

## ***Interactive comment on “Fast-freezing with liquid nitrogen preserves bulk dissolved organic matter concentrations, but not its composition” by Lisa Thieme et al.***

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We thank referee #2 for the constructive comments. Please find below our response

1. In section “Abstract”, the last sentence highlight important findings “We recommend fast-freezing with liquid nitrogen for preservation of bulk DOC concentrations of samples from terrestrial sources, whereas immediate measuring is preferable to preserve spectroscopic properties of DOM.” However, the last part of the sentence was also suggested by the study of Santos et al. (2010) for bulk deposition samples (rainwater samples), which show that such study should be used in the discussion of the present manuscript.

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We will consider and cite Santos et al (2010) in the revised version of our manuscript (see point 3 and 7).

2. In section “1 Introduction”, page 2, reformulate the sentence “In addition to cDOM in samples from aqueous systems, water-extractable soil organic matter and cDOM in soil pore water samples (Otero et al., 2007; Hur et al., 2014; Traversa et al., 2014) were investigated using EEMs plus PARAFAC as well as isolated humic substances from soil and litter (Kalbitz et al., 1999; D’Orazio et al., 2014).” The study of Otero et al. (2007) did not used the EEMs plus PARAFAC as well as isolated humic substances from soil and litter.

Thank you for the hint. We will reorganized the introduction and rephrase the sentence into: “Spectroscopic methods like UV-vis absorption and fluorescence spectroscopy used as single excitation/emission scans, synchronous scans and excitation-emission matrices (EEMs) in combination with different indices and/or parallel factor analysis (PARAFAC) are increasingly applied to characterize chromophoric dissolved organic matter (cDOM) in various environments (e.g., Murphy et al., 2008; Yamashita et al., 2010; Stedmon and Markager, 2005; Graeber et al., 2012; Otero et al., 2007, Traversa et al., 2014, Kalbitz et al., 1999).”

3. In section “1 Introduction”, page 3, I suggest to add also the reference of Santos et al. (2010) to the following sentence “For these reasons it is recommended to directly filter samples after collection and store them in the cold and dark prior to measurement as soon as possible (Spencer and Coble, 2014)”.

We strongly agree with the reviewer on the importance of immediate filtration, as well as cold and dark storage. In our experiment samples were immediately filtered and stored cold in the dark. The reference Santos et al. (2010) will be added in the respective sentence in front of “Spencer and Coble, 2014”.

4. In section “2 Material and methods”, subsection “2.2 Sampling and sample preparation”, page 4, the first and fourth sentences seems to be contradictory, because it is

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presented that samples were collected on 17 and 18 June 2014, and then is presented that bottles were biweekly used. Please, clarify.

The samples for the cDOM storage experiment described in this manuscript were taken within a biweekly sampling routine of above and belowground water samples. It takes two days to collect samples from all research sites. Therefore we state in the Materials and Methods section that samples were taken on the 17th and 18th of June. For the in-field sample collection we use the same PE bottles for the same sample every 14 days. We will rephrase the respective paragraph into: "For the experiments, we collected solution samples from five forest and three grassland plots on 17 and 18 June 2014 within a bi-weekly 2 day sampling routine of above and below-ground water samples in the DFG priority program "Biodiversity Exploratories". Together we collected 27 samples for the freezing experiment including six throughfall (TF), five stemflow (SF), five forest litter leachate (LL) as well as six top- and five subsoil solution samples. Volume-weighted composite samples were produced from replicated samplers of the same type (e.g. throughfall collectors, shallow suction cups) of one plot for the experiment in "aged" 500 ml PE bottles. The bottles were bi-weekly used in the field for the same samples, after washing in the dishwasher and with deionised water."

5. In section "2 Material and methods", subsection "2.2 Sampling and sample preparation", page 4: why were not used glass bottles and vials to store the samples? Glass material should be used to avoid contaminations. Blanks of procedure were performed?

Since we froze the samples, glass bottles could not be used because they could break when the water sample expands during the freezing process. For collecting the samples, we used aged HDPE bottles, which do not release detectable amounts of DOM according to our experience. We had blanks for all steps of the experiment. For all of them, no detectable DOC release (concentrations) and fluorescence was detectable. We will add this information to the Materials and Methods section of the revised manuscript and the data in table form in the supporting information.

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6. In section “3 Results”, subsection “3.2 PARAFAC fluorescence components”, reformulate the sentence “The maximum increase was +10% (-18âC) and +12% (N2)”. Remove the plus sign and extend the sentence with the types of freeze.

Thank you for the indication, we will rephrase the sentence accordingly.

7. In section “4 Discussion”, the reference of Santos et al. (2010) should be used together with the reference to Spencer et al. (2007) to the following sentence “This is in contrast to the results of Spencer et al. (2007), which could be related to similar fluorescence characteristics, but different chemical composition of proteinaceous fluorescence material from aquatic sources and the solutions from terrestrial ecosystems tested in this study.”

The reference Santos et al. (2010) will be added. We will rephrase the sentence into: “This is in contrast to the results of Spencer et al. (2007) and Santos et al. (2010), which could be related to similar fluorescence characteristics, but different chemical composition of proteinaceous fluorescence material from aquatic sources, rainwater and the solutions from terrestrial ecosystems tested in this study.”

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