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Microbial Vesicle-Mediated Communication: Convergence to understand interactions within and between domains of life

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Environmental Significance Statement

Cells secrete extracellular vesicles (EVs), nanoscale biological packages that contain complex mixtures of molecular cargo. The multiple roles of microbial EVs include their function as carriers for molecular messengers that facilitate interspecies communication and have been studied extensively in mammalian systems. For environmental systems, however, the prevalence, characteristics, and functions of these biological particles are only now being revealed. Here, we argue that the study of microbial EVs in the environment requires biochemical insights from studies of donor and receiving organisms as well as knowledge of soft colloid mobility and interactions with other components of the environment. Such questions of EV function, transport, and environmental impact can be addressed best by harnessing theories and methodologies developed by the biological, colloid, and geochemical sciences.

Abstract

All cells produce extracellular vesicles (EVs). These biological packages contain complex mixtures of molecular cargo and have a variety of functions, including interkingdom communication. Recent discoveries highlight the roles microbial EVs may play in the environment with respect to interactions with plants as well as nutrient cycling. These studies have also identified molecules present within EVs and associated with EV surfaces that contribute to these functions. In parallel, studies of engineered nanomaterials have developed methods to track and model small particle behavior in complex systems and measure the relative importance of various surface features on transport and function. While studies of EV behavior in complex environmental conditions have not yet employed transdisciplinary approaches, it is increasingly clear that expertise from disparate fields will be critical to understand the role of EVs in these systems. Here, we outline how the convergence of biology, soil

geochemistry, and colloid science can both develop and address questions surrounding the basic principles governing EV-mediated interkingdom interactions.

Introduction

In every ecosystem, at all scales, life forms communicate constantly with each other and their environment, often using secreted components to react to, affect, and exchange information about their surroundings. These interactions ultimately shape higher order organismal and ecosystem function. Combinations of chemical, physical, and biological processes govern this interkingdom communication and, as such, these processes can be understood only through a synergistic combination of methods and insight from multiple disciplinary perspectives. Indeed, with theory and methodology from just one field, it would be impossible to develop or investigate critical questions about these complex interactions in the context of environmental systems. Despite the obvious need for collaboration, thus far studies of interkingdom communication have remained relatively constrained to singular or similar disciplines. This predicament is exemplified in studies of extracellular vesicles (EVs).

EVs are nanoscale proteoliposomes that are secreted by all forms of life and are a ubiquitous part of every environment (1, 2). Compared to other cellular secretions, EVs uniquely enable the simultaneous interaction of a broad mixture of molecular cargo embedded and enclosed within the EV with the surroundings (2-8). These biologically complex nanostructures present a convergence challenge since a complete understanding of their function requires expertise from an array of disciplines. Here, we discuss how a multidisciplinary approach can address this challenge in bacterial EV studies.

To date, most bacterial EV studies focus on their activities in mammalian systems (9, 10) whereas EV research in environmental systems is still emerging, with only a few studies considering EV's roles in microbial communities and plant systems (11-16). Despite the recent expansion of EV research into plant systems, interactions between EVs and surrounding soil systems remain under-investigated. Our understanding of EVs in complex conditions can be expanded by investigating the soil rhizosphere environment. Rhizosphere ecosystem functions rely on interkingdom interactions, soil composition, primary production, and major element cycling in natural and managed terrestrial landscapes. Thus, understanding EV roles in this complex environment requires a rich area for interdisciplinary collaboration (Figure 1). Substantial advancement in understanding the role of EVs in the environment will require answers to fundamental questions: What are the relevant characteristics of EVs to relate their biogenesis, transport, and function? How do these change in response to environmental conditions? How do these changes impact ecosystem function?

Having diameters as small at 20 nm, many EVs may be considered naturally occurring biotic nanoparticles as nanomaterials are defined as any material that possesses at least one dimension between 1-100 nm. Questions tackled under the umbrella of the environmental health and safety of nanomaterials (nanoEHS) exemplify the advantages of a convergence approach to science. Here, findings rooted in areas such as colloid science, environmental biogeochemistry, ecology and toxicology provided theoretical frameworks and methods to inform first principles investigations (17). NanoEHS research has highlighted the fact that environmental conditions surrounding nanoscale and microscopic materials control their surface chemical properties and subsequently can govern their transport and attachment to other surfaces (18). Intriguingly, linking EV properties to findings in NanoEHS research, recent work on biological EVs has revealed how function relies on properties of the EV exterior for transport and attachment to other surfaces, and that the environmental context of the cells governs what is exposed on the EV surface (14, 19-22). We anticipate that an intersection of disciplinary domains will generate clarity and novel concepts in the understanding of the roles and activities of EVs.

Significant new understanding of EV transport, fate, and function could be gained by studying biological phenomena of EVs in the context of nanomaterial and biogeochemical processes in the environment. Here we introduce some foundational and emerging insights that will contribute to our transdisciplinary approach to understanding, predicting, and ultimately harnessing EV-mediated interkingdom communication in soils.

EV Surfaces, Cargo, and Biological Function

While EVs require considerable energy to produce, they also play important roles in inter-kingdom communication (10, 23-26). EVs include membrane vesicles, outer membrane vesicles, and exosomes, among other similar structures (Figure 2). In microbial systems, EVs bud from the outermost cell membrane and are packaged with specific cargo (9, 27-30). In mammalian and potentially plant systems, EVs can be produced by budding from the cell membrane or through pathways originating at intracellular, multivesicular bodies (31-34). While there are innumerable differences in the generation, composition, and biological function amongst these different types of vesicles, many overarching similarities exist, enabling high-level comparisons of disparate EV populations.

The universal concepts of EV functional features that are governed by their surface composition and cargo can be seen using Gram-negative bacterial EVs as a case study. Termed outer membrane vesicles (OMVs) based on their bacterial outer membrane origin, these EVs are typically much smaller (20-200 nm in diameter) than eukaryotic EVs (20-1000 nm in diameter) (Figure 2). Their cargo of enriched, depleted, and "bulk flow" amounts of cellular envelope components relative to the producing cell reveal that they are products of a regulated secretory process (9, 35-37). OMVs are secreted for a variety of reasons, including secretion of misfolded proteins, membrane remodeling, nutrient acquisition, communication, and as decoys for phage and antibiotic molecules (9, 38, 39). Additionally, host-pathogen studies show that bacterial EVs can deliver cocktails of virulence factors into host cells and activate host immune responses (5, 28, 40-42). Importantly, bacterial EV-elicited immunogenicity has been exploited for use in vaccine development (38, 43).

A few studies have begun to elucidate bacterial EV function in plant systems or the natural environment (13, 44). Just like all other bacteria, phytobacteria produce EVs that have the potential to interact with plants in the phyllosphere and rhizosphere. Several phytobacterial EV proteomes have been characterized, revealing that these EVs contain numerous metabolites as well as virulence factors (16, 45-48). Additionally, some of this cargo, such as cellulases and xylanases, retains its ability to digest plant cell wall components (45, 49). As in mammalian systems, bacterial EVs also activate plant immune responses. EVs from several different bacterial plant pathogens activate general plant innate immune responses including a reactive oxygen species burst and transcription of pattern recognition receptors (11). This work also showed some dependence on the co-receptors BAK1 and SOBIR1 for EV-mediated immune activation, though a complete mechanism has not yet been identified (11). Recent studies extended these initial findings to demonstrate that EVs from the model plant pathogen *Pseudomonas syringae* activate plant immune responses including *ICS1* transcription and salicylic acid production that result in protection from future pathogen attack (15, 16). Furthermore, it was shown for the first time that EVs from plant beneficial bacteria such as *Pseudomonas fluorescens* also lead to protection from pathogen attack, though they do so through plant immune pathways different from those activated by plant pathogen EVs (15). Together, these findings add a new layer of complexity to plant-microbe interactions and reveal a previously unstudied role for EVs in the environment, likely highlighting only a very small part of EV's overall functional contributions.

Surface properties mediate the physical interactions between bacterial EVs and the plant host and consequently can substantially impact the host-pathogen relationship. OMV surfaces consist of a subset of outer membrane components, lipopolysaccharide, phospholipids, membrane proteins, and associated small molecules and metal ions (9, 35). This composition mediates the ability of bacterial EVs to adhere to plant surfaces, which contributes to increased bacterial spread and virulence (14, 21, 22, 50). For example, OMVs from the pathogenic bacterium *Xylella fastidiosa* adhere to xylem cell walls, limiting the ability of the *X. fastidiosa* cells to attach and form bacterial communities (biofilms), and facilitating spread throughout the xylem, which leads to increased infection of plant tissue (14). Additionally, secreted hydrophobic molecules are found in association with OMVs along with lipases, esterases, and other cargo that could aid in plant cell wall degradation and virulence (21, 22). OMV surface components may also confer a particular charge, which could result in attraction to or repulsion from plant cell wall components as well as components of the phyllosphere and rhizosphere environment. While early studies reveal the potential of bacterial EVs to adhere to surfaces, future work incorporating EV charge and surface composition should be useful to predict transport and fate in biological systems.

In the natural environment, microbes must adapt to chemical and physical changes. A notable role for EV production in microbial adaptation to changing environments is highlighted from studies of *X. fastidiosa* OMVs (14). *X. fastidiosa* is transmitted via sharpshooter insect vectors and, therefore, survives by interacting differently with surfaces in two very different environments: the insect gut and the plant xylem (51, 52).

In the insect, the bacterium must adhere tightly to the host to withstand significant sheer force as the insect feeds and sap flows through its mouth parts (14, 53, 54). In contrast, if the bacteria attached firmly to the xylem cell wall, it would be unable to spread throughout the host plant (14). Thus, EV production presents an opportunity through which *X. fastidiosa* modulates its attachment in response to its environment (14, 22). Several other reports further document the use of EVs as microbial tools for responding to environment change (55-58).

These studies have revealed several governing properties of EV function in complex systems within the phyllosphere – namely, that cargo within the EV and associated with the EV surface influences interactions with hosts and attachment to biological surfaces, and that EVs play a role in survival of the producing cell during environmental transitions. The same factors likely play a role in the context of the soil rhizosphere, influencing how EVs interact with and are changed by plant roots, soil particles, nutrients, and groundwater. It remains largely unknown what factors govern EV packaging and release into the environment, and how EV cargo may affect the abiotic or living constituents in the environment. These questions pertain not only to the rhizosphere, but also to other habitats that harbor active microbial communities.

EVs in the Context of Element Biogeochemical Cycles

Within the emerging understanding of EV characteristics and biological function, the roles of EVs in environmental processes remain at an early stage of exploration. Nevertheless, evidence is mounting that EVs could influence the soil rhizosphere, for example by participating in the biogeochemical cycling of major and trace elements (59-64). Microbes play critical roles in nutrient cycling by inducing changes to various soil elements through processes like nitrogen fixation. More specifically, it is the membranes of the microbes that are critical in performing a variety of these critical functions, including sequestering limited nutrients, such as iron and trace metals, from the environment for delivery into the cell, and contributing to the role microorganisms play by regulating the concentrations and availability of soil constituents (63, 65-68). Membrane properties of bacteria and their secreted EVs are similar, as many of the same molecules found in the outermost microbial membrane are also found in EVs (19, 35, 69, 70). Therefore, it is important to consider whether EVs may play supplementary, complementary, or redundant roles in biogeochemical regulation and cycling. As with other biological functions, the production of EVs with such functionalities entails great energetic costs for microorganisms in terms of expelling macromolecular complexes, especially when compared to similar cellular processes enabled by membrane-bound proteins at the cell envelope (61, 63). Thus, the concept of EVs with scavenging and element cycling functionalities needs to be investigated in the context of competitive advantages compared to parent cell capabilities.

EV involvement in element biogeochemical processes has recently been revealed in several microbial species. For example, *Geobacter sulfurreducens* produces EVs with membrane proteins capable of extracellular electron transfer (62), a process known to occur at the cell envelope of the parent organism. Likewise, the pathogen *Mycobacterium tuberculosis* produces EVs packaged with membrane-bound siderophores that have high affinity to bind iron, similar to those associated with the cell envelope (61). These EVs are produced under iron-limited conditions as a mechanism to sequester the metal and deliver it to the EV-producing cell or a nearby cell of the same species (61). Similar phenomena for acquisition of sparingly soluble elements also occur in soil-relevant microorganisms such as *Pseudomonas aeruginosa*, which uses EVs as an intermediate step in iron acquisition (63).

EVs also contribute to biogeochemical cycling of carbon and major substrate nutrients. For example *Prochlorococcus* species, a ubiquitous marine cyanobacterium that is responsible for a notable proportion of Earth's photosynthetic activity, produce EVs in abundances that are 1 to 10 times the number of whole cells in sea water (64). These EVs are able to sustain and promote the growth of heterotrophic bacteria in culture, indicating the potential of EVs to function as an intermediary of ecological carbon transfer in surface oceans. Additionally, EVs play a critical role in the valorization of lignin, a particularly recalcitrant sink of the terrestrial carbon cycle (71).

In addition to EV roles in nutrient cycling, EVs may also play a role in physically altering the mineral phases surrounding the parent organisms as a means for promoting microbial survival. For example, heterotrophic bacteria isolated from a black shale deposit in southwestern Poland produce EVs that are the primary component of biofilms and sites of phosphate, carbonate and sulphate mineral precipitation (60). Mineral particles containing Cu, P, Mg, Si, Al and Ca were observed to form within vesicles from these organisms, suggesting an active and targeted role for vesicles in element cycling in geological deposits (60). Similarly, *Shewanella oneidensis* reduces soluble uranium species, which leads to formation of sparingly soluble uraninite mineral precipitates (59). EV production in this species functions as a protective response for the cells by enabling them to shed these uraninite crusts that would otherwise inhibit overall cellular function (59).

Whereas such studies highlight the roles EVs play in soil environments with respect to element cycling, mineral interactions, extracellular electron transfer, and nutrient sequestration, EVs are generally considered the alternative to better-known processes that occur directly at the cell envelope. Thus, the relative importance of EVs in these functions remains unknown. Evaluation of EV contributions to interactions with the organism's surroundings therefore should include comparisons to the cell-bound processes that accomplish the same function for the organism. As-yet-unappreciated advantages to functionalizing EVs include prolonged environmental persistence and increased distribution of these nanoscale particles compared to EV-producing cells. Uncovering principles governing EV mobility within the environment as well as how environmental conditions affect that mobility will be essential to develop a comprehensive understanding of how EVs impact biogeochemical processes. In the future, our understanding of the roles EVs play in these processes and where they act may provide opportunities to engineer or manage the soil environment for enhanced plant productivity among other desirable outcomes.

EVs as Colloids: Transport and Attachment

From a biophysical perspective, EVs can be considered colloids: small particles (typically less than 1 μm) dispersed in liquid medium. While their complex and sometimes multi-tasking functionalities have begun to be revealed, the colloidal properties of EVs have thus far received relatively little attention. EVs likely have similar properties to those of more simplistic phospholipid vesicles; however, given their complex composition, EVs may exhibit unexpected colloidal properties (72). Some of this work has been conducted in the context of soft matter particles, using for example quartz crystal microbalance measurements to understand lipid particle attachment (73, 74). Although not a direct comparison to EVs, these reports do suggest that both the surfaces of lipid particles and the surrounding environmental conditions influence the fate and persistence of these particles, specifically relating to attachment and possibly transformation or uptake as well. Studying EVs holistically in the context of their surrounding environment (i.e. as a collection of particles in a complex system) will provide fresh insights into vesicle fate and function (27, 29, 75). To do this, phenomena understood for colloid behavior may be helpful in predicting what governs the fate of EVs on their journey from parent cell through their surrounding environments.

Particle transport depends on size, i.e. smaller particles will be transported differently than larger ones. In particular, nanoparticles tend to be more sensitive to thermal forces, and thus Brownian motion, while larger particles tend to be more sensitive to shear or gravitational forces (76). Analogously, we predict that EV transport will be distinct from that of cellular colloids. Furthermore, as described above, EVs and EV-producing cells have distinct compositional and surface characteristics, implying that the chemical as well as the physical factors controlling particle transport will be different for EVs compared to their parent cells. While the physical transport of particles based on size is well-studied (77), knowledge of the biochemical composition of EV surfaces has rarely been directly connected to their mobilization and deposition potential. Hence, the determination of a metric to characterize surface chemistry is necessary to properly evaluate EVs as colloidal particles.

An additional consideration is that all types of particles, including biological particles (78-103), may undergo aggregation or deposition in environmental and physiological systems. Both aggregation and deposition can be considered two-step processes where largely physical phenomena transport particles to the vicinity of a surface (including another particle) and near-field chemical factors (i.e. chemical factors that are only experienced by a particle when in close proximity of the surface in question) determine whether particle attachment occurs. For example, if ten collisions of a particle with a surface result on average in only one particle attaching, the attachment efficiency of that particle would be 0.1. Therefore, the attachment (or sticking) efficiency of a particle (α) reflects its relative affinity for the surfaces it encounters. The α value of small particles such as colloidal EVs may predict their tendency to disperse in environmental systems. This concept has already been applied to bacterial systems, where simple models for colloid deposition have been applied to describe bacterial deposition in porous media (104-106).

Beyond particle attachment and transport into organisms, the attachment efficiency potentially also determines particle absorption, distribution, metabolism, and excretion behavior in organisms. This suggests that attachment efficiency could also determine these behaviors in EVs, as has been observed in the case of engineered nanoparticles (ENPs) When examined from a colloid chemistry perspective, this may lead to a wide range of environmental and biological interactions (107) that include bio-uptake (ENPs entering into cells or organisms) (108), biomagnification (ENPs observed at greater concentrations farther up the food chain) (109) trophic transfer (ENPs transferred up the food chain) (110) and maternal transfer (ENPs taken up by one generation of organism and passed on to the next generation) (111, 112). It is also possible that EVs could exhibit prolonged persistence in the environment compared to the EV-producing cell, which could result in accumulation at high levels and have unexpected implications for ecosystem function. Although we have yet to measure EV persistence in environmental conditions, EVs are remarkably stable when exposed to extreme conditions and physiological stress (15, 113-116), lending to possible biouptake or trophic transfer. Furthermore, EVs commonly possess genetic material which can propagate throughout environmental systems or food chains (117-123). Although this is not a direct colloid interaction, attachment could be the first step to allow for the genetic disruption that would then influence ecosystems indirectly through biomagnification or trophic transfer.

As noted above, complex and variable surface properties mediate EV attachment to biological surfaces, which is critical for bacterial EV interactions with plant hosts. While theoretical models for the attachment of particles to surfaces (124-126) provide useful guidelines for overall trends, quantitative predictions for the deposition of both mineral and biological particles are hampered by complexities such as surface heterogeneity and interactions with solutes and adsorbing molecules. As a result, evaluation of the attachment efficiency, α, in these systems has been largely empirical, relying on either column deposition studies (127-129) or, more recently, batch aggregation studies (130-132). The latter method is particularly well-suited to determining attachment efficiency in complex systems, which allows for direct parameterization of models to predict particle fate and serves as a “functional assay” to describe a wide range of behaviors in complex systems (133). For example, one study used laboratory determinations of values for α to predict the fate of ENPs in simulated wetlands using a simple transport model (134). The approach has also been applied to systems ranging from activated sludge (131) and soils (130) to river basins (135). As a functional assay, the parameter α encompasses a large number of underlying variables ranging from temperature, pH, and van der Waals interactions, to steric repulsion, ion strength and surface composition. Hence, α allows us to account for multiple variables in a single parameter, making this sort of assay amenable to predicting outcomes in complex systems.

Applying measurements of particle attachment behavior to EVs in the rhizosphere demonstrates the potential strength of combining theory, expertise, and methodology from colloid science, biology, and soil geochemistry to predict the fate of EVs in the environment. The resulting insights may even help us to retroactively relate functional assay-derived transport properties of EVs to their biological properties and impacts by connecting biological function with resultant surface properties and thus delivery. This systems-level understanding can only be achieved by thorough integration of our otherwise discrete fields.

Discussion

Understanding how cells communicate and impact one another across different kingdoms and across physical space within complex natural environments such as the rhizosphere presents significant research challenges. Scientific approaches in the seemingly disparate areas of biology, geochemistry and colloid theory have each independently developed a deep understanding of fundamental concepts pertinent to this undertaking. For example, soil biogeochemistry studies have characterized fundamental aspects of nutrient cycling, colloid science research has developed models to predict transport of nanomaterials, and the fields of molecular biology and biochemistry have revealed EV functionalities in simplified systems. Harnessed together, these initial findings and understanding will allow us to describe and make predictions about physiologically relevant EV properties, transport, and function in the rhizosphere. This focus on a shared thread of inquiry is a proven core to successful convergence research (136).

Convergence enables the ability to take advantage of available experimental strategies across our represented disciplines. However, multi- and transdisciplinary dialogues thus far have already revealed new challenges, highlighting the need to develop novel methods and approaches.

For instance, in transitioning colloid chemistry methodologies from NP applications to the characterization of EVs, methodological challenges arise. First, EVs are comprised primarily of organic biomolecular 'soft' materials. Therefore, in many environmental systems, EVs are difficult to differentiate from other material in the soil matrix, presenting a significant barrier to many traditional methods for colloid study. Second, the ability to validate proposed models of EV transport is severely limited by a lack of existing data. Notably, these two categories of challenges – the obfuscating nature of the environment and a paucity of data - were also among the first hurdles within the convergent field of nanoEHS. We anticipate that solutions adapting approaches across fields may prove useful both for detection and validation of EV behavior in complex systems. For example, using biochemical techniques to fluorescently label lipids or proteins in EVs, or using genetic techniques to tag particular EV cargo may benefit our colloidal studies of EVs by facilitating transport, aggregation, and deposition measurements.

Similarly, in biological studies of EVs there is a notable need to track these nanoscale packages and predict their movement and function in various environments. Using modeling principles from colloid science and a detailed understanding of soil composition from soil geochemistry, we may be able to predict which conditions will be most interesting and environmentally relevant to test experimentally and which cargo play critical roles in transport and function. Specifically, in the context of the rhizosphere, soil biogeochemistry has developed some understanding of how microbes and, perhaps, EVs play active roles in element cycling and biogeochemical processes.

EVs may function differently than microbes in these processes due to their smaller size, relative increase in surface area, and potential to travel further and persist longer in soil. Modeling techniques from colloid science and molecular characterization from biological studies will significantly advance understanding based on biogeochemical approaches alone by providing the necessary variables to define functional mechanisms. Transdisciplinary approaches to the design stage as well as in driving analyses will be important so that models may be informed appropriately and can be validated experimentally. This convergent approach could solve a common problem in biological experimentation where the number of conditions, controls and variables needed to address increasingly complex questions exceeds that which is possible or practical in a wet-lab environment.

Another major challenge in the EV field arises from technical limitations of purification methods. Current approaches for and limitations of EV purification from mammalian, bacterial, and plant cells have been discussed recently (43, 137-143). The most common purification methods involve sequential ultracentrifugation and filtration steps to pellet EVs followed by density purification to remove contaminants. One notable limitation of this approach is the inability to separate EVs based on their molecular cargo. Although some techniques like affinity purification can isolate groups of EVs that all contain one similar cargo protein, currently there are no methods to isolate EVs with the same complex mixture of cargo. A convergent approach to EV research could reveal new ways to isolate EVs based on cargo or surface properties. Alternatively, using the techniques from multiple disciplines may reveal new ways to track and measure EVs either individually or as collective populations that bypass the need to isolate specific populations of EVs. For example, perhaps discrete populations of EVs could deposit in sediment and percolate to deeper layers, while others stay more on the surface of sediment, stay suspended in a water body, or are even taken up by various organisms preferentially (108, 144, 145). EV research could be extended to current studies in mesocosms (simulated and controlled environmental systems) to evaluate whether these differences exist and the extent to which they could be used to isolate various EV populations.

Considering the challenges of studying EV-mediated interkingdom communication from three different perspectives reveals many questions that are now possible to explore through shared and co-developed methodologies (Figure 1). For example, which vesicle properties affect surface chemistry and thus the fate and persistence of EVs in relevant environments? The transport and attachment portion of the EV journey during interkingdom communication requires an understanding of how vesicle surface composition (and variations in surface composition) impact surface charge, steric interactions, and other colloidal properties of EV suspensions. Biology, chemical biology, and analytical chemistry, among other related disciplines, can provide the tools needed to identify and characterize these surface-associated molecules and inform transport models. Further, we wonder how variations in physical properties of a larger scale, like size or shape, influence the mechanisms by which EVs are transported. Combining the predictive power of colloid modeling with the ability to test these predictions experimentally presents an excellent opportunity to move all three fields forward.

An improved understanding of EV-mediated interkingdom communication in the rhizosphere would inform a myriad of future applications. Depending on EV persistence and transport profiles, potential agricultural applications might include naturally derived protective sprays, irrigation additives, or seed treatments to reduce disease incidence either by boosting plant immune responses or by interacting directly with the pathogens. Naturally produced or engineered EVs could potentially also be used to improve nutrient uptake in plants by packaging macro- and micro-nutrients in more bioavailable forms. Importantly, results from studies of naturally produced EVs and their functions will likely reveal specific cargo that results in desirable interactions and properties. This cargo could then be added to artificial lipid vesicles to improve their functionality. EVs may also prove useful as biomarkers for a variety of conditions, for example in crop disease and soil quality, as has been shown analogously in mammalian systems (31). For environmental management, a thorough understanding of EV composition, biogenesis, fate, and transport may enable optimal compositions of ENPs that deposit specific nutrients or that sequester heavy metals and other contaminants. While these applications have direct implications for agriculture and environmental management, studies of the properties governing EV function and fate will also impact our understanding of EVs in mammalian systems as well as microbial processes and complex microbial communities. The combination of theory and methods at the intersection of biology, colloid science, and geochemistry shows significant potential to advance our understanding of EV composition and function as well as potential to jumpstart a variety of environmentally, agriculturally, and economically important applications, thus providing an example of impactful convergent research.

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Declaration of Interests

The authors declare no conflicting interests.

Figure Legends

Figure 1. Convergence to address EV-mediated inter-kingdom communication. A schematic describing the different compartments of the natural environment in which EVs may be found, and the questions associated with the roles of EVs in these compartments. Top Left. How do EVs released by microorganisms influence the function of both plants and other microorganisms in the natural environment? Bottom left. How do EVs interact with leaf and root cells? Top right. In what ways do time and environmental stressors affect EV viability? Middle right. What roles do EVs have in

inter-microbial communication? Bottom right. How can EVs impact nutrient cycling in the environment?

Figure 2. "Extracellular vesicle" encompasses a variety of secreted cellular structures. Although we focus on microbial EVs throughout this perspective, the general term "EV" can refer to a wide array of vesicle structures. In general, terminology for EVs is determined according to their size and mechanism of biogenesis. This schematic depicts the various routes of EV biogenesis from mammalian and microbial cells. A detailed depiction of mammalian EVs, including specific cargo, can be found in recent reviews (2, 146-149). Depictions of cargo in EVs from bacterial and fungal species are also available (9, 38, 40, 140, 150-154). A) Top: Gram-negative bacteria produce outer membrane vesicles (OMVs) and outer inner membrane vesicles (OIMVs). Bottom: Explosive outer membrane vesicles can also result from explosive cell lysis. OM: Outer Membrane. PG: Peptidoglycan. IM: Inner Membrane. B) Gram-positive bacteria produce cytoplasmic membrane vesicles (CMVs). Fungal cells produce EVs presumably via a similar biogenesis pathway. C) Left: Mammalian cells produce a variety of EVs including exosomes, exomeres, microvesicles, migrosomes, and oncosomes. EE: Early Endosome. Lys: Lysosome. MVB: Multi-vesicular Body. ER: Endoplasmic Reticulum. Right: Apoptotic bodies are formed when mammalian cells undergo apoptotic cell death. Size in nm indicates diameter.

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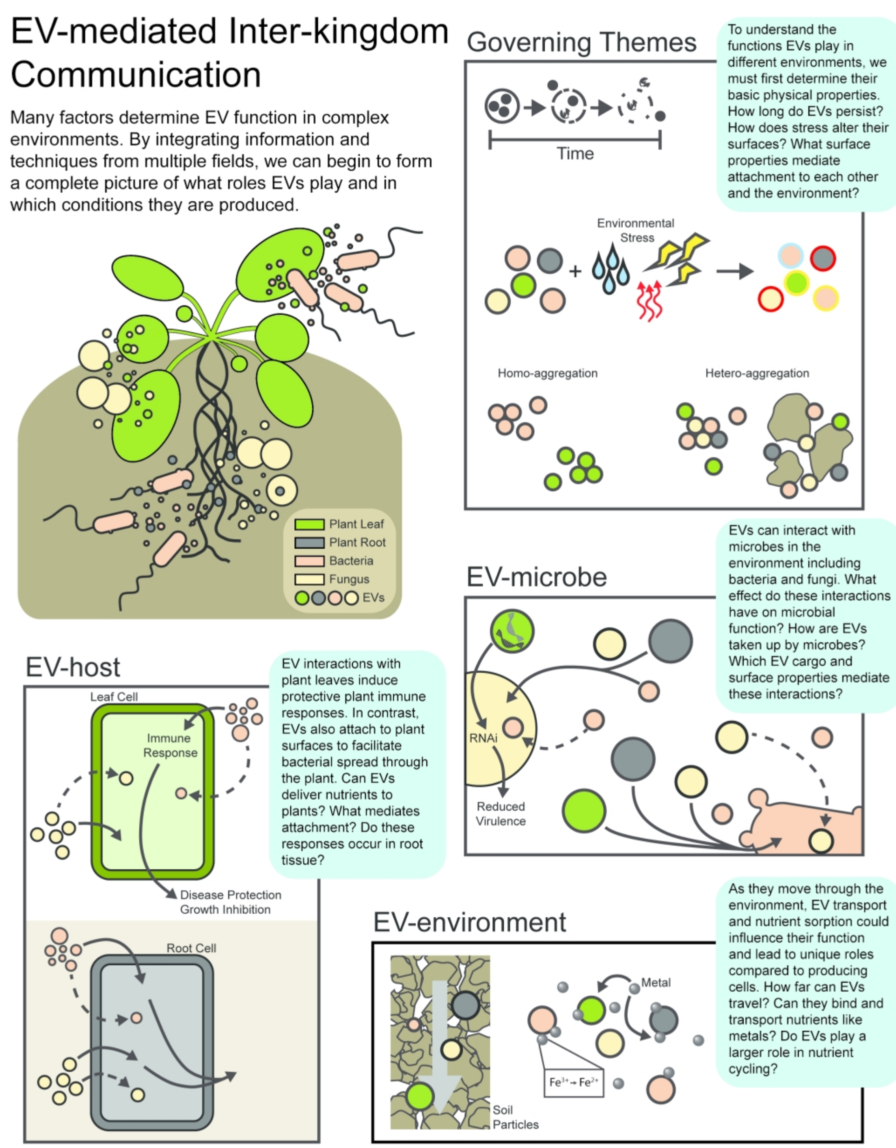
Figure 1



Caption : Figure 1. Convergence to address EV-mediated inter-kingdom communication. A schematic describing the different compartments of the natural environment in which EVs may be found, and the questions associated with the roles of EVs in these compartments. Top Left. How do EVs released by microorganisms influence the function of both plants and other microorganisms in the natural environment? Bottom left. How do EVs interact with leaf and root cells? Top right. In what ways do time and environmental stressors affect EV viability? Middle right. What roles do EVs have in inter-microbial communication? Bottom right. How can EVs impact nutrient cycling in the environment?

Figure 2

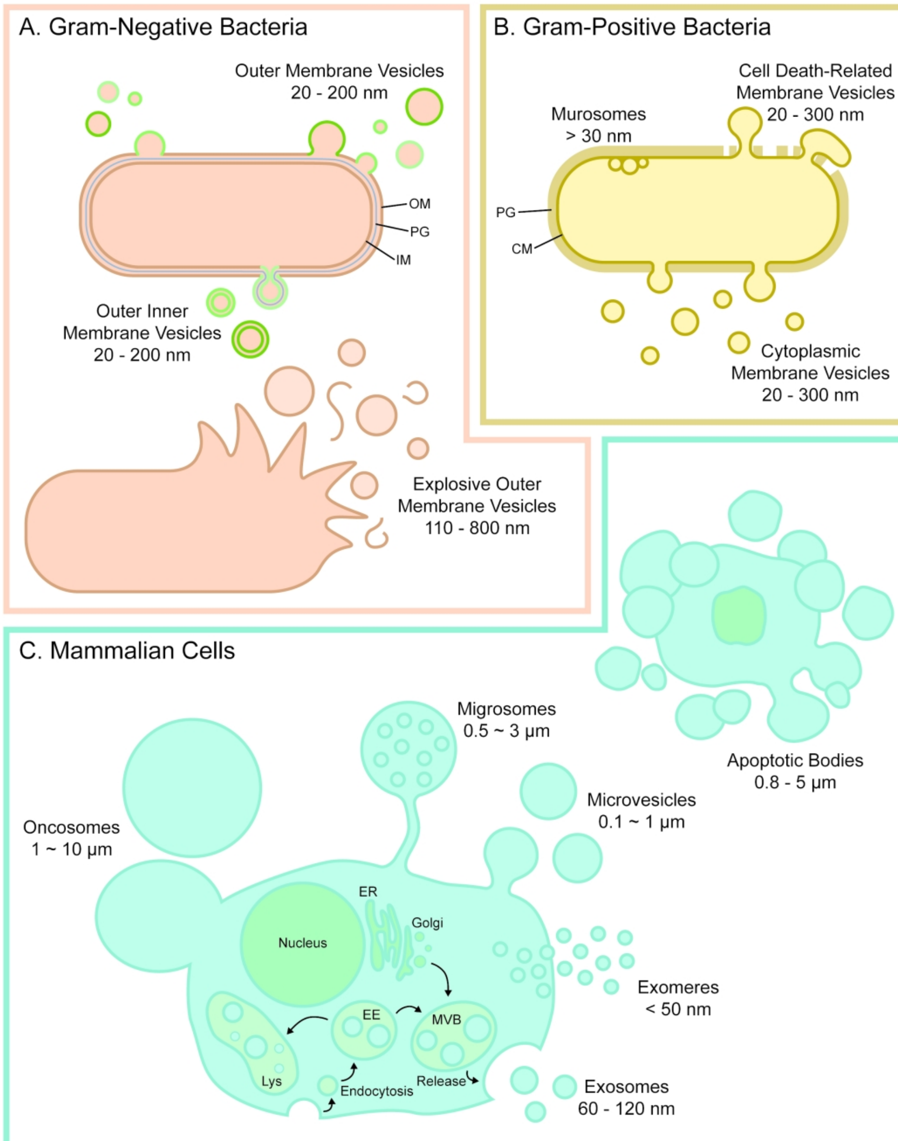


Figure 2. “Extracellular vesicle” encompasses a variety of secreted cellular structures. Although we focus on microbial EVs throughout this perspective, the general term “EV” can refer to a wide array of vesicle structures. In general, terminology for EVs is determined according to their size and mechanism of biogenesis. This schematic depicts the various routes of EV biogenesis from mammalian and microbial cells. A detailed depiction of mammalian EVs, including specific cargo, can be found in recent reviews (2, 146-149). Depictions of cargo in EVs from bacterial and fungal species are also available (9, 38, 40, 140, 150-154). A) Top: Gram-negative bacteria produce outer membrane vesicles (OMVs) and outer inner membrane vesicles (OIMVs). Bottom: Explosive outer membrane vesicles can also result from explosive cell lysis. OM: Outer Membrane. PG: Peptidoglycan. IM: Inner Membrane. B) Gram-positive bacteria produce cytoplasmic membrane vesicles (CMVs). Fungal cells produce EVs presumably via a similar biogenesis pathway. C) Left: Mammalian cells produce a variety of EVs including exosomes, exomeres, microvesicles, migrosomes, and oncosomes. EE: Early Endosome. Lys: Lysosome. MVB: Multi-vesicular Body. ER: Endoplasmic Reticulum. Right: Apoptotic bodies are formed when mammalian cells undergo apoptotic cell death. Size in nm indicates diameter.