

1 **Steering soil microbiomes to suppress aboveground insect pests**

2

3 **Ana Pineda^{1*}, Ian Kaplan² and T. Martijn Bezemer^{1,3}**

4 ¹Department of Terrestrial Ecology, NIOO-KNAW, Postbus 50, 6700 AB

5 Wageningen (The Netherlands)

6 ²Department of Entomology, Purdue University, 901 W. State Street, IN 47907 West

7 Lafayette (USA)

8 ³Institute of Biology, Section Plant Ecology and Phytochemistry, Leiden University,

9 PO Box 9505, 2300, RA Leiden, The Netherlands

10

11 *Correspondence: a.pineda@nioo.knaw.nl (A. Pineda)

12 Twitter account: @ana_pineda_

13

14 **Keywords:** phytobiomes, induced resistance, herbivores, insects, microbe-plant-insect

15 interactions, plant-soil feedbacks

16

17 **Abstract**

18 Soil-borne microbes affect aboveground herbivorous insects through a cascade of
19 molecular and chemical changes in the plant, but knowledge of these microbe-plant-
20 insect interactions is mostly limited to one or a few microbial strains. Yet, the soil
21 microbial community consists of thousands of unique taxa interacting in complex
22 networks—the so-called microbiome—that provide plants with multiple beneficial
23 functions. The role and management of whole microbiomes in plant-insect
24 interactions are almost unexplored, calling for the integration of this complexity in
25 aboveground-belowground research. Here, we propose holistic approaches to select
26 soil microbiomes that can be used to protect plants from aboveground attackers.

27

28

29 **Microbes conferring immunity in the phytobiome**

30 The late entomologist, Thomas Eisner [1], once famously stated, “Bugs are not going
31 to inherit the earth. They own it now”. In light of on-going discoveries in microbial
32 taxonomy and ecology, however, we can probably affirm that in fact “Microbes own
33 the earth”. The complex network of microorganisms inhabiting an area (e.g., soil,
34 plant, animal), referred to as the **microbiome** (see Glossary), imparts crucial functions
35 in all living organisms. For instance, the chemical defences that were previously
36 considered an innate genetic feature of many animals and plants are actually produced
37 by microbial symbionts [2, 3] and we expect more examples to be revealed in the near
38 future. In humans, immunity, and even behaviour, are influenced by the intestinal
39 microbiome [4, 5]. Interestingly, the **rhizosphere**, a thin interface between roots and
40 soil, can be considered the plant equivalent to the human intestinal tract [6].

41

42 The soil is the major source of microbes, which determine the plant-associated
43 microbiome [7]. Soil microbes are crucial for enhancing plant survival, growth, and
44 tolerance to abiotic stress, but also induce systemic resistance (ISR) against pathogens
45 and insects both aboveground [8-11] and belowground [12]. The soil microbiome has
46 thus emerged as a key component of plant immunity [8, 9, 13], and shapes how plants
47 interact with their abiotic and biotic environments, in the so-called **phytobiome** [14,
48 15]. Most of the work on aboveground plant defence, so far, focuses on the impact of
49 individual microbial species or strains. This is in sharp contrast with DNA-sequencing
50 techniques that are revealing an astonishing taxonomic diversity in soils, especially in

51 the rhizosphere, but also the plant itself [7, 16, 17]. Because the beneficial effects for
52 the plant are often provided by a consortium of microbes [18], there is an urgent need
53 for approaches that incorporate the wider diversity that exists in nature into microbe-
54 mediated plant protection strategies [19].

55

56 **Impact of soil microbiomes on aboveground herbivores**

57 Evidence for how belowground microbial communities, as a whole, impact
58 aboveground insects is scarce; however, given the typically strong responses to only
59 one or two experimentally augmented microbes, we anticipate that the community-
60 wide effects are substantial. Soil microbiomes can impact aboveground insects
61 indirectly through plant-mediated mechanisms, or directly through pathogenic or
62 mutualistic interactions. A recent study showed that the population increase of the
63 specialist foliar feeding aphid *Aphis jacobaea*, depended on the composition of
64 microbial communities inhabiting the soil used by its host plant ragwort (*Senecio*
65 *jacobaea*). The soils maintained different fungal communities that influenced the
66 concentration of amino acids in the phloem sap, which the authors proposed, in turn,
67 influenced the aphids [20]. Similarly, inoculation of distinct microbiomes collected
68 from soils with different plant species altered the leaf metabolome of arabidopsis
69 (*Arabidopsis thaliana*) and resistance of the plant to the caterpillar *Trichoplusia ni*
70 [21]. This study further confirmed via removal of the majority of microorganisms
71 using a filter of 0.45 μm , the contribution of the microbial component of the soil
72 (instead of the presence of chemical compounds that could pass the filter) to plant
73 performance. These studies illustrate that exposure to particular microbiomes alters
74 the resistance of plants to aboveground insects (Figure 1, Key Figure). However, the
75 underlying molecular plant mechanisms in microbiome-**induced systemic resistance**
76 (ISR, Box 1) are probably more complex than predicted.

77

78 Soil microbes can have direct interactions with aboveground herbivores. Recent
79 studies have shown that leaf and soil microbiomes are linked [22-24], and soils could
80 thus influence the composition of insect pathogenic or symbiotic microbes present in
81 or on the leaves. Entomopathogenic fungi such as *Beauveria bassiana* and
82 *Metarhizium anisopliae*, for example, are common in the soil but also exhibit an
83 **endophytic** phase that can promote plant growth and insect resistance [25].
84 Remarkably, these fungi not only provide a benefit to plants by killing their

85 herbivores, but can even translocate nitrogen from aboveground insect cadavers to the
86 plant via fungal mycelia [26]. Other fungi historically considered to be limited to soils
87 (e.g., *Trichoderma*) are now known to colonize leaves as endophytes where they can
88 suppress insect pests such as thrips [27]. Insect symbionts provide their host with
89 functions such as the ability to suppress plant defences or mobilize nutrients [28, 29],
90 and these symbionts can be acquired via the soil. For example, the soybean insect pest
91 *Riptortus pedestris* acquires *Burkholderia* strains from the soil that metabolize an
92 organophosphate, conferring resistance to the insecticide [30].

93
94 Given the substantial evidence that soil communities affect aboveground plant
95 interactions, we argue that agricultural scientists should start to think far more about
96 reshaping microbiomes to increase crop resistance to insect pests. Managed systems
97 allow a large amount of flexibility in inputs or other design strategies that shape soil
98 life. Here, we focus on three specific strategies that are known to generate
99 community-scale impacts on microbiomes and thus can be adapted for sustainable
100 pest control aboveground.

101

102 **Transplanting new microbiomes into the soil**

103 A major advancement in microbe-plant interaction research was the development and
104 commercialization of microbial inoculants for agricultural use. These inocula usually
105 consist of one to several species that are phylogenetically clustered within a few
106 genera (e.g., *Bacillus*, *Trichoderma*). However, many of these microbial inoculants
107 that are successful under laboratory conditions fail when applied in the field. Recent
108 studies have argued that this is probably due to competition of single strains with the
109 existing microbiome in the donor soil [9, 31]. A potential solution to this problem
110 would be to inoculate microbiomes that are more complex than currently used [19].
111 Large-scale cultivation of microbes and their introduction in complex synthetic
112 microbiomes may aid in maximizing the beneficial functions of certain microbes by
113 introducing taxa interactions [22, 32]. For instance, some microbes alter their
114 metabolism when involved in microbial interactions, and produce compounds (e.g.
115 volatiles, antibiotics) that are not produced when growing as single strains. These
116 compounds could for example act antagonistically to other microbes that are
117 prohibiting the establishment, enhance plant colonization, or have a direct effect on
118 plant growth or resistance [33]. Soil microbial diversity is a major driver of ecosystem

119 multifunctionality [34, 35] and due to the contribution of soil microbes to multiple
120 functions such as nutrient cycling, biological control or food production, soil
121 microbiomes are a multifunctional component of terrestrial ecosystems. Entire
122 microbiomes can also be introduced via soil transplantation. A recent field experiment
123 showed that introducing a thin layer of soil (5 mm) resulted in accelerated nature
124 restoration in a degraded ecosystem, and that composition of the bacterial and fungal
125 communities six years after application was still different from those where no soil was
126 added [36]. Other studies show that soils with disease-suppressive properties can be
127 successfully transplanted and remain disease suppressive in the new area [9, 17].
128 Agricultural soils, in particular in commercial glasshouses, are regularly sterilized,
129 e.g., by steaming. This practice eradicates much of the existing microbial community
130 [37], a situation that is ideal for introduction of a new microbiome.

131

132 There is a unique opportunity here to forge collaborative and mutually beneficial
133 relationships among those studying plant and animal microbiomes. Faecal microbiota
134 transplantation is now frequently used to suppress diseases and alter immune
135 responses in humans while soil inoculation and transplantation is still in its infancy.
136 Hence, those studying human health consequences of gut microbiome transplantation
137 are far ahead of those working in plant health. Yet, the two approaches, while
138 differing in practical aspects of implementation, are identical in theory. In fact, direct
139 analogies between these two areas have been highlighted for characteristics such as
140 nutrient uptake, pathogen defence, and immune function [6, 38].

141

142 **Steering existing soil microbiomes**

143 Apart from introducing a new microbiome, the residing soil community can also be
144 steered to a desired beneficial state [39, 40]. This could be accomplished by
145 stimulating particular subgroups of the microbiome via manipulations of
146 environmental factors such as soil temperature or moisture levels [37], via the
147 application of chemical compounds or manipulating resource availability through
148 organic amendments. It is well known that amelioration of soils with manure or plant
149 residues alters the soil microbiome, thereby suppressing belowground pathogens [9,
150 40, 41]. Different studies have shown that addition of biochar, pyrolyzed plant
151 residues, to soil, for example, increases bacterial diversity and microbial biomass [42],
152 as well as resistance of plants against aboveground pests and diseases [43, 44].

153 Interestingly, the plant response that biochar causes to the pathogen *Botrytis cinerea*
154 highly resembles microbial-ISR, including **priming** of defence-related genes
155 associated with the early oxidative burst via the jasmonic acid (JA) signalling
156 pathway [43]. These set of studies highlight how a soil amendment could impact
157 aboveground attackers through changes in the soil microbiome and in plant defences.
158 However, evidence linking how soil amendments alter the soil microbiome, and how
159 this cascades to induce systemic resistance in plants is still missing.

160

161 Certain “keystone” microbes are highly connected with other taxa and play a key
162 ecological role in the microbiome. By targeting keystone species the entire microbial
163 network can be adapted and recent discoveries support this idea [32, 45, 46].

164 Introduction of the oomycete pathogen *Albugo* sp. and the basidiomycete yeast fungus
165 *Dioszegia* sp., for example, alters the microbiome network in the **phyllosphere** of
166 arabidopsis [45]. The important role of these keystone taxa suggests that they should
167 be present in high abundance in the microbiome. However, keystone species can also
168 play an important role at low densities and even rare microbes, which have been
169 shown to induce resistance against aphids [47], can act as keystone players in
170 microbiomes [48]. Whether a microbial function such as induced systemic resistance
171 after introducing a keystone taxa is driven by changes in the microbiome network,
172 rather than by the introduced taxa itself, is still unknown.

173

174 **Using plants to steer the soil microbiome**

175 By growing in the soil, plants modify the microbiome, either directly, or indirectly via
176 influencing the abiotic environment [7]. Host factors such as plant species, ontogeny,
177 and exposure to antagonists all shape microbiomes. Even different genotypes imprint
178 unique microbial signatures on the soil [7, 24, 49-51]. Plant roots release compounds
179 such as sugars, organic acids, phytohormones, and secondary metabolites, and this
180 **exudation** influences the soil community [52, 53]. For instance, specific compounds
181 (e.g., malic acid, benzoxiacinoids, strigolactones) can enhance or recruit certain
182 beneficial soil microbes in the rhizosphere [54-57]. Interestingly, the exudation of
183 some of these compounds increases following aboveground herbivory, suggesting this
184 is an active strategy whereby plants recruit beneficial microbes for protection. The
185 impact of herbivory on the soil can also influence the susceptibility of plants that are
186 later exposed to this microbiome [58, 59]. For example, the soil fungal community in

187 the rhizosphere of ragwort that suffered from belowground or aboveground herbivory
188 differed considerably from communities in unexposed plants. Plants that grew later in
189 the soil with a belowground herbivory legacy displayed higher resistance to the leaf
190 chewer *Mamestra brassicae*, and this was associated with a modified profile of
191 pyrrolizidine alkaloids in the foliage [59]. These two examples illustrate a closed
192 feedback loop in interactions between plants, soil microbes, and insects, a term that
193 we propose to call “**plant-soil-insect feedbacks**”.

194

195 The concept of plants changing the soil microbiome, which subsequently influences
196 the performance of other plants that grow later in the soil is one of the main
197 mechanisms of “**plant-soil feedback**” [60, 61] and is the basis for ancient agricultural
198 practices such as crop rotation, intercropping or cover crops. However, this concept
199 has primarily been used in the context of avoidance of soil pathogen build-up and
200 autotoxicity, or to increase nutrient availability by using leguminous crops. We argue
201 that plants displaying positive feedback effects on crop immunity to pests through
202 their effect on the soil microbiome, should be selected for and included in rotation
203 systems, as “engineers” of beneficial soil microbiomes. These plants that create a
204 beneficial microbiome with positive effects on plant health can also be used to
205 produce inocula that can be then be introduced during or at the start of cultivation.
206 Surprisingly, the contribution of soil microbiomes to plant-soil feedbacks and their
207 application in agriculture is largely unknown [62]. There is an urgent need for studies
208 that improve our understanding of the mechanisms by which plants influence soil
209 microbiomes and that predict how plants respond to these changes (see Box 2). This
210 will enable us to design optimal combinations of succeeding plants in rotation
211 schemes and enable breeding for optimal crop responses to soil manipulations [63].

212

213 The genetic traits that underlie the responses of plants to changes in soil microbiomes
214 are also largely unknown. However, a recent genome-wide association study in
215 arabidopsis identified ten genetic loci that were highly associated with the ability of
216 the plant to respond to the growth-promotion effect of volatiles from a soil derived
217 *Pseudomonas simiae* strain [64]. In crop plants, breeding for resistance to pathogens
218 in combination with high inputs of fertilizers and pesticides that suppress pathogens
219 and herbivores, may have selected for poorly responding genotypes, and even for
220 genotypes that suppress beneficial microbes [49, 65]. Therefore incorporating

221 knowledge about microbiomes during the crop selection process may improve traits
222 such as plant productivity and resistance. By growing plants repeatedly in the soil and
223 selecting in each generation for specific plant traits such as early onset of flowering or
224 more efficient induction of defences, beneficial soil microbiomes can be selected and
225 therefore further steered, so that they become more effective [17, 66, 67].

226

227 **Concluding remarks and future perspectives**

228 Unravelling the mechanisms that govern species interactions is a major challenge in
229 ecology. In this opinion we have emphasized that soil microbiomes can be
230 manipulated to enhance plant performance and resistance to aboveground pests, and
231 that plants play pivotal roles in this. The mechanisms can be diverse, as soil
232 microbiomes are complex entities, and include priming for enhanced defensive
233 responses, induction of plant secondary metabolites, as well as direct interactions
234 between soil microbes and insects (via direct contact of insects with the soil or via
235 colonization of plant by soil microbes). We propose three areas for future research
236 that are essential if we aim to steer microbiomes to alter aboveground plant-insect
237 interactions (see also Outstanding Questions).

238

239 First, fundamental knowledge on the mechanisms of how plants shape soil and plant
240 microbiomes will help to develop new approaches and products. For instance,
241 cultivars emitting higher levels of compounds that enrich certain groups of beneficial
242 microbes could be selected, or products based on those of compounds could be
243 developed. Also, breeding programs could select plants to enhance microbe-mediated
244 functions, from leaving positive soil legacies to strongly respond to these legacies by
245 increasing growth or inducing resistance in aboveground tissues [63]. Therefore,
246 knowledge about soil, plant and insect microbiomes should be integrated into
247 established research on insect-plant interactions to fully understand the functioning of
248 these interactions within the phytobiome.

249

250 Second, in a similar way as gut microbiome transplantation in humans has been a
251 major breakthrough in overcoming recurrent *Clostridium difficile* infection [68], we
252 propose that soil microbiome transplantation can be successful to induce resistance in
253 plants against insects. Plant-soil feedback concepts can be used to create specific
254 donor soils. We envisage that in agriculture, plants will be grown with a clear purpose

255 of conditioning soil that can be transplanted, or that soil conditioning will be
256 incorporated in crop rotation systems. A major challenge is to predict which plant
257 species or genotypes can be used to obtain desired soils. Understanding microbiome
258 assembly and function in different plants, coupled with empirical knowledge on
259 agricultural practices, and on microbe-plant-insect interactions, will be essential for
260 the development of such predictive models.

261

262 Third, we propose that since insect herbivores can severely impact productivity in
263 terrestrial ecosystems, plant resistance to insects should be seen as a key service of
264 microbiomes, and microbiome-insect interactions should be included in agricultural
265 management strategies. Many of the ecosystem services of soil microbiomes may not
266 be effective under current production systems with high input of pesticides and
267 chemical fertilizers, and only become apparent when plants are exposed to abiotic
268 stress conditions [19]. Based on current global changes in agriculture and nutrient
269 supplies, we expect that beneficial soil microbiomes will play an even more important
270 role in plant productivity in the future. The increased availability of nutrients in
271 agriculture has been the basis for the first green revolution that led to a boost in yields
272 worldwide. We are now at the verge of a second green revolution, which utilizes the
273 potential of microbiomes to boost plant health and productivity [69, 70]. The service
274 of plant and soil microbiomes to induce resistance in plants to insect pests should be
275 an essential part of this second green revolution.

276

277 **Acknowledgements**

278 Research activities of A.P. are supported by the Netherlands Organization for
279 Scientific Research (NWO, project no. 870.15.080), and of M. B. by a Vici grant from
280 NWO (grant no. 865.14.006). I.K. was funded by sabbatical grants from KNAW,
281 NWO and PE&RC. We thank Nurmi Pangesti, and two anonymous reviewers for
282 constructive comments on an earlier version of this manuscript.

283

284 **References**

- 285 1. Berenbaum, M.R. (2011) Thomas Eisner: interpreter extraordinaire of nature's
286 chemistry. *Proc. Natl. Acad. Sci. USA* 108, 19482-19483
- 287 2. Florez, L.V. et al. (2015) Defensive symbioses of animals with prokaryotic and
288 eukaryotic microorganisms. *Nat. Prod. Rep.* 32, 904-936

- 289 3. Saikkonen, K. et al. (2013) Chemical ecology mediated by fungal endophytes in
290 grasses. *J. Chem. Ecol.* 39, 962–968
- 291 4. Thaïss, C.A. et al. (2016) The microbiome and innate immunity. *Nature* 535, 65-74
- 292 5. Eisenstein, M. (2016) Microbiome: bacterial broadband. *Nature* 533, 104-106
- 293 6. Selosse, M.-A. et al. (2014) Microbial priming of plant and animal immunity:
294 symbionts as developmental signals. *Trends Microbiol.* 22, 607-613
- 295 7. Bulgarelli, D. et al. (2013) Structure and functions of the bacterial microbiota of
296 plants. *Annu. Rev. Plant Biol.* 64, 807-838
- 297 8. Pieterse, C.M.J. et al. (2016) The soil-borne supremacy. *Trends Plant Sci.* 21, 171-
298 173
- 299 9. Raaijmakers, J.M. and Mazzola, M. (2016) Soil immune responses. *Science* 352,
300 1392-1393
- 301 10. Pineda, A. et al. (2010) Helping plants to deal with insects: the role of
302 beneficial soil-borne microbes. *Trends Plant Sci.* 15, 507-514
- 303 11. Martínez-Medina, A. et al. (2016) Recognizing plant defense priming. *Trends*
304 *Plant Sci.* 21, 818-822
- 305 12. Martínez-Medina, A. et al. (2017) Shifting from priming of salicylic acid- to
306 jasmonic acid-regulated defences by *Trichoderma* protects tomato against the root
307 knot nematode *Meloidogyne incognita*. *New Phytol.* 213, 1363-1377
- 308 13. Alvisatos, A.P. et al. (2015) A unified initiative to harness Earth's
309 microbiomes. *Science* 350, 507-508
- 310 14. Leach, J.E. et al. (2017) Communication in the Phytobiome. *Cell* 169, 587-596
- 311 15. Beattie, G.A. (2015) Microbiomes: curating communities from plants. *Nature*
312 528, 340-341
- 313 16. Lundberg, D.S. et al. (2012) Defining the core *Arabidopsis thaliana* root
314 microbiome. *Nature* 488, 86-90
- 315 17. Mendes, R. et al. (2011) Deciphering the rhizosphere microbiome for disease-
316 suppressive bacteria. *Science* 332, 1097-1100
- 317 18. Santhanam, R. et al. (2015) Native root-associated bacteria rescue a plant from
318 a sudden-wilt disease that emerged during continuous cropping. *Proc. Natl. Acad.*
319 *Sci. USA* 112, E5013-E5020
- 320 19. Busby, P.E. et al. (2017) Research priorities for harnessing plant microbiomes
321 in sustainable agriculture. *PLoS Biol.* 15, e2001793

- 322 20. Kos, M. et al. (2015) Species-specific plant–soil feedback effects on above-
323 ground plant–insect interactions. *J. Ecol.* 103, 904-914
- 324 21. Badri, D.V. et al. (2013) Potential impact of soil microbiomes on the leaf
325 metabolome and on herbivore feeding behavior. *New Phytol.* 198, 264-273
- 326 22. Bai, Y. et al. (2015) Functional overlap of the *Arabidopsis* leaf and root
327 microbiota. *Nature* 528, 364-369
- 328 23. de Souza, R.S.C. et al. (2016) Unlocking the bacterial and fungal communities
329 assemblages of sugarcane microbiome. *Sci. Rep.* 6, 28774
- 330 24. Wagner, M.R. et al. (2016) Host genotype and age shape the leaf and root
331 microbiomes of a wild perennial plant. *Nat. Commun.* 7, 12151
- 332 25. Jaber, L.R. and Enkerli, J. (2016) Effect of seed treatment duration on growth
333 and colonization of *Vicia faba* by endophytic *Beauveria bassiana* and *Metarhizium*
334 *brunneum*. *Biol. Control* 103, 187-195
- 335 26. Behie, S.W. et al. (2012) Endophytic insect-parasitic fungi translocate
336 nitrogen directly from insects to plants. *Science* 336, 1576-1577
- 337 27. Muvea, A.M. et al. (2014) Colonization of onions by endophytic fungi and
338 their impacts on the biology of *Thrips tabaci*. *PLoS ONE* 9, e108242
- 339 28. Chung, S.H. et al. (2013) Herbivore exploits orally secreted bacteria to
340 suppress plant defenses. *Proc. Natl. Acad. Sci. USA* 110, 15728-15733
- 341 29. Giron, D. et al. (2017) Influence of microbial symbionts on plant–insect
342 interactions. In *Adv. Bot. Res.* (Nicolas Sauvion, D.T. and Paul-André, C., eds), pp.
343 225-257, Academic Press
- 344 30. Kikuchi, Y. et al. (2012) Symbiont-mediated insecticide resistance. *Proc. Natl.*
345 *Acad. Sci. USA* 109, 8618-8622
- 346 31. Gadhave, K.R. et al. (2016) Developing soil microbial inoculants for pest
347 management: can one have too much of a good thing? *J. Chem. Ecol.* 42, 348-356
- 348 32. Niu, B. et al. (2017) Simplified and representative bacterial community of
349 maize roots. *Proc. Natl. Acad. Sci. USA* 114, 2450-2459
- 350 33. Schmidt, R. et al. (2015) Volatile affairs in microbial interactions. *ISME J* 9,
351 2329-2335
- 352 34. Wagg, C. et al. (2014) Soil biodiversity and soil community composition
353 determine ecosystem multifunctionality. *Proc. Natl. Acad. Sci. USA* 111, 5266-
354 5270

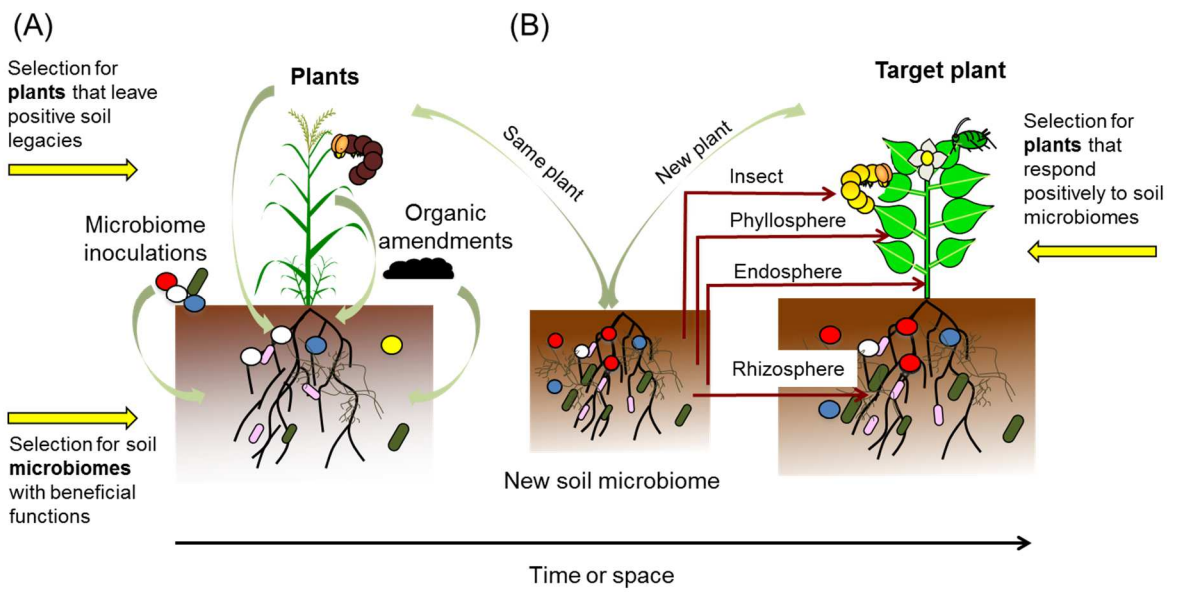
- 355 35. Delgado-Baquerizo, M. et al. (2016) Microbial diversity drives
356 multifunctionality in terrestrial ecosystems. *Nat. Commun.* 7, 10541
- 357 36. Wubs, E.R.J. et al. (2016) Soil inoculation steers restoration of terrestrial
358 ecosystems. *Nature Plants* 2, 16107
- 359 37. van der Voort, M. et al. (2016) Impact of soil heat on reassembly of bacterial
360 communities in the rhizosphere microbiome and plant disease suppression. *Ecol.*
361 *Lett.* 19, 375–382
- 362 38. Berendsen, R.L. et al. (2012) The rhizosphere microbiome and plant health.
363 *Trends Plant Sci.* 17, 478–486
- 364 39. Chaparro, J.M. et al. (2012) Manipulating the soil microbiome to increase soil
365 health and plant fertility. *Biol. Fertility Soils* 48, 489-499
- 366 40. Dessaux, Y. et al. (2016) Engineering the Rhizosphere. *Trends Plant Sci.* 21,
367 266-278
- 368 41. Mazzola, M. et al. (2015) *Brassica* seed meal soil amendments transform the
369 rhizosphere microbiome and improve apple production through resistance to
370 pathogen reinfestation. *Phytopathology* 105, 460-469
- 371 42. Kolton, M. et al. (2017) Biochar-stimulated plant performance is strongly
372 linked to microbial diversity and metabolic potential in the rhizosphere. *New*
373 *Phytol.* 213, 1393-1404
- 374 43. Mehari, Z.H. et al. (2015) Induced systemic resistance in tomato (*Solanum*
375 *lycopersicum*) against *Botrytis cinerea* by biochar amendment involves jasmonic
376 acid signaling. *Plant Soil* 395, 31-44
- 377 44. Hou, X. et al. (2015) Biochar amendment to soils impairs developmental and
378 reproductive performances of a major rice pest *Nilaparvata lugens* (Homoptera:
379 Delphacidae). *J. Appl. Entomol.* 139, 727-733
- 380 45. Agler, M.T. et al. (2016) Microbial hub taxa link host and abiotic factors to
381 plant microbiome variation. *PLoS Biol.* 14, e1002352
- 382 46. van der Heijden, M.G.A. and Hartmann, M. (2016) Networking in the plant
383 microbiome. *PLoS Biol.* 14, e1002378
- 384 47. Hol, W.H.G. et al. (2010) Reduction of rare soil microbes modifies plant–
385 herbivore interactions. *Ecol. Lett.* 13, 292-301
- 386 48. Jousset, A. et al. (2017) Where less may be more: how the rare biosphere pulls
387 ecosystems strings. *ISME J* 11, 853-862

- 388 49. Bulgarelli, D. et al. (2015) Structure and function of the bacterial root
389 microbiota in wild and domesticated barley. *Cell Host Microbe* 17, 392-403
- 390 50. Peiffer, J.A. et al. (2013) Diversity and heritability of the maize rhizosphere
391 microbiome under field conditions. *Proc. Natl. Acad. Sci. USA* 110, 6548-6553
- 392 51. Chaparro, J.M. et al. (2014) Rhizosphere microbiome assemblage is affected
393 by plant development. *ISME J* 8, 790-803
- 394 52. van Dam, N.M. and Bouwmeester, H.J. (2016) Metabolomics in the
395 rhizosphere: tapping into belowground chemical communication. *Trends Plant Sci.*
396 21, 256-265
- 397 53. Bais, H.P. et al. (2006) The role of root exudates in rhizosphere interactions
398 with plants and other organisms. *Annu. Rev. Plant Biol.* 57, 233-266
- 399 54. Rudrappa, T. et al. (2008) Root-secreted malic acid recruits beneficial soil
400 bacteria. *Plant Physiol.* 148, 1547-1556
- 401 55. Neal, A.L. et al. (2012) Benzoxazinoids in root exudates of maize attract
402 *Pseudomonas putida* to the rhizosphere. *PLoS ONE* 7, e35498
- 403 56. Lakshmanan, V. et al. (2014) Functional soil microbiome: belowground
404 solutions to an aboveground problem. *Plant Physiol.* 166, 689-700
- 405 57. López-Ráez, J.A. et al. (2017) Strigolactones in plant interactions with
406 beneficial and detrimental organisms: the yin and yang. *Trends Plant Sci.* 22, 527–
407 537
- 408 58. Bezemer, T.M. et al. (2013) Above- and below-ground herbivory effects on
409 below-ground plant–fungus interactions and plant–soil feedback responses. *J. Ecol.*
410 101, 325-333
- 411 59. Kostenko, O. et al. (2012) Legacy effects of aboveground–belowground
412 interactions. *Ecol. Lett.* 15, 813-821
- 413 60. van der Putten, W.H. et al. (2013) Plant–soil feedbacks: the past, the present
414 and future challenges. *J. Ecol.* 101, 265-276
- 415 61. Kulmatiski, A. et al. (2008) Plant–soil feedbacks: a meta-analytical review.
416 *Ecol. Lett.* 11, 980-992
- 417 62. Dias, T. et al. (2015) Accounting for soil biotic effects on soil health and crop
418 productivity in the design of crop rotations. *J. Sci. Food Agric.* 95, 447-454
- 419 63. Gopal, M. and Gupta, A. (2016) Microbiome selection could spur next-
420 generation plant breeding strategies. *Front. Microbiol.* 7, 1971

- 421 64. Wintermans, P.C. et al. (2016) Natural genetic variation in *Arabidopsis* for
422 responsiveness to plant growth-promoting rhizobacteria. *Plant Mol. Biol.* 90, 623-
423 634
- 424 65. Pérez-Jaramillo, J.E. et al. (2016) Impact of plant domestication on
425 rhizosphere microbiome assembly and functions. *Plant Mol. Biol.* 90, 635-644
- 426 66. Panke-Buisse, K. et al. (2015) Selection on soil microbiomes reveals
427 reproducible impacts on plant function. *ISME J* 9, 980-989
- 428 67. Mueller, U. and Sachs, J. (2015) Engineering microbiomes to improve plant
429 and animal health. *Trends Microbiol.* 23, 606-617
- 430 68. Fuentes, S. et al. (2014) Reset of a critically disturbed microbial ecosystem:
431 faecal transplant in recurrent *Clostridium difficile* infection. *ISME J* 8, 1621-1633
- 432 69. Pieterse, C.M.J. et al. (2014) Induced systemic resistance by beneficial
433 microbes. *Annu. Rev. Phytopathol.* 52, 347-375
- 434 70. Bender, S.F. et al. (2016) An underground revolution: biodiversity and soil
435 ecological engineering for agricultural sustainability. *Trends Ecol. Evol.* 31, 440-
436 452
- 437 71. Pangesti, N. et al. (2016) Jasmonic acid and ethylene signaling pathways
438 regulate glucosinolate levels in plants during rhizobacteria-induced systemic
439 resistance against a leaf-chewing herbivore. *J. Chem. Ecol.* 42, 1212-1225
- 440 72. van de Mortel, J.E. et al. (2012) Metabolic and transcriptomic changes
441 induced in *Arabidopsis* by the rhizobacterium *Pseudomonas fluorescens* SS101.
442 *Plant Physiol.* 160, 2173-2188
- 443 73. Pangesti, N. et al. (2014) Variation in plant-mediated interactions between
444 rhizobacteria and caterpillars: potential role of soil composition. *Plant Biol.* 17,
445 474-483
- 446 74. van Oosten, V.R. et al. (2008) Differential effectiveness of microbially
447 induced resistance against herbivorous insects in *Arabidopsis*. *Mol. Plant-Microbe*
448 *Interact.* 21, 919-930
- 449 75. Zebelo, S. et al. (2016) Rhizobacteria activates (+)- δ -cadinene synthase genes
450 and induces systemic resistance in cotton against beet armyworm (*Spodoptera*
451 *exigua*). *Plant, Cell Environ.* 39, 935-943
- 452 76. Aziz, M. et al. (2016) Augmenting sulfur metabolism and herbivore defense in
453 *Arabidopsis* by bacterial volatile signaling. *Front. Plant Sci.* 7, 458

- 454 77. Megali, L. et al. (2013) Fertilization with beneficial microorganisms decreases
455 tomato defenses against insect pests. *Agronomy for Sustainable Development* 34,
456 649-656
- 457 78. Fernández de Bobadilla, M. et al. (2017) Does drought stress modify the
458 effects of plant-growth promoting rhizobacteria on an aboveground chewing
459 herbivore? *Insect Sci.*, doi: 10.1111/1744-7917.12477
- 460 79. Bardgett, R.D. and Wardle, D.A. (2010) *Aboveground-belowground linkages:
461 biotic interactions, ecosystem processes, and global change*. Oxford University
462 Press
- 463 80. Baxendale, C. et al. (2014) Are plant–soil feedback responses explained by
464 plant traits? *New Phytol.* 204, 408-423
- 465 81. Fitzpatrick, C.R. et al. (2017) Phylogenetic relatedness, phenotypic similarity
466 and plant–soil feedbacks. *J. Ecol.* 105, 786–800
- 467 82. Mehrabi, Z. and Tuck, S.L. (2015) Relatedness is a poor predictor of negative
468 plant–soil feedbacks. *New Phytol.* 205, 1071-1075
- 469 83. Anacker, B.L. et al. (2014) Phylogenetic conservatism in plant-soil feedback
470 and its implications for plant abundance. *Ecol. Lett.* 17, 1613-1621
- 471
- 472

473 **Figure 1**



474
475

476 **Figure legend**

477

478 **Figure 1.** Soil microbiome manipulation to induce resistance in plants against
479 aboveground insects. (A) Soil microbiomes can be steered by different strategies such
480 as inoculating new microbiomes, adding organic amendments, or by growing certain
481 plants. Interactions of the plant with antagonists such as aboveground insect
482 herbivores will further shape the soil microbial community. Different components of
483 the system can be selected for desirable traits. For instance, through plant breeding,
484 cultivars that recruit beneficial soil microbiomes can be developed. Soil microbiomes
485 can also be engineered, selecting through several generations those soils that confer
486 plants with certain functions. (B) The new microbiome can affect plant growth and
487 resistance to aboveground attackers of the plant that is already growing in the soil, but
488 also that of plants growing later in the soil. The new soil microbiome will be an
489 important source for the microbial assembly of the rhizosphere, endosphere, and
490 phyllosphere of plants. Microbes inhabiting those habitats can suppress aboveground
491 insect pests, either directly (e.g. insect pathogens) or indirectly via changes in the
492 immunity of the host plant. Cultivars that show strong positive responses (in terms of
493 plant growth, resistance, etc.) to soil microbiomes could be developed. The suggested
494 pattern of events could happen along a temporal (e.g. in a crop rotation system) or
495 spatial axis (e.g. during intercropping).

496

497

498

499 **Glossary:**

500 **Endophytic:** that colonizes inside above- and/or belowground plant organs, without
501 causing evident disease symptoms.

502 **Endosphere:** microbial habitat inside plant organs.

503 **Induced systemic resistance (ISR):** enhanced resistance in the entire plant against
504 pathogens and herbivores, characterized by priming, and triggered by beneficial
505 microbes.

506 **Microbiome:** totality of microbial genomes present in a particular environment, for
507 example soil, rhizosphere, phyllosphere or endophytic compartment.

508 **Phyllosphere:** the surface of aerial plant organs, dominated by the leaves.

509 **Phytobiomes:** plants, their environment, and their associated communities of
510 organisms, including microbes, animals, and other plants.

511 **Plant-soil feedbacks:** changes by a plant in the biotic and abiotic characteristics of
512 the soil they grow in that influence the next generation of plants growing in the same
513 soil.

514 **Plant-soil-insect feedbacks:** plant-soil feedbacks that have effects on insects, or that
515 are affected by insect feeding on the plant creating the soil legacy.

516 **Priming:** alert state after certain stimulus that allows plants to mount a stronger
517 and/or faster defensive response upon attack.

518 **Rhizosphere:** thin layer of soil in contact with roots, that is under direct influence of
519 root exudates and soil microbes.

520 **Root exudates:** molecules released by plant roots and that among others, include
521 organic acids and sugars.

522

523

524

525 **Box 1. Microbial-induced systemic resistance against insects**

526 Plants can induce several types of resistance upon interacting with herbivores,
527 pathogens, or beneficial microbes. From those, induced systemic resistance (ISR) is
528 the enhanced defensive capacity of the entire plant against a broad spectrum of
529 attackers triggered upon local induction by beneficial microbes [69]. Plants then enter
530 in a primed state that allows them to respond faster and stronger upon herbivore or
531 pathogen attack [11]. Our knowledge on the molecular mechanisms of ISR against
532 insects has substantially increased in recent years. Several microbes, including plant-
533 growth promoting rhizobacteria, mycorrhizal fungi, and free-living fungi such as
534 *Trichoderma*, can trigger ISR against insect herbivores and especially against
535 generalist leaf chewers. Interestingly, the mechanisms seem to be conserved across
536 microbial groups. However, although in most cases ISR against insects is regulated by
537 JA- and ET-signalling pathways [69, 71], some microbial strains require other
538 signalling pathways to be functional [72]. Genes such as *LOX2*, *PDF1.2*, and *HEL*,
539 are often more strongly induced after herbivory in arabidopsis plants that are
540 inoculated with plant growth-promoting rhizobacteria [71, 73, 74]. However, the
541 effects and underlying mechanisms of microbes on insects are highly diverse, and two
542 aspects in particular suggest that the established paradigm of ISR needs to be re-
543 evaluated: (i) *Direct induction instead of priming*: Soil microbes can also directly
544 induce plant defence responses in the absence of an attacker. Genes in the ET-
545 pathway such as *ORA59* and *PDF1.2*, for example, are induced by rhizobacteria
546 colonization in arabidopsis [71], or the JA-regulated genes *GhAOS*, *GhLOX1* and
547 *GhOPR3* in cotton [75]. Associated with this, plant growth-promoting rhizobacteria or
548 their volatiles directly induced the synthesis of glucosinolates in arabidopsis [71, 72,
549 76] and gossypol in cotton [75]. (ii) *Induced systemic susceptibility*: insect
550 performance often increases upon soil inoculation with beneficial microbes. This is
551 especially common in phloem feeders such as aphids and whiteflies, probably due to
552 their behaviour that avoids damaging cells and feeding on phloem sap with lower
553 levels of defensive compounds than the overall leaf tissue [10]. But microbe-induced
554 susceptibility has also been observed in generalist caterpillars [77, 78]. Elucidating
555 the factors causing this variability will be a major breakthrough in the knowledge and
556 application of microbe-plant-insect interactions. Similar to microbial interaction
557 networks, insects and plants are also structured in interaction networks. Systems
558 approaches coupling microbial, insect and plant signalling networks will allow

559 scientists to design predictive models of microbiome-plant-insect interactions.

560

561

562 **Box 2. Plant-soil and plant-soil-insect feedbacks**

563 Plants as primary producers provide the basic resources for soil biota, including
564 insects, nematodes and microbes [79]. They contribute litter originating from dead
565 shoots or roots to the soil, and living plant roots release an array of metabolites. Via
566 these effects, plants shape soil biotic communities that use these compounds or are
567 influenced by them, and alter the physical and chemical properties of soils. These
568 plant-mediated changes of the soil can influence the performance of other plants that
569 grow later in the soil [60, 61]. This phenomenon is called plant-soil feedback and is
570 now receiving considerable attention because of its relevance in vegetation dynamics
571 and invasion ecology. Plants can affect individuals of the same species (known as
572 direct or conspecific feedback) or of different species (indirect or heterospecific
573 feedback). Most examples of conspecific plant-soil feedbacks are negative, but
574 heterospecific soil feedbacks are often positive, since many species perform better in
575 soil conditioned by others than by its own species [60, 61]. Outcomes also vary
576 widely between plant species and soils, and more research is needed to predict these
577 patterns. Plant functional traits such as growth rate, specific root length, and even
578 aboveground characteristics such specific leaf area, have been used to predict plant
579 soil feedbacks in natural ecosystems. For instance, soil conditioned by fast-growing
580 plant species or those with higher belowground biomass produced more positive
581 feedbacks due to increased nitrogen availability [80, 81]. One of the most
582 straightforward predictions is that closely related plant species have a higher chance
583 to be attacked by similar pathogenic microbes, and negative feedbacks would be
584 expected in this case. However, studies so far show inconsistent effects of the
585 relationship between phylogenetic relatedness and plant-soil feedbacks [81-83].
586 Another layer of complexity in plant-soil feedbacks are the presence of herbivorous
587 insects attacking the plants involved in the feedback, a concept that we would like to
588 define as plant-soil-insect feedbacks. A first possibility is that herbivory on the plants
589 that condition the soil alters soil legacies [59]. The second possibility is that plant-soil
590 feedback effects cascade to insects interacting with the responding plant during the
591 feedback phase [20]. Both scenarios may occur in a single plant-insect system [59].
592 Ecological knowledge of plant-soil feedback effects on natural enemies of plants has
593 strong potential for future implementation in agricultural ecosystems.

594

595 Outstanding Questions Box.

- 596
- Can we develop a universal approach to manage soil and plant microbiomes to
- 597 achieve higher yield, tolerance to abiotic stress and enhanced resistance to
- 598 pests?
- What genetic, molecular, and chemical plant mechanisms are responsible for
- 600 how plants shape and respond to soil microbiomes?
- What are the mechanisms that underlie microbiome-induced systemic
- 601 resistance to aboveground attackers and what are the consequences for higher
- 602 trophic levels?
- 603
- How do soil microbiomes interact with plant- and herbivore-associated
- 604 microbiomes to influence plant-insect interactions?
- 605
- 606

607 Trends box.

- 608 • Soil microbes are a major source of the plant microbiome and recent advances
609 show that they are key component of plant resistance against aboveground
610 attackers
- 611 • However, most of our knowledge on how belowground microbes affect
612 aboveground pests is limited to single strain effects, calling for research that
613 incorporates the full potential of the entire soil microbiome.
- 614 • Soil microbiomes can be manipulated, as done for centuries through
615 agricultural practices as crop rotation or the use of amendments. Conditioned
616 soils can be transplanted to restore ecological functions in other ecosystems.
- 617 • The role of the plant in shaping soil microbiomes and in how they respond to
618 them can be maximized but we need to increase our mechanistic
619 understanding at genetic, physiological and ecological levels.
620