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## Microbial ecology of the atmosphere

Tina Šantl-Temkiv, Pierre Amato, Emilio Casamayor, Patrick Lee, Stephen Pointing

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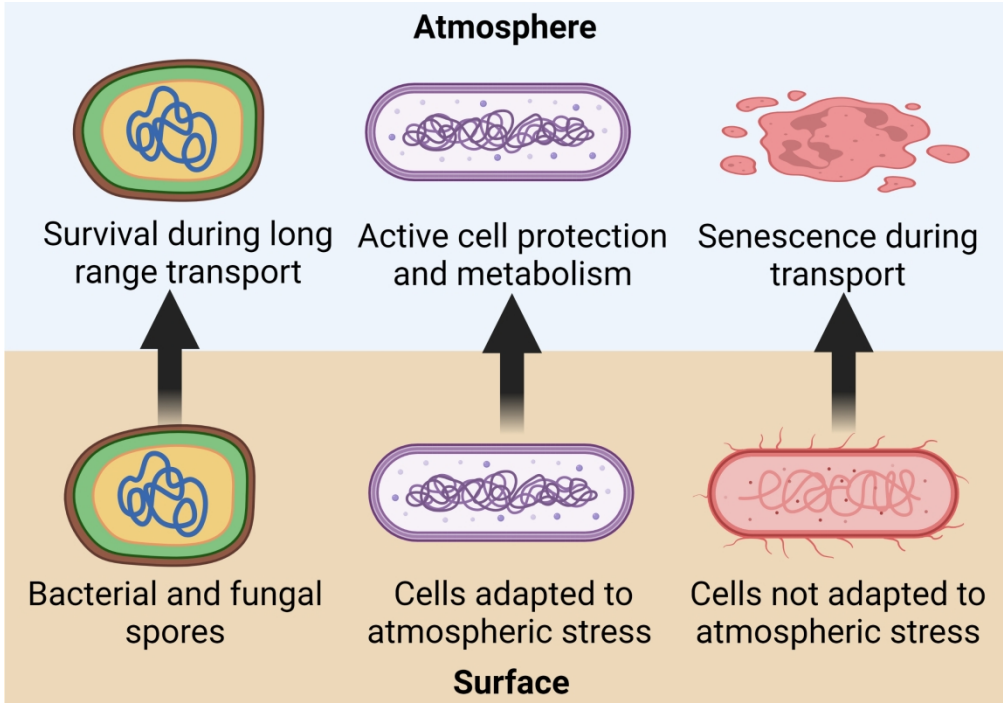
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### Microbial ecology of the atmosphere

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3 **1 Microbial ecology of the atmosphere**  
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7 3 Tina Šantl-Temkiv<sup>1,2</sup>, Pierre Amato<sup>3</sup>, Emilio O. Casamayor<sup>4</sup>, Patrick K.H. Lee<sup>5,6</sup>, Stephen

8  
9 4 B. Pointing<sup>7,8\*</sup>  
10

11 5

12  
13  
14 6 <sup>1</sup> *Department of Biology, Aarhus University, Aarhus, Denmark*

15  
16  
17 7 <sup>2</sup> *Stellar Astrophysics Centre, Department of Physics and Astronomy, Aarhus University,*

18  
19 8 *Aarhus, Denmark*

20  
21 9 <sup>3</sup> *Institut de Chimie de Clermont-Ferrand, SIGMA Clermont, CNRS, Université Clermont*

22  
23 10 *Auvergne, Clermont-Ferrand, France*

24  
25 11 <sup>4</sup> *Centre for Advanced Studies of Blanes, Spanish Council for Research (CSIC), Blanes, Spain*

26  
27 12 <sup>5</sup> *School of Energy and Environment, City University of Hong Kong, Hong Kong, China*

28  
29 13 <sup>6</sup> *State Key Laboratory of Marine Pollution, City University of Hong Kong, Hong Kong,*

30  
31 14 *China*

32  
33 15 <sup>7</sup> *Yale-NUS College, National University of Singapore, Singapore*

34  
35 16 <sup>8</sup> *Department of Biological Sciences, National University of Singapore, Singapore*

36  
37 17 \*e-mail: [stephen.pointing@yale-nus.edu.sg](mailto:stephen.pointing@yale-nus.edu.sg)

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## 19 **Summary**

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

## 26 **Keywords**

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

## 30 **Abstract**

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

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3 43 impacts of anthropogenic forcing on the atmospheric microbiota and ecological  
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5 44 outcomes are identified.  
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## 10 46 **Introduction**

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12  
13 47 Microorganisms have been extensively studied in terrestrial and aquatic natural  
14  
15 48 environments where an implicit and persistent assumption for observed ecological  
16  
17 49 patterns has been that atmospheric transport between habitats is essentially a neutral  
18  
19 50 process due to the extensive aerosolization and long-range transport of cells. This was  
20  
21 51 first embodied in the classic “everything is everywhere, but, the environment selects”  
22  
23 52 statement by Lourens Baas Becking and has driven hypotheses in microbial ecology  
24  
25 53 research for decades (de Wit and Bouvier 2006). Evidence for the distribution of  
26  
27 54 microorganisms in the atmosphere (aeromicrobiology) has been reported since the  
28  
29 55 earliest days of modern life science, with visionaries including Louis Pasteur and  
30  
31 56 Charles Darwin identifying airborne microorganisms (Schwartz 2001; Gorbushina *et al.*  
32  
33 57 2007).  
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39 58 A major challenge for aeromicrobiology has been that traditional cultivation  
40  
41 59 based enumeration is strongly biased towards cultivable taxa (Šantl-Temkiv *et al.*  
42  
43 60 2020), and even the most modern high-throughput sequencing approaches that more  
44  
45 61 fully capture environmental diversity are subject to bias from contaminant signal due to  
46  
47 62 the ultra-low biomass of microorganisms in air (Salter *et al.* 2014). Recent advances in  
48  
49 63 high-volume air mass recovery and application of bioinformatics approaches to  
50  
51 64 decontamination of environmental sequence data have led to significant novel insight,  
52  
53 65 e.g. (Cáliz *et al.* 2018; Gusareva *et al.* 2019; Tignat-Perrier *et al.* 2019; Archer *et al.*  
54  
55 66 2021), thus theoretical frameworks in ecology that challenge the traditional notion of  
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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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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  
39  
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  
51  
52 88 its ecological relevance to other biomes is considered. The scope encompasses  
53  
54 89 microorganisms that occur in outdoor atmospheric air for extended periods or travel  
55  
56 90 across regional or inter-continental distances. The short-range dispersal of human  
57  
58 91 pathogens facilitated by exhaled respiratory droplets in air (Zhang *et al.* 2020) and  
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3 92 airborne transmission within the indoor built environment (Gilbert and Stephens 2018)  
4  
5 93 are excluded. Specific focus is given to how insight from environmental metagenomics  
6  
7 94 has identified atmospheric microbiology as key to understanding the interdependence  
8  
9 95 of ecosystems via flux of diverse taxa between atmosphere and surface habitats, and  
10  
11 96 critical examination of the genetic and physiological evidence for microbial activity in  
12  
13 97 the atmosphere. Furthermore, given the atmosphere is facing profound change due to  
14  
15 98 anthropogenic emissions, consideration is given to how this may impact its  
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17 99 microbiology in future.  
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### 24 101 **Boundaries for habitability in the atmosphere**

25  
26 102 The atmosphere comprises a series of vertically delineated concentric layers whose  
27  
28 103 altitude and depth are primarily defined by thermal properties (FIG. 1a). The  
29  
30 104 troposphere is the lowermost atmospheric layer that contains 75% of its molecular and  
31  
32 105 gaseous mass as well as most water vapour and atmospheric particles (Barry and  
33  
34 106 Chorley 2010), including the majority of microbial cells (FIG. 1b). This is where water  
35  
36 107 cycles through clouds, precipitation and surface environments and it includes the  
37  
38 108 atmospheric boundary layer (a.k.a. planetary boundary layer), which is in direct contact  
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40  
41 109 with the surface, and the free troposphere, which is of great relevance for regional and  
42  
43 110 global dispersal due to long-range movement of air. Vertical mixing above the  
44  
45 111 troposphere is limited due to thermal inversion. The bulk of atmospheric ozone is  
46  
47  
48 112 located in the lower stratosphere and this is thought to represent the altitudinal limit to  
49  
50 113 survival for the majority of microbial cells due to lethal UV exposure at higher  
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52  
53 114 altitudes as a function of reduced attenuation by ozone and extended residence time for  
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56 115 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  
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5 117 habitable environmental gradient for microorganisms. The presence of sufficient liquid  
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8 118 water in cells – a fundamental pre-requisite for any microbial activity – is subject to  
9  
10 119 atmospheric relative humidity that is determined by physical processes and  
11  
12 120 evapotranspiration. In the troposphere, relative humidity is generally bimodal, with  
13  
14  
15 121 higher values in the lower and upper troposphere and a drier mid-troposphere (FIG. 1b)  
16  
17 122 (Gettelman *et al.* 2006). The lifted condensation level (LCL) is the altitude at which  
18  
19 123 vapour pressure of an air parcel becomes saturated and above the LCL, at slight  
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21  
22 124 supersaturations, the water vapour will condense on aerosol particles and form clouds.  
23  
24 125 LCL varies widely depending on temperature and water vapour content, which fluctuate  
25  
26 126 with latitude, location and time, determining the cloud patterns (FIG. 1b). Global  
27  
28 127 differences reflect atmospheric circulation, surface climate and cloud cover, and range  
29  
30 128 from saturation at equatorial latitudes to near-zero above some polar locations.  
31  
32  
33 129 Microbial exploitation of low moisture in xeric environments (Pointing and Belnap  
34  
35 130 2012; Lebre, De Maayer and Cowan 2017) suggests that sub-saturated relative humidity  
36  
37 131 levels throughout the troposphere are not inhibitory to adapted microbial taxa although  
38  
39 132 the lower limit of relative humidity that supports active metabolism has not been  
40  
41  
42 133 determined. In contrast, only regions of high relative humidity and clouds are likely to  
43  
44 134 support the majority of microorganisms not specifically adapted to low water activity.  
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47 135 Cloud droplets provide aqueous microenvironments (Joly *et al.* 2015), also the thin  
48  
49 136 layer of liquid water that occurs at the surface of deliquescent aerosol particles may be  
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52 137 sufficient to sustain microbial activity (Stevenson *et al.* 2015a), but future research will  
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54 138 be key to understand the relationship between hygroscopicity and activity of airborne  
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56 139 cells.  
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3 140 Among other major challenges to microbial survival and proliferation in the  
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5 141 troposphere are the level of photo-oxidative and thermal stress (FIG. 1b). A strong  
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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  
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17 147 be a limiting factor to radiation-tolerant taxa. Air temperature decreases with altitude in  
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19 148 the troposphere at the rate of approximately 6.5°C per km from ambient near-surface  
20  
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  
36  
37 157 unlikely to inhibit potential microbial metabolism or survival in the troposphere.

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39 158 A peculiarity of the atmosphere contrasting with other environments such as  
40  
41 159 soils and oceans is its highly dynamic nature, with high cell turnover rates (Burrows *et*  
42  
43 160 *al.* 2009). Temperature, humidity, and liquid water content can change within minutes,  
44  
45 161 exposing cells to repeated thermal and osmotic shock, and freeze-thaw cycles during  
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47 162 residence in air. Additionally, as air density decreases with altitude so does the spatial  
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49 163 proximity of cells and their potential substrates. The limited availability of surfaces in  
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51 164 the atmosphere is also a challenge to microbial community development as it limits  
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3 165 conventional biofilm formation and the possibility for microbial interactions, e.g. via  
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5 166 quorum sensing (Monier and Lindow 2003). Overall, the atmosphere has been  
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8 167 described as possessing qualities of a chaotic system in terms of its physicochemical  
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10 168 complexity (Hochman *et al.* 2019), and this underlines its heterogeneity as a microbial  
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12 169 environment.  
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### 17 171 **Diversity and biogeography of atmospheric microorganisms**

19 172 The physical volume of the troposphere dwarfs that of the equivalent microbially  
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22 173 inhabited layers of the ocean (i.e. the photic zone) or land (i.e. topsoil) although  
23  
24 174 microbial abundance is several orders of magnitude lower (FIG. 2). On a global scale,  
25  
26 175 modelled atmospheric cell concentrations fall within the range of  $10^0$ - $10^5$  per  $m^3$  for  
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28 176 bacteria and fungi (Burrows *et al.* 2009; Hoose, Kristjánsson and Burrows 2010;  
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30 177 Spracklen and Heald 2014), and observed geographic patterns in relative abundance  
31  
32 178 are broadly congruent with modelled estimates of biogeography for cell numbers  
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34 179 (Mayol *et al.* 2017; Tignat-Perrier *et al.* 2019; Archer *et al.* 2021) (FIG. 2a,b). Estimates  
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36 180 from fresh snowfall and from cloud water suggest  $4 \times 10^2 - 4 \times 10^3$  bacterial cells per  $m^3$   
37  
38 181 and  $4 \times 10^0 - 4 \times 10^2$  eukaryotic cells per  $m^3$  assuming condensed water content of 0.4 g  
39  
40 182 per  $m^3$  (Christner *et al.* 2008b; Tesson, Skjøth and Šantl-temkiv 2016; Amato *et al.* 2017;  
41  
42 183 Hu *et al.* 2018; Šantl-Temkiv *et al.* 2019). Cells associated with aerosolized desert dust  
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44 184 may reach  $10^7$  per  $m^3$  (Maki *et al.* 2016). Aggregations of cells may create localised  
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46 185 elevated concentrations of free biomass or on surfaces of airborne particulates.  
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52 186 Localised concentrations of microorganisms within clouds and particulate dust  
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54 187 plumes are likely to be significant: The global total mass of clouds has been estimated at  
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56 188 194,000 Tg (Pruppacher and Jaenicke 1995), and the fraction of the atmosphere under  
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58 189 cloud cover has been estimated at 67% globally (King *et al.* 2013). Up to two billion  
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3 190 tonnes of desert dust is transported in the atmosphere annually (Shepherd *et al.* 2016),  
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5 191 and a comparable volume of soil particulates are also emitted to the atmosphere by  
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8 192 arable lands every year (Borrelli *et al.* 2017). Attempts to extrapolate these numbers to  
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10 193 yield global atmospheric cell abundance have a high level of uncertainty and have not  
11  
12 194 been able to adequately encompass variability between boundary layer, troposphere,  
13  
14 195 stratosphere, clouds and dust. Nonetheless, a global scale meta-analysis of bacterial  
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16 196 abundance across biomes indicated approximately  $5 \times 10^{19}$  cells may occur globally in  
17  
18 197 the atmospheric boundary layer (Whitman, Coleman and Wiebe 1998). This estimate  
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20 198 was several orders of magnitude lower than those for ocean, soil and subsurface  
21  
22 199 biomes, and reflects the extremely low density of cells in the atmosphere.  
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26 200 Direct cell counting, e.g. (Mayol *et al.* 2017), estimates from environmental  
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28 201 metagenomics, e.g. (Bryan *et al.* 2019; Gusareva *et al.* 2019; Tignat-Perrier *et al.* 2019;  
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30 202 Archer *et al.* 2021), and historical cultivation approaches (Després *et al.* 2012),  
31  
32 203 collectively indicate that the most abundant microorganisms within the atmospheric  
33  
34 204 boundary layer globally are bacteria and fungi. Other microbial components such as  
35  
36 205 archaea and protists appear to comprise a relatively minor component in air (Gusareva  
37  
38 206 *et al.* 2019; Archer *et al.* 2021). Few studies have focused on viruses although there is  
39  
40 207 evidence that a significant volume of free and attached viral particles occurs in air  
41  
42 208 within and above the atmospheric boundary layer (Whon *et al.* 2012; Reche *et al.* 2018).  
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44 209 Cell-free microbial metabolites and cell fragments constitute a significant portion of  
45  
46 210 suspended particulate organic matter in air, above both terrestrial and ocean surfaces  
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48 211 where they are important for cloud ice formation (Wilson *et al.* 2015; Tobo *et al.* 2019).  
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54 212 Microbiological diversity estimations for the atmosphere have been strongly  
55  
56 213 influenced by methodological biases (Šantl-Temkiv *et al.* 2020). Direct comparisons of  
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58 214 cultivation versus molecular ecological approaches have indicated very few  
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3 215 atmospheric microorganisms are cultivable, e.g. (Temkiv *et al.* 2012), and this is  
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5 216 supported by order of magnitude higher diversity estimates from environmental  
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7 217 metagenomics surveys, e.g. (Cáliz *et al.* 2018; Tignat-Perrier *et al.* 2019). The recent  
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9 218 emergence of portable high-volume air sampling devices has greatly helped in recovery  
10  
11 219 of sufficient biomass for environmental metagenomics studies with sample sizes of  
12  
13 220 several cubic metres of air now possible during short timeframes (Šantl-Temkiv *et al.*  
14  
15 221 2020). A persistent issue for diversity estimation has been in distinguishing genuine  
16  
17 222 microbial signatures from the unavoidable contaminants that occur from equipment  
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19 223 and reagents when interrogating ultra-low biomass samples. Assessment of  
20  
21 224 contamination during sequence-based diversity estimation indicates that at sample  
22  
23 225 DNA template concentrations encountered in air contaminants may form a significant  
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25 226 component of sequences (Salter *et al.* 2014). For this reason a rigorous approach to  
26  
27 227 mitigation is required during experimental and bioinformatic workflows (Eisenhofer *et*  
28  
29 228 *al.* 2019). Therefore, it is also necessary to strongly nuance findings where  
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31 229 decontamination effort and outcome were not fully reported. Nonetheless, a growing  
32  
33 230 body of recent research indicates a growing consensus in the understanding of  
34  
35 231 microorganisms in bulk air (i.e. including free cells, aggregates and particulate-  
36  
37 232 associated cells). Taxa adapted to survival under high UV irradiance, moisture limitation  
38  
39 233 and other challenges are over-represented in atmospheric communities which is  
40  
41 234 indicative of strong environmental filtering. This is supported by evidence that  
42  
43 235 atmospheric microbial communities exhibit distinct phylogenetic structuring within the  
44  
45 236 boundary layer and at higher altitudes (Tignat-Perrier *et al.* 2019; Archer *et al.* 2021).  
46  
47 237 Across broad geographic, altitudinal and temporal scales in the troposphere taxonomic  
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49 238 diversity broadly reflects that of underlying surface source habitats, and commonly  
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51 239 encountered bacterial taxa belong to the Actinobacteria, Alphaproteobacteria,

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3 240 Firmicutes and Gammaproteobacteria (FIG. 2c); and commonly encountered fungi in air  
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5 241 largely comprise spores of Agaricomycetes and Dothidiomycetes (Cáliz *et al.* 2018; Els  
6  
7 242 *et al.* 2019b; Gusareva *et al.* 2019; Tignat-Perrier *et al.* 2019; Archer *et al.* 2021) (FIG.  
8  
9  
10 243 2c).

11  
12 244 Global air circulation patterns have been proposed to define boundaries of  
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14 245 biogeographic regions for atmospheric microorganisms (FIG. 1a) (Womack, Bohannan  
15  
16 246 and Green 2010). This is supported by recent evidence for regionalisation (Uetake *et al.*  
17  
18 247 2020) and biogeographic patterns in atmospheric microbial diversity on a global scale  
19  
20 248 (Archer *et al.* 2021). A high microbial species richness within a given air mass in the  
21  
22 249 atmospheric boundary layer reflects recruitment from a variety of terrestrial and  
23  
24 250 marine sources, and source tracking has indicated combined influence of local and  
25  
26 251 distantly recruited taxa to observed diversity (Archer *et al.* 2021). Diversity estimates  
27  
28 252 for near-ground air within the atmospheric boundary layer at various locales have  
29  
30 253 indicated bacterial and fungal communities that were correlated with local abiotic  
31  
32 254 variables such as temperature, precipitation and humidity or land use/surface cover,  
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34 255 reflecting the role of local cell emissions (Bowers *et al.* 2011; Fröhlich-Nowoisky *et al.*  
35  
36 256 2012; Tignat-Perrier *et al.* 2019; Archer *et al.* 2021; Spring *et al.* 2021). Thus, diversity  
37  
38 257 of fungi is relatively higher above regions with a well-developed phyllosphere  
39  
40 258 (Lymperopoulou, Adams and Lindow 2016; Archer *et al.* 2021; Zhou *et al.* 2021), and  
41  
42 259 lowest above oceans (Mayol *et al.* 2017; Archer *et al.* 2020; Uetake *et al.* 2020) and low  
43  
44 260 productivity terrestrial systems such as drylands and polar/alpine regions where  
45  
46 261 underlying surface communities are less diverse and microbially dominated by bacteria  
47  
48 262 (Archer *et al.* 2019, 2021). Several studies have also related diversity to factors  
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50 263 associated with dispersal such as history of air masses and UV radiation, suggesting  
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52 264 combined influence of predominant remote sources and conditions during transit  
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3 265 (*Bowers et al.* 2012; *Cáliz et al.* 2018; *Archer et al.* 2019, 2020; *Els et al.* 2019b; *Uetake*  
4  
5 266 *et al.* 2019; *Tignat-Perrier et al.* 2020).

7  
8 267 Temporal patterns in local atmospheric microbiota reflect seasonal changes in  
9  
10 268 underlying surface cover and source of incoming air mass. A pronounced diel variation  
11  
12 269 of specific bacterial and fungal groups was found in the equatorial tropics using  
13  
14 270 metagenomics (*Gusareva et al.* 2019). Inter-seasonal variation in the atmospheric  
15  
16 271 boundary layer was found to be absent (*Gusareva et al.* 2019), weak (*Tignat-Perrier et*  
17  
18 272 *al.* 2020), pronounced for some taxa (*Bowers et al.* 2012) or stochastic (*Els et al.*  
19  
20 273 2019b), depending on the location. Still, a large number of studies have identified  
21  
22 274 differences in bacterial and/or fungal communities in summer versus winter at  
23  
24 275 temperate and sub-tropical terrestrial locations (*Bowers et al.* 2012, 2013; *Woo et al.*  
25  
26 276 2013; *Barberán et al.* 2014; *Cáliz et al.* 2018; *Els et al.* 2019b; *Uetake et al.* 2019). One of  
27  
28 277 the scarce continuous long-term studies demonstrated a clear and well-delineated  
29  
30 278 seasonal pattern in bacterial and fungal communities (*Woo et al.* 2013). At polar  
31  
32 279 latitudes elevated cell abundance in air corresponded to summer and the growing  
33  
34 280 season (*Šantl-Temkiv et al.* 2019). These studies strengthen the view that the  
35  
36 281 underlying phyllosphere is a major determinant of atmospheric microbial diversity. As  
37  
38 282 the elevation above ground increases, the source area of influence widens (*Schmid*  
39  
40 283 2002; *Hsieh and Katul* 2009), and the small-scale spatial and temporal heterogeneities  
41  
42 284 observed near the ground blur (*Archer et al.* 2019, 2021; *Els et al.* 2019a). Above the  
43  
44 285 atmospheric boundary layer long-range air movements progressively decouple  
45  
46 286 atmospheric diversity from its emission sources (*Burrows et al.* 2009) (*Smith et al.*  
47  
48 287 2018).

49  
50 288 Whilst most research has focused on the atmospheric boundary layer and higher  
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52 289 altitudes within the troposphere, understanding the biogeographic limit of microbial

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3 290 occurrence and survival at higher altitudes is also of interest. It has been postulated that  
4  
5 291 limited microbial survival and transport may occur in the stratosphere (DasSarma and  
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7  
8 292 DasSarma 2018), but the role of the stratosphere in microbial dispersal may be limited  
9  
10 293 due to the combined effects of extreme environmental stress and extended residence  
11  
12 294 times (Bryan *et al.* 2019). Several studies have employed balloon and aircraft platforms  
13  
14 295 to recover stratospheric air samples and they have described a limited diversity and  
15  
16 296 viability of bacterial and fungal taxa (Wainwright *et al.* 2003; Smith, Griffin and  
17  
18 297 Schuergel 2010; Smith *et al.* 2011, 2018; Bryan *et al.* 2019), with taxa not markedly  
19  
20 298 differentiated from those occurring in the troposphere below (Smith *et al.* 2018).  
21  
22 299 Estimates of abundance vary greatly but an upper estimate concluded that bacterial  
23  
24 300 abundance in the troposphere and lower stratosphere may be comparable (Bryan *et al.*  
25  
26 301 2019). However, extreme caution is required during interpretation of results from  
27  
28 302 stratospheric air samples due to the major uncertainty surrounding effective exclusion  
29  
30 303 of the unavoidable contaminants that occur when interrogating the ultra-low biomass  
31  
32 304 system. There is currently insufficient evidence to draw robust conclusions about the  
33  
34 305 occurrence, transport, or viability of microorganisms in the stratosphere. The discipline  
35  
36 306 of astrobiology has considered the potential role of the stratosphere and upper  
37  
38 307 atmosphere for interplanetary dispersal of microorganisms (panspermia) as free cells  
39  
40 308 or via mineral vectors (Yang, Yokobori and Yamagishi 2009) but this will require major  
41  
42 309 investigative effort to be further considered.  
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52 311 **What are the determinants of microbial flux between the atmosphere and surface**  
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54 312 **habitats?**  
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57 313 The atmosphere has long been identified as a major conduit for microbial transport  
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59 314 between surface habitats, e.g. (Womack, Bohannan and Green 2010), and this has a  
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3 315 profound impact on the function and resilience of terrestrial and marine surface  
4  
5 316 communities due to its contribution to gene flow, community assembly, and dispersal of  
6  
7 317 pathogens. Recent developments in ecological theory have begun to challenge the view  
8  
9 318 that microbial transport is a neutral process (Lowe and McPeck 2014), and recent  
10  
11 319 evidence from large scale biogeographic (Mayol *et al.* 2017; Archer *et al.* 2021) and  
12  
13 320 temporal (Cáliz *et al.* 2018; Tignat-Perrier *et al.* 2020) studies of atmospheric  
14  
15 321 microbiota increasingly support this view. Deterministic influence due to  
16  
17 322 environmental filtering and microbial stress tolerance both play a significant role in  
18  
19 323 shaping atmospheric microbiota (Archer *et al.* 2021), and hence the composition of  
20  
21 324 assemblages that are exchanged between atmosphere and surface habitats.

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25  
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  
31  
32 328 and a major factor is the nature of the interface with air, such as the composition of  
33  
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  
39  
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  
43  
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  
51  
52 338 that the total annual emissions were in the range of  $7.6 \times 10^{23}$  -  $3.5 \times 10^{24}$  cells largely  
53  
54 339 originating from terrestrial environments, i.e. crops, grasslands and shrubs (Burrows *et*  
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3 340 *al.* 2009, 2013). This equated to an estimated annual emission of 470–1100 Gg bacterial  
4  
5 341 biomass (Burrows *et al.* 2013). Fungal emissions occur predominantly as free spores  
6  
7 342 and were estimated at  $9.5 \times 10^{23}$  total spores from terrestrial surfaces per year which  
8  
9 343 amounts to ~50 Tg of fungal biomass annually (Elbert *et al.* 2007).

12 344 The major marine aerosolization process is initiated by breaking waves which  
13  
14 345 produces small air bubbles that scavenge microorganisms and organic compounds from  
15  
16 346 the sea column and transport them to the sea surface (Aller *et al.* 2005)(Carlucci and  
17  
18 347 Williams 1965), where they burst to eject small droplets containing salts, biogenic  
19  
20 348 material and microorganisms (Blanchard and Syzdek 1970), into the atmosphere (Afeti  
21  
22 349 and Resch 1990)(Blanchard 1989). Even though oceans are thought to represent weak  
23  
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  
27  
28 352 enrichment of organic compounds and microbial cells has been shown experimentally  
29  
30 353 (Rastelli *et al.* 2017). In addition, large spatial and temporal variability in marine  
31  
32 354 emissions and effects on atmospheric processes may occur due to biological processes  
33  
34 355 and meteorological factors (Wang *et al.* 2015; Wilson *et al.* 2015; DeMott *et al.* 2016).

37 356 Survival during transit in air is influenced by biotic traits related to stress  
38  
39 357 tolerance, and particularly with regard to mitigating the effects of low water activity  
40  
41 358 (Stevenson *et al.* 2015b), desiccation (Potts 1994), photo-oxidative UV (Daly 2009;  
42  
43 359 Sharma *et al.* 2017) and general oxidative stress (Ziegelhoffer and Donohue 2009;  
44  
45 360 Ezraty *et al.* 2017), and low temperatures/freezing (Amato 2013) (FIG. 4). Dormancy  
46  
47 361 and sporulation are strategies that provide protection against major stressors and  
48  
49 362 functional metagenomics of air has indicated genes associated with these traits are  
50  
51 363 over-represented in atmospheric communities compared to underlying surface habitats  
52  
53 364 (Aalismail *et al.* 2019; Archer *et al.* 2021). Many spores as well as dormant and active  
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3 365 cells possess pigment compounds such as melanins, mycosprines and scytonemins that  
4  
5 366 protect against oxidative and desiccation stress largely due to scavenging of reactive  
6  
7 367 species (Gao and Garcia-Pichel 2011). A number of biodiversity surveys in air have  
8  
9 368 revealed taxa known to produce protective pigments are over-represented in air  
10  
11 369 compared to underlying terrestrial and marine habitats (Fröhlich-Nowoisky *et al.* 2012;  
12  
13 370 Tignat-Perrier *et al.* 2019; Archer *et al.* 2021), and this implies a survival advantage.  
14  
15 371 Additional strategies to counter stress may include the secretion of extracellular  
16  
17 372 polymeric substances to mitigating both low water activity via hygroscopic  
18  
19 373 carbohydrate polymers as well as extracellular protection from oxidative stress  
20  
21 374 (Matulová *et al.* 2014). Aerosolization of cells as aggregations, biofilms or attached to  
22  
23 375 particulates may also confer survival advantages during dispersal (Monier and Lindow  
24  
25 376 2003; Morris and Monier 2003) (FIG. 3), but may also reduce residence time in air due  
26  
27 377 to increased size and density (Burrows *et al.* 2009). Metagenomic profiling of  
28  
29 378 atmospheric communities has begun to reveal prevalence of genes encoding cold shock,  
30  
31 379 oxidative stress, and UV repair enzymes (Aalismail *et al.* 2019; Archer *et al.* 2021), and  
32  
33 380 metatranscriptomics of cloud water has revealed active metabolic responses to counter  
34  
35 381 oxidative, osmotic and thermal stresses (Amato *et al.* 2019). Physiological responses to  
36  
37 382 counter simulated atmospheric stress have also been observed for taxa isolated from  
38  
39 383 the atmosphere. Strains of *Pseudomonas syringae* isolated from rain and cloud water  
40  
41 384 withstood aerosolization, freezing, osmotic shock, short-term exposure to UVA and UVB  
42  
43 385 radiation (Alsved *et al.* 2018; de Araujo *et al.* 2019; Ling *et al.* 2021), and oxidative  
44  
45 386 stress imposed by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (Wirgot *et al.* 2019). Metabolomic studies  
46  
47 387 on *P.syringae* also revealed a suite of responses to cold shock under simulated  
48  
49 388 atmospheric conditions (Jousse *et al.* 2017), including production of cryoprotectants,  
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51 389 antioxidants, alkaloids and metabolites involved in energy metabolism.  
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3 390 The deposition of microorganisms occurs through a combination of wet and dry  
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5 391 deposition (Després *et al.* 2012). The wet deposition is affected by a combination of in-  
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8 392 cloud partitioning during cloud droplet / ice particle formation and below-cloud aerosol  
9  
10 393 scavenging processes, which can both contribute to segregation of taxa between the  
11  
12 394 wet and dry phases (Bauer *et al.* 2003; Moore *et al.* 2020; Woo and Yamamoto 2020).  
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14  
15 395 In particular, cloud condensation ability was modelled to shorten the average bacterial  
16  
17 396 residence time in air (and hence dispersal distance) from ~8d to 3d (Burrows *et al.*  
18  
19 397 2009) and ice nucleating microbial cells were shown to be efficiently precipitated  
20  
21 398 (Amato *et al.* 2015; Stopelli *et al.* 2015) Wet deposition rates of  $10^7 - 5 \times 10^9$  bacteria  
22  
23 399 cells  $m^{-2} d^{-1}$  have been reported (Reche *et al.* 2018; Woo and Yamamoto 2020). In  
24  
25 400 contrast, dry deposition velocity is largely determined by allometric considerations,  
26  
27 401 such as PBAP size, shape, and density (FIG. 3d). Dry deposition rates of  $\sim 10^7 - 5 \times 10^8$   
28  
29 402 bacteria cells  $m^{-2} d^{-1}$  have been reported but with no significant difference in estimated  
30  
31 403 overall wet and dry deposition (Reche *et al.* 2018; Woo and Yamamoto 2020). Patterns  
32  
33 404 in deposition of fungal spores and pollen are thought to follow the same pattern where  
34  
35 405 overall wet and dry deposition contributions are similar (Aylor 2017; Woo and  
36  
37 406 Yamamoto 2020), although taxon-specific variation is likely.

38  
39 407 Aside from the emission and deposition processes, cell fluxes are also affected by  
40  
41 408 the altitude that the cells reach, which is a deterministic factor related to stress  
42  
43 409 exposure (FIG. 3b). A very large fraction (97%) of depositing bacterial cells have been  
44  
45 410 found to be particle associated (Reche *et al.* 2018), and with a large mean diameter ( $\sim 9$   
46  
47 411  $\mu m$ ) (Woo and Yamamoto 2020), thus supporting the idea that the mixing state of cells  
48  
49 412 in air, i.e. free cells, aggregates or cells vectored on particulate matter, has a significant  
50  
51 413 effect on cell residence time (FIG. 3c). It should also be noted that processes affecting  
52  
53 414 atmospheric cell fluxes are highly variable and subject to stochastic influence from  
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3 415 events such as dust storms (González-Toril *et al.* 2020) and wildfires (Moore *et al.*  
4  
5 416 2021).

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7  
8 417 A pertinent question for atmospheric communities is the extent to which the  
9  
10 418 complex variation in emission and deposition flux, together with environmental filtering  
11  
12 419 during transit, influence the assembly of terrestrial and marine surface communities.  
13  
14 420 The flux of diversity and biomass from air to soil or oceans is likely smaller by  
15  
16 421 magnitudes than that of standing communities but given the ability of microorganisms  
17  
18 422 to rapidly exploit favourable ecological niches, atmospheric flux should be viewed as a  
19  
20 423 critical control point in aspects of connectivity and ecological resilience across biomes  
21  
22 424 (Dumbrell *et al.* 2010; Lowe and McPeck 2014; Zhou and Ning 2017). This occurs by  
23  
24 425 facilitating microbial dispersal between spatially separated natural systems (Kellogg  
25  
26 426 and Griffin 2006), contributing to maintenance of diversity via recruitment (Jones and  
27  
28 427 Lennon 2010), and in promoting dispersal of dormant propagules that may act as  
29  
30 428 microbial seed banks (Lennon and Jones 2011).  
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### 37 38 430 **What is the evidence for long distance atmospheric pathogen dispersal?**

39  
40 431 Some evidence exists for regional dispersal of pathogens without any obvious  
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42 432 adaptation to the environmental stress of atmospheric transport, e.g. foot and mouth  
43  
44 433 diseases virus (Sorensen *et al.* 2000; Björnham, Sigg and Burman 2020). However, long-  
45  
46 434 range atmospheric dispersal of pathogens (i.e. beyond short-range inter-organismal  
47  
48 435 transmission in air) typically requires protection from environmental stress, whether  
49  
50 436 facilitated by pre-adaptation as spores, or due to association with particulate or  
51  
52 437 biological vectors. Correlations have been made between atmospheric dust vectoring  
53  
54 438 and regional trans-continental scale pathogen dispersal (Tobias *et al.* 2019), and  
55  
56 439 consistent inter-annual dynamics for specific taxa may exist, which make them  
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3 440 foreseeable over time with dominance of plant pathogens over those of animal and  
4  
5 441 humans (Triado-Margarit, Caliz and Casamayor 2021).  
6  
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8 442 Fungal spores are particularly well suited to long-range dispersal and are  
9  
10 443 problematic as a threat to agriculture and global food security (Brown and Hovmøller  
11  
12 444 2002; Savary *et al.* 2019). Many plant pathogens have historically displayed distinct  
13  
14 445 regionalisation but globalisation of agriculture has exacerbated range shifts (Bebber,  
15  
16 446 Holmes and Gurr 2014). Airborne fungal pathogens have been detected above land with  
17  
18 447 agricultural land use (Nicolaisen *et al.* 2017; Archer *et al.* 2021), and some fungal  
19  
20 448 pathogens are able to disperse across inter-continental distances and re-establish  
21  
22 449 infections even when host plants are seasonally absent (Brown and Hovmøller 2002). A  
23  
24 450 striking example is the re-emergence of wheat stem rust as a threat to global food  
25  
26 451 supply. The disease caused by the basidiomycete fungus *Puccinia graminis* f.sp. *tritici* is  
27  
28 452 dispersed readily in air by basidiospores. It was virtually eradicated in the twentieth  
29  
30 453 century, however, a highly pathogenic new strain, Ug99 and its variants, emerged in the  
31  
32 454 last decade and have threatened to devastate global wheat cultivation (Singh *et al.*  
33  
34 455 2011). Combination of disease surveillance and dispersal modelling has resulted in  
35  
36 456 strong evidence for atmospheric transmission as a major determinant of wheat rust  
37  
38 457 epidemiological zones (Meyer *et al.* 2017).  
39  
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41  
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45 458 Evidence for long-range atmospheric dispersal of human pathogens also exists.  
46  
47 459 Seasonal outbreaks of meningococcal meningitis in sub-Saharan Africa have been  
48  
49 460 correlated with bacterial vectoring on dust (Jusot *et al.* 2017), and this also appears to  
50  
51 461 occur for fungal diseases such as Valley Fever caused by *Coccidioides* spores transmitted  
52  
53 462 on desert dust across the south western United States and northern Mexico (Kollath,  
54  
55 463 Miller and Barker 2019). Bacterial plant pathogens may have significantly higher  
56  
57 464 relative abundance than fungi during intercontinental dust intrusions from Africa to  
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3 465 Europe, although predominant western air mass may also bring *Coccidioides* spp., a  
4  
5 466 potentially severe and understudied fungal pathogen endemic from arid and semiarid  
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7 467 regions of North and Central America, to Europe (Triado-Margarit, Caliz and Casamayor  
8  
9 468 2021). Vectoring on biological surfaces also provides a protected means of long-  
10  
11 469 distance dispersal. Wind-borne transport of *Anopheles* mosquitoes carrying the  
12  
13 470 *Plasmodium* parasite was recorded over several hundred kilometres in the Sahel region  
14  
15 471 of Africa (Huestis *et al.* 2019), and pollen has been demonstrated as a vector for  
16  
17 472 bacteria and bacterial allergens (Oteros *et al.* 2019).  
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22 473

#### 23 24 474 **What is the evidence for metabolically active atmospheric microorganisms?**

25  
26 475 The majority of cells in the atmosphere are unlikely to be metabolically active but are  
27  
28 476 simply undergoing transport between source and sink destinations with a high turnover  
29  
30 477 (Burrows *et al.* 2009). Given that most fungi in air occur as dormant spores it is likely  
31  
32 478 that the active fraction of atmospheric microbiota is comprised almost exclusively of  
33  
34 479 bacteria that possess adaptation to atmospheric environmental stress (Archer *et al.*  
35  
36 480 2021) (FIG. 4). Putative generation times for bacteria in cloud water have been  
37  
38 481 estimated at 3.6-19.5 days (Sattler, Puxbaum and Psenner 2001), which fit within their  
39  
40 482 modelled airborne residence times (Burrows *et al.* 2009). However, whether metabolic  
41  
42 483 transformations *in situ* under the highly fluctuating atmospheric conditions are  
43  
44 484 sufficient to sustain only quasi-dormancy, or if cell homeostasis and reproduction are  
45  
46 485 energetically feasible within the short timeframe of favourable conditions offered  
47  
48 486 during atmospheric transport remains unexplored.

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54 487 Evidence from environmental samples has demonstrated that atmospheric  
55  
56 488 microorganisms possess the potential to transform substrates encountered in the  
57  
58 489 atmosphere (FIG. 4). Metagenomic surveys of cloud water (Amato *et al.* 2019), free air

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2  
3 490 (Gusareva *et al.* 2019; Archer *et al.* 2021), and airborne desert dust plumes (Aalismail *et*  
4  
5 491 *al.* 2019) indicate diverse autotrophic and heterotrophic metabolic pathways in  
6  
7 492 atmospheric assemblages that broadly reflect source environments. Photosynthesis  
8  
9 493 may be limited in atmospheric microorganisms due to light-inhibition (Barber and  
10  
11 494 Andersson 1992), but clouds and desert dust provide shading against damaging levels  
12  
13 495 of direct UV radiation and diverse viable and stress-tolerant microalgae and  
14  
15 496 cyanobacteria have been reported from clouds (Tesson, Skjøth and Šantl-temkiv 2016).  
16  
17 497 A range of pathways indicating atmospheric trace gas metabolism including hydrogen,  
18  
19 498 methane and carbon monoxide were widespread among atmospheric metagenomes  
20  
21 499 (Archer *et al.* 2021) and methane oxidation has been demonstrated by atmospheric  
22  
23 500 isolates of *Methylocystis* and *Methylosinus* (Šantl-Temkiv *et al.* 2013), although it is  
24  
25 501 unclear if this is energetically feasible under atmospheric conditions. Most research has  
26  
27 502 focused on the potential for heterotrophic metabolism of simple organic compounds in  
28  
29 503 clouds as these are envisaged as “hotspots” for atmospheric microbial activity.

30  
31 504 Microbial assemblages in cloud water have revealed general markers of  
32  
33 505 metabolic activity under simulated atmospheric conditions including ATP, high  
34  
35 506 ribosomal content, transcribed RNA, enzymatic and respiratory activities (Klein *et al.*  
36  
37 507 2016; Šantl-Temkiv *et al.* 2018; Amato *et al.* 2019). Under conditions of favourable  
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39 508 water activity, temperature, substrate availability and reduced stress exposure,  
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41 509 atmospheric microbial assemblages appear capable of active heterotrophic metabolism  
42  
43 510 as evidenced by chemical speciation in recovered cloud water (Vaitilingom *et al.* 2013;  
44  
45 511 Bianco *et al.* 2019). A comparative metagenomic and metatranscriptomic study of cloud  
46  
47 512 water assemblages highlighted that active metabolism in clouds is closely coupled with  
48  
49 513 the energetic demands of active stress response metabolism and particularly with  
50  
51 514 regard to mitigating oxidative stress (Amato *et al.* 2019). A complex metabolism was



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3 515 envisaged that also included synthesis of cryoprotectant and extracellular polymeric  
4  
5 516 substances implicated in a protective role. The study identified C1 and C2 compounds as  
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7  
8 517 important substrates and ammonium as the major nitrogen source (Amato *et al.* 2019).  
9  
10 518 Another combined metatranscriptomic and metabolic screening of cloud water  
11  
12 519 provided strong evidence for phenol metabolism by bacteria, and this was envisaged as  
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14  
15 520 an important atmospheric substrate derived from anthropogenic emissions (Lallement  
16  
17 521 *et al.* 2018).

19 522         Several studies have demonstrated the ability of atmospheric isolates to  
20  
21 523 metabolise compounds encountered in clouds: A screening of sixty atmospheric isolates  
22  
23 524 including the bacterial genera *Bacillus*, *Pseudomonas*, *Micrococcus* and *Sphingomonas*  
24  
25 525 plus fungal yeasts from cloud water revealed that under laboratory conditions all were  
26  
27 526 capable of degrading diverse carboxylic acids and volatile organic compounds (Amato  
28  
29 527 *et al.* 2007). Atmospheric bacterial isolates of *Bacillus* sp, *Frigoribacterium* sp,  
30  
31 528 *Pseudomonas* sp. and the methanotrophic genera *Methylocystis* sp. and *Methylosinus* sp.  
32  
33 529 have been demonstrated to oxidize common atmospheric volatile organic compounds  
34  
35 530 including formaldehyde and methanol (Husárová *et al.* 2011; Šantl-Temkiv *et al.* 2013).  
36  
37 531 *Rhodococcus enclensis* isolated from cloud water was shown to metabolise catechol and  
38  
39 532 phenol under simulated atmospheric conditions at rates comparable to that of chemical  
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41 533 transformations in the atmosphere (Jaber *et al.* 2020). Larger molecules may also serve  
42  
43 534 as substrates, for example a *Bacillus* strain from cloud water was demonstrated to  
44  
45 535 metabolise a range of pentose and hexose sugars a range of mono- and disaccharides  
46  
47 536 (Matulová *et al.* 2014).

54 537         Based on bulk cloud water measurements, which were unable to account for the  
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56 538 multiphase structure and high dynamic nature of clouds, it has been proposed that  
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58 539 heterotrophic microbial activity could be the main driver for the oxidation of common  
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3 540 atmospheric organic compounds in air during night and could compete with  
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5 541 photochemistry during the day (Vaithilingom *et al.* 2010). Modeling has indicated that in  
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7 542 clouds where only a small fraction ( $\sim 10^{-4}$ ) of spatially separated microdroplets contains  
8  
9 543 microbial cells, the quantitative contribution of microbial activity to the transformation  
10  
11 544 of atmospheric compounds is likely to be low (Fankhauser *et al.* 2019), although this  
12  
13 545 needs to be verified with experimental approaches simulating *in situ* atmospheric  
14  
15 546 conditions. Modelling of biodegradation potential for organic compounds by bacteria  
16  
17 547 in clouds has indicated that acetate and formate are the most utilised substrates and  
18  
19 548 that overall bacterial biodegradation may represent an underestimated sink for  
20  
21 549 atmospheric organics (Khaled *et al.* 2020).  
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### 29 551 **What is the microbial role in atmospheric biophysical processes?**

30  
31 552 Microbial involvement in atmospheric biophysical transformations occurs  
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33 553 independently from the metabolic activity that is thought to be rare during short  
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35 554 residence times in the extreme environment. Biological ice nucleation contributes to  
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37 555 cloud formation, precipitation, and the hydrological cycle, as well as impacting  
38  
39 556 microbial flux between atmospheric and surface habitats, and recent advances have  
40  
41 557 shed new insight on the mechanistic basis for this. Diverse microorganisms and  
42  
43 558 biomolecules, including bacteria (particularly Gammaproteobacteria) (Joly *et al.* 2013),  
44  
45 559 fungi (Fröhlich-Nowoisky *et al.* 2014), microalgae (Tesson and Šantl-Temkiv 2018) and  
46  
47 560 microbially-derived cell-free ice nucleation proteins (INpro) (O'Sullivan *et al.* 2015) can  
48  
49 561 nucleate ice in super-cooled cloud droplets at temperatures much warmer than other  
50  
51 562 atmospheric particles (between -13 and -1°C) and have been implicated in cloud  
52  
53 563 processes. This property has been linked with specific surface- or excreted proteins and  
54  
55 564 is essentially a biophysical process that occurs independently of cell viability or  
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3 565 association (FIG. 5). Whilst terrestrial sources are dominant on a global level, marine  
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5 566 sources associated with INpro excreted by marine microalgae (Wilson *et al.* 2015) may  
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7  
8 567 be important in remote regions unaffected by continents (e.g. Southern Ocean) (DeMott  
9  
10 568 *et al.* 2016).

11  
12 569 Among identified biological ice nucleation active molecules, the best understood  
13  
14 570 are bacterial INpro, which are large membrane-associated proteins (>120 kDa) that  
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16  
17 571 structure interfacial water molecules at low temperature promoting ice formation  
18  
19 572 (Roeters *et al.* 2021) (FIG. 5). Bacterial INpro are long repetitive proteins that present  
20  
21 573 on the surface of the outer membrane and nucleate ice through a yet to be fully resolved  
22  
23 574 mechanism (Pandey *et al.* 2016; Hudait *et al.* 2018; Lukas *et al.* 2020). Monomers or  
24  
25 575 small oligomers nucleate ice at temperatures from -7 to -10 °C and are known as Type C  
26  
27 576 INpro; and higher order oligomers of INpro form at well-defined sizes that nucleate ice  
28  
29 577 between -2 to -5 °C and are described to as Type A INpro (Wex *et al.* 2015; Ling *et al.*  
30  
31 578 2018). Laboratory studies using cloud simulation chambers have shown that the  
32  
33 579 activity of bacterial INpro is independent of cell viability (Hartmann *et al.* 2013; Amato  
34  
35 580 *et al.* 2015). Fungal spores and hyphae also carry INpro that exhibit different molecular  
36  
37 581 structure and properties to those in bacteria and they are yet to be fully characterised.  
38  
39 582 Fungal spores are the dominant state for fungi in the atmosphere and support surface-  
40  
41 583 associated INpro and ice nucleating activity (Pouleur *et al.* 1992; Morris *et al.* 2013)  
42  
43 584 (FIG. 5). Fungal hyphae occur very rarely in the atmosphere and so the major  
44  
45 585 contribution arises from dissociation of fungal INpro from cell walls and adhesion to soil  
46  
47 586 particulates that subsequently become aerosolized (O'Sullivan *et al.* 2015) (FIG. 5). The  
48  
49 587 ubiquitous soil fungal genera *Fusarium* and *Mortierella* have been demonstrated to  
50  
51 588 excrete INpro into the environment and soil particles with associated INpro were  
52  
53 589 capable of ice nucleation (Fröhlich-Nowoisky *et al.* 2014; O'Sullivan *et al.* 2015).  
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3 590           Once they induce freezing, Microbial INpro can also contribute to the formation  
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5  
6 591   of secondary ice particles in clouds which enhances the impact of low abundance  
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8 592   biological INPs through ice-multiplication, particularly at high sub-zero temperatures  
9  
10 593   (Korolev and Leisner 2020) (Fig. 5). Rain that is produced via the ice phase through the  
11  
12 594   Hallett–Mossop process dominates over the midlatitude oceans and continents  
13  
14 595   (Mülmenstädt *et al.* 2015). This process occurs in mixed phase clouds, in which some of  
15  
16 596   the supercooled cloud droplets freeze due to the presence of ice nucleating particles,  
17  
18 597   promoting the deposition of water vapor on thus-formed ice particles and allowing  
19  
20 598   them to grow to precipitation sizes (Fig. 5). Precipitation induced by ice-nucleation  
21  
22 599   active bacteria, whose natural habitats are plant leaf surfaces, contributes to the  
23  
24 600   bioprecipitation cycle by enhancing the growth of vegetation and epiphytic  
25  
26 601   microorganisms, which in turn leads to enhanced emissions of ice-nucleation active  
27  
28 602   bacteria resulting in a positive feedback (Fig. 3d) (Morris *et al.* 2014). Bioprecipitation  
29  
30 603   may be important above regions with a well-developed phyllosphere where emissions  
31  
32 604   of bacterial INpro are highest, and accompanied by relatively low levels of abiotic INPs  
33  
34 605   (Huffman *et al.* 2013; Healy *et al.* 2014). Other precipitation-related feedbacks that  
35  
36 606   increase bioaerosol generation and thus enhance the atmospheric INpro include  
37  
38 607   immediate and delayed interactions of rain with soil and plants (Huffman *et al.* 2013;  
39  
40 608   Joung, Ge and Buie 2017). These positive feedbacks occur alongside non-biological ice  
41  
42 609   nucleation and precipitation and the significance of these processes for meteorology  
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44 610   and climate is yet to be quantified.  
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### 612 **How might anthropogenic activities affect atmospheric microbiota?**

57 613           The atmosphere is a primary sink for anthropogenic emissions of greenhouse  
58  
59 614   gases, chemical pollutants and particulates from fossil fuel combustion (Archer and

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2  
3 615 Pointing 2020). Additional particulate emissions occur as a result of biomass burning  
4  
5 616 due to land clearance and wildfires, soil destabilization due to land use change and  
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8 617 expansion of intensive agriculture, and climate-change induced desertification (Archer  
9  
10 618 and Pointing 2020). Most of these emissions are concentrated in the troposphere where  
11  
12 619 long-range microbial transport, metabolism and biophysical transformations occur.  
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14  
15 620 Atmospheric forcing due to emissions has been acute in the industrial age and this has  
16  
17 621 the potential to create positive and negative impacts on the dispersal of microorganisms  
18  
19 622 in air (Archer and Pointing 2020) (FIG. 6). A recent modelling study suggests that  
20  
21 623 overall microbial diversity in the atmosphere may decline under future climate  
22  
23 624 scenarios and this may severely impact ecosystem connectivity and function (Ontiveros  
24  
25 625 *et al.* 2021).

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29 626 Gaseous emissions from fossil fuel combustion including SO<sub>x</sub> and NO<sub>x</sub> have been  
30  
31 627 shown to inhibit cell function and survival of *Pseudomonas* strains isolated from clouds  
32  
33 628 (Kondakova *et al.* 2016). Greenhouse gaseous, e.g. methane (Šantl-Temkiv *et al.* 2013),  
34  
35 629 can be metabolised by atmospheric bacteria. However, as *in situ* microbial activity and  
36  
37 630 its influence on atmospheric chemistry is still not well constrained, it also remains  
38  
39 631 unclear if elevated substrate levels in the atmosphere have the potential to significantly  
40  
41 632 affect microbial activity and its outcomes (Archer and Pointing 2020). The ice  
42  
43 633 nucleation activity of PBAP may also become more important to precipitation patterns  
44  
45 634 in a warming world because they are more efficient at warmer temperatures from -10°C  
46  
47 635 to 0°C, compared to mineral particulates (Christner *et al.* 2008a). The potential impacts  
48  
49 636 from direct and indirect anthropogenic particulate emissions are more apparent and  
50  
51 637 widespread. Anthropogenic particulates including brown carbon from biomass  
52  
53 638 combustion and black carbon from fossil fuel combustion account for approximately  
54  
55 639 20% of global emissions (Chen *et al.* 2018), and climate change increases mineral dust  
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3 640 aerosolization due to desertification (Pointing and Belnap 2014). Elevated  
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5 641 concentrations of anthropogenic and desert dust particulates have been shown to  
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7 642 influence atmospheric microbial diversity (Maki *et al.* 2019), and this may have  
8  
9 643 implications for dispersal outcomes at deposition sites. The transport of  
10  
11 644 microorganisms in plumes of airborne particulates is envisaged to provide additional  
12  
13 645 protection from UV damage during atmospheric transport (González-Toril *et al.* 2020).  
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15 646 Particulates arising from combustion are more hygroscopic than natural mineral  
16  
17 647 particles and whilst this may be viewed as facilitating greater survival and even  
18  
19 648 potentially enhanced microbial activity, it must be balanced against the often toxic  
20  
21 649 levels of PAH associated with these particulates (Hayakawa *et al.* 2018). Anthropogenic  
22  
23 650 forcing through particulate emissions may also affect ice nucleation patterns in clouds  
24  
25 651 and thus impact microbial flux and deposition in the atmosphere more generally  
26  
27 652 through changes in precipitation. Finally, airborne microorganisms themselves may  
28  
29 653 become increasingly prevalent due to climate-change forcing or pollution, e.g. marine  
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31 654 phytoplankton blooms (Lewis, van Dijken and Arrigo 2020). This includes strong  
32  
33 655 emission from point sources for pathogenic microorganisms, antibiotic resistance genes  
34  
35 656 and allergens, e.g. wastewater treatment plants (Han *et al.* 2020) and urban centres  
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37 657 (Brodie *et al.* 2007; Woo *et al.* 2013), and these have direct public health relevance  
38  
39 658 although dispersal appears to be localised. Assessing the extent of anthropogenic  
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41 659 impacts remains somewhat speculative as more dedicated studies are needed both to  
42  
43 660 provide a better constrained understanding of current processes and to investigate  
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45 661 impacts of highly complex anthropogenic changes to the atmospheric gaseous and  
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47 662 particulate composition.

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664 **Concluding remarks**

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3 665 The discipline of aeromicrobiology has benefitted enormously from recent advances in  
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5 666 methodology that overcome the limitations associated with the study of ultra-low  
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8 667 biomass habitats, and we view this is beneficial in a wider sense to improved ecological  
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10 668 understanding of linkages between the atmosphere and surface terrestrial and marine  
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12 669 biomes. Specifically, the ability to accurately estimate microbial diversity in air through  
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14 670 rigorous decontamination of environmental metagenomic data has revealed that the  
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16 671 atmospheric boundary layer supports a diverse microbiota. Atmospheric microbial  
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18 672 assemblages display taxonomic structuring that reflects strong environmental filtering,  
19  
20 673 as well as distinct spatio-temporal biogeographic signals that reflect a complex  
21  
22 674 interplay of local and regional recruitment to the atmosphere. The long-held ecological  
23  
24 675 view of atmospheric transport as neutral to dispersal outcomes is therefore no longer  
25  
26 676 supportable, and a revision of ecological assumptions about the influence of  
27  
28 677 atmospheric microbial transport is warranted. Knowledge gaps that impede greater  
29  
30 678 understanding in this regard include observational data on flux to and from surface  
31  
32 679 environments and the atmosphere, and a focus on accurate quantitative methods should  
33  
34 680 be a priority for future research.

40 681 The biophysical role of microorganisms in ice nucleation and precipitation  
41  
42 682 formation is supported by convincing evidence and the molecular mechanisms that  
43  
44 683 underpin biogenic ice nucleation are becoming better resolved. Given the temperature  
45  
46 684 range at which biogenic ice nucleation occurs is relevant to a warming climate we urge  
47  
48 685 greater focus and inclusion of microbial ice nucleation in research to predict future  
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50 686 impacts on the water cycle. In the absence of evidence for widespread metabolic  
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52 687 activity that influences biogeochemical outcomes, lack of microbial interactions and  
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54 688 food webs or evidence for cell proliferation, we conclude that microbial biochemical  
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56 689 transformations make a very limited contribution to overall atmospheric chemistry  
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3 690 although they may be important in localised habitable areas such as clouds or plumes of  
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5 691 hydrated particles. The notion of a metabolically active atmospheric microbiome must  
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8 692 therefore be contemplated with a high degree of nuance. Development of improved  
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10 693 laboratory simulations for atmospheric conditions and *in situ* measurements will assist  
11  
12 694 with further understanding of atmospheric microbial metabolism and its boundaries in  
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15 695 air.

16  
17 696 In a broader sense we advocate that estimates of the pan-global microbiota and  
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19 697 its ecological relevance require greater consideration of biomass in the gas-phase  
20  
21 698 atmospheric microbial environment alongside solid-phase terrestrial and liquid-phase  
22  
23 699 marine habitats. The increasing threats to environmental, organismal and human health  
24  
25 700 due to changes in atmospheric transport of microorganisms will be more clearly  
26  
27 701 understood when empirical considerations of the atmospheric microbiota are  
28  
29 702 incorporated into analytical frameworks. We therefore urge expansion of atmospheric  
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31 703 microbiology monitoring and inclusion as part of global scale atmospheric data  
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33 704 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  $\mu\text{m}$  and fungal spore diameter of 5  $\mu\text{m}$  (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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5 Classes are ranked in order of relative abundance in soil to emphasize those that were over-  
6 represented in air.  
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11 FIG. 3 | Factors affecting atmospheric microbial transport and macroecological outcomes.

12  
13 a) Emission and recruitment of microorganisms from continental areas to the atmosphere is  
14 strongly dependent on the source habitat and growing season. For example, the  
15 phyllosphere is the major contributor in high productivity environments, whereas in low  
16 productivity environments with reduced plant cover and in areas affected by intense  
17 agricultural practices, the mobilisation of soil particulates becomes an increasingly  
18 important emission source. A range of biotic, e.g., passive versus active spore release; and  
19 abiotic factors, e.g., rainfall-induced aerosolization from soil, can also influence emissions.  
20 Marine surfaces emit microorganisms through bubble-bursting processes driven by wind  
21 speed, but the extent of marine emissions on a per surface area basis is low compared to  
22 terrestrial sources. The effect of their spatiotemporal determinants and patterns on PBAP  
23 emissions remain largely unknown and may include areas of relative high emission in coastal  
24 and highly productive marine locations (Wang et al. 2015). b) Above terrestrial and marine  
25 surfaces at local scales transport occurs largely within the atmospheric boundary layer over  
26 relatively short distances. At larger spatial scales microbial transport involves transit above  
27 the boundary layer and this results in longer residence times and greater environmental  
28 filtering (Mayol et al. 2017; Archer et al. 2021). c) The association of cells with clouds or  
29 particulates directly impacts survival and transport. Combustion particulates may be less  
30 efficient vectors than mineral particles from desert dust and soil because they co-aerosolize  
31 with lower numbers of microorganisms, and they are associated with toxic combustion  
32 products (Archer and Pointing 2020). d) Deposition of cells from the atmosphere occurs via  
33 wet deposition as rainfall, hail, and snow, and via dry deposition that relies on  
34 sedimentation of cells due to gravity and occurs at lower velocities than wet deposition  
35 (Woo and Yamamoto 2020). Wet deposition over continents is typically induced by ice  
36 nucleation, which leads to preferential deposition of ice-nucleation active cells and  
37 contributes to bioprecipitation and other feedbacks (Huffman et al. 2013; Morris et al. 2014;  
38 Jung, Ge and Buie 2017).  
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50 FIG. 4 | Stress response and potential substrate utilization by atmospheric microorganisms.

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52 Microbial spores are pre-adapted to survival during atmospheric transport and are over-  
53 represented in the atmosphere compared to underlying surface habitats (Mayol et al. 2017;  
54 Tignat-Perrier et al. 2019; Archer et al. 2021). Cells that are not adapted to environmental  
55 stress undergo strong environmental filtering upon emission to the atmosphere. Cells  
56 adapted to environmental stress possess a suite of stress response mechanisms which may  
57 reflect source environment stress (Archer et al. 2021). A range of intracellular protective  
58 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.

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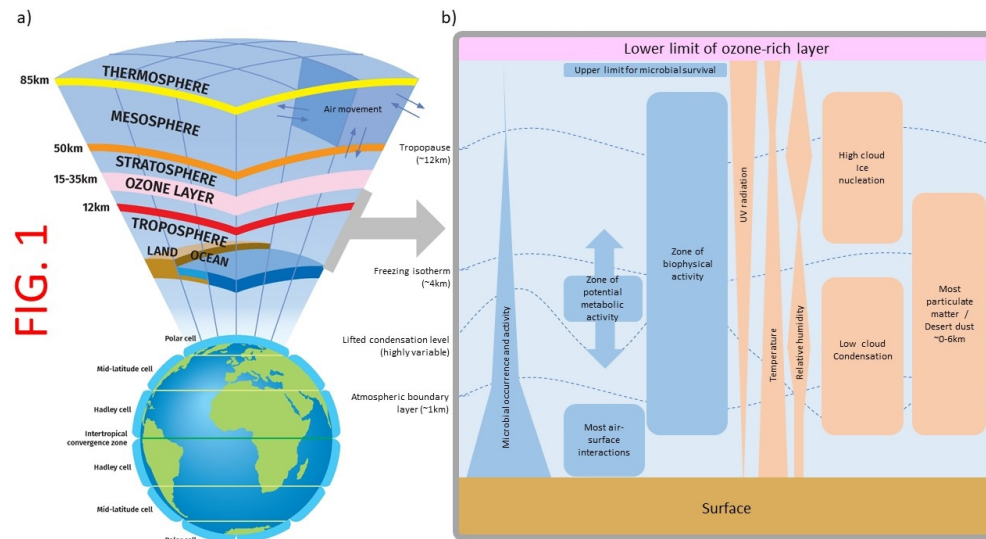


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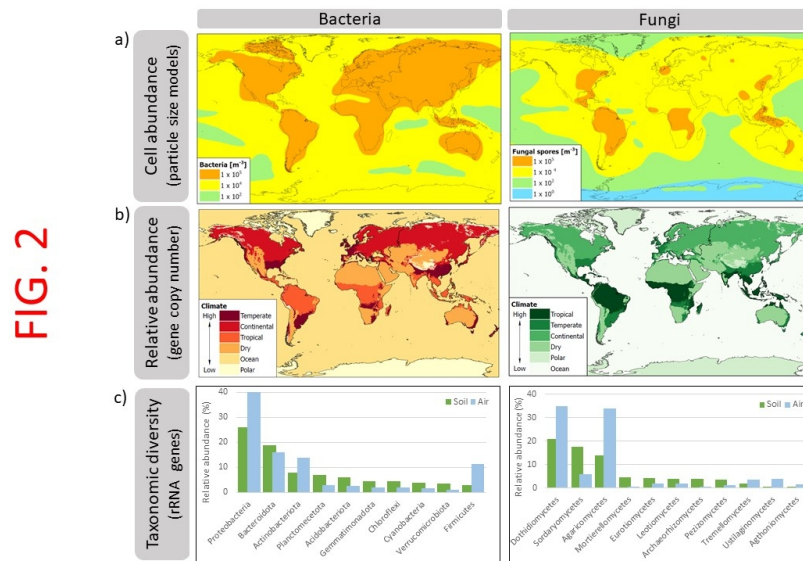


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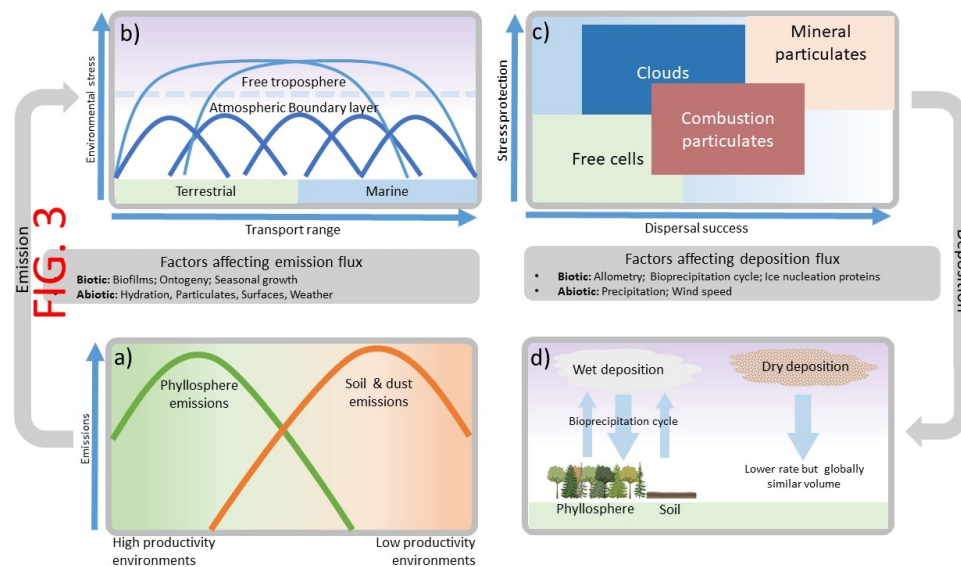


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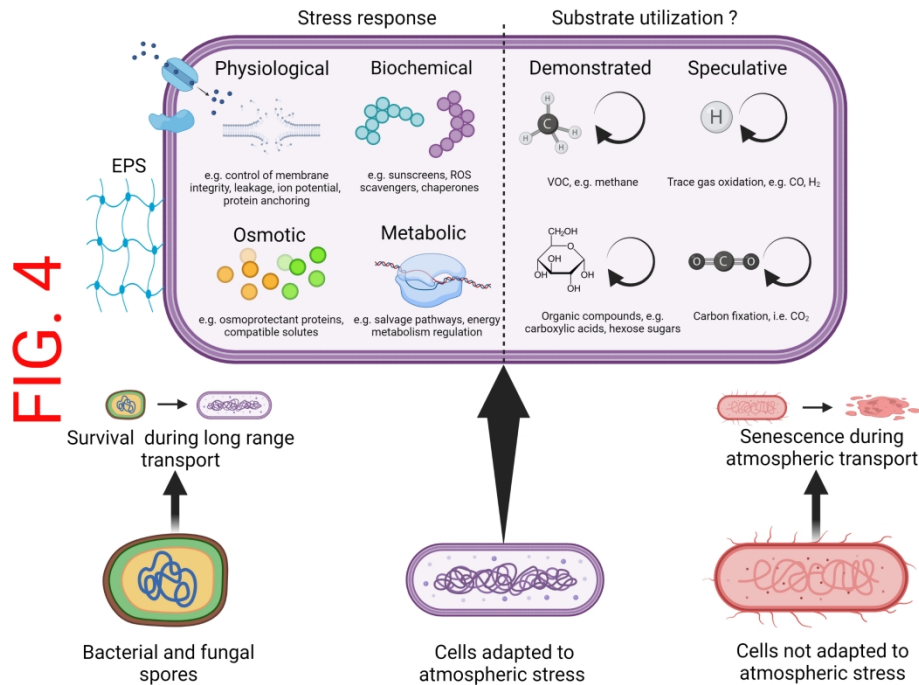


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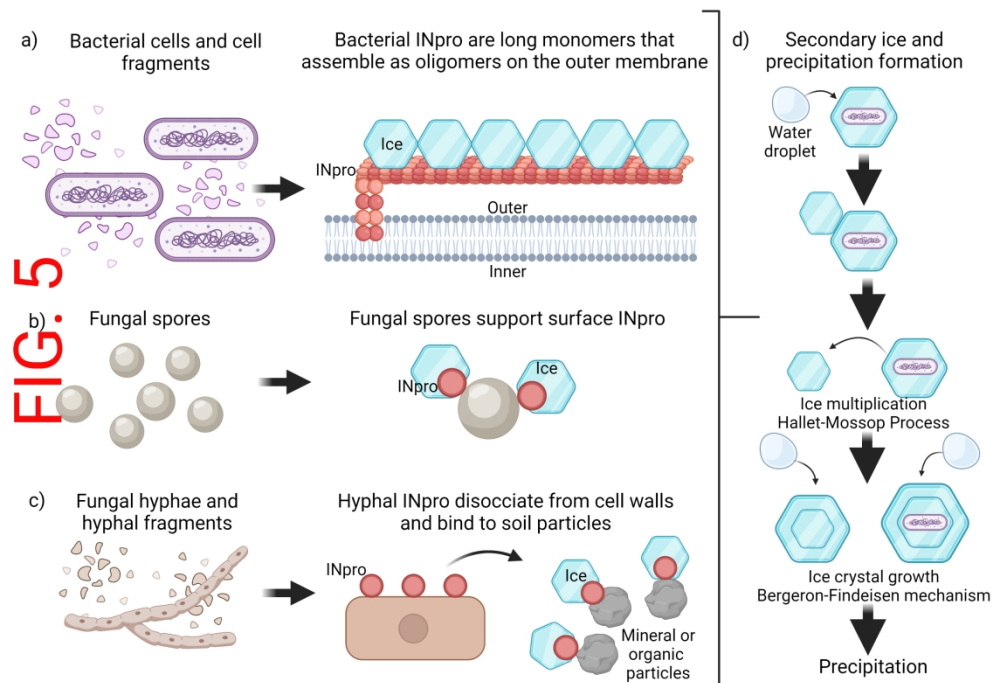


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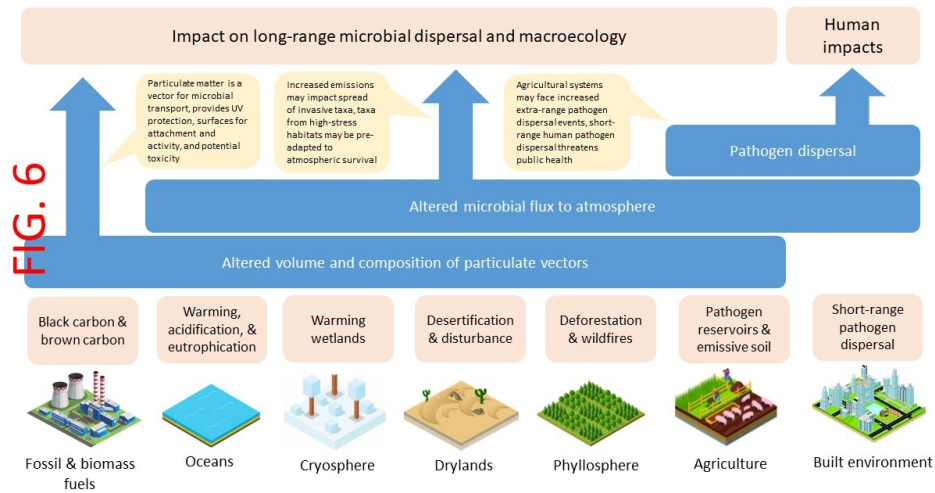


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