We deeply appreciate for the detailed and valuable comments from the reviewers on our manuscript "Vertical and seasonal variations in airborne endotoxins in a coastal megacity of North China: insights from 3-hydroxy fatty acids" (MS No.: **egusphere-2025-2269**). We have revised the manuscript accordingly and we believe the quality of the manuscript has been improved. Each suggested revision and comment brought forward by the reviewers was accurately incorporated and considered. Our responses to the Reviewers' comments are described as follows. The revised highlighted version of the manuscript and supporting information are also provided below.

Response to Reviewer#1's comments

General comments

This study reported a comprehensive chemical analysis of 3-hydroxy fatty acids (3-OH-FAs; C_8–C_{18}) in PM_{10} sample measured at two heights (2 m and 220 m) above the ground level in four seasons at an urban location in the coastal megacity of Tianjin. The study used ultra-high performance liquid chromatography mass spectrometry combined with isotope labelling to characterize the 3-OH-FAs and used them as proxies for endotoxin, gram-negative bacteria, and bioactive endotoxin. First, the authors examined the vertical and temporal (i.e., diurnal and seasonal) differences in the concentrations of each 3-OH-FAs (C_8–C_{18}) homologue and endotoxin. The concentration data showed vertical and seasonal differences, with endotoxin concentrations peaking at 2 m in winter. Secondly, the authors performed a correlation test to examine the relationships between 3-OH-FAs, endotoxins, and environmental factors (meteorological parameters, physicochemical parameters, and air pollutants). The results showed that anthropogenic pollutants (e.g., organic carbon) were mainly associated with the long-chain 3-OH-FAs (C_{14}–C_{18}) homologues. Overall, some conclusions, particularly in Section 3.5 Influencing factors of airborne endotoxins, are very weak based on the methods and data analysis. I could not recommend the manuscript for publication in the Atmospheric Chemistry and Physics journal without improved data analysis method, at least one innovation findings, and environmental implications from this work for a broader perspective.

Response: Thank you for your careful reading of our manuscript and valuable comments. We understand your concerns and have undertaken a substantial revision to address these points. Major improvements are summarized below (page/line numbers refer to the marked-up version): new analyses were applied to strengthen key conclusions. Positive matrix factorization (PMF, v5.0) analysis of 19 measured aerosol components identified 5 factors that accounts for 3-OH-FAs in Tianjin urban aerosol (Section 3.6, Figure 8). Relative methods and quality control steps are detailed in a new Section 2.7 Positive matrix factorization (PMF). With source apportionment results resolved by PMF, novel findings of 3-OH-FAs source contributions in Tianjin urban aerosols were observed: The primary sources of both mid/long-chain 3-OH-FAs (C_{10} – C_{18}) are microorganisms; the contribution of secondary sources and natural sources is significant to mid-chain 3-OH-FAs (C_{10} – C_{13}), while anthropogenic combustion sources contribute more to long-chain homologues (C_{14} – C_{18}). Meanwhile, practical emission-control options (e.g., biomass burning) were implied, making the environmental implications of this study broader. In addition, we re-organized and clarified the Results and Discussion section to

sharpen the separation between observational correlations and potential mechanistic interpretations. We polished language, definitions, and figure/table captions for improved precision and readability as well.

We hope these revisions satisfy your comment for stronger analysis, a clear innovation, and explicit environmental significance, and that we believe the manuscript is now suitable for publication in the journal of *Atmospheric Chemistry and Physics*.

My specific comments are as follows:

Major comments:

Comment 1.

Please consider reporting all statistics to the same decimal place.

Response: Thank you for your comment. All statistical values (means, SD, SE, p-values, R^2, etc.) have been rounded to **one decimal places** throughout the manuscript, tables, and figures.

Comment 2.

3-OH-FAs (C8–C18) homologue group definition

In the Abstract, the authors indicate two groups: short-chain (C_8–C_{13}) and long-chain (C_{14}–C_{18}) 3-OH-FAs. The authors also indicate three groups: short-chain (C_8, C_9), mid-chain (C_{10}–C_{13}), and long-chain (C_{14}–C_{18}) 3-OH-FAs, according to hierarchical cluster analysis in Line 352.

Please consider applying a single homologue group definition for the measured 3-OH-FAs (C_8–C_{18}) homologues throughout the manuscript.

Response: Thank you for spotting this inconsistency. To ensure uniform terminology, we have adopted the **three-group scheme derived from the hierarchical-cluster analysis—short-chain (C_8 – C_9), mid-chain (C_{10} – C_{13}), and long-chain (C_{14} – C_{18})—throughout the entire manuscript**, including the Abstract, main text, figure captions, and Supplement.

Comment 3.

Section 2.6 Concentration-weighted trajectory (CWT) analysis and Figure 2

Please consider performing the CWT analysis at both sampling heights (i.e., 2 m and 220 m), given that the study examined the vertical concentration distribution of 3-OH-FAs. Additionally, please explicitly state the arrival height of the trajectories at the receptor location (i.e., the sampling location) during the sampling period.

Response: The meteorological condition at the ground level is highly sensitive to boundary layer processes. The accuracy of the HYSPLIT model at ground level is influenced by multiple factors, including meteorological data resolution, vertical parameterization, terrain complexity, and temporal dynamics (Su et al., 2015). Due to the spatial and vertical resolution (e.g., 1° or 2.5° GDAS data) of inputted meteorological data for the HYSPLIT model, trajectory accuracy starting at the height below 50 m is much lower (Godlowska et al., 2015; Hernandez-Ceballos et al., 2014). So it is generally recommended to set a higher above ground level (usually >100 m) to reduce calculating errors and perform HYSPLIT model and subsequent analysis. However, here we **presented the CWT analysis at the observation site (39°08'N; 117°22'E)**

of air mass backward trajectories arriving heights of 50 m, 100 m and 220 m separately, and it seemed there weren't much different between these heights.

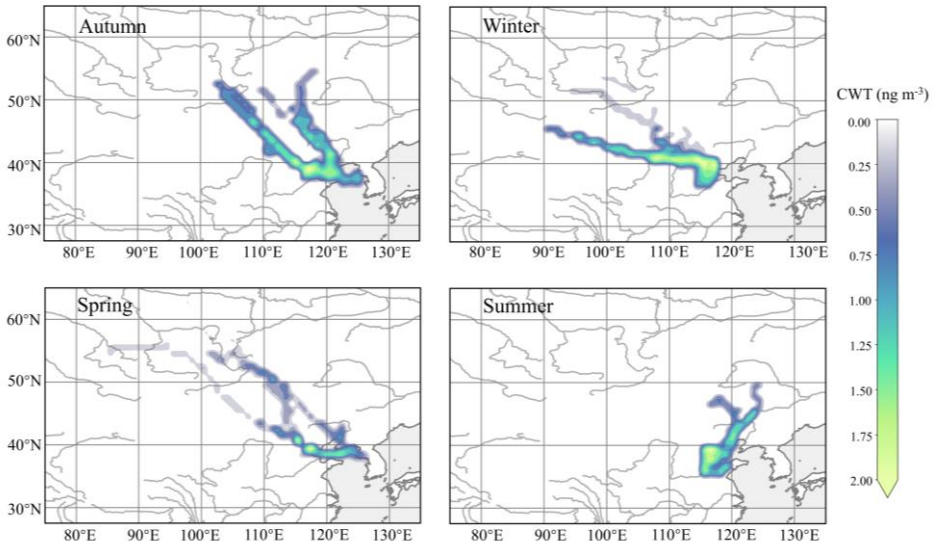

Figure R1. CWT analysis of air mass backward trajectories with an arriving height of 50 m at the observation site.

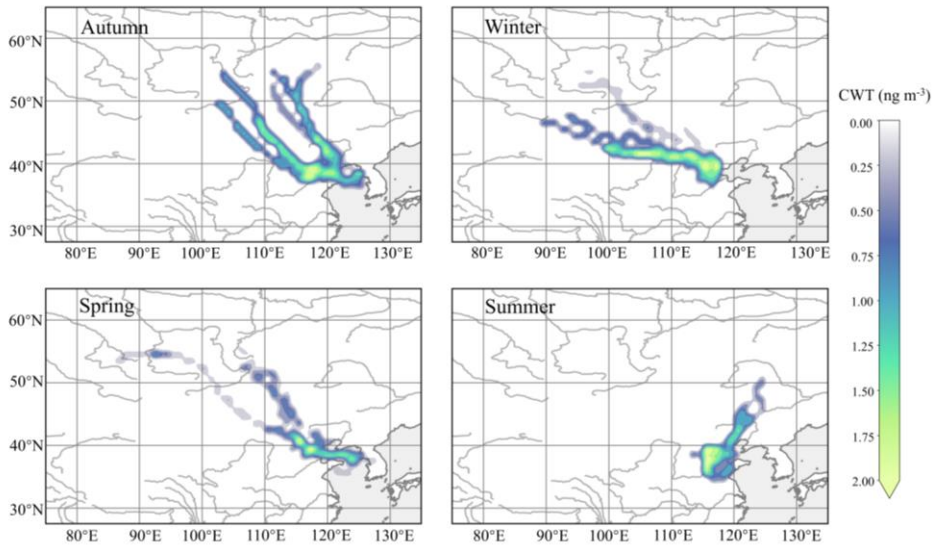

Figure R2. CWT analysis of air mass backward trajectories with an arriving height of 100 m at the observation site.

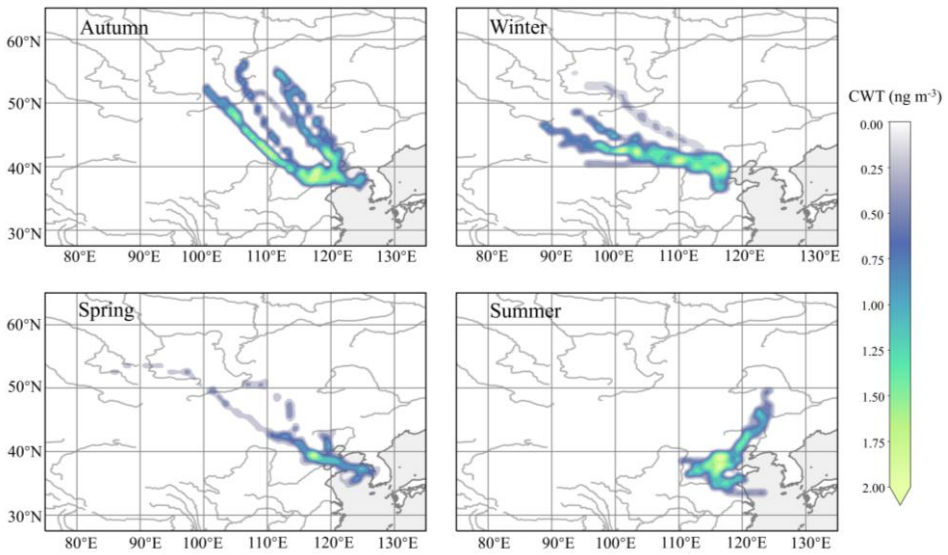

Figure R3. CWT analysis of air mass backward trajectories with an arriving height of 220 m at the observation site.

Lines 170–174, 182, 226, and so on

Please consider performing a statistical test to determine the concentration differences, particularly for the diurnal and seasonal differences in 3-OH-FAs concentrations. It is difficult to distinguish these temporal differences solely based on Figures 1(a) and 1(b).

Response: Thank you for your comment. Here we included the *p*-values from the paired sample (diurnal samples at 220 m/2 m) t-test results in Figures 1(a) and 1(c) (Table R1). We also presented t-test to illustrate seasonal variations of 3-OH-FAs in Figure 1(b) (Table R2). In addition, we added asterisks in Figures 1(a) and 1(c) to indicate the concentrations were significantly different.

Table R1. The *p*-values from the paired sample (diurnal samples) t-test results in Figures 1(a) and 1(c).

	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18
220 m	0.286	0.974	0.312	0.503	0.735	0.558	0.173	0.890	0.092	0.669	0.654
2 m	0.388	0.375	0.053	0.159	0.186	0.807	0.099	0.053	0.845	0.002*	0.140
2 m - 220 m	0.766	0.125	0.315	0.483	0.197	0.409	0.004*	0.010*	0.025*	0.015*	0.031*

Note: * The difference is significant ($p < 0.05$).

Table R2. The *p*-values from seasonal variation of 3-OH-FAs t-test results in Figures 1(b).

	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18
A-W (220 m)	0.001*	0.000*	0.015*	0.088	0.212	0.275	0.028*	0.331	0.006*	0.212	0.110
A-Sp (220 m)	/	/	/	/	/	/	/	/	/	/	/

A-Su (220 m)	0.114	0.001*	0.111	0.000*	0.000*	0.000*	0.001*	0.175	0.421	0.946	0.594
W-Sp (220 m)	/	/	/	/	/	/	/	/	/	/	/
W-Su (220 m)	0.003*	0.000*	0.000*	0.000*	0.000*	0.000*	0.735	0.812	0.012*	0.309	0.078
Sp-Su (220 m)	/	/	/	/	/	/	/	/	/	/	/
A-W (2 m)	0.016*	0.000*	0.034*	0.910	0.745	0.480	0.079*	0.276	0.036*	0.112	0.102
A-Sp (2 m)	0.014*	0.001*	0.026*	0.403	0.325	0.023*	0.344	0.003*	0.069	0.000*	0.002*
A-Su (2 m)	0.004*	0.010*	0.064	0.000*	0.000*	0.000*	0.094*	0.644	0.428	0.259	0.411
W-Sp (2 m)	0.811	0.110	0.920	0.565	0.625	0.171	0.460	0.004*	0.006*	0.000*	0.002*
W-Su (2 m)	0.960	0.006*	0.000*	0.000*	0.000*	0.000*	0.537	0.515	0.013*	0.028*	0.040*
Sp-Su (2 m)	0.730	0.173	0.000*	0.000*	0.000*	0.000*	0.772	0.006*	0.252	0.007*	0.047*

Note: The "A", "W", "Sp" and "Su" represent for "Autumn", "Winter", "Spring" and "Summer" separately; * The difference is significant ($p < 0.05$).

Lines 183–186, 226, 242, and so on:
Please provide the standard deviations for all values (concentration, ratio, percentage, etc.). Notably, the 3-OH-FAs (C_{16}) showed significant variations in winter based on Figure 1(b), so the standard deviations should be explicitly stated.
Response: Thanks for the advice. We added the standard deviation for all values mentioned above, as concentrations in line 213–216 and 254–257; ratios in line 257–258, 275–278.

Section 3.2 Spatial-temporal patterns of airborne endotoxin levels
Please consider rephrasing the section title. With only one sampling location, this section cannot support arguments regarding the spatial distribution of endotoxin levels.
Response: Thank you for pointing this out. Our intention was to emphasize the *vertical* (2 m vs. 220 m) and *temporal* (diurnal/seasonal) variability observed at a single urban site, rather than a broader horizontal (multi-site) spatial analysis. To avoid any misunderstanding, we have **revised the title of Section 3.2 to "Vertical-temporal patterns of airborne endotoxin levels."**

Lines 352, 354, and so on:
Please include the methods for the mental test and hierarchical cluster analysis in the Section 2 Materials and methods, they are used in the Section 3 Results and discussion.
Response: Thank you for your comment. We have **added a dedicated subsection entitled "2.7 Statistical analyses"** that now present both the *Mantel test* and the *hierarchical-cluster*

analysis as follows: "Mantel test was performed between 3-OH-FA homologues and those of meteorological/pollution variables using Spearman's correlation coefficient (Spearman's r) with 999 permutations, computed with the vegan package (v2.6-4) in R (v4.3.2). Significance was accepted at $p < 0.05$. Hierarchical-cluster analysis was performed via Ward's minimum-variance method on Euclidean distance using OriginPro."

Lines 364–365, 383–388, and so on:
The results regarding the influencing factors of variations in endotoxin concentrations are primarily correlational rather than causal. The wording should be carefully made. It would be an overstatement to interpret the different correlation directions and strengths as suggesting varying reaction, release, and presence mechanisms of 3-OH-FAs and endotoxin. Please consider tone down the arguments when elucidating how environmental parameters, particularly pollutants, affect the variations of endotoxin concentrations.

Response: We agree that our original wording risked overstating causality. The statistical relationships we report are indeed correlational. We have therefore **revised line 364–365 (now 403–407) into** "These meteorological conditions were favorable for photochemical reactions to occur (Huo et al., 2024; Pavuluri et al., 2015). While the data alone cannot prove a direct photochemical production route, the coincidence between intense photochemistry and the increase in these short/mid-chain (C_8 – C_{13}) 3-OH-FAs may point to photochemically driven secondary pathways of fatty acids that have been hypothesized in previous studies (Bikkina et al., 2019; Wakeham, 1999; Tyagi et al., 2015)."

We **revised line 383–388 (now 427–434) into** "Endotoxin mass concentrations were positively correlated with total oxidants in winter but negatively correlated in spring (Fig. 7e, f). These opposite signs most likely reflect season-specific controlling factors rather than a single universal mechanism. During winter the boundary layer experienced moderate oxidizing conditions (O_x=68–98 μg m^{-3}); such environments have been linked to oxidative stress and lysis of Gram-negative bacteria, which could increase aerosol-phase lipopolysaccharide (Hwang and Park, 2019; Mahapatra et al., 2018). In contrast, springtime O_x levels were even higher (78–126 μg m^{-3}), yet endotoxin decreased. One plausible explanation is a shift in microbial community composition: oxidant-tolerant taxa may dominate, while overall richness declines (Yin et al., 2021), leading to lower net release of endotoxin."

We believe that these changes adequately temper the interpretation and clearly distinguish correlation from causation. Thank you for helping us improve the balance of our discussion.

Lines 409–413
The results indicate that all 3-OH-FAs measured near ground level were significantly correlated with ions and air pollutants, suggesting their mixed sources of natural and anthropogenic origins, which are too general. Please consider performing a source apportionment analysis (e.g., Positive Matrix Factorization Model) of these measured chemical species data to better elucidate their sources.
Response: Thanks for the suggestion. Here we resolved 5 factors that accounts for 3-OH-FAs in Tianjin urban aerosol by applying **positive matrix factorization (PMF, v5.0) analysis**. Relative source apportion were discussed in Section 3.6 (Figure 8) (**line 466–489**). Methods

and quality control steps are detailed in a new Section 2.7 Positive matrix factorization (PMF) (**line 166–189**.

"2.7 Positive matrix factorization (PMF)

Source apportionment of 3-OH-FAs was carried out with EPA PMF 5.0 (U.S. EPA, 2014). PMF solves $X \approx GF$ by weighted least-squares minimization of:

$$Q = \sum_{i=1}^{n} \sum_{j=1}^{m} \left(\frac{x_{ij} - \sum_{k=1}^{p} g_{ik} f_{kj}}{u_{ij}} \right)^2 \quad (5)$$

where x is the observed concentration matrix, u the corresponding uncertainty matrix, G the factor-contribution matrix, and F the factor-profile matrix (Paatero and Tapper, 1994; Bhandari et al., 2022). Uncertainties (u) were calculated as recommended by the EPA guidance:

If the concentration is less than or equal to the species-specific method detection limit (MDL) provided, the uncertainty is calculated using a fixed fraction of the MDL (Equation 6).

If the concentration is greater than the MDL provided, the calculation is based on a user provided fraction of the concentration and MDL (Equation 7).

$$if\ x \leq MDL,\ u = \frac{5}{6} \times \mathrm{MDL} \quad (6)$$

$$\text{if } x > MDL,\ u = \sqrt[2]{(Error\ Fraction\ \times Concentration)^2 + (0.5 \times MDL)^2} \quad (7)$$

An error fraction of 0.2 was adopted for most species; values up to 0.6 were assigned to constituents near the detection limit.

A total of 19 measured species in 87 samples were used, including bulk carbonaceous fractions (OC, EC), major inorganic ions (K^+, Na^+, Ca^{2+}, Mg^{2+}, NH_4^+, NO_3^-, SO_4^{2-}, Cl^-) and nine 3-OH-FA homologues ($C_{10} - C_{18}$). Species selection followed three criteria: (1) Signal-to-noise ratio (S/N)—species with S/N ≤ 0.5 were excluded; 0.5 < S/N ≤ 1 were down-weighted ("weak") (Paatero and Hopke, 2003). Accordingly, C_{12} 3-OH-FA was removed. (2) Goodness-of-fit (R^2)—after trial runs, variables with persistently low R^2 between modelled and observed concentrations were discarded. (3) Q evaluation—models with 4–9 factors (p) were explored; the lowest Q value at five factors when moving from four to nine factors. the solution giving Qtrue/Qexp ratio closest to 1 was selected (Bhandari et al., 2022). The final configuration (five factors) yielded stable and physically interpretable profiles."

"3.6 Sources of 3-OH-FAs in Tianjin urban aerosols

Based on the PMF factor profiles, we identified 5 source factors (microorganisms, secondary nitrate formation process, secondary sulfate formation process, biomass burning, and dust) of PM_{10} from Tianjin.

Factor 1 was highly loaded on 3-OH-FAs, thus was mainly identified as microbial sources. Factor 2 was characterized by high loading of K^+ and Cl^-, which were mainly emitted from biomass burning (Srivastava et al., 2021; Hays et al., 2005). Factor 3 was heavily weighted by NH_4^+ and NO_3^-, which was typical of secondary nitrate formation process (Wang et al., 2019). Factor 4 presented high loaded of two crustal elements Ca^{2+} and Mg^{2+}, mainly emitted from dust sources (Huang et al., 2016). Factor 5 indicated secondary sulfate formation process, which was identified by high concentrations of NH_4^+ and SO_4^{2-}.

The contributions of different sources to 3-OH-FAs in PM_{10} were estimated (Fig. 8). Mid-chain 3-OH-FAs (C_{10}, C_{11}, C_{13}) were predominantly contributed by microorganisms (42.6–54.4 %), and also associated with secondary sulfate formation process (17.3–29.7 %) and dust sources (20.6–27.7 %). This result indicates substantial inputs from microorganisms associated with soil dust resuspension

and aging of primary aerosols. Long-chain 3-OH-FAs (C_{14} – C_{18}) were likewise primarily originated from microorganisms (41.5–57.1 %), with notable contribution from biomass burning (16.5–31.4 %) and associated with secondary nitrate formation process (5.2–23.2 %). Overall, microorganisms represent the primary source of both mid- and long-chain 3-OH-FAs (C_{10} – C_{18}), whereas secondary processes and biomass burning provide additional influences.

The different behaviors of sulfate and nitrate further highlight source-specific processes. According to Guo et al. (2010), sulfate aerosol forms predominantly on a regional scale, while nitrate formation is typically controlled by local processes. The observed ratio of water-soluble K^+/SO_4^{2-} in Tianjin PM_{10} (0.04 at 2 m, 0.03 at 220 m) were much lower than one in the primary biomass burning particles (Kleeman et al., 1999), indicating a regional transport origin. Moreover, higher relative contributions of mid-chain 3-OH-FAs at 220 m (Fig. 4b) support their attribution to regional transport process similar to sulfate, whereas the enrichment of long-chain homologues at 2 m (Fig. 4b), together with their association with nitrate formation process, suggests a stronger influence from local combustion and secondary formation.

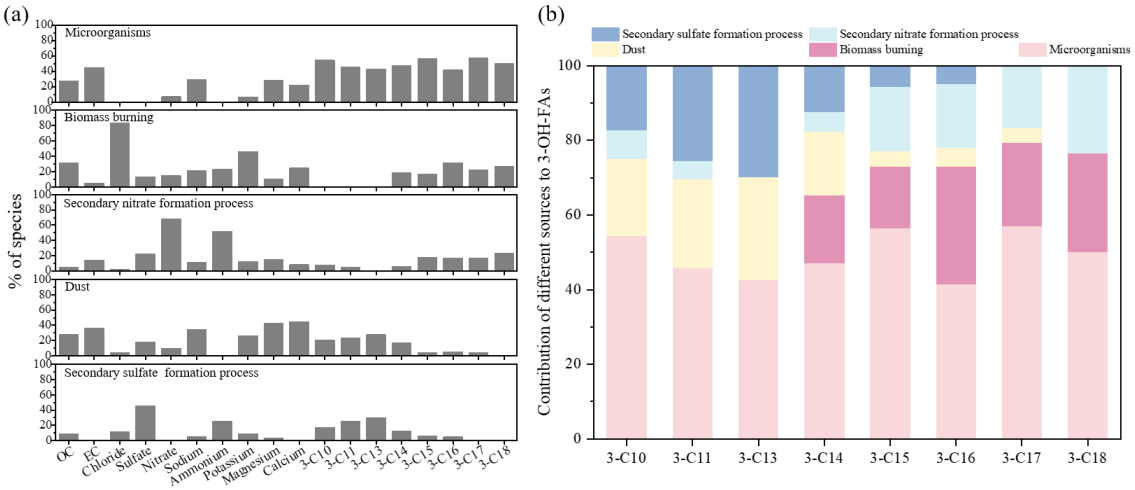

Figure 8. Source apportionment of 3-OH-FAs in Tianjin urban aerosols. (a) Factor profiles for the 5-factor solution from PMF analysis. (b) Contribution of different sources to 3-OH-FAs."

Figure 1:

Please include the meaning of the small figures (C_8 – C_{12}, 3-OH-FAs) in both Figures 1(c) and 1(d).

Response: The small figures in Figure 1(c) and 1(d) are the enlarged view of species with small concentration differences, namely 3-OH-FAs (C_8–C_{12}). We have **added the meaning in the figure caption.**

Minor comments

Line 27: Please define the acronyms "OC" and "WSOC" in the abstract for the benefit of general audiences.

Response: We spelled out OC in the abstract and deleted WSOC for simplicity. We also **define both "OC" and "WSOC" in the following section Materials and methods (line 147).**

Lines 92, 109, 122, 146, and so on: Please include a space between number and unit (i.e., 255 m, 450 °C, 4 °C, ±10 %, and so on).

Lines 111 and so on: Please use a minus sign to express the negative number (i.e., –20 °C).
Lines 129–130: Ranges need an en dash and no spaces between start and end (i.e., 0–3).
Lines 146–147, and 149: Please state the instrument company's state if it is a U.S. company.
Line 148: Please use a superscript minus sign to state the negative charges of the anions (i.e., Cl^-, NO_3^-, and SO_4^{2-}).
Lines 154–155: Please define the acronym "HYSPLIT" for the benefit of general audiences and include the reference (Stein et al., 2015) for HYSPLIT.
Line 159: Coordinates need a space when naming the direction (i.e., 20–80° N and 60–150° E).
Lines 167, 208, and so on: Ranges need an en dash and no spaces between start and end (i.e., minimum–maximum, 2020–2021, and so on).
Line 209: Please include a space before and after the sign (i.e., $p < 0.05$).
Line 370: Please consider replacing "gaseous pollutants" as "trace gas", as the study includes CO_2.
Response: Thanks for pointing out issues with writing format. We have **corrected the writing problems above.** We **added the definition of the acronym "HYSPLIT"** in line 159 and included the reference. As for the main focus of the article is on other gaseous pollutants (O_3, SO_2, NO_2, and CO), CO_2 is not a trace gas and hardly mentioned in the results and discussion. we would like to keep the original wording.

Figure 4
1) Please explain the meaning of the horizonal dash line in figure 4(a).
Response: Thank you for noting that the caption needed clarification. The horizontal dashed line represents the season-average endotoxin concentration. We have **amended the caption accordingly: "The horizontal dashed line denotes the seasonally averaged PM_{10} endotoxin level."**

Figure 6
1) Please explain the meaning of the square size in the figure caption for the benefit of the general audiences.
Response: We agree the legend required more detail. The caption now **states that square area is proportional to the absolute value of Spearman's r: larger squares indicate stronger correlations, and smaller squares indicate weaker correlations.**

Table 2:
1) Please define the acronym "TSP"
2) The column names "Sample types" and "References" should be provided in singular form.
Response: Thanks for the reminder. We **added "TSP, total suspended particle" to the note of Table 2. The column names now read "Sample type" and "Reference" throughout Table 2.**

Tables S1 and S2:
The column name "Analytes" should be provided in singular form.
Response: Thanks for the reminder. The column name **"Analytes" were carefully changed into singular form "Analyte".**

Figure S2:

2) Please explain the meaning of the circle size and asterisk in the figure caption for the benefit of the general audiences.

3) The labels and circles are overlapping along the diagonal line. Please consider moving the text a little further away from the circles.

Response: Thanks for the reminder. The caption now **clarifies that circle diameter is proportional to the correlation coefficient and that asterisks mark significant correlations at $p <= 0.05$.** We have **shifted the labels to eliminate overlap,** improving readability of Figure S2.

Response to Reviewer#2's comments

General comments

Zhang et al. analysed the 3-hydroxy fatty acids in inhalable particles from urban Tianjin at 2m and 200m in different seasons to investigate the vertical, seasonal and diurnal variations in airborne endotoxins. They then identified the potential influencing factors of 3-hydroxy fatty acids and endotoxin levels. They found higher total endotoxin concentrations at ground level compared to 200 m, peaking in winter due to the combined contributions of near-ground emissions from both natural and anthropogenic sources, as well as different control factors affecting the levels of short-chain and long-chain 3-hydroxy fatty acids and endotoxins. The study aligns with the journal's scope. However, there are a few shortcomings (outlined below) in the current version of the manuscript that require attention.

Response: Thank you very much for your thoughtful evaluation of our manuscript. We are pleased that you find the topic aligned with the journal's scope and appreciate your concise summary of our main findings. We have carefully considered each shortcoming you have outlined and have revised the manuscript accordingly. Below, we provide a detailed, point-by-point response, indicating where each revision appears in the tracked-changes manuscript. We hope these changes satisfactorily address your concerns and enhance the overall quality of the paper.

Major comments:

Comment 1.

Lines 216-217, The authors concluded that "the endotoxins at 220 m in Tianjin might be originated either from the vertical transport of ground emissions or long-range transport from marine areas". However, the conclusion drawn here differs from or contradicts the concentration-weighted trajectory (CWT) analysis in Section 3.1. The evidence should support one another, and the authors should present a consistent statement throughout the manuscript.

Response: Thank you for catching this inconsistency. Here we **modified the wording in line 247-249to** "Therefore, the endotoxins at 220 m in Tianjin might be originated from the vertical transport of ground emissions, long-range transport from northwest of Tianjin or from marine emissions (e.g., Bohai Sea) as depicted in CWT results in Section 3.1." With these changes, the discussion of vertical vs. transported sources is now fully consistent across Sections 3.1 and 3.2. We appreciate the reviewer's suggestion, which has improved the coherence of our manuscript.

Comment 2.

From Table 2, we can see that the concentrations of endotoxin in PM_{10}, $PM_{2.5\text{-}10}$, and $PM_{2.5}$ from the same region are significantly different. Therefore, it is not reasonable to make a direct comparison of the endotoxin concentrations of samples from different regions with different sample types.

Response: We fully agree that particle-size fraction strongly affects measured endotoxin concentrations; comparing different fractions (e.g., $PM_{2.5}$ vs. PM_{10}) across regions can therefore be misleading. Consequently, we now **restrict our inter-regional discussion to PM_{10} data and cite TSP values only as an upper limit reference. Lines 345-349** have been revised as follows: "Dry-mass concentrations of GNB in Tianjin PM_{10} are markedly higher than those reported for Hong Kong PM_{10} in 2002–2003 (Lee et al., 2004) and even exceed GNB levels measured in TSP at remote marine sites such as Chichijima (Japan) (Bikkina et al., 2021b), Tokyo (Japan) (Bikkina et al., 2021a), and Gosan (Korea) (Tyagi et al., 2017). These findings underscore the exceptionally heavy bioaerosol burden in Tianjin and highlight the urgency of mitigating biological—as well as chemical—particulate pollution."

Comment 3.

Although both NO_2 and O_3 are oxidants, their effects on the concentration of endotoxins appear to be contrasting (Figures 7b and c). In this case, I would not suggest combining them when considering the seasonal variation of endotoxin with oxidant.

Response: We appreciate the reviewer's point. The opposing correlations we observe—positive with NO_2, negative with O_3—indeed show that a composite O_x (= NO_2 + O_3) can mask the distinct behavior of its individual components. However, in our study O_3–endotoxin correlation is weaker ($r = -0.41$), suggesting that NO_2 largely drives the composite signal in our dataset. Meanwhile, O_x is a widely used proxy for the overall oxidizing capacity of the lower troposphere. Thus, we insist on using the O_x as total oxidants to see the synergistic effect of oxidants on endotoxins.

Specific comments

Lines 74-76, According to Binding et al., 2004, it is easier to produce unsaturated 3-fatty acids with acid hydrolysis rather than alkaline hydrolysis.

Response: Though according to the reference, the main purpose was to address that "using alkaline instead of acidic hydrolysis and cool on-column instead of split/splitless injection, elimination was reduced to an acceptable level", it was also proposed "the formation of 14:1 FA (fatty acids) still could not be avoided". Thus, here we **quoted it just to illustrate the possibilities that traditional alkaline hydrolysis process may cause the formation of unsaturated fatty acids, not to compare with acid hydrolysis.**

Lines 115-119, Abbreviations of EtOAc, ACN, CMPI, TEA and DMED should be introduced for their first occurrence.

Response: Thanks for the advice. We have **added the full forms of these abbreviations** for their first occurrence in **line 121-123**.

Line 208, The author states that there was no obvious diurnal difference in total endotoxin levels; however, the variation is actually very large, especially in Winter.

Response: Thank you for your comment. Here we attached the *p* values of the paired t-test (two-tailed) of daytime and nighttime endotoxin levels grouped by sampling heights and seasons. As we present here, all *p* values are larger than 0.05, thus we drew the conclusion that the diurnal differences in total endotoxins are not significant.

Table R3. The *p* values of the paired t-test (two-tailed) of daytime and nighttime endotoxin levels at different heights during different seasons.

	Autumn	Winter	Spring	Summer
220 m	0.456	0.091	-	0.369
2 m	0.483	0.718	0.148	0.119

Line 322-323, This sentence is not clear to me, please rephrase it.

Response: Sorry for the unclear expressions. Here, we **rephrased the sentence in line 363-367 into** "It was previously assumed that pyrogenic carbon or smoke produced by biomass burning provides temporary habitat for microbes aerosolized from soils and vegetations (Bonfantine et al., 2024; Kobziar and Thompson, 2020). Meanwhile, particulate matter in smoke confers attenuation of ultraviolet-B (UVB) and UV-A radiation, further leading to increasing bioaerosol viability and higher microbial cell concentration (Mims et al., 1997; Moore et al., 2021)."

Figure 5, Please explain the TSP and DECOS in the figure caption.

Response: Thanks for the advice. We have **added the full forms of TSP and DECOS** in the figure caption.

Response to Reviewer#3's comments

General comments

The manuscript presents a valuable investigation into the vertical and seasonal distribution of airborne endotoxins in Tianjin, China, using 3-hydroxy fatty acids (3-OH-FAs) as biomarkers. The study addresses an important gap in understanding endotoxin dynamics in urban boundary layers, particularly in coastal megacities. The methodology is robust, combining advanced analytical techniques (UHPLC-MS) with comprehensive environmental monitoring. However, several aspects require clarification or improvement to enhance the manuscript's impact and readability.

Response: Thank you for your positive assessment of our work and for recognizing its contribution to understanding vertical and seasonal endotoxin dynamics in an urban coastal boundary layer. We appreciate your remarks on the robustness of our UHPLC-MS methodology and the comprehensive environmental dataset. At the same time, we value the constructive suggestions you offered to improve clarity and impact. We hope these revisions below address your concerns and further strengthen the manuscript. A detailed point-by-point response follows on the next pages.

Major comments:

Comment 1.

Justify the use of the factor "4" (lipid A carries 4 mol 3-OH-FAs) and "8000" (average endotoxin molecular weight). Cite specific literature for these assumptions, especially the factor "4".

Response: We applied these assumptions in previous literature we cited, and here we **added (Laitinen et al., 2001; Rietschel et al., 1984), which clearly pointed out the use of the factor "4".** Those two factors were commonly used in other literatures while estimation endotoxins by biomarkers 3-OH-FAs (Cheng et al., 2012; Lee et al., 2004; Bikkina et al., 2021a; Tyagi et al., 2016).

Comment 2.

The higher endotoxin levels at 2 m vs. 220 m are attributed to "near-surface emissions," but specific sources (e.g., soil, traffic, marine) should be quantified or discussed.

Response: Thanks for your valuable suggestion. We have **added further discussion to line 452–459:** "According to Figure S2, endotoxins at the near ground level (2 m) attributed by long-chain 3-OH-FAs (C_{15} – C_{18}) are correlated ($p \leq 0.05$) with chloride (Cl^-) and sodium (Na^+). Na^+ is generally used to characterize the impact of marine aerosols, as their usual source is sea salt, with Cl^-/Na^+ mass ratio of 1.8 (Martens et al., 1973; Wang and Shooter, 2001). The mass ratio of Cl^-/Na^+ (2.9 ± 3.5) at the near ground level was much higher than that of sea salt aerosols. This result indicated the impact of anthropogenic emissions, especially combustion emissions (Wang et al., 2005; Wang et al., 2012), on those 3-OH-FAs. Moreover, the positive correlation ($p \leq 0.05$) of endotoxins with secondary inorganic species (SO_4^{2-}, NO_3^-, NH_4^+) and crustal species (Ca^{2+}, Mg^{2+}) separately, suggesting the possible sources of 3-OH-FAs (C_{10} – C_{18}) from atmospheric secondary processes (Jung et al., 2009) and soil dust (Huang et al., 2006)."

Comment 3.

The near-ground winter peak in PM_{10} from urban Tianjin is linked to "enhanced emissions," but is this due to local heating (biomass/coal combustion) or meteorological inversions?

Response: Thanks. We **checked the meteorological parameters, and temperature inversion during winter was taken as main meteorological factor.** Inversions were observed in all seasons. However, only 2 nights during the observation period in winter were under the influence of inversions. While during autumn and summer, 3 nights with inversions were observed. In addition, as in Figure S2, the PM_{10} concentrations were positively correlated to OC, K^+, Cl^-, and SO_4^{2-} (as well as the relative high concentration of SO_2 in winter in Figure 3(d)), regarded as biomass or coal combustion indicators in most cases (Hays et al., 2005; Wang et al., 2005). Thus, we conclude **the near-ground winter peak in PM_{10} from urban Tianjin is linked to enhanced local heating.**

Comment 4.

Figure 1: The diurnal/nocturnal variations in 3-OH-FAs are intriguing but lack statistical significance testing (e.g., p-values for day/night differences).

Response: Here we **included the *p* values from the paired sample t-test results (diurnal samples at 220 m/2 m) in Figures 1(a) and 1(c)**. And we added asterisks to areas where there were significant differences in Figures 1(a) and 1(c).

Table R4. The *p*-values from the paired sample (diurnal samples) t-test results

	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18
220 m	0.286	0.974	0.312	0.503	0.735	0.558	0.173	0.890	0.092	0.669	0.654
2 m	0.388	0.375	0.053	0.159	0.186	0.807	0.099	0.053	0.845	0.002*	0.140
2 m - 220 m	0.766	0.125	0.315	0.483	0.197	0.409	0.004*	0.010*	0.025*	0.015*	0.031*

Note: * The difference is significant ($p < 0.05$).

Comment 5.

Figure 5: Include error bars for literature data where available to facilitate comparison.

Response: Thanks. Here we **use the value of standard derivation for error bar, and we have included data available in the literature**, however for one literature we quoted, which compared endotoxin concentrations of three sites (Nansha, Guangzhou, Hongkong), the standard derivations and data set were not given.

Minor comments

Update citations to include recent studies on urban endotoxins.

Response: Thanks for the advice. We have **updated citations** to include recent studies in **line 40, line 44 and line 422-423.** "Endotoxins may transport via adhering to the surface of fine inorganic particles (Zhang et al., 2024; Hwang et al., 2021)." "LPS can be oxidized by ozone and the resulting reactant greatly enhanced inflammatory anemia (Liu et al., 2025)." "When

exposed to higher O_3, oxidation of the hydroxyl group in the 3-OH-FAs may turn fatty acid chain in to carbonyl group, thus cause the decrease in detected 3-OH-FAs and estimated endotoxins (Liu et al., 2025)."

Delete (b-c) in second line of Figure 4.
Response: Thanks for the advice. We have fixed that.

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