

Dear Dr Koji Suzuki

Our manuscript received two thorough reviews from the experts in the field, and we carefully revised the text, figures and tables following their comments and suggestions; we agree with most of them.

Please note that we would like to add to previously missing members of our team, Dr. Taissia Vorobyeva and Mrs. Natalia Neverova, who greatly contributed at the revision stage but also actively participated in relevant field and experimental work. However, if this is against the policy of the journal (some journals do not allow addition of co-authors at the revision stage) then please accept my apologies and disregard this request.

All the changes in the manuscript are highlighted. We thank you for giving us an opportunity to revise our work and we look forward hearing from you.

Sincerely

Oleg S Pokrovsky

on behalf of co-authors

Responses to Reviewer No 1

In this manuscript, Chupakov et al. investigate the degradation (bio- and photo-) of DOC in boreal waters. The findings are mainly discussed based on spatial and temporal trends, CO₂ production and concentration of trace metals. Given the current trends in global surface temperature, there is the potential of increasing DOC degradation in surface waters from the Arctic regions, which has strong implications for global C cycling. Thus, the findings presented here could be of interest for the scientific community in the field.

We are grateful for positive evaluation of our work.

In general, the manuscript is well-structured. However, scientific consistency and clearness can be substantially improved. For example, the authors investigate two different biogeochemical processes (biodegradation and photodegradation), at four contrasting locations (piezometer, peatland pool, stream and lake), and using different time intervals. The interpretation of the dataset is then presented in three sections: rates and UV/fluorescence indexes, CO₂ production, and trace elements. The discussion of the latter seems rather unnecessary, as the main research question appears to be the degradation rates.

The part on trace elements (TE) partitioning during bio- and photodegradation represents a strong novelty of the present study. The link between DOC and TE is straightforward: in humic waters of peatlands, most TE (except probably some alkalis and oxyanions) are strongly (> 80%) complexed to DOM (Pokrovsky et al., 2012, 2016). As a result, any DOM transformation processes may directly control TE. From the other hand, some TE may be photosensitive (Mn, Fe), toxic (Al, Cu, As, Cd, Pb), or potentially limiting micronutrients (Zn, Co, Ni, Mo) for the bacteria and therefore affect the overall rate of photo- and/or bio-degradation.

In fact, the relationship between trace elements and DOC cycling is barely mentioned in the introduction (*lines 142-145*), even after the stating the hypothesis.

A working hypothesis here is that removal of DOM via photo- or bio-degradation will inevitably change the partitioning of trace elements, which are strongly bound to DOM (such as divalent transition metals), or incorporated into organo-mineral (Fe, Al) colloids. The former might either remain in solution (during photodegradation), hence not modifying their total dissolved concentration, or being taken up by growing bacteria during bio-degradation. Both bio- and photo-degradation are capable of co-precipitating

TE with Fe and Al hydroxides hence removing trace metals from the aqueous solution. We added necessary explicative sentences to the revised Introduction (L 142-157).

I would encourage the authors to remove this section of the manuscript (*section 4.3 and related figures*) and instead focus on explaining the drivers of DOC degradation and improving readability. Perhaps the discussion of the trace elements could be moved to the supplemental material.

We thank the reviewer for constructive suggestions. However, we would like to be able to show the results of trace elements for an interesting reader. We revised the Discussion following specific hypothesis stated in the revised Introduction (see section 4.3).

Comments:

1. Is there something missing in the title of the manuscript? In my version the title shows as "*High seasonal and spatial dynamics of bio- and photodegradation in boreal humic waters*" **We modified the title as “Seasonal and spatial pattern of bio- and photodegradation in boreal humic waters” for clarity.**

2. Why were the water samples filtered for the biodegradation experiments? Filtration removes particulate attached microorganisms that play a major role in DOC removal from the water column; there is a vast amount of literature on this topic. Please check Keskitalo et al. *Environmental Research Letters*, 2022.

We thank the reviewer for pointing out this very important work, and we carefully cited it in the revised version. The present study was devoted to degradation of DOC, because we intended to compare the bio- and photodegradation process. Further, the role of POC degradation, incontestably high in large fluvial system such as the Kolyma River, or mountainous streams, is lower in POC-poor lakes and bog waters like those studied in this work. We totally agree that future research in such systems should take into account the POC bio- and photo-degradation, and this work is currently in progress. We commented on this important issue in the revised version and provided the relevant citation (L 77-81).

3. Please indicate the temperature in line 186.
4° C, added accordingly.

4. Please correct Specific Conductivity in line 211.
Specific Conductivity, corrected

5. Please clarify the exposure time in line 241 because the incubation time for the biodegradation experiments was 21 days, not 16 days (line 202). Why were different time periods used for both experiments?

Good point. The photodegradation experiments run over 16 days essentially for logistical constraints, which is still consistent with biodegradation experiments and other studies on photodegradation under sunlight, which typically ranges from 15 to 70 days (Moran et al., 2000; Vähätalo and Wetzel, 2004; Mostofa et al., 2007; Chupakova et al., 2018); L 252-255 of the revised text.

6. Please indicate the meaning of GET.
Geoscience and Environment Toulouse; revised.

7. Please indicate the units of the green/blue scale in figure 1.
This is depth of the lake, m. Added accordingly.

8. Figure 4 can be misleading as the reader can interpret zero degradation for Dec-May or Oct-May. No data is presented for those months. Please improve the figure.

This figure illustrates not only obtained results, but the entire approach to assess annual degradation. During ice-covered period, no emission of CO₂ could be assessed and hence we considered bio- and photodegradation as nil. Indeed, there was no sampling from December to April and no photodegradation has been studied in July. We added this explanation in the revised figure caption (L 1094-1095).

9. DIC and nutrient data are not discussed.

Good point. Unfortunately, due to small volume of reactor volume (60 to 100 mL), we decided to follow only general (pH, S.C., optical), target (DOC), control (TBC) and highly informative multiple parameters (TE) that did not require large volume of the fluid phase for analyses. Note that only total dissolved phosphorus (P_{tot}) has been measured via ICP MS, but there was no significant evolution of this parameter in the course of incubation, hence it was not presented in Table 2 and not discussed in the text. The same is true for dissolved Si. Measurements of possible DIC production and other nutrient (N) evolution would require specially designed study. We keep it in mind for future experiments and we thank the reviewer for pointing this out.

10. Please include *F* and *p*-values where ANOVA was used.

The ANOVA treatment was not warranted because of too low number of samples. We removed the mentioning of this method from section 2.4.

11. Please provide a plausible explanation for the positive degradation rates in figure 2b during October at Lake Temnoe. In line 527 onwards, it is mentioned that there was not photodegradation. Instead, an increase in DOC concentration is observed. What are the possible mechanisms that could led to an increase in DOC concentration under those experimental conditions?

This is very good point. It is not uncommon that, instead of stable or decreasing DOC concentration in laboratory incubation experiments, the researchers observe an increase in DOC concentration in the course of bio- or photodegradation. Among possible reasons, dark CO₂ fixation and exometabolites release, or the presence of pico-cyanobacteria (that passed through 0.22 µm filter) and produce extracellular DOM under sunlight, can be invoked. This is extensively described in seminal work of J Vonk on unified protocol for DOC degradation experiments. In the present study, following the recommendations of this unified protocol (Vonk et al., 2015), an increase in DOC during incubation has been postulated as zero degradation. We added an explicatory sentence to the revised figure caption (L 1062-1063). As it is stated in Fig. 2 caption, “Positive values signify nil photodegradation (experimental artifacts of DOC production).”

12. Please indicate the analysis that support the statement that water temperature is of secondary importance for DOC biodegradation (*line 491-495*).

There was no statistically significant (at $p < 0.05$) correlation between water temperature and BDOC parameters (overall magnitude and rate). Added to revised text (first paragraph of section 4.1) accordingly.

On a general note, the maximal potential of biodegradation was observed during autumn period when the water temperature for this particular horizon was minimal. If the water

temperature would be a governing factor, maximal effects on BDOC would be seen at highest temperatures, which are most favorable for microbial heterotrophic processing of DOM. From the other hand, experimental modeling of biodegradation during different seasons was performed at constant temperature (20 to 25 °C). In the lake, the water temperature at 5 and 10 m depth is quite similar (5.8 and 4.4 °C). This supports the idea that the differences in DOM biodegradation measured in our experiments at different horizons of Temnoe Lake do not depend on water temperature.

On this note, I wonder why the authors did not carry out a regression analysis to try to explain the main drivers of DOC degradation using, for example, the data presented in Table 1.

This comment is well taken. Due to rather small number of samples and minor variations in various parameters with sometimes substantial uncertainties of replicates, it was not warranted to run robust statistical analysis of regressions.

Nevertheless, in response to this important comment, we performed Pearson correlation treatment at $p < 0.05$, between the percentage of BDOC or PDOC change during incubation and main physio-chemical parameters of the media, including DOM quality via optical ratios (Table R1).

We found only weak positive correlation between effect of photodegradation and $SUVA_{254}$. All other parameters of aqueous solution did not exert any significant relationship with bio- and photo-degradation intensity. The use of other, non-parametric methods of correlation analysis (Spearman, Kendall Tau), yielded similar results.

Table R1. Matrix of Pearson correlations coefficients ($p < 0.05$) of bio and photodegradation yields with main physio-chemical parameters of aqueous solution. Marked (*) correlations are significant at $p < 0.05$.

	T, °C	O ₂	pH	DOC	DIC	SUVA ₂₅₄	E _{254/365}	E _{470/665}	E _{254/436}	E _{365/470}
ΔBDOC %	0.22	0.08	-0.41	0.05	0.07	-0.34	0.00	-0.42	-0.07	-0.20
ΔPDOC%	-0.23	0.00	0.17	-0.03	-0.31	0.60*	-0.15	0.30	-0.11	-0.02

To avoid the confusion of the reader and to save the space (given that the manuscript is already on a long side), we would like not to present and discuss the lack of correlations.

13. Some figures in the supplemental material should be moved to the main text. In particular, those related to DOC degradation.

We agree that it would be nice to have more data on DOM pattern in the main text, notably Figs S1 and S2 (DOC concentration) and Fig S3 (SUVA). However, this would add three additional pages to already long manuscript. We would like to leave this decision at the Editor's discretion.

14. Some error bars are outside the scale limits in figure 6a and 6d.

This is not totally true. In these plots, the bottom error bars of some symbols simply coincide with the X axis. We adjusted the scales in the way to present all data points obtained in this study.

15. The authors assumed that all CO₂ is produced by biodegradation. Is this actually the case? Are there any other sources of CO₂ in the Lake? Please clarify, I think the authors need to present some isotope data to support this statement at the very least.

We totally agree that there are multiple sources of CO₂ in the lake waters, other than DOC bio- or photodegradation, which were assessed in the present study. These additional sources include but not limited to: POC bio- and photodegradation, whose importance can strongly exceed that of DOC (e.g., Attermeyer et al., 2018; Keskitalo et al., 2022), sediment respiration, plankton and periphyton diel photosynthetic cycle, underground water discharge at the lake bottom, and delivery of DOC and CO₂-rich waters via lateral surface and shallow subsurface influx. Given that the contribution of each CO₂ source can vary among different water bodies and across seasons, the assessment of DOM bio- and photodegradation contribution to overall CO₂ flux in the present study should be considered as highly conservative. We alerted the reader about the complexity of C fluxes in the end of section 4.2 of revised Discussion (L 598-607).

Unfortunately, we do not have ¹³C DIC/CO₂ isotope data to constrain the mass balance of organic and inorganic carbon in the lake, and this was clearly outside the objectives of our study.

Please also check the statement in line 646.

Good point. We revised as “in the shallow bog continuum, the sum of bio- and photodegradation were not sufficient to provide CO₂ flux, hence suggesting additional source of CO₂ such as subsurface water influx from peat layers”

16. Please use an alternative word for "destruction" in line 543.

Replaced by ‘degradation’

17. Please standardize units throughout the manuscript (g or mmol of C?)

We agree that the units of CO₂ fluxes should be standardized throughout the manuscript and we retained mmol CO₂ in the revised version.

We thank the reviewer for very insightful and constructive comments.

Responses to Reviewer No 2

This paper studies the competitive effects of microbial and light-induced degradation of DOM and trace element (TE) behavior in boreal rivers and lakes. This study addresses the biogeochemical C and TE cycles in boreal aquatic system, which is important for CO₂ emission. There are some interesting results found in this study, but I do not think this paper is ready to publish in Biogeoscience at this stage.

We thank the reviewer for evaluation of our work. We carefully revised the manuscript following his/her comments and questions and provided detailed answers to all inquiries.

Overall comments:

1. This paper addresses two parts, C and TE dynamics in biological and photochemical processes. In my opinion, in this paper, no obvious connection can be found between these two parts. The authors did not integrate these the C and TE cycle well. The authors emphasize that there is no previous assessment of coupled C-TE biogeochemical cycles. But I did not find the meaningful to put these two cycles in one paper. What is the connection?

We understand the concern of the reviewer. First of all, we have to state that the part on trace elements (TE) partitioning during bio- and photodegradation represents a strong novelty of the present study. The link between DOC and TE is as following: in humic surface waters of peatlands studied in this work, most TE, which include divalent transition metals (Cu, Ni, Co, Zn, Mn) and toxicants (Be, Cr, Cd, Pb), trivalent and tetravalent hydrolysates (Al, Ga, Y, REE, Ti, Zr, Hf, Th), except probably some alkalis and oxyanions, are strongly (> 80%) complexed to DOM (Pokrovsky et al., 2012, 2016). As a result, any DOM transformation processes, be it bio- or photo-degradation, may directly control the concentration pattern of TE. From the other hand, some TE may be photosensitive (Mn, Fe), toxic (Al, Cu, As, Cd, Pb), or potentially limiting micronutrients (Zn, Co, Ni, Mo) for the bacteria and hence affect the temporal pattern of DOC concentration via affecting the overall rate of photo- or bio-degradation. Our working hypothesis was that removal of DOM via photo- or bio-degradation will inevitably change the partitioning of trace elements, which are strongly bound to DOM (such as divalent transition metals, micronutrients and toxicants), or incorporated into organo-mineral (Fe, Al) colloids (such as trivalent and tetravalent hydrolysates). The former might either remain in solution (during photodegradation), hence not modifying their total dissolved concentration, or being taken up by growing bacteria during bio-degradation. Both mechanisms of DOM transformation are capable of co-precipitating with Fe and Al hydroxides hence removing relevant trace elements from the aqueous solution.

Note that the entire section 4.3 of the manuscript is devoted to discussion of the impact of DOM bio- and photo transformation on trace element cycling. In the revised manuscript, this section has been strongly reorganized and restructured around the general principles of trace metal – DOM interaction, following our working hypotheses.

2. The “Abstract” was too long and hard to follow. I am not sure whether Biogeosciences has word limitation for the abstract.

Good point. We shortened our Abstract as following, so it fits the usual format of the journal:

“Studying competitive effects of microbial and light-induced degradation of dissolved organic matter (DOM) is crucially important for understanding the factors controlling aquatic carbon (C) and trace element (TE) transformation in boreal waters. Here we conducted a seasonally-resolved assessment on the degree of DOM and related major and

TE transformation under biotic activity and sunlight using conventional incubations of humic surface waters from the European subarctic: along an ombrotrophic peatbog continuum and in a stratified forest lake from the same region. Along the bog water continuum, biodegradation rate was the highest in subsurface waters collected via piezometer and the lowest in the acidic peatland pool. Photodegradation was similar for piezometrically collected subsurface waters and the stream, but was not detectable in the peatland pool. The waters of the forest lake exhibited a strong seasonal effect of biodegradation, which was the highest in October and the lowest in June. Overall, the biodegradation was capable of removing between 1 and 7 % of initial DOC, being the highest in the forest lake in October and in peatland pool in summer. The photolysis was capable of degrading a much higher proportion of the initial DOC (10-25 %), especially in the forest lake during June and the bog stream during July. Only a few trace metals were sizably affected by both photo- and biodegradation of DOM (Fe, Al, Ti, Nb and light REE), whereas V, Mn, Co, Cu and Ba were affected solely by biodegradation. A likely mechanism of TE removal was their coprecipitation with coagulating Fe(III) hydroxides. Compared to typical CO₂ emissions from inland waters of the region, biodegradation of DOM can provide the totality of C-CO₂ evasion from lake water surfaces whereas bio- and photodegradation are not sufficient to explain the observed fluxes in bog water continuum. Overall, these results demonstrated strong spatial and seasonal variability in bio- and photodegradation of DOM and organic TE complexes, and call for the need of a systematic assessment of both processes across seasons with high spatial resolution.”

3. P12: What methods you have used for microbial cell counting? At least give the name of the methods, only give the reference is not enough.

Good point; the method is described as following:

Active bacteria number count (colony forming units, CFU mL⁻¹) was performed using Petri dishes inoculation (0.1 to 1.0 mL of lake water in three replicates) performed in a laminar hood box immediately prior the experimental incubation start and upon each sampling. Samples in duplicates were inoculated on Nutrient Agar (5 g L⁻¹ beef extract, 5 g L⁻¹ gelatine peptone, 15 g L⁻¹ bacteriological agar, pH=6.8±0.2 at 25 °C) to determine the total number of heterotrophic bacteria. Difco® agar (granulated powder, Lot No 6290083) inoculation was used to assess the number of oligotrophic bacteria. Inoculation of blanks was routinely performed to assure the absence of contamination from external environments. We added necessary details to the revised manuscript (L 303-311).

4. Figure 1: no longitude and latitude in the map.

This is schematic map where only the general boundaries of NW Europe are shown. Detailed position of sampling point is provided in Table 1 as following:

	Piezometer	Lake Severnoe (peatland pool)	Stream Chernyi
GPS coordinates	N64.328694° E40.612556°	N64.334361° E40.609667°	N64.330982° E40.653352°

Lake Temnoe: N64.47683° E041.74533°

5. The language of this paper is not easy to understand, please revise. Some sentences are too long. For instance, P23 Line 568 “Assuming the entire water column studied in this work (10 m depth of Lake Temnoe) participates in DOM biodegradation and CO₂ emission, the integral flux amounts to 0.2-0.4 g C-CO₂ m⁻² d⁻¹ across the seasons”.

We corrected the language in main occasions in the manuscripts. Please note that the text received full proofread by an English speaking scientist (Chris Benker).

Furthermore, if the manuscript is accepted, the APC will cover thorough English proofread. We do agree that the above given sentence is poorly constrained and we revised it as following:

“Therefore, integral flux from 10 m deep water layer amounts to 17 – 33 mmol CO₂ m⁻² d⁻¹ across the seasons”

6. There is a input error in P27 Line 661, The ar should be are.

Thanks for catching this; we fixed this misprint accordingly.

We thank the reviewer for very insightful and constructive comments.