

## ***Interactive comment on “Reproducible determination of dissolved organic matter photosensitivity” by Alec W. Armstrong et al.***

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We appreciate the thoughtful comments from the referee. The most pressing issue presented in this review is how to justify the effort involved. Our main goal was to compare the sensitivity of different DOM sources to photochemical alteration to better understand potential influences on DOM composition in natural waters. There are many approaches to characterizing the optical properties different DOM sources and an established tradition of photochemical experimentation on DOM. However, DOM in natural waters is subject to many potential transformation processes that may compete and interact on different temporal scales, so reliable comparison of degradation kinetics seemed like the most promising approach to characterize photosensitivity under environmentally relevant photon exposures. For example, changes to DOM composi-

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tion that slowly over the equivalent of several days of photodegradation may be less important than changes occurring in a few hours in an environment where water is moving or mixing and subject to other processes like microbial transformations. Much of the current manuscript is devoted to the methodological considerations we encountered in our effort to reliably compare degradation kinetics, many of which are also relevant to other approaches used to study DOM photodegradation and thus broadly interesting to this community. Our specific research findings were that wetland DOM photo-sensitivity varied in space and time with potential implications for understanding DOM composition in this setting generally, and that our method identified particular aspects of photodegradation kinetics that might represent links between DOM composition, environmental phenomena, and photodegradation. This represents an initial foray into understanding the role of photodegradation in shaping DOM composition in our study area, and we hope it illustrates aspects of DOM photodegradation that may be applicable elsewhere and interest the broader research community.

The referee raised several methodological questions. We acidified our samples to pH 3 because we had to control pH - pH changes produce fluorescence changes that confound the fluorescence decay due to photodegradation reactions under investigation. We did not wish to use chemical buffers, as some of these may also interfere with fluorescence and may have unknown effects on degradation kinetics. Active control of pH during experiments using autotitration is another option we did not use because the electrode contaminated samples during prolonged irradiations. Therefore, we needed to be sure that pH would be consistent between samples and during photodegradation of each sample. Starting samples at pH 3 allowed the full protonation of most organic acids (Ritchie and Perdue, 2003), which should prevent solution pH change due to the photoproduction of CO<sub>2</sub>. We recognize that this itself presents an obstacle to reproducibility or comparison with other studies, as kinetics are greatly affected by pH, but this seemed the most reliable way to avoid pH change during photodegradation to allow comparisons between our samples. Another methodological question involved filtration choices. We appreciate the careful attention of the reviewer here,

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which helped us discover that we inadvertently failed to note an important step in our methods – all samples, whether raw water or solid-phase extracts redissolved in water, were filtered through syringe-mounted 0.2  $\mu\text{m}$  cellulose acetate filters that were pre-rinsed with > 30 mL ultrapure C-free water. GFF filters were used as a pre-filter immediately after samples were returned to the lab, before either short-term storage or solid-phase extraction. We apologize for the omission and ensuing confusion. Additionally, as mentioned to reviewer 1, because 0.7  $\mu\text{m}$  filters were combusted, their true pore size is probably smaller than 0.7  $\mu\text{m}$  and likely be comparable 0.2  $\mu\text{m}$  filters (Nayar and Chou, 2003).

The referee's methodological questions included several about comparability of our results to natural, un-processed DOM. These included questions about effects of extraction, the means used during evaporation of methanol extracts, details on the experiment showing effects of storage, use of freeze-dried IHSS standards, and questions about the collection and initial characterization of natural DOM sources. A few of these questions were addressed in the text The methanol extracts were evaporated in an open vial under a fume hood under a stream of high-purity N<sub>2</sub> gas – the gas flow created turbulence in methanol, facilitating its evaporation. We used IHSS SRNOM standard as the DOM source in many of our experiments because it has been extensively studied and provides a benchmark against which other photodegradation kinetics experiments can compare results. Reconstituted freeze-dried SRNOM and its filtered source water showed very similar molar absorptivity at 280 nm and fluorescence index values when collected (Kuhn et al., 2014). While this may not translate to identical kinetics of photodegradation, it suggests optical properties are not radically transformed during freeze-drying and reconstitution. We believe the advantages of using a standardized material in wide circulation outweigh any artifact of freeze-drying. The experiment showing the effects of storage on photodegradation kinetics was conducted according to different protocols than following experiments – samples had higher DOC concentrations, and a different flow cell with shorter path length was used. This experiment was the first conducted, chronologically, of those presented

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here, and its failures of replication motivated further work and helped us develop our protocols. We apologize if introducing these protocols sowed confusion, and will edit the manuscript to ensure it is clear which protocols applied to which experiments. The referee also asked whether EEMs and other measures were available for comparison between the solid-phase extracts used throughout the paper and their original raw water DOM sources. While these are not available (at least in comparable form) for many of the samples used to show the range of variability across a wide gradient of DOM sources (e.g. the stream, coastal ocean, and Sargassum sp.), we did collect EEMs and absorbance spectra for the “large” and “small” wetlands sampled in different seasons at the time of collection. We will revise the manuscript to include comparisons between source water EEMs and extract EEMs where possible.

The referee ended their specific comments by requesting “some kind of discussion. . .on how comparable the results [from extracts studied in the lab] will be to what may happen in situ.” We appreciate the utility of this kind of information – inference on the processes at work in the natural setting is the original motivation for our entire effort. While we were able to determine that extracts behaved differently than raw water (demonstrated with SRNOM), we cannot identify the mechanistic basis of these differences with the current evidence. The difference between in situ conditions and our lab results might be useful to expound further in a future manuscript, however, this is not a trivial task. For instance, we used solid phase extracts to minimize matrix effects and controlled pH to look at differences in photochemistry between DOM samples and SRNOM. Cations and in particular magnesium, may actually enhance DOM fluorescence signals (Stichak et al. 2019) and pH also influences sample optical properties, as mentioned previously. Metals like iron are known to enhance absorbance measurements and potentially quench fluorescence (Poulin et al. 2014). Halides may also enhance photodegradation rates (Grebel et al. 2009). These potential matrix effects (see Li and Hur, 2017 for a detailed discussion) make observed differences in whole water optical properties difficult to interpret. SPE allows for comparisons with minimal or entirely without potential interferences and matrix effects. While sample

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matrix is indeed different in situ, our method may best be described as measuring potential photodegradation of DOM itself, which is why we describe it as photosensitivity. One of our key results shows the utility of our method in this regard – we are able to measure the relative differences in fast degradation processes (described by  $f_L$  and  $k_L$ ), which may be more environmentally relevant than the slower processes in the context of constantly changing DOM subject to a panoply of other transformation processes and constantly in motion. We thank the referee for asking for this kind of discussion, and will revise the manuscript to make the relationships between our lab approach and in situ inferences more clear.

The referee's final question was about Figure 9. There is an error in this figure that may have produced additional confusion – the left plot is of PARAFAC component 3 while the right plot is of PARAFAC component 4. We will correct this in the manuscript. The referee's question concerned the provenance of the multiple curves shown for the wetland samples. These plots show the full data available for all different DOM sources subjected to the final protocol. This includes the three replicates of extracts from the same "small wetland" DOM source and two trials with extracts from "small wetland" DOM collected at different times, three trials with extracts from "large wetland" DOM collected at different times, and the three replicates of SRNOM extracts. As described in our response to RC1, we recognize the need to clarify the different dimensions of these multiple trials for each site/source, especially in Figure 10 and Table 2. It may be useful to further clarify here as well, though as this figure shows rawer data (PARAFAC model component fluorescence change over time) rather than biexponential model parameters for each curve shown here, this may be less pressing for this figure.

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