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Comparison of optical properties of chromophoric dissolved organic matter (CDOM) in alpine lakes above or below the tree line: Insights into sources of CDOM

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Abstract

Here we investigated absorption and fluorescence properties of chromophoric dissolved organic matter (CDOM) in 15 alpine lakes located below or above the tree line to determine its source and composition. The results indicate that the concentrations of CDOM in below-tree-line lakes are significantly higher than in above-tree-line lakes, as evidenced from the absorption coefficients of $a250$ and $a365$. The intensities of the protein-like and humic-like fluorescence in below-tree-line lakes are higher than in above-tree-line lakes as well. Three fluorescent components were identified using Parallel Factor Analysis (PARAFAC) modelling. Component 1 is probably associated with biological degradation of terrestrial humic component. The terrestrial humic-like component 2 is only found in below-tree-line lakes. The protein-like or phenolic components 3 are dominant in above-tree-line lakes, which are probably more derived from autochthonous origin. In this study, (1) higher $a250/a365$ and $S_{275\text{-}295}$ values indicate smaller molecular weight of CDOM in above-tree-line lakes than in below-tree-line lakes, and smaller molecular weight at the surface than at 2.0 m; (2) SUVA254 and FI_{255} results

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provide evidences of lower percent aromaticity of CDOM in above-tree-line lakes; and (3) FI_{310} and FI_{370} suggest a strong allochthonous origin at the surface in below-tree-line lakes, and more contribution from autochthonous biological and aquatic bacterial origin in above-tree-line lakes.

Keywords: Chromophoric dissolved organic matter; Absorption characteristics; Fluorescent properties; Alpine lakes; Tree line

1. Introduction

Ice cover can limit the penetration of sunlight in high-mountain environments. During the last few decades, a decrease in the duration of the cold season caused by global warming would decrease ice cover and significantly increase the exposure of alpine lakes to UV radiation. The impacts of future changes in UV radiation on high-altitude aquatic ecosystems are particularly alarming because the response to radiation variation is expected to be most pronounced at high elevations.[1] High altitude combined with a low concentration of UV-absorbing substances fosters increased penetration of harmful UV radiation in the water column.[2] Although some aquatic organisms may develop strategies to adapt to climate change,[3] alpine lakes are most likely to suffer from increased UV penetration since UV radiation influences organic matter input and is dependent on the amount of ice cover.[4,5]

UV radiation increases with altitude, resulting in enhanced photochemical degradation of chromophoric dissolved organic matter (CDOM). CDOM is defined as the coloured fraction of dissolved organic matter (DOM), which is responsible for absorption of solar radiation and re-emission of it as fluorescence. CDOM is also considered to be a representative organic matter with characteristics similar to humus. In alpine lakes, small changes in CDOM absorption could have a significant influence on UV penetration in the water column.[6] Photodegradation of CDOM has an adverse effect on its optical and chemical properties.[7,8] Exposure of freshwater CDOM to natural sunlight changes its concentration and composition[9] and subsequently enhances its bio-availability.[10] Although the optical properties and ecological significance of CDOM have been studied in plateau and high-mountain lakes,[2,11] limited information is available on the sources and composition of CDOM in these vulnerable environments. High altitudes limit human activity and, thus, decrease anthropogenic CDOM input. In addition, the natural export of CDOM from terrestrial to aquatic ecosystems decreases as terrestrial productivity decreases with increasing altitude.[18] Considering that the influence of catchment runoff, in-water productivity, and atmospheric deposition on alpine lakes varies across different mountain environments (dominated by forests, meadows, or rocks), we expect significant differences in the source and optical properties of CDOM. [12,13] Thus, we hypothesize that there are significant differences in the optical properties of CDOM. Considering that sampling remote alpine lakes at high altitude can be difficult, the investigation and comparison of CDOM optical properties between lakes above and below the tree line will enhance our understanding of the sources and biogeochemistry of CDOM/DOM in alpine lakes.

CDOM mainly originates from two sources. The first is the microbial decomposition of plant matter and aquatic organisms, which represents an autochthonous source.[14] The second is the allochthonous input of organic matter from surrounding terrestrial environments and precipitation.[15] Both autochthonous and allochthonous sources are likely to make a significant contribution to aquatic ecosystems, and research on the contribution of each of these sources to aquatic ecosystems has recently become an emerging field.[16] Nevertheless, the sources and transformation of CDOM are not well understood. Owing to the ability of CDOM to absorb UV and short-wavelength visible light, optical techniques, including spectrophotometry and three-dimensional excitation-emission matrix spectra (EEMs), have been developed to provide abundant information about CDOM sources and composition.[17,18]

Generally, CDOM absorption decreases exponentially with increasing wavelength.[19] It has been proposed that the ratio of absorption at 250 nm to 365 nm ($a250/a365$) can be utilized to track the relative molecular size of CDOM.[20] The ratio of $a250/a365$ should decrease with an increase in molecular size, which is attributed to the stronger absorption of longer wavelength light by high-molecular-weight CDOM. Furthermore, a parameter called specific UV absorbance (SUVA254), which is defined as UV absorbance (in 1 m) at 254 nm divided by the concentration of dissolved organic carbon (DOC) (mg L^{-1}), has been shown to correlate strongly with aromaticity and humification of DOC.[21,22] In addition, EEMs is another effective technique for determining the composition and molecular size of CDOM because of its sensitivity.[23,24] Based on EEMs, various fluorescence indices have been proposed to distinguish between aquatic and terrestrial CDOM sources.[25-28]

In the present study, we focus on comparing the differences in the sources and composition of CDOM between above-tree-line and below-tree-line alpine lakes. The lakes are located in different environments and are mainly surrounded by forests, meadows, or exposed rocks. We investigated the absorption and fluorescence of CDOM in 15 above/below-tree-line alpine lakes with the following main objectives: (1)

characterizing CDOM components based on absorbance and fluorescence properties and (2) tracing the potential sources of CDOM using various optical proxies.

2. Methods

2.1. Description of study lakes and sampling

Study lakes―The above-tree-line lakes (Lakes Huanghai, Heihai, Zihai, Meihai, Fuhai, Qihai, and Zhuhai) are located on Haba Snow Mountain (27°10′-27 °24′N, 100 °02′-100 °14′E), Yunnan, southwest China, with high elevations (approx. 4,200 m) above sea level. The below-tree-line lakes (Lakes Tiancai (TC), Rencai (RC), Zihu (ZH), Meihu (MH), Shengmuhu (SMH), Gulonghu (GLH), Dulonghu (DLH), and Sanxuanhu (SXH)) are located on Yunnan Laojun Mountain (26°38′-27 °15′N, 99 °07′-100 °00′E), with elevations lower than 3,860 m above sea level. These two mountains are geographically close, and the tree line at the sites is roughly 4,100 m above sea level. The surface area of each lake is rather small (<5 km^2). Two example pictures of lakes are displayed in Supporting Information Fig. S1. The study lakes have not been affected much by human activities owing to their high altitude. The lake water is mainly supplied by precipitation, seasonal streams, and slope flow. The sampling work was performed on a boat in the centre of the lakes in October 2012 and September 2013. The water depth was measured in situ with an ultrasonic depth finder. Water at the surface (0–5 cm) was obtained by flat plate, and at 2.0 m depth water was sampled using a 1 L Niskin water sampler with a 10 cm diameter and a height of 21 cm. Samples were collected in several 5 L acid-washed polypropylene plastic bottles wrapped with aluminium-foil paper to minimize light exposure and were transported to the laboratory under ice. After being taken back to the lab, samples were immediately filtered and measured.

2.2. Absorption measurement

To obtain CDOM, water samples were first filtered with a 0.7 μm Whatman GF/F filter (Ø47 mm) pre-combusted at 450°C for 4 h, followed by filtration with a pre-rinsed 0.22 μm Millipore membrane cellulose filter (Ø47 mm). The absorption spectra of the filtered water was measured between 220 and 900 nm at 1 nm intervals using a Lambda35 UV-Vis spectrophotometer (Perkin Elmer) with a 1 cm quartz cuvette. The absorption coefficients of CDOM were calculated using the following equation:[29]

$$\alpha_{\lambda} = 2.303 D_{\lambda} / r \qquad (1)$$

where a_{λ} is the corrected CDOM absorption coefficient, D_{λ} is the corrected optical density at wavelength λ, and r is the cuvette path length in units of m. The absorption coefficients were corrected by the average optical density value at 700–800 nm to minimize scattering effects.[30] In this study, the concentration of CDOM was expressed using both *a*250 and *a*365 because of its chemical complexity.[17] SUVA254 was determined by dividing the absorbance (in 1 m) at 254 nm by the concentration of DOC (mg L^{-1}).[21] To determine the concentration of DOC, the lake water was filtered through a 0.22 μm Millipore membrane and then stored in the dark at -20°C until subsequent analysis. The DOC concentration was measured with a high temperature static pressure concentration analysis method (Torch total organic carbon analyzer, Teledyne Tekmar). The instrument was furnished with a No Diffusion Infrared detector for high sensitivity analysis.

In addition, it has been proposed that $S_{275\text{-}295}$ is a good proxy for the molecular weight of CDOM.[31] Moreover, it facilitates comparison between different water types including CDOM-rich and CDOM-poor waters. The spectral slope of the interval between 275 and 295 nm was adopted to indicate the molecular weight and the photo-oxidative state of CDOM.[31] The spectral slope of $S_{275\text{-}295}$ was calculated using nonlinear regression (Origin software) as follows:[17]

$$\alpha_{CDOM}(\lambda) = \alpha_{CDOM}(\lambda_0)\exp[S(\lambda - \lambda_0)] \quad (2)$$

where $a(\lambda)$ and $a(\lambda_0)$ are the absorption coefficients at wavelengths λ and λ_0, respectively. Here λ_0 was the reference wavelength, and S is the exponential spectral slope.

2.3. Three-dimensional fluorescence characterization

EEMs of CDOM were determined by a Hitachi F-7000 fluorescence spectrometer (Hitachi High-Technologies) with a 700-voltage xenon lamp. The scanning range was set at 200–450 nm for excitation and 250–600 nm for emission. The bandwidths were set as 5 nm intervals for excitation and 1 nm intervals for emission. A Milli-Q water blank was subtracted to eliminate the water Raman scatter peaks. Fluorescence intensity was calibrated using quinine sulphate unit (1 QSU), which represents the maximum fluorescence intensity of 0.01 mg L^{-1} for quinine in 1 N H_2SO_4 close to the position of the excitation wavelength (Ex; nm)/emission wavelength (Em; nm) = 355/450.[32] In this work, the humic-like fluorescence was represented as *F*n355, with an excitation wavelength of 355 nm and an emission peak of 450 nm.[33,34] The protein-like fluorescence was expressed using *F*n280 with an excitation wavelength of 280 nm and an emission peak of 350 nm.[34] To facilitate comparison with other studies using different fluorometers, correction files provided by the manufacturer Hitachi (Hitachi F-7000 Instruction Manual) were utilized to correct for the EEMs. [35]

To eliminate the inner-filter effect, the EEMs were corrected for absorbance by multiplying each value in the EEMs with a correction factor as described by Ohno.[36] The

EEMs contour figures for the measured samples were drawn using Origin 8.0. In addition, statistical boxplots were drawn with Statistical Program for Social Sciences (SPSS) 16.0 software. In the boxplots, the top and bottom line segment represent the maxima and minima, respectively. The upper and lower line of the boxplot indicates the third quartile and the first quartile, respectively, and the middle bold line indicates the median value. The significance of differences in parameters between above-tree-line and below-tree-line lakes was examined with an independent samples *t*-test using a *p*-value of 0.05.

2.4. Parallel Factor Analysis (PARAFAC) modelling

PARAFAC statistically decomposes the EEMs of DOM into individual fluorescent components. The data signal can be decomposed into a set of three linear terms and a residual array.[37] EEMs combined with PARAFAC offers useful information to characterize DOM extracted from terrestrial and marine aquatic environments.[18,26,38] The reported number of fluorescent components decomposed using PARAFAC normally ranges from 4 to 13 for diverse freshwater and marine aquatic environments.[26,38,39] Stedmon and Bro described the procedure for characterization of DOM fluorescence using PARAFAC, including a split-half analysis to verify the decomposed fluorescent components.[40] Split-half analysis divides the data set into two random and equal-sized groups and then establishes a PARAFAC model for both halves independently. The loadings from both models will be the same when the correct number of components is determined. The PARAFAC analysis in this work was performed with matrix laboratory (MATLAB) using the dissolved organic matter fluorescence (DOMFluor) toolbox for MATLAB as described by Stedmon and Bro.[40] For PARAFAC modelling, excitation wavelengths of 200–220 nm and emission wavelengths of 250–300 nm were deleted from each data group because of the uncertainty of data in these regions.

2.5. Calculation of fluorescence index

To characterize CDOM in alpine lakes, we used three indices: FI_{255},[41] FI_{310},[27,42] and FI_{370}.[26] The humification index (FI_{255}) is defined, under the excitation wavelength of 255 nm, as the ratio between the average fluorescence intensity from 435 nm to 480 nm divided by the sum of 300~345 nm and 435~480 nm (λ_{Ex}=255 nm, $\Sigma F_{435–480}/(\Sigma F_{300–345} + \Sigma F_{435–480})$).[43] The increasing degree of CDOM aromaticity suggests a decrease in the ratio of H:C, which causes a red-shift in the emission spectrum excited at 255 nm, thus increasing FI_{255}. Therefore, a high FI_{255} value corresponds to complex molecules, such as aromatics with high molecular weight.[41] The index of FI_{310} was calculated as the ratio of emission intensity at 380 nm to the maximum emission intensity observed between 420 and 435 nm for an excitation wavelength of 310 nm. This corresponds to the ratio of newly produced CDOM to total CDOM, which provides information to quantitatively assess the bioactivity of a littoral area and to estimate the stage of biological productivity. A high FI_{310} value corresponds to a high CDOM degradation degree and the formation of autochthonous carbon.[27] Another useful index is FI_{370} (λ_{Ex}=370 nm, F_{470}/F_{520}), which represents the relative contribution of aromatic amino acids and non-aromatic substances to fluorescence intensity. Thus, it has been used as a tracer of CDOM source and degradation degree.[36,44]

2.6. Regular water-quality parameters

Water temperature was recorded by using a multi-parameter water quality measuring instrument (YSI 556 MPS). Total nitrogen (TN) and total phosphorus (TP) were measured by a Shimadzu UV2401 UV-Vis spectrophotometer using alkaline potassium persulphate digestion and the molybdenum blue method, respectively. Ammonia nitrogen (NH_4-N) and orthophosphate (PO_4-P) concentrations were analysed using the spectrophotometer after water samples were passed through Whatman GF/F filters. To determine chlorophyll *a* (Chl *a*), water samples were filtered over Whatman glassfibre GF/C filters, followed by extraction with ethanol (90%) at 80°C and spectrophotometric analysis at 750 nm and 665 nm.[45]

The Trophic State Index (TSI) is an indicator that integrates the physical, chemical, and biological conditions of lakes on a system level and has been used in CDOM research.[18] Here, Chl *a* (in μg L^{-1}), total nitrogen (TN in mg L^{-1}), total phosphorus (TP in mg L^{-1}), and transparency in Secchi disk depth (in m) were applied to calculate TSI in the study lakes according to the equations reported by Cai et al.[46]

3. Results and discussion

3.1. General characteristics

The calculated TSI results show that all study lakes are oligotrophic. Actually, there are rarely eutrophic lakes higher than 4000 m because of the natural changes in catchment properties and low human activities.[18] It is well known that nutrients (TN and TP) have a significant influence on the primary productivity, concentration, and decomposition of DOM. From Table 1, we can see that there is no significant difference in the concentrations of nutrients between above-tree-line lakes and below-tree-line lakes. The concentrations of Chl *a* at the surface are slightly lower than at 2.0 m depth in most study lakes. The upper water layer is believed to be more influenced by terrestrial input and photodegradation, and the deeper layer more affected by bacterial degradation of phytoplankton.[47] In fact, the interpretation that the deeper layer is more affected by bacterial degradation of phytoplankton does not mean that bacterial degradation in the deeper layer is stronger than at the surface. In this work, bacterial abundance at the surface of study lakes was significantly higher than at 2.0 m depth ($p < 0.001$) (data not shown). Therefore, the influence of photodegradation and microbial decomposition on Chl *a* concentrations at the surface should have been stronger than at 2.0 m depth. However, it should be noted that although bacterial abundance at 2.0 m depth was lower than at the surface, bacterial degradation of phytoplankton still plays a pivotal role in determining the optical properties of CDOM in the deeper layer. This means that in the deeper layer, the influence of bacterial degradation is more important than solar irradiation and terrestrial input. The correlation between Chl *a* and CDOM absorption will be discussed later.

(Table 1)

3.2. Optical absorption analysis

Fig. 1 shows that the CDOM absorption coefficients (a_g, m^{-1}) for all study lakes versus wavelength (nm) exhibit approximately exponential decay.[48] Moreover, a_g values at the water surface in most of below-tree-line lakes were remarkably higher than in above-tree-line lakes. It is significant that a_g values for both the water surface and 2.0 m depth in below-tree-line Lakes SXH and GLH was higher than in other lakes. This suggests that there is a higher concentration of CDOM in these two lakes. These two lakes are surrounded by thick pinewood, evergreen broad-leaf forest, and spruce forest, and the ambient soil presents dark-brown colour, resulting in a certain humic characteristic. Although surrounded by the same abundant vegetation, Lakes ZH, MH, and SMH are partly encircled by a heap of rocks due to geological movement. This may cause less terrestrial input of CDOM in these three lakes than other below-tree-line lakes and, thus, result in lower absorption coefficients than in other below-tree-line lakes. In contrast, above-tree-line lakes are surrounded by open fields with scattered shrub tussock. As a result, their concentrations of CDOM were much lower. Among these above-tree-line lakes, Lakes Huanghai and Zhuhai are mainly subsidized by snow melt, which causes large amounts of terrigenous substances to be transported into the lakes. Therefore, the absorption coefficients at the water surface and 2.0 m water depth in these two lakes were similar. Meanwhile, Lake Heihai lies below Huanghai and Zhuhai, and they are linked via brooks. Thus, the absorption coefficient of Heihai is slightly lower than that of Huanghai and Zhuhai.

In alpine regions, climatic change will exert an important influence on the balance between water sources (rainfall, ice-melt, snowmelt, and groundwater) and aquatic systems, particularly altering the relative contributions of meltwater, groundwater, and rain to lakes.[49] Glaciers and ice sheets represent the second largest reservoir of water in the global hydrologic system. As a direct consequence of further warming, snow cover duration will likely decrease. In 2009, Hood et al. pointed out that the bioavailability of DOM to marine microorganisms is significantly correlated with increasing ^{14}C age.[50] They believed that glacial runoff is a quantitatively important source of labile carbon to marine ecosystems. Moreover, they concluded that climatically driven changes in glacier volume could alter the age, quantity, and reactivity of DOM entering coastal oceans. Based on the previous reports, we infer that increasing snowmelt driven by climatic changes is a driver of CDOM optical properties in the lakes.

In addition, it is interesting to note that curve shapes of absorption coefficients versus wavelength presents a small shoulder around 275 nm. This feature is in accordance with previous reports.[48,51] That is, the trend in the waveband of 250–280 nm runs contrary to the whole trend. Furthermore, the shoulder in the spectrum around 275 nm is particularly evident in below-tree-line lakes in which CDOM concentrations are much higher. This result is different from some previous work, where it was reported that the shoulder was more evident for more offshore stations where CDOM concentrations were lowest.[52] This spectral feature could be related to absorption by dissolved DNA[53] or degradation products of tannins and lignins.[54] In addition, the shoulder could also be ascribed to phenolic compounds since humic associated phenolic compounds were predominant in below-tree-line lakes and the absorbance of phenol peaks is around 270 nm as well. The shoulder at 275 nm will be discussed later with the combined consideration of the fluorescence and absorbance data.

(Fig. 1)

Zhang et al. pointed out that there was a significant negative linear relationship between log-transformed altitude and log-transformed *a*280 (absorption coefficient at 280 nm);[18] however, they also stated that no significant difference was found between *a*280 at three different altitudes when raw data were analysed (p value was not shown). Nevertheless, in this work, significant differences in the raw CDOM concentrations were observed between above-tree-line and below-tree-line lakes (Fig. 2). The relative concentrations of CDOM expressed using *a*250 and *a*365 are shown in Fig. 2a and 2b, respectively. The values of both *a*250 and *a*365 in below-tree-line lakes were much higher than in above-tree-line lakes ($p < 0.001$). However, this does not necessarily mean that the concentrations of CDOM in below-tree-line lakes are higher than in above-tree-line lakes. If the absorption coefficients are roughly a linear function of DOC,[21,22] the large differences between the above and below tree line lakes may merely be the result of low DOC water in the former and higher DOC levels in the latter. Taking this into account, we plotted the linear relation between DOC concentration and the ratios of *a*250 and *a*365 (see the embedded figures in Fig. 2a and 2b); the ratios of *a*250 and *a*365 are not a linear function of DOC. Thus, *a*250 and *a*365 values represent the CDOM concentrations. Therefore, we can conclude that the CDOM concentrations in below-tree-line lakes are significantly higher than in above-tree-line lakes.

Note that the median values of the surface water are slightly higher than those of water at 2.0 m depth. Although photo-oxidation can result in lower absorption values of CDOM in surface water, the upper water layer is also greatly influenced by terrestrial inputs, and the deeper layer is more affected by bacterial degradation of phytoplankton.[17,25] In this work, we investigated seasonal variations in the CDOM concentration of Lake Tiancai and found that CDOM concentration was significantly influenced by terrestrial source. The influence of terrestrial source varied seasonally, and the smallest variation occurred in October. This indicates that the difference in CDOM composition in October was relatively small and its source was relatively simple. Consequently, although photo-oxidation can decrease CDOM absorption values, a large input of terrestrial CDOM into lakes can counteract the effect of photo-oxidation. Moreover, phytoplankton decomposition through photodegradation and microbial activity can also contribute to the elevated CDOM at the surface.

Fig. 2c and 2d show that the intensities of *F*n280 and *F*n355 for above-tree-line lakes are substantially lower than for below-tree-line lakes ($p < 0.05$), suggesting that above-tree-line lakes have a lower intensity of the protein-like and humic-like fluorescence. In above-tree-line lakes, there is no remarkable difference in the intensity of protein-like fluorescence (*F*n280) between surface water and 2.0 m depth. In contrast, a higher median value of the humic-like fluorescence (*F*n355) was observed at the surface relative to 2.0 m depth. In below-tree-line lakes, the median value of *F*n280 at the surface was significantly lower than at 2.0 m depth. In contrast, most *F*n355 values at the surface were higher than at 2.0 m, although the median values of *F*n355 were very similar at these two depths. This is contrary to results by Mopper et al,[55] who reported that protein-like fluorescence was dominant in the upper water column, while humic-like fluorescence was dominant in the deeper samples. Chen et al. suggested that the upper water layer is influenced more by terrestrial input and photodegradation, and the deeper layer is more affected by bacterial degradation of phytoplankton.[47] A large amount of terrestrial input at the surface of below-tree-line lakes causes the accumulation of humic substances, which are considered refractory. Thus, CDOM at the surface of below-tree-line lakes is probably dominated by humic-like substances. In contrast, at 2.0 m depth in below-tree-line lakes, CDOM contains more fresh protein produced by phytoplankton and bacteria.[56] In addition, phytoplankton tends to avoid the surface layer in high-mountain lakes to avoid harmful UV-B radiation, which could contribute to the low concentration of protein-like substances at the surface in below-tree-line lakes.[57] However, this would not explain the opposite result observed for above-tree-line lakes. Actually, phytoplankton biomass in these lakes is also related to nutrients and zooplankton, which will be discussed later.

In this work, the lower intensity of *F*n280 at the surface in below-tree-line lakes could be due to photodegradation of the labile protein-like substances. In high-altitude lakes, protein-like substances are easily subjected to degradation by extremely strong solar irradiation and utilization by bacterioplankton.[8,56] The photodegradation of the protein-like substances is mainly caused by UV irradiation together with the induced generation of reactive oxygen species. These species can further accelerate indirect photochemical reactions involving CDOM.[58] In addition, note that *F*n355 shows a trend contrary to that of *F*n280 in below-tree-line lakes. This difference could be explained by the fact that the humic-like substances are normally considered to be refractory. The higher concentration of humic-like substances at the surface is more likely caused by leaching of humic substances from forested soil, while their decreased concentration at 2.0 m could be attributed to bacterial degradation.

The embedded graph in Fig. 2d shows that *a*250 is strongly and positively correlated with *F*n355 for below-tree-line lakes ($r^2 = 0.95$, $p < 0.001$) (i.e., the trends of *a*250 and *F*n355 are synchronous). Nevertheless, *a*250 is only weakly and negatively correlated with *F*n280 ($r^2 = 0.14$, $p < 0.05$) (the embedded graph in Fig. 2c). This result is in agreement with prior studies, which demonstrated a stronger correlation between CDOM absorption and humic-like fluorescence than between CDOM absorption and protein-like fluorescence.[18,59] This indicates that CDOM in below-tree-line lakes is primarily derived from humic-like substances. The continuous terrestrial input of fallen leaves and/or humus soil can result in the enhanced accumulation of humic-like substances. Additionally, a positive linear relationship was found between *a*250 and *F*n280 (*F*n355) for above-tree-line lakes ($r^2 = 0.24$ and 0.20, respectively, $p < 0.005$) (see the embedded graph in Fig. 2c and 2d). A part of the humic-like substances can be produced through bacterial utilization of phytoplankton.[60] Considering the relatively high concentrations of Chl *a* observed in above-tree-line lakes, the positive linear relationship between *a*250 and *F*n280 (*F*n355) suggests that the protein-like substances make an important contribution to CDOM in these lakes, and, consequently, a part of the humic-like substances were formed when bacteria utilized phytoplankton. Of special note is that the intensities of *F*n280 and *F*n355 at the water surface in Zhuhai were remarkably higher than in other above-tree-line lakes, corresponding to higher concentrations of protein-like and humic-like substances in this lake. This is mainly because of the autochthonous contribution of microbially degraded aquatic plants and algae, which will be discussed in detail in the following section.

(Fig. 2)

Fig. 3a displays the ratio of absorption at 250 nm to absorption at 365 nm (*a*250/*a*365), which can be related to the relative molecular size of CDOM.[20] The increasing proportion of humic acid (HA) to fulvic acid (FA) in CDOM suggests that the average molecular weight of CDOM increased, which caused stronger light absorption by high-molecular-weight CDOM at longer wavelengths. Therefore, the ratio of *a*250/*a*365 will decrease with increasing molecular size. In this work, the values of *a*250/*a*365 at the water surface in most of above-tree-line lakes were higher than in below-tree-line lakes, suggesting a relatively small molecular size of CDOM in above-tree-line lakes, especially in Zhuhai (outlier in Fig. 3a). We infer that this is partly attributed to the photochemical degradation of CDOM at the surface.[8] Note that there was around 70% submerged vegetation coverage in Zhuhai, and Chl *a* concentration at the water surface was also higher than at 2.0 m depth (1.8 μg L^{-1}, Table 1). Thus, the smaller molecular size of

CDOM at the surface of Zhuhai was partly caused by photodegradation and in situ microbial decomposition of aquatic plant, algae, and extracellular material released by aquatic organisms.[8] Even in Heihai (outlier in Fig. 3a), the molecular size of CDOM at 2.0 m depth was smaller than at the same depth in other above-tree-line lakes, which is still larger than at its surface. As previously mentioned, Lake Heihai receives brook outflow from Huanghai and Zhuhai. Thus, its upper water layer is primarily supplied by runoff.[47] Before entering into Heihai, water from Huanghai and Zhuhai underwent photochemical and biological degradation to a certain extent. In addition, the sampling work was performed in September and October, and Heihai is a relative deep lake and was thermally stratified during sampling. This is very important as most of the study lakes were completely mixed during sampling, and it would explain some of the differences in optical properties with depth. A mixed lake would likely show little difference in optical properties with depth, while a stratified lake would likely have a distinct profile.

The values of $a250/a365$ in above-tree-line lakes display much higher variability than below the tree line, and this variability is more than observed for relative values that tend to be similar on average. One possible reason for such high variability might be that the decrease in ice cover could significantly increase UV exposure for above-tree-line lakes. Since lakes above the tree line are mainly fed by meltwater and precipitation, CDOM from glacial ice and meltwater contains significant levels of terrestrial material, which may be highly labile [50] or be the least reactive.[61] Consequently, there is a much higher variability in $a250/a365$ values for above-tree-line lakes surrounded by alpine meadows or rocky environments. In contrast, the input of humic substances into below-tree-line lakes is relatively stable in the case of forested soil.[13] Thus, the values of *a*250/*a*365 are less variable.

It has been reported that SUVA254 is strongly correlated with compounds containing unsaturated carbon bonds, such as refractory aromatic compounds, and the decrease in SUVA254 indicates the transformation of humus to non-humus.[21,22] Here, SUVA254 in most above-tree-line lakes was significantly lower than in below-tree-line lakes ($p < 0.02$), as shown in Fig. 3b. This suggests that the percent aromaticity of CDOM in most above-tree-line lakes is lower than in below-tree-line lakes. Moreover, for most above-tree-line lakes, SUVA254 at the surface is a little lower than at 2.0 m, indicating a lower percent aromaticity at the surface. This is consistent with the conclusions drawn from *a*250/*a*365, which suggested smaller molecular size at the surface in above-tree-line lakes. As previously mentioned, photodegradation and microbial transformation can lower the percent aromaticity of CDOM at the surface. However, this phenomenon is not remarkable in below-tree-line lakes, which is similar to the finding that little difference in *a*250/*a*365 was observed between the surface and 2.0 m depth in below-tree-line lakes (Fig. 3a). Note that in some of the box and whisker plots, the error bars appear to overlap and show that the differences between above and below-tree-line lakes are not significant for *a*250/*a*365 ratios and SUVA254 (Fig. 3a and 3b). As previously mentioned, the value of *a*250 in the above tree line lakes was very small and appeared to be statistically different from below-tree-line lakes, but for SUVA (essentially absorbance normalized to DOC) these values are much more similar. However, the molecular size of CDOM is independent of CDOM concentration. Rather, the molecular size of CDOM is more connected to the source and composition of CDOM. Thus, we still believe that the a250 and a365 numbers reflect the differences in CDOM concentration.

The concentration of Chl *a* in the study lakes is shown in Fig. 3c, which reveals that Chl *a* was lower in below-tree-line lakes than in above-tree-line lakes ($p < 0.02$). Zhang et al. concluded that phytoplankton biomass is mainly controlled by nitrogen concentration and that nitrogen is the probable limiting factor for phytoplankton growth in high altitude lakes.[18] In this work, we found that there is significantly higher zooplankton biomass in below-tree-line lakes than in above-tree-line lakes (data not shown). Theoretically, this is of great importance for the lacustrine food web in which zooplankton feed on phytoplankton (i.e., phytoplankton biomass can be regulated by top-down forces).[62] The higher Chl *a* concentration in above-tree-line lakes suggests that autochthonous phytoplankton should make an important contribution to CDOM in these lakes. The above-mentioned regression analysis corroborates this observation, meaning there is a positive correlation between *a*250 and *F*n280 for above-tree-line lakes. Phytoplankton accumulation and decomposition is considered to mainly contribute to low molecular weight carbohydrates, proteins, and lipids, which can be transformed into CDOM.[60,63-65] This provides more evidence that CDOM in above-tree-line lakes has a smaller molecular size and a lower percent aromaticity than in below-tree-line lakes, similar to a previous analysis (Fig. 3(a-c)).

Fig. 3d displays the spectral slope of $S_{275\text{-}295}$ for all study lakes. The median values of $S_{275\text{-}295}$ for above-tree-line lakes were significantly higher than those of below-tree-line lakes, regardless of the sampling depth ($p < 0.005$). Like the ratio of *a*250/*a*365, $S_{275\text{-}295}$ is largely independent of CDOM concentration. In this work, $S_{275\text{-}295}$ appeared to be positively correlated to *a*250/*a*365, indicating that it is inversely related to the molecular weight of CDOM. A similar trend was observed in a previous study,[31] suggesting that a larger proportion of humic acids corresponds to a higher value of $S_{275\text{-}295}$ and a larger CDOM molecular size. Based on this, the mean molecular weight of CDOM in above-tree-line lakes was likely lower than in below-tree-line lakes. This result supports the conclusion drawn from $a250/a365$. In addition, steeper slopes of CDOM in above-tree-line lakes corresponds to a more rapid decrease in absorption with increasing wavelength.[31] This could be attributed to the relatively strong sunlight penetration and small molecular weight in clear above-tree-line lakes. Regarding the much higher variability of $S_{275\text{-}295}$ values for above-tree-line lakes, the same explanation can be made for $a250/a365$.

Moreover, Fig. 3d shows that the median value of $S_{275\text{-}295}$ at the water surface is slightly higher than at 2.0 m depth, indicating a smaller molecular weight of CDOM at the surface. Indeed, photo-oxidation can result in lower molecular weight CDOM, especially at the surface, as evidenced from the $a250/a365$ result. It has been mentioned that the indicators of molecular weight ($a250/a365$ and $S_{275\text{-}295}$) are independent of the concentration of CDOM ($a250$ or $a365$)[20, 31]. The molecular weight of CDOM is more closely related to its source and composition. Therefore, the higher absorption of CDOM at the surface does not contradict its apparently small weight molecular weight.

(Fig. 3)

3.3. Fluorescence properties

EEMs of Lake Zihai and Tiancai were recorded, and four marked fluorescent peaks were identified based on the traditional 'peak picking' method (Supporting Information Fig. S2 and Table S1). The peaks were the tryptophan-like fluorescent component S1

(Ex$_{max}$/Em$_{max}$: 230–245/285–295(310–375)), the tyrosine-like fluorescent component S2 (Ex$_{max}$/Em$_{max}$: 210–220/265–270(285–295)), the tryptophan-like or phenolic (polyphenolic) fluorescent component S3 (Ex$_{max}$/Em$_{max}$: 230 (300)/338), and the UV humic-like fluorescent component S4 (Ex$_{max}$/Em$_{max}$: 300–340/400–450). Component S1 presents two Em$_{max}$ (285–295 and 310–375), which are similar to the tryptophan-like fluorophores found in other studies.[26,37,39,66,67] Component S2 displays two Em$_{max}$ (265–270 and 285–25) as well, which are close to the reported tyrosine-like fluorophores, possibly corresponding to peptides.[37,66,67] Component S3 shows one Em$_{max}$ (338) and two Ex$_{max}$ (230 and 300), similar to the reported tyrosine-like fluorophores.[38] Maie et al. showed a detailed analysis of T-peaks (excitation/emission maximum at 280/325 nm) of DOM in coastal wetland and estuarine environments.[68] They pointed out that its constituents can be separated into two classes of compounds, proteinaceous materials with high MW and humics associated with phenolic compounds with small MW. In many of lakes, water possesses polyphenols leached from senescent plant materials. While these polyphenols can promptly decompose, the residual phenolic groups in their humic-like diagenetic products will still contribute to phenolic compounds with small MW. In the present work, amino acid data were not shown, and, thus, the possibility that fluorescence in the low excitation and emission region could be attributed to phenolic or polyphenolic compounds fluorescence should be noted. In addition, component S4 has excitation and emission properties similar to terrestrial humic-like fluorophores.[37,39,69,70]

Three fluorescent components were identified by PARAFAC according to the split-half validation procedure (Fig. 4). Fig. 4 shows that for each component these three lines are nearly superimposed, the very similar shape of the components provides a nearly perfect validation of the fluorescence signatures. The overlapping excitation and emission loadings of the three components are presented in Fig. 4. These fluorescent components showed single or multiple excitation and emission maxima. The identified excitation and emission characteristics of the CDOM components in this study and their potential sources as well as examples of matching components of CDOM EEMs from previous work are provided in Table 2. The three components we identified were the terrestrial humic-like substances (Ex_{max}/Em_{max}: 235 (300)/430, component 1), the UV humic-like fluorescent peak (Ex_{max}/Em_{max}: 260 (305)/470, component 2), and the tryptophan-like or phenolic fluorescent peak (Ex_{max}/Em_{max}: ≤225 (295)/330–350, component 3).

Component 1 (C1) displays excitation and emission properties close to marine humic-like fluorophores with excitation maxima in the ultraviolet region (235 nm and 300 nm) and emission maxima in the visible region (430 nm).[15,18,39,69,70] Component 1 is traditionally considered to be a biological degradation humic component and was named the marine humic-like component in marine environments. Component 2 has two excitation maxima (260 nm and 305 nm) with a single emission maximum (475 nm), Normally, C2 can be extensively detected in terrestrial humus with large molecular size, especially in forest and wetland environment.[18,37] Component 3 shows two excitation maxima (≤225 nm and 295 nm) with a single emission maximum (330–350 nm), and was considered to be the reported tryptophan-like or phenolic fluorophores, representing terrestrial or autochthonous protein-like or humic associated compounds.[39,68-70]

In the study lakes, C1 falls in the transition zone between terrestrial and marine humic-like components.[37] The excitation and emission maxima of component 1 are similar to those of peak M and peak N,[70] which was likely related to marine humic-like fluorophores or to be associated with phytoplankton productivity. [18,69,70] However, the study lakes are not connected to any marine DOM source. Zhang et al. pointed out that component 1 is of biological origin and is not exclusively a marine component. They believed that component 1 is derived from phytoplankton degradation, which was deduced from the finding that there was a remarkable increase of component 1 in the transition from the mesotrophic to eutrophic state.[18] Nevertheless, all lakes in this study are oligotrophic state and have low phytoplankton productivity. Based on this, component 1 in this work may not be strongly related to phytoplankton degradation. Actually, component 1 recorded falls in the transition zone between terrestrial and marine humic-like components.[37] It is thus inferred that component 1 is mainly derived from a biological degradation of terrestrial humic component.

The protein-like or phenolic components (C3) were dominant in above-tree-line lakes. These components can be derived from various sources, including terrestrial, autochthonous, and microbial sources. Considering the environmental condition and the relatively high Chl *a* concentration in above-tree-line lakes, we infer that the protein-like components were primarily sourced from phytoplankton in these lakes. The terrestrial humic-like component (C2) was only found in below-tree-line lakes, suggesting that the leaching of humic substances into lake water was higher in the case of forested soil compared to alpine meadows or rocky environments.[13] As mentioned in the absorbance spectra, the shoulder at 275 nm in the absorbance spectra is particularly evident in below-tree-line lakes, in which humic associated phenolic compounds are predominant. This is because polyphenols leached from senescent plant materials are important precursors of humic substances.[68] Although these polyphenols could be promptly decomposed after entering into natural environments, the residual phenolic groups in their humic-like diagenetic products will still contribute to component 2. With the combined consideration of the fluorescence and absorbance data, the shoulder at 275 nm could be attributed to protein or aromatic amino acid (e.g., tryptophan) or phenolic compounds in the waters as a result of significant microbial activities. However, the small hump at 275 nm in this work needs independent corroboration to identify specific chemical species that contribute to it.

In general, for alpine lakes above the tree line, the CDOM fluorescence was predominantly contributed by protein-like components rather than by the humic-like components. This is consistent with results reported by Zhang et al.[18] In their study, they explained that autochthonous biological degradation of CDOM could account for the predominant protein-like components in the total fluorescence. It was also suggested that DOC derived from algae and bacteria could be an important source of CDOM in above-tree-line lakes.[71] In addition, we measured UV attenuation in the study lakes during sampling work and found that the UV irradiation attenuated much faster in below-tree-line than in above-tree-line lakes (data not shown). This is mainly attributed to the higher concentration of CDOM in below-tree-line lakes. The strong UV penetration in above-tree-line lakes can lead to rapid photodegradation of phytoplankton, which can indirectly contribute the protein-like components to CDOM.

(Fig. 4)

3.4. Fluorescence index analysis

Fig. 5a shows that the values of FI_{255} ranged from 0.13 to 0.98. In above-tree-line lakes, the mean value of FI_{255} at the water surface was 0.56 ± 0.29, and the value at 2.0 m was 0.55 ± 0.29. In below-tree-line lakes, the mean value of FI_{255} at the surface was 0.79 ± 0.15, and the value at 2.0 m was 0.66 ± 0.27. The mean values of FI_{255} for below-tree-line lakes were significantly higher than those of above-tree-line lakes ($p < 0.05$). It has been proposed that high FI_{255} values correspond to complex molecules, such as aromatics with high molecular weight compounds.[41] This provides additional evidence confirming that CDOM in below-tree-line lakes has a larger molecular size and higher percent aromaticity than in above-tree-line lakes.

As stated previously, the intensities of *F*n355 in below-tree-line lakes were remarkably higher than in above-tree-line lakes (Fig. 2d). Normally, FI_{255} values are not only correlated with the concentration of CDOM, but also with CDOM source and composition. Zhang et al. proposed that in the Yungui Plateau lakes, the values of FI_{255} below 1.5 correspond to CDOM with biological or aquatic bacterial origin; FI_{255} values between 1.5 and 3.0 indicate weak humic character and important recent autochthonous component; and FI_{255} values between 3.0 and 6.0 indicate strong humic character and weak recent autochthonous components.[18] Here, the study lakes were also located in the Yungui Plateau, and all values of FI_{255} were below 1.5, which seems to suggest that material of biological or aquatic bacterial origin made a crucial contribution to CDOM in the studied oligotrophic alpine lakes. This inference is true for lakes above the tree line, but contradicts earlier conclusions for lakes below the tree line. Zhang et al. reported that a significant negative linear relationship was observed between log-transformed altitude and both FI_{310} and FI_{370}, but no significant correlation was found between log-transformed altitude and FI_{255}, indicating that FI_{255} is not appropriate to characterize the effects of altitude on CDOM.[18] For this reason, the two other indices of FI_{310} and

FI_{370} were introduced to further compare the fluorescence properties of CDOM between above-tree-line lakes and below-tree-line lakes.

The FI_{310} index can be used to express the proportion of newly produced CDOM to the whole stock of CDOM. Furthermore, it can provide information to estimate aquatic biological activity and productivity level.[42] FI_{310} values will increase with an increase in the degradation degree of CDOM and new autochthonous carbon production.[27] The values of FI_{310} in the study lakes ranged from 0.36 to 1.43 (Fig. 5b), which fall into the reported range.[18] The mean value of FI_{310} in above-tree-line lakes was 0.71 ± 0.20 at the surface and 0.76 ± 0.18 at 2.0 m depth; in below-tree-line lakes it was 0.57 ± 0.19 at the surface and 0.82 ± 0.46 at 2.0 m depth. Huguet et al. reported that FI_{310} values between 0.6 and 0.7 indicate a low autochthonous component in DOM; values between 0.7 and 0.8 indicate intermediate autochthonous origin; and values between 0.8 and 1.0 correspond with a strong autochthonous component.[72] In this study, mean values of FI_{310} were close to 0.7–0.8, except for in surface samples from below-tree-line lakes. This is potentially related to two aspects: (1) an intermediate autochthonous component in the newly produced CDOM and (2) a highly degradable older/ancient CDOM component. At the surface in below-tree-line lakes, the lower mean value of FI_{310} corresponds to allochthonous/terrestrial origin. This conclusion is in accordance with the prior analysis for identified fluorescent peaks (Fig. 4); the protein-like components were predominant in above-tree-line lakes and partly found in below-tree line lakes (especially at 2.0 m), and the humic-like components were only identified in below-tree line lakes.

It is very interesting to note that recent studies showed that DOM from glacial ice and meltwater contains significant levels of terrestrial material, which may be highly bioavailable [50] or be the least reactive.[61] These studies provided definitive evidence of allochthonous DOM in these high elevation glaciers as a source of ancient DOM. The study lakes in this work, especially for above-tree-line lakes, are mainly supplied by meltwater and precipitation. The age-reactivity relationships of CDOM in these lakes need further investigation. In addition, it should be pointed out that the identification of carbon sources using FI_{310} values is based on estuarine water rather than inland water.[71] Moreover, Huguet et al. applied the FI_{310} index to trace DOM sources instead of CDOM sources,[72] which is obtained with a 0.7 μm filter rather than a 0.22 μm filter. Consequently, we stress that FI_{310} and other fluorescence indices should be used in combination to better assess CDOM sources for alpine lakes.

The FI_{370} index is applied to determine the contribution of terrestrial, microbial aromatic amino acids, and non-aromatic substances to CDOM fluorescence intensity. Thus, it can be used to investigate the sources and degradation of CDOM.[36,44] It has been suggested that FI_{370} values greater than 1.8 indicate autochthonous microbial and algal origin; and FI_{370} values less than 1.2 indicate terrestrially derived CDOM.[36] In this study, most values of FI_{370} fell into the range of 1.3–1.7 (Fig. 5d), which is close to previously reported values[18,36] and suggests that both autochthonous and allochthonous sources contribute to CDOM in these lakes. Exceptionally, FI_{370} values at the surface of Lake Zhuhai (2.45, outlier in Fig. 5d) and Fuhai (1.94) are larger than 1.8, corresponding to a higher autochthonous source contribution. This result is supported by evidence from the high ratio of $a250/a365$ (Fig. 3a), and the latter indicates that there is a relatively small molecular size of CDOM in these two lakes. Note that the mean value of FI_{370} in above-tree-line lakes is significantly higher than that in below-tree-line lakes regardless of sampling depth ($p < 0.05$); the mean value of FI_{370} in above-tree-line lakes was closer to 1.8 (1.68 ± 0.44 at the surface, 1.57 ± 0.17 at 2.0 m), revealing that in above-tree-line lakes there was a higher contribution from autochthonous microbial and algal material than from allochthonous material. The mean value of FI_{370} in below-tree-line lakes was closer to 1.2 (1.45 ± 0.05 at the surface, 1.45 ± 0.07 at 2.0 m), possibly suggesting a higher contribution from allochthonous than from autochthonous sources. Overall, these indices support each other and suggest similar conclusions for this study. Consequently, to compare the fluorescence properties of CDOM between above-tree-line lakes and below-tree-line lakes, we suggest that FI_{255}, FI_{310}, and FI_{370} should be used in combination to best assess CDOM sources and composition.

The characteristics of CDOM associated with absorption and fluorescent indices in this work are shown in Table 3. In summary, (1) higher $a250/a365$ and $S_{275\text{-}295}$ values indicate a smaller molecular weight of CDOM in above-tree-line lakes than in below-tree-line lakes and a smaller molecular weight at the surface than at 2.0 m depth; (2) SUVA254 and FI_{255} results provide evidence of a lower percent aromaticity of CDOM in above-tree-line lakes; and (3) FI_{310} and FI_{370} suggest a strong allochthonous origin at the surface in below-tree-line lakes, and a higher contribution from autochthonous biological and aquatic bacterial origin in above-tree-line lakes.

(Fig. 5 and Table 3)

4. Conclusions

In this study, we investigated absorption and fluorescence properties of CDOM in 15 alpine lakes located below or above the tree line to determine CDOM sources and composition. The results indicate that the concentrations of CDOM in below-tree-line lakes are significantly higher than in above-tree-line lakes, as evidenced from the absorption coefficients of $a250$ and $a365$. The intensities of the protein-like and humic-like fluorescence in below-tree-line lakes were also higher than in above-tree-line lakes. In below-tree-line lakes, $a250$ was strongly and positively correlated with $Fn355$, but weakly and negatively correlated with $Fn280$, indicating that CDOM in below-tree-line lakes was primarily derived from humic-like substances. In above-tree-line lakes, a weak positive linear relationship was also found between $a250$ and $Fn280$ ($Fn355$).

Three fluorescent components were identified using Parallel Factor Analysis (PARAFAC) modelling. Component 1 is probably associated with biological degradation of terrestrial humic component. The terrestrial humic-like component 2 was only found in below-tree-line lakes. The protein-like or phenolic component 3 was dominant in above-tree-line lakes, which are probably derived from autochthonous material.

In summary, (1) higher $a250/a365$ and $S_{275\text{-}295}$ values indicate smaller molecular weights of CDOM in above-tree-line lakes than in below-tree-line lakes, and smaller molecular weight at the surface than at 2.0 m; (2) SUVA254 and FI_{255} results provide evidence for lower percent aromaticity of CDOM in above-tree-line lakes; and (3) FI_{310} and FI_{370} suggest a strong allochthonous origin at the surface in below-tree-line lakes, and a higher contribution from autochthonous biological and aquatic bacterial material in above-tree-line lakes. These indices should be used in combination to best assess CDOM sources and composition in alpine lakes. The data presented in this paper will enhance our understanding of the sources and biogeochemistry of CDOM/DOM in alpine lakes.

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Fig. 1. The absorption coefficient (a_g) of CDOM at the water surface in 15 lakes located above or below the tree line.

Fig. 2. (a) CDOM absorption coefficient at the wavelength of 250 nm (a250), and the embedded graph is scatter plot and linear regression for the relationship between a250 and DOC; (b) CDOM absorption coefficient at the wavelength of 365 nm (a365), and the embedded graph is scatter plot and linear regression for the relationship between a365 and DOC; (c) the intensity of the protein-like fluorescence (Fn280), and the embedded graph is scatter plot and linear regression for the relationship between a250 and Fn280; and (d) the intensity of the humic-like fluorescence (Fn355), and the embedded graph is scatter plot and linear regression for the relationship between a250 and Fn355. Fluorescence intensity is calibrated using quinine sulfate unit (1 QSU).

Fig. 3. (a) The ratio of absorption at 250 nm to 365 nm (a250/a365); (b) specific UV absorbance of SUVA254; (c) the concentration of Chl a in 15 alpine lakes; and (d) the spectra slope for the interval of 275-295 nm ($S_{275\text{-}295}$) determined by nonlinear regression.

Fig. 4. The PARAFAC model output presenting fluorescence signatures of three fluorescent components. (a-c) The contour plots present spectral shapes of excitation and emission. (d-f) The line plots present split-half validation results. Excitation (left) and emission (right) spectra were estimated from two independent halves of data set (red and green lines), and the complete data set (blue lines). For each component these three lines are nearly superimposed, the very similar shape of the components provides a nearly perfect validation of the fluorescence signatures.

Fig. 5. The fluorescence indices of (a) FI_{255}; (b) FI_{310}; and (c) FI_{370} for 15 alpine lakes

above or below the tree line.

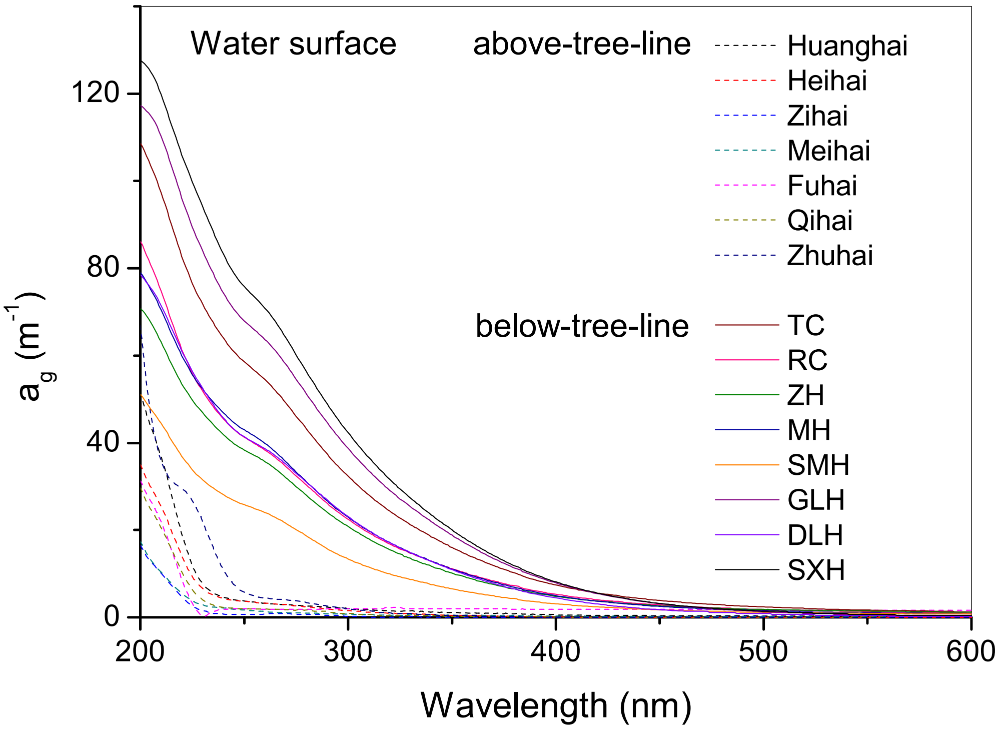
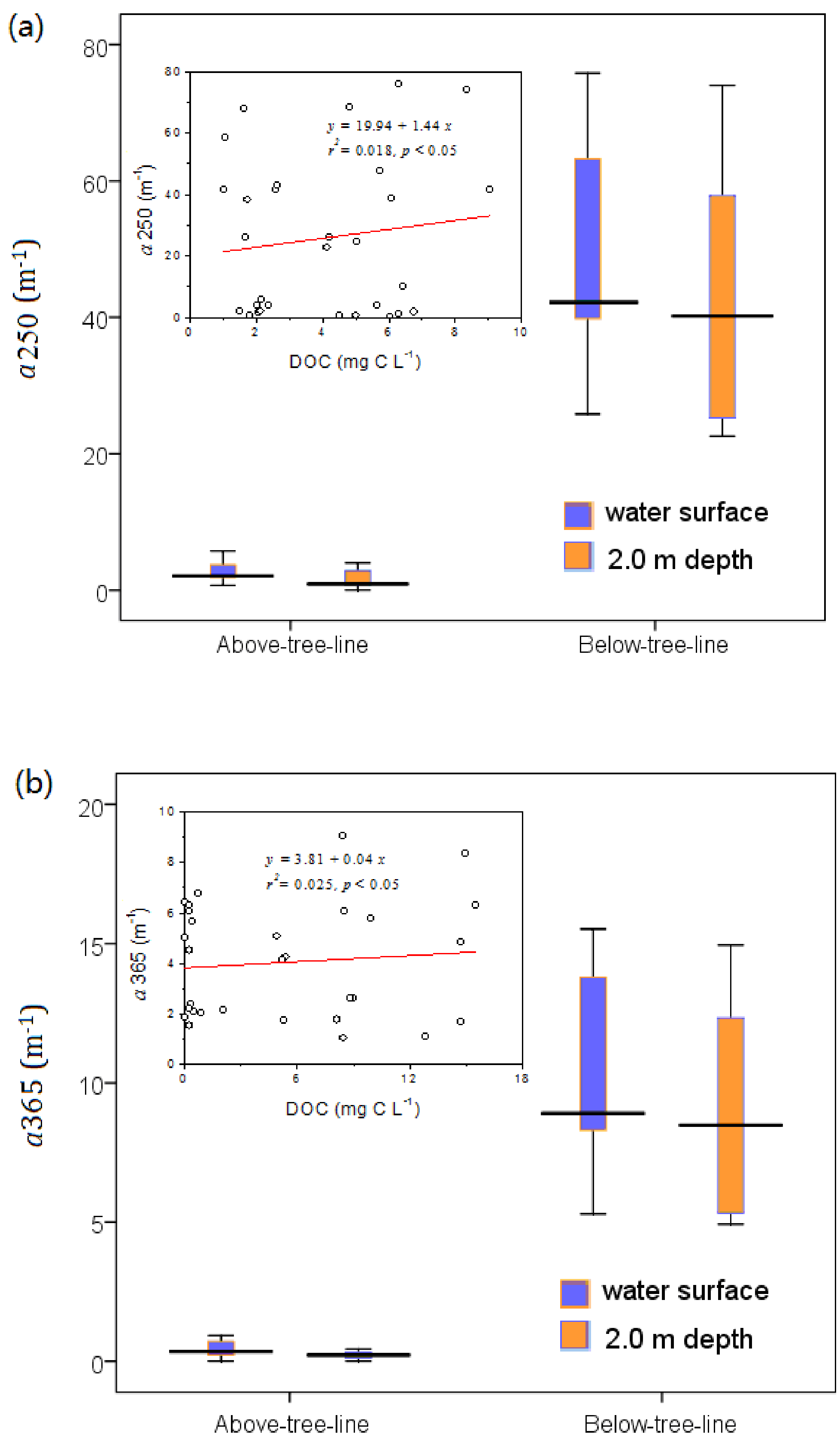


Fig. 1


(a)
80
60
40
20
0
a250 (m-1)
80
60
40
20
0
0
2
4
6
8
10
a 250 (m-1)
y = 19.94 + 1.44 x
r2 = 0.018, p < 0.05
DOC (mg C L-1)
water surface
2.0 m depth
Above-tree-line
Below-tree-line
(b)
20
15
10
5
0
a365 (m-1)
10
8
6
4
2
0
0
6
12
18
a 365 (m-1)
y = 3.81 + 0.04 x
r2 = 0.025, p < 0.05
DOC (mg C L-1)
water surface
2.0 m depth
Above-tree-line
Below-tree-line

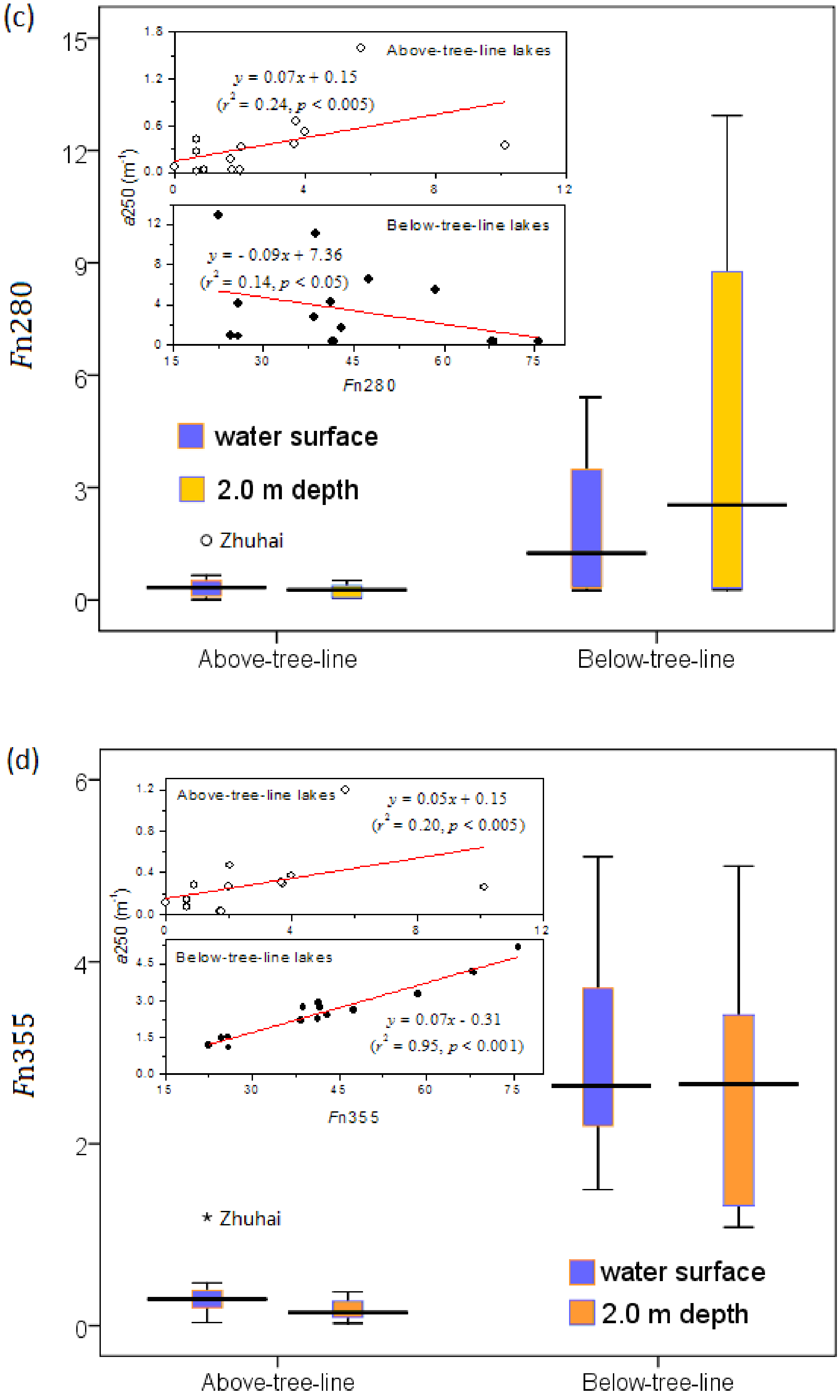
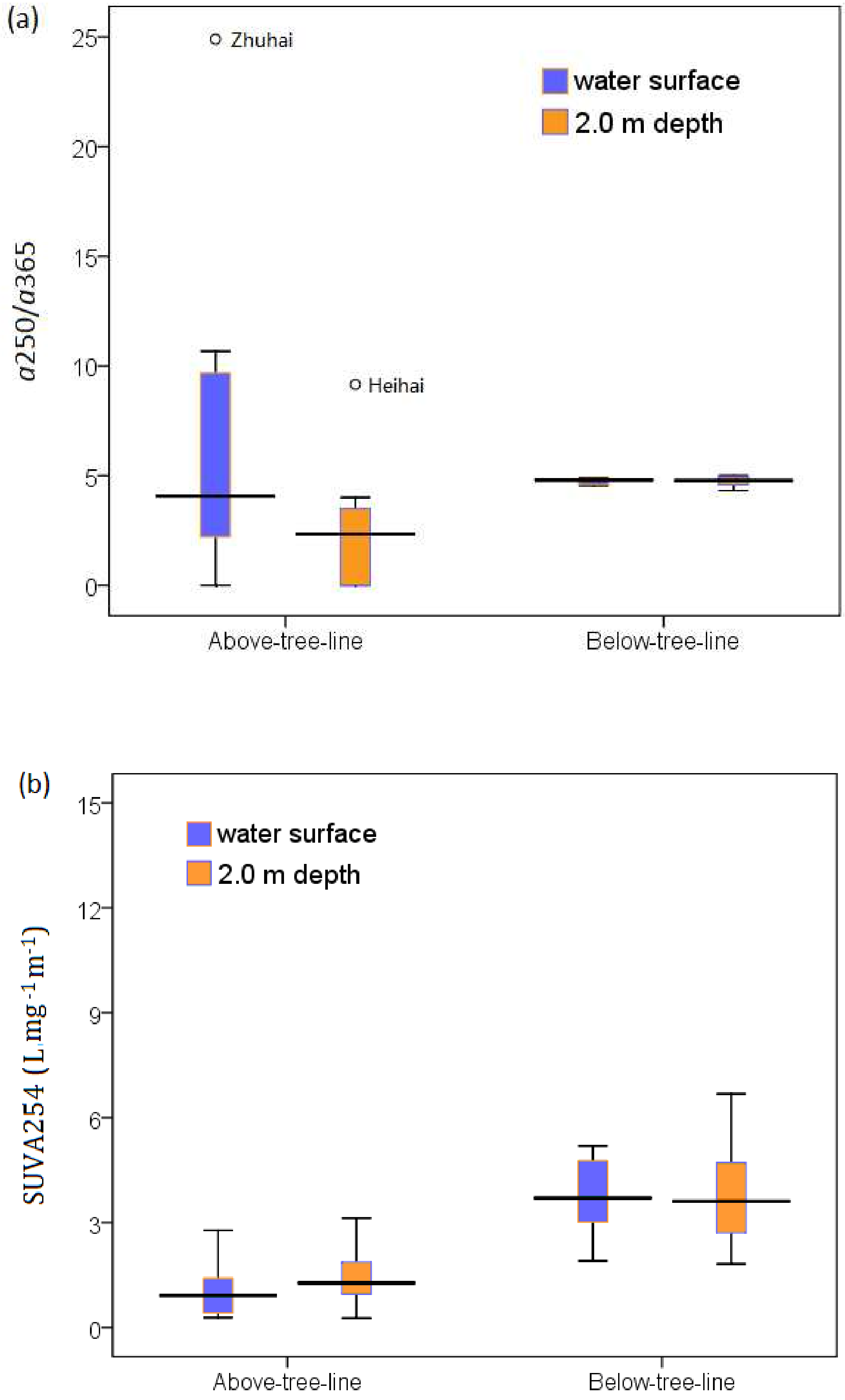

(c)
Fn280
15
12
9
6
3
0
a250 (m⁻¹)
Above-tree-line lakes
y = 0.07x + 0.15
(r² = 0.24, p < 0.005)
Below-tree-line lakes
y = - 0.09x + 7.36
(r² = 0.14, p < 0.05)
Fn280
water surface
2.0 m depth
Zhuhai
Above-tree-line
Below-tree-line
(d)
Fn355
6
4
2
0
a250 (m⁻¹)
Above-tree-line lakes
y = 0.05x + 0.15
(r² = 0.20, p < 0.005)
Below-tree-line lakes
y = 0.07x - 0.31
(r² = 0.95, p < 0.001)
Fn355
Zhuhai
water surface
2.0 m depth
Above-tree-line
Below-tree-line

Fig. 2


(a)
25
20
15
10
5
0
a250/a365
Zhuhai
Heihai
water surface
2.0 m depth
Above-tree-line
Below-tree-line
(b)
15
12
9
6
3
0
SUVA254 (L mg-1 m-1)
water surface
2.0 m depth
Above-tree-line
Below-tree-line

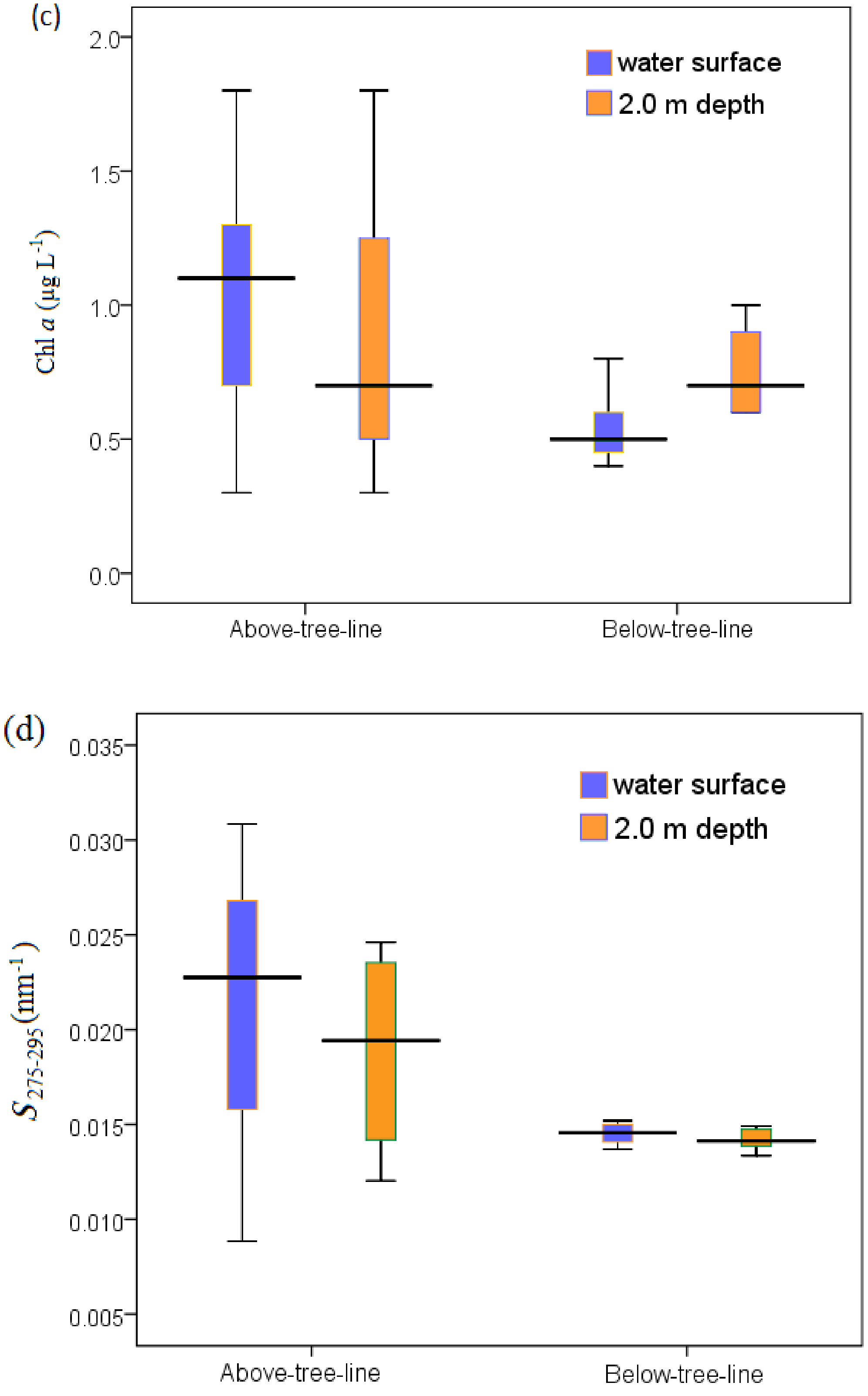
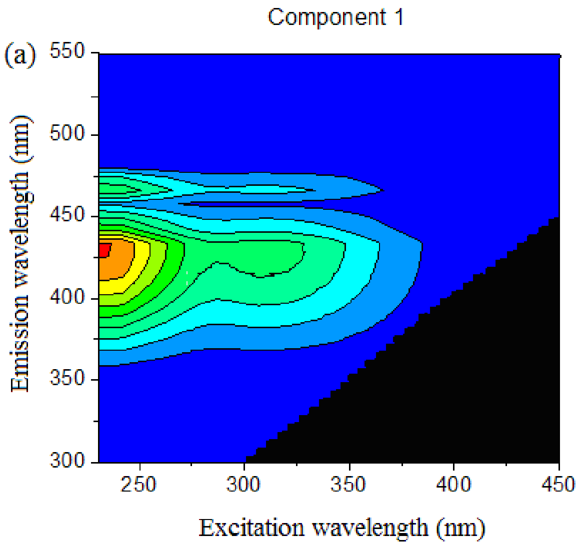
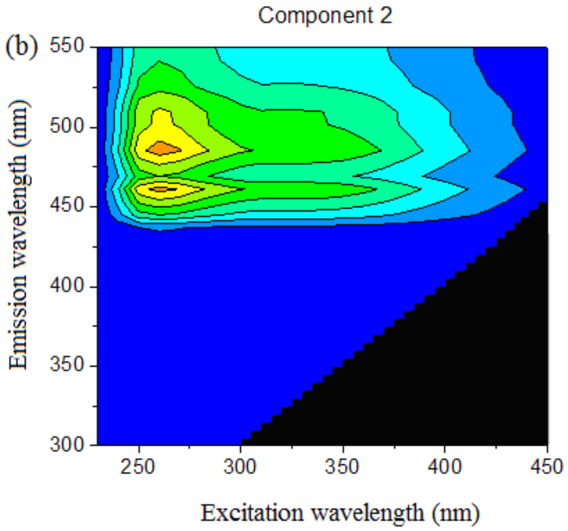
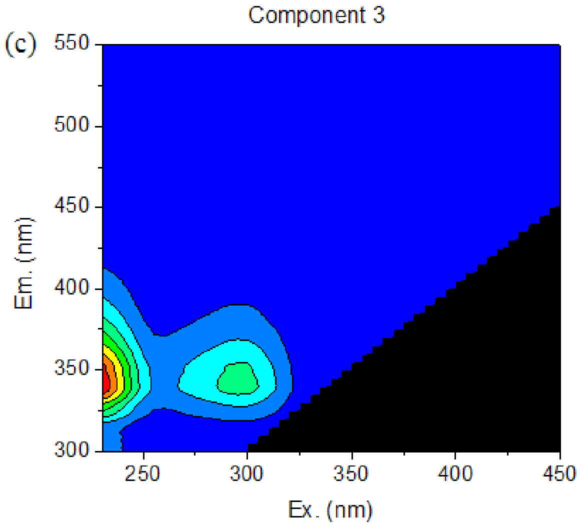

(c)
Chl a (μg L^{-1})
2.0
1.5
1.0
0.5
0.0
water surface
2.0 m depth
Above-tree-line
Below-tree-line
(d)
$S_{275\text{-}295}$ (nm^{-1})
0.035
0.030
0.025
0.020
0.015
0.010
0.005
water surface
2.0 m depth
Above-tree-line
Below-tree-line

Fig. 3


Component 1
(a)
Emission wavelength (nm)
550
500
450
400
350
300
250
300
350
400
450
Excitation wavelength (nm)


Component 2
(b)
Emission wavelength (nm)
550
500
450
400
350
300
250
300
350
400
450
Excitation wavelength (nm)


Component 3
(c)
Em. (nm)
550
500
450
400
350
300
250
300
350
400
450
Ex. (nm)

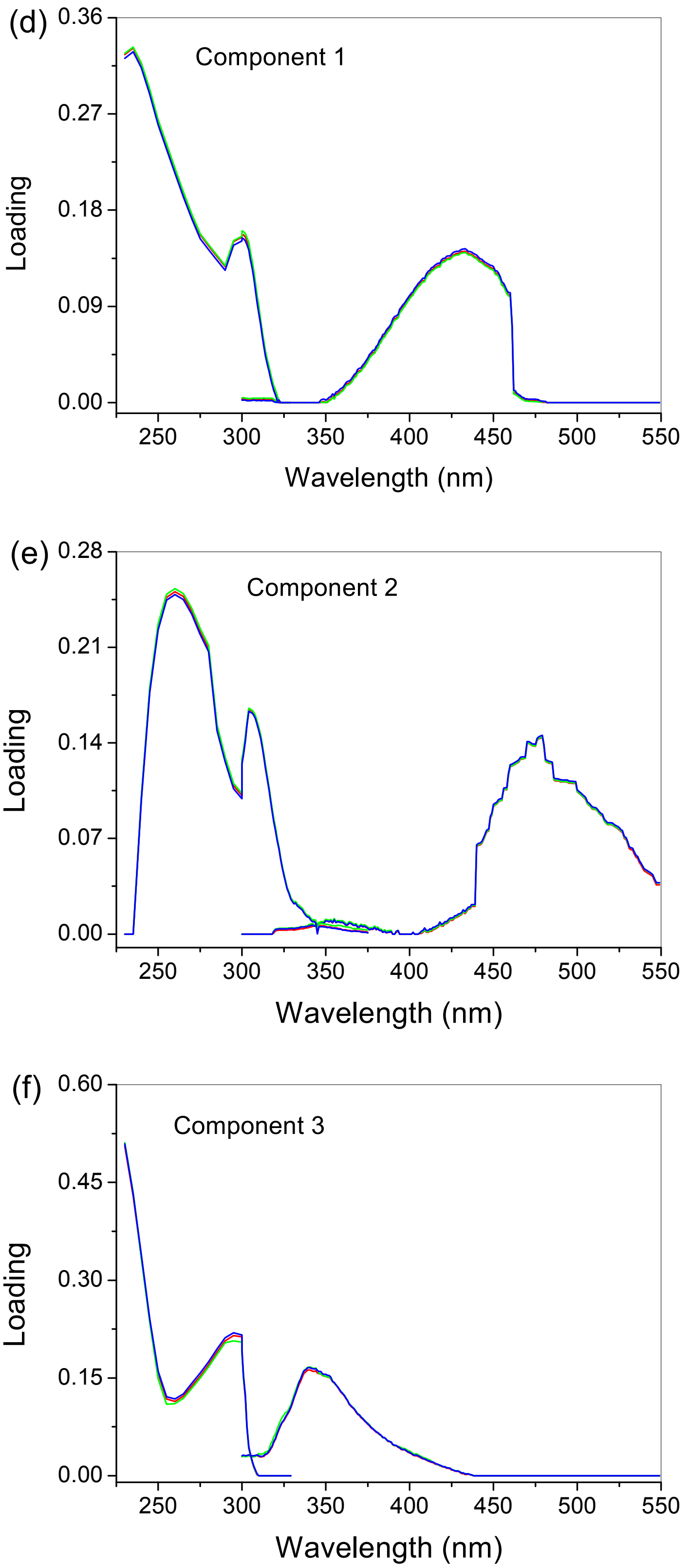
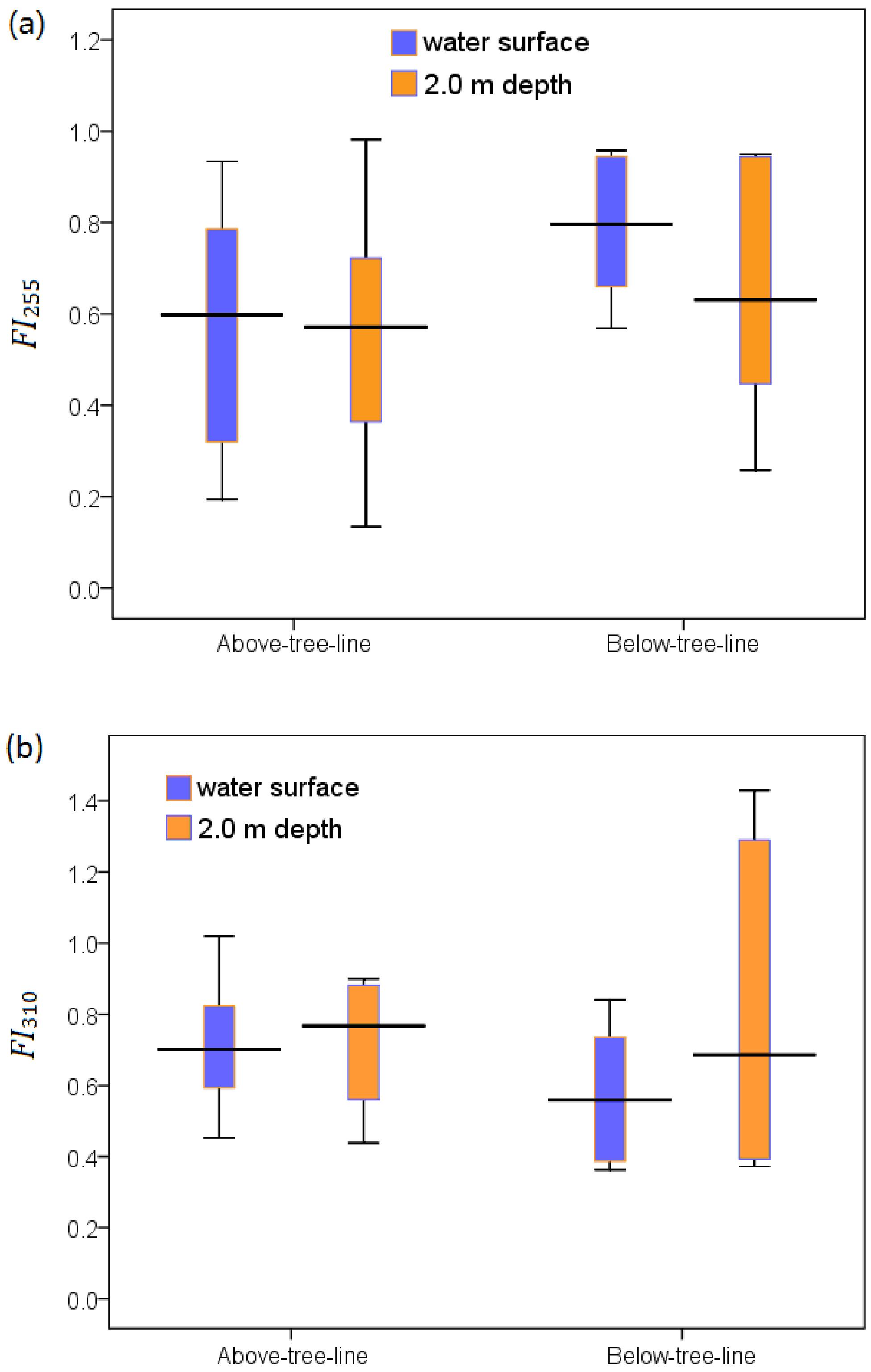

(d)
Component 1
Loading
0.36
0.27
0.18
0.09
0.00
250
300
350
400
450
500
550
Wavelength (nm)
(e)
Component 2
Loading
0.28
0.21
0.14
0.07
0.00
250
300
350
400
450
500
550
Wavelength (nm)
(f)
Component 3
Loading
0.60
0.45
0.30
0.15
0.00
250
300
350
400
450
500
550
Wavelength (nm)

Fig. 4


(a)
water surface
2.0 m depth
FI_{255}
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Above-tree-line
Below-tree-line
(b)
water surface
2.0 m depth
FI_{310}
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Above-tree-line
Below-tree-line

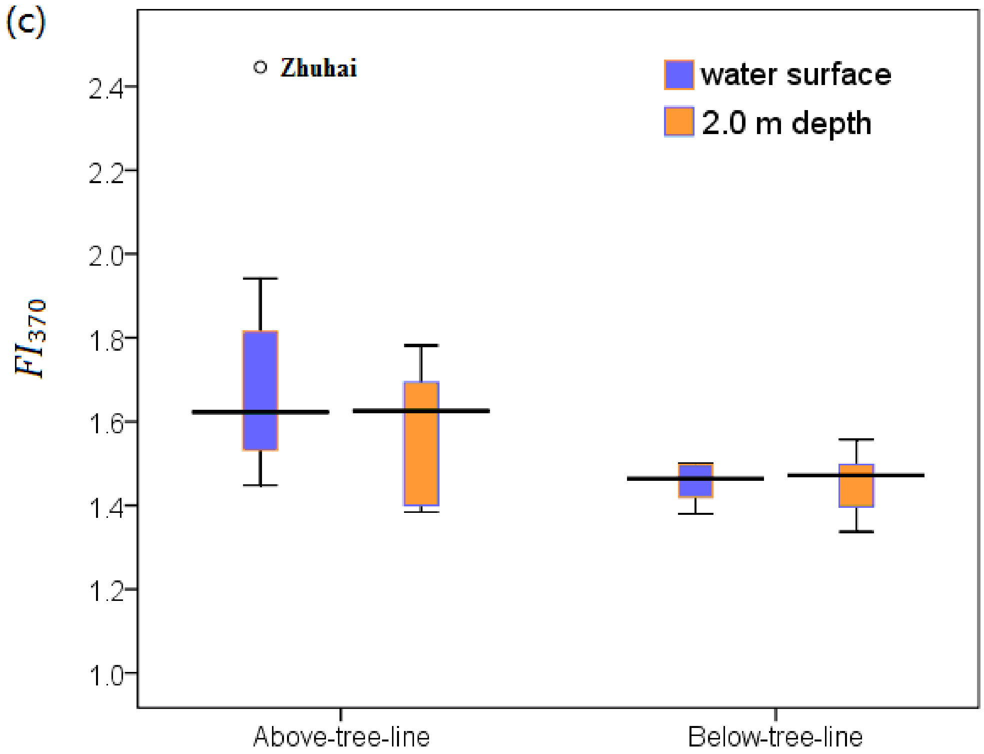

(c)
○ Zhuhai
water surface
2.0 m depth
FI_{370}
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
Above-tree-line
Below-tree-line

Fig. 5

Table 1. Mean values of regular water-quality parameters. The lakes were sampled at the surface and 2.0 m. The calculated results show that all the lakes were oligotrophic. n = no data.

Lake	Transparency (m)	Temperature (^{o}C)	TN (mg L^{-1})	TP (mg L^{-1})	Chl *a* (μg L^{-1})	DOC (mg C L^{-1})
Huanghai	2.1	7.10*	0.26	0.006	1.3	2.02
		6.84**	n	0.008	1.8	2.36
Heihai	8.2	5.29	0.19	0.006	0.5	1.82
		5.10	0.21	0.006	0.7	2.08
Zihai	4.2	7.25	0.55	0.009	1.1	2.13
		7.93	n	n	n	1.51
Meihai	5.4	6.20	0.23	0.004	0.3	2.17
		5.82	0.15	0.006	0.3	1.08
Fuhai	1.2	5.25	1.13	0.012	1.3	2.60
		4.10	n	n	n	1.74
Qihai	4.1	6.78	0.57	0.01	0.9	2.62
		6.56	n	n	n	1.69
Zhuhai	5.0	5.28	1.00	0.011	1.8	1.63
		4.44	n	n	n	1.02
TC	4.5	7.53	0.23	0.009	0.8	6.29
		6.65	0.23	0.011	1.0	4.50
RC	2.5	8.51	0.19	0.010	0.4	5.66
		6.78	0.24	0.011	0.6	5.01
ZH	2.5	10.02	0.22	0.008	0.5	6.76
		8.18	0.26	0.011	0.9	6.05
MH	3.4	10.35	0.20	0.014	0.6	6.42
		8.89	0.23	0.015	0.7	6.29
SMH	3.0	9.93	0.12	0.013	0.4	6.08
		6.78	0.19	0.009	0.6	5.75
GLH	1.8	11.24	0.90	0.015	0.5	4.22
		9.11	n	n	n	4.14
DLH	1.2	10.68	0.83	0.017	0.5	5.05
		10.40	n	n	n	4.82
SXH	1.8	10.92	0.75	0.019	0.6	9.06
		8.62	n	n	n	8.34

*Data of sample at the water surface.

**Data of sample at 2.0 m depth.

Table 2. Spectral characteristics Ex_{max} and Em_{max} of 3 components identified using PARAFAC, compared with previous identified fluorescence peaks.

Component No.	Ex_{max} (nm) [*]	Em_{max} (nm)	Peaks in other previous work [*]	Property and probable source
C1	235 (300)	430	Ex_{max}: 235 (290), Em_{max}: 397[a]; Ex_{max}: 295, Em_{max}: 398[b]; Ex_{max}: 315, Em_{max}: 418[c]; Ex_{max}: 290-310, Em_{max}: 370-420[d,e].	Terrestrial humic-like substances
C2	260 (305)	475	Ex_{max}: 255 (350), Em_{max}: 471[a]; Ex_{max}: 270 (360), Em_{max}: 478[f];	(T) UV humic-like fluorescence
C3	≤225 (295)	330-350	Ex_{max}: 280, Em_{max}: 338 (344)[b,h]; Ex_{max}: 225-230 (275), Em_{max}: 340-350[d,e].	(T, A) Tryptophan-like or phenolic fluorescence

[*] Secondary excitation band is listed in brackets.

[**] T: Terrestrial source, A: Autochthonous source.

[a] Zhang et al. (2010)

[b] Stedmon and Markager (2005a).

[c] Murphy et al. (2008).

[d] Coble (1996).

[e] Coble et al. (1998).

[f] Stedmon and Markager (2003).

[g] Stedmon and Markager (2005b).

[h] Maie et al. (2007).

Table 3. Absorption and fluorescence indices to assess the source and composition of CDOM in the two lake systems

Absorption and fluorescence indices	Mean value of above-tree-line lakes		Mean value of below-tree-line lakes		Characteristics of CDOM
	0	2 m	0	2 m	
*a*250/*a*365	7.536	4.624	4.751	4.745	Smaller molecular weight at the surface in above-tree-line lakes, smaller molecular weight at the surface than at 2.0 m
SUVA254	1.090	1.471	4.758	3.817	Lower percent aromaticity in above-tree-line lakes
$S_{275\text{-}295}$	0.021	0.019	0.015	0.014	Similar to *a*250/*a*365
FI_{255}	0.56 ± 0.29	0.55 ± 0.29	0.79 ± 0.15	0.66 ± 0.27	Larger molecular size and higher percent aromaticity in below-tree-line lakes
FI_{310}	0.71 ± 0.20	0.76 ± 0.18	0.57 ± 0.19	0.82 ± 0.46	Intermediate autochthonous component in the newly produced CDOM in above-tree-line lakes and at 2.0 m depth in below-tree-line lakes; and strong allochthonous origin at the surface in below-tree-line lakes
FI_{370}	1.68 ± 0.44	1.57 ± 0.17	1.45 ± 0.05	1.45 ± 0.07	More contribution from autochthonous biological and aquatic bacterial origin in above-tree-line lakes, and higher contribution from allochthonous source in below-tree-line lakes