Ideas and perspectives: Microorganisms in the air through the lenses of atmospheric chemistry and microphysics

Barbara Ervens[1], Pierre Amato[1], Kifle Aregahegn[1,2], Muriel Joly[1], Amina Khaled[1], Tiphaine Labed-Veydert[1], Frédéric Mathonat[1], Leslie Nuñez López[1], Raphaëlle Péguilhan[1,3], and Minghui Zhang[1,4]

[1]University Clermont Auvergne, CNRS, Institute of Chemistry Clermont-Ferrand, 63000 Clermont-Ferrand, France.
[2]now at: Department of Chemistry, York University, Toronto, ON, Canada.
[3]now at: Department of Chemical and Biochemical Engineering, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark.
[4]now at: Minerva Research Group, Max Planck Institute for Chemistry, 55128 Mainz, Germany.

Correspondence: Barbara Ervens (barbara.ervens@uca.fr)

Abstract. Microorganisms in the atmosphere comprise a small fraction of the Earth' microbiome. A significant portion of this aeromicrobiome consists of bacteria that typically remain airborne for a few days before being deposited. Unlike bacteria in other spheres (e.g., litho-, hydro-, phyllo-, cryospheres), atmospheric bacteria are aerosolized, residing in individual particles and separated from each other. In the atmosphere, bacteria encounter chemical and physical conditions that affect their stress
5   levels and survival. This article goes beyond previous overviews by placing these conditions in the context of fundamental chemical and microphysical concepts related to atmospheric aerosols. We provide ranges of water amounts surrounding bacterial cells both inside and outside clouds and suggest that the small volumes of individual cloud droplets lead to nutrient and oxidant limitations. This may result in greater nutrient limitation but lower oxidative stress in clouds than previously thought. Various chemical and microphysical factors may enhance or reduce microbial stress (e.g., oxidative, osmotic, UV-induced),
10   affecting the functioning and survival of atmospheric bacteria. We illustrate that these factors could impact stress levels under polluted conditions, indicating that conclusions about the role of pollutants in directly causing changes to microbial abundance can be erroneous. The perspectives presented here aim to motivate future experimental and modeling studies to disentangle the complex interplay of chemical and microphysical factors with the atmospheric microbiome. Such studies will help to comprehensively characterize the role of the atmosphere in modifying the Earth' microbiome, which regulates the stability of global
15   ecosystems and biodiversity.

1   Introduction

Microorganisms are the most abundant organisms on Earth and comprise about 15% of the total biological mass (Bar-On et al., 2018). This 'unseen majority' of life (Whitman et al., 1998) is an essential actor of ecosystem functioning, biogeochemical nutrient cycling, soil formation, and decomposition processes, and crucial for preserving the health of the planet (Cardinale
20   et al., 2012). Diversity within microbial communities usually leads to higher resilience towards environmental disturbances, such as pollution, climate change, and may beneficial to more robust and healthy systems (Robinson and Breed, 2023).

A significant portion of Earth's microbiome consists of bacteria; Figure 1 provides an overview of the bacteria concentrations and abundance in major aquatic and terrestrial 'spheres' of the Earth. The highest (average) bacteria cell concentrations are found in soil (10^{15} cells m^{-3}), and concentrations that are lower by several orders of magnitude in vegetation (phyllosphere), water (hydrosphere) and ice (cryosphere). The surfaces of these spheres are connected by the atmosphere that harbors significantly smaller cell concentrations, both related to air volume ($\sim 10^4$ cells m^{-3}) and in absolute numbers ($\sim 10^{19}$ cells), which comprise an apparently negligible fraction ($< 10^{-9}$ %) of the total Earth microbiome ($> 10^{30}$ cells).
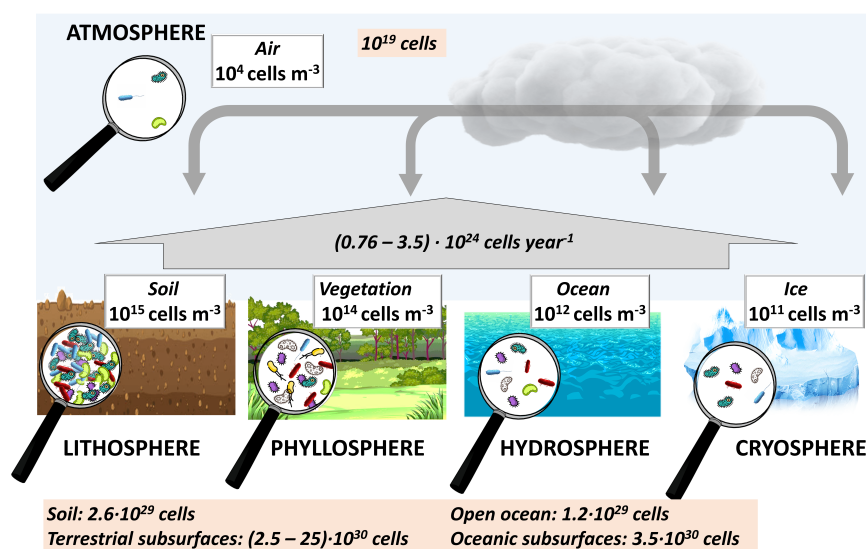
Figure 1. Schematic of bacteria cell concentrations in the spheres of the Earth. References: soil (prokaryotes) (Whitman et al., 1998), vegetation (Lindow and Brandl, 2003), assuming leaf volume-area ratio of $\sim$500 cm^{-3}m^{-2} (Poorter et al., 2009), surface waters (prokaryotes): (Whitman et al., 1998), sea ice (Boetius et al., 2015), atmosphere (Burrows et al., 2009b); the values in the orange box at the bottom are from Whitman et al. (1998), emissions to the atmosphere from Burrows et al. (2009a), atmospheric cell number from Šantl-Temkiv et al. (2022).

In comparison to the microbially more densely populated spheres, the atmospheric microbiome ('aeromicrobiome') is relatively poorly characterized. In the past, the main focus on studies of airborne microorganisms addressed their role role as pathogens (Pasteur, 1861). The rapid air movements and emission/deposition cycles in the highly dynamic atmosphere lead to efficient transport and displacement of microorganisms. While close to the ground, the atmospheric microbial diversity resembles that of the underlying surface ('footprint'), air masses mix at higher altitudes resulting in complex mixtures of microbial populations. Microbes follow major air movements along 'microbial highways'. However, the exact patterns of such aerobiological trajectories cannot be resolved to date since samples are relatively sparse (Smith et al., 2018). A fundamental difference in the atmosphere - as opposed to microbial environments in other Earth' parts - is the fact that microorganisms are aerosolized, i.e., they are surrounded by a finite hydration shell limiting their access to nutrients and water. Aerosol particles are continuously exposed to light, trace gases, oxidants and other chemical pollutants, which leads to unique conditions that

rapidly change and may expose the microorganisms to different levels of stress on a variety of spatial and temporal scales. These particular conditions, that greatly differ from those in the (more) homogeneous aquatic and terrestrial environments, are usually not taken into account in atmospheric microbiological studies.

The present article fills this gap by providing a new perspective on temporal and spatial scales of atmospheric chemical and microphysical processes that may affect the atmospheric microbiome. In Section 2, we discuss various microscale (physico)chemical aerosol properties relevant for the atmospheric bacterial environments (the underlying fundamental equations and parameters are summarized in the supplemental information). Each subsection is introduced by a question we neither attempt nor intend to comprehensively answer; instead, these questions are posed to motivate future studies to explore factors that potentially control microbial stress, survival and diversity in the atmosphere. Section 3 places the preceding considerations into the context of atmospheric scenarios, specifying various environmental factors and their potential role for microbial stress under polluted and/or cloudy conditions. In the concluding Section 4, we give recommendations for future studies to advance our understanding of the atmosphere in shaping the microbiome. We point out the need of interdisciplinary efforts merging atmospheric (aerosol) sciences, microbial ecology and aerobiology.

2 Atmospheric bacteria: temporal and spatial scales

2.1 Aerosolized bacteria: does social distancing between cells matter for their functioning?

The formation of agglomerates and/or biofilms in aquatic and terrestrial habitats provides multiple advantages to bacteria in terms of protection and collective resources. Such social traits enable microbes to adapt to diverse environments, optimize resource utilization, and enhance their survival and reproductive success. Bacteria in the atmosphere are detached from such community structures as they can only be airborne upon aerosolization. Bacteria-containing particles contain a single - or at most a few - bacteria cell(s). The settling velocity of particles approximately scales with the square of particle size; thus, doubling the number of cells (of same size) in a single particle may decrease their settling time by a factor of 4 (Section S1.1, supplemental information). In addition to gravitational settling, other particle properties and processes affect the atmospheric residence time, including the ability to act as cloud condensation or ice nuclei . Burrows et al. (2009a) demonstrated that bacteria acting as cloud condensation nuclei (CCN) have a global atmospheric residence time that is approximately half as long as bacteria not activated into droplets.

Fewer than 1 out of 1000 atmospheric aerosol particles contain a bacteria cell. Accordingly, it may be concluded that the number fraction of bacteria-containing droplets is on a similar order of magnitude. Zhang et al. (2021) showed that such low CCN number concentrations likely have a negligible effect on the formation and properties of warm clouds. However, in the same study, it was pointed out that information on the hygroscopic properties of bacteria is essential as it determines the volume of the aquatic environment that is important for microbial growth, survival, and functioning (Section 2.2).

The low bacteria concentrations in the atmosphere imply that bacteria cells are separated by considerable distances. Figure 2 shows schematically the average distance between cells (δ_{cell}) for typical atmospheric bacteria cell concentrations (0.001 cm^{-3} < N_{cell} < 0.1 cm^{-3}) (Section S1.2 in the supplemental information). The schematic shows that bacteria can be expected to
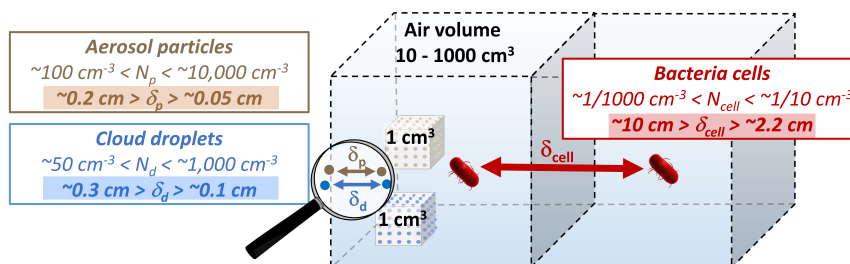
Figure 2. Schematic illustration of the distance between bacteria cells vs aerosol particles and cloud droplets (δ_{cell}, δ_p, δ_{dr}) for typical atmospheric concentrations (N [cm^{-3}]); the distance δ [cm] corresponds to $N^{-1/3}$ (for calculation and wider ranges, cf Section S1.2 in the supplemental information).

be separated on average by several centimeters ($\sim$2.2 - 10 cm), whereas other aerosol particles or cloud droplets are apart by several millimeters (δ_p, δ_{dr}). This social distancing of bacteria impairs the collective traits and functions that bacteria can benefit in other environments (Ross and Whiteley, 2020). The role of such substantial distances between cells in the atmosphere has not been explored yet as lab experiments are usually performed on bulk samples. For example, Vaïtilingom et al. (2010, 75 2011) derived biodegradation rates in cloud water samples ($\sim$100 mL) that combined billions of droplets containing 10,000s of cells. Such bulk experiments are convenient and often the only way to monitor signals above the detection limit, but they do not reflect the same conditions that microorganisms experience in dispersed cloud droplets (Sections 2.3 and 2.4.2). In addition, the chemical microstructure in individual droplets may be different than in bulk solutions (Wei et al., 2018) Novel experimental set-ups that allow the investigation on levitated droplets containing microorganisms seem promising to overcome 80 current methodological shortcomings (Fernandez et al., 2019).

2.2 Water availability: are atmospheric microbial oases limited to clouds?

All organisms, including microorganisms, need water for their biological functioning. The water content of surface waters is unlimited (at least - presumably - from a microbial perspective); the water content of soils is typically on the order of several volume percent. Clouds represent air masses with the largest amounts of liquid water in the atmosphere, but yet it 85 only comprises 10^{-5} - 10^{-4} volume%, corresponding to typical liquid water contents of 0.1 < LWC$_{cloud}$ < 1 g m^{-3}. Even in the absence of clouds, aerosol particles are never completely dry. In fact, aerosol water is often a substantial fraction of a particle mass depending on particle hygroscopicity and the surrounding relative humidity (RH). The total aerosol water content might amount to several 100s μg$_{H2O}$ m$_{air}$$^{-3}$), corresponding to $\sim$10^{-8} volume%.

The water content of atmospheric aerosol particles is often expressed by means of the hygroscopicity parameter κ. Strictly, 90 the concept of hygroscopicity does not apply to bacteria cells as they do not fully dissolve in water; their water uptake may be triggered by the formation of biosurfactants that partition to the air-water interface that attract water and slow down water evaporation to trap the water in the immediate surroundings of the cell (Gill et al., 1983).
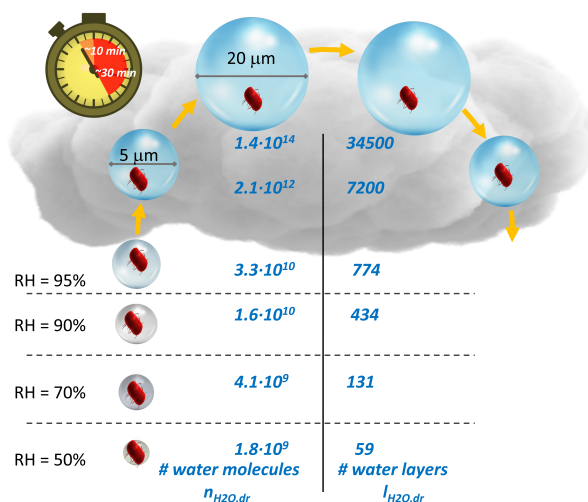
Figure 3. Number of water molecules ($n_{H2O,dr}$) and water layers ($l_{H2O,dr}$) under subsaturated conditions (RH < 100%) and for cloud droplets with diameters of 5 μm and 20 μm (Section S2.1, supplemental information). The schematic suggests that under a wide range of atmospheric RH conditions, bacteria cells are surrounded by at least ~60 water layers which corresponds to about 20 mass% of the water in a bacteria cell ($m_{cell,H2O} \sim 1.3 \cdot 10^{10}$ molecules water per cell). Since typical lifetimes of cloud droplets are on the order of 10 - 30 minutes, the largest water volumes may only represent temporally very limited 'oases' (Section S2.2, supplemental information).

Using κ_{bact} = 0.1 to estimate the amount of water around a cell, the number and layers of water molecules, n_{H2O} and l_{H2O}, can be derived (Figure 3 and Section S2.1 in the supplemental information): at RH ~90%, the water masses inside and outside of a cell are approximately equal (~10^{10} water molecules). At RH ~50%, there are several tens of layers of water around a bacteria cell (l_{H2O} = 59), corresponding to about 20% of the water inside a bacteria cell. Figure 3 summarizes n_{H2O} and l_{H2O} values the range of RH or water activities ($a_w \sim$ RH/100%), in which microbial activity, including cell division, has been observed (Stevenson et al., 2015, 2017). The presented n_{H2O} and l_{H2O} values are possibly biased low as they imply hygroscopic growth that often occurs only immediately after particle emission in the atmosphere. After their first deliquescence event, particles usually stay in a metastable state above their efflorescence RH which implies that they retain condensed water due to hysteresis of evaporation. In addition, during their residence time in the atmosphere, hygroscopic material likely condenses on bacteria-containing particles, resulting in even more water uptake.

When RH exceeds 100%, cloud droplets may form, depending on the supersaturation that is a function of the cooling rate (vertical velocity) as a source and the available drop surface area as a condensational sink. While clouds can exist for several hours or even days, they are highly dynamic systems. Cloud droplets form near cloud base and grow while ascending, followed by shrinking in descending (i.e., warming) air masses (Figure 3). These up- and downward motions constrain the lifetime of an individual cloud droplet to about 10 - 30 min, depending on cloud thickness and vertical velocity (Section S2.2 in the Supplemental Information). Thus, such high-water-volume conditions may only exist for very short times. during which bacteria need to adapt to the in-cloud environment that greatly differs from that outside clouds in terms of water availability,

110   light, temperature etc. Lab experiments in (artificial) cloud water revealed lag times of several hours before bacteria started to efficiently biodegrade organic cloud water constituents (Vaïtilingom, 2013). This lag time is comparable to that as observed for bacteria growth after rewetting of soil ('Birch effect') (Leizeaga et al., 2022). If indeed conditions of high bacterial activity were limited to the in-cloud time, hysteresis effects might impede enhanced microbial activity inside clouds since droplets evaporated before the end of the lag period.

115   Clouds have been previously termed 'microbial oases' in the atmosphere (Amato et al., 2017), implying that they provide the most ideal atmospheric conditions for bacterial activity in a fugace habitat. However, given the very rapid changes over short time scales in the environmental conditions, clouds may actually trigger several stress responses that differ from those under lower RH (20 - 90%) conditions (Péguilhan et al., 2023; Peguilhan et al., 2024, submitted). Provided that bacteria are surrounded by a considerable amount of water at $\gtrsim$90% RH, the range of biologically favorable conditions might actually

120   extend to much longer temporal and spatial scales than estimated previously (Ervens and Amato, 2020). Such considerations might be relevant for regions and/or periods of high relative humidity (e.g., tropics, polar regions and/or during night-time). It remains to be explored whether microorganisms in the atmosphere adjust their stress responses and biological functions over the full continuum of relative humidity (RH) conditions, rather than switching behaviors at specific RH thresholds (e.g. inside/outside clouds).

125   ## 2.3   Accessibility to nutrients: are nutrient levels in droplets high enough?

In most natural waters the concentrations of organic nutrients are sufficiently high that they do not lead to carbon-limiting conditions for heterotrophs (Eiler et al., 2003). In oligotrophic ocean regions or lakes, bacteria develop strategies to adapt to the lower nutrient levels, including the optimization of their energy use by producing less bacterial secondary mass, while enhancing respiration (del Giorgio and Cole, 1998). The main organic nutrients for bacteria in rivers are low molecular weight

130   compounds (LMW, $\lesssim$ 500 Da) that comprise a major fraction of the total organic carbon (TOC) (Catalán et al., 2017). The total concentration of biodegradable LMW compounds in surface waters is on the order of 50 μmol L^{-1} (0.005 - 0.4 μmol L^{-1} for individual compounds; Table S3), which overlaps with the range as found for individual compounds in fog and cloud water (0.1 - 10 μmol L^{-1}, Herckes et al. (2013)). This similarity is not surprising as volatile, water-soluble compounds are continuously emitted and/or formed in the connected spheres, followed by (thermodynamic) partitioning at the atmosphere/water interfaces

135   of cloud droplets and surface waters. The incorporation of bacteria cells into the aqueous phase does not follow the same thermodynamic principles, but, yet, bacteria cell concentrations in river and sea water ($\sim$10^3 - 10^5 cell mL^{-1}, are comparable to those in cloud water (10^4 - 10^6 cell mL^{-1}, Amato et al. (2017)).

The similarity in organic nutrient levels and bacteria cell concentrations in the different water phases may suggest equivalent organic-to-cell ratios. However, this conclusion only applies to the comparison of bulk aqueous volumes, e.g., cloud water

140   samples where all bacteria-containing and bacteria-free cloud droplets are combined (Figure 4b). In contrast, within the microcosms of individual bacteria-containing cloud droplets, the organic-to-cell ratio is much smaller since the cell concentration is 1 per droplet (0 in > 99.9% of the droplets) while the solute concentration [mol L^{-1}] is that of the bulk cloud water sample. This results in organic-to-cell ratios in cloud droplets of < 10^{-15} moles per cell as opposed to a range of 10^{-13} to 10^{-10} in surface
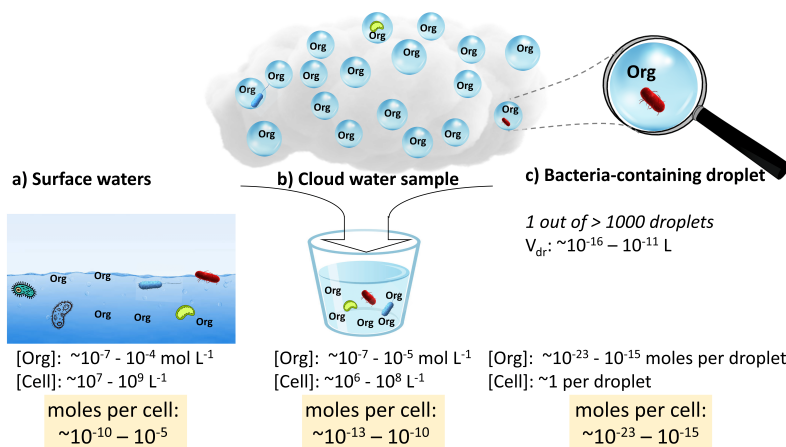
Figure 4. Typical concentrations of bacteria cells and organics in surface waters (Table S3) and cloud water samples (Herckes et al., 2013) and resulting organic-to-cell ratios. Given that 1 out of > 1000 cloud droplets contains a bacteria cell, this bulk ratio is reduced by several orders of magnitude in individual bacteria-containing cloud droplets (< 10^{-23} - 10^{-15} moles per cell; Section S3 in the supplemental information), potentially suggesting limited availability of organic nutrients in cloud droplets.

waters (Figure 4c and Section S3). As a consequence, the steady-state nutrient levels per cell in the bacteria-containing droplets are at least 2, possibly up to 10, orders of magnitude lower than those in other aquatic environments.

Previous lab experiments to derive biodegradation rates in cloud water used organic-to-cell ratios in the range of 10^{-12} - 10^{-10} moles cell^{-1} (Vaïtilingom et al., 2010, 2011). Process models explored the role of biodegradation on chemical budgets in the atmospheric multiphase system (Khaled et al., 2021; Nuñez López et al., 2024) and found that the nutrient uptake from the gas phase may not be sufficiently fast to replenish biodegraded organics in the small subset of droplets, in which microorganisms are present and potentially active, resulting in even lower nutrient-to-cell ratios. In the same studies, it was shown that simplified model assumptions of an 'averaged cell concentration' in each droplet (i.e., < 0.001 cell droplet^{-1}, e.g., Pailler et al. (2023)) may lead to wrong conclusions regarding the role of biodegradation, as the nutrient-to-cell ratio is strongly overestimated. In light of these considerations, we suggest that lab and model studies should be performed for droplet-relevant organic-to-cell ratios to systematically explore potential limitation thresholds for individual bacteria strains and nutrients. Results from such studies would lead to a more accurate categorization of the atmosphere in terms of the trophic level. They could be also used to support (or refute) the classification of bacteria in rain as 'extremophiles' as put forward by Guillemette et al. (2023) based on much lower TOC levels in rain as compared to marine surface waters and in the deep sea.

Extrapolating the conclusions as made above for clouds to potential biological activity in aerosol particles (diameter $\lesssim 1$ μm) possibly suggests even greater nutrient limitation at RH < 100%. However, the partitioning of LMW volatile organics into aerosol water can greatly differ from that to (relatively) dilute droplets. For example, the fraction of formic acid partitioned to aerosol water is up to 7 orders of magnitude higher than that predicted based on Henry's law (Liu et al., 2012). Such strong

partitioning could compensate for the lower water volume. Thus, one may hypothesize that nutrient-to-cell ratios in wet aerosol particles and cloud droplets are similar. However, since the water and oxidant contents are different in aerosol particles and cloud droplets (Sections 2.2 and 2.4.2), the resulting osmotic and oxidative stress levels in these two different aqueous regimes might lead to different nutrient utilization rates.

2.4 Sunlight and oxidants: is the high photochemical activity in the atmosphere always stressful?

2.4.1 Solar radiation

The photic zone of surface waters, i.e., the layer penetrated by sunlight, is limited to the first few meters below the air-water interface; in soil, this layer is even shallower and restricted to a few milli- to centimeters. In all environments, including the atmosphere, the photolysis of dissolved organic matter results in LMW compounds that are often more biodegradable. At the same time, products of photochemical reactions include reactive oxygen species (ROS), such as the OH radical, that oxidize organics reducing the concentration levels of organic nutrients (Scully et al., 2003). In addition, ROS cause oxidative stress to bacteria cells as they can damage the cellular structure. Therefore, sunlight has both beneficial and adverse effects on microbial functioning and survival. Airborne particles are exposed to sunlight about half of their atmospheric residence time (during the day), as opposed to the much lower photochemical activity in surface environments. DNA damage due to UV light in the atmosphere can be parameterized as a function of irradiance and bacteria type (Madronich et al., 2018).

The actinic flux inside clouds represents a particular challenge as it can be enhanced or reduced as compared to cloud-free air depending on cloud optical density and the height in cloud (Ryu et al., 2017). Enhanced actinic fluxes occur when light is scattered or reflected multiple times inside 'bright' clouds with low optical thickness and few and/or small droplets. Conversely, sunlight cannot readily penetrate through dense, 'dark' cloud, resulting possibly in lower UV-induced stress for bacteria. Thus, the macro- and microstructures of clouds (e.g., vertical profiles of density) have to be taken into account to estimate the time scales during which bacteria are exposed to detrimental UV conditions. To counteract such UV-induced stress, bacteria may develop strategies mechanisms such as the formation of pigments as a protective shield. However, such mechanisms may only become effective after some lag time that has not been quantified under atmospheric conditions.

2.4.2 OH radical

The OH radical is one of the most powerful oxidants and 'cleansing agent' in the atmosphere and aquatic environments. Its main formation pathways in surface water include the direct photolysis of hydrogen peroxide (H_2O_2) and the Fenton reaction (Fe(II) oxidation by H_2O_2). In the atmosphere, it is mainly formed in the gas phase via the photolysis of ozone and HONO but also by the reaction of the hydroxy peroxy radical (HO_2) with NO. The rate of the latter process is significantly reduced in cloudy air masses, since the highly soluble HO_2 partitions into cloud water where it is quickly consumed, while the less soluble NO remains in the gas phase. This reactant separation leads to significantly lower total HO_2 and OH concentrations in the presence of clouds ($\leq 70\%$ and $\leq 80\%$, respectively, Ervens (2015)).
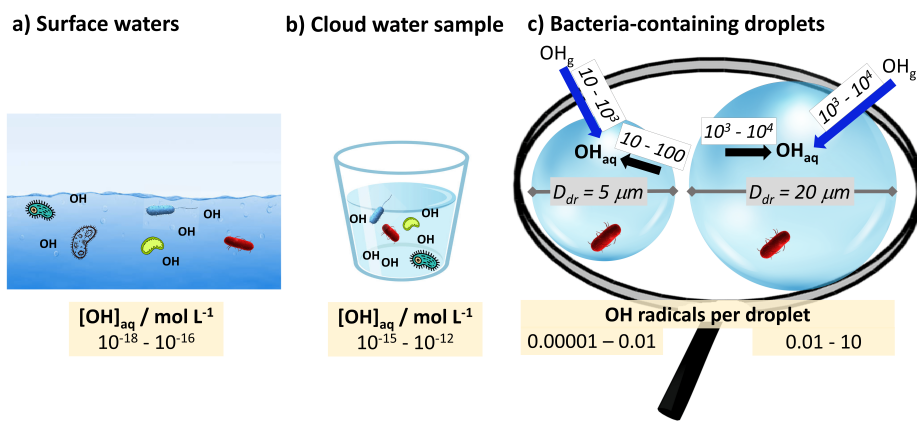
Figure 5. Concentrations of the OH radical in a) sea water (Mopper and Zhou, 1990); b) cloud water (Arakaki et al., 2013); c) individual cloud droplets (D_{dr} = 5μm, 20μm). The blue arrows in panel c denote the uptake rates of OH radicals per second, the black arrows denote their production rate inside a droplet (cf Figure S6c for individual reactions).

At typical cloud liquid water contents, less than 0.1% of all atmospheric OH radicals reside in cloud water. The aqueous phase concentration in cloud water [mol L^{-1}] is greater by about 3 orders of magnitude than in sea water (Figure 5a *vs.* b). This

195   comparison may imply - on a first sight - higher oxidative stress due to OH in the atmosphere. However, this conclusion may need to be carefully reconsidered if one defines the number of OH radicals in the immediate surrounding of a bacteria cell as a direct measure of the OH-oxidative stress for bacteria. Figure 5c shows exemplary that the steady-state OH concentration per droplet only exceeds 1 in droplets with diameters $D_{dr} > 20$ μm (Section S4 in the supplemental information). It should be kept in mind, though, that steady-state concentrations are a result of OH source and loss rates that usually (nearly) cancel.

200   OH sources in cloud droplets include the direct uptake from the gas phase and chemical reactions with rates of $\sim$10 - 10000 OH radicals per second (Figure 5c), with the Fenton being one of the main sources (Figure S6b). However, unlike the other aqueous phase OH sources (e.g. H_2O_2 or NO_3^- photolysis), the Fenton reaction only occurs in the subset of cloud droplets that contain iron (($\sim$10%), Khaled et al. (2022); Ervens (2022)). Thus, the oxidative stress due to Fenton chemistry as observed in other aquatic environments (Cabiscol et al., 2000), may be much lower in the atmosphere and only occur in 0.01% of all

205   droplets or particles (= 10% $\times$ 0.1%assuming a statistical distribution of iron and bacteria across a drop population). The OH uptake from the gas phase is often not sufficiently fast to replenish the consumed radicals and reach equilibrium concentrations ($\sim$10^{-12} mol L^{-1}), resulting in the lowest OH concentrations in large droplets (Ervens et al., 2014).

Microorganisms apply strategies to scavenge oxidants and respond to oxidative stress. The conditions under which oxidant scavenging rates cannot be compensated by defense mechanisms depend on numerous factors, including the bacteria

210   species, oxidant (concentration and species) and environmental conditions, such as temperature, relative humidity and nutrient availability. Systematic studies to constrain such conditions have not been performed yet in dispersed droplets. The aqueous photooxidation experiments of living bacteria in the presence of OH by Liu et al. (2023) might serve as a first indication;

however, the observed maximum survival time of 6 hours in a large aqueous phase volume likely represents a lower limit as compared to realistic in-cloud processing times during which OH-induced stress levels may be lower.

2.4.3   Other ROS and oxidants (e.g., $^3C^*$, $^1O_2$, H_2O_2)

While the OH radical is one of the most powerful chemical oxidants in the atmosphere, other oxidants are present at high concentrations as well, including H_2O_2, HO_2, triplet states ($^3C^*$), singlet oxygen ($^1O_2$) and ROS formed by photosensitizers which all cumulatively contribute to the oxidative stress of bacteria in clouds. Due to its high total concentration and solubility, H_2O_2 represents (one of) the most abundant ROS in cloud droplets. Wirgot et al. (2019) showed that microorganisms can metabolize H_2O_2 in aqueous solution with a chemical composition similar to that of cloud water. The experiments were conducted in a closed system; thus, it was not assessed whether biodegradation was sufficiently fast to completely detoxify a droplet that is continuously exposed to a gas phase H_2O_2 reservoir. Simultaneous measurements of H_2O_2 in the atmospheric gas and aqueous phases usually show thermodynamic equilibrium. However, such measurements reflect the conditions in the bulk cloud water; they do not allow the detection of small deviations due to biodegradation in a few droplets.

The concentrations of the more reactive $^3C^*$ and $^1O_2$ in cloud droplets are 1 to 2 orders of magnitude higher than OH (Kaur and Anastasio, 2018). Thus, scaling up the results shown for the OH radical in Figure S6a by a factor 10 - 100 suggests that there a few $^3C^*$ and $^1O_2$ in each cloud droplet. The formation rates of these oxidants under typical cloud conditions have not been fully characterized and implemented in multiphase chemistry models, and their metabolic consumption are even less constrained. Thus, the extent cannot be assessed to which the particular oxidant mix in cloud water - that is likely different from that in other aquatic environments - affects oxidative stress levels and ultimately, metabolic functioning and survival in the atmosphere. As compared to cloud droplets, the concentrations of $^3C^*$, $^1O_2$ and H_2O_2 in aerosol water are higher by 1 to 2 orders of magnitude (Ma et al., 2023; Arellanes et al., 2006) whereas OH concentrations are similar in both systems. However, the chemical conversion rates of the oxidants might be much higher than in droplets due to higher precursor concentrations (e.g. NO_3^-). At the same time, the microorganisms are exposed to higher ionic strength in the much less dilute particles that might alter their ability to apply efficient antioxidative defense mechanisms. Such mechanisms could include the formation of biosurfactants that slow down or even impede uptake from or evaporation to the gas phase (Gill et al., 1983). Due to the lack of kinetic and mechanistic data to describe chemical formation and loss rates of ROS species in aerosol particles, the ROS budgets in aerosol water cannot be speciated on a molecular level. Therefore, dedicated lab experiments and process model studies should be designed to constrain oxidant levels in the atmospheric aqueous phases to ultimately determine their effect on microbial oxidative stress levels.

3   Trends of atmospheric microorganisms and pollutants: correlation, causation or coincidence?

Observations of atmospheric trends between chemical concentrations and bacterial abundance and community structure (including diversity) are to a large extent determined by air mass types, history and age (Innocente et al., 2017; Gandolfi et al., 2013). Several atmospheric studies aimed at identifying atmospheric chemical or physical parameters that influence the abun-

245 dance, diversity and/or viability of atmospheric microorganisms. Such studies often resulted in contradictory conclusions regarding the impact of specific atmospheric conditions (e.g., pollution or clouds) on the atmospheric microbiome. Lebowitz and O'rourke (1991) cautioned against concluding on links between aerobiological and chemico-physical contaminants in determining factors that potentially trigger adverse health effects. In view of the microscale chemical and microphysical factors discussed in Section 2, similarly we emphasize the importance of not confusing causation with correlation regarding atmo-
250 spheric parameters affecting microbial stress.
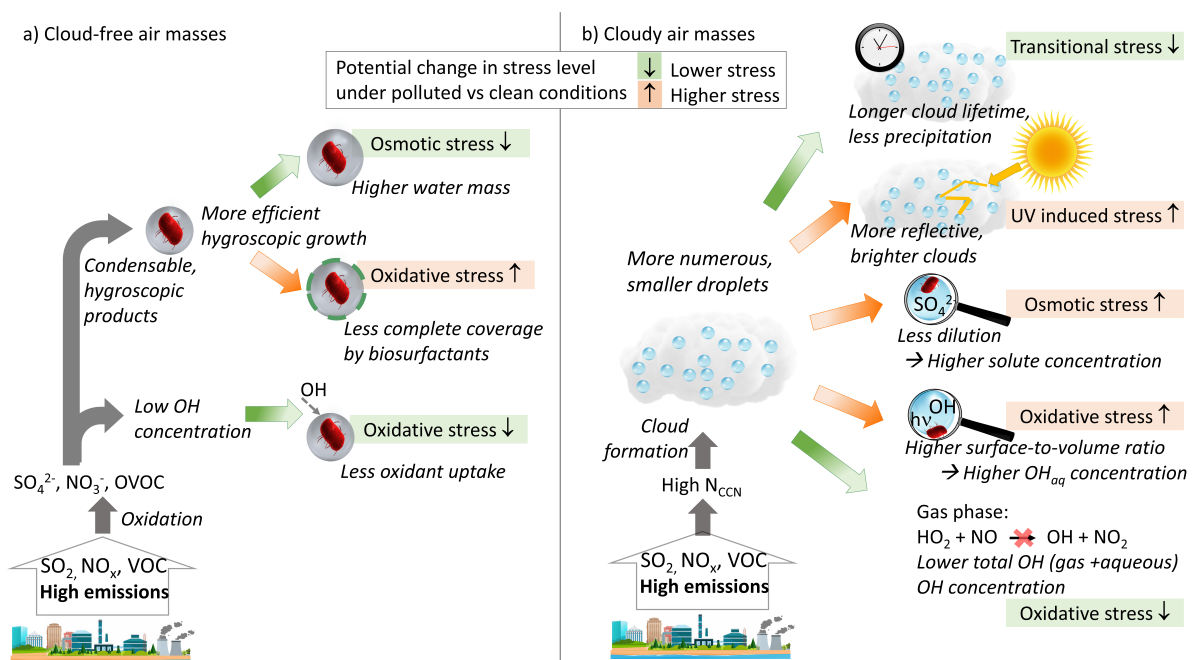
Figure 6. Potential microbial stress responses due to chemical or microphysical processes in polluted air masses. It is not implied that each of these factors is necessarily important or significant to have beneficial or adverse effects on bacteria. Instead, the illustration intends to demonstrate that correlations between atmospheric pollutants do not imply causation. a) Cloud-free air masses: osmotic stress may be lowered due to addition of hygroscopic mass that attracts more water (Section 2.2) whereas oxidative stress may increase if particles exceed sizes too large to be covered by biosurfactants to shield the particles from oxidant uptake. Total atmospheric oxidant levels may be comparably small in a polluted atmosphere since OH is efficiently titrated (Section 2.4.2). b) Cloudy air masses: high aerosol number concentrations lead to clouds with more numerous but smaller cloud droplets ('cloud lifetime effect'). Such clouds are less likely to precipitate but are at the same time less dense and brighter as light reflections between droplets are amplified (Section 2.4.1). Smaller droplets typically contain higher solute and oxidant (OH) concentrations which may enhance both osmotic and oxidative stress. The latter may be (partially) compensated for by the fact that total (gas + aqueous) OH and HO_2 concentrations are usually much lower in the presence of clouds due to smaller formation rates in the gas phase (Section 2.4.2).

Figure 6 summarizes chemical or microphysical factors in polluted air masses that may affect different types of microbial stress levels (oxidative, osmotic, UV induced, transitional). None of the shown effects imply a direct adverse or beneficial influence by a specific pollutant (e.g., SO_2, NO_x VOCs or their oxidation products) on bacteria viability, diversity or abundance. We do not suggest that such effects do not exist; however, to the best of our knowledge, corresponding biomarkers (specific response genes, enzymes) have not been identified yet. Also we do not propose that indeed all listed chemical or microphysical parameters trigger significant stress responses or that any of the indicated responses dominate or cancel each other. Instead, the schematic may be used as a qualitative - or even speculative - illustration to motivate studies to quantify the potential role of the individual factors in targeted experiments. The results of such studies may then be used to conclude on the role of each of these factors to derive robust cause-and-effect relationships. Falsely derived conclusion of causation may ultimately result in inappropriate strategies to maintain healthy ecosystems and biodiversity in a changing atmosphere.

In cloud-free air masses, an increase in the hygroscopicity of the bacteria-containing particle may lead to lower osmotic stress, but possibly to more efficient ROS uptake from the gas phase (Figure 6a). Whether such a protection mechanism is significant at all or negligible as compared to the lower OH levels that exist in polluted air masses remains to be seen.

As pointed out in the previous sections, many microphysical processes determine the micro- and macroscopic structure of clouds, including droplet size distributions that determine droplet lifetime (smaller droplets are less likely to precipitate) and cloud optical thickness (Section 2.4.1), which may have opposite effects on stress levels (Figure 6b).

In turn, higher particle concentrations in polluted air masses result in smaller, more numerous droplets with higher solute and OH concentrations, i.e., enhancing osmotic and oxidative stress. However, since OH (and several other oxidant) levels are reduced in the presence of clouds (Section 2.4.2), an apparent lower stress level in the presence of clouds could be possibly simply ascribed to exposure to lower oxidant levels.

Such considerations are likely just a small subset of bio-physico-chemical feedbacks in the atmospheric multiphase system to explain observed trends. Adding to the vagueness is the lack of comprehensive, speciated bacterial emission maps and patterns. Bacteria are detached from the surface and lifted by mechanical forces (e.g., strong wind), just like any other primary particle. In addition, there might be biological selection criteria that trigger aerosolization and emission of specific microorganisms. Since bacteria emission fluxes into the atmosphere are poorly constrained, observed trends between bacteria-related parameters and other environmental factors may be just coincidental since the atmospheric composition is a result of mixing of air masses of different origin and age. 'Chemical clocks', e.g., the ratio of co-emitted but differently reactive compounds, are often used to determine the 'chemical age of air'. Equivalent measures to determine similar indicators for the 'biological age of air' are still lacking. Such indicators would be useful to not only determine the viability and survival along air mass trajectories but it would also allow to conclude on emission patterns at different locations.

4 Conclusions

Atmospheric microbiology is an interdisciplinary research field at the intersections of atmospheric sciences, aerobiology and microbial ecology. Figure 7 illustrates their overlap and lists some shared research objectives (biogeochemical cycles, microbial

diversity and dispersion, and atmospheric transport, respectively). Scientific concepts of all three areas have to be taken into
285 account for a comprehensive characterization of atmospheric microorganisms as a small, but yet important, dynamically rapidly
changing and evolving fraction of Earth' microbiome.

Numerous previous studies focused on the role of microorganisms on the atmosphere for impacting clouds or chemical
concentrations. By now, there is general consensus that this role may be limited to specific regions, conditions and processes
such as ice nucleation near 0°C, or biodegradation of selected compounds as a significant atmospheric sink. However, despite
290 the limited global influence of microorganisms on the atmosphere and climate, further studies of the microbial bio-physico-
chemical properties are needed to explore the ability of microorganisms to take up water and/or be incorporated in clouds
(hygroscopicity, ice nucleation ability). This information will be important to correctly estimate their atmospheric processing
times of microorganisms and therefore their fate. Such studies can apply the same methodologies used for other atmospheric
particles, but should be motivated by research questions targeted to understand the role of the atmosphere on microorganisms,
295 rather than the reverse. Similarly, the rationale for exploring biodegradation rates in cloud water could be extended from
focusing on potential impacts on chemical budgets to consequences of limited nutrient availability on microorganism starvation
and survival.
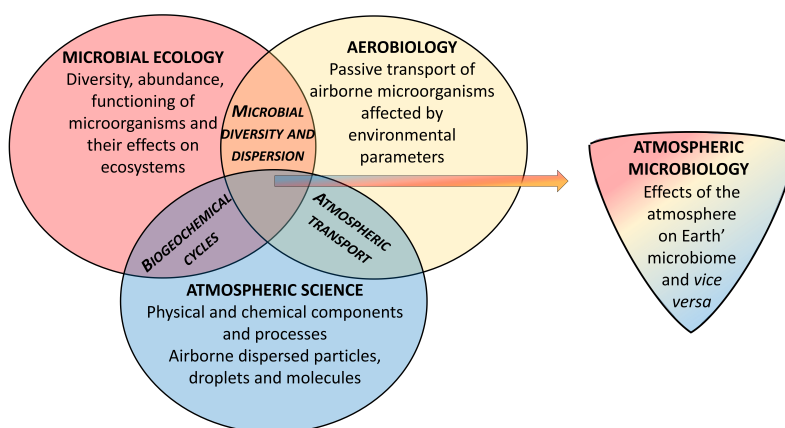
Figure 7. Atmospheric microbiology forms the intersection of atmospheric sciences, microbial ecology and aerobiology

Studies on microbial optimization strategies for water uptake or nutrient utilization are not new; they have been conducted for
many decades in the context of aquatic and terrestrial environments. Transferring such established methods to the atmospheric
300 microbiome is challenging for (at least) two reasons: Firstly, atmospheric bacteria number concentrations are extremely low.
Thus, collecting statistically meaningful samples requires large volumes and/or long time scales. This complicates the identi-
fication of patterns and sources as air masses efficiently mix over time. Secondly, unlike in other environments where bacteria
can form aggregates and biofilms or are suspended in water, atmospheric bacteria are aerosolized before they are lifted and
dispersed in air. The immediate environment of airborne bacteria is constrained by particle or drop volumes.

305 We highlight such specific conditions due to bacteria aerosolization, that imply distances of several centimeters between cells, which makes their living environments different from those in (more) homogeneous environments. Previously, conclusions on potential stress factors or nutrient availability in the atmosphere were drawn based on the concentration levels in atmospheric bulk samples where $\lesssim$0.1% bacteria-containing droplets are mixed with $\gtrsim$99.9% bacteria-free droplets (particles). In dispersed droplets (particles), the concentration ratios of cells to nutrients or oxidants, respectively, should be considered on a

310 per-droplet basis. The much lower cell-to-nutrient ratio in an individual droplet ($\lesssim$1/0.1%) as compared to that in bulk samples implies a much lower trophic regime for bacteria than previously thought. The atmosphere is considered a harsh environment for microorganisms due to numerous factors, including high photochemical activity. However, droplets and particles contain only a few oxidant molecules (e.g., OH, $^3C^*,^1O_2$) that are quickly transformed chemically but possibly also metabolized. To describe the particular mixtures of nutrients and oxidants that bacteria are exposed to in the aerosol/droplet microcosms, pro-

315 cess models should be applied with detailed atmospheric multiphase chemistry, microphysics and data on biological processes (e.g., biotransformation of nutrients or ROS). However, this latter data is largely lacking, in particular from studies on individual (e.g., levitated) droplets or particles. Given the challenges associated with such experimental set-ups, we recommend that bulk experiments should at least take into account realistic solute mixtures.

In summary, we provide a new perspective on atmospheric microbiology within the context of atmospheric chemistry and

320 microphysics. We emphasize the importance of specific spatial and temporal scales of microbial microcosms in individual particles and droplets. Such considerations are essential to enhance our understanding of the atmosphere as an extension of the more well-characterized microbial environments such as oceans and soil. Ultimately, this will lead to a complete characterization of the Earth microbiome and its cycling that ensures global ecological stability and functioning.

Code and data availability. The supplement contains all equations that were applied for calculations using the cited literature values.

325 *Author contributions.* BE developed the idea and concept of the study. All co-authors contributed to discussions and to the writing of the manuscript.

Competing interests. The authors do not have any competing interests to declare.

Acknowledgements. We thank Z. Bourhane, A.-M. Delort, F. Rossi and J. Vyskocil for useful discussions.

Financial support. This work has been supported by the French National Research Agency (ANR) (grant no. ANR-17-MPGA- 0013).

References

Amato, P., Joly, M., Besaury, L., Oudart, A., Taib, N., Moné, A. I., Deguillaume, L., Delort, A., and Debroas, D.: Active microorganisms thrive among extremely diverse communities in cloud water, PLOS One, 12, https://doi.org/10.1371/journal.pone.0182869, 2017.

Arakaki, T., Anastasio, C., Kuroki, Y., Nakajima, H., Okada, K., Kotani, Y., Handa, D., Azechi, S., Kimura, T., Tsuhako, A., and Miyagi, Y.: A general scavenging rate constant for reaction of hydroxyl radical with organic carbon in atmospheric waters, Environmental Science & Technology, 47, 8196–8203, https://doi.org/10.1021/es401927b, 2013.

Arellanes, C., Paulson, S. E., Fine, P. M., and Sioutas, C.: Exceeding of Henry's Law by Hydrogen Peroxide Associated with Urban Aerosols, Environ. Sci Technol., 40, 4859–4866, https://doi.org/10.1021/es0513786, 2006.

Bar-On, Y. M., Phillips, R., and Milo, R.: The biomass distribution on Earth, Proceedings of the National Academy of Sciences, 115, 6506–6511, https://doi.org/10.1073/pnas.1711842115, 2018.

Boetius, A., Anesio, A. M., Deming, J. W., Mikucki, J. A., and Rapp, J. Z.: Microbial ecology of the cryosphere: sea ice and glacial habitats, Nature Reviews Microbiology, 13, 677–690, https://doi.org/10.1038/nrmicro3522, 2015.

Burrows, S. M., Butler, T., Jöckel, P., Tost, H., Kerkweg, A., Pöschl, U., and Lawrence, M. G.: Bacteria in the global atmosphere – Part 2: Modeling of emissions and transport between different ecosystems, Atmospheric Chemistry and Physics, 9, 9281–9297, https://doi.org/10.5194/acp-9-9281-2009, 2009a.

Burrows, S. M., Elbert, W., Lawrence, M. G., and Pöschl, U.: Bacteria in the global atmosphere – Part 1: Review and synthesis of literature data for different ecosystems, Atmospheric Chemistry and Physics, 9, 9263–9280, https://doi.org/10.5194/acp-9-9263-2009, 2009b.

Cabiscol, E., Tamarit, J., and Ros, J.: Oxidative stress in bacteria and protein damage by reactive oxygen species., International microbiology : the official journal of the Spanish Society for Microbiology, 3, 3–8, 2000.

Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., Narwani, A., Mace, G. M., Tilman, D., Wardle, D. A., Kinzig, A. P., Daily, G. C., Loreau, M., Grace, J. B., Larigauderie, A., Srivastava, D. S., and Naeem, S.: Biodiversity loss and its impact on humanity, Nature, 486, 59–67, https://doi.org/10.1038/nature11148, 2012.

Catalán, N., Casas-Ruiz, J. P., von Schiller, D., Proia, L., Obrador, B., Zwirnmann, E., and Marcé, R.: Biodegradation kinetics of dissolved organic matter chromatographic fractions in an intermittent river, Journal of Geophysical Research: Biogeosciences, 122, 131–144, https://doi.org/10.1002/2016JG003512, 2017.

del Giorgio, P. A. and Cole, J. J.: Bacterial growth efficiency in natural aquatic systems, Annual Review of Ecology and Systematics, 29, 503–541, https://doi.org/10.1146/annurev.ecolsys.29.1.503, 1998.

Eiler, A., Langenheder, S., Bertilsson, S., and Tranvik, L. J.: Heterotrophic Bacterial Growth Efficiency and Community Structure at Different Natural Organic Carbon Concentrations, Applied and Environmental Microbiology, 69, 3701–3709, https://doi.org/10.1128/AEM.69.7.3701-3709.2003, 2003.

Ervens, B.: Modeling the Processing of Aerosol and Trace Gases in Clouds and Fogs, Chemical Reviews, 115, 4157–4198, https://doi.org/10.1021/cr5005887, 2015.

Ervens, B.: Average cloud droplet size and composition: Good assumptions for predicting oxidants in the atmospheric aqueous phase?, Journal of Physical Chemistry A, 126, 8295–8304, https://doi.org/10.1021/acs.jpca.2c0552, 2022.

Ervens, B. and Amato, P.: The global impact of bacterial processes on carbon mass, Atmospheric Chemistry and Physics, 20, 1777–1794, https://doi.org/10.5194/acp-20-1777-2020, 2020.

Ervens, B., Sorooshian, A., Lim, Y. B., and Turpin, B. J.: Key parameters controlling OH-initiated formation of secondary organic aerosol in the aqueous phase (aqSOA), Journal of Geophysical Research - Atmospheres, 119, 3997–4016, https://doi.org/10.1002/2013JD021021, 2014.

Fernandez, M. O., Thomas, R. J., Garton, N. J., Hudson, A., Haddrell, A., and Reid, J. P.: Assessing the airborne survival of bacteria in populations of aerosol droplets with a novel technology, Journal of The Royal Society Interface, 16, 20180 779, https://doi.org/10.1098/rsif.2018.0779, 2019.

Gandolfi, I., Bertolini, V., Ambrosini, R., Bestetti, G., and Franzetti, A.: Unravelling the bacterial diversity in the atmosphere, Applied Microbiology and Biotechnology, 97, 4727–4736, https://doi.org/10.1007/s00253-013-4901-2, 2013.

Gill, P. S., Graedel, T. E., and Weschler, C. J.: Organic films on atmospheric aerosol particles, fog droplets, cloud droplets, raindrops, and snowflakes, Reviews of Geophysics, 21, 903–920, https://doi.org/10.1029/RG021i004p00903, 1983.

Guillemette, R., Harwell, M. C., and Brown, C. A.: Metabolically active bacteria detected with click chemistry in low organic matter rainwater, PLOS ONE, 18, e0285 816, https://doi.org/10.1371/journal.pone.0285816, 2023.

Herckes, P., Valsaraj, K. T., and Collett Jr, J. L.: A review of observations of organic matter in fogs and clouds: Origin, processing and fate, Atmos. Res., 132–133, 434–449, https://doi.org/10.1016/j.atmosres.2013.06.005, 2013.

Innocente, E., Squizzato, S., Visin, F., Facca, C., Rampazzo, G., Bertolini, V., Gandolfi, I., Franzetti, A., Ambrosini, R., and Bestetti, G.: Influence of seasonality, air mass origin and particulate matter chemical composition on airborne bacterial community structure in the Po Valley, Italy, Science of The Total Environment, 593-594, 677–687, https://doi.org/10.1016/j.scitotenv.2017.03.199, 2017.

Kaur, R. and Anastasio, C.: First Measurements of Organic Triplet Excited States in Atmospheric Waters, Environmental Science & Technology, 52, 5218–5226, https://doi.org/10.1021/acs.est.7b06699, 2018.

Khaled, A., Zhang, M., Amato, P., Delort, A.-M., and Ervens, B.: Biodegradation by bacteria in clouds: an underestimated sink for some organics in the atmospheric multiphase system, Atmospheric Chemistry and Physics, 21, 3123–3141, https://doi.org/10.5194/acp-21-3123-2021, 2021.

Khaled, A., Zhang, M., and Ervens, B.: The number fraction of iron-containing particles affects OH, HO$_2$ and H$_2$O$_2$ budgets in the atmospheric aqueous phase, Atmospheric Chemistry and Physics, 22, 1989–2009, https://doi.org/10.5194/acp-22-1989-2022, 2022.

Lebowitz, M. D. and O'rourke, M. K.: The significance of air pollution in aerobiology, Grana, 30, 31–43, https://doi.org/10.1080/00173139109427766, 1991.

Leizeaga, A., Meisner, A., Rousk, J., and Bååth, E.: Repeated drying and rewetting cycles accelerate bacterial growth recovery after rewetting, Biology and Fertility of Soils, 58, 365–374, https://doi.org/10.1007/s00374-022-01623-2, 2022.

Lindow, S. E. and Brandl, M. T.: Microbiology of the phyllosphere., Applied and environmental microbiology, 69, 1875–1883, https://doi.org/10.1128/AEM.69.4.1875-1883.2003, 2003.

Liu, J., Zhang, X., Parker, E. T., Veres, P. R., Roberts, J. M., de Gouw, J. A., Hayes, P. L., Jimenez, J. L., Murphy, J. G., Ellis, R. A., Huey, L. G., and Weber, R. J.: On the gas-particle partitioning of soluble organic aerosol in two urban atmospheres with contrasting emissions: 2. Gas and particle phase formic acid, Journal of Geophysical Research: Atmospheres, 117, D00V21, https://doi.org/10.1029/2012jd017912, 2012.

Liu, Y., Lee, P. K. H., and Nah, T.: Emerging investigator series: Aqueous photooxidation of live bacteria with hydroxyl radicals under clouds-like conditions: Insights into the production and transformation of biological and organic matter originating from bioaerosols, Environmental Science: Processes & Impacts, https://doi.org/10.1039/D3EM00090G, 2023.

Ma, L., Worland, R., Tran, T., and Anastasio, C.: Evaluation of Probes to Measure Oxidizing Organic Triplet Excited States in Aerosol Liquid Water, Environmental Science & Technology, 57, 6052–6062, https://doi.org/10.1021/acs.est.2c09672, 2023.

405 Madronich, S., Björn, L. O., and McKenzie, R. L.: Solar UV radiation and microbial life in the atmosphere, Photochemical & Photobiological Sciences, 17, 1918–1931, https://doi.org/10.1039/C7PP00407A, 2018.

Mopper, K. and Zhou, X.: Hydroxyl radical photoproduction in the sea and its potential impact on marine processes., Science (New York, N.Y.), 250, 661–664, https://doi.org/10.1126/science.250.4981.661, 1990.

Nuñez López, L., Amato, P., and Ervens, B.: Bacteria in clouds biodegrade atmospheric formic and acetic acids, Atmos. Chem . Phys., 24,
410 5181 – 5198, https://doi.org/10.5194/acp-24-5181-2024, 2024.

Pailler, L., Wirgot, N., Joly, M., Renard, P., Mouchel-Vallon, C., Bianco, A., Leriche, M., Sancelme, M., Job, A., Patryl, L., Armand, P., Delort, A.-M., Chaumerliac, N., and Deguillaume, L.: Assessing the efficiency of water-soluble organic compound biodegradation in clouds under various environmental conditions, Environmental Science: Atmospheres, 3, 731–748, https://doi.org/10.1039/D2EA00153E, 2023.

415 Pasteur, L.: Mémoire sur les corpuscles organisés qui existent dans l'atmosphère, examen de la doctrine des générations spontanées, Masson, 1861.

Péguilhan, R., Rossi, F., Joly, M., Nasr, E., Batut, B., Enault, F., Ervens, B., and Amato, P.: Clouds, oases for airborne microbes – Differential metagenomics/ metatranscriptomics analyses of cloudy and clear atmospheric situations, bioRxiv, p. 2023.12.14.571671, https://doi.org/10.1101/2023.12.14.571671, 2023.

420 Peguilhan, R., Rossi, F., Joly, M., Nasr, E., Batut, B., Enault, F., Ervens, B., and Amato, P.: Clouds influence the functioning of airborne microorganisms, Biogeosciences, 2024, submitted.

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I. J., and Villar, R.: Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis, New Phytologist, 182, 565–588, https://doi.org/10.1111/j.1469-8137.2009.02830.x, 2009.

Robinson, J. M. and Breed, M. F.: The aerobiome–health axis: a paradigm shift in bioaerosol thinking, Trends in Microbiology, 31, 661–664,
425 https://doi.org/10.1016/j.tim.2023.04.007, 2023.

Ross, B. N. and Whiteley, M.: Ignoring social distancing: advances in understanding multi-species bacterial interactions, Faculty Reviews, 9, 23, https://doi.org/10.12703/r/9-23, 2020.

Ryu, Y.-H., Hodzic, A., Descombes, G., Hall, S., Minnis, P., Spangenberg, D., Ullmann, K., and Madronich, S.: Improved modeling of cloudy-sky actinic flux using satellite cloud retrievals, Geophysical Research Letters, 44, 1592–1600, https://doi.org/10.1002/2016GL071892,
430 2017.

Šantl-Temkiv, T., Amato, P., Casamayor, E. O., Lee, P. K. H., and Pointing, S. B.: Microbial ecology of the atmosphere, FEMS Microbiology Reviews, p. fuac009, https://doi.org/10.1093/femsre/fuac009, 2022.

Scully, N. M., Cooper, W. J., and Tranvik, L. J.: Photochemical effects on microbial activity in natural waters: the interaction of reactive oxygen species and dissolved organic matter, FEMS Microbiology Ecology, 46, 353–357, https://doi.org/10.1016/S0168-6496(03)00198-
435 3, 2003.

Smith, D. J., Ravichandar, J. D., Jain, S., Griffin, D. W., Yu, H., Tan, Q., Thissen, J., Lusby, T., Nicoll, P., Shedler, S., Martinez, P., Osorio, A., Lechniak, J., Choi, S., Sabino, K., Iverson, K., Chan, L., Jaing, C., and McGrath, J.: Airborne Bacteria in Earth's Lower Stratosphere Resemble Taxa Detected in the Troposphere: Results From a New NASA Aircraft Bioaerosol Collector (ABC) , Frontiers in Microbiology, 9, 1752, https://doi.org/10.3389/fmicb.2018.01752, 2018.

440   Stevenson, A., Burkhardt, J., Cockell, C. S., Cray, J. A., Dijksterhuis, J., Fox-Powell, M., Kee, T. P., Kminek, G., McGenity, T. J., Timmis, K. N., Timson, D. J., Voytek, M. A., Westall, F., Yakimov, M. M., and Hallsworth, J. E.: Multiplication of microbes below 0.690 water activity: implications for terrestrial and extraterrestrial life, Environmental Microbiology, 17, 257–277, https://doi.org/10.1111/1462-2920.12598, 2015.

Stevenson, A., Hamill, P. G., O'Kane, C. J., Kminek, G., Rummel, J. D., Voytek, M. A., Dijksterhuis, J., and Hallsworth, J. E.:
445   Aspergillus penicillioides differentiation and cell division at 0.585 water activity, Environmental Microbiology, 19, 687–697, https://doi.org/10.1111/1462-2920.13597, 2017.

Vaïtilingom, M.: Potential impact of microbial activity on the oxidant capacity and organic carbon budget in clouds, Proceedings of the National Academy of Sciences USA, 110, https://doi.org/10.1073/pnas.1205743110, 2013.

Vaïtilingom, M., Amato, P., Sancelme, M., Laj, P., Leriche, M., and Delort, A.-M.: Contribution of Microbial Activity to Carbon Chemistry
450   in Clouds, Applied and Environmental Microbiology, 76, 23–29, https://doi.org/10.1128/AEM.01127-09, 2010.

Vaïtilingom, M., Charbouillot, T., Deguillaume, L., Maisonobe, R., Parazols, M., Amato, P., Sancelme, M., and Delort, A. M.: Atmospheric chemistry of carboxylic acids: microbial implication versus photochemistry, Atmospheric Chemmistry and Physics, 11, 8721–8733, https://doi.org/10.5194/acp-11-8721-2011, 2011.

Wei, H., Vejerano, E. P., Leng, W., Huang, Q., Willner, M. R., Marr, L. C., and Vikesland, P. J.: Aerosol microdroplets exhibit a stable pH
455   gradient, Proceedings of the National Academy of Sciences USA, 115, 7272–7277, https://doi.org/10.1073/pnas.1720488115, 2018.

Whitman, W. B., Coleman, D. C., and Wiebe, W. J.: Prokaryotes: The unseen majority, Proceedings of the National Academy of Sciences, 95, 6578–6583, https://doi.org/10.1073/pnas.95.12.6578, 1998.

Wirgot, N., Lagrée, M., Traïkia, M., Besaury, L., Amato, P., Canet, I., Sancelme, M., Jousse, C., Diémé, B., Lyan, B., and Delort, A.-M.: Metabolic modulations of Pseudomonas graminis in response to H_2O_2 in cloud water, Scientific Reports, 9, 12 799,
460   https://doi.org/10.1038/s41598-019-49319-2, 2019.

Zhang, M., Khaled, A., Amato, P., Delort, A.-M., and Ervens, B.: Sensitivities to biological aerosol particle properties and ageing processes: potential implications for aerosol–cloud interactions and optical properties, Atmospheric Chemistry and Physics, 21, 3699–3724, https://doi.org/10.5194/acp-21-3699-2021, 2021.