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Lake browning influences periphyton biomass and chlorophyll dynamics

Master's Programme in Ecology and Evolutionary Biology

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Master's thesis

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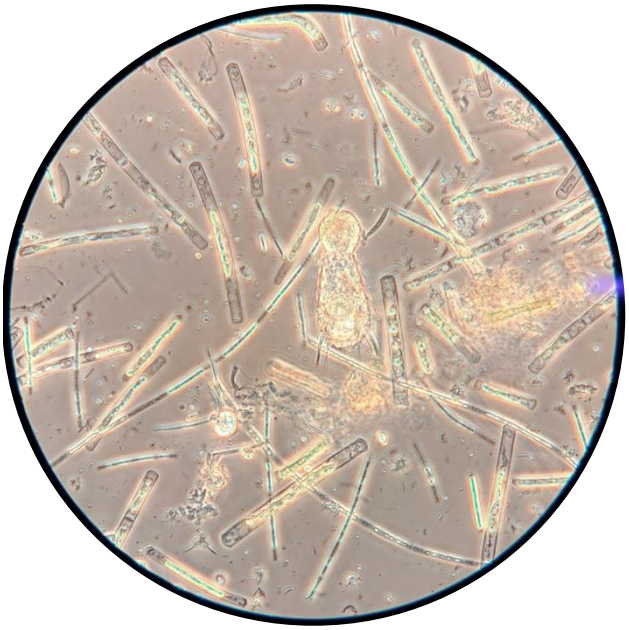

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Abstract:

The browning of freshwater lakes changes the distribution of light and heat within the water column and shrinks the available photosynthetic zone, disrupting primary production. Despite the growing concern of lake browning there are no widely established methods for monitoring its effect on lake ecosystems. Periphytic algae serve as key producers in lake communities and changes in their community composition and chlorophyll production may serve as bioindicators for browning. I compared periphyton in 12 Finnish lakes along a gradient of water color as well as a vertical gradient in the water column at three depths: 20, 50, and 80 cm. Using a dataset of samples collected in June 2024, I used mixed models to investigate the effect of water color, dissolved organic carbon (DOC) and nutrients on periphyton community structure (composition, biodiversity, functional diversity) and functioning (biomass and chlorophyll concentration). Overall algae structure remained relatively stable across the browning gradient, although biodiversity increased at the deepest sample depth. Periphyton biomass initially increased with the input of DOC and peaked at moderate color levels where light limitation became the dominating factor, but this relationship was dependent on depth and taxa. I found evidence that periphytic algae may adapt to browning by increasing the concentration of chlorophyll and I propose the ratio of chlorophyll a : biomass as an effective bioindicator of browning. My thesis explores the influence of browning on periphyton dynamics in a boreal system and highlights the need for further research on a larger spatial and time scale.

Contents

1 Introduction

1.1 Lake Browning

Lake browning is a growing concern worldwide (Blanchet et al., 2022). Browning refers to an increase in brown appearance of water color, typically as a result of dissolved organic matter (DOM) entering the water body (Solomon et al., 2015). DOM comes from the decomposition of plants and animals. A large portion of DOM is composed of humic substances whose complex chemical structure prevents further microbial degradation and, therefore, remains in the aquatic system for a long time (Kellerman et al., 2015). DOM is often measured by its primary component, dissolved organic carbon (DOC) (Pilla & Couture, 2021). While DOC is the most notable cause of browning, studies have shown that iron (Fe) can bind to DOC and further increase water color until saturation is reached at an iron concentration of 1.0 – 1.5 mg L^{-1} (Kritzberg & Ekström, 2012; Weyhenmeyer et al., 2014). It has also been shown that the influence of iron on color is strongest at DOC concentrations below 14.0 mg L^{-1} (Horppila et al., 2023). DOC and iron absorb solar radiation, reducing the depth to which light and heat can penetrate in the vertical water column (Maloney et al., 2005; Pilla & Couture, 2021).

The amount of brown lakes is growing due to increased input of DOC, largely as a result of human actions (Evans et al., 2005). Land use practices such as forestry, agriculture, ditching, and peat mining increase the external loading of DOC into lakes (Estlander et al., 2021; Kritzberg, 2017; Kritzberg et al., 2020). Warm temperatures and heavy rainfall associated with climate change may extend the growing season and production of vegetation, as well as increase the leaching of organic matter into lakes (de Wit et al., 2016; Finstad et al., 2016). The rise in DOC can also be indicative of acidification recovery; environmental initiatives and policy changes to reduce industrial pollution have decreased atmospheric sulphate, which has led to higher lake pH levels and increased solubility of organic matter (Meyer-Jacob et al., 2019; Monteith et al., 2007).

The impacts of browning are widespread and can cause financial, public health, and environmental issues. Lakes and other surface waters are major providers of drinking water, but DOM can increase the cost of water treatment, transport heavy metals and pollutants, promote bacterial growth, and even produce harmful byproducts during the treatment process (Kritzberg et al., 2020; Lavonen et al., 2013; Ledesma et al., 2012). Public surveys show that browning can also lessen recreational enjoyment of lakes (Albrecht et al., 2023).

Within lakes, browning causes heat to be absorbed more quickly, leading to a steeper, shallow, and more stable thermal gradient (Horppila et al., 2023; Kling, 1988). The warmer surface layer of brown lakes loses heat faster, leading to an overall colder lake and impacting photosynthetic and metabolic rates (Brown et al., 2004). This stable thermocline reduces the mixing of dissolved oxygen and other chemicals, which can create anoxic conditions and limit biological access to internally recycled nutrients. Depleted oxygen levels can increase the amount of carbon emitted as CO_2 and methane, exacerbating climate change and, in turn, increasing the loading of organic matter into lakes (Knoll et al., 2018; Zhang et al., 2024).

The physical and chemical changes to lakes associated with browning can affect the food web structure and interactions between organisms (Horppila et al., 2025; Williamson et al., 2015). Organisms that are less constrained by changes to the distribution of light, heat, oxygen, and nutrients may begin to dominate, leading to a reduction in biodiversity and an overall simplification of community structure across multiple trophic levels (Brüsecke et al., 2023; Murdoch et al., 2021; Urrutia-Cordero et al., 2017). In particular, the absorption of light heavily influences primary producers. In dark lakes, light can be depleted within 1.5 meters of water (Eloranta, 1978). This constricts the photic zone, in which effective photosynthesis is possible. Phytoplankton are restricted to the water surface, resulting in increased competition for resources. The influx of nutrients from organic matter can initially increase primary production until an approximate DOC concentration of 11 – 16 mg L^{-1} and water color of ca. 106 mg Pt L^{-1}, at which point light, rather than nutrients, becomes the limiting growth factor (Bergström & Karlsson, 2019; Horppila et al., 2023, 2024).

Primary producers are a key component of the food web and control ecosystems from the bottom-up. Algae contribute to a major portion of aquatic production and can take the form of large seaweeds or single-celled phytoplankton. Algae is commonly used as a bioindicator for aquatic health because of their rapid response to environmental change. Current research is investigating the use of a variety of bioindicators for browning, of which one possibility is periphyton (Estlander et al., 2024; Estlander & Horppila, 2023; Rajala et al., 2024, 2026). Periphyton is a community of photosynthetic algae and cyanobacteria, along with non-photosynthetic microbes, fungi, and small invertebrates that attaches to submerged rocks, plants, and debris. Since periphyton affix to a surface, as opposed to free-floating phytoplankton, the effect of a light gradient can be measured at different depths.

One thing every photosynthetic organism has in common is chlorophyll *a* (chl a), the main photoreceptor used in photosynthesis to absorb energy from sunlight. While photosynthesis requires chl a, the concentration can vary between species and is dependent on environmental factors like light, nutrients, and temperature (Finenko et al., 2003). The accessory chlorophyll pigments that aid in energy capture also vary between species. Chlorophyll *b* (chl b) is found mostly in green algae and has been shown to more effectively capture high energy light than chl a and c (Kume et al., 2018). Phycobilins are accessory pigment-protein complexes found in red algae and cyanobacteria. Cyanobacteria (commonly known as blue-green algae) are a class of photosynthetic bacteria which have been shown to be well-adapted to low-light conditions (Gan & Bryant, 2015); some species also contain chlorophyll d and f, which aid in the capture of low-energy light. Cyanobacteria are of additional environmental interest because some species produce toxins that can be harmful to humans and wildlife (Bláha et al., 2009). Most algae also contain carotenoids, another light harvesting accessory pigment that contain antioxidants that protect cells against UV damage in high-light environments (Sandmann, 2019; Young, 1991). These accessory pigments all capture different ranges of light and may give certain species functional advantages in brown lakes.

1.2 Research aims and questions

Despite the growing problem of lake browning, it is not explicitly addressed by the European Union's Water Framework Directive and current monitoring tools and restoration methods are not specifically designed to capture the browning processes. Most current monitoring is focused on eutrophication, which is the excessive input of nutrients, usually nitrogen (N) and phosphorus (P), in water bodies, leading to increased production (Smith et al., 1999). While these two problems often co-occur due to increased organic loading and can share similar effects, the focus of browning is on light limitation due to DOC and iron. Current monitoring techniques use the concentration of chl a as a metric of production; less chl a can indicate a less eutrophic lake. However, browning may influence chlorophyll concentration independent of biomass and a darker water color limits production, which in some cases may lead to a brown lake being classified as "good condition". One possible solution is to use the ratio of various chlorophyll pigments, which could have distinct responses to browning and better inform on the condition of lakes.

The aim of my thesis is to better understand the effects of browning on periphyton communities and chlorophyll dynamics as well as to investigate possible bioindicators. My research questions are as follows:

Question 1: Does browning influence the community structure of periphyton producers?

To answer this, I will look at the biodiversity and functional diversity of periphytic algae at different light gradients. In brown lakes, I expect an overall reduction in diversity and a higher competitive fitness of algae that can absorb a greater range of light (i.e. algae with chl b and cyanobacteria) (Gan & Bryant, 2015; Kume et al., 2018; Urrutia-Cordero et al., 2017).

Question 2: How does browning affect periphyton biomass?

I will address this by looking at how light influences the total periphyton biomass (i.e. all periphyton organisms) and the periphytic algae biomass (i.e. only the photosynthetic periphyton species). I expect to see a unimodal relationship between biomass and DOC

concentration, with a light-limitation threshold at moderate DOC and color levels (Bergström & Karlsson, 2019; Horppila et al., 2023, 2024).

Question 3: Can chlorophyll be used as a bioindicator for browning?

To answer this, I will look at whether chlorophyll concentration is increased to compensate for low light conditions and if there is a reliable shift in chlorophyll ratios that can be used as an effective bioindicator. I will investigate three ratios: chl a to chl b (chl a:b), chl a to carotenoids (chl a:car), and chl a relative to algae biomass (chl a:bm). My predictions are as follows:

a) Since chl b expands the range of light that is able to be absorbed, I would expect chl a:b to decrease in low light conditions (Kume et al., 2018; Yamazaki et al., 2005).
b) As carotenoids are protective in high light conditions, I would expect chl a:car to increase in low light conditions (Sandmann, 2019; Young, 1991).
c) If more chl a is produced to compensate for low light, I would expect chl a:bm to increase in low light conditions (Finenko et al., 2003).

2. Materials and Methods

2.1 Sample Collection

The environmental samples in this dataset were collected from 12 lakes in southern Finland from June – July 2024 as part of the HUMI project. Polycarbonate plates (10 x 15 cm) were suspended with an artificial apparatus at a depth of 20, 50, and 80 cm at the deepest part of each lake; three replicate plates were used per depth. The plates remained for 21 days to allow for periphyton growth. During plate removal, three replicate water samples were collected from the surface water and analyzed for water color, DOC, iron, total phosphorus (TP) and total nitrogen (TN). Color was measured with a Shimadzu UV-1800 spectrophotometer at 410 nm (SFS-EN ISO 7887 standard) (Finnish Standard Association SFS, 2011). DOC was measured with a Shimadzu TOC 5000A analyzer (SFS-EN 1484 standard) (Finnish Standards Association SFS, 1997). Iron was measured with a Varian SpectrAA 220FS spectrophotometer (SFS 3044 standard) (Finnish Standards

Association SFS, 1980). TP and TN were measured with a SEAL AA5000 segmented flow analyzer.

The periphyton biomass was weighed after filtration through 2 µm Whatman GF/C filters. Algae was identified to the genus level microscopically using the Utermöhl technique and algae biomass was calculated using taxon-specific weight estimates (Utermöhl, 1958). Chlorophyll was extracted in ethanol from the filtered and macerated biomass and the concentration of each pigment was measured according to (Lichtenthaler & Wellburn, 1983). I was provided with the completed dataset for analysis; detailed sampling methods and materials can be found in Rajala et al. (2026).

2.2 Research Ethics

No permits were required for sample collection and all animals collected were invertebrates. Nonetheless, sampling was done in such a way to mitigate any disturbance to the lake ecosystems.

Use of large language models: I used ChatGPT as a supplementary tool to help develop code in R.

2.3 Data Analysis

I used multiple model types to investigate the influence of environmental parameters on periphyton biomass and chlorophyll. Water color, DOC, and depth were used as predictors to represent a change in light conditions. I compared the effects of color and DOC in separate models to determine their strength as independent predictors and to avoid collinearity between the variables. TN and TP were also included as predictors to analyze the effect of nutrients. When possible, models were generated using replicates nested within lakes as a random effect to account for the unmeasured variation within and between lakes.

To improve the interpretation of model intercepts and reduce multicollinearity, the variables color, DOC, Fe, TN and TP were mean-centered prior to analysis. When variables of different scales were included in the same model, the centered values were also scaled

by division with their standard deviation. Depth was used at a factor level rather than as a continuous variable since it was measured only at three levels. When predictor variables were determined to have no significant effect or meaningfully improved the model fit, I excluded them from the final model.

I performed all analysis using R (version 4.5.1) (R Core Team, 2025) and relevant packages are listed in appendix Table A2. I assessed model assumptions and fit with residual diagnostic plots. Influential observations were identified using Cook's distance and their influence was evaluated by refitting the model without these points. I retained the observation unless the removal meaningfully changed parameter estimates or model conclusions. I compared model structures using Akaike Information Criterion (AIC) on models fit using maximum likelihood estimation (MLE). I included predictors in the final model based on model fit and ecological relevance. Final linear regression models were fit with MLE; generalized additive models (GAM) and Linear Mixed Effect Models (LMM) were fit with restricted maximum likelihood method (REML).

2.4 Models

I used a linear regression model to determine how DOC and iron influence water color. TP and TN do not directly influence color and were excluded as predictors. I further explored the influence of iron at different DOC concentrations with a Johnson-Neyman interval analysis. I used Pearson's and Spearman's correlation tests to detect relationships between DOC and nutrients: TP and TN.

I used the biomass of each algae class to analyze community composition. I performed a Hellinger square root transformation on the algae biomass to better account for the relative abundance of each taxa and to preserve biomass values of zero. I used permutational multivariate analysis of variance models (PERMANOVA : Bray-Curtis method) to identify community wide trends. I analyzed the community structure within each lake to determine the independent effect of depth. I also analyzed the community structure between lakes at each depth profile. I checked model assumptions with beta

dispersion and assessed the strength of the models with an NMDS stress test, using a stress of below 0.2 to indicate a fair fit (Clarke, 1993).

The Shannon-Wiener diversity index (H') is an assessment of community diversity and was calculated using the number of each algae genus (S) and proportional biomass (p) according to the formula:

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

I analyzed diversity trends with LMM. I also performed a post-hoc comparison of the estimated marginal means between the depth levels, which uses a Tukey-adjusted p-value.

To measure functional diversity, I grouped each identified algae class by their accessory chlorophyll pigments (Table 1). For each sample, I summed the biomass per pigment group and calculated its proportional contribution to the total sample biomass. I then used a LMM to look for trends in algae containing chl b.

Cyanophyceae occurrence was relatively rare, thus, rather than directly measuring the proportional biomass, I performed a Hurdle model to account for the zero-inflated data. I used a generalized linear mixed model (family = binomial) to determine presence and absence and subset only the positive biomass values to use in a truncated LMM model.

Table 1: Classification of algae observed in study lakes.

Class	Common Type Name	Characteristic Pigments
Charophyceae	Green algae	b
Chlorophyceae	Green algae	b
Euglenophyceae	Mixtotrophic flagellates	b
Trebouxiophyceae	Green algae	b
Zygnematophyceae	Green algae	b
Bacillariophyceae	Diatom	c

Chrysophyceae	Golden-Brown algae	c
Dinophyceae	Dinoflagellate	c
Mediophyceae	Diatom	c
Prymnesiophyceae	Haptophyte	c
Raphidophyceae	Raphidophyte	c
Xanthophyceae	Yellow-Green algae	c
Cryptophyceae	Cryptophytes	c, phycobilins
Eustigmatophyceae	Yellow-Green algae	None
Cyanophyceae	Cyanobacteria (blue-green algae)	phycobilins, d, f.

I used a natural log to transform biomass and chlorophyll concentrations to reduce skew and fit a normal distribution. I calculated the chlorophyll ratios using the raw values before transforming with a natural log.

I used a LMM with quadratic terms to model total periphyton biomass. For the algae biomass I used a more flexible GAM to fully capture the different trendlines at each depth. For both total periphyton and algae biomass, I used the best fitting model to estimate the DOC and color values at which peak biomass occurs. To do so, I generated model predictions across the observed DOC or color range, excluding random effects as to determine the system-wide optimum. The DOC or color value corresponding to the peak biomass was numerically identified for each depth that showed a non-linear response.

When analyzing the response of chlorophyll pigments, I included total algae biomass as a covariate in addition to environmental variables to account for differences in algae abundance. I used a GAM to analyze the combined response of each chlorophyll pigment and well as each chlorophyll ratio. Chl a:b and a:car were initially fit assuming a Gaussian distribution of residual errors but showed significant deviations after diagnostic evaluation. I refit the models with a scaled t-distribution (family = scat), which allows for more extreme variability and improved the residual diagnostics.

3 Results

3.1 Water Quality

Lake water color ranged from 22 to 531 mg Pt L^{-1} (median 162 mg Pt L^{-1}), DOC ranged from 6.2 to 40.3 mg L^{-1} (median 18.1 mg L^{-1}), and iron ranged from 0.04 to 3.53 mg L^{-1} (median 0.74 mg L^{-1}). Valkea Mustajärvi had the highest water clarity, with the lowest values of water color, DOC, and iron as well as relatively low nutrient concentrations. Kangas-Lyly has the highest water color, DOC, and the second highest iron concentration as well as relatively high nutrient content (Table 2). The ratio of TN:TP is above 10 in all lakes, suggesting these lakes are likely P limited (Chiaudani & Vighi, 1974). TP and TN increased linearly with one another (Pearson's $r_{(10)} = 0.93$, $p < 0.0001$). TN was moderately positively correlated with DOC (Spearman's $r_s = 0.61$, $p = 0.04$), but there was no evidence of a significant association between TP and DOC (Spearman's $r_s = 0.43$, $p = 0.16$).

Table 2: Physiochemical properties of study lakes.

Lake	Water Color (mg Pt L^{-1})	DOC (mg L^{-1})	Fe (mg L^{-1})	TN (µg L^{-1})	TP (µg L^{-1})	TN:TP
Valkea Mustajärvi	22	6.23	0.04	370	9	41.1
Iso Valkjärvi	44	7.73	0.06	430	18	23.9
Sulkuejärvi	98	17.10	1.26	1733	81	21.4
Neva-Lyly	119	12.89	0.16	453	21	21.6
Hokajärvi	130	13.37	0.83	337	9	37.4
Kynnäröjärvi	156	18.05	1.28	1057	61	17.3
Rahtijärvi	162	17.43	0.51	547	19	28.8
Tiponen	237	25.78	0.74	740	26	28.5
Kärppäjärvi	241	21.57	3.53	488	33	14.8
Käkilammi	278	28.45	1.03	1147	47	24.4
Kalliojärvi	319	30.66	0.42	590	14	42.1
Kangas-Lyly	531	40.33	2.49	690	40	17.3

DOC was a strong predictor of water color and alone accounted for 94% of the variation in color. However, a model including both DOC and iron better explained color variation based on AIC ($\Delta AIC = 7.3$; $F_{3,8} = 120.1$, $R^2 = 0.97$, $p < 0.001$; Table A3; Figure 1). Iron alone did not have a significant relationship with color, but it did amplify the effects of DOC on color. Johnson-Neyman interval analysis indicated the effect of iron became significant only at DOC values above 25 mg L^{-1}. There was no indication that this effect was dependent on iron concentration nor that saturation was reached at high iron values. These models support DOC as the main predictor of color and its use as a reliable proxy; however, at high DOC concentrations, iron may increase the effect of DOC on color.

Both models were sensitive to one high-leverage data point based on cook's distance, however, diagnostic checks with simulated residuals showed no violation of model assumptions and model results were consistent when the data point was excluded.

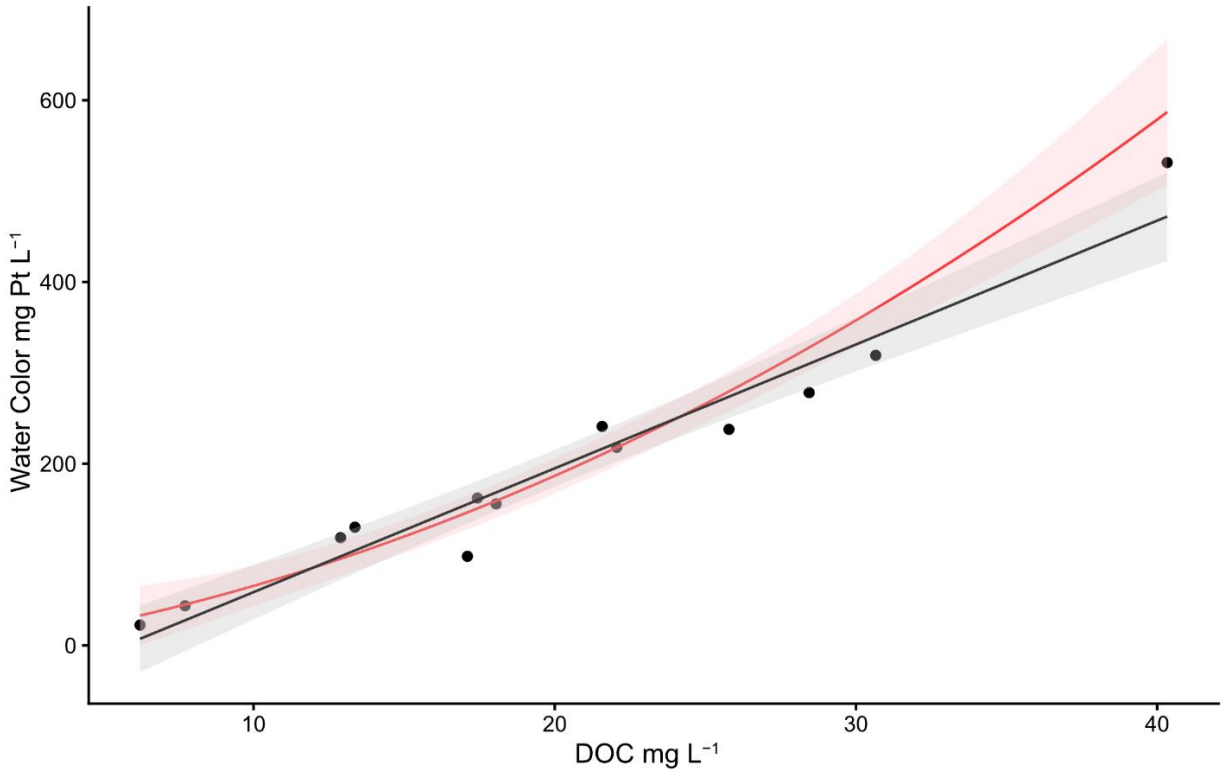

Figure 1: Water color as predicted by DOC only (black) and predicted by DOC and iron (red). The shaded areas represent the 95% CI.

3.2 Algae Community Composition

A total of 15 algae classes were identified in these lakes (Figure 2). The overall dominant algae class was Zygnematophyceae, which accounted for 58.9% of the total algae biomass and was the dominant class in eight lakes. Bacillariophyceae (17.1%) and Dinophyceae (16.1%) were also common.

Within each lake, the algae community composition remained constant and there was no strong influence of depth (PERMANOVA $R^2 = 0.04$, $F_{2,33} = 0.73$, $p = 0.15$; Table A4). Comparing communities between lakes at each depth profile indicated a borderline effect of color on community composition at the deepest sample depth (80 cm: PERMANOVA $R^2 = 0.20$, $F_{1,9} = 2.5$, $p = 0.05$; Table A5).

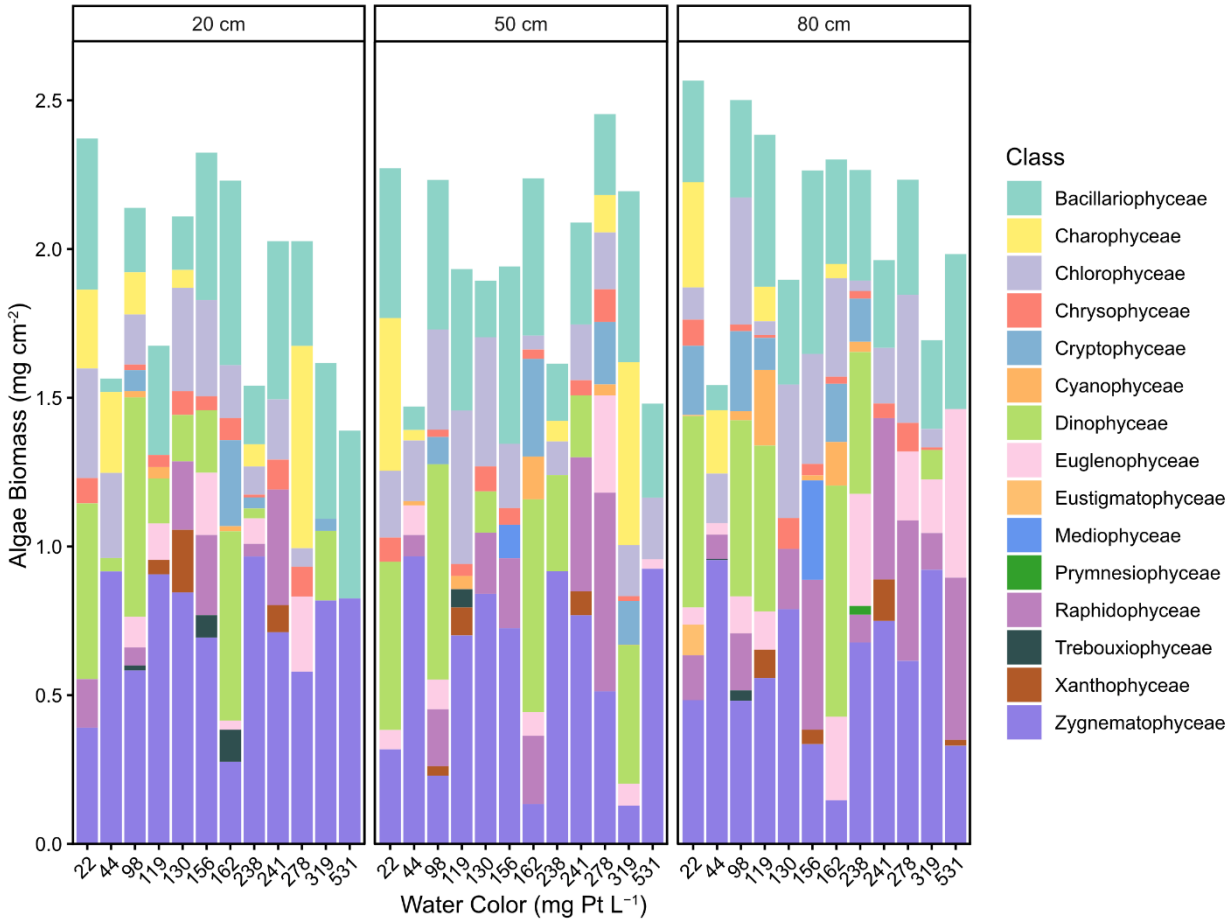

Figure 2: Square-root transformed biomass values of periphytic algae classes in order of increasing water color (left to right) at depths of 20 cm, 50 cm, and 80 cm.

I further investigated changes in functional diversity based on accessory pigments. Algae containing chl b accounted for 64.9% of the total algae biomass and their proportional contribution did not change with increasing color or depth (all $p > 0.05$, Marginal $R^2 = 0.04$, Conditional $R^2 = 0.66$; Table A6). Although chl b algae dominated the community, this pattern does not indicate an increased competitive advantage under low-light conditions. Cyanophyceae occurred in nine of the twelve lakes, and its presence was not dependent on color or nutrient gradients (all $p > 0.05$, Marginal $R^2 = 0.22$, Conditional $R^2 = 0.41$; Table A7(a): Results of Hurdle-Binomial Analysis for the Effect of Color and Depth on the Occurrence of). However, in lakes where it was present, Cyanophyceae biomass increased with depth and there was a positive relationship with color at 80 cm ($\beta = 0.07 \pm 0.02$, $t_{4.19} = 3.02$, $p = 0.037$, Marginal $R^2 = 0.43$, Conditional $R^2 = 0.71$; Table A7(b)). This suggests that although occurrence was not color-dependent, abundance increased under low-light conditions, indicating a possible advantage. Despite this pattern, Cyanophyceae represented only 0.11% of total algae biomass and was not dominant in any sampled lake.

Algae community diversity (H') did not change with color, DOC, or nutrients. However, a LMM showed a positive impact of the deepest sample level on diversity (Marginal $R^2 = 0.10$, Conditional $R^2 = 0.60$, $p = 0.009$; Table A8). Pairwise comparison confirmed diversity was significantly higher at 80 cm than at 20 cm (mean difference = 1.80 ± 0.62, $t_{22} = -2.89$, Tukey $p = 0.02$). Pairwise comparison also revealed the overall algae biomass was lower at depth 80 cm compared to 20 cm (mean difference = 1.06 ± 0.37, $t_{22} = 2.88$, Tukey $p = 0.02$).

3.3 Periphyton Biomass

Total periphyton biomass showed a unimodal relationship at all depths with increasing DOC (Marginal $R^2 = 0.35$, Conditional $R^2 = 0.78$; Table A9). DOC was a stronger predictor of this relationship than color ($\Delta AIC = 37.6$, Δ marginal $R^2 = 0.047$). This curve shape remained consistent at all depths, but the slope slightly flattened with depth. Depth also had a strong negative effect on biomass (50 cm: $\beta = -0.28 \pm 0.13$, $p = 0.032$; 80 cm: $\beta = -0.55 \pm 0.13$, $p = <0.001$). Combining these factors suggests the variance of periphyton biomass between depths is most extreme in brown lakes, represented by the biomass decrease

between 20 and 50 cm, which is 5.5-fold greater in the most brown lake compared to the clearest lake. The predicted biomass peaks occurred at DOC concentrations of 25.3, 22.7, and 21.9 mg L^{-1} (corresponding to color values of ca. 267, 232, 221 mg L^{-1}) at depths of 20, 50, and 80 cm respectively. This illustrates how the light-limitation threshold shifts to lower DOC and color concentrations with increasing depth and light attenuation (Figure 3).

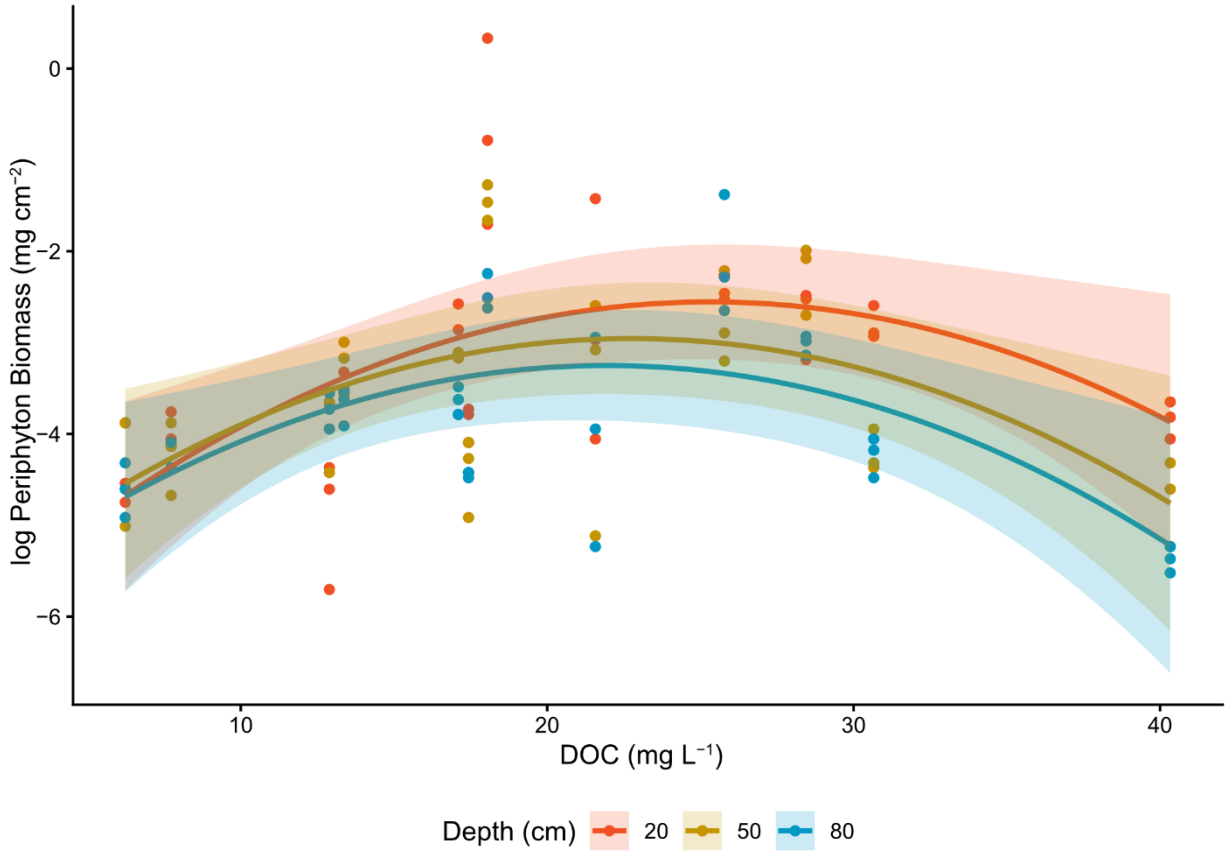

Figure 3. The effect of DOC concentration on the log-transformed total periphyton biomass depth of 20 cm (p=0.021), 50 cm (p=0.028), and 80 cm (p=0.004). Shaded areas represent the 95% confidence intervals.

The GAM indicated a moderately strong non-linear relationship between color and algae biomass (R^2 = 0.52, deviance explained = 58.5%; Table A10). Color provided a slightly better fit than DOC (ΔAIC = 4.8, ΔR^2 = 0.017, Δdeviance explained = 1.3%). A depth of 80 cm had a significant negative effect on the baseline algae biomass (β = -0.95 ± 0.28, p = 0.001), and the effect of color on biomass varied with depth. Biomass showed a non-linear

relationship with color at 20 cm, a moderately negative linear relationship at 50 cm, and a strong linear decline at 80 cm (Figure 4). The predicted maximum biomass at 20 cm occurred at a color of 359 mg Pt L^{-1} (corresponding to a DOC of ca. 31 mg L^{-1}).

In both total periphyton and algae biomass models, the variation between sample replicates within lakes was negligible, whereas unmeasured between-lake differences accounted for most of the biomass variance.

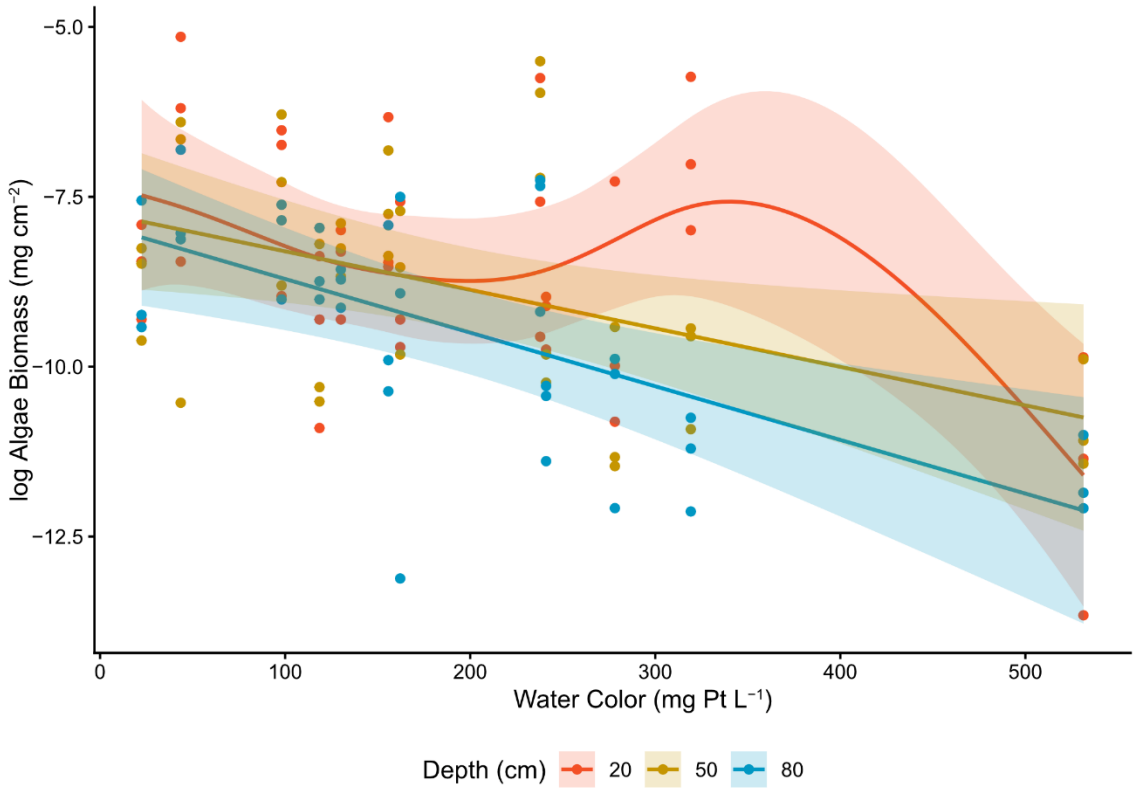

Figure 4. Effect of water color on the log-transformed algae biomass at depth of 20 cm (p=0.004), 50 cm (0.016), and 80 cm (p<0.001). Shaded areas represent the 95% confidence intervals.

3.4 Chlorophyll

DOC and color performed equally well in explaining chlorophyll variation, and the following results are presented using color models. Chlorophyll concentrations showed a strong

non-linear response to color and depth (R^2 = 0.88, deviance explained = 88.4%; Table A11). The baseline concentration of chl a was higher than chl b and carotenoids and had an average chl a:b ratio of 4.3 and chl a:car ratio of 4.7 across all sample lakes. However, including pigment specific smooth terms did not improve model fit (ΔAIC = 26) which indicates the response of chlorophyll to the light gradient was consistent across pigment type. The overall chlorophyll concentration strongly decreased with depth (50 cm: β = -0.32 ± 0.07, p = <0.001; 80 cm: β = -0.76 ± 0.07, p = <0.001). The effect of color on chlorophyll concentration varied by depth level. At 20 cm, chlorophyll displayed a significant non-linear trend across the color gradient, with a predicted chlorophyll peak at color 382 mg Pt L^{-1}(ca. DOC 33 mg L^{-1}). At depth 50 cm, chlorophyll had a mostly linear significant relationship with color while depth 80 cm showed a linear, but non-significant, relationship (Figure 5). Algae biomass, TP and TN had no significant influence on the variation in chlorophyll concentration.

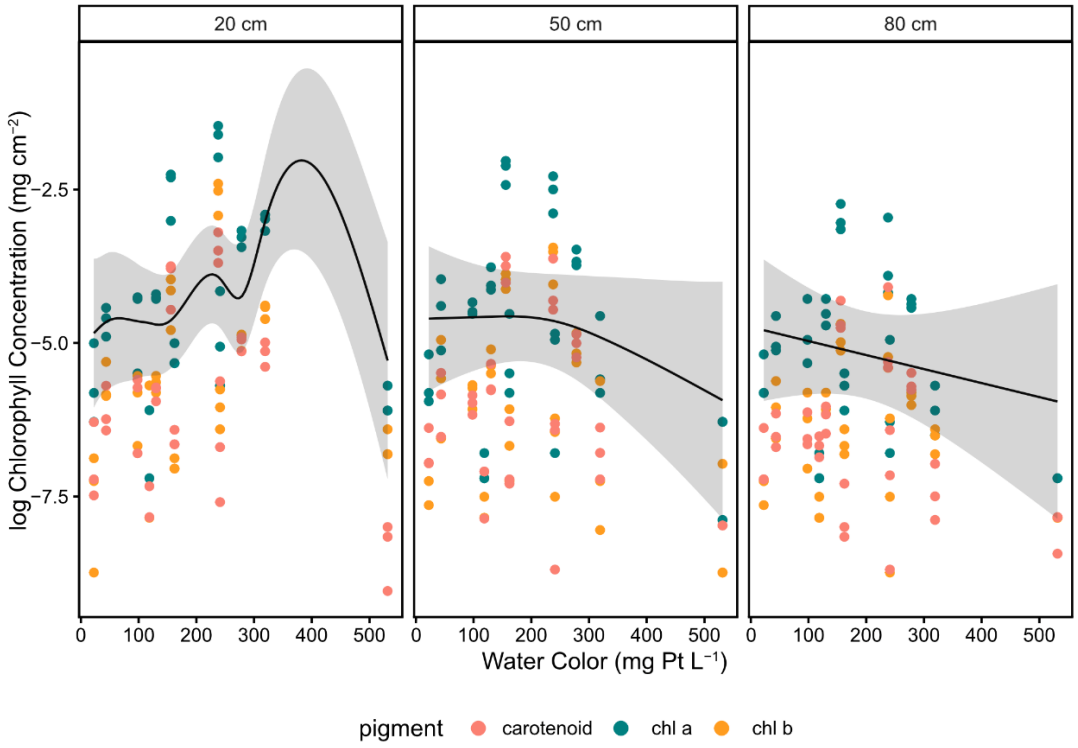

Figure 5. Overall log-transformed chlorophyll concentration response to color at depths (left to right) 20 cm (p < 0.001), 50 cm (p=0.040), and 80 cm (p = 0.387). The shaded area represents the 95% confidence interval.

The baseline chl a:b ratio did not vary with depth, but its relationship with color differed among depth levels (R^2 = 0.16, deviance explained = 54.1%; Table A12). At 20 cm, the ratio had a complex non-linear response to color, whereas at 50 cm it remained stable until increasing at color values above 310 Pt mg L^{-1} (ca. DOC 28 mg L^{-1}). At 80 cm there was no significant relationship with color (Figure 6).

Chl a:car also did not have a consistent relationship between color and depth (R^2 = 0.73, deviance explained = 58.4%; Table A13). At a depth of 20 cm, the ratio was constant at low colors and began to increase when color was greater than 172 Pt mg L^{-1}(ca. DOC 18 mg L^{-1}). There was no significant relationship with color at deeper levels, however, the baseline ratio did decrease strongly with depth (50 cm: $\beta = -0.10 \pm 0.04$, $p = 0.012$; 80 cm: $\beta = -0.20 \pm 0.04$, $p < 0.001$).

The ratio of chl a:bm had a consistent positive linear relationship with color at all depths (R^2 = 0.46, deviance explained = 52.9%; Table A14) though this relationship was only significant at the shallowest depth. Additionally, there was no change in the baseline ratio between depths. There was a clear and consistent pattern of increased chl a concentration relative to biomass at darker colors, indicating a possible adaptation of algae to the environmental changes with browning.

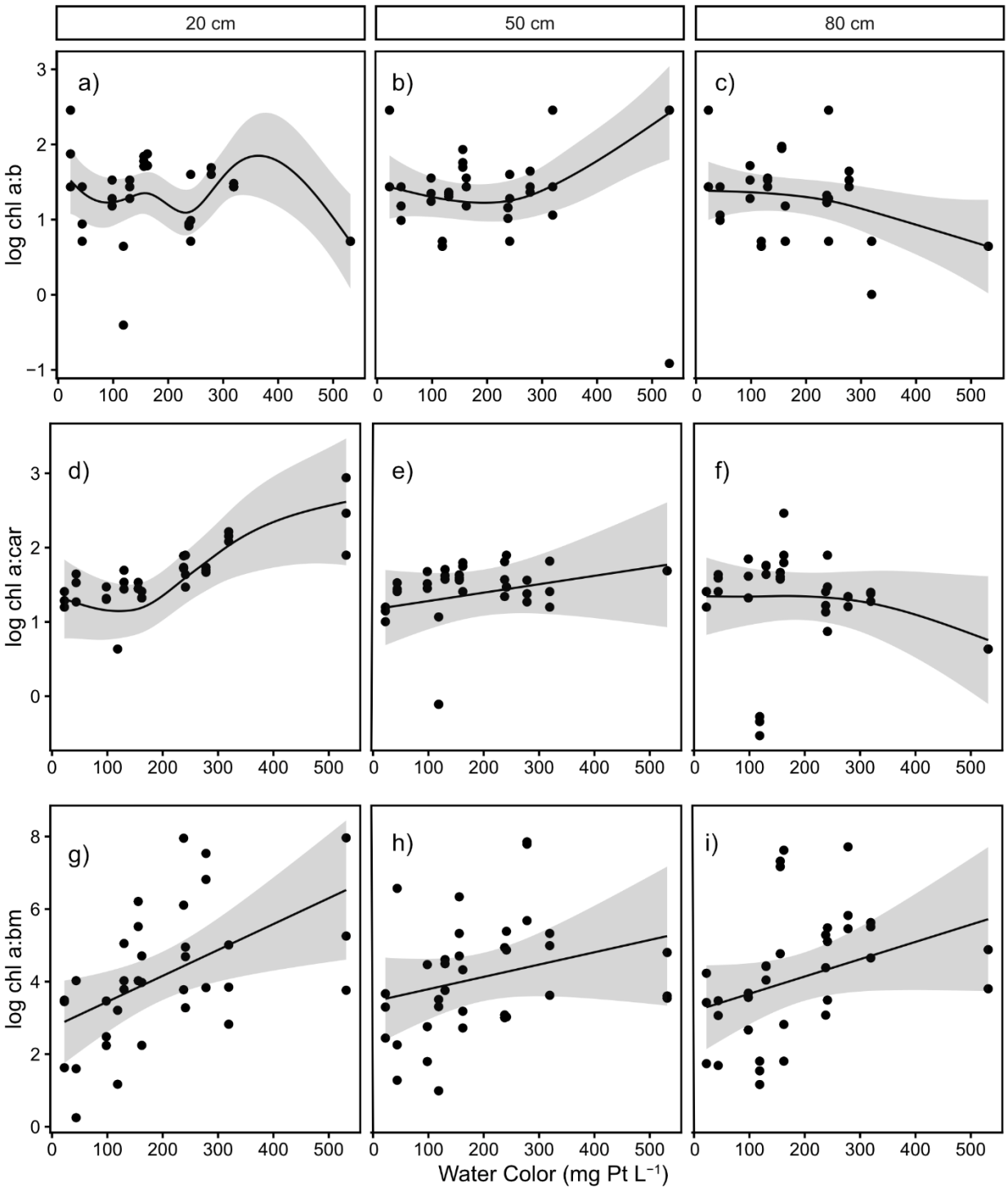

Figure 6. Relationships between log-transformed pigment ratios and water color at three depths (20, 50, and 80 cm). Rows represent different pigment ratios: chl a:b (a-c), chl a:car (d-f), and chl a:biomass (g-i). Columns represent depth levels. Shaded areas indicate 95% confidence intervals. Significance of trends: a: p=0.003, b: p=0.003, c: p=0.137, d: p<0.001, e: p=0.329, f: p=0.089, g: p=0.010, h: p=0.216, i: p=0.092.

4. Discussion

4.1 Water Quality

Water quality varied widely among the study lakes, particularly in regard to DOC and color, and this variation provides important context for interpreting the periphyton and algal responses. In many temperate lake studies, DOC concentrations above 15 mg L^{-1} are relatively uncommon (Horppila et al., 2023) and shifts in light and nutrient conditions are often most pronounced at the lower end of the DOC gradient (Creed et al., 2018; Jones & Arvola, 1984; Solomon et al., 2015). While it is still uncertain at what DOC concentration the threshold shift from nutrient to light limitation is reached, most lakes in my study are above the estimated 11 – 16 mg L^{-1} range (Bergström & Karlsson, 2019; Horppila et al., 2023, 2024). This suggests my study lakes may already be within a "brown-water" regime where additional DOC inputs primarily intensify light limitation rather than fundamentally altering water chemistry. This helps explain why DOC and color were strong predictors of light-related responses (periphyton biomass, chlorophyll), while other water quality variables played a comparatively minor role.

My model of water color suggests that iron increased color at high DOC levels, contrary to previous studies which show iron only influences color at low DOC (Horppila et al., 2023). Nonetheless, for the most part DOC and color performed equally well in models as predictors of periphyton response, indicating that the inclusion of iron did not significantly enhance the use of color as a proxy for estimating light attenuation.

I also did not find a strong relationship between DOC and other nutrients: TP and TN. A positive correlation has been described in other studies, suggesting that DOM loading does increase the total amount of these nutrients in the lake (Corman et al., 2018). However, the ratio of DOC:P and DOC:N may decline over time (Stetler et al., 2021) as P and N are more readily taken up by producers while humic DOC is retained in the lake, decoupling the relationship between them. Additionally, in this study P content was a measurement of the total inorganic and organic forms (TP) but phosphate $[PO_4]^{3-}$ is the only form that is readily available for uptake by algae. TP is easier to measure and informs of the

total P loading within lakes but does not directly indicate what percentage of P is bioavailable and, therefore, more likely to influence periphyton growth.

Another limitation of this study was its short time frame. The strength of nutrient limitation may change with seasonality, as the bioavailable nutrients are more quickly depleted (Isles et al., 2020). The quality of the DOC in these study lakes may play a role, as one study showed a stronger response of phytoplankton to autochthonous and freshly released DOM (Ai et al., 2024). Future research could investigate how the quality of DOC varies with catchment type and if this influences the sensitivity of lakes to browning.

4.2 Algae Communities

In contrast to several studies reporting strong browning-induced shifts in algal community composition (Senar et al., 2021), the communities in our study remained relatively stable across the browning gradient. Most of my study lakes had DOC concentrations above the range where major compositional changes are observed (4 – 13 mg L^{-1}), which may explain the limited structural response. This further supports the idea that aquatic communities may be more sensitive to browning in clear lakes and stabilize in high DOC lakes. Instead, the main changes in my study occurred in chlorophyll production and diversity patterns, suggesting that physiological adjustments rather than taxonomic turnover dominated the response to reduced light availability.

Furthermore, I did not see any shifts in the proportion of algae containing chl b to indicate an increased competitive advantage under browning. Other studies have shown a dominance of green algae and diatoms in low DOC lakes before competitive exclusion at high DOC by mixotrophic species (Senar et al., 2021; Urrutia-Cordero et al., 2017). In the case of strong light attenuation, trophic level may play a stronger functional role than chlorophyll pigment type (Wilken et al., 2018).

Additionally, the response of cyanobacteria to browning is uncertain in literature; cyanobacteria biomass generally dominate in high-nutrient lakes (O'Neil et al., 2012) but some studies on temperate lakes have shown either no effects of browning (Urrutia-Cordero et al., 2017), species specific responses (Ekvall et al., 2013), or a shift to

cyanobacteria dominance only between 4 – 8 mg L^{-1} DOC (Senar et al., 2021). In my study, Cyanophyceae abundance was relatively low compared to other classes and biomass only showed a positive response to browning at the deepest sample level, suggesting that only when competition from other algae is already low was cyanobacteria favored. I recommend additional research to better understand these trends and predict how cyanobacteria will respond to browning.

The only indication of community structure and diversity changes occurred at the deepest sample level. The increased algae diversity may be a result of decreased competition due to the overall lower biomass at depth 80 cm (Wisheu & Keddy, 1992), as well as greater fluctuations in light availability preventing competitive exclusion by certain species (Flöder et al., 2002; Litchman et al., 2004). However, while there was weak evidence that structural changes occurred in response to color in my community model, the increased diversity happened in all lakes, regardless of browning.

4.3 Periphyton Biomass

The observed unimodal response of periphyton biomass to increasing DOC aligns with previous work showing that browning initially stimulates aquatic production through nutrient inputs before light limitation becomes the primary driver (Horppila et al., 2023; Karlsson et al., 2009; Seekell et al., 2015). The depth-specific patterns further support this mechanism: as light attenuation increases down the water column, the positive nutrient-driven phase weakens and biomass declines more profoundly, consistent with studies that demonstrated strong vertical gradients in benthic productivity under brown water conditions (Vasconcelos et al., 2016). This is highlighted by the shift in peak biomass, as the threshold of light limitation was reached at lower DOC and color values with increasing depth.

In contrast, the algae biomass only displayed this non-linear increase at the shallowest depth, where lakes may be either nutrient or light limited, and a more direct relationship at lower depths as biomass decreased with browning. Compared to the total periphyton biomass, which includes multiple animal, fungi, and microbe taxa, the algae biomass

appeared to be more strongly impacted by light limitation down the water column. Interestingly, the peak algae biomass occurred at a higher color and DOC concentration than the total periphyton biomass. While this could indicate a difference in where the light-limitation threshold occurs for the two communities, which could be explained by variation in trophic level, tolerance to biochemical and physical stressors, and ability to access nutrients (Blanchet et al., 2022; Chen et al., 2025; Creed et al., 2018), it is more likely a variation due to small sample size. Additionally, the sample plates were removed after three weeks of incubation and it can not be confidently known whether the periphyton in this study was grazed upon prior to collection.

Furthermore, all observed thresholds in this study occurred at higher DOC and color concentrations than predicted by other studies (Bergström & Karlsson, 2019; Horppila et al., 2023; Seekell et al., 2015). At what DOC concentration light limitation occurs is specific to each lake and dependent on factors such as lake size and DOM composition (Kelly et al., 2018). While determining one "browning threshold" across all lakes is likely futile, future studies that include a greater sample range of brown lakes can help better estimate the DOC range at which fundamental community changes transpire.

4.4 Chlorophyll Dynamics

Light-driven adjustments in chlorophyll concentration and pigment composition are well-documented responses of algae to browning and reduced underwater irradiance (Felip & Catalan, 2000; Thrane et al., 2014). Consistent with these studies, chlorophyll concentration in my dataset showed the strongest response at shallow depths where light availability was highest, including a non-linear increase with color and a peak at moderate DOC levels. The offset between the chlorophyll peak and the peak in algal biomass has also been observed in other browning studies and reflects the capacity of algae to increase pigment content per cell under low-light conditions (Finenko et al., 2003; Geider et al., 1997). This is further illustrated by the linear increase in the chl a:bm ratio and suggests physiological acclimation to declining light, with algae increasing chlorophyll production even as biomass declined in darker lakes. However, this pattern weakened and the overall

chlorophyll concentration decreased with depth as the strength of light limitation exceeded the compensation capacity of the algae.

The depth-specific responses in the pigment ratios further support the interpretation that algae may change pigment content with light intensity. For example, the increase in chl a:car at shallow depths aligns with findings that carotenoid investment decreases when photoprotection is less necessary in shaded environments (Pizarro & Stange, 2009). The chl a:b relationship with color was highly variable with depth and contradicted my initial prediction of a decreasing ratio. Although previous studies have shown decreased chl a:b in shaded conditions (Yamazaki et al., 2005), others have shown only weak associations or increased ratios and suggest that changes in chl a:b may be species specific (Beneragama & Goto, 2011).

In terms of overall suitability as a bioindicator, chl a:b and chl a:car showed inconsistent responses at each depth profile. Additionally, the non-linearity of the patterns increases the complexity of interpretation and diminishes their validity as bioindicators for certain ranges of lake color. Chl a:bm had a consistent baseline ratio and linear increase at all depths and is, in my opinion, the strongest candidate to act as a quantitative bioindicator for browning. Furthermore, because color and DOC were equally good predictors for the chlorophyll models, the ratio can be compared against either parameter. The strongest response of chl a:bm was seen at shallow depths so I recommend samples be taken near the water surface.

4.5 Conclusions

Overall, my results suggest that browning primarily alters algal physiology rather than community composition, with periphytic algae showing stable taxonomic structure and support for earlier findings that show clear pigment-based adjustments under reduced light (Finenko et al., 2003; Thrane et al., 2014). Consistent with studies reporting unimodal productivity responses to DOC, periphyton biomass peaked at moderate color levels and declined under stronger light limitation (Horppila et al., 2023; Karlsson et al., 2009; Seekell et al., 2015). These patterns highlight that physiological acclimation via increased

chlorophyll production may be a key mechanism enabling algae to persist in brown-water environments. This adaptive response is represented by the increased chl a:bm ratio along a color gradient and I propose the use of this ratio as a possible bioindicator for the impact of browning on aquatic producers. The feasibility of these bioindicators should be further studied in broader contexts as this study was limited in spatial and temporal scope. Browning is more widely studied in low and mid color boreal lakes, but to robustly understand the effects, lakes should be sampled in a variety of biomes, catchment types, and with a large range of DOC and color content. The response of aquatic communities to browning may also vary with seasonality and more long-term studies are needed. Browning has become recognized as a serious issue for aquatic ecosystems and requires more research into monitoring and restoration methods.

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References

Ai, Y., Cheng, H., Quan, W., Wang, Q., Gong, Y., Yang, K., Wu, Y., Zhang, J., & Huang, D. (2024). Response of phytoplankton community to dissolved organic matter composition and lake trophic state. *Journal of Environmental Management*, *370*, 122793. https://doi.org/10.1016/j.jenvman.2024.122793

Albrecht, E., Hannonen, O., Palacín-Lizarbe, C., Suni, J., Härkönen, L. H., Soininen, N., Kukkonen, J., & Vainikka, A. (2023). Browning of boreal lakes: Do public perceptions and governance align with the biological foundations? *Ecological Applications*, *33*(5), 1–23.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Beneragama, C., & Goto, K. (2011). Chlorophyll *a*: *B* Ratio Increases Under Low-light in "Shade-tolerant" *Euglena gracilis*. *Tropical Agricultural Research*, *22*(1), 12. https://doi.org/10.4038/tar.v22i1.2666

Bergström, A.-K., & Karlsson, J. (2019). Light and nutrient control phytoplankton biomass responses to global change in northern lakes. *Global Change Biology*, *25*(6), 2021–2029. https://doi.org/10.1111/gcb.14623

Bláha, L., Babica, P., & Maršálek, B. (2009). Toxins produced in cyanobacterial water blooms – toxicity and risks. *Interdisciplinary Toxicology*, *2*(2), 36–41. https://doi.org/10.2478/v10102-009-0006-2

Blanchet, C. C., Arzel, C., Davranche, A., Kahilainen, K. K., Secondi, J., Taipale, S., Lindberg, H., Loehr, J., Manninen-Johansen, S., Sundell, J., Maanan, M., & Nummi, P. (2022). Ecology and extent of freshwater browning—What we know and what should be studied next in the context of global change. *Science of The Total Environment*, *812*, 152420. https://doi.org/10.1016/j.scitotenv.2021.152420

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a Metabolic Theory of Ecology. *Ecology*, *85*(7), 1771–1789. https://doi.org/10.1890/03-9000

Brüsecke, J., Muotka, T., Huttunen, K.-L., Litjo, S., Lepo, W.-P., & Jyväsjärvi, J. (2023). Drainage-induced browning causes both loss and change of benthic biodiversity in headwater streams. *Limnology and Oceanography Letters*, *8*(4), 620–627. https://doi.org/10.1002/lol2.10302

Chen, J., Cheng, H., Yan, Y., Zhu, J., Zhang, M., Xie, S., Xu, J., & Wang, H. (2025). Primary producers in freshwater ecosystem respond differently to multiple environmental stressors: A mesocosm study. *Environmental Research*, *272*, 121145. https://doi.org/10.1016/j.envres.2025.121145

Chiaudani, G., & Vighi, M. (1974). The N:P ratio and tests with Selenastrum to predict eutrophication in lakes. *Water Research*, *8*(12), 1063–1069. https://doi.org/10.1016/0043-1354(74)90149-3

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, *18*(1), 117–143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x

Corman, J. R., Bertolet, B. L., Casson, N. J., Sebestyen, S. D., Kolka, R. K., & Stanley, E. H. (2018). Nitrogen and Phosphorus Loads to Temperate Seepage Lakes Associated With Allochthonous Dissolved Organic Carbon Loads. *Geophysical Research Letters*, *45*(11), 5481–5490. https://doi.org/10.1029/2018GL077219

Creed, I. F., Bergström, A.-K., Trick, C. G., Grimm, N. B., Hessen, D. O., Karlsson, J., Kidd, K. A., Kritzberg, E., McKnight, D. M., Freeman, E. C., Senar, O. E., Andersson, A., Ask, J., Berggren, M., Cherif, M., Giesler, R., Hotchkiss, E. R., Kortelainen, P., Palta, M. M., … Weyhenmeyer, G. A. (2018). Global change-driven effects on dissolved organic matter composition: Implications for food webs of northern lakes. *Global Change Biology*, *24*(8), 3692–3714. https://doi.org/10.1111/gcb.14129

de Wit, H. A., Valinia, S., Weyhenmeyer, G. A., Futter, M. N., Kortelainen, P., Austnes, K., Hessen, D. O., Räike, A., Laudon, H., & Vuorenmaa, J. (2016). Current Browning of Surface Waters Will Be Further Promoted by Wetter Climate. *Environmental Science & Technology Letters*, *3*(12), 430–435. https://doi.org/10.1021/acs.estlett.6b00396

Ekvall, M. K., de la Calle Martin, J., Faassen, E. J., Gustafsson, S., Lürling, M., & Hansson, L.-A. (2013). Synergistic and species-specific effects of climate change and water colour on cyanobacterial toxicity and bloom formation. *Freshwater Biology*, *58*(11), 2414–2422. https://doi.org/10.1111/fwb.12220

Eloranta, P. (1978). Light Penetration in Different Types of Lakes in Central Finland. *Holarctic Ecology*, *1*(4), 362–366.

Estlander, S., & Horppila, J. (2023). Effects of dissolved organic carbon gradient on epilimnetic zooplankton communities in lakes. *Hydrobiologia*, *850*(18), 4015–4027. https://doi.org/10.1007/s10750-023-05284-6

Estlander, S., Nurminen, L., Rajala, S., Olin, M., & Horppila, J. (2024). Rotifer functionality as a potentially useful indicator of lake browning. *Limnologica*, *108*, 126194. https://doi.org/10.1016/j.limno.2024.126194

Estlander, S., Pippingsköld, E., & Horppila, J. (2021). Artificial ditching of catchments and brownification-connected water quality parameters of lakes. *Water Research*, *205*, 117674. https://doi.org/10.1016/j.watres.2021.117674

Evans, C. D., Monteith, D. T., & Cooper, D. M. (2005). Long-term increases in surface water dissolved organic carbon: Observations, possible causes and environmental impacts. *Environmental Pollution, Recovery from Acidificationin the UK: Evidence from 15 Years of Acid Waters Monitoring*, *137*(1), 55–71. https://doi.org/10.1016/j.envpol.2004.12.031

Felip, M., & Catalan, J. (2000). The relationship between phytoplankton biovolume and chlorophyll in a deep oligotrophic lake: Decoupling in their spatial and temporal maxima. *Journal of Plankton Research*, *22*(1), 91–106. https://doi.org/10.1093/plankt/22.1.91

Finenko, Z. Z., Hoepffner, N., Williams, R., & Piontkovski, S. A. (2003). Phytoplankton carbon to chlorophyll a ratio: Response to light, temperature and nutrient limitation. *Morskyi Ekolohichnyi Zhurnal (Marine Ecological Journal)*, *2*(2), 40–64.

Finnish Standard Association SFS. (2011). *Examination and determination of colour* (Standard SFS-EN ISO 7887; Water Quality).

Finnish Standards Association SFS. (1980). *Metal content of water, sludge and sediment determined by atomic absorption spectroscopy, atomization in flame* (Standard SFS 3044; General Principles and Guidelines).

Finnish Standards Association SFS. (1997). *Guidelines for the determination of total organic carbon and dissolved organic carbon.* (Standard SFS-EN 1484; Water Analysis).

Finstad, A. G., Andersen, T., Larsen, S., Tominaga, K., Blumentrath, S., de Wit, H. A., Tømmervik, H., & Hessen, D. O. (2016). From greening to browning: Catchment vegetation development and reduced S-deposition promote organic carbon load on decadal time scales in Nordic lakes. *Scientific Reports*, *6*(1), 31944. https://doi.org/10.1038/srep31944

Flöder, S., Urabe, J., & Kawabata, Z. (2002). The influence of fluctuating light intensities on species composition and diversity of natural phytoplankton communities. *Oecologia*, *133*(3), 395–401. https://doi.org/10.1007/s00442-002-1048-8

Gan, F., & Bryant, D. A. (2015). Adaptive and acclimative responses of cyanobacteria to far-red light. *Environmental Microbiology*, *17*(10), 3450–3465. https://doi.org/10.1111/1462-2920.12992

Geider, R., Macintyre, H., & Kana, T. (1997). Dynamic model of phytoplankton growth and acclimation: Responses of the balanced growth rate and the chlorophyll a:carbon ratio to light, nutrient-limitation and temperature. *Marine Ecology Progress Series*, *148*, 187–200. https://doi.org/10.3354/meps148187

Hartig, F. (2024). *DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models*. https://doi.org/10.32614/CRAN.package.DHARMa

Horppila, J., Keskinen, S., Nurmesniemi, M., Nurminen, L., Pippingsköld, E., Rajala, S., Sainio, K., & Estlander, S. (2023). Factors behind the threshold-like changes in lake ecosystems along a water colour gradient: The effects of dissolved organic carbon and iron on euphotic depth, mixing depth and phytoplankton biomass. *Freshwater Biology*, *68*(6), 1031–1040. https://doi.org/10.1111/fwb.14083

Horppila, J., Nurminen, L., Rajala, S., & Estlander, S. (2024). Making waves: The sensitivity of lakes to brownification and issues of concern in ecological status assessment. *Water Research*, *249*, 120964. https://doi.org/10.1016/j.watres.2023.120964

Horppila, J., Nurminen, L., Rajala, S., & Estlander, S. (2025). The Effect of Water Colour on Fish Communities in Boreal lakes. *Ecosystems*, *28*(1), 3. https://doi.org/10.1007/s10021-024-00954-5

Isles, P. D. F., Jonsson, A., Creed, I. F., & Bergström, A.-K. (2020). Does browning affect the identity of limiting nutrients in lakes? *Aquatic Sciences*, *82*(2), 45. https://doi.org/10.1007/s00027-020-00718-y

Jones, R. I., & Arvola, L. (1984). Light penetration and some related characteristics in small forest lakes in Southern Finland. *SIL Proceedings, 1922-2010*, *22*(2), 811–816. https://doi.org/10.1080/03680770.1983.11897390

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

Kellerman, A. M., Kothawala, D. N., Dittmar, T., & Tranvik, L. J. (2015). Persistence of dissolved organic matter in lakes related to its molecular characteristics. *Nature Geoscience*, *8*(6), 454–457. https://doi.org/10.1038/ngeo2440

Kelly, P. T., Solomon, C. T., Zwart, J. A., & Jones, S. E. (2018). A Framework for Understanding Variation in Pelagic Gross Primary Production of Lake Ecosystems. *Ecosystems*, *21*(7), 1364–1376. https://doi.org/10.1007/s10021-018-0226-4

Kling, G. W. (1988). Comparative transparency, depth of mixing, and stability of stratification in lakes of Cameroon, West Africa1. *Limnology and Oceanography*, *33*(1), 27–40. https://doi.org/10.4319/lo.1988.33.1.0027

Knoll, L. B., Williamson, C. E., Pilla, R. M., Leach, T. H., Brentrup, J. A., & Fisher, T. J. (2018). Browning-related oxygen depletion in an oligotrophic lake. *Inland Waters*, *8*(3), 255–263. https://doi.org/10.1080/20442041.2018.1452355

Kritzberg, E. S. (2017). Centennial-long trends of lake browning show major effect of afforestation. *Limnology and Oceanography Letters*, *2*(4), 105–112. https://doi.org/10.1002/lol2.10041

Kritzberg, E. S., & Ekström, S. M. (2012). Increasing iron concentrations in surface waters – a factor behind brownification? *Biogeosciences*, *9*(4), 1465–1478. https://doi.org/10.5194/bg-9-1465-2012

Kritzberg, E. S., Hasselquist, E. M., Škerlep, M., Löfgren, S., Olsson, O., Stadmark, J., Valinia, S., Hansson, L.-A., & Laudon, H. (2020). Browning of freshwaters: Consequences to ecosystem services, underlying drivers, and potential mitigation measures. *Ambio*, *49*(2), 375–390. https://doi.org/10.1007/s13280-019-01227-5

Kume, A., Akitsu, T., & Nasahara, K. N. (2018). Why is chlorophyll b only used in light-harvesting systems? *Journal of Plant Research*, *131*(6), 961–972. https://doi.org/10.1007/s10265-018-1052-7

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, *82*(13), 1–26. https://doi.org/10.18637/jss.v082.i13

Lavonen, E. E., Gonsior, M., Tranvik, L. J., Schmitt-Kopplin, P., & Köhler, S. J. (2013). Selective Chlorination of Natural Organic Matter: Identification of Previously Unknown Disinfection Byproducts. *Environmental Science & Technology*, *47*(5), 2264–2271. https://doi.org/10.1021/es304669p

Ledesma, J. L. J., Köhler, S. J., & Futter, M. N. (2012). Long-term dynamics of dissolved organic carbon: Implications for drinking water supply. *Science of The Total Environment*, *432*, 1–11. https://doi.org/10.1016/j.scitotenv.2012.05.071

Lenth, R. V., & Piaskowski, J. (2026). *emmeans: Estimated Marginal Means, aka Least-Squares Means*. https://doi.org/10.32614/CRAN.package.emmeans

Lichtenthaler, H. K., & Wellburn, A. R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions*, *11*(5), 591–592. https://doi.org/10.1042/bst0110591

Litchman, E., Klausmeier, C. A., & Bossard, P. (2004). Phytoplankton nutrient competition under dynamic light regimes. *Limnology and Oceanography*, *49*(4part2), 1457–1462. https://doi.org/10.4319/lo.2004.49.4_part_2.1457

Lüdecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P., & Makowski, D. (2021). performance: An R Package for Assessment, Comparison and Testing of Statistical Models. *Journal of Open Source Software*, *6*(60), 3139. https://doi.org/10.21105/joss.03139

Maloney, K. O., Morris, D. P., Moses, C. O., & Osburn, C. L. (2005). The Role of Iron and Dissolved Organic Carbon in the Absorption of Ultraviolet Radiation in Humic Lake Water. *Biogeochemistry*, *75*(3), 393–407. https://doi.org/10.1007/s10533-005-1675-3

Meyer-Jacob, C., Michelutti, N., Paterson, A. M., Cumming, B. F., Keller, W. (Bill), & Smol, J. P. (2019). The browning and re-browning of lakes: Divergent lake-water organic carbon trends linked to acid deposition and climate change. *Scientific Reports*, *9*(1), 16676. https://doi.org/10.1038/s41598-019-52912-0

Monteith, D. T., Stoddard, J. L., Evans, C. D., de Wit, H. A., Forsius, M., Høgåsen, T., Wilander, A., Skjelkvåle, B. L., Jeffries, D. S., Vuorenmaa, J., Keller, B., Kopácek, J., & Vesely, J. (2007). Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature*, *450*(7169), 537–540. https://doi.org/10.1038/nature06316

Murdoch, A., Gray, D. K., Korosi, J., Vucic, J. M., Cohen, R. S., & Sharma, S. (2021). Drivers of fish biodiversity in a rapidly changing permafrost landscape. *Freshwater Biology*, *66*(12), 2301–2321. https://doi.org/10.1111/fwb.13834

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Borman, T., Carvalho, G., Chirico, M., Caceres, M. D., … Weedon, J. (2026). *vegan: Community Ecology Package*. https://doi.org/10.32614/CRAN.package.vegan

O'Neil, J. M., Davis, T. W., Burford, M. A., & Gobler, C. J. (2012). The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae, Harmful Algae--The Requirement for Species-Specific Information*, *14*, 313–334. https://doi.org/10.1016/j.hal.2011.10.027

Pilla, R. M., & Couture, R.-M. (2021). Attenuation of photosynthetically active radiation and ultraviolet radiation in response to changing dissolved organic carbon in browning lakes: Modeling and parametrization. *Limnology and Oceanography*, *66*(6), 2278–2289. https://doi.org/10.1002/lno.11753

Pizarro, L., & Stange, C. (2009). Light-dependent regulation of carotenoid biosynthesis in plants. *Ciencia e Investigación Agraria*, *36*(2), 143–162. https://doi.org/10.4067/S0718-16202009000200001

R Core Team. (2025). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Rajala, S., Estlander, S., Nurminen, L., Sainio, K., & Horppila, J. (2024). Seasonal fluctuations in pigment content of macrophytes: Implications for monitoring brownification. *Hydrobiologia*, *851*(3), 633–648. https://doi.org/10.1007/s10750-023-05358-5

Rajala, S., Horppila, J., Nurminen, L., & Estlander, S. (2026). Periphyton pigment shifts in lakes with varying water colour: A possible monitoring method for freshwater Browning. *Ecological Indicators*, *183*, 114621. https://doi.org/10.1016/j.ecolind.2026.114621

Sandmann, G. (2019). Antioxidant Protection from UV- and Light-Stress Related to Carotenoid Structures. *Antioxidants*, *8*(7), 219. https://doi.org/10.3390/antiox8070219

Seekell, D. A., Lapierre, J., Ask, J., Bergström, A., Deininger, A., Rodríguez, P., & Karlsson, J. (2015). The influence of dissolved organic carbon on primary production in northern lakes. *Limnology and Oceanography*, *60*(4), 1276–1285. https://doi.org/10.1002/lno.10096

Senar, O. E., Creed, I. F., & Trick, C. G. (2021). Lake browning may fuel phytoplankton biomass and trigger shifts in phytoplankton communities in temperate lakes. *Aquatic Sciences*, *83*(2), 21. https://doi.org/10.1007/s00027-021-00780-0

Smith, V. H., Tilman, G. D., & Nekola, J. C. (1999). Eutrophication: Impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution*, *100*(1), 179–196. https://doi.org/10.1016/S0269-7491(99)00091-3

Solomon, C. T., Jones, S. E., Weidel, B. C., Buffam, I., Fork, M. L., Karlsson, J., Larsen, S., Lennon, J. T., Read, J. S., Sadro, S., & Saros, J. E. (2015). Ecosystem Consequences of Changing Inputs of Terrestrial Dissolved Organic Matter to Lakes: Current Knowledge and Future Challenges. *Ecosystems*, *18*(3), 376–389. https://doi.org/10.1007/s10021-015-9848-y

Stetler, J. T., Knoll, L. B., Driscoll, C. T., & Rose, K. C. (2021). Lake browning generates a spatiotemporal mismatch between dissolved organic carbon and limiting nutrients. *Limnology and Oceanography Letters*, *6*(4), 182–191. https://doi.org/10.1002/lol2.10194

Thrane, J.-E., Hessen, D. O., & Andersen, T. (2014). The Absorption of Light in Lakes: Negative Impact of Dissolved Organic Carbon on Primary Productivity. *Ecosystems*, *17*(6), 1040–1052. https://doi.org/10.1007/s10021-014-9776-2

Urrutia-Cordero, P., Ekvall, M. K., Ratcovich, J., Soares, M., Wilken, S., Zhang, H., & Hansson, L.-A. (2017). Phytoplankton diversity loss along a gradient of future warming and brownification in freshwater mesocosms. *Freshwater Biology*, *62*(11), 1869–1878. https://doi.org/10.1111/fwb.13027

Utermöhl, H. (1958). Zur Vervollkommnung der quantitativen Phytoplankton-Methodik: Mit 1 Tabelle und 15 abbildungen im Text und auf 1 Tafel. *SIL Communications, 1953-1996*, *9*(1), 1–38. https://doi.org/10.1080/05384680.1958.11904091

Vasconcelos, F. R., Diehl, S., Rodríguez, P., Hedström, P., Karlsson, J., & Byström, P. (2016). Asymmetrical competition between aquatic primary producers in a warmer and browner world. *Ecology*, *97*(10), 2580–2592. https://doi.org/10.1002/ecy.1487

Weyhenmeyer, G. A., Prairie, Y. T., & Tranvik, L. J. (2014). Browning of Boreal Freshwaters Coupled to Carbon-Iron Interactions along the Aquatic Continuum. *PLOS ONE*, *9*(2), e88104. https://doi.org/10.1371/journal.pone.0088104

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. https://ggplot2.tidyverse.org

Wickham, H., François, R., Henry, L., Müller, K., & Vaughan, D. (2026). *dplyr: A Grammar of Data Manipulation*. https://doi.org/10.32614/CRAN.package.dplyr

Wilken, S., Soares, M., Urrutia-Cordero, P., Ratcovich, J., Ekvall, M. K., Van Donk, E., & Hansson, L.-A. (2018). Primary producers or consumers? Increasing phytoplankton bacterivory along a gradient of lake warming and browning. *Limnology and Oceanography*, *63*(S1), S142–S155. https://doi.org/10.1002/lno.10728

Williamson, C. E., Overholt, E. P., Pilla, R. M., Leach, T. H., Brentrup, J. A., Knoll, L. B., Mette, E. M., & Moeller, R. E. (2015). Ecological consequences of long-term browning in lakes. *Scientific Reports*, *5*(1), 18666. https://doi.org/10.1038/srep18666

Wisheu, I. C., & Keddy, P. A. (1992). Competition and Centrifugal Organization of Plant Communities: Theory and Tests. *Journal of Vegetation Science*, *3*(2), 147–156. https://doi.org/10.2307/3235675

Wood, S. N. (2003). Thin-plate regression splines. *Journal of the Royal Statistical Society (B)*, *65*(1), 95–114. https://doi.org/10.1111/1467-9868.00374

Wood, S. N. (2004). Stable and efficient multiple smoothing parameter estimation for generalized additive models. *Journal of the American Statistical Association*, *99*(467), 673–686. https://doi.org/10.1198/016214504000000980

Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society (B)*, *73*(1), 3–36. https://doi.org/10.1111/j.1467-9868.2010.00749.x

Wood, S. N. (2017). *Generalized Additive Models: An Introduction with R* (2nd ed.). Chapman and Hall/CRC.

Wood, S. N., Pya, N., & Säfken, B. (2016). Smoothing parameter and model selection for general smooth models (with discussion). *Journal of the American Statistical Association*, *111*, 1548–1575. https://doi.org/10.1080/01621459.2016.1180986

Yamazaki, J., Suzuki, T., Maruta, E., & Kamimura, Y. (2005). The stoichiometry and antenna size of the two photosystems in marine green algae, Bryopsis maxima and Ulva pertusa, in relation to the light environment of their natural habitat. *Journal of Experimental Botany*, *56*(416), 1517–1523.

Young, A. J. (1991). The photoprotective role of carotenoids in higher plants. *Physiologia Plantarum*, *83*(4), 702–708. https://doi.org/10.1111/j.1399-3054.1991.tb02490.x

Zhang, T., Zhou, L., Zhou, Y., Zhang, Y., Guo, J., Han, Y., Zhang, Y., Hu, L., Jang, K.-S., Spencer, R. G. M., Brookes, J. D., Dolfing, J., & Jeppesen, E. (2024). Terrestrial dissolved organic matter inputs accompanied by dissolved oxygen depletion and declining pH exacerbate CO2 emissions from a major Chinese reservoir. *Water Research*, *251*, 121155. https://doi.org/10.1016/j.watres.2024.121155

Appendices

Table A1: Glossary of Terms

Term	Definition
Chl a	Chlorophyll *a*
Chl b	Chlorophyll *b*
Chl a:b	The ratio of chlorophyll *a* to chlorophyll *b*
Chl a:car	The ratio of chlorophyll *a* to carotenoids
Chl a:bm	The ratio of chlorophyll *a* to periphytic algae biomass
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
Fe	Iron
GAM	Generalized additive model
LMM	Linear mixed effect model
Periphytic Algae	The portion of periphyton samples that contained photosynthetic organisms: algae and cyanobacteria
Periphyton	All photosynthetic and non-photosynthetic organisms (algae, microbes, fungi, invertebrates) that attach to submerged substrate
PERMANOVA	Permutational multivariate analysis of variance
TN	Total Nitrogen
TP	Total Phosphorus

Table A2: R packages used during data analysis

R Package	Use	Source
DHARMa	Model diagnosis	(Hartig, 2024)
Dplyr	Data manipulation	(Wickham et al., 2026)
Emmeans	Estimated marginal means	(Lenth & Piaskowski, 2026)
Ggplot2	Visualization	(Wickham, 2016)
Lme4	Linear Mixed Effect models	(Bates et al., 2015)
lmerTest	Linear Mixed Effect models	(Kuznetsova et al., 2017)
MGCV	Generalized Additive models	(Wood, 2003, 2004, 2011, 2017; Wood et al., 2016)
Performance	Model diagnosis	(Lüdecke et al., 2021)
Vegan	PERMANOVA; Hellinger sqrt transformation	(Oksanen et al., 2026)

Table A3: Results of the Linear Regression Analysis for the Effects of DOC and Fe on Water Color

Coefficients	Estimate	Std. Error	t	p
Intercept	182.61	7.93	23.03	**< 0.0001**
DOC	12.97	0.85	15.20	**< 0.0001**
Fe	6.11	8.30	0.74	0.48
DOC x Fe	2.51	0.78	3.24	**0.012**

Adj R^2 = 0.97; Residual SE = 24.12; $F_{3,8}$ = 120.1; p < 0.001. Statistically significant p-values are in bold. DOC and Fe were mean-centered prior to analysis (DOC mean = 19.97 mg L^{-1}; Fe mean = 1.03 mg L^{-1}). Intercept represents predicted water color at average DOC and Fe values; main effects represent the effect of each predictor when the other is at its mean.

Table A4: Results of the PERMANOVA for the Effects of Depth on Community Structure Within Lakes (Hellinger square-root transformed data)

Predictor	df	Sum of Sqs	R^2	F	p
Depth	2	0.149	0.042	0.73	0.15
Residual	33	0.958			

PERMANOVA based on Bray-Curtis dissimilarity with 199 permutations; permutations constrained within lake.

Table A5: Results of the PERMANOVA for the Effects of Color and TP on Community Structure Between Lakes at Each Depth (Hellinger square-root transformed data)

Depth (cm)	Predictor	df	R^2	F	p
20	Color	1	0.176	2.01	0.082
	TP	1	0.049	0.57	0.744
50	Color	1	0.039	0.37	0.839
	TP	1	0.026	0.25	0.926
80	Color	1	0.195	2.51	**0.049**
	TP	1	0.090	1.17	0.364

PERMANOVA based on Bray-Curtis dissimilarity with 9,999 permutations; Effects are marginal (type III). Statistically significant p-values are in bold. Water color and TP were centered and scaled prior to analysis (mean = 0, SD = 1) to allow for comparison of effect sizes.

Table A6: Results of Linear Mixed-Effect Analysis for the Effects of Color and Depth on the Proportion of Chlorophyll-b Containing Periphytic Algae

Predictor	Estimate	Std. Error	df	t	p
Intercept (depth 20 cm)	0.659	0.082	16.42	8.00	**<0.001**
Depth 50 cm	-0.069	0.070	20.00	-1.00	0.331
Depth 80 cm	-0.113	0.070	20.00	-1.62	0.121
Color	0.00025	0.00062	16.42	0.40	0.694
Depth 50 cm x Color	0.00012	0.00052	20.00	0.23	0.818
Depth 80 cm x Color	-0.00021	0.00052	20.00	-0.40	0.694

Conditional R^2 = 0.66, Marginal R^2 = 0.04. Lake is used as a random intercept (variance = 0.052 ± 0.229). Depth at 20 cm is the reference level. Water color was mean-centered prior to analysis (color mean = 194.87 mg Pt L^{-1}); estimates represent predicted response at average color values.

Table A7(a): Results of Hurdle-Binomial Analysis for the Effect of Color and Depth on the Occurrence of Cyanophyceae

Predictor	Estimate	Std. Error	z	p
Intercept (depth 20 cm)	-0.924	0.808	-1.14	0.253
Depth 50 cm	0.506	0.977	0.52	0.605
Depth 80 cm	0.739	1.051	0.70	0.482
Color	-0.005	0.007	-0.74	0.460
Depth 50 cm x Color	0.004	0.008	0.470	0.639
Depth 80 cm x Color	-0.007	0.010	-0.713	0.476

Conditional R^2 = 0.407, Marginal R^2 = 0.223. Lake is used as a random intercept (variance = 1.02 ± 1.01). Depth at 20 cm is the reference level. Water color was mean-centered prior to analysis (color mean = 194.87 mg Pt L^{-1}); estimates represent predicted response at average color values.

Table A7(b): Results of Hurdle-Truncated Linear Mixed Effect Analysis for the Effect of Color and Depth on the Biomass of Cyanophyceae (ln-transformed data).

Predictor	Estimate	Std. Error	df	t	p
Intercept (depth 20 cm)	-18.57	1.63	8.91	-11.42	**<0.001**
Depth 50 cm	3.49	1.85	8.07	1.89	0.095
Depth 80 cm	6.50	1.68	3.44	3.88	**0.024**
Color	-0.056	0.023	8.66	-2.39	**0.042**
Depth 50 cm x Color	0.042	0.026	8.83	1.63	0.139
Depth 80 cm x Color	0.071	0.024	4.19	3.02	**0.037**

Conditional R^2 = 0.708, Marginal R^2 = 0.433. The truncated model includes only the positive biomass values. Statistically significant p-values are in bold. Lake is used as a random intercept (variance = 3.54 ± 1.88). Depth at 20 cm is the reference level. Water color was mean-centered prior to analysis (color mean = 194.87 mg Pt L^{-1}); estimates represent predicted response at average color values.

Table A8: Results of Linear Mixed-Effect Analysis for the Effect of Depth on Shannon-Diversity Index of Periphytic Algae Community

Predictor	Estimate	Std. Error	df	t	p
Intercept (depth 20 cm)	5.16	0.66	20.53	7.86	**<0.001**
Depth 50 cm	0.70	0.62	22.00	1.13	0.273
Depth 80 cm	1.80	0.62	22.00	2.89	**0.009**

Conditional R^2 = 0.60, Marginal R^2 = 0.10. Statistically significant p-values are in bold. Lake is used as a random intercept (variance = 2.86 ± 1.69). Depth 20 cm is the reference level.

Table A9: Results of Linear Mixed Effect Analysis for the Effect of DOC and Depth on the Total Periphyton Biomass (ln-transformed data)

Predictor	Estimate	Std. Error	df	t	p
Intercept (depth 20 cm)	-2.722	0.296	10.21	-9.19	**<0.001**
Depth 50 cm	-0.279	0.128	71.43	-2.19	**0.032**
Depth 80 cm	-0.553	0.127	70.66	-4.34	**<0.001**
DOC (linear)	0.596	0.249	10.79	2.39	**0.036**
DOC^2 (quadratic)	-0.533	0.191	9.00	-2.80	**0.021**
Depth 50 cm x DOC	-0.287	0.128	70.68	-2.25	**0.028**
Depth 80 cm x DOC	-0.376	0.128	70.66	-2.94	**0.004**

Conditional R^2 = 0.778, Marginal R^2 = 0.346. Statistically significant p-values are in bold. Replicates (variance = 0.061 ± 0.247) nested within lake (variance = 0.51 ± 0.71) are included as random effects. Depth at 20 cm is the reference level. DOC was mean-centered prior to analysis (DOC mean = 19.97 mg L^{-1}); estimates represent predicted response at average color values.

Table A10: Results of Generalized Additive Analysis for the Effect of Color and Depth on the Periphytic Algae Biomass (ln-transformed data)

Parametric Effects				
Predictor	Estimate	Std. Error	t	p
Intercept (depth 20 cm)	-8.505	0.307	-27.68	**<0.001**
Depth 50 cm	-0.335	0.281	-1.19	0.236
Depth 80 cm	-0.953	0.281	-3.39	**0.001**
Smooth Terms				
Term	edf	Ref. df	F	p
Color (20 cm)	3.45	4.05	4.12	**0.004**
Color (50 cm)	1.00	1.00	6.07	**0.016**
Color (80 cm)	1.00	1.00	11.79	**<0.001**
Lake (random eff)	7.89	10.00	3.98	**<0.001**
Replicate (random eff)	0.001	34.00	0.00	0.502

Adj R^2 = 0.516, Deviance explained = 58.5%. GAM (Gaussian errors, identity link) fit using REML. Statistically significant p-values are in bold. Lake and replicate were included as random effects using smooth terms. Depth at 20 cm is the reference level. Water color was mean-centered prior to

analysis (color mean = 194.87 mg Pt L^{-1}); estimates represent predicted response at average color values.

Table A11: Results of Generalized Additive Analysis for the Effect of Color and Depth on Chlorophyll Concentration (ln-transformed data)

Parametric Effects				
Predictor	Estimate	Std. Error	t	p
Intercept (chl a, 20 cm)	-4.417	0.355	-12.45	**<0.001**
chl b	-1.301	0.074	-17.60	**<0.001**
carotenoid	-1.436	0.074	-19.46	**<0.001**
Depth 50 cm	-0.318	0.074	-4.30	**<0.001**
Depth 80 cm	-0.764	0.074	-10.31	**<0.001**
Smooth Terms				
Term	edf	Ref. df	F	p
Color (20 cm)	6.58	7.47	9.03	**<0.001**
Color (50 cm)	2.38	2.87	2.90	**0.040**
Color (80 cm)	1.00	1.00	0.75	0.387
Lake (random eff)	9.89	10.00	112.75	**<0.001**
Replicate (random eff)	0.77	2.00	0.64	0.224

Adj R^2 = 0.875, Deviance explained = 88.4%. GAM (Gaussian errors, identity link) fit using REML. Statistically significant p-values are in bold. Lake and replicate were included as random effects using smooth terms. Chl a at depth 20 cm is the reference level. Water color was mean-centered prior to analysis (color mean = 194.87 mg Pt L^{-1}); estimates represent predicted response at average color values.

Table A12: Results of Generalized Additive Analysis for the Effect of Color and Depth on Chlorophyll a:b Ratio (ln-transformed data)

Parametric Effects				
Predictor	Estimate	Std. Error	z	p
Intercept (depth 20 cm)	1.284	0.103	12.43	**<0.001**
Depth 50 cm	0.110	0.058	1.88	0.060
Depth 80 cm	-0.036	0.058	-0.61	0.542
Smooth Terms				
Term	edf	Ref. df	χ^2	p
Color (20 cm)	4.80	5.61	20.16	**0.003**
Color (50 cm)	2.58	3.00	13.98	**0.003**
Color (80 cm)	1.66	1.82	5.04	0.137
Lake (random eff)	8.85	10.00	131.63	**<0.001**
Replicate (random eff)	0.18	2.00	0.22	0.285

Adj R^2 = 0.161, Deviance explained = 54.1%. GAM (scaled t-distribution, identity link) fit using REML. Statistically significant p-values are in bold. Lake and replicate were included as random effects using smooth terms. Depth at 20 cm is the reference level. Water color was mean-centered prior to

analysis (color mean = 194.87 mg Pt L^{-1}); estimates represent predicted response at average color values.

Table A13: Results of Generalized Additive Analysis for the Effect of Color and Depth on Chlorophyll a:car Ratio (ln-transformed data)

Parametric Effects				
Predictor	Estimate	Std. Error	z	p
Intercept (depth 20 cm)	1.485	0.155	9.57	**<0.001**
Depth 50 cm	-0.102	0.041	-2.51	**0.0124**
Depth 80 cm	-0.195	0.041	-4.76	**<0.001**
Smooth Terms				
Term	edf	Ref. df	χ^2	p
Color (20 cm)	3.66	4.33	28.69	**<0.001**
Color (50 cm)	1.00	1.00	0.954	0.329
Color (80 cm)	2.24	2.72	5.37	0.089
Lake (random eff)	9.84	10.00	683.43	**<0.001**
Replicate (random eff)	0.891	2.00	1.93	0.137

Adj R^2 = 0.728, Deviance explained = 58.4%. GAM (scaled t-distribution, identity link) fit using REML. Statistically significant p-values are in bold. Lake and replicate were included as random effects using smooth terms. Depth at 20 cm is the reference level. Water color was mean-centered prior to analysis (color mean = 194.87 mg Pt L^{-1}); estimates represent predicted response at average color values.

Table A14: Results of Generalized Additive Analysis for the Effect of Color and Depth on Chlorophyll a:bm Ratio (ln-transformed data)

Parametric Effects				
Predictor	Estimate	Std. Error	t	p
Intercept (depth 20 cm)	4.04	0.381	10.60	**<0.001**
Depth 50 cm	0.038	0.300	0.126	0.900
Depth 80 cm	0.154	0.303	0.509	0.612
Smooth Terms				
Term	edf	Ref. df	F	p
Color (20 cm)	1.00	1.00	6.87	**0.010**
Color (50 cm)	1.00	1.00	1.55	0.216
Color (80 cm)	1.00	1.00	2.90	0.092
Lake (random eff)	8.54	10.00	5.73	**<0.001**
Replicate (random eff)	0.848	2.00	0.74	0.185

Adj R^2 = 0.454, Deviance explained = 52.9%. GAM (Gaussian errors, identity link) fit using REML. Statistically significant p-values are in bold. Lake and replicate were included as random effects using smooth terms. Depth 20 cm is the reference level. Water color was mean-centered prior to analysis (color mean = 194.87 mg Pt L^{-1}); estimates represent predicted response at average color values.