Opinion

From seed to seed: the role of microbial inheritance in the assembly of the plant microbiome

Ahmed Abdelfattah,1,2,3,* Ayco J.M. Tack,2,@ Carolina Lobato,3,@ Birgit Wassermann,3 and Gabriele Berg1,3,4,@

Despite evidence that the microbiome extends host genetic and phenotypic traits, information on how the microbiome is transmitted and maintained across generations remains fragmented. For seed-bearing plants, seeds harbor a distinct microbiome and play a unique role by linking one generation to the next. Studies on microbial inheritance, a process we suggest including both vertical transmission and the subsequent migration of seed microorganisms to the new plant, thus become essential for our understanding of host evolutionary potential and host–microbiome coevolution. We propose dividing the inheritance process into three stages: (i) plant to seed, (ii) seed dormancy, and (iii) seed to seedling. We discuss the factors affecting the assembly of the microbiome during the three stages, highlight future research directions, and emphasize the implications of microbial inheritance for fundamental science and society.

Introduction

Spermatophytes (seed plants) have evolved as an adaptation to the terrestrial lifestyle during the late Devonian period. Seeds served as the plant’s means of survival during harsh periods, facilitated dispersal across the landscape, and provided plant embryos with the protection and nourishment necessary to withstand external disturbances, especially desiccation. During the evolution of seed plants, plants formed symbiotic relationships with microorganisms, which facilitated their establishment on land and subsequent evolutionary divergence [1,2]. Seeds themselves contain diverse microbial communities, which are present within their tissues (seed endophytes) and on their surface (seed epiphytes) [3–12]. The existence and function of the seed microbiome, however, has remained unnoticed for decades [13–15]. The first suggestions regarding the presence of microorganisms in seeds came from studies that focused on pathogenic fungi, giving the impression that seeds free of microorganisms reflected higher seed quality. This gave rise to the widespread use of physical and chemical treatments of crop seeds. These activities, on the one hand, resulted in loss of some microbial taxa and, on the other hand, might make plants more vulnerable and dependent on fertilizers and pesticides to compensate for the lost functions of the natural seed microbiome [9,16–18]. Early evidence that seeds harbor beneficial microorganisms was described in a study on the symbiosis between endophytic fungi of the family Clavicipitaceae and tall fescue Festuca arundinacea [19,20]. These findings were pivotal in changing our view on seed-borne microorganisms and altered the notion that seed sterilization processes would necessarily result in a healthier crop [3,20]. Since then, seed microbial communities – including fungi, bacteria, and archaea – have been repeatedly shown to be a reservoir of specialized microorganisms [9]. Currently it is evident that seeds contain mainly plant-beneficial microorganisms [21] – many of which are involved, directly or indirectly, in fundamental physiological processes.

Highlights

The microbiome has a strong influence on its host. With evidence of microbial inheritance mounting, we might extend the concept of host evolutionary potential to include the microbiome.

Seeds play a unique role in the inheritance process by linking plant generations and represent a bottleneck in the continuity of the plant microbiome.

Plant microbial inheritance encompasses three stages: plant to seed, seed dormancy, and seed to seedling. Each stage is governed by different processes.

Although research on the seed microbiome is still at its infancy, it has the potential of advancing our understanding of the natural world, with implication in fundamental and applied sciences and society.

1Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Max-Eyth Allee 100, 14469 Potsdam, Germany
2Department of Ecology, Environment and Plant Sciences, Stockholm University, Svante Arrhenius väg 20A, Stockholm, SE-106 91, Sweden
3Institute of Environmental Biotechnology, Graz University of Technology, Petersgasse 12, Graz 8010, Austria
4Institute for Biochemistry and Biology, University of Potsdam, 14476 Potsdam OT Golm, Germany
processes, such as seed dormancy and germination, environmental adaptation, resistance and tolerance against diseases, and growth promotion [22–26]. Yet, compared to other plant compartments, such as the phyllosphere and rhizosphere, studies on the seed microbiome are lagging.

Previous reviews have comprehensively summarized knowledge on the seed microbiome and its origin [7,11,27,28], the processes of vertical transmission and horizontal acquisition [29,30], and the importance of the seed microbiome for plant health [7,9,31,32]. In this Opinion, we focus on microbial inheritance, and define it as a process that encompasses vertical transmission and the subsequent migration of seed microorganisms to the new plant. We thereby divide the inheritance process into three stages: (i) from plant to seed, (ii) seed dormancy, and (iii) from seed to plant. We focus on seed endophytes, as those are likely to be inherited and are expected to have an intimate host–microbe relationship. However, in cases where literature on endophytes is lacking, we refer to studies that include both endophytes and epiphytes. We follow the journey of the microbiome from plant to seed to plant, discuss the factors affecting the assembly of the microbiome during the three stages, and highlight future challenges and promising research directions.

Seed microbial diversity and function
The diversity of the seed microbiome greatly varies across plant lineages, from a handful to thousands of microbial species [33]. The variation in diversity among plant species can be explained in large part by plant speciation, domestication, and plant breeding, which have left a strong imprint on the seed microbiome [32,34,35]. For example, studies on phyllosymbiosis of the seed microbiome demonstrate that the distance between plant lineages and the composition of the microbiome are congruent [36]. The impact of domestication on the diversity of the seed microbiome seems to be specific for the plant lineage. For instance, domestication was shown to decrease the diversity of seed-associated microorganisms in wheat, whereas some domesticated cereals and rice harbor higher microbial diversity than their wild relatives [5,36]. Yet, reported differences in species richness of the seed microbiome might also depend on methodological differences. For example, several seed microbiome studies combine both epiphytes and endophytes while others focused on one or the other. Similarly, the number of seeds pooled to make one biological replicate was found to positively correlate with species richness detected in seeds [33]. That is, samples consisting of one seed showed lower microbial diversity compared to samples consisting of more seeds [12,37,38]. Regardless of the plant species and methodology, it is a general pattern that seeds harbor less microbial species compared to other plant compartments, sometimes by up to 100-fold [12]. This drastic reduction in species richness from adult plant tissue to seeds is considered a major bottleneck in the continuity of the plant microbiome from one generation to the next [12,39–41].

Despite the variation in species richness among seeds belonging to different species, some microbial groups are commonly found in seeds, and are collectively referred to as the seed core microbiome. These include members of the orders Capnodiales and Pleosporales for fungi, Pseudomonadales and Enterobacteriales for bacteria, as well as very low abundances of the phyla Thaumarchaeota and Euryarchaeota for archaea [6,42–46]. In a meta-study on 50 plant species, Cladosporium perangustum, Alternaria sp., Pantoea agglomerans, Pseudomonas viridiflava, and Pseudomonas fluorescens were found to be the dominant seed-associated taxa [33]. When assessing microbial function through the perspective of the plant’s fitness and survival, several functional characteristics were found to be congruent among seed endophytes. Those include phosphorus solubilization, nitrogen fixation, antibiotic, acetoin secretion, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, the production of growth-promoting hormones such as indole acetic acid (IAA), siderophore production to transport iron inside the plant, as well as the ability to suppress growth of plant-pathogenic microorganisms.
Interestingly, both members and functions of the seed microbiota are typical for plant-beneficial microbes [47–49].

**Microbial inheritance and transmission routes**

The relative stability of the plant microbiome across generations has only been recently brought to attention [50–54]. Seeds play a unique role by linking one generation to the next, thereby ensuring the continuous transmission of endophytes. The seed microbiome can, consequently, be viewed either as the apex of what was achieved during the dynamic assembly in the mother plant or as the starting point of what will be established in the developing seedling. In this Opinion, we define microbial inheritance as the direct transfer of microbial species from one plant generation to the next. In other words, microbial inheritance in Spermatophyta includes vertical transmission, the direct transfer of microbial species from parents to seed, and the migration of these species to the developing seedling [12]. Microbial inheritance excludes horizontal acquisition, where environmental and non-host microorganisms can colonize the host tissues [30]. Although the relative proportion and identity of species transmitted vertically to horizontally is largely unknown, studies indicate that most of the crop microbiome is assembled from the surrounding environment [4,30].

Microbial inheritance encompasses two consecutive yet distinct stages: from parents to seed and from seed to seedling. Additionally, since seeds of several plant species do not germinate immediately after maturation – either due to natural or induced dormancy – this period can be considered as a separate stage in the inheritance process. This argument is supported by the fact that the assembly mechanisms during these three stages will greatly vary. For instance, priority effects may play an important role during germination; however, this effect is expected to be minimal during dormancy. Similarly, the bottleneck effect in each of the three stages is governed by different factors. Therefore, a distinction among the three stages – that is, (i) plant to seed, (ii) seed dormancy, and (iii) seed to seedling – is important to fully understand the mechanisms of microbial inheritance (Figure 1). In the following section, we discuss each of these steps and the factors and mechanisms that govern the transmission of microorganisms, or lack thereof, from the host’s and the symbionts’ point of view.

**From plant to seed**

To understand the transmission of the microbiome from plant to seed, it is important to identify the possible microbial sources and their transmission route, as well as differentiating between inherited and acquired microorganisms. Sources of the seed microbiome can be categorized as originating from the (i) endosphere of the parents, (ii) the environment, and this is limited to atmospheric microorganisms (air and rain) since soil microbes have to first pass the parent filter, and (iii) pollinators, which could introduce foreign microorganisms to the developing seed through the flower. The transmission route of plant endophytes to seeds has been extensively reviewed and can be divided into two groups: sexual or asexual routes. The asexual route includes the vascular system and intracellular cavities connecting the maternal plant to the developing seed through the funiculus, chalaza, and micropyle (Figure 1A). The sexual route exclusively includes microorganisms transmitted via male and female gametophytes, which subsequently colonize the embryo and endosperm. The transmission through gametophytes, however, is only hypothesized based on studies showing that flowers, pollen, and ovary contain microbial species similar to those found in seeds. Neither the actual transmission during sexual fertilization nor the subsequent fate and distribution of these microorganisms has been documented. If proven true, we should expect variations in the seed microbiome assembly depending on plant mating systems, especially between uni- and bi-parental fertilization. In which case, one may argue that bi-parental or cross-fertilization should result in seeds with higher variability in their community composition compared to self-fertilized plants.
The role of aerial and rain dispersal, as well as dispersal with biotic vectors such as pollinators, in transmitting microbes to seeds is poorly understood as well (Figure 1B). Prado et al. assessed the impact of different pollination methods on the seed microbiome; the authors reported that honeybee visitations were the only method to successfully transmit bee-associated taxa to the seed [55]. In another study on wheat, maize, soy, and pepper, the persistence of bacterial inoculants along the plant’s life cycle was tested. Bacteria were sprayed either on flowers, or directly on mature seeds. Only when applied through the floral pathway, the next-generation seeds contained those bacteria [56]. The relative importance of the different transmission routes is still not elucidated and might differ among plant species. For instance, some plant species, such as radish, have a filtering mechanism that eliminates microorganisms that enter through the floral pathway during an early stage in seed development [37]. Those are then replaced by another set of species that originated from the asexual route. These reports indicate that the transmission from plant to seed is dependent on the compatibility between plants and their symbionts. Importantly, also the within-seed distribution of the microbiome is not random (Figure 1C), which has been
reported, for example, in oak, melon, spruce, and rice \[41,57,58\]. In the case of oak and melon, the authors showed that embryonic tissues, including the cotyledons, harbored more diverse microbial communities compared to the seed coat.

**Factors affecting the transmission from plant to seed**

Several biotic and abiotic factors are likely to affect the assembly of the seed microbiome. For instance, host genetics of the parental lines might determine the seed phenotype, including seed anatomy, immune response, and seed chemical composition, which in turn can affect the assembly of the seed microbiome \[59,60\]. Domestication, and breeding, is one of the major factors shaping the seed microbiome \[4,8,12,34,60,61\]. A recent study showed that the seed microbiome of oilseed rape can be predicted based on the microbiome of parental lines and the germination performance of the offspring is determined by the male contribution to the microbiome \[60\]. Other biotic factors such as the mother plant’s age, health status, and rate of seed maturation are expected to play a major role. During seed maturation, seed colonizers are subjected to loss of water and high osmotic pressure, which can result in a shift in the composition of the microbial community \[37\]. Besides plant genetics and physiology, several abiotic factors such as climatic conditions, including drought, precipitation, and temperature can affect seed development and thereby the microbiome transmission.

**Seed dormancy**

Here we refer to dormancy as the period between seed maturation and germination, including both natural and induced dormancy (Figure 1D). Dormancy thereby includes seed storage, seed banks, and the period the seed remains in the environment or inside the animal’s gut. The effect of dormancy on the seed microbiome has rarely been studied. The main impact on the survival of seed endophytes is expected to come from the duration and conditions of dormancy. Studies focusing on storage duration of seeds of agricultural crops reported a reduction of microbial diversity and a shift towards certain microbial taxa, especially *Enterobacteriaceae* and saprophytic fungi, through time. We still do not understand how microorganisms survive during seed dormancy, and whether and how they enter and exit dormancy with their host. Bacteria have developed different mechanisms to survive harsh conditions including oligotrophic environments. For instance, some bacterial species are able to enter a dynamic non-proliferative state during long periods of starvation. Others have developed a ‘feast and famine’ strategy consisting of alternating cycles of growth and starvation \[62\].

**Factors affecting the microbiome at dormancy**

Under natural conditions, seeds spend most of their dormant period exposed to a diverse community of microorganisms during which seeds can be colonized by a variety of microorganisms (Figure 1D). Seed dispersers such as birds and mammals that consume seeds or seed-containing fruits have been suggested to affect the seed microbiome by increasing its microbial diversity \[7\]. Although the passage of seeds through animal guts has been shown to increase seed germination and growth, there is no direct evidence showing that microorganisms present in animal guts can be transmitted to the seed or affect seed microbial diversity. In agricultural systems, the seed microbiome may be affected by seed treatments, production management, post-harvest handling, storage conditions, and mixing of seeds during sowing \[16,17\]. Studies focusing on the effect of storage and seed banks on the seed microbiome are scarce \[63\]. Few studies have shown that storage of maize can have an impact on seed microbial diversity and composition \[54\]. Another study by Solanki et al. tested the effect of both storage and chemical fumigation on wheat grain; they reported that phosphine fumigation, despite being an insecticide, had a significant effect on the seed microbiome, whereas grain storage for up to 6 months had little to no effect on microbial diversity or community composition \[17\].
Seed to plant

A seed comprises the plant embryo, and, depending on the plant species, other compartments such as seed coat, endosperm, and perisperm. These compartments were previously shown to contain distinct communities of microorganisms, some of which can be transmitted to the developing seedling [11,12]. A recent study on oak showed that seeds transmit a distinct community of microorganisms to the emerging seedling’s phyllosphere and roots (Figure 1E). This suggests that the seed microbiome contains niche-specialized microbes that then migrate to their respective tissues (Figure 1C). Speculatively, the distinct migration routes could be due to differences in the anatomical and physiological properties between the leaves and roots of the developing seedling that can favor certain members of the vertically transmitted microbiome to migrate belowground, aboveground, or in both directions. The distinct migration routes also raise the question about the difference in microbial diversity and abundance that is transmitted to above- and belowground compartments. A study by Wang et al. [65] reported higher fungal diversity in rice seedling stems than in roots, while bacterial diversity was higher in roots than in stems. Yet, we need more studies to generalize this finding to other plant species.

Regardless of the migration route, the stage from seed to seedling represents yet another bottleneck, for the inherited microbiome. Several studies have found that seeds harbor higher microbial diversity than seedlings, which can be explained by the fact that not all inherited microorganisms can pass from the seed to the seedling. We can thus differentiate between transient microorganisms that are inherited from the parents and present in the seed but not passed onto the seedling, and persistent seed endophytes that are found later in the developing seedling. The fate of the seed-transmitted microorganisms after seed germination and into later developmental stages is not yet fully known. Working on tomato, Bergna et al. showed that some microbes could persist from seed to seedling for two generations [21]; they concluded that microorganisms beneficial to the plant were transmitted by seeds.

Horizontal acquisition of microorganisms must be also considered during this phase. As the seed imbibes, plant exudates, that is, signaling molecules including primary and secondary metabolites, are released, which are expected to attract certain soil microorganisms that subsequently colonize the seedling [28,66]. In fact, seeds of the same plant genotype grown in different soils/environments were found to host a distinct microbiome [28,67,68]. Few recent studies have shown that soil contributes a larger fraction of the diversity of the seedling-assembled community compared to inherited microorganisms [4,67,68]. Nonetheless, the persistence of at least a subset of the seed-borne community is pivotal in ensuring the continuity of the plant microbiome to subsequent plant generations [12]. The factors that discriminate between transient and persistent microbes are still unknown; yet, the persistent seed microbiome is thought to be a key determinant in shaping the community structure in the seedling due to priority effects over environmental microbes [11].

Factors affecting the transmission from seed to plant

Seed health status, including diseases induced by pathogenic microorganisms and abiotic disorders, can affect the composition and diversity of the seed microbiome, and thus, the available species pool to be transmitted from seed to plant. Similarly, domestication has been suggested to have negative consequences for the transmissibility of the seed microbiome to offspring [69]. Moreover, soil and climatic conditions, water regimes, and temperature can affect the survival of the seed-associated microorganisms or their ability to migrate to the developing plant [70]. For example, leaves of oak seeds germinated under drought conditions contained higher microbial diversity and abundance than those grown under normal conditions (Abdelfattah, unpublished).
**An outlook on the future of microbial inheritance**

Research on plant microbial inheritance represents a new venue for innovative applications in agriculture. For instance, there is a growing body of research suggesting that hybridization of parental breeding lines results in a predictable microbiome composition and function in their progeny [34,60]. This line of research can lead, in the near future, to the implementation of microbiome-targeted breeding approaches [34,35,60]. Another promising application is the introduction of beneficial microorganisms at flowering or as a seed coating. Several studies have shown already that flower treatment with some beneficial microorganisms resulted in seed and seedlings harboring these microorganisms [56]. Similarly, microbial seed coating containing one or a consortium of bacterial and fungal species facilitates the colonization of the emerging seedling by those microbes [56,71]. Differentiating between inherited microorganisms and those acquired horizontally is important to develop tailored applications to deliver microorganisms to the developing seedlings. Since seeds have been the latest plant organ to be studied, they likely still harbor microorganisms that are either unknown or offer novel functions. Therefore, studying the seed microbiome is expected to result in the discovery of new microorganisms as well as new molecules with promising biotechnological features.

From a natural perspective, the ecological consequences of inheritance of microorganisms are unexplored, and might turn out to be of major importance for our understanding of spatial and temporal variation in microbiome community structure, plant–microbe interactions, and plant fitness, with consequences for community dynamics and ecosystem functioning. From an evolutionary perspective, microbial inheritance is expected to play a role in evolutionary changes and speciation in both plants and microbes and affect host–symbiont coevolutionary dynamics [2]. From an applied conservation perspective, this offers new ways for the actual topic of ecosystem restoration and microbiome stewardship in preventing biodiversity loss [72].

For the field of microbial inheritance to move forward, fundamental knowledge gaps should be filled. Here, we highlight for each of the stages of the microbial inheritance process the gaps and solutions to overcome technical limitations as well as future directions of microbial inheritance research.

The plant-to-seed stage is perhaps the most challenging stage due to the presence of the multiple sources of microbes in the plant environment, including soil, air, rainwater, and pollinators, which hinders to precisely identify the origin of the seed microbiome. The issue of multiple sources is also accompanied by other methodological constraints such as the inability of some culture-independent techniques to distinguish between closely related taxa and detect the low abundance and diversity of microbial species in flowers and seeds. Until sequencing technologies can overcome these issues, future studies may rely on integrating other methods such as advanced microscopy, such as live-cell imaging, and the use of tagged microorganisms under controlled or semi-controlled conditions. Live-cell imaging can be of particular value to investigate microbial inheritance at a cellular level, especially during sexual fertilization. Another important gap is the lack of quantitative studies investigating the male and female contribution to the seed microbiome, and whether the inherited microbiome is influenced by different mating systems. Such studies can improve our understanding of the evolutionary outcome of plant mating systems and may show that microbial inheritance can be predicted with implications in evolutionary ecology and agriculture.

During seed dormancy and storage, specific research is needed to demonstrate how the inherited microbiome can undergo dormancy with their host. While dormancy in prokaryotic cells including chloroplasts by suppressing protein synthesis has been well studied [73], less is
known about seed microorganisms, their response to plant physiological signaling, and metabolic interplay. Understanding how seed microorganisms undergo dormancy with their host can contribute to the development of microbiome-based seed formulation recipes to ensure the viability/dormancy of microbial inoculants. As we move forward with culture-independent studies, it will be crucial to differentiate between live and dead microbial cells, for example, by live/dead staining in microscopy observations, the use of a dye such as propidium monoazide (PMA) that binds to relic DNA or DNA of dead cells, or the use of rRNA instead of rDNA [38,74–76].

Finally, research focusing on the transmission of the seed microbiome from seed to seedling is needed to understand the process that governs the migration of microorganisms to different compartments. Understanding the function of those microbes during germination and seedling establishment could be translated into novel agricultural bioinoculants, targeting microbial seed treatments to improve plant health and crop quality. A characteristic drawback of studying the seed microbiome is the invasive nature of the methods, which precludes the analysis of the originating seedling. In this regard, the use of synthetic communities and tagged strains under controlled conditions, such as microcosms, can represent valuable assets in inheritance studies.

Concluding remarks
Understanding the assembly of the seed microbiome as well as its structure and function can lay the foundation for sustainable crop production and ecosystem restoration in the future, either by making use of the native seed microbiome or by ensuring the adaptation and integration of selected strains into the host plant (see Outstanding questions). Despite the rapid growth of the microbiome research and the unprecedented wealth of knowledge we are collecting, the study of microbial inheritance is still in its infancy. We expect that studies of microbial inheritance will improve our understanding of the seed microbiome assembly and the importance of sexual reproduction for the plant’s health, evolution, and the survival of both the microbiome and its host. Showing that sexual reproduction is not only important for ensuring passing genetic information but also microbial species to offspring opens new venues for understanding host–microbe co-evolution, breeding programs, and disease management strategies.

Author contributions
A.A. and C.L. conducted the literature review. A.A. conceptualized and wrote the first draft of the manuscript. A.J.M.T. made a major contribution to the writing of the manuscript. G.B. and B.W. contributed to the interpretation, conclusions, and writing. All authors have read and agreed on the content of the manuscript.

Declaration of interests
No interests are declared.

References
8. Rybakova, D. et al. (2017) The structure of the Brassica napus seed microbiome is cultivar-dependent and affects the interactions of symbionts and pathogens. Microbiome 5, 104

Outstanding questions
From plant to seed
Is the plant’s microbiome inherited during sexual fertilization via pollen and/or ovules?
What is the relative contribution of the male and female parent to the inherited microbiome?
Did the evolution of different mating systems result in different assemblage processes of the seed microbiome?

Seed dormancy
How is the microbiome distributed among the seed tissues?
How does the inherited microbiome undergo exit dormancy with the seed? And what alternative strategies do microorganisms have to survive during seed dormancy?
What is the effect of seed dormancy on microbial composition and functional diversity?

From seed to seedling
Which process determines the migration route of the seed microbiome to different plant compartments? Does the functional diversity differ between microorganisms that are transmitted to the roots and leaves?
What is the fate of the inherited microbiome after germination?

For the whole process of microbial inheritance
What is the relative contribution of horizontal versus vertical transmission to the origin of the microbiome?
What stage is the largest bottleneck for vertical transmission? And during what stage is there more horizontal transmission?
Which microbial traits determine transmission during the different stages of the microbial inheritance process? And do the microbial traits necessary to be inherited depend on the host traits?
Is the inherited microbiome similar across generations, or does it depend...
47. Rochefort, A. et al. (2021) Transmission of seed and soil microbiota to seedling. mBio 6, e00446-00421

on environmental conditions? And if it depends on environmental conditions, is it variation in the composition and functional diversity of the inherited microbiome adaptive from the plant’s perspective?
74. Lennon, J.T. et al. (2018) How, when, and where relic DNA affects microbial diversity. mBio 9, e00637-00618
76. De Vrieze, J. et al. (2018) The active microbial community more accurately reflects the anaerobic digestion process: 16S rRNA (gene) sequencing as a predictive tool. Microbiome 6, 63