Response to the Referee's comments on "Influence of anthropogenic pollution on the molecular composition of organic aerosols over a forest site in the Qinling Mountains region of central China"

by Xin Zhang and Yuemei Han

We thank the referee #2 for taking time and providing further critical feedbacks on our manuscript (ID: egusphere-2025-519-R1). We have seriously taken into account of all the concerns raised by the referee and carefully revised the manuscript accordingly. Please find below our detailed point-to-point responses to all those concerns.

I-1. This manuscript presents observational evidence of the seasonal variability in organic aerosol composition. However, it is important to note that the electrospray Ionization interface that couples the UPLC and Orbitrap MS is known for its varying ionization efficiencies across different compounds. The sensitivity of this technique can vary substantially by orders of magnitude, depending on the compound structure, functional group, and the characteristics of the sample matrix.

Response: We thank the referee for pointing out the inherent limitation of ESI–HRMS technique regarding the ionization efficiency issue. However, in fact, this technique remains one of the most promising and powerful techniques in the research field of atmospheric and aerosol chemistry, despite some inherent drawbacks that need to be improve progressively. The claimed issue here itself could be applicable for any ESI–HRMS instruments, whereas cautions should be paid to utilize it for specific research topics. The ionization efficiency issue is in particular crucial for studies reporting the absolution mass concentrations of organic compounds. In our present study, however, the absolution mass concentrations of individual organic compounds were not a primary focus. Also, it is not possible to do this especially for a dataset derived from non-target screening of massive organic species measured by the HRMS, unless using some prediction approaches yet still with large uncertainties. Therefore, depending on the features of the dataset, our present study aims to investigate the influence of anthropogenic pollution on organic aerosol composition based on the parallel comparison of variation trends in species number and peak area between different seasons. The relative changes in the seasonal variation patterns of species number and peak area did reflect the actually changes of organic species, rather than caused by the varied ionization efficiency.

I-2. Consequently, the signal intensity in an ESI spectrum does not necessarily serve as a direct and reliable indicator of compound abundance. This variability poses challenges in accurately correlating ESI+ and ESI- ion abundances with the actual abundance of the compounds in a given sample.

Response: It is obvious that we did not simply use the signal intensity in an ESI spectrum for analysis in the current study. Rather, by combining the results of UHPLC chromatogram with HRMS mass spectra, the organic species number and peak area were obtained for discussion, particularly in respect of their variation trends from parallel comparison of different samples. Also, for each individual organic compound, given that the ESI–Orbitrap MS operation parameters being controlled consistently for all the samples in our study, the obtained peak area should linearly correlate with their real abundance in the atmosphere. Therefore, the observed relative variations in the species number and peak area between different samples cannot be simply explained by the changing ionization efficiency.

I-3. The high-resolution spectra offered by the Orbitrap MS only provide elemental composition information about the molecules. It is a major leap to connect this data to molecular identities, and discussions regarding functional groups or chemical details could be subjected to large uncertainties.

Response: This statement apparently underestimates the powerful capabilities of HRMS and also ignores the tremendous efforts that have been made by the entire HRMS research community thus far. Based on the HRMS-derived exactive mass and elemental composition of organic molecules, along with other parameters such as chromatogram retention time and peak area, a variety of reliable analytical methods and algorithms have been well-established in the past decades, those including but not limited to: elemental ratios, carbon oxidation states, ring and double-bond equivalent, aromaticity equivalent, maximum carbonyl ratio, van Krevelen diagrams, Kendrick mass defect, and so on (e.g., Nizkorodov et al., 2011; Nozière et al., 2015; Johnston and Kerecman, 2019; Zhang et al., 2023; Lin et al., 2025). These methods and relevant analyses have already provided extensive valuable information for understanding the detailed molecular identities and characteristics of organic species in the atmospheric and aerosol research field.

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I-4. Overall, I perceive this manuscript as lacking of scientific rigor, with conclusions and findings that are short of specificity and novelty.

Response: We disagree with this viewpoint. Given that all the raised concerns are focused on the instrumental technical and methodology aspects, while those are not really applicable to our present study, it has no scientific basis and irrational to claim that our study are short of specificity and novelty. As we clearly addressed in the 1st round of response, the specificity and novelty of this study rely primarily on the unique scientific question and findings in the studied Qinling Mountains region with significant geographical and climatic features, rather than to develop technique or methodology itself.

The specificity and novelty of this study are summarized as follows: (1) It first provided a comprehensive insight on the chemical characteristics of organic aerosols at the molecular level in the Qinling Mountains region, which has unique and significant geographical and climatic features; (2) It first demonstrated the substantial influence of anthropogenic pollution on the organic molecular composition in this region; (3) It advanced our knowledge on the anthropogenic and biogenic interactions as well as elucidated its seasonal variabilities and underlying influencing factors. We have revised the following statement to further highlight the novelty of this study:

"So far to our knowledge, this study for the first time reports the overall molecular characteristics of organic aerosols between contrasting seasons **and provides direct evidence for the large influence of anthropogenic pollution** in the Qinling Mountains region based on high-resolution Orbitrap mass spectrometry." (Lines 76–78)

In addition, we also noticed that the referee's evaluation on "Scientific significance" of this study was even degraded in this 2nd round, comparing with those in the 1st round of review process, although we kept on improving our manuscript according to the referees' comments. We would be grateful if this study could be evaluated more objectively.

II-1. This study employs a characterization approach based on exact mass formulae in combination with literature references. While high-resolution mass spectrometry offers clear advantages in molecular formula determination, its application to a highly complex mixture such as that investigated here—comprising a large number of compounds with

varying degrees of oxidation—presents significant challenges and uncertainties. These include, but are not limited to, mass spectral data processing strategies (e.g., background and noise subtraction), adduct formation and identification, charge state determination, accurate mass measurements, elemental composition assignments, and complex data-dependent acquisition setups.

Response: We thank the referee for highlighting the inherent challenges and uncertainties associated with the molecular characterization of highly complex mixtures by HRMS. The raised concerns here would influence the data interpretation in certain cases. However, by implementing the rigorous procedures for data processing in this study, we are confident that the presented results are robust and reliably represents the chemical complexity of organic species in all the samples. The following explanations are specified to address each of these points:

a. Mass spectral data processing strategies: We utilized the MZmine software with carefully optimized parameter settings for the processing of raw data from UHPLC–HRMS analysis. This software has been well-developed and widely used in the HRMS research community, offering core MS data processing procedures such as peak detection, shoulder peak filtering, chromatogram builder and deconvolution, deisotoping, adducts and peak complex searching, identification, duplicate peak filtering, and other functions (Pluskal et al., 2010; Schmid et al., 2023). On the other hand, we have established reliable parameters and procedures based on a number of previous studies in our research group (e.g., Lin et al., 2022; Han et al., 2023; Li et al., 2024; Zhang et al., 2025) and also further optimized these parameters for the current study. We have also added a relevant statement on the background and noise subtraction for aerosol samples in the revised manuscript, as follows:

"**The field blanks were also extracted and chemically analyzed using the same procedures, the results of which were used to correct any potential artifacts and backgrounds for the aerosol samples.**" (Lines 122–123)

b. Adduct formation and identification: The adduct annotation has already been taken into account for the raw mass spectral analysis using the MZmine software. We have constrained the formula assignments by specifically searching for common adducts such as protonated, deprotonated, and other ions (e.g., $[M + H]^+$ and $[M + Na]^+$ in the positive ESI mode; $[M - H]^-$ in the negative ESI mode) to minimize ambiguity.

c. Charge state determination: The ESI source used in our study primarily produced single charged ions, which was confirmed by isotopic pattern matching in the MZmine software analysis procedures. This is also very common for the ESI sources producing single charged ions, in particular for atmospheric organic aerosol composition in the studied mass ranges at *m*/*z* 50–750.

d. Accurate mass measurements: As stated in our manuscript, the Orbitrap HRMS instrument was externally calibrated using Thermo Scientific Pierce standard calibration solutions before the measurement. Also, all the molecular formula assignments in this study were performed using an accurate mass tolerance of 2 ppm. Therefore, these procedures have guaranteed the high reliability of the mass accuracy measurement.

e. Elemental composition assignments: The organic molecular formulae were constrained to containing C, H, O, N, S elements with a mass tolerance of 2 ppm in this study, following the commonly acceptable HRMS constraints for atmospheric organic aerosols. Also, the assigned molecular species were further screened using elemental ratios of H/C (0.3–3.0), O/C (0–3), N/C (0–1.3), and S/C (0–0.8) to ensure their presence in nature. The formulas disobeying the nitrogen rule for even electron ions were also excluded from the assignment lists. These procedures ensure a high degree of precision in the assignment of elemental composition in the current study.

f. Complex data-dependent acquisition setups: In our current study, we did not employ the traditional data-dependent acquisition setup in the HRMS measurement. Rather, in order to capture the overall profiles of organic aerosols, we employed a non-target screening in full MS scan mode without fragmentation. As stated in the manuscript: "The mass range was set at m/z 50–750 **in full MS scan mode**, with the mass resolution of approximately 140,000 at m/z 200." (Lines 113–114). We have also carefully tuned the experimental conditions and acquisition parameters to ensure the reproducibility and minimal bias during the HRMS analysis.

References:

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II-2. In the present manuscript, structural elucidation relies solely on high-resolution mass spectra without MS/MS (including MS^2 and MS^3) validation for standards and key compounds in field samples, and without the provision of chromatograms, mass spectra, or MS/MS spectra for the main tracer species. These omissions limit the robustness and verifiability of the reported conclusions.

Response: It might be true in terms that the proposed MS/MS validation for standards and specific compounds would further strengthen the molecular structural elucidation of organic aerosols. In the current study, however, the extensive datasets were obtained from non-target screening in full MS scan mode using the HRMS. The non-target screening analysis herein allows for a more comprehensive investigation of massive organic species, rather than target screening analysis only for a limited number of specific compounds. Here the molecular structural elucidation is mainly related to the results regarding the aliphatics and aromatics in Figure 8 and relevant discussions in section 3.3. As summarized in Tables S1–S5 of the Supplement, hundreds of tracer species were identified in this study and also reported in literatures. The molecular structures of organic species were deduced according to the commonly reported aliphatic and aromatic regimes within the van Krevelen diagrams. In fact, the distributions of known tracer species did agree well with the two regimes, as seen in Figure 8a. Therefore, the method used for molecular formular elucidation in this study is different from those assumed by the referee, while the latter one is unrealistic for hundreds of organic compounds based on non-target screening analysis. Nevertheless, we have provided the chromatogram retention time to further identify each of those tracer species from the UHPLC–HRMS analysis, as presented in Tables S1–S5 of the Supplement.

II-3. Addressing these concerns would require substantial additional experimental work, including targeted MS/MS validation and supplementary analyses, which appear to go beyond the scope of a standard revision. In light of this, I believe the manuscript, in its current form, is not yet suitable for publication in the journal.

Response: As addressed above, the data processing strategies for the UHPLC–HRMS dataset were rigorously performed in our study, and the structural elucidation relies primarily on the commonly used approach, van Krevelen diagram analysis, which is more suitable for the non-target screening of massive organic molecules detected in full MS scan mode. While the referee proposed targeted MS/MS would to some extent further strengthen the structural elucidation, our analyses were constrained by stringent mass accuracy, isotopic pattern matching, and cross-validation with previous studies, which significantly enhances the reliability of the results.

Meanwhile, we hope the referee can understand that scientific research should be essentially proceeded step by step. It is not possible for a single study to cover multiple topics, instead, each study should have its own primary focus and research emphasis. In our current study, the main objective was to provide a comprehensive molecular-level overview of organic aerosol profiles and in-depth understand the influence of anthropogenic pollution on their chemical composition in the Qinling Mountains region, for which the non-target screening with HRMS is a powerful and well-adopted approach. Considering its novel findings and significance in the unique Qinling region, along with the rigorous procedures for data processing and careful interpretation, we believe that this study provides sufficient values for publication in ACP journal.

1. Majority of discussions in this work are based on the variation or comparison of different compound categories, while the ionization efficiency of different organic compounds varied a lot. I wonder if the authors consider or evaluate the influence of varied ionization efficiency of different compounds. How much the variations of molecular characterization across different seasons are caused by the changing of ionization efficiency?

Response: We believe that the referee's viewpoints here might have overlooked the fact that we did not report the absolute mass concentrations in the present study. Since we seriously understand the inherent technical limitation of ESI with the varied ionization efficiency (IE) for individual organic compounds, we did not directly refer the observed results and relevant discussions to their absolute mass concentrations throughout the entire study. There are some previous studies tried predicting the IE of different organic compounds from ESI-HRMS measurement (e.g., Bieber et al., 2023; Evans et al., 2024; Wang et al., 2025), however, the reported methods still have many uncertainties and further optimizations are required to improve their robustness in practical applications. Give that this is still an open question and no consensus conclusion reached in the entire HRMS research community so far, especially for studies using non-target screening methods, we therefore primarily focused on the variation patterns and comparison of organic species in terms of their species number and peak area in the present study.

Since the ionization efficiency was not applied to estimate the absolute mass concentration of individual compounds in our current study, the observed seasonal variations between summer and winter were exclusively caused by the actual changes of organic species, rather than the changing of ionization efficiency. Furthermore, the ESI-Orbitrap MS operation parameters in our study were controlled consistently during the measurement procedures for all samples, the reported peak area for individual organic compound was not likely affected substantially by the changing of ionization efficiency. Therefore, based on the parallel comparison of results derived from all the samples in the same measurement conditions, the seasonal changes of organic species can be well captured from this analysis.

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2. Line 375-380: In Figure 8b, the authors present example results for two days—August 30 (summer) and January 26 (winter)—in ESI- mode. However, the rationale for selecting these specific days is not clearly explained. Are these days representative in terms of meteorological conditions, pollution levels, or biogenic emissions? Without a clear

justification, it is difficult to assess whether the observed seasonal differences can be generalized. The authors should clarify the criteria used for day selection and explain how these examples reflect the broader trends discussed in Figure 8c.

Response: We could not agree with the referee regarding this point. The reason is explained as follows. Here the analyses of two individual days in Figure 8b is mainly used to establish the analytical method, whereas, more importantly, the final results based on this analysis for all the days across the entire study periods in both negative and positive ESI modes are summarized and presented in Figure 8c. Technically, there was (and should be) no specific criteria used for selecting these two example days. Give that it is impossible to present a massive dataset for all the individual days in a single graph, we therefore only exemplified the results of these two days on August 30 and January 26 for illustration purpose herein. Regardless of which days were selected to be present in Figure 8b, this will not affect the final results and conclusions drawn from the Figure 8c for the entire periods. Actually, the results of averaged species number and peak area fractions with error bars for all the samples across the entire study periods in Figure 8c did clearly reveal that the observed seasonal differences are generalized and reflect the broader trends between the two summer and winter seasons.

Therefore, to avoid any possible ambiguous understanding, we have revised and added the following statements in the main text to highlight the key points of Figure 8b and 8c:

"Figure 8b presents the example results **obtained in the ESI− mode** on two different days (that is, August 30 and January 26) **for illustration**." (Lines 375–376)

"**A statistical analysis was further performed for all the summer and winter samples detected in both ESI− and ESI+ modes across the entire study periods, as shown in Figure 8c, from which similar seasonal variation tendencies were also obtained as those of the above two example days.**" (Lines 380–382)

3. Lines 507–512 and Lines 336–339: The comparison of tracer species and the identification of clear indicators for organosulfates and nitrooxy organosulfates are noted.

However, in HR-MS methods as the one used in this study, several structures proposed by the software can be associated with each formula and confusing and challenging decisions should be made for structural elucidation when hundreds if not thousands of compounds are present in the sample. Although these techniques can provide important information, I believe challenges are still presents when using these analytical techniques and special care should be used when interpreting data and elucidating structure from HR mass spectra. Therefore, additional analysis (e.g. MS/MS) should be conducted!

Response: Since our extraction experiments and the UHPLC–HRMS analysis of aerosol filter samples in this study were performed around two years ago, there are currently no samples from the same campaign available for the MS/MS analysis. Nevertheless, in order to clarify the referee's concern here, we have performed another control experiment recently using the aerosol samples collected at the same site but in the second year, extracted the organic compounds using the same experimental procedures, and then analyzed the samples with the UHPLC–HRMS in both full MS mode and targeted MS/MS mode. An example result of the chromogram, MS1 spectrum, MS2 spectrum for one of the organosulfate compounds analyzed this time are presented below. Overall, for all the ten samples analyzed this time, the results showed good agreements between the assigned organosulfate (including nitrooxy-organosulfate) compounds from the accurate mass analysis and those detected from by the MS2 analysis. Specifically, a number of 665–779 organic species possessed the $-OSO_3H$ groups in the molecules for individual samples, which were roughly defined as organosulfates; within them, 560–570 of which were eventually further confirmed as organosulfates (such as with HSO_4^-, SO_4^-, and SO_3^- ion fragments) based on their MS2 analysis, accounting for 73%–85% of those proposed from the structural elucidation. This percentage should be even higher for the dataset used in our current study, because only the key tracer species were used for analysis and discussion in the manuscript. Therefore, based on the control experiment, we believe that the following statement regarding the structural elucidation method and relevant discussions are certainly reliable:

"Among these, organosulfate and nitrooxy-organosulfate compounds (abbreviated as OSs hereafter), roughly defined as those with sufficient oxygen atoms to assign the $-OSO_3H$ and

$-ONO_2$ groups in the molecules (Brüggemann et al., 2020; Lin et al., 2012b), were important components in both seasons. Note that the designated OSs here would correspond to those at the upper limit, since no further fragmentation spectra **were analyzed herein**.” (Lines 336–339)

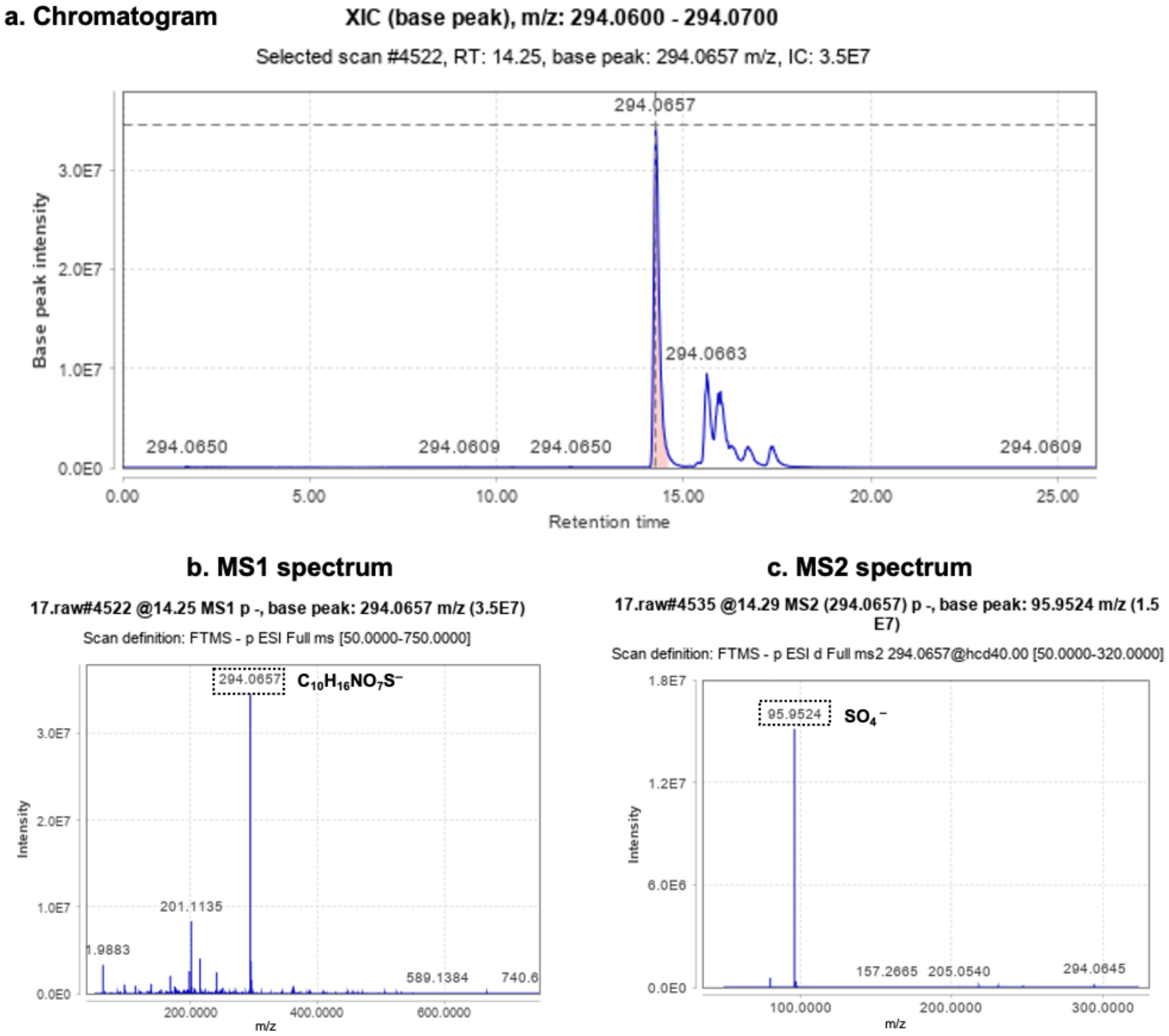

Figure. An example result of the chromogram, MS1 spectrum, and MS2 spectrum for an organosulfate compound, $C_{10}H_{17}NO_7S$, derived from the UHPLC–HRMS analysis in the control experiment.