

22 Thanks to the anonymous reviewer for their constructive comments on the manuscript
23 and helpful suggestions for further improvement. Please find detailed responses below
24 in blue-color font.

25

26 **Response to Anonymous Referee #1**

27

28 **Summary**

29 In this preprint, the authors utilized excitation-emission matrix (EEM) fluorescence
30 spectroscopy and Fourier transform ion cyclotron resonance mass spectrometry (FT-
31 ICR MS) to examine the molecular composition and formation mechanisms of water-
32 soluble organic carbon (WSOC) in atmospheric aerosols gathered from Karachi,
33 Pakistan. They identified three parallel factor (PARAFAC) components, consisting of
34 two humic-like (C1 and C2) and one protein-like (C3). They further investigated the
35 connection between PARAFAC components and possible brown carbon (BrC)
36 fluorescent species, along with absorbance characteristics, which could aid in
37 broadening our knowledge of BrC in the atmosphere. With a few adjustments,
38 clarifications, and a thorough language revision for clarity, this study has the potential
39 to contribute to the field and be suitable for publication in ACP.

40 [Response: Thank you for appreciating our work and providing valuable suggestions.](#)
41 [We have obtained professional language editing and have revised the manuscript](#)
42 [according to your comments. The revised sections are highlighted for easy reference.](#)

43

44 **Strengths**

45 The authors leveraged a blend of advanced analytical techniques, such as EEM
46 spectroscopy and FT-ICR MS, to characterize WSOC in aerosols.

47

48 By identifying three PARAFAC components and examining their molecular
49 relationships, the study provides a more in-depth understanding of the formation
50 processes and properties of fluorescent species in the atmosphere.

51

52 The insights gained on the molecular compositions and formation mechanisms of
53 atmospheric fluorescent components can prove valuable for future investigations that
54 employ EEM-PARAFAC to study atmospheric BrC.

55 [Response: We are grateful to the reviewer for recognizing our work and providing](#)
56 [valuable suggestions. The suggestions would help us improve the manuscript.](#)

57

58 **Areas for improvement**

59 The study focuses on aerosols from Karachi, Pakistan, so the conclusions might not
60 represent other geographical areas. Although there are comparisons with results from
61 Bakersfield and Guangzhou (among others), it would be beneficial for the authors to
62 critically discuss the broad applicability of their overall findings to other locations in
63 the conclusion, or suggest future studies using their methodologies to include aerosols
64 from diverse regions.

65 Response: Thanks. This study investigated the molecular compositions and formation
66 pathways of water-soluble PARAFAC components in this interesting geographical
67 location in Karachi, Pakistan. Our findings can potentially be applied to other locations,
68 highlighting two main aspects. First, we obtain the molecular characteristics of
69 PARAFAC components that are consistent with their emission wavelength maximum.
70 The trend of DBE, O/C, \overline{OS}_C , and AI_{mod} of molecular compounds assigned to C1–3 is
71 as follows: C2 (494 nm) > C1 (415 nm) > C3 (337 nm). Similar patterns were observed
72 in the molecular formulas of humic-like components compared to those of protein-like
73 components in Guangzhou, a city in southern China by He et al. (2023). These findings
74 suggest that the maximum emission wavelength of fluorescent components may serve
75 as an indicator of the chemical characteristics of BrC chromophores, such as the level
76 of unsaturation and oxidation. Second, the pathway of oxidation formation was
77 identified as an important process for the formation of C1 and C2. This is specific to
78 their assigned CHON compounds. The deduced N_2O_5 oxidation reaction, which formed
79 organic nitrates, accounts for a significant fraction of CHON compounds assigned to
80 C1 and C2. While C2 has more precursors of aromatic/condensed aromatic compounds
81 for CHON compared to C1, suggesting that this component contains more nitro-
82 aromatic compounds. Furthermore, C2 is assigned a large number of compounds that
83 match the “potential BrC” region and overlap with BrC-associated formulas and
84 exhibits a close correlation with MAE_{365} . Taken together, these findings imply that a
85 group of compounds that contribute to C2 fluorescence may significantly contribute to
86 the light absorption of BrC. According to these findings, we inferred that this
87 component likely represents a class of strongly absorbing substances, specifically nitro-
88 aromatic compounds. More discussions were shown in lines 820-897 in the revised
89 manuscript.

90

91 In Section 3.3, the authors attempt to explain the differences in oxidation pathways
92 (depending on precursor types) for the formation of C1 and C2 components. It would
93 be helpful if the authors could elucidate this explanation (Lines 520-522) and
94 incorporate a more detailed summary in both the conclusion (Lines 586-587) and
95 abstract.

96 Response: We draw the conclusion (original version in lines 520-522) based on the
97 elemental compositions of the overlapping molecules assigned to C1 and C2, which
98 consist mainly of CHON and CHO compounds. Additionally, the formation pathways
99 of these overlapping molecules were found to be a significant part of oxidation-derived
100 pathways, with CHON compounds primarily attributed to N₂O₅ oxidation and the CHO
101 compounds were highly detected in the SOA formed from limonene ozonolysis. For the
102 revised sections, please refer to lines 763-778 in the revised manuscript.

103 In addition, the detailed summary was further discussed in the conclusion (lines 849-
104 857) and mentioned in the abstract (lines 46-50).

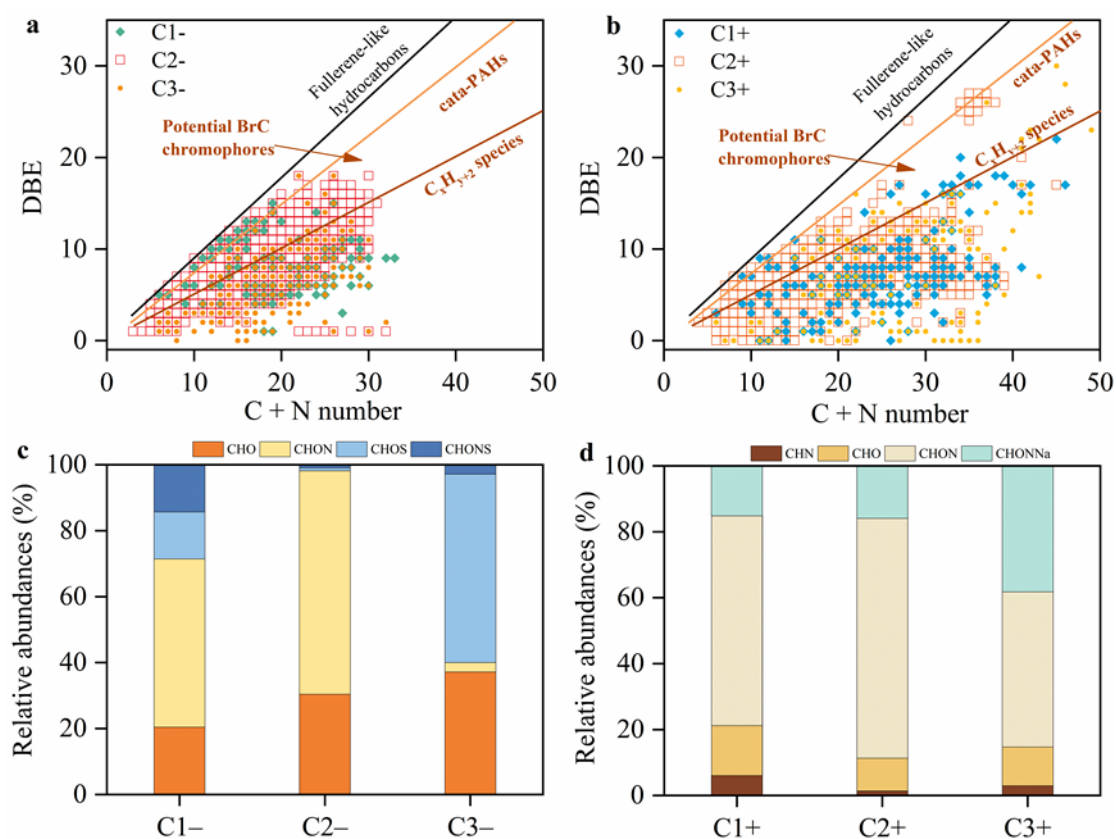
105

106 Although the study identifies molecular families associated with each of the three
107 components, their specific molecular formulas are not detailed, potentially limiting the
108 study's replicability. Providing more information on abundant (in terms of MS signal
109 intensity) molecular formulas associated with each component would allow other
110 researchers to expand upon these findings. In addition, based on Lines 537-540 (Section
111 3.4) and Figure 5, the reviewer is particularly interested in knowing the molecular
112 formulas of major (potential) BrC species (nitro-aromatics, CHO species) within the
113 C2 component.

114 Response: In fact, the data on correlation analysis is accessible on Harvard Dataverse
115 (<https://doi.org/10.7910/DVN/RWIJZT>, Tang, 2023) in the data availability section for
116 the convenience of other researchers. The reader can easily obtain the detailed
117 molecular formulas that were assigned to each PARAFAC component (or Abs₃₆₅,
118 optical indices, and fluorescence indices) in lines 903-904 in the revised manuscript.

119 In addition, we have revised the plot and incorporated subgroups of potential BrC
120 compounds, illustrated in the new Figure 4. It was found that CHO and CHON
121 contributed a significant portion (98 %) of C2-assigned formulas located in the “BrC
122 domain”, with CHON accounting for 68% of them. Furthermore, 80% of potential BrC
123 CHON compounds assigned to C2 have O/N ≥ 3, indicating that they are species of
124 nitro-aromatics. For more detailed information, please refer to the Harvard Dataverse
125 (<https://doi.org/10.7910/DVN/RWIJZT>, Tang, 2023). The updated discussions were
126 presented in lines 544-557 in the revised manuscript.

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136 Line 562: It may be worthwhile to include an alternate version of this figure that
 137 represents the number of carbon and nitrogen atoms (C+N) on the x-axis, and discuss
 138 any discrepancies with the current version. This alternate figure might be more pertinent
 139 to BrC, as nitroaromatic groups (if present) in CHON are potential chromophores.
 140 When considering C+N, the data points for N-containing compounds in this figure
 141 would shift to the right.

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Figure 4. Plot of the double bond equivalent (DBE) vs the number of C + N atoms of PARAFAC components-assigned molecular formulas, and the corresponding subgroups of potential BrC components in the ESI- (a, c) and ESI+ (b, d) modes. Lines indicate DBE reference values of linear conjugated polyenes C_xH_{x+2} with $DBE = 0.5 \times C$ (brown solid line), *cata*-condensed PAHs (yellow solid line), and fullerene-like hydrocarbons with $DBE = 0.9 \times C$ (black solid line). Data points inside the lines are potential BrC chromophores (Lin et al., 2018).

Response: Thank you for your suggestions. We have revised this plot by adding a subgroup plot of the potential BrC components assigned to C1–3 (Figure 4). The DBE values of CHON and CHONS compounds shift right by 1-4 units due to the sum number of carbon and nitrogen atoms. The new discussions were presented in lines 544-557 in the revised manuscript.

148 Several sentences are difficult to understand, and their meanings are unclear. The
149 authors should rectify any remaining language issues to enhance the manuscript's
150 readability. After these improvements, another review might be required to ensure the
151 precision of all statements in the revised manuscript.

152 **Response:** We apologize for the low quality of the language in our manuscript. We spent
153 a long time revising the manuscript, which involved repeatedly adding and removing
154 sentences and paragraphs. This led to a significant decrease in readability. We have
155 worked on improving both the language and the readability of the document.
156 Additionally, we have invited fellow experts to make professional language editing for
157 the manuscript. We hope that the clarity and language sophistication has been
158 significantly improved.

159

160 **Additional comments and suggested edits**

161 Lines 1-3: Consider rearranging the title for clarity: "Molecular signatures and
162 formation mechanisms of water-soluble chromophores in particulate matter from
163 Karachi (Pakistan) in South Asia."

164 **Response:** Thank you. The title has been modified according to your recommendations.

165

166 Line 56: Change to "originates."

167 **Response:** We have revised it (in line 65).

168

169 Line 58: Change to "formation processes, such as aqueous-phase reactions from
170 anthropogenic or biogenic emissions."

171 **Response:** We have revised it (in lines 67-68).

172

173 Lines 60-61: Change to "Light-absorbing organic (or brown) carbon (BrC) is an
174 important component of WSOC..."

175 **Response:** We have revised it (in lines 69-71).

176

177 Line 74: Change to "...absorb light at certain wavelengths..."

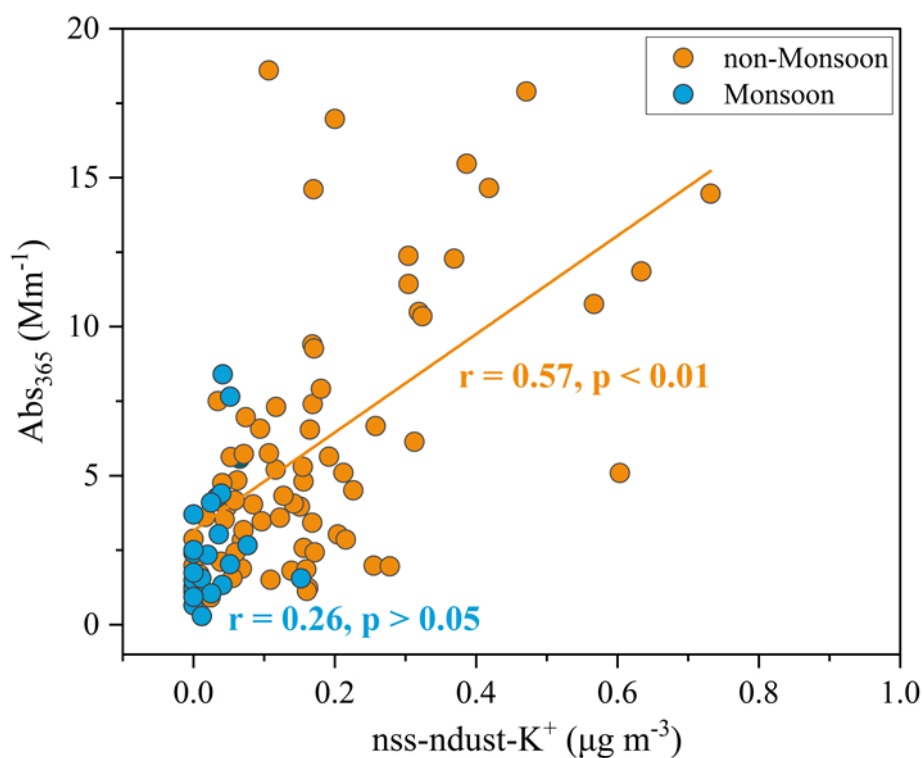
178 **Response:** We have revised it (in line 85).

179

180 Lines 115-116: Could you elaborate on whether primary or aged biomass burning
181 emissions are anticipated and how they might influence the interpretation of BrC results?

182 **Response:** Of course. In the revised paragraph, nss-ndust-K⁺ (the fraction unrelated to
183 sea salt and mineral dust, nss-ndust) was used as a marker of biomass burning to
184 investigate its influences (Pio et al., 2007; Zhou et al., 2019). A good linear correlation

185 was found between Abs_{365} (a proxy for BrC) and $nss-ndust-K^+$ ($r = 0.57$, $p < 0.01$, Fig.
186 S5) during non-monsoon seasons. In contrast, the absence of a linear relationship
187 between Abs_{365} and $nss-ndust-K^+$ in the monsoon season (Fig. S5) indicates no
188 influence of biomass burning emissions. Consistently, MAE_{365} was higher during non-
189 monsoon seasons than the monsoon season, which suggests that biomass burning has
190 an important influence on water-soluble BrC during non-monsoon seasons. Please refer
191 to lines 366-372 in the revised manuscript.



192

193 **Figure S5.** Correlation between $nss-ndust-K^+$ and Abs_{365} in non-monsoon and monsoon seasons.

194

195 Line 126: Change to "Total suspended particulate matter (TSP) was collected..."

196 Response: We have revised it (in line 155).

197

198 Line 148: Change to "PARAFAC."

199 Response: We have revised it (in line 184).

200

201 Line 285: Please clarify what the error bars signify in panels (c) and (d).

202 Response: The error bars shown in panels c and d represent the standard deviation. We
203 have added after the plot in lines 388-389 in the revised manuscript.

204

205 Lines 310-312: Could the authors provide insights as to whether the molecular formulas
206 that showed no correlation with any PARAFAC component are predominantly aliphatic

207 and sugar compounds, which are not typically expected to exhibit light absorption or
208 fluorescence?

209 Response: Of course. The molecular formulas that did not correlate with any of the
210 PARAFAC components are predominantly aliphatic compounds and highly unsaturated
211 and phenolic compounds (a total of 87% of the unassigned molecules in the ESI- mode
212 and 83% in the ESI+ mode, respectively). Please refer to lines 417-420 in the revised
213 manuscript.

214

215 Line 362: Change to "saturated."

216 Response: We have revised it (in line 485).

217

218 Line 364: Change to "lower."

219 Response: We have revised it (in line 488).

220

221 Line 604: Summarize Text S7 briefly.

222 Response: Thank you for your suggestions. We have revised it as follows: "Furthermore,
223 the degree of molecular similarity between fluorescent components in various systems
224 was examined. Despite the similarity of fluorescent components in the atmosphere and
225 aquatic environment, their respective molecular formulas have substantial differences.
226 The fluorescence characteristics derived from aquatic environments may not be suitable
227 as references for studying atmospheric WSOC, as discussed in Text S8." Please refer to
228 lines 887-893 in the revised manuscript.

229

230 **Comments on the SI**

231 Text S3: Please explain why liquid chromatography was not employed for the
232 separation of compounds prior to the MS analysis, and discuss any potential effects this
233 decision might have on the current study.

234 Response: This is because our aerosol samples were extracted using ultrapure water,
235 while the bulk samples were measured directly using a fluorometer (Aqualog; Horiba
236 Scientific, USA). Therefore, maintaining consistency of the corresponding fraction is
237 crucial to obtain the molecular formulas using FT-ICR MS.

238 Your suggestions are very valuable. If we choose liquid chromatography to separate
239 compounds before MS analysis, we must simultaneously obtain the EEM spectra of the
240 separated fractions, such as the method described by Lin et al. (2015; 2016). They
241 combine HPLC-PDA-HRMS to simultaneously obtain the light absorption and
242 molecular compositions of BrC chromophores. In addition, this proposal provides new
243 knowledge for investigating the molecular compositions of fluorescent components. It

244 is unavoidable that analyzing bulk samples will result in some missed underlying
245 information. For example, Spranger et al. (2019) used a 2D-liquid chromatographic
246 fractionation method, coupled with direct infusion electrospray ionization FT-ICR MS,
247 and observed a 2.3-fold increase in the number of molecular formulas detected in the
248 fractionated sample (18144) compared to bulk sample analysis without fractionation
249 (7819). So that will be our focus in the future.

250

251 References:

252 Lin, P., Aiona, P. K., Li, Y., Shiraiwa, M., Laskin, J., Nizkorodov, S. A., and Laskin, A.: Molecular
253 Characterization of Brown Carbon in Biomass Burning Aerosol Particles, *Environ. Sci. Technol.*,
254 50, 11815-11824, <https://doi.org/10.1021/acs.est.6b03024>, 2016.

255 Lin, P., Laskin, J., Nizkorodov, S. A., and Laskin, A.: Revealing Brown Carbon Chromophores
256 Produced in Reactions of Methylglyoxal with Ammonium Sulfate, *Environ. Sci. Technol.*, 49,
257 14257-14266, <https://doi.org/10.1021/acs.est.5b03608>, 2015.

258 Spranger, T., Pinxteren, D. V., Reemtsma, T., Lechtenfeld, O. J., and Herrmann, H.: 2D Liquid
259 Chromatographic Fractionation with Ultra-high Resolution MS Analysis Resolves a Vast Molecular
260 Diversity of Tropospheric Particle Organics, *Environ. Sci. Technol.*, 53, 11353-11363,
261 <https://doi.org/10.1021/acs.est.9b03839>, 2019.

262

263 Lines 149 & 154: Please elucidate the distinction between the carbon oxidation state
264 and the nominal oxidation state of carbon. Also, provide a rationale for the application
265 of both these metrics in this manuscript.

266 Response: These two parameters represent the oxidation state of compounds. The
267 nominal oxidation state of carbon (NOSC) is estimated from the chemical formula by
268 assuming that all other elements are in their initial oxidation states (H = +1, O = -2, N
269 = -3, and S = -2) using the following equation (1) (Riedel et al., 2012), which is
270 commonly used in aquatic and soil systems.

271
$$\text{NOSC} = 4 - [(4c + h - 3n - 2o - 2s) / c] \quad (1)$$

272 The carbon oxidation state ($\overline{\text{OS}}_{\text{C}}$) is calculated using equation (2) (Kroll et al., 2011),
273 which is commonly used for atmospheric aerosols.

274
$$\overline{\text{OS}}_{\text{C}} = -\sum_i \text{OS}_i \frac{n_i}{n_{\text{C}}} \quad (2)$$

275 Where, OS_i is the oxidation state associated with element i , and n_i/n_{C} is the molar ratio
276 of element i to carbon. Furthermore, if we choose the initial oxidation states of H, O,
277 N, and S atoms, equation (2) is equal to equation (1), as used in the study by Hettiyadura

278 et al. (2021) ($\overline{\text{OS}}_{\text{C}} = \frac{2o}{c} + \frac{3n}{c} + \frac{2s}{s} - h/c$). However, predicting the average nitrogen

279 oxidation state is a very difficult or even impossible task. It is known that highly
280 oxidized organic nitrates as well as low oxidized amines, amino acids, and other N-
281 containing organics such as imidazoles and other N-heterocyclic compounds are present
282 in aerosol samples. Spranger et al. (2019) assumed different oxidation states for N and
283 S atoms, resulting in different \overline{OS}_C values. To avoid this error, we choose \overline{OS}_C to
284 describe the oxidation state of the compounds, and make assumptions about the OS_i of
285 the different atoms: $OS_O = -2$; $OS_H = 1$; $OS_N = 0$; $OS_S = 0$, which refers to the study by
286 Spranger et al. (2019). Thus, the carbon oxidation state (\overline{OS}_C) of individual compounds
287 was calculated as $\overline{OS}_C = 2 \cdot O/C - H/C$.

288

289 References:

290 Hettiyadura, A. P. S., Garcia, V., Li, C., West, C. P., Tomlin, J., He, Q., Rudich, Y., and Laskin, A.:
291 Chemical Composition and Molecular-Specific Optical Properties of Atmospheric Brown Carbon
292 Associated with Biomass Burning, *Environ. Sci. Technol.*, 55, 2511-2521,
293 <https://doi.org/10.1021/acs.est.0c05883>, 2021.

294 Riedel, T., Biester, H., and Dittmar, T.: Molecular Fractionation of Dissolved Organic Matter with
295 Metal Salts, *Environ. Sci. Technol.*, 46, 4419-4426, <https://doi.org/10.1021/es203901u>, 2012.

296 Spranger, T., Pinxteren, D. V., Reemtsma, T., Lechtenfeld, O. J., and Herrmann, H.: 2D Liquid
297 Chromatographic Fractionation with Ultra-high Resolution MS Analysis Resolves a Vast Molecular
298 Diversity of Tropospheric Particle Organics, *Environ. Sci. Technol.*, 53, 11353-11363,
299 <https://doi.org/10.1021/acs.est.9b03839>, 2019.

300

301 Line 209: Remove "using."

302 Response: We have revised it (in line 249).

303

304 Line 232: Remove the first "that."

305 Response: We have revised it (in line 312).

306

307 Line 233: Change to "may not have the same character as in..." or similar.

308 Response: We have revised it (in line 315).

309

310 Lines 249-251: Rewrite the sentence for clarity.

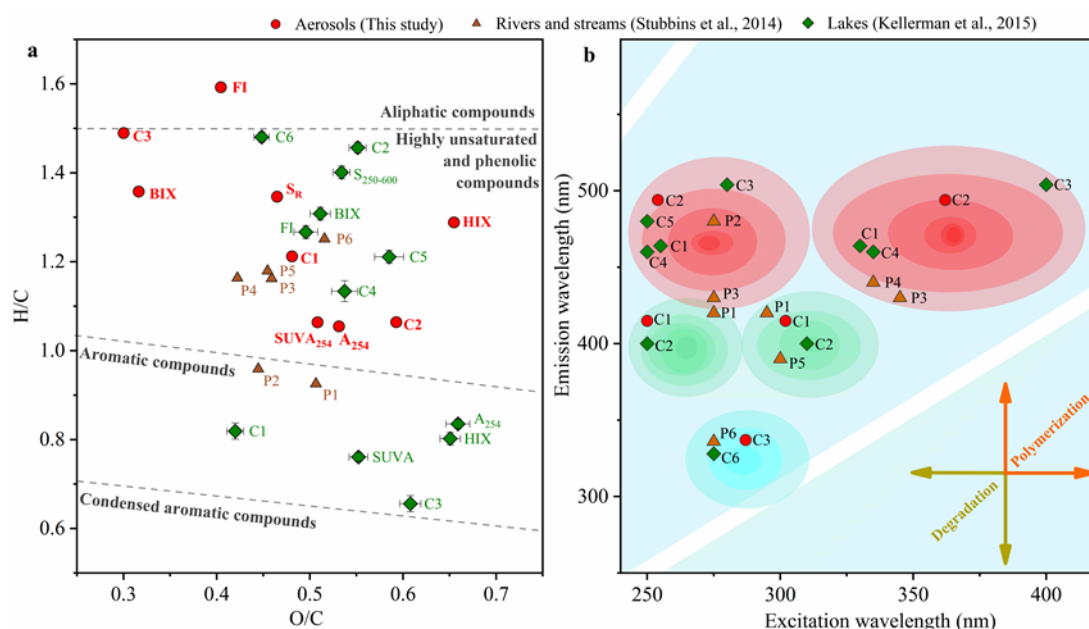
311 Response: We have rewritten it as follows: ...whereas C1 formulas (in lakes) grouped
312 in aromatic compounds region. This implies that although some PARAFAC
313 components have similar fluorescence patterns and excitation/emission maximum, their
314 chemical properties vary in different environments. For instance, the degree of

315 unsaturation in environment can affect them. Please refer to lines 334-339 in the revised
316 supplement.

317

318 Line 380: Correct "streams" in the figure legend.

319 Response: We apologize for this error. We have corrected the plot as follows.



320

321 **Figure S15. (a)** Comparison of the average chemical characteristics of molecules assigned to C1,
322 C2, and C3, as well as the optical indices of WSOC in the aerosols (marked as red dot), DOM from
323 rivers and streams (brown triangle, fluorescent component P1–6) and lakes (green diamond,
324 fluorescent component C1–6, and FI, BIX, HIX, SUVA, A₂₅₄, and S₂₅₀₋₆₀₀); **(b)** the corresponding
325 excitation and emission maxima in the EEM spectra (Stubbins et al., 2014; Kellerman et al., 2015).
326 Note that the fluorescent components associated with molecular data **(a)** of DOM in rivers and
327 streams were re-extracted from their supplement and the error bars were not shown. The error bars
328 in this study are shown in Table S5. The range of the red circle, green circle, and blue circle **(b)**
329 represent the range of HULIS-2, HULIS-1, and PLOM fluorescence, respectively. The arrows
330 indicate polymerization and degradation defined according to the study (Chen et al., 2016).

331

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333