

Author response to comments by Referee #1

All referee comments are shown in black, our author responses in blue; suggested new manuscript text is indicated in red.

This perspective was an interesting and enjoyable to read contribution that identifies and more closely examines some of the most salient challenges that microbes in the atmosphere encounter. A deeper examination of these challenges reveals that some of them may be as or more challenging than previously believed, while some may be less relevant, due to the unique circumstances of the atmosphere, and in cloud and aerosol phases. The conclusions presented are mainly based on modelling, but based on realistic approximations and our currently knowledge. As yet, little empirical data exists to validate many of these suppositions. However, the authors seem to be cognizant of these limitations (in referencing literature where unexpected findings were made e.g. Liu et al. 2012). Nevertheless, the perspective builds a more detailed and nuanced examination of these factors than I have seen elsewhere.

Author response: We thank the referee for their very positive assessment and the constructive suggestions. We address the comments below.

Specific comments:

1) While microbial interactions can have beneficial outcomes, there can also be negative outcomes/antagonistic interactions. The physical separation of cells that is more prevalent in the atmosphere than in other environments will not only reduce/eliminate beneficial interactions, but will reduce/eliminate negative interactions such as competition and direct antagonism. The net outcome on this may be beneficial or detrimental, which likely depends on the impacted organism and the context.

Author response: We thank the referee for raising this aspect. To the best of our knowledge, there are no specific studies that explored the benefits of the interbacterial antagonism in the atmosphere. Therefore, we add some general discussion of the concept of antagonism at the end of Section 2.1:

In addition to such mutualistic behavior, bacteria also exhibit antagonistic interactions in communities, i.e., benefiting from cell separation (Russel et al., 2017; Peterson et al., 2020). Such antagonistic effects include the competition for limited resources, in particular among metabolically similar species as encountered in the atmosphere. The functioning and survival of bacterial communities is usually due to a balance between mutualism and antagonism. However, the specific conditions facilitating such balance in different ecosystems may shift under atmospheric conditions, and therefore influencing bacterial metabolism and survival. Reduced antagonism in the atmosphere due to the physical separation of cells through aerosolization, may partly explain the sustained activity and survival of atmospheric bacteria despite the overall harsh environmental conditions.

2) I appreciated the discussion on water activity, and the calculations estimating the potential hydration shell that might exist under different conditions. One aspect that was missing for me in this perspective is the impact of pH in aerosols and clouds on microbial cells – most microbes are not well adapted to living at pH 5, so this presents another stressor for microbes in the atmosphere.

Author response: Firstly, regarding the water uptake, we would like to note that we became aware of a new paper by Nielsen et al. (2024) that adds valuable information on water uptake by *Pseudomonas sp.* We will refer to it in Section 2.1 and in the supplemental information.

Secondly, we agree with the referee that the pH is a very important parameter that affects microbial activity and survival. Numerous studies point to the fact that environmentally relevant bacteria show highest diversity and richness near neutral pH values (Fierer and Jackson, 2006). However, bacteria developed numerous strategies to survive other pH conditions outside this pH range. They include intracellular buffering to maintain the pH within the cytoplasm (Kobayashi et al., 2000) and adjustments of the proton concentrations in their surroundings (Ratzke and Gore, 2018). Such buffering may also explain the low dependence of biodegradation rates in cloud water on pH (within a range of 3 - 6) as observed in lab studies, since such processes occur inside the cell even though the extracellular pH may vary over several orders of magnitude (Vařtilingom et al., 2010; Nuñez López, 2024). In line of the considerations in the present paper, we add the following text as a new subsection in section 2.4:

pH value: Indeed, numerous studies point to the fact that atmospherically relevant bacteria (e.g., *Pseudomonas sp.*) show highest growth and activity rates at only mildly acidic or neutral pH values. However, they have developed numerous strategies to survive other pH conditions outside these ranges. They include intracellular buffering to maintain the pH within the cytoplasm or proton pumps that regulate the intracellular proton concentrations (Lund et al., 2020; Kobayashi et al., 2000). Such mechanisms may explain the weak dependence of biodegradation rates on pH as found in lab studies on (artificial) cloud water (Vařtilingom et al., 2010) as the intracellular pH is kept at (near) neutral values.

In the limited volume of a cloud droplet or aqueous aerosol particle, the number of protons is small as compared to that in bulk aqueous phases. Thus, the individual cells in such volumes have to 'combat' a limited number of protons (supplemental figure). Thus, the adjustment of the pH of the surrounding aqueous phase as observed in other environments (Ratzke and Gore, 2018), might be easier in the small droplet or particle volumes. Such rates of buffering agent production and proton transfer likely depend on the bacteria types and environmental factors and therefore should be explored for atmospheric conditions.

3) Several studies are beginning to build convincing links between environmental factors (e.g. UV, temperature) on microbial community structure and diversity in the aerosol phase (e.g. Archer et al. 2019 Nature Microbiology, Gusareva et al. 2019 PNAS). Developing a controlled experimental system for forming these links is difficult to conceptualize, but environmental sampling is challenged by the numerous confounding variables. With sufficiently well-resolved and controlled environmental sampling, disentangling the impacts of specific variables should be feasible, for at least some environmental factors.

Author response: We agree with the referee that controlled environmentally sampling with a statistical meaningful number of samples may be a promising strategy to constrain the role of individual environmental factors. Such sampling strategies can and should be accompanied by suitable lab or chamber experiments to test hypotheses under even more controlled conditions. We will highlight the need of such experimental strategies in the conclusion section:

Despite the difficulties associated with the statistically relevant sampling of atmospheric microorganisms, individual environmental factors have been started to be identified that control the concentration (Archer et al., 2019; Gusareva et al., 2019), diversity (Tong and Lighthart, 1997; Bryan et al., 2019) and selection (Smith et al., 2011; Joly et al., 2015) of atmospheric microorganisms. While it remains difficult to perform sampling with sufficiently high resolution and frequency, dedicated strategies should be developed to constrain the role of individual environmental parameters (e.g., UV light, temperature) for microbial diversity and survival in the atmosphere. Such studies should be accompanied by suitable lab or chamber studies under controlled conditions to test hypotheses that may be formed based on ambient observations.

4) The calculation on settling velocity of microbial cells does not take into account air currents. In addition, it is not impossible that some microbes or microbial spores have evolved for long-range dispersal by air – this has long been known for plant seeds, and there is mounting evidence of this for fungi (e.g. Borgmann-Winter et al. 2023, Ecology 104), therefore it is not unlikely that this is relevant for prokaryotes. Previous modelling work has suggested that long-range microbial dispersal is likely (Wilkinson et al. 2012 J. Biogeography 39).

Author response: We thank the referee for pointing out this aspect. We are aware of several studies that indicate long-range transport of bacteria. Such transport is included in the calculation of the atmospheric residence time as derived from the global model study by Burrows et al. (2009). This model takes into account both vertical and horizontal transport processes of air masses containing gases and particulate matter (including bacteria). The fact that the residence time is not a linear function of the settling velocity but is affected by numerous other factors, will be more clearly pointed out in Section 2.1 and in the caption of Figure S1:

Section 2.1 (new text in **bold**): In addition to gravitational settling, other particle properties and processes affect the atmospheric residence time, **including horizontal transport and** the ability to act as cloud condensation or ice nuclei. Burrows et al. (2009a) demonstrated that bacteria acting as cloud condensation nuclei (CCN) **are generally deposited faster and, thus,** have a global atmospheric residence time that is approximately half as long as bacteria not activated into droplets. **Therefore, the distances that CCN- and/or IN-active bacteria travel in the atmosphere are generally comparably short.**

Figure S1 (new text in **bold**): τ_{atmos} denotes the mean atmospheric residence times for CCN-active bacteria as **estimated derived from a global atmospheric model study** by Burrows et al. (2009).

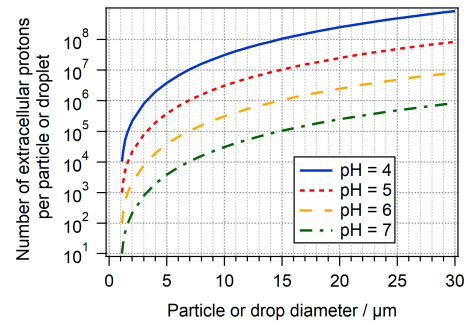


Figure 1: Number of protons in the aqueous volume of a particle or droplet surrounding a bacteria cell with a diameter of $1 \mu\text{m}$

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