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

CINDY E. MORRIS*†, FRANZ CONEN‡, J. ALEX HUFFMAN§, VAUGHAN PHILLIPS¶, ULRICH PÖSCHL‖ and DAVID C. SANDS†

*INRA, UR0407 Pathologie Végétale, Montfavet Cedex F-84143, France, †Department Plant Sciences and Plant Pathology, Montana State University, 119 Plant Bioscience Bldg., Bozeman MT 59717-3150, USA, ‡Department of Environmental Sciences, University of Basel, Basel 4056, Switzerland, §Department of Chemistry and Biochemistry, University of Denver, Denver, CO 80208, USA, ¶Department of Physical Geography and Ecosystem Science, Lund University, Solvtäjan 12, Lund 223 62, Sweden, ‖Biogeochemistry and Multiphase Chemistry Departments, Max Planck Institute for Chemistry, Mainz 55128, Germany

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

Received 28 April 2013; revised version received 2 October 2013 and accepted 13 October 2013

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
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
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 thousandfold 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; Pernni 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 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 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 °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 cells m^{-2} 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 microbi-
ological culture media, Lindemann and colleagues could 
detect as few as 0.001 bacterial cells l\(^{-1}\). When Garcia 
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 magni-
tude above their own detection limit and 4 orders of 
magnitude more than the concentrations observed by 
Lindemann and colleagues during the crop growth sea-
son. 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) ther-
mal 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 associ-
ated 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 sug-
ests that air samples might not necessarily reflect the 
spatial and temporal variability of atmospheric micro-
obial 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 atmo-
spheric circulation began about 100 million years ago 
with the break-up of Pangea during the Cretaceous pe-
riod. 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 Cretae-
ceous 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 bac-
teria catalyze freezing at the warmest temperatures of 
all the INA materials regularly found in the environ-
ment (Murray et al., 2012). An outer membrane protein 
orient 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: Xanthomon-
dales, 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 (Batti-
stuzzi 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 evolu-
tion of the γ-Proteobacteria. A hallmark of natural 
selection on a trait is that it causes the trait to be mark-
edly less variable among strains within specific genetic 
lineages of a population than between different genetic
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 milieux 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

© 2013 John Wiley & Sons Ltd, Global Change Biology, 20, 341–351
**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 vastatrix*, 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 m$^{-2}$ 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; Sesaric & 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 cultivable 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 midday, 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.

© 2013 John Wiley & Sons Ltd, Global Change Biology, 20, 341–351
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 microbial effects of human activity, such as land-use change, on changes in regional weather patterns. 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.

Acknowledgements

The authors thank the Epicurus Fund at Donors Trust for funding to cover travel for authors to meet. J. A. H. acknowledges internal faculty funding from the University of Denver. V. P. acknowledges support from US Department of Energy’s BER Program for an award supporting modeling research into aerosol impacts on glaciated clouds. C. E. M. thanks the US NSF Division of Atmospheric and Geospace Sciences for funding to stimulate scientific communication on the interaction of bioaerosols with atmospheric processes.

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.

References

Agron GN (2005) Plant Pathology. Academic Press, Elsevier, Amsterdam.
Amato P, Parazols M, Sancelme M, Laj P, Mailhot G, Delort A-M (2007) Microorganisms isolated from the water phase of tropospheric clouds at the Puy de Dôme: major groups and growth abilities at low temperatures. FEMS Microbiology Ecology, 59, 242–254.
Ansmann A, Tesche M, Althausen D et al. (2008) Influence of Saharan dust on cloud glaciation in southern Morocco during the Saharan Desert Mineral Dust Experiment. Journal of Geophysical Research, 113, D04210.

Ashley WS, Bentley ML, Stallins JA (2011) Urban-induced thunderstorm modification in the Southeast United States. Climatic Change, 113, 481–498.
Attard E, Yang H, Delort A-M et al. (2012) Effects of atmospheric conditions on ice nucleation activity of Pseudomonas. Atmospheric Chemistry and Physics, 12, 10667–10677.
Bates TN, Lamb BK, Gaebner A, Dignon J, Stoiber RE (1992) Sulfur emissions to the atmosphere from natural sources. Journal of Atmospheric Chemistry, 14, 315–337.
Battistuzzi FU, Feijao A, Hedges SB (2004) A genomic timescale of prokaryote evolution: insights into the origin of methanogenesis, phototrophy, and the colonization of land. BMC Evolutionary Biology, 4, 44.
Bigg EK (1958) A long period fluctuation in freezing nucleus concentrations. Journal of Meteorology, 15, 561–562.
Bower K, Moss S, Johnson D et al. (1996) A parametrization of the ice water content observed in frontal and convective clouds. Quarterly Journal of the Royal Meteorological Society, 122, 1815–1844.
Bowers RM, Lauber CL, Wiedinmyer C et al. (2009) Characterization of airborne microbial communities at a high-elevation site and their potential to act as atmospheric ice nucleators. Applied and Environmental Microbiology, 75, 5121–5130.
Bowers RM, McLechic S, Knight R, Fiener N (2012) Spatial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments. The ISME journal, 5, 601–612.
Burrows SM, Butler T, Joéckel P, Tost H, Kerkweg A, Pöschl U, Lawrence MG (2009) Bacteria in the global atmosphere – Part 2: modeling of emissions and transport between different ecosystems. Atmospheric Chemistry and Physics, 9, 9281–9297.
Christner BC, Morris CE, Foreman CM, Cai R, Sands DC (2008) Ubiquity of biological ice nucleators in snowfall. Science, 319, 1214.
Conen F, Morris CE, Leifeld J, Yakutin MV, Ahelel C (2011) Biological residues define the ice nucleation properties of soil dust. Atmospheric Chemistry and Physics, 11, 9643–9648. doi:10.5194/acp-11-9643-2011, 2011.
Conrad R (2009) The global methane cycle: recent advances in understanding the microbial processes involved. Environmental Microbiology Reports, 1, 285–292.
Constantinidou HA, Hinzman LS, Baker LS, Upper CD (1990) Atmospheric dispersal of ice-nucleation-active bacteria: the role of rain. Phytopathology, 80, 934–937.
DeAngelis A, Dominguez F, Fan Y, Robock A, Kustu MD, Robinson D (2010) Evidence of enhanced precipitation due to irrigation over the Great Plains of the United States. Journal of Geophysical Research, 115, 1–14.
DeConto RM (2008) Plate tectonics and climate change. In: Encyclopedia of Paleoclimatology and Ancient Environments (ed. Garnitz V), pp. 784–797. Springer-Verlag, Dordrecht.
DeMott PJ (1996) An exploratory study of ice nucleation on soot aerosols. Journal of Applied Meteorology, 29, 1072–1079.
DeMott PJ, Prenni AJ (2010) New Directions: need for defining the numbers and sources of biological aerosols acting as ice nuclei. Atmospheric Environment, 44, 1944–1945.
DeMott PJ, Cziczo DJ, Prenni AJ et al. (2003) Measurements of the concentration and composition of nuclei for cirrus formation. Proceedings of the National Academy of Sciences, 100, 14655–14660.
DeMott PJ, Möhler O, Stetzer O et al. (2011) Resurgence in ice nuclei measurement research. Bulletin of the American Meteorological Society, 92, 1623–1635.
Despres VR, Huffman JA, Burrows SM et al. (2012) Primary biological aerosol particles in the atmosphere: a review. Tellus B, 64, 13598. doi:10.3402/tellusb.v64i0.13598.
D’Souza NA, Kawasaki Y, Gantz JD et al. (2013) Diatom assemblages promote ice formation in large lakes. ISME Journal, 7, 1632–1640.
Elbert W, Taylor PE, Andreae MO, Pöschl U (2007) Contribution of fungi to primary biogenic aerosols in the atmosphere: wet and dry discharged spores, carbohydrates, and inorganic ions. Atmospheric Chemistry and Physics, 7, 4569–4588.
Fahy GM (1995) The role of nucleation in cryopreservation. In: Biological Ice Nucleation and Its Applications (eds Lee RJ, Warren GJ, Gusta LV), pp. 315–336. APS Press, St. Paul, MN, USA.
Fal R, Schnell RC (1985) Association of an ice-nucleating pseudomonad with cultures of the marine dinoflagellate, Heterocapsa triquetra. Applied and Environmental Microbiology, 22, 857–867.
Friesen TL, de Wolf ED, Frankl LJ (2001) Source strength of wheat pathogens during combine harvest. Annu. Rev. Phytopathol, 39, 293–299.
Hirano SS, Upper CD (2000) Bacteria in the leaf ecosystem with emphasis on
Harrison RM, Jones AM, Biggins PDE
Hirano SS, Baker LS, Upper CD (1996) Raindrop momentum triggers growth of leaf-
Hirst JM, Stedman OJ, Hogg WH (1967) Long-distance spore transport: methods of
Garcia E, Hill TC, Prenni AJ, DeMott PJ, Franc GD, Kreidenweis SM (2012) Biogenic
350
M Huffman JA, Sinha B, Garland RM
Huffman JA, Pohlker C, Premi AJ (2013) High concentrations of biological aerosol
particles and ice nuclei during and after rain. Atmospheric Chemistry and Physics,
13, 1767–1793.
Iannone R, Chernoff DJ, Pringle A, Martin ST, Bertram AK (2011) The ice nucleation
ability of one of the most abundant types of fungal spores found in the at-
mosphere. Atmospheric Chemistry and Physics, 11, 1191–1201.
Joly M, Attard E, Sancimat M et al. (2013) Ice nucleation activity of bacteria isolated
from cloud water. Atmospheric Environment, 70, 390–400.
Jones AM, Harrison RM (2004) The effects of meteorological factors on atmospheric
bioaerosol concentrations—a review. Science of the Total Environment, 326, 151–180.
Kajava AV (1995) Molecular modeling of the three-dimensional structure of bacterial
inosa proteins. In: Ice Nucleation: Its Applications (eds Lee RE Jr, Warren CJ, Gaeta LV), pp. 101–114. APS Press, St. Paul.
Kanur T, Seifert P, Amann A, Kämpfer P, Althausen D, Casici A, Rohwer E (2011) Contrasting the impact of aerosols at northern and southern midlatitudes on heterogeneous ice formation. Geophysical Research Letters, 38, 1–5.
Kasting JF, Seifert JL (2002) Life and the evolution of Earth’s atmosphere. In: The Early Earth: its consequences on climate and microbial evolution. In: Yearbook of the History of Geosciences, 1, 2000–0487.
Kajava AV (1995) Molecular modeling of the three-dimensional structure of bacterial
inosa proteins. In: Ice Nucleation: Its Applications (eds Lee RE Jr, Warren CJ, Gaeta LV), pp. 101–114. APS Press, St. Paul.
Kanur T, Seifert P, Amann A, Kämpfer P, Althausen D, Casici A, Rohwer E (2011) Contrasting the impact of aerosols at northern and southern midlatitudes on heterogeneous ice formation. Geophysical Research Letters, 38, 1–5.
Kasting JF, Seifert JL (2002) Life and the evolution of Earth’s atmosphere. In: The Early Earth: its consequences on climate and microbial evolution. In: Yearbook of the History of Geosciences, 1, 2000–0487.
Prenni AJ, Tobo Y, Garcia E et al. (2013) The impact of rain on ice nuclei populations at a forested site in Colorado. Geophysical Research Letters, 40, 227–231.

Raddatz RL (2007) Evidence for the influence of agriculture on weather and climate through the transformation and management of vegetation: illustrated by examples from the Canadian Prairies. Agricultural and Forest Meteorology, 142, 186–202.

Reid A (2011) Incorporating Microbial Processes into Climate Models. American Academy of Microbiology, Available at http://academy.asm.org/images/stories/documents/Incorporating_Microbial_Processes_into_Climate_Models.pdf (accessed 9 November 2013).

Respondek PS, Flossmann AI, Alheit RR, Pruppacher HR (1995) A theoretical study of the wet removal of atmospheric pollutants. 5. The uptake, redistribution, and deposition of (NH₄)₂SO₄ by a convective cloud containing ice. Journal of the Atmospheric Sciences, 52, 2121–2132.

Rossow WB, Schiffer RA (1999) Advances in understanding clouds from ISCCP. Bulletin of the American Meteorological Society, 80, 2261–2287.

Sands DC, Langhans VE, Scharen AL, de Smet G (1982) The association between bacteria and rain and possible resultant meteorological implications. Journal of the Hungarian Meteorological Service, 86, 148–152.

Santi-Temkiv T, Finster K, Dittman T, Hansen BM, Thyrrhaug R, Nielsen NW, Karlson UG (2013) Hailstones: a window into the microbial and chemical inventory of a storm cloud. PLoS ONE, 8, e53550.

Sesartic A, Dallafior TN (2011) Global fungal spore emissions, review and synthesis of literature data. Biogeochemistry, 8, 1181–1192.

Szymier W, Zawadzki J (1997) Biogenic and anthropogenic sources of ice-forming nuclei: a review. Bulletin of the American Meteorological Society, 78, 209–228.

Tobo Y, Prenni AJ, DeMott PJ et al. (2013) Biological aerosol particles as a key determinant of ice nuclei populations in a forest ecosystem. Journal of Geophysical Research: Atmospheres, 118, 10100–10110.

Upper CD, Hirano SS (1991) Aerial dispersal of bacteria. Biotechnology (Reading Mass.), 15, 75–93.

Wolber PK (1993) Bacterial ice nucleation. Advances in Microbial Physiology, 34, 203–237.

Zachariassen KE, Kristiansen E (2000) Ice nucleation and antinucleation in nature. Cryobiology, 41, 257–279.

Zeng N, Yoon J (2009) Expansion of the world’s deserts due to vegetation-albedo feedback under global warming. Geophysical Research Letters, 36, L17401.

Zhou J, Poulsen CJ, Rosenbloom N, Shields C, Briegleb B (2012) Vegetation-climate interactions in the warm mid-Cretaceous. Climate of the Past, 8, 560–576.