Title : Ecological patterns of seed microbiome diversity, transmission, and assembly

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15 **Abstract**

16 Seeds are involved in the transmission of microorganisms from one plant generation to another and
17 consequently act as the initial inoculum for the plant microbiota. The purpose of this mini-review is
18 to provide an overview of current knowledge on the diversity, structure and role of the seed
19 microbiota. The relative importance of the mode of transmission (vertical vs horizontal) of the
20 microbial entities composing the seed microbiota as well as the potential connections existing
21 between seed and other plant habitats such as the anthosphere and the spermosphere are discussed.
22 Finally the governing processes (niche vs neutral) involved in the assembly and the dynamics of the
23 seed microbiota are examined.

24

25 **Introduction**

26 Plants harbor multiple microbial taxa, known as microbiota, which influence a number of
27 plant traits such as biomass accumulation [1], metabolite production [2], drought tolerance [3] and
28 flowering time [4,5]. In addition, the composition of the plant microbiota can enhanced host
29 resistance to aboveground [6,7] and belowground [8,9] pathogens. The promise of plant benefits
30 from plant microbiota opens new possibilities for increasing plant productivity or wellness through
31 manipulation of the composition of these assemblages. However, this requires a fundamental
32 knowledge on the processes that drive assembly of the plant microbiota throughout the plant
33 developmental cycle.

34 To date many studies have investigated the composition and structure of microbiota
35 associated with the phyllosphere and rhizosphere (**Box 1**) of numerous plant species during their
36 vegetative or reproductive stages [10]. In contrast, analyses of microbiota associated with other
37 plant habitats such as the anthosphere [11], the carposphere [12-15], the seed habitat [16] and the
38 spermosphere [17,18] have been relatively less studied. Nonetheless, these habitats are important
39 for plant fitness since they are directly related to the production of offspring and then potentially
40 associated to vertical transmission of the plant microbiota.

41

42 **1 - Why study the seed microbiota?**

43 Historically, the seeds* have been traditionally viewed as a passive means of microbial
44 dispersion, via contact of micro-organisms located in the surrounding environment (e.g. threshing
45 residues or soil) with the seed surface [19]. Seed surface-sterilization methods classically employed
46 in reductionist approach to study specific plant-microbial strain interactions has led to the concept
47 of “germfree plants” [20]. However, seed-surface disinfection does not guarantee seed sterility since
48 (i) seeds can produce numerous antimicrobial compounds that inhibit culture-dependent detection

of micro-organisms [21], (ii) microbiota located in the integument, endosperm or embryo (**Fig. 1**) could still survived robust disinfection procedures [22].

The composition of the seed microbiota [23,24] can have direct impacts on seed quality. Perhaps the most frequent observation is related to the negative effect of microbial load on seed germination [25]. In contrast, some seed-borne microorganisms can promote homogenous germination rate through the release of seed dormancy *via* cytokinin production [26]. Occasionally, seed transmissions of mycotoxin (e.g. *Aspergillus flavus*) or Shiga toxin (e.g. *Escherichia coli* STEC) producers could dramatically impacted the sanitary quality of seed samples [27,28]. From an epidemiological angle, seed transmission of plant pathogens serves as a major method of dispersal and is therefore important for predicting disease emergence and spread. Indeed, very low degrees of seed contamination by bacterial pathogens can lead to efficient plant colonization [29]. Furthermore, seed transmission of phytopathogens can take place on non-host plants [30,31], which can then serve as a reservoir of plant pathogens.

2- Vertical versus horizontal inheritance of the plant microbiota

Members of the plant microbiota can be transmitted either horizontally (acquired from the surrounding environment) or vertically (acquired directly from the parent) [32]. For plants, the relative importance of horizontal and vertical transmission remain unclear [33], but both modes are likely to contribute to the ultimate composition of the seed microbiota (**Fig. 2**). The horizontal or vertical transmission of any microbial entity is, in part, linked to the seed transmission pathway employed by this micro-organism. To date, three main transmission pathways have been described: (i) the internal pathway through the xylem or nonvascular tissue of the mother plant, (ii) the floral pathway via the stigma of the mother plant and (iii) the external pathway where seed is contaminated through contact with microbial inoculum presents on fruits or threshing residues [34]. The relative importance of these transmission pathways in the composition of the seed microbiota varies according to the structure of seeds [35]. Schematically, the internal pathway can be view as a

75 means of vertical transmission [36], with the exception of environmentally-acquired microbial
76 entities that invade the vascular tissues and then the seed (e.g. phytopathogenic micro-organisms).
77 Floral pathway concerns micro-organisms that are acquired from the environment [37,38], however
78 the mode of transmission (horizontal vs. vertical) is dependent on the selection exerted by the plant.
79 Finally the external pathway is associated to horizontal transmission. According to the seed
80 transmission pathway employed, the microorganisms are then located in distinct seed location such
81 as the surface of the embryo (e.g. the embryonic axis and the cotyledons), the storages tissues (e.g.
82 endosperm and/or perisperm) and the seed coat [35], that can be view as different microhabitats
83 (**Fig. 1**).

84 Fungal vertical inheritance is recognized as common mechanism of transfer across
85 generations [39] and vertically transmitted fungal symbionts have been implicated in providing
86 direct benefit to their host plant, such as in the case of improved drought tolerance of *Festuca rubra*
87 given its fungal endophyte *Epichloë festucae* [40]. Furthermore, some studies have reported
88 vertical transmission rates of greater than 90% for many fungal endosymbionts [41]. In comparison,
89 the number of vertically transmitted bacterial isolates seems to vary greatly between seed samples
90 [42] and could even be absent in certain genotypes of *Arabidopsis* [43]. However, some evidence
91 suggests vertical transmission of bacterial entities in various plants such as rice [44], wheat [22],
92 maize [45], switchgrass [46] and *Arabidopsis* populations [47]. Though these studies have not
93 directly observed vertical transmission, they collectively provide evidence that this mode of
94 transmission contributes to a cross-generationally persistent seed microbiota.

95 Proving the mode of transmission and determining transmission rates are technically
96 challenging, as many members of the seed microbiota overlap with microorganisms that are also
97 commonly detected in rhizosphere soils, bulk soils, plant systemic tissue, and air [10,39,48-51].
98 Thus, analysis of overlap among microbiota members across plant compartments or generations
99 planted in the field is not sufficient to prove vertical transmission, even if seeds are specifically
100 investigated. Tracking studies of GFP-labeled bacteria entering the seed from the parent plant

101 [52,53] offer one of the most precise methods for determining vertical transmission, though it
102 requires genetic manipulation of strains and tools that largely are unavailable for yet-uncultivable,
103 non-model microorganisms.

104 3- Assembly of the seed microbiota

105 3.1 Connection between the anthosphere and the seed habitat

106 Since some micro-organisms can be transmitted by the floral pathway, an overlap in taxa
107 associated with floral structures and seeds has been reported in several plants, including rice [54],
108 grapevine [55], pumpkin [14], apple tree [56], *Metrosideros polymorpha* [57] almond, grapefruit
109 and tree tobacco [58]. The proximal relationship between a developing seed and a plant floral
110 structure has importance for the mode, efficacy, and the specificity of microbial transmission to the
111 seed. As the floral pathway of bacterial transmission to the seed is more effective and less
112 specialized as compared to vascular transmission [59], it suggests that microorganisms that do not
113 have intimate symbiotic (or pathogenic) relationships with the host plant may have an improved
114 success in colonizing the seed through the floral structures. Additionally, host and non-host
115 transmission could happen through the floral pathway of common bean [30]. Because both of these
116 studies facilitated floral-to-seed transmission by depositing the bacteria directly onto the flowers,
117 these results collectively suggest that dispersal mechanisms to flowers that inundates the floral
118 structures with immigrants (e.g., after a heavy rain event) may be a very successful mechanism for
119 transmission of non-specialized microorganisms to flowers, and ultimately, to developing seeds.
120 However, it also suggests that, in the absence of such effective and non-discriminating dispersal
121 events, the probability of success of non-specialist microbial transmission from the flower to the
122 seed may be low. The ephemerality of flowers (e.g., as discussed in [41]) provides an additional
123 constraint on the timing of the “perfect storm” of an inundating microbial dispersal event. This
124 further supports a hypothesis that such events likely are not the most effective in maintaining a
125 persistent transmission of non-specialist microbiota from the flower to the seed, and across plant
126 generations.

127 Floral traits will also have influence on the transmission of microbiota to the seed. McArt et
128 al. 2014 discussed floral traits that have importance for transfer of plant pathogens, but this
129 framework is also applicable to non-pathogenic microbiota [60]. Specifically, relevant floral traits
130 for floral-to-seed transmission include those that affect microbial establishment in flowers, such as
131 flower longevity and age, style length and other morphological traits, and nectar characteristics such
132 as sugar content, antimicrobial properties, and reactive oxygen species [60]. More foundational
133 work is needed to understand how specific floral traits may facilitate, or even select for, microbiota
134 that are ultimately transmitted to the seed, for example, by investigating the success and
135 composition of microbiota transfer to seeds among plant genotypes exhibiting variation in floral
136 traits.

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138 3.2 Relative importance of niche *vs* neutral processes in assembly of the seed microbiota

139 An outstanding ecological question for the assembly of seed microbiota is the relative
140 importance of different community assembly processes in determining the ultimate seed microbial
141 community structure. Community assembly processes include dispersal, drift, selection, and
142 diversification [61,62], and can be further specified by niche and neutral processes (**Box 2**; [63]).

143 Seed microbiota is ecologically interesting in that it represents both an endpoint for
144 community assembly within the seed, and also a starting point for community assembly of the new
145 seedling microbiome. When considering the seed microbiota as an endpoint, we can hypothesize
146 that internal and floral transmission may provide pioneer taxa, while external pathways provide
147 secondary colonizers. It is unknown whether the pioneer taxa in seeds drive priority effects (**Box 2** ;
148 [64]), but, given that cultivable seed microbial communities are considered to be limited in their
149 size [42] and also that many members of the seed microbiota may be inactive or dormant [65,66] , it
150 seems unlikely that priority effects play a major role in a seed ultimate community structure, though
151 this remains to be tested specifically. Still, we can hypothesize the general drivers that may be niche
152 or neutral in seed microbiota assembly. Niche processes would include biotic interactions, such as

153 plant recruitment from soil via seed exudates [18], soil context [67], insect driven dispersal to
154 flowers [38], and internal seed environmental conditions. For example, Mano et al. 2006 tracked
155 cultivable bacteria inside and outside the seed over maturation, and found that endophytes
156 inhabiting the mature seed had higher tolerance for osmotic pressure, suggesting that the seed
157 habitat can exhibit strong selective pressures [68]. Furthermore, Rezki et al. 2016 found that radish
158 seed bacterial communities were resistant to invasion by either a bacterial or fungal pathogen, while
159 seed fungal communities were resistant to a bacterial but not to a fungal invader [69]. High
160 community stability can suggest a selection by the host to maintain its core members (e.g., [70]) or
161 unavailability of niche spaces for the invader, among other possibilities. Thus, an extrapolation of
162 this study may be that seed bacterial communities are more driven by niche processes than fungal
163 communities. In contrast, neutral processes for seed assembly may include dispersal-driven
164 colonization of microbiota to flowers that then are transmitted to the seed, for example via air or
165 rain events. Cultivation-dependent approach performed on seed endophytes in corn and common
166 bean showed substantial variability in the populations recoverable from the same cob and pod,
167 respectively [71], therefore suggesting that these plants may not provide a strong selective
168 environment for seed microbiota.

169

170 **4- Dynamics of the seed microbiota during germination and emergence**

171 Seed-associated microorganisms could be either viewed as transient members of the seed
172 habitat (seed-borne microorganisms) or as persistent members of the plant microbiota (seed-
173 transmitted microorganisms). The persistence of microbiota during the first plant developmental
174 stages is strongly dependent on capabilities to efficiently colonize the aerial part of the seedling
175 and/or to outcompete soil microbial residents. Indeed, quickly after seed imbibition, various
176 primary and secondary metabolites are released into the neighboring soil [17,18], resulting in a zone
177 of “elevated microbial activity around a germinating seed”, which has been coined spermosphere
178 [72] (**Fig. 2**).

179 The potential influence of priority effects initiated by the seed microbiota may become
180 preponderant for community assembly of the spermosphere. Indeed, due to the high concentrations
181 of plant-provided resources within the spermosphere, microbiota located on or within seeds may
182 have a competitive advantage over microorganisms associated to the surrounding bulk soil simply
183 because of proximity and access to those resources. Thus, we may expect that spermosphere-
184 associated microbiota would be strongly shaped by the pioneer members of the seed microbiota.
185 However, to date, most studies have shown that the composition of spermosphere-associated
186 bacterial assemblages is mostly driven by soil types [73], since most seed-borne bacterial entities
187 are not found in root samples [74,75]. In comparison, the potential translocation of seed-borne
188 micro-organisms in the aerial part of the seedlings has been less investigated [76]. A closer
189 investigation of these microbiota is needed, as many of these studies used surface-sterilized seeds
190 [75] and less-precise fingerprinting techniques such as FAME [73] and DGGE [74]. Irrespective of
191 their origins (seed-borne or soil-borne), microbes must deal with a rapidly changing habitat during
192 seed germination due to exudation of multiple low-molecular weight compounds [18]. Hence,
193 efficient nutrient scavenging system coupled to rapid utilization of resources can provide a crucial
194 competitive advantage for microbial taxa in the spermosphere [77,78] and would potentially
195 explain why the relative abundance of copiotrophs is increased during germination and seedling
196 emergence [79]. Of course, a number of other traits are involved in spermosphere competence
197 including chemotaxis [80,81], attachment to seeds [59,82,83] biofilm formation [84-86], and
198 production of antimicrobial compounds such as phenazines or 2,4-diacetylphloroglucinol [17]

199 The relatively low influence of seed-associated microbiota on assembly of the plant
200 microbiota, could be explained in part by the role of dormancy [87] in determining seed microbiota
201 viability. For the packaging of seed microbiota to benefit the next generation, the beneficial
202 microorganisms must be able to withstand unknown periods with limited resources (including
203 water) and space prior to seed germination. A classic study showed that overwintered seeds had
204 comparably fewer cultivable isolates than what was recoverable from non-overwintered seeds, but

205 that both groups had overall low recovery (typically, only one colony-forming unit per seed given
206 their cultivation conditions ; [42]). A more recent study showed that bacterial endophytes were less
207 viable from old seeds than fresh [88]. Given that seeds remain viable for seemingly indefinite
208 periods [89], it would be interesting to investigate if the microbiota of very old seeds are also
209 viable, and if so, to ask if those surviving members also offer a benefit to the seedling.

210

211 **5- Conclusions – Futures Directions**

212 Seed transmission of microorganisms may play an important role on plant productivity
213 through influencing the primary composition of the plant microbiota. Nevertheless, the diversity
214 and structure of seed-associated microbiota have often been ignored. In addition, we have a limited
215 understanding on how microbial taxa are assembled during seed development and maturation, and
216 to interpret those patterns of assembly in light of current ecological models inclusive of
217 niche/neutral and deterministic/stochastic processes. To our opinion, assembly patterns could be
218 assessed for different plant species by coupling community profiling approaches (e.g., high-
219 throughput sequencing) and fluorescent in situ hybridization protocols on microbial populations
220 associated to seeds, germinating seeds and seedlings. This will allow researchers to define a core
221 microbiota associated to specific seed genotypes. Studying the stability of this core seed microbiota
222 to biological disturbances (or invasion) due to seed transmission of plant pathogens is a first step for
223 designing biocontrol based strategies [16].

224 Based on a number of taxonomic and functional diversity surveys performed on a range of
225 microbial habitats, it seems clear that assemblies of other host-associated microbial communities
226 are frequently based on function rather than on its community structure [90-93]. Indeed, microbial
227 taxa sharing similar ecological function probably compete for resource use and/or space, which
228 often lead to mutual exclusion. Hence, predicting the functional repertoire of the seed microbiota by
229 metagenomics and metatranscriptomics approaches seems appropriate and timely. For instance,
230 comparison of genetic content and transcript abundance during seed development and/or

231 germination would provide a list of microbial traits involved in the successful colonization of these
232 habitats, and would also provide information about the importance of dormancy strategies for
233 microbial persistence within the seed. These traits would be of interest for subsequent selection of
234 microbial strains that benefit the plant.

235

236 *In this document, the term seed will be restricted to sexually derived structures of spermatophytes,
237 which can under favorable conditions germinate and produce new plants [19].

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246 **Figure 1: Localization of some seed-associated microorganisms**

247 Schematic representation of the three main compartments of mature seed: embryo (green),
248 endosperm/perisperm (orange) and seed coat (grey). Of particular note is that this schematic
249 representation does not reflect non-endospermic seeds (e.g. Fabaceae), which are devoid of storage
250 tissues. Micro-organisms located in the embryo compartment have been mostly found (with the
251 exception of viruses) at the surface of the embryonic axis.

252

253 **Figure 2: Vertical and horizontal inheritance of the plant microbiota**

254 Transmission of micro-organisms from plant (1) to seed (2) could occurred via vertical inheritance
255 of endophytic micro-organisms (red), transfer of flower-associated micro-organisms (blue) and
256 contact of the seed with microbial entities located on fruits and leaves (yellow), or in soil (black).
257 Once the seed germinate (3), numerous exudates are released, which creates a zone of intense
258 microbial activity called the spermosphere. It is generally accepted that seed-borne micro-organisms
259 are preferentially associated to the seedling phyllosphere (4), while soil-derived micro-organisms
260 are mainly located in the rhizosphere. The symbols represent different microbial phylotypes.

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Box 1 : Plant-associated microbial habitats

- **Anthosphere:** Microbial habitat associated to flowers.
- **Carposphere:** Microbial habitat associated to fruits.
- **Caulosphere:** Microbial habitat associated to stems.
- **Endosphere:** Microbial habitat located within plant tissues.
- **Phyllosphere:** Microbial habitat associated to leaves, includes phylloplane and endosphere.
- **Rhizoplane:** Microbial habitat associated to root surface.
- **Rhizosphere:** Zone of soil under the direct influence of the root system.
- **Spermosphere:** Zone of soil under the direct influence of germinating seeds.

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Box 2 : Community Assembly Terms [61-64]

- **Determinism:** Non-probabilistic differences in community members.
- **Dispersal:** The movement of members between communities over space; can contribute to either deterministic or stochastic processes, depending on the situation.
- **Diversification:** Acquisition of genetic variation in populations that may eventually lead to speciation.
- **Drift:** Fluctuations in the relative abundances or abundances of community members over time; contributes to stochastic processes.
- **Neutral:** Ecological equivalence of community members.
- **Niche:** Ecological inequivalence among community members (e.g., driven by abiotic or biotic fitness differences).
- **Priority effects:** the first taxa to assemble in a community (pioneers) have implications for the community's successional trajectory and/or ultimate community structure.
- **Selection:** Fitness differences among community members; Contributes to deterministic processes.
- **Stochasticity:** Probabilistic differences attributed to demographic variability in birth, death, and dispersal.

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