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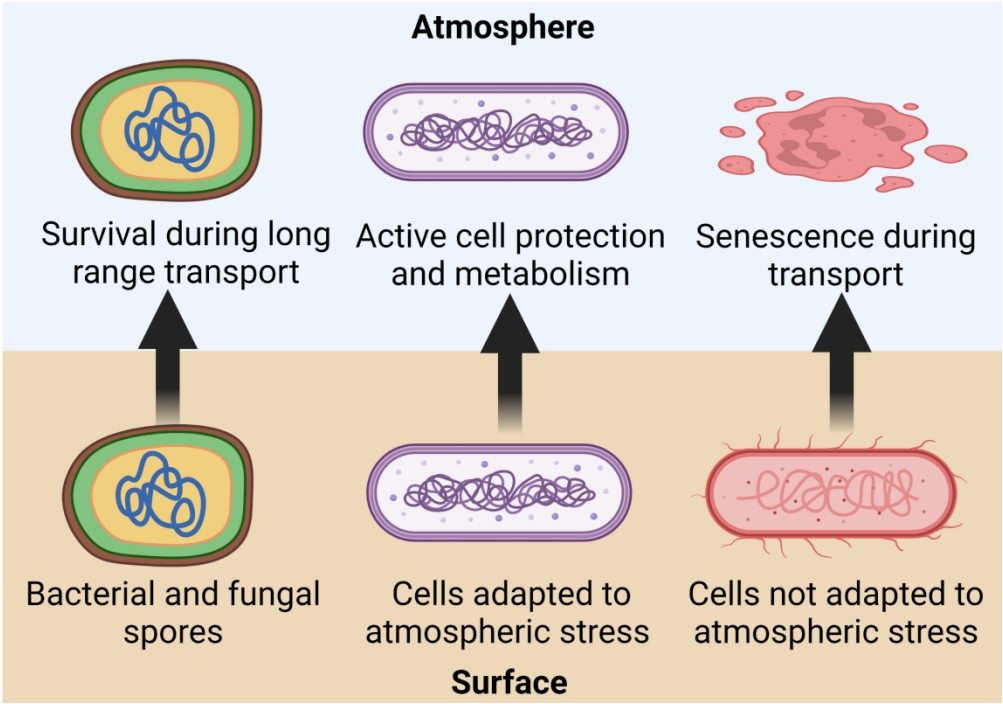
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Microbial ecology of the atmosphere

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Summary

This review highlights the ecological importance of atmospheric microorganisms with particular focus on recent advances in environmental metagenomics, a critical assessment of the evidence for microbially-mediated atmospheric biochemical and biophysical transformations and consideration of potential impacts due to anthropogenic forcing.

Keywords

Aeromicrobiology, Atmospheric microbiology, Ecosystems ecology, Microbial biogeography, Microbial ecology, Microbial dispersal

Abstract

The atmosphere facilitates connectivity between microbial habitats across multiple spatial scales via airborne transport of cells, propagules and biomolecules. Airborne microorganisms are also implicated in biochemical transformation of atmospheric compounds and biophysical ice nucleation that influence climate processes. Recent advances in methodology for interrogating ultra-low biomass habitats such as air have led to significant advances in understanding. In this review, the contribution of environmental metagenomics to atmospheric diversity estimation, evidence for diverse metabolic transformations, and elucidation of ice nucleation mechanisms are critically assessed, and knowledge gaps identified. The central importance of the atmospheric microbiota to macroecological outcomes including long-distance pathogen dispersal is highlighted. The potential for a metabolically active atmospheric microbiome is evaluated and microbial influence on the water cycle is highlighted. Finally, possible

43 impacts of anthropogenic forcing on the atmospheric microbiota and ecological
44 outcomes are identified.

46 Introduction

47 Microorganisms have been extensively studied in terrestrial and aquatic natural
48 environments where an implicit and persistent assumption for observed ecological
49 patterns has been that atmospheric transport between habitats is essentially a neutral
50 process due to the extensive aerosolization and long-range transport of cells. This was
51 first embodied in the classic “everything is everywhere, but, the environment selects”
52 statement by Lourens Baas Becking and has driven hypotheses in microbial ecology
53 research for decades (de Wit and Bouvier 2006). Evidence for the distribution of
54 microorganisms in the atmosphere (aeromicrobiology) has been reported since the
55 earliest days of modern life science, with visionaries including Louis Pasteur and
56 Charles Darwin identifying airborne microorganisms (Schwartz 2001; Gorbushina *et al.*
57 2007).

58 A major challenge for aeromicrobiology has been that traditional cultivation
59 based enumeration is strongly biased towards cultivable taxa (Šantl-Temkiv *et al.*
60 2020), and even the most modern high-throughput sequencing approaches that more
61 fully capture environmental diversity are subject to bias from contaminant signal due to
62 the ultra-low biomass of microorganisms in air (Salter *et al.* 2014). Recent advances in
63 high-volume air mass recovery and application of bioinformatics approaches to
64 decontamination of environmental sequence data have led to significant novel insight,
65 e.g. (Cáliz *et al.* 2018; Gusareva *et al.* 2019; Tignat-Perrier *et al.* 2019; Archer *et al.*
66 2021), thus theoretical frameworks in ecology that challenge the traditional notion of

1
2
3 67 ubiquitous microbial distribution in the atmosphere, e.g. (Dumbrell *et al.* 2010; Lowe
4
5 68 and McPeck 2014) are being validated and revised.
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7

8 69 It is now apparent that the atmosphere supports vast and highly variable
9
10 70 numbers of microbial cells, pollen, propagules, cell fragments and biomolecules
11
12 71 (Després *et al.* 2012), which comprise the primary biological aerosol particles (PBAP)
13
14 72 estimated to account for 25% of all particles larger than 200 nm (Jaenicke 2005).
15
16 73 Overall, the majority of cells in the atmosphere are likely metabolically inactive and are
17
18 74 undergoing transport between source and sink destinations with a high turnover
19
20 75 (Burrows *et al.* 2009). The fate of the viable fraction of PBAP is important to dispersal
21
22 76 outcomes in terrestrial and marine ecosystems globally (Hanson *et al.* 2012), where the
23
24 77 heterogenous air microbiota may strongly influence ecological outcomes, e.g. affecting
25
26 78 resilience through acting as microbial “seed banks” (Lennon and Jones 2011). In
27
28 79 addition, the recognition that some microorganisms mediate biochemical (Deguillaume
29
30 80 *et al.* 2008) and biophysical transformations (Morris *et al.* 2008) during brief residence
31
32 81 periods in the atmosphere offers the prospect of an active microbial contribution to
33
34 82 atmospheric processes. Finally, PBAP have important health-related effects, through the
35
36 83 dispersal of animal (Lai, Emberlin and Colbeck 2009) and plant (Brown and Hovmøller
37
38 84 2002) pathogens, gene-flow of antibiotic resistance genes (Pal *et al.* 2016), and
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40 85 microbially derived allergens (Woo *et al.* 2013).
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48 86 In this review, the unique complexity of the atmospheric environment and its
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50 87 microbiota is identified and the extent and limitations in our current understanding of
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52 88 its ecological relevance to other biomes is considered. The scope encompasses
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54 89 microorganisms that occur in outdoor atmospheric air for extended periods or travel
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56 90 across regional or inter-continental distances. The short-range dispersal of human
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58 91 pathogens facilitated by exhaled respiratory droplets in air (Zhang *et al.* 2020) and
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airborne transmission within the indoor built environment (Gilbert and Stephens 2018) are excluded. Specific focus is given to how insight from environmental metagenomics has identified atmospheric microbiology as key to understanding the interdependence of ecosystems via flux of diverse taxa between atmosphere and surface habitats, and critical examination of the genetic and physiological evidence for microbial activity in the atmosphere. Furthermore, given the atmosphere is facing profound change due to anthropogenic emissions, consideration is given to how this may impact its microbiology in future.

Boundaries for habitability in the atmosphere

The atmosphere comprises a series of vertically delineated concentric layers whose altitude and depth are primarily defined by thermal properties (FIG. 1a). The troposphere is the lowermost atmospheric layer that contains 75% of its molecular and gaseous mass as well as most water vapour and atmospheric particles (Barry and Chorley 2010), including the majority of microbial cells (FIG. 1b). This is where water cycles through clouds, precipitation and surface environments and it includes the atmospheric boundary layer (a.k.a. planetary boundary layer), which is in direct contact with the surface, and the free troposphere, which is of great relevance for regional and global dispersal due to long-range movement of air. Vertical mixing above the troposphere is limited due to thermal inversion. The bulk of atmospheric ozone is located in the lower stratosphere and this is thought to represent the altitudinal limit to survival for the majority of microbial cells due to lethal UV exposure at higher altitudes as a function of reduced attenuation by ozone and extended residence time for suspended biological particles at higher altitudes (Bryan *et al.* 2019).

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3 116 The troposphere presents an extremely challenging but nonetheless potentially
4
5 117 habitable environmental gradient for microorganisms. The presence of sufficient liquid
6
7 118 water in cells – a fundamental pre-requisite for any microbial activity – is subject to
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9 119 atmospheric relative humidity that is determined by physical processes and
10
11 120 evapotranspiration. In the troposphere, relative humidity is generally bimodal, with
12
13 121 higher values in the lower and upper troposphere and a drier mid-troposphere (FIG. 1b)
14
15 122 (Gettelman *et al.* 2006). The lifted condensation level (LCL) is the altitude at which
16
17 123 vapour pressure of an air parcel becomes saturated and above the LCL, at slight
18
19 124 supersaturations, the water vapour will condense on aerosol particles and form clouds.
20
21 125 LCL varies widely depending on temperature and water vapour content, which fluctuate
22
23 126 with latitude, location and time, determining the cloud patterns (FIG. 1b). Global
24
25 127 differences reflect atmospheric circulation, surface climate and cloud cover, and range
26
27 128 from saturation at equatorial latitudes to near-zero above some polar locations.
28
29 129 Microbial exploitation of low moisture in xeric environments (Pointing and Belnap
30
31 130 2012; Lebre, De Maayer and Cowan 2017) suggests that sub-saturated relative humidity
32
33 131 levels throughout the troposphere are not inhibitory to adapted microbial taxa although
34
35 132 the lower limit of relative humidity that supports active metabolism has not been
36
37 133 determined. In contrast, only regions of high relative humidity and clouds are likely to
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39 134 support the majority of microorganisms not specifically adapted to low water activity.
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41 135 Cloud droplets provide aqueous microenvironments (Joly *et al.* 2015), also the thin
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43 136 layer of liquid water that occurs at the surface of deliquescent aerosol particles may be
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45 137 sufficient to sustain microbial activity (Stevenson *et al.* 2015a), but future research will
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47 138 be key to understand the relationship between hygroscopicity and activity of airborne
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49 139 cells.
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3 140 Among other major challenges to microbial survival and proliferation in the
4
5 141 troposphere are the level of photo-oxidative and thermal stress (FIG. 1b). A strong
6
7 142 altitude-dependent gradient occurs in the troposphere due to the attenuation by ozone,
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9 143 oxygen, water vapour and particulates, and overall UV-A and UV-B radiation increases
10
11 144 approximately 10-20% with every km in altitude (Barry and Chorley 2010). Whilst UV
12
13 145 radiation exposure *per se* is a strong environmental filter to microbial survival and
14
15 146 influences diurnal bacterial occurrence in air (Tong and Lighthart 2006), it is unlikely to
16
17 147 be a limiting factor to radiation-tolerant taxa. Air temperature decreases with altitude in
18
19 148 the troposphere at the rate of approximately 6.5°C per km from ambient near-surface
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21 149 temperatures to -50°C or lower at the tropopause (NOAA, NASA and USAirForce 1976)
22
23 150 in the absence of inversions. The freezing isotherm occurs at variable altitudes between
24
25 151 ground level and about 5km according to season, latitude, terrain, and atmospheric
26
27 152 conditions (FIG. 1b). This delineates the mechanism of precipitation formation and the
28
29 153 altitudinal limit for non-psychrophilic microbial metabolism. Microbial metabolism has
30
31 154 been demonstrated at temperatures below those encountered even in the uppermost
32
33 155 region of the troposphere (Amato and Christner 2009), and so whilst the freezing
34
35 156 isotherm undoubtedly represents a physiological boundary, temperature *per se* is
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37 157 unlikely to inhibit potential microbial metabolism or survival in the troposphere.
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45 158 A peculiarity of the atmosphere contrasting with other environments such as
46
47 159 soils and oceans is its highly dynamic nature, with high cell turnover rates (Burrows *et*
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49 160 *al.* 2009). Temperature, humidity, and liquid water content can change within minutes,
50
51 161 exposing cells to repeated thermal and osmotic shock, and freeze-thaw cycles during
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53 162 residence in air. Additionally, as air density decreases with altitude so does the spatial
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55 163 proximity of cells and their potential substrates. The limited availability of surfaces in
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57 164 the atmosphere is also a challenge to microbial community development as it limits
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conventional biofilm formation and the possibility for microbial interactions, e.g. via quorum sensing (Monier and Lindow 2003). Overall, the atmosphere has been described as possessing qualities of a chaotic system in terms of its physicochemical complexity (Hochman *et al.* 2019), and this underlines its heterogeneity as a microbial environment.

Diversity and biogeography of atmospheric microorganisms

The physical volume of the troposphere dwarfs that of the equivalent microbially inhabited layers of the ocean (i.e. the photic zone) or land (i.e. topsoil) although microbial abundance is several orders of magnitude lower (FIG. 2). On a global scale, modelled atmospheric cell concentrations fall within the range of 10⁰-10⁵ per m³ for bacteria and fungi (Burrows *et al.* 2009; Hoose, Kristjánsson and Burrows 2010; Spracklen and Heald 2014), and observed geographic patterns in relative abundance are broadly congruent with modelled estimates of biogeography for cell numbers (Mayol *et al.* 2017; Tignat-Perrier *et al.* 2019; Archer *et al.* 2021) (FIG. 2a,b). Estimates from fresh snowfall and from cloud water suggest 4 × 10² – 4 × 10³ bacterial cells per m³ and 4 × 10⁰ – 4 × 10² eukaryotic cells per m³ assuming condensed water content of 0.4 g per m³ (Christner *et al.* 2008b; Tesson, Skjøth and Šantl-temkiv 2016; Amato *et al.* 2017; Hu *et al.* 2018; Šantl-Temkiv *et al.* 2019). Cells associated with aerosolized desert dust may reach 10⁷ per m³ (Maki *et al.* 2016). Aggregations of cells may create localised elevated concentrations of free biomass or on surfaces of airborne particulates.

Localised concentrations of microorganisms within clouds and particulate dust plumes are likely to be significant: The global total mass of clouds has been estimated at 194,000 Tg (Pruppacher and Jaenicke 1995), and the fraction of the atmosphere under cloud cover has been estimated at 67% globally (King *et al.* 2013). Up to two billion

tonnes of desert dust is transported in the atmosphere annually (Shepherd *et al.* 2016), and a comparable volume of soil particulates are also emitted to the atmosphere by arable lands every year (Borrelli *et al.* 2017). Attempts to extrapolate these numbers to yield global atmospheric cell abundance have a high level of uncertainty and have not been able to adequately encompass variability between boundary layer, troposphere, stratosphere, clouds and dust. Nonetheless, a global scale meta-analysis of bacterial abundance across biomes indicated approximately 5×10^{19} cells may occur globally in the atmospheric boundary layer (Whitman, Coleman and Wiebe 1998). This estimate was several orders of magnitude lower than those for ocean, soil and subsurface biomes, and reflects the extremely low density of cells in the atmosphere.

Direct cell counting, e.g. (Mayol *et al.* 2017), estimates from environmental metagenomics, e.g. (Bryan *et al.* 2019; Gusareva *et al.* 2019; Tignat-Perrier *et al.* 2019; Archer *et al.* 2021), and historical cultivation approaches (Després *et al.* 2012), collectively indicate that the most abundant microorganisms within the atmospheric boundary layer globally are bacteria and fungi. Other microbial components such as archaea and protists appear to comprise a relatively minor component in air (Gusareva *et al.* 2019; Archer *et al.* 2021). Few studies have focused on viruses although there is evidence that a significant volume of free and attached viral particles occurs in air within and above the atmospheric boundary layer (Whon *et al.* 2012; Reche *et al.* 2018). Cell-free microbial metabolites and cell fragments constitute a significant portion of suspended particulate organic matter in air, above both terrestrial and ocean surfaces where they are important for cloud ice formation (Wilson *et al.* 2015; Tobo *et al.* 2019).

Microbiological diversity estimations for the atmosphere have been strongly influenced by methodological biases (Šantl-Temkiv *et al.* 2020). Direct comparisons of cultivation versus molecular ecological approaches have indicated very few

215 atmospheric microorganisms are cultivable, e.g. (Temkiv *et al.* 2012), and this is
216 supported by order of magnitude higher diversity estimates from environmental
217 metagenomics surveys, e.g. (Cáliz *et al.* 2018; Tignat-Perrier *et al.* 2019). The recent
218 emergence of portable high-volume air sampling devices has greatly helped in recovery
219 of sufficient biomass for environmental metagenomics studies with sample sizes of
220 several cubic metres of air now possible during short timeframes (Šantl-Temkiv *et al.*
221 2020). A persistent issue for diversity estimation has been in distinguishing genuine
222 microbial signatures from the unavoidable contaminants that occur from equipment
223 and reagents when interrogating ultra-low biomass samples. Assessment of
224 contamination during sequence-based diversity estimation indicates that at sample
225 DNA template concentrations encountered in air contaminants may form a significant
226 component of sequences (Salter *et al.* 2014). For this reason a rigorous approach to
227 mitigation is required during experimental and bioinformatic workflows (Eisenhofer *et*
228 *al.* 2019). Therefore, it is also necessary to strongly nuance findings where
229 decontamination effort and outcome were not fully reported. Nonetheless, a growing
230 body of recent research indicates a growing consensus in the understanding of
231 microorganisms in bulk air (i.e. including free cells, aggregates and particulate-
232 associated cells). Taxa adapted to survival under high UV irradiance, moisture limitation
233 and other challenges are over-represented in atmospheric communities which is
234 indicative of strong environmental filtering. This is supported by evidence that
235 atmospheric microbial communities exhibit distinct phylogenetic structuring within the
236 boundary layer and at higher altitudes (Tignat-Perrier *et al.* 2019; Archer *et al.* 2021).
237 Across broad geographic, altitudinal and temporal scales in the troposphere taxonomic
238 diversity broadly reflects that of underlying surface source habitats, and commonly
239 encountered bacterial taxa belong to the Actinobacteria, Alphaproteobacteria,

Firmicutes and Gammaproteobacteria (FIG. 2c); and commonly encountered fungi in air largely comprise spores of Agaricomycetes and Dothidiomycetes (Cáliz *et al.* 2018; Els *et al.* 2019b; Gusareva *et al.* 2019; Tignat-Perrier *et al.* 2019; Archer *et al.* 2021) (FIG. 2c).

Global air circulation patterns have been proposed to define boundaries of biogeographic regions for atmospheric microorganisms (FIG. 1a) (Womack, Bohannan and Green 2010). This is supported by recent evidence for regionalisation (Uetake *et al.* 2020) and biogeographic patterns in atmospheric microbial diversity on a global scale (Archer *et al.* 2021). A high microbial species richness within a given air mass in the atmospheric boundary layer reflects recruitment from a variety of terrestrial and marine sources, and source tracking has indicated combined influence of local and distantly recruited taxa to observed diversity (Archer *et al.* 2021). Diversity estimates for near-ground air within the atmospheric boundary layer at various locales have indicated bacterial and fungal communities that were correlated with local abiotic variables such as temperature, precipitation and humidity or land use/surface cover, reflecting the role of local cell emissions (Bowers *et al.* 2011; Fröhlich-Nowoisky *et al.* 2012; Tignat-Perrier *et al.* 2019; Archer *et al.* 2021; Spring *et al.* 2021). Thus, diversity of fungi is relatively higher above regions with a well-developed phyllosphere (Lymperopoulou, Adams and Lindow 2016; Archer *et al.* 2021; Zhou *et al.* 2021), and lowest above oceans (Mayol *et al.* 2017; Archer *et al.* 2020; Uetake *et al.* 2020) and low productivity terrestrial systems such as drylands and polar/alpine regions where underlying surface communities are less diverse and microbially dominated by bacteria (Archer *et al.* 2019, 2021). Several studies have also related diversity to factors associated with dispersal such as history of air masses and UV radiation, suggesting combined influence of predominant remote sources and conditions during transit

(Bowers *et al.* 2012; Cáliz *et al.* 2018; Archer *et al.* 2019, 2020; Els *et al.* 2019b; Uetake *et al.* 2019; Tignat-Perrier *et al.* 2020).

Temporal patterns in local atmospheric microbiota reflect seasonal changes in underlying surface cover and source of incoming air mass. A pronounced diel variation of specific bacterial and fungal groups was found in the equatorial tropics using metagenomics (Gusareva *et al.* 2019). Inter-seasonal variation in the atmospheric boundary layer was found to be absent (Gusareva *et al.* 2019), weak (Tignat-Perrier *et al.* 2020), pronounced for some taxa (Bowers *et al.* 2012) or stochastic (Els *et al.* 2019b), depending on the location. Still, a large number of studies have identified differences in bacterial and/or fungal communities in summer versus winter at temperate and sub-tropical terrestrial locations (Bowers *et al.* 2012, 2013; Woo *et al.* 2013; Barberán *et al.* 2014; Cáliz *et al.* 2018; Els *et al.* 2019b; Uetake *et al.* 2019). One of the scarce continuous long-term studies demonstrated a clear and well-delineated seasonal pattern in bacterial and fungal communities (Woo *et al.* 2013). At polar latitudes elevated cell abundance in air corresponded to summer and the growing season (Šantl-Temkiv *et al.* 2019). These studies strengthen the view that the underlying phyllosphere is a major determinant of atmospheric microbial diversity. As the elevation above ground increases, the source area of influence widens (Schmid 2002; Hsieh and Katul 2009), and the small-scale spatial and temporal heterogeneities observed near the ground blur (Archer *et al.* 2019, 2021; Els *et al.* 2019a). Above the atmospheric boundary layer long-range air movements progressively decouple atmospheric diversity from its emission sources (Burrows *et al.* 2009) (Smith *et al.* 2018).

Whilst most research has focused on the atmospheric boundary layer and higher altitudes within the troposphere, understanding the biogeographic limit of microbial

occurrence and survival at higher altitudes is also of interest. It has been postulated that limited microbial survival and transport may occur in the stratosphere (DasSarma and DasSarma 2018), but the role of the stratosphere in microbial dispersal may be limited due to the combined effects of extreme environmental stress and extended residence times (Bryan *et al.* 2019). Several studies have employed balloon and aircraft platforms to recover stratospheric air samples and they have described a limited diversity and viability of bacterial and fungal taxa (Wainwright *et al.* 2003; Smith, Griffin and Schuerger 2010; Smith *et al.* 2011, 2018; Bryan *et al.* 2019), with taxa not markedly differentiated from those occurring in the troposphere below (Smith *et al.* 2018). Estimates of abundance vary greatly but an upper estimate concluded that bacterial abundance in the troposphere and lower stratosphere may be comparable (Bryan *et al.* 2019). However, extreme caution is required during interpretation of results from stratospheric air samples due to the major uncertainty surrounding effective exclusion of the unavoidable contaminants that occur when interrogating the ultra-low biomass system. There is currently insufficient evidence to draw robust conclusions about the occurrence, transport, or viability of microorganisms in the stratosphere. The discipline of astrobiology has considered the potential role of the stratosphere and upper atmosphere for interplanetary dispersal of microorganisms (panspermia) as free cells or via mineral vectors (Yang, Yokobori and Yamagishi 2009) but this will require major investigative effort to be further considered.

What are the determinants of microbial flux between the atmosphere and surface habitats?

The atmosphere has long been identified as a major conduit for microbial transport between surface habitats, e.g. (Womack, Bohannon and Green 2010), and this has a

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2
3 315 profound impact on the function and resilience of terrestrial and marine surface
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5 316 communities due to its contribution to gene flow, community assembly, and dispersal of
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8 317 pathogens. Recent developments in ecological theory have begun to challenge the view
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10 318 that microbial transport is a neutral process (Lowe and McPeck 2014), and recent
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12 319 evidence from large scale biogeographic (Mayol *et al.* 2017; Archer *et al.* 2021) and
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14 320 temporal (Cáliz *et al.* 2018; Tignat-Perrier *et al.* 2020) studies of atmospheric
15
16 321 microbiota increasingly support this view. Deterministic influence due to
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19 322 environmental filtering and microbial stress tolerance both play a significant role in
20
21 323 shaping atmospheric microbiota (Archer *et al.* 2021), and hence the composition of
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23 324 assemblages that are exchanged between atmosphere and surface habitats.

26 325 The major determinants of microbial fluxes in the atmosphere between source
27
28 326 and sink habitats are emission, survival during transport, and deposition (FIG. 3).
29
30 327 Emission from the surface, known as aerosolization, is dependent upon the substrate
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32 328 and a major factor is the nature of the interface with air, such as the composition of
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34 329 plant cover (Zhou *et al.* 2021), cohesiveness of soil (Joung, Ge and Buie 2017; Archer *et*
35
36 330 *al.* 2021), or chemistry of the water-surface microlayer (Michaud *et al.* 2018) (FIG. 3a).
37
38 331 Biotic microbial traits that affect aerosolization are also important, including cell
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40 332 hydrophobicity and membrane composition (Michaud *et al.* 2018), extracellular
41
42 333 polymeric substance (EPS) production (Morris and Monier 2003), spore production and
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44 334 release (Lagomarsino Oneto *et al.* 2020), and allometry (Norros *et al.* 2014). Bacterial
45
46 335 cells occur as free cells or are attached to particulate matter whereas the fungal spores
47
48 336 likely occur mostly as freely suspended particles. Modelling emissions for particulates
49
50 337 1-3µm diameter as a proxy for single and particle-associated bacterial cells revealed
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52 338 that the total annual emissions were in the range of 7.6×10^{23} - 3.5×10^{24} cells largely
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54 339 originating from terrestrial environments, i.e. crops, grasslands and shrubs (Burrows *et*

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2
3 340 *al.* 2009, 2013). This equated to an estimated annual emission of 470–1100 Gg bacterial
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5 341 biomass (Burrows *et al.* 2013). Fungal emissions occur predominantly as free spores
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7 342 and were estimated at 9.5×10^{23} total spores from terrestrial surfaces per year which
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9 343 amounts to ~50 Tg of fungal biomass annually (Elbert *et al.* 2007).
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12 344 The major marine aerosolization process is initiated by breaking waves which
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14 345 produces small air bubbles that scavenge microorganisms and organic compounds from
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16 346 the sea column and transport them to the sea surface (Aller *et al.* 2005) (Carlucci and
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18 347 Williams 1965), where they burst to eject small droplets containing salts, biogenic
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20 348 material and microorganisms (Blanchard and Syzdek 1970), into the atmosphere (Afeti
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22 349 and Resch 1990) (Blanchard 1989). Even though oceans are thought to represent weak
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24 350 sources of airborne microorganisms on a global per surface area basis and bioaerosols
25
26 351 largely comprise bacteria and viruses (Wilson *et al.* 2015), selective transfer and
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28 352 enrichment of organic compounds and microbial cells has been shown experimentally
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30 353 (Rastelli *et al.* 2017). In addition, large spatial and temporal variability in marine
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32 354 emissions and effects on atmospheric processes may occur due to biological processes
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34 355 and meteorological factors (Wang *et al.* 2015; Wilson *et al.* 2015; DeMott *et al.* 2016).
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40 356 Survival during transit in air is influenced by biotic traits related to stress
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42 357 tolerance, and particularly with regard to mitigating the effects of low water activity
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44 358 (Stevenson *et al.* 2015b), desiccation (Potts 1994), photo-oxidative UV (Daly 2009;
45
46 359 Sharma *et al.* 2017) and general oxidative stress (Ziegelhoffer and Donohue 2009;
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48 360 Ezraty *et al.* 2017), and low temperatures/freezing (Amato 2013) (FIG. 4). Dormancy
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50 361 and sporulation are strategies that provide protection against major stressors and
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52 362 functional metagenomics of air has indicated genes associated with these traits are
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54 363 over-represented in atmospheric communities compared to underlying surface habitats
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56 364 (Aalismail *et al.* 2019; Archer *et al.* 2021). Many spores as well as dormant and active
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cells possess pigment compounds such as melanins, mycosprines and scytonemins that protect against oxidative and desiccation stress largely due to scavenging of reactive species (Gao and Garcia-Pichel 2011). A number of biodiversity surveys in air have revealed taxa known to produce protective pigments are over-represented in air compared to underlying terrestrial and marine habitats (Fröhlich-Nowoisky *et al.* 2012; Tignat-Perrier *et al.* 2019; Archer *et al.* 2021), and this implies a survival advantage. Additional strategies to counter stress may include the secretion of extracellular polymeric substances to mitigating both low water activity via hygroscopic carbohydrate polymers as well as extracellular protection from oxidative stress (Matulová *et al.* 2014). Aerosolization of cells as aggregations, biofilms or attached to particulates may also confer survival advantages during dispersal (Monier and Lindow 2003; Morris and Monier 2003) (FIG. 3), but may also reduce residence time in air due to increased size and density (Burrows *et al.* 2009). Metagenomic profiling of atmospheric communities has begun to reveal prevalence of genes encoding cold shock, oxidative stress, and UV repair enzymes (Aalismail *et al.* 2019; Archer *et al.* 2021), and metatranscriptomics of cloud water has revealed active metabolic responses to counter oxidative, osmotic and thermal stresses (Amato *et al.* 2019). Physiological responses to counter simulated atmospheric stress have also been observed for taxa isolated from the atmosphere. Strains of *Pseudomonas syringae* isolated from rain and cloud water withstood aerosolization, freezing, osmotic shock, short-term exposure to UVA and UVB radiation (Alsved *et al.* 2018; de Araujo *et al.* 2019; Ling *et al.* 2021), and oxidative stress imposed by hydrogen peroxide (H₂O₂) (Wirgot *et al.* 2019). Metabolomic studies on *P.syringae* also revealed a suite of responses to cold shock under simulated atmospheric conditions (Jousse *et al.* 2017), including production of cryoprotectants, antioxidants, alkaloids and metabolites involved in energy metabolism.

The deposition of microorganisms occurs through a combination of wet and dry deposition (Després *et al.* 2012). The wet deposition is affected by a combination of in-cloud partitioning during cloud droplet / ice particle formation and below-cloud aerosol scavenging processes, which can both contribute to segregation of taxa between the wet and dry phases (Bauer *et al.* 2003; Moore *et al.* 2020; Woo and Yamamoto 2020). In particular, cloud condensation ability was modelled to shorten the average bacterial residence time in air (and hence dispersal distance) from ~8d to 3d (Burrows *et al.* 2009) and ice nucleating microbial cells were shown to be efficiently precipitated (Amato *et al.* 2015; Stopelli *et al.* 2015). Wet deposition rates of $10^7 - 5 \times 10^9$ bacteria cells $\text{m}^{-2} \text{d}^{-1}$ have been reported (Reche *et al.* 2018; Woo and Yamamoto 2020). In contrast, dry deposition velocity is largely determined by allometric considerations, such as PBAP size, shape, and density (FIG. 3d). Dry deposition rates of $\sim 10^7 - 5 \times 10^8$ bacteria cells $\text{m}^{-2} \text{d}^{-1}$ have been reported but with no significant difference in estimated overall wet and dry deposition (Reche *et al.* 2018; Woo and Yamamoto 2020). Patterns in deposition of fungal spores and pollen are thought to follow the same pattern where overall wet and dry deposition contributions are similar (Aylor 2017; Woo and Yamamoto 2020), although taxon-specific variation is likely.

Aside from the emission and deposition processes, cell fluxes are also affected by the altitude that the cells reach, which is a deterministic factor related to stress exposure (FIG. 3b). A very large fraction (97%) of depositing bacterial cells have been found to be particle associated (Reche *et al.* 2018), and with a large mean diameter ($\sim 9 \mu\text{m}$) (Woo and Yamamoto 2020), thus supporting the idea that the mixing state of cells in air, i.e. free cells, aggregates or cells vectored on particulate matter, has a significant effect on cell residence time (FIG. 3c). It should also be noted that processes affecting atmospheric cell fluxes are highly variable and subject to stochastic influence from

415 events such as dust storms (González-Toril *et al.* 2020) and wildfires (Moore *et al.*
416 2021).

417 A pertinent question for atmospheric communities is the extent to which the
418 complex variation in emission and deposition flux, together with environmental filtering
419 during transit, influence the assembly of terrestrial and marine surface communities.
420 The flux of diversity and biomass from air to soil or oceans is likely smaller by
421 magnitudes than that of standing communities but given the ability of microorganisms
422 to rapidly exploit favourable ecological niches, atmospheric flux should be viewed as a
423 critical control point in aspects of connectivity and ecological resilience across biomes
424 (Dumbrell *et al.* 2010; Lowe and McPeck 2014; Zhou and Ning 2017). This occurs by
425 facilitating microbial dispersal between spatially separated natural systems (Kellogg
426 and Griffin 2006), contributing to maintenance of diversity via recruitment (Jones and
427 Lennon 2010), and in promoting dispersal of dormant propagules that may act as
428 microbial seed banks (Lennon and Jones 2011).

430 **What is the evidence for long distance atmospheric pathogen dispersal?**

431 Some evidence exists for regional dispersal of pathogens without any obvious
432 adaptation to the environmental stress of atmospheric transport, e.g. foot and mouth
433 diseases virus (Sorensen *et al.* 2000; Björnham, Sigg and Burman 2020). However, long-
434 range atmospheric dispersal of pathogens (i.e. beyond short-range inter-organismal
435 transmission in air) typically requires protection from environmental stress, whether
436 facilitated by pre-adaptation as spores, or due to association with particulate or
437 biological vectors. Correlations have been made between atmospheric dust vectoring
438 and regional trans-continental scale pathogen dispersal (Tobias *et al.* 2019), and
439 consistent inter-annual dynamics for specific taxa may exist, which make them

foreseeable over time with dominance of plant pathogens over those of animal and humans (Triado-Margarit, Caliz and Casamayor 2021).

Fungal spores are particularly well suited to long-range dispersal and are problematic as a threat to agriculture and global food security (Brown and Hovmøller 2002; Savary *et al.* 2019). Many plant pathogens have historically displayed distinct regionalisation but globalisation of agriculture has exacerbated range shifts (Bebber, Holmes and Gurr 2014). Airborne fungal pathogens have been detected above land with agricultural land use (Nicolaisen *et al.* 2017; Archer *et al.* 2021), and some fungal pathogens are able to disperse across inter-continental distances and re-establish infections even when host plants are seasonally absent (Brown and Hovmøller 2002). A striking example is the re-emergence of wheat stem rust as a threat to global food supply. The disease caused by the basidiomycete fungus *Puccinia graminis* f.sp. *tritici* is dispersed readily in air by basidiospores. It was virtually eradicated in the twentieth century, however, a highly pathogenic new strain, Ug99 and its variants, emerged in the last decade and have threatened to devastate global wheat cultivation (Singh *et al.* 2011). Combination of disease surveillance and dispersal modelling has resulted in strong evidence for atmospheric transmission as a major determinant of wheat rust epidemiological zones (Meyer *et al.* 2017).

Evidence for long-range atmospheric dispersal of human pathogens also exists. Seasonal outbreaks of meningococcal meningitis in sub-Saharan Africa have been correlated with bacterial vectoring on dust (Jusot *et al.* 2017), and this also appears to occur for fungal diseases such as Valley Fever caused by *Coccidioides* spores transmitted on desert dust across the south western United States and northern Mexico (Kollath, Miller and Barker 2019). Bacterial plant pathogens may have significantly higher relative abundance than fungi during intercontinental dust intrusions from Africa to

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Europe, although predominant western air mass may also bring *Coccidioides* spp., a potentially severe and understudied fungal pathogen endemic from arid and semiarid regions of North and Central America, to Europe (Triado-Margarit, Caliz and Casamayor 2021). Vectoring on biological surfaces also provides a protected means of long-distance dispersal. Wind-borne transport of *Anopheles* mosquitoes carrying the *Plasmodium* parasite was recorded over several hundred kilometres in the Sahel region of Africa (Huestis *et al.* 2019), and pollen has been demonstrated as a vector for bacteria and bacterial allergens (Oteros *et al.* 2019).

What is the evidence for metabolically active atmospheric microorganisms?

The majority of cells in the atmosphere are unlikely to be metabolically active but are simply undergoing transport between source and sink destinations with a high turnover (Burrows *et al.* 2009). Given that most fungi in air occur as dormant spores it is likely that the active fraction of atmospheric microbiota is comprised almost exclusively of bacteria that possess adaptation to atmospheric environmental stress (Archer *et al.* 2021) (FIG. 4). Putative generation times for bacteria in cloud water have been estimated at 3.6-19.5 days (Sattler, Puxbaum and Psenner 2001), which fit within their modelled airborne residence times (Burrows *et al.* 2009). However, whether metabolic transformations *in situ* under the highly fluctuating atmospheric conditions are sufficient to sustain only quasi-dormancy, or if cell homeostasis and reproduction are energetically feasible within the short timeframe of favourable conditions offered during atmospheric transport remains unexplored.

Evidence from environmental samples has demonstrated that atmospheric microorganisms possess the potential to transform substrates encountered in the atmosphere (FIG. 4). Metagenomic surveys of cloud water (Amato *et al.* 2019), free air

(Gusareva *et al.* 2019; Archer *et al.* 2021), and airborne desert dust plumes (Aalismail *et al.* 2019) indicate diverse autotrophic and heterotrophic metabolic pathways in atmospheric assemblages that broadly reflect source environments. Photosynthesis may be limited in atmospheric microorganisms due to light-inhibition (Barber and Andersson 1992), but clouds and desert dust provide shading against damaging levels of direct UV radiation and diverse viable and stress-tolerant microalgae and cyanobacteria have been reported from clouds (Tesson, Skjøth and Šantl-temkiv 2016). A range of pathways indicating atmospheric trace gas metabolism including hydrogen, methane and carbon monoxide were widespread among atmospheric metagenomes (Archer *et al.* 2021) and methane oxidation has been demonstrated by atmospheric isolates of *Methylocystis* and *Methylosinus* (Šantl-Temkiv *et al.* 2013), although it is unclear if this is energetically feasible under atmospheric conditions. Most research has focused on the potential for heterotrophic metabolism of simple organic compounds in clouds as these are envisaged as “hotspots” for atmospheric microbial activity.

Microbial assemblages in cloud water have revealed general markers of metabolic activity under simulated atmospheric conditions including ATP, high ribosomal content, transcribed RNA, enzymatic and respiratory activities (Klein *et al.* 2016; Šantl-Temkiv *et al.* 2018; Amato *et al.* 2019). Under conditions of favourable water activity, temperature, substrate availability and reduced stress exposure, atmospheric microbial assemblages appear capable of active heterotrophic metabolism as evidenced by chemical speciation in recovered cloud water (Vaitilingom *et al.* 2013; Bianco *et al.* 2019). A comparative metagenomic and metatranscriptomic study of cloud water assemblages highlighted that active metabolism in clouds is closely coupled with the energetic demands of active stress response metabolism and particularly with regard to mitigating oxidative stress (Amato *et al.* 2019). A complex metabolism was

515 envisaged that also included synthesis of cryoprotectant and extracellular polymeric
516 substances implicated in a protective role. The study identified C1 and C2 compounds as
517 important substrates and ammonium as the major nitrogen source (Amato *et al.* 2019).
518 Another combined metatranscriptomic and metabolic screening of cloud water
519 provided strong evidence for phenol metabolism by bacteria, and this was envisaged as
520 an important atmospheric substrate derived from anthropogenic emissions (Lallement
521 *et al.* 2018).

522 Several studies have demonstrated the ability of atmospheric isolates to
523 metabolise compounds encountered in clouds: A screening of sixty atmospheric isolates
524 including the bacterial genera *Bacillus*, *Pseudomonas*, *Micrococcus* and *Sphingomonas*
525 plus fungal yeasts from cloud water revealed that under laboratory conditions all were
526 capable of degrading diverse carboxylic acids and volatile organic compounds (Amato
527 *et al.* 2007). Atmospheric bacterial isolates of *Bacillus* sp, *Frigoribacterium* sp,
528 *Pseudomonas* sp. and the methanotrophic genera *Methylocystis* sp. and *Methylosinus* sp.
529 have been demonstrated to oxidize common atmospheric volatile organic compounds
530 including formaldehyde and methanol (Husárová *et al.* 2011; Šantl-Temkiv *et al.* 2013).
531 *Rhodococcus enclensis* isolated from cloud water was shown to metabolise catechol and
532 phenol under simulated atmospheric conditions at rates comparable to that of chemical
533 transformations in the atmosphere (Jaber *et al.* 2020). Larger molecules may also serve
534 as substrates, for example a *Bacillus* strain from cloud water was demonstrated to
535 metabolise a range of pentose and hexose sugars a range of mono- and disaccharides
536 (Matulová *et al.* 2014).

537 Based on bulk cloud water measurements, which were unable to account for the
538 multiphase structure and high dynamic nature of clouds, it has been proposed that
539 heterotrophic microbial activity could be the main driver for the oxidation of common

atmospheric organic compounds in air during night and could compete with photochemistry during the day (Vaithilingom *et al.* 2010). Modeling has indicated that in clouds where only a small fraction ($\sim 10^{-4}$) of spatially separated microdroplets contains microbial cells, the quantitative contribution of microbial activity to the transformation of atmospheric compounds is likely to be low (Fankhauser *et al.* 2019), although this needs to be verified with experimental approaches simulating *in situ* atmospheric conditions. Modelling of biodegradation potential for organic compounds by bacteria in clouds has indicated that acetate and formate are the most utilised substrates and that overall bacterial biodegradation may represent an underestimated sink for atmospheric organics (Khaled *et al.* 2020).

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551 **What is the microbial role in atmospheric biophysical processes?**

552 Microbial involvement in atmospheric biophysical transformations occurs independently from the metabolic activity that is thought to be rare during short residence times in the extreme environment. Biological ice nucleation contributes to cloud formation, precipitation, and the hydrological cycle, as well as impacting microbial flux between atmospheric and surface habitats, and recent advances have shed new insight on the mechanistic basis for this. Diverse microorganisms and biomolecules, including bacteria (particularly Gammaproteobacteria) (Joly *et al.* 2013), fungi (Fröhlich-Nowoisky *et al.* 2014), microalgae (Tesson and Šantl-Temkiv 2018) and microbially-derived cell-free ice nucleation proteins (INpro) (O'Sullivan *et al.* 2015) can nucleate ice in super-cooled cloud droplets at temperatures much warmer than other atmospheric particles (between -13 and -1°C) and have been implicated in cloud processes. This property has been linked with specific surface- or excreted proteins and is essentially a biophysical process that occurs independently of cell viability or

association (FIG. 5). Whilst terrestrial sources are dominant on a global level, marine sources associated with INpro excreted by marine microalgae (Wilson *et al.* 2015) may be important in remote regions unaffected by continents (e.g. Southern Ocean) (DeMott *et al.* 2016).

Among identified biological ice nucleation active molecules, the best understood are bacterial INpro, which are large membrane-associated proteins (>120 kDa) that structure interfacial water molecules at low temperature promoting ice formation (Roeters *et al.* 2021) (FIG. 5). Bacterial INpro are long repetitive proteins that present on the surface of the outer membrane and nucleate ice through a yet to be fully resolved mechanism (Pandey *et al.* 2016; Hudait *et al.* 2018; Lukas *et al.* 2020). Monomers or small oligomers nucleate ice at temperatures from -7 to -10 °C and are known as Type C INpro; and higher order oligomers of INpro form at well-defined sizes that nucleate ice between -2 to -5 °C and are described to as Type A INpro (Wex *et al.* 2015; Ling *et al.* 2018). Laboratory studies using cloud simulation chambers have shown that the activity of bacterial INpro is independent of cell viability (Hartmann *et al.* 2013; Amato *et al.* 2015). Fungal spores and hyphae also carry INpro that exhibit different molecular structure and properties to those in bacteria and they are yet to be fully characterised. Fungal spores are the dominant state for fungi in the atmosphere and support surface-associated INpro and ice nucleating activity (Pouleur *et al.* 1992; Morris *et al.* 2013) (FIG. 5). Fungal hyphae occur very rarely in the atmosphere and so the major contribution arises from dissociation of fungal INpro from cell walls and adhesion to soil particulates that subsequently become aerosolized (O'Sullivan *et al.* 2015) (FIG. 5). The ubiquitous soil fungal genera *Fusarium* and *Mortierella* have been demonstrated to excrete INpro into the environment and soil particles with associated INpro were capable of ice nucleation (Fröhlich-Nowoisky *et al.* 2014; O'Sullivan *et al.* 2015).

Once they induce freezing, Microbial INpro can also contribute to the formation of secondary ice particles in clouds which enhances the impact of low abundance biological INPs through ice-multiplication, particularly at high sub-zero temperatures (Korolev and Leisner 2020) (Fig. 5). Rain that is produced via the ice phase through the Hallett–Mossop process dominates over the midlatitude oceans and continents (Mülmenstädt *et al.* 2015). This process occurs in mixed phase clouds, in which some of the supercooled cloud droplets freeze due to the presence of ice nucleating particles, promoting the deposition of water vapor on thus-formed ice particles and allowing them to grow to precipitation sizes (Fig. 5). Precipitation induced by ice-nucleation active bacteria, whose natural habitats are plant leaf surfaces, contributes to the bioprecipitation cycle by enhancing the growth of vegetation and epiphytic microorganisms, which in turn leads to enhanced emissions of ice-nucleation active bacteria resulting in a positive feedback (Fig. 3d) (Morris *et al.* 2014). Bioprecipitation may be important above regions with a well-developed phyllosphere where emissions of bacterial INpro are highest, and accompanied by relatively low levels of abiotic INPs (Huffman *et al.* 2013; Healy *et al.* 2014). Other precipitation-related feedbacks that increase bioaerosol generation and thus enhance the atmospheric INpro include immediate and delayed interactions of rain with soil and plants (Huffman *et al.* 2013; Joung, Ge and Buie 2017). These positive feedbacks occur alongside non-biological ice nucleation and precipitation and the significance of these processes for meteorology and climate is yet to be quantified.

How might anthropogenic activities affect atmospheric microbiota?

The atmosphere is a primary sink for anthropogenic emissions of greenhouse gases, chemical pollutants and particulates from fossil fuel combustion (Archer and

Pointing 2020). Additional particulate emissions occur as a result of biomass burning due to land clearance and wildfires, soil destabilization due to land use change and expansion of intensive agriculture, and climate-change induced desertification (Archer and Pointing 2020). Most of these emissions are concentrated in the troposphere where long-range microbial transport, metabolism and biophysical transformations occur. Atmospheric forcing due to emissions has been acute in the industrial age and this has the potential to create positive and negative impacts on the dispersal of microorganisms in air (Archer and Pointing 2020) (FIG. 6). A recent modelling study suggests that overall microbial diversity in the atmosphere may decline under future climate scenarios and this may severely impact ecosystem connectivity and function (Ontiveros *et al.* 2021).

Gaseous emissions from fossil fuel combustion including SO_x and NO_x have been shown to inhibit cell function and survival of *Pseudomonas* strains isolated from clouds (Kondakova *et al.* 2016). Greenhouse gaseous, e.g. methane (Šantl-Temkiv *et al.* 2013), can be metabolised by atmospheric bacteria. However, as *in situ* microbial activity and its influence on atmospheric chemistry is still not well constrained, it also remains unclear if elevated substrate levels in the atmosphere have the potential to significantly affect microbial activity and its outcomes (Archer and Pointing 2020). The ice nucleation activity of PBAP may also become more important to precipitation patterns in a warming world because they are more efficient at warmer temperatures from -10°C to 0°C, compared to mineral particulates (Christner *et al.* 2008a). The potential impacts from direct and indirect anthropogenic particulate emissions are more apparent and widespread. Anthropogenic particulates including brown carbon from biomass combustion and black carbon from fossil fuel combustion account for approximately 20% of global emissions (Chen *et al.* 2018), and climate change increases mineral dust

aerosolization due to desertification (Pointing and Belnap 2014). Elevated concentrations of anthropogenic and desert dust particulates have been shown to influence atmospheric microbial diversity (Maki *et al.* 2019), and this may have implications for dispersal outcomes at deposition sites. The transport of microorganisms in plumes of airborne particulates is envisaged to provide additional protection from UV damage during atmospheric transport (González-Toril *et al.* 2020). Particulates arising from combustion are more hygroscopic than natural mineral particles and whilst this may be viewed as facilitating greater survival and even potentially enhanced microbial activity, it must be balanced against the often toxic levels of PAH associated with these particulates (Hayakawa *et al.* 2018). Anthropogenic forcing through particulate emissions may also affect ice nucleation patterns in clouds and thus impact microbial flux and deposition in the atmosphere more generally through changes in precipitation. Finally, airborne microorganisms themselves may become increasingly prevalent due to climate-change forcing or pollution, e.g. marine phytoplankton blooms (Lewis, van Dijken and Arrigo 2020). This includes strong emission from point sources for pathogenic microorganisms, antibiotic resistance genes and allergens, e.g. wastewater treatment plants (Han *et al.* 2020) and urban centres (Brodie *et al.* 2007; Woo *et al.* 2013), and these have direct public health relevance although dispersal appears to be localised. Assessing the extent of anthropogenic impacts remains somewhat speculative as more dedicated studies are needed both to provide a better constrained understanding of current processes and to investigate impacts of highly complex anthropogenic changes to the atmospheric gaseous and particulate composition.

Concluding remarks

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The discipline of aeromicrobiology has benefitted enormously from recent advances in methodology that overcome the limitations associated with the study of ultra-low biomass habitats, and we view this is beneficial in a wider sense to improved ecological understanding of linkages between the atmosphere and surface terrestrial and marine biomes. Specifically, the ability to accurately estimate microbial diversity in air through rigorous decontamination of environmental metagenomic data has revealed that the atmospheric boundary layer supports a diverse microbiota. Atmospheric microbial assemblages display taxonomic structuring that reflects strong environmental filtering, as well as distinct spatio-temporal biogeographic signals that reflect a complex interplay of local and regional recruitment to the atmosphere. The long-held ecological view of atmospheric transport as neutral to dispersal outcomes is therefore no longer supportable, and a revision of ecological assumptions about the influence of atmospheric microbial transport is warranted. Knowledge gaps that impede greater understanding in this regard include observational data on flux to and from surface environments and the atmosphere, and a focus on accurate quantitative methods should be a priority for future research.

The biophysical role of microorganisms in ice nucleation and precipitation formation is supported by convincing evidence and the molecular mechanisms that underpin biogenic ice nucleation are becoming better resolved. Given the temperature range at which biogenic ice nucleation occurs is relevant to a warming climate we urge greater focus and inclusion of microbial ice nucleation in research to predict future impacts on the water cycle. In the absence of evidence for widespread metabolic activity that influences biogeochemical outcomes, lack of microbial interactions and food webs or evidence for cell proliferation, we conclude that microbial biochemical transformations make a very limited contribution to overall atmospheric chemistry

although they may be important in localised habitable areas such as clouds or plumes of hydrated particles. The notion of a metabolically active atmospheric microbiome must therefore be contemplated with a high degree of nuance. Development of improved laboratory simulations for atmospheric conditions and *in situ* measurements will assist with further understanding of atmospheric microbial metabolism and its boundaries in air.

In a broader sense we advocate that estimates of the pan-global microbiota and its ecological relevance require greater consideration of biomass in the gas-phase atmospheric microbial environment alongside solid-phase terrestrial and liquid-phase marine habitats. The increasing threats to environmental, organismal and human health due to changes in atmospheric transport of microorganisms will be more clearly understood when empirical considerations of the atmospheric microbiota are incorporated into analytical frameworks. We therefore urge expansion of atmospheric microbiology monitoring and inclusion as part of global scale atmospheric data networks.

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Microbial ecology of the atmosphere

Figure legends

FIG. 1 | Defining the atmosphere as an ecological niche.

a) The atmosphere comprises a series of vertically delineated concentric layers that are primarily defined by thermal properties. The lowermost layer, the troposphere contains 75% of the molecular and gaseous mass of the atmosphere and almost all the water vapour, clouds and aerosolised particles (Barry and Chorley 2010) and including microorganisms. b) Four boundaries that occur at varying altitude within the troposphere are important in terms of atmospheric microbial ecology: The atmospheric boundary layer delineates the region of air closest to the surface where the bulk of surface-atmosphere interactions occur, and this includes exchange of microorganisms with terrestrial and marine ecosystems. The lifted condensation level (LCL) delineates the layer above which cloud formation occurs. It is variable with temperature and humidity and delineates the thermal limit for condensation of saturated water vapour on particulate surfaces. The freezing isotherm delineates the layer above which freezing occurs and is also the limit for active non-psychrophilic microbial metabolism. We note that the relationship between the atmospheric boundary layer, the LCL and the freezing isotherm, which are highly dynamic, is significantly more complex than presented in the figure, e.g., both the LCL and the freezing isotherm can sit at ground level well within the boundary layer. The boundary between the LCL and freezing isotherm represent the theoretical range for mesophilic microbial metabolic activity, with potential for psychrophilic and xerophilic activity to extend beyond this zone. The tropopause marks the upper limit of the troposphere and mixing with upper layers is limited by the thermal inversion in the stratosphere. The bulk of atmospheric ozone is in the lower stratosphere and this likely delineates the altitudinal limit of survival for many atmospheric microorganisms (Bryan et al. 2019).

FIG. 2 | Patterns of microbial diversity and abundance in the atmosphere.

a) Simulated concentration of bacterial cells and fungal spores in the near surface atmospheric boundary layer (adapted from ref (Hoose, Kristjánsson and Burrows 2010)). Models assumed a uniform bacterial cell diameter of 1 μm and fungal spore diameter of 5 μm (Burrows et al. 2009), and emission rates were estimated from observed and modelled data (Burrows et al. 2009; Heald and Spracklen 2009). b) Predicted global relative abundance of bacteria and fungi in the near surface atmospheric boundary layer from observed quantitative PCR of rRNA genes in air above locations within different climatically defined biomes (estimated using data from ref (Archer et al. 2021)). Estimates are relative and cannot be used to directly infer absolute cell abundance, they are also domain-specific and cannot be directly compared between bacteria and fungi. c) Taxonomic composition of the ten most abundant classes of bacteria and fungi in the near surface atmospheric

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boundary layer inferred from rigorously decontaminated environmental rRNA gene sequence data from globally distributed locations (adapted from (Archer et al. 2021)). Classes are ranked in order of relative abundance in soil to emphasize those that were over-represented in air.

FIG. 3 | Factors affecting atmospheric microbial transport and macroecological outcomes.

a) Emission and recruitment of microorganisms from continental areas to the atmosphere is strongly dependent on the source habitat and growing season. For example, the phyllosphere is the major contributor in high productivity environments, whereas in low productivity environments with reduced plant cover and in areas affected by intense agricultural practices, the mobilisation of soil particulates becomes an increasingly important emission source. A range of biotic, e.g., passive versus active spore release; and abiotic factors, e.g., rainfall-induced aerosolization from soil, can also influence emissions. Marine surfaces emit microorganisms through bubble-bursting processes driven by wind speed, but the extent of marine emissions on a per surface area basis is low compared to terrestrial sources. The effect of their spatiotemporal determinants and patterns on PBAP emissions remain largely unknown and may include areas of relative high emission in coastal and highly productive marine locations (Wang et al. 2015). b) Above terrestrial and marine surfaces at local scales transport occurs largely within the atmospheric boundary layer over relatively short distances. At larger spatial scales microbial transport involves transit above the boundary layer and this results in longer residence times and greater environmental filtering (Mayol et al. 2017; Archer et al. 2021). c) The association of cells with clouds or particulates directly impacts survival and transport. Combustion particulates may be less efficient vectors than mineral particles from desert dust and soil because they co-aerosolize with lower numbers of microorganisms, and they are associated with toxic combustion products (Archer and Pointing 2020). d) Deposition of cells from the atmosphere occurs via wet deposition as rainfall, hail, and snow, and via dry deposition that relies on sedimentation of cells due to gravity and occurs at lower velocities than wet deposition (Woo and Yamamoto 2020). Wet deposition over continents is typically induced by ice nucleation, which leads to preferential deposition of ice-nucleation active cells and contributes to bioprecipitation and other feedbacks (Huffman et al. 2013; Morris et al. 2014; Joung, Ge and Buie 2017).

FIG. 4 | Stress response and potential substrate utilization by atmospheric microorganisms.

Microbial spores are pre-adapted to survival during atmospheric transport and are over-represented in the atmosphere compared to underlying surface habitats (Mayol et al. 2017; Tignat-Perrier et al. 2019; Archer et al. 2021). Cells that are not adapted to environmental stress undergo strong environmental filtering upon emission to the atmosphere. Cells adapted to environmental stress possess a suite of stress response mechanisms which may reflect source environment stress (Archer et al. 2021). A range of intracellular protective and repair strategies to reactive oxygen species (ROS) and other stressors may be

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3 complemented by extracellular protection due to extracellular polymeric substances (EPS)
4 that also confer protection against xeric, thermal, and ultraviolet radiation (UVR) stress
5 (Lebre, De Maayer and Cowan 2017). Bacterial isolates from the atmosphere have been
6 demonstrated to oxidize volatile organic compounds (VOC) such as methane and a range of
7 small organic compound carboxylic acids, pentose and hexose sugars, amino acids, and
8 phenolic compounds (Bianco et al. 2019). It has been speculated that oxidation of other
9 atmospheric trace gases and carbon fixation may also be potential pathways for activity in
10 the atmosphere although supporting data is currently lacking (Archer and Pointing 2020).
11 Finally, storage compounds accumulated in source environments could support basal
12 activity in airborne bacteria, providing the energy required to preserve cell integrity
13 (Cifuentes et al. 2019).
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21 FIG. 5 | Biophysical role of atmospheric microorganisms in ice nucleation.

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23 An important biophysical interaction of atmospheric microorganisms with cloud formation is
24 ice nucleation by specific microbial proteins that contributes to precipitation and the
25 hydrological cycle, as well as impacting microbial negative flux, i.e., deposition from the
26 atmospheric to surface habitats. a) Bacterial ice nucleating proteins (INpro) are long
27 repetitive proteins that are anchored on the surface of the outer membrane and nucleate
28 ice through a yet to be fully resolved mechanism (Pandey et al. 2016; Hudait et al. 2018;
29 Lukas et al. 2020). They may be cell-associated or retain activity in cell fragments.
30 Monomers or small oligomers nucleate ice at temperatures from -7 to -10 oC and higher-
31 order oligomers of INpro form at well-defined sizes that nucleate ice between -2 to -5 oC
32 (Wex et al. 2015; Ling et al. 2018). Fungal spores and hyphae are associated with INpro that
33 exhibit different molecular structure and properties to those in bacteria, but they are yet to
34 be fully described. b) Fungal spores are the dominant state for fungi in the atmosphere and
35 support surface associated INpro and ice nucleating activity (Pouleur et al. 1992; Morris et
36 al. 2013). c) Fungal hyphae occur very rarely in the atmosphere and so the major
37 contribution arises from fungal INpro that are easily dissociated from cell walls and adhere
38 to soil particulates that subsequently become aerosolized (O'Sullivan et al. 2015). d) Low
39 concentrations of atmospheric INpro nucleate cloud ice and may induce subsequent
40 secondary ice formation via different mechanisms, of which the best understood is riming
41 and splintering (Hallet-Mossop process) (Korolev and Leisner 2020). This process involves
42 freezing of supercooled cloud droplets that encounter primary ice particles (riming) and
43 subsequent ejection of frozen droplets that form independent ice particles (splintering).
44 Primary and secondary ice particles form precipitation through the Bergeron-Findeisen
45 mechanism where ice growth occurs at the expense of surrounding water droplets. Images
46 in panels a-d are not to scale, in a)-c) hexagonal shapes represent ice embryos forming on
47 INpro and in d) hexagonal shapes represent ice particles in clouds.
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59 FIG. 6 | Anthropogenic risks to the atmospheric microbiology.
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Direct and indirect anthropogenic forcing of the atmosphere will potentially impact the emission and deposition flux, atmospheric burden, biodiversity, biophysical, and metabolic activity of microorganisms in the atmosphere (Archer and Pointing 2020). We identify three main outcomes of anthropogenic forcing that are of potential concern, although these are likely inter-linked and overlap. They are: Altered atmospheric transport arising from changes to particulate matter (PM) burden in the atmosphere; shifts in atmospheric microbial emission and deposition flux due to change in surface microbial communities; and impacts on human systems due to agricultural and human pathogen dispersal. We advocate for future research focus to address these impacts.

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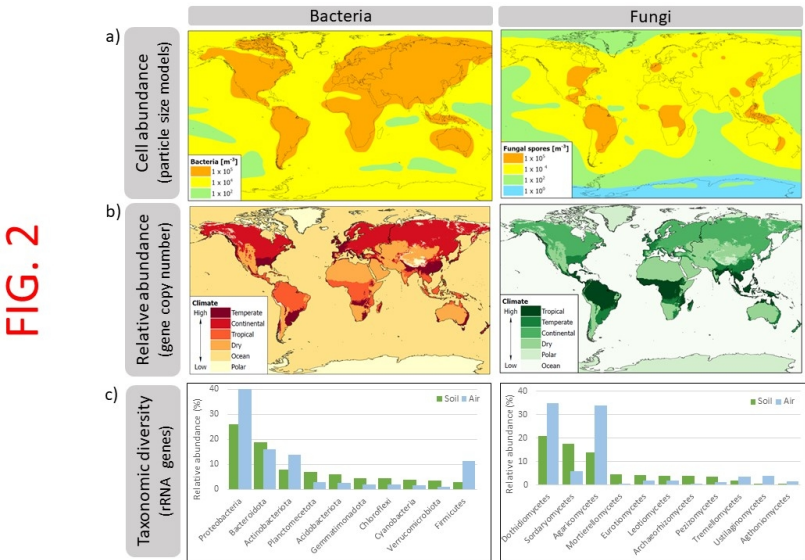


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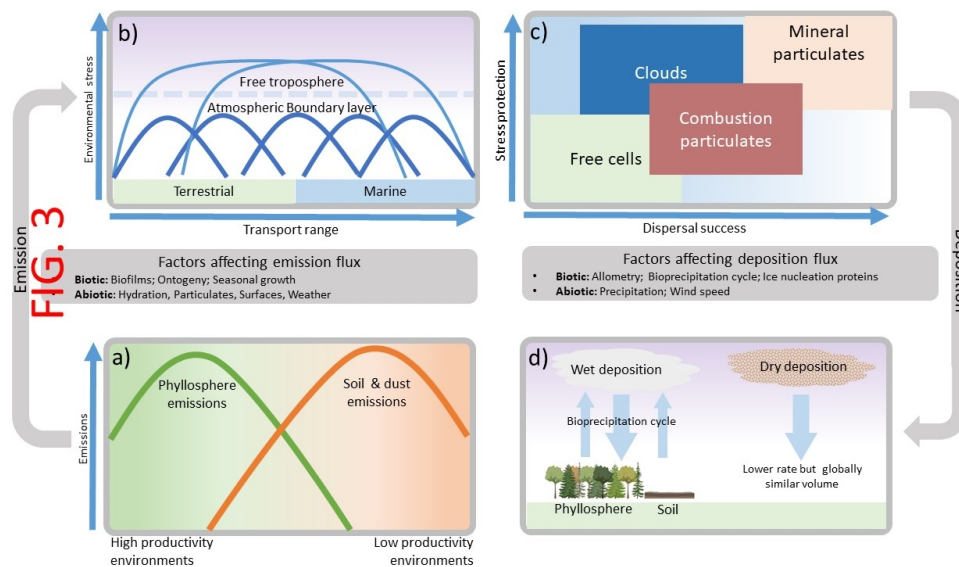


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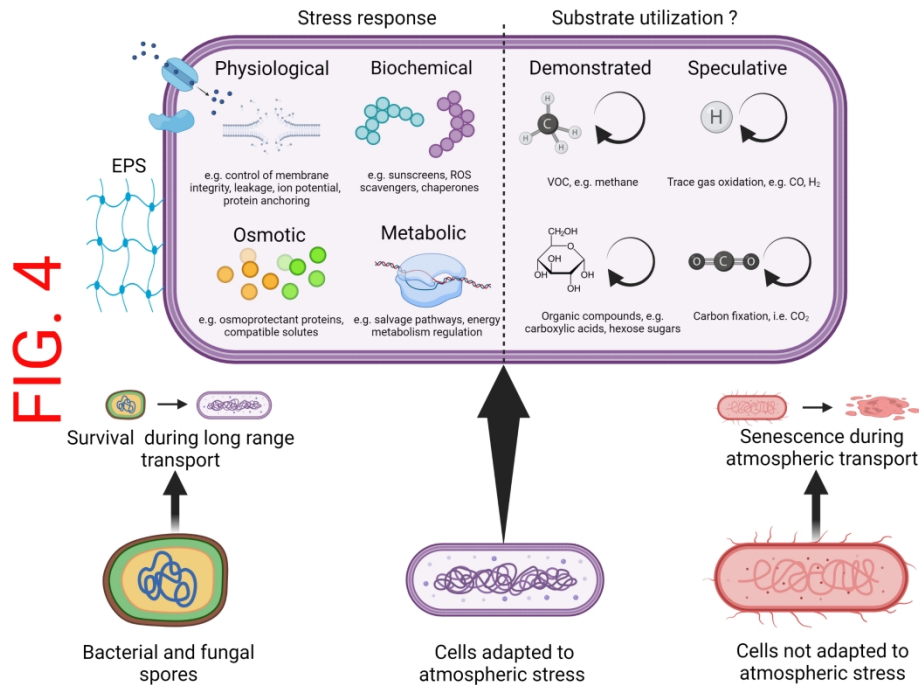


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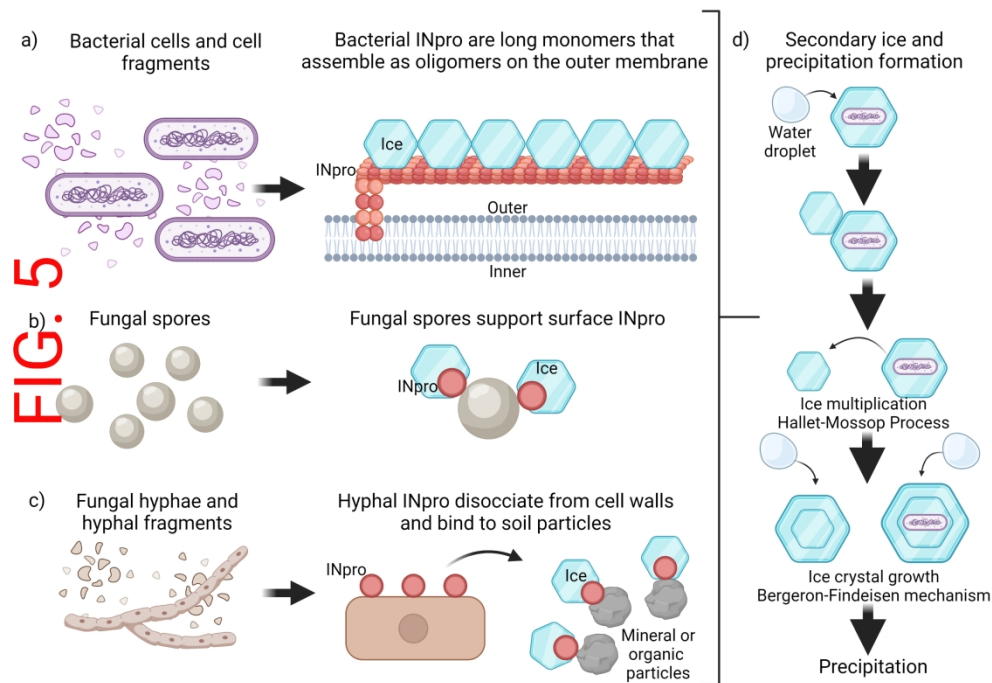


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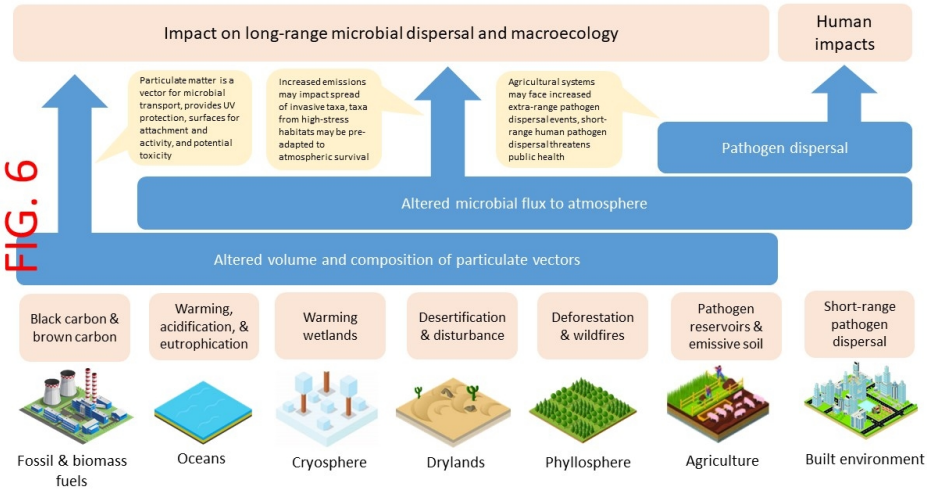


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