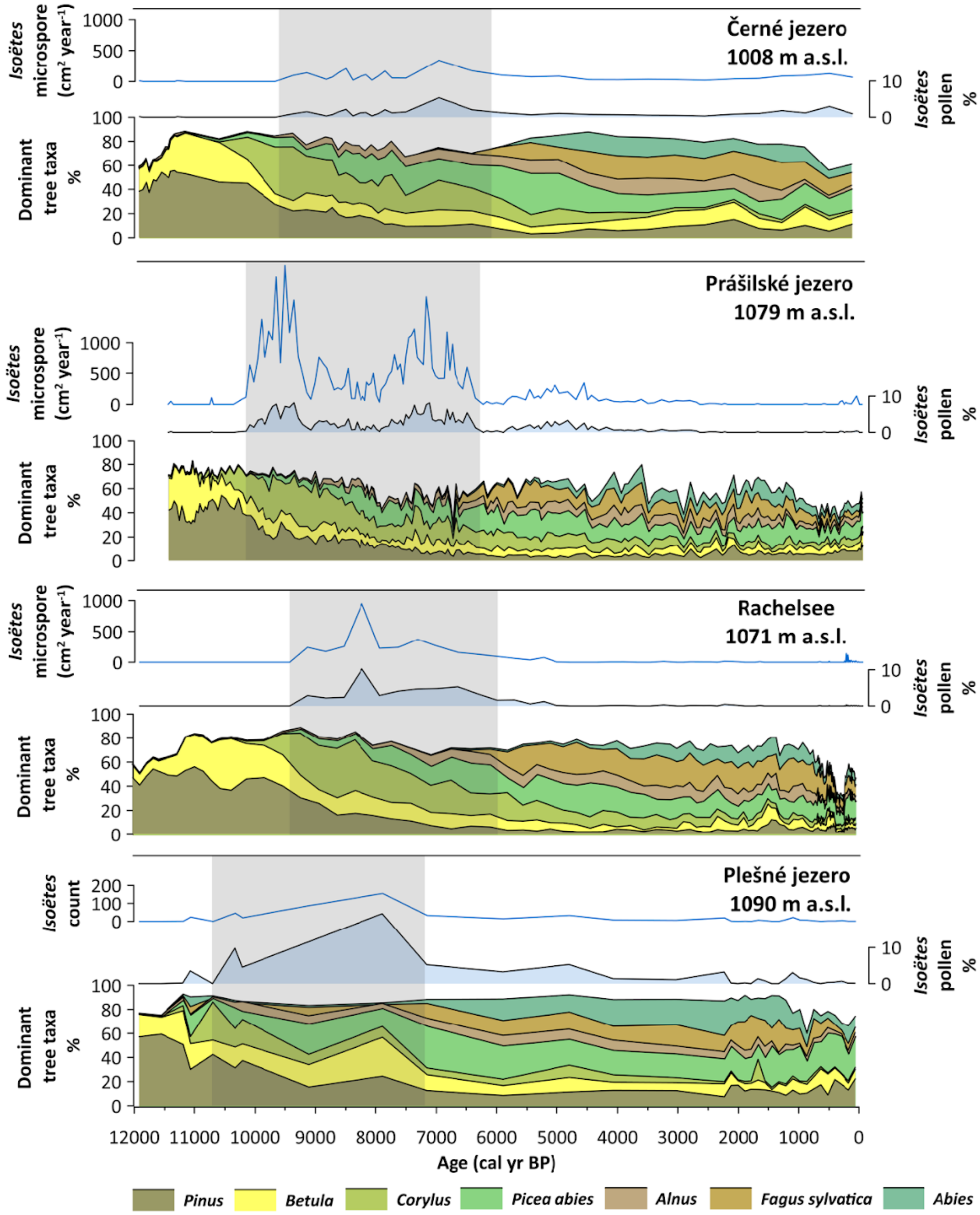

Fig. 5 Reconstructed *Isoëtes* population dynamics illustrate concurrent patterns throughout the Bohemian Forest Ecosystem. Blue lines and blue silhouettes illustrate both microspore percentages and microspore influx/counts, while coloured silhouettes illustrate the relative proportion of dominant tree taxa for Černé jezero (Carter et al., 2018a), Prášilské jezero (Carter et al., 2018b), Rachelsee (van der Knaap et al., 2019), and Plešné jezero (Jankovská, 2006). Grey shading highlights periods of increased *Isoëtes* influx/counts for each record.

12 Discussion

When reconstructing past aquatic macrophytes, core location is critical as lake morphometry, inlets and outlets and prevailing winds can all affect the preservation of fossil remains (e.g. Birks, 2001; Luoto, 2010). Heggen et al. (2012) analysed a series of surface sediment samples from five small lakes in Norway to investigate the within-lake distribution of diverse subfossil assemblages (including plant macrofossils), and concluded that the core taken from the deepest part of a small lake contains the most representative record. Given the small size, the round shape and the steep slopes of Prášilské jezero, we consider the central core (Pra-15) taken from the deepest part of the lake to best represent changes in lake *Isoëtes* populations. Furthermore, we consider microspore over megaspore data as a better proxy for the quantitative reconstruction of population dynamics because larger plant macrofossils (megaspores) tend to be unevenly dispersed across all parts of the lake (Zhao et al., 2006). Whereas, microspores are abundant in deep water and disperse well into the centre of the lake (Edwards et al., 2000).

12.1 *Isoëtes* establishment in Prášilské jezero

Isoëtes established in Prášilské jezero around 10,300 cal yr BP (Fig. 2) as a result of catchment afforestation which induced soil stabilization (Fig. 4). Prior to 10,300 cal yr BP, *Isoëtes* establishment was likely not possible due to frequent erosional events inducing turbulent conditions within the lake. Aquatic proxies, namely *Aulacoseira pfaffiana*, which is a heavily silicified diatom (Fig. 3), indicate intense water mixing as well as low light conditions and higher silica availability (Kilham et al., 1996; Leira et al., 2015). High chironomid abundance, particularly the presence of profundal taxa sensitive to oxygen depletion (Fig. 3), also illustrates intense water column mixing. Turbulent conditions in Prášilské jezero were the result of a relatively open catchment with high abundances of early-successional trees, such as *Pinus* and *Betula* (Fig. 4). High sediment accumulation rates (Fig. 2d) indicate that soils in the catchment were poorly developed and less stabilized, which led to increased clastic input from weathered bedrock into the lake, as indicated by peaks in rubidium (Fig. S1).

Erosion and the input of clastic materials (Fig. S1) both decreased as a result of forest expansion ~10,300 cal yr BP (see NAP:AP, Fig. 4). Subsequently, reduced wave action and improved light conditions in Prášilské jezero may have facilitated *Isoëtes* establishment at this time. This is evidenced by the continuous presence and increase of both microspores and megaspores (Fig. 2), and decline in *Aulacoseira* diatoms (Passy, 2007) (Fig. 3). However, there might be other environmental factors affecting *Isoëtes* establishment at Prášilské jezero. Our data show an interesting pattern of *Isoëtes* expansion accompanied by a decline in *Pediastrum* at Prášilské jezero during the Early Holocene (Fig. 3). This trend is also recorded at other lacustrine deposits across Europe, i.e. Kråkenes, Norway (Birks et al., 2000), Jezioro, Poland (Fajer et al., 2012), Rotmeer, Germany (Lotter and Höltzer, 1994), Racou mire, Spain (Guiter et al., 2005) and Pian di Gembro, Italy (Pini, 2002). At Kråkenes Lake, *Pediastrum* declined synchronously with an increase in dissolved humic content and the accumulation of organic lake sediments (i.e.more gyttja), which was likely a result of the development of a birch woodland in the catchment (Birks et al., 2000). While *Pediastrum* was not identified to the species level at Prášilské jezero, our results do show a similar increase in dissolved humic content before 10,300 cal yr BP, as evidenced by the establishment of diatoms typical for dystrophic waters (e.g. *Brachysira brebissonii*, *Eunotia incisa*, *Frustulia krammeri*, Fig. S2) (Lange-Bertalot et al., 2011; Cantonati et al., 2017) and also by the presence of acid-tolerant caddisfly fauna (Vondrák et al., 2019). Thus, it is also possible that the pattern of *Isoëtes* establishment and *Pediastrum* decline after the forest expanded at Prášilské jezero ~10,300 cal yr BP reflects the accumulation of CO_2-rich organic lake sediments required for *Isoëtes* nutrition (Rørslett and Brettum, 1989).

12.2 Vegetation change affects lake water quality of Prášilské jezero causing *Isoëtes* to either decline or collapse

Isoëtes populations declined at ~9300 and again at ~6400 cal yr BP, and eventually collapsed at ~4300 cal yr BP in response to gradual lake dystrophication induced by changes in vegetation cover. Specifically, the immigration of late-successional tree taxa, namely *Picea abies*, and later *Fagus sylvatica* and *Abies alba* and *Sphagnum* expansion, suggest that the Prášilské jezero catchment progressively podzolized with increasing forest canopy cover. This caused the gradual dystrophication and oxygen depletion of the lake as evidenced by an increase in strongly acidophilous diatom taxa and decrease in chironomid abundance.

The first *Isoëtes* decline ~9300 cal yr BP corresponds with the Early Holocene rise of *Picea abies* and *Alnus*, which likely triggered catchment soil podzolization (Fig. 4). Modern studies demonstrate that *Picea abies* produces considerable amounts of hard-to-decompose needle litter, exerting a strong acidifying effect on the soil (Augusto et al., 2002; Błońska et al., 2016). *Alnus* might magnify this effect due to its ability to symbiotically fix atmospheric nitrogen which enhances soil acidification rates through the formation of organic acids, proton production, and leaching of cations (Van Miegroet and Cole, 1984). Subsequently, the expansion of *Sphagnum* ~8500 cal yr BP may have caused additional changes in soil chemistry within the Prášilské jezero catchment (Fig. 2; 4); a known process in Finnish boreal lakes where the proportion of peatland (i.e. *Sphagnum*) within a lake catchment and resulting input of organic carbon affects lake water properties (Kortelainen, 1993; Seppä and Weckström, 1999). While our results may suggest the beginning of soil podzolization in the catchment, our results do not indicate any abrupt shift in water quality at Prášilské jezero ~9300 cal yr BP. Nevertheless, aquatic proxies indicate the gradual lake dystrophication (Fig. 3). This might be related with the amount of time needed for podzol to form, which typically requires 1000–6000 years (Mokma et al 2004; Starr and Lindroos 2006). Additionally, high fire activity during the early Holocene may have mitigated soil podzolization (see the following discussion section). Regardless of not having enough time encompass between the expansion and *Picea abies*, *Alnus* and *Sphagnum* and soil podzolization, our results illustrate *Isoëtes* populations were negatively impacted by the arrival of these taxa. The first *Isoëtes* decline may be further related to the 9.3 ka cal BP event as some studies suggest an increase in water-level within mid-European lakes (Magny et al. 2004) which would lead to reduction of suitable habitat for *Isoëtes*. However, our results do not illustrate any change in water-levels.

Between the first and second *Isoëtes* decline ~8000 cal yr BP, the gradual dystrophication trend was briefly interrupted as *Isoëtes* briefly recovered. The recovery is likely connected with 8.2 ka event, characteristic by change in atmospheric circulation, which brought more precipitation to mid-latitudes in Europe (Magny et al. 2003). An increase in chironomid abundance, a decrease in C:D ratio (Fig. 3) and an increase in the sediment accumulation rate (Fig. 2d) suggest increased water mixing, which would have benefited *Isoëtes*. Higher precipitation reconstructed for 8.2 ka event (Magny et al. 2003) may have been the cause for increased water mixing at Prášilské jezero.

The second *Isoëtes* decline at Prášilské jezero occurred ~6400 cal yr BP as a result of lake dystrophication, leading to water browning and anoxia. The increase in moderate-to-strongly acidophilous benthic diatoms ~6400 cal yr BP in Prášilské jezero denote dystrophic conditions with high concentrations of organic acids moreover supported by the presence of *Brachysira brebissonii*, an indicator of humic waters (Cantonati et al., 2017). Additionally, the rise in dominance of *Aulacoseira alpigena* further illustrates dystrophic waters with low conductivity (Falasco and Bona, 2011; Houk, 2003). High concentrations of organic acids, particularly dissolved organic carbon (DOC), commonly cause water browning which attenuate the availability of photosynthetically active radiation within a lake (Williamson et al. 2015). Bociag and Szmeja (2004) compared the populations of submerged isoetids between oligohumic and polyhumic lakes in Poland and found that individuals growing in polyhumic lakes produce fewer microspores and megaspores. Based on these results, our multi-proxy reconstruction of Prášilské jezero, a dystrophic lake, illustrates decreasing water transparency was the main driver of *Isoëtes* decline ~6400 cal yr BP. Similarly, Mariani et al. (2018) conclude that poor light penetration in Dove Lake, Tasmania, a highly dystrophic lake, resulted in *Isoëtes* decline. Water browning is commonly associated with thermal stratification (Williamson et al. 2015) which is also recorded in Prášilské jezero. Thermal stratification of Prášilské jezero is indicated by the rising proportion of chrysophycean stomatocysts (C:D ratio) and decreasing abundances of non-motile phytoplankton (planktonic diatoms, *Botryococcus*) (Fig. 3; P 3). Chrysophytes (contrary to non-motile phytoplankton) can migrate for light or nutrients through the water-column (Werner and Smol, 2005), and escape from surface waters high in UV light (Vincent and Pienitz, 1996). Additionally, lake stratification often induces oxygen depletion near the lake bottom, which in turn negatively affects macrozoobenthos communities, thereby decreasing species diversity and abundance (i.e. Klimaszyk and Heymann, 2011). The decline in total chironomid abundance, especially in taxa that inhabit well-oxygenated profundal habitats, as well as decreases in cladocerans (*Daphnia longispina*), bryozoans (*Plumatella*) (Fig. 3), and caddisflies (Vondrák et al., 2019) in Prášilské jezero around 6400 cal yr BP suggest low oxygen concentrations. Gacia and Ballestros (1996) studied the effect of dam construction on *I. lacustris* populations in Lake Baciver, a high Pyrenean mountain lake, and concluded that total bottom anoxia caused the demise of *I. lacustris* populations. Thus, it is plausible that reduced water transparency coupled with low oxygen concentrations in Prášilské jezero around 6400 cal yr BP negatively affected *Isoëtes* populations and caused their decline. These environmental processes related to Prášilské jezero dystrophication were linked with the expansion of *Fagus sylvatica*, which resulted in both a decrease in fire activity, as well as a closing of the forest canopy in the Prášilské jezero catchment (Fig. 4). This is likely due to denser forests altering microclimatic conditions and promoting a higher accumulation of organic matter on the forest floor, thus increasing the production of organic acids and input of dissolved organic carbon (DOC) into the lake (Meyer-Jacob et al., 2015).

Isoëtes collapsed in Prášilské jezero at ~4300 cal yr BP (Fig. 2; Population collapse) as a result of the final stages of dystrophication and a decrease in water transparency. This is documented by a rapid bloom of *Asterionella ralfsii* (Fig. 3, S2; Population collapse), an euplanktonic diatom that prefers acidic and dystrophic waters with high concentrations of DOC (Stabell, 1993; Gensemer et al., 1993). The increase of *A. ralfsii* abundances dramatically reduced the C:D ratio while chrysophyte abundances did not respond rapidly (see Fig. S4). Therefore, in this case, we assign the C:D ratio decrease to water browning connected to dystrophication rather than changes in mixing regime. Additionally, continuing thermal stratification is supported by the extremely low abundance of profundal oxygen-demanding chironomids. Reconstructed terrestrial vegetation dynamics show that *Isoëtes* collapse and the final stage of Prášilské jezero dystrophication were linked with the expansion of *Abies alba* (Fig. 4; Population collapse).

12.3 The effect of local fire activity on *Isoëtes* dynamics in Prášilské jezero

High fire activity during the Early Holocene likely positively influenced *Isoëtes* in Prášilské jezero. Specifically, we found that the second major decline in *Isoëtes* ~6400 cal yr BP coincided with a decrease in local fire activity (Fig. 4, P 3). A study from southern Switzerland found a significant positive correlation between aquatic macrophytes and biomass burning, which likely induced lake eutrophication by in-washing ash and soil during and after fires (Tinner et al., 1999). However, our diatom record does not suggest nutrient levels increased during the Early Holocene (Fig. 3, S2) when fire activity was high in the Prášilské jezero catchment (Fig. 4). Rather, we suggest that the high fire activity during the Early Holocene mitigated podzolization and lake dystrophication at Prášilské jezero by reducing forest floor organic matter and subsequent outflow of organic acids, and/or by inducing soil surface dryness. Several palaeoecological studies document a general slow-down of soil podzolization related to long-term and frequent fire activity (Schaffhauser et al., 2016; Bobek et al., 2018). Similarly, palaeolimnological research demonstrates fires' ability to neutralize both the chemical and physical properties of lake water (Korhola et al., 1996; Rosén and Hammerlund, 2007). Our results visually show a positive relationship between high fire activity and *Isoëtes* abundance (Fig. 2 and 4), which differs from the findings at Grizzly Lake, Alaska, where a significant negative relationship between *Isoëtes* microspores and both microscopic and macroscopic charcoal were found (Tinner et al., 2006; 2015). This discrepancy in *Isoëtes* behaviour at the two sites is difficult to explain because both microscopic and macroscopic charcoal results indicate increased local to regional fire activity (mainly fire frequency; Tinner et al., 1998; Adolf et al., 2018). However, the *Isoëtes* decline at Grizzly Lake, Alaska, was most likely driven by low (diatom-inferred) lake levels as a result of drier climatic conditions rather than fire activity and its effect on lake water properties (Tinner et al., 2006).

12.4 Spatial-temporal patterns in *Isoëtes* dynamics across neighbouring Central European lakes

The *Isoëtes* microspore records from Prášilské jezero, Černé jezero (Carter et al., 2018a), Rachelsee (van der Knaap et al., 2019) and Plešné jezero (Jankovská, 2006), show concurrent patterns of population dynamics throughout the Bohemian Forest Ecosystem (Fig. 5). We suggest that climatic controls in this region were partially driving these common trends but as evidenced by other studies from Central Europe, local-scale vegetation changes and geographical setting significantly influenced *Isoëtes* population dynamics by altering lake water chemistry.

The widespread *Isoëtes* decline after ~6500 cal yr BP in the Bohemian Forest Ecosystem lakes corresponds with regional climate change (Brooks, 2012) which induced cooler temperatures and brought higher precipitation in the Bohemian Forest Ecosystem (Carter et al., 2018b), as well as across Europe (Mauri et al., 2015). Modern observations document a positive relationship between the amount of precipitation and lake watercolour due to increased leaching of organic acids from the upper forest soils and a slow-down of DOC photo-degradation in lakes (Schindler et al., 1997; Hongve et al., 2004). Thus, it is plausible that this climatic transition ~6500 cal yr BP accelerated water dystrophication in the Bohemian Forest lakes.

However, our results are not unique in Central Europe. Reconstructed *Isoëtes* dynamics in the Black Forest, a mountain range in Southern Germany, showed a similar pattern to that in the Bohemian Forest lakes, though the regional decline started later around ~5500 cal yr BP (Rösch, 2009; 2015). The later decline in the Black Forest might be due to lower altitudes of the lakes (ranging from 830–910 m a.s.l.) compared to the Bohemian Forest lakes (Table 1; Fig. 5) and related differences in vegetation histories. Specifically, in the Bohemian Forest, *Picea abies* has dominated since ~9,000 cal yr BP (Fig. 5; Carter et al., 2018a; van der Knaap et al., 2019), while in the Black Forest, broad-leaved species such as *Quercus* and *Corylus*, with more readily decomposable and nutrient-rich litter, dominated up to ~5500 cal yr BP when *Abies alba* established and *Isoëtes* declined as a consequence of lake dystrophication (Rösch, 2015). Therefore, it is likely that podzolization of forest soils in the

Black Forest started later due to different dominant vegetation, which might also explain the delayed lake dystrophication, and *Isoëtes* decline, respectively.

Along with lake dystrophication, *Isoëtes* decline might have been caused by lake eutrophication as shown by both recent phytocenological surveys and palaeoecological studies from European lowland lakes (Kowalewski et al., 2013; Engels et al., 2017, Klimaszyk et al., 2020). In highly eutrophic lakes, *Isoëtes* individuals suffer from interspecific competition. If nutrient levels increase, faster-growing communities of planktonic algae and/or floating-leaved and helophytic macrophytes overgrow the slow-growing *Isoëtes* species (Arts, 2002; Smolders et al., 2002). Except for one *Sparganium angustifolium/natans* seed found in one sample from the littoral core (Pra-16-lit7) of Prášilské jezero dated to 4700 cal yr BP (unpublished data), we did not record any other macrofossils suggesting the local presence of other aquatic macrophytes in Prášilské jezero. Additionally, the diatom-based reconstruction does not indicate any increase in nutrient concentrations during the *Isoëtes* decline in Prášilské jezero. Thus, our results show that neither lake eutrophication nor interspecific competition affected *Isoëtes* populations in Prášilské jezero.

12.5 Coexistence of both *Isoëtes* species (*I. echinospora* and *I. lacustris*), and their extinction at Prášilské jezero

We recorded both *I. echinospora* and *I. lacustris* in Prášilské jezero; *I. echinospora* appeared ~9500 cal yr BP, *I. lacustris* established 500 years later (Fig. 2). Based on previous palaeoecological studies, *I. echinospora* is considered an Early Holocene colonizer of lakes in Greenland (Fredskild, 1992), Norway (Birks, 2000), and Southern Germany (Rösch, 2015). Our results confirm *I. echinospora* as an early colonizer at Prášilské jezero. However, these results differ from those from Kråkenes Lake, which document the extinction of *I. echinospora* following the establishment of *I. lacustris* (Birks, 2000). Our megaspore record evidences that both *Isoëtes* species coexisted at Prášilské jezero for at least 2300 years (Fig. 2).

After ~2,000 cal yr BP, there were several points in time when neither *Isoëtes* microspores nor megaspores were recorded, suggesting the near extinction of *Isoëtes* populations (Fig. 2). The maximum life span of modern *Isoëtes* individuals is unclear, but field observations suggest that they can live more than 50 years (Čtvrtlíková et al., 2014; 2016). Thus, the ‘near-extinction’ might have been the result of unfavourable conditions such as changes in lake water level which would have affected the population’s depth range, or a decrease in light availability which may have slowed down plant growth and/or suppressed spore production (Gacia and Ballesteros, 1996).

After ~1947 AD, we did not find any spores in sediments collected from Prášilské jezero suggesting that *Isoëtes* became locally extinct, which is also confirmed by our recent diving expedition in 2019. We hypothesize, based on our results on watercolour and transparency, macro-algae depth range and experience from many clear-water lakes in Europe, that both *I. echinospora* and *I. lacustris* could currently only inhabit depths between 0.5 and 1.0 m, which are out of wave or ice action risks and provide suitable light conditions. Such a narrow depth range in the upper littoral zone might be unsuitable for any rooted aquatic plant and probably prevented the survival of the *Isoëtes* in Prášilské jezero at present.

13 Conclusion

Our multi-proxy palaeoecological study demonstrates the sensitivity of *Isoëtes* species to changes in lake water quality induced by long-term terrestrial vegetation succession, soil development and disturbance dynamics. *Isoëtes* populations established at Prášilské jezero in the Bohemian Forest Ecosystem ~10,300 cal yr BP in response to afforestation inducing soil stabilization. By ~9300 cal yr BP, *Picea abies*-dominated forests with an admixture of *Alnus*, coupled with peatland expansion triggered gradual catchment podzolization. This vegetation change caused the lake to become dystrophic; however, dystrophication was likely buffered and/or attenuated by frequent fire activity during the Early Holocene. When *Fagus sylvatica* and *Abies alba* expanded and fire activity decreased during the Mid-Holocene (~6400–4300 cal yr BP), podzolization processes accelerated, dystrophication increased, and *Isoëtes* declined as a result of insufficient light availability and depleted oxygen levels at the lake bottom, as indicated by dominance of *Asterionella ralfsii* diatom species and decrease in chironomid abundance. Similar patterns of *Isoëtes* decline were documented from other glacial lakes in the Bohemian Forest Ecosystem, suggesting common climatic factors driving their population dynamics in this mountain region.

14 Acknowledgements

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15 Data Availability Statement

Pollen, plant macrofossils and macro-charcoal data supporting the results are archived in Neotoma database:

http://apps.neotomadb.org/Explorer/?datasetid=47518;
http://apps.neotomadb.org/Explorer/?datasetid=47519;
http://apps.neotomadb.org/Explorer/?datasetid=47520.

Diatom and chironomid data are available on request from the corresponding author. These data are not publicly available now because they are going to be presented as a part of a PhD project of AT. After the PhD thesis defence, these data will be publicly available.

Chapter 4:

Surface water brownification altered ecosystem functioning of a mountain lake for millennia – paleolimnological evidence from Central Europe

Abstract

Water brownification has long altered freshwater ecosystems. Recent surface water brownification was preceded by long-lasting browning effect of humic substances in many catchments. To disentangle a cascade of browning-induced environmental stressors, multi-proxy paleolimnological studies can contribute substantially. Here we present a millennial-scaled multi-proxy record from a small forest mountain lake in the Bohemian Forest, Czechia. A long core retrieved from the central part of the lake was dated using ^{14}C and ^{210}Pb, subsampled and analyzed for diatoms and zoological indicator (chironomids, planktonic cladocerans) remains. X-ray fluorescence (XRF) provided a record of elements sensitive to biogeochemical processes connected to browning and catchment development (P, Ti, Al/Rb, Fe/Ti, Mn/Ti, Si/Ti).Three detected thresholds in diatom and chironomid successions ~10.7, ~5.5 and ~4.2 cal. ky BP were likely driven by processes closely related to water browning. Whereas afforestation of the catchment ~10.7 cal. ky BP sustained thermal stratification of water column, soil saturation and spread of peatlands since ~6.8 cal. ky BP resulted in unprecedented shading and possibly nutrient limitation of the lake ecosystem. Intensive in-wash of dissolved organic matter was probably reduced after ~4.2 cal. ky BP, when paludified soils became permanently anoxic. Two temporary negative and positive anomalies of browning progress were caused by the "8.2 ka event" and the "4.2 ka event", respectively. The key role of peatlands presence in the catchment was manifested in millennial-scaled browning process, which agrees with spatial trends observed in scope of recent browning monitoring. The profound climatic driving of long-lasting browning was suggested by coincidence with moistening of climate across the northern hemisphere after ~6 cal. ky BP. Further comparison with long-term trends could help with reliable assessment of range and magnitude of recent progressive brownification of surface waters.

16 Introduction

Water brownification, i.e. increased brown coloration, stems mainly from heightened terrestrial input of humic substances measured as dissolved organic carbon (DOC) (Roulet & Moore, 2006; but see Kritzberg & Ekström, 2012). Brownification has gripped an attention of the scientific community, as fresh waters across the Northern Hemisphere have been getting browner for the last 30 years (Kritzberg, 2017; Meyer-Jacob et al., 2019). Proposed driving factors of higher DOC levels involve recent climate change (Fee et al., 1996; Creed et al., 2018), land-use (Mattsson et al., 2009; Meyer-Jacob et al., 2015; Sankar et al., 2020) or recovery from anthropogenic acid deposition from the atmosphere (Monteith et al., 2007; Hruška et al., 2009). Although recovery from acid deposition cannot explain the hemispherical extent of recent surface water brownification (Clark et al., 2010; Kritzberg, 2017), the recovery-based hypothesis builds on the important point that many freshwater bodies were probably brown naturally before the period of anthropogenic acidification. Only lacustrine sedimentary records offer the potential to study the progress of water brownification over these millennial scales. Such long-term records are needed to disentangle the differing mechanisms attributed to brownification, as recovery from anthropogenic acidification can bias correlation between spatial and decadal-scaled studies (Gavin et al., 2018; Stetler et al., 2021).

In unproductive (oligotrophic) lakes, the heightened concentration of DOC triggers a series of interconnected environmental shifts with direct impact such as (i) lower pH caused by the dominant proportion of humic acids in DOC (Thurman, 1985) and (ii) effective light attenuation (Vincent & Pienitz, 1996; Wetzel, 2001), resulting into indirect changes in e.g. (iii) water column thermal stratification by enhanced heating of surface layer (Fee et al., 1996; Snucins & Gunn, 2000), (iv) anoxic bottom conditions sustained by thermal stratification limiting oxygen replenishment (Nürnberg & Shaw, 1999; Brothers et al., 2014) and (v) altered nutrient availability (Nürnberg & Shaw, 1999; Sanders et al., 2015; Corman et al., 2018). Through this complex of stressors, water brownification can exhibit a severe impact on aquatic organisms (Karlsson et al., 2009; Solomon et al., 2015; Vasconcelos et al., 2016), which can further strengthen the effect of brownification by biotic processes (Brothers et al., 2014). On the other hand, promotion of growth of aquatic biota by allochthonous input of humic compounds was documented (Pienitz & Vincent, 2000; Kissman et al., 2013; Daggett et al., 2015). A hypothesis of a unimodal relationship between primary production and DOC compiles the above-mentioned contradicting observations to depict the responses to brownification as a trade-off between increasing input of nutrients from dissolved organic matter (DOM) and decreasing light availability (Jones, 1992; Kelly et al., 2018). The degree of fertilizing ability of DOM varies with differences in stoichiometry of nutrients contained in the organic matter (Tipping et al., 2016) or change in co-export mechanism of DOM and soil-bounded nutrients during leaching (Kopáček et al., 2011). Soil capacity to DOM-bounding alters substantially with redox changes on the gradient of its saturation by water (Olivie-

Lauquet et al., 2001; Possinger et al., 2020). A positive correlation was observed between concentrations of DOC in fresh waters and proportion of wetlands in catchments (Kortelainen, 1993; Laudon et al., 2011; Rantala et al., 2016) or rainfall (Brothers et al., 2014; Ejarque et al., 2018).

The paired impacts of precipitation dynamics and long-term soil development, e.g., podzolization and paludification, could be traced on the millennial scale and elucidate mechanisms of natural brownification. In the Post-glacial, vegetational succession mediated the effects of climate on brownification by providing sources of organic matter and interacting with soil biogeochemistry and hydrology (Korsman et al., 1994; Huvane & Whitehead, 1996; Pienitz et al., 1999; Engstrom et al., 2000). Soil formation and subsequent hardening of soil horizons during podzolization made dissolved organic matter (DOM) available for a transport into lakes (Steinberg, 1991; Engstrom et al., 2000). Humic acids could play an important role in acidification on deglaciated base-poor bedrocks (Ford, 1990; Ampel et al., 2015), despite depletion of base cations acted prominently in initial phases of glacial lake ontogeny (Boyle, 2007; Boyle et al., 2013). Steps in the progress of Holocene-scale brownification often occurred in phase with pulses of climate humidity (Brodin, 1986). Whereas the cold and dry Younger Dryas stadial (~12.9–11.7 ky BP) interrupted water brownification in some lakes due to temperatures limiting vegetation growth and frozen soil water (Norton et al., 2011; Ampel et al., 2015), lack of leaching water during the dry Mid-Holocene "climatic optimum" (~8–5 cal. ky BP) probably reduced brownification for some catchments (Huvane & Whitehead, 1996; Itkonen et al., 1999).

Conversely, a regional shift to a wetter climate after ~6 cal. ky BP, known as Mid-Holocene Climate Transition (Magny et al., 2006; Wanner et al., 2008), was able to accelerate brownification in many boreal catchments (Pienitz et al., 1999; Solovieva & Jones, 2002; Myrstener et al., 2021).This pronounced climatic threshold was connected with the inception and expansion of peatlands, i.e. paludification (Bauer et al., 2003; Le Stum-Boivin et al., 2019; Myrstener et al., 2021), which further supplied headwaters with DOC (Belyea & Malmer, 2004). The extent to which the formation of impermeable soil horizons (podzolization) conditioned this regional paludification remains in some doubt (Payette et al., 2012; Schaffhauser et al., 2017). Similarly, uncertainties accompany understanding of the progress of brownification outside the well-studied boreal regions of northern Europe and the North America in the Holocene. In particular, new paleolimnological records from mid-latitude Europe could alter the picture of Holocene brownification, given both the differences in the Holocene trajectory of climate humidity compared to northern Europe (Mauri et al., 2015; Florescu et al., 2019) and the acidic base-line of catchments prior to anthropogenic acidification (Jüttner et al., 1997; Sienkiewicz, 2016). Unfortunately, studies from Central Europe tracking specifically the progress of brownification in mountain lakes through the Holocene are rare (e.g., Steinberg, 1991).

Past DOC levels can be reconstructed by diatom-based transfer functions (Pienitz & Vincent, 2000) or by total organic carbon concentration in sediments (Meyer-Jacob et al., 2017; Russell et al., 2019). However, an increase of humic content in boreal lakes during the Holocene was most often detected indirectly in paleorecords as a decrease in ecosystem productivity or water pH, hence the terms "dystrophication" and "natural acidification" remain rooted in paleolimnological studies. The various scale and complexity of feedback mechanisms during brownification invite the application of a multi-proxy approach (Birks & Birks, 2006) to disentangle particular driving factors behind and impacts on aquatic biota during millennial-scaled brownification. Paleolimnological studies can employ numerous complementary geochemical and biological indicators that can disentangle their sensitive responses to the multiple impacts of brownification, including:

i) acidification by humic acids can be detected using changes in diatom communities (Curtis et al., 2009; Battarbee et al., 2010; Birks & Simpson, 2013),
ii) shading of the water column unbalances primary production of planktonic and benthic communities (Karlsson et al., 2009),
iii) thermal stratification imprints in change of ratio between chrysophycean cysts and diatom valves – C:D ratio (Werner & Smol, 2005; Rivera-Rondón & Catalan, 2017),
iv) anoxic bottom water conditions affects chironomid indicators and concentrations of their remains (e.g., Quinlan & Smol, 2001; Ursenbacher et al., 2020) and can be reflected in redox-sensitive element ratios such as Mn/Fe, Mn/Ti and Fe/Ti (Davison, 1993; Kylander et al., 2013; Makri et al., 2021),
v) altered nutrient availability affects the taxonomic composition of both diatom (Hall & Smol, 2010; Rivera-Rondón & Catalan, 2020) and chironomid communities (Lindegaard, 1995; Brodersen & Quinlan, 2006).

We assess the evidence and explore the range of causal factors for lake water brownification using the sediments of a small mountain lake (Prášilské Lake) located in the Bohemian Forest of Central Europe. This type of lake with poorly-buffered soils and boreal-type vegetation hold the catchment properties constant providing the opportunity to compare brownification in Central Europe with that in the more widely studied boreal region. The data generated facilitate inferring trends in pH using diatom-based transfer functions, estimating the intensity of light limitation of algal life-forms in the water by separate assessment counting of periphytic and euplanktonic diatoms, using chironomid assemblage composition and trends redox-sensitive elements (e.g., Mn/Ti and Fe/Ti) to reveal phases of anoxia, and exploring the nutrient dynamics during brownification episodes using changes in diatom-based transfer functions (e.g., total phosphorus) and sediment geochemistry (e.g., P, Si/Ti). Specifically, we: (i) track the onset and extent of brownification in what appears to be a naturally humic and small catchment lake, (ii) reconstruct the impacts of brownification on the lacustrine ecosystem using remains of aquatic organisms (i.e., diatoms and chironomids) and sediment geochemistry, and (iii) explore the main factors causing brownification during the Holocene and contrast the ecosystem functioning of this lake in Central Europe with those in the wider boreal regions.

17 Material and methods

17.1 Study site

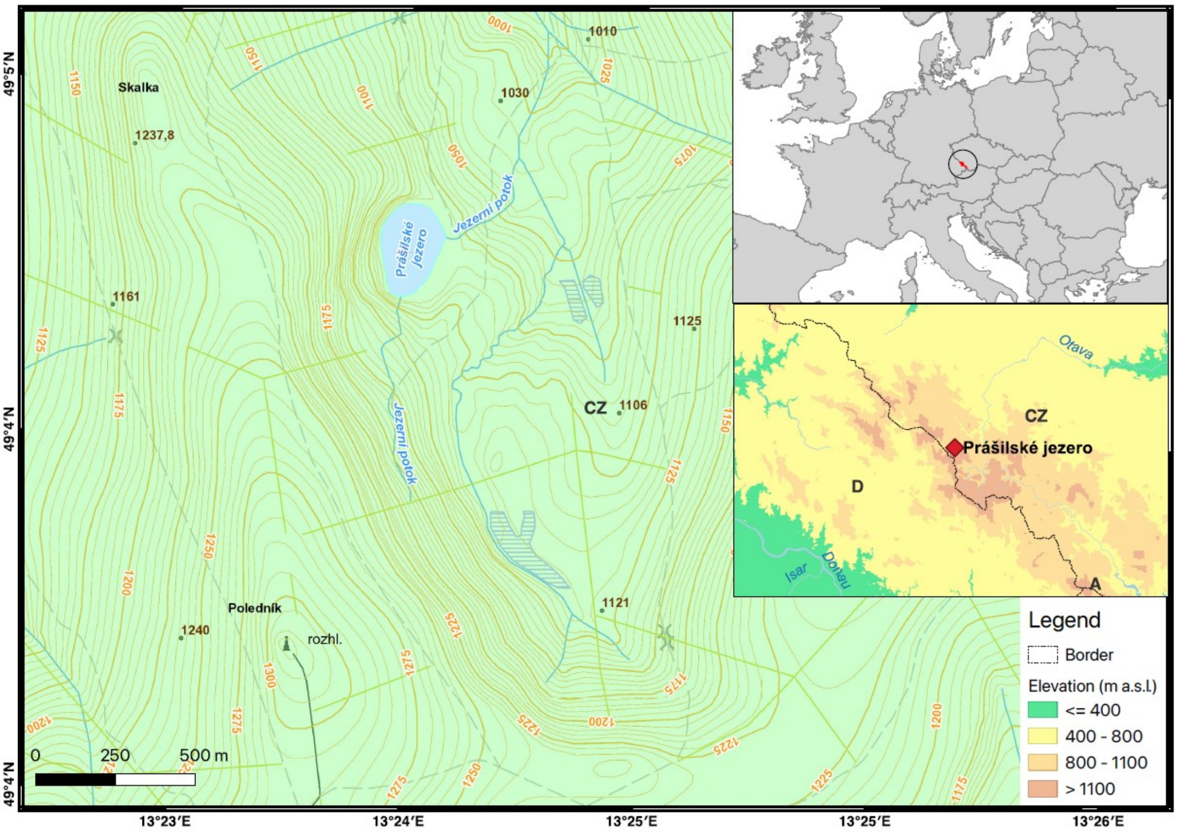

Fig. 1: Map of the Prášilské jezero catchment and the location of Prášilské jezero, the study site, (red diamond) in the Bohemian Forest mountain range.

Eight glacial lakes are situated along the Czechia-Germany-Austria border in the Bohemian Forest (Central Europe) and analyses of their sediments document the last phases of the deglaciation in this low mountain range (Mentlík et al., 2013; Vondrák et al., 2019a; Vondrák et al., 2021) (Fig. 1). Shallow soils, siliceous bedrock (gneiss, mica-schist, granite, quartzite) and presence of Norway spruce (*Picea abies*) as the dominant tree taxon, have enhanced the sensitivity of lake watersto acidifying effects, thereby both pre-industrial acidification by humic substances (Steinberg et al., 1991; Pražáková et al., 2006; Vondrák et al., 2019b; Moravcová et al., 2021) and anthropogenic acidification by mineral acids (Steinberg et al., 1988; Schmidt et al., 1993; Vesely et al., 1993; Fott et al., 1994; Vrba et al., 2003) were documented in the Bohemian Forest lakes. All lakes in the Bohemian Forest were reported as brown-water prior to anthropogenic acidification (see Vrba et al., 2000 for review), thereupon atmospheric acid deposition (1960's–1990's) increased their water-transparency by in-lake precipitation of humic substances (Steinberg, 1991). During a recent recovery from anthropogenic acidification (Nedbalová et al., 2006; Vrba et al., 2003a, 2016), water color has become darker in most of the lakes in the area (Vrba et al., 2000).

Prášilské Lake (49°05´N, 13°24´E; Fig. 1), studied here, is the smallest dimictic lake in the region (Vrba et al., 2003b; Šobr & Janský, 2016) with relatively higher DOC and TP concentrations displayed among the other Bohemian Forest's lakes (Tab 1; see Vrba et al., 2000). Six small inlets and one outlet drain the semicircular cirque that contains Prášilské Lake. Owing to moderate anthropogenic acidification of the lake leading to low concentrations of toxic aluminium, crustacean zooplankton survived there (Fott et al., 1994) and Prášilské Lake´s biota has undergone an earlier recovery from acidification compared to larger lakes (Vrba et al., 2006, 2016). Prášilské Lake is considered as naturally fishless due to absence of any historical observation of a fish population and steepness of the outlet prohibiting immigration (Veselý, 1994; Kubečka et al., 2000).

Tab. 1: Parameters of Prášilské Lake. ANC – acid neutralizing capacity, TP – total phosphorus, DOC – dissolved organic carbon, Al_T – total reactive aluminium. Parameters after Soldán et al. (2012) and Šobr & Janský (2016), chemical variables measured in September 2007.

Altitude	1079	m a.s.l.
Surface lake area	0.042	km^2
Max. depth	17.2	m
Lake volume	0.35	10^6 m^3
Catchment area	0.65	km^2
Lake retention time*	0.54	years
pH	4.96	
ANC	-10	mmol l^{-1}
TP	5.8	µg l^{-1}
DOC	5.5	mg l^{-1}
Al_T	167	µg l^{-1}

*rough estimate of lake retention time given by ratio of lake volume/catchment area [m^3 m^{-2}] (Vrba et al., 2000)

17.2 Sampling and chronology

The profile retrieved from the central part of Prášilské Lake consisted of three parallel drives Pra-15-2-1, Pra-15-1-2 and Pra-15-2-2 taken by a Russian-style corer, and one drive Pra-15-GC2 taken by a gravity corer during a fieldwork in August 2015. All cores were taken from a stable floating platform and comprise overlapped 1.5 × 0.07 m core lengths (Russian corer) and the intact sediment water interface sampled by a gravity corer (Boyle, 1995). All drives were correlated using matching of core scan data collected using the Liverpool Geotek Multi-sensor Core Logger (MSCL), and an age-depth model generated for the sediment record using ^{14}C and ^{210}Pb age measurements (for details on dating see Carter et al. (2018a) and age-depth model in Fig. A.1).

17.3 Geochemistry

For correlation of the cores and interpretation of sediment characteristics, all long-cores cores were photographed at 15 µmpixel resolution using the Line-scan camera fitted to the Liverpool GeotekMulti-sensor Core Logger (MSCL), and then they were covered with 6µm thickness polypropylene film and measured at 5mm intervals on a wet sediment basis using an Olympus Delta energy dispersive µXRF mounted on the Liverpool Geotek MSCL (Boyle et al., 2015; Schillereff et al., 2015). For the gravity core major and trace element concentrations were determined using a Bruker S2Ranger ED-XRF, with the samples were hand pressed and measured under a He atmosphere under combined Pd and Co excitation radiation and using a high resolution, low spectral interference silicon drift detector. Both XRFs undergo daily standardization and regular laboratory consistency checks using 18 certified reference materials (e.g., Boyle et al., 2015). The same device was used for each layer from the gravity core Pra-15-GC2.

17.4 Diatom and chrysophyte analyses

Remains of siliceous algae were isolated from 36 samples at 2–10 cm intervals through the profile using standard procedures (Battarbee et al., 2001). Dry sediment (~0.1 g) was boiled in 30% hydrogen peroxide. After the solution cooled, a drop of 35 % hydrochloric acid was added. Solutions were rinsed with distilled water five times. Precise volume of diluted sample solution (50 µl) and distilled water (550 µl) together with a specific concentration of divinylbenzene microspheres (100 µl, 30.65×10^4 particles/ml) and drop of ammonia were permanently mounted onto cover slides using Naphrax mounting resin. The only exception represents the most basal sample concentration (1698–1698.5 cm) with the 20 µl of sample solution with 580 µl of distilled water.

Using a light microscope, at least 400 diatom valves were counted under 1000× magnification. To retain sufficient resolution of diatom periphyton for further quantitative analyses, euplanktonic dominant *Asterionella ralfsii* was suppressed. In samples where *A. ralfsii* dominated (1567–1500.5 cm), only 100 valves of *A. ralfsii* were counted concurrently to periphytic diatoms, then its counting was stopped and at least additional 400 valves of periphytic diatoms were counted in the sample. We use broadly accepted term "periphyton" (Wetzel, 2001) for any other diatom life-form than euplanktonic following the definition that periphyton includes "all the microscopic algae, bacteria, and fungi on (or associated with) substrata" (Stevenson et al., 1996). Diatom periphyton was classified into guilds designed by Passy (2007) regarding supplementary suggestions and species database in Rimet & Bouchez (2012). We separated four guilds: low-profile, high-profile, motile and facultatively planktonic (= tychoplanktonic).

Chrysophyte to diatom periphyton ratio ($C:D_{per}$ ratio) was estimated by concurrent counting of chrysophytestomatocysts and valves of periphytic diatoms. We use diatom sums without euplanktonic *A. ralfsii* in order to use $C:D_{per}$ ratio as a proxy for pelagic to littoral primary productivity ratio (Rivera-Rondón & Catalan, 2017). $C:D_{per}$ ratio was expressed as a percentage of chrysophyte cysts while total count of periphytic diatom valves represented 100%. Diatom and chrysophyte accumulation rates were estimated using the divinylbenzene microspheres as reference particles.

Diatoms were identified using Krammer and Lange-Bertalot (1986, 1988, 1991a, b), Lange-Bertalot and Metzeltin (1996), Lange-Bertalot (2001), Krammer (2000, 2002, 2003), Houk (2003), Houk and Klee (2007) and Lange-Bertalot et al. (2011, 2017). Diatom nomenclature was updated and unified according to AlgaeBase (Guiry & Guiry, 2021).

17.5 Chironomid and planktonic cladoceran analyses

In total, 123 sediment samples with average wet volume of 3.2 ml were processed. The samples were sieved over 100 μm mesh size using distilled water. Chironomid head capsules (HCs) were picked from a Sedgwick-Rafter counting cell using a stereoscopic microscope at 40–50× magnification, fine forceps, and steel needles, then dehydrated in 90% ethanol, and mounted in Euparal mounting medium to prepare permanent slides. Due to very low average head capsule abundances in the studied material, samples with less than 50 identified HCs were merged with adjacent samples to exceed the minimum count level. This approach allowed plotting of relative abundance data (%) and more robust ecological interpretation of the subfossil assemblages (Heiri & Lotter, 2001). The taxonomic identification of HCs and related ecological preferences of the individual taxa followed mostly Wiederholm (1983) and Brooks et al. (2007). Finally, we distinguished four chironomid ecological groups – (i) taxa of oxygenated profundal, (ii) other bottom substrate taxa, (iii) semi-terrestrial and rheophilic taxa (including taxa associated with seepages, moss, and small streams), and (iv) taxa associated with aquatic macrophytes.

The same samples were also used for the identification of remains of planktonic cladocerans (Daphniidae and Bosminiidae). Resting eggs of genus *Daphnia* and g. *Ceriodaphnia* were concurrently observed and counted during the HCs picking, whereas head shields and shells of g. *Bosmina* were analyzed qualitatively, i.e., *Bosmina* remains very abundant or common (1) versus rare or absent (0). Identification of the selected cladoceran remains followed the atlas by Szeroczyńska & Sarmaja-Korjonen (2007).

17.6 Statistical analyses

Zonation proceeded from three cluster analyses of Hellinger transformed relative abundances of diatom and chironomid taxa. Two types of species data were entered for diatom clustering: (i) whole diatom assemblage and (ii) periphytic assemblage excluding euplanktonic *A. ralfsii*. Constrained Incremental Sums of Squares (CONISS) (Grimm, 1987) (Fig. A.2) tested by Broken-stick model (MacArthur, 1957; Legendre & Legendre, 1998) produced significant assemblage zones using packages “analogue”, “rioja” and “vegan”

(Oksanen et al., 2008; Juggins, 2012; Simpson & Oksanen, 2013) in R 4.1.2 software (R Core Team, 2021).

Diatom-inferred pH and total phosphorus (DI-pH, DI-TP) were calculated using combined training sets and weighted-averaging with inverse (for pH) and classical (for TP) deshrinking available at the European Diatom Database (EDDi) (Juggins, 2001). Relative abundances of periphytic diatom taxa entered the DI-pH and DI-TP reconstructions, excluding the euplanktonic *A. ralfsii* with questionable indicator value (discussed in Section 4.2). The periphytic-based estimations can provide a relevant signal of chemical changes in lakes (Philibert & Prairie, 2002; Werner & Smol, 2005; DeNicola & Kelly, 2014), if we consider their potential bias towards littoral habitats (Pla-Rabés & Catalan, 2018). The combined training sets and two regional training sets for pH (ALPE) and TP (NW Europe dataset) proved sufficient analogy with our periphytic diatom assemblages when the squared chi-squared distance to the closest analogue obtained by MAT (Modern Analogue Technique, five nearest analogues) served as a measure of analogy for the training sets (Juggins, 2001). Finally, the combined training sets for both pH and TP were favoured for the reconstruction, because of absence of *A. ralfsii* in "ALPE" and "NW Europe" regional datasets. Even though we excluded this species from the weighted averaging, we preserved its influence at least in the MAT step. See the comparison of the inferred environmental variables from combined and regional datasets in the Fig. A.3.

Hill's N2 (Hill, 1973) was calculated using C2 software version 1.5 (Juggins, 2007) in order to assess "effective number of taxa" (Jost, 2006) for every total diatom assemblage, diatom non-planktonic assemblage, diatom facultatively planktonic assemblage, and total chironomid assemblage.

18 Results

18.1 Zonation

In total 4 zones were determined based on the clustering of diatom and chironomid relative abundances in assemblages: Zone 1a, Zone 1b, Zone 2, and Zone 3 (see Fig. 2, 3 and A.2). Zones 1, 2 and 3 were supported by significant clustering of diatom assemblages, based on the whole diatom assemblage (Zone 2/Zone 3; depth of 1566.25 cm) and periphytic diatom assemblage (Zone 1/Zone 2; depth of 1585.25 cm). Zone 1 was subdivided based on the first non-significant chironomid splitting accompanied by the second diatom non-significant splitting (Zone 1a/ Zone 1b; depth of 1675.25 cm).

18.2 Zone 1a (11.4–10.7 cal. ky BP)

Diatom and chironomid assemblages were dominated strongly by few taxa characterized by low Hill's N2 (Fig. 2, 3). Facultatively planktonic *Aulacoseira pfaffiana* and *A. alpigena* proliferated considerably (Fig. 2, A.4, A.5), so that the total influx of diatom valve concentrations peaked in this zone (~$16.5*10^9$ valve particles cm^{-2} y^{-1}; Fig. 4) and outnumbered chrysophyte stomatocysts resulting in the lowest $C:D_{per}$ ratio (10.5%) within the record (Fig. 4). Si/Ti ratio reflected this trend of siliceous algae influx with excess biogenic Si relative to lithogenic elements (Fig. 4; Fig. A.7). Chironomid assemblages were dominated by taxa inhabiting bottom substrate in well-oxygenated profundal waters (31–57%; Fig. 3), namely *Heterotrissocladius grimshawi*-type, *Micropsectra insignilobus*/*contracta*-type, and *Tanytarsus lugens*-type (Fig. A.6). Concentrations of chironomid head capsules (HCs) reached values higher by order of magnitude than in the rest of the core, with up to 131 HCs cm^{-3} compared with values 0–38 HCs cm^{-3} in Zones 1b–3 (Fig. 4). Concentrations of *Daphnia longispina*-type ephippia increased during the zone and *Bosmina longispina* remains were abundant (Fig. 3). Average DI-pH values of 5.82 were affected by a low value 5.54 in a sample with maximal *A. pfaffiana* dominance (Fig. 4). This sample showed the very high standard error in estimation of DI-TP, which averaged ~1.5 µg l^{-1} in this zone. Ti concentrations decreased sharply from ~680 to ~260 ppm reflecting a decline in supply of lithogenic elements to the lake (Fig. 4). Trends in Mn/Ti and Fe/Ti broadly corresponded in the first half of the zone reflecting the gradual decline of Mn, Fe and Ti since the Holocene onset, but then diverged as Fe increases in concentration (Fig.4).

18.3 Zone 1b (10.7–5.5 cal. ky BP)

Here, diatom assemblage peaked in the effective number of diatom taxa (Hill's N2 ~22, ~9 cal. ky BP; Fig. 2). Increasing relative abundances of the low-profile guild were disrupted by a bloom of facultatively planktonic diatoms and a dominant *Aulacoseira lirata* between ~8.1 and ~7.6 cal. ky BP (Fig. 2). This event is evidenced increased Ti concentrations and synchronous harmonization of trends in Mn/Ti and Fe/Ti from the previous distinct excess of Fe relative to Ti contrasting Mn/Ti (Fig. 4). Also, concentrations of other biotic remains responded sensitively around 8.1 cal. ky BP, namely an increase in chironomid HCs concentration with a distinct peak of bottom substrate species; especially *Heterotrissocladius marcidus*-type; Fig. 3, 4, A.6) and decrease in the siliceous algae remains (Fig. 4) and *Daphnia longispina*-type ephippia (Fig. 3). Substantial drop in chrysophyte stomatocyst influx reduced the $C:D_{per}$ ratio (Fig. 4). Simultaneous decrease in Si/Ti was preceded by an oscillation to higher values ~8.2 cal. ky BP (Fig. 4). DI-pH shifted from the values averaging 6.04 before the event to slightly higher values averaging 6.16 after the event to the end of the zone (Fig. 4). DI-TP increased up to ~7.5 µg l^{-1} simultaneously with the peak of low-profile diatoms ~9 cal. ky BP, afterwards decreased during the event (~8.1–7.6 cal. ky BP) and then the average DI-TP value reduced to ~3.1 µg l^{-1} until the end of the zone ~7.5–5.8 cal. ky BP (Fig. 4). In the immediate aftermath of the ~8.1–7.6 cal. ky BP event there was a return to the distinct excess of Fe relative to Ti contrasting patterns Mn/Ti driven by elevated Fe concentrations. Phosphorus concentration in sediment exhibited the first non-zero values in this zone with the highest values ~7.5–5.8 cal. ky BP (Fig. 4).

Besides the ~8.1–7.6 cal. ky BP event, chironomid concentrations were very low (2–18 HCs cm^{-3}) and the assemblages were characterized by increased portion of semi-terrestrial and rheophilic taxa (e.g., *Georthocladius luteicornis*-type) and taxa associated with aquatic macrophytes (e.g., *Corynoneura edwardsi*-type) (Fig. 3, A.6). Bottom substrates species were dominated by *Procladius* (5–38% of the total assemblage), a taxon tolerant to oxygen depletion that did not reach such high relative abundances in the other zones (Fig. 3). Trends of Mn/Ti and Fe/Ti harmonized with reduced Fe and elevated Mn concentrations, and Al/Rb and Al/Ti started to fluctuate to their highest values after ~6.8 cal. ky BP (Fig. 4). At the very end of the zone, influx of chrysophyte stomatocysts (~$9.3*10^9$ particles cm^{-2} y^{-1}; Fig. 4) and the effective number of chironomid taxa (~18; Fig. 3) peaked. At the same time, effective number of diatom taxa decreased, mainly because of *A. alpigena* dominance (max. ~46%; Fig. 2). *Bosmina longispina* remains were numerous in all samples of the Zone 1b, but *Ceriodaphnia* ephippia were still absent, except of a one single finding (Fig. 3).

18.4 Zone 2 (5.5–4.2 cal. ky BP)

The decline in planktonic species that characterizes the Zone 2 involves decreasing the relative abundance of facultatively planktonic diatoms (Fig. 2), concentrations of *D. longispina*-type ephippia (Fig. 3) and influxes of chrysophyte stomatocysts (Fig. 4). Euplanktonic diatom *Asterionella ralfsii* and a cladoceran of genus *Ceriodaphnia* became established at low concentrations (Fig. 2, 3). *Bosmina longispina* remains continued to be very abundant (Fig. 3). Effective numbers of chironomid taxa decreased (Fig. 3), whereas the effective number of taxa in the total diatom assemblage increased (Fig. 2), which coincides with lack of dominant planktonic diatoms. Relative abundances of non-planktonic diatom periphyton increased together with the proportion of non-planktonic Hill's N2 as the dominants within assemblages (Fig. 2). The influx of siliceous algae decreased towards the zone 2/3 border, but Si/Ti and C:D_{per} both peaked ~4.2 cal. ky BP on the border (Fig. 4). Chironomid concentrations became almost negligible (max. ~8, average ~3 HC cm^{-3}; Fig. 4) and the assemblages comprised high portions of semi-terrestrial and rheophilic taxa (9–27%, dominated by *G. luteicornis*-type) and taxa associated with aquatic macrophytes (29–35%; dominated by *P. sordidellus/psilopterus*-type) (Fig. A.6). DI-pH showed a distinct decreasing trend (Fig. 4) and DI-TP remained low values averaging ~2.5 µg l^{-1} (Fig. 4). Phosphorus concentration in sediment decreased during the zone interrupted by zero values and shifted permanently to zero values at the end of the zone. Ti concentrations declined to ~100 ppm pattern that is mirrored in other conservative lithogenic elements (e.g., Rb) towards the top of zone 2 except for a distinct peak (~4.7 cal. ky BP; Fig. 4, A.7). The apparent synchrony of Mn/Ti and Fe/Ti was disrupted at the Ti peak ~4.7 cal. ky BP, with excess Fe concentrations relative to Ti contrasting the Mn/Ti ratio (Fig. 4). Al/Rb and Al/Ti ratios continue fluctuating in synchrony, with the peaks reaching their highest values particularly at the zone 2/3 boundary.

18.5 Zone 3 (4.2 cal. ky BP–recent)

A bloom of *Asterionella ralfsii* dominates the diatom relative abundances and minimizes the effective number of diatom taxa taken from the total assemblage (Fig. 2). However, the effective number of diatom taxa from non-planktonic and facultatively planktonic periphytic assemblages has increased from those in Zone 2. Non-planktonic diatom periphyton prevail over facultatively planktonic periphyton throughout the Zone 3, particularly the relative abundances of motile, high-profile, and low-profile guilds that peaked successively through ~1.7–0.2 cal. ky BP (Fig. 2). Relative abundances of semi-terrestrial and rheophilic chironomids peaked (max. of 36.5%; ~3.2 cal. ky BP; mainly *Limnophyes* and *G. luteicornis-type*) followed by a peak of chironomids associated with aquatic macrophytes (max. of 47.1%; ~1.2 cal. ky BP; mainly morphotypes of genus *Corynoneura*) (Fig. 3, A.6). Chironomid concentrations further decreased to the average of ~2 HC cm^{-3} until a further increase towards the top of zone 3 (Fig. 4), where bottom associated taxa rise due to a peak of *Tanytarsus pallidicornis*-type 2 (Fig. 3). *Ceriodaphnia* ephippia increase in concentrations and were present in all merged samples between 4.2 and ~1.3 cal. ky BP, whereas *Bosmina longispina* remains suddenly disappear from the record ~3.5 cal. ky BP (Fig. 3). While influxes chrysophyte stomatocystsdecrease, the influx of periphytic diatoms reaches a minimum, which led to maximal values for C:D_{per} (max. ~840%; ~4.1 cal. ky BP) (Fig. 4). Si/Ti gradually decreased from its maximum at the Zone 2/3 boundary ~4.2 cal. ky BP reaching minimum values ~1.4 cal. ky BP (Fig. 4). DI-pH decreased to 5.51 ~4.1 cal. ky BP, remained <6 through Zone 3, and finally decreasing to its minimum (5.06) in the uppermost sample representing (sub)recent conditions (Fig. 4). DI-TP increased from the base of zone 3 averaging ~5 µg l^{-1} through the zone and peaking in the uppermost sample at 11 µg l^{-1} admittedly with large uncertainties (Fig. 4, A.3). Al/Rb and Al/Ti ratios declined from ~3.9 cal. ky BP and Ti remained at low concentrations except for minor peaks ~0.35 cal. ky BP and ~0.28 cal. ky BP (Fig. 4). Mn/Ti and Fe/Ti retained opposite trends through zone 3 reflecting excess Fe concentrations relative to Ti, which contrasts the Mn/Ti ratio except for ~0.5–0.3 cal. ky BP with the highest Mn/Ti values (Fig. 4).

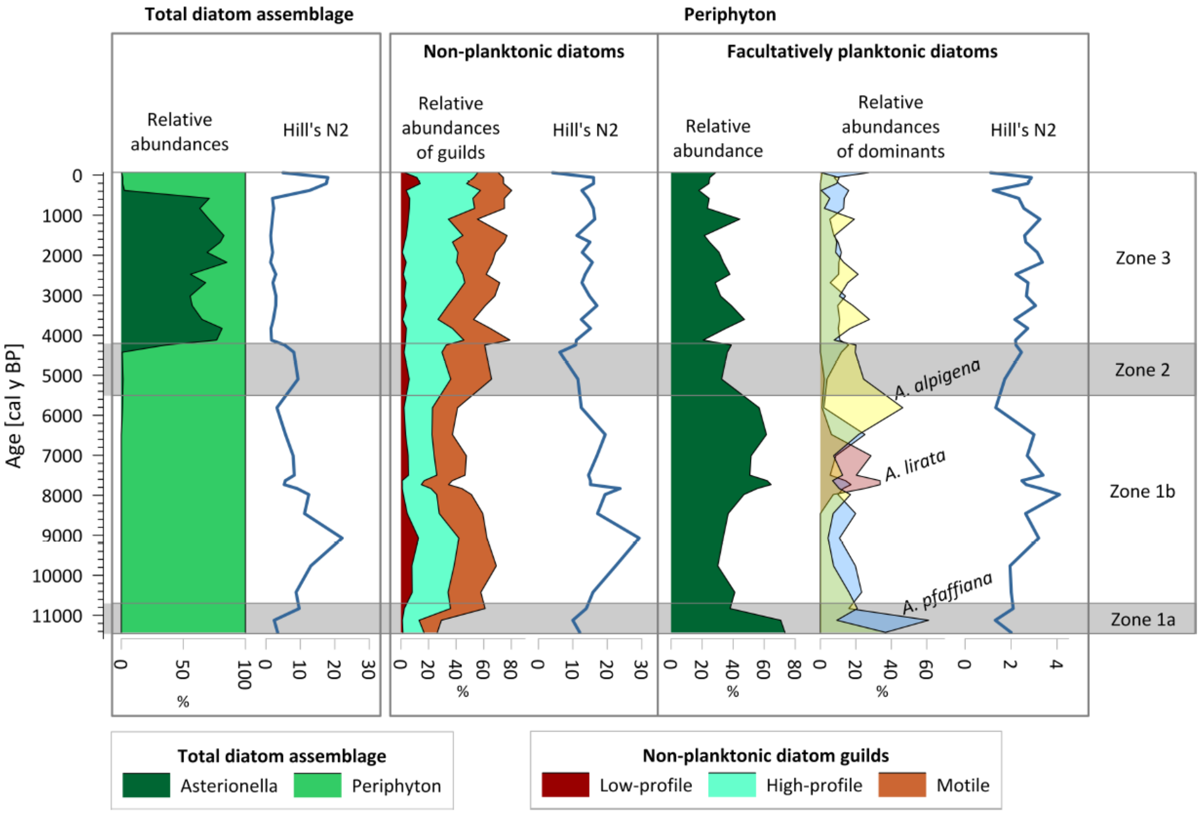

Fig. 2: Diatom stratigraphical diagram showing proportion (%) of euplanktonic *Asterionella ralfsii* (dark green silhouette) against diatom periphyton (light green s.) and relative abundances of particular guilds of the diatom periphyton: low-profile (dark red s.), high-profile (turquoise s.), motile (ginger s.), facultatively planktonic (= tychoplanktonic) (dark green s.). All three most abundant periphytic species originate from facultatively planktonic guild (plotted overlaying each other): *Aulacoseira alpigena* (yellow s.), *Aulacoseira lirata* (pink s.), *Aulacoseira pfaffiana* (blue s.). "Effective number of diatom taxa" (Hill's N2) (dark blues lines) is plotted for each group. Zonation (Zone 1a, Zone 1b, Zone 2, and Zone 3) is defined by clustering of diatom and chironomid assemblages using CONISS (stratigraphically constrained cluster analysis) and Broken-Stick model.

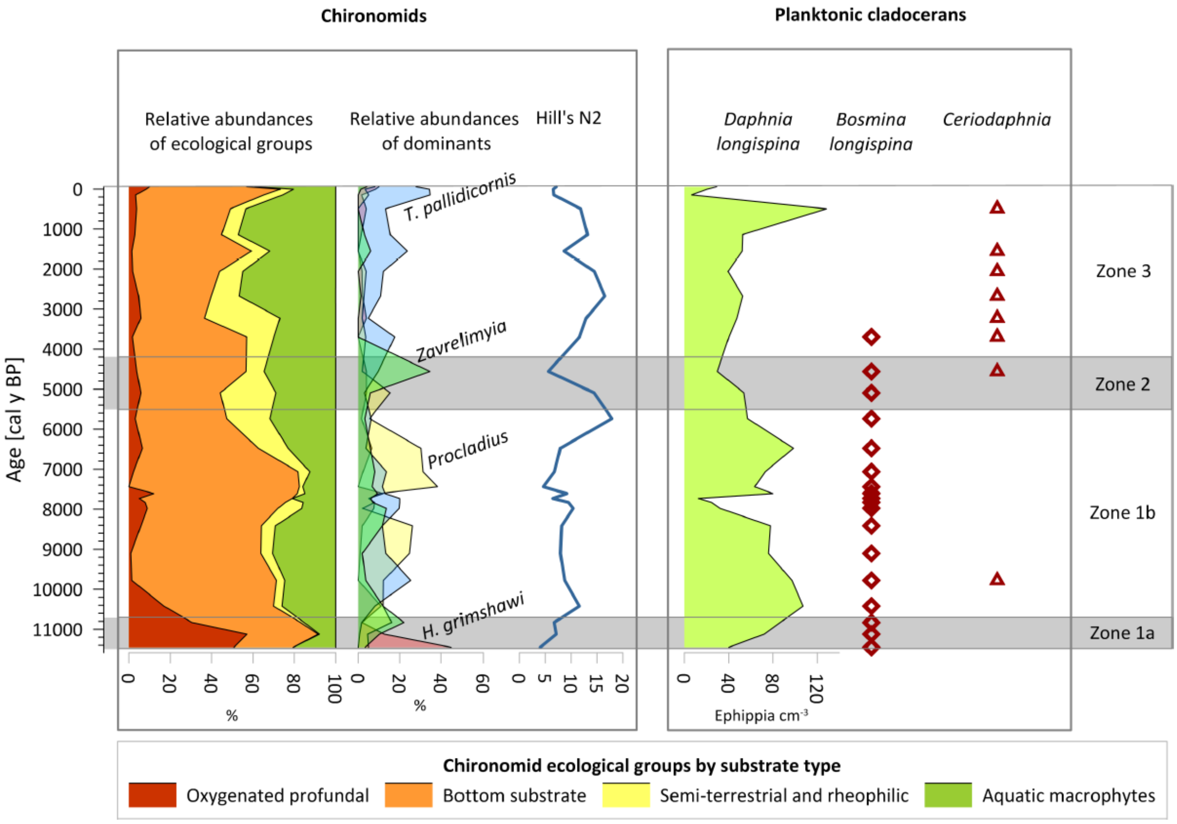

Fig. 3: Freshwater invertebrate stratigraphical diagram. Relative abundances of chironomid ecological groups (left): taxa of oxygenated profundal (red silhouette), other bottom substrate taxa (orange s.), semi-terrestrial and rheophilic taxa (including taxa associated with seepages, moss, and small streams) (yellow s.), and taxa associated with aquatic macrophytes (green s.). Four most abundant chironomid species are plotted overlaying each other: *Tanytarsus pallidicornis* type 2 (blue s.), *Zavrelimyia* type 2A (green s.), *Procladius* (yellow s.), *Heterotrissocladius grimshawi*-type (red s.). “Effective number of chironomid taxa” (Hill’s N2) is plotted in dark blue lines. Planktonic cladoceran taxa remains (right) are plotted in volumetric concentrations (remains per 1 cm^3) (light green s.) or presence/absence data (dark red symbols). Zonation (Zone 1a, Zone 1b, Zone 2, and Zone 3) is defined by clustering of diatom and chironomid assemblages using CONISS (stratigraphically constrained cluster analysis) and Broken-Stick model.

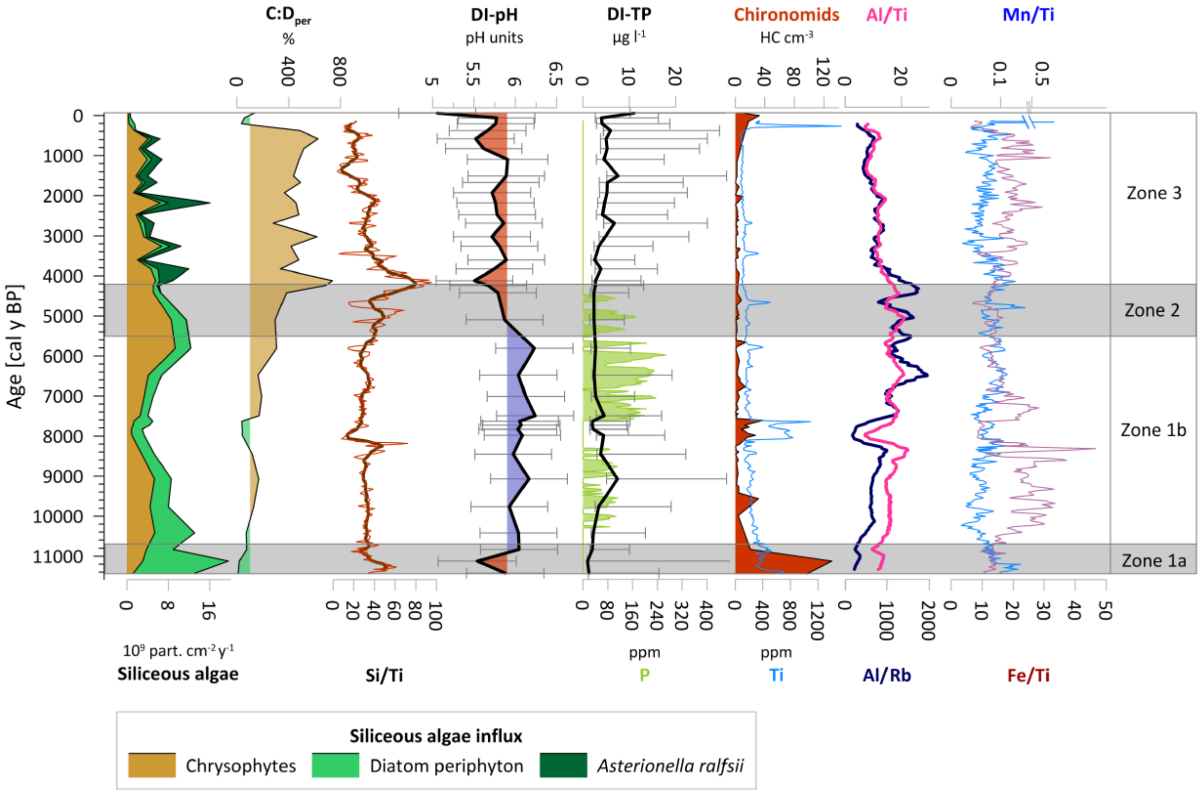

Fig. 4: Summary stratigraphical diagram of main proxies for reconstruction of brownification in Prášilské Lake showing (from left to right): influx of siliceous algae (chrysophytes – golden silhouette, diatom periphyton – light green s., euplanktonic diatom *A. ralfsii* – dark green s.), C:D$_{per}$ – ratio between chrysophyte stomatocyst and periphytic diatom valves (golden shaded values > 1, green shaded values < 1), Si/Ti ratio (orange line) with running average-window width 11 samples (dark brown bold line), DI-pH – diatom-inferred pH (black line; red shaded values – mean value >5.9, blue shaded values – mean value< 5.9) with standard errors (light grey bars), DI-TP – diatom-inferred total phosphorus (black line) with standard errors (light grey bars), P – phosphorus concentration (ppm) in sediment (green shaded area), concentration of chironomid head capsules (HC cm^{-3}) (red silhouette), Ti concentration (ppm) (light blue line), Al/Rb and Al/Ti ratio running average-window width 11 samples (Al/Rb - dark blue line, Al/Ti - pink), Fe/Ti ratio (red line) and Mn/Ti ratio (light blue line). Zonation (Zone 1a, Zone 1b, Zone 2, and Zone 3) is defined by clustering of diatom and chironomid assemblages using CONISS (stratigraphically constrained cluster analysis) and Broken-Stick model.

19 Discussion

19.1 Ecosystem functioning thresholds at Prášilské Lake

One hundred and fifty years of hydrobiological research of Bohemian Forest lakes have documented complex changes in their water chemistry, including changes in water color and transparency (e.g., Veselý et. al., 1994, Vrba et al. 2000). However, that longer-term perspective on millennial-scale water changes in brownification had remained elusive before the application of paleolimnogical methods. Vondrák et al. (2019b) and Moravcová et al. (2021) have already shown that Prášilské Lake, one of the Bohemian Forest lakes, likely experienced high DOC concentrations during the Holocene. Here, we focus on more detailed history of brownification episodes at Prášilské Lake and various related environmental drivers. Brownification of Prášilské Lake was inferred using biotic and geochemical proxy records revealing (i) the direct impacts of high humic acid concentrations – water acidification and light limitation, and (ii) indirect impacts – enhanced thermal stratification, bottom water anoxia and shifts in nutrient availability in the lake. There have been three main alterations in species composition of the aquatic biota in Prášilské Lake, and these occurred around ~10.7, ~5.5 and ~4.2 cal. ky BP (see Fig. 5) suggesting significant changes in the lacustrine ecosystem. Here, we discuss the contribution of particular brownification processes to these thresholds in ecosystem function, and finally, we estimate the intensity of brownification with time in the context of regional climatic changes and other drivers.

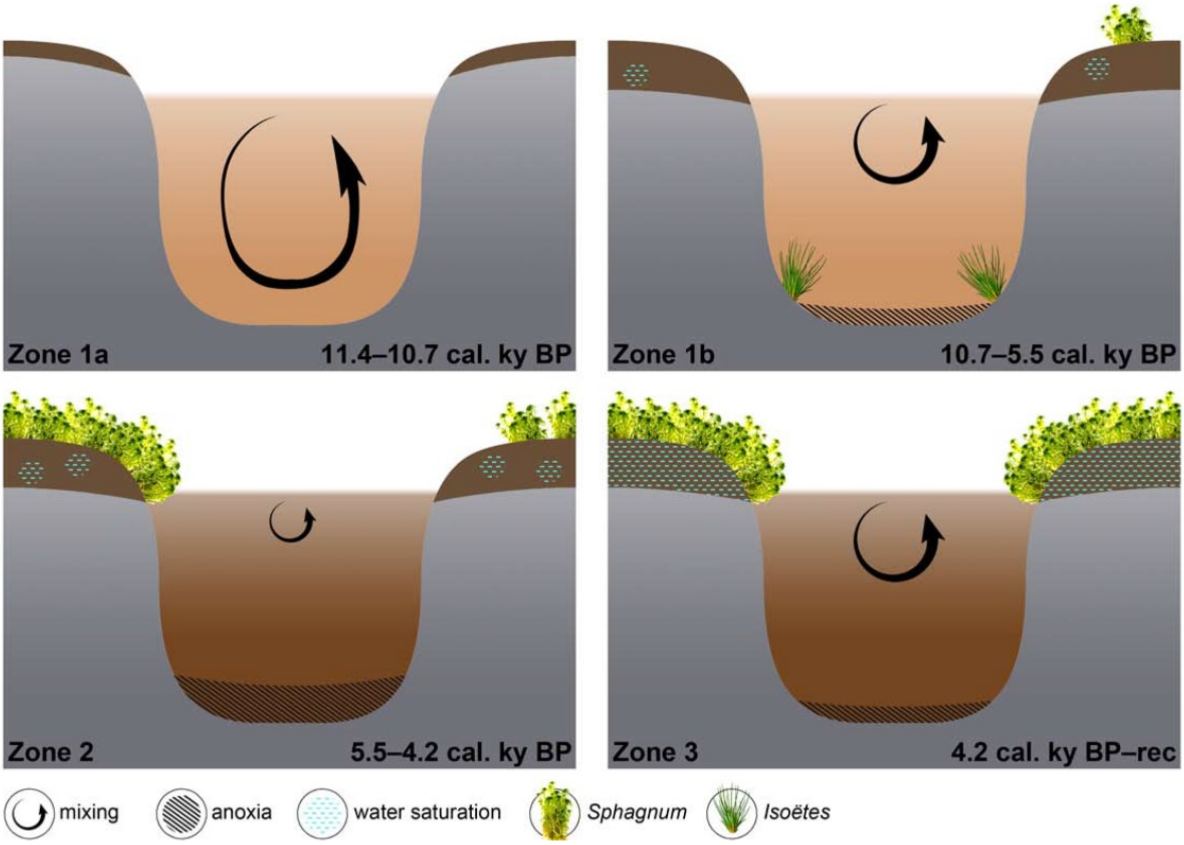

Fig. 5: Model of main phases of Prášilské Lake water brownification excluding changes connected to the "8.2 ka event" and last 500 years.

19.2 Natural acidification and light limitation

The aquatic assemblages testify to brown and humic acid rich character of Prášilské Lake throughout the postglacial period, which aligns with previous findings of abundant acid-tolerant and acid-resistant caddisflies (Vondrák et al., 2019b) and acidophilous diatoms typical of low conductivity waters rich with humic acids (Moravcová et al., 2021). The diatom species composition presented here reveals greater variety on sequence of environmental changes in the evolution of Prášilské Lake, that said diatom species throughout were always from the pool of taxa preferring acidic and typically dystrophic waters.

Humic compounds have influenced the Prášilské Lake's ecosystem at the latest since ~10.7 cal. ky BP when the productivity of periphytic diatoms started to decrease (influx of diatom periphyton; Fig. 4), suggesting possible shading during water browning (Karlsson et al., 2009; Vasconcelos et al., 2016). However, the drop in diatom productivity was caused mainly by a bloom of *Aulacoseira pfaffiana* before ~10.7 cal. ky BP and its decline afterwards (Fig. 2). As heavily-silicified *Aulacoseira* taxa can cope with strong light limitation if they are intermittently floated by turbid mixing (Reynolds, 2006; Rühland et al., 2015), we suggest strong mixing of the Prášilské Lake waters rode over the shading effects of DOC ~10.7 cal. ky BP. The Holocene sequence at Prášilské Lake involves a first stage in the aquatic succession that was driven mainly by stratification of water column after an initial more turbid phase (Zone 1a) that potentially obscured inference of pH and TP from the diatom record before ~10.7 cal. ky BP. The subsequent gradual shift from diatoms of circumneutral to weakly acidic waters to those preferring moderate to strongly acidic waters (Moravcová et al. 2021) suggests little or no change in concentration of humic acids, and the presence of caddisfly *Limnephilus coenosus* points to peaty waters in Zone 1a (Vondrák et al., 2019b). Therefore, we expect broadly consistent relatively humic-rich conditions and no sharp step in brownification around 10.7 cal. ky BP (Fig. 5).

Conversely, distinct falls in pH at Prášilské Lake are suggested by decreases in DI-pH between ~5.5 and ~4.2 cal. ky BP (Zone 2, Fig. 4). At this time, all (sub)dominants in the diatom assemblage (Fig. 2, A.4; successively *Aulacoseira alpigena*, *Brachysira brebissonii*, *Asterionella ralfsii*) belong to taxa typical of waters acidified by humic acids (Pappas & Stoermer, 2001; Houk, 2003; Lange-Bertalot et al., 2017). This succession shows the transition from periphytic (*A. alpigena*, *B. brebissonii*) to pelagic (*A. ralfsii*) primary production, further supported by increases in the proportion of chrysophytes ($C{:}D_{per}$, Fig. 4) as chrysophytes are mostly planktonic at these lakes (Rivera-Rondón & Catalan, 2017). These new evidences confirm the major role of light attenuation in a collapse of aquatic macrophytes (*Isoëtes* spp.) at Prášilské Lake ~4.2 cal. ky BP (Moravcová et al., 2021) because the decrease in primary production in deeper water benthic habitats is an aquatic ecosystem response typical of light limitation during brownification and a feature of other nutrient-poor boreal lakes (Karlsson et al., 2009; Brothers et al., 2014; Vasconcelos et al., 2016). There is at Prášilské Lake a temporary increase in the relative abundance of non-planktonic diatom periphyton (including *B. brebissonii*) ~5.5–4.2 cal. ky BP, which resembles periphytic dominance due to lower UV exposure caused by DOC shading in a Canadian Shield lake (Pienitz & Vincent, 2000). In contrast, our diatom productivity decreased as the periphyton to euplankton proportion increase, which suggests light attenuation by DOC reached levels that limited both planktonic and periphytic primary production (Jones, 1992; Carpenter et al., 1998) as well as that of herbivorous planktonic cladocerans (Fig. 3). However, other factors such as less intensive water mixing and/or reduced nutrient availability also influence the dynamics of phytoplankton at Prášilské Lake.

Other factors complicate the pH-indicative value of the bloom of *A. ralfsii* ~4.2–0.5 cal. ky BP. *A. ralfsii* has often served as an indicator of acidification by humic acids given an association with very low pH dystrophic waters (Liehu et al., 1986; Stabell, 1993; Jüttner et al., 1997), but sensitivity to effective silica-uptake and/or Al concentrations complicates this interpretation of a competitive advantage in humic waters (Stokes, 1986; Gensemer et al., 1993a, 1993b). Unfortunately, the physiological experiments exploring this were focused only on the variety *A. ralfsii* var. *americana* (Gensemer et al., 1993a, 1993b). Nonetheless, *A. ralfsii* at Prášilské Lake does define a period of enhanced concentrations of DOC. Moreover, our DI-pH record decreased simultaneously with the onset of *A. ralfsii* bloom in Prášilské Lake ~4.2 cal. ky BP, and is independent of *A. ralfsii* though constrained by being based only on periphytic species. *Bosmina longispina* disappeared after ~3.7 cal. ky BP from Prášilské Lake, indicating a possible collapse of a potential fish population (see Johnsen & Raddum, 1987). Starving of fish after the loss of periphytic resources has been attributed to light limitation in humic lakes (Karlsson et al., 2009; Brothers et al., 2014; Kankaala et al., 2019). *A. ralfsii* suddenly decreases in abundance ~0.5 cal. ky BP probably reflecting changes in lake's hydrology connected to intensifying human impact in last 500 years (Vrba et al., 2000) and the decrease in periphytic DI-pH in the top sample probably reflects anthropogenic acidification at Prášilské Lake.

Overall, although we observed the first signs of browning after ~10.7 cal. ky BP, other processes such as a water-level change or wave mixing of the water column contributed to the "delayed onset" of pronounced thermal stratification influencing the lacustrine biota (Fig. 5). Significantly, we found strong evidence of a critical threshold in humic substances, acidification and light limitation of the water column at Prášilské Lake that was crossed between ~5.5 and ~4.2 cal. ky BP (Zone 2) and these conditions persisted to the subrecent.

19.3 Lake water mixing and bottom anoxia regimes

Turbid waters most likely affected aquatic biota during the initial phase of postglacial lacustrine development (Zone 1a; Fig. 4) as indicated by the bloom of heavily-silicified *Aulacoseira pfaffiana* (Fig. 2), which was probably kept afloat by water mixing (Reynolds, 2006; Rühland et al., 2015). Several independent proxies point to prolonged thermal stratification with periods of bottom anoxia in Prášilské Lake since ~10.7 cal. ky BP. The abrupt decrease of chironomid concentrations (Fig. 4), including the fall in relative abundances of taxa indicating well-oxygenated profundal zone (Fig. 3), supports the near-bottom anoxia along with the inverse trends in Mn/Ti and Fe/Ti reflecting greater relative concentrations of Fe in the sediment (Fig. 4). Reducing Mn in sediment produces highly mobile Mn^{2+} ions while Fe^{3+} ions became immobile in complexes under anoxic conditions (Kylander et al., 2013).

The chironomid taxa mentioned above as indicating availability of dissolved oxygen in the profundal waters are only abundant in Zone 1a (Fig. 3). Three of them, *Heterotrissocladius grimshawi*-type, *Micropsectra insignilobus*/*conctracta*-type, and *Tanytarsus lugens*-type, exceed 19 % (57% cumulatively) of the total chironomid assemblage (Fig. A.6) but they do not appear again at these high relative abundances and the latter two taxa become very rare. Similar Early-Holocene chironomid fauna with *H. grimshawi*-type and *M. insignilobus*/*conctracta*-type was recorded in sediments at Plešné Lake nearby in the Bohemian Forest (Tátosová et al., 2006), but T. *lugens*-type was not recorded and replaced by *Procladius*. *Procladius* is known to be common in lakes with near-bottom oxygen depletion (Brooks et al. 2007), and we interpret its presence as a sign of profundal oxygen limitation. This process occurred earlier in Plešné Lake likely due to its mesotrophic character (granitic bedrock) whereas *Procladius* became the dominant profundal taxa in Zone 1b at Prášilské Lake, i.e. after ~10.7 cal. ky BP. Bottom water anoxia at Plešné Lake has never eliminated *Procladius* from Holocene chironomid assemblages unlike Prášilské Lake. These differences between nearby lakes are likely due to differences in bathymetry (Šobr & Janský, 2016) that prevented long periods of anoxia in elongated basin of Plešné Lake with the deepest part close to the outflow and on the contrary that enabled prolonged anoxia in more symmetric circular basin of Prášilské Lake.

Development of catchment soils through Zone 1a (decrease in Ti and Rb input; Fig. 4, A.7) contributed greater transport of DOC to the lake and restricted seepage through the moraine dam, with reduced water clarity encouraging more pronounced thermal stratification ~10.7 cal. ky BP and this was accompanied by a probable lake-level rise. Additionally, the denser forest canopy around the lake after ~10.7 cal. ky BP (Carter, et al., 2018a) would mitigate wind-induced currents inhibiting lake mixing and turnover (Klaus et al., 2021). Lake-level rise flooding fresh substrates could also increase the relative abundance, effective number of species (Fig. 2), and influx (Fig. A.5) of low-profile diatoms known to be effective colonizers (Leira et al., 2015; Pla-Rabés & Catalan, 2018). Although C:D ratios often display positive relationships with water depth in mountain lakes (Rivera-Rondón & Catalan, 2017; Buczkó et al., 2018), the influence of thermal stratification could outweigh the effect of lake morphology (Rivera-Rondón & Catalan, 2017). Flagellated chrysophytes command a distinct competitive advantage in stratified waters as they can actively migrate to secure light and nutrients (Werner & Smol, 2005). Therefore, we interpret the increasing C:D$_{per}$ ratio at Prášilské Lake (Zone 1a, 1b, Fig.4) rather owing to a strengthening of thermal stratification, changes that were potentially aided by lake-level rise.

Two anomalies interrupted the otherwise continual record of dystrophic waters and likely thermal stratification in Prášilské Lake. The first ~8.1–7.6 cal. ky BP, besides harmonized Mn/Ti and Fe/Ti ratios reflecting lower relative concentrations of Fe in the sediment, occurred as distinct shifts across nearly all recorded proxies, which suggests a complete change of the lacustrine ecosystem. Disturbance of the water column was visible in the temporary recovery of oxygen-demanding deep-water chironomids (namely *Heterotrissocradius grimshawi*-type) (Fig. 3), increased total chironomid concentrations and increased total caddisfly concentrations and diversity (Vondrák et al., 2019b), and concurrent trends of Mn/Ti and Fe/Ti. A coincident decrease in the proportion of chrysophyte stomatocysts (C:D_{per} ratio; Fig.4) probably resulted from losing their competitive advantage in a well-mixed water column (Werner & Smol, 2005). Additionally, the dominance of heavily-silicified diatom *Aulacoseira lirata* (Fig. 2) suggests facilitating of its floating by intensive water mixing (Reynolds, 2006; Rühland et al., 2015).

Whereas we consider the 8.1–7.6 cal. ky BP period to represent a direct signal of a change in the mixing regime in Prášilské Lake and from increased flux of clastic material from the catchment, the second anomaly ~6.8–4.2 cal. ky BP requires more complex explanation. Although trends of Mn/Ti and Fe/Ti harmonized in this time period, none of the other indicators of water mixing recovered, e.g., chironomids (and especially typical lake taxa) remain at very low concentrations (Fig.4) and show the highest proportion of semiterrestrial and rheophilic taxa. These represent inhabitants of the very shallow part of the littoral zones, inflows, and wet habitats in the catchment (Brooks et al., 2007) (Fig.3., A.6) and C:D_{per} ratio even gradually increased (Fig. 4). This event is also characterized by peaking *Aulacoseira* species (Fig. 2); however, *A. alpigena* could rather indicate decline in water conductivity than a physical disturbance of the water column (Houk, 2003; Leira et al., 2015). Altogether, biotic proxies indicated persistent thermal stratification and in-lake anoxia ~6.8–4.2 cal. ky BP (Fig. 5), which points to another cause of the relative enrichment by Mn in the sedimentary record. Potential mechanisms employ processes connected with the brownification of lake water supposed in Prášilské Lake for this period. First, as dark color of humic waters can trap radiation in the surface layers and reduce the mixing depth by heating the epilimnion in small lakes (Fee et al., 1996; Wetzel, 2001), more frequent meromixis (i.e., very limited mixing of the hypolimnion) likely appeared in Prášilské Lake. Consequent pronounced or nearly permanent bottom anoxia could reverse otherwise higher Mn mobility and result in the diagenetic formation of Mn compounds (Makri et al., 2021). Potential diagenetic precipitation of Mn was probably muted with the release from the most intense thermal stratification after ~4.2 cal. ky BP. Another mechanism lies in the eventual input of Mn^{2+} from soils in the catchment. If oxygen depletion occurred in soils, e.g., due to water saturation, highly mobile Mn^{2+} would in-wash from soils to the lake and further compensate for the in-lake deficiency of Mn. This preferential reduction and mobilization of Mn^{4+} against

Fe^{3+} was observed under anoxic conditions in flooded and wetland soils (Patrick Jr. & Jugsujinda, 1992; Du Laing et al., 2009; Kröpfelová et al., 2009). Moreover, in acidic soils, lower pH can facilitate reduction of Mn^{4+} under lower levels of redox potential (E_H), i.e. less oxygen depleted conditions (Frohne et al., 2011). When the supply of Mn in the eluvial soil horizon was depleted in the Prášilské Lake catchment, a terrestrial flux of Mn^{2+} would become insufficient to replenish the in-lake stock, which probably resulted in the renewal of inverse trends in Mn/Ti and Fe/Ti ratios since ~4.2 cal. ky BP. The tendency of Mn to deplete in soil systems is illustrated by a the dependence of Mn loads on supply from silt deposits in boreal streams while soils on till in upper parts of catchments already failed to release Mn (Björkvald et al., 2008). Additionally, a sharp downward decrease of Mn was documented from podzolized soil profiles (Riise et al., 2000), which indicates that Mn is released from the soils instead of precipitation in the illuvial spodic horizon.

A return to more frequent mixing of the water column was detected ~0.5 cal. ky BP in Prášilské Lake (Fig. 4) and coincided with human activities in the highest part of the Bohemian Forest. Although the first invasive anthropogenic interventions with the lake level were evidenced only since 19th century (Švambera, 1914; Holcová et al., 2020), other artificial activities, such as logging, started ca. 500 years ago in the proximity of Bohemian Forest lakes (Vrba et al., 2000). Nevertheless, the regional human impact has been growing at least for the last 1000 years as supported by continuous record of cereal pollen and opening of forest canopy (Carter et al., 2018a; Kozáková et al., 2021). The changes in lake's hydrology culminated at the end of the 19th century and were followed by the onset of anthropogenic acidification (Fott et al., 1994; Vrba et al., 2015).

19.4 Nutrient availability

Prášilské Lake was probably nutrient-poor throughout its postglacial evolution (DI-TP<10 µg l^{-1}, Fig. 4), hence the reconstructed changes in phosphorus concentration acted within restricted range. We suggested the increase of phosphorus availability based on DI-TP after ~10.7 cal. ky BP (peaking ~9.1 cal. ky BP) and after ~4.2 cal. ky BP. Slight fertilization of the lake could have resulted from co-export of DOC and phosphorus (Kopáček et al., 2011), as heightened input of dissolved organic matter was interpreted for Prášilské Lake at these times. However, a more complicated explanation requires a mechanism behind the DI-TP ~7.5–4.2 cal. ky BP decrease, since simultaneous strengthened effects of water brownification were recorded in Prášilské Lake. Three possible driving processes are proposed:

(i) The concentration of available phosphorus could decrease by its sedimentation and limited recycling from sediments. However, anoxic bottom conditions, which release phosphorus from sediments (Petticrew & Arocena, 2001), were indicated by chironomid species composition (decrease in taxa inhabiting profundal and deep littoral habitats), very low total chironomid HC concentrations, and divergent trends in Mn/Ti vs. Fe/Ti ~6.8–4.2 cal. ky BP in Prášilské Lake. Nevertheless, precipitation with Al hydroxides prevent phosphorus recycling even under bottom anoxic conditions (Kopáček et al., 2005; Vrba et al., 2006). Increased Al/Rb and Al/Ti (Fig. 4) suggest the input of Al into Prášilské Lake ~6.8–3.9 cal. ky BP, likely associated with humic compounds (Paludan & Jensen, 1995; Norton et al., 2011; Possinger et al., 2020). Although XRF-measured phosphorus exhibites many zero values, it shows the highest values exactly in the period ~7.5–4.2 cal. ky BP (Fig. 4), which further supports the probably strengthened phosphorus retention in the sediments.

(ii) Onset of strong light limitation could prevent diatoms from utilizing nutrients and bias their transfer function based signal for concentrations of available phosphorus (Rivera-Rondón & Catalan, 2020). Nevertheless, brownification more often acts ambiguously favouring planktonic primary production by nutrients and reducing benthic phototrophs by shading (Nürnberg & Shaw, 1999; Karlsson et al., 2009), although planktonic algae can be suppressed by light attenuation in lakes rich with humic compounds (Jones, 1992; Carpenter et al., 1998). Note a temporary collapse of planktonic algae ~5.5–4.2 cal. ky BP (Fig. 2, this study; *Botryococcus*, Fig. 3, Moravcová et al. 2021), whereas after ~4.2 cal. ky BP pelagic primary production partly recovered (*A. ralfsii*, Fig. 2, this study; *Botryococcus*, Fig. 3, Moravcová et al. 2021). This pattern suggests nutrient limitation of pelagic production until ~4.2 cal. ky BP and subsequent slight increase in nutrient availability, however, the strong shading of pelagic habitats and later recovery potentially became involved.

(iii) Finally, a potential change in the stoichiometry between DOC and TP for in-washed terrestrial dissolved organic matter (DOM) should not be neglected. Stetler et al. (2021) suggested three mechanisms of decreasing the P:DOC ratio during brownification (acidification of soils, storage of phosphorus in terrestrial vegetation, and change in the quality of DOM), and all of them could occur at Prášilské Lake. The ability of soils to bind phosphates decreases with increasing soil pH (Kopáček et al., 2011, 2015). As the increase in pH is an essential condition for reductive dissolution of organic matter (Grybos et al., 2009), leaching of DOC is accompanied by phosphorus originating from organic matter and binding sites in soils (Kopáček et al., 2011). Frequent disruption of reducing conditions in soils, e.g., during dry episodes (Evans et al., 2006), could result in a lower P:DOC ratio, since the phosphorus mobilization can delay recovery from acidifying (oxidizing) disturbance

(Kopáček et al., 2015; Stuchlík et al., 2017). Acidification of soils was supported by higher mobility of Al ~6.8–3.9 cal. ky BP (Fig. 4) to Prášilské Lake, and is a feature of soil podzolization processes (Ewing & Nater, 2002). The second mechanism, immobilization of phosphorus in more intensively growing terrestrial vegetation proposed in the context of recent climatic warming by Stetler et al. (2021), corresponds in part with the timing of the Mid-Holocene "climatic optimum" (~8–5.5 cal. ky BP). Lastly, change in DOM quality could operate along with the progress of soil saturation in the catchment, as nutrient proportion probably decreased in soil organic matter with the onset of anoxic conditions in poorly-drained soils (Tipping et al., 2016). Saturation of soils in the catchment of Prášilské Lake was suggested by the flux of mobilized Mn (Fig. 4).The spectrum of potential mechanisms illustrates how complex the phosphorus dynamics are and it is beyond this study's compass to disentangle them fully. Regardless, the DI-TP corresponds well with the geochemical proxies for pedogenetic and hydrologic development and together they fit the hypothesised biogeochemical mechanisms of phosphorus dynamics during brownification. Therefore, we consider our DI-TP data for Prášilské Lake as a realistic signal of decreased phosphorus availability ~7.5–4.2 cal. ky BP. An inferred value from the topmost sample (5.8 $\mu g\ l^{-1}$) overlaps within uncertainties recent measurements at the lake (7 $\mu g\ l^{-1}$; Tab.1), though this is the largest standard error among all samples (Fig. 4, A.3).

After this sensitive response to reduced phosphorus, diatoms can also be limited by concentrations of available silica (SiO_2) (Battarbee et al., 2001). Therefore it is worth considering silica's role in diatom succession, although limitation by other nutrients usually precedes silica-limitation in oligotrophic lakes (Kilham, 1971). Hence, in the oligotrophic/dystrophic conditions of Prášilské Lake, the simple gradient of Si or Si/P ratio available in the water should fail to explain diatom succession unlike at eutrophic lakes (Kilham, 1971; Tilman et al., 1982). Nevertheless, the diatom succession from genus *Aulacoseira* to g. *Asterionella* (and in subdominant level also to g. *Fragilaria* and g. *Tabellaria*) observed in Prášilské Lake (Fig. 2, A.4) is similar to that reflecting Si depletion in more productive lakes (Kilham, 1971; Tilman et al., 1982). The role of Si in Prášilské Lake's diatom succession ~5.5–4.2 cal. ky BP was probably mediated by a combination of strengthened thermal stratification and light limitation. An absence of pronounced mixing prevented heavily-silicified *Aulacoseira* taxa from reaching the insolated epilimnion (Rühland et al., 2015) along with strong shading of the silica-rich upper hypolimnion, which silica-demanding diatoms such as *Aulacoseira* taxa used to occupy (Cantonati & Lowe, 2014; Pla-Rabés & Catalan, 2018). The resulting lack of suitable habitats likely led to the decrease in the relative abundance of *Aulacoseira* taxa starting ~5.5 cal. ky BP in Prášilské Lake.

Moreover, epilimnion became probably depleted in silica soon without its recycling from deeper parts of the lake as this mechanism is the only effective supply of silica to the surface waters besides an allochthonous input (Lampert & Sommer, 2007). The episodic in-wash layers (Ti, Zone 2–3, Fig. 4) are low magnitude and less frequent than in the earlier Holocene suppressing the supply of detrital silica. Then, effective competitors for silica, such as *Asterionella ralfsii* (Stokes, 1986), dominated the epilimnetic diatom production. Overall, we can read the switch from *Aulacoseira* to *Asterionella* in Prášilské Lake as the suppression of diatom production in silica-rich habitats by strong light limitation.

The elevated Si/Ti ~6.3–3 cal. ky BP (peaking ~4.2 cal. ky BP) in Prášilské Lake, unsupported by similar increase in production of siliceous algae (Fig. 4) or heightened sedimentation of sand fraction (low Zr/Rb ratio, Fig. A.7), opens the possible influence of humic acids bounded supply of Si. Although Si was traditionally considered to form complexes with humic substances (Wetzel, 2001), the substantial extent of Si-humic complexes in natural waters is still disputed (Viers et al., 1997; Pokrovski & Schott, 1998; Kubicki & Heaney, 2003). Thus, we downplay this scenario of in-lake precipitation of available Si via the allochthonous input of humic substances. The peak of Si/Ti could result from processes connected to brownification without direct complexation with humic substances. Increased in-wash of dissolved organic carbon as well as silica has been observed after snowmelt pulses to lakes (Dyson et al., 2011; Schaetzl et al., 2015). The potential climatic shift to higher winter precipitation could result in enhanced chemical weathering in soils and explain the increased input of Si without signs of more intense erosional activity in Prášilské Lake sediments. Higher input of Si coincided loosely with an increase in Al at Prášilské Lake ~6.8–3.9 cal. ky BP (Fig. 4), both elevated mobility of Al and Si can stem from intensified podzolization process supported generally by humid climatic conditions (Lundström et al., 2000).

19.5 Climatic and regional context

Brownification of surface waters likely contributed to all three main shifts in species composition of aquatic biota in Prášilské Lake at ~10.7, ~5.5 and ~4.2 cal. ky BP. Early input of humic acids into the Bohemian Forest lakes after the Pleistocene-Holocene transition (Steinberg et al., 1991; Pražáková et al., 2006; Vondrák et al., 2019b; Moravcová et al., 2021) was probably induced by regional afforestation ~10.5-10.3 cal. ky BP (Vočadlová et al., 2015; Carter et al., 2018a). Heightened input of humic compounds from dead vegetation and side-effects of the afforestation, i.e., soil stabilization and the beginning of podzolization increasing the soil carbon pool, contributed alongside a shift from a more energetic water body (Zone 1a, Fig. 4, 5) into a more stable water column with prolonged stratification and anoxia after ~10.7 cal. ky BP in Prášilské Lake. Probably lake-level rise after ~9.3 cal. ky BP potentially facilitated greater thermal stratification, which corresponds with observations of an increase in C:D_{per} ratio (Fig. 4) and successful colonization by the low-profile diatom guild (Fig. 2). This rise in lake level after ~9.3 cal. ky BP has been identified as a plausible cause of the decline in submerged macrophytes (*Isoëtes spp.*) at Prášilské Lake (Moravcová et al., 2021).

Interrupted briefly by the greater in-wash of detrital materials from the catchment ~8.1–7.6 cal. ky BP probably linked to the "8.2 ka climatic event" (Tinner & Lotter, 2001), the succession at Prášilské Lake demonstrates further thermal stratification. Signs of favourable conditions and potential longer vegetational season appeared as algal primary production generally increased ~7.6–5.5 cal. ky BP with both silliceous algae (Fig. 4) and *Botryococcus* (Fig. 3; Moravcová et al. 2021). This period corresponds with the Mid-Holocene "climatic optimum" indicated in the region of central Czech Republic (Bohemia) ~8–5.5 cal ky BP by records of thermophilous plant species (Pokorný et al., 2015; Houfková et al., 2017) and strong growth of calcareous tufa deposits (Žák et al., 2002).

The onset of profound suppression of aquatic algal flora driven by surface water brownification occurred as two steps at ~5.5 cal. ky BP and ~4.2 cal. ky BP. The step-wise character of the succession stems probably from increased saturation of catchment soils with podzolization and paludification with wetter climates of the late Holocene. More humid climatic conditions were suggested by the macrophysical climate model (Dreslerová, 2012; Carter et al., 2018b) and encouraged lower forest fire activity (Carter et al., 2018b; Florescu et al., 2019) in Bohemia during a more widespread climatic transition ~6 cal. ky BP (Magny et al., 2006; Wanner et al., 2008). Inception of peat development in the catchment of Prášilské Lake occured ~8.2 cal. ky BP as evidenced by increased proportion of the *Sphagnum* spores from the total pollen sum (Moravcová et al., 2021). Then peatland gradually expand and reached probably littoral waters ~6.5 cal. ky BP as show moss-inhabiting diatoms that increased in abundance at these times (e.g., *Chamaepinnularia mediocris* and *Eunotia paludosa*; Fig. A.4, A.5). Recently, *Sphagnum*-covered wet forest floors with shallow peats occupy substantial extent of the catchment of Prášilské Lake, especially at flatter parts close to inlets.

Although impermeable horizons in podzolic soil profiles are probably not the only prerequisite of paludification (Payette et al., 2012; Schaffhauser et al., 2017), intensified podzolization is suggested by the increase in Al mobility ~6.8–3.9 cal. ky BP (Fig. 4). Until paludification established stable anoxic conditions after ~4.2 cal. ky BP, unstable shifts between oxidizing and reducing conditions with changing soil saturation had probably mobilized both redox-sensitive Mn (Fig. 4) and DOC within the catchment. For efficient mobilization of DOC, the regular alternation between oxic and anoxic conditions in catchment soils is the most favourable mechanism, and is a feature of the seasonal dynamics in wetlands (Olivie-Lauquet et al., 2001) and experiments saturating forest soils (Possinger et al., 2020). Inputs of DOC into lakes has been a feature of wetlands after drainage or other degradation (Liehu et al., 1986; Brothers et al., 2014), essentially reflecting perturbation of stable anoxic conditions. Decreases in Fe/Ti ~6.8–4.6 cal. ky BP and subsequent falls in both Al/Rb and Al/Ti after ~4.2 cal. ky BP in Prášilské Lake (Fig. 4) further characterise this transition from a fluctuating to more stable soil saturation regime, because pedogenic organic matter interactions can shift from Al to Fe^{3+} with decreasing frequency of saturation (Possinger et al., 2020).

Short-term episodes with saturated soils of the Prášilské Lake catchment were probably driven by oscillations between wet and dry climate after ~6.5 cal. ky BP (Žák et al., 2002). Initially, what were probably relatively unsaturated and more oxidized soils were overtaken by paludification from ~6.8 cal. ky BP, and then more permanently saturated soils established after ~4.2 cal. ky BP. In this greater qualities of soil organic matter began to accumulate under these persistent anoxic conditions (Kayranli et al., 2010; Sutfin et al., 2016), with decreases in the portion of DOC leached into the lake.

In summary, the observed transitional character of ecosystem succession ~6.8–4.2 cal. ky BP in Prášilské Lake likely reflected changes in mechanisms of brownification before and after ~4.2 cal. ky BP, and these coincide with timing of the Middle to Late Holocene transition. Synchronous onset of enhanced lake-water brownification and catchment paludification ~6.8 cal. ky BP corresponds with a moist climatic phase in Central Europe suggested by increased lake water levels (Magny, 2004; Starkel et al., 2013; Pleskot et al., 2018; Theueurkauf et al., 2021). Paludification at higher elevations of Central European mountain ranges was reported after ~6 cal. ky BP (Obidowicz, 1996; Beug et al., 1999) as well as natural acidification of regional montane forest lakes (Pražáková et al., 2006; Sienkiewicz, 2016). Peak acidity was potentially accompanied by the highest input of colloidal Si ~4.2 cal. ky BP in Prášilské Lake, and this coincides with unprecedented in-wash of organic matter and/or Si at several Central European lakes (Steinberg, 1991; Schütze et al., 2018; Pleskot et al., 2020). The regional character of these changes suggests climate drivers probably associated with the "4.2 ka climate oscillation"specifically greater seasonality and increased higher winter precipitation (Pleskot et al., 2020) as greater snowmelt fluxes produce pulses of acidity and chemical weathering (Schmidt et al., 2002; Dyson et al., 2011; Schaetzl et al., 2015).

After ~4.2 cal. ky BP, more saturated soils and advanced paludification in the Prášilské Lake catchment caused probably that the input of DOC to the lake decreased slightly and lake ecosystem remained relatively stable until the subrecent disturbances ~0.5 cal. ky BP. Such a conservation of lake conditions has been reported from northern Europe when peatlands took a full control of the biogeochemical processes in those catchments (Solovieva & Jones, 2002; Myrstener et al., 2021). Time lags between the initial peat-forming (~8.2 cal. ky BP), intensified pedogenesis with lateral spread of peatlands (~6.8 cal. ky BP), and full paludification of soils (~4.2 cal. ky BP) is likely factor governing the step-wise character of brownification at Prášilské Lake. Similar timing to the pattern of paludification in boreal regions (Bauer et al., 2003; Le Stum-Boivin et al., 2019) supports the value of Central European lakes on poorly-buffered bedrocks in tracking climatically-driven brownification. This ecosystem functioning has significance because regional-scale brownification is likely with successional changes in terrestrial vegetation and submerged macrophytes at lakes across the Bohemian Forest (Carter et al., 2018a; Moravcová et al., 2021).

The strong role of soil saturation and peatland development in surface water brownification over millennial time scales confirms importance of extent and long-term trends for wetland areas in these catchments (Kortelainen, 1993; Laudon et al., 2011; Rantala et al., 2016), information that is not tractable from decadal-scale environmental monitoring (Stetler et al., 2021). Moreover, the association of climate and intensive browning leading to substantial impacts on aquatic ecosystem functioning has implications for discussion about brownification across a range of time scales (Clark et al., 2010). The absence of a widespread trend in the precipitation regime is the only reservation for hydrological driving of recent brownification (Evans et al., 2006). Using this millennial scale perspective, there is a correspondence of brownification and hemispheric climate moistening after ~6 cal. ky BP (Routson et al., 2019).

20 Conclusions

Browning of surface waters occurred as three successive steps at Prášilské Lake and varied in terms of impacts and probable driving factors. The lake experienced elevated input of humic compounds from formation at the onset of the Holocene. The first step in browning appeared ~10.7 cal. ky BP and probably originated from soil stabilization with the catchment afforestation. We propose that browning supported thermal stratification and bottom anoxia of the lake, but shading effects were probably negligible until ~6.8 cal. ky BP. Since then, the mineralized organic matter washed in from peatlands developing in the lake proximity resulted in stronger light limitation, and probably also reduced nutrient availability. After ~4.2 cal. ky BP, soils in the catchment of Prášilské Lake became fully paludified and muted the formerly effective leaching of DOC.

Climate contributed to this brownification in part through major events or oscillations known as the "8.2 ka and 4.2 ka events", with the former interrupting the gradual browning with substantial catchment erosion and water mixing and the latter temporarily enhancing leaching of organic matter to the lake during the "4.2 ka event". The onset of the distinct browning ~6.8 cal. ky BP at Prášilské Lake corresponds with the general moistening of climate across central Europe and northern hemisphere (Magny, 2004; Wanner et al., 2008; Routson et al., 2019). Mid-Holocene climate humidity sustained greater saturation of soils and paludification in the catchment establishing these processes as key factors in brownification at Prášilské Lake.

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Chapter 5:

General conclusions

Our records from two Central-European lakes complemented the general view of post-glacial lake trophic trajectories based otherwise mainly on experience from boreal regions. Diatom analysis added important details about past nutrient dynamics and overall ecosystem succession.

The large shallow lowland lake, Komořanské jezero (**Chapter 2**), confirmed the tendency of this lake type to become eutrophic early. The lake integrates into the pattern of several other Central-European lakes that its ecosystem altered before the onset of the Holocene, probably in response to the warmer half of the Younger Dryas. Interestingly, during the Late-Glacial/Holocene transition, the main ecosystem shift consists of a sharp increase in in-lake primary production and a coincident decrease in the relative abundance of nutrient-demanding species. It suggests that one of the main drivers of lake community structure was the autochthonic in-lake process (competition for nutrients) in the period of the distinct climatic change. This lake represents an interesting example of a highly self-controlled ecosystem in terms of nutrient availability.

The small mountain forest lake, Prášilské jezero (**Chapter 3, 4**), lies on the opposite side of the gradient of autochthonous–allochthonous control responding sensitively to the vegetation (**Chapter 3**) and soil development in the catchment (**Chapter 4**). Climatically-induced input of terrestrial DOC resulted in profound changes in lake ecosystem functioning: fatal shading of littoral primary producers (diatom periphyton, aquatic macrophytes) (**Chapter 3, 4**), acidification by humic acids (**Chapter 3**), altered mixing regime and nutrient availability (**Chapter 4**). A key driving factor of water browning in Prášilské jezero was paludification of the catchment accelerated by the climate moistening after ~6 cal. ky BP, which corresponds to the timing and mechanisms of long-lasting dystrophication of boreal lakes. Our results from Prášilské jezero manifest the comparative potential of lakes from poorly-buffered bedrocks of Hercynian mountain ranges with boreal regions in tracking the processes of hemispherical scale.

References

Adolf C, Wunderle S, Colombaroli D, et al. (2018) The sedimentary and remote-sensing reflection of biomass burning in Europe. *Global Ecology and Biogeography* 27(2): 199–212. DOI: 10.1111/geb.12682.

Ammann B, Birks HJB, Brooks SJ, Eicher U, Grafenstein U Von, Hofmann W, et al. (2000) Quantification of biotic responses to rapid climatic changes around the Younger Dryas — a synthesis. 159: 313–347.

Ammann B, Van Raden UJ, Schwander J, Eicher U, Gilli A, Bernasconi SM, et al. (2013) Responses to rapid warming at Termination 1a at Gerzensee (Central Europe): Primary succession, albedo, soils, lake development, and ecological interactions. *Palaeogeography, Palaeoclimatology, Palaeoecology* 391: 111–131.

Ampel L, Kylander ME, Steinthorsdottir M and Wohlfarth B (2015) Abrupt climate change and early lake development – the Lateglacial diatom flora at Hässeldala Port, southeastern Sweden. *Boreas* 44: 94–102: doi:10.1111/bor.12081.

Anderson NJ (1995) Naturally eutrophic lakes: Reality, myth or myopia? *Trends in Ecology and Evolution* 10(4): 137–138: doi:10.1016/S0169-5347(00)89019-9.

Anderson NJ and Renberg I (1992) A palaeolimnological assessment of diatom production responses to lake acidification. *Environmental Pollution* 78: 113–119.

Anderson NJ, Rippey B and Gibson CE (1993) A comparison of sedimentary and diatom-inferred phosphorus profiles: implications for defining pre-disturbance nutrient conditions. *Hydrobiologia* 253: 357–366.

Apolinarska K, Woszczyk M and Obremska M (2012) Late Weichselian and Holocene palaeoenvironmental changes in northern Poland based on the Lake Skrzynka record. *Boreas* 41: 292–307: doi:10.1111/j.1502-3885.2011.00235.x.

Arts GHP (2002) Deterioration of atlantic soft water macrophyte communities by acidification, eutrophication and alkalinisation. *Aquatic Botany* 73(4): 373–393. DOI: 10.1016/S0304-3770(02)00031-1.

Augusto L, Ranger J, Binkley D, et al. (2002) Impact of several common tree species of European temperate forests on soil fertility. *Annals of Forest Science* 59(3): 233–253. DOI: 10.1051/forest:2002020.

Barley EM, Walker IR, Kurek J, Cwynar LC, Mathewes RW, Gajewski K, et al. (2006) A northwest North American training set: distribution of freshwater midges in relation to air temperature and lake depth. *Journal of Paleolimnology* 36: 295–314: doi:10.1007/s10933-006-0014-6.

Battarbee RW (1986) Diatom analysis. In Berglund BE (ed) *Handbook of Holocene Palaeoecology and Palaeohydrology*. Chichester: John Wiley & Sons Ltd., 527–570.

Battarbee RW, Anderson NJ, Jeppesen E and Leavitt PR (2005) Combining palaeolimnological and limnological approaches in assessing lake ecosystem response to nutrient reduction. *Freshwater Biology* 50: 1772–1780: doi:10.1111/j.1365-2427.2005.01427.x.

Battarbee RW and Bennion H (2011) Palaeolimnology and its developing role in assessing the history and extent of human impact on lake ecosystems. *Journal of Paleolimnology* 45: 399–404: doi:10.1007/s10933-010-9423-7.

Battarbee RW, Charles DF, Bigler C, Cumming BF and Renberg I (2010) Diatoms as inidicators of surface water acidity. In Smol JP and Stoermer EF (eds) *The Diatoms: Applications for the Environmental and Earth Sciences*. Cambridge University Press, 98–121.

Battarbee RW, Jones VJ, Flower RJ, Cameron NG, Bennion H, Carvalho L, et al. (2001) Diatoms. In Smol JP, Birks HJB and Last WM (eds) *Tracking Environmental Change Using Lake Sediments, Vol. 3: Terrestrial, Algal and Siliceous Indicators*. Kluwer Academic Publishers, 155–202.

Bauer IE, Gignac LD and Vitt DH (2003) Development of a peatland complex in boreal western Canada: lateral site expansion and local variability in vegetation succession and long-term peat accumulation. *Canadian Journal of Botany* 81: 833–847: doi:10.1139/B03-076.

Belyea LA and Malmer N (2004) Carbon sequestration in peatland: patterns and mechanisms of response to climate change. *Global Change Biology* 10: 1043–1052: doi:10.1111/j.1365-2486.2004.00783.x.

Bennion H (1995) Surface-sediment diatom assemblages in shallow, artificial, enriched ponds, and implications for reconstructing trophic status. *Diatom Research* 10(1): 1–19: doi:10.1080/0269249x.1995.9705326.

Bennion H, Davidson TA, Sayer CD, Simpson GL, Rose NL and Sadler JP (2015) Harnessing the potential of the multi-indicator palaeoecological approach: an assessment of the nature and causes of ecological change in a eutrophic shallow lake. *Freshwater Biology* 60: 1423–1442: doi:10.1111/fwb.12579.

Bennion H, Juggins S and Anderson NJ (1996) Predicting Epilimnetic Phosphorus Concentrations Using an Improved Diatom - Based Transfer Function and Its Application to Lake Eutrophication Management. *Environmental Science and Technology* 30(6): 2004–2007: doi:10.1021/es9508030.

Bennion H, Sayer CD, Tibby J and Carrick HJ (2010) Diatoms as indicators of environmental change in shallow lakes. In Smol JP and Stoermer EF (eds) *The Diatoms: Applications for the Environmental and Earth Sciences*. Cambridge University Press, 152–173.

Bešta T, Novák J, Dreslerová D, Jankovská V, Bernardová A, Lisá L, et al. (2015) Mid-Holocene history of a central European lake: Lake Komořany, Czech Republic. *Boreas* 44: 563–574: doi:10.1111/bor.12119.

Bešta T, Šafránková J, Pouzar M, Novák J and Nováková K (2009) Late Pleistocene-early Holocene transition recorded in the sediments of a former shallow lake in the Czech Republic. *Hydrobiologia* 631: 107–120: doi:http://dx.doi.org.ezproxy.is.cuni.cz/10.1007/s10750-009-9804-y.

Beug HJ (2004) *Leitfaden der Pollenbestimmung fur Mitteleuropa und angrenzende Gebiete*. Munchen: Friedrich Pfeil.

Beug HJ, Henrion I and Schmüser A (1999) *Landschaftsgeschichte im Hochharz. Die Entwicklung der Wälder und Moore seit dem Ende der letzten Eiszeit*. Papierflieger, Clausthal-Zellerfeld.

Birks HH (2000) Aquatic macrophyte vegetation development in Kråkenes Lake, western Norway, during the late-glacial and early-Holocene. *Journal of Paleolimnology* 23(1): 7–19. DOI: 10.1023/A:1008025116936.

Birks HH (2007) Plant macrofossil introduction. In *Encyclopedia of Quaternary science*. Elsevier, pp. 2266–2288. DOI: 10.1016/B0-44-452747-8/00215-5.

Birks HJB (2014) Challenges in the presentation and analysis of plant-macrofossil stratigraphical data. *Vegetation History and Archaeobotany* 23(3): 309–330. DOI: 10.1007/s00334-013-0430-2.

Birks HH and Ammann B (2000) Two terrestrial records of rapid climatic change during the glacial–Holocene transition (14,000 – 9,000 calendar years B. P.) from Europe. *Proceedings of the National Academy of Sciences of the USA* 97(4): 1390–1394.

Birks HH, Battarbee RW and Birks HJB (2000) The development of the aquatic ecosystem at Kråkenes Lake, western Norway, during the late-glacial and early-Holocene – a synthesis. *Journal of Paleolimnology* 23: 91–114: doi:10.1023/A.

Birks HH and Birks HJB (2006) Multi-proxy studies in palaeolimnology. *Vegetation History and Archaeobotany* 15: 235–251: doi:10.1007/s00334-006-0066-6.

Birks HJB, Heiri O, Seppä H and Bjune AE (2010) Strengths and Weaknesses of Quantitative Climate Reconstructions Based on Late-Quaternary Biological Proxies. *The Open Ecology Journal* 3: 68–110.

Birks HJB and Simpson GL (2013) 'Diatoms and pH reconstruction' (1990) revisited. *Journal of Paleolimnology* 49: 363–371: doi:10.1007/s10933-013-9697-7.

Björk S (2010) The Evolution of Lakes and Wetlands. In Eiseltová M (ed) *Restoration of Lakes, Streams, Floodplains, and Bogs in Europe. Wetlands: Ecology, Conservation and Management, vol. 3*. Dordrecht: Springer, 25–35: doi:10.1007/978-90-481-9265-6.

Björkvald L, Buffam I, Laudon H and Mörth C-M (2008) Hydrogeochemistry of Fe and Mn in small boreal streams: The role of seasonality, landscape type and scale. *Geochimica et Cosmochimica Acta* 72: 2789–2804: doi:10.1016/j.gca.2008.03.024.

Blais JM and Kalff J (1995) The influence of lake morphometry on sediment focusing. *Limnology and Oceanography* 40(3): 582–588.

Błońska E, Lasota J and Gruba P (2016) Effect of temperate forest tree species on soil dehydrogenase and urease activities in relation to other properties of soil derived from loess and glaciofluvial sand. *Ecological Research* 31(5): 655–664. DOI: 10.1007/s11284-016-1375-6.

Bobek P, Šamonil P and Jamrichová E (2018) Biotic controls on Holocene fire frequency in a temperate mountain forest, Czech Republic. *Journal of Quaternary Science* 33(8): 892–904. DOI: 10.1002/jqs.3067.

Bonk A, Müller D, Ramisch A, Kramkowski MA, Noryśkiewicz AM, Sekudewicz I, et al. (2021) Varve microfacies and chronology from a new sediment record of Lake Gościąz. *Quaternary Science Reviews* 251: 106715: doi:10.1016/j.quascirev.2020.106715.

Bos JAA, De Smedt P, Demiddele H, Hoek WZ, Langohr R, Marcelino V, et al. (2017) Multiple oscillations during the Lateglacial as recorded in a multi-proxy, high-resolution record of the Moervaart palaeolake (NW Belgium). *Quaternary Science Reviews* 162: 26–41: doi:10.1016/j.quascirev.2017.02.005.

Bos JAA, Geel B Van, Plicht J Van Der and Bohncke SJP (2007) Preboreal climate oscillations in Europe: Wiggle-match dating and synthesis of Dutch high-resolution multi-proxy records. *Quaternary Science Reviews* 26: 1927–1950: doi:10.1016/j.quascirev.2006.09.012.

Boyle J, Chiverrell R, Plater A, Thrasher I, Bradshaw E, Birks H, et al. (2013) Soil mineral depletion drives early Holocene lake acidification. *Geology* 41(4): 415–418: doi:10.1130/G33907.1.

Boyle JF (2001) Inorganic geochemical methods in palaeolimnology. In Last WM and Smol JP (eds) *Tracking environmental change using lake sediments. Volume 2: Physical and geochemical methods*. Dordrecht: Kluwer, 83–142.

Boyle JF (2007) Loss of apatite caused irreversible early-Holocene lake acidification. *The Holocene* 17(4): 543–547.

Boyle JF, Chiverrell RC and Schillereff D (2015) Approaches to water content correction and calibration for µXRF core scanning: Comparing X-ray scattering with simple regression of elemental concentrations. In Croudace I and Rothwell R (eds) *Micro-XRF Studies of Sediment Cores. Developments in Paleoenvironmental Research, vol. 17*. Dordrecht: Springer, 373–390: doi:https://doi.org/10.1007/978-94-017-9849-5_14.

Bradshaw EG, Anderson NJ, Jensen JP and Jeppesen E (2002) Phosphorus dynamics in Danish lakes and the implications for diatom ecology and palaeoecology. *Freshwater Biology* 47: 1963–1975: doi:10.1046/j.1365-2427.2002.00938.x.

Bradshaw EG, Jones VJ, Birks HJB and Birks HH (2000) Diatom responses to late-glacial and early-Holocene environmental changes at Kråkenes, western Norway. *Journal of Paleolimnology* 23: 21–34.

Bradshaw EG, Rasmussen P, Nielsen H and Anderson NJ (2005) Mid- to late-Holocene land-use change and lake development at Dallund Sø, Denmark: trends in lake primary production as reflected by algal and macrophyte remains. *The Holocene* 15(8): 1130–1142.

Bratt AR, Finlay JC, Welter JR, Vculek BA and Van Allen RE (2020) Co-limitation by N and P characterizes phytoplankton communities across nutrient availability and land use. *Ecosystems*. Springer US 23: 1121–1137: doi:10.1007/s10021-019-00459-6.

Brodersen KP and Lindegaard C (1999) Classification, assessment and trophic reconstruction of Danish lakes using chironomids. *Freshwater Biology* 42: 143–157.

Brodersen KP, Odgaard B V., Vestergaard O and John Anderson N (2001) Chironomid stratigraphy in the shallow and eutrophic Lake Søbygaard, Denmark: Chironomid-macrophyte co-occurrence. *Freshwater Biology* 46(2): 253–267: doi:10.1046/j.1365-2427.2001.00652.x.

Brodersen KP and Quinlan R (2006) Midges as palaeoindicators of lake productivity, eutrophication and hypolimnetic oxygen. *Quaternary Science Reviews* 25: 1995–2012: doi:10.1016/j.quascirev.2005.03.020.

Brodin YW (1986) The postglacial history of lake Flarken, southern Sweden, interpreted from subfossil insect remains. *Internationale Revue der gesamten Hydrobiologie und Hydrographie* 71(3): 371–432: doi:10.1002/iroh.19860710313.

Bronk Ramsey C (2008) Deposition models for chronological records. *Quaternary Science Reviews* 27: 42–60.

Bronk Ramsey C (2013) OxCal 4.2.4 online program. OxCal Project web. https://c14.arch.ox.ac.uk/oxcal/OxCal.html.

Bronk Ramsey C and Lee S (2013) Recent and Planned Developments of the Program Oxcal. *Radiocarbon* 55(2–3): 720–730.

Brooks N (2012) Beyond collapse: climate change and causality during the Middle Holocene Climatic Transition, 6400–5000 years before present. *Geografisk Tidsskrift-Danish Journal of Geography* 112(2): 93–104. DOI: 10.1080/00167223.2012.741881.

Brooks SJ, Langdon PG and Heiri O (2007) *The Identification and Use of Palaearctic Chironomidae Larvae. Palaeoecology, Technical Guide No. 10.* London: Quaternary Research Association.

Brooks SJ, Lowe JJ and Mayle FE (1997) The Late Devensian Lateglacial palaeoenvironmental record from Whitrig Bog, SE Scotland. 2. Chironomidae (Insecta: Diptera). *Boreas* 26: 297–308.

Brothers S, Köhler J, Attermeyer K, Grossart HP, Mehner T, Meyer N, et al. (2014) A feedback loop links brownification and anoxia in a temperate, shallow lake. *Limnology and Oceanography* 59(4): 1388–1398: doi:10.4319/lo.2014.59.4.1388.

Břízová E (2012) Historical Influence of Man on the Vegetation Record in the Prášilské Lake Sediments (Bohemian Forest, Czech Republic). *Folia Musei rerum naturalium Bohemiae occidentalis. Geologica et Paleobiologica* 46(1–2): 15–30. DOI: 10.2478/fbgp-2013-0002.

Buczkó K, Ács É, Báldi K, Pozderka V, Braun M, Kiss T, et al. (2019) The first high resolution diatom record from Lake Balaton , Hungary in Central Europe. *Limnetica* 38(1): doi:10.23818/limn.38.25.

Buczkó K, Szurdoki E, Braun M and Magyari E (2018) Reconciling diverse diatom-based lake responses to climate change in four mountain lakes in the South-Carpathian Mountains during the last 17 kyrs. *Quaternary International* 477: 117–137: doi:10.1016/j.quaint.2017.03.013.

Camarero L and Catalan J (2012) Atmospheric phosphorus deposition may cause lakes to revert from phosphorus limitation back to nitrogen limitation. *Nature Communications*. Nature Publishing Group 3: 1118: doi:10.1038/ncomms2125.

Canfield Jr. DE and Jones JR (1984) Assessing the trophic status of lakes with aquatic macrophytes. *Lake and Reservoir Management* 1(1): 446–451: doi:10.1080/07438148409354554.

Cantonati M and Lowe RL (2014) Lake benthic algae: toward an understanding of their ecology. *Freshwater Science* 33(2): 475–486: doi:10.1086/676140.

Cantonati M, Scola S, Angeli N, Guella G and Frassanito R (2009) Environmental controls of epilithic diatom depth-distribution in an oligotrophic lake characterized by marked water-level fluctuations. *European Journal of Phycology* 44(1): 15–29: doi:10.1080/09670260802079335.

Cardoso AC, Solimini A, Premazzi G, Carvalho L, Lyche A and Rekolainen S (2007) Phosphorus reference concentrations in European lakes. *Hydrobiologia* 584: 3–12: doi:10.1007/s10750-007-0584-y.

Carlson RE and Simpson J (1996) *A Coordinator's Guide to Volunteer Lake Monitoring Methods*. North American Lake Management Society.

Carpenter SR, Cole JJ, Kitchell JF and Pace ML (1998) Impact of dissolved organic carbon, phosphorus, and grazing on phytoplankton biomass and production in experimental lakes. *Limnology and Oceanography* 43(1): 73–80.

Carter VA, Chiverrell RC, Clear JL, Kuosmanen N, Moravcová A, Svoboda M, et al. (2018a) Quantitative Palynology Informing Conservation Ecology in the Bohemian/Bavarian Forests of Central Europe. *Frontiers in Plant Science* 8: 2268: doi:10.3389/fpls.2017.02268.

Carter VA, Moravcová A, Chiverrell RC, Clear JL, Finsinger W, Dreslerová D, et al. (2018b) Holocene-scale fire dynamics of central European temperate spruce-beech forests. *Quaternary Science Reviews* 191: 15–30: doi:10.1016/j.quascirev.2018.05.001.

Chmara R, Banaś K and Szmeja J (2015) Changes in the structural and functional diversity of macrophyte communities along an acidity gradient in softwater lakes. *Flora - Morphology, Distribution, Functional Ecology of Plants* 216: 57–64. DOI: 10.1016/j.flora.2015.09.002.

Clark JM, Bottrell SH, Evans CD, Monteith DT, Bartlett R, Rose R, et al. (2010) The importance of the relationship between scale and process in understanding long-term DOC dynamics. *Science of the Total Environment*. Elsevier B.V. 408: 2768–2775: doi:10.1016/j.scitotenv.2010.02.046.

Cohen AS (2003) *Paleolimnology: The History and Evolution of Lake Systems*. Oxford University Press.

Conley DJ and Schelske CL (2001) Biogenic silica. In Smol JP, Birks HJB and Last WM (eds) *Tracking Environmental Change Using Lake Sediments, Volume 3: Terrestrial, Algal, and Siliceous Indicators*. Dordrecht: Kluwer Academic Publishers, 281–294.

Corman JR, Bertolet BL, Casson NJ, Sebestyen SD, Kolka RK and Stanley EH (2018) Nitrogen and Phosphorus Loads to Temperate Seepage Lakes Associated With Allochthonous Dissolved Organic Carbon Loads. *Geophysical Research Letters* 45: 5481–5490: doi:10.1029/2018GL077219.

Creed IF, Bergström A-K, Trick CG, Grimm NB, Hessen DO, Karlsson J, et al. (2018) Global change-driven effects on dissolved organic matter composition: Implications for food webs of northern lakes. *Global Change Biology* 24: 3692–3714: doi:10.1111/gcb.14129.

Cremer H, Buijse AD, Lotter AF, Oosterberg W and Staras M (2004) The palaeolimnological potential of diatom assemblages in floodplain lakes of the Danube Delta, Romania: A pilot study. *Hydrobiologia* 513: 7–26: doi:10.1023/B:HYDR.0000033150.69898.30.

Čtvrtlíková M, Znachor P and Vrba J (2014) The effect of temperature on the phenology of germination of *Isoëtes lacustris*. *Preslia* 86: 279–292.

Čtvrtlíková M, Hejzlar J, Vrba J, et al. (2016) Lake water acidification and temperature have a lagged effect on the population dynamics of *Isoëtes echinospora* via offspring recruitment. *Ecological Indicators* 70: 420–430. DOI: 10.1016/j.ecolind.2016.06.042.

Curtis CJ, Juggins S, Clarke G, Battarbee RW, Kernan M, Catalan J, et al. (2009) Regional influence of acid deposition and climate change in European mountain lakes assessed using diatom transfer functions. *Freshwater Biology* 54: 2555–2572: doi:10.1111/j.1365-2427.2009.02317.x.

Daggett CT, Saros JE, Moraska B, Simon KS and Amirbahman A (2015) Effects of increased concentrations of inorganic nitrogen and dissolved organic matter on phytoplankton in boreal lakes with differing nutrient limitation patterns. *Aquatic Sciences*. Springer Basel 77: 511–521: doi:10.1007/s00027-015-0396-5.

Davison W (1993) Iron and manganese in lakes. *Earth-Science Reviews* 34: 119–163.

Deevey ES (1984) Stress, strain and stability in lacustrine ecosystems. In Haworth EY and Lund JWG (eds) *Lake Sediments and Environmental History*. Leicester: Leicester University Press.

Demiddele H, Finke P and Crombé P (2016) Diatom-based palaeoecology of a late-glacial palaeolake in the Moervaart area (northwestern Belgium) in relation to its prehistoric occupation. *Notae Praehistoricae* 36: 29–46.

DeNicola DM and Kelly M (2014) Role of periphyton in ecological assessment of lakes. *Freshwater Science* 33(2): 619–638: doi:10.1086/676117.

Denys L (1991) *A checklist of the diatoms in the Holocene deposits of the western Belgian coastal plain with a survey of their apparent ecological requirements. I. Introduction, ecological code and complete list*. Professional Paper Belgische Geologische Dienst 246.

Denys L (2007) Water-chemistry transfer functions for epiphytic diatoms in standing freshwaters and a comparison with models based on littoral sediment assemblages (Flanders, Belgium). *Journal of Paleolimnology* 38: 97–116: doi:10.1007/s10933-006-9064-z.

Denys L, Verbruggen C and Kiden P (1990) Palaeolimnological aspects of a Late-Glacial shallow lake in Sandy Flanders, Belgium. *Hydrobiologia* 214: 273–278: doi:10.1007/BF00050961.

Dong X, Bennion H, Battarbee R, Yang X, Yang H and Liu E (2008) Tracking eutrophication in Taihu Lake using the diatom record: potential and problems. *Journal of Paleolimnology* 40: 413–429: doi:10.1007/s10933-007-9170-6.

Drakare S, Blomqvist P, Bergström A-K and Jansson M (2002) Primary production and phytoplankton composition in relation to DOC input and bacterioplankton production in humic Lake Örträsket. *Freshwater Biology* 47: 41–52.

Dreslerová D (2012) Human Response to Potential Robust Climate Change around 5500 cal BP in the Territory of Bohemia (the Czech Republic). *Interdisciplinaria Archaeologica Natural Sciences in Archaeology* 3: 43–55.

Dreßler M, Schwarz A, Hübener T, Adler S and Scharf BW (2011) Use of sedimentary diatoms from multiple lakes to distinguish between past changes in trophic state and climate: evidence for climate change in northern Germany during the past 5, 000 years. *Journal of Paleolimnology* 45: 223–241: doi:10.1007/s10933-010-9494-5.

Dreßler M, Selig U, Dörfler W, Adler S, Schubert H and Hübener T (2006) Environmental changes and the Migration Period in northern Germany as reflected in the sediments of Lake Dudinghausen. *Quaternary Research* 66: 25–37: doi:10.1016/j.yqres.2006.02.007.

Drzymulska D, Fiłoc M, Kupryjanowicz M, Szeroczyńska K and Zieliński P (2015) Postglacial shifts in lake trophic status based on a multiproxy study of a humic lake. *Holocene* 25(3): 495–507: doi:10.1177/0959683614561881.

Du Laing G, Rinklebe J, Vandecasteele B, Meers E and Tack FMG (2009) Trace metal behaviour in estuarine and riverine floodplain soils and sediments: A review. *Science of the Total Environment*. Elsevier B.V. 407: 3972–3985: doi:10.1016/j.scitotenv.2008.07.025.

Dyson KE, Billett MF, Dinsmore KJ, Harvey F, Thomson AM, Piirainen S, et al. (2011) Release of aquatic carbon from two peatland catchments in E . Finland during the spring snowmelt period. *Biogeochemistry* 103: 125–142: doi:10.1007/s10533-010-9452-3.

Edwards AWF and Cavalli-Sforza LL (1965) A Method for Cluster Analysis. *Biometrics* 21(2): 362–375. DOI: 10.2307/2528096.

Edwards ME, Bigelow NH, Finney BP, et al. (2000) Records of aquatic pollen and sediment properties as indicators of late-Quaternary Alaskan lake levels. *Journal of Paleolimnology* 24(1): 55–68. DOI: 10.1023/A:1008117816612.

Eide F (1981) Key for northwest european Rosaceae pollen. *Grana* 20(2): 101–118.

Ejarque E, Khan S, Steniczka G, Schelker J, Kainz MJ and Battin TJ (2018) Climate-induced hydrological variation controls the transformation of dissolved organic matter in a subalpine lake. *Limnology and Oceanography* 63: 1355–1371: doi:10.1002/lno.10777.

Elser JJ, Andersen T, Baron JS, Bergström A, Jansson M, Kyle M, et al. (2009) Shifts in Lake N:P Stoichiometry and Nutrient Limitation Driven by Atmospheric Nitrogen Deposition. *Science* 326: 835–838.

Engels S, van Oostrom R, Cherli C, et al. (2018) Natural and anthropogenic forcing of Holocene lake ecosystem development at Lake Uddelermeer (The Netherlands). *Journal of Paleolimnology* 59(3): 329–347. DOI: 10.1007/s10933-017-0012-x.

Engstrom DR, Fritz SC, Almendinger JE and Juggins S (2000) Chemical and biological trends during lake evolution in recently deglaciated terrain. *Nature* 408: 161–166.

Enters D, Kirilova E, Lotter AF, Lücke A, Parplies J, Jahns S, et al. (2010) Climate change and human impact at Sacrower See (NE Germany) during the past 13,000 years: A geochemical record. *Journal of Paleolimnology* 43(4): 719–737: doi:10.1007/s10933-009-9362-3.

European Diatom Database – EDDI (2010). . Available at: http://craticula.ncl.ac.uk/Eddi/jsp/index.jsp.

Evans CD, Chapman PJ, Clark JM, Monteith DT and Cresser MS (2006) Alternative explanations for rising dissolved organic carbon export from organic soils. *Global Change Biology* 12: 2044–2053: doi:10.1111/j.1365-2486.2006.01241.x.

Ewing HA and Nater EA (2002) Holocene soil development on till and outwash inferred from lake-sediment geochemistry in Michigan and Wisconsin. *Quaternary Research* 57: 234–243: doi:10.1006/qres.2001.2303.

Faegri K and Iversen J (1989) *Textbook of Pollen Analysis. 4th Edition*. Chichester: John Wiley & Sons.

Fajer M, Waga JM, Rzetala M, et al. (2012) The Late Vistulian and Holocene evolution of Jezioro Lake: a record of environmental change in southern Poland found in deposits and landforms. *Journal of Paleolimnology* 48(4): 651–667. DOI: 10.1007/s10933-012-9634-1.

Falasco E and Bona F (2011) Diatom community biodiversity in an Alpine protected area: a study in the Maritime Alps Natural Park. *Journal of Limnology* 70(2): 157–167. DOI: 10.4081/jlimnol.2011.157.

Farmer AM and Spence DHN (1986) The growth strategies and distribution of isoetids in Scottish freshwater lochs. *Aquatic Botany* 26: 247–258. DOI: 10.1016/0304-3770(86)90025-2.

Fee EJ, Hecky RE, Kasian SEM and Cruikshank DR (1996) Effects of lake size, water clarity, and climatic variability on mixing depths in Canadian Shield lakes. *Limnology and Oceanography* 41(5): 912–920: doi:10.4319/lo.1996.41.5.0912.

Filippelli GM (2008) The Global Phosphorus Cycle: Past, Present, and Future. *Elements* 4: 89–95.

Finsinger W, Fevre J, Orbán I, et al. (2018) Holocene fire-regime changes near the treeline in the Retezat Mts. (Southern Carpathians, Romania). *Quaternary International* 477. Providing long environmental records of Late Quaternary climatic oscillations in the South Carpathian Retezat Mountains (PROLONG): 94–105. DOI: 10.1016/j.quaint.2016.04.029.

Finstad AG, Ingeborg PH, Ugedal O, Hesthagen T and Hessen DO (2014) Unimodal response of fish yield to dissolved organic carbon. *Ecology Letters* 17: 36–43: doi:10.1111/ele.12201.

Florescu G, Brown KJ, Carter VA, Kuneš P, Veski S and Feurdean A (2019) Holocene rapid climate changes and ice-rafting debris events reflected in high-resolution European charcoal records. *Quaternary Science Reviews* 222: 105877: doi:10.1016/j.quascirev.2019.105877.

Ford MS (Jesse) (1990) A 10 000-Yr History of Natural Ecosystem Acidification. *Ecological Monographs* 60(1): 57–89.

Fott J, Pražáková M, Stuchlík E and Stuchlíková Z (1994) Acidification of lakes in Šumava (Bohemia) and in the High Tatra Mountains (Slovakia). *Hydrobiologia* 274: 37–47.

Francis DR (2001) Bryozoan Statoblasts. In Smol JP, Birks HJB, and Last WM (eds) *Tracking environmental change using lake sediments: volume 4: zoological indicators*. Developments in Paleoenvironmental Research. Dordrecht: Springer Netherlands, pp. 105–123. DOI: 10.1007/0-306-47671-1_6.

Fredskild B (1992) The Greenland limnophytes - their present distribution and Holocene history. *Acta Botanica Fennica* (144): 93–113.

Frohne T, Rinklebe J, Diaz-Bone RA and Du Laing G (2011) Controlled variation of redox conditions in a floodplain soil: Impact on metal mobilization and biomethylation of arsenic and antimony. *Geoderma*. Elsevier B.V. 160: 414–424: doi:10.1016/j.geoderma.2010.10.012.

Fuhrmann A, Fischer T, Lücke A, Brauer A, Zolitschka B, Horsfield B, et al. (2004) Late Quaternary environmental and climatic changes in central Europe as inferred from the composition of organic matter in annually laminated maar lake sediments. *Geochemistry, Geophysics, Geosystems* 5: 11: doi:10.1029/2004GC000763.

Gacia E and Ballesteros E (1996) The effect of increased water level on *Isoetes lacustris* L. in Lake Baciver, Spain. *Journal of Aquatic Plant Management* 34: 57–59.

Gałka M, Tobolski K and Bubak I (2015) Late Glacial and Early Holocene lake level fluctuations in NE Poland tracked by macro-fossil, pollen and diatom records. *Quaternary International* 388: 23–38: doi:10.1016/j.quaint.2014.03.009.

Gavin AL, Nelson SJ, Klemmer AJ, Fernandez IJ, Strock KE and McDowell WH (2018) Acidification and Climate Linkages to Increased Dissolved Organic Carbon in High-Elevation Lakes Water Resources Research. *Water Resources Research* 54: 5376–5393: doi:10.1029/2017WR020963.

Gensemer RW, Smith REH and Duthie HC (1993a) Comparative effects of pH and aluminum on silica-limited growth and nutrient uptake in Asterionella ralfsii var. americana (Bacillariophyceae). *Journal of Phycology* 29: 36–44.

Gensemer RW, Smith REH and Duthie HC (1993b) pH Tolerance and Metal Toxicity in Populations of the Planktonic Diatom Asterionella: Influences of Synthetic and Natural Dissolved Organic Carbon. *Canadian Journal of Fisheries and Aquatic Sciences* 50: 121–132: doi:10.1139/f93-014.

Goslar T, Kuc T, Ralska-Jasiewiczowa M, Rózánski K, Arnold M, Bard E, et al. (1993) High-resolution lacustrine record of the Late Glacial/Holocene transition in Central Europe. *Quaternary Science Reviews* 12: 287–294.

Gottschalk S and Kahlert M (2012) Shifts in taxonomical and guild composition of littoral diatom assemblages along environmental gradients. *Hydrobiologia* 694(1): 41–56: doi:10.1007/s10750-012-1128-7.

Grimm EC (1987) CONISS: A FORTRAN 77 program for stratigraphically constrained cluster analysis by the method of incremental sum of squares. *Computers & Geosciences* 13(1): 13–35: doi:10.1016/0098-3004(87)90022-7.

Grybos M, Davranche M, Gruau G, Petitjean P and Pédrot M (2009) Increasing pH drives organic matter solubilization from wetland soils under reducing conditions. *Geoderma*. Elsevier B.V. 154: 13–19: doi:10.1016/j.geoderma.2009.09.001.

Grzybowski M, Endler Z and Jaworska B (2008) Disappearance of *Isoëtes lacustris* localities and changes in the phytolittoral of Lake Długie near Łukta. *Limnological Review* 8: 69–77.

Guiry MD and Guiry GM (2021) *AlgaeBase. World-wide electronic publication. National University of Ireland, Galway*. Available at: https://www.algaebase.org.

Guiter F, Andrieu-Ponel V, Digerfeldt G, et al. (2005) Vegetation history and lake-level changes from the Younger Dryas to the present in Eastern Pyrenees (France): pollen, plant macrofossils and lithostratigraphy from Lake Racou (2000 m a.s.l.). *Vegetation History and Archaeobotany* 14(2): 99–118. DOI: 10.1007/s00334-005-0065-z.

Hájková P, Pařil P, Petr L, Chattová B, Grygar TM and Heiri O (2016) A first chironomid-based summer temperature reconstruction (13-5 ka BP) around 49 N in inland Europe compared with local lake development. *Quaternary Science Reviews* 141: 94–111: doi:10.1016/j.quascirev.2016.04.001.

Hakanson L and Boulion V (2001) Regularities in Primary Production , Secchi Depth and Fish Yield and a New System to Define Trophic and Humic State Indices for Lake Ecosystems. *International Review in Hydrobiology* 86(1): 23–62.

Hall RI and Smol JP (2010a) Diatoms as indicators of lake eutrophication. In Stoermer E and Smol JP (eds) *The Diatoms: Applications for the Environmental and Earth Sciences*. Cambridge University Press, 122–151.

Hall RI and Smol JP (2010b) Diatoms as indicators of lake eutrophication. In Smol JP and Stoermer EF (eds) *The Diatoms: Applications for the Environmental and Earth Sciences*. Cambridge University Press, 122–151.

Hamburger K, Dall PC and Lindegaard C (1994) Energy metabolism of Chironomus anthracinus (Diptera: Chironomidae) from the profundal zone of Lake Esrom, Denmark, as a function of body size, temperature and oxygen concentration. *Hydrobiologia* 294: 43–50.

Havens KE and Nürnberg GK (2004) The Phosphorus-Chlorophyll Relationship in Lakes: Potential Influences of Color and Mixing Regime. *Lake and Reservoir Management* 20(3): 188–196: doi:10.1080/07438140409354243.

Haworth EY (1976) Two Late-Glacial (Late Devensian) Diatom Assemblage Profiles from Northern Scotland. *The New Phytologist* 77(1): 227–256.

Heggen MP, Birks HH, Heiri O, et al. (2012) Are fossil assemblages in a single sediment core from a small lake representative of total deposition of mite, chironomid, and plant macrofossil remains? *Journal of Paleolimnology* 48(4): 669–691. DOI: 10.1007/s10933-012-9637-y.

Heinsalu A, Luup H, Alliksaar T, Nõges P and Nõges T (2008) Water level changes in a large shallow lake as reflected by the plankton : periphyton-ratio of sedimentary diatoms. *Hydrobiologia* 599: 23–30: doi:10.1007/978-1-4020-8379-2.

Heiri O, Brooks SJ, Renssen H, Bedford A, Hazekamp M, Ilyashuk B, et al. (2014) Validation of climate model-inferred regional temperature change for late-glacial Europe. *Nature Communications* 5: 1–7: doi:10.1038/ncomms5914.

Heiri O and Lotter AF (2001) Effect of low count sums on quantitative environmental reconstructions: an example using subfossil chironomids. *Journal of Paleolimnology* 26: 343–350.

Heiri O, Lotter AF and Lemcke G (2001) Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. *Journal of Paleolimnology* 25: 101–110.

Hill MO (1973) Diversity and Evenness: A Unifying Notation and Its Consequences. *Ecology* 54(2): 427–432.

Hobbs WO, Edlund MB, Umbanhowar CE, et al. (2017) Holocene evolution of lakes in the forest-tundra biome of northern Manitoba, Canada. *Quaternary Science Reviews* 159: 116–138. DOI: 10.1016/j.quascirev.2017.01.014.

Hofmann AM, Geist J, Nowotny L and Raeder U (2020) Depth-distribution of lake benthic diatom assemblages in relation to light availability and substrate: implications for paleolimnological studies. *Journal of Paleolimnology*. Springer Netherlands 64: 315–334: doi:10.1007/s10933-020-00139-9.

Holcová K, Suchánek V, Heřmanová Z and Vondrák D (2020) The first evidence of bioerosion in chitinous lacustrine microfossils from glacial lakes (Lake Pleistocene and Holocene, Bohemian Forest). *Palaios* 35: 317–326.

Hongve D, Riise G and Kristiansen JF (2004) Increased colour and organic acid concentrations in Norwegian forest lakes and drinking water – a result of increased precipitation? *Aquatic Sciences* 66(2): 231–238. DOI: 10.1007/s00027-004-0708-7.

Hošek J, Pokorný P, Kubovčík V, Horáček I, Žáčková P, Kadlec J, et al. (2014) Late glacial climatic and environmental changes in eastern-central Europe: Correlation of multiple biotic and abiotic proxies from the Lake Švarcenberk, Czech Republic. *Palaeogeography, Palaeoclimatology, Palaeoecology* 396: 155–172: doi:10.1016/j.palaeo.2013.12.024.

Houfková P, Bešta T, Bernardová A, Vondrák D, Pokorný P and Novák J (2017) Holocene climatic events linked to environmental changes at Lake Komořany Basin, Czech Republic. *The Holocene* 27(8): 1132–1145: doi:10.1177/0959683616683250.

Houk V (2003) *Atlas of Freshwater Centric Diatoms with a Brief Key and Descriptions, Part I. Melosiraceae, Orthoseiraceae, Paraliaceae and Aulacoseiraceae*. Palacký University Olomouc.

Houk V, Klee R (2007) Atlas of freshwater centric diatoms with a brief key and descriptions, Part II. Melosiraceae and Aulacoseiraceae (Supplement to Part I). *Fottea* 7:85–255

Hruška J, Krám P, McDowell WH and Oulehle F (2009) Increased Dissolved Organic Carbon (DOC) in Central European Streams is Driven by Reductions in Ionic Strength Rather than Climate Change or Decreasing Acidity. *Environmental Science and Technology* 43: 4320–4326.

Hu FS, Finney BP and Brubaker LB (2001) Effects of Holocene Alnus Expansion on Aquatic Productivity, Nitrogen Cycling, and Soil Development in Southwestern Alaska. *Ecosystems* 4: 358–368: doi:10.1007/s10021.

Hurník S (1969) Příspěvek ke geologické problematice tzv. Komořanského jezera [Contribution to the geology of so-called Lake Komořany]. *Mostecko-Litvínovsko, Regionální Studie* 6(5): 5–14.

Hutchinson GE (1975) *A Treatise on Limnology, Vol. 3: Limnological Botany*. 2nd Printing edition. New York: John Wiley & Sons Inc.

Huvane JK and Whitehead DR (1996) The paleolimnology of North Pond: watershed-lake interactions. *Journal of Paleolimnology* 16(3): 323–354: doi:10.1007/BF00207576.

Int Panis L, Goddeeris B and Verheyen RF (1995) On the relationship between the oxygen microstratification in a pond and the spatial distribution of the benthic chironomid fauna. In Cranston P (ed) *From genes to ecosystems*. Canberra: C.S.I.R.O., 482.

Itkonen A, Marttila V, Meriläinen JJ and Salonen V (1999) 8000-year history of palaeoproductivity in a large boreal lake. *Journal of Paleolimnology* 21: 271–294.

Jankovská V (1983) Palynologische Forschung am ehemaligen Komořany-See (Spätglazial bis Subatlantikum). *Věstník Ústředního ústavu geologického* 58(2): 99–107.

Jankovská V (1984) Radiokarbondatierung der Sedimente aus dem ehemaligen Komořany-See (NW-Böhmen). *Věstník Ústředního ústavu geologického* 59(4): 235–236.

Jankovská V (1988) Palynologische Erforschung archäologischer Proben aus dem Komořanské jezero-See bei Most (NW-Böhmen). *Folia Geobotanica et Phytotaxonomica* 23: 45–77.

Jankovská V (2006) Late Glacial and Holocene history of Plešné Lake and its surrounding landscape based on pollen and palaeoalgological analyses. *Biologia* 61(20): S371–S385. DOI: 10.2478/s11756-007-0064-x.

Jankovská V and Komárek J (2000) Indicative value of *Pediastrum* and other coccal green algae in palaeoecology. *Folia Geobotanica* 35(1): 59–82. DOI: 10.1007/BF02803087.

Jankovská V and Pokorný P (2013) Reevaluation of the palaeoenvironmental record of the former Komořanské jezero lake: late-glacial and Holocene palaeolimnology and vegetation development in north-western Bohemia,Czech Republic. *Preslia* 85: 265–287.

Jeppesen E, Sondergaard M, Sondergaard M and Christoffersen K (1998) *The Structuring Role of Submerged Macrophytes in Lakes. Ecological Studies*. Springer-Science+Business Media, LLC: doi:10.1007/978-1-4612-0695-8.

Jin Z, Cao J, Wu J and Wang S (2006) A Rb/Sr record of catchment weathering response to Holocene climate change in Inner Mongolia. *Earth Surface Processes and Landforms* 31: 285–291: doi:10.1002/esp.1243.

Johnsen GH and Raddum GG (1987) A morphological study of two populations of Bosmina longispina exposed to different predation. *Journal of Plankton Research* 9(2): 297–304.

Jones RI (1992) The influence of humic substances on lacustrine planktonic food chains. *Hydrobiologia* 229: 73–91.

Jost L (2006) Entropy and diversity. *Oikos* 113(2): 363–375.

Juggins S (2001a) *European Diatom Database*. . Available at: http://craticula.ncl.ac.uk/Eddi/jsp/.

Juggins S (2001b) *The European Diatom Database - User Guide*. .

Juggins S (2007) C2 Version 1.5: User guide. Software for ecological and palaeoecological data analysis and visualisation. Newcastle upon Tyne: Newcastle University.

Juggins S (2012) Rioja: analysis of Quaternary science data. R package version 0.7-3.

Juggins S (2013) Quantitative reconstructions in palaeolimnology: New paradigm or sick science? *Quaternary Science Reviews*. Elsevier Ltd 64: 20–32: doi:10.1016/j.quascirev.2012.12.014.

Juggins S and Birks HJB (2012) Quantitative environmental reconstructions from biological data. In Birks HJB, Lotter AF, Juggins S and Smol JP (eds) *Tracking Environmental Change Using Lake Sediments, Vol. 5: Data Handling and Numerical Techniques*. Springer, 431–494.

Jüttner I, Lintelmann J, Michalke B, Winkler R, Steinberg CEW and Kettrup A (1997) The acidification of the Herrenwieser See, Black Forest, Germany, before and during industrialisation. *Water Research* 31(5): 1194–1206: doi:10.1016/S0043-1354(96)00381-8.

Kalis AJ, Merkt J and Wunderlich J (2003) Environmental changes during the Holocene climatic optimum in central Europe - human impact and natural causes. *Quaternary Science Reviews* 22: 33–79.

Kankaala P, Arvola L, Hiltunen M, Huotari J, Jones RI, Nykänen H, et al. (2019) Ecosystem responses to increased organic carbon concentration: comparing results based on long-term monitoring and whole-lake experimentation. *Inland Waters*. Taylor & Francis 9(4): 489–502: doi:10.1080/20442041.2019.1600938.

Karlsson J, Byström P, Ask J, Ask P, Persson L and Jansson M (2009) Light limitation of nutrient-poor lake ecosystems. *Nature* 460(7254): 506–509: doi:10.1038/nature08179.

Kayranli B, Scholz M, Mustafa A and Hedmark Å (2010) Carbon storage and fluxes within freshwater wetlands: a critical review. *Wetlands* 30: 111–124: doi:10.1007/s13157-009-0003-4.

Kelly MG, King L, Jones RI, Barker PA and Jamieson BJ (2008) Validation of diatoms as proxies for phytobenthos when assessing ecological status in lakes. *Hydrobiologia* 610: 125–129: doi:10.1007/s10750-008-9427-8.

Kelly MG, Phillips G, Juggins S and Willby NJ (2020) Re-evaluating expectations for river phytobenthos assessment and understanding the relationship with macrophytes. *Ecological Indicators*. Elsevier 117: 106582: doi:10.1016/j.ecolind.2020.106582.

Kelly PT, Solomon CT, Zwart JA and Jones SE (2018) A framework for understanding variation in pelagic gross primary production of lake ecosystems. *Ecosystems*. Springer US 21: 1364–1376: doi:10.1007/s10021-018-0226-4.

Killick R, Fearnhead P and Eckley IA (2012) Optimal detection of changepoints with a linear computational cost. *Journal of the American Statistical Association* 107(500): 1590–1598. DOI: 10.1080/01621459.2012.737745.

Kilham P (1971) A hypothesis concerning silica and the freshwater planktonic diatoms. *Limnology and Oceanography* 16(1): 10–18.

Kilham SS, Theriot EC and Fritz SC (1996) Linking planktonic diatoms and climate change in the large lakes of the Yellowstone ecosystem using resource theory. *Limnology and Oceanography* 41(5): 1052–1062. DOI: 10.4319/lo.1996.41.5.1052.

Kirilova E, Heiri O, Enters D, Cremer H, Lotter AF, Zolitschka B, et al. (2009) Climate-induced changes in the trophic status of a Central European lake. *Journal of Limnology* 68(1): 71–82: doi:10.4081/jlimnol.2009.71.

Kissman CEH, Williamson CE, Rose KC and Saros JE (2013) Response of phytoplankton in an alpine lake to inputs of dissolved organic matter through nutrient enrichment and trophic forcing. *Limnology and Oceanography* 58(3): 867–880: doi:10.4319/lo.2013.58.3.0867.

Klamt A-M, Poulsen SP, Odgaard BV, Hübener T, McGowan S, Jensen HS, et al. (2021) Holocene lake phosphorus species and primary producers reflect catchment processes in a small, temperate lake. *Ecological Monographs* 91: 3: doi:10.1002/ecm.1455.

Klaus M, Karlsson J and Seekell D (2021) Tree line advance reduces mixing and oxygen concentrations in arctic-alpine lakes through wind sheltering and organic carbon supply. *Global Change Biology* 27(18): 4238–4253: doi:10.1111/gcb.15660.

Kletetschka G, Vondrák D, Hruba J, Prochazka V, Nabelek L, Svitavská-svobodová H, et al. (2018) Cosmic-Impact Event in Lake Sediments from Central Europe Postdates the Laacher See Eruption and Marks Onset of the Younger Dryas. *Journal of Geology* 126(6): 561–575: doi:10.1086/699869.

Klimaszyk P and Heymann D (2011) Vertical distribution of benthic macroinvertebrates in a meromictic lake (Lake Czarne, Drawienski National Park). *Oceanological and Hydrobiological Studies* 39: 99–106. DOI: 10.2478/v10009-010-0048-y.

Klimaszyk P, Borowiak D, Piotrowicz R, et al. (2020) The effect of human impact on the water quality and biocoenoses of the soft water lake with isoetids: Lake Jeleń, NW Poland. *Water* 12(4). DOI: 10.3390/w12040945.

Kołaczek P, Mirosław-Grabowska J, Karpińska-Kołaczek M and Stachowicz-Rybka R (2015) Regional and local changes inferred from lacustrine organic matter deposited between the Late Glacial and mid-Holocene in the Skaliska

Basin (north-eastern Poland). *Quaternary International* 388: 51–63: doi:10.1016/j.quaint.2014.04.024.

Komárek J and Jankovská V (2001) Review of the green algal genus Pediastrum; Implication for pollen-analytical research. *Bibliotheca Phycologica* 108.

Kopáček J, Hejzlar J, Porcal P, and Nedoma J (1999) Chemistry of Prášilské Lake and its tributaries during the 1998 summer temperature stratification. *Silva Gabreta 3:* 33–47.

Kopáček J, Stuchlík E, Veselý J, et al. (2002) Hysteresis in reversal of Central European mountain lakes from atmospheric acidification. *Water, Air and Soil Pollution: Focus* 2(2): 91–114. DOI: 10.1023/A:1020190205652.

Kopáček J, Borovec J, Hejzlar J, Ulrich K-U, Norton SA and Amirbahman A (2005) Aluminum Control of Phosphorus Sorption by Lake Sediments. *Environmental Science and Technology* 39: 8784–8789.

Kopáček J, Hejzlar J, Kana J, et al. (2009) Trends in aluminium export from a mountainous area to surface waters, from deglaciation to the recent: effects of vegetation and soil development, atmospheric acidification, and nitrogen-saturation. *Journal of inorganic biochemistry* 103(11): 1439–1448. DOI: 10.1016/j.jinorgbio.2009.07.019.

Kopáček J, Hejzlar J, Kaňa J, Norton SA and Stuchlík E (2015) Effects of Acidic Deposition on in-Lake Phosphorus Availability: A Lesson from Lakes Recovering from Acidification. *Environmental Science and Technology* 49: 2895−2903: doi:10.1021/es5058743.

Kopáček J, Hejzlar J, Vrba J and Stuchlík E (2011) Phosphorus loading of mountain lakes: Terrestrial export and atmospheric deposition. *Limnology and Oceanography* 56(4): 1343–1354: doi:10.4319/lo.2011.56.4.1343.

Korhola A, Virkanen J, Tikkanen M, et al. (1996) Fire-induced pH rise in a naturally acid hill-top lake, southern Finland: a palaeoecological survey. *Journal of Ecology* 84(2): 257–265. DOI: 10.2307/2261361.

Korsman T, Renberg I and Anderson NJ (1994) A palaeolimnological test of the influence of Norway spruce (Picea abies) immigration on lake-water acidity. *The Holocene* 4(2): 132–140: doi:10.1177/095968369400400203.

Kortelainen P (1993) Content of total organic carbon in Finnish lakes and relationship to catchment characteristics. *Canadian Journal of Fisheries and Aquatic Sciences* 50: 1477–1483.

Kozáková R, Bobek P, Dreslerová D, Abraham V and Svobodová-Svitavská H (2021) The prehistory and early history of the Šumava Mountains (Czech Republic) as seen through anthropogenic pollen indicators and charcoal data. *The Holocene* 31(1): 145–159: doi:10.1177/0959683620961484.

Krammer K (2000) Diatoms of Europe. Diatoms of the European inland waters and comparable habitats. The Genus *Pinnularia* (vol. 1). A.R.G. Gantner Verlag K.G, Ruggell.

Krammer K (2002) Diatoms of Europe. Diatoms of the European inland waters and comparable habitats. *Cymbella* (vol. 3). A.R.G. Gantner Verlag K.G, Ruggell.

Krammer K (2003) Diatoms of Europe. Diatoms of the European inland waters and comparable habitats. *Cymbopleura*, *Delicata*, *Navicymbula*, *Gomphocymbellopsis*, *Afrocymbella* (vol. 4). A.R.G. Gantner Verlag K.G, Ruggell.

Krammer K, Lange-Bertalot H (1986) Bacillariophyceae, 1. Teil: Naviculaceae. In Ettl H, Gerloff J, Heynig H, et al. (eds), *Süßwasserflora von Mitteleuropa (Band 2/1)*. Gustav Fischer Verlag, Jena.

Krammer K, and Lange-Bertalot H (1988) Bacillariophyceae, 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae. In Ettl H, Gerloff J, Heynig H, et al. (eds) *Süßwasserflora von Mitteleuropa (Band 2/2)*. Jena: Gustav Fischer Verlag.

Krammer, K., and Lange-Bertalot, H. (1991a). Bacillariophyceae, 3. Teil: Centrales, Fragilariaceae, Eunotiaceae. In Ettl H, Gerloff J, Heynig H, et al. (eds) *Süßwasserflora von Mitteleuropa (Band 2/3)*. Jena: Gustav Fischer Verlag.

Krammer, K., and Lange-Bertalot, H. (1991b). Bacillariophyceae, 4. Teil: Achnanthaceae, Kritische Ergänzungen zu *Navicula* (Lineolatae) und Gomphonema. In Ettl H, Gerloff J, Heynig H, et al. (eds) *Süßwasserflora von Mitteleuropa (Band 2/4)*. Jena: Gustav Fischer Verlag.

Kritzberg ES (2017) Centennial-long trends of lake browning show major effect of afforestation. *Limnology And Oceanography Letters* 2: 105–112: doi:10.1002/lol2.10041.

Kritzberg ES and Ekström SM (2012) Increasing iron concentrations in surface waters – a factor behind brownification? *Biogeosciences* 9: 1465–1478: doi:10.5194/bg-9-1465-2012.

Kröpfelová L, Vymazal J, Švehla J and Štíchová J (2009) Removal of trace elements in three horizontal sub-surface flow constructed wetlands in the Czech Republic. *Environmental Pollution* 157: 1186–1194: doi:10.1016/j.envpol.2008.12.003.

Kubečka J, Frouzova J, Cech M, Peterka J, Ketelaars H, Wagenwoort A, et al. (2000) Hydroacoustic assessment of pelagic stages of freshwater insects. *Aquatic Living Resources* 13(5): 361–366.

Kubicki JD and Heaney PJ (2003) Molecular orbital modeling of aqueous organosilicon complexes: Implications for silica biomineralization. *Geochimica et Cosmochimica Acta* 67(21): 4113–4121: doi:10.1016/S0016-7037(03)00093-3.

Kubovčík V, Hošek J, Heiri O, Rojik F, Vaterková S, Trubač J, et al. (2021) Chironomid-based temperature and environmental reconstructions of the Last Glacial Termination in southern Bohemia, Czech Republic. *Palaeogeography, Palaeoclimatology, Palaeoecology* 567: 110239: doi:10.1016/j.palaeo.2021.110239.

Kuneš P, Odgaard BV and Gaillard M (2011) Soil phosphorus as a control of productivity and openness in temperate interglacial forest ecosystems. *Journal of Biogeography* 38: 2150–2164.

Kuneš P, Pelánková B, Chytrý M, Jankovská V, Pokorný P and Petr L (2008) Interpretation of the last-glacial vegetation of eastern-central Europe using modern analogues from southern Siberia. *Journal of Biogeography* 35: 2223–

2236: doi:10.1111/j.1365-2699.2008.01974.x.

Kylander ME, Klaminder J, Wohlfarth B and Löwemark L (2013) Geochemical responses to paleoclimatic changes in southern Sweden since the late glacial: The Hässeldala Port lake sediment record. *Journal of Paleolimnology* 50: 57–70: doi:10.1007/s10933-013-9704-z.

Laird KR, Kingsbury M V and Cumming BF (2010) Diatom habitats, species diversity and water-depth inference models across surface-sediment transects in Worth Lake, northwest Ontario, Canada. *Journal of Paleolimnology* 44: 1009–1024: doi:10.1007/s10933-010-9470-0.

Lambers H, Raven JA, Shaver GR and Smith SE (2008) Plant nutrient-acquisition strategies change with soil age. *Trends in Ecology and Evolution* 23(2): 95–103: doi:10.1016/j.tree.2007.10.008.

Lampert W and Sommer U (2007) *Limnoecology*. Oxford University Press.

Lange-Bertalot H (2001) Diatoms of Europe. Diatoms of Europeaen inland waters and comparable habitats. *Navicula* sensu stricto, 10 Genera Separated from *Navicula* sensu lato, *Frustulia* (vol. 2). Ruggell: A.R.G. Gantner Verlag K.G.

Lange-Bertalot H, Metzeltin D (1996) Indicators of oligotrophy. 800 taxa representative of three ecologically distinct lakes. Carbonate buffered – oligodystrophic - weakly buffered soft water. *Iconographia Diatomologica* 2: 1–390.

Lange-Bertalot H, Bąk M, and Witkowski A (2011) Diatoms of Europe. Diatoms of the european inland waters and comparable habitats. *Eunotia* and some related genera (vol. 6). Ruggell: A.R.G.Gantner Verlag K.G.

Lange-Bertalot H, Hofmann G, Werum M and Cantonati M (2017) *Freshwater benthic diatoms of Central Europe: over 800 common species used in ecological assessments*. Koeltz Botanical Books.

Laudon H, Berggren M, Agren A, Buffam I, Bishop K, Grabs T, et al. (2011) Patterns and dynamics of dissolved organic carbon (DOC) in boreal streams: The role of processes, connectivity, and scaling. *Ecosystems* 14: 880–893: doi:10.1007/s10021-011-9452-8.

Laurion I, Ventura M, Catalan J, Psenner R and Sommaruga R (2000) Attenuation of ultraviolet radiation in mountain lakes: Factors controlling the among- and within-lake variability. *Limnology and Oceanography* 45(6): 1274–1288.

Le Stum-Boivin É, Magnan G, Garneau M, Fenton NJ, Grondin P and Bergeron Y (2019) Spatiotemporal evolution of paludification associated with autogenic and allogenic factors in the black spruce–moss boreal forest of Québec, Canada. *Quaternary Research* 91(2): 650–664: doi:10.1017/qua.2018.101.

Leavitt PR, Findlay DL, Hall RI and Smol JP (1999) Algal responses to dissolved organic carbon loss and pH decline during whole-lake acidification: Evidence from paleolimnology. *Limonology And Oceanography* 44(3): 757–773: doi:10.4319/lo.1999.44.3_part_2.0757.

Legendre P and Legendre L (1998) *Numerical Ecology*. Amsterdam: Elsevier.

Leira M, Filippi ML and Cantonati M (2015) Diatom community response to extreme water-level fluctuations in two Alpine lakes: a core case study. *Journal of Paleolimnology* 53: 289–307: doi:10.1007/s10933-015-9825-7.

Lewis WM and Wurtsbaugh WA (2008) Control of lacustrine phytoplankton by nutrients: Erosion of the phosphorus paradigm. *International Review in Hydrobiology* 93(4–5): 446–465: doi:10.1002/iroh.200811065.

Leys B, Higuera PE, McLauchlan KK, et al. (2016) Wildfires and geochemical change in a subalpine forest over the past six millennia. *Environmental Research Letters* 11. DOI: 10.1088/1748-9326/11/12/125003.

Liehu A, Sandman O and Simola H (1986) Effects of peatbog ditching in lakes: Problems in paleolimnological interpretation. *Hydrobiologia* 143(1): 417–424: doi:10.1007/BF00026692.

Likens GE (ed) (1972) *Nutrients and eutrophication: the limiting-nutrient controversy*. American Society of Limnology and Oceanography.

Lindegaard C (1995) Classification of water-bodies and pollution. In Armitage PD, Cranston P and Pinder LCV (eds) *The Chironomidae: Biology and Ecology of Non-Biting Midges*. London: Chapman & Hall, 385–404.

Losert H (1940) Beiträge zur spät- und nacheiszeitlichen Vegetationsgeschichte Innerböhmens, I. Der Kommerner See. *Beihefte zum Botanischen Centralblatt* 60B: 346–394.

Lotter AF and Hölzer A (1994) A high-resolution late-glacial and early Holocene environmental history of Rotmeer, southern Black Forest (Germany). *Dissertationes Botanicae* (234): 365–388.

Lotter AF (1999) Late-glacial and Holocene vegetation history and dynamics as shown by pollen and plant macrofossil analyses in annually laminated sediments from Soppensee, central Switzerland. *Vegetation History and Archaeobotany* 8: 165–184.

Lotter AF, Birks HJB, Hofmann W and Marchetto A (1998) Modern diatom, cladocera, chironomid, and chrysophyte cyst assemblages as quantitative indicators for the reconstruction of past environmental conditions in the Alps. II. Nutrients. *Journal of Paleolimnology* 19: 443–463.

Lühne V (1897) Über ein subfossiles Vorkommen von Diatomaceen in Böhmen. *Österreichische botanische Zeitschrift* 9.

Lundström US, Van Breemen N and Bain D (2000) The podzolization process. A review. *Geoderma* 94: 91–107.

Luoto TP (2010) Hydrological change in lakes inferred from midge assemblages through use of an intralake calibration set. *Ecological Monographs* 80(2): 303–329. DOI: 10.1890/09-0498.1.

MacArthur RH (1957) On the relative abundance of bird species. *Proceedings of the National Academy of Sciences* 43:

293–295.

Magny M (2004) Holocene climate variability as reflected by mid-European lake-level fluctuations and its probable impact on prehistoric human settlements. *Quaternary International* 113(1): 65–79: doi:10.1016/S1040-6182(03)00080-6.

Magny M, Bégeot C, Guiot J, et al. (2003) Contrasting patterns of hydrological changes in Europe in response to Holocene climate cooling phases. *Quaternary Science Reviews* 22(15): 1589–1596. DOI: 10.1016/S0277-3791(03)00131-8.

Magny M, Leuzinger U, Bortenschlager S and Haas JN (2006) Tripartite climate reversal in Central Europe 5600–5300 years ago. *Quaternary Research* 65: 3–19: doi:10.1016/j.yqres.2005.06.009.

Makri S, Rey F, Gobet E, Gilli A, Tinner W and Grosjean M (2020) Early human impact in a 15,000-year high-resolution hyperspectral imaging record of paleoproduction and anoxia from a varved lake in Switzerland. *Quaternary Science Reviews*. Elsevier Ltd 239: 106335: doi:10.1016/j.quascirev.2020.106335.

Makri S, Wienhues G, Bigalke M, Gilli A, Rey F, Tinner W, et al. (2021) Variations of sedimentary Fe and Mn fractions under changing lake mixing regimes, oxygenation and land surface processes during Late-glacial and Holocene times. *Science of the Total Environment* 755: 143418: doi:10.1016/j.scitotenv.2020.143418.

Mariani M, Beck KK, Fletcher M-S, et al. (2018) Biogeochemical responses to Holocene catchment-lake dynamics in the Tasmanian World Heritage Area, Australia. *Journal of Geophysical Research: Biogeosciences* 123(5): 1610–1624. DOI: 10.1029/2017JG004136.

Matěna J (1989) Seasonal dynamics of a Chironomus plumosus (L.) (Diptera, Chironomidae) population from a fish pond in southern Bohemia. *Internationale Revue der gesamten Hydrobiologie und Hydrographie* 74(6): 599–610.

Mattsson T, Kortelainen P, Laubel A, Evans D, Pujo-Pay M, Räike A, et al. (2009) Export of dissolved organic matter in relation to land use along a European climatic gradient. *Science of the Total Environment*. Elsevier B.V. 407: 1967–1976: doi:10.1016/j.scitotenv.2008.11.014.

Mauri A, Davis BAS, Collins PM and Kaplan JO (2015) The climate of Europe during the Holocene: a gridded pollen-based reconstruction and its multi-proxy evaluation. *Quaternary Science Reviews* 112: 109–127: doi:10.1016/j.quascirev.2015.01.013.

McLauchlan KK, Williams JJ, Craine JM and Jeffers ES (2013) Changes in global nitrogen cycling during the Holocene epoch. *Nature*. Nature Publishing Group 495: 352–355: doi:10.1038/nature11916.

Mendyk Ł, Markiewicz M, Bednarek R, Świtoniak M, Gamrat WW, Krześlak I, et al. (2016) Environmental changes of a shallow kettle lake catchment in a young glacial landscape (Sumowskie Lake catchment), North-Central Poland. *Quaternary International* 418: 116–131: doi:10.1016/j.quaint.2015.10.008.

Mentlík P, Minár J, Břízová E, et al. (2010) Glaciation in the surroundings of Prášilské Lake (Bohemian Forest, Czech Republic). *Geomorphology* 117(1): 181–194. DOI: 10.1016/j.geomorph.2009.12.001.

Mentlík P, Engel Z, Braucher R, Léanni L and Team A (2013) Chronology of the Late Weichselian glaciation in the Bohemian Forest in Central Europe. *Quaternary Science Reviews* 65: 120–128: doi:10.1016/j.quascirev.2013.01.020.

Meyer-Jacob C, Michelutti N, Paterson AM, Cumming BF, Keller W (Bill) and Smol JP (2019) The browning and re-browning of lakes : Divergent lake-water organic carbon trends linked to acid deposition and climate change. *Scientific Reports*. Springer US 9: 16676: doi:10.1038/s41598-019-52912-0.

Meyer-Jacob C, Michelutti N, Paterson AM, Monteith D, Yang H, Weckström J, et al. (2017) Inferring Past Trends in Lake Water Organic Carbon Concentrations in Northern Lakes Using Sediment Spectroscopy. *Environmental Science and Technology* 51: 13248–13255: doi:10.1021/acs.est.7b03147.

Meyer-Jacob C, Tolu J, Bigler C, Yang H and Bindler R (2015) Early land use and centennial scale changes in lake-water organic carbon prior to contemporary monitoring. *Proceedings of the National Academy of Sciences* 112: 6579–6584: doi:10.1073/pnas.1501505112.

Meyers PA and Ishiwatari R (1993) Lacustrine organic geochemistry - an overview of indicators of organic matter sources and diagenesis in lake sediments. *Organic Geochemistry* 20(7): 867–900.

Meyers PA and Lallier-Vergès E (1999) Lacustrine sedimentary organic matter records of Late Quaternary paleoclimates. *Journal of Paleolimnology* 21: 345–372.

Mirosław-Grabowska J and Zawisza E (2018) Reaction of the lake environment to the Holocene warming depending on the distance to the maximum extent of the Vistulian ice sheet. *Catena* 171: 494–504: doi:10.1016/j.catena.2018.07.033.

Monteith DT, Stoddard JL, Evans CD, Wit HA De, Forsius M, Hogasen T, et al. (2007) Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature* 450: 537–541: doi:10.1038/nature06316.

Moore PD, Webb JA and Collinson ME (1999) *Pollen Analysis*. Oxford: Blackwell Science.

Moravcová A, Tichá A, Carter VA, Vondrák D, Čtvrtlíková M, Van Leeuwen JFN, et al. (2021) Mountain aquatic Isoëtes populations reflect millennial-scale environmental changes in the Bohemian Forest Ecosystem, Central Europe. *The Holocene* 31(5): 746–759: doi:10.1177/0959683620988060.

Moss B, Kosten S, Meerhoff M, Battarbee RW, Mazzeo N, Havens K, et al. (2011) Allied attack: climate change and eutrophication. *Inland Waters* 1(2): 101–105: doi:10.5268/IW-1.2.359.

Myrstener E, Ninnes S, Meyer-Jacob C, Mighall T and Bindler R (2021) Long-term development and trajectories of inferred lake-water organic carbon and pH in naturally acidic boreal lakes. *Limnology and Oceanography* 66: 2408–2422: doi:10.1002/lno.11761.

Nagell B and Landahl C-C (1978) Resistance to anoxia of Chironomus plumosus and Chironomus anthracinus (Diptera) Larvae. *Holarctic Ecology* 1(4): 333–336.

National Academy of Sciences (1969) *Eutrophication: Causes, consequences, correctives.* National Academy of Sciences.

Naumann E (1929) The scope and chief problems of regional limnology. *Internationale Revue der gesamten Hydrobiologie und Hydrographie* 22(1): 423–444.

Nedbalová L, Vrba J, Fott J, Kohout L, Kopáček J, Macek M, et al. (2006) Biological recovery of the Bohemian Forest lakes from acidification. *Biologia* 61(20): 453–465: doi:10.2478/s11756-007-0071-y.

Neustupný E (1985) On the Holocene Period in the Komořany Lake Area. *Památky archeologické* 76: 9–70.

Nõges P, Nõges T, Tuvikene L, Smal H, Ligeza S, Kornijów R, et al. (2003) Factors controlling hydrochemical and trophic state variables in 86 shallow lakes in Europe. *Hydrobiologia* 506–509: 51–58: doi:10.1023/B:HYDR.0000008541.36537.51.

Norton SA, Perry RH, Saros JE, Jacobson Jr. GL, Fernandez IJ, Kopáček J, et al. (2011) The controls on phosphorus availability in a Boreal lake ecosystem since deglaciation. *Journal of Paleolimnology* 46: 107–122: doi:10.1007/s10933-011-9526-9.

Nürnberg GK and Shaw M (1999) Productivity of clear and humic lakes: nutrients, phytoplankton, bacteria. *Hydrobiologia* 382: 97–112.

Obidowicz A (1996) A Late Glacial-Holocene history of the formation of vegetation belts in the Tatra Mts. *Acta Paleobotanica* 36(2): 159–206.

Oksanen J, Kindt R, Legendre P, O'Hara B, Simpson G, Solymos P, et al. (2008) Vegan: community ecology package. R package version 1.15-1.

Olivie-Lauquet G, Gruau G, Dia A, Riou C, Jaffrezic A and Henin O (2001) Release of trace elements in wetlands: role of seasonal variability. *Water Research* 35(4): 943–952.

Olsson H and Pettersson A (1993) Oligotrophication of Acidified Lakes -A Review of Hypotheses. *Ambio* 22(5): 312–317.

Orbán I, Birks HH, Vincze I, Finsinger W, Pál I, Marinova E, et al. (2018) Treeline and timberline dynamics on the northern and southern slopes of the Retezat Mountains (Romania) during the late glacial and the Holocene. *Quaternary International* 477: 59–78: doi:10.1016/j.quaint.2017.03.012.

Paludan C and Jensen HS (1995) Sequential extraction of phosphorus in freshwater wetland and lake sediment: significance of humic acids. *Wetlands* 15(4): 365–373.

Pappas L and Stoermer F (2001) Asterionella Taxonomic Hassall (Heterokontophyta , Bacillariophyceae) history and quantitative methods as an aid to valve shape differentiation. *Diatom* 17: 47–58.

Passy SI (2007) Diatom ecological guilds display distinct and predictable behavior along nutrient and disturbance gradients in running waters. *Aquatic Botany* 86(2): 171–178: doi:10.1016/j.aquabot.2006.09.018.

Patrick Jr. WH and Jugsujinda A (1992) Sequential reduction and oxidation of inorganic nitrogen, manganese, and iron in flooded soil. *Soil Science Society of America Journal* 56: 1071–1073.

Paull TM, Finkelstein SA and Gajewski K (2017) Interactions between climate and landscape drive Holocene ecological change in a High Arctic lake on Somerset Island, Nunavut, Canada. *Arctic Science* 3(1): 17–38. DOI: 10.1139/as-2016-0013.

Payette S, Garneau M, Delwaide A and Schaffhauser A (2012) Forest soil paludification and mid-Holocene retreat of jack pine in easternmost North America: Evidence for a climatic shift from fire-prone to peat-prone conditions. *The Holocene* 23(4): 494–503: doi:10.1177/0959683612463099.

Pedziszewska A, Tylmann W, Witak M, Piotrowska N, Maciejewska E and Latałowa M (2015) Holocene environmental changes reflected by pollen, diatoms, and geochemistry of annually laminated sediments of Lake Suminko in the Kashubian Lake District (N Poland). *Review of Palaeobotany and Palynology* 216: 55–75: doi:10.1016/j.revpalbo.2015.01.008.

Petr L, Žácková P, Grygar TM, Píšková A, Krížek M and Treml V (2013) Šúr, a former late-glacial and Holocene lake at the westernmost margin of the Carpathians. *Preslia* 85(3): 239–263.

Petticrew EL and Arocena JM (2001) Evaluation of iron-phosphate as a source of internal lake phosphorus loadings. *The Science of the Total Environment* 266: 87–93.

Philibert A and Prairie YT (2002) Is the introduction of benthic species necessary for open-water chemical reconstruction in diatom-based transfer functions? *Canadian Journal of Fisheries and Aquatic Sciences* 59(6): 938–951: doi:10.1139/f02-061.

Phillips G, Pietiläinen O-P, Carvalho L, Solimini A, Solheim AL and Cardoso AC (2008) Chlorophyll–nutrient relationships of different lake types using a large European dataset. *Aquatic Ecology* 42: 213–226: doi:10.1007/s10452-008-9180-0.

Pienitz R, Smol JP and MacDonald GM (1999) Paleolimnological reconstruction of Holocene climatic trends from two boreal treeline lakes, Northwest Territories, Canada. *Arctic, Antarctic, and Alpine Research* 31(1): 82–93: doi:10.2307/1552625.

Pienitz R and Vincent WF (2000) Effect of climate change relative to ozone depletion on UV exposure in subarctic lakes. *Nature* 404: 484–487: doi:10.1038/35006616.

Pini R (2002) A high-resolution Late-Glacial – Holocene pollen diagram from Pian di Gembro (Central Alps, Northern Italy). *Vegetation History and Archaeobotany* 11(4): 251–262. DOI: 10.1007/s003340200038.

Pla-Rabés S and Catalan J (2018) Diatom species variation between lake habitats: implications for interpretation of paleolimnological records. *Journal of Paleolimnology* 60: 169–187: doi:10.1007/s10933-018-0017-0.

Pleskot K, Apolinarska K, Kołaczek P, Suchora M, Fojutowski M, Joniak T, et al. (2020) Searching for the 4.2 ka climate event at Lake Spore, Poland. *Catena* 191: 104565: doi:10.1016/j.catena.2020.104565.

Pleskot K, Tjallingii R, Makohonienko M, Nowaczyk N and Szczuciński W (2018) Holocene paleohydrological reconstruction of Lake Strzeszyńskie (western Poland) and its implications for the central European climatic transition zone. *Journal of Paleolimnology* 59: 443–459: doi:10.1007/s10933-017-9999-2.

Płóciennik M, Self A, Birks HJB and Brooks SJ (2011) Chironomidae (Insecta: Diptera) succession in Żabieniec bog and its palaeo-lake (central Poland) through the Late Weichselian and Holocene. *Palaeogeography, Palaeoclimatology, Palaeoecology* 307: 150–167: doi:10.1016/j.palaeo.2011.05.010.

Pokorný O (1963) Několik poznámek k historickému vývoji Komořanského jezera [Some remarks to the historical development of Lake Komořany]. *Sborník Československé společnosti zeměpisné* 1: 52–57.

Pokorný P (2002) A high-resolution record of Late-Glacial and Early-Holocene climatic and environmental change in the Czech Republic. *Quaternary International* 91: 101–122.

Pokorný P, Chytrý M, Juřičková L, Sádlo J, Novák J and Ložek V (2015) Mid-Holocene bottleneck for central European dry grasslands: Did steppe survive the forest optimum in northern Bohemia, Czech Republic? *The Holocene* 25(4): 716–726: doi:10.1177/0959683614566218.

Pokorný P and Jankovská V (2000) Long-term vegetation dynamics and the infilling process of a former lake (Švarcenberk, Czech Republic). *Folia Geobotanica* 35: 433–457.

Pokrovski GS and Schott J (1998) Experimental study of the complexation of silicon and germanium with aqueous organic species: Implications for germanium and silicon transport and Ge/Si ratio in natural waters. *Geochimica et Cosmochimica Acta* 62(21/22): 3413–3428.

Poniewozik M, Wojciechowsk W and Solis M (2011) Dystrophy or eutrophy: Phytoplankton and physicochemical parameters in the functioning of humic lakes. *Oceanological and Hydrobiological Studies* 40(2): 22–29: doi:10.2478/s13545-011-0013-8.

Possinger AR, Bailey SW, Inagaki TM, Kögel-Knabner I, Dynes JJ, Arthur ZA, et al. (2020) Organo-mineral interactions and soil carbon mineralizability with variable saturation cycle frequency. *Geoderma*. Elsevier 375: 114483: doi:10.1016/j.geoderma.2020.114483.

Potapova MG, Charles DF, Ponader KC and Winter DM (2004) Quantifying species indicator values for trophic diatom indices: a comparison of approaches. *Hydrobiologia* 517: 25–41.

Prairie YT, Duarte CM and Kalff J (1989) Unifying nutrient-chlorophyll relationships in lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 46: 1176–1182.

Pražáková M, Veselý J, Fott J, Majer V and Kopáček J (2006) The long-term succession of cladoceran fauna and palaeoclimate forcing : A 14,600–year record from Plešné Lake, the Bohemian Forest. *Biologia* 61(20): S387–S399: doi:10.2478/s11756-007-0072-x.

Procházka F (2000) Šumavské šídlatky – mýty a skutečnost. Quillwort species in the Czech Bohemian Forest (Sumava Mts.) – fables and reality. *Silva Gabreta* (5): 83–92.

Punt W (1976) *The Northwest European Pollen Flora: Parts 1–7*. Amsterdam: Elsevier.

Punt W and Blackmore S (1991) *The Northwest European Pollen Flora: Parts 44–51*. Amsterdam: Elsevier.

Punt W, Blackmore S and Clarke G (1988) *The Northwest European Pollen Flora: Parts 38–43*. Amsterdam: Elsevier.

Punt W, Blackmore S and Hoen P (1995) *The Northwest European Pollen Flora: Parts 52–56*. Amsterdam: Elsevier.

Punt W, Blackmore S, Hoen P and Stafford PJ (2003) *The Northwest European Pollen Flora: Parts 57–68*. Amsterdam: Elsevier.

Punt W and Clarke G (1980) *The Northwest European Pollen Flora: Parts 8–20*. Amsterdam: Elsevier.

Punt W and Clarke G (1981) *The Northwest European Pollen Flora: Parts 21–28*. Amsterdam: Elsevier.

Punt W and Clarke G (1984) *The Northwest European Pollen Flora: Parts 29–37*. Amsterdam: Elsevier.

Punt W and Hoen P (2009) *The Northwest European Pollen Flora: Part 70*. Amsterdam: Elsevier.

Quinlan R and Smol JP (2001) Chironomid-based inference models for estimating end-of-summer hypolimnetic oxygen from south-central Ontario shield lakes. *Freshwater Biology* 46: 1529–1551.

Ralska-Jasiewiczowa M, Goslar T, Rózański K, Wacnik A, Czernik J and Chróst L (2003) Very fast environmental changes at the Pleistocene/Holocene boundary, recorded in laminated sediments of the Lake Gościąż, Poland. *Palaeogeography, Palaeoclimatology, Palaeoecology* 193: 225–247: doi:10.1016/S0031-0182(03)00227-X.

Ralska-Jasiewiczowa M, Latalowa M, Wasylikowa K, Tobolski K, Madeyska E, Wright HE, et al. (eds) (2004) *Late Glacial and Holocene history of vegetation in Poland based on isopollen maps*. Krakow: W. Szafer Institute of

Botany, Polish Academy of Sciences.

Rantala M V., Nevalainen L, Rautio M, Galkin A and Luoto TP (2016) Sources and controls of organic carbon in lakes across the subarctic treeline. *Biogeochemistry*. Springer International Publishing 129(1–2): 235–253: doi:10.1007/s10533-016-0229-1.

Rasmussen SO, Bigler M, Blockley SP, Blunier T, Buchardt SL, Clausen HB, et al. (2014) A stratigraphic framework for abrupt climatic changes during the Last Glacial period based on three synchronized Greenland ice-core records : re fi ning and extending the INTIMATE event stratigraphy. *Quaternary Science Reviews*. Elsevier Ltd 106: 14–28: doi:10.1016/j.quascirev.2014.09.007.

R Core Team (2016, 2019, 2021) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Řeháková Z (1983) Diatom succession in the post-glacial sediments of the Komořany Lake , North-West Bohemia, Czechoslovakia. *Hydrobiologia* 103: 241–245.

Řeháková Z (1986) The Postglacial history of diatom-bearing sediments of the former Lake Komorany (North-West Bohemia). *Antropozoikum* 17: 87–134.

Reimer PJ, Bard E, Bayliss A, Beck JW, Blackwell PG, Bronk C, et al. (2013) Intcal13 and Marine13 radiocarbon age calibration curves 0–50,000 years cal BP. *Radiocarbon* 55(4): 1869–1887.

Reynolds CS (2006) *The Ecology of Phytoplankton*. Cambridge University Press.

Rieradevall M and Brooks SJ (2001) An identification guide to subfossil Tanypodinae larvae (Insecta: Diptera: Chrironomidae) based on cephalic setation. *Journal of Paleolimnology* 25(1): 81–99. DOI: 10.1023/A:1008185517959.

Riise G, Hees P Van, Lundström U and Strand LT (2000) Mobility of different size fractions of organic carbon, Al, Fe, Mn and Si in podzols. *Geoderma* 94: 237–247.

Rimet F and Bouchez A (2012) Life-forms, cell-sizes and ecological guilds of diatoms in European rivers. *Knowledge and Management of Aquatic Ecosystems* 406(01): doi:10.1051/kmae/2012018.

Rivera-Rondón CA and Catalan J (2017) The ratio between chrysophycean cysts and diatoms in temperate, mountain lakes: some recommendations for its use in paleolimnology. *Journal of Paleolimnology* 57: 273–285: doi:10.1007/s10933-017-9946-2.

Rivera-Rondón CA and Catalan J (2020) Diatoms as indicators of the multivariate environment of mountain lakes. *Science of the Total Environment*. Elsevier B.V. 703: 135517: doi:10.1016/j.scitotenv.2019.135517.

Rodhe W (1969) Crystallization of eutrophication concepts in Northern Europe. *Eutrophication: Causes, consequences, correctives*. National Academy of Sciences, 50–64.

Rørslett B and Brettum P (1989) The genus *Isoëtes* in Scandinavia: An ecological review and perspectives. *Aquatic Botany* 35(3): 223–261. DOI: 10.1016/0304-3770(89)90001-6.

Rösch M (2009) Botanical evidence for prehistoric and medieval land use in the Black Forest. In Klápště J and Sommer P (eds) *Medieval Rural Settlement in Marginal Landscapes*. Turnhout: Brepols Publishers, pp. 335–343. DOI: 10.1484/M.RURALIA-EB.3.1181.

Rösch M (2015) Abies alba and Homo sapiens in the Schwarzwald – a difficult story. *Interdisciplinaria Archaeologica* (6): 47–62.

Rosén P and Hammarlund D (2007) Effects of climate, fire and vegetation development on Holocene changes in total organic carbon concentration in three boreal forest lakes in northern Sweden. *Biogeosciences* 4(6): 975–984. DOI: https://doi.org/10.5194/bg-4-975-2007.

Roulet NT and Moore TR (2006) Browning the waters. *Nature* 444: 283–284.

Routson CC, Nicholas P, Kaufman DS, Erb MP, Goosse H, Shuman BN, et al. (2019) Mid-latitude net precipitation decreased with Arctic warming during the Holocene. *Nature*. Springer US 568: 83–87: doi:10.1038/s41586-019-1060-3.

Rudolph K (1926) Pollenanalytische Untersuchungen im thermophilen Florengebiet Böhmens: Der 'Kommerner See' bei Brüx. *Berichten der Deutschen Botanischen Gesellschaft* 4: 239–248.

Rühland KM, Paterson AM and Smol JP (2015) Lake diatom responses to warming: reviewing the evidence. *Journal of Paleolimnology* 54: 1–35: doi:10.1007/s10933-015-9837-3.

Russell FE, Boyle JF and Chiverrell RC (2019) NIRS quantification of lake sediment composition by multiple regression using end-member spectra. *Journal of Paleolimnology*. Springer Netherlands 62: 73–88: doi:10.1007/s10933-019-00076-2.

Sanchini A, Szidat S, Tylmann W, Vogel H, Wacnik A and Grosjean M (2020) A Holocene high-resolution record of aquatic productivity, seasonal anoxia and meromixis from varved sediments of Lake Łazduny, North-Eastern Poland: insight from a novel multi-proxy approach. *Journal of Quaternary Science* 35(8): 1070–1080: doi:10.1002/jqs.3242.

Sand-Jensen K and Borum J (1991) Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. *Aquatic Botany* 41: 137–175: doi:10.1016/0304-3770(91)90042-4.

Sanders RW, Cooke SL, Fischer JM, Fey SB, Heinze AW, Jeffrey WH, et al. (2015) Shifts in microbial food web structure and productivity after additions of naturally occurring dissolved organic matter: Results from large-scale lacustrine mesocosms. *Limnology and Oceanography* 60: 2130–2144: doi:10.1002/lno.10159.

Sankar MS, Pasmanava D, Lu Y, Mercer AE, Turnage G, Shoemaker CM, et al. (2020) Land use and land cover control on the spatial variation of dissolved organic matter across 41 lakes in Mississippi , USA. *Hydrobiologia* 847: 1159–1176.

Sayer CD (2001) Problems with the application of diatom-total phosphorus transfer functions: examples from a shallow English lake. *Freshwater Biology* 46: 743–757.

Sayer CD, Davidson TA, Jones JI and Langdon PG (2010) Combining contemporary ecology and palaeolimnology to understand shallow lake ecosystem change. *Freshwater Biology* 55(3): 487–499: doi:10.1111/j.1365-2427.2010.02388.x.

Sayer CD, Roberts N, Sadler J, David C and Wade PM (1999) Biodiversity changes in a shallow lake ecosystem: A multi-proxy palaeolimnological analysis. *Journal of Biogeography* 26(1): 97–114: doi:10.1111/j.1365-2699.1999.00298.x.

Schaetzl RJ, Luehmann MD and Rothstein D (2015) Pulses of podzolization: The relative importance of spring snowmelt, summer storms, and fall rains on spodosol development. *Soil Science Society of America Journal* 79: 117–131: doi:10.2136/sssaj2014.06.0239.

Schaffhauser A, Payette S, Garneau M and Robert EC (2017) Soil paludification and Sphagnum bog initiation: the influence of indurated podzolic soil and fire. *Boreas* 46: 428–441: doi:10.1111/bor.12200.

Scheffer M (1990) Multiplicity of stable staes in freshwater systems. *Hydrobiologia* 200: 475–486: doi:10.1007/BF02530365.

Scheffer M (2004) *Ecology of Shallow Lakes*. Kluwer Academic Publishers: doi:10.1016/S0304-3770(00)00103-0.

Scheffer M and Van Nes EH (2007) Shallow lakes theory revisited: Various alternative regimes driven by climate, nutrients, depth and lake size. *Hydrobiologia* 584: 455–466: doi:10.1007/s10750-007-0616-7.

Schillereff D, Chiverrell RC, Croudace I and Boyle JF (2015) An Inter-comparison of µXRF Scanning Analytical Methods for Lake Sediments. In Croudace I and Rothwell R (eds) *Micro-XRF Studies of Sediment Cores. Developments in Paleoenvironmental Research, vol. 17*. Dordrecht: Springer, 583–600: doi:https://doi.org/10.1007/978-94-017-9849-5_24.

Schindler DW (1977) Evolution of phosphorus limitation in lakes. *Science* 195: 260–262.

Schindler DW (2012) The dilemma of controlling cultural eutrophication of lakes. *Proceedings of the Royal Society of London, Series B, Containing Papers of a Biological Character* 279: 4322–4333.

Schindler DW, Curtis PJ, Bayley SE, et al. (1997) Climate-induced changes in the dissolved organic carbon budgets of boreal lakes. *Biogeochemistry* 36(1): 9–28. DOI: 10.1023/A:1005792014547.

Schindler DW, Carpenter SR, Chapra SC, Hecky RE and Orihel DM (2016) Reducing phosphorus to Curb Lake eutrophication is a success. *Environmental Science and Technology* 50: 8923–8929: doi:10.1021/acs.est.6b02204.

Schmidt R, Arzet K, Facher E, Fott J, Irlweck K, Řeháková Z, et al. (1993) *Acidification of Bohemian Lakes: Recent trends and historical development. The final report of the Ost-West Programm*. .

Schmidt R, Koinig KA, Thompson R and Kamenik C (2002) A multi proxy core study of the last 7000 years of climate and alpine land-use impacts on an Austrian mountain lake (Unterer Landschitzsee, Niedere Tauern). *Palaeogeography, Palaeoclimatology, Palaeoecology* 187: 101–120.

Schütze M, Tserendorj G, Pérez-Rodríguez M, Rösch M and Biester H (2018) Prediction of Holocene mercury accumulation trends by combining palynological and geochemical records of lake sediments (Black Forest, Germany). *Geosciences* 8(358): doi:10.3390/geosciences8100358.

Seddon AWR, Mackay AW, Baker AG, Birks HJB, Breman E, Buck CE, et al. (2014) Looking forward through the past: Identification of 50 priority research questions in palaeoecology. *Journal of Ecology* 102: 256–267: doi:10.1111/1365-2745.12195.

Seekell D, Lapierre J-F and Karlsson J (2015) Trade-offs between light and nutrient availability across gradients of dissolved organic carbon concentration in Swedish lakes: implications for patterns in primary production. *Canadian Journal of Fisheries and Aquatic Sciences* 11: 1663–1671.

Seppä H and Weckström J (1999) Holocene vegetational and limnological changes in the Fennoscandian tree-line area as documented by pollen and diatom records from Lake Tsuolbmajavri, Finland. *Écoscience* 6(4): 621–635. DOI: 10.1080/11956860.1999.11682562.

Seppä H, Alenius T, Muukkonen P, et al. (2009) Calibrated pollen accumulation rates as a basis for quantitative tree biomass reconstructions. *The Holocene* 19(2): 209–220. DOI: 10.1177/0959683608100565.

Shinneman ALC, Umbanhowar CE, Edlund MB, Hobbs WO, Camill P and Geiss C (2016) Diatom assemblages reveal regional-scale differences in lake responses to recent climate change at the boreal-tundra ecotone, Manitoba, Canada. *Journal of Paleolimnology*. Springer Netherlands 56: 275–298: doi:10.1007/s10933-016-9911-5.

Sienkiewicz E (2016) Post-glacial acidification of two alpine lakes (Sudetes Mts., SW Poland), as inferred from diatom analyses. *Acta Palaeobotanica* 56: 65–77: doi:10.1515/acpa-2016-0002.

Simpson G and Oksanen J. (2013) Analogue: analogue matching and modern analogue technique transfer function models. R package version 0.10-0.

Smol JP, Wolfe AP, Birks HJB, Douglas MS V, Jones VJ, Korhola A, et al. (2005) Climate-driven regime shifts in the biological communities of arctic lakes. *Proceedings of the National Academy of Sciences* 102(12): 4397–4402.

Smolders AJP, Lucassen ECHET and Roelofs JGM (2002) The isoetid environment: biogeochemistry and threats. *Aquatic Botany* 73(4). Acidification and restoration of soft water lakes and their vegetation: 325–350. DOI: 10.1016/S0304-3770(02)00029-3.

Snucins E and Gunn J (2000) Interannual variation in the thermal structure of clear and colored lakes. *Limnology and Oceanography* 45(7): 1639–1646.

Šobr M and Janský B (2016) The morphometric parameters of glacial lakes in the Bohemian Forest. *Silva Gabreta* 22: 31–61.

Soldán T, Bojková J, Vrba J, Bitušík P, Chvojka P, Papáček M, et al. (2012) Aquatic insects of the Bohemian Forest glacial lakes: Diversity, long-term changes, and influence of acidification. *Silva Gabreta* 18(3): 123–283.

Solomon CT, Jones SE, Weidel BC, Buffam I, Fork ML, Karlsson J, et al. (2015) Ecosystem consequences of changing inputs of terrestrial dissolved organic matter to lakes: current knowledge and future challenges. *Ecosystems* 18: 376–389: doi:10.1007/s10021-015-9848-y.

Solovieva N and Jones VJ (2002) A multiproxy record of Holocene environmental changes in the central Kola Peninsula, northwest Russia. *Journal of Quaternary Science* 17(4): 303–318: doi:10.1002/jqs.686.

Søndergaard M, Jensen JP and Jeppesen E (2003) Role of sediment and internal loading of phosphorus in shallow lakes. *Hydrobiologia* 506–509: 135–145: doi:10.1023/B:HYDR.0000008611.12704.dd.

Stabell B (1993) A pre-industrial acidification event shown by diatom analysis of a recently acidified lake in Southern Norway. *Diatom Research* 8(1): 145–158: doi:10.1080/0269249x.1993.9705246.

Starkel L, Michczyńska DJ, Krapiec M, Margielewski W, Nalepka D and Pazdur A (2013) Progress in the Holocene chrono-climatostratigraphy of Polish territory. *Geochronometria* 40(1): 1–21: doi:10.2478/s13386-012-0024-2.

Steinberg C (1991) Fate of organic matter during natural and anthropogenic lake acidification. *Water Research* 25(12): 1453–1458.

Steinberg C, Hartmann H, Arzet K and Krause-Dellin D (1988) Paleoindication of acidification in Kleiner Arbersee (Federal Republic of Germany, Bavarian Forest) by chydorids, chrysophytes, and diatoms. *Journal of Paleolimnology* 1: 149–157.

Steinberg CEW, Saumweber S and Kern J (1991) Paleolimnological trends in total organic carbon indicate natural and anthropogenic sources of acidity in Grosser Arbersee, Germany. *The Science of the Total Environment* 107: 83–90.

Sterner RW (2008) On the phosphorus limitation paradigm for lakes. *International Review in Hydrobiology* 93(4–5): 433–445: doi:10.1002/iroh.200811068.

Stetler JT, Knoll LB, Driscoll CT and Rose KC (2021) Lake browning generates a spatiotemporal mismatch between dissolved organic carbon and limiting nutrients. *Limnology And Oceanography Letters* 6: 182–191: doi:10.1002/lol2.10194.

Stevenson J (2014) Ecological assessments with algae: A review and synthesis. *Journal of Phycology* 50: 437–461: doi:10.1111/jpy.12189.

Stevenson RJ, Bothwell ML and Lowe RL (eds) (1996) *Algal Ecology. Freshwater Benthic Ecosystems*. Academic Press.

Stockmarr J (1971) Tablets with spores used in absolute pollen analysis. *Pollen et Spores* 13: 615–621.

Stokes PM (1986) Ecological effects of acidification on primary producers in aquatic systems. *Water, Air, and Soil Pollution* 30(1–2): 421–438: doi:10.1007/BF00305212.

Stuchlík E, Bitušík P, Hardekopf DW, Hořická Z, Kahounová M, Tátosová J, et al. (2017) Complexity in the biological recovery of Tatra Mountain lakes from acidification. *Water, Air and Soil Pollution* 228: 184: doi:10.1007/s11270-017-3362-0.

Stuchlík L (2001) *Atlas of Pollen and Spores of the Polish Neogene*. Krakow: W. Szafer Institute of Botany.

Stuiver M, Grootes PM and Braziunas TF (1995) The GISP2 δ^{18}O climate record of the past 16,500 years and the role of the sun, ocean, and volcanoes. *Quaternary Research* 44: 341–354.

Sümegi P, Gulyás S and Jakab G (2008) Holocene paleoclimatic and paleohydrological changes in Lake Balaton as inferred from a complex quantitative environmental historical study of a lacustrine sequence of the Szigliget embayment. *Documenta Praehistorica* 35: 33–44.

Sutfin NA, Wohl EE and Dwire KA (2016) Banking carbon: a review of organic carbon storage and physical factors influencing retention in floodplains and riparian ecosystems. *Earth Surface Processes and Landforms* 41: 38–60: doi:10.1002/esp.3857.

Švambera V (1914) Šumavská jezera III. Prášilské jezero. *Rozpravy České akademie císaře Františka Josefa. Class II* 23(16): 1–28.

Szeroczyńska K and Sarmaja-Korjonen K (2007) *Atlas of subfossil Cladocera from Central and Northern Europe*. Świecie: Friends of Lower Vistula Society.

Szmeja J and Bociąg K (2004) The disintegration of populations of underwater plants in soft water lakes enriched with acidic organic matter. *Acta Societatis Botanicorum Poloniae* 73(2): 165–173. DOI: 10.5586/asbp.2004.023.

Tabacchi E, Lambs L, Guilloy H, Planty-Tabacchi A-M, Muller E and Decamps H (2000) Impacts of riparian vegetation on hydrological processes. *Hydrological Processes* 14: 2959–2976: doi:10.1002/1099-1085(200011/12)14.

Talbot MR (2001) Nitrogen isotopes in palaeolimnology. In Last WM and Smol JP (eds) *Tracking environmental change using lake sediments. Volume 2: Physical and geochemical methods*. Dordrecht: Kluwer Academic Publishers, 401–440.

Tarrats P, Cañedo-Argüelles M, Rieradevall M and Prat N (2018) The influence of depth and macrophyte habitat on paleoecological studies using chironomids: Enol Lake (Spain) as a case study. *Journal of Paleolimnology* 60: 97–107: doi:10.1007/s10933-018-0026-z.

ter Braak CJF and Barendregt LG (1986) Weighted averaging of species indicator values: its efficiency in environmental calibration. *Mathematical Biosciences* 78: 57–72.

ter Braak CJF and Juggins S (1993) Weighted averaging partial least squares regression (WA-PLS): an improved method for reconstructing environmental variables from species assemblages. *Hydrobiologia* 269/270: 485–502.

ter Braak CJF and Looman CWN (1986) Weighted averaging, logistic regression and the Gaussian response model. *Vegetatio* 65(1): 3–11.

Theuerkauf M, Bos JAA, Jahns S, Janke W, Kuparinen A, Stebich M, et al. (2014) Corylus expansion and persistent openness in the early Holocene vegetation of northern central Europe. *Quaternary Science Reviews* 90: 183–198: doi:10.1016/j.quascirev.2014.03.002.

Theueurkauf M, Blume T, Brauer A, Dräger N, Feldens P, Kaiser K, et al. (2021) Holocene lake-level evolution of Lake Tiefer See, NE Germany, caused by climate and land cover changes. *Boreas*: doi:10.1111/bor.12561.

Thevenon F, Adatte T, Spangenberg JE and Anselmetti FS (2012) Elemental (C/N ratios) and isotopic (δ15Norg, δ13Corg) compositions of sedimentary organic matter from a high-altitude mountain lake (Meidsee, 2661 m a.s.l., Switzerland): Implications for Lateglacial and Holocene Alpine landscape evolution. *The Holocene* 22: 1129–1136: doi:10.1177/0959683612441841.

Thurman EM (1985) *Organic Geochemistry of Natural Water*. Boston: Dr W. Junk Publishers.

Tilman D, Kilham SS. and Kilham P (1982) Phytoplankton community ecology: the role of limiting nutrients. *Annual Review of Ecology and Systematics* 13: 349–372.

Tinner W, Conedera M, Ammann B, et al. (1998) Pollen and charcoal in lake sediments compared with historically documented forest fires in southern Switzerland since AD 1920. *The Holocene* 8(1): 31–42. DOI: 10.1191/095968398667205430.

Tinner W, Hubschmid P, Wehrli M, et al. (1999) Long-term forest fire ecology and dynamics in southern Switzerland. *Journal of Ecology* 87(2): 273–289. DOI: 10.1046/j.1365-2745.1999.00346.x.

Tinner W and Lotter AF (2001) Central European vegetation response to abrupt climate change at 8.2 ka. *Geology* 29(6): 551–554.

Tinner W, Hu FS, Beer R, et al. (2006) Postglacial vegetational and fire history: pollen, plant macrofossil and charcoal records from two Alaskan lakes. *Vegetation History and Archaeobotany* 15(4): 279–293. DOI: 10.1007/s00334-006-0052-z.

Tinner W, Beer R, Bigler C, et al. (2015) Late-Holocene climate variability and ecosystem responses in Alaska inferred from high-resolution multiproxy sediment analyses at Grizzly Lake. *Quaternary Science Reviews* 126: 41–56. DOI: 10.1016/j.quascirev.2015.08.019.

Tipping E, Somerville CJ and Luster J (2016) The C:N:P:S stoichiometry of soil organic matter. *Biogeochemistry* 130: 117–131: doi:10.1007/s10533-016-0247-z.

Tu L, Gilli A, Lotter F, Vogel H, Moyle M, Boyle JF, et al. (2021) The nexus among long-term changes in lake primary productivity, deep-water anoxia, and internal phosphorus loading, explored through analysis of a 15, 000-year varved sediment record. *Global and Planetary Change* 207: 103643: doi:10.1016/j.gloplacha.2021.103643.

Turner F, Pott R, Schwarz A and Schwalb A (2014a) Response of Pediastrum in German floodplain lakes to Late Glacial climate changes. *Journal of Paleolimnology* 52: 293–310: doi:10.1007/s10933-014-9794-2.

Turner F, Pott R, Schwarz A and Schwalb A (2014b) Response of Pediastrum in German floodplain lakes to Late Glacial climate changes. *Journal of Paleolimnology* 52: 293–310: doi:10.1007/s10933-014-9794-2.

Turner F, Tolksdorf JF, Viehberg F, Schwalb A, Kaiser K, Bittmann F, et al. (2013) Lateglacial/early Holocene fluvial reactions of the Jeetzel river (Elbe valley, northern Germany) to abrupt climatic and environmental changes. *Quaternary Science Reviews*. Elsevier Ltd 60: 91–109: doi:10.1016/j.quascirev.2012.10.037.

Ursenbacher S, Stötter T and Heiri O (2020) Chitinous aquatic invertebrate assemblages in Quaternary lake sediments as indicators of past deepwater oxygen concentration. *Quaternary Science Reviews* 231: 106203: doi:10.1016/j.quascirev.2020.106203.

Vadeboncoeur Y, Jeppesen E, Zanden MJ Vander, Christoffersen K and Lodge DM (2003) From Greenland to green lakes: Cultural eutrophication and the loss of benthic pathways in lakes. 48(4): 1408–1418.

Vadeboncoeur Y, Peterson G, Vander Zanden MJ and Kalff J (2008) Benthic Algal Production across Lake Size Gradients: Interactions among Morphometry, Nutrients, and Light. *Ecology* 89(9): 2542–2552.

Van Dam H, Mertens A and Sinkeldam J (1994) A coded checklist and ecological indicator values of freshwater diatoms from The Netherlands. *Netherlands Journal of Aquatic Ecology* 28(1): 117–133: doi:10.1007/BF02334251.

van der Knaap WO, van Leeuwen JFN, Fahse L, et al. (2019) Vegetation and disturbance history of the Bavarian Forest National Park, Germany. *Vegetation History and Archaeobotany*. DOI: 10.1007/s00334-019-00742-5.

Vander Zanden MJ, Vadeboncoeur Y and Chandra S (2011) Fish reliance on littoral–benthic resources and the distribution of primary production in lakes. *Ecosystems* 14: 894–903: doi:10.1007/s10021-011-9454-6.

Van Miegroet H and Cole DW (1984) The impact of nitrification on soil acidification and cation leaching in a red alder ecosystem 1. *Journal of Environmental Quality* 13(4): 586–590. DOI: 10.2134/jeq1984.00472425001300040015x.

Vasconcelos FR, Diehl S, Rodríguez P, Hedström P, Karlsson J and Byström P (2016) Asymmetrical competition between aquatic primary producers in a warmer and browner world. *Ecology* 97(10): 2580–2592.

Vermaire JC, Greffard M-H, Saulnier-Talbot É and Gregory-Eaves I (2013) Changes in submerged macrophyte abundance altered diatom and chironomid assemblages in a shallow lake. *Journal of Paleolimnology* 50: 447–456: doi:10.1007/s10933-013-9737-3.

Vermaire JC, Prairie YT and Gregory-Eaves I (2011) The influence of submerged macrophytes on sedimentary diatom assemblages. *Journal of Phycology* 47: 1230–1240: doi:10.1111/j.1529-8817.2011.01069.x.

Veselý J (1994) Investigation of the nature of the Šumava lakes: a review. *Časopis Národního Muzea, Řada Přírodovědná* 163: 103–120.

Veselý J, Almquist-Jacobson H, Miller LM, Norton SA, Appleby P, Dixit AS, et al. (1993) The history and impact of air pollution at Čertovo Lake, southwestern Czech Republic. *Journal of Paleolimnology* 8(3): 211–231.

Viers J, Dupré B, Polvé M, Schott J, Dandurand J-L and Braun J-J (1997) Chemical weathering in the drainage basin of a tropical watershed (Nsimi-Zoetele site, Cameroon): comparison between organic-poor and organic-rich waters. *Chemical Geology* 140: 181–206.

Vincent WF and Pienitz R (1996) Sensitivity of high-latitude freshwater ecosystems to global change: temperature and solar ultraviolet radiation. *Geoscience Canada* 23(4): 231–236.

Vočadlová K, Petr L, Žáčková P, Křížek M, Křížová L, Hutchinson SM, et al. (2015) The Lateglacial and Holocene in Central Europe: a multi-proxy environmental record from the Bohemian Forest, Czech Republic. *Boreas* 44: 769–784: doi:10.1111/bor.12126.

Vöge M (2003) Environmentally related demography: field studies on *Isoëtes lacustris* L. (Lycophyta, Isoëtaceae) in Europe. In Chandra S and Srivastava M (eds) *Pteridology in the new millennium: NBRI golden jubilee volume*. Dordrecht: Springer Netherlands, pp. 233–260. DOI: 10.1007/978-94-017-2811-9_19.

Vöge M (2015) Monitoring the vitality of *Isoëtes lacustris* by using a non-destructive method. *Limnological Review* 14(3): 153–158. DOI: 10.1515/limre-2015-0005.

Vondrák D, Prach J and Houfková P (2015) Sediments of postglacial lakes in the Czech Republic-unique natural archives overlooked by (Czech) limnology. In Rádková V and Bojková J (eds) *XVII. konference České limnologické společnosti a Slovenskej limnologickej spoločnosti 'Voda-věc veřejná' - Sborník příspěvků*. Brno: ČLS and Masarykova univerzita v Brně, 162–167.

Vondrák D, Kopáček J, Kletetschka G, Chattová B, Suchánek V, Tátosová J, et al. (2019a) Lithostratigraphy and age of the Bohemian Forest lake sediments: A first assessment. *Geoscience Research Reports* 52(1): 75–83: doi:10.3140/zpravy.geol.2019.13. [in Czech with English summary and English figures and a table]

Vondrák D, Schafstall NB, Chvojka P, Chiverrell RC, Kuosmanen N, Tátosová J, et al. (2019b) Postglacial succession of caddisfly (Trichoptera) assemblages in a central European montane lake. *Biologia* 74(10): 1325–1338.

Vondrák D, Krause D, Hrubá J and Kletetschka G (2021) The paleolake Stifter Hollow and signs of the last stage of Quaternary glaciation in the Ple š né Lake cirque. *Silva Gabreta* 27: 1–16.

Vrba J, Bojková J, Chvojka P, Fott J, Kopáček J, Macek M, et al. (2016) Constraints on the biological recovery of the Bohemian Forest lakes from acid stress. *Freshwater Biology* 61: 376–395: doi:10.1111/fwb.12714.

Vrba J, Kopáček J, Bittl T, Nedoma J, Štrojsová A, Nedbalová L, et al. (2006) A key role of aluminium in phosphorus availability, food web structure, and plankton dynamics in strongly acidified lakes. *Biologia* 61(20): S441–S451: doi:10.2478/s11756-007-0077-5.

Vrba J, Kopáček J and Fott J (2000) Long term limnological research of the Bohemian Forest lakes and their recent status. *Silva Gabreta* 4: 7–28.

Vrba J, Kopáček J, Fott J, Kohout L, Nedbalová L, Pražáková M, et al. (2003a) Long-term studies (1871–2000) on acidification and recovery of lakes in the Bohemian Forest (central Europe). *The Science of the Total Environment* 310: 73–85.

Vrba J, Kopáček J, Fott J, Kohout L, Nedbalová L, Pražáková M, et al. (2003b) Long-term studies (1871–2000) on acidification and recovery of lakes in the Bohemian Forest (central Europe). *Science of The Total Environment* 310(1–3): 73–85: doi:10.1016/S0048-9697(02)00624-1.

Vrba J, Kopáček J, Tahovská K and Šantrůčková H (2015) Long-term ecological research of glacial lakes in the Bohemian Forest and their catchments. *Silva Gabreta* 21(1): 53–71.

Wanner H, Beer J, Bütikofer J, Crowley TJ, Cubasch U, Flückinger J, et al. (2008) Mid- to Late Holocene climate change: an overview. *Quaternary Science Reviews* 27: 1791–1828: doi:10.1016/j.quascirev.2008.06.013.

Waters MN, Piehler MF, Smoak JM and Bianchi TS (2012) Algal community responses to shallow lake dystrophication.

Canadian Journal of Fisheries and Aquatic Sciences 69: 1433–1443: doi:10.1139/F2012-021.

Werner P and Smol JP (2005) Diatom-environmental relationships and nutrient transfer functions from contrasting shallow and deep limestone lakes in Ontario, Canada. *Hydrobiologia* 533(1): 145–173: doi:10.1007/s10750-004-2409-6.

Wettstein R (1896) Über ein subfossiles Vorkommen von Trapa natans in Böhmen. *Lotos* 44.

Wetzel RG (2001) *Limnology. Lake and River Ecosystems*. Academic Press: doi:10.1046/j.1529-8817.2001.37602.x.

Wiederholm T (1983) Chironomidae of the Holarctic Region. Keys and Diagnoses. Part 1. Larvae. *Entomologica Scandinavica* 19: 1–457.

Williamson CE, Morris DP, Pace ML and Olson OG (1999) Dissolved organic carbon and nutrients as regulators of lake ecosystems: Resurrection of a more integrated paradigm. *Limnology and Oceanography* 44(3): 795–803.

Williamson CE, Overholt EP, Pilla RM, et al. (2015) Ecological consequences of long-term browning in lakes. Scientific Reports 5: 18666. DOI: 10.1038/srep18666.

Wolfe AP and Härtling JW (1996) The late Quaternary development of three ancient tarns on southwestern Cumberland Peninsula, Baffin Island, Arctic Canada: paleolimnological evidence from diatoms and sediment chemistry. *Journal of Paleolimnology* 15: 1–18.

Yu Z (2000) Ecosystem response to Lateglacial and early Holocene climate oscillations in the Great Lakes region of North America. *Quaternary Science Reviews* 19(17): 1723–1747. DOI: 10.1016/S0277-3791(00)00080-9.

Žák K, Ložek V, Kadlec J, Hladíková J and Cílek V (2002) Climate-induced changes in Holocene calcareous tufa formations , Bohemian Karst , Czech Republic. *Quaternary International* 91: 137–152.

Zawiska I, Apolinarska K and Woszczyk M (2019) Holocene climate vs. catchment forcing on a shallow, eutrophic lake in eastern Poland. *Boreas* 48: 166–178: doi:10.1111/bor.12347.

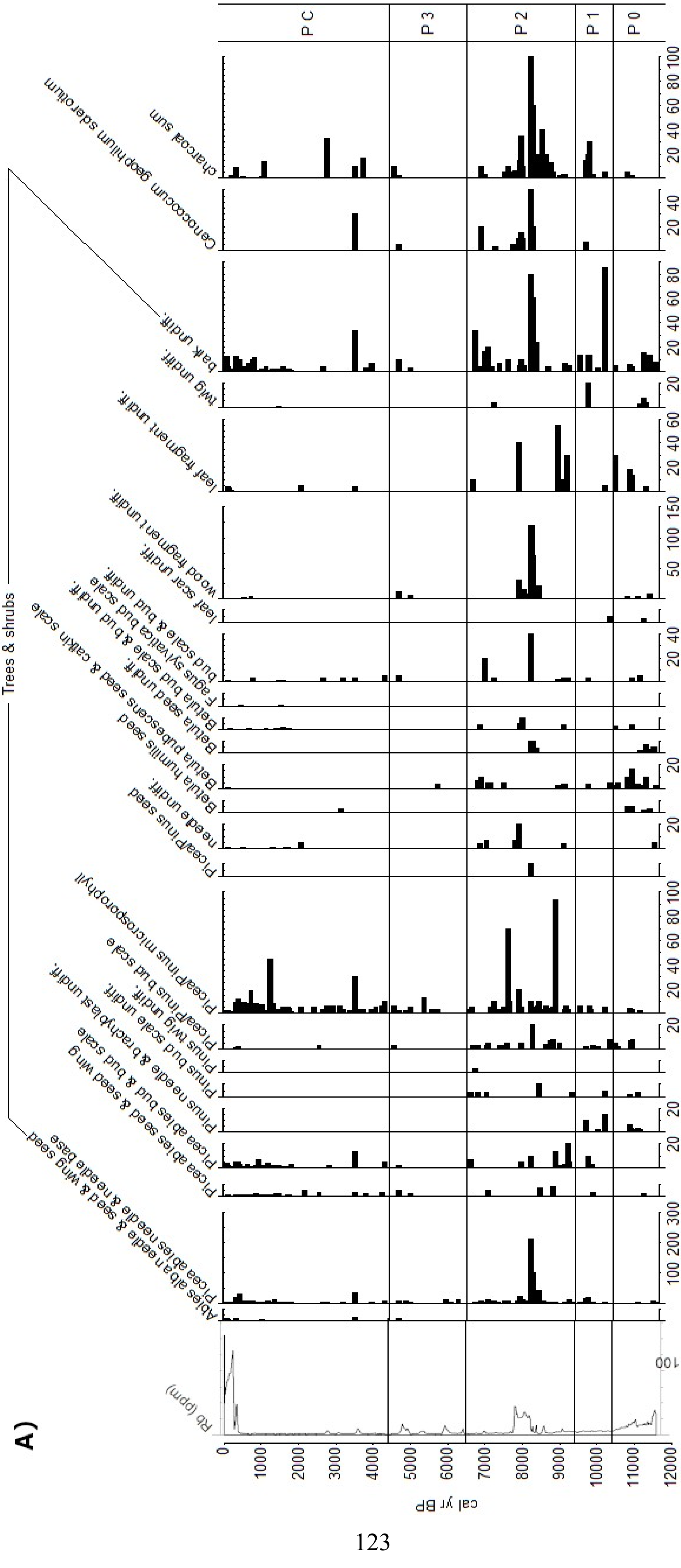
Zawiska I, Słowiński M, Correa-Metrio A, Obremska M, Luoto T, Nevalainen L, et al. (2015) The response of a shallow lake and its catchment to Late Glacial climate changes — A case study from eastern Poland. *Catena* 126: 1–10: doi:10.1016/j.catena.2014.10.007.

Zhao Y, Sayer CD, Birks HH, et al. (2006) Spatial representation of aquatic vegetation by macrofossils and pollen in a small and shallow lake. *Journal of Paleolimnology* 35(2): 335–350. DOI: 10.1007/s10933-005-1336-5.

Zwart JA, Craig N, Kelly PT, Sebestyen SD, Solomon CT, Weidel BC, et al. (2016) Metabolic and physiochemical responses to a whole-lake experimental increase in dissolved organic carbon in a north-temperate lake. *Limnology and Oceanography* 61: 723–734: doi:10.1002/lno.10248.

Supplement 1:

Supporting information to Chapter 3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cal yr BP
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4000
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Trees & shrubs
Abies alba needle & seed & wing seed
Picea abies needle & needle base
Picea abies seed & seed wing
Picea abies bud & bud scale
Pinus needle & brachyblast undiff.
Pinus bud scale undiff.
Pinus twig undiff.
Picea/Pinus bud scale
Picea/Pinus microsporophyll
Picea/Pinus seed
needle undiff.
Betula undiff.
Betula humilis seed
Betula pubescens seed & catkin scale
Betula seed undiff.
Betula bud scale & bud undiff.
Fagus sylvatica bud scale
bud scale & bud undiff.
leaf scar undiff.
wood fragment undiff.
leaf fragment undiff.
twig undiff.
bark undiff.
Cenococcum geophilum sclerotium
charcoal sum
100 200 300
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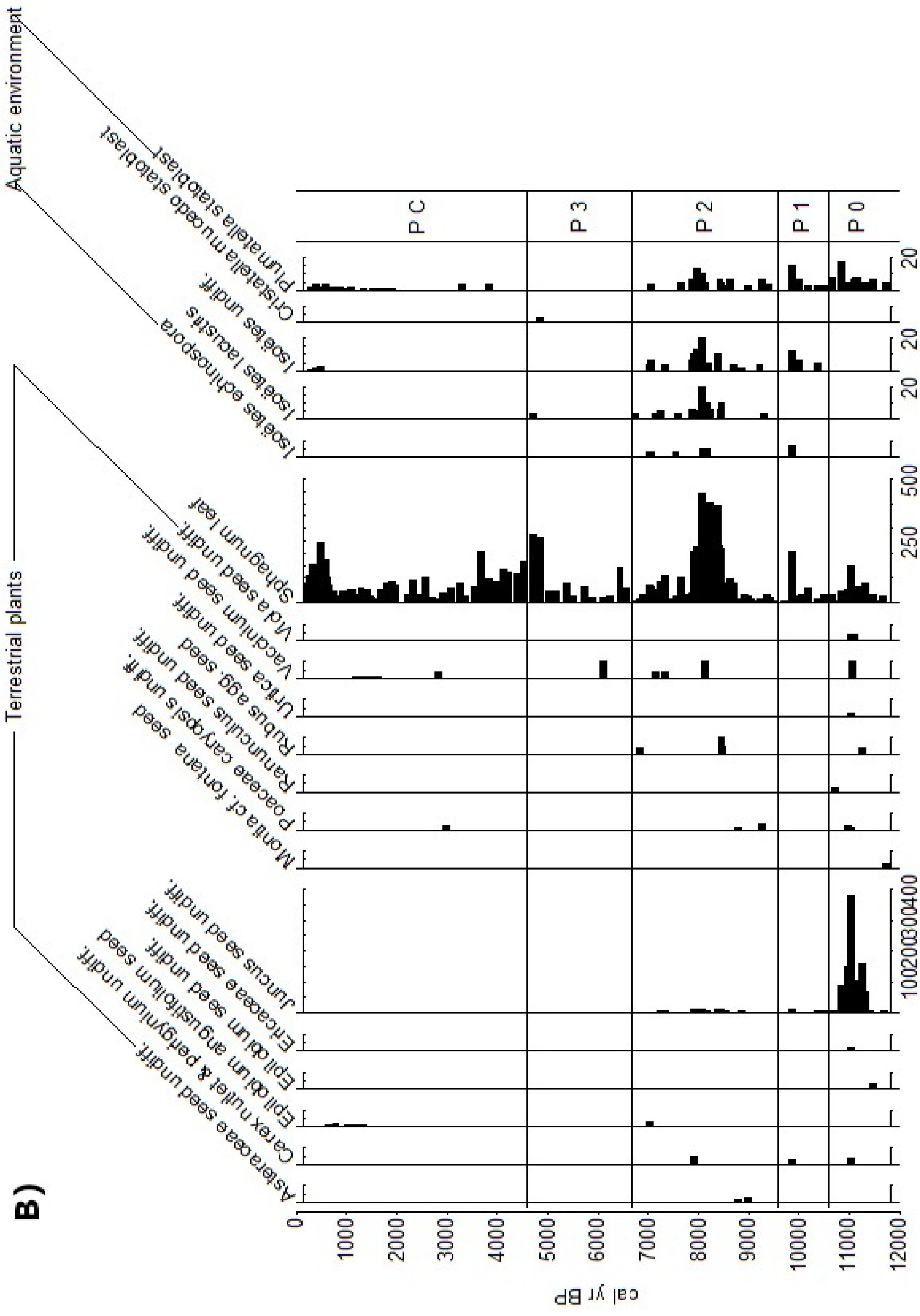
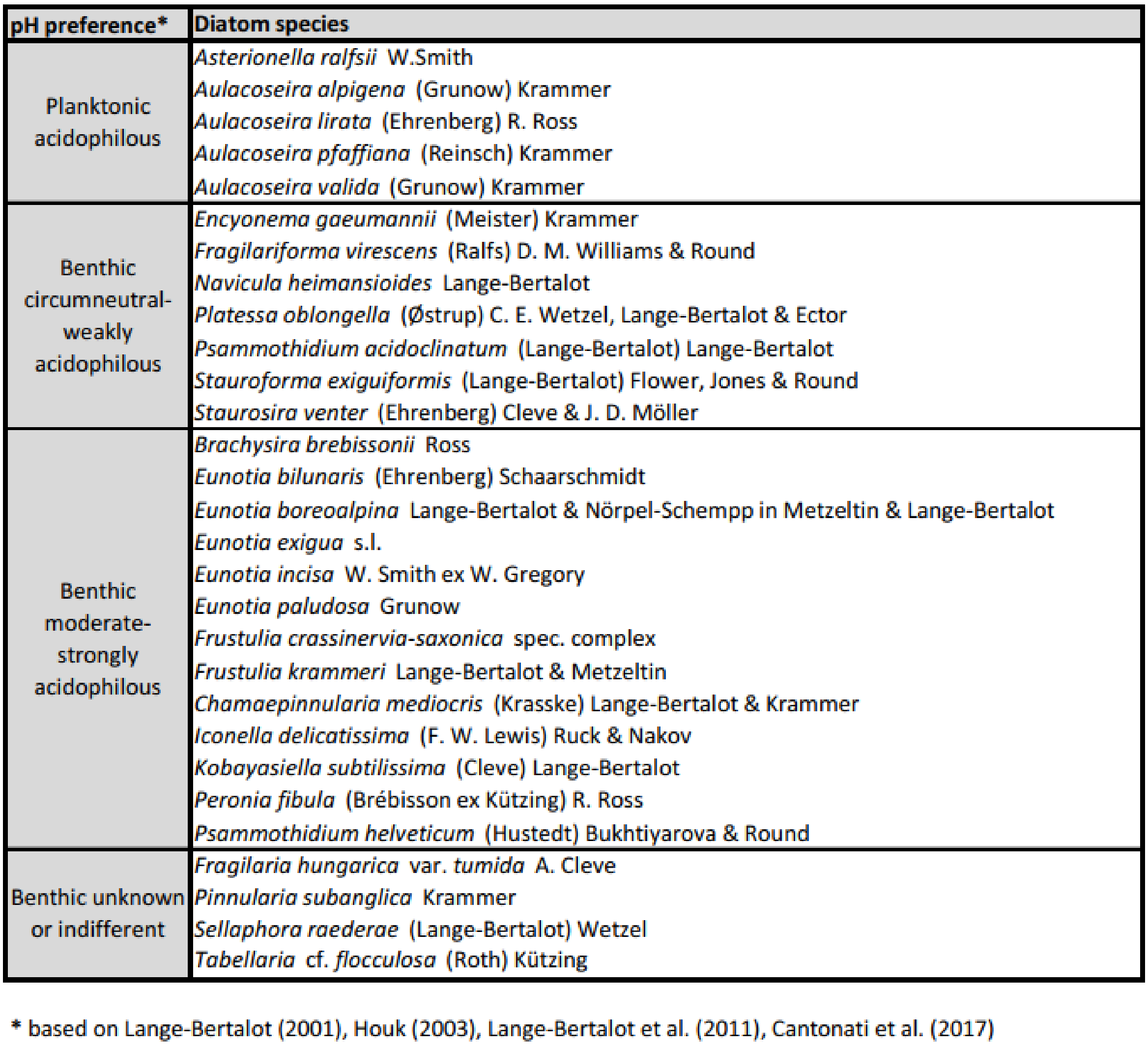


Fig. S1: Plant-macrofossil concentration (black bars) diagram and concentration of rubidium element (black curve) from the central core of Prášilské jezero (Pra-15). A): trees, shrubs, *Cenoccocum geophilum* and macrocharcoals; B): terrestrial plants and aquatic biota. The concentration of plant macrofosssils were standardized to a constant volume of 10 cm^3. Zones follow changes in *Isoëtes* microspore infix values based on change point analysis.



pH preference*	Diatom species
Planktonic acidophilous	*Asterionella ralfsii* W.Smith
	Aulacoseira alpigena (Grunow) Krammer
	Aulacoseira lirata (Ehrenberg) R. Ross
	Aulacoseira pfaffiana (Reinsch) Krammer
	Aulacoseira valida (Grunow) Krammer
Benthic circumneutral-weakly acidophilous	*Encyonema gaeumannii* (Meister) Krammer
	Fragilariforma virescens (Ralfs) D. M. Williams & Round
	Navicula heimansioides Lange-Bertalot
	Platessa oblongella (Østrup) C. E. Wetzel, Lange-Bertalot & Ector
	Psammothidium acidoclinatum (Lange-Bertalot) Lange-Bertalot
	Stauroforma exiguiformis (Lange-Bertalot) Flower, Jones & Round
	Staurosira venter (Ehrenberg) Cleve & J. D. Möller
Benthic moderate-strongly acidophilous	*Brachysira brebissonii* Ross
	Eunotia bilunaris (Ehrenberg) Schaarschmidt
	Eunotia boreoalpina Lange-Bertalot & Nörpel-Schempp in Metzeltin & Lange-Bertalot
	Eunotia exigua s.l.
	Eunotia incisa W. Smith ex W. Gregory
	Eunotia paludosa Grunow
	Frustulia crassinervia-saxonica spec. complex
	Frustulia krammeri Lange-Bertalot & Metzeltin
	Chamaepinnularia mediocris (Krasske) Lange-Bertalot & Krammer
	Iconella delicatissima (F. W. Lewis) Ruck & Nakov
	Kobayasiella subtilissima (Cleve) Lange-Bertalot
	Peronia fibula (Brébisson ex Kützing) R. Ross
	Psammothidium helveticum (Hustedt) Bukhtiyarova & Round
Benthic unknown or indifferent	*Fragilaria hungarica* var. *tumida* A. Cleve
	Pinnularia subanglica Krammer
	Sellaphora raederae (Lange-Bertalot) Wetzel
	Tabellaria cf. *flocculosa* (Roth) Kützing

* based on Lange-Bertalot (2001), Houk (2003), Lange-Bertalot et al. (2011), Cantonati et al. (2017)

Table S1: Assignment of diatom taxa to ecological groups based on their pH preferences used to infer water quality changes in Prášilské jezero.

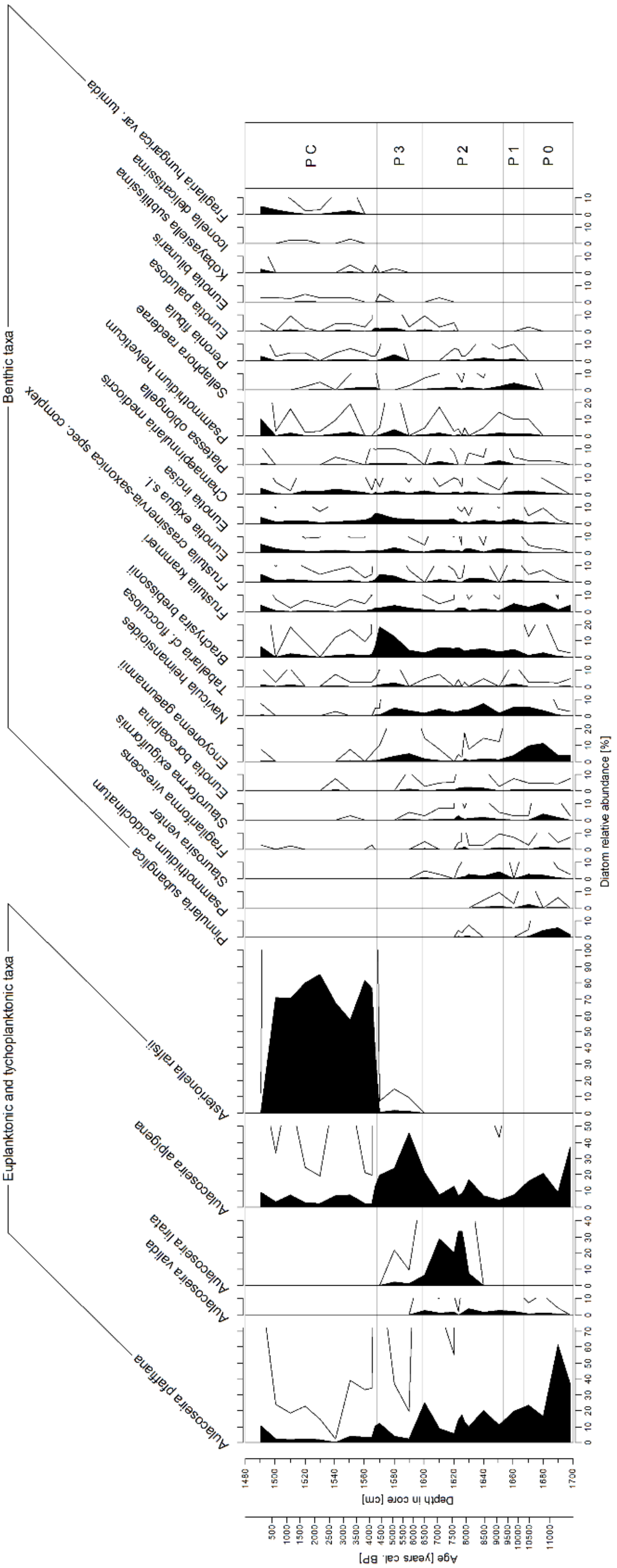


Fig. S2: Dominant diatom taxa found in Pra-15. Black silhouettes illustrate proportions (%); white areas represent 10x exaggeration. Zones follow changes in *Isoëtes* microspore infix values based on change point analysis conducted on the central core (Pra-15).

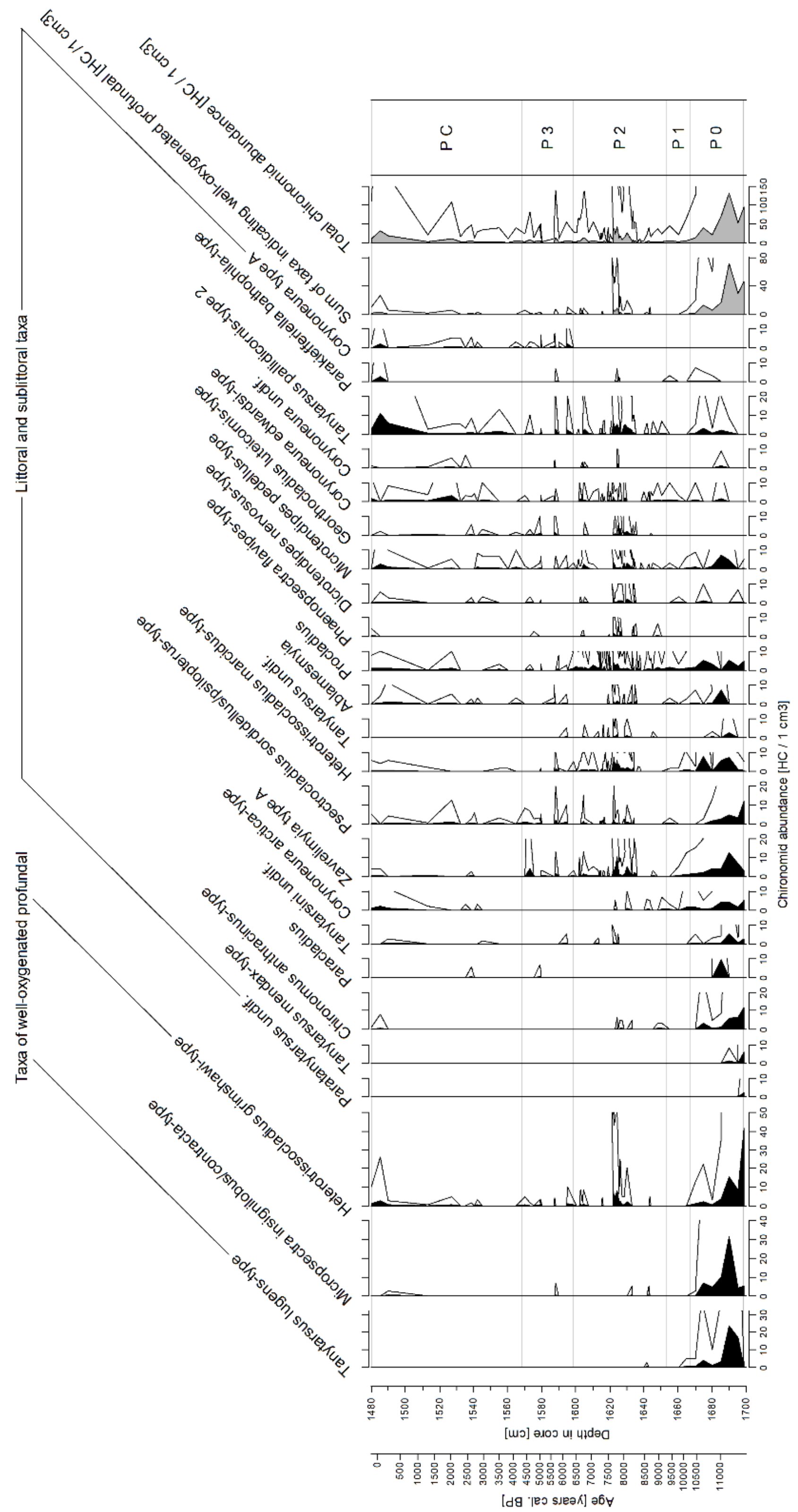


Fig. S3: Concentrations of chironomid headcapsules (HC) recorded in 1 cm^3 sediment from Pra-15. Black silhouettes illustrate the distribution of dominant chironomid taxa (at least 2 HC per 1 cm^3 in one layer), grey silhouettes show the abundance of taxa indicating well-oxygenated profundal zone and total chironomid abundance; white represents 10x exaggeration. Zones follow changes in *Isoëtes* microspore infix values based on change point analysis conducted on the central core (Pra-15).

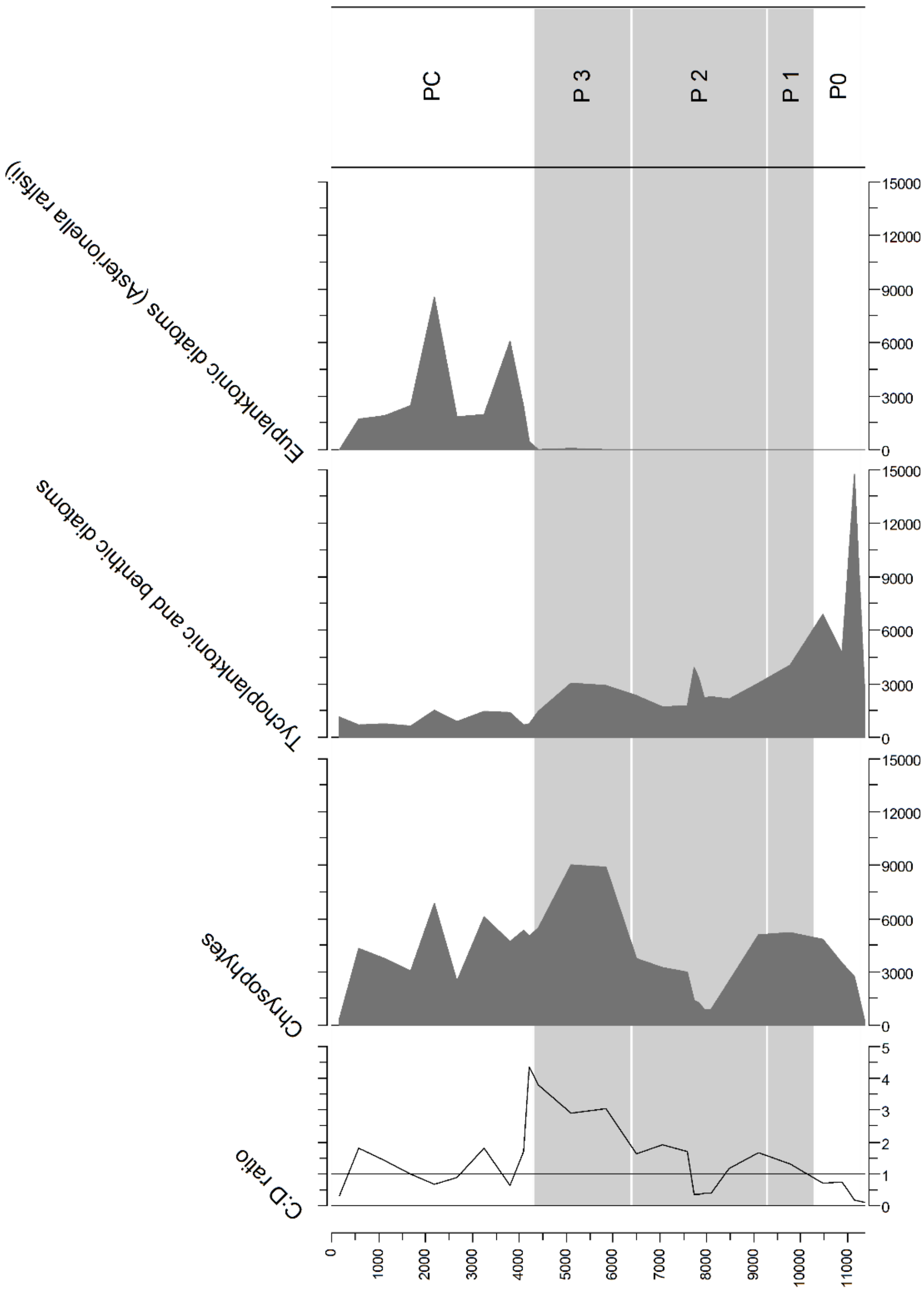


Fig. S4: Comparison of chrysophyte and diatom abundance. Black line represents the ratio of chrysophycean stomatocysts and diatom valves (C:D); grey silhouettes illustrate chrysophyte and diatom influxes (millions particles/cm^2 *year). Zones follow changes in *Isoëtes* microspore influx values based on change point analysis conducted on the central core (Pra-15).

Supplement 2:

Supporting information to Chapter 4

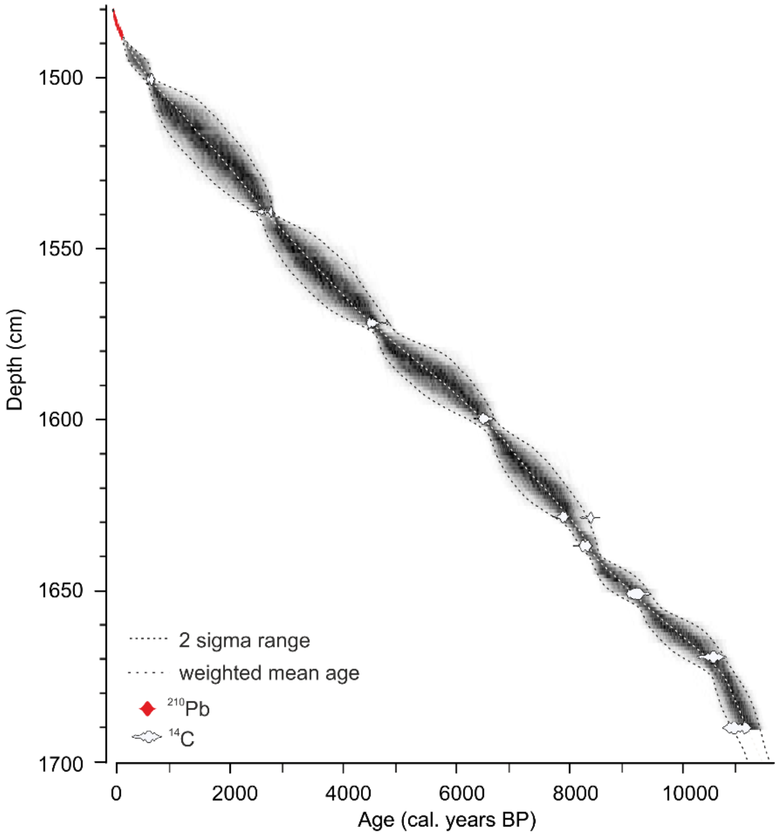
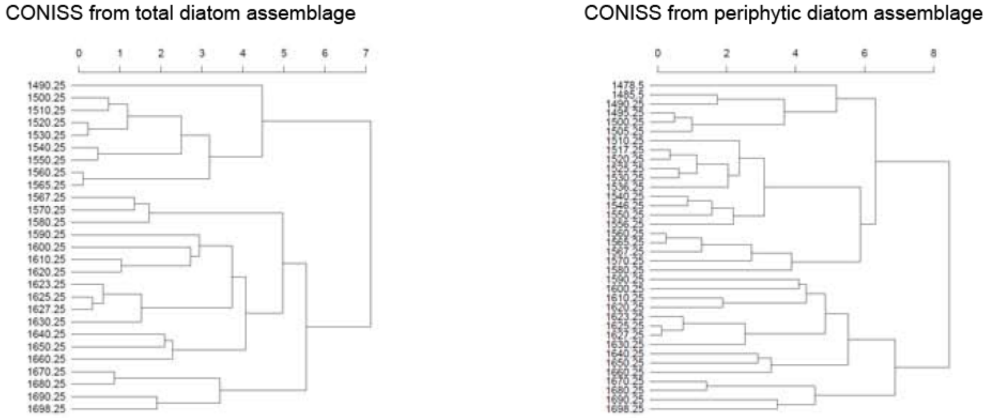


Fig. A.1: Bayesian age-depth model for the Prášilské Lake lake sediment profile. Nine ^{210}Pb-dated layers and eleven ^{14}C dates implemented.


CONISS from total diatom assemblage

CONISS from periphytic diatom assemblage

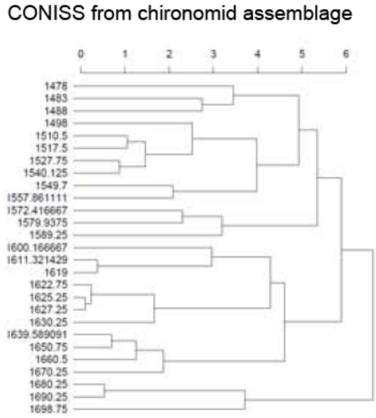

CONISS from chironomid assemblage

Fig. A2: Dendrograms of Constrained Incremental Sums of Squares (CONISS) based on various assemblages.

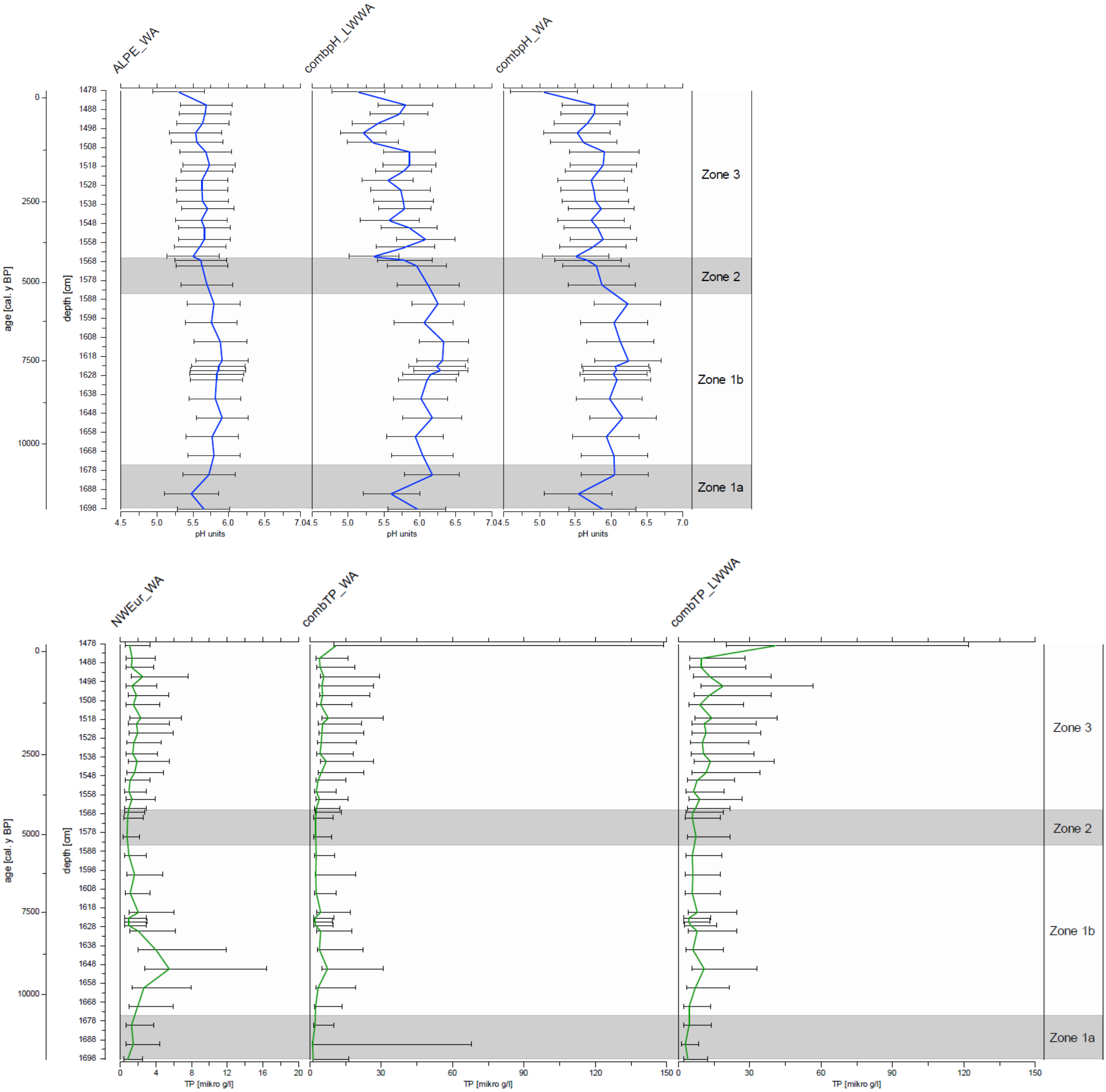


Fig. A3: Diatom-inferred pH and total phosphorus (TP) from regional and combined training sets selected by modern analogue technique (MAT) according ; WA – weighted averaging, LWWA – locally-weighted weighted averaging, ALPE – ALPE diatom-pH calibration dataset, combpH – combined diatom-pH calibration dataset, NWEur - Northwest European diatom-TP calibration dataset, combTP - combined diatom-TP calibration dataset.

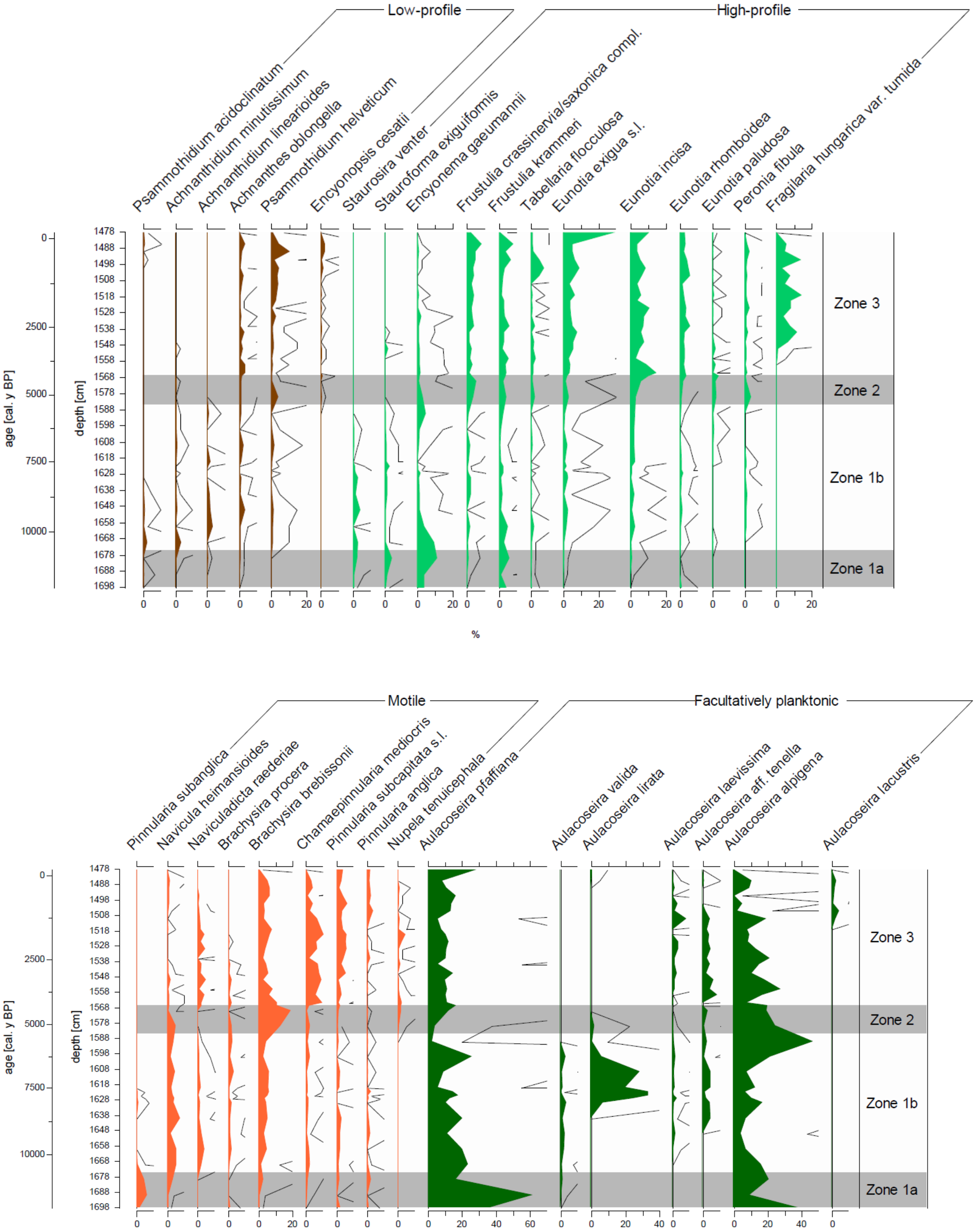


Fig. A4: Relative abundances of periphytic diatom taxa arranged into guilds – low profile (>2%), high-profile (>3%), motile (>3%), and facultatively planktonic (>3%).

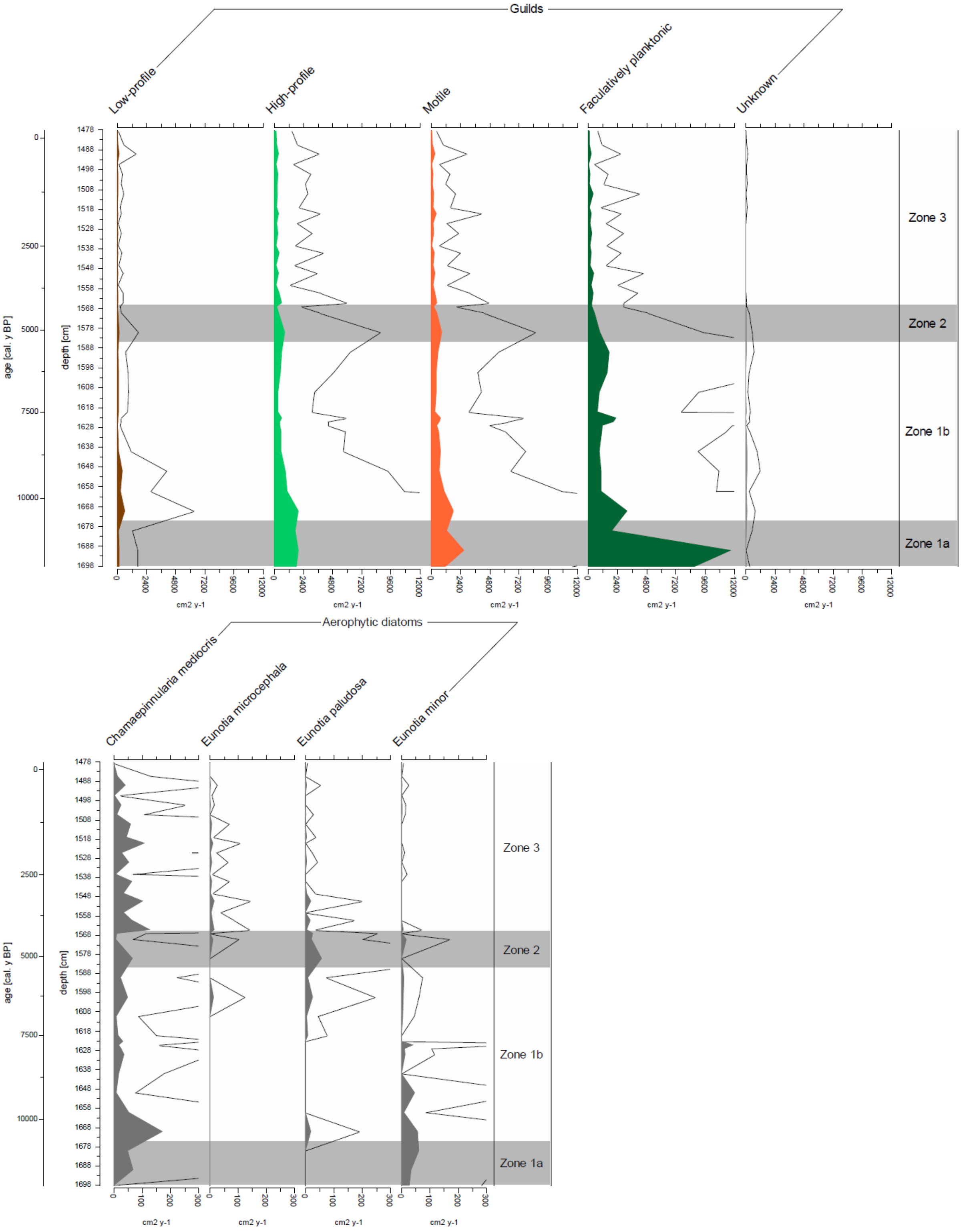


Fig. A5: Influx of important diatom ecological groups – diatom guilds (low-profile, high-profile, motile, facultatively planktonic, unknown) and aerophytic diatoms (taxa with relative abundance >1%).

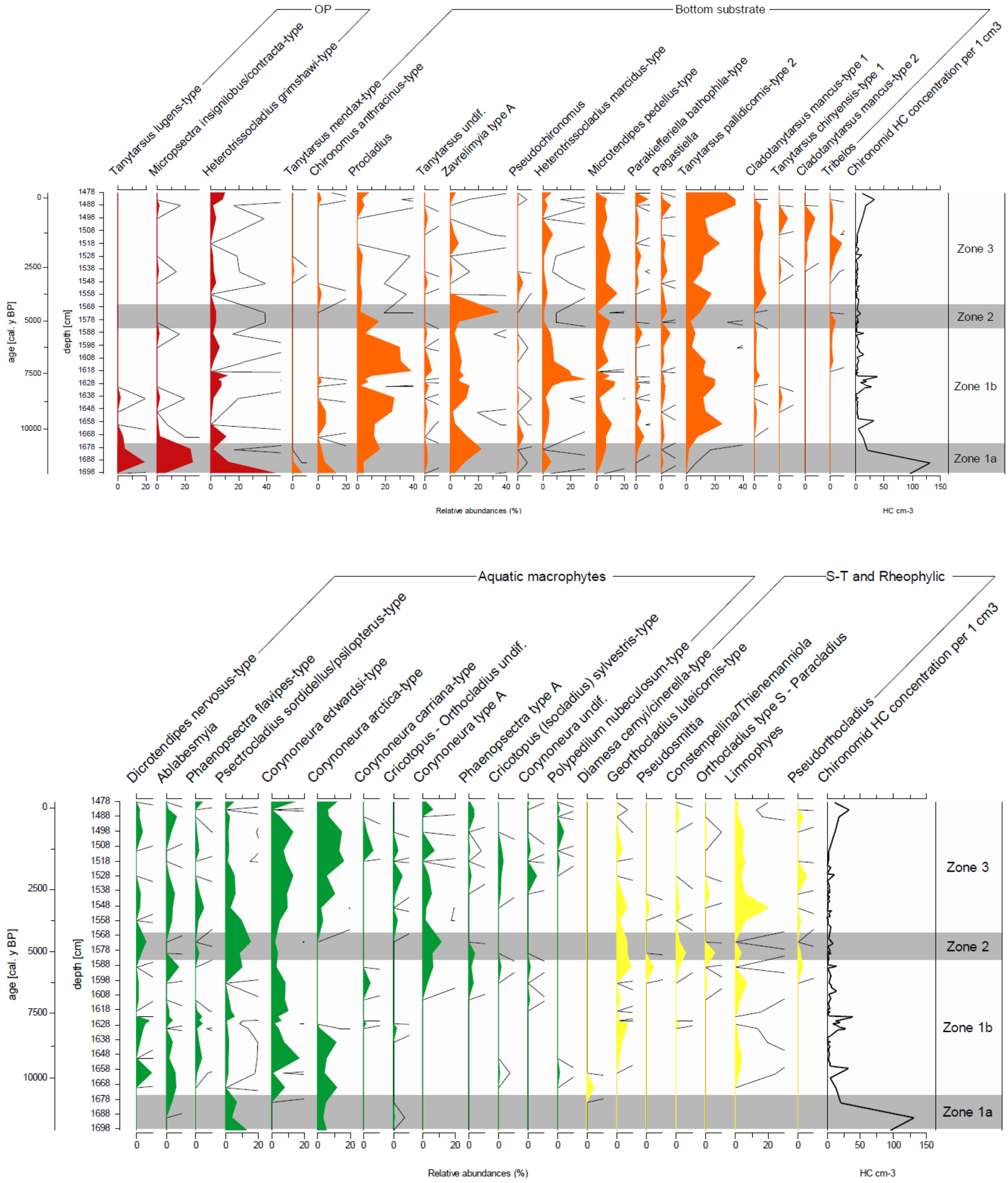


Fig. A.6: Relative abundances of chironomid taxa (>3%) arranged to ecological groups (oxygenated profundal, bottom substrate, aquatic macrophytes, semi-terrestrial and rheophylic) and concentration of chironomid head capsules (HC).

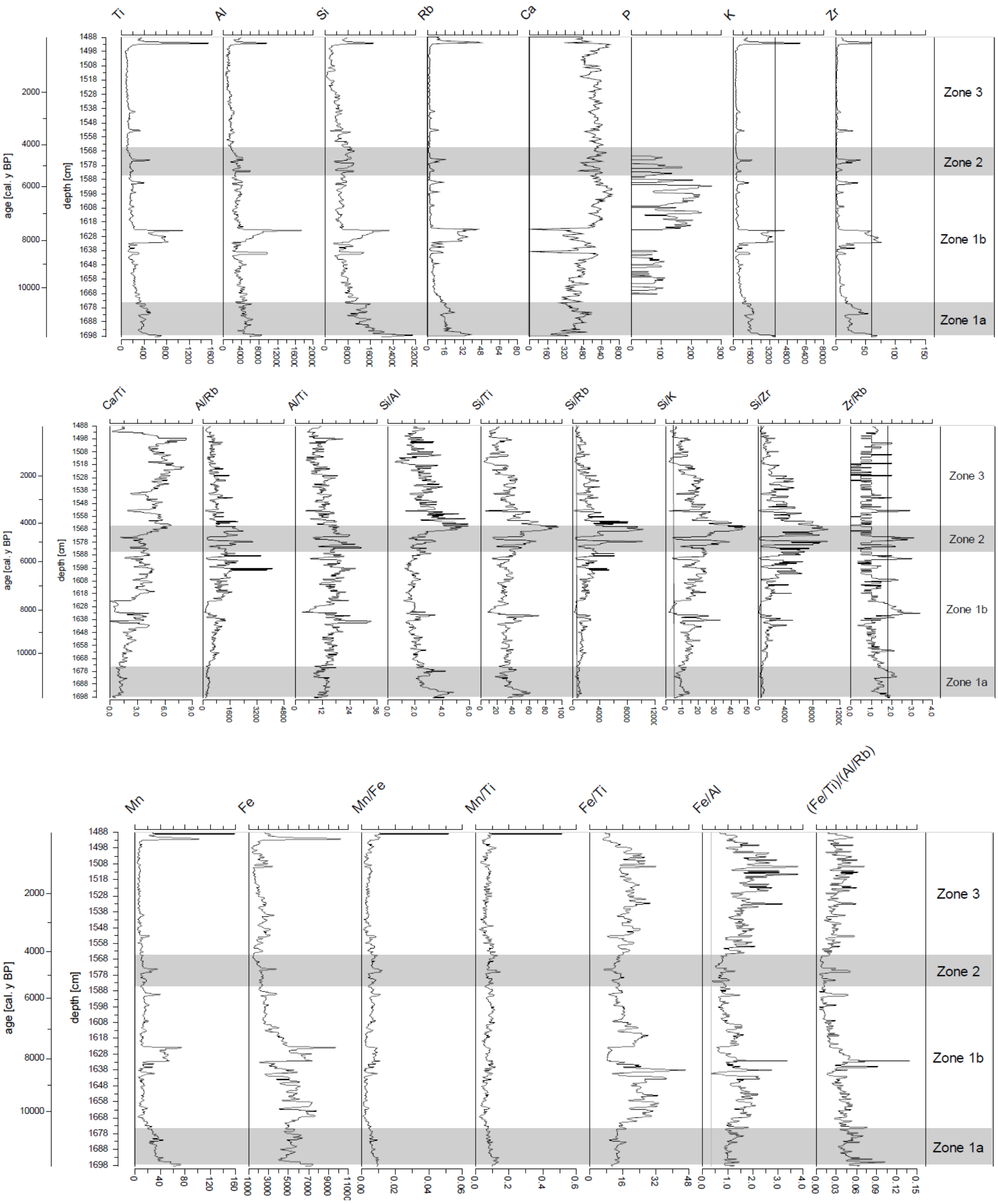


Fig. A.7: Trends of important elements and ratios (ppm).