Photochemical reactivities of dissolved organic matter (DOM) in a
sub-alpine lake revealed by EEM-PARAFAC: an insight into the fate of
allochthonous DOM in alpine lakes affected by climate change

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Abstract: Due to climate change, tree line advance is occurring in many alpine regions. Within the next 50 to 100 years, alpine lake catchments are expected to develop increased vegetation cover similar to that of sub-alpine lake catchments which currently exist below the tree line. Such changes in vegetation could trigger increased allochthonous DOM inputs to alpine lakes. To understand the fate of allochthonous DOM in alpine lakes impacted by climate change, the photochemical reactivity of DOM in sub-alpine Lake Tiancai (located 200 m below the tree line) was investigated by excitation emission matrix fluorescence combined with parallel factor analysis (EEM-PARAFAC) and UV-Vis spectra analysis. With photo-exposure, a decrease in apparent DOM molecular weight was observed and 32% DOM was photomineralized to CO₂. Interestingly, the aromaticity of DOM increased after photodegradation, as evidenced by increases in both the specific UV absorbance at 254 nm (SUVA₂₅₄) and the humification index (HIX). Five EEM-PARAFAC components were identified, including four terrestrially-derived substances (C1, C2,

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C3 and C4; allochthonous) and one tryptophan-like substance (C5; autochthonous). Generally, allochthonous DOM represented by C2 and C3 exhibited greater photoreactivity than autochthonous DOM represented by C5. C4 was identified as a possible photoproduct with relatively high aromaticity and photorefractive tendencies and contributed to the observed increase in SUVA₂₅₄ and HIX. UV light facilitated the photodegradation of DOM and had the greatest effect on the removal of C3. This study provides information on the transformation of EEM-PARAFAC components in a sub-alpine lake, which is important in understanding the fate of increased allochthonous DOM inputs to alpine lakes impacted by climate change.

Keywords: photoreactivity; DOM; allochthonous; sub-alpine/alpine lake; EEM-PARAFAC

1. Introduction

Dissolved organic matter (DOM) is an assemblage of heterogeneous organic molecules with diverse biogeochemical and ecological roles and is ubiquitous in aquatic ecosystems (Findlay and Sinsabaugh 2003). DOM is sourced from both allochthonous(e.g., soil runoff) and autochthonous(e.g., microbial exudates) biomass (Castillo et al., 2010; Romera-Castillo et al., 2011). DOM comprises a major mobile fraction of organic carbon and is an intimate link between terrestrial and aquatic environments(Hedges et al., 1997; Benner et al., 2004). Annually, global inland waters receive approximately 1.9 Pg C from the terrestrial landscape, of which about 0.2 Pg C is buried in aquatic sediments, 0.8 Pg C is returned to the atmosphere and the remaining 0.9 Pg C is delivered to the oceans (Cole et al., 2007). Although they cover a relatively small proportion of the Earth's surface, inland waters (such as lakes) are key contributors to the processing and global cycling of DOM and associated DOC (Battin et al., 2008; Tranvik et al., 2009).

Alpine lakes are located at elevations above the tree line, which is defined as the edge of the habitat at which trees are capable of growing, and generally have small catchment areas with poorly developed soils and sparse terrestrial vegetation (Sommaruga 2001). Consequently, DOM in alpine lakes is mainly autochthonous. However, tree line advance is occurring in many alpine regions in response to changes in climate and precipitation (Harsch et al., 2009; Hagedorn et al., 2014). It has been proposed that, within the next 50 to 100 years, climate change could alter the vegetation of lake catchments at higher altitudes (Sommaruga et al., 1999; Larsen et al., 2011). As such, alpine lake catchments are expected to develop increased vegetation cover and adopt characteristics similar to that of sub-alpine lake catchments immediately below the tree line. Such climate-triggered changes in catchment vegetation could result in considerable increases in allochthonous DOM input to alpine lakes. Since the reactivity of DOM is closely related to its chemical character, a shift in the dominant DOM source (from autochthonous to allochthonous) would greatly impact the ecological and biogeochemical processing of DOM in alpine lakes (Benner 2002). It has been reported that allochthonous DOM is more resistant to microbial degradation than autochthonous DOM in alpine lakes (Pérez and Sommaruga 2006). However, photochemical transformations likely represent key degradation pathways for terrestrial DOM in alpine and sub-alpine lakes due to the intensive solar irradiation at higher altitudes (Sommaruga 2001). The photodegradation of DOM results in the alteration of its molecular structure and mineralization of DOC to CO₂ (Kaiser and Sulzberger 2004; Piccini et al., 2009; Osburn et al., 2011; Santos et al., 2014). The overall effects of photodegradation, depend largely on the irradiation conditions and the origin and composition of the DOM exposed to sunlight (Stedmon and Markager 2005b; Zhang et al., 2013).

Because alpine lake catchments are expected to adopt characteristics similar to that of sub-alpine lake catchments under climate change scenario, the photoreactivity of DOM in current sub-alpine lakes can be assessed to help predict the degradation and fate of increased allochthonous DOM inputs to alpine lakes impacted by climate change. Sub-alpine lakes are remote and difficult to access, therefore studies assessing the photodegradation of DOM in such high-altitude lakes are relatively limited.

Optical properties are easily-measured proxies for DOM composition, therefore absorbance and fluorescence spectroscopy has been extensively applied to elucidate the chemical nature and sources of DOM in aquatic systems (McKnight et al., 2001; Weishaar et al., 2003; Helms et al., 2008). Especially, three dimensional excitation emission matrices combined with parallel factor analysis (EEM-PARAFAC) has been widely used for the characterization of fluorescent DOM. PARAFAC modeling statistically deconvolute complex EEMs into individual components and determines the relative contribution of each component to the total EEM fluorescence of DOM samples (McKnight et al., 2001; Fellman et al., 2010). EEM-PARAFAC analyses has been applied to photodegradation studies in eutrophic lakes (Xu and Jiang 2013; Zhang et al., 2013) and marine environments (Stedmon et al., 2007).

To assess the reactivity and fate of increased allochthonous DOM inputs to climate change-impacted alpine lakes, the *in-situ* photodegradation of DOM in a sub-alpine lake (Lake Tiancai on the Yunnan-Guizhou Plateau, China; 200 m below the tree line) was investigated in this study. EEM-PARAFAC was applied to trace the photodegradation of fluorescent DOM components in Lake Tiancai. The effects of UV light on EEM-PARAFAC components were revealed by exposing lake DOM to three different light treatments (visible, visible+UVA, visible+UVA+UVB). The results provided insight into the fate of allochthonous DOM inputs to alpine lakes,

which is expected to increase with changes in high altitude climate and catchment vegetation cover.

2. Methods

2.1. Study sites

Lake Tiancai (26° 38' 2.436"N~26° 38' 6.198"N, 99° 42' 55.2060"E~99° 43' 3.3996"E) is located in Laojun Mountain on the Yunnan-Guizhou Plateau, China (Figure S1). Lake Tiancai is a sub-alpine lake situated at 3880 m above sea level, approximately 200 m below the tree line (which is roughly 4100 m above sea level). The lake has an area of approximately 20000 m² and a maximum water depth of 6.5 m. Lake Tiancai is surrounded by ancient forest where the dominant vegetation consists of spruce and *Rhododendron lapponicum*. The lake water is supplied by precipitation and surface runoff from the catchment. General characteristics and DOM properties of Lake Tiancai are shown in Table 1.

(Table 1)

2.2. Microcosm experimental design

Water samples (10L) were collected at 0.5 m below the surface in the center of Lake Tiancai (26° 38' 4.57"N, 99° 42' 59.21"E) using a plexiglass sampler (Institute of Hydrobiology, Chinese Academy of Sciences). The depth of 0.5 m was chosen because UV light could not penetrate at this depth (Figure S2). The sample was initially filtered through a precombusted (450 °C, 4 h) 0.7 µm glass fiber filter (Whatman GF/F) and subsequently passed through a prerinsed 0.22 µm membrane filter (Millipore, GVWP02500) to remove suspended particles and bacteria. The filtered water with DOM was used for the photodegradation experiments. Effects of microbial degradation during the photodegradation were assumed to be minimal (Stedmon et al., 2007; Zhang et al., 2013). The photodegradation experiments of DOM

were carried out in Lake Tiancai, about 15 m from the shore.

Aliquots of filtered samples (150 mL each) were placed in 200 mL sealed Whirl-Pak® bags (B00992WA, NASCO Whirl-Pak, USA) and positioned horizontally on a wire frame (~5 cm depth, see Figure S1(b)). Whirl-pak bags transmit solar radiation at wavelengths above 220 nm (Holm-Hansen and Helbling 1993), as shown in Figure S3. Control experiments using pure water confirmed negligible contamination of aromatic compounds from the bag, as shown in Figure S4. Three light treatments were applied: (1) visible (Vis)+UVA+UVB (total irradiation), Vis+UVA (exclusion of UVB) and Vis (exclusion of UVA and UVB). Light treatments were achieved using cutoff filters which block certain wavelengths: triacetate fiber A (Lucky Film Corp., China, transmits Vis+UVA+UVB); PET-C(Sui On insulating materials Corp., China, transmits Vis+UVA) and triacetate fiber B (Lucky Film Corp. China, transmits visible light only). Spectral transmittance characteristics of these sheets were confirmed using a UV-Vis spectrophotometer (PekinElmer, Lambda 35) (Figure S3). Photodegradation experiments were performed in triplicate and exposed to sunlight for 4 days (18:00, 17th June, 2013 to 18:00, 21th June, 2013). Every triplicate bags for each treatment were taken out in the evening (18:00-19:00) on June 18, 19, 20 and 21 in 2013. Samples were filtered within 24 h, serially through pre-combusted (450 °C for 4 h) 0.7 µm glass fiber filters (Whatman GF/F) and prerinsed 0.22 µm membrane filters (Millipore, GVWP02500). The filtered subsamples were stored under refrigeration and measured within 3 weeks of collection.

2.3. Analytical methods

Irradiance was measured using a SAM 8180 scanning spectroradiometer (TriOS, Germany). Figure S2 shows the irradiance of natural sunlight on the water surface of

Lake Tiancai in June 19, 2013. By integrating the spectrum, the light intensities for visible light (Vis, 400-800 nm), UVA (320-400 nm) and UVB (280-320 nm) regimes were calculated as 329.8 W/m², 25.8 W/m² and 1.2 W/m², respectively.

DOC was measured by high temperature combustion with a Shimadzu TOC-5000 analyzer.

The absorption spectra of DOM were recorded between 200 nm and 800 nm at 1 nm intervals, using a UV-Vis spectrophotometer (PekinElmerLamba 35). Milli-Q water was used as reference. The absorbance (A) was baseline-corrected, by subtracting the absorbance at 700 nm. The absorption coefficients (a) were calculated using Eq. (1), in which L is the pathlength of the quartz cell (0.01 m).

$$a_{(\lambda)} = 2.303A_{(\lambda)} / L \quad (1)$$

Specific UV absorbance at 254 nm (SUVA₂₅₄) values were determined by dividing the UV absorbance measured at 254 nm by the DOC concentration and reported in the units as liter per milligram carbon per meter (L/mg C·m). SUVA₂₅₄ was developed to estimate the dissolved aromatic carbon content of DOM (Weishaar et al., 2003). Absorption coefficients were used to determine the spectral slopes over 275-295 nm (S₂₇₅₋₂₉₅), which serve as an optical proxy for the molecular weight distribution of DOM (Helms et al., 2008).

EEMs were measured using a Hitachi F-7000 fluorescence spectrometer (Hitachi High-Technologies) equipped with a 700-voltage xenon lamp. Emissions scans were collected at excitation wavelengths from 200 to 450 nm at 5 nm intervals. Emission wavelengths were scanned from 250 to 550 nm at 1 nm intervals. Instrument corrections were performed according to the procedure recommended by the Hitachi F-7000 Instruction Manual (Zhang et al., 2010). Several post-acquisition steps were also carried out for correction and standardization of EEM spectra: (1) inner filter

effect corrections were performed using UV-Vis absorbance spectra data according to McKnight et al. (McKnight et al., 2001); (2) blank subtraction using Milli-Q water; (3) calibration and normalization of daily fluorescence intensity variations using water Raman units (RU).

The corrected fluorescence data were used to calculate the fluorescence index (FI; Cory and McKnight 2005) and humification index (HIX; Huguet et al., 2009). FI was calculated as the ratio of the fluorescence intensity from 470 nm to 520 nm at excitation wavelength 370 nm. HIX was calculated as the ratio of the average fluorescence intensity from 435 nm to 480 nm, divided by the average intensity from 300 nm to 345 nm, at excitation wavelength 255 nm.

PARAFAC modeling was applied to statistically deconvolute the complex EEM spectra into discrete components. PARAFAC analysis was carried out following the commendation of Stedmon and Bro (2008) in MATLAB R2008a (Mathworks) with the DOMFluor toolbox (Stedmon and Bro 2008). A five-component model (C1-C5) was validated using split-half analysis and random initialization methods.

Statistical Analyses

Statistical analyses were performed with Statistical Program for Social Sciences (SPSS19.0) software. Changes in parameters with respect to the reaction time and the light treatment were assessed with repeated measures analysis of variance.

3. Results and discussion

3.1. Change of DOC and absorbance under irradiation

The waters of Lake Tiancai were enriched with terrestrial-type DOM, as evidenced by relatively low FI, high SUVA₂₅₄ and the relative abundance of PARAFAC components (see section 3.3). Due to the input of allochthonous DOM, Lake Tiancai exhibits high DOC concentrations (> 10 mg/L) compared to the alpine

lakes in the same region (1.4~1.8 mg/L; data collected our survey in 2013 but not published).

During the solar irradiation, DOC was significantly removed ($p < 0.001$) and the absorbance of DOM significantly reduced with the reaction time ($p < 0.001$) in Lake Tiancai (Figure 1), implying that DOM underwent both photomineralization and photobleaching. After four days of photoexposure under the Vis+UVA+UVB treatment, DOC decreased from 10.33 mg/L to 7.00 mg/L (32.2% loss). The loss of DOC suggested that, under natural solar irradiation, some DOM in Lake Tiancai was photomineralized to CO_2 , which indicates that photoreactivity of sub-alpine lake DOM may impact local carbon cycles. The DOM absorption coefficient at 254 nm (a_{254}) of Lake Tiancai was reduced from 63.8 m^{-1} to 57.2 m^{-1} after four days of irradiation. Since the photo-removal of DOC was greater than a_{254} , SUVA_{254} was shown to increase significantly ($p < 0.001$) with the reaction time (Figure 1(c)). With the Vis+UVA+UVB treatment, SUVA_{254} increased from 2.83 to 3.54 $\text{L}/\text{mg C}\cdot\text{m}$ after four days solar exposure. Previous research has shown that photodegradation of Suwannee River fulvic acid and eutrophic lake-derived DOM resulted in an overall decrease in SUVA_{254} (Hur et al., 2011; Xu and Jiang 2013). The reduction in SUVA_{254} was explained by the preferential removal of aromatic chromophores and/or degradation of aromatic compounds to generate non-UV-absorbing DOM photo-products. Interestingly, the current study indicates that the relative aromaticity of DOM from Lake Tiancai increased with photodegradation. The increase in SUVA_{254} may have occurred for several reasons: (1) some aromatic chromophores were resistant to photodegradation and were therefore preserved relative to other DOM compounds or (2) aromatic DOM compounds were formed during the irradiation experiments. In this study, the increase in SUVA_{254} with photodegradation appeared to

be a result of the generation of aromatic DOM components(as evidenced by the application of EEM-PARAFAC; see section 3.3). The aromatic content of DOM has been shown to be negatively correlated to its bioavailability (Khadka et al., 2015). Thus, increases in $SUVA_{254}$ during DOM photodegradation indicated that photo-altered DOM may be less easily assimilated by bacteria in Lake Tiancai.

(Figure 1)

When UV light was excluded (Vis treatment), the photo-removal of DOC and DOM absorbance was less efficient. After 4 days of photo-exposure, 28% of the DOC was removed. Since the decrease in a_{254} and DOC in the Vis+UVA+UVB and Vis+UVA treatments were similar, UVB appeared to have a minimal impact on the photobleaching and photomineralization of DOC. This may be, in part, due to the low light intensity of UVB (1.2 W/m^2) compared to UVA (25.8 W/m^2) and visible (329.8 W/m^2) regions of the irradiation spectrum.

3.2 Photochemical alternation of DOM optical characteristics

The spectral slope ($S_{275-295}$) increased significantly with reaction time ($p<0.001$) within all three light treatments (Figure 2 (a)). For example, $S_{275-295}$ increased from 0.0137 to 0.0165 after 4 days of exposure under the Vis+UVA+UVB treatment. Spectral slope has been established as a proxy for DOM molecular weight, where high values indicate lower molecular weight distributions (Helms et al., 2008). The observed drop in spectral slope suggested that a photo-induced decrease in DOM molecular weight occurred with photodegradation, even during the Vis treatment. The photo-sensitivity gradient of $S_{275-295}$ followed the order of Vis+UVA+UVB>Vis+UVA>Vis, which indicates that UV light accelerated the decomposition of high molecular weight DOM into relatively low molecular weight DOM.

The FI is an indicator of DOM source and has a value of ~1.9 for microbially-derived fulvic acids, and ~1.4 for terrestrially-derived fulvic acids (Cory and McKnight 2005). The FI value of DOM in Lake Tiancai prior to photo-exposure was 1.52, which suggested a dominance of terrestrial DOM. The results of the repeated measures analysis of variance showed that the changes of FI with respect to reaction time and light treatments were both significant ($p < 0.001$). With the Vis+UVA+UVB treatment, FI values decreased from 1.51 to 1.35 after 4-day exposure reflecting the relative enrichment of terrestrial DOM during the photodegradation. Interestingly, FI values increased from 1.52 to 1.57 with the Vis treatment, exhibiting an opposite trend to the Vis+UVA+UVB treatment. This demonstrated the heterogeneous reactivities of different DOM components to UV and visible light. The photoreactivities of individual EEM-PARAFAC components could help to explain the contrasting changes of FI under different light treatments.

HIX is directly proportional to the humic content of DOM, similar to $SUVA_{254}$. HIX values around 1-2 have been associated with non-humified plant material, while fulvic acid extracts have been reported with HIX values >10 (Zsolnay et al., 1999; Ohno 2002). In this study, HIX values increased significantly ($p < 0.001$) with the time, but the changes of HIX among the three light treatment showed no significant difference ($p > 0.05$). HIX values increased from 4.25 to 6~7 during the three light treatments (Figure 2(c)). The increase of HIX values followed a similar trend as $SUVA_{254}$, which further confirmed the enhanced aromaticity of photodegraded DOM in Lake Tiancai.

(Figure 2)

3.3 Changes of EEM-PARAFAC components in three light treatments

In this study, a PARAFAC model consisting of five components was developed.

The EEMs of each component are shown in Figure 3 along with the excitation and emission loadings for each half and the whole dataset. C1 (Component 1) displayed two excitation maxima (<250 nm and 295 nm) corresponding to a single emission maximum (412 nm). C1 is similar to C2 (ex<240/300, em 420) in the 6-component model for the Neuse River Estuary (NRE model), which was a novel find and described as a possible photodegradation product in NRE model (Osburn et al., 2012). C2 (Component 2; Ex/Em=255(345)/498), C3 (Component 3; Ex/Em=<250(325)/438) and C4 (Component 4; Ex/Em=<250(310)/438) all had longer emission wavelengths than C1 and have been categorized as terrestrial humic-like components (Cory and McKnight 2005; Stedmon and Markager 2005a; Stedmon et al., 2007; Osburn et al., 2012). C5 (Component 5; Ex/Em=275/330) corresponds to tryptophan-like fluorescence (Osburn et al., 2012). The excitation and emission wavelengths, description and photolability of these five components are shown in Table 2.

(Figure 3)

(Table 2)

The intensities of C1 decreased significantly with time ($p<0.001$) in the Vis+UVA+UVB treatment (Figure 4(a)). After 4-day exposure, the intensities of C1 decreased from 1.66 to 1.42 and 1.53 RU for Vis+UVA+UVB and Vis+UVA treatments, respectively. The reduction of C1 for DOM under the Vis+UVA+UVB light treatment (0.24 RU) was nearly two times that of DOM under the Vis+UVA treatment (0.13 RU). UVB appeared to have a considerable effect on the photodegradation of C1, despite its relatively low intensity. Interestingly, during the Vis treatment, the intensity of C1 increased from 1.66 to 1.81 RU after 4-days of exposure. The net increase of C1 with the Vis treatment suggested that C1 might be a photodegradation product of terrestrially-derived DOM. C2 in the NRE model

(C2_{NRE}), which is similar to C1 in the current model, was also suggested to be a degradation product of terrestrial organic matter. However, it was uncertain whether photo or biological processes led to the generation of C2_{NRE} (Osburn et al., 2012). Our study suggests that C1 represents DOM that is photoproducted and photolabile when exposed to UV light.

C2 is a terrestrially-derived humic-like substance with emission maxima at long wavelengths (~500 nm). PARAFAC components similar to C2 have been identified in a variety of environments (e.g., lakes, streams, estuaries, coastal waters and the open ocean) and have been shown to be susceptible to photochemical degradation (Murphy et al., 2006; Stedmon et al., 2007). In the current study, C2 intensities decreased significantly ($p < 0.001$) with the reaction time under all light treatments. According to the photodegradation mechanism (Schmitt-Kopplin et al., 1998; Xu et al., 2011), our results highlight the strong photolability of C2. Among three light treatments, the reduction of C2 was insignificantly different ($p > 0.05$), which indicated the apparent ineffectiveness of UV light on the degradation of C2 in the current study.

C3 was also identified as a terrestrially-derived humic-like component with shorter excitation and emission wavelengths than C2. C3 degraded with the reaction time significantly ($p < 0.001$) and showed similar photodegradation trends for the Vis+UVA and Vis+UVA+UVB treatments, in which approximately 34.1% was removed after 4 days of exposure. However, when UV light was filtered out, C3 remained relatively consistent, which indicated that visible light had little effect on the degradation of C3. The photoreactivity of C3 was opposite to that of C2, which is sensitive to visible light. This maybe because C3 has shorter excitation wavelengths than C2, thus it could not be excited by visible light which has lower energies than UV light.

The intensities of C4 were found to increase significantly with the reaction time ($p<0.001$) (Figure 4(d)). During the irradiation, C4 intensity was slightly higher for the Vis+UVA+UVB than for the Vis+UVA treatment, and much significantly higher than for the Vis treatment. After 4days of exposure, the net increase of C4 intensities were 0.49, 0.82 and 0.85 RU for Vis, Vis+UVA and Vis+UVA+UVB treatments, respectively. The spectral characteristics of C4 are similar to components identified in previous reports (Murphy et al., 2006; Stedmon et al., 2007), which suggested that C4 may be a photoproduct of terrestrially-derived DOM. In the current study, two terrestrially-derived DOM components (C2 and C3) were identified. For DOM under the Vis treatment, the generation of C4 and preservation of C3 were observed, therefore it is likely that C4 was a photoproduct of C2, not of C3. In addition, C1 could also be a product of C2. Although C1 and C4 both appeared to be photoproducts, C1 was also effectively degraded by UV light, whereas C4 was consistently photo-refractory, even under UV irradiation.

C5, a tryptophan-like component, was degraded significantly with the reaction time ($p<0.001$) in the three light treatments (Figure 4(e)). After 4-days exposure, 16.7%, 20.6% and 14.7% of C5 was removed in the Vis, Vis+UVA and Vis+UVA+UVB treatments, respectively. Like C2, UV light seemed to have little influence on the degradation of C5. However, components exhibiting similar spectral characteristics as C5 have been shown to be degrade dunder UVA irradiation (Stedmon et al., 2007). In the current study, the apparent ineffectiveness of UV light on the degradation of C2 and C5 was likely due to competition with other components, in which the UV light was primarily absorbed by C1 and C3.

(Figure 4)

The above results demonstrate the diverse photoreactivities of each of the five

EEM-PARAFAC components in response to UV and visible light. The photodecomposition of DOM is thought to proceed firstly via direct photolysis, which involves energy and electron transfer after photon absorption by DOM (Xu et al., 2011). The match of the absorbance of DOM and the light wavelength determined whether DOM could be photodegraded via the direct photolysis. Thus, the EEM-PARAFAC components with different optical characteristics showed the heterogeneities to UV and visible light. In current study, allochthonous-type DOM (C2 and C3) was more efficiently photodegraded than autochthonous-type DOM (C5). When lake DOM was exposed to the Vis+UVA+UVB treatment (total radiation), the intensities of C2 and C3 were reduced by 27% and 34% respectively, whereas only 14% of C5 intensity was reduced after 4 days exposure. Despite both components being seemingly representative of terrestrial-type DOM, C2 and C3 had different photoreactivities to visible and UV light, in which C2 was sensitive to visible light and C3 was only degraded when exposed to UV light. The autochthonous-type DOM component, C5, was degraded by visible light. C1 appeared to represent products of DOM photodegradation, but was effectively removed by UV light. C4 was suggested to be a photoproduct of C2 and was photorefractory when exposed to UV light. For DOM exposed to UV irradiation, photodegradation was generally more effective under UVA compared to UVB, likely due to the higher intensity of UVA. One exception was C1, which was much more sensitive to UVB light than UVA. The photoreactivities of each EEM-PARAFAC component are summarized in Table 2.

The photosensitivity of each of the five EEM-PARAFAC components to UV and visible light could also be related to observed changes in the optical indices. FI is determined as the ratio of the fluorescence intensity at 470 nm to 520 nm, excited at 370 nm, and showed an opposite trend during photodegradation under Vis and

Vis+UV treatments. According to excitation and emission loadings for each component (Figure 3), the fluorescence intensity at em 520 nm and ex 370 nm corresponded to C2 and fluorescence intensity at em 470 nm and ex 370 nm was associated with C3. Thus, FI was positively correlated with the ratio of C3 to C2. In Lake Tiancai, visible light degraded C2 but had little effect on C3, which resulted in an increase in FI under visible light. C3 was more easily degraded than C2 by UV light, which resulted in an observed decrease in FI during the Vis+UV treatments. The increase in HIX and SUVA₂₅₄ could be related to the production of C4. HIX is calculated by the intensity of emission from 435 to 480 nm divided by emission from 300 to 345 nm, at an excitation wavelength of 254 nm. C2, C3 and C4 contributed to the fluorescence at emission wavelengths between 435 and 480nm, excited at 254 nm; while C1 contributed to the emission from 300 to 345 nm. Since C2 and C3 were both photodegraded to a greater degree than C1, the generation of C4 contributed to the increase of HIX and SUVA₂₅₄. In addition, the HIX value for C4 is calculated as high as 16 according to the excitation and emission loadings. Thus C4, a presumed photoproduct of C2, likely represents DOM with high aromatic content. This finding suggests that the effect of photodegradation on DOM aromaticity depends on the composition of DOM exposed to sunlight. The photodegradation of Suwannee River fulvic acid and phytoplankton-derived DOM (Hur et al., 2011; Xu and Jiang 2013) resulted in an overall decrease in DOM aromaticity, however the PARAFAC models employed to characterize these DOM types did not contain a component resembling that of C2 which is presumed to generate C4-type components with photo-exposure.

3.4. Environmental implications and perspective

Previous studies have indicated that climate change will lead to tree line advance, thereby altering the land cover around alpine lakes (Harsch et al., 2009; Hagedorn et

al., 2014). It has been proposed that within the next 50-100 years, the catchments of alpine lakes will come to resemble those of sub-alpine lakes, therefore it is expected that inputs of allochthonous-type DOM to these lakes will increase. In order to predict the reactivity of DOM in alpine lakes impacted by tree line advance, the photodegradation of DOM in a sub-alpine lake was assessed. The results indicated that approximately 30% of allochthonous DOM was photomineralized after 4 days of solar exposure, however, a portion of aromatic-type DOM was photo-produced during or photo-resistant to photodegradation as evidenced by an increase in $SUVA_{254}$ and HIX with irradiation. Such evidence suggests that a part of allochthonous DOM would be possibly stored in alpine lakes. This would change the local carbon cycle because the stored DOM is difficult to be photo- or bio-mineralized to CO_2 . This looks like different from the aquatic systems dominated by the autochthonous DOM. In the eutrophic lakes dominated by the autochthonous DOM, DOM photodegradation was found to facilitate bioavailability (Xu and Jiang 2013; Zhang et al., 2015), which indicated that after photodegradation, DOM became more easier assimilated by bacteria thus carbon as the form of CO_2 could be feedback into the atmosphere. However, the current study is a preliminary investigation, in which the photodegradation of DOM is monitored over a short duration (4-days). To accurately predict the fate of allochthonous DOM in sub-alpine/alpine lakes, further investigations into the photodegradation of DOM over longer periods of time and possible changes in the bioavailability of photo-altered allochthonous DOM are warranted.

4. Conclusions

Lake Tiancai is dominated by terrestrially-derived DOM with FI and HIX values of 1.51 and 6.74, respectively. During photodegradation under the Vis+UVA+UVB

treatment, 32.2% of the DOC was photomineralized. $S_{275-295}$ increased from 0.0137 to 0.0167, indicating that photodegradation generated relatively low molecular weight DOM. Five EEM-PARAFAC components, including four terrestrially-derived humic-like substances (C1, C2, C3 and C4) and one tryptophan-like substance (C5) were identified. The five components had diverse photoreactivities to UV and visible light. Components representing allochthonous-type DOM (C2 and C3) were more effectively photodegraded than components representing autochthonous-type DOM (C5). In the Vis+UVA+UVB treatment (total irradiation), the reduction in C2 and C3 after 4 days exposure were 27% and 34%, respectively, while only 14% C5 was removed. C1 and C4 were suggested to be photoproducts of C2, however C1 was also degraded by UV light upon formation, which caused a decrease in FI during Vis+UV treatment. C4 was characterized as having high aromatic content and appeared to be photorefractory, thus the generation of C4 resulted in an increase in $SUVA_{254}$ and HIX. The results suggested that a deal of allochthonous DOM could be stored in alpine lakes because only a fraction of allochthonous DOM was photomineralized and highly aromatic DOM was photogenerated which was presumed to be relatively biorefractory.

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Figure captions:

Figure 1 Changes of DOC(a), a_{254} (b) and SUVA₂₅₄(c) in three treatments

Figure 2 Changes of $S_{275-295}$ (a), FI(b) and HIX(c) of DOM in Lake Tiancai during the three light treatments

Figure 3 The PARAFAC model output showing fluorescence signatures of the five fluorescent components. (A–E: The contour plots present spectral shapes of excitation and emission; F–J: The line plots present split-half validation results; excitation (left) and emission (right) spectra were estimated from two independent halves of the data set (black and green lines), and the complete data set (red lines)).

Figure 4 Changes of five EEM-PARAFAC components during three light treatments

Table 1 Chemical and optical characteristics of Lake Tiancai.

Table 2 Excitation and emission maxima, the possible sources and photoreactivities of the five EEM-PARAFAC components.

Table 1 Chemical and optical characteristics of Lake Tiancai

pH	Water Temperature(°C)	TN (mg/L)	TP (µg/L)	DO C (mg/L)	SUV A ₂₅₄	FI	HI X	C1 %	C2 %	C3 %	C4 %	C5 %
7.91	11.49	0.54	14.44	10.33	3.7	1.51	4.25	28.60	18.94	29.25	4.4	18.97

Table 2 Excitation and emission maxima, the possible sources and photoreactivities of the five EEM-PARAFAC components

Component	Ex _{max} (nm)	Em _{max} (nm)	Assignment	Photolability	
				UV light	Visble light
C1	<250 (295)	412	photodegradation product	Y	N
C2	255(345)	498	terrestrially-derived humic-like	-	Y
C3	<250(325)	438	terrestrially-derived humic-like	Y	N
C4	<250(310)	438	photodegradation product	N	N
C5	275	330	tryptophan-like components	-	Y

*"Y" denotes "Yes"; "N" denotes "No"; "-"denotes "not sure"

Figure 1 Changes of DOC(a), a_{254} (b) and $SUVA_{254}$ (c) in Vis+UVA+UVB treatment

Figure 2 Changes of $S_{275-295}$ (a), FI(b) and HIX(c) of CDOM in TC lake during the three light treatments

Figure 3: The PARAFAC model output showing fluorescence signatures of the five fluorescent components. (A–E: The contour plots present spectral shapes of excitation and emission; F–J: The line plots present split-half validation results; excitation (left) and emission (right) spectra were estimated from two independent halves of the data set (black and green lines), and the complete data set (red lines)).

Figure 4 Changes of five PARAFAC-Components during three light treatments

photoreactivities of
components

alteration
properties of DOM

Increase in $SUVA_{254}$, decreasing bioavailability