

OPINION

Bioprecipitation: a feedback cycle linking Earth history, ecosystem dynamics and land use through biological ice nucleators in the atmosphere

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Abstract

Landscapes influence precipitation via the water vapor and energy fluxes they generate. Biologically active landscapes also generate aerosols containing microorganisms, some being capable of catalyzing ice formation and crystal growth in clouds at temperatures near 0 °C. The resulting precipitation is beneficial for the growth of plants and microorganisms. Mounting evidence from observations and numerical simulations support the plausibility of a bioprecipitation feedback cycle involving vegetated landscapes and the microorganisms they host. Furthermore, the evolutionary history of ice nucleation-active bacteria such as *Pseudomonas syringae* supports that they have been part of this process on geological time scales since the emergence of land plants. Elucidation of bioprecipitation feedbacks involving landscapes and their microflora could contribute to appraising the impact that modified landscapes have on regional weather and biodiversity, and to avoiding inadvertent, negative consequences of landscape management.

Keywords: aerobiology, biological ice nucleation, cloud physics, ice multiplication, *Pseudomonas syringae*, rainfall

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Microorganisms have long played important roles in the Earth system. For example, the most important change in the history of Earth's climate, and the most significant for life on our planet, was the rise in atmospheric O₂ concentration from 2% about 2.3 billion years ago (Gya) to the current concentration of 20% (Kasting & Siefert, 2002), caused primarily by microorganisms. They are also currently responsible for about 70% of the greenhouse gas methane released into the atmosphere (Conrad, 2009), and for production of nitrogen oxides and of the precursor of dimethylsulfide, a major source of atmospheric sulfur (Bates *et al.*, 1992). In light of these and other roles in Earth system processes, the American Academy of Microbiology recently and justifiably pointed out the need to integrate microbiology more closely with the study of climate processes (Reid, 2011). Their report called for enhanced interdisciplinary collaboration on a range of biogeochemical processes, yet it neglected processes whereby microorganisms influence climate (cloud and

rainfall patterns in particular) via their impact on atmospheric chemistry and physics.

Over the past decade interest has grown in the so-called bioprecipitation hypothesis: a feedback cycle whereby land plants generate small air-borne particles (aerosols) containing plant-associated microorganisms that influence formation and evolution of clouds via their ice nucleation activity, leading to precipitation that in turn is beneficial for plant and microbial growth (Sands *et al.*, 1982) (Fig. 1). The renewed interest in this hypothesis has been ignited, in part, by the surging need for more precise quantification of cloud evolution for models of global climate change and by recurring droughts and desertification. Part of this renewed effort has been to assemble the evidence that ice nucleation-active (INA) microorganisms indeed affect the microphysical processes in clouds leading to rainfall (Szyrmer & Zawadzki, 1997; Möhler *et al.*, 2007; DeMott & Prenni, 2010; Morris *et al.*, 2011; Murray *et al.*, 2012). The effect of microorganisms on the microphysical processes in clouds is one essential component of bioprecipitation. However, there has been little effort to

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illustrate how it is part of greater Earth system processes. In this study, we go beyond previous reviews of microbial ice nucleation by bringing together current information supporting that microbial-induced ice nucleation in clouds is directly linked to a feedback cycle with plants, and by illustrating how this cycle benefits from auxiliary processes that reinforce ice nucleation activity in microorganisms. As landscapes are modified by human activities, and as the ranges of habitats of plants change with the changing climate of the Earth, feedback cycles could lead to unexpected consequences. Elucidation of precipitation feedbacks involving landscapes and their microflora could contribute to appraising the impact that modified landscapes have on regional weather and biodiversity, and to avoiding inadvertent, negative consequences of landscape management. Therefore, we believe that concerted effort for such elucidation is needed.

The essential components of a bioprecipitation cycle

Knowledge of well-known environmental processes led Sands and colleagues (Sands *et al.*, 1982) to propose the bioprecipitation cycle over 30 years ago. Recently, there have been reports of additional phenomena associated with the two basic components of this feedback cycle that add credibility to its existence.

Component 1

Plants emit water vapor and release microorganisms into the atmosphere. The water vapor emitted from plants is essential for cloud formation and ice nuclei are critical for rainfall. Among the microorganisms released are INA strains of various species of bacteria and fungi (Morris *et al.*, 2004; Murray *et al.*, 2012), and these microorganisms can be transported to the altitude of clouds as aerosols. A wide range of microorganisms has been observed directly in cloud water, including bacteria and fungi known to be INA (Amato *et al.*, 2007; Joly *et al.*, 2013). Laboratory studies have shown that the harsh conditions endured during ascent and residence in the atmosphere are not detrimental to bacterial ice nucleation activity (Attard *et al.*, 2012). The potential efficiency of microbial ice nuclei in this role has been the subject of the numerous reviews indicated above.

Component 2

Rainfall provides water for growth of plants and for multiplication of microorganisms. The latter benefit from increased plant mass and directly from the availability of water. Plant water vapor and microorganisms can then feed back into the first component of the cycle. The idea of the bioprecipitation cycle arose from the

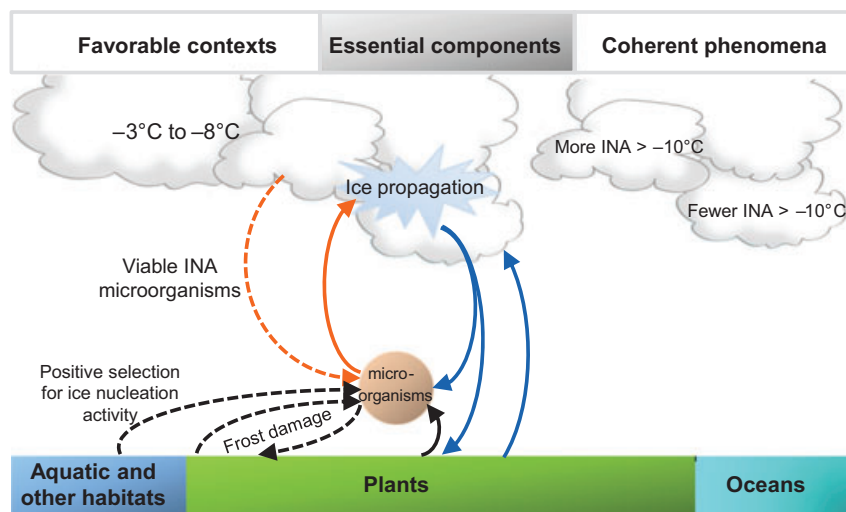


Fig. 1 Environmental phenomena contributing to the bioprecipitation cycle. There are two essential components of bioprecipitation: (i) water vapor (blue lines) and ice nucleation active (INA) microorganisms (orange lines) are emitted from plants into the atmosphere where they participate in the process leading to rain and snowfall and (ii) the resulting precipitation (direct rainfall or snow melt that feeds into groundwater) enhances the growth of plants and microorganisms. Plants are the major source of INA microorganisms in the atmosphere. This is reflected in the greater abundance of ice nuclei active at temperatures $> -10^{\circ}\text{C}$, typical of microbial ice nuclei, over-vegetated land masses than over oceans and is coherent with phenomena involved in the bioprecipitation cycle. Ice nucleation activity of microorganisms is positively selected in various ecosystems and environmental contexts (dotted lines) including aquatic habitats where freezing occurs, on frost damaged plants and with precipitation itself. These phenomena ensure the positive evolution of ice nucleation activity in certain microorganisms and provide a favorable context for bioprecipitation.

obvious beneficial impact of rain on plant growth and the subsequent enhanced growth of microorganisms due to the increased availability of plant tissue. Furthermore, it is well-known that wet conditions favor nearly all diseases of leaf tissue caused by fungi or bacteria (with the exception of the powdery mildews that proliferate in dry weather) including those caused by INA microorganisms that are also plant pathogens (bacteria and rust fungi, for example) (Agrios, 2005). More specifically, studies of molecular mechanisms of plant disease resistance have revealed, for the case of the INA bacterium *Pseudomonas syringae* in particular, that plants interfere with the growth of bacterial pathogens by restricting their access to water at the cellular level (Freeman & Beattie, 2009), further illustrating the critical role of water limitation in the multiplication of plant-associated bacteria. As a corollary to these phenomena, heavy rainfalls (≥ 20 mm) result in thousand-fold increases in population sizes of *P. syringae* on leaves within 48 h, and these increased densities can persist for weeks before declining (Hirano *et al.*, 1996). It is reasonable to assume that as population sizes of microorganisms on leaves increase, the emission of microorganisms from these leaves increases. However, there has not been a formal demonstration of this phenomenon, mostly due to the paucity of studies of microbial flux in the atmosphere (Morris *et al.*, 2013a). Nevertheless, in one of the few studies of microbial flux, upward flux over crops was the highest over the crops having the most dense microbial populations on their leaves (Lindemann *et al.*, 1982). Overall, plant quality affects the abundance of biological ice nuclei in the atmosphere, with the greatest concentrations being observed over agricultural land than over suburban land or forests (Bowers *et al.*, 2011). Finally, rainfall is followed by enrichment of ice nuclei in the atmosphere (Bigg, 1958; Constantinidou *et al.*, 1990; Huffman *et al.*, 2013; Prenni *et al.*, 2013; Tobo *et al.*, 2013) some of which are microbial.

The framework for the bioprecipitation cycle has been largely inspired by knowledge of the emission of ice nucleation-active microorganisms such as *P. syringae* and rust fungi from plants and their transport through the atmosphere and clouds. Nevertheless, this cycle is pertinent for any biological INA particle whose abundance increases as a feedback response to the precipitation that it initiated. Therefore, INA particles implicated in a bioprecipitation cycle would have to multiply by reproduction at least during some part of the cycle, although they could be functionally incapable of reproduction by the time in the cycle that emission or glaciation occurred. Many different types of biological ice nuclei have been detected in the atmosphere but, to date, only a few of them have been

specifically linked to the full set of processes involved in the bioprecipitation cycle (Fig. 1).

The impact of biological ice nuclei on cold clouds – a contentious issue

The real impact of INA microorganisms on cloud microphysics and subsequent precipitation has been a subject of contention. There is generally agreement that the atmospheric processes leading to interaction of ice nuclei and supercooled cloud droplets could set INA microorganisms into the appropriate context to catalyze freezing, and this has been substantiated by direct observation of such microorganisms in clouds as indicated above. However, the crux of the debate is whether there are sufficient numbers of biological ice nuclei in clouds to set off a process that renders measurable precipitation.

There are two main pathways by which precipitation forms in natural clouds. The 'warm rain process' involves collisions between cloud droplets of differing sizes and fall speeds, resulting in coalescence. The warm rain process tends to dominate precipitation production in deep convective clouds with warm bases and rapid ascent $>1 \text{ m s}^{-1}$ aloft (Phillips *et al.*, 2001, 2005). The 'ice crystal process' involves nucleation of ice followed by vapor growth of crystals, riming of supercooled droplets and aggregation of ice. This type of precipitation is sensitive to active concentrations of ice nuclei (Phillips *et al.*, 2003). Horizontally extensive stratiform (nonconvective) clouds that contain ice and are mixed-phase (liquid and ice coexisting), such as frontal nimbostratus clouds, can generate precipitation by this ice-crystal process. In the present-day climate, mixed-phase nimbostratus clouds (and thus the cold-rain process) are more common at mid- and high latitudes than in the tropics and subtropics (Rossow & Schiffer, 1999; Hahn *et al.*, 2001). Their slow ascent ($<1 \text{ m s}^{-1}$) and longevity (lifetimes of many hours) allow ice crystals to fall, to grow by vapor deposition and consequently to become precipitation in the form of ice that may melt.

When the cloud top is warmer than about -37°C , nimbostratus precipitation is predicted to be boosted by higher total concentrations of all active ice nuclei – biological, mineral or others (Phillips *et al.*, 2003). It is under such conditions that INA microorganisms could be involved in the freezing process and hence in precipitation. A role for INA microorganisms is especially expected for glaciated clouds with tops too warm for INA dust to be prolific (warmer than about -15 to -20°C). For clouds with colder tops, the few estimates available suggest that the abundance of active bacterial ice nuclei (those active at warmer than ca. -10°C) in

the remote free troposphere over continental regions (Bowers *et al.*, 2009) is typically at least two orders of magnitude lower than that of mineral or other nonbiological ice nuclei active at much colder temperatures (colder than -20°C) (DeMott *et al.*, 2003) and might be insufficient to influence precipitation. However, INA microorganisms can benefit from a process of ice multiplication that is restricted to a very specific temperature range, if their primary ice can grow to become ice precipitation aloft. Between -3 and -8°C , the number of ice particles in clouds can be amplified by the Hallett-Mossop (H-M) process of ice multiplication (Hallett & Mossop, 1974; Harris-Hobbs & Cooper, 1987), which involves rime splintering of ice precipitation leading to ice concentrations that are several orders of magnitude greater than what would be expected due to primary ice nucleation alone (Bower *et al.*, 1996). Indeed, such impacts from biological ice nuclei on the H-M process have been simulated (Phillips *et al.*, 2009). Virtually none of the nonbiological ice nuclei naturally present in the atmosphere, such as mineral dust (active when colder than -10 to -20°C) (Ansmann *et al.*, 2008; DeMott *et al.*, 2011) or soot (active when colder than -20°C) (DeMott, 1990), can prolifically catalyze ice formation in the temperature range of the H-M process (Hallett & Mossop, 1974), whereas INA bacteria clearly catalyze ice formation in this temperature range and under conditions simulating clouds (Möhler *et al.*, 2008).

One of the first global modeling studies of biological ice nuclei predicted that their contribution to the global average ice nucleation rate is very small (10^{-5} – 0.6%) (Hoose *et al.*, 2010). However, this study does not take ice multiplication effects such as the H-M effect or rime splintering into account, and there is much uncertainty about how to simulate biological ice nuclei. This global study was based on properties of a specific strain of *P. syringae* recently shown to have IN efficiencies much lower (by at least about three orders of magnitude) than many other strains of this bacterium (Murray *et al.*, 2012). Moreover, a separate empirical parameterization of ice nucleation by aerosol species predicts that 3–6% of all active ice nuclei at -30°C are biological in the continental background troposphere based on observations of very large samples of many strains of naturally occurring *P. syringae* (Phillips *et al.*, 2008, 2013). This parameterization was validated against coincident field measurements of active biological ice nuclei and bio-aerosol particles in Colorado (Phillips *et al.*, 2013). At least over certain continental regions such as the tropical rainforest basin in the Amazon, the formation of ice and precipitation in deep stratiform clouds and convective clouds with cold bases and tops warmer than about -15 to -20°C (ice nuclei from dust have

little activity at temperatures warmer than this), and the glaciation of deep convective clouds with warm bases, are all likely triggered by biological particles. In such clouds, biological ice nuclei are present and in some cases may prevail in the overall initiation of primary ice (Prenni *et al.*, 2009; Pöschl *et al.*, 2010).

The growing pool of data on abundance of microbial ice nuclei in the atmosphere portends the emergence of more studies illustrating regional impacts of these ice nuclei on precipitation. (Phillips *et al.*, 2013; Prenni *et al.*, 2013). However, there will be further points of contention to resolve. First, not all particles carrying surfaces of biological origin and capable of catalyzing ice formation at warm temperatures ($>-10^{\circ}\text{C}$) are microorganisms launched from plant surfaces. Another form of biological IN includes decomposed organisms, or parts thereof, attached to soil minerals (Conen *et al.*, 2011; O'Sullivan *et al.*, 2013). Soil organic matter typically has a turnover time of decades to centuries. Abundance and IN activity associated with it are unlikely affected by precipitation events in the short term and therefore unlikely to play a role in bioprecipitation as we understand it. Second, the presence of INA microorganisms in clouds is proof that they are transported up to the altitude of clouds (Amato *et al.*, 2007; Joly *et al.*, 2013). But there are few data available about how this happens – about the conditions that are favorable for this transport and about real measured rates of transport from different sources. Furthermore, some of the available data seem to contradict each other. In the only reported direct measurement of flux of INA microorganisms into the atmosphere, they ascended at $72\text{ cells m}^{-2}\text{ min}^{-1}$. The bacterium *P. syringae* contributed 75% of this INA aerosol (Lindemann *et al.*, 1982). Under these same conditions, INA *P. syringae* was found in aerosols at canopy height at concentrations of about 6 cells m^{-3} in a snap bean field having on average 2.7×10^6 INA *P. syringae* per gram of leaf. This microbial aerosol concentration is at the limit of sensitivity of sampling and detection methods available in aerobiology and illustrates why there have been such few reports of successful detection of *P. syringae* or other INA microorganisms in dry aerosols. Under conditions where INA *Pseudomonas* spp. were present on plant canopies, Garcia and colleagues failed to detect these bacteria in most samples of aerosols collected downwind of the suspected source fields and were successful only during combine harvesting of the fields (Garcia *et al.*, 2012). Although the authors were surprised by this result, it can be explained by the differences in detection thresholds of the technique they used compared to that of Lindemann and colleagues. Garcia and colleagues used a direct molecular detection method based on polymerase chain reaction amplification of

the gene for ice nucleation activity (the *ina* or the *ice* gene) in *Pseudomonas* spp. The detection threshold of this technique, according to the reported methods, was 0.1 copies of the targeted gene per liter of air. It can be assumed that cells of *Pseudomonas* spp. carry one copy of the *ina* or *ice* gene per cell, therefore this threshold is equivalent to 0.1 cells l^{-1} . In contrast, using microbiological culture media, Lindemann and colleagues could detect as few as 0.001 bacterial cells l^{-1} . When Garia and colleagues detected INA bacteria in air samples during combine harvesting of the agricultural fields at their experimental site, a context where high quantities of aerosols are generated, the concentrations were about 19 cells l^{-1} . This represented 2 orders of magnitude above their own detection limit and 4 orders of magnitude more than the concentrations observed by Lindemann and colleagues during the crop growth season. Hence, it is likely that atmospheric concentrations of INA bacteria were below the limits of detection for most of the sampling times in the study of Garcia and colleagues thereby giving the impression that they were not present as aerosols. The sensitivity of detection methods for INA microorganisms in the atmosphere is not the only problem that will complicate the debate about the role of these microorganisms in precipitation. As described in previous work (Morris *et al.*, 2012) thermal plumes can have an important role in the upward flux of microorganisms from plant surfaces. These plumes are 'packages' of air that can uplift the associated aerosols without marked dilution and they are not uniformly distributed across a landscape. The results of such a phenomenon have in fact been observed in the dissemination of fungi (Hirst *et al.*, 1967). The resulting uneven and aggregated distribution of aerosols suggests that air samples might not necessarily reflect the spatial and temporal variability of atmospheric microbial concentrations.

Favorable environmental contexts for the emergence and maintenance of a bioprecipitation cycle

Atmospheric circulation of heat and water vapor and the distribution of clouds have changed over Earth's history. The dramatic changes leading to modern atmospheric circulation began about 100 million years ago with the break-up of Pangea during the Cretaceous period. Poleward shifts of land masses and the uprising of mountains contributed to ascent and adiabatic cooling of rising air over the newly created continents (DeConto, 2008) thereby leading to subzero temperatures in clouds at mid- and high latitudes over these continents in contrast to the arid desert that had covered much of the interior of Pangea previously. The changing climatic conditions were accompanied by an expansion

across mid- and high latitudes of terrestrial angiosperms and, in particular, temperate forests (Zhou *et al.*, 2012). This set in place conditions that could have been exploited by plant-associated INA microorganisms. Data on the evolutionary history of the gene conferring ice nucleation activity in bacteria and of the major class of bacteria that possess this gene, the γ -Proteobacteria, suggest that as of the Cretaceous period of Earth's history all of the fundamental conditions were in place for INA γ -Proteobacteria to play a role in ice-induced rainfall.

Although the ability to catalyze the freezing of water above the temperature of spontaneous freezing (ca. $-37^{\circ}C$) (Murray *et al.*, 2010) is a property of various organisms, nonliving organic compounds and minerals (Mossop, 1963; Morris *et al.*, 2004; Murray *et al.*, 2012), the most is known about this capacity for the class of bacteria called the γ -Proteobacteria. Strains of these bacteria catalyze freezing at the warmest temperatures of all the INA materials regularly found in the environment (Murray *et al.*, 2012). An outer membrane protein orients water molecules into the cubic form of ice that spontaneously converts to hexagonal ice (Kajava, 1995) in a process that resembles the recently discovered shift from cubic to hexagonal ice that can occur during the spontaneous freezing of water in the atmosphere (Murray *et al.*, 2005). The most plausible origin of the gene for the INA protein in the γ -Proteobacteria is from a common ancestor of the highly divergent orders of bacteria in which it has been described: Xanthomonadales, Pseudomonadales, Enterobacteriales (Wolber, 1993). This allows us to estimate that the gene has been part of the γ -Proteobacteria since sometime between the emergence of this class of bacteria [ca. 1.75 Gya (Battistuzzi *et al.*, 2004)] and the diversification of some of the youngest genera in this class [0.5 Gya (Lerat *et al.*, 2003)]. During this part of Earth's history, there were significant periods of glaciation (Fig. 2). This provided millions of years of opportunity for the INA protein to interact with freezing water.

One of the most intriguing aspects of bacterial ice nucleation activity is its efficiency relative to that of mineral particles or of other biological ice nuclei. INA γ -Proteobacteria have orders of magnitude more ice active sites per surface area of their cells than fungi or birch pollen or when compared in terms of surface area to inert particulate matter such as ash, dust, soot and other INA minerals (Murray *et al.*, 2012). It is likely that the remarkable ice nucleation activity of these bacteria results from cycles of natural selection during the evolution of the γ -Proteobacteria. A hallmark of natural selection on a trait is that it causes the trait to be markedly less variable among strains within specific genetic lineages of a population than between different genetic

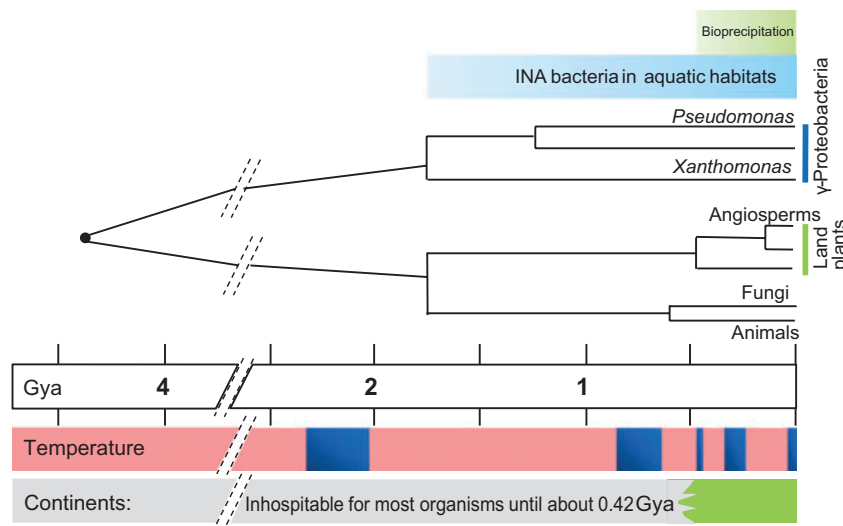


Fig. 2 Evolution of ice nucleation active (INA) bacteria in the timeline of Earth's history. The bacterial ice nucleation protein originated from a common ancestor of the γ -Proteobacteria, a class of bacteria that emerged about 1.75 billion years ago (Gya). This group of bacteria witnessed about 250 million years of glaciation (blue periods in the temperature bar) before the colonization of land by higher plants. Ice nucleation activity was advantageous most probably due to the latent heat released during freezing thereby enhancing survival in cold aquatic habitats before landmasses were inhabitable. After their association with land plants, INA bacteria were readily swept up into the atmosphere and into clouds. The cycle of aerosols of INA bacteria from modern landscapes being washed back to Earth's surface with precipitation represents a process that has been occurring since at least the time of instauration of modern atmospheric circulation patterns and cloud formation (about 100 million years) and is facilitated by a protein with several hundred million more years of history of interaction with ice.

lineages (Nielsen, 2005). Interestingly, INA is not a uniform trait across the γ -Proteobacteria class or even within the *P. syringae* species complex nor does it seem to be a randomly distributed capacity among the different strains of these groups. Certain genetic lineages of *P. syringae*, for example, clearly have higher frequencies of very efficient INA strains than other lineages (Morris *et al.*, 2010) corroborating the importance of positive natural selection in the evolution of this trait.

There are several ways in which catalysis of freezing could have been an advantage for the fitness of bacteria in the γ -Proteobacteria class. It should be noted that there is no evidence for other functions of the ice nucleation protein beyond being INA (Wolber, 1993) and thus the fitness advantages of freezing would have, in themselves, led to positive natural selection of ice nucleation activity. During their early evolution before the emergence of life on land, the γ -Proteobacteria were aquatic organisms. The advantage of INA in aquatic habitats has likely been due to the enhanced survival gained by managing ice formation outside of the cell wall. Nucleation of ice outside of the cell, whether provoked by an organism itself or induced during procedures of cryoconservation, is an effective method of protecting cells during freezing events in the ambient environment (Fahy, 1995; Zachariassen & Kristiansen, 2000). In aquatic habitats, freezing might also be a

means to increase access to nutrients. It has been shown that microbial INA is responsible for the formation of frazil ice in aggregates of diatoms and bacteria. This is thought to benefit the bacteria associated with the aggregates because the frazil ice floats to the surface where the diatoms have greater access to light thereby producing more photosynthates that are food for the associated bacteria (D'Souza *et al.*, 2013). Presently, aquatic milieus are still habitats for INA bacteria. INA *Pseudomonas* spp. have been found in salt water (Fall & Schnell, 1985) and in a supraglacial stream in Antarctica (Foreman *et al.*, 2013), and INA *P. syringae* are abundant in fresh water (Morris *et al.*, 2008, 2010). When INA bacteria became associated with land plants, one likely impact of ice nucleation activity has been the gain in fitness from nutrients released from leaf tissue damaged during ice formation (Wolber, 1993).

It has been proposed that, by inducing precipitation, INA microorganisms also procure a gain in fitness that leads to positive selection of ice nucleation activity (Wolber, 1993; Morris *et al.*, 2010, 2012). This idea is supported by evidence from ecological studies of *P. syringae*, one of the most environmentally predominant INA microbial species. However, it should be kept in mind that precipitation is one of several processes of natural selection that have honed the ice nucleation activity of this species. The percent of cells of the entire

P. syringae global population that actually attains cloud height is unknown and might be very small. Hence, precipitation alone has probably not been the main driver in the evolution of ice nucleation activity. Nevertheless, the enrichment of INA *P. syringae* in rain and snowfall compared to its population in clouds would be important evidence for the role of this bacterium in the processes leading to precipitation.

P. syringae is wide-spread in habitats associated with the cycle of fresh water, from clouds to precipitation to snow pack, alpine prairies and litter, lakes, rivers, epilithon, and a range of wild plants as well as crops (Morris *et al.*, 2008, 2010; Monteil *et al.*, 2012). Its populations generally consist of a mixture of lines of cells of different genotypes and phenotypes, some of which are INA and others not. However, out of all sources of this bacterium studied, it is only in snowfall – a form of precipitation that is always initiated by freezing – that all of the strains are markedly INA at warm temperatures (≥ -6 °C) (Morris *et al.*, 2008). Furthermore, INA strains of *P. syringae* constitute from 60% to 100% of the populations of this bacterium that fall with rain (Morris *et al.*, 2013b). These observations reveal a clear enrichment of ice nucleation activity when compared to the populations in cloud water for which fewer than 10% of strains of this bacterium are INA (Joly *et al.*, 2013). It should be noted that the regular presence of *P. syringae* in rain is not likely the result of scrubbing of aerosols below clouds because this process is very inefficient for particles smaller than several microns in diameter (McDonald, 1962; Respondek *et al.*, 1995). Furthermore, the traits of *P. syringae* strains collected in rainfall above a plant canopy were distinct from those on the plant canopy indicating that the strains in precipitation came from a source other than the local plant canopy (Constantinidou *et al.*, 1990). It has been proposed that, by falling with precipitation, the bacterium avoids a long residence time in the atmosphere that could lead to death from desiccation or overexposure to UV (Morris *et al.*, 2013b). Ice nucleation activity in *P. syringae* is significantly correlated with various other traits allowing it to grow readily in a wide range of environments (Morris *et al.*, 2010). This capacity would be necessary for a bacterium that is deposited with precipitation into diverse habitats.

Interestingly, other species of microorganisms whose life cycle depends critically on air-borne dissemination and deposition via rainfall, obligate parasitic rust fungi such as *Puccinia* spp. and *Hemileia vastratrix*, have recently been shown to be highly INA (Morris *et al.*, 2012). Dry deposition of rust spores rarely occurs once the spores have been transported up to several hundred meters. Fallout from the atmosphere is assured by rainfall (Nagarajan & Singh, 1990). In contrast, none of the

microorganisms that are dominant in the atmosphere have been shown to be ice nucleation active at temperatures > -10 °C (Mortazavi *et al.*, 2008; Bowers *et al.*, 2009; Iannone *et al.*, 2011). These observations are strong arguments for the positive selection of precipitation on ice nucleation activity of certain microorganisms. The ensemble of processes that are forces for positive natural selection of microbial ice nucleation activity contribute to the capacity of these microorganisms to participate in a bioprecipitation feedback cycle. If processes other than precipitation are the main forces for positive selection of ice nucleation activity, then environmental changes that markedly affect these processes could alter the availability of microbial ice nuclei and subsequently the bioprecipitation cycle.

Landscapes, aerosols and phenomena coherent with bioprecipitation

Earth's land masses are the primary source of microbial aerosols in the atmosphere. Based on a broad literature survey and numerical simulations using a global climate model, the average emission rate of bacteria over land is about $200 \text{ m}^{-2} \text{ s}^{-1}$ (Burrows *et al.*, 2009). These emission rates are consistent with simulated and observed atmospheric number concentrations of bacteria-containing particles in the range of 10^4 – 10^5 m^{-3} over most continental regions. Emission rates and average atmospheric concentrations for fungal spores are estimated to be on the same order of magnitude (Elbert *et al.*, 2007; Sesartic & Dallafior, 2011; Despres *et al.*, 2012; Huffman *et al.*, 2012; Šantl-Temkiv *et al.*, 2013). Vegetation is implicated as the main source of air-borne microorganisms, and more so than bare soil (Lindemann *et al.*, 1982; Lighthart, 1997; Burrows *et al.*, 2009). In a study of the composition of hailstones, the culturable bacterial community was skewed toward groups of bacteria most common on plants, whereas their dissolved organic matter content was mainly from soils (vs. plants) (Šantl-Temkiv *et al.*, 2013). Overall, concentrations of bacteria and other bioaerosols are generally much lower over aquatic environments than above terrestrial ones (Burrows *et al.*, 2009). Furthermore, vegetated land masses are stronger sources of biological ice nuclei than water masses and nonvegetated regions (Christner *et al.*, 2008). Likewise, clouds that are warmer than -10 °C contain ice more frequently when they are over vegetated continental regions than at coastal stations or over the open ocean (Kanitz *et al.*, 2011) suggesting that biological ice nuclei, possessing the unique capacity to catalyze ice formation at these temperatures, are responsible for this ice formation.

The specific mechanisms involved in the emissions of microbial particles into the atmosphere from their

sources are mostly unknown. Some mechanisms, for example, involving active emissions from dry and wet surfaces have been well documented for fungi. Active release is prevalent, however, among fungi in only certain species in a limited number of phyla (Meridith, 1973; Elbert *et al.*, 2007). For bacteria and also for a wide range of fungi that do not deploy active release of spores, the processes of emission are linked mainly to the physical conditions providing the forces for removal and release of particles. This involves energy input from an external source provided by wind shear, convection, rain splash, agricultural practices such as combining, etc. (Upper & Hirano, 1991; Jones & Harrison, 2004). Bacterial launch, in particular, is most efficient during rain, when droplets shake leaves and release poorly attached particles (Upper & Hirano, 1991), or at mid-day, when leaves are dry and solar heating promotes convection (Lindemann *et al.*, 1982). Evidence exists for meteorological correlation between bacterial concentrations and atmospheric conditions (e.g. wind-speed and temperature) (Jones & Harrison, 2004; Harrison *et al.*, 2005). Although there is much that remains to be revealed about the specific mechanisms of emission, land-use practices have been implicated in the rates of emission. One influential factor is the type of crop cover which not only influences the rate of emissions (Lindemann *et al.*, 1982) but clearly influences the diversity of microorganisms that can potentially be emitted into the atmosphere. Another markedly important factor is harvesting of a crop (Lighthart, 1997) which can be responsible for the release of up to 10^{13} spores of the wheat rust fungus, for example, for every 5 ha of slightly diseased wheat that are harvested with a combine tractor (Friesen *et al.*, 2001).

In light of the variability in the abundance of INA microbial populations on different plant species and in different types of vegetated ecosystems, and of the effect of land-use practices on their emission, it is reasonable to suspect that the marked changes in regional precipitation patterns over the last century have been partly affected by changes in emission patterns of microbial ice nuclei resulting from drastic human-induced land-use changes over this same timescale (Lioubimtseva *et al.*, 2005; Pielke *et al.*, 2007; Raddatz, 2007; Zeng & Yoon, 2009; DeAngelis *et al.*, 2010).

Future scenarios: changing landscapes, changing rainfall

Land-use changes constitute inadvertent interventions to the bioprecipitation cycle. Alterations to type, density, and seasonality of vegetation cover can severely change the potential source of biological ice nucleators

emitted into the atmosphere (Hirano & Upper, 2000) and thus the patterns of cloud extent and precipitation downwind. This, in turn, must affect the net fluxes of radiation entering the top of the atmosphere and reaching the ground, with possible effects on the regional climate. A shift in land use often affects soil moisture and modifies heat, moisture and momentum transfer to the atmosphere with consequences for local and regional weather (Raddatz, 2007). Large areas subjected to land-use change constitute islands on a continental surface that leave an atmospheric imprint upon the more nearly uniform background surrounding them. If this imprint could be broken down into all factors causing it, the effect of biological ice nucleators may become apparent. Three examples indicate where elucidation of feedbacks involving biological ice nucleators from managed or natural landscapes could contribute to appraising their impact on regional weather and to (possibly) avoiding nefarious inadvertent consequences.

A spectacular growth during the second half of the 20th century in irrigated areas in Central Asia has significantly increased local precipitation in large oases (Lioubimtseva *et al.*, 2005). Similar growth in the irrigated area of the American Great Plains has, since its establishment in the early 20th century, increased downwind precipitation during the month of July by 15–30%. Only part of the additional precipitation can be explained by the increased moisture contributed by irrigation (DeAngelis *et al.*, 2010). The addition of irrigation not only changes the type of crop cover, its density and seasonality, it also changes the emission of biological ice nucleators. Yet, the contribution of crop irrigation to altered regional precipitation patterns is unknown, and has not been the subject of much research.

There have also been land-use changes over large areas on the fringes of the major deserts subjected to overgrazing, where vegetation cover is reduced, desertification progresses, albedo increases, and evaporation declines. This change leads to a feedback loop involving a loss of water vapor from evapotranspiration, where loss of vegetation cover and reduction in precipitation reinforce each other (Zeng & Yoon, 2009). But INA microbial aerosols are also potentially being lost as vegetation declines, and their loss could further accelerate desert expansion.

A third example is the effect of urbanization on atmospheric processes. Urban areas, heat islands with increased surface roughness, enhance local convection and are also significant sources of aerosols. Combined, these effects result in more frequent and persistent thunderstorm activity above large cities, compared to surrounding regions (Ashley *et al.*, 2011). Recent modeling of thunderstorms over São Paulo City indicates a potentially significant role of biological ice nucleators

in cloud development over the cityscape (Gonçalves *et al.*, 2012). Consequently, damage caused by hail or flooding could potentially be reduced by modifying the sources of biological ice nucleators from a cityscape by planting, for example, trees that harbor and release sufficient populations of microbial ice nucleators.

In all three examples, human activities have visible effects on local or regional weather regimes. As anthropogenic activity and climate change further alter vegetated landscapes, increasingly extreme repercussions to weather are expected. In weather processes, physical aspects are studied most intensively. More recently, the role of vegetation is also being considered. Although the potential of biological ice nucleators to modify cloud development has been the subject of numerous investigations (e.g. Phillips *et al.*, 2009; Gonçalves *et al.*, 2012), we still lack the first example of a study linking physical, biological and microbiological effects of human activity, such as land-use change, on changes in regional weather pattern. This begs for concerted interdisciplinary research on this intersection between microbiology, climatology and land-use ecology to elucidate the extent to which microorganisms drive yet another biogeochemical cycle.

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Author contributions

All authors contributed to the conception and writing of this paper. They are all aware of the full contents and give their consent to its publication.

Conflict of interest

The authors declare no conflict of interest.

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