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LETTER

Vulnerable but not equal: Mountain lakes exhibit heterogeneous patterns of phytoplankton responses to climate change

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Scientific Significance Statement

Climate change affects the phytoplankton both locally and worldwide, and its effects are exacerbated in mountain environments due to their faster warming rate and limited biodiversity. However, because of the polyphyletic complexity of phytoplankton, its response to climate change is heterogeneous, and numerous studies argue for taxon-specific responses to warming. Understanding how the community's structure and composition influence the algal response to warming is essential to predicting its impact at the ecosystem level. Our multi-year study of 24 alpine communities reveals three major phytoplankton assemblages with contrasting dominant taxa and biovolumes. The three phytoplankton assemblages are partly determined by the environmental setting and exhibit distinct diversity and biomass responses to increasing temperature, providing insight into potential changes in community structure in the context of climate change.

Abstract

While climate change affects the phytoplankton biodiversity at both local and global scales, predicting phytoplankton community responses to warming is impaired by their polyphyletic complexity. High mountain lakes are highly vulnerable systems, partly due to their limited biodiversity, and forecasting their ecological trajectories is a key challenge for scientists and conservation managers. We evaluated the phytoplankton's sensitivity to temperature in 24 high-altitude lakes over a multi-year (average 7-year) study. We detected assemblagespecific responses to warming, with different trends in biovolume and diversity observed among the diatomdominant, mixed-mixotrophs dominant, and colonial-green dominant assemblages. The environmental

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Data Availability Statement: The data that support the findings of this study are openly available on the website of the Observatory and experimentation on Lakes (OLA) at https://si-ola.inra.fr.

settings partly governed assemblage responses, highlighting the role of the landscape filters in determining the response to warming. The biological stability of lakes, that is, their ability to resist shifts in their phytoplankton assemblage, is therefore determined both by the lake characteristics and warming intensity.

Phytoplankton plays a central role in the functioning of aquatic ecosystems by producing organic matter and transferring it to higher trophic levels. Important common tendencies for phytoplankton species loss emerged in previous studies (Hillebrand et al. 2008), and an acceleration of the homogenization of algal communities with nutrient enrichment and climate warming has been observed in lakes of the North temperate zone (Huo et al. 2022). However, other studies argued for taxon-specific responses to climate change (Thackeray et al. 2008, 2016), reflecting a non-consensual link between climate change and phytoplankton diversity. For example, the growth and biomass of warming-sensitive taxa such as Chlorophytes, pico-algae, and Cyanobacteria are expected to increase significantly compared to other groups (Rasconi et al. 2015, 2017; Salmaso and Tolotti 2021). At the local scale, however, the effect of temperature increase on phytoplankton richness shows mixed trends, with studies finding declines (Hillebrand et al. 2012; Verbeek et al. 2018), increases (Maberly et al. 2022), or no discernible trends (Cermeño et al. 2008; Stomp et al. 2011), mainly due to differences in local species composition and ecosystem properties (Bestion et al. 2021; Gruner et al. 2017).

In highly variable thermal environments, such as highaltitude lakes, phytoplankton biodiversity is expected to bear an even greater influence on ecosystem functioning (Bestion et al. 2021). At the same time, phytoplankton diversity is notoriously limited in high-altitude lakes (Stomp et al. 2011), and the limited functional redundancy may render the phytoplankton community of mountain ecosystems particularly vulnerable to the impacts of climate change (Moser et al. 2019; Schmeller et al. 2022). Besides, the warming rate is typically stronger in high-elevation environments (Mountain Research Initiative Edw Working Group 2015), with atmospheric temperatures rising twice faster in the European Alps than in adjacent lowland sites (Gobiet et al. 2014; O'Reilly et al. 2015; Wang et al. 2014). As a result of both greater exposure to climate warming and greater biological vulnerability, the phytoplankton community of high-altitude lakes is expected to be particularly affected by climate change.

Paleolimnological records (Catalan et al. 2013; Rühland et al. 2008, 2015) and the few available data on recent community changes in high-altitude lakes (González-Olalla et al. 2018; Przytulska et al. 2017) confirmed high sensitivity of phytoplankton assemblages to climate variability, with yet highly idiosyncratic responses (Buchaca and Catalan 2024). For instance, an experimentally induced water warming (+5.3 \pm 0.9°C) promoted the Cyanobacteria in Canadian high-altitude lakes (Przytulska et al. 2017). Yet, in mountain lakes of the Spanish Sierra Nevada, Chlorophytes' biomass increased and mixotrophic phytoflagellates declined after increased phosphorus deposition and atmospheric warming (González-Olalla et al. 2018). While functionally, higher water temperatures shall promote smaller sizes of phytoplankton (Litchman et al. 2010; Machado et al. 2019), the link between temperature and colonial/filamentous forms is still unclear: studies found positive (Duan et al. 2018) or negative (M. Li et al. 2014) effects of warming on the formation of Cyanobacteria colonies, with other concomitant factors (community succession, grazing pressure, nutrient availability, stratification) possibly explaining the observed summer dominance of large colonies in some lakes (Sommer et al. 2012; Žutinić et al. 2014).

The high disparities in phytoplankton response render predictions of the future of biodiversity difficult: heterogeneous phytoplankton response to warming may not be captured by global trends, while species-specific approaches lack potential for generalization. An intermediate level of complexity, through assemblages, coupled with a functional approach, may offer an integrated comprehension of the phytoplankton response to climate change, providing comparable information that can be used for management strategies.

In this study, we used a multi-year dataset (2–11 year-coverage) of 24 high-altitude lakes to better characterize the functional response of phytoplankton to climate warming. In particular, we aimed to identify assemblage-specific sensitivities of phytoplankton to climate change, providing generalizable response trends while accounting for disparities across lakes and time. We expected that temperature increase would significantly impact the phytoplankton communities, but not equally, and specific sensitivities of phytoplankton to warming should be observed according to the composition of the phytoplankton assemblage. In particular, we expected that the most diverse assemblages show a higher resistance (lower response) to climate change and aimed at formulating hypotheses of possible community compositions in a warming world.

Methods

Study sites and data

Data from the 24 lakes located in the French Alps and Pyrenees were obtained through the harmonized protocol from the Network *Lacs sentinelles* (*see* Text panel S1 for details). The lakes cover a wide gradient of altitude (1645–2759 m a.s.l.), catchment size (7.1–1290 ha) and cover (percentage of rock, grass, and glacier), and water depth (6–52 m; major characteristics in Table 1). The 24 lakes have typically low dissolved inorganic nitrogen (DIN) and total phosphorus

indicated t	the numb	er of yea	ars with <u>F</u>	<u>ohytoplan</u>	ikton data,	temperati	ure data, and	both data available.	-		'n	'n	
			Alt.	Lake area	Depth	Cat. area	Drainage	Number of years available for phytoplankton temperature	Cat. rock	Cat. grass	Cat. alacier	Phytoplankton biovolume	T Aug
ID LAKE	Lat.	Long.	(m)	(ha)	(m)	(ha)	ratio	both	(%)	(%)	(%)	(µg L ⁻¹)	(°C)
IZOU	42.69	1.50	1645	33	35	1290	39.09	5 5 2	45	55	0	587 ± 154	16.4 ± 0.7
PORM	45.96	6.79	1945	4.6	6	56	12.17	9 7 5	81.7	18	0	3503 ± 1548	16.9 ± 0.5
ANT	45.99	6.80	2063	11.6	13	278	23.97	11 8 8	58.8	41.2	0	$\textbf{256}\pm\textbf{51}$	13.7 ± 0.8
PETA	44.80	6.17	2095	2.45	20	131	53.47	6 10 6	81.8	15.4	0	1040 ± 456	15.5 ± 0.5
CORNE	45.22	6.08	2098	8.17	27	97	11.87	8 6 5	74.9	24.1	0	$15,437 \pm 6790$	15.9 ± 0.4
MUZ	44.95	6.10	2105	9.68	18	442	45.66	8 7 5	80.4	14	6.2	297 ± 58	11.2 ± 0.5
BREV	45.93	6.83	2159	2.95	20	14	4.75	11 6 6	64.9	33.9	0	$\textbf{7422} \pm \textbf{1101}$	16 ± 0.5
VO	45.76	6.73	2173	7.5	8	333	44.4	9 7 5	76.8	22.3	0	1464 ± 154	10.2 ± 0.9
COS	45.23	6.08	2183	19.8	52	292	14.75	6 6 3	94.7	5.2	0	$19,647 \pm 16,751$	13.9 ± 0.2
AUMAR	42.84	0.15	2192	25	24	308	12.32	5 6 2	28	42	0	461 ± 244	18 ± 0.3
PLAN	44.96	6.04	2250	5.16	8.5	72	13.95	6 9 6	71.8	27.1	0	388 ± 106	14.2 ± 0.5
CORNU	45.96	6.85	2276	5.3	22	7.1	1.34	9 7 5	92.5	7.5	0	2359 ± 924	13 ± 1.0
LAU	44.38	6.87	2284	3.8	7	497	130.79	4 8 3	74.5	24.8	0	80 ± 19	11.9 ± 0.3
BRAM	45.20	6.18	2447	15	39	180	12	2 3 2	75	25	0	$\textbf{279} \pm \textbf{54}$	13.5 ± 1.0
MER	45.36	6.64	2452	4.92	29	63	12.8	4 6 4	72.2	27.7	0	1091 ± 339	13.6 ± 0.5
BRESINF	44.16	7.24	2458	1.16	13	10	8.62	5 7 4	96.9	3.1	0	2232 ± 1039	15.6 ± 0.3
BLANBR	45.19	6.17	2470	10.8	14	300	27.78	2 3 2	75	25	0	131 ± 36	$\textbf{12.0} \pm \textbf{1.0}$
PIS	44.72	6.38	2490	1.68	7	79	47.02	8 10 7	9.9	87.8	0	608 ± 204	12.5 ± 0.6
RABU	44.27	6.98	2500	16.34	50	313	19.16	6 8 4	96	1.1	0	491 ± 285	13.8 ± 0.3
BRESSUP	44.16	7.24	2501	0.95	12	35	36.84	5 7 4	100	0	0	$\textbf{450} \pm \textbf{113}$	14.5 ± 0.2
ARP	45.32	6.78	2666	3.72	17	339	91.13	4 8 4	31.4	0.1	72.8	188 ± 87	10 ± 2.0
MONC	45.32	6.64	2672	2.48	10	80	32.26	3 7 3	98.9	0.4	0	1664 ± 695	$\textbf{11.6}\pm\textbf{0.4}$
BLANCA	45.42	7.13	2754	4.1	9	270	65.85	3 8 3	85.8	1.9	10.1	40 ± 9	10.7 ± 0.7
NOIR	45.42	7.12	2759	1.18	11	49	41.53	4 8 4	95.5	4.5	0	1168 ± 12	$\textbf{12.8}\pm\textbf{0.5}$

Table 1. Characteristics of the studied lakes, organized by elevation. Latitude (Lat.), longitude (Long.), and elevation (Alt.) are provided, as well as the catchment (Cat.) area, and the percentage of rock, grass, and glacier cover estimated on the catchment. Phytoplankton biovolume and T_Aug are averaged by

(TP) concentrations, with a mean and SD of 0.41 \pm 0.03 and 0.011 \pm 0.001 mg L^{-1} , respectively (Table S2).

The monitoring protocol includes continuous highfrequency monitoring of the water column temperature, with data loggers (Tinytag Aquatic2, HOBO U22-001) moored at different depths, measuring temperature at 1-h intervals, and one annual sampling campaign at the end of summer, when the phytoplankton communities can be assumed to be in a similar and stable successional stage (Buchaca and Catalan 2024). The annual sampling campaign consists of discrete measurements from pooled samples within the euphotic zone, at the deepest point of each lake (see Text panel S1 for details). Phytoplankton identification and counts were performed according to the standardized Utermöhl (1958) method (Table S1; Text panel S1). Alpha-diversity (α-diversity) was estimated by the richness index (S) as the number of taxa, the Shannon index (H'), and the Pielou evenness (J), all calculated at the taxonomic (class) and functional (MFG groups > 1%, Salmaso and Padisák 2007) levels.

Phytoplankton diversity links with thermal metrics

The mean surface water temperature in August (T_Aug, from August 01–31) was calculated from the data of the sensor located at 1.5–2 m below the surface. The growing degree-days (GDD), that is, the number of degrees (°C) over the number of days, above a certain temperature threshold below which the process of interest does not progress (Deng et al. 2014; McMaster and Wilhelm 1997), was calculated as (Eq. 1):

$$GDD_n = \sum_{i=1}^n \left(\left[\frac{T_{max_i} + T_{min_i}}{2} \right] - T_{base} \right) \tag{1}$$

where T_{max_i} is the maximum daily temperature of day *i*, T_{min_i} is the minimum daily temperature of day *i*, and T_{base} is the base temperature at which development is thought to occur. T_{base} was set at 4°C, that is, the expected surface temperature during spring mixing (Christianson and Johnson 2020). The GDD was calculated from surface water temperatures over 22 d prior to sampling. The relative thermal resistance (RTR), that is, the potential of stratification of a water body, was computed by the density (computed from water temperature) difference at 1 m above the bottom and 1 m below the surface compared to the density difference between 4 and 5°C (Kalff 2002).

Change in surface water temperature (T_Aug and GDD) over time was tested using mixed-effect linear models (Kuznetsova et al. 2017), including lakes and years as random predictors to control for the unbalanced number of years across lakes (2–11). Similarly, the links between surface water temperature (GDD) and phytoplankton metrics (log-transformed biovolume and Shannon diversity), across the whole community or among assemblages, were tested using mixed-effect linear models, accounting for the unbalanced number of years available among lakes (from 2 to

11 consecutive years of data). Further bootstrapping tests were performed to ensure that data gaps did not alter the robustness of our conclusions (*see* the detailed description of the bootstrap procedure in Fig. S1).

Phytoplankton communities were separated according to their composition into assemblages using hierarchical cluster analysis with Ward's method. Cluster numbers were fixed through the optimization of Dunn's index and silhouette, while having evenly distributed observations in each cluster (Fig. S2). A Similarity Percentages (SIMPER) analysis was performed to evaluate the contribution of the phytoplankton taxa in each assemblage, and pairwise comparisons of diversity and biovolume among assemblages were performed with the emmeans package in R (Lenth et al. 2018). The biological stability of the lakes, thereafter referred to as Turnover, was assessed by calculating a turnover value based on the previously-identified assemblages: Turnover = (Na + T)/Ny, where Na is the total number of different assemblages observed during the studied period, T is the number of times the assemblage changed, and Ny is the number of years available. Turnover is therefore an indicator of the ability of each lake to have a stable phytoplankton assemblage over the years, with lower values reflecting a more stable assemblage.

The effect of the environmental variables on the community composition was assessed using non-parametric multivariate analysis of variance (PERMANOVA) and visualized by Non-metric Multidimensional Scaling (NMDS), both performed from Bray-Curtis matrices calculated from the biovolumes of taxa at the taxonomic and functional level. Environmental variables were fitted to the configuration by vector fitting. The contribution of lake characteristics, climate, and chemical drivers to phytoplankton biovolume and composition was identified by using Generalized Boosted Regression Models with the *gbm* package of R (Ridgeway 2007) and variation partitioning, respectively, after a dimension reduction for explanatory variables using a Principal Component Analysis.

Finally, conditional inference tree (ctree) analysis, conducted with the *party* R package (Hothorn et al. 2015), was used to predict which conditions should promote the different assemblages of phytoplankton, using two independent predictors previously identified as the most influential variables by the Generalized Boosted Regression Models.

Results

Temporal trends of temperature and link with phytoplankton

T_Aug increased significantly over the years (df = 140.63, F = 35.98, *p*-value < 0.001), with an average increase of 0.32°C/yr between 2012 and 2022. However, the warming trend varied significantly between lakes (df = 23, F = 2.5, *p*-value < 0.001, range 0.18–1.8°C per year) (Fig. 1). Consistently, the GDD significantly increased with time (df = 80.7,



Fig. 1. Temporal trends of temperature (T_Aug) over lakes. Different colors for each lake are used to establish quick visual references with other figures.

F = 25.39, *p*-value < 0.001), with a variability across lakes that followed that of T_Aug (df = 23, F = 1.9, *p*-value = 0.02). The phytoplankton biovolume increased significantly with GDD (df = 19.9, F = 5.31, *p*-value = 0.03) while there was no significant relationship between GDD and the Shannon diversity, both at the taxonomic (df = 17.6, F = 0.36, *p*-value = 0.55) and functional (df = 13.3, F = 0.003, *p*-value = 0.9) levels.

Typology of phytoplankton assemblages

The cluster analysis identified three phytoplankton assemblages: GP1 (N = 40) was dominated by Diatoms, especially small centric (MFG 7a) and pennate diatoms (MFG 6b and 7b) (Figs. 2A and S3). GP2 (N = 54) was dominated by flagellated potential mixotrophs (mainly Dinophyceae and Chrysophyceae, MFG 1a, 1b, and 2b), and several colonial Chlorophytes (mainly Chlorophyceae and Trebouxiophyceae, MFG 11b and 11a). Finally, GP3 (N = 49) was dominated by filamentous Conjugatophytes and colonial Chlorophytes (MFG 10b, 11a, and 11b). The three assemblages differed in their total biovolume (df = 2, F = 103.02, p-value < 0.001) and their taxonomic (df = 2, F = 6.19, p-value = 0.002) and functional (df = 2, F = 7.8, p-value < 0.001) diversity (Fig. 2A). GP3 was characterized by the highest biovolume and the lowest diversity, while GP2 was characterized by the lowest biovolume and the highest diversity. GP1 presented intermediate values of biovolume and diversity (Fig. S4).

The functional and taxonomic composition of the phytoplankton was significantly influenced by lake environmental settings (Table S3). The centric and pennate diatoms that dominate in GP1 (6b, 7a, 7b) were highly correlated with high mineral concentrations, such as Si, Mg, and Ca (Figs. 2B and S5), as well as low temperatures, high depths, and altitudes.



Fig. 2. (A) Phytoplankton biovolume of the different functional groups in each assemblage, reflecting the differences in functional richness and evenness. (B) Ordination of samples based on the biovolume of functional groups in the 24 lakes studied in relation to environmental variables. Ellipses of the three phytoplankton assemblages are projected on the ordination plot. The stress value of the non-metric multidimensional scaling was 0.162.

The mixotrophic flagellated Chrysophytes (MFG 1a), abundant in GP2, were related to large drainage ratios and catchment areas. The filamentous Conjugatophytes, dominant in GP3, were correlated with higher water temperatures. The variation partitioning analysis demonstrated that the lake characteristics (longitude, latitude, altitude, catchment area, drainage ratio, depth, lithology, percentage of grass) explained 19% of the functional composition and 13% of the

taxonomic composition, while climate (RTR and GDD) explained 6% and 7%, respectively (Fig. S6).

Evolution of phytoplankton assemblages over the years and lakes

Despite some detected environment-composition associations, phytoplankton assemblages were weakly tied to the lake morphological characteristics, as 15 lakes out of the 24 have had phytoplankton communities that switched assemblage at least once over the study period (Fig. 3) and in four lakes (MONC, JOV, AUMAR, and PLAN) the phytoplankton community shifted from GP1 to GP3 or moved among the three assemblages. For 6 out of the 9 lakes that did not shift, the time series were too short to assert stability. In all cases but MONC, the years when the phytoplankton assemblage shifted toward GP3 matched with maximal values of phytoplankton biovolume (e.g., JOV shifted to GP3 in 2017 and PORM shifted to GP3 in 2014-2015). The three lakes characterized by a low turnover (T < 0.15) (i.e., lakes with stable assemblage for a relatively long period) systematically belonged to GP1 or GP3.

The ctree analysis of thermal predictor (GDD) and lake characteristics (catchment area) showed a dominance of GP1

in lakes characterized by small catchment areas (area ≤ 180 ha) with low GDD (GDD $\leq 187.5^{\circ}$ C) (Fig. 4). The dominance of GP3 was observed in lakes characterized by small catchment areas (area ≤ 131 ha) but high GDD (GDD > 187.5^{\circ}C). The dominance of GP2 was observed in lakes characterized by large catchment areas (area > 131 ha).

The mixed-effect model showed that the effect of GDD on phytoplankton metrics differed among the assemblages. The total biovolume of phytoplankton increased with the GDD in GP2 (df = 36, F = 13.8, p-value < 0.001), and in GP3 (df = 31, F = 7.3, p-value = 0.01), while the effect was not significant in GP1 (df = 28, F = 0.002, p-value = 0.95) (Fig. S7). The taxonomic diversity of phytoplankton increased with the GDD in GP2 (df = 34, F = 4.05, p-value = 0.05), while the effect was not significant in GP1 and GP3. The effect of GDD was not significant on the functional diversity (Fig. S7).

Discussion

The $+0.32^{\circ}$ C/yr warming trend observed herein between 2012 and 2022 for the 24 alpine lakes of the study is one order of magnitude greater than the global average annual trend for lake surface temperature reported by O'Reilly et al.

Fig. 3. Total biovolume of phytoplankton (left axis) and GDD (right axis) over time within each lake. The turnover (T) is indicated for each lake with more than 2 yr of data.

Fig. 4. Result of conditional inference tree analyses performed with thermal predictor (GDD) and lake characteristic (catchment area) on the three phytoplankton groups.

(2015), confirming that high-altitude lakes are particularly exposed to the threat posed by climate change. The warming trend for surface waters was, however, highly variable among the lake local disparities and in response to the complex micrometeorological, topological, and cryogenic features of their catchment, echoing results from Livingstone et al. (2005) on Swiss altitude lakes. As a result, predicting the evolution of phytoplankton assemblages in high-altitude lakes with warming has to contend with two levels of difficulty: (i) the variability in the water warming rate between lakes (exposure); (ii) the polyphyletic complexity of phytoplankton responses to increased temperature (Huertas et al. 2011).

Herein, we could demonstrate a consistent trend of increased phytoplankton biomass with greater GDD, in line with results from Redmond (2018) on lakes and ponds in the Rocky Mountains. Still, the assemblages' responses to a similar GDD increase were governed by the environmental setting. The assemblage shifts observed over the studied period for most of the lakes confirm that the environmental settings only weakly control the phytoplankton assemblage in mountain lakes, as already observed (Buchaca and Catalan 2024), and other factors, such as temperature variations, caused the observed shifts in phytoplankton assemblages. Interestingly, the shifts toward the colonial-green assemblage (GP3) corresponded to higher phytoplankton biovolumes. Moreover, values of community turnovers suggest that the mixedmixotrophs assemblage (GP2) represents an "intermediate state". Consistently, the functional diversity of GP2, that is, taxa tolerant to a wide range of environmental conditions with diverse nutrient acquisition and resource utilization strategies (Ward 2019), may be advantageous in highly dynamic environments.

Among the abiotic predictors that drive the variation in the phytoplankton community, the drainage ratio and the catchment area were of primary importance for both the biovolume and the community composition. The thermal variables, represented by the GDD and the RTR, also explained a large proportion of the variation of the phytoplankton variation. These results are in line with the conceptual model of Blenckner (2005) and more recent conclusions by Jacquemin et al. (2019), highlighting that the landscape filters (i.e., catchment characteristics and lake morphology) determine the response of lakes to climate and environmental changes. Chemical variables linked to nutrient concentrations were the less influential drivers, probably related to the oligotrophic nature of the lakes (Li and Chesson 2016), and that many measurements of nutrient concentrations were below the detection limits, making it impossible to decipher between the potential impact of very fine nutrient variations year by year.

Greater GDD did not influence the three phytoplankton assemblages equally, validating our hypothesis of assemblagespecific response to warming. However, the mixed-mixotroph dominant assemblage (GP2) responded more actively to warming, with both an increase in biovolume and diversity, refuting expectations of a lower response (i.e., higher resistance) in the more diverse assemblages, as well as the traditional view of a loss of diversity in response to warming (Hillebrand 2011; Hillebrand et al. 2012). This result could be explained by an increase of phytoplankton taxa better adapted to warmer temperatures, initially present in the community but in low proportions (like filamentous Conjugatophytes and colonial Chlorophytes), increasing both total biovolume and evenness. Additionally, increased summer temperature and longer ice-free season may enlarge the window of opportunity for phytoplankton taxa and increase the richness at the end of summer (Salmaso et al. 2012). Finally, the mixotrophs, which represent a high proportion of the phytoplankton assemblage, can adapt their nutritional strategy, giving them an adaptive advantage when environmental conditions change (Medina-Sánchez et al. 2022).

Greater GDD increased the phytoplankton biovolume in the colonial-green assemblage (GP3) without affecting diversity. Phytoplankton taxa present in this assemblage were associated with high temperatures and previously found in warmer water (Salmaso and Padisák 2007; Tolotti et al. 2012). We can assume that those of the dominating warm-adapted taxa determine the response of the entire assemblage. Finally, the diatom-dominant assemblage (GP1), which showed the lowest response to temperature increase without any effect on phytoplankton biovolume and diversity, was primarily composed of taxa associated with colder environments and higher altitudes, thus potentially less exposed to warming. In addition, the taxa associated with this assemblage are often found in deeper lakes, thus likely more stratified and more limited by nutrients, which can decrease their temperature sensitivity (Brookes and Carey 2011; Dory et al. 2024). As reported by the results of the ctree analysis, if the diatom-dominant assemblage is less responsive to temperature increase compared to the other assemblages, this one is also the most likely to be lost as lake temperature increases, especially in lakes with small catchments. On the contrary, the mixedmixotroph assemblage is more responsive to temperature but should remain dominant in lakes with large catchment areas even under warming. The colonial-green assemblage presents an intermediate response to warming and should dominate under a warming scenario in lakes with small catchments. Overall, our results demonstrated that a unique conclusion about any potential positive or negative

effect of warming on phytoplankton cannot be established, highlighting how complex the response of phytoplankton is to temperature.

We provide the first analysis of these phytoplankton assemblages data, whose collection was initiated by French National Parks and Nature preserve scientists over a decade ago, intending to know the lakes of these remote areas better and to support their management strategies with scientific findings. Management implications of this study are twofold. First, the results bring a new insight into under-studied ecosystems, allowing to classify them based on their biodiversity, offering a new approach to assess their vulnerability to climate change compared to the traditionally used classifications (i.e., by their characteristics, e.g., presence of glacier, or/and the existing practices, e.g., fish stocking). Second, the findings highlighted assemblage-specific responses to temperature, meaning that knowledge about the phytoplankton dominant taxa and functions (mixotrophy, colonial-green, diatom dominance) may be used by managers as indicators of the potential sensitivity of the community to future warming.

Author Contributions

Flavia Dory: Conceptualization, data curation, formal analysis, investigation, methodology, visualization. writing - original draft. Florent Arthaud: Validation, writing - review & editing. Vincent Augé: Conceptualization, validation, writing - review & editing. Sonia Baillot: Validation, writing - review & editing. Céline Bertrand: Writing review & editing. Carole Birck: Writing - review & editing. Rosalie Bruel: Conceptualization, data curation, formal analysis, writing - review & editing. Laurent Cavalli: Conceptualization, validation, writing - review & editing. Evelyne Franquet: Validation, writing – review & editing. Frédérick Jacob: Validation, writing – review & editing. Clotilde Sagot: Validation, writing - review & editing. Marine Souchier: Writing – review & editing. Raphaelle Napoleoni: Validation, writing - review & editing. Marie-Elodie Perga: Conceptualization, data curation, formal analysis, methodology, visualization, writing - original draft.

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Conflicts of Interest

None declared.

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Supporting Information

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

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