Interactive comment on "Spatiotemporal transformation of dissolved organic matter along an alpine stream flowpath on the Qinghai-Tibetan Plateau: importance of source and permafrost degradation" *by* Yinghui Wang et al.

Yinghui Wang et al.

ypxu@shou.edu.cn

We appreciate the anonymous reviewer to supply valuable comments. We accepted most of those comments and made changes in the revised manuscript. The follows are our point by point response, and the changes are marked in green in the revised manuscript. Please see the attached file for details.

General comments: The manuscript describes the use of multi techniques (UV, AMS, and FT-ICR MS) to characterize dissolved organic matter (DOM) and discuss the tem-

poral and spatial transformation of DOM along an alpine stream. The methodologies are state-of-the-art, the study area will be of interest to readership, the discussion is appropriate, and the manuscript is well-written. There are some issues, listed below, to address prior to publication.

Specific comments: Comment 1: Figure 5 was not mentioned in the context, which is my most concern. The "highly unsaturated compounds" was classified into L and H, how to define the classification?

Response: The reviewer is correct here. In our manuscript there is no distinction of between the unsaturated low oxygen (O/C < 0.5) from the unsaturated high oxygen (O/C > 0.5), throughout the manuscript both high and low oxygen are grouped together. So, we have grouped the 'U.H' and 'U.L' together in figure 5 and provides a new figure 5e in the revised manuscript.

Comment 2: From the distributions of H/C and O/C ratios shown in the van Krevelen diagrams in Figure 5, I trust the assignment of compounds is not credible. The authors directly used the data processing results from the software (EnviroOrg), however, the molecule assignment based on accurate mass value cannot guarantee a correct identification on the mass peak, especially on the high mass end.

Response: We somewhat agree with the reviewer. Assignment of molecular formulas in a complex mixture is challenging and this process would be nearly impossible, especially at relatively high m/z as the reviewer points out, if formulas were assigned to individual signals without context. However, in addition to exact mass, the compositional continuum of natural organic matter has been used for the past 15-20 years to assign molecular formulas to natural organic matter (Kujawinski ,E.B., 2002; Stenson, A., et al. 2003). The compositional patterns (homologous series) in natural organic matter enable us to assign molecular formulas to compounds associated with high accurate signals at low m/z where there is only one possible formula. We then extrapolate formula assignment to those at high m/z (in combination with the use of accurate

mass), as many researchers did. We added the references listed above to line 174 of the text, also at lines 513 and 568 as Kujawinski, E.B., (2002). Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI FT-ICR MS): Characterization of Complex Environmental Mixtures. Environ. Forensics 3, 207-216., Stenson, A.C.; Marshall, A.G.; Cooper, W.T., (2003) Exact masses and chemical formulas of individual Suwannee River fulvic acids from ultrahigh resolution electrospray ionization Fourier transform ion cyclotron resonance mass spectra, Anal. Chem. 75, 1275-1284.

Comment 3: Most "compounds" classified into the CA area (O/C<0.2) should be incorrect assignment of mass peaks with high mass values. This does not mean the data processing and the discussion are wrong, in fact, most published papers in the past years have this problem.

Response: There have been some papers that show "islands" or isolate pockets (in van Krevelen space) of formulas that are classified as condensed aromatic compounds. In these cases, we agree with the reviewer that they might be misassigned formulas that are associated with signals of compounds at relatively high m/z. However, this is not the case here. In addition to using homologous series (see above) that is afforded to us by the compositional continuum of natural organic matter (NOM) to assign molecular formulas, we also ensure that the formulas assigned are continuous in carbon number molecular weight, aromaticity, and heteroatom content. The combination of these parameters along with homologous series and accurate mass provide several layers of verification for accurate formula assignments for complex mixtures. The van Krevelen diagrams in Figure 5 show that there are no discontinuities (islands) in the compositional continuum, what's more, the number of condensed aromatic formulas have strong correlation with SUVA 254 ($r2 = 0.9679$, $p<0.01$), which all indicated that the formulas are assigned correctly.

Comment 4: In Table 1 and Table 2, as well as many places in the context, the number of assigned molecular formulas was used to discuss the composition of DOM and the

degradation along the stream. This is not rigorous and could lead to misunderstanding for readers both on the mass spectrometry analysis and the environmental interpretation. Not like most other analysis instrument detectors, the limit of detection (LOD) of ESI FT-ICR MS for DOM analysis is uncertain, it partially determined by the most abundant peak in the spectrum. Briefly, less mass peaks do not means the composition of the sample is "simple" and compounds not detected in the sample does not means these compounds must in lower concentration. I suggest the authors provide some raw mass spectrometry data in the supporting information, such as the broad bound mass spectra, expanded mass scale mass spectra.

Response: We agree with the reviewer that interpreting environmental relevance based solely on changes in the number of assigned molecular formulas could be ambiguous. We also agree that changes in the number of assigned formulas is not indicative of compounds at lower concentration as FT-ICR MS is better regarded as a qualitative rather than quantitative method. However, in our study, thousands of specific signals were detected in headwater samples but not detected in downstream waters, providing an unambiguous evidence on compositional change of DOM along the stream. In addition, DOC concentrations of the SPE extracts were normalized for FT-ICR MS analysis. This step largely removed the difference in background matrix between samples. Given these fact, we are confident that the peaks lost in downstream are not caused by the detection limit. In addition, optical and radiocarbon data also showed substantial changes along stream, supporting the changes observed by FT-ICRMS. Nevertheless, we have changed the term "molecular richness" to "chemodiversity" (as described by Kellerman et al. 2014) in lines 227 and 237 for clarity. Frankly speaking, we do not see any necessity of the addition of raw mass spectrum in supporting information. There are over ten thousand peaks in raw mass spectrum for leachate DOM, which reveal little useful information to readers without further data processes. As such, we decided not to do so. But we provided the raw data in the excel sheet in supporting information.

Line 220. The numbers include the isotope formulae?

Response: We did not include the isotopologues here.

Line 221. Elemental analysis was not mentioned in the context.

Response: We changed 'elemental' to 'molecular-level' for clarity.

Line 232. I don't think it is a good manner to compare the changes in MW and AI with percentage values.

Response: We removed the percentages from MW and AI.

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2018-182/bg-2018-182-AC2-supplement.zip

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-182, 2018.