Interactive comment on “New insights into mechanisms of sunlight-mediated high-temperature accelerated diurnal production-degradation of fluorescent DOM in lake waters” by

Yijun Liu et al.

Dear Prof. Dr. Koji Suzuki, Associate Editor, Biogeosciences:

Anonymous Referee #1 Received and published: 2 June 2020.

We are very grateful to Review#1 for the valuable and constructive comments on our manuscript. We are submitting the manuscript and Figures revised according to the Reviewer comments. We have considered duly all Reviewer comments, providing more examples, which could contribute to better understanding our FDOM research. Thank you.

Itemized responses (R) to Reviewer comments below:

Note: all line numbers refer to the revised manuscript.

Major comments

The authors measured diurnal changes in CDOM components, DOC concentrations, and nutrients concentrations in two small lakes in Tianjin University, China to identify the biogeochemical processes controlling the diurnal DOM variation which is feasibly related to global warming. I think the research topics described in the manuscript would be of great interests to readers in Biogeosciences. However, I also think that the manuscript is not clearly written and difficult to follow, not technically sound and not appropriately discussed in the context of previous literature. Some of figures are not clear. Please see comments listed below

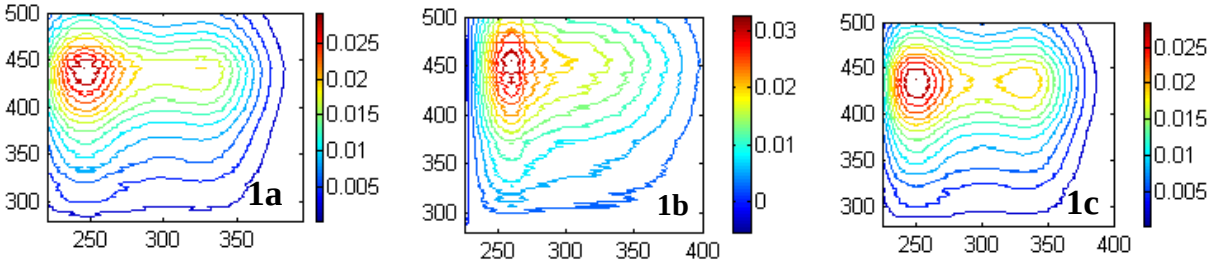
(1) The authors ran PARAFAC modeling to determine fluorescent components for each time period and compare the components among time periods. First of all, since there is no description regarding how the authors determined the number of components and validated PARAFAC models, I cannot evaluate whether the conclusion derived from PARAFAC are scientifically/technically sound or not. My opinion from our experiences of PARAFAC modeling is that it is not reasonable to apply PARAFAC for a dataset comprising the small number of samples ($n < 20$). It seems that the authors used small data set for PARAFAC modeling (lines197-200; I cannot understand the sentence though...). While, since the validity depends on

the dataset for PARAFAC modeling, the authors should describe the validation method to identify the number of PARAFAC components and show the results of the validation.

R-1. "**Number of Samples"**. Our research group has provided ample previous evidence that only two EEM spectra from two samples are enough to identify the authentic PARAFAC components for freshwater and seawater samples (Mostofa et al., 2019; Mostofa et al., 2018-conference paper). In the 2019-EST paper and earlier studies, i.e. Mostofa et al. 2010; Mostofa et al. 2013, we have clearly assessed that "two freshwater samples, six inshore seawater samples and 12 offshore seawater samples are sufficient to run the PARAFAC model" . My research group is doing FDOM research since 1999 and I have presented EEM data as a Keynote speaker in the Conference on Organic Matter Spectroscopy 2018 (WOMS18), held on 23-27 October 2018, in Carqueiranne City, France. Website: http://woms18.univ-tln.fr/moftofa-abstract/. In that presentation the number of samples issue, along with many other issues have been discussed providing substantial evidence. Three examples are provided below for further supporting the number of samples used in our PARAFAC model.

(i) The PARAFAC model applied to upstream water (Namitaki, site-KR 1) of Kurose River was fitted by only one component (terrestrial humic-like substances) in three cases, i.e. all ten individual EEM data sets on samples from May 2002 to Feb 2003 (Fig. 1a), two EEM data sets on summer (May and July) samples (Fig. 1b) and two EEM data sets on winter (January and February) samples (Fig. 1c). Details about sampling can be found in Mostofa KMG et al. (2005) Geochemical J 39: 257-271.

Terrestrial humic-like substances



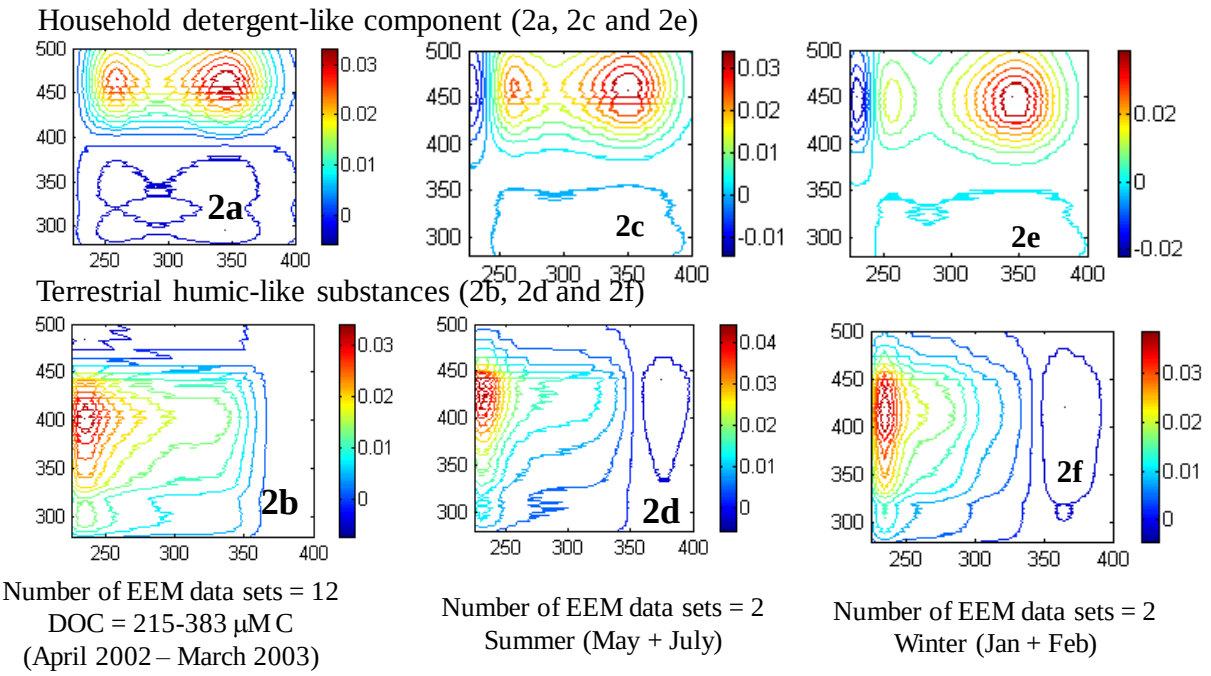
Number of EEM data sets = 10
DOC = 47-146 µM C
(May 2002 – Feb 2003)

Number of EEM data sets = 2
Summer (May + July)

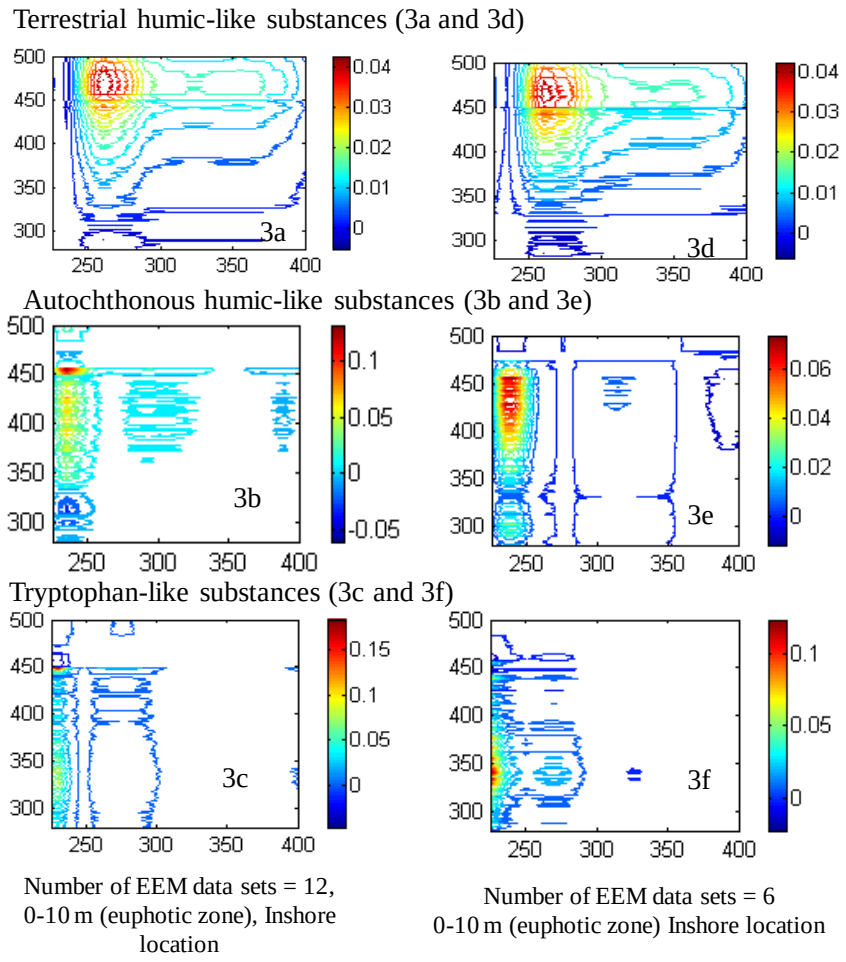
Number of EEM data sets = 2
Winter (Jan + Feb)

(ii) The PARAFAC model applied to sewerage-impacted downstream water (Izumi, site-KR 5) of Kurose River was fitted two components in three cases, i.e. all 12 individual EEM data sets

on samples from May 2002 to Feb 2003 (Fig. 2a-b), two EEM data sets on summer (May and July) samples (Fig. 2c-d), and two EEM data sets on winter (January and February) samples (Fig. 2e-f). Details about sampling can be found in Mostofa KMG et al. (2005) Geochemical J 39: 257-271.

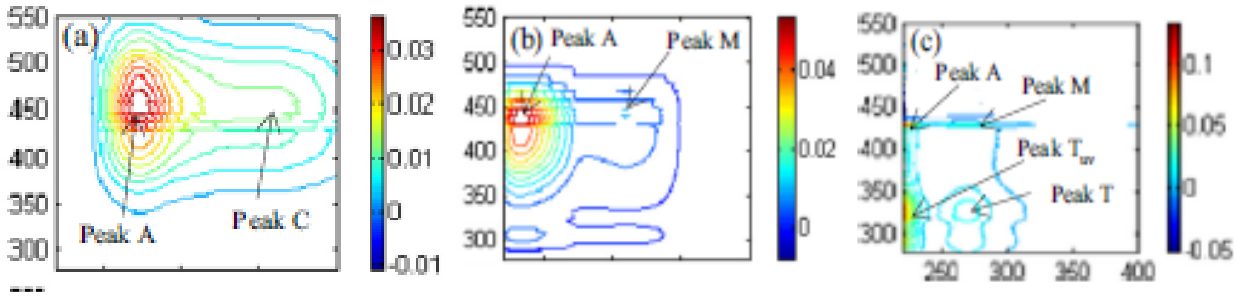


(iii) The PARAFAC model applied to inshore seawater of Seto Inland Sea was fitted by three components in two cases, i.e. all 12 individual EEM data sets (Fig. 3a-b-c) and six EEM data sets (Fig. 2d-e-f). Details about sampling can be found in Mostofa KMG et al. (2019) Environ Sci and Technol 53: 561-563.



R-1. 'Components and Model Validation'. First of all, we wish to escribe our knowledge of DOM and FDOM compositions in diverse surface waters. Without understanding of DOM or FDOM composition, without which we cannot dicuss about our views on "components and model validation'. FDOM are primarily originated from three key sources.

First, the terrestrial source, i.e. FDOM derived from soil/land, which is predominantly detected in streams and rivers, and is largely decomposed in lakes/reservoirs/ponds/oceans (Coble, 1996; Mostofa et al. 2013; Mostofa et al. 2019). It is well known that extracted soil humic substances are composed of three key components that include humic acids (HA), fulvic acids (FA) and protein-like fluorofores (PLF), with soil PLF entirely different from those detected in surface waters (Fig. below- soil HA, FA and PLF of forest soil origin in Mohinuzzaman et al., 2020).



HA FA PLF

Second, the autochthonous source, i.e. FDOM originated from phytoplankton, which is mostly detected in stagnant waters such as lakes/reservoirs/ponds (Zhang et al., 2009; Parlanti et al. 2000; Mostofa et al. 2013; Yijun et al.- this study). However, some rivers/streams in winter dry season can present stagnant water areas where phytoplankton can grow and produce autochthonous FDOM. As phytoplankton cannot grow in streams or upstream rivers where water is only released from groundwater/terrestrial land, these waters do not contain autochthonous source-FDOM. It is well known that the production of microorganisms/phytoplankton needs time (several days) and stagnant waters for their growth under sunlight conditions. Therefore, stream/upstream river FDOM can only be of terrestrial source and not autochthonous source.

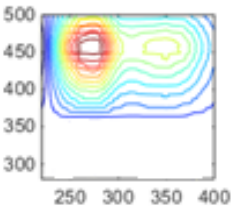
Third, an anthropogenic source or sewerage-derived FDOM (e.g. detergent-like components), is detected only in untreated sewerage-impacted rivers in specific site (Baker et al. 2001; Mostofa et al. 2005; Mostofa et al. 2010).

Fourth, another important issue is that seawater FDOM is strongly affected by salinity, pH and photochemical degradation, which are completely different from those of freshwater FDOM. For example, salinity can cause the peak C of terrestrial humic-like substances to shift at longer wavelength region (red-shifted phenomena), together with other changes of other FDOM components with respect to river freshwaters (Coble 1996; Yamashita and Zaffé, 2008; Yamashita et al. 2010-Deep-Sea Res-II, 57, 1478-1485; Mostofa et al. 2010; Mostofa et al. 2013; Mostofa et al. 2019).

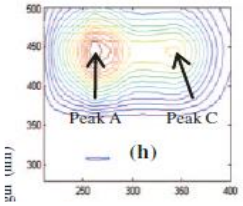
Fifth, in this study (Yijun et al. 2020; see Figure-below) and other studies (Zhang et al., 2009; Mostofa et al. 2013; Parlanti et al. 2000). Terrestrial humic acid and terrestrial humic-like substances (C type) show fluorescence properties (peak positions and EEM images) similar to those of autochthonous humic-like substances (C type) of phytoplankton origin The two

autochthonous and terrestrial sources of FDOM components cannot be distinguished by PARAFAC analysis when all diverse samples are mixed together. This is the reason why one cannot distinguish between these two autochthonous sources.

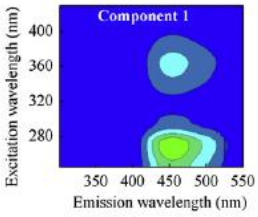
Autochthonous humic like substances (C type) of phytoplankton origin



Yijun et al.-current study

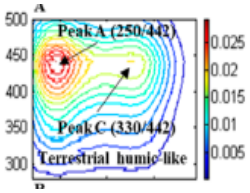


Phytoplankton origin (Mostofa et al. 2013)

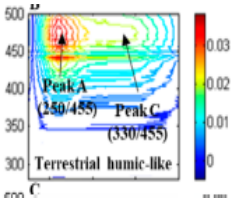


Phytoplankton origin (Zhang et al. 2009)

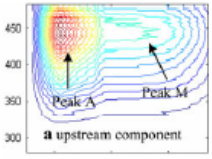
Terrestrial humic-like substances (C type) of land origin



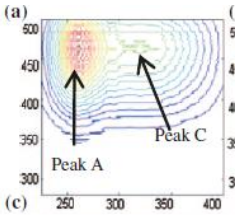
Stream water, Japan (Mostofa et al. 2019; Mostofa et al. 2005)



Another stream water, Japan (Mostofa et al. 2019; Mostofa et al. 2005)



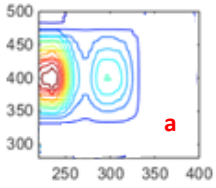
Upstream water, China (Mostofa et al. 2010)



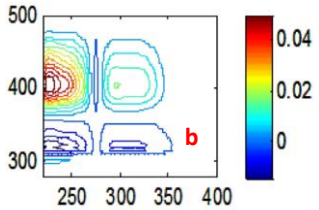
Standard Suwannee River Fulvic Acids (Mostofa et al. 2013)

Sixth, in this and other studies (Yijun et al. 2020; see Figure-below; Zhang et al., 2009; Mostofa et al. 2013; Shammi et al. 2017) terrestrial fulvic acid and terrestrial humic-like substances (M type) (Mostofa et al. 2019; Mostofa et al. 2013; Mohinuzzaman et al. 2020) show fluorescence properties (peak positions and EEM images) similar to autochthonous humic-like substances (M type) of phytoplankton origin). The two autochthonous (Fig. a-d) and terrestrial (Fig. e-g) sources of FDOM components cannot be distinguished by PARAFAC analysis when all diverse samples are mixed together. This is the reason why one cannot distinguish between these two autochthonous sources.

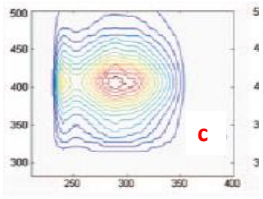
Autochthonous humic like substances (M type) of phytoplankton origin (a-d)



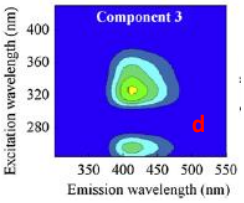
Yijun et al.-current study (Oct, Jingya lake)



Origin from EPS under sunlight conditions (Shammi et al. 2017-Scientific Report)

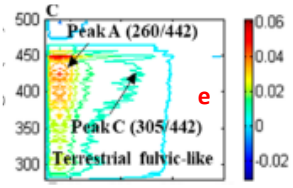


Origin from lake phytoplankton after resuspenssions in Milli-Q water under dark incubation (Mostofa et al. 2013)

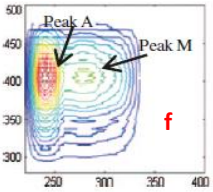


Phytoplankton origin under dark incubation (Zhang et al. 2009)

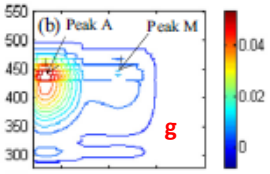
Terrestrial humic-like substances (M type) of land origin (e-g)



Upstream water, site KR-2, Kurose River, Japan (Mostofa et al. 2019; Mostofa et al. 2005)



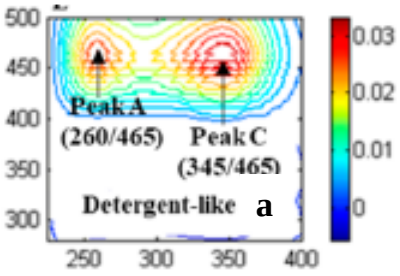
Water samples collected from Yellow River, China (Mostofa et al. 2013)



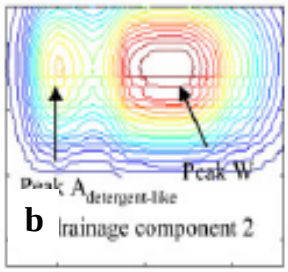
Soil fulvic acid after water extraction from forest soil (Mohinuzzaman et al. 2020)

Seventh, untreated sewerage-affected river could transport detergent-like substances and standard household detergents into river waters (see Fig. a-c; below; Mostofa et al. 2013; Mostofa et al. 2010; Mostofa et al. 2005; Baker et al. 2001).

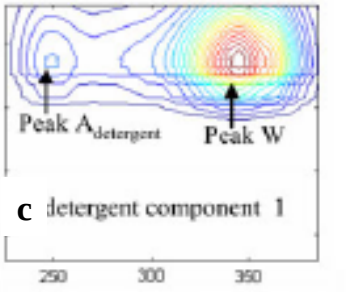
Detergent-like components (a-c)



Detergent-like component detected in Kurose River water (Mostofa et al. 2019)



Detergent-like component detected in city drainage waters before enter in mainstream river (Mostofa et al. 2010)



Standard household detergent component after dissolved in Milli-Q water (Mostofa et al. 2010)

Based on all seven different types of FDOM sources, it is very important to choose "Selective Characteristic Samples" that can represent authentic FDOM components (Mostofa et al. 2019), whereas mixing all seven types of FDOM sources together in a PARAFAC model cannot evaluate authentic FDOM. Stedmon et al. (2003) firstly used the PARAFAC model on a total of 90 samples covering all major terrestrial sources of DOM, streams and estuary DOM mixed with

seawater from the adjacent Kattegat, which resulted in a five component model. Since the study of Stedmon et al. (2003), the use of mixed samples in the PARAFAC model has been repeated in several other studies to date (Murphy et al., 2013; Kulkarni et al., 2017; Wu et al., 2018; Yue et al., 2019). However, artifact due to mixing. Mostofa et al. (2019) provided evidence that these components were artifacts due to mixing.

"Model Validation". Stedmon et al. (2003) and Murphy et al. (2013) have used the split-half analysis method for validation of the PARAFAC components. This method consists in dividing the EEM data set into two random, typically equal sized groups and then pplying the PARAFAC model to both data halves independently (Stedmon et al., 2003). The procedure of mixing all diverse samples appears to be inappropriate to validate the PARAFAC model. Furthermore, this model validation based on spilt-half analysis of unselected EEM data does not allow to identify and distinguish the authentic fluorescent components in the PARAFAC model Thus, we believe that a correct model validation should be based on the authentic identification and characterization of fluorescent components of the individual seven sources mentioned above. In this study, we have validated the authentic fluorescent components on the basis of our earlier experiences on the identification an classification of EPS and their released photomicrobial components (Shammi et al. 2017a, 2017b, 2017c; Sheng and Yu, 2006), autochthonous humic-like substances (C-type and M-type) of phytoplankton origin (Zhang et al., 2009; Mostofa et al., 2013), as well as aromatic amino acids (e.g. tryptophan, tyrosine and phenylalanine) of phytoplankton origin and standard substances (Yamashita and Tanoue, 2003; Zhang et al., 2009; Mostofa et al., 2013, 2018a, 2018b, 2019).

According to the comments above, we have revised the manuscript with addition of a new paragraph "The number of components and validation of PARAFAC model were performed based on the following criteria. Firstly, we applied 'Selective Characteristic Sample' EEM data to the PARAFAC model that could be continued from 1 to 8 components until achieving negative *a*-values (see Eqs 1-2). When two successive models (e.g. the 5- and 6-component model) showed one or two negative *a*-values, the model could be fitted up to 4 components and there was no need to go further by checking for a 7- or 8-component model. Then, the valid models were subjected to the next step for their validation by considering the highest number of fluorescent components and comparing with their respective EEM images and fluorescence

peaks of standard and extracted substances (or FDOM) (Yamashita and Tanoue, 2003; Sheng and Yu, 2006; Zhang et al. 2009; Mostofa et al., 2013; Shammi et al. 2017a, 2017b, 2017c; Mostofa et al., 2019). For example, if we consider a 4-component model where the image and fluorescence peaks of one of the four fluorescent components does not correspond to the respective peak position appearing outside the usual DOM fluorescence (*i.e*. Rayleigh and water Raman peak positions), then the model should be rechecked for 3 components. Differently, if all components are verified with those of standard substances or specific field/experimental observations, the model and the respective fluorescent components are validated. Finally, the fluorescent components obtained from the model can be characterized and classified (Yamashita and Tanoue, 2003; Sheng and Yu, 2006; Zhang et al. 2009; Mostofa et al., 2013; Shammi et al. 2017a, 2017b, 2017c; Mostofa et al., 2019)." in *lines 206-225*.

We believe that it is important to find out the various sources of FDOM which can be related to biogeochemical facts and their significances. To clarify the principles on which the PARAFAC model is based, we have also added an additional paragraph "The parallel factor (PARAFAC) analysis is a three-way multivariate method that can be applied to EEM data of DOM to decompose them into trilinear components (Harshman. 1970; Caroll and Chang, 1970). For any fluorophore of FDOM, the emission intensity, x_{jk}, at a specific wavelength, j, which corresponds to excitation at the wavelength, k, can be expressed by the following equation (Harshman. 1970):

$$x_{jk} = ab_j c_k \qquad \text{……..…….} (1)$$

wherea is the concentration (in arbitrary units, a.u.) of the analyte (fluorophore or FDOM), b_j is the relative emission at the wavelength j, and c_k is the relative amount of light absorbed at the excitation wavelength k. For any number of analytes and samples, the PARAFAC model can be expressed as a set of trilinear terms and a residual array as (Stedmon et al., 2003):

$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + \varepsilon_{ijk}, \; i = 1,..,I; \; j = 1,..,J; \; k = 1,..,K \qquad \text{…….(2)}$$

where x_{ijk} is the fluorescence intensity of the i^{th} sample at the emission wavelength j and excitation wavelength k, a_{if} is directly proportional to the concentration (in a.u.) of the analyte f

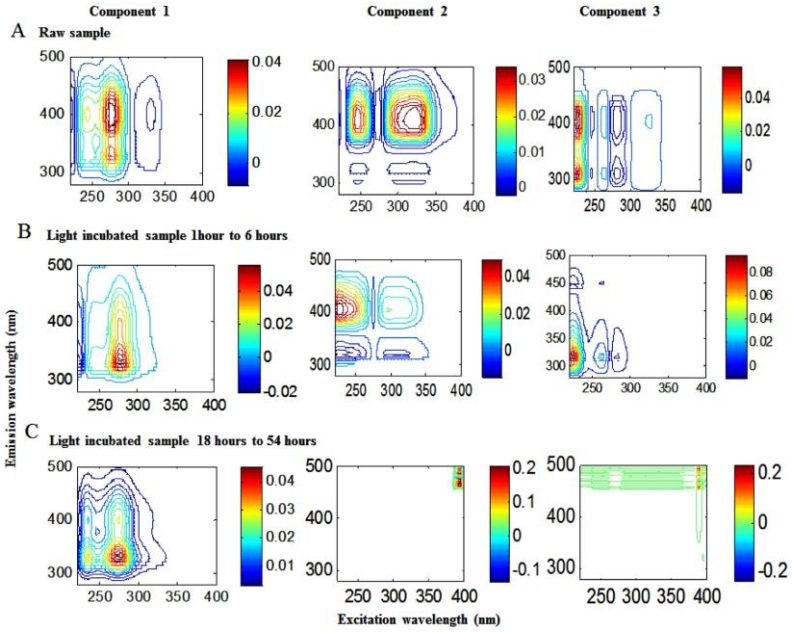
in sample *i*. b_{jf} is directly proportional to the quantum efficiency of fluorescence of the analyte *f* at the emission wavelength *j*, c_{kf} is linearly related to the specific absorption coefficient at the excitation wavelength *k*, *F* is the number of components in the model, and ε_{ijk} is the residual matrix that indicates the variability not accounted for by the model." in *lines 182-198.*

(2) The authors concluded that some components were disappeared over a 24-h diurnal period (e.g., lines 29-32) from the qualitative analysis by PARAFAC. I do not agree with this conclusion, because it may be results of artifact by PARAFAC modeling. For example, if the authors apply new PARAFAC with slightly large number of samples, the disappeared components may appear. If the authors want to conclude disappearance of components quantitatively, the authors should use single PARAFAC model and determine diurnal changes in fluorescence intensity of individual PARAFAC components. I also disagree the conclusion of "degraded into four FDOM components (lines 32-35)" with the same reason.

R-2. We have extensively discussed above that this study does not include any artifact components. A large number of water samples from different sources can produce artifact components from their mixing (Mostofa et al. 2019). The disappearance of FDOM occurred only in July samples under strong sunlight and high water/air temperature conditions and did not occur in October and June samples. Disappearance of FDOM is possibly due to its conversion into low molecular weight DOM which usually do not show any fluorescence. Furthermore, terrestrial humic-like substances (C-type) do not degrade and only autochthonous FDOM can degrade as we showed in this study. The July changes in FDOM can be further evidenced from inconsistent changes of DOC concentrations that significantly increased at noon (18%) and then decreased again (15%) at night (Fig. 6). This DOC alteration can be attributed to the net production and mineralization effect considering a constant DOM production from phytoplankton and its simultaneous degradation.

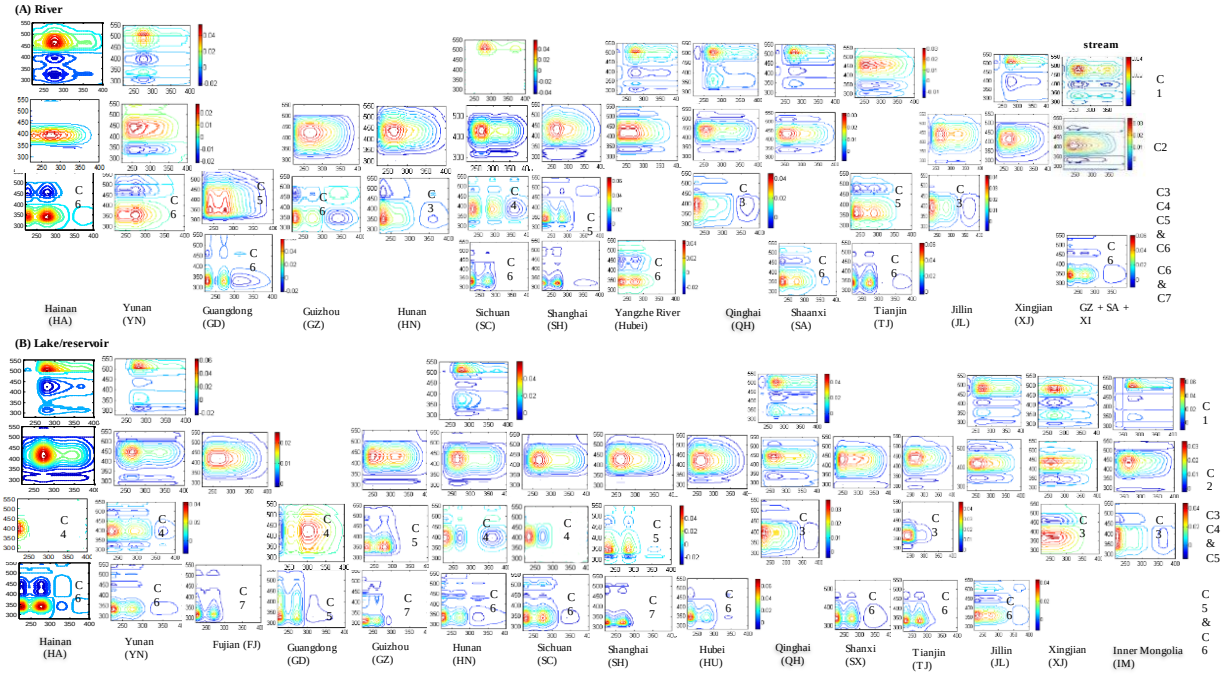
Furthermore, EPS is the first component that we have comprehensively discussed in the manuscript. Earlier results (Shammi et al. 2017a, 2017b) demonstrated that raw EPS (A: 3 components) can convert rapidly into three new components in one to six-hours irradiation, which is supported by results of this current study. Then, after 18 to 54 hours, two components

are completely degraded. In our July samples, degradation occurred at the highest air temperature (41.13 °C) and water temperature range (29.2-33.5 °C).



The subsequent conversion of raw EPS into FDOM components were similar to results of this study and some other unpublished data for river and lakes (manuscript in preparation) (see Figure below).

C1 = Terrestrial humic acid-like; C2 = Terrestrial fulvic acid-like; C3 = Terrestrial protein-like fluorophore; C4 = Autochthonous humic-like substances; C5 = Extracellular polymeric substances; C6 = Protein-like/tryptophan-like & C7 = tyrosine-like substances



To better clarify these aspects, we have revised the relevant sentence "at the highest air and water temperatures (respectively, 41.1 and 33.5°C)" in *lines 31-32. Full sentence:* In this work sunlight-mediated high-temperature was shown to accelerate the production of FDOM, but also its complete disappearance over a 24-h diurnal period in July at the highest air and water temperatures (respectively, 41.1 and 33.5°C), but not in lower temperature months*).*

(3) The authors described temporal changes in PARAFAC components quantitatively (e.g., lines 232-234). However, I cannot follow the time periods which involving the temporal changes. Again, it is difficult to quantitatively compare abundance of PARAFAC components derived from different PARAFAC models.

R-3. We have rearranged the sentences and revised properly "In particular: (a) newly-released PLS with a peak T at 280/351 nm appeared and the T_{UV} peak disappeared, whereas the intensity of peak T of newly-released PLS increased by 49%; (c) the fluorescence peak C of C type-AHLS was partly shifted to longer wavelength, i.e. 325/430 nm; (d) peak M of M type-AHLS showed a 3.2-fold intensity increase; and (e) the fluorescence intensity of peak C of C type-AHLS and peak T of TLS decreased, respectively, by about 3% and 63%." in *lines 266-270*.

The changes of FDOM components occur due to microbial respiration/ sunlight degradation at every moment, which simultaneously altered nutrients (NO3-, NH4+, etc.) and DOC contents. FDOM components are changing with changes in sunlight intensity during day time and microbial degradation at night time. Therefore, PARAFAC model on time-specific water samples are important, as well as the seven-specific FDOM components, to conduct PARAFAC analysis individually. Otherwise, we cannot observe the biogeochemical changes of FDOM components which are related to changes in nutrients, DOC concentration and phytoplankton.

(4) The authors seemed to conclude that EPS produced during early morning partially degraded at midday and remineralized at night time (lines 32-35). Is this conclusion consistent with temporal changes in DOC concentrations and nutrients concentrations (Fig. 6)? I don't think so. To verify the authors' conclusion, careful, quantitative, and statistical comparisons of diurnal

changes in PARAFAC components with diurnal changes in DOC and nutrients concentrations should be made.

R-4. We have partly clarified this issue in the reply to comment 2. This issue is based on the various biogeochemical changes that occur at each timescale and involves the following processes:

- Phytoplankton via photosynthesis is constantly produced during sunlight period.
- Photoinduced and microbial respiration can release various FDOM from phytoplankton , which changes (generally increases) DOC and DON contents.
- The occurrence of photodegradation by photo-induced reactive oxygen species ($O_2^{\bullet -}$, H_2O_2 and $^{\bullet}OH$) during day time varies depending on sunlight intensity.
- The occurrence of microbial degradation of FDOM can alter FDOM, nutrients and DOC.
- The conversion of FDOM by photochemical and microbial processes into low molecular weight (LMW)-non-fluorescent DOC and nutrients.
- The complete mineralization of DOC occurs constantly under photo-microbial processes.
- Nitrification, denitrification and anaerobic ammonium oxidation processes are occurring constantly.

As all of these processes are occurring actively at every moment, the FDOM, DOC and nutrients we have measured at specific times (e.g. 10 am) are the net existing amounts deriving from all the mentioned biogeochemical changes, in ***"4.2 Biogeochemical processes involving DOC and nutrients in lake Jingye"***

In July, DOC concentration varied hourly from a minimum of 815 μM C achieved in the night (from 21.00 to 6.00) to a maximum of 963 μM C reached during the day (from 10.00 to 16.00), with the highest DOC fluctuation of 18% (Table S2, Fig. 6). In October, the DOC content was generally higher than in July and varied from low night values with a minimum of 975 μM C at early morning (6:00) to a maximum of 2989 μM C during the day-time (10.00-15.00) with an increase of approximately 3.07-fold at 14:00 (Table S2, Fig. 6). The trends of DOC concentration were paralleled by those of nutrients which in October were generally higher and more fluctuating along the day than in July (Table S2, Fig. 6). In particular, in July the NO_3^- content decreased of about 20% from early morning (6.00-9.00), to middle day (10.00-15.00) and subsequently increased (1.0-7.8%) in all successive sub-diurnal samples, whereas in October, it increased of

about 10.0% during morning from 6 to 15.00, then remained nearly constant and increased again of about 16.0% during night time (2.00-6.00). Differently, NO_2^- increased substantially (70.0-94%) during day-time in October compared to July (0.5-14.4%), but then decreased significantly (5.0-5.8%) during night-time, which suggested a rapid turnover rate of NO_2^- during phytoplankton growth. Similarly, NH_4^+ substantially increased in October (117.0-257.0%) compared to the same time interval in July (4.1-41.7%), with the highest increase in the middle of the day (10.00-15.00). The DON content increased (7.6-31.0%) between 6.00-9.00 in July, whereas it was not detected in samples collected in October. The PO_4^{3-} content decreased significantly in July during the 16.00-20.00 period and then increased (18.0-34%) in night-time, whereas in October it decreased (11.4-40.0%) in night- time, showing the highest concentrations in the 16.00-20.00 time period. In July, DSi decreased (3-17%) from early morning (6:00-9:00) to late day, with an early night (21.00-1.00) increase of 23%, whereas it was absent in samples collected in October, which suggested its complete uptake by phytoplankton during the strong growth period occurring in this month.

The highest production of nutrients in October than in July was estimated to be approximately of 16.0, 28, 4.0 and 23.8-fold respectively for NO_3^-, NO_2^-, NH_4^+, and PO_4^{3-} (Fig. 6). In particular, in October the highest NH_4^+ concentration paralleled the highest DOC concentration detected at the same time (14.00) (Table S2, Fig. 6), which suggested a rapid biogeochemical transformation of organic matter into DOC and nutrients under ambient lake conditions. Apparently, the NH_4^+ formation in October was followed by its subsequent rapid nitrification and denitrification at same time (14.00) (Table S2, Fig. 6). Similarly, the lowest level of NH_4^+ at 10.00 was consistent with the lowest level of NO_3^-. Further, the occurrence of nitrification was confirmed by the decrease of NH_4^+ contents occurring simultaneously to the increase of NO_3^- in early morning samples (Table S2, Fig. 6).

Further evidence of photosynthetic activities of phytoplankton was provided by the significant shifting of the $\delta^{15}N$ value of NO_3^-. In particular, in July the $\delta^{15}N$ value decreased from +0.67‰ at 10:00 to −0.02‰ at 12:00, which corresponded to the increase of SI from 2.33 MJ m^{-2} to 2.95 MJ m^{-2} (for a total of 8.14 MJ m^{-2}), whereas the $\delta^{18}O$ value of NO_3^- increased from +5.28‰ at 10:00 to +9.3‰ at 12:00 under conditions of elevated SI and high WT (36.5-39.9 °C) and AT (31.0-32.7 °C). The decrease of $\delta^{15}N$ and increase of $\delta^{18}O$ in NO_3^- with increasing SI from 10:00

to 12:00 indicated the uptake of lighter $\delta^{16}O$-containing NO_3^- by phytoplankton with increasing photosynthesis. This effect was confirmed by the 20% decrease of NO_3^- from 9:00 to15:00 in July, whereas in samples collected in October the disappearance of DSi with the corresponding high production of DOC and nutrients would indicate the occurrences of high photosynthetic activity with a high production of phytoplankton during day-time from both photoinduced processes and microbial respiration of planktonic organisms, which confirmed earlier results (Fan and Glibert, 2005; Guidi et al. 2016; Parsons et al. 2017; Condon et al. 2010; Carpenter et all. 1998; Elser et al. 1995). Thus, the activity of photosynthetic planktonic communities in sunlit surface water with the simultaneous release of DOC and nutrients could represent the driving force of the overall biogeochemical processes and functions occurring in surface lake waters."

We have also partly discussed this issue based on the reviewer#2 comments in the new addition "***4.4. High-temperature production of FDOM from phytoplankton and its sequential entire degradation at 24-h diurnal scale***

The complete degradation of FDOM after its formation from phytoplankton can be ascribed to the gradual increase in SI (from 1.36 to 2.97 MJ/m^2 for a total of 15.26 MJ/m^2) at the highest WT (30.2-33.7 °C) and AT (35.4-41.8 °C) (Fig. 2). These environmental conditions are directly responsible of the rapid production of FDOM from phytoplankton and its corresponding rapid degradation by day-time sunlight-induced and night-time microbially-induced processes. Such entire sequential diurnal degradation of FDOM was not observed in October, May and June samples (Figs. 3-5) when solar intensity, AT and WT were relatively low compared to July samples (Fig. 2). As discussed previously, the production and degradation of FDOM are related to simultaneous significant fluctuations in the contents of NO_3^-, NH_4^+, NO_2^-, DON, PO_4^{3-}, DSi and DOC (Fig. 6; Table S2). Photochemical and microbial processes can transform EPS into various forms of FDOM (Shammi et al., 2017a, 2017b) that are related to field observations, which show that different components of EPS vary over different timescales and temperatures (Shammi et al., 2017c; Sheng and Yu, 2006). Many studies provided evidence that photochemical and microbial degradation processes increase with increasing temperature and light intensity (Matsumoto et al., 2007; Weston and Joye, 2005; Malinverno and Martinez, 2015; Whelan and Rhew, 2015; McKay and Rosario-Ortiz, 2015; Grannas et al., 2006; Farias et al., 2007). In turn, these results would indicate that high SI, WT and AT would accelerate the complete transformation of autochthonous

FDOM into LMW DOM and other mineralization end-products on a 24-h diurnal cycle, which could be further increased by the influence of future GW. Such changes can to be reasonably expected to occur in the future on the basis of increasing water temperature, extended summer season and increased water stratification in response to the predicted GW from 1.5 to 2.0°C (Huisman et al., 2006; Watanabe et al., 2011; Rogelj et al., 2019).”

(5) It seems that the authors generally consider/discuss degradation of EPS to explain daytime changes in PARAFAC components. Why don’t the authors consider DOM production including the PARAFAC components with primary production during daytime?

R-5. DOM is well known to comprise many types of organic substances, which include EPS that can gradually convert into various FDOM components, non-fluorescent photoproducts that can also be produced from FDOM, non-fluorescent microbial products, non-fluorescent LMW substances (aldehydes, ketones), etc. Besides terrestrial sources, autochthonous DOM is a key fraction of DOM compositions in lakes and reservoirs, but it is still uncertain how it is released from phytoplankton. Our research group has gained a wide experience on EPS extraction from large volume of surface waters, and its photo-microbial products, which is the key to better understand the relation between FDOM and DOM. We believe that results of this study will contribute to understand the sequential release of FDOM from EPS and their subsequent degradation (Figs. 8-9; this study). As commonly reported in all other studies, in this manuscript DOM is discussed as DOC changes.

Other comments

I listed some other comments below. Similar errors with some of the comments were found throughout the manuscript.

Lines 69-72: What is “key DOM components”? It’s not clear.

R. The sentence has been revised according to this comment with addition of “Key DOM components include terrestrial humic substances (fulvic and humic acids), EPS, and aromatic

amino acids (Coble, 1996; Yamashina and Tanoue, 2003; Yamashina and Tanoue, 2004; Shammi et al. 2017a, 2017b, 2017c; Zhang et al., 2009).

Lines 99-101: I could not understand the message of the sentence. The authors would like to mention "diurnal degradation processes depended on the photosynthetic activity of primary producers"?

R: Yes. The sentence has been rearranged according to this comment as "Diurnal day-time (sunlight) and night-time (microbial) degradation processes are a natural phenomenon that depends on the photosynthetic activity of primary producers in surface waters which is ultimately related to daily biogeochemical changes of C, N and P cycling".

Lines 102-105: "a significant decrease of its fluorescence intensity with increasing water depth" is due to photoinduced degradation? I don't agree with it.

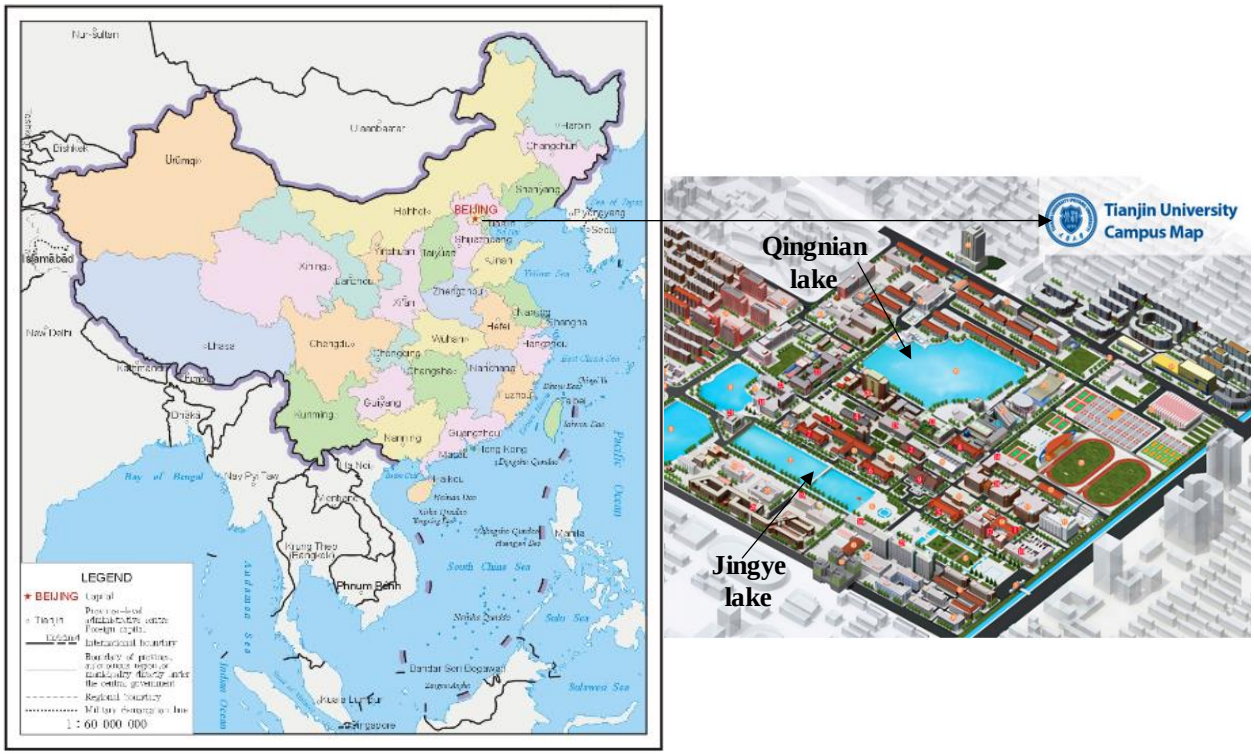
R. Yes, we agree. The sentence has been revised properly in *lines 108-109*.

Line 105: Which overall day-night degradation of FDOM corresponds "such"?

R. The sentence has been moved earlier sentence, as it is connected with the previous sentence. "Diurnal day-time (sunlight) and night-time (microbial) degradation processes are a natural phenomenon that depends on the photosynthetic activity of primary producers in surface waters which is ultimately related to daily biogeochemical changes of C, N and P cycling (Guidi et al., 2016; Segschneider and Bendtsen., 2013; Huisman et al., 2006; Carpenter et al., 1998). Such overall day-night degradation of FDOM is caused from diurnal photo-microbial transformations."

Fig. S1: The figure is not clear. It may be better to show a campus map with map of Tianjin city.

R. The maps have been replaced as suggested.



Line 134: Do seagrasses occur in inland closed lakes

R. Yes, there are some seagrasses in the Qingnian lake.

Line 149: Add filter name and company name supplied it.

R. Revised as suggested (…..0.45-μm glass-fiber filter (GF/F, Shanghai Xin Ya Purification Equipment Co. Ltd) previously cleaned…..).

Lines 152-153: "a combustion total organic carbon (TOC) auto-sampler analyzer" is awkward.

R. Revised as "…. using a total organic carbon (TOC) analyzer (OI Analytical Aurora,…….".

Lines 154-155 & 168-169: The authors described a method for UV-Vis absorbance twice. In addition, the authors described different spectrophotometers, respectively.

R. Revised as "A UV-VIS spectrophotometer (UV-2700, Shimadzu) was used to estimate absorption properties of chromophoric DOM (CDOM). Ultra-pure water was simultaneously used in the reference cell as a blank.". We deleted the duplication in former lines 167-168.

Lines 171-180: How did the authors calibrate the fluorescence intensity? With quinine sulfate? Also, what is "700 v" in line 174.

R. Arbitrary units have been used in this study. '700 v' has been revised as "….and a photomultiplier voltage of 700 v." in *lines 175-176*.

Line 209: Define "early stage DOM".

R. The text has been revised as suggested by addition of "Generally, EPS are considered an early-stage DOM, defined as "dissolved organic substances newly-formed and not yet converted to individual organic components", which ……..".

Lines 223-226: I think no reference cited here describe "FDOM production by degradation of EPS would occur during the gradual increase of SI".

R. All references in *line 261* have been deleted from the mentioned sentences "FDOM production by degradation of EPS would occur during the gradual increase of SI (from 1.36 to 2.97 MJ/m^2 for a total = 15.26 MJ/m^2) at WT of 30.2-33.7 °C and AT of 35.4-41.8 °C (Fig. 2).".

Lines 245-247: I could not follow how the authors estimate the abundance of EPS quantitatively.

R. The text has been revised as "….. In particular, the decrease in fluorescence intensity of EPS in this time period was approximately 3% lower……".

Line 254: The citation of Ma and Green (2004) is not appropriate for the sentence, because their work was carried out Lake Superior.

R. Reference deleted in *line 288-289* and revised according to our own results with addition of "(b) night-time extended microbial degradation, which was also supported by the net decrease of DOC along with nutrients at night period, which will be comprehensively discussed in the next section.".

Line 415: What is "photoinduced respiration of phytoplankton"? In addition, it seems that the authors discuss the photosynthesis rather than respiration at the rest of this paragraph.

R. The text has been revised as "Further evidence of photosynthetic activities of phytoplankton was provided by the significant shifting of the $\delta^{15}N$ value of NO_3^-." .

Lines 439-465: I think the authors do not discuss "A global view of production and degradation pathways of FDOM in lake waters" in this paragraph.

R. For reviewer's kind information. Our group published previously on EPS extraction at large scales using 40 liters of surface waters and on the subsequent photo-microbial experiments that can sequentially release FDOM from EPS (Shammi et al. 2017a, 2017b, 2017c). The results of our current study, as well as some unpublished data, appear to confirm all previous results on FDOM, EPS and their byproducts. Although it is till now unclear how autochthonous FDOM originates from phytoplankton, the results of this study demonstrated that EPS are produced in the morning and then convert into many autochthonous FDOM components that are ubiquitously detected in surface waters, as previously reported in experimental observations (Zhang et al., 2009). The paragraph in question attempts to provide a conceptual model based on our research findings by describing the sequential release of FDOM, which ultimately describes dynamics of DOM that originates from phytoplankton. This global view contributes to further understanding autochthonous FDOM and its degradation processes.

Line 435: I could not find Figs 12 and 13.

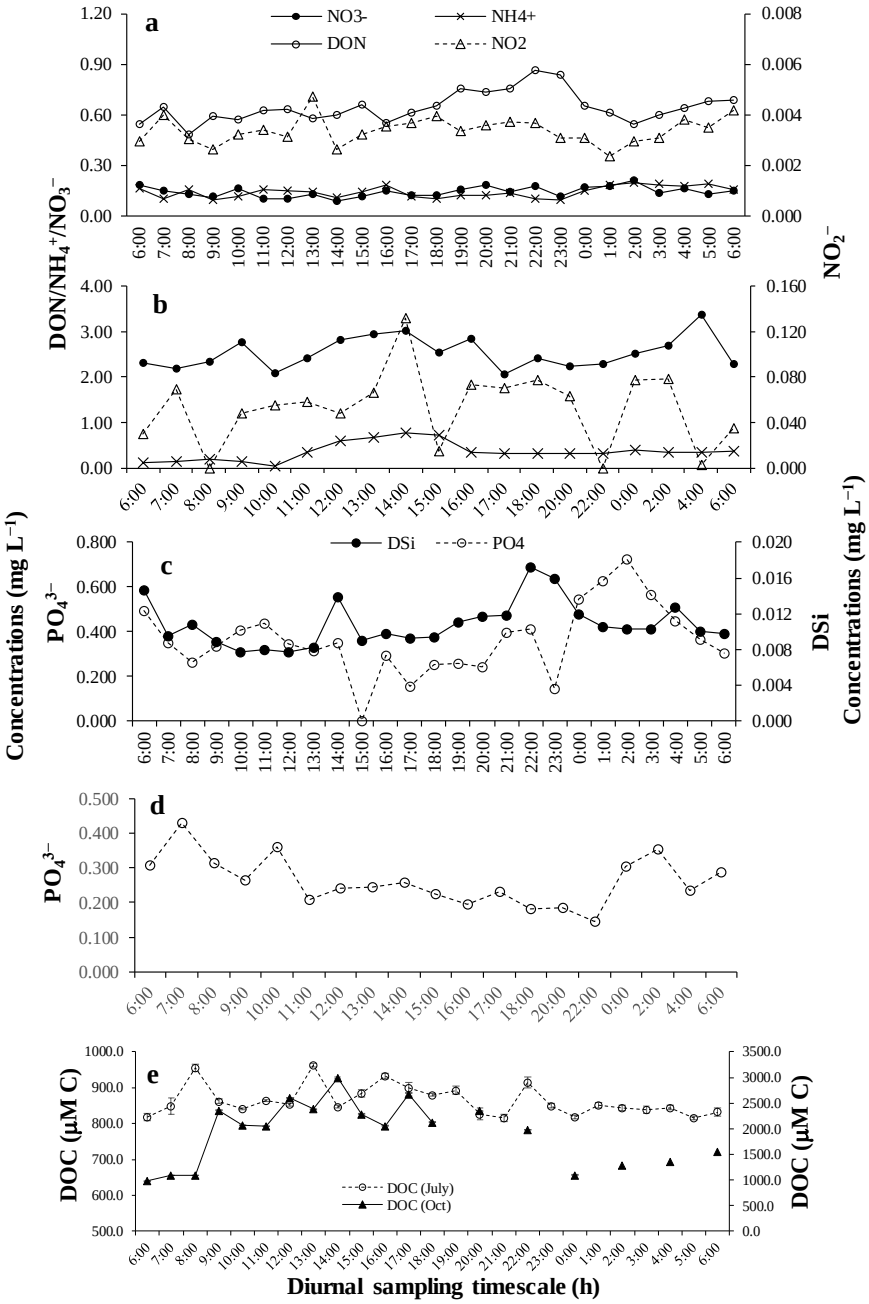
R. Sorry, these are Fig. 8 ad 9 as "……can be illustrated in the pathway shown in Fig. 7 and detailed in Figs. 8 and 9.".

Fig. 2: The figure caption, in particular about (b), does not explain the figure.

R. Figure caption corrected.

Fig. 6: Describe which Y-axis corresponds to each plot in all figures except the figure (e). Why does the Y-axis on right side of Fig. 6d show negative values?

R. Figure 6 has been revised as suggested, and negative values for dissolved Si have been deleted.


a
NO3-
NH4+
DON
NO2
b
c
DSi
PO4
d
e
DOC (July)
DOC (Oct)
Concentrations (mg L⁻¹)
DON/NH4+/NO3−
NO2−
PO4 3−
DSi
DOC (μM C)
Diurnal sampling timescale (h)