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Gontran Arnault, Cendrine Mony, Philippe Vandenkoornhuysse

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## **Plant microbiota dysbiosis and the Anna Karenina Principle**

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7 Gontran Arnault, Cendrine Mony &amp; Philippe Vandenkoornhuysen

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9 Université de Rennes 1, CNRS, UMR6553 ECOBIO, Campus Beaulieu, 35042 Rennes

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12 **KEYWORDS:** plant microbiota, dysbiosis, Anna Karenina Principle, stress, disease, plant13 **holobiont**

14 **ABSTRACT:**

15

16           Microorganisms are associated with all plants, recently leading to the hologenome concept.  
17 We reviewed the assembly processes of plant microbiota and analyzed its structure during the  
18 emergence of dysbioses. In particular, we discussed the Anna Karenina Principle (AKP) based on  
19 Leo Tolstoy’s assertion applied to plant microbiota: “all healthy microbiota are alike; each disease-  
20 associated microbiota is sick in its own way”. We propose the AKP to explain how stochastic  
21 processes in plant microbiota assembly due to several external stressors could lead to plant diseases.  
22 Finally, we propose the AKP to conceptualize plant dysbioses as a transitory loss of host capacity to  
23 regulate its microbiota, implying a loss of function that leads to a reduction of host’s fitness.

## 24 MAIN TEXT

### 25 **Plant microbiota associated with plant health**

26 Microbes can be associated with all higher organisms, from animals to plants, and are  
27 involved in many functions linked to the health, behavior or fitness of their host [1,2]. In plants,  
28 microbes promote plant nutrition [3,4], increase plant tolerance to abiotic stresses [5,6] and prevent  
29 plant diseases [7–10] by inducing higher immunity responses [5] or by fighting the pathogen directly  
30 [11]. The plant microbiota is composed of many microorganisms including eubacteria [12], fungi  
31 [13,14], archaea [15], viruses [16] and even protists [17]. Much evidence highlights the importance  
32 of microorganisms during plant evolution [18].

33 Dysfunctions of this **microbiota** (see Glossary) can lead to **dysbiosis** (Box 1), which is  
34 generally seen as detrimental for the host. The dysbiosis concept is not clearly defined [19,20], which  
35 can lead to several disagreements among researchers and studies. We define and consider dysbiosis  
36 as a transitory loss of host capacity to regulate its microbiota, implying a loss of function that leads  
37 to a reduction of host's fitness (Box 1). The loss of function can be linked to processes involved in  
38 plant-protection against stresses, nutrition intake or reproduction. We discuss here the causality  
39 between modifications of the microbiota and the appearance of symptoms within the **hologenome**  
40 concept (i.e. the idea that the plant fitness relies not solely on the plant genome but also the  
41 microbiota). One of the hypotheses that predicts the results of healthy versus disease-associated  
42 microbiota is the **Anna Karenina Principle (AKP)** [23,24]. It states that disease-associated  
43 microbiota are more dissimilar than healthy ones. We analyze to what extent the AKP is transposable  
44 to plant microbiota. We propose the AKP as an interpretation of the increased stochasticity observed  
45 in dysbiosis state. Reasoning on the AKP would become a way to identify and interpret the type of  
46 transient dysbiotic state of the plant **holobiont**, i.e. the type of microbiota disorder.

47

### 48 **Origin of the healthy plant microbiota and determinist processes of assembly: a microbiota** 49 **composition under control**

50 Plant microbiota includes microbial communities in the **phyllosphere**, **rhizosphere** and  
51 **endosphere** mainly, that are very distinct in their composition (Fig1) [25–27]. Part of the plant  
52 microbiota can be transmitted during sexual reproduction through seeds [28] or during clonal  
53 reproduction [29]. Recent studies have shown that plant genetics determines a plant's ability to  
54 interact with a specific microbiota [30,31]. Chemical interactions between the plant and the  
55 microorganisms are numerous and shape the composition of the rhizosphere [32]. These deterministic  
56 filtration processes of the plant microbiota (Fig. 1D) [33–35] are more intense in the endosphere than

57 in the rhizosphere, resulting in less stochasticity in the compartment where the plant-microorganism  
58 association is the most intimate. For instance, the rhizoplane community is the potential source of  
59 close to 90% of the root endosphere [36]. The root endosphere contributes more than 60% of the leaf  
60 endosphere composition [36]. In both leaf and root microbiota, multiple parameters can alter  
61 microbiota composition during the life of the plant: microbial interactions, plant neighbors, insect  
62 vectors and climate [37–39]. Thus, a determinist selection of microorganisms by a host-plant shapes  
63 at least in part the plant microbiota composition [40]. In other words, the host controls microbiome  
64 assembly tightly and low stochasticity in the microbiota composition is supposed to be observed.  
65 Variations among host-plants observed could be explained for instance by spatial heterogeneity  
66 within the recruitable soil microbial reservoir. It can also be explained by the host microbial  
67 colonization dynamics. A **priority effect**, primed at an early stage of microbiota composition, could  
68 lead to microbial variation between hosts [41]. In this situation, the apparent heterogeneity could be  
69 driven by microbe-microbe determinist interactions through evolution of dependencies among  
70 microorganisms [42]. Nevertheless, the host-plant could likely be the driver of a ‘healthy’ microbiota  
71 resulting from host-plant active microbial filtration to positively influence the plant holobiont fitness.  
72

### 73 *Plants can present dysbioses*

74 Conversely, dysbiosis is detrimental for (i) plant nutrition [3] (ii) modulating stress tolerance  
75 [5] and/or (iii) protecting the plant against pathogens [10] leading to negative effect on host’s fitness.  
76 This dysbiosis can be both the cause or the consequence of stress or disease. That is why the concept  
77 of dysbiosis has been criticised [19,20]. It is thus necessary to define and conceptualize dysbiosis  
78 (Box 1) which in this opinion paper, uses the AKP to identify and describe the dysbiosis types (Box  
79 2) and related processes.

80 Several studies in plant sciences have shown the occurrence of dysbioses within the holobiont,  
81 but the term ‘dysbiosis’ only started to be used [22,31,45–47]. Dysbioses can appear at different levels  
82 of the plant microbiota: the phyllosphere [31,37,45,48], the rhizosphere [49] and the endosphere [50].  
83 Dysbioses within plant holobiont can lead to diseases with diverse symptoms, such as the formation  
84 of galls [50,51], leaf necrosis [31], growth decline [31], bleeding canker disease [48] or lethal wilt  
85 disease [52]. According to the Koch principle, a pathogen drives a disease and is tacitly causal of the  
86 uncontrolled microbiota composition, i.e. a dysbiosis [53]. Examples exist but a key issue to consider  
87 is the resistance to the pathogen attack is controlled at least in part by the microbiota community  
88 composition and niche occupancy. Thus, shifts in microbiota composition is a signature of an attack.

89 The first evidence of shifts in microorganism composition in stress-associated microbiota  
90 compared to healthy ones was provided for soil [54]. Recent studies showed that the effect of stress

91 was higher in the rhizosphere and endosphere than in soil [35]. This observation suggests that not  
92 only niche parameters are responsible for the shift in the microbial community, but that plant selection  
93 is also involved. Interestingly, plant dysbiosis may sometimes share characteristics with dysbioses  
94 associated with inflammatory bowel diseases in humans, with an increase in Proteobacteria and a  
95 decrease in Firmicutes [31,55]. This similarity may point at common processes of microbiota  
96 homeostasis in healthy individuals of both plants and animals [21,38], calling for a possible  
97 transposition of the patterns of dysbiosis between the two kingdoms.

98

## 99 **Plant dysbioses**

100 —

### 101 **Box 1: Dysbiosis redefined and conceptual construction**

102 Despite the existing definition of dysbiosis of Levy et al. (2017), a '*compositional and*  
103 *functional alteration of the microbiome*' [56], we prefer to consider a refined dysbiosis definition, i.e.  
104 a transitory loss of host capacity to regulate its microbiota, implying a loss of function that implicitly  
105 leads to a reduction of the holobiont fitness. Dysbiosis could be a consequence of either biotic  
106 [11,37,50,51,57], or abiotic [6,33,34,54] stressors and could impact the host, the microbiota or both.  
107 Dysbiosis could be both causal [52,58] or a consequence of the symptoms development [11,53].

108 It is important to consider that there isn't a single form of dysbiosis and process. Among them,  
109 the most intuitive dysbiosis process is a loss of controls in the microbiota community composition  
110 from the host or/and from changes in microbe-microbe interactions. This stochastic dysbiosis  
111 signature of microbial assemblages is seen herein to follow the Anna Karenina Principle (Box 2 &  
112 Fig 3) and be paired with a fitness decrease of the plant holobiont.

113

114 *—END OF BOX 1*

115

116 Dysbiotic microbiota can lead to diseases or symptoms i.e. a cause [11,31,40,52,58,59]. Chen  
117 et al argued that 'true dysbiosis implies a causative role of altered microbiota in inducing symptoms'  
118 [31]. Microbiota transplantation methods and the use of synthetic microbial communities make it  
119 possible to test the causality between dysbioses and symptoms [60,61]. A work on tomato bacterial  
120 wilt disease revealed dysbiosis to induce the lack of the protective gram-positive bacterial community  
121 (composed of 4 major beneficial bacteria) which increases disease incidence [49]. Another study of  
122 the same tomato bacterial wilt disease confirmed that the lack of particular species of bacteria leads  
123 to wilt disease [52]. In this case, the pre-existing community might favor pathogens' appearance  
124 through priority effects [41].

125 Reversely, external abiotic stressors or pathogens can lead to dysbiosis, i.e. a consequence.  
126 For instance, inoculation of *Rhizoctonia solani*, a fungal pathogen, is responsible for a deep  
127 modification of lettuce microbiome, linked with a decrease of plant fitness, i.e. a dysbiosis [53].  
128 Another fungal plant pathogen, *Verticillium dahliae*, uses effector proteins to manipulate tomato  
129 (*Solanum lycopersicum*) and cotton (*Gossypium sp*) microbiomes. This pathogen suppresses  
130 antagonist bacteria from the host microbiome, which facilitates its colonization and the appearance  
131 of symptoms [11]. Koch's postulates describe this phenomenon, i.e. when a single known pathogen  
132 led to disease. However, much evidence suggests that the pathogen establishment depends on both  
133 plant immunity and microbiome ability to ward off a given pathogen.

134 Therefore, dysbiosis can be both the cause and the consequence of stress or disease. That is  
135 why the concept of dysbiosis is often criticized [19,20]. It is thus necessary to conceptualize dysbiosis  
136 which in this opinion paper, uses the AKP to identify and describe dysbiosis as a transient loss of host  
137 capacity to regulate its microbiota, implying a loss of function that leads to a reduction of host's  
138 fitness (Fig 2).

139

#### 140 *Characteristics of plant dysbioses*

141 Dysbiosis can be characterized by a change in  **$\alpha$ -diversity** of microbial communities  
142 [31,59,62,63]. A change in  $\alpha$ -diversity can be explained by the drastic changes in the niche  
143 parameters, where only few opportunistic microorganisms are able to survive [64]. Because among  
144 samples either from the same or different individuals the new niches are chaotically colonized  
145 possibly from opportunistic microorganisms living nearby, an increased  **$\beta$ -diversity** is expected. This  
146 process can be explained by a loss of control of microbiota composition by the host and/or by  
147 modification of microbe-microbe interactions and related cascading effect.

148

#### 149 *From transient dysbiotic state to plant resilience: the cry for help strategy*

150

151 Pathogens and abiotic stressors can cause varying degrees of change to the composition of the  
152 microbiota. Recruitment of microorganisms is an important toolbox for a plant to buffer stress [65].  
153 This process expected to be determinist enables the plant to fight or resist the stress leading to a new  
154 equilibrium, highlighting resilience (Fig 2). This resilience process attenuating the holobiont fitness  
155 decrease was called 'cry for help strategy' [66,67].

156 First evidence for this strategy was through disease suppressive soils studies [68–70]. There  
157 is growing evidence that plants recruit beneficial microorganisms through the emission of volatile  
158 organic compounds [71,72] or emitted secondary metabolites from roots as among others, coumarins,  
159 which can modulate the root microbiota to face iron deficiency [67,73]. Secretion of salicylic acid,

160 malic acid, flavonoids and strigolactones have also been shown to shape root microbiota assembly  
161 [74–77].

162 In many cases the symbiosis is considered to be an adaptive response to stressors [59]. Cha et  
163 al (2016) showed that Actinobacteria have a key role during the attack of strawberry (*Fragaria*  
164 *ananassa*) roots by *Fusarium oxysporum*. They have an antifungal activity using thiopeptide  
165 antibiotic [78]. Plants can also recruit beneficial microorganisms that modulate their defense capacity  
166 by activating the jasmonic acid and salicylic acid signaling pathways both involved in the host  
167 immune surveillance [79,80]. Especially, jasmonic acid pathway is also known to be activated during  
168 plant response to stress [81] and is supposed to be involved in the filtration of microbiota components  
169 [82]. When the protective effect against a pathogen is extended to the next generation, this process is  
170 called “the soil born legacy” [83,84].

171

172 Thus, to face and buffer an environmental constraint, the host-plant can activate mechanisms  
173 of attraction, recruitment and exclusion (i.e. filtration) of microbial components. This allows the quick  
174 mobilisation of hundreds of genes to ‘passively’ adapt to the constraint. The transient maladaptive  
175 state is herein conceptualized as the dysbiotic phase.

176

### 177 **The Anna Karenina Principle (AKP) as a dysbiotic transient state**

178

179 The previous section on plant dysbiosis highlighted the need to compare a microbiota  
180 imbalance with normal heterogeneity (i.e. balanced/healthy microbiota) [21,22]. In this sense, the  
181 Anna Karenina Principle could be the appropriate conceptual framework to describe microbiota  
182 modification between two equilibria in stress conditions. The Anna Karenina principle (AKP) asserts  
183 that disease-associated microbiota present more stochasticity in their assembly processes compared  
184 to healthy ones.

185

#### 186 *AKP: from animal to plant diseases*

187 The AKP has already been described for animal microbiota [24], mostly for humans [23] but  
188 also for coral microbiota [88]. However, to our knowledge, the AKP has never been described and  
189 conceptualized for plant dysbiosis, both being the main aims of this paper. For soil and plant  
190 associated microbiota,  $\beta$ -diversity increases in abiotic stress conditions [54], validating, at least in  
191 part, the AKP assumptions for abiotic stressors. Other studies on biotic stress also confirmed the AKP  
192 effect, with a global increase in  $\beta$ -diversity in disease-associated microbiota compared to healthy-  
193 associated microbiota [11,48,49]. Gao et al. showed that  $\beta$ -diversity was higher in diseased chili



194 pepper (*Capsicum annuum* L.) for both fungal and bacterial communities [85]. As in animals, the loss  
195 of host control increases the stochasticity during microbiota assembly, leading to an AKP effect  
196 [24,89].

197 A few studies also highlighted the reciprocal anti-AKP effect, a deterministic microbiota  
198 modification induced by the pathogen, can also be identified (see next section ‘methods to identify  
199 the AKP’). Masson et al (2020) described the gallobiome, a microbiome associated with nematode  
200 infection of rice roots [51]. In this case, the pathogen stress drastically altered the niche parameters,  
201 with a very new, diverse (increased  $\alpha$ -diversity) and specific (decreased  $\beta$ -diversity) microbial  
202 community. A study on oak **pathobiome** highlighted the importance of the intensity of stress on  
203 microbial community dispersion [90]. Interestingly, when the level of infection was low (0-15%) the  
204 AKP was confirmed with high  $\beta$ -diversity, whereas when the level of infection was higher (20-30%),  
205 anti-AKP was confirmed. Liu et al (2022) showed a significant negative correlation between  $\beta$ -NTI  
206 (Beta Nearest Taxon Distance) and relative abundance of *Ralstonia solanacearum*, a bacterial  
207 pathogen of tomato [91]. This negative correlation means that the more pathogens there are, the more  
208 deterministic the processes are, leading to a lower species turnover compared to null model  
209 expectation. In these three examples, the pathogen attack might induce a transitory AKP effect. Then,  
210 by niche modification, the microbial community tended to be drastically selected by the habitat  
211 changes and/or pathogen manipulation, resulting in an anti-AKP effect (Fig 2B scenario A). More  
212 broadly, deep changes in microbiota habitat conditions resulting from either biotic or abiotic stress  
213 may induce rapid changes in the composition of the microbiota as a consequence of derived  
214 microbial-fitness modifications [24,54].

215

#### 216 *Methods to identify the AKP*

217 The higher the  $\beta$ -diversity, the greater the differences between samples of the same group. At  
218 first glance,  $\beta$ -diversity can be a very efficient tool to identify the effects of AKP. Indeed, if the  
219 microbiota dissimilarity is significantly higher in the stressed group than in the healthy one, it would  
220 point to an AKP effect, while if the contrary is observed (less dissimilarity in the stressed group) it  
221 would point to an anti-AKP effect (Box 2). The different kinds of dissimilarity between healthy-  
222 associated microbiota and disease-associated microbiota can be visualized through ordination  
223 methods (Fig 3). Under the AKP hypothesis, samples with healthy-associated microbiota will be more  
224 clustered than disease-associated ones.  $\beta$ -diversity is an index of differentiation between 2 local  
225 communities, but does not directly reflect underlying communities’ assembly processes.

226 The microbiota assembly rules result from a balance between stochastic and deterministic  
227 processes (Fig 1). The AKP predicts the rise of stochastic processes during the microbial community

228 assembly within the holobiont [24]. This rise of stochasticity could lead to two different scenarii (Fig  
229 2B). Scenario A predicts a decrease of  $\beta$ -diversity and scenario B predicts an increase of  $\beta$ -diversity.  
230 In scenario A, deterministic forces drive microbial communities to be more dissimilar than null model  
231 expectation in the healthy microbiota (e.g. through exclusive competition processes). However, in  
232 scenario B, it means that deterministic forces drive microbial communities to be more similar than  
233 null model expectation in the healthy microbiota [23,92]. At the opposite, a dysbiosis would follow  
234 anti-AKP processes if the deterministic part of the balance increases in the stressed group compared  
235 to in the healthy group (see Box 2 for more details). Using a null-model approach, Stegen et al (2012)  
236 developed an analytical framework that intended to infer the ecological processes that govern the  
237 microbial community assembly [93]. In particular, the Beta Nearest Taxon Distance ( $\beta$ -NTI)  
238 quantifies the turnover in phylogenetic composition between different microbial communities [94].  
239  $\beta$ -NTI values enable to estimate the relative importance of ecological drift (i.e. stochastic process)  
240 and selection (i.e. deterministic process) (Fig 2B). A  $\beta$ -NTI between -2 and 2 reveals a dominance of  
241 stochastic processes and a  $|\beta$ -NTI| > 2 reveals the dominance of deterministic processes. If  $\beta$ -NTI >  
242 2, it reveals a higher turnover and if  $\beta$ -NTI < -2, it reveals a lower turnover compared to null model  
243 expectation. Thus,  $\beta$ -NTI could be useful to detect if diseased microbiota do present an increased  
244 dominance of stochastic processes compared to healthy ones (see Box 2 for more details). More  
245 recently, Ning et al. (2019) developed a framework to quantify the proportion of stochastic versus  
246 deterministic processes during microbial community assembly. In particular, their normalized  
247 stochasticity ratio (NST) could be relevant to test the AKP on plants [92]. Also, a microbial  
248 community modification would not follow AKP if the stochastic versus deterministic balance is the  
249 same between the healthy and disease-associated microbiota.

250 Finally, an increased  $\beta$ -diversity is not necessarily linked to an increased stochasticity and  
251 loss of host control. This increased  $\beta$ -diversity could also be driven by deterministic processes and  
252 be revealed thanks to a  $\beta$ -NTI > 2. In this sense, we suggest to use both  $\beta$ -diversity and  $\beta$ -NTI (or  
253 NST) indices to highlight an AKP effect (Fig 2B) and distinguish the different scenarii.

## 254 **BOX 2: No-AKP vs AKP vs anti-AKP processes in plant dysbiosis**

255 Using both  $\beta$ -diversity and  $\beta$ -NTI estimates we can distinguish different cases of AKP, no  
256 AKP and anti-AKP effect.

### 257 **No AKP**

258 A dysbiosis would present no AKP if the  $\beta$ -diversity of the stressed group and the healthy  
259 group do not present any significant difference. Indeed, the balance between the deterministic and the  
260 stochastic processes would be the same between the healthy microbiota and the disease-associated

261 microbiota. In this case, both  $\beta$ -diversity and  $\beta$ -NTI would be the same between healthy and dysbiotic  
262 microbiota.

### 263 **AKP**

264 A dysbiosis following the AKP results in a higher proportion of stochastic processes in  
265 microorganism assembly compared to healthy microbiota, meaning that the new microbiota induced  
266 by an external stress is less predictable. As healthy microbiota are expected to be mostly driven by  
267 deterministic processes, there are different possibilities. The first case is when the healthy microbiota  
268 has a  $\beta$ -NTI  $< -2$ , *i.e.* deterministic processes leading to microbial communities that are more similar  
269 than null model expectation (Fig 2B, first step of scenario B). In this first case, an AKP effect would  
270 be detected if the  $\beta$ -NTI increases until 2, leading thus to an increase of both stochasticity and  $\beta$ -  
271 diversity. The second case is when healthy microbiota has a  $\beta$ -NTI  $> 2$ , *i.e.* deterministic processes  
272 leading to microbial communities that are more dissimilar than null model expectation (Fig 2B, first  
273 step of scenario A). In this second case, an AKP effect would be detected if the  $\beta$ -NTI decreases until  
274  $-2$ , leading thus to an increase of stochasticity and a decrease of  $\beta$ -diversity. This second case  
275 highlights the importance of using both  $\beta$ -diversity and  $\beta$ -NTI to describe microbial shift as an AKP  
276 effect.

277 What is hiding under the AKP effect? In plants, an AKP effect could be due to a reduction in  
278 host immune control of its microbiota composition from modifications of the **PTI** and **ETI** immune  
279 response, allowing stochastic colonization of opportunistic bacteria and possibly pathogens [95,96].  
280 This random establishment of microorganisms might be explained by several types of ecological  
281 interactions. For instance, if two microbial species have mutual inhibitory actions, the one that is  
282 more dominant at the beginning will exclude the other. This initially dominant species may differ in  
283 another individual, which explains why the two hosts can present different kinds of dysbiosis.  
284 Another possible explanation is that stochastic changes in the biotic and abiotic environmental  
285 conditions may have different impacts on each individual along with the accessible reservoir of  
286 recruitable microorganisms. Step by step, these tiny random modifications at the individual scale may  
287 lead to progressively divergent communities. It may also be possible that ecological processes as (i)  
288 the epidemic development of a given microorganism with a high competitive ability, or (ii)  
289 microorganisms that release compounds that are toxic to part of the existing community [11]. Thus,  
290 behavioral processes may explain AKP effect not only from the microbial side but also through  
291 modifications of plant-microorganisms' interactions as with limited transfer of organic compounds  
292 from the plant to its symbionts or conversely a modification of a symbiotic microorganism becoming  
293 cheater [64].

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## Anti-AKP

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Conversely, a dysbiosis following anti-AKP processes would be related to changes in the microbial community driven by deterministic processes. In such conditions, there are two different possibilities, (i) a  $\beta$ -NTI < -2, i.e. a lower microbial turnover than expected by null model or (ii) a  $\beta$ -NTI > 2, i.e. a higher microbial turnover.

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In the first case, it means that in a population, the same stress with the same intensity would modify the microbial community in the same way among individuals (Fig 2B, last step of scenario A). These deterministic processes can be driven by deep modifications of the host's physiology, morphology and hence ecological niche allowing the establishment of a new microbial community [51]. This process was demonstrated by Rocca et al. (2019). Indeed, these authors showed that diesel and overgrazing stressors may strengthen ecological selection and increase the importance of deterministic shifts in the composition of the microbial community [54]. We hypothesize that the more drastic the stress, the more deterministic the shift, because the niche will correspond to highly selective environmental conditions, where only specialized microorganisms would be able to grow. The anti-AKP effect detected could also be due to a strong environmental filtering, and not so much the selection of the host itself leading to convergent recruitment. More often, anti-AKP effect is expected to be detected after the dysbiotic transient state. In this case, the increased deterministic process is the strong selection by the host-plant of beneficial microorganisms, as through the 'cry for help' strategy [66]. One outstanding issue is to be able to distinguish the environmental filtering from the plant selection of specific microorganisms.

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In the second case, the anti-AKP effect would be characterized by an increased microbial turnover (Fig 2B, last step of scenario B). In this case, it could be a plant strategy that tends to deterministically favor microbial turnover in order to optimize chances of being colonized by beneficial microorganisms [6,33–35].

319

*–END OF BOX 2*

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*Using the AKP conceptual framework to study microbial shift under stress conditions*

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AKP is a conceptualization of the plant transient state that follows the stress and that may precede the resilience of the plant holobiont (Fig 2). Increased  $\beta$ -diversity in a stress group emphasizes the high degree of dissimilarity between disease-associated microbiota. This increased  $\beta$ -diversity is interpreted as an increase in some of the stochastic processes during microbiota assembly in stress conditions (Fig 3B-D). In normal conditions, deterministic processes of plant microbiota assemblage are expected to dominate (Fig 3A-C). However, stress can limit the plant's ability to select beneficial microorganisms and fight other microorganisms. This loss of ability

329 enables many different microorganisms to colonize the plant, explaining the marked dissimilarities  
330 observed between disease-associated microbiota (Fig 3B).

331 Using AKP to analyze dysbiosis has many advantages as discussed above (e.g.  
332 conceptualization of dysbiosis from which hypotheses can be tested leading to new interpretation of  
333 dysbiosis and/or rejection of the concept under particular conditions). Issues in the dysbiosis  
334 methodological characterization can also be pushed forward to exemplify the new outputs and  
335 interpretations that could permit the dysbiosis conceptualization. For instance, in response to drought  
336 conditions, different studies reported higher  $\beta$ -diversity in stress-associated microbiota, but in all of  
337 them, the Actinobacteria phylum increased in response to stress [6,33–35]. Even if  $\beta$ -diversity  
338 increases at the species level, a functional redundancy within these Actinobacteria could be an  
339 explanation for their recruitment by the plant to survive drought conditions. Thus, this apparent  
340 stochastic process could in fact be interpreted as determinist since the observed increase in a specific  
341 phylum occurred across independent studies, a case that might be detectable with a  $\beta$ -NTI > 2 (i.e.  
342 higher apparent microbial community diversity/turnover than expected). Omae et al. 2022 argued in  
343 this sense, as the recruitment of Actinobacteria in drought conditions is conserved between plant  
344 species and could be thus interpreted as deterministic [97]. Elsewhere,  $\beta$ -diversity can also increase  
345 due to (i) opportunistic microorganisms even if the plant selects specific bacteria in a deterministic  
346 manner [78,79], (ii) genetic innovation that modifies the fitness of a given microorganism within the  
347 microbiota and/or to the host. Because dysbiosis induced by a stress-effector could be a transitory  
348 state, the proportion of stochastic versus deterministic processes could also vary over time. To avoid  
349 these seemingly stochastic processes, further analyses such as phylogenetic factorization methods can  
350 be useful to highlight the deterministic selection of microbial clades by the plant [98]. Another  
351 possibility would be the analysis of functional gene profiles [58]. This functional gene profile analysis  
352 shows deterministic processes of gene expression in response to stress. However, these limits to the  
353 AKP conceptual framework can lead us to think and interpret microbial community perturbation more  
354 deeply and provide new opportunities for future research.

355

## 356 **Conclusions and prospects**

357 Many studies highlight the key role of microorganisms on plant health (Fig 1). The plant  
358 holobiont is dynamic in its composition and can change over time in response to external biotic or  
359 abiotic parameters. Under stress conditions, the microbiota can either resist or be disrupted leading  
360 to dysbiosis within the holobiont (Fig 2). However, through deterministic selection of beneficial  
361 microorganisms, the holobiont can increase its resistance to these external stressors. These processes  
362 highlight holobiont resilience. Thus, we propose the Anna Karenina Principle (AKP) to describe and

363 interpret ecological stochasticity associated with the transient state of dysbiosis (Fig 3). The AKP  
364 highlights the difference between the dominant deterministic processes of healthy microbiota  
365 composition versus the dominant stochastic processes of sick microbiota. Studying the AKP effect  
366 could be a good way to estimate plant microbiota health.

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567 **GLOSSARY:**

568

569 **Anna Karenina Principle:** hypothesis asserting that stress/disease associated microbiota  
570 display more variability among themselves than healthy ones. This increased variability is due  
571 to an increase in stochastic processes during microbial community assembly.

572 **Deterministic processes:** ecological processes such as environmental filtering, competition,  
573 trophic interaction or plant selection that drive microbial community assembly.

574  **$\alpha$ -diversity:** mean species diversity at the local scale.

575  **$\beta$ -diversity:** species diversity differentiation between 2 local communities.

576  **$\beta$ -NTI:** Beta Nearest Taxon Distance quantifies the turnover in phylogenetic composition  
577 between different microbial communities.  $\beta$ -NTI values enable to estimate the relative  
578 importance of ecological drift (i.e. stochastic process) and selection (i.e. deterministic  
579 process). A  $\beta$ -NTI between -2 and 2 reveals a dominance of stochastic processes and a  $|\beta$ -  
580 NTI| > 2 reveals the dominance of deterministic processes.  $\beta$ -NTI > 2, it reveals a higher  
581 turnover and if  $\beta$ -NTI < -2, it reveals a lower turnover compared to null model expectation.

582 **Dysbiosis:** In this paper, we define dysbiosis as a transitory loss of host capacity to regulate  
583 its microbiota, implying a loss of function that implicitly leads to a reduction of the holobiont  
584 fitness.

585 **Endosphere:** the inside of the plant, that constitutes a habitat for microorganisms.

586 **ETI (Effector-Triggered Immunity):** second layer of plant immunity based on the recognition  
587 of effectors by R proteins.

588 **Holobiont:** association between a host and its microbiota that is considered as a biological  
589 unit.

590 **Hologenome:** a level of biological organization resulting from the association between a host  
591 genome and its microbiota genomes. This concept is based on the importance of the  
592 microbiota for host fitness, evolution processes and the related genetic controls driving  
593 microbiota assembly.

594 **Microbiota:** all microorganisms associated with a host. Many are beneficial, being involved  
595 in many host functions and thus increasing host fitness.

596 **Pathobiome:** host microbiota associated with a disease induced by a pathogen.

597 **Phyllosphere:** the aboveground habitat including both epiphytic- (i.e. the phylloplane) and  
598 endophytic habitat. No consensual definition exists yet, this one being close to the  
599 etymological definition.

600 **Priority effect:** impact of priori arrival of a species on subsequent community development.

601 **PTI** (PAMP-Triggered Immunity): first layer of plant immunity based on pathogen-  
602 associated-molecular patterns recognition by pattern recognition receptors (PRR).

603 **Rhizosphere:** close to the roots, the microorganism-habitat under the influence of the plant  
604 notably through the emission of root exudates by the plant.

605 **Stochastic processes:** all the processes that cannot be predicted by a model, in this case, the  
606 random probability distribution of the microbial community assembly; not subjected to  
607 selection. In ecology, stochastic processes include probabilistic dispersal (random  
608 colonization to a new site) and ecological drift (random shift in abundance).

609 **Stress:** abiotic or biotic factors that impact organisms (decreasing fitness).

610

611 Figure legends:

612 **Figure 1: Overview of the plant microbiota, its establishment, and its role in plant holobiont**  
613 **fitness.**

614 The plant microbiota is composed of the phyllosphere, the rhizosphere and the endosphere  
615 (A). The phyllosphere is the region near the leaves and is established from the seeds, the air bulk or  
616 interaction with the rhizosphere (D). Most leaf microorganisms are found outside the plant, with a  
617 role in stress tolerance and pathogenic resistance (B). The rhizosphere is the region where plant roots  
618 and soil microorganisms have an impact on each other (C). The rhizosphere is composed of a fraction  
619 of the soil microbial reservoir passively or actively recruited by the release of rhizodeposits (i.e.  
620 various C compounds emitted by plants at the root surface) and by transmission from the seeds (A).  
621 Root microorganisms are essential for water and ion intake, nitrogen fixation and protection against  
622 pathogens (C). Ordination analyses unable to identify clear differences among soil and air bulk,  
623 rhizosphere, root endosphere, phyllosphere and leaf endosphere microbiota with deterministic  
624 processes of selection by the plant. Moreover, there is less stochasticity during microbiota assembly  
625 in the endosphere when plant-microorganism interactions are more intimate. (Figure created with  
626 Biorender).

627

628 **Figure 2: Stress, dysbiosis and adaptation of the plant holobiont.**

629 (A) A plant is in a healthy state when nothing affects its fitness (1). External stressors can be both  
630 abiotic (e.g. drought, temperature, pollutant) and biotic (e.g. pathogens) (2). If the plant holobiont is  
631 resistant to these stressors, the initial healthy state will be maintained (3). However, these stressors  
632 can affect host fitness (physiology, survival, reproduction, etc.) and the composition of the  
633 microbiota, leading to dysbiosis (3'). In response to stress, the host can select beneficial  
634 microorganisms from the environment to strengthen stress tolerance (4). This modification of the  
635 microbiota leads to an adaptation of the holobiont to the external stress, highlighting holobiont  
636 resilience (5) and leads to a new healthy state (6). (Figure created with Biorender).

637 (B) Method of identification of AKP / anti-AKP using  $\beta$ -diversity and  $\beta$ -NTI and related possible  
638 scenarii. In scenario A, the deterministic processes of assembly of the healthy microbiota increase  
639 microorganisms' turnover compared to null model expectation. The loss of host control increases the  
640 stochasticity leading to an AKP effect. This AKP effect is expected to be transient, as the plant could  
641 recover its control on microbiota composition (solid arrow), or alternatively (dotted arrow), new types  
642 of deterministic processes could lead to a decrease of  $\beta$ -diversity (anti-AKP). In scenario B, the  
643 deterministic processes of assembly of the healthy microbiota decrease microorganisms' turnover  
644 compared to null model expectation. The loss of host control increases the  $\beta$ -diversity (AKP effect).  
645 After the transient AKP effect, the plant could recover its original control (solid arrow), or

646 alternatively new types of deterministic processes could lead to an increase of  $\beta$ -diversity (either plant  
647 strategy or stress effect).

648

649

650 **KEY FIGURE:**

651 **Figure 3: Plant dysbiosis and the Anna Karenina Principle (AKP).**

652 The AKP asserts that “all healthy microbiota are alike; each disease-associated microbiota is sick in  
653 its own way”. Thus, it postulates that disease-associated microbiota present more dissimilarities  
654 among themselves (B) (e.g., higher  $\beta$ -diversity) than healthy ones (A). These dissimilarities can be  
655 visualized with ordination representation, with a higher/stronger dissimilarity among disease-  
656 associated microbiota (D) than among healthy ones (C). Also, the AKP predicts an increase in  
657 stochastic processes during the microbial community assembly of disease-associated microbiota (D)  
658 compared to healthy ones (C). (Figure created with Biorender).

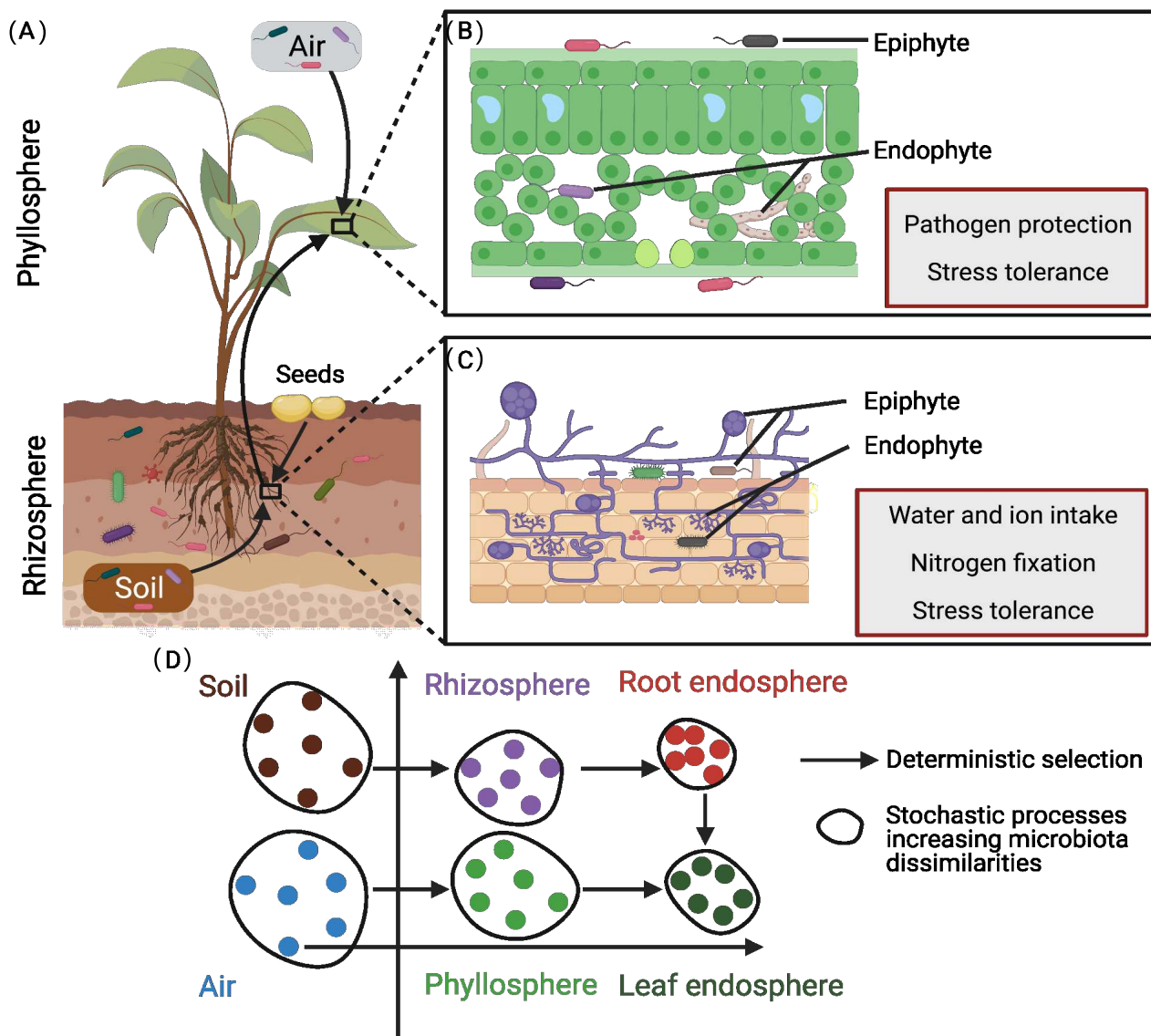
**OUTSTANDING QUESTIONS:**

- What is the relative proportion of deterministic and stochastic processes during microbial community assembly (MCA) of healthy *vs* stressed plants?
- How can we distinguish between environmental filtering and plant deterministic selection of microorganisms and what is the relative proportion of these deterministic processes during environmental MCA?
- How can we avoid apparent stochasticity through functional redundancy tools?
- As stress can impact each microorganism differently, what is the definition of stress for a whole microbiota?
- What is an altered plant microbiota?
- What is the relevance of the transposition of the Anna Karenina Principle to the understanding of the plant microbiota?

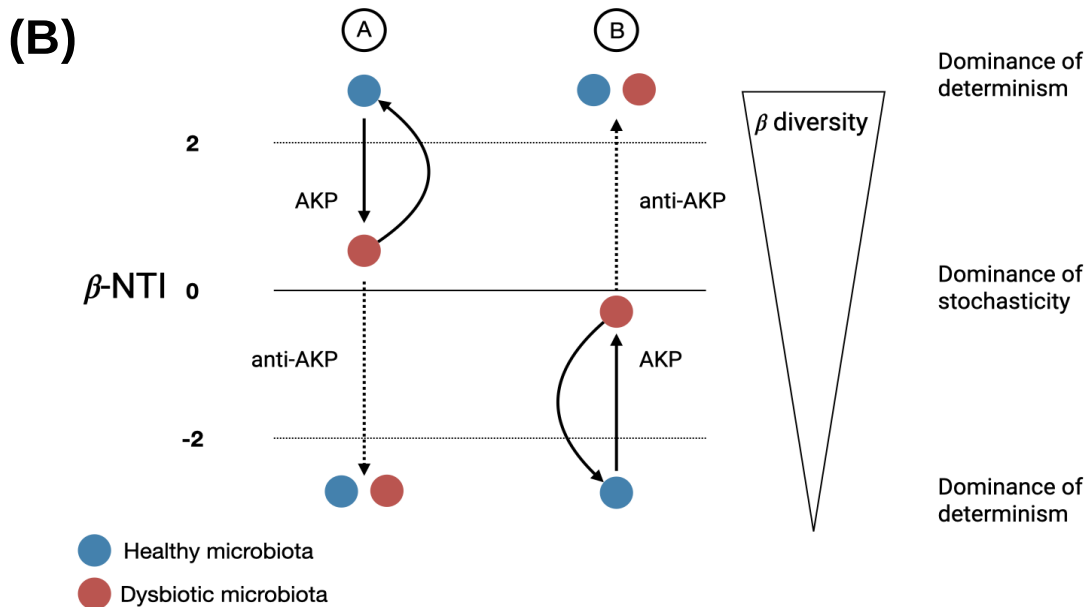
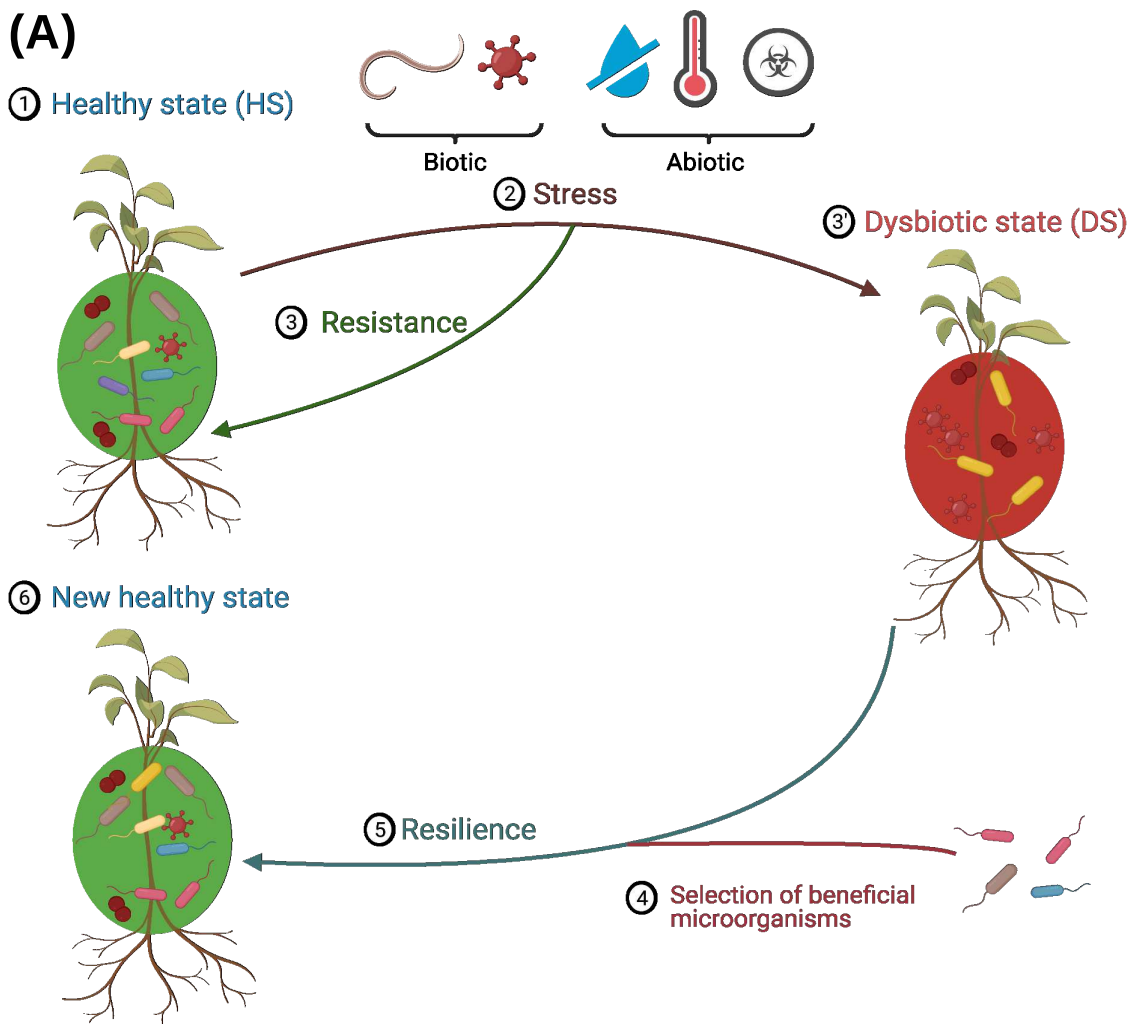
**HIGHLIGHTS:**

- The involvement of plant microbiota in many plant functions, including resistance to abiotic or biotic stressors, water and nutrient intake, recently led to the holobiont concept.
- Shifts in microbiota composition linked to stress or disease is termed dysbiosis, but whether the shifts are a cause or a consequence of the stress / disease remains unknown.
- The Anna Karenina Principle (AKP) describes how microbiota are affected by external stressors and asserts that dysbiotic microbiota vary more among themselves than healthy microbiota. From this AKP, different dysbiosis scenarii can be identified by analysing the  $\beta$ -diversity and evaluating the proportion of stochasticity in the community assembly.
- In response to external stress, the plant holobiont can either resist or recruit beneficial microorganisms from its environment. This latter mechanism emphasizes plant holobiont resilience.

Figure 1







Scenario A: from increased stochasticity (AKP) to niche selection (anti-AKP)

Scenario B: from increased stochasticity (AKP) to driven changes leading to diversification (anti-AKP)

In both scenarios, AKP effect is expected to be a transient state.

