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1 **Woody Plant declines. What's wrong with the microbiome?**

2

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33 **Abstract**

34 Woody plant (WP) declines have multi-factorial determinants as well as a biological and  
35 economic reality. The vascular system of WPs involved in the transport of carbon, nitrogen  
36 and water from sources to sinks has a seasonal activity, which places it at a central position  
37 for mediating plant–environment interactions from nutrient cycling to community assembly  
38 and for regulating a variety of processes. To limit effects and to fight against declines, we  
39 propose (i) to consider the WP and its associated microbiota as an holobiont and as a set of  
40 functions, (ii) to consider simultaneously, without looking at what comes first, the  
41 physiological or pathogenic disorders and (iii) to define pragmatic strategies including  
42 preventive and curative agronomical practices based on microbiota engineering.

43

44 **Woody plants in ecosystem functioning**

45 Plants can be simply divided into annual, biennial (life cycle from seed to seed lasts  
46 one or two years) and perennial plants (can survive more than 2 years). Woody plants (WP)  
47 are perennial plants characterized by the presence during the non-growing season of  
48 persistent aboveground dormant parts, as well as ligneous material (mainly in vascular  
49 tissues).

50 WPs play a major role in the functioning of the ecosystem. For example, they actively  
51 participate in water and CO<sub>2</sub> cycles, especially within complex ecosystems such as forests [1].  
52 WPs render many ecosystem services, either directly when providing economically  
53 important goods and services (*e.g.* wood, fiber and fruits) or indirectly as reservoir of  
54 biodiversity [1]. In a context of global warming, their carbon (C) sequestration capacity is  
55 also of particular interest [2].

56 In their tissues or organs, WPs host a set of symbiotic and free-living microorganisms  
57 (*i.e.* archae, bacteria, fungi, oomycetes, protists and viruses) commonly referred to as the  
58 **microbiota**. Although biomolecular techniques for detecting the diversity of microorganisms  
59 are improving, host-microbiota interaction mechanisms are still poorly understood [3–5].  
60 The emergence of the “**holobiont**” concept, *briefly defined as a “functional entity formed by*  
61 *a macrobe and its long- and short-term associations with microbes and viruses”* [6], opened  
62 the possibility to view an entire system.

63 In the present review, we first discuss the origin of WP declines with a focus on the  
64 link between flow and storage of carbon (C), nitrogen (N) and water in WPs and their

65 transfer to/from associated microbes, in healthy or declining conditions. Then, we introduce  
66 definitions and concepts about the interactions between WPs and their microbiota, often  
67 inspired by knowledge on animal-associated microbiota. Finally, we discuss prospects of  
68 microbiota engineering to enhance tolerance of WPs to declines.

69

## 70 **Woody plant declines: pathological and physiological disorders**

### 71 *Decline versus dieback*

72 In the last decades, WP declines are affecting more and more species [7]. They can be  
73 defined as a long-term degeneration of plant tissues following a succession of multiple  
74 negative events (*e.g.* abiotic stresses, climate deregulation, emergence of new pathogens,  
75 biological invasions and agricultural strategies). WP declines may also be seen as a decrease,  
76 over multiple years, of wood or fruit productivity, leading or not to a sudden death, the  
77 latter being occasionally referred to as dieback (**Box 1**). Several authors use ‘decline’ strictly  
78 for the progressive reduction of the vigor of the WP, whatever its causes and symptoms,  
79 while they would associate ‘dieback’ to the drying of the plant that starts at the tips [8].  
80 However, the distinction between ‘decline’ and ‘dieback’ is not always made or accepted as  
81 such by forest pathologists [8,9]. For better readability, we will retain only the term ‘decline’  
82 later in this review.

83

### 84 *Origin of the declines and impact on productivity and ecosystem services*

85 Individual tree mortality, estimated by the ICP forest network ([www.icp-forests.net](http://www.icp-forests.net)  
86 ; [10]), has doubled in Europe’s temperate forests over the last three decades [11]. Per  
87 year, about 0.5 to 1.5% of trees died owing to different factors such as fungi, fire, grazing,  
88 climate change. Today, the impact of WP declines on ecosystem services has reached an  
89 apparent peak and brought to our attention the necessity to identify determinants and  
90 mechanisms leading to their occurrence [12]. However, the main mortality factor remains  
91 undetermined (**Box 2**).

92 Longevity and late sexual maturity of WPs make their physiology, development and  
93 ecology different from annual plants. WPs are especially vulnerable to pathogens since  
94 exposure lasts for a longer period of time compared to annual plants [13]. WPs are also  
95 increasingly exposed to pathogens due to silvicultural and fruit farming techniques, and

96 especially globalization, favoring fast dispersal of microbe species along commercial routes  
97 [14].

98         Compared to less complex or non-multifactorial diseases, it is difficult to define a  
99 state of decline based on the observed symptoms, as these vary widely according to the  
100 species or individuals concerned. Declines may affect different compartments and the  
101 symptoms may be diverse (*i.e.* twigs drying, leaves yellowing, wood rotting). Since declines  
102 result from the combination of various factors, the association of given symptoms to their  
103 origin is challenging. In most declines, the whole plant is damaged including woody parts and  
104 leaves. A given decline can first appear in a specific compartment, then spread to others, and  
105 finally affect the balance of the whole holobiont. After a while, it leads to visible symptoms  
106 such as a significant loss of biomass as shown in poplar [15] and ash [16,17], or a decrease in  
107 the quantity and quality of fruits (*e.g.* grapevine or olive tree) [18,19]. In addition to the  
108 economic impact (*e.g.* loss of quality, quantity, yield), forest diseases can affect some  
109 ecosystem services either directly (*e.g.* C sequestration, water purification, soil stability) or  
110 indirectly (*e.g.* associated biodiversity, recreational and cultural aspects) [20,21].

111

112 *Carbon, nitrogen and water in WP declines: fueling and fluxes problems?*

113         WP species exhibit a wide range of gas exchange responses and hydraulic strategies,  
114 depending on whole-tree conductance and rooting depth. Photosynthesis essentially takes  
115 place in leaves during the light period. The Rubisco-fixed C is partly accumulated (i) as starch  
116 in the chloroplast to sustain metabolism during the dark period and (ii) as hexoses and  
117 sucrose in the cytosol for both daily metabolism and plant long distance transport. The  
118 excess of C is exported for long-term storage in the trunk and roots. Within the plant,  
119 carbohydrate are transported from photosynthetic and storage tissue (sources) to areas of  
120 active growth and metabolism (sinks). This phenomenon is at least partially regulated by a  
121 large number of monosaccharide- and sucrose-specific transporters, identified in WPs as  
122 poplar and grapevine [22,23]. Besides C, nitrogen (N) is required in significant quantities as it  
123 constitutes 1–5% of the WP dry weight [24]. N compounds are taken-up directly from the  
124 soil either under organic or inorganic forms directly through the plant roots- or the  
125 mycorrhizal-pathway. They are then transferred to the upper parts in the form of amino  
126 acids *via* an interconnection of the xylem and the phloem (**Box 4**) [23,25]. Because N-

127 containing compounds are necessary for C assimilation, N mobilization and C assimilation  
128 are probably tightly co-regulated to allow the optimal development of WPs [26,27].

129 C storage organs, varying depending on the season [28], play a key role in annual and  
130 seasonal metabolism and growth variations in WPs [26], as it represents resources that build  
131 up in the plant and can be mobilized in the future. This is especially true in deciduous species  
132 where in spring remobilization of reserves is necessary for budburst and growth of new  
133 leaves, allowing functional photosynthesis [29]. N is stored before leaf fall. Two thirds of the  
134 foliar N, mainly as Rubisco, which is primarily a storage protein, is recycled during the leaf  
135 senescence process, then transported and stored as amino acids and proteins in the  
136 perennial parts of the tree [30]. C and N stored in autumn in the trunk and roots are used for  
137 wood formation, especially for the phloem [31], before N root uptake is efficient.

138 As C, N and water transport depends on source and sink activity, any disturbance  
139 could influence WP functions, from growth and allocation to defense and reproduction.  
140 Leaves, trunks and roots perform unique functions, necessary for a healthy development.  
141 Interruptions caused by short, long term or successive stresses, in any of the functions  
142 performed by leaves (decrease of photosynthesis leading to less food and fewer energy  
143 storage), trunks (damage to vascular tissues reduces or inhibits C, N and water transport)  
144 and roots (improper soil conditions and mechanical damages lead to lesser root extension)  
145 may lead to plant starvation. These interruptions, combined with defenses against other  
146 diseases (physiological or pathogenic), could finally lead to decline. In addition, several  
147 groups have recently proposed to link forest declines with microbiome modification or  
148 imbalance [32].

149

## 150 **The WP holobiont**

151 The recent explosion of the number of studies on human gut microbiota highlighted  
152 links between altered microbiota (dysbiosis) and development of immune-mediated and  
153 metabolic pathologies such as obesity, type-2 diabetes, or cancer [33]. In this context, one  
154 could imagine a transposition to the study of links between taxonomic composition and  
155 functioning of the WP microbiota and decline outcomes.

156

157 *Key concepts on the plant holobiont*

158           Plants do not live alone, but are colonized and surrounded by microbes. Microbial  
159           colonization, whether **epiphytic** or **endophytic**, vary between plant tissues. A wide diversity  
160           of microbial taxa is involved in these associations going from pathogenic to beneficial  
161           through neutral [34,35] (**Figure 1**) as revealed by more and more sophisticated methods (see  
162           for instance Mercado-Blanco, 2018 [3] and **Outstanding question 1**). The microbiota  
163           corresponds to all microbes associated with a plant and the pool of microbial genes  
164           corresponds to the **microbiome** [4,36]. In addition, it appears that (i) from one individual to  
165           another the microbiota and the microbiome are different, (ii) within the same individual,  
166           several microbiomes or microbiota co-exist among different tissues [37,38] and (iii) microbes  
167           might support various plant functions. Therefore, to investigate a potential link between the  
168           microbiota or microbiome and declines, it is necessary to (i) analyze the diversity of  
169           associated microbes, (ii) determine how microbes interact amongst themselves and with the  
170           plant and (iii) highlight their respective potential functions and their impact on WP  
171           metabolism and development.

172           The short and long-term interactions between host-microbe and microbe-microbe  
173           mediate both host and microbial properties and are essential for the health of the host  
174           [3,34,39]. Plant microbiota is carried out either by vertical transmission through the seeds or  
175           by horizontal transmission through environmental sources [40]. Within plant microbiota,  
176           beneficial microbes (i) provide or extend major metabolic functions of plants, especially in  
177           terms of nutrient acquisition [3,41,42], (ii) promote plant resistance against biotic and  
178           abiotic stresses [43,44], (iii) mediate metabolites production and (iv) support seed  
179           germination [45]. Considering the importance of the gained functions, some authors account  
180           microbiome for a 'second plant genome'. Combination of plant host and microbiota is thus  
181           referred to as 'holobiont' (**Figure 1**), which can be defined as 'the genomic reflection of the  
182           complex network of symbiotic interactions that link an individual of a given taxon with its  
183           associated microbiome' [42,46,47]. The holobiont concept applied to plants allows a new  
184           interpretation of the **facilitation** concept in plant ecology, the microbiota being considered  
185           as a 'facilitator' element providing additional functions to the holobiont and adjusting to  
186           environmental conditions that are optimal for survival and to keep or reinforce plant  
187           **homeostasis** [48].

188

189           The **hologenome** theory takes the holobiont as a unit of selection in evolution [49],  
190 where the microbiome is associated with the plant genome both evolving in parallel. Pioneer  
191 descriptions of plant microbiota have been published for model plants such as *Arabidopsis*  
192 *thaliana*, *Medicago truncatula* and *Lotus japonicus* [50–52]. Microbiota of WPs has been  
193 described on poplar [53–55], oak [56,57] and grapevine [5], either on a plant compartment,  
194 including root system [34], trunk [58], **phyllosphere** [55], fruits [59], or as a comprehensive  
195 analysis [54,60].

196

197    *Concept of ‘core microbiota’: any bacterial or fungal species shared in healthy and diseased*  
198 *plants*

199           Plant diseases, either physiological or ecological, are often associated with changes  
200 or compositional deviations from a ‘core microbiota’ of the holobiont [50] (**Figure 2**). The  
201 core microbiota concept emerged with the discovery that plant microbiota differs from  
202 rhizospheric microbiota. Briefly, healthy plants favor colonization by commensal microbes  
203 and avoid its colonization by pathogens [3,50]. The core microbiota varies with plant age and  
204 compartment as well as soil properties [61,62]. The idea of a ‘functional core microbiota’  
205 playing a central role in plant physiology and health is now admitted [63], and is often  
206 described as the sum of ‘the good’ (plant beneficial microbes), the ‘bad’ (plant pathogenic  
207 microbes) and ‘the ugly’ (human pathogenic microbes) [35]. From one plant to another, core  
208 microbiota does not consist of identical microbial taxa, but rather different taxa that ensure  
209 the same set of essential functions for the holobiont fitness [63]. **Key or hub species** of this  
210 core microbiota can be affected during the onset of declines, making the holobiont  
211 ineffective against the spread of symptoms.

212

213    *The holobiont functioning: exchange of good practices between partners?*

214    **A chemical and molecular dialogue within holobiont.** Within the holobiont, plant-microbe  
215 and microbe-microbe communicate mainly through chemical and molecular signaling [64]. A  
216 large part of microbiome studies focused on the **rhizosphere** were dedicated to quorum  
217 sensing (strictly microbe-microbe communication) and signaling molecules [65] such as  
218 volatile organic compounds, phytohormones or antimicrobial peptides [66]. Different  
219 communication tools are used to recruit beneficial microbes, to activate desirable microbe  
220 traits, induce systemic defense, coordinate microbial population behavior and activity, shape



221 rhizomicrobiome or establish mutualistic symbiosis with fungi or bacteria [66]. For instance,  
222 mycorrhizal fungi form mutualistic associations called **mycorrhizas** [57,67–70], able to  
223 transfer signaling molecules and nutrients between plants through **common mycorrhizal**  
224 **networks** [71] (**Box 4**). Disruption of this communication tools could be linked to the  
225 evolution of declines in WP (see **Outstanding question 2**).

226

227 ***Around the roots: a top exchange zone.*** The rhizosphere and the mycorrhizosphere refer to  
228 the zone of influence created by roots alone or with the mycelium of mycorrhizal fungi,  
229 influencing the composition and function of its microbiota through their exudates, and *vice*  
230 *versa* [71,72]. The soil is subjected to numerous abiotic and biotic stresses, affecting the  
231 diversity, abundance and activity of living organisms in the soil and consequently the  
232 functioning of the ecosystem [73]. Soil microbial community can affect plant survival, growth  
233 and tolerance to biotic and abiotic stresses and induce systemic resistance against pests and  
234 pathogens both in leaves and roots [34,74,75]. The microbial lifestyle could switch from  
235 rhizospheric to mycorrhizospheric or from epiphytic to endophytic depending on  
236 environmental factors [76].

237 C and N are the main fuels for plant development and the basis of **rhizodeposition** by  
238 root exudation of primary metabolites. Rhizodeposition describes the C-root loss from  
239 plants, ranging from 10 to 40 % of assimilated C [77]. The flux of root-derived C (sugars,  
240 sugar alcohols, amino acids and phenolics) constitutes a substantial nutrient release in soils,  
241 with sugars the main C source for microbes as mycorrhizal fungi and bacteria [78,79]. It was  
242 suggested that the more C translocated from leaves to roots, the more C ought to be exuded  
243 from roots (*e.g.* [80]) and that C exudation by roots is controlled by factors drawing C into  
244 the rhizosphere, such as root colonization by fungi (*e.g.* [81]). In addition to sugars, released  
245 amino acids are used by microbial communities as C-N source, but are also recognized by  
246 microbial chemoreceptors involved in the early root colonization [82]. Several authors have  
247 proposed that differences in sugars and amino acids concentration between root cells and  
248 root vicinity mediate root exudation [83], but mechanisms and transport systems remain  
249 largely unknown.

250

251 *C-N pools, WP declines and associated microbes: a vicious circle?*

252 WP storage is highly sensitive to variations in environmental factors. Pathogens  
253 involved in declines accentuate some of these physiological dysfunctions in C-N production  
254 and flow. In several WP declines, physiological functions needed to assure proper storage  
255 are disrupted, although such disruptions might be associated with other type of diseases. For  
256 example, in the olive tree CoDiRO disease (**Box 1**), leaves are subjected to chlorosis or  
257 wilting, leading to the reduction of the leaf surface area and a lower photosynthesis rate. In  
258 *Eutypa dieback* or Esca disease in grapevine [84], anatomical analyses have shown a  
259 degradation of the photosynthetic systems (*e.g.* chloroplast alteration, thylakoid and  
260 endomembrane deformation). In addition, leaf degradation leads to a reduction of stomatal  
261 conductance, gas exchanges between atmosphere and the plant, and hydraulic lift from the  
262 soil to the leaves [84].

263 C and N storage can also be reduced after vascular system degradation or plug-  
264 blocking. Cankers in the trunk disturb water flux by degrading xylem vessels. The bacterium  
265 *Xylella fastidiosa*, involved in Pierce disease and CoDiRO, forms plugs and blocks xylem sap  
266 circulation [85]. In the case of horse chestnut bleeding canker, presence of *Pseudomonas*  
267 *syringae* is related to the stopping of water flow through the trunk [13]. In grapevine, *in vitro*  
268 tests showed disappearance of all starch reserves after 18 months of *Eutypa lata* activity  
269 [86]. Following the damage, three allocations for the non-structural sugars are possible: (i)  
270 storage for re-growth when the threat has passed (*i.e.* poplar; [87]), (ii) growth of sink  
271 tissues such as fine roots or young leaves, and (iii) synthesis of defensive compounds  
272 (poplar: [88]; *Quercus ilex*: [89]). Similar reactions following the damage were recorded for  
273 N allocation on common milkweed and red oak [90,91]. N deprivation in the previous  
274 autumn (N blocked in roots and woods) leads to budburst disturbance, C resource depletion  
275 and tree decline in spring. Finally, root architecture could be modified leading to the  
276 reduction of water and N uptake [92].

277 Finally, the question of which came first, the physiological or ecological disease, is still open  
278 (Figure 3). Is it possible that a monofactorial disease, readily identifiable, leads to a  
279 weakening of the WP? This would make the affected WP more susceptible to other stresses  
280 leading to an overall decline. Conversely, it can be assumed that multiple stresses applied to  
281 a WP weaken it, lowering its innate immune response when a specific pathogen arrives,  
282 acting like an opportunist (**Box 2**).

283 This question is still open depending on the type of pathogen involved and the type of stress  
284 applied. Indeed, a strict biotrophic agent can be disadvantaged during the infection of a  
285 plant already stressed and deficient, which cannot provide the necessary nutrients for its  
286 metabolism. On the contrary, saprophytes (*e.g.* the GTD responsible fungi) will be favored  
287 within already weakened plants. These saprophytic pathogens are often latent members of  
288 the plant holobiont, but their virulence would only be expressed during a modification of the  
289 microbial community due to the previous weakening of the plant (with for example an  
290 exclusion of beneficial endophytes when the carbon status of the plant no longer allows the  
291 survival of these).

292

293 Nevertheless, we know that imbalances of microbiota are observed in many declines  
294 (**Boxes 2 & 3**). In Acute Oak Decline, comparison between diseased and healthy trees  
295 revealed microbiota modifications, with a predominance of *Brenneria goodwinii*, *Rahnella*  
296 *victoriana* and *Gibbsiella quercinecans* in diseased tissues ([57,67–70]. In silver fir dieback,  
297 the link between modifications of the microbiota and the presence of disease is not clearly  
298 established, even if tree decline has an impact on soil microbiota composition [93]. Decline  
299 of photosynthesis rate leads to modify roots and mycorrhizal exudation, changing bacterial  
300 growth and community composition as well as nitrification process in and near  
301 the rhizosphere and mycorrhizosphere [94–96].

302

### 303 **Engineering the microbiota, a realistic perspective to cure WPs from declines?**

304 Plant microbiota protect the plant against pathogens *via* direct (production of  
305 bacterial toxins) or indirect effect (stimulation of the host immune response and  
306 competition for ecological niches) [13,36,39]. Cultural practices are known to impact the  
307 composition of WP associated microbiota [97–99]. Therefore, adjusting these practices  
308 might be a straightforward option to reduce, prevent or even cure declines [3].

309 The development of agroecological practices, based on the valorization of ecosystem  
310 services and of natural biological regulations, responds to a strong societal demand [100]. In  
311 this context, beneficial microbes promoting the growth, nutrition and health of plants  
312 constitute the basis for a model of functional community useful in the development of  
313 agroecological practices [101]. The majority of the results on these approaches come from  
314 crops, mostly annual plants like rice, tobacco, tomato, maize or potato (for review, see

315 Compant et al., 2019 [102]). Transferring this knowledge for WP management, requires  
316 specific adaptation to perennial plants:

- 317 ■ *Development of new rootstock-graft assemblies and ungrafted plants*: having a better  
318 tolerance to diseases using beneficial synthetic communities within the selection  
319 process. It is tempting to hypothesize that better ecosystem services might be explained  
320 by roots and rootstock capacity to associate with different microbiota [103]. The  
321 physiological aspects of the rootstock-graft interactions may affect the physiology of the  
322 plant and it is possible to optimize the assembly between the two vegetative parts [104–  
323 106]. In addition, WP habitat can be also dependent on the local microbiome and  
324 especially on symbiotic organisms [107].
- 325 ■ *Association of tree species with beneficial microbiota*: sharing the benefits of the  
326 microbiota from one individual to another can be considered directly by pooling the  
327 microbiota in the soil (especially thanks to the mycorrhizae, see **Box 4**) or indirectly by  
328 chemical communication between microorganisms. After identification of the  
329 microbiota associated with a tree species and the functional benefits associated with it  
330 (*i.e.* root growth promotion), it will be possible to select species and associate them with  
331 others for a “microbial complementarity”.

332

### 333 *Conserving microbial functions, an important issue for microbiota engineering*

334 Plant-associated microbiota is a key factor to buffer the effects of biotic and abiotic  
335 constraints [65]. However, the description of the taxonomic composition of plant microbiota  
336 remains incomplete with the usual and current molecular tools and the processes regulating  
337 their assembly need to be further studied [108]. The understanding of the assembly of the  
338 microbiota is a research theme of fundamental importance to understand the phenomena of  
339 facilitation and rapid adaptation of plants to local environmental constraints. In this line of  
340 research, Lapsansky and collaborators [109] proposed the concept of “soil memory” : from  
341 one plant generation to another, a given soil would hold its associated rhizospheric and  
342 edaphic microbiota. The fitness of perennial plants of the n+1 generation (or of a new woody  
343 plant set up in a plot replanting context) would thus be improved, taking advantage of the  
344 pre-existing beneficial microbes for their development [109]. Time scales are often  
345 considered on an annual or biannual basis for agronomic plants with a short life cycle, but

346 this concept can also be applied to the time scales necessary for the renewal of a forest, a  
347 vineyard or at least an orchard. The concept of soil memory could be exploited artificially for  
348 example by sampling a given well-balanced forest soil microbiota, multiply it in controlled  
349 conditions, before introducing it into nursery greenhouses in plantlets soil.

350

351 *Acting directly on the microbiota composition by microbial inoculation*

352 Some bacteria, endophytic and mycorrhizal fungi can be used as microbial inoculants,  
353 owing to their functional properties of antibiosis, lysis, competition or nutrient acquisition,  
354 induced resistance or hormonal stimulation. Their functions depend on both abiotic and  
355 biotic context [110]. With an annual growth rate of about 10 %, the economic market of  
356 microbial inoculants increases rapidly [111]. Although field results on annual crops  
357 compared to market gardening are often disappointing compared to promising advances  
358 under controlled conditions, we can already try to modify root microbiota of woody species  
359 such as oak for Acute Oak Decline [56]. For example, the beneficial role of *Fraxinus ornus*  
360 fungal endophytic microbiota against ash decline was suggested, although results need to be  
361 further supported [112].

362 Microbial inoculation (see **Outstanding question 3**) could be achieved:

- 363 ▪ in nursery by inoculating a single or a cocktail of microbial strains by selecting of native  
364 or locally adapted inoculants (considering both biotic and abiotic context) or strains  
365 with a marketing authorization [113]
- 366 ▪ on established or mature WP by direct addition of bacterial strains or cocktails of  
367 strains by spraying (rather for endophytes) or by reintroduction of mycelial strains as  
368 spores or mycelium directly in the soil (*i.e.* mycorrhizal fungi, [114]). Such additions  
369 could be repeated until the microbial imbalance ends.
- 370 ▪ by indirect regular supplementation by complantation (*i.e.* replacing dead plants, a  
371 cultural practice largely used in vineyards) or by using plant cover as a reservoir of  
372 diversity [115].

373 A key issue is that the introduction of beneficial microbes into an established  
374 holobiont is not easy to sustain over time. The microbial populations introduced by humans  
375 can decrease over time and thus their beneficial effects disappear. They can also eliminate  
376 native beneficial microbial species, changing the functional balance of the ecosystem.

377 Nursery manipulations (early stages of plant development) would reduce the odds of  
378 disturbing an established microbial equilibrium [109]. Additionally, it can be difficult to  
379 switch from an ‘unbalanced microbiota state’ to a ‘healthy microbiota state’, without coming  
380 back to the original microbial communities because of the resilience of the system, which  
381 would make it difficult to keep the new equilibrium over time [116]. Additionally, when  
382 microbial strains are characterized as beneficial for plants, the question of the specificity of  
383 certain strains for targeted cultivars will prevail. Results from lab experiments may be  
384 difficult to implement in the field [39]. Finally, it becomes clearly necessary to include  
385 microbiota in plant breeding strategies and in the analysis of varieties and rootstocks  
386 performance to limit the loss of microbial genetic diversity, inherent in current agricultural  
387 systems and practices.

388

389 *Acting indirectly on the microbiota composition: impact of agricultural practices.*

390 Some agricultural methods are specific of WPs agro-management and might act indirectly on  
391 microbiota composition. For example, yearly dead parts of woody plants are often removed  
392 because they are thought to contain detrimental microorganisms. However, they represent  
393 a valuable carbon source and their removal would contribute to the reduction of organic  
394 compounds within the soil which in turn affect soil microbiome [117,118]. If the risks of  
395 contamination are too high for some declines and prevent application of grinding dead  
396 wood, another option might be to spread dead parts after complete or partial combustion.

397

398 Grafting is a technique widely used within WPs, such as grapevine, olive and citrus  
399 trees [73]. Some cultivars or rootstocks as well as clones are more tolerant to pathogens or  
400 are physiologically different: the nature of the host influences the susceptibility to a disease  
401 (*e.g.* GTDs in grapevine [119]), an apoplectic disorder or a physiological disturbance (*e.g.* the  
402 decline of the grapevine associated with the use of the rootstock 161-49C, see **Box 1**)  
403 [120,121]. As for GTDs, some olive tree cultivars are more susceptible to CoDiRO than  
404 others. For example, cultivar ‘Leccino’ seems to be more resistant [122]. For the grapevine  
405 Syrah decline, it has been shown that even if all rootstocks are concerned, 110R and 99R are  
406 more sensitive than others, so their use is not recommended. A very strong clone effect was  
407 also observed [123]. These differences in susceptibility could be related to differences in  
408 associated microbiota. Rhizodeposition, which is related to the recruitment of rhizospheric

409 microbes, has been shown to depend on the genotype of the rootstock in apple trees [124].  
410 Within other plant compartments, an influence of the rootstock-scion combination genotype  
411 and of WP genotype on microbial diversity is also suggested (*e.g.* the endophytic microbiota  
412 in apple trees [125], the bacterial endophytes in olive trees [126], the rhizospheric  
413 microbiota in grapevine [127] or the arbuscular mycorrhizal community in citrus [128].

414

415 It has been shown that the diversity of organisms (plants, animals and microbes) is positively  
416 affected in organically managed or low-farming systems, compared to systems under  
417 integrated management [102]. Soil diversity has been studied, particularly in vineyards,  
418 where a link was found between soils under green manure and highest soil microbiota  
419 diversity, compared to other organic or biodynamic agro-management practices [129].

420 Finally, since microbiota associated with plants can vary from one species to another,  
421 and even from one individual to another within the same species, strategies of association of  
422 different species within the same agricultural system can be considered [130]. Thus, a plant  
423 could take advantage of the beneficial effects of the microbiota of the neighboring plant, in  
424 particular the rhizospheric microbiota, and *vice versa* (see **Outstanding questions 4 and 5**).

425

#### 426 **Concluding remarks and future perspectives**

427 Improving longevity of WPs is a major challenge considering their declines. A major feature  
428 would be to understand, not only the physiology and the ecology of WPs, but also of their  
429 holobionts. Microbiota and by extension the microbiome could offer new engineering  
430 solutions to prevent and even cure WP declines only if (i) we take into account the specific  
431 physiology of WPs, especially fluxes of C, N and water between sources and sinks that are  
432 changing over seasons and years and (ii) the chemical and molecular dialogues between  
433 microbes and between the WP and microbes. Keeping the homeostasis of WP holobiont  
434 would necessitate to carefully balance its resources between growth, storage, reproduction  
435 and defense. From nursery to fruit and berry plantations, considering the use of microbiota  
436 portfolio adapted to specific conditions (*i.e.* climatic, chemical and biological stresses) will  
437 constitute a major step towards a better understanding and management of the agro-  
438 ecological outcome of WPs holobionts (see **Outstanding questions**).

439

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448

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757

## 758 **Text Boxes**

759

### 760 **Box 1. WP declines and consequences on the productivity of fruits**

761 The question of which came first, the pathological or physiological disorder, is difficult to  
762 answer. Most of the time, WPs are considered as asymptomatic as no visual declines are  
763 observed and some plant compartments (*i.e.* vessels, roots) are difficult to access. So, WP  
764 weakening is already under way when the first symptoms of the disease or decline appear.  
765 In addition, there is a bias on several species since we are more focused on plants producing  
766 wood, fibers, berries and fruits for human nutrition. Grapevine (*Vitis vinifera* L.) and olive  
767 trees (*Olea europaea* L.) are worldwide distributed crops of significant economic importance  
768 and are currently facing several diseases, due to physiological imbalance of WP tissues and  
769 to fungal or bacterial agents. The rootstock 161-49C in grapevine develops limited C storage  
770 and displays disturbance in xylem (*i.e.* thyllose) and phloem (*i.e.* smaller diameter) leading up  
771 to 50% of unproductive vineyard [121]. The main declines affecting mature grapevine plants  
772 are grapevine trunk diseases (GTDs) caused by several fungal species [86,131]. GTDs are  
773 currently affecting vineyards throughout the world. Approximately 13 % and 14% of  
774 respectively the French and Californian vineyards are unproductive. The Olive Quick Decline  
775 Syndrome (or CoDiRO, for Complesso del disseccamento rapido dell'olivo), whose symptoms  
776 look like those of grapevine declines, is mainly caused by the bacterium *Xylella fastidiosa*.  
777 CoDiRO, originally detected in the olive groves of Salento in Italy in the late 2000s, is

778 expected to expand to the rest of the Mediterranean basin and California [132]. Worldwide  
779 olive production has recently decreased by about 25% and in Italy by 50%.

780

781 **Box 2. Link between WP declines, pathogens and holobiont imbalance**

782 It seems that pathogenic agents (fungal, bacterial or viral) can trigger diseases on healthy  
783 trees acting as “primary factors” when using biotic diffusion vectors or as “secondary  
784 factors” when the pathogen benefits from the weakening of the tree (*e.g.* another disease,  
785 pruning wounds). This weakening can result from significant climatic (*i.e.* drought, extreme  
786 fluctuation in weather conditions), chemical (*i.e.* excessive fertilizers) and biological stresses  
787 (*i.e.* insects), poor plant selection for the location, physical constraints, improper planting  
788 practices, poor soil conditions and physical injury. It is likely that a given disease is not  
789 always related to the presence of a particular pathogen alone, but would result from a  
790 combination of adverse factors and the presence of one or several pathogens. In addition,  
791 external factors are necessary to influence the transition from an endophytic to a pathogenic  
792 state of some fungi in asymptomatic tissues state [133]. The intensity of the disease related  
793 to pathogen leads to the modification of the holobiont and depends on either the WP (*i.e.*  
794 cultivar) or the balance of microbes (*i.e.* epiphytic and endophytic microbiota) [134].

795

796 **Box 3. Are some microbial species always present in the microbiota of woody plants**  
797 **exhibiting different declines related to pathogens?**

798 The same pathogenic species are often involved in declines affecting various WPs and act as  
799 opportunists. Similar fungal species from the *Botryosphaeriaceae* (endophytes, saprophytes  
800 and some pathogenic) are associated with many declines [135] and found in GTDs, Coast  
801 Redwood Trees Decline [136,137] and Branch Dieback of olive [138,139]. More than 300  
802 species of plants are susceptible to *Xylella fastidiosa* in the world [85,140] and some strains  
803 carried by xylem-sap feeding insects (*e.g.* leafhoppers and spittlebugs), cause fatal diseases  
804 in grapevine (Pierce disease), olive and citrus trees. The bacterium *Pseudomonas syringae*  
805 infect orchards (*e.g.* cherry, pear, plum and apricot) weakened by water stress or injury and  
806 are involved in horse chestnut bleeding canker [13,141].

807

808 **Box 4. Common mycorrhizal networks: a role of sentinel in prevention of decline?**

809 Plant–plant interactions (*e.g.* facilitation, competition) are among the fundamental  
810 processes that shape structure and functioning of plant communities. Within the fungal  
811 component of the root microbiota, mycorrhizal fungi form mutualistic symbiotic associations  
812 called **mycorrhizas** [142]. Around 90 % of terrestrial plant species including fruit and olive  
813 trees, grapevine and tree species cultivated for their wood are mycorrhized. Mycorrhizal  
814 fungi are able to form **common mycorrhizal networks (CMN)** linking roots from the same or  
815 different species. CMNs benefit to host plant in many ways, including plant-plant facilitation  
816 or nutrition [3]. Since the highlighting of intra-kingdom communication between plants and  
817 microbes, especially between plants and bacteria, it has been shown that molecules other  
818 than nutrients can pass between microbes and their host plant. Bidirectional nutrient  
819 transfers of carbon (C) between ectomycorrhizal tree species such as birch and Douglas fir  
820 [143] and nutrient exchanges between endomycorrhizal plants [71] have been shown. We  
821 thus define a CMN formed by ecto- or endo-mycorrhizal fungi that link plants together.  
822 Transfer of different molecules may be bidirectional between plants and CMN can improve  
823 interplant nutrition and growth through plant-plant facilitation. CMNs can also induce plant  
824 defense responses and plant communication through phytohormones (*e.g.* jasmonic acid,  
825 methyl jasmonate, zeatin riboside) and therefore have a strong potential as a biocontrol  
826 agent. The role of arbuscular mycorrhizal fungi as potential bridge between different plants  
827 in the spread of a disease has recently been hypothesized, for example with Potato virus Y or  
828 following a necrotrophic fungus attack [144]. Indeed, in addition to the competitive role of  
829 arbuscular mycorrhizal fungi against pathogens by **allelopathy**, CMN can act as a conduit  
830 carrying chemical signaling compounds emitted by the attacked plant. These links make it  
831 possible for a plant to send warning signals during an insect or pathogenic attack to the  
832 network of plants connected by the CMN ‘informing them’ of the attack. In this context, an  
833 interesting perspective would be to study how this CMN would transfer the information of a  
834 pathogen attack for a ‘sentinel’ plant to a neighboring plant, which could preventively  
835 activate their defense mechanisms and enhance their immunity.

836

### 837 **Figure legends**

#### 838 **Figure 1. The woody plant holobiont.**

839 It is essential to consider the woody plant (WP) as a “holobiont”, *e.g.* the tree and its  
840 microbial continuum from soil to leaves. The microbiota resides on (epiphytic microbiota) or



841 within (endophytic microbiota in the phyllosphere, the lignosphere and the rhizosphere) any  
842 of a number of WP tissues. Interactions exist between the WP and its associated microbes,  
843 and among microbes. These microbes can be beneficial (*e.g.* better fitness, nutrition,  
844 immune defense), pathogenic (*e.g.* diseases) or neutral for the WP.

845

## 846 **Figure 2. Woody plant declines: a role of the microbiota**

847 The woody plant (WP)-associated microbiota is made up of bacteria, fungi, viruses, protists,  
848 archae and oomycetes. The microbiota could either enrich or weaken some physiological  
849 functions of the WP. We hypothesize that the taxonomic composition and functions of the  
850 WP-associated microbiota can influence the WP physiological status. The plant stays healthy  
851 with a given balance of microbiota. On the contrary, an imbalance of microbiota (as cause or  
852 consequence of the decline), leads to visible symptoms, whose origin can be physiological,  
853 cultural and pathological. Various options may be used to cure some WP declines: a direct  
854 inoculation of specific microbial species (*e.g.* involved in phosphorus mobilization) in order  
855 to restore the balance, or by providing specific microbial species with targeted nutrients to  
856 promote their growth.

857 In this figure, pie charts only reflect the distribution of the microbiota and the microbiome.  
858 We have chosen some genes, not an exhaustive list, encoding proteins involved in functions  
859 of WP metabolism. Similarly, a balanced microbiota may correspond to different proportions  
860 of microorganisms belonging to each taxon. Taxonomic diversity does not necessarily mirror  
861 the functional diversity.

862

863

## 864 **Figure 3. Tag cloud of physiological and ecological diseases related to woody plant declines**

865 Woody plant (WP) declines have multi-factorial determinants (*e.g.* cultural practices,  
866 parasites, abiotic stresses) and their importance is not clearly established, and may depend  
867 on the WP species. This tag cloud (built using <https://nuagedemots.co/>) depicts factors and  
868 keywords commonly mentioned on WP declines in references used in this review, without  
869 any causal relationships. In WP declines, it would appear that microbiota could be a reliable  
870 indicator.

871

## 872 **Glossary**

873

874 **Allelopathy:** the chemical (excretory products, metabolites) inhibition or regulation of one  
875 organism by another.

876

877 **Common mycorrhizal network:** A continuum network formed by both endo- and  
878 ectomycorrhizal fungi connecting plants with each other *via* their mycelium. Common  
879 mycorrhizal network allows exchanges of nutrients and other chemical substances between  
880 connected plants.

881

882 **Core microbiota:** All microbial species (bacteria, fungi, viruses, protists) which predominate  
883 at surface or inside a living organism (here plants). Plant core microbiota could be defined by  
884 a set of microbial species always found in plant of different genus.

885

886 **Core microbiome:** Set of essential functions provided by the microbiota of a plant found  
887 from one plant to another.

888

889 **Decline:** Gradual reduction of growth, vigor and productivity in a woody plant due to  
890 multiple abiotic (water or thermic stresses...) and biotic stresses (pathogens, insects...) and  
891 likely to an unbalanced microbiota.

892

893 **Dieback:** Progressive death of twigs and branches which generally starts at the tips.

894

895 **Endophyte:** Microorganism (mostly bacteria or fungi) that lives inside tissues of other  
896 organisms, in a symbiotic way, with beneficial effects or at least with no negative  
897 consequences for the host fitness. Microorganisms involved can therefore be commensal or  
898 beneficial, but they can also be saprobes or latent pathogens.

899

900 **Endosphere:** Internal regions of plant tissues that can be colonized by microorganisms.

901

902 **Epiphyte:** Microorganism (mostly bacteria or fungi) that lives on the surface of a given  
903 organism.

904

905 **Facilitation:** A positive interaction that can contribute to assembling ecological communities  
906 and preserving global biodiversity.

907

908 **Holobiont:** Sum of the different species that forms an ecological unit. Here, we limit our  
909 definition to the plant and all its symbiotic microbiota.

910

911 **Hologenome:** The host and microbiome genomes. The host genome is highly conserved with  
912 slow genetic changes. The microbiome genome is dynamic (*e.g.* horizontal gene transfer,  
913 mutation) and can change rapidly by modifying microbial populations in response to  
914 environmental changes.

915

916 **Homeostasis:** The maintenance of a relatively steady state or equilibrium in a biological  
917 system by intrinsic regulatory mechanisms.

918

919 **Key or hub species:** A small number of taxa that are strongly interconnected and having a  
920 severe effect on communities.

921

922 **Lignosphere:** Botanically, it is the micro-ecosystem of the trunk and branches surface,  
923 considered as a possible habitat for microbes.

924

925 **Microbiome:** Set of genes brought by microbes forming the microbiota within a holobiont.

926

927 **Microbiota:** Community of microbes associated with the plant (bacteria, archaea, fungi,  
928 viruses, protists and other microeukaryota).

929

930 **Mycorrhiza:** From *mukes*, fungus; and *rhiza*, root. Intimate association between roots from  
931 90% of land plants and specialized soil fungi. At least seven types of mycorrhiza exist, but  
932 ectomycorrhiza and arbuscular mycorrhiza are the most common.

933

934 **Phyllosphere:** Botanically, it is the micro-ecosystem of the leaf surface, considered as a  
935 possible habitat for microbes.

936

937 **Rhizosphere:** Volume of soil surrounding living plant roots that is influenced by root activity  
938 and hosting specific rhizospheric microbes.

939

940 **Rhizodeposition:** The release of materials from plant roots into the rhizosphere, including  
941 soluble and insoluble exudates, lysates and gases.

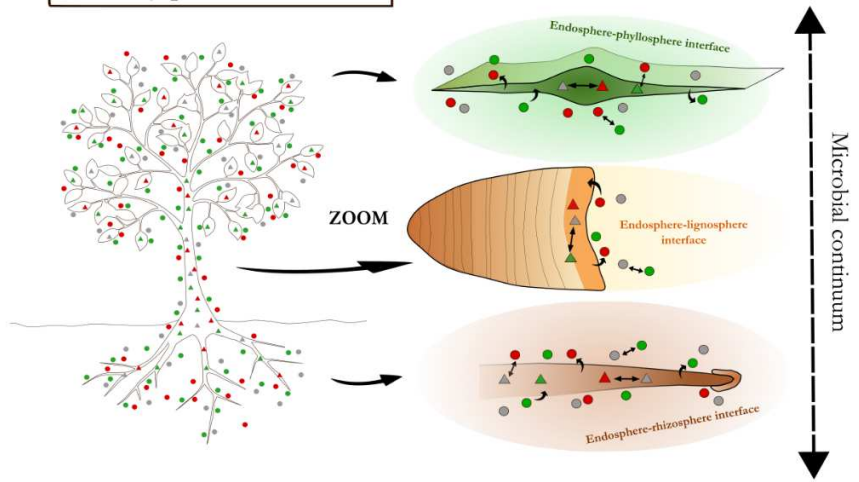
942

943 **Symbiont:** Organism establishing a close and long-term interaction with its host (here the  
944 plant).

# Woody plant holobiont

△ Endophytic } beneficial, neutral or  
 ○ Epiphitic } pathogenic microbiota

↔ Microorganisms-microorganisms interactions:  
 toxin production, competition for ecological niche  
 ↷ Microorganisms-plants interactions:  
 growth promoting (nutrition *via* mineral nutrients or exudates),  
 immunity modulation



Woody plant declines: A role of the microbiota ?

