We thank Reviewer 1 for their positive assessment of the novelty and scientific value of this work and for their thoughtful critique. Below, we provide point-by-point responses (in blue), with cited or newly added text indicated in *italic.* Crossed-out text indicates content that has been removed through tracked modifications. We hope that our responses significantly improved the clarity of the manuscript.

Reviewer 1 general comments: This study represents a highly novel and scientifically valuable attempt to experimentally investigate interactions between atmospheric PFAS and bioaerosols (Pseudomonas fluorescens) under controlled chamber conditions. However, there are substantial limitations in how the wastewater treatment plant (WWTP) environment is represented by the experimental design, particularly the use of a 40:60 water–methanol mixed solvent and aerosol generation via a Collison nebulizer. The reduced surface tension resulting from the presence of methanol creates a physical environment that differs markedly from natural bubble-bursting processes in wastewater aeration basins. As a consequence, the aerosol size distributions observed in this study appear to be strongly governed by the imposed experimental conditions rather than by intrinsic physicochemical properties of the PFAS compounds themselves. The authors are clearly aware of these limitations and have discussed them to some extent, which is appropriate.

Response: We appreciate Reviewer's positive evaluation of the novelty and scientific merit of this work, as well as their constructive comments. We believe there is misunderstanding regarding the scope of the work, which was not to replicate WWTP aerosol formation or internal mixing conditions found at WWTPs, but to carry out a controlled laboratory investigation focused on isolating fundamental aspects of PFAS interactions with biological particles introduced as a seed aerosol (as stated in the title, objectives and throughout the text), e.g. "The aim of this study was to advance understanding of PFAS aerosol formation and size distribution *under controlled laboratory conditions, with particular focus on the potential role of bioaerosols as carriers.*" Lines (105-106), original document.

WWTPs are referenced in the Introduction solely as an example of environments where PFAS and bioaerosols may co-occur, rather than as systems intended for experimental reproduction. The study is therefore framed as a mechanistic investigation in which bacterial particles are introduced as a pre-existing particulate surface to evaluate their influence on PFAS aerosol formation and size-resolved behaviour**.**

To improve clarity regarding the scope and intent of the work, we have revised the statements related to WWTPs and expanded the contextual framing of the present study in the Introduction:

> "*Experiments were conducted under controlled chamber conditions using a water–organic solvent system, in the absence/presence of the model bacterium Pseudomonas fluorescens seed,* ***to investigate the potential influence of microbial presence on PFAS behaviour*** ~~*representative of wastewater-impacted environments*~~." Line 18, revised document.
>
> " *Understanding whether and how biological matter influences PFAS aerosol behaviour is therefore essential for* ~~*accurately*~~ *assessing emission pathways from engineered systems,* ~~*such as*~~ ***including but not limited*** *to WWTPs.*" Lines 73-74, revised document.

"*WWTPs represent one example of environments in which PFAS and biological aerosols may co-occur, highlighting the broader relevance of understanding PFAS–bioaerosol interactions, rather than serving as a system directly replicated in this study*." Lines 74-78, revised document.

"*Pseudomonas fluorescens is a ubiquitous freshwater bacterium, frequently reported in rivers, lakes, and surface waters, where it occurs in the water column, sediments, and biofilms, supporting its relevance as a representative organism for aquatic and wastewater-influenced systems (Baum et al., 2009; Batrich et al., 2019*)." Lines 113-114, revised document.

Reviewer Comment: The interpretation of the MPPD modeling results raises concerns. The authors conclude that the largely similar size distributions observed across most PFAS, regardless of molecular structure, are a consequence of physical constraints imposed by the nebulization process. If this is indeed the case, then the resulting modeled respiratory deposition should likewise be viewed primarily as an artifact of the experimental setup, rather than as compound-specific behavior. In this context, the subsequent discussion of differential inhalation risks among individual PFAS appears insufficiently justified.

I therefore encourage the authors to more critically acknowledge the dominant role of the experimental configuration in shaping the results and to revise the scope and framing of their interpretation accordingly. On this basis, I recommend Major Revision.

Also similar/relevant comment #3 from the same reviewer: The MPPD modeling results are inherently driven by particle size. According to the manuscript, particle size in this study was strongly influenced by experimental conditions such as solvent composition and the use of a nebulizer. Given this, it is unclear whether interpreting the modeled deposition results on a compound-specific basis is appropriate.

Moreover, while the authors acknowledge that the experimental setup does not fully represent real environmental conditions, it remains questionable whether predicting the magnitude and regional distribution of respiratory deposition is truly meaningful or environmentally representative. The limitations associated with applying the MPPD model to aerosol size distributions generated under these experimental constraints should therefore be explicitly discussed, and the interpretation of the modeling results should be framed accordingly.

Response: We thank the reviewer for this comment and for the opportunity to clarify the intent and interpretation of the MPPD modelling.

We agree that respiratory deposition predicted by the MPPD model is primarily governed by aerosol size. The MPPD results are not intended to predict absolute exposure levels for specific environmental scenarios. Instead, they translate experimentally measured size distributions into size-resolved deposition patterns, allowing comparison across experimental conditions and compounds. This size-based interpretation remains valid regardless of the specific aerosol source, because deposition in the respiratory tract is determined by particle size rather than by the process by which the particles are generated.

For PFAS in particular, inhalation exposure remains poorly constrained relative to ingestion pathways, and size-resolved deposition data are largely absent. The modelling therefore provides a first-order framework for interpreting how aerosolised PFAS associated with submicron particles may deposit within the respiratory tract. These results can be used as

reference points for future field measurements and for evaluating the relevance of different aerosol generation processes that produce similar particle size ranges.

To ensure clarity, we revised the manuscript (both Results and Discussion and Conclusion sections) to explicitly state that the MPPD modelling is used to examine size-resolved respiratory deposition behaviour based on the measured particle size distributions, rather than to directly quantify population-level exposure under specific environmental scenarios:

> "*The modelling is not intended to represent population-level exposure or the full range of environmental conditions, but rather to evaluate size-resolved respiratory deposition behaviour for the measured aerosol populations*." Lines 465-467, revised document.

> "*It must be noted that MPPD modelling serves to contextualise size-resolved respiratory deposition for the observed aerosol populations, not to represent population-level exposure or comprehensive environmental scenarios. However, our results can be used as reference points for future field measurements and for evaluating the relevance of different aerosol generation processes that produce similar particle size ranges*." Lines 493-496, revised document.

Comment 1: A key concern is that the mixing state between bacteria and PFAS in the chamber experiments differs fundamentally from the environmental scenario described in the Introduction. Bacteria and PFAS were aerosolized separately using different generators, resulting in external mixing in the chamber air. In contrast, wastewater treatment plant aeration produces internally mixed aerosols because microorganisms and PFAS are already mixed in the aqueous phase prior to bubble bursting. Given the low particle concentrations and short residence times in chamber studies, the probability of meaningful interaction between separately generated bacterial aerosols and PFAS droplets in air is very low. Therefore, the reported lack of bacterial influence on PFAS aerosol formation may be a consequence of the experimental design rather than a true absence of interaction.

The authors should more clearly acknowledge this limitation and restrict the scope of their conclusions, or modify the experimental setup to better represent internal mixing conditions relevant to wastewater environments.

Response: We thank the reviewer for the comment and agree that the mixing state between bacteria and aerosols can influence aerosol formation and interactions. We would like to emphasise that the aim of this study was not to replicate WWTP aerosol formation or internal mixing conditions, but to investigate potential PFAS interaction with biological particles introduced as a seed aerosol.

As clarified above, WWTPs are referenced in the Introduction solely as an example of environments where PFAS and bioaerosols may co-occur, rather than as systems intended for experimental reproduction. Co-nebulisation of PFAS and bacteria from the same solution would increase the likelihood of PFAS–bacteria interactions during aerosol generation due to shear and rapid deformation of the liquid at the nebuliser nozzle, combined with PFAS lipophilic properties, favouring their "in-solution" association prior to aerosolisation and resulting in internally mixed droplets. Such conditions would complicate separation of aqueous-phase and aerosol-phase processes and hinder mechanistic interpretation.

Separate aerosolisation was also necessary, because PFAS required dissolution in a methanol–water mixture to minimise losses due to their strong surface activity, and premixing

this solution with live bacteria would have compromised bacterial viability. While internal mixing of PFAS and biological material prior to aerosolisation may occur in some environmental systems, this represents a different mechanism that was beyond the scope of the present study.

As noted by the reviewer, particle concentrations and residence times can influence interaction probability. In this study, PFAS aerosol concentrations (< 0.15 ng/m^3) were close to those typically encountered under ambient environmental conditions, while bacterial aerosol number concentrations were approximately 29 ± 1 cm^{-3}. Aerosols were sampled continuously over chamber residence times of up to about 1 h, with dilution during operation taken into account. The absence of a measurable influence of bacterial particles on PFAS aerosol formation or size-resolved behaviour under these conditions suggests that PFAS–bacteria interactions in the aerosol phase are unlikely to represent a dominant process under environmentally relevant concentrations and aerosol lifetimes.

Importantly, we acknowledge that internal mixing between bacterial material and PFAS in environmental systems may lead to outcomes that differ from those reported here: "*These results suggest that biological material exerts only a minor influence on PFAS partitioning through the airborne pathway examined here; however, aqueous-phase sorption or complexation before aerosolisation may still contribute to water-to-air transfer and warrants further investigation*." Lines 454-457, original manuscript.

To improve clarity regarding the scope and intent of the work, we have revised the statement around WWTPs in the Introduction and refined the framing of the Conclusions accordingly:

> "*Experiments were conducted under controlled chamber conditions using a water–organic solvent system, in the absence/presence of the model bacterium Pseudomonas fluorescens seed,* ***to investigate the potential influence of microbial presence on PFAS behaviour*** ~~*representative of wastewater-impacted environments*~~." Lines 16-18, revised document.
>
> " *Understanding whether and how biological matter influences PFAS aerosol behaviour is therefore essential for* ~~*accurately*~~ *assessing emission pathways from engineered systems* ~~*such as*~~ ***including but not limited*** *to WWTPs*." Lines 73-74, revised document.
>
> "*WWTPs represent one example of environments in which PFAS and biological aerosols may co-occur, highlighting the broader relevance of understanding PFAS–bioaerosol interactions, rather than serving as a system directly replicated in this study*." Lines 74-76, revised document.
>
> *"We emphasise that in environmental systems where PFAS and biological matter may also be internally mixed in aqueous sources prior to droplet formation, different interaction mechanisms may occur."* Lines 415-417, revised document.

Comment 2: Wall loss is likely to vary as a function of PFAS chain length, yet the manuscript appears to assume that relative comparisons remain valid because all compounds experience wall loss. It is not clear that this assumption is justified. If wall-loss efficiencies differ systematically with chain length, then the observed trends may be influenced, at least in part, by differential losses rather than intrinsic behavior. Therefore, in sections where chain-length-

dependent trends are discussed, this limitation should be explicitly acknowledged and its potential impact on the interpretation of the results should be clearly addressed.

Response: We thank the reviewer for this comment. We agree that the current phrasing could lead to misinterpretation and have therefore rephrased the statement in the Methods section to explicitly reflect potential limitations associated with wall losses:

> *"Moreover, wall-loss efficiencies may vary with PFAS chain length and could influence absolute recoveries. All experiments were therefore conducted under consistent chamber configuration and operating conditions to minimise variability in wall-loss behaviour and to allow relative comparisons within a common experimental framework."* Lines 233-236, revised document.

It is also worth noting that the highest filter-collected PFAS masses were observed for long-chain PFCAs, consistent with previously reported behaviour of PFAS (Pandamkulangara Kizhakkethil et al., 2024; Pandamkulangara Kizhakkethil and Kourtchev, 2025). Preferential wall losses would be expected to reduce the filter-collected mass of long-chain PFAS (Folorunsho et al., 2024). Such losses would therefore act in the opposite direction to the observed trend and would introduce a negative bias in the measured magnitude rather than produce the reported pattern. This point has also been clarified in the Results and Discussion:

> "*While a clear chain-length-dependent increase in aerosol-phase PFCA concentrations is observed, any preferential wall losses of longer-chain compounds would act to reduce their measured recovery and therefore bias the magnitude of this increase towards lower values*." Lines 264-266, revised document.

Comment #3: The MPPD modeling results are inherently driven by particle size. According to the manuscript, particle size in this study was strongly influenced by experimental conditions such as solvent composition and the use of a nebulizer. Given this, it is unclear whether interpreting the modeled deposition results on a compound-specific basis is appropriate.

Moreover, while the authors acknowledge that the experimental setup does not fully represent real environmental conditions, it remains questionable whether predicting the magnitude and regional distribution of respiratory deposition is truly meaningful or environmentally representative. The limitations associated with applying the MPPD model to aerosol size distributions generated under these experimental constraints should therefore be explicitly discussed, and the interpretation of the modeling results should be framed according**ly.**

Response: See response for MPPD comment above.

Minor comments

1. Line 85-99 The current discussion appears to rely heavily on listing information rather than synthesizing it. Reorganizing and more clearly integrating the key points would help condense the content and improve overall readability.

Response: As suggested, this paragraph has been rewritten.

2. Line 127 Please check the superscripts.

Response: Corrected, thank you.

3. Line 170 Please check whether 109 cells/mL is correct.

Response: It should be 10^9, thank you.

4. Line 156 and 174 Both L/min and lpm are used in the manuscript. It would be preferable to use a single, consistent unit throughout.

Response: The units have now been harmonised. Thank you for spotting this.

5. Figure 1. For readers who are not familiar with PFAS abbreviations, it may be difficult to infer chain length from compound names alone. You may want to consider reorganizing the compounds by grouping them into the same classes (e.g., PFCAs, PFSAs) and arranging them in order of chain length within each group to improve clarity and readability.

Response: As requested, the PFAS in Figure 1 have been rearranged along the x axis according to carbon chain length. This is clarified in the figure caption: "*PFAS are arranged along the x axis according to carbon chain length*."

6. Figures. Please consider expanding the figure caption slightly. At present, the caption mainly describes what is shown in the figure. Adding one sentence that highlights the key takeaway or main message of the figure would help readers better understand its significance.

Response: We have added a brief interpretative sentence to figure captions where a clear and unambiguous takeaway can be provided, in line with Copernicus guidelines.

> Figure 1. …"*Overall, similar aerosol-phase PFAS concentrations are observed with and without biological material, indicating no systematic bacterial influence*."
>
> Figure 2. …" *The dominant fraction of Pseudomonas fluorescens present in the chamber was observed around 0.6 µm*."
>
> Figure 3…" *Most PFAS exhibit a unimodal mass–size distribution centred near 0.3 µm, indicating dominant fine-mode aerosol association across compounds*."

References:

Batrich, M., Maskeri, L., Schubert, R., Ho, B., Kohout, M., Abdeljaber, M., Abuhasna, A., Kholoki, M., Psihogios, P., Razzaq, T., Sawhney, S., Siddiqui, S., Xoubi, E., Cooper, A., Hatzopoulos, T., and Putonti, C.: Pseudomonas Diversity Within Urban Freshwaters, Front Microbiol., 10, 195, https://doi.org/10.3389/fmicb.2019.00195, 2019.

Baum, M. M., Kainović, A., O'Keeffe, T., Pandita, R., McDonald, K., Wu, S., and Webster, P.: Characterization of structures in biofilms formed by a Pseudomonas fluorescens isolated from soil, BMC Microbiol., 9, 103, https://doi.org/10.1186/1471-2180-9-103, 2009.

Folorunsho, O., Kizhakkethil, J. P., Bogush, A., and Kourtchev, I.: Effect of short-term sample storage and preparatory conditions on losses of 18 per- and polyfluoroalkyl substances (PFAS) to container materials, Chemosphere, 363, 142814, https://doi.org/10.1016/j.chemosphere.2024.142814, 2024.

Pandamkulangara Kizhakkethil, J., Shi, Z., Bogush, A., and Kourtchev, I.: Aerosolisation of per- and polyfluoroalkyl substances (PFAS) during aeration of contaminated aqueous solutions, Atmos. Environ., 334, 120716, https://doi.org/10.1016/j.atmosenv.2024.120716, 2024.

Pandamkulangara Kizhakkethil, J. and Kourtchev, I.: Aerosolisation of new generation perfluoroalkyl ether carboxylic and sulfonic acids from aeration of contaminated aqueous solutions, Atmos. Environ., 352, 121218, https://doi.org/10.1016/j.atmosenv.2025.121218, 2025.

We thank Reviewer 2 for their positive assessment of the novelty and scientific value of this work and for their critique. Below, we provide point-by-point responses (in blue), with cited or newly added text indicated in *italic.* We hope that our responses significantly improved the clarity of the manuscript.

Reviewer 2 comments:

PFAS are synthetic organofluorine compounds widely used in industrial and consumer applications, with ubiquitous prevalence in air, water, and soil. However, limited knowledge on atmospheric transport behavior of PFAS is hindering further evaluation of their impacts on the ecosystem. The manuscript prepared by Kourtchev et al studied the aerosolisation and size-resolved behaviour of 25 PFAS covering short-, medium-, and long-chain perfluoroalkyl carboxylic acids (PFCA), perfluoroalkane sulfonates, fluorotelomer sulfonates and emerging alternatives with the absence/presence of the model bacterium Pseudomonas fluorescens seed. Results from this work provided implications for understanding the physical droplet and evaporation controlled PFAS aerosol behavior in mixed-solvent systems, especially for wastewater treatment plants (WWTPs) enriched with both abundant PFAS and bioaerosol. By illustrating the water-air transformation behavior of PFAS, MPPD calculation was also performed based on the measured PFAS aerosol size distributions, which predicted substantial deposition of aerosol PFAS in the pulmonary region, particularly for PFAS ultrafine particles. In general, this study investigated a novel topic regarding PFAS aerosol with Pseudomonas fluorescens seed during atmospheric transportation, which would benefit future improvements in atmospheric models and exposure assessments. However, the current version of manuscript fits more with a methodological development journal, discussions can be elaborated with in-depth mechanisms to enhance the overall scientific rigor before it can be considered for publication.

Response: We are grateful to the reviewer for recognising the relevance of the novelty of this work for future atmospheric modelling and exposure assessment. However, we respectfully disagree with the assessment that the manuscript is primarily methodological in scope. According to the scope of Atmospheric Chemistry and Physics (https://www.atmospheric-chemistry-and-physics.net/about/aims_and_scope.html), the journal explicitly covers studies addressing aerosol properties, including chemical composition and microphysical behaviour, aerosol processes and chemistry.

Our controlled chamber experiments systematically explore how PFAS molecular structure influence size-resolved aerosol formation and interactions with bioaerosols under defined physicochemical conditions. These mechanistic aspects are woven into the experimental rationale and interpretation of results (see Introduction and Discussion).

Throughout the manuscript, we link observed size distributions to physicochemical properties of PFAS (e.g., chain length, functional groups) and to established theories of aerosol interfacial behaviour. We explicitly discuss how variations in volatility, surface activity and condensation dynamics influence aerosol formation and potential human exposure, supported by relevant literature.

The study deliberately applies established aerosol and chamber methodologies (thus it does not fit to “methodological development journal“) to interrogate physical and chemical processes governing PFAS aerosol behaviour. This approach ensures robustness and reproducibility, while enabling isolation and interpretation of aerosol microphysical and

compositional effects. We therefore consider the manuscript to align closely with the core scientific scope of Atmospheric Chemistry and Physics.

Reviewer 2 comment: Evidence on the mixing state of PFAS aerosol with bioaerosol seed inside the chamber would help interpreting the results.

Direct evidence of the aerosol mixing state at the single-particle level cannot be easily obtained with the offline, size-segregated instrumentation used in this study. Within the current setup, mixing can only be assessed indirectly by comparing PFAS concentrations and size distributions in experiments conducted with and without the bioaerosol seed. The strong overlap in PFAS mass concentrations, size-resolved profiles, and relative standard deviations, together with the absence of PFAS enrichment at the bacterial size mode, indicates that PFAS and bioaerosol particles did not interact.

Moreover, the chamber mixing time (~2 min, as reported in Massabò et al., 2018) ensures rapid spatial homogenisation of aerosols but does not imply chemical or interfacial mixing between PFAS and bacterial aerosols. The lack of observable PFAS enhancement at the "bacterial mode", therefore, suggests that condensation, coagulation, or adsorption of PFAS onto bioaerosol particles was not significant under the applied conditions.

Reviewer 2 comment: The authors concluded that the presence of Pseudomonas fluorescens as an aerosol seed did not enhance PFAS aerosolisation or alter modal diameters. This was derived from the methanol:water 4:6 experiments, right? Is this relevant with real waste water treatment environment? How would the authors consider the impacts from experimental PFAS:bioaerosol ratio on this conclusion?

We thank the reviewer for this comment and would like to clarify a key point regarding the scope of the study. The work does not aim to investigate or replicate wastewater treatment plant (WWTP) aerosolisation. As stated in the original manuscript (lines 105-106): "The aim of this study was to advance understanding of PFAS aerosol formation and size distribution under *controlled laboratory conditions*, with particular *focus on the potential role of bioaerosols as carriers*." References to WWTPs are provided only as illustrative examples of environments where PFAS and bioaerosols may co-occur and have now been further clarified to avoid misinterpretation.

> *"WWTPs represent one example of environments in which PFAS and biological aerosols may co-occur, highlighting the broader relevance of understanding PFAS–bioaerosol interactions, rather than serving as a system directly replicated in this study*." Lines 74-76, revised document.

The conclusion that the presence of *Pseudomonas fluorescens* as an aerosol seed did not enhance PFAS aerosolisation or alter modal diameters is not derived from a methanol–water aerosol system per se. PFAS were introduced using a small volume (≈3 mL) of a methanol-containing (40:60 v.v.) solution into a 2.2 m³ chamber solely to ensure PFAS solubility and stable aerosol generation. Considering the low injected solvent volume and the high vapour pressure and volatility of methanol, it is expected to evaporate rapidly following aerosol generation, such that aerosols present in the chamber are dominated by water and PFAS rather than by the solvent itself. However, the effect of methanol on PFAS particle properties was acknowledged in the original manuscript (lines 302-304): "*Methanol substantially reduces surface tension (from 71.7 dyne cm^{-1} for pure water to 38.7 dyne cm^{-1} at 40 % v/v methanol*

at 25 °C; Cheong and Carr, 1987), which promotes droplet formation and minimises differences in surface activity among PFAS, thereby obscuring potential molecular-specific effects on aerosol behaviour."

Reviewer 2 comment: Also, would experiment temperature or RH make a difference to these results?

Response: Variations in temperature and relative humidity can influence aerosol evaporation dynamics, water content, and phase state, and could therefore affect PFAS partitioning under certain conditions. In the present study, temperature and relative humidity were deliberately held constant to isolate the effect of biological aerosol seeding on PFAS aerosolisation and size distributions. As stated in the manuscript (lines 124–125), temperature and relative humidity inside ChAMBRe were continuously monitored and maintained at 23 ± 3 °C and 40 ± 6 %, respectively, ensuring that comparisons between experiments with and without bacterial seeding were made under identical thermodynamic conditions.

To acknowledge this point, the following statement has been added to the Conclusions:

> "*Variability in temperature and relative humidity may influence PFAS aerosol behaviour through effects on aerosol water content and evaporation dynamics and should be considered when extrapolating these findings to broader atmospheric conditions*." Lines 502-504, revised document.

Reviewer 2 comment: The authors stated that PFAS are known to interact with surfaces and can partition during drying, passing the aerosol through additional tubing or drying devices (e.g., diffusion dryers/denuders) would introduce unnecessary interfaces and increase the risk of losses.

Response: We thank the reviewer for raising this point. The decision to limit tubing or drying devices was motivated by the well-documented tendency of PFAS to interact with surfaces and, which would introduce additional interfaces and increase the risk of uncontrolled losses (Folorunsho et al., 2024).

Reviewer 2 comment: But the authors used a fan and different mixing periods for PFAS/bioaerosol in the chamber for a better mixing efficiency. I wonder how these factors would bring uncertainties to the results.

Response: Thank you for this comment. The use of active mixing reflects processes that occur naturally in environmental systems, where turbulence, convection, and air movement promote rapid mixing of gases, particles, and bioaerosols rather than stagnant conditions. In the chamber, mixing was achieved using a low-speed fan and a short, defined mixing period of 2 min to ensure spatial homogeneity prior to sampling. This approach was chosen to minimise concentration gradients and does not introduce additional physical or chemical processes beyond homogenisation. Importantly, the absence of any observable PFAS enhancement in the bacterial size mode suggests that condensation, coagulation, or adsorption of PFAS onto bioaerosol particles was not significant under the applied conditions. Importantly, the same mixing protocol, residence times, and chamber configuration were applied consistently across all experiments, constraining any effects associated with chamber mixing to be systematic.

Reviewer 2 comment: Also, it would be helpful to characterize the chamber wall loss of PFAS aerosol.

Direct quantification of PFAS aerosol wall losses would require time-resolved measurements of aerosol-phase PFAS concentrations throughout the chamber residence time. This is not technically feasible for PFAS at slightly higher than environmentally relevant concentrations used in these experiments, as no online measurement techniques are available to quantify ionic PFAS in the aerosol phase in the lab. Offline determination of wall losses would therefore necessitate repeated sampling at multiple time points to resolve concentration gradients within the chamber. Considering the low aerosol-phase PFAS concentrations obtained from short, sequential filter sampling, such an approach would introduce substantial analytical uncertainty.

Using higher PFAS concentrations to quantify wall losses is also problematic, as wall-loss processes are known to be concentration- and size dependant (e.g. Wang et al., 2018), such that results obtained under elevated concentrations are not necessarily representative of losses potentially occurring in our experiments.

To reflect on this point, we have added a statement to the manuscript acknowledging the inability to directly quantify wall losses and clarifying that, while wall losses may influence absolute recoveries, the reported trends represent conservative estimates of aerosol-phase PFAS behaviour under the conditions studied:

> "*Direct quantification of aerosol-phase PFAS wall losses was not performed, as it would require time-resolved aerosol-phase PFAS measurements that are not technically feasible at the concentrations applied, given the absence of online measurement techniques for ionic PFAS and the need for extended offline sampling to achieve sufficient analytical sensitivity, especially in size-resolved samples.*" Lines 228-231, revised manuscript.

We also acknowledged limitations and support of the approach resulting from this phenomenon:

> "*Moreover, wall-loss efficiencies may vary with PFAS chain length and could influence absolute recoveries. All experiments were therefore conducted under consistent chamber configuration and operating conditions to minimise variability in wall-loss behaviour and to allow relative comparisons within a common experimental framework."* Lines 233-236, revised document.

> "*While a clear chain-length-dependent increase in aerosol-phase PFCA concentrations is observed, any preferential wall losses of longer-chain compounds would act to reduce their measured recovery and therefore bias the magnitude of this increase towards lower values*." Lines 264-266, revised document.

Reviewer 2 comment: A Figure of PFAS solubility and loss according to different methanol and ultrapure water ratios would help explaining why this 40:60 (v/v) methanol and ultrapure water ratio was eventually adopted for the experiments. Also, why methanol instead of other organic solvent was used here? Have the authors tried different types of organic solvents to see the different aerosolization efficiencies of PFAS?

Response: We thank the reviewer for this comment. The 40:60 (v/v) methanol–ultrapure water mixture was selected to minimise PFAS losses to system surfaces during aerosol

generation while maintaining sufficient solubility and appropriate evaporation behaviour. Previous studies demonstrated that PFAS adsorption to container materials, including plastic and glass components, also used in the nebulisation system here, is substantially reduced by the addition of methanol to aqueous PFAS solutions (Kourtchev et al., 2022, Folorunsho et al., 2024, Mancini et al., 2023). In particular, studies by Mancini et al. (2023) reported a marked reduction in PFAS losses at methanol contents of approximately 30% (v/v). To provide a conservative margin relative to these findings, and to account for additional losses associated with aerosolisation and contact with stainless steel components within the nebulisation system, the methanol fraction was increased to 40% (v/v) in the present study.

With regards to the solvent choice, methanol is fully miscible with water, has a relatively high vapour pressure compared to water, and is expected to evaporates rapidly during aerosol generation, thereby minimising its persistence in the particle phase while enabling reproducible formation of PFAS-containing droplets. This behaviour is advantageous for aerosol studies, as it limits residual organic solvent effects on particle size, phase state, and surface properties. Methanol is also routinely used for PFAS preparation and analysis due to its compatibility with PFAS standards, low background PFAS contamination (this is one of the main factors), and minimal chemical interaction with PFAS relative to less volatile or more surface-active organic solvents.

Different organic solvents were not tested, as the study deliberately employed a single, well-characterised PFAS-free solvent (methanol, LiChrosolv®, Supelco) to ensure consistent aerosol generation across experiments.

The rationale for solvent selection has now been clarified in the Methods section:

> "*A 40:60 (v/v) methanol–ultrapure water mixture was used based on prior evidence that methanol additions of approximately 30% (v/v) substantially reduce PFAS losses to glass (e.g. Mancini et al., 2023) and polymer surfaces, including glass components of the nebulising system; the higher methanol fraction was used here to provide a conservative margin accounting for additional losses during aerosolisation and contact with both glass and stainless steel components of the system. Methanol was selected due to its full miscibility with water, rapid evaporation during aerosolisation, low background PFAS contamination, and established compatibility with PFAS analytical workflows (Kourtchev et al., 2022), and was used consistently to ensure reproducible aerosol generation conditions.*" Lines 160-167, revised document.

Reviewer 2 comment: MPPD simulations was adopted in this work. However, the results from MPPD are basically based on particle size distribution, and I deduce this would vary with a different solvent (methanol to water ratio, given that they did not dry the particles), experimental flow rate, and even chamber size/tubing length for PFAS aerosol generation. I wonder if the authors have considered/evaluated such variance from experimental conditions in their study. In other words, more characteristic impacts from PFAS chemical composition as well as potential toxicity should be stated in the discussion.

Response (this point was also addressed in response to Reviewer 1): We thank the reviewer for this comment and for the opportunity to clarify the intent and interpretation of the MPPD modelling.

We agree that respiratory deposition predicted by the MPPD model is primarily governed by aerosol size. The MPPD results are not intended to predict absolute exposure levels for specific environmental scenarios. Instead, they translate experimentally measured size distributions into size-resolved deposition patterns, allowing comparison across experimental conditions and compounds. This size-based interpretation remains valid regardless of the specific aerosol source, because deposition in the respiratory tract is determined by particle size rather than by the process by which the particles are generated.

For PFAS in particular, inhalation exposure remains poorly constrained relative to ingestion pathways, and size-resolved deposition data are largely absent. The modelling therefore provides a first-order framework for interpreting how aerosolised PFAS associated with submicron particles may deposit within the respiratory tract. These results can be used as reference points for future field measurements and for evaluating the relevance of different aerosol generation processes that produce similar particle size ranges.

To ensure clarity, we revised the manuscript (both Results and Discussion and Conclusion sections) to explicitly state that the MPPD modelling is used to examine size-resolved respiratory deposition behaviour based on the measured particle size distributions, rather than to directly quantify population-level exposure under specific environmental scenarios:

> "*The modelling is not intended to represent population-level exposure or the full range of environmental conditions, but rather to evaluate size-resolved respiratory deposition behaviour for the measured aerosol populations*." Lines 465-467, revised document.
>
> "*It must be noted that MPPD modelling serves to contextualise size-resolved respiratory deposition for the observed aerosol populations, not to represent population-level exposure or comprehensive environmental scenarios. However, our results can be used as reference points for future field measurements and for evaluating the relevance of different aerosol generation processes that produce similar particle size ranges*." Lines 493-496, revised document.

Reviewer 2 comment: The author stated that this is the first time to explore PFAS aerosol formation with bioaerosol seed. It would be helpful to have a schematic of experimental setup in the method section of this manuscript.

Response: The requested schematic of the experimental design has been added to the supplementary information section.

Reviewer 2 comment: Line 161–164: change to "increase the risks of losses"

Response: Changed.

Reviewer 2 comment: Line 127: "5 × 10-2" is a typo

Response: Addressed. Thank you.

Reviewer 2 comment: Line 197: should mention the storage duration before extraction

Response: Although, this is irrelevant in the context of PFAS="forever chemicals" stability, this information is now added to the text. *"..approximately for 60 days at –20 °C until extraction*." Line 210, revised document.

Reviewer 2 comment: Line 367–368: typo in “fluorescens-seeded (average 0.105±0.0043 ng m-3, n=3) and unseeded (0.099±0.008 ng m-3, n=3) “

Response: Addressed. Thank you.

Reviewer 2 comment: Figure 3: should be error bars on each data point

Response: Since dm/dlog dp is a bin normalised quantity defined per logarithmic diameter interval, conventional error bars would be misleading. Therefore, we have retained the figure in its current form.

References:

Cheong, W. J. and Carr, P. W.: The Surface Tension of Mixtures of Methanol, Acetonitrile, Tetrahydrofuran, Isopropanol, Tertiary Butanol and Dimethyl-Sulfoxide with Water at °C, J. Liq. Chromatogr., 10, 561-581, https://doi.org/10.1080/01483918708069009, 1987.

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We thank Reviewer 3 for their comments and suggestions. We have carefully considered these and provide point-by-point responses below. Responses are given in blue, with cited or newly added text indicated in *italic*.

Reviewer 3

General comments

PFAS are present in atmospheric aerosols; however, their interfacial chemistry at aerosol surfaces remains poorly understood. This study investigated the behaviour of PFAS in aerosols in both the absence and presence of Pseudomonas fluorescens as a biological seed. By illustrating a unimodal aerosol PFAS mass distribution, the study showed association of PFAS with the fine particle mode. No effect of Pseudomonas fluorescens addition on aerosol PFAS concentration was observed. The study applied Multiple-Path Particle Dosimetry (MPPD) modeling, based on the measured particle size distributions, to evaluate particle deposition in the respiratory tract.

Response: We thank the reviewer for this summary of the study.

Research comments (Reviewer 3): The PFAS-containing mixture introduced into the chamber would be expected to exhibit compound-specific wall deposition, which should be monitored prior to the start of the experiment. Were wall loss deposition rates measured? Stainless steel chambers are known to exhibit significant wall deposition for organic acids and peroxides; could this have affected the experiments? Please comment in detail on the possible contribution of wall losses in relation to compound vapor pressure.

Response: We would like to thank the reviewer for this comment. Wall losses were not measured and as stated in the original submission not applied for data correction (lines 214-215). Direct quantification of PFAS aerosol wall losses would require time-resolved measurements of aerosol-phase PFAS concentrations throughout the chamber residence time. This is not technically feasible for PFAS at slightly higher than environmentally relevant concentrations used in our experiments, as no online measurement techniques are available to quantify ionic PFAS in the aerosol phase in the lab. Offline determination of wall losses would therefore necessitate repeated sampling at multiple time points to resolve concentration gradients within the chamber. Considering the low aerosol-phase PFAS concentrations obtained from short, sequential filter sampling, such an approach would introduce substantial analytical uncertainty.

Using higher PFAS concentrations to quantify wall losses is also problematic, as wall-loss processes are known to be concentration- and size dependant (Wang et al., 2018), such that results obtained under elevated concentrations are not necessarily representative of losses potentially occurring in our experiments.

To reflect on this point, we have added a statement to the manuscript acknowledging the inability to directly quantify wall losses and clarifying that, while wall losses may influence absolute recoveries, the reported trends represent conservative estimates of aerosol-phase PFAS behaviour under the conditions studied:

> "*Direct quantification of aerosol-phase PFAS wall losses was not performed, as it would require time-resolved aerosol-phase PFAS measurements that are not technically feasible at the concentrations applied, given the absence of online*

measurement techniques for ionic PFAS and the need for extended offline sampling to achieve sufficient analytical sensitivity, especially in size-resolved samples." Lines 228-231, revised manuscript.

We also acknowledged limitations and support of the approach resulting from this phenomenon:

"*Moreover, wall-loss efficiencies may vary with PFAS chain length and could influence absolute recoveries. All experiments were therefore conducted under consistent chamber configuration and operating conditions to minimise variability in wall-loss behaviour and to allow relative comparisons within a common experimental framework*." Lines 233-236, revised document.

"*While a clear chain-length-dependent increase in aerosol-phase PFCA concentrations is observed, any preferential wall losses of longer-chain compounds would act to reduce their measured recovery and therefore bias the magnitude of this increase towards lower values*." Lines 264-266, revised document.

Reviewer 3 comment: How was dilution due to aerosol sampling corrected? What was the dilution rate during the experiments?

Response: Dilution due to aerosol sampling was accounted for by correcting the measured concentrations using the total sampling and dilution flow rates applied during the experiment. The corresponding clarification has now been added to the SI section:

"*Dilution due to aerosol sampling was corrected by accounting for the total sampling flow rate relative to the chamber volume, treating sampling as a first-order loss process. The total volume of ChAMBRe is 2,200 L. The instruments flow was:*

- *NanoMOUDI 10 L min^{-1};*
- *TSP: 10 L min^{-1};*
- *OPS: 1 L min^{-1};*
- *SMPS: 1 L min^{-1};*
- *WIBS: 0.3 L min^{-1};*

In the experiments with only PFAS, the instruments used were MOUDI, TSP, OPS and SMPS. The dilution factor was 12/2200 min^{-1} = 0.01 min^{-1}.

In the experiments with PFAS + bacteria, the instruments used were MOUDI, TSP, OPS, SMPS and WIBS. The dilution factor was 12.3/2200 min^{-1} = 0.0101 min^{-1}."

Reviewer 3 comment: The nebulization process may exert mechanical stress and cause fragmentation of bacteria; however, is this the only explanation for the observed fragmentation of Pseudomonas fluorescens? Could the processes differ under higher relative humidity (RH) conditions?

Response: The observed fragmentation of *Pseudomonas fluorescens* is consistent with mechanical stress induced during nebulisation; however, this is unlikely to be the only contributing mechanism. Rapid droplet evaporation at the moderate RH (~40%) used in this study likely imposed additional desiccation and osmotic stress, promoting further

fragmentation of already weakened cells. Under higher RH conditions, slower evaporation would be expected to reduce desiccation stress and preserve a larger fraction of intact cells or aggregates, leading to a shift toward larger particle sizes. Thus, bacterial fragmentation reflects a combination of mechanical and humidity-dependent physicochemical processes rather than nebulisation alone.

Reviewer 3 comment: Smaller aerosol particles experience higher internal pressure due to curvature effects enhanced when PFAS are present. How might this influence the mass accommodation of Pseudomonas fluorescens onto or into PFAS-containing aerosols?

Response: Curvature-related (Kelvin) effects are primarily relevant for very small (nm) droplets, diminishing rapidly as particle size increases beyond the tens of nanometres range (e.g. Tröstl et al., 2016). In contrast, *Pseudomonas fluorescens* cells are µm sized (reported cell length 1–2 µm; width 0.3–0.6 µm), placing them several orders of magnitude larger than the droplet sizes for which Kelvin effects are significant and at least an order of magnitude larger than the fine aerosol particles dominating the PFAS mass distribution. Furthermore, mass accommodation coefficients are defined for molecular uptake and describe the probability of gas-phase molecules entering the condensed phase, rather than interactions involving aerosolised PFAS and intact biological cells. Accordingly, curvature-induced internal pressure effects are not expected to control interactions between *Pseudomonas fluorescens* and PFAS-containing aerosols; any influence of PFAS is more plausibly related to changes in surface properties rather than Kelvin-driven accommodation.

Reviewer 3 comment: PFAS-containing aerosols may be stable at smaller diameters depending on RH. How would RH affect the reported results? Did the authors perform experiments at different RH levels, and were similar conclusions obtained? Higher RH is expected to promote stronger adhesion, whereas lower RH may result in more reversible interactions.

Response: Although relative humidity can influence aerosol water content and phase state, the present results remain important because they demonstrate that biological aerosol seeding does not measurably enhance PFAS aerosolisation or alter size-resolved PFAS mass distributions under moderate relative humidity conditions (~40%), which are representative of many atmospheric environments. The absence of a detectable biological effect at this RH places a meaningful constraint on the role of bioaerosols in PFAS atmospheric partitioning, indicating that any potential RH-dependent enhancement would need to be substantial to alter the conclusions reported here.

A systematic assessment of relative humidity and temperature effects is indeed of interest and represents a valuable avenue for further investigation; however, such an approach would require analysis of a very large number of samples due to the 13-stage size-resolved MOUDI sampling, the need for experimental replication, and the inclusion of additional test conditions, with each condition generating at least 39 samples. At an analytical cost of approximately £180 per sample, this was not feasible within the scope of the present study, but remains an important direction for future research.

To acknowledge this point, the following statement has been added to the Conclusions:

> "*Variability in temperature and relative humidity may influence PFAS aerosol behaviour through effects on aerosol water content and evaporation dynamics and should be*

considered when extrapolating these findings to broader atmospheric conditions." Lines 502-504, revised document.

Minor comments

Reviewer 3 comment: Line 51: Please check whether "and" should be used instead of "&" for ACP.

Response: Corrected to "*and*". Thank you.

Reviewer 3 comment: Line 138: Consider moving the PFAS mixture description to the Supplementary Information.

Response: We agree with the reviewer that detailed compound lists should be kept concise where possible. For this reason, only the PFAS details required to interpret the Results and Discussion are listed in the main text, while the names of isotopically labelled compounds that are not discussed later were already placed in the Supplementary Information. The remaining mixture description is retained in the main text to allow readers to follow compound-specific results without repeated cross-referencing and interrupting the narrative flow, particularly given the number of analytes and the use of abbreviations throughout.

Reviewer 3 comment: Line 174: The unit for liter is given as "L" in the text; please check the use of "lpm."

Response: Changed to L min^{-1}

Reviewer 3 comment: Line 197: Please provide information regarding the extraction time and storage period.

Response: Although this information is not directly relevant to the long-term stability of PFAS as "forever chemicals", details on storage duration and extraction timing have now been added to the text, indicating storage for ~ 60 days prior to analysis and extraction and analysis within 24 hours per batch:

"*All samples were analysed within 24 hours of extraction for each analytical batch.*" Lines 224-225, revised manuscript.

"..approximately for 60 days at –20 °C until extraction." Line 210, revised document.

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