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Quality transformation of dissolved organic carbon during water transit through lakes: contrasting controls by photochemical and biological processes

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Abstract. Dissolved organic carbon (DOC) may be removed, transformed or added during water transit through lakes, resulting in qualitative changes in DOC composition and pigmentation (color). However, the process-based understanding of these changes is incomplete, especially for headwater lakes. We hypothesized that because heterotrophic bacteria preferentially consume non-colored DOC, while photochemical processing remove colored fractions, the overall changes in DOC quality and color (absorbance) upon water passage through a lake depends on the relative importance of these two processes, accordingly. To test this hypothesis we combined laboratory experiments with field studies in nine boreal lakes, assessing both the relative importance of different DOC decay processes (biological or photo-chemical) and the loss of color during water transit time (WTT) through the lakes. We found that photo-chemistry qualitatively dominated the DOC transformation in the epilimnia of relatively clear headwater lakes, resulting in selective losses of colored DOC. However, in highly pigmented brown-water lakes (absorbance at 420 nm >7 m^{-1}) biological processes dominated, and there was no systematic relationship between color loss and WTT. Instead in situ data and dark experiments supported our hypothesis of selective microbial removal of non-pigmented DOC, mainly of low molecular weight, leading to persistent water color over time in these lakes. Our study shows that individual brown headwater lakes do not conform to the commonly reported pattern of selective removal of colored constituents in freshwaters, but rather the DOC shows a sustained degree of pigmentation upon transit through these lakes.

1 Introduction

The color of water is a defining feature of freshwater ecosystems, primarily caused by inputs of brown-pigmented dissolved organic carbon (DOC) from terrestrial runoff (Xiao et al., 2015). Recent concerns have been raised of widespread increases in color and DOC concentrations in the northern hemisphere, caused by a combination of factors involving a warmer climate (Lepistö et al., 2014;Pagano et al., 2014), an intensified hydrological cycle (Weyhenmeyer et al., 2012;Fasching et al., 2016) and release of DOC that previously was immobilized in soils due to acidification (Monteith et al., 2007). This rise in colored DOC, reviewed by Solomon et al. (2015), is predicted to reduce aquatic productivity (Karlsson et al., 2009), change food webs and population structures (Jansson et al., 2007), alter the stoichiometry and magnitude of bioavailable nutrients pools (Berggren et al., 2015b), and to cause increased freshwater CO_2 outgassing (Lapierre et al., 2013). Thus, water color is key to understanding fundamental aspects of aquatic ecosystem functioning in a changing environment.

Inland waters represent a significant component in the global carbon cycle, e.g. emitting greenhouse gases to the atmosphere at the rate of at least 1 or 2 Pg C per year (Raymond et al., 2013;Cole et al., 2007). A fundamentally important water-column process that generates carbon dioxide (CO_2) is the microbial degradation of terrestrially-derived DOC (Lapierre et al., 2013;Fasching et al., 2014). Significant amounts of DOC can also be mineralized by ultraviolet (UV) sunlight in lakes (Koehler et al., 2014) and running waters (Cory et al., 2014). However, while much research attention has been drawn to the CO_2 production from these different processes, surprisingly little is known about the relative role played by biological and photochemical processes for qualitative DOC transformations and, in particular, for the removal of color.

On large scales, color tends to decrease faster than DOC along the land-sea continuum (Creed et al., 2015;Weyhenmeyer et al., 2012), but circumstances allowing for selective removal of colored DOC in single lakes are unclear. Some studies have reported relative losses of colored DOC across lake basins with increasing theoretical residence times (Köhler et al., 2013;Curtis and Schindler, 1997), while other studies have found preferential loss of non-colored DOC in laboratory biodegradation experiments (Hansen et al., 2016) and in time-series analyses of brown headwater lakes (Berggren et al., 2009). From a process perspective, the biological degradation of DOC is unlikely a mechanism leading to selective color loss because bacteria tend to consume non-colored DOC (Asmala et al., 2014;Hansen et al., 2016). An exception is the apparent preferential use of organo-ferric colloids by bacteria, where the removed color comes from iron, not DOC (Oleinikova et al., 2017). The UV light oxidation could theoretically explain losses of colored DOC, but efficient photo-processing has been found mainly in relatively DOC poor water (Molot and Dillon, 1997) and in alkaline lakes (Reche et al., 1999), and not systematically in unproductive DOC-rich lakes (Amon and Benner, 1996;Molot and Dillon, 1997;Jonsson et al., 2001). Thus, the processing of colored DOC remains poorly understood in response to water transit through typical unproductive DOC-rich headwater systems (Weyhenmeyer et al., 2014).

Most of the studies that have addressed changes in water chemistry in response to water transit times in lakes have applied fixed theoretical mean residence time values (Köhler et al., 2013;Curtis and Schindler, 1997;Weyhenmeyer et al., 2012). However, in reality water transit times through lakes and reservoirs vary several-fold over time, as a result in temporal flow variations (Li et al., 2015;Rueda et al., 2006). Thus, the processing of DOC in response to the actual water transit time through a given lake has been overlooked, addressed only in few studies (Berggren et al., 2009;Berggren et al., 2010b). There is a knowledge gap on the ecohydrological and biogeochemical mechanisms behind DOC quality and color change in headwater lakes. In this study we therefore combine water transit time (WTT; time spent in a single lake) calculations for a range of different headwater lakes with laboratory simulations of DOC processing to determine the relative importance of different processes that remove DOC (biological or photo-chemical) and color over time. We hypothesized that heterotrophic bacteria preferentially consume non-colored DOC fractions, resulting in small overall color loss during water retention in lakes where bio-degradation represents the dominant DOC transformation process. On the contrary, selected loss of colored DOC could be expected in lakes where the DOC transformation is dominated by photo-chemical processing.

2 Methods

2.1 Study design overview

Targeting the well-studied Björntjärnarna brown-water catchment in northern Sweden (Berggren et al., 2009;Berggren et al., 2015a;Karlsson et al., 2012), a large data set for a single catchment was first compiled and analyzed using linear mixed effects regression. In total 260 samples were obtained over seven different years from the inlets, epilimnia, hypolimnia and terminal outlet of two tightly connected lakes called 'Övre Björntjärnen' and 'Nedre Björntjärnen'. These chain lakes share 92% of the catchment area, and their epilimnia are tightly connected by a short (70 m length) stream, making the same water pass through the two lakes in sequence. The purpose of this first analysis was to analyze one large pooled data set to maximize power, i.e. the chance of finding significant patterns in the response variables with increasing WTT.

For a second part of the analysis, we collected a limited number of samples (ca 10 per year and site) during 3-4 years from seven additional 'survey lakes' along a gradient of DOC and color, increasing the total number of study lakes to nine. This data were used to increase the representativeness of the study, given the variations in UV light exposure in the water column and possible differences in UV light degradation between brown-water and clear-water lakes. We selected unproductive lakes (Chl-a $< 2\ \mu g\ L^{-1}$) because the majority of lakes worldwide are located in northern regions where nutrient concentrations are low, yet lake DOC concentrations and optical conditions vary widely (Karlsson et al., 2009). In the analyses of these survey lakes, epilimnetic and hypolimnetic data were kept separate, due to the differences in light climate with different water depths. To understand the relative role played by biological and photo-chemical processes in the qualitative transformations of DOC, we performed laboratory bioassay experiments on water from three of the lakes (Table 1), where changes in optical water properties were measured in dark bacterial bioassays and under UV light irradiation, respectively. Since the two processes had systematically different impact on DOC optical properties, we could use optical indices to see which of the processes that dominated based on how the indices changed *in situ* with increasing natural WTT in the lakes.

We assumed no significant variations in color induced by iron (Fe) or pH in this study. Total unfiltered Fe concentrations are in the order of one mg L^{-1} at the inlets, epilimnia and outlets (personal communication, D Bastviken, Linköping University; measured in the four most colored lakes). Although the abundance and speciation of Fe can affect optical properties of freshwaters (Pullin et al., 2007), the effect on absorbance in the water should be marginal (Weishaar et al., 2003). According to Kritzberg and Ekström (2012), the contribution from one mg L^{-1} of Fe to absorbance at 420 nm (a_{420}) is 0.8 m^{-1}, corresponding to 5-10% of the observed a_{420} in the four lakes that have been analyzed for total Fe. Regarding pH, Pace et al. (2012) showed that the absorbance coefficients increases sharply beyond a pH of 7 due to changes in the three-dimensional structure of the DOC molecules. In this study, the observed range in pH across all sites and sampling dates was 3.4-6.8, which is below the threshold for major pH interference (Pace et al., 2012).

2.2 Study site descriptions

The nine Swedish boreal lakes that were selected (5-10 m max depth, 0.01-0.05 km^2 area) varied more than 5-fold in color and ca 3-fold in DOC (Table 1). All lakes have previously been depth-profiled using an echo sounder. Regional mean annual temperature is 1˚C and the average annual precipitation is 500-600 mm, of which half arrives as snow. Lake ice is generally present from late October to early May. The catchment areas range from 0.03 km^2 to 3.25 km^2, resulting in mean epilimnetic water transit times from 0.2-0.3 yrs to ca 1-3 yrs in the lakes with the largest and the smallest watersheds, respectively (Table 1). The catchments are mainly (>75%) covered by coniferous forest (*Picea abies, Pinus sylvestris*) and *Sphagnum*-dominated mires (<25%). Forests are managed and have widely varying age, both within and between the catchments, from 5-100 years. Location, mean optical properties and DOC concentrations of each of the study lakes are presented in Table 1.

Three of the lakes (Table 1) received inorganic N additions 2012-2014 (as part of another study) to create a slightly elevated nitrate concentration, by 0.1 mg N L^{-1} (Deininger et al., 2017). This fertilization could potentially affect the DOC decay (Berggren et al., 2007). Therefore, before initiation of the lake fertilization, we tested the potential influence of N by performing 2-week *in vitro* DOC and color loss measurements (bioassays) on water from the lake Nedre Björntjärnen, which has the lowest natural ratio of inorganic N to DOC among the nutrient amended lakes, and thus would be at the highest risk for bias. The bioassays were performed as described previously in detail (Berggren et al., 2009) at 20°C dark conditions with ambient bacterial communities and with additions of single spikes (1 mg N L^{-1} added) of ammonium nitrate at the beginning of the incubations, using epilimnetic water obtained in winter, spring, summer and fall. We found no N additions effect on DOC degradation or change in water color during these bioassays experiment (Fig. S3). Moreover, none of the N-amended lakes appeared as outliers in this study. Therefore, we assume that the lake N addition had no critical impact on the results or the conclusions drawn in this work.

2.3 Sampling and water analysis

Sampling was carried out between 2006 and 2014 (3-7 years of data per lake). Water from epilimnion and hypolimnion (mid-depths or composite samples) was collected every 2-3 weeks throughout the ice-free seasons, and occasionally under ice, at the deepest point of each lake (see sample numbers in Table 1). Additional samples for the detailed analysis of the Björntjärnarna catchments were obtained on most sampling dates at the headwater inlet and outlets of the chain lakes (Björntjärnarna). Sampled water was stored in cooling boxes until processing in the lab within 2-10 hours. Temperature profiles were obtained with electronic sensors at each sampling occasion (plus occasional additional dates) and used to calculate volumes above and below the thermocline depth, defined as the mid-depth of the transect where temperature changed >1°C m^{-1}.

Lake water was filtered with acid-washed 0.7 µm glass fiber filters (Whatman GF/F). Absorbance spectra of the filtrate were measured at room temperature in 1 cm quartz cuvettes using a Jasco V-560 UV-VIS spectrophotometer. Blank values from deionized water were subtracted from the spectra. An aliquot of 40 ml of the filtrate was acidified (50 µL 1.2M

HCl) and stored in darkness at 6˚C until DOC analysis by high-temperature catalytic oxidation using a HACH-IL 550 TOC-TN analyzer (Hach-Lange GmbH Düsseldorf, Germany).

We used the decadic absorbance coefficient a_{420} (m^{-1}) for this study, conventional in water monitoring and research in the study region (Kritzberg and Ekström, 2012;Weyhenmeyer et al., 2012). Discharge was assessed as described in Supplementary information, Text S1.

2.4 Water transit time assessments

The transit time, represented by the water that resides in a lake at a given moment, is dependent upon the retention and renewal history of that water. We assumed that WTT increases with +1 per unit of time that passes by and that it decreases in proportion to how the water volume (Vol_{total}) is replaced by new inflowing water (Flow rate), which gives a change in WTT per unit time (dWTT/dt) according to Eq. 1.

$$\frac{dWTT}{dt} = 1 - WTT * \frac{Flow\ rate * dt}{Vol_{total}} \tag{1}$$

However, this continuous function (Eq. 1) is not suited to be applied directly in this study, because our data is discrete and further involves two depth strata (epilimnion and hypolimnion) with reciprocal entrainment effects due to dislocation of the thermocline. Therefore we adapted discrete functions for the changes in epilimnetic and hypolimnetic WTT from one day (t) to the next day (t+1) (Berggren et al., 2010b;Berggren et al., 2009). Using these functions (Eq. 2-5), lake WTTs were calculated iteratively for each day in sequence from discharge (measured daily) and lake volume data (epilimnetic and hypolimnetic; daily values obtained by linear interpolations between sampling dates). To get realistic WTT values for the first day of the study period, the iteration was initiated from a date 10 years in advance. An arbitrary WTT starting value could then be chosen without impact on the calculated WTTs of the study years. For the pre-study period, the mean seasonal mixing pattern for each lake (see Fig. S1a) was used to generate daily epilimnetic and hypolimnetic proxy volumes.

For any given day (t), a certain volume of inflowing water (Vol_{inflow}) was considered to mix with a certain volume of epilimnetic lake water (Vol_{epi}; above mid-thermocline depth) and, during days with downward dislocation of the thermocline, with an additional volume of hypolimnetic water ($Vol_{hypo\ flow}$). Thus, after the day in question (t+1), the resulting new mean WTT equals the volume weighted average WTT of these different volumes that mixed during day t. In addition, the transit time also changes with '+1' per unit of time, as the water resides 1 d in the lake during the day (t). Hence the resulting WTT on day 't+1' was calculated according to Eq. 2 (days without downwards thermocline dislocation) or Eq. 3 (days with downwards thermocline dislocation). $Vol_{hypo\ flow}$ (t) was given from the decrease in the hypolimnetic volume from day t to the next day (t+1).

$$WTT_{epi}(t+1) = 1 + \frac{WTT_{in}(t) \times Vol_{inflow}(t) + WTT_{epi}(t) \times Vol_{epi}(t)}{Vol_{inflow}(t) + Vol_{epi}(t)} \tag{2}$$

$$WTT_{epi}(t+1) = 1 + \frac{WTT_{in}(t)\times Vol_{inflow}(t)+WTT_{epi}(t)\times Vol_{epi}(t)+WT\ \ _{hypo}(t)\times Vol_{hypo\ flow}(t)}{Vol_{inflow}(t)+Vol_{epi}(t)+Vol_{hypo\ flow}(t)} \quad (3)$$

After a day with reduced thermocline depth, the new hypolimnetic WTT (t+1) resulting from entrainment of epilimnetic water ($Vol_{epi\ flow}$) into the hypolimnion (Vol_{hypo}) was calculated using Eq. 4. Again, the transit time also changes with '+1', as the water resides in the lake during the day in question. After a day without reduced thermocline depth, the WTT_{hypo} was unaffected by mixing with epilimnetic water (Eq. 5). $Vol_{epi\ flow}$ (t) was given by the increase in the hypolimnetic volume from day (t) to the next day (t+1).

$$WTT_{hypo}(t+1) = 1 + \frac{WTT_{epi}(t)\times Vol_{epi\ flow}(t)+WTT_{hypo}(t)\times Vol_{hypo}(t)}{Vol_{epi\ flow}(t)+Vol_{hypo}(t)} \quad (4)$$

$$WTT_{hypo}(t+1) = 1 + WTT_{hyp}\ \ (t) \quad (5)$$

Inflowing water from the catchment (Vol_{inflow}) was assigned the WTT of 0, except for in the lower chain lake (Nedre Björntjärnen), where the WTT of inflowing water was considered to equal the WTT of outflowing (epilimnetic) water from the upper chain lake (Övre Björntjärnen). The other inlet streams represent water transit times that can be considered negligible compared to the WTT in the lakes (Berggren et al., 2009).

Our consideration that all inflowing water mixed with the epilimnion (not hypolimnion) is supported by the fact that all of the lakes with permanent inlets (five of the study lakes) have inlet streams entering shallow areas of the respective lakes, i.e. without hypolimnia, where the water is forced to mix with the epilimnion. Thus even if the inflowing stream water sometimes had a temperature (and thus density) similar to that of the hypolimnion, no down welling was likely to happen.

2.5 Response variables

Besides DOC and a_{420}, we analyzed two additional optical indices (ratios) of qualitative changes in the dissolved organic matter in response to changing WTT. Firstly, we used the absorbance ratio $a_{254} : a_{365}$, which describes a shift towards absorption in the red part of the spectrum, and thus tends to be negatively related to average molecular DOC weight (Dahlén et al., 1996) and positively correlated to low molecular weight DOC compounds (Berggren et al., 2010a). Specifically, we found that $a_{254} : a_{365}$ was correlated to the sum of 39 of the most common organic acids, free amino acids and simple carbohydrates (Fig. A2) that we could identify in the Björntjärnarna catchment, using a liquid chromatography-ion spray tandem mass spectrometry (LC-MS) system. The direction of change in this ratio is indicative of the dominant DOC transformation process: $a_{254} : a_{365}$ increases with UV light processing (Dahlén et al., 1996), but it decreases in response to bacterial DOC processing (Berggren et al., 2007). The LC-MS system consisted of a Dionex (Sunnyvale, CA, USA) ICS-2500 liquid chromatography system and an Applied Biosystems (Foster City, CA, USA) 2000 Q-trap triple quadrupole mass spectrometer. The method is described in further detail in Ström et al. (2012).

Secondly, we used the ratio a_{420} : DOC. If this ratio goes up with increasing WTT, then non-colored DOC is selectively removed (or more colored DOC added), but if a_{420} : DOC goes down, then this is either due to selective decay of colored DOC (Weyhenmeyer et al., 2012) or selective addition of low-pigmented DOC (Creed et al., 2015).

2.6 Laboratory experiments

We performed laboratory experiments on water from three catchments to disentangle the effects of microbial processing and UV light degradation through dark and irradiated experimental conditions, respectively. To simulate the microbial processing that occurs during the WTT in the study lakes (about 1 yr on average; Table 1), we used water either from the lake inlets or from the epilimnion at times when the water had only resided a short period in the lake according to our model (< 1 month on average). The unfiltered natural water samples (with ambient microbial community) were then incubated in darkness for 450 days at 20 °C in 1 L acid-washed Duran glass bottles, with a gas headspace (~100 mL) containing sufficient O_2 to theoretically oxidize all DOC in the samples. Although protozoa were not removed, it can be assumed that bacteria vastly dominate the biomass (~90%) when dark bioassays are performed on natural humic water (Daniel et al., 2005).

For the light experiments, we chose a slightly different approach. While the microbial processing happens continuously in the entire water column of the lakes, UV light processing occurs only in a thin superficial layer of the lakes, during daytime, and under ice free conditions. This means that the DOC is likely processed by microbes before getting in contact with UV light. For this reason, we used water that had first been incubated with microbes in the dark (as described above) as starting material for the UV light experiments. Hence, 10 ml filtered (0.45 µm) samples were incubated for 24h in cylindrical quartz vials placed horizontally on a spinning disc (0.67 rpm) in a 20°C climate chamber, at ca 40 cm distance from two xenon-sodium lamps. The UV irradiation of the different parts of the disk was within 3.64-6.89 W m^{-2} for UV-A and 0.06-0.1 W m^{-2} for UV-B according to radiation measurements (Spectroradiometry, International Light Inc.). Spinning of the disk ensured equal light dose received by all samples. Based on Bertilsson and Tranvik (2000), we used complete spectra for lamp irradiation and absorption to calculate that the samples absorbed a UV light dose of approximately 100 MJ m^{-3}, equivalent to at least two years of water column-integrated *in situ* UV light absorption. The bacterial DOC processing during irradiation was considered negligible, because the rate of DOC loss under light was 1-2 order of magnitudes higher than in dark control incubations with microbial degradation only.

The dark and light experiments were performed on 14 samples from Övre Björntjärnen, Lillsjöliden and Struptjärnen (4-5 per site, from spring to fall). DOC and absorbance was measured before and after each experiment.

2.7 Statistics

The response in spectrophotometric variables to changing WTT was evaluated through linear mixed effects regression in the pooled dataset from the Björntjärnarna chain lakes, using site as random factor to avoid problem with autocorrelation between the monitoring stations. However, ordinary linear regression was used in the individual analyses of the survey lakes. Temporally, all response variables showed systematic and significant ($p < 0.05$) autocorrelation for a time lag of 1 step, i.e. the 2-3 week sampling frequency (Box-Ljung autocorrelations around 0.5; software IBM SPSS 22), but there was no systematic autocorrelation for two time steps, i.e. 4-6 weeks. To compensate for loss of independent replicates in the time-series data due to temporal autocorrelation, the α for correlations between WTT and response variables was adjusted from 0.05 to 0.01. This scaling of the significance level is sufficient to take into account that only every second observation in the time-series could be assumed to be independent. The 0.05 level was considered only marginally significant.

For the laboratory experiment results, we used 2-tail paired t-tests to test for changes between initial and ending conditions. All statistics were performed using IBM SPSS 22, except for the linear mixed effects regressions performed using the statistical package 'lm4' for R. To obtain conditional and marginal R^2 estimates (R^2c and R^2m, respectively) for mixed models, the package 'MuMIn' was used, while significance of coefficients and intercepts (fixed effects) were tested with the package 'lmerTest'.

3 Results

3.1 Seasonal water transit time patterns

The different study lakes showed a coherent seasonal mixing pattern, with stable thermal stratification from mid-May to mid-September, in between of spring and fall overturns (Fig. S1). The temporal variability in WTT was partly controlled by annually recurring high-flow events that caused systematic drops in the WTT, by approximately 0.2-0.5 years in spring (due to snow melt) and 0.1-0.2 years in fall (due to rain storms). Conversely, during low flow in winter and summer all sites showed a slow but stable increase in WTT (Fig. S1).

In the site-by-site analysis of survey data from all nine lakes, the water transit time spanned from 0.2-3.1 years for epilimnetic samples from each respective site (mean across all sampling dates) and 0.3-3.7 years for the corresponding hypolimnetic samples. Within each lake, there was considerable variability in WTT, with on average twice as high epilimnetic WTT after the driest periods compared to the wettest periods. The temporal span in WTT, i.e. highest minus lowest value for each site, varied from 0.4 years (the least dynamic lake) to 1.8 years (the most dynamic lake).

3.2 Björntjärnarna chain lakes

In the pooled data set from the Björntjärnarna brown-water chain lakes, we found significant relationships (linear mixed effects regression) between spectrophotometric response variables and the WTT. The ratio a_{254} : a_{365}, which indicates relative abundance of low molecular weight DOC, showed a decreasing trend (from 4.1 to 3.8) over the span from 0 to 0.8 years of WTT in this lake system ($R^2m = 0.15$, $R^2c = 0.19$, $n = 260$; Fig. 1a). At the same time, the DOC became relatively more colored, demonstrated by the significant positive trend for the ratio a_{420} : DOC ($R^2m = 0.16$, $R^2c = 0.20$, $n = 260$; Fig. 1b). For changes in DOC (Fig. 1c) there was no significant fixed effect caused by water transit time, although the random factor (site) explained

17% of the variance (difference between $R^2c = 0.17$ and $R^2m = 0.00$), largely due to higher DOC at the inlet than in the lakes. The overall color (absorbance at 420 nm) remained remarkably constant with increasing WTT (Fig. 1d).

3.3 Survey lakes

The survey results partly conformed to the patterns in Björntjärnarna, as three out of the nine epilimnetic sites showed significant ($p < 0.01$, two cases) or marginally significant ($p < 0.05$, one case) linear decreases in $a_{254} : a_{365}$ with increasing WTT. In one case this trend was significant at the 0.01 level both above and below the thermocline (Fig. 2a). On the contrary, in the epilimnia of three clearer lakes, epilimnetic $a_{254} : a_{365}$ increased with increasing WTT (Fig. 2a). Furthermore, only two of the most colored lakes showed increases in a_{420} : DOC with increasing WTT significant at the 0.01 level (Fig. 2b), observed both above and below the thermocline in one case. On the contrary, the epilimnion of one of the lakes showed decreasing a_{420} : DOC over the WTT gradient (Fig. 2b).

When analyzing the nine lakes one by one, significant ($p < 0.01$) losses of DOC with increasing WTT were found in two of the lakes (Fig. 2c). Unexpectedly, one lake (Stortjärnen) showed a significant trend of increasing DOC with increasing WTT, indicating release of DOC from within the lake or its benthic/littoral contact surfaces. Finally, in terms of the overall color of the lakes (absolute a_{420} values), significant ($p < 0.01$) increases with increasing WTT were found in two hypolimnia and one epilimnion (Fig. 2d). In the epilimnetic water of the clearer lakes, the a_{420} tended to decrease, but no significant pattern of decreasing a_{420} was found for any hypolimnetic sites (Fig. 2d).

We further found that there were systematic shifts in the dynamics of both $a_{254} : a_{365}$ and the index a_{420} : DOC along the gradient of increasing mean color of the different lakes. In fact, the rate of change in epilimnetic $a_{254} : a_{365}$ per unit WTT was strongly negatively related to the mean a_{420} of the respective lakes ($R^2 = 0.94$, $n = 9$, $p < 0.001$; Fig. 3a). Furthermore, the rate of change in epilimnetic a_{420} : DOC per unit WTT was positively related to the mean a_{420} ($R^2 = 0.69$, $n = 9$, $p < 0.01$; Fig. 3b).

3.4 Experiments

To understand the mechanisms behind such patterns, we performed laboratory light and dark experiments. In the light treatment, the changes in the $a_{254} : a_{365}$ ratio (significantly positive) and in a_{420} : DOC (non-significant slightly negative) were similar to the changes observed over time in clear epilimnetic waters (Fig. 3c-d). In the dark bacterial bioassays, the changes in both of these ratios were significant in the opposite direction relative to the light treatment (Fig. 3c-d), i.e. similar to the changes observed in brown epilimnetic waters (Fig. 3a-b).

3.5 Overall color loss

Finally, we multiplied the *in situ* rate of epilimnetic color loss in the survey lakes (same as slopes in Fig 2d) with the mean water transit time for the respective sites to find out how much total change there was in water color upon transit through each lake. This showed that losses corresponding to 19-79% of mean a_{420} occurred in the four clearest lakes, whereas the a_{420} either showed no change or increased slightly in the five brownest lakes (Fig. 4). These changes in color upon lake transit largely overlapped the ranges of color loss shown in the light (22-51% loss) and dark (13% increase to 36% loss) incubation experiments, respectively. The change in color upon transit through the lakes over the gradient of increasing mean a_{420} was best described (in terms of fit) by a logarithmic curve (Fig. 4).

4 Discussion

4.1 Main findings

Our results show that individual brown headwater lakes do not conform to the generally reported patterns of efficient and selective removal of colored constituents in freshwaters. Based on a rigorous data set from the Björntjärnarna brown-water catchment (n = 260) spanning seven study years, we found that the color (a_{420}) was sustained at a constant level over water transit times from zero up to 0.8 years. Variability in hydrological turnover rates had no measureable impact on the overall level of water pigmentation upon transit through the lake. Thus, even if previous studies have shown that recent inputs of humic materials from the catchment represent a relatively photo-reactive DOC source (Lindell et al., 2000;Vachon et al., 2016), the quantitative photo-bleaching in the Björntjärnarna catchment apparently was not sufficient to cause a significant net loss of color (a_{420}) over time in single lakes. Moreover, we found no selective removal of colored DOC; on the contrary the ratio between a_{420} and DOC increased significantly over time, supporting our hypothesis.

In the multi-lake comparison along a gradient of increasing DOC and color our results demonstrate contrasting a_{420} dynamics for different types of lake ecosystems. In the brownest lakes ($a_{420} > 7\ m^{-1}$), the color was either unaffected or tended to increase with longer WTT. In contrast, epilimnetic waters of relatively clear lakes showed negative change in a_{420} with WTTs, i.e., such that the waters conversely became browner in response to water renewal, a pattern also found elsewhere in Sweden (Müller et al., 2013;Weyhenmeyer et al., 2012). In the clearest of the nine survey lakes, both DOC and color decreased strongly and significantly with increased WTT.

4.2 Possible bias

A factor that could potentially bias these interpretations is that the chemistry of source water is variable over time, leading to seasonal changes in the color of inflowing water to the lakes (Ågren et al., 2008). For example, during low flow it has been shown that headwater streams in the region can have unusually high concentrations of colored wetland-derived DOC (Laudon et al., 2011). However, headwater streams show relatively small variability in water chemistry during episodic flow, which represent a majority of the annual DOC export from small catchments (Laudon et al., 2011) and thus also the majority of the DOC which was processed in the study lakes during subsequent low flow periods. Similar to what has been reported elsewhere for headwaters (Wilson et al., 2013;Boyer et al., 1997), the low flow periods in our study contributed to a negligible part of the total water and thus DOC budget (Figure S1b). Therefore, it appears unlikely that the patterns in DOC and optical properties with increasing WTT in the lakes would be primarily driven by temporal variations in inflowing water.

Another potential bias is represented by new sources of DOC, i.e. other than catchment runoff, which can contribute to the development of lake color over time (Creed et al., 2015). For example, DOC can be added internally within lakes by release from phytoplankton, sediments, macrophytes or littoral peats and marshes in direct contact with the lake water (Wetzel, 2001). The lakes in this study are unproductive with small contributions from primary producers, exemplified by the negligible role of primary production in a previous organic carbon budget for Övre Björntjärnen (Karlsson et al., 2012). However, one of the study lakes (Stortjärnen) has littoral peatlands along roughly half of its shoreline, with peat virtually floating in the lake water thus potentially releasing DOC without hydrological inputs. This particular lake showed significant increases in DOC with increasing WTT (Fig 2C, the lines with positive slopes), possible due to direct inputs from the littoral peat. Moreover, since peatlands in an area close to the Stortjärnen lake have been shown to have particularly high DOC concentrations during low flow periods (Laudon et al., 2011), it is possible that Stortjärnen makes a special case where even small diffuse hydrological inputs from the peatlands surrounding the lake was sufficient to raise the DOC concentration of the lake during low flow. Nonetheless, while it is interesting to note that color and DOC in absolute terms can increase with residence time in individual lakes, result from this study are too limited to generalize such patterns.

4.3 Organic carbon transformation processes

To adequately understand our results it is necessary to bring the mechanisms of DOC decay in the lakes to attention. Previous studies in the region have shown that non-pigmented low molecular weight carbon (LMWC) is selectively used by bacteria in brown-water streams and lakes (Berggren et al., 2010a;Berggren et al., 2010b). In agreement, the LMWC indicator $a_{254} : a_{365}$ (see relationship between LMWC and $a_{254} : a_{365}$ in Fig. S2) decreased significantly with WTT in brown-water lakes in this study, which together with increasing a_{420} : DOC of the same lakes suggests that low-pigmented fractions were favored over more colored bacterial substrates. It is also possible that the microbial processing increased the pigmentation of DOC by the excretion of humic-like chromophoric molecules by bacteria (Shimotori et al., 2009;Tranvik, 1993;Guillemette and del Giorgio, 2012), contributing to the increase in a_{420} : DOC and the strong decrease in $a_{254} : a_{365}$ found coherently in both our dark biological decay experiment and in the brown-water lakes in situ. The fact that these lakes showed the same in situ optical changes over time as shown in the dark bioassays indicates that dark biological degradation processes dominated the DOC transformation in the brown-water lakes, most likely because of high optical density which prevented photo-degradation from most of the water column.

For the clearest lakes, the prevailing mechanism behind the DOC transformation was obviously different than that in the brownest lakes, at least in the epilimnetic waters exposed to sunlight. The clear epilimnetic lake waters showed increases in $a_{254} : a_{365}$, which is the expected pattern for systems where UV light transformation dominates (Dahlén et al., 1996) and where LMWC is produced by photochemical processes (Bertilsson and Tranvik, 2000). In agreement, we also found that $a_{254} : a_{365}$ increased in the laboratory UV light experiment. Thus, the relative role that UV light processing played for the qualitative DOC transformations was much larger in clear lakes than in highly colored lakes. It should be mentioned, however, that previous studies have found that the absolute light-induced color loss may be similar for brown and clear lakes, given equal incoming surface irradiation, even if this loss in color is distributed in different ways over the water column (Granéli et al., 1996;Koehler et al., 2014). In our study the mean absolute a_{420} loss in the four clearest epilimnetic sites was 1.0 m^{-1} yr^{-1} (Fig. 2D). Based on Granéli et al. (1996) it can be speculated that the same processing happened also in the most colored of our lakes, but that this change was too small to be distinguished from the high background a_{420} level of 11-13 m^{-1} in these lakes.

4.4 Conceptual implications

Altogether our results suggest a conceptual framework for DOC transformation (Fig. 5) with distinctly different color trajectories under 'brown-water' and 'clear-water' regimes, respectively. In the brown-water regime biological processes dominate, leading to small changes in color over time. In a clear-water state, photochemical processes play a relatively much larger role, resulting in substantial decreases over time in both DOC concentrations and in color. From this proposed concept (Fig. 5), the circumstances which allow for development of a lake into a brown-water or clear-water system, respectively, can be discussed. Considering that boreal watercourses show at least two orders of magnitude spatial variation in color (Lapierre et al., 2013;Temnerud et al., 2014), the color of the inflowing catchment runoff water is beyond doubt a key factor determining the regime. Additionally, it can be speculated on the potential for regime shifts to occur in lakes that are close to the border between the clear and brown-water regime, e.g. by rapidly increased input of unprocessed DOC from the catchment during extreme discharge episodes such as described by Raymond et al. (2016). However, tipping over a brown regime into a clear regime should be relatively difficult, given the inefficient color loss in brown headwater lakes (Fig. 5). This view is consistent with laboratory studies indicating that biological decay needs to proceed for a few years before substantial DOC and color exhaustion in brown water lakes (Koehler et al., 2012). Nonetheless, the fact that we only found relatively clear lakes among the sites with water residence times above two years suggests that the clear-water regime eventually takes over, if the water transit time through the lake is sufficiently long.

Our results do not contradict the findings from previous studies that have proposed selective loss of pigmented DOC in freshwater networks (Ilina et al., 2014;Weyhenmeyer et al., 2012;Köhler et al., 2013). Rather, this study can help explain why previous studies have not been able to detect the decreasing color with increasing water residence time in headwaters (Müller et al., 2013), where many of the lakes presumably follow the dynamics of the brown-water regime (see Fig. 5). The decreasing absorbance with increasing water residence time has mainly been possible to model at non-headwater sites with a relatively low color level (Müller et al., 2013;Weyhenmeyer et al., 2012). For such sites, decrease in color per unit DOC could be expected from photo-processing (Molot and Dillon, 1997), iron flocculation (Weyhenmeyer et al., 2014) or DOC replenishment along the aquatic continuum, e.g. from algal sources that selectively adds carbon of low degree of pigmentation (Creed et al., 2015).

5 Summary and conclusions

In summary, our results demonstrate how brown headwater lakes may not conform to the general reported pattern of selective removal of colored constituents in freshwaters, but rather show sustained level of pigmentation regardless of WTT variations. Thus change in WTT, e.g. due to a potentially wetter future climate, has no universal effect on lake color. However, if combined with changes in the absorbance of catchment runoff water, an intensified hydrological cycle could possibly cause regime shifts in headwater lakes, where e.g. clear-water lakes renewed with more colored water relatively quickly will transform into brown-water systems. Conceptually, our study challenges the view of the aquatic network as a single continuum of DOC processing. In headwaters, the functioning of different aquatic networks depend on which DOC transformation process that dominates.

Competing interests

The authors have no conflict of interest to declare.

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Available supplementary information contains extended methods (Text S1) and three figures (Figures S1-S3).

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Table 1. Characteristics and sampling details for the nine study lakes. Variables from left to right: decimal degrees (DD) WGS84 latitude (Lat) and longitude (Lon) coordinates, water transit time (WTT), area of lake (A_{lake}), area of catchment ($A_{catchment}$), ratio between the absorbance at the wavelengths of 254 nm and 365 nm ($a_{254} : a_{365}$), carbon specific absorbance at 420 nm (a_{420} : DOC), dissolved organic carbon (DOC), absorbance at 420 nm (a_{420}), number of epilimnetic and hypolimnetic samples (n), and study years. The WTT and chemical characteristics are shown as mean epilimnetic values across all sampling dates.

Site name	Lat (DD)	Lon (DD)	WTT (yrs)	A_{lake} (km^2)	$A_{catchment}$ (km^2)	$a_{254} : a_{365}$	a_{420} : DOC	DOC (mg C L^{-1})	a_{420} (m^{-1})	n (epi/hypo)	Years (20XX)
Fisklösan[*]	64.150	18.800	0.94	0.017	0.089	4.55	0.32	7.7	2.5	38/28	11-14
Nästjärnen	64.160	18.777	3.13	0.010	0.034	4.50	0.35	7.6	2.7	40/30	11-14
Mångstrettjärn	64.251	18.762	1.44	0.018	0.141	4.12	0.48	11.5	5.5	41/31	11-14
Lapptjärn[*]	64.237	18.790	0.67	0.020	0.168	4.12	0.48	13.1	6.3	41/31	11-14
Lillsjöliden[‡]	63.845	18.616	0.19	0.008	0.254	4.10	0.49	15.7	7.6	35/33	12-14
Nedre Björntjärnen[*,#]	64.122	18.785	0.30	0.032	3.249	4.02	0.57	18.9	10.7	59/29	06-07, 11-14
Struptjärnen[‡]	64.023	19.489	0.34	0.031	0.791	4.03	0.55	21.4	11.7	38/29	12-14
Övre Björntjärnen[#,‡]	64.123	18.779	0.19	0.048	2.840	4.04	0.55	21.9	11.9	72/45	06-07, 09, 11-14
Stortjärnen	64.261	19.763	0.41	0.039	0.817	3.81	0.63	20.9	13.3	31/29	12-14

[*] Elevated inorganic N concentrations 2012-2014, by 0.1 mg N L^{-1}.

[#] Lake included in the focal study of the Björntjärnarna catchment

[‡] Lake selected for laboratory incubation experiments

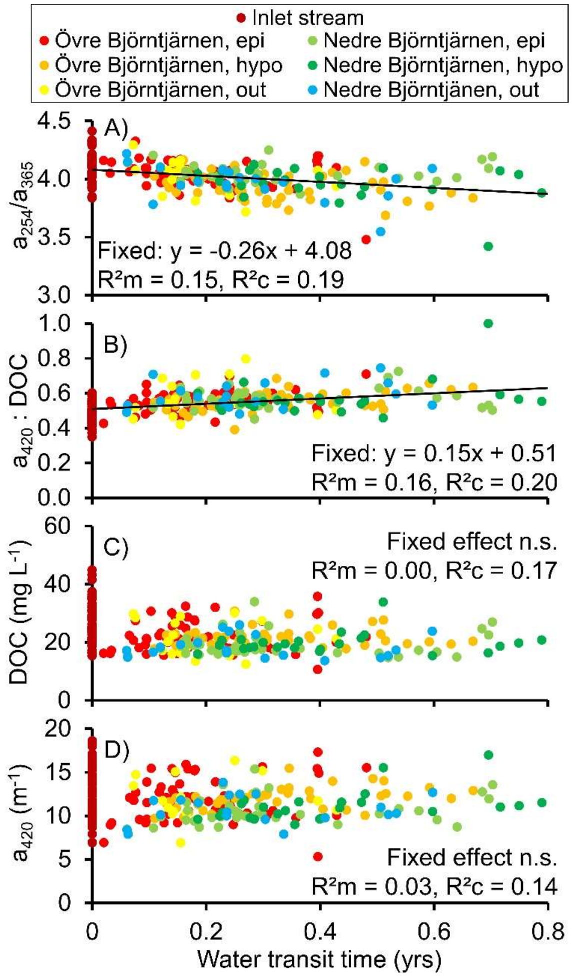

Figure 1. Relationships between dissolved organic carbon (DOC) properties and the water transit time in two chain lakes with 92% shared catchment area. The y-axis variables are (A) ratio between the absorbance at the wavelengths of 254 nm and 365 nm, (B) ratio between absorbance at 420 nm and DOC, (C) DOC and (D) absorbance at 420 nm (n = 260, study years 2006-2014). Solid lines are based on significant ($p < 0.01$) fixed effects coefficients and intercepts. The R^2m shows marginal R^2 for fixed effects and the R^2c refers to conditional mixed effects models with site as random effect. Inlet samples during drought (lower 5 percentiles of flow) are not included since drought inflow makes a negligible contribution to the water that resides in the lakes.

Abbreviations: n.s. – not significant; epi – epilimnion; hypo – hypolimnion; out – outlet.

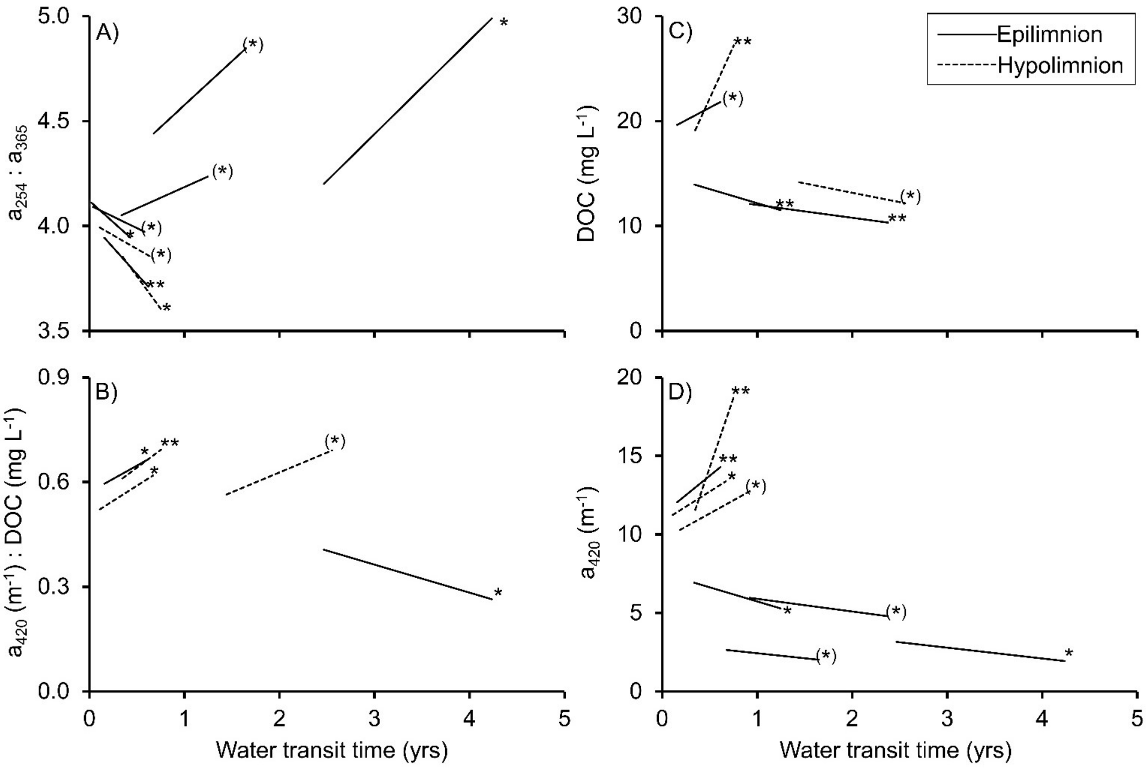

Figure 2. Linear regression lines for significant† relationships between different properties of DOC and the water transit time, shown for individual epilimnia (solid lines) and hypolimnia (dashed lines) of nine lakes in northern Sweden. Y-axis variables are: (A) the absorbance ratio $a_{254} : a_{365}$; (B) ratio between absorbance at 420 nm and DOC concentration; (C) DOC concentration and; (D) absorbance at 420 nm. Non-significant regression lines are not shown.

†Significance: * $p < 0.01$; ** $p < 0.001$; $^{(*)}$ marginally significant $p < 0.05$

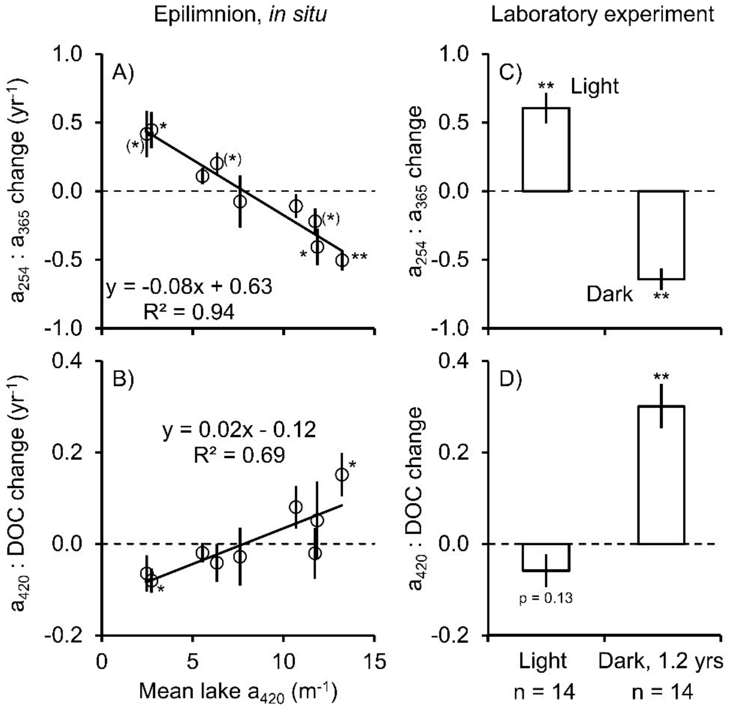

Figure 3. Changes in DOC quality over time as indicated by the optical indices a_{254} : a_{365} (unitless) and a_{420} : DOC (m^{-1} : mg L^{-1}), shown separately for (A-B) the lake epilimnia and (C-D) laboratory experiments. In panels A-B, the y-axis value of each symbol (± error bar) shows the linear slope (± standard error) of a_{254} : a_{365} or a_{420} : DOC as function of the water transit time in a certain lake[#]. Symbols with asterisks represent individual changes (*in situ* data regression slopes; see Fig. 2) that are significant[†]. The x-axis values show mean absorbance (a_{420}) of the lakes. Panels C-D show changes (mean ± standard error) in a_{254} : a_{365} and a_{420} : DOC during light (~100 MJ absorbed per m^3 of water) and dark (1.2 yrs) experiments performed on water from three of the study lakes. Bars with asterisks show changes that are significant[†] (2-tailed paired t-test, comparing initial and ending conditions).

[#] See Table 1 for sample numbers and descriptions of each lake

[†] Significance: * $p < 0.01$; ** $p < 0.001$; (*) marginally significant $p < 0.05$

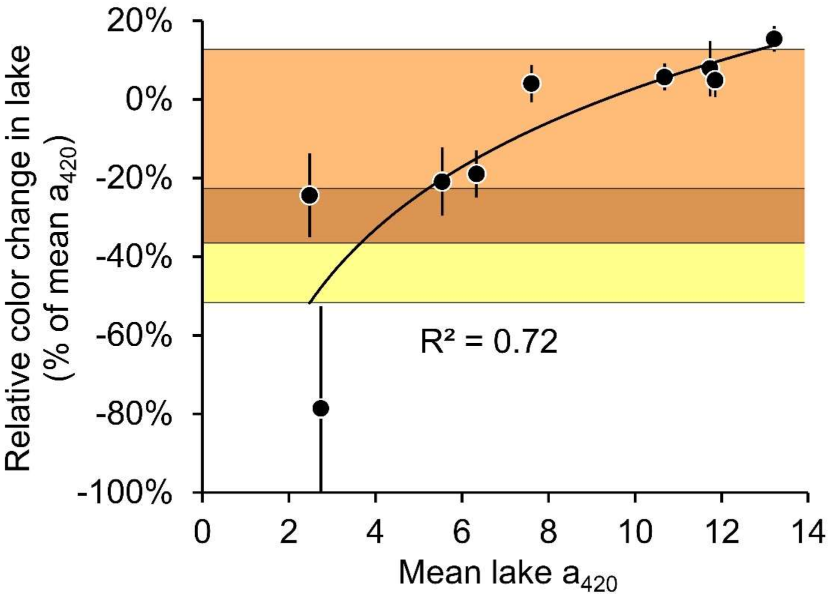

Figure 4. Percent change in epilimnetic color (a_{420}; filled circles) during water transit through nine boreal lakes, plotted over a gradient of increasing mean lake color ($y = 0.39\ln(x) - 0.87$, $p < 0.01$). The change in color (± error bar) is based on the *in situ* data regression slopes (± SE), multiplied by the mean water transit time in each lake. For a qualitative comparison, yellow and brown areas, respectively 5 (overlapping in the dark brown area), indicate ranges in a_{420} change observed during light (~100 MJ absorbed per m^3 of water) and dark (1.2 yrs incubation) experiments performed in the laboratory.

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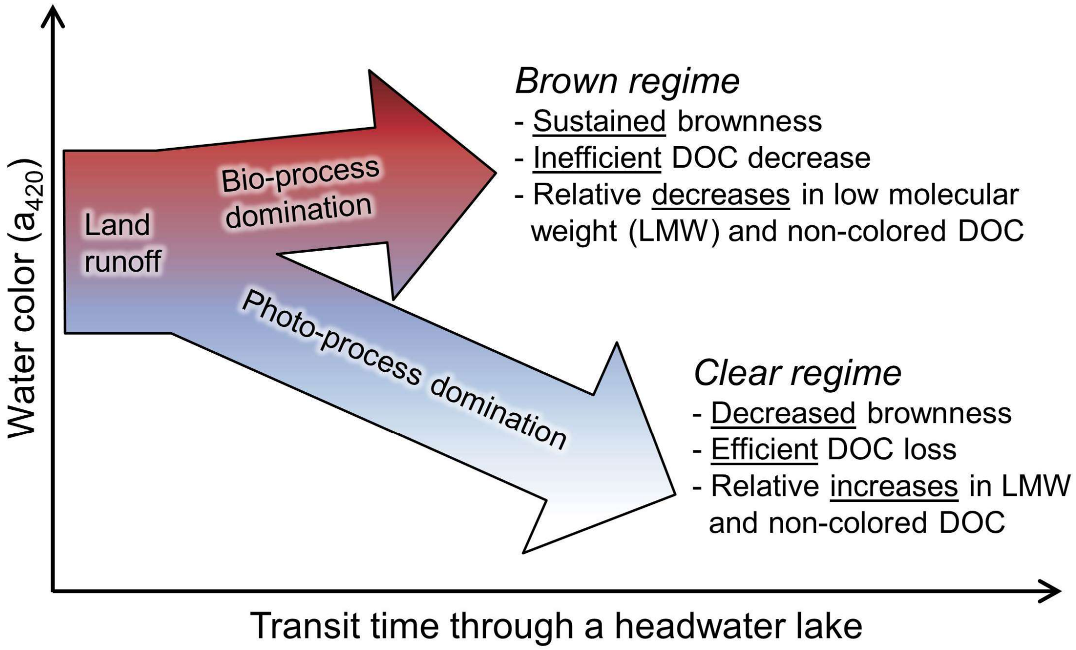

Figure 5. Conceptual representation of the trajectory of color and dissolved organic carbon (DOC) characteristics in headwater lakes with increasing residence time under brown and clear lake regimes, respectively. The figure is based on the observations from this study, where DOC processing in brown lakes is characterized by microbial consumption of low molecular weight (LMW) and non-colored DOC. Only the clear-water lakes show efficient reductions in color and DOC over time, facilitated by photochemical processing that remove colored DOC to a larger extent than microbial processes. Compare with original data from Figs. 2-4.