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Stratification strength and light climate explain variation in chlorophyll *a* at the continental scale in a European multilake survey in a heatwave summer

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Additional Supporting Information may be found in the online version of this article.

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Abstract

To determine the drivers of phytoplankton biomass, we collected standardized morphometric, physical, and biological data in 230 lakes across the Mediterranean, Continental, and Boreal climatic zones of the European continent. Multilinear regression models tested on this snapshot of mostly eutrophic lakes (median total phosphorus [TP] = 0.06 and total nitrogen [TN] = 0.7 mg L^{-1}), and its subsets (2 depth types and 3 climatic zones), show that light climate and stratification strength were the most significant explanatory variables for chlorophyll *a* (Chl *a*) variance. TN was a significant predictor for phytoplankton biomass for shallow and continental lakes, while TP never appeared as an explanatory variable, suggesting that under high TP, light, which partially controls stratification strength, becomes limiting for phytoplankton development. Mediterranean lakes were the warmest yet most weakly stratified and had significantly less Chl *a* than Boreal lakes, where the temperature anomaly from the long-term average, during a summer heatwave was the highest (+4°C) and showed a significant, exponential relationship with stratification strength. This European survey represents a summer snapshot of phytoplankton biomass and its drivers, and lends support that light and stratification metrics, which are both affected by climate change, are better predictors for phytoplankton biomass in nutrient-rich lakes than nutrient concentrations and surface temperature.

Globally, temperature, light, and nutrients are key drivers of phytoplankton blooms, but their relative importance in determining algal biomass strongly depends on the role of thermal stratification, that is, water column stability (Sverdrup 1953; Cloern 1996; Ptacnik et al. 2003; Carvalho et al. 2016). As a matter of fact, the relative importance of these drivers and interactive mechanisms between them cannot be fully resolved without including thermal stability (Winslow et al. 2017). This is particularly relevant under global processes of eutrophication and climate warming (Sinha et al. 2017) as some research foresees an allied impact of eutrophication and climate change effects in promoting harmful cyanobacterial blooms (Moss et al. 2011).

Stratification suppresses the exchange of heat and dissolved substances between the epi- and hypolimnion by reducing turbulent motions that otherwise would facilitate transport (Wüest and Lorke 2003). While the vertical structure of the water column constitutes the first response to temperature fluctuations (Sahoo et al. 2016), it also regulates the development of phytoplankton biomass by affecting light and nutrient availability (Yang et al. 2016), as well as phytoplankton settling, and therefore exerts a strong control on lake ecosystem functioning (Scheffer et al. 2001; Bartosiewicz et al. 2015).

Especially when nutrients are not limiting (e.g., in eutrophic lakes), light climate and stratification strength likely play dominant roles in regulating phytoplankton biomass (Fig. 1), and this role of light as a limiting resource has been suggested since the early days of eutrophication research (Mur et al. 1977). In general, by controlling light and nutrient availability, the underwater light climate and stratification strength determine phytoplankton growth conditions. When stratification is strong, thus suppressing fluxes from the deeper layers, mixing is restricted to the surface layer. Under such conditions, phytoplankton is constantly maintained within the euphotic zone, promoting algal growth until nutrients are depleted or other factors as grazing and sedimentation take over in controlling phytoplankton biomass (Fig. 1a; Camacho 2006; Reynolds 2006; Yankova et al. 2017). When stratification is weak, water column mixing can reach deep and nutrient rich waters, however potentially taking the algal communities beyond the euphotic zone that would limit their growth (Ibelings et al. 1994; Fig. 1b). One other ecological consequence of a strongly stratified lake is that phytoplankton may have reduced access to nutrients that remain locked in the hypolimnion (Nürnberg 1984; Posch et al. 2012; Salmaso et al. 2020). Yet, while the strength of stratification is determined primarily by light climate and heat exchange, other factors too can affect the extent and duration of the stratification, such as lake morphology (i.e., basin geometry, maximum depth and surface area) (Thompson and Schmidt 2005; Kirillin and Shatwell 2016; Magee and Wu 2017) as well as the dissolved organic and inorganic carbon content of the water, wind orientation and sheltering. Dissolved organic matter in general can have a huge

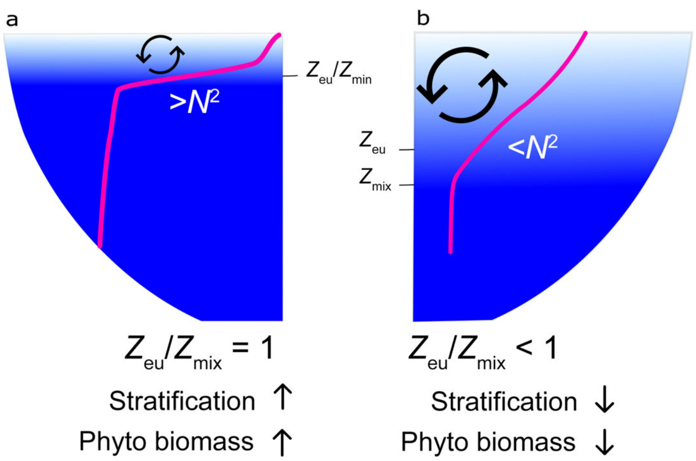

Fig 1. Schematic overview of how lake N^2 and light climate (Z_{eu}/Z_{mix}) may define phytoplankton biomass in nutrient-rich lakes. (**a**) A strong stratification (> N^2) allows phytoplankton to circulate well within the euphotic zone ($Z_{eu}/Z_{mix} \geq 1$)—promoting growth. (**b**) A weaker stratification (< N^2) allows deeper mixing, hence phytoplankton communities are highly diluted—eventually below the euphotic zone ($Z_{eu}/Z_{mix} < 1$).

impact on stratification by influencing light penetration, and consequently surface heating, as seen in humic boreal lakes (Heiskanen et al. 2014). Wind and convection, acting on the surface mixed layer (SML), control a lake's interior diffusive fluxes regulating the physical environment experienced by phytoplankton. Important properties of the SML, such as its depth, vary widely among lakes as the result of a specific balance between factors that strengthen stratification (surface warming), and factors that disrupt or deepen the layer, such as wind shear and surface cooling (Imberger 1985; Imboden and Wüest 1995; Boehrer and Schultze 2008).

Stratification of lakes is changing under the impact of eutrophication, re-oligotrophication and climate warming (Flaim et al. 2016). For instance, in recent decades, the strength of stratification of lakes in northeastern North America has clearly increased (Richardson et al. 2017); a phenomenon that might be further enhanced by a trend of atmospheric stilling (Woolway and Merchant 2019). Analyses of the 2007 National Lake Assessment, NLA dataset (Pollard et al. 2018) showed that synergistic interactions between nutrients and temperature promoting algal or cyanobacterial developments are probable, especially in the eutrophic and hypereutrophic subsets of NLA lakes (Rigosi et al. 2014). Kosten et al. (2012) provided more support for synergistic interactions between nutrients and temperature in determining chlorophyll *a* (Chl *a*) and cyanobacterial dominance in a multilake survey along a latitudinal gradient stretching from the tip of South America to the equator. However, no lake physical variables other than surface temperature, such as density gradient or stratification strength, were included in these large-scale studies on drivers of algal biomass.

To further our understanding of the main drivers and their interactions on phytoplankton biomass across continental climatic gradients, the "grassroots" European Multi Lake Survey (EMLS) was organized during summer 2015, which coincided with the period of maximum stratification in most of the examined lakes. Data from the EMLS are publicly available (Mantzouki et al. 2018). Here, we report on the difference in Chl *a* as a proxy for phytoplankton biomass between 230 of the EMLS lakes to: (1) determine the dependency of phytoplankton biomass at the continental scale on a set of ecosystem drivers, including growth conditions (total phosphorous [TP], total nitrogen [TN], lake temperature, and light) and morphophysical properties (lake depth, surface area, light climate, and stratification strength); and (2) investigate potential interactions between these predictors that influence phytoplankton biomass.

Methods

EMLS organization

During the EMLS in summer 2015, 230 lakes were sampled across major geographical and climatic regions in Europe for various chemical, physical, and biological parameters using highly standardized sampling protocols (Mantzouki et al. 2018; Mantzouki and Ibelings 2018). All key variables were analyzed centrally (by one person on one machine) in dedicated laboratories to ensure data comparability and a fully integrated dataset.

The lake sampling site was selected as either the historical sampling point, for which long-term records exist, or the geographic center of the lake. The sampling period was defined as the warmest 2-week period of the summer, based on long-term (minimum 10 yr) air temperature data of each region. An in situ temperature profile carried out on the sampling day was used to identify and characterize the thermocline as the point where there was ≥ 1°C change of temperature per meter lake depth. An integrated water sample was obtained from 0.5 m depth to the bottom of the thermocline using a water sampler that could effectively sample the whole volume without creating intervals. In nonstratified shallow lakes, an integrated sample was drawn from 0.5 m below the lake surface to 0.5 m above the lake bottom.

Nutrient analyses

Total phosphorus and nitrogen concentrations were assessed in unfiltered samples. Sample bottles were acid washed overnight in 1 M HCl and rinsed with demineralized water before usage. Nutrients were measured using a Skalar SAN+ segmented flow analyzer (Skalar Analytical BV, Breda, the Netherlands) with UV/persulfate digestion integrated in the system. The limit of detection was 0.02 mg L^{-1} for TP and 0.2 mg L^{-1} for TN. TP was analyzed following NEN (1986) and TN according to NEN (1990). All nutrient analyses were performed at the University of Wageningen, the Netherlands.

Pigment analyses

Pigment analysis, modified from the method described by Van der Staay et al. (1992), was carried out to determine concentrations of Chl *a* and Zeaxanthin (Zea). Measurement of Zea concentrations in the EMLS lakes were carried out with the aim of investigating cyanobacterial biomass, alongside to the general phytoplankton biomass estimate obtained with Chl *a*. Filters (45 mm diameter GF/C or /F) were freeze-dried for 6 h and then cut in half, placed in separate Eppendorf tubes, and kept on ice. A number of 0.5 mm beads and 600 μL of 90% acetone were added to each tube. To release the pigments from the phytoplankton cells and increase the extraction yield, tubes were placed on a bead-beater for 1 min and then in an ultrasonic bath for 10 min. To ensure complete extraction of the total pigment content of the filters, the bead-beater and ultrasonic bath steps were performed twice. To achieve binding of the pigments during the high-performance liquid chromatography (HPLC) analysis, 300 μL of a tributyl ammonium acetate (1.5%) and ammonium acetate (7.7%) mix were added to each tube. Lastly, samples were centrifuged at 15,000 rpm and 4°C for 10 min. Next, 35 μL of the supernatant from Eppendorf tubes were transferred into glass HPLC sampling vials. Pigments were separated on a Thermo Scientific ODS Hypersil column (250 mm × 3 mm, particle size 5 μm) in a Shimadzu HPLC, using a KONTRON SPD-M2OA diode array detector. The different pigments were identified based on their retention time and absorption spectrum and quantified by means of pigment standards. Pigment analysis was performed at the University of Amsterdam, the Netherlands.

Lake groups

Lake classification was based on climatic zone and depth type. Predicted climatic zones based on different IPCC scenarios (2000–2025; Rubel and Kottek 2010) were used to avoid the inconsistency in available digital maps, especially for areas such as the Alpine region (Rubel et al. 2017). The climatic zones were defined using the Köppen-Geiger's classification (Köppen 1900). This classification regards the main climate of the region (C = warm temperate, D = alpine), precipitation levels (f = fully humid, s = summer dry), and mean temperature (a = hot summers, b = warm summers). For easier interpretation and more statistical power, climatic regions that were of the same main climate and precipitation level were combined in three main ones: Mediterranean (Csa and Csb, $n = 54$ lakes), Continental (Cfa and Cfb, $n = 128$ lakes), and Boreal (Dfb and Dfc, $n = 48$ lakes) (Fig. 2). This way, only the mean temperature varied within each of the combined groups, which allowed for testing of a temperature gradient. The selection of climatic zones has a clear advantage over a latitudinal analysis, as several lakes within the Continental region are classified as Boreal lakes based on their climatic characteristics rather than their position on a latitudinal gradient (*see* Table S1 for list of EMLS lakes and corresponding climatic zone).

The EMLS lakes were categorized into shallow (< 6 m maximum depth, $n = 93$ lakes) and deep (> 6 m maximum depth, $n = 137$ lakes). This classification was used in previous snapshot surveys as an approximation for weakly or strongly thermally stratified systems (Kosten et al. 2012; Beaulieu et al. 2013).

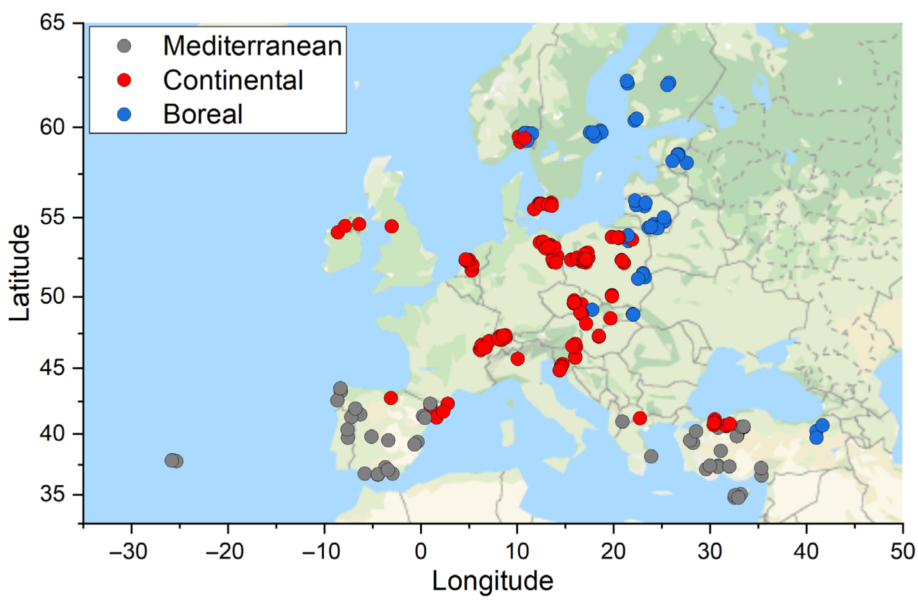

Fig 2. Location of the 230 EMLS lakes distributed over the main climatic zones of the European continent (Rubel and Kottek 2010). The Mediterranean region ($n = 54$) consists of Csa and Csb classes (C, warm temperate; s, summer dry; a, hot summer; b, warm summer), the Continental region ($n = 128$) of Cfa and Cfb (f, fully humid; rest as above), and the Boreal region ($n = 48$) of Dfb and Dfc (D, snow; c, cool summer; rest as above).

Table 1. List of lake variables with their units, range of values, means, medians, and standard deviations for the 230 EMLS lakes. Variables with * are included in the linear models.

Variable		Units	Range	Mean ± SD	Median
Maximum depth	maxD	m	1–310	23±41	10.00
Surface area*	SurfA	km^2	0.001–580	19±69	5
Total nitrogen*	TN	mg L^{-1}	0.1–5	1.0±0.8	0.70
Total phosphorus*	TP	mg L^{-1}	0.02–1	0.1±0.1	0.06
Surface temperature*	SurfT	°C	14.6–33	23±3.4	22.4
Average temperature	AvT	°C	13.4–33	21±3.5	20.6
Secchi depth	SD	m	0.16–10	1.8±1.7	1.19
Light climate*	Z_{eu}/Z_{mix}	-	0.02–11	1.0±1.0	0.63
Stratification strength *	N^2	s^{-2}	$3{\cdot}10^{-5}$–$3{\cdot}10^{-2}$	$5{\cdot}10^{-3}$±$4{\cdot}10^{-3}$	4.10^{-3}
Chlorophyll *a**	Chl *a*	μg L^{-1}	0.03–933	44±110	9.98
Zeaxanthin	Zea	μg L^{-1}	0.00–90	3±9.7	0.68

Statistical analysis

To disentangle the importance of various drivers of phytoplankton biomass, we applied linear regression models to six lake groups: all, deep, shallow, Mediterranean, Continental, Boreal. We (1) assessed the quality of the statistical models after excluding collinear and nonsignificant variables, (2) included groups of interactions and nominal variables as environmental predictors, and (3) discussed the three most important predictors for each model.

Response variable and environmental predictors

The response variable of all regression models was the concentration of Chl *a* obtained from the HPLC analysis, which was used as a proxy for total phytoplankton biomass (Pinckney et al. 2001; Tamm et al. 2015) and tested with the following single predictors: maximum depth (maxD), surface area (SurfA), TN, TP, surface temperature (SurfT), average temperature (AvT), Secchi disk depth (SD), light climate (Z_{eu}/Z_{mix}), and maximum buoyancy frequency (stratification strength, N^2) (Table 1).

Surface and average temperatures were determined via a water column profile with a temperature probe, taking respectively the temperature of the top 0.5 m of the water column and the average of the full profile.

Light climate was defined as the ratio of euphotic depth over mixing depth (Z_{eu}/Z_{mix}), which describes the light that phytoplankton experience while circulating through the water column (Scheffer et al. 1997). The equation $Z_{eu} = 2 \times$ SD (Secchi depth) was used to calculate Z_{eu} (equation selected as an average estimate from the range of constants reported in literature, e.g., Koenings and Edmundson 1991; Salmaso 2002; Brentrup et al. 2018). In stratified lakes, Z_{mix} was determined as the depth of the steepest density gradient (Winslow et al. 2017). In nonstratified shallow lakes, Z_{mix} matched the maximum depth and sampling depth. Water density was calculated according to the combined effects of salinity (set to 0) and water temperature based on the method of Millero and Poisson (1981).

Lake stratification is the density-induced layering of the water column (Boehrer and Schultze 2008). Strength of water column stratification was determined by the N^2 given by the Brunt Väisälä equation or buoyancy frequency, N (s^{-1}).

$$N = \sqrt{-\frac{g}{\rho}\left(\frac{\partial \rho}{\partial z}\right)}; N^2 = -\frac{g}{\rho}\left(\frac{\partial \rho}{\partial z}\right) \quad (1)$$

Buoyancy frequency is greater than zero, when a water volume (of density ρ) that is displaced vertically (z) from its initial position without heat transfer, experiences a restoring force. If $N^2 < 0$ instead, the water parcel tends to be displaced away from its initial position and the vertical water column is locally unstable. Here, we use the symbol N^2 to indicate the maximum value over the entire water column. By suppressing vertical turbulent eddies, density stratification determines the water column stability so that, in general, the greater the density gradient, the slower the diffusive exchange of water constituents between the hypolimnion and the epilimnion (Boehrer and Schultze 2008).

Three groups of interactions between some of the aforementioned variables, selected based on ecological theory and previous literature, were included as additional predictors in the models. Namely the interaction between (1) nutrients and surface temperature (Rigosi et al. 2014), (2) stratification strength and light climate (Graff and Behrenfeld 2018), and (3) surface area and light climate.

Analysis of variance

Differences in mean values of the selected variables within climatic zones and depth types were tested using one-way ANOVA. Homogeneity of variance was tested using the Levene's test from the car R package (Fox and Weisberg 2011). In case of heterogeneity, a Kruskal–Wallis test was used instead of ANOVA. Post hoc pairwise comparisons for unequal sample sizes were performed using Tukey HSD (Honest Significant Difference) or Games–Howell test (userfriendlyscience R package; Peters et al. 2018) for homogeneous or heterogeneous variance, respectively.

Multiple linear regression model

All variables were log-transformed (natural logarithm) to obtain a normal and homogeneous distribution. Stepwise selection (backwards and forward) was used for model selection where the AIC scores were compared based on a modified equation that corrects for unequal sample size among categories (R code provided by Statoo Consulting, Switzerland). If the interaction term was significant ($p \leq 0.05$), the lower order terms were included in the equation. The most parsimonious model, in which elimination or addition of any other predictors would not improve the model by ΔAIC > 2, was used for the ANOVA. The metric "lmg" of the relaimpo R package (Grőmping 2006) was used to decompose the overall R^2 of each final model into the absolute contributions of each predictor term and their interaction terms (similarly done in Rigosi et al. 2014). The relative contribution of each predictor was normalized, by forcing the sum to 100%. A bootstrapping approach was used to replicate the observed data 9999 times and determine if there were any clear differences between the predictors of the interaction terms with regards their relative contribution to the interaction term (Grőmping 2006). If those differences included zero, it indicated that the predictors were not significantly different from each other, meaning that they contributed similarly to the interaction term. When the interaction term had a significant value of $p < 0.05$ and was positive, it was interpreted as a synergistic interaction.

To avoid multicollinearity between the interactions and their main effects, we checked the variance inflation factor (VIF). If VIFs were exhibiting high numbers (VIF > 3, threshold according to (Zuur et al. 2010), we centered the interaction term with the mean of the raw variables which alleviated the collinearity problem.

We applied multiple linear regression models to test the relative importance of the selected response variables in explaining Chl a variance. The model applied was:

$$\mathrm{Chl}\,a = A_0 + A_1 X_{\mathrm{SurfA}} + A_2 X_{N2} + A_3 X_{\mathrm{SurfT}} + A_4 X_{\mathrm{TN}} + A_5 X_{\mathrm{Zeu/Zmix}} + A_6 X_{N2*\mathrm{Zeu/Zmix}} + \varepsilon \qquad (2)$$

where A_0 represents the intercept term, A_1–A_6 are model parameters for each respective predictor in the models, "*" denotes the interaction between two terms, and ε is an error term. Two multiple linear regression models were applied to the entire EMLS group of lakes. Apart from the full set of environmental predictors, each of these two models included the nominal variable "depth type" or "climatic zone" (see Supplementary Material for more

Table 2. List of applied models and relative metrics. AIC does not apply correctly if number of observations is not the same, for which we rely on R^2.

Lake group	Multilinear model	N lakes	R^2	AIC
(1) All-a	Chl $a = -9.06 - 0.23$ (SurfA) $- 0.31$ (N^2) $+ 3.36$ (SurfT) $+ 0.46$ (TN) $+ 0.47$ (Z_{eu}/Z_{mix}) $+ 0.18$ (N^2*Z_{eu}/Z_{mix}) $-$ 0.90 (Cont) $-$ 2.06 (Med)	230	35%	842.43***
(2) All-b	Chl $a = -2.44 - 0.15$ (SurfA) $- 0.11$ (N^2) $+ 1.12$ (SurfT) $+ 0.31$ (TN) $+ 0.45$ (Z_{eu}/Z_{mix}) $+ 0.19$ (N^2*Z_{eu}/Z_{mix}) $+ 1.17$ (Shallow)	230	30%	856.65**
(3) Shallow	Chl $a = 0.33$–0.05 (SurfA) $- 0.17$ (N^2) $+ 0.48$ (SurfT) $+ 0.78$ (TN) $- 0.09$ (Z_{eu}/Z_{mix}) $+ 0.12$ (N^2*Z_{eu}/Z_{mix})	93	31%	Na
(4) Deep	Chl $a = -0.65 - 0.14$ (SurfA) $+ 0.07$ (N^2) $+ 0.84$ (SurfT) $+ 0.01$ (TN) $+ 1.13$ (Z_{eu}/Z_{mix}) $+ 0.29$ (N^2*Z_{eu}/Z_{mix})	137	12%	Na
(5) Med.	Chl $a = -17.035 - 0.23$ (SurfA) $- 0.029$ (N^2) $+ 5.26$ (SurfT) $+ 0.40$ (TN) $+ 0.83$ (Z_{eu}/Z_{mix}) $+ 0.22$ (N^2*Z_{eu}/Z_{mix})	54	45%	Na
(6) Cont.	Chl $a = -5.40 - 0.26$ (SurfA) $- 0.33$ (N^2) $+ 1.88$ (SurfT) $+ 0.47$ (TN) $+ 0.23$ (Z_{eu}/Z_{mix}) $+ 0.12$ (N^2*Z_{eu}/Z_{mix})	128	25%	Na
(7) Bor.	Chl $a = -15.65 - 0.15$ (SurfA) $- 0.005$ (N^2) $+ 6.05$ (SurfT) $+ 0.41$ (TN) $+ 2.77$ (Z_{eu}/Z_{mix}) $+ 0.62$ (N^2*Z_{eu}/Z_{mix})	48	43%	Na

* P ≤ 0.05.
** P ≤ 0.01.
*** P ≤ 0.001.

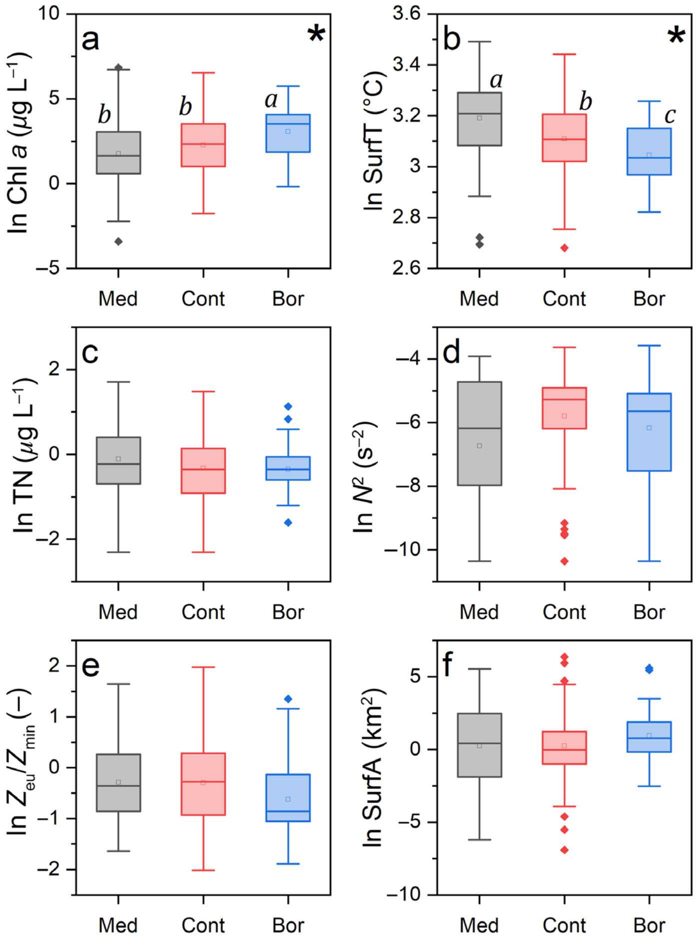

Fig 3. EMLS log-transformed response variable, (**a**) Chl *a*, and significant predictors: (**b**) surface temperature, (**c**) total nitrogen, (**d**) maximum buoyancy frequency (stratification strength), (**e**) light climate (Z_{eu}/Z_{mix}), and (**f**) surface area, averaged over climatic zones. Significant differences at the 0.05 level are marked with *. Different italic letters indicate significant differences among categories (Tukey test; $p < 0.05$).

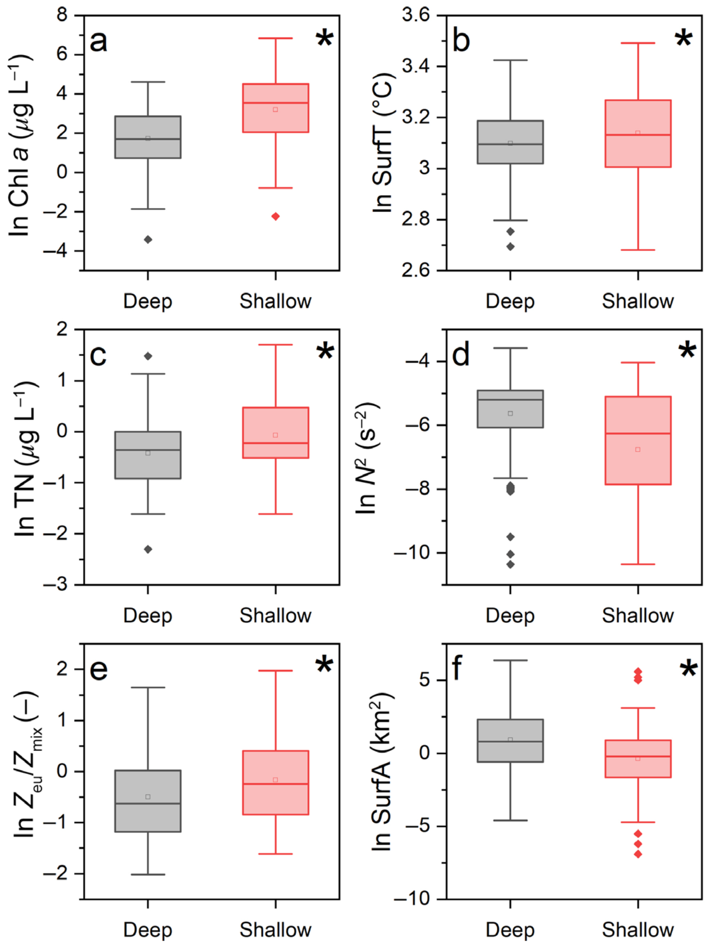

Fig 4. EMLS log-transformed response variable (**a**) Chl *a*, and significant predictors: (**b**) surface temperature, (**c**) total nitrogen, (**d**) maximum buoyancy frequency (stratification strength), (**e**) light climate (Z_{eu}/Z_{mix}), and (**f**) surface area, averaged over depth type. Significant differences at the 0.05 level are marked with *.

detail). These nominal variables comprehend the lake subsets to which the same multilinear regression model that was further applied, that is, deep, shallow, Mediterranean, Continental, Boreal (Table 2).

Results

Response variable and environmental predictors

The EMLS lake data cover a wide range of morphological, physical, chemical, and biological values (Table 1). The median measured TP was 60 μg L^{-1}, and according to Carlson trophic state index (TSI) 85% of the lakes were classified as eutrophic (TSI > 50). EMLS lakes were largely represented by eutrophic conditions (70%) also when calculating the TSI on basis of Secchi disk depth (median SD = 1.2 m), while TSI based on Chl *a* concentration (median Chl *a* = 10 μg L^{-1}) leads to 54% of lakes being classified as eutrophic.

Significant collinearity was observed between maximum depth and surface area, and between surface temperature and average temperature (Fig. S1). VIFs of maximum depth and average temperature were higher than 3, thus they were removed from subsequent analyses. Secchi depth was also removed in favor of using the light climate variable, Z_{eu}/Z_{mix}.

All the variables were found to be significant, except for TP, and the interactions TN*SurfT and SurfA*Z_{eu}/Z_{mix}, which therefore never appeared as Chl *a* variance predictors.

Lake groups: Climatic zone and depth type

Climatic zone

ANOVA was performed on the three climatic zone groups, composed by 54 Mediterranean, 128 Continental, and 48 Boreal lakes (Fig. 3; Table S2).

Mean Chl *a* concentrations were significantly higher in the Boreal lakes (mean ln ± 1 SD, 3 ± 1 μg L^{-1}) compared to

Continental (2.2 ± 1 μg L^{-1}) and Mediterranean (1.7 ± 2 μg L^{-1}), while no significant difference was found between Continental and Mediterranean lakes (Fig. 3a; Table S2).

Depth type

The EMLS dataset is composed of 93 shallow and 137 deep lakes (> 6 m). Response variable, Chl *a*, and all of the predictors used in the statistical models of the EMLS significantly differed between deep and shallow lakes (Fig. 4; Table S2). Chl *a*, SurfT, TN, and Z_{eu}/Z_{mix} were all higher for shallow lakes, whereas deep lakes showed a stronger stratification strength (N^2) and greater surface area than shallow ones.

Drivers explaining Chl *a* at the continental scale

The applied models significantly explain a proportion of the variability in Chl *a* ($p \leq 0.001$; Table 2), with the model applied to Mediterranean lakes explaining the highest variability ($R^2 = 45\%$), closely followed by the model applied to Boreal lakes ($R^2 = 43\%$) with Continental lakes further behind ($R^2 = 25\%$), while the model applied to deep lakes explained the lowest variability ($R^2 = 12\%$), compared to $R^2 = 31\%$ for shallow lakes. Based on AIC comparison, the nominal variable "climatic zone" is more significant than "depth type" in explaining the variance of algal biomass (Table 2). Nevertheless, the lake group "depth type" explained more of the overall R^2 compared to "climatic zone" (37% vs. 26%; Table S3).

When the model included the nominal variable "climatic zone" among the predictors, it resulted as the strongest predictor for algal biomass with 26% of the model R^2 explained, closely followed by stratification strength (24%), and with a significant but smaller contribution of TN (13%; Table S3). Similarly, when "depth type" was included, it resulted as the most significant predictor (37%); however, it was much more important than the second most significant predictor (stratification strength, 18%), that was closely followed by light climate (15%; Table S3).

The 230 lakes dataset allows us to carry out the same analysis separately on each group of lakes corresponding to the explanatory categories, climatic zone, and depth type, to gain more insights on the summer drivers of phytoplankton biomass for this set of lakes.

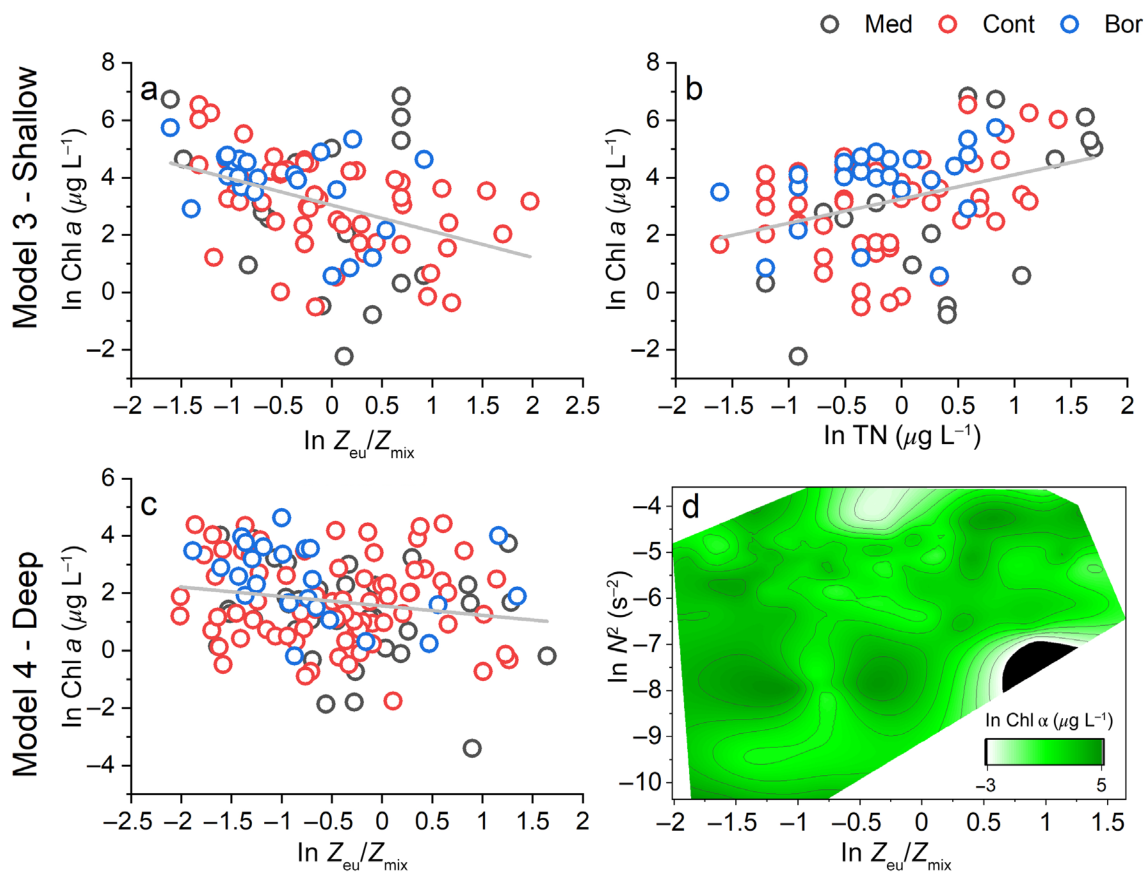

Fig 5. First two significant predictors for Chl *a* in lake group model 3 (shallow) and model 4 (deep). (**a**, **b**) Light climate, Z_{eu}/Z_{mix}, and TN explain respectively 46% and 33% of Chl *a* variance in EMLS shallow lakes (model $R^2 = 31\%$). (**c**, **d**) Light climate, Z_{eu}/Z_{mix}, and its interaction with stratification strength, $N^2 * Z_{eu}/Z_{mix}$, explain respectively 32% and 29% of Chl *a* variance in EMLS deep lakes (model $R^2 = 12\%$). All variables are plotted as to the statistical models, that is, natural logarithm (ln). See Table S4 for relative contribution and significance of all predictors.

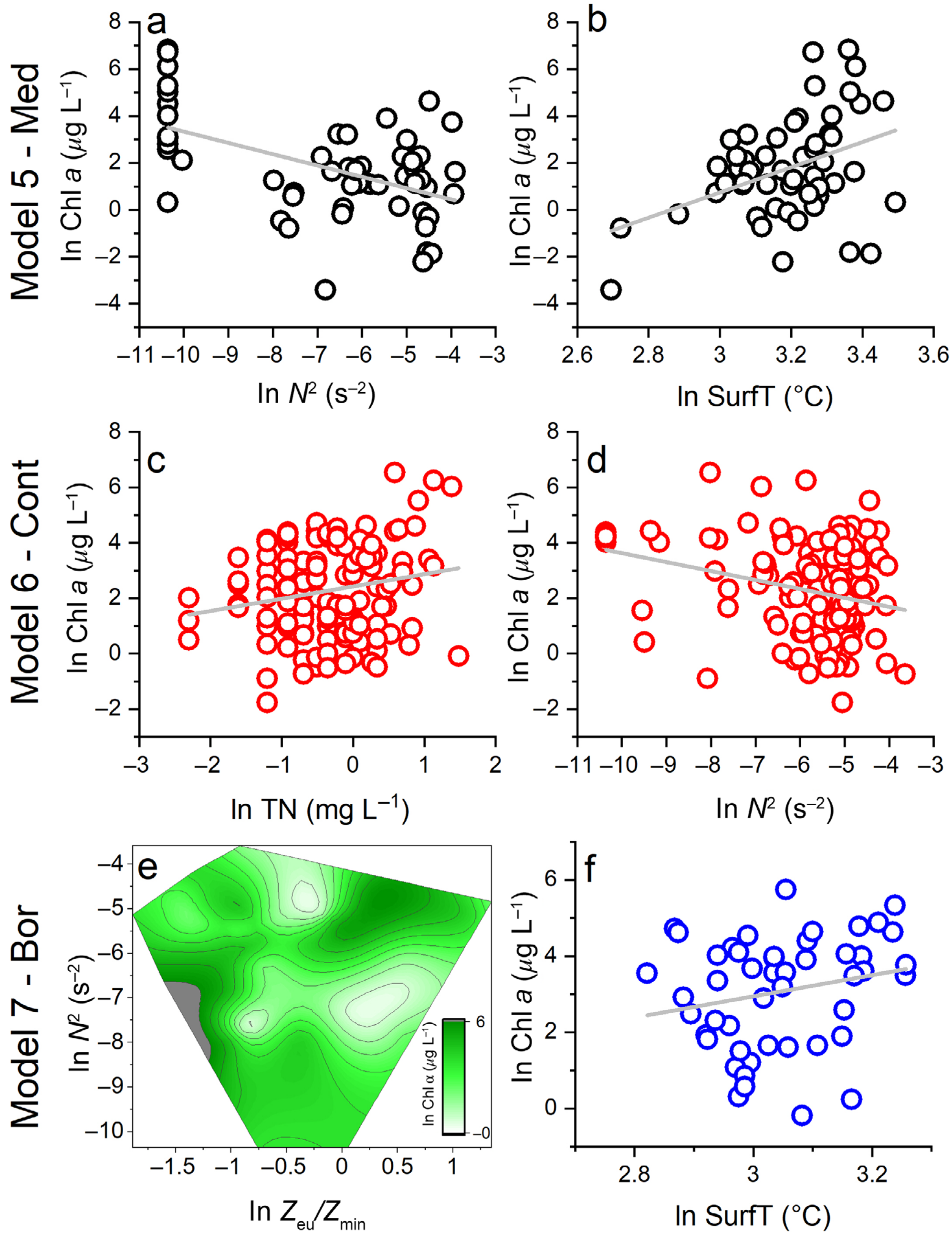

Fig 6. First two significant predictors for Chl a in lake group model 5 (Mediterranean), model 6 (Continental), and model 7 (Boreal). (**a**, **b**) Stratification strength, N^2, and surface temperature, SurfT, explain respectively 46% and 24% of Chl a variance in EMLS Med lakes (model $R^2 = 45\%$). (**c**, **d**) TN and N^2 explain respectively 35% and 29% of Chl a variance in EMLS Cont lakes (model $R^2 = 25\%$). (**e**, **f**) Light climate, interaction with stratification strength, $N^2 * Z_{eu}/Z_{mix}$, and SurfT explain respectively 34% and 21% of Chl a variance in EMLS Boreal lakes (model $R^2 = 43\%$). All variables are plotted as to the statistical models, that is, natural logarithm (ln). See Table S5 for relative contribution and significance of all predictors.

Shallow vs. deep lakes

Light climate was the most important variable explaining Chl a variance in both shallow and deep lake subsets (46% and 32%, respectively; Fig. 5a,c). Stratification strength was also a significant contributor for both lake types, either individually (14%, shallow lakes; Table S4), or in synergistic interaction with light climate (29%, deep lakes; Fig. 5d). However, for shallow lakes, TN played a more significant role than stratification (33%; Fig. 5b) while not appearing as a significant predictor of algal biomass in the deep lakes subset.

Mediterranean vs. Continental vs. Boreal lakes

When applying the model to the different climatic zones, the strength of the stratification appeared as a strong

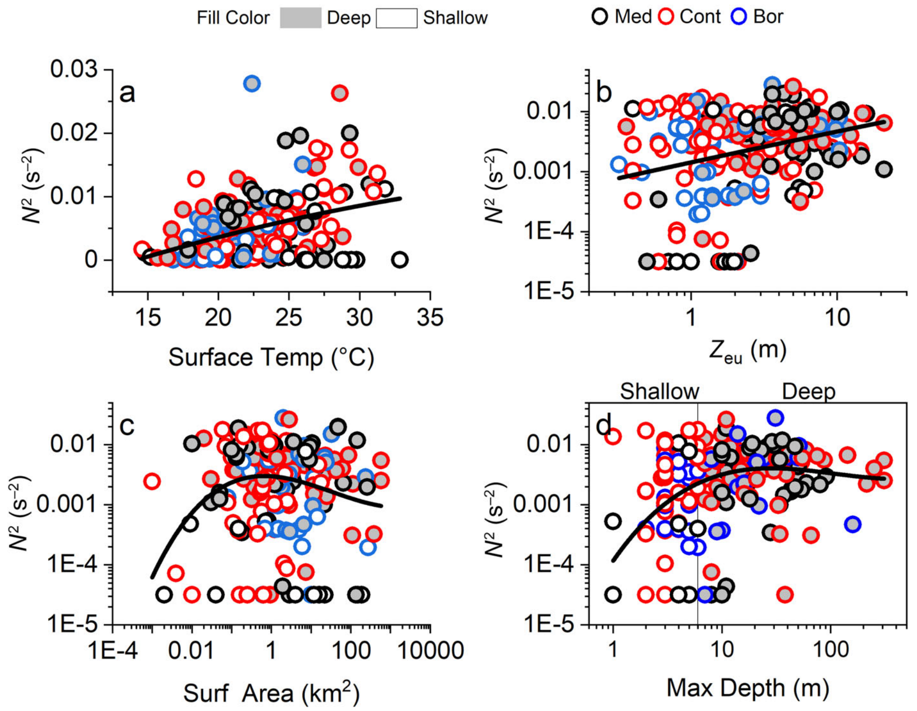

Fig 7. Relationships between N^2 and (**a**) surface temperature, 2[nd]-order polynomial fit, $R^2 = 0.14$; (**b**) euphotic depth, 2[nd]-order polynomial fit, $R^2 = 0.06$; (**c**) surface area, 3[rd]-order polynomial fit, $R^2 = -0.08$; (**d**) maximum depth, 4[th]-order polynomial fit, $R^2 = 0.2$. All polynomial fit are significantly better than function $y =$ constant at the 0.05 level.

predictor, either individually (Med. 46% and Cont. lakes 29%; Fig. 6a,d) or in interaction with light climate (Boreal lakes 34%; Fig. 6e). In Mediterranean and Boreal lakes, surface temperature was also a strong predictor of algal biomass (24% and 21%, respectively; Fig. 6b,f) but not for Continental lakes (Table S5). Instead, nutrients were the most significant predictors of Chl *a* (34%) for Continental (Fig. 6c), while being less important for Boreal lakes (14%) and not important for Mediterranean lakes (Table S5).

Relationship between stratification metrics

Within the EMLS lakes, we analyzed the relationship between stratification strength and some of the drivers, that is, temperature, light penetration, and lake morphology. As already shown, Mediterranean lakes, while being on average the warmest, did not have the highest average stratification strength (Fig. 3d). When looking at the entire dataset (Fig. 7), the polynomial fit between the maximum N^2 and surface temperature was significant ($p < 0.001$) but weak ($R^2 = 0.14$; Fig. 7a), indicating that only for a relatively small number of the EMLS lakes, higher surface water temperatures at the sampling time corresponded to a stronger stratification. An even weaker relationship ($R^2 = 0.06$) was observed between stratification strength and light penetration depth (Z_{eu}; Fig. 7b). As for the morphological features, the relationship observed between maximum N^2 and surface area (Fig. 7c), was much weaker ($R^2 = 0.08$) than between maximum N^2 and maximum lake depth ($R^2 = 0.2$). Here, the 4[th]-order polynomial fit followed the effect of temperature on N^2 for increasing lake depths, reaching a plateau for lakes deeper than ~ 20 m (Fig. 7d).

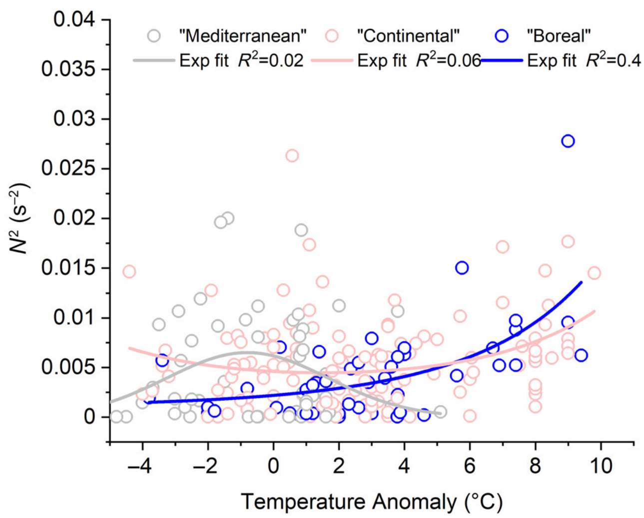

Fig 8. Eight-day average temperature anomaly at the sampling site and sampling period in relation to the lake stratification strength for the EMLS climatic zone subsets, Continental (gray), Mediterranean (light pink), and Boreal (blue). All best fits are given by exponential curves, with the one for Boreal lakes being the most significant ($R^2 = 0.4$).

Air temperature anomaly

The summer of 2015 was the third warmest summer (after 2003 and 2010) since 1880 in Europe (GISTEMP, NOAA online data). During the sampling period in 2015, 70% of EMLS lakes experienced a positive temperature anomaly of 1.9°C ± 3.4°C (average ± 1 SD, based on each lake 8-d temperature average compared to 10-yr average for the same 8 d). However, when looking at each climatic zone separately, 96% of Continental lakes and 87% of the Boreal lakes experienced a positive temperature anomaly of 3.8°C ± 2.6°C and 3.7°C ± 2.9°C, respectively. In contrast, only 53% of the Mediterranean lakes experienced a positive temperature anomaly, of 1.4°C ± 1°C. The remaining 30% of the total, 4% of Continental, 13% of Boreal and 47% of Mediterranean are lakes that showed a negative deviation from the long term average.

Hence, at the time of sampling, the great majority of Continental and Boreal lakes experienced a strong temperature increase compared to the long-term average levels, which was not the case for Mediterranean lakes. Compared to the other regions, Boreal lakes as well showed the strongest exponential relationship between the experienced temperature anomaly and stratification strength (Fig. 8).

Pigments analysis

Measurement of Zea concentrations in the EMLS lakes were carried out with the aim of investigating cyanobacterial biomass. A strong linear relationship was found between Zea and Chl *a* (R^2 = 0.6; Fig. S3) indicating that higher Chl *a* concentrations systematically corresponded with higher concentrations of Zea.

Discussion

Drivers explaining phytoplankton biomass at the continental scale

Several studies have focused on the effects of nutrients and warming on phytoplankton in more than one lake (table S1 in Salmaso and Tolotti 2021). This is of particular concern for resolving the climate warming effect on lakes and the positive feedbacks on eutrophication of lakes (Sinha et al. 2017; Deng et al. 2018). However, thermal stratification, which will likely increase with climate warming (Woolway and Merchant 2019), is an important feature governing lake ecosystems as it affects both nutrient availability and light climate (Schwefel et al. 2016), generating complex feedbacks for the biota (Mesman et al. 2021). The importance of these factors may dominate when lakes are not nutrient limited.

We have applied a set of multiple regression models to 230 European lakes (54–85% of which were eutrophic depending on the criterion applied) to test the dependency of Chl *a* on phytoplankton growth resources (nutrients, temperature, and light climate) and morphophysical lake properties (surface area, stratification strength), including interactions between specific predictors. Our results indicate that physical properties of a lake, such as stratification strength and light climate (expressed as the ratio of euphotic to mixing depth), are the strongest ecosystem drivers for phytoplankton biomass for this set of mostly nutrient-rich lakes, at the sampling time. It is possible, however, that a different result would be obtained from the same dataset in a different time of the year.

In a similar fashion to the present work, an earlier study on 1076 US lakes (Rigosi et al. 2014), showed that surface temperature, nutrients, and their interaction were the main phytoplankton biomass predictors. Interestingly, their results showed that the largest part of the variance in Chl *a* for the subset of eutrophic and hypereutrophic lakes was explained by a synergistic interaction between nutrients and temperature. Our study moves a step forward and highlights the fact that additional variables need to be considered when collecting lake "snapshots" at a continental scale. The analysis presented here indicates that nutrients, temperature, and light should not be the only algal growth conditions to be considered. We show that when lake stratification metrics are included, we can gain insights into the lake physics mechanisms that promote phytoplankton biomass growth and potentially improve the development of predictive tools.

Moreover, our statistical analysis indicates that surface temperature alone should not be used as a proxy for stratification strength. Indeed for a multilake survey, it is necessary to estimate lake stability (N^2) as a variable that comprises the lake thermal "history," and therefore gives insight into the environmental conditions that the phytoplankton have experienced during the recent past. Such information is easily attained with a temperature profile and is extremely relevant when looking at ecosystem functioning, as thermal structure and light penetration determine the physical constraints of the photosynthetic biomass distribution in the water column. These constraints also determine to what extent specific phytoplankton features adapted to life in a stable water column, such as the pigment composition (e.g., presence of phycoerythrin in deep chlorophyll maxima), and buoyancy regulation (e.g., gas vesicles, motility, shape adaptations) may favor specific algal groups.

Shallow vs. deep lakes

In the EMLS, most of the lakes were eutrophic which may explain the predominant importance of light climate (Z_{eu}/Z_{mix}) for algal biomass variance in both shallow and deep lakes. We therefore assume that, for nutrient-rich lakes, phytoplankton rather than inorganic suspended solids determine underwater light extinction (Scheffer et al. 1997), which subsequently determines phytoplankton biomass.

Although light climate was the most important factor for both EMLS depth types, we observed a relatively greater importance of light climate in shallow rather than deep lakes (explaining 46% and 32% of the variation, respectively), which may be explained by the fact that shallow lakes exist in two clearly distinct states, clear vs. turbid. Mechanisms directly linked to the underwater light climate, for example, high cyanobacterial biomass and benthivorous fish stirring up the sediment, provide varying degrees of resilience to the turbid state (Scheffer et al. 1997). In contrast, macrophytes stabilize the clear water state, and light penetration that reaches the sediment is vital for their development (Ibelings et al. 2007). With 72% of the shallow EMLS lakes having a Secchi depth of less than 0.8 m, we could argue that the majority are in a turbid state, be it stable or not. This may go some way to explain the critical role of light in determining biomass of algae in EMLS shallow lakes.

TN is the second-most important predictor for Chl *a* in shallow lakes (33%) which, together with the general absence of TP as significant predictor for Chl *a* variance, suggests that for the 230 EMLS lakes, the commonly found linear relationship between TP and Chl *a* does not hold true (Vollenweider 1968). This is in line with previous studies on nutrient-rich lakes suggesting that (1) a positive linear TP–Chl *a* relationship exists only at intermediate concentrations of TP (0.004–0.23 mg L^{-1}; Quinlan et al. 2020) and (2) nitrogen becomes limiting for phytoplankton under high TP, especially over shorter temporal scales (Filstrup et al. 2014).

A eutrophic status of a lake, however, does not mean that nutrients cannot be limiting for dense phytoplankton, with a large demand to sustain a high biomass. Yet, the condition of nutrient limitation (in our case nitrogen) could be seen as an effect driven by Z_{eu}/Z_{mix} (first predictor). Especially for shallow lakes, when this ratio becomes smaller, the mixed layer exceeds the euphotic zone and nutrients from the sediment are likely to be resuspended. We may easily see a more direct relationship between Chl *a* and light climate than with the nutrient abundance, because light climate, by revealing the recent mixing history, is a more integrative indicator of nutrient availability than the nutrient content of a single water sample, especially for productive shallow lakes.

For deep lakes, light climate and its synergistic interaction with water column stability had a similarly important contribution to the overall R^2, explaining Chl *a* variance (32% and 29% for Z_{eu}/Z_{mix} and N^2, respectively). High algal biomass increases turbidity, which can increase water temperature in the surface layer through increased heat absorption (Ibelings et al. 2003), and thus reinforce stratification (Paerl and Huisman 2008). Reinforced stratification through increased turbidity implies that phytoplankton is maintained within the euphotic zone offering a potential explanation of how light climate can interact synergistically with water column stability ($Z_{eu}/Z_{mix} > 1$; Fig. 1a). However, in a strongly stratified lake, nutrients may remain available in the hypolimnion even when they are depleted in the epilimnion, so that deeper mixing, also of short duration, enhances the likelihood that phytoplankton gains access to this pool of nutrients. In deep, well-stratified lakes, it is also relatively common to find algal biomass maxima (a.k.a. deep chlorophyll maximum [DCM]) at the crossroads of light from above and nutrients from below (Leach et al. 2018). On the other hand, if stratification is weak and mixing can reach deeper layers, it will take the algal communities beyond the euphotic zone reducing algal growth ($Z_{eu}/Z_{mix} < 1$; Fig. 1b). A deeper mixed layer will allow light to reach greater depths by diluting epilimnetic phytoplankton over a larger volume of lake water, thus increasing light penetration. This extended euphotic depth will likely, however, not make up for light limitation due to a deeper mixing depth, so the ratio Z_{eu}/Z_{mix} would still decrease when water column stability decreases (Fig. 1b), exacerbating the light limitation of phytoplankton growth.

In contrast to the shallow lakes, in EMLS deep lakes neither TP nor TN appeared as a significant predictor of algal biomass, possibly because of the higher likelihood of light limitation mentioned above. Interestingly, another difference between EMLS shallow and deep lakes was that the surface area explained a significant 22% of the overall Chl *a* variance of deep lakes, while did not explain the Chl *a* variance for the shallow lakes (Table S4). This might be due to the fact that the surface area becomes important considering its direct relationship with lake wind exposure, which can influence the water column mixing depth in deep lakes, hence the availability of light and nutrients for phytoplankton (Fig. 1). Although wind exposure was not included in this study, EMLS lake area correlated with depth (Fig. S1a), and was therefore indirectly related to the water column thermal structure. Indeed, EMLS lakes with larger surface areas tended to be deeper (Fig. 4f) and more stable (Fig. 4d), and this may have favored phytoplankton's access to light, in particular when nutrients are not—or less of—a limiting factor, for example, when DCMs are formed where phytoplankton has access to nutrients in the hypolimnion (Leach et al. 2018).

Mediterranean vs. Continental vs. Boreal lakes

When EMLS lakes were clustered by climatic zone, stratification strength appeared as a strong predictor for Chl *a*, either individually (Mediterranean 46% and Continental lakes 29% variation explained) or in synergistic interaction with light climate (Boreal lakes 34%). Stratification strength was thus a dominant factor promoting phytoplankton optimal growth conditions, interacting with the availability of nutrients and light, as discussed. Light climate interaction with water column stratification was a strong factor for Boreal lakes phytoplankton growth (Fig. 6e) possibly due to their tendency to be richer in humic substances and consequently darker (Kutser et al. 2005; Kelly et al. 2018).

Phytoplankton biomass in Continental lakes seemed to exhibit a higher degree of nitrogen dependency (Fig. 6c); however, we cannot exclude that those lakes in other regions were in a similar state, since as discussed above, predictors like light climate can possibly encompass nutrient limitation. On the other hand, the comparatively lower Chl *a* content of Mediterranean lakes (Fig. 3a) seems to indicate that, at the time of sampling, these lakes were experiencing a better nutrient–phytoplankton balance than Continental lakes.

The best predictor for algal biomass: Stratification strength or lake depth?

Stratification strength decreased in importance when splitting the dataset into depth types, which may indicate that depth-type itself explained algal biomass variance. This is also suggested by the fact that all predictors were significantly different between deep and shallow lakes (Fig. 4), and some important environmental factors have a different effect on these two clusters. Wind has generally a larger effect on temperature structure and stability of shallow lakes, because the wind-induced mixing allows heat to be transferred throughout the entire water column (Nõges et al. 2011). Furthermore, shallow lakes respond more directly to short-term weather variations (Arvola et al. 2009; Deng et al. 2018). For deep lakes that have a higher heat retention and potential energy, greater wind speeds are required to drive mixing during the summer months, resulting in greater stability (Boehrer and Schultze 2008). Fetch and dominant wind direction and intensity are also important in determining stratification strength in deep lakes (Wetzel 2001), although these data were not collected for this study. However, given the consistently higher N^2 observed for EMLS deep lakes (Fig. 4d), we can assume that sufficiently strong and long-lasting winds were not present at each sampling site during—or shortly prior to—the sampling period to modify the aforementioned scenario of deep lakes that are more strongly stratified than shallow lakes.

Depth and N^2 may therefore be confounding variables because, at least for this dataset, lake depth can explain most of the variation in stratification trends. Nevertheless, whether lake maximum depth or stratification strength is actually the most significant predictor of Chl *a* in the overall EMLS dataset, the message remains unchanged: lake morphophysical properties are essential when investigating phytoplankton biomass responses to environmental changes.

Relationship between stratification metrics

Given the importance of stratification strength as a predictor of Chl *a* variance at the European continental scale, we analyzed the relationship between this variable, represented by maximum N^2, and the environmental and morphological characteristics that act on the density gradient of a lake (surface temperature, light penetration depth, maximum depth, and surface area).

Surface temperature

Stratification strength responds directly to changes in water temperature, yet each lake will need a certain number of warm days with relatively low wind speed to develop stratification, which also depends on lake morphological factors. The reason for the weak correlation observed between maximum N^2 and surface temperature (Fig. 7a) may be that deep and shallow lakes are equally represented, and while deep lakes are more strongly stratified, the shallow lakes had the highest surface temperature (Fig. 4b,d). The absence of a strong correlation between stratification strength and surface temperature is further confirmed by the absence of any trend between stratification strength and climatic zone (Fig. 4d).

Moreover, the fact that shallow and Mediterranean lakes had the highest surface temperature, but the weakest stratification confirms that surface temperature can be a misleading indicator for stratification strength, especially for snapshot surveys as shown in previous studies on large datasets (Read et al. 2014; Winslow et al. 2017).

Light penetration depth

Changes in light absorption by the dissolved and suspended content of a lake affect the vertical distribution of heat and resulting stratification (Andrew et al. 2008; Rinke et al. 2010). We did not observe, however, a distinct relationship between stratification strength and light penetration (Fig. 7b). The reason why this relationship is not stronger may be that the effect of light on stratification is more evident in time series than in spatial gradients. This is because light-induced heat diffusion in the water column and its temporal variability has a stronger effect on the duration of the stratification than on its absolute value. Indeed, more transparent lakes (Secchi transparency > 5 m) tend to maintain a seasonal thermal stratification for a longer duration than more turbid ones (Richardson et al. 2017), therefore remaining stable longer. Assessing whether this is the case is not possible with a summer snapshot sampling design, although it was observed that light penetration can drive the depth of the mixed layer. This is suggested for the EMLS dataset by a moderate linear relationship ($R^2 = 0.35$) between the depth of the epilimnion, or mixed layer, and the euphotic depth (Fig. S2).

Maximum depth and surface area

We observed a relationship between N^2 and both the lake maximum depth and lake surface area (Fig. 7c,d). The shape of the polynomial fit shows that the linearity of stratification strength with lake maximum depth holds until lake depths of ~ 20 m, because of the physical limit dictated by the thermal diffusivity of water. This relationship may confirm that N^2 and depth are interdependent in determining resource availability for the algal communities.

EMLS lakes with a greater area were on average deeper and had a more stable water column (Fig. 4d,f). Therefore, surface area of the EMLS dataset was directly correlated with maximum depth and was used as the only morphological variable in the statistical models. However, the relationship between surface area and N^2 was not strong (Fig. 7c), possibly because a larger surface area does not necessarily mean a greater wind exposure, which is largely determined by the lake's orientation toward the dominant wind direction and lake topography. Clearly, as for the underwater light regime, analyzing the effect of wind exposure on the thermal structure is not possible with a single observation, but would require a water column temperature time series.

Temperature anomaly: 2015, an unusually hot summer

As expected, Mediterranean lakes had higher surface temperatures than Boreal ones (Fig. 3a,b). However, Boreal lakes exhibited a significantly higher Chl *a* concentration than Mediterranean lakes, while lakes in both climatic zones had comparable nutrient concentrations (Table S2). This seems to confirm the importance of factors other than temperature (lower in Boreal lakes) or nutrients (similar) driving phytoplankton biomass, especially water column stability in relation to the light climate. Indeed, while Boreal lakes are known to stratify intermittently during summer (Kirillin and Shatwell 2016; Woolway and Merchant 2019), the heat wave likely intensified the stratification strength in the Boreal lakes more strongly than in other regions, given that the region experienced the largest temperature anomaly (Fig. 8). This may have favored the conditions shown in Fig. 1a and supported by our model results, that is, the interaction between light climate and stratification strength is the main Chl *a* driver for Boreal lakes (Fig. 6e).

As several studies addressed the relationship between light, nutrients, and temperature effects on primary producers in Boreal regions (Zwart et al. 2016; Bergstrom and Karlsson 2019), alternative explanations may apply too. Although it is not possible to generalize, such observations are crucial to generate ideas and stimulate future research. It is possible that a higher abundance of mixotrophs in Boreal lakes may help to explain the higher Chl *a* in that region, since Hansson et al. (2019) demonstrated that the success of mixotrophs is correlated with the elevated colored dissolved organic matter content of Boreal lakes. It is also interesting to note that Mantzouki et al. (2018) found that for the same EMLS dataset, the variety of toxins produced by cyanobacteria increased with latitude, which possibly may have reduced the grazing pressure in Boreal lakes, contributing to higher Chl *a*.

Cyanobacteria like it warmer?

Zea is frequently used as a pigment to indicate cyanobacterial biomass (Bianchi et al. 2000; Glibert et al. 2004; Przytulska et al. 2017; Ewing et al. 2020), although it is found both in cyanobacteria and in chlorophytes (Deshpande et al. 2014; Ibelings et al. 2016). In this study, we do not provide microscopy results to confirm the correspondence between cyanobacteria and Zea; therefore, the following discussion is presented with a degree of caution, and mainly serves to stimulate further ideas, eventually contributing to a deeper understanding of the worldwide increase in cyanobacterial blooms.

The strong correlation between Zea and Chl *a* EMLS (Fig. S3) indicated that higher Chl *a* concentrations systematically corresponded with higher concentrations of Zea, which may suggest that water column stratification and light climate are the main drivers for cyanobacterial growth in eutrophic lakes, as they are for overall phytoplankton. Moreover, cyanobacteria have evolved specific traits like buoyancy and accessory pigments that renders them specifically well adapted to stably stratified conditions (Huisman et al. 2018). Consequently, the fact that light climate was the main driver for both lake depth types (Fig. 4) may confirm that at high nutrient levels, light becomes limiting for cyanobacterial development (Ganf and Oliver 1982; Bouterfas et al. 2002; Huisman et al. 2004).

Considering the high temperature anomaly experienced in Boreal regions at the sampling time, the significance of a positive interaction between water column stability and light climate in promoting cyanobacteria in the Boreal lakes during a record hot summer supports the general observation that "blooms like it hot" (Paerl and Huisman 2008). In the context of climate change—and rapid warming at high latitudes—perhaps a more appropriate rephrasing is "blooms like it warmer than usual," since the Boreal lakes were still cooler than the Mediterranean lakes, yet the temperature anomaly was higher as were Zea levels. Among the EMLS subset of lakes with detectable Zea (which are 172 over the total 230 of this study), almost all (95%) of the Boreal lakes experienced a higher positive T-anomaly ($\sim 4°C \pm 2.5°C$). Evidently, more detailed integrated lab-field studies, including both ecological and evolutionary aspects, are needed to resolve this issue.

Future scenario and management strategies

Among the Chl *a* predictors of this study, lake surface temperature and water column stratification are expected to have the strongest impact on lake ecosystems in a warming future (O'Reilly et al. 2015; Kraemer et al. 2017). Both variables were significant drivers for trends in phytoplankton biomass across climatic gradients in Europe. Thus, since lake water column stability will likely increase with a warming climate (Oleksy and Richardson 2021), bloom-forming cyanobacteria in particular will be further promoted given their typical dependence on buoyancy that makes them particularly well adapted to a stable water column (Steinberg and Hartmann 1988; Paerl and Paul 2012).

Although we concur with Ibelings et al. (2016) that any sustainable approach controlling cyanobacterial blooms has to be rooted in nutrient reduction, our present analysis underlines the potential effectiveness of additional measures that weaken the future strengthening of lake stratification, which is demonstrated here to play such a critical role in determining differences in lake phytoplankton and cyanobacterial biomass. It may be essential, for instance, to include measures like artificial lake mixing (Visser et al., 2016) to mitigate algal (and cyanobacterial) blooms.

Conclusions

Nutrients and light are the fundamental resources for phytoplanktonic biomass, even in nutrient rich lakes, such as the ones represented in this study; however, results from the EMLS analysis show that Chl *a* variance is better predicted by light climate and stability metrics. These predictors are also strong indicators of the epilimnetic nutrient load and of the light experienced by the algal biomass prior to sampling. This explains why in this nutrient-rich lake dataset, light climate was the most important variable explaining Chl *a* variance in both shallow and deep lakes, with the difference that, only for deep lakes the optimum condition for photosynthetic biomass was obtained when stratification operated in a synergistic interaction with light climate. The dominance of light climate and the absence of TP as significant predictor for Chl *a* variance confirms that: (1) when TP levels are high as in the average EMLS, light and nitrogen become limiting resources for phytoplankton and (2) light climate, as metric for the recent history of water column mixing, is a powerful indicator for nutrient availability, and needs to be included in similar studies.

Furthermore, our analysis of this pan-European dataset shows that shallow and Mediterranean lakes exhibit the highest surface temperature, although the weakest stratification, confirming that lake surface temperature does not necessarily correlate with lake stratification strength. Consequently, especially for snapshot surveys, a lake temperature profile should be preferred over surface temperature data as it is a more sensible indicator for stratification strength and ecological response to warming.

Finally, among the 230 European lakes, we observed a significant exponential correlation between temperature anomaly and the stratification strength only for Boreal lakes that, incidentally, had the highest Chl *a* concentrations; a notion deserving further attention in light of most rapid increases in warming taking place at high latitude regions. This, coupled with the fact that, for mildly to hyper-eutrophic lakes, light climate and water column stratification are the most important drivers determining phytoplankton biomass, may serve to better plan and implement lake management and mitigation strategies.

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Conflict of Interest

None declared.

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Supplementary Figures

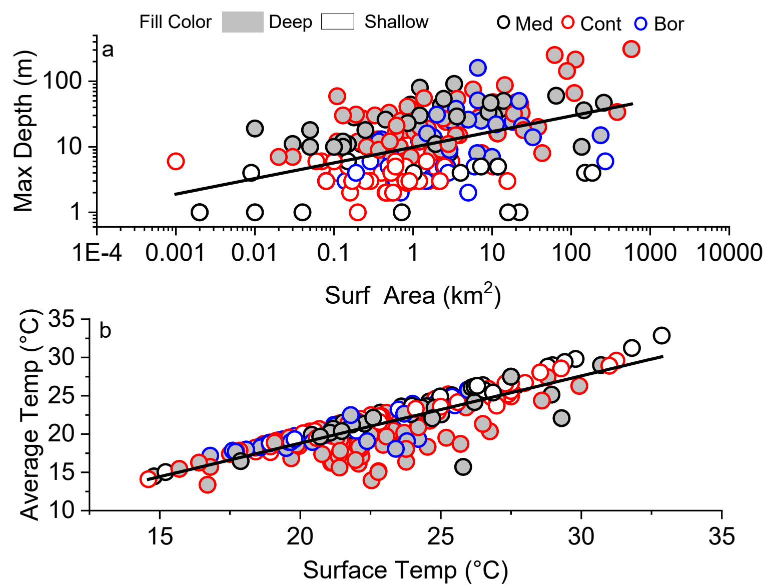

Figure S1. (a) Maximum depth and surface area of EMLS lakes showed high collinearity (linear fit, Pearsons' r =0.46, R^2=0.21), such as (b) average and surface temperature (linear fit, Pearsons' r =0.84, R^2= 0.72)

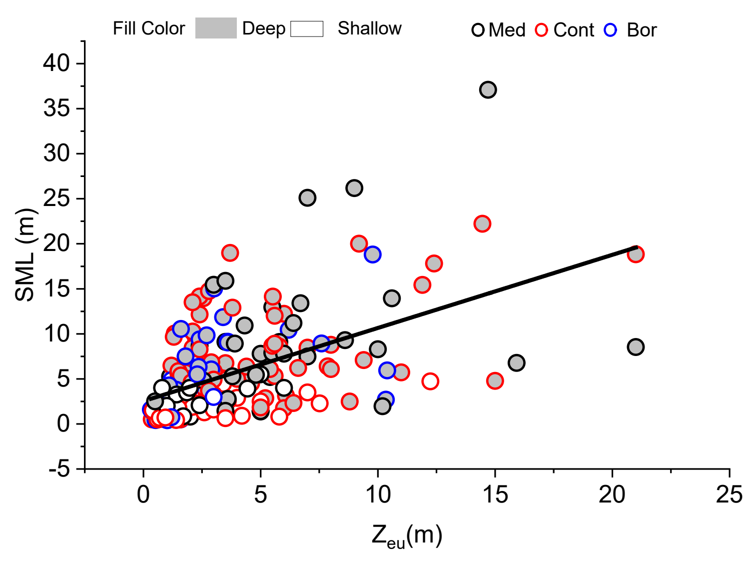

Figure S2. Significant linear relationship between EMLS lake's euphotic depth (Z_{eu}) and mixed layer depth (SML), R^2 =0.3.

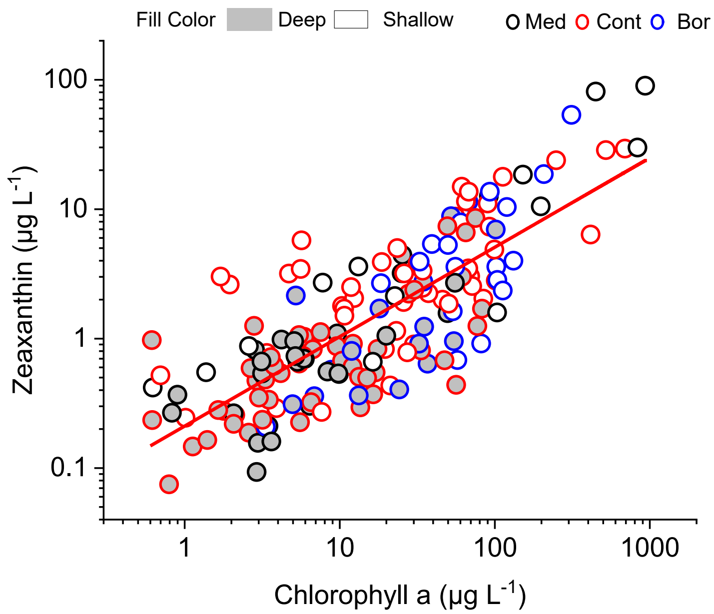

Figure S3. Scatter plot of Chl-*a* and Zeaxanthin, showing a strong linear relationship (R^2=0.6).

Supplementary Tables

Table S1. List of EMLS lakes and corresponding climatic zones

Lake Name	Latitude	Longitude	ClimZone
Brenet	46.67375	6.322252778	Continental
Bret	46.51416111	6.773583333	Continental
Divonne	46.35461667	6.152488889	Continental
Geneva_GE3	46.29200278	6.213036111	Continental
Greifen	47.33993333	8.678416667	Continental
Hallwil	47.28744722	8.2115	Continental
Joux	46.63895278	6.286463889	Continental
Lucerne	47.02083333	8.355516667	Continental
Morat	46.92760833	7.074027778	Continental
Ober	47.20716389	8.821936111	Continental
Pfaffikon	47.35079167	8.782402778	Continental
Sempacher	47.14369444	8.150269444	Continental
Geneva_SHL2	46.44721944	6.583597222	Continental
Sopen	47.0899	8.080633333	Continental
Zurich	47.32166667	8.565	Continental
Akaki_Malounda	35.03	33.17	Mediterranean
Asprokremmos	34.73955556	32.55866667	Mediterranean
Kannaviou	34.92888889	32.58938889	Mediterranean
Kouris Reservoir	34.74636111	32.92194444	Mediterranean
Boskovice	49.496334	16.696983	Continental
Brnenska prehrada	49.237133	16.511232	Continental
Hamry	49.737267	15.914233	Continental
Luhacovice	49.121517	17.774866	Boreal
Matejovsky	49.535168	15.882933	Continental
Nesyt	48.77795	16.742849	Continental
Nove Mlyny II	48.893284	16.643499	Continental
Novovesky	48.934191	16.529216	Continental
Velke Dako	49.632233	15.90435	Continental
Bikowsee	53.14811	12.8870556	Continental
Carwitzersee	53.315693	13.452971	Continental
Grosser Dabelowsee	53.254358	13.204838	Continental
Dollgenesee	52.60658	14.1712222	Continental
Kleiner Wentowsee	53.07803	13.1714444	Continental
LangerSee	52.2445	13.78544444	Continental
Muggelsee	52.441171	13.657738	Continental
Oberuckersee	53.174916	13.856108	Continental

Peetschsee	53.237915	12.861389	Continental
Pfsarrsee	53.28372	13.1958611	Continental
Plauersee	53.487471	12.349336	Continental
Roofensee	53.11067	13.0350222	Continental
Scharmutzelsee	52.21683	14.0235	Continental
Schwedtsee	53.18828	13.1496111	Continental
Tiefwarensee	53.527362	12.691713	Continental
Wittwesee	53.12653	12.9364167	Continental
Wurlsee	53.22131	13.2936111	Continental
Bagsvaerd	55.77266667	12.45663889	Continental
Bastrup	55.81802778	12.2695	Continental
Farum	55.80522222	12.36719444	Continental
Fure	55.81305556	12.44313889	Continental
Haraldsted	55.48463889	11.76422222	Continental
Lyngby	55.77472222	12.47911111	Continental
Elistvere	58.57116667	26.71133333	Boreal
Lammijarv	58.12	27.575	Boreal
Raigastvere	58.588889	26.655	Boreal
Saadjarv	58.53608333	26.64883333	Boreal
Vortsjarv	58.21191667	26.10275	Boreal
Abegondo-Cecebre	43.28138889	-8.295	Mediterranean
Albufera de Valencia	39.34433333	-0.338916667	Mediterranean
Embalse de Arcos de la Frontera	36.75222222	-5.793888889	Mediterranean
Azutan	39.77533611	-5.088780556	Mediterranean
Baxe reservoir	42.60694444	-8.616111111	Mediterranean
Boadella	42.348167	2.821567	Continental
Basturs petit	42.14402778	1.016833333	Continental
Basturs	42.14327778	1.020361111	Continental
Laguna Canas	36.67293333	-4.455225	Mediterranean
Cedillo	39.66731111	-7.532625	Mediterranean
Embalse del Cubillas	37.2787	-3.675683333	Mediterranean
Doninos	43.491886	-8.312181	Mediterranean
Laguna Eucaliptal	36.671994	-4.452602	Mediterranean
El Foix	41.255951	1.6490111	Continental
Laguna Grande	36.67131	-4.45601	Mediterranean
Laguna Grande de Villafranca	39.44975	-3.338555556	Mediterranean
Laguna Honda	36.75555	-2.9464	Mediterranean
Mequinenza Reservoir	41.37067	0.2675556	Mediterranean
Montcortes	42.331207	0.994539	Mediterranean
Ribarroja Reservoir	41.24938889	0.429194444	Mediterranean

Laguna Rio Viejo	36.67361944	-4.455016667	Mediterranean
Sobron Resrbvoir	42.76691944	-3.102138889	Continental
Embalse de Tous	39.13372222	-0.647388889	Mediterranean
Vallfornes	41.72095	2.341644444	Continental
Laguna de las Yeguas	37.05641667	-3.379533333	Mediterranean
Harkmerifjarden	62.18833333	21.43888889	Boreal
Jyvasjarvi	62.23918889	25.77259167	Boreal
Kakskerranjarvi	60.355	22.20833333	Boreal
Littoistenjarvi	60.45138889	22.39527778	Boreal
Muuratjarvi	62.12474444	25.56038889	Boreal
Stortrasket	62.31777778	21.38027778	Boreal
Marathonas	38.17156667	23.90511111	Mediterranean
Bajer	45.30633333	14.71333333	Continental
Njivice	45.16963889	14.56338889	Continental
Peto Jezero	45.83161111	16.02616667	Continental
Ponikve	45.07794444	14.55833333	Continental
Savica	45.769	16.03244444	Continental
Vransko	44.84002778	14.39241667	Continental
Patkai Reservoir	47.267605	18.48562	Continental
Zamolyi	47.30188889	18.46486111	Continental
Templehouse	54.09972222	-8.589444444	Continental
Iseo	45.68722222	10.06722222	Continental
Gaustvinis	55.65575	23.20038889	Boreal
Gineitiskes	54.73747222	25.18572222	Boreal
Gulbinas	54.79386111	25.30755556	Boreal
Jieznas	54.59152778	24.18508333	Boreal
Lukstas	55.71286111	22.335	Boreal
Mastis	55.97755556	22.24886111	Boreal
Niedulis	54.39172222	24.36605556	Boreal
Pabezninkai	54.34941667	24.571	Boreal
Rekyva	55.86075	23.31230556	Boreal
Simnas	54.39433333	23.643	Boreal
Sitvys	54.98566667	25.21661111	Boreal
Udrija	54.43508333	23.85044444	Boreal
Dojran	41.19861111	22.73333333	Continental
Prespa	40.9525	20.91888889	Mediterranean
Amstelveense	52.30002778	4.840333333	Continental
Empelse put	51.72298	5.337262	Continental
Ertveldplas	51.70669444	5.288194444	Continental
Henschotermeer	52.080702	5.373029	Continental
Kinselmeer	52.38566667	5.017111111	Continental

Meerwijkplas	52.34833333	4.664083333	Continental
Molenplas	52.344	4.655111111	Continental
Sloterplas	52.36366667	4.817222222	Continental
Westbroekplas	52.42558333	4.669416667	Continental
Akersvannet	59.24499167	10.32690083	Continental
Arungen	59.68997417	10.74331722	Boreal
Hillestadvannet	59.5143325	10.15597611	Continental
Mjaer	59.69832931	11.05206361	Boreal
Ostensjovannet	59.68844944	10.82986111	Boreal
Saebyvannet	59.42701722	10.98318778	Continental
Skulerudsjoen	59.66792389	11.54612917	Boreal
Vansjo-Storefjorden	59.39440806	10.83571972	Continental
Tunevannet	59.29807833	11.08458833	Boreal
Vansjo-Vanemfjorden	59.44232083	10.75360167	Continental
Bartezek	53.83109167	19.84415	Continental
Biale Sosnowickie	51.53272222	23.0425	Boreal
Bninskie	52.20016667	17.11708333	Continental
Bukowieckie Dute	52.400966	15.620132	Continental
Bukowieckie Mate	52.381964	15.603918	Continental
Czarne	53.78222222	20.45444444	Continental
Czerniakowskie	52.19305556	21.07138889	Continental
Debno	52.29361111	16.69880556	Continental
Dziekanowskie	52.36722222	20.84694444	Continental
Goreckie	52.26575	16.79672222	Continental
Ilinskie	53.79708611	19.84148611	Continental
Kielpinskie	52.36166667	20.87583333	Continental
Lednica	52.52302778	17.37905556	Continental
Leknenskie	52.842467	17.292447	Continental
Lodzko Dymaczewskie	52.24905556	16.75191667	Continental
Lubosinskie	52.52777778	16.3825	Continental
Lusowskie	52.42986667	16.65826667	Continental
Majcz	53.77388889	21.46	Boreal
Maltanski Reservoir	52.401944	16.970556	Continental
Mikolajskie	53.78194444	21.59222222	Boreal
Nidzkie	53.57555556	21.54888889	Boreal
Niepruszewskie	52.38363333	16.61503333	Continental
Pniewskie	52.51138889	16.24083333	Continental
Podkamycze_1	50.08638889	19.83377778	Continental
Podkamycze_2	50.08322222	19.83483333	Continental
Probarskie	53.82322222	21.37822222	Boreal
Rogozinskie	52.750756	17.007086	Continental

Ros	53.67	21.91861111	Continental
Rusalka	52.42469444	16.88380556	Continental
Rynskie	53.90972222	21.48916667	Boreal
Skanda	53.75666667	20.53055556	Continental
Swarzndzkie	52.413611	17.065	Continental
Syczynskie	51.28755556	23.23813889	Boreal
Tomaszne	51.46658333	23.00238889	Boreal
Track	53.78916667	20.54	Continental
Tyniec_1	50.02986111	19.82761111	Continental
Tyniec_2	50.02447222	19.81325	Continental
Ukiel_2	53.78638889	20.42916667	Continental
Uzarzewskie	52.448056	17.133333	Continental
Zemborzycki	51.18844444	22.52969444	Boreal
Azibo	41.57561111	-6.897638889	Mediterranean
Azul	37.87288333	-25.76991667	Mediterranean
Furnas	37.758206	-25.332376	Mediterranean
Gostei	41.78597222	-6.820972222	Mediterranean
Miranda	41.49486111	-6.26925	Mediterranean
Peneireiro	41.29397222	-7.175222222	Mediterranean
Peixao	40.33604	-7.5926361	Mediterranean
Serra Serrada	41.96166667	-6.772	Mediterranean
Lagoa Verde	37.8428	-25.78891667	Mediterranean
Malaren Ekoln	59.73298	17.547852	Boreal
Erken	59.84007	18.62512	Boreal
Limmaren	59.73056667	18.73476667	Boreal
Eastern Ringsjon	55.86308333	13.54197222	Continental
Sepond_1	55.77933333	13.3615	Continental
Sepond_2	55.73913889	13.20922222	Continental
Vallentunasjon	59.50832	18.0448	Boreal
Valloxen	59.73936111	17.84322222	Boreal
Vombsjon	55.68172222	13.59277778	Continental
Gajsevsko	46.53325278	16.12036667	Continental
Ledavsko	46.74987778	16.040175	Continental
Pernica	46.58318611	15.73239444	Continental
Kuchajda	48.17016667	17.14206667	Continental
Malinec	48.52186667	19.66493333	Continental
Vinne	48.81695	21.98628333	Boreal
Zemplinska	48.79675	22.02086667	Boreal
Acarlar	41.1199	30.490133	Continental
Akyatan	36.58658333	35.31869444	Mediterranean
Aladag	40.60611111	31.67638889	Continental

Catalan	37.21497222	35.31344444	Mediterranean
Cubuk	40.48027778	30.83444444	Mediterranean
Caygoren	39.252586	28.232628	Mediterranean
Eber	38.6283	31.11801667	Mediterranean
Eymir	39.82583333	32.8325	Mediterranean
Golcuk	40.65416667	31.62527778	Continental
Golhisar	37.12083333	29.59811667	Mediterranean
Gumerdigin	40.45808333	33.26580556	Mediterranean
Ikizcetepeler	39.473607	27.929277	Mediterranean
Kucuk Akgol	40.87833333	30.43194444	Continental
Karaoren	40.50208333	33.23177778	Mediterranean
Karacaoren_1	37.40211667	30.84833333	Mediterranean
Karacaoren_2	37.30864722	30.81348056	Mediterranean
Karadere	40.50197222	33.48494444	Mediterranean
Karatas	37.38566667	29.96963333	Mediterranean
Kuzgun	40.196153	41.050553	Boreal
Mogan	39.77888889	32.79611111	Mediterranean
Mollakoy	40.688141	30.389838	Continental
Palandoken	39.67133333	41.02433333	Boreal
Poyrazlar	40.83833333	30.46888889	Continental
Saraykoy	40.52980556	33.46688889	Mediterranean
Sabanozu	40.52286111	33.26955556	Mediterranean
Seydi	40.57358333	33.46125	Mediterranean
Sugla	37.32455556	31.99213889	Mediterranean
Taskisigi	40.87055556	30.40111111	Continental
Tortum	40.64923333	41.64139722	Boreal
Uluabat	40.19072222	28.54881667	Mediterranean
Yenicaga	40.77861111	32.02527778	Continental
Erne	54.48544444	-7.843722222	Continental
Grasmere	54.44869167	-3.019344444	Continental
Neagh	54.58241667	-6.396222222	Continental

Table S2. A) One-way-ANOVA or post-hoc pairwise comparisons for all lake log-transformed variables among climatic zones (Mediterranean, Continental, Boreal) and depth types (Deep and Shallow). Non-significant differences at 0.05 level are marked in red. Variables with * are included in the linear models. b) Climatic zones Tukey multiple comparisons, where difference between variable's mean refers to the natural logarithm.

(a)

Variable		ANOVA - climatic zones	ANOVA - depth types
Maximum depth	maxD	$F_{2,210}$= 0.74; p=0.474	
Surface area*	SurfA	Chi^2= 4.85 ; p=0.088	$F_{1,228}$= 17.725; p=3.68E-5
Total nitrogen*	TN	$F_{2,227}$= 1.91; p=0.150	$F_{1,228}$= 13.3; p=3.20E-4
Total phosphorus*	TP	$F_{2,227}$= 1.98; p=0.140	$F_{1,228}$= 1.32; p=0.250
Surface Temperature *	SurfT	$F_{2,226}$= 13.39; p=3.17E-6	Chi^2= 3.88; p=0.048
Average Temperature	AvT	$F_{2,227}$= 14.73; p= 9.6E-7	$F_{1,228}$= 31.09; p=6.92E-8
Secchi Depth	SD	$F_{2,227}$= 6.64;p=0.001	$F_{1,228}$= 89; p=4.74E-18
Light climate*	Z_{eu}/Z_{mix}	$F_{2,227}$= 2.93; p=0.054	$F_{1,228}$= 8.53; p=0.003
Maximum buoyancy frequency*	N^2	Chi^2= 5.18; p=0.075	Chi^2= 16.5; p<0.0001
Chlorophyll a *	Chl-a	$F_{2,227}$= 7.00 ; p=0.001	$F_{1,228}$= 43.65; p=2.73E-10
Zeaxanthin	Zea	$F_{2,169}$= 2.20; p=0.113	Chi^2= 51.2; p<0.0001

(b)

Variable	climatic zones					
	Cont-Bor		Med-Bor		Med-Cont	
	Diff (ln)	p	Diff (ln)	p	Diff (ln)	p
maxD	-	-	-	-	-	-
SurfA	-	-	-	-	-	-
TN	-	-	-	-	-	-
TP	-	-	-	-	-	-
SurfT	0.064	0.023	0.146	<0.0001	0.081	0.002
AvT	0.011	0.906	0.138	<0.0001	0.127	<0.0001
SD	0.357	0.04	0.625	0.0009	0.267	0.139
Z_{eu}/Z_{mix}	-	-	-	-	-	-
N^2	-	-	-	-	-	-
Chl-a	-0.797	0.02	-1.302	0.0007	-0.505	0.185
Zea	-	-	-	-	-	-

Table S3 *Model 1 and 2 predictors for Chl-a and their contribution (%) and significance (p) explaining the overall R^2. Non-significant predictors at the 0.05 level are marked red.*

Model 1		
Predictor	**% of model R^2**	**p**
"Climatic Zone"	26	<0.0001
Stratification strength - N^2	24	<0.0001
Total Nitrogen – TN	13	<0.0001
Surface area – SurfA	12	<0.001
Light climate - Z_{eu}/Z_{mix}	10	0.002
Surface temperature - SurfT	9	0.06
Interaction N^2*Z_{eu}/Z_{mix}	4	0.02
Model 2		
Predictor	**% of model R^2**	**p**
"Depth Type"	37	<0.0001
Stratification strength - N^2	18	<0.0001
Light climate – Z_{eu}/Z_{mix}	15	0.004
Total Nitrogen – TN	10	<0.0001
Surface area – SurfA	8	<0.001
Interaction N^2*Z_{eu}/Z_{mix}	6	0.01
Surface temperature - SurfT	3	0.06

Table S4. *Model 3 and 4 predictors for Chl-a and their contribution (%) and significance (p) explaining the overall R^2. Non-significant predictors at the 0.05 level are marked red.*

Model 3 - Shallow lakes		
Predictor	**% of model R^2**	**p**
Light Climate - Z_{eu}/Z_{mix}	46	0.0002
Total Nitrogen – TN	33	0.0001
Stratification strength - N^2	14	0.02
Interaction $N^2* Z_{eu}/Z_{mix}$	3	0.29
Surface area – SurfA	2	0.83
Surface Temperature – SurfT	2	0.71

Model 4 - Deep lakes		
Predictor	% of model R^2	p
Light Climate - Z_{eu}/Z_{mix}	32	0.04
Interaction $N^2 * Z_{eu}/Z_{mix}$	29	0.03
Surface area -SurfA	22	0.02
Stratification strength - N^2	14	0.13
Surface Temperature – SurfT	3	0.43

Table S5. Model 5,6 and 7 predictors for Chl-a and their contribution (%) and significance (p) explaining the overall R^2 . Non-significant predictors at the 0.05 level are marked red.

Model 5 - Mediterranean lakes		
Predictor	% of model R^2	P
Stratification strength - N^2	46	<0.0001
Surface Temperature – SurfT	24	0.05
Surface area – SurfA	16	0.03
Light Climate - Z_{eu}/Z_{mix}	5	0.11
Interaction $N^2 * Z_{eu}/Z_{mix}$	3	0.15
Model 6 - Continental lakes		
Predictor	% of model R^2	P
Total Nitrogen -TN	35	0.004
Stratification strength - N^2	29	0.001
Surface area – SurfA	17	0.0002
Light Climate - Z_{eu}/Z_{mix}	10	0.28
Surface Temperature – SurfT	6	0.06
Interaction $N^2 * Z_{eu}/Z_{mix}$	3	0.28
Model 7 - Boreal lakes		
Predictor	% of model R^2	p
Interaction $N^2 * Z_{eu}/Z_{mix}$	34	0.002
Surface Temperature – SurfT	21	0.004
Total Nitrogen – TN	14	0.01

Light Climate - Z_{eu}/Z_{mix}	18	0.12
Surface area – SurfA	9	0.18
Stratification strength - N^2	4	0.94

Supplemental note

Multiple linear regression model

At first, we have looked at all continuous variables, without nominal ones (i.e. climatic zone and depth type), and found that maximum N^2 was the most significant predictor (note that TP is excluded because it is not significant).

Table S6. *Model (without nominal variables) significant predictors for Chl-a and their contribution (%) and significance (p) explaining the overall R^2.*

Chl-a ~ N^2 + TN + ZeuZmix + SurfT + SurfA + N^2*ZeuZmix + TN*SurfT + SurfA *ZeuZmix (R^2 23%; p<0.001)		
Predictor	**% of R^2**	**p**
Stratification strength - N^2	33	<0.0001
Total Nitrogen – TN	19	0.0004
Light Climate - Z_{eu}/Z_{mix}	17	0.0002
Surface area – SurfA	16	0.01
N^2* Z_{eu}/Z_{mix}	7	0.02

However, we had to exclude some variables due to collinearity (e.g. Air Temp collinear with Surf Temp and Max Depth with Surf Area), thus we decided to use continuous variables (environmental predictors) and nominal variables (categories). This allowed us to keep both surface temperature and climatic zone – otherwise we had to exclude either SurfT or AirT. Similarly, by using the nominal variable “depth type” we could keep Surface Area otherwise collinear with Max Depth.

We decided to not include the first approach (as in Table S6) in the main text because the resulting message does not change: stratification strength is the most important environmental predictor for the overall group (all lakes).

COMMENTS (and answers) TO THE AUTHORS

It was not clear to me why categories were used rather than continuous data. Given that there is actual climate data available for these locations, is the climatic grouping necessary or would a continuous approach provide more information, using annual air temperature and precipitation for example? This might be more meaningful in terms of thinking about lakes across a gradient, especially given that climate change may have moved lakes out of their historical classification of Mediterranean, Continental, Boreal?
We have added the Supplemental note to clarify on the variables choice (cathegories vs continuous).
We believe we acknowledge the second point (climate change vs classification) as an important issue. To consider climate change the Koppen-Geiger classification used here was based on 25-year averages of temperature and precipitation data from state-of the art regional climate model predictions for the years 2011–2100. We have also looked at recent literature on shifted climatic zones (Maberly , 2020) but given that we combined regions that were of the same main climate and precipitation level (Mediterranean = Csa and Csb, Continental= Cfa and Cfb and Boreal = Dfb and Dfc), the difference with new projections is negligible.

- Global lake thermal regions shift under climate change Maberly, S. 2020 DOI: http://dx.doi.org/10.1038/s41467-020-15108-z
- C = warm temperate, D = alpine, f = fully humid, s = summer dry, a = hot summers, b = warm summers

It seems like the statistical analyses could perhaps be more sophisticated and synthetic if continuous variables were used.
We are convinced that different statistical approaches can be applied here, which all have their merits. We went through several statistical approaches before concluding that, for this sampling design (snapshot), the simplest statistics are preferable (e.g. a multilinear model). We could have probably used a more sophisticated approach, but when we tried we found that the core message was the same without substantial gain (and then Occam`s razor should apply). We thus firmly believe that the statistical approach we finally chose is not only easier to digest but the results are robust, and the take home message more straight forward for the reader.

I was also very surprised to see that TP did not appear to play a stronger role, especially given recent synthesis papers on chlorophyll (Quinlan 2020, Shuvo 2021).
Is this just because the lakes are mostly eutrophic with high TP concentrations?
Yes, indeed. It has been shown in other publications that, although log-linear models nicely predict Chl-a from TP in some lakes or regions, they tended to over-predict Chl-a at high TP across large TP gradients, using global datasets. Chl-a–TP relationships are in fact better described as sigmoidal functions (Filstrup et al., 2014).
For the present study both Chl-a and TP concentrations were about 50% greater than for the two studies show in Table 2, which are studies carried out on global lake datasets.

Table 2. Chl- a and TP mean concentrations for the aforementioned studies and EMLS

	Chl-a (ug/L)	TP (ug/L)
Quinlan 2020	19	62
Shuvo 2021	16	55
This study- EMLS	**44**	**110**

When looking at nutrient rich lakes, Filstrup, et al., 2014 suggest that, “at increasing TP another resource, such as nitrogen (N) or light, becomes limiting for phytoplankton under high TP, especially over shorter temporal scales.” “Similar to our findings, Jones et al. (2008b) found that significant TN terms resulted in a slight improvement in the amount of variance explained by Chl-a predictive models compared to TP”.

We include this observation in the abstract, in the conclusions and in the discussion where we emphasize that for the EMLS study there are no data on nutrient availability at other times of the year, and we therefore limit the discussion on nutrients to the summer, the period with maximum stratification. These summer snapshot data are however still very useful as further investigation of Chl-a, TN, and TP relationships from hypereutrophic waters is required given a risk of future increased eutrophication linked to climate change.

Filstrup, C. T., et al. (2014). "Regional variability among nonlinear chlorophyll-phosphorus relationships in lakes." Limnology and Oceanography **59**(5): 1691-1703.

Shuvo, A., et al. (2021). "Total phosphorus and climate are equally important predictors of water quality in lakes." Aquatic Sciences **83**(1).

Quinlan, R., et al. (2020). "Relationships of total phosphorus and chlorophyll in lakes worldwide." Limnology and Oceanography **66**(2): 392-404.

At the very least this is worth substantial discussion, it may be that the study set here is really only representative of lakes where nutrients are not limiting, and if so, the paper should be written in that context.

We add to discussion in line 593-598:

“Total Nitrogen is the second most important predictor for Chl-a in shallow lakes (33%), even though EMLS lakes are mostly eutrophic. This outcome, together with the general absence of TP as significant predictor for Chl-a variance, is in line with previous studies on nutrient-rich lakes suggesting that 1) a positive linear TP–Chl-a relationship exists only at intermediate concentrations of TP (0.004–0.23 mg L^{-1},(Quinlan et al. 2020) and 2) nitrogen becomes limiting for phytoplankton under high TP, especially over shorter temporal scales (Filstrup et al. 2014).”

It would be helpful to consistently be specific in the text (e.g. line 509 refers to ‘nutrients’ as the most significant predictors of Chl a – is this TN, TP, or both?)

We checked carefully and specified Total Nitrogen when needed. We also highlighted that our results pertain to a nutrient rich data set in the abstract.

As a side note, the reason for this is that individual countries contributed to the EMLS from lakes that they routinely sample, which often tend to have a history of eutrophication. Nevertheless, even the lakes that were sampled for the first time were also found to fall within the hyper-eutrophic limits (TP > 30 µg/L). The bias towards productive lakes is a potential disadvantage of the grassroots nature of the EMLS, compared to a centrally organized survey like the NLA, where a Generalized Random Tessellation Stratified Survey Design was used. However, scarcity of non-eutrophic lakes in a multi-lake survey also reflects the reality of the European continent, where eutrophic lakes still are more common (EEA, 2018), despite decades of lake restoration efforts.

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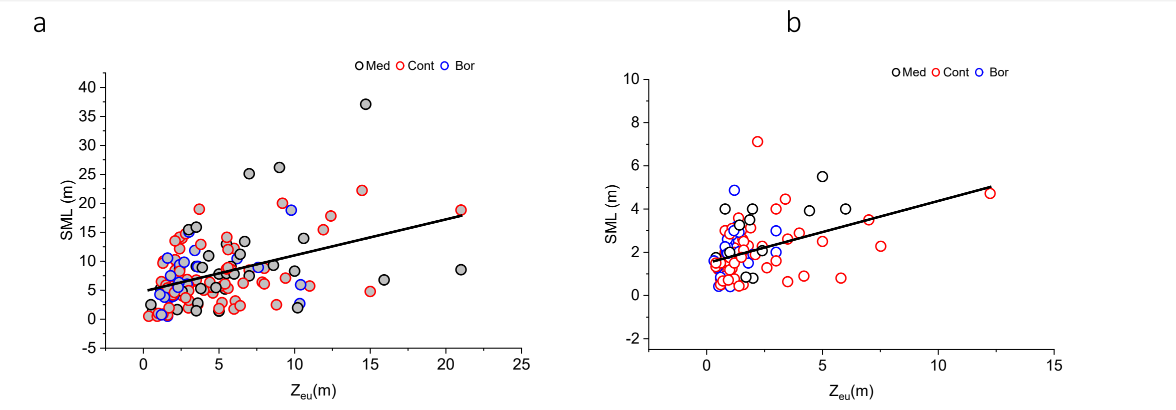
I was specifically asked by the editor to provide feedback on the stratification aspect of this manuscript. Overall, I think this is of sufficient standard. Notably, the metric used to characterise stratification strength is widely used in the limnological community. There are also many other metrics that one could have used, such as the vertical density gradient and so on… but N2 is equally suitable. One additional lake characteristic which would strengthen this aspect of the work, would be the lake mixing regime. Specifically, as the data only provides a snapshot of the seasonal cycle, it is unclear if the strength of stratification provides a similar mechanistic description across the lakes. For example, what if some of the lakes are discontinuous polymictic that only stratify intermittently during the warmest part of the year (which is studied here)? Notably, if the lake was mixed up until that point, would stratification strength still be expected to have a dominant influence, or is this simply considered as 'noise' in the study?

R1 rightfully points out the greatest tradeoff in this kind of approach, that is space for time.

Using a variable to include lake mixing regimes would have been very interesting indeed, but quite hard to obtain for the EMLS. Many of these lakes were visited for the first time and most of them have not been studied for lake physics, water column structure and mixing regime. Even if data were partially available, for a subset of lakes, it may have been a variable that we would have excluded from the statistical analysis because it would have considerably shrunken the sample size.
We could however look at mixing regimes in a "gedankenexperiment" and try to seek an explanation for a specific "outlier". It is possible – especially for shallow lakes - that a mixing event disrupts the stratification that only few days before sampling boosted phytoplankton's growth (by keeping algae in the optimum light regime). The biomass would still be there (and would have been sampled on the "snapshot" day) but the N^2 from a few days before, would no longer be present. Thus, the relationship between biomass and N2 that would be recorded would not be representative. So yes, R1 is correct when hypothesizing that this case would be

“considered as ‘noise’ in the study”. All in all this kind of events must have been sufficiently rare to not nullify the value of our space for time approach (see References in Mantzouki et al, 2018, Snapshot surveys for lake monitoring, more than a shot in the dark, Front Ecol and Evol, https://doi.org/10.3389/fevo.2018.00201).

We have also looked at the mixed layer (SML) - euphotic depth (Zeu) relationship (Fig. S2). A significant positive linear relationship between these two variables tells us that the majority of the lakes in our sample did not experience a strong mixing event (Zeu< SML) in the days before the sampling was carried out, and there is no substantial difference between shallow and deep lakes on this regard. (Figures below)



Significant linear relationship between EMLS lake’s euphotic depth (Z_{eu}) and mixed layer depth (SML), R^2 =0.2 for deep (a) and R^2 =0.15 shallow lakes (b) .

As I’m sure the authors have already considered, this study does not take into account the interacting effects of lake physical processes. Notably, one could argue that the timing of stratification onset in seasonally stratifying lakes is both an indirect and even direct driver of phytoplankton biomass, but cannot be investigated here (this also feeds into my comment below).

Absolutely. That is why the above-mentioned relationships are (significant but) not strong. We think that reason is exactly what R1 highlights, i.e. the timing of stratification. We investigate the relationship SML/Zeu, but the effect of light on stratification is revealed by time series. We elucidate this in lines 702-708 of the revised manuscript: “Light induced heat diffusion in the water column and its temporal variability has a stronger effect on the duration of the stratification than on its absolute value. (…) more transparent lakes tend to maintain a seasonal thermal stratification for a longer duration than more turbid ones, therefore being stable for a longer period. Assessing whether this is the case is not possible with a summer snapshot sampling design.”

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My major concern is that there is not enough attention given to the reasons that stratification metrics (Zeu/Zmix, N2) are better predictors in than nutrient availability (as TN or TP, for example) in these mostly eutrophic lakes.

We have revised the introduction to better introduce these concepts and come back to the relevance of Zeu/Zmix and N2 in the discussion.

It is almost intuitive that when nutrients are abundant, resulting in dense phytoplankton biomass, light for photosynthesis becomes a critical resource, that can take over from nutrients like N and P in setting a limit to phytoplankton development. References from the literature that we refer to in our text underline this (Filstrup et al. 2014). Light availability for phytoplankton in a lake is the outcome of the ratio between mixing depth Zmix and euphotic depth Zeu, and it is this ratio Zeu/Zmix that we have used to define light availability in the EMLS lakes. Clearly deep mixing will result in lower light availability for phytoplankton, and this explains why water column stability in deep eutrophic lakes plays such a critical role. Stability limits mixing, reduces Zmix and enhances light availability.

Lines 754-780 section "Cyanobacteria like it hotter": This section has potential but at the end of the discussion is seems to come out of the blue. What were the a prior expectations about Zeaxanthin across these lakes? I would suggest adding more information to the Introduction to introduce some research questions to the reader before digging into all of this literature.

We understand perfectly the suggestion as cyanobacteria are a very interesting and important topic. However, although Zeaxanthin is a pigment found in cyanobacteria, as we explain, it can be found in chlorophytes. Since we do not support our pigment analysis with microscopy results, we cannot be sufficiently sure that Zea=cyano. That is why we do not use Zea as a response variable as for Chl-a, and why we cannot formulate an hypothesis.

We thus add a results section, to inform earlier the reader about the Zeaxanthin – as suggested - and discuss the caution needed to interpret the data.

What does stratification strength say about nutrient availability?
I would love to see regression between the stratification metrics and nutrient variables across the lake depth types and region types to help the reader gain a more mechanistic understanding of how stratification influences both the physical environment and nutrient conditions that ultimately fuel phytoplankton growth.

We understand and agree on the potential of such relationship, although we do not see a significant relationship between N^2 and TN or TP. Possibly the explanation lays within the drawback of a snapshot survey. LightClimate*N2 interaction may better express nutrients availability for Chl-a than nutrient concentration on a single time sample- especially for shallow lakes.