Clouds Provide Atmospheric Oases for Microbes

Estimated to total 10^{19} cells, microorganisms in clouds appear sufficient to affect physicochemical processes in the global atmosphere

Pierre Amato

When thermodynamic conditions prove favorable in the atmosphere, water vapor condenses on aerosol particle surfaces, forming micrometer-sized droplets or ice crystals constituting clouds. Even though clouds gather only 0.03% of the fresh water on Earth, they are important components of climate, acting as filters to solar and infrared radiation entering and leaving the planet. Moreover, they provide a multiphasic mixture of liquid, solid, and gas to support chemical reactions affecting the composition of the atmosphere.

When scientists began collecting air samples at high altitudes from mountains, balloons, and, later, airplanes, they learned that living bacteria and fungi are present within the atmosphere. Because condensed water can protect airborne microbial cells against desiccation, aerobiologists consider clouds atmospheric oases. On a global scale, the total number of microorganisms in clouds reaches about 10^{19}. Although this estimate seems low compared with the 10^{26} microorganisms estimated to occupy lakes and rivers and to the 10^{29} microorganisms in oceans, microbial levels in clouds appear sufficient to affect physicochemical processes in the atmosphere. Additionally, clouds could play a major role in disseminating microbes over long distances.

Summary

- Although low in number compared to the 10^{29} microbes estimated in oceans, the 10^{19} microbial cells in clouds are sufficiently plentiful to affect the atmosphere.
- Bacteria in clouds actively metabolize nutrients—for example, about 1 million tons of organic carbon per year—but only about 1% of such cells can be cultured.
- Even though clouds play an important role dispersing microbes over long distances, they apparently do not serve as long-term microbial reservoirs.
- In theory, a single ice-nucleating bacterium within a cloud can induce precipitation and thus cause its own deposition.
- Little is known about rates of emission of bacteria from surfaces into the atmosphere, and such data are not easy to generate.

Clouds Host Metabolically Active Cells

The concentration of microorganisms in clouds typically ranges from 10^2 to 10^5 cells per milliliter. Although only a small fraction—typically less than 1%—of such cells can be recovered by culture, bacteria actively grow in clouds, according to Birgit Sattler and colleagues of the University of Innsbruck in Austria. Because active growth entails the uptake of nutrients, living cells presumably change the chemistry in clouds, acting through processes that are likely driven by sunlight and that generate free radicals, notably hydroxyl and superoxide, $OH^{\cdot}$ and $HO_2^{\cdot}$, respectively.

Clouds are acidic, with pH ranging from 3 to 7, and have conductivity values ranging from 1 to 300 μ S cm^{-1}. This chemistry results from compounds from gas and aerosols dissolving into the aqueous phase of clouds, and varies with underlying local ter-

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FIGURE 1

The 1,465-m puy de Dôme mountain, situated near Clermont-ferrand in France, has hosted an atmospheric observatory for more than a century; it is also being used as a sampling site and field laboratory for studying microorganisms in clouds.

restrial sources. The main ions within clouds—nitrates, sulfates, chloride, ammonium, and sodium—are present at micromolar concentrations. Cloud water also contains organic compounds, including carboxylic acids, aldehydes, and alcohols, from natural and anthropic origins that bacteria can use as nutrients. Additionally, other elements, including phosphorus, iron, copper, and magnesium, are dissolved within cloud water and can sustain microbial metabolism.

In 2003, my colleagues and I in Clermont-Ferrand, France, began sampling cloud water from samples that we collect along the puy de Dôme summit, which is 1,465 m above sea level. We are studying interactions between organic compounds and microbes in those samples, addressing whether cloudborne microbes affect atmospheric chemistry.

To estimate biodegradation rates in clouds, we constructed microcosms in solution whose chemical compositions approximate what occurs in cloud water. We inoculated these microcosms with microorganisms isolated from cloud water, and then monitored their behavior as well as changes in organic compounds by ^{1}H and ^{13}C NMR and by ion chromatography. Our observations surprised us: In some cases, microbes metabolize organic compounds at rates similar to or higher than they are changed by simulated solar light, on the order of 10^{-11} M s^{-1}.

The relative contributions of biology and photochemistry vary for each chemical species, ranging from 0 to 100%. Where the two types of reactions combine, the rates are additive, as is the case in natural situations. Microorganisms catalyze some reactions exclusively—for instance, reducing formaldehyde to methanol. However, cloud-borne microbes do not take up oxalate, which was degraded exclusively photochemically.

In addition and perhaps more importantly, microorganisms partly shut off photochemical reactions by lowering the concentration of hydrogen peroxide, the major source of free radicals in the atmosphere, likely degrading it via oxidative stress enzymes and antioxidant species. Although we continue to refine our estimates, we estimate that microorganisms in clouds metabolize about 1 million tons of organic carbon each year on a global scale.

Clouds Disseminate Microbes, Which Can Drive Precipitation

Even though individual microbial cells are active in cloud droplets, microbial communities likely do not form within this, for them, transitory environment. Thus, clouds are not long-term microbial reservoirs, even though clouds can play an important role dispersing microbes over long distances. Indeed, wet deposition is the main process leading to removal of 1-μm particles, the size range of bacteria, from the atmosphere, and clouds likely are the best "shuttles" for moving airborne microorganisms back to the ground.

To disseminate by air means that microbes must survive the harsh conditions that they en-

Amato: Head in the Clouds Investigating Microbes, Feet Treading Forested Mountains

Friends of Pierre Amato tell him that he has his head is in the clouds. "It is teasing, of course, but at the same time it relates to something poetic, and maybe even romantic in some ways," he says, adding a more serious note: "Increasing concerns related to epidemiology and bioterrorism have aroused a particular attention to the atmosphere as a conveyor of microbes, and clouds appear to be essential."

Amato, a staff scientist at the Institut de Chimie de Clermont-Ferrand in France studies microorganisms in clouds. "My work should lead us to better understand and predict the formation of clouds, their behavior, and the chemical processes taking place in these floating aquatic environments," he says. "What motivates me the most is the role these environments may have as filters to the long-distance dissemination of organisms. Some can survive, some can't, and I believe this . . . is contributing, to the evolution of microorganisms."

Amato, 33, grew up near where he works, in a small village called Bonnac, whose population totals about 30. "This is, to my mind, an idyllic place, with a small river running through it, and surrounded by mountains, forests, and meadows, including sheep and cows managed by shepherd dogs," he says. His parents, now in their 60s, moved there during the 1970s, seeking a home "far from the civilized world," he says. His father was born in Tunisia of Italian parents who moved to France in the 1950s. He has an older sister and a younger brother. His mother taught French, and his father held a variety of jobs, including artisan and forest manager, and later began doing social work. "My mother was a teacher of French in high school, and she loves literature and books in general, so we had tons of books at home," Amato says. "My father used to listen to good music most of the time, so I had [an early] musical education."

Living in that village "certainly shaped my vision of the world and of the place of humans on Earth," Amato says. As a child, he spent considerable time outdoors, especially around the nearby rivers and lakes. "I had to have a real aquatic bestiary at home: salamanders, crawfishes, a frog that I kept more than a year feeding it with flies before letting it go, and many kinds of fish, including even trout and a baby pike that I watched growing up," he says. He still hikes the mountains near his home, goes fishing, gathers forest mushrooms, and continues to "dig into lakes and rivers to look for their inhabitants."

Amato received a bachelor's degree in science in 1996 from the Lycée Lafayette, Brioude, and then a series of graduate degrees leading to a doctorate in 2006, all from Blaise Pascal University in Clermont-Ferrand. Amato then moved to Louisiana State University in Baton Rouge to do postdoctoral research in 2007–2008, studying microbial activity in ice, including an excursion to the Dry Valleys, Antarctica. He spent the next two years studying landscape, microclimate, and dynamics of microbial populations of plants at the French Food Research Institute in Avignon.

While a Ph.D. student, Amato recalls taking samples from clouds from an observatory at the summit of the Puy de Dôme, a volcano near Clermont-Ferrand. "There are places like this that you know have a history," he says. "The wind, the fog, the cold, the ruins of the Roman temple of Mercury just in front of the building, the inside of the building, with old furniture and an old drawing of Emile Alluard, the director of the observatory at the earliest stages, made it very mystical. I guess this contributed to my desire to know more about what was going on in those clouds."

Marlene Cimons

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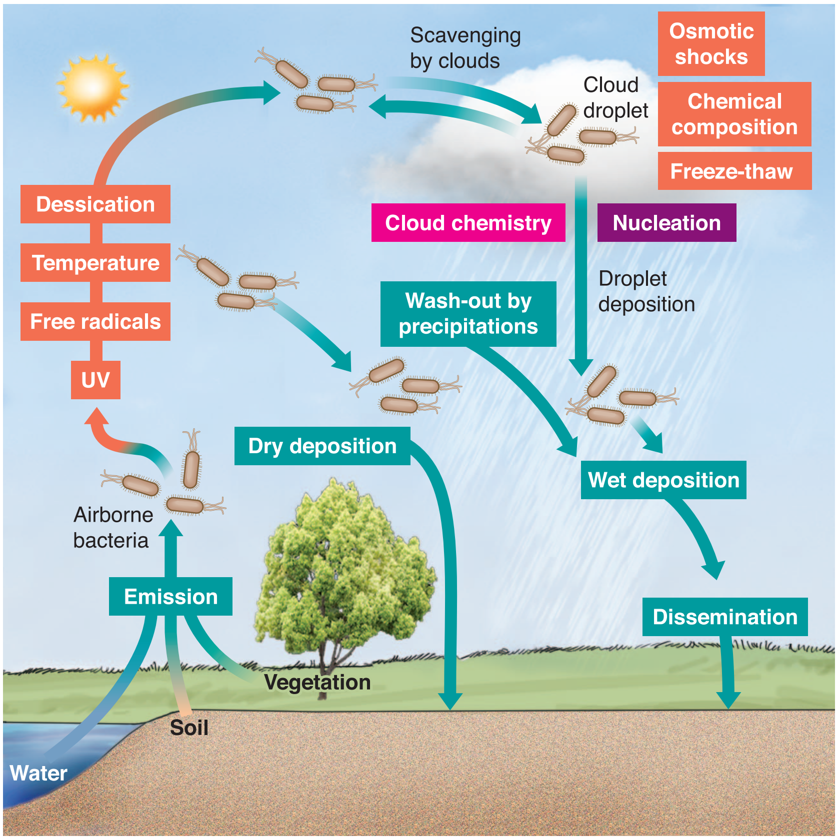
counter in the high atmosphere, including low temperature, desiccation, high levels of UV light, repeated freeze-thaw cycles, and osmotic shock. Hence, it is not surprising that microbes in clouds resemble those recovered from other harsh environments where such stresses occur, including along plant surfaces and glaciers. Two genera of bacteria frequently recovered alive from cloud water collected at the puy de Dôme summit are *Sphingomonas* spp. and *Pseudomonas* spp. These hardy, cloud-borne microbes use the atmosphere and clouds as conveyors to reach distant terrestrial environments.

The *Sphingomonas* species are oligotrophic

FIGURE 2



Schematic representation of the life cycle of microorganisms in the atmosphere, of which the main steps are indicated in grey boxes. Microbes are emitted from surfaces (water, soil, vegetation), get airborne and transported upward by turbulent fluxes. They are subject to environmental conditions (indicated in red boxes) in the atmosphere that likely filter for the more resistant of them. In cloud droplets, viable microorganisms can participate to the degradation of organic compounds, and some species can nucleate freezing and, in theory, induce precipitations. They are finally wet deposited and in a position for colonizing new environments.

organisms that produce yellow and red pigments, which contribute to their high resistance to UV. They also resist cold and salinity, and tolerate relatively high concentrations of oxidants. These traits help to explain how *Sphingomonas* species can survive in clouds until rain or snow brings them back to the Earth's surface.

By contrast, *Pseudomonas* species typically grow using a wide variety of carbon compounds, which can be advantageous in clouds where nutrient availability is limited. These bacteria are capable of autotrophy under some conditions, and some species produce siderophores to acquire iron. However, *Pseudomonas* species are less resistant to UV and oxidants than are many of the *Sphingomonas* spp. that we isolate from clouds. Some *Pseudomonas* species from clouds have properties that enable them to induce clouds to form and rain or snow to precipitate. Such cells produce biosurfactants, which facilitate the condensation of water on their surface, thus improving the chances of cells acting as cloud condensation nuclei (CCN).

In addition, thanks to a protein embedded in their surfaces and triggered by low temperatures and nutrient limitations, *Pseudomonas syringae* cells can induce freezing of supercooled water at temperatures as "warm" as −2°C, acting as ice nuclei (IN); typical abiologic aerosols act as IN around −10°C. Because freezing generally induces precipitation at mid-latitudes, through the Bergeron-Findeisen and Hallett-Mossop processes by which ice crystals grow and multiply within the cloud, respectively, these microbes could induce precipitation.

In theory, a single ice-nucleating bacterium within a cloud can induce precipitation and thus cause its own deposition. This idea of bioprecipitation was launched in the 1970s by Gabor Vali of the University of Wyoming, Laramie, and supported by Dave Sands and Cindy Morris from Montana State University in Bozeman. Sands and Morris, who continue to study the abundance of the plant pathogen *Pseudomonas syringae* in water systems such as rivers, showed that its life cycle is intimately linked with and perhaps drives the water cycle.

Unsettled Questions about Microbes in Clouds

With many open questions concerning microorganisms in the atmosphere and clouds, numeric models will be useful for developing insights and answers. A key but difficult-to-obtain parameter that these models require is concentrations of airborne microbes. One recent aerobiology reference work, published in 2009 by Susannah Burrows and her collaborators at the Max Planck Institute for Chemistry in Mainz, Germany, presents airborne microbial communities as material transported from the surface via turbulent fluxes. When this concept and published concentrations of airborne microorganisms are used in atmospheric models, the high spatial variability of the airborne biomass is not satisfyingly portrayed due to the lack of experimental data.

Notably, we know very little about rates of emission of bacteria from surfaces (fluxes), and these data are not easy to generate for several reasons. For one, many different types of surfaces emit microbes, and they all behave differently. To develop a better understanding, we need to generate large datasets, and this effort necessitates the use of instruments that can continuously count bacterial aerosols. Several groups are developing such instruments, which are expected to be operational soon.

Another challenge is that measuring flux requires one to make several meteorological measurements simultaneously, including sensible heat flux. This parameter can be measured directly using three-dimensional anemometers or approximated by monitoring horizontal wind speed and temperatures at two levels above the ground. Measuring emission fluxes of bacteria from surfaces and predicting their concentrations in clouds will entail complex experimental setups over different types of surfaces. Other challenging measurements include survival rates of microbes and modulations of their metabolic activity under different stresses in the atmosphere.

Although investigators long ago acknowledged that microbes are "everywhere," it still took a while for them to recognize that microbes occupy clouds. Thus, we are in the early stages of thinking about the atmosphere as an extension of the biosphere, in which clouds appear to play an essential role distributing microbial species around the globe. This research has broad implications. While high levels of UV and the numerous selection pressures in the Earth's envelope probably drove early microbial evolution, the arrival of humans doubtless changed those early dynamics. For instance, chemical data suggest that carbon is likely the main limiting nutritional factor in clouds, whereas it is considered to be closer to optimal concentrations in other aquatic environments. However, because recent anthropogenic atmospheric emissions are resulting in acidification and increased concentrations of organic carbon in cloud water, it is legitimate to ask what impact that has on microbes in clouds. A key question is whether humans are responsible for the "eutrophication" of clouds or are rendering them more hostile to microbial life.

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