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Effect of plant-derived microbial soil legacy in a grafting system—a turn for the better

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Abstract

Background Plant-soil feedback arises from microbial legacies left by plants in the soil. Grafting is a common technique used to prevent yield declines in monocultures. Yet, our understanding of how grafting alters the composition of soil microbiota and how these changes affect subsequent crop performance remains limited. Our experiment involved monoculturing ungrafted and grafted watermelons to obtain conditioned soils, followed by growing the watermelons on the conditioned soils to investigate plant-soil feedback effects.

Results Ungrafted plants grew better in soil previously conditioned by a different plant (heterospecific soil) while grafted plants grew better in soil conditioned by the same plant (conspecific soil). We demonstrated experimentally that these differences in growth were linked to changes in microorganisms. Using a supervised machine learning algorithm, we showed that differences in the relative abundance of certain genera, such as *Rhizobium*, *Chryseobacterium*, *Fusarium*, and *Aspergillus*, significantly influenced the conspecific plant-soil feedback. Metabolomic analyses revealed that ungrafted plants in heterospecific soil enriched arginine biosynthesis, whereas grafted plants in conspecific soil increased sphingolipid metabolism. Elsewhere, the metagenome-assembled genomes (MAGs) of ungrafted plants identified in heterospecific soil include *Chryseobacterium* and *Lysobacter*, microorganisms having been prominently identified in earlier research as contributors to plant growth. Metabolic reconstruction revealed the putative ability of *Chryseobacterium* to convert D-glucono-1,5-lactone to gluconic acid, pointing to distinct disease-suppressive mechanisms and hence distinct microbial functional legacies between grafted and ungrafted plants.

Conclusions Our findings show a deep impact of the soil microbial reservoir on plant growth and suggest the necessity to protect and improve this microbial community in agricultural soils. The work also suggests possibilities of optimizing microbiota-mediated benefits through grafting herein, a way that "engineered" soil microbial communities for better plant growth.

Keywords Grafting, Metabolomics, Metagenomics, Microbial composition, Plant-soil feedback, Soil legacy

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Introduction

The term "plant-soil feedback" includes changes to the soil environment made by plants that regulate the performance of other plants that grow later, including their own offspring [1]. Plant-soil feedback can be either negative or positive. Negative plant-soil feedback, including allelopathy, results from plants emitting compound(s) into the soil that are toxic to neighboring plants [2]. It can also occur in continuous monoculture, with, in this case, a decrease in the productivity of the monoculture over time that may be due to the increased prevalence of pathogenic microorganisms able to infect plant roots [3]. Positive plant-soil feedback can occur when access to resources increases or mutualists become abundant. A typical example is the release of signaling molecules by legumes that attract nitrogen-fixing rhizobia, which form root nodules and can enhance nitrogen availability and plant growth [4].

In agricultural systems, plant-soil feedback is often exploited in the form of crop rotation, in which the sequence of crops is adjusted to provide the best possible soil conditions for crop yield and sustainability [5]. Although continuous monocropping is often associated with negative effects due to the accumulation of pathogens, it can also lead to an enrichment of beneficial microorganisms that act as antagonists to these pathogens [6]. Both negative and positive effects can be a consequence of microbial legacies left by plants. This means that plants growing at specific locations influence the community of soil microorganisms, creating a reservoir of microbes that can be beneficial or detrimental to future crops [7]. It is worth noting that in agriculture, crop rotation is not the only way to avoid biomass decline, and other agronomic practices, such as grafting, are also used to avoid negative effects. Little is known about plant-soil feedback in the context of grafting. It has been demonstrated that the process of converting a natural grapevine to a chimeric state drives modifications in the root-associated microbial composition and structure, depending on the type of rootstock [8]. Grafting is thus expected to influence plant-soil feedback by modifying microbial community composition. Specifically, we suggest that the microbial legacy effects are modified by growing grafted plants.

The outcome of plant–soil feedback depends on both the direct effects, where a focal plant modifies the soil influencing itself or its offspring, and the indirect effects, where a neighboring plant influences the soil in ways that impact a focal plant [1]. Most studies of plant-soil feedback have focused on the direct effects caused by the individual focal plant [9, 10]. Research has shown that the outcomes of plant-soil feedback depend largely on the effects from neighboring plants (i.e., the indirect effects). For instance, the invasive forb *Lespedeza cuneata* modifies soils to benefit its own fitness more than that of native plants [11, 12]. Therefore, if a focal plant is competing with another plant in a soil conditioned by one of them, the focal plants may experience different plant-soil feedback effects compared to the plant grown individually. However, it is unknown whether the indirect effects of plant-soil feedback are applicable between grafted and ungrafted plants and their microbiological processes.

The interactions between plants and their associated soil organisms in the rhizosphere, at least partially depend on the composition of root-associated metabolites [13, 14]. Metabolites produced by plant roots can directly influence (i) the composition and activity of soil microbial communities [15, 16] and (ii) plant microbiota composition [17]. These plant-emitted compounds can drive plant-soil feedback by modifying microbial communities [18], while plant-associated microbes can also influence the production and composition of root metabolites by interacting with the plant roots, altering metabolite production [19]. For instance, the planting history can influence the current composition of the microbial community in the peanut (*Arachis hypogaea*) rhizosphere, likely resulting in the down-regulation of genes associated with auxin production in the roots [20]. Thus, a better understanding of the relationship between the rhizosphere microbiota composition and root metabolites is required.

Previous studies have elucidated the broad contribution of microbial communities to plant-soil feedback [21] and a role demonstrated for specific communities (i.e., functional guilds): for instance, enrichment in fungal pathogens correlates with reduced diversity of mycorrhizal fungi, resulting in strong suppression of plant growth in soil occupied by the same plant species [22]. Beyond the effects mediated by changes in microbial community composition, some keystone microbes can also modify plant-soil feedback. However, the specific links between keystone microorganisms and plant-soil feedback remain to be identified.

In the agricultural context, we still do not know why grafted plants are generally less susceptible to negative plant-soil feedback. Thus, in the present study, we examined the dynamic growth patterns of grafted and ungrafted watermelons, grown in isolation and in competition exposed to grafted watermelon-conditioned soil and ungrafted watermelon-conditioned soil. We hypothesize that (1) soil microbiota influenced by grafting is modulated by, and responds to, different soil legacies; (2) grafting-induced changes in plant-soil feedbacks are significantly influenced by the indirect effects of neighboring plants; (3) soil legacy determines reprogramming of root metabolites; (4) a plant's reliance on rhizosphere

microbes are manifested through the functional specificities of keystone microbes. Our results provide novel insights into the impacts of continuous monoculture of grafted plants on soil microbiota and into the consequences of the presence of different soil microbiota for plant growth.

Materials and methods

Plant resources

An annual ungrafted watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai var. Zaojia 8424, Nanjing Institute of Vegetable Science, China] and a grafted watermelon, a chimeric plant composed of the scion from the watermelon and rootstock from the bottle gourd [*Lagenaria siceraria* (Molina) Standl. var. dayehuzi, Nanjing Institute of Vegetable Science, China], were used in this study.

Soil feedback conditioning stage

The soil feedback conditioning stage with either ungrafted or grafted watermelon consisted of continuous monocultures (using the same varieties each year) for 6 years in a single field (the same initial soil) of Nanjing Institute of Vegetable Science, Hengxi, China (N31°43′, E118°47′) (Fig. 1a). The experimental field was initially homogenized through thorough tillage to ensure uniform soil conditions across the site. Following homogenization, the field was randomly divided into different plots. Each treatment, including both the ungrafted and grafted watermelon, consisted of two randomized plots to minimize any inherent variability in soil conditions.

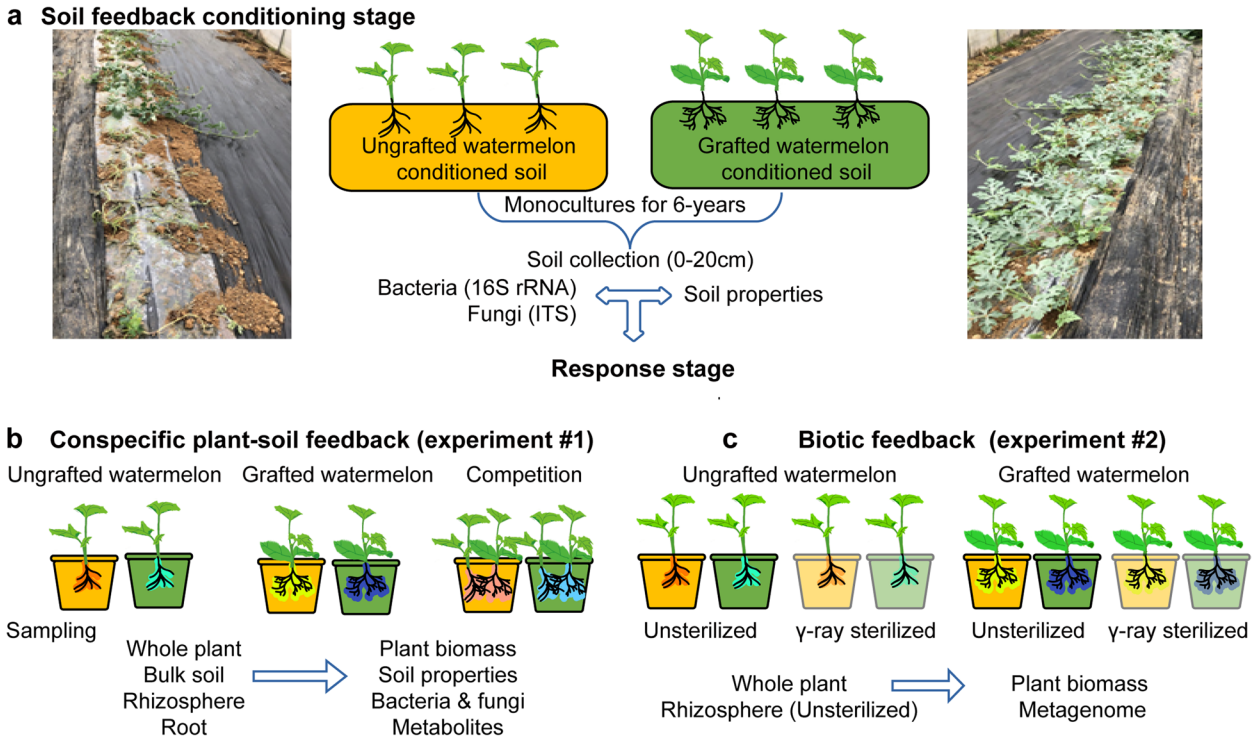

Fig. 1 Schematic representation of the experimental setup used to assess the plant-derived soil microbial legacy effects. **a** During the conditioning stage, ungrafted watermelon and grafted watermelon were grown in fields in continuous monoculture for 6 years. Samples of the bulk soil in the two treatments were collected to assess the soil properties and for bacterial and fungal amplicon sequencing. The same soils were also used for the response stage experiment. **b** In the conspecific plant-soil feedback experiment, ungrafted and grafted watermelon plants were grown in isolation and competition on both the ungrafted watermelon-conditioned soil and grafted watermelon-conditioned soil. After the plants had been grown for 18 days, the plants were harvested at 3-day intervals and the biomass was determined, i.e., a total of 10 harvests ($n=3$). At the last harvest time point (45 days after transplanting), bulk soils were sampled for assessment of their soil properties, and rhizosphere soils were sampled for bacterial and fungal amplicon sequencing ($n=3$). Root samples were collected to determine the composition of metabolites ($n=4$). **c** Ungrafted-conditioned soil and grafted-conditioned soil used for the biotic feedback experiment were each divided into two parts. One part was unsterilized and the other part was gamma-ray sterilized, giving a total of 4 soil treatments. Ungrafted watermelon and grafted watermelon were grown on both soils, giving a total of 8 treatments. These plants were also harvested 45 days after transplanting, and their biomass was determined ($n=9$ for ungrafted watermelon, $n=10$ for grafted watermelon). The rhizosphere soil of the unsterilized treatments was kept for metagenomic sequencing ($n=5$)

The ungrafted watermelon field developed replant disease over time, which is evident in the appearance of the plants (Fig. 1a). In contrast, the grafted watermelon field remained healthy. The two fields were managed according to conventional farming practices, including the use of chemical fertilizers (270 kg N urea, 140 kg P_2O_5 superphosphate, and 300 kg K_2O muriate of potash per hectare applied with water 12 times throughout plant growth). After 6 years of monoculture, soil samples were randomly collected from a 0–20 cm layer in each plot. The soil taken from each plot was uniformly mixed after the removal of visible plant root fragments and then homogenized for pot cultivation experiments in a greenhouse. Based on their conditioning stage, the collected soils were termed "ungrafted watermelon conditioned soil" (hereafter called "ungrafted-conditioned soil") and "grafted watermelon conditioned soil" (hereafter called "grafted-conditioned soil"). The two conditioned soils were collected for physicochemical analysis (Supplementary information 1.1), and the data was summarized in Table S1. Aliquots of the two individual soils were used for bacterial and fungal community analyses. The rest was used for greenhouse experiments.

Experimental setup to assess soil legacy

To explore the role of soil microbiota in the plant-soil feedback effects, both the ungrafted-conditioned soil and grafted-conditioned soil samples were split into two for two different experiments. The first experiment aimed to investigate the conspecific plant-soil feedback by examining the plant performance in conspecific soil (soil conditioned by roots of the same plant) and in heterospecific soil (soil conditioned by roots of another plant), with a focus on how microbial community structure impacts plant growth (Fig. 1b). The second experiment aimed to test the hypothesis that a plant's reliance on rhizosphere microbes is reflected through the functional specificities of keystone microbes, by comparing plant performance in unsterilized and sterilized soil and analyzing microbial functional potential (Fig. 1c).

Experiment no. 1: Conspecific plant-soil feedback experiment

In the conspecific plant-soil feedback experiment (Fig. 1b), when each ungrafted watermelon and grafted watermelon seedling (grown in isolation) had 3 true leaves, it was individually transplanted to ungrafted-conditioned soil or grafted-conditioned soil. In parallel, both ungrafted watermelon and grafted watermelon were grown together (i.e., grown in competition) in the two conditioned soils. This means there were 6 treatments (2 soil types × 3 plant growing types) in the conspecific plant-soil feedback experiment. To ensure that root growth in competing plants was not restricted, sufficiently large pots were used, each containing approximately 1 kg of soil. All the pots were watered daily (50 ml) to maintain adequate moisture, and 25 ml once a week with Hoagland solution [23] to avoid nutrient deficiency. The nitrogen concentration in this nutritive solution is 210 mg L^{-1}. Pot-cultures were randomized in the greenhouse. Eighteen days after transplanting, plants were harvested at 3-day intervals to assess the plant-growth dynamics, specifically focusing on plant biomass. There were 10 harvest time points in all. At each time point and for each treatment, 3 randomly selected replicates were sampled (i.e., 6 treatments × 3 reps = 18 pots for each time point). At each harvest time point, plant material was collected and oven-dried at 70 °C for at least 48 h and then weighed. At the 10th harvest time point (after 45 days of culture), the rhizospheric soil was collected. All the plants grown (i.e., in either single- or mixed-plant conditions) were sampled as single individuals. Each individual plant sampled was shaken vigorously to remove loose soil from the root system. The root systems of each individual plant taken from the single condition, and those of mixed plants taken from the competition condition, were placed separately in a sterile 50-ml tube. Individual rooting systems were washed in 20 ml of sterile distilled H_2O, vortexed for 1 min, and centrifuged for 3 min to suspend the rhizospheric soil. The rhizosphere soil suspension was used for DNA extraction and bacterial (16S rRNA) and fungal (ITS) community sequencing. The root samples were kept for metabolomic analyses (detailed information can be found in Supplementary information 1.2).

Experiment no. 2: Biotic feedback experiment

In the biotic feedback experiment (Fig. 1c), each of the two types of conditioned soil was further divided into two sets of aliquots, either gamma-ray sterilized (50 kGy) or not. Each pot contained conditioned soil that was tailored to either ungrafted- or grafted-conditioned soil and further divided into unsterilized and sterilized treatments. Individual ungrafted watermelon and grafted watermelon plants were planted in separate pots. This means there were 8 treatments (i.e., 2 conditioned soils × 2 (unsterilized and sterilized) × 2 plant types (grafted and ungrafted)), and within each treatment, 9 replicates of ungrafted plants and 10 replicates of grafted plants (different number of replicates due to different survival rates of seedlings post-transplantation). Plant biomass was measured at the end of the experiment, i.e., after 45 days of growth. Plants were harvested and oven-dried at 70 °C for at least 48 h, then weighed. The rhizospheric microbial community of the ungrafted and grafted

watermelon grown on the unsterilized soil was analyzed using metagenomic sequencing.

Sample preparation and amplicon sequencing

DNA was extracted from bulk soils sampled at the end of the soil feedback conditioning stage and from the rhizospheric soil at the end of the conspecific plant-soil feedback experiment (i.e., experiment no. 1). The Internal Transcribed Spacer (ITS) region and the 16S rRNA gene (V4-V5) were amplified and sequenced to characterize the composition of fungal and bacterial communities, respectively. DNA was extracted from soils using a MoBio PowerSoil™ DNA isolation kit (MoBio Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. The bacterial 16S rRNA gene was amplified using the primer pairs 515F/907R (5′-GTG CCA GCM GCC GCG GTA A-3′/5′- CCG TCA ATT CMT TTR AGT TT-3′) [24, 25]. Primers ITS3/ITS4 (5′-GCA TCG ATG AAG AAC GCA GC-3′/5′-TCC TCC GCT TAT TGA TAT GC-3′) were used for amplification of the fungal ITS2 region [26, 27]. PE-250 sequencing was conducted by Personal Biotechnology Co., Ltd. (Shanghai, China) on an Illumina MiSeq platform.

Raw bacterial and fungal sequences were processed and analyzed using the USEARCH (version 10) pipeline [28]. After forward and reverse reads were merged, low-quality sequences with a quality score below 30 were removed. Next, the fastx_uniques command was used to remove redundant sequences, and denoising was conducted by Unoise3 to remove any chimeras and obtain the zero-radius operational taxonomic units (zOTUs). Finally, the representative sequences of each zOTU were matched against the RDP 16S rRNA database for bacteria and the UNITE Fungal ITS database for fungi.

Metagenome sequencing, annotation, and metagenome-assembled genomes (MAGs)

At the end of the biotic feedback experiment (i.e., experiment no. 2), the rhizosphere of ungrafted watermelon and grafted watermelon grown on unsterilized soil were collected (2 unsterilized conditioned soils × 2 plant types (grafted and ungrafted watermelon)). DNA was extracted like in experiment no. 1 for metagenomic analysis to assess the functional differences of the microorganisms left in the soil. Specifically, DNA shotgun libraries were prepared using the KAPA Hyper Prep Kit following the manufacturer's recommendations at Luojie Information Technology Co., Ltd. (Jinan, China). All the libraries were sequenced simultaneously into an Illumina PE-150 NovaSeq run following the manufacturer's recommendations. Only high-quality reads (i.e., cleaned reads after Trimmomatic v0.39 filtration) were used. Taxonomic classification of the sequence reads was performed using Kraken2 v2.0.8b with the Kraken PlusPFP database.

Metagenome-assembled genomes (MAGs) were analyzed using MegaHit version 1.2.9 with the "meta-sensitive" preset. MegaHit-assembled contigs and MetaBat2 version 2.15 were used to create contig bins to reconstruct single genomes. The contig bins were de-replicated using dRep version 3.2.0. The completeness and contamination of the resulting MAGs were assessed using checkM and bins with more than 70% completeness and less than 10% contamination was retained as MAGs for further analysis. MAG annotations were performed with GTDB-Tk (version 2.1.1). Phylogenetic trees for MAGs were generated by the gtdbtk infer module in GTDB-Tk. The phylogenetic tree of these MAGs was visualized by iTOL (https://itol.embl.de/).

Statistical analyses

Microbial community composition was analyzed from the zOTU normalized contingency table. The richness and Shannon diversity index of the bacteria and fungi were calculated. Using the VEGAN package in R, principal coordinates analysis (PCoA) was performed to analyze the β-diversity of the microbial communities based on Bray–Curtis distances. Permutational multivariate analysis of variance (PERMANOVA) was conducted to test the significance of microbial community dissimilarity using the "adonis" functions in R.

In the conspecific plant-soil feedback experiment (i.e., experiment no. 1), the two-way ANOVA was used to test the effect of conditioned soil type and time on the plant biomass. The conspecific plant-soil feedback was calculated as the difference between ln-transformed biomass of specific plants in conspecific soil, i.e., soil conditioned by the same plant, and heterospecific soil, i.e. soil conditioned by another plant [29].

$$\text{Conspecific plant-soil feedback} = \ln\left(\frac{\text{biomass of specific plants in conspecific soil}}{\text{biomass of specific plants in heterospecific soil}}\right)$$

The conspecific plant-soil feedback effect was calculated using full pairwise combinations of plant replicates in conspecific versus heterospecific soil for each plant and at each sampling occasion. Specifically, we had three replicates for each treatment, resulting in all possible pairwise differences being calculated between the replicates (i.e., $3 \times 3 = 9$ independent data points) for each

plant at each sampling time. One sample *t*-test was carried out to check that the value of feedback differed significantly from zero for each sampling time. To identify the correlation between microbial taxa abundance and plant growth, we utilized random forest analysis to correlate the variations in relative abundances of microbial taxa between plants grown in different plant-conditioned soils and the resulting conspecific plant-soil feedback.

A volcano plot was used to visualize rhizosphere microbial taxa associated with the differences in competition between ungrafted-conditioned soil and grafted-conditioned soil, as identified by DESeq2 analysis. A pie chart was drawn to visualize the taxonomic composition of the enriched zOTUs. Faprotax [30] and FUNGuid [31] were used to provide functional annotations of the enriched bacterial and fungal zOTUs, respectively. To compare the metabolic profiles of the specific plants grown on conspecific and heterospecific soil, differentially represented metabolites were determined based on the value of the VIP (variable importance in projection, VIP > 1), i.e., the importance of each variable in the projection used in OPLS-DA (orthogonal partial least-squares discrimination analysis) to discriminate groups of multivariate data and to identify response variables in a regression model. These analyses were performed using the ropls package in R. Differential metabolites were also calculated by Welch's *t* test. Only metabolites that satisfied VIP > 1 and had a *P* value < 0.05 were retained. The MetaboAnalyst online tool (http://www.metaboanalyst.ca) was used to analyze the metabolic pathways for the enriched metabolites.

To further validate the functional capabilities of the microbial keystone species, i.e. the predictors inferred from the random forest at the zOTU level identified in the conspecific plant-soil feedback experiment (experiment no. 1), we performed metagenomic sequencing of the treatments applied to unsterilized soils in the biotic feedback experiment (experiment no. 2) to get the metagenome-assembled genomes (MAGs). Then, these zOTUs were matched to the MAGs based on the common annotation name of the genus, and the MAGs sharing the genus information were considered to be the species capable of influencing plant growth and the subsequent metabolic function analyses. Prokka was used to predict open reading frames (ORFs) within the MAGs and annotated them using the KEGG database to obtain comprehensive information on all the KEGG pathways. The compound IDs (cpd numbers) of the metabolites detected in the metabolic analysis were compared to the KEGG database to determine the corresponding ko numbers. By combining the KEGG annotations of the MAGs with the KEGG annotations of metabolites, the multi-dataset integration produced a set of ko numbers shared by the MAGs and metabolites. These shared ko numbers indicated the corresponding MAGs have the metabolic potential to utilize these specific metabolites. We then entered the cpd numbers of the metabolites and their corresponding shared ko numbers in the iPATH3 website to screen for specific metabolites that matched the metabolic potential of the MAGs.

Results

Grafting significantly diverged plant-soil feedback and altered microbial composition

At the end of the soil feedback conditioning stage (Fig. 1a), we characterized changes in bulk soil microbiota composition between the ungrafted- and grafted-conditioned soil (Figure S1). The bacterial Shannon diversity index and richness in ungrafted-conditioned soil were significantly lower ($P < 0.001$ for both indexes) than in grafted-conditioned soil (Figure S1a, b). Fungal richness was also lower in the ungrafted-conditioned soil ($P < 0.05$) (Figure S1f). Soil conditioning explained 92% and 47% of the variation in the bacterial and fungal communities, respectively (Figure S1c, g). The composition of bacteria and fungi differed between the ungrafted- and grafted-conditioned soil (Figure S1d, h). The soil parameters pH, available phosphorus (AP), and total carbon (TC) contents were higher in ungrafted-conditioned soil (Table S1). The differences in AP and TC between the two conditioned soils disappeared at the end of the conspecific plant-soil feedback experiment (experiment no. 1, Fig. 1b) (Table S1).

When we compared plant performance in the conspecific plant-soil feedback experiment (experiment no. 1, Fig. 1b), isolated ungrafted watermelon plants produced more biomass in grafted-conditioned soil than in the ungrafted-conditioned soil (Figure S2a, Table S2, $P < 0.001$). Isolated grafted watermelon plants also grew better in the grafted-conditioned soil than in the ungrafted-conditioned soil (Figure S2b, Table S2, $P < 0.001$). Conspecific plant-soil feedback in experiment no. 1 was calculated: *ln (biomass of specific plants in conspecific soil)–ln (biomass of specific plants in heterospecific soil)*, so negative values mean that plants grew better in heterospecific soil. Isolated ungrafted watermelon plants exhibited negative conspecific feedback, and the strength of the feedback effect did not change over time (Fig. 2a). Isolated grafted watermelon plants always exhibited significantly positive conspecific feedback (Fig. 2b). Soil conditioned by grafted watermelon plants always induced a positive feedback effect on the growth of both grafted and ungrafted watermelon plants.

At the end of the conspecific plant-soil feedback experiment (i.e., experiment no. 1), the composition of the

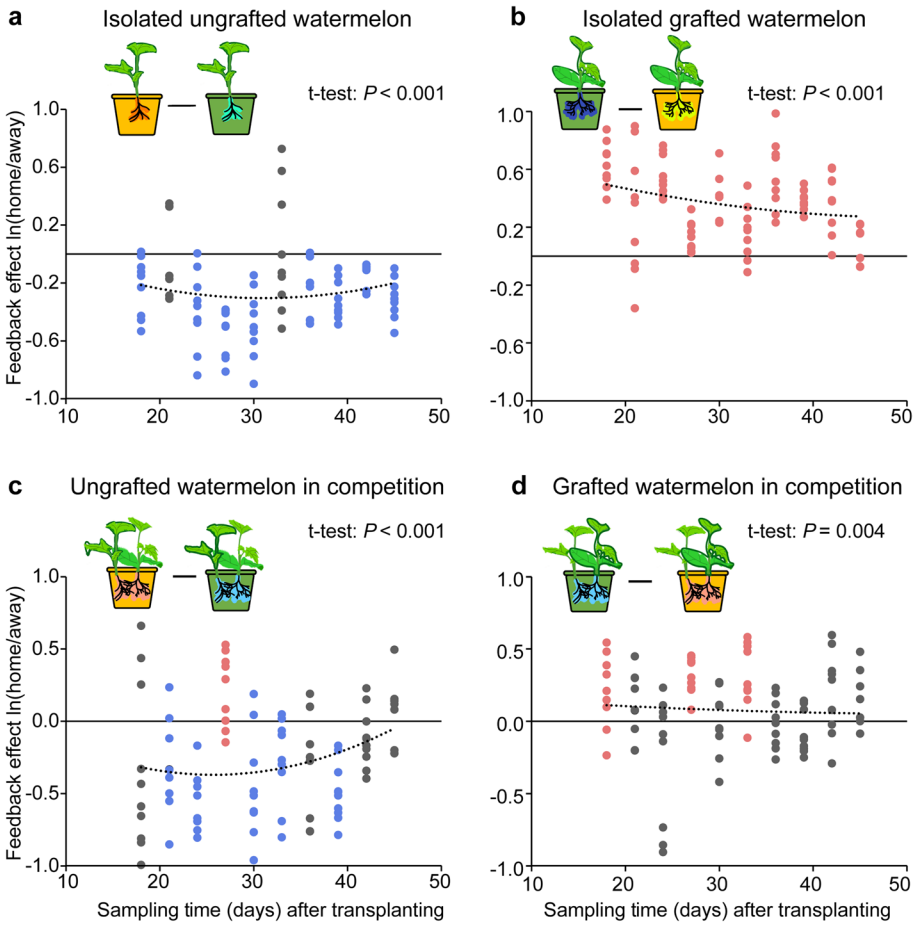

Fig. 2 Temporal dynamics of the conspecific plant-soil feedback effects in ungrafted and grafted watermelon grown in isolation and in competition. Conspecific plant-soil feedback was calculated as all pairwise differences between ln-transformed dry biomass of plants in conspecific soil (home) and in heterospecific soil (away) ($n = 3$ for each plant grown in each conditioned soil). The dotted lines represent the mean feedback effect, calculated by fitting a polynomial trendline to the biomass data points over time. The red dots indicate that the conspecific plant-soil feedback was significantly higher than zero (*t* test, $P < 0.05$). The blue dots indicate that the conspecific plant-soil feedback was significantly lower than zero (*t* test, $P < 0.05$). Positive values in each panel indicate greater plant growth in soil conditioned by a conspecific plant. Negative values in each panel indicate greater plant growth in soil conditioned by a heterospecific plant. The *P* value in the upper left of the panel was calculated from all the feedback values compared to zero by one sample *t* test

rhizosphere microbiota among treatments was characterized (Figure S3). No statistically significant differences in microbial α-diversity were found among treatments (Figure S3a, b, e, f). Concerning bacterial community variation, it was mainly explained by the conditioned soil type (ungrafted- and grafted-conditioned soil) (21%; $F = 2.6$; $P = 0.007$) rather than plant type (ungrafted or grafted plants) (7%; $F = 0.9$; $P = 0.59$) (Figure S3c, Table S3). For the fungal community, it was also mainly dominated by the conditioned soil type (17%; $F = 2.0$; $P = 0.01$) rather than the plant type (7%; $F = 0.8$; $P = 0.82$) (Figure S3g, Table S3).

Potential keystone microbial taxa influenced plant growth

To examine how microbial community composition affects plant growth, random forest analysis was used to assess how variations in the abundances of bacterial and fungal genera between soils conditioned by the same plant (conspecific soil) and another plant (heterospecific soil) influence the conspecific plant-soil feedback values. The variations in bacterial genus abundances were strongly associated with these feedback values ($R^2 = 88\%$, $P = 0.001$; Fig. 3a). Similarly, variations in fungal genus abundances significantly predicted the conspecific feedback values ($R^2 = 81\%$, $P = 0.001$; Fig. 3b). Among Proteobacteria, *Phenylobacterium*, *Ensifer,* and *Rhizobium* had a significant effect on the conspecific feedback. Among Bacteroidetes, *Chryseobacterium* also played a critical role in influencing the conspecific feedback (Fig. 3a). Among fungi, *Monosporascus* and *Fusarium* played an important role in predicting the conspecific plant-soil feedback (Fig. 3b).

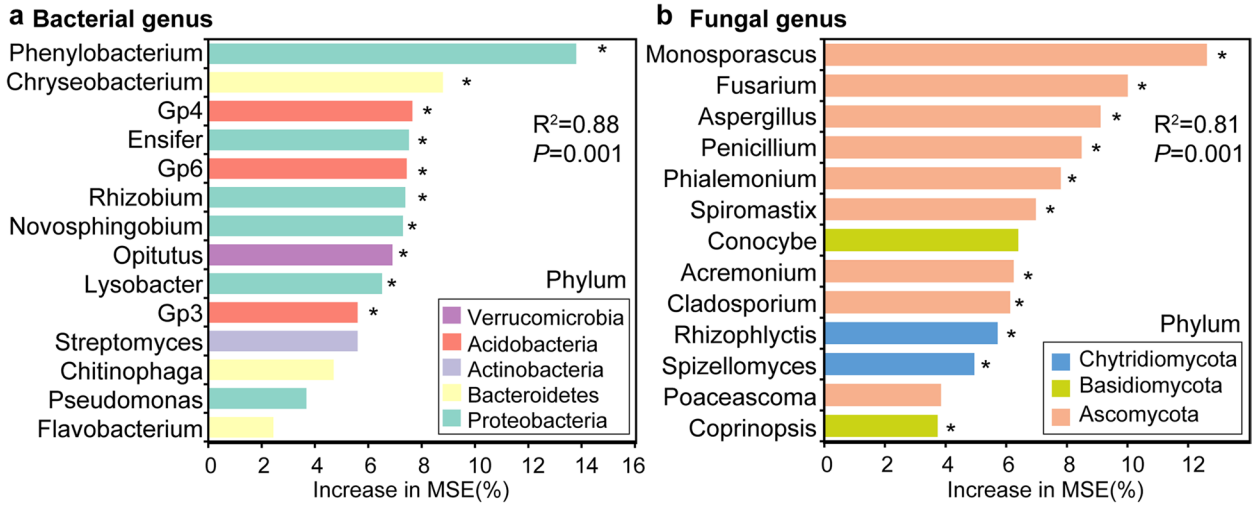

Fig. 3 Potential contributions of the difference in microbial taxa at genus level to conspecific plant-soil feedback. Random forest mean predictor importance (percentage of increase of mean square error, MSE) of differences in the relative abundance of rhizospheric bacteria (**a**) and fungi (**b**) as drivers of conspecific plant-soil feedback. Model accuracy was computed for each decision tree and averaged over the forest ($n = 3$). Percentage increases in the MSE of variables were used to estimate the importance of predictors. Percentage increases in the MSE of variables were used to estimate the importance of these predictors, and higher MSE% values imply more important predictors. *: $P < 0.05$. Potential contributions of the differences in the relative abundance of microbial zOTUs to conspecific plant-soil feedback are also shown in a random forest model in Table S4

The two next most important fungal genera in predicting the conspecific feedback were *Aspergillus* and *Penicillium*.

Plant competition impacted plant-soil feedback outcomes

To address hypothesis no. 2, which states that graft-driven changes to the microbial legacy effect depend on whether the plant is under competition or not, plant performance was compared between the conspecific and heterospecific soil during competitive growth. In competition, individual ungrafted watermelons had much higher biomass in the grafted-conditioned soil (Figure S2c, Table S2, $P = 0.008$) and there was a significant difference in biomass between grafted watermelons grown in two conditioned soils (Figure S2d, Table S2, $P = 0.04$). In competition, the strength of negative feedback of ungrafted watermelon decreased in the late growth stage and even became positive (Fig. 2c) as the plants aged. The positive feedback observed in isolated grafted watermelon plants was significantly reduced when the plants were in competition (Fig. 2d, Figure S4b). However, this feedback remained significantly higher than zero (*t* test, $P = 0.004$, Fig. 2d), indicating that the feedback, although weakened, still contributed positively to plant growth.

In the volcano plot, we identified two distinct microbial communities enriched for competition in the ungrafted- and grafted-conditioned soil (Figure S5). Concerning bacteria, the enriched zOTUs belonging to the grafted-conditioned soil were members of Acidobacteria. However, the ungrafted-conditioned soil enriched more Bacteroidetes. From Faprotax function annotation, the grafted-conditioned soil promoted putative nitrification, ureolysis, and aerobic nitrite oxidation, while the ungrafted-conditioned soil exhibited methylotrophy, methanol oxidation, fermentation, and dark hydrogen oxidation, as well as a higher nitrate reduction (Figure S5a). Concerning the fungal community, both the ungrafted- and grafted-conditioned soil comprised a large proportion of Ascomycota. The grafted-conditioned soil was composed of Chytridiomycota while the ungrafted-conditioned soil was enriched in Mucoromycota. As for the putative functional guilds, more saprophytes were found in the ungrafted-conditioned soil (Figure S5b).

Soil microbial legacy altered metabolic profiling of plant root

We hypothesized that microbial legacy effects extend to altering the composition of the root metabolome and expected distinct metabolic profiles in plants grown in soils conditioned by different plants. The root-associated metabolic profiles of ungrafted watermelon grown in ungrafted- and grafted-conditioned soil were compared, as well as those of grafted watermelon grown in the two conditioned soils. Variable importance in projection (VIP) identified the ungrafted watermelon

metabolites that best explained the observed variance in OPLS-DA: the metabolites identified as driving the difference in the ungrafted-conditioned soil modality included for example, D-glucoheptose, N-acetyl-beta-alanine, succinic acid, and in the grafted-conditioned soil modality, including for example, Beta-sitosterol, cyclohexane-1,2-diol, and urea (Fig. 4a). Metabolome mapping revealed that the enriched metabolites in ungrafted watermelon grown on ungrafted-conditioned soil were associated with sulfur metabolism pathways (Fig. 4b) but the same ungrafted plant grown on grafted-conditioned soil, led to enriched metabolites related to arginine biosynthesis pathways (Fig. 4c). VIP analysis identified key metabolites in grafted watermelon that contributed to the observed variance between the different conditioned soils. In the ungrafted-conditioned soil, grafted watermelon showed higher levels of metabolites such as albendazole, mannose, and purine riboside. Conversely, in the grafted-conditioned soil, they exhibited higher levels of aminomalonic acid, cetadiol, phytosphingosine, and mucic acid (Fig. 4d). Metabolome mapping indicated that sulfur metabolism was overrepresented in grafted watermelon grown on the ungrafted-conditioned soil and sphingolipid metabolism was overrepresented in grafted-conditioned soil (Fig. 4e, f).

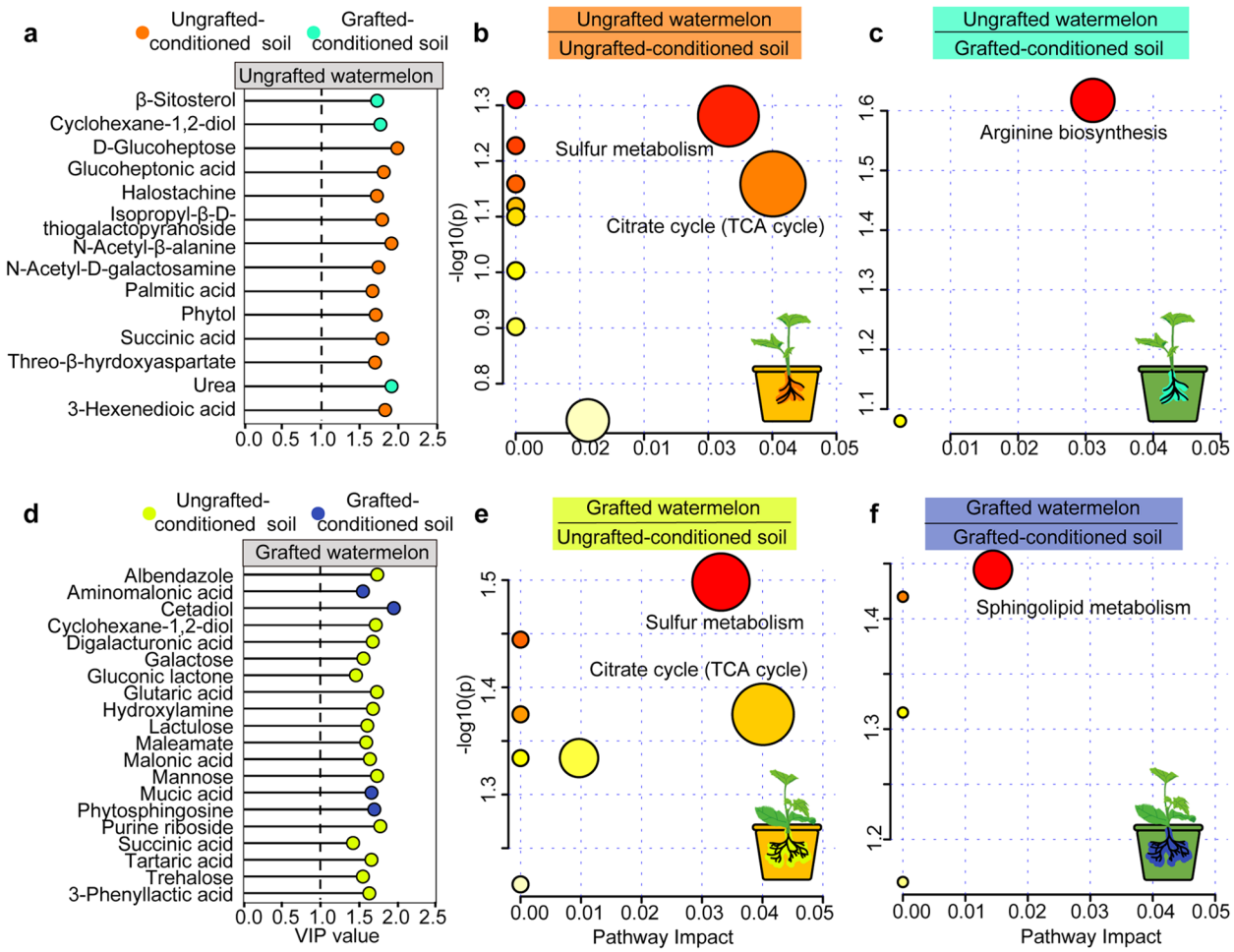

Fig. 4 The soil legacy alters metabolic profiling. **a** Difference in the metabolites of ungrafted watermelon grown on grafted- and ungrafted-conditioned soil based on the OPLS-DA VIP value. **b** Pathway analysis of metabolites identified as being overrepresented in ungrafted watermelon grown on the ungrafted-conditioned soil. **c** Pathway analysis of metabolites identified as being overrepresented in ungrafted watermelon grown on the grafted-conditioned soil. **d** Difference in the metabolites of grafted watermelon grown on the grafted- and ungrafted-conditioned soil based on the OPLS-DA VIP value. **e** Pathway analysis of metabolites identified as being overrepresented in grafted watermelon grown on the ungrafted-conditioned soil. **f** Pathway analysis of metabolites identified as being overrepresented in grafted watermelon grown on the grafted-conditioned soil. A higher VIP value denotes metabolites that explain the observed variance. The *X* axis represents the pathway impact, and the *Y* axis represents the pathway enrichment ($n = 4$). Larger dots represent increased pathway impact values; darker colors represent higher pathway enrichment (**b**, **c**, **e**, **f**)

Soil sterilization affected plant growth and microbial community composition

To investigate the causal connection between the soil microbiota and plant growth, we conducted a biotic feedback experiment (i.e. experiment no. 2, Fig. 1c) by growing ungrafted and grafted watermelon in the same conditioned soils as in experiment no. 1. The conditioned soils were used either in their "native" form (unsterilized) or subjected to gamma-ray sterilization to disrupt the taxonomic and functional configurations of the microbiota (Fig. 1c). As expected, ungrafted watermelon grown on the unsterilized ungrafted-conditioned soil displayed a growth deficit compared to unsterilized grafted-conditioned soil, and these differences disappeared using the gamma-ray sterilized soils (Fig. 5). This confirmed that the unsterilized conditioned soil microbiota plays a key role in plant performance.

Metagenomic analyses were deployed to investigate the changes in the composition and functions of microbial communities associated with ungrafted and grafted watermelon grown in unsterilized soils (i.e. ungrafted- or grafted-conditioned soil) (Figure S6). No marked effect of past cultivation on the richness of the rhizospheric communities profiled ($P > 0.05$; Figure S6b). The Shannon index in the rhizosphere of ungrafted watermelon grown on grafted-conditioned soil was lower than in the other treatments, and in particular, it was significantly lower than that in the rhizosphere of ungrafted watermelon in ungrafted-conditioned soil (Figure S6a). This result is consistent with the trend of diversity results from amplicon sequencing (Figure S3a), except for the difference in the significance of the statistical results. The type of conditioned soil (14%; $F = 3.6$; $P = 0.011$), the type of plant (14%; $F = 3.7$; $P = 0.007$) and their interaction (12%; $F = 3.2$; $P = 0.015$) significantly explained the observed difference in the composition of the bacterial communities (Figure S6c, Table S3).

Metagenome-assembled genome reconstructions linked to metabolomic profiling

From metagenome sequencing data, we aimed to reconstruct MAGs, i.e., putative genomes of keystone bacteria that proliferate in the rhizosphere of both ungrafted and grafted watermelon plants grown on the unsterilized soil (ungrafted- or grafted-conditioned soil). This resulted in the reconstruction of 100 metagenome-assembled genomes (MAGs) (Fig. 6) with more than 70% completeness and a proportion of contamination of less than 10%. Nineteen MAGs associated with ungrafted watermelon were grown on ungrafted-conditioned soil and 34 on grafted-conditioned soil. Considering grafted watermelon, respectively 25 and 22 MAGs on ungrafted- and

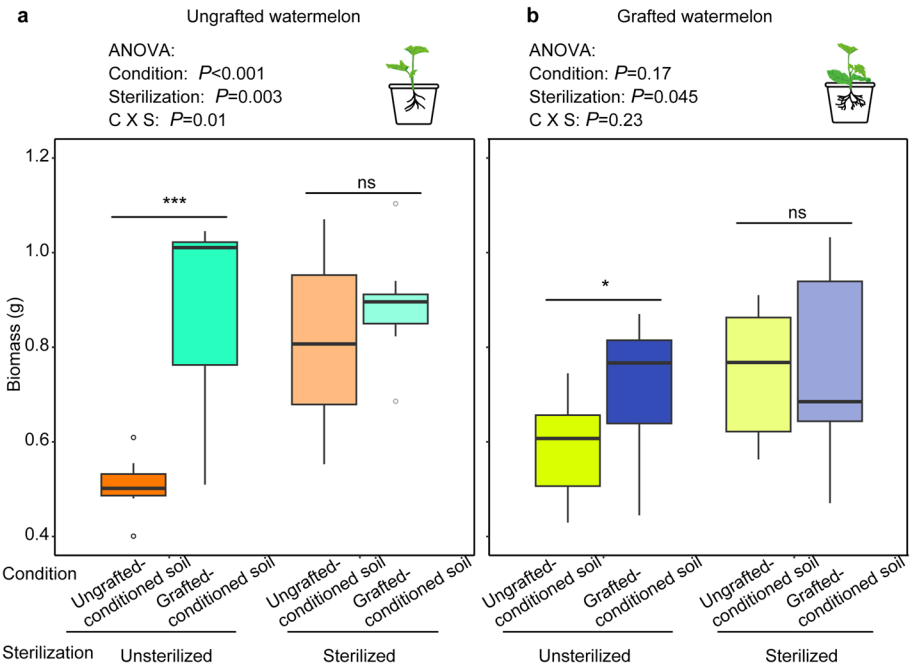

Fig. 5 Soil microbiota are necessary and sufficient factors to trigger changes in plant performance. Performance of ungrafted watermelon (**a**) and grafted watermelon (**b**) plants growing in soils previously conditioned by ungrafted watermelon or by grafted watermelon. Soils were either left unsterilized (native) or sterilized by gamma-ray. Asterisks indicate significant differences within a given soil treatment (ANOVA, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$). ns., not significant ($n = 9$ for ungrafted plants; $n = 10$ for grafted plants)

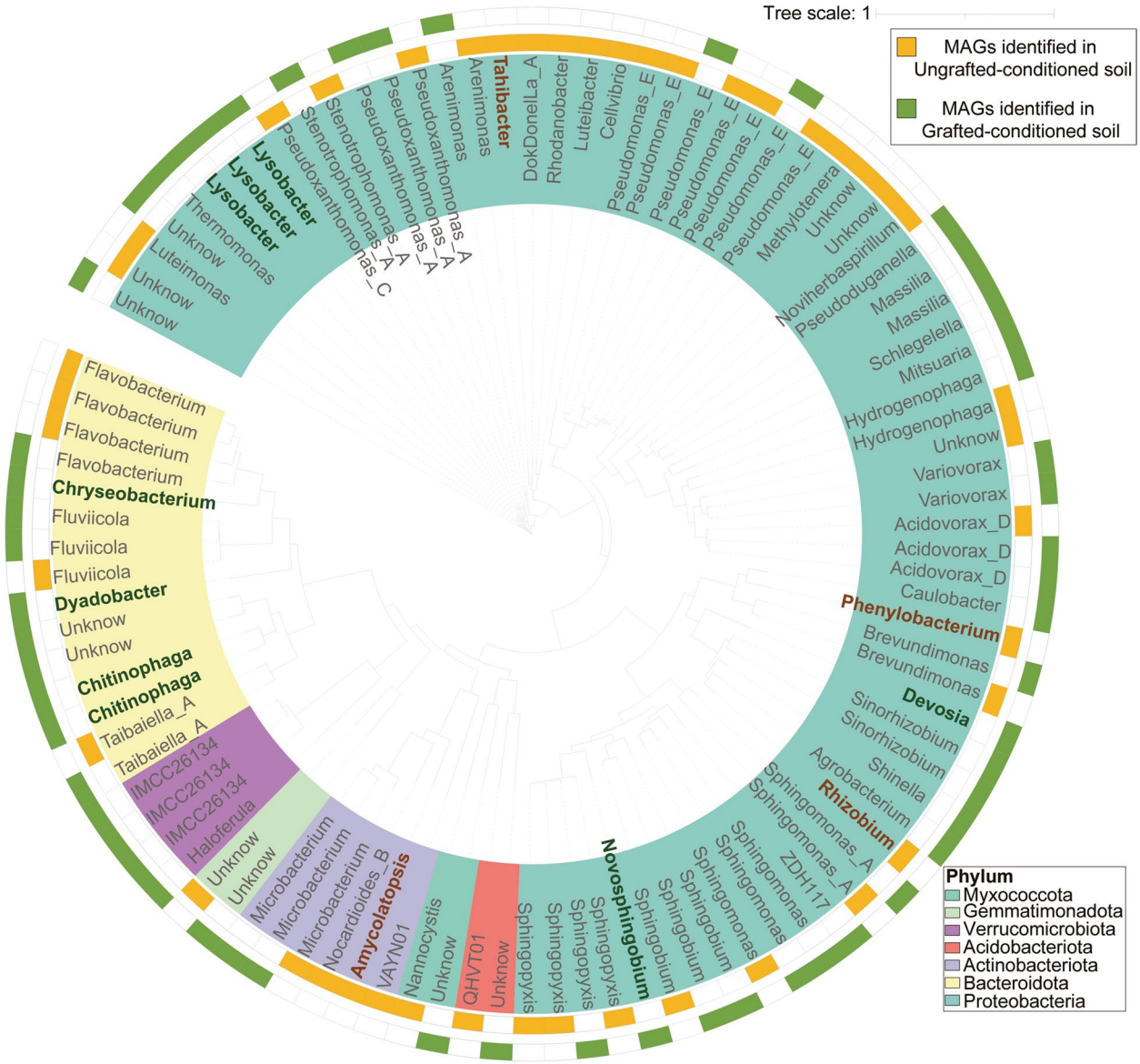

Fig. 6 Phylogenetic tree of the MAGs identified in the rhizosphere of ungrafted and grafted watermelon grown on the ungrafted- and grafted-conditioned soil. In the biotic feedback experiment (experiment no. 2), ungrafted watermelon and grafted watermelon grow in unsterilized soils previously conditioned by ungrafted watermelon or grafted watermelon. The rhizosphere soil was collected for metagenome sequencing and the metagenome-assembled genomes (MAGs) were constructed ($n = 5$). The phylogenetic tree was constructed from all the MAGs (100) with > 70% completeness and < 10% contamination. The colored middle ring represents the phylum to which each MAG belongs. The two outer rings of colored bars represent the presence or absence (white) of each MAG in watermelon plants grown on the ungrafted- (orange) and grafted-conditioned soil (green), respectively. The phylogenetic tree labels only provide genus-level information. Genus-level names in bold represent the intersection between the genus names of significant predictors from the zOTU-level random forest model (Table S4) and the genus names of the MAGs identified in each treatment

grafted-conditioned soil were reconstructed. These MAGs were taxonomically affiliated with seven different bacterial phyla (Fig. 6).

To clarify the function of microorganisms that can predict conspecific plant-soil feedback, the microorganisms identified by random forest analysis (Table S4) were intersected with MAGs from each treatment to detect the putative keystone species and metabolic features of the shared microorganisms. Two MAGs from the rhizosphere of the ungrafted watermelon grown on ungrafted-conditioned soil were found to be affiliated with *Tahibacter* and *Amycolatopsis* (Fig. 7a, b),

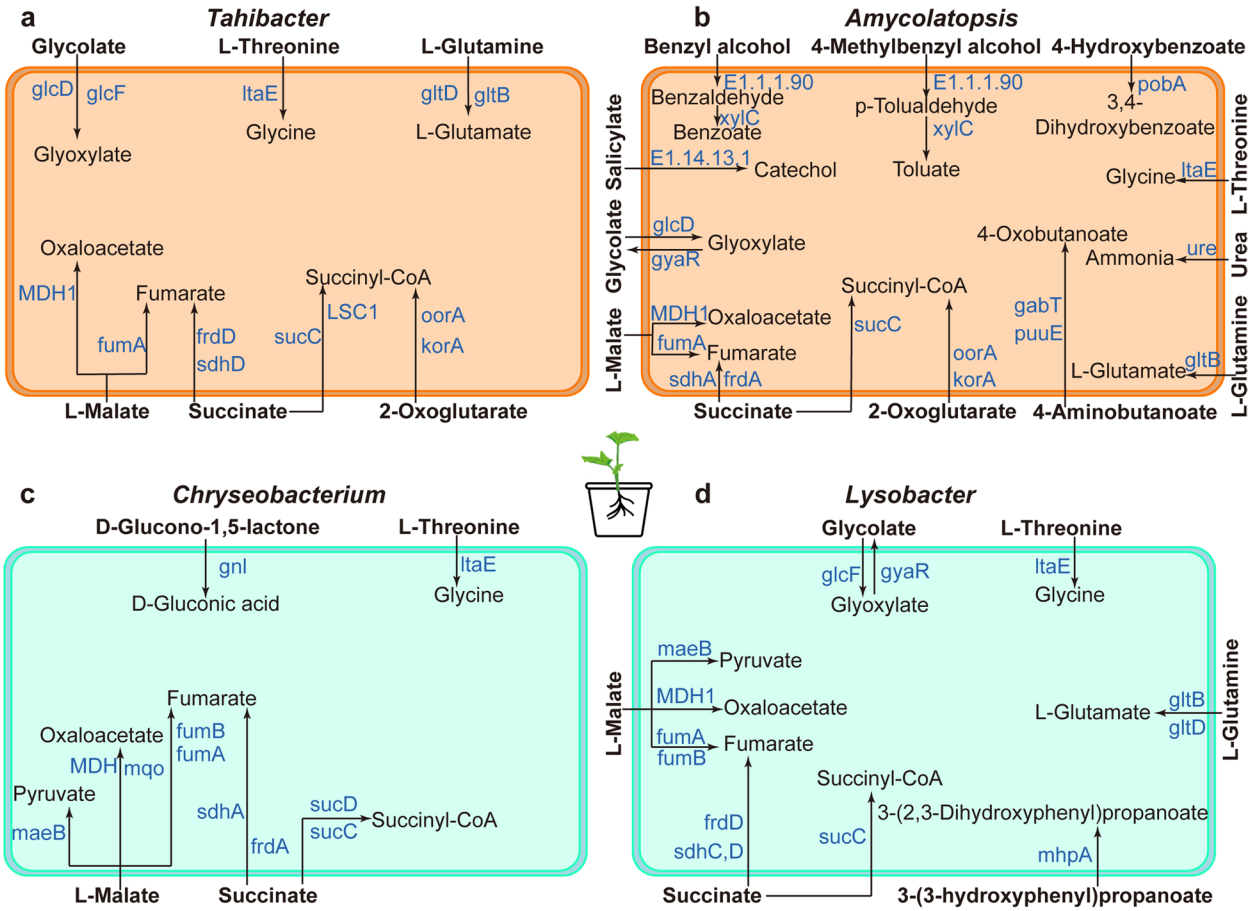

Fig. 7 Metabolic features of four MAGs from ungrafted watermelon grown on the ungrafted-conditioned soil and grafted-conditioned soil. **a**, **b** The MAGs identified in the rhizosphere of ungrafted watermelon grown on the ungrafted-conditioned soil were compared with significant predictors identified by a random forest model at the zOTU level (Table S4), and with MAGs selected by intersection with the shared genus name. **c**, **d** The MAGs identified in the rhizosphere of ungrafted watermelon grown on the grafted-conditioned soil were also compared with predictors identified by a random forest model at the zOTU level (Table S4), and with MAGs selected by intersection with the shared genus name. The metabolic features of the four MAGs reconstructed in this study are highlighted ($n = 5$). Gene symbols are shown in blue font inside the cells

and two other MAGs affiliated with *Chryseobacterium* and *Lysobacter* when the same ungrafted watermelon was grown in the heterospecific soil (Fig. 7c, d). Metabolic and ecological inferences derived from the MAGs were performed for these four MAGs (Fig. 7). *Tahibacter* and *Amycolatopsis* were shown to be putatively able to use the L-malate, succinate, 2-oxoglutarate, glycolate, L-threonine, benzyl alcohol, 4-methylbenzyl alcohol, and 4-hydroxybenzoate (Fig. 7a, b). *Chryseobacterium* and *Lysobacter* were also shown to be putatively able to use the same compounds but not the last three: benzyl alcohol, 4-methylbenzyl alcohol, and 4-hydroxybenzoate. The specific metabolic pathways of *Chryseobacterium* and *Lysobacter* were D-glucono-1,5-lactone degradation and 3-(3-hydroxyphenyl) propanoate (3HPP) metabolism, respectively (Fig. 7c, d). D-glucono-1,5-lactone undergoes degradation by *Chryseobacterium*, resulting in the formation of gluconic acid.

Concerning the grafted watermelon grown on ungrafted-conditioned soil, the intersection of predictor species obtained from random forest analysis with MAGs objectified the putative *Rhizobium* and *Phenylobacterium* (Figure S7) while the same intersection analysis of grafted watermelon grown on the conspecific grafted-conditioned soil, objectified *Chitinophaga*, *Lysobacter*, *Dyadobacter*, *Devosia* and *Novosphingobium* (Figure S8). Among the MAGs, the main shared putative biochemical processes included the metabolism of L-malate, succinate, glycolate, L-threonine, L-glutamine. Especially, D-galactonate could putatively be only metabolized by *Chitinophaga*, *Lysobacter*, *Dyadobacter*, and *Devosia* in the grafted-conditioned soil.

Discussion

In this study, we examined the growth performance of grafted and ungrafted plants grown in isolation and in competition in soil conditioned by their conspecific or heterospecific plants. The fact that the growth performance of grafted and ungrafted plants grown in monoculture diverged makes the experimental system ideal for testing hypotheses related to the plant-derived microbial soil legacy effect.

Soil microbial legacy modified by plant grafting influences plant growth

Interestingly, our results revealed that grafting modulates plant growth performance by significantly modulating soil microbial legacy, confirming the predictions outlined in our hypothesis no. 1. In the conspecific plant-soil feedback experiment (experiment no. 1) ungrafted watermelon exhibited negative plant-soil feedback, i.e., a negative effect of ungrafted watermelon induced by the microbial soil legacy (Fig. 2). This is reminiscent of the observation that long-term rice domestication alters rhizosphere bacteria and has a negative effect on rice seedling vigor [32]. Prolonged cultivation of the same plant species is assumed to influence the rhizosphere microbiota [33], thereby impeding plant growth if there is enrichment in microorganisms that have a negative effect on plants, for instance, pathogens. At the end of the soil feedback conditioning stage, grafting increased the microbial richness and influenced the composition of the rhizosphere microorganisms, consistent with the results of other studies. For example, the bacterial diversity was greater in a grafted tomato system compared to the nongrafted control and the rootstock types determined the bacterial community's composition [34]. The changes in the rhizosphere microbial community caused by grafted plants (Figure S1) are assumed to contribute to the observed positive effects on conspecific plant growth (experiment no. 1, Fig. 2). It is noteworthy that although many studies have shown that different rootstocks can improve plant growth and disease resistance [35, 36], the direction and strength of the plant-soil feedback dependent on the type of rootstock used, which is not yet fully understood. Understanding this relationship will be of great benefit in selecting the most suitable rootstocks for future agricultural production.

To gain further insights into the links between these changes in microbial community composition and plant growth, a supervised machine learning algorithm, a random forest analysis, was used. Specifically, the analysis made it possible to identify the abundance of specific genera that could affect the conspecific plant-soil feedback. For bacteria, *Phenylobacterium*, and *Rhizobium*, and for fungi, both potential pathogens and beneficial fungi were identified as having significant importance in influencing plant growth (Fig. 3). A study of wilt disease under continuous cropping reported that *Phenylobacterium* was notably more present in diseased soil than in healthy soil [37] while *Rhizobium* was found to be involved in non-legume wheat resistance to stress thereby promoting plant growth [38]. Although no root rot symptoms were observed in our experiment, *Monosporascus*-related sequences, a group of fungi, known to be primary pathogens of root rot disease in melons have been identified [39]. In the *Fusarium* genus, sequences of *Fusarium oxysporum* are also found, a fungus known as a pathogen that causes *Fusarium* wilt, a destructive soil-borne plant disease [40]. *Aspergillus* is known for its ability to solubilize inorganic phosphates and hence to promote plant growth [41]. *Penicillium* was shown to have a vast potential for the production of secondary metabolites [42]. These findings highlighted the correlation between key species we identified and plant growth performance. Taken together, the abundance changes of these genera, which can affect plant growth, support hypothesis no. 1 that the microbial legacy effects are modified by grafting. While this correlation suggests potential strategies for disease management, further experimental studies are necessary to confirm the causal relationships and underlying mechanisms.

Soil sterilization suppressed differences in plant performance in the two different plant-conditioned soils (Fig. 5), supporting the hypothesis that changes in soil microbiota are among the first determinants of plant performance, as previously shown by Hu et al. [18]. Another experiment examined how the size-selective removal of soil microbiota affects plant–soil feedback of *Jacobaea vulgaris*, also emphasizing the crucial role of microbiota in influencing plant growth performance [43]. This highlights the significant role of soil microbiota in influencing plant growth, leading to a closer examination of the differences in microbial communities and their functions between various conditioned soils (Figure S5). The grafted conditioned soil, which is enriched with Acidobacteria, significantly enhances soil fertility by increasing the availability of nitrogen through processes such as nitrification [44]. Conversely, the ungrafted conditioned soil, enriched with Bacteroidetes, emphasizes microbial activities such as a higher rate of nitrate reduction [45]. This reduction in nitrate availability can limit the nitrogen supply

for plant uptake, thereby potentially restricting plant growth.

Grafting-mediated indirect effects alter plant-soil feedback outcomes

Our work showed that grafting has a legacy effect mediated by soil microbiota and corroborates the role of keystone microbial species (i.e., random forest analysis) in altering plant-soil feedback. Among the key ecological questions that emerge from these conclusions, one aspect that is presumed to influence the plant-soil feedback effect is plant-plant competition. Experiment no. 1 demonstrated that the plant-soil feedback effect is significantly influenced by whether or not the plant is in competition with other plants. In support of this hypothesis, we observed that the negative conspecific feedback of ungrafted watermelon decreased in strength when subject to competition, consequently weakening the positive conspecific feedback of the competing grafted watermelon. This finding is in line with the result of previous studies indicating that competition does not exacerbate the negative conspecific feedback [46], and can even mitigate the negative feedback effects of conspecific soil pathogens when plants are grown alongside heterogeneous plants [47]. Moreover, a recent study showed that the dilution of pathogens drives productivity benefits from diversity in plant mixtures when pathogen hosts are buffered by unrelated neighbors, diluting pathogen impacts [48].

However, based on the results of a study examining competition between *Jacobaea vulgaris* and *Holcus lanatus*, the negative conspecific feedback of *J. vulgaris* became more pronounced in the presence of interspecific competition in its own soil. This inconsistent result can primarily be attributed to the weak competitiveness of *J. vulgaris*, resulting in poor performance in its conspecific soil [29]. The outcome of plant interactions is influenced by the extent to which soil microbes generate differences in species' average competitive abilities (termed "fitness differences"). This opinion was supported by a comprehensive meta-analysis encompassing 518 pairs of plant species, which revealed that different plants with varying degrees of fitness ultimately influence the outcome of plant-soil feedback [49]. The meta-analysis found that soil microbial communities could enhance or diminish plant fitness depending on the species and their interactions. For example, soil microbes sometimes increase the competitive ability of dominant species, suppressing less competitive ones. In other cases, microbes facilitated the growth of less competitive species by providing nutrients or protection from pathogens, promoting coexistence.

Soil microbial legacy reprograms root metabolic composition

The results of our study provide valuable insights into the significance of soil microbial legacy in shaping the reprogramming of root metabolites (hypothesis no. 3), thereby exerting a direct influence on plant growth. While it is well-established that each plant species has a distinct metabolic network producing specific metabolites, our study revealed that plants exhibit diverse metabolic responses in different ecological environments. This is consistent with the results of a previous study showing that the metabolome of *Centaurea jacea* and *Leucanthemum vulgare* can be altered by soil conditioned by different plant species, underscoring the importance of soil microbial legacy [50]. Examples in the literature show that signaling molecules released outside the roots allow the colonization to pre-filter and condition the rhizospheric microorganisms thus to control which microorganisms from the "conditioned" rhizosphere could later be recruited within the endosphere microbiota. Genetic differences between plants can lead to variations in root characteristics such as size and structure, which subsequently affect microbiome composition [51]. At first sight, these phenomena can be interpreted as being under the control of the plant itself, according to its needs. However, recent studies show that the plant's associated microbiota is capable of reprogramming the metabolic pathways of its host, leading to changes in the production of primary and secondary metabolites secreted by the plant [19]. The microbiota therefore appears to influence its own succession.

To compare the differences in metabolite responses in different soil legacies, the impact of soil biota on the metabolic composition of plant roots was investigated (Fig. 4). Since the biomass of plants grown on ungrafted-conditioned soil was reduced, we assumed that the enriched metabolic pathways found in the roots of these plants may be detrimental to their growth. Our result showed that the sulfur metabolism pathways were enriched in both ungrafted and grafted plants grown on ungrafted-conditioned soil. Sulfur is essential for all organisms and plays a critical role in plant nitrogen uptake [52, 53]. The increase of sulfide in the rhizosphere can enhance phosphate mobility by reacting with iron phosphates to release soluble phosphates, thereby improving plant assimilation and growth. However, it is important to note that excessive sulfide can lead to toxicity and limit phosphorus retention by binding with iron [54, 55]. In grafted-conditioned soil, we observed enriched metabolites involved in arginine biosynthesis pathways in ungrafted plants and in sphingolipid metabolism pathways in grafted plants. Arginine is known to play a pivotal role in plants

[56], contributing to various essential cellular processes. Arginine decarboxylation leads to the production of putrescine, which is crucial for cell expansion and other growth-related functions [57]. The enrichment of arginine biosynthesis pathways in grafted-conditioned soil suggests that these mechanisms are actively engaged, supporting healthy plant development. Sphingolipids act as essential structural components and signaling molecules in plants. They play a crucial role in maintaining cell membrane integrity and fluidity, which is vital for proper cell function and growth [58]. The mechanisms underlying the observed positive feedback in grafted plants and negative feedback in ungrafted plants revolve around specific metabolic pathway activation, which could contribute to the observed differences in plant performance.

Metagenomic and metabolomic integration reveals microbial impact on plant growth

Understanding the mechanisms by which the keystone microbial taxa influence plant growth can provide deep insights into their functional roles and potential applications in sustainable agricultural practices. A review of plant grafting shows that grafting can maximize the interaction between beneficial microorganisms and plants, and that configuring the core microbiome through grafting can enhance crop sustainability [59], which is consistent with our view of focusing on the functions of keystone species. Our study revealed that the key microbial genomes carried the function genes associated with plant growth promotion (thereby supporting hypothesis no. 4).

Notably, in the case of ungrafted watermelons exhibited better growth on heterospecific soil, *Chryseobacterium* and *Lysobacter* were identified as significant contributors to plant growth (Fig. 7c, d). These species have garnered attention for their exceptional beneficial functions [60, 61] and have shown their innate ability to synergistically cooperate with other favorable bacteria, for instance, *Bacillus* [62]. *Chryseobacterium* is involved in a specific metabolic pathway that includes the degradation of D-glucono-1,5-lactone (Fig. 7c). This pathway entails the hydrolysis of D-glucono-1,5-lactone by gluconolactonase, resulting in the formation of gluconic acid. Gluconic acid produced by *Chryseobacterium* may influence *Fusarium* growth, similar to what has been observed in soils resistant to the wheat pathogen *Gaeumannomyces graminis* var. *tritici*. In these soils, gluconic acid produced by certain rhizobacterial populations of *Pseudomonas fluorescens* in the wheat rhizosphere reduces disease incidence [63, 64]. *Lysobacter* are potential synthesizers of (novel) antibiotics [65] and antifungal weapons [66]. Characterization of the antibiotic profile of *Lysobacter* suggests it plays the role of biological control agent of plant pathogenic microorganisms [67]. Comparative genomics profiling of the genus *Lysobacter* revealed that its genomes contain a large number of genes encoding extracellular enzymes, including chitinases, glucanases, and peptidases, which inhibit the hyphal growth of soil-borne pathogenic fungi [68]. Both *Chryseobacterium* and *Lysobacter* exhibit putative specific metabolic pathways that are likely to have contributed to their high microbial antagonistic performance, making them key contributors to the observed variations in plant-soil feedback. Despite the significant advancements made in identifying key genes and metabolic processes through the integration of metabolomics and metagenome analysis, the actual activity and expression levels of these genes remain largely unexplored. Further research, particularly when combined with metatranscriptomic analysis, is essential to pinpoint which genes are actively expressed. Such studies will provide a deeper understanding of gene functionality and their roles in metabolic processes.

Overall, we observed significant variations in the associated microbial communities and growth responses to soil legacy trajectories between ungrafted and grafted plants. As the microbial composition of the rhizosphere was impacted by the long-term monoculture, comprehensive knowledge of the rhizosphere microbial community will help understand how members of the community affect plant growth. The growth of grafted plants led to the enrichment of particular microorganisms in the long-term monoculture soil that had a positive impact on plant biomass. In this way, continuous long-term cropping using grafted plants can mitigate the detrimental influence of the microbial community. One can envisage taking advantage of plant species with desired microbiota phenotypes to control the abundance of at least some microbial taxa and designing microbiome-based communities for the improvement of future crop production. Deeper knowledge of the characteristics of these beneficial microorganisms is still required to understand their ecological relationships with plants and with other microbial taxa, not only to control pathogen density but also to explore possible synergistic effects on plant performance. This opens up promising research avenues for innovative strategies that maximize agricultural productivity while minimizing reliance on chemical inputs.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40168-024-01938-2.

Supplementary Material 1: Supplementary information 1.1: Analysis of soil properties. Supplementary information 1.2: Metabolomics analyses. Table S1. Abiotic characteristics of soils collected at the end of the soil feedback conditioning stage and at the end of conspecific plant-soil feedback experiment (experiment #1). Table S2. Two-way ANOVA results for the effects of conditioning soil type (ungrafted-conditioned soil, grafted-conditioned soil) and growth time on plant biomass. Table S3. Two-way ANOVA results for the effects of conditioning soil type (ungrafted-conditioned soil, grafted-conditioned soil) and plant type (ungrafted watermelon, grafted watermelon) on microbial community composition. Table S4. Importance values of predictive features for the microbiota from random forest analysis at the zOTU level. Figure S1. Bulk soil microbial community composition at the end of the soil feedback conditioning stage. Figure S2. Temporal dynamics of biomass of ungrafted watermelon and grafted watermelon in the conspecific plant-soil feedback experiment (i.e. experiment #1). Figure S3. Rhizosphere microbial composition at the end of the conspecific plant-soil feedback experiment (experiment #1). Figure S4 Comparison of conspecific plant-soil feedback on isolated and competition-grown watermelon plants: Ungrafted watermelon (a) vs Grafted watermelon (b). Figure S5. The rhizosphere microbial composition and functional characteristics of the competition treatments compared between the ungrafted- and grafted-conditioned soil. Figure S6. Rhizosphere metagenomic microbial composition of the plants grown on unsterilized soil in the biotic feedback experiment. Figure S7 Metabolic features of two MAGs identified in grafted watermelon grown on the ungrafted-conditioned soil. Figure S8 Metabolic features of five MAGs in grafted watermelon grown on the grafted-conditioned soil.

Acknowledgements
We are very grateful to He Zhang and Ling Li at Nanjing Agricultural University for their help with data analysis and Huiling Ning for her help with experiments.

Authors' contributions
NL designed the methodology of the study. TTW carried out the experiment and analyzed the data with the help of QCX and YR. TTW wrote the paper with the help of PV. QRS contributed to the project scope. NL, PV, YR, and QCX contributed to the review and editing of the manuscript. All authors contributed critically to the interpretation of the results and gave final approval for publication.

Funding
This study was supported by the National Nature Science Foundation of China (32302670) and Nanjing Agricultural University (XUEKEN2023039).

Availability of data and materials
The 16S rRNA and ITS amplicons raw sequencing data have been deposited in the NCBI database under BioProject ID PRJNA1050607 and PRJNA1050612, respectively. The shotgun metagenomics sequencing data have been deposited in the NCBI database under BioProject ID PRJNA1054185.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 3 April 2024 Accepted: 23 September 2024
Published online: 14 November 2024

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