

## ALGAL COMMUNITY CHANGE IN MOUNTAIN LAKES OF THE ALPS REVEALS EFFECTS OF CLIMATE WARMING AND SHIFTING TREELINES<sup>1</sup>

Wolfgang Kuefner<sup>2,†</sup> , Andrea M. Hofmann 

Department for Life Science Systems, Aquatic Systems Biology Unit, Limnological Research Station Iffeldorf, Technical University of Munich, Hofmark 1-3, D-82393 Iffeldorf, Germany

Juergen Geist 

Department for Life Science Systems, Aquatic Systems Biology Unit, Technical University of Munich, Mühlenweg 22, D-85354 Freising, Germany

Natalie Dubois

Department Surface Waters – Sedimentology, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Überlandstrasse 133, CH-8600 Dübendorf, Switzerland

and Uta Raeder

Department for Life Science Systems, Aquatic Systems Biology Unit, Limnological Research Station Iffeldorf, Technical University of Munich, Hofmark 1-3, D-82393 Iffeldorf, Germany

The biological communities of mountain lakes are suspected to be highly sensitive to global warming and associated catchment changes. To identify the parameters determining algal community responses, subfossil pigments from 21 different mountain lakes in the Bavarian-Tyrolean Limestone Alps were investigated. Sediment cores were radio-isotopically dated, and their pigment preservation evaluated. General additive models (GAM) of pigment compositions were calculated with temperature as the explanatory variable and generalized linear models with several lake parameters explaining log-transformed GAM *P*-values. Lake depth and trophic state were identified as major control variables of the algal community and productivity changes. Shifts in a deep oligotrophic alpine lake ( $\lg(P) = -1.04$ ) were half as strong as in a shallow mesotrophic alpine lake ( $\lg(P) = -1.86$ ) with faster warming and higher productivity forcing the development of biomass. Phytoplankton and macrophyte pigments increased clearly with warming, at lower altitudes, and decreased at the treeline, so that periphytic pigments dominated alpine sediments. This pattern is probably the result of interactions of UV radiation and allochthonous inputs of DOM. Our findings suggest that (sub)alpine shallow lakes with higher

nutrient levels are most vulnerable to climate change-driven changes whereas deep, nutrient-poor lakes appear more resilient.

**Key index words:** altitude; global warming; periphyton; pigments; plankton; sediment cores; UV radiation; vulnerability

**Abbreviations:** AIC, Akaike information criterion; CDOM, chromophoric dissolved organic matter; D+D, diadinoxanthin plus diadinochrome; DOC, dissolved organic carbon; GAM, general additive model; GLM, generalized linear model; LOI, loss-on-ignition;  $\bar{\theta}$  T<sub>Air</sub>, annual average air temperature;  $\bar{\theta}$  T<sub>SiVa</sub>, diatom-inferred summerly average lake surface temperatures; OM, organic matter; PC, principal component; REML, restricted maximum likelihood; TP, total phosphorous; WLF, water level fluctuations

The abiotic conditions of habitats govern the composition of biological communities. Conversely, biological community structures can be used as bioindicators mirroring environmental conditions such as temperature, nutrients, or light conditions (Battarbee et al. 2001, Leavitt and Hodgson 2001, Smol and Cumming 2008). Primary producers such as algae and macrophytes have been employed as indicators of trophic state, lake mixing, and bathymetry (Lotter et al. 1997, 1998, Catalan et al. 2013, Melzer and Schneider 2014). For instance, plankton algae such as cryptophytes and chrysophytes benefit from and are thus indicators of warmer waters and longer periods of thermal stratification (Lee 1999, Lami et al. 2000, Buchaca and Catalan 2007,

<sup>1</sup>Received 16 September 2020. Accepted 4 February 2021.

<sup>2</sup>Author for correspondence: e-mail wolfgang.kuefner@tum.de.

<sup>†</sup>Dr. Wolfgang Kuefner defended his PhD thesis on April 22, 2021 with honours. He wanted to enjoy this great success in his beloved mountains, where he died in a fatal accident on April 26, 2021, at the age of only 31. We mourn the loss of a promising young scientist, a much appreciated colleague and a very good friend.

Editorial Responsibility: M. Roleda (Associate Editor)

Buchaca and Catalan 2008). In addition, algal communities in lakes are also influenced by dissolved substances and UV radiation (UVR), as well as by dissolved organic matter (DOM), as it mitigates the exposure to UVR (Vinebrooke and Leavitt 1996, 1999a, Leavitt et al. 2003, Xenopoulos and Frost 2003, Chen et al. 2015). In that context, the composition of algal communities and their remains as subfossil pigment assemblages vary as a function of temperature, nutrients, and dissolved substances (Guilizzoni et al. 2012, Michalak 2016, Zhen et al. 2016, Zhang et al. 2019). However, the compositions being deposited in the sediment also depend on the pigment stability and preservation conditions. These are affected by lake characteristics such as temperature, transparency, oxygen conditions, irradiance and food web composition, as well as lake depth, that is, losses during sinking (Sanger 1988, Sun et al. 1993, Cuddington and Leavitt 1999, Buchaca and Catalan 2008). Altogether, the continuous sedimentation of biological communities onto the lake bottom provides chronological records, which allow the reconstruction of past changes in these systems in relation to processes such as eutrophication and climate change (Catalan et al. 2013).

Climate and human effects in lakes appear to vary along altitudinal gradients in mountainous systems. At higher altitudes, the biological communities of mountain lakes are highly adapted to the extreme environments of the harsh boreo-alpine climate (Sala et al. 2000, Smol et al. 2005, Fjellheim et al. 2009, Williamson et al. 2010). These climates are characterized by short growing seasons, cold temperatures, long ice, and snow covers as well as strong UVR. This damaging radiation increases by 10–20% per 1,000 m of altitude (Blumenthaler et al. 1992) and can suppress algal growth, particularly in lakes above the treeline (Vinebrooke and Leavitt 1996, 1999a, Xenopoulos and Frost 2003). Small changes in these parameters can result in strong responses of biological diversity, community composition, and productivity in mountain lake phototrophs, making them highly sensitive indicators of small environmental changes (Moser et al. 2019). Mountain lakes and their biota can therefore serve as valuable sentinels for global warming (Mueller et al. 2009, Rogora et al. 2018, Moser et al. 2019). In particular, sedimentary pigments from primary producers may act as bioindicators that integrate climate- and human-driven changes (Michalak 2016, Toride et al. 2018) and serve as a powerful bellwether of the responses of individual mountain lakes (Leavitt and Hodgson 2001, Catalan et al. 2013).

Lakes at high altitudes may be particularly affected by climate change despite being physically remote from most direct anthropogenic impacts (Sala et al. 2000, Smol et al. 2005, Mueller et al. 2009, Michelutti et al. 2015, Rühland et al. 2015, Weckström et al. 2016). For example, mountain

regions, such as the Alps, are undergoing among the greatest rates of warming in the world (EEA 2009, Warscher et al. 2019). Such elevation-dependent warming can arise because of accompanying environmental changes such as snow cover and albedo feedback loops (Pepin et al. 2015). Changes in thermal regime can affect water-column mixing behavior and duration of summer stratification, which in turn influence phytoplankton abundance. These habitats also experience significant changes in nutrient and light conditions (Koinig et al. 1998, Lotter et al. 1999, Sommaruga et al. 1999, Sommaruga and Augustin 2006). Changes in soil development (Arnaud et al. 2016), erosion, and increased incidences of heavy precipitation events (Bogataj 2007) can in turn alter the influxes of nutrients and dissolved organic matter (Leavitt et al. 2003).

The presence of sharp ecotonal boundaries such as treeline and soil presence may result in nonlinear shifts in primary producer communities. For example, warming can favor uphill migration of many plant species (Pauli et al. 2012, Rosbakh et al. 2014). The establishment of trees and sparse forests in formerly treeless alpine meadows can critically affect the allochthonous loads of organic material entering the lakes at these altitudes (Lotter et al. 1999, Sommaruga et al. 1999) that alter or even mask climate change effects (Moorhouse et al. 2018). Such a transition between alpine and montane lakes, which are located within established dense forests, can have strong effects on most aspects of lake chemistry and biology (Lotter et al. 1999, Sommaruga et al. 1999, Bunting et al. 2010). For instance, macrophytes in mountain lakes of the Alps were found to be restricted mainly to lakes below the treeline (Kuefner et al. 2020b). Therefore, analyses of subfossil pigment biomarkers and reconstructed primary production can reveal a nonlinear or even sigmoid response to global warming depending on the altitude of a mountain lake, with the treeline playing a special role (Vinebrooke and Leavitt 1999). Accordingly, contents of subfossil pigments from phototrophs, which benefit from higher loads of DOM and thus mitigated UVR, in particular groups such as macrophytes and phytoplankton, is supposed to correlate with tree densities in the catchment as well.

Atmospheric and catchment effects on mountain lakes can be further modified by individual lake characteristics. For example, lake depth and trophic state are the most important variables that modulate the response of mountain lakes to environmental changes (Kuefner et al. 2020b). In deeper lakes, hypolimnetic conditions can mitigate climate change-related developments, as the warming and biological responses are mostly limited to the epilimnion (Rühland et al. 2015, Niederist et al. 2018, Kuefner et al. 2020b). In contrast, in shallow lakes the entire water body and biota are affected by

changes in energy fluxes. Additionally, warming further stimulates the already high biomass production of eutrophic lakes and promotes the remobilization of nutrients from the sediments (Schwoerbel and Brendelberger 2013). If these lakes are polymictic and shallow, these nutrients can be immediately distributed in the water body and support elevated primary productivity. In deep stratified lakes, however, nutrients can be trapped at the lake bottom and limit phototrophic shifts (Berthon et al. 2014, Rühland et al. 2015, Kuefner et al. 2020b). Consequently, lake response to climate is expected to vary with both individual basin characteristics and along altitudinal gradients.

The main objective of this study was to analyze changes in algal communities from 21 mountain lakes in the Bavarian-Tyrolean Limestone Alps to evaluate how the abundance and composition of primary producers (as subfossil pigments) varied as a function of climate change in the past decades and centuries. Specifically, we tested the hypotheses that: (i) the shifts and increases in subfossil phototrophic pigments due to regional warming are generally strongest in shallow mountain lakes that have higher nutrient concentrations, are located at subalpine and alpine altitudes, and describe the strongest succession of substrata; (ii) most significant community shifts coincide with the elevation of the treeline; and (iii) warming-driven shifts in primary producer communities are mainly mediated by specific guilds, which are limited by altitude and prefer the warmer waters of lower elevations. To test these hypotheses, the individual history and development of phototrophic communities in mountain lakes were reconstructed using subfossil pigments (Hodgson et al. 1997). The pigments were extracted from dated sediment cores of 21 Bavarian (Germany) and Tyrolean (Austria) mountain lakes located along a wide gradient of altitudes, phosphorous concentrations, and depths.

#### MATERIALS AND METHODS

**Study sites.** The 21 mountain lakes investigated are located in the Northern Limestone Alps along the German-Austrian border (47°17' to 47°45' N, 10°15' to 13°1' E; Fig. 1). The sites range in altitude from 922 to 2,060 m a.s.l. and vary in area from 0.4 to 12 ha and in depth from 1.3 to 20.7 m (cf. Kuefner et al. 2020a,b). Lakes are categorized according to their catchment characteristics into three altitudinal levels: montane (900–1,250 m a.s.l.), subalpine (1,250–1,650 m a.s.l.), and alpine (1,650–2,100 m a.s.l.). Montane lakes are surrounded by mixed forests, consisting mainly of spruce (*Picea abies*) and beech (*Fagus sylvatica*), subalpine areas are characterized by sparse coniferous forests (spruce and larch *Larix decidua*) and alpine pasture, while alpine catchments consist only of sparse vegetation such as alpine meadows (e.g., evergreen sedge *Carex sempervirens*, hairy alpenrose *Rhododendron hirsutum*) and patches of dwarf pine (*Pinus mugo*). Accordingly, the treeline in the study area is currently located around 1,650 m a.s.l. separating subalpine from alpine lakes. No glaciers remain in the catchments studied. Geologically, dolomite, limestone, and glacial deposits

(lodgement, moraine deposits) predominate substrates in the study area. Two lakes are postglacial (doline and landslide lakes), while all others are of glacial origin (cirque and moraine lakes). All the mountain lakes are well-buffered hard water lakes with a mean pH of 8.4 and an electrical conductivity of 240  $\mu\text{S} \cdot \text{cm}^{-1}$  (at 25°C). The lakes range from ultra-oligotrophic to mesotrophic with an average total phosphorous concentration (TP) of 7.6  $\mu\text{g} \cdot \text{L}^{-1}$  (0.1–17.3  $\mu\text{g} \cdot \text{L}^{-1}$ ). The mean water temperatures reached 16°C (10.8–22.6°C) in late-August 2016 at the surface and 9.7°C (4.6–18.4°C) at the bottom. On average, Secchi depth was on average 5.3 m with a maximum of 15.5 m, although light penetrated to the bottom substrata in most of the shallow lakes. The presence of macrophytes and pronounced water level fluctuations (WLF), that is, WLF > 1.5 m, were also documented. Kuefner et al. (2020a,b) provide a detailed description of both the Bavarian and Tyrolean mountain lakes' hydrophysics and hydrochemistry.

**Core sampling and dating.** Sediment cores were taken from the deepest area of each lake using a gravity corer (Uwitech, Mondsee, Austria) fitted with a 6-cm diameter tube. The sediment cores were immediately hermetically sealed in dark foil and stored at 4°C for no longer than 3 months. The cores were then subsampled at intervals of 1 cm under darkened conditions.

For sediment dating, subsamples of one core half were freeze-dried, ground, and then analyzed by gamma spectrometry (High-purity Germanium Well Detector, HPGe, Canberra, USA) in the Surface Waters Department of the EAWAG (Dübendorf, Switzerland). Calculated accumulation rates were based on the activities of the  $^{210}\text{Pb}$  and  $^{137}\text{Cs}$  radionuclides.  $^{210}\text{Pb}$  chronologies refer to unsupported isotopes, assuming a constant rate of supply (CRS model, Appleby 2001). The CRS model was further confirmed or adjusted using anthropogenic peaks of  $^{137}\text{Cs}$  corresponding to nuclear weapons testing (1963) and the Chernobyl disaster (1986; Appleby 2001, Kirchner 2011). Full dating results are presented in Kuefner et al. (2020c).

**Pigment analysis.** The pigment extraction procedure primarily followed Leavitt and Hodgson (2001). Sediment samples for pigment analyses were immediately wrapped in aluminum foil and stored at –18°C before being freeze-dried in the dark. Pigments were extracted by adding 0.3 g of each sample to 4 mL of ROTISOLV HPLC acetone (Carl Roth, Karlsruhe, Germany) in the dark at –20°C for 24 h. Solutions were then centrifuged for 15 min at 5532g in a Heraeus Fresco 17/21 microcentrifuge (Thermo Fisher Scientific, Schwerte, Germany) and clear supernatant pushed through using a 0.2  $\mu\text{m}$  pore PTFE filter. Samples were stored at –80°C before being analyzed by reverse phase HPLC using the Jasco MD-2015 system (Jasco, Pfungstadt, Germany). Known aliquots (50–200  $\mu\text{L}$ ) were separated by HPLC with a flow rate of 1  $\text{mL} \cdot \text{min}^{-1}$  and a visible spectrum recorded for 350–750 nm. The HPLC solvents and pigment separation program followed Bidigare et al. (2005). The system was calibrated to authentic standards of eleven pigments: alloxanthin,  $\beta$ -carotene, chl *a* and *b*, canthaxanthin, diatoxanthin, fucoxanthin, lutein, neoxanthin, peridinin, zeaxanthin, obtained from VWR (Darmstadt, Germany) and DHI (Hørsholm, Denmark). Calibration and pigment quantifications refer to the 450 nm channel, except for chl *a*, which refers to the 436 nm line.

Sedimentary pigment concentrations were related to sample dry weight and expressed as  $\mu\text{g} \cdot \text{g}^{-1}$  for calibrated compounds. For other detected pigments, spectra and peak time data were compared with data from Wright et al. (1991) to tentatively identify the pigment. Their approximate concentrations were expressed as a function of the peak area and sediment mass, so that these pigments were given in  $\text{mV} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ . Changes in preservation environment were estimated using the ratio

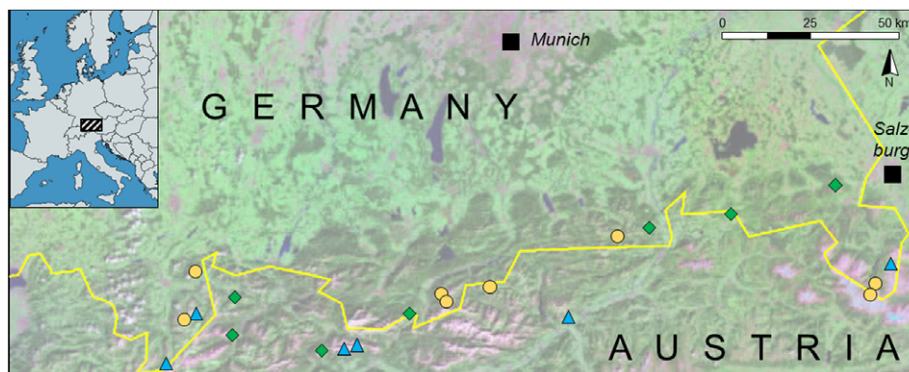


FIG. 1. Study area and location of the 21 mountain lakes. The different symbols represent the altitudinal class of the lakes: diamonds—montane; circles—subalpine; triangles—alpine. [Color figure can be viewed at wileyonlinelibrary.com]

between chl *a* and its derivate phaeophytin *a* (Chl/Phe), a ratio commonly used as a measure of pigment conservation (Leavitt 1993, Cuddington and Leavitt 1999, Waters et al. 2005, Chen et al. 2015, Tonnö et al. 2019). Individual indications of phototrophic pigments and taxonomic affinities are shown in Table 1.

*Environmental proxies.* Temperature is one of the major drivers of global change triggering environmental and meteorological changes in mountain habitats (Luoto and Nevalainen 2016). Accordingly, the regional average air temperature ( $\bar{T}_{Air}$ ) was calculated from local weather station data for Munich, Hohenpeißenberg, Innsbruck, Salzburg, and Zugspitze provided by the meteorological services in Germany (DWD) and Austria (ZAMG). Mean temperatures were only calculated for the period since 1900 as no continuously measured temperature data are generally available before that time. For cores comprising more than 120 y, only meteorological data from Hohenpeißenberg were used, as these have been available continuously since 1781.

Summer lake temperatures were also included in statistical analyses of climate effects on lakes as they can deviate from

air temperatures due to basin-specific differences in hydrology and physical structure (Niederist et al. 2018). As there is no physical data available for the mountain lakes, the temperatures ( $\bar{T}_{SiVa}$ ) were inferred from subfossil diatom assemblages by applying the silicification value (SiVa) described by Kuefner et al. (2020a; Fig. S1 in the Supporting Information). The corresponding transfer-function (eq. 1) is based on surface sediments from 43 mountain lakes of the same study area and refers to the negative correlation between lake temperature and average silicifications of diatom assemblages (Kuefner et al. 2020c). These integrate individual species abundances and species-specific valve silicification.

$$T_{SiVa} [^{\circ}C] = 25.956 - 4.7155 \cdot SiVa \quad (RMSEP = 0.976) \quad (1)$$

Additionally, the data of subfossil diatom assemblages were used to estimate the historical evolution of mountain lake substrata. This value is represented by the  $\beta$ -diversity of the compositional turnover of diatom assemblages, as many diatoms prefer specific substrata, and is referred to as

TABLE 1. The 14 most frequently occurring pigments in Bavarian and Tyrolean mountain lake sediments.

Pigment	Unit	Maximum concentration	Average concentration	Number of lakes	Taxonomic affinity
Alloxanthin	[ $\mu\text{g} \cdot \text{g}^{-1}$ ]	95.17	8.42	20	Cryptophytes <sup>1-4,13</sup>
$\beta$ -Carotene	[ $\mu\text{g} \cdot \text{g}^{-1}$ ]	279.56	44.80	21	Total primary production <sup>6-8</sup>
Chlorophyll <i>a</i>	[ $\mu\text{g} \cdot \text{g}^{-1}$ ]	838.22	49.70	21	Total primary production <sup>6-8</sup>
Chlorophyll <i>b</i>	[ $\mu\text{g} \cdot \text{g}^{-1}$ ]	152.81	14.04	20	Chlorophytes, macrophytes <sup>4,6,11,13</sup>
Diadinochrome	[ $\text{mV} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ ]	192.52	32.62	17	Derivative of diadinoxanthin <sup>14</sup>
Diadinoxanthin	[ $\text{mV} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ ]	309.54	11.52	11	Siliceous algae, mainly diatoms <sup>4-6,11,13</sup>
Diatoxanthin	[ $\mu\text{g} \cdot \text{g}^{-1}$ ]	136.04	15.20	20	Diatoms <sup>13</sup> (also chrysophytes) <sup>1-5</sup>
Fucoxanthin	[ $\mu\text{g} \cdot \text{g}^{-1}$ ]	1,003.15	41.91	21	Mainly diatoms (also chrysophytes) <sup>1-5</sup>
Lutein	[ $\mu\text{g} \cdot \text{g}^{-1}$ ]	220.07	28.32	20	Chlorophytes <sup>1-5,13</sup> , higher plants; e.g., macrophytes <sup>11</sup>
Neoxanthin	[ $\mu\text{g} \cdot \text{g}^{-1}$ ]	225.77	10.73	20	Chlorophytes <sup>1,13</sup>
Peridinin	[ $\mu\text{g} \cdot \text{g}^{-1}$ ]	22.62	1.180	13	Dinoflagellates <sup>1-5</sup>
Phaeophytin	[ $\text{mV} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ ]	106.93	12.20	21	Degraded derivate of Chl <i>a</i> <sup>4,9,10</sup>
Prasinoxanthin	[ $\text{mV} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ ]	181.48	28.27	17	Prasinophytes <sup>12</sup>
Zeaxanthin	[ $\mu\text{g} \cdot \text{g}^{-1}$ ]	364.81	41.90	21	Cyanobacteria <sup>11</sup> , but with Canthaxanthin <sup>1-6,12</sup>

Maximum and average amounts per dry sediment are shown, the numbers of lake sediments in which each pigment was found as well as their taxonomic affinities. Numbers in italics represent the values of uncalibrated pigments. Averages are arithmetic means. Minimum values are not shown, as each pigment (except Chl *a*) fell below the detection limit in at least one sample. References of taxonomic affinity (superscripted numbers) are 1: Goodwin (1980), 2: Züllig (1982), 3: Hurley and Armstrong (1990), 4: Leavitt (1993), 5: Olaizola et al. (1994), 6: Leavitt and Hodgson (2001), 7: Lotter et al. (2002), 8: Das et al. (2005), 9: Cuddington and Leavitt (1999), 10: Waters et al. (2005), 11: Lami et al. (2000), 12: Wright et al. (1991), 13: Buchaca and Catalan (2008), 14: Krajewska et al. (2017).

'substratum succession proxy' in the following. The diatom data derived from Kuefner et al. (2020b).

**Numerical analyses.** Changes in pigment preservation were evaluated using two reference criteria: a strong increase in the Chl/Phe ratio of >25 units or independent and disproportionate increases of chl *a* compared to the more chemically stable  $\beta$ -carotene, both being biomass markers. Massive increases in pigment concentrations due to ongoing degradation would strongly bias the results toward exaggeration of the expected impact in relation to climate change and are most common in surface sediment deposits. Accordingly, the uppermost centimeters that fulfilled the criteria, indicating that degradation was still in progress, were excluded from further analysis. The pigment assemblages of all the sediment cores were combined and reduced to a main set of principal pigments that were detected in the majority of lakes, while rare pigments found in only one or two cores were excluded from further analysis. To quantify the temporal variances of the pigments, a PCA was conducted for each lake using all sediment samples together. The significance of the PCA axes was calculated by applying the broken-stick model in the scree plot. The PCA axes, which significantly explained the pigment variances, were then correlated against temperature proxies.

General additive models (GAM) were computed to evaluate how much historical variability in pigment composition can be explained by temperature change. GAMs are a non-parametric extension of generalized linear regression models and use smoothing splines instead of linear coefficients as covariates. This makes GAMs a more effective and appropriate method for addressing nonlinear relationships between response and explanatory variables (Hastie and Tibshirani 1990, Pearce et al. 2011), as the pigment patterns were translated into principal components, which do not necessarily follow linear functions (Makri et al. 2019, Tonnö et al. 2019). GAM smoothing was calculated using restricted maximum likelihood (REML). At the same time, the scores of significant PCA axes were tested for their dependence on temperature proxies, using Spearman correlations. Finally, the significance levels of the correlations and model calculations were used in this study to quantify the degree of vulnerability of the phototrophic communities to warming (i.e., the 'strictness' of biological response). Then, environmental parameters were detected that modulate this strictness. For this purpose, generalized linear models (GLM) with an AIC-based stepwise backward selection were calculated using the correlation and GAM *P*-values as response variables and strictness proxy with a set of environmental proxies as explanatory variables. The response *P*-values were preliminarily log-transformed to normalize the distribution and allow a Gaussian GLM approach. Consequently, negative model estimates indicate a positive correlation with temperature-driven lake reactions. Altitude, lake surface area, total phosphorous (TP), Secchi depth, and the 'substratum succession proxy' were implemented as independent variables, and depth and lake volume as dependent parameters. Additionally, GLM calculations were performed exclusively for mountain lakes with an equal pigment preservation (i.e., Chl/Phe ratios) in order to avoid distorting lake depth effects, such as differential losses during variable sinking distances.

The GAM analyses were calculated using the 'mgcv' package (Wood 2019) and the GLMs with the standard package in R, version 3.6.3 (R Core Team 2020). PCA and correlation analyses were computed in PAST 3.25 (Hammer et al. 2001). Significance levels (*P*-value) of  $\leq 0.05$  were considered significant and are marked with asterisks; *P*-values  $\leq 0.1$  were also highlighted.

## RESULTS

**Sediment core characteristics.** The length of most sediment cores was 9–55 cm, revealed an average sedimentation rate of  $0.27 \text{ cm} \cdot \text{a}^{-1}$  ( $0.08$ – $0.60 \text{ cm} \cdot \text{a}^{-1}$ ), and dated back to the year 1899 (1,649–1,971). On the other hand, the 10 cm core of the doline lake indicated a CRS of  $1.17 \text{ cm} \cdot \text{a}^{-1}$  and only dated back to the year 2010. This core was not used in further statistical analyses. For detailed sediment dating results, see supplementary material in Kuefner et al. (2020c).

**Pigment analysis.** The HPLC analyses detected ten of the eleven calibrated pigments in the mountain lake samples (Fig. 2, Figs. S2-S19 in the Supporting Information). Of these, nine pigments were found in almost every sediment core, with neoxanthin exhibiting only low concentrations in most cores. Peridinin occurred in only 13 lakes, whereas canthaxanthin could not be detected in any sample, which may also be the result of overlaps with neighboring peaks. Besides those, peaks with specific run times and spectral patterns possibly originated from eleven more pigments. Owing to highly constant peak characteristics, the chl *a*-derivative phaeophytin as well as diadinoxanthin, and its derivate diadinochrome ('D+D') could be identified with high probability in most of the cores, as could prasinoxanthin albeit less frequently. The fact that other studies already detected D+D (Repeta 1989, Brotas and Plante-Cuny 2003, Krajewska et al. 2017) and prasinoxanthin in sediments (Sampere et al. 2011, Kang et al. 2016, Zhang et al. 2019) supports the tentative identification of these pigments. Hence, D+D were summarized, and all four pigments included in the following analysis. Moreover, seven more peaks resembled specific pigments, but with less certainty and rare occurrences: antheraxanthin, ethyl 8'-beta apocarotenoate, the neoxanthin-isomers neochrome and 9'-cis-neoxanthin, siphonaxanthin, siphonene, and violaxanthin. They were not considered in further analysis.

The highest subfossil pigment concentrations were recorded for the ubiquitous pigments chl *a* and  $\beta$ -carotene, as well as fucoxanthin, with maximum values of  $838 \mu\text{g} \cdot \text{g}^{-1}$  (chl *a*),  $280 \mu\text{g} \cdot \text{g}^{-1}$  ( $\beta$ -C.), and  $1,003 \mu\text{g} \cdot \text{g}^{-1}$  (fuco.) of dry sediment, respectively (Table 1, Fig. 2). Together with the chl-derivative phaeophytin *a*, these pigments were detected in every mountain lake and most samples. In contrast, the other pigments were absent from (ultra)oligotrophic lakes or were present only in individual mountain lakes. For example, alloxanthin was found predominantly in deeper mountain lakes with the highest concentration of  $95 \mu\text{g} \cdot \text{g}^{-1}$  detected in Lake Taubensee.

The total pigment quantities were highly divergent among lakes. Total pigment concentrations were the highest in the uppermost centimeters of most cores, while the highest amounts in the

■ Alloxanthin ■  $\beta$ -Carotene ■ Chlorophyll *a* ■ Chlorophyll *b* ■ Diatoxanthin ■ Fucoxanthin ■ Lutein  
 ■ Neoxanthin ■ Peridinin ■ Zeaxanthin

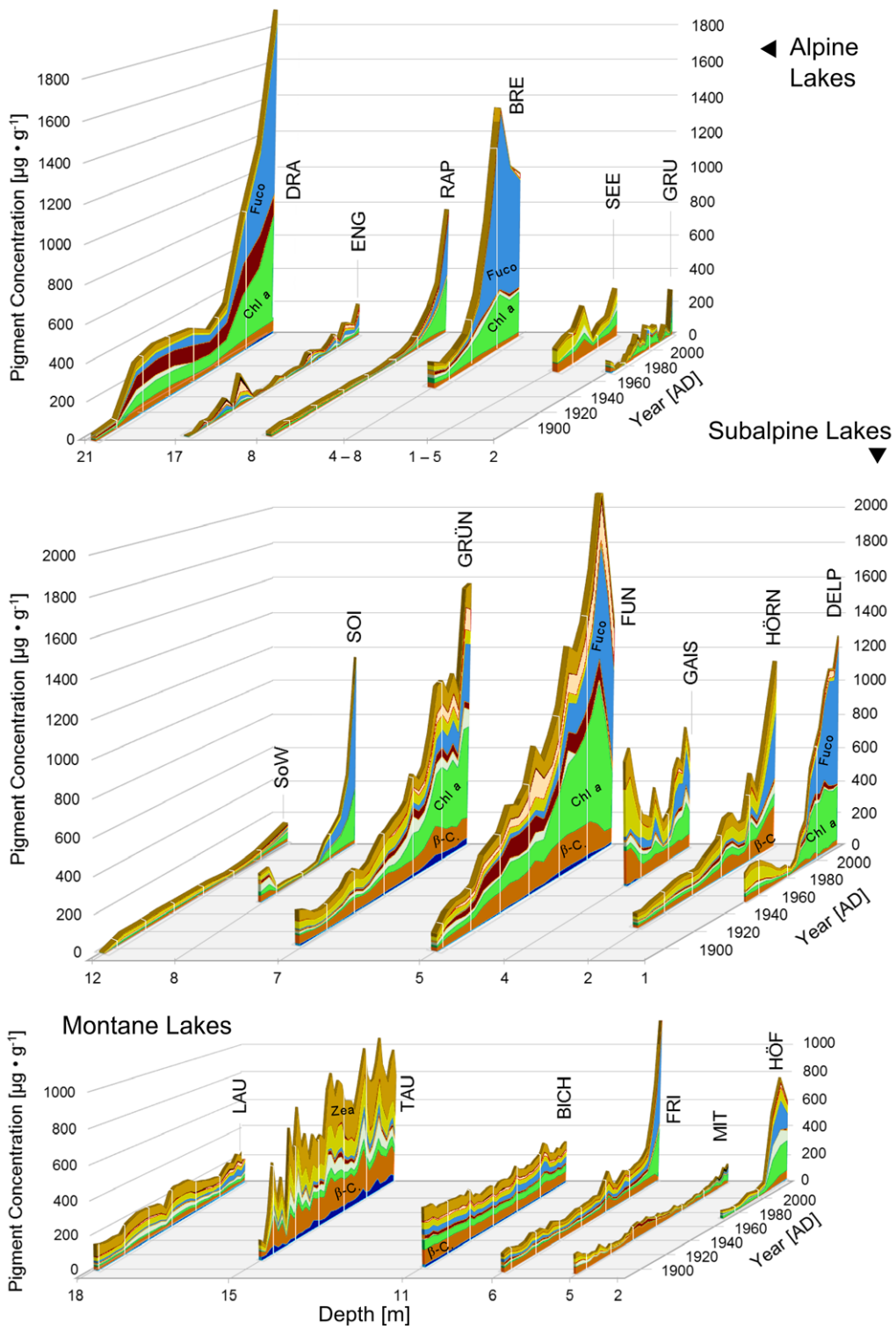


FIG. 2. Subfossil pigments in the sediment cores of montane (900–1,250 m a.s.l.) and subalpine (1,250–1,650 m a.s.l.) and alpine mountain lakes (1,650–2,100 m a.s.l.) in the Bavarian Alps. Mountain lakes are ordered on the *x*-axis according to their depth, while pigment concentration is given on the *y*-axis in µg per g sediment dry weight, and sample age is given on the *z*-axis. Pigment stratigraphies in each lake are always ordered from alloxanthin (bottom) to zeaxanthin (top). [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

sediments of alpine lakes were mostly  $<200 \mu\text{g} \cdot \text{g}^{-1}$ , those of subalpine and montane lakes  $>1,000 \mu\text{g} \cdot \text{g}^{-1}$  (Fig. 2). Pigment preservation was highly variable when the water body bottom was illuminated and aerobic, as indicated by the strong increase in Chl/Phe ratio. This means that the pigments in the uppermost centimeters of the sediment core, which comprise approximately the last six years, were often still subject to ongoing degradation (Koinig et al. 2002, Hyodo et al. 2008). The youngest sediment sample(s) were therefore excluded from further analysis in the event of strongly increasing Chl/Phe ratios. Additionally, six out of the 13 shallow lakes ( $<10 \text{ m}$ ) revealed significantly elevated Chl/Phe ratios of 13.8 on average (medians: 12.2–15.5), whereas the medians of the remaining lakes averaged 3.9 (1.9–7.6). Accordingly, separate GLMs were calculated for the latter group of 14 lakes (Fig. S20 in the Supporting Information). Details of the pigment stratigraphies of each sediment core can be found in the supplement, together with all detected pigments, Chl/Phe ratios, and numbers of excluded sediment samples.

**PCAs and GAMs.** The first two axes of the PCA of all sediment samples together explained 82.2% of all variance (Fig. 3). The pigments Chl *a*, D+D, diatoxanthin, fucoxanthin, and prasinoxanthin are well represented by PC 1, whereas alloxanthin,  $\beta$ -carotene, Chl *b*, lutein, phaeophytin, and zeaxanthin are more oriented along PC 2 (for axis loadings, see Fig. S21 in the Supporting Information). PC 1 and 2, in turn, correlated well with temperature proxies. PC 1 displayed a significant positive correlation with the regional air temperature ( $\rho_s = 0.172$ ,  $P = 0.003$ ), but it was negative with PC 2 ( $\rho_s = -0.212$ ,  $P < 0.001$ ). In contrast, both axes revealed strong positive correlations with the diatom-inferred summer lake temperature (PC 1:  $\rho_s = 0.721$ , PC 2:  $\rho_s = 0.642$ , both:  $P < 0.001$ ).

In the lake specific PCAs, most variance was explained by the first axis averaging 86.3% (Table 2). According to the scree plots (Figs. S22 and S23 in Supporting Information), PC 2 only contributed significantly to the sample ordination in L. Gaisalpsee and L. Lautersee, explaining a further 34.1% and 21.1%, respectively. Apart from the short cores with pigment data from less than ten samples remaining for further analysis (L. Brendlsee, L. Höfersee, L. Seeleinsee, L. Sieglsee; Fig. 2), a majority of PC1 scores of the mountain lake samples correlated significantly with temperature data (Table 1).

Core analyses with GAMs explained significant deviances in down-core pigment composition with  $T_{\text{Air}}$  in all but one of the lakes (L. Soiernsee [E]). On average, GAM with air temperature and diatom-inferred temperature explained 50.8% and 34.4% of the deviances in sedimented pigments, respectively. Most of the deviances along the time scale that were not explained by the temperature proxies were

restricted to recent decades. This pattern was most pronounced in the Funtensee, Grünsee, Rappensee, and Soinsee lakes, where the data deviated by  $>1$  from the temperature models (Fig. 4).

GLM analyses revealed that the influence of temperature on pigment development, expressed as correlation and GAM *P*-values, was significantly related to the total phosphorous concentration and lake volume (Table 3). This was observed for both, the general and the exclusive models, which only implemented lakes of similar pigment preservation. According to the model estimates, the predictive strength of temperature increases with TP and declines with the size of the water body. In most GLMs, the lake depth was retained and exhibited negative effects in pigments in the final model, but with weaker significance levels than the lake volume. Altitude and diatom  $\beta$ -diversity constituted positive estimates in the GLM, if significant in the final model, whereas lake area and Secchi depth were rejected during backward selection or remained insignificant.

#### DISCUSSION

Pigment analyses of sediments taken from Bavarian and Tyrolean mountain lakes identified greater nutrient concentrations and smaller water bodies as amplifiers of mountain lake responses to climate warming. Most of the lakes revealed a close relationship between the pigment compositions and concentrations both with regional annual air temperatures and the summer surface temperatures of the lakes. On the level of algal groups, this was reflected by different compositions and developments of algae assemblages at different altitudes. The diversity of algae, in general, and the abundance of phytoplankton, in particular, decreased with the altitude of the lakes so that shifts in alpine lakes were currently still limited to periphytic taxa. In conclusion, the biggest changes such as algae composition and biomass shifts occurred in lakes at subalpine altitudes, that is, the range of the current treeline.

*Primary drivers of algal community changes in mountain lakes.* Supporting the first hypothesis, which stated strongest responses in shallow and nutrient-rich lakes, climate effects appeared most pronounced in more productive mountain lakes (Table 3). These lakes can produce a higher abundance of plankton and macrophytes, which is then reflected by increasingly organic sediment deposits that tend to correlate with climate warming. Ecological metabolic theory (Allen et al. 2005) would actually suggest that warming decreases the biomass owing to increased metabolic rates in freshwater systems such as the studied mountain lakes (Daufresne et al. 2009, Yvon-Durocher et al. 2011). The resulting metabolic deficit of algae, however, may be better compensated for in phosphorous-rich lakes

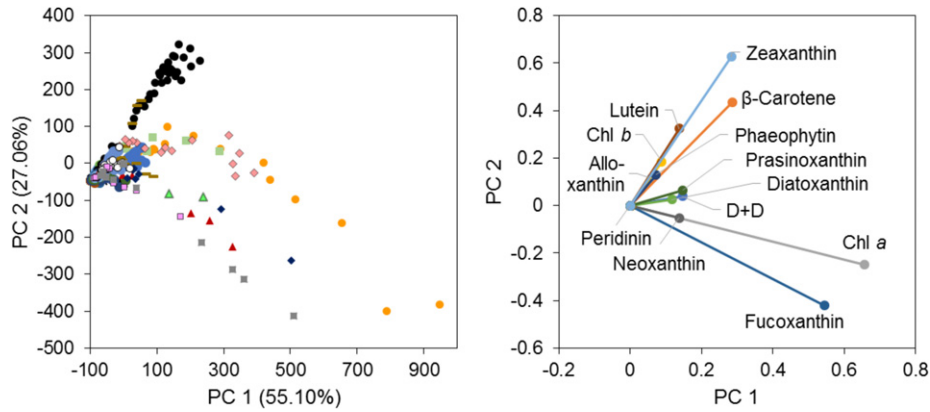


FIG. 3. PCA of pigments of all the mountain lake sediment samples together (left) with the organization of the pigment axes (right). Different symbols in the left-hand graph stand for the different mountain lakes. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2. PCA and significance of Spearman correlations and GAMs with the first principal component of the mountain lake pigment data.

Alt. level	Lake code	Depth	PC 1's explained variance	GAM's explained deviance		Spearman correlation ( <i>P</i> )		GAM ( <i>P</i> )	
				Ø T <sub>AIR</sub>	Ø T <sub>SiVA</sub>	Ø T <sub>AIR</sub>	Ø T <sub>SiVA</sub>	Ø T <sub>AIR</sub>	Ø T <sub>SiVA</sub>
Montane	Sieg	20.2	99.6%	–	–	0.782	0.919	–	–
	Lau	18.0	49.5%	49.8%	19.4%	0.002**	0.276	<0.001***	0.152
	Tau	15.5	78.0%	32.9%	61.4%	<0.001***	0.002**	0.001***	<0.001***
	Bich	11.0	80.5%	33.1%	33.4%	<0.001***	<0.001***	0.003**	<0.001***
	Fri	5.5	79.5%	53.9%	7.8%	<0.001***	0.338	<0.001***	0.537
	Mit	4.7	70.8%	70.8%	6.5%	0.033*	0.003**	0.083(*)	0.007**
Subalpine	Höf	1.9	98.9%	–	–	0.139	0.046*	–	–
	SoW	11.5	92.8%	93.5%	0.8%	0.244	0.871	<0.001***	0.736
	Soi	8.2	98.9%	33.0%	30.3%	0.067(*)	0.726	0.082(*)	0.099(*)
	Grün	6.9	93.4%	46.3%	34.2%	<0.001***	<0.001***	<0.001***	0.048*
	SoE	5.5	94.1%	16.1%	–	0.085(*)	–	0.155	–
	Fun	4.5	89.7%	79.9%	–	<0.001***	–	<0.001***	–
	Gais	4.1	78.0%	21.8%	23.3%	0.459	0.106	0.092(*)	0.302
Alpine	Hörn	1.8	91.0%	74.1%	92.3%	<0.001***	<0.001***	0.001***	<0.001***
	Delp	1.3	92.6%	72.2%	–	0.016*	–	<0.001***	–
	Dra	20.7	93.8%	69.8%	36.5%	0.018*	0.009**	<0.001***	0.123
	Eng	17.3	83.6%	61.4%	25.8%	<0.001***	0.553	<0.001***	0.065(*)
	Bre	8.2	99.2%	–	–	<0.001***	0.012*	–	–
	Rap	7.8	92.7%	87.3%	41.1%	0.096(*)	0.195	<0.001***	0.081(*)
	See	5.4	74.2%	–	–	0.356	0.444	–	–
	Gru	0.5	81.2%	32.3%	41.9%	0.014*	<0.001***	0.002**	<0.001***

Mountain lakes are ordered according to their altitude and depth. Significance levels (*P*-values) of the correlation and GAM analyses are given for the average annual air temperature and diatom-related summer lake surface temperature. Dashes represent nonapplicable diatom-based temperature reconstruction sediment cores or datasets that are too short for reliable GAM calculation. To bypass the latter, longer weather data from the Hohenpeißenberg weather observatory were used in a recalculation, given in italics. Significance levels: \*\*\**P* < 0.001, \*\**P* < 0.01, \**P* < 0.05, (\*) *P* < 0.1.

(Kraemer et al. 2017a) as TP is the main driver of most freshwater primary production (Blomqvist et al. 2004). Additionally, these lakes possibly experience more external nutrient inputs that alleviate the trapping of nutrients during lake stratification as well as further metabolic deficits (Kraemer et al. 2017a). In contrast, nutrient-poor lakes are more challenged by metabolic deficits and nutrient limitation, reducing the algal carrying capacity, so that changes in subfossil assemblages are restricted to taxonomic shifts within major algal groups rather

than increases in biomass. Altogether, these processes also affect physical lake warming (Luoto and Nevalainen 2016, Shatwell et al. 2016), as nutrient-dependent phytoplankton densities affect water transparency and color, thus long-wave absorptivity in lakes (Pepe et al. 2001, Schmid et al. 2014). Extreme plankton turbidities of eutrophic lakes, which were absent in the study area, completely absorb solar radiation already in the uppermost centimeters of a lake and only the immediate surface of the water body is warmed (Schmid et al. 2014,



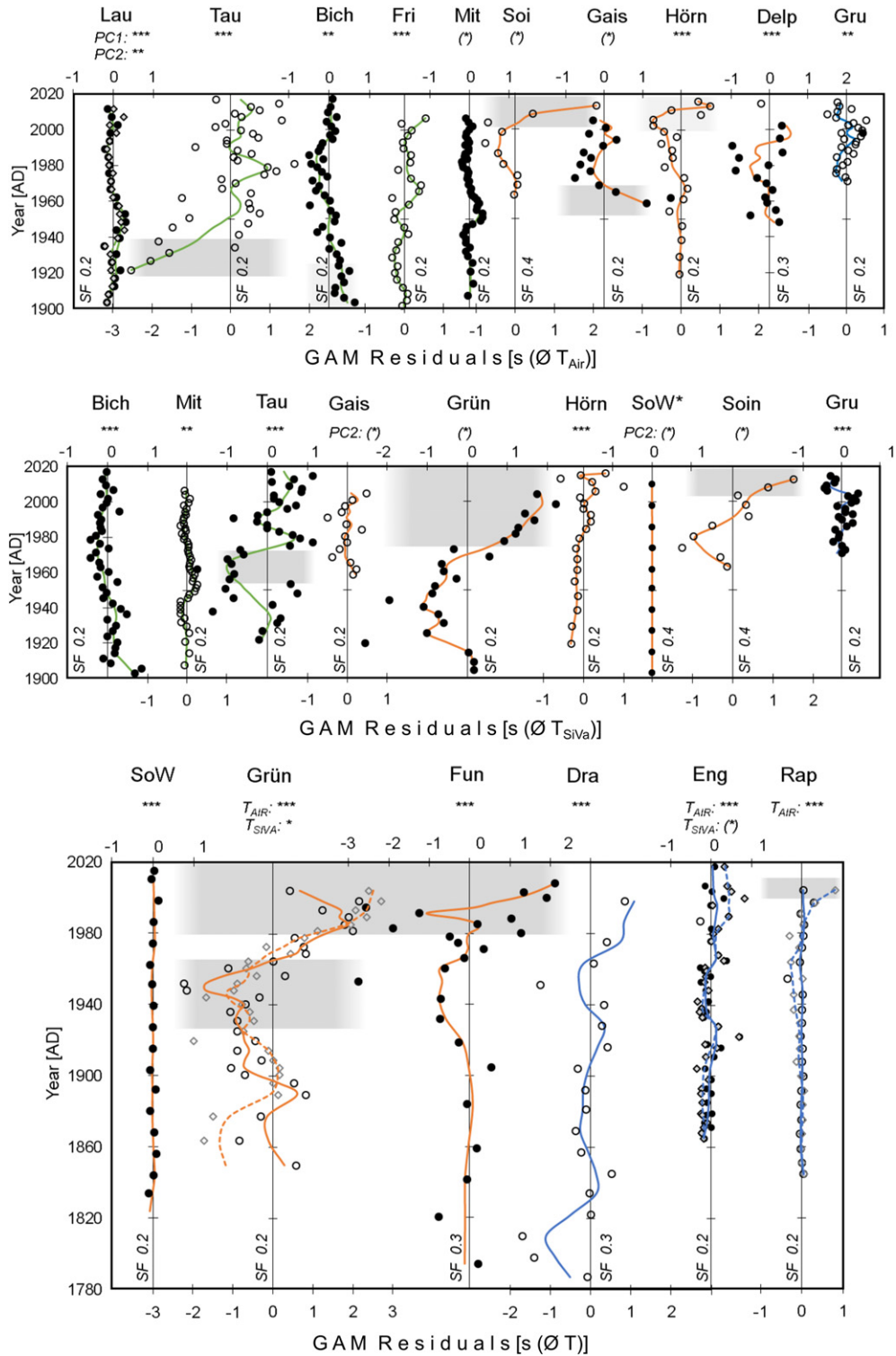


FIG. 4. GAM residuals of sedimentary mountain lake pigments. Top: Air temperature models; Center: diatom-inferred summer temperature models; Bottom: models for sediment cores that incorporated periods of >120 y. Mountain lakes are ordered from montane (Bich, Fri, Lau, Mit, Tau) to subalpine (Delp, Fun, Gais, Gru, Grün, Hörn, Soi(n), SoW) to alpine (Dra, Eng, Rap) according to depth from deep to shallow within the altitudinal levels. Dots: respective sediment sample; Lines: visualization of trend using Loess smoothing and smoothing factor (SF), shown at the bottom of each diagram. Only mountain lakes whose GAMs were significantly correlated with temperature proxies are shown. Significance levels are given beneath the titles: \*\*\* <0.001, \*\* <0.01, \* <0.05, (\*) <0.1. Gray areas highlight pigment variances >1 that are not explained by temperature models. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

TABLE 3. GLMs of Spearman correlation's and GAM's significance levels of subfossil mountain lake pigments with local temperature proxies.

	Regional average air temperature				Diatom-inferred summer lake temperature			
	<i>P</i> (Spearman)		<i>P</i> (GAM)		<i>P</i> (Spearman)		<i>P</i> (GAM)	
Intercept	-5.439	(0.023)*	-4.071	(<0.001)***	-2.602	(0.096)(*)	4.246	(0.327)
Altitude	-5.098	(0.002)**	4.837	(0.291)	-1.883	(0.565)	12.030	(0.029)*
	2.40•10 <sup>-3</sup>	(0.127)	-4.85•10 <sup>-3</sup>	(0.075)(*)	-1.24•10 <sup>-3</sup>	(0.257)	8.40•10 <sup>-3</sup>	(0.012)*
Area	-	-	-	-	-	(0.055)(*)	-	(0.171)
	-	-	-	-	-1.172	-	-1.274	-
Depth	-0.238	(0.142)	-	(0.734)	-0.185	(0.160)	-1.371	(0.008)**
	-	-	-6.84•10 <sup>-2</sup>	-	-1.79•10 <sup>-2</sup>	(0.926)	-1.823	(0.003)**
Volume	4.83•10 <sup>-6</sup>	(0.079)(*)	-	(0.134)	4.24•10 <sup>-6</sup>	(0.046)*	20.45•10 <sup>-6</sup>	(0.005)**
	2.72•10 <sup>-6</sup>	(0.048)*	-2.94•10 <sup>-5</sup>	-	2.14•10 <sup>-5</sup>	(0.078)(*)	3.56•10 <sup>-5</sup>	(0.012)*
Secchi depth	-	-	-	-	-	(0.339)	-	(0.259)
	-	-	-	-	-0.174	-	0.324	-
TP	-0.247	(0.085)(*)	-	-	-0.235	(0.049)*	-1.400	(0.004)**
	-0.276	(0.017)*	-	-	-0.290	(0.189)	-1.909	(0.003)**
Diatom β div.	0.766	(0.103)	-	-	0.639	(0.051)(*)	-	-
	1.296	(0.009)**	-	-	-	-	-	-
Depth * Volume	-	-	-	(0.135)	-	(0.369)	-	-
	-	-	1.54•10 <sup>-6</sup>	-	-4.78•10 <sup>-7</sup>	-	-	-
AIC	86.574	-	87.625	-	59.778	-	73.040	-
	60.689	-	74.412	-	37.375	-	46.501	-

Black numbers: all lakes except the doline lake; italicized numbers: only lakes with similar pigment preservation conditions. Left-hand value of each column: estimate; Right-hand value: significance of the environmental parameter with asterisks if significant (\*\* <0.01, \* <0.05, (\*) <0.1). The first row represents the intercept of the linear model and its significance, the last row the model's AIC.

Richardson et al. 2017). This dramatically increases pigment preservation as it overrides photooxidation (Sanger 1988), so that elevated trophic levels can even superimpose climate change effects (Moorhouse et al. 2018).

The models further indicated that smaller water bodies caused progressively stronger responses of algal communities in the mountain lakes to climate warming, as revealed by analyses of their pigments. These bases on the positive estimates of lake volume, where it significantly contributed to the models (Table 3). However, the results also imply some uncertainty of the hypothesized role of basin-specific proxies and their influence on lake response to warming. In fact, lake depth significantly contributed to two models with negative estimates (*P*<sub>GAM</sub> with diatom-inferred lake temperatures; Table 3) although this proxy actually correlates with the lake volume. On the one hand, the volume's effects, which confirm the first hypothesis, are supported by diatom-based analyses in the same study area (Kuefner et al. 2020b) as well as the thermodynamic influence of the size of water bodies on warming (Kraemer et al. 2017b, Kuefner et al. 2020c; Fig. S24 in the Supporting Information). Accordingly, both, shifts in subfossil diatom assemblages and warming of surface waters, increased with smaller lake volumes. Additionally, warming-driven tipping points of substratum succession particularly concern the littoral of mountain lakes (Kuefner et al. 2020b). Thus, they most

strongly affect shallower lakes that are completely euphotic, whereas they are diluted in the large water body of deep lakes. On the other hand, the subfossil pigment concentrations crucially depend on the location of the coring sites in a lake basin. Longer sinking distances of pigments and exposure to degradation, that is, on-site pigment production on littoral sediments versus a translocation before being deposited in profundal sediments (Cuddington and Leavitt 1999), may overstate shallow lake responses to warming. Nevertheless, additional models were calculated that only included lakes of similar pigment preservation patterns and still indicated the suggested influence of lake sizes (Table 3).

The expected influence of the altitude could not be validated by the GLMs, while two models even contradicted the first hypothesis (Table 3). Similarly, the diatom β-diversities, which were used to estimate the substratum succession in mountain lakes, was negatively correlated with temperature-driven responses of the algal communities. Diatom β-diversities in turn tended to correlate with the altitude in the studied lakes with similar Chl/Phe ratios (*P* = 0.07). Hence, algal shifts are probably driven by other factors than warming at higher elevations, which disguise or even uncouple temperature-pigment correlations. As such, the recent abandonment of alpine pasture (Lotter et al. 1999, Sommaruga et al. 1999, Gehrig-Fasel et al. 2007), as well as changes in precipitation, erosion, water transparency (Pauli et al. 2012, Catalan et al. 2013,

Rosbakh et al. 2014), and thus UVR penetration (Laurion et al. 2000, Leavitt et al. 2003; Fig. 5a) probably still constitute the main determinants of phototrophic compositions in alpine lakes (DeNicola 1996, Koinig et al. 2002). In contrast, algal communities in montane lakes thrive in a balanced environment with a completed succession of the catchment, the aquatic flora and substrata. Therefore, these lakes revealed the strongest responses to warming despite showing the least  $\beta$ -diversities because temperature remained as the major driver. Nevertheless, compositional shifts were hypothesized to climax around the treeline, that is, within the range of subalpine and alpine lakes. This cannot be reflected by linear models such as the GLM, but GAM calculations to test a nonlinear influence of the altitude could still explain only ca. 11% of the deviances (Fig. S25 in the Supporting Information).

*Responses of ecological algal guilds to climate change.* In contrast to the model results, the analyses on individual algal pigments and groups supported the hypothesized role of the altitude with most pronounced shifts of phototrophic communities in mountain lakes at the treeline elevation. They revealed the most significant transition from planktic and periphytic algal communities to exclusively periphyton to occur between subalpine and alpine lakes, that is, at the treeline. In detail, the pigments D + D, diatoxanthin, and fucoxanthin gain in

importance in shallow lakes and at higher altitudes (Fig. 2). They mainly derive from diatoms and chrysophytes (Züllig 1982, Hurley and Armstrong 1990, Leavitt 1993), but at alpine altitudes, they probably originate almost exclusively from periphytic algae. In montane lakes, planktic diatoms and chrysophytes reached  $1.8$  and  $2 \text{ g} \cdot \text{m}^{-3}$ , respectively, but nearly disappeared at alpine altitudes (Mayr 2018, Kuefner et al. 2020b). Interestingly, Vinebrooke and Leavitt (1999) observed the same transition in the littoral zone at the forest line in Canadian mountain lakes, which is, however, located at the same altitude as the Bavarian treeline at around  $1,600 \text{ m a.s.l.}$  This indicates the likely suppression of algae growth by UVR (Vinebrooke and Leavitt 1996, 1999a, Leavitt et al. 2003, Chen et al. 2015). In conclusion, periphytic algae such as benthic diatoms are among the most robust algae that can cope with cold climates and high-radiative conditions in alpine lakes. However, these lakes above the treeline are more vulnerable to future climate change (Sommaruga et al. 1999, Vinebrooke and Leavitt 1999, Jiménez et al. 2015) because macrophytes and phytoplankton will reach higher lakes with increasing temperatures and shrink the periphyton dominance.

Accordingly, the most drastic shifts in subfossil pigments and algal compositions are mainly triggered by taxa that benefit from warmer waters of

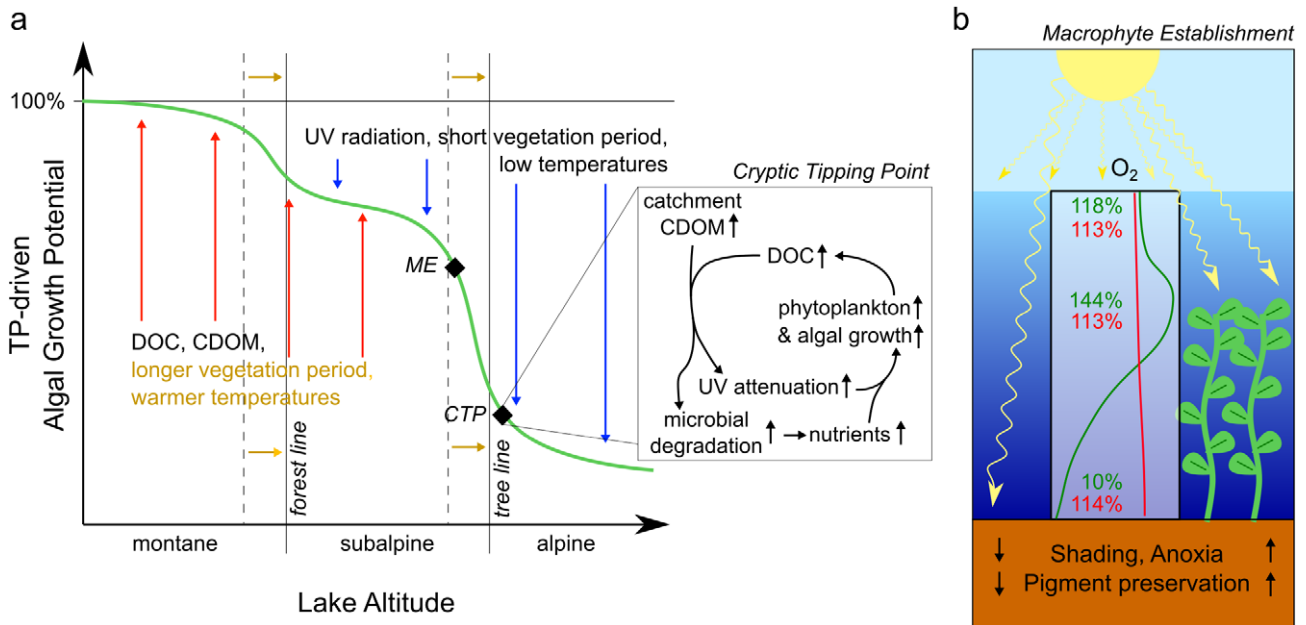


FIG. 5. Scheme of stressors and tipping points of climate warming driving algal growth and pigment conservation in mountain lakes. (a) Highlighted text and horizontal arrows indicate direct results of climate change, for example, uphill migration of forest and treeline. Potential algal growth (curved line) is suppressed by UVR and cooler climates in higher altitudes, which is outcompeted by DOC, CDOM and warmer conditions at lower altitudes. Major changes occur in subalpine lakes owing to the treeline shifts and macrophyte establishment (ME) after crossing the cryptic tipping point (CTP), which triggers a positive feedback loop. (b) Consequences of macrophyte establishment on oxygen saturation and sediment conditions in the alpine L. Brendlsees (straight line from central graph) and subalpine L. Hörnlsees (curved line from central graph) in summer 2016. Macrophytes support shading and hypoxia above the sediment, therefore pigment preservation in the sediment. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

lower altitudes confirming the third hypothesis. As such, lutein as a marker pigment, inter alia, of periphytic and planktic chlorophytes attained their lowest levels in alpine lakes (i.e., above the treeline) but generally increased below probably as a response to climate warming (Vinebrooke and Leavitt 1999, Lami et al. 2000, Buchaca and Catalan 2008). Similarly, lutein is probably a marker of macrophytes in these lakes (Lami et al. 2000), as almost no other higher aquatic plants were documented in the study area. It is likely that these primary producers can either not cope with the cold and harsh conditions and/or the elevated UVR in alpine lakes (Laurion et al. 2000, Leavitt et al. 2003, Kuefner et al. 2020b). They may therefore benefit from increases in lake temperatures and allochthonous inputs (i.e., DOC and UVR absorbing matter) enabling them to already thrive well in montane in subalpine lakes.

Similarly, pigments of specific phytoplankton communities showed overall increases over time with climate change (Fig. 2). In the studied mountain lakes, alloxanthin from the planktic cryptophytes was the most representative phytoplankton pigment and reached highest amounts in lakes below the treeline. Vinebrooke and Leavitt (1999) already indicated negative correlations of alloxanthin with altitude. These algae are among the most sensitive in terms of water temperature (Barone and Naselli-Flores 2003) and benefit from warmer waters, thermal stability, and DOM (Züllig 1982, Lami et al. 2000, Buchaca and Catalan 2008). Despite the fact that phytoplanktic cryptophytes and prasinophytes typically prefer deeper lakes, cryptophyte remains as well as the peaks of the prasinoxanthin-like pigment also appear in the younger sediments of shallow lakes (Fig. 2). Both indicate developments triggered by warming and further support to identify the latter as prasinoxanthin, which was also found to increase in other sediments (Sampere et al. 2011, Kang et al. 2016, Zhang et al. 2019).

In addition to the previous pigments, peridinin was mainly detected in low amounts predominantly in the top sediments of deep montane lakes (Fig. 2). Instead of warming-driven increases in phytoplanktic dinoflagellates, however, analyses on the mountain lake plankton were highly divergent. They revealed that dinoflagellates, particularly *Ceratium hirundinella* and *Peridinium inconspicuum*, dominated the subalpine and alpine phytoplankton throughout the vegetation period reaching densities of up to  $12 \text{ g} \cdot \text{m}^{-3}$  and 94% of the total phytoplankton biomass (Hofmann and Raeder 2014, Mayr 2018). Accordingly, peridinin suffers lability, intense degradation in euphotic lakes, and therefore serves as a weak palaeolimnological marker pigment, which is in line with Makri et al. (2019).

Also, zeaxanthin was detected in the sediments. In combination with canthaxanthin and echinenone,

this would indicate cyanobacteria (Hurley and Armstrong 1990, Leavitt 1993, Buchaca and Catalan 2008), but these pigments were either not detected in any sample or masked by neighboring peaks. Nevertheless, the former is in line with Daly et al. (2019) and previous plankton studies (Hofmann and Raeder 2014, Mayr 2018) that showed that cyanobacteria and their pigments were virtually absent in (ultra)oligotrophic lakes. The optimum temperature for cyanobacteria is higher than for eukaryotic algae (Havens and Paerl 2015, Helbling et al. 2015), so that the typically low cyanobacteria production at high altitudes releases a negligible signal in relation to the benthic algal communities (Kamenik et al. 2000, Lami et al. 2000, Buchaca et al. 2005, Jiménez et al. 2015).

*Secondary mediators of primary drivers.* The pigment analyses suggest that the location of mountain lakes relative to the treeline is crucial for their algal communities and responses to climate warming. This in turn crucially determines the influx rates of allochthonous organic matter, which influences levels of nutrients, DOC, and CDOM (Vinebrooke and Leavitt 1996, 1999a, Bunting et al. 2010), thus UVR absorptivity of lakes (Laurion et al. 2000, Leavitt et al. 2003, Rose et al. 2009). Consequently, the phototrophic responses in mountain lakes to warming are indirectly mediated by drivers of the catchment as well. There, a warming climate triggers decreases in snow covers, increases in heavy rainfall events, and thus, several succession and erosion processes particularly in typically snowy areas at higher elevations. The succession processes represent a cascade of enhanced weathering, soil formation, and the establishment of vegetation (Baron et al. 1991, Bogataj 2007, Arnaud et al. 2016, Michalak 2016) and eventually pushes the treeline uphill (i.e., the subalpine–alpine–transition). At these altitudes, where UVR is the strongest in the study area, increases in phototrophs that benefit from warmer waters (e.g., phytoplankton) can therefore be the result of elevated DOC and CDOM concentrations, but also rising lake trophic levels (Ossyssek et al. 2020). In that context, the former may also influence abundances of heterotrophic bacteria, which transfer DOM into inorganic nutrients and finally cause the latter for the phototrophs (Vinebrooke and Leavitt 1998).

However, increasing phytoplankton abundances as well as single macrophytes were already observed in alpine lakes previously as well as in this study despite their catchments are still treeless (Kuefner et al. 2020b). Probably, processes of succession and erosion already represent decisive stepping-stones of warming that finally culminate in a ‘cryptic tipping point’. Together with bacterial effects on nutrients (Vinebrooke and Leavitt 1998), this may in turn trigger a positive feedback loop (Fig. 5a) and further promote the warming-driven pigment increases and developments in alpine lakes in addition to

changes in thermal regime and lake stratification (Figs. S16-S19). Similarly, the positive correlations of algal shifts with diatom-inferred lake temperatures may be overestimated, as they derive from similar developments in the DOM and light regime.

In contrast, warming also increases the frequency of heavy precipitation events and, in particular, temporarily increased inorganic sedimentation especially after mudslides. Owing to higher turbidity and weaker light conditions, this can dilute and decrease pigment contents, as observed exemplarily in several mountain lakes. In Lake Soiernsee (W), 5 cm of inorganic sediment were deposited after an extreme heavy rainfall event in 2015 (A. Hofmann, unpub. data; Fig. S11), while the superregional flood in summer 2002 caused pigment minima in the sediments of the lakes Taubensee and Gaisalpsee (Figs. S5 and S12).

In mountain lakes themselves, the temperature increase primarily changes the lake freezing and mixing regimes, but secondarily the oxygen conditions. Changes in freezing can particularly affect the littoral flora <2 m (Peterson 1996, Vinebrooke and Leavitt 1999), while thick mats of periphyton can grow undisturbed in deeper lakes (Stanford and Prescott 1988). Further, warming supports macrophytes in mountain lakes, which shade the sediment, increase its saprobity and combined with prolonged summer stratification promote sediment hypoxia (Fig. 5b). This results in improved pigment preservation (Sanger 1988), increases subfossil pigment contents, and possibly overstates the mountain lake responses to climate change, especially in littoral areas and therefore in entirely euphotic shallow lakes (Figs. S2-S19).

#### CONCLUSIONS

Analysis of subfossil pigments in Bavarian and Tyrolean mountain lakes showed that climate change effects on algal communities are most pronounced in shallow and nutrient-rich lakes. These lakes can transfer a growing amount of thermal energy into biomass, which is mirrored by increased shifts in algal compositions and depositions of pigment-containing organic sediments. In contrast, deep lakes may dilute such developments in the larger water body and hold greater amounts of cold hypolimnetic water, buffering increased temperatures in summer and trapping nutrients during stratification. Thus, deeper and clearer lakes can be considered more resilient to warming. This highlights the need to protect oligotrophic lakes and to promote the development of lakes towards this state.

Warming-driven algal community shifts also depended on basin-specific changes along the altitudinal scale. Owing to the sparse vegetation in catchments at higher altitudes, alpine lakes receive low amounts of allochthonous inputs. Therefore, little developments in the catchment such as changes in snow cover, precipitation, and consequent initial

catchment succession processes can have significant consequences for the lakes. Preceding the treeline shifts, these developments may already cause significant increases in pigment contents and compositional shifts in some alpine lakes although their catchments are still treeless.

Altogether, the pigment analyses in combination with previous diatom studies identified several factors that are suggested to be the most important drivers of the past and future development of mountain lakes. Moreover, the importance of ecotonal boundaries and the accompanying processes was highlighted, which allows a worldwide transfer of the presented results to similar lakes and areas.

This study was funded by the Bavarian State Ministry of the Environment and Consumer Protection as a part of the 'Climate Change in Bavarian mountain lakes' project (TKP01 KPB-70807). We are grateful to Prof. Tanja Gschlößl for her continuous support of this project. We would also like to thank Dr Markus Hoffmann for assisting with the HPLC analysis and Pascal Rüenzi for dating and interpreting the sediment cores. Moreover, we would like to thank Melina Stegbauer and Sabrina Pitschi for their enthusiastic help with the pigment extractions. Open Access funding enabled and organized by Projekt DEAL.

#### CONFLICT OF INTEREST

All authors have approved this article and have no conflicts of interest to declare.

#### AUTHOR CONTRIBUTIONS

**W. Kuefner:** Conceptualization (supporting); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); visualization (lead); writing-original draft (lead). **A. Hofmann:** Conceptualization (supporting); data curation (supporting); investigation (supporting); methodology (supporting); writing-review & editing (equal). **J. Geist:** Formal analysis (equal); project administration (equal); supervision (lead); writing-review & editing (lead). **N. Dubois:** Data curation (equal); investigation (equal); methodology (equal). **U. Raeder:** Conceptualization (lead); funding acquisition (lead); methodology (supporting); project administration (lead); resources (lead); supervision (lead); validation (equal); writing-review & editing (lead).

#### DATA AVAILABILITY STATEMENT

Detailed data are visualized in the supplemental material and can be derived from the corresponding author.

- Allen, A. P., Gillooly, J. F. & Brown, J. H. 2005. Linking the global carbon cycle to individual metabolism. *Funct. Ecol.* 19:202–13.
- Appleby, P. G. 2001. Chronostratigraphic techniques in recent sediments. In Last, W. M. & Smol, J. P. [Eds.] *Tracking Environmental Change using Lake Sediments*. Kluwer Academic Publisher, Dordrecht, Netherlands, pp 171–203.

- Arnaud, F., Pulenard, J., Giguët-Covex, C., Wilhelm, B., Révillon, S., Jenny, J. P., Revel, M. et al. 2016. Erosion under climate and human pressures: an alpine lake sediment perspective. *Quat. Sci. Rev.* 152:1–18.
- Baron, J., McKnight, D. & Denning, A. S. 1991. Sources of dissolved and particulate organic material in Loch Vale Watershed, Rocky Mountain National Park, Colorado, USA. *Biogeochemistry* 15:89–110.
- Barone, R. & Naselli-Flores, L. 2003. Distribution and seasonal dynamics of cryptomonads in Sicilian water bodies. *Hydrobiologia* 502:325–9.
- Battarbee, R. W., Jones, V. J., Flower, R. J., Cameron, N. G., Bennion, H., Carvalho, L. & Juggins, S. 2001. Diatoms. In Smol, J. P., Birks, H. J. & Last, W. M. [Eds.] *Tracking Environmental Change using Lake Sediments, Volume 3: Terrestrial, Algal and Siliceous Indicators*. Kluwer Academic Publishers, Dordrecht, Netherlands, 371 pp.
- Berthon, V., Alric, B. & Rimet, F. 2014. Sensitivity and responses of diatoms to climate warming in lakes heavily influenced by humans. *Freshw. Biol.* 59:1755–67.
- Bidigare, R. R., Van Heukelem, L. & Trees, C. C. 2005. Analysis of algal pigments by high-performance liquid chromatography. In Andersen, R. A. [Ed.] *Algal Culturing Techniques*. Academic Press, New York, pp 327–45.
- Blomqvist, S., Gunnars, A. & Elmgren, R. 2004. Why limiting nutrient differs between temperate coastal seas and freshwater lakes: a matter of salt. *Limnol. Oceanogr.* 49:2236–41.
- Blumenthaler, M., Ambach, W. & Rehwald, W. 1992. Solar UV-A and UV-B radiation fluxes at two alpine stations at different altitudes. *Theor. Appl. Climatol.* 46:39–44.
- Bogataj, L. K. 2007. How will the Alps respond to climate change? Scenarios for the future of alpine water. *Alpine Space* 3: 43–51.
- Brotas, V. & Plante-Cuny, M. P. 2003. The use of HPLC pigment analysis to study microphytobenthos communities. *Acta Oecol.* 24:S109–S115.
- Buchaca, T. & Catalan, J. 2007. Factors influencing the variability of pigments in the surface sediments of mountain lakes. *Freshw. Biol.* 52:1365–79.
- Buchaca, T. & Catalan, J. 2008. On the contribution of phytoplankton and benthic biofilms to the sediment record of marker pigments in high mountain lakes. *J. Paleolimnol.* 40:360–83.
- Buchaca, T., Felip, M. & Catalan, J. 2005. A comparison of HPLC pigment analyses and biovolume estimates of phytoplankton groups in an oligotrophic lake. *J. Plankton Res.* 27:91–101.
- Bunting, L., Leavitt, P. R., Weidman, R. P. & Vinebrooke, R. D. 2010. Regulation of the nitrogen biogeochemistry of mountain lakes by subsidies of terrestrial dissolved organic matter and the implications for climate studies. *Limnol. Oceanogr.* 55:333–45.
- Catalan, J., Pla-Rabés, S., Wolfe, A. P., Smol, J. P., Rühland, K. M., Anderson, N. J., Kopaček, J., Stuchlík, E., Schmidt, R., Koinig, K. A. & Camarero, L. 2013. Global change revealed by paleolimnological records from remote lakes: a review. *J. Paleolimnol.* 49:513–35.
- Chen, Q., Nie, Y., Liu, X., Xu, L. & Emslie, S. D. 2015. An 800-year ultraviolet radiation record inferred from sedimentary pigments in the Ross Sea area, East Antarctica. *Boreas* 44:693–705.
- Cuddington, K. & Leavitt, P. R. 1999. An individual-based model of pigment flux in lakes: implications for organic biogeochemistry and paleoecology. *Can. J. Fish. Aquat. Sci.* 56:1964–77.
- Daly, M., Kurek, J., Gregory-Eaves, I. & Patoine, A. 2019. Reorganization of aquatic communities from low-nutrient lakes in northwestern New Brunswick, Canada. *J. Paleolimnol.* 61:185–200.
- Das, B., Vinebrooke, R. D., Sanchez-Azofeifa, A., Rivard, B. & Wolfe, A. P. 2005. Inferring sedimentary chlorophyll concentrations with reflectance spectroscopy: a novel approach to reconstructing historical changes in the trophic status of mountain lakes. *Can. J. Fish. Aquat. Sci.* 62:1067–78.
- Daufresne, M., Lengfellner, K. & Sommer, U. 2009. Global warming benefits the small in aquatic ecosystems. *Proc. Natl. Acad. Sci. USA* 106:12788–93.
- DeNicola, D. M. 1996. Periphyton responses to temperature at different ecological levels. In Stevenson, R. J., Bothwell, M. L. & Lowe, R. L. [Eds.] *Algal Ecology: Freshwater Benthic Ecosystems*. Academic Press, London, UK, pp 150–81.
- EEA 2009. *Regional Climate Change and Adaptation: The Alps Facing the Challenge of Changing Water Resources*. European Environment Agency Report 8. Copenhagen, pp. 143.
- Fjellheim, A., Raddum, G. G., Vandvik, V., Cogălniceanu, D., Boggero, A., Brancelj, A., Galas, J. et al. 2009. Diversity and distribution patterns of benthic invertebrates along alpine gradients. A study of remote European freshwater lakes. *Adv. Limnol.* 62:167–90.
- Gehrig-Fasel, J., Guisan, A. & Zimmermann, N. E. 2007. Treeline shifts in the Swiss Alps: climate change or land abandonment? *J. Veg. Sci.* 18:571–82.
- Goodwin, T. W. 1980. *The Biochemistry of the Carotenoids: Plants*, Vol. 1. Chapman & Hall, London, UK, 349 pp.
- Guilizzoni, P., Marchetto, A., Lami, A., Gerli, S. & Musazzi, S. 2012. Use of sedimentary pigments to infer past phosphorus concentration in lakes. *J. Paleolimnol.* 45:433–45.
- Hammer, Ø., Harper, D. A. T. & Ryan, P. D. 2001. PAST: paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* 4:9.
- Hastie, T. J. & Tibshirani, R. J. 1990. *Generalized Additive Models*. Chapman & Hall, London, UK, 352 pp.
- Havens, K. E. & Paerl, H. W. 2015. Climate change at a crossroad for control of harmful algal blooms. *Environ. Sci. Technol.* 49:12605–6.
- Helbling, E. W., Banaszak, A. T. & Villafañe, V. E. 2015. Global change feed-back inhibits cyanobacterial photosynthesis. *Sci. Rep.* 5:14514.
- Hodgson, D. A., Wright, S. W. & Davies, N. 1997. Mass spectrometry and reverse phase HPLC techniques for the identification of degraded fossil pigments in lake sediments and their application in palaeolimnology. *J. Paleolimnol.* 18:335–50.
- Hofmann, A. M. & Raeder, U. 2014. *Bergseen im Klimawandel - Einfluss und Anpassung anhand der Verschiebung der Primärproduzenten als Indikator des Klimawandels. Final report of the pilot study TK01KPB*, Technical University of Munich/Bavarian State Ministry of the Environment and Consumer Protection, Munich/Iffeldorf, pp. 107.
- Hurley, J. P. & Armstrong, D. E. 1990. Fluxes and transformations of aquatic pigments in Lake Mendota, Wisconsin. *Limnol. Oceanogr.* 35:384–98.
- Hyodo, F., Tsugeki, N., Azuma, J. I., Urabe, J., Nakanishi, M. & Wada, E. 2008. Changes in stable isotopes, lignin-derived phenols, and fossil pigments in sediments of Lake Biwa, Japan: implications for anthropogenic effects over the last 100 years. *Sci. Total Environ.* 403:139–47.
- Jiménez, L., Romero-Viana, L., Conde-Porcuna, J. M. & Pérez-Martínez, C. 2015. Sedimentary photosynthetic pigments as indicators of climate and watershed perturbations in an alpine lake in southern Spain. *Limnetica* 34:439–54.
- Kamenik, C., Koinig, K. A., Schmidt, R., Appleby, P. G., Dearing, J. A., Lami, A., Thompson, R. & Psenner, R. 2000. Eight hundred years of environmental changes in a high Alpine lake (Gossenköllesee, Tyrol) inferred from sediment records. *J. Limnol.* 59:43–52.
- Kang, Z., Yu, R., Kong, F., Wang, Y., Gao, Y., Chen, J., Guo, W. & Zhou, M. 2016. Records of bulk organic matter and plant pigments in sediment of the “red-tide zone” adjacent to the Changjiang River estuary. *Chin. J. Ocean. Limnol.* 34:915–27.
- Kirchner, G. 2011. <sup>210</sup>Pb as a tool for establishing sediment chronologies: examples of potentials and limitations of conventional dating models. *J. Environ. Radioact.* 102:490–4.
- Koinig, K. A., Kamenik, C., Schmidt, R., Agustí-Panareda, A., Appleby, P., Lami, A., Prazakova, M. et al. 2002. Environmental changes in an alpine lake (Gossenköllesee, Austria) over the last two centuries – the influence of air temperature on biological parameters. *J. Paleolimnol.* 28:147–60.

- Koinig, K. A., Schmidt, R., Sommaruga-Wöger, S., Tessadri, R. & Psenner, R. 1998. Climate change as the primary cause for pH shifts in a high alpine lake. *Water Air Soil Pollut.* 104:167–80.
- Kraemer, B. M., Anneville, O., Chandra, S., Dix, M., Kuusisto, E., Livingstone, D. M., Rimmer, A. et al. 2017b. Morphometry and average temperature affect lake stratification responses to climate change. *Geophys. Res. Lett.* 42:4981–8.
- Kraemer, B. M., Mehner, T. & Adrian, R. 2017a. Reconciling the opposing effects of warming on phytoplankton biomass in 188 large lakes. *Sci. Rep.* 7:10762.
- Krajeswka, M., Szymczak-Żyła, M. & Kowalewska, G. 2017. Algal pigments in Hornsund (Svalbard) sediments as biomarkers of Arctic productivity and environmental conditions. *Pol. Polar Res.* 38:423–43.
- Kuefner, W., Hofmann, A. M., Ossyssek, S., Dubois, N., Geist, J. & Raeder, U. 2020b. Composition of highly diverse diatom community shifts as response to climate change: a down-core study of 23 central European mountain lakes. *Ecol. Indic.* 117:106590.
- Kuefner, W., Hofmann, A. M., Geist, J. & Raeder, U. 2020c. Evaluating climate change impacts on mountain lakes by applying the new silicification value to paleolimnological samples. *Sci. Tot. Environ.* 715:136913.
- Kuefner, W., Ossyssek, S., Geist, J. & Raeder, U. 2020a. The silicification value: a novel diatom-based indicator to assess climate change in freshwater habitats. *Diatom Res.* 35:1–16.
- Lami, A., Guilizzoni, P. & Marchetto, A. 2000. High resolution analysis of fossil pigments, carbon, nitrogen and Sulphur in the sediment of eight European Alpine lakes: the MOLAR project. *J. Limnol.* 59:15–28.
- Laurion, I., Ventura, M., Catalan, J., Psenner, R. & Sommaruga, R. 2000. Attenuation of ultraviolet radiation in mountain lakes: factors controlling the among- and within-lake variability. *Limnol. Oceanogr.* 45:1274–88.
- Leavitt, P. R. 1993. A review of factors that regulate carotenoid and chlorophyll deposition and fossil pigment abundance. *J. Paleolimnol.* 9:109–27.
- Leavitt, P. R., Cumming, B. F., Smol, J. P., Reasoner, M., Pienitz, R. & Hodgson, D. A. 2003. Climatic control of UV radiation effects on lakes. *Limnol. Oceanogr.* 48:2062–9.
- Leavitt, P. R. & Hodgson, D. A. 2001. Sedimentary pigments. In Smol, J. P., Birks, H. J. B. & Last, W. M. [Eds.] *Tracking Environmental Change Using Lake Sediments. Volume 3: Terrestrial, Algal, and Siliceous Indicators*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 295–325.
- Lee, R. E. 1999. *Phycology*. Cambridge University Press, Cambridge, p 535.
- Lotter, A. F., Appleby, P. G., Bindler, R., Dearing, J. A., Grytnes, J. A., Hofmann, W., Kamenik, C. et al. 2002. The sediment record of the past 200 years in a Swiss high-alpine lake: Hagelseewli (2339 m a.s.l.). *J. Paleolimnol.* 28:111–27.
- Lotter, A. F., Birks, H. J. B., Hofmann, W. & Marchetto, A. 1997. Modern diatom, cladocera, chironomid, and chrysophyte cyst assemblages as quantitative indicators for the reconstruction of past environmental conditions in the Alps. I. Climate. *J. Paleolimnol.* 18:395–420.
- Lotter, A. F., Birks, H. J. B., Hofmann, W. & 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. *J. Paleolimnol.* 19:443–63.
- Lotter, A. F., Pienitz, R. & Schmidt, R. 1999. Diatoms as indicators of environmental change near arctic and alpine treeline. In Stoermer, F. & Smol, J. P. [Eds.] *The Diatoms. Applications for the Environmental and Earth Sciences*. Cambridge University Press, New York, pp 205–26.
- Luoto, T. P. & Nevalainen, L. 2016. Solar and atmospheric forcing on mountain lakes. *Sci. Total Environ.* 566–567:168–74.
- Makrī, S., Lami, A., Lods-Crozet, B. & Loizeau, J. J. 2019. Reconstruction of trophic state shifts over the past 90 years in a eutrophic lake in western Switzerland, inferred from the sedimentary record of photosynthetic pigments. *J. Paleolimnol.* 61:129–45.
- Mayr, J. 2018. *Phytoplanktonentwicklung ausgewählter Bergseen unterschiedlicher Höhenlagen im Jahresverlauf*. Student's Project Thesis, Technical University of Munich, Munich/Iffeldorf. pp. 69.
- Melzer, A. & Schneider, S. 2014. Submersed Makrophyten als Indikatoren der Nährstoffbelastung von Seen. In Calmano, W., Hupfer, M., Fischer, H. & Klapper, H. [Eds.] *Handbuch Angewandte Limnologie: Grundlagen – Gewässerbelastung – Restaurierung – Aquatische Ökotoxikologie – Bewertung – Gewässerschutz*. Wiley-VCH Verlag, Weinheim, p 3136.
- Michalak, A. M. 2016. Study role of climate change in extreme threats to water quality. *Nature* 535:7–84.
- Michelutti, N., Cooke, C. A., Hobbs, W. O. & Smol, J. P. 2015. Climate-driven changes in lakes from the Peruvian Andes. *J. Paleolimnol.* 54:153–60.
- Moorhouse, H. L., McGowan, S., Taranu, Z. E., Gregory-Eaves, I., Leavitt, P. R., Jones, M. D., Barker, P. & Brayshaw, S. A. 2018. Regional versus local drivers of water quality in the Windermere catchment: the dominant influence of wastewater pollution over the past 200 years. *Glob. Change Biol.* 24:4009–22.
- Moser, K. A., Baron, J. S., Brahney, J., Oleksy, I. A., Saros, J. E., Hundey, E. J., Sadro, S. A. et al. 2019. Mountain lakes: eyes on global environmental change. *Global Planet. Change* 178:77–95.
- Mueller, D. R., Van Hove, P., Antoniadis, D., Jeffries, M. & Vincent, W. F. 2009. High Arctic lakes as sentinel ecosystems: cascading regime shifts in climate, ice-cover, and mixing. *Limnol. Oceanogr.* 54:2371–85.
- Niederist, G. H., Psenner, R. & Sommaruga, R. 2018. Climate warming increases vertical and seasonal water temperature differences and inter-annual variability in a mountain lake. *Clim. Change*. 151:473–490.
- Olaizola, M., Roche, J., Kolber, Z. & Falkowski, P. G. 1994. Non-photochemical fluorescence quenching and the diadinoxanthin cycle in a marine diatom. *Photosynth. Res.* 41:357–70.
- Ossyssek, S., Geist, J., Werner, P. & Raeder, U. 2020. Identification of the ecological preferences of *Cyclotella comensis* in mountain lakes of the northern European Alps. *Arct. Antarct. Alp. Res.* 52:512–23.
- Pauli, H., Gottfried, M., Dullinger, S., Abdaladze, O., Akhalkatsi, M., Alonso, J. L. B., Coldea, G. et al. 2012. Recent plant diversity changes on Europe's mountain summits. *Science* 336:353–5.
- Pearce, J. L., Beringer, J., Nicholls, N., Hyndman, R. J. & Tapper, N. J. 2011. Quantifying the influence of local meteorology on air quality using generalized additive models. *Atmos. Environ.* 45:1328–36.
- Pepe, M., Giardino, C., Borsani, G., Cardoso, A. C., Chiaudani, G., Premazzi, G., Rodari, E. & Zilioli, E. 2001. Relationship between apparent optical properties and photosynthetic pigments in the sub-alpine Lake Iseo. *Sci. Total Environ.* 268:31–45.
- Pepin, N., Bradley, R. S., Diaz, H. F., Baraer, M., Caceres, E. B., Forsythe, N., Fowler, H. et al. 2015. Elevation-dependent warming in mountain regions of the world. *Nat. Clim. Chang.* 5:424–30.
- Peterson, C. G. 1996. *Response of Benthic Algal Communities to Natural Physical Disturbance. Algal Ecology: Freshwater Benthic Ecosystems*. Academic Press, London, UK, pp 375–402.
- R Core Team 2020. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Repeta, D. J. 1989. Carotenoid diagenesis in recent marine sediments: II. Degradation of fucoxanthin to loliolide. *Geochim. Cosmochim. Acta* 53:699–707.
- Richardson, D. C., Melles, S. J., Pilla, R. M., Hetherington, A. L., Knoll, L. B., Williamson, C. E., Kraemer, B. M. et al. 2017. Transparency, geomorphology and mixing regime explain variability in trends in lake temperature and stratification across Northeastern North America (1975–2014). *Water* 9:442.
- Rogora, M., Frate, L., Carranza, M. L., Freppaz, M., Stanisci, A., Bertani, I., Bottarin, R. et al. 2018. Assessment of climate

- change effects on mountain ecosystems through a cross-site analysis in the Alps and Apennines. *Sci. Total Environ.* 624:1429–42.
- Rosbakh, S., Bernhardt-Roemermann, M. & Poschod, P. 2014. Elevation matters: contrasting effects of climate change on the vegetation development at different elevations in the Bavarian Alps. *Alp. Bot.* 124:143–54.
- Rose, K. C., Williamson, C. E., Saros, J. E., Sommaruga, R. & Fischer, J. M. 2009. Differences in UV transparency and thermal structure between alpine and subalpine lakes: implications for organisms. *Photoch. Photobio. Sci.* 8:1244–56.
- Rühland, K. M., Paterson, A. M. & Smol, J. P. 2015. Lake diatoms responses to warming: reviewing the evidence. *J. Paleolimnol.* 54:1–35.
- Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E. et al. 2000. Global biodiversity scenarios for the year 2100. *Science* 287:1770–4.
- Sampere, T. P., Bianchi, T. S., Allison, M. A. & McKee, B. A. 2011. Burial and degradation of organic carbon in Louisiana shelf/slope sediments. *Estuar. Coast. Shelf Sci.* 95:232–44.
- Sanger, J. E. 1988. Fossil pigments in paleoecology and paleolimnology. *Palaeogeogr. Palaeoclimato. Palaeoecol.* 62:343–59.
- Schmid, M., Hunziker, S. & Wüest, A. 2014. Lake surface temperatures in a changing climate: a global sensitivity analysis. *Clim. Change* 124:301–15.
- Schwoerbel, J. & Brendelberger, H. 2013. *Einführung in die Limnologie. Stoffhaushalt – Lebensgemeinschaften – Technologie.* Springer Spektrum, Heidelberg, p 386.
- Shatwell, T., Adrian, R. & Kirillin, G. 2016. Planktonic events may cause polymictic-dimictic regime shifts in temperate lakes. *Sci. Rep.* 6:24361.
- Smol, J. P. & Cumming, B. F. 2008. Tracking long – term changes in climate using algal indicators in lake sediments. *J. Phycol.* 36:986–1011.
- Smol, J. P., Wolfe, A. P., Birks, H. J. B., Douglas, M. S. V., Jones, V. J., Korhola, A., Pienitz, R. et al. 2005. Climate-driven regime shifts in the biological communities of arctic lakes. *Proc. Natl. Acad. Sci. USA* 102:4397–402.
- Sommaruga, R. & Augustin, G. 2006. Seasonality in UV transparency of an alpine lake is associated to changes in phytoplankton biomass. *Aquat. Sci.* 68:129–41.
- Sommaruga, R., Psenner, R., Schaffner, E., Koinig, K. & Sommaruga-Wögrath, S. 1999. Dissolved organic carbon concentration and phytoplankton biomass in high-mountain lakes of the Austrian Alps: potential effect of climatic warming on UV underwater attenuation. *Arct. Antarct. Alp. Res.* 31:247–53.
- Stanford, J. A. & Prescott, G. W. 1988. Limnological features of a remote alpine lake in Montana, including a new species of *Cladophora* (Chlorophyta). *J. N. Am. Benthol. Soc.* 7:140–51.
- Sun, M. Y., Lee, C. & Aller, R. C. 1993. Laboratory studies of oxic and anoxic degradation of chlorophyll-a in Long Island Sound sediments. *Geochim. Cosmochim. Acta* 57:147–57.
- Tonnö, I., Nauts, K., Belle, S., Nömm, M., Freiberg, R., Kõiv, T. & Alliksaar, T. 2019. Holocene shifts in the primary producer community of algae, shallow European Lake Peipsi, inferred from sediment pigment analysis. *J. Paleolimnol.* 61:403–17.
- Toride, K., Cawthorne, D. L., Ishida, K., Kayvas, M. L. & Anderson, M. L. 2018. Long-term trend analysis on total and extreme precipitation over Shasta dam watershed. *Sci. Total Environ.* 626:244–54.
- Vinebrooke, R. D. & Leavitt, P. R. 1996. Effects of ultraviolet radiation on periphyton in an alpine lake. *Limnol. Oceanogr.* 41:1035–40.
- Vinebrooke, R. D. & Leavitt, P. R. 1998. Direct and interactive effects of allochthonous dissolved organic matter, inorganic nutrients, and ultraviolet radiation on an alpine littoral food web. *Limnol. Oceanogr.* 43:1065–81.
- Vinebrooke, R. D. & Leavitt, P. R. 1999a. Differential responses of littoral communities to ultraviolet radiation in an alpine lake. *Ecology* 80:223–37.
- Vinebrooke, R. D. & Leavitt, P. R. 1999b. Phyto-benthos and phytoplankton as potential indicators of climate change in mountain lakes and ponds: a HPLC-based pigment approach. *J. N. Am. Benthol. Soc.* 18:15–33.
- Warscher, M., Wagner, S., Marke, T., Laux, P., Smiatek, G., Strasser, U. & Kunstmann, H. 2019. A 5 km resolution regional climate simulation for central Europe: performance in high mountain areas and seasonal, regional and elevation-dependent variations. *Atmosphere* 10:682.
- Waters, M. N., Schelske, C. L., Kenney, W. F. & Chapman, A. D. 2005. The use of sedimentary algal pigments to infer historic algal communities in Lake Apopka, Florida. *J. Paleolimnol.* 33:53–71.
- Weckström, K., Weckström, J., Huber, K., Kamenik, C., Schmidt, R., Salvenmoser, W., Rieradevall, M., Weisse, T., Psenner, R. & Kurmayer, R. 2016. Impacts of climate warming on Alpine lake biota over the past decade. *Arct. Antarct. Alp. Res.* 48:361–76.
- Williamson, C. E., Salm, C., Cooke, S. L. & Saros, J. E. 2010. How do UV radiation, temperature and zooplankton influence the dynamics of alpine phytoplankton communities? *Hydrobiol.* 648:73–81.
- Wood, S. 2019. *Package 'mgcv'*. CRAN R-project, pp. 309.
- Wright, S. W., Jeffrey, S. W., Mantoura, R. F. C., Llewellyn, C. A., Bjornland, T., Repeta, D. & Welschmeyer, N. 1991. Improved HPLC methods for the analysis of chlorophylls and carotenoids from marine phytoplankton. *Mar. Ecol. Prog. Ser.* 77:183–96.
- Xenopoulos, M. A. & Frost, P. C. 2003. UV radiation, phosphorus, and their combined effects on the taxonomic composition of phytoplankton in a boreal lake. *J. Phycol.* 39:291–302.
- Yvon-Durocher, G., Montoya, J. M., Trimmer, M. & Woodward, G. 2011. Warming alters the size spectrum and shifts the distribution of biomass in freshwater ecosystems. *Glob. Chan. Biol.* 17:1681–94.
- Zhang, H., Huo, S., Yeager, K. M., He, Z., Xi, B., Li, X., Ma, C. & Wu, F. 2019. Phytoplankton response to climate changes and anthropogenic activities recorded by sedimentary pigments in a shallow eutrophied lake. *Sci. Total Environ* 647:1398–409.
- Zhen, Y., Min, Z., Shi, X., Kong, F., Ma, R. & Yang, Y. 2016. Nutrient reduction magnifies the impact of extreme weather on cyanobacterial bloom formation in large shallow Lake Taihu (China). *Water Res.* 103:302–10.
- Züllig, H. 1982. Untersuchungen über die Stratigraphie von Carotinoiden im geschichteten Sediment von 10 Schweizer Seen zur Erkundung früherer Phytoplankton-Entfaltungen. *Schweiz. Z. Hydrol.* 44:1–98.

### Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

**Figure S1.** Determination of the Silicification Value (SiVa) using light microscopy pictures. Determining the darkness of the valve edges relative to the background, grey values can be calculated. With these grey values of a diatom valve, the SiVa of a diatom species can be estimated using the bottom scheme. For more information, see Kuefner et al. (2020a).

**Figure S2.** Subfossil pigment concentrations of the montane Lake Höfersee (1.9 m depth, 1192 m a.s.l.). Pigments with black areas are calibrated pigments in  $\text{mg} \cdot \text{g}^{-1}$ , with white areas uncalibrated and shown in  $\text{mV} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ . Chl/Phe ratio is given in the right graph based



on the ratio of both areas. The two y-axes show sediment age (left) and sediment depth (right). The orange box represents the uppermost layers that were excluded from further analyses due to indications for a changed preservation.

**Figure S3.** Subfossil pigment concentrations of the montane Lake Mittersee (left, 4.7 depth, 1082 m a.s.l.) and Lake Sieglsee (right, 20.2 m depth, 1207 m a.s.l.). For further explanation, see Figure S2. For Lake Sieglsee, pigment concentrations are triple scaled and no Chl/Phe ratio could be calculated since could phaeophytin could be detected throughout the core.

**Figure S4.** Subfossil pigment concentrations of the montane Lake Bichlersee (11 m depth, 955 m a.s.l.). For further explanation, see Figure S2.

**Figure S5.** Subfossil pigment concentrations of the montane Lake Taubensee (14.6 m depth, 1138 m a.s.l.). For further explanation, see Figure S2.

**Figure S6.** Subfossil pigment concentrations of the montane Lake Lautersee (18 m depth, 1013 m a.s.l.). For further explanation, see Figure S2.

**Figure S7.** Subfossil pigment concentrations of the montane Lake Frillensee (5.5 m depth, 973 m a.s.l.). For further explanation, see Figure S2.

**Figure S8.** Subfossil pigment concentrations of the subalpine Lake Hörnlesee (1.9 m depth, 1601 m a.s.l.). For further explanation, see Figure S2.

**Figure S9.** Subfossil pigment concentrations of the subalpine Lake Funtensee (4.5 m depth, 1601 m a.s.l.). For further explanation, see Figure S2. Pigment concentrations are half-scaled.

**Figure S10.** Subfossil pigment concentrations of the subalpine Lake Soirnsee (E; 5.5 m depth, 1552 m a.s.l.). For further explanation, see Figure S2. Pigment concentrations are triple scaled and no Chl/Phe ratio could be calculated since phaeophytin could not be detected throughout the core.

**Figure S11.** Subfossil pigment concentrations of the subalpine Lake Delpssee (I m depth, 1600 m a.s.l.) and Soiernsee (W; right, 11.5 m depth, 1558 m a.s.l.). For further explanation, see Figure S2. The uppermost 5 cm of the Soiernsee (W) sediment core had to be excluded due to a fast deposition event in 2015. Older cores lacked this new inorganic layer. Thus, the core starts at cm 5.

**Figure S12.** Subfossil pigment concentrations of the subalpine Lake Gaisalpsee (4.1 m depth,

1508 m a.s.l.). For further explanation, see Figure S2. Pigment concentrations are half-scaled.

**Figure S13.** Subfossil pigment concentrations of the subalpine Lake Grünsee (6.9 m depth, 1474 m a.s.l.). For further explanation, see Figure S2. Pigment concentrations are half-scaled.

**Figure S14.** Subfossil pigment concentrations of the subalpine Lake Soirnsee (8.2 m depth, 1458 m a.s.l.). For further explanation, see Figure S2.

**Figure S15.** Subfossil pigment concentrations of the alpine lakes Seeleinsee (left, 5.4 m depth, 1809 m a.s.l.) and Grubersee (right, 3.5 m depth, 2060 m a.s.l.). For further explanation, see Figure S2.

**Figure S16.** Subfossil pigment concentrations of the alpine Lake Rappensee (7.8 m depth, 2047 m a.s.l.). For further explanation, see Figure S2.

**Figure S17.** Subfossil pigment concentrations of the alpine Lake Drachensee (20.7 m depth, 1874 m a.s.l.). For further explanation, see Figure S2.

**Figure S18.** Subfossil pigment concentrations of the alpine Lake Engeratsgundsee (17.3 m depth, 1876 m a.s.l.). For further explanation, see Figure S2.

**Figure S19.** Subfossil pigment concentrations of the alpine Lake Brendlsee (6.2 m depth, 1903 m a.s.l.). For further explanation, see Figure S2.

**Figure S20.** Pigment preservation expressed as Chl/Phe ratio in relation to the mountain lake depths. Uppermost centimetres, where the ratio indicated variable preservation within a sediment core, are excluded. Red box: excluded lakes, blue box: lakes with homogenous pigment preservation included for exclusive GLM calculations. Only box plots with median, 1. and 3. Quantile are given to ease readability of the graph.

**Figure S21.** Loadings plot of the first PC axis (left) and data table (right) showing the loadings of the implemented pigments in first three axes of the general pigment PCA.

**Figure S22.** Scree plots of the PCAs with subfossil pigment assemblages since 1900. The broken-stick models (red line) indicate the number of principal components that significantly explain the assemblage shifts. L. Lautersee is the only one with two significant components. Green names: Montane lakes, orange names: Subalpine lakes.

**Figure S23.** Scree plots of the PCAs with subfossil pigment assemblages since 1900. The broken-stick models (red line) indicate the number of principal components that significantly explain

the assemblage shifts. L. Gaisalpsee is the only one with two significant components. Orange names: Subalpine lakes, blue names: Alpine lakes.

**Figure S24.** Epilimnetic temperature increase in Bavarian mountain lakes during a 6-d-period of constant warming in late-Aug 2016. Lakes deeper than 10 m warmed significantly weaker than shallower lakes.

**Figure S25.** GAMs of strictness of algal response to warming (i.e., Spearman and GAM  $P$ -values) in relation to altitude, depth and TP as predictor variables.  $P$  values and percentage of explained deviances are given for each model.