Referee 2

1. *Abstract: I think that it would be useful to indicate the mean residence/life times estimated for E. coli (baseline conditions).*

The Referee is right. We will add this information in the abstract of the revised manuscript.

2. *The manuscript is likely aimed at non-microbiologists, and so very basic protocols for culturing bacteria and characterizing their growth and numbers are given in great detail. I leave these to authors' choice, but these can be found in any handbook of basic microbiology that could eventually be referred to in order to shorten the manuscript. Examples include lines 401-419 regarding bacterial growth (which appeared in addition quite out-of-subject here), or lines 422-438 regarding preparation of cell suspension and estimation of viable cell numbers by dilution-platting. I thought that Figure 6 as well is unnecessary in the context of the study and could be put in supplement. Section 2.3.1 could therefore ultimately be greatly shortened and merged with another section (e.g. 2.3.2).*

The Referee is right, the manuscript is aimed at non-microbiologists, therefore we have decided to describe the protocols for culturing bacteria in detail, considering useful this part to the potential reader. We would prefer to keep this part in the main text; however, we leave the decision to the Editor (to maintain this section in the manuscript or move to supplement).

3. *Lines 28-29 (abstract): the sentence "This figure quantifies the protocol sensitivity as well to changes in viability when bacteria are exposed in other (e.g., polluted) conditions" is not clear (which figure? Conditions other than what?). In addition, "exposed in" should be "exposed to".*

The Referee is right, the sentence is not clear. We will modify the sentence in the revised manuscript as follows:

"The whole procedure showed an estimated life time of total (T) and viable (V) E. coli of about 153 and 32 minutes, respectively, and a V:T ratio lifetime of 40 ± 5 minutes when ChAMBRe is held in a reference "baseline" condition. The coefficient of variation of 13% shows how sensitive the protocol is also to changes in viability when the bacteria are exposed to other (e.g., polluted) conditions."

4. *Lines 43-45: DMS and other VOCs are given as examples of SBAs, but these are not aerosols! Literally, SBA would correspond to fragments of larger biological particles, material released from cells (disruption, excretion…), nucleated biogenic gases, or cells "born" in the air from microbial multiplication.*

We will modify the sentence in the revised manuscript as follows: “SBA are fragments of larger biological particles, material released from cells (disruption, excretion…), nucleated biogenic gases, or cells “born” in the air from microbial multiplication (Morris et al., 2014, Ervens et Amato, 2020).”

5. *Lines 46 and 47: the two “can” are not necessary. Moreover, do they really “vary” in size (at the individual scale)? Or do they rather fall within a range of sizes?*

We will modify the sentence in the revised manuscript as follows: “The PBAs vary in size depending on the specific biological material being aerosolized; they range from several nanometers (e.g., viruses, cell fragments) to a few hundred micrometers in aerodynamic diameter (e.g., pollen, plant debris) (Pöschl, 2005).”

6. *Line 57: It is said that bacteria are at concentration “usually greater than 104 cell/m3” over land. The reference Bauer et al2002 is not appropriate here for such statement. Rather cite the review by (Burrows et al., 2009) where Table 1 summarizes well literature data as a function of land use. In addition, the statement that concentrations in air over sea tend to be lower needs a reference, and it is not fully supported by literature (see for instance (Mayol et al., 2014), Table 1). This whole sentence thus needs revision.*

The Referee is right. In the revised manuscript, we will replace Bauer et al 2002 with Burrows et al., 2009. The sentence will be revised and replaced with: “They are ubiquitous in the atmosphere, and their presence and abundance can vary depending on factors such as location, season, and local environmental conditions: usually, over the land, the concentration in atmosphere is greater than $10^4$ cells m-3 (Burrows et al., 2009) while our understanding of airborne microbes over oceans, is indeed limited compared to the knowledge we have about microbes in terrestrial and aquatic environments. In a recent work (Mayol et al., 2014), the airborne prokaryotic abundance over the North Atlantic ocean ranged from about 3000 to 20000 prokaryotes $m^{-3}$ (average about 8000 cells $m^{-3}$).”

7. *Lines 61-62: “Bacteria have a relatively long atmospheric residence time, of the order of several days or more, compared to larger particles and can be transported over long distances, up to thousands of km (Després et al., 2012)” is barely a plagiarism of the cited reference, that says: “Due to their small size, bacteria have a relatively long atmospheric residence time (on the order of several days or more) compared to larger particles and can be transported over long distances (up to thousands of kilometres).” This may need rephrasing.*

We will rephrase the sentence in the revised manuscript as follows: “Bacteria, as small airborne particles or aerosols, can have relatively long atmospheric residence times compared to larger particles. This is due to their small size and low settling velocity, which allows them to remain suspended in the air for prolonged periods. (Després et al., 2012).”

8. *Lines 69-72: Is this statement about the lack of data on abundance and diversity of microbes in the air really relevant here? It would be more helpful to develop a bit on the subject of the paper: viability, and the impacts of environmental conditions, for which even more data is lacking.*

In the revised manuscript, we will add a sentence in line 78 as follows: “In particular, bacterial viability, the proportion of viable to total bacteria concentration, can act as Cloud Condensation Nuclei (CCN) thanks to the hygroscopic properties of their surfaces (Delort et al., 2010). Additionally, the near-surface atmosphere's viable bacteria can have a significant impact on human health, including allergies, acute toxic effects, and infections (Bolashikov and Melikov 2009).”

9. *Line 79-80: I would suggest to use more recent references here about the potential impacts on cloud chemistry, such as (Khaled et al., 2021; Jaber et al., 2021, 2020; Fankhauser et al., 2019).*

In the revised manuscript, we will replace the original references with those suggested by the Referee.

10. *Lines 86-88: what is “direct deposition”, and how does this differ from precipitation? Do you rather mean “wet or dry deposition”. Also, can you develop what “multifaceted” means here when referring to the mechanisms that govern transport, survival and activity. Eventually cite (Amato et al., 2023) which discusses the fact that biologically- and physically-driven processes are indeed intertwined.*

In the revised manuscript, we will rephrase the sentence as follows:

“Once in the air, they are carried upwards by air currents and may remain in the atmosphere for many days before being removed by wet or dry deposition onto surfaces. Indeed, the mechanisms that govern the transport, survival, and activity of bacteria in the atmosphere are complex and multifaceted. Understanding these mechanisms is crucial for various scientific disciplines, including microbiology, atmospheric science, and public health. This complexity is related to some key factors such as aerosolization, transport and dispersion, survival, hygroscopicity, interactions with other particles, droplet nucleation, deposition, activation of ice nucleation, impacts on cloud formation and chemistry and all these processes are indeed intertwined (Amato et al., 2023).”

11. *Line 88-90: what kind of impacts are in question here?*

We will add the words climate and health in the revised manuscript.

12. *Line 98-104: In addition of the references cited, I think that a number of other (and early) studies that have investigated the survival and activity of bacteria using simulation chambers should be referenced: (Ehrlich et al., 1970; Krumins et al., 2014; Wright et al., 1969).*

In the revised manuscript, we will add the sentence as follows: "Few studies have investigated bacterial survival and activity using simulation chambers, and some of them are old (Wright et al., 1969; Ehrlich et al., 1970; Krumins et al., 2014)."

13. *Line 177: provide the reference of the nebulizer inlet please.*

In the revised manuscript, we will add the reference at the paragraph 2.2.

14. *Line 180: I do not see the point of mentioning "(or in the laboratory)". Please specify (or remove).*

In the revised manuscript, we will remove "in the laboratory".

15. *Line 342: quartz filters can be used for offline analysis. Please consider that this is also the case with other sampling methods (impingers and cultures cannot be considered "online" methods).*

The referee is right: in the revised manuscript, at the line 327 we will add the following sentence: "All the methods described below allow to perform offline analyses". We will remove "allowing subsequent offline laboratory analysis" from line 338 and "for offline analysis" from line 342.

16. *Lines 351-352: "The hood is equipped with HEPA filter and UV-light laminar is created inside the cabinet" probably needs rephrasing (what is UV-light laminar?).*

In the revised manuscript, we will change the sentence as follows: "The hood is equipped with HEPA filter and an UV-light lamp allows the sterilization of the illuminated surfaces inside the hood."

17. *Line 358: specify that the spectrophotometer is dedicated to liquid samples, to avoid confusion.*

In the revised document, we will add the sentence: "designed for liquid samples."

18. *Line 364: same as previous comment, regarding the shaker: it might not be obvious for non-microbiologists that these are intended for liquid cultures.*

In the revised document, we will add the sentence: "designed for liquid samples."

19. *Line 446: "the results of the logistic fit are only shown" might rather read "the results of the logistic fit only are shown".*

We will apply the correction to the revised manuscript.

20. *Line 472: "centrifugated" should be centrifuged.*

We will apply the correction to the revised manuscript.

21. *Line 468-481: this is quite useless as presented as many details are missing: what is the volume of culture used? And that volume of liquid were cells resuspended into after centrifugation? Dilution step from what (line 480)?*

We will revise the part from line 469 to 481 to explain better the preparation and providing more details. In the revised manuscript this part will be replaced by:
"To prepare the inoculum for the chamber experiments, the E. coli is grown in 30 ml of fresh TSB nonselective medium, in a shaking incubator at 37 °C and 200 rpm and its growth is followed by checking the OD600nm value until the mid-exponential phase. When OD600nm ~ 0.5, 20 ml of this liquid preparation is centrifuged at 3000 rpm for 10 min. Afterward, the bacteria pellet, separated by surnatant, is resuspended in 20 ml of sterile physiological solution (NaCl 0.9 % w/v) to prepare a suspension of approximately $10^8$ CFU $mL^{-1}$, as verified by standard dilution plating. To retrieve the bacterial concentration, the average of CFU counting on agar plates and the uncertainty are calculated following the same metric described in 2.3.1 paragraph.
For the experiments performed at ChAMBRe, the typical bacterial concentration in the inoculum is $10^7$ CFU ml-1: to reach this concentration, a further dilution step is needed (i.e., typically 1:10 or 1:5) before the injection (see Massabò et al, 2018 for details)."

22. *Line 489 "relative standard deviation" and Lines 500-501 "the ratio between standard deviation and mean": aren't these actually coefficients of variation?*

In the revised manuscript we will replace "relative standard deviation" and "the ratio between standard deviation and mean" with coefficient of variation.

23. *Line 546: nebulized*

The correction will be done in the revised manuscript.

24. *Line 547: Indicate if these are true independent replicates of experiments (i.e. led from separate cultures? So somehow accounting for biological variability), or just consecutive repeats from same cultures.*

Thanks for the comment: we will add the sentence "led from separate cultures" in the Results section in the revised manuscript.

25. *Line 562-565: check language ("the same of", "this indicating").*

In the revised manuscript we will rephrase the sentence as follows: "particles in the same size range of E.Coli (1-2 μm) and τ of 2-3 hours".

26. *Line 570: what is a "roughly complete cell mortality"? It is either complete, or incomplete…*

In the revised manuscript we will delete "roughly" word since we had a complete cell mortality.

27. *Table 3?*

We don't understand the request of referee.

28. *Lines 588-589: NO is less toxic than NO2. It would be nice to develop on this (in the discussion section): why could this be? what are the processes of toxicity of these compounds? What does this imply for natural situations? What is known about it?*

In the revised manuscript, we plan to go deeply into the discussion of toxicity effect of NO and NO2 on *E.coli*. Below, the phrases we would like to add:
"The increase of the NO concentration did not correspond to a decrease in the E. coli viability, as observed with NO2: these results suggest a greater toxic effect of NO2 than of NO on E. coli. The literature of a comparison of the toxic effects of NO and NO2 on E coli is poor. Some research articles have demonstrated negative effects of these two gases on bacterial strains: Kosaka et al. 1986 found a decrease in E.coli viability with increasing NO2 concentration. Janvier et al, 2020 highlighted a significant adverse effect of NO2 on some commensal skin bacterial strains. Mancinelli and McKay, 1983 found that a low concentration

of NO is bacteriostatic for some organisms but not for others. It is worth noting that NO has a strong antimicrobial property, being an endogenously produced molecule that is critical for critical infection defence (Fang, 1997), although some bacteria are able to escape this NO action (Privett et al., 2012).

29. *Line 598: "30 min", or less, given the data shown. This is difficult to tell from Figure 9, maybe showing it on log-scale would be more appropriate? (using the 1/10 detection limit to represent "0" for instance).*

The Referee is right. In the revised manuscript we will rephrase the sentence as follows: "The viable concentration collapses quickly, reaching zero after 30 minutes.  The comparison between V:T ratio obtained for dark and light baseline is shown in Figure 9."

30. *Line 600: the reference should be "Figure 9 "here.*

In the revised manuscript we will change the reference.

31. *Lines 614 and 621: "next future" probably should read "near future".*

We will modify "next future" in "near future" in the revised manuscript.

32. *The perspectives section could be developed based on other sampling possibility. Notably the possibility of using methods other than cultures to evaluate viability would be relevant if this is the case (live/dead staining, RT-qPCR, and others), in particular as non-culturable state is common in viable bacteria (as mentioned line 608). Moreover, neither SMPS data nor their potential use are exploited. It could be mentioned for instance that investigations about the relationship between particle size and survival could be performed, using cascade impactors as samplers for instance.*

In the revised manuscript, we will add the following sentence:
"It is well known in the literature that the viable but non-culturable condition (VBNC) is a survival strategy of many bacteria in the environment in response to adverse environmental conditions (e.g., solar radiation). There is a growing scientific interest in studying VBNC cells, including to understand novel public health implications of VBNC cells. In our simulated experiments, we are investigating alternative methods to detect bacterial viability and VBNC state, such as "live and dead staining" by fluorescence microscopy. This assay can be used to monitor the viability of bacterial populations as a function of cell membrane integrity using different fluorescent dyes.

Further experiments with “flow cytometry” could certainly be more beneficial not only to enumerate live and dead bacteria, but also to evaluate the health and viability of bacterial cells by determining the activity of bacterial oxidases and reductases.”

References cited and added in the revised manuscript:

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