

Response to Reviewer No 1

This reviewer noted that the manuscript “needs a bit of work and clarification to reassure readers that these results are not anomalous”.

The first major issue raised by this reviewer is the following «One of the core issues partly addressed by this ms is that experimental treatments for DOM incubations are designed to isolate specific processes to answer the questions at hand, but of course in the natural environment these processes don't operate independently. A lot of controlled laboratory studies have shown that in stream processing of DOM is important, but it is not well demonstrated how this actually occurs in the field given the often unrealistic conditions of incubation (e.g. doi:10.5194/bg-15-7141-2018).» We thank the reviewer for pointing out this important reference. Consistent with recent work of Dean et al (2018), in our work, we used several filter sizes to prepare the inoculum for biodegradation (0.8 µm, 3 µm), essentially to avoid the artifacts linked to unrealistic incubation.

His/her further issue is that “Biodegradation doesn't occur entirely in the absence of photo-oxidation, priming from fresh plant/soil leachates, or the full breadth of bacterial and viral community dynamics. The separation of these processes may to some degree explain the results presented here, and this should be addressed more fully in the discussion.”

We are confident that in this study we did separate bio- and photo-degradation. First, our biodegradation assays followed the standardized protocol for assessing biodegradable DOC in Arctic waters (Vonk et al., 2015). Second, all incubation were run in the absence of light, in bottles wrapped in Al foil, in the dark. For photodegradation, sterile filtered (< 0.22 µm) water was used and this is the only suitable method for biodegradation assays. However, we do agree with the reviewer and seminal papers of R. Cory et al that biodegradation can be enhanced in previously photodegraded samples, and the separation of two processes in natural settings is not possible. Yet, the present study is purely experimental, aimed at separating bio- and photodegradation in the laboratory.

Another issue with this study is the uncertainty. In the methods, the authors present uncertainty values which are roughly equal to the degree of degradation observed in the current study. It is not clear how these uncertainties were calculated and propagated through the results, and therefore whether these rather high uncertainties explain the limited response of the incubations.

This is very good point. The response is below 10%, and this is the main result of the present study. We present true uncertainties and 5-10% is the best what an experiment can provide. It is important to distinguish these 5-10% of experimental uncertainties from 1-2% of analytical uncertainties. Below we present detailed explanation of the uncertainties and we added this part to revised version (section 2.3, L 281-295):

To assess the variability of results, shown as vertical uncertainties in the graphs, we used the percentage ratio of standard deviation of n replicates at the i -th day of exposure to the initial DOC concentration following:

$$SD_i = \sqrt{((BDOC_i^1 - BDOC_i^{mean})^2 + (BDOC_i^2 - (BDOC_i^{mean})^2 + \dots + (BDOC_i^n - BDOC_i^{mean})^2)/n)}$$

$$\%SD = (SD_i/DOC_0) \times 100$$

The results were presented as %BOD_i± SD_i

To assess the uncertainties during photodegradation experiments, we used the percentage of standard deviation on n replicates at the i -th day of exposure to the DOC concentration in the dark (blank control) reactors as

$$PDOC_i^n = DOC_i^{blank} - DOC_i^n$$

$$PDOC_i^{mean} = (PDOC_i^1 + PDOC_i^2 + \dots + PDOC_i^n) / n$$

$$\%PDOC_i = (PDOC_i^{mean} / DOC_i^{blank}) \times 100$$

$$SD_i = \sqrt{((PDOC_i^1 - PDOC_i^{mean})^2 + (PDOC_i^2 - PDOC_i^{mean})^2 + \dots + (PDOC_i^n - PDOC_i^{mean})^2) / n}$$

$$\%SD = (SD_i / DOC_i^{blank}) \times 100$$

The results were presented as $\%PDOC_i \pm SD_i$

In full agreement with Vonk et al. (2015), the negative values of %BOD or %PDOC were assigned to zero.

Further, this reviewer stated that “this study stresses how different the study site is to previous studies which have shown degradation of DOM in the water column to be an important contribution to CO₂ fluxes. A more robust comparison than the general terms currently used would be very useful to more clearly explain the site differences and enhance the discussion on why these might cause such divergent results.”

We believe that relating DOM degradation and CO₂ emission fluxes across the Arctic was beyond the scope of this work. Note that currently we are preparing a report on CO₂ concentration and emission fluxes from inland waters of Bolshezemelskaya tundra, performed over a wide range of season and geographical coverage. Further, there is an excellent review summarizing most available studies (Vonk et al., 2015) and we related to this when discussing the broad significance of our results. Nevertheless, in the revised manuscript, we reorganized and extended our discussion as recommended by reviewer.

The reviewer also stated “Regardless, these are very interesting results that should be of great interest to the community. It will be interesting to see if other studies find similar results beyond the somewhat limited spatial coverage of Arctic inland water DOM degradation sites to date.” We thank the reviewer for pointing this out. There is a clear need of implementing standardized procedure of bio- and photo-degradable DOC assessment across the Arctic. Currently, we are working on bringing together the BDOC measurements in frozen peatland waters, from N Sweden (Abisko) to western Siberia.

Reply to Specific Comments of Reviewer No 1:

L44. 10% is generally measurable, is it not? See later comments on uncertainty. The term “measurable” is not suitable here; we changed it to “within the experimental resolution”. Please distinguish here the analytical uncertainty (which is 1-2%) and experimental reproducibility (which is rarely below 5-10%).

L61-64. Needs to be clear this conclusion is only for high latitude systems, Vonk et al 2015 didn't look at systems outside of the permafrost zone.

This is not totally correct. Vonk et al demonstrated zero BDOC loss in aquatic systems without permafrost.

L78. These studies aren't from mountain regions. They are tundra ecosystems with peat soils overlying the mineral substrate on the Alaskan north slope. See comments below on characterizing the study sites of previous work. We thank the reviewer for pointing this out and modified the text accordingly (L73-74).

L86. A key point here is not that photo-oxidation will convert much DOC directly to CO₂, but that it can transform DOM molecular structures into more (or sometimes less) biolabile forms (see Cory and Kling 2018). The interaction between photo- and bio-degradation is an important aspect, not just the individual processes themselves. The combination of the two processes is not explored by this experimental design, and so cannot be ruled out.

We totally agree about the importance of synergy between photo- and bio-degradation and added relevant sentence in the text as recommended (L88-91). However, it was beyond the scope of this work to study the combination of both.

L87. I wouldn't say they are controversies, more that there is an emerging paradigm which may not be as consistent across the Arctic as previously thought. We agree and revised the text accordingly (L92-93)

L96. Citation needed. Added « Tarnocai et al., 2009; Raudina et al., 2018 »

L103-104. I think you are generalising too broadly here. The Mann et al. 2015 study was conducted in the yedoma region on the eastern Siberian Arctic where drainage flows through upper peat layers as well as frozen yedoma soils.

We revised that “Numerous experiments in permafrost-bearing and permafrost-free aquatic environments including both organic and mineral soil substrates relatively poor in DOC demonstrated...”

L107. Photo-oxidation and biodegradation are important components of peatlands also (e.g. doi:10.5194/bg-14-1793-2017; doi:10.1016/j.jhydrol.2013.03.016; doi:10.1029/2018JG004650).

We thank the reviewer for pointing out these important references and we added their proper citations in revised text (L 111-113). Note however that all three studies were performed in temperate (Scotland, UK) peat mires, certainly different in environmental context from frozen peatlands in NE Europe of this work.

L122-125. This does not belong in the introduction. We removed this sentence from Introduction.

L169. "All" - Fixed

L180. Add citation to support this (e.g. doi:10.5194/bg-15-7141-2018). We thank the reviewer for pointing this out; added respectively.

L183. Did you measure DOC concentrations of 3µm filtrate?

Yes, of course. The DOC concentration was similar within $\pm 2\%$ between 3 μm , 0.7 μm and 0.2 μm poresize (L320-323, section 3.1). We are aware that some studies reported notable differences for poresizes, but this depends on environmental context (such as phytoplankton bloom) which was not the case in waters investigated in our work.

L185. *There is not a lot of carbon in the 0.22 um to 0.7 um size range (see discussion in doi:10.5194/bg-15-7141-2018 and references therein).*

We agree and added this reference to revised text.

L206-209. *Which samples got which treatment? I would have thought a consistent treatment of all samples would make more sense, so please justify and clearly explain each incubation process in more detail.*

All photo-degradation samples received one single treatment: 0.2 µm-sterile filtration and sunlight exposure in quartz reactors in the outdoor pool as stated in L 223-229.

L210. *So they were all incubated in an outdoor pool? This makes the previous sentence confusing as to why it was written that way.*

Yes, this is most conventional methodology. All manipulations for setting up the incubation and sampling at each time interval were performed in laminar hood box to insure minimal external bacterial contamination. We reorganized the presentation of photo-degradation method for clarity.

L211. *So the headspace was a closed system, i.e. no O₂ was able enter nor CO₂ exit the incubation vessels? Is this standard protocol? Can you justify whether this method would prevent O₂ limitation from slowing photo-oxidation?*

There was no O₂ limitation because, as it is stated in L338-339 of submitted manuscript, the exposed water remained oxygenated.

L237. *This is not a common method for measuring dissolved CO₂ in aquatic systems. How long was the probe submerged for? How did you ensure it was in equilibrium with the water being measured? Did you measure replicates, or attempt to constrain local variability in your measurements?*

This is new and highly reliable method. Typically, the probe was submerged for half an hour to ensure the equilibrium with water, and the measurements were done in several spots of the water body thus providing necessary replicates. We added references to recent papers of our group (Serikova et al., 2018, 2019) that discuss methodological aspects.

L241-242. *Add a sentence here to explain why you selected these wavelengths and what they can tell the reader about DOM structure. Later you mention E4:E6 ratios as well, introduce them here.*

The specific UV-absorbency (SUVA₂₈₀, L mg⁻¹ m⁻¹) is used as a proxy for aromatic C, molecular weight and source of DOM (Weishaar et al., 2003; Ilina et al., 2014 and references therein), added to L 251-253. We also introduced the E4:E6 ratios and explained them here (section 2.3).

L273. *How were these uncertainties calculated? This becomes very important because in your results you do not present any changes that greatly exceed these uncertainties, and in the abstract 10% is highlighted as below detectable - these limits need to be carefully and quantitatively justified in order to support the main conclusions of this study.*

We added a big deal of information on uncertainties calculation in section 2.3, L 281-295 as requested.

L279. *Which statistical package did you use for these analyses?*

All calculations were performed in STATISTICA ver. 10 (StatSoft Inc.,Tulsa) at p = 0.05), added to L307-308.

L309. This is not well worded. The inability to detect this change was due to the instrument limitations, not the high DOC content. We thank the reviewer for pointing this out and revised this part of the text as "...due to instrument limitation (the intrinsic uncertainty on NPOC analyses (ca. 2% of 15 mg/L of DOC)...", L336-337

L338. This data should be presented for transparency, but is fine to go in the supplement - same goes for the EC data, DIC and O2 data. We added the raw data (Table S1) in the revised version.

L362 and 366. Again, why are these not presented somewhere, even if in the supplement? This does not help transparency of the study methods. The concentration of all pertinent trace elements were added to supplementary table, consistent with the policy of the journal.

L378-379. Yes, but according to your uncertainties, these values would also mostly lie outside of the detectable limit.

We cannot judge statistical significance of the differences between the present study and other works. Vonk et al reported 3 to 18% (mean 13%) in continuous permafrost sites and 5 to 15% (mean 14%) in discontinuous permafrost sites on mineral soils. Our study adds another important and representative site to this list, where the BDOC ranges from 0 to 10%.

L381. It feels like this point is being over sold, and that the differences between the study site and the previous work in high latitude permafrost systems are not that great, and what differences there are currently are not well constrained. I would recommend that the authors develop a framework to present these inter-site differences, for example a table with soil C-content, soil depths, climate, elevation etc. This would not be a big effort, and would much more strongly back up the claim that these sites are so different.

Such a review goes a bit above the scope of this work. The compilation is available in Vonk et al (Table S1 of their article), so we do not see any interest of reporting it again. Moreover, we believe that the main factor controlling BDOC and pCO₂ pattern in surface waters is not C soil, climate and vegetation per se but the pathway of soil DOC delivery to the surface reservoirs, via suprapermafrost flow (Raudina et al., 2018). Depending whether the suprapermafrost flow passes through mineral or organic horizons and the residence time of water in depressions and lakes, the bio- and photodegradable DOC will be dramatically different. At present, this soil hydrological information is not available for most of sites used in BDOC assessment and as such a straightforward comparison is not warranted.

L389. 0-1% does not equal the 0-10% seen in this study. Maybe this is just an artifact of the way you present these values, i.e. were the majority of the BDOC values closer to 0 than to 10%? Maybe rethink how you present this number when contextualizing.

The value in Vonk et al (2015) is between 0 and 1%. The experimental reproducibility is between 5 and 10%. Indeed, the majority of our BDOC values were closer to 0 than to 10% - but this is still within 5-10% of experimental uncertainty. Such an uncertainty is merely inevitable, because we deal with the difference of two large values (high DOC concentration in humic waters). In oligotrophic low-DOC waters, the analytical and experimental resolution would allow for much higher precision of delta DOC measurements.

L406. This isn't well explained - there isn't any clear impact of priming here because to investigate priming, an interaction between the fresh vegetation/soil leachate and the recalcitrant organic matter pool has to occur in the experimental design. It's also not clear what direct relevance the priming question is to the current study design. This is pertinent comment. We removed the discussion of priming effect from revised version.

L428. This is also partially supported by the findings of doi:10.5194/bg-15-7141-2018, and it may also be worth noting that bacterial communities are not just shaped by size fractionation by filtration, but also the presence or absence of bacterial grazers. This is totally correct, and we happily added this reference to revised text (L 457-458)

L438. While I can see the interest in including a 37°C treatment to help answer the question of degradability, it cannot be argued that surface waters in the Arctic would ever be expected to reach those temperatures. Consistent 23°C in surface waters is already unlikely in that part of the world. This doesn't change the point presented here, but I think it's important to not misrepresent the experimental design.

We only partially agree with this. Russian Arctic inland waters are really different from other regions of the world, and we encountered water temperatures around 25-26°C in Bolshezemelskaya Tundra in July 2015 (in preparation), and thermokarst lakes of western Siberia discontinuous permafrost zone had water temperatures around 27-29°C in July 2012 (Pokrovsky et al., 2013, Biogeosciences). As stated in Table 1 of this manuscript, the actual measured water temperatures during sampling were 24-25°C in the depression, small stream and thermokarst lake.

L472. See my comments above for L381, but that said the discussion here is strong and well supported

We thank the reviewer for pointing this out, but the comparison between sites is not trivial. The key issue is how the soil and plant litter DOM are delivered to lakes and rivers via supra-permafrost waters. If these waters drain through mineral or organic surface soil horizons, this will determine the difference between sites. Such information on the exact position of active layer within a mineral or organic soils is rarely available except several case studies (i.e., Raudina et al., 2017 Biogeosciences; Raudina et al., 2018 Sci. Total Env.). Today, without hydrological model of water objects (and they are rarely available for BDOC studies), straightforward comparison between sites considering just general soil and climate context is not warranted.

L478. "hours - Fixed.

L487. This proposed mechanism requires the water column to be well mixed, with no photo-degradable DOM present in water deeper than 0.5m. Is this reasonable at the study site? Is this the key difference between this site and the other cited in the literature? Again, this could be addressed with a more in depth exploration of the site differences (see comment re. L381). The depth of majority of thermokarst lakes is < 0.5 m. Yes, the water column of lakes and rivers in BZT are well mixed and well oxygenated.

L517. How does "sizeable" compare to other studies?

Sizeable here means 20 to 50%. A comparison with other studies seems not feasible because, to our knowledge, this is the only study where P, Fe and trace metals were monitored during photolysis of humic waters from permafrost zone.

L558. Based on the discussion above, residence time in the soil sounds like the most important control, given that the authors argue that degradation in the soil means almost no degradable DOM is entering the aquatic systems in the study area.

Yes, we hypothesize that water residence time in soil exerts primary control on bio- and photo-degradability. However, it is possible that the degradation happens very fast, once the soil fluids enter the open waters (as actually indicated by this reviewer in his/her comment to L 538). In that case, it is the balance between half-life time of soil BDOC (minutes to hours) and water residence time in surface reservoirs (days to weeks), which determines the biodegradability potential of DOM in sampled waters.

Fig S1. The upper panel does not give a good regional context of where the site is. I suggest presenting this location in the context of the whole Arctic.

We would like to note that our map encompasses huge part of the Arctic, from 35 to 75°E. The studied territory is between the Scandinavia and Yamal Peninsula and its position is clearly indicated by rectangle. Following the recommendation, we extended this map in revised version.

We thank reviewer No 1 for very insightful and constructive comments.

Response to Reviewer No 2.

We thank the reviewer for acknowledging that “the experimental design for the photodegradation experiments was logic and reproducible.”

The reviewer issued the following critical comment: “*For the microbial degradation experiments I had a few reservations/concerns. In many studies investigating the degradability of DOM people have used an inoculum to jump start the incubation as well as nutrient additions in order to exclude nutrient limitation as the dominant factor controlling DOM decomposition. The question is was the bacterial population reduced too much and nutrient limited, artificially reducing the degree of degradation relative to natural conditions. Because the authors have data on DAPI counts of bacterial cell numbers they should present the actual cell numbers they found in the experiments and compare that to the number of bacteria found in the natural environment before filtration. Total counts are more informative than CFU in this case.*”

Therefore, the reviewer suggested present the actual cell numbers in the experiment and compare them to the number of bacteria in the natural experiment before filtration. This is valuable proposition. Such data are indeed available as shown in table R1 below. The total counts in natural environments are within a factor of 2 to 3 similar to the values measured in the experiment. As such we argue that the bacterial population is not at all reduced compared to natural settings. We added the results of DAPI counts in revised Table 1 of the manuscript, and also extended presentation of bacterial counts in Fig. S3 via adding DAPI counts for all experiments.

Table R1. Total bacterial count in the sampled waters prior the experiment.

Sample I.D.	Description	TBC*10 ⁶ , cell ml ⁻¹	Cocci, %	Rods, %
BZ-2-17 67°36'48,8"N, 53°54'29,8"E	Depression in peatbog, $S_{\text{area}} = 7.5$ m ²	0.81	89.1	10.9
BZ-24-17 , 67°36.53'N 53°50.26'E	Stream in frozen peatland, $S_{\text{watershed}} =$ 7.5 km ²	5.72	76.5	23.5
BZ-12 67°36'47,7"N 53°54'38,5"E	Thermokarst lake (Isino), $S_{\text{area}} = 0.005$ km ²	5.36	92.2	7.8
P5 67°40'09,4" 52°39'30,8"	r. Pechora, $S_{\text{watershed}} =$ 322,000 km ²	3.51	84.3	23.5

Further, as it is stated in the text (section 4.1), “the total bacterial number in studied surface waters $(0.5-5) \times 10^6$ cell mL⁻¹ is in excellent agreement with other studies of thermokarst peatland lake waters (Deshpande et al., 2016).” This further confirms the representability of laboratory experiments to actual natural waters of peatland environments.

The reviewer also suggested that we present the concentrations of inorganic nutrients like phosphate, nitrate and ammonia.

A relevant table R2 represents the data, now included in revised Table 1 of the manuscript. The concentrations of nutrients are generally low because the peat is essentially oligotrophic and contains very little nutrients. Together with refractory nature of OM, it could be a cause of low biodegradability of DOM, but this is typical condition for large territory of frozen peatlands in Northern Eurasia.

Table R2. Inorganic nutrient concentrations in sampled waters.

Sample I.D.	Description	PO ₄ , µg P/L	P _{tot} , µg P/L	NO ₂ , µg N/L	NO ₃ , µg N/L	NH ₄ , µg N/L	N _{tot} , µg N/L	Si, µg/L
BZ-2-17 67°36'48,8"N, 53°54'29,8"E	Depression in peatbog, S _{area} = 7.5 m ²	2,28	14,6	14,6	14,6	13	228	22,0
BZ-24-17 , 67°36.53'N 53°50.26'E	Stream in frozen peatland, S _{watershed} = 7.5 km ²	9,8	-	5,0	-	152	-	392
BZ-12 67°36'47,7"N 53°54'38,5"E	Thermokarst lake (Isino), S _{area} = 0.005 km ²	4,42	7,3	3,6	76,6	117	200	100,0
P5 67°40'09,4" 52°39'30,8"	r. Pechora, S _{watershed} = 322,000 km ²	26,7	37,52	1,67	111,2	36,5	438	2689

The third argument of this reviewer is that “The major weakness of the current manuscript is the fact that all experiments were performed in one season only. The late summer has been shown to be a low activity period for these aquatic systems and many studies about DOM degradation have been published for this time period.” We would like to point out that July in these regions is not at all the late summer but the middle summer. We did perform extensive field measurements during early summer (spring) and autumn but discussing and presenting these results go above the scope of this paper. The present study matches the period used by other researchers for biodegradation assays, and we stated this in revised text (L 134-135, section 2.1). We agree that seasonal aspect should be reflected in revised version of the paper, and that comparison with other studies should be done bearing in mind this seasonal context. Following the reviewer’s remark, we alerted the reader that the lack of seasonally-based data set does not allow sufficient representability of results for the pan Arctic boreal environment (section 4.3, L 561-563).

We took into account all minor issues noted by this reviewer: we revised the map showing the sampling/experimental sites and better referenced this map in the text (L150-151, section 2.1), corrected the misprints (found, hours). We thank the reviewer for insightful comments.

Response to comment of Dr Laurion:

We totally agree that, in order to challenge the paradigm, we have to test both processes (photo- and bio-degradation) at the same time. The present paper was designed to rigorously evaluate them independently, which represents only a first step in this direction. We are aware of the importance of photo-produced organic ligands. In fact, low molecular weight organic carbon production upon sunlight exposure of surface waters is well known since pioneering works of Zafiriou et al. (EST, 18(12), 358A-371A, 1984) and confirmed over past decade in the Arctic waters (Cory et al., 2007, 2014). In our experiments on humic waters from permafrost-free zone of NW Russia, these photochemically produced organic ligands were <1 kDa as followed from the increase of LMW< 1 kDa concentration of organic carbon in quartz reactors in the course of sunlight irradiation (Oleinikova et al., 2017, GCA, 211, 97-114)). For example, over 10 days of solar irradiation, the bog water from N. Karelia produced a 2-fold increase in concentration of low molecular aliphatic acids (acetic, formic, oxalic and citric) and benzol-carbonic acids. This increase (ca. 0.2 mg/L of acids), however, represented less than 10% of overall DOC increase in the LMW< 1 kDa fraction, which itself represents < 30% of all DOC. As such, we believe that the overall photoproduction of biolabile organic ligands does not exceed a few percent of the initial DOC and as such cannot account for more than 5% of bio-degradation. This is consistent with maximum 5 ± 5 % of bio and photo-degradable DOC reported in the present work.

Your comment on dark DOM chemical oxidation that can be important in iron-rich organic-rich waters facing redox oscillation is well taken. Concerning Bolshezemelskaya Tundra environments, we do not expect sizeable redox oscillations in aquatic systems, be it large Pechora River or shallow (< 0.5 m) oxygenated thaw pond. We acknowledged anaerobic C mineralization in thermokarst lake sediments (L547-549 of first version) as a possible mechanisms of CO₂ production and added a few lines on dark DOM oxidation together with pertinent citation of Page et al (L583-586 of revised text).

You stated the importance of delay between sampling and experiment, and we seriously took this issue into account during design of our experiments. This time was minimized to several hours employing fastest transfer of refrigerated samples to the laboratory. Note that the DOC concentration at the moment of sampling and at the beginning of experiments was identical thus suggesting the lack of transformations.

The issue of DIC evolution is important. However we were not able to detect any change in DIC concentration in the course of experiment, i.e., within 5% of analytical and experimental uncertainty. We presented the DIC data in supplementary information of revised paper (Table S1).

Finally, the comment on total bacteria count (DAPI method) is pertinent, and we also addressed it in our response to Reviewer No 2. The TBC increased over the course of experiment as illustrated in revised Fig. S3 of the supplement.

We thank I. Laurion for very pertinent comments.