Author’s Response to Referee #2

We would like to thank the Referee for the helpful and constructive comments, which will greatly improve the manuscript. Our detailed responses to the comments are listed below.

 # Specific comments:

 # The study is intended to elucidate the DOM quality and dynamics in two contrasting catchments. The authors expect “water level, temperature and precipitation to be the main controls on stream DOM…”. I would acknowledge if the authors provide a more specifically defined working question/hypothesis along which the results and discussion can be organized. How can the DOM of both catchments differ?

Response: Also in line with referee #1 we will rephrase our objectives in the introduction to clarify our hypothesis. Main objectives/hypotheses were: I) To test if spectrofluorometric indices can be used to track origin and dynamics of DOM. II) That DOM quality is highly variable in a headwater stream depending on hydrologic conditions and season. We expect short-term DOM quality changes due to high discharge events, which cause changes in hydrologic connectivity of different DOM pools and possible leachate effects of labile DOM during strong rain events. This short-term pattern is expected to be overlain by seasonal DOM changes due to changes of DOM production and consumption over the year. III) We expect general differences in DOM quality between the bog and forested riparian zone catchment. The riparian zone is characterized by accelerated water level fluctuations and a nutrient-richer vegetation than at the bog site, which leads to the hypothesis that DOM quality is strongly affected by changes in hydrologic conditions and is more labile than at the bog site. Additionally, we will review our result and discussion section in term of hypothesis consistency.

 # Samples were taken by an automated sampler in a six day interval and the filtered samples were stored at 4°C in the dark. How long were the samples stored until analysis? Although filtered, the samples were probably not sterile. Bacteria can degrade organic carbon even at low temperatures. Have the authors tested if the DOC composition remained unchanged?

Response: The storage time of our samples depended upon the type of measurement. DOC concentration and UV-Vis analytics were conducted at the TU Braunschweig. We could therefore measure samples more or less immediately (within a week). Immediate fluorescence measurements were impossible, as those were conducted at the University of Münster, which necessitated measurement campaigns in about three months intervals. To address the problem of conservation we tested UV-VIS changes, which were inconspicuous over that time-period. Furthermore, due to the longtime experience of the ecohydrology group in Münster in fluorescence measurements we came to the conclusion that we produce less analytic artefacts by storing the samples at low temperatures over that time period than freeze them for conservation. We also refer to following literature: Santos et al. 2010, Hudson et al. 2009, Graeber et al. 2012. There it also is recommended that samples should be frozen, if storage time is longer than one year.

 # UV absorption characteristics were used as indicators of DOM composition (absorbance at 254 nm, spectral slope ratio). Besides DOM, however, dissolved iron exhibits significant UV absorption. The dynamics of iron is also related to hydrology, i.e. flood events can be associated with high iron concentrations. Changes in UV absorption may reveal changes in DOM quality but at the same time they can reflect different contributions of dissolved iron. How the authors think about that?

Response: This is an important point. We are aware of biases of UV-VIS and fluorescence DOM characterization by Fe concentration or pH changes. We therefore consulted literature for critical Fe concentration levels, which cause interferences (Weishaar et al 2003; Xiao et al. 2013 and Poulin et al. 2014). We concluded that in our case Fe interference can be neglected as we measured maximum concentrations of 500 µg L^{-1} Fe in our water samples. This results in a Fe_{mg}/C_{mg} ratio of about 0.01, which is very low. Additionally, samples were measured in 6 or 8 fold dilutions resulting in maximum absolute concentrations during measurements of about 60 or 80 µg L^{-1}. Possible biases from pH were assessed by continuous pH measurements, which resulted in a constant pH of about 3.8 over the whole sampling period.

 # The fluorescence index (FI) differentiates between vascular plant derived (FI 1.3 – 1.4) and microbially derived (1.7-2.0) DOM. FI values >1.7 were interpreted as microbial DOM (p. 5 lines 15-20). Can the authors exclude that non-vascular plants (mosses) contributed to DOM generation? Later in the manuscript a contribution of Sphagnum is specifically discussed with respect to SUVA but not to FI (p. 9 lines 15-31).

Response: We are glad that the referee pointed out this apparent limitation of this approach, which also caught our attention before. We do not exclude non-vascular plants, indeed we assume that bog derived DOM does also origin from prevailing Sphagnum vegetation. This fact should not modify assumptions that are made by the fluorescence index. The study of Wickland et al. (2007) published FI values of leachates of plant material. The FI of Sphagnum were around 1.1-1.4, which is similar to vascular plants signatures. We will rephrase the sentence to improve clarity here.

 # In the results and also in the discussion sections, fluorescence indices and absorption values were often reported to be higher or lower when sites or situations were compared. However, I missed statistics providing a little more confidence if these differences are significant. I

recommend including a table/figure, summarizing the main results as well as the levels of significance. This table/figure along with a hypothesis can be used to guide the reader through the results and discussion. I found it less convenient to work through the detailed description of the results. The figures 2, 3, 5, 6, 7 and 8 look more or less similar, which makes it not easy to keep the most important results in mind.

Response: We agree that the current presentation was not easy to follow. We thus extend our study to clear statistical evaluations in the revised version. We will prepare descriptive statistic tables (planned as table 1 in the revised manuscript), which include mean, median, standard deviation and minimum and maximum values of the concentrations and indices. We will consider providing box plots to condense information and acknowledge the currently unclear presentation. Differences have now been tested with appropriate statistic tests like Mann-Whitney or Kruskal-Wallis tests and results are ready to be included in a revised version.

 # Further comments:

 # The title: Please refer more directly to the outcome of the study

Response: We will substantiate our title.

 # p.1 lines 22-24: The concluding sentence of the abstract "Our study demonstrated that DOM export dynamics are not only a passive mixing of different hydrological sources, but…" is unclear. Now the reader expects a statement why it is not a passive mixing or how the process can be characterized instead. However, it is only concluded that "…assessing DOM quality can greatly improve our understanding…". Please include here the most relevant result that improved our understanding. The sources and the quality of DOM appeared to be highly variable within events depending on runoff generation. What are the consequences for sampling/monitoring programs?

Response: We will revise the abstract also in view of the comments of referee #1. We will refer stronger to the outcome of this study, which is the mentioned high variability of DOM quality. This implies that monitoring programs have to consider not only changes due to seasonality, but need to cover different hydrologic conditions as well. Furthermore, the study shows that bog DOM quality is less susceptible to changes in hydrologic conditions than the peaty riparian zones. Additionally, the used spectrofluorometric indices proofed to be a useful tool to track DOM origin and dynamics in this headwater stream.

 # p. 2 line 28: "aromatic or humic", is there a difference?

Response: This is a tricky question. Well for our understanding the term “aromatic” refers to the specific ring structure in an organic molecule, while humic or humic substances rather describes a large fraction of DOM mainly defined as complex, high molecular weight, chromophoric molecules derived from decomposition of plant and animal residues. These substances comprise an aromatic fraction. We used those terms in account with the general usage in literature, especially in literature reporting fluorescence results (e.g. Inamdar et al. 2012).

 # p. 2 line 33: groundwater DOM is of smaller size and mostly of microbial origin, please include a reference

Response: We will include a reference (e.g. Inamdar et al. 2012, Singh et al. 2012).

 # p. 4 line 29: The near UV includes light from 300 – 400 nm, 254 nm is in the middle UV range

Response: The referee is right and we will change this.

 # p. 7 line 9: Figure 5 instead of Figure 6?

Response: The referee is right. We will change this.

 # p. 8 lines 10 and 16-17: I had problems to relate these statements to the figures

Response: We will illustrate that statement with box plot figures and tests of significant differences (Mann-Whitney or Kruskal-Wallis test).

 # p. 9 lines 6-9: A confirmation of the suitability of fluorometric indices appears difficult without independent methods (e.g. isotopes, mass spectrometry). It is problematic to conclude that an increase in aromaticity is caused by an increase in apparent molecular size if the latter is not measured.

Response: Regarding the first part of this comment we agree with the referee that this statement might be misleading. This statement was not intended to refer to the suitability of fluorometric indices to represent specific DOM fractions or molecules. We want to express that the spectrofluorometric approach seems an appropriate tool to distinguish relevant hydrological compartments based on DOM quality. We will rephrase that.

Regarding the second part we conclude this from a negative correlation of the SUVA index for aromaticity and S_R which is an index for molecular size as confirmed in available studies (see Helms et al. 2008). This is why we explicitly stated "apparent" molecular size to state that this is a molecular size derived from S_R. Maybe this was unclear. We will strengthen this assumption by a significant statistical correlation in the revised manuscript and will rephrase this conclusion in order of the method limitations.

 # p. 14 line 1: "The export of labile, protein-like DOM was specific…" I suggest being more cautious with characteristics of DOC that have not measured directly (e.g. "labile"). See also P. 9 line 5 "…specific strong microbial DOM signature…".

Response: While in general the suitability of comparable fluorometric indices has been confirmed in existing studies (see e.g. Fellman et al. 2008), we agree that the method has inherent limitations and we will adjust these statements accordingly.

 # In Figures 1, 3, 5 and 6, the individual symbols in highlighted boxes (events) were difficult to distinguish.

Response: We will consider this limitation in our revision of the figures. We will rework our figures to improve clarity.

References:

Fellman, J.B., D'Amore, D. V., Hood, E. and Boone, R. D.: Fluorescence characteristics and biodegradability of dissolved organic matter in forest and wetland soils from coastal temperate watersheds in southeast Alaska, Biogeochemistry, 88, 169-184, doi:10.1007/s10533-008-9203-x, 2008

Graeber, D., Gelbrecht, J., Pusch, M. T., Anlanger, C., and Schiller, D. von: Agriculture has changed the amount and composition of dissolved organic matter in Central European headwater streams, The Science of the total environment, 438, 435–446, doi:10.1016/j.scitotenv.2012.08.087, 2012.

Helms, J. R., Stubbins, A., Ritchie, J. D., Minor, E. C., Kieber, D. J., and Mopper, K.: Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter, Limnol Oceanogr, 53, 955–969, doi:10.4319/lo.2008.53.3.0955, 2008.

Hudson, N., Baker, A., Reynolds, D. M., Carliell-Marquet, C., and Ward, D.: Changes in freshwater organic matter fluorescence intensity with freezing/thawing and dehydration/rehydration, J. Geophys. Res., 114, doi:10.1029/2008JG000915, 2009.

Inamdar, S., Finger, N., Singh, S., Mitchell, M., Levia, D., Bais, H., Scott, D., and McHale, P.: Dissolved organic matter (DOM) concentration and quality in a forested mid-Atlantic watershed, USA, Biogeochemistry, 108, 55–76, 2012.

Poulin, B. A., Ryan, J. N., and Aiken, G. R.: Effects of Iron on Optical Properties of Dissolved Organic Matter, Environ Sci Technol, 48, 10098–10106, doi:10.1021/es502670r, 2014.

Santos, P. S. M., Otero, M., Santos, E. B. H., and Duarte, A. C.: Molecular fluorescence analysis of rainwater: effects of sample preservation, Talanta, 82, 1616–1621, doi:10.1016/j.talanta.2010.07.048, 2010.

Singh, S., Inamdar, S., Mitchell, M., and McHale, P.: Seasonal pattern of dissolved organic matter (DOM) in watershed sources: influence of hydrologic flow paths and autumn leaf fall, Biogeochemistry, 118, 321–337, doi:10.1007/s10533-013-9934-1, 2014

Weishaar, J. L., Aiken, G. R., Bergamaschi, B. A., Fram, M. S., Fujii, R., and Mopper, K.: Evaluation of Specific Ultraviolet Absorbance as an Indicator of the Chemical Composition and Reactivity of Dissolved Organic Carbon, Environ Sci Technol, 37, 4702–4708, doi:10.1021/es030360x, 2003.

Wickland, K. P., Neff, J. C., and Aiken, G. R.: Dissolved Organic Carbon in Alaskan Boreal Forest: Sources, Chemical Characteristics, and Biodegradability, Ecosystems, 10, 1323–1340, doi:10.1007/s10021-007-9101-4, 2007.

Xiao, Y.-H., Sara-Aho, T., Hartikainen, H., and Vähätalo, A. V.: Contribution of ferric iron to light absorption by chromophoric dissolved organic matter, Limnol Oceanogr-Meth, 653–662, 2013.