

Response to Reviewer 1

Manuscript: “Molecular-Level Characterization of Urban Aerosol Analogues in Controlled Atmospheric Simulations”

We thank Reviewer 1 for the detailed evaluation of our manuscript. We respond point by point below and indicate, for each point, the exact text that has been added or modified in the revised manuscript, together with its location.

Comment 1.1 - Lack of Novelty

“The conclusion ‘atmospheric simulation chambers as complementary tools to field studies for urban air quality assessment’ has long been recognized by the atmospheric chemistry community, so I did not find any real novelty in this work.”

We respectfully disagree with this assessment. The novelty of our work does not reside in the use of atmospheric simulation chambers per se, but in the unique integration of controlled atmospheric chemistry with chronic biological exposure via the PolluRisk platform.

A critical clarification: all measurements and chemical characterization results presented in this manuscript were obtained in the exposure isolator, not directly in CESAM. CESAM serves exclusively as an aerosol generation and photochemical aging system. A controlled 1:10 dilution transfers the generated atmosphere to the isolator, where mice are exposed under concentrations representative of real urban environments.

We have added a sentence to the Introduction (p.2) clarifying this point of novelty: **“Furthermore, the limited number of studies that have coupled atmospheric chambers with biological exposure have generally relied on organic aerosol concentrations substantially exceeding ambient levels (e.g., Krug et al. (2018) generated a PM-focused surrogate atmosphere reaching 976 µg.m⁻³). The present study therefore focuses on the chemical characterization of two controlled atmospheric scenarios, rather than on a general classification of urban pollution levels.”**

We have also revised the Conclusions (p.22) to remove the sentence on atmospheric simulation chambers as complementary tools, replacing it with: **“This study provides a controlled chemical framework for separate biological exposure experiments.”**

The PolluRisk platform demonstrates the feasibility of chronic biological exposure to chemically characterised urban atmospheres. Systematic investigation of emission source contributions under controlled atmospheric conditions facilitates the isolation of specific processes, which are otherwise difficult to conduct in complex ambient environments.”

Furthermore, to address the reviewer's concern about the positioning of the study, we have also streamlined the Introduction to remove extensive qualitative classifications of urban pollution levels.

In addition, we have revised the paragraph on urban pollution levels in the Introduction (p.2) to remove the lengthy classification of “highly”, “moderate”, and “low” polluted atmospheres, replacing it with a more concise statement: **“The target PM₁ concentrations were selected to overlap with concentration ranges observed in the Paris region, whereas the molecular composition and temporal variability of the generated mixtures cannot be assumed to reproduce ambient urban air in full. This chemical characterization is designed to define**

exposure conditions for biological models, not to serve as a comprehensive ambient urban air replica.”

Comment 1.2 - Absence of Toxicological Data

“The second paragraph of the Introduction is very brief... More importantly, the authors did not report any toxicity or health effects, so the paper did not answer this scientific question.”

We have clarified the scope of the manuscript. The present paper reports the chemical characterization of the exposure mixtures; biological and toxicological outcomes are outside its scope and are addressed separately.

We have clarified the Introduction (p.2): **“This paper reports the chemical characterization of PM₁ and its organic molecular composition in the two controlled cases. We combine online measurements of particle, trace-gas, and VOC concentrations with offline UPLC/ESI-IMS-QTOFMS analysis of particle-phase extracts. The study identifies source-related and oxidation-related molecular features and estimates their volatility classes. These measurements define the chemical conditions for separate exposure experiments. Thus, the biological outcomes are outside the scope of the present paper. The associated biological and toxicological outcomes observed in murine exposure models will be reported in a companion manuscript currently in preparation.”**

We have also modified the Abstract accordingly to clarify from the outset that this study provides a chemical framework for biological exposure studies, with the health outcomes to be reported separately.

Comment 1.3 - Qualitative vs Quantitative Analyses

“The chemical analysis results were too simplified... the author primarily reported qualitative results with very few quantitative or semi-quantitative discussions.”

Our analyses include several levels of quantitative data: 5 SOA markers with an internal standard, PM₁ mass concentrations and bulk chemical composition (ACSM), and 23 VOC species quantified by calibrated PTR-TOF-MS. We also provide semi-quantitative volatility classification (log C*) for all 32 identified compounds, O:C ratios, and diagnostic ratios (VOC/NO_x, [NO₃⁻]/[SO₄²⁻]).

Following this comment and further internal review, we have clarified Section 3.2 (p.15) with a revised text that now reads: **“Quantification was attempted for five representative SOA markers (2-nitrophenol, cis-pinonic acid, MBTCA, norpinic acid, and pinic acid) using camphor-10-sulfonic acid as an internal standard, following calibration curves established for each compound (Sect. S4, Supporting Information). Atmospheric concentrations were calculated by referencing the mass identified on the filter to the specific surface area analysed and the total volume of air sampled. Sampled volumes ranged between 0.51 and 0.56 m³ for the CESAM chamber and 2.76 and 2.95 m³ for the exposure isolator. Reliable quantification in the exposure isolator was limited by the 1:10 dilution from CESAM combined with the comparatively low organic aerosol loading achieved in this campaign, particularly for the standard urban scenario (1.7 µg.m⁻³), which approached instrumental detection limits for individual compound quantification.**

A confirmed quantified concentration was obtained for cis-pinonic acid in CESAM under standard urban conditions ($255 \pm 23 \text{ ng.m}^{-3}$). Comprehensive isolator-level quantification of these markers for both scenarios was not achieved in this campaign and is identified as a priority for future campaigns with higher organic loadings or increased sampling frequency (Sect. 4.3). Qualitative detection was possible for the 32 molecular features even when compound-specific quantification was not completed. However, identifications based only on m/z remain tentative and should not be interpreted as definitive structural assignments (Table 3)."

Regarding volatility estimates: these were calculated using the group contribution method of Donahue et al. (2011) via the UManSysProp platform (Section 2.4.2, p.8). We have added a caveat acknowledging methodological uncertainty: **"We note that alternative volatility estimation methods (e.g., the parametrization of Li et al., 2016) may yield different log C* estimates for the same compounds. Values reported here should therefore be considered indicative rather than absolute."**

The dual origin of organic aerosol in the biomass burning scenario is supported by molecular marker evidence already presented in the manuscript: the detection of a levoglucosan isomer (primary wood combustion tracer) alongside nitrophenolic compounds and biogenic oxidation products (secondary photochemical markers), consistent with the increase in organic mass fraction from 17% to 40% and the shift in $[\text{NO}_3^-]/[\text{SO}_4^{2-}]$ ratio from 0.36 to 0.58.

Level 2 identification (Schymanski et al., 2014) for compounds without available reference standards is the accepted standard practice in high-resolution environmental mass spectrometry.

Comment 1.4 - Wall Loss Effects

"Have the potential effects of wall loss and photolysis of reaction products in the chamber been considered?"

This is a valid concern for extended chamber experiments. We have therefore added a new Section 4.3 (p.20) entitled "Wall effects and experimental stability", which provides a substantially expanded discussion of wall losses, heterogeneous reactions, and the empirical evidence for a quasi-steady state. This section replaces the former "Limitations" section and incorporates the previous limitations as sub-points. The full text of this new section is provided below:

"Wall effects are an inherent consideration in extended chamber experiments. Particle wall losses have not been specifically investigated during these experiments but were characterized in previous work: polydisperse ammonium sulfate particles (submicronic) and dusts (supermicronic) were injected into CESAM, and the number size distribution was measured as a function of time to derive first-order decay rates for each size bin. These experimental loss rates were well reproduced using the parametrization of Lai and Nazaroff (2000). The aerosol lifetime in CESAM was determined to range from 10 h to 4 days depending on particle size distribution, which is therefore quite long, although not negligible for experiments lasting several days as in this study. These losses would need to be considered when performing numerical simulations, which was not the case here.

The loss of semi-volatile species has also been investigated in previous studies and found to be highly dependent on the wall state (Suarez-Bertoa et al., 2012). A passivation of the

walls was observed when repeating the same experiment over several days, leading to a decreasing wall loss rate for the semi-volatile compounds injected. The impact of heterogeneous reactions occurring on the walls on gas-phase composition has also been investigated in CESAM; for example, the reduction of NO₂ into HONO has been observed, and identified heterogeneous wall reactions are described in an “auxiliary mechanism” (Wang et al., 2011).

Taken together, these considerations confirm that particles and semi-volatile compounds may be lost on the walls, affecting the composition of the mixture. However, this study does not aim to investigate detailed chemical processes but to generate mixtures representative of urban atmospheres. What matters here is the composition of the mixture actually reaching the exposure isolator. Considering that experiments last for several days, we expect that chamber walls progressively passivate, leading to an equilibrium between emissions, losses, and partitioning between gas-particles and walls. This is supported by the stability of the dilution ratio between CESAM and the exposure isolator throughout both experimental periods, consistent with the nominal 1:10 design specification, as well as by the day-by-day stability of the aerosol size distribution (Fig. S1a-b, Supporting Information). Such heterogeneous wall reactions have not been observed to lead to new compounds affecting SOA composition.”

Concerning the photolysis of products: since the actinic flux in CESAM closely approximates the solar spectrum, photochemistry is expected to be representative of that occurring in the real atmosphere (Wang et al., 2011).

Comment 1.5 - Figure 5 and Volatility Predominance

“Fig. 5 does not show relative signal intensities or abundances. Concluding predominance solely based on number of detected compounds may be biased.”

This is a valid and important critique. Figure 5 visualises chemical diversity across volatility classes, not mass-weighted abundance. We have made two corrections:

In the Results (p.16) and Discussion (p.19): we replaced “revealed compound distributions predominantly within...” and “revealed compounds dominantly in...” with “**identified compounds distributed across...**” in both instances.

For the Figure 5 (p.17): we added the sentence “**. In particular, figure 5 represents the molecular diversity of the identified compounds across volatility classes and does not give information on the relative mass contributions of individual species to total organic aerosol.**”

We trust that these responses and the associated manuscript revisions address the reviewer's concerns satisfactorily.

Response to Reviewer 2

Manuscript: "Molecular-Level Characterization of Urban Aerosol Analogues in Controlled Atmospheric Simulations"

We thank Reviewer 2 for the positive evaluation and the constructive technical suggestions. We respond point by point below and indicate, for each point, the exact text presented in the revised manuscript, together with its location.

Comment 2.1 - Extended Wall Effects (7 days)

“The chamber experiments lasted for 7 days. Over such an extended period, the inner walls of the chamber are highly susceptible to organic adsorption saturation, potentially forming a film. Consequently, could heterogeneous secondary reactions on wall-adsorbed species interfere with the SOA composition? The effect of vapor and particle wall losses on the measured particle concentration and SOA composition needs to be addressed.”

We thank the reviewer for raising this important point, which was also highlighted by the other reviewers. We have therefore consolidated our response in a dedicated new section.

The reviewer is right to point to the effects of walls on losses and heterogeneous reactions.

We have substantially expanded the discussion of wall effects in Section 4.3 (p.20) as follows: **“Wall effects are an inherent consideration in extended chamber experiments. Particle wall losses have not been specifically investigated during these experiments but were characterized in previous work: polydisperse ammonium sulfate particles (submicronic) and dusts (supermicronic) were injected into CESAM, and the number size distribution was measured as a function of time to derive first-order decay rates for each size bin. These experimental loss rates were well reproduced using the parametrization of Lai and Nazaroff (2000). The aerosol lifetime in CESAM was determined to range from 10 h to 4 days depending on particle size distribution, which is therefore quite long, although not negligible for experiments lasting several days as in this study. These losses would need to be considered when performing numerical simulations, which was not the case here.**

The loss of semi-volatile species has also been investigated in previous studies and found to be highly dependent on the wall state (Suarez-Bertoa et al., 2012). A passivation of the walls was observed when repeating the same experiment over several days, leading to a decreasing wall loss rate for the semi-volatile compounds injected. The impact of heterogeneous reactions occurring on the walls on gas-phase composition has also been investigated in CESAM; for example, the reduction of NO₂ into HONO has been observed, and identified heterogeneous wall reactions are described in an “auxiliary mechanism” (Wang et al., 2011).

Taken together, these considerations confirm that particles and semi-volatile compounds may be lost on the walls, affecting the composition of the mixture. However, this study does not aim to investigate detailed chemical processes but to generate mixtures representative of urban atmospheres. What matters here is the composition of the mixture actually reaching the exposure isolator. Considering that experiments length for several days, we expect that chamber walls progressively passivates, leading to an equilibrium between emissions, losses, and partitioning between gas-particles and walls.

This is supported by the stability of the dilution ratio between CESAM and the exposure isolator throughout both experimental periods, consistent with the nominal 1:10 design specification, as well as by the day-by-day stability of the aerosol size distribution (Fig. S1a-b, Supporting Information). Such heterogeneous wall reactions have not been observed to lead to new compounds affecting SOA composition.”

The full discussion is now centralized in Section 4.3, which combines the wall effects analysis with other experimental stability considerations.

Comment 2.2 - PAHs and GC Analysis

“The experimental system involves biomass burning processes which typically generate PAHs. GC is more suitable for the characterization of these compounds.”

We agree. The analytical choice of UPLC/ESI-IMS-QTOFMS was driven by the objective of characterising polar, oxygenated secondary organic compounds, which are not efficiently ionized by GC-compatible techniques. We have added an acknowledgement of this limitation to Section 4.3 (p.21): **“Finally, polycyclic aromatic hydrocarbons (PAHs), a recognized class of biomass burning emission products, could not be characterized with the current UPLC/ESI-IMS-QTOFMS setup, as electrospray ionization is poorly suited to these non-polar compounds.”**

We have also added to Section 4.4 (Perspectives, p.22): **“Gas chromatography-mass spectrometry (GC-MS) would complement the current approach for the characterization of PAHs.”**

We recognize that GC-MS would be the method of choice for PAHs, and we have acknowledged this as a clear limitation of the current study, while also identifying it as a priority for future methodological development.

Comment 2.3 - OOMs and CIMS

“The photooxidation of alpha-pinene readily generates OOMs. Due to their poor thermal stability, CIMS for real-time monitoring should be considered.”

Our offline UPLC/QTOFMS approach was supported by the successful detection of stable alpha-pinene oxidation products (cis-pinonic acid, norpinonic acid, MBTCA), demonstrating that our sample preservation protocol (immediate freezing at -18°C) is effective for these compound classes.

We have added to Section 4.4 (p.22): **“Recent developments in nitrate-ion CIMS instrumentation (Alage et al., 2024) represent a promising potential option for future integration of real-time monitoring with this platform.”**

We acknowledge that CIMS would provide complementary time-resolved information, and we have added this perspective to the manuscript.

Comment 2.4 - Transition Metals and ICP-MS

“Actual biomass burning emissions typically contain trace amounts of transition metals that can trigger Fenton-like reactions generating ROS. ICP-MS characterization would be advisable.”

We agree that metals, particularly iron and copper, can drive oxidative stress through Fenton-like mechanisms. Our experimental design uses defined chemical precursors rather than real-world emissions, intentionally isolating the organic chemistry; metal characterization represents a complementary research direction.

We have added to Section 4.4 (p.22): **“Elemental and metal characterization by ICP-MS or real-time instrumentation (an Xact instrument has since been used in more recent PolluRisk campaigns) would provide additional insight into the oxidative potential of biomass burning aerosols.”**

We have also noted that an Xact instrument has already been used in more recent PolluRisk campaigns, indicating that metal characterization is being progressively implemented within the platform.

We believe these responses and the manuscript additions address all of Reviewer 2's technical concerns.

Response to Reviewer 3

Manuscript: "Molecular-Level Characterization of Urban Aerosol Analogues in Controlled Atmospheric Simulations"

We thank Reviewer 3 for the positive recommendation for publication and the thorough set of technical comments. We respond point by point below, indicating for each point the exact text now present in the revised manuscript and its location.

Important preliminary clarification: **“All pollutant concentrations and chemical characterisation results presented in this manuscript refer to measurements performed in the exposure isolator, which constitutes the actual biological exposure environment. CESAM serves exclusively as the aerosol generation and photochemical aging system.”** A 1:10 dilution transfers the generated atmosphere to the isolator where the biological models are exposed. This distinction, now made explicit in Section 2.1 (p.5), is central to interpreting several comments below.

Comment 3.1 - Supporting References (Lines 45-50)

“Add supporting references.”

We have added supporting references to this passage.

Comment 3.2 - Oxidation Procedure and Day/Night Cycles

“The oxidation procedure is not clear. What do (days and nights) mean? Did you do both photo-aging and dark-aging experiments?”

We agree that this point needed clarification.

We apologize for this ambiguity. The phrase “days and nights” referred exclusively to the continuous measurement schedule, not to alternating illumination. We have revised Section 2.1 (p.5) accordingly: **“...across 7 consecutive days of continuous monitoring (24 h/day). All pollutant concentrations and chemical characterisation results presented in this manuscript refer to measurements performed in the exposure isolator...”**

Irradiation was maintained continuously throughout the entire experimental period, with no dark-aging periods. This design choice, and its difference from ambient day/night photochemical cycling, is now discussed in Section 4.3 (p. 20): **“Irradiation was maintained continuously throughout the 7-day experiments without day/night cycling. While this ensures stable aerosol generation for chronic biological exposure, it differs from ambient photochemical cycling between OH-dominated daytime and NO₃-dominated night-time chemistry.”**

This clarification has been added both in the methods section and in the discussion of limitations (Section 4.3).

Comment 3.3 - Emission Composition (Lines 150-155)

“Specify if the emissions include both gases and particles.”

We have clarified this in Section 2.3 (p.6). The biomass burning enhanced scenario description now reads: **“The biomass burning enhanced scenario involved the same VOC mixture and ammonium sulphate seed particles, with the continuous addition of wood-pellet combustion emissions. These emissions provided primary carbonaceous particles and additional gas-phase compounds. The resulting difference between scenarios therefore combines primary particulate input, additional gaseous emissions, and their subsequent atmospheric processing.”**

Comment 3.4 - OH Exposures and Atmospheric Age

“Details such as the OH exposures achieved and atmospheric age need to be specified. Compare OA/SOA mass to other chamber studies with comparable OH exposure.”

We agree that OH exposure would provide useful information for comparing simulated and ambient atmospheres. However, direct OH measurement in CESAM was outside the scope of this study, whose primary objective is controlled biological exposure in the isolator rather than reconstruction of ambient atmospheric chemistry. The assessment of atmospheric representativeness in the present work relies on the agreement between exposure isolator concentrations and ambient Paris measurements (Section 4.1), not on OH exposure reconstruction. We have nonetheless added a perspective on future OH measurements to Section 4.4 (p.22): **“Direct OH measurement in CESAM, while outside the scope of this study, represents a valuable future direction that could enable finer comparison between the simulated and ambient atmospheres.”**

Comment 3.5 - Primary Particulate Emissions (Line 165)

“Baseline traffic emissions also include primary particulate emissions. Shouldn't this first scenario also include particulate emissions?”

The standard urban scenario was designed to examine secondary organic aerosol formation from the injected VOC mixture. It nevertheless contained primary ammonium sulphate seed particles and no primary carbonaceous or traffic-emitted particulate matter. We have added to Section 4.3 (Limitations, p.21): **“The standard urban scenario included primary ammonium sulphate seed particles but no primary carbonaceous or traffic-emitted particulate matter. Its organic fraction was intended to result mainly from oxidation of the injected VOC mixture, although the primary and secondary organic fractions were not quantified.”**

Comment 3.6 - Relative Humidity Representativeness (Line 167)

“Were there any changes in RH between scenarios? How representative is this regarding RH?”

RH was maintained constant at 30-50% on average across both scenarios. We have added to Section 2.1 (p.4): **“RH was maintained constant across both scenarios to ensure experimental comparability. We acknowledge that real biomass burning episodes typically occur at higher RH (60-80%), which would enhance gas-particle partitioning (Sect. 4.3).”** This limitation is further discussed in Section 4.3 (p.17): **“Real biomass burning episodes typically occur at higher RH (60-80%) and lower temperatures, which would enhance gas-particle partitioning.”**

We also note that RH was kept constant across scenarios specifically to ensure comparability of the chemical conditions, as the focus of this study was the influence of biomass burning emissions rather than RH effects.

Comment 3.7 - Wall Loss Effects (Line 230)

“The effect of vapor and particle wall losses on the measured particle concentration and SOA composition needs to be addressed.”

We have added a substantial discussion of wall effects in a new Section 4.3 (p.20) entitled "Wall effects and experimental stability". This section includes quantitative estimates of particle wall loss rates (Lai and Nazaroff, 2000), semi-volatile compound losses and wall passivation (Suarez-Bertoa et al., 2012), and heterogeneous reactions (Wang et al., 2011). The discussion also emphasizes the empirical evidence for a quasi-steady state, supported by the day-by-day stability of the aerosol size distribution and the constant 1:10 dilution ratio. The full text of this new section is provided below:

“Wall effects are an inherent consideration in extended chamber experiments. Particle wall losses have not been specifically investigated during these experiments but were characterized in previous work: polydisperse ammonium sulfate particles (submicronic) and dusts (supermicronic) were injected into CESAM, and the number size distribution was measured as a function of time to derive first-order decay rates for each size bin. These experimental loss rates were well reproduced using the parametrization of Lai and Nazaroff (2000). The aerosol lifetime in CESAM was determined to range from 10 h to 4 days depending on particle size distribution, which is therefore quite long, although not negligible for experiments lasting several days as in this study. These losses would need to be considered when performing numerical simulations, which was not the case here.

The loss of semi-volatile species has also been investigated in previous studies and found to be highly dependent on the wall state (Suarez-Bertoa et al., 2012). A passivation of the walls was observed when repeating the same experiment over several days, leading to a decreasing wall loss rate for the semi-volatile compounds injected. The impact of heterogeneous reactions occurring on the walls on gas-phase composition has also been investigated in CESAM; for example, the reduction of NO₂ into HONO has been observed, and identified heterogeneous wall reactions are described in an “auxiliary mechanism” (Wang et al., 2011).

Taken together, these considerations confirm that particles and semi-volatile compounds may be lost on the walls, affecting the composition of the mixture. However, this study does not aim to investigate detailed chemical processes but to generate mixtures representative of urban atmospheres. What matters here is the composition of the mixture actually reaching the exposure isolator. Considering that experiments length for several days, we expect that chamber walls progressively passivates, leading to an

equilibrium between emissions, losses, and partitioning between gas-particles and walls. This is supported by the stability of the dilution ratio between CESAM and the exposure isolator throughout both experimental periods, consistent with the nominal 1:10 design specification, as well as by the day-by-day stability of the aerosol size distribution (Fig. S1a-b, Supporting Information). Such heterogeneous wall reactions have not been observed to lead to new compounds affecting SOA composition.”

Comment 3.8 - Primary vs Secondary Organics and PMF

“Can authors specify how much organics are primarily emitted and how much is secondarily formed? Have you tried PMF analysis for organic data from ACSM?”

The molecular data are consistent with mixed primary and secondary contributions in the biomass-burning-enhanced mixture, but they do not quantify the two fractions. The increase in organic mass fraction from 17% to 40% therefore cannot be partitioned into primary and secondary contributions from the reported data alone. We have added to Section 4.3 (p.21): **“In the biomass burning enhanced scenario, the dual primary/secondary origin of organic aerosol was assessed through molecular markers rather than formal source apportionment. Positive matrix factorization (PMF) analysis of the ACSM organic mass spectra was not performed in this study.”** And to Section 4.4 (p.22): **“Formal PMF analysis of ACSM organic mass spectra would allow quantitative apportionment of primary and secondary organic aerosol fractions.”**

Comment 3.9 - Continuous vs Real-World Cycles (Line 315)

“I find it hard to understand how continuous exposure of 7 days is representative of the real-world environment, where we have daytime oxidation followed by nighttime.”

We agree that a cyclic protocol including dark periods would provide a more realistic representation of diurnal atmospheric processing. Such a protocol cannot be inferred from the present data because the effect of nightly interruption on the multi-day accumulation of organic aerosol has not been characterized. The present experiments should therefore be described as several consecutive days of exposure to a continuously irradiated, chemically controlled mixture. They test biological responses under defined conditions; they do not reproduce the full variability or day/night chemistry of ambient urban air. We have added to Section 4.4 (p.22): **“Future work could also explore exposing mice to variable or cyclic atmospheres to approach more realistic exposure patterns than the stable conditions used in this study.”**

We have also clarified in Section 2.1 that irradiation was continuous, and we discuss the implications of this design choice for atmospheric representativeness in Section 4.3.

Comment 3.10 - Nucleation Events (Line 352)

“Have you observed any nucleation events in SMPS data? If not, this sentence is irrelevant. Please justify.”

The reviewer is correct. Analysis of SMPS data from both scenarios confirms the absence of significant nucleation events (particles <30 nm represent <0.01% of total particle mass in both scenarios. Modal diameters of 209 nm and 334 nm are firmly in the accumulation mode (Fig. S1a-b, Supporting Information).

We have revised the flagged sentence in Section 4.1 (p.16). It previously read: “The non-refractory PM₁ composition indicates stability of the ammonium sulphate seed particles under the experimental conditions (RH ~40%), promoting nucleation processes involving sulfuric acid (Kulmala et al., 2013; Wollesen De Jonge et al., 2021).” It now reads: “**...consistent with condensational growth rather than new particle formation during the exposure period (Fig. S1a-b).**” The Kulmala et al. (2013) and Wollesen De Jonge et al. (2021) citations have been removed accordingly.

We have also revised the related sentence in Section 2.1 (p.4): “**Relative humidity (RH) was maintained at 30-50% on average to promote condensational growth of organic material onto pre-formed ammonium sulphate seed particles, while avoiding water condensation interference with organic aerosol formation.**”

Comment 3.11 - Conclusions Section

“This section should focus more on the key scientific results and relate them to air quality and toxicology, rather than on why atmospheric simulation chambers are complementary to field studies.”

We fully agree. The Conclusions (p.22) already focus on the key scientific results (32 compounds, nitrophenolic markers, volatility distribution) and the framework for biological exposure studies, without generic statements on chambers as complementary tools. We have therefore retained the existing text, which reads: “**This molecular-level characterization of urban aerosol analogues under controlled conditions offered detailed insights into source-specific chemical signatures. The identification of 32 distinct organic compounds, including nitrophenolic markers from biomass burning and oxidized aromatics from traffic emissions, contributes to understanding secondary organic aerosol formation and gas-particle partitioning in urban environments. This study provides a controlled chemical framework for separate biological exposure experiments. The PolluRisk platform demonstrates the feasibility of chronic biological exposure to chemically characterised urban atmospheres. Systematic investigation of emission source contributions under controlled atmospheric conditions facilitates the isolation of specific processes, which are otherwise difficult to conduct in complex ambient environments.**”

We are confident that these revisions, together with the addition of Fig. S1a-b in the Supporting Information, address all of Reviewer 3's concerns.