Pigments in surface sediments of South American shallow lakes as an integrative proxy for primary producers and their drivers

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Pigments in surface sediments of South American shallow lakes as an integrative proxy for primary producers and their drivers

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Summary

1. Pigments in lake surface sediments integrate both benthic and pelagic primary producers composition at the whole-lake ecosystem level.

2. We gathered a comprehensive set of water chemistry, morphometric, physical and biological lake variables, catchment and climate characteristics, and geographical descriptors. We used multiple regressions to relate whole-lake algal pigment concentration to environmental conditions and multivariate statistical analyses to assess the factors most associated with photoautotrophic algal and bacterial community composition. The relative influence of environmental factors and spatial distribution was assessed by variance partitioning analysis (VPA) among three groups of variables: (i) in-lake factors, (ii) external factors and (iii) geographic descriptors.

3. Pigment concentration and community composition were most sensitive to in-lake factors. Pigment-inferred abundance of algae, including cyanobacteria, tended to be highest in shallower systems with high nutrient concentrations, independent of the latitudinal temperature or irradiance gradients. Pigment-inferred community composition was best explained by nutrients and biotic composition (zooplankton and fish communities). Only in maritime temperate lakes did a link with regional location occur due to their low DIN to soluble reactive phosphorus (SRP) ratios (1.5 atomic ratio), suggesting nitrogen limitation of phytoplankton growth; accordingly, the sediment pigments revealed cyanobacteria to be the dominant group, although this may also be a consequence of the overall high nutrient levels in these lakes.

4. Despite the large climate gradient covered, in-lake rather than external factors were associated with the patterns observed in pigment concentration (from benthic and pelagic microorganisms) and inferred composition and abundance in these shallow lakes. Our results suggest that pigment assemblages in sediments, which integrate both benthic and pelagic microbial photoautotrophic community and processes, are valuable indicators of changes in shallow (0.75 - 12 m depth) lake ecosystems.

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Introduction

Shallow lakes react rapidly to changes in nutrients and temperature and are therefore considered
sentinels of global change (Moss, 1998). The emerging pattern is that climate change will further
enhance eutrophication symptoms in northern European shallow lakes (Jeppesen, Søndergaard,
& Jensen, 2003; Paerl & Huisman, 2009; Wagner & Adrian, 2009), lakes in the Mediterranean
region (Gyllström et al., 2005; Jeppesen et al., 2009; Romo et al., 2004) and lakes located along
wider climate gradients from the sub-Arctic to the tropics (Kosten et al., 2012). One of the effects
typically described is a temperature- and nutrient-related increase in the proportion of
cyanobacteria to total phytoplankton biomass (Jeppesen et al., 2009; Kosten et al., 2012).
Temperature has a direct effect on the rate of photosynthesis and, under nutrient-saturated
conditions, may lead to an annual primary production in tropical lakes twice as high or even
higher than in their temperate counterparts (Lewis, 1996).

For several decades, limnologists have emphasised that periphyton may contribute substantially
to the whole-lake primary production in shallow lakes (Kairesalo, 1980; Wetzel, 1964), but much
less is known about the effects of the global change on the whole-lake primary production when
integrating pelagic and benthic production. However, these effects may be considerable.

Eutrophication, for instance, not only favours pelagic but often also benthic cyanobacteria,
including highly toxic species (Dasey et al., 2005; Paerl & Paul, 2012). Periphyton dominates
whole-lake primary production in clear-water and shallow arctic and temperate lakes
(Liboriussen & Jeppesen, 2003; Vadeboncoeur et al., 2003) and can also constitute a substantial
proportion in large subtropical shallow lakes, accounting for up to 40% of the chlorophyll-a at
certain times of the year (Steinman, Meeker, Rodusky, Davis, & McIntire, 1997). Under these
conditions, periphyton influences nutrient lake dynamics (Hansson, 1992; Zhang, Li, Jeppesen,
& Li, 2013) and may control sediment-water column fluxes of organic and inorganic nitrogen

compounds that regulate the benthic-pelagic coupling (Tyler, McGlathery, & Anderson, 2003).

It also affects the release of methane from the lake sediment through production of oxygen, facilitating methane oxidation (King, 1990). Periphyton produces a substantial part of the food resources for consumers, including fish (Karlsson & Byström, 2005; Liboriussen & Jeppesen, 2003; Mendonça et al., 2013; Vander Zanden & Vadeboncoeur, 2002). Despite recognition of the existence of major direct and indirect links between the benthic and the pelagic habitats, most published studies focus on the pelagic habitat. Furthermore, studies of benthic communities are often restricted to the summer or dry seasons. Therefore, proxies that integrate the response of both pelagic and benthic habitats to stressors of the lake ecosystem at a larger timescale (seasons to years) are particularly valuable.

Surface sediment pigments derive from planktonic and benthic phototrophic bacteria, cyanobacteria and algae. Although aquatic macrophytes as well as plant material from the surrounding catchment may contribute to the sediment pigments, their share is generally low (Leavitt & Hodgson, 2001). Therefore, when collected from the deepest part of the lake, pigments from lake surface sediments integrate habitats (pelagic and benthic) (Buchaca & Catalan, 2008; Leavitt & Carpenter, 1989; Steinman, Havens, Louda, Winfree, & Baker, 1998) and temporal variation (seasons to years) (Buchaca & Catalan, 2007; Leavitt, Carpenter, & Kitchell, 1989; Leavitt & Findlay, 1994) and represent different groups of in-lake microbial primary producers. Revelation of longer-term trends (decades to centuries or millenia) in controls of algal abundance and composition in shallow lakes has been endeavoured through palaeolimnological studies (Buchaca et al., 2011; McGowan et al., 2005; Waters, Schelske, & Brenner, 2015). Amongst the different pigments present in the sediment, chlorophyll-a (Chl-a) and β -carotene are used as proxies for total algal biomass, including cyanobacteria, while carotenoids are biomarkers of different taxa, including phototrophic bacteria (Liaaen-Jensen, 1964; Rowan, 1989). In addition, a-phorbin composition, including Chl-a and transformation

products of Chl-a such as phaeophytins and phaeophorbides, reveals trophic interactions and diagenetic processes (Buchaca & Catalan, 2007; Leavitt & Hodgson, 2001). The general view that the sediment surface is a deposition area of pigmented signatures from plankton and littoral benthic algae is certainly valid for deep lakes (Leavitt & Hodgson, 2001). However, in highly transparent oligotrophic lakes (Buchaca & Catalan, 2007) or in shallow lakes – where sufficient light reaches the bottom – a phototrophic biofilm may develop, providing an additional pigment signature (Leavitt & Carpenter, 1989). Therefore, the pigment signature of surface sediments strongly depends on the relative contribution of planktonic and benthic algae and phototrophic bacteria to the production (Buchaca & Catalan, 2007, 2008).

How the relative contribution of spatial factors and local conditions affect microorganisms such as phytoplankton and bacteria remains ambiguous (Finlay, 2002; Kruk et al., 2009; Lami et al., 2009; Souffreau et al., 2015). The main drivers of primary production in lakes are nutrients, light, grazing pressure and fish (Reynolds, 1984), while substrate type is also included for benthic communities (Zhang et al., 2013). These drivers are broadly modulated by climatic conditions, residence time and mixing regimes (De Senerpont Domis et al., 2013; Reynolds, 1998). Spatial surveys measuring pigments in surface sediments have described which factors influence the algal composition and production in various ecosystem types. Littoral habitats of shallow and deep temperate mountain lakes were found to be regulated by dissolved organic carbon, inorganic nutrients and exposure to ultraviolet radiation (UV) (McMaster & Schindler, 2005; Vinebrooke & Leavitt, 1999), while surface sediments from the profundal zone of temperate deep mountain lakes (Buchaca & Catalan, 2007), Antarctic shallow lakes (Hodgson et al., 2004) and Arctic and sub-Arctic shallow lakes (Reuss, Leavitt, Hall, Bigler, & Hammarlund, 2010) identified lake depth as one of the main environmental factors explaining algal composition as a result of its influence on the light climate. Indeed, oligotrophic lakes are often dominated by benthic communities that can be nutrient-sufficient and limited by other factors such as light and

the availability and type of habitats (Bonilla, Villeneuve, & Vincent, 2005; Vadeboncoeur et al., 2003). However, to our knowledge, no such spatial surveys have been conducted on broad latitudinal gradients of tropical or subtropical lakes.

The aim of our study was to assess the relative importance of climate (particularly temperature) and local factors in explaining benthic and pelagic primary producers abundance and composition (as major groups, i.e. division/class level). The study was based on analyses of surface sediment sampled from 83 shallow lakes along a 6000 km latitudinal gradient in eastern South America. The relative influence of local factors, climate and spatial distribution on community composition was assessed by organising the conditioning variables in three groups: (i) in-lake biotic and abiotic factors, (ii) external factors, including climatic region and catchment characteristics, and (iii) geographic descriptors. We hypothesised that the proportion of cyanobacteria to the total biomass of microbial in-lake primary producers, including pelagic and benthic sources, would increase with an increase in nutrients and temperature, as described in previous analyses of pelagic primary producers (Jeppesen et al., 2009; Kosten et al., 2012), thereby showing that local factors (e.g. nutrients) are at least as strongly associated with patterns in primary producer assemblages as climatic factors (temperature).

Methods

Lake selection criteria and study area

The 83 lakes studied covered a large climatic gradient in South America (5-55° S; Figure 1). The lakes were selected to resemble each other as closely as possible in morphology and altitude and to vary as much as possible in trophic state within each climate zone (Kosten, Huszar, et al., 2009). All lakes were shallow (maximum depth range: 0.7-12 m), had a surface area between 0.09 and 2.53 km² and were located no higher than 500 m above sea level (Table 1). The lakes were grouped into five different categories based on the prevailing climate characteristics

following the Köppen (1936) climate classification system digitised by Leemans and Cramer (1991): tropical-*i* (19 lakes), tropical (19 lakes), subtropical (18 lakes), maritime temperate (16 lakes) and tundra (11 lakes) (Figure 1). Tropical-*i* is an isothermal subzone in the tropics, which has a smaller annual temperature range than the tropical zone.

Sediment sampling and pigment analyses

The study lakes were sampled once during the summer (subtropical, temperate and tundra regions) or the dry season (tropical zones) (between November 2004 and March 2006). These are the periods exhibiting the highest production, thus having the strongest influence on sediment pigment composition as well (De Senerpont Domis et al., 2013). Sediment cores were taken with a gravity corer. The cores were obtained at the deepest point and extruded in the field; the top 5 cm surface sediments were sub-sampled at 1 cm intervals. We used the top 1 cm for analysis of pigments and organic matter. Samples for pigment analysis were kept dark and cool before freezing (-20° C). The frozen sediment samples were freeze-dried and pigments were extracted and analysed by HPLC following the method described in Buchaca & Catalan (2007).

Pigments of phototrophic bacteria and algae including cyanobacteria

The pigments identified were classified into eight major phylogenetic groups at the following division/class level: brown Chlorobiaceae (isorenieratene), green Chlorobiaceae (chlorobactene), Cyanobacteria (zeaxanthin, aphanizophyll, echinenone, oscillaxanthin, canthaxanthin), Cryptophyta (alloxanthin, α -carotene), Chlorophyta (lutein, zeaxanthin), Bacillariophyceae and Chrysophyceae (fucoxanthin, diatoxanthin, diadinoxanthin), and Dinophyta (diadinoxanthin). In addition, we found astaxanthin, a pigment occasionally occurring in some Chlorophyta genera under N-limited conditions but only present in sediment when accumulated in invertebrate tissues (Leavitt & Hodgson, 2001), and the UV-screen pigment scytonemin, which is present in cyanobacteria (Garcia-Pichel & Castenholz, 1991; Leavitt,

Vinebrooke, Donald, Smol, & Schindler, 1997) and in some dinoflagellates exposed to high irradiances (T. B. pers. obs.). A list of marker carotenoids included in the study and taxonomic affinities can be found in Appendix A3 (Supporting Information).

Diagenetic processes

We also analysed chlorophyll-a (Chl-a) and derivative products of Chl-a including chlorophyllide *a*, two allomers and two epimers of Chl-a, four phaeophorbides and two phaeophytins (Appendix A4, Supporting Information). We defined the sum of Chl-a and Chl-a derivatives as a-phorbin. Phaeophorbide are indicative of grazing processes and phaeophytin of senescence (Leavitt & Hodgson, 2001, Buchaca & Catalan, 2007). Furthermore, we calculated a Chl-a preservation index as the Chl-a:a-phorbin ratio, the index is scaled from 0 (low Chl-a preservation) to 1 (high Chl-a preservation) (Buchaca & Catalan, 2007).

Cyanobacteria and algal pigment concentration

Cyanobacteria and algal abundance in the system (planktonic and benthic) was inferred from the quantification of the ubiquitous β -carotene in the sediment instead of Chl-a because β -carotene preserves better than Chl-a (Leavitt & Hodgson, 2001, Buchaca & Catalan, 2007, 2008). Subsequently, we used β -carotene divided by sediment organic matter (nmol g^{-1} OM) as a proxy for the importance of planktonic and benthic primary producers compared with other carbon sources, derived from autochthonous and allochthonous matter and detritus. By using β -carotene as a proxy we assume that differences in β -carotene due to the contribution of algae with a higher β -carotene content per cell are minor compared with the differences encountered by using the sum of carotenoids in that we have a variable number of marker pigments per algal group.

Environmental descriptors

The environmental descriptors considered were: (i) in-lake factors, including biotic (phytoplankton total abundance, biovolume and richness, macrophyte coverage, zooplankton biomass, fish functional composition and abundance) and abiotic descriptors (water chemistry, lake morphometry and physical characteristics) of the lake system, (ii) external factors, including climatic region classification, lake altitude, categorical catchment composition, lake distance to coast and soil organic carbon density at 1 m depth (kg C m^{-2}), and (iii) geographic descriptors compiled in a matrix of distance-based Moran eigenvector maps (dbMEM) (Dray, Legendre, & Peres-Neto, 2006). It is important to emphasise that some variables like biotic descriptors, physical characteristics and water chemistry are snapshots of the sampling day, while others like lake morphometry and external descriptors are long-term constant variables. The analytical methods of environmental descriptors are detailed in Kosten et al. (2009a), and a list of all descriptors can be found in Appendices A1 and A2 (Supporting Information).

Nitrogen (N) or phosphorus (P) often limits phytoplankton growth in lakes. The absolute concentrations as well as the balance between fractions of N and P affect the phytoplankton growth rate, abundance and community composition (Wetzel, 2001). The trophic state of lakes generally increases with increases in their total N (TN) and total P (TP) concentrations, the balance between TN and TP (the atomic TN:TP ratio) varying along the trophic gradient. The TN:TP ratio has been used in studies to determine whether phytoplankton growth in aquatic systems is mainly N or P limited (Downing & McCauley, 1992; Elser, Andersen, & Baron, 2009; Guildford & Hecky, 2000). TP includes all bioavailable pools of P. However, the most commonly available forms of N are ammonium and nitrate, comprising the bulk of dissolved inorganic nitrogen (DIN) (Wetzel, 2001). In lakes, significant amounts of TN often consist of refractory dissolved organic nitrogen (DON) compounds (Lewis & Wurtsbaugh, 2008; Wetzel, 2001). Therefore, TN might be a poor indicator of bioavailable N, and the use of TN:TP ratios as indicators of N and P limitation for phytoplankton can be questioned (Lewis & Wurtsbaugh,

2008). In this study, we used DIN:SRP ratios as an indicator of N- or P-limited conditions. Following Kosten et al. (2009a), a DIN:SRP (atomic ratio) below 13 indicates that algal growth is potentially N limited, whereas algal growth is potentially P limited at ratios above 50.

Statistical analysis

Differences between the five climatic areas in water temperature, lake depth, TN, TP, DIN, DIN:SRP, planktonic Chl-a and β -carotene in lake surface sediment were tested with a one-way ANOVA (Legendre & Legendre, 1998) following Tukey HSD or Tamhane's T2 *post hoc* test using SPSS version 23 for Mac.

To identify which combination of environmental variables best explained β -carotene concentrations in surface sediment, we used multiple regression and stepwise procedure by including 33 in-lake variables in the initial model (S-PLUS 6.1 for Windows). Variance partitioning analyses (VPA) of β -carotene were carried out using partial linear regression analyses (Legendre & Legendre, 1998) with in-lake variables after log transformation to better approximate normality of residuals.

Marker pigment data, expressed as percentages of total carotenoids in the sample, was analysed using multivariate statistical techniques. We used the relative composition because we assumed that the carotenoids degrade at comparable rates in the lakes studied. Most of the carotenoids included in the study have less than 4 functional groups in their molecule. There is only one carotenoid, fucoxanthin, that has 5 functional groups and in this case we show how the signal of this pigment is higher in subtropical lakes and relate this to *in situ* production (in the surface sediment itself). An exploratory detrended correspondence analysis showed that the gradients of pigment responses were short and, as a consequence, linear models were used in unconstrained

(principal component analysis; PCA) and constrained (redundancy analysis; RDA) ordinations (Ter Braak, 1994). Computations were performed using CANOCO (Ter Braak & Šmilauer, 2002). Constrained and partial canonical ordinations were used to evaluate the relationships between three categories of explanatory variables and changes in the percentage of marker pigments along the latitudinal gradient. A complete list of the variables included can be found in Appendices 1 and 2 (Supporting Information). The three categories were: in-lake, external and spatial factors. The in-lake factors included water chemistry, morphometric and physical lake parameters (28 variables) and biotic factors like phytoplankton, macrophytes, zooplankton and fish (23 variables). The external factors comprised the Köppen climate classification system and catchment characteristics including distance to coast (14 variables). The spatial category included geographic descriptors (33 filters) compiled in a matrix of distance-based Moran eigenvector maps (dbMEM) (Dray et al., 2006). The dbMEM were calculated using the R package Vegan (Oksanen et al., 2010) by determining the minimum spanning Euclidean distances among all sampling sites and truncating distances larger than the largest minimum spanning distance to four times this distance (Daniel Borcard, Legendre, & Gillet, 2011). This truncated matrix was then submitted to a PCo analysis, which yielded 33 positive axes (dbMEM spatial variables). The first half of the axes represents large-scale spatial structure (~among regions) and the latter half small-scale spatial structure (~within regions). As such, one can determine the relative importance of small-scale to large-scale spatial structure in the data during a forward selection procedure in RDA (Daniel Borcard, Legendre, Avois-Jacquet, & Tuomisto, 2004) by estimating which variables contribute most to the overall explained variance. RDA with forward selection was used to reduce redundancy in categories with multiple variables, and the significance of each remaining variable was tested with Monte Carlo permutation (999 permutations, $p < 0.005$). During the forward selection, we used the Blanchet procedure (Blanchet, Legendre, & Borcard, 2008) to reduce the number of variables and avoid any spurious

variance explanations. Variation inflation factors (VIF) <20 indicated no collinearity in the explanatory variables (Ter Braak, 1994).

To partition the variation in pigment composition accounting for the three influencing factors: in-lake (L), including biotic and abiotic, external (E) and spatial (S), variance partitioning analysis (VPA) was carried out using partial RDA (pRDA) (Borcard, Legendre, & Drapeau, 1992; Kernan & Helliwell, 2001) where the relation between response and explanatory variables is analysed after removing the influence of 'covariables'. The relative importance of individual and joint effects was quantified.

Results

Environmental gradient

Lakes were sampled along a latitudinal temperature gradient including five climatic regions: tropical-*i*, tropical, subtropical, maritime temperate and tundra (Figure 1). Lake surface water temperatures within the sampling period ranged from 30 °C in the tropical-*i* zone to 10 °C in the tundra region (Table 1 and Figure 2a).

The maritime temperate lakes surveyed were significantly shallower ($p < 0.05$, Tamhane T2 *post hoc* test; Figure 2b) and had higher water column concentrations of TP and TN (Figure 2c, 2d) than lakes from the other climatic zones, although DIN was slightly higher in the tropical lakes (Figure 2e). Median TP values were above 0.06 mg L⁻¹, corresponding to predominantly eutrophic conditions in most of the sampled lakes (Table 1). The median value of the DIN:SRP atomic ratio was below 13 in the tropical-*i*, subtropical and maritime temperate lakes, indicating a higher potential for N than P limitation of phytoplankton in these lakes (Table 1 and Figure 2f). Despite the fact that lakes were all shallow (median values of maximum depth < 4.6 m; Table 1), light penetration was generally low, with median values of percentage subsurface light

at lake maximum depth, $I(Z_{\max})$, below 0.5% in the sample set for the sampling period (Table 1).

Algal abundance variability

Chl-a in the water column was significantly higher in lakes in the maritime temperate region than in the other climate regions ($p < 0.05$, Tamhane T2 *post hoc* test; Figure 3a). Accordingly, the concentration of β -carotene in the sediment, used as a proxy for the algal abundance of the whole system, including both planktonic and benthic sources, showed the highest values in the maritime temperate lakes and the lowest in the subtropical lakes (Figure 3b). Maritime temperate and subtropical lakes had significantly higher and lower values, respectively, of β -carotene than tropical-*i*, tropical and tundra lakes ($p < 0.05$, one way ANOVA Tamhane T2 *post hoc* test) (Figure 3b).

A stepwise linear regression with β -carotene in the surface sediment as the dependent variable was performed. We excluded from the model any proxy of total algae (water column Chl-a and phytoplankton biovolume). Of the initial number of explanatory variables included in the model (33 variables), nine had a significant contribution. Conductivity, sediment and water column total nitrogen and dissolved inorganic carbon were positively associated with β -carotene in the sediment, while bottom oxygen, dissolved organic carbon and benthic-pelagic omnivorous and piscivorous fish were negatively associated with β -carotene. Overall, there was a high percentage of explained variance (64% of total variance, $p < 0.001$, $n = 79$; Table 2). When including phytoplankton biovolume in the model the variance explained did not increase (64%, $p < 0.001$) and when including planktonic Chl-a and phytoplankton biovolume variance explained was slightly higher (68%, $p < 0.001$) (see Supporting Information).

Factors associated with the degradation patterns observed using the a-phorbin composition

Redundancy analysis (RDA) of a-phorbin composition displayed two main environmental gradients related to Chl-a preservation and grazing-senescence processes (Figure 4; RDA1, eigenvalue 0.13; RDA2, eigenvalue 0.064). The relative Chl-a composition (Chl-a:a-phorbin) is indicative of Chl-a preservation. The variables best explaining the a-phorbin composition were pH (7.1%, $p=0.001$), subtropical (5.2%, $p=0.001$), sum of nitrate and nitrite in the water column (NO_3NO_2 ; 2.8%, $p=0.005$), large-scale spatial structure (MEM4; 2.9%, $p=0.003$ and MEM7; 2.1%, 0.034), suspended solids (SS; 2.6%, $p=0.015$), lake bottom temperature (botT; 2.6%, $p=0.012$) and benthic omnivorous fish (FishB-O, 2.3%, $p=0.011$) (Figure 4). Together, these variables explained 28.2% of the variance in the a-phorbin composition (20% adjusted).

Chl-a preservation was highest in the tundra lakes, in most of the maritime temperate and in some of the subtropical lakes (Figure 3c and Figure 4). High concentrations of suspended solids (SS) and high pH were the main variables positively correlated with Chl-a preservation, while high lake bottom temperature and benthic omnivorous fish were negatively correlated with Chl-a preservation.

The first axis of the RDA furthermore indicated that chlorophyllide-a and two epimers of Chl-a were positively related to the Chl-a preservation proxy. Senescence marker pigments like phaeophythin (Phyta1, -2), most grazing marker pigments (Phida2, -3, -4, -5) and allomer-2 were negatively correlated with the preservation axis. All grazing marker pigments were positively correlated with the second axis, while senescence marker pigments (Phyta1, -2) had a negative correlation with the second axis.

Relationship between marker pigment composition and environmental factors

In a principal component analysis (PCA), marker pigments (as a fraction of total carotenoids in the sample) of Bacillariophyceae and Chrysophyceae (fucoxanthin, diatoxanthin and diadinoxanthin) were positively correlated with the first axis (PCA1, eigenvalue 0.166) and were the predominant group in surface sediments of subtropical lakes, while the marker pigments of Chlorophyta (lutein, zeaxanthin, antheraxanthin) and green Chlorobiaceae (chlorobactene) were negatively correlated with the first axis and were broadly distributed in the lakes of the other climatic zones (Figure 5). Cyanobacteria marker pigments (aphanizophyll, oscillaxanthin, echinenone, canthaxanthin and zeaxanthin) and Cryptophyta marker pigments (alloxanthin and α -carotene) were negatively correlated with the second axis (PCA2, eigenvalue 0.136), while scytonemin (UV-screen pigment) and marker pigments of Dinophyta, Chrysophyceae and Bacillariophyceae (diadinoxanthin) were positively correlated with the second axis (Figure 5). Based on the marker pigments, nearly all subtropical lakes were characterised by high proportions of diatoms and chrysophytes, and many of the maritime temperate lakes were characterised by high proportions of cyanobacteria. The other three climatic zones were distributed along the rest of the bi-dimensional space of the PCA, indicating that they had a relatively wide range of marker pigment compositions.

The relationships between the three categories of explanatory variables (in-lake, external and spatial) and changes in marker pigment composition along the latitudinal gradient were explored with RDA. A preliminary RDA revealed that between one to eight environmental variables per category explained a significant amount of the variance (Table 3). The variables selected from the in-lake factors explained 29.5% of the variance and included cyclopoid copepod biomass, benthic detritivorous fish abundance, dissolved inorganic carbon, benthic omnivorous fish abundance, three different forms of nitrogen in the water column and total phosphorus and nitrogen in the sediment. The selected external factors explained 18.3% of the variance and included subtropical, maritime temperate and tropical climate, and lake altitude. The variables

selected from the spatial structure category explained 5.5% of the variance and were reduced to MEM2, indicating the importance of the large-scale spatial structure in the data (~ among regions), since the axis selected is in the first half group of the axes (<15 of a total of 33). In a final RDA with each group of previously selected environmental descriptors, explanatory variables accounted for 33.9% of the variance in pigment composition and were reduced to climate-related variables (subtropical and tropical), lake altitude, cyclopoid copepod biomass, functional groups of fish associated with the benthic habitat, total nitrogen in the sediment and dissolved inorganic carbon and nitrogen forms in the water column (Table 3).

RDA of the marker pigment composition demonstrated two main environmental gradients related to trophic state and biotic composition (Figure 6). Pigments of cryptophytes (alloxanthin, α -carotene) and chlorophytes (lutein, zeaxanthin, antheraxanthin) were associated with lakes with higher nutrient sources (DIC, DIN and sediment TN), while those of chrysophytes and diatoms (fucoxanthin, diadinoxanthin and diatoxanthin) were more abundant at lower nutrient levels (RDA1, eigenvalue 0.101). Along the trophic gradient, subtropical lakes emerged as a separate typology from the lakes in the other climatic zones. Pigments of diatoms were the dominant signal in the surface sediment of subtropical lakes that are characterised by higher abundances of benthic detritivorous fish, pointing to stronger relevance of the benthic habitat in these lakes. Pigments of cyanobacteria (aphanizophyll, oscillaxanthin, echinenone, canthaxanthin and zeaxanthin) were positively correlated with a second gradient defined by biotic composition with higher biomass of cyclopoid copepods and higher abundance of omnivorous fish (RDA2, eigenvalue 0.084). Interestingly, the maritime temperate lakes were highly productive and had very low DIN:SRP atomic ratios (Table 1), suggesting nitrogen limitation of phytoplankton growth and pigments of cyanobacteria were dominant in most of the lakes.

Variance partitioning analysis (VPA) revealed that in-lake factors explained a higher percentage of the variance in the phototrophic bacteria and algal composition (10.1%; $p=0.001$) than external (2.8%; $p=0.001$) or spatial factors ($<0.1\%$; $p=0.001$) (Table 3). Additive effects of the three categories and higher order interactions accounted for an additional 11.7% of the variance. Overall, the total percentage variance captured using VPA was 24.7%.

Discussion

Our analysis shows clear relationships between whole-lake microbial photoautotroph community traits (pigment concentration and composition) and environmental variables using information on surface sediments of shallow lakes sampled along a wide latitudinal gradient in South America. Although our results suggest some differences among groups of photoautotrophs, in most cases in-lake processes appeared to be the most important driving factors. The β -carotene per unit of organic matter, which in some studies has been found to be related to lake phytoplankton production (Guilizzoni, Bonomi, Galanti, & Ruggiu, 1983) or total algal biomass (Leavitt & Findlay, 1994), showed no latitudinal pattern and was best described by local environmental variables, including conductivity, bottom oxygen, lake water dissolved inorganic and organic carbon, total nitrogen in the sediment and water column and abundances of benthic-pelagic omnivorous and piscivorous fish. Similarly, in-lake factors including chemistry and biotic composition explained a significantly higher percentage of variance in the pigment-derived phototrophic community composition (phototrophic bacteria and algae) than external factors or spatial descriptors. Chl-a preservation, on the other hand, exhibited a relationship with latitude, with increasing Chl-a preservation towards the pole, implying better conservation at lower temperatures. Our pigment results concur with previous analyses of pelagic primary producers from the same lakes, in which phytoplankton community assemblies were also explained by local variables rather than by climate and showed a rather similar predictive power (Kosten et al., 2009; Kruk et al., 2011). However, the pigment analyses of

sediments provide a more complete picture of the microbial photoautotrophic communities (by integrating benthic and pelagic signals) than the former analyses of contemporary planktonic algae.

Indicative value of Chl-a derivative products

Preservation of Chl-a (as estimated from the ratio of Chl-a:a-phorbins) was highest in maritime temperate and tundra lakes, suggesting a relationship with latitude (temperature). The degree of net pigment degradation in the sediment depends on factors relating to, among others, the depositional environment such as temperature, light penetration, pH, degree of anoxia or grazing pressure (Buchaca & Catalan, 2007; Carpenter, Elser, & Elser, 1986). Higher temperatures and benthic-omnivorous fish pressure in the tropics (González-Bergonzoni et al., 2012) may result in higher degradation, while higher production, lower bottom oxygen levels and relatively low temperatures may have helped maintain a relatively high Chl-a preservation index in the maritime temperate lakes. However, in tundra and subtropical lakes with higher bottom-water oxygen concentrations and a higher light climate at the bottom, the apparently high Chl-a preservation may reflect a higher *in situ* production (benthic production) rather than actual preservation *per se*. The presence of other Chl-a derivatives, such as chlorophyllide-a, supports this view. Indeed, chlorophyllide-a is a Chl-a degradation product formed by chlorophyllase enzyme activity (Daley & Brown, 1973) and is more active in some diatom species (Jeffrey & Hallegraeff, 1987) that are known to be abundant in biofilms (Lowe, 1996). Temperature-induced higher decomposition and the higher grazing pressure are therefore likely the cause of the overall picture of a decrease in Chl-a preservation towards the equator. At more local scale, however, the Chl-a preservation may be inflated by benthic production. Our results stress the role of regulatory factors (e.g. light, grazing, algal recruitment, oxygen) in the surface sediment pigment record and concur with the findings using modelling approaches of pigment flux in lakes (Cuddington & Leavitt, 1999).

Inferred algal abundance

465 β -carotene concentration was mainly associated with local conditions, including dissolved organic and inorganic carbon, total nitrogen and biological interactions (abundance of benthopelagic omnivorous and piscivorous fish) that had no relationship with the latitudinal gradient. We found the highest values in maritime temperate lakes, which are characterised by high nutrient concentrations, higher phytoplankton biomass, higher DOC concentrations and thus 470 lower transparency than the surveyed lakes in the other climate regions (Kosten, Huszar, et al., 2009; Kruk et al., 2012). Consequently, at the time of sampling, light does not reach the bottom in the deepest part of the maritime temperate lakes. These lakes also had the lowest oxygen concentration near the sediment and the highest chlorophyll-a preservation index. Therefore, in the surveyed maritime temperate shallow lakes, pigments likely mainly reflect pelagic 475 production. Tundra lakes had the second highest values of β -carotene and were characterised by having the highest water transparency, the highest deep oxygen concentration and the highest planktonic chlorophyll-a concentration as well as a high chlorophyll-a preservation index. The combination of these factors suggests that the main source of pigments in tundra lakes was benthic algae. The close distance between pigment sources to the sediment suggests that the use 480 of β -carotene concentrations as a proxy for whole lake algal abundance likely leads to an overestimation in that particular case.

The β -carotene concentration was negatively correlated with the abundances of benthic-pelagic omnivorous fish, suggesting a suppressing effect of fish on production or accumulation. The 485 abundances of benthic-pelagic omnivorous and piscivorous fish were particularly high in the subtropical lakes that had the lowest values of inferred algal abundance and intermediate nutrient concentrations. This suggests the existence of a trophic cascade effect, favouring primary

consumers (e.g. algal grazers) that may selectively consume benthic algae (González-Bergonzoni et al., 2012; Meerhoff et al., 2007). This would reduce algal abundance to a lower concentration than expected from the nutrient levels, leading to an apparently lower production of algae than that actually occurring.

Nitrogen seemed to play a larger role than phosphorus for algal abundance as the total nitrogen concentration in the sediment was positively correlated with β -carotene. This relationship was not biased by the choice to use β -carotene to infer algal abundance since β -carotene and Chl-a showed good correlations in surface sediment (see A6 in Supporting Information). Nitrogen limitation has been described to be more common in tropical than in temperate systems (Talling & Lemoalle, 1998), possibly due to greater P supply by chemical weathering of rocks and greater internal N loss at higher temperatures (Lewis, 2002). The role of nitrogen has been questioned by other authors, showing that P can be a limiting nutrient to phytoplankton growth in freshwaters at any latitude (Elser et al., 2007; Huszar, Caraco, Roland, & Cole, 2006; Kosten et al., 2009a). We observed a potentially higher N limitation in the maritime temperate than in the tropical region. The maritime temperate lakes were highly productive and had low DIN:SRP atomic ratios (<13), suggesting phytoplankton nitrogen-limited growth (Morris & Lewis, 1988), and cyanobacteria pigments dominated in many of them. In accordance with this, the dominant cyanobacteria in the water column were those able to fix atmospheric nitrogen (Kosten et al., 2009a). A high percentage of livestock-based agriculture in the lake catchments in this region may have enhanced the P inputs and thus intensified the eutrophication (Kosten et al., 2009a; Sharpley, 1994). High eutrophication may therefore have contributed to or determined the dominance of cyanobacteria in this region.

Macrophyte coverage (as PVI) was not selected as a predictor variable of β -carotene in the sediment. Macrophytes play a central role in shallow temperate lakes by keeping nutrient concentrations low, thereby creating higher water transparency (Jeppesen et al., 1997; Søndergaard et al., 2010). The absence of an effect of macrophytes on β -carotene may perhaps reflect the fact that the overall effect of vegetation on water clarity in the same set of lakes was relatively weak compared with findings of studies conducted in the northern hemisphere (Kosten et al., 2009b).

Importance of climatic and environmental gradient vs. spatial gradient in explaining algal composition

Trophic state (nutrients and biotic components) explained approximately ten percent of the variance in pigment-derived photoautotrophic algal and bacterial composition obtained from the surface sediments along the latitudinal gradient and climatic and catchment characteristics explained a lower percentage (2.8 %), while the spatial components were of minor importance, except in the subtropical lakes. Pigment-inferred diatoms were the dominant algal group in the benthic-pelagic integrated signal of these subtropical lakes characterised by higher light penetration and thus higher contribution from the benthic habitats. In the subtropical lakes, diatoms co-occurred in similar proportions as cyanobacteria at the time of sampling in the pelagic (V. Huszar pers. comm.). Pigments of cryptophytes (alloxanthin, α -carotene) and chlorophytes (lutein, zeaxanthin) were associated with lakes with higher nutrient concentrations (DIC, DIN and sediment TN), while chrysophytes and diatoms (fucoxanthin, diadinoxanthin and diatoxanthin) were more abundant at the opposite extreme of the trophic gradient. Numerous studies on phytoplankton communities (Huszar et al., 2015; Mazaris, Moustaka-Gouni, Michaloudi, & Bobori, 2010; Phillips, Willby, & Moss, 2016; Vanormelingen et al., 2008) have indicated that algal group composition is mainly affected by local environmental conditions.

However, lake depth was not significant in explaining pigment composition variability in these shallow eutrophic lakes contrary to what has been found in more oligotrophic shallow also deeper lakes (Buchaca & Catalan, 2007; Hodgson et al., 2004; Reuss, Leavitt, Hall, Bigler, & Hammarlund, 2010). The key role of in-lake factors is also in accordance with previous studies on other major trophic groups from bacteria to fish in freshwaters (Beisner, Peres-Neto, Lindström, Barnett, & Longhi, 2006). A parallel study (Souffreau et al., 2015) conducted in a subset of the lakes in our study (those from the tropical-*i*, tropical and tundra regions) also showed environmental rather than spatial factors to be of key importance in structuring the bacterioplankton community.

Pigments of cyanobacteria (aphanizophyll, oscillaxanthin, echinenone, canthaxanthin and zeaxanthin) were positively related with the biomass of cyclopoid copepods and abundances of omnivorous fish and low light availability. The biomass of cyclopoid copepods was higher in most of the highly productive maritime temperate lakes, which is a pattern commonly found in field studies and experiments with high fish predation and associated low zooplankton grazing on phytoplankton (Hansson, Gyllstrom, Ståhl-Delbanco, & Svensson, 2004; Jeppesen, Jensen, Sondergaard, Lauridsen, & Landkildehus, 2000). So, low light in such lakes likely reflects both less grazing on phytoplankton and higher concentrations of suspended solids (more phytoplankton and more fish-induced resuspension), which may favour the competitive capacity of shade-tolerant species, such as some cyanobacteria, most notably Oscillatoriales (oscillaxanthin) as evidenced by both controlled experiments and field data (Scheffer, Rinaldi, Gragnani, Mur, & vanNes, 1997).

Conclusions

Our results suggest that pigment assemblages in sediments, which integrate both benthic and pelagic microbial photoautotrophic community and processes, are valuable indicators of changes

in shallow (0.75 - 12 m depth) lake ecosystems. We found that whole lake algal abundance (expressed as the β -carotene concentration) as well as community composition were best described by in-lake factors such as water chemistry and biotic interactions rather than by external factors including climatic regions or spatial descriptors. Maritime temperate lakes were associated with a low DIN:SRP ratio, likely reflecting a more intensive live-stock based agriculture than in the other regions.

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Table 1. Median, standard error (SE), minimum (min) and maximum (max) of the main physical and chemical features of the 83 sampled lakes. I ($Z_{\max}$) is light intensity at lake maximum depth as a percentage of subsurface irradiance.

	Tropical i n=19			Tropical n=19			Subtropical n=18			Maritime temperate n=16			Tundra n=11		
	median	SE	(min-max)	median	SE	(min-max)	median	SE	(min-max)	median	SE	(min-max)	median	SE	(min-max)
Altitude (m. a. s. l.)	35	20	(1-318)	1	2.2	(1-36)	17	2	(1-31)	68	22	(2-220)	400	44.2	(105-500)
Area (ha)	43	7	(9-124)	34	6.7	(11-127)	63	13	(16-243)	78	14	(25-242)	37	21	(9-253)
Max. depth (m)	4.6	0.4	(1.4-7.7)	3.2	0.3	(1.4-5.2)	2.5	0.3	(1.5-7)	1.5	0.1	(0.75-2.7)	2.25	1.2	(0.7-12)
Temperature (°C)	28.2	0.2	(28-30)	25.9	0.2	(24-27)	24.9	0.4	(22-28)	24	0.7	(19-28.6)	13.3	0.7	(10.4-18.7)
Surface oxygen (mg/L)	7.5	0.1	(6.1-8.9)	7.4	0.3	(3.3-10)	7.7	0.2	(6.4-9.3)	9	1	(5-21)	10.4	0.2	(9.7-11.3)
Bottom oxygen (mg/L)	7.7	0.4	(2.8-9)	7.2	0.5	(1.8-8.9)	7.6	0.2	(6-9)	5.8	0.6	(1.5-12)	10.9	0.2	(9.7-11.5)
Chlorophyll-a (µg/L)	3.5	4.7	(0.5-79)	3.2	2	(1-26)	3.6	2.9	(0.9-56)	96	70	(6-1124)	4.3	261	(1.2-2889)
I ($Z_{\max}$) (%)	0.11	0.6	(0-8)	0.04	0.1	(0-2.3)	0.18	0.1	(0-1.2)	0.0002	0.9	(0-12.2)	0.5	0.4	(0-4.6)
pH	6.8	0.2	(5.7-8.7)	6.7	0.2	(4-8.5)	7	0.2	(5.5-8.9)	9	0.1	(7.6-9.7)	8.5	0.2	(7.2-9.2)
Conductivity (µS/cm)	154	128	(46-2100)	129	65	(69-1021)	104	24	(53-397)	1059	307	(584-4930)	254	90	(38-982)
DIC (mgC/L)	0.22	0.1	(0.1-1.8)	0.33	0.1	(0.16-2)	0.03	0.1	(0.03-1.4)	7.6	17	(1-271)	1.7	0.5	(0.25-4.5)
DOC (mgC/L)	9.7	1.7	(3.1-24.2)	8.7	2.4	(6.1-48.5)	7.4	0.6	(2.6-11.7)	28	6	(11-86)	13.4	7.2	(1.8-84)
DIN (µgN/L)	30	10	(3-130)	80	20	(10-470)	30	10	(20-200)	30	20	(0.4-250)	30	20	(20-2040)
SRP (µgP/L)	6.04	0.8	(2.1-14)	10	13	(4.1-254)	5.6	3.6	(4.2-70.9)	58	75	(11-1118)	3.8	609	(0.5-6711)
TN (mgN/L)	0.35	0.1	(0.01-1.7)	0.44	0.1	(0.1-1)	0.23	0.1	(0.01-1.04)	3.1	1.7	(1-26)	0.4	2.05	(0.01-23)
TP (mgP/L)	0.06	0.01	(0.002-0.13)	0.07	0.01	(0.002-0.3)	0.13	0.01	(0.07-0.19)	0.26	0.1	(0.1-1.7)	0.07	0.8	(0.02-9.1)
DIN : SRP (atomic)	9	5.2	(2.4-90)	18.3	11.2	(1-207)	13	4.3	(3.4-79)	1.5	0.7	(0.001-8.3)	17	8.8	(0.7-99)

Table 2. Variance partitioning analysis (VPA) of surface sediment β -carotene (nmol g⁻¹OM) using a multiple linear regression analysis with in-lake variables. All variables except bottom oxygen (botO2) were log10 transformed. Bottom oxygen (botO2), conductivity (Cond), dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), water column total nitrogen (TN), sediment total nitrogen (SedTotN), benthopelagic detritivorous fish (FishBP-Detr), benthopelagic omnivorous fish (FishBP-O) and benthopelagic piscivorous fish (FishBP-Pisc).

	Coefficient	Pr(> t)	Explained variance (%)
Intercept	3.6	0.0055	
botO2	-0.1	0.0121	3.5
Cond	0.3	0.0944	1.5
DIC	0.3	0.0019	5.5
DOC	-0.6	0.0716	1.8
TN	0.3	0.0615	1.9
SedTotN	0.3	0.0013	5.9
FishBP-Detr	0.2	0.1248	1.3
FishBP-O	-0.1	0.0025	5.2
FishBP-Pisc	-0.1	0.0531	2
Shared variance			35.5
Total explained variance			63.9
Unexplained variance			36.1
F _{9,69} = 13.56, P<<0.001			

Table 3. Pigment composition explained variation obtained in the redundancy analysis (RDA) and variance partitioning analysis (VPA, pRDA) of in-lake (L), external (E) and spatial (S) components. Cyclopoid copepod biomass (BCyclo), benthic detritivorous fish (FishB-Detr), dissolved inorganic carbon (DIC), benthic omnivorous fish (FishB-O), sum of nitrate and nitrite in the water column (NO₃NO₂), ammonium in the water column (NH₄), sediment total phosphorus (SedTotP), sediment total nitrogen (SedTotN), lake altitude (Alt). Percentage explained variation (%var) and percentage explained variation adjusted for the number of predictors in the model (% var (adj)) are given.

			% var	% var (adj)	p-value
preliminary RDA by groups	[L]	All variables	29.5	21.4	0.001
		BCyclo	6.8		0.001
		FishB-Detr	4.9		0.001
		DIC	4.2		0.001
		FishB-O	2.9		0.002
		NO ₃ NO ₂	2.9		0.001
		NH ₄	2.7		0.002
		SedTotP	2.5		0.002
		SedTotN	2.6		0.005
	[E]	All variables	18.3	13.9	0.001
		Subtropical	8.4		0.001
		Temperate	4.4		0.001
		Tropical	2.9		0.002
		Alt	2.6		0.006
	[S]	All variables	5.5	4.3	0.001
		MEM2	5.5		0.001
final RDA	Total explained		33.9	24.1	0.001
	Subtropical		8.4		0.001
	BCyclo		6.5		0.001
	Tropical		3.2		0.002
	FishB-O		2.6		0.002
	NH ₄		2.5		0.003
	SedTotN		2.5		0.005
	FishB-Detr		2.3		0.007
	Alt		2.3		0.007
	NO ₃ NO ₂		1.9		0.008
	DIC		1.7		0.039
VPA pRDA	Fraction		% var (adj)	DF	Mean square
	[L+E+S]	Total explained	24.7	13	0.029
	[L E+S]	In-lake	10.1	8	0.022
	[E L+S]	External	2.8	4	0.016
	[S L+E]	Spatial	<0.1	1	0.01
	L∩E∩S	Shared	11.7		
	[U]	Not explained	75.3		

Figure Captions

Figure 1. Location of the 83 shallow lakes surveyed in east South America in this study. Also shown is the prevailing climate following the Köppen classification system.

Figure 2. Boxplots of a) lake surface temperature, Temp; b) lake maximum depth, MaxDepth; c) water column total phosphorus concentration, TP; d) water column total nitrogen concentration, TN, and e) water column dissolved inorganic nitrogen concentration, DIN; f) DIN:SRP atomic ratios, in different climate zones. Trop=tropical, Subtr=subtropical, Temp=maritime temperate, Tund=tundra. Lines inside the boxplots are mean values, boundaries indicate the 25th and 75th percentile. Whiskers above and below indicate the 90th and 10th percentiles. Black dots with horizontal lines are outlying points. Letters indicate groups with significantly similar medians (Tamhane T2 post hoc test $p>0.05$).

Figure 3. Boxplots of a) pelagic Chl-a concentration (Chl-a), b) surface sediment β -carotene concentration per unit biomass (b-carot) and c) Chl-a:a-phorbin ratio where a-phorbin are the sum of Chl-a and derivative products of Chl-a, which is an indicator of Chl-a preservation. Trop=tropical, Subtr=subtropical, Temp=maritime temperate, Tund=tundra. Lines inside the boxplots are mean values, boundaries indicate the 25th and 75th percentiles. Whiskers above and below indicate the 90th and 10th percentiles. Black dots with horizontal lines are outlying points. Letters indicate groups with significantly similar medians (Tamhane T2 post hoc test $p>0.05$).

Figure 4. Ordination diagram showing the linear combination of environmental variables describing the main gradients of variation in the relative a-phorbin

composition (%) along the two first axes of the redundancy analysis (RDA). Pigments are represented by blue arrows: chlorophyll-a (*Chl-a*), chlorophyllide a (*Chlide-a*), Chl-a allomer-1 and -2 (*Allom1* and *Allom2*), Chl-a epimer-1 and -2 (*Epim1* and *Epim2*), phaeophythin-a1 and -a2 (*Phyta1* and *Phyta2*), phaeophorbide-a2, -a3, -a4, -a5 (*Phida2*, *Phida3*, *Phida4*, *Phida5*). Environmental variables are represented by red arrows: subtropical climate (Subtropical), lake bottom temperature (botT), benthic-omnivorous fish (FishB-O), nitrite and nitrate in the lake water (NO₃NO₂), suspended solids (SS), filters 4 and 7 of the Moran eigenvector maps (MEM4 and MEM7). Tropical-i (red circles), tropical (orange squares), subtropical (green diamonds), maritime temperate (blue triangles), tundra (grey crosses).

Figure 5. Principal component analysis (PCA) of marker pigment concentration expressed as a percentage of total carotenoids. Diadinoxanthin (Diadin), fucoxanthin (Fucox), Diatoxanthin (Diato), canthaxanthin (Cantha), astaxanthin (Astax), aphanizophyll (Aphan), alloxanthin (Allox), oscillaxanthin (Oscill), , echinenone (Echin), isorenieratene (Isoren), zeaxanthin (Zeax), a-carotene (a_carot), antheraxanthin (Anthera), lutein (Lut), chlorobactene (Chlorob), scytonemin-I (Scyt-I). Tropical-i (red circles), tropical (orange squares), subtropical (green diamonds), maritime temperate (blue triangles), tundra (grey crosses).

Figure 6. Ordination diagram showing the linear combination of environmental variables describing the main gradients of variation in the relative carotenoid composition (expressed as a percentage of total carotenoids) along the two first axes of the redundancy analysis (RDA). Pigments are represented by blue arrows, abbreviations as in figure 5. Environmental variables are represented by red arrows:

cyclopoid copepod biomass (BCyclo), dissolved inorganic carbon (DIC), tropical climate (Tropical), lake altitude (Alt), sum of nitrate and nitrite in the lake water (NO3NO2), ammonium in the lake water (NH4), subtropical climate (Subtropical), benthic detritivorous fish (FishB-Detr), benthic omnivorous fish (FishB-O). Tropical-i (red circles), tropical (orange squares), subtropical (green diamonds), maritime temperate (blue triangles), tundra (grey crosses).

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Supporting Information A1. In-lake descriptors.

ACRONYM	DESCRIPTION	UNITS
Physico-chemical descriptors		
1 Larea	Area	m ²
2 Lperim	Perimeter	m
3 Lvol	Volume	m ³
4 MDepth	Maximum depth	m
5 surfT	Surface temperature	celcius
6 botT	Bottom temperature	celcius
7 surfO2	Surface oxygen	mg L ⁻¹
8 botO2	Bottom oxygen	mg L ⁻¹
9 Kd	Calculated light extinction coefficient	m ⁻¹
10 I(Z _{max})	Calculated percent light in surface sediment	%
11 Turb	Turbidity	NTU
12 pH	pH	
13 Cond	Conductivity	μS cm ⁻¹
14 CO3	Carbonate	mg L ⁻¹
15 HCO3	Bicarbonate	mg L ⁻¹
16 ABC	Acid binding capacity	meq L ⁻¹
17 DIC	Dissolved inorganic carbon	mg L ⁻¹
18 DOC	Dissolved organic carbon	mg L ⁻¹
19 NO3NO2	Nitrate and nitrite	mg L ⁻¹
20 NH4	Ammonium	mg L ⁻¹
21 SRP	Soluble reactive phosphorus	μg L ⁻¹
22 TN	Total nitrogen (water column)	mg L ⁻¹
23 TP	Total phosphorus (water column)	mg L ⁻¹
24 Si	Silicate	mg L ⁻¹
25 SS	Suspended solids	mg L ⁻¹
26 SOM	Organic matter (suspended)	mg L ⁻¹
27 SedTotN	Total nitrogen (sediment)	g g ⁻¹ sediment dry weight
28 SedTotP	Total phosphorus (sediment)	g g ⁻¹ sediment dry weight
Biotic descriptors		
1 pChla	Planktonic Chlorophyll-a	μg/L
2 flPlant	Floating plant coverage	%
3 emergPlant	Emergent plant coverage	%
4 submerPlant	Submergent plant coverage	%
5 PVI	Percentage of lake volume filled with submerged vegetation	%
6 RichnessNP	Phytoplankton species richness without picoplankton (>1μm)	taxa per sample
7 ABunNP	Phytoplankton abundances without picoplankton (>1μm)	cell mL ⁻¹
8 BVolNP	Phytoplankton biovolume without picoplankton (>1μm)	mm ³ L ⁻¹
9 BRotif	Rotifera biomass	μg dry weight L ⁻¹
10 BCalan	Calanoidea copepoda biomass	μg dry weight L ⁻¹
11 Bcyclop	Cyclopoidea copepoda biomass	μg dry weight L ⁻¹
12 Bclado	Cladocera biomass	μg dry weight L ⁻¹
13 Bharpa	Harpacticoidea copepoda biomass	μg dry weight L ⁻¹
14 BiomZooTotal	Macrozooplankton total biomass	μg dry weight L ⁻¹
15 FishBP-H	Benthic/Pelagic herbivorous fish	ind. net ⁻¹ 12h
16 FishB-Detr	Benthic detritivorous fish	ind. net ⁻¹ 12h
17 FishBP-Detr	Benthic/Pelagic detritivorous fish	ind. net ⁻¹ 12h
18 FishB-O	Benthic omnivorous fish	ind. net ⁻¹ 12h
19 FishBP-O	Benthic/Pelagic omnivorous fish	ind. net ⁻¹ 12h
20 FishP-O	Pelagic omnivorous fish	ind. net ⁻¹ 12h
21 FishBP-Pisc	Potential Benthic/Pelagic piscivorous fish	ind. net ⁻¹ 12h
22 FishD-Pisc	Potential Demersal piscivorous fish	ind. net ⁻¹ 12h
23 FishP-Pisc	Potential Pelagic piscivorous fish	ind. net ⁻¹ 12h

Supporting Information A2. External descriptors.

ACRONIM	DESCRIPTION	UNITS
Climatic descriptors		
1 Subtropical	tempCF	categorical variable
2 MaritimeTemperate	temp CF, temp CFb	categorical variable
3 Tropical	Tropical AM, tem CF, tropical AW	categorical variable
4 Tropic-i	Tropical Ami	categorical variable
5 Tundra	Polar ET, CFc, Cc, CSc, BSK', CFb	categorical variable
Catchment descriptors		
6 Alt	Altitude	m
7 GeolRock	Rock	categorical variable
8 GeolCarb	Carbonate bedrock	categorical variable
9 GeolPeat	Peat	categorical variable
10 GeolSand	Sand	categorical variable
11 GeolOrg	Organic matter	categorical variable
12 GeolClay	Clay	categorical variable
13 soilcarb	Soil carbon	kg C m ⁻² to 1 meter depth
14 DistCoast	Distance to the coast	Decimal degrees

Legend Koeppen	
CF	temp CF = subtropical
Cfb	temp Cfb = 'temperate <i>Marine West Coast Mild</i> '
AM	Am - Monsoon type. Short dry season but sufficient moisture to keep ground wet throughout the year
AW	Aw - Distinct dry season. One month with precipitation < 60 mm.
AMi	Tropical AMi (isothermal subtype, annual range of T<5 degrees C)
ET	Polar ET (Tundra)
CFc	CFc (temperate cool)
Cc	Cc temperate cool
CSc	CSc temperate cool
BSk'	BS k' :Dry and cool (temperature of the warmest month < 18 °C).

Supporting Information A3. Marker carotenoids included in the study and taxonomic affinities.

ACRONYM	PIGMENT NAME	TAXONOMIC AFFINITY
Carotenoids		
1 b_carot	β -carotene	Cyanobacteria, eukaryotic algae and vascular plants
2 a_carot	α -carotene	Cryptophyta
3 Allox	Alloxanthin	Cryptophyta
4 Diadin	Diadinoxanthin	Dinoflagellates, Chrysophyceae and Bacillariophyceae
5 Fucox	Fucoxanthin	Chrysophyceae and Bacillariophyceae
6 Diato	Diatoxanthin	Bacillariophyceae
7 Lut	Lutein	Chlorophyta
8 Anthera	Antheraxanthin	Chlorophyta
9 Zeax	Zeaxanthin	Chlorophyta and Cyanobacteria
10 Oscill	Oscillaxanthin	Cyanobacteria (Oscillatoriaceae)
11 Aphan	Aphanizophyll	Cyanobacteria (N ₂ -fixing groups)
12 Cantha	Canthaxanthin	Cyanobacteria and zooplankton
13 Echin	Echinenone	Cyanobacteria and zooplankton
14 Astax	Astaxanthin	Zooplankton
15 Chlo	Chlorobactene	Phototrophic bacteria (green Chlorobiaceae)
16 Isoren	Isorenieratene	Phototrophic bacteria (brown Chlorobiaceae)
17 Scyt-I	Scytonemin-I	Photosynthetic organisms exposed to high UV radiation

Supporting Information A4. a-phorbins included in the study.

ACRONYM	PIGMENT NAME	AFFINITY
1 Chla	Chlorophyll-a	Cyanobacteria, eukaryotic algae and vascular plants
2 Chlide_a	Chlorophyllide-a	Chl-a derivative, abundant in diatom cells
3 Allom1	Chla-allomer1	Chl-a derivative
4 Allom2	Chla-allomer2	Chl-a derivative
5 Epim1	Chla-epimer1	Chl-a derivative
6 Epim2	Chla-epimer2	Chl-a derivative
7 Phyta1	Phaeophythin-a1	Chl-a derivative (senescence)
8 Phyta2	Phaeophythin-a2	Chl-a derivative (senescence)
9 Phida2	Phaeophorbide-a2	Chl-a derivative (grazing)
10 Phida3	Phaeophorbide-a3	Chl-a derivative (grazing)
11 Phida4	Phaeophorbide-a4	Chl-a derivative (grazing)
12 Phida5	Phaeophorbide-a5	Chl-a derivative (grazing)

Supporting Information A5.

Table 2a. Variance partitioning analysis (VPA) after stepwise of surface sediment β -carotene ($\text{nmol g}^{-1}\text{OM}$) using a multiple linear regression analysis with in-lake variables ($n=33$). All variables except bottom oxygen (botO2) were LOG10 transformed to approximate normality of residuals. Refer to table A1 for variable abbreviations.

	Coefficient	Pr(> t)	Explained variance (%)
Intercept	3.4	0.0000	
pChla	0.5	0.0000	22.5
botO2	-0.1	0.0000	9.4
DIC	0.3	0.0002	7.4
SedTotN	0.3	0.0012	5.3
FishBP-O	-0.1	0.0109	3.4
FishBP-Pisc	-0.1	0.0146	2.9
DOC	-0.6	0.0156	2.8
PVI	0.1	0.0248	2.4
Shared variance			11.8
Total explained variance			67.7
Unexplained variance			32.3
$F_{8,70} = 18.34, P < 0.001$			

Table 2b. Variance partitioning analysis (VPA) after stepwise of surface sediment β -carotene ($\text{nmol g}^{-1}\text{OM}$) using a multiple linear regression analysis with in-lake variables; pChl-a was excluded from the model ($n=32$). All variables except bottom oxygen (botO2) were LOG10 transformed to approximate normality of residuals. Refer to table A1 for variable abbreviations.

	Coefficient	Pr(> t)	Explained variance (%)
Intercept	1.8	0.0000	
FishBP-O	-0.1	0.0043	4.4
FishB-Detr	-0.1	0.0115	4.2
BVoINP	0.2	0.0055	4.1
SedTotP	0.3	0.0641	1.8
SedTotN	0.3	0.0053	4.2
DIC	0.3	0.0000	10.9
botO2	-0.1	0.0013	6.8
Shared variance			36.3
Total explained variance			64.4
Unexplained variance			35.6
$F_{7,71} = 18.36, P < 0.001$			

Supporting Information A6.

β -carotene and Chl-a showed good correlations in surface sediment indicating that the relationship between nitrogen in the sediment and β -carotene was not biased by the choice to use β -carotene to infer algal abundance.

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