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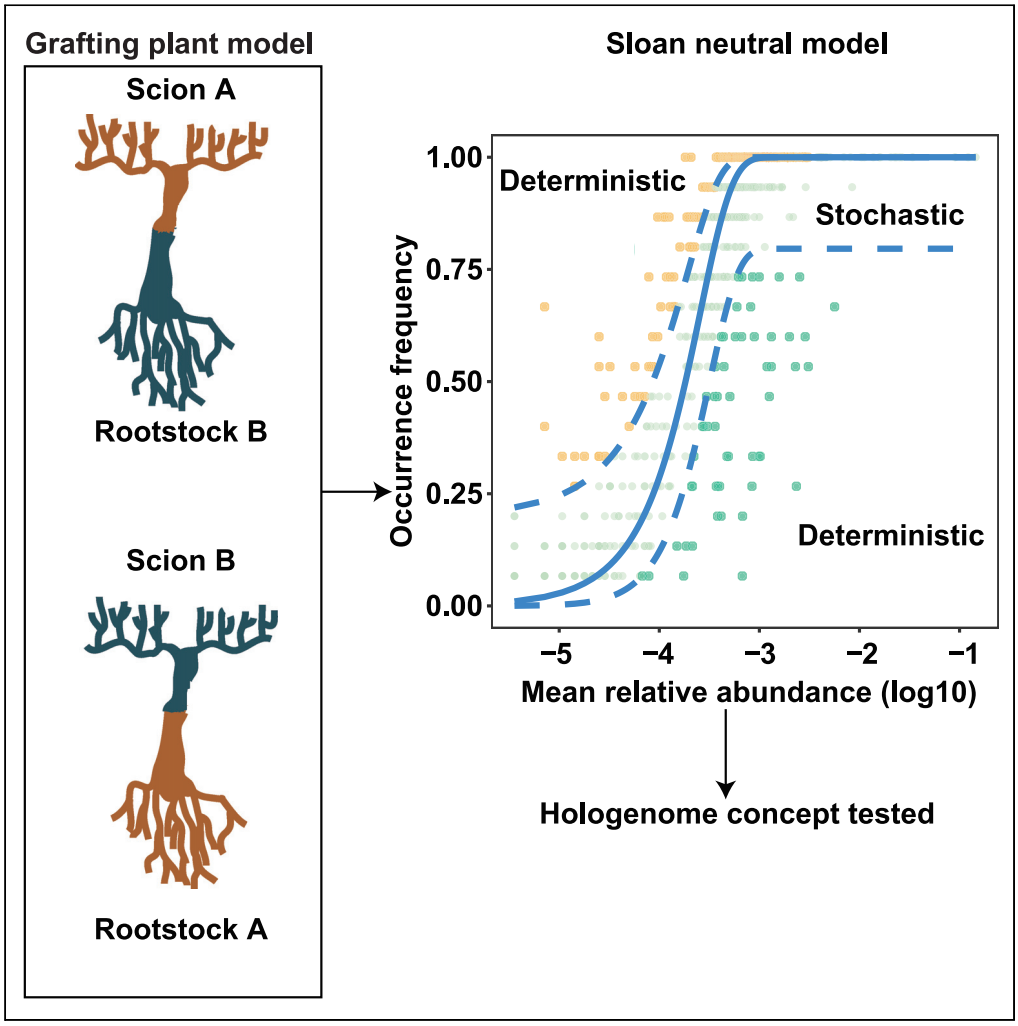

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Highlights

Hologenome research is still in its infancy and hologenome concept needs to be tested

The study demonstrates experimentally that the hologenome concept cannot be excluded

Root endosphere microbial composition in grafted-plants is driven by rootstock mainly

The study calls for experimental evaluation to identify limits of hologenome theory

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Evaluating the hologenome concept by analyzing the root-endosphere microbiota of chimeric plants

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SUMMARY

The hologenome concept considers the entity formed by a host and its microbiota, the holobiont, as new level of hierarchical organization subject to neutral and selective forces. We used grafted plants to formally evaluate the hologenome concept. We analyzed the root-endosphere microbiota of two independent watermelon and grapevine plant systems, including ungrafted and reciprocal-grafting combinations. Grafted and ungrafted hosts harbor markedly different microbiota compositions. Furthermore, the results indicate a non-random assembly of bacterial communities inhabiting the root endosphere of chimeric plants with interactive effect of both the rootstock and scion on the recruitment of microorganisms. Because chimeric plants did not have a random microbiota, the null hypothesis that holobionts assemble randomly and hologenome concept is an intellectual construction only can be rejected. The study supports the relevance of hologenome as biological level of organization and opens new avenues for a better fundamental understanding of plants as holobionts.

INTRODUCTION

Multicellular organisms, both plants and animals, associate with different types of microorganisms and no exception has yet been encountered in nature.[1] These associations vary along the spectrum of symbiotic interactions (beneficial, neutral, or detrimental relationships).[2] The interpretation of macroorganisms as holobionts and hologenome concept captures these inevitable partnerships by defining the holobiont as the emerging entity resulting from multivarious associations between a host macrobe and its microbial symbionts, and the hologenome as the genetic content of the holobiont.[3] It has repeatedly been shown that the symbionts of a given host can deeply impact the realized phenotype of host-animals[3,4] and plants.[5,6] According to neo-Darwinian theory, natural selection acts on the organism's traits[7] and its realized phenotypes.[8–10] The evolution of many phenotypes can thus not be understood without considering the many different ways in which the holobiont and the hologenome impact phenotypes that can be under selection.[8–10] This is the cornerstone of the hologenome concept[11] but this concept has yet to be conclusively demonstrated despite a developing knowledge and theoretical bases about the evolution and selection processes acting on the host that is associated with its microbiome.[12,13]

The hologenome concept encompasses the potential for both selective and neutral forces to shape population-level changes in holobiont phenotypes over time. These variations create a base of holobiont diversity upon which natural selection can then act. Despite the reasonable development of the holobiont and hologenome, their legitimacy and value have been the subject of debate, at least partially because of misunderstandings or the incorrect attribution of definitions,[14] but also because the conceptual framework is new and parts of the theory have yet to be challenged experimentally.[14]

In this framework, the plant microbiota that form complex associations with plants can contribute to plant fitness and productivity, for instance by influencing host nutrition and/or by enhancing plant resistance and resilience during stressful conditions.[15,16] As sessile entities, plants can also adjust their phenotype to adapt to environmental oscillations using epigenetic mechanisms and by recruiting microorganisms.[6] Plants are thus strongly dependent on evolving interactions with their microbiota that may directly

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contribute capacities to acclimate responses and phenotypes to environmental pressures,[5,6] and are good models to address hypotheses related to the hologenome concept.

It is well established that a host plant can actively recruit specific microorganisms from local reservoirs, for example, legumes recruit Rhizobia, and more widely, plants recruit arbuscular mycorrhiza.[5] More generally, plants are colonized by a wide range of bacteria, fungi and archaea[17–19] recruited from the environment.[5] Have all these microorganisms coevolved with their host? Put another way, do these horizontally transmitted microorganisms colonize their host simply by chance or in response to deterministic forces shaped by the genetic background of the host? This question is important because in plants and in a large proportion of animals, the majority of microorganisms that form the microbiota are acquired horizontally.[5,20,21] The question of the active or passive recruitment of these horizontally acquired microbial components within the microbiota is thus key to evaluating the nature of the holobiont. Passive acquisition is assumed to be central if coevolution processes are not at play. Thus, "stochastic recruitment", the possibility for any microorganisms living close to the roots to be part of the root microbiota endosphere by chance, yet obeying the rules of microbial community assembly (i.e., synergies, antagonisms, competition), is assumed to control horizontal recruitment. Thus, the null hypothesis to be tested is that the holobiont is only an intellectual construction because neutrality principally shapes microbiota assembly in the host plant. Under this assumption, the root microbiota can also be considered as an independent ecosystem that ultimately renders services to its host.

Marked heterogeneity among microorganism communities has been observed in the roots of co-occurring individual plants.[22] At first sight, this heterogeneity suggests that neutrality shapes the plant microbiota, i.e., microorganisms functionally equivalent to each other being stochastically replaceable.[23] However, the heterogeneity could also be the consequence of a locally heterogeneous soil microbial reservoir. Observed heterogeneity is thus not necessarily paired with stochastic recruitment (see definition above). Furthermore, under the assumption of stochastic recruitment, there is no reason for such high filtration between the soil microbial rhizosphere (i.e. the community of microorganisms living close to the roots) and the root endosphere.[5] To evaluate the significance of neutrality and selection in shaping the holobiont, we experimentally manipulated the host plant by creating chimeras (i.e. grafted plants); specifically, we grew plant *A*, plant *B*, plant *AB* (grafted scion from *A* on a rootstock from *B*), and plant *BA* (reciprocal grafting) in a common garden setting and characterized the resultant composition and structure of the microbiota across host plants.

To formally evaluate the hologenome concept we hypothesized that (1) the pattern of recruitment is only passive (null hypothesis, plant holobionts cannot be consistent units of selection) and, as a consequence, high heterogeneity in the microbiota community composition among plants is expected. (2) Alternatively, the pattern of recruitment is shaped deterministically by the host plant (i.e. genetically determined) and, as a consequence, the root microbiota endosphere of *A* differs from that of *B* but grafting *AB* and *BA* would be expected to produce similar microbiota that are close to *A* and *B*. (3) Grafting (creating a chimeric plant) is known to modify the recruitment of microorganisms,[24] and microbiota in *AB*and *BA* that differ from those of the controls are therefore expected. If this is true, the chimeric plants would be a proxy of passive vs active recruitment, and hence enable estimation of the proportion of the community that is actively recruited. To test these hypotheses, we analyzed the root-endospheric bacteria in two experiments involving two different plant systems. The results of the experiments will enable us to determine if the artificially created selection obtained from plant grafting systems intentionally disrupted the recruitment of plant root endophytes, with potential significance for holobiont manipulation in horticulture and agriculture.

RESULTS

The degree of stochastic versus deterministic assembly in the root-endosphere microbiota was analyzed using the same experimental design but with two levels of biological integration: grafted species (in the Chinese experiment with watermelon and bottle-gourd), and grafted cultivars/genotypes (in the French experiment with grapevine) (Figure 1). For the watermelon and bottle-gourd (*W*&*B*) experiment, it includes four different conditions: ungrafted watermelon (*W*), ungrafted bottle gourd (*B*), watermelon grafted on bottle gourd (*WB*), and reciprocal grafting (*BW*). For the different grapevine genotype experiments, it was divided into two cases. The first one was Fercal and Chenin (*F*&*C*) combinations: ungrafted Chenin grapevine (*C*), ungrafted Fercal grapevine (*F*), Chenin grapevine grafted on Fercal grapevine (*CF*), and reciprocal grafting (*FC*). The second one is 101-14 and Chenin (*101*&*C*) combinations: ungrafted Chenin grapevine (*C*), ungrafted American "wild" hybrid grapevine (*101*), Chenin grapevine grafted on American "wild" hybrid grapevine (*C101*), and

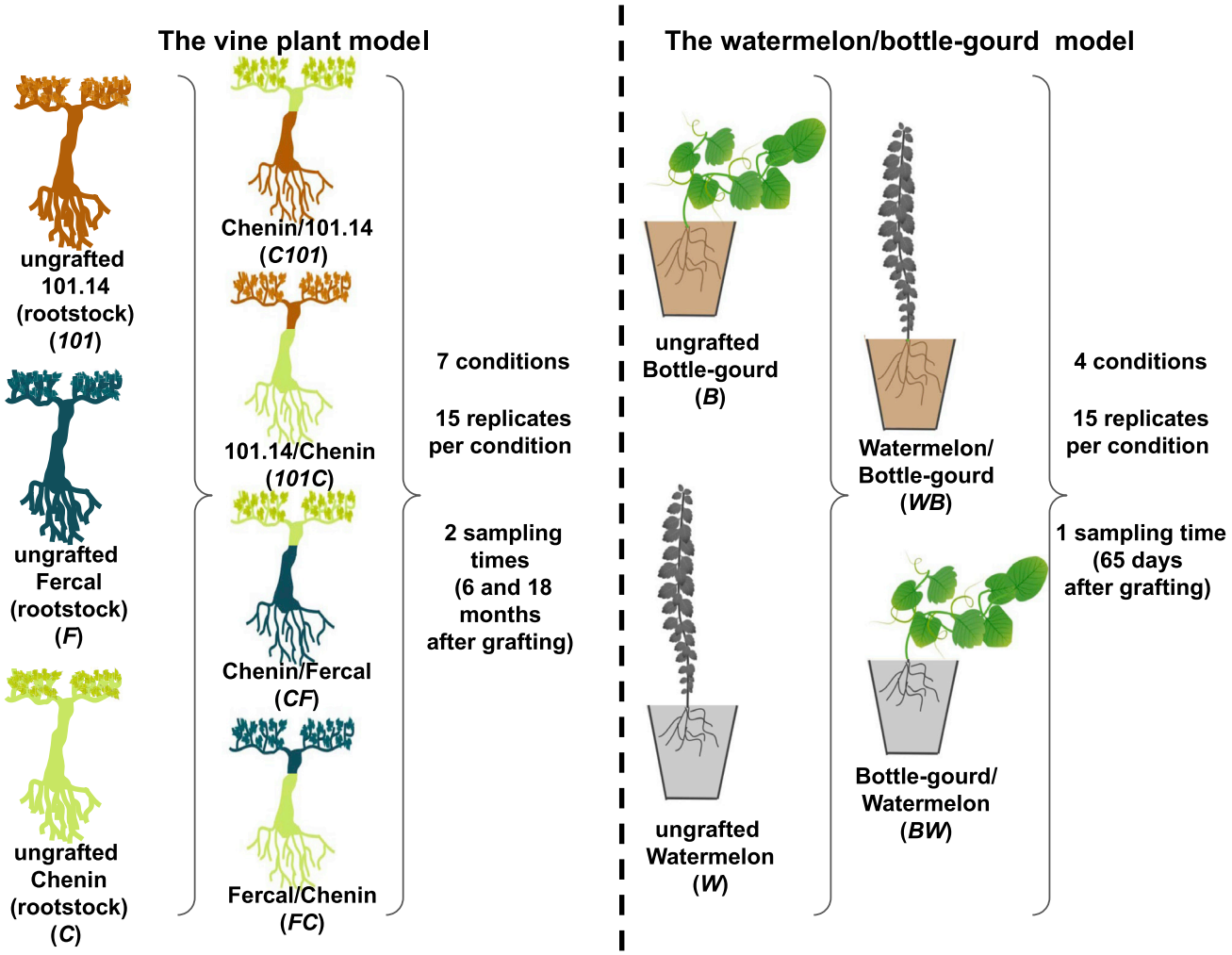

Figure 1. Experimental design and sampling scheme

This study comprised two biological models: grapevine (on the left); watermelon and bottle-gourd (on the right). The grapevine model (perennial and ligneous plant) comprised two sub-cases (*101&C* and *F&C*) with four combinations: ungrafted *101* (usual 101.14 rootstock), ungrafted *C* (usual scion Chenin), grafted *C101* (*C*: scion-S; *101*: rootstock), grafted *101C* (*101*: scion; *C*: rootstock) and ungrafted *F* (usual Fercal rootstock), ungrafted *C* (usual scion Chenin), grafted *CF* (*C*: scion; *F*: rootstock) and grafted *FC* (*F*: scion; *C*: rootstock). Roots of each condition (15 replicates) were collected on two occasions (6 (T1) and 18 (T2) months after grafting). For the grapevine model, T1 and T2 refer to a 1-to 3-inch shoots stage and a 10-to 16-inch shoots stage respectively. The watermelon and bottle-gourd model (annual plant) comprised two ungrafted conditions: *B* (bottle-gourd), *W* (watermelon) and two grafted combinations: *WB* (*W*: scion; *B*: rootstock) and *BW* (*B*: scion; *W*: rootstock). Roots of each condition (15 replicates) were collected on one occasion (65 days after grafting).

reciprocal grafting (*101C*) (Figure 1). Samples were collected for the grapevine experiment at two time points (the first and second sampling hereafter named as T1 and T2, see STAR Methods section).

The α-diversity of the root endospheric bacterial communities of chimeric plants were similar to those of ungrafted plants

The composition of the root-endosphere bacterial communities in the two grapevine experimental genotype combinations (*F&C; 101&C*) were found to be similar at the Class level and were dominated by Gammaproteobacteria and Alphaproteobacteria at T1 and T2, respectively (Figures S1A, S1B, S1D, and S1E). These bacterial communities differed from those in the root-endosphere in the watermelon and bottle-gourd (*W&B*) experiments (Figure S1C), as expected. We analyzed root endospheric bacterial α-diversity (richness, Pielou evenness and Shannon diversity index) between the ungrafted and grafted combinations (Figure S2). In the *W&B* experiment, the Wilcoxon test showed that no significant differences in α-diversity were found between the different combinations of rootstock and scion (Figures S2C, S2H, and S2M). In the grapevine experiments, two-way PERMANOVA (permutational multivariate analysis of variance) and Wilcoxon test clarified that roots

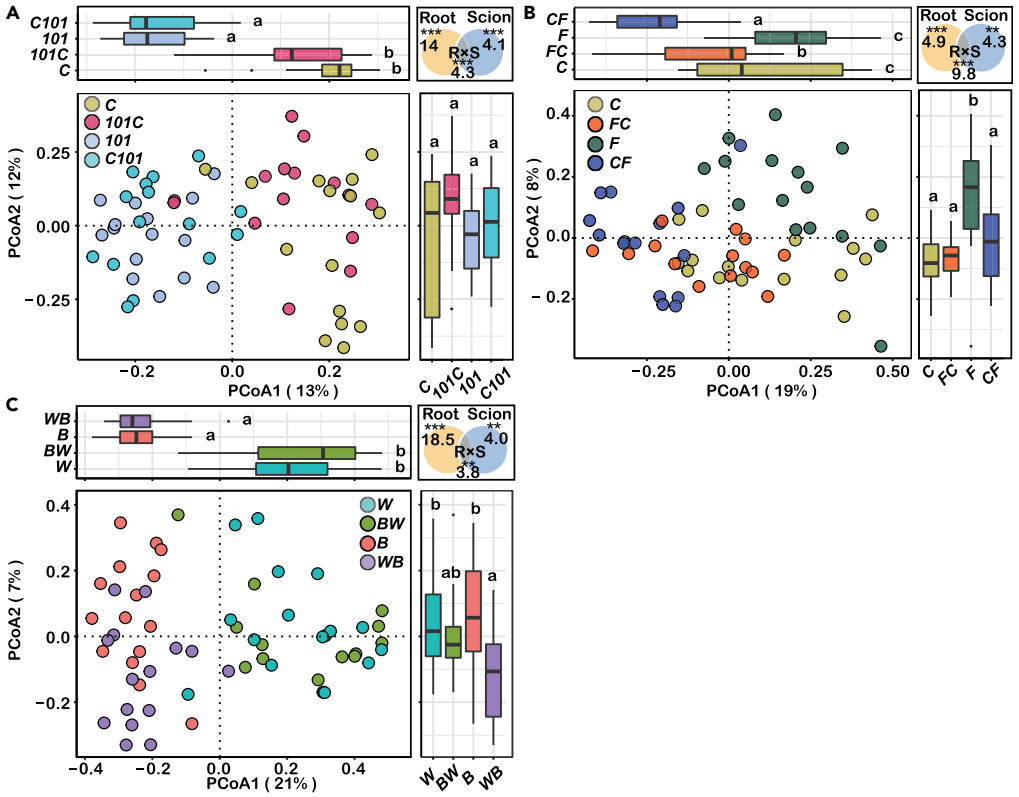

Figure 2. Bacterial community composition of the two models

Principal coordinate analysis (PCoA) plots depict the bacterial communities (zOTUs composition) of the grapevine models: *101*&*C* grapevine experiment case and *F*&*C* grapevine experiment case at T1 sampling time (A, B respectively) and the watermelon & bottle-gourd (*W*&*B*) model (C). Results of the sampling 18 months after grafting (i.e., grapevine experiment) are provided in Figure S3. Results of the PERMANOVA statistical tests analyzing the effect of the rootstock (Root), the scion (Scion) and the interaction of the two variables (R x S) (**: $p < 0.01$; ***: $p < 0.001$) and R^2, percentage of each explained variance, are provided on the top right of each panel included in the plots. Boxplots show the overall distribution of PC1 and PC2 scores within each condition. Different small letters indicate significant differences between conditions (Tukey's test for multiple comparisons).

of the grafted grapevines Fercal and Chenin combination (i.e., *CF* and *FC*) and grafted grapevines 101-14 and Chenin combination (i.e., *C101* and *101C*) harbored significantly higher richness than ungrafted Chenin (*C*) at T1 only (Figures S2A and S2B; no significant differences were found at T2, Figures S2D, S2E and Table S1). Two-way PERMANOVA and VPA (variation partition analysis) showed that the rootstock had a significant effect on the richness in the *C*&*101*case at T1. For both the *C*&*101* and *C*&*F* cases, the richness index result showed a significant interaction between rootstock and scion. The significant interaction also occurred in the Shannon index only for the *C*&*101*case. For the watermelon and bottle gourd experiment, we did not record a significant interaction effect (Table S1). This indicated that in the grapevine experiment, especially at the early stage of grapevine growth, the differences of richness between the rootstock also varied according to the scion type.

The rootstocks, scion and their interaction impacted the composition of the root microbiota of chimeric plants

We used principal coordinate analysis (PCoA) and two-way PERMANOVA to test for potential shifts in bacterial community composition at the zOTU level depending on grafting conditions (Figures 2 and S3). Overall, we demonstrated differences among rootstock and scion combinations with distinct groups between conditions with the same rootstocks (Figures 2 and S3). We found that the rootstock and scion exerted a significant influence on bacterial community composition in all experiments (*101*&*C* grapevine experiment case 1 in Figure 2A; *F*&*C* grapevine experiment case 2 in Figure 2B; *W*&*B* experiment in Figure 2C and Table S1). Meanwhile the interaction between the rootstock and scion also significantly affected β-diversity, explaining 3.8–9.8% of the observed variation (Table S1). This effect was confirmed on the two root-sampling dates of grapevine experiment (T1 and T2) (Figures 2 and S3). Variation partition analysis

(VPA) was carried out to quantify the relative contributions of rootstock, scion and the interactions between these two factors to the bacterial communities (Table S1). These factors explained 16.8–31.7% of the observed variation. Of these two independent factors, rootstock exhibited the largest significant impact on the microbial community in the grapevine *101&C* (T1 and T2) and *W&B* experiments.

Deterministic assemblages of the root-endosphere microbiota of the chimeric plants

We conducted Sloan neutral community model analyses (NCM) to identify the stochastic and deterministic drivers of the bacterial communities on the roots of the grafted chimeric plants (Figures 3 and S4). For this purpose, we used ungrafted metacommunities (*B* and *W*; *101* and *C*; *F* and *C*) as source communities for each experiment. Overall, the occurrence frequency of bacterial taxa fitted Sloan neutral community model analyses (T1: *101C*: $R^2 = 0.44$; *C101*: $R^2 = 0.42$-T2: *101C*: $R^2 = 0.67$; *C101*: $R^2 = 0.60$-T1: *FC*: $R^2 = 0.39$; *CF*: $R^2 = 0.50$-T2: *FC*: $R^2 = 0.61$; *CF*: $R^2 = 0.55$-*WB*: $R^2 = 0.12$; *BW*: $R^2 = 0.09$-Figures 3 and S4; graphs on the left). Thus, we can determine the relative importance of stochastic versus deterministic processes for community assembly. In all the experiments, some taxa occurred more or less frequently than predicted by the neutral models, the neutrality envelope being the vectorial space between the two dashed lines (Figures 3 and S4). Above the neutrality interval, the yellow bacterial taxa (Figures 3 and S4) overlapped more consistently in grafted plants than expected, i.e. were more abundant than expected, and were interpreted as being actively maintained and selected by the host plant, whereas the green taxa below the neutrality interval, (Figures 3 and S4) overlapped less than expected in grafted plants, i.e., were less abundant than expected, implying active counter-selection by the host (Figures 3 and S4). These taxa occurring more frequently than expected are mainly predicted as chemoheterotrophs, and to a lower extent as 'intracellular parasites', or involved in Nitrogen cycling especially through ureolysis and nitrogen fixation processes (Figure S5).

Across the two biological experiments, from a minimum of ~20% (T1 -*101C*, Figure 3A) to more than 80% (T1-*FC*, Figure 3C) of the bacterial taxa detected in the grafted plants were outside the neutrality interval. Thus, the more-and less-abundant taxa than expected from neutral distribution represent a proportion that is far from negligible. Among the root endosphere taxa, Alphaproteobacteria were consistently overrepresented (i.e. dominant) in the two independent biological independent experiments (Figures S6 and S7). The taxa that comprised the more abundant fraction than expected were consistently largely shared between ungrafted and grafted plants (Figures S6 and S7), whereas a very limited number of taxa were specific to a grafting mode. However, differences in the composition of taxa that were more abundant than expected were observed within and between the experiments (Figures S6 and S7), for instance see Bacillus in the watermelon and bottle-gourd experiment (Figures S6E and S6F).

A Bayesian approach was used to estimate the proportion of recruited taxa (located above neutrality interval, Figure 3) originating from each source (i.e., ungrafted related). The results of source tracking invariably indicated that both the rootstock and scion explained a fraction of the root endosphere taxa that were selectively hosted, hence a detectable specific effect of both parts of the plants on the recruited microbiota (bar charts in Figures 3 and S4). Source tracking analysis also shows that the largest proportion of selectively hosted taxa could not be explained (unknown in the bar charts, Figures 3 and S4) because both sources contained the taxa considered and the higher frequency of the taxa in comparison to the null model expectation could be the consequence of the scion or the rootstock or of both parts of the chimeric plant. This does not presume that these particular zOTUs are not important, but rather could be part of a stable core microbiota which could result from a strong selection process within this fraction of the host endosphere.

Taken altogether, these results indicated a non-random assembly of bacterial communities inhabiting the root endosphere of the chimeric plants with a greater effect of the rootstock on actively recruited taxa.

DISCUSSION

In the holobiont framework, if selective pressures shape the composition of the holobiont, it can be predicted that a large proportion of host associated microorganisms will be specific to the genetic background of the hosts and that the heterogeneity observed among microbiota of genetically identical hosts is related to differences in the reservoir of recruitable microorganisms. Alternatively, if holobionts are only intellectual constructions, i.e., objects that do not actually exist, their microbiota would be assumed to result primarily from neutral forces and heterogeneous and stochastic microbiota compositions would be expected across different individual hosts. For a broad scientific impact, two plant types (perennial and annual plants) and two levels of analyses, the genotype level (with the grapevine manipulation) and the species level (with the

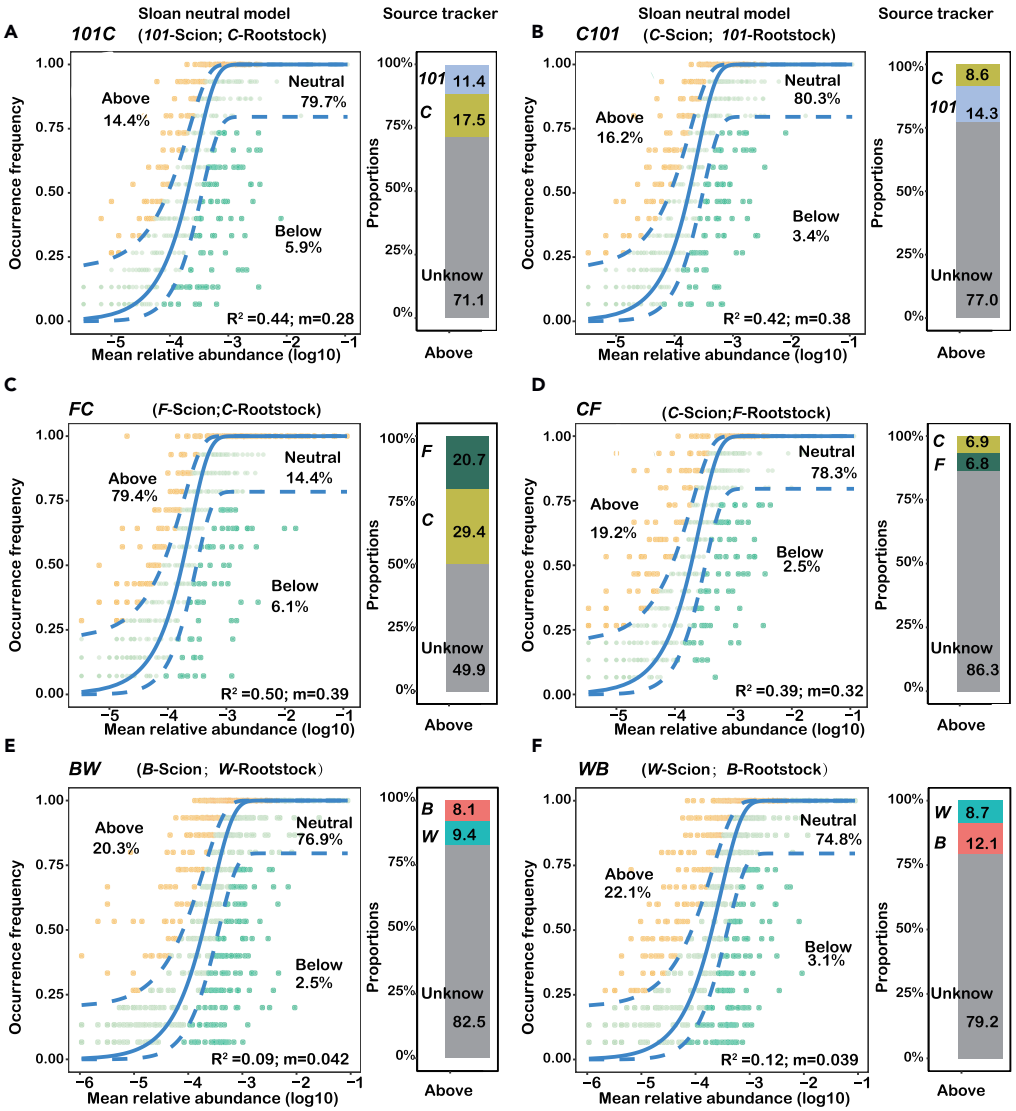

Figure 3. Partitioning of deterministic and stochastic processes of bacterial community assembly and source tracker results

The first graphs (curves on the left) show the occurrence frequencies predicted by the neutral community model (NCM) at T1 sampling time. Results of sampling 18 months after grafting are provided in Figure S4. The taxa occurring more frequently than expected are shown as yellow dots whereas those occurring less frequently are in green. The solid blue line indicates the best fit of the model and the dashed lines indicate the 95% confidence intervals. The dashed lines indicate the neutrality envelope. Goodness of statistical fit R^2 and m (i.e., migration rate) are both indicated on the graphs. Percentages on the graphs indicate cumulative taxa occurrence frequencies Above, Neutral and Below fractions. The second graphs (bar charts on the right) show the results of the source tracker analysis indicating origin proportions of the above partitions of the NCM. The 'unknown' status within the source tracker analysis refers to these zOTUs that can't be defined to belong to a given source. For the below partitions of the NCM representing a low proportion of the abundances (i.e., from 2.5% in panels D and E, to up to 6.1% only in panel C) made it statistically impossible to do the same source tracker analysis. The figure shows the results obtained for 101 grafted on Chenin (A) and reciprocally (B), Fercal grafted on Chenin (C) and reciprocally (D) and Bottlegourd grafted on Watermelon (E) and reciprocally (F).

watermelon and bottle-gourd manipulation), were used to evaluate key predictions for the assembly of holobionts in the context of the hologenome concept. Most of the published studies on the microbiota of grafted (grapevine) plants have focused on the rhizosphere compartment.[25] However, to limit the passive effect of soil on the rhizosphere composition we have chosen to focus herein on the root-endosphere, an intimate plant microbiota compartment better fitted to test our hypotheses.

Non-random recruitment of the root endosphere microbiota

A sophisticated experimental design including different models of ungrafted and grafted plants was developed and set up to evaluate the hologenome concept (Figure 1). The first hypothesis stating that the pattern of recruitment is only passive was invalidated. Consequently, the hologenome concept applied to plants remains valid (i.e., is not here rejected). Independent analyses of the root-endospheric microbiota demonstrated differentiation among the microbial communities that colonized the roots in the two experimental systems (Figures 2 and S3). Hypothesis 2 was thus validated. These results confirm both previous and very recent studies. For instance, a greenhouse bioassay showed clear modifications in root microbial community structure depending on the identity of the plant species (*Baccharis pilularis, Ceanothus thyrsiflorus*, or *Pinus muricata*).[26] Host preference signatures of commensal bacterial communities of the root endosphere of two different plant species (*Lotus japonicus, Arabidopsis thaliana*) were demonstrated in greenhouse experiments and synthetic communities (SynCom) specific to each plant species.[27] Further evidence of host-plant preference effect was obtained using different mixes of neighboring plant species.[28,29]

Chimeric plants were used for in-depth assessment of the randomness of the microbiota composition by comparing ungrafted relatives. If the microbiota of *A* differs from *B* as demonstrated here in the two experimental systems, and if holobionts are persistent enough across generations to be consistent units of selection, grafting *AB* and *BA* are expected to be similar and close to *A* and *B* if the molecules produced by *A* and *B* determine the microbiota community composition. Our results support this interpretation of the consequence of considering plants as holobionts and also provide information concerning the drivers of root-microbiota composition. Far from negligible, determinism in the microbiota assembly of grafted plants was demonstrated across all the experiments (Figures 3 and S4) supporting the hypothesis that plants should be considered as holobiont objects and further evaluated in an evolutionary perspective.

Rootstock, scion and the interaction drive the root endosphere microbial communities in chimeric plants

In grafted plants, root associated microbiota composition can be responsive to rootstock genotypes,[30,31] but the existence of interaction between scion and rootstock in the root microbiota recruitment is often neglected. Recent studies on grafted grapevine plants have however shown the effect of both scion and rootstock on the rhizosphere composition.[32,33] In our study, the interactive effect of rootstock and scion on β-diversity were found for all experiments whereas it was detected for α-diversity only for the grapevine experiment at T1 (Table S1). For the bottle gourd/watermelon experiment, only rootstock drives microbiota species α-diversity (Figure S2 and Table S1). Our results on the microbiota endosphere of grafted grapevine are consistent with a previous study focusing on rhizosphere microbial communities' composition showing the influence by both scion and rootstock.[32] Arbuscular mycorrhizal Fungal (AMF) community's composition in *Citrus* root was significantly impacted by both *Citrus* scion and rootstock, but rootstock genotype exerted a greater impact on the AMF community than scion.[34]

The determinants by which either rootstock or scion effect the composition of a microbial community are unknown.[35] However, we demonstrated herein that the determinism in the root endosphere microbiota recruitment mediated by rootstock or scion in the chimeric plant was uneven (Figures 3 and S4). We also showed dominance in the effect of the rootstock over the scion in the recruitment of the root microbiota (i.e., higher proportion of zOTUs being under the recruitment influence of rootstock (Figures 3 and S4)). This determinant did not depend on the plant-genotype used and was strikingly observed in reciprocal grafts (i.e. *101C* versus *C101* conditions showed similar trends with the rootstock shaping root endosphere microbiota, Figure 3).

Despite the importance of root endosphere microbiota in plant functioning,[16,36,37] these microorganisms have been overlooked so far. The triggering effect for selective recruitment of root-associated microbiota is likely conferred by root secretions. Although the carbon skeleton of the secreted molecules is synthesized by photosynthesis (i.e., by the scion), the root traits determine the quantity and quality of root secretions, which can be changed or made more elaborate according to the origin of the root. This may explain why active recruitment is dominated by the rootstock.

Unless similar plant-genetic materials are used in reciprocal grafting different root endospheric microbiota are observed

Our experimental results clearly demonstrate differentiation of the bacterial endosphere communities among the reciprocal grafted plants (Figure 2). Thus, validating hypothesis 3, a different microbiota in

AB and *BA* was observed based on the assumption of induced perturbation of the signaling molecules. The difference in the endospheric microbiota between *AB* and *BA* was found repeatedly across the two experiments. The effect of the scion on the determinist recruitment was also significant but was not as strong as that of the rootstock (Figures 2 and 3). Considering that the largest fraction of the root endosphere microbiota originates from the rhizosphere,[38,39] it is likely that signal molecules emitted outside the roots in the form of exudates are synthesized in the roots themselves and to a lesser extent in the shoot, and/or the recognition signals of a given microorganism permitting the root endosphere colonization are controlled by the roots.

Plants are known to synthesize secondary metabolites they emit at the level of the roots, including coumarins,[40] glucosinolates, benzoxazinoids,[41,42] triterpenes,[43,44] as well as other less complex compounds such as citric acid, malic acid, succinic acid that have regulatory effects on root colonization, nutrient acquisition and root volume.[45] In addition to these compounds, miRNA emitted outside the roots can mediate the plant-microorganism interactions.[46] Thus, the predominantly deterministic nature of the composition of the plant microbiota may be explained at least in part by these kinds of molecules produced and emitted by the plant that determine the composition of the microbiota. Other processes including microbe-microbe interactions either being antagonistic-(i.e., competition for niche colonization) or synergistic-interactions (e.g., evolution of dependencies among microorganisms) can occur[47] and can also explain part of the observed determinism.

According to the literature, the succession in the composition of the microbiota observed over time in the grapevine experiment also seems to be correlated with a modification in secondary metabolites in the roots.[48] Intuitively, one can interpret these changes as being under the intrinsic control of the plant itself and to depend on the plant's physiological needs and stage of growth. However, it has also been documented that the endospheric microbiota of the plant and its composition reprogram the metabolic pathways, thereby changing the composition of the primary and secondary metabolites produced by the host plant.[49] The microbiota is therefore hypothesized to influence its own succession. To summarize, the determinism of the root endosphere microbiota composition could possibly be under the control of the host-plant and components of its microbiota, hence under the control of the plant holobiont. Further important analyses tracking the microbial migration from a plant compartment to another would need to be set up to demonstrate the continuity among microbial reservoirs. From a microbial culture collection of the studied plant endosphere compartments and GfP transformed lines, gnotobiotic microbial communities manipulations or inoculations of zOTUs that were assigned to a given source, would allow to highlight the microorganism recruitment and fate within the plant holobiont.

Conclusions limits and prospects

To our knowledge it is the first time that the hologenome concept is formally tested. Our experiments demonstrated that the hologenome concept cannot be rejected, nor can the hypothesis of plant holobionts as units of selection. Despite two independent experiments have led to this important conclusion, the main limit of the study is its novelty. Because the hologenome concept deeply modifies our perception of individuality for a macro-organism including both animals and plants, not solely from a philosophical but also from a scientific point of view, this paper calls for further evaluation of the hologenome concept and for the identification of the limits of the hologenome theory using hypothesis driven research in the different eco-evolutionary dimensions. Understanding how this functional entity (holobiont) is built and how, together, the plant and the microbiota, are capable of buffering variations in the environment, is one of the main challenges facing plant management in the context of sustainable agriculture. Conceiving plants as holobionts might modify practices in agriculture with plant breeding and seed production strategies.

Our work also calls for elucidation of the mechanistic processes behind the determinism of microbiota recruitment, filtering, and assembly. Holobiont and hologenome research is still in its infancy. Plants are probably the best organisms to test further predictions related to the hologenome concept because they are easier to use than animals if the composition of the microbiota has to be manipulated. For instance, gnotobiotic strategies where a plant is inoculated by a synthetic community have been successfully developed mainly using *A. thaliana*.[50–52] One important avenue of research would be to investigate how particular compounds produced by a host plant, by components of the microbiota, or by both, can shape and change the microbiota and hence modify holobiont functioning. Based on the manipulated plants (i.e., chimeric plants), we anticipate a new understanding of coevolution between host and

microorganisms and also among microorganisms, with possible impacts on the future of plant-and health-science stimulated by future holobiont-and hologenome-based research.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.106031.

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AUTHOR CONTRIBUTIONS

M.B., T.W., N.L., and P.V. conceived the project with the support and the expertise of K.T. and C.M. M.B., T.W., C.M., N.L., and P.V. set up the methodology of the study. M.B. and P.V. sampled the grapevine and T.W. sampled the watermelon. M.B. and T.W. conducted the lab experiments and analyzed the sequence data (with the help of X.Q. for data processing and analyses). M.B. and T.W. performed the statistical analyses with the help of C.M., X.Q., and L.L. M.B., T.W., and P.V. wrote the first draft of the paper. All the authors contributed critically to interpret the results and gave their final approval for publication.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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REFERENCES

1. Morris, J.J. (2018). What is the hologenome concept of evolution? F1000Res. *7*. F1000 Faculty Rev-1664. https://doi.org/10.12688/f1000research.14385.1.

2. Mendes, R., Garbeva, P., and Raaijmakers, J.M. (2013). The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol. Rev. *37*, 634–663. https://doi.org/10.1111/1574-6976.12028.

3. Rosenberg, E., Sharon, G., Atad, I., and Zilber-Rosenberg, I. (2010). The evolution of animals and plants via symbiosis with microorganisms. Environ. Microbiol. Rep. *2*, 500–506. https://doi.org/10.1111/j.1758-2229.2010.00177.x.

4. Blanton, L.V., Charbonneau, M.R., Salih, T., Barratt, M.J., Venkatesh, S., Ilkaveya, O., Subramanian, S., Manary, M.J., Trehan, I., Jorgensen, J.M., et al. (2016). Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children. Science *351*, aad3311. https://doi.org/10.1126/science.aad3311.

5. Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., and Dufresne, A. (2015). The importance of the microbiome of the plant holobiont. New Phytol. *206*, 1196–1206. https://doi.org/10.1111/nph.13312.

6. Vannier, N., Mony, C., Bittebière, A.K., and Vandenkoornhuyse, P. (2015). Epigenetic mechanisms and microbiota as a toolbox for plant phenotypic adjustment to environment. Front. Plant Sci. *6*, 1159. https://doi.org/10.3389/fpls.2015.01159.

7. Scheiner, S.M. (1993). Genetics and evolution of phenotypic plasticity. Annu. Rev. Ecol. Syst. *24*, 35–68.

8. Rosenberg, E., Sharon, G., and Zilber-Rosenberg, I. (2009). The hologenome theory of evolution contains Lamarckian aspects within a Darwinian framework. Environ. Microbiol. *11*, 2959–2962. https://doi.org/10.1111/j.1462-2920.2009.01995.x.

9. Zilber-Rosenberg, I., and Rosenberg, E. (2021). Microbial-driven genetic variation in holobionts. FEMS Microbiol.Rev. *45*, fuab022. https://doi.org/10.1093/femsre/fuab022.

10. Zilber-Rosenberg, I., and Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. FEMS Microbiol. Rev. *32*, 723–735. https://doi.org/10.1111/j.1574-6976.2008.00123.x.

11. Bordenstein, S.R., and Theis, K.R. (2015). Host biology in light of the microbiome: ten principles of holobionts and hologenomes. PLoS Biol. *13*, e1002226. https://doi.org/10.1371/journal.pbio.1002226.

12. Kopac, S.M., and Klassen, J.L. (2016). Can they make it on their own? Hosts, microbes, and the holobiont niche. Front. Microbiol. *7*, 1647. https://doi.org/10.3389/fmicb.2016.01647.

13. Huitzil, S., Sandoval-Motta, S., Frank, A., and Aldana, M. (2018). Modeling the role of the microbiome in evolution. Front. Physiol. *9*, 1836. https://doi.org/10.3389/fphys.2018.01836.

14. Theis, K.R., Dheilly, N.M., Klassen, J.L., Brucker, R.M., Baines, J.F., Bosch, T.C.G., Cryan, J.F., Gilbert, S.F., Goodnight, C.J., Lloyd, E.A., et al. (2016). Getting the hologenome concept right: an eco-evolutionary framework for hosts and their microbiomes. mSystems *1*, 00028-16. https://doi.org/10.1128/mSystems.00028-16.

15. Trivedi, P., Leach, J.E., Tringe, S.G., Sa, T., and Singh, B.K. (2020). Plant-microbiome interactions: from community assembly to plant health. Nat. Rev. Microbiol. *18*, 607–621. https://doi.org/10.1038/s41579-020-0412-1.

16. Compant, S., Samad, A., Faist, H., and Sessitsch, A. (2019). A review on the plant microbiome: ecology, functions, and emerging trends in microbial application. J. Adv. Res. *19*, 29–37. https://doi.org/10.1016/j.jare.2019.03.004.

17. Bulgarelli, D., Rott, M., Schlaeppi, K., Ver Loren van Themaat, E., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E., et al. (2012). Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. Nature *488*, 91–95. https://doi.org/10.1038/nature11336.

18. Lundberg, D.S., Lebeis, S.L., Paredes, S.H., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektson, A., Kunin, V., Del Rio, T.G., et al. (2012). Defining the core *Arabidopsis thaliana* root microbiome. Nature *488*, 86–90. https://doi.org/10.1038/nature11237.

19. Vannier, N., Mony, C., Bittebiere, A.K., Michon-Coudouel, S., Biget, M., and Vandenkoornhuyse, P. (2018). A microorganisms' journey between plant generations. Microbiome *6*, 79. https://doi.org/10.1186/s40168-018-0459-7.

20. Baltrus, D.A. (2017). Adaptation, specialization, and coevolution within phytobiomes. Curr.Opin. Plant Biol. *38*, 109–116. https://doi.org/10.1016/j.pbi.2017.04.023.

21. Frank, A.C., Saldierna Guzmán, J.P., and Shay, J.E. (2017). Transmission of bacterial endophytes. Microorganisms *5*, 70. https://doi.org/10.3390/microorganisms5040070.

22. Lê Van, A., Quaiser, A., Duhamel, M., Michon-Coudouel, S., Dufresne, A., and Vandenkoornhuyse, P. (2017). Ecophylogeny of the endospheric root fungal microbiome of co-occurring *Agrostis stolonifera*. PeerJ *5*, e3454. https://doi.org/10.7717/peerj.3454.

23. Rosindell, J., Hubbell, S.P., and Etienne, R.S. (2011). The unified neutral theory of biodiversity and biogeography at age ten. Trends Ecol. Evol. *26*, 340–348. https://doi.org/10.1016/j.tree.2011.03.024.

24. Marasco, R., Rolli, E., Fusi, M., Michoud, G., and Daffonchio, D. (2018). Grapevine rootstocks shape underground bacterial microbiome and networking but not potential functionality. Microbiome *6*, 3. https://doi.org/10.1186/s40168-017-0391-2.

25. Darriaut, R., Lailheugue, V., Masneuf-Pomarède, I., Marguerit, E., Martins, G., Compant, S., Ballestra, P., Upton, S., Ollat, N., and Lauvergeat, V. (2022). Grapevine rootstock and soil microbiome interactions: keys for a resilient viticulture. Hortic.Res. *9*, uhac019. https://doi.org/10.1093/hr/uhac019.

26. Duhamel, M., Wan, J., Bogar, L.M., Segnitz, R.M., Duncritts, N.C., and Peay, K.G. (2019). Plant selection initiates alternative successional trajectories in the soil microbial community after disturbance. Ecol. Monogr. *89*, e01367. https://doi.org/10.1002/ecm.1367.

27. Wippel, K., Tao, K., Niu, Y., Zgadzaj, R., Kiel, N., Guan, R., Dahms, E., Zhang, P., Jensen, D.B., Logemann, E., et al. (2021). Host preference and invasiveness of commensal bacteria in the *Lotus* and *Arabidopsis* root microbiota. Nat. Microbiol. *6*, 1150–1162. https://doi.org/10.1038/s41564-021-00941-9.

28. Mony, C., Gaudu, V., Ricono, C., Jambon, O., and Vandenkoornhuyse, P. (2021). Plant neighbours shape fungal assemblages associated with plant roots: a new understanding of niche-partitioning in plant communities. Funct. Ecol. *35*, 1768–1782. https://doi.org/10.1111/1365-2435.13804.

29. Vannier, N., Bittebiere, A.-K., Mony, C., and Vandenkoornhuyse, P. (2020). Root endophytic fungi impact host plant biomass and respond to plant composition at varying spatio-temporal scales. Fungal Ecol. *44*, 100907. https://doi.org/10.1016/j.funeco.2019.100907.

30. Poudel, R., Jumpponen, A., Kennelly, M.M., Rivard, C.L., Gomez-Montano, L., and Garrett, K.A. (2019). Rootstocks shape the rhizobiome: rhizosphere and endosphere bacterial communities in the grafted tomato system. Appl. Environ. Microbiol. *85*, 01765-18. https://doi.org/10.1128/AEM.01765-18.

31. Liu, J., Abdelfattah, A., Wasserman, B., Wisniewski, M., Droby, S., Fazio, G., Mazzola, M., and Wu, X. (2022). Contrasting effects of genotype and root size on the fungal and bacterial communities associated with apple rootstocks. Hortic.Res. *9*, uhab013. https://doi.org/10.1093/hr/uhab013.

32. Vink, S.N., Dini-Andreote, F., Höfle, R., Kicherer, A., and Salles, J.F. (2021). Interactive effects of scion and rootstock genotypes on the root microbiome of grapevines (*Vitis spp. L.*). Appl. Sci. *11*, 1615. https://doi.org/10.3390/app11041615.

33. Marasco, R., Alturkey, H., Fusi, M., Brandi, M., Ghiglieno, I., Valenti, L., and Daffonchio, D. (2022). Rootstock-scion combination contributes to shape diversity and composition of microbial communities associated with grapevine root system.

Environ. Microbiol. *24*, 3791–3808. https://doi.org/10.1111/1462-2920.16042.

34. Song, F., Bai, F., Wang, J., Wu, L., Jiang, Y., and Pan, Z. (2020). Influence of *Citrus* scion/rootstock genotypes on arbuscular mycorrhizal community composition under controlled environment condition. Plants *9*, 901. https://doi.org/10.3390/plants9070901.

35. Liu, J., Abdelfattah, A., Norelli, J., Burchard, E., Schena, L., Droby, S., and Wisniewski, M. (2018). Apple endophytic microbiota of different rootstock/scion combinations suggests a genotype-specific influence. Microbiome *6*, 18. https://doi.org/10.1186/s40168-018-0403-x.

36. Compant, S., Cambon, M.C., Vacher, C., Mitter, B., Samad, A., and Sessitsch, A. (2021). The plant endosphere world - bacterial life within plants. Environ. Microbiol. *23*, 1812–1829. https://doi.org/10.1111/1462-2920.15240.

37. Müller, D.B., Vogel, C., Bai, Y., and Vorholt, J.A. (2016). The plant microbiota: systems-level insights and perspectives. Annu. Rev. Genet. *50*, 211–234. https://doi.org/10.1146/annurev-genet-120215-034952.

38. Xiong, C., Zhu, Y.G., Wang, J.T., Singh, B., Han, L.L., Shen, J.P., Li, P.P., Wang, G.B., Wu, C.F., Ge, A.H., et al. (2021). Host selection shapes crop microbiome assembly and network complexity. New Phytol. *229*, 1091–1104. https://doi.org/10.1111/nph.16890.

39. Guo, J., Ling, N., Li, Y., Li, K., Ning, H., Shen, Q., Guo, S., and Vandenkoornhuyse, P. (2021). Seed-borne, endospheric and rhizospheric core microbiota as predictors of plant functional traits across rice cultivars are dominated by deterministic processes. New Phytol. *230*, 2047–2060. https://doi.org/10.1111/nph.17297.

40. Voges, M.J.E.E.E., Bai, Y., Schulze-Lefert, P., and Sattely, E.S. (2019). Plant-derived coumarins shape the composition of an *Arabidopsis* synthetic root microbiome. Proc. Natl. Acad. Sci. USA *116*, 12558–12565. https://doi.org/10.1073/pnas.1820691116.

41. Lahrmann, U., Strehmel, N., Langen, G., Frerigmann, H., Leson, L., Ding, Y., Scheel, D., Herklotz, S., Hilbert, M., and Zuccaro, A. (2015). Mutualistic root endophytism is not associated with the reduction of saprotrophic traits and requires a noncompromised plant innate immunity. New Phytol. *207*, 841–857. https://doi.org/10.1111/nph.13411.

42. Cotton, T.E.A., Pétriacq, P., Cameron, D.D., Meselmani, M.A., Schwarzenbacher, R., Rolfe, S.A., and Ton, J. (2019). Metabolic regulation of the maize rhizobiome by benzoxazinoids. ISME J. *13*, 1647–1658. https://doi.org/10.1038/s41396-019-0375-2.

43. Huang, A.C., Jiang, T., Liu, Y.X., Bai, Y.C., Reed, J., Qu, B., Goossens, A., Nützmann, H.W., Bai, Y., and Osbourn, A. (2019). A specialized metabolic network selectively modulates *Arabidopsis* root microbiota. Science *364*, eaau6389. https://doi.org/10.1126/science.aau6389.

44. Jacoby, R.P., Chen, L., Schwier, M., Koprivova, A., and Kopriva, S. (2020). Recent advances in the role of plant metabolites in shaping the root microbiome. F1000Res. *9*, 151. https://doi.org/10.12688/f1000research.21796.1.

45. Noman, M., Ahmed, T., Ijaz, U., Shahid, M., Azizullah, Li, D., Manzoor, I., and Song, F. (2021). Plant-microbiome crosstalk: dawning from composition and assembly of microbial community to improvement of disease resilience in plants. Int. J. Mol. Sci. *22*, 6852. https://doi.org/10.3390/ijms22136852.

46. Middleton, H., Yergeau, É., Monard, C., Combier, J.P., and El Amrani, A. (2021). Rhizospheric plant-microbe interactions: miRNAs as a key mediator. Trends Plant Sci. *26*, 132–141. https://doi.org/10.1016/j.tplants.2020.09.005.

47. Mataigne, V., Vannier, N., Vandenkoornhuyse, P., and Hacquard, S. (2021). Microbial systems ecology to understand cross-feeding in microbiomes. Front. Microbiol. *12*, 780469. https://doi.org/10.3389/fmicb.2021.780469.

48. Dang, H., Zhang, T., Wang, Z., Li, G., Zhao, W., Lv, X., and Zhuang, L. (2021). Succession of endophytic fungi and arbuscular mycorrhizal fungi associated with the growth of plant and their correlation with secondary metabolites in the roots of plants. BMC Plant Biol. *21*, 165. https://doi.org/10.1186/s12870-021-02942-6.

49. Kaur, S., and Suseela, V. (2020). Unraveling arbuscular mycorrhiza-induced changes in plant primary and secondary metabolome. Metabolites *10*, 335. https://doi.org/10.3390/metabo10080335.

50. Finkel, O.M., Salas-González, I., Castrillo, G., Conway, J.M., Law, T.F., Teixeira, P.J.P.L., Wilson, E.D., Fitzpatrick, C.R., Jones, C.D., and Dangl, J.L. (2020). A single bacterial genus maintains root growth in a complex microbiome. Nature *587*, 103–108. https://doi.org/10.1038/s41586-020-2778-7.

51. Hou, S., Thiergart, T., Vannier, N., Mesny, F., Ziegler, J., Pickel, B., and Hacquard, S. (2021). A microbiota-root-shoot circuit favours *Arabidopsis* growth over defence under suboptimal light. Nat. Plants *7*, 1078–1092. https://doi.org/10.1038/s41477-021-00956-4.

52. Kremer, J.M., Sohrabi, R., Paasch, B.C., Rhodes, D., Thireault, C., Schulze-Lefert, P., Tiedje, J.M., and He, S.Y. (2021). Peat-based gnotobiotic plant growth systems for *Arabidopsis* microbiome research. Nat. Protoc. *16*, 2450–2470. https://doi.org/10.1038/s41596-021-00504-6.

53. Edgar, R.C. (2010). Search and clustering orders of magnitude faster than BLAST. Bioinformatics *26*, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.

54. Vandenkoornhuyse, P., Mahé, S., Ineson, P., Staddon, P., Ostle, N., Cliquet, J.-B., Francez, A.-J., Fitter, A.H., and Young, J.P.W. (2007). Active root-inhabiting microbes identified by rapid incorporation of plant-derived carbon into RNA. Proc. Natl. Acad. Sci. USA *104*, 16970–16975. https://doi.org/10.1073/pnas.0705902104.

55. Beckers, B., Op De Beeck, M., Weyens, N., Boerjan, W., and Vangronsveld, J. (2017). Structural variability and niche differentiation in the rhizosphere and endosphere bacterial microbiome of field-grown poplar trees. Microbiome *5*, 25. https://doi.org/10.1186/s40168-017-0241-2.

56. Mitter, E.K., de Freitas, J.R., and Germida, J.J. (2017). Bacterial root microbiome of plants growing in oil sands reclamation covers. Front. Microbiol. *8*, 849. https://doi.org/10.3389/fmicb.2017.00849.

57. Edgar, R.C. (2016). UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. Preprint at bioRxiv. https://doi.org/10.1101/081257.

58. Sloan, W.T., Lunn, M., Woodcock, S., Head, I.M., Nee, S., and Curtis, T.P. (2006). Quantifying the roles of immigration and chance in shaping prokaryote community structure. Environ. Microbiol. *8*, 732–740. https://doi.org/10.1111/j.1462-2920.2005.00956.x.

59. Elzhov, T.V., Mullen, K.M., Spiess, A.N., and Bolker, B. (2015). minpack.lm: R Interface to the Levenberg-Marquardt Nonlinear Least-Squares Algorithm Found in MINPACK, Plus Support for Bounds. http://CRAN.R-project.org/package=minpack.lm.

60. Burns, A.R., Stephens, W.Z., Stagaman, K., Wong, S., Rawls, J.F., Guillemin, K., and Bohannan, B.J. (2016). Contribution of neutral processes to the assembly of gut microbial communities in the zebrafish over host development. ISME J. *10*, 655–664. https://doi.org/10.1038/ismej.2015.142.

61. Letunic, I., and Bork, P. (2021). Interactive Tree of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res. *49*. W293–w296. https://doi.org/10.1093/nar/gkab301.

62. Louca, S., Parfrey, L.W., and Doebeli, M. (2016). Decoupling function and taxonomy in the global ocean microbiome. Science *353*, 1272–1277. https://doi.org/10.1126/science.aaf4507.

63. Knights, D., Kuczynski, J., Charlson, E.S., Zaneveld, J., Mozer, M.C., Collman, R.G., Bushman, F.D., Knight, R., and Kelley, S.T. (2011). Bayesian community-wide culture-independent microbial source tracking. Nat. Methods *8*, 761–763. https://doi.org/10.1038/nmeth.1650.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
DNeasy PowerSoil® DNA Isolation Kit	Qiagen, Germany	CAS: 12888
Deposited data		
Raw and analyzed data	This paper	NCBI: PRJNA798611
Code	This paper	https://doi.org/10.5281/zenodo.7514321
Experimental models: Organisms/strains		
Grape Vine	University of Rennes 1 Lab	N/A
Watermelon	Nanjing Agricultural University Lab	N/A
Bottle gourd	Nanjing Agricultural University Lab	N/A
Oligonucleotides		
Primers:799F (5′-AACMGGATTAGATACCCKG-3′) and 1223R (5′-CCATTGTAGTACGTGTGTA-3′)	Vannier et al.[19]	N/A
Software and algorithms		
Usearch	Edgar et al.[53]	https://drive5.com/
R	This Paper	https://www.r-project.org/
Scripts	This paper	https://github.com/wangtingting0104/HolobionT-Dataset

RESOURCE AVAILABILITY

Lead contact

Philippe Vandenkoornhuyse (philippe.vandenkoornhuyse@univ-rennes1.fr).

Material availability

This study did not generate new unique reagents.

Data and code availability

Raw sequencing data derived from plant root samples have been deposited at European Nucleotide Archive (ENA), and accession numbers (PRJNA798611) are listed in the key resources table.

All original code has been deposited at Zenodo and is publicly available as of the date of publication. DOIs are listed in the key resources table https://doi.org/10.5281/zenodo.7514321.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

To test our hypotheses, we set up an equivalent experimental scheme (Figure 1) on two biological models traditionally used as grafted plants: grapevine (France) and watermelon grafted with bottle-gourd (China). Watermelon (*Citrullus lanatus*) (*W*) and the bottle-gourd (*Lagenaria siceraria*) (*B*) are both annual plants whereas grapevine, including the Chenin grapevine (*Vitis vinifera*) (*C*), the American "wild" hybrid grapevine (101.14 Millardet et deGrasset-*Vitis rupestris* x *Vitis riparia*) (*101*) and the Fercal grapevine (*Vitis vinifera* x *Vitis berlandieri* x *Vitis longii*) (*F*) are perennial ligneous plants. Figure 1 summarizes the experimental design and details of the different combinations of grafting. We split the two genotype rootstock conditions into two separate sub-cases: 101-14&Chenin (case 1, named *101&C*) and Fercal & Chenin (case 2,

named *F*&C). We tested four combinations at two times points (T1 and T2): (1) ungrafted Chenin grapevine (*C*); (2) ungrafted American "wild" hybrid grapevine (*101*); (3) Chenin grapevine grafted on American "wild" hybrid grapevine (*C101*); (4) American "wild" hybrid grapevine grafted on Chenin grapevine (*101C*); and (1') ungrafted Chenin grapevine (*C*); (2') ungrafted Fercal grapevine (*F*); (3') Chenin grapevine grafted on Fercal grapevine (*CF*); (4') Fercal grapevine grafted on Chenin grapevine (*FC*) (Figure 1). For the watermelon and bottle-gourd experiment, four different conditions have been tested at one time point: (1) ungrafted watermelon (*W*); (2) ungrafted bottle gourd (*B*); (3) watermelon grafted on bottle gourd (*WB*); (4) bottle gourd grafted on watermelon (*BW*) (Figure 1). The watermelons were grown in a greenhouse using soil collected from Jiangsu Province in China. The grapevines, produced by Pépinière deSalettes (France) were grown outdoors (Saint-Geours-de-Maremne, France).

METHOD DETAILS

Root sampling and DNA extraction

For each condition, we sampled the roots of 15 replicates 65 days after grafting in the watermelon and bottle-gourd model (1 sampling time (T1) for the annual plants) and 6 and 18 months after grafting (2 sampling times (T1 and T2) for the perennial plant) in the grapevine model (Figure 1). For the grapevine model, T1 and T2 refer to a 1-to 3-inch shoots stage and a 10-to 16-inch shoots stage respectively. Roots were sampled, sorted, and placed individually in hermetic bags and in coolers for transport to the laboratory. Because of the differences in root structure and toughness of the two biological models, the washing steps were different. The grapevine roots were first thoroughly washed in tap water, then immersed in a 5‰ Triton X100 solution for 10 minutes in a sterile 20 ml polycarbonate analysis container and finally thoroughly rinsed several times in sterile ultra-pure water.[54] The watermelon roots were surface sterilized with 70% ethanol for 1 min and then immersed in 2% sodium hypochlorite for 3 min. The watermelon roots were then washed in 70% ethanol for 30 seconds and rinsed five times in sterile ultra-pure water. Aliquots (100 μL) of the final rinse water were spread on TSA plates at 28°C for 5 days to confirm complete sterilization.[55,56] All the roots of similar diameters were collected in different places to obtain a representative sample of the rooting system. After cleaning, the roots were placed in sterile micro-tubes and stored at −80 C until DNA processing.

Total watermelon root DNA was extracted from 0.25 g of homogenized root tissue sample using the DNeasy PowerSoil® DNA Isolation Kit to remove the PCR inhibitors (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Total grapevine-root DNA was extracted and quantified by the GENTYANE platform (Clermont-Ferrand, France) using magnetic bead technology (Sbeadex) on an Oktopure LGC Genomics automat, using Hoechst 33258 reagent, and a Tecan Infinite 1000 instrument. All the DNAs were then stored at −20 °C until amplicon production.

16S rRNA gene sequencing

All the DNAs were normalized at 10 ng/ul to guarantee equivalent amplification of all the samples. Based on previous optimization experiments, endophytic bacteria were analyzed from PCR amplification of the V5–V7 hypervariable region of the bacterial 16S rRNA gene using the primer pairs 799F (5'-AACMGGATTAGA TACCCKG-3') and 1223R (5'-CCATTGTAGTACGTGTGTA-3').[19] Paired-end (2×250 cycles) sequencing was performed on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) by Sangon Biotech Co., Ltd for the watermelon experiment and by the EcogenO platform (Rennes, France) for the grapevine experiments.

Raw data processing and affiliation

Raw reads were processed using USEARCH.[53] First, paired ends were merged using USEARCH fastq_mergepairs command (fastq_maxdiffs = 2, with the maximum number of mismatches in the alignment set at 2 bp), and were then truncated to 410 bp (USEARCH fastq_filter: fastq_trunclen = 410, fastq_maxee = 0.01). Only the remaining quality-filtered sequences were used to find the set of unique sequences (USEARCH fastx_uniques). Unique sequences were then denoised using the UNOISE algorithm,[57] which both corrects errors and removes chimeras (USEARCH unoise3: unoise_alpha = 2 and minsize = 200), to obtain a more restricted list of zero-radius operational taxonomic units (zOTUs) (100% identity).[57] A taxonomic classification of each zOTU was performed using the RDP classifier with a minimum bootstrap threshold of 80% on the whole fragment. Eighteen reads annotated to the chloroplasts were removed from the datasets. To obtain an equivalent sequencing depth for each plant species for downstream analyses, the watermelon samples and the grapevine samples were always analysed independently and

rarefied to 31000 and 10000 sequences with the *otutab_rare* command in USEARCH respectively and a check was performed to be sure that all curve slopes asymptotically converged to the plateau (Figure S8). The watermelon and the grapevine contingency tables contained 808 and 958 bacterial zOTUs, respectively. All the statistical analyses were performed from these 2 contingency tables.

QUANTIFICATION AND STATISTICAL ANALYSIS

Description of the bacterial communities

The α-diversity (richness, Shannon diversity index, Pielou evenness index) of each bacterial community were calculated using the *alpha_div* command in USEARCH. We performed two-way ANOVA to test the effect of rootstock, scion and their interaction on microbiota diversity index. Homoscedasticity and normality of residuals for each model were checked graphically that residuals followed a normal law. When significant Tukey's contrast tests were done to check for significant differences. The principal coordinate analysis (PCoA) was used to analyze the β-diversity of the bacterial communities between samples based on the Euclidean distance matrix with Hellinger transformation using the "ape" and "Vegan" packages in R. PERMANOVA test were done to test for the effects of rootstock, scion and their interaction in root endospheric composition. In α- and β-diversity analyses, we used Variation partitioning analysis to quantify the effects of the rootstock, scion and their interaction on root endospheric microbiota α-diversity and community composition.

Neutral community model and source tracking

To determine the relative importance of stochastic and deterministic processes of community assembly, we used the Sloan Neutral Community Model (NCM) to predict the relationship between the frequency with which taxa occur in a set of local communities (in this case in the bacterial community of an individual grafted plant root) and their abundance across the wider metacommunity (the community of both the ungrafted plants of the scion and of the rootstock).[58] This model evaluates whether the microbial assembly from a metacommunity follows a neutral model or a niche-based process as a function of the metacommunity log abundance. In this model, m is an estimate of dispersal between communities. The fitting of this parameter was performed in R using non-linear least-squares fitting and the minpack.lm package.[59] The 95% confidence interval around all fitting statistics was calculated by bootstrapping with 1 000 replicates. The taxa were subsequently separated into three fractions depending on whether they occurred more frequently than (above fraction), less frequently than (below fraction), or within the 95% confidence interval of the NCM predictions (neutrality interval). To analyze deviations from the NCM predictions, we compared the composition of the neutral and non-neutral (above and below) fractions and estimated the migration rate (m) of the bacterial communities. All computations were performed in R (version 4.0.2). The R code used for this analysis originated from Burns.[60]

From the Sloan model, we extracted the taxa of grafted roots communities that occurred more frequently than the 95% confidence interval (the above fraction) of the NCM predictions. The taxa of the above fraction were then used to construct the phylogenetic tree using the Interactive Tree of Life (iTOL).[61] Taxa unique to the chimeric plants were defined as zOTU that were specific. The part that coexisted in the chimeric plant (Sink) and the two natural plants (Sources) were called common taxa, and the part that only coexisted with one of the natural plants (Sources) were defined as share taxa. The potential function of the taxa belonging to the above fraction was predicted by the Functional Annotation of Prokaryotic Taxa (FAPROTAX).[62] In addition, the taxa of the above fraction were extracted and used for source tracker analysis. Here, the taxa of the above fraction in the grafted condition were called sink and the ungrafted plants bacterial community were called source. The Bayesian-based SourceTracker[63] was used to quantify the extent of the contribution of potential ungrafted plants root community source to the chosen grafted plants root community sink.